Article

An Alternative Focus for Route Design for the Synthesis of Antibody-Drug Conjugate Payloads

Arnaud Charles Tiberghien, Philip W. Howard, William Robert Fraser Goundry, Marc McCormick, and Jeremy Stephen Parker

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.8b02876 • Publication Date (Web): 02 Jan 2019

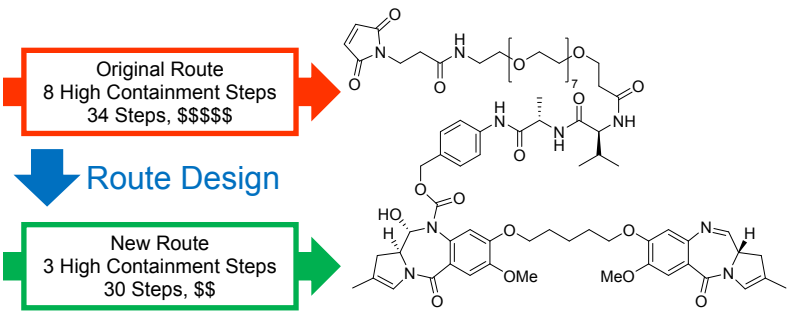
An Alternative Focus for Route Design for the Synthesis of Antibody-Drug Conjugate Payloads

*Arnaud C. Tiberghien†, Philip W. Howard†, William R. F. Goundry‡, Marc McCormick‡ and Jeremy S. Parker‡**

†Spirogen, QMB Innovation Centre, 42 New Road, E1 2AX London, United Kingdom

‡Early Chemical Development, Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Macclesfield, United Kingdom

TOC Graphic

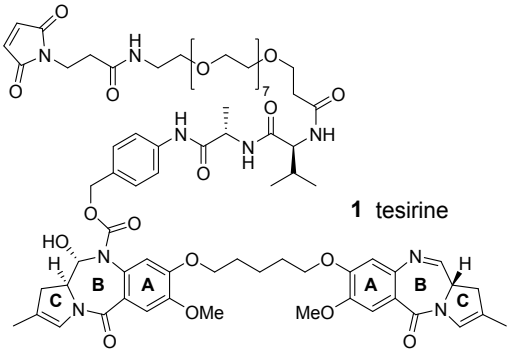

Original Route
8 High Containment Steps
34 Steps, $$$$$
Route Design
New Route
3 High Containment Steps
30 Steps, $$

Abstract

An analysis of Antibody-Drug Conjugate Payload manufacturing has revealed that the majority of the cost is associated with the use of high-containment facilities for the latter stages of the synthesis. To make a significant reduction in the Cost of Goods (CoGs), a new approach to route design has been introduced which focusses on minimizing the number of steps that require high-containment. This approach has been exemplified in a new synthesis of tesirine, including the first application of a ring-closing copper (I)/TEMPO aerobic oxidation to the pyrrolobenzodiazepine ring system, affording a 60% reduction in CoGs.

Introduction

Antibody-Drug Conjugates (ADCs) are an important new class of oncology therapeutics, combining a tumor-targeting antibody with a cell-killing cytotoxic drug (payload).[1] Significant activity in this new field has resulted in the approval of brentuximab vedotin (Adcetris)[2] in 2011 and trastuzumab emtansine (Kadcyla)[3] in 2013, followed by the 2017 approvals of inotuzumab ozogamicin (Besponsa) and gemtuzumab ozogamicin (Mylotarg). Extensive clinical programs continue to evaluate potential commercial candidates, with further launches expected in the next few years.



1 tesirine

The cytotoxic drugs employed as ADC payloads are typically based on natural products, exemplified by the auristatins[4] (based on dolastatin 10)[5] which have been used in Adcetris, and the maytansinoids[6] (based on maytansine)[7] used in Kadcyla.

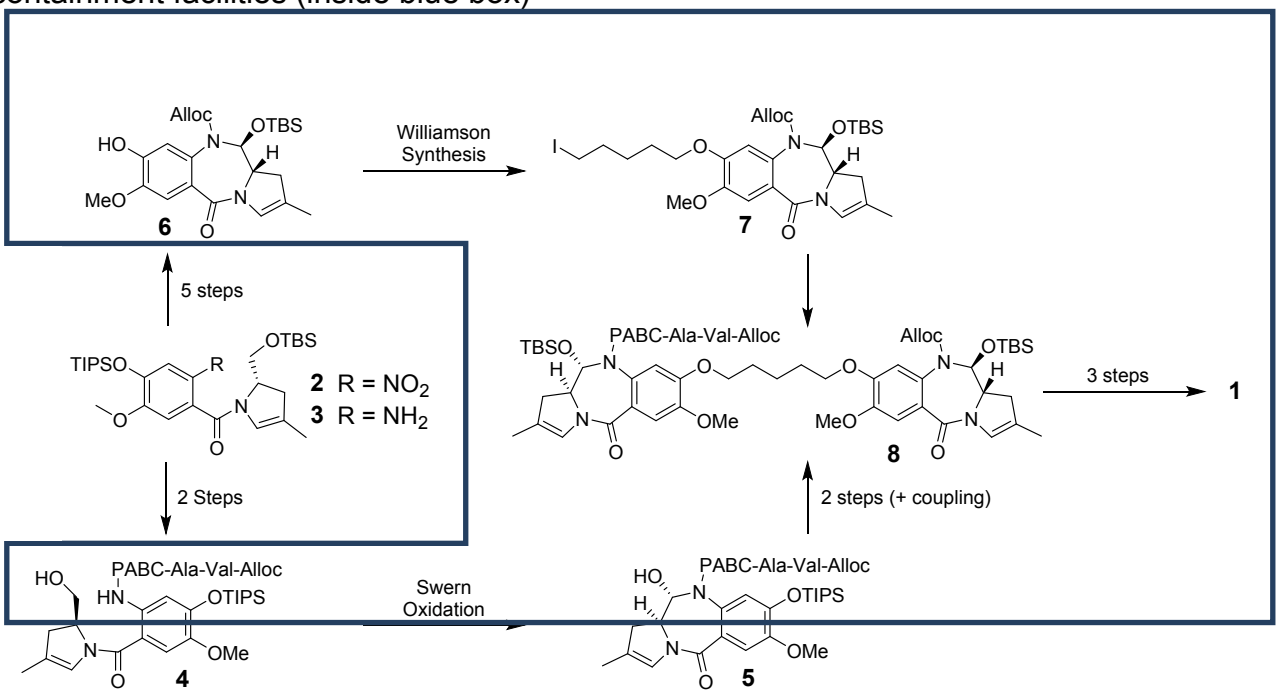
These molecules have high synthetic complexity, and their discovery and development has required significant contributions from the field of synthetic organic chemistry. Recent interest in new payloads has been focused on the pyrrolobenzodiazepines (PBDs),[8] which have been employed by Seattle Genetics, Stemcentrx, ADC Therapeutics and MedImmune in their clinical ADC programs. The leading PBD linker-payload is tesirine (**1**, SG3249),[9] which is utilized in rovalpituzumab tesirine (Rova-T)[10] for the treatment of small cell lung cancer and loncastuximab tesirine (Lonca-T)[11] for the treatment of B-cell non-Hodgkin lymphoma,[11] as well as in a range of further programs. The initial synthesis of tesirine has been described previously,[12] as well as a detailed description of the synthetic development and scale-up of this route.[13]

As part of a detailed review of the route following the delivery of a 60g batch of tesirine, a full analysis of costs, timings and yields was completed to provide greater insight into areas for further development. Overall the original route is high yielding and has been successfully scaled-up, delivering multiple campaigns on multi-gram scales. However, one area that the analysis highlighted was the cost breakdown between the chemistry steps performed in high-containment and standard facilities. High-

containment is required for molecules that are cytotoxic and these specialist facilities include features such as controlled air handling (including airlocks); controlled entry and exit of materials and personnel; specialist containment equipment (gloveboxes for solids, fume hoods/downflow booths for liquids) and planned cleaning and decontamination systems. The use of such facilities is expensive and is a major contributor to any campaign of a cytotoxic linker-payload.

For the original route to tesirine (Scheme 1), the final 8 out of the 34 synthetic steps are performed in a high containment facility (Figure 1). An analysis of the 60g delivery revealed that this part of the synthetic campaign accounted for 90% of the cost of campaign, with the initial 26 synthetic steps, performed in standard facilities, only accounting for 10% of the cost. This cost breakdown is in direct contrast to the synthesis of a comparable non-cytotoxic molecule prepared using a route of similar synthetic length and complexity, where the early part of the manufacturing is more expensive due to the need to use pilot-plant or kilo-lab facilities, whereas the latter steps can be completed in laboratory glassware.

Scheme 1. Original synthesis of tesirine detailing chemistry conducted in high-containment facilities (inside blue box)



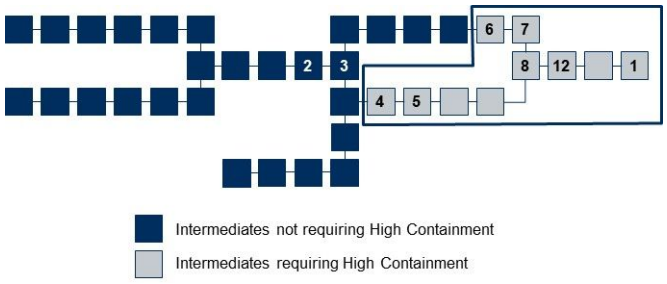


Figure 1. Schematic of original synthesis of tesirine (each block representing an intermediate) showing portion conducted in high containment (inside blue box).

This analysis clearly demonstrated that in order to make a significant impact on the cost of manufacturing tesirine, a reduction in the use of high-containment facilities would be required. To facilitate this, a new approach to route design has been introduced, focusing on reduction in the number of synthetic steps requiring high-containment facilities. Route design typically focusses on number of steps, convergency and yield, but for the large-scale preparation of cytotoxic payloads (and more generally for cytotoxic molecules), a focus on reduction in the number of steps requiring high-containment facilities should be a key consideration, both in terms of cost reduction and improving campaign flexibility and timings.

With the completion of the analysis, work was initiated looking to redesign the route with a focus on reduction in the number of steps requiring high-containment facilities. Success in this approach offered the potential to afford a new, more efficient route in terms of cost, time and synthetic efficiency.

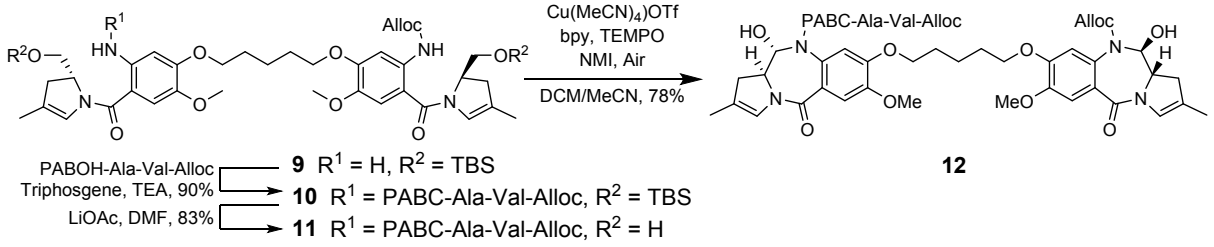
Results and Discussion

The key structural features imparting cytotoxicity to the PBDs are well understood,[14] with a requirement for the A-, B- and C-rings of the molecule to be in place. In the original synthesis of tesirine, high-containment facilities were required during construction of the two halves of molecule and for the dimerization and subsequent stages (Figure 1). For the half of the molecule containing the dipeptide trigger, high containment was required for the oxidative cyclisation of **4** to **5** to afford the completed ABC ring system. For the other half of the molecule, a number of the tricyclic intermediates were tested and found to be non-toxic, but attachment of the 5-carbon linker to **6** to afford **7** required high-containment. In total, 8 stages needed to be undertaken in high-containment facilities.

To minimize the number of cytotoxic intermediates and thus the number of steps requiring high-containment facilities we reasoned that construction of the B-rings should be moved to as late as possible in the synthesis. To this end, we considered what would be suitable final steps for the synthesis. Oxidative cyclisation of both B-rings was

considered as the final step, but based on previous experience this chemistry would be incompatible with the sensitive maleimide group. It was thus decided to retain the alloc-deprotection and attachment of the PEG-maleimide linker as the final two steps, and investigate the oxidative cyclisation of both B-rings as the antepenultimate step (Scheme 2). Thus, starting from the previously reported mono-protected dimer (**9**),[15] the alloc-protected Val-Ala-PAB trigger was attached affording **10**, following which the TBS protecting groups were removed affording diol (**11**).

Scheme 2. Demonstration of the closure of the B-rings as the antepenultimate step.



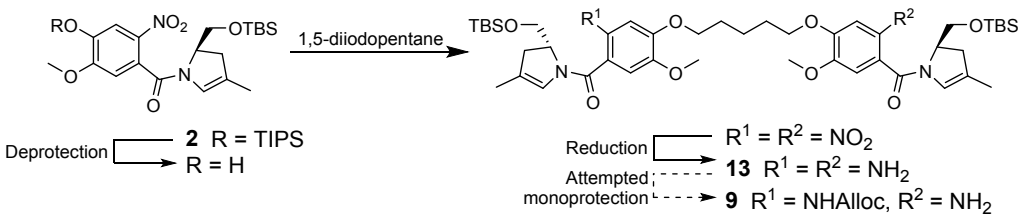
Identifying effective oxidation conditions which would reliably deliver full cyclisation of both B-rings proved challenging. A wide-ranging initial screen showed low conversion with methods such as Swern,[16] Pfitzner-Moffat[17] and TEMPO/bleach,[18] but over-

oxidation with Dess_Martin periodinane[19] and sulfur trioxide pyridine.[20] TEMPO/bis(acetoxy)iodobenzene (BAIB)[21] or Stahl conditions ([$Cu(MeCN)_4$]OTf, bpy, TEMPO, NMI)[22] were identified as the most promising options to get clean oxidative cyclisation of both B-rings. Attempted optimization of the TEMPO/BAIB showed significant variability in the reaction and in the purity of the product, thus development was directed to the use of Stahl conditions. Work focused on optimizing the equivalents of reagents and ensuring that effective air-sparging was scalable. These conditions gave consistent and selective oxidations, with >95% conversion to the required product (**12**). This intermediate then intercepts the original route for the final two steps to tesirine (Figure 1).

This changed route requires 3 steps to be completed in high-containment facilities, in contrast to the original route which requires 8 steps. To quantify the full benefits of this revised route in terms of cost reduction, it was decided to develop the required transformations to allow the newly demonstrated final steps to be used in a manufacturing campaign.

To this end, efficient routes to bis-TBS ether (**10**) were evaluated starting from the key intermediate **2**. Synthesis via the mono-protected dimer (**9**) was evaluated in detail (Scheme 3), where **2** was deprotected and alkylated with 1,5-diiodopentane following which nitro-reduction afforded a bis-aniline (**13**). For this approach to demonstrate synthetic efficiency, an efficient mono-alloc protection of **13** was required. This transformation was investigated in detail, including varying reagents, stoichiometries and conditions (including the use of biphasic systems), but no significant improvement could be made on the statistical ratio of 1:2:1 bis-aniline:mono-alloc protected product:bis-alloc protected product. Thus, separate preparation of two halves of the PBD-dimer was targeted as a more efficient method of preparing **10**.

Scheme 3. Approach to bis-TBS ether (**10**) via bis-aniline (**13**).



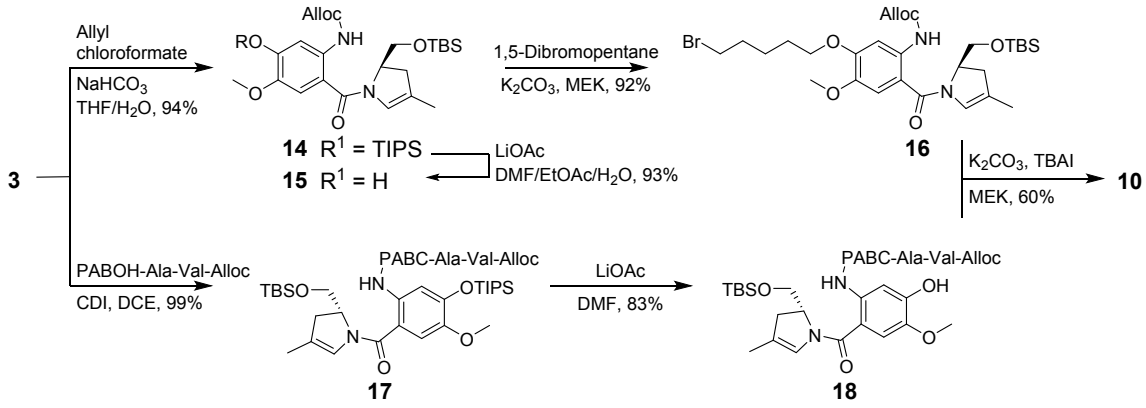
Key intermediate (**2**) was reduced to aniline (**3**) using zinc and ammonium acetate in ethanol. Alternative reagents for this transformation were evaluated and the use of dithionate initially looked promising, but more detailed analytical work showed that although effective for completing the reduction, the reagent also caused O- to N-migration of the TIPS group. Thus the original zinc conditions were adopted and the resultant material used without further purification. From this intermediate (**3**), work started on the separate functionalisation of the two halves of the dimer. For the alloc-protected half of the dimer, the alloc protecting group was introduced using allyl chloroformate and sodium hydrogen carbonate in aqueous THF and the product (**14**) used without further purification. Investigation into removal of the TIPS-group demonstrated that transformation could be achieved using potassium carbonate/methanol as well as the more standard lithium acetate/DMF, although the latter conditions were used for manufacturing as they gave a cleaner reaction profile. The product phenol (**15**) was purified using a silica plug. Introduction of the 5-carbon linker was then evaluated using a range of 1,5-dihalopentanes. The concept of using 1-bromo-5-chloropentane was investigated as it was reasoned that less of this reagent

would be required due to the difference in reactivity between the two ends. However, it was not possible to develop reproducible conditions using this approach, and the more standard 1,5-dibromopentane was employed. The reaction conditions and solvent were evaluated using factorial experimental design, with the optimized conditions using potassium carbonate in MEK affording >98% conversion. The obtained crude material was purified by flash column chromatography affording the product (**16**) with 98% Area HPLC purity.

The half of the PBD dimer with the linker-payload trigger portion was prepared by coupling **3** with Alloc-Val-Ala-PAB-OH, using CDI as a safer, more convenient alternative to the standard phosgene conditions to afford the carbamate (**17**) in high yields. The TIPS-group was deprotected using lithium acetate/DMF and the resulting phenol (**18**) was used without further purification. The two halves of the PBD-dimer were then coupled together to afford the required bis-TBS ether (**10**). This reaction was also optimized using factorial experimental design, with the best conditions utilizing potassium carbonate in MEK with the addition of tetrabutylammonium iodide (TBAI). The reaction was slow, typically taking 4-5 days, but a conversion of >98% could be

obtained and the resulting crude material was purified by flash column chromatography to afford **10** in good purity. Removal of the TBS-groups was achieved simply using TsOH in aqueous THF, and the resulting diol (**11**) could be obtained as solid following precipitation from methyl-THF and heptane. The oxidative cyclisation was then completed using the previously developed Stahl conditions and the product purified by reverse-phase HPLC to afford dimer (**12**). The final steps, common to the original route, involved alloc-deprotection and coupling of the PEG-maleimide linker, and these utilized conditions developed in previous campaigns, including purification using reverse-phase HPLC, to afford tesirine with 98.9% Area HPLC purity. The manufacturing campaign started with 1.3kg of intermediate **2**, and successfully delivered 151g of tesirine (Scheme 4).

Scheme 4. Central section of the revised synthesis of tesirine



With the new route having been demonstrated on a manufacturing scale, an analysis of the benefit of applying route design with a focus on reduction in the number of steps requiring high-containment was completed. A direct comparison of Cost of Goods (CoGs), utilizing $/g for tesirine delivered, showed that the new route afforded material that was 40% of the cost of the original route. It is worth noting that although the number of high-containment steps has been minimized to 3 steps (Figure 2, total number of synthetic chemistry steps in the new route is 30), these steps still represent 50% of the overall cost of the campaign.

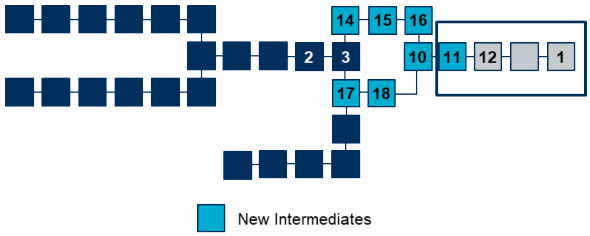


Figure 2. Schematic of revised synthesis of tesirine (each block representing an intermediate) showing portion conducted in high containment (inside blue box).

As noted previously, the concept of applying route design to minimize the number of steps requiring high-containment is applicable to the synthesis of all cytotoxic molecules. This concept is of significance for large-scale/commercial synthesis of cytotoxic molecules, but will be of interest to all chemists working on designing and developing improved routes to such molecules.

Conclusions

In summary, a new approach to route design for the synthesis cytotoxic payloads has been presented which focusses on the reduction in the number of synthetic chemistry steps requiring high-containment facilities. This has been exemplified by the development of an improved synthesis of the ADC linker-payload tesirine, where reducing the number of high-containment steps from 8 to 3 has afforded a 60% reduction in CoGs. This approach has broader applicability to the synthesis of cytotoxic molecules and provides an important focus for the development of new and improved routes to molecules with commercial potential.

Experimental Section

General Procedures. All solvents and reagents were commercially obtained and used without purification. Advanced intermediates were prepared using previously reported routes by dedicated Contract Manufacturing Organisations. NMR spectra were recorded on a Bruker 500MHz spectrometer. Accurate mass spectra analyses were recorded on a Waters LC-MS ZQ 2000 system in positive electrospray ionization mode (ESI).

*(S)-(2-amino-5-methoxy-4-(triisopropylsilyloxy)phenyl)(2-((tert-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (**3**).*[12] To a solution of (*S*)-(2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-(triisopropylsilyloxy)phenyl)methanone (**2**, 237g, 97.8% w/w, 0.40mol) in EtOH (2400mL) was charged zinc dust (234g, 3.58mol, 8.95eq). A solution of ammonium acetate (285g, 3.60mol, 9.00eq) in water (360mL) was added at a rate to control the exotherm, maintaining the reaction between 15 and 30°C. When full conversion was achieved the mixture was filtered to remove the inorganic solids. The

residual solids were washed with EtOH (200ml) and the filtered washings added to the filtered reaction solution. The solution was then concentrated, removing approximately 1500mL of EtOH affording a two-phase mixture. EtOAc (2000mL) and water (1000mL) were added and the layers separated. The organic phase was washed with 6% w/w aqueous sodium hydrogen carbonate solution. The combined aqueous phases were extracted with EtOAc (1000mL) and the organic phases combined. The combined EtOAc layers were washed with water (2 × 1000mL) and 10% w/w aqueous sodium chloride solution (2 × 1000mL) then the solvent was removed in vacuo affording the product (**3**, 214g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 97.6%, assay corrected yield 209g, 95%) as a clear orange oil. ^{1}H NMR (500 MHz, DMSO) -0.03 – 0.05 (m, 6H), 0.84 (s, 9H), 1.04 (s, 9H), 1.05 (s, 9H), 1.22 (hept, J = 7.4 Hz, 3H), 1.63 (s, 3H), 2.32 – 2.44 (m, 1H), 2.70 (dd, J = 16.0, 10.4 Hz, 1H), 3.60 (s, 3H), 3.63 – 3.89 (m, 2H), 4.41 – 4.52 (m, 1H), 4.97 (s, 2H), 6.03 – 6.22 (m, 1H), 6.31 (s, 1H), 6.61 (s, 1H).

*(S)-allyl 2-(2-((tert-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-(triisopropylsilyloxy)phenylcarbamate (**14**).*[12] To a solution of

(*S*)-(2-amino-5-methoxy-4-(triisopropylsilyloxy)phenyl)(2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (**3**, 200g, 97.6% w/w, 0.36mol) and sodium hydrogen carbonate (54.6g, 0.65mol, 1.80eq) in THF (1000mL) and water (500mL) was added allyl chloroformate (43.0g, 0.36mol, 1.00eq) at a rate to control gas evolution. When full conversion was achieved the phases were separated and orange organic upper phase dried over sodium sulfate, filtered and the solvent removed in vacuo affording the product (**14**, 227g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 93.0%, assay corrected yield 211g, 94%) as a clear orange oil. ^{1}H NMR (500 MHz, DMSO) -0.06 – 0.1 (m, 6H), 0.76 – 0.88 (m, 9H), 0.98 – 1.1 (m, 18H), 1.11 – 1.29 (m, 3H), 1.59 (s, 3H), 2.3 – 2.41 (m, 1H), 2.59 – 2.76 (m, 1H), 3.6 – 3.75 (m, 4H), 3.75 – 3.86 (m, 1H), 4.36 – 4.56 (m, 3H), 5.16 (dd, J = 10.5, 1.4 Hz, 1H), 5.25 (d, J = 17.4 Hz, 1H), 5.87 (ddt, J = 15.9, 10.6, 5.4 Hz, 1H), 5.99 (s, 1H), 6.77 (s, 1H), 7.17 (s, 1H), 8.95 (s, 1H).

*(S)-allyl 2-(2-((tert-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-5-hydroxy-4-methoxyphenylcarbamate (**15**):* To a solution of (*S*)-allyl 2-(2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-

methoxy-5-(triisopropylsilyloxy)phenylcarbamate (**14**, 304g, 72.4% w/w, 0.35mol) in DMF (290mL) and EtOAc (190mL) was added lithium acetate dihydrate (11.6g, 0.11mol, 0.32eq) in water (40mL). When full conversion was achieved the reaction mixture was diluted with EtOAc (2200mL), then washed with 0.1M citric acid solution (2000mL), water (2 × 2000mL) and saturated sodium chloride solution (1000mL). The solvent was removed in vacuo and the crude material dissolved in MeCN (1500mL) which was extracted with heptane (6 × 1000mL). The solvent was removed from the MeCN solution in vacuo and the crude product dissolved in MTBE (500mL) and petroleum ether (500mL) and purified by filtration through a silica plug (200g of silica), eluting with 1:1 EtOAc:petroleum ether (6000mL). The solvent was removed in vacuo affording the product (**15**, 206g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 75.0%, assay corrected yield 155g, 93%) as a yellow oil. ^{1}H NMR (500 MHz, DMSO) -0.08 – 0.09 (m, 6H), 0.74 – 0.91 (m, 9H), 1.60 (s, 3H), 2.32 – 2.41 (m, 1H), 2.6 – 2.74 (m, 1H), 3.59 – 3.86 (m, 5H), 4.38 – 4.55 (m, 3H), 5.17 (dd, J = 10.5, 1.4 Hz, 1H), 5.27 (dd, J = 17.5, 1.4 Hz, 1H), 5.89 (ddd, J = 22.5, 10.6, 5.4 Hz, 1H), 6.07 (s, 1H), 6.74 (s, 1H), 7.13 (s, 1H), 8.98 (s, 1H), 9.65 (s, 1H). ^{13}C{^{1}H} NMR (126 MHz,

DMSO) -5.5, -5.4, 17.8, 25.6, 31.2, 36.0, 55.9, 58.5, 62.1, 64.7, 110.1, 112.1, 117.2, 120.4, 125.2, 130.0, 133.2, 143.6, 148.4, 153.2, 164.0. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{37}N_2O_6Si$ 477.2416; Found 477.2418.

*(S)-allyl 5-(5-bromopentyloxy)-2-(2-((tert-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenylcarbamate (**16**):* A mixture of (*S*)-allyl 2-(2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-5-hydroxy-4-methoxyphenylcarbamate (**15**, 303g, 84.2% w/w, 0.54mol), 1,5-dibromopentane (714g, 3.11mol, 5.80eq) and potassium carbonate (129g, 0.93mol, 1.74eq) in MEK (2000mL) was stirred at 70°C. After 4 hours, in-process analysis indicated 54% conversion and additional potassium carbonate (111g, 0.81mol, 1.50eq) was added. After 6 hours, in-process analysis indicated 84% conversion and additional potassium carbonate (106g, 0.77mol, 1.43eq) was added. After 8 hours, in-process analysis indicated >98% conversion and the reaction mixture was cooled and filtered. The solvent was removed from the MEK solution in vacuo and the crude product slurried with silica gel (857g) and heptane (1000mL) then concentrated to dryness to give the adsorbed crude product as a powder. This powder was loaded into a glass

sinter funnel and eluted with heptane (50L) to remove the remaining 1,5-dibromopentane, then 1:3 EtOAc:heptane (32L). The solvent was removed from the product factions in vacuo affording the product (**16**, 352g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 84.6%, assay corrected yield 298g, 92%) as a yellow oil. ^{1}H NMR (500 MHz, DMSO) -0.08 – 0.09 (m, 6H), 0.73 – 0.87 (m, 9H), 1.44 – 1.54 (m, 2H), 1.59 (s, 3H), 1.67 – 1.79 (m, 2H), 1.84 (dt, J = 14.4, 6.8 Hz, 2H), 2.3 – 2.4 (m, 1H), 2.54 – 2.77 (m, 1H), 3.52 (t, J = 6.7 Hz, 2H), 3.58 – 3.76 (m, 4H), 3.76 – 3.89 (m, 1H), 3.93 (t, J = 6.4 Hz, 2H), 4.38 – 4.59 (m, 3H), 5.12 – 5.21 (m, 1H), 5.21 – 5.32 (m, 1H), 5.88 (ddd, J = 22.5, 10.6, 5.4 Hz, 1H), 6.00 (s, 1H), 6.76 (s, 1H), 7.22 (s, 1H), 9.01 (s, 1H). ^{13}C{^{1}H} NMR (126 MHz, DMSO) -5.5, -5.4, 17.8, 24.3, 25.62, 27.63, 31.2, 31.9, 35.0, 36.0, 55.8, 58.4, 62.0, 64.7, 68.1, 111.5, 117.2, 120.4, 125.0, 129.5, 133.2, 144.9, 149.2, 153.4, 163.6. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{29}H_{46}BrN_2O_6Si$ 625.2304; Found 625.2309.

*N-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-N-[4-({[(2-[(2S)-2-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-4-methoxy-5-{[tri(propan-2-yl)silyl]oxy}phenyl)carbamoyl]oxy}methyl)phenyl]-L-alaninamide (**17**):*

To a solution of (*S*)-(2-amino-5-methoxy-4-(triisopropylsilyloxy)phenyl) (2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (**3**, 350g, 90.2% w/w, 0.57mol) in DCE (2500mL) at 23°C was added CDI (113g, 0.68mol, 1.18eq). The mixture was stirred for 30 min then allyl (*S*)-1-((*S*)-1-(4-(hydroxymethyl)phenylamino)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate (241g, 98.3% w/w, 0.63mol, 1.09eq) was added. The reaction mixture was then heated to 80°C and stirred for 5 days at which time 98% conversion was observed. The reaction was cooled to ambient temperature and filtered through silica gel (250g). The silica gel was washed with DCE (500mL), the solutions combined and the solvent removed *in vacuo* to afford the product (**17**, 650g, w/w assay by ^{1}H NMR [$CDCl_3$, dimethylsulfone internal standard] 84.0%, assay corrected yield 546g, 99%) as an orange foam. ^{1}H NMR (500 MHz, DMSO) -0.02 – 0.09 (m, 6H), 0.89 (s, 9H), 0.91 – 1.13 (m, 27H), 1.35 (d, J = 6.8 Hz, 3H), 1.64 (s, 3H), 1.94 – 2.11 (m, 1H), 2.36 – 2.51 (m, 1H), 2.63 – 2.79 (m, 1H), 3.78 (d, J = 8.0 Hz, 3H), 3.91 – 3.96 (m, 1H), 3.98 (t, J = 5.8 Hz, 1H), 4.35 – 4.41 (m, 1H), 4.42 – 4.58 (m, 4H), 5 – 5.13 (m, 2H), 5.21 (d, J = 10.5 Hz, 1H), 5.27 – 5.41 (m, 1H), 5.95 (ddt, J = 15.8, 10.5, 5.3 Hz, 1H), 6.03 – 6.26 (m, 1H),

7.25 (d, J = 1.1 Hz, 1H), 7.3 – 7.32 (m, 1H), 7.35 (dd, J = 8.4, 3.3 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 7.0 Hz, 1H), 8.93 – 9.13 (m, 1H), 10.08 (s, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO) -5.5, -5.4, 17.7, 17.8, 18.1, 19.2, 25.6, 30.3, 31.9, 36.2, 44.2, 49.0, 55.9, 59.0, 59.9, 62.1, 64.4, 65.5, 116.9, 118.89, 118.91, 119.6, 121.0, 127.9, 128.56, 128.62, 131.2, 131.3, 133.6, 134.9, 137.2, 137.6, 138.75, 138.81, 156.0, 164.0, 171.0, 171.1. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{49}H_{78}N_5O_{10}Si_2$ 952.5282; Not Found (only mass for **18** found).

*(N-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-N-(4-{[({2-[(2S)-2-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-hydroxy-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (**18**):* To a solution of *N*-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-*N*-[4-({[(2-[(2*S*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-4-methoxy-5-{[tri(propan-2-yl)silyl]oxy}phenyl)carbamoyl]oxy}methyl)phenyl]-L-alaninamide (**17**, 641g, 84.0% w/w, 0.57mol) in DMF (3200mL) and water (64mL) at ambient temperature was added LiOAc (46.0g, 0.45mol, 0.80eq). When full conversion was achieved the reaction mixture was diluted with MTBE (3000mL), then washed water (3000mL then

2000mL). The aqueous washes were back extracted with MTBE (1500mL) and the combined organic portions filtered through silica gel (190g). The silica gel was washed with MTBE (1000mL) and the organic portions combined. The solvent was removed in vacuo and the crude material dissolved in MeCN (1250mL) which was washed with petroleum ether (5 × 1250mL). The solvent was removed from the MeCN solution in vacuo affording the product (**18**, 503g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 75.0%, assay corrected yield 377g, 83%) as an orange waxy solid. ^{1}H NMR (500 MHz, DMSO) -0.05 – 0.07 (m, 6H), 0.81 – 0.92 (m, 15H), 1.31 (d, J = 7.1 Hz, 3H), 1.62 (s, 3H), 1.98 (dq, J = 13.4, 6.7 Hz, 1H), 2.38 (d, J = 15.5 Hz, 1H), 2.62 – 2.72 (m, 1H), 3.65 – 3.77 (m, 4H), 3.77 – 3.86 (m, 1H), 3.86 – 3.94 (m, 1H), 4.36 – 4.56 (m, 4H), 4.97 – 5.07 (m, 2H), 5.17 (d, 1H), 5.31 (dd, 1H), 5.91 (ddt, J = 15.8, 10.5, 5.3 Hz, 1H), 6.11 (s, 1H), 6.7 – 6.85 (m, 1H), 7.19 (s, 1H), 7.25 (d, J = 8.7 Hz, 1H), 7.31 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 8.17 (d, J = 7.0 Hz, 1H), 9.02 (s, 1H), 9.69 (s, 1H), 10.01 (s, 1H). ^{13}C{^{1}H} NMR (126 MHz, DMSO) -5.5, -5.4, 17.80, 17.82, 18.1, 19.2, 25.6, 30.3, 36.0, 49.0, 55.9, 58.5, 59.9, 62.1, 64.4, 65.5, 112.1, 116.9, 118.9, 120.5,

125.2, 128.6, 130.1, 131.2, 138.8, 143.5, 148.4, 153.4, 156.0, 164.0, 171.0, 171.1. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{40}H_{58}N_5O_{10}Si$ 796.3948; Found 796.3949.

*N-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-N-(4-{[({2-[(2S)-2-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-[(5-{4-[(2S)-2-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-2-methoxy-5-({[(prop-2-en-1-yl)oxy]carbonyl}amino)phenoxy}pentyl)oxy]-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (**10**):* To a solution of *N*-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-*N*-(4-{[({2-[(2*S*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-hydroxy-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (**18**, 236g, 82.4% w/w, 0.24mol) and (*S*)-allyl 5-(5-bromopentyloxy)-2-(2-((*tert*-butyldimethylsilyloxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxyphenylcarbamate (**16**, 214g, 76.7% w/w, .0.26mol, 1.07eq) in MEK (2500mL) was added potassium carbonate (34.6g, 0.25mol, 1.03eq) and tetrabutyl ammonium iodide (90.6g, 0.23mol, 0.96eq) and the mixture was stirred at 50°C. After 29 hours, additional potassium carbonate (17.4g, 0.13mol, 0.52eq) was added. After an additional 43 hours, further potassium carbonate

(17.4g, 0.13mol, 0.52eq) was added. After a total reaction time of 118 hours, the reaction was cooled and water (1500mL) added. The layers were separated and the organic layer washed with saturated aqueous sodium hydrogen carbonate solution (600mL) and saturated aqueous sodium chloride solution (300mL). The solvent was removed in vacuo and the residue dissolved in MTBE (1500mL) and washed with water (1000mL). The organic layer was filtered through a plug of silica gel (340g), eluting with MTBE (1800mL) and the solvent removed from the filtrate in vacuo affording a tan foam. The material was dry-loaded onto silica gel (600g) using MTBE and purified in portions using flash column chromatography (80g of dry-loaded silica gel, 330g of silica gel, gradient of 45% to 100% EtOAc in petroleum ether). Impure fractions were collected and further purified using the same procedure. The purified material was combined affording the product (**10**, 226g, w/w assay by ^{1}H NMR [$CDCl_3$, dimethylsulfone internal standard] 87.0%, assay corrected yield 196g, 60%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO) -0.24 – 0.22 (m, 12H), 0.90 (m, 24H), 1.34 (d, J = 7.0 Hz, 3H), 1.53 – 1.77 (m, 9H), 1.79 – 1.92 (m, 4H), 2.02 (q, J = 7.2, 6.8 Hz, 1H), 2.42 (d, J = 15.5 Hz, 2H), 2.63 – 2.81 (m, 2H), 3.63 – 4.12 (m, 15H), 4.39 – 4.65 (m, 7H), 5.00 – 5.13 (m, 2H), 5.15

– 5.29 (m, 2H), 5.34 (d, J = 17.2 Hz, 2H), 5.95 (ddt, J = 20.2, 9.7, 5.2 Hz, 2H), 6.08 (s, 2H), 6.82 (s, 2H), 7.31 (m, 4H), 7.63 (d, J = 8.5 Hz, 2H), 8.20 (d, J = 7.0 Hz, 1H), 9.09 (s, 2H), 10.04 (s, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO) -5.5, -5.4, 13.4, 17.9, 18.1, 19.2, 22.3, 25.7, 28.3, 30.4, 36.1, 39.2, 49.0, 55.8, 58.5, 59.9, 62.1, 64.4, 64.8, 65.6, 68.2, 111.5, 116.9, 117.3, 118.9, 120.6, 125.0, 128.6, 129.7, 131.2, 133.2, 133.6, 138.8, 144.9, 149.4, 153.5, 153.6, 156.0, 163.7, 171.0, 171.1. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{69}H_{102}N_7O_{16}Si_2$ 1340.6917; Found 1340.6915.

N-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-N-(4-{[({2-[(2S)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-[(5-{4-[(2S)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-2-methoxy-5-({[(prop-2-en-1-yl)oxy]carbonyl}amino) phenoxy}pentyl)oxy]-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (11): To a solution of *N*-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-*N*-(4-{[({2-[(2*S*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-[(5-{4-[(2*S*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-2-methoxy-5-({[(prop-2-en-1-yl)oxy]carbonyl}amino)phenoxy}pentyl)oxy]-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (**10**, 402g, 89.4% w/w,

0.27mol) in THF (2000mL) was added water (200mL) then para-toluenesulfonic acid monohydrate (47.4g, 0.26mol, 0.90eq). The mixture was stirred at ambient temperature for 8 hours then diluted with EtOAc (5000mL). The organic solution was washed with water (2 × 4000mL), saturated aqueous sodium hydrogen carbonate solution (1200mL) and saturated aqueous sodium chloride solution (600mL). The organic solution was dried over sodium sulfate and the solvent removed in vacuo to afford a colourless solid. This solid was slurried in MTBE (2000mL), isolated by filtration and washed with MTBE (1000mL). The solid was dried at ambient temperature under vacuum, then slurry washed in MTBE:petroleum ether (1:1, 2000mL). The resulting solid was further slurry washed in petroleum ether (2000mL) then dried at ambient temperature under vacuum. The solid was dissolved in 2-MeTHF (1000mL) and this solution was added to n-heptane (3500mL) affording a white precipitate. The solid was isolated by filtration and dried at ambient temperature under vacuum affording the product (**11**, 304g, w/w assay by ^{1}H NMR [CD_3OD, dimethylsulfone internal standard] 93.6%, assay corrected yield 284g, 95%) as an off-white solid. ^{1}H NMR (500 MHz, DMSO) 0.83 – 0.90 (m, 3H), 0.93 (d, J = 6.8 Hz, 3H), 1.35 (d, J = 7.0 Hz, 3H), 1.54 – 1.78 (m, 8H), 1.76 – 1.92 (m, 4H),

2.02 (dq, J = 13.5, 6.6 Hz, 1H), 2.45 (d, J = 16.6 Hz, 2H), 2.63 – 2.79 (m, 2H), 3.56 (s, 2H), 3.68 (s, 2H), 3.74 – 3.85 (m, 6H), 3.9 – 3.99 (m, 1H), 4.03 (t, J = 6.3 Hz, 4H), 4.35 – 4.68 (m, 7H), 4.90 (s, 2H), 5.01 – 5.16 (m, 2H), 5.16 – 5.28 (m, 2H), 5.28 – 5.42 (m, 2H), 5.87 – 6.09 (m, 4H), 6.90 (s, 2H), 7.20 – 7.32 (m, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 8.21 (d, J = 6.9 Hz, 1H), 9.07 (s, 2H), 10.05 (s, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO) 13.5, 18.1, 19.2, 22.3, 26.8, 28.3, 30.3, 36.3, 48.7, 49.0, 55.9, 59.1, 59.9, 61.0, 64.4, 64.8, 65.6, 68.2, 111.7, 116.9, 117.2, 118.9, 120.6, 125.0, 128.6, 129.2, 131.3, 133.2, 133.6, 138.8, 145.0, 149.2, 153.6, 153.8, 156.0, 163.8, 171.0, 171.1. HRMS (ESI/QTOF) m/z: $[M+H]^+$ Calcd for $C_{57}H_{74}N_7O_{16}$ 1112.5187; Found 1112.5171.

*(11S)-allyl 8-(5-((11S)-10-((4-((S)-2-((S)-2-(allyloxycarbonylamino)-3-methylbutanamido)propanamido)benzyloxy)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-yloxy)pentyloxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**12**):*[13] *N*-{[(prop-2-en-1-yl)oxy]carbonyl}-L-valyl-*N*-(4-{[({2-[(2*S*)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl]-5-[(5-{4-[(2*S*)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-

carbonyl]-2-methoxy-5-({[(prop-2-en-1-yl)oxy]carbonyl}amino) phenoxy}pentyl)oxy]-4-methoxyphenyl}carbamoyl)oxy]methyl}phenyl)-L-alaninamide (**11**) (230g, 0.207mol) was dissolved in DCM (4600mL) at 23°C and the mixture sparged with air. Tetrakisacetonitrile copper(I) triflate (27.4g, 0.073mol, 0.35eq) and freshly prepared TEMPO Stahl solution (363mL, 0.2M in MeCN, 0.072mol, 0.35eq) were added and the mixture stirred at ambient temperature with an air sparge for 24h. The reaction mixture was then washed with 5% w/v aqueous sodium thiosulfate solution (2300mL) and the aqueous wash back extracted with DCM (1150mL). The combined organic portions were washed with saturated aqueous ammonium chloride solution (2 × 4600mL) and 1% aqueous sodium chloride solution (2 × 2070mL) and the solvent removed in vacuo. The resulting crude material was dissolved in DCM (4600mL) and the solvent removed in vacuo three times, then the final crude material was dissolved in DCM (4830mL). The solution was purified in portions using high pressure liquid chromatography (eluting with DCM/MeOH 95.5/4.5 (v/v)). The purified material was combined and the solvent removed in vacuo affording the product as a colourless oil. This oil was redissolved in DCM to give the product (**12**, 4450g, w/w assay by HPLC 4.0%, assay corrected yield

178g, 78%, area% purity by HPLC 99%) as a DCM solution. ^{1}H NMR (400 MHz, Isolated Sample, DMSO) 0.85 (dd, J = 18.5, 6.8 Hz, 6H), 1.29 (d, J = 7.0 Hz, 3H), 1.56 (d, J = 7.8 Hz, 2H), 1.67 – 1.9 (m, 10H), 2.50 (p, J = 1.8 Hz, 2H), 2.91 (dd, J = 17.3, 10.4 Hz, 2H), 3.66 (td, J = 9.8, 3.3 Hz, 2H), 3.79 (d, J = 8.0 Hz, 6H), 3.85 – 4.03 (m, 4H), 4.33 – 4.52 (m, 4H), 4.58 (d, J = 13.6 Hz, 1H), 4.83 (d, J = 12.4 Hz, 1H), 4.97 – 5.22 (m, 4H), 5.29 (dd, J = 17.2, 1.9 Hz, 1H), 5.58 (s, 2H), 5.71 – 6.01 (m, 2H), 6.57 – 6.71 (m, 4H), 6.73 (s, 1H), 6.80 (s, 1H), 7.05 (s, 1H), 7.07 (s, 1H), 7.19 (dd, J = 17.4, 8.4 Hz, 3H), 7.53 (d, J = 8.0 Hz, 2H), 8.14 (d, J = 7.0 Hz, 1H), 9.97 (s, 1H).

Associated Content

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Factorial experimental design optimization studies for the preparation of **16** and **10**

Optimization studies for the preparation of **12**

Copies of ^{1}H and ^{13}C NMR spectra

Author Information

Corresponding Author

*E-mail: jeremy.parker@astrazeneca.com.

ORCID

William Goundry: 0000-0003-2869-5855

Jeremy Parker: 0000-0002-4758-3181

Arnaud Tiberghien: 0000-0002-2936-0515

Notes

The authors declare no competing financial interest.

Acknowledgements

Luke Masterson (Advice and Guidance); Matt Welham (Project Management); Elfyn Jones, Mikael Hillgren (Scale-Up of **11**); Sandrine Ballot (Scale-Up of **12**).

References

(1) (a) Beck, A.; Goetsch, L.; Dumontet, C.; Corvaia, N. Strategies and challenges for the next generation of antibody–drug conjugates. *Nat. Rev. Drug Discovery* **2017**, *16*, 315-337; (b) Joubert, N.; Denevault-Sabourin, C.; Bryden, F.; Viaud-Massuard, M. C. Towards antibody-drug conjugates and prodrug strategies with extracellular stimuli-responsive drug delivery in the tumor microenvironment for cancer therapy. *Eur. J. Med. Chem.* **2017**, *142*, 393-415.

(2) Senter, P. D.; Sievers, E. L. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat. Biotechnol.* **2012**, *30*, 631-637.

(3) Lambert, J. M.; Chari, R. V. J. Ado-trastuzumab Emtansine (T-DM1): An Antibody–Drug Conjugate (ADC) for HER2-Positive Breast Cancer. *J. Med. Chem.* **2014**, *57*, 6949-6964.

(4) Maderna, A.; Leverett, C. A. Recent Advances in the Development of New Auristatins: Structural Modifications and Application in Antibody Drug Conjugates. *Mol. Pharm.* **2015**, *12*, 1798-1812.

(5) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. The Isolation and Structure of a Remarkable Marine Animal Antineoplastic Constituent: Dolastatin 10. *J. Am. Chem. Soc.* **1987**, *109*, 6883-6885.

(6) Erickson, H. K.; Lambert, J. M. ADME of Antibody-Maytansinoid Conjugates. *AAPS Journal* **2012**, *14*, 799-805.

(7) Morris Kupchan, S.; Komoda, Y.; Court, W. A.; Thomas, G. J.; Smith, R. M.; Karim, A.; Gilmore, C. J.; Haltiwanger, R. C.; Bryan, R. F. Tumor Inhibitors. LXXIII. Maytansine,

a Novel Antileukemic Ansa Macrolide from Maytenus ovatus. *J. Am. Chem. Soc.* **1972**, *94*, 1354-1356.

(8) Mantaj, J.; Jackson, P. J.; Rahman, K. M.; Thurston, D. E. From Anthramycin to Pyrrolobenzodiazepine (PBD)-Containing Antibody–Drug Conjugates (ADCs). *Angew. Chem., Int. Ed.* **2017**, *56*, 462-488.

(9) Hartley, J. A.; Flynn, M. J.; Bingham, J. P.; Corbett, S.; Reinert, H.; Tiberghien, A.; Masterson, L. A.; Antonow, D.; Adams, L.; Chowdhury, S.; Williams, D. G.; Mao, S.; Harper, J.; Havenith, C. E. G.; Zammarchi, F.; Chivers, S.; van Berkel, P. H.; Howard, P. W. Pre-clinical pharmacology and mechanism of action of SG3199, the pyrrolobenzodiazepine (PBD) dimer warhead component of antibody-drug conjugate (ADC) payload tesirine. *Sci. Rep.* **2018**, *8*, 10479.

(10) Lashari B. H.; Kolandra L.; Hamid M.; Vallatharasu Y.; Uprety D. Rovalpituzumab Tesirine: A Novel DLL3-Targeting Antibody-Drug Conjugate. *Drugs R D*, **2018**, *18*, 255-258.

(11) O'Connor, O. A.; Kahl, B. S.; Hamadani, M.; Caimi, P.; Reid, E.; Feingold, J.; Havenith, K.; He, S.; Mould, D. R.; Freedman, I.; Boni, J. Elucidating Exposure-Response (Safety and Efficacy) of ADCT-402 (Loncastuximab Tesirine), a Novel Pyrrolobenzodiazepine-Containing Antibody Drug Conjugate, for Recommended Phase 2 Dose Determination in Patients with Relapsed or Refractory Non-Hodgkin Lymphoma. *Blood* **2017**, *130*, 2543.

(12) Tiberghien, A. C.; Levy, J. N.; Masterson, L. A.; Patel, N. V.; Adams, L. R.; Corbett, S.; Williams, D. G.; Hartley, J. A.; Howard, P. W. Design and Synthesis of Tesirine, a Clinical Antibody–Drug Conjugate Pyrrolobenzodiazepine Dimer Payload. *ACS Med. Chem. Lett.* **2016**, *7*, 983-987.

(13) Tiberghien, A. C.; von Bulow, C.; Barry, C.; Ge, H.; Noti, C.; Collet Leiris, F.; McCormick, M.; Howard, P. W.; Parker, J. S. Scale-up Synthesis of Tesirine. *Org. Process Res. Dev.* **2018**, *21*, 1241-1256.

(14) Vlahov, I. R.; Qi, L.; Kleindl, P. J.; Santhapuram, H. K.; Felten, A.; Parham, G. L.; Wang, K.; You, F.; Vaughn, J. F.; Hahn, S. J.; Klein, H. F.; Vetzel, M.; Reddy, J. A.;

Nelson, M.; Nicoson, J.; Leamon, C. P. Latent Warheads for Targeted Cancer Therapy: Design and Synthesis of pro-Pyrrolobenzodiazepines and Conjugates. *Bioconjugate Chem.* **2017**, *28*, 2921-2931.

(15) Howard, P W. Pyrrolobenzodiazepines And Conjugates Thereof. *WO 2014/057074 A1* **2014**.

(16) Mancuso, A. J.; Swern, D. Activated Dimethyl Sulfoxide: Useful Reagents for Synthesis. *Synthesis* **1981**, *3*, 165–185.

(17) Pfitzner, K. E.; Moffatt, J. G. A New and Selective Oxidation of Alcohols. *J. Am. Chem. Soc.* **1963**, *85*, 3027-3028.

(18) Vogler, T.; Studer, A. Applications of TEMPO in Synthesis. *Synthesis* **2008**, *13*,1979-1993.

(19) Dess, D. B.; Martin, J. C. Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. *J. Org. Chem.* **1983**, *48*: 4155-4156.

(20) Parikh, J. R.; Doering, W. v. E. Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

(21) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. A Versatile and Highly Selective Hypervalent Iodine (III)/2,2,6,6-Tetramethyl-1-piperidinyloxyl-Mediated Oxidation of Alcohols to Carbonyl Compounds. *J. Org. Chem.* **1997**, *62*, 6974-6977.

(22) Hoover, J. M.; Stahl, S. S. Highly Practical Copper(I)/TEMPO Catalyst System for Chemoselective Aerobic Oxidation of Primary Alcohols. *J. Am. Chem. Soc.* **2011**, *133*, 16901-16910.