Aminoacylation of Nucleosides with FMOC Amino Acid Fluorides[1]

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Introduction

Aminoacylated nucleosides and oligonucleotides are important molecules in bioorganic chemistry. Aminoacylated nucleotides are key intermediates in the chemical synthesis of aminoacylated tRNAs.[2,3] Furthermore, aminoacyl adenylates are intermediates in the ribosome-mediated biosynthesis of peptides, and related adenylates show pharmacological activity.[4] Therefore, efficient methods for the synthesis of aminoacylated nucleosides, nucleotides, and nucleic acid derivatives are highly desirable.

Our interest in models of ribosomal and nonribosomal peptide synthesis on monolayer films required the synthesis of a number of aminoacylated nucleoside derivatives. A survey of methods for aminoacylating nucleosides and nucleoside derivatives revealed a large number of published procedures, none of which provided a high yielding and easy route to the desired products. On the other hand we found that the N-FMOC amino acid fluorides used by Carpino[5] for the synthesis of peptides, are superior reagents for the aminoacylation of nucleosides.

Background

A number of methods for the preparation of 5′-O-, 3′-O-, or 2′(3′)-O, or 5′-OP-aminoacyl derivatives have appeared. Early methods focussed on the aminoacylation of the 5′-phosphate of adenosine 5′-monophosphate by amino acid acyl chlorides,[6] thiophenol esters of amino acids,[7] or DCC-mediated couplings.[8] Methods for the aminoacylation of 2′(3′)-O have utilized amino acid anhydrides[9] or cyanomethyl esters.[10] Alternatively, the amino acid has been activated *in situ* by a number of coupling agents.[11] Most of these synthetic procedures proceed in extremely low yield. The prime exception,[10] while proceeding in higher yield, is limited by the nature of the protecting groups that can be placed on the amino acid. Although aminoacylation of nucleosides has also been accomplished with enzymes,[12] catalytic antibodies,[13] and recently an RNA molecule that accelerates the aminoacylation of its 2′(3′) terminus,[14] these are not routine synthetic methods at this time.

The 9-fluorenylmethoxycarbonyl (FMOC) group has been found to be an advantageous protecting group for the aminoacylation of dinucleotides.[15] Both the dimethoxytrityl (DMT) and FMOC protecting groups are readily removed without deacylation of an aminoacylated nucleotide.[11b,15] Therefore we have chosen to study activated N-FMOC protected amino acids as acylating reagents. In particular, the N-FMOC amino acid fluorides show several advantages over other aminoacylation reagents.[16] These derivatives are stable crystalline compounds, yet readily react with nucleophiles. Our initial results using these activated amino acids to aminoacylate nucleosides are described below.

Results

The synthesis of the N-FMOC amino acid fluorides **2**−**4** proceeded as described.[16a] The acid fluorides were recrystallized from methylene chloride/hexane and stored as white crystals at 4 °C. We chose N-FMOC-phenylalanyl fluoride (**4**) as the model reagent in order to verify that a sterically demanding amino acid would be coupled. In addition we used a similarly derivatized alanine (**3**) in order to test selectivity for monoacylation in the coupling of a small amino acid with a ribonucleoside which contains both 2′ and 3′ free hydroxyls.

The aminoacylation is simple to perform and proceeds in high yield (Scheme 1). A representative procedure for aminoacylation of the nucleosides involves the addition of an N-FMOC amino acid fluoride to a solution of the protected nucleoside in methylene chloride and pyridine. The solution is stirred at room temperature for 4−12 h followed by removal of the solvent and chromatography. Table 1 presents data on yields for representative transformations. Both pyrimidine and purine bases are compatible with this chemistry though exocyclic amines, if present on the base, must be protected. An aminoacylation reaction performed on 5′-DMT-2′-deoxycytidine resulted in a complex mixture of products. Although the material was not characterized, it was assumed that other products resulted from acylation of N-4 as well as the 3′ hydroxy group. The fluorenylmethyloxy group has been used to protect bases when necessary, though other commonly used protecting groups such as *N*-benzoyl or *N*-isobutyryl are presumably compatible.

(1) Abbreviations used: FMOC, 9-fluorenylmethoxycarbonyl; T, thymidine; U, uridine; A^{FMOC}, N^6-(9-fluorenylmethoxycarbonyl)adenin-9-yl; CFMOC, N^4-(9-fluorenylmethoxycarbonyl)cytos-1-yl; GFMOC, N^2-(9-fluorenylmethoxycarbonyl)guanin-9-yl.

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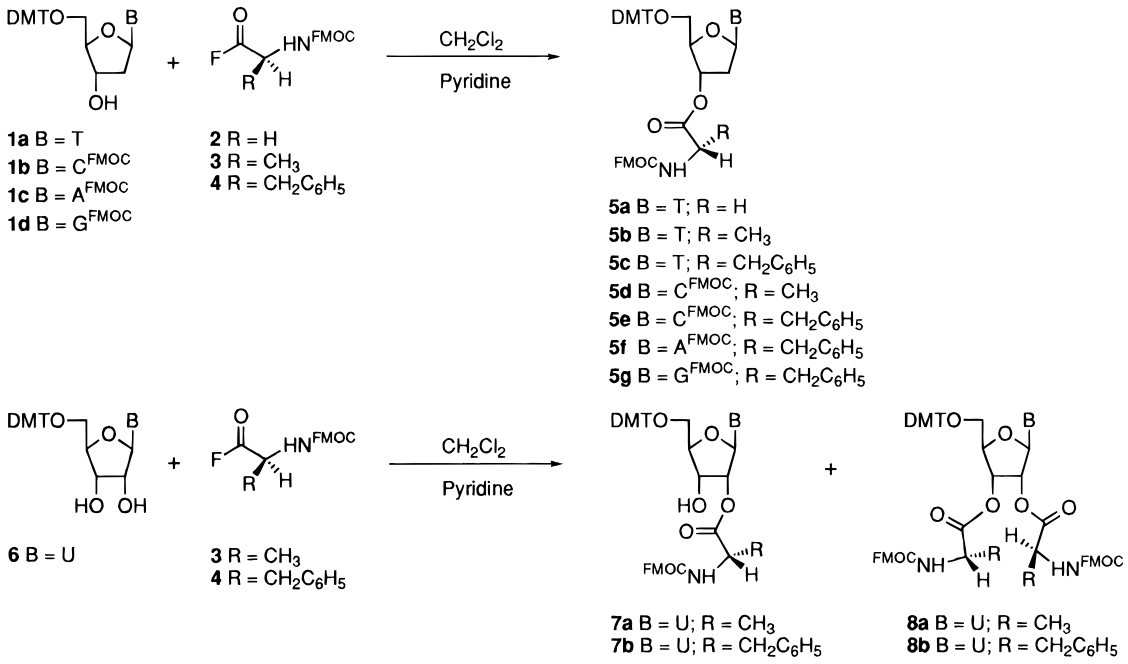
Scheme 1. Synthesis of Aminoacylated Nucleosides



1a B = T
1b B = C^{FMOC}
1c B = A^{FMOC}
1d B = G^{FMOC}

2 R = H
3 R = CH_3
4 R = $CH_2C_6H_5$

5a B = T; R = H
5b B = T; R = CH_3
5c B = T; R = $CH_2C_6H_5$
5d B = C^{FMOC}; R = CH_3
5e B = C^{FMOC}; R = $CH_2C_6H_5$
5f B = A^{FMOC}; R = $CH_2C_6H_5$
5g B = G^{FMOC}; R = $CH_2C_6H_5$

6 B = U

3 R = CH_3
4 R = $CH_2C_6H_5$

7a B = U; R = CH_3
7b B = U; R = $CH_2C_6H_5$

8a B = U; R = CH_3
8b B = U; R = $CH_2C_6H_5$

Table 1. Aminoacylated Nucleosides Synthesized

nucleoside	amino acid (equiv)	time (h)	product (% yield[a])
5′DMT-T	Gly (2)	4	**5a** (97)
	Ala (2)	4	**5b** (97)
	Phe (1)	4	**5c** (71)
	Phe (2)	4	**5c** (96)
DMT-FMOC-C	Ala (2)	4	**5d** (70)
		12	**5d** (90)
	Phe (2)	4	**5e** (67)
		12	**5e** (85)
DMT-FMOC-A	Phe (2)	4	**5f** (67)
		12	**5f** (90)
DMT-FMOC-G	Phe (2)	12	**5g** (74)
	Ala (2)	4	**7a** (57), **8a** (41)
DMT-U	Phe (1)	4	**7b** (55), **8b** (12)
	Phe (1.5)	4	**7b** (60), **8b** (15)
	Phe (2)	4	**7b** (51), **8b** (31)

[a] Based on isolated pure compound.

Some interesting features of this reaction are evident. In the case of 2′-deoxynucleosides **1a**−**1d** the sterically bulky amino acid phenylalanine is condensed in high yield. The use of only 2 equiv of activated amino acid results in a high yield of the aminoacylated product **5a**−**5g**. The reaction of amino acid fluorides with 5′-*O*-(dimethoxytrityl)thymidine (**1a**) is complete in 4 h and isolated yields are high. The reaction of the FMOC protected deoxynucleosides **1b**−**1d** on the other hand is incomplete after 4 h. Extension of the coupling time to 12 h resulted in higher yields.

In the case of ribonucleoside **6,** there are two hydroxy groups which can be aminoacylated. Aminoacylation at either the 2′- or 3′-position gives a single monoacylated product (**7**) in which the amino acid is rapidly exchanging between the two hydroxy groups. Alternatively, both hydroxy groups can be aminoacylated to give a diacylated nucleoside (**8**). The mono- and diacylated products can be easily separated by chromatography. For ribonucleosides with free 2′ and 3′ hydroxyl groups, the yield of mono aminoacylated product increases with ratios of amino acid fluoride to ribose up to about 1.5. With more than 1.5 equiv of the derivatized amino acid, the amount of diacylated nucleosides becomes significant. Higher yields for monoaminoacylation of the ribonucleoside could undoubtedly be obtained by using a nucleoside with a protecting group at the 2′ position. However the yield of monoacylated ribonucleoside is high enough without prior 2′ protection that overall yields would suffer if protective groups were incorporated.

Conclusions

Our present work describes a convenient method to obtain aminoacylated derivatives of 2′-deoxy- and ribonucleosides from N-protected amino acid fluorides. The procedure is straightforward and proceeds in high yield.

Experimental Section

Pyridine and dichloromethane were refluxed over and then distilled from CaH_2. The following chemical reagents were purchased: thymidine, uridine, cytidine, 4, 4′-dimethoxytrityl chloride, 9-fluorenylmethyl choroformate, pyridine, *N*-(9-fluorenylmethoxycarbonyl)-L-alanine monohydrate, *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine, and all solvents were purchased from Aldrich. Cyanuric fluoride was from Alfa and was used without further purification. Glycine was from General Biochemicals. Trimethylchlorosilane and 2′-deoxyadenosine were from Fluka. The 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyguanosine was from Sigma. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and the spots were examined with UV light. Column chromatography was carried out on 200−400 mesh silica gel. Melting points are reported uncorrected.

5′-Dimethoxytrityl derivatives of uridine and thymidine were prepared following the procedure of Meier *et al*.[17] The synthesis of 6-*N*-(9-Fluorenylmethoxycarbonyl)-2′-deoxyadenosine was carried out as described previously.[18]

5′-*O*-(Dimethoxytrityl)-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine (1b). 4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine (0.5 g, 1.11 mmol) was placed in an oven-dried round bottom flask (25 mL) and anhydrous pyridine (10 mL) was added. 4, 4′-Dimethoxytrityl chloride (0.428 g, 1.2 mmol) was added to the solution, and the reaction was stirred at room temperature for 3 h. Solvent was removed from the reaction mixture under vacuum, and the crude material was purified by flash column chromatography (silica gel, step gradient of 30:1, 20:1, 15:1 CH_2Cl_2/MeOH) to give 0.55 g (65%) of a light yellow

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solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.15 (1H, m), 2.77 (1H, m), 3.41 (2H, m), 3.72 (6H, s), 4.20−4.25 (2H, m), 4.38-4.49 (4H, m), 6.30 (1H, app t, J = 5.7 Hz), 6.82 (4H, d, J = 8.1 Hz), 6.99 (1H, d, J = 6.8 Hz), 7.16−7.41 (13H, m), 7.53 (2H, dd, J_1= 3.1 Hz, 3.1 Hz), 7.71 (2H, d, J = 7.5 Hz), 8.23 (1H, d, J = 7.4 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 41.9, 46.5, 55.0, 62.8, 67.8, 70.8, 77.2, 86.5, 86.7, 87.2, 94.9, 113.2, 119.9, 124.9, 126.9, 127.1, 127.8, 127.8, 128.1, 129.9, 129.9, 135.3, 135.5, 141.1, 143.2, 143.3, 144.2, 144.3, 152.3, 155.2, 158.5, 162.4; HRMS (FAB, NBA/NaI) calcd for $[C_{45}H_{41}N_3O_8 + Na]^+$ 774.2791, found 774.2789.

5′-*O*-(Dimethoxytrityl)-6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine (1c). 6-*N*-(9-Fluorenylmethoxycarbonyl)-2′-deoxyadenosine (0.12 g, 0.25 mmol) was placed in an oven-dried round bottom flask (15 mL). Anhydrous pyridine (3 mL) was added by syringe. After the solid had dissolved 4, 4′-dimethoxytrityl chloride (0.0990 g, 0.279 mmol) was added. The reaction was stirred at room temperature for 3 h. Solvent was removed from the reaction mixture under vacuum, and the crude product was purified by flash column chromatography on silica gel as for **1b** to give (0.095 g, 48%) of a white powdery solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.54 (1H, m), 2.82 (1H, m), 3.10 (1H, br s), 3.39 (2H, m), 3.73 (6H, s), 4.17 (1H, m), 4.30 (1H, t, J = 6.6 Hz), 4.60 (2H, d, J = 6.8 Hz), 4.70 (1H, br s), 6.44 (1H, t, J = 6.4 Hz), 6.76 (4H, d, J = 8.7 Hz), 7.13−7.40 (13H, m), 7.62 (2H, d, J = 7.3 Hz), 7.74 (2H, d, J = 7.5 Hz), 8.10 (1H, s), 8.67 (1H, s), 8.81 (1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 40.2, 46.9, 55.2, 63.6, 67.7, 72.4, 77.2, 84.7, 86.2, 86.6, 113.2, 120.0, 122.3, 125.0, 126.9, 127.1, 127.8, 127.8, 128.1, 130.0, 135.6, 141.3, 141.3, 143.5, 144.4, 149.2, 150.8, 151.0, 152.6, 158.6; HRMS (FAB, NBA/NaI) calcd for $[C_{46}H_{41}N_5O_7 + Na]^+$ 798.2904, found 798.2910.

5′-*O*-(Dimethoxytrityl)-2-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyguanosine (1d). 5′-*O*-(Dimethoxytrityl)-2′-deoxyguanosine (0.12 g, 0.21 mmol) was placed in an oven-dried round bottom flask (15 mL), and anhydrous pyridine (3 mL) was added. Trimethylchlorosilane (0.06 mL, 0.47 mmol) was added dropwise by syringe to the solution over a 3-min period. The reaction mixture was stirred at room temperature for 1 h followed by addition of 9-fluorenylmethyl choroformate (0.06 g, 0.23 mmol). Stirring was continued for an additional 1.5 h. Hydrolysis of excess reagent and trimethysilyl protecting groups was effected by addition of water (2 mL) and stirring for 20 min. The solution was poured into saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The organic fractions were combined, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (silica gel, 12:1 $CHCl_3$/MeOH) gave 65 mg (39%) of chromatographically pure **1d** as a white solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.64 (2H, m), 3.43 (2H, br s), 3.61 (6H, s), 3.90 (1H, t, J = 7.4 Hz), 4.14−4.36 (3H, m), 5.18 (1H, br s), 5.51 (1H, br s), 6.23 (1H, app t, J = 5.5 Hz), 6.63 (4H, d, J = 7.8 Hz), 6.97−7.50 (17H, m), 7.70 (1H, s), 9.92 (1H, br s), 11.40 (1H, br s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 40.1, 46.4, 55.1, 64.5, 68.2, 71.1, 77.2, 84.5, 86.3, 86.8, 113.0, 120.0, 120,0, 120.8, 124.8, 125.1, 126.7, 126.8, 127.0, 127.6, 127.9, 128.2, 129.9, 130.0, 135.9, 136.0, 138.2, 141.0, 141.1, 142.6, 143.1, 144.6, 146.7, 148.3, 154.1, 155.7, 158.4; HRMS (FAB, PEG600/NBA/NaOAc) calcd for $[C_{46}H_{41}N_5O_8 + Na]^+$ 814.2853, found 814.2864.

Representative Procedure for the Synthesis of FMOC Amino Acid Fluorides. The procedure employed was as described by Carpino et al.[16] *N*-(9-Fluorenylmethoxycarbonyl)-phenylalanine (0.7906 g, 2 mmol) was dissolved in dry dichloromethane (15 mL) and dry pyridine (0.162 mL, 2 mmol). Cyanuric fluoride (0.35 mL, 4 mmol) was added to the mixture, and the reaction was refluxed for 3 h. During the reaction a water soluble, white solid precipitated from the reaction. The crude reaction mixture was poured into dichloromethane (30 mL) and then extracted with cold water (3 × 15 mL). The organic layer was dried with sodium sulfate, and the solvent was evaporated to give a white glassy solid. The solid was recrystallized from dichloromethane/hexane to give a 0.64 g (82%) of a powdery white solid. TLC as described by Carpino in CH_2Cl_2/MeOH 9:1 was used to verify that the product was free of contaminating starting material. Mp 116−120 °C; IR (CaF_2 cell) cm^{-1} 3427.5, 3033.3, 2955.6, 1847.0, 1728.4, 1607.9, 1509.5, 1450.9, 1400.2; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 3.13 (2H, m), 4.15 (1H, t, J = 6.7 Hz), 4.39 (2H, m), 4.79 (1H, m), 5.19 (1H, d, J = 8.1 Hz), 7.09−7.75 (13H, m); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 36.84, 47.03, 53.43, 54.03, 67.22, 119.97, 124.88, 124.91, 127.04, 127.68, 127.75, 128.95, 129.12, 134.25, 141.28, 143.45, 143.55, 155.41, 159.87, 163.54.

Representative Procedure for the Aminoacylation Reaction. 5′-*O*-(4,4′-Dimethoxytrityl-2′(3′)-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)iridine (7b) and 5′-*O*-(4,4′-Dimethoxytrityl)-2′, 3′-*O*,*O*-bis(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)uridine (8b). 5′-*O*-Dimethoxy-trityluridine (**6**) (0.110 g, 0.201 mmol) was added to an oven-dried round bottom flask (10 mL) under argon and coevaporated with anhydrous pyridine (2 × 3 mL). Dry dichloromethane (1.5 mL) and dry pyridine (0.33 mL) were added under argon. *N*-(9-Fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (**4**) (0.118 g, 0.303 mmol) was added to the solution and the mixture stirred at room temperature under argon for 4 h. The reaction was concentrated and the crude material purified by flash chromatography (silica gel, step gradient 8:1; 6:1; 3:1 CH_2Cl_2/acetone) to give 0.110 g (60%) of **7b** and 0.038 g (15%) of **8b** as chromatographically pure, white solids. A similar reaction containing **6** (55 mg, 0.101 mmol) and **4** (39 mg, 0.101 mmol) gave 51 mg (55%) of **7b** and 16 mg (12%) of **8b**. A reaction containing **6** (55 mg, 0.101 mmol) and **4** (79 mg, 0.202 mmol) gave 47 mg (51%) of **7b** and 40 mg (31%) of **8b**.

7b: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.11 (2H, m), 3.43 (2H, m), 3.74 (3H, s), 3.76 (3H, s), 3.97−4.78 (6H, m), 5.34-5.42 (2H, m), 5.75 (0.75H, d, J = 6.2 Hz), 5.96 (0.25H, d, J = 8.1 Hz), 6.06 (0.75H, d, J = 4.5 Hz), 6.11 (0.25H, d, J = 4.5 Hz), 6.83 (4H, d, J = 7.9 Hz), 7.12−7.38 (18H, m), 7.48 (2H, d, J = 7.0 Hz), 7.69−7.78 (3H, m), 9.31 (0.25H, br s), 10.04 (0.75H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz). δ 37.4, 38.1, 46.9, 47.1, 55.2, 55.5, 62.3, 62.6, 67.0, 67.4, 69.9, 73.4, 74.6, 77.2, 81.7, 83.8, 86.2, 87.2, 87.3, 88.2, 102.7, 102.8, 113.3, 113.4, 119.9, 124.9, 125.0, 127.0, 127.1, 127.2, 127.2, 127.3, 127.7, 127.7, 128.1, 128.2, 128.6, 128.8, 129.1, 129.2, 130.0, 130.1, 130.1, 135.0, 135.0, 135.1, 135.6, 135.8, 136.0, 139.9, 140.0, 141.3, 143.6, 143.7, 144.1, 149.7, 150.3, 150.8, 156.0, 158.7, 158.7, 158.8, 163.2, 163.8, 170.4, 170.8. HRMS (FAB, NBA/NaI) calcd for $[C_{54}H_{49}N_3O_{11} + Na]^+$ 938.3265, found 938.3277.

8b: ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 3.05−3.37 (6H, m), 3.76 (6H, s), 3.88−4.30 (7H, m), 4.64 (2H, m), 5.37 (1H, d, J = 57.9 Hz), 5.58 (2H, br s), 5.78 (1H, d, J = 8.2 Hz), 5.85 (1H, d, J = 8.2 Hz), 6.08 (1H, d, J = 4.3 Hz), 6.85 (4H, d, J = 8.4 Hz), 7.18−7.44 (31H, m), 7.67 (5H, m), 9.21 (1H, br s). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 37.1, 38.1, 46.8, 55.1, 55.2, 55.2, 62.3, 67.0, 67.2, 71.4, 73.9, 77.2, 81.7, 86.5, 87.5, 102.9, 113.4, 119.8, 124.9, 125.1, 126.9, 127.1, 127.2, 127.3, 127.6, 128.1, 128.6, 128.6, 129.1, 130.0, 130.1, 134.7, 134.8, 135.6, 136.0, 139.8, 141.1, 143.6, 143.6, 143.8, 150.3, 155.8, 156.0, 158.8, 158.8, 162.8, 170.3, 170.9; HRMS (FAB, PEG600/NBA/NaOAc) calcd for $[C_{78}H_{68}N_4O_{14} + Na]^+$ 1307.4626, found 1307.4619,

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)glycyl)thymidine (5a). Reaction of protected nucleoside **1a** (100 mg, 0.184 mmol) and *N*-(9-fluorenylmethoxycarbonyl)glycyl fluoride (110 mg, 0.368 mmol) for 4 h followed by flash chromatography (silica gel, step gradient 0−1% MeOH in $CHCl_3$) gave 147 mg (97%) of chromatographically pure **5a** as a white solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.39 (3H, s), 2.47 (2H, br s), 3.47 (2H, br s), 3.77 (6H, s), 4.00 (2H, d, J = 5.6 Hz), 4.14 (1H, br s), 4.22 (1H, t, J = 6.9 Hz), 4.41 (2H, d, J = 7.0 Hz), 5.48 (2H, br s), 6.44 (1H, app t), 6.83 (4H, d, J = 8.8 Hz), 7.25−7.39 (13H, m), 7.59 (3H, d, J = 7.3 Hz), 7.74 (2H, d, J = 7.3 Hz), 9.41 (1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 11.6, 37.8, 42.7, 47.0, 55.2, 55.2, 63.5, 67.2, 76.5, 77.2, 83.7, 84.2, 87.2, 111.7, 113.3, 120.0, 125.0, 127.0, 127.2, 127.7, 128.0, 128.0, 130.0, 130.0, 135.0, 135.1, 135.2, 141.2, 143.7, 144.1, 150.5, 156.3, 158.7, 158.7, 163.8, 169.6; HRMS (FAB, NBA/NaI) calcd for $[C_{48}H_{45}N_3O_{10} + Na]^+$ 846.3003, found 846.3008.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-alanyl)thymidine (5b). Reaction of protected nucleoside **1a** (100 mg, 0.184 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-alanyl fluoride (116 mg, 0.368 mmol) for 4 h followed by flash chromatography (silica gel, step gradient 0−1% MeOH in $CHCl_3$) gave 149 mg (97%) of chromatographically pure **5b** as a white solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.39−1.42 (6H, m), 2.47 (2H, m), 3.47 (2H, br s), 3.77 (6H, s), 4.10 (1H, br s), 4.22 (1H, t, J = 6.8 Hz), 4.39 (3H, m), 5.39 (1H, d, J = 7.5 Hz), 5.52 (1H, br s), 6.44 (1H, app t), 6.83 (4H, d, J = 8.9 Hz), 7.23−7.41 (13H, m), 7.59 (3H, br s), 7.75 (2H, d, J = 7.4 Hz), 9.10 (1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 11.6, 18.2, 37.7, 47.1, 49.6, 55.2, 55.2, 63.5, 67.0, 76.2, 77.2, 84.0, 84.3, 87.2, 111.7, 113.3,

120.0, 125.0, 127.0, 127.2, 127.7, 128.0, 128.0, 130.0, 135.1, 135.2, 135.2, 141.3, 143.7, 143.8, 144.1, 150.4, 155.6, 158.8, 163.6, 172.6; HRMS (FAB, NBA/NaI) calcd for $[C_{49}H_{47}N_3O_{10} + Na]^+$ 860.3159, found 860.3162.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)thymidine (5c). Reaction of protected nucleoside **1a** (100 mg, 0.184 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (72 mg, 0.184 mmol) for 4 h followed by flash chromatography (silica gel, step gradient 0−1% MeOH in $CHCl_3$) gave 119 mg (71%) of chromatographically pure **5c**. An identical reaction containing 2 equiv of *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (144 mg, 0.368 mmol) gave 161 mg (96%) of chromatographically pure **5c** as a white solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.37 (3H, s), 2.32−2.43 (2H, br s), 3.10 (2H, m), 3.39 (2H, m), 3.77 (6H, s), 3.83 (1H, s), 4.20 (1H, t, J = 6.7 Hz), 4.41 (2H, m), 4.63 (1H, m), 5.38 (2H, d, J = 7.5 Hz), 6.31 (1H, app. t), 6.84 (4H, d, J = 8.9 Hz), 7.00−7.42 (18H, m), 7.54−7.57 (3H, br s), 7.75 (2H, d, J = 7.3 Hz), 9.17 (1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 11.6, 37.7, 38.3, 47.1, 54.8, 55.2, 63.5, 67.0, 76.5, 77.2, 83.6, 84.2, 87.2, 111.6, 113.3, 120.0, 125.0, 127.0, 127.2, 127.3, 127.7, 128.0, 128.0, 128.7, 129.1, 130.0, 135.0, 135.2, 135.2, 135.3, 141.3, 143.6, 143.8, 144.1, 150.4, 155.5, 158.8, 158.8, 163.7, 171.3; HRMS (FAB, NBA/NaI) calcd for $[C_{55}H_{51}N_3O_{10} + Na]^+$ 936.3472, found 936.3473.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxy-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-alanyl)cytidine (5d). Reaction of protected nucleoside **1b** (100 mg, 0.133 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-alanyl fluoride (83 mg, 0.26 mmol) for 12 h followed by flash chromatography (silica gel, step gradient 0−3% MeOH in $CHCl_3$) gave 125 mg (90%) of chromatographically pure **5d** as a white solid. An identical reaction which was allowed to react for 4 h yielded 97 mg (70%) of **5d**. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.40 (3H, d, J = 7.1 Hz), 2.32 (1H, m), 2.81 (1H, m), 3.45 (2H, br s), 3.76 (6H, s), 3.19−3.28 (3H, m), 4.38−4.47 (5H, m), 5.37−5.44 (2H, m), 6.29 (1H, t, J = 6.2 Hz), 6.83 (4H, d, J = 7.9 Hz), 6.95 (1H, d, J = 7.2 Hz), 7.25−7.40 (17H, m), 7.57 (4H, d, J = 7.0 Hz), 7.75 (4H, d, J = 7.3 Hz), 8.07 (2H, br s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 18.2, 39.2, 46.6, 47.1, 49.6, 55.2, 55.2, 62.9, 67.0, 68.0, 75.2, 77.2, 84.5, 87.0, 87.1, 94.6, 113.3, 119.9, 120.1, 124.9, 125.0, 127.0, 127.2, 127.7, 128.0, 128.0, 129.9, 130.0, 135.0, 135.2, 141.3, 143.1, 143.2, 143.7, 143.8, 144.0, 144.1, 152.1, 154.7, 155.6, 158.7, 162.2, 172.4; HRMS (FAB, NBA/NaI/2-nitrophenyl octyl ether) calcd for $[C_{63}H_{56}N_4O_{11} + Na]^+$ 1067.3843, found 1067.3832.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxy-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)cytidine (5e). Reaction of protected nucleoside **1b** (100 mg, 0.133 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (101 mg, 0.26 mmol) for 12 h followed by flash chromatography (silica gel, step gradient 0−3% MeOH in $CHCl_3$) gave 126 mg (85%) of chromatographically pure **5e** as a white solid. An identical reaction which was allowed to react for 4 h yielded 100 mg (67%) of **5e**; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.24 (1H, m), 2.77 (1H, m), 3.08 (2H, d, J = 6.2 Hz), 3.38 (2H, br s), 3.74 (6H, s), 4.01 (1H, br s), 4.21 (2H, m), 4.42 (4H, m), 4.65 (1H, m), 5.31 (1H, d, J = 5.0 Hz), 5.48 (1H, d, J = 8.0 Hz), 6.31 (1H, t, J = 6.3 Hz), 6.81 (4H, d, J = 7.6 Hz), 6.97 (1H, d, J = 7.2 Hz), 7.07−7.45 (22H, m), 7.56 (4H, d, J = 7.2 Hz), 7.74 (4H, d, J = 7.4 Hz), 8.04 (4H, d, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 38.2, 39.2, 46.6, 47.1, 54.8, 55.1, 63.0, 67.0, 67.9, 75.8, 77.2 84.3, 87.1, 94.6, 113.3, 119.9, 120.1, 123.7, 124.9, 125.0, 127.0, 127.1, 127.7, 127.9, 128.0, 128.0, 128.6, 129.1, 129.2, 129.9, 130.0, 135.0, 135.2, 135.5, 135.9, 141.3, 143.2, 143.2, 143.7, 143.8, 144.0, 149.7, 152.2, 154.7, 155.5, 158.7, 162.3, 171.0. HRMS (FAB, NBA/NaI/2-nitrophenyl octyl ether) calcd for $[C_{69}H_{60}N_4O_{11} + Na]^+$ 1143.4156, found 1143.4153.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxy-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)adenosine (5f). Reaction of protected nucleoside **1c** (120 mg, 0.155 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (**4**) (181 mg, 0.465 mmol) for 12 h followed by flash chromatography (silica gel, step gradient 0−1% MeOH in $CHCl_3$) gave 160 mg (90%) of chromatographically pure **5f** as a white solid. An identical reaction containing **1c** (85 mg, 0.110 mmol) and **4** (85 mg, 0.219 mmol) yielded 84 mg (67%) of **5f**. A similar reaction of **1c** (54 mg, 0.0696 mmol) and **4** (54 mg, 0.139 mmol) which was allowed to react for 4 h yielded 40.3 mg (51%) of **5f**. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.56 (1H, dd, J_1 = 7.2 Hz, 7.2 Hz), 3.05 (3H, m), 3.43 (2H, m), 3.75(6H, s), 4.05 (1H, br s), 4.21 (1H, t, J = 6.7 Hz), 4.31 (1H, t, J = 6.6 Hz), 4.36−4.50 (2H, m), 4.60−4.68 (3H, m), 5.33 (1H, d, J = 8.1 Hz), 5.48 (1H, br s), 6.26 (1H, app t), 6.78 (4H, d, J = 8.8 Hz), 6.94−7.41 (22H, m), 7.55−7.64 (4H, m), 7.75 (4H, d, J = 7.4 Hz), 8.05 (1H, s), 8.52 (1H, s), 8.6(1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 37.5, 38.4, 46.9, 47.1, 54.9, 55.2, 63.4, 67.0, 67.7, 76.5, 77.2, 84.1, 84.5, 86.7, 113.1, 113.2, 120.0, 122.4, 125.0, 127.0, 127.0, 127.1, 127.3, 127.7, 127.8, 127.9, 128.1, 128.7, 129.2, 129.9, 130.0, 135.4, 135.4, 141.1, 141.3, 143.5, 143.6, 143.7, 144.3, 149.3, 151.0, 151.0, 152.7, 155.5, 158.6, 171.0; HRMS (FAB, NBA/NaI) calcd for $[C_{70}H_{60}N_6O_{10} + Na]^+$ 1167.4269, found 1167.4286.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxy-3′-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl)guanosine (5g). Reaction of protected nucleoside **1d** (50 mg, 0.064 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl fluoride (**4**) (50 mg, 0.128 mmol) for 12 h followed by flash chromatography (silica gel, step gradient 0−1% MeOH in $CHCl_3$) gave 54 mg (74%) of chromatographically pure **5g** as a white solid. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 2.40 (1H, m), 2.83 (1H, m), 3.08 (2H, m), 3.27 (2H, br s), 3.74 (6H, s), 3.94 (1H, br s), 4.20 (2H, m), 4.39−4.65 (5H, m), 5.37 (2H, m), 5.92 (1H, m), 6.79 (4H, m), 7.09−7.55 (27H, m), 7.67−7.76 (5H, m), 11.20 (1H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 37.4, 38.3, 46.6, 47.1, 55.0, 55.2, 63.5, 67.2, 68.4, 76.3, 77.2, 83.7, 84.0, 86.7, 113.3, 120.0, 120.2, 121.5, 124.8, 124.9, 125.0, 127.0, 127.1, 127.3, 127.3, 127.8, 128.0, 128.1, 128.7, 129.2, 130.0, 135.4, 135.5, 136.9, 141.3, 141.3, 142.8, 142.8, 143.6, 143.6, 144.3, 146.2, 148.1, 153.2, 155.3, 155.7, 158.7, 171.1; HRMS (FAB, NBA/triethanolamine/NaI) calcd for $[C_{70}H_{60}N_6O_{11} + Na]^+$ 1183.4218, found 1183.4229.

5′-*O*-(4,4′-Dimethoxytrityl)-2′(3′)-*O*-(*N*-(9-fluorenylmethoxycarbonyl)-L-alanyl)uridine (7a) and 5′-*O*-(4,4′-Dimethoxytrityl)-2′, 3′-*O*,*O*-bis(*N*-(9-fluorenylmethoxycarbonyl)-L-alanyl)uridine (8a). Reaction of protected nucleoside **6** (110 mg, 0.202 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-alanyl fluoride (**3**) (127 mg, 0.405 mmol) for 4 h followed by flash chromatography (silica gel, step gradient 14−25% acetone in CH_2Cl_2) gave 97.4 mg (58%) of **7a** and 94.1 mg (41%) of **8a** as chromatographically pure, white solids.

7a: ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.43 (3H, m), 3.45 (2H, m), 3.75 (3H, s), 3.76 (3H, s), 4.00−4.50 (5H, m), 4.63 (1H, m), 5.40 (2H, m), 5.88 (0.25H, d, J = 7.4 Hz), 5.98 (0.75H, d, J = 6.3 Hz), 6.09 (0.75H, d, J = 4.5 Hz), 6.15(0.25H, d, J = 4.5 Hz), 6.82 (4H, d, J = 8.7 Hz), 7.17−7.36 (13H, m), 7.53 (2H, m), 7.69−7.85 (3H, m), 9.45 (0.25H, br s), 10.06 (0.75H, br s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 17.4, 18.4, 46.9, 47.1, 49.9, 50.2, 55.2, 55.2, 62.2, 67.0, 67.4, 69.8, 72.8, 74.6, 77.2, 81.6, 83.7, 86.5, 87.2, 87.3, 88.7, 102.7, 109.6, 113.3, 113.4, 119.9, 124.9, 125.0, 127.0, 127.1, 127.2, 127.7, 127.7, 128.0, 128.1, 130.0, 130.1, 134.9, 135.0, 135.2, 140.0, 141.2, 141.3, 143.7, 143.8, 144.0, 144.1, 150.4, 150.8, 156.1, 156.9, 158.7, 163.3, 163.9, 171.4, 172.0; HRMS (FAB, NBA/NaI) calcd for $[C_{48}H_{45}N_3O_{11} + Na]^+$ 862.2952, found 862.2961.

8a: ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 1.40 (3H, d, J = 7.0 Hz), 1.46 (3H, d, J = 6.8 Hz), 3.43 (2H, br s), 3.76 (6H, s), 4.09−4.43 (9H, m), 5.36 (1H, d, J = 7.7 Hz), 5.54 (1H, m), 5.68−5.77 (2H, m), 6.00 (1H, d, J = 7.7 Hz), 6.17 (1H, d, J = 4.9 Hz), 6.83 (4H, d, J = 8.6 Hz), 7.19−7.37 (17H, m), 7.49 (4H, m), 7.67 (5H, m), 9.24 (1H, br s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 17.7, 18.0, 46.9, 49.6, 49.6, 55.2, 55.2, 62.1, 67.1, 71.0, 74.1, 77.2, 81.7, 86.7, 87.4, 103.0, 113.4, 119.8, 125.0, 127.0, 127.3, 127.6, 127.6, 128.1, 130.0, 130.1, 134.7, 134.8, 139.6, 141.2, 141.2, 143.6, 143.7, 143.8, 150.3, 155.9, 156.0, 158.8, 158.8, 162.8, 171.5, 171.9; HRMS (FAB, NBA/NaI) calcd for $[C_{66}H_{60}N_3O_{14} + Na]^+$ 1155.4004, found 1155.3983.

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Supporting Information Available: Proton and carbon NMR spectra are available for all compounds described in the experimental section (31 pages). This material is contained in libraries on microfiche, immediately follows 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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