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Note

Synthesis of 1-(5-amino-3,5-dideoxy-3-methoxycarbonylmethyl-β-D-ribofuranosyl)thymine

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Abstract

In order to synthesize amide-linked antisense oligonucleotides, a general chemical approach was developed for the synthesis of building blocks in which an amino group and a carboxylic group are contained at the 5′ and 3′ positions of a nucleoside.

Keywords: Thymidine analogue; Oligonucleotides; Stereoselective synthesis

Antisense oligonucleotides having amide linkages replacing the phosphodiester linkage have some advantages over the naturally occurring oligonucleotides [1,2]. The amide unit is compatible with conditions required for solid-phase synthesis, and is also more stable than the phosphodiester linkage under physiological conditions. Furthermore, the lower overall charge of oligonucleotides containing neutral amide groups should facilitate penetration of cell membranes. The amide moiety is readily accessible and achiral.

Amide-linked dimers thus far reported, which were inserted into oligonucleotides, were synthesized by combining one deoxynucleoside having an amino group with another deoxynucleoside having a carboxylic acid group. Chemical synthesis of a monomer that has both an amino group and a carboxylic acid group in the same nucleoside has not been reported until now.

Recently, a new type of antisense oligonucleotide having a 2′-O-substituted group has shown significant stability toward enzymes in vivo and maintains the property of hybridization to a target sequence [3]. We are interested in the synthesis of oligoribonucleotides having amide linkages, and a general chemical approach has been developed for the synthesis of building blocks in which an amino group and a carboxylic group are contained at the 5′ and 3′ positions of a nucleoside. In this paper, we report the synthesis of such a monomer: 1-(5-amino-3,5-dideoxy-3-methoxycarbonylmethyl-β-D-ribofuranosyl)thymine (**10**).

In order to provide generality for different bases, D-xylose was used as the starting material. The synthetic route is depicted in Scheme 1.

D-Xylose was treated sequentially with acetone and then benzoyl chloride to give 5-*O*-

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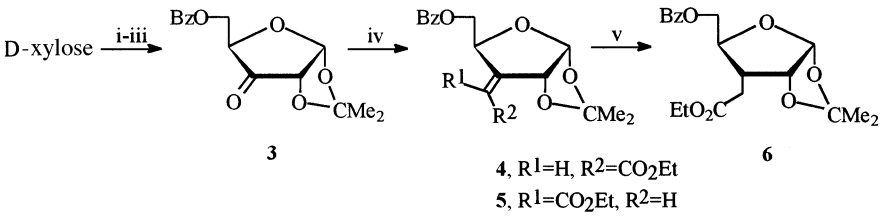


i. conc. H_2SO_4, acetone; 75.7%; ii. BzCl / Py; 87.3%; iii. CrO_3, Py, CH_2Cl_2, Ac_2O; 83.5%; iv. Wittig reagent, BuOK, DMF; 64.9%; v. 5% Pd-C/ H_2; 96.8%

Scheme 1.

Table 1
Distances between hydrogens in optimized conformations of the 3-(*S*)- and 3-(*R*)-isomers of **6**

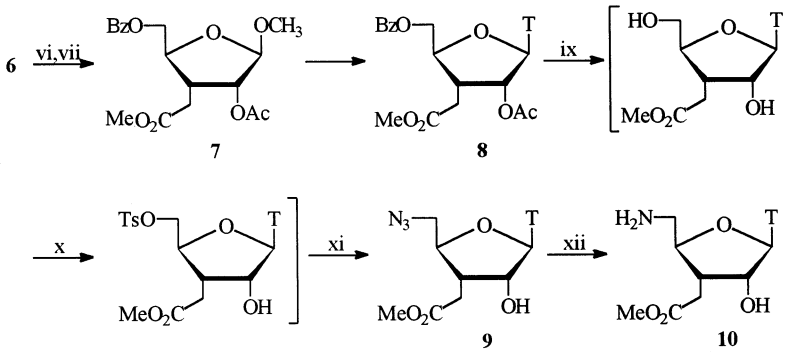
	3-(*S*) (Å)	3-(*R*) (Å)
6-CH_2···5-CH_2	2.64, 3.72, 3.89, 4.33	3.62, 4.60, 5.45, 4.33
3-H···5-CH_2	2.98, 3.98	3.06, 3.77
6-CH_2···2-H	3.78, 2.29	2.83, 3.53
1-H···6-CH_2	4.37, 2.96	4.90, 4.76

benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**2**) in 66.1% yield [4]. Oxidation of compound **2** with chromium trioxide–pyridine–acetic anhydride in dichloromethane produced the glycos-3-ulose derivative **3** in 83.5% yield [5]. 5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose (**3**) was converted by a Wittig reaction into the isomeric alkenes **4** and **5**, which were stereoselectively reduced to **6** by hydrogenation in presence of 5% palladium–carbon as catalyst.

The configuration of **6** was determined by 2D NOE. The enhancement was observed between H-3 and H-1, H-2, H-4, H-5, indicating that H-3 is above the furanosyl ring, and that compound **6** has the 3-(*R*)-configuration.

The configuration of **6** was further confirmed by computer-aided molecular mechanics analysis. The data (Table 1) are consistent with the NOE interpretation and support the 3-(*R*)-configuration.

Deprotection of the 1,2-*O*-isopropylidene group in compound **6** was efficiently achieved by hydrolysis with 1% HCl in MeOH. Compound **7** was obtained from **6** after acetylation of the 2-OH group. Compound **7** was treated with silylated thymidine and trimethylsilyl trifluoromethane sulfonate as catalyst to afford **8**. Because of the neighboring group effect of the 2-acetyl group, **8** was obtained as the β anomer in 75.3% yield [4,5]. For synthesis of compound **9**, compound **8** was deprotected under basic conditions, and then reacted sequentially with TsCl and NaN_3 to afford **9** in 43.7% yield in three steps. Compound **9** was directly reduced with hydrogen over 5% palladium–carbon catalyst, and the desired compound **10** was obtained in 65.2% yield (see Scheme 2).



vi. 1%HCl-CH_3OH; vii. Ac_2O, Py; 40.6%;

viii. silylated nucleobase, $CF_3SO_3SiC(CH_3)_3$; 75.2%; ix. K_2CO_3-CH_3OH;

x. TsCl / Py; xi. NaN_3, DMF,80 ℃; 43.7%; xii. 5%Pd-C/ H_2; 65.2%.

Scheme 2.

1. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer 243B polarimeter. IR spectra were recorded on a DE-983G spectrophotometer in KBr pellets. Mass spectra were obtained on ZAB-HS mass spectrometers. NMR spectra were recorded on VXR-300, INOVA-500 or DPK-400 spectrometers with Me_4Si as an internal standard. Exchangeable protons were detected by addition of D_2O. Column chromatography was performed on silica gel (200–300 mesh) and GF_{254} used for TLC was purchased from the Qingdao Chemical Company, China.

5-O-Benzoyl-3-(Z)-ethoxycarbonylmethylene-1,2-O-isopropylidene-α-D-ribofuranose (**4**) *and 5-O-benzoyl-3-(E)-ethoxycarbonylmethylene-1,2-O-isopropylidene-α-D-ribofuranose* (**5**). —To a stirred solution of 29.0 g (67 mmol) of Wittig reagent ($Ph_3P^+CH_2CO_2OEtBr^-$) in 150 mL of DMF, potassium *tert*-butoxide 5.6 g (50 mmol) and compound **3** 14.32 g (50 mmol) in 50.0 mL of DMF were added at 0 °C. After 10 h, H_2O (100 mL) was poured into the solution then the organic layer was extracted with CH_2Cl_2 (3×100 mL). After drying (Na_2SO_4) and concentration, the residue was applied to a column of silica gel that was eluted with petroleum ether–EtOAc (30:1–15:1) to give compound **4** 1.325 g (7.3%) and compound **5** 10.4 g (57.6%).

Compound **4**: $[\alpha]_D$ +260.7° ($CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.947 (2 H, m, Ar-H), 7.562 (1 H, m, Ar-H), 7.442 (2 H, m, Ar-H), 6.209 (1 H, t, *J* 2.0 Hz, H-1), 6.004 (1 H, d, *J* 5.0 Hz, H-6), 5.848 (1 H, t, *J* 2.0 Hz, H-2), 5.159 (1 H, m, H-4), 4.685 (1 H, dd, *J* 11.5, 3.0 Hz, H-5a), 4.506 (1 H, dd, *J* 11.5, 2.5 Hz, H-5b), 4.206 (2 H, m, $-OCH_2CH_3$), 1.438, 1.425 (each 3 H, s, $>C(CH_3)_2$), 1.313 (3 H, t, *J* 7.0 Hz, $-OCH_2CH_3$); FABMS: *m/z* 363 ($[M+1]^+$). Anal. Calcd for $C_{19}H_{22}O_7$: C, 62.98; H, 6.12. Found: C, 63.18; H 6.35.

Compound **5**: $[\alpha]_D$ +143.2° ($CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.796 (2 H, m, Ar-H), 7.520 (1 H, m, Ar-H), 7.402 (2 H, m, Ar-H), 5.981 (1 H, t, *J* 2.0 Hz, H-6), 5.952 (1 H, d, *J* 4.0 Hz, H-1), 5.751 (1 H, m, *J* 1.5, 4.5 Hz, H-2), 5.141 (1 H, m, H-4), 4.546 (1 H, dd, *J* 4.0, 12.0 Hz, H-5a), 4.397 (1 H, dd, *J* 5.0, 12.0 Hz, H-5b), 4.198 (2 H, q, *J* 7.0 Hz, $-OCH_2CH_3$), 1.480, 1.401 (each 3 H, s, $>C(CH_3)_2$), 1.255 (3 H, s, $-OCH_2CH_3$); FABMS: *m/z* 363 ($[M+1]^+$). Anal. Calcd for $C_{19}H_{22}O_7$: C, 62.98; H, 6.12. Found: C, 62.77; H, 6.16.

5-O-Benzoyl-3-deoxy-3-ethoxycarbonylmethyl-1,2-O-isopropylidene-α-D-ribofuranose (**6**).—To a solution of the mixture of **4** and **5** (7.50 g, 20.7 mmol) in EtOH (100 mL), 5% Pd–C (0.75 g) was added. The solution was stirred under 0.4 MPa hydrogen pressure at room temperature (rt) until TLC showed the completion of reaction ($\sim$20 h). The mixture was filtered to give **6** as the single product, 7.30 g (96.8%); $[\alpha]_D$ +56.25° ($CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.050 (2 H, m, Ar-H), 7.562 (1 H, m, Ar-H), 7.437 (2 H, m, Ar-H), 5.882 (1 H, d, *J* 4.0 Hz, H-1), 4.839 (1 H, t, *J* 4.0 Hz, H-2), 4.576 (1 H, dd, *J* 2.5, 12.5 Hz, H-5a), 4.356 (1 H, dd, *J* 5.0, 12.5 Hz, H-5b), 4.148 (3 H, m, H-4 and $-OCH_2CH_3$), 2.760 (1 H, dd, *J* 10.0, 17.0 Hz, H-6a), 2.507 (1 H, dd, *J* 4.5, 17.0 Hz, H-6b), 2.416 (1 H, m, H-3), 1.520, 1.333 (each 3 H, s, $>C(CH_3)_2$), 1.254 (3 H, t, *J* 7.5 Hz, $-OCH_2CH_3$); FABMS: *m/z* 365 ($[M+1]^+$), 387 ($[M+Na]^+$). Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.63; H, 6.64. Found: C, 62.39; H, 6.27.

Methyl 2-O-acetyl-5-O-benzoyl-3-deoxy-3-methoxycarbonylmethyl-β-D-ribofuranoside (**7**).—Compound **6** 7.30 g (20.1 mmol) was dissolved in a solution of 1% HCl in MeOH (100 mL). Stirring was continued for 12 h at rt, and then pyridine (10 mL) was added to stop the reaction. After evaporation, the residue was dissolved in anhyd pyridine (60 mL) and Ac_2O (5.0 mL) was added dropwise. After completion of the reaction, water (50 mL) was added. The mixture was extracted with CH_2Cl_2 (3×50 mL), and after drying and concentration, the residue was applied to a column of silica gel, and the title compound was eluted with petroleum ether–acetone (20:1–10:1) in a yield of 40.6% (2.98 g); $[\alpha]_D$ +11.45° ($CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.078 (2 H, m, Ar-H), 7.567 (1 H, m, Ar-H), 7.445 (2 H, m, Ar-H), 5.226 (1 H, d, *J* 4.5 Hz, H-2), 4.852 (1 H, s, H-1), 4.548 (1 H, dd, *J* 3.0, 12.0 Hz, H-5a), 4.316 (1 H, dd, *J* 5.0, 12.0 Hz, H-5b), 4.243 (1 H, m, H-4), 3.671 (3 H, s, $-OCH_3$), 3.336 (3 H, s, $-OCH_3$), 2.954 (1 H, m, H-3), 2.614 (1 H, dd, *J* 8.5, 16.0 Hz, H-6a),

2.512 (1 H, dd, *J* 6.0, 16.0 Hz, H-6b), 2.097 (3 H, s, OOCCH_3); FABMS: *m/z* 334 ([M − CH_3OH]$^+$). Anal. Calcd for $C_{18}H_{22}O_8$: C, 59.01; H, 6.05. Found: C, 59.25; H, 5.98.

1-(2-O-Acetyl-5-O-benzoyl-3-deoxy-3-methoxycarbonylmethyl-β-D-ribofuranosyl)thymine (**8**).—A mixture of thymidine (0.15 g, 1.2 mmol) and $(NH_4)_2SO_4$ (10 mg) in hexamethyldisilazane (HMDS) (6.0 mL) was refluxed at 125 °C to give a clear solution. The excess of HMDS was removed by evaporation at 50–60 °C. A solution of compound **7** (0.18 g, 0.49 mmol) in anhydrous MeCN (5.0 mL) was added to the residue. After the addition of trimethylsilyl trifluoromethanesulfonate (0.25 mL, 1.3 mmol), the solution was refluxed for 2.5 h. The mixture was cooled to rt, $CHCl_3$ (20 mL) was added, and the solution was poured into saturated $NaHCO_3$ solution. After stirring, filtration and concentration, the residue was applied to a column of silica gel and compound **8** was eluted by petroleum ether–acetone (5:1–3:1) to give pure **8** in 75.3% yield (170 mg); $[\alpha]_D$ +4.00° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.813 (1 H, br, H-3), 8.052 (2 H, d, Ar-H), 7.604 (1 H, m, Ar-H), 7.475 (2 H, m, Ar-H), 7.134 (1 H, d, *J* 1.0 Hz, H-6), 5.809 (1 H, d, *J* 2.5 Hz, H-1′), 5.527 (1 H, dd, *J* 2.5, 7.0 Hz, H-2′), 4.723 (1 H, dd, *J* 2.0, 12.5 Hz, H-5′a), 4.491 (1 H, dd, *J* 4.5, 12.5 Hz, H-5′b), 4.259 (1 H, m, H-4′), 3.685 (3 H, s, $-OCH_3$), 3.003 (1 H, m, H-3′), 2.625 (1 H, dd, *J* 8.5, 17.0 Hz, H-6′a), 2.536 (1 H, m, H-6′b), 2.176 (3 H, s, $OCOCH_3$), 1.672 (3 H, s, 5-CH_3); FABMS: *m/z* 461 ([M + 1]$^+$), 335 ([M + 1 − basyl]$^+$). Anal. Calcd for $C_{22}H_{24}N_2O_9$: C, 57.39; H, 5.25; N, 6.08. Found: C, 57.55; H, 5.53; N, 5.72.

1-(5-Azido-3,5-dideoxy-3-methoxycarbonylmethyl-β-D-ribofuranosyl)thymine (**9**).—Compound **8** (270 mg, 0.58 mmol) was dissolved into a mixture of MeOH (10.0 mL) and K_2CO_3 (30 mg). The solution was stirred for 26 h at rt. Acetic acid was added dropwise to the solution to keep the pH at 7. After concentration, the residue and TsCl (550 mg, 2.88 mmol) were dissolved in anhydrous pyridine (5.0 mL). After stirring for 36 h, MeOH (3.0 mL) was added to decompose the excess of TsCl. After concentration, the residue was mixed with NaN_3 (300 mg, 4.62 mmol) in DMF (10.0 mL), and the mixture was refluxed for 12 h at 80–90 °C. The solution was cooled and H_2O (5.0 mL) was added and the organic layer was extracted by CH_2Cl_2 (5 × 10 mL), EtOAc (3 × 10 mL). After concentration, the residue was chromatographed on silica gel and eluted by petroleum ether–acetone (3:1–2:1) to give compound **9** in 43.7% yield (87 mg); IR ν_{max} (cm^{-1}): 3424, 3202, 3062, 2922, 2851, 2106, 1785, 1689; ^{1}H NMR (Me_2SO-d_6): δ 11.454 (1 H, s, D_2O exchangeable, 3-NH), 7.527 (1 H, s, H-6), 5.881 (1 H, s, H-1′), 5.215 (1 H, d, *J* 6.9 Hz, H-2′), 4.037 (1 H, br, H-4′), 3.709 (1 H, d, *J* 13.0 Hz, H-5′a), 3.476 (1 H, dd, *J* 6.0, 13.2 Hz, H-5′b), 3.415–3.382 (4 H, m, H-3′ and $-OCH_3$), 3.173 (1 H, dd, *J* 7.8, 17.0 Hz, H-6′a), 2.817 (1 H, dd, *J* 9.0, 17.0 Hz, H-6′b), 2.552 (1 H, s, D_2O exchangeable, 2′-OH), 1.175 (3 H, s, 5-CH_3); FABMS: *m/z* 340 ([M + 1]$^+$). Anal. Calcd for $C_{13}H_{17}N_5O_6$: C, 46.01; H, 5.05; N, 20.63. Found: C, 45.64; H, 5.10; N 20.05.

1-(5-Amino-5,3-dideoxy-3-methoxycarbonylmethyl-β-D-ribofuranosyl)thymine (**10**).—Compound **9** (25 mg, 0.074 mmol) was added to MeOH (10.0 mL) which was acidified to pH 5–6 with acetic acid. Hydrogenation was carried out in the presence of 5% Pd–C (10 mg) as catalyst under 0.3 MPa hydrogen pressure for 30 min. After filtration and concentration, the residue was chromatographed by PTLC eluted by CH_2Cl_2–CH_3OH (10:1) to afford the title compound (15 mg, 65.2%); $[\alpha]_D$ −11.05° (CH_3OH); ^{1}H NMR (Me_2SO-d_6): δ 7.623 (1 H, s, H-6), 5.874 (1 H, s, H-1′), 5.182 (1 H, d, *J* 7.0 Hz, H-2′), 4.480 (2 H, br, 5′-NH_2), 3.851 (1 H, m, H-4′), 3.387 (4 H, overlapped, $-OCH_3$ and H-5′a), 3.169 (1 H, dd, *J* 7.0, 17.0 Hz, H-5′b), 3.001 (1 H, dd, *J* 3.5, 14.0 Hz, H-6′a), 2.921 (1 H, dd, *J* 7.5, 13.5 Hz, H-6′b), 2.858 (1 H, q, *J* 8.5 Hz, H-3′), 1.785 (3 H, s, 5-CH_3); High-resolution FABMS: *m/z* Calcd. for $C_{13}H_{20}N_3O_6$ ([M + 1]$^+$): 314.13521; Found: 314.13465. Calcd. for $C_{13}H_{19}N_3O_6Na$ ([M + Na]$^+$): 336.11716; Found: 336.11578.

Acknowledgements

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References

[1] J. Lebreton, A. Waldner, V. Fritsch, R.M. Wolf, A.D. Mesmaeker, *Tetrahedron Lett*., 35 (1994) 5225–5228.
[2] A.D. Mesmaeker, A. Waldner, J. Lebreton, P. Hoffmann, V. Fritsch, R.M. Wolf, S.M. Freier, *Angew. Chem., Int. Ed. Engl*., 33 (1994) 226–229.
[3] T.H. Keller, R. Haner, *Nucleic Acids Res*., 21 (1993) 4499–4505.
[4] C. Mathe, G. Gosselin, M.-C. Bergogne, A.-M. Aubertin, G. Obert, A. Kirn, J.-L. Imbach, *Nucleosides Nucleotides*, 14 (1995) 549–550.
[5] D.H.R. Barton, S.D. Gero, B. Quiclet-Sire, M. Samadi, *Tetrahedron Lett*., 30 (1989) 4969–4972.