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Practical synthesis of a potent indolocarbazole-based, DNA topoisomerase inhibitor

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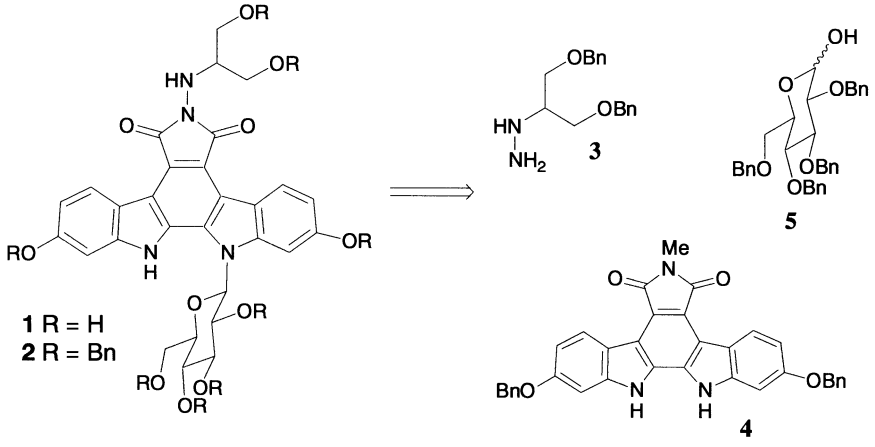
Abstract—DNA topoisomerase I inhibitors are currently under investigation as cancer chemotherapy agents of which indolocarbazole glycoside (**1**) has been identified as a promising candidate. A practical, scalable synthesis of **1** that limits the isolation of cytotoxic compounds to only that of the final product is described. The convergent process features a novel phase transfer-promoted glycosylation of aglycone core (**4**); subsequent hydrolysis provides anhydride (**8**). The hydrazine fragment (**3**), which is coupled with **8**, is synthesized via a modification of existing procedures. The coupled product (**2**) is subsequently hydrogenated to provide **1** in excellent purity via direct crystallization (>99.3 A%).

1. Introduction

The development of DNA topoisomerase I (topo I) inhibitors as cancer chemotherapy agents is currently an active area of research.[1] Specifically, the indolocarbazole glycoside class of compounds, such as the rebeccamycin analogs,[2] have been identified as attractive clinical targets.[3] Among them, **1** has emerged based on its potent cytotoxic activity against human cancer cells[4] and its wide safety margin and is currently in clinical trials. We required a practical route to **1** in support of developmental studies as the existing route[5] was not amenable to larger scale synthesis. The overriding strategy in this second-generation synthesis was to minimize the handling of topoisomerase-active intermediates. As the chemistry in the early steps dictated using fully protected compounds, coupled with the knowledge that the perbenzylated analog of **1** was not topo I active,[6] we sought to remove the protecting groups as the last step of the synthesis. Thus, the perbenzylated analog (**2**) became our penultimate target.



Scheme 1.

Keywords: indolocarbazole; topoisomerase; glycosylation.
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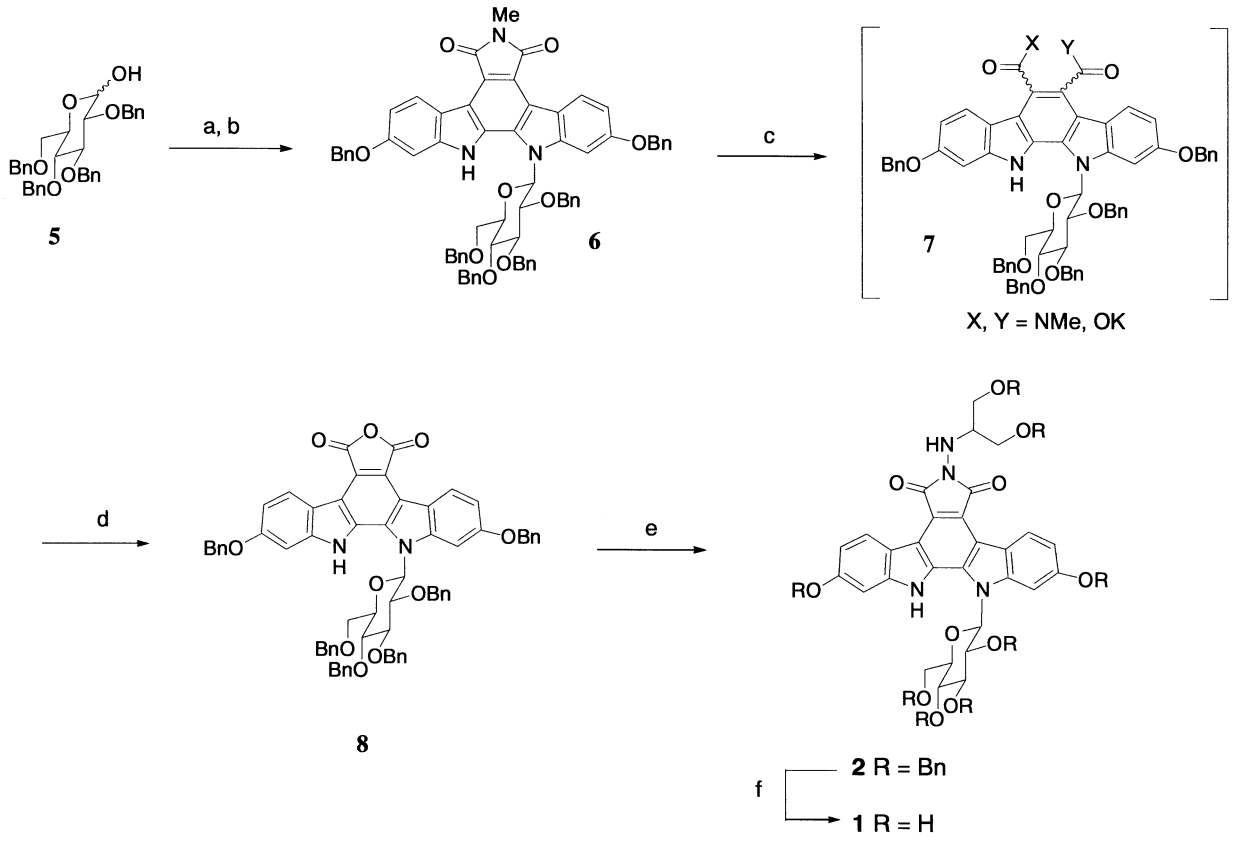


Scheme 2. *Reagents and conditions*: (a) thionyl chloride, DMF 5–25°C/2 h (>95%); (b) **4**, 48% aqueous KOH, Aliquat 336, MTBE, rt/3 h (83%); (c) 48% KOH, EtOH, toluene, 30°C/16 h; (d) Aqueous citric acid to pH 8, 25°C/6 h (82%); (e) **3a**, TEA, DMA, 65°C/3 h (99%); (f) H_2 (40 psi), 10% Pd/C, THF, IPA, aqueous HCl, 40°C/14 h (82%).

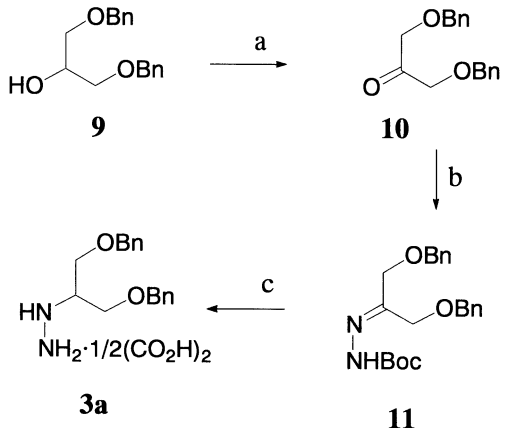
A retrosynthetic analysis of **2** reveals 1,3-dibenzyloxy-2-hydrazinopropane (**3**), indolocarbazole core (**4**)[5] and perbenzylated glucopyranose derivative **5** as accessible building blocks. Herein, we disclose a practical, convergent synthesis of **1** from readily-available starting materials utilizing this strategy (Scheme 1).

2. Results and discussion

2.1. Glycosylation/hydrolysis

The direct glycosylation of indolocarbazoles remains a formidable challenge due to the low nucleophilicity of the nitrogen atoms and the necessity for high levels of stereoselectivity. Prior glycosylations of protected indolocarbazoles have utilized Mitsunobu or Königs–Knorr (silver or mercury-based reagents) conditions[2b,4,5] to achieve the necessary levels of stereoselectivity. These approaches suffer from low-modest yields, and the inclusion of undesirable reagents and/or by-products. The use of powdered KOH, *tert*-BuOK and other strong bases under anhydrous conditions has been demonstrated to provide the β-*N*-glycosides in high yield as well.[4] The need to prepare the former reagent immediately prior to use and the extreme water sensitivity of the reactions rendered them less attractive. Alternatively, we sought to implement phase transfer agents in combination with an aqueous base to overcome the shortcomings of the prior methodologies. While the phase transfer-mediated alkylation of indoles and related systems is known,[7] to our knowledge there are no examples using indolocarbazole substrates in an aqueous medium. Accordingly, after treatment of **5** with thionyl chloride in DMF and subsequent isolation of the glycosyl chloride in MTBE, a series of common phase transfer catalysts conditions were screened in the reaction with indolocarbazole **4**. Subsequently, the use of Aliquat 336 (tricaprylylmethylammonium chloride; 1.4 equiv.) in combination with 48% aqueous KOH and MTBE (25°C, 2.5 h) provided an 83% yield of glycoside **6** after crystallization from MTBE/MeOH. The ratio of anomers was >150:1 (β:α). The use of sub-stoichiometric amounts of Aliquat 336 led to incomplete reactions. Although the role of this reagent is not clear, we postulate that the first equivalent forms a complex with **4**, whereas the remainder works as a phase transfer catalyst; for upon addition of 1.0 equiv. Aliquat 336 to a slurry of **4** in MTBE in the presence of base, an orange powder precipitates whose solid-state NMR differs from that of **4**. Indeed, addition of base and glycosyl chloride to this mixture does not initiate the reaction; further addition of Aliquat 336 is necessary. The formation of the di-glycoside was not observed under these Aliquat 336-promoted conditions (Scheme 2).

The conversion of **6** to anhydride **8** was accomplished by treatment with aqueous KOH in toluene/EtOH for 16 h at 30°C. The reaction initially generates a mixture of regioisomeric amic acid intermediates (**7**), which upon adjustment to pH 7.5–8 at 5°C with aqueous citric acid undergoes dehydration to form the anhydride over the



Scheme 3. *Reagents and conditions*: (a) aqueous NaOCl, cat. TEMPO, MeCN, 5°C/1 h; (b) Boc-NH-NH_2, heptane/toluene, 70°C/1 h (86% from **9**); (c) (i) $NaBH_4$, BF_3–etherate, THF, 0°C/1 h; (ii) 6N HCl, 60°C/4 h; (iii) 0.5 equiv. oxalic acid, MTBE, EtOH, 20–40°C/12 h (79% overall).

course of 6 h at 25°C. This pH adjustment is critical as failure to keep the pH above 7 led to higher levels of **6** (>2 area% by HPLC), the result of the retro reaction. Extraction into MTBE followed by crystallization from MeCN/MeOH provided **8** in 82% yield.

2.2. Synthesis of hydrazine oxalate (3a)

The synthesis of the hemioxalate salt of **3** (**3a**), based on a prior methodology,[8] began with conversion of commercially-available 1,3-dibenzyloxy-2-propanol (**9**) to ketone **10** via a TEMPO-catalyzed oxidation with bleach as the co-oxidant in MeCN/aqueous $NaHCO_3$.[9] The use of the $NaHCO_3$ buffer was critical to avoid over-oxidation of relatively unstable **10**. The ketone was carried forward into the next step as a slurry in heptane, obviating the need for isolation, whereupon it was treated with a solution of Boc-hydrazine in toluene. Condensation was complete within 3 h at 70°C to afford hydrazone **11** in 85% overall yield from **9** upon direct crystallization from the cooled reaction mixture (Scheme 3).

Subsequent reduction of hydrazone **11** with commercial lots of borane–THF suffered from variable yields, but in-situ formation from sodium borohydride and boron trifluoride–etherate (1.5 equiv.) in THF proved more reliable. The reaction proceeded to completion in 1 h at 0–5°C. Subsequent treatment with 6N aqueous HCl, followed by heating to 65°C for 3 h served to quench the reaction and remove the Boc group. Following neutralization, the product was crystallized as the hemioxalate salt **3a** from a MTBE/THF/EtOH solvent system at 40°C, giving colorless plates in 79% overall yield. It is noteworthy that the free base of **3a** decomposes upon exposure to air, forming H_2NNH_2 that complicates the subsequent steps. Thus the use of deoxygenated/degassed solvents and handling under a nitrogen atmosphere were necessary upon extraction until the salt formation.

2.3. Endgame-synthesis of 1

The coupling of hydrazine **3a** with anhydride **8** was run in deoxygenated dimethylacetamide (DMA) with triethylamine at 65°C. The triethylamine was added at 65°C to minimize decomposition of **3** to H_2NNH_2, which can also couple with **8** to form a *N*-amino-maleimide impurity. The cooled reaction mixture was partitioned between water and MTBE. The inability to crystallize **2** led to its introduction into the next step as a THF solution (quantitative yield) after a solvent switch from MTBE.

Hydrogenation of **2** in *i*-PrOH/THF in the presence of aqueous HCl proceeded to completion (>99.8% conversion) in 4–14 h at 40°C with 10% Pd–C to provide **1**. Under these conditions, no N–N bond cleavage was observed. Removal of the catalyst through solka floc was sufficient to minimize residual Pd in the final product (<15 ppm). The filtrate was pH adjusted to 2.5 with 1 M triethylamine in *i*-PrOH. Water was then added to the filtrate to avoid precipitation of the amorphous form of **1** during the subsequent concentration to remove the THF and toluene. The water content was first lowered to 20% via azeotropic drying and seed was added. Interestingly, the requisite level of crystal growth, as measured by supernatant concentration, was achieved only after prolonged aging (9 h) at 70°C. The water content was then lowered to 3% by means of *i*-PrOH addition and azeotropic drying. Crystalline **1** was isolated in 82% yield on cooling to 22°C.

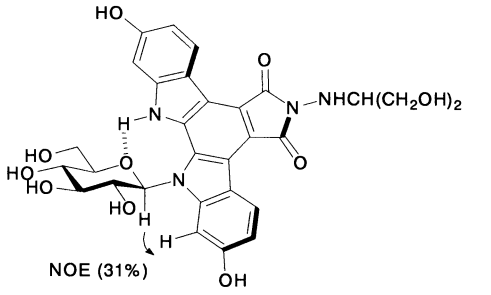
2.4. NMR analysis of 1 and related compounds

Interest in the conformational control of the indolocarbazole glycosides, which stems from the theory that it plays a role in pre-organizing the molecule for biological activity,[10] led us to study the solution-phase behavior of **1**. ^{1}H NMR analysis of **1**, in DMSO-d_6, shows the presence of two conformers (95/5 ratio) corresponding to rotational isomers arising from restricted bond rotation between the indolo nitrogen and the anomeric carbon of the sugar. Gradient-enhanced NOE (Nuclear Overhauser Effect) experiments[11] were used to assign these rotamers and the diagnostic NOEs are shown in Fig. 1.

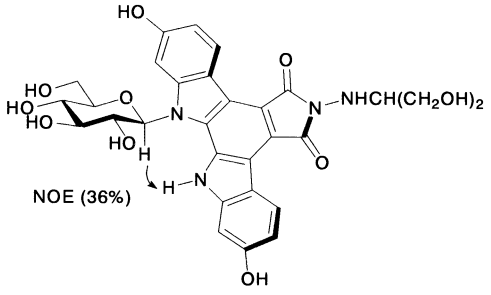
Similar NMR studies for **2** show the same major rotamer although the ratio has become 82/18. Essentially the same rotamer distribution is also seen for **6** in DMSO-d_6. However, when **6** is run in $CDCl_3$ there is only one rotamer observed. NOE studies show that this rotamer is the same as the major rotamer seen in DMSO-d_6 solution. The solvent dependance of the rotameric ratio is due to the ability of DMSO to hydrogen bond with the NH proton, whereas chloroform cannot. The strong preference for the observed conformation ('closed') in glycosides **1**, **2** and **6** is attributed to hydrogen bonding between the indole NH and the ethereal oxygen of the pyranose, a common feature in this class of molecules.[10]

3. Conclusion

We have developed a convergent, readily-scalable synthesis of **1** in 56% overall yield from **4** which minimizes the isolation of cytotoxic intermediates to only that of the final compound. Highlights of the process include the use of a highly stereoselective, phase transfer-promoted glycosylation and the direct crystallization of **1** from the reaction



1 (major rotamer)



1' (minor rotamer)

Figure 1.

mixture of sufficient purity (>99.3 area% by HPLC) to obviate the need for further purification. NMR analysis indicates that indolocarbazole glycosides described herein adopt the expected 'closed' conformation.

4. Experimental

4.1. General methods

All reactions were carried out under a nitrogen atmosphere. All commercial chemicals were used without further purification; 2,3,4,6-tetra-*O*-benzyl-D-gluco-pyranose and Aliquat 336 were purchased from Sigma/Aldrich. 1,3-Dibenzyloxy-2-propanol was purchased from Dixie Chemical (Texas, USA). Degassed solvents were prepared by placing under vacuum followed by purging with nitrogen or by sparging with nitrogen. Indolocarbazole **4** was prepared according to Ref. 5. NMR spectra were recorded on Bruker AM-300, DPX-400 and DRX-600 spectrometers. Spin–spin coupling constants (*J*) are reported in Hertz.

4.1.1. Chlorination of 2,3,4,6-tetra-*O*-benzyl-D-gluco-pyranose (5). $SOCl_2$ (3.71 kg, 31.2 mol) was added slowly to a chilled solution of 2,3,4,6-tetra-*O*-benzyl-D-gluco-pyranose (**5**) (14.7 kg, 27.2 mol) and DMF (59 L) in a 80-L glass vessel, keeping the internal temperature below 5°C. The resulting mixture was warmed to 25–30°C and aged for 2 h. MTBE (66 L) was added to the vessel followed by cooling below 0°C. 0.5N aqueous NaOH (210 L) was slowly added to the vessel, keeping the internal temperature below 10°C. The resulting mixture (pH 12.5) was warmed to 25°C, and the organic layer separated and washed with water (2×38 L) and 25% brine (27 L). The organic layer was then diluted with MTBE to make a 70-L solution and used as is in the next step. ^{1}H NMR was consistent with previously reported values.[12]

4.1.2. Synthesis of indolocarbazole glycoside (6). The MTBE solution (70 L) of 2,3,4,6-tetra-*O*-benzyl-D-gluco-pyranose chloride was added to a well-dispersed suspension of **4**[5] (2,10-dibenzyloxy-12,13-dihydro-5*H*-indolo[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-6-methyl-5,7-(6*H*)-dione) (10.0 kg, 18.1 mol), MTBE (70 L) and 48% aqueous KOH (50 L) in a 600-L tank at 25°C. After 0.5 h, 48% aqueous KOH (50 L) was added to the vessel. The resulting biphasic yellow slurry was stirred for 30 min at 22–25°C, followed by the rapid addition of a solution of Aliquat 336 (10.3 kg, 25.4 mol) in MTBE (50 L) to the mixture. The resulting reddish-brown suspension was further stirred at 20–25°C for 2.75 h, at which time the color became reddish-purple with increased viscosity. The organic layer was separated, washed with water (50 L) and cooled below 5°C. 1N aqueous HCl (20 L) was then added slowly to the mixture, keeping the internal temperature below 15°C. The resulting biphasic mixture was warmed to 20–25°C, and the pH adjusted to 1.5–2.5 with additional 1N aqueous HCl (1 L). The organic layer was separated and washed with water (50 L) and 25% brine (50 L). The filtered organic solution was concentrated in vacuo to the 140-L level, and MeOH (20 L) was added to the mixture at 20–23°C followed by a seed of product, which initiated crystallization. The mixture was aged for 2 h at 20–25°C, followed by the addition of MeOH (80 L) over 1 h. The resulting suspension was aged at room temperature overnight, and the crystals were isolated by filtration, washed with a 4:1 (v/v) mixture of MeOH/MTBE (150 L) and dried in vacuo to afford **6** (16.2 kg, 83%, 99.4 area% by HPLC) as a yellow powder: mp 114–115°C; ^{1}H NMR (400.25 MHz, $CDCl_3$-selected data) δ 10.67 (s, 1H), 9.25 (d, J=9.2 Hz, 1H), 9.14 (d, J=9.2 Hz, 1H), 7.03 (m, 1H), 6.91 (t, J=7.6 Hz, 2H), 6.22 (d, J=7.6 Hz, 2H), 5.86 (d, J=8.8 Hz, 1H), 5.19 (overlapping m, 3H), 5.09 (d, J=11.6 Hz, 1H), 4.99 (d, J=10.4 Hz, 1H), 4.90 (d, J=10.8 Hz, 1H), 4.87 (d, J=10.8 Hz, 1H), 4.76 (d, J=13.1 Hz, 1H), 4.68 (d, J=10.4 Hz, 1H), 4.58 (d, J=13.1 Hz, 1H), 4.34 (t, J=9.6 Hz, 1H), 4.04 (overlapping m, 2H), 3.93 (overlapping m, 2H), 3.88 (d, J=9.6 Hz, 1H), 3.81 (dd, J=10.0, 2.0 Hz, 1H), 3.32 (s, 3H), 3.01 (d, J=9.6 Hz, 1H); ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 170.3, 170.2, 159.3, 159.3, 143.1, 142.9, 137.9, 137.6, 136.9, 136.9, 136.7, 136.1, 130.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.3, 126.7, 126.4, 120.1, 119.3, 118.9, 118.2, 116.8, 116.7, 110.3, 96.6, 96.3, 85.7, 84.7, 80.8, 76.0, 75.9, 75.4, 74.8, 73.9, 70.6, 70.4, 66.7, 23.7; HRMS (FAB) 1073.4242 (M, 1073.4251 calcd for $C_{69}H_{59}N_3O_9$).

4.1.3. Synthesis of anhydride (8). EtOH (145 L) was added over 30 min to a biphasic mixture of **6** (16.1 kg, 14.9 mol), toluene (58 L) and 48% aqueous KOH (43.1 kg) in a 600-L tank at 20°C, keeping the internal temperature below 30°C. The resulting dark red mixture was stirred at 20–30°C for 16 h, during which time the mixture became a homogeneous

red solution. The mixture was aged at −10°C for 1 h, followed by the slow addition of 10% aqueous citric acid (240 L), keeping the internal temperature below 5°C. The resulting mixture (pH 7.7–8.0) was stirred at 25–30°C for 6 h, during which time an additional 10% aqueous citric acid (22 kg) was periodically added to maintain pH at 7.5–8.0. The mixture was then extracted with MTBE (135 L), and the separated organic layer was washed with 3% aqueous NaCl (2×33 L) and 25% aqueous NaCl (33 L), and treated with carbon (Darco G-60, 1.61 kg, room temperature, 1 h). The filtered solution was concentrated in vacuo to the 70 L level, and MeCN flushes (2×70 L) were performed, each time concentrating in vacuo to a 70 L batch volume (residual toluene: 3.6%). The mixture was then diluted with MeCN to make a 240 L solution, and MeOH (66 L) was added slowly over 30 min at 22–25°C followed by a seed of product, which initiated crystallization. The resulting slurry was aged at this temperature range for 1 h, followed by the slow addition of MeOH (257 L) over 1 h. The resulting yellow suspension was aged at 22–25°C for 1 h followed by aging at 0–5°C for 2 h. The crystals were isolated by filtration, washed with a 9:1 (v/v) mixture of MeCN/MeOH (2×60 L) and dried in vacuo to afford **8** (13.1 kg, 82%, 99.3 area% by HPLC) as a yellow powder: mp 95–96°C; ^{1}H NMR (400.13 MHz, DMSO-d_6-selected data-major rotamer) δ 11.06 (s, 1H), 8.80 (d, J=8.8 Hz, 1H), 8.67 (d, J=8.8 Hz, 1H), 7.74 (d, J=2.0 Hz, 1H), 6.97 (m, 1H), 6.81 (m, 2H), 6.62 (d, J=9.2 Hz, 1H), 6.11 (m, 2H), 5.26 (d, J=11.6 Hz, 1H), 5.21 (s, 2H), 5.00 (d, J=11.6 Hz, 1H), 4.91 (d, J=10.8 Hz, 1H), 4.90 (d, J=13.8 Hz, 1H), 4.81 (s, 2H), 4.66 (d, J=13.8 Hz, H), 4.62 (d, J=11.6 Hz, 1H), 4.30 (m, 2H), 4.12 (m, 1H), 3.90 (overlapping m, 4H); ^{13}C NMR (100.61 MHz, DMSO-d_6-major rotamer) δ 165.0, 164.9, 159.7, 159.5, 143.7, 142.7, 138.5, 138.4, 137.6, 137.1, 137.1, 136.7, 130.4, 129.0, 129.0, 128.9, 128.8, 128.7, 128.6, 128.6, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.1, 125.1, 125.0, 119.0, 118.2, 118.2, 116.7, 115.6, 115.2, 111.8, 111.3, 97.7, 96.7, 84.8, 83.3, 81.1, 76.6, 76.4, 75.3, 74.7, 74.0, 73.3, 70.5, 69.9, 66.9; HRMS (FAB) 1060.3937 (M, 1060.3935 calcd for $C_{68}H_{56}N_2O_{10}$).

4.1.4. Preparation of hydrazone (11). 8.9% aqueous NaOCl (titrated with aqueous $Na_2S_2O_3$ prior to use; 18.4 kg, 22.0 mol) was added dropwise over 2 h to a stirred mixture of 1,3-dibenzyloxy-2-propanol (**9**; 5.00 kg, 18.4 mol), 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO; 287 g, 1.84 mol), MeCN (54.0 kg) and 3% aqueous $NaHCO_3$ (50.8 kg, 18.4 mol) in a 200-L glass lined tank at 0°C, keeping the internal temperature at 0–5°C. The resulting mixture was stirred for an additional 1 h at 0–5°C and extracted with MTBE (100 kg) below 10°C. The separated organic layer was washed with 10% aqueous Na_2SO_3 (16.7 kg) below 10°C followed by 5% aqueous NaCl (10.5 kg) and 20% aqueous NaCl (15.0 kg) at room temperature. The solution was then placed in the 200-L vessel and concentrated in vacuo (50°C/60–150 mmHg) to ca. 25 L and flushed with *n*-heptane (3×50 L) followed by dilution with *n*-heptane (68.2 kg). The mixture was then warmed to 50°C, and a solution of Boc-$NHNH_2$ (3.15 kg, 23.9 mol) in toluene (4.3 kg) was added. The resulting mixture was stirred above 70°C for 1 h, followed by cooling to 60°C. A seed of product was added, and the resulting mixture was aged at 60°C for 1 h to initiate crystallization. The mixture was cooled to room temperature and aged overnight. The crystals were isolated by filtration, washed with *n*-heptane (10.3 kg) followed by a 9:1 v/v mixture of *n*-heptane/*t*-BuOH (12.5 L) and *n*-heptane (2×10.3 kg), and dried in vacuo at 50°C to provide **11** (6.05 kg, 86%, 99.98 area% by HPLC) as colorless needles: mp 92–93°C; ^{1}H NMR (400.13 MHz, DMSO-d_6) δ 9.86 (br s, 1H) 7.24–7.38 (br m, 10H), 4.49 (s, 2H), 4.46 (s, 2H), 4.31 (s, 2H), 4.06 (s, 2H), 1.43 (s, 9H); ^{13}C NMR (100.61 MHz, DMSO-d_6)) δ 152.7, 148.6, 138.4, 137.9, 128.7, 128.1, 128.1, 128.1, 127.9, 80.1, 72.6, 72.0, 65.5, 28.4. Anal. Calcd for $C_{22}H_{28}N_2O_4$: C, 68.73; H, 7.34; N, 7.29. Found: C, 68.75; H, 7.33; N, 7.26.

4.1.5. Synthesis of the hemioxalate salt of 1,3-dibenzyloxy-2-hydrazinopropane (3a). A stirred suspension of $NaBH_4$ (1.326 kg, 35.01 mol) and THF (30 L) was treated slowly with a solution of hydrazone **11** (6.00 kg, 15.61 mol) in THF (42 L) in a 200-L tank at 0°C, keeping the internal temperature below 5°C. $BF_3{\cdot}OEt_2$ (3.321 kg, 23.40 mol) was then added dropwise to the resulting mixture, keeping the internal temperature below 10°C. The resulting colorless suspension was stirred at 0–5°C for 1 h (reaction can be properly monitored by HPLC after mixing the sample with EtOH and subsequent reflux for 4 min to decomplex boranes from the product), at which time 6N aqueous HCl (17.09 kg) was added dropwise over 1 h, keeping the internal temperature below 20°C (*caution*: vigorous gas evolution). The resulting colorless suspension was warmed to 60–65°C and aged for 3.5 h, at which time the gas evolution ceased. Degassed 2N aqueous NaOH (50.9 L) was then added slowly to the mixture at 3°C, keeping the internal temperature below 20°C, followed by warming the resulting mixture to room temperature and extraction with degassed MTBE (100 L). The separated organic layer was washed with degassed water (24 L) followed by degassed brine (26 kg). Toluene (604.9 g) was then added (internal HPLC standard) to the organic layer, followed by stirring the resulting mixture for 5 min. The concentration of the free base measured by a potentiometric titration: 26.6 mg/mL so that 4.12 kg (14.4 mol) of free base was estimated in the mixture (batch volume=155 L). The organic layer was then diluted with degassed MTBE to form a 180-L solution. Then EtOH (90 L) was added, and the solution was heated to 40–45°C. A seed of product was added followed by a solution of oxalic acid (649 g, 7.21 mol, 0.5 equiv.) and degassed MTBE (22.5 L) dropwise over 1.5–2 h at 40–45°C, which crystallized the product. The resulting colorless slurry was stirred at 40–45°C for 30 min followed by cooling to 20–25°C over 20 min. The suspension was stirred at room temperature for 12 h, and the crystals isolated by filtration, washed with a 2:1 v/v MTBE/EtOH (17.72 kg, 9.40 kg) and dried under positive pressure of nitrogen followed by in vacuo drying for 19 h at 40°C to provide **3a** (4.07 kg, 79%, 99.9 area% by HPLC) as colorless plates: mp 128–129°C; ^{1}H NMR (300.13 MHz, DMSO-d_6) δ 7.22–7.34 (m, 10H), 5.95–6.35 (br, 4H exchangeable protons), 4.47 (s, 4H), 3.56 (s, 2H), 3.54 (s, 2H), 3.37 (m, 1H); ^{13}C NMR (75.47 MHz, DMSO-d_6) δ 164.9, 138.5, 128.7, 128.1, 128.0, 72.9, 68.1, 58.6. Anal. Calcd for $C_{18}H_{23}N_2O_4$: C, 65.24; H, 7.00; N, 8.45. Found: C, 65.17; H, 7.03; N, 8.43.

4.1.6. Synthesis of penultimate intermediate (2). A 22-L vessel was charged with DMA (8.3 L), anhydride **8** (1.00 kg, 0.94 mol) and hydrazine hemioxalate **3a** (350 g, 1.06 mol) at 22°C. The resulting slurry was degassed with stirring by applying vacuum to the vessel (40–80 torr) for 5 min and filling with nitrogen (three cycles). The contents were heated to 65°C over 30 min during which time the solution became homogeneous. Triethylamine (146 mL, 1.05 mol) was added rapidly and the solution aged at 65°C for 3 h. The contents were cooled to 45°C and transferred to a 50-L cylindrical vessel containing nitrogen-sparged MTBE (17.0 L) held at 10°C. The contents of the vessel were again cooled to 10°C and nitrogen-sparged water (4.7 L) was added over 10 min to keep the internal temperature below 30°C. 2N aqueous HCl (440 mL) was added to the biphasic mixture at 22°C. After agitation at 22°C for 10 min, the layers were separated and the organic layer washed with water (3×3.8 L). The organic layer was concentrated in vacuo to the 5 L level (20–25°C), and multiple THF flushes were performed to remove MTBE. The solution concentration was adjusted to 175 g/L with THF and utilized in the next reaction. Assay of the solution indicated 1.25 kg of product (>99% yield). A portion of the solution was evaporated to dryness to facilitate characterization. ^{1}H NMR (600.13 MHz, DMSO-d_6-selected data-major rotamer) δ 10.89 (s, 1H), 9.10 (d, J=8.7 Hz, 1H), 9.01 (d, J=8.7 Hz, 1H), 7.74 (d, J=1.9 Hz, 1H), 6.99 (m, 1H), 6.83 (t, J=7.6 Hz, 2H), 6.58 (d, J=8.3 Hz, 1H), 6.13 (d, J=7.6 Hz, 2H), 5.71 (d, J=2.6 Hz, 1H), 5.31 (d, J=11.7 Hz, 1H), 5.26 (s, 2H), 5.06 (d, J=11.7 Hz, 1H), 4.92 (d, J=11.0 Hz, 1H), 4.81 (d, J=13.2 Hz, 1H), 4.80 (s, 2H), 4.66, 4.64 (overlapping d's, 2H), 4.48 (d, J=11.7 Hz, 2H), 4.41 (d, J=11.7 Hz, 1H), 4.39 (d, J=11.7 Hz, 1H), 4.29 (m, 2H), 4.09 (t, J=8.7 Hz, 1H), 3.93 (d, J=10.6 Hz, 1H), 3.86 (m, 2H), 3.81 (d, J=10.0 Hz, 1H), 3.62 (m, 4H), 2.90 (d, J=10.0 Hz, 1H); ^{13}C NMR (150.90 MHz, DMSO-d_6-major rotamer) δ 168.6, 168.5, 159.0, 158.8, 143.3, 142.3, 138.1, 138.0, 138.0, 138.0, 137.2, 136.8, 136.8, 136.3, 129.3, 128.5, 128.4, 128.4, 128.4, 128.2, 127.9, 127.9, 127.9, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 127.4, 127.2, 125.6, 125.5, 118.2, 117.7, 117.5, 115.8, 115.7, 115.4, 110.8, 110.3, 97.1, 96.2, 84.4, 82.8, 80.7, 76.0 (2C), 74.9, 74.3, 73.6, 72.8, 72.4, 72.3, 70.0, 70.0, 69.8, 69.5, 66.5, 57.5.

4.1.7. Synthesis of 1. *Caution*: The product of the reaction is cytotoxic!

10% Pd on carbon (50% wet, 112 g) was charged to a five gallon autoclave, followed by a THF solution of **5** (175 g/L solution; 6.4 L, 1.12 assay kg), *i*-PrOH (7.9 L) and 3N aqueous HCl (224 mL). The contents were hydrogenated at 40°C/40 psi with rapid agitation (900 rpm) for 14 h during which time 110% of the theoretical amount of hydrogen was absorbed. The contents were cooled to 25°C, and the reaction mixture filtered over a pad of solka floc which was then rinsed with a 3:2 v/v mixture of *i*-PrOH/THF (1×3 L). The filtrate pH was adjusted to 2.5 with 1N triethylamine in *i*-PrOH (ca. 600 mL) followed by the addition of water (4.0 L). The batch was concentrated at atmospheric pressure to the 7.5 L level. Distillation was continued at a constant batch volume while feeding in a 4:1 v/v *i*-PrOH/water (6.5 L). The water content was lowered to 20% (w/v) by feeding *i*-PrOH (ca. 9 L) to the vessel while keeping the batch volume at 7.5 L. The contents were cooled to 70°C and a seed of **1** (5.0 g) was added as a slurry in *i*-PrOH (50 mL). The batch was held at 70°C for 1 h followed by the addition of *i*-PrOH (5.0 L) over 90 min. The batch was aged at 70°C for 9 h during which time the bulk of the product crystallized. A constant volume distillation feeding in *i*-PrOH (17 L) was performed that resulted in lowering the water content to 3% (w/v). The slurry was aged at 70°C for 3 h (supernatant concentration=5.3 g/L) followed by cooling to 22°C and aging for 1 h. The slurry was filtered and the cake was washed with *i*-PrOH (2.5 L) and then methanol (1.5 L), followed by drying in vacuo at 38°C for 6 h to provide **1** (419 g, 82%, 99.4 area% by HPLC) as an orange solid: mp 330°C (dec); $[\alpha]^{23}_{D}$=+117 (*c* 0.8, 1/1 MeCN/water); ^{1}H NMR (400.13 MHz, DMSO-d_6-major rotamer) δ 11.23 (s, 1H), 9.80 (s, 1H), 9.77 (s, 1H), 8.90 (d, J=8.4 Hz, 1H), 8.82 (d, J=8.4 Hz, 1H), 7.21 (br s, 1H), 7.01 (br s, 1H), 6.84 (overlapping m, 2H), 6.00 (d, J=8.0 Hz, 1H), 5.88 (t, J=3.6 Hz, 1H), 5.57 (d, J=2.4 Hz, 1H), 5.34 (d, J=4.4 Hz, 1H), 5.13 (d, J=4.4 Hz, 1H), 4.94 (d, J=4.4 Hz, 1H), 4.56 (t, J=5.6 Hz, 2H), 4.04 (dd, J=11.2, 3.2 Hz, 1H), 3.95 (overlapping m, 2H), 3.81 (dd, J=10.4, 4.0 Hz, 1H), 3.53 (overlapping m, 6H); ^{13}C NMR (100.64 MHz, DMSO-d_6-major rotamer) δ 169.0, 168.9, 157.8, 157.6, 144.4, 143.1, 129.5, 127.9, 125.2 (2C), 118.9, 117.6, 115.9, 114.3, 114.2, 113.9, 110.3, 110.2, 97.5, 97.5, 84.5, 78.4, 76.8, 72.9, 67.5, 62.6, 60.5 (2C), 58.3.

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