Semisynthesis of an Antifungal Lipopeptide Echinocandin

Michel Journet,* Dongwei Cai,* Lisa M. DiMichele, David L. Hughes, Robert D. Larsen, Thomas R. Verhoeven, and Paul J. Reider

Department of Process Research, Merck Research Laboratories, P.O. Box 2000, RY80E-100, Rahway, New Jersey 07065-0900

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Compound **1** is a macrocyclic lipopeptide belonging to the echinocandin family, which has been effective in treating systemic fungal infections. In this paper, we report a unique regio-, chemo-, and stereoselective synthesis of **1** in four steps from the deacylated nucleus **3** in an impressive 83% overall yield. Highlights of this synthesis include a selective reduction of the amide to the amine and a highly stereoselective (99:1 α/β) introduction of the 2-aminoethyl hemiaminal. In addition, the synthesis of the naphthoyl side chain was accomplished in three steps in 79% overall isolated yield from commercially available 6-bromo-2-naphthol.

Introduction

The need for new drugs to treat systemic fungal infections has intensified due to the increase in the immunocompromised-patient population.[1] The echinocandins are a class of fungicidal cell-wall active lipopeptides that are specific inhibitors of β-(1,3)-D-glucan synthesis.[2] Recently, the discovery of their activity against *Pneumocystis carinii*[3] and *Aspergillus*[4] has broadened interest in their development. The pneumocandins are a subset of the echinocandins, which are produced by the fungus *Glarea lozoyensis*. Their isolation, structure elucidation, and biological evaluation have recently been reported.[5] The fermentation product pneumocandin B_0 **2**[6] has since become the nucleus of structure−activity relationship (SAR) studies at Merck with the identification of **1** as a drug candidate.[7] The structure is modified at three points (Table 1): a naphthoyl moiety R_3 replaces the 10,12-dimethylmyristoyl side chain, the primary amide is reduced to the primary amine (R_1), and an ethanolamine substituent R_2 is present at the hemiaminal position.[6]

The key to the effective conversion of **2** to **1** was the determination of the optimal point to remove the myristyl side chain. With a prior reduction of the amide to the amine at R_1 and/or incorporation of the ethanolamine hemiaminal at R_2, a protection/deprotection sequence would be necessary as these amines are more reactive in the reacylation than the desired amine at R_3. In fact, protection of the amines at R_1 and R_3 as the Cbz groups in an earlier approach gave a mixture of products that required chromatography. With this scheme the overall conversion to **1** proved to be a low-yielding process. Instead, deacylation of **2** as the first step in the synthesis affording **3** was the most efficient point, which obviated the protection of the amines.

The deacylation was carried out by enzymatic hydrolysis. The details of this procedure will be published elsewhere.[8] After the reacylation of **3** reduction of the primary amide was to follow. The one-step reduction of the amide group to the amine with hydrides, such as Red-Al, DIBALH, LAH, etc., failed to give a clean reaction. Borane·dimethyl sulfide afforded the desired primary amine; however, an unstirrable gel was formed and only a 50% conversion was obtained.[9] Consequently, a two-step, albeit more effective, procedure was developed through dehydration to the nitrile followed by a catalytic hydrogenation. As the introduction of the ethanolamine at the hemiaminal position was best accomplished with Cbz-protected ethanolamine, the hydrogenation was run as the last step to cleave the Cbz and to reduce the nitrile in a one-pot operation.

Here, we wish to report these three regio-, chemo-, and stereoselective transformations from the deacylated nucleus **3** to produce **1** in four steps in 83% overall yield (Scheme 1): (i) reacylation (98%); (ii) dehydration (92%); (iii) Cbz-protected ethanolamine insertion (100%); and

* To whom correspondence should be addressed. E-mail: michel_journet@merck.com or dongwei_cai@merck.com.

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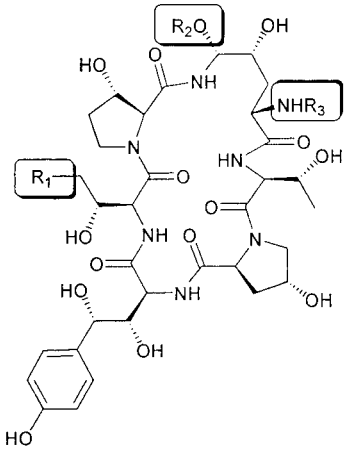
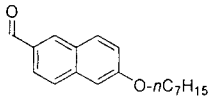
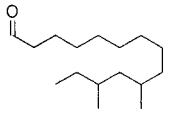
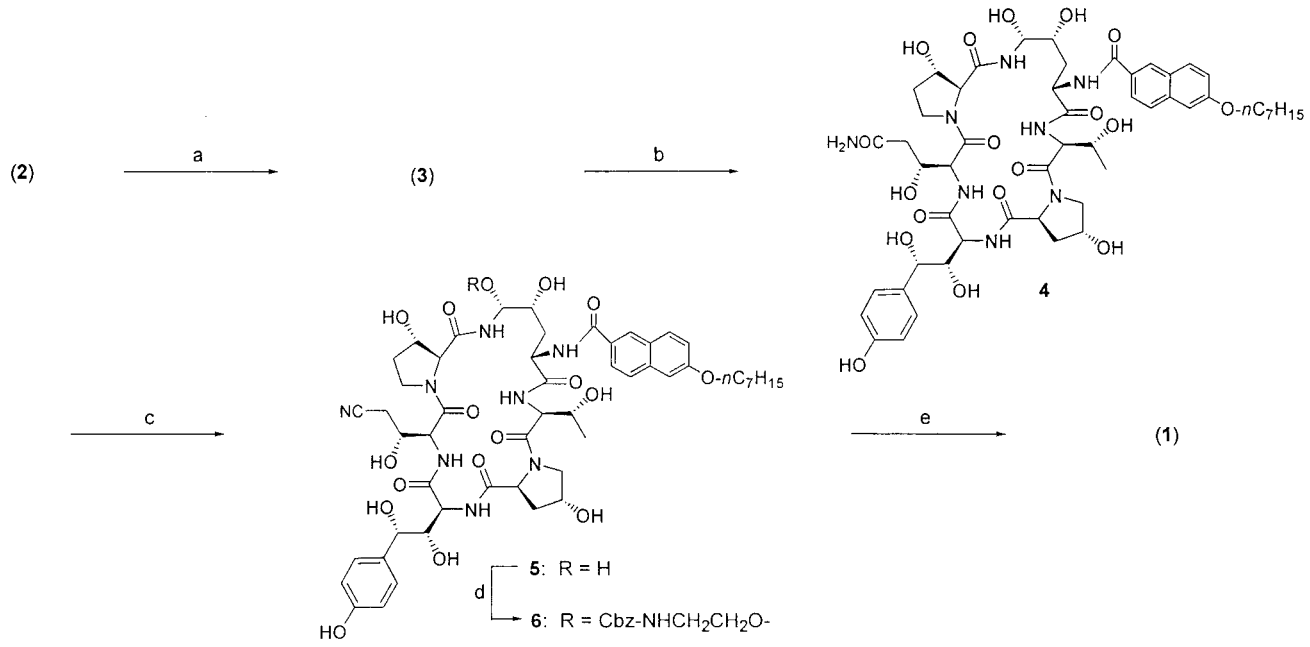
Table 1



Compound	R_1	R_2	R_3
(1)	CH_2NH_2	$(CH_2)_2NH_2$	
(2)	$CONH_2$	H	
(3)	$CONH_2$	H	H

Scheme 1[a]



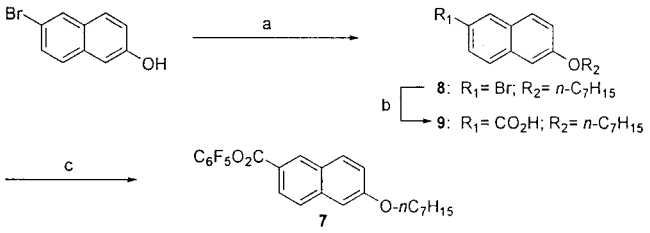
[a] Key: (a) enzymatic hydrolysis;[8] (b) **7**, Et_3N, DMF; (c) cyanuric chloride, H_2O (2.5 equiv), DMF, −30 °C; (d) (i) phenylboronic acid, THF, (ii) benzyl *N*-hydroxyethylcarbamate, Cl_3CCO_2H, CH_3CN, 5 °C; (e) NH_4OAc, *i*-PrOH/H_2O (9:1), 5% AcOH, Pd/Al_2O_3,Rh/Al_2O_3, H_2(40 psi).

(iv) simultaneous catalytic hydrogenation of the nitrile and the Cbz-protecting group (92%). In addition, the preparation of the naphthoyl side chain **7** was accomplished in three steps off line in 79% overall isolated yield from commercially available 6-bromo-2-naphthol.

Results and Discussion

Synthesis of the Naphthoyl Side Chain and Reacylation of 3. The preparation of the naphthoyl side chain involved a three-step procedure from commercially avail-

Scheme 2[a]



[a] Key: (a) 2.5 N NaOH, n-$C_7H_{15}Br$, DMSO, 75 °C, 3 h, 97%; (b) 3 mol % [$Pd(OAc)_2$, dppp], DIPEA, CO (60 psi), DMSO/H_2O (4:1), 80 °C, 12 h, 86%; (c) DCC, C_6F_5OH, THF, 5 °C, 16 h, 95%.

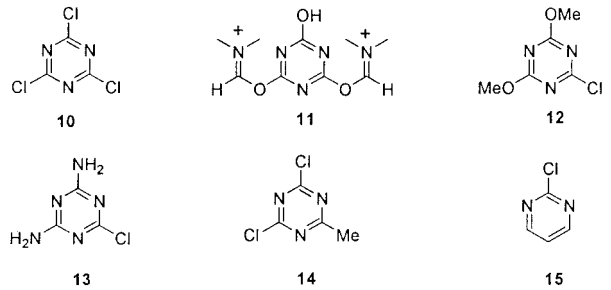


Figure 1.

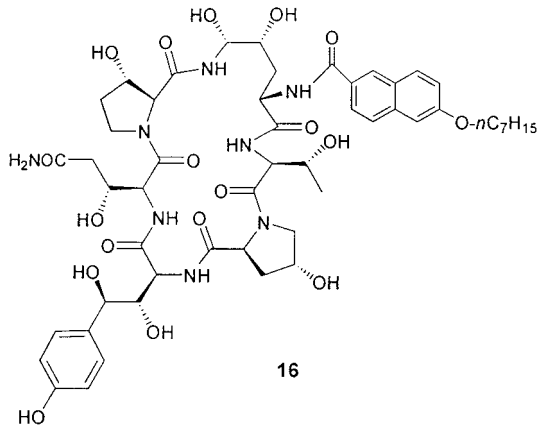
able 6-bromo-2-naphthol with 79% overall isolated yield (Scheme 2). Etherification with 1-bromoheptane in DMSO at 75 °C with sodium hydroxide as the base provided **8** in 97% isolated yield. Palladium-catalyzed carbonylation in a 4:1 mixture of DMSO/water at 80 °C for 12 h gave the carboxylic acid **9** in 86% yield with 5% of the reduced derivative (H replaced Br).

The use of phenyl esters and mixed anhydrides for problematic amide bond-forming reactions[10] prompted the preparation of the pentafluorophenyl ester, commonly used in peptide bond formation. The crude acid was reacted with pentafluorophenol in THF under standard DCC coupling conditions to yield the pentafluorophenyl-ester **7** in 95% yield.

The amine **3** as a TFA salt was reacylated with 3 equiv each of **7**[11] and triethylamine in DMF at room temperature. The reaction was complete within 3 h and was quenched with a 10% solution of aqueous acetic acid at 0 °C. Isolation of the reacylated cyclic hexapeptide **4** was accomplished by solid-phase extraction (SPE) in 98% overall yield. To circumvent precipitation of the side chain, an extraction with hexane was necessary to remove pentafluorophenol and the excess side chain. After recrystallization, this afforded a 90% recovery of **7** in >99% purity, which could be recycled.

Amide Dehydration. The dehydration of the primary amide **4** to the nitrile **5** was carried out as part of the reduction to the amine. Of the dehydrating reagents tested only cyanuric chloride **10**[12] in DMF gave a clean reaction (Figure 1). However, this dehydration proved to be tricky and required the use of 2.5 equiv of cyanuric chloride as opposed to the theoretical 0.33 equiv. At room temperature, the reaction was complete within 5 min, but unfortunately, compound **5** totally decomposed within 1 h. Obviously, the high reactivity of the reagent and the associated instability of the product posed a real problem. Two major factors—temperature and water—had a tremendous impact on the rate of the dehydration. Cooler temperatures (−30 °C) resulted in a much slower reaction (1 h), but again decomposition was observed upon further aging. No reaction occurred at −45 °C. Interestingly, with "wet" **4** and 2 equiv of cyanuric chloride at −30 °C, the dehydration was achieved with >98% conversion over 30 h and minimal decomposition. The need for water (ca. 250 mol %) indicates that cyanuric chloride might be converted in situ to a new reagent, such as **11**, that is less reactive. With this in mind, other chlorinated-triazine derivatives **12**−**14** and a chloropyrimidine **15** were tested, but none of them gave the desired reaction, only unreacted starting material.

The reaction was optimized by using 2.0 equiv of cyanuric chloride in the presence of water (1000−1100 μg of H_2O/mL) in DMF (40 mL/g) at −30 °C over 30 h. Under these conditions, a reproducible 92% isolated yield of **5** was obtained. The product mixture contained 4% of a byproduct that was isolated by preparative HPLC and identified as the epi isomer **16** at the benzylic position.



The formation of this byproduct increased substantially when cyanuric chloride was used in a larger excess, indicating that the formation of HCl was not responsible for this side reaction but the reagent itself.[13] For example, a reaction mixture with no cyanuric chloride remaining was completely stable for more than 1 week at −30 °C (pH ∼2).[14] To avoid increased epimerization, the reaction mixture was quenched when 98% conversion was reached with an equal volume of water at −30 °C. The product mixture was warmed to room temperature and loaded on the C-18 resin IMPAQ RG 10150 (10:1 loading) to give **5** in 92% yield along with 4% of the inseparable epi isomer **16**.

(10) (a) Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1989**, *54*, 3640. (b) Kisfaludy, L.; Roberts, J. E.; Johnson, R. H.; Mayers, G. L.; Kovacs, J. *J. Org. Chem.* **1970**, *35*, 3563. (c) Kovacs, J.; Mayers, G. L.; Johnson, R. H.; Cover, R.; Ghatak, U. *J. Org. Chem.* **1970**, *35*, 1810.

(11) One equivalent of the side chain could be used. However, an excess ensured complete conversion of the reaction in less than 3 h at room temperature. In addition, the excess could be recovered afterwards.

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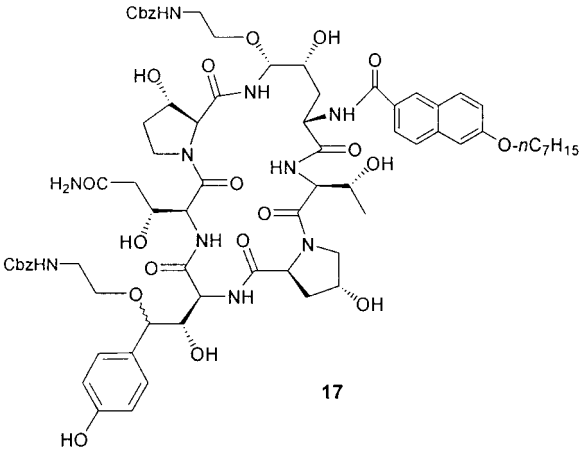
(13) Cyanuric chloride was reported to be a hydrochlorinating reagent for alcohols: Sandler, S. R. *J. Org. Chem.* **1970**, *35*, 3967. Chlorination could have occurred at the benzylic position to give the epichlorohydrin. Solvolysis or epoxide formation followed by ring opening with water would explain the epimerization of **5** to **16**.

(14) This was demonstrated by the following set of experiments: Pure **5** was treated in DMF at −30 °C with (i) HCl/H_2O (6 equiv); (ii) cyanuric acid (2 equiv); (iii) HCl/H_2O (6 equiv) with cyanuric acid (2 equiv); or (iv) cyanuric chloride (2 equiv). Formation of the epi isomer **16** occurred only with the cyanuric chloride treatment (40% decomposition after 24 h); under the other conditions (i−iii) **5** was found to be completely stable.

Ethanolamine Insertion. The control of the regio-, chemo-, and stereoselectivity at the hemiaminal position in the aminal step was challenging. The substitution required acidic conditions. With ethanolamine as the hydrochloride salt only a 3:1 α/β selectivity was achieved. Also, the reaction took days with substantial decomposition. Protection of the nitrogen was considered. Of the protecting groups tested (*N*-9-fluorenylmethoxycarbonyl-, 2,2,2-trichloroethoxycarbonyl-), the carbobenzyloxy (Cbz) group gave the best results. This had further advantages, since the deprotection could be achieved during the reduction of the nitrile to the primary amine. In anhydrous acetonitrile with 15% TFA and 8 equiv of benzyl *N*-(2-hydroxyethyl)carbamate at 5 °C the aminal **6** was formed within 3.5 h as a 99:1 α/β mixture in 90% yield. However, 10% of the bis-ethanolamine adduct **17**, where again the reactivity of the benzylic position has come into play, was observed as a 2:1 mixture of diastereomers.

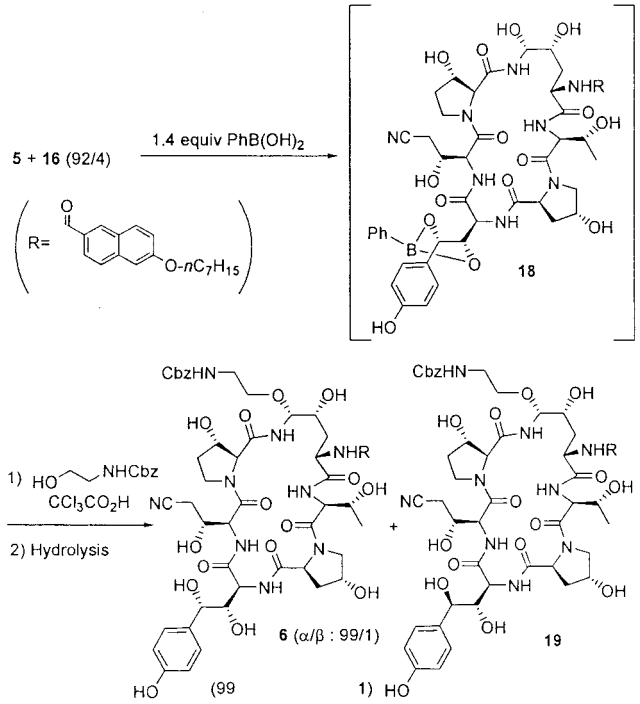


17

To overcome this reactivity, the syn 1,2-diol was selectively and quantitatively protected as the phenylborate **18** (Scheme 3).[15] Also, trichloroacetic acid gave slightly better results than TFA. Under these new conditions, crude nitrile **5**, containing 4% of **16**, was converted to **6** in 100% isolated yield with 99:1 α/β selectivity. The higher-than-expected yield is due to the reequilibration[16] of **16** in the reaction. Interestingly, under the reaction conditions 75% of the epi impurity **16** was recovered during the aminal reaction to afford an added 3% yield to the desired penultimate intermediate **6**. Only 1% of the epi isomer **19** contaminated the product mixture. Upon hydrolysis with water, the borate was cleaved and the product was isolated again by SPE.

Hydrogenation. The reduction of the nitrile and removal of the Cbz group remained to complete the synthesis. Hydrogenation was expected to effect both transformations, perhaps in one pot. Parameters, such as catalyst, catalyst support, catalyst loading, solvent, and pH, were important. As the support, alumina was crucial for this transformation. For example, Rh/Al_2O_3 (5% Rh) reduced **6** efficiently, whereas Rh/C gave no reaction at all. Ruthenium gave no reaction, and the conversion was very low with PtO_2. Palladium gave

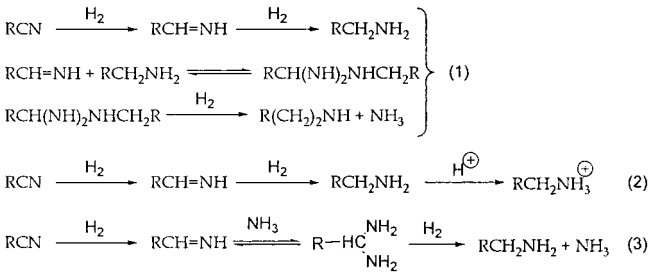
Scheme 3



encouraging results, but the reduction did not reach more than 70% conversion. Although rhodium on alumina was the best catalyst, cleavage of the Cbz-protecting group was quite sluggish. By using Pd/Al_2O_3 as a cocatalyst to cleave the Cbz group with Rh/Al_2O_3 to reduce the nitrile at 8 and 16 wt % (5 and 10 mol %) loading, respectively, the transformation proceeded very efficiently.[17]

The solvent also proved to be a key in this reaction. For instance, when **6** was hydrogenated in methanol only a 50% conversion (30% yield) was observed. A large amount of rhodium (up to 60 mol %) was needed to reach completion with a moderate 75% yield. In addition, formation of the epi isomer (at the benzylic position) occurred upon aging the reaction. By adding water to an alcohol solvent a good conversion and yield were achieved. With optimization a 9:1 mixture of 2-propanol/water offered the best solvent system. In particular, no epi isomer was formed.

The pH of the reaction was investigated as a means to control the formation of a secondary amine byproduct (eq 1). This dimerization can be prevented or minimized by



carrying out the hydrogenation in the presence of acid to trap the amine through salt formation (eq 2) or excess ammonia (≥6 equiv) to remove the imine intermediate

(15) Phenylboronic acid reacted selectively and quantitatively with the syn 1,2-diol on the bottom part of the molecule within 5 min, whereas the protection of the anti 1,2-diol on the top part of the molecule required a 24 h reaction. Belyk, K. M.; Bender, D. R.; Hughes, D. L.; Leonard, W. Unpublished results.

(16) Compound **5** reacts with $PhB(OH)_2$ but **16** does not. As a result, **16** reequilibrates to **5** under the reaction conditions.

(17) Hydrogen transfer (ammonium formate) with Pd/C gave **1** cleanly; however, 300 mol % of Pd/C was needed.

from the reaction mixture (eq 3).[18] The substrate **6** was not stable to strong mineral acids leading to epimerization at the benzylic position and cleavage of the Cbz-ethanolamine moiety. Acetic acid could be used, but was not optimal. On the other hand, the intermediate is stable to ammonia in alcohol solutions, but the hydrogenation stopped at 20% conversion with Rh/Al_2O_3 as the catalyst.[19] Raney nickel reduced the nitrile, but a 100 wt % loading was required and >20% of the secondary amine was formed. Interestingly, the reaction rate increased in the presence of ammonium acetate. Ammonium chloride gave no reaction. By moderating the acid/base combination a suitable reaction was attained.

The yield of the reaction was highly dependent on the ammonium acetate. Under the optimized conditions, ammonium acetate (35 equiv) was added to **6** in a 9:1 mixture of 2-propanol/water (20 mL/g) with 5% acetic acid. The catalyst system of 8 wt % of Pd/Al_2O_3 (5% Pd) and 16 wt % of Rh/Al_2O_3 (5% Rh) was added. The resulting black slurry was treated with hydrogen at 40 psi for 12 h at room temperature, reducing the nitrile and removing the Cbz group. The crude product was isolated by SPE in 92% yield with only 2% of the secondary amine. A final preparative HPLC using a Zorbax RX-C8 column and eluting with an 87:13 mixture of water (0.15% AcOH)/acetonitrile removed the byproduct. The rich cuts were lyophilized to give the pure **1** as a bis-acetate salt in 95% recovery as an amorphous white solid.

Conclusion

In summary, an efficient regio-, chemo-, and stereoselective semisynthesis of the echinocandin analogue **1** has been developed from the cyclic hexapeptide **3** in four linear steps in 83% overall yield. A number of key discoveries were critical to the successful transformations of these very sensitive molecules. The preliminary dehydration of the amide to the cyano group overcame the problematic direct reduction. A highly stereoselective (99:1) and quantitative introduction of the ethanolamine at the hemiaminal position was achieved by blocking the nitrogen as the Cbz derivative. Through in situ protection of the syn vicinal diol with phenylboronic acid the epimerization of the benzylic position was overcome. Finally, an interesting one-pot catalytic hydrogenation of the penultimate intermediate was achieved through a mixed catalyst of rhodium and palladium on alumina in the presence of ammonium acetate to reduce the nitrile and cleave the Cbz-protecting group.

Experimental Section

General Methods. Reactions were performed under a positive atmosphere of dry nitrogen. Prior to use, the solvents were dried over 4 Å molecular sieves to <100 μg H_2O/mL. Water content was determined by Karl Fisher titration (KF). Commercially available reagents were purchased from Aldrich Chemical Co. and used as received. Melting points are uncorrected. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. HR-MS analyses were performed by electrospray ionization. All reactions were monitored by reversed-phase HPLC with Metachem inertsil ODS-3 column (250 × 4.6 mm) with a flow rate of 1.5 mL/min and using UV detection at 220 nm. The solvent systems were (A) H_2O (0.1% $HClO_4$) and (B) acetonitrile. A pure sample of each intermediate of the synthesis was prepared by preparative HPLC and used as a standard for the determination of the weight %. Solid-phase extractions were performed with C-18 resin IMPAQ RG10150 (100 Å, 150 μm) purchased from Dupont and was reused after having been washed with 100% methanol.

6-Bromo-2-*n*-heptyloxynaphthalene (8). To a stirred solution of 6-bromo-2-naphthol (100 g, 448 mmol) in DMSO (1 L) was added sodium hydroxide (2.5 N aqueous, 206 mL, 515 mmol) over ca. 5 min, maintaining the temperature at 20−25 °C. The cold bath was removed, and 1-bromoheptane (81 mL, 515 mmol) was added in one portion. The resulting yellow solution was heated at 75 °C for 3 h and cooled to room temperature, whereupon the ether precipitated at 45−50 °C. The solid was filtered and washed with water (2 L) to give 143 g of **8** in 97% yield as an off-white solid. Analytical HPLC conditions: isocratic elution 5% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of 6-bromo-2-naphthol, 2.55 min; **8**, 9.25 min: mp 54−55 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.90 (d, J = 1.8 Hz, 1 H), 7.65−7.56 (m, 2 H), 7.48 (dd, J = 8.7, 1.9 Hz, 1 H), 7.15 (dd, J = 8.9, 2.2 Hz, 1 H), 7.08 (d, J = 2.2 Hz, 1 H), 4.05 (t, J = 7.1 Hz, 2 H), 1.84 (quint, J = 7.1 Hz, 2 H), 1.56−1.33 (m, 8 H), 0.90 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 157.3, 133.0, 129.7, 129.4, 129.3, 128.2, 119.9, 116.7, 106.3, 67.9, 31.7, 29.1, 29.0, 26.0, 22.5, 14.0. Anal. Calcd for $C_{17}H_{21}BrO$ (321.26): C, 63.56; H, 6.59. Found: C, 63.52; H, 6.58.

6-*n*-Heptyloxy-2-naphthoic Acid (9). To a stirred autoclave were added 6-bromo-2-heptyloxynaphthalene **8** (140 g, 436 mmol), palladium acetate (2.93 g, 13.1 mol), 1,3-bis-(diphenylphosphino)propane (5.4 g, 13.1 mmol), DMSO/H_2O (4:1, 870 mL), and *N*,*N*-diisopropylethylamine (152 mL, 872 mmol). The reaction mixture was heated at 80 °C under 60 psi of CO for 12 h (monitored by CO uptake). The resulting black mixture was cooled, partitioned between ethyl acetate (2 L) and aqueous HCl (1.2 N, 1 L), and filtered through a pad of solka-floc. The layers were separated, and the organic extract was washed with brine (1 L). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to give 117 g of the crude acid **9** (107 g assay) in 86% yield as a pale-yellow solid containing 5% of the reduced bromide. Analytical HPLC conditions: isocratic elution 5% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **9**, 3.90 min; reduced bromide, 8.70 min: mp 159−160 °C (standard); ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.90 (br s, 1 H), 8.50 (d, J = 1.7 Hz, 1 H), 7.99 (d, J = 9.0 Hz, 1 H), 7.92 (d, J = 1.7 Hz, 1 H), 7.90 (d, J = 1.7 Hz, 1 H), 7.39 (d, J = 2.5 Hz, 1 H), 7.22 (dd, J = 9.0, 2.5 Hz, 1 H), 4.10 (t, J = 6.9 Hz, 2 H), 1.77 (quint, J = 6.9 Hz, 2 H), 1.51−1.30 (m, 8 H), 0.85 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 167.6, 158.5, 136.8, 130.9, 130.4, 127.5, 126.9, 125.8, 125.7, 119.6, 106.5, 67.7, 31.3, 28.6, 28.5, 25.6, 22.1, 14.0. Anal. Calcd For $C_{18}H_{22}O_3$ (286.37): C, 75.50; H, 7.74. Found: C, 75.42; H, 7.73 (standard).

Pentafluorophenyl 6-*n*-Heptyloxy-2-naphthoate (7). To a stirred solution of the crude acid **9** (92 wt %, 107 g assay, 374 mmol) in THF (1.2 L) were added at 5 °C pentafluorophenol (75.8 g, 412 mmol) and 1,3-dicyclohexylcarbodiimide (77.5 g, 374 mmol). The reaction was stirred at 5 °C for 16 h. The mixture was filtered to remove the spent urea and concentrated under reduced pressure to give **7**. The crude ester was redissolved in methyl *tert*-butyl ether (MTBE) (500 mL). The mixture was filtered to remove additional spent urea and concentrated under reduced pressure to dryness to afford a pale yellow solid. Recrystallyzation from a 2:1 mixture of 2-propanol/water at 20 mL/g afforded 160 g of **6** (99.5 wt %) in 95% yield as a white shiny crystalline solid. Analytical HPLC conditions: isocratic elution 5% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **7**, 10.35 min: mp 81−82 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.69 (d, J = 1.7 Hz, 1 H), 8.09 (dd, J = 8.6, 1.8 Hz, 1 H), 7.87 (d, J = 9.1 Hz, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.22 (dd, J = 9.0, 2.5 Hz, 1 H), 7.16 (d, J = 2.5 Hz, 1 H), 4.10 (t, J = 7.1 Hz, 2 H), 1.86 (quint, J = 7.1 Hz, 2 H), 1.62−1.33 (m, 8 H), 0.88 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (62.5

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MHz, $CDCl_3$) δ 162.9, 160.0, 138.2, 132.8, 131.2, 127.7, 127.4, 126.1, 121.5, 120.6, 106.5, 68.3, 31.8, 29.2, 29.1, 26.1, 22.7, 14.1, MS *m*/*z* 453.1 $(M + H)^+$. Anal. Calcd for $C_{24}H_{21}F_5O_3$ (452.42): C, 63.72; H, 4.68. Found: C, 63.66; H, 4.75.

Reacylation of 3 to 4. To a stirred solution of **3**[8] (TFA salt, 55 wt %, 5.23 g assay, 5.56 mmol) in DMF (85 mL) were added pentafluorophenyl 6-*n*-heptyloxy-2-naphthoate **7** (7.55 g, 16.7 mmol) and triethylamine (2.35 mL, 16.7 mmol). The resulting homogeneous solution was stirred at room temperature for 3 h and cooled to 0−5 °C. The reaction mixture was quenched with 10% aqueous acetic acid (125 mL), and the product was extracted with hexane (120 mL). The aqueous phase (pH ∼5) was loaded on the C-18 resin IMPAQ RG10150 (70 g). The column was washed with three volumes of a 90:10 mixture of water/methanol (650 mL). Compound **4** was recovered by eluting with 2 volumes of methanol (400 mL). The methanolic fraction was concentrated under reduced pressure to dryness to give 6.86 g of crude **4** (95 A%, 87 wt %, 5.97 g assay) in 98% yield as a white solid that was used in the next step without further purification. Analytical HPLC conditions: isocratic elution 57% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **3**, 1.85 min (overlaps with DMF); **4**, 6.25 min: ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 0.9 Hz, 1 H), 7.78 (dd, J = 8.6, 1.7 Hz, 1 H), 7.74 (d, J = 9.1 Hz, 1 H), 7.65 (d, J = 8.7 Hz, 1 H), 7.17−7.11 (m, 2 H), 7.15 (d, J = 8.5 Hz, 2 H), 6.76 (d, J = 8.5 Hz, 2 H), 5.41 (d, J = 2.4 Hz, 1 H), 5.11 (d, J = 4.2 Hz, 1 H), 5.04 (d, J = 3.1 Hz, 1 H), 4.75 (dd, J = 12.7, 4.7 Hz, 1 H), 4.63−4.51 (m, 4 H), 4.43−4.39 (m, 1 H), 4.34−4.23 (m, 4 H), 4.21 (td, J = 7.9, 2.2 Hz, 1 H), 4.08 (t, J = 6.4 Hz, 2 H), 4.01−3.91 (m, 2 H), 3.81−3.75 (m, 2 H), 2.91 (dd, J = 17.0, 3.7 Hz, 1 H), 2.58 (dd, J = 15.3, 9.9 Hz, 1 H), 2.45−2.41 (m, 1 H), 2.29−2.10 (m, 2 H), 2.04 (td, J = 12.4 and 3.4 Hz, 1 H), 1.97−1.93 (m, 1 H), 1.83 (quint, J = 6.9 Hz, 2 H), 1.52 (quint, J = 8.2 Hz, 2 H),1.42−1.32 (m, 6 H), 1.22 (d, J = 6.0 Hz, 3 H), 0.92 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.25 ($CONH_2$), 174.7, 173.5, 172.9, 172.8, 172.5, 169.9, 169.2, 160.1, 158.5, 138.0, 133.0, 131.6, 129.7, 129.2, 129.0, 127.9, 125.5, 120.8, 116.3, 107.4, 77.0, 75.8, 74.3, 74.0, 71.4, 71.0, 70.8, 69.8, 69.2, 68.3, 62.5, 58.5, 57.1, 56.3, 55.7, 52.3, 47.1, 39.7, 38.5, 34.5, 33.0, 30.4, 30.3, 27.2, 23.7, 23.7, 19.8, 14.5; HR-MS calcd for $C_{52}H_{70}N_8O_{18}$ 1094.4808, found 1094.4773.

Dehydration of the Amide 4 to the Nitrile 5. A solution of **4** (87 wt %, 5.97 g assay, 5.45 mmol) in dry DMF (250 mL) was chilled to −30 °C. The water content was measured by KF and was adjusted to ca. 1000 μg H_2O/mL (ca. 0.25 g, 13.6 mmol). Cyanuric chloride (2.01 g, 10.9 mmol) was added in one portion. The resulting pale yellow solution was stirred at −30 °C. When 98% conversion (ca. 30 h) was reached (HPLC), water (250 mL) was added over 10 min, and the mixture was warmed to room temperature. The crude mixture (1:1 DMF/H_2O; pH ∼2; 500 mL) was loaded on the C-18 resin IMPAQ RG10150 (70 g), and the column was washed with a 90:10 mixture of water/methanol (1.5 L). The nitrile **5** was eluted by washing the column with methanol (500 mL). This fraction was concentrated under reduced pressure to dryness to give 6.43 g of **5** (92 A%, 84 wt %, 5.40 g assay) in 92% yield as a white solid containing 4% of epi isomer **16** and 2% of nonreacted **4**. This mixture was used as is in the next step without further purification. Analytical HPLC conditions: isocratic elution 57% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **5**, 10.25 min; **16**, 11.95 min: ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (d, J = 1.3 Hz, 1 H), 7.93 (dd, J = 8.6, 1.8 Hz, 1 H), 7.76 (d, J = 9.1 Hz, 1 H), 7.69 (d, J = 8.8 Hz, 1 H), 7.19 (d, J = 2.2 Hz, 1 H), 7.14 (dd, J = 9.0, 2.2 Hz, 1 H), 7.13 (d, J = 8.6 Hz, 2 H), 6.75 (d, J = 8.6 Hz, 2 H), 5.42 (d, J = 2.3 Hz, 1 H), 5.04 (t, J = 3.3 Hz, 2 H), 4.71 (dd, J = 11.0, 6.4 Hz, 1 H), 4.61−4.53 (m, 3 H), 4.38−4.33 (m, 3 H), 4.28 (qd, J = 8.0, 1.4 Hz, 2 H), 4.22 (d, J = 4.1 Hz, 1 H), 4.16 (td, J = 7.9, 2.2 Hz, 1 H), 4.09 (t, J = 6.4 Hz, 2 H), 3.98−3.86 (m, 2 H), 3.82−3.74 (m, 2 H), 2.88 (dd, J = 17.0, 3.7 Hz, 1 H), 2.77 (dd, J = 17.0, 8.4 Hz, 1 H), 2.46−2.41 (m, 1 H), 2.31−2.23 (m, 1 H), 2.19−2.14 (m, 2 H), 2.06 (td, J = 12.4, 3.4 Hz, 1 H), 1.99−1.92 (m, 1 H), 1.84 (quint, J = 6.9 Hz, 2 H), 1.52 (quint, J = 8.2 Hz, 2 H),1.44−1.32 (m, 6 H), 1.26 (d, J = 6.2 Hz, 3 H), 0.91 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.6, 173.5, 173.0, 172.9, 172.7, 172.6, 170.0, 168.5, 160.1, 158.5, 138.0, 133.0, 131.6, 129.9, 129.7, 129.2, 129.0, 127.9, 125.6, 120.8, 119.8 (CN), 116.2, 107.4, 77.2, 75.7, 74.2, 74.2, 71.4, 71.2, 69.9, 69.7, 69.6, 69.2, 68.5, 62.6, 58.8, 57.1, 56.1, 55.0, 52.4, 47.0, 38.6, 35.0, 34.5, 33.0, 30.4, 30.3, 27.2, 23.7, 23.6, 19.7, 14.4; HR-MS calcd for $C_{52}H_{68}N_8O_{17}$ 1076.4702, found 1076.4702.

Cbz-Protected Ethanolamine Insertion. Preparation of 6. To a solution of **5** (84 wt %, 5.40 g assay, 5.01 mmol) in THF (100 mL) was added phenylboronic acid (853 mg, 7.0 mmol) at room temperature. The solution was concentrated under reduced pressure to dryness to remove the water. This azeotropic distillation was repeated twice. The resulting borate was slurried in dry CH_3CN (175 mL, KF ∼50 μg H_2O/mL) with benzyl *N*-(2-hydroxyethyl)carbamate (7.8 g, 40 mmol), and the reaction mixture was cooled to 5 °C. A solution of trichloroacetic acid (60 g, 367 mmol) in dry CH_3CN (75 mL) was added over 5 min at <5 °C. The solution was stirred for 3.5 h at 5 °C and quenched into water at 5 °C (375 mL). The mixture was loaded on a C-18 resin IMPAQ RG10150 (70 g). The column was washed with a 60:40 mixture of water/acetonitrile (1.5 L) to remove the excess benzyl *N*-(2-hydroxyethyl)carbamate and phenylboronic acid. Compound **6** was then eluted with methanol (600 mL). This fraction was concentrated under reduced pressure to dryness to afford 7.31 g of **6** (91 A%, 86 wt %, 6.28 g assay) in 100% yield as a white solid containing 1% of the epi isomer **19**. The product was used as is in the next step without further purification. Analytical HPLC conditions: isocratic elution 45% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **6**, 10.15 min; **19**, 11.25 min: ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (br s, 1 H), 7.86 (dd, J = 8.6 and 1.7 Hz, 1 H), 7.79 (d, J = 8.9 Hz, 1 H), 7.72 (d, J = 8.6 Hz, 1 H), 7.28−7.21 (m, 6 H), 7.16−7.13 (m, 1 H), 7.13 (d, J = 8.5 Hz, 2 H), 6.75 (d, J = 8.5 Hz, 2 H), 5.33 (br s, 1 H), 5.05 (t, J = 3.5 Hz, 2 H), 4.92 (br s, 2 H), 4.73 (dd, J = 12.6, 4.8 Hz, 1 H), 4.61−4.55 (m, 3 H), 4.36−4.30 (m, 2 H), 4.26 (dd, J = 12.6, 4.3 Hz, 2 H), 4.18 (td, J = 7.9, 2.2 Hz, 1 H), 4.09 (t, J = 6.4 Hz, 2 H), 3.98−3.87 (m, 2 H), 3.83−3.78 (m, 2 H), 3.63−3.59 (m, 1 H), 3.54−3.49 (m, 1 H), 3.29−3.23 (m, 2 H), 2.86 (dd, J = 17.0, 3.8 Hz, 1 H), 2.77 (dd, J = 17.0, 8.4 Hz, 1 H), 2.46−2.41 (m, 1 H), 2.28−2.03 (m, 4 H), 2.00−1.93 (m, 1 H), 1.84 (quint, J = 6.9 Hz, 2 H), 1.52 (quint, J = 8.2 Hz, 2 H), 1.44−1.30 (m, 6 H), 1.24 (d, J = 6.2 Hz, 3 H), 0.90 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.2, 173.7, 173.5, 172.8, 172.8, 170.0, 168.4, 160.2, 158.5, 138.1, 133.1, 131.6, 129.9, 129.7, 129.4, 129.3, 129.0, 128.9, 128.7, 128.1, 125.6, 120.9, 119.7 (CN), 116.2, 107.4, 80.6, 77.2, 75.7, 74.7, 71.4, 70.3, 69.9, 69.5, 69.2, 68.4, 68.0, 67.4, 62.6, 58.8, 57.1, 56.1, 54.9, 51.8, 47.0, 41.7, 38.6, 35.1, 34.7, 33.0, 30.4, 30.3, 27.2, 23.7, 23.6, 19.6, 14.4; HR-MS calcd for $C_{62}H_{79}N_9O_{19}$ 1253.5492, found 1253.5534.

Catalytic Hydrogenation of 6 to 1. To a solution of **6** (86 wt %, 6.28 g assay, 5.01 mmol) in a 9:1 mixture of 2-propanol/water (130 mL) were added acetic acid (6.5 mL), ammonium acetate (13.5 g, 175.0 mmol), Pd/Al_2O_3 (5% Pd, 525 mg, 0.25 mmol), and Rh/Al_2O_3 (5% Rh, 1.05 g, 0.5 mmol). The resulting black slurry was treated with hydrogen (40 psi) at room temperature for 12 h, diluted with water (400 mL), and filtered through a pad of solka-floc. The filtrate was loaded on the C-18 resin IMPAQ RG10150 (70 g) and washed with 90:10 water/methanol (1.5 L). The product was eluted with methanol (600 mL). This fraction was concentrated under reduced pressure to dryness to give 7.26 g of crude **1** as the bis-acetate salt (86 A%, 79 wt %, 5.73 g assay) in 92% yield as a white solid. Crude **1** was dissolved in a 90:10 mixture of water (0.15% AcOH)/CH_3CN (250 mL), and the mixture was loaded on a preparative HPLC column (75 × 500 mm E. Merck A/E column containing 1.1 kg of Zorbax 10 μm C8 material, detection at 220 nm, 100 mL/min). The product was eluted with 13% acetonitrile/water (0.15% AcOH). The rich cuts were lyophilized to give 5.50 g of the pure **1** as the bis-acetate salt (99.5 A%, 99 wt %, 5.45 g assay) in 95% recovery as an amorphous white solid. Analytical HPLC conditions: isocratic elution 65% H_2O (0.1% $HClO_4$) in acetonitrile; retention time of **1**, 9.80 min: ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (br s, 1 H), 7.85−7.76 (m, 3 H), 7.25 (d, J = 1.2 Hz, 1 H), 7.14 (dd, J = 8.9, 1.2 Hz, 1 H), 7.13 (d, J = 8.6 Hz, 2 H), 6.75 (d, J = 8.6 Hz, 2 H), 5.25 (br. s, 1 H), 5.03−4.93 (m,

2 H), 4.75 (dd, J = 12.7, 5.0 Hz, 1 H), 4.62−4.54 (m, 3 H), 4.34−4.23 (m, 7 H), 4.11 (t, J = 6.4 Hz, 3 H), 3.99−3.57 (m, 7 H), 3.15−3.12 (m, 2 H), 2.95−2.93 (m, 2 H), 2.45−2.41 (m, 1 H), 2.29−1.95 (m, 7 H), 1.88 (s, 6 H), 1.87−1.82 (m, 2 H), 1.51 (quint, J = 7.3 Hz, 2 H), 1.39−1.32 (m, 6 H), 1.22 (d, J = 4.9 Hz, 3 H), 0.92 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3-OD) δ 174.4, 174.2, 173.5, 172.8, 172.8, 170.3, 169.1, 160.3, 158.6, 138.1, 133.0, 131.6, 129.7, 129.6, 129.2, 129.0, 128.1, 125.4, 121.1, 116.3, 107.4, 80.4, 77.3, 75.6, 74.9, 72.3, 71.4, 69.9, 69.3, 69.1, 68.3, 66.7, 62.8, 58.4, 57.1, 56.4, 56.1, 51.8, 47.1, 41.2, 39.1, 38.5, 34.6, 33.0, 31.2, 30.4, 30.2, 27.2, 24.3, 23.7, 20.1, 14.4; HR-MS calcd for $C_{54}H_{77}N_9O_{17}$ 1123.5437, found 1123.5428.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**, **4**, **5**, and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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