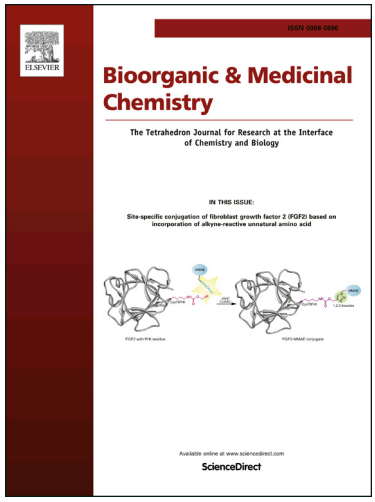

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Development of a cyclosporin A derivative with excellent anti-hepatitis C virus potency

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Abstract:

Synthetic modification of cyclosporin A at P3-P4 positions led to the discovery of NIM258, a next generation cyclophilin inhibitor with excellent anti-hepatitis C virus potency, with decreased transporter inhibition, and pharmacokinetics suitable for coadministration with other drugs. Herein is disclosed the evolution of the synthetic strategy to from the original medicinal chemistry route, designed for late diversification, to a convergent and robust

development synthesis. The chiral centers in the P4 fragment were constructed by an asymmetric chelated Claisen rearrangement in the presence of quinidine as the chiral ligand. Identification of advanced crystalline intermediates enabled practical supply of key intermediates. Finally, macrocyclization was carried out at 10% weight concentration by a general and unconventional "slow release" concept.

Keywords:

Cyclosporin A derivative, Hepatitis C Virus, Cyclophilin inhibitor

Introduction and background

Cyclosporin A (CsA) was initially discovered at Sandoz while searching for novel antifungal agents.[1a] It was later found to have immunosuppression properties[1b] and its introduction revolutionized the field of organ transplantation.[1c] Cyclosporin has since found additional applications in the treatment of psoriasis, rheumatoid arthritis, and uveitis.[2] The immunosuppressive effects of cyclosporin can be attributed to the formation of a ternary complex between the compound, cyclophilin A, and the two subunits of the phosphatase calcineurin. Formation of this complex decreases the production of inflammatory cytokines by T-lymphocytes.[3]

Cyclosporin can be synthetically modified to decrease its immunosuppressive properties while retaining cyclophilin binding.[4] Such non-immunosuppressive cyclophilin inhibitors have been explored for the treatment other diseases.[5] In particular, alisporivir, NIM811, and SCY-635 (Figure 1) have demonstrated clinical efficacy in patients infected with hepatitis C virus (HCV).[6] Alisporivir was the first oral non-immunosuppressive cyclophilin inhibitor to enter clinical trials, where administration alone or in combination with pegylated-interferon α resulted in a

significant decrease in viral RNA.[7] As host-targeted agents, cyclophilin inhibitors maintain activity against all major HCV genotypic variants, as well as exhibit a much higher barrier to resistance development than most direct-acting antiviral agents.[8] In a subset of patients, however, alisporivir was associated with an increase in circulating levels of conjugated and unconjugated bilirubin.[7] This hyperbilirubinemia was attributed to inhibition of transporters.[9] We therefore undertook a program to address the transporter liability associated with alisporivir.

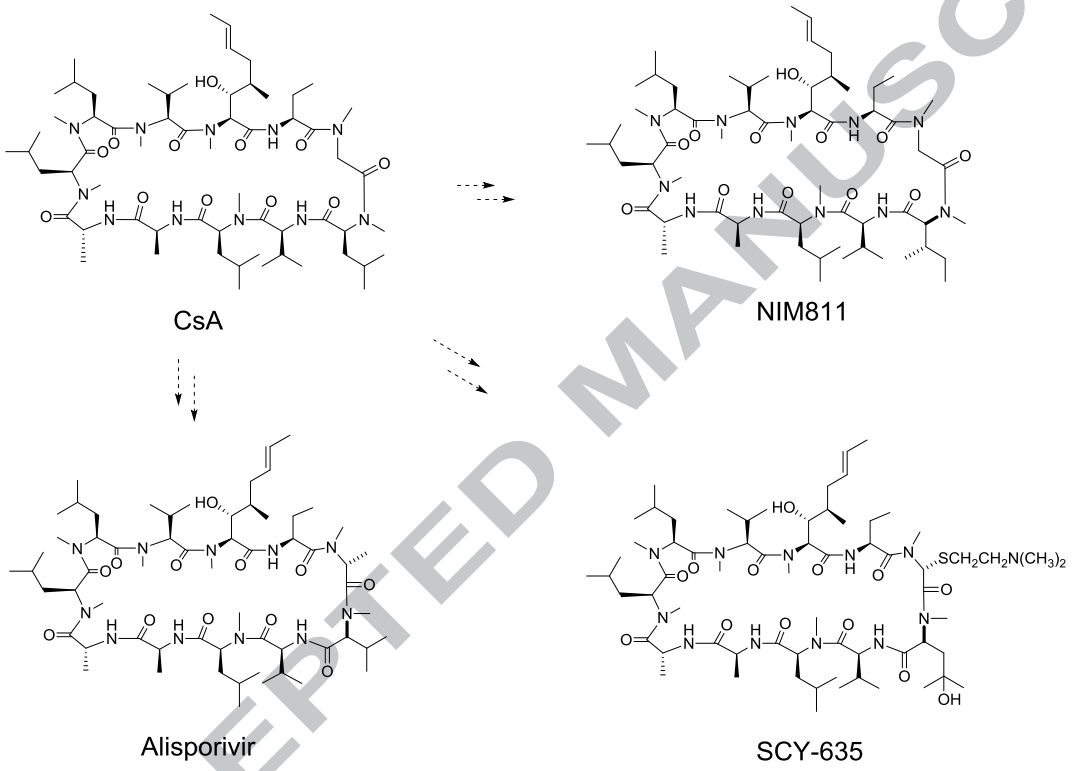


Figure 1. Cyclosporin related active pharmaceutical ingredients (API): cyclosporin A (CsA), NIM811, alisporivir, and SCY-635.

Medicinal Chemistry background

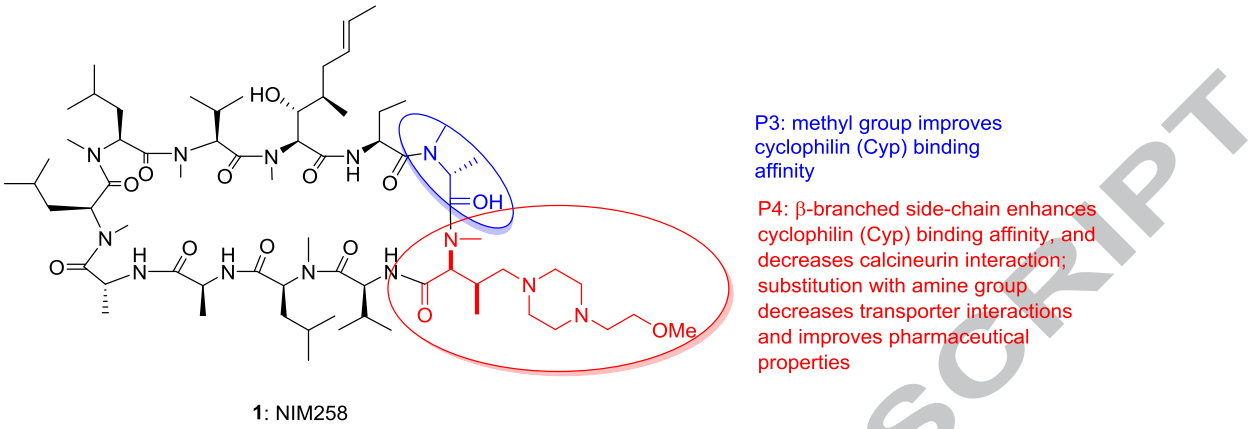


Figure 2. Synthetic modifications on CsA led to NIM258.

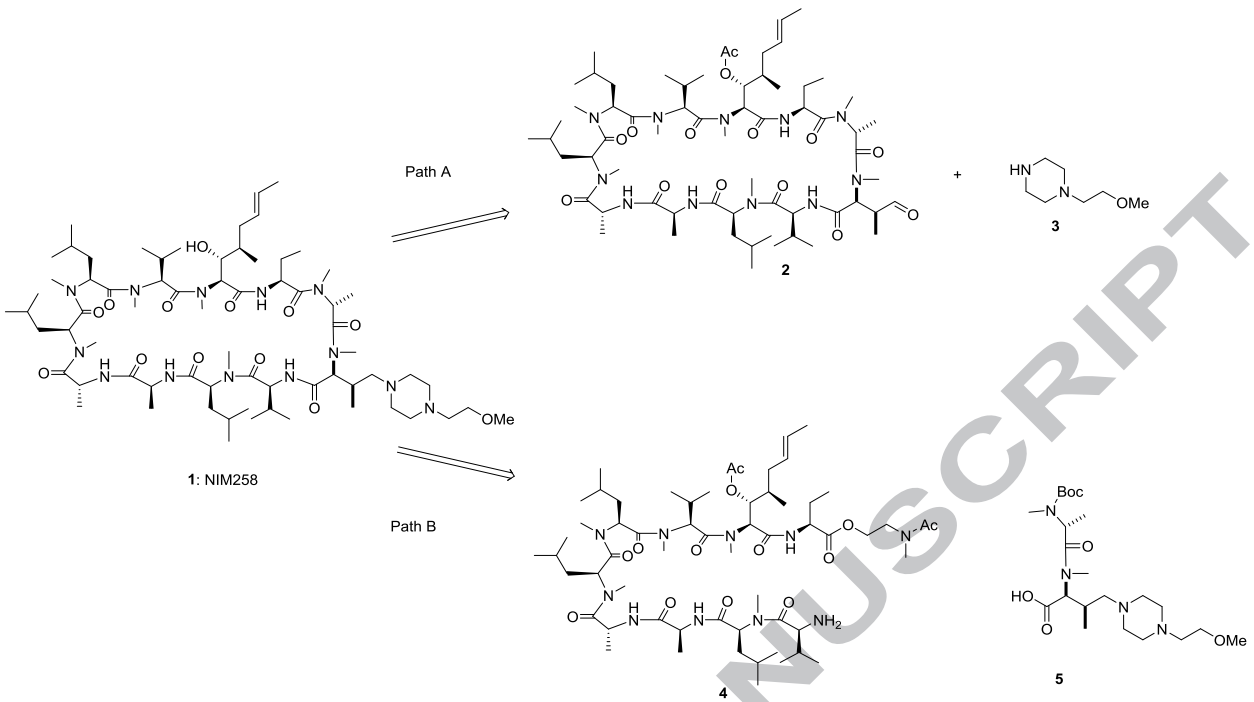
Since its discovery, cyclosporin A has been subjected to extensive synthetic modifications.[10] Our medicinal chemistry approach was therefore defined by existing structure-activity relationships and largely focused on P3-P4 modification (Figure 2).[11] An extensive survey of Novartis cyclosporin analogs containing P3 groups has revealed that small, non-branched groups such as CH_3 or CH_2OH increase cyclophilin binding affinity. At the P4 position, branching enhances potency as well as decreases calcineurin interaction.[12] Furthermore, polar groups could reduce MDR1 inhibition by cyclosporin,[13] while decreasing lipophilicity can improve pharmaceutical properties, such as solubility and plasma protein binding.

In an effort to maintain antiviral potency comparable to alisporivir, while decreasing transporter activities, we focused on analogs with D-alanine at the P3 position and a branched P4 with appended polar groups. Our initial survey of polar groups included hydroxy, alkoxy, cyano, sulfone, and amines. While most analogs retained cyclophilin binding affinity and cellular

potency, only the amine analogs achieved a substantial reduction in OATP1B1 inhibition. Further optimization on the amine moiety led to the discovery of NIM258, a next generation cyclophilin inhibitor with excellent anti-HCV potency, decreased transporter inhibition, and pharmacokinetics suitable for coadministration with other drugs.

Retrosynthetic analysis

NIM258 is derived from the fermentation product cyclosporin A *via* hemi-synthesis.[11] Two strategies were utilized during the development of the compound. The first one relied on late introduction of the side-chain **3** at late-stage stage through reductive amination with aldehyde **2**, a strategy well amenable to medicinal chemistry derivatization (path A). The second strategy emerged once the lead compound had been selected and relied on the convergent construction of the dipeptide **5**, that was directly coupled with nonapeptide **4** and then cyclized to provide NIM258 (**1**) (path B). Both strategies were enabled by the practical access to advanced crystalline intermediates identified in an earlier efforts.[14] The dipeptide fragment **5** synthesis also turned to be a major endeavor, as will be discussed in more details in the next section.



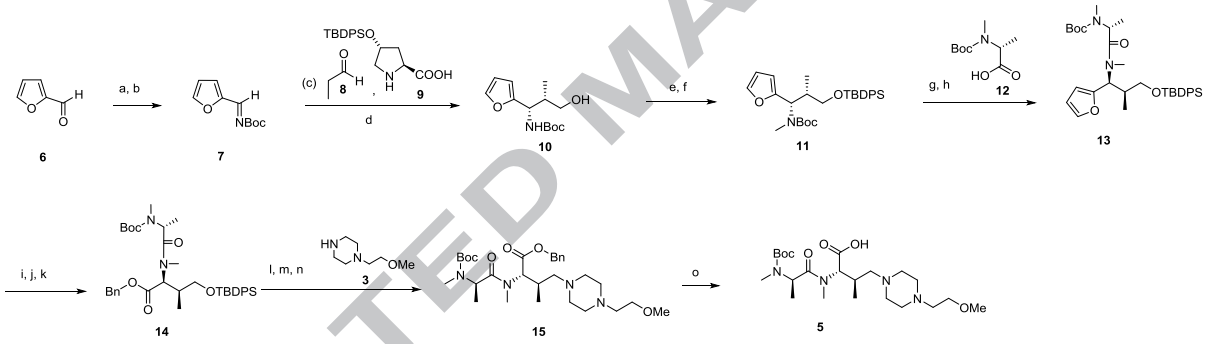
Scheme 1. Original and final retrosynthetic analysis

Synthesis of the dipeptide fragment

In the initial synthesis of dipeptide **5**, the chiral centers in P4 were introduced in an asymmetric organocatalytic propionaldehyde **8** *syn*-addition to an activated imine **7** mediated by (2*S*,4*R*)-4-(tert-butyldiphenylsilyloxy)-pyrrolidine-2-carboxylic acid **9** to give aldehyde **10** with modest diastereoselectivity (70-90% d.r.) and good enantioselectivy (>95% e.e. for the major diastereoisomer).[15] Reduction of aldehyde resulted in alcohol **10**, which was obtained in isomerically pure form after crystallization. Subsequent silylation of alcohol and N-methylation provided protected amine **11**. Removal of Boc group, coupling with Boc-protected D-alanine provided **13** in 90% yield over two steps. The furan was then oxidatively cleaved to result first in the alpha-keto acid, subsequently cleaved to the desired acid, later masked as the benzyl ester

14. Desilylation of the TBDPS-protected precursor **14**, followed successively by oxidation, reductive amination afforded **15**. Benzyl ester was then removed under hydrogenation condition to give key dipeptide **5** later used in our final assembly.

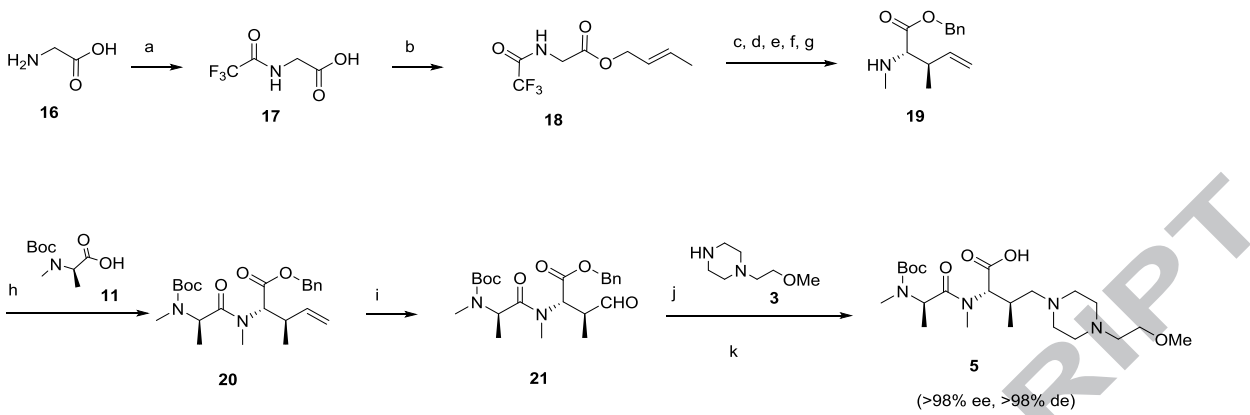
This long linear sequence was sufficiently expedient and productive for small quantities of dipeptide, but scale-up issues were soon encountered and especially envisioned for larger scale. For example, formation of the activated imine was extremely slow as was the organocatalytic propionaldehyde addition. The selectivity was at best moderate. Cleavage of the furan was particularly undesired from a health, safety and environment standpoint. Most intermediates were oils, which led to challenging purifications. This resulted in an overall low yield (<1.5%).



Conditions: a) NH_2Boc, $PhSO_2Na$, HCOOH, MeOH, H_2O; b) K_2CO_3, Na_2SO_4, THF, 50 % over 2 steps; c) propionaldehyde, (2*S*,4*R*)-4-((tert-butyldiphenylsilyl)oxy)pyrrolidine-2-carboxylic acid, MeCN, 70-90% dr, >95% ee of major diastereomer; d) $NaBH_4$, MeOH; e) TBDPSCl, imidazole, DMF, 40% yield over 3 steps; f) NaH, MeI, THF, DMF, 80% 2 steps; g) HCl, dioxane; h) **12**, HATU, DIPEA, 90% yield over 2 steps; i) $RuCl_3$, $NaIO_4$, heptane, EtOAc, water; j) H_2O_2, $NaHCO_3$; k) BnOH, EtOAc, water, 87% crude yield over 2 steps; l) TBAF, THF; m) $Py{\cdot}SO_3$, DIPEA, DMSO; n) **3**, $NaBH(OAc)_3$, AcOH, DCE, 42% yield over 3 steps; o) H_2, $Pd(OH)_2$, 71 % yield.

Scheme 2. Original route to the dipeptide **5**.

We therefore envisioned a *de novo* synthesis based on more readily scalable chemistry, and on high control of the dia and enantio-selectivity.[16] The sequence started with glycine **16**. The terminal amine was masked as the trifluoroacetamide **17**, and the crotyl ester **18** was prepared. An asymmetric chelated Claisen rearrangement in the presence of chiral ligands (quinidine) was then performed. Although a significant cost-driver, quinidine could easily be recovered and proved to be the best and most expedient ligand for optimal selectivity, providing in a straight manner good enantiomeric and diastereoisomeric excess (94.2% ee, 88.4% dr). Chiral upgrade was achieved *via* recrystallization with (*R*)-(+)-α-methyl benzyl amine and the α-amino ester **19** was finally obtained with >98% ee, >96% de and >98% chemical purity after the rearrangement and benzylation and *N*-methylation. The second amino acid **11** was introduced using propyl phosphonic anhydride (T_3P) as the coupling reagent, to allow for minimal amount of epimerization. A vinyl moiety was used as an alternative to furan and masked aldehyde. The advantage was the more convenient unmasking *via* a simple ozonolysis, conducted in the presence of excess propionaldehyde to prevent over-oxidation. While purification of product **5** was previously carried out by column chromatography, this could now be avoided after the introduction of the desired piperazine and direct crystallization of the resulting intermediate. Other methods of introduction of the piperazine *via* nucleophilic substitution strategies were evaluated but all led to lower yield mostly due to β-elimination, or epimerization. Overall, a more efficient and asymmetric synthesis of the fully functionalized dipeptide in 10 steps had been demonstrated.



Conditions: (a) TFA, Tf_2O, 81% yield; (b) oxalyl chloride, DMF, DCM; crotyl alcohol, 68%; (c) 5.5 equiv. LiHMDS, 1.1 equiv $Al(OiPr)_3$, 2.5 equiv quinidine, THF, then recrystallization with (*R*)-(+)-α-methyl benzyl amine, 23% overall; (d) BnBr, K_2CO_3, DMF, 92%; (e) MeI, K_2CO_3, DMF, 95%; (f) $NaBH_4$, MeOH, 70%; (h) **11**, T_3P,Et_3N, 78%; (i) ozone, DCM; (j) **3**, $NaBH(OAc)_3$, AcOH, 40% yield 2 steps; (k) H_2, $Pd(OH)_2$, 71%, >98% ee, >98% de.

Scheme 3. Revised route to the dipeptide **5**.

This chromatography-free revised strategy resulted in a *ca*. 2% overall yield, high optical purity, and enabled rapid and reliable access to the targeted dipeptide and proved suitable for early stages of development, including the first multi-kg supply.

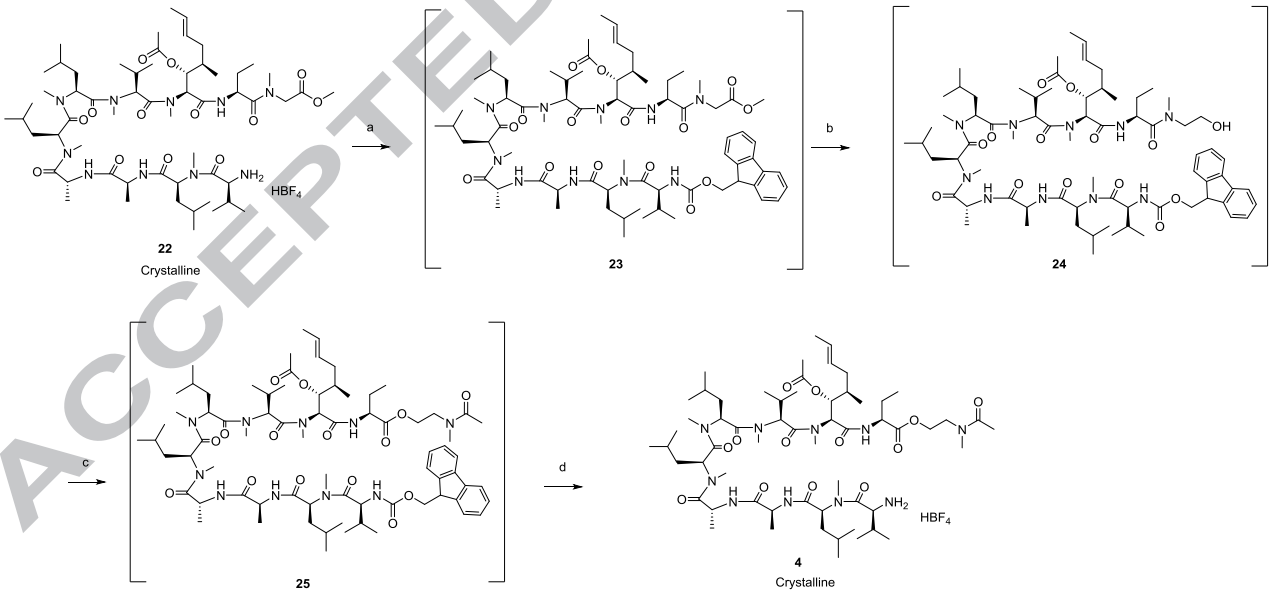
Preparation of key crystalline intermediate

Earlier efforts on similar scaffolds had showed the importance of crystalline intermediates, and more particularly or tetrafluoroborate salt of complex acyclic polypeptides.[14] Such compounds not only facilitate the rapid access to advanced intermediates, but also enabled

efficient scale-up for supply in development. We again endeavored at finding such crystalline compounds.

Tetrafluoroborate salt of basic amine acyclic polypeptide **22** had resulted in crystalline products in a related synthesis, and we had capitalized on this to generate our starting point for this project. Initial attempts to identify rapidly more advanced crystalline compounds failed. However, we noticed some transient crystallinity on the rearranged depsipeptide **4**, an unusual potential intermediate that could nevertheless serve our purposes. Crystalline **4** would greatly help to access a readily hydrolyzable ester, on the way to the coupling fragment. No other intermediate up to this compound displayed any crystallinity. We therefore constructed our synthetic strategy through this crystalline intermediate, and hoped to develop chemistry as selective and clean as possible, to avoid generating further process-related impurities.

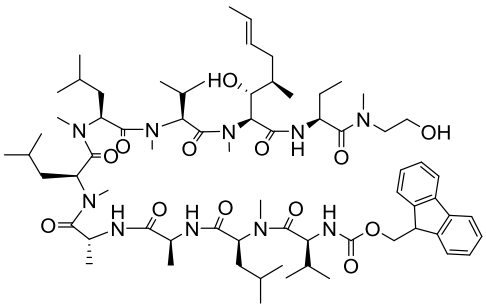
Scheme 4. From the advanced crystalline intermediate to the final acyclic intermediate **4**



Conditions: (a) Fmoc *N*-hydroxysuccinimide ester, Na_2CO_3, water/toluene; (b) $NaBH_4$, glycine, MeOH/toluene; (c) $MeSO_3H$, *i*Pr-OH, then Pyridine, Ac_2O, 52 % over 3 steps; (d) tris(2-aminoethyl)amine, toluene, then HBF_4, MeOH/water, quantitative yield.

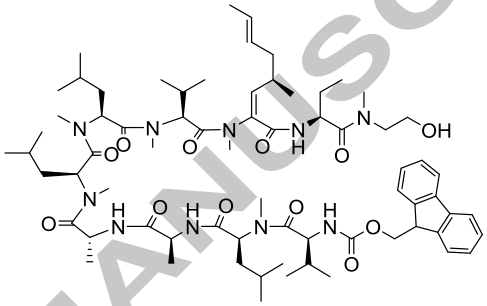
The sequence starting first with Fmoc protection, followed by reduction, acidic rearrangement, and capping as an acetamide turned to be the most selective sequence. A scalable and reliable protocol was developed that led to crystalline material of high purity > 92%. The critical point for the crystallization was to carefully control the water content during the crystallization from a well-defined wet MeTHF/TBME solvent system. For the earlier steps, the dichloromethane previously used in the Fmoc protection step could be substituted by toluene when working under biphasic conditions and resulted in the generation of compound **23** in quantitative yield, and purity of *ca*. 90%, the product being used directly as solution in toluene for the next step. The reduction to **24** turned out to be very complex, with competing deacylation (product **26**) and elimination (product **27**) (see Figure 3). We required here selective methyl ester reduction in the presence of an acetate. Des-acetylation and elimination were observed but could be minimized by using sodium borohydride in THF and toluene with glycine. While numerous control experiments were done to fully understand the role of glycine, it is still not yet fully clear how it affects positively the outcome of the transformation. It is nevertheless unambiguous that it acts as a buffer in our case. The product was finally obtained as a solution in *i*PrOH and used as such in the next step. The purity of **24** had nevertheless dropped significantly to <80%. It is worth pointing out that activation with some methanol was critical to favor a smooth and complete reduction. One potential rationale is that a more reactive dimethoxy-borohydride species is formed *in situ*. Additional parameters which caused some

practical challenges were the hydroxide content and particle size of the sodium borohydride, that varied with the lots. These challenges could be partly solved when dissolving the powder fully. The best solution was found with glymes, which we however did not consider as a viable option due the severe long-term limitations coming from undesirable reprotoxicity and SVHC restriction.[17] Ultimately, tight specifications on particle size and minimum sodium hydroxide content were introduced to ensure robustness in the process.



26

des-acetyl impurity



27

elimination impurity

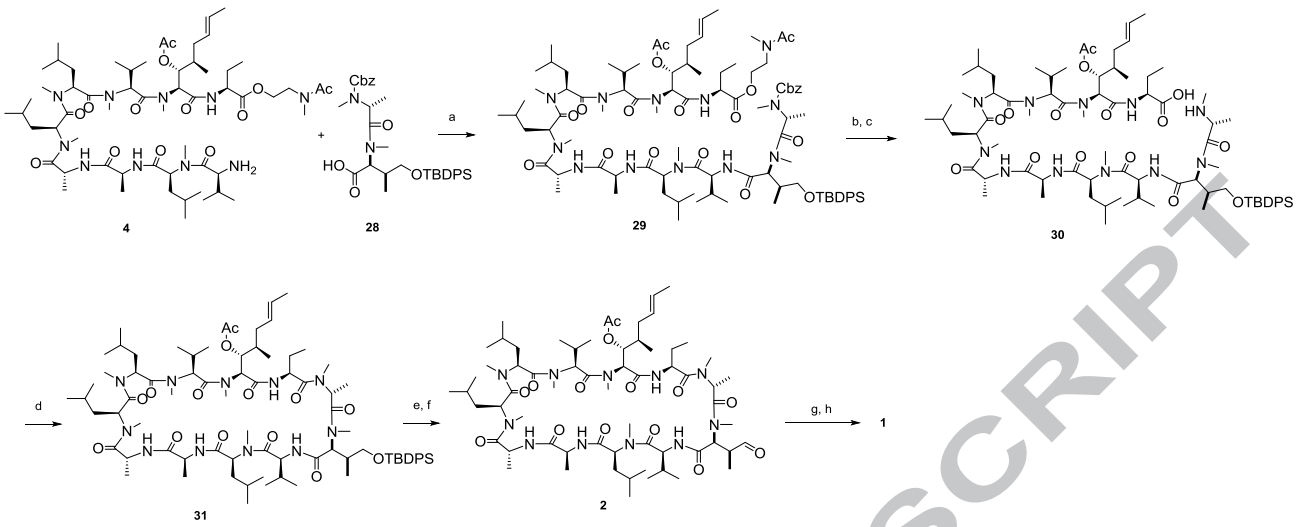
Figure 3. Major impurities from the reduction step.

The subsequent rearrangement step turned out to be challenging from a robustness and scalability standpoint. This step required very careful monitoring of the pH and temperature to promote rearrangement of the amino-ester into the *N*-Acetyl protected derivative **25**. Proper stirring proved crucial to generate reliably material of high purity (87-92%), in acceptable yields (55%). We rationalized that local variation of pH should be minimized, hence the importance of good stirring. Fmoc removal was then carried out under standard conditions using tris-ethylamine, followed by HBF_4 salt formation to result in the crystalline tetrafluoroborate salt **4** in 92% yield and high purity (92-95%). To our delight, we had identified a chromatography-free

process up to this key intermediate which allowed us to generate large quantities in a streamlined manner This crystalline salt was again pivotal for practical access to advanced acyclic derivatives of cyclosporin A as we could tolerate modest purity throughout the sequence and purify at the end of the sequence by crystallization.

Final assembly to the API

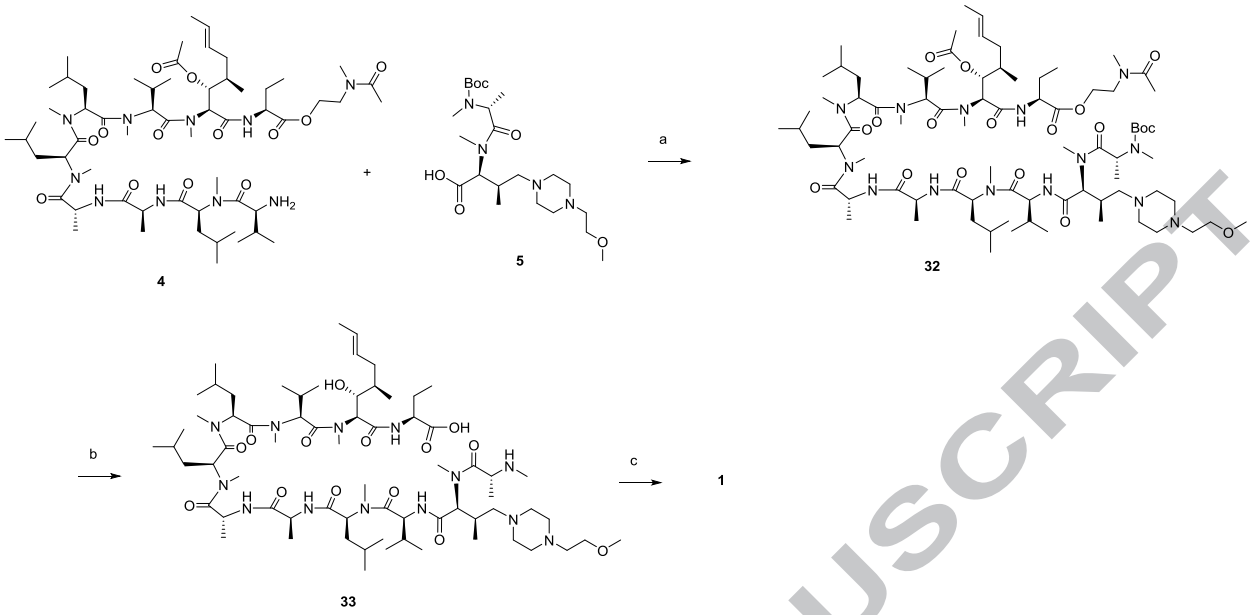
At this stage, whether for the early multigram lots or the multi-kilogram ones, two last key bonds remained to be formed, whether on the partial dipeptide **28** utilized in the early lots, or on the fully functionalized dipeptide **5**. In both cases, the chances to easily purify the intermediates were deemed as very low, as it would be experienced ultimately. Indeed, no crystalline acyclic polypeptide could be obtained, and direct crystallization of the crude macrocycle turned out to be unfruitful despite major efforts. For path A, the decapeptide **4** was first coupled to dipeptide **28** under standard HATU conditions to result in dodecaptide **29** in 70% yield. We then set the stage for the macrocyclization after palladium-mediated silane cleavage of the benzyloxy carbamate and mild ester hydrolysis of the terminal fragment ethylene glycol methyl acetamide to **30**. Cyclization was carried out under dilute conditions using BOP and DMAP and resulted in macrocyclic polypeptide **31** in 58% yield for the three steps. The synthesis was completed by fluoride silyl ether deprotection, oxidation to an aldehyde and reductive amination with the piperazine moiety **3**, followed by basic hydrolysis of the acetate in 20% overall for the 8 final steps.



Conditions: (a) HATU, DIPEA, DCM, 70%; (b) $Pd(OAc)_2$, TEA, Et_3SiH, DCM; (c) NaOH, THF, water; (d) BOP, DMAP, DCM, 58% over 3 steps; (e) TBAF, THF; (f) $Py{\cdot}SO_3$, DIPEA, DMSO, DIPEA; (g) 1-(2-methoxyethyl)piperazine, AcOH, $NaBH(OAc)_3$, DCE; (h) Me_4NOH, MeOH, 48% over 3 steps.

Scheme 5. End-game for path A.

Such a lengthy sequence for an overall mediocre isolated proved particularly costly and sub-optimal for the long-term. We therefore rapidly turned our attention to the direct introduction of the fully decorated dipeptide **5** obtained *via* either the original synthesis (scheme 2) or the revised one (scheme 3). Incorporation of the dipeptide **5** worked optimally when utilizing HATU as the activating agent added in solution in DMF at -10 °C in 1-2 h to a solution of both peptides and NMM in dichloromethane. This resulted in crude material **32** that was purified by chromatography in toluene/ethanol/ ammonium hydroxide to afford the product in 82% yield and 91% purity for the supply of the first few grams. Later, the crude material was used as such for the cyclization.



Conditions: (a) HATU, NMM, CH_2Cl_2/DMF, 82%; (b) H_2SO_4, MeOH/toluene, then $BnNMe_3OH$, MeOH, 75%; (c) Cl-HOBT, DCC, Li_2CO_3, toluene/TBME (3:1), 74 % crude yield.

Scheme 6. End-game to the API via path B.

The subsequent ester hydrolysis was triggered under acidic transesterification with sulfuric acid in methanol, followed by very mild caustic hydrolysis of both methyl ester and hydroxyl acetate functionality utilizing benzyltrimethylammonium hydroxide. The reaction proceeded quantitatively to **33** and resulted in material of *ca*. 78% purity.

The final macrocyclization was originally conducted with 6-chloro-1-hydroxybenzotriazole (Cl-HOBT) and dicyclohexylcarbodiimide (DCC), under a traditional slow addition of the acyclic polypeptide in a very dilute mixture of dichloromethane and toluene. The slow addition over 10 h at 30 °C minimizes accumulation of the activated intermediate and the resulting competing oligomerization (concentration *ca*. 0.5% wt)[18] while maintaining the low

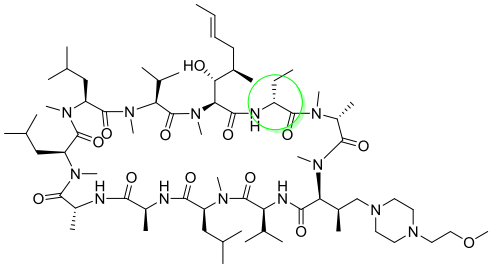
concentration necessary for minimal formation of oligomer, which is controlled to a total level below 3%. This strategy enabled the generation of the first few grams but rapidly showed limitations upon scale-up. It indeed requires large amount of solvent and particularly of the undesirable methylene chloride, long overall processing time, which contribute to major expenses. The seminal review by Yudin[18a] reports some of the recent and elegant approaches to solve the entropic challenge associated with such a disfavored macrocyclization event. Another elegant alternative approach relied on the pre-organization of the acyclic precursor into the conformation favoring macrocyclization via either complexation or constraining the system into a more desirable conformation.[19] Such an approach was however not pursued as it requires substantial experimentation with a rather uncertain success. Our preferred approach was based on early successes on related macrocyclic systems, where we engineered the process to follow a dual slow-release / slow-addition process. Indeed, we targeted avoidance of the accumulation of the acyclic precursor, which would lead to oligomers at too high concentration, *via* the standard slow-addition technique, but also *via* an uncommon slow-release concept. A prerequisite was obviously to accelerate the reaction to minimize the required addition time. This was achieved in a traditional manner by standard screening of reagent conditions that confirmed dicyclohehylcarbodiimide (DCC) and 6-Chloro-Hydroxybenzotriazole (Cl-HOBt) to be the optimal reagent combination.

The main challenge was the choice of reaction medium (solvent composition and ratio). Out of an extensive variety of solvents (toluene, xylenes, anisole, TBME, *i*Pr_2O, MeTHF, EtOAc, *i*PrOAc, acetone, acetonitrile) and combinations thereof, toluene and TBME in a 3 to 1 ratio by volume were demonstrated to both promote the transformation and ensure a proper dissolution profile of the acyclic polypeptide precursor. The ratio proved critical to ensure suitable outcome (yield > 70%, total amount of oligomers < 5%), although sufficient tolerance

for robustness was observed. We indeed demonstrated that the toluene/TBME ratio could vary from 1:2 to 1:4 with minimal variability in the outcome. A more detailed report of our screening, and design of experiment approach to identify this set of conditions will be reported shortly. It was besides critical that **33** displays suitable physical properties in the medium. The suspension of **33** in toluene itself tended for example to aggregate, something that would render the large scale dosing unmanageable. It also tended to oil out if local supersaturation was reached in the course of the addition, as observed when we added **33** in TBME directly into Toluene.

Base, concentration, addition time, temperature, and additive effect were then optimized in a standard way to ultimately result in the final conditions, namely addition of compound **33** in suspension in toluene/TBME (3/1) in 5 h, to a mixture of DCC (2.5 eq), Cl-HOBT (0.15 eq), Li_2CO_3 (3 eq) (overall concentration 2.2 $mol.L^{-1}$), at 40 °C, which resulted in 74% isolated yield, and below 3.5% total oligomers. Such an approach was later proven to be general in terms of scope of polypeptide that undergoes macrocyclization, which will be thoroughly reported from a process standpoint in a coming venue.

Overall, this new process allowed us to increase the concentration of the macrocyclization from 0.5% of acyclic starting material by weight to *ca*. 10%, with the same yield, and selectivity (*ca*. 3% oligomers, *ca*. 5% epimerization). The main process-related impurities resulted from epimerization (**epi-1** in Figure 4) or oligomerization.

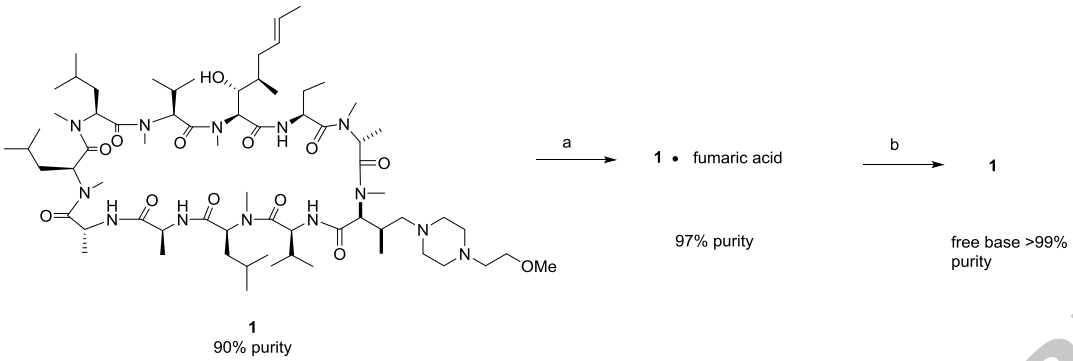


Epimer (**epi-1**)

Figure 4. Epimeric impurity generated during the macrocyclization step.

Purification of the drug substance

With the crude Drug substance in hand, the first and most expedient option relied on purification by reverse-phase column chromatography. The crude material was thus purified by preparative chiral column chromatography and resulted in material of 93% purity in 45% yield, and was further purified by RP-LC on Daiso-RP-8 column to upgrade the quality to 98.5% and a recovery of 78%. This strategy proved useful to supply the first few grams but rapidly showed limitations in terms of productivity and required investment of substantially large columns and high flow preparative systems. Thus, we tried to identify (a) crystalline form(s) and develop robust processes enabling the practical generation of high quality drug substance by crystallization. We aimed to utilize the basic piperazine side-chain for salt formation. A rapid solid state screening identified the fumarate salt and the free form exhibited sufficient crystallinity to be built into our purification strategy. The best outcome was obtained when the crude was first submitted to formation of the fumarate salt in acetonitrile/water which could upgrade the quality from *ca*. 90% to *ca*. 97%, in 85 to 90% yield, although in a very slow crystallization process (5 days). Subsequent free base formation and recrystallization in TBME/heptane allowed for the isolation of a transient crystalline form, that becomes amorphous upon drying. The process nevertheless allowed us to increase the purity to > 99% with *ca*. 80% yield. This novel process, although long overall, enabled the larger scale production of the desired drug substance in high quality.



Conditions: (a) fumaric acid (1.0 eq), acetonitrile/water, 55 ^{o}C to rt; (b) K_2CO_3, MeTHF, then TBME/water/heptane, 80 % overall yield.

Scheme 7. Purification to the API.

Conclusion

Overall, we have disclosed the evolution of the synthetic strategy to the target compound NIM258 (**1)** from the original medicinal chemistry route, designed for late diversification, to the development approach, built for increased convergency, expediency, and robustness. Critical to this was the identification of advanced crystalline intermediates that enabled practical supply of advanced intermediates and of the drug substance, and an unprecedented macrocyclization approach to the best of our knowledge, that allowed for high throughput.

Experimentals

^{1}H/^{13}C NMR spectra were recorded on 400’ 54 ascend purchased from Bruker Biospin AG, operating at 400/100 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard.

The LC-HRMS analyses were performed by using electrospray ionization in positive ion modus after separation by liquid chromatography (Nexera from shimadzu). The elemental composition was derived from the mass spectra acquired at the high resolution of about 30'000 on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific). The high mass accuracy below 1 ppm was obtained by using a lock mass.

Reaction solvents were obtained commercially, and used without further purification. Commercial reagents were used as received. Reaction were monitored by thin-layer chromatography (TLC) on 0.25mm precoated Merck Silica Gel 60 F254, visualizing with ultraviolet light.

N-(2,2,2-Trifluoroacetyl)glycine (**17**)

To a 30 L reactor equipped with a mechanical stirrer, a thermometer, a reflux condenser, an addition funnel, and nitrogen inlet-outlet was charged TFA (2 L), glycine **16** (2.0 kg, 26.6 mol) with stirring while keeping the inner temperature below 0 ^{o}C resulting in a suspension. Then to the suspension was added dropwise TFAA (6.6 kg, 31.4 mol) with stirring while keeping the inner temperature below 70 ^{o}C. After addition, the reaction mixture was kept at 70 ^{o}C and stirred for another 2 h until all solid was dissolved. Completion was demonstrated by disappearance of the starting material by HPLC after 2 h. The mixture was concentrated to dryness under vacuum at 50 ^{o}C to obtain the crude **17** as a light yellow solid. The crude material was purified by re-crystallization from EtOAc (6 L) and heptane (12 L) to afford pure **17** (3.7 kg, 81 %) as a white solid: mp 120-121 °C. ^{1}H NMR (400 MHz, D_2O) δ 4.01 (s, 2 H). ^{13}C NMR (101 MHz, D_2O) δ 40.86, 117.08, 159.37, 171.66; HRMS (ESI) $[C_4H_4F_3NO_3-H]^-$) calcd 170.0065, found 170.0044.

(2E)-But-2-en-1-yl N-(2,2,2-trifluoroacetyl)glycinate (**18**)

To a 30 L reactor equipped with a mechanical stirrer, a thermometer, a reflux condenser, an addition funnel, and nitrogen inlet-outlet was charged CH_2Cl_2 (20 L), **17** (3.4 kg, 19.6 mol), DMF (0.01 L) with stirring to afford a clear solution. And then to this solution was added dropwise oxalyl chloride (2.8 kg, 21.6 mol) for 30 min at 15 °C under nitrogen protection. After addition, the reaction mixture was stirred at 40 ^{o}C for 4 h. Completion was demonstrated by disappearance of the starting material by HPLC after 4 h. The solvent was removed under vacuum at 40 °C to afford 2-(2, 2, 2-trifluoroacetamido) acetyl chloride (3.7 kg, 100%) as a brown oil which was directly used in the next step without purification.

To a stirred solution of crotyl alcohol (1.4 kg, 18.7 mol) in CH_2Cl_2 (20 L) was added dropwise a solution of 2-(2, 2, 2-trifluoroacetamido) acetyl chloride (3.7 kg, 19.7 mol) in CH_2Cl_2 (1 L) at 25 °C over 30 min. After addition, the reaction mixture was stirred at 30 °C overnight. At completion, the mixture was washed with H_2O (3 x 5 L) and 5% $NaHCO_3$ aqueous solution (3 x 5 L). The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness to obtain the crude product **18** which was purified by re-crystallization from CH_2Cl_2: THF: heptane (0.5 L: 0.5 L: 10 L) to obtain purified **18** (3.0 kg, 68%) as a white solid: mp 48-49 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 1.70 (br d, *J* = 6.36, 3 H), 4.08 (br d, *J* = 5.26, 2 H), 4.58 (br d, *J* = 6.48, 2 H), 5.45 - 5.65 (m, 1 H), 5.72 -5.90 (m, 1 H), 7.32 (br s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) 17.53, 41.21, 66.62, 117.01, 123.91, 132.78, 157.57, 168.07; HRMS (ESI) $[C_8H_{10}F_3NO_3{-}H]^{-}$ calcd 224.0535, found 224.0545.

(2S,3R)-3-Methyl-2-(2,2,2-trifluoroacetamido)pent-4-enoic acid

To a solution of **18** (4.9 kg, 21.7 mol), $Al(OiPr)_3$ (4.9 kg, 23.0 mol) and quinidine (16.2 kg, 54.4 mol) in THF (192.0 kg) was added dropwise a solution of LiHMDS in THF (1 M, 93.0 kg) at -78

°C for 3 h. After addition, the reaction mixture was then warmed to 15-25 °C with stirring for 12 h. Completion was demonstrated by disappearance of the starting material by HPLC after 12 h. 420.0 kg of 15% $KHSO_4$ aqueous solution was added dropwise to the reaction mixture at 0-15 °C (pH <2). The aqueous layer was extracted with EtOAc (2 x 75 kg). The combined organic phase was washed with 64 kg of 15% $KHSO_4$ aqueous solution, and then concentrated under vacuum to dryness to obtain the crude product (1.7 kg, 34.6%) which was directly used in the next step without purification.

A solution of crude product above (1.7 kg, 7.5 mol) in EtOAc (10.0 kg) was washed with 7% $NaHCO_3$ aqueous solution (3 x 6 kg). The combined aqueous layer was extracted with EtOAc (2 kg). The aqueous layer was acidified with $KHSO_4$ (2.5 kg) until pH <2 and extracted with IPAc (3 x 7 kg). The organic phase was washed with 3 kg of 25% NaCl aqueous solution and concentrated to about 3-5 kg. IPAc (5 kg) was charged to the vessel and concentrated to 3-5 kg to make sure the water content is below 0.1%. Then the solution was heated to 60 °C and a solution of R-(+)-α-methyl benzyl amine (0.9 kg, 7.0 mol) in IPAc (1 kg) was added dropwise over 1 h. The solution was stirred for 1 h and n-heptane (14.0 kg) was added dropwise at 60-65 °C. The mixture was stirred at 60-65 °C for 2 h and cooled to 0 °C and stirred at 0 °C for 6 h. The mixture was filtered and washed with a mixture solution (4 kg) of n-heptane/IPAc=6/1. The chiral salt was obtained with 99.9 % ee and 96.8 % dr. The solid was then dissolved in IPAc (8 kg) and 15 kg of 10 % $KHSO_4$ aqueous solution was added. The two layers were separated and the aqueous layer was extracted with IPAc (3 kg). The combined organic phase was washed with 3 kg of 25% NaCl aqueous solution and concentrated to dryness to obtain the purified product (1.1 kg, 23.1 %) as a yellow liquid: ee 99.1 %; de 96.9 %; ^{1}H NMR (400 MHz, $CDCl_3$) 1.15 (d, *J* = 6.97, 3 H), 2.76 - 2.88 (m, 1 H), 4.68 (dd, *J* = 8.56, 4.89, 1 H), 5.09 - 5.19 (m, 2 H), 5.62 -

5.80 (m, 1 H), 7.00 (br d, J = 8.31, 1 H), 8.84 (br s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) 14.99, 40.13, 56.11, 117.02, 117.54, 136.99, 157.33, 173.88; HRMS (ESI) [$C_8H_{10}F_3NO_3$−H]$^-$) calcd 224.0535, found 224.0510.

Benzyl (2S,3R)-3-methyl-2-(methylamino)pent-4-enoate (**19**)

To a solution of the above product (1.9 kg, 8.2 mol) in DMF (8 L) was added K_2CO_3 (1.1 kg, 8.2 mol) and BnBr (1.4 kg, 8.2 mol) at -10 °C (approximately 2 h) and stirred for 18 h at room temperature under nitrogen atmosphere. Completion was demonstrated by disappearance of the starting material by HPLC after 18 h. To the reaction mixture was added K_2CO_3 (3.4 kg, 24.6 mol) and CH_3I (2.3 kg, 16.4 mol) at -10 °C (approximately 2 h) and stirred for 18 h at room temperature under nitrogen atmosphere. At completion, water (2 L) and methyl tert-butyl ether (5 L) were added and the mixture was stirred for 1 h. The organic layer was washed with water (3 x 5 L), then brine (3 x 5 L). The aqueous layer successively extracted with methyl tert-butyl ether (3 x 5 L) and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the crude product (2.6 kg, 95.0%) as a light-brown oil which was directly used in the next step without purification.

To the above solution (2.0 kg, 6.1 mol) in MeOH (9 L) and CH_2Cl_2 (9 L) was added $NaBH_4$ (0.8 kg, 21.2 mol) slowly at 0 ^{o}C for 2 h. After addition, the mixture was stirred for 30 min at 0 ^{o}C. Completion was demonstrated by disappearance of the starting material by HPLC after 1 h. To the reaction mixture was added dropwise H_2O (1 L) over 20 min at 20 ^{o}C with stirring, and then concentrated under vacuum to 20 kg. To the residue was added dropwise an aqueous HCl solution (2.0 N, 7 L) over 20 min at 20 ^{o}C with stirring. After addition, the mixture was extracted with EtOAc (2 x 10 L). The pH of the aqueous layer was adjusted to 10 by adding 10%

K_2CO_3 solution, and then extracted with EtOAc (3 x 10 L). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to afford crude **19** (1.5 kg, crude, quantitative) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) 1.04 (d, *J* = 6.97, 3 H), 2.26 - 2.40 (m, 3 H), 2.41 - 2.59 (m, 1 H), 3.12 (d, *J* = 5.87, 1 H), 4.90 - 5.11 (m, 2H), 5.13 - 5.22 (m, 2 H), 5.58 - 5.84 (m, 1 H), 7.30 - 7.43 (m, 5 H); ^{13}C NMR (101 MHz, $CDCl_3$) 16.02, 34.79, 41.18, 66.03, 67.65, 115.38, 128.06, 128.21, 128.30, 135.60, 139.56, 173.80; HRMS (ESI) $[C_{14}H_{19}NO_2+H]^+$ calcd 234.1494, found 234.1491.

Benzyl *N*-(tert-butoxycarbonyl)-N-methyl-D-alanyl-(2S,3R)-3-methyl-2-(methylamino)pent-4-enoate (**20**)

To a mixture of **19** (1.5 kg, crude from earlier step) and **11** (1.6 kg, 7.7 mol) in EtOAc (15 L) was added Et_3N (2.6 kg, 25.7 mol). T3P as a 50% solution in EtOAc (3.1 kg, 9.7 mol) was then added dropwise over 20 min at 0 ^{o}C. The reaction mixture was stirred at 10 ^{o}C overnight. Completion was demonstrated by disappearance of the starting material by HPLC after 16 h. To the mixture was added EtOAc (5 L) and then quenched by adding water (2 L) dropwise over 20 min at 20 ^{o}C. The organic layer was washed with H_2O (2 x 10 L), brine, dried over anhydrous Na_2SO_4, and concentrated to afford **20** (2.0 kg, 78.0 %) as a yellow oil which was directly used in the next step without purification.

Benzyl *N*-(tert-butoxycarbonyl)-N-methyl-D-alanyl-(2S,3S)-3-methyl-2-(methylamino)-4-oxobutanoate (**21**)

To a stirred solution of **20** (1.28 kg, 3.1 mol) in CH_2Cl_2 (13 L) was added propionaldehyde (355 g, 6.0 mol) at 25 ^{o}C. The reaction mixture was cooled to -78 ^{o}C, and then to the reaction mixture was bubbled O_3 for 40 min at -78 ^{o}C. The reaction mixture was slowly warmed to -10 ^{o}C. To the

reaction mixture was added triethylamine (400 g, 4 mol) over 10 min at -10 ^{o}C. After addition, the mixture was stirred at -10 ^{o}C for 1 h, and then washed with NH_4Cl (2 x 0.3 L). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to obtain **21** (1.29 kg) as a yellow oil which was directly used in the next step without purification.

Benzyl *N*-(tert-butoxycarbonyl)-N-methyl-D-alanyl-(2S,3R)-4-[4-(2-methoxyethyl)piperazin-1-yl]-3-methyl-2-(methylamino)butanoate

To a stirred solution of **21** (882 g, 6.1 mol) in CH_2Cl_2 (18 L) was added AcOH (294 g, 4.9 mol) and $NaBH(OAc)_3$ (3.12 kg, 15 mol) at 25 ^{o}C. The reaction mixture was cooled to -10 ^{o}C and compound **3** (1.28 kg, 3.0 mol) was then added. The reaction mixture was stirred at -10 ^{o}C for 16 h. Completion was demonstrated by disappearance of the starting material by HPLC after 16 h. The reaction mixture was quenched with NH_4Cl (3 L), and washed with NH_4Cl (2 x 3 L), $NaHCO_3$ (2 x 3 L) and brine (2 x 3 L). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to obtain the crude product, which was filtered through a plug of silica gel (ca. 1 kg, elution with CH_2Cl_2) to afford the product (651 g, 40 % yield) as a light yellow oil. ^{1}H NMR (400 MHz, DMSO-d6) 0.46 - 0.83 (m, 3 H); 0.99 - 1.19 (m, 3 H); 1.23 - 1.52 (m, 9 H); 2.07 - 2.46 (m, 13 H); 2.54 - 3.04 (m, 5 H); 3.21 (s, 3 H); 3.37 - 3.50 (m, 3 H); 4.73 - 5.36 (m, 4 H); 7.16 - 7.48 (m, 5 H); ^{13}C NMR (101MHz, DMSO-d6) 14.28 - 15.72, 21.83 - 23.62, 28.18 - 28.37, 28.41, 28.55, 29.15, 29.26 - 29.99, 31.26, 47.92 - 52.51, 53.43, 53.25 - 53.47, 53.49 - 53.57, 53.58, 53.69, 55.37, 57.53, 58.44, 60.12, 62.31 - 64.38, 65.41 - 67.13, 70.35, 79.06 - 81.24, 127.90, 128.34 - 128.46, 128.50, 128.85, 128.92, 135.50 - 137.21, 153.98 - 156.15, 168.79 - 170.36, 170.89 - 173.14; HRMS (ESI) $[C29H_{48}N_4O_6+H]^+$ calcd 549.3652, found 549.3621.

N-(tert-Butoxycarbonyl)-N-methyl-D-alanyl-(2S,3R)-4-[1-(2-methoxyethyl)piperazin-1-yl]-3-methyl-2-(methylamino)butanoic acid (***5***)

To a solution of the above product (220.0 g, 401.0 mmol) in EtOH (0.2 L00 mL) was added $Pd(OH)_2$/C 10 wt% (41.2 g). The reaction mixture was stirred at 25 °C under 1 atm of H_2 for 16 h. Completion was demonstrated by disappearance of the starting material by HPLC after 16 h. The reaction mixture was filtered and concentrated to dryness. To this residue was added EtOAc (0.3 L) followed by heptane dropwise (2 L) over 20 min at 10 °C. After addition, the mixture was stirred at 10 °C for 40 min. The slurry was filtered and concentrated to dryness to obtain **5** (130 g, 71 %) as a white solid, mp 94-96 °C. ^{1}H NMR (400 MHz, DMSO-d6) 0.73 (br d, *J* = 5.75, 3 H), 1.05 - 1.21 (m, 3 H), 1.33 - 1.47 (m, 9 H), 2.39 (br s, 2 H), 2.55 - 2.97 (m, 15 H), 3.16 - 3.28 (m, 3 H), 3.43 - 3.58 (m, 2 H), 3.97 (br d, *J* = 8.68, 1 H), 4.59 (br s, 1 H), 4.70 - 5.26 (m, 1 H), 4.77 - 5.22 (m, 1 H); ^{13}C NMR (101MHz, DMSO-d6) 14.60 - 16.69, 28.39, 28.44, 28.45 - 28.49, 28.55, 28.77, 29.21, 29.45, 51.52, 51.62, 51.81, 51.85 - 52.07, 55.86 - 57.01, 8.42, 58.44, 62.30, 65.32, 68.29 - 71.40, 79.25 - 80.26, 154.45 - 155.22, 170.84 - 171.42, 171.67 - 172.56; HRMS (ESI) $[C_{22}H_{42}N_4O_6+H]^+$ calcd 459.3183, found 459.3159.

Methyl *N*-{[(9H-fluoren-9-yl)methoxy]carbonyl}-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enoyl-(2S)-2-aminobutanoyl-*N*-methylglycinate (**23**)

To a 3 L double-jacketed reactor at rt charged with sodium carbonate (101.7 g), in water (0.63 kg) and toluene (1.1 kg) was added portionwise solid Methyl L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enoyl-(2S)-2-aminobutanoyl-N-methylglycinate—trifluoroborane—hydrogen fluoride (1/1/1) **22** (0.25 kg, 0.20 mol, *ca*. 90% purity). The lumps

went into an emulsion within a few minutes of vigorous stirring. (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (61.5 g, 0.18 mol) was added portionwise in *ca*. 30 min, and the transfer line was rinsed with toluene (0.05 L). The resulting mixture was stirred for 1 h, until completion of the transformation. Methanol (0.05 L) was added, the emulsion was warmed to 50 °C and stirred for 1 h. Stirring was discontinued and the layers were separated. The organic phase was cooled to rt, and a 1 M $NaHSO_4$ aqueous solution (1 L) was added slowly under gentle stirring (Caution CO_2-evolution!). Stirring was discontinued and the layers were separated. The organic extract was washed with water (1 L), and the yellowish turbid organic layer was concentrated at *ca*. 50 °C and reduced pressure 250 to 500 mbar to a concentrated solution of *ca*. 500 g. An aliquot was further dried under high vacuum to provide an off-white solid and calculate the corresponding yield (278 g by HPLC quantitative assay compared to pure reference, *ca*. 93.5% purity). $C_{73}H_{114}N_{10}O_{15}$: ^{1}H NMR (600 MHz, DMSO-d6): one proton is missing (broad line) due to conformer mixture and very broad line proton spectra: one proton couldn't be located precisely. 2.29 3H 7.17 2H,7.24 2H, 7.14 1H, Carbon 21.47 1C 129.40 2C,128.64 2C and 125.75 1C) 0.50 - 0.97 (m, 36H), 1.09 - 1.20 (m, 6H), 1.30 - 1.46 (m, 4H), 1.45 - 1.78 (m,7H), 1.59 (br d, 3H), 1.93 (m, 3H), 2.02 (m, 1H), 2.14 (m, 1H), 2.20 (m, 1H), 2.64 - 3.14 conformer mixture of singulets (s, 18H), 3.63 and 3.68 (s, 3H), 3.87 (d, J= 17.10, 1H), 4.14 - 4.44 (m, 6H), 4.60 - 4.72 (m, 2H), 5.04 (d, *J* =10.52, 1H), 5.09 (m, 1H), 5.21 - 5.31 (m, 2H), 5.31 - 5.48 (m, 6H), 7.31 (m, 2H), 7.40 (t, *J* = 7.4, 2H), 7.58 (d, *J* = 7.46, 1H),7.65 (d, *J* = 8.76, 1H), 7.71 (br, 2H), 7.88 (d, *J* = 7.62, 2H), 8.04 - 8.28 (m, 2H); ^{13}C NMR (151 MHz, DMSO): due to conformers mixture at room temperature, carbon signal could be splitted into 2 different signal, 10.14, 10.25, 16.13, 16.32, 17.51, 18.24, 18.32, 18.72, 19.06, 19.38, 19.56, 21.07, 21.49, 21.90, 22.33, 22.78, 23.00, 23.33, 23.57, 24.60 to 24.76, 27.32, 29.95, 30.18,

30.26, 30.30, 31.22, 32.67, 34.66, 34.84, 35.00, 36.47, 37.01, 38.00, 38.03, 45.18, 17.13, 48.48, 49.69, 48.85, 50.25, 51.21, 51.51, 52.11, 52.51, 54.13, 56.27 (brs), 56.72, 57.85, 66.20, 72.63, 73.47, 74.64, 120.47 to 102.55 (br.s), 121.83, 125.68, 125,86, 126.66, 127.44, 127.49, 127.74, 129.72, 137.80, 141.14, 141.16, 144.35, 156.64, 167.74, 167.91, 169.70, 169.79, 170.01, 170.34, 170.40, 170.98, 171.09, 171.58, 171.79, 172.05, 172.05, 172.47, 173.13; HRMS for $[C_{73}H_{114}N_{10}O_{15}+2H]^+$ calcd 1372.86106, obsd 1372.86096.

(2S)-2-[{[(9H-Fluoren-9-yl)methoxy]carbonyl}-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enamido]-*N*-hydroxyethyl-*N*-methylbutanamide (**24**)

A suspension of sodium borohydride (14.5 g, 0.38 mol) in THF (0.4 L) was added at rt, to a 2 L double-jacketed reactor equipped with an impeller stirrer, a temperature sensor, an addition funnel and Argon inertization bubbler and the mixture was heated to ca. 45 ^{o}C. To the slightly turbid thin suspension cooled to 15 °C was added glycine (14.4 g, 0.19 mol), then the freshly prepared 50% wt solution of **23** into toluene (276 g, ca. 138 g, 0.10 mol **23**) *via* the addition funnel in *ca*. 30 min. At the end of the addition, the transfer line was rinsed with toluene (10 mL). Within 1 h, the reduction started. A mixture of toluene and methanol (0.05 L each) were added continuously within 3 h, to allow for continuous hydrogen release and controlled gas release, at which point the reaction reached > 99% conversion, as monitored by HPLC. The reaction mixture was then cooled to 0 ^{o}C, and added to a second 3 L double-jacketed reactor equipped with an impeller stirrer, a temperature sensor, an addition funnel and Argon inertization bubbler, charged with water (0.83 L), and acetic acid (69 g) at 0 ^{o}C. Care should be taken as gas evolution ensued. The reaction vessel and the transfer line was rinsed with toluene

(0.2 L), and the resulting white emulsion was stirred overnight at rt. Stirring was discontinued, and the phases were separated. The organic layer was washed twice with water (0.25 L), and concentrated at *ca*. 50 °C and reduced pressure to a viscous colorless solution. Isopropyl alcohol (200 mL) was added and the concentration process was repeated one more time until the water content was below 1% and toluene < 0.1 % as measured by Karl Fischer titration and GC. The solution was then diluted further to provide a 67% wt solution of **24** (333 g, ca. 225 g, and 80% purity). ^{1}H NMR (600 MHz, DMSO-d6): 0.50 - 1.00 (m, 36H), 1.10 - 1.21 (d, *J* = 6.9, 6H), 1.31 - 1.43 (m, 5H), 1.44 - 1.73 (m, 6H), 1.60 (br s, 3H), 1.93 (m, 3H), 2.03 (m, 1H), 2.13 (m, 1H), 2.21 (m, 1H), 2.68 to 3.10 conformer mixture of singlets (s, 18H), 3.18 and 3.23 (m, 1H), 3.45 and 3.59 (m, 1H), 3.47 and 3.54 (m,2H), 4.14 - 4.21 (m, 2H), 4.21 - 4.33 (m, 3H), 4.52 - 4.72 (m, 3H), 4.80 (br s, 1H), 5.01 - 5.16 (m, 2H), 5.21 - 5.53 (m, 7H), 7.31 (m, 2H), 7.41 (t, *J* = 7.3, 2H), 7.51 - 7.68 (m, 2H), 7.71 (m, 2H), 7.87 (d, *J* = 7.5, 2H), 8.09 (m, 2H); ^{13}C NMR (151 MHz, DMSO) due to conformer mixtures at room temperature, carbon signal could be splitted into 2 different signal. 9.76, 9.93, 15.73, 15.85, 17.07, 17.81, 17.88, 18.27, 18.62, 18.94, 19.10, 19.13, 20.62 (br s), 21.46, 21.90, 22.33, 22.41, 22.56, 22.86 (br s), 23.13, 24.18, 24.24, 24.32, 24.53, 24.93, 26.87, 29.51, 29.73, 29.82, 29.86, 30.78, 32.24 (br s), 32.44 (br s), 33.62, 34.26 (br s), 34.40, 34.46, 34.56, 35.97, 37.58 (br s), 41.86, 44.74, 46.69, 48.05 (br s), 49.52, 49.99, 50.13, 50.77, 51.07, 53.70, 55.88 (br s), 56.28, 57.34, 57.41, 58.49, 58.67, 65.77, 74.26, 74.41, 119.85, 119.94, 120.02, 120.09 (br s), 121.38, 124.16, 125.24, 125.42, 126.29, 126.78, 126.97, 127.00, 127.04, 127.15, 127.29, 127.63, 128.92, 129.18 (br s), 139.91, 140.72 (br s), 143.71, 143.90, 148.64, 156.20, 167.21, 167.28, 169.23, 169.34, 169.91, 169.96, 170.35, 170.57, 170.63, 170.68, 170.79, 171.61, 172.03, 172.69; HRMS for $[C_{72}H_{114}N_{10}O_{14}+NH_4]^+$ calcd 1360.88543, obsd 1360.88538.

2-(*N*-Methylacetamido)ethyl (2S)-2-[*N*-{[(9H-fluoren-9-yl)methoxy]carbonyl}-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enamido]butanoate (**25**)

A 3 L double-jacketed reactor at rt charged with **24** (1.8 kg, 80% purity, 1.1 mol) in 2-propanol (9 L) was warmed to 50 °C, and methanesulfonic acid (185 g, 1.9 mol) was added in a few minutes *via* an addition funnel. The reaction mixture was stirred at this temperature until completion monitored by HPLC (2 days), cooled to rt, and submitted to pyridine (456 g, 5.8 mol), and acetic anhydride (294 g, 2.9 mol), until the terminal hydroxyl was fully capped as determined by HPLC (*ca*. 18 h). The reaction mixture was then filtered to remove the solid salts, rinsed with toluene (5 L), and the organic phase was concentrated at 50 °C under reduced pressure to ca. 2.5 L solution. Solid salts precipitated again and the above described operation was repeated. The resulting toluene solution was further diluted with toluene (2.5 L), and washed with 5% aqueous HCl solution (1 L). The organic layer was washed with 2.5% aqueous HCl solution (0.6 L), then water (1 L), then a 5% sodium hydrogen carbonate solution (1 L), and lastly with water (1 L). The resulting solution was concentrated at 50 °C nuder reduced pressure and resulted into **25** as a yellowish foam (1.3 kg, purity *ca*. 61%, 52% yield). ^{1}H NMR (600 MHz, DMSO-d6) 0.69 (d, *J* = 6.77, 3H), 0.75 (m, 6H), 0.78 - 0.96 (m, 27H), 1.12 - 1.18 (m, 6H), 1.31 - 1.47 (m, 5H), 1.47 - 1.64 (m, 9H), 1.70 (m, 2H), 1.91 - 2.01 (multiple s, 6H), 2.02 (m, 1H), 2.19 (m, 2H), 2.75 - 3.01 (multiple s, 18H), 3.45 - 3.58 (m, 2H), 4.04 - 4.33 (m, 8H), 4.66 (m, 1H), 5.03 (d, *J*=10.18, 1H), 5.09 (m, 1H), 5.26 - 5.45 (m, 6H), 7.30 (m, 2H), 7.40 (t, *J* = 7.19, 2H), 7.58 (d, *J* = 7.4, 1H), 7.64 (d, *J* = 8.50, 1H), 7.70 (m, 2H), 7.88 (d, *J* = 7.5, 2H), 8.09 (d, *J* = 7.3, 1H), 8.38 - 8.51 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d6) due conformers mixture

at room temperature, carbon signal could be splitted into 2 different signal. δ 10.21, 14.10, 15.87, 17.07, 17.83, 17.90, 18.27, 18.63, 18.94, 19.06, 20.69, 20.77, 21.16, 21.46, 21.58, 21.90, 22.36, 22.55, 22.89, 23.14, 23.60, 23.65, 24.16, 24.24, 24.32, 26.94, 29.54, 29.75, 29.81, 29.85, 30.79, 32.11 (brs), 32.77, 34.13, 34.38, 36.58, 36.65, 37.55, 37.59, 44.75, 45.81, 46.69, 48.04, 48.37, 50.78, 51.07, 53.57, 53.70, 56.28, 57.37, 58.07, 59.78, 61.98, 65.76, 74.10 (brs), 120.09, 120.11, 121.40, 125.25, 125.42, 126.29, 127.01, 127.06, 127.65, 129.25, 140.70, 140.72, 143.70, 143.91, 156.20, 167.83, 167.87, 169.49, 169.74, 169.90, 169.98, 170.12, 170.19, 170.51, 170.65, 171.15, 171.21, 171.62, 172.03, 172.70; HRMS for $[C_{74}H_{116}N_{10}O_{15}+4H]^{+}$, calcd 1386.87672, obsd 1386.87684.

2-(*N*-Methylacetamido)ethyl (2S)-2-[*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enamido]butanoate—trifluorborane—hydrogen fluoride (1/1/1) (**4**)

25 (1.3 kg, purity *ca*. 61%,0.57 mol), dissolved into toluene (6 L) was charged to a 15 L double-jacketed reactor. A solution of Tris(2-aminoethyl)amine (116.6 g, 0.80 mol, 1.4 eq) in toluene (1.5 L) was added at rt in *ca*. 0.5 h and the resulting mixture was stirred at rt overnight until completino of the reaction as monitored by HPLC. At completion, the reaction mixture was added to water (11 L) at rt charged in 0.5 h to a 30 L double-jacketed reactor. The transfer lines were rinsed with toluene/water (1 L, 1:1), stirring was discontinued and the phases were separated. The organic phase a second time with water (11 L). Water (15 L) and methanol (6 L) were then added, followed by a 48% aqueous solution of HBF_4 (*ca*. 0.5 kg, 2.85 mol, 5 eq).The resulting mixture was stirred for 0.5 h, stirring was discontinued, the layers were separated. The lower Methanol-water phase was extracted with toluene (5 L), and the Methanol-water phase

was concentrated at *ca*. 50 ^{o}C under reduced pressure to ca. 2 L, and cooled down to rt. The product was extracted twice with 2-MeTHF (8 L and 2 L), and the combined organic extracts were washed with water (8 L and 2 L). The combined organic extracts were concentrated at ca. 50 ^{o}C under vacuum. Azeotropic distillation with 2-MeTHF was repeated until the water content was measured below 0.2% by weight, typically obtained when adding a total of *ca*. 20 L 2-MeTHF, and a *ca*. 7 L solution is then obtained. To this solution at *ca*. 40 ^{o}C, was added TBME (5 L), and seeds of 4 salt (2 g). The resulting suspension was brought back to rt in 3 h, resulting in a thick, white suspension, collected by filtration on a Nutsche filter. The cake was washed with TBME/2-MeTHF (1:1, 5 L), and the solid was dried under vacuum at 60 ^{o}C overnight, resulting into 0.79 kg enriched **4** in purity > 90% as a white solid (quantitative yield). ^{1}H NMR (600 MHz, DMSO-d6): 0.69 (d, *J* = 6.55, 3H), 0.75 (d, *J* = 6.46, 3H), 0.78 - 0.96 (m, 27H), 0.99 (d, *J* = 6.85, 3H), 1.16 (d, *J* = 6.26, 3H) and 1.16 (d, *J* = 6.65, 3H), 1.29 - 1.53 (m, 6H), 1.54 – 1.66 (m, 8H), 1.71 (m, 2H), 1.92 – 2.01 (multiple s, 6H), 2.08 (m, 1H), 2.20 (m, 1H), 2.67 -3.03 (multiple s, 18H), 3.44-3.58 (m, 2H), 4.03-4.49 (m, 6H), 4.66 (m, 1H), 5.01 (d, *J* = 10.74, 1H), 5.08 (t, *J* = 7.96, 1H), 5.26-5.57 (m, 6H), 7.85 (d, *J* = 7.50, 1H), 7.99 (br s, 2H), 8.08 and 8.18 (d, *J* = 7.27, 1H), 8.39 to 8.56 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d6) due conformers mixture at room temperature, carbon signal could be splitted into 2 different signals. δ 10.24, 15.92, 16.30, 16.88, 17.08, 17.85, 17.94, 18.19, 18.48, 18.73, 18.76, 19.07, 20.71, 21.17, 21.60, 21.71, 21.96, 22.09, 22.36, 22.57, 22.92, 23.08, 23.59, 23.65, 24.15, 24.25, 23.35, 26.96, 29.30, 29.55, 29.76, 29.81, 30.77, 32.03, 32.78, 34.08, 34.37, 36.55, 36.65, 37.59, 44.73, 45.80, 48.12, 48.39, 50.82, 51.09, 53.60, 54.15, 54.45, 55.58, 57.42, 60.63, 61.96, 62.01, 74.05, 126.30 (brs), 129.26, 167.85, 167.88, 169.10, 169.52, 169.56, 169.76, 169.86, 170.15, 170.48, 170.63, 171.15, 170.48,

170.63, 171.15, 171.21, 171.64, 172.09; HRMS for $[C_{59}H_{106}N_{10}O_{13}+H]^+$, calcd 1162.80136, obsd 1162.80139.

2-(*N*-Methylacetamido)ethyl (2S)-2-{*N*-(tert-butoxycarbonyl)-*N*-methyl-*D*-alanyl-(2S,3R)-4-[4-(2-methoxyethyl)piperazin-1-yl]-3-methyl-2-(methylamino)butanoyl-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(2S,3R,4R,6E)-3-(acetyloxy)-4-methyl-2-(methylamino)oct-6-enamido}butanoate (**32**)

The solid dipeptide **5** (66.3 g, 0.14 mol) was charged to a reactor along with **4** (118.0 g, 0.094 mol), and dissolved into dichloromethane (0.9 L). The resulting mixture was cooled to 0 ^oC, NMM (48.0 g, 0.47 mol) was added dropwise while maintaining the temperature around 0 ^oC. The temperature of the reaction mixture was reduced to -15 ^oC, then HATU (50.0 g, 0.13 mol) dissolved into DMF (0.2 L) was added within 1.5 h, and the mixture was stirred until completion at that temperature (2 h, as determined by HPLC). Water (1 L) was added and the temperature was raised to *ca*. 5 ^oC. The heavy organic phase was removed and concentrated at *ca*. 50 ^oC under reduced pressure until a residue was obtained. It was then azeotropically distilled with toluene (1 L) to provide the crude product **32** (157 g, *ca*. 85% purity).

$C_{81}H_{146}N_{14}O_{18}$: ^1H NMR (600 MHz, DMSO-d6): due to conformer mixture and very broad line proton spectra, 4 protons couldn't be located precisely. 069 (d, J = 6.27,3H), 0.75 (d, J = 6.21, 6H), 0.78 - 0.92 (m, 33H), 1.16 (m, 6H), 1.37 (broad peak, 3H), 1.37 and 1.42 (s, 9H), 1.40 and 1.5 (m, 2H), 1.54 - 1.77 (m, 9H), 1.96 (m, 1H), 1.92 to 2.0 (s, 6H), 2.04 - 2.59 (very broad signal, 12H), 2.20 (m, 2H), 2.64 - 3.0 (multiple conformer of singulet, 24H), 3.23 (broad s, 3H), 3.45 (m, 2H), 3.47 - 3.57 (m, 2H), 4.08 (m, 1H), 4.11 - 4.20 (m, 2H), 4.23 (m, 1H), 4.41 and 4.49 (m, 1H), 4.63- 4.73 (m, 2H), 4.88 and 5.01 (m, 1H), 5.03 (m, 1H), 5.11 and 5.15 (m, 1H),5.29 (m, 1H), 5.31 (m, 1H),5.35 and 5.39 (m, 1H), 5.41 (m, 1H), 5.43 (m, 1H), 7.64 (m, 1H), 7.78 and

8.02 (m, 1H), 8.08 and 8.31 (m, 1H), 8.43 and 8.48 (d, J = 6.34, 1H); ^{13}C NMR (151 MHz, DMSO): due conformers mixture at room temperature, carbon signal could be splitted 2 different signal. δ 10.20, 14.63, 14.70, 15.87, 17.08, 17.81, 17.88, 18.21, 18.95, 19.04, 20.67, 21.14, 21.56, 21.70, 21.79, 21.91, 22.33, 22.53, 22.87, 23.14, 23.59, 23.63, 24.14, 24.23, 24.33, 26.93, 27.94, 28.07, 29.02, 29.51, 29.73, 29.78, 30.15, 30.60, 32.01, 32.75, 34.09, 34.36, 36.64, 36.72, 37.56, 37.59, 44.74, 45.80, 48.08, 48.36, 50.76, 53.48, 53.56, 55.55, 55.60, 56.05, 57.37, 58.03, 61.97, 74.04, 79.11, 79.52, 125.31, 126.23, 126.25, 128.20, 128.89, 129.25, 154.57, 167.81, 167.84, 169.45, 169.69, 169.83, 169.86, 170.07, 170.45, 170.59, 171.12, 171.18, 171.58, 171.81, 171.99; HRMS for $[C_{81}H_{148}N_{14}O_{18}+4\ H]^+$ calcd 1605.10850, obsd 1605.10926.

(2S)-2-{*N*-Methyl-*D*-alanyl-(2S,3R)-4-[4-(2-methoxyethyl)piperazin-1-yl]-3-methyl-2-(methylamino)butanoyl-*L*-valyl-*N*-methyl-*L*-leucyl-*L*-alanyl-*D*-alanyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-leucyl-*N*-methyl-*L*-valyl-(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)oct-6-enamide}butanoic acid (**33**)

Compound **32** (565 g, 0.35 mol) was charged to a 2 L stirred vessel and dissolved with methanol (0.8 L) to make for a *ca*. 40% wt **32** solution. A 10 L double-jacketed reactor equiped with anchor stirrer, chiller and dosing-pump was flushed with nitrogen was charged with methanol (0.7 L), and toluene (0.7 L), and 37% sulfuric acid (155.45 mL, 84.5 g) dropwise. The temperature rose from rt to *ca*. 40 °C and the slightly turbid emulsion was heated to 50 °C. To the reaction mixture at this temperature was added the solution of starting material in 1 h. At completion determined by HPLC (3 h), the temperature was reduced to 15 °C, and a 40% solution of benzyltrimethylammonium hydroxide (2.3 kg, 13.8 mol, 39 eq) methanol at 15 °C was added within 1 h, then water (0.4 L). At completion within 3 h, the temperature was brought

back to rt, the reaction mixture was diluted with more water (1.2 kg), and the pH was brought to 7.1 with dropwise addition of sulfuric acid (1.11 kg). Methanol (0.25 L) was added, and the resulting mixture was concentrated at 45 °C under reduced pressure until ca. 2 kg raw mixture, which was redissolved into ethyl acetate (3 L), and washed with water twice (1 L). The organic extract was concentrated at *ca*. 35 ^{o}C under reduced pressure to provide **33** as a white foam (459 g, in 78% purity, 75% yield), redissolved into THF (0.5 L), and added slowly to cold heptane (4.5 L) to precipitate the desired crude product **33**, which was isolated by filtration, and dried under high vacuum at 60 ^{o}C.

conformer mixture $C_{68}H_{125}N_{13}O_{14}$: ^{1}H NMR (600 MHz, DMSO-d6): 1NH and 2 OH not detectable at Room temperature 0.61-0.72 (m, 6H), 0.72-0.95 (m 30H), 1.13 - 1.31 (m, 12H), 1.30 (br.s, 1H), 1.33 - 1.43 (m, 3H), 1.45 - 1.73 (m, 9H),1.57 and 1.78 (m,2H), 1.93 - 2.14 (m, 3H), 2.19 (m, 1H), 2.22 - 2.34 (m, 4H), 2.35 - 2.48 (m, 11H), 2.73 - 2.78 (s, 3H), 2.83 - 2.86 (s, 6H), 2.87 - 2.94 (s, 3H), 2.95 - 3.03 (br.s, 3H), 3.09 - 3.17 (s, 3H), 3.21 (s, 3H), 3.39 (m, 2H), 3.87 - 4.02 (m, 4H), 4.02 - 4.17 (m, 1H), 4.25 (m, 1H), 4.44 - 4.58 (t, *J* = 7.5,1H), 4.65 (m, 1H), 4.76 and 5.04 (d, *J* = 9.7, 1H), 5.08 - 5.17 (m, 2H), 5.28 - 5.43 (m, 4H), 7.49 - 7.72 (m, 2H), 8.13 (m, 1H), 8.27 and 8.44 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): due conformer mixtures at room temperature carbon signal could be splitted into 2 different signals δ 11.08, 11.18, 15.01, 15.38, 15.48, 15.67, 15.80, 16.78, 17.43, 17.46, 18.10, 18.19, 18.24, 18.34, 18.38, 18.70, 18.74, 18.87, 19.32, 19.43, 19.84, 19.94, 20.38, 22.07, 22.32, 22.50, 22.75, 22.82, 23.06, 23.11, 23.21, 23.44, 23.67, 24.54, 24.64, 24.79, 27.06, 29.78, 29.96, 30.12, 30.36, 30.58, 30.96, 31.16, 32.27, 32.73, 33.77, 36.06, 36.19, 36.42, 37.19, 37.29, 38.02, 45.07, 45.16, 48.46, 51.24, 51.69, 53.57, 53.69, 53.99, 54.62, 54.67, 55.88, 57.48, 57.56, 58.44, 58.63, 59.67, 61.02, 62.05, 70.39, 70.47,

75.01, 126.14, 130.37, 169.05, 170.11, 170.17, 170.17, 170.23, 170.37, 171.01, 171.25, 171.78, 171.88, 172.08, 172.15, 172.45, 172.50, 175.44, 175.94.

(3S,6S,9S,12R,15S,18S,21S,24S,27R,30S,33S)-30-Ethyl-33-[(1R,2R,4E)-1-hydroxy-2-methylhex-4-en-1-yl]-24-{(2R)-1-[4-(2-methoxyethyl)piperazin-1-yl]propan-2-yl}-1,4,7,10,12,15,19,25,27,28-decamethyl-6,9,18-tris(2-methylpropyl)-3,21-di(propan-2-yl)-1,4,7,10,13,16,19,22,25,28,31-undecaazacyclotritriacontan-2,5,8,11,14,17,20,23,26,29,32-undecone (**1**)

Solid **33** from above (550 g, 0.32 mol, 75 % purity) suspended in a solution of TBME and Toluene (1:1, 3 kg each) is added portionwise over 10-12 h to a mixture of Li_2CO_3 (72 g, 0.98 mol, 3 eq), Dicyclohexylcarbodiimide (167 g, 0.80 mol, 2.5 eq) and 6-chloro-HOBt (10 g, 0.05 mol , 0.15 eq) in toluene (6.2 kg) at 40 °C. After an additional 2 hours aging, the solid DCU formed was removed by filtration. The filtrate was concentrated, filtered again and precipitated over Heptanes. The crude solid obtained (497 g, 74% yield, ca. 65% purity) was dissolved in 4 L Acetonitrile at 55 °C resulting into a yellowish solution. To the yellowish solution, 50 mL Water and Fumaric acid (25.5 g, 1.10 mol, 5 eq) were added. The thin suspension was stirred at 55 °C for 15 min until it turned into a clear yellowish solution. This solution was seeded with a few seed-crystal and the thin suspension was allowed to cool to rt within 10 h, and stirred at that temperature overnight. The slightly yellowish suspension was filtered. The collected white solid was washed with 200 mL Acetonitrile at rt, and further dried at 45 °C, under full vacuum over night. It resulted into 301 g of crystalline fumarate salt (purity > 97%). The resulting solid was charged to a reactor, and suspended into 1.5 L 2-Methyl-THF at rt. The mixture was heated to 45 ^{o}C . To the white suspension was added a 10% K_2CO_3 solution in 30 min. A clear solution was

observed within one third of the addition and a two layers at the end. The mixture was then cooled to rt, and the aqueous phase was removed. The organic layer was concentrated under reduced pressure, heated to 50 ^{o}C and distilled with TBME (2 x 3 L) to dryness. Further addition of TBME (3 L) and washing with water (2.5 L). MeTHF (0.75 L), then TBME (0.72 L), and water (30 mL) were added at 50 oC and the resulting mixture was cooled to 0 oC in ca. 10 h. Once a thick suspension was observed, heptane (0.4 L) was added dropwise and the resulting mixture was stirred for an additional 2 h. The solid was removed by filtration, washed with 0.75 L cold TBME/heptane (2:1), and dried under vacuum at 50 ^{o}C overnight to result into 236 g of material of purity > 99% (80% isolated yield). ^{1}H NMR (600 MHz, DMSO-d_6) 0.67 (d, *J* = 6.6, 3H), 0.80 - 0.70 (m, 16H), 0.82 (d, *J* = 6.6, 6H), 0.91 - 0.85 (m, 12H), 0.95 (d, *J* = 6.3, 3H), 1.07 (d, *J* = 6.5, 3H), 1.18 (d, *J* = 6.9, 3H), 1.24 (d, *J* = 7.1, 3H), 1.22 (m, 1H), 1.35 – 1.30 (m, 1H), 1.29 (m, 1H), 1.48 (m, 4H), 1.43 (br.s, 1H), 1.61 (d, *J* = 6.1, 3H), 1.55 (m, 1H), 1.68 - 1.63 (m, 1H), 1.80 - 1.68 (m, 3H), 1.90 – 1.84 (m, 1H), 1.93 (br.d, *J* = 9.9, 1H), 2.05 (t, *J* = 11.1, 1H), 2.27 - 2.10 (m, 6H), 2.38 (br.s, 4H), 2.42 (t, *J* = 5.8, 2H), 2.62 (s, 3H), 2.68 (s, 3H), 2.75 (s, 3H), 2.78 (s, 3H), 2.81 (s, 3H), 2.96 (s, 3H), 3.08 (s, 3H), 3.21 (s, 3H), 3.39 (t, *J* = 5.8, 2H), 3.95 (br.d, *J* = 8.7, 1H), 4.16 - 4.09 (m, 1H), 4.20 - 4.16 (m, 1H), 4.20 (br.s., 1H), 4.69 (q, *J* = 7.6, 1H), 4.78 (p, *J* = 6.9, 1H), 4.85 (d, *J* = 10.9, 1H), 5.14 (br.s, 1H), 5.06 (d, *J* = 10.7, 1H), 5.25 - 5.18 (m, 1H), 5.30 - 5.26 (m, 1H), 5.35 - 5.30 (m, 1H), 5.43 - 5.35 (m, 2H), 5.73 - 5.57 (m, 1H), 7.54 (d, *J* = 6.0, 1H), 8.19 (d, 1H), 8.20 (d, 1H), 8.64 (d, *J* = 6.1, 1H); ^{13}C NMR (151 MHz, DMSO) δ 10.19, 14.47, 14.68, 14.96, 17.45, 17.72, 17.93, 18.02, 18.53, 19.42, 20.74, 21.78, 22.08, 23.30, 23.56, 23.85, 24.24, 24.81, 24.98, 25.61, 29.15, 29.37, 29.47, 29.56, 29.78, 31.25, 31.37, 34.59, 34.91, 36.30, 36.34, 37,16, 37.58, 43.34, 48.20, 49.51, 49.91, 51.96, 53.32, 53.46, 55.09, 57.09, 57.14, 58.01, 58.13, 60.00, 69.96, 74.16, 126.79, 127.19, 168.55, 169.87, 170.23, 170.28, 170.30,

170.39, 170.54, 171.20, 171.70, 172.21, 172.79; HRMS for [$C_{69}H_{125}N_{13}O_{13}$+$H_3O^+$] calcd 1362.96982, obsd 1362.97058.

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Graphical abstract