

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 Oligomers¹

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Received December 11, 1996[®]

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 ¹H 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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[®] Abstract published in *Advance ACS Abstracts*, July 1, 1997.

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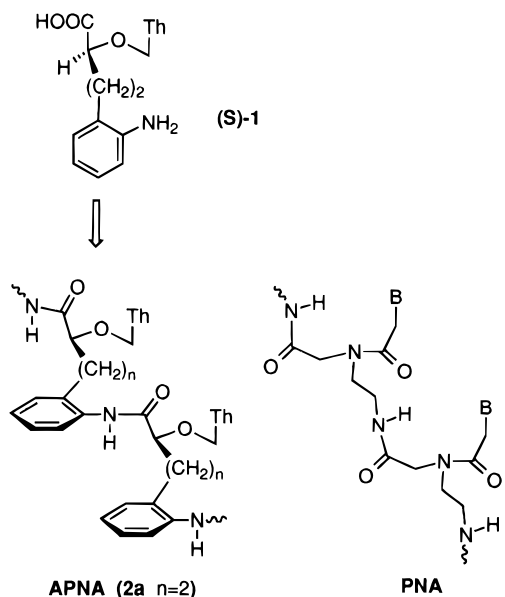
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Scheme 1. Structural Comparison of APNA and PNA Analogs



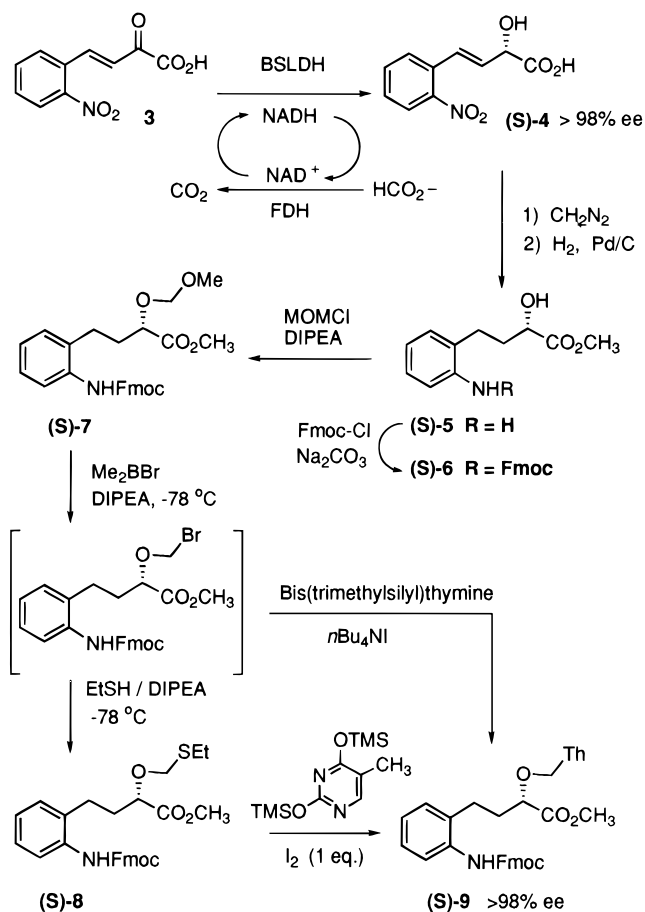
potentially form stabilizing intramolecular interactions with the center of the adjacent benzene ring (dipole/cation π interactions¹² or hydrogen bonds¹³) thus contributing to the precise conformation and stability of the APNA macromolecules in a hybridized duplex or triplex, analogous to that proposed 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 ¹H 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 dehydrogenase¹⁶ gave the (*S*)-

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



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

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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(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 measurements of optical purity.

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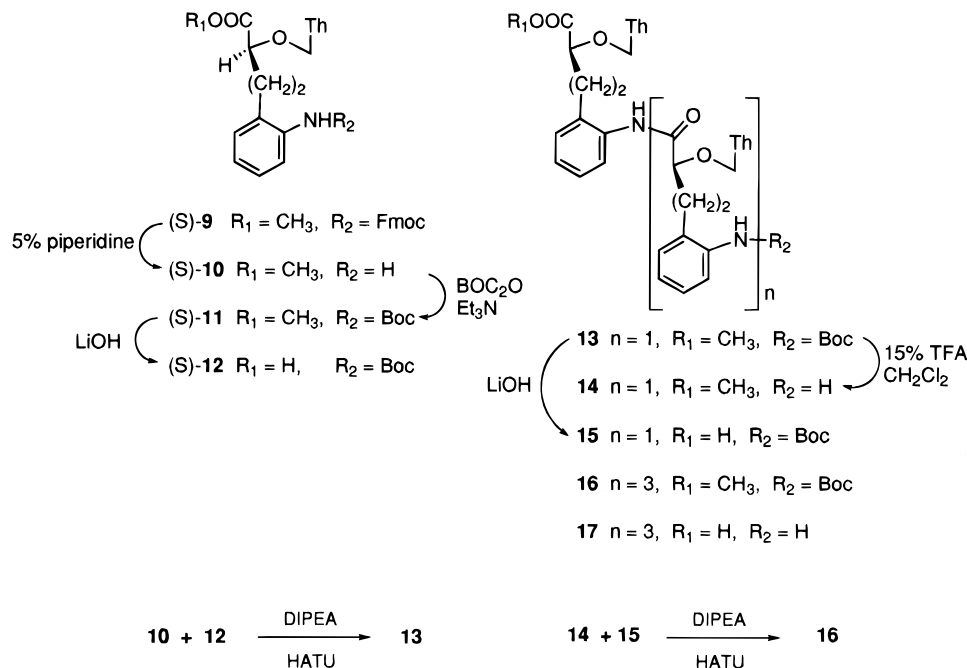
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Scheme 3. Synthesis of the Thymidine APNA Oligomers



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*-Bu₄NI, 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)thymine²⁴ 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

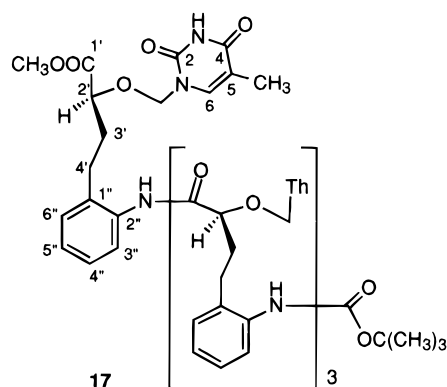
The Fmoc protecting group was partly unstable to conditions required for ester hydrolysis,²⁶ thus it was replaced with the base stable Boc group using standard literature procedures (Scheme 3). Peptide bond formation between the aniline monomer (*S*)-**10** and the Boc-protected free acid (*S*)-**12** 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 ¹³C 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 ¹³C (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 D₂O 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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 (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-D₂O (1:1) at rt.
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 (28) The diastereomeric mixture of **13** was prepared from racemic monomers **10** and **12**, and examined by ¹H NMR (300 MHz, CDCl₃). 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), -CO₂CH₃ (δ 3.64 and 3.69), Th-CH₃ (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, CDCl₃) showed only one set of signals [δ 8.53 (NHCO), 3.69 (CO₂CH₃), 1.91 (Th-CH₃), 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. ¹H (500 MHz) and ¹³C (150 MHz) NMR Data of APNA Tetramer **17** in CDCl₃

assignment	¹³ C (δ)	¹ H (δ)	int, mult, J (Hz)	assignment	¹³ C (δ)	¹ H (δ)	int, mult, J (Hz)
1' (CO ₂ CH ₃)	172.51			4	164.04		
(CO ₂ CH ₃)	52.90	3.64	3H, s		164.22		
2'	77.94	4.17–4.23 ^b	4H, m		164.43		
	78.22			5	164.73		
	78.76				112.03		
3'	79.88	1.90–2.24 ^b	8H, m		112.17 ^c		
	32.46				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-CH 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-NH-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-NH-Boc		8.71	1H, bs		124.50	7.74	1H, d, J = 8.1
Ph-NH-CO	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-CO ₂ C(CH ₃) ₃	151.94				127.32		
Ph-NH-CO ₂ C(CH ₃) ₃	80.76				127.33 ^c		
Ph-NH-CO ₂ C(CH 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 ¹³C resonances, these chemical shifts assignments were based on the HMQC and HMBC NMR data. ^b Due to overlapping resonances in the ¹H 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 ¹³C resonances.

Table 2. ¹H Chemical Shifts of Monomer **1**, Dimer **16**, and Tetramer **18** in D₂O^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 ¹H NMR spectrum, the exact chemical shifts were obtained from the 1D COSY spectra.

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

Table 3. UV Absorbance at 264 nm in CH₃OH

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

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. ¹H and ¹³C 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, CH₂Cl₂ was distilled from P₂O₅, 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 dithiothriitol (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 N₂ 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 ¹H NMR (98% yield): mp 123–125 °C. [α]_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/H₂O), *R*_f = 0.32. ¹H 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, H^{4'}), 7.70–7.73 (tm, *J* = 8.0 Hz, H^{5''}), 7.81 (dd, *J* = 1.5, 8.0 Hz, H^{6''}), 7.93 (dd, *J* = 1.0, 8.0 Hz, H^{3''}). ¹³C NMR (67.5 MHz, CDCl₃): 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: [α]_D +55 (*c* 0.40, CHCl₃). TLC (3:2 Hex/EtOAc): *R*_f = 0.23. ¹H NMR (270 MHz, CDCl₃): δ 3.85 (s, OCH₃), 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, H^{4'}), 7.37–7.45 and 7.55–7.59 (2m, 3H, Ar), 7.93 (d, *J* = 7.9 Hz, H^{3''}).

Hydrogenation of both the nitro group and the double bond was achieved by reacting the methyl ester (3.3 g) with H₂ 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 ¹H 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: [α]_D +28 (*c* 0.40, CHCl₃). TLC (1:2 Hex/EtOAc): *R*_f = 0.25. ¹H NMR (270 MHz, CDCl₃): δ 1.85–2.14 (2m, 2H³), 2.55–2.75 (m, 2H⁴), 3.72 (s, OCH₃), 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). ¹³C NMR (67.5 MHz, CDCl₃): δ 25.99, 33.54, 52.34, 69.48, 115.87, 118.83, 125.34, 127.17, 129.70, 144.14, 175.30. MS (NH₃)CI *m/z* (assignment): 210 (base, MH⁺), 209 (M⁺), 178 (M – OCH₃⁺), 177 (M – CH₃OH⁺), 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 Na₂CO₃ (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 H₂O (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 MgSO₄. 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. ¹H NMR (270 MHz, CDCl₃): δ 1.89–2.15 (m, 2H³), 2.65–2.89 (m, 2H⁴), 3.09 (bs, OH), 3.70 (s, OCH₃), 4.06 (dd, *J* = 8.6, 3.7 Hz, H²), 4.27, (t, *J* = 6.9 Hz, NHCOOCH₂CH), 4.48 (d, *J* = 6.9 Hz, NHCOOCH₂CH), 7.04–7.78 (12H, Ar and Fmoc-Ar). ¹³C NMR (67.5 MHz, CDCl₃): 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 N₂. 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 MgSO₄ 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: [α]_D –19.6 (*c* 0.30, CHCl₃). TLC (2:1 Hex/EtOAc): *R*_f = 0.24. ¹H NMR (300 MHz, CDCl₃): δ 2.06–2.13 (m, 2H³), 2.73 (t, *J* = 7.2 Hz, 2H⁴), 3.35 (s, CH₂OCH₃), 3.68 (s, COOCH₃), 4.19 (t, *J* = 6 Hz, H²), 4.29 (t, *J* = 6.9 Hz, Fmoc-CHCH₂), 4.48–4.60 (m, Fmoc-CHCH₂), 4.64 (d, *J* = 6.9 Hz, A part of AB, 1H, OCH₂O), 4.71 (d, *J* = 6.9 Hz, B part of AB, 1H, OCH₂O), 6.90–7.64 (13H, Ar, Fmoc-Ar, NH). ¹³C NMR (75 MHz, CDCl₃): 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 – H₂O)⁺].

Synthesis of (Methylthio)ethyl Ether Intermediate (S)-8. Compound (S)-7 (1.52 g, 3.2 mmol) was dissolved in freshly distilled CH₂Cl₂ (40 mL) and cooled to –78 °C under N₂. A solution of dimethylboron bromide in CH₂Cl₂ (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 NaHCO₃ (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 MgSO₄ and

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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, CHCl₃). TLC (4:1 Hex/EtOAc): $R_f = 0.19$. ¹H NMR (300 MHz, CDCl₃): δ 1.24 (t, $J = 7.5$ Hz, SCH₂CH₃), 2.07–2.14 (m, 2H₃'), 2.60 (q, $J = 7.4$ Hz, SCH₂CH₃), 2.73 (t, $J = 6.6$ Hz, 2H₄'), 3.67 (s, OCH₃), 4.31 (t, OCH₂CH-Fmoc), 4.40 (t, $J = 5.5$ Hz, H₂'), 4.45–4.61 (m, OCH₂CH-Fmoc), 4.67 (d, $J = 11$ Hz, A part of AB, 1H, OCH₂S), 4.87 (d, $J = 11$ Hz, B part of AB, 1H, OCH₂S), 7.06–7.81 (13H, ArH, NH). ¹³C NMR (75 MHz, CDCl₃): 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 – SE)⁺].

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 I₂ (109 mg, 0.43 mmol), and the reaction mixture was stirred at rt under N₂ 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 H₂O (45 mL) and saturated NaCl (45 mL). The organic layer was then dried with anhydrous MgSO₄ 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): $R_f = 0.25$. ¹H NMR (270 MHz, CDCl₃): δ 1.84 (s, CH₃), 2.00–2.18 (m, 2H₃'), 2.61–2.74 (m, 2H₄'), 3.65 (s, OCH₃), 4.20 (dd, $J = 5.2$ Hz, H₂'), 4.29 (t, $J = 9$ Hz, -CHCH₂- of Fmoc), 4.58–4.68 (m, -CHCH₂- of Fmoc), 4.90 (d, $J = 12$ Hz, A part of AB, 1H, OCH₂Th), 5.13 (d, $J = 12$ Hz, B part of AB, 1H, OCH₂Th), 6.9–8.3 (14H, Ar, NH, H₆). ¹³C NMR (75 MHz, CDCl₃): 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, 0.81 mmol) 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 H₂O (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 MgSO₄ 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): $R_f = 0.20$. ¹H NMR (300 MHz, CD₃OD): δ 1.87 (d, $J = 1.2$ Hz, Th-CH₃), 1.83–2.13 (m, 2H₃'), 2.59 (t, $J = 7.5$ Hz, 2H₄'), 3.68 (s, OCH₃), 4.17 (dd, $J = 3.6, 8.4$ Hz, H₂'), 5.14 (d, A part of AB, $J = 11.1$ Hz, OCH₂Th), 5.29 (d, B part of AB, $J = 11.1$ Hz, 1H, OCH₂Th), 6.59 (dt, $J = 7.5, 1.2$ Hz, 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, $J = 1.2$ Hz, H₆). ¹³C NMR (75 MHz, CD₃OD): 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 H₂O (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 MgSO₄ 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*-enantiomers are 23.8 and 27.7 min, respectively: $[\alpha]_D -34$ (*c* 0.40, MeOH). TLC (3:1 EtOAc/Hex): $R_f = 0.30$. ¹H NMR (300 MHz, CD₃OD): δ 1.58 (9H, tBoc), 1.95 (Th-CH₃), 1.94–2.16 (m, 2H₃'), 2.67–2.74 (m, 2H₄'), 3.75 (s, OCH₃), 4.30 (dd, $J = 6$ Hz, H₂'), 5.18 (d, A part of AB, $J = 10.5$ Hz, 1H, OCH₂Th), 5.39 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH₂Th), 7.04–7.27 (m, 5H, ArH, H₆), 9.35 (s, NHBoc). ¹³C NMR (75 MHz, CD₃OD): 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 μ L, 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 H₂O (10 mL) at pH 3 and extracted with EtOAc (3 × 20 mL). The organic layer was dried with anhydrous MgSO₄ 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 C₁₈-silica (2:1 MeOH/H₂O): $R_f = 0.53$. ¹H NMR (300 MHz, CD₃OD): δ 1.50 (s, C(CH₃)₃), 1.88 (d, $J = 1.2$ Hz, Th-CH₃), 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, OCH₂Th), 5.39 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH₂Th), 7.05–7.32 (m, 4H, ArH), 7.48 (d, $J = 1.2$ Hz, H₆), 8.21 (s, NH). ¹³C NMR (75 MHz, CD₃OD): 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 CH₂Cl₂ (2 mL) and stirred at rt under N₂ for 15 min. The fully deprotected monomer **1** was obtained in high purity after evaporation of the reaction mixture to dryness. C₁₈-TLC (3:1 MeOH/H₂O): $R_f = 0.77$. ¹H NMR (300 MHz, D₂O): δ 1.80 (d, $J = 1.2$ Hz, Th-CH₃), 1.77–1.93 and 2.05–2.15 (2m, 2H₃'), 2.57 (t, $J = 7$ Hz, 2H₄'), 3.85–3.89 (dd, $J = 9.3, 3.6, \text{H}_2$ '), 5.01 (d, A part of AB, $J = 11$ Hz, 1H, OCH₂Th), 5.22 (d, B part of AB, $J = 11$ Hz, 1H, OCH₂Th), 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 C₁₅H₁₇N₃O₅ + 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 N₂. Diisopropylethylamine (80 μ L, 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 H₂O (10 mL), and the product was extracted with EtOAc (3 × 20 mL). Purification *via* C₁₈ reversed phase chromatography using a solvent gradient from 100% H₂O 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 C₁₈-silica (1:1 MeOH/H₂O): $R_f = 0.35$. ¹H NMR (300 MHz, CD₃OD): δ 1.47 (s, C(CH₃)₃), 1.84 (d, $J = 1.5$ Hz, Th-CH₃), 1.88 (d, $J = 1.5$ Hz, Th-CH₃), 1.88–2.18 (2m, 4H₃'), 2.6–2.8 (2m, 4H₄'), 3.60 (s, OCH₃), 4.16–4.28 (m, 2H₂'), 5.08 (d, A part of AB, $J = 10.5$ Hz, 1H, OCH₂Th), 5.17 (d, B part of AB, $J = 10.5$ Hz, 1H, OCH₂Th), 5.27 (d, A part of AB, $J = 10.2$ Hz, 1H, OCH₂Th), 5.35 (d, B part of AB, $J = 10.2$ Hz, 1H, OCH₂Th), 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). ¹³C NMR (300 MHz, CD₃OD): 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 CH₂Cl₂ (2 mL) and stirred at rt under N₂ 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% H₂O 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: [α]_D -43 (c 0.17, MeOH). TLC on C₁₈-silica (2:1 MeOH/H₂O): *R*_f = 0.30. ¹H NMR (300 MHz, CD₃OD): δ 1.82 (d, *J* = 1.2 Hz, Th-CH₃), 1.87 (d, *J* = 1.2 Hz, Th-CH₃), 1.9–2.21 (m, 4H₃'), 2.56–2.80 (m, 4H₄'), 3.58 (s, OCH₃), 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, OCH₂Th), 5.17 (d, B part of AB, *J* = 10.8 Hz, 1H, OCH₂Th), 5.27 (d, A part of AB, *J* = 10.2 Hz, 1H, OCH₂Th), 5.34 (d, B part of AB, *J* = 10.2 Hz, 1H, OCH₂Th), 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'). ¹³C NMR (75 MHz, CD₃OD): 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**: [α]_D -25 (c 0.25, MeOH). TLC on C₁₈-silica (2:1 MeOH/H₂O): *R*_f = 0.38. ¹H NMR (300 MHz, CD₃OD): δ 1.47 (s, C(CH₃)₃), 1.84 (bs, Th-CH₃), 1.88 (bs, Th-CH₃), 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, OCH₂Th), 5.20 (d, B part of AB, *J* = 11.1 Hz, 1H, OCH₂Th), 5.26 (d, A part of AB, *J* = 10.2 Hz, 1H, OCH₂Th), 5.33 (d, B part of AB, *J* = 10.2 Hz, 1H, OCH₂Th), 7.05–7.35 (8H, m, Ar), 7.38 (bs, H₆'), 7.55 (bs, H₆'), 8.25 (s, NH). ¹³C NMR (75 MHz, CD₃OD): 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 CH₂Cl₂ (2 mL) and stirred at rt under N₂ for 15 min. The fully deprotected dimer **16** was obtained after evaporation of the reaction mixture to dryness. C₁₈-TLC (2:1 MeOH/H₂O): *R*_f = 0.71. ¹H NMR (300 MHz, D₂O): δ 1.83 (bs, Th-CH₃), 1.87 (bs, Th-CH₃), 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 OCH₂Th), 5.26 (d, A part of AB, *J* = 11.1 Hz, 1H, OCH₂Th), 5.32 (d, B part of AB, *J* = 11.1 Hz, 1H, OCH₂Th), 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 C₆₀H₆₂N₁₉O₁₇ + Cs⁺ = 1355.341022]. HRFAB

MS *m/z*: 621.23111 (M + H)⁺, calcd mass for C₃₀H₃₂N₆O₉ + H⁺ = 621.230902.

Synthesis 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 C₁₈ reversed phase flash column chromatography using a gradient from 100% H₂O to 100% MeOH. The desired APNA tetramer **17** eluted with 70–80% aqueous MeOH: [α]_D -31 (c 0.08, MeOH). TLC on C₁₈-silica (2:1 MeOH/H₂O): *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 C₁₈-TLC (3:1 MeOH/H₂O): *R*_f = 0.50. ¹H NMR (500 MHz, D₂O) showed resonances in the expected chemical shifts regions (based on the ¹H NMR spectra of compound **17**). However, due to the great extent 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 C₆₀H₆₂N₁₉O₁₇ + Cs⁺ = 1355.341022.

Acknowledgment. This work was supported by grants to Y.S.T. from the Banting Research Foundation, the Canadian Foundation for AIDS Research, Natural Sciences and Engineering Research Council of Canada, and Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec. We are grateful to Dr. Tsuguo Ikebe, Yoshitomi Pharmaceutical Industries, LTD., Fukuoka, Japan, for his generous gift of compound **2**. We thank Dr. Daniel Boismenu and Dr. Orval Mamer of the Biomedical Mass Spectrometry Unit for assistance with the ES MS and HRFAB MS data. We also thank Dr. Phan Viet Minh Tan (Regional Center for high field NMR at the Université de Montréal) for recording the ¹³C NMR (150 MHz) of **16** and Dr. Françoise Sauriol (Chemistry Department of McGill University) for recording the ¹H, COSY, HMQC, and HMBC NMR spectra of **16**.

Supporting Information Available: ¹H and ¹³C 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.

JO962326P