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Scale-up synthesis of tesirine

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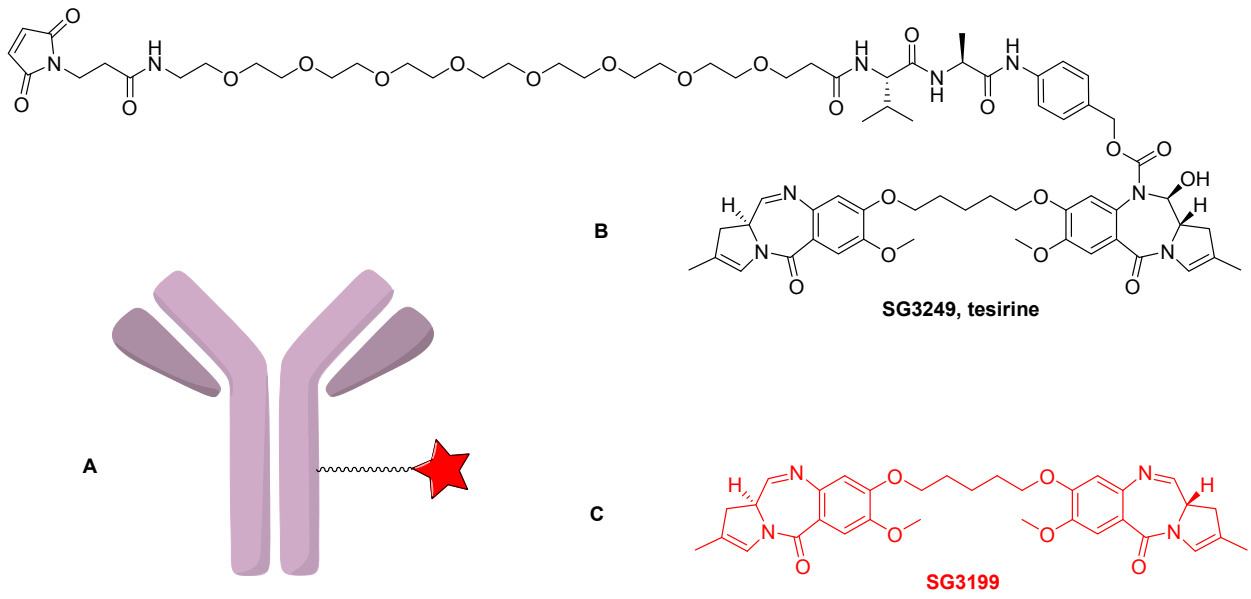
TOC Graphic



ABSTRACT

This work describes the enabling synthesis of tesirine, a pyrrolobenzodiazepine antibody drug conjugate drug-linker. Over the course of four synthetic campaigns, the discovery route was developed and scaled-up to provide a robust manufacturing process. Early intermediates were produced at kilogram scale and high purity, without chromatography. Mid stage reactions were optimized to minimize impurity formation. Late stage material was produced and purified using a small number of key high-pressure chromatography steps, ultimately resulting in a 169 g batch after 34 steps. At the time of writing, tesirine is the drug-linker component of 8 antibody-drug conjugates in multiple clinical trials, 4 of them pivotal.

KEYWORDS

Pyrrolobenzodiazepine

Antibody Drug Conjugate

High pressure chromatography

Loncastuximab tesirine

Camidanlumab tesirine

INTRODUCTION

During the last 10 years, antibody-drug conjugates (ADCs) have made a welcome addition to our arsenal in the fight against cancer. In 2011, Adcetris (Seattle Genetics) was approved for CD30 positive AML, followed by Kadcyla (Genentech/Roche) in 2013 to treat HER-2 positive breast cancers. Last year, two further approvals (Mylotarg and Besponsa from Pfizer) validated the ADC approach, where a potent anticancer agent is delivered specifically to an antigen-expressing tumour target. The ADC field is now expanding rapidly, with more than 60 agents in clinical trials and many more in pre-clinical development.[1-2]

An antibody-drug conjugate is typically represented as a three component system: the targeting antibody, the linker, and the active drug (Figure 1a). Although it is desirable to optimize and tune each and every one of these components, the linker and the drug can be treated as a single small molecular entity: the drug-linker. In addition, antibody-drug conjugates are modular systems; the same antibody may be used to deliver different classes of drugs. Similarly, the same drug-linker may be conjugated to any appropriate tumor-targeting antibody.

In 2012, tesirine (**SG3249**) was developed by Spirogen, as a drug-linker combining a set of desired properties: fast and straightforward conjugation to antibody cysteines by maleimide Michael addition, good solubility in aqueous/DMSO (90/10) systems, and a traceless cleavable linker system delivering the highly potent pyrrolobenzodiazepine (PBD) DNA crosslinker **SG3199** (Figure 1b,c).[3 4]

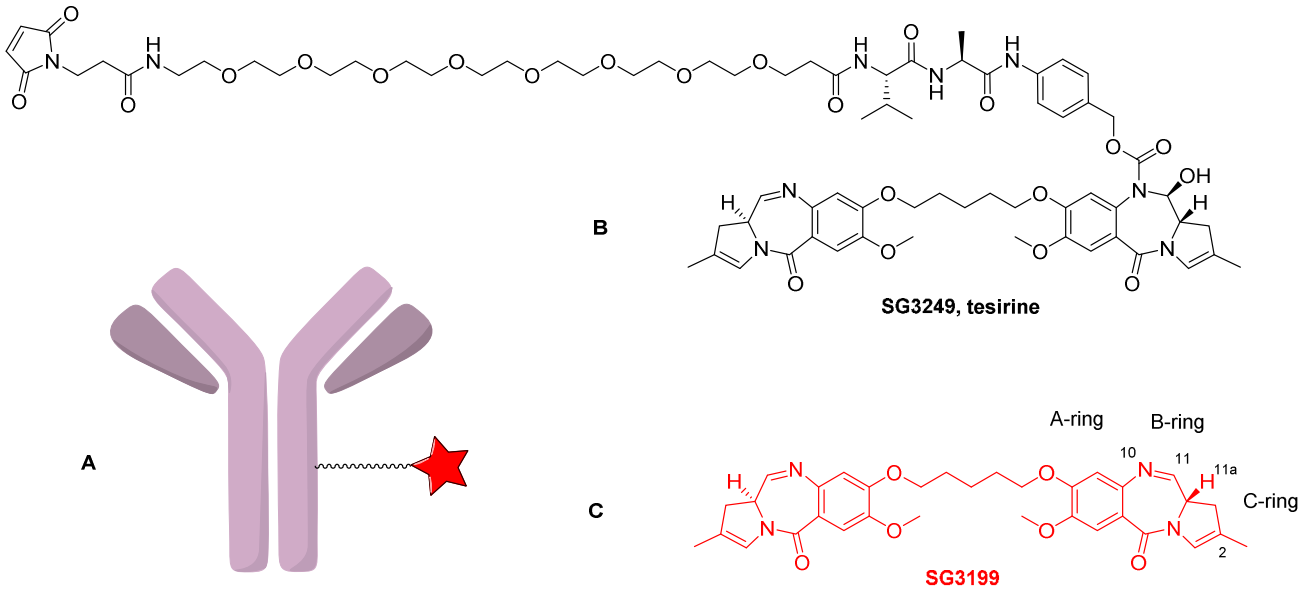


Figure 1: A) Schematic depiction of an antibody-drug conjugate; B) tesirine, a drug-linker featuring a dipeptide trigger; C) **SG3199**, the active drug released by tesirine cleavage in the presence of peptidases.

Antibodies conjugated to tesirine have been shown to be highly efficacious in preclinical studies.[5-8] As a result of the modular nature of antibody drug conjugates, and the wide interest for efficacious drug-linkers, tesirine was licensed to a number of companies. At the time of writing, tesirine was the drug-linker component of more than 15 clinical trials, either in solid or liquid tumours (see Table 1), and there is a considerable requirement for robust processes providing tesirine on scale.

Sponsor	Indication	Target	Phase
Medimmune/AZ	MM/AML	ASCT2	I
Medimmune/AZ	Prostate	PSMA	I
Medimmune/AZ	MM	BCMA	I
Abbvie	SCLC	DLL3	II Trinity
Abbvie	SCLC	DLL3	III Meru
Abbvie	SCLC	DLL3	III Tahoe
Abbvie	NETS	DLL3	I
Abbvie	Ovarian	DPEP3	I
Abbvie	Melanoma	DLL3	I/II
ADCT	Lymphoma	CD25	I
ADCT	AML/ALL	CD25	I
ADCT	B-NHL	CD19	I
ADCT	DLBCL	CD19	II
ADCT	B-ALL	CD19	I

Table 1: Some examples of key tesirine antibody-drug conjugates clinical trials.

In 2016, we reported the synthesis of tesirine on discovery scale.[9] Despite being modular and robust, the synthesis was not scalable without optimization. Indeed, flash chromatography was still used in the initial stages of the synthesis (where kilograms of intermediates are typically required to supply a phase I study), and excessively so in the later stages. A few key reactions

suffered from low yields, and other were the source of impurities difficult to separate from the desired material.

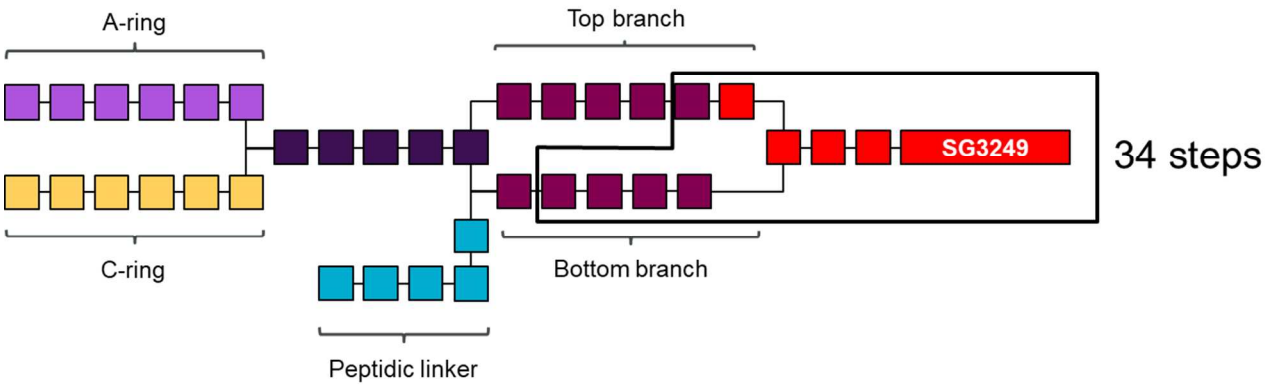


Figure 2: Block synthesis of **SG3249**. Boxed intermediates were synthesized in facilities equipped for high-potency cytotoxics. The longest linear sequence is 20 steps long.

In this article we describe the work carried out over four synthetic campaigns, to optimize this synthetic route on scale. Chromatographic steps were eliminated as often as possible, and especially in the early stages of the route. Synthetic processes were simplified, and often telescoped. Individual yields were improved, and impurities levels were controlled. Analytical methods for each intermediate and final product were optimized and performance tests were conducted to ensure accurate monitoring of the reactions and determine the purity of the products. Ultimately, these optimizations resulted in a synthesis with improved robustness and manufacturability, a purity increase from an original 85% to 97%, and the production of a 169 g batch. This batch size appears relatively small, but because of the potency of **SG3199**, the drug

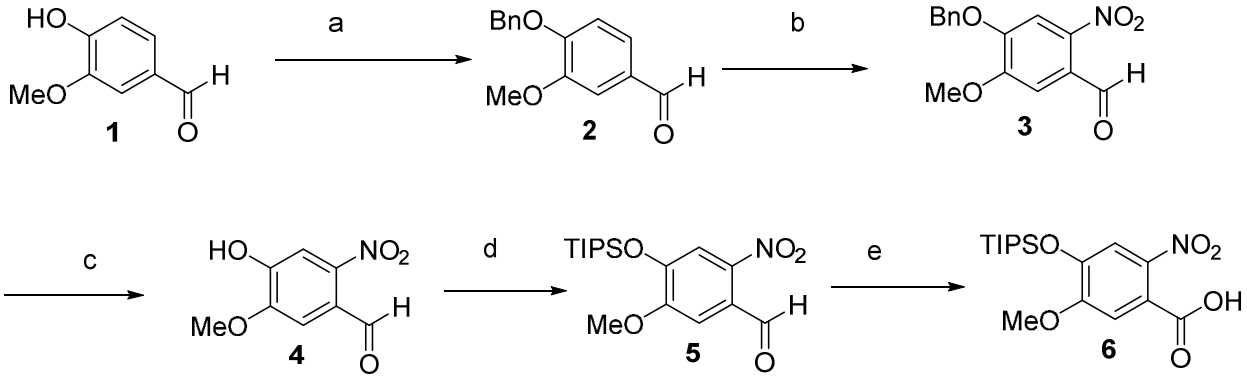
delivered by **SG3249**, only very small amounts are enough to achieve a pharmacological effect. To put this into context, a 169 g batch of **SG3249** would be theoretically sufficient to provide more than 100 000 doses of Lonca-T at the current clinical schedule (120 µg/kg q3w x2).[6, 10]

RESULTS AND DISCUSSION

In the early stages of the synthesis (Aromatic A-ring, proline C-ring, and Val-Ala peptide trigger), we sought to optimize the process for larger scale production. In particular, great efforts went into producing pure material whilst eliminating all chromatography steps.

Nitro-aromatic acid A-ring 6

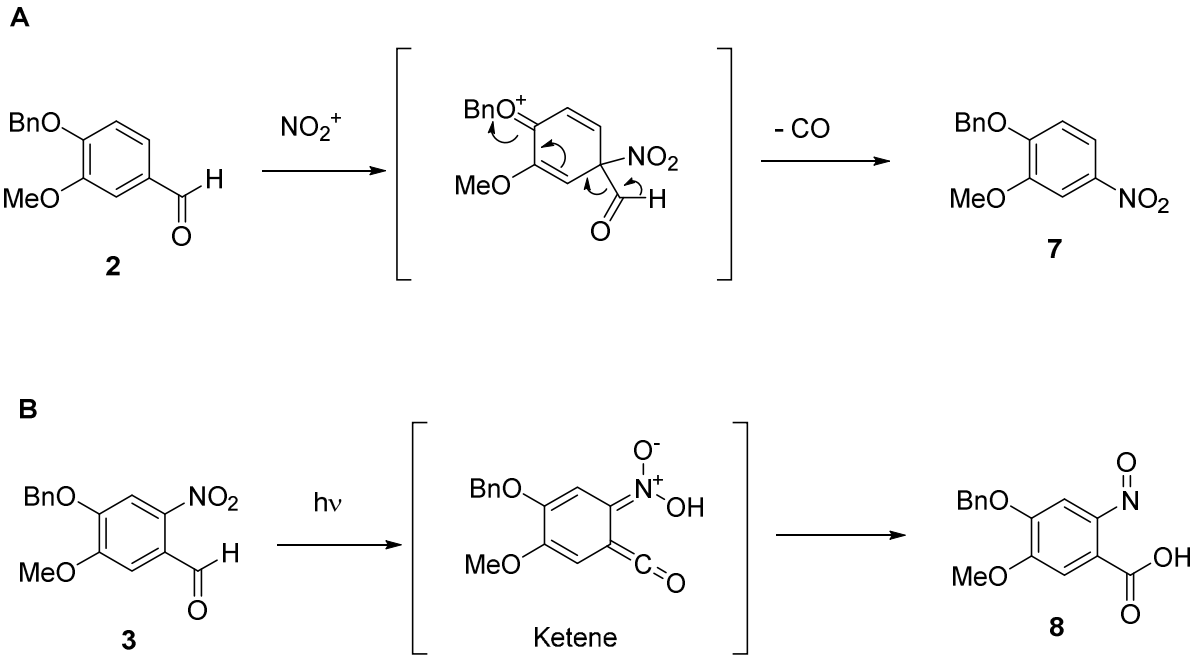
Scheme 1: Improved synthesis of TIPS-protected 6-nitrovanillic acid **6**



Reagents and conditions: (a) K_2CO_3, NMP, BnBr, 60 °C, 93%; (b) AcOH, HNO_3, 22 °C, 75%; (c) TFA, AcOH, 80 °C, 85%; (d) TIPSCl, triethylamine, THF, 10 °C, 100%; (e) Sulfamic acid, NH_4OH, NaH_2PO_4, $NaClO_2$, THF, water, 0 °C, 68%.

Benzylvanillin **2** is commercially available, but it was found to be more economical to produce it on site from vanillin and benzyl bromide. Originally, DMF was used as the solvent, but a screen revealed NMP to be kinetically advantageous, with good conversions in 1h at 60 °C. Isolation was straightforward after precipitation in water. Nitration of benzylvanillin was initially performed at 12 °C, aiming to minimize by-product formations, and in particular, the *ipso*-nitrodeformylation[11] product **7** (Scheme 2a). This side product is explained by the position of the formyl *para* to the benzyloxy group. In 1996, Cotelle and Cateau[12] postulated that *ipso*-nitrodeformylation can occur if the formyl group occupies the most (or one of the most) electron-rich position of the aromatic ring, thus explaining competition with nitration in position 6.

Scheme 2: a) *Ipso*-nitrodeformylation of benzylvanillin; b) Photodecomposition of 6-nitrobenzyvanillin



The nitration of benzylvanillin was found to be exothermic, and concerns over thermal accumulation on scale forced us to reassess the process. The order of addition was reversed

(adding a solution of benzylvanillin to the nitrating mixture), and the operational temperature was changed to 22 °C. At this temperature, nitration occurred almost immediately with very little thermal accumulation, and with a manageable level of **7** (12% by HPLC). An reaction calorimetry test (RC1) on 587 g of benzylvanillin (Figure 3) found heat generation to be easily controlled by the rate of addition. The heat of the process was 85.35 kJ and the corresponding molar enthalpy (ΔHr) was 147 kJ/mol. These parameters, together with the onset decomposition temperature of product **3** at 244 °C validated process safety for kg scale production. Precipitation in water followed by recrystallisation in ethyl acetate afforded the 6-nitrobenzylvanillin **3** in good purity (96.6%) and 75% yield. The product was protected from light, since *o*-nitrobenzaldehydes are known to photochemically convert to *o*-nitrosobenzoic acid (Scheme 2b).[13] Next, the benzyl ether was cleaved with TFA at 80 °C. The volume of TFA was kept to a minimum (2 V) because of its cost and potential environmental impact. AcOH (1V) was added to ensure efficient stirring. Phenol **4** was cleanly obtained by precipitation with heptane. With an overall yield of 59% and a purity of 98.7%, the results for this 3 steps sequence were found in line with the reports of Rakshit and co-workers.[14]

On research scale, we had protected phenol **4** in a solvent-free reaction; effectively melting TIPS-Cl and imidazole at 100 °C. Although this approach is attractive in terms of solvent volume reduction, the process was difficult to control on scale, and the reaction was potentially reversible. Instead, a base and solvent screen showed high conversions with triethylamine and TIPS-Cl in DCM. Crude aldehyde **5** was used directly in the Pinnick oxidation to provide carboxylic acid **6**. Here, the chlorine scavenger was swapped from hydrogen peroxide, to sulfamic acid, as described by Lindgren in his original conditions.[15] This has many advantages including improved safety (no oxygen production, scavenging of chlorine dioxide), and clean

removal in the aqueous phase during work-up. After slurrying in heptane, pure A-ring **6** was thus obtained in 40% yield over 5 steps, on kg scale, and avoiding any chromatography.

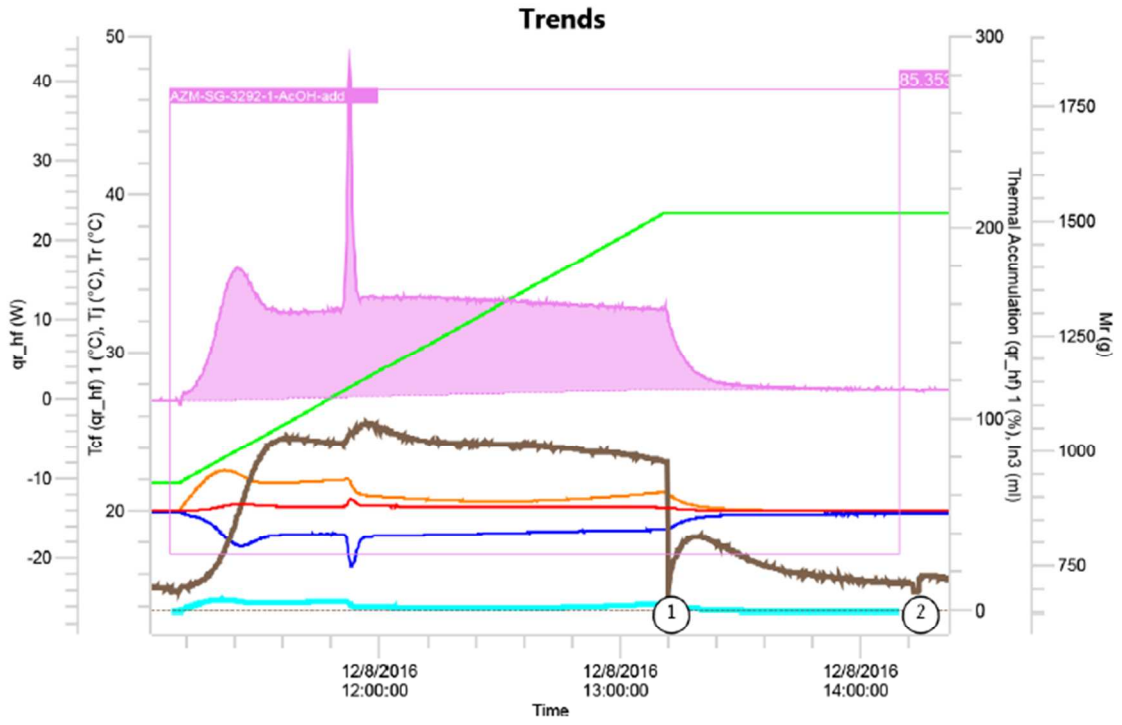
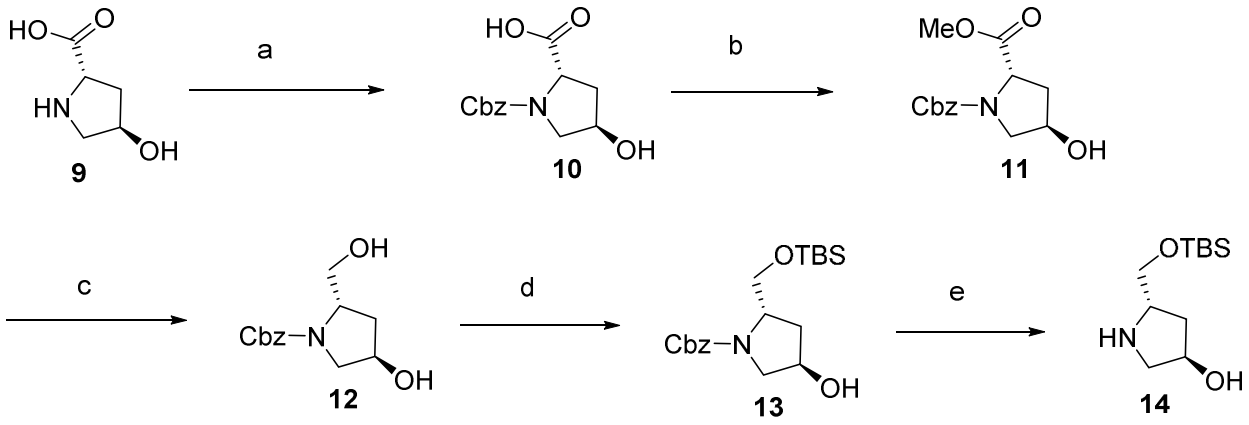


Figure 3: Reaction calorimetry test on benzylvanillin. 587 g of **2** (23.8 w/w %) was dosed into the reaction mixture at 20 °C during 2 h. The mixture was stirred for an additional 1h at the same temperature. Heat generation in pink. The sharp peak is caused by precipitation of the product.

Pyrrolidine C-ring 14

Scheme 3: Improved synthesis of Pyrrolidine C-ring **14**



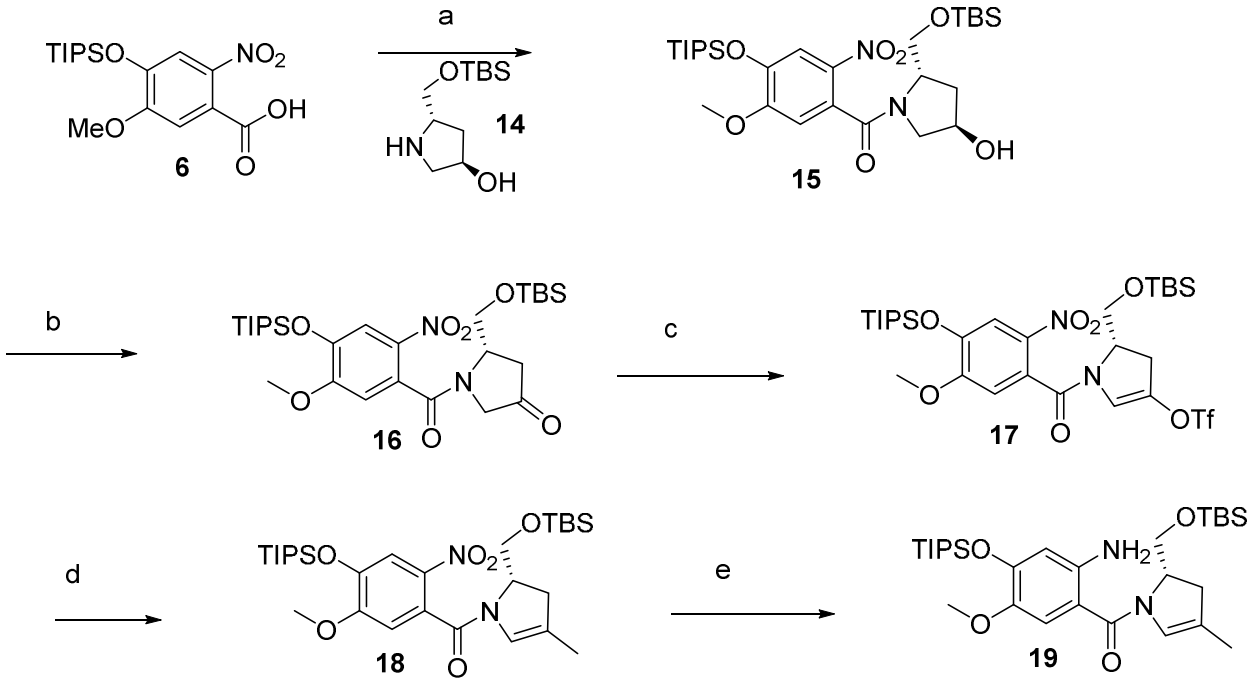
Reagents and conditions: (a) K_2CO_3, MTBE, water, Cbz-Cl, 15 °C, 100%; (b) MeOH, DCM, H_2SO_4, 40 °C, 81%; (c) THF, Water, LiCl, $NaBH_4$, 17 °C, 71%; (d) Toluene, triethylamine, TBSCl, 25 °C, 100%; (e) IPA, 10% Pd/C, H_2, 30 °C, 61%.

The synthesis of pyrrolidine C-ring **14** was conducted in parallel. The route described by Gregson and co-workers[16] was adapted on scale (Scheme 3). Benzyloxycarbonyl protection of *trans*-hydroxyproline **9** was achieved more advantageously in a MTBE/water system rather than toluene/water, with a high purity grade of Cbz-Cl in 100% yield. Classical esterification conditions employing methanol and catalytic sulfuric acid were found difficult to work-up on kg scale. Instead a 5/1 mixture of DCM/methanol was used, allowing for a convenient aqueous

work-up with $NaHCO_3$. Ester **11** was isolated in 81% yield (7.8 kg) after solvent evaporation. The reduction of the methyl ester to alcohol **12** had been readily achieved with $LiBH_4$ on research scale. However, the quantities of reactive $LiBH_4$ involved on kg scale posed a fire hazard, thus substitution with the more stable $NaBH_4$ was desirable. Trials with $NaBH_4$ alone showed that the reaction rate was much slower than with $LiBH_4$. Lithium chloride was added to produce $LiBH_4$ *in situ* thus conserving the initial reaction kinetics and low number of equivalents.[17] Next, achieving the selective silylation of a primary alcohol in the presence of secondary alcohol **12** was found to be key to avoiding chromatography on scale. Historically, this improvement of selectivity had been achieved by replacing imidazole with the bulkier DBU, and a low number of equivalents of TBS-Cl (0.77 equiv); altogether with moderate success. Chromatography was still required to remove the bis-silylated product, and recycle the starting material. Here, further screenings revealed triethylamine to be a more appropriate base for this transformation.[18] The rate of the reaction was reduced, but higher selectivity ratios were obtained (i.e.: SM/Primary silylation/Bis-silylation 6/89/5). The chromatography stage was eliminated and the crude **13** was used directly in the next step. After hydrogenolysis of benzyl carbamate **13**, the resulting amine was purified by precipitation as its oxalate salt. The impurities were removed in the filtrate, and amine **14** was isolated as its free base in 61% yield over two steps after a basic aqueous work-up. Altogether, **14** was synthesized in 35% over 5 steps, with high purity (99.5%), on kg scale without chromatographic purification. It was later established that high purity was necessary, both for A-ring **6** and C-ring **14**, to achieve good yields in the subsequent amide coupling.

Branching A and C ring aniline 19

Scheme 4: Improved synthesis of key intermediate **19**

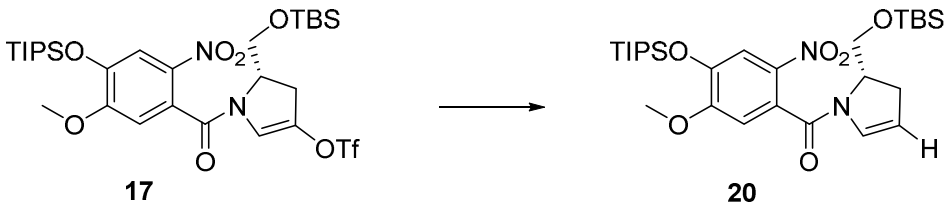


Reagents and conditions: (a) EDCI, HOPO, DCM, 15 °C, 82%; (b) TEMPO, KBr, NaOCl, $NaHCO_3$, 3 °C, 92%; (c) Tf_2O, 2,6-lutidine, toluene, -40 °C, (d) $MeB(OH)_2$, $Pd(dppf)Cl_2.CH_2Cl_2$, K_3PO_4, toluene, 65 °C, 44% (two steps); (e) Zn, AcOH, EtOH, water, 5 °C, 81%.

The conditions for the coupling of **6** and **14** reported in the research paper[9] (Scheme 4) suffered from a number of issues; DCC could not be removed easily by work-up, and HOBt is classed as a desensitized explosive, causing shipping restrictions and supply chain concerns. Instead, EDCI and HOPO[19] were used. Experiments conducted without HOBt or HOPO were substantially lower yielding, thus demonstrating the activating properties of these agents. The high purity of the starting materials, together with careful temperature control meant that very little impurities were produced during the reaction. The coupled product **15** could be isolated in 82% yield and 99.8% purity after crystallization from ethanol/water, on a 5 kg scale. In the next step, the secondary alcohol was oxidized to a ketone with TCCA/TEMPO. This combination is very efficient, but can result in undesired chlorination of aromatic groups and alkenes. We successfully substituted TCCA/TEMPO with DMP (89% on 4 kg batches). However, DMP is costly, shock sensitive and the work-up can be challenging. With this in mind, the team selected the simpler and cleaner Anelli-Montanari process[20] (TEMPO/Bleach in buffered biphasic conditions). Ketone **16** was obtained cleanly in 92% yield. Next, ketone **16** was transformed to the thermodynamic enol triflate **17** (with the unsaturation conjugated with the nitrogen in position 2,3). Although the conditions of this reaction were not dramatically altered, subtle changes in the number of equivalents of triflic anhydride (1.5 equiv instead of 3 equiv), and 2,6-lutidine (2 equiv instead of 4 equiv) meant that the amount of 2,6-lutidine triflate by-product was considerably reduced. This by-product acted as a poison in the subsequent Suzuki coupling and had to be controlled to a low level. The solvent was switched from DCM to toluene, allowing telescoping of the triflation with the next step. Introduction of the methyl group in C2 by sp^2-sp^3 Suzuki coupling was particularly challenging. The operational temperature of 65 °C is very close to the decomposition temperature of the triflate. Additionally, undesired reduction of **17** resulted

in triflate elimination to yield **20** (Scheme 5) which is difficult to separate from the product, throughout the remainder of the synthesis, even by chromatography. A conditions screen looking at the influence of bases, solvent, catalysts and temperature was conducted.

Scheme 5: Impurity formation during the sp^2-sp^3 Suzuki coupling



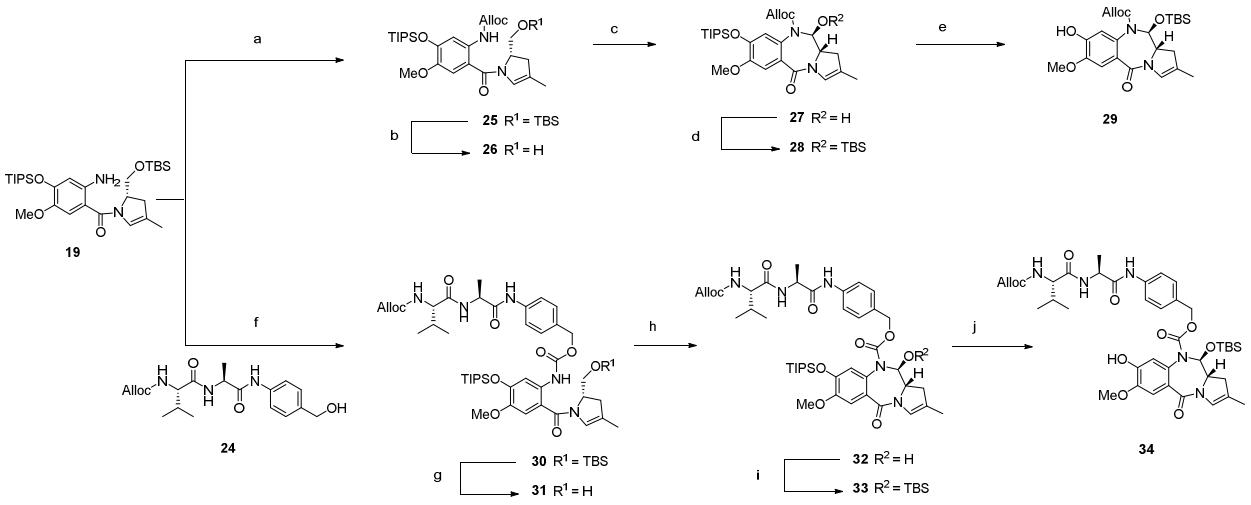
$Pd(dppf)Cl_2.CH_2Cl_2$ was found to be a superior catalyst to the original bis(benzonitrile)palladium(II) chloride, $Pd(PPh_3)_2Cl_2$, or the combination of palladium acetate and RuPhos. In 1984, Hayashi and co-workers[21] had suggested that the high activity of $Pd(dppf)Cl_2$ could be ascribed to the large P-Pd-P angle of the catalyst (99°). This, in turn, may explain the improved selectivity and reduction in side-products such as **20** caused by β-hydride elimination.[22] Potassium phosphate remained the best base, but a lower number of equivalents was used (3 equiv instead of 6 equiv) to avoid overloading the reactor with solid material. Toxic triphenylarsine and solid silver oxide were eliminated, thereby considerably improving the work-up. Other methyl donors such as trimethylboroxine or MeZnCl were explored with varying degrees of success, but did not afford improved conditions. Finally, a chromatographic step removed most impurities and controlled the level of by-product **20** down to 0.7%. As a result of

these improved conditions, batches of C2-methyl product **18** could be produced in 44% yield and over two steps, on kg scale. Next, the nitro functionality was reduced with Zn and AcOH to provide aniline **19**. Instead of pre-activating the zinc by washing it with dilute HCl, it was found more convenient to activate the zinc *in situ* by adding 5% water in the solvent (ethanol). Under these conditions, the reaction is rapid, even at low temperature (5 °C), and the exotherm can be controlled by the rate of addition of **18** to the reducing mixture. Amine **19** was isolated in 81% yield and 97% purity, (27% over 5 steps, 1 chromatography) and was used rapidly in the next steps due to its relatively low stability.

Carbamate protection of Amine 19

Amine **19** occupies a key position in the synthesis as it is the starting material for two parallel branches (Figure 2): the top branch with a simple alloc protection (Scheme 6), and the bottom branch with the inclusion of the Val-Ala peptidic trigger. Both branches rejoin later in a dimerization step to form **39**. Amine **19** was therefore split in two batches. A split factor was calculated based on subsequent yields and number of equivalents used in the dimerization. Initially, this split ratio was fixed at 0.39/0.61 top branch/bottom branch. However, as the yields improved and the number of equivalents in the dimerization step was optimized from 1.5 to 1.2 equiv, the split ratio changed to 0.58/0.42. It is likely that the ratio will be further optimized by subsequent campaigns improving the process robustness, and providing ever more reliable yields and equivalence factors. In any case, both branches follow the same chemistry, except for the carbamate formation with Alloc-Val-Ala-PAB-OH **24**.

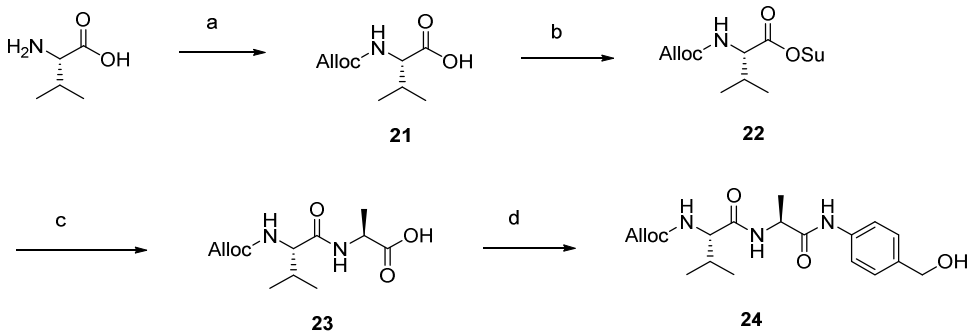
Scheme 6: Improved syntheses of carbamate protected PBD monomers **29** and **34**



Reagents and conditions: (a) Allyl chloroformate, pyridine, DCM, -5 °C, 84%; (b) PTSA, THF, Water, 35 °C, 87%; (c) Oxalyl chloride, DMSO, triethylamine, DCM, -70 °C, 81%; (d) TBS-OTf, 2,6-lutidine, DCM, 25 °C, 76%; (e) LiOAc, DMF, Water, 25 °C, 76%; (f) Triphosgene, **24**, triethylamine, DCM, 25 °C, 69%; (g) PTSA, THF, Water, 25 °C, 68%; (h) Oxalyl chloride, DMSO, triethylamine, DCM, -60 °C; (i) TBS-OTf, 2,6-lutidine, DCM, -15 °C; (j) LiOAc, DMF, Water, 40 °C, 71% (3 steps and 1 chromatography).

We have previously reported[23] the synthesis of peptide trigger building block **24**, following the studies of Dubowchik [24] and Jeffrey.[25] In this work, we have optimized the synthesis to allow production of highly pure material on 500 g scale (Scheme **7**). Most notably, in step **a**, different bases were screened to avoid formation of double alloc, or dimerisation impurities. The combination of NaOH/ Na_2CO_3 showed an improved purity profile. In step **d**, prolonged slurrying with MTBE controlled the by-product level of EEDQ condensation (quinoline) to very low levels. Compound **24** was obtained at a purity of 98.9%, in 48% yield over 4 steps and did not require chromatography. The chiral purity was found to be 99.9% by chiral HPLC.

Scheme 7: Improved synthesis of Alloc-Val-Ala-PAB-OH (**24)**



Reagents and conditions: (a) Allyl chloroformate, NaOH, Na_2CO_3, water, MTBE, 20 °C, 97%; (b) HOSu, DCC, DCM, 20 °C, 92%; (c) L-alanine, Na_2CO_3, THF, water, 25 °C, 79% (d) 4-aminobenzyl alcohol, EEDQ, THF, 25 °C, 68%.

Protection of amine **19** with allyl chloroformate and pyridine was straightforward, even on kg scale, and did not require further improvements. However, the hydrolysis of primary TBS ether

25 originally relied on AcOH, in a mixture of water, THF and methanol. The removal of AcOH on scale during the work-up was difficult. Instead, a method relying on low number of equivalents of acid was investigated. Ultimately, 0.6 equiv of tosic acid (PTSA) in wet THF was used, thus considerably simplifying the work-up. Flash chromatography purification was performed here, in order to provide a high grade of material **26** going into the next reaction. Oxidation of the primary alcohol gives an aldehyde which spontaneously ring-closes to form the B-ring of the PBD system (step c, Scheme 6). The Swern reaction is well suited to this transformation[26], and more so on manufacturing scale than on research scale. Indeed, the number of equivalents of oxalyl chloride had to be precisely controlled, or impurities such over-oxidized lactam and unreacted started material were observed. The best outcomes were obtained after a number of trial reactions, narrowing down on the optimum number of equivalents of oxalyl chloride. It goes without saying that the moisture content of the starting material was a key parameter, and Karl-Fisher determination helped inform the team when selecting a range of oxalyl chloride equivalences. Typically, a small excess was used to compensate for the remaining moisture. In this case, with a 0.23% water content (0.07 equiv water), 1.03 equiv of oxalyl chloride was used and the product **27** was found pure enough to be used as such in the next step (starting material / product / over-oxidation, 0.5 / 94.3 / 3.9). Protection of the secondary alcohol with TBS triflate and 2,6-lutidine did not require further optimization. Similarly, cleavage of the phenolic silyl ether with lithium acetate in wet DMF, proved to be a remarkably smooth, mild, high yielding and scalable method. A simple slurrying sequence in hexane and ethyl acetate provided key PBD monomer **29** with 99% purity and 34% yield over 5 steps, with a single chromatographic purification.

The second PBD monomer **34** was synthesized in an almost identical sequence, apart from the peptide trigger introduction by isocyanate chemistry. The chloroformate of benzylic alcohol **24** could not be used, since these types of compounds tend to eliminate CO_2 and form their chlorobenzyl analogues. On the other hand, aromatic aniline **19** is not nucleophilic enough to easily react with activated carbonates (although it must be noted that Smith and co-workers recently reported the clean condensation of an analogue of **19**, at room temperature over a period of 6 days, with a pentafluorophenyl carbonate[27]). For these reasons, the research route relied on formation of an isocyanate intermediate by reaction of amine **19** with triphosgene (a solid, safer alternative to phosgene gas). Several attempts were made to substitute triphosgene with low toxicity reagents, but in these instances, were found inferior to the isocyanate and alcohol condensation. A key improvement to this reaction was the simple solvent switch from (hygroscopic) THF to DCM.[28] Moisture levels had to be stringently controlled or urea side-products were observed as a result of isocyanate hydrolysis and self-condensation. Peptide **24** had a lower solubility in DCM than THF, but this was not found to be a limiting factor. These improved conditions allowed the number of equivalents of **24** to be lowered from 1.5 to 1.05, which in turn considerably simplified the work-up and chromatography. (An excess of **24** prevents silica gel chromatography by forming insoluble gel networks). Carbamate **30** was thus obtained in 69% yield. TBS deprotection with PTSA revealed primary alcohol **31** as above.

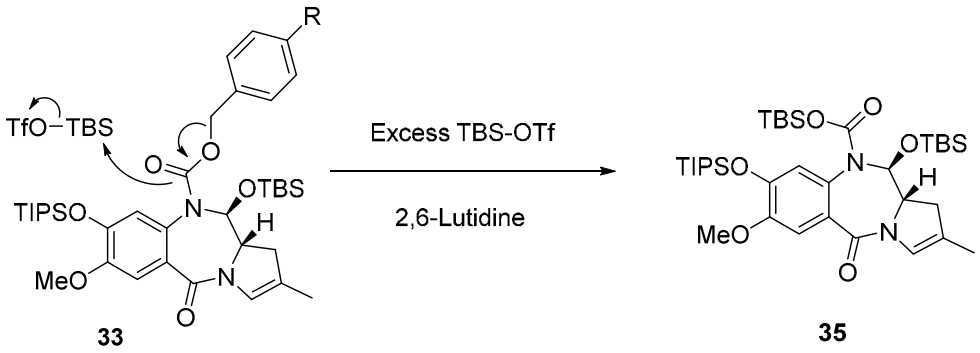
Structure-activity relationship (SAR) of PBD species highlighted the importance to treat the synthetic intermediates with caution from this point onward. Unless forming part of a pro-drug strategy, ring-opened PBDs lacking an imine moiety (or equivalent carbinolamine and aldehydes) are relatively non-toxic[29], and so are PBDs protected with biologically non-cleavable carbamates such as Alloc. For example, the IC_{50} of alloc protected phenol **29** could not be

measured (>10 μM, K562 CellTiter96 (MTS), 96 h incubation). But ring-closed intermediate **32** is protected by an enzymatically-cleavable dipeptide trigger, and could exert cytotoxicity *in-vivo.*

A change of strategy was therefore applied. The synthesis was completed in facilities equipped to handle highly-potent compounds. Batch splitting and chromatography were permitted if necessary, and high performance preparative chromatography was considered as a purification option.

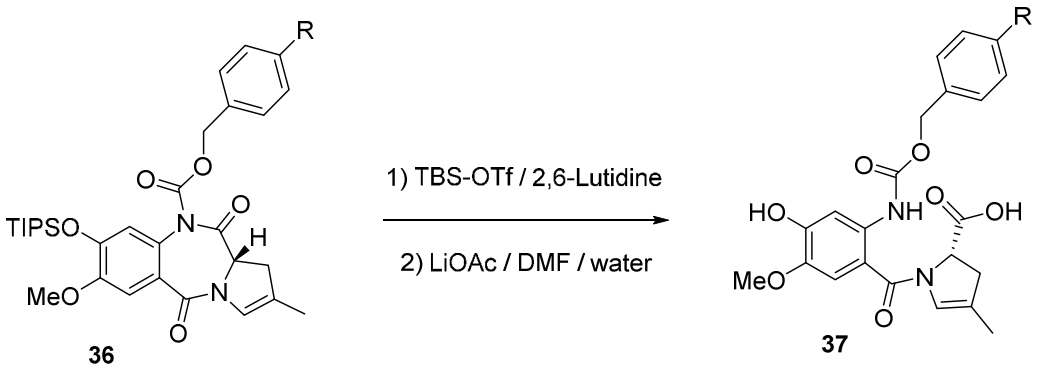
Primary alcohol **31** was ring-closed by Swern oxidation as described for compound **26**. Again, the oxalyl chloride equivalence was key, and in direct relation with the moisture content of the starting material. For example, if thorough water azeotroping with dry toluene was conducted, typical water levels would be at 0.06% w/w, and 1.03 equiv of oxalyl chloride were used. On the other hand, when azeotroping with toluene was not used, and the water content was measured at 0.2% w/w, 1.2 equiv of oxalyl chloride was used to fully consume the starting material and limit the formation of over-oxidized impurity **36** (Scheme 9). The use of lower temperature could not be considered to control over-oxidation due to lower solubility of the starting material in DCM. Because of the cytotoxic properties of these PBD intermediates, isolation in solid form was not a preferred option. Instead, the material was kept in solution (based on appropriate stability data in solution) and used directly in the next step. Protection of secondary alcohol **32** with TBS-OTf and 2,6-lutidine initially gave unacceptable levels of impurities. Mass analysis of the crude LC profile showed up to 13% of TBS-OTf mediated carbamate cleavage product **35**, presumably in a reaction analogous to the BOC cleavage described by Sakaitani and Ofhune[30] (Scheme 8).

Scheme 8: Postulated benzyl carbamate cleavage with TBS-OTf and 2,6-lutidine.



A conditions screen looking at temperature and number of equivalents concluded that -15 °C was low enough to avoid this side reaction, whilst allowing the protection to proceed (albeit slowly). An excess of TBS-OTf (5 equiv) and 2,6-lutidine (6 equiv) was used to obtain full conversion in 20h. The solubilization of TBS-OTf in DCM proved to be useful for a better control of the addition. Again, this step was telescoped with the next one to avoid any solid isolation. The phenolic triisopropylsilyl ether was cleaved with lithium acetate in wet DMF as before, but it was found that the reaction kinetics could be improved at 40°C without any degradation. The extraction solvent was changed from ethyl acetate to Me-THF to avoid carrying traces of DMF in the organic phase, which would have negatively impacted on the subsequent normal phase chromatography. The removal of acid **37** (resulting from over-oxidation during the Swern reaction, Scheme 9) was efficient with additional $NaHCO_3$ wash. Remarkably, this three steps sequence yielded pure phenol **34** after high pressure chromatography and precipitation in 71% yield. This key intermediate was isolated as a solid.

Scheme 9: Postulated conversion of over-oxidation impurity **36** to acid **37**.

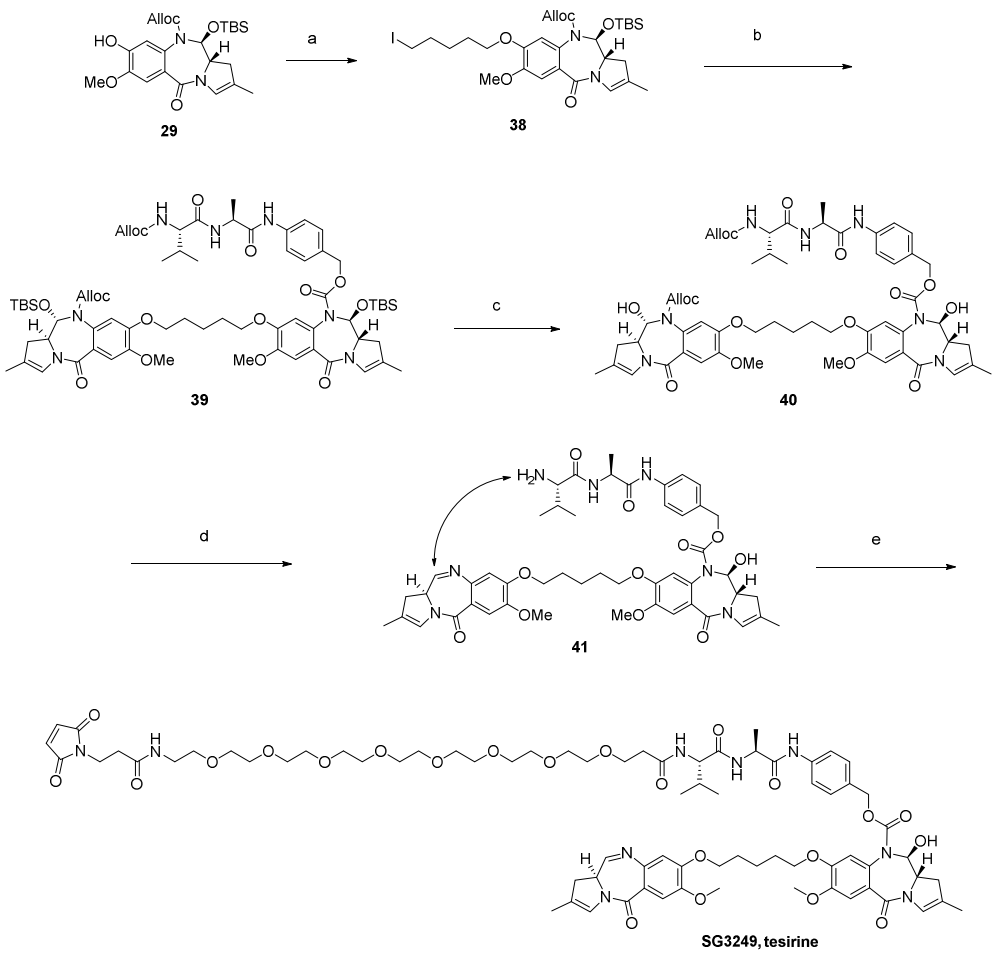


36 37

Next, the two phenolic monomers **29** and **34** were dimerized in two separate Williamson etherification steps. In both steps, acetone was replaced with MEK to allow higher operating temperatures, and a fine grade (typically ≤250 μm) of potassium carbonate was used to improve reaction kinetics. First, iodoalkane **38** was made by reacting phenol **29** with 5 equivalents of diiodopentane. This high number of equivalents not only drives the reaction kinetics forward, but also helps control the amount of homodimerization to below 10%. An aqueous work-up was implemented to remove the reaction salts. Iodoalkane **38** was isolated by high pressure chromatography and was stored in solution in ethyl acetate and used as such. This strategy presents some advantages on scale due to the oily nature of **38** which prevents straightforward transfers. In the second Williamson etherification, **38** and **34** were condensed under similar conditions. An important factor here is the excess of iodoalkane **38** versus phenol **34**. The discovery route employed 1.5 equiv of **38** to fully consume **34** and ensure straightforward chromatography. However, as was discussed above, this equivalence factor is taken into account 7 steps earlier to calculate the proportion of amine **19** to commit to each branch. When the yields of the lower branch were dramatically improved, the amount of iodoalkane **38** became unbalanced with the quantities of phenol **34** available. Fortunately, the dimerization was found to work cleanly with a reduced equivalence of **38** (1.2 equiv), which partially restored the balance

between the two branches of the route. A good conversion was obtained and crude dimer **39** was taken directly to the next step without any purification.

Scheme 10: Final stages. Synthesis of tesirine (**SG3249**).

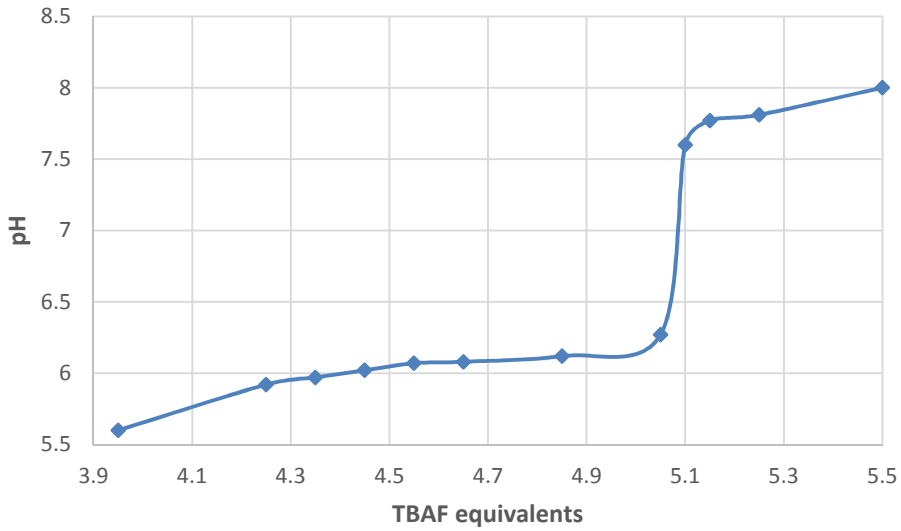


Reagents and conditions: (a) Diiodopentane, K_2CO_3, MEK, 75 °C, 88%; (b) **34**, K_2CO_3, MEK, 75 °C; (c) TBAF, AcOH, THF, 20 °C, 89% (2 steps); (d) $Pd(PPh_3)_4$, pyrrolidine, DCM, 20 °C; (e) Mal-$(PEG)_8$-acid, EDCI, DCM/MeOH, 20 °C, 75% (2 steps).

We have previously described how unbuffered fluoride deprotection of the secondary TBS ethers in **39** caused partial racemization of a key stereocenter in C11a.[9] This base driven mechanism can be prevented by buffering the mixture with a mild acid such as AcOH. Although this represented a successful solution to the problem, we quickly noticed that the reaction kinetics dropped in line with the pH – the reaction being unacceptably slow at acidic pH (< 4). This

meant that a relatively narrow and optimum pH range (around 6.0) was controlled by the precise equivalence of acid and TBAF. To add to our difficulties, and to our surprise, the actual fluoride content of the TBAF solutions varied considerably between batches. These inconsistencies were observed on multiple occasions, in different countries.

Figure 4: AcOH titration of a weak batch of TBAF



Note: Titration of 4 equiv of AcOH with a weak batch of TBAF in THF/water 80/20.

For example, when the reaction would not reach completion during one of the synthetic campaigns, an acid-base titration (Figure 4) revealed that 5 “equiv” of this batch of TBAF were required to neutralize 4 equiv of AcOH. The ratio used during the experiment was 4/ “3.9” AcOH/TBAF which at this pH (5.5) prevented the reaction proceeding rapidly. The actual concentration of the TBAF solution was found to be 0.72M (by anion-exchange chromatography; see supporting information)[31] instead of 1.0M, thus explaining these inconsistencies. During a subsequent campaign, the reaction proceeded very rapidly, and partial

racemization was detected in the final step. This time, the concentration was calculated to be 1.09 M instead of 1.0 M. On addition, fluoride precipitation can occur in bottles stored at 2 to 3 °C. With such wide error margin existing between real concentrations, and stated concentrations, we introduced a strict quality control of the TBAF solutions, to determine their exact concentrations before proceeding with the reaction (see supporting information for titration method). In order to ensure the robustness of this critical step, a DoE study (supporting information) was carried out to evaluate the impact of the main parameters (number of equivalents of TBAF per TBS, and ratio TBAF/AcOH) on the conversion and diastereoisomer content. The ratio TBAF/AcOH was the most important parameter to control the diastereoisomer content. A low variability was found in the defined range 0.5-0.8, where the model was stable. The amount of TBAF had a low impact on the diastereoisomer content. On the other hand, the model was found to be linear for the conversion response. The amount of TBAF can be increased to improve the kinetics of the reaction, while keeping a constant ratio of TBAF/AcOH. This DoE screen revealed that the reaction kinetics could be improved by adding further TBAF/AcOH, and that a margin of safety could be conserved by carrying out the reaction with a slight excess of AcOH. The final conditions were 1.5 equiv of TBAF per TBS, and 0.63 equiv of TBAF per AcOH. In these conditions, the reaction proceeded quite slowly (48 h), but guaranteed a low level of racemization (<1%). High performance normal phase chromatography provided product **40** in 88% yield over two steps.

Next, the alloc carbamates were deprotected with *tetrakis*(triphenylphosphine)palladium(0) (0.02 equiv instead of 0.06 equiv) as described previously. Even with this reduced number of equivalents, the reaction was rapid (30 min) and clean. A point of interest was the solubility limit of the product in DCM, which became an issue upon scale-up. Relatively large volumes (27 V

for the reaction, and 90 V for the extraction) had to be used to avoid losses upon precipitation. To mitigate this issue, amine **41** was stored in a DCM / methanol mixture (95 / 5; 15 V) for improved solubility and transfer purposes, and was used relatively rapidly. Prolonged solution storage has been shown to favor the macrocyclic form of **41**. Interestingly, this macrocycle cannot be observed under the acidic aqueous HPLC conditions. However, it may be observed as a streak on the TLC as it opens-up on the mildly acidic silica gel. (Figure S1, supporting information). Understanding the different conditions controlling the equilibrium towards the ring-opened (reactive) or ring-closed (unreactive) forms of **41** proved crucial to drive the amide bond formation to completion in the final step. A moderate excess of EDCI and Mal-$(PEG)_8$-acid (1.2 equiv) in DCM/MeOH (95/5) created a mildly acidic environment, which favored the opening of the macrocycle and a full conversion. Many commercial batches of Mal-$(PEG)_8$-acid were found to contain shorter Peg chains impurities such as Mal-$(PEG)_7$-acid which can impact the final purity of **SG3249.** As a result, careful quality monitoring of the Mal-$(PEG)_8$-acid was introduced to select the purest (99%) material. Crude **SG3249** was purified by high performance reverse phase chromatography, in mildly acidic conditions (0.01 % AcOH). These conditions afforded a product of high purity, by removing any trace of starting materials and other conjugable impurities. It is worth noting that more aggressive conditions (higher acid concentration, heat) can trigger the aromatization of the PBD C-ring; an impurity with reduced biological activity[32]. Finally, high purity solid **SG3249** was obtained after extraction and concentration under vacuum (169 g, 75% yield over two steps, one chromatography).

CONCLUSIONS

This article summarizes the work accomplished over four scale-up campaigns to provide clinical grade tesirine (**SG3249**), in quantities large enough to support multiple clinical trials. Although the overall yield of the longest linear synthetic sequence (20 steps) was moderately improved from 2.02% to 2.22%, the yield of the 7 linear steps carried out in high-potency facilities was more than doubled, at 46.2% from an original 19.6%. The final purity was improved from 85% in the first discovery batches, to above 97% in this work. Significantly, the number of chromatographic steps was reduced from 17 to 8. Vanillin and proline-derived early stage intermediates **6** and **14** were produced safely on multi-kg scale without relying on chromatography. The process robustness was considerably improved, with a better understanding of the reactions conditions and side-products. At the time of writing, tesirine is the drug-linker component of 8 antibody-drug conjugates in multiple clinical trials, 4 of them pivotal.

EXPERIMENTAL SECTION

From compound **32** onwards, syntheses were performed in facilities equipped to handle highly potent agents. These intermediates were typically stored in solution to minimize chances of exposure. Compound **34** and **SG3249** were isolated as solids. The purity stated was determined by HPLC area % (absolute area). The concentration of key late stage intermediates held in solution was determined by quantitative HPLC.

All anhydrous solvents, reagents and solvents were commercially obtained and used without purification. Pd(dppf)Cl2.CH2Cl2 was produced on site by Pharmaron and used as such. 1N

TBAF in THF was purchased from Sigma Aldrich. Early stage analytical HPLC were acquired on an Agilent 1260 equipped with a Photo Diode Array detector (PDA) detector. Late stage analytical HPLC were acquired on a Waters Alliance 2695 equipped with a Waters 2996 PDA. Late stage UPLC analyses were performed using a Waters UPLC H-class system with a PDA detector and a QDA mass detector equipped with an electrospray ionization (ESI) interface. Late stage mass spectra analyses were recorded on a Waters LC-MS ZQ 2000 system in positive electrospray ionization mode (ESI). Water content was determined by titration in a Metronhm 917 titro processor according to the reference method of Karl Fisher. HYDRANAL coulomat AG (Riedel-de-Haën) and Water standard 1.00 (Riedel-de-Haën) were used as solvent and reagent for titration. Impurities structures were postulated from mass spectroscopy analysis and known synthetic pathways.

Experimental procedure. *4-(Benzyloxy)-3-methoxybenzaldehyde (**2**).* Potassium carbonate powder (5.12 kg, 37.1 mol, 1.2 equiv) was added to a mixture of vanillin (4.7 kg, 30.9 mol), in anhydrous N-methyl-2-pyrrolidone (NMP, 18.8 L, 4 V). The reaction mixture was pre-heated to 45 °C. Benzyl bromide (3.86 L, 32.4 mol, 1.05 equiv) was added dropwise to the reaction mixture. An exotherm was allowed to proceed, and the temperature maintained at 60 °C for 1h or until completion. After cooling to 25 °C, water (37.6 L, 8.0 V) was added and the reaction mixture was stirred for 1h. The solids were isolated by filtration and washed with water (4.7 L, 1.0 V). The crude product was dissolved in EtOAc (7.05 L, 1.5 V) at 55 °C. Heptane (23.5 L, 5.0 V) was then added to the mixture at 50 °C. The mixture was cooled to 5 °C. The solids were collected by filtration and dried under vacuum at 40 °C to give benzylvanillin (4-(benzyloxy)-3-methoxybenzaldehyde **2** (7.0 kg, 100 HPLC area % purity, 93% yield). t_R: 6.3 min.

*4-(Benzyloxy)-5-methoxy-2-nitrobenzaldehyde (**3**).* AcOH (3.3 L, 1V) was added to 70% nitric acid (13.2 L, 17 equiv, 4V) at 22 °C in a 50L reactor. A solution of benzylvanillin (3.3 kg, 13.6 mol) in AcOH (9.9 L, 3V) was added dropwise whilst controlling the temperature at 22 °C (+/- 2 °C). The reaction mixture was stirred for 1h at 22 °C. The mixture was poured on ice/water (66 L, 20 V) and stirred for 1h. The solids were isolated by filtration and washed with water (9.9 L, 3V). The crude product was dissolved in EtOAc (6.6 L, 2 V) at 75 °C and stirred for 1h. The solution was cooled slowly to 15 °C and allowed to stand for 2h. The solids were collected by filtration and dried under vacuum at 40 °C to yield 4-(benzyloxy)-5-methoxy-2-nitrobenzaldehyde **3** (2.95 kg, 96.6 HPLC area % purity, 75% yield). t_R: 6.9 min.

*4-Hydroxy-5-methoxy-2-nitrobenzaldehyde (**4**).* AcOH (5.8 L, 1V), followed by TFA (11.6 L, 2V), was loaded in a 50L reactor under nitrogen at room temperature. *O*-benzyl-6-nitrovanillin (5.8 kg, 20.2 mol) was loaded, and the reaction mixture was heated at 80°C for 4h, when completion was observed by HPLC. After cooling to 25 °C, the reaction mixture was added to heptane (29 L, 5V), and stirred for 1h. The product was isolated by filtration, washed with heptane (5.8 L, 1V), and dried under vacuum at 50 °C to yield 6-nitrovanillin **4** (3.4 kg, 98.7 HPLC area % purity, 85% yield). t_R: 3.7 min.

*5-Methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzaldehyde (**5**).* 6-nitrovanillin **4** (3.3 kg, 16.7 mol) was dissolved in THF (23.1 L, 7 V) in a 50 L reactor under nitrogen, and cooled to 5 °C. Triethylamine (2.57 L, 18,4 mol, 1.1 equiv) was added, followed by TIPSCl (3.55 kg, 18.4 mol, 1.1 equiv) batchwise. The reaction mixture was stirred at 10 °C for 2h, when reaction completion was observed by HPLC. The solids were removed by filtration, and washed with THF (5 L, 1.5 V). The filtrate was used directly in the next step (total THF volume 8.5 V). (98.4 HPLC area % purity). t_R: 10.4 min.

*5-Methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoic acid (**6**).* Water (33L, 10 V), sulfamic acid (1.13 kg, 11.7 mol, 0.7 equiv), ammonium hydroxide (345 mL, 5.0 mol, 0.3 equiv), and sodium phosphate monobasic (NaH_2PO_4) (3.0 kg, 25.0 mol, 1.5 equiv) and sodium chlorite (4.34 kg, 38.4 mol, 2.3 equiv, 80% pure) were loaded in sequence to a 50 L reactor at room temperature, and the reaction mixture was cooled to 0 °C. The previously prepared THF solution of 5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzaldehyde (16.7 mol, 1 equiv) was added dropwise whilst controlling the temperature at 0 °C (+/- 5°C). The reaction mixture was stirred for 1h at 0 °C (+/- 5°C), at which point HPLC showed reaction completion. The reaction was quenched with saturated aqueous sodium thiosulfate (4 V) at 0 °C (+/- 5°C). The pH was adjusted to pH 3 to 4 with conc aqueous HCl. The reaction mixture was extracted with ethyl acetate (39.6 L, 12 V) x 2. The organic layers were combined and washed with brine (26.4 L, 8 V). The mixture was concentrated under vacuum (6.6 L, 2 V) and slurried with hexane (13.2 L, 4 V). The product was isolated by filtration, washed with hexane (3.3 L, 1 V) and dried under vacuum at 40 °C to yield 5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoic acid **6**. (2.15 kg, 98.7 HPLC area % purity, 68% yield (2 steps)). t_R: 9.3 min.

*(2S,4R)-1-((Benzyloxy)carbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (**10**)*

trans-4-Hydroxy-L-proline (4.5 kg, 34.3 mol) and potassium carbonate (5.92 kg, 42.9 mol, 1.25 equiv) were added to a mixture of MTBE (18 L, 4 V) and water (22.5 L, 5 V). Benzyl chloroformate (5.39 L, 37.7 mol, 1.1 equiv) was added dropwise at 15 °C. After 3h, completion was observed by HPLC. The organic phase was discarded. The aqueous phase was stripped with ethyl acetate (18 L, 4 V). The organic phase was discarded. The aqueous phase was mixed with ethyl acetate (18 L, 4 V) and was acidified with conc. HCl dropwise until pH = 2 at 15 °C. The organic phase was collected, and the aqueous phase was extracted with a further 18 L (4 V) of

ethyl acetate. The organic phases were combined and concentrated down to a volume of 13.5 L (3 V). Ethyl acetate (13.5 L, 3 V) was added and the combined organic were concentrated down to a volume of 13.5 L (3 V). This step was repeated with methanol (13.5 L, 3 V) twice, and the solution was used as such in the next step. (97.3 HPLC area % purity). t_R: 5.2 min. (100 Chiral HPLC area % purity) (t_R: 6.9 min; Enantiomer t_R: 8.3 min).

*1-Benzyl 2-methyl (2S,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (**11**)*

Methanol (9 L, 2 V) and DCM (45 L, 10V) were added to the methanolic solution of **10** (13.5 L, 3 V, 34.3 mol). Sulfuric acid (286 mL, 5.15 mol, 0.15 equiv) was added, and the reaction mixture was heated at 40 °C for 12 h. Reaction completion was verified by HPLC. The reaction mixture was neutralized with 5% aqueous $NaHCO_3$ (22.5 L, 5 V) to pH = 7-8 at 20 °C. The organic phase was washed with water (22.5 L, 5 V), and dried over sodium sulfate. The solvents were removed by evaporation to yield **11**. (7.8 kg, 100 HPLC area % purity, 81% yield (2 steps)). t_R: 6.7 min. (100 Chiral HPLC area % purity) (t_R: 10.4 min; Enantiomer t_R: 14.1 min).

*Benzyl (2S,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidine-1-carboxylate (**12**)*

Water (1.7 L, 0.66 V) and lithium chloride (513 g, 12.1 mol ,1.3 equiv) was added to a solution of ester **11** (2.6 kg, 9.3 mol) in tetrahydrofuran (26 L, 10 V). Sodium borohydride (458 g, 12.1 mol ,1.3 equiv) was added batchwise at 17 °C and the reaction mixture was stirred for 5h at 17 °C. Reaction completion was verified by HPLC. 2N HCl (13 L, 5 V) was added dropwise, whilst keeping the temperature below 15 °C. The pH was adjusted to 7-8 with saturated aqueous $NaHCO_3$ (15.6 L, 6 V). The mixture was concentrated (28 .6 L, 11 V) below 45 °C, and extracted with ethyl acetate 3 x (13 L, 5 V). The organic phases were combined, washed with

brine (13 L, 5 V), and dried over sodium sulfate. The volatiles were removed by evaporation to yield **12**.

(1.67 kg, 98.0 HPLC area % purity, 71% yield). t_R: 5.4 min. (100 Chiral HPLC area % purity) (t_R: 18.4 min; Enantiomer t_R: 13.4 min).

*Benzyl (2S,4R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1-carboxylate (**13**)*

12 (5 kg, 19.9 mol) was added to a mixture of triethylamine (4.16 L, 29.8 mol, 1.5 equiv) and toluene (29 L, 5.8 V). tert-Butyldimethylsilyl chloride (3.6 kg, 23.9 mol, 1.2 equiv) was then added at room temperature. The mixture was stirred for 18h at 60°C. Water (20 L, 4 V) was added and the reaction mixture was allowed to stir for 1h at 25 °C. The organic phase was separated and washed with brine (16 L, 3.2 V). The volatiles were removed by evaporation. The residue was dissolved in isopropanol (25 L, 5 L). The final volume was adjusted to 20 L (4 V) by evaporation. The solution of **13** was used as such in the next step. (90.5 HPLC area % purity). t_R: 10.4 min. (100 Chiral HPLC area % purity) (t_R: 30.2 min; Enantiomer t_R: 27.1 min).

*(3R,5S)-5-(((tert-Butyldimethylsilyl)oxy)methyl)pyrrolidin-3-ol (**14**). N.B.: This hydrogenolysis was split in four batches of identical sizes. Weight and volumes are given for the total.* The solution of **13** in isopropanol (19.9 mol, 1 equiv) was diluted with isopropanol (4.5 x w/w) and loaded in an autoclave. 10% Pd/C (5% w/w, water content: 56%) was added, and the mixture was hydrogenated at 1.0 Mpa for 5h at 30 °C. Reaction completion was observed by HPLC. The mixture was allowed to cool and the solids were removed by filtration. The filtrate was concentrated to 7.5 L (1.5 V) by evaporation, followed by dilution with DCM (75 L, 15 V). A solution of oxalic acid (806 g, 8.95 mol, 0.45 equiv) in isopropyl acetate (15 L, 3 V) was added

dropwise at 25 °C. The mixture was allowed to stir for 2h. The oxalate salt was isolated by filtration, and washed with DCM (10 L, 2 V). The salt was dissolved in a water (15 L, 3 V) / DCM (75 L, 15 V) mixture. Potassium carbonate (3.57 kg, 25.9 mol, 1.3 equiv) was added batchwise at 20 °C and the reaction mixture was stirred for 5h. The aqueous layer was extracted with DCM (25 L, 5 V). The organic layers were combined and concentrated to 7.5 L (1.5 V). Heptane (25 L, 5 V) was added and the mixture was concentrated to 7.5 L (1.5 V). Heptane (15 L, 3 V) was stirred for 3 h at 0 °C. The solids were isolated by filtration and dried under vacuum at 35 °C to give compound **14**. (2.8 kg, 61% yield (2 steps)).

*((2S,4R)-2-(((tert-Butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidin-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone (**15**).* EDCI (2.8 kg, 14.6 mol, 1.2 equiv) was added to a solution of acid **6** (4.5 kg, 12.18 mol), and 2-pyridinol 1-oxide (HOPO) (1.49 kg, 13.4 mol, 1.1 equiv) in DCM (24 L, 6 V) at 0 °C. The reaction was allowed to proceed for 1h at 15 °C, at which time a cold solution (- 5 °C) of C-ring amine **14** (3.1 kg, 13.4 mol, 1.1 equiv) and triethylamine (2.12 L, 15.2 mol, 1.25 equiv) in DCM (24 L, 6 V) was added at -10 °C. The reaction mixture was allowed to stir at 15 °C for 1h. Reaction completion was observed by HPLC. The reaction mixture was washed with water (16 L, 4 V), followed by cold aqueous HCl (0.1 M) until the pH was adjusted to 4 or 5. The organic phase was then washed with saturated aqueous $NaHCO_3$ (12 L, 3 V), then water (16 L, 4 V). The volatiles were removed under vacuum and the residue was slurried with hexane (16 L, 4 V). The solids were isolated by filtration, washed with hexane (8 L, 2 V) and dried. The solids were dissolved in ethanol (14 L, 3.5 V) at 50 °C. Water (10 L, 2.5 V) was added slowly, and the mixture was stirred for 30 min and cooled to 15 °C. The solids were isolated by filtration and dried under vacuum at 40 °C to give **15** (5.2

kg, 99.8 HPLC area % purity, 82% yield). t_R: 15.1 min. (100 Chiral HPLC area % purity) (t_R: 13.8 min; Enantiomer t_R: 9.1 min).

*(S)-5-(((tert-Butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)pyrrolidin-3-one (**16**).* TEMPO (21 g, 134 mmol, 0.015 equiv) was added to a stirred solution of **15** (5.2 kg, 8.95 mol), potassium bromide (16 g, 134 mmol, 0.015 equiv) and $NaHCO_3$ (752 g, 8.95 mol, 1.0 equiv) in DCM (31.2 L, 6 V) at 3 °C Aqueous sodium hypochlorite (10.5% (w/w) NaClO aqueous solution, 8.45 kg, 11.6 mol, 1.3 equiv) was added dropwise at 3 °C, and the mixture was allowed to stir for 1h at 3 °C. Reaction completion was observed by HPLC. The reaction was quenched with 5% aqueous sodium bisulfite (15.6 L, 3V). The aqueous phase was extracted with DCM (15.6 L, 3V). The organic phases were combined, washed with brine (36.4 L, 7 V), dried over sodium sulfate and concentrated to dryness (4.78 kg, 97.0 HPLC area % purity, 92% yield). t_R: 16.0 min. (100 Chiral HPLC area % purity) (t_R: 10.4 min; Enantiomer t_R: 8.3 min).

*(S)-5-(((tert-Butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)-4,5-dihydro-1H-pyrrol-3-yl trifluoromethanesulfonate (**17**).* Triflic anhydride (2.08 L, 12.4 mol, 1.5 equiv) was added dropwise to a mixture of 2,6-lutidine (1.92 L, 16.5 mol, 2 equiv) and ketone **16** (4.8 kg, 8.26 mol) in dry toluene (28.8 L, 6 V) at - 40 °C. The reaction mixture was allowed to stir for 2 h at -35 °C (+/- 5 °C). Reaction completion was observed by HPLC. The mixture was washed with water (14.4 L, 3 V), 1N aqueous HCl (14.4 L, 3 V) to pH 3, followed by saturated aqueous $NaHCO_3$ (14.4 L, 3 V), and brine (14.4 L, 3 V). The organic phase was dried over sodium sulfate, filtered, and used as such in the next step. (90.3 HPLC area % purity). t_R: 21.1 min.

*(S)-(2-(((tert-Butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone (**18**).* The toluene solution of triflate **17** (8.26 mol), followed by $Pd(dppf)Cl_2.CH_2Cl_2$ (337 g, 0.41 mol, 0.05 equiv) were added to a suspension of potassium phosphate tribasic (10.5 kg, 49.6 mol, 6 equiv) in toluene (28.8 L, 6 V) under nitrogen atmosphere at 65 °C. Methylboronic acid (1.98 kg, 33.0 mol, 4.0 equiv) was added batchwise and the reaction mixture was allowed to stir for 30 min at 65 °C. Reaction completion was observed by HPLC. The mixture was cooled to 30 °C and poured in water (24 L, 5 V). The organic phase was collected and concentrated under vacuum. The residue was purified by flash chromatography (heptane / ethyl acetate gradient from 98/2 to 90/10) to afford **18**. (2.1 kg, 99.0 HPLC area % purity, 44% yield (2 steps)). t_R: 20.2 min. (100 Chiral HPLC area % purity) (t_R: 6.2 min; Enantiomer t_R: 4.7 min).

*(S)-(2-Amino-5-methoxy-4-((triisopropylsilyl)oxy)phenyl)(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (**19**).* Zinc powder (5.43 kg, 83.1 mol, 37 equiv) was added to a mixture of ethanol (10.4 L, 8V), water (600 mL, 0.5 V) and AcOH (600 mL, 0.5 V) at 0 °C. The reaction mixture was stirred at 5 °C for 30 min. A solution of **18** (1.3 kg, 2.25 mol) in ethanol (2.6 L, 2 V) was added dropwise at 5 °C. The reaction was allowed to proceed at 5 °C for 30 min. Reaction completion was observed by HPLC. The solids were removed by filtration. The filtrate was diluted with ethyl acetate (26 L, 20V) and washed with water (26 L, 20 V), saturated aqueous $NaHCO_3$ (26 L, 20 V), and brine (26 L, 20 V). The organic phase was dried over sodium sulfate, filtered and the solvent removed by rotary evaporation under reduced pressure to afford the product **19** as a brown oil**.** (1.0 kg, 97.6 HPLC area % purity, 81.3% yield). t_R: 19.5 min.

*((Allyloxy)carbonyl)-L-valine (**21**).* Sodium hydroxide (210.2 g, 5.25 mol, 1.76 equiv) and sodium carbonate (174.1 g, 1.64 mol, 0.55 equiv) were dissolved in water (4.55 L, 13 V) at 10 °C. L-Valine (350 g, 2.99 mol) was added and the mixture was stirred for 30 min. A solution of allyl chloroformate (349 mL, 3.28 mol, 1.1 equiv) in MTBE (1.75 L, 5 V) was added dropwise at 10 °C. The reaction was allowed to proceed at 20 °C for 1 h. Reaction completion was observed by HPLC. The organic phase was discarded. The aqueous phase was acidified to pH 3 with 6N HCl at 10 °C and extracted with DCM 3 x (1.4 L, 4 V). The combined organic phases were washed with brine (1.4 L, 4 V) and concentrated to dryness under vacuum to yield 583 g (97%) of yellow oil. (583 g, 89.9 HPLC area % purity, 97.0% yield). t_R: 7.35 min. (100 Chiral HPLC area % purity) (t_R: 9.6 min; Enantiomer t_R: 11.9 min).

*2,5-Dioxopyrrolidin-1-yl ((allyloxy)carbonyl)-L-valinate (**22**).* N-Hydroxysuccinimide (348.4 g, 3.03 mol, 1.05) and ((allyloxy)carbonyl)-L-valine **21** (580g, 2.88 mol) were dissolved in DCM (5.8 L, 10 V) at 5 °C. DCC (653.1 g, 3.16 mol, 1.1 equiv) was added portionwise and the reaction mixture was allowed to stir for 2 h at 20 °C. Reaction completion was observed by HPLC. The solids were removed by filtration and washed with DCM (0.58 L, 1 V). The filtrate was washed with water (2.8 L, 5 V) and concentrated under vacuum to dryness. The residue was slurried with petroleum ether (1.1 L, 2 V) and stirred for 1 h. The solids were isolated by filtration and dried to give **22** as a white solid. (791 g, 89.9 HPLC area % purity, 92.0% yield). t_R: 10.04 min. (100 Chiral HPLC area % purity) (t_R: 19.0 min; Enantiomer t_R: 20.2 min).

*((Allyloxy)carbonyl)-L-valyl-L-alanine (**23**).* L-alanine (259.3 g, 2.91 mol, 1.1 equiv) was dissolved in a mixture of sodium carbonate (308.8 g, 2.91 mol, 1.1 equiv), THF (3.2 L, 4 V) and water (7.9 L, 10 V). A solution of **22** (790 g, 2.64 mol) in THF (2.4 L, 3 V) was added dropwise at 0 °C, and the reaction was allowed to proceed at 25 °C for 5 h. Reaction completion was

observed by HPLC. The mixture was concentrated under vacuum to 7.9 L (10V). The solids were removed by filtration. The filtrate was acidified to pH = 3 with 6N HCl (0.79 L, 1 V). The solids were collected by filtration, redissolved in MeTHF (7.9 L, 10 V) and washed with water (4.0 L, 5 V). The organic phase was concentrated to dryness under vacuum to give the product **23** as a white solid (567 g, 97.1 HPLC area % purity, 78.6% yield). t_R: 6.6 min. (100 Chiral HPLC area % purity) (t_R: 1.3 min; RS t_R: 1.8 min, RR t_R: 2.3 min, SR t_R: 7.2 min).

*Allyl ((S)-1-(((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**24**).* EEDQ (538.8 g, 2.18 mol, 1.05 equiv) was added to a mixture of **23** (565 g, 2.07 mol) and 4-aminobenzyl alcohol (268.2 g, 2.18 mol, 1.05 equiv) in THF (11 L, 20 V) under nitrogen. The reaction was allowed to proceed at 25 °C for 16 h. Reaction completion was observed by HPLC. The mixture was concentrated to dryness under vacuum. The residue was slurried with MTBE (11.3 L, 20 V) and stirred for 5 h at 35 °C The solids were isolated by filtration and dried under vacuum at at 35 °C for 12 hours to give the product **24** as a white solid (530 g, 98.9 HPLC area % purity, 67.7% yield). t_R: 8.0 min. (99.9 Chiral HPLC area % purity) (t_R: 7.4 min; RS t_R: 4.9 min, RR t_R: 9.6 min, SR t_R: 5.6 min).

*(S)-Allyl-(2-(2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (**25**).* Pyridine (486 mL, 6.01 mol, 2.2 equiv) was added to a solution of **19** (1.5 kg, 2.73 mol) in DCM (12 L, 8 V), and cooled to -10 °C. Allyl chloroformate (319 mL, 3.00 mol, 1.1 equiv) was added dropwise at - 5 °C. The reaction was allowed to proceed at - 5 °C for 1h. Reaction completion was observed by HPLC. The reaction mixture was washed with 10% aqueous citric acid (12 L, 8 V), followed by saturated aqueous $NaHCO_3$ (6 L, 4 V), and brine (6 L, 4 V). The organic phase was dried over sodium sulfate, filtered and the solvent removed by rotary evaporation under reduced pressure at

40 °C to afford the product **25** as a brown oil (1.45 kg, 93.8 HPLC area % purity, 83.8% yield). t_R: 23.6 min.

*(S)-Allyl-(2-(2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (**26**).* *Para*-toluenesulfonic acid hydrate (252 g, 1.33 mol, 0.6 equiv) was added to a solution of **25** (1.4 kg, 2.21 mol) in THF (8.4 L, 6 V) and water (0.42 L, 0.3 V). The reaction mixture was allowed to stir for 1h at 22 °C. Reaction completion was observed by HPLC. The mixture was diluted with ethyl acetate (14 L, 10 V), and washed with water (5.6 L, 4 V). The organic phase was washed with brine (5.6 L, 4 V), dried over sodium sulfate, and concentrated under vacuum at 35 °C. The residue was purified by flash chromatography (heptane / ethyl acetate; 98/2 up to 90/10) to afford the product **26**. (1.0 kg, 98.2 HPLC area % purity, 87.2% yield). t_R: 13.0 min.

*(11S,11aS)-Allyl-11-hydroxy-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**27**).* Anhydrous dimethyl sulphoxide (171 mL, 2.4 mol, 2.5 equiv) was added dropwise to a solution of oxalyl chloride (84 mL, 0.99 mol, 1.03 equiv) in dry DCM (4.5 L, 9 V)) at -75 °C. After 30 min, a solution of **26** (500 g, 0.96 mol) in dry DCM (4 L, 8 V) was added slowly whilst maintaining the temperature at -70 °C. After 30 min, triethylamine (672 mL, dried over 4 Å molecular sieves, 4.82 mol, 5 equiv) was added dropwise and the temperature was allowed to reach -50°C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Completion was observed by HPLC. The reaction mixture was stirred with 5% aqueous citric acid (5 L, 10 V) for 15 min. The organic phase was washed with saturated aqueous $NaHCO_3$ (2.5 L, 5 V), water (2.5 L, 5 V) and dried over sodium sulfate. Concentration under vacuum at 35 °C gave the crude

product **27** which was used in the next step without further purification. (400 g, 94.3 HPLC area % purity, 81.3% yield). t_R: 11.8 min.

*(11S,11aS)-Allyl-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**28**).* *Tert*-butyldimethylsilyltriflate (533 mL, 2.32 mol, 1.5 equiv) was added to a mixture of compound **27** (800 g, 1.55 mol) and 2,6-lutidine (451 mL, 3.87 mol, 2.5 equiv) in dry DCM (8 L, 10 V) at 0 °C. The reaction mixture was allowed to stir at 5 °C for 30 min, followed by 3 h at 25 °C. Completion was observed by HPLC. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (4 L, 5 V), water (4 L, 5 V) and dried over sodium sulfate. Concentration under vacuum at 35 °C gave the crude product **28** which was used in the next step without further purification. (750 g, 93.8 HPLC area % purity, 76.0% yield). t_R: 22.6 min.

*(11S,11aS)-Allyl-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**29**).* Lithium acetate (78.4 mol, 1.19 mol, 1 equiv) was added to a solution of compound **28** (750 g, 1.19 mol) in wet dimethylformamide (7.5 L, 49/1 DMF/water). The reaction was allowed to proceed for 6 h at 25 °C. Completion was observed by HPLC. The reaction mixture was diluted with ethyl acetate (15 L, 20 V) and washed with water (7.5 L, 10 V). The aqueous phase was extracted with ethyl acetate (5.25 L, 7 V). The combined organic phases were washed with 5% aqueous citric acid (7.5 L, 10 V), brine (7.5 L, 10 V) and dried over sodium sulfate (2.8 kg). The volatiles were removed under vacuum at 35 °C. The residue was slurried in heptane (1.5 L, 2 V), and the solids were isolated by filtration. The crude product was slurried in ethyl acetate (1.12 L, 1.5 V) and stirred for 1.5 h. The solids were isolated by filtration and dried under vacuum at 40 °C to yield **29** as a white powder. (430 g, 99.5 HPLC area % purity, 76.2% yield). t_R: 10.6 min.

*Allyl-3-(2-(2-(4-((((2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamoyl)oxy)methyl)phenyl)hydrazinyl)propanamido)-4-methyl-2-oxopentanoate (**30**).* Triphosgene (233 g, 787 mmol, 0.36 equiv) was added to a stirred solution of amine **19** (1.2 kg, 2.19 mol) in anhydrous DCM (12 L, 10 V) at -20 °C. Triethylamine (670 mL, 4.81 mol, 2.2 equiv) was added dropwise at -20 °C. The mixture was allowed to stir for 30 min at -20 °C. Formation of the isocyanate was monitored by HPLC analysis by quenching an aliquot with methanol. A mixture of **24** (866 g, 2.29 mol, 1.05 equiv) and triethylamine (457 mL, 3.28 mol, 1.5 equiv) in anhydrous DCM (12 L, 10 V) was added rapidly at -20 °C. The reaction was allowed to proceed for 16 h at 25 °C. Reaction completion was observed by HPLC. The reaction mixture was washed with water (4.8 L, 4 V). The organic phase was dried over sodium sulfate and concentrated under vacuum at 35 °C. The residue was purified by flash chromatography (hexane / ethyl acetate 3 /1) to give product **30** as a yellow solid. (1.43 kg, 96.9 HPLC area % purity, 68.5% yield). t_R: 18.4 min.

*Allyl-3-(2-(2-(4-((((2-((S)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamoyl)oxy)methyl)phenyl)hydrazinyl)propanamido)-4-methyl-2-oxopentanoate (**31**). Para*-toluenesulfonic acid hydrate (256 g, 1.35 mol, 0.6 equiv) was added to a solution of **30** (2.14 kg, 2.25 mol) in THF (12.8 L, 6 V) and water (0.64 L, 0.3 V) at 15 °C. The reaction mixture was allowed to stir for 30 min at 25 °C. Reaction completion was observed by HPLC. The mixture was diluted with ethyl acetate (21.4 L, 10 V), and washed with water (8.56 L, 4 V). The organic phase was washed with saturated $NaHCO_3$ (8.56 L, 4 V), brine (5.6 L, 4 V), dried over sodium sulfate, and concentrated under vacuum at 35 °C. The residue was

purified by flash chromatography (hexane / ethyl acetate 50 /50) to afford the product **31** as a yellow solid (1.28 kg, 98.0 HPLC area % purity, 67.9% yield). t_R: 12.2 min.

*(11S,11aS)-4-(2-(1-((1-(Allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-hydroxy-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**32**)*

Dimethyl sulphoxide (53 mL, 747 mmol, 2.5 equiv) was added dropwise to a solution of oxalyl chloride (2.03 M in DCM, 177 mL, 359 mmol, 1.2 equiv) in dry DCM (1.76 L, 7 V) at -65 °C under nitrogen. After 15 min, a solution of **31** (250.5 g, 299 mmol) in dry DCM (1.5 L, 6 V) was added slowly whilst maintaining the temperature at -65 °C. The reaction mixture was allowed to stir for 15 min at -60 °C. Triethylamine (208 mL, 1.49 mol, 5 equiv) was added dropwise and the reaction mixture was allowed to stir for 1 h at -60 °C. The reaction mixture was allowed to warm to 10 °C and washed with cold HCl (0.2 N, 4.0 L, 16 V), aqueous $NaHCO_3$ (5%, 4.0 L, 16 V) and aqueous sodium chloride (1%, 4.0 L, 16 V). The organic phase was concentrated under vacuum. DCM (3.76 L, 15 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. The crude product **32** was kept as a solution in DCM (2.5 L, 10 V) at 5 °C and used directly in the next step. (2 x 250 g, 91.7 HPLC area % purity). t_R: 9.893 min.

*(11S,11aS)-4-(2-(1-((1-(Allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**33**).* 2,6-Lutidine (210 mL, 1.79 mol, 6 equiv) was added to a solution of **32** (249.9 g, 299 mmol) in DCM (3.0 L, 12 V) at -15 °C. The mixture was cooled to -50 °C. *Tert-*

butyldimethylsilyltriflate (395 mL, 1.49 mol, 5 equiv) as a solution in DCM (2.0 L, 8 V) was added dropwise. The mixture was stirred for 20 min at -50 °C. The reaction was allowed to proceed at -15 °C for 20 h. Completion was observed by HPLC. The mixture was allowed to warm to 5 °C and was washed sequentially with 0.1 N aqueous citric acid (4.0 L, 16 V), 5% aqueous $NaHCO_3$ (4.0 L, 16 V) and 1% aqueous sodium chloride (4.0 L, 16 V). The organic phase was concentrated under vacuum. DCM (3.75 L, 15 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. Inorganic solids were removed by filtration and the crude product **33** was kept as a solution in DCM (3.75 L, 15 V) at 5 °C and used directly in the next step. (2 x 284 g, 83.1% HPLC area % purity batch 1; 87.2% HPLC area % purity batch 2). t_R: 23.675 min.

*(11S,11aS)-4-(2-(1-((1-(Allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**34**)* A solution of previously prepared crude **33** (568 g, 716 mmol, 85% pure) in DCM was concentrated under vacuum. The residue was dissolved in dimethylformamide (4.5 L, 8 V) at 20 °C. A solution of lithium acetate dihydrate (60.9 g, 716 mmol, 1 equiv) in water (171 mL, 0.3 V) was added, and the reaction mixture was stirred at 40 °C for 2 h. Completion was observed by HPLC. The mixture was allowed to cool to room temperature and was diluted with Me-THF (8.5 L, 15 V). The mixture was washed sequentially with 0.1 N aqueous citric acid (8.5 L, 15 V), 5% aqueous $NaHCO_3$ (5.7 L, 10 V), 1% aqueous sodium chloride (5.7 L, 10 V), and water (5.7 L, 10 V). The organic phase was concentrated under vacuum. Me-THF (8.5 L, 15 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. Inorganic solids were removed by filtration and the crude product was kept as a solution in DCM

(5.0 L, 8.8 V) at 5 °C. The solution was purified by high pressure chromatography using Hipersep Novasep automatic purification system (Daiso Si 100 Å, 10 µm; Heptane / EtOAc, 25 / 75). The pure fractions were combined and evaporated three times with ethyl acetate (9.5 L, 20 V), down to a final volume of 4.4 L. The solution was diluted with DCM (10.25 L, 22 V). (Storage in Ethyl acetate / DCM 30/70 at 5 °C). The DCM was removed by evaporation. Heptane (12.3 L, 48 V) was added slowly. The reaction mixture was allowed to stir for 15 min at room temperature. The precipitate was isolated by filtration, rinsed with heptane (2 x 10 L), and dried under vacuum at 40 °C to give **34** as a pale yellow solid. (338 g, 99.8% HPLC area % purity, 70.2% yield (three steps)). t_R: 23.102 min.

*(11S,11aS)-allyl 11-((tert-Butyldimethylsilyl)oxy)-8-((5-iodopentyl)oxy)-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**38**).*

Diiodopentane (313 mL, 682 g, 2.10 mol, 5 equiv) and potassium carbonate (75.7 g, 548 mmol, 1.3 equiv) were added to a solution of phenol **29** (200 g, 421 mmol) in MEK (2.4 L, 12 V, Water content $\leq$ 0.1 % (1000 ppm)). The reaction mixture was heated to 75 °C and stirred for 24 h under nitrogen. Completion was observed by HPLC. The mixture was cooled to 10 °C and was diluted with water (2.4 L, 12 V) and ethyl acetate (3.0 L, 15 V). The organic phase was washed with 1% aqueous sodium chloride (2.4 L, 12 V). The organic phase was concentrated under vacuum. Ethyl acetate (2.0 L, 10 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. Inorganic solids were removed by filtration and the crude product was kept as a solution in ethyl acetate / heptane 50 / 50 (1.6 L, 8 V) at 5 °C (261 g estimated by assay). The solution was purified by high pressure chromatography using Hipersep Novasep automatic purification system (Daiso Si 100 Å, 10 µm; Heptane / EtOAc, 70 /

30). The pure fractions were combined and evaporated three times with ethyl acetate (2.6 L, 10 V). The pure product **38** was stored as a solution in EtOAc (2.6 L, 10V) at 5 °C.

(251 g, 98.1% HPLC area % purity, 88% yield by quantitative HPLC). t_R: 22.874 min.

*(11S)-Allyl 8-((5-(((11S)-10-(((4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl)oxy)carbonyl)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**39**).* A solution of iodo **38** (251 g, 375 mmol, 1.2 equiv) in ethyl acetate was concentrated under vacuum. The residue was dissolved in MEK (1.49 L, 6 V). A solution of phenol **29** (248.1 g, 312.5 mmol) in MEK (1.98 L, 8 V) was added, followed by potassium carbonate (47.5 g, 344 mmol, 1.1 equiv). The reactor was rinsed with further MEK (248 mL, 1 V). The reaction was allowed to proceed at 75 °C for 24 h. Completion was observed by HPLC. The mixture was cooled to 10 °C and was diluted with water (3.72 L, 15 V) and ethyl acetate (4.96 L, 20 V). The organic phase was washed sequentially with 1% aqueous sodium chloride (3.72 L, 15 V), and water (3.72 L, 15 V). The organic phase was concentrated under vacuum. Ethyl acetate (3.72 L, 15 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. Tetrahydrofuran (2.5 L, 10 V) was added and the resulting solution was again concentrated under vacuum. The crude product **39** was stored as a solution in THF (2.1 L, 8.5V) at 5 °C and used as such in the next reaction. (418 g, 80.9% HPLC area % purity). t_R: 21.696 min.

(11S)-Allyl-8-((5-(((11S)-10-(((4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-

*5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**40**).* A buffered solution of tetra-*n*-butylammonium fluoride (0.67 M in THF, 1.54 L, 1.03 mol, 3.3 equiv) and AcOH (93 mL, 1.62 mol, 5.2 equiv) was added to a solution of **39** (418 g, 312 mmol) in tetrahydrofuran (2.1 L, 6 V). The reaction was allowed to proceed at 20 °C for 48 h. Completion was observed by HPLC. The mixture was diluted with water (2.5 L, 6 V) and ethyl acetate (8.36 L, 20 V). The organic phase was washed sequentially with 5% aqueous $NaHCO_3$ (2.5 L, 6 V), 1% aqueous sodium chloride (2.5 L, 6 V), and water (2.5 L, 6 V). The organic phase was concentrated under vacuum. Ethyl acetate (6.27 L, 15 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. DCM (8.36 L, 20 V) was added and the resulting solution was stored at 5 °C (329 g estimated by assay). The solution was purified by high pressure chromatography using Hipersep Novasep automatic purification system (Kromasil Si 60 Å, 13 µm; DCM / methanol, 96 / 4). The pure fractions were combined, evaporated three times with DCM (3.29 L, 10 V), and stored at 5 °C in DCM (6.58 L, 20 V).

(308 g, 99.0% HPLC area % purity, 88% yield by quantitative HPLC (2 steps)). t_R: 10.641 min.

*(11S)-4-(2-(1-((1-Amino-3-methyl-1-oxobutan-2-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl 11-hydroxy-7-methoxy-8-((5-((7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**41**).*

Tetrakis(triphenylphosphine)palladium(0) (3.5 g, 3.0 mmol, 0.02 equiv) was added to a solution of **40** (167 g, 151 mmol) and pyrrolidine (31.4 mL, 377 mmol, 2.5 equiv) in DCM (4.51 L, 27 V)

under nitrogen at 20 °C. The reaction was allowed to proceed at 20 °C for 30 min. Completion was observed by HPLC. The mixture was diluted with DCM (15 L, 90 V). The reaction mixture was washed with saturated aqueous ammonium chloride (5 L, 30 V). The aqueous phase was re-extracted with DCM (15 L, 90 V). The combined organic phases were washed with 1% aqueous sodium chloride (10 L, 60 V) and concentrated under vacuum. DCM (5.0 L, 10 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. The residue was dissolved in a mixture of DCM / methanol (95 /5; 2.44 L, 14.65 V) and the resulting solution was stored at -15 °C. The yield was assumed to be 100% (139 g) and the solution was used as such in the next step.

t_R: 9.886 min.

(11S,11aS)-4-((2S,5S)-37-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl 11-hydroxy-7-methoxy-8-((5-(((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate ***SG3249, Tesirine***. A solution of Mal-$(PEG)_8$-acid (107.1 g, 181 mmol, 1.2 equiv) in DCM (306 mL, 2.2 V) was added to a solution of **41** (139 g, 151 mmol) in DCM / methanol (95 /5; 2.92 L, 21 V) at 20 °C. 1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI, 34.7 g, 181 mmol, 1.2 equiv) was added portionwise at 20 °C. The reaction was allowed to proceed at 20 °C for 18 h. Completion was observed by HPLC. The mixture was diluted with DCM (1.39 L, 10 V). The reaction mixture was washed with 1% aqueous sodium chloride (1.11 L, 8 V) and concentrated under vacuum. DCM (1.39 L, 10 V) was added and the resulting solution was again concentrated under vacuum. This operation was repeated twice. The residue was dissolved in DCM (420 mL, 3 V) and the resulting solution

was stored at -15 °C (219 g estimated by assay). The solution of crude product was purified by high pressure chromatography using Hipersep Novasep automatic purification system (C18 Daiso SP120-15 ODS BP 120 Å, 15 µm; acetonitrile / water, 65 / 35, + 0.01 % AcOH, collection at 230 nm). The pure fractions were extracted twice with DCM (2 x 1.5 L). The combined organic phases (46 runs) were washed sequentially with 0.1% aqueous $NaHCO_3$ (46 x 1.5 L), and twice with water (2 x 46 x 1.5 L). The resulting solution was concentrated under vacuum. DCM (2 L) was added and the solution was concentrated under vacuum. This operation was repeated twice. The product was stored as a solution in DCM (1.5 L, 7.19 V) at 5 °C (188 g estimated by assay). The solution was concentrated to dryness under vacuum. The solids were transferred and dried at 35 °C under vacuum for 24 h to give **tesirine** as a pale yellow powder.

(169 g, 97.9% HPLC area % purity, 77% yield (2 steps)). t_R: 13.447 min. Diastereoisomers content 0.95% (HPLC area). t_R: 13.313 min

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ASSOCIATED CONTENT

Supporting Information.

The following file is available free of charge.

Scale up synthesis of tesirine: HPLC methods, TBAF titration method, TBAF/AcOH DoE (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

HER-2, human epidermal growth factor receptor 2, AML, Acute myeloid leukaemia, MM, Multiple myeloma, SCLC, Small cell lung cancer, NETS, Neuroendocrine tumours, ALL, Acute lymphoblastic leukaemia, NHL, Non-Hodgkin lymphoma, DLBCL, Diffuse Large B-Cell Lymphoma, EDCI (EDAC), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, HOPO, 2-Pyridinol 1-oxide, HOBt, 1-Hydroxybenzotriazole hydrate, Pd(dppf)Cl_2, [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), TCCA, Trichloroisocyanuric acid, DMP, Dess-Martin periodinane, Tf_2O, Trifluoromethanesulfonic anhydride, PTSA, *para*-toluenesulfonic acid, EEDQ, 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, HOSu, N-Hydroxysuccinimide, MTS, [3-(4,5-dimethylthiazol-2-yl)- 5-(3-carboxymethonyphenol)-2-(4-

sulfophenyl)-2H-tetrazolium, inner salt, IC_{50}, the half maximal inhibitory concentration, Me-THF, 2-MeTHF, 2-Methyltetrahydrofuran, MEK, Methyl ethyl ketone, PEG, Polyethylene glycol, DoE, Design of Experiments, Alloc, Allyloxycarbonyl, Mal, Maleimide, PAB, 4-Aminobenzyl alcohol, Val, valine, Ala, Alanine.

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