Stereoselective Synthesis of a Thymine Derivative of (*S*)-2-Hydroxy-4-(2-aminophenyl)butanoic Acid. A Novel Building Block for the Synthesis of Aromatic Peptidic Nucleic Acid Oligomers[1]

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The synthesis of a thymine derivative of (*S*)-2-hydroxy-4-(2-aminophenyl)butanoic acid, compound **1**, was achieved in high enantiomeric purity. The acyclic pyrimidine analog (*S*)-**1** is a useful building block for the synthesis of a novel class of oligomers, the aromatic peptide nucleic acids (APNA, Scheme 1). The APNA tetramer **18** was prepared from the amino acid monomer (*S*)-**1** using classical peptide synthesis. UV absorption spectra and ^{1}H NMR data of this tetramer suggested that base stacking interactions in the APNA oligomers may be favorable.

Introduction

Numerous synthetic analogs of natural oligonucleotides (ODN) have been described in the literature for their promising applications in antisense chemotherapy.[2] The fidelity of the base-pairing interactions in the formation of DNA−DNA or RNA−DNA duplexes (Watson−Crick base pairing) is governed by an array of complementary hydrogen bonds between the nucleotide bases. However, the major stabilizing force for the formation of a duplex is the electrostatic/hydrophobic attraction between the stacked aromatic rings of the purine and pyrimidine bases.[3] Thus, ODN derivatives possessing structural features which enhance π-stacking interactions exhibit stronger hybridization affinity with a complementary single strand of RNA or DNA (*antisense*),[4] as well as with double-stranded DNA (*antigene*, Hoogsteen or reversed Hoogsteen base pairing),[5] in the formation of a helix.

Two of the critical requirements for both antisense and antigene applications, nuclease stability and enhanced binding affinity of a synthetic ODN analog, were addressed in the design and synthesis of the first peptide nucleic acids by Nielson's group (PNAs, Scheme 1).[6] Although PNAs do not cross cell membranes readily, their nuclear microinjection into cells has been shown to result in the efficient and site-specific termination of both transcription and translation.[6c,7] Contrary to studies showing that incorporation of flexible acyclonucleosides into synthetic ODNs decrease the stability of duplex formation,[8] the PNA oligomers exhibit a remarkable affinity for both complementary DNA and RNA. Molecular mechanics calculations,[9] based on the solution NMR data of a hexameric PNA−RNA duplex,[10] suggested that interresidue hydrogen-bonding interactions in the backbone of the PNAs provide conformational stability in the duplexes and triplexes formed with these molecules.

A new class of peptide oligonucleotide analogs, the aromatic peptide nucleic acids (APNA), having general structure **2** (Scheme 1), is currently under investigation in our laboratory. These molecules were designed in order to investigate possible π-stacking interactions in the backbone of the APNA oligomers and their imparting stabilizing effect on a duplex or triplex structure.[11] We have speculated that the backbone N−H protons could

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(1) The abbreviation APNA for aromatic peptide nucleic acids has been assigned to this new class of analogs.

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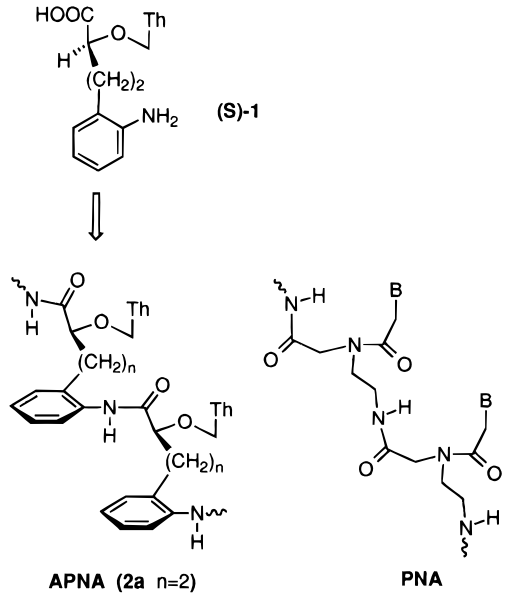
Scheme 1. Structural Comparison of APNA and PNA Analogs



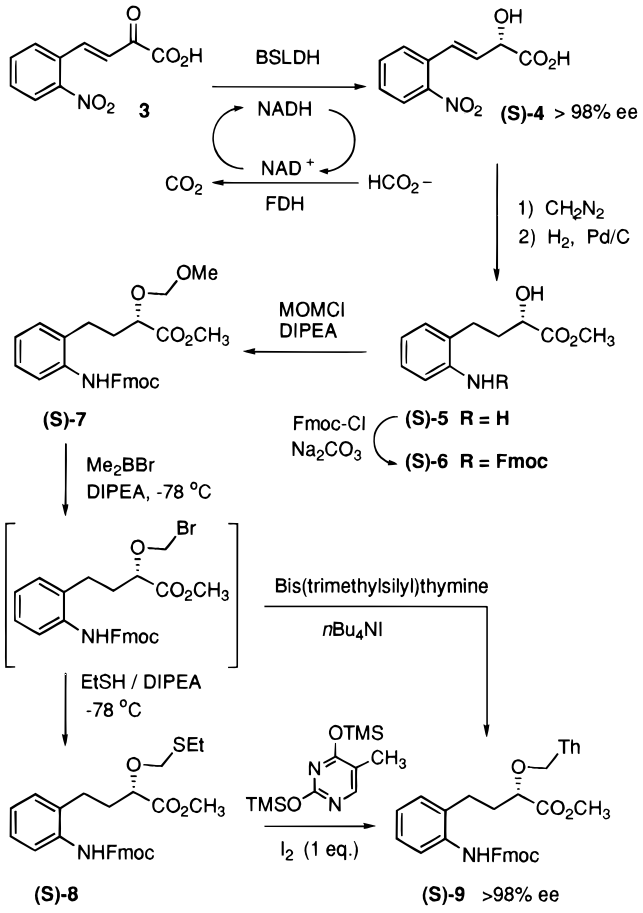
potentially form stabilizing intramolecular interactions with the center of the adjacent benzene ring (dipole/cation π interactions[12] or hydrogen bonds[13]) thus contributing to the precise conformation and stabilty of the APNA macromolecules in a hybridized duplex or triplex, analogous to that proposed for the PNA oligopeptides.[9]

The APNA analogs were designed to have a distance of three bonds between the nucleobases and the backbone, as in the case of natural oligonucleotides. Since three of the bonds in the aniline moieties of the APNA oligomers are coplanar, the spatial distance between the bases would greatly depend on the conformation and presence, or absence, of π-stacking interactions along the backbone. Therefore, the most favorable number of bonds between the bases in these new analogs remains to be determined. This report outlines the stereocontrolled synthesis of a thymine derivative of (*S*)-2-hydroxy-4-(2-aminophenyl)butanoic acid (**1**), the first monomer in the series of analogs which will be used as building blocks in the synthesis of APNA oligomers (**2a**). The dimer and tetramer of **1** were also prepared using classical peptide synthesis, and their conformation in solution was examined by ^{1}H NMR and UV spectroscopy. The distance between nucleobases in analog **2a** is seven bonds along the backbone; thus it is homomorphous to the acetamidate and carboxymethyl-linked ODN analogs; the latter are known to form stable duplex structures with complementary RNA.[14]

Results and Discussion

The novel acyclic thymidine analog (*S*)-**1** was obtained using the α-keto acid **3** as the key starting material (Scheme 2).[15] Enzymatic reduction of **3** using *Bacillus stearothermophilus* lactate dehydrogenase[16] gave the (*S*)-α-hydroxy acid **4** in high chemical yield (98%) and enantiomeric purity (>98% ee).[17] Compound (*S*)-**4** was subsequently converted to the methyl ester with diazomethane and reduced to (*S*)-**5** by catalytic hydrogenation,[18] and the resulting aniline was protected to give the Fmoc derivative (*S*)-**6** in good overall yield (>70% from **4**).[19]

Scheme 2. Synthesis of the Thymidine Acyclonucleoside Analog (*S*)-1



Initial efforts to extend the α-hydroxy moiety of **6** to the (methylthio)methyl ether using methyl sulfide and benzoyl peroxide,[20] or DMSO in a Pummerer type rearrangement,[21] gave primarily the α-keto ester and only 15−20% yield of the desired product. However, the synthesis of the analogous intermediate (ethylthio)methyl ether (*S*)-**8** was easier to achieve in reasonable yields (55−60%) by converting the MOM ether (*S*)-**7** first to an

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(13) (a) Levitt, M; Perutz, M. F. *J. Mol. Biol.* **1988**, *201*, 751. (b) Adams, H.; Harris, K. D. M.; Hembury, G. A.; Hunter, C. A.; Livingstone, D.; McCabe, J. F. *Chem. Commun.* **1996**, 2531. (c) Adams, H.; Carver, F. J.; Hunter, C. A.; Osborne, N. J. *Chem. Commun.* **1996**, 2529.
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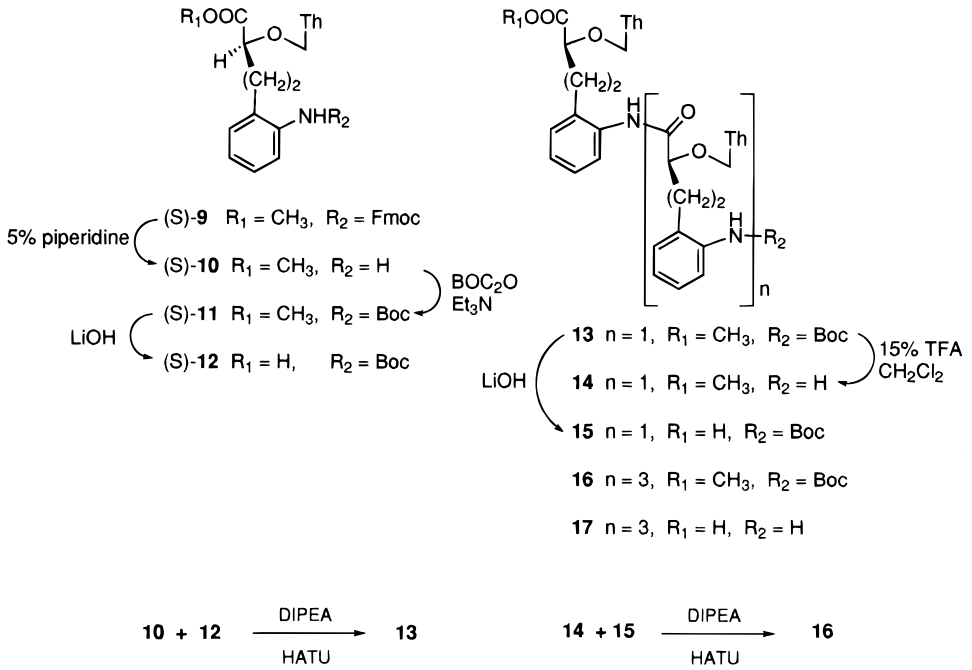
(17) The enantiomers of **4** could not be separated by chiral HPLC. Thus, compound **4** was first converted to **5** and then the optical purity of **5** was determined from its ^{1}H NMR in the presence of (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol. (a) Pirkle, W. H.; Rinaldi, P. L *J. Org. Chem.* **1978**, *43*, 4475. (b) Tsantrizos, Y. S.; Ogilvie, K. K. *Can. J. Chem.* **1991**, *69*, 772.
(18) Reduction of both the α-ketone and the β,γ-olefin of **3** with a L-proline/$NaBH_4$ complex gave (*R*)-2-hydroxy-4-(2-nitrophenyl)butanoic acid, with ~50% ee. The β,γ-olefin reduction has been shown to involve conjugation with the ortho nitro group in the phenyl ring: Kitajima, H.; Ikebe, T.; Murakami, S. Unpublished results. After reaction with CH_2N_2 and catalytic hydrogenation, the (*R*)-2-hydroxy-4-(2-nitrophenyl)butanoic acid was then converted to **5**. This sample of (*R*)-**5** (~50% ee) was subsequently used to produce the *R* enantiomers of all key intermediates in Scheme 2; these compounds were used as reference samples in measurments of optical purity.
(19) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
(20) Medina, J. C.; Salomon, M.; Kyler, K. S. *Tetrahedron Lett.* **1988**, *29*, 3773.
(21) Pojer, P. M.; Angyal, S. J. *Aust. J. Chem.* **1978**, *31*, 1031.

Scheme 3. Synthesis of the Thymidine APNA Oligomers



α-bromo ether with dimethylboron bromide and subsequently displacing the bromide with ethanethiol.[22] Several attempts to prepare **9** directly from **7** (one-pot reaction), by reacting the unstable α-bromo ether intermediate with bis(trimethylsilyl)thymine in the presence of *n*-Bu_4NI, led to a very low yield of the desired compound **9** (~5% yield), with alcohol **6** being the only other detectable product.[23] In contrast, coupling of (*S*)-**8** with bis(trimethylsilyl)thymine[24] in the presence of iodine gave the acyclonucleoside thymidine analog (*S*)-**9** in 58% yield, and the unreacted (*S*)-**8** was recovered (based on the recovery of **8**, compound **9** was formed with 98% efficiency). The enantiomeric purity of compound (*S*)-**9** was analyzed by chiral HPLC and found to be greater than 98% ee.[25]

The Fmoc protecting group was partly unstable to conditions required for ester hydrolysis;[26] thus it was replaced with the base stable Boc group using standard literature procedures (Scheme 3). Peptide bond formation between the aniline monomer (*S*)-**10** and the Boc-protected free acid (*S*)-**12** was easily achieved using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent in the presence of diisopropylethylamine (DIPEA).[27] The addition and removal of the Boc group as well as the hydrolysis of the methyl ester were almost quantitative, whereas the peptide coupling reaction gave typically a 70% yield. The preparation of dimer (*S,S*)-**13** and tetramer (*S,S,S,S*)-**17** were achieved using the same reaction conditions (Scheme 3).[28]

The structural identity of all new compounds was confirmed by their MS and NMR data. The ^1H and ^{13}C chemical shifts of all new compounds were assigned after extensive analysis of their high-field 2D NMR spectra. The chemical shift assignments of tetramer **17** were based on its ^1H, COSY, NOESY, HMQC, HMBC (500 MHz), and ^{13}C (150 MHz) NMR spectra and are summarized in Table 1. In addition, the high-resolution FAB MS data of the fully deprotected APNA monomer, dimer, and tetramer (**1**, **16**, **18**) confirmed the elemental composition of these new molecules.

The fully deprotected compounds, monomer **1**, dimer **16**, and tetramer **18** were found to be completely water soluble. Thus, the ^1H resonances of the thymine moieties in D_2O were compared, in the hope of gaining some insight into the preorganization of these molecules in an aqueous solution.[29] Small upfield shifts of both the H6 and methyl resonances in the tetramer, as compared to those of the dimer were observed (Table 2). This phenomenon is generally attributed to the ring-current magnetic anisotropy effect of the purine and pyrimidine bases and is usually observed in conformations involving intramolecular base-stacking interactions. Unfortunately, the monomer did not show the expected chemical shifts, downfield from those of the dimer. We have speculated that this discrepancy may be due to the presence of two chromophores in the APNA monomer which can π-stack with each other. We have further

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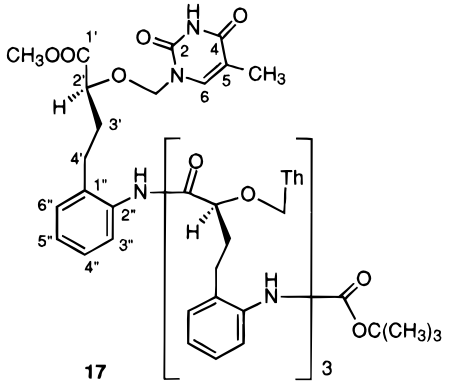
(23) When menthol was used as a model secondary alcohol to test the reaction conditions, the desired thymine derivative was produced in 90% yield.
(24) Nishimura, T.; Iwai, I. *Chem. Pharm. Bull.* **1964**, *12*, 352.
(25) The two enantiomers of racemic **9** were clearly separated on a chiralcel OD HPLC column using 10% ethanol in hexane at a flow rate of 1 mL/min.
(26) Hydrolysis of the methyl ester did not lead to any racemization under the reaction conditions used; deuterium exchange of the α-proton was not observed when the reaction was carried out using 3 equiv of LiOH in dioxane−D_2O (1:1) at rt.
(27) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(28) The diastereomeric mixture of **13** was prepared from racemic monomers **10** and **12**, and examined by ^1H NMR (300 MHz, $CDCl_3$). Two sets of diastereomeric protons were clearly observed (so that integrals could be obtained with a high degree of accuracy) for the following resonances: backbone-NHCO (δ 8.24 and 8.53), $-CO_2CH_3$ (δ 3.64 and 3.69), Th-CH_3 (δ 1.91 and 1.95), and the Boc group (δ 1.50 and 1.53). However, the ^1H NMR of compound (*S,S*)-**13** (300 MHz, $CDCl_3$) showed only one set of signals [δ 8.53 (NHCO), 3.69 (CO_2CH_3), 1.91 (Th-CH_3), and 1.50 (Boc)]. Since it is highly unlikely that coupling of the enantiomerically pure *S* monomers would lead to the formation of the (*R,R*) dimer **13**, we feel fairly confident that the peptide coupling reaction does not suffer from racemisation to any significant extent under the reaction conditions used.
(29) The chemical shifts were obtained from COSY NMR spectra using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the reference standard.

Table 1. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR Data of APNA Tetramer 17 in $CDCl_3$



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assignment	^{13}C (δ)	^{1}H (δ)	int, mult, J (Hz)	assignment	^{13}C (δ)	^{1}H (δ)	int, mult, J (Hz)
1′ (**C**O_2CH_3)	172.51			4	164.04		
(CO_2**C**H_3)	52.90	3.64	3H, s		164.22		
2′	77.94	4.17−4.23[b]	4H, m		164.43		
	78.22				164.73		
	78.76			5	112.03		
	79.88				112.17[c]		
3′	32.46	1.90−2.24[b]	8H, m		112.24		
	33.07			Th-CH_3	12.50[c]	1.86	9H, bs
	33.43				12.50[c]	1.88	3H, bs
	33.75			6	139.64	7.05−7.18[b]	
4′	26.65	2.62−2.75[b]	8H, m		139.81		
	26.78				140.02[c]		
	26.90			1″	131.63		
	27.11				131.64		
O-**C**H_2-Th	76.40[a]	5.12[b]	2H, bs		132.97		
	76.71[a]	5.06[b]	2H, AB quartet		133.43		
	77.03[a]	5.18[b]	2H, AB quartet	2″	134.66		
	77.03[a]	5.26[b]	2H, AB quartet		134.83		
Ph-N**H**-CO		8.62	1H, bs		135.03		
		8.72	1H, bs		136.34		
		8.84	1H, bs	3″	123.42	7.66	1H, bs
Ph-N**H**-Boc		8.71	1H, bs		124.50	7.74	1H, d, J = 8.1
Ph-NH-**C**O	170.33				124.55	7.61	1H, d, J = 7.3
	170.72				124.87	7.54	1H, d, J = 7.3
	170.80			4″	127.24	7.15−7.20[b]	
Ph-NH-**C**$O_2C(CH_3)_3$	151.94				127.32		
Ph-NH-CO_2**C**$(CH_3)_3$	80.76				127.33[c]		
Ph-NH-CO_2C(**C**H_3)$_3$	28.57	1.47	9H, s	5″	125.11	6.98−7.01[b]	
2	151.94				126.09		
	152.00				126.27		
	152.16				126.48		
	152.23			6″	129.35	7.09−7.19[b]	
Th-NH		9.95	1H, bs		129.61		
		10.02	1H, bs		129.78		
		10.16	1H, bs		129.87		
		10.27	1H, bs				

[a] Due to overlapping ^{13}C resonances, these chemical shifts assignments were based on the HMQC and HMBC NMR data. [b] Due to overlapping resonances in the ^{1}H NMR spectrum, the exact chemical shifts and coupling constants could not be determined; the values given were obtained from the HMQC and HMBC data. [c] The strong intensity of the signal suggested overlapping ^{13}C resonances.

Table 2. ^{1}H Chemical Shifts of Monomer 1, Dimer 16, and Tetramer 18 in D_2O[a]

assignment	**1** (δ)	**16** (δ)	**18** (δ)
H6	7.34	7.54, 7.41	7.45, 7.41, 7.38, 7.30[b]
Th-CH_3	1.80	1.87, 1.83	1.81, 1.80 (2Me), and 1.76

[a] DSS was used as the reference standard. [b] Due to overlapping resonances in the ^{1}H NMR spectrum, the exact chemical shifts were obtained from the 1D COSY spectra.

Table 3. UV Absorbance at 264 nm in CH_3OH

compound	molar concentration	absorbance
thymine	2.0×10^{-4}	1.525
compound **6**	2.0×10^{-4}	~0 (UV_{max} = 232 nm)
monomer **11**	2.0×10^{-4}	1.222
dimer **15**	1.0×10^{-4}	1.789
tetramer **17**	0.5×10^{-4}	1.418

explored this possibility by comparing the UV absorption spectra of pure thymine to the APNA molecules at 264 nm (Table 3). Although the absorbance value of the monomer was lower than that of the free thymine, it was once again inconsistent with respect to the dimer and tetramer. The APNA tetramer showed significant hypochromism compared to the dimer, indicative of a stacked structure. Duplex formation between the APNA tetramer **18** and a complementary DNA tetramer [$(dA)_4$] could not be detected at temperatures as low as 5 °C. However, duplex formation is not usually observed between two complementary tetramers, even with natural oligonucleotides; an octameric (dT)−(dA) complex would be the minimum length required in order to properly measure a T_m value.

In conclusion, the chiral APNA tetramer **18** constitutes a new class of synthetic nucleic acid analogs prepared

from the novel amino acid monomer (*S*)-**1** using classical peptide synthesis. It is expected that the APNA analogs will be stable to nuclease and protease degradation and the lipophilic character of their novel backbone may have a positive effects on the cell permeability of these compounds. It is also encouraging that these analogs are fairly soluble in aqueous media. A thorough evaluation of their influence on the hybridization stability of a duplex or a triplex requires the synthesis of longer APNA homopolymers, as well as DNA−APNA chemical chimeras; this studies are currently in progress in our laboratory.

Experimental Section

Instrumentation and General Methods. NMR spectra were obtained at 20−22 °C. ^{1}H and ^{13}C NMR chemical shifts are quoted in ppm and are referenced to the internal deuterated solvent unless otherwise indicated. Mass spectral data were obtained at McGill University, Biomedical Mass Spectrometry Unit, and the Department of Chemistry. All reactions were run under a nitrogen atmosphere using oven-dried syringes and glassware when appropriate. THF was distilled from Na/benzophenone, CH_2Cl_2 was distilled from P_2O_5, MeOH were distilled from Mg turnings, and DMF was distilled from CaO. Reagents and solvents were purchased from Aldrich Chemical Co. and VWR Scientific of Canada, respectively. The enzymes BSLDH and FDH were purchased from Genzyme (Cambridge, MA) and Boeringer Mannheim (Montreal, Quebec), respectively. Reversed phase flash column chromatography was carried out on silica gel reacted with *n*-octadecyltrichlorosilane, following previously reported procedures.[30]

Synthesis of α-Hydroxy Acid (*S*)-4. An aqueous suspension of compound **2** was titrated to pH 7 with 1 N NaOH and freeze-dried to obtain the sodium salt as a yellow powder. The salt (2.42 g, 10 mmol) was dissolved in TRIS·HCl buffer (5 mM, pH 6, 500 mL) containing sodium formate (2.3 equiv, 23 mmol, 1.56 g), NADH (0.02 equiv, 0.2 mmol, 150 mg), and dithiothrietol (0.005 equiv, 0.05 mmol, 7.8 mg), and the solution was degassed under vacuum for at least 30 min. Lyophilized powders of the two enzymes, BSLDH (600 units) and FDH (50 units), were added, and the mixture was gently stirred at rt under N_2 for 24 h with a periodic addition of acid (0.2 N HCl) to maintain the pH at 6.0−6.2. The solution was then acidified to pH 3 with 1 N HCl and extracted with EtOAc (4 × 300 mL). The organic layer was concentrated to give the desired compound as a light brown solid which was found to be fairly pure by ^{1}H NMR (98% yield): mp 123−125 °C. $[\alpha]_D$ +43.2 (*c* 0.10, MeOH), >98% ee. The enantiomers of **4** could not be separated by chiral HPLC; however, the enantiomeric purity was estimated from the % ee of compound **5**. TLC on normal silica gel (1:1 MeOH/EtOAc): R_f = 0.56; on C18 reversed phase silica gel (1:1 MeOH/H_2O), R_f = 0.32. ^{1}H NMR (270 MHz, acetone-d_6): δ 4.94 (dd, J = 2.0, 4.9 Hz, H2′), 6.53 (dd, J = 4.9, 15.8 Hz, H3′), 7.21 (dd, J = 2.0, 15.8 Hz, H4′), 7.53 (dt, J = 7.9, 1.5, H4″), 7.70−7.73 (tm, J = 8.0 Hz, H5″), 7.81 (dd, J = 1.5, 8.0 Hz, H6″), 7.93 (dd, J = 1.0, 8.0 Hz, H3″). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 72.24, 125.30, 127.25, 129.62, 129.65, 132.84, 133.36, 134.18, 149.61, 175.00.

Synthesis of Aniline Methyl Ester (*S*)-5. The α-hydroxy acid (*S*)-**4** was dissolved in methanol and reacted with excess diazomethane at rt until the evolution of gas had ceased. Pure methyl ester (*S*)-**5** was obtained after flash column chromatography in 90% yield: $[\alpha]_D$ +55 (*c* 0.40, $CHCl_3$). TLC (3:2 Hex/EtOAc): R_f = 0.23. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.85 (s, OCH_3), 4.89 (dd, J = 1.7, 5.2 Hz, H2′), 6.22 (dd, J = 5.2, 15.8 Hz, H3′), 7.29 (dd, J = 1.7, 15.8 Hz, H4″), 7.37−7.45 and 7.55−7.59 (2m, 3H, Ar), 7.93 (d, J = 7.9 Hz, H3″).

Hydrogenation of both the nitro group and the double bond was achieved by reacting the methyl ester (3.3 g) with H_2 gas (3 atm) in the presence of 10% Pd/C (0.33g) in EtOH for 15 h. The solution was filtered through Celite and concentrated to give a 92% yield of the desired product as a light yellow oil. The enantiomeric purity of the (*S*)-enantiomer was measured to be >98% ee from its ^{1}H NMR observed in the presence of 1 equiv of (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol; (*R*)-**5** having ∼50% ee was also synthesized and used for reference in the NMR analysis of the optical purity: $[\alpha]_D$ +28 (*c* 0.40, $CHCl_3$). TLC (1:2 Hex/EtOAc): R_f = 0.25. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.85−2.14 (2m, 2H3′), 2.55−2.75 (m, 2H4′), 3.72 (s, OCH_3), 3.78 (bs, OH), 4.21 (dd, J = 8.2, 3.7 Hz, H2′), 6.65−6.78 (m, 2H, Ar), 7.00−7.05 (m, 2H, Ar). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 25.99, 33.54, 52.34, 69.48, 115.87, 118.83, 125.34, 127.17, 129.70, 144.14, 175.30. MS (NH_3)CI *m*/*z* (assignment): 210 (base, MH^+), 209 (M^+), 178 (M − OCH_3^+), 177 (M − CH_3OH^+), 149 (177 − CO).

Synthesis of Fmoc-Protected (*S*)-6. Compound **5** (3.06 g, 14.66 mmol) was dissolved in a mixture of 10% aqueous Na_2CO_3 (17.6 mL, 1 equiv) and dioxane (10 mL) and cooled to 0 °C. Fluorenylmethyl chloroformate (3.79 g, 1 equiv, 14.66 mmol) was dissolved in dioxane (20 mL) and added dropwise *via* a syringe, giving a white milky reaction mixture. The mixture was stirred at 0 °C for 1.5 h and allowed to warm up to rt for an additional hour. The reaction was then quenched with H_2O (30 mL), acidified to pH 3, and extracted with EtOAc (4 × 30 mL); the combined organic layers were washed with saturated NaCl (120 mL) and dried over anhydrous $MgSO_4$. Flash column chromatography using 3:2 Hex/EtOAc as the solvent system led to the isolation of the desired product **6** as a white solid in 84% yield. TLC (1:1 Hex/EtOAc): R_f = 0.35. Mp: 119−120 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.89−2.15 (m, 2H3′), 2.65−2.89 (m, 2H4′), 3.09 (bs, OH), 3.70 (s, OCH_3), 4.06 (dd, J = 8.6, 3.7 Hz, H2′), 4.27, (t, J = 6.9 Hz, $NHCOOCH_2CH$), 4.48 (d, J = 6.9 Hz, $NHCOOCH_2CH$)), 7.04−7.78 (12H, Ar and Fmoc-Ar). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 25.87, 34.26, 47.09, 52.57, 66.85, 68.75, 119.88, 124.51, 125.05, 127.00, 127.14, 127.63, 129.82, 135.88, 141.24, 143.81, 154.27, 175.04.

Synthesis of Methoxymethyl Ether (*S*)-7. Compound (*S*)-**6** (1.1 g, 2.3 mmol) was dissolved in freshly distilled THF (20 mL) at 0 °C under N_2. Chloromethyl methyl ether (4.0 mL, 20 equiv, 53.2 mmol) was added, followed by the dropwise addition of dry diisopropylethylamine (1.4 mL, 3 equiv, 0.8 mmol). The reaction mixture was allowed to warm up to rt and to stir for 18 h. The reaction was quenched with the addition of water (50 mL), followed by extraction with EtOAc (3 × 50 mL); the organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a light amber oil. Purification by flash column chromatography using 2:1 Hex/EtOAc as the solvent system gave product **7** as a light yellow oil in 90% yield: $[\alpha]_D$ −19.6 (*c* 0.30, $CHCl_3$). TLC (2:1 Hex/EtOAc): R_f = 0.24. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06−2.13 (m, 2H3′), 2.73 (t, J = 7.2 Hz, 2H4′), 3.35 (s, CH_2OCH_3), 3.68 (s, $COOCH_3$), 4.19 (t, J = 6 Hz, H2′), 4.29 (t, J = 6.9 Hz, Fmoc-$CHCH_2$), 4.48−4.60 (m, Fmoc-$CHCH_2$), 4.64 (d, J = 6.9 Hz, A part of AB, 1H, OCH_2O), 4.71 (d, J = 6.9 Hz, B part of AB, 1H, OCH_2O), 6.90−7.64 (13H, Ar, Fmoc-Ar, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.37, 33.13, 47.41, 52.24, 56.30, 66.99, 74.40, 96.34, 120.15, 122.87, 124.72, 125.25, 127.26, 127.39, 127.90, 129.76, 131.70, 135.79, 141.49, 143.97, 144,03, 154.38, 172.67. EIMS *m*/*z* (assignment): 476 $(MH)^+$, 475 (M^+), 297 [(M − Fmoc + $COOH)^+$], 279 [(297 − $H_2O)^+$].

Synthesis of (Methylthio)ethyl Ether Intermediate (*S*)-8. Compound (*S*)-**7** (1.52 g, 3.2 mmol) was dissolved in freshly distilled CH_2Cl_2 (40 mL) and cooled to −78 °C under N_2. A solution of dimethylboron bromide in CH_2Cl_2 (1.5 M, 7.6 mL, 11.2 mmol) was added along with diisopropylethylamine (0.04 mL, 0.1 equiv, 0.32 mmol). After 20 min, more diisopropylethylamine (1.2 mL, 6.4 mmol) was added followed by ethanethiol (0.8 mL, 9.6 mmol), and the resulting mixture was stirred for 2 h at −78 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL), and the mixture was allowed to warm up to rt. The aqueous layer was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with saturated NaCl (150 mL). The organic layer was then dried with anhydrous $MgSO_4$ and

(30) (a) Tsantrizos, Y. S.; Ogilvie, K. K.; Watson, A. K. *Can. J. Chem.* **1992**, *70*, 2276. (b) Blunt, J. W.; Calder, V. L.; Fenwick, G. D.; Lake, R. J.; McCombs, J. D.; Munro, M. H. G.; Perry, N. B. *J. Nat. Prod.* **1987**, *50*, 290.

concentrated to dryness. Pure compound (*S*)-**8** was isolated as a light yellow oil (450 mg) after flash column chromatography using 4:1 Hex/EtOAc as the solvent system. The average yield of this reaction was 55−60%, based on the amount of recovered alcohol **6**: $[\alpha]_D$ −54 (*c* 1.80, $CHCl_3$). TLC (4:1 Hex/EtOAc): $R_f = 0.19$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, $J = 7.5$ Hz, SCH_2CH_3), 2.07−2.14 (m, 2H3′), 2.60 (q, $J = 7.4$ Hz, SCH_2CH_3), 2.73 (t, $J = 6.6$ Hz, 2H4′), 3.67 (s, OCH_3), 4.31 (t, OCH_2CH-FMoc), 4.40 (t, $J = 5.5$ Hz, H2′), 4.45−4.61 (m, OCH_2CH-Fmoc), 4.67 (d, $J = 11$ Hz, A part of AB, 1H, OCH_2S), 4.87 (d, $J = 11$ Hz, B part of AB, 1H, OCH_2S), 7.06−7.81 (13H, ArH, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.01, 25.29, 26.55, 32.99, 47.48, 52.37, 67.19, 73.04, 73.90, 120.26, 124.78, 125.36, 127.36, 127.51, 128.00, 129.85, 135.87, 141.58, 144.04, 144.12, 154.43, 172.64. FAB MS *m/z* (assignment): 506 (MH^+), 505 (M^+), 444 [$(M - SEt)^+$].

Synthesis of Thymine Analog (*S*)-9. Methoxymethyl ethyl thioether (*S*)-**8** (218 mg, 0.43 mmol) was dissolved in dry THF (1 mL) in the presence of activated molecular sieves (3 Å). A solution of bis(trimethylsilyl)thymine (1.4 mL, 1.5 M in dry THF) was added, followed by the addition of I_2 (109 mg, 0.43 mmol), and the reaction mixture was stirred at rt under N_2 for 48 h. The mixture was then poured into a 5% aqueous sodium sulfite solution (10 mL) and extracted with EtOAc (3 × 15 mL), and the organic layer was washed with H_2O (45 mL) and saturated NaCl (45 mL). The organic layer was then dried with anhydrous $MgSO_4$ and concentrated. Purification by flash column chromatography, using 1:2 Hex/EtOAc as the eluting solvent, led to the isolation of the desired product in 58% yield (98% based on recovery of starting material). TLC (1:2 Hex/EtOAc): $R_f = 0.25$. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.84 (s, CH_3), 2.00−2.18 (m, 2H3′), 2.61−2.74 (m, 2H4′), 3.65 (s, OCH_3), 4.20 (dd, $J = 5.2$ Hz, H2′), 4.29 (t, $J = 9$ Hz, -$CHCH_2$- of Fmoc), 4.58−4.68 (m, -$CHCH_2$- of Fmoc), 4.90 (d, $J = 12$ Hz, A part of AB, 1H, OCH_2Th), 5.13 (d, $J = 12$ Hz, B part of AB, 1H, OCH_2Th), 6.9−8.3 (14H, Ar, NH, H6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.46, 26.31, 32.81, 47.50, 52.55, 66.83, 76.38, 77.42, 111.87, 120.26, 125.21, 125.23, 127.31, 127.39, 127.57, 128.03, 129.81, 135.66, 139.58, 141.56, 143.89, 144.03. MALDI MS *m/z* (assignment): 592 [$(M + Na)^+$].

Preparation of Aniline Analog (*S*)-10. Compound (*S*)-**9** (460 mg, 0.81mmol) was treated with 5% piperidine in DMF (13 mL) at rt for 20 min. The DMF was then removed by evaporation, and the crude mixture was partitioned between H_2O (10 mL) and EtOAc (20 mL). The aqueous layer was extracted with more EtOAc (3 × 20 mL), the combined organic layers were dried with anhydrous $MgSO_4$ and concentrated to give a light yellow oil. Purification by flash column chromatography using 5:1 EtOAc/Hex afforded compound **10** in 98% yield: $[\alpha]_D$ −37 (*c* 0.2, MeOH). TLC (5:1 Hex/EtOAc): $R_f = 0.20$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.87 (d, $J = 1.2$ Hz, Th-CH_3), 1.83−2.13 (m, 2H3′), 2.59 (t, $J = 7.5$ Hz, 2H4′), 3.68 (s, OCH_3), 4.17 (dd, $J = 3.6$, 8.4 Hz, H2′), 5.14 (d, A part of AB, $J = 11.1$ Hz, OCH_2Th), 5.29 (d, B part of AB, $J = 11.1$ Hz, 1H, OCH_2Th), 6.59 (dt, $J = 7.5$, 1.2 1H, Ar), 6.68 (dd, $J = 8.1$, 1.2 Hz, 1H, Ar), 6.89 (dd, $J = 7.2$, 1.2 Hz, 1H, Ar), 6.94 (dt, $J = 8.1$, 1.2 Hz, 1H, Ar), 7.48 (q, $J = 1.2$ H6). ^{13}C NMR (75 MHz, CD_3OD): δ 12.36, 27.55, 32.96, 52.73, 77.80, 77.86, 112.05, 117.29, 119.51, 126.27, 128.36, 130.63, 142.31, 146.40, 153.38, 166.85, 174.48. MS EI *m/z* (assignment): 347 (M^+), 348 (MH^+).

Preparation of Boc-Protected Analog (*S*)-11. Compound (*S*)-**10** (52 mg, 0.15 mmol) was dissolved in 10% triethylamine in MeOH (1 mL) and mixed with di-*tert*-butyl dicarbonate (66 mg, 0.30 mmol). The mixture was stirred at rt for 17 h. The solvent was then evaporated, and the residue was partitioned between H_2O (10 mL) and EtOAc (20 mL). The aqueous layer was extracted with more EtOAc (3 × 20 mL), and the combined organic layers were dried with anhydrous $MgSO_4$ and concentrated to give the Boc-protected analog (**11**) as a light yellow foam with a 92% yield and 98−99% ee. The enantiomeric purity of **11** was determined by HPLC using a Chiralcel OD column with 10% ethanol in hexane as the eluting solvent and a flow rate of 1 mL/min. Under these conditions, base line separation of the two enantiomers was observed with a reference sample containing both enantiomers;[14] the retention times for the *S*- and *R*-enantiomer are 23.8 and 27.7 min, respectively: $[\alpha]_D$ −24 (*c* 0.40, MeOH). TLC (3:1 EtOAc/Hex): $R_f = 0.30$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.58 (9H, tBoc), 1.95 (Th-CH_3), 1.94−2.16 (m, 2H3′), 2.67−2.74 (m, 2H4′), 3.75 (s, OCH_3), 4.30 (dd, $J = 6$ Hz, H2′), 5.18 (d, A part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 5.39 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 7.04−7.27 (m, 5H, ArH, H6), 9.35 (s, NHBoc). ^{13}C NMR (75 MHz, CD_3OD): δ 12.51, 26.44, 28.59, 32.66, 52.52, 76.44, 77.44, 80.68, 112.02, 122.97, 124.41, 127.38, 129.59, 131.09, 136.14, 139.52, 151.52, 153.69, 164.20, 172.12.

Preparation of the Free Acid Analog (*S*)-12. To a solution of compound **11** (105 mg, 0.234 mmol) in 3:1 THF/MeOH (5 mL) was added aqueous LiOH (250 μL, 1.4 M), and the reaction mixture was stirred at rt for 3 h. The mixture was subsequently evaporated to dryness, and the resulting residue was dissolved in H_2O (10 mL) at pH 3 and extracted with EtOAc (3 × 20 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated to give a fairly pure sample of the free acid analog (*S*)-**12** in 94% yield: $[\alpha]_D$ −15 (*c* 0.37, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): $R_f = 0.53$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.50 (s, $C(CH_3)_3$), 1.88 (d, $J = 1.2$ Hz, Th-CH_3), 1.86−2.14 (m, 2H3′), 2.62−2.81 (m, 2H4′), 4.10−4.15 (m, H2′), 5.14 (d, A part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 5.39 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 7.05−7.32 (m, 4H, ArH), 7.48 (d, $J = 1.2$ Hz, H6), 8.21 (s, NH). ^{13}C NMR (75 MHz, CD_3OD): δ 12.40, 28.28, 28.87, 34.11, 77.69, 77.86, 81.11, 111.93, 126.94, 127.49, 127.96, 130.91, 136.92, 137.30, 142.33, 153.29, 156.83, 166.84, 175.60.

Preparation of the Fully Deprotected Monomer (*S*)-1. Boc-protected monomer **12** (∼10 mg) was dissolved in an anhydrous solution of 15% trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at rt under N_2 for 15 min. The fully deprotected monomer **1** was obtained in high purity after evaporation of the reaction mixture to dryness. C_{18}-TLC (3:1 MeOH/H_2O): $R_f = 0.77$. ^{1}H NMR (300 MHz, D_2O): δ 1.80 (d, $J = 1.2$ Hz, Th-CH_3), 1.77−1.93 and 2.05−2.15 (2m, 2H3′), 2.57 (t, $J = 7$ Hz, 2H4′), 3.85−3.89 (dd, $J = 9.3$, 3.6, H2′), 5.01 (d, A part of AB, $J = 11$ Hz, 1H, OCH_2Th), 5.22 (d, B part of AB, $J = 11$ Hz, 1H, OCH_2Th), 6.72−7.10 (m, 4H, ArH), 7.34 (d, $J = 1.2$ Hz, H6). HRFAB MS *m/z*: 320.12457 $(M + H)^+$, calcd mass for $C_{15}H_{17}N_3O_5 + H^+ = 320.124646$.

Synthesis of APNA Dimer (*S,S*)-13. Free acid monomer **12** (96 mg in 2 mL of dry DMF, 0.22 mmol) was added to a solution of the HATU coupling reagent (101 mg in dry DMF, 0.27 mmol) at 0 °C under N_2. Diisopropylethylamine (80 μL, 0.44 mmol) was added, and the reaction was allowed to stir for 10 min. The free aniline monomer **10** (100 mg in 1 mL of dry DMF, 0.29 mmol) was then added via a syringe, and the resulting solution was stirred at rt for 24 h. The reaction was quenched by dilution with H_2O (10 mL), and the product was extracted with EtOAc (3 × 20 mL). Purification *via* C_{18} reversed phase chromatography using a solvent gradient from 100% H_2O to 100% MeOH led to the isolation of the desired product in 70% yield as a pale yellow solid (eluted from column in ∼55% aqueous MeOH): $[\alpha]_D$ −24 (*c* 0.40, MeOH). TLC on C_{18}-silica (1:1 MeOH/H_2O): $R_f = 0.35$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.47 (s, $C(CH_3)_3$), 1.84 (d, $J = 1.5$ Hz, Th-CH_3), 1.88 (d, $J = 1.5$ Hz, Th-CH_3), 1.88−2.18 (2m, 4H3′), 2.6−2.8 (2m, 4H4′), 3.60 (s, OCH_3), 4.16−4.28 (m, 2H2′), 5.08 (d, A part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 5.17 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH_2Th), 5.27 (d, A part of AB, $J = 10.2$ Hz, 1H, OCH_2Th), 5.35 (d, B part of AB, $J = 10.2$ Hz, 1H, OCH_2-Th), 7.0−7.35 (8H, m, ArH), 7.38 (q, $J = 1.5$ Hz, H6), 7.57 (q, $J = 1.5$ Hz, H6), 7.9 (s, NH). ^{13}C NMR (300 MHz, CD_3OD): δ 12.37, 12.24, 28.17, 28.26, 28.90, 34.38, 34.81, 52.81, 78.04, 78.18, 78.30, 79.71, 81.18, 111.95, 112.17, 127.06, 127.54, 127.93, 128.03, 128.14, 128.18, 131.03, 131.08, 142.31, 153.32, 156.89, 166.78, 173.52, 174.16, MALDI MS *m/z* (assignment): 786 [$(M + Na)^+$].

Preparation of Free Aniline Dimer (*S,S*)-14. Boc methyl ester dimer **13** (53 mg, 0.07 mmol) was dissolved in an anhydrous solution of 15% trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at rt under N_2 for 15 min. The reaction mixture was subsequently evaporated to dryness and partitioned by chromatography on a dianion HP20 column using a solvent

gradient from 100% H_2O to 100% MeOH. The aniline dimer **14** eluted from the column with 50−60% aqueous MeOH; 40% yield of pure **14** was isolated. However, some unreacted starting material was detected by TLC: $[\alpha]_D$ −43 (*c* 0.17, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): $R_f = 0.30$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.82 (d, $J = 1.2$ Hz, Th-CH_3), 1.87 (d, $J = 1.2$ Hz, Th-CH_3), 1.9−2.21 (m, 4H3′), 2.56−2.80 (m, 4H4′), 3.58 (s, OCH_3), 4.20 (dd, $J = 3.9$, 8.1 Hz, H2′), 4.24 (dd, $J = 3.9$, 8.1 Hz, H2′), 5.07 (d, A part of AB, $J = 10.8$ Hz, 1H, OCH_2Th), 5.17 (d, B part of AB, $J = 10.8$ Hz, 1H, OCH_2Th), 5.27 (d, A part of AB, $J = 10.2$ Hz, 1H, OCH_2Th), 5.34 (d, B part of AB, $J = 10.2$ Hz, 1H, OCH_2Th), 6.62 (dt, $J = 7.5$, 1.2 Hz, 1H, Ar), 6.70 (dd, $J = 7.2$, 1.2 Hz, 1H, Ar), 6.91−7.00 (m, 2H, Ar), 7.17−7.23 (m, 3H, Ar), 7.29−7.30 (m, 1H, Ar), 7.32 (q, $J = 1.2$ Hz, H6), 7.50 (q, $J = 1.2$ Hz, H6). ^{13}C NMR (75 MHz, CD_3OD): δ 12.38, 12.42, 27.92, 28.34, 33.77, 34.44, 52.81,78.04, 78.27, 78.38, 79.80, 111.96, 112.19, 117.29, 119.60, 126.67, 128.05, 128.17, 128.26, 128.42, 130.75, 131.10, 136.01, 137.67, 142.31, 146.49, 153.32, 153.35, 166.81, 173.78, 174.15. FAB MS *m*/*z* (assignment): 663 (M^+).

Preparation of Free Acid Dimer (*S,S*)-15. Hydrolysis of methyl ester dimer **13** to the corresponding free acid **15** (∼99% yield) was carried out using the same reaction conditions as in the preparation of the free acid analog (*S*)-**12**. **15**: $[\alpha]_D$ −25 (*c* 0.25, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): $R_f = 0.38$. ^{1}H NMR (300 MHz, CD_3OD): δ 1.47 (s, $C(CH_3)_3$), 1.84 (bs, Th-CH_3), 1.88 (bs, Th-CH_3), 2.00−2.15 (m, 4H3′), 2.60−2.85 (m, 4H4′), 4.16−4.25 (m, 2H2′), 5.08 (d, A part of AB, $J = 11.1$ Hz, 1H, OCH_2Th), 5.20 (d, B part of AB, $J = 11.1$ Hz, 1H, OCH_2Th), 5.26 (d, A part of AB, $J = 10.2$ Hz, 1H, OCH_2Th), 5.33 (d, B part of AB, $J = 10.2$ Hz, 1H, OCH_2-Th), 7.05−7.35 (8H, m, Ar), 7.38 (bs, H6). 7.55 (bs, H6), 8.25 (s, NH). ^{13}C NMR (75 MHz, CD_3OD): δ 12.42, 12.45, 24.37, 28.10, 28.49, 28.90, 30.83, 34.54, 34.74, 77.92, 78.17, 79.66, 81.18, 111.86, 112.16, 127.06, 127.52, 127.85, 128.00, 128.08, 128.17, 131.00, 131.20, 136.00, 137.09, 137.33, 137.70, 142.37, 153.29, 156.90, 166.70, 173.54, 175.73, 210.21. FAB MS *m*/*z* (assignment): 749 (M^+).

Preparation of Fully Deprotected Dimer (*S,S*)-16. Boc-protected dimer **15** (∼15 mg) was dissolved in an anhydrous solution of 15% trifluoroacetic acid in CH_2Cl_2 (2 mL) and stirred at rt under N_2 for 15 min. The fully deprotected dimer **16** was obtained after evaporation of the reaction mixture to dryness. C_{18}-TLC (2:1 MeOH/H_2O): $R_f = 0.71$. ^{1}H NMR (300 MHz, D_2O): δ 1.83 (bs, Th-CH_3), 1.87 (bs, Th-CH_3), 1.70−2.33 (4m, 4H3′), 2.63−2.71 and 2.84−2.91 (2m, 4H4′), 4.00−4.04 and 4.23−4.27 (2m, 2H2′), 5.08 (s, 2H OCH_2Th), 5.26 (d, A part of AB, $J = 11.1$ Hz, 1H, OCH_2Th), 5.32 (d, B part of AB, $J = 11.1$ Hz, 1H, OCH_2Th), 7.05−7.43 (8H, m, Ar), 7.41 (bs, H6). 7.54 (bs, H6). HRFAB MS *m*/*z*: 1355.3408 $(M + Cs)^+$, calcd mass for $C_{60}H_{62}N_{19}O_{17} + Cs^+ = 1355.341022$]. HRFAB MS *m*/*z*: 621.23111 $(M + H)^+$, calcd mass for $C_{30}H_{32}N_6O_9 + H^+ = 621.230902$.

Sythesis of APNA Tetramers (*S,S,S,S*)-17 and -18. The peptide coupling reaction between dimers **14** and **15** was achieved using the same reaction procedure as in the coupling of their respective monomers. The crude product was purified by C_{18} reversed phase flash column chromatography using a gradient from 100% H_2O to 100% MeOH. The desired APNA tetramer **17** eluted with 70−80% aqueous MeOH: $[\alpha]_D$ −31 (*c* 0.08, MeOH). TLC on C_{18}-silica (2:1 MeOH/H_2O): $R_f = 0.13$. NMR data in Table 1. ES MS *m*/*z* (assignment): 1392.6 (M^+), 1393.6 (MH^+). Hydrolysis of the methyl ester followed by removal of the Boc group (using standard conditions in both cases) led to the isolation of tetramer **18** which was purified by preparative C_{18}-TLC (3:1 MeOH/H_2O): $R_f = 0.50$. ^{1}H NMR (500 MHz, D_2O) showed resonances in the expected chemical shifts regions (based on the ^{1}H NMR spectra of compound **17**). However, due to the great extend of overlapping resonances, the exact chemical shifts of all resonances were not assigned; only those resonances associated with the methyls and H6 protons of the thymine moieties were assigned from the 1D COSY NMR spectra. HRFAB MS *m*/*z*: 1355.3408 $(M + Cs)^+$, calcd mass for $C_{60}H_{62}N_{19}O_{17} + Cs^+ = 1355.341022$.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds (24 pages). This material is contained in libraries on microfiche, immediately following this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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