Stereoselective Total Syntheses of Piericidins, the Neuritogenic Steroid Withanolide A and the Development of Photolabile Surface Anchors

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Prof. Dr. Jörg Schibler
Dekan

Für Sandra und
meine Familie

Ever tried. Ever failed. No matter.
Try again. Fail again. Fail better.

Samuel Becket (1906-1989)

Table of Content

This cumulative PhD-Thesis is based on the following publications and manuscripts submitted for publication:

Chapter 2:

Synthesis of Withanolide A, Biological Evaluation of Its Neuritogenic Properties, and Studies on Secretase Inhibition, C. K. Jana, J. Hoecker, T. M. Woods, H. J. Jessen, M. Neuburger, K. Gademann, *Angew. Chem.* **2011**, *123*, 8557-8561; *Angew. Chem. Int. Ed.* **2011**, *50*, 8407-8411.

Withanolide A: Synthesis and Structural Requirements for Neurite Outgrowth, J. Hoecker, R. Liffert, C. K. Jana, T. M. Woods, P. Burch, H. J. Jessen, M. Neuburger, K. Gademann, *Chem. Sci*. **2013**, *4*, 2851-2857.

Chapter 3:

Nitrocatechols as Tractable Surface Release Systems, J. Hoecker, R. Wehlauch, K. Gademann, *ChemPlusChem* **2012**, *77*, 1071-1074.

Chapter 4:

Caged Retinoids as Photoinducible Activators: Implication for Cell Differentiation and Neurite Outgrowth, J. Hoecker, R. Liffert, P. Burch, R. Wehlauch, K. Gademann, *Org*. *Biomol. Chem.* **2013**, *11*, 3314-3321.

Chapter 5:

Enantioselective Total Syntheses and Absolute Configuration of JBIR-02 and Mer-A2026B, J. Hoecker, K. Gademann, *Org. Lett.* **2013**, *15*, 670-673.

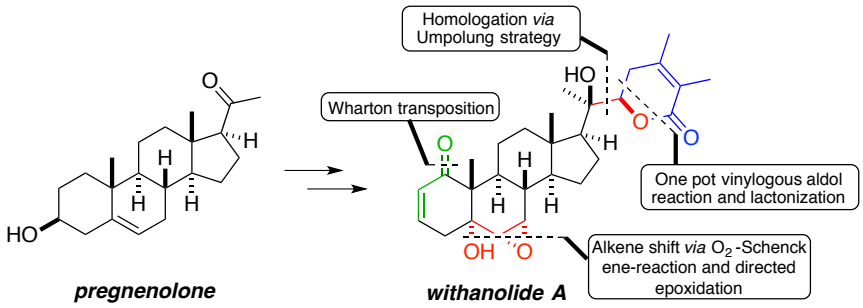
A Unified Synthetic Strategy for the Total Synthesis of Piericidins and Related Natural Products, J. Hoecker, G. Meier, H. Gsellinger, D. Häussinger, M. Neuburger, K. Gademann, *manuscript in preparation.*

Abstract

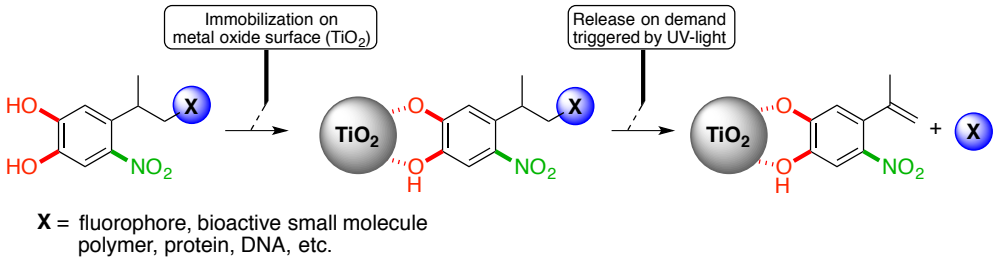
This thesis is divided into five chapters where each corresponds to different research projects inspired and influenced by each other. Every chapter highlights one of the multiple aspects of modern organic synthesis, the application of the obtained targets either for the elucidation of biological phenomena or as a tool to shine a different light on the chemistry of life. The corresponding current state of the research is also enclosed in each chapter along with a brief introduction in this particular domain. Experimental data, spectroscopic analysis and appendices can be found at the end of this thesis.

Chapter 1 starts with a general introduction on the use of natural products and its relationship to *total synthesis* in a historical progression.

Chapter 2 includes a detailed description of the stereoselective synthesis of the neuritogenic steroid lactone withanolide A. Key for the successful total synthesis were mostly strategic considerations for the sequential order of the installment of the enone moiety. The total synthesis was achieved by singlet oxygen ene-reaction, Wharton transposition, a Corey-Seebach homologation and a vinylogous aldol reaction. Biological evaluation demonstrated neurite outgrowth, which further supports the potential neuritogenic role of this compound in traditional Indian medicine.

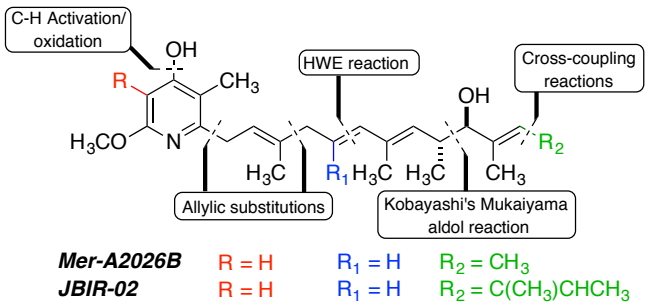


In **Chapter 3** the development of a molecular surface modification platform based on biomimetic nitrocatechol derivatives is reported. This allowed for small molecule functionalization on TiO_2 under mild aqueous conditions and efficient release triggered by light therefore uncaging a small molecule cargo on demand. Small molecules may be markers which become fluorescent upon decaging as shown in the proof-of-principle study or bioactive small molecules for drug release as shown in the following **Chapter 4**.



Here the use of caged retinoic acid derivatives as controllable probes for nerve cell differentiation is explained. These investigations should allow to navigate the axonal growth cones by an extrinsic guidance therefore directing neurite outgrowth by the developed release strategy.

The first total syntheses of the piericidin natural products JBIR-02 and Mer-A2026B, and related piericidins are the topic of **Chapter 5**. Key features of the unified synthetic strategy involved an Ir-catalyzed one pot C-H activation/oxidation procedure for the preparation of multiple hydroxypyridines, a vinylogous Mukaiyama aldol reaction and a Negishi cross-coupling of an advanced pyridine intermediate with the fully elaborated allylic side chain. Following the successful total synthesis, the absolute configurations of Mer-A2026B and JBIR-02 were established as (9*R*,10*R*).



Chapter 6 describes briefly the synthesis of electron-rich 2,2'-bipyridine-systems as sensors for proton coupled electron transfer. The heteroaromatic core structure from the natural product JBIR-02 was successfully implemented to generate various bipyridines.

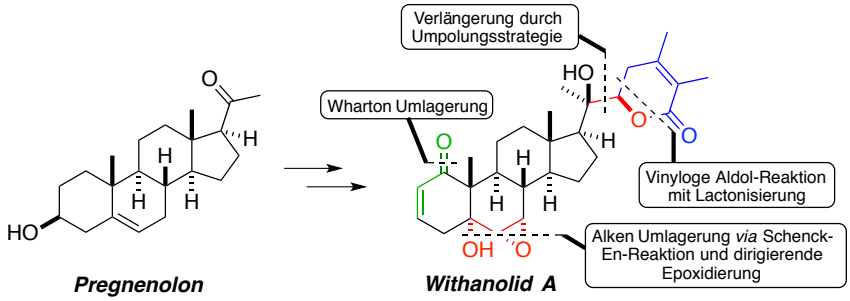
Keywords: Natural Products, Total Synthesis, Neurite Outgrowth, Photocleavable Surface Hybrids, Steroids, Piericidins, Retinoids and Bipyridines

Kurzbeschreibung

Die vorliegende Doktorarbeit gliedert sich in fünf individuelle Teile, die sich gegenseitig mehr oder weniger inspirieren und beeinflussen. Jedes Kapitel beschreibt einen der zahllosen Aspekte moderner organischer Synthesechemie mit Schwerpunkt auf der Anwendung des jeweiligen Synthesetargets, entweder zur Untersuchung komplexer biologischer Systeme oder als "Hilfsmittel", um die Chemie des Lebens in einem anderen Licht zu sehen. Jedes Kapitel enthält eine kurze Einführung zum aktuellen Stand der Forschung im jeweiligen Gebiet. Am Ende der Doktorarbeit sind die experimentellen Vorschriften als auch die entsprechenden spektroskopischen Daten aller Verbindungen in einem eigenen Kapitel angehängt.

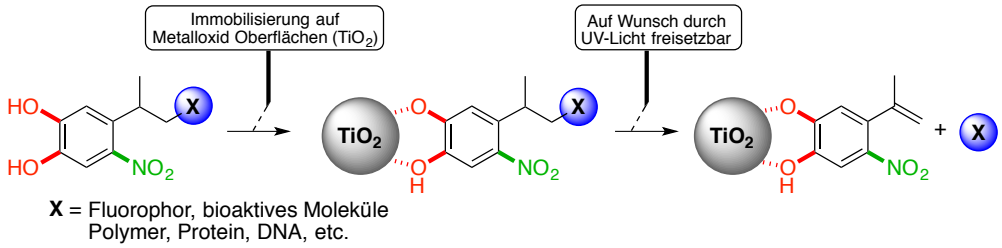
Kapitel 1 startet mit einer Einleitung zur Verwendung von Naturstoffen und deren Verbindung zum Gebiet der Totalsynthese im historischen Kontext.

Die stereoselektive Synthese des Steroidlactons Withanolid A ist das Thema des **2**. **Kapitel.** Schlüssel für die erste Totalsynthese waren vor allem strategische Überlegungen zur Installierung des sensitive A Rings. Schlussendlich führten eine Corey-Seebach-Homologisierung, eine vinyloge Aldolreaktion, eine regioselektive Schenk-En-Reaktion unter Verwendung von Singulett-Sauerstoff sowie eine Wharton-Verschiebung zur erfolgreichen Herstellung. Biologische Untersuchungen demonstrieren Neuritenwachstum, was die potenziell neuroaktive Wirkung dieses Naturstoffs in der traditionellen indischen Medizin stützt.



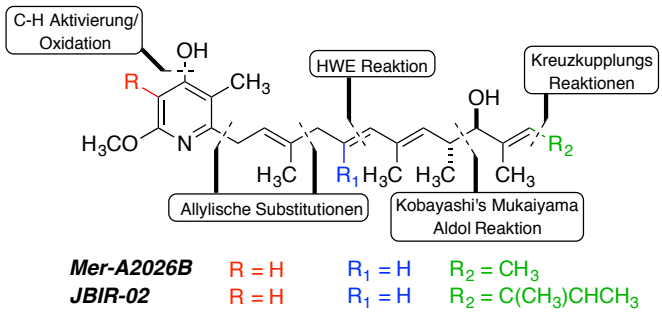
In **Kapitel 3** wird die Entwicklung einer Oberflächenmodifizierung auf molekularem Level beschrieben. Basierend auf biomimetischen Nitrocatecholen erlaubt diese Ankerstrategie TiO_2-Partikel unter milden Bedingungen mit gewünschten Verbindungen zu funktionalisieren, um diese bei Bedarf durch Licht wieder freizusetzen. Die verwendeten Verbindungen (small molecules) können Fluorophore sein wie in einer

ersten konzeptionellen Untersuchung gezeigt oder aber auch bioaktive Moleküle, die bei Bedarf freigesetzt werden, siehe dazu Kapitel 4.



In **Kapitel 4** wird die Verwendung von photoaktivierbaren Retinoiden als Proben für die Zelldifferenzierung erklärt. Die Synthese dieser Verbindungen sollte es erlauben, die Ausbreitung von Axonen zu beeinflussen und damit letztendlich Neuritenwachstum auf Oberflächen zu dirigieren.

Die ersten Totalsynthesen der piericidinen Naturstoffe JBIR-02 und Mer-A2026B sowie verwandter Piericidine sind in **Kapitel 5** zusammengefasst. Zu den Schlüsselschritten dieser Strategie zählen neben einer Ir-katalysierten C-H Aktivierung-Oxidations Sequenz, die zur Herstellung einer Vielzahl von 4-Hydroxypyridinen führt, eine vinyloge Mukaiyama Aldolreaktion sowie eine Negishi-Kreuzkupplung eines hochfunktionalisierten Pyridins mit dem sorgfältig konstruierten Seitenkettenvorläufer. Zusätzlich wurden durch die erfolgreichen Totalsynthesen erstmals die absoluten Konfigurationen von JBIR-02 und Mer-A2026B als (9*R*,10*R*) etabliert.



Kapitel 6 beschreibt die Synthese von elektronenreichen 2,2'-Bipyridin-Systemen als Sensoren für protonengekoppelten Elektronentransfer. Dabei wurde die Verwendung des Heteroaromaten aus Kapitel 5 erfolgreich implementiert.

Schlüsselwörter: Naturstoffe, Totalsynthese, Neuritenwachstum, Fotospaltbare Oberflächenhybride, Steroide, Piericidine, Retinoide und Bipyridine

1 INTRODUCTION

1.1 Natural Products: Inherent Evolutionary Wisdom

Natural products and their sources have been used by mankind ever since and have been tightly associated with the evolution of our culture. Owing to the diverse biological benefits and medicinal potentials of natural products, every civilization has collected experience of their use as aliment, drug or poison e.g. Indian *Ayurveda* or traditional Chinese medicine.[1] The earliest records come from ancient Mesopotamia, circa 2600 BC, describing the use of approximately 1,000 plants and plant-derived substances for the treatment of various illnesses. Other natural compounds originate from microbial or marine sources, usually effecting their isolation and characterization challenging due to very small available quantities.[1]

Nature has optimized biological active compounds in an evolutionary process - its own high-throughput screening - over millions of years to function in the required way as messenger, repellent or attractant.[2,3] At present, natural product motifs still contribute to more than 40% of pharmaceutical lead-structures in drug-discovery, whereas only one new molecular entity from a combinatorial research program was approved as a drug in the last 25 years.[1] Thus, synthesis of natural products as potent drug targets remains even in times of modern biotechnology essential to gain an understanding of their inherent pharmaceutical properties by structure-activity relationship (SAR) studies.

1.2 Total Synthesis: Blending Organic Synthesis and the Game of Chess

Synthetic chemistry (Greek: *synthesis* = the process of putting together) is the handcraft of constructing molecules from simple available precursors. Through which aspects these precursors are chosen, availability, costs or quality, and in which way

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1 D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022-1037.

2 I. Paterson, E. A. Anderson, *Science* **2005**, *310*, 451-453.

3 a) K. Gademann, *Chimia* **2006**, *60*, 841-845; b) H.-F. Ji, X.-J. Li, H.-Y. Zhang, *EMBO Rep.* **2009**, *10*, 194-200; c) A. Eschenmoser, *Angew. Chem*. **2011**, *123*, 12618-12681.

connectivity is established is the purpose of organic chemistry especially when the target molecule is a natural product of *total synthesis*.

Total synthesis can be best compared to the game of chess sharing some of the same characteristics: frustrations, dead-ends and glorious moments.[4,5] The object of chess is to outmaneuver the opponent's king by a combination of moves with the ensemble of chessmen. Similarly in *total synthesis*, the goal is to reach the target compound by a foresighted strategy (using synthetic transformations) to overcome nature's hurdles (complex architectures). In both cases, the effects of the opening moves are felt right to the end game and can be hardly anticipated before.[6] The correspondence of both disciplines is also pictured in the following statement of the former chess world champion Emanuel Lasker (1868-1941): 'When you see a good move, look for a better one,' which inspires chemist to fine-tune synthetic routes towards the same target or molecular framework over and over.

While the game of chess in its todays form is around 300 years old, the birth of *total synthesis* is marked with the synthesis of urea (**1.1**)[7] by F. Wöhler in 1828 when it became apparent that molecules originating from living organisms could be created by men using inorganic 'death' starting materials (**Figure 1.1**). Headed by the German laboratories in Giessen (J. von Liebig, 1803-1873) and Berlin (A. W. von Hofman, 1818-1892) a prospering synthetic period started mainly to understand fundamental rules of connectivity. Later the first simple amino acids (Strecker, 1850),[8] aromatics (Reimer and Tiemann, 1876),[9] sugars (E. Fischer, 1891)[10] and steroids were obtained by means of synthesis to confirm the structures of isolated natural products. Sir R. Robinson's (1886-1975) tropinone synthesis (**1.2**)[11] highlights

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4 K. C. Nicolaou, E. J. Sorensen, *Classics in Total Synthesis*, Wiley-VCH, **1995**, pp. 2-17.

5 'A chess game is divided into three stages: the first, when you hope you have the advantage, the second when you believe you have an advantage, and the third ... when you know you are going to lose!' Savielly Tartakower (1887-1956)
'Chess is not relaxing; it is stressful even if you win.' Jennifer Shahade (born 1980)

6 In chess around 1,300 different named openings are classified: D. Hooper, K. Whyld, *The Oxford Companion to Chess*, Oxford University Press, **1984**.

7 F. Wöhler, *Ann. Phys. Chem*. **1828**, *12*, 253-256.

8 A. Strecker, *Liebigs Ann. Chem.* **1850**, *75*, 27-45.

9 K. Reimer, F. Tiemann, *Ber. Deutsch. Chem. Ges.* **1876**, *9*, 824-828.

10 E. Fischer, *Ber. Deutsch. Chem. Ges.* **1890**, *23*, 799-805.

11 R. Robinson, *J. Chem. Soc.* **1917**, 762-768.

another milestone in the history of total synthesis through its innovative simplicity and biomimetic character.

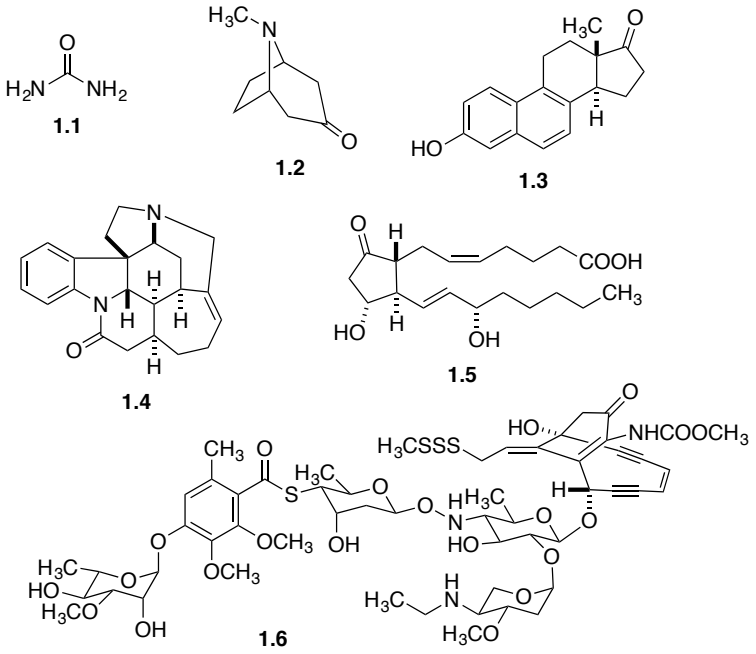

Figure 1.1. Milestones in the field of total synthesis: urea (**1.1**, F. Wöhler, 1828)[7], tropinone (**1.2**, Robertson, 1917)[11], equilenin (**1.3**, W. E. Bachmann, 1939)[12], strychnine (**1.4**, R. B. Woodward, 1954)[13], prostagladine E_2 (**1.5**, E. J. Corey, 1969)[14] and calicheamicin γ_1^I (**1.6**, K. C. Nicolaou, 1992; S. J. Danishefsky, 1994)[15].

After World War II, due to the more sophisticated analytical toolbox (NMR and X-ray structure analysis), first R. B. Woodward (1917-1979) with his foresight and later E. J. Corey (born 1928) with his systematical retrosynthetic disconnections of complex architectures, raised total synthesis to another level. At this stage nature's challenges represented no longer a puzzle about the right structure but the drive for a new generation of chemists equipped with a variety of new methods, reagents and catalysts developed for special strategic maneuvers as for example enantioselective reactions. The following area in the early 1990s was towered by K. C. Nicolaou (born 1946), D. A. Evans (born 1941), S. J. Danishefsky (born 1936) and their co-workers who demonstrated that nearly every natural product, being a 'small molecule' (e.g.

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[12] W. E. Bachmann, W. Cole, A. L. Cole, *J. Am. Chem. Soc.* **1939**, *61*, 974-975.

[13] R. B. Woodward, M. P. Cava, W. D. Ollis, A. Hunger, H. U. Daeniker, K. Schenker, *J. Am. Chem. Soc.* **1954**, *76*, 4749-4751.

[14] E. J. Corey, N. M. Weinshenker, T. K. Schaaf, W. Huber, *J. Am. Chem. Soc.* **1969**, *91*, 5675-5677.

calicheamicin γ_1^I , **1.6**)[15] can be accessed by synthetic organic chemists in a reasonable timeframe.

In this regard *total synthesis* has reached a dead-end street where the performance of synthetic chemistry itself is no longer the sole motivation to pursue a *total synthesis* of a molecule (except for a few famous examples). But instead it requires additional justification, supplementary to the proof of a structure, the assignment of stereogenic centers or the challenge of a complex assembly.

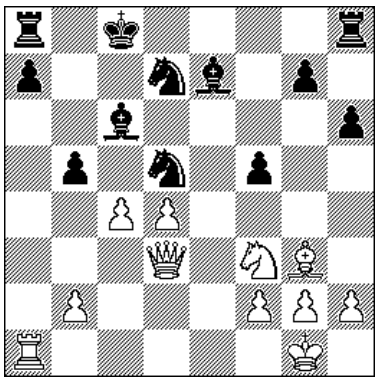

Figure 1.2. Chessboard. Final position in Game 6: J. Kasparov *vs.* Deep Blue.

Similarly, the development of more powerful computers has significantly changed the game of chess in the last two decades. Analogous to *total synthesis* a blockade was reached when grandmasters were no longer able to compete with machines and programs once developed by men started to become dominant (**Figure 1.2**).[16]

1.3 Modern Synthetic Chemistry

Nevertheless, the future for synthetic organic chemistry looks bright. It has the enormous advantage over most other scientific disciplines that it can create its own object, what is reflected in the famous words of French chemist M. Berthelot (1827-1907):[17] 'This creative faculty, similar to arts itself, distinguishes it essentially from

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[15] a) K. C. Nicolaou, C. W. Hummel, E. N. Pitsinos, M. Nakada, A. L. Smith, K. Shibayama, H. Saimoto, *J. Am. Chem. Soc.* **1992**, *114*, 10082-100084; b) S. A. Hitchcock, S. H. Boyer, M. Y. Chu-Moyer, S. H. Olson, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **1994**, *33*, 857-862.

[16] The reigning chess word champion Garry Kasparov lost against the chess-playing computer Deep Blue developed by IBM on March 11, 1997 with 3½:2½ (in 6 games).

[17] French original: « La chimie crée son objet. Cette faculté créatrice, semblable à celle de l'art lui-même, la distingue essentiellement des sciences naturelles et historiques. »

natural and historical sciences.' Because of this innovative power, new disciplines such as chemical biology were established investigating fundamental interaction of bioactive small molecules, being a natural product or not, with its biological environment.[18] Here, sophisticated tools for a better understanding of the most fundamental and complex processes at the cutting edge of biology and chemistry are established by synthetic chemists and applied in an interdisciplinary fashion by other researchers leading for example to 'high-tech drugs' as seen in **Figure 1.3** for a hybrid of calicheamicin γ_1^I and a target-specific antibody (**1.6**).[19]

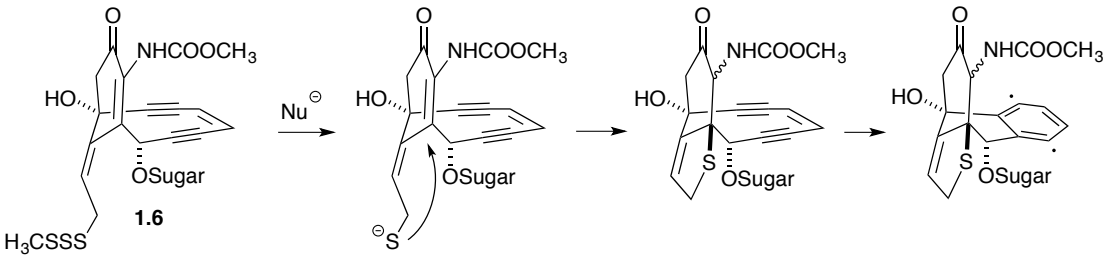

Figure 1.3. Proposed mechanism for the action of calicheamicin γ_1^I (**1.6**): Formation of a reactive aromatic diradical through a cascade reaction (accompanied with a sp^2-sp^3 hybridisation change) induces double-strand cleavage of dublex DNA, thereby causing apoptosis.[20] Delivery of this highly cytotoxic agent by a selective antibody resulted in the development of a recently approved anti-cancer drug.

Another prominent example improving the pharmacological properties of a natural product by synthetic chemistry is the recently developed anticancer drug eribulin or E7389 (**1.8**, **Figure 1.4**).[21] This molecule is a simplified analog of the mitotic inhibitor halichondrin B (**1.7**), a secondary metabolite from the marine sponge *Halichondria okadai*. Eribulin was first synthesized in collaboration of the Kishi group and Eisai Co. Ltd (Tokyo) in 1992 and was approved by the US FDA in 2010 for the treatment of late-stage breast cancer.[22] The structurally complexity of the

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[18] H. Waldmann, *Angew. Chem. Int. Ed*. **2012**, *51*, 6284-6285.

[19] F. E. Koehn, G. T. Carter, *Nature Drug Disc. Rev.* **2005**, *4*, 206-220.

[20] R. C. Hawley, L. L. Kiessling, S. L. Schreiber, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1105-1109.

[21] A. Kirschning, F. Hahn, *Angew. Chem. Int. Ed.* **2012**, *51*, 4012-4022.

[22] a) T. D. Aicher, K. R. Buszek, F. G. Fang, C. J. Forsyth, S. H. Jung, Y. Kishi, M. C. Matelich, P. M. Scola, S. K. Spero, S. K. Yoon, *J. Am. Chem. Soc.* **1992**, *114*, 3162-3164; b) D.-S. Kim, C.-G. Dong, J. T. Kim, H. Guo, J. Huang, P. S. Tiseni, Y. Kishi, *J. Am. Chem. Soc*. **2009**, *131*, 15636-15641; c) C.-G. Dong, J. A. Henderson, Y. Kaburagi, T. Sasaki, D.-S. Kim, J. T. Kim, D. Urabe, H. Guo, Y. Kishi, *J. Am. Chem. Soc*. **2009**, *131*, 15642-15646.

western fragment was substituted by a simplfied amino alcohol subunit, retaining its biological activity and modifying the structure a bit more druglike. Here contemporary synthesis shows its capacity to meet the requirement of supplying this fully synthetic molecule incorporating 18 stereogenic centers in gramm quantities.

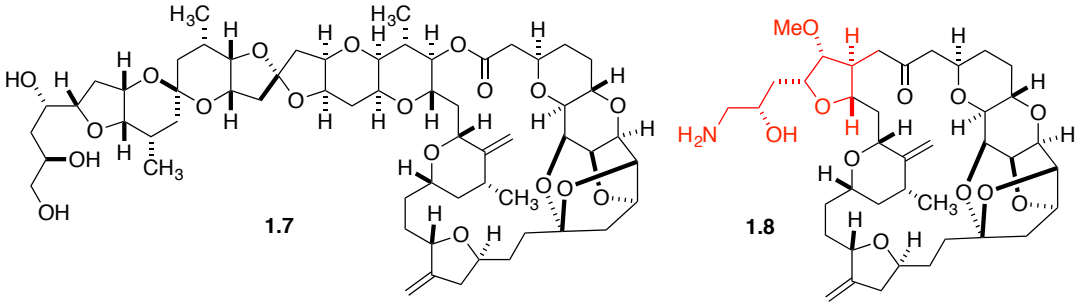

Figure 1.4. Structure of halichondrin B (**1.7**) and eribulin (**1.8**, the structural simplification with respect to halichondrin B is highlighted in red).

Similar the immunosuppressive polyketide discodermolide **1.9** was prepared by S. J. Mickel and coworkers at Novartis in 2002 on process-scale (>100 g of natural product).[23] In their campaign to obtain quantities of the natural product (**1.9**) for clinical development the asymmetric *total synthesis* was performed as biotechnology approaches utilizing polyketide synthatases have not yet been demonstarted on such a significant scale. Examination of various published synthetic approaches[24] with particulary view on the scale-up lead in less then 20 months to a 36 steps route with an overall yield of 0.2% and only 15 chromatographic purification necessary. Unfortunately unexpected toxicity of the compound in clinical trials did not lead to the manufactoring of the drug, but set a new standard for what is possible though *total synthesis* especially in an industrial research program.

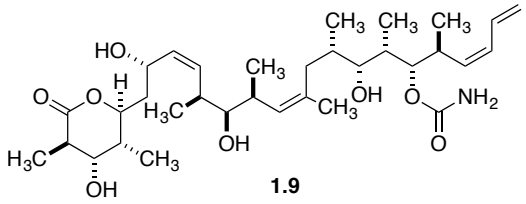

Figure 1.5. Structure of discodermolide (**1.9**).

[23] a) S. J. Mickel, *Strategies and Tactics in Organic Synthesis, Vol. 6*, Elsevier, **2005**, pp. 269-317; b) S. J. Mickel, *Pure Appl. Chem*. **2007**, *79*, 685-700.

[24] For reviews: a) A. B. Smith III, B. S. Freeze, *Tetrahedron* **2008**, *64*, 261-298; b) G. J. Florence, N. M. Gardner, I. Paterson, *Nat. Prod. Rep.* **2008**, *25*, 342-375.

A more recent scafold investigated in the Waldmann group is the natural product family of secoyohimbanes to which the epimeric rhynchophylline (**1.10**) and isorhynchophylline (**1.11**) belong.[25] These spiro indol alkaloids exhibit potential neuromodulatory activities, which is reflected in a substaintial increase of single neurite length and a rise of the number of neurites per neuron. Through an asymmeric catalytic domino Michael-Mannich reaction a small collection of natural products analogues of type **1.12** was obtained an evaluated in rat hippocampal neurons as well as in mouse models to investigate on their strucural activity relationship.[25d]

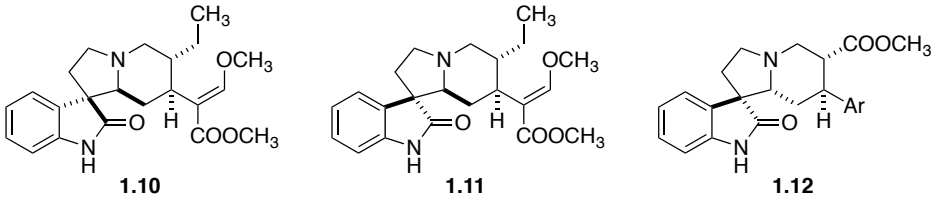

Figure 1.6. Structures of neuroactive rhynchophylline (**1.10**), isorhynchophylline (**1.11**) and the core structure of analogues (**1.12**) from the Waldmann group.

To summarize, organic chemists of the 21st century especially the ones dealing with the *total synthesis* of natural products do not singularly face problems associated with the development of reliable, economical and well-designed syntheses, but also involve in applying their obtained targets to related disciplines such as biology, pharmacology, medicine or even physics.

This thesis will present the possible use of natural products obtained by the means of *total synthesis* to elaborate questions from neuroscience in the context of cell differentiation as well as navigation of these differentiated neurites. Furthermore, it will be shown how modern organic chemistry can generate simple 'tools' adjustable to a particular demand e.g. a new innovative surface anchors inspired by natures structures and functionalities. Finally, new synthetic tactics for the *total synthesis* of structurally complex natural products will be disclosed and pragmatically applied in related research.

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[25] For the total synthesis: a) R. Stahl, H. J. Borschberg, P. Acklin, *Helv. Chim. Acta* **1996**, *79*, 1361-1378; b) A. Lerchner, E. M. Carreira, *J. Am. Chem Soc*. **2002**, *124*, 14826-14827; c) A. Deiters, M. Pettersson, S. F. Martin, *J. Org. Chem*. **2006**, *71*, 6547-6561; d) A. P. Antonchick, S. López-Tosco, J. Parga, S. Sievers, M. Schürmann, H. Preut, S. Höing, H. R. Schöler, J. Sternckert, D. Rauh, H. Waldmann, *Chemistry & Biology* **2013**, *20*, 500-509.

2 WITHANOLIDE A: SYNTHESIS AND EVALUATION ON ITS NEURITOGENIC PROPERTIES

2.1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by amyloid plaques formation, dysfunctional neurons and loss of cognitive functions.[26] The numbers of people suffering from this disease is increasing at an alarming rate, with death occurring approximately 9 years after first diagnosis.[27] The pathogenesis of AD is yet to be fully understood, with both genetic and environmental factors contributing to the progress of the disease.[28] Currently, dementia is treated mainly with acetylcholine esterase inhibitors that delay the progression rather than actually restoring the brain function. Giving about the prevalence of AD and unmet long-term therapies, there is a demanding requirement for the evaluation of new drug leads for AD and associated diseases.[26] Additional to the inherent challenge for CNS drugs to bypass the blood-brain-barrier, clinical studies to observe significant differences in patients *vs.* control requires an undefined length as the initiation and the manifestation of the disease are usually separated by decades.

Figure 2.1. *Withania somniferia* (on an Indian stamp): Highlight the value of this traditional plant and the *Ayurvedic* medicine in general for the Indian society.

Ashwagandha (from the roots of *Withania somniferia*, also known as *Indian Ginseng*) is one of the most prominent drugs in traditional *Ayurvedic* medicine. It is

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[26] Alzheimer's Disease International, *World Alzheimer Report* **2012**, 1-13.

[27] Review: P. Williams, A. Sorribas, M.-J. R. Howes, *Nat. Prod. Rep.* **2011**, *28*, 48-77.

[28] D. K. Lahiri, B. Maloney, M. R. Basha, Y. W. Ge, N. H. Zawia, *Curr. Alzheimer Res.* **2007**, *4*, 219-228.

used as nootropic to improve the intellectual performance of the elderly[29] in the context of anti-aging, memory reconstruction and more recently as cognitive enhancers for healthy subjects.[30]

One of the most active constituents of the methanolic extracts, withanolide A **2.1** showed very promising neuroactive properties in human neuroblastoma cell model as well as in mice: axonal outgrowth, regeneration of neurites and recovery of damaged synapses.[31,32]

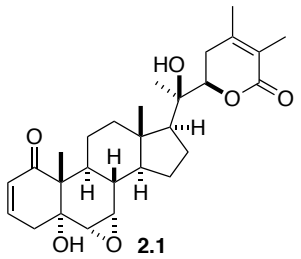

Figure 2.2. Structure of withanolide A (**2.1**).

These remarkable biological properties are further extended by a recent study documenting that withanolide A (**2.1**) modulates several secretase targets with regard to neurodegenerative diseases. Chan and co-workers lately demonstrated that this steroid lactone **2.1** downregulates BACE1 and upregulates ADAM10 in primary rat cortical neurons.[33] These combined reports provide a strong pharmacological rationale for synthetic investigation of withanolide A (**2.1**), for which no preparation from simple steroid precursors has yet been reported.[34]

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[29] Selected articles: a) J. Harris, R. C. Kessler, M. Gazzaniga, P. Campbell, M. J. Farah, *Nature* **2008**, *456*, 702-705; b) M. J. Farah, J. Illes, R. Cook-Deegan, H. Gardner, E. Kandel, P. King, E. Parens, B. Sahakian, P. R. Wolpe, *Nature Neurosci. Rev.* **2004**, *5*, 421-425.

[30] http://www.cerebralhealth.com/brainhealthsupplements.php

[31] For neuroactive properties of withanolide A: a) T. Kuboyama, C. Tohda, J. Zhao, N. Nakamura, M. Hattori, K. Komatsu, *Neuroreport* **2002**, *13*, 1715-1720; b) J. Zhao, N. Nakamura, M. Hattori, T. Kuboyama, C. Tohda, K. Komatsu, *Chem. Phar. Bull.* **2002**, *50*, 760-765; c) T. Kuboyama, C. Tohda, K. Komatsu, *Brit. J. Pharmacol.* **2005**, *144*, 961-971.

[32] For neuroactive properties of *ashwagandha*: S. Jain, S. D. Shukla, K. Sharma, M. Bathnagar, *Phytother. Res.* **2001**, *15*, 544-548.

[33] S. P. Patil, S. Maki, S. A. Khedkar, A. C. Rigby, C. Chan, *J. Nat. Prod.* **2010**, *73*, 1196–1202.

[34] P. Neogi, M. Kawai, Y. Butsugan, Y. Mori, M. Suzuki, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4479-4481.

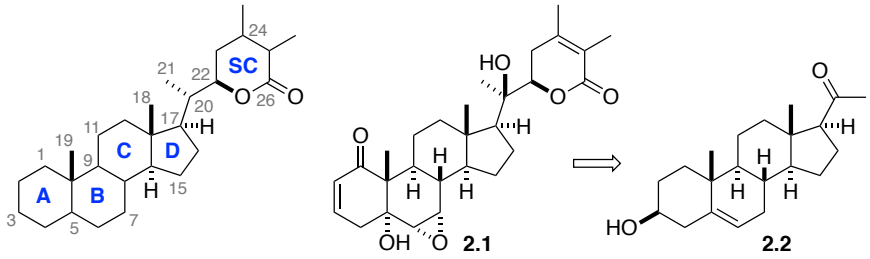

Figure 2.3. General numbering for steroid backbones and structure of the commercially available retrosynthetic precursor pregneolone (**2.2**)

Withanolide A is a highly oxygenated steroidal lactone offering several challenges in the context of a total synthesis. The natural product family consists of the A, B, C and D ring and the lactone side chain (SC, **Figure 2.3**).[35,36] Withanolide A possess a reactive enone moiety in the A ring, an epoxy alcohol in the B ring, and a tetrasubstituted unsaturated lactone as the side chain. It is obvious that the main synthetic challenges arise in the stereoselective construction of the side chain and the oxidation pattern of the A and B ring, but the diastereoselective installment of a tertiary alcohol at C-20 was also found to be not-trivial. Approaching the synthesis of a steroid, it was proposed to start from the commercially available and inexpensive steroidal precursor pregnenolone **2.2**.

The following chapters will disclose the synthetic evolution observed through different approaches undertaken towards the first total synthesis of withanolide A, including those that were unsuccessful.[37] Furthermore, we envisioned that the synthesis of withanolide A derivatives should be accessible using semi-synthetic transformations starting from the natural product. Besides the acquired knowledge concerning the reactivity pattern of the natural product for its total synthesis, these compounds should help to better understand the pharmacological properties of **2.1**. The derivatization should be mainly focused on the A ring of withanolide A as its

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[35] Reviews on withanolide structures, bioactivities and synthetic approaches: a) L.-X. Chen, H. He, F. Qiu, *Nat. Prod. Rep.* **2011**, *28*, 705-740; b) I. Kirson, E. Glotter, *J. Nat. Prod.* **1981**, *44*, 633-647; c) N. V. Kovganko, Z. N. Kahkan, *Chem. Nat. Compd.* **1997**, *33*, 133-145.

[36] Synthetic investigations on withanolides: a) K. Gamoh, M. Hirayama, N. Ikekawa, *J. Chem. Soc., Perkin Trans. I* **1984**, 449-454; b) A. Perez-Medrano, P. A. Grieco, *J. Am. Chem. Soc.* **1991**, *113*, 1057-1059; c) E. Glotter, S. Kumar, M. Sahai, A. Goldman, I. Kirson, M. Medelovici, *J. Chem. Soc., Perkin Trans. I* **1991**, 739-745; d) M. Ishiguro, A. Kajikawa, T. Haruyama, Y. Ogura, M. Okubayashi, M. Morisaki, N. Ikekawa, *J. Chem. Soc., Perkin Trans. I* **1975**, 2295-2302.

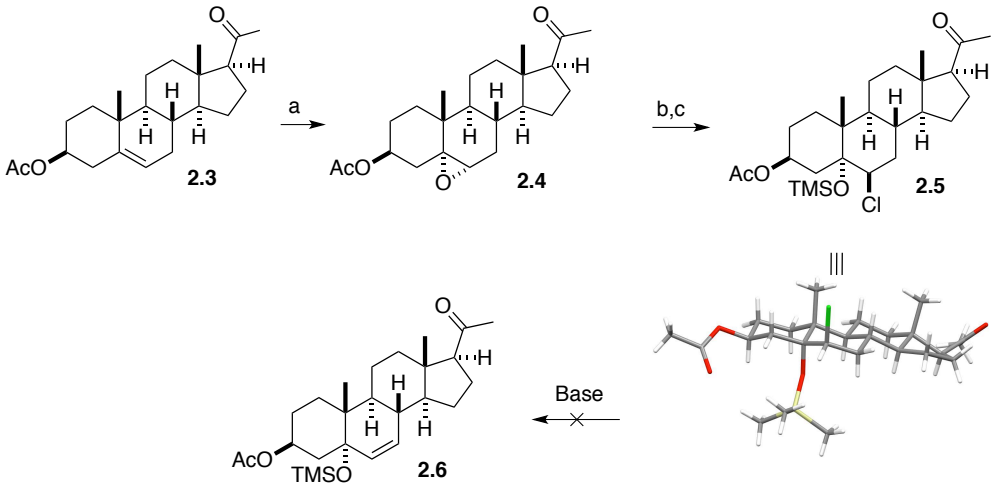
[37] T. Woods, Research report, EPF Lausanne, **2010**.

reactivity showed to be critical for successful transformations, what will be explained in more detail in the following sections. Finally, the natural product as well as its analogues will be tested in cell assays to improve the understanding of the biological mode of action regarding their neurite outgrowth abilities.

2.2 Results and Discussion

2.2.1 1st Generation Approach: The B Ring Hurdle[37]

The first generation approach commenced with the investigation towards the synthesis of the B ring. It was envisioned that an epoxidation of the 5,6-double bond followed by the opening of the corresponding epoxide with a nucleophile would furnish the desired tertiary alcohol at C-5, thereby allowing functionalization of the B ring first.



Scheme 2.1. Investigations on the B ring synthesis of withanolide A: a) *m*-CPBA, CH_2Cl_2, RT, 99%, (*dr* 3:1); b) $BiCl_3$, dioxane, 80 °C, 76%; c) i) TMSOTf, 2,6-lutidine, CH_2Cl_2, -15 °C to RT; ii) HCl in THF, 90%.

Thus, commercially available pregnenolone acetate **2.3** was treated with *meta*-chloroperoxybenzoic acid (*m*-CPBA) in CH_2Cl_2 to afford epoxide **2.4** as a 3:1 mixture of the α- and β-epoxides in quantitative yield (**Scheme 2.1**). Recrystallization from EtOAc/hexane gave the desired α-isomer. Following the procedure of Pinto *et al.*,[38] the epoxide **2.4** was opened with the use of $BiCl_3$ in dioxane at elevated temperatures giving the expected regioselectivity and provided chlorohydrin in a 76% yield. Tri-

[38] R. M. A. Pinto, J. A. R. Salvador, C. Le Roux, *Tetrahedron* **2007**, *63*, 9221-9228.

methylsilylation was performed using TMSOTf to form the TMS ether **2.5** in 90% yield. During the cause of this reaction, the formation of the enol ether at the methyl ketone (C-20) was initially observed, followed by much slower alcohol protection. However, once all the starting material was converted to the *bis*-silyl intermediate, the crude material was exposed to HCl in THF, and resulting in mono-desilylation to give the TMS protected alcohol **2.5**. With chlorohydrin **2.5** in hands, it was expected that the elimination process of **2.5** to give **2.6** would be a facile step by using a strong base.

Unfortunately, all attempts for this transformation proved unsuccessful. X-ray analysis of chloride **2.5** confirmed its structure and more importantly, the diaxial arrangement of the chloride and hydrogen needed for E2 elimination. Attempts to form the analogous bromohydrin and iodohydrin were unsuccessful and consequently this approach was abandoned.[37]

2.2.2 2nd Generation Approach: Elaboration of the A and B Ring[37]

Before exploring the chemistry of the B ring, the homologation required for the side chain was carried out using a known Corey-Seebach umpolung strategy.[39] Thus, the 3β-OH of pregnenolone **2.2** was protected as TBS ether under standard reaction conditions (**Scheme 2.2**). The methyl ketone was treated with the lithiated dithiane to afford the tertiary alcohol **2.7** in a 95% yield (*dr* 15:1). The removal of the dithiane moiety by treatment with $HgCl_2$ afforded the globally deprotected α-hydroxy aldehyde, which was found to be unstable and partial decomposition (probably *via* a 1,2-rearrangement) was observed. The reprotection of the aldehyde was necessary for the oxidative modifications in the A and B rings and was achieved by using propanediol, methylorthoformate and catalytic amount of $Sc(OTf)_3$ and to afford the acetal **2.8** in reasonable yield. With **2.8** available, functionalization of the B ring could then be explored.

Accordingly, diol **2.8** was epoxidized with *m*-CPBA in CH_2Cl_2 in a 95% yield with a 4.5:1 diastereomeric ratio in favour of the desired α-epoxide **2.9**.[37] Several attempts were made to affect the key epoxide-allylic-alcohol-rearrangement to allylic alcohol **2.10** without success using strong bases such as LDA or KO*t*-Bu (**Scheme 2.2**). It was

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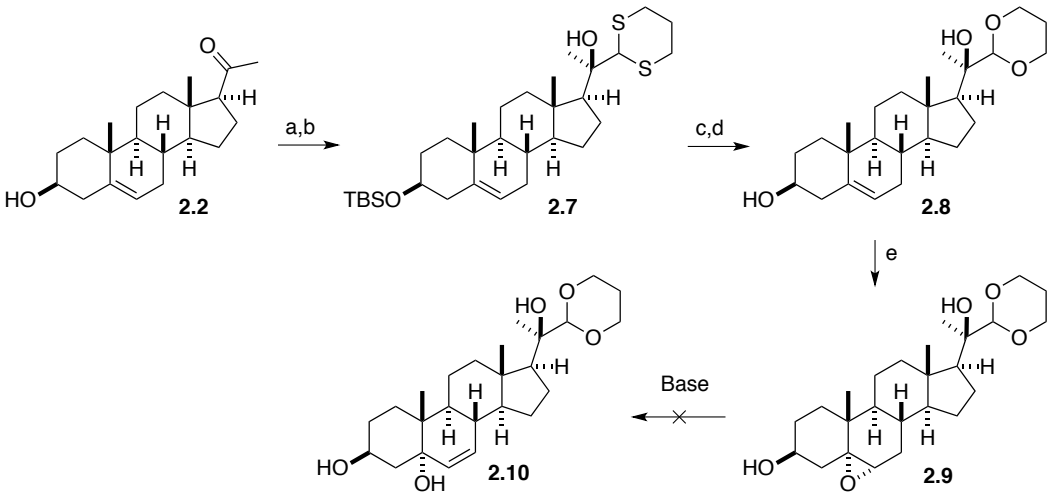
[39] B. B. Shingate, B. G. Hazra, V. S. Pore, R. G. Gonnade, M. Bhadbhade, *Tetrahedron* **2007**, *63*, 5622-5635.

thought that the presence of two unprotected hydroxy functions might be the reason for the failure.

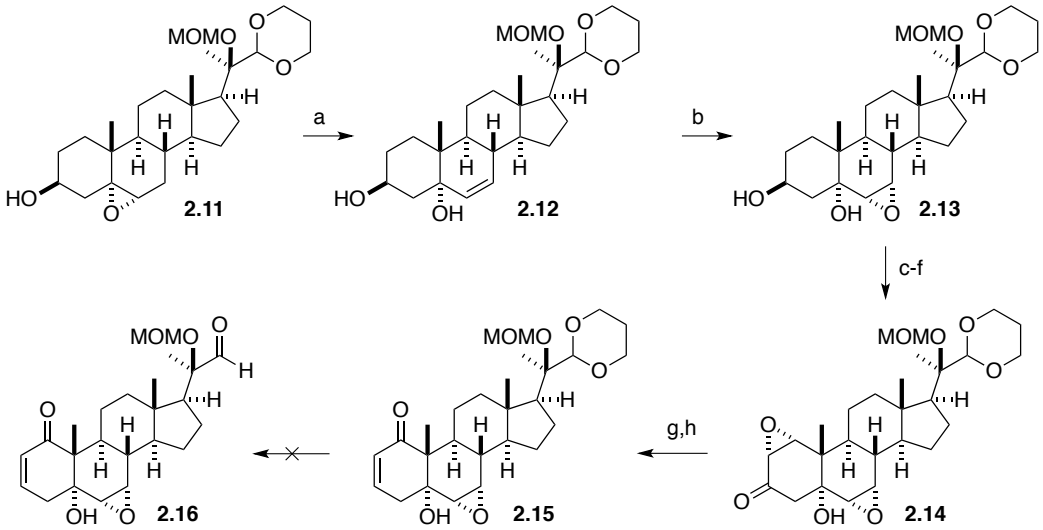


Scheme 2.2. Second generation approach towards the total synthesis of diol **2.10**: a) TBSCl, imidazole, THF; b) 1,3-dithiane, *n*-BuLi, THF, -78 °C to RT, 82% (2 steps, *dr* 15:1); c) $HgCl_2$, HgO, CH_3CN, H_2O; d) $Sc(OTf)_3$, $(MeO)_3CH$, 1,3-propandiol, 47% (2 steps); e) *m*-CPBA, CH_2Cl_2, RT, 95% (*dr* 4.5:1).

To address this problem the tertiary alcohol was protected as methoxymethyl (MOM) ether **2.11** after the removal of the dithiane protecting group (similar to **Scheme 2.2**). Following a method described by Sharpless *et al*. for the oxidative opening of epoxides,[40] a reduction of $(PhSe)_2$ with $NaBH_4$ in EtOH was followed by treatment with MOM protected epoxide and H_2O_2, to afford the desired allylic alcohol **2.13** in 42% yield (79% *brsm*, **Scheme 2.3**). The undesired β-epoxide was not reactive towards these conditions and separation was easily achieved. Allylic alcohol **2.12** was sensitive to acidic conditions and was prone to rearrange to the presumably more stable trisubstituted olefin. The allylic alcohol **2.12** was then transformed to the epoxy alcohol **2.13** using *m*-CPBA in 88% yield. In this reaction, the allylic hydroxyl group adequately directed the epoxidation to achieve a high diastereoselectivity. Next, the secondary alcohol **2.13** was oxidized using tetrapropylammonium perruthenate (TPAP) and *N*-methyl morpholine *N*-oxide (NMO), and the resulting ketone was converted to its silyl enol ether using TIPSOTf. After allylic oxidation with DDQ and

[40] K. B. Sharpless, R. F. Lauer, *J. Am. Chem. Soc.* **1973**, *95*, 2697-2699.

t-BuOOH turned out to be unsuccessful,[41] Saegusa oxidation of the enol ether was performed instead.[42] The TIPS enol ether was treated with 1 eq. of $Pd(OAc)_2$ in CH_3CN and the desired α,β-unsaturated ketone was isolated in quantitative yield. Epoxidation of the unsaturated ketone under basic conditions gave the *bis*-epoxide **2.14** in 79% yield with complete diastereoselectivity. K_2CO_3 was found to be the best choice of base, whereas stronger bases such as NaOH and KO*t*-Bu gave complex mixtures of products.



Scheme 2.3. MOM protected tertiary alcohol as precursor for the installment of the A and B ring: a) *i)* $(PhSe)_2$, $NaBH_4$, EtOH; *ii)* **2.11**, THF; *iii)* H_2O_2, 42% (79% *brsm*); b) *m*-CPBA, CH_2Cl_2, 88%; c) TPAP, NMO, CH_2Cl_2; d) TIPSOTf, Et_3N, CH_2Cl_2; e) $Pd(OAc)_2$, CH_3CN, 63% (3 steps); f) K_2CO_3, H_2O_2, THF, H_2O, 79%; g) $N_2H_4 \cdot HCl$, Et_3N, CH_3CN, 52%; f) TPAP, NMO, CH_2Cl_2, 85%.

Having synthesized the keto epoxide **2.14** the stage was set for the Wharton transposition. Nevertheless, Wharton's original conditions ($N_2H_4 \cdot H_2O$, AcOH) afforded the desired allylic alcohol in poor yield (28%).[43] However in 1989, Dupuy and Luche reported revised conditions for the Wharton transposition using basic conditions.[44] Satisfyingly, these conditions gave the allylic alcohol in an improved yield of 52%. Oxidation of allylic alcohol again with TPAP, NMO afforded the

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41 J.-Q. Yu, H.-C.Wu, E. J. Corey, *Org. Lett.* **2005**, *7*, 1415-1417; b) A. J. Catino, R. E. Forslund, M. P. Doyle, *J. Am. Chem. Soc.* **2004**, *126*, 13622-13623.

42 Y. Ito, T. Hirao, T. Saegusa, *J. Org. Chem*. **1978**, *43*, 1011-1013.

43 P. S. Wharton, D. H. Bohlen, *J. Org. Chem*. **1961**, *26*, 3615-3616.

44 C. Dupuy, J. L. Luche, *Tetrahedron* **1989**, *45*, 3437-3444.

α,β-unsaturated ketone **2.15** in 85% yield and therefore completed the construction of the A and the B rings.

The following plan was to unmask the aldehyde (**2.16**) and to introduce the eastern side chain in order to form the desired lactone. However, the removal of the acetal protecting group was proved to be extremely difficult. Almost all sets of conditions attempted initially removed the MOM protecting group, whereas others were too harsh and led to complex mixtures of products due to decomposition. This approach had to be abandoned at this stage and a search for suitable protecting group was necessary to complete the target, which is outlined below.

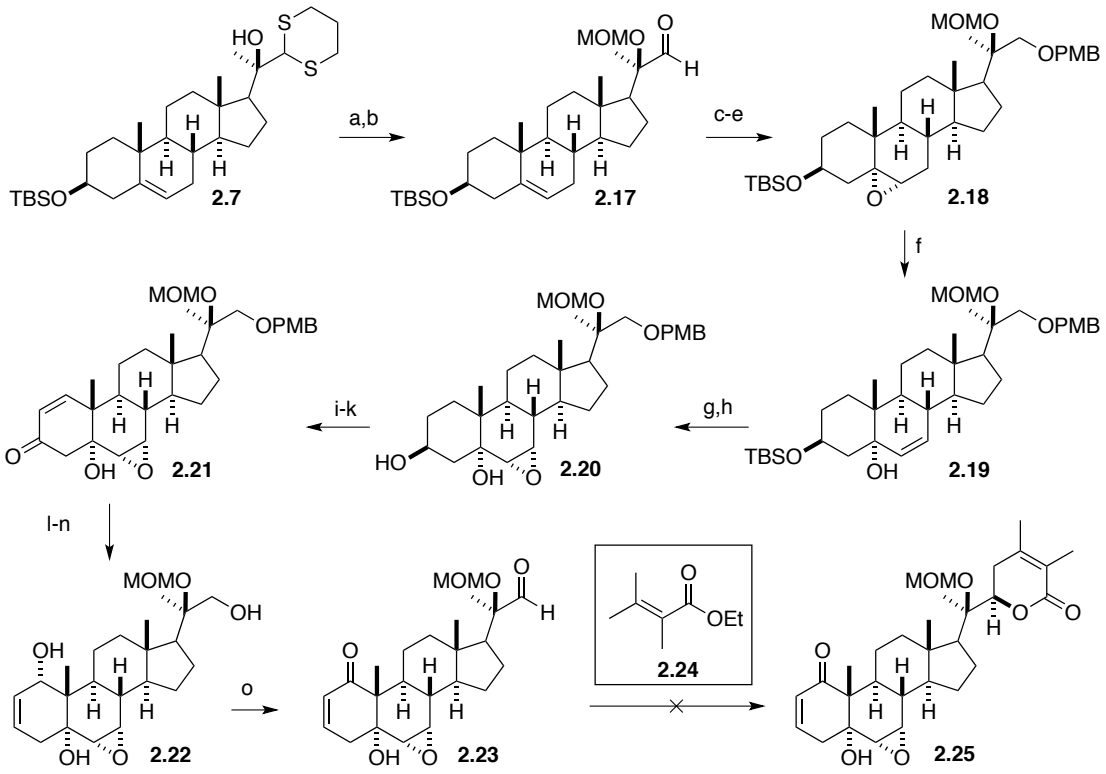
2.2.3 3rd Generation Approach: New Protecting Group Strategy[37]

Careful consideration of alternative protecting groups resulted in the use of the *para*-methoxybenzyl (PMB) group, which would require the reduction of the aldehyde prior to its attachment. The chemistry of this approach is analogous to the one of the second generation, which had proven reliable. It was further anticipated that we could use a procedure developed by Ikekawa to form the lactone from a simple steroidal aldehyde by a vinylogous Mukaiyama aldol reaction in the last step.

Several methods were investigated to induce the cleavage of the dithiane moiety in **2.7**, with the main concern being the stability of the TBS protecting group. Conditions based on $HgCl_2$, NBS and I_2 both with and without addition of base were unsuccessful and resulted in either desilylation, formation of the thioacetal or more complex mixtures. Pleasingly, simple treatment of dithiane **2.7** with NCS in a CH_2Cl_2/H_2O mixture in an open vessel at room temperature afforded the desired α-hydroxy-aldehyde in 73%.

The resulting alcohol was MOM-protected using *N*,*N*-diisopropylethylamine (DIPEA) and MOMI in 94%. The conversion of the reaction was found to be depended on the quality of the starting materials, in particular, the NaI was dried under high vacuum at 100 °C for several hours and the MOMCl was freshly distilled before use. A simple filtration of the crude reaction mixture through a short pad of silica was sufficient to provide material for the next step. Subsequent, reduction of aldehyde **2.17** with $NaBH_4$ in MeOH furnished the desired alcohol as a white solid, which was used in the next step without further purification.The PMB protection was then performed utilizing PMBCl and a catalytic amount of tertabutylammonium

iodide (TBAI) to furnish benzyl ether **2.18**. Epoxidation of the olefin proceeded smoothly affording a 4.1:1 diastereomeric ratio of the desired α-epoxide **2.18** in 71% yield over three steps. The inseparable diastereomers were used as a mixture in the next step, at which point the desired product was separated by column chomatography. Rearrangement of epoxide **2.18** to allylic alcohol **2.19** was carried out as described earlier,[40] affording a 48% yield of compound **2.19**, and the remaining unreacted starting material could be recycled. The second epoxidation of allylic alcohol **2.19** with *m*-CPBA afforded the allylic epoxide with complete selectivity and in high yield (96%).



Scheme 2.4. Third generation approach for the total synthesis of withanolide A: a) NCS, CH_2Cl_2, H_2O; b) NaI, MOMCl, DIPEA, DME, 69% (2 steps); c) $NaBH_4$, MeOH; d) NaH, PMBCl, TBAI, THF, DMF; e) *m*-CPBA, CH_2Cl_2, 71% (3 steps, *dr* 4.1:1); f) *i)* $(PhSe)_2$, $NaBH_4$, EtOH; *ii)* **2.18**, THF, *iii)* H_2O_2, 48% (72% *brsm*); g) *m*-CPBA, CH_2Cl_2; h) TBAF, THF, 82% (2 steps); i) TPAP, NMO, CH_2Cl_2; j) TIPSOTf, Et_3N, CH_2Cl_2; k) $Pd(OAc)_2$, CH_3CN, 43% (3 steps); l) K_2CO_3, H_2O_2, THF, H_2O; m) $N_2H_4 \cdot HCl$, Et_3N, CH_3CN; n) DDQ, CH_2Cl_2, H_2O, 27% (3 steps); o) TPAP, NMO, CH_2Cl_2, 75%.

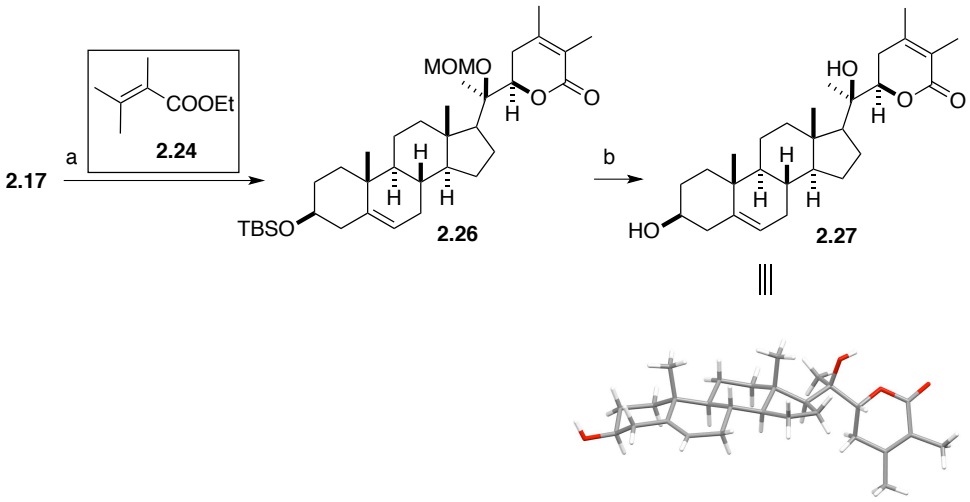
For the synthesis of western A ring, the TBS group was deprotected using tetra-butylammonium fluoride (TBAF) to deliver alcohol **2.20**. Following oxidation, silyl enol ether formation, and Saegusa oxidation, enone **2.21** was prepared in 43% over

3 steps. Epoxidation of the enone **2.21** with basic H_2O_2 afforded the *bis*-epoxide, which was converted under the Wharton transposition conditions using $N_2H_4 \cdot HCl$ and Et_3N. Subsequent PMB deprotection using DDQ afforded the primery alcohol **2.22** in 27% yield over 3 steps. The primary and secondary dialcohols at C-1 respectively at C-22 were then oxidized simultaneously with TPAP, NMO to complete the synthesis of the A ring and to furnish the desired aldehyde **2.23**.

The final two steps in the synthesis, vinylogous aldol reaction and MOM deprotection, turned out to be problematic and no promising results were obtained.[37] It was anticipated that this is most likely due to the reactivity of the enone moiety and therefore an alternative approach was proposed as follows.

2.2.4 Final Approach – Total Synthesis of Withanolide A

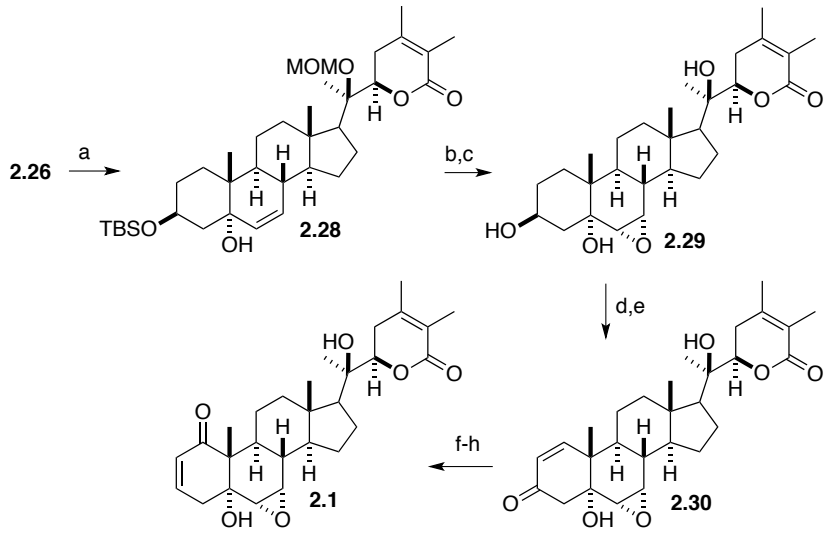
The problematic lactonisation step in presence of the α,β-unsaturated ketone was addressed by reversing the order of the synthetic steps. After semi-synthetic investigations on the isolated natural product, the stablility of the eastern lactone became apparent as formation of the derived lactam could not be accomplished. So, prior to the functionalization of the B ring and especially the sensitive A ring, the lactone moiety was succesfully introduced *via* the previously mentioned Mukaiyama aldol reaction (**Scheme 2.5**).



Scheme 2.5. Diasterostereoselective lactonisation: a) *i)* **2.24**, LiHMDS, DMPU, THF, -78 °C; *ii)* **2.17**, THF, -78 °C to RT, 87% (*dr* 93:7); b) HCl, H_2O, THF, 74%.

The aldehyde **2.17** was reacted with the vinylogous enolate, derived from ethyl 2,3-dimethylbut-2-enoate (**2.24**) and LiHMDS to undergo a stereoselective aldol

reaction and delivered the unsaturated lactone **2.26** in high yield and stereoselectivity (87%, *dr* 93:7).[45] It should also be mentioned that the protection of the tertiary alcohol as MOM ether was a crucial manipulation, ensuring minimized side reaction and allowing the unhindered nucleophilic attack during the aldol process. The correct diastereselective installment of the side chain was unambigiously confirmed by X-ray crystal structure of the globaly deprotected steroid (**2.27**).



Scheme 2.6. Total synthesis of withanolide A: a) *i)* Na-lamp, O_2, TPP, pyridine; *ii)* PPh_3, 61%; b) *m*-CPBA, CH_2Cl_2; c) HCl, THF, 74% (2 steps); d) TPAP, NMO, CH_2Cl_2; e) IBX, Et_3N, CH_2Cl_2, 77% (2 steps); f) H_2O_2, Triton B; g) $N_2H_4 \cdot HCl$, Et_3N, CH_3CN; h) PDC, CH_2Cl_2, 30% (3 steps)

Our next plan was to address the correct oxidation pattern in the B ring of the steroid **2.1**. As previously mentioned, the challenge consisted in the regioselective installment of the epoxy alcohol in the presence of the unsaturated lactone. Considering the limitations associated with phenylselenium-chemistry, we recognized that the singlet oxygen mediated photo-oxygenative olefin migration (Schenck-ene reaction) could be a straightforward method for the synthesis of the allylic alcohol **2.28**.[46] Therefore, the olefin **2.26** was allowed to react with singlet oxygen generated *in situ* from O_2 in the presence of *meso*-tetraphenylporphyrin (TPP) as sensitizer under irradiation with Na-light. The formed hydroperoxide was reduced using PPh_3 to

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[45] M. Ishiguro, M. Hirayama, H. Saito, A. Kajikawa, N. Ikekawa, *Heterocyles* **1981**, *15*, 823-834.

[46] a) G. O. Schenck, DE-B 933925, **1943**; b) G. O. Schenck, *Naturwissenschaften* **1948**, *35*, 28-29.

the allylic alcohol **2.28** in satisfying 61% yield.[47] Directed epoxidation of allylic alcohol **2.28** mediated by *m*-CPBA proceeded as anticipated to provide after removal of the protecting groups (exposure to HCl) the desired epoxy alcohol **2.29** in excellent yield.

Having established the correct functionalization of both B ring and the side chain, we then focused on the synthesis of the A ring. The initial oxidation of the triol **2.29** using TPAP and NMO to the ketone was followed by exposure to 2-iodoxybenzoic acid (IBX) to afford the corresponding enone **2.30** (81%).[48] Saegusa oxidation in this case provided lower yields whereas DDQ did not result in the formation of enone **2.30**. The unsaturated ketone **2.30** was converted to the epoxy ketone by basic aqueous H_2O_2 (Triton B).[49] Finaly, the stage was now set for the Wharton transposition of the epoxy ketone. The resulting epoxy ketone was reacted with hydrazine (NH_2NH_2) in the presence of Et_3N to give the rearranged allylic alcohol. This reaction proceeded smoothly to furnish, after subsequent oxidation by pyridinium dichromate (PDC), withanolide A **2.1** in moderate 30% yield over 3 steps.

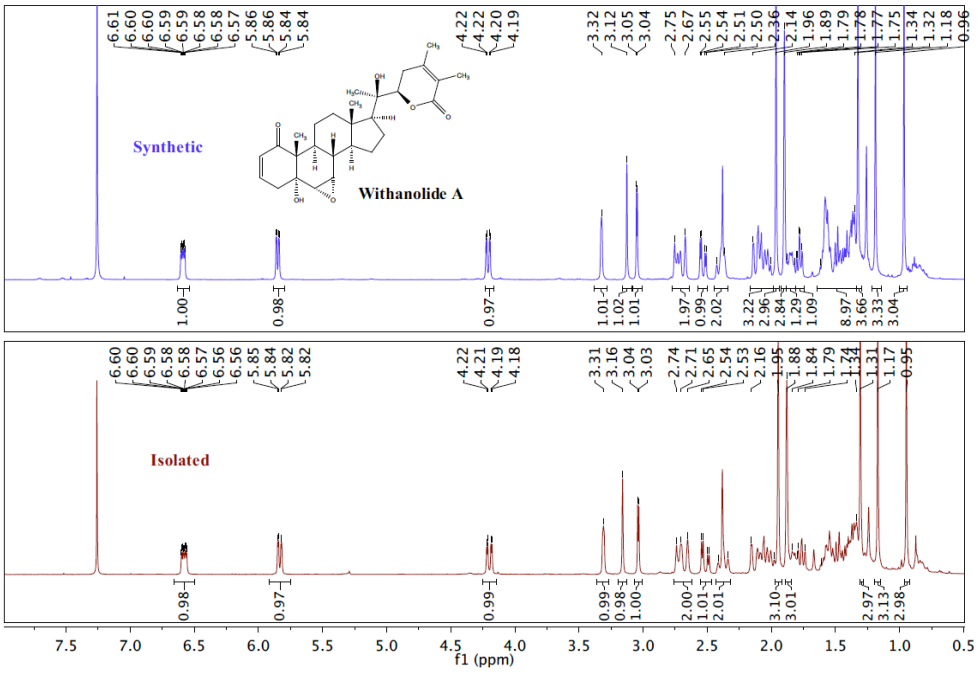


Figure 2.4. ^{1}H NMR spectra of the synthetic and the isolated withanolide A.

[47] a) W. Adam, E. Staab, *Liebigs Ann. Chem.* **1988**, 757-759; b) Review: M. Prein, W. Adam, *Angew. Chem. Int. Ed.* **1996**, *35*, 477-494.

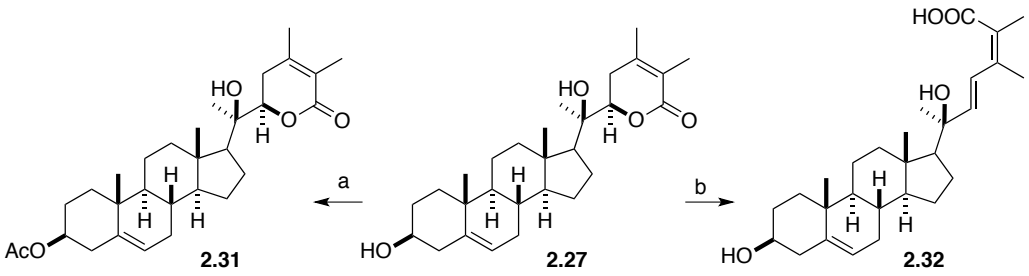
[48] K. C. Nicolaou, T. Montagnon, P. S. Baran, *Angew. Chem. Int. Ed.* **2002**, *41*, 993-996.

[49] M. T. Barros, C. D. Maycock, M. R. Ventura, *Tetrahedron* **1999**, *55*, 3233-3244.

All analytical data (^{1}H NMR (**Figure 2.4**) and ^{13}C NMR, optical rotation, UV, M.p.) for synthetic and isolated natural product were found to be in full agreement. The final proof of identity of synthetic and isolated natural sample (**2.1**) was established by co-injection and analysis of both samples by HPLC.

2.2.5 Natural Product Hybrids

After the completion of the synthesis of withanolide A, gaining insight into the intrinsic structural features of the natural product, the project was extended to investigate on the mode of action. For this purpose we proposed to use the strategy of natural products hybrids referring sometimes to chemical or molecular editing.[50]



Scheme 2.7. Protection of the diol as its acetate: a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 85%; b) NaH, BnBr, THF.

In this regard, the alcohol intermediate **2.27** was acetylated to the protected secondary alcohol **2.31** to evaluate the feasibility of this intermediate for further SAR studies. Treatment with stronger bases such as NaH to introduce various other substituents for example an benzyl group at the same position resulted in decomposition and in the opening of the lactone to the double unsaturated side chain **2.32**.

Further, diol **2.27** was linked to the catechol-anchoring unit (for details on nitrocatechols see **Chapter 3**) or fluorescent dansyl markers to elaborate on the location of the biological target in cells (**Scheme 2.8**). Diol **2.27** was first treated with succinic anhydride to afford carboxylic acid **2.33** as a colorless solid (M.p. = 200-202 °C) in 85% yield, which was then converted to desired hybrid by simple use of peptide

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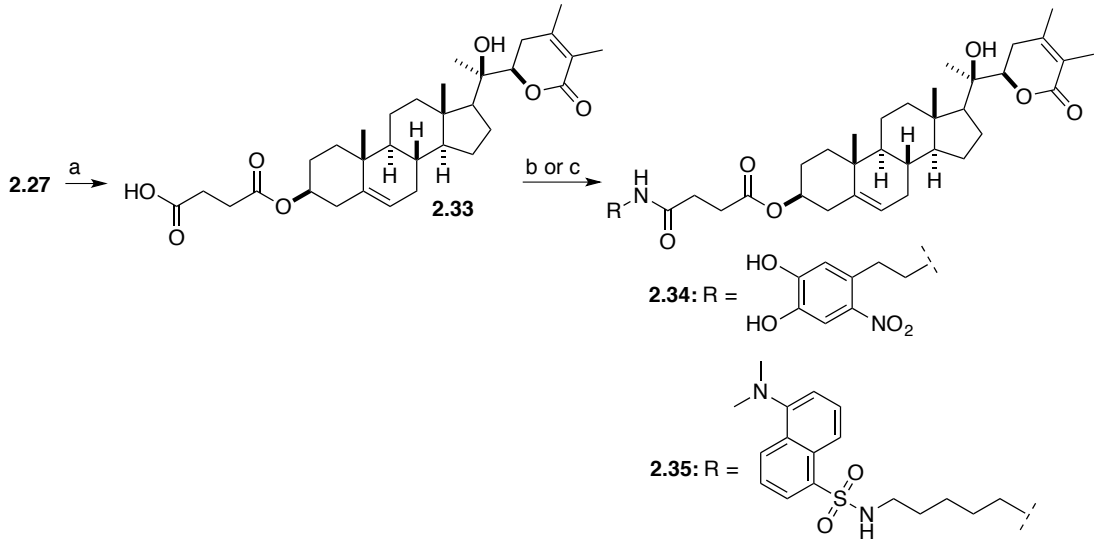
[50] a) J.-Y. Wach, K. Gademann, *Synlett* **2012**, 163-170; b) A. Fürstner, E. Kattnig, G. Kelter, H. H. Fiebig, *Chem. Eur. J.* **2009**, *15*, 4030-4043; c) A. Fürstner, D. Kirk, M. D. B. Fenster, C. Aissa, D. de Souza, O. Müller, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8103-8108; d) T. Oskarsson, P. Nagorny, I. J. Krauss, L. Perez, M. Mandal, G. Yang, O. Ouerfelli, D. Xiao, M. A. S. Moore, J. Massagué, S. J. Danishefsky, *J. Am. Chem. Soc.* **2010**, *132*, 3224-3228; e) A. M. Szpilman, E. M. Carreira, *Angew. Chem. Int. Ed.* **2010**, *49*, 9592-9628.

coupling chemistry: Catechol **2.33** obtained using nitrocatechol hydrosulfate was and *N*,*N*'-disuccinimidy carbonate (DSC) in 14% yield after reverse phase HPLC purification, whereas dansyl derivative **2.35** was formed by the use of 1-(3-dimetyl-aminopropyl)-3-ethylcarbodiimid (EDC) and 1-hydroxybenzotriazol (HOBt) in 71%.



Scheme 2.8. Formation of natural product hybrids: a) succinic anhydride, Et_3N, DMAP, CH_2Cl_2, RT, 85%; b) dansyl-NH_2, HOBt, EDC, Et_3N, CH_3CN, RT, 71%; c) nitrodopamine, DSC, pyridine, CH_3CN, RT, 14%.

Both hybrids were evaluated in cell assays (for details see **Chapter 2.3**), but exhibit unfortunately cytotoxic activities and therefore could not provide further insight into the complex phenomenon of cell differentiation. Future candidates will be derived directly from the natural product with additional hydroxy and epoxy functionalities in the B ring, therefore more closely mimicking the natural steroid.

2.2.6 Semisynthetic Studies

In order to access such derivatives for biological studies, we initiated semi-synthetic studies starting from withanolide A (**2.1**).[51] The steroidal lactone was therefore isolated from the dried and powdered roots of *Withania somnifera* (*Ashwagandha*) by a modification of the literature procedure:[52] First, dried roots were percolated with MeOH several times. The MeOH extracts were washed with pentane to remove grease and non-polar components. Rotary evaporation gave a residue which

[51] R. Liffert, Master Thesis, Basel, **2012**.

[52] S. S. Subramanian, P. D. Sethi, E. Glotter, I. Kirson, D. Lavie, *Phytochemistry* **1971**, *10*, 685-688.

was resuspended in EtOAc, washed with H_2O to remove the more polar ingredients and concentrated to a brown oil, which was purified by multiple silica gel chromatography to afford steroid **2.1** as a colorless solid in 0.025% yield from the mass of the dried roots (**Figure 2.5**).

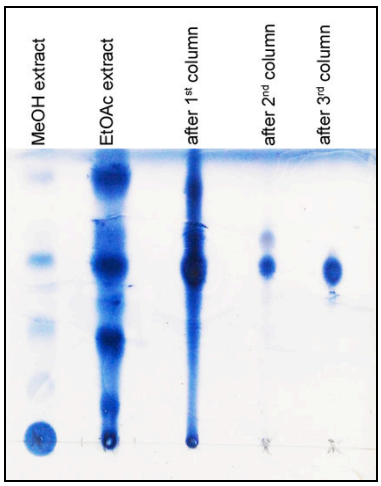
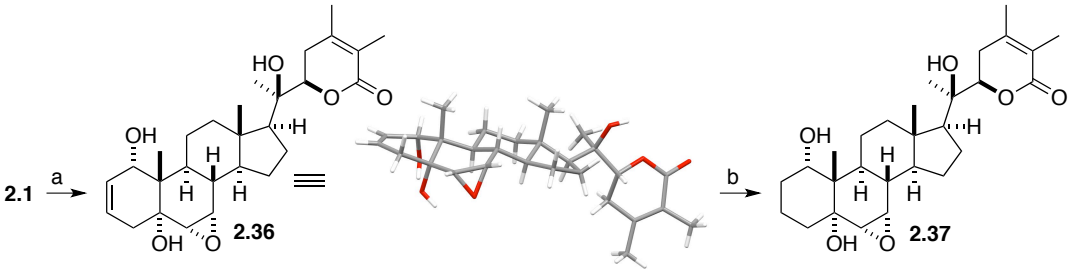


Figure 2.5. TLC (SiO_2, EtOAc:$CHCl_3$:hexane 8:1:1) of the individual purifications for the extraction-process of withanolide A (**2.1**) from the dried roots of *Ashwagandha*.

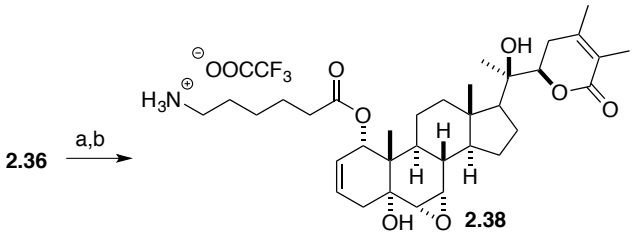
After an extensive survey of different approaches commonly employed in semi-synthesis to functionalize complex natural products, we notized that only the A ring of withanolide A (**2.1**) was feasible for chemical modification. In addition, A ring analogues, bearing a 3β-hydroxy group comparable with sominone - an aglycon of withanoside IV, were found to display neuritogenic properties as shown from the work of Tohda and co-workers.[53]



Scheme 2.9. Regioselective reduction of withanolide A: a) $CeCl_3$, $NaBH_4$, $CHCl_3$, MeOH, 75%; b) Crabtree's catatyst, H_2, CH_2Cl_2, *quant*.

[53] a) T. Kuboyama, C. Tohda, K. Komatsu, *Eur. J. Neurosci.* **2006**, *23*, 1417-1426; b) C. Tohda, E. Joyashiki, *Brit. J. Pharmacol*. **2009**, *157*, 1427-1440.

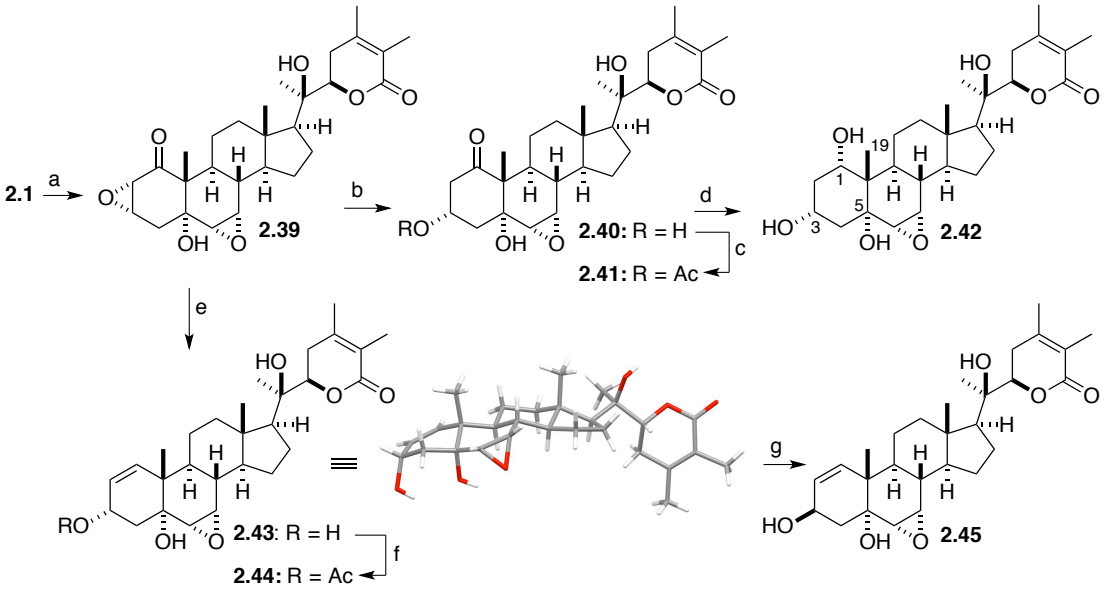
We first targeted the regio- and stereoselective Luche reduction of withanolide A resulting in the allylic alcohol **2.36**, which was envisioned to serve as building block to a variety of new functionalities at C-1 (**Scheme 2.9**). Surprisingly, the same diastereoselective outcome was observed when the reaction was performed in the absence of $CeCl_3$. Regioselective hydrogenation of the 2,3-double bond was performed by using 2 mol % Crabtree's catalyst under a hydrogen atmosphere of 10 bar to obtain the saturated alcohol **2.37**. Unfortunately, all attempts to further functionalize the C-1 hydroxy group or acetylation reactions were unsuccessful, except the acylation with Boc protected amino acid (**Scheme 2.10**), which upon exposure to TFA afforded a primary amine function (**2.38**) suitable for further transformations.



Scheme 2.10. Acylation of the Luche reduction product to form a primary amine: a) $BocNH(CH_2)_5COCl$, DIPEA, $AgNO_3$, CH_2Cl_2, RT; b) TFA, CH_2Cl_2, RT, 68% (2 steps).

Due to the reluctance of allylic alcohol **2.36** to undergo further functionalization, a new strategy for the derivatization of the natural product needed to be developed.[51] Inspired by the SAR studies on sominone by Tohda *et al.*,[53] we developed a synthetic route starting from natural withanolide A to introduce hydroxyl substituents at C-3. We thought to epoxidize the natural product followed by an epoxide opening under reductive conditions, developed earlier for the B ring functionalization, which would lead to the alcohol **2.40** (**Scheme 2.11**). Given the less sterical hinderance of alcohol **2.40** compared to **2.36**, we predicted it should be facilitated to introduce various substituents at this position. Hence, the same procedure for the epoxidation as in the successful total synthesis of withanolide A was applied. A small-scale approach for the epoxidation with aqueous H_2O_2 in the presence of triton B yielded **2.39** already in 48%. Unfortunately, up-scaling these conditions resulted in a dramatic reduced yield (11% for 100 mg of starting material, **2.1**). Additionally, the long reaction time of 24-48 h and the formation of the epoxidized lactone as a side-product were disadvantages of the developed protocol. After extensive investigation of different conditions

(solvent, base and oxidizing agent),[54] we found fluoride-promoted epoxidation of α,β-unsaturated carbonyl compounds described by Yoshikoshi and co-workers was compatible with our system.[55] In their protocol the use of TBAF as a base and H_2O_2 or *tert*-butyl hydroperoxide (*t*-BuOOH) as an oxidation reagent in DMSO proved to be superior. The adapted conditions enriched the yield reliable to 84% (on a 150 mg scale) when the solvent was finally changed to CH_2Cl_2 and *t*-BuOOH was used as an oxidant.



Scheme 2.11. Semisynthetic transformations of withanolide A to access A ring derivatives:[51] a) *t*-BuOOH, TBAF, CH_2Cl_2, 84%; b) *i)* $(PhSe)_2$, $NaBH_4$, EtOH; *ii)* **2.39**, AcOH, CH_2Cl_2, 80%; c) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 77%; d) $NaBH_4$, $CHCl_3$, MeOH, 70%; e) $N_2H_4 \cdot H_2O$, AcOH, MeOH, CH_2Cl_2, 61%; f) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 87%; g) *i)* 4-$NO_2(C_6H_4)$COOH, DEAD, PPh_3, CH_2Cl_2; *ii)* NaOH, TBAH, THF, CH_2Cl_2, 50%.

After completion of this building block, the organoselenium-mediated reduction of the α,β-epoxy ketones **2.39** to the corresponding β-hydroxy ketone was investigated. Alcohol **2.40** was obtained in good yields when adding the ethanolic solution of the active reagent ($Na[PhSe(OEt)_3]$) and AcOH to a solution of epoxy ketone **2.39** in

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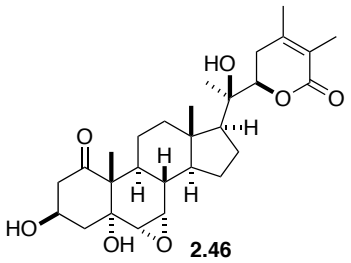
[54] a) F. E. Ziegler, K.-J. Hwang, J. F. Kadow, S. I. Klein, U. K. Pati, T.-F. Wang, *J. Org. Chem.* **1986**, *51*, 4573-4579; b) F. J. Moreno-Dorado, F. M. Guerra, F. J. Aladro, J. M. Bustamante, Z. D. Jorge, G. M. Massanet, *Tetrahedron* **1999**, *55*, 6997-7010; c) R. Takagi, K. Tojo, M. Iwata, K. Ohkata, *Org. Biomol. Chem.* **2005**, *3*, 2031-2036.

[55] M. Miyashita, T. Suzuki, A. Yoshikoshi, *Chem. Lett.* **1987**, 285-288.

CH_2Cl_2.[56] The chemical shift of the equatorial H-3 in **2.40** was found at 4.11 ppm, whereas the axial one is reported for the C-3 diastereomeric natural product (**2.46**)[57] surprisingly at 4.91 ppm (**Figure 2.5**).



2.46

Figure 2.6. Natural 3-hydroxy withanolide (**2.46**) isolated from *Withania somnifera*.[57]

Efficiently, acetylation of alcohol **2.40** under Steglich conditions (Ac_2O, DMAP, Et_3N in CH_2Cl_2) delivered acetate **2.41** in 77% yield without detectable amount of eliminated side-product (**2.1**). This example demonstrates the opportunity to introduce carboxylate residues of choice at this position. Reduction of **2.40** was performed using $NaBH_4$ and a new polar product was observed by TLC analysis. Although the formation of tetraol **2.42** was confirmed by mass spectrometry, the isolation of this hydrophilic compound turned out to be difficult. Nevertheless, work-up with saturated Rochelle's solution liberated tetraol **2.42** and its structure was elucidated by NMR spectroscopy. The stereochemical outcome of **2.42** was anticipated from the $NaBH_4$ mediated reduction of withanolide A **2.1** and could be confirmed by NOE analysis which showed interactions of H-1 with H-2, H-4 and H-19.

Focusing on the introduction of substituents at C-3, another possibility was the transformation of epoxy ketone **2.39** to allylic alcohol **2.43** (**Scheme 2.11**). For this manipulation, the Wharton carbonyl transposition was reinvestigated using the conditions from the final key step of the successful total synthesis. Consequently, epoxy ketone **2.39** was treated with $NH_2NH_2 \cdot HCl$ in the presence of Et_3N in CH_3CN, but no product formation was observed. To our surprise, Wharton transposition under acidic conditions improved the yield to 40%.[58] Due to the low solubility of **2.39** in

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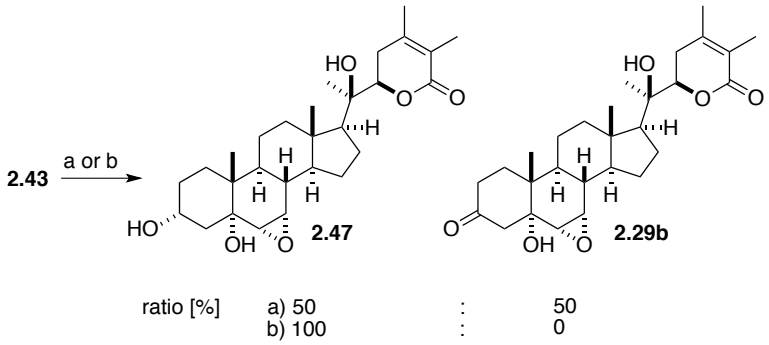
[56] a) M. Miyashita, T. Suzuki, A. Yoshikoshi, *Tetrahedron Lett.* **1987**, *28*, 4293-4296; b) M. Miyashita, M. Hoshino, A. Yoshikoshi, *Tetrahedron Lett.* **1988**, *29***,** 347-350.

[57] M. I. Choudhary, S. Yousuf, S. A. Nawaz, S. Ahmed, A. Rahman, *Chem. Pharm. Bull.* **2004**, *52*, 1358-1361.

[58] H. Y. Wang, G. A. O'Doherty, *Chem. Commun.* **2011**, *47*, 10251-10253.

methanol, the reaction was further optimized and the best conditions were to conduct the reaction in a 1:1 mixture of methanol and CH_2Cl_2 to obtain the allylic alcohol **2.43** in 61% yield. The structure of **2.43** was confirmed by X-ray crystal structure analysis and the stereochemistry of the 3α-hydroxyl group was herein retained from the epoxidation step of withanolide A.

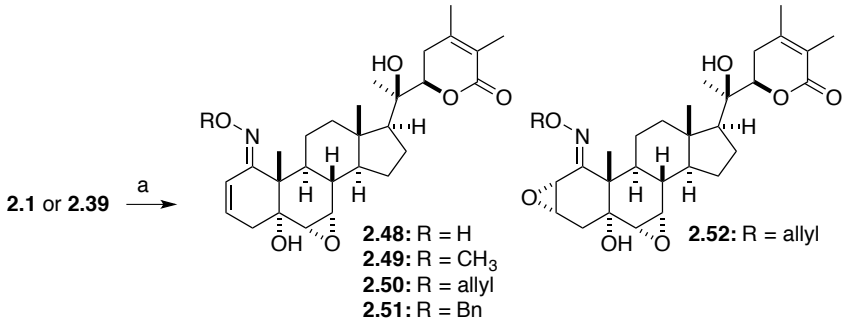
Next, we investigated the introduction of functional groups at the newly formed secondary alcohol in order to probe if **2.43** can be used as a chemical probe (see **Chapter 2.2.5**). Acetylation of allylic alcohol **2.43** was performed again under standard acetylation conditions and afforded acetate **2.44** in good yield. Inversion of the C-3 hydroxyl group in **2.43** was accomplished using the Mitsunobu procedure. Esterification with *para*-nitrobenzoic acid, followed by saponification with aqueous NaOH in the presence of tetrabutylammonium hydroxide (TBAH) as phase-transfer catalyst afforded 3β-allylic alcohol **2.45** in 50% yield over the two steps.



Scheme 2.12. Hydrogenation of allylic alcohol **2.43**: a) 10 mol% Pd/C, H_2 (30 bar), EtOAc, *quant.*; b) 2 mol% Crabtree's catalyst, H_2 (10 bar), CH_2Cl_2, *quant.*

To further access novel derivatives, the double bond of allylic alcohol **2.43** was reduced with 10 mol % Pd on activated charcoal. Quantitative conversion provided alcohol **2.47** as an inseparable 1:1 mixture with the isomerized saturated ketone **2.29b** – an intermediate from the total synthesis (**Scheme 2.12**).[59] Taking into account that we were interested in alcohol **2.47**, Crabtree's catalyst turned out to be efficient for hydrogenation of **2.43** to quantitatively yield alcohol **2.47**.

[59] R. Uma, C. Crévisy, R. Grée, *Chem. Rev.* **2003**, *103*, 27-51.



Scheme 2.13. Formation of oxime analogues of withanolide A: a) $RONH_2 \cdot HCl$, pyridine, 70 °C, from **2.1**: R = H, 58%, R = CH_3, 58%, R = allyl, 72%, R = Bn, 63%, from **2.39**: R = allyl, 58%.

Oxime derivatives of withanolide A as prodrugs may improve the solubility as well as the bioavailability *in vivo*. Furthermore, oximes turned out to be the only possible functionalization of the C-1 position of **2.1** (**Scheme 2.13**). A series of oximes **2.48-2.52** were easily obtained by heating withanolide A or the derived epoxide **2.39** with the corresponding hydroxylamine hydrochlorides in pyridine at 70 °C for 24-48 h.

2.3 Biological Evaluation

The natural product **2.1** and several semi-synthetic derivatives were tested in order to determine their neuritogenic properties according to the previously published method.[31b,60] Briefly, human SH-SY5Y neuroblastoma cells (ca. 4 x 10^4 cells/cm^2) were grown on collagen I coated 24-well plates in minimal essential medium (MEM) with 5% fetal bovine serum (FBS). After incubation for 4 days in the presence of the corresponding compounds (1 μM, 0.1% DMSO), the cells were examined under a phase contrast microscope after fixation (5% formaldehyde in PBS buffer solution), stained (modified Giemsa stain), and the differentiated cells were counted. For each compound nine pictures from random areas of three different wells were taken. At least 500 cells were counted for each individual compound. The criterion for a differentiated cell was at least one neurite with a length of more than 50 μm. In control experiments DMSO (0.1%) was used as negative control. To obtain more accurate results for withanolide A, more than 1500 cells were evaluated.

[60] All cell-assays were performed by Dr. H. J. Jessen or P. Burch at the University of Basel. Analysis of the results was performed either by Dr. H. J. Jessen, R. Liffert or myself.

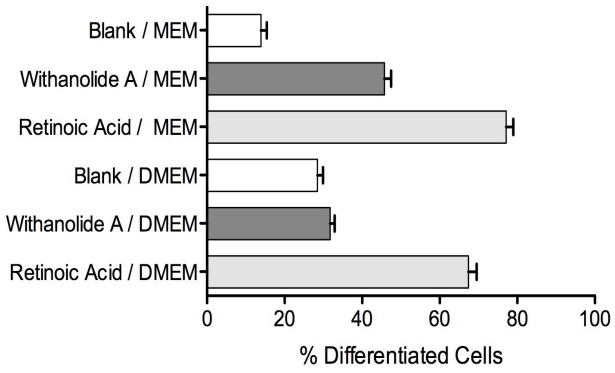


Figure 2.7. Neurite outgrowth induced by withanolide A (**2.1**), negative (DMSO) and positive (retinoic acid) controls in human SH-SY5Y cells in minimal essential medium (MEM) and Dulbecco's modified Eagle medium (DMEM). Error bars denote SEM. For representive micrographs see **Figure 2.7 D**-**F** and **G**-**I**.

Interestingly, when we used conditions published by Tohda *et al.*[31b] we were able to reproduce their results (DMSO vehicle 12% differentiated cells *vs.* withanolide A 22%, data not shown). We then examined the same compounds on collagen-coated 24-well plates to support adhesion of the cells under identical conditions. The vehicle control experiments (0.1% DMSO) displayed a dramatically different phenotype compared to withanolide A or retinoic acid (positive control) treated cells (**Figure 2.7**). In the former case, we almost exclusively observed large cell aggregate formation with only few isolated cells. Withanolide A treated cells formed aggregates to a reduced extent, with many viable isolated cells that were often found to be differentiated (**Figure 2.8**). Finally, treatment with retinoic acid completely suppressed aggregate formation and led to fully differentiated phenotypes. We then incubated the cells in Dulbecco's modified Eagle's medium (DMEM, 10% FBS, antibiotics) in collagen-coated wells with the compounds and controls as recommended by the supplier. In these cases, the large difference obtained for negative control experiments in MEM compared to withanolide A (14% vs. 46%) was reduced to a non significant difference (28% vs. 32%).

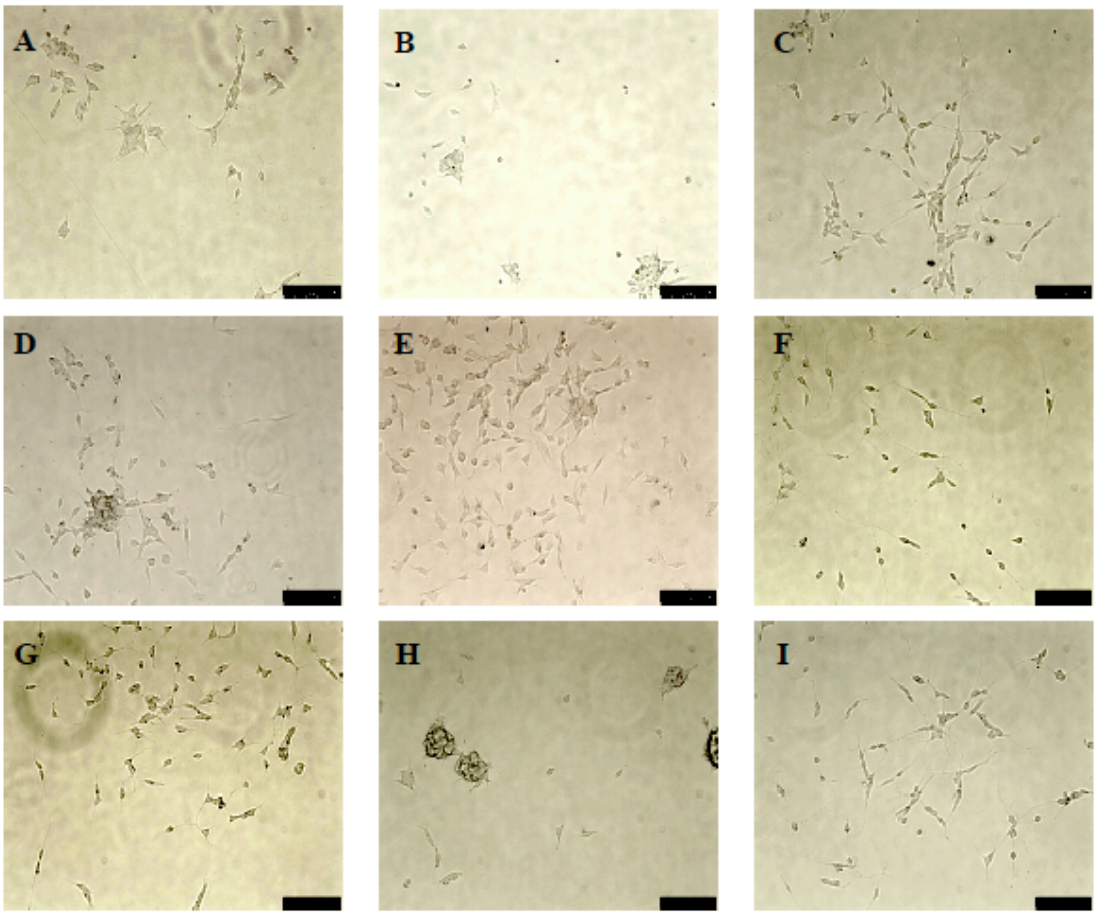


Figure 2.8. Representative micrographs of the performed cell-assays (reffering to the conditions described in **Figure 2.6**). **A**, **D**, **G** withanolide A (**1.1**), **B**, **E**, **H** negative control, **C**, **F**, **I** positive control. **A-C** MEM, uncoated wells, **D-F** DMEM coated wells, **G-I** MEM, coated wells. Indicated scale bar = 250 µm.

Also in rat cortical neurons, a reassessment of the neuritogenic activity has been proposed recently. In the presence of DMEM, isolated non-differentiated cells appear to be more viable, thus levelling the observed ratios. These experiments therefore suggest that the neuritogenic phenotype observed in SY5Y cells appears to be conditional related to medium (MEM *vs.* DMEM) or coating of the wells.

Following these investigations, the results for the semi-synthetic derivatives are summarized in **Figure 2.9**. While it is difficult to draw quantitative conclusions from the biological assays as already mentioned, several facts became apparent: Compounds **2.36**, **2.43**, **2.45**, and **2.48** showed similar activities when compared to withanolide A with regard to neurite outgrowth stimulating activity in SH-SY5Y cells. The compounds **2.39**, **2.44**, and **2.49-2.52** showed in fact less activity than withanolide A, to the extent that some compounds can be hardly distinguished from

the solvent control in these phenotypic assays. It appears that there is a correlation between the size of the attached group and their activity. For example, the hydroxylamine **2.48** shows increased activities than the corresponding and sterically more demanding oximes **2.49**-**2.52**, what can also be attributed to the more non-polar nature of these compounds. The same trend was observed for allyl alcohol **2.43** and its acetate **2.44**. Additionally, the chemically reactive enone moiety of withanolide A in the A ring might not be required for neuroactive properties, as the allylic alcohols **2.36** and **2.43** retain activity.

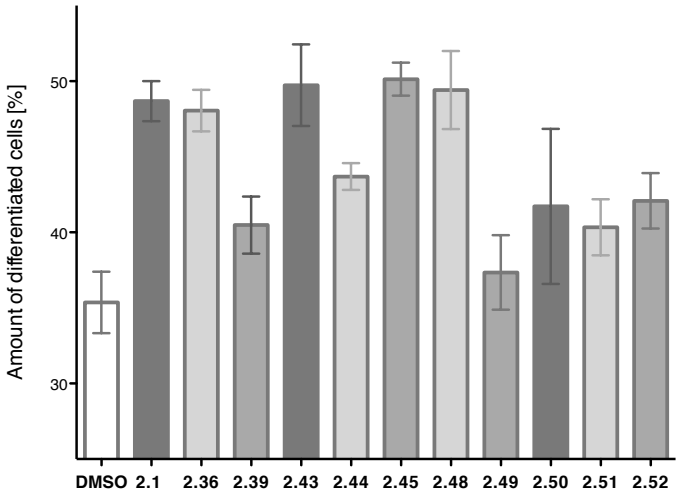


Figure 2.9. Neurite outgrowth activity of DMSO blank, withanolide A and derivatives in human SH-SY5Y cells with MEM as cell-medium. Error bars denote SEM.

Concerning the mechanism of action of **2.1**, a recent study by Chan and co-workers[33] showed that this steroid lactone is able to modulate several secretase targets of relevance to neurodegeneration. Moreover, this study reported the docking of withanolide A into beta-secretase 1 (BACE1) suggesting enzyme inhibition by binding. We have evaluated the binding of withanolide A (**2.1**) against several proteases of potential relevance to neurodegenerative diseases (**Table 2.1**), however, in these assays withanolide A and derivatives **2.27** and **2.31** were found to be inactive up to a concentration of 100 μM.[61]

Only very weak activity was found against plasmepsin I (compound **2.27**) and plasmepsin I, II and IV (compound **2.31**). While it remains feasible that withanolide A

[61] Enzymatic assays were carried out and evaluated by Dr. Solange Meyer, Actelion Pharmaceuticals Ltd.

is able to modulate the expression of BACE1, as demonstrated by Chan and coworkers,[33] direct interaction of **2.1** with BACE1 as suggested appears very unlikely based on the values reported in this study.

Table 2.1. Determined IC_{50} values (μM) of compounds **2.1**, **2.27** and **2.31** against protease targets potentially relevant to neurodegenerative diseases (BACE and cathepsin (Cath)) and control proteases (plasmepsin (PM)).

Comp.	BACE1-GST	Cath D	Cath E	PM I	PM II	PM III
2.1	>100	>100	>100	>100	>100	>100
2.27	>100	>100	>100	67 ± 4	>100	>100
2.31	>100	>100	>100	46 ± 14	52 ± 5	31 ± 7

2.4 Conclusion

In conclusion, we have developed the first successful synthesis of the pharmacologically important steroid withanolide A. Semi-synthetic investigations on the natural product provided essential understanding on its reactivity and successfully elaborated the synthetic route. Notable features of this synthesis include a highly diastereoselective Schenck-ene reaction, a minimal protecting group strategy exploiting the inherent reactivity pattern in the endgame and a Wharton transposition for the A ring formation. Furthermore, we have synthesized more than 15 derivatives of withanolide A directly from the isolated natural product. Consequently, we developed two new synthetic routes that allow the insertion of a hydroxyl group at C-3 and thereby opening the door for further functionalization. Both the β-hydroxy ketone **2.40** and the allylic alcohol **2.43** were synthesized in high yields and complete diastereoselectivity from **2.1**.

Biological studies of neurite outgrowth in human SH-SY5Y cells demonstrated the dependence of neuritogenic properties on the size rather than the functionality in the A ring of the evaluated compounds. Three compounds showed similar neurite outgrowth activity in cell assays than withanolide A itself. Further investigations towards the insertion of functional groups at C-3 of withanolide A could possibly give access to more potent candidates and allow for the manipulation of properties such as solubility or bioavailability.

3 DEVELOPMENT OF NITROCATECHOLS AS PHOTOLABILE SURFACE ANCHORS

3.1 Introduction

This chapter describes the development of a molecular surface modification platform based on nitrocatechols that allows for small molecule functionalization of TiO_2 under mild aqueous conditions and efficient release triggered by light - therefore uncaging a small immobilized molecule cargo on demand.[62] Altering a molecular surface with additional tailored properties and functionalities while maintaining the ones inherent in the bulk material has found applications in a wide variety of fields in modern surface coating technology.[63] The combination of well designed and characterized molecules as anchoring units or coating device with erratic surfaces is fast growing and very promising research field, despite the problems associated with the transfer of molecular understanding towards material science and the multiple techniques required to characterize this symbiosis.

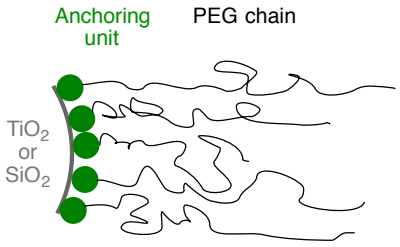


Figure 3.1. Strategy for the conception of biocompatible antifouling surfaces (SiO_2 or TiO_2) using immobilized PEG chains.

One famous application, which is of high importance for devices in medicinal environment, is the development of antifouling surfaces. The term biofouling characterizes the random absorption of proteins as well as microorganisms such as fungi or bacteria on surfaces and represents a major issue in hospitals.[64] When

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[62] R. Wehlauch, Master Thesis, Basel, **2012**.

[63] Reviews: a) Q. Ye, F. Zhou, W. Liu, *Chem. Soc. Rev*. **2011**, *40*, 4244-4258; b) J. L. Dalsin, P. B. Messersmith, *Mater. Today* **2005**, *8*, 38-46.

[64] F. Brétagnol, A. Valsesia, G. Ceccone, P. Colpo, D. Gilliland, L. Ceriotti, M. Hasiwa, F. Rossi, *Plasma Process. Polym*. **2006**, *3*, 443-455.

exposed to sea- or freshwater containing microorgansims or protein solutions basically all surfaces suffer from biofouling. One promising strategy to prevent this is the immobilization of polymers e.g. water soluble polyethylene-glycol (PEG) on TiO_2 or glass surfaces, inhibiting the settlement of the microorganisms due to their dynnamic and flexible architecture.

A central challenge remains the nature of the molecular anchor that links coating and surface. In this respect, catechols display unique properties due to their ability to strongly bind to metal oxides in aqueous media. Originally found in mussel adhesive proteins,[65] L-DOPA or dopamine derived catechols were successfully used in surface modifications despite their sensitivity to oxidation.[66] Additionally this biomimetic approach benefits from the fact that a single functional group - the catechol unit - adjustable by external ambience as pH, ions or temperature regulates the reactivity of the anchor. The binding mode itself is strongly depended on environmental ions, leading to toughness by chelation of metal oxides and gaining robustness by oxidative cross-linking to form polymeric surface coating. Beneficial catechols allow to bind and unbind under water in a cooperative fashion, what is used by mussels to change locations.

We have introduced a related anchoring catechol with electron-withdrawing substituents based on the iron chelator anachelin (**3.1**).[67,68] This natural product was isolated from cyanobacteria and was shown to be essential for the iron uptake of these bacteria. Again, the catechol moiety was suspected to be responsible for this strong

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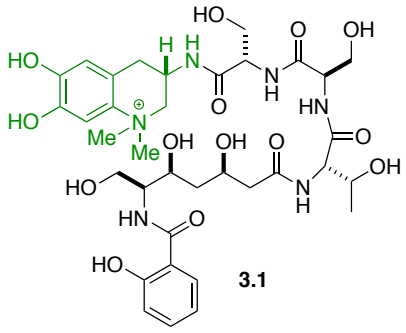
[65] For recent examples: a) B. Geiseler, L. Fruk, *J. Mater. Chem.* **2012**, *22*, 735-741; b) A. S. Goldmann, C. Schödel, A. Walther, J. Yuan, K. Loos, A. H. E. Müller, *Macromol. Rapid Commun.* **2010**, *31*, 1608-1615.

[66] a) H. Zhao, J. H. Waite, *J. Biol. Chem.* **2006**, *281*, 26150-26158; b) H. Lee, N. F. Scherer, P. B. Messersmith, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12999-13003; c) J. H. Waite, *Nature Mater.* **2008**, *7*, 8-9; d) H. Zeng, D. S. Hwang, J. N. Israelachvili, J. H. Waite, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12850-12853; e) H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426-430; f) J. Moser, S. Punchihewa, P. P. Infelta, M. Grätzel, *Langmuir* **1991**, *7*, 3012-3018.

[67] S. Zürcher, D. Wäckerlin, Y. Bethuel, B. Malisova, M. Textor, S. Tosatti, K. Gademann, *J. Am. Chem. Soc*. **2006**, *128*, 1064-1065.

[68] a) A. E. Walsby, *Br. Phycol. J.* **1974**, *9*, 371-381; b) A. E. Walsby, *Br. Phycol. J.* **1974**, *9*, 383-391; c) H. Breiderbeck, K. Taraz, H. Budzikiewicz, A. E. Walsby, *Z. Naturforsch. C. Biosci.* **2000**, *55*, 681-687; d) Y. Itou, S. Okada, M. Murakami, *Tetrahedron* **2001**, *57*, 9093-9099; e) Y. Ito, K. Ishida, S. Okada, M. Murakami, *Tetrahedron* **2004**, *60*, 9075-9080.

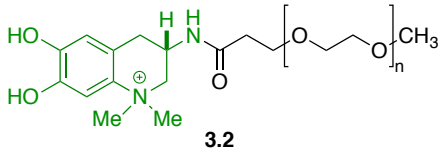
binding event. Iron as essential nutrition for microorganism is highly prevalent in nature but only as insoluble iron oxides. In order to obtain enough iron, cyanobacteria produce siderophores to chelate metals ions and make them bioavailable.



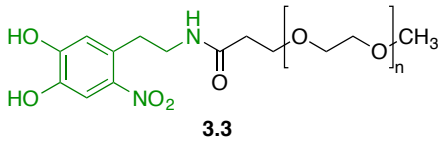
3.1

Figure 3.2. Structure of siderophore anacheline (**3.1**), surface anchoring moiety indicated in green.

Using this knowledge, we were able to show that the affinity to bind to metal oxide surfaces in particularly to iron and titanium oxide, could lead to new innovative surface modifications based on the anachelin siderophore. Substitution of the natural side chain by a polymeric PEG chain led to protein-resistant surfaces (**Figure 3.3**), which remained stable to multiple washings. This surface alteration was achieved by an operationally simple dip-and-rinse procedure, incubating with TiO_2 coated silicon wafers in catechol solution with a high salt buffer to achieve dense packing of the PEG chains.[69]



3.2



3.3

Figure 3.3. Design of antifouling surfaces consist of anachelin (**3.2**) or nitrocatechol (**3.3**) anchoring moiety and a biocompatible PEG chain.

Furthermore, we also showed that the positively charged nitrogen substituent is essential for improved stability compared to the unsubstituted dopamine present in

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[69] J.-Y. Wach, B. Malisova, S. Bonazzi, S. Tosatti, M. Textor, S. Zürcher, K. Gademann, *Chem. Eur. J.* **2008**, *14*, 10579-10584

mussels. A second-generation design consisted of nitrocatechols such as nitro-dopamine (**3.3**) due to the ease of preparation,[70] improved adhesion properties and stability towards oxidation. All these anchors have found widespread applications.[71] We have shown that such catechols can be used to functionalize surfaces with complex antibiotics (vancomycin) to generate anti–microbial surfaces and expose them to biofilm forming bacteria, which will be killed.[72]

3.2 Concept of the Light-Tunable Anchors

In addition to immobilization, the release of small molecules from a modified surface triggered by an external stimulus would be highly desirable. Potential applications would range from drug delivery, small molecule microarrays to selective probes in chemical biology.

In particular in the latter area, such an approach would combine the well-known strategy of temporarily disabling the biological activity of a small molecule ('caging') with the possibility of controlled release on demand. This would allow for both high spatial control (through immobilization) and temporal control (through caging) of the biological activity of a small molecule, two key elements in chemical biology approaches.[73,74] In here, we describe the development of such a molecular platform that allows for the spatio-temporal control of small molecule release. In particular, we propose a bio-inspired approach that leverages the presence of a catechol with the versatility of the nitrophenyl system (**Figure 3.4**).

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[70] B. Malisova, S. Tosatti, M. Textor, K. Gademann, S. Zürcher, *Langmuir* **2010**, *26*, 4018-4026

[71] a) S. Saxer, C. Portmann, S. Tosatti, K. Gademann, S. Zürcher, M. Textor, *Macromolecules* **2010**, *43*, 1050-1060; b) Review: K. Gademann, J. Kobylinska, J.-Y. Wach, T. M. Woods, *Biometals* **2009**, *22*, 595-604.

[72] J.-Y. Wach, S. Bonazzi, K. Gademann, *Angew. Chem. Int. Ed.* **2008**, *47*, 7123-7126.

[73] T. Tachikawa, Y. Asanoi, K. Kawai, S. Tojo, A. Sugimoto, M. Fujitsuka, T. Majima, *Chem. Eur. J.* **2008**, *14*, 1492-1498.

[74] X. Wang, S. Werner, T. Weiß, K. Liefeith, C. Hoffmann, *RSC Adv.* **2012**, *2*, 156-160.

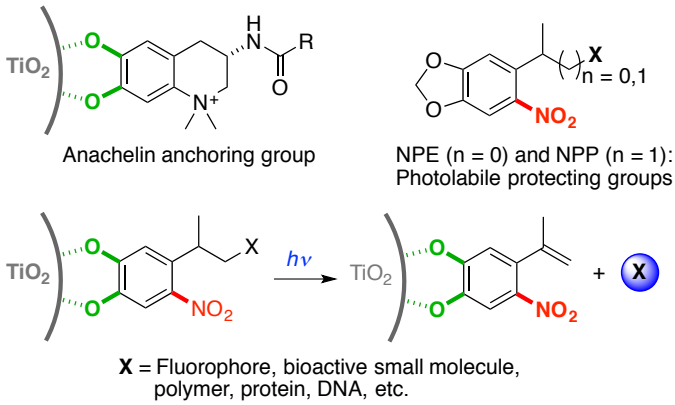

Figure 3.4. Concept of the light induced surface release of carrier molecules for the photolabile NPP protecting group.

Among the most commonly used photocleavable protecting groups are several nitrophenyl derivatives, including (2-nitrophenyl)ethyl (NPE) and (2-nitrophenyl)propyl (NPP) derivatives.[75,76] However, upon inspection of the literature, we were surprised to find that no free nitrocatechols have been utilized as photocleavable groups and the surface functionalization and release properties of such systems have not been investigated.[77] We have targeted both NPE and NPP systems **3.4** (n = 0) and **3.5** (n = 1), respectively, and demonstrated that controlled bonding and release is achievable for the latter derivatives.

3.3 Synthesis of the Caged Anchors

3.3.1 NPE Derivatives

The first target compound constituted for the 2-nitrophenyl ethyl derivative **3.4**. Its synthesis started with the preparation of nitrocatechol **3.7** by nitration of commercially available 3,4-(methylenedioxy)acetophenone (**3.6**) using HNO_3 in acetic acid (**Scheme 3.1**). Subsequent treatment with $AlCl_3$ at low temperature was followed by hydrolysis with concentrated HBr gave nitrocatechol **3.7** in satisfying yield. Catechol

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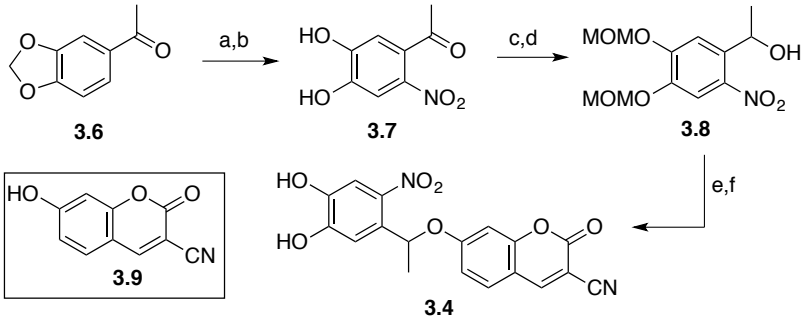
[75] Reviews: a) G. Mayer, A. Heckel, *Angew. Chem Int. Ed.* **2006**, *45*, 4900-4921; b) D. Puliti, D. Warther, C. Orange, A. Specht, M. Goeldner, *Biorg. Med. Chem.* **2011**, *19*, 1023-1029; c) D. Warther, S. Gug, A. Specht, F. Bolze, J.-F. Nicoud, A. Mourot, M. Goeldner, *Biorg. Med. Chem.* **2010**, *18*, 7753-7758; d) C. G. Bochet, *J. Chem. Soc., Perkin Trans. 1* **2002**, 125-142.

[76] S. Walbert, W. Pfleiderer, U. E. Steiner, *Helv. Chim. Acta* **2001**, *84*, 1601-1611.

[77] Z. Shafiq, J. Cui, L. Pastor-Pérez, V. San Miguel, R. A. Gropeanu, C. Serrano, A. del Campo, *Angew. Chem. Int. Ed.* **2012**, *51*, 4332-4335.

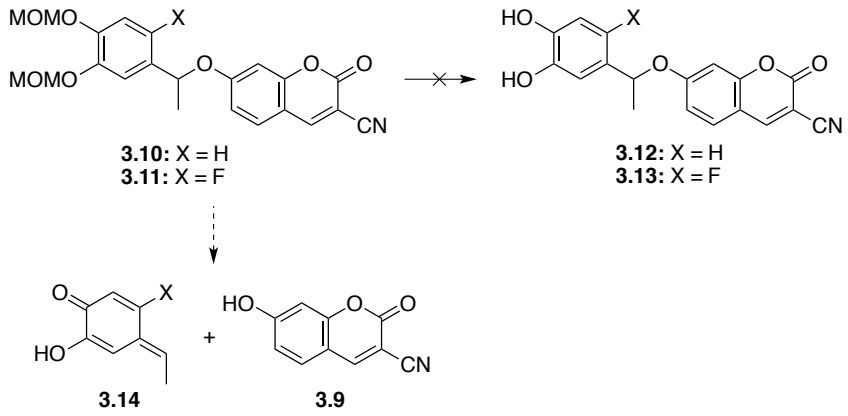
3.7 was then protected as MOM ether and $NaBH_4$ reduction furnished benzylic alcohol **3.8**. In the context of this synthesis, it was found necessary to protect the catecholate OH groups, in order to prevent attachment of this compound to silica during chromatographic purification. The alcohol **3.8** was then attached to the fluorophore[78] **3.9** *via* Mitsunobu reaction in moderate yields. For the final coupling reaction was performed under exclusion of light to avoid decomposition of the formed product. During the isolation of the photolabile compound from the reaction mixture, it became already apparent that the uncaging process could be induced, when TLC plates were exposed to UV light at 366 nm (see videos on the enclosed CD for further information). The MOM protecting group were finally removed using aqueous TFA, leading to the NPE derivative **3.4**.



Scheme 3.1. Preparation of NPE conjugate **3.4** for surface modification: a) HNO_3, AcOH, 0 °C to RT, 2.5 h, 59%; b) $AlCl_3$, DCE, -5 °C, 1 h, then 48% HBr, RT, 24 h, 84%; c) MOMCl, K_2CO_3, MeCN, 0 °C to RT, 3 h, 87%; d) $NaBH_4$, MeOH, 0 °C to RT, 3.5 h, 99%; e) **3.9**, PPh_3, DIAD, THF, 0 °C to RT, over night, 41%; f) TFA, H_2O, RT, 46%.

There are at least two fundamentally different pathways possible for the cleavage of such catechols from titania, either *via* (1) photocatalytic oxidation to the quinone or (2) *via* nitroaryl mediated bond cleavage.[77] In order to identify the cleavage products and to delineate the role of the nitro substituent, we planed to prepare control compounds **3.10** (X = H) and **3.11** (X = F) lacking this substituent, where only photocatalytic oxidation to the quinone would be possible.

[78] F. Fringuelli, O. Piermatti, F. Pizzo, *Synlett* **2003**, 2331-2334.



Scheme 3.2. Deprotection studies of fluorinated and unsubstituted catechols.

We were surprised to discover that the deprotection of either **3.10** or **3.11** under a variety of conditions proved to be impossible, as only decomposition of the starting material and the free fluorophore could be detected. This could be explained by the formation of reactive quinone methides (**3.14**) by cleavage of the fluorophore at the benzylic position.[79]

3.3.2 NPP Derivatives

In order to circumvent the decomposition *via* the quinone methide pathway, we wanted to investigate the corresponding homologated propyl substituted nitrophenols **3.5**. Therefore, acetonide **3.16** was successfully prepared from catechol **3.15** by using 2,2-dimethoxypropane and catalytic amounts of *p*-TsOH in refluxing benzene. Subsequent nitration of the acetonide with half-concentrated HNO_3 afforded **3.16** in high yield. To our delight, these strongly acidic, aqueous conditions did not induce the deprotection of the acetonide group. A X-ray crystal structure of **3.16** was obtained, which in turn confirmed the correct installment position of the nitro group (**Figure 3.5**). The reverse synthetic order, first nitration followed by protection, failed as no acetonide formation could be detected. The crucial elongation reaction of **3.16** with paraformaldehyde and Triton B gave the desired product **3.17**,[80] even though only a moderate conversion was achieved (with 40% starting material **3.16** recovered).

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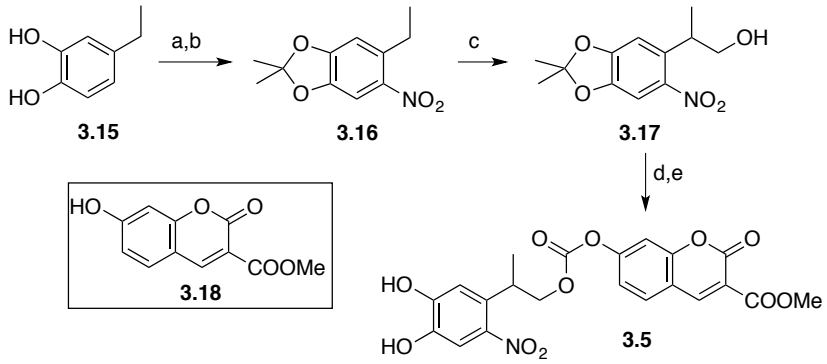
[79] a) M. Sugumaran, V. Semensi, *J. Biol. Chem.* **1990**, *266*, 6073-6078; b) M. Sugumaran, V. Semensi, S. J. Saul, *Arch. Inst. Biochem. Phys*. **1988**, *9*, 269-281; c) M. Sugumaran in *Biological Oxidation Systems*, *Vol. 1*, Academic-Press, **1990**, pp. 347-363.

[80] K. R. Bhushan, *Org. Biomol. Chem.* **2006**, *4*, 1857-1859.



Scheme 3.3. Preparation of NPP conjugate **3.5** for surface modification: a) 2,2-dimethoxypropane, cat. *p*-TsOH, benzene, reflux, overnight, b) HNO_3, H_2O, 0 °C to RT, 1.5 h, 86% (over 2 steps); c) CH_2O, Triton B in MeOH, 85 °C, 65 h, (80% brsm); d) i) triphosgene, Et_3N, THF; 0 °C, 25 min, ii) **3.18**, pyridine, CH_2Cl_2, 0 °C to RT, 1.5 h; e) TFA, H_2O, RT, over night, 68% (over 2 steps).

With the catechol protected as the acetonide, carbonate formation using triphosgene and triethylamine in THF led to the desired chloroformate in quantitative yield within 25 min at 0 °C.[81] To prevent decomposition, the chloroformate was coupled immediately to coumarin **3.18** to afford the desired carbonate, which was subsequently deprotected to the target catechol **3.5** using neat TFA.

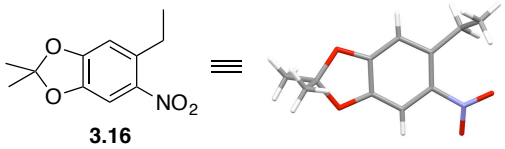


Figure 3.5. Crystal structure of protected nitrocatechol **3.16** confirming the correct installment of the nitro group.

In total, the preparation of **3.5** was achieved in five steps using only two purifications rendering **3.5** an easily accessible and attractive candidate for further investigations. In particular, we expect that the alcohol **3.17** could serve as an ideal starting point for the attachment of various cargo compounds by coupling chemistry.

3.4 Evaluation of the Physical Properties

The stability of the different caged compounds was then investigated using a variety of methods and assays. Gratifyingly, the NPP linker **3.5** was considerably

[81] a) G. H. McGall, A. D. Barone, M. Diggelmann, S. P. A. Fodor, E. Gentalen, N. Ngo, *J. Am. Chem. Soc.* **1997**, *119*, 5081-5090; b) A. Gautier, D. P. Nguyen, H. Lusic, W. An, A. Deiters, J. W. Chin, *J. Am. Chem. Soc.* **2010**, *132*, 4086-4088.

stable to hydrolysis in aqueous medium (MOPS buffer) at pH 5.5 in the dark for at least 72 h. This is contrast to the ethyl derivative **3.4**, which displayed only limited stability. Photocleavage of catechol **3.5** was investigated by detection of the evolving fluorescence during the release of coumarin **3.18** to determine the half-life time of **3.5** in aqueous solution (MOPS buffer) under near UV-irradiation. As expected, a rapid increase in fluorescence was detected during the first minutes of irradiation and the fluorescence maximum was identified at 454 nm. However, after approximately 10 min, the detected fluorescence intensity was decreasing, indicating that photo-bleaching or [2+2] addition of the coumarin **3.18** occurred, which could be confirmed by control experiments using solely the coumarine in solution.

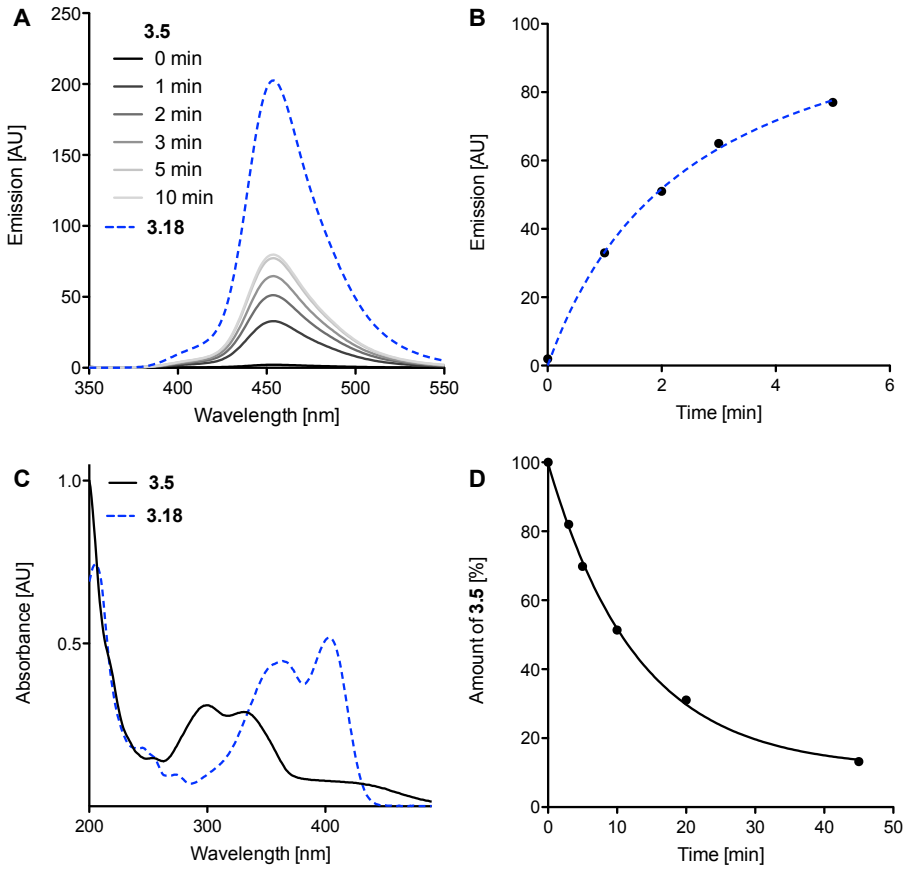
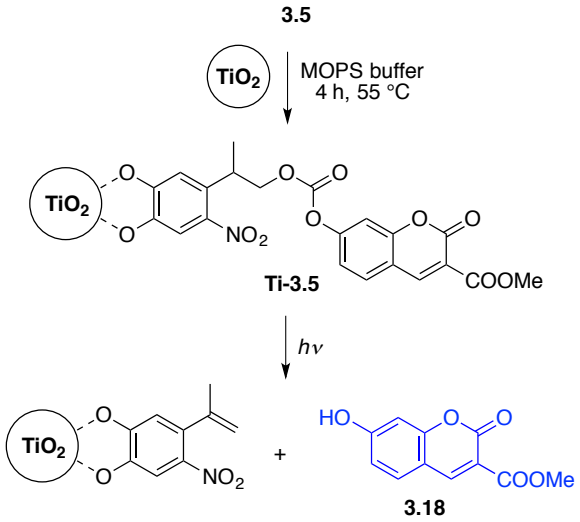


Figure 3.6. A) Fluorescence spectra of **3.5** and **3.18** after certain irradiation times; B) Fluorescence emission intensity at 454 nm depending on the irradiation time at 366 nm of **3.2** as a $5 \cdot 10^{-5}$ M solution in MOPS buffer; C) UV spectra of **3.5** and **3.18**; D) Decay of **3.2** upon UV irradiation at 366 nm, determined by HPLC-MS.

Quantification by HPLC-MS determined the half-life time of **3.5** to be around 12 min at a concentration of $5 \cdot 10^{-5}$ M in buffer. This is an attractively short period for cleavage, since the light intensity of a simple laboratory UV-lamp is rather low (~ 20 mW·cm^{-2}).



Scheme 3.4. Immobilization of nitrochatechol **3.5** on TiO_2 microparticles to deliver **Ti-3.5** and release of the cargo **3.18** upon irradiation.

To evaluate the surface release properties, TiO_2 particles (1.0-2.0 µm) were functionalized with nitrocatechol **3.5** in MOPS buffer at 55 °C according to previously developed conditions.[82] After the incubation, the functionalized particles **Ti-3.5** were washed three times with CH_3CN, and HPLC analysis of the washing solutions determined only small amounts of fluorophore **3.18** and no free **3.5** present.

[82] a) J. Gomes, A. Grunau, A. Lawrence, L. Eberl, K. Gademann, *Chem. Comm*. **2013**, *49*, 155-157; b) J. Gomes, ongoing PhD Thesis, Basel **2013**.

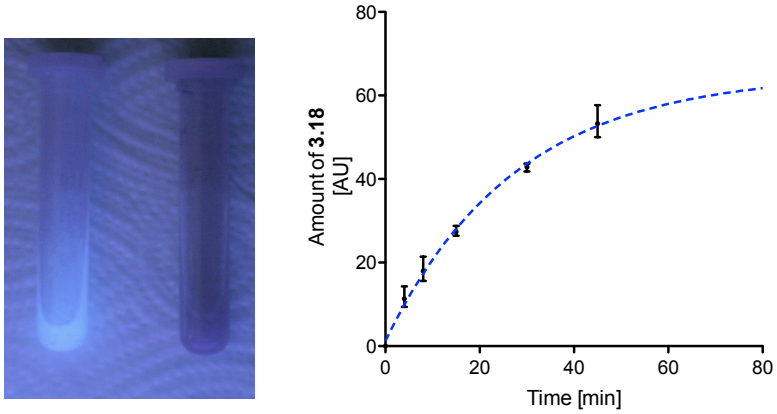


Figure 3.7. Ti-3.5 (left) and negative control (uncoated TiO_2 particles, right) in MOPS buffer upon UV irradiation; Release kinetics of **3.18** from **Ti-3.5** upon UV irradiation at 366 nm in MOPS buffer.

The release of the fluorescent cargo from the functionalized beads was investigated next. The functionalized TiO_2 beads **Ti-3.5** were suspended in MOPS buffer and the mixture was irradiated. Fluorescence became immediately visible (**Figure 3.7A**). Aliquots were taken after certain irradiation time intervals and analyzed by HPLC at 366 nm. The obtained data demonstrates that no catechol **3.5** is present and the amount of **3.18** was increasing over the irradiation time (**Figure 3.7B**), providing evidence that photocleavage of surface-adsorbed nitrocatechol **3.5** and concomitant release of coumarin **3.18** has occured. In this reaction setup, a half-life time of ca. 19 min was determined for the release of **3.18**.

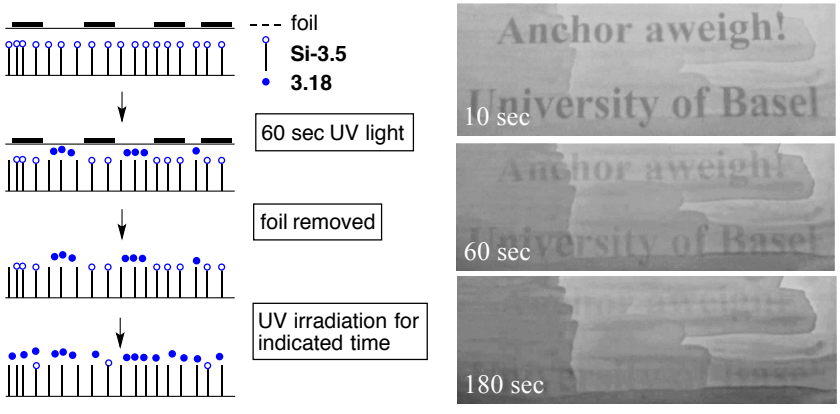


Figure 3.8. TLC plate coated with nitrocatechol **3.5** (**Si-3.5**) and irradiated for 60 sec at 366 nm under a printed foil, after 10 sec exposure to UV light without foil, after 60 sec and after 180 sec UV irradiation at 366 nm (see videos).

Qualitatively, these observations could be corroborated by the immobilization of **3.5** on silica (**Si-3.5**, TLC plate) and controlled release under UV light (**Figure 3.8A**

and **B**; see videos on the enclosed CD for further information). In this simple application, a printed foil was used as template to generate the desired surface pattern, which could be unmasked by release of the fluorophore *via* irradiation.

3.5 Conclusion and Outlook

In conclusion, we have successfully developed a bio-inspired surface modification platform based on nitrocatechols that allows for the controlled release of small molecules under an external stimulus. Salient features of this method involve (1) ease of functionalization of TiO_2 particles under an operationally simple dip-and-rinse procedure, (2) stability of the resulting functionalized particles to repeated washing and (3) rapid release of the small molecule cargo under an external stimulus, *i.e.* UV light. We think that this method displays advantages with regard to controlled release. This surface modification platform might find applications in drug delivery, as caged probes in chemical biology or for direct assays 'on chip'.

The detailed photocleavage process and binding properties of the designed system need to be further investigated in detail. The effect of the tether bridging the coumarine and the caging group should also have an influence of the kinetic release of the cargo. Furthermore it is envisioned to use iron oxide particles instead of TiO_2, so an additional handle for transport namely the magnetic field would be appropriate. Applications of the system to immobilize bioactive small molecules will be investigated in our group.

4 CAGED RETINOIDS AS PHOTOINDUCIBLE ACTIVATORS: IMPLICATION FOR CELL DIFFERENTIATION

4.1 Introduction

Neurodegenerative disorders such as Alzheimer's, Parkinson's or Huntington's disease are becoming more and more prevalent in our aging society as already discussed in the second chapter.[83] In this respect, small molecules could give detailed insight into the progress of neurodegenerative diseases, regarding complex phenomena as plaque formation, loss of connectivity between synapses or neuron-neuron communication.[84] Additionally, treatment with a small molecule is a more convenient way to treat CNS injuries than with gene therapy or neurotrophins, since its use in principle could allow for simple oral administration.[85] In our current research program directed towards neuropharmacological properties of such small molecules, we recently described the total syntheses of the neuroactive steroid withanolide A (**Chapter 2**) as well as several pyridone alkaloids of the militarinone type and evaluated their neuritogenic activity in different cell lines,[86] aiming to control and direct neurite formation and navigate the axonal growth cones by an extrinsic guidance.[87]

Related - signalling molecules, in particularly all-*trans* retinoic acid (ATRA, **4.1**) are established by many pharmacological studies to initiate such a complex 'growth program' resulting in differentiation, neurite outgrowth and axonal elongation, and

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[83] Review: A. Wimo, B. Winblad, H. Aguero-Torres, E. von Strauss, *Alzheimer Dis. Assoc. Disord.* **2003**, *17*, 63-67.

[84] Review: M. P. Mattson, *Nature* **2004**, *430*, 631-639.

[85] a) J. Corcoran, P.-L. So, R. D. Barber, K. J. Vincent, N. D. Mazarakis, K. A. Mitrophanous, S. M. Kingsman, M. Maden, *J. Cell Sci*. **2002**, *115*, 3779-3786; b) M. Agudo, P. Yip, M. Davies, E. Bradbury, P. Doherty, S. McMahon, M. Maden, J. Corcoran, *Neurobiol. Disease* **2010**, *37*, 147-155.

[86] a) H. J. Jessen, A. Schumacher, T. Shaw, A. Pfaltz, K. Gademann, *Angew. Chem. Int. Ed.* **2011**, *50*, 4222-4226; b) H. J. Jessen, A. Schumacher, F. Schmid, A. Pfaltz, K. Gademann, *Org. Lett.* **2011**, *13*, 4368-4370; c) F. Schmid, H. J. Jessen, P. Burch, K. Gademann, *Med. Chem. Commun.* **2013**, *4*, 135-139; d) F. Schmid, ongoing PhD Thesis, Basel, **2013**.

[87] B. M. Marsick, K. C. Flynn, M. Santiago-Medina, J. R. Bamburg, P. C. Letourneau, *Devel. Neurobiol*. **2010**, *70*, 565-588.

therefore represent a standard control substance.[88,89] *Corcoran* and *Maden* have examined that the vitamin A metabolite is critical for neurite regeneration and that its synthesis is regulated by the neurotrophin nerve growth factor (NGF) and *vice versa*.[90]

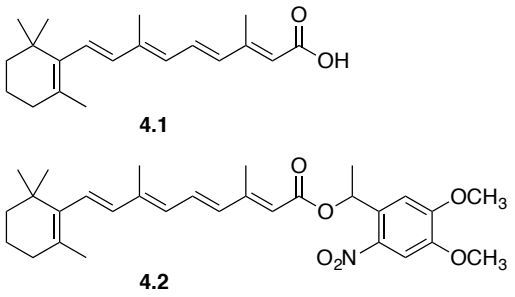

Figure 4.1. Structures of retinoic acid (ATRA, **4.1**) and the caged analog (**4.2**) used by Jullien *et al.* in zebrafish embryos.[91]

Recently, *Jullien* reported on photo-released ATRA in zebrafish embryos by one and two-photon excitation with UV light, to induce teratogens in the vertebrate (**Figure 4.1**).[91] While the photo-regulation effect could be verified after passive diffusion into the cells, photo-isomerization and degradation of the light-sensitive polyene side chain of ATRA (**4.2**) were recognized as drawbacks, besides the lack of selectivity to one specific receptor.

The use of selective agonists showed that RARβ agonists stimulate neurite outgrowth, proposing ATRA acting *via* RARβ2 in the outgrowth of neurites.[92] The binding mode of ATRA can be rationalized from crystal structures of the ligand-binding domain of RARs (or related RXRs, **Figure 4.2**)[93] with different retinoids suggesting an attraction for the carboxylic acid moiety and inducing a 'mouse-trap' mechanism. Therefore an appropriate ligand should fulfil two criteria: 1) contain a

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88 V. Perissi, M. G. Rosenfeld, *Nature* **2005**, *6*, 542-554.

89 Review: M. Clagett-Dame, E. M. McNeill, P. D. Muley, *J. Neurobiol*. **2006**, *66*, 739-756.

90 J. Corcoran, M. Maden, *Nat. Neurosci*. **1999**, *2*, 307-308.

91 P. Neveu, I. Aujard, C. Benbrahim, T. Le Saux, J.-F. Allemand, S. Vriz, D. Bensimon, L. Jullien, *Angew. Chem. Int. Ed.* **2008**, *47*, 3744-3746.

92 J. Corcoran, B. Shroot, J. Pizzey, M. Maden, *J. Cell Sci*. **2000**, *113*, 2567-2574.

93 a) P. Germain, S. Kammerer, E. Pérez, C. Peluso-Iltis, D. Tortolani, F. C. Zusi, J. Starrett, P. Lapointe, J.-P. Daris, A. Marinier, A. R. de Lera, N. Rochel, H. Gronemeyer, *EMBO Rep.* **2004**, *5*, 877-882; b) J.-P. Renaud, N. Rochel, M. Ruff, V. Vivat, P. Chambon, H. Gronemeyer, D. Moras, *Nature* **1995**, *378*, 681-689; c) A. R. de Lera, W. Bourguet, L. Altucci, H. Gronemeyer, *Nature Drug Disc. Rev.* **2007**, *6*, 811-820.

carboxylic acid functionality for the electrostatic attraction and 2) must not exceed a certain length to keep its agonistic properties.

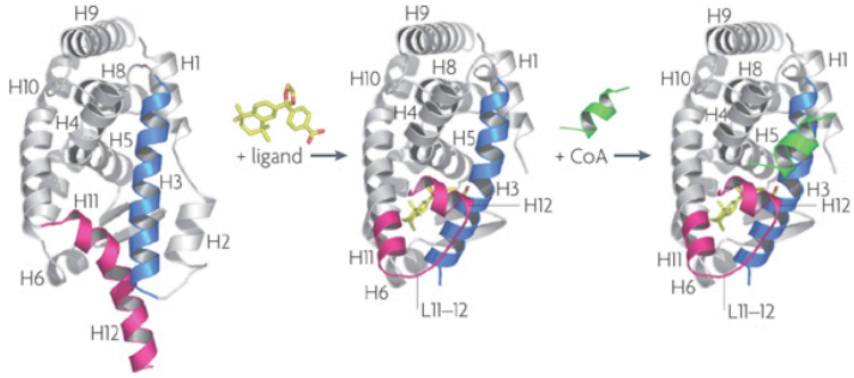

Figure 4.2. X-ray crystal structure of the related RXR binding domain, co-crystallized with RXR agonist (SR11237). (adapted by permission from *Nature Drug Disc. Rev.* **2007**, *6*, 811-820). Upon binding of the agonistic ligand induced conformational change occurs to display a hydrophobic surface selectiv for short helical motif of LXXLL. L = leucine; X = any amino acid.

Based on the third generation SAR study of *Carpentier*,[94] we wanted to use retinoid **4.3** as a temporally tuneable activator for neurite outgrowth. Compound **4.3** (**Figure 2.2**; RARα (695±248 nM), RARβ (21±6 nM), RARγ (72±2 nM)) has an attractive activity and selectivity profile towards RARβ as required. Further, the alcohol functionality in **4.3** offers the possibility to bind an adjustable protecting group to the agonist, thereby increasing the length and temporally inactivating its biological activity.[95] Upon uncaging with an external stimulus, *e.g.* irradiation with UV light of a photolabile protecting group, the concentration of free ligand **4.3** should increase, postulated binding to RARβ should occur and thereupon lead to the active conformation of the protein, resulting in desired neurite outgrowth.

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[94] a) B. Charpentier, J.-M. Bernardon, J. Eustache, C. Millois, B. Martin, S. Michel, B. Shroot, *J. Med. Chem.* **1995**, *38*, 4993-5006; b) B. Charpentier, Centre International de Recherches Dermatologiques Galderma, US-Patent: US5547983 A1, **1996**, 1-18.

[95] K. H. Link, F. G. Cruz, H.-F. Ye, K. E. O'Reilly, S. Dowdell, J. T. Koh, *Bioorg. Med. Chem.* **2004**, *12*, 5949-5959.

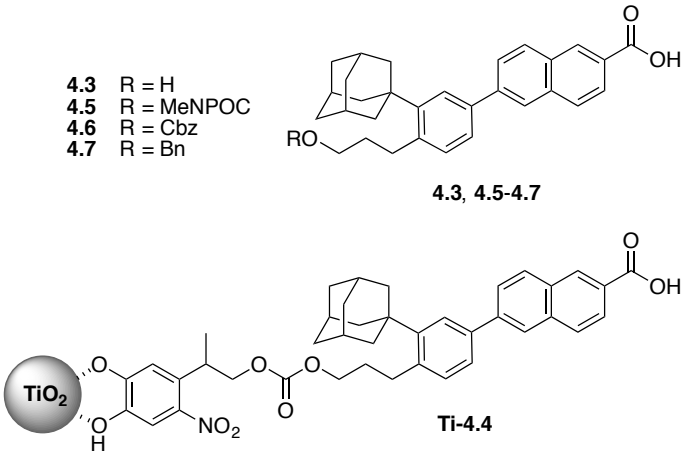


Figure 4.3. Retinoids as agonists or non-agonists for RARβ. MeNPOC = [(α-methyl-2-nitropiperonyl)oxy] carbonyl; Cbz = carboxybenzyl; Bn = benzyl.

As photolabile protecting group the established [(α-methyl-2-nitropiperonyl)oxy]carbonyl (MeNPOC)[96] was chosen, because its photochemical properties are well understood and its chemical stablility under physiological conditions. Similarly, free nitrocatechol allows the attachment of small molecules to a solid support (**Ti-4.4**), and thereby provides access to direct neurite outgrowth also in a spatially controllable fashion. This methodology was introduced in **Chapter 3** in a proof-of-concept study, using a caged fluorophore to investigate light mediated release properties from a solid support.[77]

Herein, the development of caged retinoids as photoinducible activators to temporally and spatially control neurite outgrowth is presented. Release of the active agonist by UV irradiation leads to significant neurite outgrowth and confirmed the selectivity of the ATRA receptors.

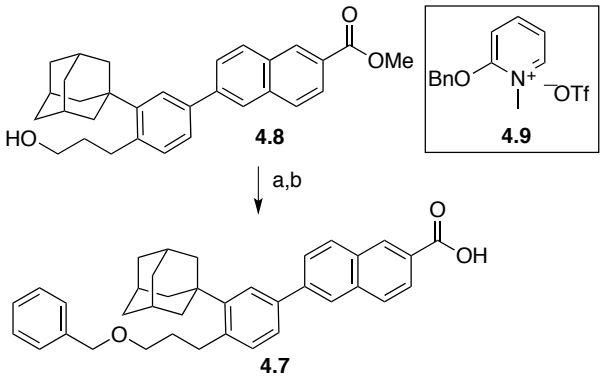
4.2 Results and Discussion

4.2.1 Synthesis

The synthesis of the caged retinoids **4.4** and **4.5** as well as control substances **4.6** and **4.7** started from the known route to acid **4.3** by *Carpentier* and co-workers.[94] For the introduction of the photolabile protecting group to the primary hydroxy group different strategies were investigated. Etherifications of the alcohol group of the methyl ester **4.8**, a synthetic intermediate in the established 10 step sequence to

[96] J. H. Kaplan, B. Forbush III, J. F. Hoffman, *Biochemistry* **1978**, *17*, 1929-1935.

retinoid **4.3**, with nitrophenyl derivatives was not successful under a variety of conditions (Williamson ether synthesis, glycosylation strategy using trichloro-acetimidate, Lewis acid catalyzed etherification).[97] Except for the benzylation under neutral conditions using BnOPT (**4.9**),[98] which resulted in full conversion to the desired ether (**Scheme 4.1**), and upon hydrolysis with aqueous LiOH in THF, led to the free acid **4.7**.



Scheme 4.1. Synthesis of control compound **4.7**: a) **4.9**, MgO, DCE, rfx, 95%; b) LiOH, H_2O, THF, RT, *quant*.

In order to circumvent problems associated with the etherification we used a protocol to introduce the photolabile MeNPOC group as its carbonate.[99] Subsequent hydrolysis to set free acid **4.5** would cleave the carbonate linkage even by use of mild LiOH. So, we first formed the *tert*-butyl ester **4.10** by exposing acid **4.3** to DMF di-*tert*-butyl acetal in a mixture of refluxing benzene and THF (**Scheme 4.2**). Due to the low solubility of this acid **4.3** only moderate conversion was accomplished, but a simple acid-base-extraction could re-isolate the remaining starting material.

To reach the desired caged retinoid **4.5**, *tert*-butyl ester **4.10** was added to a mixture of MeNPOC chloroformate in pyridine/ CH_2Cl_2 at 0 °C to yield the corresponding carbonate in excellent 95% yield. Upon exposure to a mixture of trifluoro-acetic acid **4.5** was obtained in quantitative yield as an off-white solid, decomposing at temperatures above 125 °C. All operations involving the light-sensitive MeNPOC

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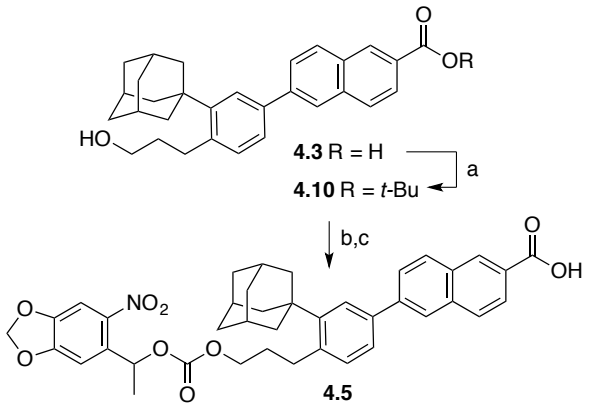
97 V. J. Patil, *Tetrahedron Lett*. **1996**, *37*, 1481-1484.

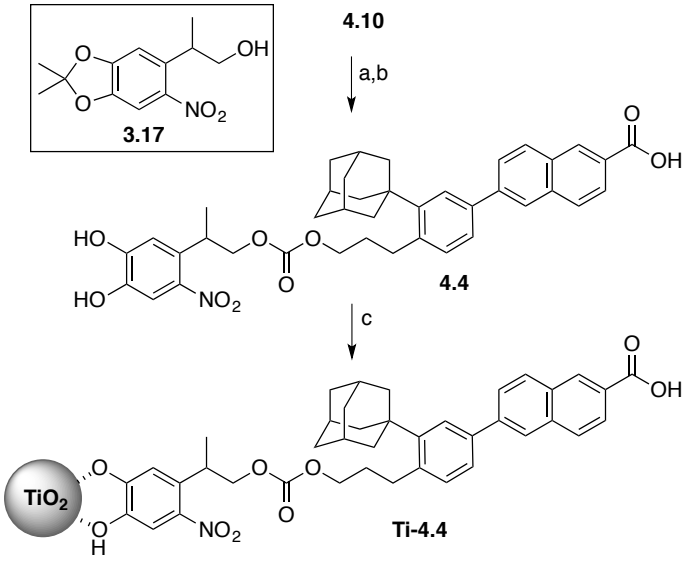
98 W. C. K. Poon, S. E. House, G. B. Dudley, *Synlett* **2005**, *20*, 3142-3144.

99 G. H. McGall, A. D. Barone, M. Diggelmann, S. P. A. Fodor, E. Gentalen, N. Ngo, *J. Am. Chem. Soc.* **1997**, *119*, 5083-5090.



Scheme 4.2. Synthesis of caged retinoid **4.5**: a) DMF di-*tert*-butyl acetal, benzene, THF, 90 °C, 23% (99% *brsm*); b) MeNPOC chloroformate, pyridine, CH_2Cl_2, 0 °C to RT, 95%; c) TFA, TIPS, CH_2Cl_2, RT, 99%.

group were performed in the dark, although photo-deprotection was not detectable for short exposure to daylight. The same reaction sequence starting from ester **4.10** was used to synthesize benzyloxycarbonyl (Cbz) protected retinoid **4.6**, as a non-agonistic control substance in 53% yield over two steps.



Scheme 4.3. Synthesis of the caged retinoid **4.4** and its immobilization on TiO_2 surface to **Ti-4.4**: a) *i)* **3.15**, triphosgene, Et_3N, THF; 0 °C, 25 min; *ii)* **4.10**, pyridine, CH_2Cl_2, 0 °C to RT, 1.5 h; b) TFA, H_2O, RT, over night, 42% (over 2 steps); c) TiO_2, MOPS buffer, 50 °C, 4 h.

Following our protocol for the immobilization of small molecules on metal oxide surfaces by an operationally simple dip-and-rinse procedure (**Chapter 3**), we examined the synthesis of nitrocatechol **4.4** (**Scheme 4.3**). Nitroarene alcohol **3.17** was converted with triphosgene and triethylamine in 25 min at 0 °C to the chloro-

formate in quantitative yield. Subsequently, the previously established conditions (pyridine, CH_2Cl_2 at 0 °C to room temperature) were used to generate the carbonate, containing small amounts of the homo-coupled nitrophenyl carbonate as an inseparable side-product. Exposure to TFA supplemented with a small amount of water gave finally access to the free catechol **4.4** as a yellow solid in moderate yield (42% over 2 steps) after reverse-phase HPLC purification.

Immobilization on TiO_2 microparticles (1.0-2.0 μm) to **Ti-4.4** was accomplished by incubation of **4.4** in MOPS buffer at 50 °C for 4 h. After incubation, the functionalized particles **Ti-4.4** were washed three times with CH_3CN and once with MOPS buffer; HPLC analysis of the last washing solution determined no free agonist **4.3**. This synthesis demonstrated the versatility of the nitroarene alcohol **3.17** for subsequent chemical transformations. This simple combination of two functionalities in compound **3.17**, namely the catechol unit and the simple nitroarene, allows for the caging on solid support and controlled release on demand by non-invasive UV light at 366 nm.

4.2.2 Studies on Photocleavage

The ability of both photolabile compounds **Ti-4.4** and **4.5** to liberate free agonist **4.3** was investigated next by spectroscopic means (HPLC-MS and UV). First, it was demonstrated that both compounds were stable to the appropriate solvent environment over a period of at least 3 days incubation in the dark, and no free alcohol **4.3** could be detected by HPLC.

Then, MeNPOC derivative **4.3** was irradiated with a common laboratory UV lamp (~20 $mW \cdot cm^{-2}$) at 366 nm in cell medium (minimal essential medium, MEM) in a 24-well plate covered with a glass disk in order to filter off undesired short-wave radiation. Aliquots were taken after indicated irradiation time intervals and analysed by HPLC with the help of their UV absorption. The half-life could be determined for the cleavage of **4.3** in MEM with an irradiated surface area of approximately 2.5 cm^2 per 1 mL solvent volume to 8.3 min (**Figure 4.4B**).

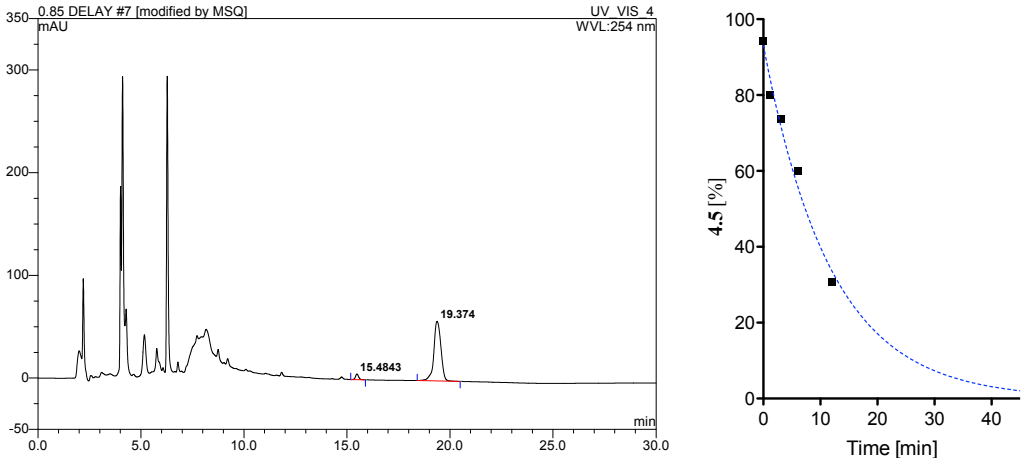
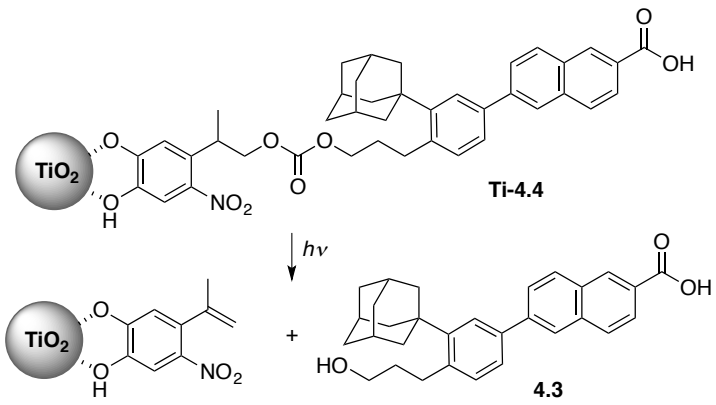


Figure 4.4. A) Representative HPLC diagram for the cleavage process after 30 s irradiation: UV traces at 254 nm, retention times for retinoid **4.3** (t_R = 15.484 min) and caged-retinoid **4.5** (t_R = 19.374 min); minor peaks from 2 to 10 min derive from the cell medium (MEM); B) Uncaging process of retinoid **4.5** to **4.3** during exposure to UV light at 366 nm; cell medium, 24-well plate: volume = 1 mL, irradiated surface area = 254.5 mm^2.

The uncaging properties of **Ti-4.4** were determined in a similar setup. The incubated TiO_2 particles **Ti-4.4** were resuspended in cell medium in a 24-well plate and irradiated with a common UV lamp. Aliquots were taken after the indicated period and analysed by HPLC-MS. The HPLC analysis indicates release of free agonist **4.3** occurred in detectable quantities after 5 to 10 min of irradiation (HPLC traces and UV spectra). Longer exposure to 366 nm UV light for extended periods (1 h to 15 h) strongly increased the liberation of free agonist **4.3**.



Scheme 4.4. Cleavage of **Ti-4.2** induced by UV irradiation at 366 nm to liberate agonist **1** from a solid support.

4.2.3 Investigations of the Neuritogenic Activity

We then investigated the target and control compounds for their ability to induce neurite outgrowth in the human neuroblastoma SH-SY5Y cellular model.[60] To achieve this goal, free alcohol **4.3**, caged acid **4.5**, carbonate **4.6** and ether **4.7** were evaluated in human SH-SY5Y cells according to the method described and optimized in **Chapter 2**.[31b] Briefly, cells were grown on collagen coated 24-well plates in minimum essential medium (MEM without an indicator) supplemented with 5% fetal bovine serum in the presence of the corresponding compounds (1 μM, 0.1% in DMSO) at 37°C in humidified atmosphere (CO_2 content 5%). In control experiments, cells were treated with DMSO as a negative or ATRA as a positive control. After incubation for four days, cells were fixed, stained and examined under a phase contrast microscope. The differentiated cells from three random areas of the well were counted. In each experiment at least 500 cells were counted, except for DMSO and caged compound **4.5**. Those having at least one neurite with a length of more than 50 μm were counted as positive.

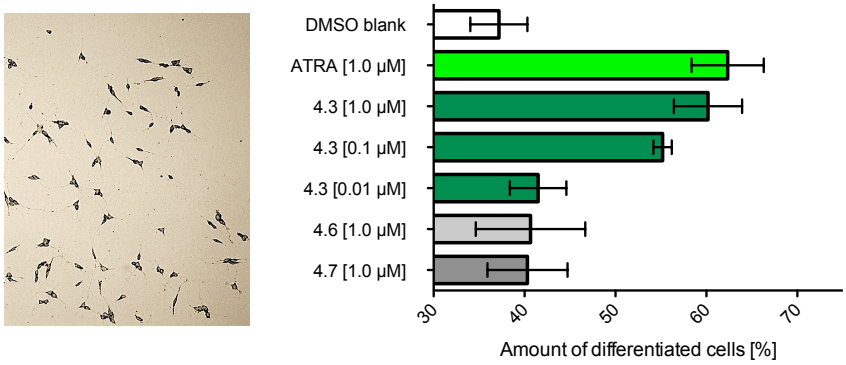


Figure 4.5. A) Neurite outgrowth activity of ATRA, DMSO blank, retinoids **4.3**, **4.6** and **4.7** in human SH-SY5Y cells with MEM as cell-medium. Error bars denote SEM. B) Representative micrograph for retinoid **4.3** (0.1 μM).

The results are summarized in **Figure 4.5B** and representative micrographs are given in the **Appendices**. The results of the experiments confirms our expectations that retinoid **4.3** acts as agonist and significantly induces neurite outgrowth down to a concentration as low as 0.1 μM, on a scale comparable to ATRA itself. The observed phenotypes (micrograph; **Figure 4.5A**) display the same characteristics as for ATRA: almost complete differentiation, indicated by formation of several long neurites for each individual cell, no aggregate formation and a high cell-density on the well

surface. Below a concentration of 0.1 µM retinoid **4.3** does not display significant activity. The control-compounds **4.6** and **4.7** (concentration = 1 µM) do not act as agonists and their phenotypes strongly resemble the one observed for vehicle (DMSO) as negative control. In addition, high cell aggregate formation complicates the evaluation and a low cell-density on the surface is observed. The low observed cell density can be explained by the removal of non-differentiated cells by washing before the fixation step.

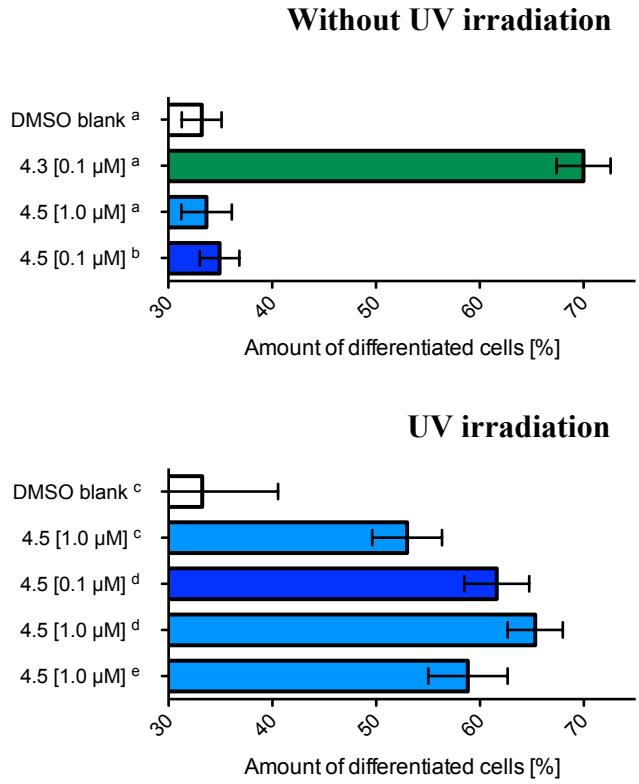


Figure 4.6. Neurite outgrowth activity of retinoid **4.3** (0.1 µM), DMSO blank and caged retinoid **4.5** (0.1 µM or 1.0 µM) in human SH-SY5Y cells with MEM as medium; A) **without UV irradiation**: [a] performed as control without UV irradiation; [b] incubated for 24 h, prewashed without irradiation; B) **UV irradiation**: [c] irradiated for 60 s at 366 nm without prewash; [d] irradiated for 180 s at 366 nm without prewash; [e] irradiated for 180 s at 366 nm after incubation for 24 h prewash. Error bars denote SEM.

A second assay based on the kinetics of the investigated uncaging process of **4.5** to **4.3** was performed to temporally control neurite outgrowth. Again, the vehicle control experiment (0.1 % DMSO) displayed a completely different phenotype compared to cells treated with ATRA or **4.3**. We almost exclusively observed large cell aggregates with only a few isolated, non-differentiated cells. The same picture could be drawn

for the evaluation of caged retinoid **4.5**, as long as no UV irradiation liberated the free agonist **4.3**. This successful control experiment indicates that the caged compound does not act as agonist as required for the experiment-setup, as anticipated after the results of control compounds (**Figure 4.6A**).

Upon UV irradiation of caged compound **4.5** for 60 or 180 s neurite outgrowth with familiar phenotypes was induced (**Figure 4.6B**). To simulate intracellular uncaging, cells were incubated for 24 h with caged retinoid **4.5**, but prior to irradiation the cell medium was removed, cells were washed twice with fresh media and directly irradiated for 60 s. Again differentiation was the read-out as in the cases of no prewash before illumination.

Ti-4.4 was not suitable for our assay conditions, as it did not allow us the visual elaboration of the neurite outgrowth due to a lack of background contrast from the TiO_2 microparticles. In further studies a more convenient surface consistence based on biocompatible TiO_2 should be investigated to proof our concept to navigate axonal growth cones by an extrinsic guidance on surface.

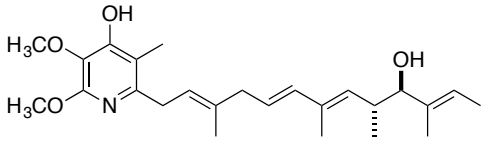
4.3 Conclusion

In summary we were able to show that retinoid **4.3** exhibits significant neurite outgrowth activities as anticipated for a RARβ agonist. For caged retinoid **4.5**, as well as for the non-agonistic control compounds **4.6** and **4.7**, no significant outgrowth could be determined in the cell assay. The controlled release of retinoid agonist **4.3** from the caged precursor **4.5** was achieved using non-invasive UV light (366 nm) in cell-medium. As a result, neuron differentiation and neurite outgrowth could be temporally controlled by using our caged retinoid probes in solution. Furthermore, anchor **4.4** was successfully synthesized and the agonist was caged and immobilized on TiO_2 particles (**Ti-4.4**). Although the release properties of **Ti-4.4** have been investigated by spectroscopy, further studies should investigate if **Ti-4.4** can direct neurite outgrowth on surfaces triggered by light under certain assay conditions or by carefully choosing an appropriate surface texture.

5 TOTAL SYNTHESES OF JBIR-02 AND RELATED PIERICIDINS

5.1 Classification and Biological Activities of Piericidins

Piericidins belong to a family of antibiotics that are produced dominantly by various *Streptomyces* strains. Piericidin A_1 the prototypical exemplar was isolated in 1963 from *Streptomyces mobaraensis* by Takahashi and co-workers (**Figure 5.1**).[100] The elucidation of the structure remained in those early days of NMR spectroscopy problematic,[101] even though more and more members of the piericidin family were isolated.[102] Additionally, the originally attributed configuration had to be reassigned several times[103] and was not established until the first total synthesis of piericidin A_1 and B_1 in 2005 by Boger and Schnermann.[104]



5.1

Figure 5.1. Piericidin A_1 (**5.1**) parent structure of the piericidin family.

Still today, the isolation of novel piericidins remains an active field[105] and multiple biological targets besides the long known inhibitory effects of electron transport chain[106] attract the attention of researchers from various disciplines. Up to now more

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[100] S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki, J. Nagatsu, *Agr. Biol. Chem.* **1963**, *27*, 576-582.

[101] N. Takahashi, A. Suzuki, Y. Kimura, S. Miyamoto, *Tetrahedron Lett.* **1967**, *21*, 1961-1964.

[102] S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe, N. Takahashi, *Agr. Biol. Chem.* **1977**, *41*, 855-862.

[103] R. Jansen, G. Höfle, *Tetrahedron Lett.* **1983**, *24*, 5485-5486.

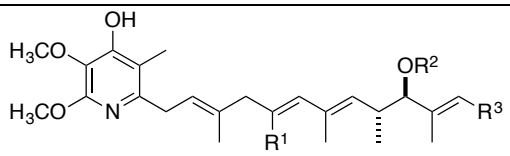
[104] a) M. J. Schnermann, D. L. Boger, *J. Am. Chem. Soc.* **2005**, *127*, 15704-15705; b) M. J. Schnermann, F. A. Romero, I. Hwang, E. Nakamura-Ogiso, T. Yagi, D. L. Boger, *J. Am. Chem. Soc*. **2006**, *128*, 11799-11807; c) M. J. Schnermann, PhD Thesis, La Jolla, **2008**.

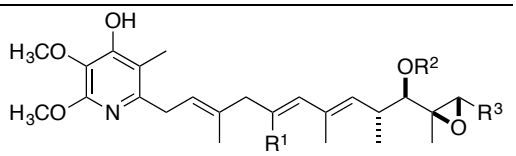
[105] K. A. Shaaban, E. Helmke, G. Kelter, H. H. Fiebig, H. Laatsch, *J. Antibiot.* **2011**, *64*, 205-209.

[106] a) D. L. Nelson, M. M. Cox in *Lehninger Principles of Biochemistry*, Worth, **2000**; Chapter 19; b) D. Voet, J. G. Voet in *Biochemie*, Wiley-VCH, **1994**, pp. 527-545.

then twenty different piericidins have been isolated (**Table 5.1**),[107] sharing some characteristics in their structural arrangement.

Table 5.1. Core structures of the piericidin class. Natural products have been isolated (colorless), putative natural products (light grey) and natural products labeled with * have been synthesized by total synthesis.





	R^1	R^2	R^3		R^1	R^2	R^3
A_1*	H	H	Me	C_1	H	H	Me
A_2	Me	H	Me	C_2	Me	H	Me
A_3	H	H	*i*-Pr	C_3	H	H	*i*-Pr
A_4	Me	H	*i*-Pr	C_4	Me	H	*i*-Pr
A_5	H	H	Et	C_5	H	H	Et
A_6	Me	H	Et	C_6	Me	H	Et
A_7	H	H	C_4H_7	C_7	H	H	C_4H_7
A_8	Me	H	C_4H_7	C_8	Me	H	C_4H_7
B_1*	H	Me	Me	D_1	H	Me	Me
B_2	Me	Me	Me	D_2	Me	Me	Me
B_3	H	Me	*i*-Pr	D_3	H	Me	*i*-Pr
B_4	Me	Me	*i*-Pr	D_4	Me	Me	*i*-Pr
B_5	H	Me	Et	D_5	H	Me	Et
B_6	Me	Me	Et	D_6	Me	Me	Et
B_7	H	Me	C_4H_7	D_7	H	Me	C_4H_7
B_8	Me	Me	C_4H_7	D_8	Me	Me	C_4H_7

The compounds named A_7 and A_8 for systematic reasons in this table correspond to IT-143-A and IT-143-B.[108]

Interestingly for each individual member novel biochemical properties were reported presumably due to a lack of material for a necessary comparisons of the individual compounds. Moreover, the implication from **Table 5.1** is that the established structural motif is highly preserved in nature and in most cases only small

[107] G. P. Meier, Master Thesis, Basel, **2012**.

[108] A. Urakawa, T. Sasaki, K.-I. Yoshida, T. Otani, Y. Lei, W. Yun, *J. Antibiot.* **1996**, *49*, 1052-1055.

post-modifications in the biosynthesis lead to relatives of the parent natural product piericidin A_1 (methylation of the secondary alcohol for class B and D or epoxidation of the adjacent double-bond for class C and D). More significant variations that have been isolated include (**Figure 5.2**) altered heterocycles (**5.2** and **5.3**), tailored (**5.4**) or glycosilated side chains (**5.5**) and the corresponding *N*-oxides of the pyridine moiety (**5.6**).

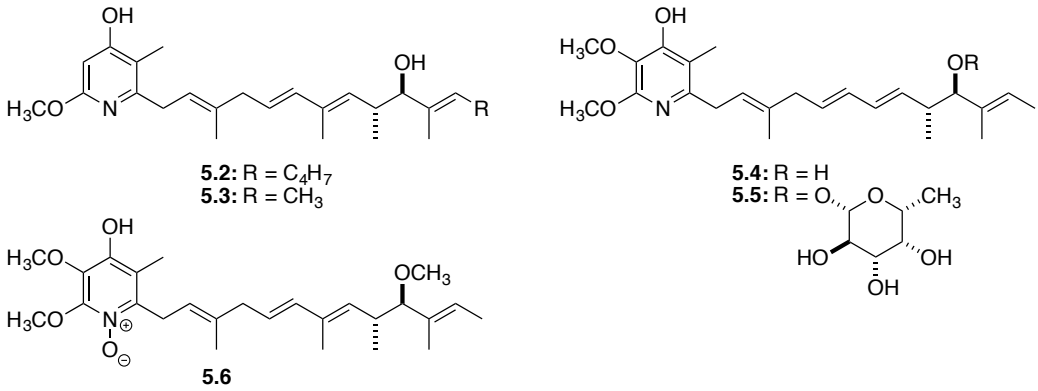


Figure 5.2. Structures of some isolated variations of piericidins A_1: JBIR-02 (**5.2**)[109] and Mer-A2026B (**5.3**),[110] will be the object of the following total synthesis, 7-de-methylpiericidin A_1 (**5.4**),[111] 7-demethyl-3'-rhamnopiericidin A_1 (**5.5**),[111] piericidin B_1 *N*-oxide (**5.6**).[112]

The prominent pharmaceutical feature of piericidins is their ability to mimic ubiquinone (CoQ, **5.7**). Due to their high structural similarity, inhibition of complex I of the ubiquitous mitochondrial NADH dehydrogenase (K_i = 0.6-1.0 μM) is accomplished through which they exhibit their strong antimicrobial, antifungal and antitumor activities. Complex I, the first enzyme of the energy transducting enzymes in the electron transport chain is mainly responsible to build an electrochemical potential.

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[109] J. Ueda, T. Togashi, S. Matukura, A. Nagai, T. Nakashima, H. Komaki, K. Anzai, S. Harayama, T. Doi, T. Takahashi, T. Natsume, Y. Kisu, N. Goshima, N. Nomura, M. Takagi, K. Shin-ya, *J. Antibiot.* **2007**, *60*, 459-462.

[110] a) K. Kominato, Y. Watanabe, S.-I. Hirano, T. Kioka, T. Terasawa, T. Yoshioka, K. Okamura, H. Tone, *J. Antibiotics* **1995**, *48*, 99-102; b) K. Kominato, Y. Watanabe, S.-I. Hirano, T. Kioka, T. Terasawa, T. Yoshioka, K. Okamura, H. Tone, *J. Antibiot.* **1995**, *48*, 103-105.

[111] K. Kimura, H. Takahashi, N. Miyata, M. Yoshihama, M. J. Uramoto, *J. Antibiot.* **1996**, *49*, 697-699.

[112] H. Nishioka, M. Imoto, T. Imaoka, T. Sawa, T. Takeuchi, K. Umezawa, *J. Antibiot.* **1994**, *47*, 447-452.

The following reaction is catalyzed by complex I:

$$NADH + H^+ + CoQ + 4H^+_{in} \rightarrow NAD^+ + CoQH_2 + 4H^+_{out}$$

In this process, the complex translocates four protons facilitated by ubiquinone across the inner membrane, what creates a proton gradient used to produce ATP from ADP. Other famous natural inhibitors of the respiratory chain are rotenone (**5.8**), which was used as fishing poison in the 17th century[113] and members of the myxalamide family (**5.9** and **5.10**).

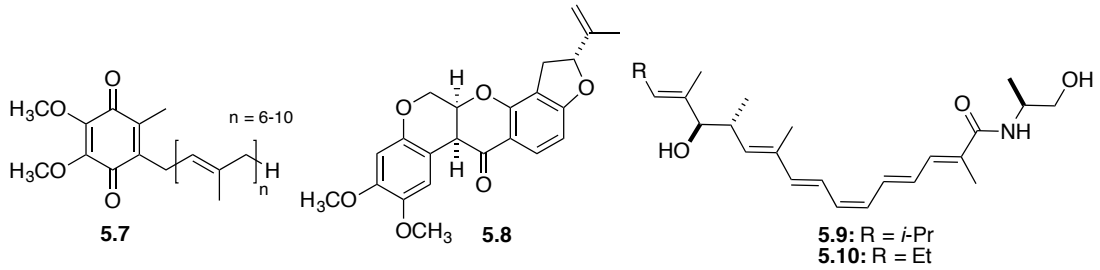


Figure 5.3. Structures of ubiquinone (coenzyme CoQ, **5.7**) and further potent inhibitors of the electron transport chain in cells: rotenone (**5.8**) and myxalamide B (**5.9**, R = *i*-Pr) & D (**5.10**, R = Et).

The symbiosis of beewolf digger wasps with certain strains of *Streptomyces* to incooperate piericidins into the larval cocoon of their offspring is a related phenomenon that recently caused much attention.[114]

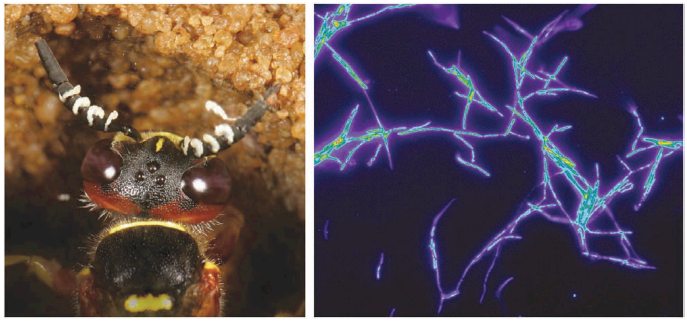


Figure 5.4. Left: Beewolf digger wasp secreting *Streptomyces* with their glands. Right: Fluorescently labeled symbiotic *Streptomyces* strains. (adapted by permission from *Nat. Chem. Biol*. **2010**, *6*, 261-263).

[113] C. Moretti, P. Grenand, *J. Ethnopharmacol*. **1982**, *6*, 139-160.

[114] a) J. Kroiss, M. Kaltenpoth, B. Schneider, M.-G. Schwinger, C. Hertweck, R. K. Maddula, E. Strohm, A. Svatos, *Nat. Chem. Biol*. **2010**, *6*, 261-263; b) S. Koehler, J. Doubsky, M. Kaltenpoth, *Front. Zool.*, **2013**, **doi**:10.1186/1742-9994-10-3.

The cooperative effect of this piericidin cocktail provides an astonishing vital defense against competitors such as fungi or bacteria that grow preferable in the warm and humid environment of the larvae. Female beewolves cultivate certain strains of *Streptomyces* in their unique antennal glands and administer them on top of the brood thereby creating an antimicrobial, antifungal coating.

5.2 Biosynthetic Pathways for the Formation of Piericidins

Taking in account these potent biological activities, investigations on the biosynthesis of piericidin were already performed as early as 1968. First, simple feeding experiments with ^{14}C labeled propionate and acetate established the origin of the side chain pattern after degradation analysis.[115] The arragenement follows the well investigated polketide pathway wherein malonyl-CoA or methyl malonyl-CoA is incooperated into the growing chain *via* acyl carrier proteins (ACPs). However, it remained unclear until last year how and when the aromatic core structure is formed.

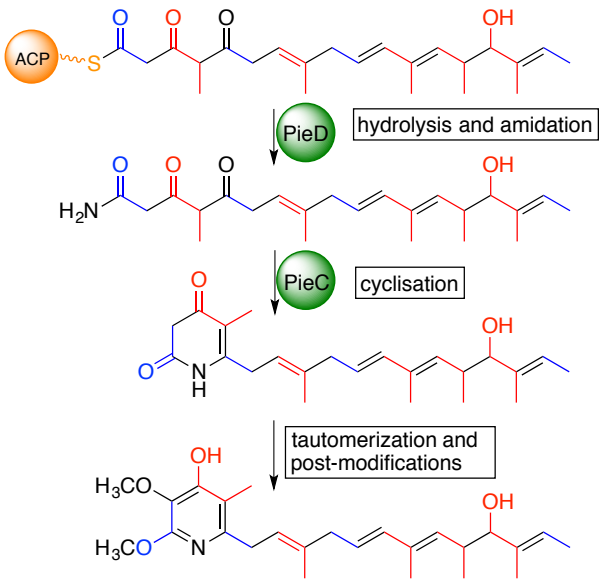


Figure 5.5. Constitution of piericidin A_1 (**5.1**) and its biosynthetic precursors, propionate and acetate units are labeled red respectively blue. ACP = acyl carrier protein; PieD = polyketide synthase (PKS; here: amidotranferase); PieC = PKS (here: cyclase).

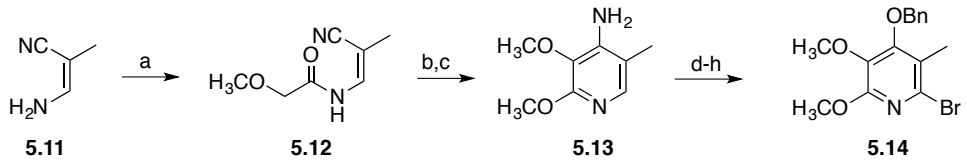
In 2012 You and co-workers sequenced and identified several gen cluster located in *Streptomyces piomogeues var.* and revealed a pathway for the cyclisation to form

[115] N. Takahashi, Y. Kimura, S. Tamura, *Tetrahedron Lett.* **1968**, *45*, 4659-4662.

the pyridine ring.[116] In their analysis the identification of all polyketide synthase (PKS) genes, responsible for the elongation of the side chain, as well as hydrolase, amidotransferase (PieD) and cyclase (PieC) could be verified and compared to existing gene clusters known from the biosynthesis of other natural products (**Figure 1.1**). Post-modifications, involving the hydroxylation of the aromatic moiety (PieE) and the two *O*-methylation (PieB1 and PieB2) have not yet been completely clarified. Studies towards the origin of methyl groups (CH_3O-) point towards S-adenosyl-methionine (SAM) mediated methyl transfer (PieB1) which was earlier emphazised by ^{13}C-enriched NMR studies,[117,118] however the responsible gene cluster has not yet been isolated.

5.3 Previous Synthetic Contributions

The initial reports on the first synthesis of the piercidin structures date back to 1977 when Schmidtchen and Rapoport accomplished natural product analogs featuring simplified side chains.[119] For the construction of the heteroaromatic core they started from 3-amino-2-methylacrylonitrile (**5.11**) which was condensed with methoxyacetylchloride to acrylonitrile **5.12** (**Scheme 5.1**). Cyclisation of the enamide by NaH and methylation furnished aminopyridine **5.13**.



5.11 **5.12** **5.13** **5.14**

Scheme 5.1. Synthesis of the heteroaromatic moiety by Rapoport *et al.*:[119] a) methoxy-acetyl chloride, *n*-BuLi, THF, 59%; b) NaH, dioxane/THF, 40%; c) $(CH_3)_3OBF_4$, CH_2Cl_2, 69%; d) 4-$NO_2(C_6H_4)COCl$, pyridine, 80%, e) Br_2, NaOAc, AcOH, 68%; f) KOH, CH_2OHCH_2OH, 110 °C, 86%; g) $NaNO_2$, AcOH, H_2SO_4, H_2O, 0 °C, 84%; h) *O*-benzyldiisopropylisourea, 100 °C, 90%.

To overcome stability problems associated with the diazotation and the bromination, pyridine **5.13** was first acetylated using nitrobenzoylchloride, sub-

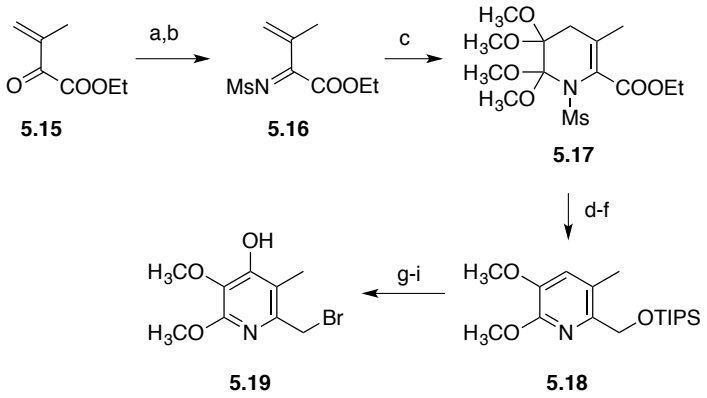
[116] Q. Liu, F. Yao, Y. H. Chooi, Q. Kang, W. Xu, Y. Li, Y. Shao, Y. Shi, Z. Deng, Y. Tang, D. You, *Chemistry & Biology* **2012**, *19*, 243-253.

[117] Y. Kimura, N. Takahashi, S. Tamura, *Agric. Biol. Chem*. **1969**, *33*, 1507-1516.

[118] M. Tanabe, H. Seto, *J. Org. Chem*. **1970**, *35*, 2087-2088.

[119] F. P. Schmidtchen, H. Rapoport, *J. Am. Chem. Soc*. **1977**, *99*, 7014-7019.

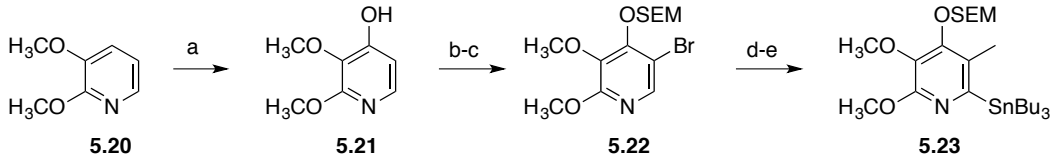
sequently brominated using Br_2 in AcOH. Deacylation, diazotation and finaly protection as the benzylic hydroxypyridine ether **5.14** gave the coupling precursor. Although the overall yield for 8 steps is relatively high, this synthesis does not allow for different substitution pattern at the aromatic rings and the introduction of the 4-hydroxy group was difficult to realize.



Scheme 5.2. Synthesis by Boger *et al.*:[104] a) $NH_2OH{\cdot}HCl$, EtOH, 96%; b) $MeSO_2Cl$, Et_3N, CH_2Cl_2; c) tetramethoxyethene, toluene, 50 °C, 64% (over 2 steps); d) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 88%; d) DIBAlH, CH_2Cl_2, 92%; e) TIPSCl, imidazole, DMF, 95%; f) *i)* *n*-BuLi, THF; *ii)* $B(OMe)_3$, AcOOH, H_2O, 88%; g) TBAF, THF, 96%; h) CBr_4, PPh_3, CH_2Cl_2, 84%.

In 2005, the Boger group reported the first total synthesis of piericidin A_1, the prototypical member of the natural family itself.[104] Their synthesis of the hetero-aromatic core started with an inverse electron demanding Diels-Alder reaction of the formed sulfonylimine **5.16** with tetramethoxyethene (**Scheme 5.2**), a methodology developed by Boger group several years before.[120] Lewis acid ($BF_3{\cdot}Et_2O$) mediated aromatization of **5.17** was followed by DIBAlH reduction and silyl protection to afford pyridine **5.18**. To introduce the 4-hydroxy function it was envisioned to use a directed lithiation-borylation-oxidation sequence, but additionally to the desired outcome a reverse Brook rearrangement of the TIPS group was observed. Therefore the obtained compound was exposed to TBAF prior to the formation of its benzyl bromide (**5.19**) under Appel conditions.

[120] a) D. L. Boger, K. C. Cassidy, S. J. Nakahara, *J. Am. Chem. Soc.* **1993**, *115*, 10733-107041; b) D. L. Boger, O. Hüter, K. Mbiya, M. Zhang, *J. Am. Chem. Soc.* **1995**, *117*, 839-849.



5.20 5.21 5.22 5.23

Scheme 5.3. Synthesis by Phillips *et al.*:[121] a) *i)* *n*-BuLi, THF; ii) $B(OMe)_3$, AcOOH, H_2O, 70%; b) SEMCl, Ag_2CO_3, CH_2Cl_2, 97%; c) *i)* *t*-BuLi, hexane, -78 °C; *ii)* $CBrCl_2CBrCl_2$, THF, -78 °C, 82%; d) *i)* LiTMP, THF, -78 °C to -40 °C; *ii)* CH_3I, -40 °C, 85%, e) *i)* *t*-BuLi, THF, -78 °C; *ii)* Bu_3SnCl, 96%.

In 2006, Keaton and Phillips realized the synthesis of natural 7-demethylpiericidin A_1 (**5.4**) containing the same aromatic motif (**Scheme 5.3**).[121] They started from commericially available 2,3-dimethoxypyridine (**5.20**) which was converted to the 4-hydroxypyridine **5.21** by the earlier mentioned lithiation-borylation-oxidation protocol. Afterwards, the free hydroxy group was protected selectively using Ag_2CO_3 and SEMCl, which led to the next directed lithiation to introduce a bromine substituent with NBS as electrophile (**5.22**). The synthesis was completed by a halogen dance reaction,[122] where the greater stability of the intermediate formed 3-lithio-2-bromo-pyridine which was used to introduce the lacking methyl group. Substitution of the bromine by Bu_3SnCl led to the final coupling precursor (**5.23**). This elegant route was later adapted by the Akita group in 2009 to finaly reward their efforts towards the natural piericidin A_1 which they originally started in 1997 with the completion of the side chain.[123]

The pyridinol synthesis of Lipshutz and Amorelli from 2009 (**Scheme 5.4**)[124] was inspired by the chemistry of Takahashi in the early 1980s.[125] Dieckmann cyclisation of the acylated aminotiglate **5.25** resulted in the formation of 4-hydroxypyridone **5.26** which was PMB-deprotected by exposure to TFA at 110 °C. Specific silylation of the sterically more shielded 4-hydroxy-group in presence of the 2-hydroxy-group afforded surprisingly after methylation (Ag_2CO_3, CH_3I) pyridine **5.27**. Deprotonation at the benzylic position by the assistance of *t*-BuLi in THF at -78 °C, led after

[121] A. J. Phillips, K. A. Keaton, *J. Am. Chem. Soc*. **2006**, *128*, 408-409.

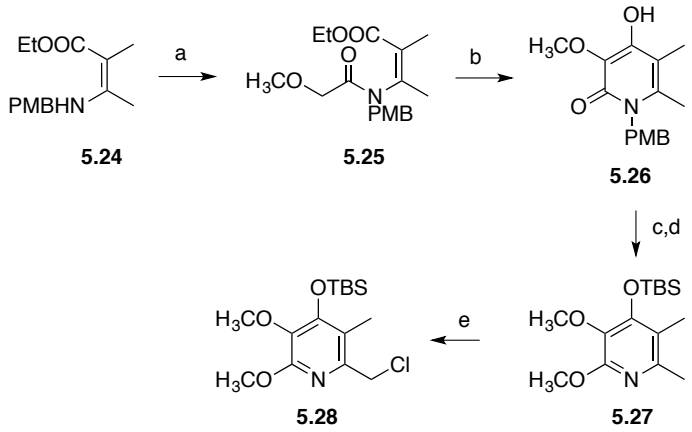
[122] For reviews on halogen dance reactions: a) V. Snieckus, *Chem. Rev.* **1990**, *90*, 879-933; b) J. F. Bunnett, *Acc. Chem. Res.* **1972**, *5*, 139-147; c) J. Froehlich in *Progress in Heterocyclic Chemistry*; Pergamon, **1994**, pp. 1-35.

[123] a) R. Kikuchi, M. Fujii, H. Akita, *Tetrahedron: Asymmetry* **2009**, *20*, 1975-1983; b) M. Ono, N. Yoshida, Y. Kokubu, E. Sato, H. Akita, *Chem. Pharm. Bull.* **1997**, *45*, 1428-1434.

[124] B. H. Lipshutz, B. Amorelli, *J. Am. Chem. Soc*. **2009**, *131*, 1396-1397.

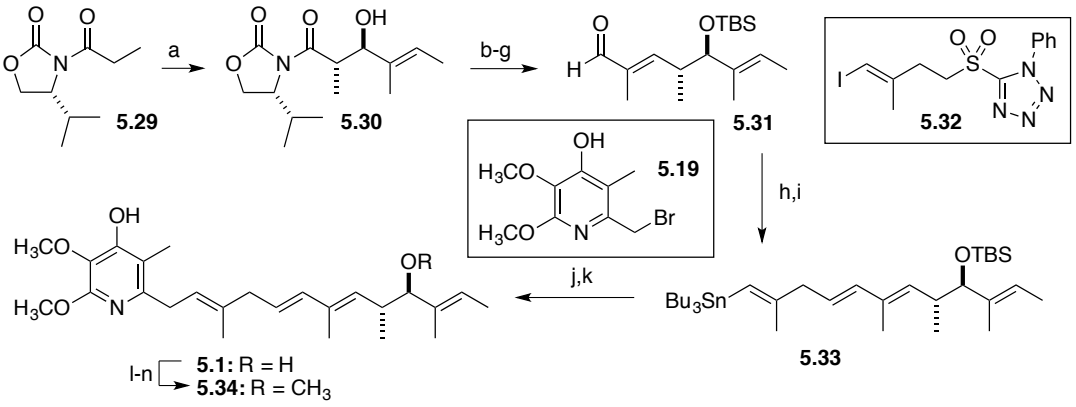
[125] S. Yoshida, Y. Nagao, N. Takahashi, *Agric. Biol. Chem.* **1980**, *44*, 2913-2920.

quenching with the electrophilic chlorine reagent CCl_3CCl_3 to the fully elaborated aromatic coupling partner **5.28**.



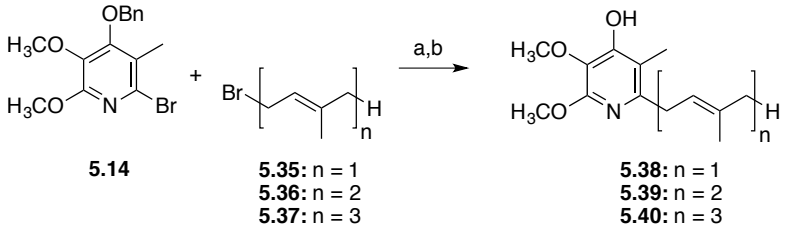
Scheme 5.4. Lipshutz' synthesis:[124] a) methoxyacetyl chloride, pyridine, CH_2Cl_2, 73%; b) Na, EtOH, 71%; c) TFA, 110 °C, 86%; d) TBSCl, Et_3N, CH_2Cl_2, 95%; d) Ag_2CO_3, CH_3I, CH_2Cl_2, 90%; e) *i)* *t*-BuLi, hexane, 0 °C; *ii)* CCl_3CCl_3, THF, -78 °C, 82%.

Obviously, after a closer look on these examples, the preparation of the pyridine moiety seems more challenging as originally anticipated. The unusual substitution pattern in combination with the typical problems of heterocyclic chemistry did not allow to synthesize this part of the molecule under five steps from commercially available precursors. For the construction of the side chain modern transformations developed in the last 10 to 15 years were necessary as shown in the following section.



Scheme 5.5. Total synthesis by Boger *et al.*:[104] a) tiglic aldehyde, Bu_2BOTf, DIPEA, CH_2Cl_2, 0 °C, 81%; b) $H_2NMeOMe{\cdot}HCl$, $AlMe_3$, THF, 0 °C; c) TBSCl, imidazole, DMF, 66% (2 steps); d) DIBAlH, CH_2Cl_2, -78 °C, 60%; e) $PO(OEt)_2CHCH_3COOEt$, NaH; f) DIBAlH, THF, -78 °C, 72% (2 steps); g) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 99%; h) **5.32**, KHMDS, DME, -78 °C, 60%; i) *i)* *n*-BuLi, THF, -78 °C; *ii)* Bu_3SnCl, 96%; j) **5.19**, $Pd_2(dba)_3$, *t*-Bu_3P, LiCl, dioxane, 70 °C, 74%; k) TBAF, THF, 50 °C, 93%; l) PivCl, HSO_4NBu_4, NaOH, CH_2Cl_2, 92%; m) NaH, MeI, DMF, 78%; n) *t*-BuONa, MeOH, 60 °C, 88%.

The enantioselective side chain synthesis of piericidin A_1 was first performed by Boger *et al.* (**Scheme 5.5**) as following:[104] Acylated Evans auxiliary **5.29** was submitted to tiglic aldehyde ($CHOCCH_3CHCH_3$) in presence of Bu_2BOTf to promote the *anti*-selective aldol reaction.[126] The formed alcohol **5.30** was protected and further converted to the aldehyde (*via* the Weinreb amide), which was then elongated by a Horner-Wadsworth-Emmons (HWE) reaction. After additional redox manipulations the aldehyde **5.31** could be coupled *via* an (*E*)-selective Julia reaction with sulfone **5.32**, which upon lithium-halogen exchange followed by treatment with Bu_3SnCl afforded the vinyl stannane **5.33**. The final coupling was achieved using Fu's catalyst system:[127] $Pd_2(dba)_3$ and *t*-Bu_3P in 74% yield. The natural product was finally obtained after desilylation and established the absolute configuration by the synthesis of the related piericidin B_1 (**5.34**) due to its higher optical rotation (α_D = -7.3° in MeOH, respectively α_D = -1.1° in MeOH for piericidin A_1).



5.14

5.35: n = 1
5.36: n = 2
5.37: n = 3

5.38: n = 1
5.39: n = 2
5.40: n = 3

Scheme 5.6. Rapoports synthesis of piericidin analogs:[119] a) *i) n*-BuLi, THF, HMPA, -78 °C; *ii)* allyl bromide, THF, -78 °C, 60-68%; b) BuSNa, DMF, 80 °C, 60-70% (for n = 1, 2, 3).

Rapoport studied alternative coupling strategies by using simple allylic bromides derived form prenol (**5.35**, n = 1), geraniol (**5.36**, n = 2) and farnesol (**5.37**, n = 3), with *n*-BuLi in a mixture of THF and HMPA at -78 °C (**Scheme 5.6**).[119] This transformation was realized without detectable amount of isomerization of the allylic fragment (^{1}H NMR analysis). Cleavage of the benzylic protecting group was accomplished by treatment with sodium butanthiolate at elevated temperatures to afford **5.38**-**5.40** as colorless oils in 40-60% yield over 2 steps.

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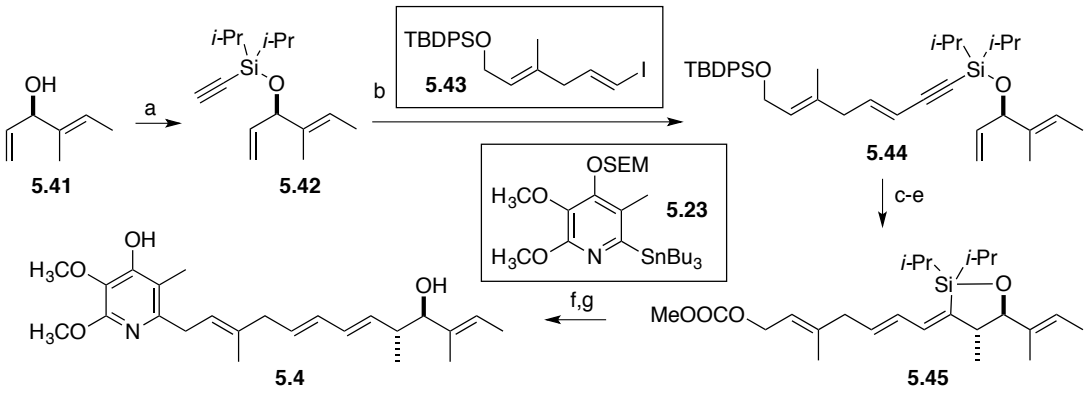
[126] Y. Nakao, W. Y. Yoshida, Y. Takada, J. Kimura, L. Yang, S. L. Mooberry, P. J. Scheuer, *J. Nat. Prod.* **2004**, *67*, 1332-1340.

[127] A. F. Littke, G. Fu, *J. Am. Chem. Soc*. **2002**, *124*, 6343-6348.



Scheme 5.7. Total synthesis by Phillips *et al.*:[121] a) ethynyl(diisopropyl)bromosilane, Et_3N, DMAP, CH_2Cl_2, 78%; b) $PdCl_2(PPh_3)_2$, CuI, Et_3N, CH_3CN, 87%; c) $ClTi(O\textit{i}\text{-}Pr)_3$, *i*-PrMgCl, Et_2O, 52%; d) HF·pyridine, THF, 84%; e) MeOOCOCl, pyridine, CH_2Cl_2, 97%; f) **5.23**, $Pd_2(dba)_3$, LiCl, DMF, 55% (*E*:*Z* 2.5:1); g) TBAF, DMF, 75 °C, 70%.

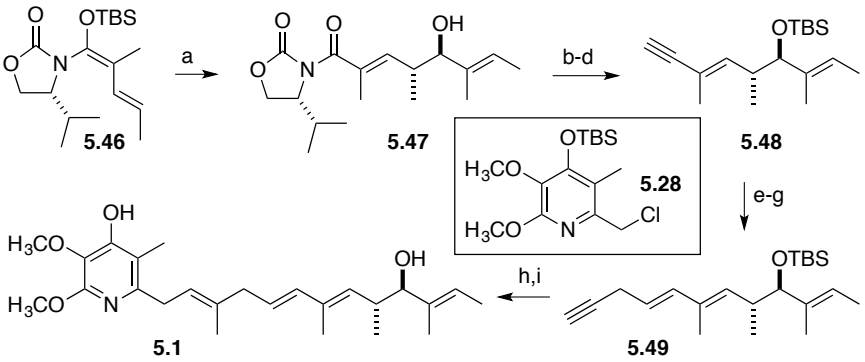
Next, the completed synthesis of the Phillips group is shown:[121] Chiral allylic alcohol **5.41** (obtained from vinylmagnesium bromide addition to tiglic aldehyde followed by kinetic resolution) was silylated using ethynylbromosilane to **5.42** (**Scheme 5.7**). Sonogashira-coupling with vinyl iodide **5.43** provided the precursor for the Ti(II)-mediated key step **5.44** - the eneyne cyclisation.[128] Transformation of the TBDPS protected alcohol into its carbonate were performed to connect in a final Stille-coupling both entities in 55% yield with a *E*:*Z* ratio of 2.5:1, solely separable on silver coated HPLC columns. After TBAF cleavage the natural piericidin **5.4** was obtained in 9 linear steps for the longest sequence.

Lipshutz synthesis of piericidin A_1 was the second targeting the same natural product (**Scheme 5.8**).[124] Key for his successful strategy was the Mukaiyama aldol reaction developed by Kobayashi and co-workers[129] which will be discussed in the following part. The obtained alcohol **5.47** was TBS protected, converted first to the aldehyde (1 eq. DIBAlH at -78 °C) and then to the alkyne **5.48** by the Ohira-Bestmann methodology. Hydrozirconation-iodination of eneyne **5.48** followed by propargyl coupling and cleavage of the TMS protecting group yielded alkyne **5.49**. The crucial carboalumination was effected with $AlMe_3$ and catalytic amounts of Cp_2ZrCl_2 and isobutylaluminiumoxane (IBAO). Then the vinylaluminium species was

[128] G. W. O'Neil, A. J. Phillips, *Tetrahedron* **2004**, *45*, 4253-4256.

[129] S. Shirokawa, M. Kamiyama, T. Nakamura, M. Okada, A. Nakazaki, S. Hosokawa, S. Kobayashi, *J. Am. Chem. Soc.* **2004**, *126*, 13604-13605.

in situ converted by addition of Ni catalyst and the heteroaromatic core **5.28** to the protected natural hydroxypyridine. This methodology developed in the Lipshutz laboratories[130] was also used for the successful synthesis of related pyrone natural products (verticipyrone).[131] Final cleavage with TBAF afforded piericidin A_1 (**5.1**) in 11 steps and an overall yield of 8% for the longest linear sequence.



Scheme 5.8. Total synthesis by Lipshutz *et al.*:[124] a) tiglic aldehyde, $TiCl_4$, CH_2Cl_2, -35 °C, 68%; b) TBSCl, imidazole, DMF, 92%; c) DIBAlH, CH_2Cl_2, -78 °C, 60%; d) dimethyl (1-diazo-2-oxopropyl)phosphonate, K_2CO_3, MeOH, 61%; e) *i)* Cp_2ZrCl_2, DIBAlH, THF, 0 °C; *ii)* I_2, THF, -78 °C, 81%; f) TMS-propyne, *n*-BuLi, CuI, DMAP, THF, 0 °C; g) K_2CO_3, MeOH, 72% (2 steps); h) *i)* Cp_2ZrCl_2, $AlMe_3$, IBAO, CH_2Cl_2; *ii* $NiCl_2(PPh_3)_2$, *n*-BuLi, **5.28**, THF, -50 to 0 °C; h) TBAF, THF, 60 °C, 58% (2 steps).

Summarizing these achievements, the total synthesis of these complex natural products was made possible during the last ten years, when new synthetic methods for the stereoselective construction of the labile side chain were established and novel coupling startegies for merging both entities allowed for the final construction under mild conditions.

5.4 Strategic Considerations and Goal of the Study

5.4.1 Retrosynthetic Analysis

As discussed in the previous section, two potential disconnections are reasonable for the synthesis of piericidins and dictate the following synthetic considerations: either the bond at C1'-C1 or the benzylic (C1-C2) bond has to be formed. The advantage of the C1-C2 disconnection for its facilitated installment - benzylic electrophiles can solely be attacked in a S_N2 fashion, whereas in an allylic precursors

[130] B. H. Lipshutz, T. Butler, A. Lower, J. Servesko, *Org. Lett*. **2007**, *9*, 3737-3740.
[131] B. H. Lipshutz, B. Amorelli, *Tetrahedron Lett*. **2009**, *50*, 2144-2146.

both attacks a S_N2 or a S_N2' are possible - is paid off later for the preparation of SAR libaries in which allylic precursors are easier accessible than their vinylic counterparts (**Figure 5.6**).

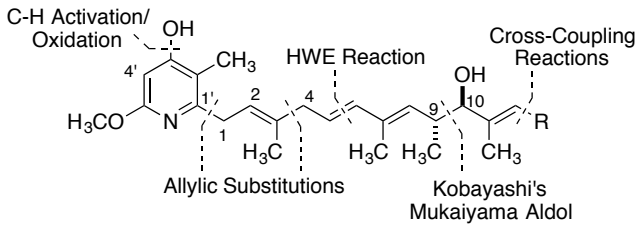


Figure 5.6. Retrosynthetic analysis of the 4'-demethoxy-piericidin structure.

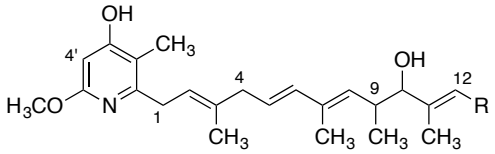
On the first glance the most difficult part of the synthesis appears to be the sensitive polyene side chain with its isolated 1,4-diene units from C1' to C2 and C3 to C5. However the 4-hydroxypyridine or 4-pyridone ring with its ambident reactivity and its uncommon substitution pattern[132] in combination with the final coupling of both elaborated parts represent the 'mysterious' challenge in the synthesis of these substrates. This synthetically delicate maneuver led in our investigation as well as in the published attempts from the Akita group to major problems.[123] The side chain was envisioned to be constructed from small molecular entities allowing a more flexible route for further SAR investigations, implementing cross-coupling reactions for the terminal part and selective elongation strategies to build the polyene moiety. At last, the heteroaromatic core was proposed to come from a C-H activation-oxidation procedure to form 4-hydroxypyridines in one step from unfunctionalized pyridine precursors.

5.4.2 Significance of the Proposed Project

Interestingly, the piericidin JBIR-02 (**5.2**, **Figure 5.7**) derived from mycelium *Streptomyces sp. ML55* (isolated from a soil sample collected in Taketomi Island, Japan)[109] was reported to act as a nuclear export inhibitor by blocking the interaction between nuclear transport protein (CRM1) and the nuclear export signal of the cargo. Similarly, leptomycin and other nuclear export inhibitors can undergo a Michael-type addition of Cysteine-529 on the exportin CRM1. This well understood covalent

[132] M. Breugst, H. Mayr, *J. Am. Chem. Soc*. **2010**, *132*, 15380-15389.

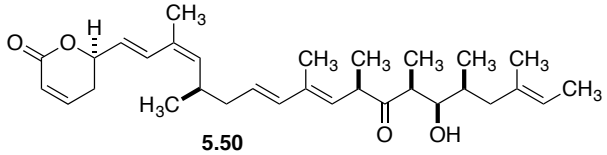
modification inhibits binding between the nuclear export mediator (CRM1) of hundreds of proteins and the NES cargo.



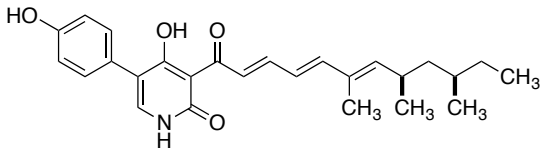
5.2: R = $C(CH_3)CHCH_3$
5.3: R = CH_3

Figure 5.7. Structures of isolated JBIR-02, **5.2** (R = C_4H_7) and Mer-A2026B, **5.3** (R = CH_3).

In the context of our program directed towards controlling protein transport in living cells, the pyridone polyene **5.2** was identified as a promising lead structure, despite its low activity (20 μM), caused by the loss of aromaticity when attacked covalently by a nucleophile. Still, **5.2** is a rather attractive target to find alternative heterocyclic derivatives, since members of the leptomycin family (e.g. anguinomycin A, **5.50**, **Figure 5.8**)[133] have been evaluated in clinical trials, but have failed in phase II due to unfavorable toxicity issues.



5.50



5.51

Figure 5.8. Structures of anguinomycin A (**5.50**) and militarinone D (**5.51**).

In addition, we have synthesized natural pyridone polyenes of the militarinone type (**5.51**) with the goal to induce and understand neurite outgrowth.[86,134] In the current approach we like to crosslink both aspects in terms of the identification of new biological targets for this phenomenon. Further, this synthesis should allow us to

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[133] a) S. Bonazzi, S. Güttinger, I. Zemp, U. Kutay, K. Gademann, *Angew. Chem. Int. Ed.* **2007**, *46*, 8707-8710; b) S. Bonazzi, O. Eidam, S. Güttinger, J.-Y. Wach, I. Zemp, U. Kutay, K. Gademann, *J. Am. Chem. Soc*. **2010**, *132*, 1432-1442.

[134] Reviews on pyridone natural products: H. J. Jessen, K. Gademann, *Nat. Prod. Rep.* **2010**, *27*, 1168-1185.

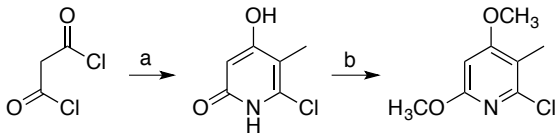
understand the role of the side chain length and substitution pattern for the recognition-processes. The combination of these structural features with the reported mechanism of action for these compounds lead to the hypothesis that small variations on the pyridone core could result in a large alteration of selectivity for biological targets.[86,134,135]

As there are, to the best of our knowledge, no published reports on compounds lacking the 4'-methoxy group, we wanted to develop synthetic access to JBIR-02 and Mer-A2026B, which should also allow for the assignment of the unknown absolute and relative configuration of the two stereogenic centers of these compounds.

5.5 Results and Discussion

5.5.1 1st Generation Approach: Synthetic Investigations on Pyridones and Hydroxypyridines

As pointed out in the retrosynthetic analyses the primary aim of this project was to find a suitable pyridine building block, functionalized at C1', that allows the attachment of a fully elaborated side chain under mild conditions.



5.52 **5.53**

Scheme 5.9. Construction of the dimethylated pyridone: a) EtCN, neat, RT, 54%; b) Ag_2CO_3, MeI, CH_2Cl_2, hexane, RT, 34%.

The investigations started with the formation of hydroxypyridone **5.52** in one step from ethyl nitrile and malonylchloride stirring with mechanical stirrer for three days (**Scheme 5.9**).[136a] The obtained brown solid could be easily purified by simple washing with CH_2Cl_2 to remove unreacted starting material. Then **5.52** was submitted to MeI and Ag_2CO_3 in order to obtain statistically the *O*,*O*-dimethylated hydroxy-pyridone **5.53**.[136b]

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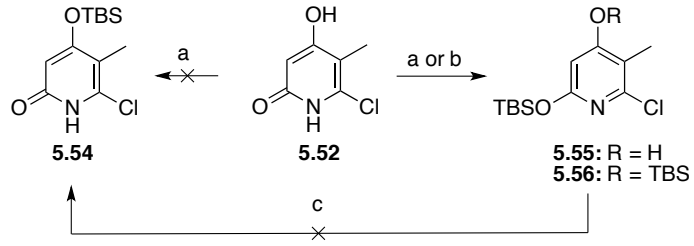
[135] K. Gademann, *Current Drug Targets* **2011**, *12*, 1574-1580.

[136] a) S. J. Davies, J. A. Elvidge, A. B. Foster, *J. Chem. Soc.* **1962**, 3638-3644; b) J. Buck, J. P. Madeley, G. Pattenden, *J. Chem. Soc. Perkin Trans. 1*, **1992**, 67-73.



Scheme 5.10. Attempts for the regioselective TBS protection: a) TIPSOTf, Et_3N, hexane, DMSO, -78 °C to 0 °C, 40% (for **5.55**); b) TBSCl, Et_3N, CH_2Cl_2, DMSO, 80% (for **5.56**); c) TBAF, KF or TFA.

Attempts to selectively protect the 4-hydroxygroup of **5.52** with a TBS protecting group (**5.54**) as it was anticipated from the Lipshutz strategy[124] were unsuccessful (**Scheme 5.10**). In contrast, the 2-hydroxy group was selectively protected when **5.52** was treated with TBSOTf at -78 °C in a mixture of hexane/DMSO in presence of base which was verified by X-ray crystal structure analysis (**5.55**, not shown). Selective de-protection methods (TBAF, Brønsted- or Lewis-acids) of the double TBS protected pyrone **5.56** to set free desired 4-mono-protected pyridone **5.54** were also unsuccesful (**Scheme 5.10**).

Nevertheless, the dimethylated pyridone **5.53** was used to evaluate the finishing coupling strategy for the construction of the allyl-aryl fragment. A Stille-coupling, developed for unreactive arylchloride by Fu and co-workers (addition of CsF to facilitate the transmetallation)[127] led to the formation of an undesired allene **5.58** in reasonable 68% yield (**Scheme 5.11**). The observed outcome of the reaction is in line with the one expected for a Hosomi-Sakurai reaction in which the propargylstannane **5.57** gets activated by fluoride facilitating the tranfer of the attached residue.[137]

We then inverted the coupling partners; the chloride subsituent was transformed by exposure to Me_6Sn_2, $Pd(PPh_3)_4$ and dppf in dioxane at 110 °C to the labile 2-pyridyl stannane **5.59**.[138] The propargylstannane **5.57** was substituted by geranylchloride to accomplish the desired allylic coupling[127] in 41% yield (**Scheme 5.11**) without detectable *E*/*Z*-isomerization of the side chain (**5.60**). Nevertheless, the limited stability of the aryl precursor in combination with the undistinguishable double methylated hydroxy groups led us to abandoned this attempt on this early stage.

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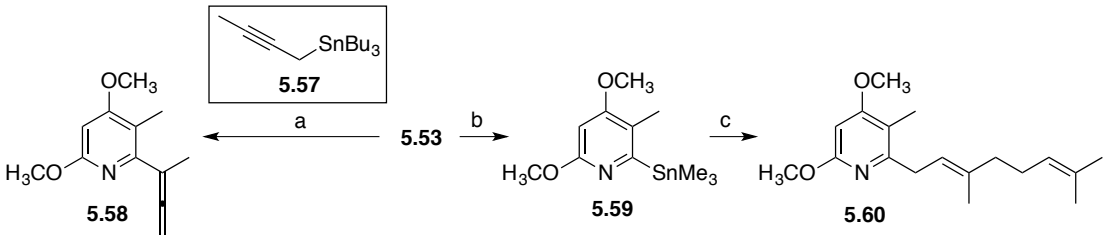
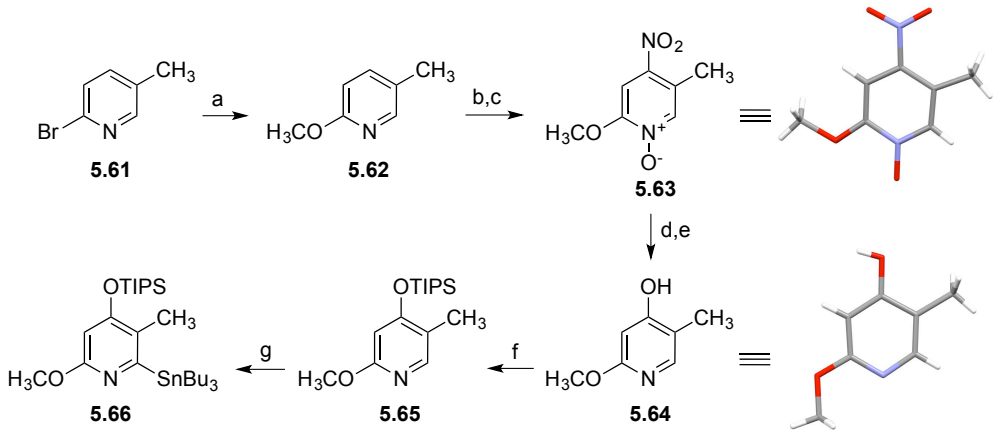
[137] N. Krause, A. S. K. Hashmi in *Modern Allene Chemistry*, Wiley-VCH, **2008**, pp.1-37.
[138] G. R. Dick, E. M. Woerly, M. D. Burke, *Angew. Chem. Int. Ed*. **2012**, *51*, 2667-2672.



Scheme 5.11. Investigations on the final union of both entities: a) Pd(P*t*-Bu$_3$)$_2$ (10 mol %), CsF, dioxane, 100 °C, 68%; b) Pd(PPh$_3$)$_4$ (10 mol %), dppf (10 mol %), Me$_6$Sn$_2$, dioxane, 110 °C, 46%; c) geranylchloride, Pd(P*t*-Bu$_3$)$_2$ (10 mol %), CuI, CsF, dioxane, 60 °C, 41%.

5.5.2 2nd Generation Approach: Ortho-Functionalization of Pyridines

In a second approach, it was envisioned to differentially protect both hydroxy groups directly from the beginning. Further, it was anticipated that the sensible organometallic species could be installed prior to the coupling by a methodology using dimethylaminoethanol as directing group for the ortho-lithiation of pyridines.



Scheme 5.12. Formation of the differently protected hydroxy-pyridone **5.66**: a) NaH, MeOH, DMF, 100 °C, *quant.*; b) *m*-CPBA, CH_2Cl_2; c) HNO_3 (fuming), H_2SO_4, 100 °C, 3 h, 17% (2 steps); d) AcOH, Fe, 100 °C, 2 h; e) $NaNO_2$, HNO_3, H_2O, 81% (2 steps); f) TIPSCl, Et_3N, CH_2Cl_2, RT, 97%; g) *i)* DMEA (4 eq.), *n*-BuLi (8 eq.), hexane, 0 °C; *ii)* **5.65**, 0 °C, 1 h, *iii)* $SnBu_3Cl$, THF, -78 °C to RT, 19%.

The sequence started with the nucleophilic substitution of commercially available bromopyridine **5.61** with sodium methanolate at 100 °C (**Scheme 5.12**). Next the *N*-oxide was formed by addition of *m*-CPBA in CH_2Cl_2,[139] or the methoxypyridine **5.62** was directly treated with aqueous H_2O_2 and AcOH. Nitration of this compound was challenging, therefore the *N*-oxide was exposed to a 3:1 mixture of fuming HNO_3

[139] J. M. Miller, *J. Heterocycl. Chem.* **1985**, *22*, 145-147.

and concentrated H_2SO_4 at 100 °C for 3 h.[140] However, the yield of **5.63** (17% over both steps) was very low and the work-up and accordingly the purification of the polar nitropyridine-*N*-oxide was found to be difficult. The correct installment of the nitro group of **5.63** was confirmed by X-ray crystal structure analysis. The following reduction mediated by AcOH and iron powder at 100 °C and diazotation ($NaNO_2$, HNO_3, H_2O) worked smoothly to afford 4-hydroxypyridine **5.64** in 81% yield over 2 steps. Throughout these synthetic studies, exclusively the 4-hydroxypyridine tautomer, and not the 4-pyridone tautomer as reported for the natural JBIR-02 (**5.2**), was observed. The C-O bond lengths in the obtained X-ray crystal structure analysis of **5.64** support this observation. The C-OMe bond has a length of 135.3 pm likewise has the C-OH a length of 134.5 pm, which is rather long for a C=O double bond (typically around 120 pm) as required for a 4-pyridone.

To prefunctionalize the heteroaromatic core for the subsequent allylic substitution, the directed lithiation strategy from Gros and Fort was adapted.[141] Deprotonation of the ortho-position in pyridines is mediated by lithium dimethylaminoethoxide. The role of the amino group to chelate the lithium ion derived from *n*-BuLi, and the electron-rich alkoxy group enhances basicity, creating at the same time a sterically more hindered and less nucleophilic superbase. Additionaly, the nitrogen lone pair of the pyridine contributes to the stabilization of the lithium base accounting for the observed selectivity. Prior to the hydrogen-lithium exchange pyridine, **5.64** was protected as TIPS ether (**5.65**) and in a first screening exposed to the deprotonation conditions present in literature. The use of 4 eq. of the formed superbase was found to be the best compromise in terms of deprotonation time and concentration, what could be analysed by ^1H NMR after quenching with D_2O. Afterwards the lithiated hetero-aromatic core was treated with 5 eq. of Bu_3SnCl and stirring continued for further 30 min at 0 °C. The choice of the used solvent, *n*-hexane and toluene had negligible effect on the reaction, as well as the temperature ranging from -20 °C until 10 °C.

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[140] a) M. Tiecco, M. Tingoli, L. Testaferri, D. Chianelli, E. Wenkert, *Tetrahedron* **1986**, *42*, 1475-1485; b) E. C. Taylor Jr, A. J. Crovetti, *Org. Synth.* **1963**, *4*, 654-655.

[141] a) H. K. Khartabil, P. C. Gros, Y. Fort, M. F. Ruiz-Lopez, *J. Am. Chem. Soc*. **2010**, *132*, 2410-2416; For Reviews: b) P. Gros, Y. Fort, *Eur. J. Org. Chem*. **2002**, 3375-3383; c) P. Gros, Y. Fort, *Eur. J. Org. Chem*. **2009**, 4199-4209.

However, even when the reaction was succesfully performed, purification of the labile ortho-stannane **5.66** remained problematic (best yield 19%). Various column chromatography strategies were examined either on Alox or deactivated silica with solvents containing additional base or degased prior to use. This approach was therefore abandoned on this stage due to the low yielding nitration and the problematic ortho-functionalization. Furthermore the following Stille-coupling, when performed with the obtained stannane **5.66** afforded the protected piericidin precursor as an inseparable mixture of *E*:*Z* isomers (see **Chapter 5.6**, **Scheme 5.21**). So another strategy for the construction of the heteroaromatic motif had to be envisioned, focusing predominantly on the facilitated formation of 4-hydroxypyridines.

5.5.3 3rd Generation Approach: Directed Bromine-Lithium Exchange

Finally, the total synthesis started with the preparation of the protected bromo pyridinol **5.71** in 5 steps as shown in **Scheme 5.13**. Commercially available 2-bromo-6-methoxypyridine (**5.67**) was converted to pyridinol **5.68** in 62% yield by a C-H activation reaction using $[Ir(COD)Cl]_2$ and dppe in neat pinacolborane (pinBH, 5 eq.) at 150 °C, followed by oxidation of the crude boronic acid intermediate with oxone in THF.[142] Improving this protocol by replacing the catalyst to 2 mol % $[Ir(COD)OMe]_2$, and the ligand to 4 mol % 4,4'-di-*tert*-butyl-2,2'-bipyridine (dtbpy) lead to a lower reaction temperature and a smaller amount of required pinBH (1.5 eq.) as the reaction was performed in hexane and improved the yield to 79%.[143]

Subsequent treatment with excess of *N*-bromosuccinimide (NBS) afforded tribromopyridinol **5.69** in 92% yield, as all attempts to selectively mono-functionalize C2' with different electrophilic bromination and iodination reagents (NBS, 1,1-dibromo-5,5-dimethylhydantoin (DBDMH) or I_2 with catalytic amounts of CAN) were unsuccessful (**Table 5.2**).

The best selectivity obtained for the C2' functionalization was 57:43 in favor of the undesired regioisomer, when SEM protected pyridine **5.72** was subjected to NBS in CH_3CN at room temperature. Analogous, 4-hydroxypyridine **5.68** was also protected as TIPS ether (**5.73**) or acetate (**5.74**) by standard conditions and evaluated for the

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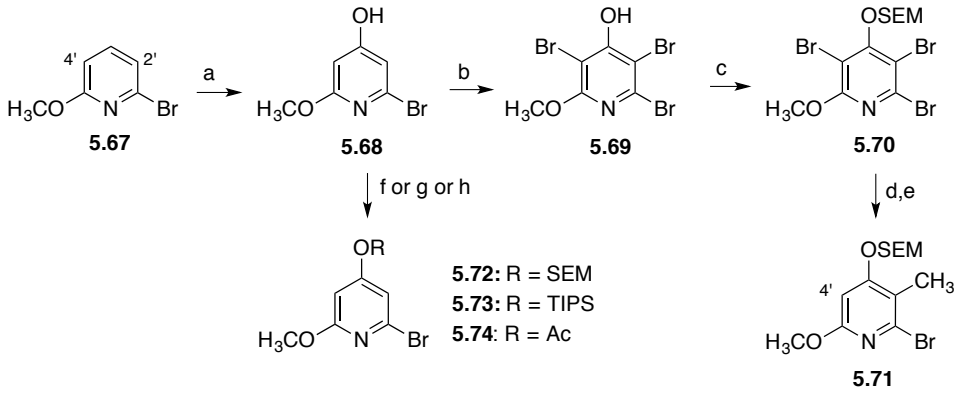
[142] R. E. Maleczka Jr., F. Shi, D. Holmes, M. R. Smith III, *J. Am. Chem. Soc.* **2003**, *125*, 7792-7793.

[143] T. Ishiyama, Y. Nobuta, J. F. Hartwig, N. Miyaura, *Chem. Commun*. **2003**, 2924-2925.

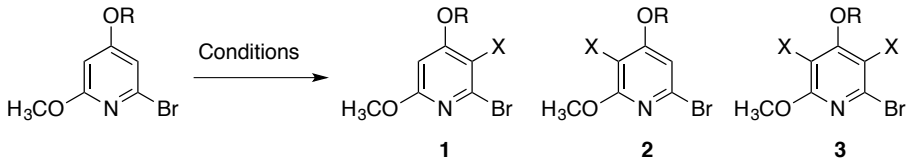
bromination reaction without effecting the selectivity. Additional investigations were performed with a smaller chloro substituted pyridinol instead of the bromo substituent without improving the selectivity.



Scheme 5.13. Synthesis of the heteroaromatic intermediate **5.71**. The positions labelled 2' and 4' refer to the piericidin nomenclature: a) *i)* $[Ir(COD)OMe]_2$ (2 mol %), dtbpy (4 mol %), HBpin, hexane, RT, *ii)* oxone, THF, H_2O, RT, 79%; b) NBS, CH_3CN, RT, 92%; c) SEMCl, DIPEA, CH_2Cl_2, 0 °C to RT, 82%; d) *i) n*-BuLi, THF, -78 °C; *ii)* MeI, -78 °C to RT; e) *i) n*-BuLi, THF, -78 °C; *ii)* MeOH, -78 °C to RT, 67% (2 steps); f) TIPSCl, Et_3N, CH_2Cl_2, 88%; g) SEMCl, Et_3N, CH_2Cl_2, 88%; h) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 84%.

Selective *O*-SEM protection of the triple brominated pyridine **5.69** to direct the aromatic functionalization in the next steps was accomplished using SEMCl and DIPEA in CH_2Cl_2. The correct substitution pattern at the heteroaromatic ring was installed by sequential two-fold lithium-halogen exchange using *n*-BuLi in THF at -78 °C: (1) Trapping of the stabilized C2'-lithiated aryl species with MeI (for C2' substitution) followed by (2) protonation with MeOH (for the C4' position) completed the target fragment **5.71** with the correct substitution pattern in 67% yield over 2 steps. Apparently, the donor capacity of the neighboring bromine is higher than the stabilizing effect of the methoxy group. This strategy was found to be superior to ortho-metalation, in which even bulky non-nucleophilic bases such as LDA or LiTMP resulted in nucleophilic substitution of the bromo substituent and could not be suppressed by varying the reaction conditions.

Table 5.2. Screening for the selective halogenation of position C2'.



	Conditions	Ratio of regio-isomers[a] **1**:**2**:**3**	Conversion[a]	Yield[b]
R = H	1.1 eq. NBS, CH_3CN	0:100:0	79%/ 60 h	63%
	1.1 eq. NBS, MeOH	0:100:0	72%/ 16 h	-
	1.1 eq. NBS, CCl_4	0:100:0	99%/ 16 h	-
	1 eq. DBDMH, THF	0:0:100	99%/ 19 h	-
	0.5 eq. DBDMH, THF	0:66:34	75%/ 18 h	-
	1 eq. I_2, 0.1 CAN, CH_3CN	0:31:69	99%/ 1.5 h	55%
	0.5 eq. I_2, 0.1 CAN, CH_3CN	0:75:25	99%/ 1 h	*quant.*
	2 eq. I_2, 0.1 CAN, CH_3CN	0:0:100	99%/ 12 h	*quant.*
R = SEM	1.1 eq. NBS, CH_3CN	42:57:0	78%/ 16 h	35%
	1.5 eq. NBS, CH_3CN, 60 °C	40:60:0[c]	99%/ 4 h	-
	1.1 eq. NBS, MeOH	0:100:0	75%/ 18 h	-
	1.1 eq. NBS, CCl_4	-	- / 18 h	-
	1.1 eq. NBS, CCl_4, 75 °C	-	- / 5 h	-
	1.0 eq. Br_2, NaOAc, AcOH	30:47:22	70%/ 1.5 h	-
	1 eq. DBDMH, THF	*Complex mixture*	99%/ 19 h	-
	0.5 eq. I_2, 0.1 CAN, CH_3CN	-	- / 16 h	-
R = Ac	1.1 eq. NBS, CH_3CN	-	- / 16 h	-
	1.1 eq. NBS, CH_3CN, 90 °C	-	- / 16 h	-
R = TIPS	1.1 eq. NBS, CH_3CN	-	- / 16 h	-
	1.1 eq. NBS, CH_3CN, 90 °C	-	- / 16 h	-

[a] Determined by ^{1}H NMR of the crude product or the reaction mixture; [b] after purification by column chromatography. Pure products were analyzed by ^{1}H NMR, ^{13}C NMR and UPLC-MS; [c] before work-up; when more then 1 eq. NBS was used double brominated side-product was obtained after evaporation of solvent.

5.5.4 The Polyene Side Chain

The polyene side chain synthesis was addressed next. As the configuration of both target compounds was unknown, we assumed the same absolute configuration as established for piericidin A_1. The preparation of the side chain started with a vinylogous Mukaiyama aldol reaction developed by Kobayashi and co-workers.[129,144] This procedure allows to induce a high degree of asymmetric information in an acyclic system. The methyl group at the α-position stabilizes the planar conformation of the acyl residue on the one hand and excludes a free rotation of the chiral auxilliary (**Figure 5.9**), leading to the almost perpendicular arrangement of oxazolidin-2-one and the nucleophilic enolate. The aldehyde activated by the Lewis-acid approaches from the less hindered side to form a C-C bond and affords the observed stereogenic outcome.

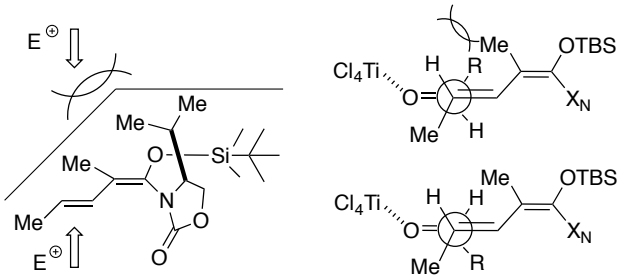
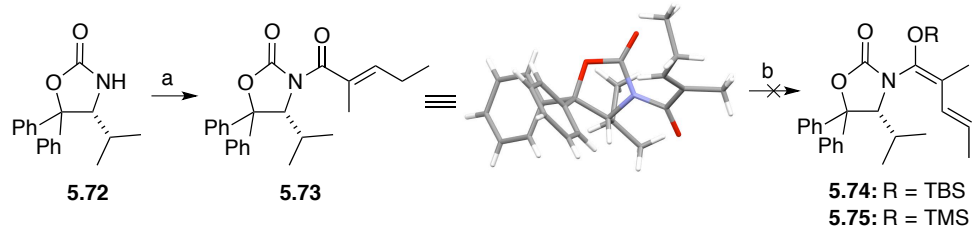


Figure 5.9. Mechanistic rational for Kobayashi's Mukaiyama aldol.[129] X_N = Evans auxiliary.

Instead of the more common Evans auxiliary, we planed to perfome the described transformation with the help of the bulkier, UV active Seebach's auxiliary (**5.72**) which can be easiliy recovered and recycled by fash column chromatography once cleaved from the side chain. Acylation with 2-methyl-pentenoic acid was performed using pivalyl chloride to form *in situ* the unsymmetric anhydride which is attacked by the cyclic carbamate (**Scheme 5.14**). Due to the help of LiCl coordinating to the lone pairs of the anhydride increasing its electrophilic character **5.73** was obtained after stirring over night at room temperature in very good yield (89%). This method

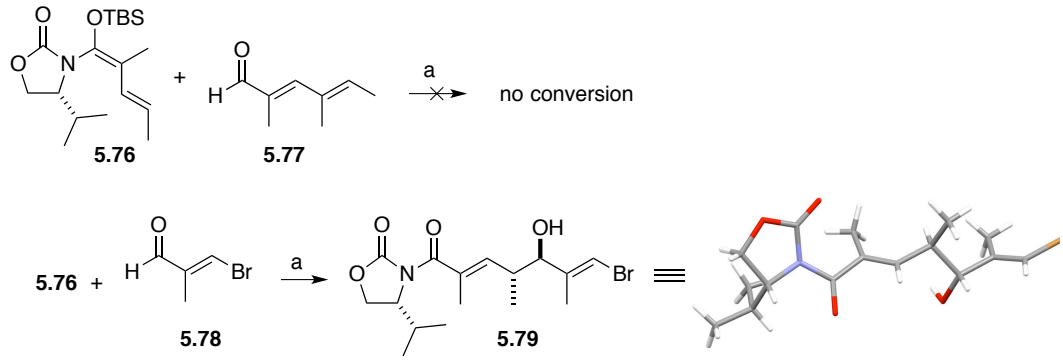
[144] For recent examples and modifications of this methodology: a) I. Paterson, S. B. J. Kan, L. J. Gibson, *Org. Lett*. **2010**, *12*, 3724-3726; b) Y. Mukaeda, T. Kato, S. Hosokawa, *Org. Lett.* ***2012***, *14*, 5298-5301; c) G. Symkenberg, M. Kalesse, *Org. Lett*. **2012**, *14*, 1608-1611; d) K. Fujita, R. Matsui, T. Suzuki, S. Kobayashi, *Angew. Chem. Int. Ed.* **2012**, *51*, 7271-7274; e) H. Tsukada, Y. Mukaeda, S. Hosokawa, *Org. Lett.* **2013**, *15*, 678-681.

displays significant advantages in terms of yield and handling compared to the regular use of acid chloride.



Scheme 5.14. Attempts for the preparation of *N*,*O*-silyl ketene acetal starting from Seebach's auxilliary: a) *i)* PivCl, Et_3N, 2-methyl-pentenoic acid; *ii)* LiCl, auxilliary, THF, -45 °C to RT, 89%; b) *i)* KHMDS; *ii)* TBSCl or TMSCl, THF, -78 °C.

Unfortunately all attemps to form the silyl ketene (**5.74** or **5.75**) acetal using TBSCl or TMSCl as protecting groups failed. This can be rationalized from the obtained X-ray crystal structure of the acylated auxiliary **5.73**: The isopropyl group is pushed by both phenyl substituents of the auxilliary into the side chain thereby prohibiting the all planar arrangement for the desired product formation. Following the literature procedure usage of the smaller Evans auxilliary led to the formation of the desired TBS enolate (**5.76**).[129]



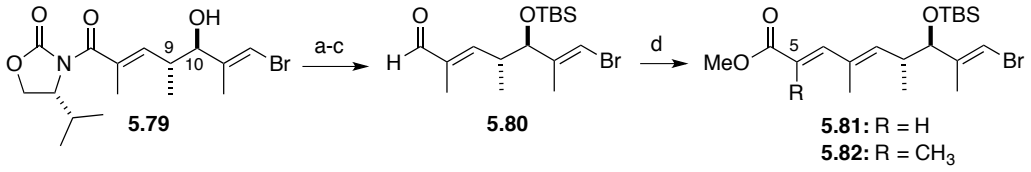
Scheme 5.15. Kobayashi's vinylogous Mukaiyama aldol reaction: a) $TiCl_4$, CH_2Cl_2, -35 °C, 18 h, 91%, *dr* > 50:1.

Treatment of *N*,*O*-silyl ketene acetal **5.76** and bromoacrylate **5.78**[145] provided the desired hydroxyl imide **5.79** *via* a $TiCl_4$ mediated C-C bond forming reaction with exclusive formation of one diastereomer (^{1}H NMR > 50:1) in 91% yield (12-mmol scale). The relative and absolute configuration at C-9 and C-10 were established by

[145] Y. Murakami, M. Nakano, T. Shimofusa, N. Furuichi, S. Katsumura, *Org. Biomol. Chem.* **2005**, *3*, 1372-1374.

the X-ray crystal structure analysis of the aldol product **5.79** (**Scheme 5.15**). The vinylogous Mukaiyama aldol reaction with the more complex substrate (2*E*,4*E*)-2,4-dimethylhexa-2,4-dienal **5.77** as coupling partner, was not successful under the previously described conditions. This finding could be explained by the extended conjugation, transforming the dienal in a rather unreactive coupling partner towards nucleophilic attack.[146]

Silylation of hydroxyl imide **5.79** with TBSOTf in the presence of 2,6-lutidine in CH_2Cl_2 at -78 °C, followed by the removal of the chiral auxiliary in a two-step process *via* reduction with $NaBH_4$ and oxidation of the allylic alcohol with activated MnO_2 afforded aldehyde **5.80** in 87% over 3 steps as our corporate building block (**Scheme 5.16**). Elongation to the unsaturated ester **5.81** was achieved by a Horner-Wadsworth-Emmons (HWE) reaction applying a protocol developed by Masamune and Roush to avoid partial epimerization at C-9.[147] Expanding the scope of this strategy, this transformation allowed the introduction of different residues at C-5 - as for example CH_3 present in natural piericidins (IT-143-B)[108] by changing the phosphonate coupling partner (**5.82**).



5.79 **5.80** **5.81:** R = H **5.82:** R = CH_3

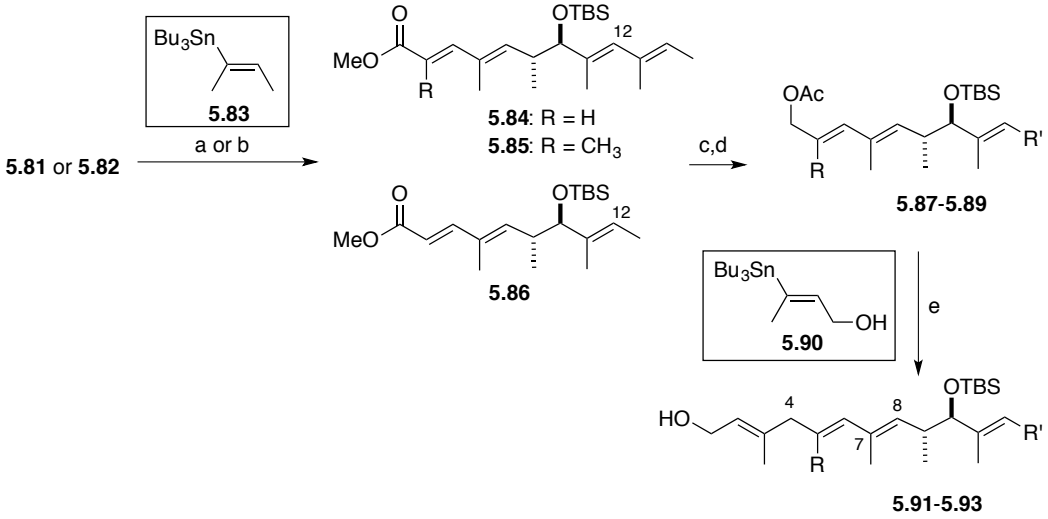
Scheme 5.16. Synthesis of the polyene side chain (part 1): a) TBSOTf, 2,6-lutidine, CH_2Cl_2, -78 °C to RT; b) $NaBH_4$, THF, H_2O, RT; c) MnO_2, CH_2Cl_2, RT, 87% (3 steps); d) $P(O)(OEt)_2CH_2COOMe$, DBU, LiCl, CH_3CN, 0 °C to RT, *quant*. (R = H) or $P(O)(OEt)_2CHCH_3COOEt$, DBU, LiCl, CH_3CN, 0 °C to RT, 85%.

Cross-coupling of vinyl bromide **5.81** (or **5.82**) under Stille conditions ($Pd(Pt\text{-}Bu_3)_2$ (10 mol %), LiCl, NMP, 80 °C) with stannane **5.83**, derived from commercially available *E*-2-bromo-2-butene by lithiation and quenching with Bu_3SnCl, afforded tetraene **5.84** (or **5.85**) in very good yield (**Scheme 5.17**). Under these conditions, isomerization of the terminal methyl group could be avoided (determined by NOE investigations). Using the opportunity to transform

[146] a) M. Bock, R. Dehn, A. Kirschning, *Angew. Chem. Int. Ed*. **2008**, *47*, 9134-9137; b) Y. Nakamura, H. Kiyota, B. J. Baker, S. Kuwahara, *Synlett*, **2005**, 635-636.

[147] M. Blanchette, W. Choy, J. T. Davis, A. P. Essenfeld, S. Masamune, W. R. Roush, T. Sakai, *Tetrahedron Lett.* **1984**, *25*, 2183–2186.

vinylbromide **5.81** by transition metal catalysis into a number of natural piericidins, the vinylbromide was elongated at C-12 by Suzuki coupling ($Pd(PPh_3)_4$ (5 mol %), $CH_3B(OH)_2$, Cs_2CO_3, dioxane, H_2O, 65 °C) to triene **5.86** in 69% yield.



Scheme 5.17. Synthesis of the polyene side chain (part 2): a) $Pd(Pt\text{-}Bu_3)_2$ (10 mol %), LiCl, NMP, 80 °C, 95% (R = H), 97% (R = CH_3); b) $Pd(PPh_3)_4$ (5 mol %), $CH_3B(OH)_2$, Cs_2CO_3, dioxane, H_2O, 65 °C, 69%; c) DIBAlH, CH_2Cl_2, -78 °C to 0 °C; d) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 0 °C, 90% (R = H, R' = C_4H_7, 2 steps), 80% (R = CH_3, R' = C_4H_7, 2 steps) and 95% (R' = CH_3, 2 steps); e) $Pd(dba)_2$ (5 mol %), LiCl, DIPEA, NMP, 45 °C, 3 h, 90% (R = H, R' = C_4H_7), 77% (R = CH_3, R' = C_4H_7) or 77% (R = CH_3), for all cases *E*:*Z* = 5:1.

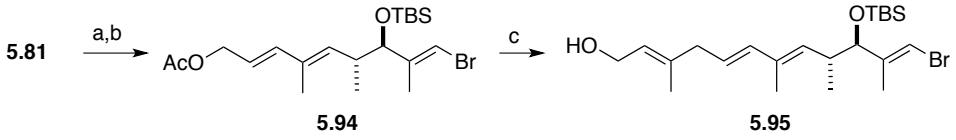
DIBAlH reduction of esters **5.84**-**5.86** followed by acetylation produced the allylic acetates **5.87**-**5.89** as precursors for the first allylic substitution (90% for **5.87,** 80% for **5.88** and 95% for **5.89** over 2 steps). In order to remove traces of AcOH, which led to significantly reduced yields, Ac_2O was filtered through a small column of Al_2O_3 before use. Next allylic substitution using $Pd(dba)_2$, LiCl, DIPEA and hydroxy stannane **5.90**[148] at 45 °C for 3 h delivered **5.91** (90%), **5.92** (77%) and **5.93** (77%) in a ratio of 5:1 as its *E*- and *Z*-isomers.

5.5.5 Isomerization Along the Chain

As the identification of the formed side product remained unknown at the beginning, bromide **5.95** was synthezised as a model compound *via* the same transformations: DIBAlH reduction, acetylation and allylic substitution (**Scheme 5.18**). This time both isomers could be easily separated by chiral preparative HPLC

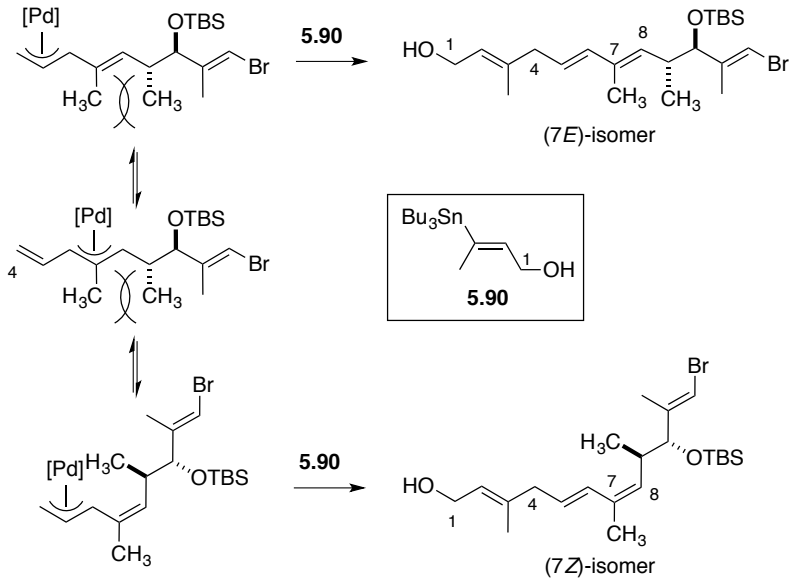
[148] J.-F. Betzer, F. Delaloge, B. Muller, A. Pancrazi, J. Prunet, *J. Org. Chem*. **1997**, *62*, 7768-7780.

(hexane/*i*-PrOH, 99.5:0.5 as solvent) and characterized by NOE experiments (**Figure 5.10** and **Appendices**).



Scheme 5.18. Synthesis of the vinyl bromide model: a) DIBAlH, CH_2Cl_2, -78 °C to 0 °C; b) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 0 °C, 83%; c) **5.90**, $Pd(dba)_2$ (5 mol %), LiCl, DIPEA, NMP, 45 °C, 3 h, 71%, *E*:*Z* 5:1.

Surprisingly, the Stille-coupling performed at 45 °C did not solely answer the identity of the formed side product but itself is a rare example in which the reactivity of an allylic and a vinylic positon in a cross-coupling reaction are compared. Obviously, the allylic position is much more reactive towards the Stille-coupling conditions affording exclusively the allylic substitution products remaining the vinylic bromide untouched.



Scheme 5.19. Plausible mechanism for the isomerization during allylic substitution with stannane **5.90**.

Interestingly, the isomerization could be observed at the olefinic linkage between C7-C8. A reasonable mechanism for the isomerization is shown in **Scheme 5.19**. Upon treatment with Pd catalyst and formation of the Pd-allyl-complex, isomerization of this complex along the chain is likely to occur. Due to the allylic strain of the adjacent methyl groups this complex could isomerize to the thermodynamically more

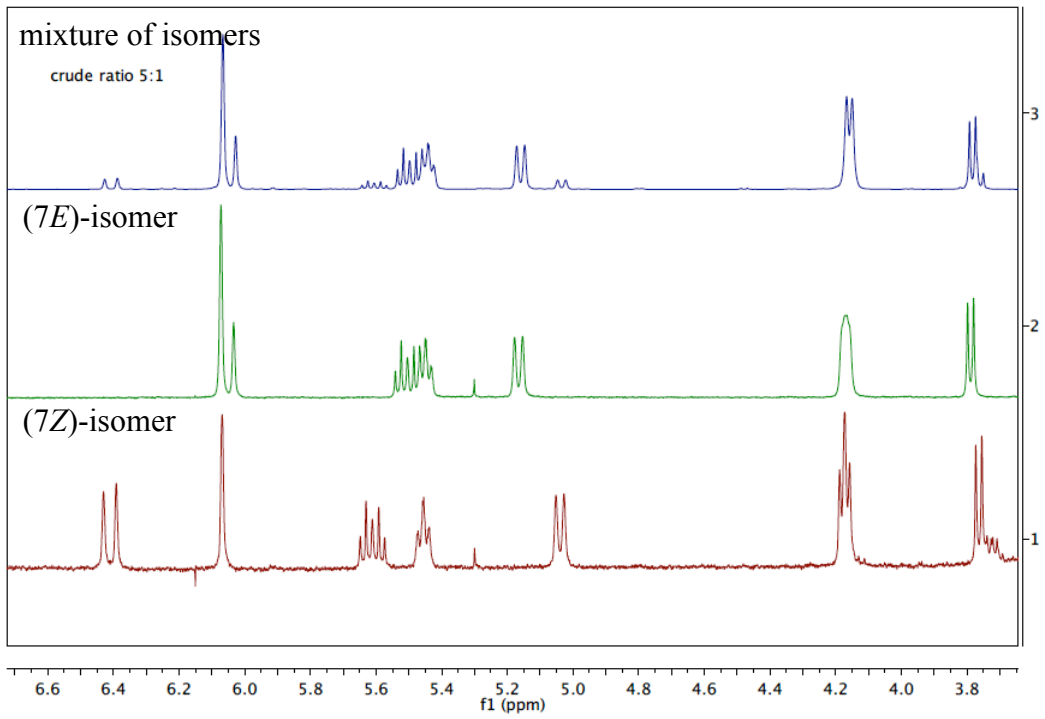


Figure 5.10. ^{1}H NMR analysis of the isomerization during the second allylic substitution with a model substrate (vinyl bromide **5.95**, crude *E*:*Z* ratio: 5:1).

stable *Z*-olefin and react further with stannane **5.90** to afford (7*Z*)- **5.95**. The *E*:*Z*-ratio could not be improved by exploring different reaction parameters such as catalyst, reaction time or temperature.

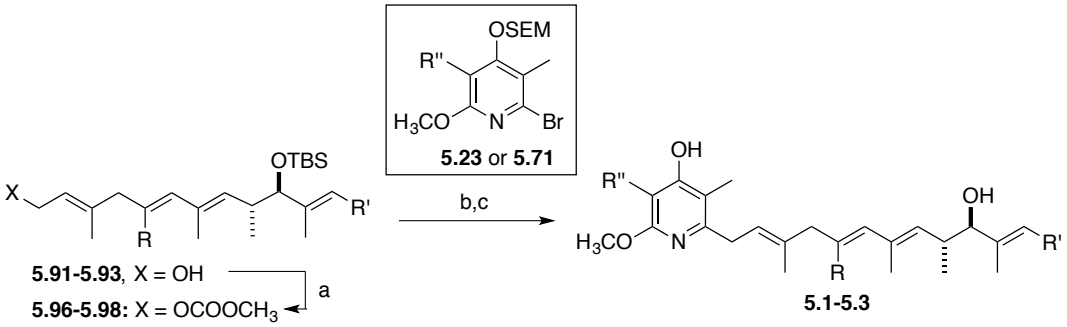
5.5.6 Total Synthesis of JBIR-02 and Mer-A2026B

Next, (7*E*)-allylic alcohols **5.91-5.93** were transformed to their corresponding carbonates **5.96-5.98** (CH_3OCOCl, Et_3N at 0 °C) thereby generating a suitable leaving group for the subsequent allylic substitution as shown in **Scheme 5.20**. The crucial connection of both moieties, the 4-hydroxypyridine core and the fully elaborated polyene chain, was realized after extensive experimentation on the Negishi cross-coupling reaction and proved to be mild enough to avoid isomerization or decomposition.[149]

The protected Br-pyridinol **5.23** or **5.71** was lithiated using *n*-BuLi in THF at -78 °C. After 10 min, $ZnCl_2$ was added and the resulting mixture was allowed to warm to room temperature over 1 h to form a relatively stable 2-pyridylzinc

[149] E. Negishi, F. Liu in *Handbook of Organopalladium Chemistry for Organic Synthesis*, Wiley, **2002**, pp. 551-589.

reagent,[150] which was exposed to a freshly prepared mixture of carbonate **5.96-5.98** and 5 mol % of $Pd(PPh_3)_4$, and warmed to 50 °C for 3 h to provide the protected natural products without detectable isomerization. Final deprotection of the succesful coupled polyenes was achieved by treatment with TBAF at elevated temperatures (70 °C, THF) to produce piericidin A_1 (**5.1**, 29% over 3 steps), JBIR-02 (**5.2**, 57% over 3 steps) and Mer-A2026B **(5.3**, 60% over 3 steps) in good yields.



Scheme 5.20. Completion of the synthesis of piericidin A_1 (**5.1**), JBIR-02 (**5.2**), Mer-A2026B (**5.3**): a) $ClCOOCH_3$, Et_3N, CH_2Cl_2, 0 °C, 30 min, *quant.*; b) *i)* **5.23** or **5.71**, *n*-BuLi, THF, -78 °C; *ii)* $ZnCl_2$, THF, -78 °C to RT; *iii)* $Pd(PPh_3)_4$ (5 mol %), **5.96** or **5.98**, THF, 50 °C, 2 h, 69% (R' = C_4H_7, R'' = H) and 65% (R' = CH_3, R'' = H), *E*:*Z* > 25:1; c) TBAF, THF, 70 °C, 30 min, 29% (over 2 steps, R' = CH_3, R'' = OCH_3), 82% (R = H, R' = C_4H_7) and 93% (R = H, R' = CH_3).

The Negishi conditions proved superior to Sn based cross-coupling, in which significant isomerization was observed (**Scheme 5.21**), what was also observed by Phillips et al. during their synthesis of natural 7-demethyl-piericidin.[121]

In the case of the Stille-coupling, the side chain was activated as its allyl chloride instead (Me_2S, NCS in CH_2Cl_2 at 0 °C) and exposed to $Pd(PPh_3)_4$, LiCl in degased DMF at 45 °C. Except the low yield and selectivety this method lacked reproduceability mainly caused by the nature of the sensitive aromatic coupling partner. Purification of the desired *E*-isomers for the natural product was neither possible with silver coated silica nor with preparative HPLC.

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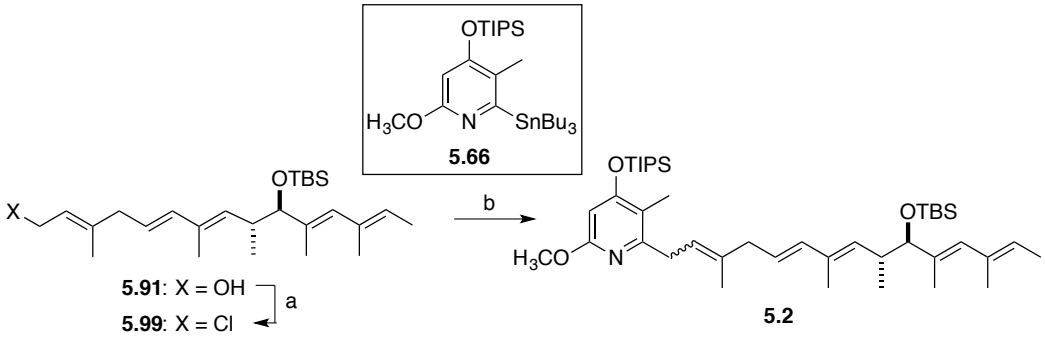
[150] For selected 2-pyridylzinc reagents: a) M. R. Luzung, J. S. Patel, J. Yin, *J. Org. Chem*. **2010**, *75*, 8330-8332; b) J. K. Klosterman, A. Linden, J. S. Siegel, *Org. Biomol. Chem*. **2008**, *6*, 2755-2764; c) U. Kiehne, J. Bunzen, H. Staats, A. Lützen, *Synthesis* **2007**, 1061-1069; d) T. Sammakia, E. L. Stangeland, M. C. Whitcomb, *Org. Lett* **2002**, *4*, 2385-2388; e) F. Mongin, F. Trécourt, O. Mongin, G. Quéguiner, *Tetrahedron* **2002**, *58*, 309-314; f) S. A. Savage, A. P. Smith, C. L. Fraser, *J. Org. Chem*. **1998**, *63*, 10048-10051.



Scheme 5.21. Final Stille cross-coupling of stannane **5.66** and allylchloride **5.99**: a) Me_2S, NCS, CH_2Cl_2, 0 °C, *quant.*; b) $Pd(dba)_2$ (10 mol %), PPh_3, LiCl, DMF, 45 °C, 45%, *E*:*Z* = 2.5:1

5.5.7 Characterization

The comparison of spectra of natural and synthetic samples was carried out next, in order to confirm the reported structures and to assign the absolute configuration.

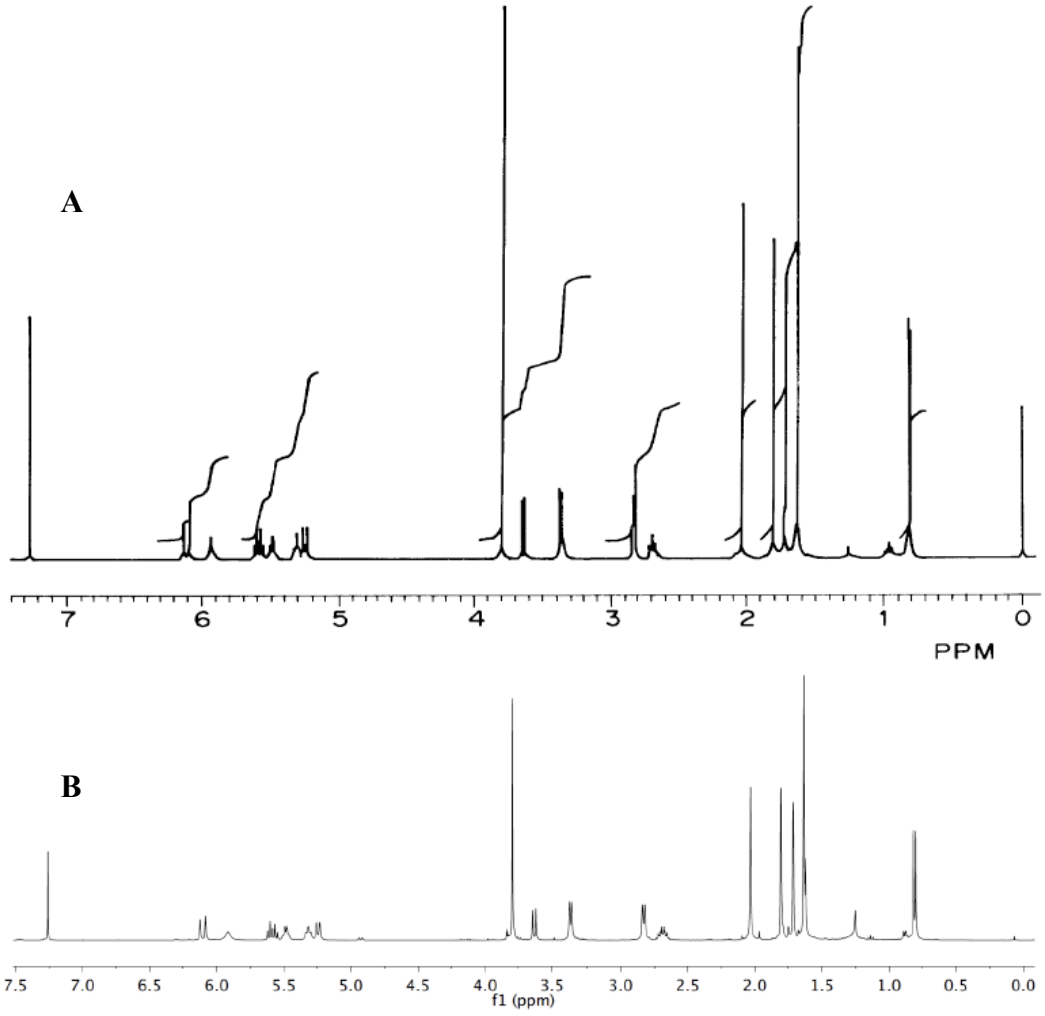
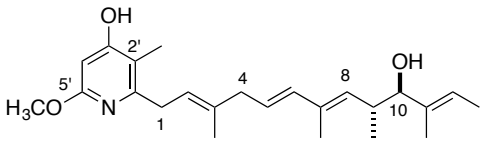


Figure 5.11. ^{1}H NMR overlay of isolated (**A**) and synthetic (**B**) Mer-A2026B (**5.3**).

Table 5.3. NMR comparison of natural and synthetic Mer-A2026B (**5.3**).



	Synthetic Mer-A2026B		**Natural Mer-A2026B**[110]	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	3.36 *d* (7.0)	29.8	3.36 *d* (6.9)	NR
2	5.31 *m*	130.0	5.32 *t* (7.0)	NR
3	-	136.4	-	NR
4	2.84 *d* (6.7)	42.9	2.83 *d* (7.0)	43.0
5	5.58 dt (15.5, 7.0)	125.4	5.58 *dt* (15.4, 7.0)	125.8
6	6.11 *d* (15.5)	136.5	6.10 *d* (15.4)	136.3
7	-	135.8	-	135.7
8	5.25 *d* (9.7)	133.8	5.24 *d* (9.9)	133.6
9	2.69 *m*	36.8	2.69 *m*	36.9
10	3.66 *d* (9.1)	82.8	3.64 *d* (9.2)	82.8
11	-	135.5	-	135.6
12	5.49 *m*	123.7	5.49 *m*	123.5
13	1.63 *d*	13.0	1.63 *d* (7.0)	13.1
1’	-	NR	-	NR
2’	-	NR	-	NR
3’	-	NR	-	NR
4’	5.87 *bs*	92.4	5.93 *s*	92.4
5’	-	NR	-	NR
3-Me	1.72 *s*	16.5	1.72 *s*	16.6
7-Me	1.81 *s*	13.0	1.81 *s*	13.1
9-Me	0.81 *d* (6.7)	17.2	0.81 *d* (6.6)	17.4
11-Me	1.64 *s*	10.4	1.64 *s*	10.6
2’-Me	2.03	9.9	2.03 *s*	10.1
5’-OMe	3.81	55.0	3.79 *s*	54.6

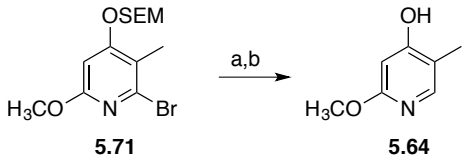
^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz in $CDCl_3$; NR = could not be observed.

The spectroscopic data of synthetic Mer-A2026B (**5.3**) were found to be identical in all respects (^{1}H NMR, **Figure 5.11**, ^{13}C NMR, UV spectra and optical rotation: $[\alpha]_D$ = -1.1° (*c* = 0.11, MeOH), lit.$[\alpha]_D$ = -1.07° (*c* = 0.36, MeOH)) to those reported in the literature for the natural product (**Table 5.3**). The same is true for piericidin A_1 which was identical to the spectra of the total syntheses of Boger,[104] Lipshutz[124] and

Akita[123] and showed only one set of peaks indicating either the pyridone or hydroxypyridine tautomer is present.

For JBIR-02 (**5.2**), confirmation of the structure proved to be more complex. At the beginning, several key signals of the aromatic core in the ^{1}H NMR spectra (**Figure 5.12**) displayed different chemical shifts when compared with the reported data of the isolated natural product (**Table 5.4**, indicated in red). Afterwards, the identity of natural and synthetic JBIR-02 was confirmed by ^{1}H, ^{13}C NMR and UHPLC measurements by Dr. Izumikawa in the laboratories of Prof. Dr. Shin-ya (National Institute of Advanced Industrial Science and Technology) and later in our laboratories by ^{1}H NMR spectroscopy. In the ^{1}H NMR of synthetic JBIR-02 was only one tautomer form observed when measured in Switzerland ($CDCl_3$; Cambridge Isotope Lab. Inc.: DLM-7-100). Whereas when measured in Japan ($CDCl_3$; Eurisotop: D007H) two tautomeric forms were detected in the synthetic sample and the spectra was in full agreement with the one from the isolated sample, what could be verified later by measuring the isolated natural product in Switzerland (with only one tautomer form visible).

In the meantime the heterocyclic precursor (**5.71**) alone was exposed to the approved coupling conditions and SEM-deprotected afterwards to prove its structure by comparison with compound **5.64** an intermediate of the second generation approach for which we obtained a X-ray crystal structure analysis (**Scheme 5.22**). As both compounds were equivalent, methyl-group transfer as side reaction under the successful Negishi cross-coupling conditions could be ruled out and the present of the correct substitution pattern was confirmed.



5.71 **5.64**

Scheme 5.22. Confirmation of the structure of the heterocyclic motif: a) *i)* *n*-BuLi, THF, -78 °C; *ii)* $ZnCl_2$, THF, -78 °C to RT; *iii)* MeOH; b) TBAF, THF, 70 °C, 30 min.

The final proof of identical constitution of synthetic and natural samples of JBIR-02 was established by co-injection of both samples and analysis by UHPLC. Apparently, the amount of water or the amount of acid in the $CDCl_3$ solution can lead to the observation of tautomerism and, therefore causes drastically different spectra.

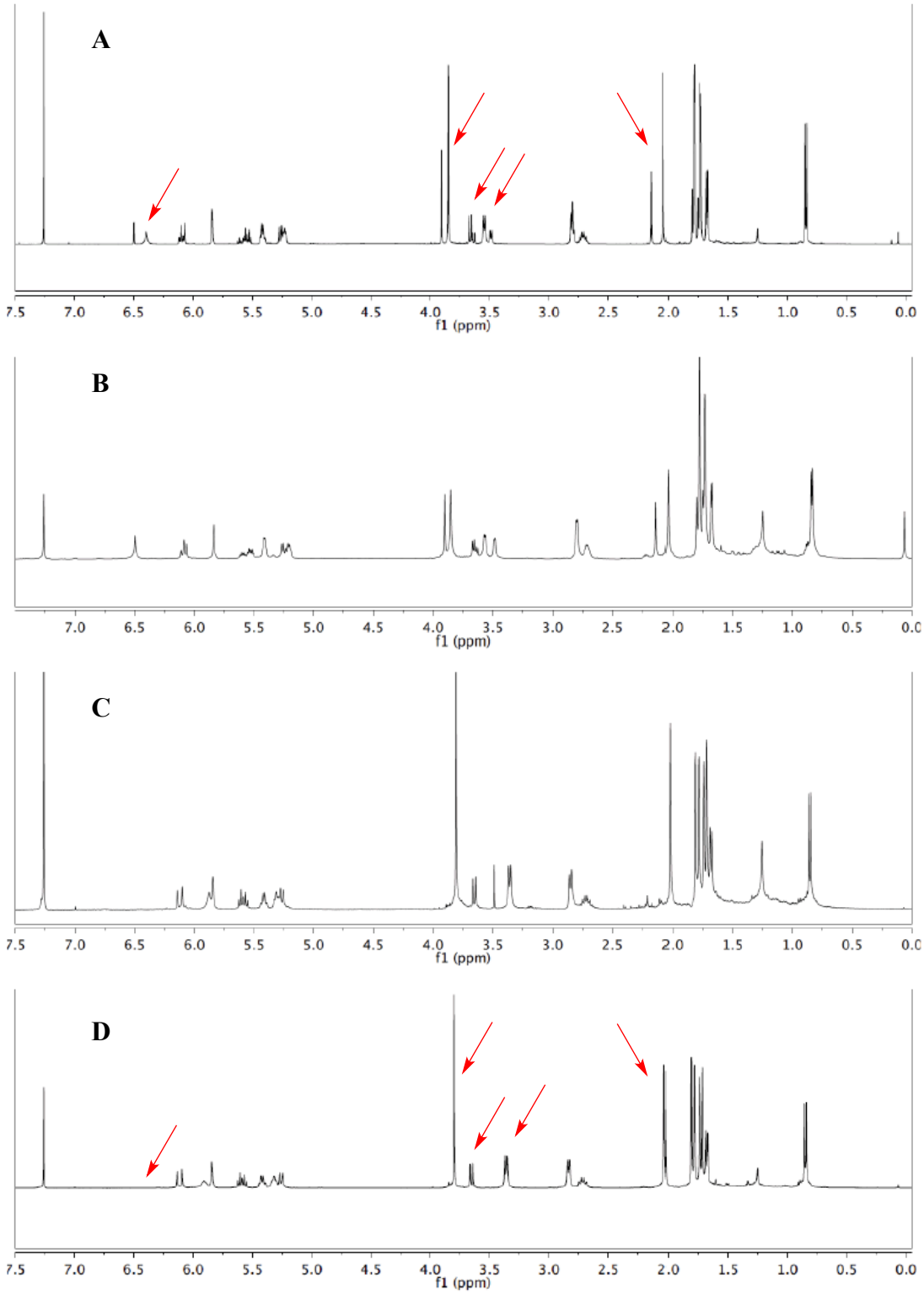
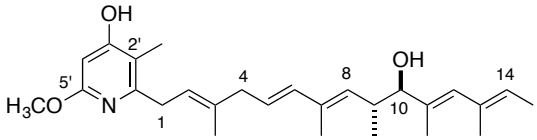


Figure 5.12. ^{1}H NMR overlay of isolated natural JBIR-02, **5.2** (**A**) and synthetic **5.2** (**B**, both measured in the laboratory of Prof. Shin-ya at the National Institute of Advanced Industrial Science and Technology (AIST), Japan), natural **5.2** (**C**) and synthetic **5.2** (**D**, both measured in the University of Basel, Switzerland); most significant differences are indicated by red arrow symbols (in **A** and **D**).

Table 5.4. NMR comparison of natural and synthetic JBIR-02 (**5.2**); red frames indicated mismatches in the first obtained ^{1}H NMR spectra of isolated (Japan) and synthesized (Switzerland) natural product.



	Synthetic JBIR-02		**Pyridone form**[109]		**Hydroxypyridine form**[109]	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	**3.36 *d* (7.1)**	30.6	**3.54 *d* (6.9)**	30.6	**3.49 *d* (6.9)**	35.1
2	**5.30 *m***	122.2	**5.23 *t* (6.1)**	121.5	[b]**5.42**	118.3
3	-	135.8	-	139.9	-	135.8
4	2.83 *d* (6.9)	42.9	2.81 *d* (6.8)	43.1	2.80 *d* (6.8)	43.2
5	5.58 dt (15.5, 7.0)	125.9	5.54 *dt* (15.7, 6.9)	125.5	5.60 *dt* (15.7, 7.0)	126.7
6	6.11 *d* (15.5)	136.3	6.09 *d* (15.7)	136.7	6.10 *d* (15.4)	136.0
7	-	135.7	-	135.6	-	136.1
8	5.25 *d* (9.6)	133.6	5.26 *d* (9.8)	134.1	5.23 *m*	133.4
9	2.71 *ddq*	36.9	2.71 *ddq*	37.1	2.71 *ddq*	37.2
10	3.65 *d* (8.9)	83.3	3.66 *d* (8.8)	83.6	3.64 *d* (9.1)	83.5
11	-	133.9	-	134.2	-	134.2
12	5.84 *s*	132.8	5.84 *s*	132.9	5.84 *s*	132.8
13	-	133.5	-	133.1	-	132.8
14	5.41 *q* (6.8)	124.7	5.41 *qd* (6.8, 1.2)	124.9	[b]5.41 *qd* (6.8,1.2)	124.8
15	1.68 *d* (6.8)	13.8	1.69 *d* (6.9)	13.8	1.69 *d* (6.9)	13.8
1’	-	NR	-	148.0	-	159.2
2’	-	[a]113.4	-	115.9	-	116.7
3’	-	NR	-	175.7	-	158.1
4’	**5.92 *bs***	92.4	**6.40 *bs***	92.2	**6.50 *bs***	100.2
5’	-	[a]158.6	-	159.6	-	162.6
3-Me	1.71 *d* (1.1)	16.8	1.74 *d* (1.2)	16.9	1.75 *s*	16.8
7-Me	1.80 *d* (1.1)	13.3	1.79 *d* (1.2)	13.3	1.80 *d* (1.2)	13.3
9-Me	0.85 *d* (6.7)	17.6	0.84 *d* (6.6)	17.7	0.84 *d* (6.6)	17.6
11-Me	1.78 *s*	12.7	1.78 *bs*	12.7	1.78 *bs*	12.7
13-Me	1.74 *s*	16.7	1.74 *bs*	16.7	1.74 *bs*	16.7
2’-Me	**2.03**	10.3	**2.04 *s***	10.1	**2.14 *s***	10.8
5’-OMe	**3.79**	54.7	**3.85 *s***	56.3	**3.91 *s***	53.8

^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz in $CDCl_3$.
[a] Low temperature (218 K) HMBC. [b] Overlap. NR = could not be observed.

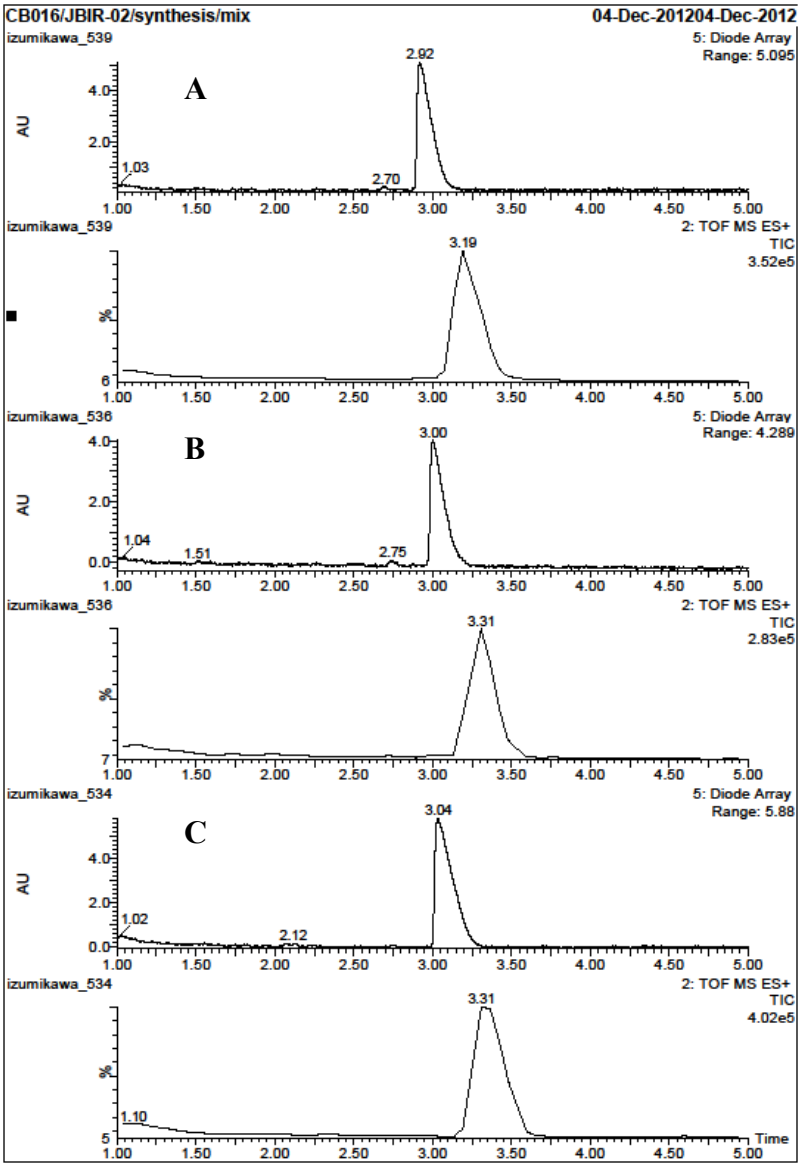


Figure 5.13. UPLC traces of (**A**) a mixture of synthetic and isolated natural JBIR-02 (**5.2**), (**B**) natural **5.2** and (**C**) synthetic **5.2** measured in the laboratory of Prof. Shin-ya (Tokyo, Japan). UPLC conditions: column: ACQUITY UPLC BEH C_{18}; 1.7 mm, 2.1 i.d. x 100 mm; flow rate: 0.4 ml/min; solvent: 50% MeCN (0.1% HCOOH).

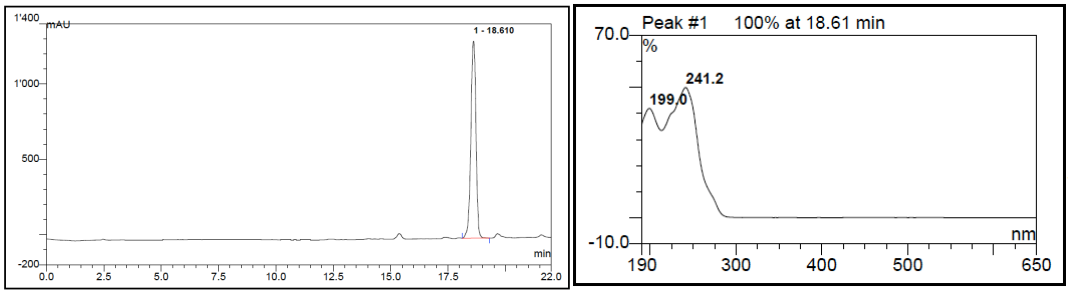
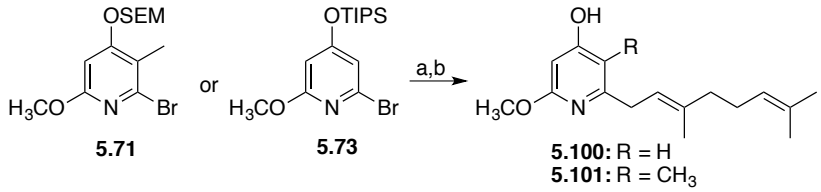


Figure 5.14. RP-HPLC (H_2O:CH_3CN) UV-trace and UV spectra of synthetic JBIR-02.

Interestingly, the pyridone form of these piericidin analogs has been never observed by any other research group (see also X-ray crystal structure in **Chapter 5.5.1**, the related work of Boger and co-workers[104] and the isolation and characterisation of natural piericidins[105,108-112]). The optical rotation ($[\alpha]_D$ = -11.1° (*c* = 0.21, MeOH)) matched its literature value ($[\alpha]_D$ = -13.0° (*c* = 0.88, MeOH)) and thereby allowed us to establish the absolute configuration as (9*R*,10*R*) for **5.2**.

5.5.8 SAR studies

Having established conditions for the final coupling of the sensitive side chain with the heteroaromatic core we aimed to use further simplified side chain analogs to investigate whether the complexity - 2 chiral centers, 1,4-diene moiety - is necessary for its biological activity. For this purpose we used geranyl carbonate as side chain mimic and performed our developed Negishi cross-coupling with different heterocyclic cores (**Scheme 5.23**). TBAF mediated deprotection afforded two piericidin analogs, differing in the aromatic substitution pattern.



Scheme 5.23. Synthesis of simplified analogs using the Negishi cross-coupling strategy: a) *i)* **5.71** or **5.73**, *n*-BuLi, THF, -78 °C; *ii)* $ZnCl_2$, THF, -78 °C to RT; *iii)* $Pd(PPh_3)_4$ (5 mol %), geranyl carbonate, THF, 50 °C, 2 h, 42% (R = H) and 72% (R = CH_3), *E*:*Z* > 25:1; c) TBAF, THF, 70 °C, 30 min, 98% (R = H) and 79% (R = CH_3).

In the future it is envisioned to create a library of different lengths similar to the one produced by Rapoport 40 years ago and evaluate the side chain recognition pattern. Biological evaluation for the obtained compounds (natural products as well as analogs) is currently carried out at Syngenta AG in Stein (Switzerland).

5.6 Conclusion

In conclusion, the first total syntheses of piercidin derivatives Mer-A2026B and JBIR-02 (longest linear sequence 12 steps, overall yield 28% for **5.2**, respectively 20% for **5.3**) were developed and the absolute configuration could be established as (9*R*,10*R*). Further piericidin A_1 **5.1** – the prototype of the natural product family itself

was synthesized using the same strategy. Salient features of this efficient and convergent synthetic route are a highly diastereoselective Kobayashi-Mukaiyama aldol reaction, a C-H activation reaction in combination with an oxidation protocol for the preparation of the highly functionalized pyridine moiety and a final Negishi cross-coupling reaction without isomerization of the labile side chain. Moreover, this approach opens up the stage for the synthesis of a number of piericidins by simply changing the coupling partner for the HWE reaction (C-5 analogs) or the organometallic species for the cross-coupling reaction (C-12 functionalization). Studies targeting these derivatives are underway in our laboratories.

6 ELECTRON-RICH BIPYRIDINES

6.1 Introduction

Inspired by the original bioactivities of piericidins as inhibitors of the respiratory chain, electron-rich 2,2'-bipyridine (bpy) systems also attracted our attention and were therefore investigated as model for the concerted proton coupled electron transfer (PCET).[151]

Light driven water oxidation in the photosystem II is an ubiquitous reaction in plants producing not solely essential oxygen but also a proton gradient across the membrane from the stroma to the lumen responsible for the producion of ATP (eq 1). This oxygen-evolving complex with the active manganese cluster is still under controversial discussion since high-energetic X-ray beams could have altered its structural analysis; therefore speculations persist on its mechanism of action. Additional justification for the investigation of model bypyridine ligands originates from the catalytic water splitting, as this seems to be the most promising energy storage technology to create *solar fuels*: H_2 from H_2O (eq 2) respectively hydrocarbons from CO_2 (eq 3).[152] These processes of transferring light to energy might participate to solve our current and future energy problems.

$$(1)\ 2\ NADP^+ + 2\ H_2O + 4\ h\nu \rightarrow 2\ NADPH + O_2 + 2\ H^+$$

$$(2)\ 2\ H_2O + 4\ h\nu \rightarrow 2\ H_2 + O_2$$

$$(3)\ 2\ H_2O + CO_2 + 8\ h\nu \rightarrow CH_4 + 2\ O_2$$

Bipyridine are currently employed for a countless number of catalytic systems as selectivity and reactivity-regulating ligands.[153] In particularly, electron-rich bipyridine systems have shown to exhibit good donor capacities by chelating a number of transition metal ions such as ruthenium, iron or iridium. As a result highly colored charge-transfer complexes can be found which have found application as sensors at present world.

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[151] C. J. Gagliardi, A. K. Vannucci, J. J. Concepcion, Z. Chen, T. J. Meyer, *Energy Environ. Sci.* **2012**, *5*, 7704-7717.

[152] K. S. Joya, H. J. M. de Groot, *Int. J. Hydrogen Energy* **2012**, *37*, 8787-8799.

[153] O. Wenger, *Chem. Rev*. **2013**, **doi**: 10.1021/cr300396p

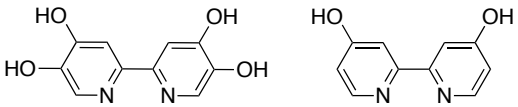


Figure 6.1. Structure of prototypic 4,4',5,5'-tetrahydroxy-2,2'-bipyridine (**6.1**) and of 4,4'-dihydroxy-2,2'-bipyridine (**6.2**).

In 2011, Paul and co-workers have extensively investigated the 4,4'-dihydroxy-2,2'-bipyridine (**6.2**) by spectroscopical means either as a mixed-ligand system Ru(bpy)$_2$(**6.2**)[154a] or as a complex Ru(**6.2**)$_3$[154b] on its oxidation capacities (**Figure 6.1**). Significantly the influence of different protonation stages was found to alter the properties of the obtained complex. Elaboration of the electronic properties with cyclic voltammetry yields a reversible Ru$^{III/II}$ wave that shifts 1.43 V to lower energy upon deprotonation of the complex.

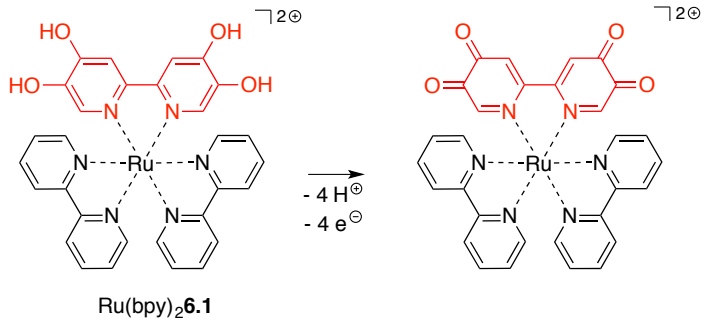


Figure 6.2. PCET in Ru(bpy)$_2$(**6.1**)-complexes

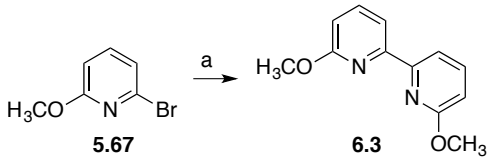
On the other hand complex Ru(bpy)$_2$**6.1** should allow the transfer of 4 protons coupled with 4 electrons in one single molecular entity. Similar to Ru(bpy)$_2$**6.2** a pH dependency for Ru(bpy)$_2$**6.1** is expected, which would allow to fine-tune the environment and optimize the desired PCET.

6.2 Synthesis of Bipyridine Ligands

In order to evaluate whether our developed cross-coupling conditions are feasible for the construction of bpy-systems, bromopyridine **5.67** was subjected to homocoupling conditions. As a result the desired product **6.3** was obtained in 91%

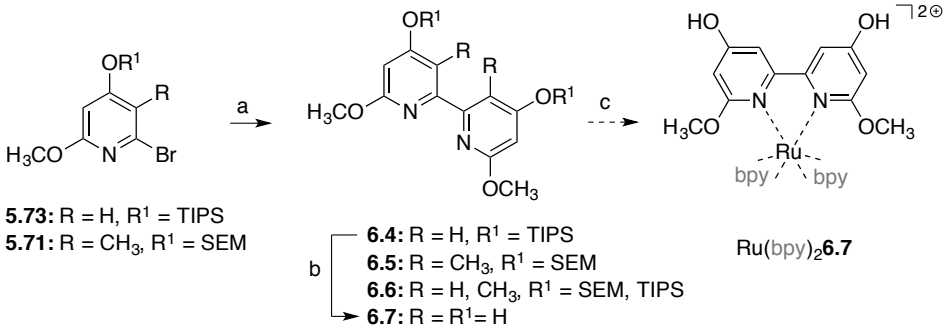
[154] a) S. Klein, W. G. Dougherty, W. S. Kassel, T. J. Dudley, J. J. Paul, *Inorg. Chem.* **2011**, *50*, 2754-2763; b) M. J. Fuentes, R. J. Bognanno, W. G. Dougherty, W. J. Boyko, W. S. Kassel, T. J. Dudley, J. J. Paul, *Dalton Trans*. **2012**, *41*, 12514-12523.

yield (**Scheme 6.1**).[155] The formed 2,2'-bipyridines with their inherent strong chelating abilities was expected to affect the desired C-C bond formation by poisoning the Pd-catalyst. In this regard, the use of Negishi coupling reaction proved superior presumably because of the chelating effect of bipyridines to zinc ions thereby preventing the catalytic activity of the palladium.[156]



Scheme 6.1. Negishi cross-coupling reaction for the formation of bpy-system (**6.3**): a) *i)* *n*-BuLi, THF, -78 °C; *ii)* $ZnCl_2$, THF, -78 °C to RT; *iii)* $Pd(PPh_3)_4$ (5 mol %), **5.67**, THF, 50 °C, 2-4 h, 91%.

Following these successful conditions were also applied for the synthesis of several electron-rich pyridines. Symmetrical bipyridine-systems were obtained in good yields (61% for **6.4**; 64% for **6.5**) starting from protected 4-hydroxypyridines **5.71** and **5.73**. The bulkier methylated pyridine **5.73** did not influence the reaction outcome (**Scheme 6.2**) and clean conversion was observed. The unsymmetrical dimer **6.6** was afforded only in poor 18% yield contaminated with both homocoupled products and debrominated starting material.



Scheme 6.2. Synthesis of electron-rich bipyridines starting from heteroaromatic core of the piericidin syntheses (for clarity bpy ligand is indicated in grey): a) *i)* *n*-BuLi, THF, -78 °C; *ii)* $ZnCl_2$, THF, -78 °C to RT; *iii)* $Pd(PPh_3)_4$ (5 mol %), **5.71** or **5.73**, THF, 50 °C, 2-4 h, 61% (R^1 = H), 64% (R^1 = CH_3) and 18% (R^1 = H and CH_3); b) TBAF, THF, 70 °C, 4-12 h, 75% (R^1 = R^2 = H); c) $Ru(bpy)_3Cl_2$, H_2O.

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[155] M. Tiecco, L. Testaferri, M. Tingoli, D. Chianelli, M. Montanucci, *Synthesis* **1984**, 736-738.

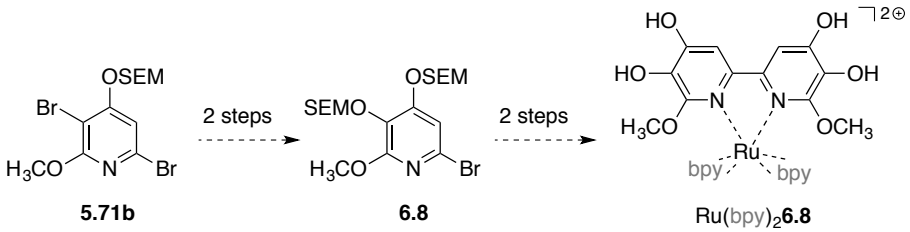
[156] J. K. Klostermann, A. Linden, J. S. Siegel, *Org. Biomol. Chem.* **2008**, *6*, 2755-2764.

Desilylation of **6.4** was achieved using TBAF in THF at elevated temperature to set free **6.7** as a colorless solid. Further studies in collaboration with the Wenger group (University Basel) should clarify, whether the electron-rich bpy-ligand **6.7** can easily substitute a dummy-ligand of the commercially available $Ru(bpy)_3$ after deprotection or the inverse synthetical order, first coordination to metal ion followed by global desilylation is more convenient.

$Ru(bpy)_2$**6.7** could also be used as a probe for PCET to an acceptor system as it should allow in principle to transfer two protons and two electrons by oxidizing the bpy system.

6.3 Conclusion and Outlook

In future experiments, the 4,4',5,5'-tetrahydroxy-2,2'-bypyridines **6.8** (**Scheme 6.3**) will be synthesized in a similar manner. Key for this construction would be the regioselective exchange of the bromine at C-4' (**5.71b**) with an electrophilic oxygen source, followed by protection and cross-coupling of both heteroaromatic moities. The Ru-complex could be obtained either by global desilylation and complexation to the transition metal or the inverse sequential order (coordination and deprotection) depending on the donor capacity of the bpy-ligand **6.8**.



5.71b **6.8** $Ru(bpy)_2$**6.8**

Scheme 6.3. Synthetic plan for the future installment of catecholic bypyridine ligands.

With this system in hands, it is invisioned to investigate the concerted transfer of four protons and four electrons across this complex to an acceptor system. Potential application would range from artificial photosynthesis to improved energy storage devices.

7 CONCLUSION

The present PhD thesis entitled *Stereoselective Total Syntheses of Natural Piericidins, the Neurotogenic Steroid Withanolide A and the Development of Photolabile Surface Anchors* should give a comprehensive impression on parts of the field of modern synthetic chemisry. The presented approaches may contribute to a certain extend for the understanding of complex biological phenomena such as cell differentiation, neurite outgrowth or nuclear export inhibition.

The general motivation for this work is very much application-driven and inspired by problems addressed earlier in our research group. Similar in chess 'if the student forces himself to examine all moves that smite, however absurd they may look at first glance, he is on the way to becoming a master of tactics.' (C. Purdy, 1906-1979). Synthetic organic chemistry in particular enables us to perform successful research not before a lot of setbacks that lead to a higher frustration tolerance, a broader structural knowledge and a better understanding of the synthetic strategy.

Chapter 2 described one of these targets in full detail with the first total synthesis of neurotogenic steroid withanolide A in which strategic considerations for the sequential order of the installation of the reactive enone moiety were the key for the succesful construction of the natural product. The synthesis was achieved starting from the commercially available pregnenolone by a Corey-Seebach homologation, a vinylogous aldol reaction, a singlet oxygen mediated Schenck ene-reaction and a Wharton transposition. In addition, semi-synthetic studies starting from the isolated natural product not only allowed to investigate the reactivity of different parts of the steroidal structure, but also resulted in the synthesis of 15 derivatives for further SAR studies. We then evaluated the neuritogenic activity of withanolide A in different cell lines, in which the shown phenotypes of neuronal differentiation in neuroblastoma cells supported the potential role of withanolide A in traditional *Ayurvedic* medicine where it is used as a tonic for the improvement of age-related cognitive disorders.

Chapter 3 dealed with the evaluation of nitrocatechols as biomimetic surface anchors and photolabile protecting groups for the controlled release of small molecules under an external stimulus, UV light. This approach still holds the potential

for applications in drug delivery, as caged probes in chemical biology or for direct assays 'on chip'. Characteristics of this method involve the ease of functionalization of TiO_2 under an operationally simple dip-and-rinse procedure. The stability of the resulting functionalized particles to repeated washing and a rapid release of the small molecule cargo under UV light stimulation at 366 nm was achieved.

In **Chapter 4** the UV light mediated release-strategy was also applied for retinoids as mediators for neurite outgrowth. The use of caged agonistic probes in solution resulted in temporally controllable neuron differentiation. The release properties of immobilized retinoids were subsequently investigated by spectroscopy. However, further studies are required to to clarify if we can direct neurite outgrowth on surfaces by the light-triggered release choosing an appropriate surface texture.

The first total syntheses of piericidin derivatives Mer-A2026B and JBIR-02 to determine the previously unknown absolute configuration of both natural products as well as to confirm their reported biological properties was the subject of **Chapter 5**. Both natural products were obtained in enantiomerically pure in 12 steps and lead to the establishment of the absolute and relative configuration as (9*R*,10*R*). Salient features of this convergent synthetic route are a highly diastereoselective Kobayashi-Mukaiyama aldol reaction, an efficient C-H activation reaction in combination with an oxidation protocol for the preparation of the functionalized 4-hydroxy-pyridine moiety and a final Negishi cross-coupling reaction merging both subunits without isomerization of the sensitive side chain.

Finally, this strategy paved the way for the convenient synthesis of electron-rich bipyridine ligands by submitting the heteroaromatic coupling partner to the developed Negishi cross-coupling conditions in **Chapter 6**. These ligands could potentially find applications in the future as probes of PCET in artificial photosynthesis systems.

8 EXPERIMENTAL PART

8.1 General Methods and Materials

Chemicals were purchased from ABCR, Acros, Alfa Acer, or Sigma-Aldrich and used without further purification if not otherwise reported. Solvents for work-up and chromatography were distilled from technical quality. Amine bases were distilled prior to use by Dieckmann distillation. Solvents used for chemical transformations were either *puriss*. quality or dried by filtration through activated aluminum oxide under nitrogen (H_2O content < 10 ppm, *Karl-Fischer* titration). Reactions involving air or moisture sensitive reagents or intermediates were performed under argon in glassware which had been heat gun dried under high vacuum. Concentration under reduced pressure was performed by rotary evaporation at 40 °C. Yields refer to purified, dried and spectroscopically pure compounds.

Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm thickness) precoated with fluorescent indicator. The developed plates were examined under UV light (254 nm or 366 nm) and stained with aqueous $KMnO_4/Na_2CO_3$ solution or Seebach's stain ($Ce(SO_4)_2$, phosphomolybdic acid, H_2SO_4 in H_2O) followed by heating.

Flash chromatography was performed using silica gel 60 (230-240 mesh) from Fluka using a forced flow eluant at 0.3-0.5 bar pressure.

All ^1H and ^{13}C NMR spectra were recorded either using Bruker Avance 400 MHz (^1H) & 101 MHz (^{13}C) or Bruker Avance DRX 500 MHz (^1H) & 126 MHz (^{13}C) spectrometer at RT. Chemical shifts (δ-values) are reported in ppm, spectra were calibrated related to solvent's residual proton chemical shift ($CHCl_3$, δ = 7.26; d_4-MeOD, δ = 4.78, 3.35; d_6-DMSO, δ = 2.50) and solvent's residual carbon chemical shift ($CDCl_3$, δ = 77.16; d_4-MeOD, δ = 49.30; d_6-DMSO, δ = 39.52), multiplicity is reported as follows: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved and coupling constant *J* in Hz.

IR spectra were recorded using a *Varian 800 FT-IR ATR Spectrometer*. The absorptions are reported in cm^{-1}.

Optical rotations $[\alpha]_D$ were measured at the sodium D line using a 1 mL cell with a 1 dm path length on a *Jasco P-2000 digital polarimeter* and the concentration *c* is

given in g/100 mL and the used solvent is MeOH or $CHCl_3$.

Melting points (M.p.) were determined using a *Büchi B-545 apparatus* in open capillaries and are uncorrected.

HPLC purification was performed on a *Dionex HPLC* with a UV-detector using a Chiralpak IA column with isocratic solvent mixture of *n*-hexane:*i*-PrOH 99.5:0.5, with a flowrate of 5 mL/min, 25 °C, UV = 254 nm, if not further specified in the **Experimental Part**.

UPLC reaction control was performed on a *Agilent 1290 Infinity*.

Mass spectra (HRMS ESI) were recorded by the Mass spectrometric Service of the University of Bern on a *Sciex QSTAR Pulsar mass spectrometer* or by H. Nadig at the University of Basel on a *Bruker maXis 4G* using electrospray ionization in both cases.

X-ray analyses: Data collections for both crystal structures were performed at low temperature (123 K) using Mo-K_a radiation on a *Bruker KappaAPEX diffractometer*. Integration of the frames and data reduction was carried out using APEX2.[157] The structures were solved by direct methods using SIR92.[158] All non-hydrogen atoms were refined using anisotropically by full-matrix least squares on *F* using CRYSTALS.[159] Hydrogen atoms were placed in calculated positions by means of the "riding" model.

Labeling of compounds was organized as following: All compound mentioned in the theoretical part were indexed with a number (e.g. **Chapter 1 - 1.X**, **Chapter 2 - 2.X** etc.). Intermediates that are not mentioned in the theoretical part, but in the **Experimental Part** referring to compounds mentioned in the theoretical part are either labeled with **Xa** (afore in the synthetic order) or **Xb** (later on).

Reaction indicated with [51],[62] and [107] were performed and characterized by

[51] Raphael Liffert, [62] Robin Wehlauch or [107] Gregor P. Meier.

[157] Bruker Analytical X-ray Systems, Inc., **2006**. *Apex2*, Version 2 User Manual, M86-E01078, Madison, WI.

[158] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst.* **1994**, *27*, 435-436.

[159] P. W. Betteridge, J. R. Carruthers, R. I. Cooper, K. Prout, D. J. Watkin, *J. Appl. Cryst.* **2003**, *36*, 1487.

8.2 List of Abbreviations, Acronyms and Symbols

Ac	acetyl
AcOH	acetic acid
AD	Alzheimer disease
aq.	aqueous
ATRA	all-*trans* retinoic acid
Bn	benzyl
Bn-OPT	benzyloxypyridinium triflate
brsm	based on recovered starting material
bpy	2,2'-bipyridine
c	concentration
CAN	ceric ammonium nitrate
cath	human cathepsin
Cbz	carboxybenzyl
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
COD	1,5-cyclooctadiene
δ	chemical shift
d	doublet
D	deuterium
DBDMH	1,1-dibromo-5,5-dimethylhydantoin
DBU	1,8-diazabicyclo[5.4.0]undec-7-en
DCE	1,2-dichloroethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMEM	*Dulbecco's modified Eagle's medium*
DMF	dimethylformamide
DMP	Dess-Martin periodinane

DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethyl sulfoxide
L-DOPA	3,4-dihydroxyphenylalanine
dppf	1,1'-bis(diphenylphosphino)ferrocene
dr	diastereomeric ratio
DMS	dimethyl sulfide
DSC	*N*,*N*'-disuccinimidy carbonate
dtbpy	4,4'-di-*tert*-butyl-2,2'-bipyridine
EDC	1-(3-dimetylaminopropyl)-3-ethylcarbodiimid
ee	enantiomeric excess
ESI	electrospray ionization
Et	ethyl
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eq.	equivalent
FTIR	Fourier transform infrared spectroscopy
HBpin	pinacolborane
HMPA	hexamethylphosphoramide
HOBt	1-hydroxybenzotriazol
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IBX	2-iodoxybenzoic acid
Imd	imidazole
J	coupling constant
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
m	multiplet
m-CPBA	*meta*-chloroperoxybenzoic acid
M.p.	melting point
Me	methyl
MeNPOC-Cl	5'-(α-methyl-2-nitropiperonyl) oxycarbonyl chloride

MeOH	methanol
MEM	minimal essential medium
MOM	methoxymethyl
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NGF	nerve growth factor
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance spectroscopy
NMP	*N*-methyl-2-pyrrolidon
NOE	nuclear Overhauser effect
NPE	(2-nitrophenyl)ethyl
NPP	(2-nitrophenyl)propyl
Oxone	$2\ KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$
PCC	pyridinium chlorochromate
PCET	proton coupled electron transfer
PDC	pyridinium dichromate
Ph	phenyl
PPh_3	triphenylphosphine
PM	plasmepsin
PMB	*para*-methoxybenzyl
ppm	parts per million
*p*TsOH	*para*-toluenesulfonic acid
q	quartet
RAR	retinoic acid receptor
R_f	retention factor
R_t	retention time
RT	room temperature
s	singlet
sat.	saturated
SEM	2-(trimethylsilyl)ethoxymethyl
t	triplet
TBAF	tetrabutylammonium fluoride

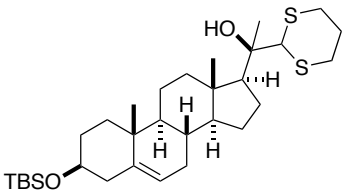
TBAH	tetrabutylammonium hydroxide
TBHP	*tert*-butyl hydroperoxide
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TPAP	tetrapropylammonium perruthenate
TPP	*meso*-tetraphenylporphyrin
Triton B	benzyltrimethylammonium hydroxide
UPLC	ultra high-performance liquid chromatography
UV	ultraviolet
ν	wavenumber

Units of measure (except Å for Angstrom) and their standard unit prefixes were used in accordance with the international system of units (SI), together with the respective prefixes for the type of physical quantity.

8.3 Withanolide A

8.3.1 Total Synthesis of Withanolide A

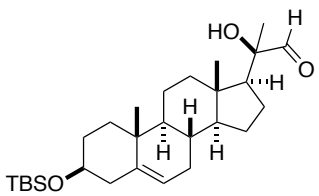
Dithian (2.7):[37] To a cold (-10 °C) solution of 1,3-dithiane (8.900 g, 0.074 mol, 2.0 eq.) in dry THF (65 mL) was added *n*-BuLi (1.6 M, 47.0 mL, 2.05 eq.) slowly and the reaction was stirred at -10 °C for 2 h. The mixture was then cooled to -78 °C and a cold (0 °C) solution of TBS protected pregnenolone (15.943 g, 0.037 mol, 1.0 eq.) in dry THF (100 mL) was added slowly *via* syringe and the resulting mixture stirred at -78 °C for 5 h then allowed to warm slowly to RT and stirred for a further 18 h. The reaction was quenched with sat. aqueous NH_4Cl, then extracted with Et_2O, the combined organics were washed with H_2O, dried over $MgSO_4$ and concentrated to afford an off-white solid (*dr* 15:1). The crude material was recrystallised from hot EtOAc/hexane (ca. 1:1) affording the dithian **2.7** as a colorless solid (16.986 g, 0.031 mol, 84%).



R_f = 0.36 (hexane:EtOAc 9:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.31 (m, 1H), 4.14 (s, 1H), 3.48 (m, 1H), 2.90 (m, 4H), 1.44 (s, 3H), 1.00 (s, 3H), 0.89 (s, 9H), 0.87 (s, 3H), 0.05 (s, 6H).

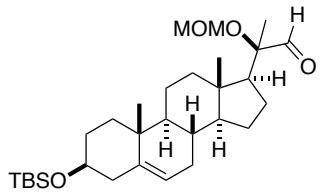
Analytical according to reference: B. B. Shingate, B. G. Hazra, V. S. Pore, R. G. Gonnade, M. Bhadbhade, *Tetrahedron* **2007**, *63*, 5622-5635.

Hydroxy aldehyde (2.7b): To a stirred solution of dithian **2.7** (10.794 g, 19.591 mmol, 1.0 eq.) in CH_2Cl_2/H_2O (10:1, 150 mL) was added *N*-chlorosuccinimide (5.363 g, 40.161 mmol, 2.05 eq.) in portions. The reaction mixture was stirred at RT for 2 h, then quenched with $Na_2S_2O_3/NaHCO_3$ solution (1:1, 100 mL) and the aqueous phase extracted with Et_2O. The combined organic layers were washed with H_2O, dried over Na_2SO_4 and concentrated to a white solid. The residue was purified by flash column chromatography (pentane:Et_2O 10:1) and afforded aldehyde **2.7** as a colorless solid (6.624 g, 14.375 mmol, 73%).



M.p.: 199-201 °C. $\mathbf{R_f}$ = 0.6 (hexane:EtOAc 4:1). **Optical rotation**: $[\alpha]_D$ = -56.8° (*c* = 0.45, $CHCl_3$). **FTIR** ν = 3375, 2936, 2858, 1728, 1470, 1385, 1358, 1254, 1080, 887, 868, 837, 772 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 9.56 (s, 1H), 5.31-5.30 (m, 1H), 3.51-3.44 (m, 1H), 3.25 (s, 1H), 2.29-2.24 (m, 1H), 2.18-2.12 (m, 2H), 1.98-1.95 (m, 1H), 1.81-1.75 (m, 2H), 1.73-1.62 (m, 3H), 1.57-1.42 (m, 6H), 1.35 (s, 3H), 1.32-1.25 (m, 1H), 1.22-1.13 (m, 1H), 1.07-1.02 (m, 2H), 1.00 (s, 3H), 0.96-0.91 (m, 1H), 0.88 (s, 9H), 0.79 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 203.7, 141.8, 121.0, 79.7, 72.7, 56.7, 55.6, 50.3, 43.4, 42.9, 40.2, 37.5, 36.7, 32.2, 31.9, 31.6, 26.1, 24.3, 23.1, 22.3, 21.1, 19.6, 18.4, 14.0, -4.4. **HRMS ESI** calc. for $C_{28}H_{47}O_3Si$ $[M-H]^-$: 459.3294, found: 459.3303.

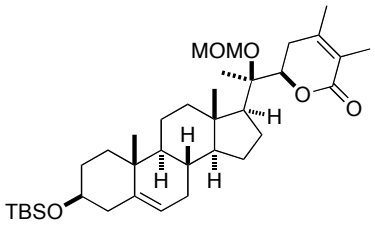
MOM protected aldehyde (2.8): To a cold (0 °C) solution of NaI (671 mg, 4.479 mmol, 4 eq., dried under high vacuum at 100 °C for 6 h) in dry DME (5 mL) was added freshly distilled MOMCl (451 mg, 5.599 mmol, 5 eq.) upon which a yellow suspension formed. The mixture was allowed to warm to RT and stirred for 10 minutes. This mixture was then added slowly *via* syringe to a cold (0 °C) solution of aldehyde **2.7b** (516 mg, 1.120 mmol, 1 eq.) and DIPEA (956 mg, 6.159 mmol, 5.5 eq.) in dry DME and the reaction mixture heated at reflux for 18 h. The reaction mixture was allowed to cool to RT and quenched with Na_2CO_3 solution (1M, 10 mL) and then extracted with Et_2O. The combined organic layers were washed with H_2O, dried over Na_2SO_4, filtered and concentrated to afford an orange solid. The crude material was subjected to flash chromatography on silica (hexane:EtOAc, 9:1) and concentrated to give protected aldehyde **2.8** (540 mg, 1.070 mmol, 94%) as an off-white solid.



M.p. : 131-132 °C. $\mathbf{R_f}$ = 0.8 (pentane:EtOAc, 4:1). **Optical rotation**: $[\alpha]_D$ = -16.3° (*c* = 1.11, $CHCl_3$). **FTIR**: ν = 2931, 1727, 1462, 1380, 1252, 1080, 1033, 869, 838, 776, 671 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.69 (s, 1H), 5.33-5.28 (m, 1H), 4.84 (d, *J* = 7.3 Hz, 1H), 4.61 (d, *J* = 7.3 Hz, 1H), 3.52-3.42 (m, 1H), 3.41 (s, 3H), 2.31-2.21 (m, 1H), 2.19-2.07 (m, 2H), 2.01-1.91 (m, 1H), 1.90-1.77 (m, 2H), 1.73-1.60 (m, 4H), 1.58-1.41 (m, 7H), 1.36 (s, 3H), 1.27-1.09 (m, 3H), 0.99 (s, 3H), 0.88 (s, 9H),

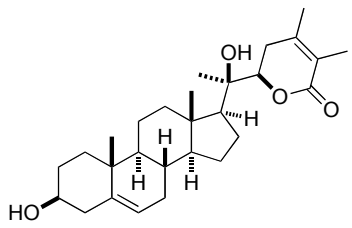
0.77 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 205.3, 141.7, 121.0, 92.0, 85.7, 72.7, 58.5, 56.2, 55.9, 50.2, 43.5, 42.9, 40.0, 37.5, 36.7, 32.2, 31.9, 31.6, 26.0, 24.1, 22.0, 21.0, 19.5, 18.8, 18.3, 14.7, -4.5. **Elementary Analyses** calc. for $C_{30}H_{52}O_4Si$: C = 71.38, H = 10.38, found: C = 71.60, H = 10.24.

Lactone (2.26): A solution of ethyl 2,3-dimethylbut-2-enoate **2.24** (384 mg, 2.70 mmol, 2.7 eq.) and DMPU (2.5 mL, distilled freshly over CaH_2) in THF (2.5 mL) was added dropwise to a solution of LiHMDS (3.0 mL 1.0 M in THF, 3.0 mmol, 3 eq.) in THF (2.5 mL) at -78 °C and the mixture was stirred for 1.5 h at that temperature. A solution of protected aldehyde **2.8** (0.5 g, 1.0 mmol, 1 eq.) in THF (3.0 mL) was then added dropwise and the resulting mixture was stirred at -78 °C for 6 h. The temperature of the reaction mixture was allowed to increase to RT overnight. Then, the reaction was quenched by addition of saturated NH_4Cl solution. The mixture was extracted with Et_2O. The combined organic layers were washed with sat. aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (pentane:Et_2O 3:1) to give lactone **2.26** (as a 93:7 mixture of diasteroisomers; 518 mg, 0.862 mmol, 87%) as colorless foam.



R_f = 0.3 (pentane:EtOAc 7:1). **Optical rotation**: $[\alpha]_D$ = +19.4° (*c* = 0.44, $CHCl_3$). **FTIR**: ν = 2931, 1714, 1463, 1381, 1318, 1253, 1083, 1016, 889, 836, 775, 670 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.32-5.29 (m, 1H), 4.97 (d, *J* = 6.6 Hz, 1H), 4.82 (d, *J* = 6.6 Hz, 1H), 4.25 (dd, *J* = 13.3, 3.3 Hz, 1H), 3.51-3.44 (m, 1H), 3.37 (s, 3H), 2.53-2.44 (m, 1H), 2.29-2.22 (m, 1H), 2.18-2.10 (m, 2H), 2.02-1.96 (m, 3H), 1.93 (s, 3H), 1.86 (s, 3H), 1.84-1.67 (m, 4H), 1.65-1.45 (m, 7H), 1.39 (s, 3H), 1.35-1.27 (m, 2H), 1.07-1.00 (m, 2H), 0.99-0.96 (m, 3H), 0.88 (s, 9H), 0.86 (s, 3H), 0.05 (s, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 166.3, 148.9, 141.8, 122.0, 121.1, 92.8, 82.3, 80.0, 72.7, 56.8, 56.4, 54.5, 50.2, 43.1, 42.9, 40.3, 37.5, 36.7, 32.2, 32.2, 31.9, 31.5, 26.1, 24.1, 22.0, 21.0, 20.6, 19.5, 18.4, 18.0, 13.9, 12.6, -4.5. **Elementary Analyses** calc. for $C_{36}H_{60}O_5Si$: C = 71.95, H = 10.06, found: C = 72.04, H = 9.86.

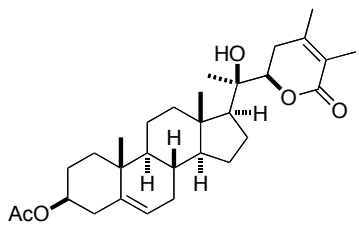
Hydroxy lactone (2.27): To a solution of lactone **2.26** (143 mg, 238 µmol) in THF (10 mL) was added 6 M aqueous HCl (2 mL) and the resulting solution was stirred for 3 h until TLC indicated full conversion. $NaHCO_3$ solution was added, the aqueous phase was extracted by CH_2Cl_2, dried over Na_2SO_4 and subjected to flash chromatography (pentane: EtOAc, 2:1) to yield hydroxy lactone **2.27** (92 mg, 208 µmol, 87%) as a colorless solid. An analytical sample was recrystallized from EtOAc for X-ray analysis.



M.p. : 243-245 °C. $\mathbf{R_f}$ = 0.3 (pentane:EtOAc 2:1). **Optical rotation**: $[\alpha]_D$ = +16.7° (*c* = 0.62, $CHCl_3$). **FTIR**: ν = 3406, 2935, 1696, 1438, 1383, 1320, 1190, 1135, 1059, 955, 833, 756, 667 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.38 – 5.31 (m, 1H), 4.23 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.62-3.46 (m, 1H), 2.49-2.41 (m, 1H), 2.34-2.19 (m, 3H), 2.16-2.09 (m, 1H), 2.07-2.02 (m, 1H), 2.01-1.97 (m, 1H), 1.95 (s, 3H), 1.94-1.91 (m, 1H), 1.89 (s, 3H), 1.87-1.80 (m, 2H), 1.66-1.60 (m, 1H), 1.56-1.42 (m, 8H), 1.28 (s, 3H), 1.25-1.04 (m, 3H), 1.01 (s, 3H), 0.99-0.88 (m, 2H), 0.87 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.3, 149.1, 141.0, 122.1, 121.7, 81.1, 75.4, 71.9, 57.0, 54.8, 50.2, 43.1, 42.4, 40.2, 37.4, 36.6, 31.9, 31.8, 31.6, 31.5, 24.0, 22.2, 21.1, 20.9, 20.7, 19.5, 13.8, 12.6. **Elementary Analyses** calc. for $C_{28}H_{42}O_4$: C = 75.98, H = 9.56, found: C = 75.91, H = 9.42. **X-ray crystal structure** is given in the appendices section.

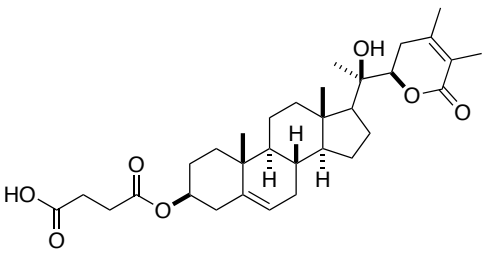
8.3.2 Semisynthetic Studies on Synthetic Precursors of Withanolide A

Acetate (2.31): To a solution of diol **2.27** (12 mg, 27 µmol, 1 eq.) in CH_2Cl_2 (2 mL) was added Et_3N (50% in CH_2Cl_2; 22 µL, 71 µmol, 3eq.), DMAP (0.3 mg, 3 µmol, 0.1 eq.) and Ac_2O (10 µL, 98 µmol, 4 eq.) and the resulting mixture was stirred at RT over night. The mixture was quenched by addition of 0.1 M HCl solution and extracted using CH_2Cl_2. The organic layers were dried over Na_2SO_4, evaporated and subjected to flash chromatography (pentane:EtOAc 4:1) to yield acetate **2.31** (11.2 mg, 23 µmol, 85%) as a colorless solid.



$\mathbf{R}_f$ = 0.2 (EtOAc:hexane:$CHCl_3$ 8:1:1). **Optical rotation**: $[\alpha]_D$ = +38.5° (*c* = 0.41, $CHCl_3$). **FTIR**: ν = 2940, 1711, 1440, 1379, 1319, 1245, 1132, 1032, 918, 758, 631 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.37 (d, *J* = 4.4 Hz, 1H), 4.66-4.54 (m, 1H), 4.22 (dd, *J* = 13.3, 3.4 Hz, 1H), 2.47-2.38 (m, 1H), 2.34-2.27 (m, 2H), 2.24 (s, 1H), 2.15-2.09 (m, 1H), 2.03 (s, 3H), 2.01-1.97 (m, 1H), 1.95 (s, 3H), 1.93-1.79 (m, 6H), 1.67-1.41 (m, 10H), 1.29 (s, 3H), 1.25-1.09 (m, 4H), 1.02 (s, 3H), 0.87 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 170.82, 166.37, 165.72, 151.42, 149.19, 139.95, 122.67, 122.21, 81.21, 75.46, 74.14, 57.03, 54.89, 50.19, 43.26, 40.29, 38.32, 37.21, 36.80, 31.99, 31.78, 31.50, 27.98, 24.08, 22.21, 21.69, 21.11, 21.10, 20.81, 19.55, 13.82, 12.71. **HRMS ESI** calc. for $C_{30}H_{45}O_5$ $[M-H]^+$: 485.3262 found: 485.3254.

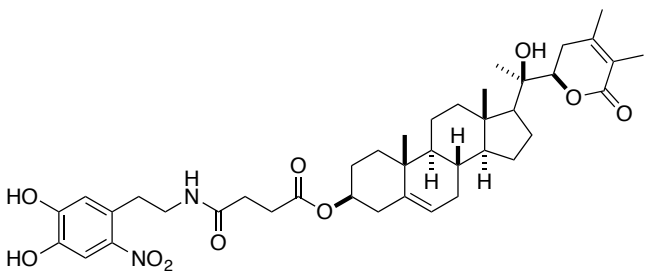
Acid (2.33): To a solution of diol **2.27** (23 mg, 52 μmol, 1eq.) in CH_2Cl_2 (2 mL) was added Et_3N (50% in CH_2Cl_2; 44 μL, 156 μmol, 3 eq.), DMAP (3 mg, 3 μmol, 0.1 eq.) and succinic anhydride (21 mg, 208 μmol, 4 eq.) and the resulting mixture was stirred at RT for 1 h. The mixture was quenched by addition of 0.1 M HCl solution and extracted using CH_2Cl_2. The organic layers were dried over Na_2SO_4, evaporated and subjected to flash chromatography (CH_2Cl_2:MeOH 10:1 + 1% TFA) to yield acid **2.33** (11.2 mg, 23 μmol, 85%) as a colorless solid.



M.p.: 200-202 °C. $\mathbf{R}_f$ = 0.2 (CH_2Cl_2:MeOH 10:1 + 1% TFA). **Optical rotation**: $[\alpha]_D$ = +24.3° (*c* = 0.24, $CHCl_3$). **FTIR**: ν = 3476, 2934, 1727, 1664, 1386, 1325, 1156, 1012, 890, 826, 635 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.94 (bs, 1H), 5.36 (d, *J* = 4.1 Hz, 1H), 4.66-4.58 (m, 1H), 4.23 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.70 (s, *J* = 4.7, 1H), 2.72-2.56 (m, 5H), 2.49-2.33 (m, 1H), 2.31 (d, *J* = 7.8 Hz, 2H), 2.13 (dd, *J* = 17.4, 2.6 Hz, 1H), 2.10-2.03 (m, 1H), 1.95 (s, 3H), 1.88 (s, 3H), 1.80-1.35 (m, 10H), 1.28 (s, 3H), 1.02 (s, 4H), 0.87 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 177.55, 171.80, 166.75, 149.53, 139.74, 122.62, 122.00, 81.22, 75.39, 74.57, 56.87, 54.73, 50.01, 43.16, 40.16, 38.05, 37.01, 36.70, 31.89, 31.67, 31.39, 29.34, 29.05, 27.76,

23.97, 22.08, 21.02, 20.94, 20.73, 19.43, 13.73, 12.53. **HRMS ESI** calc. for $C_{32}H_{46}O_7Na$ $[M\text{-}Na]^+$: 565.3136 found: 565.3144.

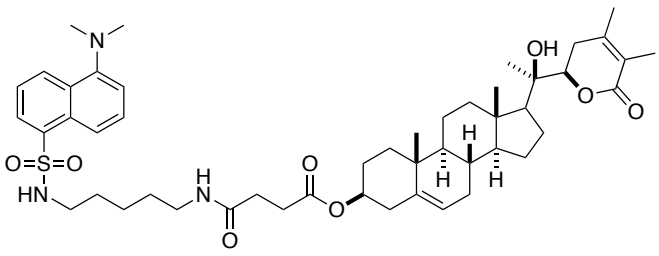
Nitrocatechol hybrid (2.34): To a solution of acid **2.33** (42 mg, 77 µmol, 1 eq.) in dry CH_3CN (2 mL) was added N,N'-disuccinimidy carbonate (22 mg, 81 µmol, 1.05 eq.) ar RT to form a white suspension. After 5 min pyridine (9.5 µL, 116 µmol, 1.5 eq.) was added at RT to form again a clear solution and stirring was continued for 6 h at RT, until TLC indicated full conversion. To the reaction mixture was added 0.2 M aqueous HCl, and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, evaporated to afford a colorless solid, which was dissolved in DMSO/THF (1:1, 2 mL). To this mixture was added nitro dopamine sulfate (21 mg, 43 µmol, 0.55 eq.) and Et_3N (55 mL, 387 µmol, 5 eq.) and stirring continued at RT over night. The mixture was quenched by addition of water and extracted using EtOAc. The organic layers were dried over Na_2SO_4, evaporated and subjected to flash chromatography (CH_2Cl_2:MeOH 10:1) and reverse phase HPLC to yield catechol **2.34** (8 mg, 11 µmol, 14%) as a orange oil.



M.p.: 140-170 °C (decomposition). $\mathbf{R_f}$ = <0.1 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +2.9° (*c* = 1.10, $CHCl_3$). **FTIR**: ν = 3362, 2942, 2696, 1527, 1437, 3883, 1288, 1192, 1052, 888, 769 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.14 (bs, 1H), 7.63 (s, 1H), 6.81 (s, 1H), 6.48 (t, *J* = 5.9 Hz, 1H), 5.33 (d, *J* = 4.4 Hz, 1H), 4.56-4.53 (m, 1H), 4.24 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.59 (dd, *J* = 12.7, 6.4 Hz, 2H), 3.08 (t, *J* = 6.7 Hz, 2H), 2.65 (t, *J* = 6.4 Hz, 2H), 2.52-2.48 (m, 2H), 2.43-2.05 (m, 8H) 1.96 (s, 3H), 1.89 (s, 3H), 1.80-1.02 (m, 10H), 1.27 (s, 2H), 0.99 (s, 3H), 0.84 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 173.22, 173.01, 166.68, 149.68, 143.63, 141.14, 139.54, 128.11, 122.82, 121.97, 118.20, 112.67, 81.16, 75.41, 75.16, 56.87, 54.77, 50.01, 43.01, 40.57, 40.05, 38.09, 37.01, 36.64, 32.76, 31.84, 31.58, 31.34, 31.07, 30.89,

29.86, 27.79, 23.92, 22.14, 20.97, 20.81, 19.40, 13.76, 12.58. **HRMS ESI** calc. for $C_{40}H_{54}O_{10}N_2Na^+$ $[M\text{-}Na]^+$: 745.3671 found: 745.3686.

Dansyl hybrid (2.35): To a solution of acid **2.33** (23 mg, 42 µmol, 1 eq.) in dry

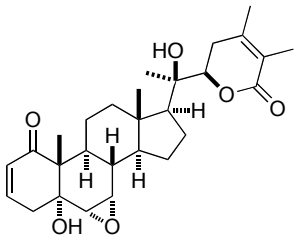


CH_3CN/DMF (5:1, 2.5 mL) was added sequentially Et_3N (21 µL, 148 µmol, 3.5 eq.), 1-hydroxy-benzotriazole (7 mg, 51 µmol, 1.2 eq.), EDC (10 mg, 51 µmol, 1.2 eq.) and dansyl amine (17 mg, 51 µmol, 1.2 eq.) and the resulting mixture stirred over night, until TLC indicated complete conversion. The volatiles were removed at HV, then 0.1 M aqueous HCl was added and the aqueous layer was extracted with EtOAc. The organic layer was dried over Na_2SO_4, evaporated and subjected to flash chromatography (CH_2Cl_2:MeOH 40:1) to afford catechol **2.35** (26 mg, 30 µmol, 71%) as yellow solid.

M.p.: 141-143 °C. $\mathbf{R_f}$ = 0.3 (CH_2Cl_2:MeOH 40:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.53 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.7 Hz, 1H), 8.23 (d, *J* = 7.3 Hz, 1H), 7.59-7.50 (m, 2H), 7.18 (d, *J* = 7.5 Hz, 1H), 5.73 (t, *J* = 5.6 Hz, 1H, *NH*), 5.34 (d, *J* = 4.1 Hz, 1H, *NH*), 5.00 (t, *J* = 6.0 Hz, 1H), 4.59 (d, *J* = 8.5 Hz, 1H), 4.22 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.09 (dd, *J* = 12.5, 6.4 Hz, 2H), 2.88 (m, 8H), 2.63 (t, *J* = 6.9 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 2.30 (d, *J* = 7.7 Hz, 2H), 1.95 (s, 3H), 1.88 (s, 3H), 2.30-1.00 (m, 27H), 1.28 (s, 3H), 1.00 (s, 3H), 0.86 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.71, 171.73, 170.58, 166.29, 152.10, 149.16, 139.76, 135.00, 130.45, 129.99, 129.76, 129.66, 128.45, 123.35, 122.58, 122.05, 118.93, 115.29, 109.71, 81.10, 75.32, 74.44, 56.89, 54.76, 50.02, 45.55, 43.12, 43.05, 40.15, 39.13, 38.15, 37.02, 36.67, 31.86, 31.65, 31.36, 31.22, 30.05, 28.98, 28.89, 27.82, 23.96, 23.42, 22.09, 20.96, 20.69, 19.42, 13.71, 12.59. **HRMS ESI** calc. for $C_{49}H_{70}O_8N_3S^+$ $[M\text{-}H]^+$: 860.4878 found: 860.4887.

8.3.3 Semisynthetic Studies on Withanolide A

Isolation Procedure of Withanolide A (2.1): The dried Ashwagandha roots (1.5 kg) were placed in a large column and percolated with MeOH (3 L) several times. The MeOH extracts were concentrated from 3 L to 1 L under reduced pressure and then washed with pentane (3 x 250 mL). The MeOH extracts were then carefully concentrated under reduced pressure affording a thick brown oil, which was suspended in EtOAc (300 mL), washed with H_2O (3 x 200 mL), dried over $MgSO_4$ and concentrated to a brown oil (4.39 g). The crude material was purified by silica gel chromatography using a graduated eluent system (40% to 100% EtOAc/pentane at intervals of 10%, 1.5 column lengths of eluent/fraction) and each fraction was collected in an Erlenmeyer and concentrated separately and analyzed by HPLC and TLC. Withanolide A was found to be present in the 70% and 80% fractions. These two fractions were combined and purified by column chromatography (CH_2Cl_2:MeOH 40:1) affording withanolide A **2.1** (340 mg, 0.722 mmol, 0.025% from mass of dried roots) as an off-white solid.



M.p.: 287-289 °C. $\mathbf{R_f}$ = 0.5 (EtOAc:hexane:$CHCl_3$ 8:1:1). **Optical rotation**: $[\alpha]_D$ = +86.8° (*c* = 1.15, $CHCl_3$). **UV**: 228.5 nm. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.58 (ddd, *J* = 10.1, 5.0, 2.1 Hz, 1H), 5.83 (dd, *J* = 10.2, 2.2 Hz, 1H), 4.20 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.31 (s, 1H), 3.16 (s, 1H, OH), 3.04 (d, *J* = 3.9 Hz, 1H), 2.76-2.62 (m, 2H), 2.51 (dd, *J* = 18.8, 5.1 Hz, 1H), 2.44-2.33 (m, 2H), 2.20-1.99 (m, 3H), 1.95 (s, 3H), 1.88 (s, 3H), 1.85-1.72 (m, 2H), 1.61-1.33 (m, 7H), 1.31 (s, 3H), 1.17 (s, 3H), 0.95 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 203.3, 166.2, 149.0, 139.8, 129.1, 122.1, 81.1, 75.2, 73.4, 57.4, 56.5, 54.5, 52.0, 51.1, 44.0, 40.5, 36.9, 35.7, 35.2, 31.8, 23.3, 21.9, 21.9, 21.2, 20.7, 14.9, 13.9, 12.6. **HPLC/MS**: R_t = 48.9 min, *m/z* (ES^+) 493.2 (MNa^+).

Analytical data according to reference: S. S. Subramanian, P. D. Sethi, E. Glotter, I. Kirson, D. Lavie, *Phytochemistry* **1971**, *10*, 685-688.

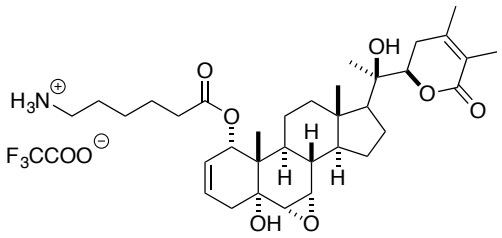
Allylic alcohol (2.36): To a cold solution (0 °C) of withanolide A (30 mg, 64 µmol, 1 eq.) in $CHCl_3$/MeOH (1.5 mL; 1:2) was added $CeCl_3 \cdot 7\ H_2O$ (47.5 mg, 128 µmol, 2 eq.). After 5 min $NaBH_4$ (24.1 mg, 640 µmol, 10 eq.) was added over a period of 4 h in 5 portions until TLC indicated full conversion. The reaction was quenched by addition of saturated NH_4Cl solution, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated. The crude product was subjected to flash chromatography (EtOAc:pentane 6:4) to yield allylic alcohol **2.36** (24.3 mg, 48 µmol, 75%) as a colorless solid. An analytical sample was recrystallized from EtOAc for X-ray analysis.

M.p.: 255-257 °C. $\mathbf{R_f}$ = 0.6 (EtOAc:hexane:$CHCl_3$ 8:1:1). **Optical rotation**: $[\alpha]_D$ = +112.2° (*c* = 0.40, $CHCl_3$). **FTIR**: ν = 3480, 2944, 1703, 1384, 1290, 1217, 1132, 1028, 910, 784 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.89 (d, *J* = 9.9 Hz, 1H), 5.72-5.66 (m, 1H), 4.18 (dd, *J* = 13.0, 3.7 Hz, 1H), 3.52 (s, 1H), 3.51 (s, 1H), 3.43 (s, 1H), 3.31-3.27 (m, 1H), 2.95 (d, *J* = 3.8 Hz, 1H), 2.40 (s, 1H), 2.35-1.95 (m, 6H), 1.92 (s, 3H), 1.85 (s, 3H), 1.84-1.72 (m, 3H), 1.63-1.31 (m, 7H), 1.28 (s, 3H), 0.93 (s, 3H), 0.75 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 166.2, 148.9, 129.8, 124.2, 122.2, 81.1, 75.2, 71.4, 70.8, 58.0, 57.5, 54.5, 52.0, 44.3, 40.3, 40.1, 35.7, 34.9, 34.7, 31.7, 23.2, 22.1, 21.2, 20.7, 20.3, 15.3, 13.8, 12.6. **HRMS ESI** calc. for $C_{28}H_{40}O_6Na$ $[M\text{-}Na]^+$: 495.2717 found: 495.2704. **X-ray crystal structure** is given in the appendix.

Alcohol (2.37):[51] Allylic alcohol **2.36** (10.0 mg, 0.021 mmol, 1.0 eq.) and Crabtree's catalyst (0.34 mg, 2 mol%) were dissolved in CH_2Cl_2 (0.2 mL) in a small vial. The vial was placed in an autoclave and the mixture was stirred at RT for 2 h under a H_2 atmosphere (10 bar). The solvent was removed, the residue filtered through a short pad of silica (EtOAc) and evaporated to yield alcohol **2.37** (10.0 mg, 0.021 mmol, *quant*.) as a colorless solid.

M.p. = 251 °C (decomposed). $\mathbf{R_f}$ = 0.74 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +46.4° (*c* = 0.5, $CHCl_3$). **FTIR**: ν = 3578, 3464, 3362, 2938, 1717, 1445, 1381, 1316, 1103, 1021, 949, 913, 854, 757, 687 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.49 (d, *J* = 10.7 Hz, 1H), 4.21 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.58-3.51 (m, 1H), 3.50 (d, *J* = 1.2 Hz, 1H), 3.28 (dd, *J* = 3.9, 2.4 Hz, 1H), 2.90 (d, *J* = 3.9 Hz, 1H), 2.43-2.31 (m, 2H), 2.18-2.00 (m, 4H), 1.95 (s, 3H), 1.89 (s, 3H), 1.86-1.33 (m, 15H), 1.31 (s, 3H), 0.94 (s, 3H), 0.80 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.14, 148.89, 122.19, 81.15, 75.24, 72.37, 72.07, 58.52, 57.80, 54.48, 52.00, 44.50, 40.25, 40.22, 35.39, 35.24, 32.53, 31.79, 28.81, 23.13, 22.17, 21.25, 20.69, 19.89, 16.06, 15.86, 13.88, 12.62. **UPLC/MS**: R_t = 2.015 min, *m/z* (ES^+) 971.5 ($2M + Na^+$).

Amine (2.38): To a solution of acyl chloride (80 mg, 317 µmol, 5 eq.) in CH_2Cl_2 (1 mL) was added a solution of diol **2.36** (30 mg, 64 µmol, 1 eq.) CH_2Cl_2 (1 mL) and DIPEA (55µL, 317 µmol, 5 eq.). The reaction mixture was stirred over night, but no conversion was monitored by TLC. So $AgNO_3$ (27 mg, 160 µmol, 2.5 eq.) was added and a white precipitate occurred. The reaction mixture was quenched by addition of aqueous 0.1 M HCl, extracted by CH_2Cl_2. The crude product was subjected to flash chromatography (CH_2Cl_2:MeOH 50:1) to afford Boc-protected amine as a colorless solid.

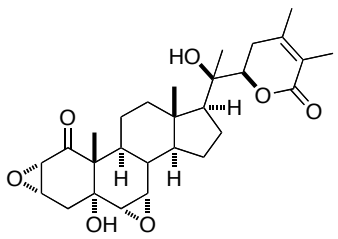


^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.97-5.78 (m, 2H), 4.81 (d, *J* = 3.8 Hz, 1H), 4.20 (dd, *J* = 13.3, 3.4 Hz, 1H), 3.27 (d, *J* = 1.9 Hz, 1H), 3.09 (d, *J* = 7.1 Hz, 1H), 2.98 (d, *J* = 3.7 Hz, 1H), 2.49-2.19 (m, 6H), 2.00-1.00 (m, 24H), 1.95 (s, 3H), 1.88 (s, 3H), 1.42 (s, 9H), 1.29 (s, 3H), 0.94 (s, 3H), 0.86 (s, 3H).

Boc protected amine was dissolved in CH_2Cl_2 (2 mL) and exposed to TFA (10 eq., as 50% solution in CH_2Cl_2) to afford after removal of all volatiles amine **2.38** (30 mg, 43 µmol, 68%) as its trifluoroacetate.

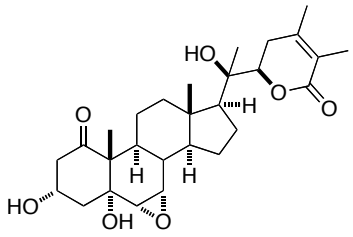
M.p. : 127-128 °C (decomposition). $\mathbf{R_f}$ = <0.1 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +91.6° (*c* = 0.28, MeOH). **FTIR**: ν = 2936, 2624, 2072, 1984, 1680, 1387, 1176, 1128, 806, 629 cm^{-1}. **^{1}H NMR** (400 MHz, d_4-MeOD) δ = 5.94-5.75 (m, 2H), 4.74 (d, *J* = 4.6 Hz, 1H), 4.26 (dd, *J* = 13.2, 3.4 Hz, 1H), 3.25 (s, 1H), 3.00-2.87 (m, 2H), 2.67-2.55 (m, 1H), 2.43-2.17 (m, 6H), 2.00-1.00 (m, 24H), 1.99 (s, 3H), 1.85 (s, 3H), 1.25 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H). **^{13}C NMR** (126 MHz, d_4-MeOD) δ = 174.84, 168.56, 153.00, 129.90, 125.01, 121.93, 82.71, 76.06, 73.21, 70.35, 58.03, 57.27, 55.57, 53.25, 44.60, 41.81, 40.78, 40.53, 36.80, 36.43, 36.00, 34.99, 32.03, 28.03, 26.68, 25.39, 23.93, 23.35, 21.74, 20.84, 20.49, 16.00, 14.48, 12.41. **HRMS ESI** calc. for $C_{34}H_{52}O_7N$ $[M-H]^+$: 586.3738 found: 586.3743.

Epoxy ketone (2.39):[51] To a solution of withanolide A (100 mg, 0.21 mmol, 1.0 eq.) and TBHP (5.5 M in decane, 100 µL, 0.55 mmol, 2.6 eq.) in CH_2Cl_2 (1.3 mL) was added dropwise TBAF (1 M in THF, 425 µL, 0.43 mmol, 2 eq.) and the mixture was stirred at RT for 15 h. The reaction was then diluted with water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/pentane, 2:1 to pure EtOAc) to give epoxy ketone **2.39** (78.4 mg, 0.16 mmol, 76%) as a colorless solid.



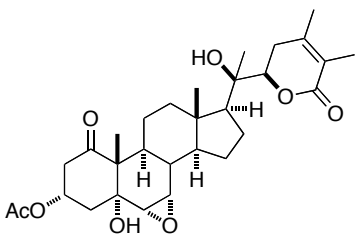
M.p. = 285 °C (decomposed). $\mathbf{R_f}$ = 0.37 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +107.1° (*c* = 0.5, $CHCl_3$). **FTIR**: ν = 3476, 2967, 2924, 1981, 1706, 1517, 1468, 1382, 1318, 1221, 1187, 1141, 1107, 1069, 1009, 952, 897, 852, 815, 757. 648 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.17 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.61-3.54 (m, 1H), 3.29 (d, *J* = 3.4 Hz, 1H), 3.26-3.18 (m, 2H), 2.94 (d, *J* = 3.8 Hz, 1H), 2.54-2.41 (m, 2H), 2.42-2.29 (m, 2H), 2.23 (dd, *J* = 15.9, 2.4 Hz, 1H), 2.14-1.97 (m, 3H), 1.94 (s, 3H), 1.87 (s, 3H), 1.84-1.21 (m, 12H), 1.10 (s, 3H), 0.92 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 203.68, 166.10, 148.97, 122.06, 81.09, 75.15, 70.75, 56.36, 54.43, 53.44, 52.77, 52.09, 51.67, 43.86, 40.23, 35.17, 34.84, 33.32, 31.77, 23.26, 21.87, 21.50, 21.21, 20.66, 13.76, 13.50, 12.56. **HRMS ESI** calc. for $C_{28}H_{39}O_7^+$ $[M+H]^+$: 487.2690, found: 487.2689.

β-Hydroxy ketone (2.40):[51] To a solution of $(PhSe)_2$ (96.2 mg) in EtOH (1.0 mL) was added $NaBH_4$ (23.3 mg) and acetic acid (10 µL). The stock solution was stirred for 10 min at RT and then added (0.2 mL, 0.123 mmol, 3 eq.) to a solution of epoxy ketone **2.39** (20 mg, 0.041 mmol, 1.0 eq.) in CH_2Cl_2 (0.2 mL). The resulting yellow mixture was stirred at RT for 1 h, then diluted with CH_2Cl_2 and washed with saturated brine. The organic phase was dried over $NaSO_4$, concentrated and subjected to flash column chromatography (EtOAc) to yield β-hydroxy ketone **2.40** (16 mg, 0.033 mmol, 80%) as a colorless solid.



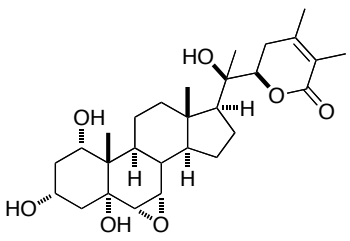
M.p. = 275-276 °C. $\mathbf{R_f}$ = 0.37 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +148.2° (*c* = 0.36, $CHCl_3$). **FTIR**: ν = 3478, 2924, 1707, 1388, 1142, 1008, 897, 814, 759, 649 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.40-4.33 (m, 1H), 4.20 (dd, *J* = 13.3, 3.5 Hz, 1H), 4.16-4.07 (m, 1H), 3.46 (d, *J* = 2.0 Hz, 1H), 3.30 (dd, *J* = 3.9, 2.2 Hz, 1H), 3.14 (dd, *J* = 8.1, 6.1 Hz, 1H), 3.10 (d, *J* = 3.9 Hz, 1H), 2.56-2.26 (m, 4H), 2.19-1.99 (m, 4H), 1.96 (s, 3H), 1.89 (s, 3H), 1.86-1.78 (m, 1H), 1.77-1.67 (m, 1H), 1.66-1.34 (m, 7H), 1.31 (s, 3H), 1.27-1.20 (m, 1H), 1.19 (s, 3H), 0.94 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 210.33, 166.13, 148.91, 122.21, 81.14, 75.21, 68.92, 57.57, 56.40, 54.53, 53.73, 51.95, 47.20, 44.32, 40.42, 38.49, 35.53, 34.83, 31.83, 23.23, 22.02, 21.65, 21.26, 20.72, 16.02, 13.98, 12.63. **HRMS ESI** calc. for $C_{28}H_{41}O_7^+$ $[M+H]^+$: 489.2847, found: 489.2837.

Acetate (2.41):[51] To a solution of β-hydroxy ketone **2.40** (10 mg, 0.021 mmol, 1 eq.) in CH_2Cl_2 (0.3 mL) was added acetic anhydride (9.6 µL, 0.102 mmol, 5 eq.), DMAP (2.5 mg, 0.021 mmol, 1 eq.), and Et_3N (29 µL, 0.210 mmol, 10 eq.). The colorless solution was stirred at RT for 45 min, then quenched with 0.1 M HCl and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (pentane/EtOAc 1:2) affording acetate **2.41** (8.4 mg, 0.016 mmol, 78%) as a colorless solid.



M.p. = 237-238 °C. $\mathbf{R_f}$ = 0.51 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +69.5° (*c* = 0.42, $CHCl_3$). FTIR: ν = 3518, 3470, 2956, 2932, 1703, 1380, 1282, 1144, 1026, 900, 813, 758, 651 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.49-5.41 (m, 1H), 4.20 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.29 (dd, *J* = 4.0, 2.0 Hz, 1H), 3.16 (d, *J* = 2.0 Hz, 1H), 3.01 (d, *J* = 3.9 Hz, 1H), 2.98 (dd, *J* = 9.6, 7.3 Hz, 1H), 2.59 (dd, *J* = 16.9, 4.9 Hz, 1H), 2.51 (ddd, *J* = 15.6, 7.3, 2.1 Hz, 1H), 2.45-2.36 (m, 2H), 2.15-1.98 (m, 4H), 2.07 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.86-1.79 (m, 1H), 1.77-1.70 (m, 1H), 1.58-1.21 (m, 8H), 1.31 (s, 3H), 1.08 (s, 3H), 0.94 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 208.89, 170.79, 166.12, 148.90, 122.19, 81.13, 75.20, 73.17, 67.58, 57.50, 56.59, 54.54, 52.54, 51.94, 44.11, 43.06, 40.43, 37.93, 35.72, 34.90, 31.83, 23.31, 21.99, 21.82, 21.48, 21.23, 20.71, 15.00, 13.90, 12.62. **UPLC/MS**: R_t = 1.785 min, *m/z* (ES^+) 1061.5 ($2M + Na^+$).

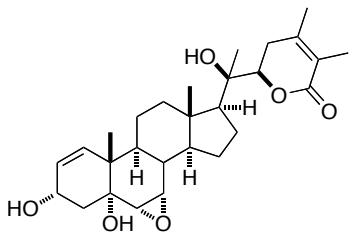
Tetraol (2.42):[51] To a solution of β-hydroxy ketone **2.40** (30 mg, 0.061 mmol, 1 eq.) in $CHCl_3$/MeOH (1.0 mL, 1:1) was added $NaBH_4$ (9.5 mg, 0.246 mmol, 4 eq.). After stirring for 1 h at RT the reaction mixture was quenched with 0.1 M HCl solution. Saturated aqueous Rochelle's solution (40 mL) and EtOAc (40 mL) were added to the mixture and stirred for 1 h. Then the layers were separated and the aqueous was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 25:1) to yield tetraol **2.42** (19.5 mg, 0.040 mmol, 65%) as a colorless solid.



M.p. = 234-235 °C. $\mathbf{R_f}$ = 0.22 (CH_2Cl_2/MeOH, 25:1). **Optical rotation**: $[\alpha]_D$ = +38.0° (*c* = 0.27, $CHCl_3$). **FTIR**: ν = 3551, 2943, 1705, 1440, 1382, 1305, 1106, 1093, 910, 843, 746 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.21 (dd, *J* = 13.3, 3.5 Hz, 1H), 4.18-4.12 (m, 1H), 3.85 (s, 1H), 3.78 (d, *J* = 10.7 Hz, 1H), 3.67 (d, *J* = 9.0 Hz, 1H), 3.62 (ddd, *J* = 10.7, 4.4, 2.0 Hz, 1H), 3.33 (dd, *J* = 3.9, 2.3 Hz, 1H), 2.98 (d, *J* = 3.8 Hz, 1H), 2.44-2.33 (m, 2H), 2.31-2.23 (m, 1H), 2.18-1.99 (m, 6H), 1.95 (s, 3H), 1.89 (s, 3H), 1.87-1.74 (m, 3H), 1.66-1.28 (m, 10H), 0.95 (s, 3H), 0.77 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.11, 148.88, 122.20, 81.11, 75.21, 73.98, 71.90, 66.82, 58.23, 57.93, 54.46, 51.93, 44.46, 40.69, 40.10, 38.44, 36.89, 35.15, 31.77,

23.13, 22.16, 21.22, 20.70, 19.91, 16.40, 13.88, 12.62. **UPLC/MS**: R_t = 1.612 min, *m/z* (ES^+) 981.6 ($2M + H^+$).

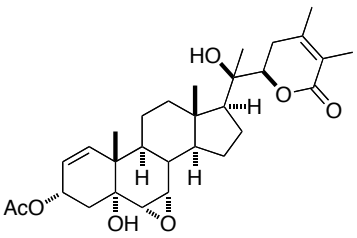
Allylic alcohol (2.43):[51] A solution of epoxy ketone **2.39** (100 mg, 0.21 mmol, 1 eq.)



in MeOH/CH_2Cl_2 (4.0 mL, 1:1) was cooled to 0 °C, and hydrazine hydrate (100 µl, 2.1 mmol, 10 eq.) was added dropwise. After stirring for 15 min, AcOH (50 µL) was added dropwise and the reaction mixture was stirred for additional 15 min at 0 °C. Then the reaction mixture was allowed to come to RT and stirred for 3 h. The slightly yellow solution was diluted with CH_2Cl_2 and quenched with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash column chromatography (EtOAc/pentane, 1:1 to 2:1) to give allylic alcohol **2.43** (59 mg, 0.13 mmol, 61%) as a colorless solid. An analytical sample was recrystallized from EtOAc for X-ray analysis.

M.p. = 248-249 °C. TLC: $\mathbf{R}_f$ = 0.51 (EtOAc). **Optical rotation**: $[\alpha]_D$ = -7.0° (*c* = 0.5, $CHCl_3$). **FTIR**: ν = 3495, 2939, 1700, 1384, 1318, 1092, 1029, 895, 748, 644 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.87-5.80 (m, 2H), 4.20 (dd, *J* = 13.3, 3.5 Hz, 1H), 4.16-4.09 (m, 1H), 3.35 (d, *J* = 11.2 Hz, 1H), 3.33-3.28 (m, 1H), 3.22 (d, *J* = 1.6 Hz, 1H), 3.09 (d, *J* = 3.8 Hz, 1H), 2.45-2.25 (m, 3H), 2.21-2.00 (m, 4H), 1.95 (s, 3H), 1.89 (s, 3H), 1.87-1.74 (m, 2H), 1.68-1.54 (m, 2H), 1.52-1.34 (m, 5H), 1.31 (s, 3H), 1.29-1.20 (m, 1H), 0.95 (s, 3H), 0.92 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.13, 148.91, 132.74, 128.48, 122.21, 81.13, 75.21, 70.14, 64.81, 57.93, 57.57, 54.45, 51.80, 44.35, 40.85, 40.23, 38.61, 36.35, 35.50, 31.85, 23.16, 22.21, 21.27, 21.16, 20.72, 13.87, 12.62. **HRMS ESI** calc. for $C_{28}H_{41}O_6^+$ $[M+H]^+$: 473.2898, found: 473.2890. **X-ray crystal structure** is given in the appendices section.

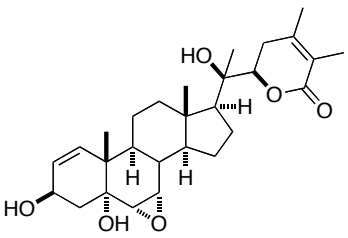
Acetate (2.44):[51] To a solution of allylic alcohol **2.43** (6 mg, 0.013 mmol, 1 eq.) in



CH_2Cl_2 (0.1 mL) was added acetic anhydride (4.8 µL, 0.052 mmol, 4 eq.), DMAP (0.16 mg, 0.001 mmol, 0.1 eq.), and Et_3N (5.4 µL, 0.039 mmol, 3 eq.). The colorless solution was stirred at RT for 45 min, then quenched with 0.1 M HCl and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (pentane/EtOAc, 1:1) affording acetate **2.44** (5.7 mg, 0.011 mmol, 87%) as a colorless solid.

M.p. = 245-246 °C. TLC: $\mathbf{R_f}$ = 0.60 (EtOAc). **Optical rotation**: $[\alpha]_D$ = -4.3° (*c* = 0.29, $CHCl_3$). **FTIR**: ν = 3502, 2922, 2324, 1718, 1462, 1374, 1310, 1248, 1139, 1100, 1015, 970, 891, 813, 754 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.07 (dd, *J* = 10.2, 1.7 Hz, 1H), 5.75-5.69 (m, 1H), 5.45-5.39 (m, 1H), 4.20 (dd, *J* = 13.4, 3.5 Hz, 1H), 3.31 (dd, *J* = 3.8, 2.4 Hz, 1H), 3.08 (d, *J* = 1.6 Hz, 1H), 3.05 (d, *J* = 3.8 Hz, 1H), 2.47-2.30 (m, 3H), 2.10 (s, 3H), 2.19-2.00 (m, 4H), 1.95 (s, 3H), 1.89 (s, 3H), 1.87-1.75 (m, 2H), 1.68-1.51 (m, 2H), 1.42 (m, 5H), 1.32 (s, 3H), 1.26-1.19 (m, 1H), 0.96 (s, 3H), 0.92 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.99, 166.13, 148.87, 136.29, 124.39, 122.22, 81.14, 75.22, 67.89, 65.88, 57.68, 57.26, 54.38, 51.81, 44.37, 40.77, 40.32, 37.15, 36.15, 35.40, 31.91, 23.16, 22.19, 21.67, 21.33, 21.13, 20.71, 20.61, 13.83, 12.62. **HRMS ESI** calc. for $C_{30}H_{43}O_7^+$ $[M+H]^+$: 515.3003, found: 515.2987.

Allylic alcohol (2.45):[51] A stirred mixture of allylic alcohol **2.43** (10.0 mg,



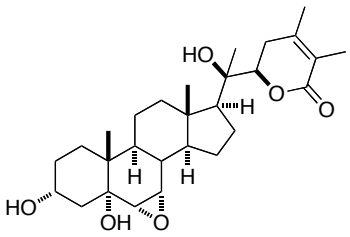
0.021 mmol, 1.0 eq.), 4-nitrobenzoic acid (14.4 mg, 0.085 mmol, 4.0 eq.) and PPh_3 (21.1 mg, 0.080 mmol, 3.8 eq.) in CH_2Cl_2 (0.2 mL) was cooled to 0 °C. To the cold solution was added DEAD (17.1 mg, 0.095 mmol, 4.5 eq.), and the mixture was stirred at RT overnight. The solvent was removed under reduced pressure and the residue subjected to flash column chromatography (pentane/EtOAc, 1:1) to yield the 4-nitrobenzoate (8.7 mg, 0.014 mmol, 66%) as a yellowish solid.

R_f = 0.79 (EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.33-8.17 (m, 5H), 6.03 (dd, *J* = 10.3, 1.8 Hz, 1H), 5.98-5.91 (m, 1H), 5.77–5.70 (m, 1H), 4.21 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.33 (dd, *J* = 3.8, 2.5 Hz, 1H), 3.17 (d, *J* = 1.5 Hz, 1H), 3.08 (d, *J* = 3.8 Hz, 1H), 2.57-2.46 (m, 1H), 2.46-2.31 (m, 2H), 2.28-1.98 (m, 5H), 1.96 (s, 3H), 1.89 (s, 3H), 1.87-1.77 (m, 2H), 1.52-1.34 (m, 6H), 1.32 (s, 3H), 1.12 (s, 3H), 0.97 (s, 3H).

To a solution of 4-nitrobenzoate (8.7 mg, 0.014 mmol, 1 eq.) in THF/CH_2Cl_2 (0.4 mL, 1:1) was added 5% aqueous NaOH solution (0.2 mL) and TBAH solution (7 µL, 1 M in methanol) and the mixture was stirred at RT for 2 h. The mixture was then diluted with CH_2Cl_2 and washed with 1 M HCl and water. The organic phase was dried over Na_2SO_4, evaporated and the residue subjected to flash column chromatography (EtOAc) to yield allylic alcohol **2.45** (5.0 mg, 0.011 mmol, 76%) as a colorless solid.

M.p. = 240 °C (decomposed). **R_f** = 0.27 (pentane/EtOAc, 1:1). **Optical rotation**: $[\alpha]_D$ = +39.1° (*c* = 0.25, $CHCl_3$). **FTIR**: ν = 3475, 2925, 1696, 1453, 1384, 1139, 1098, 1061, 1022, 923, 817, 763, 610 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.85 (dd, *J* = 10.3, 1.8 Hz, 1H), 5.67 (ddd, *J* = 10.1, 2.8, 1.1 Hz, 1H), 4.75-4.63 (m, 1H), 4.20 (dd, *J* = 13.4, 3.5 Hz, 1H), 3.30 (dd, *J* = 3.8, 2.5 Hz, 1H), 3.10 (d, *J* = 1.6 Hz, 1H), 3.06 (d, *J* = 3.9 Hz, 1H), 2.46-2.24 (m, 3H), 2.19-1.98 (m, 4H), 1.95 (s, 3H), 1.89 (s, 3H), 1.86-1.72 (m, 3H), 1.68-1.52 (m, 3H), 1.50-1.33 (m, 5H), 1.31 (s, 3H), 1.05 (s, 3H), 0.95 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.15, 148.91, 133.69, 128.84, 122.21, 81.15, 75.22, 71.52, 66.75, 57.92, 57.73, 54.43, 51.79, 44.42, 41.01, 40.28, 39.87, 36.02, 35.34, 31.88, 23.13, 22.71, 22.19, 21.29, 20.75, 20.71, 13.87, 12.62. **HRMS ESI** calc. for $C_{28}H_{41}O_6^+$ $[M+H]^+$: 473.2898, found: 473.2885.

Alcohol (2.47):[51] Allylic alcohol **2.43** (5.0 mg, 0.011 mmol, 1.0 eq.) and Crabtree's catalyst (0.17 mg, 2 mol%) were dissolved in CH_2Cl_2 (0.1 mL) in a small vial. The vial was placed in an autoclave and the mixture was stirred at RT for 2.5 h under a H_2 atmosphere (10 bar). The solvent was removed, the residue filtered through a short pad of silica (EtOAc) and evaporated to yield alcohol **2.47** (5.0 mg, 0.011 mmol, quant.) as a colorless solid.

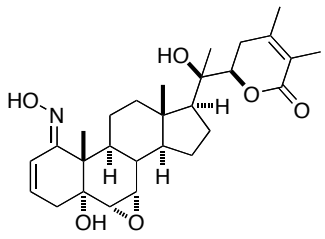


M.p. = 205-206 °C. TLC: $\mathbf{R}_f$ = 0.52 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +19.1° (*c* = 0.36, $CHCl_3$). **FTIR**: ν = 3475, 2922, 1705, 1382, 1142, 1102, 1024, 895, 855, 811, 759, 652 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.53 (d, *J* = 9.7 Hz, 1H), 4.21 (d, *J* = 9.8 Hz, 1H), 4.04-3.93 (m, 1H), 3.35 (s, 1H), 3.31-3.24 (m, 1H), 2.95 (d, *J* = 3.8 Hz, 1H), 2.46-2.31 (m, 2H), 2.17 (s, 1H), 2.14-1.97 (m, 3H), 1.95 (s, 3H), 1.92-1.90 (m, 1H), 1.89 (s, 3H), 1.85-1.65 (m, 5H), 1.50 (t, *J* = 9.5 Hz, 1H), 1.44-1.33 (m, 3H), 1.30 (s, 3H), 1.28-1.04 (m, 5H), 0.92 (s, 3H), 0.81 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.15, 148.94, 122.19, 81.10, 75.23, 71.60, 67.11, 58.07, 57.92, 54.48, 51.83, 44.31, 40.25, 38.44, 38.23, 37.94, 35.15, 31.78, 29.25, 24.29, 23.08, 22.22, 21.16, 20.72, 20.18, 14.56, 13.87, 12.62. **HRMS ESI** calc. for $C_{28}H_{43}O_6^+$ $[M+H]^+$: 475.3054, found: 475.3042.

General Procedure A for oxime ethers 2.48-2.52

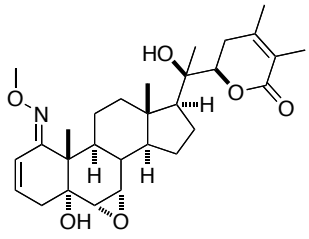
To a solution of withanolide A (1 eq.) in pyridine was added the hydroxylamine hydrochloride (2 eq.) and the mixture was heated at 70 °C for the indicated time. The solvent was removed under high vacuum, and the residue dissolved in CH_2Cl_2. Saturated aqueous NH_4Cl solution was added and the aqueous phase was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, evaporated, and the residue subjected to flash column chromatography to yield oxime ethers **2.48-2.52** as colorless solids.

Hydroxylamine (2.48):[51] Following General Procedure A: Withanolide A (20 mg, 0.04 mmol, 1 eq.) in pyridine (0.50 mL), hydroxylamine hydrochloride (5.9 mg, 0.08 mmol, 2 eq.), 70 °C for 20 h. Additional hydroxylamine hydrochloride (3.0 mg, 0.04 mmol, 1 eq.), 70 °C for 5 h, flash column chromatography (pentane/EtOAc, 1:1), 58% yield.



M.p. = 180-181 °C. $\mathbf{R}_f$ = 0.55 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +175.0° (c = 0.69, $CHCl_3$). **FTIR**: ν = 3417, 2922, 2360, 1691, 1382, 1290, 1023, 907, 815, 750, 645 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.19 (s, 1H), 6.75 (ddd, *J* = 10.3, 2.9, 1.2 Hz, 1H), 6.02 (ddd, *J* = 10.3, 4.7, 2.5 Hz, 1H), 4.21 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.30 (dd, *J* = 4.0, 2.1 Hz, 1H), 3.01 (d, *J* = 3.9 Hz, 1H), 2.98 (d, *J* = 1.5 Hz, 1H), 2.73-2.66 (m, 1H), 2.53-2.33 (m, 4H), 2.17-2.08 (m, 1H), 2.03-1.97 (m, 1H), 1.95 (s, 3H), 1.88 (s, 3H), 1.87-1.77 (m, 2H), 1.64-1.32 (m, 8H), 1.30 (s, 3H), 1.08 (s, 3H), 0.96 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.21, 159.55, 149.01, 131.00, 122.12, 117.81, 81.14, 75.24, 71.42, 57.43, 56.82, 54.61, 52.39, 45.37, 44.02, 40.52, 37.42, 36.70, 35.43, 31.79, 23.33, 22.85, 22.07, 21.15, 20.72, 15.98, 14.07, 12.61. **HRMS ESI** calc. for $C_{28}H_{40}NO_6^+$ $[M+H]^+$: 486.2850, found: 486.2839.

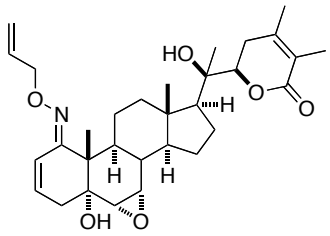
Methyloxime (2.49):[51] Following General Procedure A: Withanolide A (10 mg, 0.02 mmol, 1 eq.) in pyridine (0.25 mL), methoxylamine hydrochloride (3.6 mg, 0.04 mmol, 2 eq.), 70 °C for 3 h. Additional methoxylamine hydrochloride (1.8 mg, 0.02 mmol, 1 eq.), 70 °C for 2 d, flash column chromatography (pentane/EtOAc, 1:1), 58% yield (as an inseparable diastereomeric mixture).



M.p. = 157-158 °C. $\mathbf{R}_f$ = 0.81 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +217.1° (*c* = 0.18, $CHCl_3$). **FTIR**: ν = 3500, 2937, 2325, 1702, 1463, 1381, 1289, 1187, 1130, 1046, 896, 829, 733, 683, 614 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.67-6.60 (m, 1H), 6.01-5.94 (m, 1H), 4.21 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.87 and 3.84 (s, 3H), 3.30 (dd, *J* = 4.0, 2.1 Hz, 1H), 3.00 (d, *J* = 3.9 Hz, 1H), 2.95 (d, *J* = 1.5 Hz, 1H), 2.93-2.85 (m,

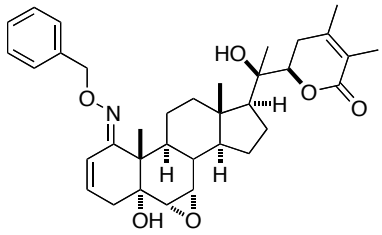
1H), 2.51-2.30 (m, 4H), 2.17-1.98 (m, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.87-1.77 (m, 1H), 1.67-1.34 (m, 8H), 1.32 (s, 3H), 1.12 and 1.07 (s, 3H, diastereomers), 0.97 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.19, 157.97, 148.89, 130.78, 122.19, 118.30, 81.17, 75.25, 71.31, 61.81, 57.46, 56.86, 54.60, 52.36, 45.19, 44.02, 40.63, 37.41, 36.74, 35.41, 31.87, 23.38, 22.74, 22.02, 21.24, 20.71, 16.10, 14.00, 12.62. **HRMS ESI** calc. for $C_{29}H_{42}NO_6^+$ $[M+H]^+$: 500.3007, found: 500.2996.

Allyloxime (2.50): Following General Procedure A: Withanolide A (50 mg, 0.11 mmol, 1 eq.) in pyridine (1.25 mL), *O*-allylhydroxylamine hydrochloride (23.3 mg, 0.21 mmol, 2 eq.), 70 °C for 14 h. Additional *O*-allylhydroxylamine hydrochloride (11.7 mg, 0.11 mmol, 1 eq.), 70 °C for 14 h, flash column chromatography (pentane/EtOAc, 1:1), 72% yield.



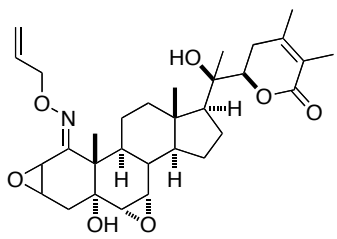
M.p. = 117-118 °C. $\mathbf{R_f}$ = 0.75 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +218.3° (*c* = 0.24, $CHCl_3$). **FTIR**: ν = 3506, 2925, 1702, 1382, 1289, 1131, 1097, 1025, 906, 826, 664 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.73-6.62 (m, 1H), 6.03-5.90 (m, 2H), 5.27-5.20 (m, 1H), 5.17-5.11 (m, 1H), 4.61-4.45 (m, 2H), 4.20 (dd, *J* = 13.3, 3.5 Hz, 1H), 3.29 (dd, *J* = 4.0, 2.1 Hz, 1H), 2.99 (d, *J* = 3.9 Hz, 1H), 2.94 (s, 1H), 2.89-2.79 (m, 1H), 2.51-2.28 (m, 4H), 2.18-1.97 (m, 4H), 1.94 (s, 3H), 1.88 (s, 3H), 1.88-1.74 (m, 2H), 1.60-1.38 (m, 5H), 1.31 (s, 3H), 1.29-1.23 (m, 1H), 1.06 (s, 3H), 0.96 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.16, 158.08, 148.88, 134.84, 130.73, 122.13, 118.43, 117.22, 81.15, 75.20, 74.86, 71.29, 57.43, 56.82, 54.55, 52.35, 45.27, 44.01, 40.57, 37.37, 36.74, 35.38, 31.83, 23.34, 22.74, 21.99, 21.21, 20.68, 16.07, 13.97, 12.59. **HRMS ESI** calc. for $C_{31}H_{44}NO_6^+$ $[M+H]^+$: 526.3163, found: 526.3148.

Benzyloxime (2.51):[51] Following General Procedure A: Withanolide A **1** (10 mg, 0.02 mmol, 1 eq.) in pyridine (0.25 mL), *O*-benzylhydroxylamine hydrochloride (6.8 mg, 0.04 mmol, 2 eq.), 70 °C for 14 h. Additional *O*-benzylhydroxylamine hydrochloride (3.4 mg, 0.02 mmol, 1 eq.), 70 °C for 14 h, flash column chromatography (pentane/EtOAc, 2:1), 63% yield (as an inseparable diastereomeric mixture).



M.p. = 106-107 °C. $\mathbf{R}_f$ = 0.79 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +174.0° (*c* = 0.37, $CHCl_3$). **FTIR**: ν = 3485, 2922, 2361, 1703, 1455, 1381, 1289, 1212, 1131, 1095, 1019, 906, 815, 753, 698, 613 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39-7.27 (m, 5H), 6.75-6.68 (m, 1H), 6.01-5.93 (m, 1H), 5.16-5.01 (m, 2H), 4.26-4.17 (m, 1H), 3.32-3.27 (m, 1H), 3.00 (d, *J* = 3.9 Hz, 1H), 2.95 (d, *J* = 1.4 Hz, 1H), 2.84-2.77 (m, 1H), 2.52-2.29 (m, 4H), 2.17-1.98 (m, 3H), 1.96 (s, 3H), 1.90 (s, 3H), 1.85-1.74 (m, 2H), 1.63-1.36 (m, 6H), 1.33 (s, 3H), 1.32-1.29 (m, 1H), 1.08 and 1.05 (s, 3H, diastereomers), 0.96 and 0.95 (s, 3H, diastereomers). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.18, 158.33, 148.90, 138.50, 130.76, 128.33, 128.30, 127.65, 122.20, 118.64, 81.18, 75.99, 75.26, 71.35, 57.46, 56.86, 54.60, 52.40, 45.33, 44.06, 40.63, 37.41, 36.77, 35.43, 31.88, 23.36, 22.77, 22.04, 21.25, 20.72, 16.10, 14.02, 12.64. **HRMS ESI** calc. for $C_{35}H_{46}NO_6^+$ $[M+H]^+$: 576.3320, found: 576.3323.

Epoxy allyloxime (2.52):[51] To a solution of epoxy ketone **2.39** (5 mg, 0.01 mmol, 1 eq.) in pyridine (0.15 mL) was added O-allylhydroxylamine hydrochloride (2.3 mg, 0.02 mmol, 2 eq.) and the mixture was heated at 70 °C for 7 h. The solvent was removed under high vacuum, and the residue dissolved in CH_2Cl_2. Saturated NH_4Cl solution was added and the aqueous phase was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, evaporated, and the residue subjected to flash column chromatography (pentane:EtOAc, 4:6) to yield the allylhydroxylamie **2.52** (2.7 mg, 0.005 mmol, 49%) as a colorless solid.

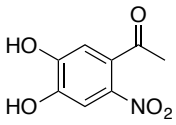


M.p. = 109-110°C. $\mathbf{R_f}$ = 0.54 (EtOAc). **Optical rotation**: $[\alpha]_D$ = +126.7° (*c* = 0.16, $CHCl_3$). **FTIR**: ν = 3495, 2922, 2362, 1704, 1462, 1383, 1315, 1261, 1096, 1006, 919, 805, 754, 665 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.08-5.89 (m, 1H), 5.33-5.23 (m, 1H), 5.22-5.14 (m, 1H), 4.67-4.56 (m, 2H), 4.20 (dd, *J* = 13.3, 3.7 Hz, 1H), 3.93 (d, *J* = 3.7 Hz, 1H), 3.51 (t, *J* = 3.2 Hz, 1H), 3.24-3.10 (m, 1H), 2.94 (s, 1H), 2.90 (d, *J* = 3.8 Hz, 1H), 2.63 (d, *J* = 11.6 Hz, 1H), 2.47-2.30 (m, 2H), 2.22-2.00 (m, 5H), 1.95 (s, 3H), 1.89 (s, 3H), 1.76-1.63 (m, 3H), 1.46 (d, *J* = 19.6 Hz, 6H), 1.32 (s, 3H), 1.04 (s, 3H), 0.96 and 0.92 (s, 3H, diastereomers). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.05, 155.33, 148.77, 134.37, 122.04, 117.63, 81.03, 75.29 and 75.11 (diastereomers), 70.35, 56.28, 55.69, 54.36, 52.17, 51.86, 46.97, 46.03, 43.87 and 43.79 (diastereomers), 40.22 and 40.07 (diastereomers), 35.74, 35.70, 35.30, 33.85, 31.74, 29.70, 23.21, 21.96 and 21.87 (diastereomers), 21.17, 20.58, 15.40, 13.75, 12.48. **HRMS ESI** calc. for $C_{31}H_{44}NO_7^+$ $[M+H]^+$: 542.3112, found: 542.3099.

Neurotogenic properties: SH-SY5Y cells (obtained from DSMZ, Germany) were cultured in minimal essential medium (MEM without phenol red (GIBCO, Invitrogen)) containing 5% fetal bovine serum (FBS, GIBCO, Invitrogen) with and Ala-Gln (406 mg/L, BioReagent, Sigma). All experiments were conducted under strictly sterile conditions and exclusion of light. Passage numbers 3 to 9 were used for the experiments. Cells were plated either on collagen coated 24-well plates (Iwaki, Asahi Glass Co., Japan). After incubating in the dark for three days at 37 °C in humidified atmosphere (CO_2 content 5%) in the presence of the compounds, e.g. withanolide A (0.1 or 1 µM), vehicle solution (DMSO, 0.1%, negative control) or ATRA (1 µM, positive control, Sigma-Aldrich), cells were examined under a phase contrast microscope (Leica, Germany). Cells were examined in three randomly chosen areas per well. More than 500 cells were examined and ranked positive, if they possess a neurite with more than 50 µM length (double cell diameter). Large cell aggregates were not counted. The ratio of neurite positive cells to total cells was calculated. Error bars are given as SEM.

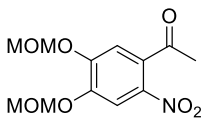
8.4 Release on Demand: Nitrocatechols

1-(4,5-dihydroxy-2-nitrophenyl)ethanone (3.7): Sublimed $AlCl_3$ (3.65 g, 27.38 mmol, 3 eq.) was added to 10 mL of cold (-5 °C), dry 1,2-dichloroethane. Then a solution of methylen protected nitroactophenone[81] (1.91 g, 9.13 mmol, 1 eq.) in 1,2-dichloroethane (9 mL) was added dropwise. The reaction was stirred at -5 to +5 °C for 1 h, then poured into cold HBr (48%; 25 mL) and stirred at RT over night. The reaction mixture was diluted with water, extracted with Et_2O and dried over Na_2SO_4. The solvent was evaporated to yield **3.7** as a green brown adhesive solid (1.86 g, 9.41 mmol, 84 %).



M.p. = 148-149 °C. $\mathbf{R_f}$ = 0.1 (pentane: Et_2O 2:1). **FTIR**: ν = 3405, 3121, 2802, 2662, 1669, 1593, 1503, 1441, 1291, 1189, 1107, 1011, 964, 893, 798, 754, 670 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 10.75 (s, 1H), 10.44 (s, 1H), 7.45 (s, 1H), 6.87 (s, 1H), 2.41 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 199.32, 151.39, 146.64, 137.27, 130.42, 113.68, 111.09, 29.88. **HRMS ESI** calc. for $C_8H_7O_5NNa^+$ $[M\text{-}Na]^+$: 220.0216, found: 220.0216.

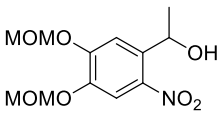
MOM protected catechol (3.7b): To a stirred solution of catechol **3.7** (1.325 g, 6.72 mmol, 1.0 eq.) and K_2CO_3 (4.644 g, 33.6 mmol, 5.0 eq.) in CH_3CN (50 mL) at 0 °C was dropwisely added MOMCl (1.69 mL, 22.2 mmol, 3.3 eq.). After 5 minutes the cooling bath was removed and stirring was continued at RT for 3 h. The brown mixture was diluted with Et_2O, washed with water and brine and dried over Na_2SO_4. The solvent was evaporated and the resulting solid triturated in pentane to afford **3.7b** (1.658 g, 5.81 mmol, 87%) as a pale yellow solid.



M.p. = 84-85 °C. $\mathbf{R_f}$ = 0.4 (pentane/Et_2O 1:1). **FTIR**: ν = 2937, 2832, 2307, 1701, 1575, 1518, 1332, 1272, 1153, 1080, 989, 917, 785, 618 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.91 (s, 1H), 7.11 (s, 1H), 5.32 (s, 2H), 5.32 (s, 2H), 3.53 (s, 3H), 3.52 (s, 3H), 2.50 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 199.51, 152.05, 147.46, 139.45,

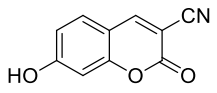
133.48, 113.10, 112.01, 95.48, 95.32, 56.79, 56.66, 30.30. **Elementary Analysis** calc. for $C_{12}H_{15}NO_7$: C = 50.53, H = 5.30, N = 4.91, found: C = 50.66, H = 5.13, N = 5.09.

Benzylic alcohol (3.8): To a stirred solution of acetophenone **3.7b** (255.0 mg, 0.894 mmol, 1.0 eq.) in methanol (2.5 mL) at 0 °C was added $NaBH_4$ (33.8 mg, 0.894 mmol, 1.0 eq.) and after 5 minutes at 0 °C stirring was continued at RT for 3.5 h. The mixture was quenched with a saturated, aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford alcohol **3.8** (254.6 mg, 0.886 mmol, 99%) as a pale yellow solid.



M.p. = 67-68 °C. **FTIR**: ν = 3284, 2932, 2829, 2072, 1577, 1515, 1328, 1262, 1151, 1080, 995, 947, 916, 845, 795, 715 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.84 (s, 1H), 7.56 (s, 1H), 5.48 (q, *J* = 6.3 Hz, 1H), 5.34 (s, 2H), 5.28 (s, 2H), 3.52 (s, 6H), 1.55 (d, *J* = 6.3 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 151.95, 145.71, 141.03, 137.49, 113.38, 113.13, 95.64, 95.20, 65.72, 56.81, 56.62, 24.25. **HRMS ESI** calc. for $C_{12}H_{17}O_7NNa^+$ $[M\text{-}Na]^+$: 310.0897, found: 310.0892.

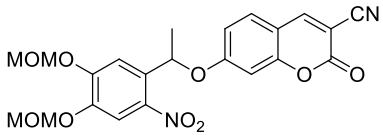
3-Cyano-7-hydroxycoumarin (3.9):[62] A mixture of 2,4-dihydroxybenzaldehyde (2.072 g, 15.0 mmol, 1.0 eq.), malononitrile (1.239 g, 18.8 mmol, 1.3 eq.) and 0.05 M aqueous $NaHCO_3$ (75 mL) was stirred at RT for 5 h. Conc. HCl (2.8 mL) was added and the mixture was heated to 90 °C for 2 h. After cooling to RT the resulting precipitate was filtered off, washed with water and dried *in vacuo* to afford coumarin **3.9** (2.397 g, 12.8 mmol, 85%) as an orange-red solid.



M.p. = 257-260 °C (lit. 273-275 °C). **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 11.33 (s, 1H), 8.79 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 6.90 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.79 (d, *J* = 2.2 Hz, 1H).

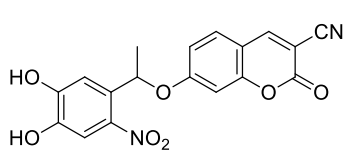
Analytical data are in agreement with the reported literature: F. Fringuelli, O. Piermatti, F. Pizzo, *Synthesis* **2003**, *15*, 2331-2334.

Benzylic ether (3.4a):[62] To a stirred solution of alcohol **3.8** (100 mg, 0.348 mmol, 1 eq.), coumarin **3.9** (130 mg, 0.696 mmol, 2.0 eq.) and PPh_3 (182 mg, 0.696 mmol, 2 eq.) in THF (2.0 mL) at 0 °C and under exclusion of light was slowly added DIAD (138 µL, 0.696 mmol, 2 eq.). After 5 min the cooling bath was removed and stirring was continued at RT overnight. The reaction mixture was concentrated under reduced pressure and the oily resin was subjected to repeated flash chromatography (CH_2Cl_2:MeOH 200:1 to pentane:EtOAc 3:1 to 1:1) to afford ether **3.4a** (65 mg, 0.142 mmol, 41 %) as a yellow oil.

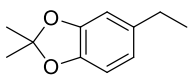


R_f = 0.5 (pentane/EtOAc 1:1). **FTIR**: ν = 2982, 2940, 1733, 1688, 1605, 1519, 1374, 1337, 1246, 1153, 1107, 1092, 1049, 1002, 923, 853, 793, 760, 639, 609 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.12 (s, 1H), 7.97 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.36 (s, 1H), 6.91 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.73 (d, *J* = 2.2 Hz, 1H), 6.27 (q, *J* = 6.2 Hz, 1H), 5.30-5.17 (m, 4H), 3.52 (s, 3H), 3.39 (s, 3H), 1.75 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 163.68, 157.00, 156.80, 152.47, 151.39, 146.47, 140.64, 133.14, 130.77, 114.89, 114.08, 113.32, 112.77, 111.49, 103.66, 99.42, 95.62, 95.26, 73.10, 56.80, 56.71, 23.28. **HRMS ESI** calc. for $C_{22}H_{21}O_9N_2^+$ $[M-H]^+$: 457.1242, found: 457.1231.

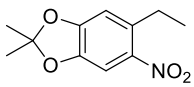
Catechol (3.4):[62] To a stirred solution of ether **3.4a** (24 mg, 52.6 µmol) in $CHCl_3$ (0.5 mL) was added TFA (0.15 mL) at 0 °C and the mixture was stirred overnight at RT under exclusion of light. Volatiles were removed under reduced pressure and the residue was subjected to preparative reversed-phase HPLC (CH_3CN:H_2O 5% to 100%, over 33 min, R_t = 17.2 min) to afford catechol **3.4** (9 mg, 24.4 µmol, 46%).



M.p. = 109-111 °C. **FTIR**: ν = 3354, 2238, 1713, 1599, 1525, 1504, 1435, 1376, 1329, 1290, 1245, 1156, 1133, 1077, 1045, 1000, 892, 860, 806, 762. **^{1}H NMR** (400 MHz, DMSO-d_6) d = 8.79 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.57 (s, 1H), 6.99 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.93 (s, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.17 (q, *J* = 6.1 Hz, 1H), 1.64 (d, *J* = 6.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) d = 162.88, 157.60, 156.14, 153.04, 152.25, 144.95, 137.97, 131.83, 130.62, 114.98, 114.41, 112.38, 112.27, 111.66, 102.61, 97.99, 72.39, 22.82. **UPLC-MS ESI** for $C_{18}H_{13}O_7N_2^+$ $[M-H]^+$: 369.1, found: 369.1.

Acetonide (3.15b):[62] A solution of ethylcatechol **3.15** (0.993 g, 7.19 mmol, 1.0 eq.),  2,2-dimethoxypropane (1.78 mL, 14.5 mmol, 2.0 eq.) and *p*-toluene-sulfonic acid (14 mg, 72.4 µmol, 1 mol %) in benzene (26 mL) was refluxed overnight. After cooling to RT the solution was poured into saturated aqueous $NaHCO_3$ solution followed by extraction with Et_2O. The combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated to afford crude acetonide **3.15b** (1.679 g) as a yellowish oil which could be used without further purification. To obtain an analytical sample of **3.15b** the crude product was subjected to flash chromatography (pentane + 1% Et_3N).

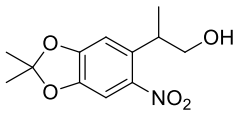
R_f = 0.6 (pentane). **FTIR**: ν = 2965, 2934, 2873, 1496, 1445, 1377, 1228, 1156, 982, 837, 808, 658, 627 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.65-6.58 (m, 3H), 2.56 (q, *J* = 7.6 Hz, 2H), 1.66 (s, 6H), 1.20 (t, *J* = 7.6 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 147.49, 145.43, 137.68, 119.87, 117.56, 108.36, 108.01, 28.80, 25.99, 16.14.

Acetonide (3.16):[62] Crude acetonide **3.15b** (ca. 1.7 g) was cooled to 0 °C and slowly  added a cold solution of conc. HNO_3 (4.18 mL) in H_2O (4.18 mL) resulting in a brown mixture. The cooling bath was removed after 5 min and stirring was continued at RT for 1.5 h. The mixture was cautiously poured into saturated aqueous $NaHCO_3$ solution followed by extraction with Et_2O. The combined organic extracts were washed with water, dried over Na_2SO_4 and

evaporated to afford nitroaryl compound **3.16** (1.381 g, 6.18 mmol, 86% over 2 steps) as a yellow solid.

M.p. = 56-59 °C. $\mathbf{R_f}$ = 0.3 (pentane). **FTIR**: ν = 2988, 2938, 2877, 1615, 1519, 1492, 1416, 1379, 1331, 1250, 1215, 981, 876, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39 (s, 1H), 6.64 (s, 1H), 2.88 (q, *J* = 7.4 Hz, 2H), 1.71 (s, 6H), 1.26 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 151.76, 145.98, 142.30, 136.26, 120.81, 109.72, 105.59, 27.23, 26.03, 15.18. **HRMS ESI** calc. for $C_{11}H_{13}O_4NNa^+$ $[M\text{-}Na]^+$: 246.0737, found: 246.0738.

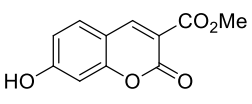
Alcohol (3.17):[62] Acetonide **3.16** (0.700 g, 3.14 mmol, 1.0 eq.) and paraformaldehyde



(1.883 g, 62.7 mmol, 20 eq.) were dissolved in benzyltrimethylammonium hydroxide (6.41 mL, 14.1 mmol, 4.5 eq., 40 wt. % solution in MeOH) and the mixture was heated to 85 °C for 65 h in a sealed flask. After cooling to RT the mixture was poured into 0.1 M aqueous HCl followed by extraction with CH_2Cl_2. The combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated. The residue was subjected to flash chromatography (pentane/EtOAc 3:2 to 1:1, 1% Et_3N) to afford alcohol **3.17** (324 mg, 1.28 mmol, 41%, 80% *brsm*) as a yellow oil.

$\mathbf{R_f}$ = 0.3 (pentane/EtOAc 3:2). **FTIR**: ν = 3335, 2988, 2927, 2878, 1519, 1493, 1379, 1335, 1256, 1214, 1033, 978, 872, 822, 759 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.24 (s, 1H), 6.44 (s, 1H), 3.81-3.70 (m, 2H), 3.67-3.59 (m, 1H), 1.71 (s, 6H), 1.27 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 151.68, 146.12, 143.94, 134.86, 121.03, 106.67, 105.31, 68.20, 36.55, 26.10, 17.84. **HRMS ESI** calc. for $C_{12}H_{15}O_5NNa^+$ $[M\text{-}Na]^+$: 276.0842, found: 276.0842.

Methyl 7-hydroxycoumarin-3-carboxylate (3.18):[62] A solution of 2,4-dihydroxy-



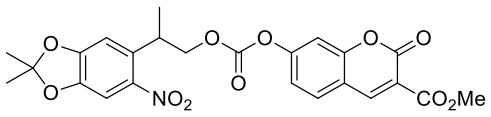
benzaldehyde (4.000 g, 29.0 mmol, 1.0 eq.), dimethyl malonate (5.000 g, 37.9 mmol, 1.3 eq.) and piperidine (0.7 mL, 7.03 mmol, 0.2 eq.) in methanol (25 mL) was heated at 90 °C. After 6 h the mixture

was allowed to cool to RT, the yellow precipitate was filtered off, washed with water and dried *in vacuo* to afford coumarin **3.18** (3.101 g, 14.1 mmol, 49%) as a yellow solid.

M.p. = 255-260 °C. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 11.09 (s, 1H), 8.69 (s, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 6.84 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.72 (d, *J* = 2.1 Hz, 1H), 3.79 (s, 3H).

Analytical data are in agreement with the reported literature: H. Valizadeh, S. Vaghefi, *Syn. Comm*. **2009**, *39*, 1666-1678.

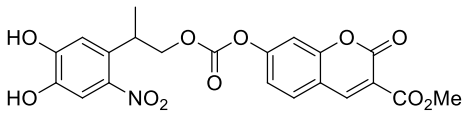
Carbonate (3.5a):[62] A solution of triphosgene (183 mg, 0.616 mmol, 1.4 eq.) in THF (0.82 mL) was cooled to 0 °C and a solution of alcohol **3.17** (130 mg, 0.513 mmol, 1.2 eq.) and Et_3N (71 µL, 0.513 mmol, 1.2 eq.) in THF (0.82 mL) dropwisely added. After stirring for 25 min at 0 °C, the mixture was filtered and volatiles were removed *in vacuo* without heating to afford the desired chloroformate in approximately quantitative yield (as indicated by ^{1}H NMR spectroscopy, see **8.4**).



Chloroformate was redissolved in CH_2Cl_2 (1.3 mL) and slowly added to a solution of coumarin **3.18** (94 mg, 0.428 mmol, 1.0 eq.) in pyridine (1.3 mL) at 0 °C. After 20 min the ice bath was removed and stirring was continued for another 60 min. The mixture was poured into water and subsequently extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and evaporated to afford crude carbonate **3.5a** (300 mg, contaminated mainly with pyridine) as a brown oil which was used without further purification. Exposure to light was avoided as much as possible during the entire procedure.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.56 (s, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.29 (s, 1H), 7.23 (d, *J* = 2.0 Hz, 1H), 7.19 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.77 (s, 1H), 4.48-4.34 (m, 2H), 3.99-3.91 (m, 1H), 3.96 (s, 3H), 1.73 (s, 3H), 1.72 (s, 3H), 1.37 (d, *J* = 7.0 Hz, 3H).

Catechol (3.5):[62] Crude carbonate **3.5a** (ca. 300 mg) was added TFA (3.0 mL) and the mixture was stirred at rt under the exclusion of light. Since after 14 h ^{1}H NMR analysis did not show complete conversion of the starting material, water (0.6 mL) and TFA (1.5 mL) was added and stirring was continued for another 24 h so ^{1}H NMR indicated full conversion. Volatiles were removed *in vacuo* without heating and the residue was subjected to preparative, reversed-phase HPLC (CH_3CN 1% to 50%, 15 min; 50% to 100%, 3.6 min) to afford nitrocatechol **3.5** (145 mg, 0.290 mmol, 68% over 2 steps) as a yellow solid.



M.p. = >65 °C (decomposed). **FTIR**: ν = 3367, 3090, 2959, 1754, 1616, 1525, 1289, 1206, 1166, 1027, 957, 800, 773 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 10.31 (s, 1H), 9.98 (s, 1H), 8.81 (s, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.38 (s, 1H), 7.27 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.93 (s, 1H), 4.42-4.31 (m, 2H), 3.86-3.77 (m, 1H), 3.83 (s, 3H), 1.27 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ = 163.04, 155.68, 155.27, 154.68, 151.96, 151.11, 148.59, 144.02, 140.47, 131.53, 130.05, 118.40, 116.75, 116.02, 114.27, 111.98, 109.39, 72.64, 52.47, 32.24, 17.52. **HRMS ESI** calc. for $C_{21}H_{17}O_{11}NNa^+$ $[M\text{-}Na]^+$: 482.0694, found: 482.0696.

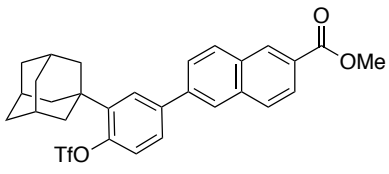
***Surface Modifications*:** Test tubes (5 mL) were cleaned with piranha solution (conc. H_2SO_4 and 30% H_2O_2 3:1), rinsed with Millipore water and oven-dried (120 °C). Portions of TiO_2 particles (10 mg, rutile, 1.0-2.0 μm) were suspended in toluene (3 mL), sonicated for 20 min at 35 °C, centrifuged (4500 rpm, 3 min) and the solvent decanted. This washing was repeated with *i*-PrOH and the particles were oven-dried (120 °C) overnight. To the TiO_2 particles (20 mg) were added MOPS buffer (3 mL) and **3.5** (2.5 mg, dissolved in a few drops of DMSO), followed by sonication for 2 min and subsequent incubation at 50 °C for 4 h under exclusion of light. During incubation TiO_2 particles were re-suspended with a syringe every 15 min. The mixture was centrifuged (4500 rpm, 4 min), the particles were washed with CH_3CN (3 x 3 mL) and MOPS buffer (3 mL), centrifugation after each washing to yield off-white **Ti-3.5**. **Ti-3.5** particles were re-suspended in 6 mL of cell medium (MEM), 1 mL of the yellow suspension was added to each well of a 24-well plate and

irradiated (irradiated surface area = 254.5 mm^3). The complete mixture of a well was taken after the indicated time, filtered and subjected to HPLC analysis, showing the qualitatively release of **3.18** from surface bond **Ti-3.5**.

***Kinetic Measurements*:** Applying a Falcon® 24 well plate, wells were loaded with millipore water (1 mL) and a 1 mM solution of **3.5** in CH_3CN (50 µL) was added. On a sheet of aluminum foil the plate was positioned under a laboratory UV lamp and irradiation at 366 nm was performed with a distance of approximately 3.5 cm between the lamp and the sample solutions. After the indicated irradiation-periode the entire solution of one well at a time was filtered and subjected to UPLC analysis. Substance ratios (between caged **3.5** and the released coumarin **3.18**) were determined by integration of their respective peaks in the UV trace recorded at 254 nm.

8.5 Caged Retinoids as Photoinducible Activators

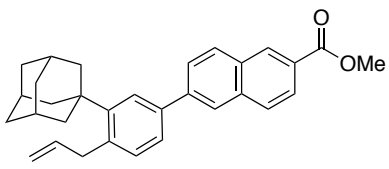
Triflate (4.8aa): To a solution of substituted phenol[94] (825 mg, 2.00 mmol, 1.0 eq.) in CH_2Cl_2 (8.0 mL) was added pyridine (0.49 mL, 6.00 mmol, 3.0 eq.) and DMAP (2.44 mg, 20.0 µmol, 1 mol %). The yellow mixture was cooled to -78 °C followed by dropwise addition of triflic anhydride (0.40 mL, 2.40 mmol, 1.2 eq.). After stirring for 2 h TLC did not show full conversion. Hence, another portion of triflic anhydride (0.2 mL, 1.19 mmol, 0.6 eq.) was added to the red mixture and stirring was continued for 2 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated. The crude material was subjected to flash column chromatography (pentane:CH_2Cl_2 2:3) to afford triflate **4.8aa** as a pale yellow solid (1.014 g, 1.86 mmol, 93%).



R_f = 0.6 (pentane:CH_2Cl_2 2:3). **M.p.** = 186–187 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.64 (s, 1H), 8.11 (dd, *J* = 8.6, 1.7 Hz, 1H), 8.05-8.03 (m, 2H), 7.95 (d, *J* = 8.7 Hz, 1H), 7.77-7.74 (m. 2H), 7.59 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 4.00 (s, 3H), 2.16 (s, 9H), 1.82 (s, 6H).

All analytical data were in agreement with reference: B. Charpentier, J.-M. Bernardon, J. Eustache, C. Millois, B. Martin, S. Michel, B. Shroot, *J. Med. Chem.* **1995**, *38*, 4993-5006.

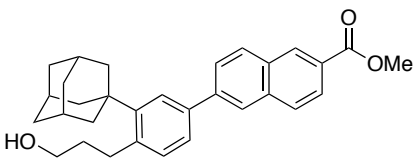
Olefin (4.8a): Allyltributylstannane (500 µL, 1.61 mmol, 1.2 eq.), triflate **4.8aa** (732 mg, 1.34 mmol, 1.0 eq.) and dried LiCl (114 mg, 2.68 mmol, 2.0 eq.) were suspended in DMF (4.6 mL) and DMSO (2 mL) and heated until a clear yellow solution was obtained. The mixture was degassed applying the freeze-thaw technique. $Pd(PPh_3)_2Cl_2$ (18.8 mg, 26.7 µmol, 0.02 eq.) was added and the stirred mixture was heated to 100 °C for 3.5 h. The reaction mixture was poured into ice water followed by extraction with Et_2O. The combined



organic extracts were washed with brine, dried over Na_2SO_4 and evaporated. The solid brown residue was subjected to flash column chromatography (pentane: CH_2Cl_2 3:2) to afford olefin **4.8a** as a colorless solid (583 mg, 1.34 mmol, 99%).

R_f = 0.3 (pentane:CH_2Cl_2 3:2). **M.p.** = 135-136 °C. **FTIR**: ν = 2902, 2678, 1921, 1712, 1632, 1434, 1287, 1219, 1183, 1095, 978, 903, 808, 747 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.63 (s, 1H), 8.08 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.05 (s, 1H), 8.01 (d, *J* = 8.6 Hz, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.82 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.70 (d, *J* = 1.9 Hz, 1H), 7.52 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 6.04 (ddt, *J* = 16.3, 10.2, 6.1 Hz, 1H), 5.13 (dd, *J* = 10.1, 1.6 Hz, 1H), 5.08 (dd, *J* = 17.0, 1.7 Hz, 1H), 4.00 (s, 3H), 3.84 (d, *J* = 6.1 Hz, 1H), 2.17 (s, 9H), 1.82 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.44, 148.43, 141.6, 138.75, 138.29, 138.25, 136.02, 133.47, 131.65, 130.98, 129.89, 128.49, 127.29, 126.71, 125.75, 125.66, 125.42, 124.82, 116.10, 52.36, 42.40, 38.68, 38.17, 37.00, 29.34. **HRMS ESI** calc. for $C_{31}H_{33}O_2$: 437.2470, found: 437.2475.

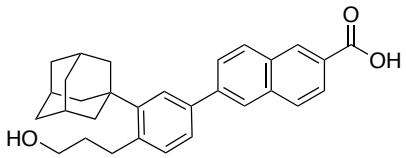
Alcohol (4.8): To a solution of **4.8a** (518 mg, 1.19 mmol, 1.0 eq.) in THF (2.4 mL) at 0 °C was added 9-BBN (0.5 M solution in THF; 4.74 mL, 2.37 mmol, 2.0 eq.). After 1 h the cooling bath was removed and stirring was continued for 2 h. As TLC did not indicate full conversion another portion of 9-BBN (0.5 M solution in THF; 2.00 mL, 1.00 mmol, 0.8 eq.) was added and again stirring was continued for 16 h. The mixture was cooled to 0 °C and subsequently quenched by addition of 1 M aqueous NaOH (3.0 mL) and 30% H_2O_2 (2.4 mL). After 20 min the cooling bath was removed and stirring was continued for 3 h. The mixture was diluted with water and extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated. The crude material was subjected to flash column chromatography (pentane:Et_2O 1:1) to afford alcohol **4.8** as a colorless solid (436 mg, 959 μmol, 81%).



R_f = 0.3 (pentane:Et_2O 1:1). **M.p.** = 175–176 °C. **FTIR**: ν = 3306, 2904, 1717, 1629, 1450, 1281, 1218, 1097, 1056, 991, 916, 882, 814, 751 cm^{-1}. **^{1}H NMR** (400 MHz,

$CDCl_3$) δ = 8.62 (s, 1H), 8.08 (dd, *J* = 8.6, 1.7 Hz, 1H), 8.05 (s, 1H), 8.01 (d, *J* = 8.6 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.82 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.69 (d, *J* = 1.9 Hz, 1H), 7.52 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 3.99 (s, 3H), 3.83 (t, *J* = 6.3 Hz, 2H), 3.13-3.09 (m, 2H), 2.17 (s, 9H), 2.01-1.94 (m, 2H), 1.82 (s, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.45, 148.25, 141.66, 140.86, 137.96, 136.03, 132.79, 131.64, 130.98, 129.89, 128.48, 127.27, 126.70, 125.74, 125.69, 125.38, 124.89, 63.18, 52.37, 42.47, 38.28, 37.01, 36.33, 30.70, 29.38.

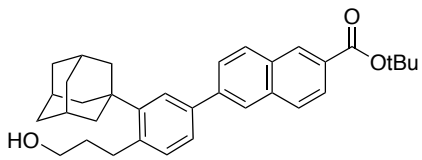
Acid (4.3): To a solution of **4.8** (10 mg, 22.0 µmol, 1.0 eq.) in THF (0.4 mL) was added LiOH (0.1 M solution in H_2O; 0.44 mL, 44.0 µmol, 2.0 eq.) and the resulting clear solution was stirred for 16 h at rt. The mixture was acidified with 0.1 M aqueous HCl to pH ~5 and extracted with Et_2O. The combined organic extracts were thoroughly washed with water, dried over Na_2SO_4 and evaporated to afford acid **4.3** as a colorless solid (10 mg, 22.0 µmol, *quant*.).



R_f < 0.1 (pentane:Et_2O 2:5). **M.p.** = 241-243 °C. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 13.04 (s, 1H), 8.61 (s, 1H), 8.25 (s, 1H), 8.17 (d, *J* = 8.7 Hz, 1H), 8.09 (d, *J* = 8.7 Hz, 1H), 7.99 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.90 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.65 (d, *J* = 1.8 Hz, 1H), 7.59 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 3.55 (m, 2H), 3.02-2.98 (m, 2H), 2.12 (s, 9H), 1.78 (s, 6H).

Analytical data according to afore mentioned reference.

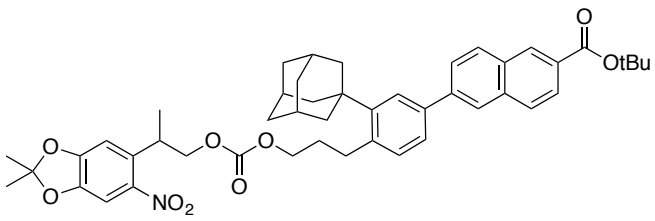
***tert*-Butyl ester (4.10):** A solution of acid **4.3** (220 mg, 0.499 mmol, 1 eq.) and *N,N*-dimethylformamide di-*tert*-butyl acetal (358 µL, 1.498 mmol, 3 eq.) in benzene-THF (1:1, 2 mL) was refluxed for 3 h. The mixture was washed with 5% aqueous Na_2CO_3, dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography



(pentane:Et_2O 1:1) to give ester **4.10** as a colorless solid (57 mg, 0.115 mmol, 23.0 %, respectively 99% based on recovered starting material).

M.p. = 77-78 °C. $\mathbf{R_f}$ = 0.3 (pentane: Et_2O 1:1). **FTIR**: ν = 3412, 2924, 2855, 2361, 1735, 1514, 1461, 1376, 1166, 1033, 824, 765, 622 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.54 (s, 1H), 8.06-8.01 (m, 2H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.80 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.52 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 3.83 (q, *J* = 6.0 Hz, 2H), 3.16-3.06 (m, 2H), 2.17 (s, 9H), 2.03-1.93 (m, 2H), 1.82 (s, 6H), 1.66 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.12, 148.20, 141.30, 140.75, 138.02, 135.81, 132.76, 131.66, 130.54, 129.81, 129.16, 128.22, 126.49, 125.84, 125.64, 125.33, 124.87, 81.27, 63.18, 42.47, 38.28, 37.02, 36.34, 30.70, 29.38, 28.44. **HRMS ESI** calc. for $C_{34}H_{40}O_2Na$: 519.2867, found: 519.2870.

Carbonate (4.4a): A solution of triphosgene (21.3 mg, 0.07 mmol, 1.4 eq.) in THF (0.4 mL) was cooled to 0 °C and a solution of alcohol **3.17** (15.3 mg, 0.06 mmol, 1.2 eq.) and Et_3N (11 µL, 0.07 mmol, 1.2 eq.) in THF (0.3 mL) dropwisely added. After stirring for 25 min at 0 °C, the mixture was filtered over *Celite* and all volatiles were removed *in vacuo* to afford the desired chloroformate in approximately quantitative yield as indicated by ^{1}H NMR spectroscopy.



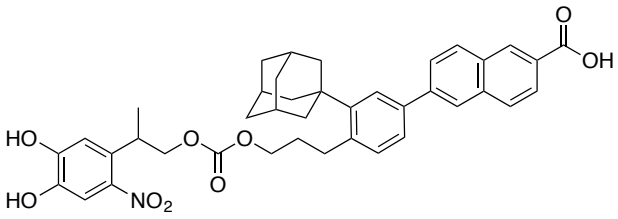
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.30 (s, 1H), 6.74 (s, 1H), 4.44 (d, *J* = 6.1 Hz, 2H), 3.89 (m, 1H), 1.73 (s, 3H), 1.72 (s, 1.72), 1.36 (d, *J* = 7.0 Hz, 3H).

The chloroformate was redissolved in CH_2Cl_2 (0.5 mL) and slowly added to a solution of *tert*-butyl ester **4.10** (25 mg, 0.05 mmol, 1 eq.) in pyridine (0.3 mL) at 0 °C. After 20 min the ice bath was removed and stirring was continued for another 60 min. The mixture was poured into water and subsequently extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and evaporated to afford a

brown oil (28 mg) which was subjected to flash column chromatography (pentane:Et_2O 1:1 + Et_3N) to afford carbonate **4.4a** (24 mg, 0.028 mmol, 58%) as a orange solid with homocoupled nitrocarbonate as an inseparable side-product. Exposure to light was avoided as much as possible during the entire procedure.

R_f = 0.2 (pentane:Et_2O 1:1). **FTIR**: ν = 2910, 2361, 1745, 1629, 1521, 1379, 1257, 1162, 1098, 978, 875, 820 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.55 (s, 1H), 8.06-8.01 (m, 2H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.68 (d, *J* = 1.8 Hz, 1H), 7.52 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.27-7.29 (d and s, 2H), 6.78 (s, 1H), 4.27-4.22 (m, 4H), 3.91-3.79 (m, 1H), 3.14-3.06 (m, 2H), 2.14 (s, 9H), 2.05 (dd, *J* = 10.7, 5.3, 2H), 1.81 (s, 6H), 1.71 (s, 9H), 1.66 (s, 9H), 1.35 (d, *J* = 6.9, 3H). **^{13}C NMR** (63 MHz, $CDCl_3$) δ = 166.10, 155.36, 151.65, 148.27, 146.37, 143.57, 141.26, 139.97, 138.25, 135.81, 133.60, 132.68, 131.69, 130.54, 129.82, 129.22, 128.23, 126.48, 125.86, 125.70, 125.36, 124.93, 121.12, 106.86, 81.24, 68.16, 65.98, 42.44, 38.23, 36.97, 33.58, 32.18, 30.61, 29.84, 29.37, 28.43, 15.41. **HRMS ESI** calc. for $C_{47}H_{53}NO_9Na$ (M-Na^+): 798.3613, found: 798.3627.

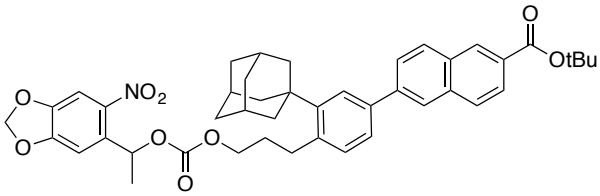
Acid (4.4): To carbonate **4.4a** (23 mg) was added TFA (1.0 mL) and H_2O (0.1 mL) and the mixture was stirred at RT under the exclusion of light for 16 h. Volatiles were removed *in vacuo* without heating and the residue was subjected to preparative, reversed-phase HPLC (CH_3CN/H_2O 1% to 50%, over 15 min, then 50% to 100%, over 3.6 min; without UV detector) to afford nitrocatechol **4.4** (14.5 mg, 0.021 mmol, 42% over two steps) as a yellow solid.



R_f < 0.1 (EtOAc). **FTIR**: ν = 3394, 2907, 2361, 2350, 1630, 1523, 1409, 1265, 1174, 1043, 788 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO) δ = 8.61 (s, 1H), 8.25 (s, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 8.8 Hz, 1H), 7.99 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.90 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.64 (d, *J* = 1.7 Hz, 1H), 7.60 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.38 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 6.89 (s, 1H), 4.17-4.23 (m, 4H), 3.80-3.67 (m, 1H), 2.98-

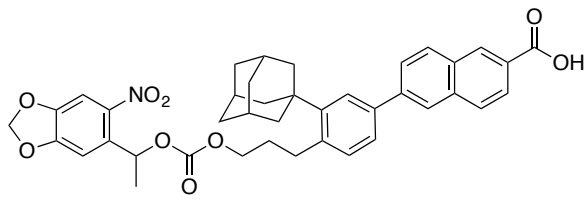
3.03 (m, 2H), 2.07 (s, 9H), 1.91 (bs, 2H), 1.74 (s, 6H), 1.23 (d, *J* = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO) δ = 167.48, 154.54, 151.20, 147.59, 143.94, 140.32 (2), 139.98, 136.95, 135.37, 132.60, 131.19, 130.41, 130.22, 129.88, 128.46, 127.98, 126.02, 125.54, 124.75, 124.68, 124.57, 114.10, 111.91, 71.16, 67.50, 41.57, 37.47, 32.39, 31.63, 29.75, 28.57, 17.71. **HRMS ESI** calc. for $C_{40}H_{41}NO_9Na^+$ ($M\text{-}Na^+$): 702.2674, found: 702.2671.

Carbonate (4.5a): The *tert*-butyl ester **4.10** (20.0 mg, 0.040 mmol, 1 eq.) was dissolved in 0.15 mL pyridine under argon and then cooled to 0 °C. A solution of MeNPOC-Cl (30.9 mg, 0.120 mmol, 3 eq.) in 0.2 mL of dry CH_2Cl_2 was then added dropwise. After 30 min, the solution was allowed to reach rt and stirred for 75 min. Then the solvent was evaporated. The crude material was taken up in Et_2O and washed with saturated aqueous NH_4Cl and water. The organic phase was dried over Na_2SO_4, evaporated and subjected to flash column chromatography (pentane:Et_2O 4:1) to afford **4.5a** as a yellowish solid (28.2 mg, 0.038 mmol, 95%).



M.p. = 80-81 °C. **R_f** = 0.7 (pentane: Et_2O 1:1). **FTIR**: ν = 2907, 2361, 1746, 1708, 1628, 1524, 1484, 1391, 1259, 1162, 1036, 984, 931, 880, 817, 737 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.55 (s, 1H), 8.06-8.02 (m, 2H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.69 (d, *J* = 1.7 Hz, 1H), 7.54-7.50 (m, 2H), 7.28 (d, *J* = 5.1 Hz, 1H), 7.10 (s, 1H), 6.31 (q, *J* = 6.4 Hz, 1H), 6.11 (dd, *J* = 7.1, 1.0 Hz, 2H), 4.32-4.18 (m, 2H), 3.13-3.05 (m, 2H), 2.16 (s, 9H), 2.09-1.99 (m, 2H), 1.83 (s, 6H), 1.68 (d, *J* = 1.6 Hz, 3H), 1.66 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.08, 154.33, 152.62, 148.24, 147.47, 141.75, 141.19, 139.82, 138.27, 135.78, 135.18, 132.61, 131.67, 130.53, 129.83, 129.21, 128.21, 126.45, 125.86, 125.72, 125.35, 124.93, 105.90, 105.38, 103.23, 81.26, 72.14, 68.22, 42.43, 38.22, 36.98, 32.10, 30.52, 29.36, 28.43, 22.26. **HRMS ESI** calc. for $C_{44}H_{47}NO_9^+$ $(M\text{-}Na)^+$: 756.3143, found: 756.3123.

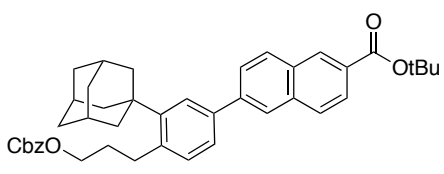
Acid (4.5): Carbonate **4.5a** (28.2 mg, 0.038 mmol, 1 eq.) was added into a mixture of



20% TFA, 5% TIPS in CH_2Cl_2 (0.4 mL) and stirred at RT for 2 h. The solvent was evaporated and the residue purified by flash column chromatography (pentane: Et_2O 1:1) to give **4.5** as a yellowish solid (25.5 mg, 0.038 mmol, 99 %).

M.p. = 127 °C (decomposed*)*. $\mathbf{R_f}$ = 0.2 (pentane: Et_2O 1:1). **FTIR**: ν = 3417, 2905, 2851, 2656, 2565, 2325, 2111, 1994, 1745, 1682, 1628, 1523, 1483, 1423, 1392, 1337, 1257, 1224, 1185, 1142, 1064, 1034, 983, 930, 877, 815, 758, 726, 684, 613 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 13.05 (s, 1H), 8.61 (s, 1H), 8.26 (s, 1H), 8.18 (d, *J* = 8.5 Hz, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.99 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.90 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.65 (d, *J* = 2.1 Hz, 1H), 7.63-7.57 (m, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 6.24 (d, *J* = 5.3 Hz, 2H), 6.04 (q, *J* = 6.4 Hz, 1H), 4.25-4.15 (m, 2H), 3.05-2.96 (m, 2H), 2.09 (s, 9H), 1.97-1.86 (m, 2H), 1.79 (m, 6H), 1.60 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6, HMBC/HMQC) δ = 167.2, 153.5, 152.0, 147.3, 147.1, 141.4, 140.1, 139.7, 136.7, 135.1, 133.1, 132.4, 131.0, 130.1, 129.7, 128.3, 127.5, 125.9, 125.4, 124.6, 124.5, 124.4, 105.5, 104.5, 103.5, 70.9, 67.5, 41.3, 37.2, 36.0, 31.4, 29.5, 28.4, 21.1. **HRMS ESI** calc. for $C_{40}H_{39}NO_9^-$ $(M-H)^-$: 676.2552, found: 676.2565.

Cbz carbonate (4.6a): The *tert*-butyl ester **7** (16.0 mg, 0.032 mmol, 1 eq.) was

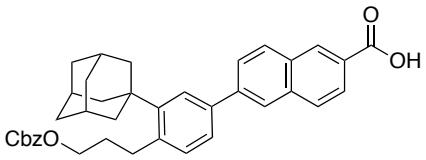


dissolved in 0.11 mL pyridine under argon and then cooled to 0 °C. A solution of CbzCl (16.5 mg, 0.097 mmol, 3 eq.) in 0.2 mL of dry CH_2Cl_2 was then added dropwise. After 30 min, the solution was allowed to reach rt and stirred for 2.5 h. TLC still indicated starting material, therefore additional CbzCl (16.5 mg, 0.097 mmol, 3 eq.) in 0.2 mL of dry CH_2Cl_2 was added dropwise and the reaction mixture was stirred over night. The solvent was evaporated and the crude material was taken up in Et_2O, washed with saturated aqueous NH_4Cl and water. The organic phase was dried over Na_2SO_4,

evaporated and subjected to flash column chromatography (pentane:Et_2O 4:1) to afford **4.6a** as a colorless solid (12.0 mg, 0.019 mmol, 59 %).

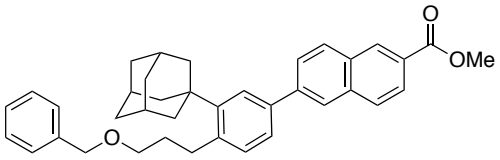
R_f = 0.9 (pentane: Et_2O 1:1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 8.55 (s, 1H), 8.05-8.02 (m, 2H), 7.99 (d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.79 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.43-7.33 (m, 5H), 7.28 (d, *J* = 7.9 Hz, 1H), 5.19 (s, 2H), 4.31 (t, *J* = 6.1 Hz, 2H), 3.13-3.09 (m, 2H), 2.14 (s, 9H), 2.10-2.04 (m, 2H), 1.80 (s, 6H), 1.66 (s, 9H).

Cbz protected acid (4.6): Carbonate **4.6a** (12.0 mg, 0.019 mmol, 1 eq.) was added into a mixture of 20% TFA, 5% TIPS in CH_2Cl_2 (0.2 mL) and stirred at RT for 2 h. The solvent was evaporated and the residue purified by flash column chromatography (pentane:Et_2O 1:1) to afford **4.6** as a colorless solid (10.0 mg, 0.017 mmol, 89 %).



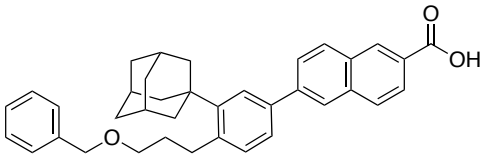
M.p. = 204-205 °C. **R_f** = 0.3 (pentane: Et_2O 1:1, 1% TFA). **FTIR**: ν = 3446, 2907, 1744, 1672, 1472, 1426, 1251, 1140, 1007, 971, 895, 817, 741, 689 cm^{-1}. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 13.06 (s, 1H), 8.61 (s, 1H), 8.26 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 7.99 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.91 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.61 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.41-7.36 (m, 5H), 7.34 (d, *J* = 8.0 Hz, 1H), 5.16 (s, 2H), 4.27 (t, *J* = 5.9 Hz, 2H), 3.08-2.99 (m, 2H), 2.08 (s, 9H), 2.01-1.90 (m, 2H), 1.81-1.68 (m, 6H). **^{13}C NMR** (126 MHz, DMSO-d6) δ = 167.2, 154.4, 147.5, 140.1, 139.8, 136.7, 135.4, 135.2, 132.5, 130.9, 130.1, 129.8, 128.4, 128.3, 128.2, 127.9, 127.6, 125.9, 125.4, 124.6, 124.5, 124.4, 68.7, 67.4, 41.4, 37.3, 36.0, 31.5, 29.5, 28.3. **HRMS ESI** calc. for $C_{38}H_{38}O_3^-$ $(M-H)^-$: 573.2646, found: 573.2654.

Benzyl ether (4.7a): A mixture of Bn-OPT **4.9** (15.4 mg, 0.044 mmol, 2 eq.), MgO (40.3 mg, 0.044 mmol, 2 eq.) and methyl ester **4.8** (10 mg, 0.022 mmol, 1 eq.) in CH_2Cl_2 (0.1 mL) was heated at 80 °C over night. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was subjected to flash column chromatography (pentane: Et_2O 10:1) and afforded benzyl ether **4.7a** as a colorless solid (11 mg, 0.021 mmol, 95%).



$\mathbf{R_f}$ = 0.7 (pentane: Et_2O 3:1). **FTIR**: ν = 3732, 2905, 2850, 2361, 1719, 1629, 1453, 1362, 1287, 1219, 1097, 1028, 911, 800, 753, 630 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.62 (s, 1H), 8.08 (dd, *J* = 8.6, 1.7 Hz, 1H), 8.05 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.93 (d, *J* = 8.6 Hz, 1H), 7.82 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.42-7.29 (m, 6H), 4.58 (s, 2H), 3.99 (s, 3H), 3.65 (t, *J* = 6.0 Hz, 2H), 3.17-3.08 (m, 2H), 2.20-2.11 (m, 9H), 2.06-1.96 (m, 2H), 1.83-1.77 (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 167.45, 148.30, 141.72, 141.12, 138.80, 137.87, 136.04, 132.78, 131.63, 130.98, 129.87, 128.49, 128.47, 127.66, 127.63, 127.26, 126.72, 125.73, 125.63, 125.36, 124.84, 73.09, 70.45, 52.36, 42.41, 38.27, 36.99, 33.44, 31.18, 29.39.

Acid (4.7): To a solution of **4.7a** (11.0 mg, 0.020 mmol, 1 eq.) in THF (0.5 mL) was added LiOH (0.1 M solution in H_2O; 0.40 mL, 0.040 mmol, 2 eq.). The solution was stirred over night, acidified with 0.1 M HCl to pH 2 and extracted with Et_2O. The combined organic phases were washed with water, dried over Na_2SO_4 and evaporated to afford **4.7** as a colorless solid (11.0 mg, 0.020 mmol, *quant.*).



M.p. = 243-244 °C. $\mathbf{R_f}$ = <0.1 (pentane:Et_2O 1:2). **FTIR**: ν = 3391, 2910, 2851, 2680, 2558, 1680, 1627, 1475, 1453, 1346, 1291, 1221, 1103, 1027, 927, 918, 816, 737 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6) δ = 13.04 (s, 1H), 8.61 (s, 1H), 8.25 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.09 (d, *J* = 8.7 Hz, 1H), 7.99 (dd, *J* = 8.5, 1.8 Hz, 1H),

7.90 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.65 (s, 1H), 7.59 (dd, *J* = 7.7, 2.0 Hz, 1H), 7.41-7.27 (m, 6H), 4.52 (s, 2H), 3.60 (t, *J* = 5.7 Hz, 2H), 3.09-3.02 (m, 2H), 2.15-2.04 (m, 9H), 1.94-1.84 (m, 2H), 1.76-1.69 (m, 6H). **HRMS ESI** calc. for $C_{37}H_{38}O_3^-$ $(M-H)^-$: 529.2748, found: 529.2747.

Kinetic measurements of cleavage: 24-Well plates were filled with **4.5** (1 µM) in cell medium (MEM) or CH_3CN/MeOH (1:1) and irradiated under a common laboratory UV lamp at 366 nm (~ 20 mW cm^{-2}) with a distance of approximately 3.5 cm between the lamp and the sample solutions for the indicated time (irradiated surface area = 254.5 mm^3). Afterwards samples were filtered through syringe filter and submitted for HPLC analysis.

Surface modifications and kinetic measurements of surface release: As already reported in section **8.3**.

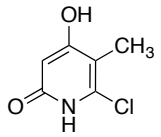
Neuritogenic properties: Cells were treated in the same manner as already reported in section **8.2**.

Additionally, In order to determine if ligand was deprotected inside cells, SH-SY5Y cells were incubated for 24 h at 37 °C in presence of active agonist **4.3** and its photo-caged analogue **4.5**. The cells were then washed twice with afore mentioned medium and replaced with ligand free medium. Immediately after the medium was changed, the cells were irradiated for 60 s and 180 s at 366 nm to liberate the free agonist. The cells were further allowed to incubate for three days and examined as described in the previous section.

8.6 JBIR-02 and Related Piericidins

8.6.1 Synthesis of the Heterocyclic Core (1st Generation Approach)

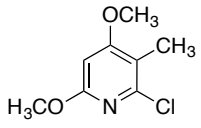
Pyridone (5.52): Malonylchloride (25.00 g, 170 mmol, 1.4 eq.) and propionitrile (6.53 g, 119 mmol, 1 eq.) were stirred under argon with a mechanical stirrer for 3 days at RT. The obtained solid was diluted with water and extensively washed with CH_2Cl_2 in order to remove unreacted starting material. The aqueous phase was then lyophilized to afford pyridone **5.52** (9.70 g, 61 mmol, 51%) as a beige solid.



M.p. = 302-303 °C. $\mathbf{R_f}$ = <0.05 (EA). **^{1}H NMR** (400 MHz, d6-DMSO) δ = 11.12 (bs, 1H, *OH*), 6.13 (s, 1H), 3.76 (bs, 1H, *OH* or *NH*), 2.00 (s, 3H).

Analytical data according to reference : S. J. Davies, J. A. Elvidge, A. B. Foster, *J. Chem. Soc.* **1962**, 3638-3644.

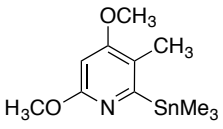
Dimethoxy pyridine (**5.53**): A mixture of pyridone **5.52** (100 mg, 0.6 mmol, 1 eq.) and Ag_2CO_3 (114 mg, 1.9 mmol, 3 eq.) was suspended in hexane (20 ml). Methyl iodide (1.6 ml, 3.57 g, 3.1 mmol, 5 eq.) was added and the mixture was stirred at RT during 36 h, then heated to reflux during 1 h. After cooling, CH_2Cl_2 (20 ml) was added and the mixture was stirred vigorously. Remaining solids were filtered and the filtrate was evaporated. The residue subjected to flash column chromatography (pentane:Et_2O 25:1 + 1% Et_3N) to afford dimethoxy pyrdine **5.53** (34 mg, 0.18 mmol, 32%) as a colorless solid.



M.p. = 92-93.5 °C. $\mathbf{R_f}$ = 0.4 (pentane:Et_2O 10:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.12 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 2.14 (s, 3H).

Analytical data according to reference: J. Buck, J. P. Madeley, G. Pattenden, *J. Chem. Soc. Perkin Trans. 1* **1992**, 67-73.

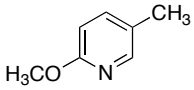
Pyridyl stannane (5.59): LiCl (20 mg, 0.45 mmol, 3 eq.) was fuse-dried in a Schlenck flask. Then pyridine **5.53** (30 mg, 0.15 mmol, 1 eq.), $Pd(PPh_3)_4$ (8.8 mg, 0.008 mmol, 5 mol %), dppf (8.7 mg, 0.015 mmol, 10 mol %) were added and dried under high-vacuum. Dry dioxane (0.5 mL) was added and finally hexamethylditin (60 mg, 0.18 mmol, 1.2 eq.) with a Hamilton-syringe. The solution was deoxygenated using the freeze-thaw method, and then stirred at 110 °C for 22 h. The orange solution was cooled to RT, water was added extracted with Et_2O and washed with water. The aqueous phase was extracted twice with Et_2O, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash column chromatography Al_2O_3 (pentane) to afford stannyl pyridine **5.59** (22 mg, 0.069 mmol, 46%) as colorless oil contaminated wit PPh_3O.



R_f = 0.8 (pentane:Et_2O 100:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.06 (s, 1H), 3.92 (s, 3H), 3.78 (s, 3H), 2.14 (s, 3H), 0.34 (m, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.03, 164.39, 163.40, 124.07, 90.77, 54.97, 53.18, 14.82, 8.36.

8.6.2 Synthesis of the Heterocyclic Core (2nd Generation Approach)

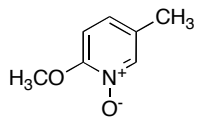
2-Methoxy pyridine (5.62): To a solution of NaH (60% dispersion in mineral oil; 2.44 g, 28.4 mmol, 2 eq.) in dry DMF (30 mL) was added 2-bromo-5-methylpyridine **5.61** (5.24 g, 14.2 mmol, 1 eq.) in small portions. Then a solution of dry MeOH (5 mL) in DMF (10 mL) was added slowly (1 h) through the reflux condenser. The resulting suspension was further heated at 100 °C for 48 h. The mixture was cooled to RT, water was carefully added, extracted with Et_2O and washed with water. The aqueous phase was extracted twice with Et_2O, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to afford the volatile 2-methoxy pyridine (5.36 g, 70% purity (Et_2O remaining), 14.2 mmol, *quant*.) as colorless oil.



R_f = 0.9 (EA). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.96 (dd, *J* = 1.5, 0.7 Hz, 1H), 7.38 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 2.24 (s, 3H).

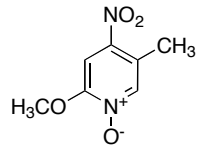
Analytical according to reference: P. M. Hatton, S. Sternhell, *Aust. J. Chem.* **1993**, *46*, 149-152.

Pyridine-*N*-oxide (5.62b): To a solution of 2-methoxy pyridine **5.61** (1.00 g, 8.12 mmol, 1 eq.) in 10 mL of AcOH was added H_2O_2 (30% in H_2O) at RT and the mixture was stirred at 75 °C for 24 h until reaction control by ^{1}H NMR indicated full conversion. The excess AcOH was removed under reduced pressure, the residue was diluted with H_2O and concentrated again. The residual mixture is cooled to 0 °C and NaOH (40%) is added slowly. The strong alkaline solution was extracted using CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness to afford *N*-oxide **5.62b** (740 mg, 5.3 mmol, 66%) as a colorless crystalline solid, which was used in the next step without further purification.



M.p. = 107-108 °C. $\mathbf{R_f}$ = 0.5 (EA). **FTIR**: ν = 3047, 2171, 1632, 1528, 1442, 1325, 1272, 1133, 1000, 882, 771, 711 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.12 (s, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 4.04 (d, *J* = 1.5 Hz, 3H), 2.25 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 156.16, 143.92, 139.64, 129.11, 110.52, 59.15, 17.62. **HRMS ESI** calc. for $C_7H_{10}NO_2^+$ $[M\text{-}H]^+$: 140.0706, found: 140.0707.

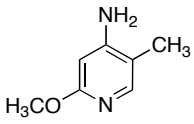
Nitropyridine (5.63): *N*-oxide **5.62b** (4.00 g, 28.7 mmol) was added to cooled (0 °C) conc. H_2SO_4 (5 mL). To this solution was added a mixture of fuming HNO_3 and conc. H_2SO_4 (3:1, 5 mL). The mixture is then heated to 100 °C for 3 h (Fume hood! Aqueous NaOH for gas adsorption). The yellow mixture is cooled to 5°C and added dropwise onto crushed ice carbonate. Extraction using $CHCl_3$ yielded an orange solid which was subjected to flash column chromatography (CH_2Cl_2:MeOH 20:1) to afford nitropyridine **5.63** as yellow crystals (710 mg, 3.9 mmol, 13%).



M.p. = 191.5-196.5 °C (decomposition). $\mathbf{R_f}$ = 0.05 (EA). **FTIR**: ν = 3053, 2473, 1612, 1562, 1506, 1458, 1299, 1220, 1057, 982, 890, 805, 753, 662 cm^{-1}. **^{1}H NMR**

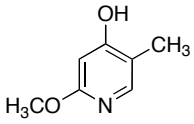
(400 MHz, $CDCl_3$) δ = 8.21 (s, 1H), 7.64 (s, 1H), 4.14 (s, 3H), 2.56 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 157.71, 143.53, 141.98, 123.96, 103.37, 58.18, 17.62. **HRMS ESI** calc. for $C_7H_9N_2O_4^+$ $[M-H]^+$: 185.0557, found: 185.0552. **X-Ray crystal structure** is given in the appendices section.

Aminopyridine (5.63b): To a solution of **5.63** (710 mg, 3.86 mmol, 1 eq.) in AcOH (22 mL) was added Fe-powder (1.50 g, 27 mmol, 7 eq.) and the brownish suspension was heated to 100 °C for 2 h until reaction control by ^{1}H NMR indicated full conversion. The crude mixture was neutralized using aqueous sat. $NaHCO_3$ solution and further basified using aqueous NH_3 solution. The alkaline solution (pH 10) was extracted using Et_2O, dried over Na_2SO_4 and evaporated to dryness to afford aniline **5.63b** (450 mg, 3.24 mmol, 84%) as colorless crystalline solid, which was used in the next step without further purification.



M.p. = 108.5-109.5°C. **R_f** = 0.35 (EA). **FTIR**: ν = 3464, 3313, 3169, 2933, 1610, 1567, 1497, 1460, 1403, 1317, 1219, 1026, 932, 841, 743 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.70 (s, 1H), 5.96 (s, 1H), 4.00 (bs, 2H), 3.86 (s, 3H), 2.04 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 164.37, 153.64, 146.61, 112.30, 93.11, 53.19, 13.44. **HRMS ESI** calc. for $C_7H_{10}N_2O^+$ $[M-H]^+$: 139.0866, found: 139.0866.

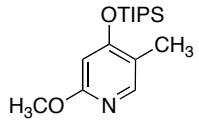
4-Hydroxypyridine (5.64): To a cold (0°C) solution of **5.63b** (200 mg, 1.45 mmol, 1 eq.) in half conc. HNO_3 (5 mL) was added $NaNO_2$ (599 mg, 8.68 mmol, 6 eq.) in 5 mL H_2O, so the solution turned immediately yellowish. Stirring was continued over night at RT. Then the reaction was poured into sat. aqueous $NaHCO_3$ solution (pH 7) and extracted with EtOAc to afford hydroxypyridine **5.64** (201 mg, 1.45 mmol, *quant*.) as colorless crystals.



M.p. = 152-153 °C. **R_f** = 0.25 (EA). **FTIR**: ν = 3484, 3311, 3173, 2361, 1638, 1570, 1499, 1403, 1321, 1221, 1030, 993, 776 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.54

(s, 1H), 6.12 (s, 1H), 3.84 (s, 3H), 2.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 172.74, 162.52, 140.06, 118.71, 94.56, 54.85, 13.07. **HRMS ESI** calc. for $C_7H_{10}NO_2^+$ $[M-H]^+$: 140.0706, found: 140.0705. **X-Ray crystal structure** is given in the appendices section.

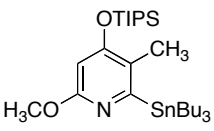
TIPS-protected hydroxy pyridine (5.65): To a cold (0 °C) suspension of pyridone



5.64 (395 mg, 2.84 mmol, 1.0 eq.) in dry CH_2Cl_2 (15 mL) was added Et_3N (574 mg, 0.8 mL, 5.68 mmol, 2 eq.) and TIPSOTf (942 mg, 2.98 mmol, 1.05 eq.) and allowed to come to RT and stirring was continued for 3 h until TLC indicated full conversion. Water was added to quench the reaction. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:Et_2O 50:1 + Et_3N) to afford TIPS protected hydroxy pyridine **5.65** as a colorless oil (790 mg, 2.67 mmol, 94%).

R_f = 0.15 (pentane:Et_2O 50:1). **FTIR**: ν = 2946, 2868, 2361, 1609, 1492, 1391, 1252, 1189, 884, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.82 (s, 1H), 6.13 (s, 1H), 3.88 (s, 3H), 2.10 (s, 3H), 1.38-1.25 (m, 3H), 1.11 (d, *J* = 7.4 Hz, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 164.45, 163.35, 147.19, 119.06, 98.90, 53.37, 18.05, 13.36, 12.97. **HRMS ESI** calc. for $C_{16}H_{30}NO_2Si^+$ $[M-H]^+$: 296.2040, found: 296.2039.

Stannane pyridine (5.66): To a cold (0 °C) solution of *N,N*-dimethylaminoethanol



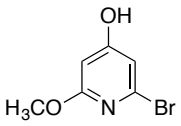
(0.8 mL, 8 mmol) in dry toluene (10 mL) was added dropwise *n*-BuLi (1.6 M in hexane; 10 mL, 16 mmol). The stock solution was stirred 30 min at 0 °C getting a clear yellow 0.4 M solution. 1.27 mL (3 eq.) of this solution was added to a second Schlenk tube followed by addition of TIPS-protected hydroxy pyridine **5.65** (50 mg, 0.17 mmol, 1 eq.) in dry toluene (0.9 mL) and stirring at 0 °C was continued for 1 h. ^{1}H NMR (D_2O quench) indicated full deprotonation. The solution was cooled to -78 °C and $SnBu_3Cl$ (165 mg, 0.51 mmol, 3 eq.) in dry THF (0.7 mL) was added dropwise. After 30 min the

reaction was allowed to warm to RT and quenched with H_2O. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash column chromatography (pentane:Et_2O 50:1 + Et_3N) to afford stannane **5.66** (10 mg, 0.02 mmol, 10%) as colorless oil.

R_f = 0.9 (pentane:Et_2O 50:1 + Et_3N). **FTIR**: ν = 2926, 2361, 1575, 1457, 1418, 1384, 1153, 1017, 961, 881, 750, 689 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.01 (s, 1H), 3.88 (s, 3H), 2.15 (s, 3H), 1.60-1.52 (m, 6H), 1.37-1.27 (m, 9H), 1.11-1.09 (m, 24H), 0.87 (t, *J* = 7.3 Hz, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 170.24, 163.00, 161.50, 126.75, 97.70, 53.20, 29.36, 27.55, 18.13, 15.97, 13.86, 13.05, 10.82. **HRMS ESI** calc. for $C_{28}H_{55}NO_2SiSn^+$ $[M-H]^+$: 586.3097, found: 586.3097.

8.6.3 Synthesis of the Heterocyclic Core (3rd Generation Approach)

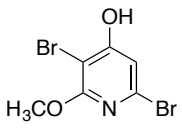
4-Hydroxy-pyridine (5.68): A dry 50 mL Schlenck tube was filled with



$[Ir(COD)OMe]_2$ (16.9 mg, 0.03 mmol, 2 mol %), dtbpy (13.8 mg, 0.05 mmol, 4 mol %) and filled with argon. To this heterogeneous mixture pinacolborane (204 mg, 1.55 mmol, 1.2 eq.) and 2-bromo-6-methoxypyridine (**5.67**, 97%, 250 mg, 1.29 mmol, 1 eq.) in *n*-hexane (2.5 mL) was added and the orange solution stirred further at rt for 3 h. Reaction control by ^{1}H NMR after 3 h indicated ca. 95% conversion The resulting mixture was diluted with THF (10 mL) and aqueous Oxone (872 mg, 1.42 mmol, 1.1 eq.; in 10 mL H_2O) was added slowly under vigorous stirring. After 10 min at rt, the reaction mixture was quenched with sat. aqueous $NaHSO_3$ solution, sat. aqueous NaCl solution and extracted with EtOAc. The aqueous phase was extracted with EtOAc, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was subjected to flash column chromategraphy (pentane:EtOAc 4:1) to afford 4-hydroxy-pyridine **5.68** (208 mg, 1.02 mmol, 79%) as colorless solid.

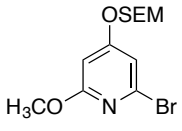
M.p. = 148-149 °C. TLC: $\mathbf{R_f}$ = 0.5 (CH_2Cl_2:EtOAc 10:1). **FTIR**: ν = 2884, 2654, 2507, 1554, 1455, 1406, 1346, 1256, 1200, 1153, 1045, 980, 796, 647 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO) δ = 11.07 (s, 1H, *OH*), 6.64 (d, *J* = 1.7 Hz, 1H), 6.12 (d, *J* = 1.7 Hz, 1H), 3.77 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ = 167.50, 164.61, 138.45, 109.68, 94.92, 53.76. **HRMS ESI** calc. for $C_6H_7O_2NBr^+$ $[M-H]^+$: 203.9655, found: 203.9659.

Dibromo-pyridone (5.68b): To a solution of pyridone **5.68** (50 mg, 0.25 mmol, 1 eq.) in CH_3CN (1.5 mL) was added NBS (48 mg, 0.27 mmol, 1.1 eq.) and stirred in the dark over the weekend at RT. ^{1}H NMR after 70 h indicated formation of a new compound. All volatiles were removed *in vacuo* and the crude orange oil subjected to flash column chromatography (CH_2Cl_2) to afford dibromo pyridone **5.68b** (55 mg, 0.19 mmol, 79%) as colorless solid.



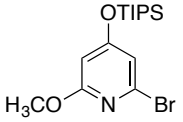
$\mathbf{R_f}$ = 0.3 (CH_2Cl_2). **FTIR**: ν = 3479, 2874, 1579, 1366, 1139, 1085, 1030, 940, 855, 772 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.80 (s, 1H), 4.00 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.51, 160.31, 137.79, 110.00, 93.62, 55.48. **HRMS MS** calc. for $C_6H_6Br_2NO_2^+$ $[M-H]^+$ = 280.8760, found: 280.8758.

SEM protected 4-pyridone (5.72): To a suspension of 4-pyridone **5.68** (50 mg, 0.25 mmol, 1 eq.) in CH_2Cl_2 was added DIPEA (52 µL, 0.37 mmol, 1.5 eq.) to form a clear-yellow solution. Then SEMCl (49 mg, 0.29 mmol, 1.2 eq.) was added in a single portion and the resulting mixture stirred at RT for 3 h, until TLC indicated full conversion. The reaction was quenched by sequential addition of sat. aqueous $NaHCO_3$ solution, sat. aqueous NaCl solution and extracted with CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was filtered through a plug of silica (CH_2Cl_2) to afford SEM protected 4-hydroxypyridine **5.72** (72 mg, 0.22 mmol, 88%) as colorless oil.



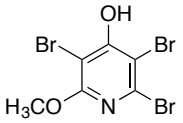
R_f = 0.7 (CH_2Cl_2). **FTIR**: ν = 2952, 2898, 1595, 1551, 1462, 1387, 1174, 1087, 1047, 984, 914, 694, 651, 622 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 6.78 (d, *J* = 1.8 Hz, 1H), 6.31 (d, *J* = 1.8 Hz, 1H), 5.20 (s, 2H), 3.91 (s, 3H), 3.72 (dd, *J* = 8.8, 7.9 Hz, 2H), 1.00-0.89 (m, 2H), 0.00 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.67, 165.16, 139.20, 110.16, 95.72, 92.73, 67.21, 54.36, 18.16, -1.30. **HRMS ESI** calc. for $C_{12}H_{20}BrNO_3SiNa^+$ $[M-Na]^+$ = 356.0289, found: 356.0288.

TIPS protected 4-pyridone (5.73): To a suspension of 4-pyridone **5.68** (150 mg, 0.74 mmol, 1 eq.) in CH_2Cl_2 was added Et_3N (0.21 mL, 1.47 mmol, 2.0 eq.) to form a clear-yellow solution. Then TIPSCl (145 mg, 0.75 mmol, 1.02 eq.) was added in a single portion and the resulting mixture stirred at RT for 3 h, until TLC indicated full conversion. The solvent was evaporated and the crude oil filtered through a short plug of silica to afford TIPS protected 4-pyridone **5.73** (257 mg, 0.65 mmol, 88%) as colorless oil.



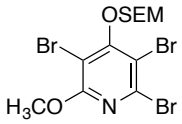
R_f = 0.8 (CH_2Cl_2). **FTIR**: ν = 2946, 2868, 1588, 1541, 1440, 1388, 1256, 1178, 1119, 1043, 974, 863, 750, 695, 654 cm^{-1}. **^1H NMR** (400 MHz, $CDCl_3$) δ = 6.63 (d, *J* = 1.8 Hz, 1H), 6.12 (d, *J* = 1.8 Hz, 1H), 3.90 (s, 3H), 1.36-1.21 (m, 3H), 1.09 (d, *J* = 7.2 Hz, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.01, 165.23, 139.28, 114.03, 99.73, 54.27, 17.92, 12.74. **HRMS ESI** calc. for $C_{15}H_{27}O_2NBrSi^+$ $[M+H]^+$: 360.0989, found: 360.0984.

2,3,5-Tribromo-4-pyridone (5.69): To a solution of 4-hydroxypyridine **5.68** (670 mg, 3.28 mmol, 1 eq.) in dry acetonitrile (12 mL) was added NBS (1227 mg, 3.28 mmol, 2.1 eq.) in a single portion and stirred for 5 h at rt in the dark until reaction control by ^1H NMR or UPLC indicated full conversion. The reaction mixture was poured in sat. aqueous $Na_2S_2O_3$ solution and extracted with EtOAc, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was subjected to flash column chromatography (pentane: EtOAc 4:1 + 1% TFA) to afford tribromo pyridine **5.69** as colorless solid. (1088 mg, 3.01 mmol, 92 %).



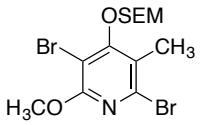
M.p. = 153-155 °C. $\mathbf{R_f}$ = 0.3 (strong tailing, pentane:EtOAc 4:1 + 1% TFA). **FTIR**: ν = 3438, 2958, 1559, 1468, 1370, 1290, 1178, 1106, 1056, 950, 870, 703, 646 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO) δ = 3.87 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ = 160.85, 158.94, 138.41, 105.58, 93.65, 55.12. **HRMS ESI** calc. for $C_6H_5O_2NBr_3^+$ $[M-H]^+$: 359.7865, found: 359.7870.

SEM-protected pyridine (5.70): To a cold (0 °C) solution of pyridine **5.69** (200 mg, 0.55 mmol, 1 eq.) in dry CH_2Cl_2 was added DIPEA (137 µL, 107 mg, 0.83 mmol, 1.5 eq.) and SEMCl (115 mg, 0.69 mmol, 1.2 eq.). The mixture was slowly allowed to reach rt; when reaction control by TLC indicated full conversion the reaction mixture was filtered through a short plug of silica using CH_2Cl_2 + 1% Et_3N as solvent to afford SEM-protected pyridine **5.70** as colorless oil (223 mg, 0.453 mmol, 82%).



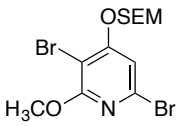
$\mathbf{R_f}$ = 0.7 (CH_2Cl_2). **FTIR**: ν = 2952, 1552, 1459, 1369, 1291, 1249, 1077, 889, 835, 705, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.33 (s, 2H), 4.00 (s, 3H), 3.96 (m, 2H), 1.00 (m, 2H), 0.04 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 161.82, 159.90, 139.77, 111.67, 101.70, 98.40, 68.87, 55.78, 18.20, -1.26. **HRMS ESI** calc. for $C_{12}H_{18}O_3NBr_3Si^+$ $[M-X]^+$: 492.0737, found: 433.8240.

2,5-Dibromo-3-methylpyridine (5.71a): To a cold (-78 °C) solution of tribromo-pyridine **5.70** (220 mg, 0.45 mmol, 1.0 eq.) in dry THF (3 mL) was added dropwisely *n*-BuLi (280 µL, 0.45 mmol, 1.0 eq., as a 1.6 M solution in cyclohexane). After stirring for 10 min at -78 °C to the deep orange solution was added MeI (635 mg, 4.47 mmol, 10 eq.) and the mixture further stirred at this temperature. After another 15 min the mixture was allowed to reach rt within 1 h and quenched by addition of sat. aqueous $Na_2S_2O_3$ solution. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was subjected to flash column chromatography (pentane: Et_2O 100:1 + 1% Et_3N) to afford dibromo-pyridine **5.71a** as colorless oil. (151 mg, 0.35 mmol, 79%).



R_f = 0.7 (pentane:Et_2O 10:1). **FTIR**: ν = 2953, 1574, 1528, 1466, 1389, 1302, 1249, 1170, 1076, 1016, 959, 907, 858 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.19 (s, 2H), 3.98 (s, 3H), 3.90 (m, 2H), 2.31 (s, 3H), 0.99 (m, 2H), 0.04 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 163.05, 158.96, 140.02, 123.69, 100.82, 98.22, 68.40, 55.29, 18.30, 16.20, -1.30. **HRMS ESI** calc. for $C_{13}H_{22}O_3NBr_2Si^+$ $[M-H]^+$: 425.9730, found: 425.9728.

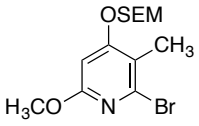
2,5-Dibromo-pyridine (5.72b): Following the procedure for 3-methylpyridine **5.70b**:



Tribromo-pyridine **5.70b** (500 mg, 1.02 mmol, 1.0 eq.) in dry THF was treated with *n*-BuLi (635 µL, 1.02 mmol, 1.0 eq., as a 1.6 M solution in cyclohexane) and MeOH (412 µL, 10.20 mmol, 10 eq.) to afford dibromo-pyridine **5.72b** (350 mg, 0.85 mmol, 83%) as colorless oil.

R_f = 0.3 (pentane: Et_2O 100:1). **FTIR**: ν = 2953, 1578, 1536, 1362, 1141, 1082, 1031, 984, 894, 854, 692 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.94 (s, 1H), 5.31 (s, 2H), 4.00 (s, 3H), 3.81-3.75 (m, 2H), 0.98-0.93 (m, 2H), 0.01 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 162.87, 160.99, 137.66, 108.90, 94.96, 93.55, 67.63, 55.41, 18.13, -1.29. **HRMS MS** calc. for $C_{12}H_{20}Br_2NO_3Si^+$ $[M-H]^+$ = 411.9574, found: 411.9572.

2-Bromo-3-methylpyridine (5.71): Following the procedure for 3-methyl pyridine

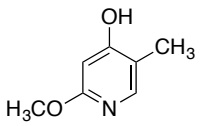


5.70b: Dibromo-pyridine **5.70b** (173 mg, 0.41 mmol, 1.0 eq.) in dry THF (3 mL) was treated with *n*-BuLi (0.25 mL, 0.41 mmol, 1.0 eq., as a 1.6 M solution in cyclohexane) and MeOH (0.16 mL, 4.05 mmol, 10 eq.) to afford bromo-pyridine **5.71** (120 mg, 0.35 mmol, 85%) as colorless oil.

R_f = 0.7 (pentane:Et_2O 10:1). **FTIR**: ν = 2953, 1598, 1555, 1473, 1408, 1358, 1249, 1206, 1160, 1094, 1053, 1012, 917, 760, 693 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.38 (s, 1H), 5.23 (s, 2H), 3.88 (s, 3H), 3.72 (m, 2H), 2.18 (s, 3H), 0.94 (m, 2H), 0.00 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 164.38, 162.68, 140.89, 117.29, 94.24,

92.94, 67.10, 54.06, 18.14, 14.53, -1.30. **HRMS ESI** calc. for $C_{13}H_{23}O_3NBrSi^+$ $[M-H]^+$: 348.0625, found: 348.0625.

4-Hydroxy-pyridine (5.64): To a cold (-78 °C) solution of **5.71** (25.8 mg, 74 µmol, 1 eq.) in dry THF (1 mL) was added *n*-BuLi (74 µL, 74 µmol, 1.0 eq., as a 1.6 M solution in cyclohexane). After stirring for 10 min at -78 °C $ZnCl_2$ (89 µL, 89 µmol, 1.2 eq., as a 1.0 M solution in THF) was added and the mixture allowed to come to rt. Then the H_2O was added and the reaction mixture was extracted with EtOAc to afford SEM protected 4-hydroxypyridine as colorless oil.



^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (s, 1H), 6.39 (s, 1H), 5.25 (s, 2H), 3.89 (s, 3H), 3.71-3.75 (m, 2H), 2.09 (s, 3H), 0.93-0.97 (m, 2H), 0.00 (s, 9H).

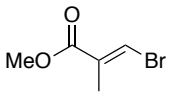
The crude oil was dissolved in dry THF (1 mL), TBAF (0.37 mL, 370 µmol, 5.0 eq., as a 1.0 M solution in THF) was added and the mixture was heated to 70 °C for 3 h, until TLC monitoring indicated full conversion. The brown solution was allowed to come to RT, 1 M HCl was added, the aqueous phase was extracted with EtOAc, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was subjected to flash column chromatography (pentane:EtOAc 3:1) to afford 4-hydroxypyridine **5.64** (8 mg, 58 µmol, 77%) as colorless solid.

M.p. = 152-153 °C. $\mathbf{R_f}$ = 0.25 (EA). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.54 (s, 1H), 6.12 (s, 1H), 3.84 (s, 3H), 2.09 (s, 3H).

Analytical data according to compound **5.64**.

8.6.4 Synthesis of the Polyene Side Chain

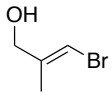
Bromo acrylate (5.78aa): To a solution of methyl methacrylate (4.90 g, 48.5 mmol, 1 eq.) in CCl_4 (10 mL) was slowly added bromine (7.74 g, 48.5 mmol, 1 eq.) in 5 mL of CCl_4 at 0 °C. After stirring at rt for 3 h, DBU (11.06 g, 72.7 mmol, 1.5 eq.) was added dropwise at 0 °C and the solution stirred over night, therby allowing to come to RT. The mixture was acidified to pH 2-3 with aqueous 1 M HCl, and the organic layers were separated. The aqueous layer was extracted with Et_2O, and the combined layers were washed with sat. aqueous NaCl solution (3 times) to afford after removal of solvent bromo acrylate **5.78aa** (8.20 g, 45.8 mmol, 95%) as yellowish oil, which was used without any further purification in the next step.



$\mathbf{R_f}$ = 0.8 (pentane:Et_2O 4:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.50 (s, 1H), 3.75 (s, 3H), 1.99 (s, 3H).

All analytical according to reference: X. Li, X. Zeng, *Tetrahedron Lett*. **2006**, *47*, 6839-6842.

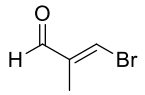
Allyl alcohol (5.78a): To a suspension of $LiAlH_4$ (1.17 g, 30.7 mmol, 1.00 eq.) in Et_2O (60 mL) at 0 °C was added a solution of ester **5.78aa** (5.50 g, 30.7 mmol, 1.00 eq.) in Et_2O (50 mL) and the resulting mixture was stirred for 1 h at RT. The mixture was cooled to 0 °C followed by addition of MeOH, water and HCl (2M aqueous solution). Saturated aqueous Rochelle's solution was added and the mixture was stirred at RT for 12 h. The resulting clear mixture was extracted with Et_2O and washed with water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford the allyl alcohol **5.78a** (4.37 g, 29.0 mmol, 94%) as colorless oil.



$\mathbf{R_f}$ = 0.2 (pentane:Et_2O 4:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.23-6.21 (m, 1H), 4.06 (s, 2H), 2.21 (s, 1H, *OH*), 1.81-1.80 (m, 3H).

Analytical data according to reference: Y. Murakami, M. Nakano, T. Shimofusa, N. Furuichi, S. Katsumura, *Org. Biomol. Chem.* **2005**, *3*, 1372-1374.

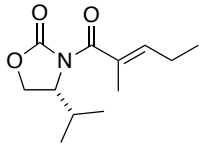
Aldehyde (5.78): To a suspension of MnO_2 (7.65 g, 88.0 mmol, 11.1 eq.) in CH_2Cl_2 (20 mL) was added allyl alcohol **5.78a** (1.20 g, 7.95 mmol, 1.00 eq.) and stirred at RT for 12 h. The black suspension was filtered through a plug of celite and carefully evaporated to afford the volatile aldehyde **5.78** (0.89 g, 5.96 mmol, 75%) as colorless oil.



$\mathbf{R_f}$ = 0.7 (CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$) ů = 9.49 (s, 1H), 7.43 (q, *J* = 1.3 Hz, 1H), 1.90 (d, *J* = 1.3 Hz, 3H).

Analytical data according to reference: Y. Murakami, M. Nakano, T. Shimofusa, N. Furuichi, S. Katsumura, *Org. Biomol. Chem.* **2005**, *3*, 1372-1374.

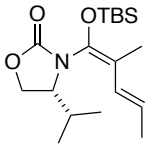
Imide (5.76a): To a cold (-40 °C, acetonitrile/dry ice) solution of 2-methyl-2-pentenoic acid (1.59 g, 13.9 mmol, 1.2 eq.) and Et_3N (5.17 mL, 36.2 mmol, 3.2 eq.) in dry THF (80 mL) was added dropwise trimethylacetyl chloride (1.80 mL, 14.6 mmol, 1.25 eq.). The resulting white slurry was stirred for 1.5 h at -40 °C. Then flame dried LiCl (0.68 g, 16.0 mmol, 1.4 eq.) and (*R*)-(+)-4-Isopropyl-2-oxazolidinone (1.50 g, 11.6 mmol, 1 eq.; R_f = 0.05 (pentane:Et_2O, 1:1)) were added in a single portion. The cooling bath was removed and the reaction mixture was allowed to stir at RT over night until TLC-control indicated full conversion. The reaction was quenched with first MeOH, then saturated aqueous NH_4Cl solution. The aqueous phase was extracted with Et_2O, washed with saturated $NaHCO_3$ solution, saturated NaCl solution and water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (pentane:Et_2O 3:1) to yield imide **5.76a** as colorless oil (2.17 g, 9.61 mmol, 83 %).



R_f = 0.8 (pentane:Et_2O 1:1). **Optical rotation**: $[\alpha]_D$ = -29.7° (*c* = 0.56, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$ δ = 6.01 (tq, *J*= 7.2 Hz, *J*= 1.3 Hz, 1H), 4.47-4.42 (m, 1H), 4.42 (t, *J*= 8.9 Hz, 1H), 4.12-4.08 (m, 1H), 2.34-2.25 (m, 1H), 2.18-2.09 (m, 2H), 1.83 (s, 3H), 0.98 (t, *J*= 7.6 Hz, 3H), 0.85 (t, *J*= 6.4 Hz, 6H).

Analytical data according to reference: S. Shirokawa, M. Kamiyama, T. Nakamura, M. Okada, A. Nakazaki, S. Hosokawa, S. Kobayashi, *J. Am. Chem. Soc.* **2004**, *126*, 13604-13605.

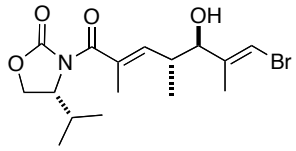
TBS enol (5.76): To a solution of imide **5.76a** (1000 mg, 4.44 mmol, 1 eq.) in dry THF (30 mL) at -78°C was added NaHMDS (1M solution in THF; 6.67 mL, 6.67 mmol, 1.5 eq.) and the resulting mixture was stirred for 1.5 h at -78 °C. Then TBSCl (2000 mg, 13.3 mmol, 3 eq.) in 10 mL dry THF was added and stirred another 30 min. Quenching by addition of first MeOH, then NH_4Cl at -78 °C, extraction with Et_2O afforded a colorless oil which was further purified by flash column chromatography (pentane: Et_2O 6:1) to afford TBS enol **5.76** as colorless oil (1497 mg, 4.41 mmol, 99%).



R_f = 0.6 (pentane:Et_2O, 3:1). **Optical rotation**: $[\alpha]_D$ = +23.6° (*c* = 0.65, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$ δ = 6.21 (d, *J*= 16.0 Hz, 1H), 5.63 (dq, *J*= 13.4, 6.6 Hz, 1H), 4.32 (t, *J*= 8.7 Hz, 1H), 4.12 (t, *J*= 8.4 Hz, 1H), 4.02-3.96 (m, 1H), 1.97-1.89 (m, 1H), 1.79-1.77 (m, 6H), 0.98 (s, 9H), 0.93 (s, 3H), 0.91 (s, 3H), 0.19 (s, 3H), 0.14 (s, 3H).

Analytical data according to reference: S. Shirokawa, M. Kamiyama, T. Nakamura, M. Okada, A. Nakazaki, S. Hosokawa, S. Kobayashi, *J. Am. Chem. Soc.* **2004**, *126*, 13604-13605.

Allylic alcohol (5.79): To a cold (-78 °C) solution of aldehyde **5.78** (7.430 g, 25.90 mmol, 2 eq.; R_f = 0.5 (pentane:EtOAc, 7:3)) in dry CH_2Cl_2 (70 mL) was added $TiCl_4$ (1 M solution in CH_2Cl_2, 13.60 mL, 13.60 mmol, 1.05 eq.) and the resulting orange solution stirred for 5 min. Then a solution of TBS enol **5.76** (4.863 g, 12.90 mmol, 1 eq.; R_f = 0.8 (pentane:EtOAc, 7:3)) in dry CH_2Cl_2 (60 mL) was added dropwise *via* additional funnel into the reaction mixture and further stirred at -35 °C (cryostate) for 18 h to form a yellow solution. The mixture was quenched by addition of pyridine (5 mL), Rochelle solution and $NaHCO_3$ solution (20 mL) and allowed to come to RT until a clear solution was formed. The aqueous layer was extracted with Et_2O, dried over Na_2SO_4 and evaporated to yield a yellow oil, then subjected to flash column chromatography (pentane:EtOAc 7:3) to afford allylic alcohol **5.79** as colorless foamy oil. (4.712 g, 11.90 mmol, 92%). An analytical sample was recrystallized from EtOAc/*n*-hexane for X-ray analysis.



M.p. = 73-75 °C. **R_f** = 0.3 (pentane:EtOAc, 7:3). **Optical rotation**: $[\alpha]_D$ = +9.3° (*c* = 0.48, $CHCl_3$). **FTIR**: ν = 3395, 3301, 3134, 2972, 2324, 1786, 1663, 1431, 1394, 1303, 1223, 1119, 1015, 966, 901, 777, 712 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.15 (d, *J* = 1.1 Hz, 1H), 5.65 (dd, *J* = 10.3, 1.5 Hz, 1H), 4.52 (ddd, *J* = 9.0, 5.6, 4.6 Hz, 1H), 4.29 (t, *J* = 9.0 Hz, 1H), 4.13 (dd, *J*= 9.1, 5.6 Hz, 1H), 3.73 (dd, *J* = 9.2, 2.2 Hz, 1H), 3.50 (d, *J* = 2.3 Hz, 1H, OH), 2.70 (ddq, *J* = 10.3, 9.2, 6.7 Hz, 1H), 2.29 (ddq, *J* = 7.0, 7.0, 4.6 Hz, 1H), 1.91 (d, *J* = 1.5 Hz, 3H), 1.79 (d, *J* = 1.3 Hz, 3H), 0.87 (d, *J* = 5.3 Hz, 3H), 0.86 (d, *J* = 5.2 Hz, 1H), 0.79 (d, *J* = 6.7, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.41, 154.73, 141.36, 140.46, 132.60, 106.30, 80.28, 63.68, 58.25, 38.12, 28.57, 18.00, 16.15, 15.34, 14.26, 14.24. **HRMS ESI** calc. for $C_{16}H_{25}BrNO_4^+$ $[M-H]^+$: 374.0950, found: 374,0961. **X-Ray structure** is given in the appendices section.

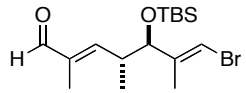
Imide (5.79b): To a cold (-78 °C) solution of alcohol **5.79** (5.360 g, 14.30 mmol, 1 eq.; R_f = 0.3 (pentane:EtOAc, 7:3)) and 2,6-lutidine (3.34 ml, 28.6 mmol, 3 eq.) in dry CH_2Cl_2 (150 mL) was added TBSOTf (4.28 ml, 18.60 mmol, 1.3 eq.) in dry CH_2Cl_2 (20 mL). The solution was allowed to reach rt and further stirred for 1 h at RT before being quenched with aqueous $NaHCO_3$ solution (50 mL), NH_4Cl solution and water. Extraction with Et_2O and evaporation yielded a colorless oil which was subjected to flash column chromatography (pentane:Et_2O 4:1) to afford imide **5.79b** as colorless oil (7.118 g, 13.87 mmol, 97%).

R_f = 0.8 (pentane: EtOAc 7:3). **Optical rotation**: $[\alpha]_D$ = -3.1° (*c* = 0.28, $CHCl_3$). **FTIR**: ν = 2960, 2857, 1785, 1684, 1463, 1386, 1298, 1204, 1077, 1007, 860, 775, 713 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.14 (s, 1H), 5.84 (dd, *J* = 9.8, 1.4 Hz, 1H), 4.47 (dt, *J* = 8.9, 4.6 Hz, 1H), 4.30 (t, *J* = 8.8 Hz, 1H), 4.18 (dd, *J* = 9.0, 4.9 Hz, 1H), 3.91 (d, *J* = 6.7 Hz, 1H), 2.68 (ddq, *J* = 9.8, 6.8, 6.8 Hz, 1H), 2.38 (ddq, *J* = 7.0, 7.0, 4.3 Hz, 1H), 1.92 (d, *J* = 1.4 Hz, 3H), 1.76 (d, *J* = 1.2 Hz, 3H), 0.91 (m, *J* = 7.9, 3.2 Hz, 9H), 0.85 (s, 9H), 0.04 (s, 3H), -0.02 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.74, 153.49, 142.99, 140.07, 131.88, 105.20, 80.55, 63.58, 58.48, 37.94, 25.87, 15.20, 15.01, 14.40, -4.73, -5.00. **HRMS ESI** calc. for $C_{22}H_{39}BrNO_4Si^+$ $[M-H]^+$: 488.1826, found: 488.1817.

Allylic alcohol (5.80a): To a solution of imide **5.79b** (2.500 mg, 4.97 mmol, 1 eq.; R_f = 0.4 (CH_2Cl_2, UV active)) in THF/H_2O (5:1, 50 mL) at rt was added $NaBH_4$ (950 mg, 24.90 mmol, 5eq.) in small portions. The reaction mixture was stirred in the dark for 12 h, two times addition of additional $NaBH_4$ (1 eq.), before being cooled to 0 °C and quenched with 0.1 M HCl. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:DCM 3:1) to afford alcohol **5.80a** as colorless oil (1.700 g, 4.67 mmol, 94%).

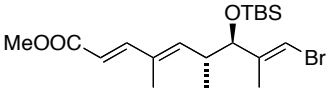
R_f = 0.3 (DCM, not UV active). **Optical rotation**: $[\alpha]_D$ = +12.5° (*c* = 0.87, $CHCl_3$). **FTIR**: ν = 3326, 2929, 2857, 1791, 1630, 1461, 1377, 1253, 1078, 1007, 940, 864, 775, 714, 671 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.08 (s, 1H), 5.22 (dd, *J* = 9.7, 1.3 Hz, 1H), 4.00 (d, *J* = 6.2 Hz, 2H), 3.80 (d, *J* = 7.2 Hz, 1H), 2.59 (ddq, *J* = 9.6, 7.2, 6.8 Hz, 1H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.67 (d, *J* = 1.3 Hz, 3H), 0.85 (s, 9H), 0.82 (d, *J* = 6.8 Hz, 3H), -0.00 (s, 3H), -0.03 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 143.48, 135.30, 128.97, 104.52, 81.24, 69.16, 37.00, 25.82, 18.24, 17.53, 15.10, 14.22, -4.67, -4.95. **HRMS ESI** calc. for $C_{16}H_{32}BrO_2Si^+$ $[M-H]^+$: 363.1713, found: 363.1703.

Aldehyde (5.80): To a suspension of MnO_2 (8.200 g, 94.00 mmol, 30 eq.) in CH_2Cl_2 (125 mL) was added alcohol **5.80a** (1.700 g, 4.68 mmol, 1 eq.; R_f = 0.3 (CH_2Cl_2)) and stirred over night. The black suspension was filtered through a plug of celite and evaporated to yield **5.80** without further purification as colorless oil (1.655 g, 4.59 mmol, 98%).



R_f = 0.8 (DCM). **Optical rotation**: $[\alpha]_D$ = +21.4° (*c* = 0.78, $CHCl_3$). **FTIR**: ν = 2956, 2858, 2416, 2257, 1687, 1462, 1256, 1077, 1007, 836, 775, 715, 672 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 9.40 (s, 1H), 6.32 (dd, *J* = 9.9, 1.3 Hz, 1H), 6.16 (s, *J* = 0.7 Hz, 1H), 3.95 (d, *J* = 7.1 Hz, 1H), 2.89 (ddq, *J* = 9.9, 7.1, 6.8 Hz, 1H), 1.80-1.73 (m, 6H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.83 (s, 9H), 0.00 (s, 3H), -0.03 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 195.39, 156.61, 142.62, 139.72, 105.54, 80.73, 38.45, 25.77, 18.20, 16.65, 15.02, 9.70, -4.62, -5.00. **UPLC-MS ESI** calc. for $C_{16}H_{29}BrO_2Si^+$ $[M-H]^+$: 360.1, found: 360.1.

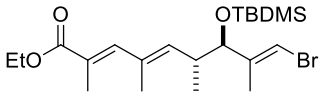
Ester (5.81): To a cold (0 °C) solution of Methyl diethylphosphonoacetate (182 mg, 0.87 mmol, 2.5 eq.) in CH_3CN (2 mL) was added DBU (114 µl, 0.76 mmol, 2.2 eq.) and stirred for 10 min. Then LiCl (44 mg, 1.04 mmol, 3 eq.) was added and the solution allowed to further stir at rt. Then aldehyde **5.80** (125 mg, 0.35 mmol, 1 eq.; R_f = 0.8 (CH_2Cl_2)) was added in CH_3CN (2 mL) and the resulting solution stirred over night (12 h) until ^{1}H NMR control indicated full conversion of the reaction. The



reaction mixture was quenched with 0.1M HCl, NH_4Cl solution. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (CH_2Cl_2) to afford ester **5.81** as colorless oil (144 mg, 0.35 mmol, *quant.*).

R_f = 0.8 (CH_2Cl_2). **Optical rotation**: $[\alpha]_D$ = +40.8° (*c* = 0.77, $CHCl_3$). **FTIR**: ν = 2929, 2857, 1720, 1623, 1461, 1377, 1310, 1253, 1168, 1077, 1014, 862, 776, 714 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.31 (dd, *J* = 15.7, 0.6 Hz, 1H), 6.10 (d, *J* = 1.7 Hz, 1H), 5.80 (d, *J* = 15.5 Hz, 1H), 5.70 (d, *J* = 9.9 Hz, 1H), 3.85 (d, *J* = 6.9 Hz, 1H), 3.75 (s, 3H), 2.72 (ddq, *J* = 9.9, 6.9, 6.8 Hz, 1H), 1.75 (dd, *J* = 11.4, 1.2 Hz, 6H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.82 (s, 9H), -0.04 (d, *J* = 6.5 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.16, 149.93, 144.48, 143.07, 133.24, 115.69, 105.04, 80.99, 51.61, 38.23, 25.80, 18.17, 17.24, 15.06, 12.80, -4.71, -4.94. **HRMS ESI** calc. for $C_{19}H_{34}BrO_3Si^+$ $[M-H]^+$: 417.1455, found: 417.1456.

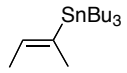
Ester (5.82):[107] To a solution of 2-(diethoxyphosphoryl)-propionic acid ethyl ester (528 mg, 0.48 mL, 2.22 mmol, 4.00 eq.) in CH_3CN (0.4 mL) at 0 °C was added DBU (294 mg, 0.29 mL, 1.94 mmol, 3.50 eq.). The resulting mixture was stirred for 10 min and LiCl (70.5 mg, 1.66 mmol, 3.00 eq.) was added. After 10 min stirring a solution of aldehyde **5.80** (206 mg, 0.56 mmol, 1.00 eq.) in CH_3CN (0.5 mL) was added dropwise. The mixture was allowed to come to RT and stirred at ambient temperature for 6 h until ^{1}H NMR showed complete conversion. The resulting yellow solution was quenched using saturated aqueous NH_4Cl solution and extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified by column chromatography (pentane:Et_2O 40:1) to afford ester **5.82** (199 mg, 0.45 mmol, 81%) as colorless oil.



R_f = 0.3 (pentane:Et_2O 40:1). **Optical rotation**: $[\alpha]_D$ +36.4° (*c* = 0.84, $CHCl_3$). **FTIR**: ν = 3890, 2932, 2358, 2109, 1706, 1628, 1462, 1378, 1251, 1008, 861, 776,

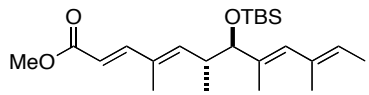
631 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.09 (s, 1H), 6.10 (s, 1H), 5.43 (d, *J* = 9.8 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.86 (d, *J* = 6.3 Hz, 1H), 2.67 (ddq, *J* = 9.8, 6.8, 6.7 Hz, 1H), 1.99 (d, *J* = 1.4 Hz, 3H), 1.82 (d, *J* = 1.3 Hz, 3H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.85 (s, 9H), 0.01 (s, 3H), -0.04 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.35, 143.22, 143.02, 138.36, 132.51, 125.70, 104.78, 80.94, 60.73, 37.77, 25.84, 18.22, 17.67, 16.93, 15.45, 14.46, 14.23, -4.64, -4.98. **HRMS ESI** calc. for $C_{21}H_{38}O_3BrSi^+$ $[M-H]^+$: 445.1768, found: 445.1760.

Stannane (5.83): To a cold (-50 °C) solution of (*E*)-2-Bromo-2-butene (95% purity; 392 mg, 2.76 mmol, 1.25 mmol) in dry THF (5 mL) was added dropwise *t*-BuLi (as a 1.9 M solution in pentane, 2.16 mL, 3.45 mmol, 1.55 eq.) and further stirred at this temperature until the yellow color of the solution disappears (1 h). Then $SnBu_3Cl$ (0.60 mL, 2.21 mmol, 1 eq.) was added as a solution in dry THF (1.5 mL) and slowly allowed to reach rt and stirred another 12 h at rt. To this white suspension was added water to obtain a clear organic layer. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to yield a light yellow oil which was further purified by flash column chromatography on Al_2O_3 (pentane) to afford stannane **5.83** as colorless oil (720 mg, 2.21 mmol, 95%).



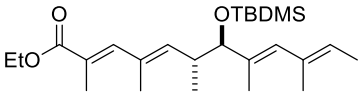
R_f = 0.9 (pentane). **FTIR**: ν = 2922, 2853, 1902, 1615, 1461, 1377, 1291, 1181, 1073, 1001, 872, 802, 664 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.68-5.43 (m, 1H), 1.75 (dd, *J* = 1.8, 1.1 Hz, 3H), 1.62 (dd, *J* = 6.4, 1.0 Hz, 3H), 1.46-0.75 (m, 27H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 138.92, 134.86, 29.35, 27.58, 18.82, 14.01, 13.86, 9.18. **Elementary Analyses** calc. for $C_{16}H_{34}Sn$: calc.: C = 55.68, H = 9.93, Sn = 34.39; found: C = 55.47, H = 9.68.

Ester (5.84): LiCl (136 mg, 3.21 mmol, 3 eq.) was dried in a Schlenk tube with a Bunsen burner. A solution of ester **5.81** (460 mg, 1.070 mmol, 1 eq.; R_f = 0.20 (pentane:Et_2O 40:1)) and stannane **5.83** (480 mg, 1.390 mmol, 1.3 eq.) in dry NMP (5 mL) was added and the mixture was degassed by the freeze-thaw procedure (cooled in liquid N_2 until solution freezes, high vacuum; allowed to reach rt still high vacuum, then Argon; this procedure was repeated 3 times). Then Pd(*t*-$Bu_3P)_2$ (54 mg, 0.107 mmol, 10 mol %) was added and the orange solution stirred 10 min at rt then 1.5 h at 80 °C. The mixture was cooled to RT, Et_2O was added and the crude black suspension filtered before washing with H_2O. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:Et_2O 40:1) to afford ester **5.84** as colorless oil (400 mg, 1.017 mmol, 95%) together with trace impurities which could not be separated by column chromatography. For analytical purposes a sample was purified using HPLC.



R_f = 0.25 (pentane:Et_2O 40:1). **Optical rotation**: $[\alpha]_D$ = -7.8° (*c* = 0.18, $CHCl_3$). **FTIR**: ν = 2955, 1722, 1622, 1460, 1378, 1308, 1252, 1168, 1067, 774, 631 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.33 (d, *J* = 15.6 Hz, 1H), 5.79 (s, 1H), 5.76 (s, 1H), 5.74 (s, 1H), 5.34 (q, *J* = 6.8 Hz, 1H), 3.75 (s, 3H), 3.71 (d, *J* = 7.4 Hz, 1H), 2.72 (ddq, *J* = 9.9, 6.9, 6.8 Hz, 1H), 1.77 (d, *J* = 1.1 Hz, 3H), 1.71 (s, 3H), 1.69 (d, *J* = 1.1 Hz, 3H), 1.67 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.82 (s, 9H), -0.04 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.29, 150.41, 146.43, 135.40, 133.15, 132.62, 131.46, 124.16, 115.07, 83.64, 51.54, 38.36, 25.89, 18.21, 17.39, 16.67, 13.77, 13.08, 12.81, -4.46, -4.89. **HRMS ESI** calc. for $C_{23}H_{40}O_3NaSi^+$ $[M-H]^+$: 415.2639, found: 415.2642.

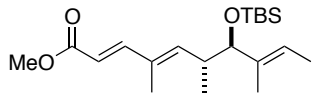
Ester (5.85):[107] To a solution of ester **5.82** (172 mg, 374 μmol, 1.00 eq.) and stannane **5.83** (194 mg, 562 μmol, 1.50 eq.) in NMP (8.0 mL) was added flame-dried LiCl (31.7 mg, 749 μmol, 2.00 eq.). This mixture was degassed three times by



freeze-thaw procedure. $Pd(P\textit{t}\text{-}Bu_3)_2$ (19.1 mg, 37.4 μmol, 0.10 eq.) was added and the resulting orange mixture was stirred at 80 °C for 6 h until TLC indicated full conversion. The resulting black suspension was filtered and washed with Et_2O. Water was added and the mixture was extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting yellow oil was purified by column chromatography (pentane:Et_2O 100:1) to afford the olefin **5.85** (153 mg, 363 μmol, 97%) as colorless oil. For analytical purposes a sample was further purified by HPLC.

$\mathbf{R_f}$ = 0.2 (pentane:Et_2O 100:1). **Optical rotation**: $[\alpha]_D$ = -9.2° (*c* = 0.06, $CHCl_3$). **FTIR**: ν = 2926, 2856, 2362, 1708, 1462, 1377, 1248, 1112, 1014, 735, 623 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.11 (s, 1H), 5.75 (s, 1H), 5.49 (d, *J* = 9.8 Hz, 1H), 5.34 (q, *J* = 6.9 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.72 (d, *J* = 6.9 Hz, 1H), 2.67 (ddq, *J* = 9.8, 6.8, 6.8 Hz, 1H), 2.00 (d, *J* = 1.3 Hz, 3H), 1.84 (d, *J* = 1.2 Hz, 3H), 1.71 (d, *J* = 3.1 Hz, 3H), 1.70 (s, 3H), 1.67 (d, *J* = 6.9 Hz, 3H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H), 0.84 (s, 9H), -0.02 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 169.45, 143.24, 140.32, 135.42, 133.10, 131.68, 130.78, 124.90, 123.88, 83.30, 60.45, 37.59, 25.69, 18.02, 17.57, 16.75, 16.45, 14.19, 13.95, 13.64, 13.23,-4.76, -5.03. **HRMS ESI** calc. for $C_{25}H_{44}O_3NaSi^+$ $[M\text{-}Na]^+$: 443.2953, found: 443.2944.

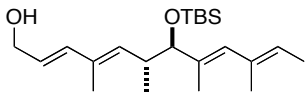
Ester (5.86): A solution of ester **5.81** (475 mg, 0.97 mmol, 1 eq.), $MeB(OH)_2$ (88 mg, 1.45 mmol, 1.5 eq.) and Cs_2CO_3 (946 mg, 2.90 mmol, 3 eq.) in dioxane (5 mL) and 0.1 mL H_2O was degassed by the freeze-thaw procedure. Then $Pd(PPh_3)_2$ (56 mg, 0.05 mmol, 5 mol %) was added and the orange solution stirred 10 min at RT then 5 h at 65 °C. The mixture was cooled to RT, Et_2O was added and the crude black suspension filtered before washing with H_2O. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:Et_2O 40:1) to afford ester **5.86** as colorless oil (238 mg,



0.67 mmol, 69%) together with trace impurities which could not be separated by column chromatography.

R_f = 0.3 (pentane:Et_2O 40:1). **FTIR**: ν = 3629, 2955, 1722, 1622, 1459, 1308, 1252, 1168, 1056, 775, 630 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.32 (dd, *J* = 15.6, 0.5 Hz, 1H), 5.82-5.70 (m, 2H), 5.35 (q, *J* = 6.4 Hz, 1H), 3.75 (s, 3H), 3.70 (d, *J* = 7.6 Hz, 1H), 2.74- 2.62 (m, 1H), 1.77 (d, *J* = 1.1 Hz, 3H), 1.58 (dd, *J* = 6.7, 0.9, 3H), 1.55 (s, 3H), 0.81-0.78 (m, 12H), -0.07 (s, 3H), -0.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 168.29, 150.44, 146.68, 136.86, 135.82, 132.50, 132.21, 121.86, 114.98, 114.23, 107.15, 83.20, 77.48, 77.16, 76.84, 72.54, 51.51, 38.21, 25.85, 18.18, 17.26, 13.06, 12.75, 11.03, -4.63, -4.94. **HRMS ESI** calc. for $C_{20}H_{37}O_3Si^+$ $[M-H]^+$: 353.2506, found: 353.2509.

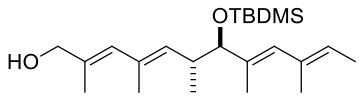
Allylic alcohol (5.87a): To a solution of ester **5.84** (107 mg, 0.27 mmol, 1 eq.) in dry CH_2Cl_2 (2.5 mL) was added at -78 °C DIBAH (1 M solution in hexane, 0.81 mL, 0.81 mmol, 3 eq.) and the solution was stirred at -78 °C until TLC indicated full conversion (0.5 h). The reaction mixture was then quenched by dropwise addition of first MeOH, Rochelle's solution and Et_2O and warmed to rt and stirred until both layers became clear solutions. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in* vacuo to afford alcohol **5.87a** (96 mg, 0.27 mmol, *quant.*) as colorless oil, which was used without further purification in the next step. For analytical purposes a sample was purified using HPLC.



R_f = 0.2 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = -17.5° (*c* = 0.12, $CHCl_3$). **FTIR**: ν = 3361, 2927, 2856, 2316, 1717, 1566, 1456, 1362, 1255, 1079, 1061, 1036, 838, 774 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.25 (dd, *J* = 15.6, 0.6 Hz, 1H), 5.75-5.67 (m, 2H), 5.38-5.31 (m, 2H), 4.20 (s, 2H), 3.67 (d, *J* = 7.5 Hz, 1H), 2.71-2.62 (m, 1H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.71 (s, 3H), 1.69 (d, *J* = 1.2 Hz, 3H), 1.67 (d, *J* = 6.8 Hz, 3H), 0.82 (m, 12H), -0.04 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 137.86, 137.47, 135.90, 133.28, 132.63, 131.08, 125.07, 123.91, 83.91, 64.28,

37.78, 25.93, 18.24, 17.76, 16.70, 13.77, 13.11, -4.48, -4.86. **HRMS ESI** calc. for $C_{22}H_{40}O_2NaSi^+$ $[M\text{-}Na]^+$: 387.2690, found: 387.2691.

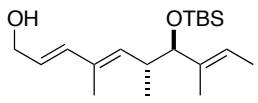
Allyl alcohol (5.88a):[107] Following the reported procedure for **5.87a**: Ester **5.85**



(92.7 mg, 220 μmol, 1.00 eq.) in dry CH_2Cl_2 (4.0 mL) was treated with DIBAL (1M solution in hexane, 661 μL, 661 μmol, 3.0 eq.) to afford the allyl alcohol **5.88a** (81.0 mg, 214 μmol, 97%) as colorless oil. For analytical purposes a sample was further purified by HPLC.

$\mathbf{R_f}$ = 0.2 (pentane :Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = +5.53° (*c* = 0.13, $CHCl_3$). **FTIR**: ν = 3336, 2929, 2856, 2361, 1460, 1377, 1252, 1069, 1033, 887, 836, 775, 669 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.86 (s, 1H), 5.74 (s, 1H), 5.33 (q, *J* = 6.8 Hz, 1H), 5.19 (d, *J* = 9.7 Hz, 1H), 4.04 (s, 2H), 3.70 (d, *J* = 6.7 Hz, 1H), 2.62 (ddq, *J* = 9.7, 6.9, 6.8 Hz, 1H), 1.82 (d, *J* = 1.3 Hz, 3H), 1.75 (d, *J* = 1.3 Hz, 3H), 1.70 (d, *J* = 3.0 Hz, 3H), 1.70 (s, 3H), 1.67 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.85 (s, 9H), -0.02 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 135.85, 134.67, 133.81, 133.17, 131.50, 130.56, 130.05, 123.70, 83.47, 69.74, 37.30, 25.67, 18.01, 17.83, 15.27, 17.15, 16.43, 13.45, 13.20, -4.68, -5.18.

Allylic alcohol (5.89a): Following the reported procedure for **5.87a**: Ester **5.86**

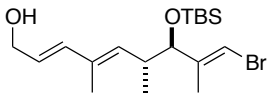


(255 mg, 0.72 mmol, 1 eq.) in dry CH_2Cl_2 (10 mL) was treated with DIBAH (1 M solution in hexane, 2.2 mL, 2.17 mmol, 3eq.) to yield alcohol **5.89a** (225 mg, 0.69 mmol, 95%) as colorless oil together with trace impurities which could not be separated by column chromatography.

$\mathbf{R_f}$ = 0.2 (pentane:Et_2O 4:1). **FTIR**: ν = 3313, 2928, 2857, 1249, 1056, 963, 860, 835, 773, 669, 630 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.25 (d, *J* = 15.6 Hz, 1H), 5.70 (dt, *J* = 15.6, 6.2 Hz, 1H), 5.38-5.28 (m, 2H), 4.19 (d, *J* = 6.0 Hz, 2H), 3.66 (d, *J* =

7.6 Hz, 1H), 2.68-2.58 (m, 1H), 1.74 (s, 3H), 1.61-1.56 (m, 3H), 1.55 (d, *J* = 1.0 Hz, 3H), 1.26 (s, 1H, OH), 0.81 (s, 9H), 0.77 (d, *J* = 6.8 Hz, 3H), -0.06 (s, 3H), -0.08 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 138.11, 137.52, 137.26, 132.50, 124.98, 121.39, 118.28, 92.42, 83.47, 64.29, 37.63, 25.90, 18.23, 17.66, 13.08, 11.09, -4.63, -4.89. **HRMS ESI** calc. for $C_{19}H_{36}O_2SiNa^+$ $[M\text{-}Na]^+$: 347.2377, found: 347.2380.

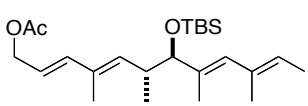
Allylic alcohol (5.94a): Following the reported procedure for **5.87a :** Ester **5.81**



(150 mg, 0.36 mmol, 1 eq.) in dry CH_2Cl_2 (5 mL) was treated with DIBAL-H (1.08 mL, 1.08 mmol, 3 eq., 1 M solution in hexane, mL, mmol, 3 eq.) to yield alcohol **5.94a** (135 mg, 0.35 mmol, 97%) as colorless oil.

R_f = 0.2 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = +25.0° (*c* = 0.70, $CHCl_3$). **FTIR**: ν = 3307, 2956, 2928, 2857, 1629, 1255, 1081, 1004, 964, 861, 836, 775, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.24 (d, *J* = 16.2 Hz, 1H), 6.08 (d, *J* = 0.6 Hz, 1H), 5.73 (dt, *J* =15.6, 6.1 Hz, 1H), 5.29 (d, *J* = 9.7 Hz, 1H), 4.20 (t, *J* = 5.6 Hz, 2H), 3.82 (d, *J* = 6.9 Hz, 1H), 2.67 (dq, *J* = 9.6, 6.8 Hz, 1H), 1.74 (d, *J* = 1.1 Hz, 6H), 1.25 (s, 1H, OH), 0.84 (m, 12H), -0.03 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 143.39, 136.86, 135.94, 133.33, 125.69, 104.56, 81.14, 64.07, 37.66, 25.81, 18.17, 17.57, 15.13, 13.07, -4.75, -4.94. **HRMS ESI** calc. for $C_{18}H_{33}BrO_2SiNa^+$ $[M\text{-}Na]^+$ = 411.1325, found: 411.1330.

Allylic acetate (5.87): To a cold (0 °C) solution of alcohol **5.87a** (88 mg, 0.24 mmol,

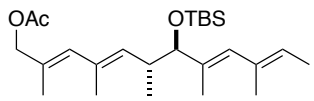


1 eq.) in dry CH_2Cl_2 (1.0 mL) was added DMAP (3 mg, 0.02 mmol, 0.1 eq.) distilled Et_3N (68 µl, 0.48 mmol, 2 eq.) and Ac_2O (28 µl, 0.30 mmol, 1.25 eq.; purified by filtration over basic Alox) and the resulting mixture was further stirred at 0 °C for 0.5 h. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ solution. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was

subjected to flash column chromatography (pentane:Et_2O 50:1) to afford acetate **5.87** as colorless oil (95 mg, 0.24 mmol, 97%). For analytical purposes a sample was purified using HPLC.

R_f = 0.8 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = -12.8° (*c* = 0.4, $CHCl_3$). **FTIR**: ν = 2929, 2857, 1741, 1452, 1378, 1027, 860, 835, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.29 (d, *J* = 15.6 Hz, 1H), 5.72 (s, 1H), 5.61 (dt, *J* = 15.5, 6.8 Hz, 1H), 5.39-5.28 (m, 2H), 4.61 (m, 2H), 3.67 (d, *J* = 7.5 Hz, 1H), 2.72-2.61 (m, 1H), 2.07 (s, 3H), 1.74 (d, *J* = 0.7, 3H), 1.71 (s, 3H), 1.68 (m, 6H), 0.82 (m, 12H), -0.04 (s, 3H), -0.06 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.09, 140.33, 138.82, 135.83, 133.26, 132.48, 131.14, 123.94, 119.69, 83.88, 65.77, 37.81, 25.91, 21.22, 18.24, 17.67, 16.69, 13.77, 13.09, 13.01, -4.48, -4.89. **HRMS ESI** calc. for $C_{24}H_{42}O_3NaSi^+$ $[M-Na]^+$: 429.2795, found: 429.2793.

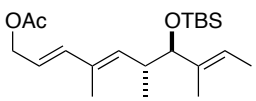
Acetate (5.88):[107] Following the reported procedure for **5.87**: Alcohol **5.88a**



(73.2 mg, 193 μmol, 1.0 eq.) in dry CH_2Cl_2 (4.0 mL) was treated with DMAP (4.72 mg, 38.6 μmol, 0.20 eq.), Et_3N (39.1 mg, 54.3 μL, 386 μmol, 2.00 eq.) and Ac_2O (24.7 mg, 22.7 μL, 241 μmol, 1.25 eq.) in CH_2Cl_2 (0.5 mL) to afford acetate **5.88** as colorless oil (67.0 mg, 159 μmol, 82%).

R_f = 0.8 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = +4.40° (*c* = 0.09, $CHCl_3$). **FTIR**: ν = 2929, 2857, 2361, 1741, 1649, 1451, 1375, 1228, 1063 1023, 836, 775, 669 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.88 (s, 1H), 5.74 (s, 1H), 5.33 (q, *J* = 6.8 Hz, 1H), 5.21 (d, *J* = 9.7 Hz, 1H), 4.49 (s, 2H), 3.69 (d, *J* = 6.9 Hz, 1H), 2.62 (ddq, *J* = 9.6, 6.9, 6.8 Hz, 1H), 2.08 (s, 3H), 1.80 (d, *J* = 1.3 Hz, 3H), 1.75 (d, *J* = 1.2 Hz, 3H), 1.71 (s, *J* = 1.0 Hz, 3H), 1.70 (d, *J* = 1.2 Hz, 3H), 1.67 (d, *J* = 6.8 Hz, 3H), 0.85 (s, 9H), 0.84 (d, *J* = 6.7 Hz, 3H), -0.02 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 171.01, 135.40, 135.32, 133.24, 133.08, 131.20, 130.62, 128.72, 123.73, 83.48, 71.03, 37.36, 25.67, 20.90, 18.07, 17.74, 17.02, 16.42, 15.54, 13.47, 13.15, -4.72, -5.24.

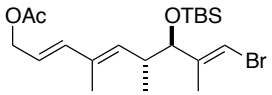
Allylic acetate (5.89): Following the reported procedure for **5.87**: Alcohol **5.89a**



(204 mg, 0.63 mmol, 1 eq.) in dry CH_2Cl_2 (7.5 mL) was treated with DMAP (8 mg, 0.06 mmol, 0.1 eq.), Et_3N (177 µL, 1.27 mmol, 2 eq.) and Ac_2O (922 µL, 0.79 mmol, 1.2 eq.) to yield acetate **5.89** (231 mg, 0.63 mmol, *quant*.) as colorless oil together with trace impurities which could not be separated by column chromatography.

R_f = 0.8 (pentane:Et_2O 4:1). **FTIR**: ν = 2925, 2857, 1740, 1232, 1228, 964, 840, 669, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.29 (d, *J* = 15.6 Hz, 1H), 5.60 (dt, *J* = 15.5, 6.7 Hz, 1H), 5.40-5.29 (m, 2H), 4.66-4.56 (m, 2H), 3.66 (d, *J* = 7.7 Hz, 1H), 2.68-2.57 (m, 1H), 2.06 (s, 3H), 1.73 (d, *J* = 1.1 Hz, 3H), 1.60-1.56 (m, 3H), 1.54 (d, *J* = 1.0 Hz, 3H), 0.81 (s, 9H), 0.77 (d, *J* = 6.8 Hz, 3H), -0.06 (s, 3H), -0.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.09, 140.38, 139.06, 137.20, 132.36, 121.45, 119.60, 83.44, 65.78, 37.66, 25.89, 21.22, 18.23, 17.58, 13.07, 12.97, 11.08, -4.62, -4.92. **HRMS ESI** calc. for $C_{21}H_{38}O_3SiNa^+$ $[M\text{-}Na]^+$: 389.2482, found: 389.2487.

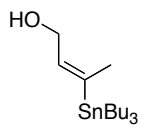
Acetate (5.94): Following the reported procedure for **5.87**: Alcohol **5.94a**



(15 mg, 38 µmol, 1 eq.) in dry CH_2Cl_2 (1 mL) was treated with DMAP (5 mg, 4 µmol, 0.1 eq.), Et_3N (11 µL, 77 µmol, 2 eq.) and Ac_2O (22 µL, 48 µmol, 1.5 eq., as 30% solution in CH_2Cl_2) to yield acetate **5.94** (14.3 mg, 33 µmol, 86%) as a colorless oil.

R_f = 0.8 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = +17.8° (*c* = 0.50, $CHCl_3$). **FTIR**: ν = 2929, 2857, 1738, 1647, 1377, 1225, 1077, 1025, 963, 835, 775, 669, 632 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.31 (d, *J* = 15.6 Hz, 1H), 6.11 (s, 1H), 5.66 (dt, *J* = 15.5, 6.7 Hz, 1H), 5.35 (d, *J* = 9.7 Hz, 1H), 4.69-4.59 (m, 2H), 3.85 (d, *J* = 7.0 Hz, 1H), 2.70 (dq, *J* = 9.6, 6.9 Hz, 1H), 2.10 (s, 3H), 1.77 (m, 6H), 0.86 (m, 12H), -0.00 (s, 3H), -0.02 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 171.06, 143.37, 139.85, 137.01, 133.18, 120.35, 104.64, 81.14, 65.62, 37.72, 25.82, 21.21, 18.20, 17.52, 15.12, 12.99, -4.72, -4.95. **HRMS ESI** calc. for $C_{20}H_{35}O_3BrSiNa^+$ $[M\text{-}Na]^+$: 453.1429, found: 453.1431.

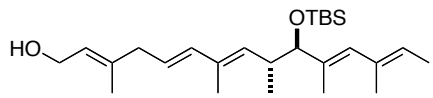
Vinyl stannane (5.90): To a suspension of CuCN (358 mg, 4 mmol, 2 eq.) in dry THF (16 mL) was added *n*-BuLi (1.6 M solution in hexane; 5.25 mL, 8.4 mmol, 4.2 eq.) at -78 °C. This mixture was stirred 10 min at -40 °C to become a clear solution. Then Bu_3SnH (2445 mg, 8.4 mmol, 4.2 eq.) was added to the mixture at -78 °C. The mixture was stirred until a yellow solution was obtained, then warmed to -40 °C for 10 min and cooled again to -78 °C. Dry MeOH (2 mL) was added at -78 °C and the temperature was warmed to -10 °C for 5 min to form a brown solution. Then the brown solution was cooled again to -78 °C and 2-butin-1-ol (97%; 145 mg, 2 mmol, 1 eq.) in 2 mL dry THF was added, and slowly warmed to 10 °C overnight. MeOH was added to quench the reaction at -20 °C, then H_2O and Et_2O. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. ^{1}H NMR of the crude mixture indicated the formation of a single product. The crude oil was subjected to flash column chromatography (SiO_2, pentane:Et_2O 4:1 + Et_3N) to afford stannane **5.90** as colorless oil (620 mg, 1.72 mmol, 86%).



R_f = 0.3 (pentane:Et_2O 4:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.75 (ddd, *J* = 6.1, 4.2, 2.1 Hz, 1H), 4.25 (t, *J* = 5.5 Hz, 2H), 2.00-1.79 (m, 3H), 1.56-1.41 (m, 6H), 1.31 (m, 6H), 0.89 (m, 15H).

Analytical data were in full agreement with literature: J.-F. Betzer, F. Delaloge, B. Muller, A. Pancrazi, J. Prunet, *J. Org. Chem*. **1997**, *62*, 7768-7780.

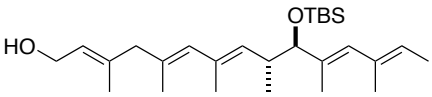
Allyl alcohol (5.91): To a solution of allyl acetate **5.87** (250 mg, 0.63 mmol, 1 eq.) and stannane **5.90** (250 mg, 0.69 mmol, 1.1 eq.) in dry NMP (1.0 mL) was added flame dried LiCl (107 mg, 2.52 mmol, 4 eq.). The suspension was degased using the freeze-thaw procedure; DIPEA (138 mg, 178 µl, 1.07 mmol, 1.7 eq.) was added and finally $Pd(dba)_2$ (36.2 mg, 0.06 mmol, 10 mol %) to give a black suspension, which was stirred at 45 °C for 3 h (until TLC indicated full conversion). Water was added to stop the reaction. The aqueous phase was extracted



with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:Et_2O 7:1) to afford allyl alcohol **5.91** as colorless oil (241 mg, 0.55 mmol, 88%) as a 5:1 mixture of *E*:*Z* isomers. Separated by HPLC (column: IA (l = 25 cm; ∅ = 1 cm), solvent: hexane: *i*-PrOH 99.5:0.5, 25 °C, flow: 6 mL/min, 2 mg/100 µL; R_t = 28 min).

R_f = 0.2 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = -40.5° (*c* = 1.00, $CHCl_3$). **FTIR** ν = 3350, 2928, 2316, 1670, 1444, 1385, 1251, 1066, 1006, 882, 775 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.06 (dd, *J* = 15.5, 0.6 Hz, 1H), 5.71 (s, 1H), 5.49 (t, *J* = 7.3 Hz, 1H), 5.47-5.43 (m, 1H), 5.34 (q, *J* = 6.8 Hz, 1H), 5.20 (d, *J* = 9.7 Hz, 1H), 4.17 (d, *J* = 7.1 Hz, 2H), 3.64 (d, *J* = 7.8 Hz, 1H), 2.78 (d, *J* = 7.1 Hz, 2H), 2.70-2.61 (m, 1H), 1.73 (d, *J* = 1.2 Hz, 3H), 1.71 (s, 3H), 1.69 (d, *J* = 1.2 Hz, 3H), 1.68 (s, 3H), 1.66 (s, 3H), 0.82 (s, 9H), 0.80 (d, *J* = 6.8 Hz, 3H), -0.04 (s, 3H), -0.06 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 139.58, 137.45, 136.00, 135.79, 133.31, 133.13, 131.09, 124.11, 123.83, 123.80, 84.14, 59.65, 43.12, 37.57, 25.93, 18.25, 17.75, 16.69, 16.45, 13.76, 13.21, 12.99, -4.49, -4.88. **HRMS ESI** calc. for $C_{26}H_{46}O_2NaSi^+$ $[M+H]^+$: 441.3159, found: 441.3157.

Allyl alcohol (5.92):[107] Following the reported procedure for **5.91**: Acetate **5.88**

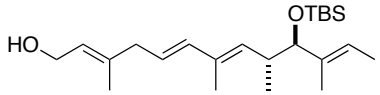


(64.8 mg, 154 µmol, 1.00 eq.) in dry NMP (0.25 mL) was treated with stannane **5.90** (66.8 mg, 185 µmol, 1.20 eq.), LiCl (26.1 mg, 616 µmol, 4.00 eq.), DIPEA (46 µL, 262 µmol, 1.7 eq.) and $Pd(dba)_2$ (17.7 mg, 30.8 µmol, 0.20 eq.) to afford allyl alcohol **5.92** (51.6 mg, 119 µmol, 77%) as colorless oil. Separated by HPLC (column: IA (l = 25 cm; ∅ = 1 cm), solvent: hexane: *i*-PrOH 99.5:0.5, 25 °C, flow: 5 mL/min, 2 mg/100 µL; R_t = 32 min).

R_f = 0.2 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = -15.4° (*c* = 0.08, $CHCl_3$). **FTIR**: ν = 3727, 3336, 2929, 2858, 2361, 1448, 1380, 1521, 1067, 1022, 837, 775, 670 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.72 (s, 1H), 5.66 (s, 1H), 5.47 (t,

J = 6.9 Hz, 1H), 5.34 (q, *J* = 6.8 Hz, 1H), 5.10 (d, *J* = 9.7 Hz, 1H), 4.18 (dd, *J* = 5.9, 5.9 Hz, 2H), 3.66 (d, *J* = 7.3 Hz, 1H), 2.70 (s, 2H), 2.60 (ddq, *J* = 9.6, 6.9, 6.8 Hz, 1H), 1.73 (d, *J* = 1.3 Hz, 3H), 1.70 (d, *J* = 3.2 Hz, 3H), 1.70 (s, 3H), 1.68 (d, *J* = 1.3 Hz, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.11 (dd, *J* = 5.5, 5.5 Hz, 1H, *OH*), 0.84 (s, 9H), 0.83 (d, *J* = 6.8 Hz, 3H), -0.02 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 138.23, 135.82, 133.77, 133.18, 132.04, 131.69, 131.61, 130.62, 124.98, 123.59, 83.81, 59.55, 51.13, 37.38, 25.78, 18.04, 17.86, 17.44, 17.24, 17.18, 15.60, 13.48, 13.11, -4.86, -5.11 **HRMS ESI** calc. for $C_{27}H_{48}O_2NaSi^+$ $[M\text{-}Na]^+$: 455.3316, found: 455.3322.

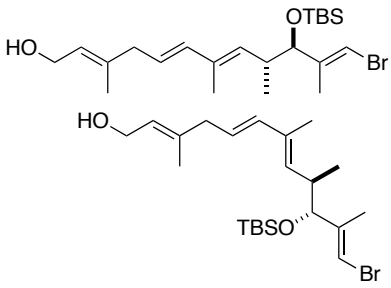
Allylic alcohol (5.93): Following the reported procedure for **5.91**: Acetate **5.89**



(180 mg, 0.49 mmol, 1 eq.) in dry NMP (0.5 mL) was treated with stannane **5.90** (195 mg, 0.54 mmol, 1.1 eq.), LiCl (83 mg, 1.96 mmol, 4 eq.), DIPEA (138 µL, 108 mg, 0.84 mmol, 1.7 eq.) and $Pd(dba)_2$ (28 mg, 0.05 mmol, 10 mol %) to yield acetate **5.93** as colorless oil (143 mg, 0.38 mmol, 77%) as a 5:1 mixture of *E*:*Z* isomers. Separated by HPLC (column: IC (l = 25 cm; ∅ = 3 cm), solvent: hexane: *i*-PrOH 99.5:0.5, 25 °C, flow: 6 mL/min, 10 mg/100 µL; R_t = 48 min).

R_f = 0.2 (pentane:Et_2O 4:1). **Optical rotation**: $[\alpha]_D$ = -14.3° (*c* = 0.43, $CHCl_3$). **FTIR**: ν = >3100, 2926, 2855, 1454, 1378, 1248, 1056, 860, 835, 774, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.06 (d, *J* = 15.5 Hz, 1H), 5.52-5.41 (m, 2H), 5.32 (q, *J* = 6.4 Hz, 1H), 5.19 (d, *J* = 9.7 Hz, 1H), 4.21-4.13 (m, 2H), 3.63 (d, *J* = 7.9 Hz, 1H), 2.78 (d, *J* = 7.1 Hz, 2H), 2.66-2.56 (m, 1H), 1.72 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 1.56 (d, *J* = 7.6 Hz, 3H), 0.80 (s, 9H), 0.76 (d, *J* = 6.8 Hz, 3H), -0.06 (s, 3H), -0.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 139.42, 137.46, 137.36, 136.02, 133.01, 124.06, 123.96, 121.36, 83.71, 59.64, 43.12, 37.42, 25.91, 18.24, 17.67, 16.46, 13.19, 13.06, 10.98, -4.63, -4.91. **HRMS ESI** calc. for $C_{23}H_{42}O_2SiNa^+$ $[M\text{-}Na]^+$: 401.2846, found: 401.2849.

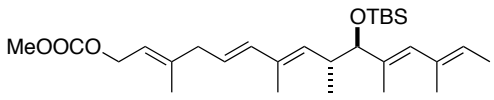
Allylic alcohol (5.95): Following the reported procedure for **5.91**: Acetate **5.94**



(24 mg, 59 µmol, 1 eq.) in dry NMP (0.5 mL) was treated with stannane **5.90** (23 mg, 65 µmol, 0.1 eq.), LiCl (10 mg, 236 µmol, 4 eq.), DIPEA (16 µL, 100 µmol, 1.7 eq.) and $Pd(dba)_2$ (3 mg, 6 µmol, 10 mol %) to yield acetate **5.95** (17 mg, 41 µmol, 71%) as a colorless oil in a 5:1 ratio of *E*/*Z*-isomers. Separated by HPLC (column: IA (l = 25 cm; ∅ = 1 cm), solvent: hexane: *i*-PrOH 99.5:0.5, 25 °C, flow: 5 mL/min, 2 mg/100 µL; R_t (*E*) = 23 min, R_t (*Z*) = 19 min).

R_f = 0.2 (pentane:Et_2O 4:1). ***E*-5.95**-isomers: **Optical rotation**: $[\alpha]_D$ = +4.8° (*c* = 0.10, $CHCl_3$). **FTIR**: ν = 3311, 2929, 1633, 1460, 1080, 1032, 778, 630 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.05 (m, 2H), 5.55-5.42 (m, 2H), 5.17 (d, *J* = 9.7 Hz, 1H), 4.17 (s, 2H), 3.79 (d, *J* = 7.3 Hz, 1H), 2.79 (d, *J* = 7.1 Hz, 2H), 2.71-2.60 (m, 1H), 1.74 (d, *J* = 1.2, 3H), 1.72 (s, 3H), 1.66 (s, 3H), 0.82 (m, 12H), -0.03 (s, 3H), -0.05 (s, 3H). ***Z*-5.95**-isomers: **^{1}H NMR** (600 MHz, $CDCl_3$) δ = 6.41 (d, *J* = 15.9 Hz, 1H), 6.07 (s, 1H), 5.63 (dt, *J* = 15,4 7.2 Hz, 1H), 5.46 (t, *J* = 6.9 Hz, 1H), 5.04 (d, *J* = 9.7 Hz, 1H), 4.17 (t, *J* = 6.0 Hz, 2H), 3.77 (d, *J* = 7.3 Hz, 1H), 2.82 (d, *J* = 7.1 Hz, 2H), 2.80-2.70 (m, 1H), 1.79 (s, 3H), 1.75 (d, *J* = 0.9 Hz, 3H), 1.68 (s, 3H), 0.82 (s, 9H), 0.81 (d, *J* = 6.9 Hz, 3H), -0.03 (s, 3H), -0.05 (s, 3H). **HRMS ESI** calc. for $C_{22}H_{39}O_2BrNaSi^+$ $[M\text{-}Na]^+$: = 465.1795, found: 465.1794,

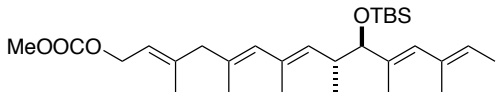
Allylic carbonate (5.96): To a cold (0 °C) solution of alcohol **5.91** (21.5 mg,



51 µmol, 1 eq.) in dry CH_2Cl_2 (2.5 mL) was added pyridine (12.3 µL, 154 µmol, 3 eq.) and methyl chloroformate (77 µL, 77 µmol, 1.5 eq., as a 1 M solution in CH_2Cl_2). After 45 min reaction time TLC indicated full conversion and saturated NH_4Cl solution was added. The aqueous phase was extracted with CH_2Cl_2, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude carbonate **5.96** (24.5 mg, 51 µmol, 99%) was directly used without any further purification in the next step.

R_f = 0.8 (CH_2Cl_2). **Optical rotation**: $[\alpha]_D$ = -36.2° (*c* = 0.61, $CHCl_3$). **FTIR**: ν = 2928, 2856, 1749, 1443, 1383, 1259, 1066, 942, 881, 835, 775 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.06 (d, *J* = 15.5 Hz, 1H), 5.71 (s, 1H), 5.50-5.38 (m, 2H), 5.34 (q, *J* = 6.7 Hz, 1H), 5.20 (d, *J* = 9.7 Hz, 1H), 4.67 (d, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 3.64 (d, *J* = 7.8 Hz, 1H), 2.80 (d, *J* = 7.0 Hz, 2H), 2.70-2.60 (m, 1H), 1.76-1.60 (m, 15H), 0.82 (s, 9H), 0.80 (d, *J* = 6.8 Hz, 3H), -0.04 (s, 3H), -0.06 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 156.03, 142.75, 137.70, 136.00, 135.94, 133.31, 133.10, 131.09, 123.84, 123.67, 118.35, 84.12, 64.91, 54.81, 43.06, 37.57, 25.91, 18.24, 17.74, 16.70, 16.68, 13.76, 13.21, 12.99, -4.49, -4.89.

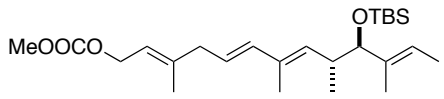
Allylic carbonate (5.97): Following the reported procedure for **5.96**: Alcohol **5.92**



(10.0 mg, 23 µmol, 1 eq.) in dry CH_2Cl_2 (1.5 mL) was treated with pyridine (6 µL, 69 µmol, 3 eq.) and methyl chloroformate (34 µL, 34 µmol, 1.5 eq., as a 1 M solution in CH_2Cl_2) to yield carbonate **5.97** (10.4 mg, 23 µmol, 99%) as colorless oil.

R_f = 0.8 (CH_2Cl_2). **Optical rotation**: $[\alpha]_D$ = -8.3° (*c* = 0.52, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 5.72 (s, 1H), 5.66 (s, 1H), 5.42 (t, *J* = 7.7 Hz, 1H), 5.34 (dd, *J* = 13.5, 6.8 Hz, 1H), 5.09 (d, *J* = 9.7 Hz, 1H), 4.68 (d, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 3.66 (d, *J* = 7.3 Hz, 1H), 2.71 (s, 2H), 2.65-2.55 (m, 1H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.71-1.68 (m, 9H), 1.67 (d, *J* = 1.2 Hz, 3H), 1.64 (s, 3H), 0.84 (s, 9H), 0.83 (d, *J* = 7.1 Hz, 3H), -0.03 (s, 3H), -0.05 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 156.03, 141.82, 136.06, 134.07, 133.35, 132.23, 132.04, 131.89, 130.82, 123.77, 119.53, 83.99, 64.95, 54.82, 51.26, 37.55, 25.95, 18.28, 18.00, 17.62, 17.42, 16.71, 15.92, 13.78, 13.25, -4.42, -4.92.

Allylic carbonate (5.98): Following the reported procedure for **5.96**: Alcohol **5.93**

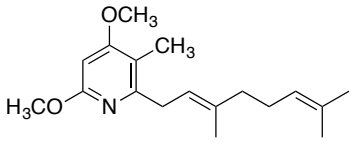


(12.4 mg, 33 µmol, 1 eq.) in dry CH_2Cl_2 (1 mL) was treated with pyridine (8 µL, 98 µmol, 3 eq.) and methyl chloroformate (49 mg, 49 µmol, 1.5 eq., as a 1 M solution in CH_2Cl_2) to yield carbonate **5.98** (14.2 mg, 33 µmol, 99%) as colorless oil.

R_f = 0.8 (CH_2Cl_2). **Optical rotation**: $[\alpha]_D$ = -12.6° (*c* = 0.71, $CHCl_3$). **FTIR**: ν = 2956, 2858, 1749, 1677, 1602, 1519, 1443, 1342, 1252, 1087, 940, 835, 780, 678 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.05 (dd, *J* = 15.5, 0.5 Hz, 1H), 5.49-5.37 (m, 2H), 5.32 (q, *J* = 6.7 Hz, 1H), 5.19 (d, *J* = 9.7 Hz, 1H), 4.66 (d, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 3.63 (d, *J* = 7.9 Hz, 1H), 2.79 (d, *J* = 7.0 Hz, 2H), 2.66-2.56 (m, 1H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.70 (s, 3H), 1.58 (dd, *J* = 6.7, 0.8 Hz, 3H), 1.55 (s, *J* = 1.0 Hz, 3H), 0.80 (s, 9H), 0.76 (d, *J* = 6.8 Hz, 3H), -0.07 (s, 3H), -0.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 156.02, 142.74, 137.72, 137.35, 136.17, 132.97, 123.57, 121.36, 118.34, 83.70, 64.91, 54.80, 43.05, 37.42, 25.89, 18.23, 17.65, 16.68, 13.18, 13.06, 10.97, -4.64, -4.93.

8.6.5 Completion of the Synthesis – Cross-Coupling Strategies

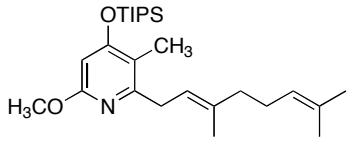
Dimethoxy pyridine (5.60): A solution of **5.59** (47 mg, 0.11 mmol, 1.25 eq.) and



geranyl chloride (15 mg, 0.08 mmol, 1 eq.) in dry DMF (1.0 mL) was added to CsF (33 mg, 0.22 mmol, 2.5 eq.), CuI (3.3 mg, 0.018 mmol, 0.2 eq.) and Pd(*t*-$Bu_3P)_2$ (4.4 mg, 0.008 mmol, 10 mol %), degased (3 x) and stirred at 60 °C over night. After 30 min reaction time the mixture became black. The mixture was cooled to RT and Et_2O was added. The aqueous phase was extracted with Et_2O, washed with water (3 x) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash column chromatography (pentane:Et_2O 50:1 to 25:1 + Et_3N) to afford dimethoxy-pyridine **5.60** (12 mg, 0.04 mmol, 41%) as colorless oil.

$\mathbf{R_f}$ = 0.2 (pentane:Et_2O 25:1). **FTIR**: ν = 2944, 1584, 1463, 1361, 1205, 1147, 1059, 822 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.04 (s, 1H), 5.38-5.31 (m, 1H), 5.07 (dddd, *J* = 8.3, 5.6, 2.8, 1.4 Hz, 1H), 3.89 (s, 3H), 3.79 (s, 3H), 3.40 (d, *J* = 6.8 Hz, 2H), 2.10-1.98 (m, 4H), 2.03 (s, 3H), 1.75 (d, *J* = 0.9 Hz, 3H), 1.65 (d, *J* = 1.0 Hz, 3H), 1.57 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 166.1, 162.7, 156.6, 135.8, 131.3, 124.2, 121.3, 112.9, 89.0, 55.2, 53.1, 39.6, 34.7, 26.5, 25.5, 17.5, 16.3, 9.8. **HRMS ESI** calc. for $C_{18}H_{28}NO_2^+$ $[M-H]^+$: 290.2115, found: 290.2117.

TIPS protected pyridone (5.101'a): A suspension of stannane **5.66** (15 mg, 26 µmol,

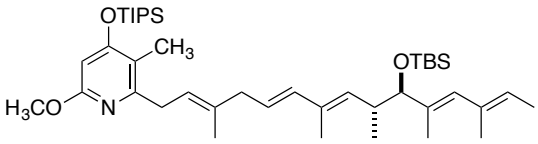


1.0 eq.) and geranyl chloride (5 mg, 28 µmol, 1.1 eq.) in dry DMF (0.2 mL) was degassed three times. To the orange mixture was added flame dried LiCl (3.5 mg, 77 µmol, 3 eq.) and $Pd_2(dba)_3$ (2.4 mg, 3 µmol, 0.1 eq.), degassing repeated (another 3 x) and the mixture was heated to 80 °C to afford a clear yellow solution. Stirring at this temperature was continued until TLC indicated full conversion (ca. 3 h), the mixture was cooled to RT and Et_2O was added. The aqueous phase was extracted with Et_2O, washed with water (3 x) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash

column chromatography (pentane:Et_2O 50:1) to afford protected pyridone **5.101'a** (8 mg, 18 μmol, 72%) as colorless oil.

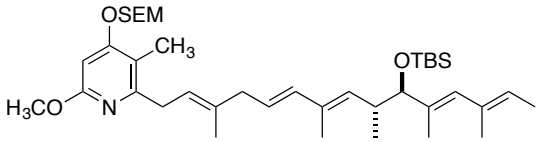
R_f = 0.2 (pentane:Et_2O 50:1). **FTIR**: ν = 2921, 2361, 1595, 1468, 1395, 1263, 1192, 1156, 909, 737, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.01 (s, 1H), 5.35 (dd, *J* = 6.8, 5.7 Hz, 1H), 5.08 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.86 (s, 3H), 3.40 (d, *J* = 6.8 Hz, 2H), 2.07 (s, 7H), 1.75 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H), 1.31 (m, 3H), 1.10 (d, *J* = 7.4 Hz, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 163.29, 162.58, 157.62, 135.86, 131.47, 124.47, 121.52, 115.79, 96.37, 53.24, 39.86, 35.36, 26.81, 25.81, 18.12, 17.80, 16.57, 13.04, 11.04. **HRMS ESI** calc. for $C_{26}H_{46}NO_2Si^+$ $[M+H]^+$: 432.3292, found: 432.3283.

Protected JBIR-02 (5.2'a): A suspension of stannane **5.66** (16 mg, 38 μmol, 1.0 eq.) and allyl chloride **5.99** (16.6 mg, 38 μmol, 1.0 eq.) in dry DMF (0.2 mL) was degassed three times. To the orange mixture was added flame dried LiCl (3 mg, 75 μmol, 2 eq.) and $Pd_2(dba)_3$ (2.3 mg, 4 μmol, 10 mol %.), degassing repeated (another 3 x) and the mixture was heated to 80 °C to afford a clear yellow solution. Stirring at this temperature was continued until TLC indicated full conversion (ca. 3 h), the mixture was cooled to RT and Et_2O was added. The aqueous phase was extracted with Et_2O, washed with water (3 x) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash column chromatography (pentane:Et_2O 50:1) and to HPLC (SiO_2; hexane:*i*-PrOH 99.5:0.5) to afford protected JBIR-02 **5.2'a** (13 mg, 19 μmol, 49%) as inseparable mixtures of (*E*:*Z* = ca. 3:2) isomers.



R_f = 0.6 (pentane:Et_2O 10:1). **HRMS ESI** for $C_{42}H_{74}NO_3Si_2^+$ $[M+H]^+$: 696.5202, found: 696.5195.

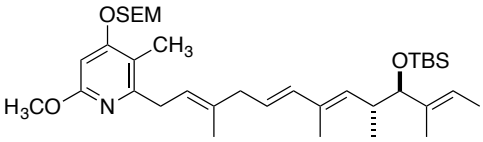
Protected JBIR-02 (5.2a): To a cold (-78 °C) solution of pyridine **5.71** (21 mg, 60 µmol, 1.2 eq.) in dry THF (0.5 mL) was added *n*-BuLi (40 µL, 65 µmol, 1.2 eq., as a 1.6 M solution in cyclohexane) dropwisely. The color of the reaction mixture changed to light orange. After 10 min at -78 °C $ZnCl_2$ (75 µL, 75 µmol, 1.5 eq., as a freshly prepared 1.0 M solution in THF) was added, stirred 10 min at -78 °C and allowed to come to RT and stirred at rt for 45 min to yield a clear solution. ^{1}H NMR (quench with H_2O and D_2O) indicated formation of the desired pyridinylzinc reagent. In a separate flask was added $Pd(PPh_3)_4$ (3 mg, 3 µmol, 5 mol-%) to a solution of carbonate **5.96** (24 mg, 50 µmol, 1.0 eq.) in dry THF (0.5 mL), and the orange solution was transferred slowly to the ArZnCl solution at 0 °C to form a colorless solution. After 5 min the mixture was removed from the ice-bath and further stirred at 50 °C for 3 h (until TLC indicated full conversion). Water was added to stop the reaction. The aqueous phase was extracted with Et_2O, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (pentane:Et_2O 50:1 + Et_3N) to afford protected JBIR-02 **5.2a** as colorless oil (23 mg, 35 µmol, 69%).



R_f = 0.6 (pentane:Et_2O 10:1). **Optical rotation**: $[\alpha]_D$ = -24.3° (*c* = 0.22, $CHCl_3$). **FTIR**: ν = 2954, 1597, 1470, 1345, 1252, 1192, 1155, 1068, 994, 783, 630 cm^{-1}. **^{1}H NMR** (600 MHz, $CDCl_3$) δ = 6.27 (s, 1H), 6.05 (d, *J* = 15.5 Hz, 1H), 5.71 (s, 1H), 5.48 (dt, *J* = 15.2, 7.0 Hz, 1H), 5.40 (td, *J* = 6.8, 1.2 Hz, 1H), 5.34 (q, *J* = 6.9 Hz, 1H), 5.22 (s, 2H), 5.17 (d, *J* = 9.7 Hz, 1H), 3.87 (s, 3H), 3.75-3.71 (m, 2H), 3.64 (d, *J* = 7.8 Hz, 1H), 3.42 (d, *J* = 6.9 Hz, 2H), 2.77 (d, *J* = 6.9 Hz, 2H), 2.68-2.61 (m, 1H), 2.06 (s, 3H), 1.73 (s, 3H), 1.71 (d, *J* = 1.2 Hz, 3H), 1.71 (s, 3H), 1.69 (d, *J* = 1.2 Hz, 3H), 1.67 (d, *J* = 6.8 Hz, 3H), 0.97-0.93 (m, 2H), 0.81 (s, 9H), 0.79 (d, *J* = 6.7 Hz, 3H), 0.00 (s, 9H), -0.05 (s, 3H), -0.06 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ = 164.00, 162.84, 157.04, 136.72, 136.06, 135.37, 135.31, 133.33, 133.28, 131.05, 125.29, 123.81, 122.04, 113.52, 92.64, 92.08, 84.17, 66.73, 53.35, 43.41, 37.55, 35.16, 25.91, 18.23, 18.15, 17.77, 16.70, 16.58, 13.77, 13.25, 12.98, 10.49, -1.28,

-4.51, -4.89. **HRMS ESI** calc. for $C_{39}H_{68}O_4NSi_2^+$ $[M+H]^+$: 670.4681, found: 670.4674.

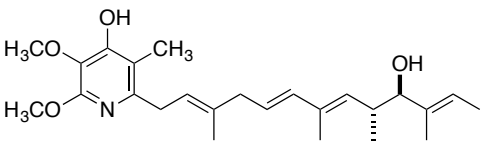
Protected Mer-A2026B (5.3a): Following the reported procedure for **5.2a**: Pyridine



5.71 (19 mg, 54 µmol, 1.25 eq.) was treated with *n*-BuLi (35 µL, 59 µmol, 1.35 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (65 µL, 65 µmol, 1.5 eq., as a 1.0 M solution in THF), carbonate **5.98** (19 mg, 44 µmol, 1 eq.) and $Pd(PPh_3)_4$ (2.5 mg, 2 µmol, 5 mol-%) in dry THF (0.5 mL) to afford protected Mer-A2026B **5.3a** (18 mg, 28 µmol, 66%) as colorless oil.

$\mathbf{R_f}$ = 0.6 (pentane:Et_2O 10:1). **Optical rotation**: $[\alpha]_D$ = -11.1° (*c* = 0.35, $CHCl_3$). **FTIR**: ν = 2954, 2856, 1598, 1471, 1381, 1345, 1249, 1058, 859, 835, 774, 631 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.27 (s, 1H), 6.04 (d, *J* = 15.5 Hz, 1H), 5.47 (dt, *J* = 15.5, 7.0 Hz, 1H), 5.40 (t, *J* = 6.8 Hz, 1H), 5.31 (q, *J* = 6.3 Hz, 1H), 5.22 (s, 2H), 5.16 (d, *J* = 9.7 Hz, 1H), 3.87 (s, 3H), 3.76-3.68 (m, 2H), 3.62 (d, *J* = 7.9 Hz, 1H), 3.42 (d, *J* = 6.8 Hz, 2H), 2.77 (d, *J* = 7.0 Hz, 2H), 2.65-2.55 (m, 1H), 2.06 (s, 3H), 1.73 (s, 3H), 1.71 (d, *J* = 0.9 Hz, 3H), 1.58 (d, *J* = 7.0 Hz, 3H), 1.55 (s, 3H), 0.98-0.91 (m, 2H), 0.79 (s, 9H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.00 (s, 9H), -0.07 (s, 3H), -0.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 164.01, 162.85, 157.05, 137.41, 136.76, 135.52, 135.37, 133.16, 125.20, 122.04, 121.30, 113.51, 92.64, 92.10, 83.74, 66.73, 53.34, 43.40, 37.41, 35.16, 25.90, 18.23, 18.15, 17.68, 16.60, 13.21, 13.06, 10.99, 10.49, -1.28, -4.65, -4.92. **HRMS ESI** calc. for $C_{36}H_{64}O_4NSi_2^+$ $[M+H]^+$: 630.4360, found: 630.4368.

Piericidin A_1 (5.1): Following the reported procedure for **5.2a**: **5.23** (9.0 mg,



24 µmol, 1.3 eq.) was treated with *n*-BuLi (16 µL, 25 µmol, 1.35 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (28 µL, 28 µmol, 1.5 eq., as a 1.0 M solution in THF), carbonate **5.98** (8 mg, 18 µmol, 1.0 eq.) and $Pd(PPh_3)_4$ (0.9 mg, 1 µmol, 5 mol %) in dry THF (0.5 mL) to afford protected

piericidin A1 **5.1a** (ca. 8 mg) as colorless oil as a inseparable 1:1 mixture with dehalogenated pyridone.

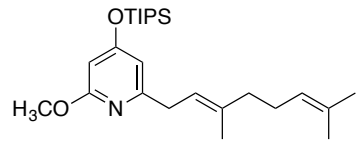
$\mathbf{R}_f$ = 0.7 (CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.05 (d, *J* = 15.5 Hz, 1H), 5.48 (dt *J* = 14.9, 7.6 Hz, 1H), 5.41 (dd, *J* = 6.9, 5.7 Hz, 1H), 5.31 (q, *J* = 6.6 Hz, 1H), 5.28 (s, 2H), 5.17 (d, *J* = 9.7 Hz, 1H), 3.94 (s, 3H), 3.83-3.79 (m, 2H), 3.78 (s, 3H), 3.62 (d, *J* = 7.9 Hz, 1H), 3.38 (d, *J* = 6.9 Hz, 2H), 2.77 (d, *J* = 7.0 Hz, 2H), 2.66-2.54 (m, 1H), 2.15 (d, *J* = 0.7, 3H), 2.12 (s, 3H), 1.72 (s, 3H), 1.71 (d, *J* = 1.1 Hz, 3H), 1.57 (d, *J* = 5.6 Hz, 3H), 1.55 (d, *J* = 1.0 Hz, 3H), 0.99-0.94 (m, 2H), 0.80 (s, 9H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.01 (s, 9H), -0.07 (s, 3H), -0.09 (s, 3H).

The crude material was directly subjected to deprotection with TBAF: Following the reported procedure for **5.2a**: A mixture of pyridine and protected piericidin **5.2a** (ca. 8.0 mg) was treated with TBAF (110 µL, as a 1.0 M solution in THF) in dry THF (0.8 mL) to afford the natural product piericidin A_1 **5.1** (2.3 mg, 5 µmol, 29%, over 2 steps) as light-yellow oil.

$\mathbf{R}_f$ = 0.4 (hexane EtOAc 1:1). **Optical rotation**: $[\alpha]_D$ = -0.5° (*c* = 0.1, MeOH); Lit.: $[\alpha]_D$ = +1.0° (*c* = 0.1, MeOH). **UV**: λ^{MeOH} = 273, 236, 203 nm. **FTIR**: ν = 3400, 2923, 2854, 1586, 1468, 1412, 1190, 1125, 776, 630 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.19 (br s, 1H), 6.09 (d, *J* = 15.0 Hz, 1H), 5.61 (dt, *J* = 15.4, 7.0 Hz, 1H), 5.48 (dd, *J* = 6.8, 2.1 Hz, 1H), 5.41 (dt, *J* = 6.9, 1.2, 1H), 5.21 (d, *J* = 9.7 Hz, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 3.62 (d, *J* = 9.1 Hz, 1H), 3.37 (s, 3H), 2.79 (d, *J* = 7.0 Hz, 1H), 2.72-2.63 (m, 1H), 2.09 (s, 3H), 1.80 (d, *J* = 1.2 Hz, 3H), 1.75 (s, 3H), 1.64 (s, 3H), 1.62 (s, 3H), 0.80 (d, *J* = 6.7 Hz, 3H). **HRMS ESI** calc. for $C_{25}H_{38}O_4N^+$ $[M-H]^+$: 416.2795, found: 416.2800.

Analytical data (except optical rotation) according to reference: M. J. Schnermann, D. L. Boger, *J. Am. Chem. Soc*. **2005**, *127*, 15704-15705.

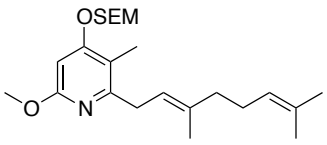
TIPS protected pyridone (5.100a): Following the reported procedure for **5.2a**:



Pyridine **5.73** (37 mg, 103 µmol, 1.0 eq.) was treated with *n*-BuLi (70 µL, 113 µmol, 1.1 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (123 µL, 123 µmol, 1.2 eq., as a 1.0 M solution in THF), geranyl carbonate (26 mg, 123 µmol, 1.2 eq.) and $Pd(PPh_3)_4$ (6 mg, 5 µmol, 5 mol-%) in dry THF (0.5 mL) to afford TIPS protected pyridone **5.100a** (18 mg, 43 µmol, 42%) as colorless oil.

R_f = 0.6 (pentane:Et_2O 10:1). **FTIR**: ν = 2945, 2868, 1598, 1454, 1408, 1362, 1262, 1158, 1056, 1009, 882, 784, 688 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.29 (d, *J* = 1.9 Hz, 1H), 6.02 (d, *J* = 1.9 Hz, 1H), 5.42 (td, *J* = 7.3 Hz, 1.2, 1H), 5.12 (t, *J* = 6.8 Hz, 1H), 3.89 (s, 3H), 3.36 (d, *J* = 7.3 Hz, 2H), 2.14-2.03 (m, 4H), 1.70 (s, 3H), 1.68 (d, *J* = 0.7 Hz, 3H), 1.60 (s, 3H), 1.30-1.19 (m, 3H), 1.09 (d, *J* = 7.2 Hz, 18H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 165.53, 165.44, 160.48, 137.41, 131.59, 124.40, 121.21, 108.52, 97.54, 53.51, 39.89, 36.71, 26.89, 25.83, 17.99, 17.80, 16.44, 12.81. **HRMS ESI** calc. for $C_{25}H_{44}NO_2Si^+$ $[M+H]^+$: 418.3136, found: 418.3132.

SEM protected pyridone (5.101a): Following the reported procedure for **5.2a**:



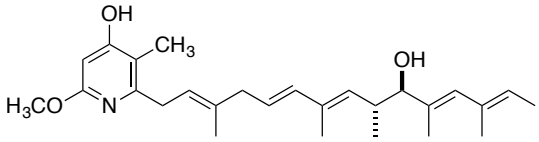
Pyridine **5.71** (21 mg, 60 µmol, 1.0 eq.) was treated with *n*-BuLi (45 µL, 72 µmol, 1.2 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (90 µL, 90 µmol, 1.5 eq., as a 1.0 M solution in THF), geranyl carbonate (26 mg, 121 µmol, 2.0 eq.) and $Pd(PPh_3)_4$ (4 mg, 3 µmol, 5 mol %) in dry THF (0.5 mL) to afford SEM protected pyridone **5.101a** (18 mg, 44 µmol, 74%) as colorless oil.

R_f = 0.6 (pentane:Et_2O 10:1). **FTIR**: ν = 2951, 2844, 1596, 1469, 1409, 1345, 1249, 1192, 1064, 991, 834, 758, 667 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.27 (s, 1H), 5.34 (t, *J* = 6.8 Hz, 1H), 5.22 (s, 2H), 5.08 (t, *J* = 6.8 Hz, 1H), 3.88 (s, 3H), 3.73 (t, *J* = 8.3 Hz, 2H), 3.41 (d, *J* = 6.8 Hz, 2H), 2.12-1.99 (m, 7H), 1.75 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H), 0.95 (t, *J* = 8.3 Hz, 2H), 0.00 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 164.00, 162.84, 157.23, 135.97, 131.46, 124.46, 121.44, 113.50, 92.62, 92.03, 66.71,

53.34, 39.86, 35.17, 26.81, 25.81, 18.14, 17.80, 16.57, 10.43, -1.29. **HRMS ESI** calc. for $C_{23}H_{40}NO_3Si^+$ $[M-H]^+$: 406.2772, found: 406.2769.

8.6.6 Deprotection

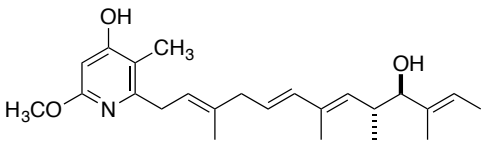
JBIR-02 (5.2): To a solution of protected JBIR-02 **5.2a** (23 mg, 34 µmol, 1.0 eq.) in



dry THF (0.5 mL) was added TBAF (170 µL, 172 µmol, 5.0 eq., as a 1.0 M solution in THF) and the brown solution was heated to 70 °C for 3.5 h until UPLC indicated no further conversion. The solution was cooled to rt, EtOAc was added and washed with H_2O. The aqueous phase was extracted three times with EtOAc and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude orange oil was subjected to flash column chromatography (hexane:EtOAc 3:1 to 1:1) to afford JBIR-02 **5.2** as colorless solid (12.1 mg, 28 µmol, 82%).

$\mathbf{R_f}$ = 0.4 (hexane:EtOAc 1:1). **Optical rotation**: $[\alpha]_D$ = -11.1° (*c* = 0.21, MeOH); Lit.: $[\alpha]_D$ = -13.0° (*c* = 0.88, MeOH). **UV**: λ^{CH_3CN} = 199, 241 nm. λ^{MeOH} = 240, 223, 202 nm. **FTIR**: ν = 3249, 2922, 2859, 1597, 1489, 1452, 1399, 1231 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.11 (d, *J* = 15.5 Hz, 1H), 5.92 (bs, 1H), 5.84 (s, 1H), 5.58 (dt, *J* = 15.5, 7.0 Hz, 1H), 5.41 (q, *J* = 6.8 Hz, 1H), 5.30 (m, 1H), 5.25 (d, *J* = 9.6 Hz, 1H), 3.79 (s, 3H), 3.65 (d, *J* = 8.9 Hz, 1H), 3.36 (d, *J* = 7.1 Hz, 2H), 2.83 (d, *J* = 6.9 Hz, 2H), 2.71 (ddq *like*, 1H), 2.03 (s, 3H), 1.80 (d, *J* = 1.1 Hz, 3H), 1.78 (s, 3H), 1.74 (s, 3H), 1.71 (d, *J* = 1.1 Hz, 3H), 1.68 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 158.6, 136.3, 135.8, 135.7, 133.9, 133.6, 133.5, 132.8, 125.9, 124.7, 122.2, 113.4, 92.4, 83.3, 54.7, 42.9, 36.9, 30.6, 17.6, 16.8, 16.7, 13.8, 13.3, 12.7, 10.3, (C1' and C3' not detected; C2' and C5' detected by low temperature 318K HMBC). **HRMS ESI** calc. for $C_{27}H_{40}NO_3^+$ $[M+H]^+$: 425.3003, found: 426.2997.

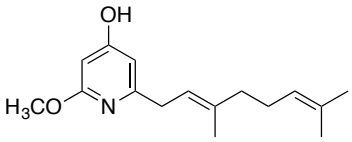
Mer-A2026B (5.3): Following the reported procedure for **5.2**: Protected Mer-A2026B



5.2a (14.0 mg, 22 µmol, 1 eq.) was treated with TBAF (110 µL, 110 mmol, 5.0 eq., as a 1.0 M solution in THF) in dry THF (0.8 mL) to afford the natural product Mer-A2026B **5.3** (8.0 mg, 21 µmol, 93%) as light-yellow oil.

R_f = 0.4 (hexane EtOAc 1:1). **Optical rotation**: $[\alpha]_D$ = -1.1° (*c* = 0.105, MeOH); Lit. $[\alpha]_D$ = -1.07° (*c* = 0.36, MeOH). **UV**: λ^{CH_3CN} = 201, 235, 272 nm. λ^{MeOH} = 238, 223, 202 nm. **FTIR**: ν = 3340, 2924, 2856, 1599, 1489, 1456, 1399, 965 cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.11 (d, *J* = 15.5 Hz, 1H), 5.87 (bs, 1H), 5.58 (dt, *J* = 15.5, 7.0 Hz, 1H), 5.31 (m, 1H), 5.25 (d, *J* = 9.7 Hz, 1H), 3.81 (s, 3H), 3.66 (d, *J* = 9.1 Hz, 1H), 3.36 (d, *J* = 7.0 Hz, 2H), 2.84 (d, *J* = 6.7 Hz, 2H), 2.69 (ddq *like*, 1H), 2.03 (s, 3H), 1.81 (s, 3H), 1.72 (s, 3H), 1.64 (s, 3H), 1.63 (d, 3H), 0.81 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ = 136.5, 136.4, 135.8, 135.5, 133.8, 130.0, 125.4, 123.7, 92.4, 82.8, 55.0, 42.9, 36.8, 29.8, 17.2, 16.5, 13.0, 13.0, 10.4, 9.9, (C1', C2', C4' and C3' not detected). **HRMS ESI** calc. for $C_{24}H_{36}O_3N^+$ $[M+H]^+$: 386.2690, found: 386.2687.

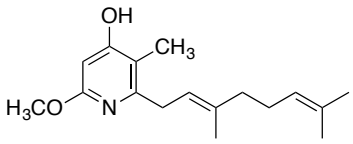
4-Hydroxy-pyridine (5.100): Following the reported procedure for **5.2**: Protected Piericidin



5.100a (18.0 mg, 43 µmol, 1 eq.) was treated with TBAF (86 µL, 86 mmol, 2.0 eq., as a 1.0 M solution in THF) in dry THF (0.8 mL) to afford 4-hydroxy-pyridine **5.100** (11 mg, 42 µmol, 98%) as light-yellow oil.

R_f = 0.4 (hexane EtOAc 1:1). **FTIR**: ν = 2916, 2674, 1612, 1489, 1361, 1229, 1152, 1073, 988, 841 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.23 (d, *J* = 1.9 Hz, 1H), 5.93 (d, *J* = 1.9 Hz, 1H), 5.30 (td, *J* = 7.3, 1.2 Hz, 1H), 5.07 (t, *J* = 6.8 Hz, 1H), 3.82 (s, 3H), 3.29 (d, *J* = 7.3 Hz, 2H), 2.16-1.97 (m, 4H), 1.67 (d, *J* = 0.6 Hz, 3H), 1.65 (s, 3H), 1.59 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 163.12, 139.50, 131.94, 124.07, 119.22, 108.19, 93.03, 54.89, 39.78, 34.06, 26.62, 25.80, 17.84, 16.38 (C1' and C3' not detected). **HRMS ESI** calc. for $C_{16}H_{24}NO_2^+$ $[M-H]^+$: 262.1802, found: 262.1802.

4-Hydroxy-pyridine (5.101): Following the reported procedure for **5.2**: Protected



piericidin **5.101a** (13.0 mg, 32 µmol, 1 eq.) was treated with TBAF (64 µL, 64 mmol, 2.0 eq., as a 1.0 M solution in THF) in dry THF (0.8 mL) to afford the title compound **5.101** (7.0 mg, 25 µmol, 79%) as light-yellow oil.

$\mathbf{R}_f$ = 0.4 (hexane EtOAc 1:1). **FTIR**: ν = 2927, 1613, 1534, 1490, 1451, 1382, 1261, 1231, 1192, 1141, 1083, 831, 695 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 5.96 (s, 1H), 5.24 (dd, *J* = 7.2 Hz, 6.2, 1H), 5.06 (dd, *J* = 4.7, 3.3 Hz, 1H), 3.81 (s, 3H), 3.38 (d, *J* = 7.2 Hz, 2H), 2.17-2.08 (m, 4H), 2.02 (s, 3H), 1.70 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 159.56, 132.34, 123.74, 118.02, 92.34, 55.39, 46.03, 39.73, 26.45, 25.79, 17.88, 16.49, 10.13, 8.77 (C1', C2', C4' and C3' not detected). **HRMS ESI** calc. for $C_{17}H_{26}NO_2^+$ $[M-H]^+$: 276.1958, found: 276.1959.

8.7 Bipyridine Ligands

6,6'-Dimethoxy-2,2'-bipyridine (6.3): To a cold (-78 °C) solution of 2-bromo-6-methoxypyridine **5.67** (70 mg, 0.36 mmol, 1 eq.) in dry THF (2 mL) was added *n*-BuLi (250 µL, 0.39 mmol, 1.1 eq., as a 1.6 M solution in cyclohexane). The reaction mixture turned orange and the solution was stirred for 10 min at this temperature. Then $ZnCl_2$ (450 µL, 0.45 mmol, 1.25 eq., as a 1.0 M solution in THF) was added and the solution slowly allowed to reach RT to form a white suspension. Then a solution of 2-bromo-6-methoxypyridine (70 mg, 0.36 mmol, 1 eq.) and $Pd(PPh_3)_4$ (21 mg, 0.02 mmol, 5 mol %) in dry THF (2 mL) at RT was added and the mixture (orange-white suspension) stirred further at 50 °C for 6 h to form an orange solution, until TLC reaction control indicated full conversion (UV active at 366 nm). The reaction mixture was quenched by addition of sat. aqueous NH_4Cl solution, EDTA solution and extracted with EtOAc. The aqueous phase was extracted with EtOAc, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was subjected to flash column chromatography (CH_2Cl_2 to CH_2Cl_2:MeOH 50:1) to afford dimethoxy-2,2'-bipyridine **6.3** (71 mg, 0.33 mmol, 91%) as colorless crystals.

M.p. = 120-121 °C. $\mathbf{R_f}$ = 0.5 (CH_2Cl_2, strong tailing). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.01 (dd, *J* = 7.4, 0.7 Hz, 2H), 7.68 (dd, *J* = 8.1, 7.5 Hz, 2H), 6.75 (dd, *J* = 8.2, 0.7 Hz, 2H), 4.04 (s, 6H).

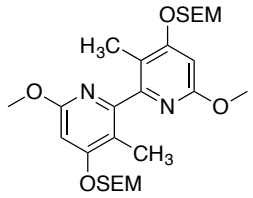
Analytical data according to reference: M. Tiecco, L. Testaferri, M. Tingoli, D. Chianelli, M. Montanucci, *Synthesis* **1984**, 736-738.

TIPS protected 2,2'-bipyridine (6.4): Following the reported procedure for **6.3**: Pyridine **5.73** (75 mg, 202 µmol, 1.0 eq.) in dry THF (2 mL) was treated with *n*-BuLi (139 µL, 222 µmol, 1.1 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (252 µL, 252 µmol, 1.25 eq., as

a 1.0 M solution in THF), pyridine **5.73** (75 mg, 202 µmol, 1.0 eq.) and $Pd(PPh_3)_4$ (11 mg, 10 µmol, 5 mol %) in dry THF (2 mL) to afford TIPS protected 2,2'-bipyridine **6.4** (92 mg, 123 µmol, 61%) as colorless oil.

$\mathbf{R_f}$ = 0.5 (CH_2Cl_2, strong tailing). **FTIR**: ν = 2945, 2867, 1583, 1463, 1418, 1378, 1275, 1172, 1047 881, 824, 633 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 7.61 (d, *J* = 2.0 Hz, 2H), 6.22 (d, *J* = 2.0 Hz, 2H), 3.99 (s, 6H), 1.32 (sep, *J* = 7.2, 6H), 1.13 (d, *J* = 7.3, 36H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.78, 165.29, 154.44, 107.66, 100.71, 53.40, 18.04, 12.81. **HRMS ESI** calc. for $C_{30}H_{53}O_4N_2Si_2^+$ $[M-H]^+$: 561.3538, found: 561.3545.

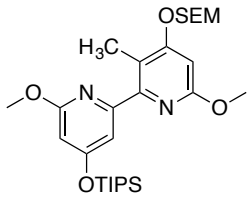
SEM protected 2,2'-bipyridine (6.5): Following the reported procedure for **6.3**:



Pyridine **5.71** (40 mg, 115 µmol, 1.0 eq.) in dry THF (1.5 mL) was treated with *n*-BuLi (79 µL, 126 µmol, 1.1 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (144 µL, 144 µmol, 1.25 eq., as a 1.0 M solution in THF), pyridine **5.71** (40 mg, 115 µmol, 1.0 eq.) and $Pd(PPh_3)_4$ (7 mg, 6 µmol, 5 mol %) in dry THF (2 mL) to afford SEM protected 2,2'-bipyridine **6.5**(40 mg, 75 µmol, 64%) as colorless oil.

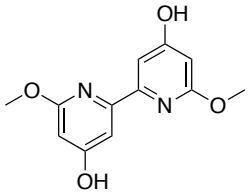
$\mathbf{R_f}$ = 0.5 (CH_2Cl_2:MeOH 50:1, strong tailing). **FTIR**: ν = 2947, 2867, 1584, 1463, 1379, 1191, 1174, 1055, 834, 693, 631 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 6.44 (s, 2H), 5.29 (s, 4H), 3.88 (s, 6H), 3.76 (dd, *J* = 8.9, 7.8 Hz, 4H), 1.93 (s, 6H), 1.00-0.92 (m, 4H), 0.02 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 164.44, 162.99, 154.70, 132.31, 132.21, 132.08, 128.70, 128.58, 115.37, 94.05, 92.67, 66.84, 53.65, 18.17, 11.32, -1.26. **HRMS ESI** calc. for $C_{26}H_{45}O_6N_2Si_2^+$ $[M-H]^+$: 537.2811, found: 537.2815.

Unsymmetrical protected 2,2'-bipyridine (6.6): Following the reported procedure for **6.3**: Pyridine **5.71** (40 mg, 115 µmol, 1.0 eq.) in dry THF (1.5 mL) was treated with *n*-BuLi (79 µL, 126 µmol, 1.1 eq., as a 1.6 M solution in cyclohexane), $ZnCl_2$ (144 µL, 144 µmol, 1.25 eq., as a 1.0 M solution in THF), pyridine **5.73** (41 mg, 115 µmol, 1.0 eq.) and $Pd(PPh_3)_4$ (7 mg, 6 µmol, 5 mol %) in dry THF (2 mL) to afford unsymmetrical protected 2,2'-bipyridine **6.6** (11 mg, 20 µmol, 18%) as colorless oil.



$\mathbf{R_f}$ = 0.5 (CH_2Cl_2:MeOH 50:1, strong tailing). **FTIR**: ν = 2947, 2867, 1585, 1565, 1401, 1379, 1250, 1176, 1158, 1061, 983, 834, 632 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.11 (d, *J* = 2.0 Hz, 1H), 6.45 (s, 1H), 6.21 (d, *J* = 2.0 Hz, 1H), 5.29 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.78-3.73 (m, 2H), 2.36 (s, 3H), 1.35-1.25 (m, 3H), 1.12 (d, *J* = 7.3, 18H), 1.00-0.94 (m, 2H), 0.01 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 165.27, 164.92, 164.64, 162.48, 157.78, 152.57, 146.79, 116.02, 111.23, 99.78, 94.37, 92.74, 66.79, 53.70, 53.31, 18.17, 18.02, 12.83, 12.03, -1.27, -1.30. **HRMS ESI** calc. for $C_{28}H_{49}O_5N_2Si_2^+$ $[M-H]^+$: 549.3175, found: 549.3181.

4,4'-Dihydroxy-2,2'-bipyridine (6.7): To a solution of TIPS protected 2,2'-bipyridine **6.4** (45 mg, 80 µmol, 1 eq.) in dry THF (2 mL) was added TBAF (400 µL, 400 µmol, 5 eq., as a 1 M solution in THF) at RT and the resulting mixture was further stirred at 50 °C until TLC indicated full conversion. The white suspension was acidified using aqueous 1 M HCl and extracted with EtOAc. The aqueous phase was extracted with EtOAc, washed with water and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oil was washed with $CHCl_3$ (3 x) to afford after removal of all volatiles 4,4'-dihydroxy-2,2'-bipyridine **6.7** (15 mg, 60 µmol, 75%) as colorless solid.



M.p. = >350 °C (decomposition). $\mathbf{R_f}$= 0.05 (EtOAc). **FTIR**: ν = 3105, 2857, 1610, 1560, 1477, 1370, 1211, 1165, 1062, 855 711 cm^{-1}. **^{1}H NMR** (400 MHz, d_4-MeOD) δ = 7.28 (d, *J* = 1.9 Hz, 2H), 6.54 (d, *J* =1.7 Hz, 2H), 4.10 (s, 6H). **^{1}H NMR**

(400 MHz, d_6-DMSO) δ = 10.69 (s, 2H), 7.45 (d, *J* = 2.0 Hz, 2H), 6.14 (d, *J* = 1.9 Hz, 2H), 3.89 (s, 6H). **HRMS ESI** calc. for $C_{12}H_{13}N_2O_4^+$ $[M-H]^+$: 249.0870, found: 249.0873.

9 APPENDICES SECTION

9.1 Crystal structures

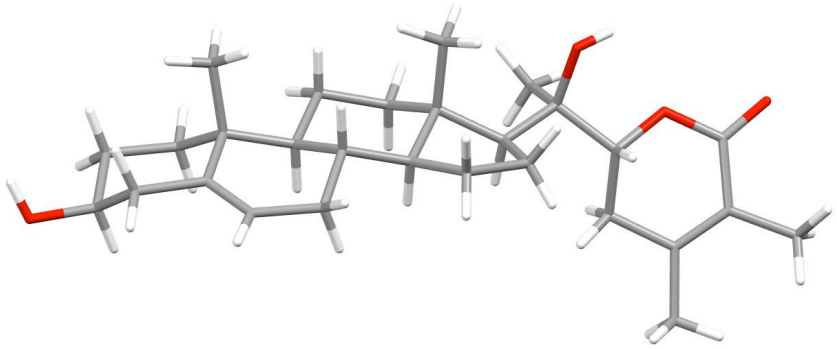


Table 9.1. Crystal data for **2.27** (CCDC 816947).

Formula	$C_{28}H_{42}O_4$
Formula weight	442.64
Z, calculated density	4, 1.174 Mg · m^{-3}
F(000)	968
Description and size of crystal	colorless, 0.08 · 0.17 · 0.23 mm^3
Absorption coefficient	0.076 mm^{-1}
Min/max transmission	0.99 / 0.99
Temperature	123 K
Radiation (wavelength)	Mo Kα (λ = 0.71073 Å)
Crystal system, space group	orthorhombic, *P* 2_1 2_1 2_1
a	8.8903(2) Å
b	11.1253(3) Å
c	25.3120(8) Å
α	90°
β	90°
γ	90°
V	2503.54(12) $Å^3$
Min/max Θ	2.000° / 37.817°
Number of collected reflections	87315
Number of independent reflections	7430 (merging r = 0.045)
Number of observed reflections	5937 (I>3.0σ(I))
Number of refined parameters	289
r	0.0451 (I>3.0σ(I))
rW	0.1064 (all data)
Goodness of fit	1.0530

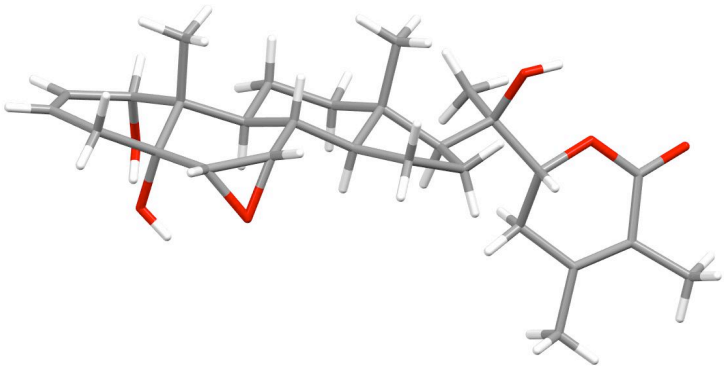


Table 9.2. Crystal data for **2.36** (CCDC 816949).

Formula	$C_{28}H_{40}O_6$, $C_4H_8O_2$
Formula weight	560.73
Z, calculated density	4, 1.273 Mg · m^{-3}
F(000)	1216
Description and size of crystal	colorless prism, 0.09 · 0.14 · 0.25 mm^3
Absorption coefficient	0.090 mm^{-1}
Min/max transmission	0.99 / 0.99
Temperature	123 K
Radiation(wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	orthorhombic, $P\,2_1\,2_1\,2_1$
a	11.8379(5) Å
b	13.3459(6) Å
c	18.5218(9) Å
α	90°
β	90°
γ	90°
V	2926.2(2) $Å^3$
Min/max Θ	1.881° / 32.358°
Number of collected reflections	65338
Number of independent reflections	5762 (merging r = 0.045)
Number of observed reflections	4785 (I>3.0σ(I))
Number of refined parameters	416
r	0.0287 (I>3.0σ(I))
rW	0.0479 (all data)
Goodness of fit	1.1101

The solvent molecule in compound **2.36** (EtOAc) has been refined using restraints in order to keep the geometrical parameters within reasonable ranges.

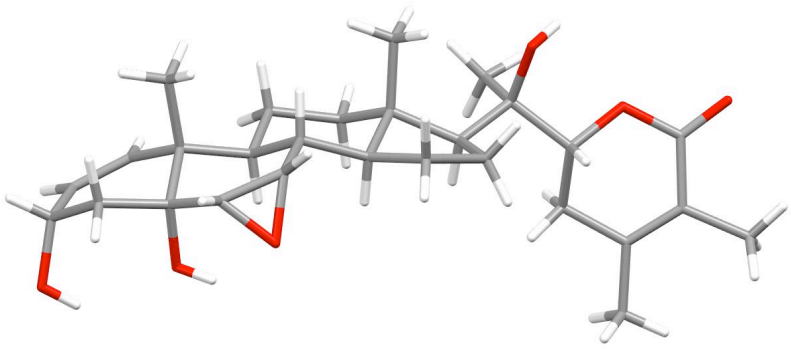


Table 9.3. Crystal data for allylic alcohol **2.43** (CCDC 924130).

Formula	$C_{28}H_{40}O_6$
Formula weight	472.62
Z, calculated density	4, 1.256 Mg · m^{-3}
F(000)	1024
Description and size of crystal	colorless, 0.030 · 0.090 · 0.270 mm^3
Absorption coefficient	0.087 mm^{-1}
Min/max transmission	0.99 / 1.00
Temperature	123 K
Radiation (wavelength)	Mo Kα (λ = 0.71073 Å)
Crystal system, space group	orthorhombic, *P* 2_1 2_1 2_1
a	11.3881(10) Å
b	13.7611(13) Å
c	15.9461(16) Å
α	90°
β	90°
γ	90°
V	2499.0(4) $Å^3$
Min/max Θ	1.955° / 36.318°
Number of collected reflections	42220
Number of independent reflections	6467 (merging r = 0.050)
Number of observed reflections	4541 (I>2.0σ(I))
Number of refined parameters	307
r	0.0394
rW	0.0673 (all data)
Goodness of fit	1.1096

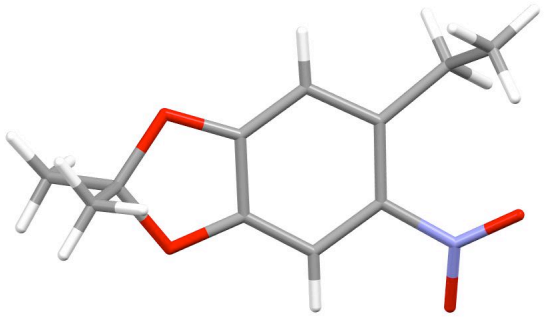


Table 9.4. Crystal data for acetonide **3.16** (CCDC881462).

Formula	$C_{11}H_{13}NO_4$
Formula weight	223.23
Z, calculated density	4, 1.374 Mg · m^{-3}
F(000)	472
Description and size of crystal	colorless, 0.030·0.110·0.230 mm^3
Absorption coefficient	0.105 mm^{-1}
Min/max transmission	0.99 / 1.00
Temperature	123K
Radiation(wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	monoclinic, $P\ 2_1/n$
a	9.3296(17) Å
b	12.264(2) Å
c	10.4903(19) Å
α	90°
β	116.015(6)°
γ	90°
V	1078.7(3) $Å^3$
Min/max Θ	2.442° / 26.371°
Number of collected reflections	9848
Number of independent reflections	2203 (merging r = 0.107)
Number of observed reflections	1516 (I>2.0σ(I))
Number of refined parameters	145
r	0.0432
rW	0.0903
Goodness of fit	0.9781

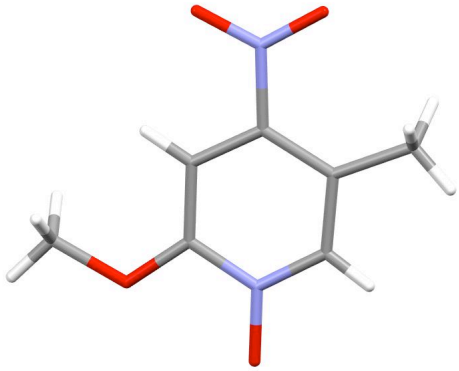


Table 9.5. Crystal data for *N*-oxide **5.63**.

Formula	$C_7H_8N_2O_4$
Formula weight	184.15 g · Mol^{-1}
Z, calculated density	12, 1.605 Mg · m^{-3}
F(000)	1152
Description and size of crystal	colorless, 0.050 · 0.080 · 0.330 mm^3
Absorption coefficient	0.134 mm^{-1}
Min/max transmission	0.99 / 0.99
Temperature	123K
Radiation (wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	monoclinic, $P\ 2_1/c$
a	11.6811(6) Å
b	10.1479(5) Å
c	19.3476(9) Å
α	90°
β	94.720(3)°
γ	90°
V	2285.66(19) $Å^3$
Min/max Θ	1.749° / 31.552°
Number of collected reflections	35833
Number of independent reflections	7601 (merging r = 0.072)
Number of observed reflections	4068 (I>2.0σ(I))
Number of refined parameters	352
r	0.0613
rW	0.1602
Goodness of fit	1.0818

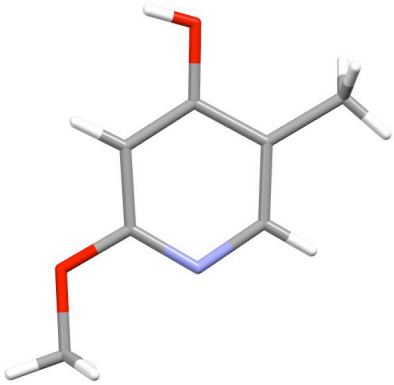


Table 9.6. Crystal data of 4-hydroxypyridine **5.64**.

Formula	$C_7H_9NO_2$
Formula weight	139.15 g · Mol^{-1}
Z, calculated density	4, 1.298 Mg · m^{-3}
F(000)	296
Description and size of crystal	colorless, 0.020 · 0.080 · 0.190 mm^3
Absorption coefficient	0.096 mm^{-1}
Min/max transmission	0.99 / 1.00
Temperature	123 K
Radiation (wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	monoclinic, $P\ 2_1/n$
a	4.6487(6) Å
b	14.2532(18) Å
c	10.8700(15) Å
α	90°
β	98.746(8)°
γ	90°
V	711.86(16) $Å^3$
Min/max Θ	2.374° / 30.104°
Number of collected reflections	7111
Number of independent reflections	2094 (merging r = 0.058)
Number of observed reflections	1035 (I>2.0σ(I))
Number of refined parameters	95
r	0.0530
rW	0.1046
Goodness of fit	1.1020
Bond length C-OMe	**1.353 Å**
Bond length C-OH	**1.345 Å**

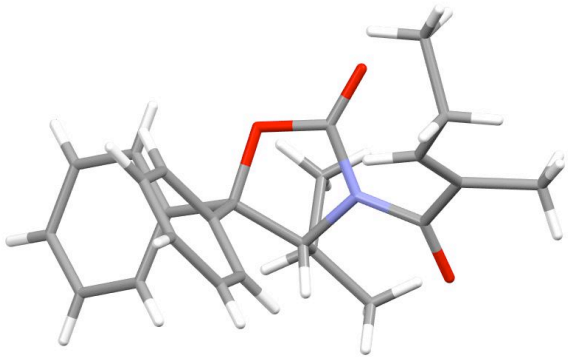


Table 9.7. Crystal data of acylated auxiliary **5.73**.

Formula	$C_{24}H_{27}NO_3$
Formula weight	377.48
Z, calculated density	4, 1.221 Mg · m^{-3}
F(000)	808
Description and size of crystal	colorless, 0.070 · 0.110 · 0.230 mm^3
Absorption coefficient	0.080 mm^{-1}
Min/max transmission	0.99 / 0.99
Temperature	123 K
Radiation (wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	orthorhombic, $P\ 2_1\ 2_1\ 2_1$
a	9.3102(9) Å
b	12.0749(12) Å
c	18.2636(18) Å
α	90°
β	90°
γ	90°
V	2053.2(3) $Å^3$
Min/max Θ	2.022° / 27.506°
Number of collected reflections	34925
Number of independent refections	2671 (merging r = 0.122)
Number of observed reflections	2113 (I>2.0σ(I))
Number of refined parameters	253
r	0.0590
rW	0.0754
Goodness of fit	1.0135

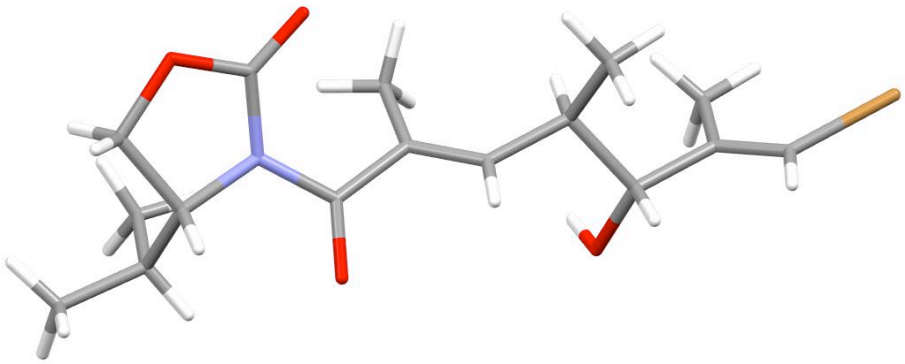


Table 9.8. Crystal data for alcohol **5.79** (CCDC916688).

Formula	$C_{16}H_{26}BrNO_5$
Formula weight	392.29 g · mol^{-1}
Z, calculated density	2, 1.436 Mg · m^{-3}
F(000)	408
Description and size of crystal	colorless, 0.030 · 0.110 · 0.350 mm^3
Absorption coefficient	2.288 mm^{-1}
Min/max transmission	0.78 / 0.93
Temperature	123K
Radiation (wavelength)	Mo K_α (λ = 0.71073 Å)
Crystal system, space group	monoclinic, P 2_1
a	11.2472(3) Å
b	6.4690(2) Å
c	13.0993(3) Å
α	90°
β	107.800(2)°
γ	90°
V	907.46(4) $Å^3$
Min/max Θ	1.902° / 31.503°
Number of collected reflections	13039
Number of independent reflections	5832 (merging r = 0.031)
Number of observed reflections	4357 (I>2.0σ(I))
Number of refined parameters	209
r	0.0283
rW	0.0316
Goodness of fit	0.9690

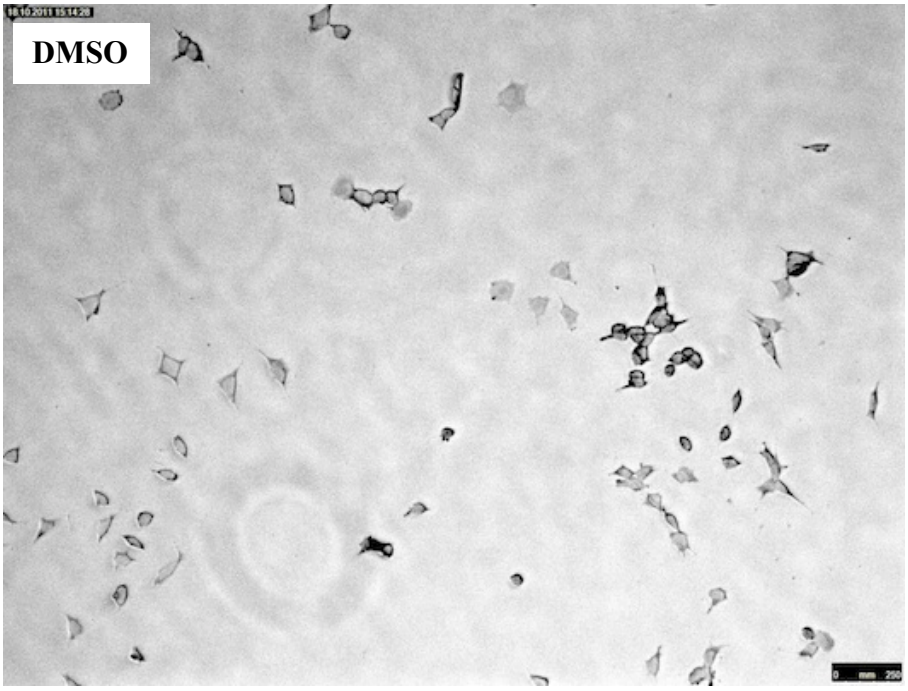
9.2 ^{1}H and ^{13}C NMR Spectra

^{1}H and ^{13}C NMR Spectra for the all characterized compounds can be found on the enclosed CD to this PhD thesis.

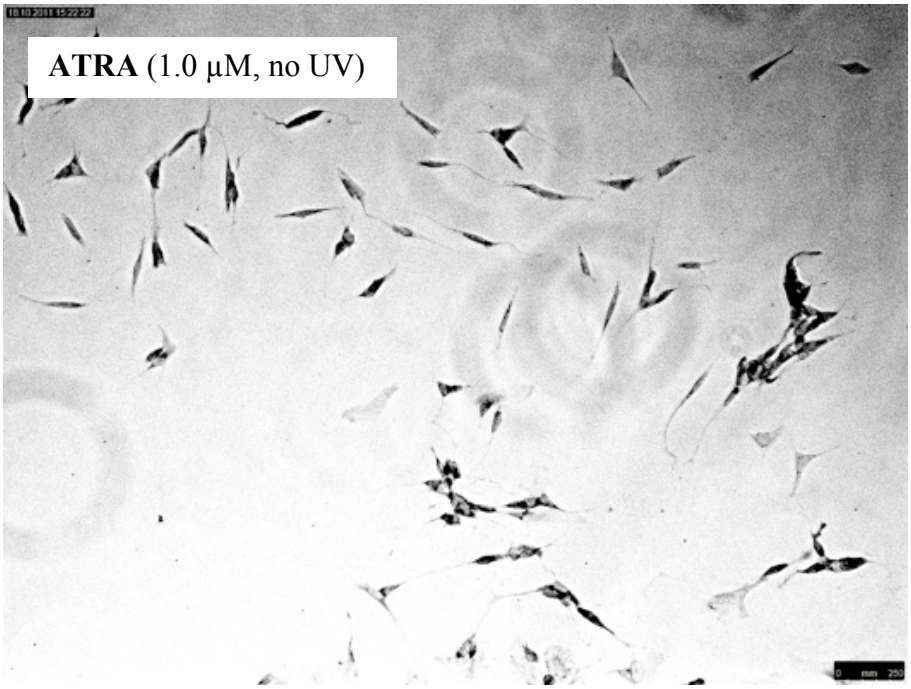
9.3 Representative Micrographs

Neurite outgrowth activity of DMSO (**A**), ATRA **4.1** (1 μM; **B**), retinoids **4.3** (1 μM; **C** and 0.1 μM; **D**), **4.6** (1 μM; **E**) and **4.6** (1 μM; **F**) in human SH-SY5Y cells with MEM as cell medium.

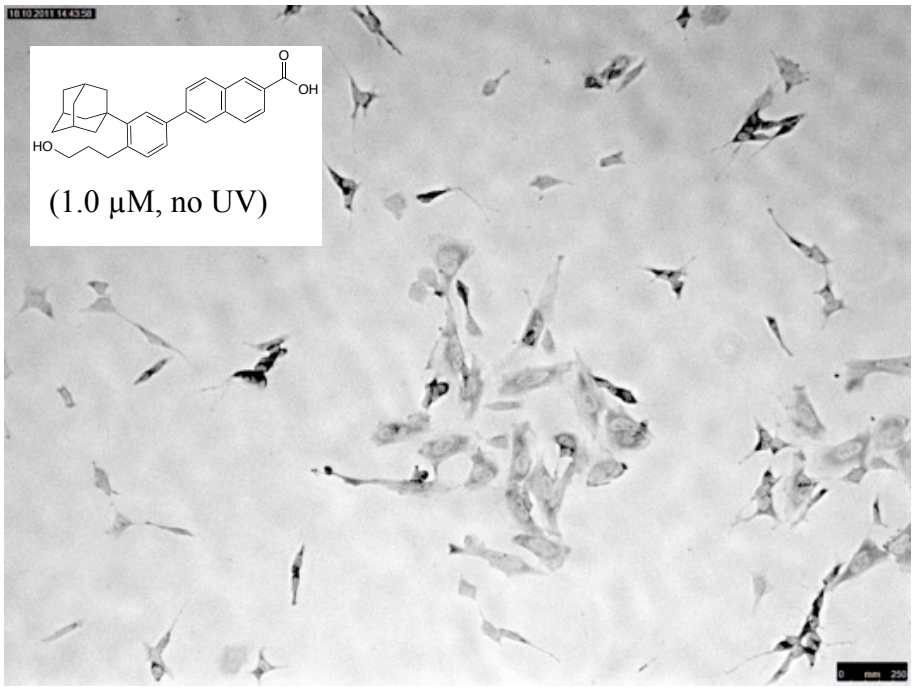
A



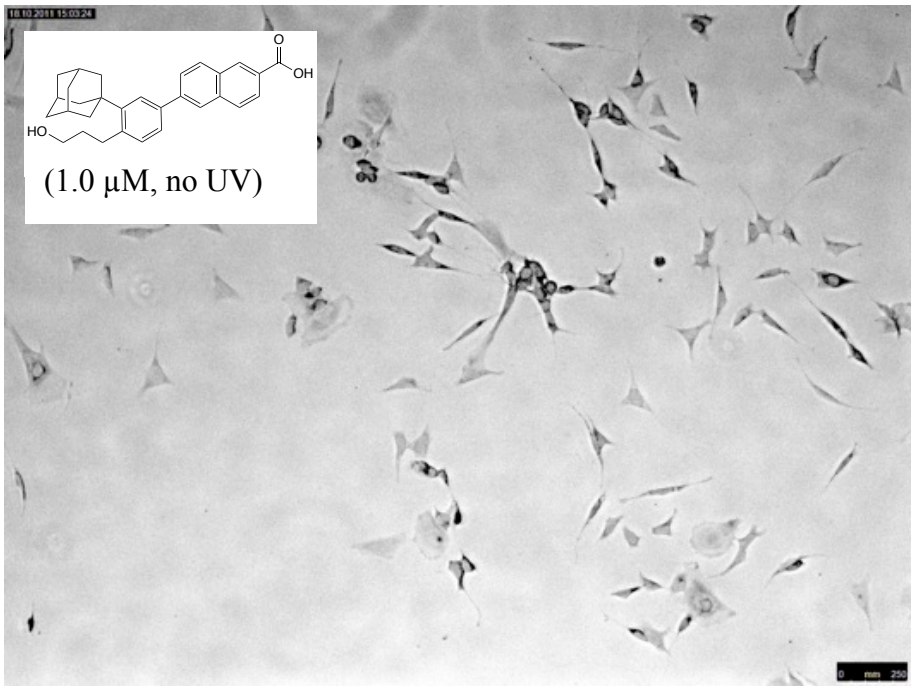
B



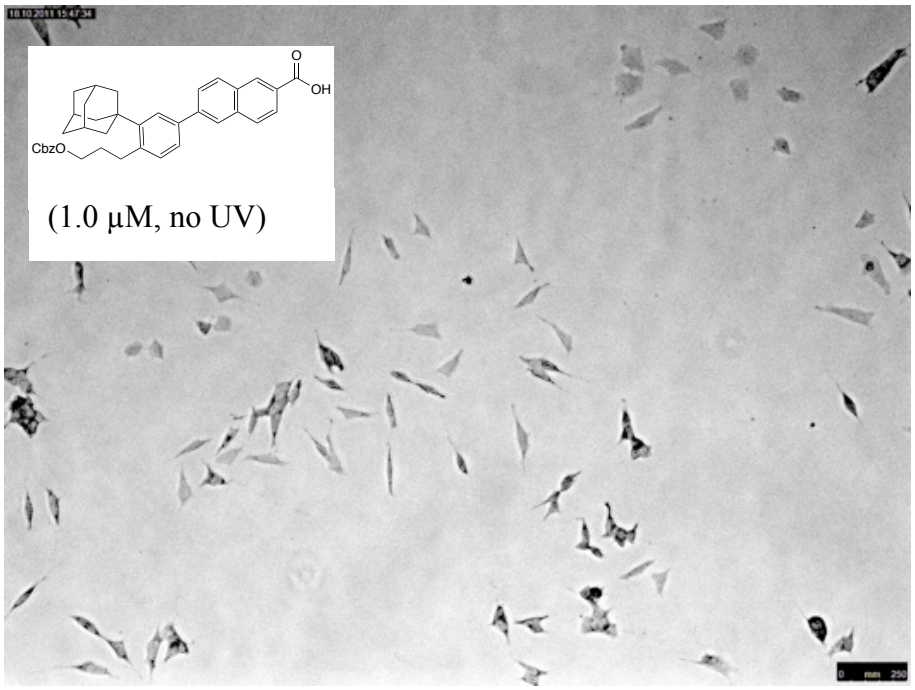
C



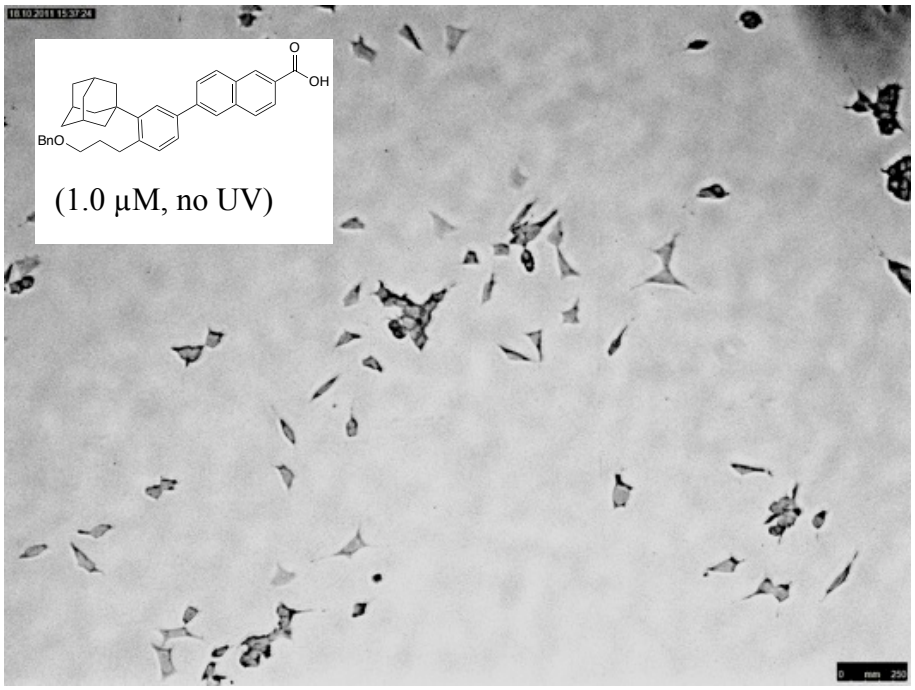
D



E

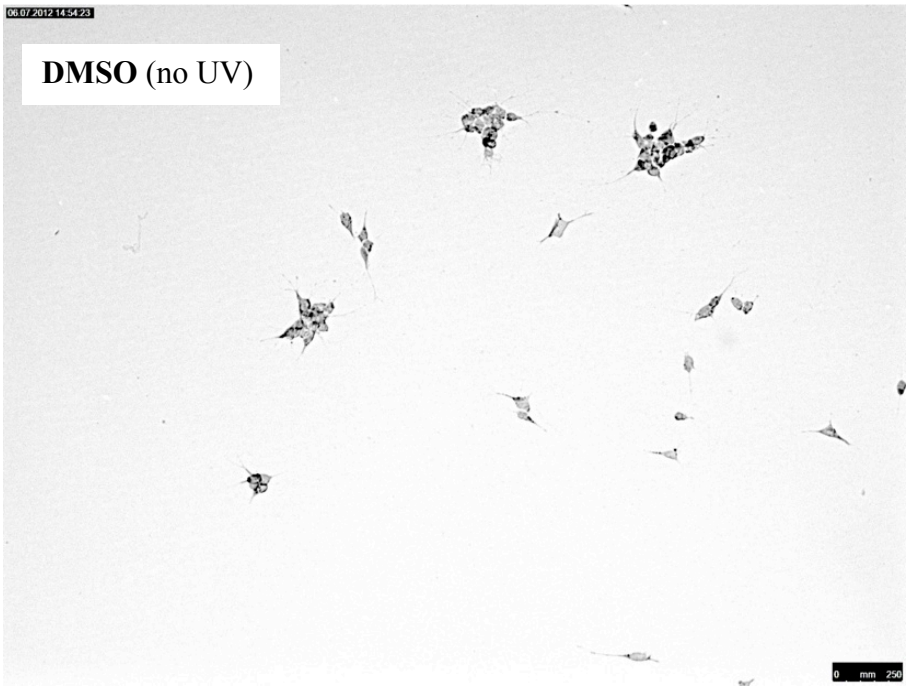


F

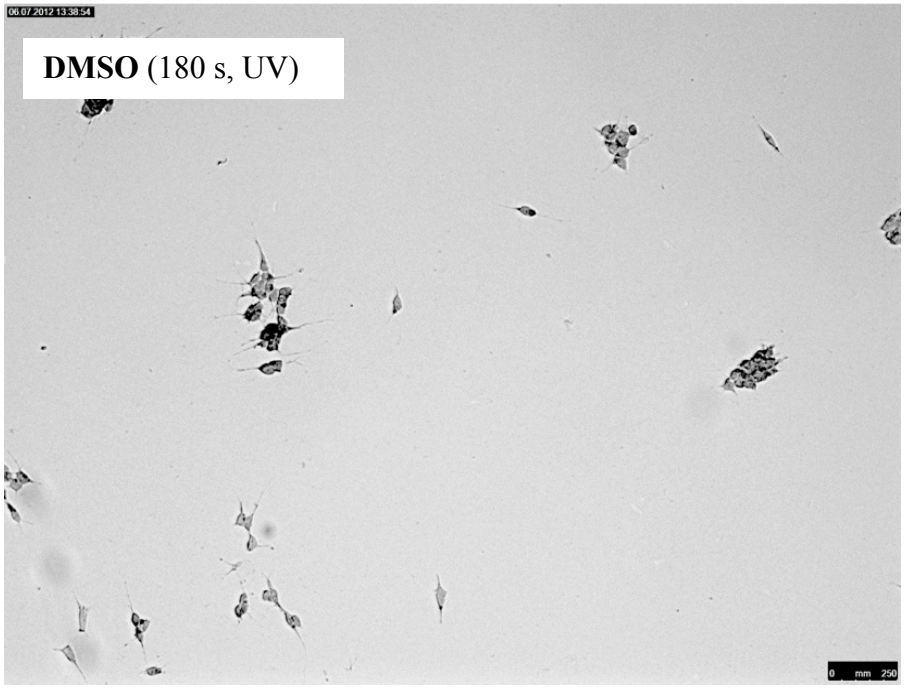


Neurite outgrowth activity of DMSO control without UV irradiation (no UV, **A**), DMSO control, with 180 s UV irradiation at 366 nm (180 s UV, **B**), retinoid **4.3** (0.1 μM, no UV, **C**), caged retinoid **4.5** (1 μM, no UV, **D**), **4.5** (1 μM, 60 s UV, **E**), **4.5** (0.1 μM, 180 s UV, **F**), **4.5** (1 μM, 180 s UV, **G**) in human SH-SY5Y cells.

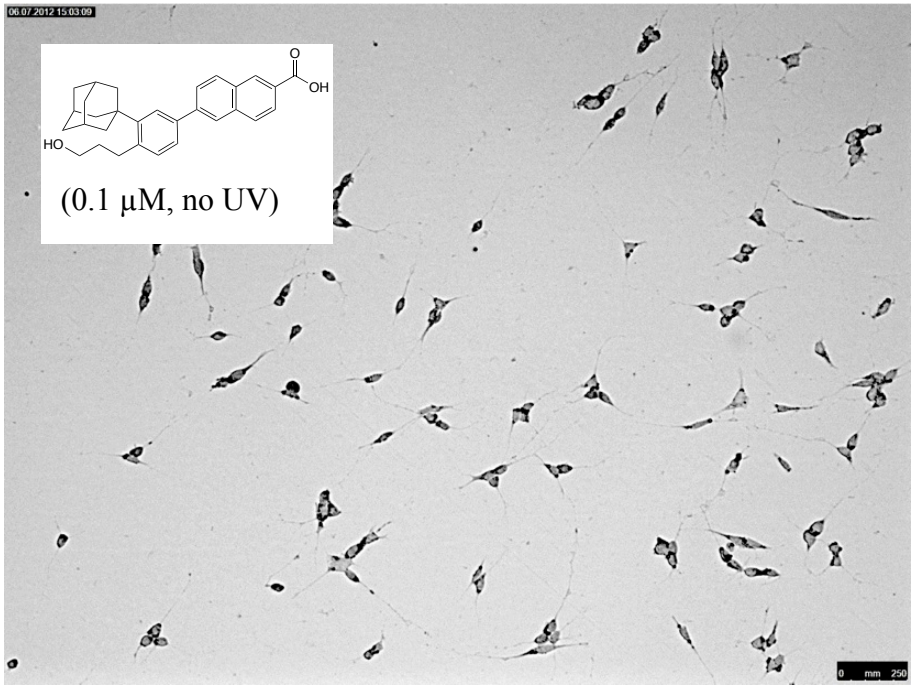
A



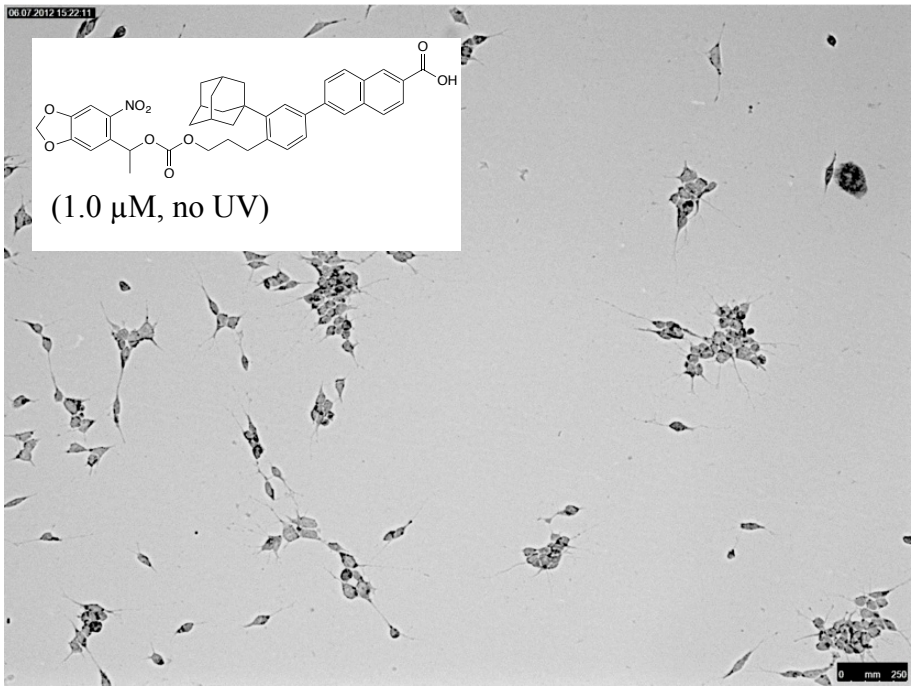
B



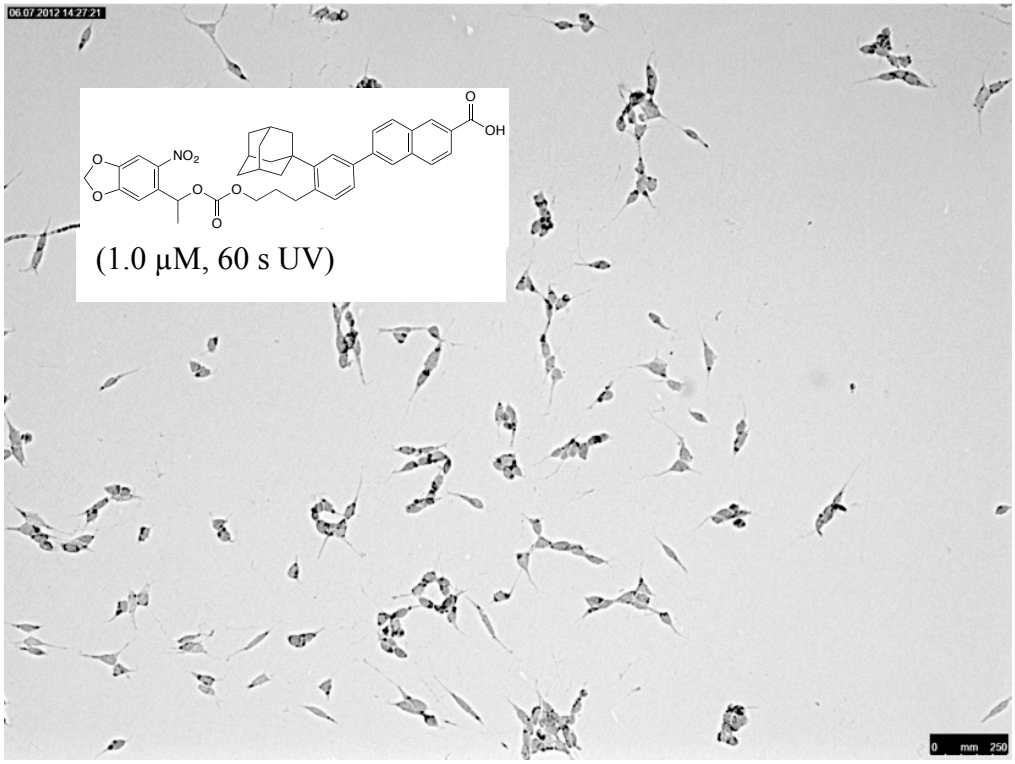
C


06.07.2012 15:03:09
HO
O
OH
(0.1 μM, no UV)
0 mm 250

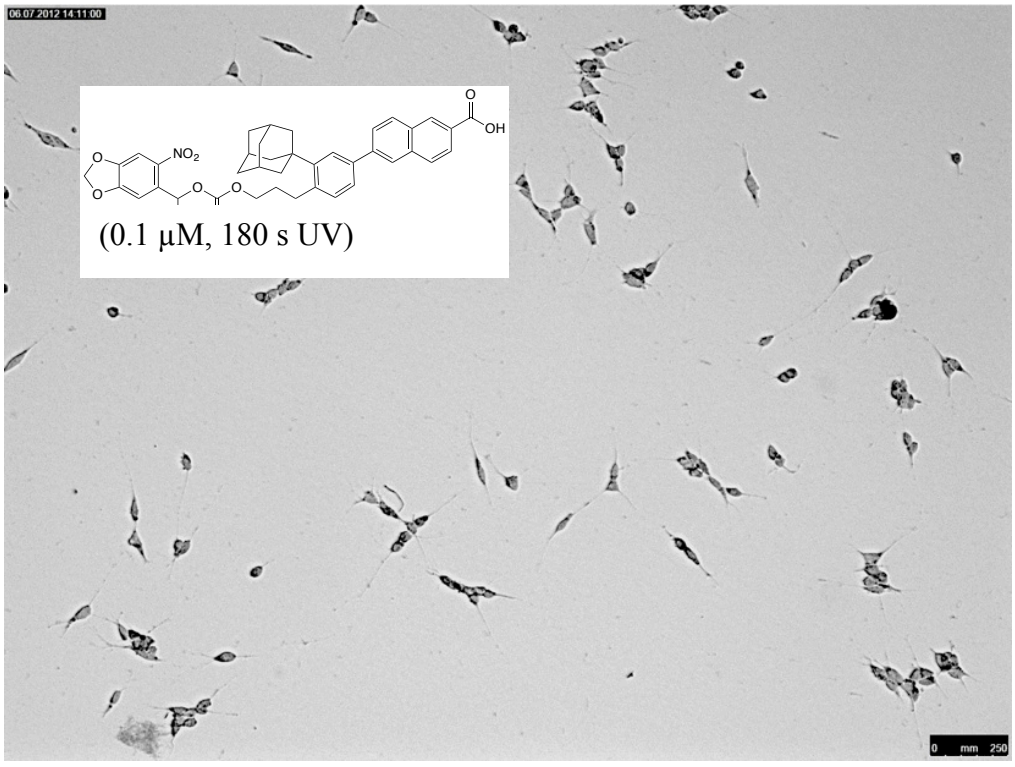
D


06.07.2012 15:22:11
O
OH
NO2
O
O
O
O
(1.0 μM, no UV)
0 mm 250

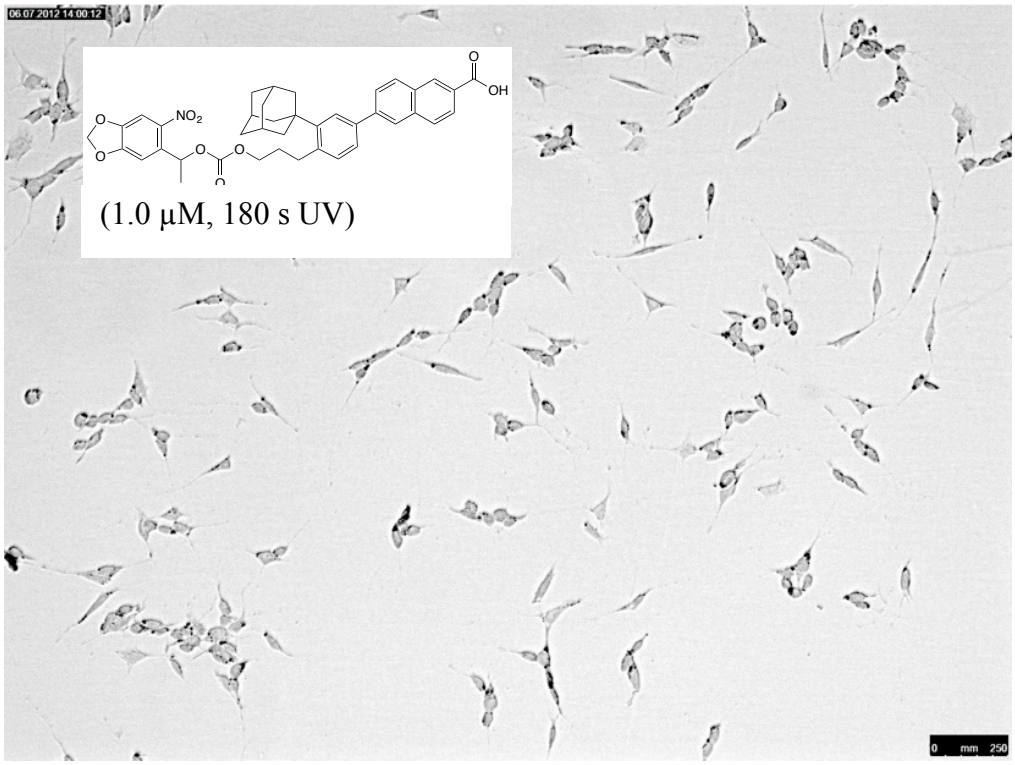
E


06.07.2012 14:27:21
NO2
O
OH
(1.0 µM, 60 s UV)
0 mm 250

F


06.07.2012 14:11:00
NO2
O
OH
(0.1 µM, 180 s UV)
0 mm 250

G



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[160] „Fussball ist wie Schach, nur ohne Würfel.“ (Eng.: Football is like chess, but without dice, J. Böhmermann)

Curriculum Vitae

Johannes David Hoecker, born 21st August 1983 in Bad Kreuznach, Germany

02.2010 – 06.2013	Dissertation at the **University of Basel** PhD thesis in organic chemistry under the supervision of Prof. Dr. K. Gademann: *Stereoselective Total Syntheses of Piericidins, the Neuritogenic Steroid Withanolide A and the Development of Photolabile Surface Anchors*
09.2009 - 01.2010	Trainee at **Actelion Pharmaceuticals Ltd** in Allschwil Drug Discovery Chemistry Department: Multi-step organic synthesis, purification and analysis of new organic compounds for the identification of potent antagonists
02.2008 - 04.2008	Trainee at **BASF SE** in Ludwigshafen am Rhein Department of Global R & D Crop Protection; research area melamine-formaldehyde resins: development of analysis methods, screening and optimization of reaction conditions
08.2007 - 01.2008	Exchange year at the **University of Aarhus** Research project in the group of Prof. Dr. H. Birkedal
04.2004 - 07.2009	Diploma in chemistry at the **University of Heidelberg** Diploma thesis in organic chemistry under the supervision of Prof. Dr. G. Helmchen: *Synthesis of Sphingosine and Azasugars through Iridium catalyzed allylic Substitution as well as Methodical Studies for Iodolactonization*
04.2003 - 01.2004	**Schmiedel Gesellschaft e.V.** in Bad Kreuznach Community service: support of behavior challenged children and teenager in a professional day-care centre
08.1994 - 03.2003	Abitur at the **Lina-Hilger-Gymnasium** in Bad Kreuznach

During my PhD at the **University of Basel,** I was teaching assistant in organic chemistry lectures (*OC I* and *OC IV*) and lab courses (*Biological* and *Pharmaceutical practicum*); supervision of three Master students during their thesis and several research students; responsible for IT, maintenance of lab equipment and inventory of chemicals

Poster Presentations

Synthesis and Biological Evaluation of the Neuritogenic Steroidal Lactone Withanolide A and its Derivativs

Frontiers in Medical Chemistry, Saarbrücken, **2011**
Heidelberg Forum of Catalysis, Heidelberg, **2011**
BioValley Science Day, Basel, **2012**

Towards the Total Synthesis and Biological Evaluation of JBIR-02

GDCh-Wissenschaftsforum Chemie, Bremen, **2011**
Fall Meeting of the Swiss Chemical Society, Lausanne, **2011**

Towards the Total Synthesis of JBIR-02 and Related Piericidiens

Fall Meeting of the Swiss Chemical Society, Zürich, **2012**

Oral Presentation

Release on Demand: Natural Products as a Tool to Understand Biological Processes
Clariant Chemistry Day, Basel, **2012**

Distinctions

2006 'Excellence in Molecular Chemistry Heidelberg' promotion to the elite in molecular chemistry at the University of Heidelberg (Vordiplom)

2008 'Dr. Sophie Bernthsen-Fond' prize for outstanding academic achievements at the University of Heidelberg (Diploma)

Initiative

Founding and organization of the first ***'Basel Chemistry Symposium'*** international symposium at the University of Basel under the umbrella of the newly established graduate community

Creation and organization of the monthly ***'Research Seminars'*** PhD student lecture series at the chemical department to promote scientific exchange within the department

Basel, June 2013