Total synthesis and biological activity of new functionalized epothilones for prodrug design and tumor targeting

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Total Synthesis and Biological Activity of New Functionalized Epothilones for Prodrug Design and Tumor Targeting

A dissertation submitted to

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For the degree of

Doctor of Sciences ETH Zürich

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Abstract

Epothilones (Fig. 1) are a highly potent class of antiproliferative natural products, which exert their activity through stabilization of cellular microtubules, leading to mitotic arrest and apoptotic cell death in cancer cells. These compounds display potent effects on tubulin polymerization in vitro and they inhibit tumor growth in animal models in vivo, including models of multi-drug-resistant human tumors. Since their discovery in 1987 and the elucidation of their mechanism of action in 1995, the epothilones have been the subject of intense research activities, which have led to a broad-based empirical understanding of their structure-activity-relationships. They have served as successful lead structures for anticancer drug discovery and several epothilone-derived agents have entered clinical trials in humans; one of these analogs (Ixempra®) has been introduced into clinical cancer treatment in 2008, marking the most tangible result of epothilone-directed research to date.

![Epothilone Structures](image)

**Fig. 1**

Like many other antiproliferative agents, however, epothilones lack any intrinsic selectivity for cancer cells over normal ones; this is the cause of side effects, which ultimately reduce the dose of epothilones that may be administered to patients. The therapeutic effectiveness of epothilones would benefit greatly, if they could be modified to preferentially kill tumor cells, thus widening their therapeutic window. A promising method to do so is the conjugation of an epothilone with a tumor-targeting moiety, which may deliver the drug preferentially to tumor cells or their immediate environment. Such targeting agents may be
conjugated to the epothilone structure either irreversibly or through an enzymatically cleavable linker, i.e. through a prodrug approach.

Fig. 2

In this context we have devised and synthesized novel modified epothilone analogs A-1 - A-3, (Fig.2) which in their aromatic side chain bear additional functional groups (compared to natural epothilones) that enable conjugation with various tumor-targeting moieties; evaluation of the biological activity of derivatives A-1- A-3 has been performed in a tubulin polymerization assay and in cytotoxicity assays against several human cancer cell lines.

Synthesis of A-1 - A-3 has been achieved through the preparation of building blocks A-4 and A-5 (Fig. 3), which have been connected via a palladium-mediated Suzuki-Miyaura coupling; a Yamaguchi macrolactonization has then been used to close the 16-membered ring.

Fig. 3
A synthesis of A-1 in hundred-mg amounts has been completed and the resulting material has been used to prepare conjugates of A-1 with folic acid. The latter was chosen as a targeting device to exploit its preferential cellular uptake pathway through the folate receptor (FR). The FR is practically absent from most healthy tissues, but overexpressed in certain types of epithelial cancers.

Two conjugates of A-1 with folic acid have been synthesized, derivatives A-6 and A-7 (Fig. 4); the former includes a linker which is expected to be stable in the cellular environment, while the latter contains a dipeptide moiety susceptible to cleavage by the enzyme cathepsin B, which is overexpressed in several types of tumors. Conjugate A-7 is then expected to behave as a prodrug and release free A-1 after cellular uptake. Biological evaluation of A-6 and A-7 is ongoing.

![A-6](image1.png)

![A-7](image2.png)

Fig. 4
Riassunto

Gli epotiloni (Fig. 1) sono una classe di composti naturali dalla potente attivita’ antiproliferativa; essi esercitano la loro azione attraverso la stabilizzazione dei microtubuli cellulari, provocando l’arresto mitotico e l’apoptosi nelle cellule colpite. Questi composti hanno un potente effetto sulla polimerizzazione della tubulina in vitro e inibiscono la crescita di tumori in vivo in modelli animali, compresi modelli di tumori umani multiresistenti ai farmaci. Dalla loro scoperta nel 1987 e dopo la dimostrazione del loro meccanismo d’azione nel 1995, gli epotiloni sono stati oggetto di intense attivita’ di ricerca, che hanno portato a un’estesa comprensione empirica delle relazioni struttura-attivita’ in questa classe di composti. Essi sono stati utilizzati con successo come strutture ‘lead’ per la ricerca di agenti anticancro, e diversi farmaci basati sugli epotiloni sono stati oggetto di studi clinici; uno di questi (Ixempra®) e’ stato introdotto nella pratica clinica nel 2008, segnando il risultato finora piu’ tangibile della ricerca basata sugli epotiloni.

![Diagram of Epothilones](image)

**Fig. 1**

Come molti altri farmaci antiproliferativi, tuttavia, gli epotiloni non possiedono alcuna selettivita’ verso le cellule tumorali rispetto a quelle normali; questo causa effetti collaterali, che in ultima analisi riducono la dose di epotilone che puo’ essere somministrata al paziente. L’efficacia terapeutica degli epotiloni verrebbe significativamente accresciuta se essi potessero essere modificati in modo da attaccare preferenzialmente le cellule tumorali, ampliando così’ la finestra terapeutica di questi farmaci. Un metodo promettente per fare cio’ e’ la coniugazione di un epotilone con un secondo agente, che interagisca preferenzialmente con le cellule tumorali e sia in grado di far accumulare il farmaco al loro interno o nelle loro immediate vicinanze. Questa seconda molecola puo’ venire legata
all’epotilone in maniera irreversibile, oppure attraverso un legame che si presti a scissione enzimatica, realizzando cioè un profarmaco.

Fig. 2

In questo scenario abbiamo progettato e sintetizzato i nuovi epotiloni **A-1 - A-3, (Fig.2)** che recano nella loro catena laterale aromatica gruppi funzionali aggiuntivi (rispetto agli epotiloni naturali), che permettono la coniugazione con varie molecole; la valutazione dell’attività biologica dei derivati **A-1 - A-3** e’ stata effettuata attraverso un saggio di polimerizzazione della tubulina e in saggii di citotossicità su varie linee cellulari tumorali umane.

La sintesi di **A-1 - A-3** e’ stata realizzata attraverso la preparazione degli intermedi **A-4 e A-5 (Fig. 3)**, che sono stati uniti per mezzo di una reazione di accoppiamento Suzuki-Miyaura mediata dal palladio; una macrolattonizzazione di Yamaguchi e’ stata poi utilizzata per chiudere il ciclo a sedici membri dell’epotilone.

![Fig. 3](image-url)

**Fig. 3**
La sintesi di A-1 è stata completata su diverse centinaia di milligrammi di materiale, che è stato utilizzato per preparare coniugati di A-1 con l’acido folico. Quest’ultimo è stato scelto per sfruttare il percorso preferenziale di endocitosi attraverso il recettore del folato (FR). FR è praticamente assente dalla maggior parte dei tessuti sani, ma è sovraespresso in vari tipi di tumore.

Due coniugati di A-1 con l’acido folico sono stati sintetizzati, i derivati A-6 e A-7 (Fig. 4); il primo è caratterizzato da un legame che ci si aspetta sia stabile all’interno delle cellule, mentre il secondo comprende un dipeptide suscettibile di scissione da parte dell’enzima catepsina B, che è sovraespresso in diversi tipi di tumore. È previsto quindi che il coniugato A-7 si comporti come un profarmaco e rilasci A-1 dopo l’ingresso nelle cellule tumorali. La valutazione dell’attività biologica di A-6 e A-7 è in corso.
1. Introduction

1.1. Tumor-targeted delivery of anticancer drugs

Cancer is a severe disease and a leading cause of death worldwide; it represents the second most frequent cause of disease-related death in most developed countries\textsuperscript{2,3} and the number of cancer cases is increasing worldwide.\textsuperscript{2} Pharmacological therapies for cancer have been investigated for at least sixty years and increasingly effective treatments have been developed for many types of malignancies;\textsuperscript{3} however, most cancer types are still characterized by a very poor prognosis.\textsuperscript{4}

Treatment with antiproliferative and cytotoxic agents is a mainstay of current anticancer therapy; however, this approach is often plagued by severe side effects and a high rate of disease recurrence.\textsuperscript{5} These shortcomings can be traced back to a significant extent to the narrow therapeutic window of the available drugs: there is a too small difference between the minimum dose which significantly affects the tumor and the maximum dosage that is tolerable to the patient because of side effects.\textsuperscript{5,6} This is largely due to the fact that most antiproliferative agents lack any inherent selectivity mechanism, which would allow them to attack malignant cells preferentially over normal ones. Their clinical efficacy relies instead mainly on the increased proliferation rate of many types of tumors in comparison to the majority of healthy tissues, since these drugs affect proliferating cells more effectively than resting ones. However, this means normal proliferating cells are affected as well, and healthy tissues inevitably receive some level of toxicity during therapy; this often causes the most significant side effects.\textsuperscript{5} Thus in many cases it becomes impossible to administer high enough doses of cytotoxic agents to eradicate the tumor without intolerable damage to the patient.

Therefore, methods that enable the widening of the therapeutic window of cytotoxic and antiproliferative drugs could greatly improve the efficacy of existing anticancer therapy.\textsuperscript{3,7} To this end, several approaches are conceivable; on one hand, one may strive to modify the characteristics of the affected cells, for instance lending additional protection to the
susceptible healthy tissues (as in regimens that include 'rescue' treatments\(^8\)), or conferring increased sensitivity to malignant cells (for example, specifically delivering enzymes that activate a certain drug,\(^9\) or co-administering drugs that thwart the detoxification mechanisms of cancer cells, such as PgP inhibitors\(^10\)). On the other hand, the characteristics of the drug may be tuned to exploit existing differences between normal and malignant cells, and between the tumor immediate environment and healthy tissues.\(^3\) In this context, a prominent strategy consists in modifying a cytotoxic drug by attaching it to a second chemical entity, which displays a specific interaction with the malignant cells or with the tumor microenvironment.\(^3,11\) If the specific biochemical behavior of this second agent results in preferential delivery of the attached drug to the tumor, such a molecule may then be called a 'tumor-targeting moiety'.

Several types of molecules lend themselves to the role of tumor-targeting moieties, through a variety of biochemical mechanisms.\(^7,11\) Some small molecules can interact with cancer cells through specific surface receptors, which are significantly overexpressed on certain types of tumor; examples of such molecules for which drug conjugates have been prepared include vitamins like folic acid,\(^12,13\) peptides\(^14\) such as somatostatins\(^15\) and bombesins,\(^16,17\) and carbohydrates\(^18\) such as hyaluronic acid.\(^19\) Such low molecular weight (LMW) targeting moieties are generally quite accessible through chemical synthesis, and amenable to relatively easy chemical modification for the preparation of drug-conjugates and for the tuning of the pharmacological properties of the conjugate. However, their use as tumor-targeting moieties depends on the existence on the tumor cells of a significantly overexpressed receptor or transport system, for which the targeting agent has a strong and selective affinity. This admittedly limits the number of available tumor-targeting small molecules and the range of tumors amenable to this strategy to certain favourable cases.

Another prominent class of potential tumor-targeting moieties is clearly represented by antibodies,\(^20\) which in principle offer a greater versatility in terms of their target tumor types, since they may be raised against a wider range of antigens overexpressed on tumor cells or their immediate environment, achieving a strong and selective binding much more easily than what can be obtained with most small-molecular ligands.\(^21\) Drug-antibody conjugates, however, have some disadvantages over low molecular weight tumor-targeting
moieties; for instance, antibodies are more likely than small molecules to elicit an unwanted immunogenic response and their large molecular weight can hinder their tissue penetration.³

Both the above approaches are based essentially on tumor-specific proteins, and their preferential interaction with certain small-molecular or macromolecular counterparts; another set of strategies relies rather on the biochemical and physiological characteristics of the tumor.²² This is the case, for instance, of delivery methods which try to exploit the enhanced permeation and retention (EPR) effect observed with high-molecular-weight polymers, which tend to accumulate at the site of solid tumors due to a combination of increased extravasation through the irregular and porous angiogenic vasculature of the tumor and reduced lymphatic drainage in the same area.²³ Drug-conjugates have been prepared with polymers²¹,²⁴ such as poly(ethylene glycol),²⁵ HPMA²⁶ and dextran.²⁷ The tumor microenvironment has also other characteristics that are often exploited for targeting,²² namely its lower pH and more reducing environment compared to the extracellular environment of healthy tissues, or the presence of specific proteolytic enzymes.²⁸ These features are the focus of approaches that use them to trigger the activation of a prodrug at the tumor site through chemical modification or drug release from a non-targeted carrier.²⁹ The combination of a tumor-targeting moiety with a tumor-specific release method can be regarded as a development from simple tumor targeting, in which two separate mechanisms contribute to enhance selectivity; some approaches go one step forward in complexity and seek to combine three independent targeting mechanisms, by loading a drug, via a tumor-specifically cleavable linker, to a high-molecular-weight polymer which also carries a tumor-targeting moiety aimed at a tumor-overexpressed protein.²¹
1.2. Epothilones

1.2.1. Discovery of the Epothilones

Epothilones are a family of macrocyclic polyketides, some of which display potent cytotoxic and antiproliferative properties. The prototype for this class of compounds is represented by the naturally occurring epothilones A and B (Epo A (1-1) and Epo B (1-2), Fig. I-1), which were discovered in 1987 by scientists led by Höflé and Reichenbach at the “Gesellschaft für Biotechnologische Forschung” (GBF) in Braunschweig, Germany.30,31 These two 16-membered lactones were isolated from the culture broth of the SoCe90 strain of the myxobacterium Sorangium cellulosum, which was found in soil samples collected along the Zambezi River in Africa. Antifungal properties were reported for both compounds as well as submicromolar cytotoxic activity against human cancer cell lines;32 initially, however, these compounds did not draw much attention from the research community.

![Fig.I-1](image1.png)  
**Fig.I-1**  
Epothilones A and B

![Fig.I-2](image2.png)  
**Fig.I-2**  
Chemical structure of Taxol
<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; [nM]</th>
<th>Taxol</th>
<th>Epo A</th>
<th>Epo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT-116 (colon)</td>
<td>2.79</td>
<td>2.51</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>PC-3M (prostate)</td>
<td>4.77</td>
<td>4.27</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>A-549 (lung)</td>
<td>3.19</td>
<td>2.67</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>MCF-7 (breast)</td>
<td>1.80</td>
<td>1.49</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>NCI/ADR *</td>
<td>9105</td>
<td>27.5</td>
<td>2.92</td>
<td></td>
</tr>
<tr>
<td>KB-31 (cervix)</td>
<td>2.31</td>
<td>2.1</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>KB-8511*</td>
<td>533</td>
<td>1.9</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

*Table I-1* Inhibition of human carcinoma cell lines by Taxol, Epo A and Epo B<sup>33</sup>; * multi-drug-resistant cell lines

Epithilones were brought into the spotlight with the publication, in 1995, of the results of a screening conducted at Merck Research Laboratories to identify novel compounds with a mechanism of action similar to that of the well established anticancer drug taxol (I-3, Fig. I-2).<sup>34</sup> This antiproliferative agent causes cell cycle arrest through stabilization of cellular microtubules and at the time was the only compound known to possess this mode of action. Epithilones A and B emerged as hits in a screening for the promotion of microtubule assembly, and on preformed microtubules showed similar effects as taxol; both compounds were shown to compete with taxol for binding to microtubules and their cytotoxic activity at nanomolar concentrations was confirmed in human cancer cell lines (Table I-1).<sup>34,35</sup> These findings triggered an intense interest in epithilones, as they displayed several potential advantages in comparison to taxol. Their chemical structures are not only unrelated, but epithilones are significantly simpler, and amenable both to total synthesis and the synthesis of analogues for structure-activity relationship (SAR) studies, a daunting and impractical task in the case of taxol. Even more interestingly, epithilones A and B retain their activity also in Pgp-overexpressing, taxol-resistant cancer cell lines,<sup>34,35</sup> as well as in the presence of certain tubulin mutations that render taxol ineffective.<sup>36</sup> Epithilones also have more attractive physico-chemical properties, in particular a higher water solubility,
which suggested the possibility of an easier formulation; taxol has in fact very limited water solubility, and is usually administered together with vehicles such as Cremophor EL (polyethoxylated castor oil), which are believed to be responsible for some of the negative side effects in taxol treatment.37

The promising pharmacological profile of epothilones spurred synthetic efforts by several research groups, which quickly led to the total synthesis of epothilones A and B38–41, and the definitive confirmation of their structures; meanwhile, their antitumor activity was confirmed by in vivo studies.42,43 At the same time, a marked interest arose in exploring the SAR of these compounds, in order to find analogs with even better pharmacological profiles and possibly simpler chemical structures; during the last fifteen years, this has prompted a number of research groups to design and prepare a vast repertoire of diverse epothilones and test them in vitro against different cancer cell lines.33 Therefore, a large body of knowledge is now available regarding the SAR of the epothilone family; moreover, some of these analogs have shown promising properties and have progressed to clinical stage testing,44 with one compound (ixabepilone, Ixempra®) already being approved for breast cancer therapy.45,46

1.2.2. Mechanism of action of the Epothilones

Epothilones exert their cellular effect by binding to and stabilizing cellular microtubules. These are protein-based filaments that form a network throughout the cell cytoplasm and contribute to the spatial organization of the cellular content, with regard to organelle position, vesicle transport, and cell polarity; possibly the most crucial role of microtubules is carried out during mitosis, when they are responsible for separating the two sets of chromosomes into the incipient daughter cells. Microtubules are hollow, cylindrical supramolecular structures formed by the noncovalent head-to-tail assembly of heterodimers of the α- and β-forms of the protein tubulin (Fig. I-3); they may measure up to several micrometers in length. They constitute a very dynamic system,47–49 in which addition and loss of heterodimers at the extremes of the filaments is constantly ongoing; the binding of new heterodimers requires energy, which is provided by the hydrolysis of tubulin-bound GTP. Microtubules can be assembled and observed in vitro in cell-free
systems, where their dynamics have been extensively studied\textsuperscript{50}, as well as in cells;\textsuperscript{51} they are characterized by so-called ‘dynamic instability’, namely the repeated switching between periods of rapid shrinking (‘catastrophic’ events) and periods of growth (‘rescue’ events), typically observed within minutes. Between these extreme events, microtubules also undergo phases of resting, in which the overall length of the tubule does not change, as the rates of loss and addition of heterodimers are approximately equal; this situation has been termed ‘treadmilling’. The two ends of each tubule display distinct behaviors, in that the $\beta$-tubulin end ((+)-end) is more dynamic than the opposite side ((-)-end), i.e. the (+)-end undergoes assembly and disassembly of new heterodimers at a higher rate than the (-)-end. In cells, where microtubules are spatially organized around microtubule organizing centres (MTOCs), this difference is even more pronounced, because the (-)-end typically remains bound to a MTOC, which may hinder its dynamics, while the (+)-end grows out and away from the organelle. During the early stages of mitosis (Fig. I-4), the microtubules become much more dynamic and rearrange to form the mitotic spindle, which spans the cell between two poles occupied by MTOCs;\textsuperscript{1} microtubules elongate and shrink repeatedly, probing the space for chromosomes, which they eventually bind at a central region, called kinetochore. At metaphase the mitotic spindle is complete, and the two sets of chromosomes are aligned in pairs along a plane in the middle of the structure. When mitosis proceeds correctly, the cell then enters anaphase, and the microtubules begin shrinking, pulling the attached chromosomes apart towards the poles of the spindle.\textsuperscript{1}
Stabilization of microtubules by epothilones and other microtubule-stabilizing agents results in altered microtubule dynamics, which leads to arrest of the cell cycle at the metaphase-anaphase transition. Chromosome segregation does not take place, and the cell remains blocked in this state (mitotic arrest); apoptotic cell death eventually follows. The specific effects of the different microtubule-targeted drugs on various parameters of polymerization dynamics are rather complex. Concentration-dependent suppression of microtubule dynamics can be observed with epothilones in interphase cells and is manifest in reduced rates and extent of both growth and shrinking events, an increased frequency of rescue events, and an increased fraction of microtubules which show no length change. Other experiments in cell-free systems have demonstrated that epothilones induce resistance of microtubules to depolymerizing conditions and increased polymerization rate of unpolymerized tubulin dimers.

In contrast to the action of taxol and epothilones, other small molecules may alter microtubule dynamics in the opposite way, i.e. by the destabilization of the microtubules; such compounds inhibit the polymerization of tubulin and, at high concentrations, induce depolymerization of assembled microtubules; they are generally known as tubulin polymerization inhibitors. Despite their opposite impact on tubulin polymerization, their ultimate effect at the cellular level is similar to that of microtubule-stabilizing agents, including mitotic arrest and apoptosis. This is unsurprising if one considers that both polymerization of tubulin and depolymerization of microtubules are
essential for the proper execution of the cell division process, and ultimately it is interference with these microtubule dynamics which is responsible for mitotic arrest, rather than the overall mass change in the polymerized tubulin present in the cell.\textsuperscript{49,54} Among tubulin polymerization inhibitors (\textbf{Fig I-5}), colchicine (I-4)\textsuperscript{55,56} is well-known mostly for historical reasons, while the Vinca alkaloids (I-5, I-6)\textsuperscript{57,58} have been successfully employed in clinical oncology for almost fifty years; several other microtubules as I-7).\textsuperscript{54}

Since the discovery of epothilones, a number of additional microtubule-stabilizing agents

\begin{align*}
\text{Colchicine (I-4)} & \\
\text{Vinblastine, } R = \text{Me (I-5)} & \\
\text{Vincristine, } R = \text{CHO (I-6)} & \\
\text{Combretastatin A-4 (I-7)} & 
\end{align*}

\textbf{Fig. I-5} Examples of tubulin polymerization inhibitors

have been identified;\textsuperscript{59} all have been isolated from natural sources, and many, like epothilones, are based on a polyketide structure. Among these, discodermolide (I-8), laulimalide (I-9),\textsuperscript{60,61} eleutheroxin (I-10),\textsuperscript{62} dictyostatin (I-11)\textsuperscript{63,64}, peloroside A (I-12)\textsuperscript{65,66} and zampanolide (I-13)\textsuperscript{67} (Fig I-6) have all shown promising \textit{in vitro} cytotoxic activity against human cancer cell lines; however, none of these natural products or their derivatives are currently in clinical development.
Several distinct tubulin binding sites have been identified for tubulin-interacting agents. Epothilones bind to the β-subunit of tubulin, in a competitive manner with respect to taxol. The microtubule-binding affinity of several epothilones has been measured against stabilized microtubules employing a competitive binding assay with a fluorescent taxol derivative (Flutax-2); the observed $K_a$ values for tubulin binding are shown in Table I-2 for epothilones A and B and taxol. Overall, binding affinity correlates both with polymerization induction and with cytotoxicity, but in spite of these general trends, for individual epothilone analogs the prediction of cellular activity from microtubule-binding or tubulin polymerization data is not wholly reliable.
Most other microtubule-stabilizing natural products also bind at the taxane binding site on β-tubulin, including discodermolide, dictyostatin and eleutherobin,\textsuperscript{69} laulimalide and peloruside A, on the other hand, share a different and as yet not identified binding site, which may be located on α-tubulin,\textsuperscript{70} yet other binding sites are utilized by the vinca alkaloids and by colchicine.\textsuperscript{49}

<table>
<thead>
<tr>
<th></th>
<th>Taxol</th>
<th>Epo A</th>
<th>Epo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a [10^{-7} \text{ M}^{-1}]$</td>
<td>$1.07 \pm 0.11$</td>
<td>$2.93 \pm 0.44$</td>
<td>$60.8 \pm 10.1$</td>
</tr>
</tbody>
</table>

\textbf{Table I-2} Binding affinities of taxol and epothilones for stabilized microtubules at 37°C\textsuperscript{68}
1.2.3. Structure and SAR of epothilones

The epothilone chemical structure is characterized by a polyketide core featuring a 16-membered lactone and a ketone at position 5, and an aromatic side chain attached at C 15 of the core ring (for the numbering of the epothilone framework used throughout this work, see Fig. I-7). In epothilones A and B, the side chain includes a thiazole, and an epoxide is fused with the main ring at C12 and C13; these features were considered when the compounds were termed “epothilones” by Reichenbach and Höflle to reflect their epoxide, thiazole and ketone moieties. The importance of the macrolactone ring substitution pattern and stereochemistry for biological activity have been extensively investigated in the context of SAR studies, as well as many modifications of the aromatic side chain; the resulting wealth of information has been amply reviewed.33,71

Fig. I-7 Numbering of the epothilone structure

1.2.3.1. C12-C13 modifications

The very first piece of SAR information about epothilones came from the comparison between the original epothilones I-1 and I-2; as shown in Table I-1, while the former has similar cytotoxicity as taxol, the latter is consistently more potent,35 up to about one order of magnitude in some cell lines, which suggested that the methyl group at C12 was beneficial for activity. Efforts have been made to explore the effect of modifications at this
position (C26);\textsuperscript{72} some of the smaller substituents (ethyl, halomethyl\textsuperscript{73}) produced activities comparable with epothilone B, while large or polar groups proved to be detrimental.

Among the first epothilone analogs to be investigated were the deoxy-derivatives of epothilones A and B, in which the epoxide ring including C12 and C13 had been replaced with a double bond; these compounds are called epothilone C (I-14) and D (I-15), respectively (Fig. I-8). Initially, I-14 and I-15 were investigated because they were available as late-stage intermediates in the total syntheses of the corresponding epoxides,\textsuperscript{40,74} but they had also been isolated, in minimal amounts, from the broth of S. cellulosum.\textsuperscript{75} In contrast to previous speculations that the epoxide would play an important role in the binding to tubulin as a hydrogen-bond acceptor,* the in vitro activity of I-14 and I-15 is only moderately attenuated in comparison to I-1 and I-2.\textsuperscript{74} Thus, I-15 induces tubulin polymerization with equal potency to I-2, and displays IC\textsubscript{50} values up to ten-fold higher in a variety of cell lines\textsuperscript{76}, including multi-drug resistant types. A similar comparison can be drawn between I-14 and I-1; it is also worth noting that the effect of the methyl group at C12 in deoxyepothilones follows the trend seen in the epoxide series, with epothilone D generally being more potent than C.\textsuperscript{74} In vivo studies showed high antitumor efficacy for epothilone D, which displayed an interesting preclinical profile, due to its high potency and reduced toxicity in comparison with I-2;\textsuperscript{76} such results warranted the advancement of I-15 to clinical development.\textsuperscript{77}

This piece of SAR information has prompted the design of several other deoxy-analogs, in light of their simplified synthesis and higher stability in physiological conditions, where the epoxide ring may react relatively easily to give inactive derivatives; moreover, the slight activity loss in deoxyepothilones may be counterbalanced with other, activity-enhancing structural modifications, for example in the side chain (\textit{vide infra}). Other efforts in the epothilone D series concerned substitution at the C26 position \textsuperscript{72} and the introduction of unsaturation in the C9-C11 region;\textsuperscript{78} combination of these modifications afforded the promising preclinical candidate fludelone (I-16, Fig I-8),\textsuperscript{78} which, however, has not been further developed.\textsuperscript{79}
The stereochemistry of the epoxide moiety was as well the object of early SAR investigations, which showed that, while the (12S, 13R) epimers (bearing a cis-epoxide of opposite stereochemistry to the natural one) are generally less active than their counterparts bearing the natural configuration, some of the analogs with a trans geometry in this region retain very potent activity. In particular, the (12S, 13S) trans-epoxide analog of epothilone A (I-17, Fig 1-8) is practically as potent as the parent compound; interestingly, the other trans-epoxide derivative, (12R, 13R)-epothilone A, is several hundred-fold less cytotoxic. Also in the deoxy-series, the trans-analogs of Epo C and Epo D retain significant cytotoxic activity.

When it became clear that the epoxide hydrogen-bond-acceptor properties where not a prerequisite for activity, its substitution by other small rings seemed an attractive variation on the epothilone framework; thus, several analogs were prepared bearing a cyclopropane...
or cyclobutane \(^{82,83}\) ring fused at the C12-C13 bond with the macrocyclic core. In particular, the cyclopropane analogs of epothilone A\(^{82}\) and B\(^{84}\) (I-18, I-19, Fig 1-8) proved to be practically equipotent with the parent compounds, suggesting that the contribution of the epoxide moiety to the activity of I-1 and I-2 may be essentially conformational. The activity of the \textit{trans}-cyclopropane derivative of Epothilone A \(^{82}\) and of its analogs with a modified side chain\(^{85}\) was particularly promising, reaching in some cases potencies comparable to that of I-2; the corresponding \textit{trans}-Epothilone B analogs exhibited instead a lower activity than their Epothilone A counterparts.\(^{85}\)

Similar considerations about the role of the epoxide ring prompted its replacement with an aziridine\(^{86}\), which produced as well a series of very active Epo A analogs, some even more potent than I-1, such as I-20 (Fig. 1-8). Moreover, the nitrogen atom at this position offers a further functionalization possibility, which has been explored with some success\(^{86}\) (see also Section 1.3.5).

1.2.3.2. Side chain modifications

The aromatic side chain is the most obvious site for structural modifications of epothilones, and has been the focus of extensive SAR studies over the years. Early investigations established that removal of the side chain abolishes activity, and that the natural stereochemistry is preferred at C15.\(^{42,80}\) Modifications of the substitution pattern of the side chain have been explored,\(^{87,88}\) and have demonstrated for example that small substituents such as hydroxymethyl,\(^{87}\) aminomethyl* or methylthio\(^{85}\) are well tolerated at position C20 and can even lead to increased potency\(^{89}\) (see also Section 1.2.4).
**Fig. I-9** Selected epothilone analogs with modified side chain

The naturally occurring thiaazole moiety has been replaced with a variety of heterocycles in the context of the Epo A-D series\textsuperscript{80,89-91} as well as in the cyclopropyl-Epo A and B series.\textsuperscript{85,89} From this wealth of information a few general conclusions can be drawn, for instance that the presence of a sulphur atom is not necessary for biological activity, as demonstrated by the high activity of oxazole analogs **I-21, I-22** (Fig. I-9)\textsuperscript{80}; the hydrogen-bond acceptor function at that position can be entirely removed without detriment to the activity, and six-instead of five-membered rings are well tolerated, as shown by the pyridine analog **I-23** (Fig. I-9), which is practically as potent as EpoB.\textsuperscript{90} The role of the nitrogen atom is more complex; several experiments indicate that its presence is of significant importance for the biological activity of epothilones, but interpretation of these data is not straightforward.\textsuperscript{33} For example, in the pyridine-Epo B series, with the nitrogen atom in meta or para position...
with respect to the attachment point of the side chain (I-24 and I-25, Fig. 1-9), a marked decrease both in cytotoxicity and in tubulin-polymerizing activity is observed with respect to the ortho analog I-23\textsuperscript{90}, apparently indicating a role of the ortho-positioned nitrogen in tubulin binding. However, in a series of Epo D analogs bearing a bicyclic side chain (I-26-I-29, Fig. 1-10)\textsuperscript{92} the position of the nitrogen atom does not affect significantly the tubulin polymerization properties of the corresponding compounds, while a substantial difference is observed in their cellular activities, again in favor of the analogs with the nitrogen atom in the natural position (I-26 and I-28). This seems to indicate that factors other than tubulin-polymerization activity have a profound effect on cell growth inhibition on the part of these Epo D derivatives; what exactly these factors may be and what role they may play in the activity of this and other types of epothilone analogs remains an open issue.\textsuperscript{33}

Fig 1-10 Selected Epo D analogs bearing a bicyclic aromatic side chain
Epothilone analogs I-26-I-29 are examples of a rather pronounced modification of the side chain, where the entire C16-C21 moiety is replaced with a bicyclic heteroaromatic system.\textsuperscript{92,93} This line of investigation stemmed from the observation that, in NMR measurements of the bioactive conformation of epothilones, the torsion angle about the

**Fig I-11** Selected epothilone B analogs bearing a bicyclic aromatic side chain

C16-C17-C18-N portion of the molecule was approximately 180°,\textsuperscript{94} therefore, incorporating this part of the structure into a planar, rigid framework was thought to be beneficial for activity.\textsuperscript{33} Work along these lines has produced benzothiazole, benzimidazole and quinoline epothilone derivatives based on epothilone B (**Fig I-11**) and D (**Fig I-10**) core structures;\textsuperscript{93} in general, these analogs indeed proved to be more potent inhibitors of human tumor cells growth than their parent compounds epothilones B and D (see for example **Table I-3**). This potency increase is significantly more pronounced in the epothilone D than in the epothilone B series; the underlying reasons for this differential effect are poorly understood so far, however, also in this case the activity enhancement does not seem to correlate with a more efficient promotion of tubulin polymerization.\textsuperscript{33,93}

<table>
<thead>
<tr>
<th></th>
<th>Epo D</th>
<th>I-26</th>
<th>I-28</th>
<th>Epo B</th>
<th>I-30</th>
<th>I-31</th>
</tr>
</thead>
<tbody>
<tr>
<td>% T</td>
<td>83</td>
<td>76</td>
<td>90</td>
<td>90</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>IC\textsubscript{50} KB-31[nM]</td>
<td>2.70</td>
<td>0.45</td>
<td>0.59</td>
<td>0.19</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>IC\textsubscript{50} KB-8511[nM]</td>
<td>1.44</td>
<td>0.23</td>
<td>0.38</td>
<td>0.18</td>
<td>0.09</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Table I-3 Tubulin polymerization activity and cytotoxicity of selected epothilones bearing a bicyclic side chain;\textsuperscript{93} \%T: \% of tubulin polymerized by each compound in 2 \, \mu M concentration, where \%T = 100 corresponds to the polymerization induced in the same conditions by 25 \, \mu M Epo B.

The increase in cellular activity in the Epothilone D series of rigidified side chain analogs is particularly pronounced in the case of the dimethylbenzimidazole analog I-33\textsuperscript{93} (Fig. I-12 and Table I-4); this, alongside with the observation of the favourable solubility properties of the benzimidazole derivatives,\textsuperscript{33} has warranted investigation of further benzimidazole-based analogs (Fig. I-12). The potency-enhancing effect of the dimethylbenzimidazole side chain has proved to be rather general, and is found also in the corresponding analogs of epothilone A and C,\textsuperscript{95} of trans-Epo A (I-35),\textsuperscript{95} and of the 3-deoxy derivatives of epothilone B (I-34) and trans-Epo A (I-36, Table I-4).\textsuperscript{96} Remarkably, in I-33 the gain in activity from the dimethylbenzimidazole moiety compensates almost entirely the loss of potency associated with removal of the epoxide, and leads to potency almost as high as that of epothilone B;\textsuperscript{93} similarly, I-34 is approximately ten-fold more potent than 3-deoxy -epothilone B (I-38, Fig. I-13).\textsuperscript{96}
**Fig. I-12** Selected epothilone analogs bearing a dimethylbenzimidazole side chain

<table>
<thead>
<tr>
<th></th>
<th>I-32</th>
<th>I-33</th>
<th>I-34</th>
<th>I-35</th>
<th>I-37</th>
<th>I-38</th>
<th>Epo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>% T</td>
<td>99</td>
<td>90</td>
<td>nd</td>
<td>95</td>
<td>nd</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>(\text{IC}_{50}) KB-31 [nM]</td>
<td>0.14</td>
<td>0.21</td>
<td>0.58</td>
<td>0.25</td>
<td>0.17</td>
<td>7.4</td>
<td>0.29</td>
</tr>
<tr>
<td>(\text{IC}_{50}) KB-8511 [nM]</td>
<td>0.38</td>
<td>0.73</td>
<td>1.89</td>
<td>1.36</td>
<td>0.13</td>
<td>4.0</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Table I-4** Tubulin polymerization activity and cytotoxicity of selected epothilones bearing a bicyclic side chain\(^{93,95-97}\) (nd: not determined); %T: % of polymerized tubulin (see **Table I-3**).

While dimethylbenzimidazole-based epothilones are generally more potent than their counterparts bearing the natural side chain in drug-sensitive cell lines (such as KB-31), they also tend to be more susceptible to P-gp-mediated drug efflux, as shown for example by the
cytotoxicity values of I-32 - I-35 against the P-gp-overexpressing cell line KB-8511 (Table I-4).\textsuperscript{93,95,96} This effect in P-gp overexpressing cells has been correlated with increased polarity (decreased clog P) of the affected derivatives with respect to the parent compound.\textsuperscript{93} Consistent with this interpretation, derivative I-37,\textsuperscript{97} where a less polar cyclopropane ring replaces the epoxide moiety and compensates for the increased polarity of the side chain, retains full activity in both drug-sensitive and P-gp-overexpressing cell lines.

1.2.3.3. Modifications in the C1-C5 region

Modifications in this region have followed two main themes: investigation of the role and importance of the C3 hydroxy group for biological activity, and the possibility of replacing the lactone function with a more physiologically stable moiety.

While inversion of stereochemistry at C3 has proved detrimental for tubulin polymerization promotion,\textsuperscript{80} removal of this hydroxy function is a better tolerated modification, which results in compounds such as I-38, which retains nanomolar cytotoxicity despite being significantly less active than Epothilone B.\textsuperscript{96}

![I-38 and I-39](image)

**Fig. I-13** 3-deoxy Epo B (I-38) and ixabepilone (I-39)

The lactone functionality has been considered by some as a potential weakness for the in vivo stability of epothilones;\textsuperscript{98} in fact, the stability of Epothilone B and D in rodent plasma has been shown to be limited,\textsuperscript{98,99} presumably due to the high esterase activity found in this setting. Plasma stability, however, is high in human plasma and is sufficient for the in vivo activity of epothilones in many mouse-based animal models.\textsuperscript{43,99} This is further underlined by the fact that epothilone B and other lactone-based analogs have been advanced to clinical trials (see Section 1.2.4). As a consequence, the vast majority of epothilone analogs
to date still feature a lactone function; one prominent exception is the lactam analog of epothilone B (ixabepilone, I-39, Fig. 1-13), for which BMS developed an interesting semisynthetic route of only three steps from I-2 itself.\cite{98} Compound I-39 shows a potent cytotoxic activity and a very favourable preclinical profile,\cite{46} and has been the most successful epothilone-based clinical candidate so far, becoming the first approved epothilone-type drug in 2007.

### 1.2.3.4. Modifications of the C6-C11 region

Early SAR investigations in this region established that changes of stereochemistry or substitution pattern at C8 or simultaneous inversion of the stereochemistry at both C6 and C7\cite{80} lead to a pronounced loss of biological activity. As a consequence, this portion of the epothilone structure has been kept unchanged in the vast majority of derivatives; one notable exception is the clinical candidate sagopilone (I-40, Fig. 1-14), which features an elongated C6 substituent (see Section 1.2.4).\cite{100}

Given its lack of functional groups, the C9-C11 region has been mainly the object of modifications aimed at improving biological properties through conformational effects on the macrocycle; epothilone analogs with both larger\cite{71} and smaller ring size\cite{42,71} have been prepared, but these modifications resulted in considerable loss of activity. Attempts to introduce unsaturation in this region, in order to stabilize the bioactive conformation of the macrolactone core, have met with more promising results; in particular, introducing a trans-double bond in the C9-C11 region resulted in some derivatives with potent \textit{in vitro} antiproliferative activity,\cite{101,102,103,104} and one such compound (KOS-1584, I-41, Fig. 1-14) is undergoing clinical development (see Section 1.2.4).\cite{105}

### 1.2.4. Epothilones in Clinical Trials

As previously mentioned, a number of epothilones have been advanced to human clinical trials (Fig. 1-14). Currently, the most advanced of these compounds is the lactam analog of epothilone B, ixabepilone (I-39),\cite{46} which has received FDA approval for the treatment of metastatic or advanced breast cancer in 2007 and is marketed under the trade name
Ixempra® by Bristol-Myers Squibb. Clinical evaluation of this drug is ongoing for a large number of other oncologic indications as well,79,106 including prostate,107 pancreatic108 and ovarian109 cancer. Epothilone B itself (patupilone, I-2), developed by Novartis, is also at an advanced stage of clinical evaluation against ovarian cancer (phase III studies),110 and is being tested against other malignancies as well.111,112 Epothilone D (I-15) has entered clinical trials sponsored by Kosan and Roche (as KOS-862); after advancing to phase II for several solid tumors,77,79 trials have been suspended,79,113 and the company has chosen to focus instead on further unsaturated epothilone D analog I-41 (KOS-1584)105, which is undergoing phase II trials for non-small-cell lung cancer114 and phase I investigations for breast cancer.79 Sagopilone100,115 (ZK-EPO, I-40), a fully synthetic epothilone analog developed by Bayer-Schering Pharma, is currently in phase II clinical trials for a number of malignancies, including prostate115, ovarian115 and lung cancer79, and melanoma79. Two other side chain modified epothilone B analogs, BMS-310705 (I-42)116 and ABJ879 (I-43)117, have been advanced to phase I clinical trials for solid tumors; however, the development of these compounds seems to have been discontinued.79,113
Fig. I-14: Epothilones that have been advanced to clinical trials.
1.3. Folic acid as a targeting agent for drug delivery

1.3.1. Folic acid

Folic acid (Fig I-15) and its close derivatives, collectively known as folates, are essential vitamins for the growth processes of all prokaryotic and eukaryotic cells; they play a crucial role in the biosynthesis of a variety of cellular components, most prominently in the synthesis of nucleotides. In nature, folates are produced by some bacteria, yeasts (for instance *S. Cerevisiae*) and plants, especially of the *Leguminosae* family. Like all animals, humans must take them up with the diet, at a recommended dose for adults of 400 μg of folic acid per day; folate deficiency is associated with a variety of pathologies, such as anaemia, cardiovascular diseases, neural tube defects in newborns, cancer, neurologic diseases and cognitive impairment. For its essential dietary role, in many countries folic acid is routinely added to livestock feed and to cereal products for human nutrition.

![Chemical structure of folic acid](image)

**Fig. I-15** Chemical structure of folic acid

Folic acid is a relatively small molecule, having a molecular weight of 441 Da. Its chemical structure comprises a relatively large aromatic part, called pteroic acid, and a single glutamate unit; the two moieties are joined through an amide bond. Pteroic acid in turn can be dissected into two moieties: the fused bicyclic aromatic motif of pterin and *p-*aminobenzoic acid. The pterin moiety is the most biologically active part of the molecule.
Folic acid has a rather low solubility in water of 1.6 mg/L (3.6 μM);\textsuperscript{124} this is presumably due in part to its zwitterionic nature, but also to the presence of the pterin motif, which is prone to forming aggregates through hydrogen bonds between its amino and amide functionalities.\textsuperscript{125} Folic acid has limited stability when exposed to light;\textsuperscript{126} it decomposes upon heating, starting around 150°C, and no melting point can be observed.\textsuperscript{127}

### 1.3.2. Biological activity of folic acid and related compounds

Folic acid was initially discovered in the context of microbiological research as an uncharacterized nutrient necessary for the growth of \textit{L. Casei} and other bacteria.\textsuperscript{128} In parallel with its full chemical characterization, its role in promoting cell growth in bacteria\textsuperscript{129} and its antianemic action in animals\textsuperscript{130} was demonstrated. Attempts at oncological applications of folic acid and its analogs followed very soon, and led to the discovery that antagonists of folic acid were useful in the treatment of some types of hematologic malignancies.\textsuperscript{131} So-called antifolates met with an early success when aminopterine (\textbf{Fig. I-16}) was found to produce remission in children with acute leukaemia.\textsuperscript{132} Methotrexate (2-amino-10-methyl-folic acid, \textbf{Fig. I-16}) was introduced for cancer treatment shortly afterwards,\textsuperscript{131} and in the following decades it became a mainstay of combination chemotherapy against a variety of cancers.\textsuperscript{133} In many cases methotrexate has been substituted with newer antitumor agents, but it is still widely used to date in the therapy of acute lymphoblastic leukaemia\textsuperscript{134} as well as other types of cancer,\textsuperscript{135-137} and other diseases such as rheumatoid arthritis\textsuperscript{138} and psoriasis.\textsuperscript{139} Meanwhile, the successes and limitations of methotrexate have prompted the search for new antifolates, which over time has generated a whole class of antimetabolite anticancer drugs.\textsuperscript{133 140-142}

![Chemical structure of early antifolates](image)

\textbf{Fig. I-16} Chemical structure of early antifolates
The exact metabolic role of folic acid was discovered a few years after its isolation. A series of studies in the 1940s and 1950s clarified the main aspects of its metabolism and especially the role of its various reduced metabolites, collectively named ‘reduced folates’, as cofactors in the synthesis of nucleotides in the cytoplasm (Fig. I-17). In fact, folic acid as such is not present to a significant extent under physiological conditions in any organism, and is not involved in any known biosynthetic reactions. When folic acid enters the cell, it is reduced stepwise to 7,8-dihydrofolate (DHF) and then tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (DHFR). THF is in turn the precursor for two metabolically active folates, 10-formyl-tetrahydrofolate (10-formyl-THF) and 5,10-methylenetetrahydrofolate (5,10-methylen-THF); these are essential one-carbon-unit donors in the de novo syntheses of purines and of thymidine nucleotides, respectively. Both these pathways may be disrupted by folate deficiency or by inhibition of folate-dependent enzymes, such as that exerted on DHFR by methotrexate and other antifolates; the subsequent depletion of deoxynucleotides may lead to miscoding, DNA strand break, arrest of DNA synthesis, and ultimately apoptosis. These alterations are the basis for antifolate antiproliferative activity; on the other hand, the increased DNA fragility that results from disruption of these pathways may account for a correlation between folate deficiency and the incidence of some malignancies.
**Fig. 1-17** Cytosolic metabolism of folates; FA: folic acid; DHFR: dihydrofolate reductase; DHF: 7,8-dihydrofolate; TS: thymidylate synthase; SAM: S-adenosylmethionine
Another metabolically active folate is 5-methyl-tetrahydrofolate (5-methyl-THF), which plays an important role as methyl donor in yet another cytosolic pathway, the homocysteine/methionine cycle.\textsuperscript{146,147} This is closely related to the synthesis of S-adenosylmethionine (SAM), a crucial C1 donor in a large array of methylation processes spanning regulation of gene expression, post-translational modifications of proteins, and lipid synthesis.\textsuperscript{148} In connection with this pathway, folate deficiency has been directly linked to increased levels of plasma homocysteine, a cardiovascular risk factor\textsuperscript{120}, as well as to reduced levels of DNA methylation.\textsuperscript{122} The latter may play a role in the activation of oncogenes, which would otherwise be silenced by methylation,\textsuperscript{122} while altered DNA or protein methylation may explain the epidemiological correlation between maternal folate deficiency and certain developmental anomalies of the central nervous system such as neural tube defects (NTDs).\textsuperscript{121} It must be noted, however, that the exact biochemical mechanisms that connect folates deficiency with the initiation and progression of NTDs, as well as cancer and other diseases, remain largely unknown.

Folate derivatives also play a role in metabolic pathways involving exchange of one-carbon-units in the mitochondrion and the nucleus.\textsuperscript{144} Folates in the mitochondrion form a distinct pool from those in the cytosol and are cofactors in amino acid interconversion and in the synthesis of formylmethionyl-tRNA, which initiates mitochondrial protein synthesis.* However, mitochondrial folate metabolism has not yet been investigated as extensively as its cytosolic counterpart. Even less is known about the role of these cofactors in the nucleus, where approximately 10% of cell folates reside, and where thymidylate synthesis may take place as well.\textsuperscript{144,149}

### 1.3.3. Transport of folates across the plasma membrane

Folic acid and related compounds do not permeate through the plasma membrane by simple diffusion to any significant extent,\textsuperscript{150} presumably because their polar character prevents them from partitioning significantly into the lipidic layer; their transport from the extracellular environment into cells occurs through a few different mechanisms that involve
specific membrane-bound proteins. These can be broadly divided into two groups: bidirectional ion transporters, such as the reduced folate carrier (RFC) and a few other less-known transporters; and the membrane-localized isoforms of the folate receptor (FR), which mediate endocytosis.

The reduced folate carrier (RFC) is the most thoroughly characterized among the various folate-transporting membrane proteins and also the one that was identified first. RFC is an 80 to 120 kDa anion carrier, which transports folates and related compounds mainly from the extracellular environment into the cell. This translocation requires energy and is likely coupled to downhill transport of other anions in the opposite direction; although various organic anions have been shown to bind to the RFC and be transported, the exact physiological counter-ions are not known. RFC also performs an efflux of folates, but this process is only of minor significance when compared to outward transport of these compounds by other proteins, such as those of the multidrug resistance transporter family.

The kinetic parameters of the RFC-mediated influx of various compounds have been measured in several studies; the efficiency of transport of different folates may be compared in terms of influx constant K_m. These are typically in the range of 1-2 μM for reduced folates such as 5-methyltetrahydrofolate, and approximately two orders of magnitude higher for folic acid; thus, folic acid has a significantly lower affinity for RFC than reduced folates. Methotrexate and many other classical antifolates are also transported by RFC, and many have influx K_m values comparable to those of physiological reduced folates. There is, however, a limit to the extent of chemical modification allowed in RFC substrates, as there are indications that covalent conjugation of folates to exogenous molecules abolishes RFC transport.

RFC is ubiquitously expressed in human tissues, both normal and malignant, as has been shown by several RNA-based assays; expression levels vary from tissue to tissue and are highest in the placenta, liver and kidney; lowest expression is found in heart and skeletal muscle. Ubiquitous expression of RFC in human tissues is further supported by immunohistochemical studies; these have also shown localized expression of RFC in epithelial tissues, among others on the apical side of the brush border membrane in the
small intestine and on the basolateral side of the epithelium of the renal tubule, consistent with a role of this transporter in folate absorption in the gut and reabsorption from the excretory pathway. For its high capacity and broad expression, RFC is considered the main route for the uptake of folates into mammalian cells, and to have an integral role in the homeostasis of folates in the tissues.\textsuperscript{159} For similar reasons, it is also instrumental to the success of classical antifolates therapy; impaired RFC-mediated transport is one of the main mechanisms of antifolate resistance.\textsuperscript{159}

In the late 1980s and early 1990s a family of high-affinity folate-binding proteins distinct from RFC was identified in various types of human epithelial cells; they are encoded by three different genes and were designated as folate receptor (FR) alpha, beta and gamma.\textsuperscript{160-163} FR-\(\alpha\) and -\(\beta\) were characterized as approximately 40 kDa receptors bound to the plasma membrane through a glycosylphosphatidylinositol anchor; they display approximately 70\% sequence homology, localize in lipid rafts on the membrane surface and are both capable of mediating folate internalization.\textsuperscript{12} The third form of these proteins, FR-\(\gamma\), is a soluble receptor which shares 79\% of its sequence with FR-\(\beta\); it is detected principally in hematopoietic cells, both normal and malignant, and is practically absent from normal circulating serum.\textsuperscript{12,162} FR-\(\alpha\) is the most widely expressed and so far best described of the three forms.\textsuperscript{12}

FR-mediated endocytosis of folates results in vesicles containing the receptor-bound folate,\textsuperscript{164} which, upon maturation into late endosomes, undergo a decrease in internal pH (although the extent of that decrease is debated\textsuperscript{165,166}) At this point, the folate is released from the receptor, which is recycled to the plasma membrane in a cycle that requires several hours.\textsuperscript{167} The exact mechanism of the translocation of folates from the endosome to the cytoplasm remains to be clarified, as well as the details of the inner environment of these cell compartments.

FR-\(\alpha\) and -\(\beta\) have a considerably higher affinity for folic acid than RFC, with a \(K_d\) in the range of 0.1 nM. Moreover, the relative affinities of the various folates for FR-\(\alpha\) follow a significantly different pattern than the \(K_m\) values for RFC: in this case, folic acid has a much lower \(K_d\) than either 5-methyltetrahydrofolate or methotrexate.\textsuperscript{154} The two routes of folate
transport also have very different capacities: RFC can transport its substrates at a rate of 10-20 molecules per minute per transporter, while each unit of FR transports only one molecule of substrate per endocytosis cycle.

The expression patterns of FRs in human tissues have been studied by several research groups by means of immunohistochemistry and by more quantitative methods based on in situ hybridization and radioactive-ligand-based assays. These methods give somewhat different numerical results; however their results are generally in agreement and consistently point to a much more limited expression of FR proteins than RFC (Table I-5).

Significant expression of FR-α is restricted to a few types of normal epithelial tissues, and some epithelial cancers. Among normal tissues, high expression of FR-α is found in the epithelium of the kidney proximal tubule, the choroid plexus, fallopian tubes, uterus and epididymis, in pneumocytes in the lung and in trophoblasts in the placenta. High expression of FR-α in tumors is limited to certain epithelial cancers and varies greatly even within these, not only according to the subtype of malignancy but also between patients and between different regions of the same tumor. Ovarian and endometrial cancers stand out among the most widespread types of epithelial tumors because they show the highest consistency in FR-expression levels, with overexpression of these receptors in over 90% of the clinical samples. In other cases, such as lung primary carcinoma, there is no significant difference between FR levels in normal and malignant tissue.

FR-β is even less widespread than the -α form, with significant levels occurring only in the placenta, in hematopoietic cells of the myelomonocytic lineage and in some types of leukaemia; as the receptor seems to be functional in activated macrophages and monocytes, but not in their resting counterparts, FR-β targeting has been proposed for the imaging and treatment of rheumatoid arthritis and other inflammatory diseases. Given their expression patterns, the physiological importance of FR receptors as transporters of folates seems to be limited to a few rather specific situations; several studies have shown modulation of FR expression in cultured cells by varying the extracellular concentration of folates, which has led to the suggestion that FRs may play a particularly important role in conditions of low folate availability.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>$[^{13}\text{H}]$-FA $^{168}$</th>
<th>IHC$^{158}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>heart</td>
<td>1.87 ± 1.05</td>
<td>-</td>
</tr>
<tr>
<td>lung</td>
<td>7.79 ± 2.99</td>
<td>+</td>
</tr>
<tr>
<td>liver</td>
<td>1.23 ± 0.42</td>
<td>-</td>
</tr>
<tr>
<td>intestine</td>
<td>2.74 ± 1.10</td>
<td>-</td>
</tr>
<tr>
<td>kidney</td>
<td>14.40 ± 6.70</td>
<td>+ (a)</td>
</tr>
<tr>
<td>spleen</td>
<td>0.55 ± 0.43</td>
<td>-</td>
</tr>
<tr>
<td>muscle</td>
<td>0.97 ± 0.41</td>
<td>-</td>
</tr>
<tr>
<td>brain</td>
<td>0.32 ± 0.28</td>
<td>-</td>
</tr>
<tr>
<td>ovary</td>
<td>1.54 ± 1.00</td>
<td>+ (b)</td>
</tr>
<tr>
<td>endometrium</td>
<td>0.95 ± 0.50</td>
<td>+</td>
</tr>
<tr>
<td>placenta</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>ovarian serous</td>
<td>34.31 ± 22.87</td>
<td>-</td>
</tr>
<tr>
<td>ov. metastatic</td>
<td>46.34 ± 49.68</td>
<td>-</td>
</tr>
<tr>
<td>ov. mucinous</td>
<td>1.83 ± 1.19</td>
<td>-</td>
</tr>
<tr>
<td>endometrial</td>
<td>9.32 ± 18.10</td>
<td>-</td>
</tr>
<tr>
<td>lung (primary)</td>
<td>6.11 ± 5.71</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1-5** Expression of FR proteins in human tissues, as detected with a $[^{13}\text{H}]$-folic acid binding assay$^{168}$ and immunohistochemistry with M0v19 antibody;$^{158}$ (a) proximal tubule only; (b) germinal epithelium only.

In recent years, indications have emerged of at least two further routes of folate transport: on one hand, absorption of folates appears to happen in the small intestine through some as yet unidentified, RFC-independent system that operates optimally at low pH, in addition to the already known transporters;$^{155,171}$ on the other hand, several organic anion transporters of the SLC21 family display affinity for folic acid and methotrexate in the range of 10 μM, comparable with the affinity of RFC for reduced folates.$^{150}$ These carriers are
expressed primarily in the epithelium of liver and kidney, but also in some types of cancer; relatively little is known about them so far, and their impact on the transport of folates and folate-derived drugs has not been elucidated yet.

1.3.4. FR-targeted delivery

The differential affinities of FR and RFC for folic acid and its conjugates, alongside the selective expression pattern of FR, suggested the idea of exploiting folic acid as a 'Trojan horse' to deliver molecules selectively to cancer cells via conjugates. Folic acid may be chemically bound to a second molecule, a payload, to form a conjugate; if this derivative retains the parent folate’s relative affinities for RFC and FR, it may be taken up by those cells which overexpress FR at a significantly greater rate than all other cells, achieving a selective delivery of the conjugate to FR-overexpressing tissues.

The chemical structure of folic acid presents an obvious opportunity for chain elongation and conjugation at the glutamic acid moiety, in the form of two carboxylic acid functions. These lend themselves to the formation of amide and ester bonds through relatively straightforward chemical methods. Moreover, the glutamate portion of the folic acid molecule does not seem to play a relevant role in the interaction with folate-transporting membrane proteins; thus, modification at this moiety may reasonably be expected to result in retention of the favourable relative affinity pattern of folic acid for RFC and FR transporters.

These considerations have led to a number of lines of research in the field that can be broadly defined as ‘FR-targeted delivery’, where a variety of small-molecular and macromolecular payloads have been conjugated to the pterolic acid moiety of folic acid, mostly via a glutamate but also through a substitute linker. Folate targeted delivery has attracted a growing amount of attention in the last fifteen years, and has been applied successfully in drug delivery and cancer imaging as well as in immunotherapeutic approaches.
1.3.5. Conjugates of folic acid

Around the time when the nature and role of FR was being elucidated, research in the Low group demonstrated that conjugation with folic acid could achieve receptor-mediated endocytosis of large proteins in a variety of cultured animal cells;\(^ {172}\) after the selective expression pattern of FR became apparent, the focus of this research shifted from general cellular delivery of macromolecules to tumor-targeted delivery of cytotoxic agents.\(^ {13}\) Early attempts still focused on proteins as payload, in particular toxins such as mormordin\(^ {177}\) and *Pseudomonas* exotoxin;\(^ {178}\) these conjugates displayed potent cytotoxic activity, however the interest of the group moved to applications with liposomes and low-molecular-weight drugs, which proved rather successful in the following years.\(^ {13}\) The folic acid-\(*Pseudomonas* exotoxin conjugate marked the first application of a disulfide-based cleavable linker in a folic acid conjugate, which would become a recurring theme in the work of the Low group; this study also highlighted the potency-enhancing effect of a cleavable linker with respect to the non-cleavable corresponding conjugate.\(^ {178}\)

Initial investigation of conjugates of folic acid with low-molecular-weight cytotoxic agents met with somewhat mixed results with regard to both the necessity of a drug-release system and their effective selectivity. Taxol was chosen for one of the first drug-folic acid conjugate attempts by the Low group;\(^ {179}\) a short ethylene glycol-based linker was introduced in the hope that this type of construct would address the low solubility of taxol as well as its tumor-selectivity. Two positions in the taxol structure were considered for conjugation, the C7 and C2’ hydroxyl groups, and the corresponding conjugates were synthesized incorporating a polyether linker (EG) and a cleavable moiety in the form of an acid labile \(\alpha\)-amino or \(\alpha\)-alkoxyester (I-44-I-47, Fig. I-18); in the case of the C7 conjugate also a non-cleavable version was prepared (I-48).\(^ {179}\) While the latter was entirely inactive \textit{in vitro}, compounds I-44-I-47 did display potent cytotoxicity, although approximately tenfold lower than that of taxol; however, no selectivity could be detected for FR-positive cell lines. While the reasons for this lack of selectivity were unclear, this study reinforced the idea that a cleavable linker is beneficial for activity in this type of conjugates. Meanwhile, a group at the University of Nebraska prepared and tested a non-cleavable conjugate of folic acid with oligomeric deoxyribonucleotide (FdU)\(_{10}\), a fluoropyrimidine-type of cytotoxic
agent that targets thymidylate synthase (I-49, Fig I-19). Contrary to the non-cleavable taxol conjugate, I-49 was about ten times more active in in vitro cellular assays than the untargeted parent compound; however, its cytotoxicity was in fact higher in FR-negative than in FR-positive cells. The effect of I-49 in the latter cell line was indeed reduced by the presence of folic acid in the medium, possibly indicating competition with the vitamin for the same transport system, but the picture at this point was far from clear.

Fig. I-18 Early folic acid-taxol conjugates
Fig. I-19 Folic acid conjugate with FdU₁₀

Fig. I-20: Folic acid-mitomycin C conjugates
Clearer and more encouraging results were achieved with the synthesis of a series of mitomycin C-folic acid conjugates,\textsuperscript{181} starting with EC-72 (I-50, Fig. I-20), a prodrug-type of conjugate which bears a cleavable disulfide-based linker.\textsuperscript{182} I-50 was highly potent in vitro against FR-overexpressing KB cell lines, and practically inactive in FR-negative lines (expressing approximately one hundred fold less FR); activity in KB cells was suppressed in the presence of folic acid, and antitumor effect was confirmed by \textit{in vivo} studies with FR-overexpressing models,\textsuperscript{181,182} where EC-72 showed remarkably less toxicity than the parent drug mitomycin C. In these studies I-50 was compared to its non-cleavable counterpart EC-110 (I-51, Fig. I-20) \textsuperscript{181}, which failed to produce any significant \textit{in vivo} antitumor activity, lending further support to the importance of drug release for the efficacy of folic acid conjugates. The same set of studies indicated that the efficacy of I-50 was reduced in large and well-established tumors, and that synergistic effects were obtained by its combination with taxol, presumably due to the P-gp suppressing effect of prolonged exposure to free mitomycin C.\textsuperscript{181} These observations prompted the design of EC-118 (I-52, Fig I-20), an analog of I-50 that includes a second cleavable moiety, an acidic-labile hydrazone introduced to improve intracellular release of the drug. I-52 displayed a similar cytotoxicity profile to I-50, and in an \textit{in vivo} study its combination with taxol was found to be superior to the I-50-taxol regimen.
I-21: Examples of disulfide-based folic acid-drug conjugates
The promising results obtained with EC-72 and EC-118 were followed by the synthesis and testing of a series of disulfide-based drug-folic acid conjugates, including payloads such as camptothecin (I-52, Fig. I-21),183 desacetylvinblastine monohydradizide (vinblastine-derivative EC-145, I-53),184,185 and maytansinoid DM1 (I-54).186 I-52 and I-53 feature a self-immolative linker containing a disulfide bond and a carbonate or carbamate moiety separated by a two-carbon spacer (according to the general structure I-55, Fig. I-22),173 this linker was designed to be cleaved in physiological conditions in the presence of glutathione, releasing the corresponding drug as a free amine or alcohol in a reaction which also produces a five-member byproduct (path a in Fig. I-22, although a second pathway, path b, is also possible). While both exploit the high glutathione concentration typical of cancer cells to release the drug, the self-immolative linker represents an improvement over a simple disulfide bond, such as that found in I-50 and I-54, in that it can be introduced easily by reaction of the versatile intermediate I-56 (Fig. I-22) with a variety of amino- or hydroxy-group containing drugs.173 Recent applications of the same strategy include an epothilone conjugate (aziridine-based derivative I-57, Fig. I-23)187 and a bifunctional construct containing vinblastine and mitomycin C (EC-0225, I-58),188 whose design is based on a combination of I-50 and I-53. I-58 represents the first example of folate-targeted dual prodrug and displayed promising activity in in vitro and in vivo preclinical evaluation; in particular, it proved more active than a combination of I-50 and I-53 in animal models, and was approximately as well-tolerated.188

Several of these folic acid conjugates have been deemed promising enough to enter clinical development. I-58 is currently in phase I for refractory or metastatic solid tumors,79,188 while epothilone derivative I-57 (epofolate) is in early clinical evaluation (phase I/II) at Bristol-Myers Squibb for the treatment of patients with advanced ovarian, renal or breast cancer;79 I-53 has reached phase II trials for several types of solid tumors.79,189.
Fig. I-22: Self-immolative linker for folic acid conjugates

Fig. I-23: Recent disulfide-based folic acid-drug conjugates
Besides the unsuccessful series of taxol derivatives I-44-I-47, conjugates with different acid-cleavable linkers have been explored as well, for instance in derivative I-59 (Fig. 1-24)\(^{190}\) bearing a hydrazone moiety alone. While its in vitro activity was comparable to that of its reducible counterpart I-53, the in vivo profile of the latter was superior. The rationale for employing an acidic labile linker was based especially on early reports on the intracellular destiny of FR-containing endocytic vesicles, which indicated a marked decrease of pH in the compartment after internalization, down to approximately pH 4.\(^ {165}\) The disappointing results with I-44-I-47 and I-59 prompted further investigations to clarify the release mechanism,\(^ {13}\) using a FRET probe closer in structure to the candidate drugs than the pH-sensitive, multiply-charged dyes employed in the early studies.\(^ {191}\) The new measurements led to a higher value for the relevant endocytic compartment of approximately pH 6, which may explain at least in part the reduced activity of the acid cleavable prodrugs, as the drug release would then be significantly less efficient than anticipated.

![Chemical structure of I-59](image)

**Fig. 1-24: Acid-sensitive conjugate I-59**

All the folic acid conjugates discussed so far feature a linkage through the carboxylic acid in position \(\gamma\) of the glutamate; in principle, conjugation through the \(\alpha\) carboxylic acid is also possible. Initial reports hinted however at the possibility that \(\gamma\)-conjugates, but not \(\alpha\)-, would be taken up by FR;\(^ {172}\) in addition, it was generally deemed preferable to work with single compounds rather than mixtures to simplify the interpretation of activity data. For these reasons, a selective solid-phase synthesis of \(\gamma\)-conjugates was developed in the Low group, and was employed for the preparation of subsequent conjugates. However, it has not been conclusively demonstrated that \(\alpha\)-conjugates are not subject to FR-mediated internalization as well.
In parallel to therapeutic applications featuring cytotoxic agents, folic acid conjugates have been the object of promising research also in the field of tumor imaging. FR-targeted radionuclide chelating systems have been at the forefront of this research; several chelating groups have been investigated, and have been complexed with a variety of nuclides for applications in different imaging techniques.\textsuperscript{13,192,193} One of the first studies to demonstrate the feasibility of this approach employed a deferoxamine-folic acid conjugate for the chelation of $^{67}$Ga (I-61, Fig. I-25);\textsuperscript{193} soon afterwards it was followed by the folate-DTPA conjugate I-62 \textsuperscript{194} (Fig. I-25), which presents a more favourable pharmacological profile and has been labelled both with $^{111}$In\textsuperscript{195} and $^{99}$Tc.\textsuperscript{196} A close analog of I-62, $^{99}$Tc-labeled EC20\textsuperscript{197} (I-63) has been recently tested on cancer patients in an exploratory study.\textsuperscript{198} While most of these conjugates feature amide-bond-based linkers, a versatile approach to the synthesis of folic acid conjugates through click-type chemistry has been recently developed,\textsuperscript{199} and has been applied to several structurally diverse imaging probes;\textsuperscript{200} this chemistry, which is based on folic acid alkyne-containing derivative I-64 (Fig. I-25), could be applied to a much wider range of structures and may become a useful tool in the preparation of folic acid conjugates with therapeutic agents as well.

While conjugates of toxins with folic acid have had some follow-up,\textsuperscript{164,201} this vitamin has been proposed as a targeting agent for other types of proteins, often in applications that do not require internalization of the payload.\textsuperscript{164} For example, conjugates of folic acid with prodrug-activating enzymes have been proposed as a tool to concentrate these enzymes on the surface of FR-positive cancer cells, in an approach similar to antibody-directed enzyme prodrug therapy (ADEPT);\textsuperscript{164} in a trial of this concept, the enzyme penicillin V amidase has been delivered to FR-positive cells and used to activate a doxorubicin prodrug.\textsuperscript{202} FR-targeted immunotherapy applications have been developed in the context of cancer\textsuperscript{176} and rheumatoid arthritis.\textsuperscript{170}
2. Aims and scope

It is the overall objective of this thesis to provide epothilone conjugates with tumor-targeting moieties. In a first phase this will require the synthesis of novel epothilone analogs that are suitably functionalized for conjugation, and the assessment of their biological properties with respect to tubulin polymerization and cytotoxicity. This is the major objective of this PhD thesis. In a second phase a series of conjugates of one or more of these derivatives with tumor-targeted agents will be prepared, and the biological activity of such conjugates will be explored. To this end, a significant amount of at least one of the targeted epothilone analogs will need to be synthesized; this amount is estimated to be in the range of 500-1000 mg.

2.1. Benzimidazole-based epothilone analogs for the synthesis of conjugates

While the mechanism of action and the potency of epothilones have brought several members of this class into clinical development, the issue of their relatively narrow therapeutic index so far has remained largely unaddressed. It is therefore reasonable, from a drug design point of view, to explore methods that may widen the epothilones’ therapeutic window. Conjugation with tumor-targeting moieties is an attractive strategy for this class of compounds, given their synthetic accessibility and the large body of synthetic and SAR knowledge that has been accumulated over the years. No tumor-targeted conjugates of epothilones had been described when this project was started; in the meantime information has been disclosed on one such derivative by Endocyte, Inc. ([-57, see Section 1.3.5], but overall the field remains largely unexplored.

When designing an epothilone derivative which could be employed as the cytotoxic payload in a tumor-targeted conjugate, two considerations are of primary importance: (1) which part or function of the epothilone may serve as an attachment point for a tumor-targeting moiety without reducing the antimitotic potency of the drug, and (2) which conjugation chemistry would be best suited for our conjugate.

Regarding the first issue, it is worth noting that the natural epothilone framework offers little opportunity for chemical modification; only two functionalities may lend themselves readily to functionalization, namely the two hydroxyl groups at C3 and C8, which might be
converted for instance to esters, carbonates or carbamates. However, functionalization of these is less straightforward than might be expected, since both of these are secondary alcohols in a relatively crowded sterical environment. Moreover, derivatization at these positions may entail a risk of an unwanted macrocycle opening through retro-aldol reactions. In addition to these drawbacks, it is not clear how much additional volume and functional modification could be imparted to the molecule at these positions without negatively affecting activity; cleavage of the appended moiety is very likely to be necessary to maintain a high potency.

While the natural epothilone side chain lacks a readily modifiable reactive group, it has been shown that the aromatic moiety can tolerate profound chemical modification without a significant loss of biological potency, and in addition, that both additional sterical bulk as well as polar groups may be introduced at certain positions in this part of the molecule without compromising antiproliferative activity.\textsuperscript{33,91} Therefore, a promising approach may be found by modifying the side chain with an additional functionality amenable to conjugation.

Among the large array of side-chain-modified epothilone analogs that have been synthesized and tested in the past years, benzimidazole-based derivatives\textsuperscript{93} are of particular interest, because the benzimidazole moiety offers a site for further modification at the N1 position. Dimethylbenzimidazole analogs of epothilone B (\textbf{A-8}, \textbf{Fig. A-1}) and epothilone D (\textbf{A-9}) both display high antiproliferative potency;\textsuperscript{93} at the beginning of the project, some indication existed that a substituent somewhat larger than the methyl group may be well tolerated, based on structural data on the $\beta$-tubulin-epothilone A complex\textsuperscript{203} (however this structural information has to be treated with caution, also in the light of subsequent literature\textsuperscript{204}) We reason that a short chain bearing a functional group can likely be introduced without severely affecting potency; should the steric tolerance for substituents at this position be very high in the tubulin-epothilone complex, the targeting moiety may not even have to be cleaved in order to preserve activity, otherwise a prodrug approach will be adopted.
Fig. A-1: Benzimidazole-based analogs of Epo B and D

The dimethylbenzimidazole side chain has displayed enhanced biological activity particularly in combination with the epothilone D macrocycle, rather than the epothilone B framework\textsuperscript{33} (see Section 1.2.3); thus A-9 is significantly more potent than epothilone D and comparable with epothilone B and A-8. Moreover, the epothilone D core structure appears more suitable for the cellular uptake pathway anticipated for at least some of the planned conjugate, namely endocytosis involving transport into relatively acidic cellular compartments; the highly acid sensitive epoxide moiety of epothilone B is likely to undergo hydrolysis under these condition, producing a mixture of inactive diols.\textsuperscript{205} Therefore the epothilone D framework has been selected for the macrocyclic part of the derivatives investigated in this project.

We envision introducing a 2-X-ethyl residue at the N1 position of the benzimidazole core, where X is any functionality that may be used for conjugation. Specifically, it is one of the objectives of this thesis to prepare epothilone analog A-1 and A-2 bearing a primary amine (X = NH\textsubscript{2}, Fig. A-2) or a primary hydroxyl function (X = OH, Fig. A-2).
2.2. Conjugates of A-1

In principle, epothilone derivatives A-1 and A-2 may be attached to any tumor targeting moiety among those mentioned in Section 1.1; because of the interesting targeting properties of folic acid and its precedents in conjugates with other antiproliferative agents (see Section 1.3.5-1.3.6) this project will focus on epothilone-folic acid conjugates. The required folic acid intermediates should be accessible through relatively straightforward synthetic methods in a limited number of steps. Based on the relevant literature, conjugation will involve the γ-carboxyl group of the glutamate portion of folic acid.¹⁷² Both a non-cleavable version A-6 (Fig. A-3), which should remain intact in the cellular environment, and a cleavable conjugate of the type A-7, which includes a dipeptide moiety (a PheGly sequence attached to a β-alanine spacer, Fig. A-3) susceptible to cleavage in the environment of the endosome under the action of the enzyme cathepsin B will be prepared. Cathepsin B is an endopeptidase overexpressed by many types of tumors;²⁰⁶ its relative rate of cleavage of different amino acid sequences has been investigated in several studies, and the PheGly moiety has been chosen as a result of the investigation of the relevant literature.²⁰⁷,²⁰⁸ The endopeptidase is expected to cleave the linker at the amide bond between glycine and the amino group of A-1, releasing the latter in the endosomal compartment. The β-alanine spacer serves to provide some further distance between the putative cleavage site and the sterically encumbered pteroic acid; the glutamate is not expected to play a significant role in the recognition of the sequence by cathepsin B.
At a later stage during the project, we realized that a carboxylic acid based epothilone analog might be interesting to investigate in the context of prodrug-type conjugates. This reasoning was based on the consideration that, for conjugates that follow an endocytic internalization pathway and find themselves in an acidic cellular compartment, translocation to the cytosol might be hindered by a free amino group, which would be charged under these conditions; a carboxylic acid moiety, on the other hand, would not carry an additional charge and might be beneficial for intracellular distribution. However, total synthesis of a third analog of A-1 and A-2 with X = COOH was beyond the time scope of this project at that point. A convenient way of preparing a similar derivative quickly was to synthesize compound A-3 (Fig. A-4).

Given that the major interest in A-3 resides with its cellular behavior as a releasable antimitotic agent in comparison to A-1, rather than as part of a non-cleavable conjugate, only the cleavable conjugate A-8 (Fig. A-5) will be prepared, where the same cathepsin B sensitive dipeptide is introduced as in A-7. The main difference between A-8 and A-7 is the
reversed orientation of the dipeptide moiety, due to the presence of a carboxylic acid rather than amine function in the epothilone part; as a consequence, the spacer at the opposite end of the linker is an ethylenediamine unit. The putative endopeptidase cleavage site is, as in A-7, at the glycine C terminus (indicated with a in Fig. A-8); however, as cathepsin B also possesses carboxypeptidase activity, glycine and phenylalanine are expected to be cleaved as well (paths b and c) after the initial hydrolysis, thus releasing free A-3.

![Chemical structure of A-3](image)

**Fig. A-4:** Carboxylic-acid derivative A-3

![Chemical structure of A-8](image)

**Fig. A-5:** Conjugate A-8
2.3. Retrosynthetic analysis of structures A-1 and A-2

Fig. A-6: Retrosynthetic analysis of A-1 and A-2: primary disconnections

The synthesis of target compounds A-1 and A-2 will follow a common approach, which contains elements of previous synthetic work done on the total synthesis of epothilones A and B by the groups of Danischeffsky,\textsuperscript{38} Nicolaou\textsuperscript{209} and Schinzer,\textsuperscript{210} and was developed by Altmann and coworkers at Novartis for the synthesis of several epothilone analogs.\textsuperscript{81} Two crucial disconnections of the macrocyclic core allow A-1 and A-2 to be traced back to two primary building blocks (Fig. A-6): on one side, either one of vinyl iodides A-9 or A-10, which include the appropriate side chain and the C12-C15 portion of the macrocycle; on the other side, alkene A-5, which contains the C1-C11 part of the targets and is a common precursor of A-1 and A-2. The connection of the southeastern moiety A-5 with either A-9 or A-10 is envisioned to be accomplished through a palladium-mediated Suzuki-Miyaura reaction; after selective deprotection of the secondary alcohol at C15 and hydrolysis of the methyl ester at C1, a macrolactonization reaction will close the 16-membered ring. From this point, completion of the synthesis of A-1 and A-2 will only require cleavage of the two silyl ethers on O3 and O7 and of the side chain protective group; ideally, this should all be accomplished in one step, most easily under acidic conditions, therefore Boc and TBS protecting groups will be the primary choice for the side chain functionalities of A-9 and A-10, respectively.
Intermediate A-5 contains four chiral centers and is the most demanding building block in terms of stereoselective synthesis. The synthesis of A-5 was already established in the literature before the start of this project. A highly efficient stereoselective synthesis of olefin A-5 from ketone A-11 (a compound first described by Schinzer and coworkers\textsuperscript{211} Fig. A-7) and aldehyde A-12 has been developed as part of a trans-epothilone A project\textsuperscript{91}, providing access to multiple g-quantities of this intermediate.

Based on these considerations, the preparation of A-5 will follow the scheme outlined in Fig. A-7: the terminal double bond at C10-C11 will be installed last, through a Grieco-Sharpless dehydration protocol on alcohol A-13; the latter is derived from the crucial aldol product A-15 though protecting group manipulations and oxidation of the primary hydroxyl goup of A-14 to introduce the carboxylic acid moiety at C1. A-15 is obtained from A-11 and A-12 through an aldol reaction known to be highly stereoselective\textsuperscript{91,210} A-11 can be obtained from β-keto-aldehyde A-16 through Oppolzer chemistry\textsuperscript{91}, while the chiral center of aldehyde A-12 will be installed by means of Evans oxazolidinone chemistry, starting from commercially available valerolactone A-17 (Fig. A-8).
Synthesis of the benzimidazole-containing vinyl iodides A-9 and A-10 (Fig. A-9) will follow essentially the same path for both derivatives with the introduction of the trisubstituted double bond via a Wittig-type reaction on aldehydes A-18 and A-19; this step establishes the required Z-geometry of the trisubstituted vinyl iodide moiety, a motif which is rather cumbersome to access otherwise.
The other key reaction in the synthesis of \textbf{A-9} and \textbf{A-10} is the introduction of the chiral center at the future C15 of the target compounds; this was devised as a stereoselective reaction on achiral aldehydes \textbf{A-20} and \textbf{A-21}, for which several methods are available in principle. The initial plan envisions an aldol-type reaction with Oppolzer’s chiral sultam \textbf{A-22},\textsuperscript{213} followed by reductive cleavage of this auxiliary to deliver aldehydes \textbf{A-18} and \textbf{A-19}. Due to unsatisfactory stereoselectivity in the first attempt (with \textbf{A-20}, see \textbf{Section 3.1.1}), a revised approach has been developed based on asymmetric Brown allylation\textsuperscript{214} and ozonolysis of the resulting olefins \textbf{A-23} and \textbf{A-24}.

![Fig. A-10: Retrosynthesis of intermediates A-20 and A-21](image)

The construction of the benzimidazole system in aldehydes \textbf{A-20} and \textbf{A-21} will involve the introduction of the ethylenediamine or 2-aminoethanol handle at a very early stage through nucleophilic aromatic substitution on either commercially available 4-flouro-3-nitrobenzoic acid \textbf{A-25} or its methyl ester (\textbf{Fig. A-10}), to give intermediates \textbf{A-26} and \textbf{A-27} respectively. This is somewhat suboptimal from a strategic point of view, since a more efficient approach would rather introduce the functionalized ethylamine ‘handle’ at a late stage of the synthesis, allowing the preparation of a range of diversely functionalized epothilones through the scale-up of one common epothilone intermediate. However, we decided to keep the ‘handle’ as short and simple as possible to avoid interfering the biological activity of the releasable final compounds; this limited our options for late-stage coupling of the ‘handle’ to an already formed benzimidazole. Forming the benzimidazole complete with handle at a late stage, on the other hand, seemed risky in that a relatively large amount of chemistry would have needed to be performed on an already advanced intermediate, in addition to the targeting-moiety conjugation chemistry.
2.4. Carboxylic acid-based derivative of A-1

Preparation of A-3 will start from advanced intermediate A-28; instead of full deprotection to A-1, selective cleavage of the Boc group and an acylation reaction with succinic anhydride, followed by cleavage of the silyl ether protective groups should afford A-3 in a straightforward manner.

![Image of A-3 and A-28]

**Fig. A-11**: Derivative A-3 and its precursor A-28

2.5. Synthetic approach to the conjugates

Retrosynthesis of A-6 and A-7 was initially designed to minimize the number of chemical steps that the valuable advanced intermediate A-29 (**Fig. A-12**, derived by selective deprotection of the amine on A-28) would need to undergo. Thus, a fully assembled and fully protected folic acid moiety A-30 (PG₁, PG₂ and PG₃ being generic protecting groups, see **Section 3.4**) will be activated and reacted with amine A-29, a crucial step followed only by cleavage of all protecting groups; use of as many simultaneously cleavable protecting groups as possible will limit the number of such deprotection steps to one or two.

Along the same lines, A-29 will be reacted with the complete folic acid-β-alanine construct A-31 (**Fig. A-12**, where AA₁AA₂ is the dipeptide PheGly, C-terminus on the left), similarly protected and activated in the same manner as A-30.
Fig. A-12: First retrosynthetic approach to A-1-folic acid conjugates

This overall strategy could not be implemented (as will be discussed in the Results and discussion section), thus a revised strategy was conceived in which the pteroic acid moiety would be attached to the rest of the construct as late as possible (Fig. A-13). This constituted a reversal of our first generation approach, in that the epithilone moiety would now undergo several conjugation and deprotection steps, particularly in the cases of A-6 and A-8; however, the relatively large amounts of A-28 that were available after completion of the first part of the project and the physico-chemical properties of the epithilone intermediates, which were significantly more favourable than those of the pteroic acid derivatives, prompted this decision. To limit, however, the number of steps from A-29 onwards, the complete linker moiety of A-7 was to be synthesized separately, and include
the glutamic acid part (Fig. A-13); after deprotection of the amino function on the glutamic acid, protected pteroiic acid A-32 would be coupled at last with the rest of the molecule, and two deprotection steps would yield the desired conjugates.

\[
\text{linker} = \text{Glu} \quad \text{A-6} \\
\text{linker} = \text{Glu-βAla-PheGly} \quad \text{A-7}
\]

Fig. A-13: Second generation approach to A-6 and A-7

The same approach was envisioned for A-8 starting from A-33 (Fig. A-14). However, the synthesis of the linker moiety for A-8 requires a different protecting group strategy than that used for the corresponding building block of A-6, given that this linker has two N-terminals (see Section 3.5).
Synthesis of protected pteroic acid A-32 will be accomplished by protection of pteroic acid A-34 with trifluoroacetic anhydride (Fig. A-15), which is expected to afford protection at the secondary amino group; it is unclear whether the guanidine moiety will be protected as well in this reaction. A-34 will be prepared directly from folic acid through enzymatic cleavage with carboxypeptidase G.
**Fig. A-15:** Approach to intermediates A-30 and A-32
3. Results and Discussion

Synthesis of the amino and hydroxy functionalized benzimidazole-based epothilone D analogs A1 and A2 (Section 2.1) was performed following the strategy outlined in Section 2.3.

3.1. Synthesis of the amino-functionalized epothilone analog

3.1.1. Synthesis of the benzimidazole building block

Synthesis of the benzimidazole building block R-8 started from commercially available 4-fluoro-3-nitrobenzoic acid (A-26) and is summarized in Scheme R-1. After protection of the carboxylic acid moiety as a methyl ester, the Boc-protected ethylenediamine moiety was introduced at the C4 position through nucleophilic aromatic substitution of fluoride ion. The necessary tert-butyl-2-aminoethyl carbamate was easily synthesized in multigram amounts by reaction of di-tert-butyl dicarbonate with excess ethylenediamine.\(^\text{215}\) These first steps were both high yielding and afforded crystalline products (R-1 and R-2), for which chromatographic purification could be avoided in the multigram amount batches.

\[ \text{Scheme R-1: a: MeOH H}_2\text{SO}_4, \text{reflux, 98%; b: BocNHCH}_2\text{CH}_2\text{NH}_2, \text{TEA, DCM, r.t., 96%; c: 1 atm H}_2, \text{Pd/C, MeOH, r.t., 99%; d: (EtO)}_3\text{CCH}_3, \text{EtOH, reflux, 96%} \]
Subsequent reduction of the nitro group of **R-2** and cyclization of the resulting diamine with triethylorthoacetate afforded the desired functionalized benzimidazole **R-3** in very good yield (88% over 4 steps). The synthesis then proceeded with reduction of the ester to alcohol **R-4** and partial re-oxidation of the same to aldehyde **A-21** (**Scheme R-2**); previous work in the group in the context of the dimethylbenzimidazole- and quinoline-based epothilone analogs had established the difficulty of controlling the reduction of compounds of the type **R-3** directly to the corresponding aldehyde. Interestingly, while in those previous cases Swern conditions had worked smoothly in the oxidation of the benzylc alcohols, this approach failed completely to convert **R-4** to **A-21**; the latter was obtained instead in excellent yield (96%) using MnO₂ as the oxidizing agent.

The next step was the crucial introduction of the chiral center at the future position C15 of the epothilone macrocycle. Our initial approach made use of Oppolzer’s bornane sultam auxiliary²¹³ in an aldol reaction with aldehyde **A-21** (**Scheme R-3**); unfortunately, the stereoselectivity of this reaction was limited, giving the product as a mixture of the two possible diastereoisomers in a ratio of approximately 2:1. After protection of the newly-formed hydroxyl group as a TBS-ether (i.e. at the level of **R-6**), chromatographic separation of the two diastereoisomers was possible. The major diastereoisomer was carried on through the synthesis, on the assumption that it would be the (S)-product represented in **Scheme R-3**, in accordance with literature precedents for the reaction of an aldehyde with the boron-enolate of the sultam **A-23**, (1S)-N-acetyl-2,10-camphorsultam.²¹⁶,²¹⁷ The identity of this isomer was later confirmed by the comparison of the material (**R-7**) obtained from the major isomer of **R-6** with the same aldehyde obtained by Brown allylation (*vide infra*); the absolute stereochemistry was proven for the latter approach by Mosher ester
analysis,\textsuperscript{218} therefore the stereochemistry of the major isomer obtained from the aldol reaction between A-21 and A-23 is the one represented in Scheme R-3 by structure R-5.

Reductive cleavage of the auxiliary from R-6 afforded aldehyde R-7 in good yield; this intermediate, however, proved to be rather unstable and prone to β-elimination of the silyl ether to give the 2,3-unsaturated derivative (complete conversion to this decomposition product was once observed upon dry storage at 4°C for two weeks). Once prepared, R-7 was therefore immediately converted into vinyl iodide R-8, which proved to be stable upon storage. The conversion of R-7 to R-8 was accomplished through a Wittig-type reaction with ylide R-9 (Scheme R-3) which is known from the literature to proceed with complete

\[
\text{Scheme R-3: a: 1) } \text{BEt}_3, \text{CF}_3\text{SO}_2\text{H, DCM, r.t.} @ 40^\circ\text{C}; 2) \text{DIPEA, R-12, 0}\,^\circ\text{C}; 3) \text{R-7, -78}\,^\circ\text{C, 15h, 78\% (2:1 mixture of diastereoisomers); b: TBSOTf, lutidine, DCM, r.t., 57\% (38\% single diastereoisomer); c: 2 eq DIBAL, DCM, -78^\circ\text{C, 83\%; d: R-13 THF, -78^\circ\text{C, 37\%}}}
\]

Z-selectivity, but also with yields limited to less than 50\%.\textsuperscript{212,219} An explanation for these observations was proposed by Smith and coworkers,\textsuperscript{220} in that one of the two possible betaine intermediates (the one which would lead to the E- Wittig-type product) would be
more likely to undergo intramolecular nucleophilic attack to form an epoxide rather than proceed along the olefination pathway (Scheme R-4); therefore, the only intermediate able to complete the Wittig-type reaction would lead to the Z-alkene, which would be found in a mixture with the epoxide by-product. Consistent with this explanation, and with Smith and coworkers’ findings on a model reaction, only one alkene product was observed alongside a second product, displaying the m/z value of the epoxide, but no efforts were made to characterize the latter.

The Z-geometry of the vinyl iodide was confirmed by NOESY spectra at the stage of the completed epothilone.
Scheme R-5: a: 1) C₃H₅MgBr, (-)-Ipc₂BCl (1.5 equiv), Et₂O, 0°C→RT (A); 2) slow addition of A to R-7, -100°C, 89%, 94% ee; b: TBSOTf, lutidine, DCM, r.t., 5h, 90%; c: TES-Cl, imidazole, DMAP, 98%; d: OsO₄, NaIO₄, 2,6-lutidine, 74%; e: 1) R-13, THF, -78°C; 2) R-10 or R-17, THF, -78°C, 42%.

Although the synthesis described above was successful in that it allowed the preparation of R-8 in sufficient amounts for the subsequent completion of the target epothilone, the low stereoselectivity of the transformation of A-21 into R-5 prompted the search for a more efficient alternative. Thus the Brown allylation protocol was applied to aldehyde A-21, and afforded allylic alcohol A-24 (Scheme R-5) in very good yield and with 94% ee. The ee was determined through Mosher ester analysis, which also established the absolute configuration of A-24 as (S). A-24 was then protected as TBS ether and transformed into aldehyde R-12 through an oxidative cleavage of the terminal double bond. Ozonolysis was employed initially, with yields that changed significantly from batch to batch; a more reliable method was found in a protocol based on OsO₄. This rather toxic reagent was used at first in stoichiometric amounts, but an improved catalytic protocol with excess of sodium periodate as the terminal oxidant was finally implemented for the synthesis of R-12. A final improvement in the synthesis of this building block was the change in the protecting group for the secondary alcohol from TBS to TES. The bulky TBS moiety was especially useful in the first approach for the chromatographic separation of R-6 and its diastereoisomer; since at this stage of the synthesis we were dealing with enantiomers, this point became irrelevant. The smaller and more labile TES group could be used instead,
which was anticipated to offer an advantage at a later point in the synthesis, when selective cleavage of this protecting group would be required in the presence of two TBS-protected secondary alcohols. Thus after the Brown allylation, TES protection afforded A-19, which was submitted to the same oxidation and Wittig protocols as the TBS analog to yield TES-protected vinyl iodide A-9.

3.1.2. Synthesis of the C1-C11 building block

Synthesis of the C7-C11 portion of the C1-C11 building block started from commercially available δ-valerolactone (A-18, Scheme R-6), which was opened in a base-catalyzed reaction in the presence of benzyl chloride to afford acid R-12; Evans chemistry was used to install the stereogenic center at C2 of R-12 (the future C7 of the epothilone structure), through methylation of the chiral oxazolidinone R-13; reductive cleavage of the chiral auxiliary and Swern oxidation of the resulting alcohol afforded aldehyde A-13.

A-13 was connected to the C1-C5 building block A-12 through an aldol reaction, which represents the key step in the assembly of the C1-C11 fragment, as both the two stereogenic centers at the future C6 and C7 of the epothilone are installed in this transformation. A-12 was first described by Schinzer\textsuperscript{211,210} and its lithium-mediated aldol reactions are known in the literature to proceed with unusually high stereoselectivity for the (6R, 7S)
diastereoisomer,\textsuperscript{81,210} this was reproduced in the present case, with a ratio of approximately 10:1 (from NMR data) between the major and minor diastereoisomer.

A number of protecting group manipulations were necessary at this point in order to obtain intermediate \textbf{A-15}, where both secondary hydroxyl groups were masked as TBS ethers while the primary hydroxyl function was made available for further transformation.

\textbf{Scheme R-7}: a) \textit{n-BuLi}, (iPr)\textsubscript{2}NH, \textbf{R-25}, -78°C; 2) \textbf{R-24}, -78°C, 88% (dr 10:1); b) PPTS, MeOH, r.t., 96%;

Oxidation at C1 was performed to install the required carboxylic acid function (\textbf{Scheme R-8}). A large excess of pyridinium dichromate (11 equivalents) afforded acid \textbf{R-18} in moderate and not entirely reproducible yields (55-73%); a two-step protocol employing Swern and Pinnick conditions was preferred in the scale-up stage of the synthesis, since it allowed to obtain \textbf{R-18} in more reliable yields while avoiding the use of large amounts of chromium.
Protection of the carboxylic acid as a methyl ester and catalytic hydrogenation to remove the benzyl ether at the C11 position afforded intermediate **A-14**, which was submitted to Grieco-Sharpless olefination conditions in order to install the terminal double bond between C10 and C11.

![Chemical structure](image)

**Scheme R-8**: a: 1) oxaly chloride, DMSO, -78°C, 86%; 2) 2-methylpropene, NaClO₂, NaH₂PO₄, H₂O, t-BuOH/H₂O, -10°C r.t., 87%; b: DCC, DMAP, MeOH, DCM, r.t., 77%; c: 1 atm H₂, Pd/C, MeOH, r.t., 98%; d: 1

A certain amount of Schinzer ketone **A-12** was available in the laboratory at the start of the project and was used for the first synthesis of the target amino-epithilone; however, scale-up of this preparation in sufficient quantities for performing further conjugation chemistry required the synthesis of more **A-12**. Fortunately, a large quantity of its chiral precursor **R-19** was available as well;¹ several grams of Schinzer ketone could then be prepared from it in a sequence of three steps (**Scheme R-9**). After hydrolytic cleavage of the sultam auxiliary, direct reduction of acid **R-20** to the corresponding primary alcohol was attempted with borane-dimethylsulfide complex and with LiAl(OtBu)₃H, but side reactions and multiple by-products were observed; conversion of **R-20** to a mixed anhydride and in situ reduction of the latter succeeded in providing the desired alcohol **R-21**, albeit in moderate yields (53%).

¹ This material was kindly provided by Novartis Pharma AG.
Finally, simultaneous cleavage of TBS and protection of the resulting diol with acetone under acid catalysis afforded building block A-12.

Scheme R-9: a: LiOH, H₂O₂, THF/water, r.t., 75%; b: 1) iBuOCl, TEA, THF, -10°C; 2) LiAl(OtBu)₃, -78°C =°C, 53%; c: 3 eq TFA, acetone, r.t., 75%
3.1.3. Connection of the building blocks and endgame

Building blocks A-11 and A-9 were connected through a hydroboration-palladium-mediated coupling sequence, in which a borane was prepared from alkene A-11 and reacted directly with vinyl iodide A-9 in a Suzuki-Miyaura reaction (Scheme R-10) in high yield (83%). The Suzuki-Miyaura cross-coupling is a powerful and well-established method for the formation of carbon-carbon bonds; it consists in a palladium-mediated reaction of organoboranes with organic halides or triflates (Scheme R-11). The mechanism of the Suzuki-Miyaura reaction, similarly to other cross-coupling reactions, consists of a catalytic cycle in four steps. In the first, the palladium complex undergoes oxidative addition of an
organic halide (or triflate) R₂-X; then an exchange of anions takes place and the anion of the base employed in the reaction replaces the halide on the Pd species (a step called metathesis). In the third step, transmetalation occurs between the Pd species and the alkylboronate complex, which is formed by the organoborane and the base; at this point, both residue R₁ and R₂ are part of the Pd complex. In the last step, reductive elimination forms the R₁-R₂ carbon-carbon bond and restores the Pd(0) species.

\[
R₁B(R)₂ + R₂-X \xrightarrow{\text{Pd(0) base, ligand}} R₁R₂ + X-B(R)₂
\]

**Scheme R-11**: Mechanism of the Suzuki-Miyaura cross-coupling

Basic hydrolysis of methyl ester R-22 was followed by cleavage of the TES group through a prolonged acidic workup, which allowed the direct isolation of seco-acid R-23 in 70% yield over three steps. The same sequence was performed as well with vinyl iodide R-11 using a separate TBAF deprotection step for the cleavage of the TBS ether, and afforded R-23 in 31% yield over three steps.
The last crucial transformation was the macrolactonization reaction to synthesize A-29 from R-23 employing a Yamaguchi protocol (Scheme R-12);\textsuperscript{225} the moderate yield in this step prompted a search for alternatives, in which the Keck\textsuperscript{226} and the Corey-Nicolaou\textsuperscript{227} methods were briefly investigated, but with less satisfactory yields. Final simultaneous cleavage of the Boc and both TBS groups was achieved under acidic conditions with trifluoroacetic acid, affording the free epothilone A-1 in 44% yield after HPLC purification (as the trifluoroacetic acid salt).

\textbf{Scheme R-12}: a: 1) Et\textsubscript{3}N, 2,4,6-trichlorobenzoyl chloride, THF, 10°C; 2) DMAP, toluene, RT, 57%; b: CF\textsubscript{3}COOH, CH\textsubscript{2}Cl\textsubscript{2}, RT, 44%.
3.2. Synthesis of the carboxylic acid-functionalized epothilone analog

Carboxylic acid derivative A-3 was synthesized from A-29 in a three step sequence depicted in Scheme R-12. Selective removal of the BOC-group was achieved with zinc bromide; while partial loss of the TBS groups was also observed, sufficiently diluted reaction conditions provided roughly 60% of the desired TBS-protected free amine product A-30.

Acylation of the amino group with succinic anhydride proceeded smoothly, affording R-24 in 92% yield; contrary to our expectations, this intermediate could be isolated relatively easily and purified by column chromatography. Deprotection under the same acidic conditions used for the deprotection of A-29 afforded A-3 (as the corresponding trifluoroacetic acid salt) in a moderate but still acceptable yield of 23% after HPLC purification.
3.3. Synthesis of the hydroxyl-functionalized epothilone analog\(^2\)

3.3.1. Synthesis of the hydroxyl-functionalized benzimidazole building block

The synthesis of the side chain containing precursor of the hydroxyl-functionalized epothilone target followed the second-generation approach described for its amine-counterpart in Section 3.1.1, starting from the same commercial precursor A-26, and is depicted in Scheme R-13. In this case nucleophilic aromatic substitution to introduce the 2- aminoethanol ‘handle’, nitro-group reduction and cyclization with triethylorthoacetate afforded functionalized benzimidazole R-26 without need for protecting groups. After TBS protection of the primary alcohol and esterification, the resulting methyl ester R-28 was submitted to a similar reduction and oxidation sequence as it had been done with its amino analog R-3, in order to obtain aldehyde A-22; interestingly, in this case Swern oxidation was successful.

In light of the experience with the corresponding amino group containing sequence (A-21 to A-9, Section 3.1.1), Brown allylation was employed to install the chiral center of A-25, with a satisfactory enantiomeric excess of 91%; TES protection, oxidative double-bond cleavage and Wittig reaction afforded the desired vinyl iodide A-10

\(^2\) The work described in this section has been the object of the master project conducted by Linda Riediker in our research group.
**Scheme R-13:**

a: ethanolamine, MeOH, r.t., 89%; b: H₂, Pd/C, EtOH, r.t., 83%; c: triethylorthoacetate, EtOH, reflux, 94%; d: H₂SO₄, MeOH, 65°C, 98%; e: TBSCl, imidazole, DMF, r.t., 90%; f: DIBAL-H, DCM, -78°C → r.t., 82%; g: (COCl)₂, DMSO, DCM, -78°C, 67%; h: 1) (-)-DIP-Cl, allyl-MgBr, Et₂O, 0°C, 1h, then -78°C (solution A); 2) R-50, Et₂O, -100°C, dropwise addition of solution A, then -100°C, 2h, 95%, ee 91%; i: TESCl, imidazole, DMAP, DMF, r.t., 92%; j: OsO₄, 2,6-lutidine, NaIO₄, DMF/water, r.t., 74%; k: [Ph₃PCH(CH₃)]I, Na-HMDS, THF, -78°C → -30°C, 1h, then -78°C, R-53, 37%.
3.3.2. Completion of the hydroxyl-functionalized benzimidazole epothilone

Scheme R-14 shows the endgame sequence for this synthesis; after a successful Suzuki-Miyaura coupling of A-11 and A-10, an unexpected difficulty arose when deprotection of the seco-acid functionalities proved to be rather unselective. We had expected the primary TBS ether to withstand basic hydrolysis conditions and to allow subsequent preferential cleavage of the secondary TES group; in fact, both the primary TBS as well as the secondary TES ether in R-31 were not stable enough under basic conditions, and cleavage occurred both at position 15 and at the ethanolamine moiety during the hydrolysis of the methyl ester. Despite our attempts to optimize the reaction conditions, the result was in all instances a mixture of derivatives (R-32 - R-36, Scheme R-14), which ultimately provided only a modest yield of the desired product R-32. The latter could be isolated from these mixtures through column chromatography, and sufficient material was eventually obtained to complete the synthesis. Selective cleavage of the TES ether in R-32 was then achieved under acidic conditions; the resulting seco-acid R-37 was cyclized successfully under Yamaguchi conditions, then deprotection with hydrofluoric acid/pyridine afforded the desired analog A-2.
Scheme R-14: a) R-33, 9-BBN, THF, r.t. (solution A); ii. AsPh₃, Cs₂CO₃, [PdCl₂(dppf)]:DCM, DMF, water, R-54, solution A, -10°C □ r.t., 2.5h, 77%; b: LiOH·H₂O, isopropanol/water, r.t., 72h, 26%; c: DCM/isopropanol/1M HCl 2:1:1, r.t, 2.5h, not purified; d) 1) 2,4,6-trichlorobenzoylchloride, triethylamine,
3.4. Synthesis of folic acid conjugates of A-1

3.4.1. Synthesis of a non-cleavable folic acid conjugate of A-1

While folic acid is easily available from commercial sources in its unprotected form, only a small selection of protected folates was commercially available at the start of this project. In particular, the choice of folic acid derivatives bearing differentially protected α- and γ-carboxylic acid functions at the glutamate moiety was limited. Availability of such building blocks was considered a necessary prerequisite for the preparation of pure γ-conjugates of folic acid, free of their α-counterparts; biological testing of mixtures of α- and γ-conjugates would be best avoided to simplify interpretation of the data, and because of early indications in the literature of their possibly different interaction with FR.\textsuperscript{172} In addition, more than one protecting group strategy was applicable in principle to the glutamate

![Chemical Structures]

**Scheme R-15:** a: Carboxypeptidase G, Tris-HCl, ZnCl\textsubscript{2}, 30°C, pH 7.3, 19d, 97%; b: trifluoroacetic anhydride, rt, 42%; c: 1) R-33, HBTU, DIPEA, DMF; 2)R-66, 90%; d: Pd/C, H\textsubscript{2}, AcOEt
moiety, depending also on the exact structure of the employed linker, which was not rigidly defined at the start of the project; therefore, it was preferable to have access to diverse protecting groups for the two carboxylic acid functions. These combined considerations led to the decision of synthesizing the required folic acid derivatives following the synthesis described in Scheme R-15.

Based on the work pioneered by Nomura, pteroic acid A-34 (Scheme R-15) was prepared from the hydrolysis of folic acid by the enzyme carboxypeptidase G in excellent yield (it must be noted, however, that this reaction is very slow and completion takes several days). Subsequent reaction with trifluoroacetic anhydride afforded protected intermediate A-33; the latter is only protected at the secondary amine function, whereas double protection of both the amine and guanidine moieties would have been a preferred outcome. This was primarily due to concerns regarding the physico-chemical properties of the pteroic acid-derived intermediates, which are generally characterized by low solubility and difficult chromatographic properties; use of protecting groups was expected to alleviate these problems. Unwanted reactivity of the guanidine moiety in subsequent steps, however, was not a great concern, based on the existing literature on the preparation of other folic acid conjugates bearing an unprotected guanidine. A-33 was therefore carried on to react with protected glutamic acid R-39 after activation with HBTU; purification of R-40 was accomplished by recrystallization from DMSO/water mixtures. The next planned step was deprotection of the $\gamma$-carboxylic acid function of R-40 through catalytic hydrogenation in ethyl acetate; however, no conversion was observed under these conditions, even with high catalytic loads, presumably due to the very low solubility of R-40. A brief survey of different solvents was undertaken, to no avail; R-33 and R-40 were in fact only soluble to a limited extent in DMSO and DMF, which are not optimal solvents for catalytic hydrogenation. An alternative protective group may have been chosen for the $\gamma$-carboxylic acid group of R-39, but it would have been limited by the necessity of a cleavage method orthogonal both to the acidic labile tert-butyl ester and to the basic sensitive trifluoroacetamide moiety. Moreover, the chromatographic properties of R-33 and R-40 were rather unfavorable, and we anticipated that purification of later intermediates would pose a significant problem en route to our planned cleavable conjugates, when mixtures of several pteroic-acid containing compounds would likely have had to be separated.
A revised strategy was the implemented through the synthesis of conjugate R-41 (Scheme R-16), in which glutamic acid derivative R-42 was activated with HATU and reacted with epothilone intermediate R-30; cleavage of the Fmoc group provided R-43 in good yield. R-33 was activated with HATU and DIPEA in DMSO/DCM 1:1 (despite the initial very limited solubility, a clear solution is obtained after a few minutes) and successfully reacted with R-43 to give the fully protected conjugate R-44; as we had hoped, its chromatographic purification was straightforward, presumably because the large protected epothilone moiety affects the physico-chemical properties of the conjugate in a beneficial way.

Scheme R-16: a) R-42, HBTU, DIPEA, DMF; 2) R-30, 88%; b: Me2NH, DMF, 84%; c: 1) R-33, HBTU, DIPEA, DMF; 2) R-43, 82%; d: K2CO3, THF/water 2:1, 71%; e: TFA, DCM, 0°C to rt, 52% (HPLC)
Cleavage of the trifluoroacetamide moiety succeeded in mild conditions in the presence of 
K₂CO₃, while complete deprotection was achieved upon exposure of R-45 to trifluoroacetic 
acid in DCM.

3.4.2. Synthesis of a cleavable folic acid conjugate of A-3

Synthesis of cleavable conjugate A-7 (Scheme R-18, see Retrosynthesis in Section 2.5) 
required the synthesis of building block R-46. This was executed as represented in Scheme 
R-17, starting from protected glutamic acid R-42, through a series of alternate amide 
coupling and deprotection steps which assembled R-46 one amino acid at a time. β-alanine 
was introduced as its methyl ester, which could be cleaved selectively in the presence of the 
Fmoc moiety thanks to its reactivity to Ba(OH)₂; the hydration state of the latter seemed to 

![Chemical diagram]

**Scheme R-17:** a: 1) HBTU, DIPEA, DMF, r.t.; 2) β-AlaOMe, 73%; b: Ba(OH)₂, THF/water, r.t. 95%; c: 1) 
HATU, DIPEA, DMF, r.t.; 2) PheOBn, 80%; d: Pd/C, H₂, r.t., 71%; e: 1) HATU, DIPEA, DMF, r.t.; 2) 
GlyOBn, 95%; f: Ba(OH)₂, THF/water 1:1 76%

play a significant role in the reaction, since activation of the barium salt through heating 
under vacuum was necessary to ensure success of the reaction. The non-activated Ba(OH)₂ 
led to slow conversion and decomposition with loss of the Fmoc group. R-48 was then 
activated with HATU and coupled with phenylalanine benzyl ester; catalytic hydrogenolysis 
of the benzyl ester afforded R-50. The amide coupling was repeated on R-50 using glycine
methyl ester as the amine partner, and Ba(OH)$_2$ mediated deprotection afforded the desired intermediate **R-46**.

Completion of the synthesis of **A-7** proceeded along the lines of the synthesis of its non-cleavable counterpart **A-6** (**Scheme R-16**) and afforded the desired conjugate in five steps from **A-30** (**Scheme R-18**).

**Scheme R-18**: a: 1) **R-46**, HATU, DIPEA, DMF; 2) **A-30**, 65%; b: Me$_2$NH, DMF, 51%; c: 1) **R-33**, HBTU, DIPEA, DMF; 2) **R-53**, %; d: K$_2$CO$_3$, THF/water 2:1, nd; e: TFA, DCM, 0°C to rt, nd
3.5. Towards the synthesis of a cleavable folic acid conjugate of A-3

The synthesis of A-9 has been planned along the lines of what has been done to prepare A-7 (Scheme R-18) and requires building block R-56. As the latter comprises the same dipeptide present in R-46 (namely PheGly), we initially devised the approach depicted in Scheme R-19 to synthesize both R-46 and R-56 from common precursors R-57 and R-42.

![Chemical structures](image)

**Scheme R-19:** Initial retrosynthetic approach to cleavable linkers R-46 and R-56

While R-57 could be easily synthesized (in one step from commercially available protected amino acids), this approach could ultimately not be implemented. In the route leading to R-46, hydrogenolysis of R-57 did not lead to isolation of R-58, but rather of the diketopiperazine formed by the latter through intramolecular amide bond formation. On the branch of the synthesis leading to R-56, preparation of R-60 was attempted from R-42 and both Cbz-diaminoethane and excess unprotected diaminoethane (Scheme R-20), but
the desired product could not be isolated in either case. In the first instance, selective deprotection of the primary amino group of **R-62** could not be achieved with catalytic hydrogenolysis without loss of the Fmoc moiety; in the second, the desired product could not be isolated from the reaction mixture, and it is possible that loss of Fmoc occurred as well under the conditions employed.

![Chemical Diagram](image)

**Scheme R-20:** a 1) HBTU, DIPEA, DMF, r.t.; 2) Cbz-diaminoethane·HCl, 50% b: Pd/C, H₂, MeOH, failed; c: HBTU, ethylenediamine, DMF, failed

In light of these difficulties, the approach depicted in **Scheme R-19** was abandoned, and separate syntheses were devised for **R-46** (see **Scheme R-17**) and **R-56** (**Scheme R-21**).

The failure to obtain **R-60** precluded a stepwise approach to **R-56** from **R-42** analogous to that described for **R-46** in **Section 3.4.2**; it was not obvious, in fact, which protecting group might successfully replace Cbz, also considering that selective deprotection of the growing N-terminus would have had to be achieved for each of the three units that were to be attached to **R-42**. The revised synthesis of **R-56** (**Scheme R-21**) started then from Boc-protected diaminoethane and employed Cbz-protected amino acids to elongate the linker, thus exploiting the well-established orthogonal reactivity of these two protecting groups to produce intermediate **R-66**. Change of the protecting group on Phe to trifluoroacetamide was based on reports of its successful cleavage in the presence of the Fmoc moiety under mild basic conditions; unfortunately, however, the final deprotection of **R-69** to **R-56** with K₂CO₃ failed to produce any conversion. While alternatives might be explored, the time...
scope for the present project had reached its end by the time of this attempt, and further investigation had to be postponed to future studies.

Scheme R-21: a: HBTU, DIPEA, DMF, r.t., 76%; b: Pd/C, H₂, MeOH, r.t., 80%; c: HBTU, DIPEA, DMF, r.t., 27%; d: Pd/C, H₂, MeOH, r.t., 71%; e: trifluoroactic anhydride, TEA, DCM, 54%; f: TFA, DCM, 36%; g: HATU, DIPEA, DMF, 46%; h: K₂CO₃, THF/water, no conversion
3.6. Biological evaluation of functionalized epothilone analogs A1, A2 and A3 and of conjugate A6

Compounds A1, A2 and A3 were evaluated in vitro for their effect on tubulin polymerization and on human cancer cell growth against different cell lines; the results are summarized in Table R-1.

In a first sep, the interactions of these epothilone derivatives with the tubulin/microtubules system were investigated in a tubulin polymerization assay. In this assay the extent of tubulin polymerization is measured at different concentrations of test compound for a fixed concentration of soluble tubulin (10 µmol in this case), and the concentration leading to 50% of the maximum observed tubulin polymerization (EC50) is determined. This value may be used as a rough comparator for the tubulin-polymerizing ability of different compounds; the method can clearly identify those compounds which are altogether devoid of effect on tubulin polymerization, while it is less suited to distinguish between compounds with good tubulin-polymerizing ability. All three epothilone analogs A1, A2 and A3 display a strong effect on tubulin polymerization in this test, with EC50 values very close to that of Epo A for A1 and A3 and even lower for A2.

As mentioned previously (Section 1.2.3), while EC50 values for the tubulin polymerization assay give an indication of the cytotoxicity of the compounds, they do not always correlate strongly with antiproliferative activity in cancer cells. In fact, comparison of the IC50 values of A1 with those of A3 highlights a significant difference (about 6- to 8-fold) in their antiproliferative potency despite almost identical EC50 values; also, they are both less active than Epo A (roughly three and twenty times respectively). This discrepancy between the effects displayed on tubulin and on cells may reflect differences in the cellular uptake of these compounds, but its precise reasons remain unclear.

The antiproliferative activity results in Table R-1 show that derivative A2 is significantly more active than A1, with 20 to 40 times lower IC50 values. In fact, A2 is significantly more potent than Epo A and only slightly less potent than Epo B (which is the most potent natural epothilone known). Although the chemical structures of A1 and A2 would seem very similar, it appears that a hydroxyethyl group is much more advantageous than an
**aminooethyl** substituent at the position N1 of the benzimidazole; any explanation of why this is the case is at this point highly speculative.

### Table 1: Tubulin-polymerizing and antiproliferative activity of epothilones A1, A2 and A3

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC₅₀ tubulin polymerization [µM]ᵃ</th>
<th>% Tubulin polymerizationᵇ</th>
<th>IC₅₀ [nM]ᵇ</th>
<th>MCF-7</th>
<th>A549</th>
<th>HCT116</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>4.3 ± 0.8ᵈ</td>
<td>n.d.</td>
<td>10.5 ± 3.0ᵈ</td>
<td>13.0 ± 4.8ᵈ</td>
<td>n. d.ᶜ</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>n. d.ᶜ</td>
<td>93</td>
<td>0.52 ± 0.018</td>
<td>0.35 ± 0.019</td>
<td>0.38 ± 0.029</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>4.1 ± 0.5ᵈ</td>
<td>n.d.</td>
<td>65 ± 12ᵈ</td>
<td>108 ± 14ᵈ</td>
<td>n. d.ᶜ</td>
<td></td>
</tr>
<tr>
<td>Epothilone A</td>
<td>3.9 ± 0.6ᵈ</td>
<td>82</td>
<td>2.9 ± 0.3ᵈ</td>
<td>5.0 ± 1.4</td>
<td>2.8 ± 0.4ᵈ</td>
<td></td>
</tr>
<tr>
<td>Epothilone B</td>
<td>3.0 ± 0.3ᵈ</td>
<td>n.a.¹</td>
<td>0.33 ± 0.01ᵈ</td>
<td>0.34 ± 0.03ᵈ</td>
<td>0.16 ± 0.01ᵈ</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Concentration required to induce 50% of the maximum tubulin polymerization achievable with the respective compound (10 µM of porcine brain tubulin). ᵇ IC₅₀-values for human cancer cell growth inhibition. MCF-7: breast; A549: lung; HCT116: colon. ᶜ n. d. = not determined. ᵈ See reference⁴ ᵉ Induction of polymerization of porcine brain microtubule protein by 2 µM of test compound relative to the effect of 25 µM Epo B. ⁻ not applicable.

The reduction in antiproliferative activity displayed by compounds A1 and A3 in comparison with natural epothilones is somewhat more pronounced than we had anticipated, based on the nanomolar cytotoxicity displayed by the dimethylbenzimidazole analog of epothilone D.⁹³ The increased bulkiness of these derivatives may be part of the explanation, although literature precedents (see **Section 1.2.3**) would indicate a relatively good tolerance to large substituents in this part of the molecule. Comparison of A1 with A2, however,
indicates that steric hindrance is very unlikely to be the only factor at play, since it should affect both compounds in a similar way. Moreover, an analog of A1 carrying a tert-butyl carbamate on the amino-group, which was also tested in the cytotoxicity assay, proved to be roughly equipotent to A1; this lends further support to the hypothesis that other factors than the size of the substituent at the N1 position of the benzimidazole must be considered.

Next to their size, the acid/base properties of the newly introduced functional groups should be considered as possible explanations for the variations in antiproliferative activity, as they may have a significant influence on the transport of these compounds into cells, as well as on their intracellular distribution. This may be relevant for all three derivatives, as the precise pKa of the benzimidazole nitrogen is unknown in these compounds; the additional ionizable groups on A1 and A3 may play a particularly important role in this context. In light of these considerations, the potency of the tert-butyl carbamates analog mentioned above might be the result of two opposite effects; the increased bulk of the side chain with respect to A1 might be somewhat detrimental to the activity, but the change from a basic amino-group to a much less ionizable carbamate moiety might be beneficial, and result in essentially unchanged potency.

Overall, all three compounds A1, A2 and A3 display potent antiproliferative activity, and, therefore, are deemed interesting candidates for the development of drug conjugates with tumor targeting moieties.

The antiproliferative activity of folic acid conjugate A6 was evaluated in the three cell lines used to test the unconjugated epothilone analogs A1, A2 and A3 (A549, MCF-7 and HCT116 for lung, breast and colon cancer respectively), which according to the literature do not overexpress the folate receptor FR; these experiments have shown no significant antiproliferative activity (IC_{50} > 30 \mu M) in all three cases. A measurable cytotoxic effect, however, has been observed with FR-positive KB cells, with an IC_{50} value of 18.4 (±1.1) \mu M. Although this result is somewhat disappointing, in that a high cytotoxicity was hoped for in FR-positive cells, it does show a difference between FR-positive and FR-negative cell lines, which may indeed reflect transport of A6 into the cells through the FR pathway. The low cytotoxicity in KB cells may be due to intracellular transport issues or to impaired
interaction with tubulin; a tubulin polymerization experiment was then performed and A6 showed a small polymerization-inducing effect, which however could not be quantified.\textsuperscript{229} This low tubulin-polymerizing activity may be the cause of the weak cytotoxicity, and may simply reflect an excessive bulk represented by folic acid, which may hinder the interaction of A6 with its target protein; in this case, a prodrug approach such as that represented by A7 should restore cytotoxicity, if free A1 were released efficiently. Indeed, in a prodrug approach the lack of cytotoxic activity of the full conjugates (A6 and, presumably, intact A7) may not be regarded as a drawback at all, in that no toxic effect should be exerted by such compounds on cells that do not overexpress the FR intake pathway.
4. Experimental Section

4.1. General Methods

All non-aqueous reactions were carried out using oven-dried or flame-dried glassware under a positive pressure of dry argon or nitrogen unless otherwise stated. Tetrahydrofuran, acetonitrile, toluene, diethyl ether, dichloroethane, N, N-Dimethylformamide, dimethyl sulfoxide and methylene chloride were purchased as anhydrous from Fluka. All chemicals were purchased from Acros, Aldrich, Fluka, Merck, Lancaster, ABCR or TCI and used as such unless otherwise stated. Deuterated solvents were obtained from Aldrich and Cambridge Isotope Labs.

Reactions were magnetically stirred if not indicated otherwise and monitored by thin layer chromatography using Merck silica gel 60 F<sub>254</sub> TLC aluminium backed plates and visualized by fluorescence quenching under UV light. In addition, TLC plates were stained with cerium molybdate or potassium permanganate stain. Chromatographic purification was performed as flash chromatography on Fluka Silica Gel 60 (230-400 mesh) using a forced flow of eluant at 0.3 bar. Technical grade solvents were employed, which were distilled prior to use. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. Purified compounds were further dried for 12 – 48 h under high vacuum (0.01 – 0.05 Torr). Yields refer to chromatographically purified and spectroscopically pure compounds, unless stated otherwise.

Melting points were measured on a Büchi B-540 melting point apparatus using open glass capillaries and are uncorrected.
Optical rotations were measured on a JASCO P-1020 digital polarimeter at the sodium D line with a 100 mm path length cell, and are reported as follows: $[\alpha]_D^T$, concentration (g/100 ml), and solvent.

NMR spectra were recorded on a Bruker AV-400 400 MHz and a Bruker DRX-500 500 MHz spectrometer at room temperature for $^1$H and $^{13}$C acquisitions, respectively. Chemical shifts (δ) are reported in ppm with the solvent resonance as the internal standard relative to chloroform (δ 7.26 ppm for $^1$H and 77.0 ppm for $^{13}$C). All $^{13}$C spectra were measured with complete proton decoupling. Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, coupling constants in Hz, integration.

IR spectra were recorded on a Jasco FT/IR-6200 instrument as thin film. Absorptions are given in wavenumbers (cm$^{-1}$).

Mass spectra were recorded by the MS service at ETH Zürich. EI-MS (m/z): EI-HIRES Micromass Autospel-ULTIMA spectrometer at 70 eV. ESI-MS (m/z): IONSPEC Ultima ESI-FT-ICR spectrometer at 4.7 T. MALDI-MS (m/z): Ion Spec Ultima HR

HPLC analyses were carried out on a Merck-Hitachi device using a Waters Symmetry column (C18, 3.5 µm, 4.6x100 mm). Preparative HPLC was carried out using Waters Symmetry columns (C18, 5 µm, 7.8x100 mm and C18, 5 µm, 19x100 mm).
4.2. Experimental Procedures and Analytical Data

4.2.1. Synthesis of the Benzimidazole Side Chain Precursors

Methyl 4-fluoro-3-nitrobenzoate (R-1):

\[
\text{H}_3\text{C} \text{OOC} \begin{array}{c} \text{F} \\ \text{NO}_2 \end{array}
\]

10 mL of 95% sulphuric acid (17.5 g, 178 mmol) were added to a solution of 15 g (108 mmol) of 4-fluoro-3-nitrobenzoic acid in 500 mL of absolute methanol and the mixture was heated to reflux for 6h. After cooling, 300 mL of ethyl acetate were added and methanol was removed under reduced pressure. The mixture was diluted with 300 mL of water and the aqueous phase was neutralized with 2N sodium hydroxide; organic layer separation, back-extraction with dichloromethane, washing with brine, drying over magnesium sulfate and evaporation afforded 15.7 g of product as a yellow solid (97%).

TLC hexane/ethyl acetate 1:1 with 1% v/v acetic acid, UV, CPS, Rf 0.75   
mp: 60.5-61.1°C  
\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta = 8.74\) (1H, dd, C2H, \(J_1=7.4\) Hz, \(J_2=2.2\) Hz); 8.32 (1H, ddd, C6H, \(J_1=8.8\) Hz, \(J_2=4.2\) Hz, \(J_3=2.0\) Hz); 7.39 (1H, dd, C5H, \(J_1=10.2\) Hz, \(J_2=8.6\) Hz); 3.98 (3H, s, COOCH\(_3\)).

\(^13\)C NMR (CDCl\(_3\), 100.6 MHz): \(\delta =\) 164.24 (C=O); 159.57 (CNO\(_2\)); 156.86 (CCO); 136.66 (d, C6H, \(J(C-F) = 10.1\) Hz); 128.00 (d, C2H, \(J(C-F) = 2.2\) Hz); 127.38 (d, CF, \(J(C-F) = 4.2\) Hz); 118.95 (d, C5H, \(J(C-F) = 21.2\) Hz); 53.06 (OCH\(_3\)).

HRMS (EI): C\(_8\)H\(_4\)FNO\(_4\); [MH]\(^+\): calc. 199.0276; found 199.0274.

IR (film): \(\nu 3076; 2956; 1729; 1615; 1540; 1439; 1351; 1276; 1233; 1197; 1116 \text{cm}^{-1}\).

Tert-butyl 2-aminoethylcarbamate:

\[
\text{H}_2\text{N} \begin{array}{c} \text{NHBOc} \\ \end{array}
\]

a solution of 1.31 g of di-tert-butyl-dicarbonate (30 mmol) in 150 mL of chloroform was added dropwise to a stirred solution of 10 mL of 1,3-diaminoethane (298 mmol) in 300 mL of chloroform at 0°C. After stirring at rt for 19h, the solution was washed with brine (6x80 mL) and dried over magnesium sulfate; evaporation of the solvent afforded 4.69 mg of colourless oil. FC: DCM/MeOH 9:1+2%TEA afforded 4.11 g of yellow oil (85%).
$^{1}$H NMR (CDCl$_3$, 400 MHz): δ = 5.00 (1H, b, NHCO); 3.14 (2H, q, NHCH$_2$, $J$=5.46Hz); 2.76 (2H, q, CH$_2$NH$_2$, $J$=5.96Hz); 1.41 (11H, bs, NH$_2$+C(CH$_3$)$_3$).

$^{13}$C NMR (CDCl$_3$, 100.6 MHz): δ = 156.22 (CO); 79.24 (C(CH$_3$)$_3$); 43.41 (NHCH$_2$); 41.86 (NH$_2$CH$_2$); 28.40 (C(CH$_3$)$_3$).

HRMS (ESI): C$_7$H$_{17}$N$_2$O$_2$; [MH]$^+$: calc. 161.1285; found 161.1284.

IR (film): 3425, 1699, 1528, 1366, 1274, 1252, 1171, 1050, 1024, 1003 cm$^{-1}$.

**Methyl 4-(2-(tert-butoxycarbonylamino)ethylamino)-3-nitrobenzoate (R-2):**

![Methyl 4-(2-(tert-butoxycarbonylamino)ethylamino)-3-nitrobenzoate (R-2)](image)

A solution of 0.53 g (2.7 mmol) of R-1 in 5 mL of dichloromethane was added to 0.48 g (3 mmol) of tert-butyl 2-aminoethylcarbamate stirred at r.t.; a yellow precipitate formed immediately. Monitoring of the reaction via TLC and portionwise addition of 0.8 ml (5.7 mmol) of triethylamine altogether lead to complete conversion after 25h total reaction time. Dilution with dichloromethane and washing with KH$_2$SO$_4$ 2 % solution (pH ca 4), back-extraction three times with dichloromethane, drying over MgSO$_4$ and solvent evaporation afforded 0.88 g of yellow solid R-2 (96%).

TLC: hexane/AcOEt 2:1, vis. (yellow), UV, CPS, Rf 0.25

mp: Mp: 116.5-117.5 °C

$^{1}$H NMR (CDCl$_3$, 400 MHz): δ = 8.89 (1H, d, 1CH, $J$=2.4 Hz); 8.50 (1H, b, ArNH); 8.07 (1H, dd, 6CH, $J_1$= 2.0 Hz, $J_2$= 9.2 Hz); 6.96 (1H, d, 5CH, 9.2 Hz); 4.85 (1H, b, NHBoc); 3.91 (3H, s, COOCH$_3$); 3.56-3.44 (4H, m CH$_2$CH$_2$); 1.46 (9H, s, C(CH$_3$)$_3$).

$^{13}$C NMR (CDCl$_3$, 100.6 MHz): δ = 165.57 (CO); 147.76 (4Cq); 136.44 (6CH); 129.54 (1CH); 117.55 (3Cq); 113.46 (5CH); 80.12 (C(CH$_3$)$_3$); 52.12 (COOCH$_3$); 43.23 (ArNHCH$_2$); 39.56 (CH$_2$NHBoc); 28.34 (C(CH$_3$)$_3$).

HRMS (ESI): C$_{15}$H$_{21}$N$_3$O$_6$; [MNa]$^+$: calc.362.1323; found 362.1327.

IR (film): v 3363; 2976; 1707; 1623; 1566; 1524; 1440; 1362; 1287; 1227; 1163 cm$^{-1}$.

**Methyl 3-amino-4-(2-(tert-butoxycarbonylamino)ethylamino)benzoate (A-27):**

![Methyl 3-amino-4-(2-(tert-butoxycarbonylamino)ethylamino)benzoate (A-27)](image)

R-2 (16.7 g, 49 mmol) was dissolved at r.t in MeOH (300 mL), after flushing 3 times with Ar, 0.8 g of Pd/C were added and flushed with 3 times with H$_2$; the mixture was rotated
vigorously for 17 h at r.t., then flushed Ar with and filtered through a celite pad; FC hexane/AcOEt 2:1 to 1:2 afforded 15.1 g of A-27 as a red oil (99%).

TLC: hexane/AcOEt 1:2, UV Rf 0.55 (UV 254 nm very bright, turns brown after some time)

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta = 7.53$ (1H, dd, 6CH, J=8.4 Hz, J=2=2 Hz); 7.36 (1H, d, 1CH, J=1.6 Hz); 6.52 (1H, d, 5CH, J=8.4 Hz); 5.04 (1H, b, NH); 3.83 (3H, s, COOCH$_3$); 3.47-3.41 (2H, m, NHCH$_2$); 3.28-3.25 (2H, m, NHCH$_2$); 1.46 (9H, s, C(CH$_3$)$_3$).

$^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta = 167.71$ (C=O); 157.09 (C=O); 142.46 (Ar); 132.43(Ar); 123.98(Ar); 118.8(Ar); 117.61(Ar); 108.94(Ar); 79.86 (C(CH$_3$)); 51.67(COOCH$_3$); 44.77(CH$_3$); 40.03(CH$_3$); 28.45((CH$_3$)$_3$).

HRMS (ESI): $C_{15}H_{21}N_3O_6$; [MH]$^+$: calc. 310.1761; found 310.1759; [MNa]$^+$: calc.332.1581; found 332.1579.

IR (film): $\nu$ 3371, 2976, 2948, 1686, 1600, 1524, 1442, 1393, 1365, 1296, 1253, 1221, 1158, 1113 cm$^{-1}$.

**Methyl 1-(2-\{tert-butoxycarbonylamino\}ethyl)-2-methyl-1H-benzo[d]imidazole-5-carboxylate (R-3):**

![Methyl 1-(2-\{tert-butoxycarbonylamino\}ethyl)-2-methyl-1H-benzo[d]imidazole-5-carboxylate (R-3):](image)

Triethylorthoacetate (0.35 mL, 1.94 mmol) was added to a solution of A-27 (100 mg, 0.33 mmol) in EtOH (6 mL) and heated to reflux (95°C) for 19 h30'. Evaporation of the solvent (dark brown solid) and FC purification AcOEt/MeOH 95:5 afforded 104 mg of white solid R-3 (96%).

TLC: AcOEt/MeOH 95:5, UV, CPS Rf 0.3

mp: 180.1-181.6°C.

$^1$H NMR(CDC$_3$, 400 MHz): $\delta = 8.32$ (1H, s, Ar-H$_a$); 7.98 (1H, dd, Ar-H$_b$, J=8.4 Hz, J=2=1.2 Hz); 7.35 (1H, d, Ar-H$_c$, J=8.4 Hz); 4.96 (1H, broad s, NH); 4.33 (2H, t, NCH$_2$, J= 5.4Hz); 3.94 (3H, s, COOCH$_3$); 3.51 (2H, q, CH$_2$NHBoc, J=5.7 Hz); 2.67 (3H, s, NCH(CH$_3$)N); 1.43 (9H, s, t-Bu).§

$^{13}$C NMR (CDCl$_3$, 100.6 MHz): $\delta = 167.54$ (COOCH$_3$); 156.32 (NHCO); 153.44 (Cq ar); 141.82 (Cq ar); 138.37 (Cq ar); 124.15 (Cq ar); 123.93 (CH ar); 120.99 (CH ar); 108.68 (CH ar); 80.08 (C(CH$_3$)$_3$); 52.08 (COOCH$_3$); 43.56 (CH$_2$); 40.29 (CH$_2$); 28.51(C(CH$_3$)$_3$); 13.92 (NCH(CH$_3$)N).


IR (film): $\nu$ 3223, 2977, 2952, 1705, 1619, 1522, 1435, 1396, 1366, 1336, 1285, 1253, 1209, 1165, 1082 cm$^{-1}$. 106
Tert-butyl 2-(5-(hydroxymethyl)-2-methyl-1H-benzo[d]imidazol-1-yl)ethylcarbamate (R-4):

A solution of DIBAL (159 mL, 1M in DCM) was added dropwise to a solution of R-3 (13.23 g, 39.7 mmol) in dichloromethane (170 mL) at -78°C; the mixture was allowed to r.t. and stirred for 17h. Addition of MeOH (100 mL, -30°C) led to formation at r.t. of a jelly solid; upon further MeOH addition (100 mL) a filterable precipitate was obtained. Paper filtration, evaporation of the filtrate and recrystallization from MeOH afforded 10.03 g of R-4 as a beige solid (78%).

TLC: AcOEt/MeOH 95:5, UV Rf 0.1
mp: 130°C (decomposition)
1H NMR (CDCl3, 400 MHz): δ = 7.50 (1H, m, CH ar); 7.24 (2H, m, 2xCH ar); 5.12 (1H, b, NH); 4.74 (2H, s, CH2OH); 4.25 (2H, t, CH2CH2NH, J=5.4 Hz); 3.50-3.44 (2H, m, CH2CH2NH); 2.57 (3H, s, NC(CH3)N); 1.44 (9H, s, C(CH3)3).
13C NMR (CDCl3, 100.6 MHz): δ = 156.16 (CO); 153.29 (NC(CH3)N); 142.63 (Cq ar); 135.41 (Cq ar); 134.57 (Cq ar); 121.95 (CH ar); 117.80 (CH ar); 109.17 (CH ar); 80.25 (C(CH3)3); 65.79 (CH2OH); 43.62 (arNCH2); 40.17 (NHCH2); 28.50(C(CH3)3); 13.84 (NC(CH3)N).
IR (film): ν 3310; 3218; 2976; 2932; 2869; 1689; 1623; 1519; 1436; 1404; 1356; 1332; 1277; 1251; 1164; 1084; 1035; 1014 cm⁻¹.

Tert-butyl 2-(5-formyl-2-methyl-1H-benzo[d]imidazol-1-yl)ethylcarbamate (A-21):

Solid manganese dioxide (704 mg, 7.8 mmol) was added to a solution of R-4 (207 mg, 0.66 mmol) in chlorophorm (1.5 mL) and refluxed for 1h. The mixture was cooled to r.t. and filtrated through a celite pad; evaporation of the solvent yielded 192.6 mg of white solid A-21 (96%).
TLC AcOEt/MeOH 95:5, UV Rf 0.5
mp: 186°C (decomposition)
\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta = 10.04\) (1H, s, CHO); 8.16 (1H, d, ArH, \(J=1.2\) Hz); 7.88 (1H, dd, ArH, \(J_1=5.2, J_2=1.2\) Hz); 7.49 (1H, d, ArH, 5 Hz); 4.87 (1H, b, NH); 4.38 (2H, m, NHCH\(_2\)); 3.54 (2H, m, NHCH\(_2\)); 2.75 (3H, s, CH\(_3\)); 1.42 (9H, s, C(CH\(_3\))\(_3\)).
\(^13\)C NMR (CDCl\(_3\), 100. MHz): \(\delta = 192.03\) (CHO); 156.21 (NCHO); 154.31 (Cq ar); 142.24 (Cq ar); 139.55 (Cq ar); 131.53 (Cq ar); 123.47 (CH ar); 122.15 (CH ar); 109.63 (CH ar); 80.20 (C(CH\(_3\))\(_3\)); 43.66 (NCH\(_2\)); 40.24 (CH\(_2\)NH); 28.49 (C(CH\(_3\))\(_3\)); 13.96 (NC(CH\(_3\))N).
HRMS (MALDI): \(C_{16}H_{21}N_3O_3; [MH]^+\) calc. 304.1656; found: 304.1654.
IR (film): \(\nu\ 3339, 2974, 2929, 2854, 2736, 1687, 1613, 1586, 1522, 1422, 1395, 1366, 1335, 1285, 1253, 1165\ cm\(^{-1}\).

\((S)-\text{tert-butyl 2-}(5-\text{-1-hydroxybut-3-enyl})\text{-1H-benzo}[d]\text{imidazol-1-yl})\text{ethylcarbamate (A-24):}\)

![Chemical Structure]

Allylmagnesium bromide 1M in diethyl ether (60 mL, 60 mmol) was added dropwise under Ar atmosphere at 0°C to a solution of (-)-diisopinocampheyl chloride (16.17 g, 50.3 mmol) in diethyl ether (100 mL) and stirred 1 h at rt. Dry pentane was added (80 mL), the mixture was cooled to -30°C and filtered under Ar through a sintered glass septum, washing with dry pentane (20 mL). The resulting clear solution was cooled to -78°C (solution A). In a separate flask, A-21 (8.99 g, 29.6 mmol) was suspended in diethyl ether (200 mL) and cooled to -100°C (suspension B). Solution A was added dropwise to B over 1 h while keeping the temperature at -100°C. The reaction mixture was stirred at the same temperature for 1 h longer, then dry MeOH (15 mL) was added and the temperature was allowed to -15°C; ethanolamine (20 mL, 0.33 mol) was added and the mixture was stirred 18 h at rt. Filtration and evaporation afforded 41.87 g of yellow crude oil. FC DCM/MeOH 20:1 (3 runs) afforded 9.15 g of A-24 as a white solid (89%). From 19F-NMR of Mosher ester: 97:3 of the 2 enantiomers (ee 94%).

TLC DCM/MeOH 20:1 UV, CPS Rf 0.2
Mp: 132.8-137.4°C
\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta = 7.29\) (1H, m, CH ar); 7.19 (2H, m, 2xCH ar); 5.84-5.70 (2H, m, \(=CH + NH\)); 5.14-5.04 (2H, m, \(=CH_2\)); 4.73 (1H, t, CHOH, \(J=6.4\) Hz); 4.25-4.16 (2H, m,
arNCH$_2$); 3.56-3.38 (2H, m, CH$_2$NH); 3.18-2.75 (1H, b, OH); 2.52 (3H, s, NC(CH$_3$)$_2$N); 2.48 (2H, t, CH$_2$CHOH, $J$=7.2 Hz); 1.45 (9H, s, C(CH$_3$)$_3$).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ = 156.25 (CO); 152.36 (Cq); 142.31 (Cq); 138.35 (Cq); 134.94 (=CH$_2$); 134.35 (Cq); 120.07 (arCH); 118.02 (=CH$_2$); 116.36 (arCH); 108.85 (arCH); 79.97 (C(CH$_3$)$_3$); 73.74 (CHOH); 44.40 (CH$_2$CHOH); 43.47 (arNCH$_2$); 40.16 (CH$_2$NH); 28.55 (C(CH$_3$)$_3$); 13.76 (NC(CH$_3$)$_2$).

HRMS (ESI) C$_{19}$H$_{27}$N$_3$O$_3$; [M]$^+$calc.: 345.2057; found: 345.2050.

$[\alpha]_p^{20}$ = -55.2° (CHCl$_3$, c=1.66)

IR (film): $\nu$ 3318, 3215, 2980, 2930, 1688, 1518, 1433, 1403, 1365, 1275, 1252, 1165, 1039 cm$^{-1}$.

**(S)-tert-butyl 2-[(5-[(triethylsilyloxy)but-3-enyl]-1H-benzo[d]imidazol-1-yl)ethylcarbamate (R-11):**

![Structure of (S)-tert-butyl 2-[(5-[(triethylsilyloxy)but-3-enyl]-1H-benzo[d]imidazol-1-yl)ethylcarbamate](image)

TES-chloride (3 mL, 17.8 mmol), imidazole (1.3 g, 19.4 mmol) and DMAP (0.2 g, 1.6 mmol) were added at r.t. to a solution of **A-24** (85.6 g, 16.2 mmol) in DMF (40 mL) and stirred for 4h. Washing with saturated NaHCO$_3$ aqueous solution and extraction with AcOEt, drying over magnesium sulfate and evaporation of the solvent afforded the crude product as 27.3 g yellow oil; FC hexane/AcOEt 4:6 to AcOEt to AcOEt/MeOH 10:1 afforded 7.3 g of **R-11** as a yellow oil which later solidified (98%).

TLC DCM/MeOH 10:1 UV, CPS Rf 0.2

Mp: 101.5-104.3°

$^1$H NMR (CHCl$_3$, 400 MHz): $\delta$ = 7.58 (1H, s, arCH); 7.23-7.20 (2H, m, 2xar-CH); 5.81-5.69 (1H, m, CH=); 5.03-4.93 82H, m, CH$_2$=); 4.78 (1H, t, CHOTES, $J$=6.5 Hz+1H, b, NH); 4.30-4.20 (2H, m, arNCH$_2$); 3.47 (2H, q, CH$_2$NH, $J$=6.0 Hz); 2.58-2.37 (5H, m, NC(CH$_3$)$_2$N+ CH$_2$CHOTES); 1.43 (9H,s, C(CH$_3$)$_3$); 0.86 (9H, t, 3xSiCH$_2$CH$_3$, $J$=7.8 Hz); 0.58-0.44 (6H, m, 3xSiCH$_2$CH$_3$).

$^{13}$C-nmr (CHCl$_3$, 100 MHz): $\delta$ = 156.05 (NHCOO); 152.14 (NC(CH$_3$)$_2$N); 142.59 (arCq); 139.63 (arCq); 135.53 (=CH); 134.45 (arCq); 120.63 (arCH); 116.78 (=CH$_2$); 116.59 (arCH); 108.66 (arCH); 80.04 (C(CH$_3$)$_3$); 75.29 (CHOTES); 46.07 (CH$_2$OTES); 43.34 (arNCH$_2$); 40.13 (CH$_2$NH); 28.45 (C(CH$_3$)$_3$); 13.87 (NC(CH$_3$)$_2$N); 6.96 (SiCH$_2$CH$_3$); 4.98 (SiCH$_2$CH$_3$).

HRMS (ESI): C$_{25}$H$_{41}$N$_3$O$_3$Si; [MH]$^+$calc.: 460.2990; found: 460.2990.

$[\alpha]_p^{20}$ = -25.7° (CHCl$_3$, c=1.06).

IR (film): $\nu$ 3293, 2954, 2875, 1701, 1521, 1453, 1433, 1404, 1365, 1326, 1276, 1254, 1170, 1166, 1082, 1036, 1004 cm$^{-1}$.
(S)-tert-butyl 2-(5-(3-oxo-1-(triethyilsilyloxy)propyl)-1H-benzo[d]imidazol-1-yl)ethylcarbamate (R-19):

\[
\text{\begin{tabular}{c}
\includegraphics[width=0.2\textwidth]{structure}
\end{tabular}}
\]

2,6-lutidine (510 µL, 4.4 mmol), OsO₄ (2.5% w/w solution in tert-BuOH, 0.6 mL, 0.1 mmol) and NaIO₄ (1.9 g, 8.8 mmol) were added successively at r.t. to a solution of R-11 (1.0 g, 2.2 mmol) in dioxane/water 3:1 (20 mL). After stirring at r.t. for 23h the mixture was washed with brine (30 mL) and extracted with Et₂O (3X30 mL). Drying over magnesium sulfate and evaporation of the solvents afforded 1.59 g of crude brown oil. FC hexane/AcOEt/MeOH 6:4:0.1 afforded 0.77 g of A-19 as brown oil (74%).

TLC AcOEt/MeOH 10:1 UV, CPS Rf 0.38

\[\text{^1}H \text{ NMR: (CDCl}_3, 400 \text{ MHz} \delta = 9.80 (1H, t, J=2.2Hz); 7.66 (1H, s, ar CH); 7.28-7.23 (m, 2H, Ar CHs); 5.34 (1H, dd, CHOTES, J=4.4, 8 Hz); 4.65 (1H, b, NHBOc); 4.27 (2H, t, aSrNCH}_2, J=5.6 Hz); 3.51-3.46 (2H, m, CH}_2NHBOc); 2.93-2.86 (1H, m, CHHCHOTES); 2.69-2.846 (1H, m, CHHCHOTES); 2.59 (3H, s, ar-CH}_3); 1.44 (9H, s, Boc); 0.86 (9H, t, SiCH}_2CH}_3 x3, J=8 Hz); 0.58-0.48 (6H, m, SiCH}_2CH}_3 x3).

\[\text{^13C} \text{ NMR: (CDCl}_3, 100 \text{ MHz} \delta = 201.66 (CO); 152.5 (ar NC(CH}_3)N); 142.71 (ar C q); 138.20 (ar C q); 134.62 (ar C q); 120.09 (ar CH); 116.27 (ar CH); 108.99 (ar CH); 80.04 (C q Boc); 70.86 (CHOTES); 54.47 (CH}_2CHOTES); 43.25 (NCH}_2CH}_2NHBOc); 40 01 (NCH}_2CH}_2NHBOc); 28.31 (C(CH}_3) Boc); 13.80 (NC(CH}_3)N); 6.71 (SiCH}_2CH}_3); 4.77 (SiCH}_2CH}_3).

HRMS (ESI): MH⁺+MeOH calc.: 494.3042; found 494.3041.

[\alpha]^{D}_{20}= -44.5° (CHCl₃, c=1.06)

IR (film): ν 3731, 3624, 2957, 2882, 1705, 1515, 1455, 1399, 1363, 1247, 1165, 1088, 1005 cm⁻¹.
(S,Z)-tert-butyl 2-(5-(4-iodo-1-(triethylsilyloxy)pent-3-enyl)-1H-benzo[d]imidazol-1-yl)ethylcarbamate (A-9):

![Chemical Structure Image]

NaHMDS 1M in THF (9.0 mL, 9.0 mmol) was added dropwise at -78°C to a suspension of [1-iodoethyl]triphenylphosphonium iodide (2.59 g, 4.76 mmol) in THF (10 mL). The reaction mixture was stirred at the same temperature for 30 min, then at -20°C for 30 min (clear red solution), then at -78°C a solution of A-19 (2.00 g, 4.33 mmol) in THF (22 mL) was added over 15 min. The mixture was stirred at -78°C for 7 h, then a saturated solution of NaHCO₃ (20 mL) was added, followed by water (100 mL). After extraction with Et₂O (120 mL), separation, washing with water (100 mL), drying over magnesium sulfate and evaporation of the solvents 4.5 g of crude A-9 were obtained. FC hexane/AcOEt/MeOH 6.4:0.1 (several runs) afforded 0.96 g of clean A-9 as a white solid (42%).

TLC hexane / DCM/MeOH 6:4:0.5, UV, CPS (product is blue, phosphinoxide is yellow) RF 0.43 (p) and 0.21 (Ph₃PO)
mp: 125.8-126.3°C

¹H NMR (CDCl₃, 400 MHz): δ = 7.62 (1H, s, Ar CH); 7.24 (2H, s, Ar CH x2); 5.45 (1H, dt, C=CH, J = 1.4Hz, 6.8Hz); 4.85 (1H, CHOTES, dd, J=5.6Hz, 6.8Hz); 4.62 (1H, b, NH); 4.26 (2H, ArNCH₂, bt, J=5.2Hz); 3.48 (2H, NHCH₂, q, J=6.0Hz); 2.59 (3H, s, CH₃C=CH); 2.59-2.43 (2H, m, CH₂CHOTES); 2.46 (3H, s, NC(CH₃)N); 1.43 (9H, s, Boc); 0.88 (9H, t, 3xCH₃ of TES, J=7.8Hz); 0.60-0.46 (6H, m, 3xCH₂ of TES).

¹³C NMR (CDCl₃, 100.6 MHz): δ = 155.8 (CO); 152.25 (ar Cq); 142.67 (ar Cq); 139.44 (ar Cq); 134.49 (ar Cq); 132.42 (CH=); 120.51 (arCH); 116.59 (arCH); 108.77 (arCH); 102.56 (=Cl); 80.17 (C(CH₃)₃); 74.22 (CHOTES); 48.32 (=CHCH₂); 43.42 (arNCH₂); 40.29 (CH₂NHBOc); 33.77 (=CCH₃); 28.49 (C(CH₃)₃); 13.96 (NC(CH₃)N); 6.97 (SiCH₂CH₃); 5.00 (SiCH₂CH₃).

[α]₀₂₀° = -5.0° (CHCl₃, c=0.96)

IR (film): ν 3212, 2954, 2913, 2877, 1705, 1520, 1457, 1432, 1401, 1363, 1283, 1251, 1170, 1068 cm⁻¹.
Alcohol **R-25**: 

![Chemical Structure](image)

4-fluoro-3-nitrobenzoic acid (15 g, 81.03 mmol) was dissolved in methanol; 2-aminoethanol (65 ml, 1.08 mol, 13.3 eq.) was added. The reaction was stirred for 4h at r.t.. After distillation of the solvent, the residue was dissolved in 170 ml of 1N HCl solution, under cooling and stirring. Concentrated HCl was added until pH3. The yellow precipitate was filtered and washed 3 times with 1N HCl; evaporation of the mother liquor afforded further material. After HV drying, 18.18 g of **R-25** (99 %) were obtained as a yellow solid.

TLC: Et₂O 2% AcOH, Ref: 4-fluoro-3-nitrobenzic acid, UV
Mp: 206.3-207.6°C

$^1$H-NMR (DMSO, 400 MHz): δ = 8.60 (1H, d, J=2.0), 8.56 (1H, t, J=5.4), 7.95 (1H, dd, $J_1$=2.0, $J_2$=9.2), 7.14 (1H, d, J=9.2), 3.66 (2H, t, J=5.4), 3.47 (2H, q, J=5.4).

$^{13}$C-NMR (DMSO, 100.6 MHz): δ = 165.92 (COOH), 147.56 (ar), 135.97 (ar), 130.40 (ar), 128.43 (ar), 117.06 (ar), 114.86 (ar), 58.91 (CH₂), 44.92 (CH₂).

HR-MS (ESI): [M]·calc: 225.05200; found: 225.05147.

IR (film): ν 3360.35, 2951.52, 1684.52, 1621.84, 1566.88, 1527.35, 1439.60, 1362.46, 1287.25, 1255.54, 1155.15, 1065.48, 923.73, 760.78, 695.21, 518.76 cm⁻¹.

Amino-alcohol **A-28**: 

![Chemical Structure](image)

**R-25** (0.5 g, 2.2 mmol) was dissolved in ethanol and transferred into a flame-dried flask filled with Ar. Pd on charcoal (100 mg) was added and the flask was filled 3 times with hydrogen. The reaction mixture was stirred vigorously for 40 min under hydrogen atmosphere. After venting the flask several times with argon, the mixture was filtered through a celite pad; after HV drying, **A-28** was isolated as a grey solid (0.39 g, 83%).

TLC: Et₂O/EtOAc 1:1 + 2% AcOH, UV
Mp: 112.7-113.0°C

$^1$H-NMR (DMSO, 400 MHz): δ = 7.19 (1H, dd, $J_1$=2.0, $J_2$=8.0), 7.16 (1H, d, J=2.0), 6.44 (1H, d, J=8.4), 5.11 (1H, t, J=2.6), 4.73 (1H, t, J=5.6), 3.60 (2H, q, J=6.0), 3.18 (2H, m).
$^{13}$C-NMR (DMSO, 100.6 MHz): $\delta$ = 167.98 (COOH), 140.45 (ar), 134.04 (ar), 120.89 (ar), 117.81 (ar), 114.76 (ar), 107.80 (ar), 59.35 (CH$_2$), 45.56 (CH$_2$).

HR-MS: [M]$^+$ calc.: 196.08456; found: 196.0844

IR (film): $\nu$ 3443.28, 3343.96, 3310.21, 3241.75, 2858.27, 2363.34, 2340.19, 1672.95, 1601.59, 144.35, 1446.35, 1309.43, 1264.11, 1222.65, 1146.47, 1055.84, 767.53 cm$^{-1}$.

Alcohol R-26

![Chemical Structure]

A-28 (10.7 g, 54.5 mmol) was dissolved in ethanol (600 ml), then triethylorthoacetate (27 ml, 148 mmol, 2.7 eq.) was added. The solution was heated to reflux (110°C) for 2h, then allowed to r.t.; some product precipitated as white solid and was filtered away; after HV-drying, 9.41 g of A-28, were obtained. Evaporation of the mother liquor afforded further 1.86 of A-28, bringing the total yield to 11.27 g (93.9%).

TLC: EtOAc/MeOH 8:2 + 2% AcOH, UV

Mp: 268.2-269.2°C

$^1$H-NMR (DMSO, 400 MHz): $\delta$ = 8.19 (1H, d, $J=1.2$), 7.80 (1H, dd, $J_1=1.6, J_2=8.0$), 7.56 (1H, d, $J=8.8$), 4.96 (1H, s), 4.27 (2H, t, $J=5.2$), 3.71 (2H, t, $J=4.8$), 2.57 (3H, s).

$^{13}$C-NMR (DMSO, 100.6 MHz): $\delta$ = 168.01 (COOH), 154.57 (ar), 141.92 (ar), 138.63 (ar), 123.74 (ar), 122.73 (ar), 119.80 (ar), 109.84 (ar), 59.57 (CH$_2$), 46.11 (CH$_2$), 13.84 (CH$_3$).

HR-MS: [M]$^+$ calc.: 220.0848; found: 220.0844.

IR (film): $\nu$ 2768.31, 2597.64, 2522.43, 2294.88, 1696.09, 1616.06, 1521.56, 1446.35, 1403.92, 1336.43, 1279.54, 1230.36, 1081.87, 911.02, 759.82 cm$^{-1}$.
Ester R-27

![Ester R-27](image)

**A-28** (9.41 g, 42.8 mmol) was dissolved in methanol (430 ml, 250 eq.) and sulphuric acid (7.1 ml, 3.1 eq.) was added; the solution was heated to reflux (75 °C) for 27h. After allowing it to r.t., ice-cold water (140 ml) was added, followed by drop-wise addition of 2N NaOH (ca. 72 ml, 132 mmol, 1.1 eq. to sulfuric acid). Extraction with 6 times 500 ml DCM, drying and of the organic layer over magnesium sulfate and evaporation of the solvents afforded after HV drying 9.83 g of **R-27** (98%).

**TLC:** DCM/EtOH 8:2 +2 % AcOH, UV

**Mp:** 182.8 - 183.2 °C.

**¹H-NMR (DMSO, 400 MHz):** δ = 8.11 (1H, d, J=0.8), 7.82 (1H, dd, J₁=1.6, J₂=8.4), 7.60 (1H, d, J=8.4), 4.96 (1H, t, J=5.4), 4.28 (2H, t, J=5.2), 3.86 (1H, s), 3.71 (2H, q, J=5.2), 2.58 (3H, s).

**¹³C-NMR (DMSO, 100.6 MHz):** δ = 166.90 (COOCH₃), 154.87 (ar), 141.94 (ar), 138.90 (ar), 122.60 (ar), 122.50 (ar), 119.64 (ar), 110.13 (ar), 98.63 (ar), 59.57 (CH₂), 51.88 (CH₃ an COO), 46.14 (CH₂), 13.83 (CH₃).

**HR-MS:** [M⁺] calc.: 234.0999; found: 234.0999.

**IR (film):** ν 3215.72, 2946.70, 2848.35, 1711.51, 1623.77, 1442.49, 1400.07, 1294.00, 1218.79, 1085.73 cm⁻¹.

Silyl ether **R-28**

![Silyl ether R-28](image)

**R-27** (9.83 g, 41.96 mmol) was dissolved in DMF (230 ml) under argon atmosphere; imidazole (5.81 g, 85.34 mmol, 2.0 eq.) and TBS-chloride (7.93 g, 52.61 mmol, 1.2 eq.) were added. The reaction mixture was stirred at r.t. for 2.5h. The solution was diluted with AcOEt and washed with saturated NaHCO₃ solution (400 ml). The aqueous layer was back-extracted twice with AcOEt, the combined organic layers were dried over magnesium
sulfate and the solvents were evaporated. After HV-drying, 15.59 g were obtained (excess weight). 0.97 g of this material were recrystallized for analytical purposes.

TLC: DCM/EtOH 9:1, UV.
Mp: 109.6-110.3 °C.
$^1$H-NMR (DMSO, 400 MHz): $\delta = 8.11$ (1H, d, $J=1.6$), 7.81 (1H, dd, $J_1=1.6$, $J_2=8.4$), 7.59 (1H, d, $J=8.4$), 4.36 (2H, $t$, $J=4.8$), 3.90 (2H, $t$, $J=4.8$), 3.85 (3H, s), 2.58 (3H, s), 0.69 (9H, t, $J=2.8$), -0.28 (6H, $t$, $J=2.8$).
$^{13}$C-NMR (DMSO, 100.6 MHz): $\delta = 167.57$ (COO), 154.71 (ar), 141.90 (ar), 122.66 (ar), 122.46 (ar), 119.69 (ar), 110.24 (ar), 61.35 (CH$_2$), 51.88 (CH$_3$ an COO), 45.66 (CH$_2$), 25.55 (TBS), 17.74 (TBS), 13.83 (CH$_3$), -5.94 (TBS).
HR-MS: [M]$^+$ calc.: 348.1864; found: 348.1860
IR (film): $\nu$ 2950.55, 2858.95, 1703.80, 1619.91, 1434.78, 1393.32, 1301.72, 1256.40, 1199.51, 1085.73, 938.20, 832.13, 767.53 cm$^{-1}$.

Alcohol R-29

R-28 (14.6 g, 41.9 mmol) was set under argon atmosphere and dissolved in DCM; DIBAL DCM solution 1M (100 ml, 100 mmol) was added drop-wise over 6h at -75 °C. The mixture was allowed to r.t. and stirred under argon atmosphere for 20h. Methanol (150 ml) was added drop-wise while cooling the mixture in an ice bath. The ensuing white precipitate was filtered away, and washed with methanol. After evaporation of the solvents, 14.84 g of an oil were obtained. FC purification (two runs, DCM/MeOH 95:5 isocratic and 98:2 to 95:5 respectively) afforded 11.0 g of R-29 (82 %) as an oil.

TLC: DCM/MeOH 95:5, UV
Mp: 134.8 - 135.3±0.1; 2°C.
$^1$H-NMR (DMSO, 400 MHz): $\delta = 7.43$ (1H, s), 7.39 (1H, d, $J=8.0$), 7.11 (1H, dd, $J_1=1.0$, $J_2=8.0$), 5.09 (1H, $t$, $J=6.0$), 4.56 (2H, $d$, $J=5.6$), 4.27 (2H, $t$, $J=5.2$), 3.88 (2H, $t$, $J=4.8$), 2.52 (3H, s), 0.75 (9H, s), 0.24 (6H, $t$, $J=2.8$).
$^{13}$C-NMR (DMSO, 100.6 MHz): $\delta = 152.27$ (ar), 142.30 (ar), 135.46 (ar), 134.11 (ar), 120.36 (ar), 116.12 (ar), 109.29 (ar), 63.40 (CH$_2$ an OH), 61.37 (CH$_2$), 45.49 (CH$_2$), 25.61 (TBS), 17.78 (TBS), 13.73 (CH$_3$), -5.87 (TBS).
IR (film): ν 3229.22, 2935.13, 2858.95, 1518.67, 1438.64, 1402.00, 1358.60, 1254.47, 1111.76, 1014.37, 940.13, 879.38, 832.13, 781.99 cm⁻¹.

Aldehyde A-22

Oxalyl chloride (5.25 ml, 60.2 mmol, 1.6 eq.) was dissolved in DCM (80 ml). DMSO (8.75 ml, 123.3 mmol, 3.3 eq.) was mixed under argon atmosphere with DCM (20 ml); this mixture was added drop-wise at −78 °C in 15 min to the oxalyl chloride solution. After stirring for 15 min, a solution of R-29 (12.04 g, 37.57 mmol, 1 eq.) in DCM (25 ml) was added drop-wise at −78 °C over 60 min. After 80 min the formation of a slightly coloured solid was observed. The reaction mixture was allowed to 0 °C and Et₂N (16.5 ml, 160 mmol, 4.3 eq.) was added drop-wise over 10 min. Water (100 ml) and DCM (100 ml) were added, he layers were separated and the aqueous phase was extracted twice with DCM. The combined organic layers were dried over magnesium sulfate; he solvent was evaporated. FC purification (Et₂O/Hexane 2:1, gradient 0% to 5% MeOH, followed by pure AcOEt) afforded 5.99 g of pure A-22 and 2.11 g of slightly unpure product (50%).

TLC: DCM/MeOH 95:5, UV R₆=0.62.
Mp: 57.8 - 60.1°C.

¹H-NMR (DMSO, 400 MHz): δ = 10.02 (1H, s), 8.09 (1H, s), 7.74 (1H, dd, J₁=1.6, J₂=8.4), 7.68 (1H, d, J=8.4), 4.38 (2H, t, J=5.2), 3.91 (2H, t, J=4.8), 2.60 (3H, s), 0.69 (9H, d, J=3.2), 0.27 (6H, t, J=3.2).

¹³C-NMR (DMSO, 100.6 MHz): δ = 192.63 (C=O), 122.15 (ar), 121.51 (ar), 110.96 (ar), 61.43 (CH₂), 45.72 (CH₂), 25.48 (TBS), 17.89 (CH₃), 13.81 (TBS), 5.95 (TBS)

HR-MS: [M-C₄H₉]⁺ calc.: 261.1054; found: 261.1053.

IR (film): ν 2937.06, 2858.95, 2734.57, 1688.37, 1612.20, 1522.52, 1440.56, 1391.39, 1332.57, 1282.43, 1254.47, 1109.83, 1007.62, 939.16, 881.31, 830.21, 810.92, 779.10, 716.43, 665.32 cm⁻¹.
Alkene A-25

(-)-DIP-chloride (13.1 g, 40.8 mmol, 1.8 eq.) was dissolved in Et₂O (80 ml) and set under argon atmosphere; then 1 M allylmagnesium bromide solution (40.8 ml, 40.8 mmol, 1.8 eq.) was added drop-wise at 0 °C. The reaction mixture was stirred 1h at r.t., then dry pentane (56 ml) was added. The resulting mixture was transferred under argon through Teflon tubing onto a sintered-glass filter and the precipitate was washed with further pentane. The solution was cooled to – 78 °C. A-22 (7.17 g, 22.5 mmol, 1 eq.) was dissolved in toluene in a separate flask and the solution was evaporated several times to dryness; the residue was suspended in Et₂O (105 ml) and cooled to -100 °C, the borane solution was added drop-wise over 40 min through Teflon tubing. The reaction mixture was stirred at -100 °C for 1.5h. Methanol (7.5 ml, 185 mmol, 8.2 eq.) was added drop-wise, the reaction mixture was allowed to 10 °C and ethanolamine (9 ml, 150 mmol, 6.7 eq.) was added. After 20h of stirring at r.t., the precipitate was filtered off and the solvents were evaporated. FC-purification (DCM/MeOH 95:5) afforded after HV-drying 5.82 g

TLC: DCM/MeOH 95:5, Rf=0.31.
Mp: 143.9 - 44.8±0.15.

1H-NMR (DMSO, 400 MHz): δ = 7.42 (1H, s), 7.37 (1H, d, J=8.0), 7.12 (1H, dd, J₁=1.2, J₂=8.4), 5.71 (1H, m), 5.17 (1H, d, J=4.4), 4.93 (2H, t), 4.65 (1H, q), 4.27 (2H, t, J=5.0), 3.88 (2H, t, J=5.0), 2.52 (3H, s), 2.40 (2H, m), 0.71 (9H, s), -0.24 (6H, d, J=2.8).

13C-NMR (DMSO, 100.6 MHz): δ = 152.18 (ar), 142 (ar), 138 (ar), 135.73 (ar), 134.17 (ar), 119.51 (ar), 116.33 (C=C), 115.35 (ar), 109.17 (ar), 72.63 (C an OH), 61.37 (CH₂), 45.45 (CH₂), 44.33 (CH₂), 25.60 (TBS), 17.77 (TBS), 13.71 (CH₃), 5.90 (TBS).


IR (film): v 3203.18, 2935.13, 2860.88, 1518.67, 1436.71, 1402.00, 1353.78, 1255.43, 1112.73, 1051.98, 1001.84, 935.31, 882.27, 833.10, 781.03, 727.03, 671.11 cm⁻¹.
4.2.2. Synthesis of the C1-C11 Building Block

**R-12** 5-benzyloxy-pentanoic acid:

![5-benzyloxy-pentanoic acid](image)

Solid KOH (76 g, 1362 mmol) was added at rt to a solution of pentane-5-lactone (25.0 mL, 239 mmol) in toluene (500 mL) under Ar; benzyl chloride (102 mL, 885 mmol) was added and upon vigorous mechanical stirring to the mixture, which was then heated to reflux for 23h. Water (350 mL) was added to the mixture; after layer separation the aqueous phase was adjusted to pH 2 and extracted with EE (3x400 mL). After drying over MgSO4, evaporation of the solvents, and drying in high vacuum, **R-12** was obtained as yellow oil (29.5 g, 59%).

TLC: MTBE/hexane 1:1, CPS, Rf=0.45

$^1$H NMR (400 MHz, CDCl₃): δ = 7.37-7.14 (m, 5H), 4.50 (s, 2H, PhCH₂O), 3.50 (t, 2H, PhCH₂OCH₂, J=6.0 Hz), 2.38 (t, 2H, CH₃COOH, J=7.4 Hz), 1.77-1.66 (m, 4H, CH₂CH₂CH₂COOH).

$^{13}$C NMR (100 MHz, CDCl₃): δ = 179.81 (CO); 138.50 (Cq ar); 128.49 (2x CH ar); 127.78 (2x CH ar); 127.70 (CH ar); 73.04 (OCH₂Ph); 69.85 (CH₂CH₂O); 33.86 (CH₂COOH); 29.13 (CH₂); 21.63 (CH₂).

IR (film): ν 2925, 2868, 1704, 1453, 1413, 1362, 1316, 1273, 1207, 1174, 1115, 1098, 1071, 1027 cm⁻¹.


**R-13:**

![R-13](image)

Triethylamine (13 mL, 93.4 mmol) and pivaloyl chloride (8 mL, 65 mmol) were added under Ar at -30°C to a solution of **R-12** (8.0 g, 38.4 mmol) in THF (mL). After 75 min LiCl (1.9 g, 45.3 mmol) and (4S)-benzylazolidin-2-one (6.8 g, 38.4 mmol) were added and the mixture was stirred at rt for 26h. The reaction mixture was washed with water (50 mL),
which was back-extracted with Et₂O (2x70 mL). The combined extracts were dried over MgSO₄, the solvents were evaporated and drying of the residue in high vacuum afforded 17.4 g of crude oil. FC: hexane/AcOEt 85:15 afforded **R-13** as yellow oil (13.46 g, 95%).

TLC: hexane/AcOEt 3:1, CPS; Rf 0.28

**¹H-NMR** (400 MHz, CDCl₃): δ = 7.27-7.11 (m, 10H), 4.59-4.55 (m, 1H, CHN), 4.43 (s, 2H, PhCH₂O), 4.09-4.07 (m, 2H, NCH₂O), 3.45 (t, 2H, PhCH₂OCH₂, J= 6.2 Hz), 3.21 (dd, 1H, J=13.2, 3.2 Hz), 2.93-2.87 (m, 2H), 2.66 (dd, 1H, J= 13.4, 9.8 Hz), 1.75-1.63 (m, 4H, OCH₂CH₂CH₂CH₂)

**¹³C NMR** (100 MHz, CDCl₃): δ = 173.26 (CO); 153.60 (CO oxazolidinone); 138.76 (Cq ar); 135.49 (Cq ar); 129.56 (CH ar); 129.10 (CH ar); 128.51 (CH ar); 127.77 (CH ar); 127.64 (CH ar); 127.48 (CH ar); 73.07 (OCH₂Ph); 70.14 (OCH₂CH₂); 66.32 (COOCH₂); 55.30 (NCH); 38.09 (PhCH₂CHN); 35.39 (CH₂CON); 29.24 (COCH₂CH₂); 21.24 (CH₂CH₂CH₂).

[α]₀²⁰ = +41.2° (CHCl₃, c = 0.62)

IR (film): ν 3029, 2919, 2858, 1776, 1698, 1496, 1480, 1454, 1385, 1352, 1291, 1248, 1209, 1098, 1051, 1027 cm⁻¹.

HRMS (ESI): C₂₂H₂₈NO₄; [MH]⁺: calcd 368.1856; found 368.1859.

**R-14:**

![Structure of R-14](image)

A solution of **R-13** (13.7 g, 37.2 mmol) in THF (80 mL) was added dropwise under Ar at -75°C into a solution of NaHDMS 1M in THF (65 mL, 65 mmol); after 1h at -78°C a solution of Mel (5 mL, 80 mmol) in THF (100 mL) was added dropwise to the reaction mixture, which was stirred at -78°C for 1h then allowed to RT. THF (50 mL), water (50 mL) and saturated NH₄Cl solution (100 mL) were added, the aqueous layer was separated and extracted with MTBE; drying of the combined organic layers over MgSO₄ and evaporation yielded 13.6 g orange oil. FC: hexane/EE 8:2 to 1:1 afforded **R-14** as colourless oil which crystallized upon standing (9.58 g, 67%). Based on NMR data, de = 93%.

TLC hexane/AcOEt 3:1, CPS; Rf = 0.48

mp 51.0-53.0°C

**¹H-NMR** (400 MHz, CDCl₃): δ = 7.34-7.19 (m, 10H, aromatics); 4.69-4.59 (m, 1H, CHN); 4.48 (s, 2H, PhCH₂O); 4.64-4.10 (m, 2H, CHCH₂COO); 3.79-3.69 (m, 1H, CHCH₃); 3.50-3.46 (m, 2H, PhCH₂OCH₂); 3.25 (dd, 1H, J=13.4, 3.4Hz); 2.76 (dd, 1H, J= 13.4, 9.6 Hz), 1.89-1.49 (3 m, 4H, OCH₂CH₂CH₂CH₂); 1.23 (d, 3H, CHCH₃, J=6.8Hz).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 177.24 (CO); 153.18 (CO oxazolidinone); 138.74 (Ar Cq); 135.49 (Ar Cq); 129.59 (CH ar); 129.08 (CH ar); 128.51 (CH ar); 127.76 (CH ar); 127.47 (CH ar); 73.00 (OCH$_2$Ph); 70.42 (PhCH$_2$OCH$_2$); 66.11 (COOCH$_2$); 55.46 (NCH); 38.06 (NCH$_2$CH$_2$Ph); 37.65 (CHCH$_3$); 30.20 (CH$_2$); 27.63 (CH$_2$); 17.69 (CH$_3$).

$[\alpha]_D^{20}$ = +63.3° (CHCl$_3$, c = 0.58)

IR (film): $\nu$ 3029, 2919, 2858, 1776, 1698, 1496, 1480, 1454, 1385, 1352, 1291, 1248, 1209, 1098, 1051, 1027 cm$^{-1}$.


Alcohol R-15:

![](image)

R-14 (23.65 g, 62 mmol) was dissolved under Ar atmosphere in 120 mL of THF, then at 2-5°C LAH 1M in THF (48 mL, 48 mmol) was added drop wise (2h); the reaction mixture was stirred at RT for 1h, then 8.5 mL of THF/H$_2$O 10:7 and 5 mL of NaOH 2N were added carefully. Filtration of the white solid (washing with abundant THF) and evaporation yielded 24.5 g of colourless oil. The residue was dissolved in AcOEt (100 mL) and washed with 80 mL each of H$_2$O, NaOH 1M, HCl 1M, and brine; all aq.layers were back-extracted twice with 100 mL of AcOEt. Drying over MgSO$_4$ and evaporation gave 25.38 g colourless oil. Destillation (2.5*10$^{-2}$ mbar, 125-130°C) afforded 10.8 g colourless oil (52 mmol, 84%).

TLC: hex/AcOEt 3:1, CPS (brown), Rf = 0.34;

$^1$H-nmr (400 MHz, CDCl$_3$): $\delta$ = 7.36-7.27 (m, 5H, ar Hs); 4.52 (s, 2H, PhCH$_2$O); 3.52-3.41 (m, 4H, CH$_2$OCH$_2$Ph+CH$_2$OH); 1.72-1.54 (m, 4H, OCH$_2$CH$_2$ CH$_2$CH$_2$);1.23-1.18 (m, 1H, CHCH$_3$); 0.93 (d, 3H, CHCH$_3$).

$^{13}$C-nmr: 138.54 (C q ar); 128.38 (2C ar); 127.68 (2C ar); 127.55 (C ar); 72.97 (PhCH$_2$); 70.68; 68.09 (CH$_2$O, CH$_2$OH); 35.60 (CHCH$_3$); 29.60; 27.13 (CH$_2$CH$_2$CHCH$_3$); 16.59 (CH$_3$).

$[\alpha]_D^{20}$ = -10.3° (CHCl$_3$, c = 0.73)

IR (film): $\nu$ 3393, 2933, 2862, 1748, 1719, 1496, 1454, 1362, 1313, 1275, 1205, 1097,1027 cm$^{-1}$.

HRMS (EI): $\text{C}_{13}\text{H}_{20}\text{O}_2$; [MH]$^+$: calcd 208.1463; found 208.1458.
Aldehyde A-13:

![Chemical structure of aldehyde A-13](image)

A solution of 5.5 mL of DMSO (78 mmol) in 5.5 mL DCM was added drop wise at -78°C (40 min) to a solution 2.74 mL of oxaly chloride (31.4 mmol) in 11 mL of DCM; after stirring at the same temperature for 15min, a solution of R-15 (3.3 g, 15.8 mmol) in 13 mL DCM was dropwise (over 1h 40min) to the mixture. The reaction was stirred at -78°C for 1h, then 13 mL of triethylamine (93.8 mmol) were added drop wise (25 min). The mixture was allowed to RT, then water was added (20 mL) and the clear biphasic system was separated; the organic phase was washed with 30 mL each of KHSO₄ 2%. NaHCO₃ aqu. sat. sol. and brine; the KHSO₄ solution was re-extracted with DCM (30 mL). Drying over MgSO₄ and evaporation of the solvent afforded 3.51 g of yellow oil. FC hexane/AcOEt 7:3 afforded 2.87 g of A-13 as yellow oil (13.9 mmol, 88%).

TLC: hexane/AcOEt 7:3, CPS, Rf = 0.86

¹H-nmr (400 MHz, CDCl₃): δ = 9.63 (d, 1H, J = 2.7 Hz, CHO); 7.38-7.27 (m, 5H, ar CH); 4.51 (s, 2H, PhCH₂); 3.50 (t, 2H, J = 6.2 Hz, OCH₂); 2.38-2.34 (m, 1H, CHCH₃); 1.88-1.43 (m, 4H, CH₂CH₂); 1.12 (d, 3H, J = 6.8 Hz, CH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 205.17 (CHO); 138.58 (Cq ar); 128.53 (CH ar); 127.78 (CH ar); 127.73 (CH ar); 73.10 (PhCH₂O); 70.08 (OCH₂CH₂); 46.21 (CHCH₃); 27.31 (CH₂); 27.30 (CH₂); 13.51 (CH₃).

[α]D<sup>20</sup> = +4.5° (CHCl₃, c = 0.30)

IR (film): ν 2938, 2866, 1702, 1454, 1416, 1362, 1316, 1274, 1238, 1174, 1099, 1027 cm⁻¹.

ESI-MS: ES+ 207.63 [MH]⁺ (C₁₃H₁₉O₂)

Aldol product A-16:

![Chemical structure of aldol product A-16](image)

30.0 mL of 1.6M solution of n-BuLi in hexane (48 mmol) were added drop wise (1h) to a solution of iPr₂NH (6.8 mL, 48 mmol) in THF (60 mL) at -10°C to 0°C. After 30' of stirring at 0°C, the mixture was cooled to -78°C and a solution of 10.3 g of A-12 (48 mmol) in 60 ml of
THF was added dropwise (2h). The mixture was stirred at the same temperature for 1h, then a solution of \textbf{A-13} (8.94 g, 43.3 mmol) in 60 ml THF was added dropwise (1h30') and the mixture was stirred at -78°C for 1h45min longer. Aqu. sat. sol. of NH₄Cl (150 mL) was added and the mixture was allowed to rt. Et₂O and H₂O were added until a clear biphasic system was formed then separation of the layers and back-extraction of the aqu. phase with 2x200 ml of Et₂O and evaporation of the solvents afforded 19.95 g of \textbf{A-16} as a mixture of diastereoisomers (38 mmol, 88%).

TLC: hexane/AcOEt 3:2, CPS, RF= 0.65 (upper, major diastereoisomer), 0.57 (minor diast.)

\begin{align*}
^{1}H\text{-NMR (CDCl}_3, \text{400 MHz): } &\delta = 7.35-7.26 \text{ (m, 5H, aromatics); 4.51 (s, 2H, PhCH}_2\text{O); 4.05 (1H, dd, J1=2.6 Hz; J2=11.6 Hz); 3.96 (1H, dt, J1=2.8 Hz, J2=12 Hz); 3.89-3.85 (1H, m); 3.53-3.44 (3H, m); 3.82 (1H, d, J=9.2 Hz); 3.29 (1H, q, J=7 Hz); 1.87-1.53 (4H, m); 1.41(3H, s); 1.34 (3H, s); 1.21 (3H, s); 1.09 (3H, s); 1.02 (3H, d, J=6.8 Hz); 0.85 (3H, d, J=6.8 Hz). \\
^{13}C\text{-nmr (100 MHz, CDCl}_3): &\delta = 215.13 \text{ (CO); 129.72 (Cq ar); 128.47 (CH ar); 127.78 (CH ar);74.94 (CHOR C3); 74.49 (CHOH C7);73.05 (PhCH}_2\text{O); 71.14 (OCH}_2\text{ C11); 64.04 (OCH}_2\text{ C1); 63.27 (CHCH}_3\text{); 51.77 (Cq); 41.44 (CHCH}_3\text{); 35.45 (CH}_2\text{); 29.90 (gem-CH}_3\text{); 25.31 (CH}_2\text{); 21.74 (gem-CH}_3\text{); 21.66 (CH}_2\text{ C2); 19.22 (gem-CH}_3\text{); (CHCH}_3\text{); 18.73 (gem-CH}_3\text{); 15.52 (CHCH}_3\text{); 9.46 (CHCH}_3\text{); (CH}_3\text{).} \\
\ldots
\end{align*}

IR (film): \nu 2938, 2866, 1702, 1454, 1416, 1362, 1316, 1274, 1238, 1174, 1099, 1027 cm⁻¹.

MS: ES⁺ 421.96 [MH]+ \(\text{[C}_{25}\text{H}_{34}\text{O}_5}\)

**Triol R-16:**

\begin{align*}
\text{2.05 g of PPTS (8 mmol) were added to a solution of 3.38 g of \textbf{A-16} (8 mmol) in 100 mL of MeOH; after stirring at r.t. for 19h, evaporation of the solvent afforded 5.2 g of colourless oil (partially solidified in the fridge). FC Et}_2\text{O yielded 2.97 g of \textbf{R-16} as yellow oil (7.8 mmol, 97%).} \\
\text{TLC: EE, CPS, Rf = 0.52 (major diast.) and 0.45 (minor diast.)} \\
^{1}H\text{-NMR (CDCl}_3, \text{400 MHz): } &\delta = 7.37-7.32 \text{ (m, 4H, ar CH); 7.31-7.26 (m, 1H, CH ar); 4.51 (s, 2H, OCH}_2\text{Ph); 4.08-4.00 (m, 1H, CHOH); 3.95-3.83 (m, 2H, CH}_2\text{OH); 3.54-3.44 (m, 2H, CH}_2\text{OCH}_2\text{Ph); 3.40 (dd, 1H, J1 = 8.8 Hz, J2 = 1.5 Hz, CHOH); 3.31-3.24 (m, 1H, CHCH}_3\text{ C6);}
\end{align*}
1.85-1.69 (m, 2H, CH₂ C9); 1.68-1.51 (m, 5H, 2xCH₂ at C10 and C2, CH at C8); 1.22 (s, 3H, gem-CH₃); 1.15 (s, 3H, gem-CH₃); 1.07 (d, 3H, J = 6.9 Hz, CHCH₃); 0.88 (d, 3H, J = 6.8 Hz, CHCH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 222.28 (CO); 138.83 (Cq ar); 128.49 (CH ar); 127.80 (CH ar); 127.62 (CH ar); 76.55 (CHOH); 74.78 (CHOH); 73.08 (PhCH₂O); 71.08 (CH₂O C11); 62.39 (CH₂ C1); 52.58 (Cq C4); 41.15 (CH C6); 35.59 (CH C8); 32.76 (CH₂); 29.30 (CH₂); 27.08 (CH₂); 21.67 (gem-CH₃); 18.75 (gem-CH₃); 15.71 (CH₃); 10.34 (CH₃).

IR (film): ν 3418, 2937, 2875, 1683, 1496, 1454, 1411, 1382, 1365, 1314, 1272, 1205, 1075, 1054, 1017, 995, 975 cm⁻¹.

HR-MS (ESI) (C₂₂H₃₆O₃) [MNa⁺]: calcd. 403.2455; found 403.2458.

**Silyl ether R-17:**

![Image of silyl ether R-17]

A solution of **R-16** (10.3 g, 27 mmol) and 2,6-lutidine (15 mL, 135 mmol) in 110 mL of DCM was prepared and cooled to 0°C; 10.1 mL of TBSOTf (95 mmol) were added dropwise over 30 min. The mixture was stirred at RT for 21 h, than concentrated in vacuo to ca 50% of the initial volume; 400 mL of AcOEt and 250 mL of sat. aqu. sol. of NaHCO₃ were added. Separation of the layers and washing of the organic phase with water, KH₂SO₄ 5% (2x) and brine (2x), drying over MgSO₄ and evaporation of the solvent afforded 24.64 g of yellow oil. FC hexane/Et₂O 97:3 (2 runs) 18.26 g of **R-17** as a single diastereoisomer (25 mmol, 93%).

TLC: Et₂O, CPS, Rf = 0.58

¹H-NMR (CDCl₃, 400 MHz): δ = 7.36-7.32 (m, 4H, CH ar); 7.30-7.27 (m, 1H, CH ar); 4.51 (s, 2H, PhCH₂O); 3.91 (dd, 1H, J₁ = 7.6 Hz, J₂ = 2.7 Hz, CHOTBS); 3.80 (dd, 1H, J₁ = 7.6 Hz, J₂ = 2.7 Hz, CHOTBS); 3.73-3.54 (m, 2H, CH₂OTBS); 3.46 (t, J = 6.50, CH₂OCH₂Ph); 3.21-3.09 (m, 1H, CHCH₃); 1.68-1.64 (m, 1H, CHCH₃); 1.64-1.32 (m, 6H, 3xCH₂ C2, C9 C10); 1.22 (s, 3H, gem-CH₃); 1.05 (d, 3H, J = 6.9 Hz, CHCH₃); 1.03 (s, 3H, gem-CH₃); 0.94 (d, 3H, J = 7.1 Hz, CHCH₃); 0.92 (s, 9H, tBu TBS); 0.91 (s, 9H, tBu TBS); 0.89 (s, 9H, tBu TBS); 0.11-0.02 (m, 24 H, 6xSiCH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 218.40 (CO); 139.13 (Cq ar); 128.47 (CH ar); 127.69 (CH ar); 127.60 (CH ar); 77.63 (CHOTBS); 74.12 (CHOTBS); 73.03 (PhCH₂); 71.05 (CH₂ C11); 61.18 (CH₂OTBS); 53.88 (Cq C4); 45.27 (CHCH₃); 38.96 (CH₂); 38.33 (CH₂); 28.22 (CHCH₃); 27.52 (CH₂); 26.41 (tBu TBS); 26.29 (tBu TBS); 26.13 (tBu TBS); 24.74 (gem-CH₃); 19.36 (gem-...
CH₃); 18.69 (Cq tBu); 18.51 (Cq tBu); 18.46 (Cq tBu); 17.76 (CHCH₃); 15.40 (CHCH₃); -3.49 (SiCH₃); -3.54 (SiCH₃); -3.57 (SiCH₃); -3.79 (SiCH₃); -5.06 (SiCH₃); -5.09 (SiCH₃).

\[\alpha\]₀^20 = -24.7° (CHCl₃, c = 0.82)

IR (film): v 2954, 2929, 2884, 2857, 1694, 1472, 1463, 1407, 1388, 1361, 1253, 1097, 1005, 985 cm⁻¹.

HR-MS (ESI) [MNa]^+ : calcd. 745.5049; found 745.5068.

Alcohol A-15:

A solution of CSA (68 mg, 0.28 mmol) in 10 mL of MeOH was added drop wise (15 min) to an ice/acetone-cooled solution (-10°C) of R-17 (1.02 g, 1.4 mmol) in 60 mL DCM/MeOH 1:1; the mixture was stirred at 0°C for 3h, then: 20 mL of sat. aqu. sol. of NaHCO₃ were added; after filtration and evaporation of the solvents, the residue was dissolved in DCM (50 mL) and washed with brine (50 mL, back-extracted with 2x50 mL of DCM); after drying over MgSO₄, evaporation and HV drying afforded 841.2 mg of crude product. FC hexane/Et₂O 2:1 yielded 655.7 mg of A-15 as colourless oil (1.08 mmol, 77%).

TLC: hexane/Et₂O 9:1, CPS Rf = 0.11

¹H-NMR (CDCl₃, 400 MHz): δ = 7.28-7.24 (m, 4H, CH ar); 7.24-7.19 (m, 1H, CH ar); 4.51 (s, 2H, PhCH₂O); 4.00 (dd, 1H, J₁ = 6.2 Hz, J₂ = 3.9 Hz, CHOTBS); 3.75 (dd, 1H, J₁ = 7.5 Hz, J₂ = 1.9 Hz, CHOTBS); 3.65 (t, 2H, J = 6.2 Hz, CH₂); 3.47 (t, 2H, J = 6.9 Hz, CH₂); 3.11-3.02 (m, 1H, CHCH₃); 1.81-1.73 (m, 1H, CHCH₃); 1.69-1.23 (m, 6H, 3CH₂); 1.14 (s, 3H, gem-CH₃); 1.01-0.97 (m, 6H, gem-CH₃ + CHCH₃); 0.86 (d, 3H, J = 6.9 Hz, CHCH₃); 0.84 (s, 9H, tBu TBS); 0.83 (s, 9H, tBu TBS); 0.06-0.02 (m, 12H, 4xSiCH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 217.60 (CO); 136.60 (Cq ar); 128.49 (CH ar); 127.69 (CH ar); 127.62 (CH ar); 77.62 (CHOTBS); 73.19 (CHOTBS); 73.04 (PhCH₂); 71.02 (CH₂ C11); 60.41 (CH₂OH); 54.02 (Cq C4); 45.35 (CHCH₃); 38.77 (CH₂); 38.60 (CH₂); 28.22 (CHCH₃); 27.34 (CH₂); 26.41 (tBu TBS); 26.23 (tBu TBS); 25.18 (gem-CH₃); 20.32 (Cq tBu); 17.92 (Cq tBu); 17.78 (gem-CH₃); 17.76 (CHCH₃); 15.87 (CHCH₃); -3.45 (SiCH₃); -3.54 (SiCH₃); -3.73 (SiCH₃); -3.76 (SiCH₃).

\[\alpha\]₀^20 = -16.9° (CHCl₃, c = 0.40)

IR (film): v 3450, 2954, 2930, 2884, 2856, 1691, 1472, 1462, 1407, 1387, 1361, 1254, 1210, 109 9, 1020, 1005, 986 cm⁻¹.

ESI-MS ES+ 631.54 [MNa]^+ (C₃H₆₄O₅S₁₂) HR-MS (ESI) [MNa]^+ : calcd. 631.4184; found 631.4188.
Aldehyde **R-100**:

![Chemical Structure]

DMSO (2.4 mL, 34.5 mmol) was added dropwise at -78°C to a solution of oxaly chloride (7 g, 11.5 mmol) in 50 ml DCM; after stirring 30min, a solution of **A-15** (7 g, 11.5 mmol) in 50 ml DCM was added drop wise and the resulting solution was stirred for 2h. After quenching with 5 mL of TEA, the mixture was allowed to -10°C; water (100 mL) and DCM (300 mL) were added, the layers were separated, the organic phase was washed with brine (back-extraction of the combined aqueous layers with 1x300 mL DCM). Drying with MgSO₄ and evaporation of the solvent afforded 7.04 g of slightly yellow oil. FC hexane/Et₂O 4:1 yielded 5.99 g of **R-100** (9.86 mmol, 86%); 0.71 g of impure product were isolated as well.

**TLC:** hex/acetone 2:1, CPS Rf 0.8

**¹H-NMR** (CDCl₃, 400 MHz): δ = 9.78-9.77 (m, 1H, CHO); 7.36-7.32 (m, 4H, CH ar); 7.32-7.25 (m, 1H, CH ar); 4.51 (s, 2H, PhCH₂O); 4.52-4.47 (m, 1H, CHOTBS C3); 3.80 (dd, 1H, J₁ = 7.6 Hz, J₂ = 2.2 Hz, CHOTBS); 3.47 (t, 2H, J = 6.7 Hz, CH₂ C11); 3.14 (quint, 1H, J = 7.3 Hz, CHCH₃ C6); 2.55-2.48 (m, 1H, ½ CH₂ C2); 2.44-2.36 (m, 1H, ½ CH₂ C2); 1.78-1.66 (m, 1H, ½ CH₂ C9); 1.56-1.38 (m, 2H, ½ CH₂ C9 + ½ CH₂ C10); 1.38-1.29 (m, 1H, CHCH₃ C8); 1.26-1.20 (m, 4H, ½ CH₂ C10 + gem-CH₃); 1.08 (s, 3H, gem-CH₃); 1.04 (d, 3H, J = 6.9 Hz, CHCH₃); 0.94 (d, 3H, J = 6.9 Hz, CHCH₃); 0.92 (s, 9H, tBu TBS); 0.89 (s, 9H, tBu TBS); 0.11-0.03 (m, 12H, 4xSiCH₃).

**¹³C-nmr** (100 MHz, CDCl₃): δ = 213.57 (CHO); 201.28 (CO C5); 138.79 (Cq ar); 128.46 (CH ar); 127.66 (CH ar); 127.61 (CH ar); 77.81 (CHOTBS C3); 73.02 (PhCH₂); 71.35 (CHOTBS C7); 70.98 (CH₂ C11); 53.65 (C5); 49.72 (C2); 45.37 (C6); 38.72 (C8); 28.20 (C9); 27.25 (C10); 26.38 (CH₃ tBu TBS); 26.06 (CH₃ tBu TBS); 24.29 (gem-CH₃); 18.68 (gem-CH₃); (18.25 Cq TBS) 17.97 (CHCH₃); 15.77 (CHCH₃); -3.46 (SiCH₃); -3.53 (SiCH₃); -3.93(SiCH₃); -4.92 (SiCH₃).

**IR(film):** v 2954, 2930, 2884, 2856, 1727, 1693, 1472, 1463, 1407, 1388, 1361, 1253, 1211, 1097, 986 cm⁻¹.

**HRMS (ESI):** (C₃₄H₆₃O₅Si₂) [M⁺] calcd. 607.4209, found 607.4227.
Acid R-18:

A solution of isobutylene (9 g, 160 mmol) in THF (29 mL) was prepared at -10°C; at 0°C a solution of R-100 (5.99 g, 9.9 mmol) in t-butanol (49 mL) was added drop wise (15 min), then 9.6 ml of H₂O were added, followed by NaClO₂ 80% (3.6 g, 31.6 mmol) and NaH₂PO₄·H₂O (2.18 g, 15.8 mmol). The mixture was stirred at r.t. (solids dissolved upon reaching r.t.; yellow solution) for 7h. At 4.5 h partial conversion was observed in TLC, therefore further NaClO₂ 80% (1.11 g, 12.3 mmol) and NaH₂PO₄·H₂O (0.68 g, 4.9 mmol) were added. The solvents were evaporated and 200 ml each of DCM and H₂O were added; pH was corrected to 4.5 and the layers were separated, back-extracting the aq. phase (2x200 mL DCM); drying with MgSO₄ and evaporation of the solvent afforded 6.78 g of crude product. FC hex/acetone 4:1 afforded 5.22 g of R-18 (8.4 mmol, 87%); 0.41 g of impure product were obtained as well.

TLC hexan/EE 2:1, CPS Rf 0.53

¹H-NMR (CDCl₃, 400 MHz): δ = 7.42-7.30 (m, 4H, CH ar); 7.30-7.26 (m, +H, CH ar); 4.50 (s, 2H, PhCH₂O); 4.41-4.39 (m, 1H, CHOTBS); 3.82-3.79 (m, 1H, CHOTBS); 3.48-3.45 (m, 2H, CH₂OCH₂Ph); 3.16-3.10 (1H, m, CHCH₃); 2.52-2.33 (m, 2H, CH₂COOH); 1.73-1.68 (m, 1H, ½ CH₂); 1.51-1.39 (m, 2H, 2x ½ CH₂); 1.38-1.29 (m,1H, CHCH₃); 1.23 (s, 3H, gem-CH₃); 1.08 (d, 3H, J = 6.4 Hz, CHCH₃); 1.05 (s, 3H, gem-CH₃); 0.99-0.82 (m, 21H, CHCH₃ + 2x tBu of TBS); 0.10-0.00 (m, 12H, 4xSiCH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 207.04 (CO C5); 176.50 (COOH); 138.64 (Cq ar); 128.32 (CH ar); 127.53 (CH ar); 127.46 (CH ar); 77.79 (CHOTBS); 73.44 (CHOTBS); 72.85 (PhCH₂); 70.86 (CH₂ C11); 53.57 (C4); 45.32 (CH C6); 40.12 (CH₂ C2); 38.58 (CH C6); 28.03 (CH₂); 27.08 (CH₂); 26.25 (CH₃ tBu TBS); 26.02 (CH₃ tBu TBS);25.65 (gem-CH₃); 23.76 (gem-CH₃); 18.89 (CHCH₃); 18.54 (Cq TBS); 17.86 (Cq TBS); 15.76(CHCH₃); -3.59 (SiCH₃); -3.67 (SiCH₃); -4.25 (SiCH₃); -4.41 (SiCH₃).

[α]₂⁰D = -27.2° (CHCl₃, c = 0.54)

HRMS (ESI): (C₃₉H₆₅O₆Si₂) [M⁺] calcd. 623.4158, found 623.4155

Ester R-31
DCC (198 mg, 0.96 mmol), DMAP (116 mg, 0.91 mmol) and MeOH (0.6 mL, 14.8 mmol) were added at -20°C to a solution of **R-30** (540 mg, 0.87 mmol) in DCM (15 ml); the mixture was allowed to r.t and stirred for 5h. Dilution with 60 mL DCM and washing with 2x30 mL of water (back-extraction of the combined aqueous phases with 2x30 mL of DCM), drying and evaporation of the combined organic layers afforded 0.82 g of crude product as grey solid. FC hexane /EE 9:1 yielded 427.2 mg of **R-31** as white oil (0.67 mmol, 77%).

**TLC hex/AcOEt 10:1, CPS Rf 0.4**

**1H-NMR (CDCl₃, 400 MHz):** δ = 7.37-7.31 (m, 4H, CH ar); 7.31-7.27 (m, +H, CH ar); 4.51 (s, 2H, PhCH₂O); 4.41 (dd, 1H, J₁ = 6.8 Hz, J₂ = 3.2 Hz, CHOTBS C3); 3.80 (dd, 1H, J₁ = 7.3 Hz, J₂ = 2.0 Hz, CHOTBS C7); 3.68 (s, 3H, COOCH₃); 3.47 (t, 2H, J = 6.7 Hz, CH₂OCH₂Ph); 3.15 (1H, quint, J = 7.2 Hz, CHCH₃); 2.47-2.40 (m, 1H, ½ CH₂ C2); 2.32-2.24 (m, 1H, ½ CH₂ C2); 1.79-1.66 (m, 1H, ½ CH₂ C9); 1.55-1.40 (m, 2H, 2x ½ CH₂ C9, C10); 1.40-1.30 (m, 1H, CHCH₃); 1.23-1.21 (m, 1H, ½ CH₂ C10); 1.22 (s, 3H, gem-CH₃); 1.07 (s, 3H, gem-CH₃); 1.05 (d, 3H, J = 7.1 Hz, CHCH₃); 0.94 (d, 3H, J = 6.9 Hz, CHCH₃); 0.92 (s, 9H, tBu of TBS); 0.88 (s, 9H, tBu of TBS); 0.12-0.05 (m, 12H, 4xSiCH₃).

**13C-nmr (100 MHz, CDCl₃):** δ = 218.15 (CO); 172.57 (CO); 138.86 (Cq); 128.47 (CH ar); 127.68 (CH ar); 127.60 (CH ar); 77.82 (CHOTBS); 73.95 (CHOTBS); 73.02 (PhCH₂); 71.04 (C11); 53.64 (C5); 51.75 (CH3); 45.44 (C6); 40.37 (C2); 38.83 (C8); 28.22 (CH2); 27.35 (CH2); 26.43 (CH3 tBu TBS); 26.16 (CH3 tBu TBS); 23.93 (gem-CH₃); 19.28 (gem-CH₃); 18.70 (Cq TBS); 18.36 (Cq TBS); 17.93 (CH3); 15.73 (CH3); -3.46 (SiCH3); -3.54 (SiCH3) - 4.42 (SiCH3); -4.43 (SiCH3).

[α]D²⁰ = -29.0° (CHCl₃, c = 0.70)

**IR (film):** ν 2953, 2930, 2884, 2856, 2120, 1742, 1694, 1472, 1463, 1436, 1361, 1296, 1254, 1196, 1174, 1094, 1020, 987 cm⁻¹.

**HRMS (ESI):** (C₃₅H₆₈O₆Si₂) [MNH₄⁺] calcd. 654.4580, found 654.4567
Alcohol A-14

Pd/C (0.4 g, 0.4 mmol) was added under Ar to a solution of R-31 (3.83 g, 6 mmol) in MeOH (50 mL), then the mixture was stirred under H₂ atmosphere at r.t. for 3.5 h. Filtration over a celite pad, evaporation of the solvent and FC (hex/diethylether 9:1->8:2->3:1->1:1) yielded 3.18 g of A-14 as oil (5.8 mmol, 98%).

TLC hexane/Et₂O 1:1, CPS Rf 0.41

¹H-NMR (CDCl₃, 400 MHz): δ = 4.43-4.39 (m, 1H, CHOTBS C3); 3.82-3.79 (m, 1H, CHOTBS C7); 3.68 (s, 3H, COOCH₃); 3.66-3.61 (m, 2H, CH₂ C11); 3.21-3.11 (m, 1H, CH C6); 2.47-2.40 (m, 1H, ¼ CH₂ C2); 2.33-2.25 (m, 1H, ¼ CH₂ C2); 1.71-1.60 (m, 1H, ½ CH₂); 1.59-1.33 (m, 3H, 2x½ CH₂+ CH) 1.23 (s, 3H, gem-CH₃); 1.12-1.06 (m, 1H, ½ CH₂); 1.11 (s, 3H, gem-CH₃); 1.08 (d, 3H, J = 7.1 Hz, CHCH₃); 0.91 (d, 3H, J = 7.0 Hz, CHCH₃); 0.91 (s, 9H, tBu of TBS); 0.88 (s, 9H, tBu of TBS); 0.13-0.08 (m, 12H, 4xSiCH₃).

¹³C-nmr (100 MHz, CDCl₃): δ = 218.19 (CO); 172.76 (COO); 77.74 (CHOTBS); 73.84 (CHOTBS); 63.49 (C11); 53.71 (C5); 51.80 (COOCH₃); 45.33 (C6); 40.32 (C2); 38.75 (C8); 31.19 (C9); 27.09 (C10); 28.38 (TBS); 20.15 (TBS); 23.88 (gem-CH₃); 19.30 (gem-CH₃); 18.68 (Cq); 18.37 (Cq); 17.78 (CH3); 15.85 (CH3); -3.51 (SiCH₃); -3.60 (SiCH₃); -4.31 (SiCH₃); -4.43 (SiCH₃).

IR (film): ν 3734, 3710, 3628, 3600, 2953, 2931, 2885, 2858, 1741, 1694, 1472, 1463, 1437, 1387, 1375, 1362, 1297, 1254, 1215, 1195, 1174, 1086, 1062 cm⁻¹.

ESI-MS ES+ 569.42 [MNa]⁺ (C₂₀H₅₈O₆Si₂)
Alkene A-11

\[
\begin{align*}
\text{Bu}_3\text{P} & \text{ (0.6 mL, 20.4 mmol) was added drop wise (20-27°C, 5 min) to a sol. of R-32 (3.48 g, 6.4 mmol) and 2-nitrophenylselenocyanate (4.63 g, 20.4 mmol) in 40 mL THF. After stirring at RT for 1h, solid NaHCO}_3 & \text{ (16 g, 192 mmol) was added followed by dropwise addition of H}_2\text{O}_2 & \text{30% (22 mL, 198 mmol, over 20min); the mixture was stirred at rt for 9 h. KHSO}_4 & \text{ 2% sol. (150 mL) was added, then extraction with Et}_2\text{O (3x100 mL, pH 9) and washing of the combined org. phases with H}_2\text{O (100 mL, back-extraction 2x60 mL Et}_2\text{O), drying over MgSO}_4, & \text{ evaporation and HV o.n. afforded red-brown oil (containing red solid). FC: hex/AcOEt 95:5, yielded 2.8 g of A-11 as yellow oil (mmol, 83%).} \\
\end{align*}
\]

TLC: hex/AcOEt 95:5, CPS Rs 0.8

\[\text{H-nmr (CDCl}_3, 400 MHz): \delta = 5.79-5.69 (1H, m, CH=CH}_2; 5.03-4.98 (2H, m, =CH}_2); 4.42 (1H, dd, C3HOTBS, J=3.2, 7.2Hz); 3.83 (1H, dd, C7HOTBS, J=2, 7.6 Hz); 3.68 (3H, s, COOCH}_3); 3.18 (1H, quint, CH(CH}_3)CO, J= 7.4 Hz); 2.44 (1H, dd, CHCOOCH}_3, J=3, 18Hz); 2.29 (1H, dd, CHCOOCH}_3, J=3, 18 Hz); 2.28-2.21 (1H, m, CHHCH=CH}_2); 1.91-1.81 (1H, m, CHHCH=CH}_2); 1.46-1.36 (1H, m, CHCH}_3); 1.24 (3H, s, gem-CH}_3); 1.10 (3H, s, gem-CH}_3); 1.07 (3H, d, C6HCH}_3, J=6.8 Hz); 0.94-0.91 (m, 12H, CHCH}_3+C(\text{CH}_3}_3); 0.88 (9H, s, C(\text{CH}_3}_3); 0.12-0.01 (12H, m, Si(\text{CH}_3}_2)x2. \\
\text{C-nmr (CDCl}_3, 100.6 MHz): \delta = 218.1 (\text{CO, C5}); 172.39 (\text{COOCH}_3); 137.78 (\text{CH}_2=); 115.68 (=CH); 77.66 (CHOTBS, C7); 73.69 (CHOTBS, C3); 53.51 (C(\text{CH}_3}_2); 51.61 (COOCH}_3); 45.63 (COOCH}_3); 40.20 (CH}_2COOCH}_3); 38.02 (CHCH}_3, C8); 35.18 (CH}_2CH=CH}_2); 26.24 (C(\text{CH}_3}_3); 25.97 (C(\text{CH}_3}_3); 23.69 (1x \text{gem-CH}_3); 19.14 (1x \text{gem-CH}_3); 18.55 (C(\text{CH}_3}_3); 18.18 (C(\text{CH}_3}_3); 18.00 (CHCH}_3, C8); 15.72 (CHCH}_3, C6); -3.53 (SiCH}_3); -3.65 (SiCH}_3); -4.50 (SiCH}_3); -4.63 (SiCH}_3). \\
\alpha_0^{25} = -33.7° (\text{Chloroform, c=1.11).} \\
\text{IR (film): v 2954, 2932, 2888, 2857, 1744, 1695, 1471, 1438, 1363, 1296, 1255, 1194, 1173, 1087 cm}^{-1}. \\
\text{HRMS (ESI): C}_{28}\text{H}_{56}\text{O}_{5}\text{Si}_2; [MNa]^*:\text{ calcld 551.3558; found 551.3568}.
\]
4.2.3. Endgame

Ester R-22

To a solution of carefully dried A-11 (1.3 g, 2.45 mmol) in 20 ml of THF were added 5 ml of a 0.5M solution of 9-BBN (2.5 mmol) under Ar at RT and the mixture was stirred for 3 h (solution A). In a separate flask a solution of 1.47 g (2.45 mmol) of vinyl iodide A-9 in 26 ml of DMF was added to a mixture of AsPh₃ (0.30 g, 0.98 mmol), Cs₂CO₃ (1.28 g, 3.92 mmol), [PdCl₂(dppf)]•CH₂Cl₂ complex (399 mg, 0.49 mmol), and H₂O (0.397 ml, 22 mmol) in 10 ml of dry DMF under Ar; this red suspension gradually turned orange-brown over a few min (B). Directly afterwards, solution A was added to B at -5 °C and the mixture was stirred at RT for 12 h. AcOEt (400 ml) and water (400 ml) were then added, the layers were separated, and the aqueous solution was back-extracted with AcOEt (1 x 300 ml). Drying of the combined organic extracts over MgSO₄, evaporation of the solvent, and purification of the residue by FC in hexane /AcOEt (10/0 → 2:8; 2 runs) gave R-22 as a white solid (2.03 g, 83%).

¹H-NMR (400 MHz, CDCl₃): δ = 7.59 (s, 1H); 7.22 (m, 2H); 5.12 (t, J = 6.8 Hz, 1H); 4.72-4.64 (m, 2H); 4.37 (dd, J = 6.9 Hz, 3.2 Hz, 1H); 4.26 (m, 2H); 3.75 (dd, J = 1.9 Hz, 6.9 Hz, 1H); 3.66 (s, 3H); 3.50-3.46 (m, 2H); 3.12 (quint, J = 6.9 Hz, 1H); 2.58 (s, 3H); 2.51-2.39 (m, 2H); 2.38-2.23 (m, 2H); 1.99-1.90 (m, 2H); 1.62 (s, 3H); 1.44 (s, 9H); 1.40-1.25 (m, 4H); 1.22 (s, 3H); 1.19-1.07 (m, 1H); 1.05 (s, 3H); 1.37 (d, J = 6.8 Hz, 3H); 0.90-0.82 (m, 12H); 0.89 (s, 9H); 0.87 (s, 9H); 0.58-0.46 (m, 6H); 0.05 (m, 12H).

¹³C-NMR (100.6 MHz, CDCl₃): δ = 217.84, 172.43, 156.04, 152.07, 140.18, 137.04, 134.27, 121.69, 120.62, 116.68, 108.58, 79.94, 77.70, 75.72, 74.10, 53.54, 51.75, 45.34, 43.23, 40.30, 40.15, 40.09, 39.02, 32.73, 31.10, 28.48, 26.38, 26.31, 26.13, 23.80, 23.64, 19.56, 18.65, 18.33, 17.80, 15.59, 13.92, 6.97, 5.01, -3.50, -3.60, -4.37, -4.42.

[α]D²⁰ = -30.6° (c = 0.60, CHCl₃).


IR (film): v 3365, 2932, 2858, 1737, 1695, 1619, 1520, 1460, 1393, 1364, 1293, 1254, 1170, 1085, 989 cm⁻¹.

Seco acid R-23

![Chemical Structure Image]

To a solution of R-22 (4.16 g, 4.16 mmol) in i-PrOH/water 4/1 (60 ml) was added solid LiOH•H₂O (1.05 g, 25 mmol) and the mixture was heated to 60 °C for 11.5 h. After cooling to RT the reaction mixture was diluted with CH₂Cl₂ (50 ml) and water (30 ml), the pH was adjusted to 2 with 1N HCl and the mixture was stirred at RT for 6 h. The organic layer was then separated and the aqueous solution was re-extracted with CH₂Cl₂ (2 x 60 ml). The combined organic extracts were dried over MgSO₄, the solvent was evaporated, and the residue was purified by FC in AcOEt/MeOH (20/1 → 10/1) to give 3.09 g (85%) of R-23 as a white solid.

¹H-NMR (400 MHz, CDCl₃): δ = 7.75 (s, 1H); 7.49-7.34 (m, 2H); 5.49 (b, 1H); 5.15 (t, J = 6.4 Hz, 1H); 4.77-4.69 (m, 1H); 4.44-4.31 (m, 3H); 3.76 (dd, J = 1.6 Hz, 1H); 3.59-3.47 (m, 2H); 3.10 (quint., J = 6.6 Hz, 1H); 2.83 (s, 3H); 2.51-2.25 (m, 4H); 2.14-2.01 (m, 1H); 1.94-1.81 (m, 1H); 1.68 (s, 3H); 1.50-1.41 (m, 2H); 1.38 (s, 9H); 1.38-1.27 (m, 1H); 1.30-1.15 (m, 4H); 1.08 (s, 3H); 1.05 (d, J = 6.8 Hz, 3H); 0.92-0.80 (m, 3H); 0.89 (s, 9H); 0.84 (s, 9H); 0.10-0.01 (m, 12H).

¹³C-NMR (CDCl₃, 100 MHz): δ = 217.88, 171.30, 156.35, 151.32, 142.63, 139.70, 135.12, 123.16, 120.41, 113.32, 110.57, 80.31, 77.38, 73.96, 73.79, 53.94, 44.38, 44.23, 40.26, 39.72, 39.49, 38.72, 32.63, 31.97, 28.46, 26.50, 26.28, 26.18, 23.75, 23.43, 19.86, 18.55, 18.37, 16.44, 16.28, 12.45, -3.79, -3.88, -3.92, -4.42.

[α]D²⁰ = -21.2° (c = 0.90, CHCl₃).

IR (film): ν 3360, 2954, 2931, 2857, 1695, 1517, 1468, 1405, 1365, 1252, 1167, 1081 cm⁻¹.


Protected macrolactone A-29
To a solution of dry **R-23** (20 mg, 0.023 mmol) in 250 µl of THF were added 16 µl of Et₃N (0.137 mmol) and 18 µl of 2,4,6-trichlorobezoyl chloride (0.114 mmol) at 10 °C under Ar. The mixture was stirred at 10 °C for 1 h and then diluted at 0 °C with 2 ml of toluene. This solution was added via syringe pump over a period of 3 h at RT to a solution of 29 mg of DMAP (0.23 mmol) in 8 ml of toluene. After 2 h of additional stirring at RT the solvent was evaporated and the residue was purified by FC in hexane/AcOEt/MeOH (25/75/0 → 15:85/0 → 0/1/0 → 0/10/1) to give 11.4 mg (57%) of **A-29** as white solid.

Mp: 108.4-109.5°C.

¹H-NMR (400 MHz, CDCl₃): δ = 7.70 (s, 1H); 7.26 (m, 2H); 5.58 (d, J = 9.2 Hz, 1H); 5.24 (t, J = 8.0 Hz, 1H); 4.66 (b, 1H); 4.25 (b, 2H); 3.95 (t, J = 5.8 Hz, 1H); 3.90 (d, J = 8.8 Hz, 1H); 3.46 (q, J = 5.7 Hz, 2H); 3.08-2.98 (m, 1H); 2.98-2.88 (m, 1H); 2.71 (d, J = 6 Hz, 2H); 2.67-2.55 (m, 1H); 2.58 (s, 3H); 2.18-2.08 (m, 1H); 1.84-1.72 (m, 2H); 1.70 (s, 3H); 1.68-1.52 (m, 2H); 1.43 (s, 9H); 1.24-1.14 (m, 1H); 1.14-1.06 (m, 10H); 0.99 (d, J = 6.8 Hz, 3H); 0.96 (s, 9H); 0.85 (s, 9H); 0.14-0.05 (m, 9H) and -0.09 (s, 3H).

¹³C-NMR (100 MHz, CDCl₃): δ = 215.08, 171.40, 155.87, 152.63, 142.85, 140.82, 136.04, 134.63, 120.85, 119.24, 116.24, 108.91, 80.07, 79.57, 77.52, 76.35, 53.37, 48.15, 43.28, 39.96, 39.36, 37.73, 36.03, 32.10, 31.55, 28.34, 27.53, 26.41, 26.16, 24.61, 24.14, 23.09, 19.36, 18.71, 18.60, 17.76, 13.74, -3.27, -3.68, -3.70, -5.68.

[α]D <sup>20</sup> = -41.3° (c=1.00, CHCl₃).

IR (film): ν 2955, 2932, 2888, 2857, 1737, 1699, 1518, 1468, 1388, 1365, 1253, 1162, 1096, 1066, 1018, 985 cm⁻¹.

HRMS (MALDI): m/z 856.5681 [MH⁺] calcd for [C₄₇H₉₁N₃O₇Si₂H] 856.5686.

**Macrolactone A-1**
Protected macrolactone **A-29** (20 mg, 0.023 mmol) was added at 0 °C to a solution of 100 μl of CF₃COOH (0.135 mmol) in DCM (200 μl). The mixture was at RT for 2 h and the solvent was evaporated to give 29 mg of a yellow oil. Purification by FC in CHCl₃/MeOH/water/AcOH 85:13:1.5:0.5 afforded after HV drying 16.8 mg of pale yellow oil; according to HPLC analysis this sample had a purity of 64%, corresponding to 90% yield. Purification by preparative RP-HPLC (A/B 80/20 → 50/50 over 6 min; A = 0.1% aqu. TFA, B = 0.1%TFA in CH₃CN) afforded 7.6 mg of **A-1** as its bis-trifluoroacetic acid salt, as a white solid (purity 99.4% according to HPLC); analytical characterization and biological evaluation are referred to this product. HPLC purification afforded also a second fraction of 2.5 mg containing the same salt of **A-1** in 62% purity (combined yield 52%).

**TLC**: CHCl₃/MeOH/water/AcOH 85:13:1.5:0.5, UV, CPS, 

**¹H-NMR** (500 MHz, DMSO-d₆): δ = 8.26-8.09 (b, 3H); 7.89-7.82 (m, 2H); 7.61 (d, J = 9.0 Hz, 1H); 5.78 (d, J = 10.5 Hz, 1H); 5.20 (t, J = 5.8 Hz, 1H); 5.14 (b, 1H); 4.59 (t, J = 6.2 Hz, 2H); 4.19 (d, J = 10.5 Hz, 1H); 3.52 (d, J = 8.1 Hz, 1H); 3.38-3.29 (m, 2H); 3.17 (quint, J = 7.2 Hz, 1H); 2.84-2.72 (m, 4H); 2.48-2.42 (m, 1H); 2.41-2.30 (m, 2H); 2.27-2.20 (m, 1H); 1.85-1.76 (m, 1H); 1.73-1.62 (m, 4H); 1.49-1.40 (m, 1H); 1.39-1.31 (m, 1H); 1.27-1.14 (m, 4H); 1.13-1.03 (m, 1H); 1.09 (d, J = 6.5 Hz, 3H); 0.93 (d, J = 6.8 Hz, 3H); 0.90 (s, 3H).

**¹³C-NMR** (125 MHz, DMSO-d₆): δ = 217.55, 170.36, 158.49 (q, J = 32 Hz), 152.74, 139.36, 138.87, 131.64, 122.85, 120.15, 116.92 (q, J = 297 Hz), 112.03, 111.76, 75.77, 75.55, 70.75, 53.14, 44.75, 41.99, 38.74, 37.53, 36.49, 35.80, 31.75, 30.12, 26.18, 23.11, 22.43, 20.38, 17.86, 16.35, 12.03.

[α]D20 = -36.9° (c = 0.97, CHCl₃).

**IR** (film): ν 3417, 2928, 1733, 1659, 1502, 1460, 1414, 1388, 1255, 1198, 1177, 1132, 1096 cm⁻¹. **HRMS (ESI)**: m/z 528.3429 [MH⁺] calcd for [C₃₀H₄₆N₃O₅+H] 528.3432.

**Partially protected macrolactone A-30**

![Diagram](attachment:image.png)

To a solution of 101 mg (0.12 mmol) of protected macrolactone **A-29** in 2.5 ml of CH₂Cl₂ were added 93 mg (0.35 mmol) of ZnBr₂ a 0-4 °C under Ar and the mixture was stirred at 0 °C for 72 h. It was then diluted with 5 ml of CH₂Cl₂ and washed with 5 ml of sat. aqu. NaHCO₃. The aqueous layer was back-extracted with CH₂Cl₂ (2 x 6 ml). The combined

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organic extracts were dried over MgSO₄, the solvent was evaporated, and the residue was purified by FC in CHCl₃/MeOH/Water/AcOH 85/13:1.5/0.5 to give 57 mg (57%) of A-30 as yellow oil. In addition, 18 mg of A-1 and 7 mg of the starting material A-29 were isolated.

TLC: CHCl₃/MeOH/water/AcOH 85:13:1.5:0.5, UV, CPS,

¹H-NMR (400 MHz, CDCl₃): δ 7.68 (m, 1H); 7.42 (d, J = 8.1, 1H); 7.29 (d, J = 8.1, 1H); 5.52 (d, J = 9.9 Hz, 1H); 5.21 (t, J = 7.6 Hz, 1H); 4.54-4.40 (m, 2H); 3.97-3.86 (m, 2H); 3.39-3.22 (m, 2H); 3.03 (t, J = 7.4 Hz, 1H); 2.95-2.82 (m, 1H); 2.80-2.50 (m, 6H); 2.15-2.02 (m, 1H); 1.83-1.70 (m, 2H); 1.70 (s, 3H); 1.69-1.50 (m, 2H); 1.20-1.00 (m, 10H); 0.99 (d, J = 6.1 Hz, 3H); 0.94 (s, 9H); 0.84 (s, 9H); 0.14-0.01 (m, 9H); -0.06--0.13 (m, 3H).

¹³C-NMR (100 MHz, CDCl₃): δ 215.16, 171.79, 152.70, 141.08, 140.97 137.08, 134.03, 121.67, 119.13, 115.79, 109.66, 79.80, 77.37, 76.53, 53.47, 48.18, 42.51, 39.45, 39.20, 37.91, 36.01, 32.27, 31.71, 27.73, 26.53, 26.27, 24.71, 24.24, 23.23, 19.40, 18.81, 18.71, 7.92, 13.57, -3.21, -3.53, -5.51, -3.53.

[α]D⁰ = -41.6° (c = 0.52, CHCl₃)

IR (film): ν 2953; 2928; 2856; 1737; 1697; 1578; 1545; 1522; 1467; 1442; 1403; 1386; 1364; 1253 cm⁻¹.

Macrolactone R-24

To a solution of 15 mg (0.020 mmol) of A-30 in 0.2 ml of DMF were added DIEA (8 μl, 0.05 mmol) and a solution of 5 mg (0.05 mmol) of succinic anhydride in 0.2 ml of DMF at RT. After stirring at RT for 2 h sat. aqu. NH₄Cl (4 ml) was added and the solution was extracted with AcOEt (3 x 4 ml). The combined organic extracts were dried over MgSO₄ and the solvent was evaporated to provide 15.4 mg (92%) of R-24 as colorless oil, which was used in the next step without further purification.

TLC: CHCl₃/MeOH/water/AcOH 85:13:1.5:0.5, UV, CPS,
¹H-NMR (400 MHz, CDCl₃): δ = 7.68 (m, 1H); 7.34 (m, 2H); 6.82 (b, 1H); 5.53 (d, J = 10.7 Hz, 1H); 5.22 (m, 1H); 4.33 (t, J = 5.3 Hz, 2H); 3.94-3.87 (m, 2H); 3.69-3.61 (m, 2H); 3.08-2.99 (m, 1H); 2.96-2.86 (m, 1H); 2.81-2.65 (m, 2H); 2.64-2.56 (m, 5H); 2.56-2.48 (m, 2H); 2.43-2.32 (m, 2H); 2.16-2.05 (m, 1H); 1.86-1.72 (m, 2H); 1.70 (s, 3H); 1.64-1.50 (m, 2H); 1.33-1.24 (m, 1H); 1.16-1.05 (m, 9H); 0.99 (d, J = 6.9 Hz, 3H); 0.95 (s, 9H); 0.84 (s, 9H); 0.14-0.04 (m, 9H); -0.09--0.12 (m, 3H).
¹³C-NMR (100 MHz, CDCl₃): δ = 215.15, 173.62, 173.05, 171.80, 153.03, 141.15, 139.91, 137.54, 133.68, 122.12, 119.13, 115.28, 109.98, 79.72, 77.37, 76.99, 53.48, 48.31, 43.28, 39.37, 38.76, 37.82, 36.70, 35.88, 32.27, 31.27, 30.03, 29.05, 26.56, 26.29, 24.85, 24.25, 23.23, 19.43, 18.84, 18.75, 17.94, 13.01, -3.18, -3.49, -3.54, -5.54.
[α]D<sub>20</sub> = -31.2° (c = 0.9, CHCl₃).
IR (film): ν 2932, 2857, 1735, 1697, 1668, 1545, 1468, 1436, 1408, 1384, 1364, 1253, 1201, 1161, 1096, 1066, 1020, 984 cm⁻¹.
Macrolactone A-3

Protected macrolactone R-24 (21 mg, 0.025 mmol) was added at RT to a solution of 30 µl of CF₃COOH (0.40 mmol) in CH₂Cl₂ (300 µl) and the mixture was stirred for 5 h. Evaporation of the solvent and purification of the residue by FC in CHCl₃/MeOH/Water/AcOH 85/13/1.5/0.5 afforded two product-containing fractions of 9.1 mg (purity 84% according to HPLC) and 7.4 mg (purity 74%; combined yield 85%). These combined factions were purified by preparative RP-HPLC (A/B 75/25 → 55/45 over 6 min; A = 0.1% aqu. TFA, B = 0.1%TFA in CH₃CN) and afforded 3.4 mg of A-30 (99% purity) as its trifluoroacetic acid salt, as a white solid; analytical characterization and biological evaluation are referred to this product. A fraction of 0.9 mg of the same product in 89% purity was also isolated (combined yield 23%).

¹H-NMR (400 MHz, DMSO-d₆): δ = 12.05 (b, 1H); 8.09 (t, J = 5.8 Hz, 1H); 7.86-7.79 (m, 2H); 7.62 (d, J = 8.9, 1H); 5.79 (d, J = 9.4 Hz, 1H); 5.20 (t, J = 7.5 Hz, 1H); 5.13 (b, 1H); 4.56-4.39 (m, 3H); 4.20 (d, J = 10.6 Hz, 1H); 3.60-3.30 (m, 3H); 3.17 (quint, J = 7.2 Hz, 1H); 2.83-2.73 (m, 4H); 2.48-2.42 (m, 1H); 2.41-2.31 (m, 2H); 2.30-2.21 (m, 3H); 2.20-2.13 (m, 2H); 1.86-1.76 (m, 1H); 1.74-1.62 (m, 4H); 1.50-1.40 (m, 1H); 1.39-1.30 (m, 1H); 1.27-1.15 (m, 4H); 1.14-1.04 (m, 4H); 0.93 (d, J = 6.6 Hz, 3H); 0.90 (s, 3H).

¹³C-NMR (125 MHz, DMSO-d₆): δ = 217.60, 173.72, 171.77, 170.35, 158.03 (q, J = 31 Hz), 152.12, 139.54, 138.83, 131.58, 122.97, 120.19, 117.04 (q, J = 298 Hz), 112.15, 111.53, 75.71, 75.54, 70.69, 53.14, 44.72, 44.26, 38.74, 37.42, 36.52, 35.76, 31.74, 30.11, 29.74, 28.68, 26.18, 23.12, 22.44, 20.35, 17.84, 16.35, 13.68. 

[α]D₃₀ = -37.6° (c = 1.13, CHCl₃).

IR (film): ν 3338, 2927, 2858, 1729, 1671, 1555, 1539, 1526, 1461, 1424, 1376, 1337, 1299, 1256, 1198, 1180, 1141 cm⁻¹.

4.2.4. Folic acid conjugates

4-((2-amino-4-oxo-3,4-dihydropyrimidin-6-yl)methylamino)benzoic acid (pteroid acid) **A-34**

![Folic Acid Conjugate A-34](image)

Folic acid (17.7 g, 40 mmol) was suspended in 1M tris-(hydroxymethyl)-aminomethane-HCl solution (pH 7.3) at 30°C; ZnCl₂ (48 mg, 0.35 mmol) and Carboxypeptidase G (5.8 mg, 10 units) were added. The suspension was stirred at the same temperature and pH for 19 days. After correction of the pH to 4.0 (with 3N HCl, mL), the precipitate was filtered and washed with NH₄Cl sat. aqu. sol.; 12.27 g of **A-34** were obtained (39 mmol, 97.5%)

TLC: NH₃ conc./propanol/EtOH 1:1:3, UV, CPS, Rf 0.6

Mp: >200°C

¹H-nmr (DMSO, 400 MHz): δ = 8.64 (s, 1H), 7.78 (d, J = 9.2 Hz, 2H), 7.14 (t, J = 6.0 Hz, 1H), 6.64 (d, J = 7.2 Hz, 2H), 4.48 (d, J = 3.6 Hz, 2H), 3.43 (s, 1H, NH).

¹³C-nmr (DMSO, 100 MHz): δ = 182.1, 175.2, 172.9, 164.0, 155.4, 150.2, 147.9, 147.3, 130.9, 129.0, 128.2, 125.1, 112.6, 63.0.

Protected pteroid acid **A-33**

![Protected Folic Acid Conjugate A-33](image)

TLC: NH₃ conc./propanol/EtOH 1:1:3, UV, CPS, Rf 0.5

Mp: >200°C

¹H-nmr (DMSO, 400 MHz): δ = 8.63 (1H, s, ar CH); 7.97 (2H, d, 2xCH ar, J=8.4 Hz); 7.62 (2H, d, 2xCH ar, J=8.4 Hz); 7.15-6.75 (2H, b, NH₂); 5.13 (2H, s, ArCH₂N).

¹³C-nmr (DMSO, 100 MHz): δ = 166.36 (COOH); 160.59 (Cq ar); 155.79 (CO of pteridine); 155.55 (q, J(C-F) = 39 Hz, COCF₃); 153.86 (Cq ar); 149.22 (Cq ar); 144.30 (Cq ar); 142.82 (Cq
PABA); 131.36 (Cq PABA); 130.17 (2xCH PABA); 128.95 (2xCH PABA); 128.11 (Cq ar); 116.05 (q, CF₃, J = 289 Hz); 53.76 (CH₂).

Epithilone derivative R-41

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\text{DIPEA (15 µL, 0.09 mmol) and HBTU (17 mg, 0.045 mmol) were added at 0°C to a solution of FmocGlu(OH)OtBu (19 mg, 0.0045 mmol) in DCM (0.3 ml) and stirred for 45 min at the same temperature, then A-30 (23 mg, 0.03 mmol) dissolved in DCM (0.3 ml) was added and the mixture was stirred at r.t. for 18h. Dilution with DCM (10 ml) and washing with brine (10 ml, back-extracted 2x 15 ml of DCM, pH 6), drying and evaporation afforded 64 mg of yellow oil. FC DCM/MéOH 95:5 afforded 34 mg of colourless oil R-41. NMR shows some tetramethylurea (1.7 eq, corrected yield: 25 mmol, 29 mg; corrected yield 80%).}

TLC DCM/MéOH 10:1, UV, CPS, RF 0.57

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\text{\textsuperscript{1}H-nmr (400 MHz, CHCl₃): δ = 7.75-7.70 (3H, m, 2xCH Fmoc + CH ar); 7.60-7.55 (2H, m, 2x CH Fmoc); 7.40-7.30 (2H, m, 2x CH Fmoc); 7.29-7.24 (4H, m, 2x CH Fmoc + 2xCH ar); 6.50 (1H, b, NHCO γ of Glu); 5.64 (1H, d, J = 7.6 Hz, NHCO α of Glu); 5.56 (1H, d, J = 9.7 Hz, CH 15); 5.23 (1H, t, J = 8.2 Hz, CH 15); 4.45-4.31 (2H, m, Fmoc-CH₂); 4.29-4.14 (4H, m, arN-CH₂ + CH Fmoc + CH α of Glu); 3.97-3.88 (2H, m, CH 3 + CH 7); 3.65-3.51 (2H, m, CH₂NH); 3.07-2.98 (1H, m, CH 6); 2.97-2.82 (1H, m, \( \frac{1}{2} \text{CH}_2 \) 14); (2.80, s, TMU); 2.75-2.68 (2H, m, CH₂ 2); 2.64-2.52 (4H, m, \( \frac{1}{2} \text{CH}_2 \) 11 + CH₃ BI); 2.27-2.16 (3H, CH₂ γ of Glu + \( \frac{1}{2} \) CH₂ β of Glu); 2.15-2.07 (1H, m, \( \frac{1}{2} \text{CH}_2 \) 14); 1.95-1.84 (1H, m, β of Glu); 1.84-1.73 (2H, m, \( \frac{1}{2} \text{CH}_2 \) 11 + \( \frac{1}{2} \) CH₂ 9 or 10); 1.71 (3H, s, =CH₃); 1.68-152 (2H, m, CH 8 + \( \frac{1}{2} \text{CH}_2 \) 9 or 10); 1.48 (9H, s, COOC(CH₃)₃); 1.33-1.26 (1H, m, \( \frac{1}{2} \text{CH}_2 \) 9 or 10); (10H, m, \( \frac{1}{2} \text{CH}_2 \) 9 or 10 + C(CH₃)₂ + CHCH₃ at C6); 1.00 (3H, d, J = 6.3 Hz, CHCH₃ at C8); 0.96 (9H, s, SiC(CH₃)₃); 0.85 (9H, s, SiC(CH₃)₃); 0.12 (3H, s, SiCH₃); 0.09 (3H, s, SiCH₃); 0.08 (3H, s, SiCH₃); -0.09 (3H, s, SiCH₃).

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\text{\textsuperscript{13}C-nmr (100 MHz, CHCl₃): δ = 215.18 (CO 5); 173.02 (CO Glu-NHCO); 171.56 (CO 1); 171.04 (COOtBu); 156.58 (CO of Fmoc); 152.34 (N=C-N); 143.99 (Cq Fmoc); 141.45 (Cq Fmoc); 140.99 (Cq=); 134.61 ((Cq of BI); 127.89 (2xCH Fmoc); 127.17 (2xCH Fmoc); 125.21 (2xCH Fmoc); 121.29 (CH BI); 120.16 (2xCH Fmoc); 119.29 (=CH); 116.10 (CH BI); 109.26}

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(CH Br); 82.86 (COOC(CH₃)₃); 79.70 (CH 7); 77.37 (CH 15); 76.54 (CH 3), 67.18 (CH₂ of Fmoc); 53.99 (CH α of Glu); 53.46 (C(CH₃)₂); 48.27 (CH 6); 47.30 (CH of Fmoc); 42.90 (arN-CH₂); 39.42 (CH₂ 2); 31.13 (CH₂NH); 37.88 (CH 8); 36.09 (CH₂ 14); 32.46 (CH₂ γ of Glu); 32.23 (CH₂ 11); 31.70 (CH₂ 9 or 10); 29.08 (CH₂ β of Glu); 28.13 (COOC(CH₃)₃); 27.69 (CH₂ 9 or 10); 26.53 (SiC(CH₃)₃); 26.29 (SiC(CH₃)₃); 24.78 (gem-CH₃); 24.21 (gem-CH₃); 23.20 (=C(CH₃)); 19.44 (CH₃ at C8); 18.82 (SiCq); 18.73 (SiCq); 17.88 (CH₃ at C6); 13.68 (N=C(CH₃)-N); -3.16 (SiCH₃); -3.55(2xSiCH₃); -5.55(SiCH₃).

[α]D²₀ = -42.2° (CHCl₃, c = 1.3)

HR-MS (ESI) C₆₆H₉₈N₄O₁₀Si₂ [MH⁺] 1163.6873 (calcd.1163.6894)

IR (film): ν 3292, 3043, 2953, 2931, 2891, 2857, 1730, 1665, 1520, 1451, 1369, 1286, 1253, 1162 cm⁻¹.

Amine R-43

![Chemical structure of R-43]

A 12% w/w dimethylamine solution in DMF (0.1 mL) was added at 0°C to a solution of R-41 (29 mg, 0.025 mmol) in DMF (0.1 mL) and stirred for 2 h at the same temperature; evaporation afforded 26 mg of colorless oil. FC DCM/MeOH 20:1 afforded 19.3 mg of R-43 (0.02 mmol, 82%).

TLC DCM/MeOH 10:1, UV, ninhydrin, Rf 0.47.

¹H-nmr (CHCl₃, 400 MHz): δ = 7.68 (1H, s, CH ar); 7.32-7.24 (2H, m, 2xCH ar); 6.80 (1H, t, J = 5.6 Hz, NHCO); 5.57 (1H, d, J = 9.4 Hz, CH 15); 5.27-5.20 (1H, m, CH=); 4.31-4.24 (2H, m, NCH₂); 3.98-3.93 (1H, m, CH 3); 3.91 (1H, d, J = 8.9Hz, CH 7); 3.63-3.55 (2H, m, CH₂NHCOC); 3.30 (1H, dd, J = 8.3, 4.5 Hz, CH α of Glu); 3.08-2.98 (1H, m, CH at C6); 2.98-2.86 (1H, m, ½ CH₂ at C14); 2.77-2.69 (2H, m, CH₂ at C2); 2.65-2.54 (4H, m, ½ CH₂ at C11 + N=C(CH₃)N); 2.36-2.26 (2H, m, NHCOCH₂); 2.20-2.09 (1H, m, ½ CH₂ at C14); 2.09-1.99(1H, m, ½ CH₂ β of Glu); 1.85-1.69 (3H, m, ½ CH₂ at C11 + ½ CH₂ β of Glu + ½ CH₂ at C9 or 10); 1.71 (3H, s, =CCH₃); 1.65-1.55 (3H, CH₂ at C 9 or 10 + CH at C8); 1.46 (9H, s, COOC(CH₃)₃); 1.24-1.17 (1H, m, ½ CH₂ at C9 or 10); 1.13 (3H, s, C(CH₃)CH₃); 1.11 (3H, s, C(CH₃)CH₃); 1.10 (3H, d, J = 6.8 Hz, CHCH₃ at C6); 1.00 (3H, d, J = 6.5 Hz, CHCH₃ at C8); 0.96 (9H, s, SiC(CH₃)₃); 0.85 (9H, s, SiC(CH₃)₃); 0.12 (3H, s, SiCH₃); 0.10-0.08 (6H, m, 2xSiCH₃); -0.08 (3H, s, SiCH₃).
$^{13}$C-nmr (CHCl$_3$, 100 MHz): δ = 215.17 (CO C5); 174.16 (COOtBu); 173.46 (NHCO); 171.59 (CO C1); 152.56 (N=C-N); 142.27 (ar Cq); 140.97 (Cq= C12); 136.28 (ar Cq); 134.84 (ar Cq); 121.00 (ar CH); 119.33 (CH C13); 116.31 (ar CH); 109.24 (ar CH); 81.90 (Cq tBu); 79.67 (CH C7); 77.67 (CH C15); 76.53 (CH C3); 54.39 (CH α of Glu); 53.49 (Cq C4); 48.30 (CH C6); 42.99 (arN-CH$_2$); 39.48 (CH$_2$ C2); 39.05 (CH$_2$NHCO); 37.90 (CH C8); 36.18 (CH$_2$ C14); 33.08 (COCH$_2$CH$_2$); 32.24 (CH$_2$ C11); 31.71 (CH$_2$ C9 or 10); 29.82 (CH$_2$ C9 or C10); 29.68 (CH$_2$ β of Glu); 28.18 (COOC(CH$_3$)$_3$); 26.54 (Si(CH$_3$)$_3$); 26.30 (Si(CH$_3$)$_3$); 24.74 (C(CH$_3$)CH$_3$); 24.28 (C(CH$_3$)CH$_3$); 23.22 (=CCH$_3$); 19.46 (CH$_3$ at C8); 18.83 (Si(CH$_3$)$_3$); 18.73 (Si(CH$_3$)$_3$); 17.89 (CH$_3$ at C6); 13.95 (NC(CH$_3$)N); -3.14 (SiCH$_3$); -3.54 (SiCH$_3$); -3.55 (SiCH$_3$); -5.33 (SiCH$_3$).

[α]$_{D}^{20} = -38.5^\circ$ (CHCl$_3$, c=0.548)


IR (film): ν 3282, 2927, 2885, 1737, 1694, 1522, 1467, 1360, 1344, 1281, 1246, 1147, 1110, 1064 cm$^{-1}$

Protected conjugate **R-44**

DIPEA (15 µL, 0.09 mmol) and HBTU (17 mg, 0.045 mmol) were added at r.t to a suspension of **R-33** (7 mg, 0.018 mmol) in DMSO/DCM 1:1 (0.5 ml) and stirred for 15 min at the same temperature; **R-43** (19 mg, 0.02 mmol) was added as solution in 0.5 ml DCM. After stirring for 21 h, dilution with DCM (10 ml) and washing with NaHCO$_3$ aqu. sat. sol. (10 ml, back-extraction 2x15 ml DCM), drying and evaporation afforded 141 mg yellow oil. FC Chloroform/MeOH/water/AcOH 85:13:1.5:0.5 (several runs) afforded 21.9 mg of **R-44** as yellow oil (0.016 mmol, 82%).

TLC CHCl$_3$/MeOH/water/AcOH 85:13:1.5:0.5, UV, Rf 0.53

$^1$H-nmr (CHCl$_3$, 400 MHz): δ = 8.74 (d, 1H, J = 8.4 Hz, NH); 8.61 (s, 1H, CH ar); 8.06 (t, 1H, J = 5.9 Hz, NH); 7.89 (d, 2H, J = 8.4 Hz, CH ar); 7.61 (d, 2H, J = 7.6 Hz, CH ar); 7.53 (s, 1H, CH ar); 7.39 (d, 2H, J = 8.4 Hz, CH ar); 7.24-7.19 (m, 1H, CH ar); 5.47 (d, 1H, J = 10.9 Hz, C15); 5.22 (t, 1H, J = 7.6 Hz, C13); 5.17-5.07 (m, 2H, CH2); 4.29-4.16 (m, 3H, CH, CH2); 3.88-3.80 (m, 2H,
C3, C7); 3.41-3.34 (m, 2H, CH2); 3.22-3.12 (m, 1H, C6); 3.12-3.03 (m, 1H, ½ CH2); 2.86-2.73 (m, 1H, ½ CH2); 2.66-2.55 (m, 3H, ½ CH2, CH2); 2.47 (s, 3H); 2.20-2.10 (m, 2H, CH2 Glu); 2.09-1.95 (m, 3H, 3x ½ CH2); 1.94-1.82 (m, 1H, ½ CH2); 1.78-1.67 (m, 2H); 1.65 (s, 3H); 1.59-1.50 (m, 1H, C8); 1.41 (s, 9H, tBu); 1.13 (s, 3H, CH3); 1.05-0.98 (m, 6H, 2xCH3); 0.96-0.89 (m, 12H, CH3, TBS); 0.80 (s, 9H, TBS); 0.13- -0.16 (TBS).

\(^{13}\text{C-nmr} \text{(CHCl}_3\text{, 100 MHz)}: \delta = \left[\alpha\right]_D^{20} = -38.1^\circ \text{ (CHCl}_3\text{, c = 0-58)}

HR-MS (ESI): 1331.6865 [MH\text{+}^\text{+}] \text{ calcd. 1331.6902}

IR (film): \( \delta \): 3730, 3627, 3597, 3291, 2929, 2856, 1733, 1695, 1660, 1607, 1538, 1503, 1461, 1406, 1369, 1290, 1253, 1206, 1157, 1099, 1064, 1022, 985.

**Conjugate R-45**

![Conjugate R-45 structure](image)

Solid K\textsubscript{2}CO\textsubscript{3} (10 mg, 72 \textmu mol) was added at r.t. to a solution of **R-43** (12 mg, 8.9 \textmu mol) in THF/water 2:1 (1.5 mL) and stirred for 1h. Dilution with DCM (10 mL) and water (5mL), separation of the layers, back-extraction with DCM (2x 10 mL), drying and evaporation of the organic layers afforded 9.9 mg of crude. FC CHCl\textsubscript{3}/MeOH/water/AcOH 85:13:1.5:0.5 afforded 7.7 mg of product as yellow oil. No further purification was performed.

HR-MS (ESI): 1235.7080 [MH\text{+}], calcd. 1235.7079.
Epithilone conjugate A-6

TFA (0.1 mL, 1.35 mmol) was added at 0°C to a solution of R-43 (7.7 mg, 0.06 mmol) and anisole (24 µl, 0.21 mmol) in DCM (0.4 mL) and stirred at the same temperature for 1 h, then for further 3 h at rt. Evaporation afforded 13.7 mg of yellow oil A6.

Preparative HPLC (A = water + 0.15 TFA; B = ACN + 0.1% TFA; t = 0 to t= 2 min A/B 57:43; t = 9 min A/B 0:90) afforded 3.1 mg of product, 4.5 mg of mixed fr. (0.035 mmol, 56%).

ESI-MS m/z 951.46

$^1$H-nmr (CHCl$_3$, 400 MHz): $\delta$ = 8.65 (s, 1H, CH ar); 8.14 (d,2H, $J = 8.9$ Hz); 8.08 (t, 1H, $J = 5.3$ Hz); 7.84-7.80 (m, 2H); 7.65-7.59 (m, 3H); 6.63 (m, 2H, 8.9 Hz); 5.77 (d, 1H, $J = 8.9$ Hz); 5.17 (t, $J = 7.2$ Hz, 1H); 4.49 (s, 2H); 4.45-4.38 (m, 2H); 4.29-4.21 (m, 1H); 4.21-4.16 (m, 1H); 3.19-3.13 (m, 1H); 2.83-2.70 (m, 5H); 2.48-2.16 (m, 4H); 2.11-2.02 (m, 2H); 1.98-1.87 (m, 1H); 1.84-1.72 8m, 2H); 1.70-1.58 (m, 7H); 1.18 8s, 3H); 1.08 (d, 3H, $J = 7.15$); 0.92 (d, 3H, $J = 7.15$).

Ester R-47

FmocGlu(OtBu)OH (500 mg, 1.18 mmol) was dissolved in DMF (3 mL), 208 µl of DIPEA (1.2 mmol) and 463 mg HBTU (1.22 mmol) were added, then the mixture was stirred at r.t. for 15 min. β-alanine methyl ester hydrochloride was added (140 mg, 1.18 mmol) followed
by 638 µl DIPEA (3.7 mmol) and stirring continued at rt for 22h. Dilution with AcOEt (10 mL) then washing with NH₄Cl (2x30 mL, pH 5.5, back-extracted 2x30 mL AcOEt) and NaHCO₃ sat. aqu. sol.(50 ml, pH 8.5, back-extracted 2x30 mL AcOEt), drying and evaporation afforded 713 mg of yellow oil. FC hex/AcOEt 4:6 afforded 482.1 mg of R-47 as colourless oil; nmr showed the presence of 0.4 eq tetramethylurea (MW 116.2) calcd. yield: 0.86 mmol, 440.6 mg, 73%.

TLC hex/AcOEt 4:6 UV, CPS Rf 0.41

1H-nmr (CDCl₃, 400 MHz): 7.77 (2H, d, J = 7.6 Hz, 2xCH Fmoc); 7.61 (2H, d, J = 7.4 Hz, 2xCH Fmoc); 7.41 (2H, d, J = 7.5 Hz, 2xCH Fmoc); 7.337.77 (2H, ddt, J = 1.2, 2.6, 7.5 Hz, 2xCH Fmoc); 6.35 (1H, b, NHCH₂); 5.59 (1H, bd, J = 7.2 Hz, NH of Glu); 4.40 (2H, d, J = 6.9 Hz, CH₂ of Fmoc); 4.28-4.19 (2H, m, CH α of Glu + CH of Fmoc); 3.69 (3H, s, COOCH₃); 3.61-3.45 (2H, m, NHCH₂); 2.62-2.48 (2H, m, CH₂COOCH₃); 2.29-2.15 (3H, m, CH₂ g of Glu + ½ CH₂ b of Glu); 2.00-1.87 (1H, m, ½ CH₂ β of Glu); 1.48 (9H, s, C(CH₃)₃).

13C-nmr (CDCl₃, 100 MHz): 172.90 (COOCH₃); 172.14 (CONH); 171.04 (CONH); 156.34 (CO of Fmoc); 143.72 (Cq of Fmoc); 141.35 (Cq of Fmoc); 127.72 (2xCH of Fmoc); 127.08 (2xCH of Fmoc); 125.11 (2xCH of Fmoc); 119.99 (2xCH of Fmoc); 82.56 (COOC(CH₃)₃); 67.03 (CH₂ of Fmoc); 53.91 (CH of Fmoc); 51.79 (COOCH₃); 47.22 (CH α of Glu); 35.04 (NHCH₂); 33.77 (CH₂COOCH₃); 32.44 (CH₂γ of Glu); 28.95 (CH₂ β of Glu); 27.99 (C(CH₃)₃).

IR (film): 3309, 3066, 297, 2948, 1721, 1654, 1524, 1475, 1447, 1368, 1323, 1248, 1227, 1152, 1105, 1050 cm⁻¹.

[α]D 20° = -10.2 ° (c = 0.99, MeOH)

ESI-MS: ES+ 532.83 (M+Na) HR-MS (ESI): 511.2444 (calc. 511.2439 for [MH]+ C₂₈H₃₅N₂O₇)

Acid R-48

\[
\text{CO}_2\text{Bu} \quad \text{Fmoc} \quad \text{N} \quad \text{HO}_2
\]

Ba(OH)₂·H₂O (378 mg, 1.2 mmol) was added at r.t. to a solution of R-47 (430 mg, 0.84 mmol) in THF/water 1.1 (50 mL) and stirred for 10 min; the pH was corrected to 3.ca (with HCl 1M) then the mixture was extracted with 6x100 mL AcOEt; drying and evaporation afforded 445 mg of crude colourless oil. FC hex/AcOEt/MeOH/ACOH 3:7:1:0 to 0:9:1:0.1 (two runs) afforded 398 mg of R-48 as colourless oil (95%).

1H-nmr (DMSO, 400 MHz): 8.34-8.26 (1H, m, NH of Phe); 7.96-7.86 (1H, m, NH of β-Ala); 7.89 (2H, d, J = 7.5 Hz, 2xCH Fmoc); 7.81 (1H, t, J = 8.9 Hz, NH of Glu); 7.77-7.71 (2H, m, 2xCH Fmoc); 7.71-7.63 (1H, b, NH of Gly); 7.42 (2H, t, J = 7.2 Hz, 2xCH Fmoc); 7.32 (2H, t, J =
7.5 Hz, 2xCH Fmoc); 7.27-7.20 (4H, m, 2x2xCH of Ph); 7.19-7.12 (1H, m, CH of Ph); 4.52-4.44 (1H, m, CH of Phe); 4.34-4.15 (3H, m, CH₂CH of Fmoc); 3.93-3.83 (1H, m, CH of Glu); 3.58-3.44 (2H, m, CH₂ of Gly); 3.21-3.11 (2H, m, NHCH₂ of β-Ala); 3.75 (1H, dd, J = 14.0, 4.1Hz, ½ CH₂ of Phe); 2.74 (1H, dd, J =14.0, 10.4 Hz, ½ CH₂ of Phe); (4H, m, CH₂CO of β-Ala and CH₂ g of Glu); (1H, m, ½ CH₂ β of Glu); (1H, m, ½ CH₂ β of Glu); (9H, s, C(CH₃)₃).

¹³C-NMR (CDCl₃, 100 MHz): 176.43 (COOH); 172.84 (CONH); 171.34 (COOtBu); 156.68 (CO of Fmoc); 143.81 (Cq of Fmoc); 141.44 (Cq of Fmoc); 127.88 (ar CH of Fmoc); 127.21 (ar CH of Fmoc); 125.26 (ar CH of Fmoc); 120.13 (ar CH of Fmoc); 82.79 (Cq of tBu); 67.27 (Fmoc CH₂); 54.21 (CH α of Glu); 47.28 (Fmoc CH); 35.14 (NHCH₂); 33.99 (CH₂COOH); 32.68 (CH γ of Glu); 28.93 (CH β of Glu); 28.09 (tBu).

[α]D₂⁰ = +7.6° (c = 0.75, MeOH)

IR (film): ν 3730, 3705, 3627, 3331, 2924, 2853, 1711, 1649, 1535, 1449, 1368, 1338, 1229, 1153, 1104, 1051 cm⁻¹.

HR-MS (ESI): 497.2281 [C₂₂H₃₃N₂O₇]⁺ calcd. 497.2282.

R-49

120 µl of DIPEA (0.7 mmol) and 91 mg HATU (0.24 mmol) were added at rt to a solution of R-48 (100 mg, 0.2 mmol) in 0.8 ml DMF and stirred for 15'. PheOBn (64 mg, 0.22 mmol) was added and the mixture was stirred at rt for 18 h. Dilution with 10 ml AcOEt, washing with NH₄Cl sat (30 mL, pH 6, back-extracted 2x30 mL AcOEt), NaHCO₃ sat (60 mL, pH 9, back-extracted with 2x50 ml AcOEt), drying and evaporation afforded 192 mg orange solid. FC hex/AcOEt/MeOH 8:2:0 to 6:4:0 to 4:6:0 to 0:1:0 to 0:10:1 afforded 130.8 mg of product. Recrystallization from MeOH/DCM 1:1 (precipitation induced with hexane) afforded, after removal of the surnatant, quick washing with MeOH twice and HV drying, 77.1 mg of white solid R-49. (NMR: 98% purity).Crystallization upon standing of the mother liquor 3d at 4°C afforded further 43.1 mg of product (120 mg, 0.16 mmol, 80%).

TLC hexane/AcOEt 1:1, UV; CPS, Rf 0.27

¹H-NMR (400 MHz, DMSO): 8.42 (1H, d, J = 8.0 Hz, NH of Phe); 7.89 (2H, d, J = 8.0 Hz, 2xCH Fmoc); 7.78 (1H, t, J = 6.0 Hz, NH of amide δ of Glu); 7.72 (2H, d, J = 8.0 Hz, 2xCH Fmoc); 7.66 (1H, d, J = 4.0 Hz, NH of Glu); 7.42 (2H, t, J = 6Hz, 2xCH Fmoc); 7.39-7.28 (5H, m); 7.27-7.24 (4H, m); 7.23-7.19 (3H, m); 5.11-5.04 (2H, m, OCH₂Ph); 4.52 (1H, q, J = 8.0 Hz, CH of Phe); 4.34-4.20 83H, m, CH₂CH of Fmoc); 3.91-3.85 (1H, m, CH α of Glu); 3.20-3.14 (2H, m,
NHCH$_2$); 3.06-2.90 (2H, m, CHCH$_2$Ph); 2.30-2.22 (2H, m, NHCH$_2$CH$_2$CO); 2.15-2.09 (2H, m, CH$_2$ $\gamma$ of Glu); 1.97-1.71 (2H, m, CH$_2$ $\beta$ of Glu); 1.39 (9H, s, C(CH$_3$)$_3$).

$^{13}$C-NMR (100 MHz, DMSO): 171.45 (COOCH$_2$Ph); 171.34 (COOtBu); 171.14 (CO d of Glu); 170.51 (CONHPhe); 156.04 (CO of Fmoc); 143.76 (2xCq of Fmoc); 140.69 (2xCq of Fmoc); 137.09 (Cq Ar of Phe); 135.74 (OCH$_2$Cq ar); 129.01 (2x CH ar of Phe); 128.34 (2xCH ar); 128.20 (2x CH ar); 127.97 (CH ar); 127.75 (2x CH ar Bn); 127.61 (2x CH Fmoc); 127.03 (2x CH ar); 126.49 (CH ar); 125.21 (2xCH Fmoc); 120.08 (2xCH Fmoc); 80.50 (Cq of tBu), 65.91 (OCH$_2$Ph); 65.61 (CH$_2$ of Fmoc); 54.16 (CH of Glu); 53.64 (CH of Phe); 46.63 (CH of Fmoc); 36.66 (CH$_2$Ph of Phe); 35.09 (NHCH$_2$CH$_2$CO); 34.99 (NHCH$_2$CH$_2$CO); 31.59 (CH$_2$ $\gamma$ of Glu); 27.61 ((CH$_3$)$_3$ of tBu); 26.69 (CH$_2$ $\beta$ of Glu).

$[\alpha]_D^{20} = -5.3^\circ$ (c = 0.376, MeOH)

IR (film): δ 3730, 3705, 3627, 3598, 3304, 2975, 2923, 2852, 1731, 1692, 1646, 1545, 1451, 1369, 1343, 1281, 1245, 1219, 1157, 1026, 993 cm$^{-1}$.

ESI-MS m/z 733.52 (M$^+$), 756.33 (MNa$^+$); HR-MS (ESI): 756.3216 [C$_{43}$H$_{47}$N$_3$NaO$_8$]$^+$ calcd 756.3255.

R-50

![Chemical Structure of R-50](image)

To a suspension of R-49 (110 mg, 0.15 mmol) in MeOH (8 mL) was added Pd/C 10% (10 mg, 9 mmol) under Ar atmosphere, then the mixture was put under hydrogen atmosphere at atmospheric pressure and stirred vigorously for 2h at rt. Filtration through a celite pad and evaporation afforded 91.4 mg of yellow solid. FC AcOEt/MeOH/AcOH 10:1:0.1 (two runs) afforded 68.4 mg of R-50 (0.11 mmol, 71%).

$^1$H-nmr (CDCl$_3$, 400 MHz): 7.75 (2H, d, J = 8.0 Hz, 2xCH Fmoc); 7.60 (2H, d, J = 4.0 Hz, 2xCH Fmoc); 7.39 (2H, t, J = 6.0 Hz, 2xCH Fmoc); 7.38-7.26 (2H, m, 2xCH Fmoc); 7.26-7.14 (5H, m, CH ar of Phe); 6.90-6.68 (2H, b, NH of Phe and NHCH$_2$CH$_2$CO); 5.89 (1H, d, J = 8.0 Hz, NH of Glu); 4.80-4.60 (1H, m, CH $\alpha$ of Phe); 4.50-4.25 (2H, m, CH$_2$ of Fmoc); 4.25-4.15 (2H, m, CH of Fmoc and CH $\alpha$ of Glu); 3.60-3.42 (1H, m, 1/2 of NHCH$_2$); 3.40-3.30 (1H, m, 1/2 of NHCH$_2$); 3.25-3.18 (1H, m, 1/2 of PhCH$_2$); 3.06-2.95 (1H, m, 1/2 of PhCH$_2$); 2.45-2.26 (2H, m, NHCH$_2$CH$_2$CO); 2.22-2.05 (3H, m, CH$_2$ $\gamma$ of Glu and 1/2 of CH$_2$ $\beta$ of Glu; 1.98-1.86 (1H, m, 1/2 of CH$_2$ $\beta$ of Glu); 1.46 (9H, s, t-Bu).

$^{13}$C-nmr (CDCl$_3$, 100 MHz): 175.62 (CO); 173.37 (CO); 172.71 (CO); 171.65 (CO); 156.65 (CO of Fmoc); 144.04 (Cq of Fmoc); 141.56 (Cq of Fmoc); 136.73 (Cq of Phe); 129.49 (2xCH of Phe); 128.83 (2xCH of Phe); 128.03 (2xCH of Fmoc); 127.40 (2xCH of Fmoc); 127.26 (CH of Phe); 125.48 (2xCH of Fmoc); 120.27 (2xCH of Fmoc); 82.82 (C(CH$_3$)$_3$); 67.42 (CH$_2$ of
Fmoc); 54.50 (CH α of Glu); 54.26 (CH α of Phe); 47.40 (CH of Fmoc); 37.42 (PhCH₂); 36.07
(CH₂CO); 35.94 (CH₂NH); 32.62 (CH₂ γ of Glu); 28.55 (CH₂ β of Glu); 28.26 (C(CH₃)₃).

[α]D²⁰ = +5.1° (MeOH, c = 1.27)

FT-IR (film): 3308, 3062, 3033, 2977, 2935, 1718, 1651, 1531, 1450, 1368, 1228, 1154, 1050, 1027, 1005 cm⁻¹.

HR-MS (ESI): 688.2611 [C₃₅H₄₀N₃Na₂O₈, M-HNa₂⁺] calcd.688.2605

R-51

DIPEA (42 µL, 0.25 mmol) and HATU (47 mg, 0.124 mmol) were added to a solution of R-50
(40 mg, 0.06 mmol) in 1 ml DMF and the bright yellow solution was stirred for 10 min.
Glycine methyl ester hydrochloride (9 mg, 0.07 mmol) was added and the mixture and
further stirred at rt for 2h. Dilution with 20 mL AcOEt and washing with NH₄Cl sat (30 ml,
acidified to pH 2 with HCl, back-extracted 2x30 ml and 2x50 ml ), drying and evaporation
afforded 102 mg of yellow oil. FC hex/AcOEt/MeOH 3:7:1 to 0:1:0 to 0:10:1 afforded 42 mg
of R-51 (after lyophilisation; 0.059 mmol, 95%).

TLC: AcOEt, UV, CPS, Rf 0.22

¹H-nmr (DMSO, 400 MHz): 8.43 (1H, t, J = 5.8 Hz, NH of Gly); 8.17 (1H, d, J = 8.5 Hz, NH of
Phe); 7.89 (2H, d, J = 7.6 Hz, 2XCH Fmoc 4'); 7.72 (2H, d, J = 7.0 Hz, 2XCH Fmoc 1'); 7.77-7.69
(1H, m, NH of β-Ala); 7.67 (1H, d, J = 8.0 Hz, NH of Glu); 7.42 (2H, t, J = 7.5 Hz, 2XCH Fmoc
3'); 7.33 (2H, t, J = 7.5 Hz, 2XCH Fmoc 2'); 7.26-7.22 (4H, m, 2x2CH ar of Phe); 7.21-7.13 (1H,
m, CH ar of Phe); 4.58-4.50 (1H, m, CH of Phe); 4.35-4.18 (3H, m, CH₂-CH of Fmoc); 3.92-
3.79 (3H, m, CH₂ of Gly + CH a of Glu); 3.63 (3H, m, COOCH₃); 3.12 (2H, m, NHCH₂ of β-Ala);
3.04 (1H, dd, J = 13.9, 4.3 Hz, ½ CH₂ of Phe); 2.73 (1H, dd, J = 13.8, 10.3 Hz, ½ CH₂ of Phe);
2.29-2.14 (2H, m, CH₂CO of β-Ala); 2.14-2.07 (2H, m, CH₂ γ of Glu); 1.96-1.85 (1H, m, ½ CH₂
β of Glu); 1.80-1.68 (1H, m, ½ CH₂ β of Glu); 1.39 (9H, s, C(CH₃)₃).

¹³C-nmr (DMSO, 100 MHz): 171.87 (COOttBu); 171.38 (CO of Phe); 171.11 (γ CO of Glu);
173.26 (CO of β-Ala); 170.19 (COOCH₃);156.07 (CO of carbamate); 143.79 (Cq of Fmoc);
140.71 (Cq of Fmoc); 138.01 (Cq of Ph); 129.09 (2xCH of Ph); 127.99 (2xCH of Ph); 127.64
(2xCH of Fmoc); 127.06 (2xCH of Fmoc); 126.19 (4-CH of Ph); 125.25 (2xCH of Fmoc);
120.12 (2xCH of Fmoc); 80.52 (Cq of tBu); 65.65 (CH₂ of Fmoc); 54.16 8CH of Glu); 53.69
(CH of Phe); 51.70 (COOCH₃); 46.64 (CH of Fmoc); 40.61 (CH₂ of Gly); 37.46 (CH₂Ph); 35.26
(NHCH₂CH₂CO); 35.14 (NHCH₂CH₂CO); 31.57 (CH₂ γ of Glu); 27.63 (tBu); 26.66 (CH₂ β of
Glu).

[α]D²⁰ = -5.4° (c=0.98, MeOH)
IR (film): v 3422, 3316, 2955, 2924, 2853, 1733, 1659, 1535, 1497, 1451, 1370, 1228, 1187, 1156, 1080, 1052, 1028, 1007, 971 cm⁻¹.
HR-MS (ESI): 715.3326 [MH⁺] calcd. 715.3338;

**R-46**

![R-46](image)

Ba(OH)₂·H₂O (22 mg, 0.07 mmol) was added at r.t. to a solution of **R-51** (36 mg, 0.05 mmol) in THF/water 1:1 (5 mL) and stirred for 18 min. The pH was corrected to 3.0 (0.1 mL HCl 1M) then the mixture was extracted with 3x20 mL AcOEt; drying and evaporation afforded 29.6 mg of crude colourless oil. FC hex/AcOEt/MeOH/ACOH 3:7:1:0 to 0:9:1:0.1 afforded 27.6 mg of **R-46** as colourless oil (0.038 mmol, 76%).

¹H-nmr (DMSO, 400 MHz): 8.34-8.26 (1H, m, NH of Phe); 7.96-7.86 (1H, m, NH of β-Ala); 7.89 (2H, d, J = 7.5 Hz, 2xCH Fmoc); 7.81 (1H, t, J = 8.9 Hz, NH of Glu); 7.77-7.71 (2H, m, 2xCH Fmoc); 7.71-7.63 (1H, b, NH of Gly); 7.42 (2H, t, J = 7.2 Hz, 2xCH Fmoc); 7.32 (2H, t, J = 7.5 Hz, 2xCH Fmoc); 7.27-7.20 (4H, m, 2x2xCH of Ph); 7.19-7.12 (1H, m, CH of Ph); 4.52-4.44 (1H, m, CH of Phe); 4.34-4.15 (3H, m, CH₂CH of Fmoc); 3.93-3.83 (1H, m, CH of Glu); 3.58-3.44 (2H, m, CH₂ of Gly); 3.21-3.11 (2H, m, NHCH₂ of β-Ala); 3.75 (1H, dd, J = 14.0, 4.1Hz, ½ CH₂ of Phe); 2.74 (1H, dd, J =14.0, 10.4 Hz, ½ CH₂ of Phe); (4H, m, CH₂CO of β-Ala and CH₂γ of Glu); (1H, m, ½ CH₂ β of Glu); (1H, m, ½ CH₂ β of Glu); (9H, s, C(CH₃)₃).

¹³C-nmr (DMSO, 100 MHz): 173.10, 171.40, 171.23, 170.52, 170.43, 156.11, 143.80, 140.72, 128.29, 129.08, 128.00, 127.66, 127.08, 126.16, 125.28, 120.13, 80.54, 65.66, 54.22, 54.11, 46.64, 43.42, 37.33, 35.30, 35.30, 31.59, 27.65.

[α]D²⁰ = -2.3° (c = 0.75, MeOH)

IR (film): ν 3418, 3270, 2926, 2854, 1720, 1654, 1605, 1540, 1449, 1398, 1370, 1250, 1155, 1054, 1027 cm⁻¹.

HR-MS (ESI): 723.0001 [MH⁺] calcd. 723.2999
HATU (14 mg, 0.036 mmol) and DIPEA (13 µL, 0.075 mmol) were added to a solution of R-46 (25 mg, 0.036 mmol) in 1 ml DMF; after stirring 5 min at r.t. A-30 (23 mg, 0.030 mmol) was added as solution in 0.5 ml DMF. The mixture was stirred at rt for 18h. Dilution with AcOEt (20 mL) then washing with 15 ml NaHCO3 sat (pH 9, back-extraction 2x10 ml AcOEt) afforded 79.6 mg yellow oil. FC DCM/MeOH 10:1 afforded 28 mg of R-52 (0.019 mmol, 65%).

\[^1\text{H-} \text{nmr (DMSO, 400 MHz)}: 8.24 (t, 1H, J = 5.6 Hz, NH); 8.19 (d, 1H, J = 8.2 Hz, NH); 8.06-7.95 (m, 2H, 2xNH); 7.88 (d, 2H, J = 8.0 Hz, 2xCH Fmoc); 7.77-7.64 (m, 3H, 2xCH Fmoc, NH); 7.55 (s, 1H, CH ar); 7.46-7.37 (m, 3H, 2xCH Fmoc, CH ar); 7.34-7.28 (m, 2H, 2xCH Fmoc); 7.27-7.20 (m, 5H, CH ar); 7.20-7.12 (m, 1H, CH ar); 5.48 (d, 1H, J = 9.7 Hz, CH C15); 5.23 (t, 1H, J = 6.8 Hz, CH C13); 4.53-4.44 (m, 1H, CH of Phe); 4.35-4.14 (m, 5H, OCH\textsubscript{2}CH Fmoc, CH\textsubscript{2}); 3.92-3.79 (m, 2H, CH C3, C7); 3.71-3.54 (m, 2H, Ch2 Gly); 3.45-3.34 (m, 2H); 3.21-2.99 (m, 5H, CH C6, CH\textsubscript{2} C2, \(\frac{1}{2}\) CH\textsubscript{2} Phe, CH Glu); 2.92-2.70 (m, 3H); 2.66-2.53 (m, 3H); 2.49 (s, 3H); 2.29-2.00 (m, 6H); 1.78-1.68 (m, 2H, CH C8, \(\frac{1}{2}\) CH2); 1.65 (s, 3H); 1.38 (s, 9H, tBu); 1.14 (s, 3H, CH3); 1.05-0.98 (m, 6H, 2xCH3); 0.98-0.88 (m, 12H, CH3, TBS); 0.82 (s, 9H, TBS); 0.13- -0.15 (12H, m, TBS).

\([\alpha]_{D}^{20}=-21.2^\circ \text{(c= 0.45 CHCl\textsubscript{3})}\)

ESI-MS 1438.71
Dimethylamine (12% w/w in DMF, 0.1 ml, 0.37 mmol) was added at 0°C to a solution of R-52 (19 mg, 0.013 mmol) in DMF (0.1 mL); the mixture was stirred at 0°C for 2h; evaporation of the solvent and FC Chloroform/MeOH/water/AcOH 85:13:15:0.5 afforded 8 mg of R-53 (0.007 mmol, 51%).

$^1$H-nmr (DMSO, 400 MHz): 8.27 (t, 1H, J = 6.0 Hz, NH); 8.23 (d, 1H, J = 7.3 Hz, NH); 8.02 (t, 1H, J = 5.7 Hz, NH); 7.73 (t, 1H, J = 5.7 Hz, NH); 7.55 (1H, s, ar CH); 7.28-7.13 (m, 7H, ar CH); 5.48 (d, 1H, J = 10.7 Hz, CH C15); 5.23 (t, 1H, J = 8.2 Hz, CH C13); 4.53-4.43 (m, 1H, CH of Phe); 4.25-4.18 (m, 2H, CH$_2$); 3.88-3.80 (m, 2H, CH C3, CH C7); 3.70-3.55 (m, 2H, CH$_2$ of Gly); 3.47-3.35 (m, 2H, CH$_2$); 3.20-3.08 (m, 4H, CH C6, CH2NH Ala, CH Glu); 3.08-2.71 (m, 2H, CH$_2$ Phe); 2.81-2.71 (m, 1H, 1/2 CH$_2$ C14); 2.66-2.55 (m, 2H, C2); 2.55-2.48 (m, 1H, ½ CH$_2$ overlapping DMSO); 2.41 (s, 3H, CH$_3$); 2.28-2.14 (m, 2H, CH$_2$CO); 2.13-2.02 (m, 3H, CH$_2$ + ½ CH$_2$); 1.81-1.68 (m, 3H, 3x1/2 CH$_2$); 1.65 (s, 3H, CH$_3$); 1.59-1.49 (m, 2H, ½ CH$_2$, CH C8); 1.42-1.36 (m, 1H, ½ CH$_2$); 1.40 (s, 9H, COOtBu); 1.14 (s, 3H, gem-CH$_3$); 1.03 (s, 3H, gem-CH$_3$); 1.01 (d, 3H, J = 7.0 Hz, CHCH$_3$); 0.96-0.90 (m, 12H, CHCH$_3$, TBS); 0.80 (s, 9H, TBS); 0.12-0.16 (m, 12H, TBS).

HRMS (ESI): C$_{65}$H$_{106}$N$_7$O$_{11}$Si$_2$ found 1216.7486 [MH$^+$] calcd. 1216.7483.

R-54
$^1$H-nmr (DMSO, 400 MHz): 8.76 (d, 1H, J = 8.4 Hz, NH); 8.55 (s, 1H); 8.35-8.26 (m, 1H, NH); 8.22 (d, 1H, J = 7.6 Hz, NH); 8.06-7.98 (m, 1H, NH); 7.88 (d, 2H, J = 9.3 Hz, CH ar); 7.81-7.72 (m, 1H, NH); 7.59 (d, 2H, J = 8.6 Hz, CH ar); 7.55 (s, 1H, CH ar); 7.43 (d, 1H, J = 10.1 Hz, CH ar); 7.28-7.19 (m, 5H, ar); 7.18-7.11 (m, 1H, CH ar); 5.48 (d, 1H, J = 9.7 Hz, CH C15); 5.23 (t, 1H, J = 7.9 Hz, CH C13); 5.09 (s, 2H, CH$_2$); 4.54-4.42 (m, 1H, CH of Phe); 4.28-4.15 (m, 3H, CH of Glu, CH$_2$); 3.88-3.79 (m, 2H, CH C3, C7); 3.70-3.55 (m, 2H, CH$_2$ Gly); 3.46-3.36 (m, 2H, CH$_2$); 3.21-2.99 (m, 5H, CH C6, CH$_2$, 2x1/2 CH$_2$); 2.86-2.70 (m, 1H, 1/2CH$_2$); 2.67-2.53 (m, 2H, 2x1/2CH$_2$); 2.48 (3H, s, CH$_3$); 2.25-2.11 (m, 4H, 2xCH$_2$); 2.11-2.00 (m, 2H, 2x1/2CH$_2$); 1.94-1.84 (m, 1H, 1/2CH$_2$); 1.77-1.68 (m, 2H, 2x1/2CH$_2$); 1.68-1.58 (m, 3H, 3x1/2CH$_2$); 1.65 (s, 3H, CH$_3$); 1.58-1.50 (2H, m, ½ CH$_2$, CH C8); 1.43 (s, 9H, tBu); 1.14 (s, 3H, gemCH$_3$); 0.94-0.98 (m, 6H, gemCH$_3$, CHCH$_3$); 0.96-0.93 (m, 3H, CH$_3$); 0.92 (s, 9H, TBS); 0.80 (s, 9H, TBS); 0.12 - 0.15 (m, 12H, TBS).

$[\alpha]_D^{20}$ = + 5.1 (C=0.92, CHCl$_3$)

HRMS (MALDI): C$_{81}$H$_{114}$F$_3$N$_{14}$O$_{14}$Si$_2$ found 1607.8188 [M$^+$] calcd. 1607.8199

**R-55**

![R-55](attachment:image.png)

K$_2$CO$_3$ (10 mg, 0.07 mmol) was added at r.t. to a solution of **R-84** (4.7 mg, 0.003 mmol) in THF/water (mL) and stirred for 1h; Dilution with DCM (10 ml) and water (5ml), separation (pH 8), back-extraction (2x 10ml), drying and evaporation of the organic layers afforded 4 mg of yellow oil. No further purification was performed.
100 µl TFA (mmol) were added at 0°C to a solution of **R-85** (mg, mmol) in 0.8 ml DCM; the mixture was stirred at 0°C for 2.5h, then the solvent was evaporated; 40 mg of yellow oil were obtained. ESI-MS m/z; 1226.86; purification pending.
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