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Org. Process Res. Dev., **Just Accepted Manuscript** • DOI: 10.1021/acs.oprd.5b00390 • Publication Date (Web): 06 Jan 2016

Just Accepted

ACS Publications

Organic Process Research & Development is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Process Development and Synthesis of Birinapant – Large Scale Preparation and Acid-mediated Dimerization of the Key Indole Intermediate

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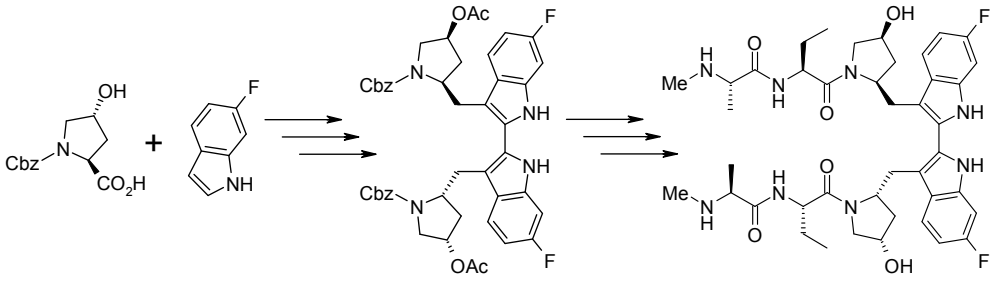


9 **12** **21** **Birinapant/TL32711 (1)**

KEYWORDS: birinapant, Smac-mimetic, peptide, biindole, hydroxyproline

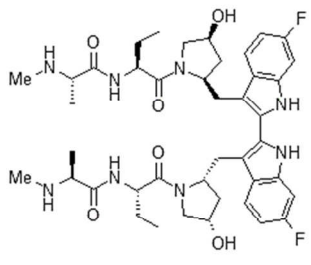
ABSTRACT

Birinapant/TL32711 (**1**) is a novel bivalent antagonist of the inhibitor of apoptosis (IAP) family of proteins which is currently in clinical development for the treatment of cancer and hepatitis B virus (HBV) infection. In this report, we present a detailed description of the **1** drug substance synthesis used to support our ongoing clinical studies. Key transformations in this process included the development of a scalable, high-yielding route to acyl indole **14** as well as a two-step dimerization/oxidation of indole **8** that afforded biindole **21** in excellent yield and purity (70% yield, 2 steps; >95 area% purity by HPLC analysis). In addition, partial defluorination of **21** was observed following hydrogen-mediated benzyloxycarbonyl (Cbz) protective group removal which was obviated by the use of HBr/HOAc for this transformation. The use of commercially-available amino acid derivatives afforded related impurities which proved difficult to purge in subsequent steps. Thus, defining the impurity specification for these reagents was critical to providing **1** drug substance of >99 area% chemical purity. Using this process, we have successfully prepared **1** drug substance multiple times on >500 g-scale in support of our clinical development program.

INTRODUCTION

Apoptosis, or programmed cell death, is required for normal cellular function, tissue development and homeostasis.[1] The inhibitor of apoptosis, or IAP, family of proteins include the X chromosome-linked inhibitor of apoptosis protein (XIAP),[2] cellular IAP 1 (cIAP1) and cIAP2,[3] and melanoma IAP (ML-IAP),[4] amongst others.[5] Many of these proteins have well-described roles in the suppression of apoptosis under normal physiological conditions.[6] Inhibition of apoptosis might also contribute to the initiation and propagation of many cancers and further the development of drug resistance.[7] Inhibiting the IAPs therefore is an attractive strategy for anticancer drug development.[8]

The second mitochondria-derived activator of caspases (Smac/DIABLO) is the endogenous antagonist of IAP proteins.[9] Structural and biological studies have shown that the interaction between the *N*-terminal tetrapeptide of Smac, AVPI, and IAPs is mediated through a shallow groove present on certain baculovirus IAP repeat (BIR) domains of the IAPs. Several small molecule mimetics of AVPI, termed IAP antagonists, are being advanced in clinical trials for the treatment of cancer.[10] Birinapant/TL32711 (**1**, Figure 1) is a novel bivalent antagonist of the IAPs which is currently in clinical trials for the treatment of cancer and HBV infection.[11,12] Here we report the development of a synthetic process for the kg-scale preparation of **1** drug substance to support early-phase clinical development.



Birinapant (**1**)

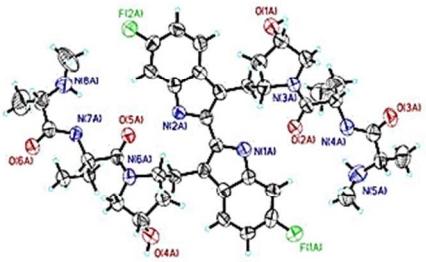


Figure 1. The chemical structure and ORTEP diagram of 1.

RESULTS AND DISCUSSION

Retrosynthetic Analysis of 1. We envisioned that the synthesis of **1** would rely on the parallel assembly of the two dipeptide chains from a central biindole-containing template represented by **A** (Figure 2). To prepare the biindole nucleus, further retrosynthetic analysis suggested the acid-catalyzed dimerization of monomer unit **B** followed by oxidation.[13] Thus, the preparation of monomer **B**, including the appropriate selection of heteroatom protective groups (PG_1 and PG_2), became the initial focus of our development efforts.

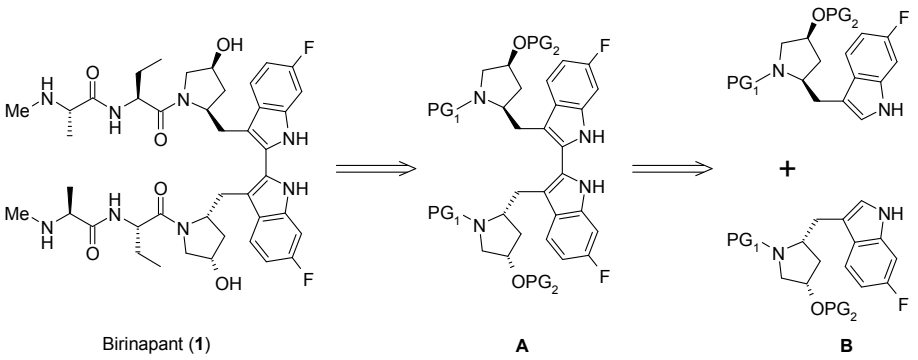
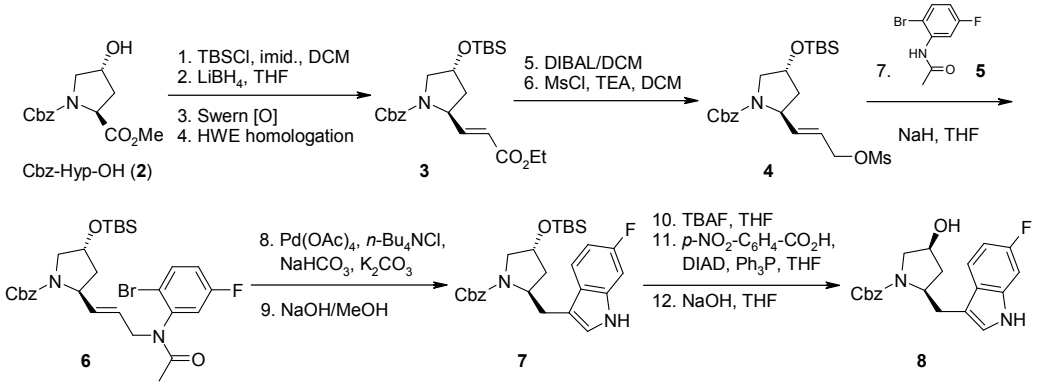


Figure 2. Retrosynthetic analysis of 1.

The Discovery Route to Indole 8. During the discovery phase of the program, we relied upon a synthetic route which allowed for the construction of multiple series of 2-arylalkyl or 2-heteroarylalkyl pyrrolidines.[14] For the eventual discovery of the biindole series of IAP antagonists represented by **1**, we identified indole **8** as a key synthetic intermediate (Scheme 1). Towards this end, Cbz-Hyp-OMe (**2**) was converted to ester **3** and then to **4** following standard procedures.[15] Alkylation of **5** with mesylate **4** afforded the desired acetanilide (**6**). Treatment of **6** with palladium acetate effected indole formation; then, removal of the pendent acetyl group provided indole **7** as an oil.[16] Inversion of the secondary alcohol was accomplished in three steps

to yield the partially-protected monomer unit (**8**). Although this synthetic route provided sufficient quantities of **8** to support the early drug discovery program, the low overall yield (ca. 10%) and reliance on several chromatographic purifications made this process unsuitable for further development.

Scheme 1. First-Generation Route to Indole 8



Process Development of Indole 8 Synthesis. The second generation retrosynthetic analysis of **8** is shown in Figure 3. We envisioned that **8** could be accessed via the reduction of ketone **C** which, in turn, would be prepared via the coupling of *O*-protected Cbz-Hyp-OH (**D**) with an organometallic species derived from 6F-indole (**E**). Macor *et al.* have described the *C3*-acylation of indoles using the acid chloride derived from Cbz-Pro-OH and indole-derived Grignard reagents in benzene[17] and this strategy has been exploited to prepare the anti-migraine agent eletriptan.[18] From literature reports, we recognized that the choice of the organometallic indole species **E** and solvent might have a profound effect on the ratio of *C3*- versus *N1*-acylation products.[19] For our program, we would also need to determine at which stage to perform the inversion of the secondary alcohol of Cbz-Hyp-OH.

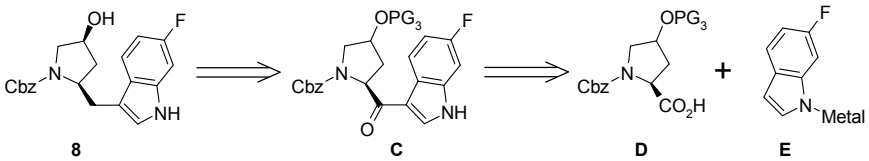
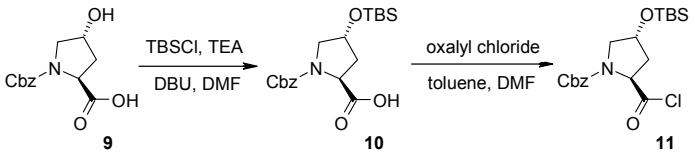


Figure 3. An alternative retrosynthetic analysis of indole 8.

As the *tert*-butyldimethylsilyl (TBS) group was employed in the first generation synthesis, commercially-available Cbz-Hyp-OH (**9**, ≥98% purity by HPLC) was reacted with TBSCl which, following work-up, afforded Cbz-Hyp(TBS)-OH (**10**, Scheme 2).[20] Although dichloromethane solvent could be employed in the acid chloride formation, subsequent reaction with indole-derived Grignard reagents is reported to require an aromatic solvent.[19,21] Thus, toluene was employed for the acid chloride formation. A toluene solution of **10** was treated with oxalyl chloride and DMF (cat.) at 25 °C to generate Cbz-Hyp(TBS)-Cl (**11**). The reaction mixture was partially-concentrated at 40-45 °C under reduced pressure to remove HCl and excess oxalyl chloride. At temperatures >50 °C, we observed lower yields of **11** which we attributed to the loss of the TBS group and subsequent polymerization of the formed Cbz-Hyp-Cl (not shown).

Scheme 2. Synthesis of Cbz-Hyp(TBS)-Cl in Toluene Solution

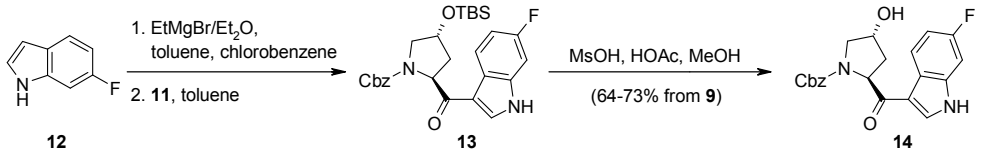


9 **10** **11**

In advance of the key indole acylation, a solution of 6F-indole (**12**) in toluene was treated with 3.0 M EtMgBr in diethyl ether to form a 6F-indoylmagnesium bromide solution (Scheme 3). The choice of solvent for the acylation reaction proved to be critical. The use of toluene as a single solvent resulted in agglomeration when toluene solutions of **11** and **12**·MgBr (2 equivs) were combined. A mixed-solvent screen consisting of diethyl ether, THF, MTBE, toluene, and chlorobenzene identified chlorobenzene as the preferred co-solvent (Supporting Information, Table S4). Under this condition, the desired acyl indole (**13**) together with 5-10% **14** formed

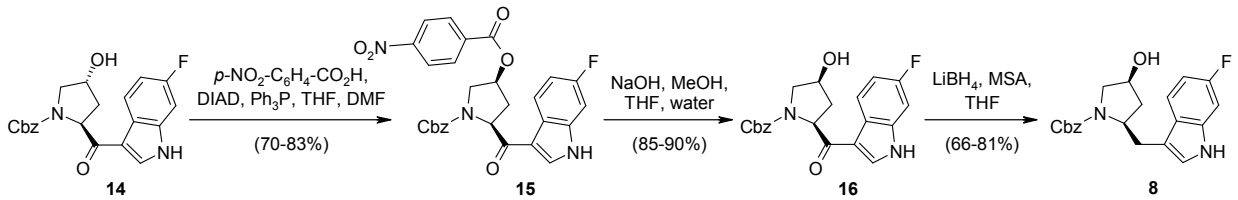
without any precipitation throughout the addition process. To obviate the separation of **13** and **14**, the homogeneous reaction mixture was further diluted with acetic acid (HOAc)/MeOH and treated with methanesulfonic acid (MSA) resulting in the precipitation of **14** directly from the reaction mixture. On kg-scale, the four-step transformation of **9** was accomplished without the isolation of any intermediates to afford **14** in ca. 73% overall yield (based on input **9**; >98 area% by HPLC).

Scheme 3. One-pot Acylation of 6-Fluoroindolylmagnesium Bromide



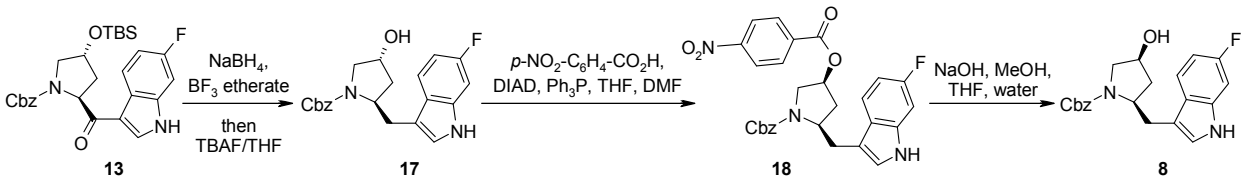
Inversion of the secondary alcohol under the Mitsunobu condition[22] gave *para*-nitrobenzoate **15** which, without isolation, was saponified to afford keto-alcohol **16** as an easily filterable white-colored solid in ca. 70-80% yield (from **14**, >98 area% by HPLC, Scheme 4). Importantly, HPLC analysis indicated no formation of the non-inverted nitrobenzoate which was prepared by independent synthesis (not shown). In addition, isolated **16** was absent of any unreacted **14** further confirming the efficiency of this two-step transformation. Carbonyl group reduction of **16** was accomplished using 2.0 M $LiBH_4$ (2.5 equivalents) and MSA (1.8 equivalents) in THF at room temperature to give **8** as an isolable solid in ca. 70-80% yield (>97 area% by HPLC).

Scheme 4. Mitsunobu Inversion of the Secondary Alcohol and Ketone Reduction



Alternative Synthesis of Indole 8. In the course of this investigation, we observed that ketone **8** was reduced using $NaBH_4/BF_3$ etherate to afford **17** after TBS group removal (1 M TBAF in THF, Scheme 5). Inversion of the secondary alcohol was accomplished using standard conditions and the resultant nitrobenzoate (**18**) was saponified to give **8**. Although these reactions proceeded satisfactorily on gram-scale, a significant disadvantage to this route was that intermediates **17** and **18** were isolated as oils. As intermediates **14**-**16** (Schemes 3 and 4) were recrystallizable solids, this alternative route was not pursued further.

Scheme 5. An Alternative Route to Indole 8

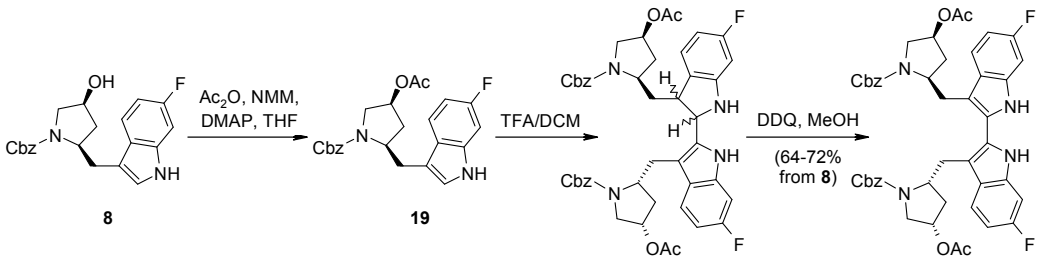


13 **17** **18** **8**

TFA-Mediated Indole Dimerization. The dimerization of tryptophan derivatives and other indoles in neat trifluoroacetic acid (TFA) has been reported.[13] Unfortunately, the TFA-mediated dimerization of **8** afforded multiple unidentified products by HPLC analysis possibly due to instability of the intermediate indoylindolines (not shown). We reasoned that the free alcohol group might be contributing to this poor outcome so we formed the acetyl ester (**19**) using acetic anhydride and *N*-methylmorpholine (NMM)/DMAP in THF in quantitative yield with only minimal (ca. 0.5%) indole *N*-acetylation (Scheme 6).[23] To facilitate transfer into the dimerization reaction, **19** was provided in DCM solution. In the reaction, **19** in DCM (3 volumes) was added to pre-cooled (-10 °C) TFA (6 volumes). Higher DCM/TFA ratios resulted in substantially reduced reaction rates. Under these optimized conditions, ca. 85% consumption of **19** (HPLC analysis) was observed at 3 h post-addition. In an effort to consume the remaining **19**, DCM was removed under reduced pressure thus allowing the reaction to proceed to >95% completion. The acidic reaction mixture was carefully quenched by reverse addition into

aqueous K_2CO_3 and the indoylindoline diastereomers (**20**) were extracted into isopropylacetate (IPAc). In advance of the DDQ oxidation, the IPAc was replaced with MeOH. Oxidation of **20** with DDQ in MeOH afforded **21** which precipitated while the DDQ by-product remained in solution. Following isolation, crude **21** was recrystallized from DMF/MeOH to provide **21** as an off-white solid in ca. 70% yield (from **8**, >98 area% by HPLC).

Scheme 6. TFA-Mediated Dimerization of Indole 19 to Form Biindole Core Template



8 **19** **20** **21**

Stereochemical Purity of Biindole 21. Biindole **21** contains four chiral centers giving rise to 16 possible stereoisomers which can be further reduced to six racemic pairs after accounting for the C_2-symmetry of the molecule (Figure 4A). To confirm the stereochemical purity of **21**, we prepared each of these stereoisomers using modifications of known methods (Supporting Information). As shown in Figure 4B, **21** was resolved from each of the other stereoisomers using reversed-phase (C18) HPLC analysis when prepared as an artificial mixture of stereoisomers. Chromatographic analysis of an authentic sample of **21** demonstrated its chemical purity and confirmed the sterochemical purity of the indole congener (**8**, Figure 4C).

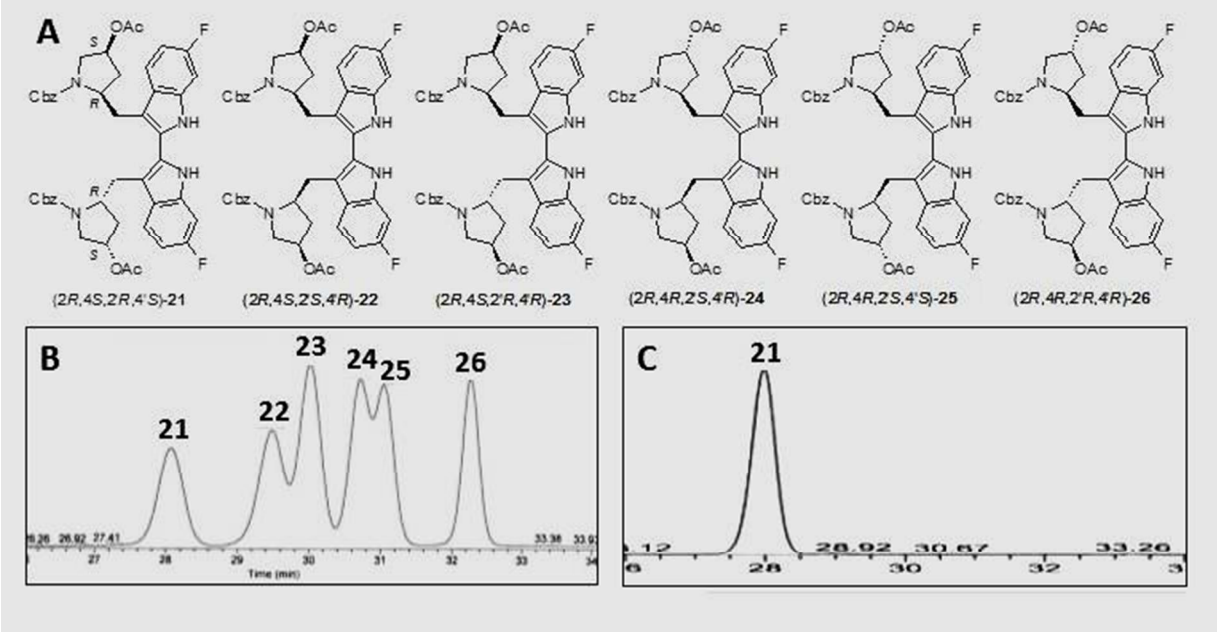
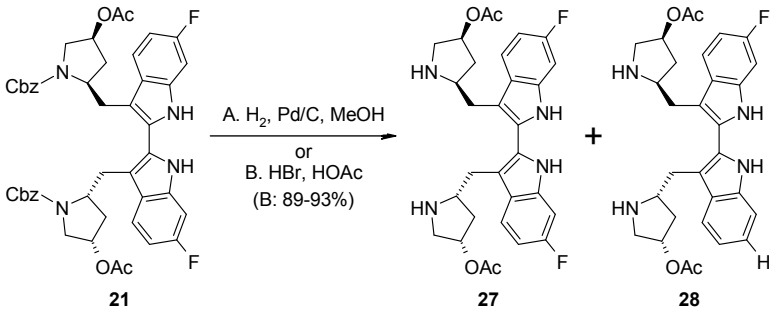


Figure 4. The stereochemical purity of 21. A. The six possible stereoisomers of 21; B. HPLC chromatogram of an artificial mixture of 21-26 demonstrating the separation of 21 from the other stereoisomers; and, C. HPLC chromatogram of authentic 21.

The First Peptide Bond Formation. Having secured a robust, kg-scale synthesis of **21**, we turned our attention to the sequential coupling with the requisite amino acid derivatives. Removal of the two Cbz groups was routinely achieved on multigram-scale using 10% Pd/C in MeOH under hydrogen atmosphere (50 psi) using a Parr Shaker-type hydrogenation apparatus which afforded **27** in quantitative yield (Scheme 7). Application of these conditions on >100 g-scale, however, resulted in ca. 1-1.5% of the mono-fluorinated derivative **28** which was confirmed by independent synthesis.[24] More importantly, we were unable to remove **28** by recrystallization of **27** or at a later stage in the synthesis. In order to identify hydrogenation conditions that either reduced or eliminated formation of **28**, we performed a catalyst screen using the ENDEAVOR® catalyst screening system (Argonaut Technologies, Foster City, CA;

Supporting Information, Tables S5). Despite identifying promising Pd catalysts, i.e., Escat™ 1931 (Strem Chemicals), we remained concerned about the generation of this difficult-to-purge impurity (**28**) on subsequent scale-up of these conditions. Thus, we opted to explore the use of HBr/HOAc for the Cbz group removal. In the event, HBr (33 wt% in HOAc) was added to **21** in DCM at ambient temperature. After approximately 24 h, the reaction mixture was quenched with water and extracted with methyl *tert*-butyl ether (MTBE) to remove benzyl bromide. The product-containing aqueous phase was made basic (pH 8.0±0.2) with 2 N NaOH and **25** was extracted into 2-MeTHF followed by a solvent exchange to MeOH which allowed **27** to be isolated as an off-white solid (93% yield, >98 area% by HPLC). Under these new conditions, we were unable to detect **28** in either the crude reaction mixture or isolated **27**.

Scheme 7. Defluorination under Hydrogenation Conditions

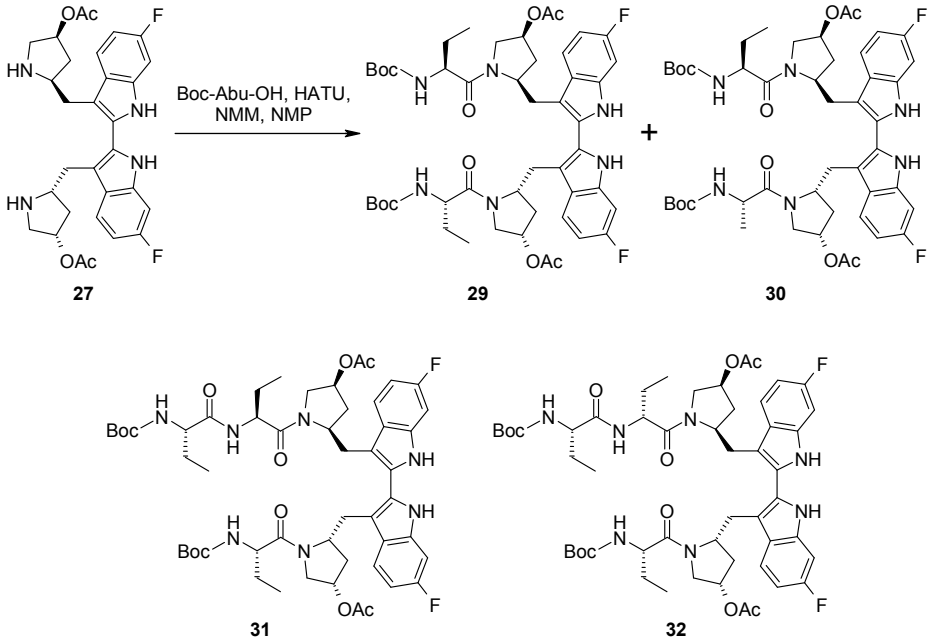


21 **27** **28**

From the outset, we selected HATU as the reagent of choice owing to its ability to couple sterically-hindered and hydrophobic amino acid derivatives with little or no epimerization.[25,26] Reaction of **27** with 2.4 equivalents of Boc-Abu-OH and excess HATU/NMM in 1-methyl-2-pyrrolidinone (NMP) gave **29** in excellent yield (Scheme 8). As expected, under these conditions we observed no epimerization of the Abu stereocenter by HPLC analysis using an authentic mono-D-Abu-**29** standard (not shown). However, ca. 5% of the des-methyl derivative (**30**) was observed in the reaction mixture. We suspected that the formation of **30** came from the

Boc-Abu-OH which was likely contaminated with Boc-Ala-OH. To confirm this hypothesis, we prepared an authentic sample of **30** (Supporting Information). During an independent preparation of **29** using a second lot of Boc-Abu-OH, we observed ca. 2% formation of Abu-insertion adducts **31** and **32** generated by the coupling of **27** with Boc-Abu-Abu-OH dipeptide. Analysis of several lots of commercially-available Boc-Abu-OH revealed various levels of contamination with H-Abu-OH, Boc-Ala-OH, and/or Boc-Abu-Abu-OH. As such, commercial lots of Boc-Abu-OH which did not achieve the desired purity specification were recrystallized (10:1.5 *n*-heptane/MTBE) prior to use. Notably, we suspect that the formation of **32** arose via *C*-terminal epimerization of HATU-activated Boc-Abu-Abu-OH intermediate.[27]

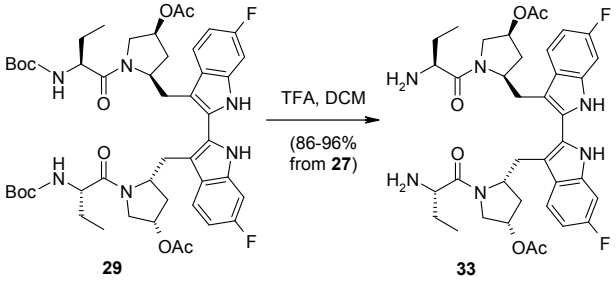
Scheme 8. By-Products 30, 31 and 32 Formed by Contaminated Boc-Abu-OH Reagent



Purification of Diamine 33. The isolation of **29** was problematic as the solid was a very fine powder and filtration was difficult. Our attempts to generate crystalline **29** with improved filtration characteristics were unsuccessful. Thus we opted to advance **29** into the next reaction without isolation. Following Boc-Abu-OH coupling to **27** (Scheme 8), the reaction mixture was

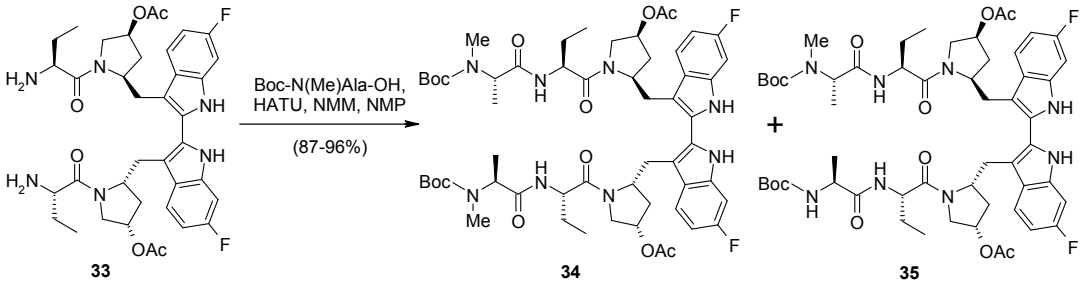
diluted with MTBE/EtOAc and subjected to aqueous workup. The MTBE/EtOAc extracts were concentrated, dissolved in DCM, and used directly in the TFA-mediated Boc group removal (Scheme 9). Gratifyingly, **33** was isolated as an easily-filterable solid in high purity (>97 area% by HPLC) after work-up. Additionally, recrystallization of **33** effectively purged the Abu-insertion products derived from **31** and **32**.

Scheme 9. Synthesis of Key Intermediate 33



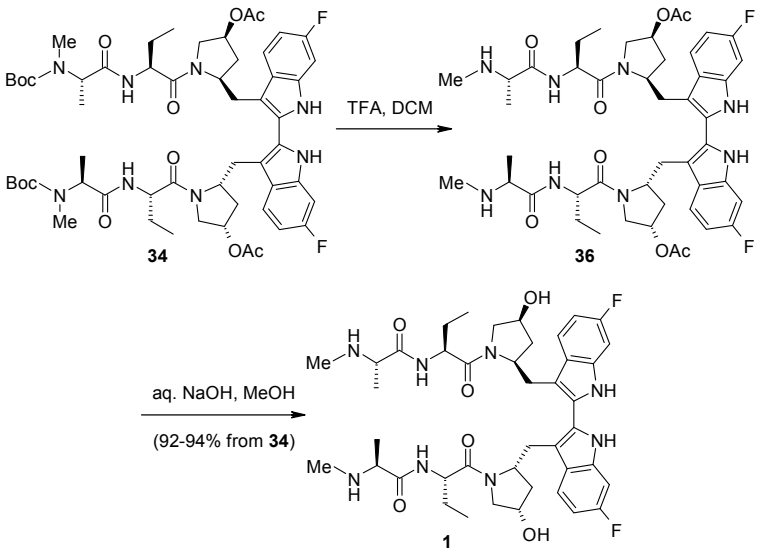
The Second Peptide Bond Formation. The successful coupling of **33** with Boc-N(Me)Ala-OH under HATU/NMM conditions afforded **34** contaminated with 1-1.5% of des-methyl species **35** (Scheme 10). As before, the genesis of **35** could be traced to contamination of commercially-available Boc-N(Me)Ala-OH with Boc-Ala-OH; recrystallization of commercial lots of Boc-N(Me)Ala-OH (15:2 *n*-heptane/MTBE) afforded high purity reagent thus eliminating the formation of **35**. Fully-protected birinapant **34** was isolated as a white solid after recrystallization from MTBE/*n*-heptane.

Scheme 10. Synthesis of Penultimate 34 and Des-Methyl By-product 35



Synthesis and Isolation of 1. The final removal of the Boc and acetyl protective groups was accomplished in two-steps without isolation of the partially-protected intermediate (**36**, Scheme 11). Fully-protected **34** was treated with TFA in DCM to afford diamine **36**. Following neutralization and extraction (2-MeTHF), the solvent was replaced with MeOH and then combined with 1 N NaOH to effect removal of the two acetyl groups. Distillation of MeOH allowed for the extraction of crude **1** into 2-MeTHF from the aqueous solution. The organic extracts were concentrated to dryness and the residue was recrystallized in IPA/EtOAc/water to provide **1** as a crystalline, off-white-colored solid.

Scheme 11. The Synthesis of Birinapant/TL32711 (1)



CONCLUSIONS

We have developed a scalable process to synthesize **1** drug substance for clinical use. A major improvement was the synthesis of intermediate **8** through acid chloride/indoylmagnesium bromide coupling which improved the yield from ca. 10% to ca. 50% from commercially-available reagents. Unexpectedly, we observed significant levels of defluorination during the hydrogenation of **21** which mandated an alternative method for Cbz group removal (i.e.,

HBr/HOAc). This program also revealed the chemical purity of the amino acid derivatives, Boc-Abu-OH and Boc-N(Me)Ala-OH, as critical quality attributes for this process. Finally, all chromatographic purifications were removed from the entire synthesis and crystalline **1** was conveniently isolated by filtration. This new synthetic process has allowed multiple >500 g lots of **1** to be produced for use in clinical trials.

EXPERIMENTAL SECTION

General. ^{1}H and ^{13}C NMR spectra were obtained at 300 and 75 MHz, respectively, using a Varian L600 spectrometer using tetramethylsilane as the internal standard. For all proline-containing analogs where two sets of resonances are observed owing to the presence of conformational isomers, the signal set for the major conformational isomer is reported. Reactions were routinely performed under a nitrogen atmosphere using standard glassware and high purity, commercial-grade solvents. Amino acid derivatives were purchased from Bachem, Fluka, Chem-Impex or other reputable suppliers and used without further purification unless stated otherwise. Unless indicated, amino acids and derivatives have the L-configuration. LC/MS analysis was performed on a Thermo-Fisher MSQ Plus instrument with a Gemini 5 μ C6-Phenyl 110 Å column (50 × 4.60 mm) using standard gradient conditions (A: water containing 0.1% HOAc v/v; B: acetonitrile containing 0.1% HOAc v/v); MS data were acquired with ESI, positive ionization; UV detection at 254 nm. Compound purifications were performed using flash silica gel chromatography or by preparative high-performance liquid chromatography using a Varian Prostar system either in normal phase (SiO_2, EtOAc/hexane, 250 × 41.4 mm) or reversed-phase (C18, 100 Å, 60 μ, 250 × 41.4 mm). Abbreviations: 2-MeTHF, 2-methyltetrahydrofuran; Abu, aminobutyric acid; ACN, acetonitrile; Ac_2O, acetic anhydride; AUC, area under the curve; Boc, *tert*-butyloxycarbonyl; Cbz or Z, benzyloxycarbonyl; DBU,

1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DDQ, 2,3-dicholoro-5,6-dicyano-1,4-benzoquinone; DIAD, diisopropylazodicarboxylate; DIPEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; EtOAc, ethyl acetate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAc, acetic acid; HPLC, high-performance liquid chromatography; Hyp, L-hydroxyproline; IPA, isopropyl alcohol; IPAc, isopropylacetate; MeOH, methanol; MSA, methanesulfonic acid; MTBE, methyl *tert*-butyl ether; NMM, 4-methylmorpholine; NMP, 1-methyl-2-pyrrolidinone; NMR, nuclear magnetic resonance; TBS-Cl, *tert*-butyldimethylsilyl chloride; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

*4R-(tert-Butyl-dimethyl-silanyloxy)-pyrrolidine-1,2S-dicarboxylic acid 1-benzyl ester (**10**).* *Z*-Hyp-OH (**9**, 3.0 kg, 11.3 mol), and *N,N*-dimethylformamide (DMF, 12.6 L) were charged to a 100 L reactor. The solution was cooled to 10-15 °C. Triethylamine (3.9 L, 28.3 mol) was added, followed by addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 170 mL, 1.1 mol). To the solution was added *tert*-butyldimethylsilyl chloride (TBS-Cl, 1.9 kg, 12.4 mol) in portions to the batch maintaining the temperature between 15–25 °C. The batch was stirred at 25 °C for 18 h. The reaction mixture was cooled to 0±5 °C and quenched with water (15.4 L) while maintaining the temperature below 20 °C. MTBE (30 L) was added and the mixture was acidified to pH 3-4 with 4 N HCl (6 L) while maintaining the batch temperature below 20 °C. The organic layer was separated and washed with water (15 L) and brine (15 L). The organic phase was concentrated to 6 L under reduced pressure at 35-40 °C. The solution was azeotropically-dried with toluene (2 × 5 L) at ≤40 °C to yield **10** (theoretical yield: 4.29 kg, >99 area% by HPLC) in toluene (6 L), which was stored at 2-8 °C and used directly in the following reaction. An analytical sample

was prepared for characterization: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (br s, 3H), 7.30 (m, 2H), 5.18 (m, 2H), 4.52 (dd, *J* = 7.5, 15.9 Hz, 1H), 5.43 (m, 1H), 3.40-3.70 (m, 2H), 2.06-2.36 (m, 2H), 0.85 (s, 9H), 0.07 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.1, 181.7, 160.8, 159.5, 141.3, 141.2, 131.4, 133.3, 133.0, 132.8, 132.5, 75.6, 72.5, 72.2, 63.1, 62.6, 60.1, 59.7, 44.8, 43.4, 30.6, 22.9, 0.07 ppm; HRMS(ESI) *m/z* 380.1891 [(M+H), calcd for $C_{19}H_{30}NO_5Si$: 380.1893].

2S-(6-Fluoro-1H-indole-3-carbonyl)-4R-hydroxy-pyrrolidine-1-carboxylic acid benzyl ester (14). **Step 1:** To the solution of Z-Hyp(OTBS)-OH **10** (4.3 kg, based on ^{1}H NMR wt% assay, 11.3 mol) in toluene (6 L) was added DMF (4 mL, cat.) followed by the addition of oxalyl chloride (1.18 L, 13.5 mol) at the rate to maintain the internal temperature below 25 °C. The batch was stirred at ambient temperature for 2 h. An aliquot (0.2 mL) of the reaction mixture was removed and added into $MeNH_2$ (2 M in THF, 2 mL). The sample was diluted with MeOH and analyzed by HPLC to confirm completion of the reaction. The homogeneous reaction mixture was concentrated under reduced pressure at 40-45 °C and dried azeotropically with toluene to provide compound **11** in toluene (12 L) as a pale yellow-colored solution.

Step 2: 6-Fluoroindole (**12**, 3.06 kg, 22.6 mol) was dissolved in anhydrous chlorobenzene (23.1 L) and toluene (15.4 L) and the solution was cooled to -5-0 °C. Ethylmagnesium bromide (3 M solution in diethyl ether, 8.3 L, 24.9 mol) was added at the rate to maintain the temperature below 5 °C. After stirring for 1 h at 5 °C, **11** in toluene (*infra*) was added at the rate to maintain the temperature below 5 °C. The reaction mixture was stirred for 3 h at <5 °C then quenched with a mixture of water (0.43 L), glacial acetic acid (2.7 L) and MeOH (19 L). The resulting **13**-containing reaction mixture was used directly in the subsequent reaction.

Step 3: To the **13**-containing reaction mixture was charged methanesulfonic acid (MSA, 0.97 L, 14.8 mol) while maintaining the internal temperature below 25 °C. The reaction mixture was stirred at ambient temperature for 16 h. The solid was collected by filtration and washed with water (8.5 L) and MTBE (2 × 8.5 L). The isolated solid was slurried in a mixture of water and MeOH (12% MeOH in water, 72.5 L) and filtered. The wet cake was washed with MTBE (2 × 8.5 L) and dried under vacuum at 45 °C for 48 h to provided compound **14** as an off-white-colored solid (3.1 kg, 73% yield from **9**, >98 area% by HPLC). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.06 (d, J = 9.3, Hz, 1H), 8.43 (dd, J = 2.1, 9.9 Hz, 1H), 8.16 (m, 1H), 7.35 (br s, 2H), 7.26 (d, J = 9.9 Hz, 1H), 7.02 (m, 3H), 5.23 (m, 1H), 5.12 (m, 1H), 5.04 (d, J = 6.9 Hz, 1H), 4.91 (q, J = 13.5 Hz, 1H), 4.30 (br s, 1H), 3.54 (m, 1H), 3.46 (br d, J = 10.8 Hz, 1H), 3.33 (br s, 1H), 2.24 (m, 1H), 1.91 (m, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6), mixture of rotamers: δ 194.4, 193.9, 160.0 (d, J_{CF} = 235.5 Hz), 154.7 (d, J_{CF} = 12.0 Hz), 137.8, 137.4, 137.3, 137.2, 137.2, 135.2, 129.1, 128.50, 128.46, 128.1, 127.9, 127.4, 123.3, 123.1, 123.0, 114.7, 114.5, 111.0, 111.0, 110.8, 110.7, 99.3, 98.9, 69.6, 68.8, 66.5, 66.4, 61.5, 61.1, 56.3, 55.8 ppm; HRMS (ESI), *m/z* 383.1401 [(M+H), calcd for $C_{21}H_{20}FN_2O_4$:383.1396].

*2S-(6-Fluoro-1H-indole-3-carbonyl)-4S-(4-nitro-benzoyloxy)-pyrrolidine-1-carboxylic acid benzyl ester (**15**).* A solution containing **14** (2.9 kg, 7.7 mol), 4-nitrobenzoic acid (1.6 kg, 9.6 mol) and triphenylphosphine (3.6 g, 13.5 mol) in anhydrous THF (49.8 L) and DMF (9.9 L) was cooled to 0-5 °C. Methanesulfonic acid (MSA, 199 mL, 3.1 mol) was added, followed by addition of diisopropylazodicarboxylate (DIAD, 2.8 L, 14.1 mol) at a rate that maintained the internal temperature below 5 °C. After 1 h at 0-5 °C, the solution was allowed to warm to ambient temperature and stirred for 16 h. MeOH (2.9 L) was added. The batch was stirred for 30 min and concentrated under reduced pressure at 45 °C. MeOH (14.7 L) was added and the

solution was concentrated again to remove residual THF. MeOH was added and the resulting heterogeneous mixture was stirred at 0 °C for 2 h. The solid was collected by filtration and washed with MeOH (8 L). The wet cake was dried under vacuum at 35 °C for 16 h to give **15** as a pale yellow-colored solid (3.4 kg, 83% yield, >97 area% by HPLC). ^{1}H NMR (300 MHz, d_6-DMSO), mixture of rotamers: δ 12.10 (br s, 1H), 8.45 (dd, J = 6.3, 3.0 Hz, 1H), 8.29-8.20 (m, 3H), 8.02 (dd, J = 2.7, 9.0 Hz, 2H), 7.65-7.50 (m, 2H), 7.30-7.42 (m, 2H), 7.26 (dd, J = 2.1, 9.9 Hz, 1H), 7.20-7.04 (m, 4H), 5.53 (br s, 1H), 5.40 (dd, J = 9.4, 14.4 Hz, 1H), 5.11 (dd, J = 12.6, 21.9 Hz, 1H), 5.04 (s, 1H), 3.97 (m, 1H), 3.71 (d, J = 12.3 Hz, 1H), 2.90 (m, 1H), 2.36 (m, 1H) ppm; ^{13}C NMR (75 MHz, d_6-DMSO), mixture of rotamers: δ 192.9, 192.5, 164.2, 160.0 (d, J_{CF} = 235.9 Hz), 154.5 (d, J_{CF} = 11.8 Hz), 150.9, 137.5 (d, J_{CF} = 2.6 Hz), 137.2 (d, J_{CF} = 12.6 Hz), 135.6, 135.2, 132.7, 132.2 (d, J_{CF} = 9.5 Hz), 131.3, 129.5, 129.4, 129.1,128.7, 128.5, 128.3, 128.2, 127.6, 124.3, 123.1, 113.7, 113.6, 111.0 (d, J_{CF} = 23.5 Hz), 99.7 (d, J_{CF} = 25.7 Hz), 75.3, 74.3, 68.5, 66.8, 66.5, 62.5, 62.2, 53.6, 53.1, 38.7, 37.7, 22.6. HRMS (ESI), *m/z* 554.1351 [(M+Na), calcd for $C_{28}H_{22}FN_3O_7Na$: 554.1359].

*2S-(6-Fluoro-1H-indole-3-carbonyl)-4S-hydroxy-pyrrolidine-1-carboxylic acid benzyl ester (**16**)*. To a suspension of **15** (6.5 kg, 12.2 mol) in THF (65 L) and MeOH (19.5 L) was added 50% aq. NaOH (0.97 L) at a rate to maintain the internal temperature below 20 °C. After 1.5 h at ambient temperature, glacial acetic acid (0.8 L) was added to adjust the pH to 6.5±0.2 and the reaction mixture was concentrated under reduced pressure at 45 °C. The solvent was exchanged with MTBE (5 × 11 L), followed by addition of water (65 L). After 1 h at ambient temperature, the solid was collected by filtration. The wet cake was washed with water and MTBE, dried under vacuum at 45 °C for 16 h to give **16** as a white-colored solid (4.1 kg, 87%, >98 area% by HPLC). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.1 (br s, 1H), 8.38 (d, J = 11.1 Hz, 1H), 8.14 (m,

1H), 7.40-7.30 (m, 2H), 7.25 (dd, J = 2.1, 9.9 Hz, 1H), 7.10-6.95 (m, 4H), 5.16-4.98 (m, 3H), 4.95 (dd, J = 13.2, 25.4 Hz, 1H), 4.26 (m, 1H), 3.75 (m, 1H), 3.31 (br s, 1H), 3.22 (m, 1H), 2.60 (m, 1H), 1.74 (m, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 193.8, 193.3, 160.0 (d, J_{CF} = 235.3 Hz) , 154.5 (d, J_{CF} = 14.6 Hz), 137.7, 137.4, 137.2, 135.2 129.1, 128.5, 128.2 (d, J_{CF} = 35.8 Hz), 128.1, 127.5, 123.3, 123.1, 114.5, 114.4, 110.9 (d, J_{CF} = 23.5 Hz), 110.8 (d, J_{CF} = 23.7 Hz), 99.1 (d, J_{CF} = 25.7 Hz), 69.5, 68.7, 66.5, 66.4, 61.5, 61.3, 55.0, 54.7 ppm. HRMS (ESI), *m/z* 383.1405 [(M+H), calcd for $C_{21}H_{21}FN_2O_4$: 383.1407].

*2R-(6-Fluoro-1H-indol-3-ylmethyl)-4S-hydroxy-pyrrolidine-1-carboxylic acid benzyl ester (**8**).* To a suspension of **16** (3.0 kg, 0.22 mol) in anhydrous THF (60 L) at -10-0° C was added $LiBH_4$ in THF (2 M, 9.8 L, 19.6 mol) at the rate to maintain the internal temperature below 0 °C. The resulting mixture was stirred at 0-5 °C for 30 min followed by addition of MSA (0.92 L, 14.0 mol) at the rate that maintained the batch temperature below 0 °C [Note: vigorous gas evolution was observed during the addition of MSA]. The reaction mixture was stirred at ambient temperature for 16 h, and then cooled to 0-5 °C before a mixture of water and THF (1:5, volume) was slowly added to quench the reaction. The mixture was concentrated under reduced pressure at <35°C. The residue was diluted with EtOAc (45 L) and water (30 L). After 15 min, the organic phase was separated and washed successively with $NaHCO_3$ solution (5 wt%), 2 N HCl, water and aq. $NaHCO_3$ (5 wt%). The organic phase was concentrated under reduced pressure. The residue was diluted with MTBE (15 L) and the solvent was exchanged with MTBE. After 1 h, the product was collected by filtration, washed with MTBE and dried under vacuum at 45 °C for 16 h to give **17** as a white-colored solid (2.3 kg, 79% yield, >97 area% by HPLC). ^{1}H NMR (300 MHz, DMSO-d_6): ~1:1 mixture of rotamers: δ 10.89, 10.87 (brs, 1H), 7.66 (dd, J = 8.1, 8.1 Hz, 0.5H), 7.50-7.30 (m, 4.5H), 7.16-7.04 (m, 3H), 6.84 (dd, J = 8.4, 8.4 Hz, 0.5H), 6.56 (dd, J

=9.0, 9.0 Hz, 0.5H), 5.13 (br s, 2H), 5.06 (br s, 1H), 4.24 (br s, 1H), 3.98 (m, 1H), 3.55 (dd, J = 4.8, 11.4 Hz, 1H), 3.26 (d, J = 11.4 Hz, 1H), 3.00 (dd, J = 10.5, 13.2 Hz, 2H), 1.79 (m, 2H) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): mixture of rotamers: δ 159.6 (d, J_{CF} = 231.8, Hz), 159.4 (d, J_{CF} = 232.4 Hz), 154.9, 138.0, 137.5, 136.8, 136.6, 129.2, 129.1, 128.8, 128.5, 128.2, 125.2, 124.9, 124.5, 124.4, 120.2 (d, J_{CF} = 27.8 Hz), 120.0 (d, J_{CF} = 27.8 Hz), 112.5, 112.4, 107.5 (d, J_{CF} = 24.3 Hz), 107.3 (d, J_{CF} = 23.7 Hz), 97.9 (d, J_{CF} = 25.5 Hz), 69.9, 69.2, 67.2, 66.4, 58.8, 58.1, 56.1, 55.6, 38.4, 37.7, 31.3, 30.1 ppm. HRMS (ESI), *m/z* 369.1602 [(M+H), calcd for $C_{21}H_{22}FN_2O_3$: 369.1603].

*4S-Acetoxy-2R-(6-fluoro-1H-indol-3-ylmethyl)-pyrrolidine-1-carboxylic acid benzyl ester (**19**)*. To a suspension of **8** (2.3 kg, 6.2 mol), 4-methylmorpholine (NMM, 1.4 L, 12.4 mol) and 4-dimethylaminopyridine (DMAP, 76 g, 0.62 mol) in THF (22.8 L) at 15-20 °C was added acetic anhydride (0.71 L, 7.5 mol) at a rate that maintained the internal temperature below 20 °C. After 1 h, the reaction mixture was quenched with water (2.3 L). The batch was concentrated under reduced pressure at 35-40 °C. The residue was diluted with DCM (23 L) and washed successively with 2 N HCl, saturated $NaHCO_3$ solution and brine. The organic phase was concentrated under reduced pressure at 35 °C. The residue containing **19** was diluted with DCM (3 vol.) and used directly in the next reaction (97.6% yield, >96 area% by HPLC). An analytical sample was prepared: ^{1}H NMR (300 MHz, DMSO-d_6), ~1:1 mixture of rotamers: δ 10.92 (br s, 1H), 7.61 (m, 0.5 H), 7.50-7.30 (m, 5H), 7.02-7.16 (m, 2.5H), 6.85 (m, 0.5H), 6.58 (m, 0.5H), 5.16 (m, 1H), 5.14 (s, 2H), 4.09 (m, 1H), 3.73 (dd, J = 4.8, 12.2 Hz, 1H), 3.58 (m, 0.5H), 3.40 (m, 1H), 3.28 (d, J = 13.5 Hz, 0.5H), 3.16 (d, J = 12.3 Hz, 0.5H), 2.80 (t, J = 12.3 Hz, 1H), 2.08 (s, 3H), 2.05 (m, 1H), 1.70-1.88 (m, 1.5H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6), mixture of rotamers: δ 170.7, 159.5 (d, J_{CF} = 226.4 Hz), 154.7, 137.7, 137.3, 136.8, 136.7, 129.1, 128.8,

128.5, 128.3, 125.0, 124.6, 123.0, 119.9, 111.8, 107.7, 107.4, 98.1 (d, J_{CF} = 23.4 Hz), 74.0, 73.2, 67.7, 67.3, 66.6, 58.3, 57.6, 55.5, 53.4, 52.9, 35.2, 34.5, 31.0, 30.0, 25.8, 21.7 ppm. HRMS (ESI), *m/z* 411.1760 [(M+H), calcd for $C_{23}H_{24}FN_2O_4$: 411.1760].

*4S-Acetoxy-2R-[3'-(4S-acetoxy-1-benzyloxycarbonyl-pyrrolidin-2R-ylmethyl)-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl]-pyrrolidine-1-carboxylic acid benzyl ester (**21**).* The solution containing **19** (2.5 kg, theoretical yield from previous step, 6.2 mol) in DCM (7.5 L) was charged to pre-cooled TFA (15.2 L) at -10 °C. After 1 h, the batch was concentrated under reduced pressure to remove DCM at 0-5 °C and stirred for another 4 h (98% conversion by HPLC). The cold reaction mixture was transferred into a pre-cooled (0-5 °C) mixture of water (64 L), K_2CO_3 (17.8 kg) and IPAc (38 L). The organic phase was separated and washed with water and concentrated under reduced pressure below 40 °C. The residue was diluted with IPAc followed by a solvent exchange to MeOH which was used directly in the next reaction. HRMS (ESI), *m/z* 821.3372 [(M+H), calcd for $C_{46}H_{47}F_2N_4O_8$: 821.3362].

To the solution containing the indoylindoline diastereomers (**20**, 3.1 mol, assuming 100% conversion from previous step) in MeOH (55 L) at 10-15 °C was added 2,3-dicholoro-5,6-dicyano-1,4-benzoquinone (DDQ, 0.7 kg, 2.8 mol) in portions. The resulting slurry was stirred at ambient temperature for 2 h before the crude product was collected by filtration. The wet cake was washed with MeOH (2 × 5 L) and dried under vacuum at 40 °C for 16 h to give crude **21** which was recrystallized with DMF (30 L) and MeOH (90 L) to provide **21** as an off-white-colored solid (1.8 kg, 72% yield, >98 area% by HPLC). $[\alpha]^{25}_D$ = +114.6° (*c* = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.29 (br s, 2H), 7.57-7.36 (m, 14H), 6.90 (app dt, *J* = 2.1, 9.3 Hz, 2H), 5.39-5.30 (m, 6H), 4.28 (t, *J* = 9.0 Hz, 2H), 3.84-3.73 (m, 4H), 3.66 (d, *J* = 13.2 Hz, 2H),

3.40 (dd, J = 12.0, 14.4 Hz, 2H), 2.31 (s, 6H), 2.17 (m, 2H), 2.05 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 160.4 (d, J_{CF} = 236.1 Hz), 156.3, 137.4 (d, J_{CF} = 13.4 Hz), 136.5, 128.9, 128.6, 128.5, 125.9, 118.7 (d, J_{CF} = 10.6 Hz), 108.8, 108.4 (d, J_{CF} = 8.3 Hz), 98.5 (d, J_{CF} = 25.8 Hz), 74.4, 68.0, 60.1, 53.5, 34.5, 28.9, 21.7 ppm. HRMS (ESI), *m/z* 819.3252 [(M+H), calcd for $C_{46}H_{45}F_2N_4O_8$: 819.3205].

*Acetic acid 5R-[3'-(4S-acetoxy-pyrrolidin-2R-ylmethyl)-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl]-pyrrolidin-3S-yl ester (**27**).* To a solution of **21** (0.64 kg, 0.78 mol) in DCM (3 L) was added HBr solution (33 wt% in acetic acid, 1.1 L, 6.3 mol) at ambient temperature. After 48 h, the reaction mixture was transferred into a mixture of water (15 L) and MTBE (9 L) at 0-5 °C. The batch was stirred for 30 min and the organic phase was removed. The aqueous phase was washed with MTBE (6.4 L) and charged with 2-MeTHF (13 L) and 2 N NaOH (11 L) to adjust the pH to 7.8-8.2 while maintaining the internal temperature below 5 °C. After 15 min, the organic phase was separated. The aqueous phase was extracted with 2-MeTHF (9.6 L). The combined organic phase was washed with 5 wt% brine and concentrated. After solvent exchange with MeOH (6.5 L), the solid was collected by filtration and washed with MeOH (2 × 0.7 L). The wet cake was dried under vacuum at 40 °C for 16 h to give **27** as an off-white-colored solid (0.72 kg, 93% yield, >98 area% by HPLC). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.06 (br s, 2H) 7.65 (dd, J = 5.4, 8.7 Hz, 2H), 7.03 (dd, J = 1.8, 9.6 Hz, 2H), 6.87 (m, 2H), 5.37 (m, 2H), 3.74 (m, 2H), 3.18-3.35 (m, 4H), 2.92-3.6 (m, 4H), 2.59 (m, 2H), 2.00-2.20 (m, 2H), 2.05 (s, 6H), 1.80-1.92 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 159.9 (d, J_{CF} = 235.8 Hz), 135.7(d, J_{CF} = 10.0 Hz), 130.3, 130.2, 125.1, 118.7 (d, J_{CF} = 10.0 Hz), 109.3, 107.5 (d, J_{CF} = 24.3 Hz), 97.4 (d, J_{CF} = 25.1 Hz), 74.9, 57.4, 51.4, 38.4, 32.6, 21.4 ppm. HRMS (ESI), *m/z* 551.2471 [(M+H), calcd for $C_{30}H_{33}F_2N_4O_4$: 551.2470].

*Acetic acid 5R-{3'-[4S-acetoxy-1-(2S-tert-butoxycarbonylamino-butyryl)-pyrrolidin-2R-ylmethyl]-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl}-1-(2S-tert-butoxycarbonylamino-butyryl)-pyrrolidin-3S-yl ester (**29**).* To a solution containing Boc-Abu-OH (0.76 kg, 3.8 mol) and HATU (1.6 kg, 4.1 mol) in anhydrous NMP (6.9 L) at 0-5 ºC was added NMM (0.62 L, 5.6 mol) followed by a solution of **27** (0.86 kg, 1.6 mol) in NMP (5.2 L). The reaction mixture was warmed to ambient temperature over 2 h. The reaction mixture was diluted with EtOAc (17 L) and MTBE (9 L) and washed with 1 N HCl (6.9 L). The layers were separated and the aqueous phase was extracted with 2:1 EtOAc/MTBE (7.8 L). The combined organic phase was washed successively with saturated $NaHCO_3$ and brine. The **29**-containing organic phase was concentrated followed by solvent exchange to DCM (10 L) and used directly in the next reaction. An analytical sample was prepared: ^{1}H NMR (300 MHz, $CDCl_3$), mixture of rotamers: δ 11.38 (br s, 2H), 7.44 (dd, *J* = 5.1, 15.8 Hz, 2H), 7.37 (d, *J* = 9.30 Hz, 2H), 6.80 (dd, *J* = 8.7, 8.7, 2H), 6.88 (br s, 2H), 5.45 (m, 2H), 4.36 (m, 4H), 4.15 (dd, J = 4.2, 12.0 Hz, 2H), 3.80 (d, *J* = 12.6 Hz, 2H), 3.15-3.40 (m, 4H), 2.32 (m, 2H), 2.27 (s, 6H), 1.90 (m, 2H), 1.74 (m, 4H), 1.53 (s, 18H), 1.02 (t, *J* = 7.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) , mixture of rotamers: δ 172.5, 170.4, 160.0 (d, J_{CF} = 235.8 Hz) , 155.9, 137.1 (d, J_{CF} = 12.9 Hz), 128.6, 125.6, 118.7 (d, J_{CF} = 9.8 Hz), 108.4 (d, J_{CF} = 24.9 Hz), 108.3, 98.3 (d, J_{CF} = 25.9 Hz), 80.6, 74.6, 60.3, 53.8, 53.5, 34.0, 28.7, 28.4, 26.2, 21.6, 10.4 ppm. HRMS (ESI), *m/z* 921.4598 [(M+H), calcd for $C_{48}H_{63}F_2N_6O_{10}$: 921.4574].

*Acetic acid 5R-{3'-[4S-acetoxy-1-(2S-amino-butyryl)-pyrrolidin-2R-ylmethyl]-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl}-1-(2S-amino-butyryl)-pyrrolidin-3S-yl ester (**33**).* A solution containing **29** in DCM (10 L) was added TFA (2.3 L) at the rate that maintained the batch temperature below 25 °C. The resulting mixture was stirred at ambient temperature for 40 h, then

slowly transferred to a mixture of 2-MeTHF (52 L) and saturated $NaHCO_3$ (28 L). After 15 min, the organic phase was separated. The aqueous phase was back extracted with 2-MeTHF (17 L). The combined organic phase was washed successively with saturated $NaHCO_3$ and brine and concentrated. The residue was diluted with MTBE (6.9 L) followed by a solvent swap to MTBE (6.9 L, 2-MeTHF ≤10%) to precipitate **33** which was collected by filtration. The wet cake was washed with MTBE (1.7 L) and dried under vacuum at 40 °C for 91 h to give crude **33** as pale yellow-colored solid which was purified by recrystallization in MeOH/2-MeTHF/MTBE to provide **33** as a white-colored solid (0.97 kg, 86% overall yield, >99 area% by HPLC). ^{1}H NMR (300 MHz, $CDCl_3$ + MeOH-d_4), mixture of rotamers: δ 7.57 (dd, J = 5.1, 9.9 Hz, 2H), 7.53 (dd, J = 2.4, 7.8 Hz, 2H), 6.89 (ddd, J = 2.4, 9.3, 13.2 Hz, 2H), 5.48 (dd, J = 4.5, 4.5 Hz, 2H), 4.52 (dd, J = 9.3, 9.3 Hz, 2H), 4.06 (dd, J = 5.1, 12.3 Hz, 2H), 3.78 (d, J = 12.3 Hz, 2H), 3.54-3.70 (m, 4H), 3.30-3.40 (m, 2H), 3.14 (s, 6H), 2.34 (m, 2H), 2.33 (s, 6H), 2.10 (m, 2H), 1.86 (m, 2H), 1.78 (m, 2H), 1.09 (t, J = 7.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + MeOH-d_4): δ 173.5, 170.9, 160.2 (d, J_{CF} = 235.8 Hz), 137.2 (d, J_{CF} = 12.8 Hz), 128.2, 128.1, 125.6, 118.7 (d, J_{CF} = 10.0 Hz), 108.6 (d, J_{CF} = 24.9 Hz), 108.0, 98.4 (d, J_{CF} = 25.7 Hz), 74.6, 60.1, 53.5, 33.5, 28.0, 21.4, 9.7 ppm. HRMS (ESI), *m/z* 721.3539 [(M+H), calcd for $C_{38}H_{47}F_2N_6O_6$: 721.3525].

*Acetic acid 5R-(3'-{4S-acetoxy-1-[2S-(2S-methyl-(tert-butoxycarbonyl)-amino-propionylamino)-butyryl]-pyrrolidin-2R-ylmethyl}-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl)-1-[2S-(2S-methyl-(tert-butoxycarbonyl)-amino-propionylamino)-butyryl]-pyrrolidin-3S-yl ester (**34**).* To a solution containing Boc-N(Me)Ala-OH (0.65 kg, 3.2 mol) and HATU (1.4 kg, 3.7 mol) in anhydrous NMP (7.6 L) at 0-5 ºC was added NMM (0.53 L, 4.8 mol) followed by addition of **33** (0.96 kg, 1.3 mol) in NMP (8.7 L). The resulting mixture was allowed to warm to ambient temperature. After 3 h, the reaction mixture was diluted with MTBE (29 L) and washed

successively with water (19 L), 1 N HCl (7.6 L), saturated $NaHCO_3$ (2 × 7.6 L), and brine (5.7 L). The organic phase was separated and concentrated to dryness. The residue was dissolved in MTBE (7.4 L) at 50 ºC followed by addition of *n*-heptane (5.0 L). After cooling to 0-5 ºC, **34** was collected by filtration. The wet cake was washed with a 3:2 MTBE/*n*-heptane (2 × 1.5 L) and dried under vacuum at 30 ºC for 28 h to provide **34** as a white-colored solid (1.3 kg, 87% yield, >99 area% by HPLC). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.57 (br s, 2H), 7.40-7.60 (m, 4H), 6.89 (ddd, J = 2.4, 4.5, 9.0 Hz, 2H), 5.50 (dd, J = 5.1, 5.1 Hz, 2H), 4.76 (m, 2H), 4.67 (q, J = 6.9 Hz, 2H), 4.50 (t, J = 9.6 Hz, 2H), 4.20 (dd, J = 3.9, 12.3 Hz, 2H) 3.85 (d, J = 12.3 Hz, 2H), 3.57 (br d, J = 13.5 Hz, 2H), 3.34 (dd, J = 12.0, 13.8 Hz, 2H), 2.89 (s, 6H), 2.34 (s, 6H), 2.30 (brs, 2H), 2.02-2.18 (m, 4H), 1.95 (td, J = 6.0, 13.8 Hz, 2H), 1.79 (td, J = 7.2, 14.1 Hz, 2H), 1.52 (s, 18H), 1.39 (d, J = 7.2 Hz, 6H), 1.03 (t, J = 7.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.6, 172.2, 172.0, 170.5, 160.3 (d, J_{CF} = 236.4 Hz) , 137.4 (d, J_{CF} = 12.9 Hz), 128.5, 128.4, 125.8, 118.6 (d, J_{CF} = 10.0 Hz), 108.6 (d, J_{CF} = 24.6 Hz), 108.1, 98.6 (d, J_{CF} = 25.7 Hz), 81.1, 74.6, 60.1, 54.0, 52.0, 33.7, 30.5, 28.6, 28.1, 25.9, 21.6, 20.9, 14.0, 9.9 ppm. HRMS (ESI), *m/z* 1091.5779 [(M+H), calcd for $C_{56}H_{77}F_2N_8O_{10}$: 1091.5629].

*Acetic acid 5R-(3'-{4S-acetoxy-1-[2S-(2S-methylamino-propionylamino)-butyryl]-pyrrolidin-2R-ylmethyl}-6,6'-difluoro-1H,1'H-[2,2']biindolyl-3-ylmethyl)-1-[2S-(2S-methylamino-propionylamino)-butyryl]-pyrrolidin-3S-yl ester (**36**).* To a solution containing **34** (1.25 kg, 0.70 mol) in DCM (11 L) was added TFA (1.3 L) at a rate maintaining the internal temperature below 25 °C. The resulting mixture was stirred at ambient temperature for 16 h and quenched by transferring to a mixture of saturated $NaHCO_3$ (38 L) and 2-MeTHF (38 L). The organic phase was separated and the aqueous phase was extracted with 2-MeTHF (13 L). The combined organic phase was washed successively with saturated $NaHCO_3$ (6 L) and brine (6 L) and

concentrated. The residue was dissolved in MeOH (7 L) and the resulting **36**-containing solution was used directly in the next reaction. An analytical sample was prepared: ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.76 (s, 2H), 8.19 (d, *J* = 8.7 Hz, 2H), 7.52 (dd, *J* = 2.1, 10.0 Hz, 2H), 7.52 (dd, *J* = 5.4, 8.9 Hz, 2H), 6.88 (ddd, *J* = 2.1, 9.2, 9.2 Hz, 2H), 5.48 (dd, *J* = 4.5, 4.5 Hz, 2H), 4.68 (m, 2H), 4.47 (m, 2H), 4.20 (dd, *J* = 5.4, 12.5 Hz, 2H), 3.86 (d, *J* = 12.3 Hz, 2H), 3.61 (dd, *J* = 1.8, 12.6 Hz, 2H), 3.30 (dd, *J* = 12.0, 14.1 Hz, 2H), 3.12 (q, *J* = 6.6 Hz, 2H), 2.56 (s, 6H), 2.34 (s, 6H), 3,27 (br d, *J* = 15.3 Hz, 2H), 2.07 (m, 2H), 1.97 (m, 2H), 1.84 (m, 2H), 1.71 (br s, 2H), 1.38 (d, *J* = 6.9 Hz, 6H), 1.08 (t, *J* = 7.5 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.2, 172.7, 170.5, 160.3 (d, J_{CF} = 235.8 Hz), 137.4 (d, J_{CF} = 12.9 Hz), 128.5 (d, J_{CF} = 3.5 Hz), 125.9, 118.5 (d, J_{CF} = 10.0 Hz), 108.5 (d, J_{CF} = 24.9 Hz), 107.8, 98.6 3 (d, J_{CF} = 25.7 Hz), 74.6, 60.9, 60.0, 53.9, 51.3, 35.8, 33.8, 27.9, 26.1, 21.6, 20.2, 10.0 ppm. HRMS (ESI), *m/z* 913.4435 [(M+Na), calcd for $C_{46}H_{60}F_2N_8O_8Na$: 913.4424].

*N-{1S-[2R-(6,6'-Difluoro-3'-{4S-hydroxy-1-[2S-(2S-methylamino-propionylamino)-butyryl]-pyrrolidin-2R-ylmethyl}-1H,1'H-[2,2']biindolyl-3-ylmethyl)-4S-hydroxy-pyrrolidine-1-carbonyl]-propyl}-2S-methylamino-propionamide (**1**).* To a solution containing **36** in MeOH (13 L) from previous step was added 1 M NaOH (3.5 L) at 0 ºC. The resulting mixture was stirred for 4 h at ambient temperature. MeOH was removed *in vacuo* at 35 ºC and the residue was diluted with water (25 L) and extracted with 2-MeTHF (25 L). The aqueous phase was separated and extracted with 2-MeTHF (13 L). The combined organic extracts were washed with water (13 L) and concentrated to dryness. The residue was suspended in MTBE (7 L) at 35 °C for 1 h and the solid was collected by filtration and washed with MTBE (2.6 L). The wet cake was dried under vacuum at 45 ºC for 16 h to provide **1** as an off-white-colored solid (0.96 kg, 99.6 area% by HPLC). The crude product (0.96 kg) was dissolved in a mixture of EtOAc (14 L), IPA (5 L)

and water (0.6 L). The volume was reduced by 50% under reduced pressure at 35-40 ºC. To the resulting mixture was added EtOAc (29 L) and the solution was concentrated at 35-40 ºC under reduced pressure until the ratio of EtOAc/IPA ≥15 by ^{1}H NMR analysis and water content ≤0.2% by KF analysis. The solution was cooled to ambient temperature. After 16 h, the solid was collected by vacuum filtration, washed with EtOAc (1 L) and dried under high vacuum at 50±5 °C for 24 h to afford **1** as an off-white-colored solid (0.87 kg, 94% overall yield, >99 area% by HPLC). $[\alpha]^{25}_{D}$ = +93.1° (*c* = 1.0, MeOH). ^{1}H NMR (500 MHz, DMSO-*d*$_6$), major rotamer: δ 11.92 (s, 2H), 8.30 (d, *J* = 7.8 Hz, 2H), 7.84 (dd, *J* = 5.5, 8.7 Hz, 2H), 7.47 (dd, *J* = 2.3, 9.8 Hz, 2H), 6.94 (dt, *J* = 2.0, 9.2 Hz, 2H), 5.66 (s, 2H), 4.55 (m, 2H), 4.49 (br s, 2H), 4.26 (br s, 2H), 3.91 (dd, *J* = 4.4, 11.1 Hz, 2H), 3.72 (d, *J* = 10.9 Hz, 2H), 3.51 (m, 5H), 3.08 (q, *J* = 6.9 Hz, 2H), 2.34 (s, 6H), 1.88 (m, 6H), 1.76 (m, 2H), 1.16 (d, *J* = 6.9 Hz, 6H), 1.00 (t, *J* = 7.4 Hz, 6H) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 174.1, 172.0, 159.3 (d, J_{CF} = 235.2 Hz), 136.5 (d, J_{CF} = 13.2 Hz), 127.6 (d, J_{CF} = 3.5 Hz), 125.7, 120.0 (d, J_{CF} = 10.0 Hz), 108.7, 107.8 (d, J_{CF} = 24.5 Hz), 97.5 (J_{CF} = 25.8 Hz), 70.1, 59.2, 59.2, 55.9, 51.2, 35.7, 34.3, 27.6, 24.8, 18.8, 9.6 ppm. HRMS (ESI), *m/z* 807.4394 [(M+H), calcd for $C_{42}H_{57}F_2N_8O_6$: 807.4369].

SUPPORTING INFORMATION

The supporting information contains data refinement for the crystallographic representation of **1**, details on the choice of co-solvent for the addition of 6F-indoylmagnesium bromide to **11** (Table S4), details on catalyst screening for the hydrogenation of **21** (Table S5), and a description of the heterodimer synthetic strategy that allowed independent synthesis of **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors contributed to the writing or editing of this manuscript. All authors contributed to the design, synthesis, or analysis of the chemistry described in this manuscript.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENTS

The authors thank Richard Staples (Michigan State University, East Lansing, MI) for solving the crystal structure of **1**.

ABBREVIATIONS

Abu, aminobutyric acid; Ala, alanine; API, active pharmaceutical ingredient; AVPI, alanine-valine-proline-isoleucine; BIR, baculovirus IAP repeat domain; Boc, *tert*-butyloxycarbonyl; Cbz,

benzyloxycarbonyl; cIAP, cellular IAP as in cIAP1 or cIAP2; DCM, dichloromethane; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMAP, 4-dimethylaminopyridine; EtOAc, ethyl acetate; EtOH, ethyl alcohol; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; HBr, hydrobromic acid; HOAc, acetic acid; HPLC, high-performance liquid chromatography; Hyp, hydroxyproline; IAP, inhibitor of apoptosis proteins; IPAc, isopropyl acetate; $LiBH_4$, lithium borohydride; MeOH, methyl alcohol; ML-IAP, melanoma IAP; MsOH, methanesulfonic acid; MTBE, methyl *tert*-butyl ether; NaOH, sodium hydroxide; NMM, *N*-methylmorpholine; NMP, 1-methyl-2-pyrrolidinone; Pd/C, palladium on carbon catalyst; PG, protective group; Smac/DIABLO, second mitochondria-derived activator of caspases/direct IAP binding protein with low pI; TBSCl, *tert*-butyldimethylsilyl chloride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; XIAP, X chromosome-linked IAP.

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