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Synthesis of δ-Amino Acids with an Ether Linkage in the Main Chain and Nucleobases on the Side Chain as Monomer Units for Oxy-Peptide Nucleic Acids

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Abstract

Syntheses of four *N*-Fmoc δ-amino acids with an ether linkage in the main chain and four different nucleobases on the side chain, Fmoc-NH-C*H(CH_2-CH_2-B)-CH_2-O-CH_2-COOH (B =thymine, uracil, N^4-benzoylcytosine, and N^2-isobutyrylguanine) are described. The δ-amino acids were prepared through 8-12 step synthesis starting from L-homoserine and could be linked together to form novel peptide nucleic acids (oxy-PNAs = OPNAs).

Keywords: Peptide and polypeptide; Nucleic acid analogues; Amino acids and derivatives

The study of peptide-based nucleic acid analogues (PNAs) takes its origin from nearly thirty years ago [1]. Jones and coworkers prepared PNA(I) (B = thymine, n ≈ 9-20) from amino acids with nucleobases attached at the β-position of alanine [2]. However, no interaction was observed between the PNA(I) and polyadenylic acid. Molecular modeling by Weller *et al.* predicted that PNA(II) may hybridize to the complementary nucleic acids [3]. But no successful result has been reported. Indeed, we have found that two types of PNA(II)s with (B = uracil, R = H) and with (B = uracil, R = Me) showed no interaction with oligodeoxyadenylic acids [4]. These results suggest that the peptide main chain and side chain may be too rigid to achieve the hybridization. To increase the side chain flexibility, the spacer between the main chain and the nucleobase was elongated by a single methylene unit as PNA(III). The latter oligopeptides containing γ-substituted homoalanines have been synthesized [5], but no hybridization has been reported. Similarly, PNA(IV) that carries B-CH_2-CH_2- side groups at the amide nitrogen did not hybridize to DNAs [6]. These results suggest that PNAs that consist of a conventional polypeptide backbone with B-CH_2- or B-CH_2-CH_2- side groups do not hybridize to DNAs, because of insufficient flexibility of the

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polypeptide chain. To relax the rigidity of the polypeptide backbone, an ester linkage was introduced as the depsipeptide PNA(V) [4]. Unfortunately, however, the ester bond was easily hydrolyzed in aqueous media.

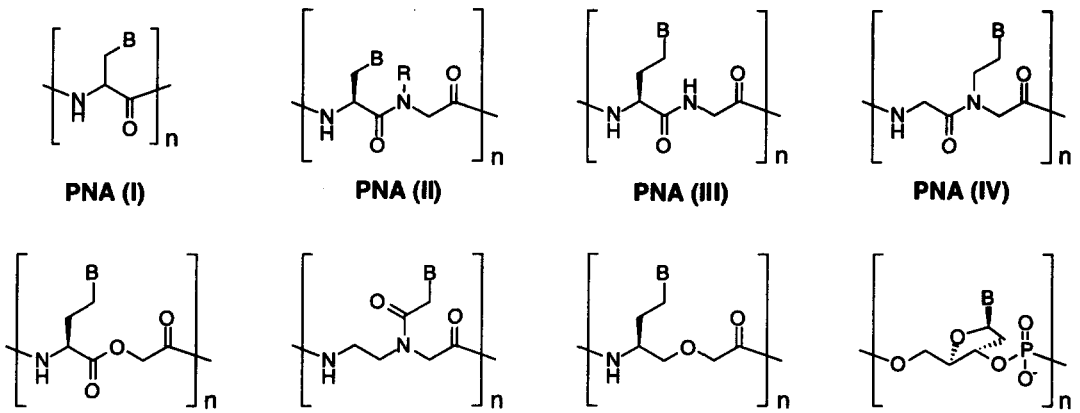
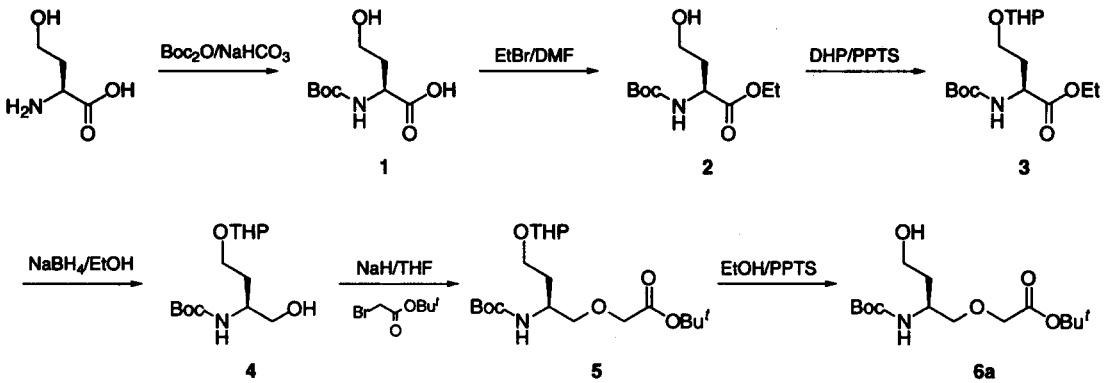


Chart 1

In 1991, Nielsen and coworkers discovered that PNAs of δ-amino acids with a [NH-CH_2-CH_2-N(CO-CH_2-B)-CH_2-CO]$_n$-type structure [PNA(VI)] hybridize to the complementary DNAs with higher affinity than the DNA-DNA counterpart [7]. The Nielsen's PNAs may have a flexible main chain compared with polypeptides chains of α-amino acids and this may allow them to hybridize to DNAs. A drawback of the Nielsen's PNA is its limited water solubility that restricts their applications to medicinal uses. After the Nielsen's PNA, several workers reported PNAs of different main chain structures [8], however, most of these new PNAs showed little interaction with nucleic acids.

Very recently, we have reported a novel PNA (oxy-PNA = OPNA) that consists of another δ-amino acids with a [NH-C*H(CH_2-CH_2-B)-CH_2-O-CH_2-CO]$_n$-type structure [9, 10]. The OPNA with 12 adenine bases showed a strong hybridization with $(dT)_{12}$ in aqueous solution. The OPNA showed improved water solubility and all-or-none-type hybridization with the complementary DNA. These characteristic features of the OPNA have been attributed to the flexible ether linkages that afford sufficient flexibility in the main chain. Another feature of the OPNA is the presence of a chiral center in the main chain that may extend their chemical diversity.

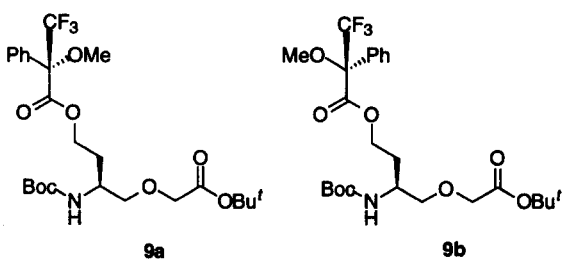
In the previous paper, synthesis of the δ-amino acid with adenine base and the preparation of an oligopeptide with 12 adenine bases [OPNA(A_{12})] have been described [9]. In this article we describe synthesis of other new *N*-Fmoc δ-amino acids bearing thymine (**8a**), uracil (**8b**), N^4-benzoylcytosine (**8c**), and N^2-isobutyrylguanine (**8d**) as the monomer units of the OPNA. These δ-amino acids were prepared through a common intermediate **6a**, that was prepared from L-homoserine in 6 steps as shown in Scheme 1.



Scheme 1

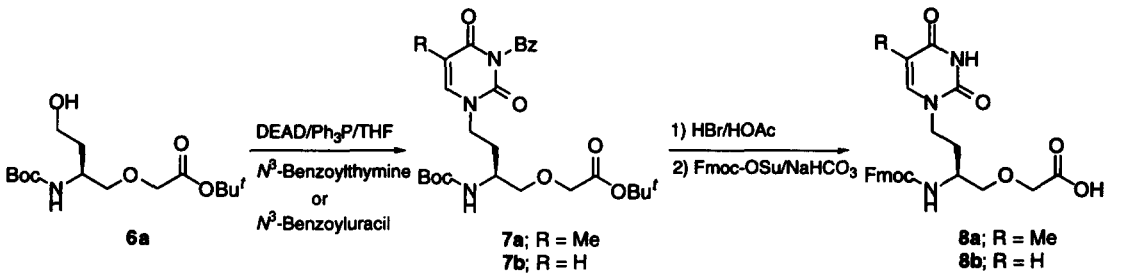
First, amino group of L-homoserine was protected with Boc group and treated with dicyclohexylamine to give dicyclohexylammonium salt of *N*-Boc-L-homoserine **1** in 83% yield. The reaction of the salt **1** with ethyl bromide gave compound **2** in 91% yield. Treatment of the ethyl ester **2** with 3,4-dihydro-2H-pyran gave *N*-Boc-*O*-tetrahydropyranyl-L-homoserine ethyl ester **3** in 87% yield. Reduction of compound **3** with $NaBH_4$ resulted in the corresponding alcohol **4** in 90% yield. The sodium alkoxide of **4** was reacted with bromoacetic acid *tert*-butyl ester to give compound **5** in 30% yield. Removal of tetrahydropyranyl group of compound **5** with pyridinium tosylate in ethanol afforded the key intermediate **6a** in an overall yield 15% from L-homoserine.

The Mosher esters **9a** and **9b** were synthesized to determine the optical purity of compound **6a**. The diastereomeric esters **9a** and **9b** were prepared from the alcohol **6a** with R(–)- and S(+)-methoxy-α-trifluoromethylphenylacetyl chloride in pyridine, respectively [11]. The ^{19}F NMR signal of the CF_3 group of compound **9a** and **9b** appeared as a single peak at –272.87 and –272.74 ppm from trichlorofluoro-methane, respectively. The single peak indicates that the optical purity of compound **6a** is > 99% ee. Since no racemization is expected in the synthetic route after the compound **6a**, the *N*-Fmoc δ-amino acids (**8a**, **8b**, **8c**, and **8d**) must be optically pure.



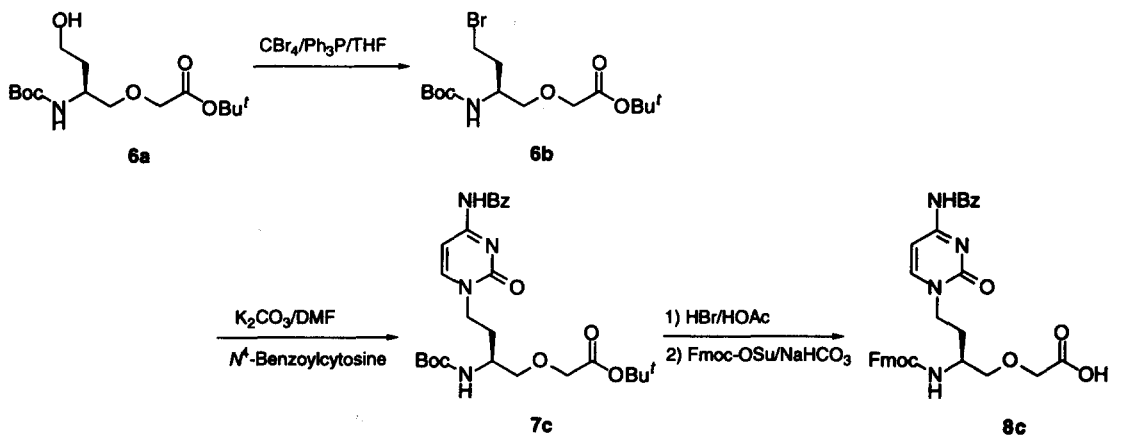
The adenine derivative was synthesized by a direct substitution of hydroxy group of the alcohol **6a** with N^6-benzoyladenine through Mitsunobu reaction [12]. The detail of the synthesis of the adenine derivative has been reported before [9]. Similar protocol was applied to the syntheses of the thymine and uracil derivative (Scheme 2). The alcohol **6a** was reacted with N^3-benzoylthymine and N^3-benzoyluracil under standard Mitsunobu conditions, respectively, to give the desired N^1-isomer **7a** and N^1-isomer **7b** in 43% and 46% yield, respectively. Compound **7a** and **7b** were treated with 25% HBr in acetic acid to remove the

Boc, OBu^t, and the exocyclic amide protecting group. The free compounds were protected again with 9-fluorenylmethyl succinimidyl carbonate to give the final products **8a** and **8b** in an overall yield of 22% and 26% from **6a**, respectively.



Scheme 2

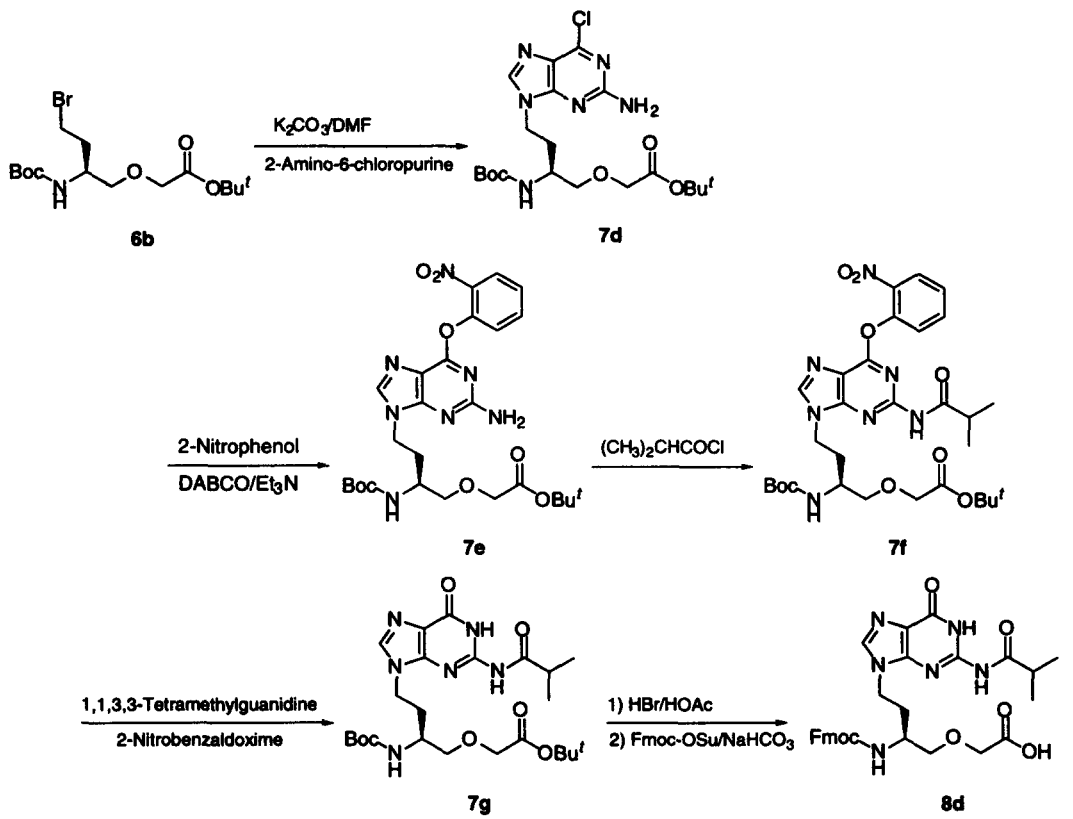
In the case of the cytosine derivative, no product **7c** was obtained by a direct substitution under standard Mitsunobu conditions, probably because of the reduced solubility of N^4-benzoylcytosine in THF. Therefore, N^4-benzoylcytosine was introduced through a bromide derivative **6b** (Scheme 3). The alcohol **6a** was treated with carbon tetrabromide and triphenylphosphine in dry THF to give the bromide **6b** in 82% yield. Nucleophilic substitution of the bromide **6b** with N^4-benzoylcytosine gave the desired N^1-isomer **7c** in 63% yield. Removal of the amino and carboxy protecting group in compound **7c** with 25% HBr in acetic acid followed by the treatment with 9-fluorenylmethyl succinimidyl carbonate gave the final product **8c** in an overall yield of 26% from **6a**. The N^4-benzoyl group has been reported to be effective for protecting cytosinyl group in the peptide synthesis [8m].



Scheme 3

Similar protocol as the cytosine derivative was applied to the synthesis of the guanine derivative (Scheme 4). Generally speaking, nucleophilic substitution of alkyl bromide with 2-amino-6-chloropurine is the most convenient method to obtain N^9-isomer of guanine derivative in high yield [13]. Alkylation of 2-amino-6-chloropurine with bromide **6b** was

performed in dry DMF for 6 h at room temperature in the presence of anhydrous K_2CO_3 to give the product **7d** in 91% yield. Replacement of the 6-chloro group in compound **7d** with an oxygen atom was accomplished according to Howarth *et al.* [5d]. Conversion of the chloro group in **7d** with a 2-nitrophenoxy group gave the product **7e** in 98% yield. Subsequently, the N^2-amino group in **7e** was protected by the isobtyryl group to avoid undesirable acylation in the peptide-coupling step. The N^2-isobutyrylamino derivative **7f** was obtained in 93% yield from **7e**. Removal of the 2-nitrophenoxy group in **7f** with a solution of 1,1,3,3,-tetramethylguanidene in acetonitrile gave the product **7g** in 80% yield. Removal of the amino and carboxy protecting group with 25% HBr in acetic acid followed by the treatment with 9-fluorenylmethyl succinimidyl carbonate gave the final product **8d** in an overall yield of 40% from **6a**. The N^2-isobutyryl group has been reported to be effective for protecting guaninyl group in the peptide synthesis [8m].



Scheme 4

All intermediates and the final products (**8a**, **8b**, **8c**, and **8d**) were characterized by ^1H and ^{13}C NMR, IR, and high resolution mass spectroscopy.

Experimental

General NMR spectra were recorded on a Varian Mercury 300 spectrometer. IR spectra were taken on a JASCO FT-IR 610 spectrophotometer. FAB Mass spectra were taken on a JEOL JMS-DX303 under FAB positive ionization mode.

***tert*-Butyl 7-hydroxy-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 6a:** Compound **6a** was prepared from L-homoserine as described above: R_f 0.38 [50:50, ethyl acetate: hexane]; ^{1}H NMR (300MHz, $CDCl_3$) δ 5.39 (d, br, 1H), 3.96 (s, 2H), 3.95–3.86 (m, 1H), 3.71–3.55 (m, 5H), 1.85–1.62 (m, 2H), 1.47 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.60, 157.07, 81.99, 79.74, 73.80, 68.72, 58.64, 47.06, 35.47, 28.29, 28.06; IR (KBr) 3451, 3313, 2980, 1725, 1253, 1168 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{15}H_{30}NO_6$ 320.2073, found 320.2081; $[\alpha]_D^{21}$ –8.6 (*c* 1.1, $CHCl_3$)

***tert*-Butyl 7-bromo-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 6b:** Compound **6a** (2.30 g, 7.2 mmol) and carbon tetrabromide (3.58 g, 10.8 mmol) were dissolved in dry THF (60 mL) and cooled to ~15 °C. To this cooled solution was added triphenylphosphine (2.83 g, 10.8 mmol), and the mixture was stirred at ~15 °C for 15 min and at room temperature overnight. The THF was removed under reduced pressure and the crude residue was chromatographed over silica with 20:80 ethyl acetate:hexane mixture as an eluting solvent. Compound **6b** was obtained as a colorless viscous oil which solidified on standing (2.25 g, 82 %): R_f 0.44 [20:80, ethyl acetate:hexane]; ^{1}H NMR (300MHz, $CDCl_3$) δ 5.09 (d, br, 1H), 3.96 (s, 2H), 3.94–3.84 (m, 1H), 3.66–3.40 (m, 4H), 2.21–2.10 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.47, 155.50, 81.90, 79.40, 72.80, 68.78, 49.36, 35.46, 29.96, 28.33, 28.07; IR (KBr) 3383, 2980, 1739, 1234, 1146 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{15}H_{29}NO_5Br$ 384.1211, 382.1229 found 384.1199, 382.1244.

***tert*-Butyl 7-(N^3-benzoylthymin-1-yl)-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7a:** Diethyl azodicarboxylate (350μL, 2.2 mmol) was added dropwise to stirred THF suspension (13mL) of N^3-benzoylthymin (1.50 g, 6.5 mmol), compound **6a** (0.70 g, 2.2 mmol) and triphenylphosphine (0.63 g, 2.4 mmol) at -15 °C. The reaction mixture was stirred for 20 h at room temperature. The resultant mixture was evaporated to dryness and the residue was chromatographed over silica with 50:50 ethyl acetate:hexane mixture as an eluting solvent. Compound **7a** was obtained as a foam (0.50 g, 43 %): R_f 0.27 [50:50, ethyl acetate:hexane]; ^{1}H NMR (300MHz, $CDCl_3$) δ 7.96–7.89 (m, 2H), 7.67–7.44 (m, 3H), 7.31 (s, 1H), 5.17 (d, br, 1H), 4.04–3.90 (m, 1H), 3.95 (s, 2H), 3.84–3.47 (m, 4H), 2.10–1.88 (m, 2H), 1.95 (s, 3H), 1.48 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.52, 169.12, 163.22, 155.72, 149.80, 140.89, 134.86, 131.62, 130.41, 129.07, 110.47, 81.98, 79.63, 73.28, 68.63, 47.88, 46.33, 31.62, 28.32, 28.07, 12.36; IR (KBr) 3357, 3068, 2979, 1748, 1701, 1657, 1250, 1168 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{27}H_{38}N_3O_8$ 532.2659, found 532.2651.

7-(Thymin-1-yl)-5S-[*N*-(fluoren-9-ylmethoxycarbonyl)amino]-3-oxaheptanoic acid 8a: Compound **7a** (0.93 g, 1.7 mmol) was treated with 10% HBr in acetic acid (10 mL) for 1 h at room temperature. The resultant mixture was evaporated to dryness and the residue was dissolved in 5% aq. $NaHCO_3$ (7 mL). A solution of 9-fluorenylmethyl

succinimidyl carbonate (0.64 g, 1.9 mmol) in acetonitrile (7 mL) was added to the aqueous solution with stirring and cooling in an ice bath. The reaction mixture was stirred for 12 h at room temperature and evaporated to dryness. The residue was dissolved in water (20 mL) and the aqueous layer was washed with diethyl ether (3 × 4 mL), acidified to pH 2 with 5% aq. $KHSO_4$, and extracted with ethyl acetate (4 × 4 mL). The extract was dried over $MgSO_4$. Filtration followed by solvent evaporation gave the crude product, which was purified by reverse-phase HPLC (C_{18} column; 20 mm I.D. × 250 mm L) to give compound **8a** as a white powder (0.45 g, 52 %): mp 99–103 °C; ^{1}H NMR (300MHz, DMSO-d_6) δ 11.23 (br, 1H), 7.88 (d, 2H), 7.71 (d, 2H), 7.45–7.25 (m, 5H), 4.42–4.19 (m, 3H), 4.01 (s, 2H), 3.75–3.30 (m, 5H), 1.95–1.56 (m, 2H), 1.72 (s, 3H); ^{13}C NMR (75.5MHz, DMSO-d_6) δ 171.80, 164.42, 155.95, 150.86, 143.95, 141.57, 140.83, 127.68, 127.16, 125.29, 120.17, 108.46, 72.61, 67.63, 65.37, 48.24, 46.90, 44.87, 30.60, 12.06; IR (KBr) 3277, 3065, 2952, 1701, 1543, 1249, 1133 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{26}H_{28}N_3O_7$ 494.1927, found 494.1929.

***tert*-Butyl 7-(*N*^{3}-benzoyluracil-1-yl)-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7b:** Diethyl azodicarboxylate (390μL, 2.5 mmol) was added dropwise to stirred THF suspension (15mL) of *N*^{3}-benzoyluracil (1.60 g, 7.4 mmol), compound **6a** (0.80 g, 2.5 mmol) and triphenylphosphine (0.71 g, 2.7 mmol) at -15 °C. The reaction mixture was stirred for 20 h at room temperature. The resultant mixture was evaporated to dryness and the residue was chromatographed over silica with 60:40 ethyl acetate:hexane mixture as an eluting solvent. Compound **7b** was obtained as a foam (0.60 g, 46 %): R_f 0.29 [60:40, ethyl acetate:hexane]; ^{1}H NMR (300MHz, $CDCl_3$) δ 7.97–7.90 (m, 2H), 7.68–7.45 (m, 4H), 5.79 (d, 1H), 5.23 (d, br, 1H), 4.06–3.92 (m, 1H), 3.94 (s, 2H), 3.83–3.46 (m, 4H), 2.11–1.88 (m, 2H), 1.47 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.47, 168.85, 162.51, 155.81, 149.76, 144.93, 134.98, 131.42, 130.41, 129.10, 101.86, 81.97, 79.63, 73.31, 68.60, 47.82, 46.68, 31.76, 28.29, 28.05; IR (KBr) 3356, 3087, 2979, 1749, 1705, 1665, 1251, 1167 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{26}H_{36}N_3O_8$ 518.2502, found 518.2480.

7-(Uracil-1-yl)-5S-[*N*-(fluoren-9-ylmethoxycarbonyl)amino]-3-oxaheptanoic acid 8b: Compound **7b** (0.95 g, 1.8 mmol) was treated with 10% HBr in acetic acid (10 mL) for 1 h at room temperature. The resultant mixture was evaporated to dryness and the residue was dissolved in 5% aq. $NaHCO_3$ (7 mL). A solution of 9-fluorenylmethyl succinimidyl carbonate (0.68 g, 2.0 mmol) in acetonitrile (7 mL) was added to the aqueous solution with stirring and cooling in an ice bath. The reaction mixture was stirred for 12 h at room temperature and evaporated to dryness. The residue was dissolved in water (20 mL) and the aqueous layer was washed with diethyl ether (3 × 4 mL), acidified to pH 2 with 5% aq. $KHSO_4$, and extracted with ethyl acetate (4 × 4 mL). The extract was dried over $MgSO_4$. Filtration followed by solvent evaporation gave the crude product, which was purified by reverse-phase HPLC (C_{18} column; 20 mm I.D. × 250 mm L) to give compound **8b** as a white powder (0.49 g, 56 %): mp 198–202 °C; ^{1}H NMR (300MHz, DMSO-d_6) d 11.24 (br, 1H), 7.89 (d, 2H), 7.71 (d, 2H), 7.55 (d, 1H), 7.50–7.25 (m, 4H), 5.54 (d, 1H), 4.45–4.18 (m, 3H), 4.01 (s, 2H), 3.78–3.27 (m, 5H), 1.96–1.55 (m, 2H); ^{13}C NMR (75.5MHz, DMSO-d_6) δ 171.71, 163.81, 155.90, 150.86, 145.68, 143.92, 140.78, 127.63, 127.11, 125.29, 120.14, 100.86, 72.52, 67.51, 65.29, 48.17, 46.84, 45.07, 30.56; IR (KBr) 3318, 3063, 2961, 1701,

1546, 1248, 1151 cm^{-1}; HRMS (FAB; M + H^+) calcd for $C_{25}H_{26}N_3O_7$ 480.1771, found 480.1797.

***tert*-Butyl 7-(N^4-benzoylcytosin-1-yl)-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7c:** A mixture of compound **6b** (0.32g, 0.83 mmol), N^4-benzoylcytosine (0.41 g, 1.9 mmol) and anhydrous K_2CO_3 (0.46 g, 3.3 mmol) in DMF (7mL) was stirred for 6 h at 75 °C. The resultant mixture was evaporated to dryness and the residue was dissolved in ethyl acetate. The ethyl acetate layer was washed with water and brine, then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica with ethyl acetate as an eluting solvent. Compound **7c** was obtained as a foam (0.27 g, 63 %): R_f 0.57 [ethyl acetate]; ^1H NMR (300MHz, $CDCl_3$) δ 9.57 (s, br, 1H), 7.98–7.82 (m, 3H), 7.56–7.32 (m, 4H), 5.34 (d, br, 1H), 4.20–4.05 (m, 1H), 3.90 (s, 2H), 3.85–3.42 (m, 4H), 2.13–1.90 (m, 2H), 1.41 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.35, 166.92, 162.41, 155.75, 154.39, 149.61, 133.02, 132.74, 128.62, 127.64, 96.54, 81.70, 79.30, 73.34, 68.57, 48.19, 47.72, 31.31, 28.18, 27.90; IR (KBr) 3328, 3148, 2978, 1746, 1697, 1560, 1246, 1169 cm^{-1}; HRMS (FAB; M + H^+) calcd for $C_{26}H_{37}N_4O_7$ 517.2662, found 517.2686.

7-(N^4-Benzoylcytosin-1-yl)-5S-[*N*-(fluoren-9-ylmethoxycarbonyl)amino]-3-oxaheptanoic acid 8c: Compound **7c** (0.91 g, 1.8 mmol) was treated with 10% HBr in acetic acid (10 mL) for 1h at room temperature. The resultant mixture was evaporated to dryness and the residue was dissolved in 5% aq. $NaHCO_3$ (7 mL). A solution of 9-fluorenylmethyl succinimidyl carbonate (0.68 g, 2.0 mmol) in acetonitrile (7mL) was added to the aqueous solution with stirring and cooling in an ice bath. The reaction mixture was stirred for 12 h at room temperature and evaporated to dryness. The residue was dissolved in water (20 mL) and the aqueous layer was washed with diethyl ether (3 × 4 mL), acidified to pH 2 with 5% aq. $KHSO_4$, and extracted with ethyl acetate (4 × 4 mL). The extract was dried over $MgSO_4$. Filtration followed by solvent evaporation gave the crude product, which was purified by reverse-phase HPLC (C_{18} column; 20 mm I.D. × 250 mm L) to give compound **8c** as a white powder (0.52 g, 51 %): mp 133–137 °C; ^1H NMR (300MHz, DMSO-d_6) δ 8.08–7.24 (m, 15H), 4.42–4.19 (m, 3H), 4.02 (s, 2H), 3.96–3.32 (m, 5H), 2.03–1.66 (m, 2H); ^{13}C NMR (75.5MHz, DMSO-d_6) δ 171.76, 167.48, 162.94, 155.97, 154.97, 150.31, 143.96, 140.82, 133.29, 132.72, 128.47, 127.66, 127.15, 125.27, 120.16, 95.86, 72.59, 67.56, 65.36, 48.31, 47.35, 46.89, 30.44; IR (KBr) 3411, 3067, 2952, 1702, 1490, 1248, 1140 cm^{-1}; HRMS (FAB; M + H^+) calcd for $C_{32}H_{31}N_4O_7$ 583.2193, found 583.2204.

***tert*-Butyl 7-(2-amino-6-chloropurin-9-yl)-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7d:** A mixture of compound **6b** (0.50g, 1.3 mmol), 2-amino-6-chloropurine (0.27 g, 1.6 mmol) and anhydrous K_2CO_3 (0.22 g, 1.6 mmol) in DMF (10 mL) was stirred for 6 h at room temperature. The resultant mixture was evaporated to dryness and the residue was dissolved in ethyl acetate. The ethyl acetate layer was washed with water and brine, then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed over silica with 80:20 ethyl acetate:hexane mixture as an eluting solvent. Compound **7d** was obtained as a colorless viscous oil (0.56 g, 91 %): R_f 0.39 [80:20, ethyl acetate:hexane]; ^1H NMR (300MHz, $CDCl_3$) δ 7.90 (s, 1H), 5.49 (s, br, 2H), 5.34 (d, br, 1H), 4.25–4.10 (m, 2H), 3.92 (s, 2H), 3.84–3.70 (m, 1H), 3.61–3.45

(m, 2H), 2.21–2.02 (m, 2H), 1.43 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.40, 159.03, 155.67, 153.69, 150.94, 142.86, 125.12, 81.90, 79.55, 73.18, 68.55, 48.05, 41.02, 32.32, 28.25, 27.99; IR (KBr) 3336, 2977, 1706, 1617, 1243, 1164 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{20}H_{32}N_6O_5Cl$ 471.2123, found 471.2146.

***tert*-Butyl 7-[2-amino-6-(2-nitrophenoxy)purin-9-yl]-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7e:** A solution of 2-nitrophenol (0.28 g, 2.01 mmol), DABCO (0.08 g, 0.64 mmol) and triethylamine (280 μL, 2.01 mmol) in anhydrous 1,2-dichloroethane (1 mL) was added to a solution of compound **7d** (0.32 g, 0.68 mmol) in anhydrous 1,2-dichloroethane (3 mL) and the mixture was stirred at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was dissolved in dichloromethane. The organic solution was washed with water, then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude product which was chromatographed over silica with ethyl acetate as an eluting solvent. Compound **7e** was obtained as a yellow foam (0.38 g, 98 %): R_f 0.46 [ethyl acetate]; ^{1}H NMR (300MHz, $CDCl_3$) δ 8.09–8.04 (m, 1H), 7.83 (s, 1H), 7.69–7.62 (m, 1H), 7.42–7.35 (m, 2H), 5.38 (d, br, 1H), 4.88 (s, br, 2H), 4.26–4.10 (m, 2H), 3.93 (s, 2H), 3.86–3.74 (m, 1H), 3.63–3.47 (m, 2H), 2.26–2.07 (m, 2H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 169.44, 158.92, 158.61, 155.71, 155.30, 145.58, 142.52, 141.22, 134.43, 125.93, 125.42, 125.31, 115.21, 81.87, 79.50, 73.23, 68.65, 48.18, 40.84, 32.53, 28.31, 28.03; IR (KBr) 3348, 2979, 1705, 1628, 1530, 1239, 1166 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{26}H_{36}N_7O_8$ 574.2625, found 574.2585.

***tert*-Butyl 7-[2-isobutyrylamino-6-(2-nitrophenoxy)purin-9-yl]-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7f:** Isobutyryl chloride (40 μL, 0.38 mmol) was added dropwise to a stirred solution of compound **7e** (0.18 g, 0.31 mmol) in anhydrous pyridine (1.6 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was dissolved in ethyl acetate. The organic solution was washed with 5% aq. citric acid and water, then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude product which was chromatographed over silica with ethyl acetate as an eluting solvent. Compound **7f** was obtained as a pale yellow foam (0.19 g, 93 %): R_f 0.49 [ethyl acetate]; ^{1}H NMR (300MHz, $CDCl_3$) δ 8.15–8.10 (m, 1H), 8.09 (s, br, 1H), 7.85 (s, 1H), 7.74–7.66 (m, 1H), 7.48–7.38 (m, 2H), 5.45 (d, br, 1H), 4.40–4.20 (m, 2H), 3.93 (s, 2H), 3.82–3.68 (m, 2H), 3.65–3.49 (m, 2H), 3.11–2.99 (m, 1H), 2.24–2.12 (m, 2H), 1.444 (s, 9H), 1.436 (s, 9H), 1.02 (d, 3H, J = 6.87), 1.01 (d, 3H, J = 6.78); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 177.04, 169.43, 158.94, 155.80, 154.47, 151.20, 145.56, 143.61, 142.19, 134.70, 126.45, 125.67, 125.49, 117.56, 81.85, 79.48, 73.24, 68.59, 48.04, 41.36, 34.34, 32.49, 28.29, 28.01, 18.85; IR (KBr) 3303, 2977, 1703, 1625, 1530, 1366, 1236, 1165 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{30}H_{42}N_7O_9$ 644.3044, found 644.3036.

***tert*-Butyl 7-(N^2-isobutyrylguanin-9-yl)-5S-[*N*-(*tert*-butoxycarbonyl)amino]-3-oxaheptanate 7g:** A solution of 1,1,3,3-tetramethylguanidine (0.44 g, 3.81 mmol) in anhydrous acetonitrile (4 mL) was add to a stirred solution of compound **7f** (0.28 g, 0.43 mmol) and 2-nitrobenzaldoxime (0.72 g, 4.33 mmol) in anhydrous acetonitrile (4 mL) at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was chromatographed over silica with 90:10 chloroform:methanol mixture as an

eluting solvent. Compound **7g** was obtained as a foam (0.18 g, 80 %): R_f 0.60 [90:10, chloroform:methanol]; ^{1}H NMR (300MHz, $CDCl_3$) δ 12.14 (s, 1H), 10.32–10.10 (br, 1H), 7.79 (s, 1H), 5.49 (d, br, 2H), 4.18–4.02 (m, 2H), 3.91 (s, 2H), 3.83–3.70 (m, 1H), 3.58–3.42 (m, 2H), 2.91–2.78 (m, 1H), 2.12–1.94 (m, 2H), 1.42 (s, 9H), 1.39 (s, 9H),), 1.23 (d, 3H, J = 6.87), 1.21 (d, 3H, J = 6.78); ^{13}C NMR (75.5MHz, $CDCl_3$) δ 179.47, 169.56, 155.81, 148.64, 147.61, 139.39, 120.74, 81.88, 79.41, 73.15, 68.58, 48.13, 41.05, 35.98, 32.57, 28.26, 27.96, 18.92; IR (KBr) 3200, 2978, 1685, 1611, 1251, 1164 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{24}H_{39}N_6O_7$ 523.2880, found 523.2861.

7-(N^2-isobutyrylguanin-9-yl)-5S-[*N*-(fluoren-9-ylmethoxycarbonyl)amino]-3-oxaheptanoic acid 8d: Compound **7g** (0.22 g, 0.42 mmol) was treated with 10% HBr in acetic acid (5 mL) for 5 min at room temperature. The resultant mixture was evaporated to dryness and the residue was dissolved in 5% aq. $NaHCO_3$ (4 mL). A solution of 9-fluorenylmethyl succinimidyl carbonate (0.15 g, 0.44 mmol) in acetonitrile (4 mL) was added to the aqueous solution with stirring and cooling in an ice bath. The reaction mixture was stirred for 12 h at room temperature and evaporated to dryness. The residue was dissolved in water (10 mL) and the aqueous layer was washed with diethyl ether (2 × 2 mL), acidified to pH 2 with 5% aq. $KHSO_4$, and extracted with ethyl acetate (4 × 2 mL). The extract was dried over $MgSO_4$. Filtration followed by solvent evaporation gave the crude product, which was purified by reverse-phase HPLC (C_{18} column; 20 mm I.D. × 250 mm L) to give compound **8d** as a white powder (0.18 g, 73 %): mp 125–129 °C; ^{1}H NMR (300MHz, DMSO-d_6) δ 12.11 (br, 1H), 11.71 (br, 1H), 8.15–7.23 (m, 9H), 4.35 (d, 2H), 4.22 (t, 1H), 4.15–4.00 (m, 2H), 4.01 (s, 2H), 3.65–3.52 (m, 1H), 3.50–3.36 (m, 2H), 2.83–2.59 (m, 1H), 2.17–1.77 (m, 2H), 1.10 (d, 3H, J = 6.87), 1.09 (d, 3H, J = 6.87); ^{13}C NMR (75.5MHz, DMSO-d_6) δ 180.22, 171.71, 155.99, 154.74, 148.52, 148.04, 143.89, 140.79, 139.71, 127.62, 127.07, 125.21, 120.13, 119.52, 72.50, 67.53, 65.31, 48.21, 46.87, 40.74, 34.75, 31.56, 18.90; IR (KBr) 3232, 2975, 1685, 1608, 1562, 1408, 1140 cm^{-1}; HRMS (FAB; M + H$^+$) calcd for $C_{30}H_{33}N_6O_7$ 589.2411, found 589.2443.

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References and notes.

[1] Inaki, Y.; Wada, T.; Mochizuki, E. *Jinkou Kakusan (in Japanese)*; IPC: Tokyo, Japan, 1992.

[2] Buttrey, J. D.; Jones, A. S.; Walker, R. T. *Tetrahedron* **1975**, *31*, 73.

[3] Weller, D. D.; Daly, D. T.; Olsen, W. K.; Summerton, J. E. *J. Org. Chem.* **1991**, *56*, 6000.

[4] Kuwahara, M.; Takeuchi, R.; Fujii, H.; Sisido, M. unpublished works.

[5] (a) Lenzi, A.; Reginato, G.; Taddei, M. *Tetrahedron Lett.* **1995**, *36*, 1713. (b) Lenzi, A.; Reginato, G.; Taddei, M.; Trifilieff, E. *Tetrahedron Lett.* **1995**, *36*, 1717. (c) Ciapetti, P.; Soccolini, F.; Taddei, M. *Tetrahedron* **1997**, *53*, 1167. (d) Howarth, N. M.; Wakelin, L. P. G. *J. Org. Chem.* **1997**, *62*, 5441.

[6] Almarsson, Ö.; Bruice, T. C.; Kerr, J.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7818.

[7] Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.

[8] (a) Garner, P.; Yoo, J. U. *Tetrahedron Lett.* **1993**, *34*, 1275. (b) Lewis, I. *Tetrahedron Lett.* **1993**, *34*, 5697. (c) Hyrup, B.; Egholm, M.; Nielsen, P. E.; Wittung, P.; Nordén, B.; Buchardt, O. *J. Am. Chem. Soc.* **1994**, *116*, 7964. (d) Kosynkina, L.; Wang, W.; Liang, T. C. *Tetrahedron Lett.* **1994**, *35*, 5173. (e) Krotz, A. H.; Buchardt, O.; Nielsen, P. E. *Tetrahedron Lett.* **1995**, *36*, 6937. (f) Diederichsen, U.; Schmitt, H. W. *Tetrahedron Lett.* **1996**, *37*, 475. (g) Hyrup, B.; Egholm, M.; Buchardt, O.; Nielsen, P. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083. (h) van der Laan, A. C.; Strömberg, R.; van Boom, J. H.; Kuyl-Yeheskiely, E.; Efimov, V. A.; Chakhmakhcheva, O. G. *Tetrahedron Lett.* **1996**, *37*, 7857. (i) Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. *Tetrahedron* **1996**, *52*, 15017. (j) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. *Angew. Chem. (Engl)* **1996**, *35*, 1939. (k) Tsantrizos, Y. S.; Lunetta, J. F.; Boyd, M.; Fader, L. D.; Wilson, M. -C. *J. Org. Chem.* **1997**, *62*, 5451. (l) Lesnik, E.; Hassman, F.; Bardeau, J.; Teng, K.; Weiler, K.; Griffey, R.; Freier, S. M. *Nucleosides Nucleotides* **1997**, *16*, 1775. (m) Lowe, G.; Vilaivan, T. *J. Chem. Soc. Perkin Trans. 1* **1997**, *4*, 555. (n) Fujii, M.; Yamamoto, K.; Hidaka, J.; Ohtsu, T. *Tetrahedron Lett.* **1997**, *38*, 417. (o) Cantin, M.; Schüz, R.; Leumann, C. J. *Tetrahedron Lett.* **1997**, *38*, 4211. (p) Diederichsen, U. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1743. (q) Bergmeier, S. C.; Fundy, S. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3135. (r) Wittung, P.; Nielsen, P. E.; Nordén, B. *J. Am. Chem. Soc.* **1997**, *119*, 3189. (s) Diederichsen, U. *Angew. Chem. (Engl)* **1997**, *36*, 1886. (t) Diederichsen, U. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 165. (u) Diederichsen, U.; Schmitt, H. W. *Angew. Chem. (Engl)* **1998**, *37*, 302. (v) Peyman, A.; Uhlmann, E.; Wagner, K.; Augustin, S.; Weser, C.; Will, D. W.; Breipohl, G. *Angew. Chem. (Engl)* **1998**, *36*, 2809. (w) Fujii, M.; Yoshida, K.; Hidaka, J.; Ohtsu, T. *Chem. Commun.* **1998**, *6*, 717. (x) van der Laan, A. C.; van Amsterdam, I.; Tesser, G. I.; van Boom, J. H.; Kuyl-Yeheskiely, E. *Nucleosides Nucleotides* **1998**, *17*, 219. (y) Efimov, V. A.; Choob, M. V.; Buryakova, A. A.; Kalinkina, A. L.; Chakhmakhcheva, O. G. *Nucleic Acids Res.* **1998**, *26*, 566. (z) Püschl, A.; Sforza, S.; Haaima, G.; Dahl, O.; Nielsen, P. E. *Tetrahedron Lett.* **1998**, *39*, 4707.

[9] Kuwahara, M.; Arimitsu, M.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 256.
[10] Kuwahara, M.; Arimitsu, M.; Sisido, M. *Bull. Chem. Soc. Jpn.* **1999**, *72*, in press.
[11] Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1968**, *33*, 2171.
[12] (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Lowe, G.; Vilaivan, T. *J. Chem. Soc. Perkin Trans. 1* **1997**, *4*, 539.
[13] Harnden, M. R.; Jarvest, R. L.; Bacon, T. H.; Boyd, M. R. *J. Med. Chem.* **1987**, *30*, 1636.