Note

Synthesis of Thymidine Dimer Containing Novel (N-Acetyl)imino Linkage

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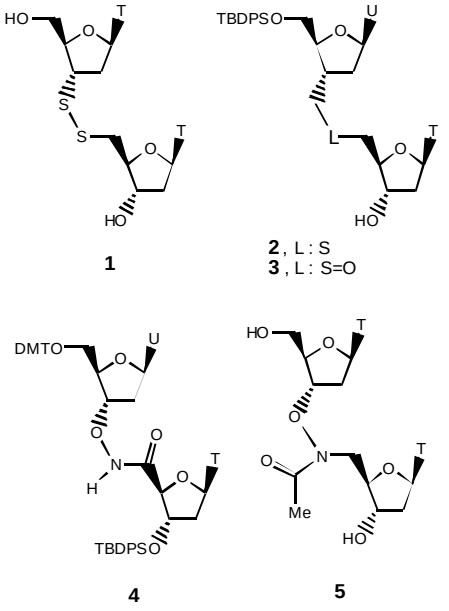
By starting with 3′-keto-5′-O-protected thymidine, 3′-O-amino-5′-t-butyl-diphenylsilyl thymidine (**9**) was prepared and coupled with 3′-O-t-butyl-diphenylsilyl-5′-formyl thymidine to form a nucleoside dimer (**11**) containing oxime linkage. The backbone of this dimer, then, underwent reduction followed by acetylation to give an (N-acetyl)imino linkage, and a novel dinucleotide (**13**) was obtained.

INTRODUCTION

Remarkable progress on the search for promising oligonucleotides as potential antisense agents has been made in the last decade.[1-5] Especially, some problems such as chirality,[2] nucleolytic degradation[4] and low binding affinity found with oligonucleotides containing phosphorus linkages have almost been overcome by adopting those containing various non-ionic, hydrolytically stable and non-chiral backbones. For example, amide-linked oligomers have proved to be fully resistant to cleavage by ribozyme[6] or have shown good binding affinity to complementary RNA.[7] On the other hand, methylene(methylimino) (MMI) linked tetramer[8] was found to be extremely stable in the presence of nucleases. These phenomena seem to imply that an internucleosidic linkage containing both imino and amide groups might be a useful modification of the backbone in antisense oligonucleotides. Although most of the reported non-natural backbones contained in dinucleotides, so far, are four-atom moieties, a few nucleoside dimers such as **1**,[9] **2**,[10] **3**[10] and **4**,[11] in which the linkages are composed of a three-atom chain (Scheme I), have also been investigated. It is noteworthy that the oligonucleotide into which the oxyamide-linked dimer[11] had been incorporated was shown to affiliate with complementary DNA as well as the sequence which possesses natural linkage only. This promising result attracted our attention and prompted us to study the synthesis of some oxyamide-like linked nucleotide dimers.

Thus, we are interested in (N-acetyl)imino linkage since it is achiral, non-ionic[12] and nearly isosteric to the oxyamide group. Furthermore, it is reasonable to anticipate that the (N-acetyl)imino group would also project itself away from the imped ing effects of the sugar moiety,[11] and the conformational rigidity[9] of the group could be in favor of the duplex helix. Herein we report the synthesis of thymidine dimer analog which contains 3′-(β)-O-N(Ac)-5′ linkage (**5**).

Scheme I

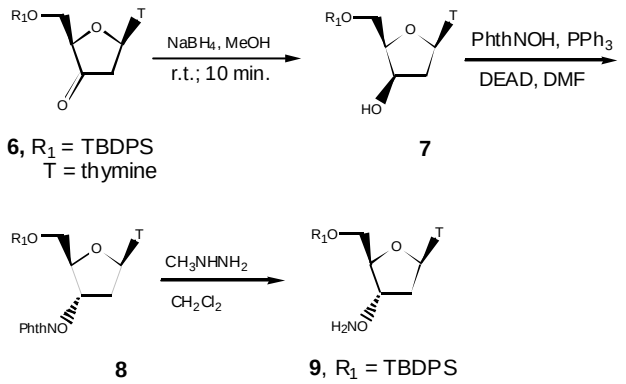


Compound **5** is expected to incorporate into an oligonucleotide, and the new strand could be applied to antisense therapy.

RESULTS AND DISCUSSION

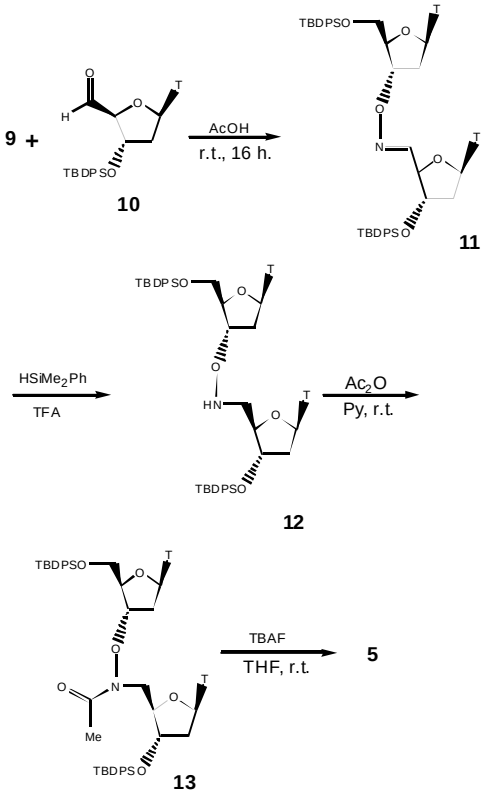
Initially, 5′-O-protected keto thymidine **6** was simply reduced with $NaBH_4$ to predominantly provide (3′-β-hydroxy)thymidine (**7**) (Scheme II) as the major product. A Mitsunobu reaction of **7** with N-hydroxyphthalimide[2] gave 3′-O-phthalimidothymidine (**8**), which was then treated with methyl hydrazine to afford 3′-O-aminothymidine (**9**). Coupling reaction of **9** with 5′-formyl thymidine (**10**)[7] proceeded smoothly at room temperature in acetic acid solution (Scheme III), and the oxime linked thymidine dimer (**11**) was obtained in 85% yield. Very recently, Gotor and coworkers[13] have just

Scheme II



reported the coupling reaction of 3′-carbazoyl thymidine with 5′-formyl-3′-O-t-butyl-dimethyl thymidine at 40 °C using methanol as the solvent and formic acid as the catalyst. We did adopt these conditions for the reaction compound **9** with compound **10**. However, product **11** was obtained in low yield. It is also noteworthy that the tert-butyl-diphenyl-silyl (TBDPS) group was a better one, compared with the trityl group or tert-butyl-dimethyl-silyl (TBDMS) group, for the protection of both 5′-O on compound **7** and 3′-O on compound **10**, since the former (TBDPS) is compatible with acetic acid at room temperature.

Scheme III



Reduction of the oxime linkage in **11** by dimethyl phenylsilane[14,15] led to the imino linkage contained in thymidine dimer **12**. We then intended to carried out acetylation reaction of the imino linkage with acetyl chloride but failed. The reaction was finally achieved by using acetic anhydride as the resource of acetyl moiety, and this resulted in the formation of dimer analogue **13** (81%). Removal of TBDPS group in **13** by TBAF furnished the target product (**5**). The N-O bond in compound **5** is stable in trifluoroacetic acid and is expected to be as stable as the N-O bond in compound **4**.[11]

In conclusion, we have synthesized a series of thymidine dimer analogues (**11**, **12**, **5**) containing 3-atom linkages. The method is facile and efficient, and the reagents adopted are inexpensive and readily available. Incorporation of the title dinucleotide into DNA sequences is being investigated and the duplex melting temperatures will be measured.

EXPERIMENTAL SECTION

General

Melting points were determined on a Fargo MP-10 melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian-200 MHz instrument, using tetramethylsilane as internal standard. Chemical shifts are given in δ values/ppm and coupling constants (*J*) are given in hertz (Hz). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm, E. Merck). Column chromatography was performed using Merck silica gel 60 (230-400 mesh). High-resolution mass spectra (HRMS) were determined on a JEOL JMX-SX/SX 102 A mass spectrometer at National Chung-Hsing University. Elemental analyses were performed in the Microanalytical Laboratory at National Taiwan University.

1-[5-O-(tert-Butyldiphenylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]thymine (7)

To a solution of **6** (3.00 g, 6.27 mmol) in methanol (100 mL), $NaBH_4$ (316 mg, 20.71 mmol) was added. The reaction mixture was stirred at room temperature for 10 min. After the solvent was removed under reduced pressure, the residue was extracted with ethyl acetate (30 mL × 3). The extracts were combined and washed with water (50 mL). The organic layer was further washed with 20% acetic acid (50 mL), 5% $NaHCO_3$ (50 mL), dried ($MgSO_4$) and concentrated. The residue was purified with silica gel column chromatography (19:1, CH_2Cl_2/MeOH) to give a 4:1 mixture of **7** (2.18 g, 72%) and its stereoisomer, 5′-O-tert-butyldiphenylsilyl thymidine (0.55 g, 18%). Properties of **7**: $[\alpha]_D^{25}$ +10.6 (c

0.35, $CHCl_3$), mp 73-74 °C; IR: 3396 (br), 2932, 1691, 1470, 1280, 1109, 1070 cm^{-1}; R_f 0.67 (19:1, CH_2Cl_2/MeOH); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.08 (s, 9H), 1.75 (s, 3H), 2.24 (dd, *J* = 14.8 Hz, 1.8 Hz), 2.55 (m, 1H), 3.59 (bs, 1H), 3.92 (dd, *J* = 7.6 Hz, 4.8 Hz), 4.04-4.13 (m, 2H), 4.51 (m, 1H), 6.20 (dd, *J* = 8.0 Hz, 2.4 Hz), 7.25-7.73 (m, 11H), 9.86 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 12.39, 19.06, 26.71, 40.88, 62.48, 70.88, 83.37, 84.79, 110.01, 127.83, 130.00, 132.47, 135.47, 137.29, 150.91, 164.24.

5′-O-tert-Butyldiphenylsilyl-3′-O-phthalimido-thymidine (8)

Diethyl azodicarboxylate (0.79 g, 4.54 mmol) was added dropwise to a mixture of **7** (1.9 g, 3.95 mmol), triphenylphosphine (1.2 g, 4.58 mmol) and N-hydroxy phthalimide (0.74 g, 4.54 mmol) in THF at 0 °C. The reaction mixture was stirred for 3 hours and then concentrated under reduced pressure. The yellowish syrup was extracted with CH_2Cl_2 (50 mL × 3), washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified with silica gel column chromatography (3:2:2, EtOAc/n-hexane/CH_2Cl_2) to give **8** as a white solid (2.18 g, 88%). $[\alpha]_D^{25}$ +102.1 (c 1.0, $CHCl_3$), mp 69-70 °C; IR: 3387 (br), 2937, 1733, 1690, 1466, 1366, 1276, 1180, 1109, 1080 cm^{-1}; R_f 0.53 (3:2:2, EtOAc/n-hexane/CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.05 (s, 9H), 1.63 (s, 3H), 2.25 (m, 1H), 2.85 (dd, *J* = 12.6 Hz, 5.2 Hz, 1H), 3.90 (dd, *J* = 12 Hz, 3Hz, 1H), 4.02 (dd, *J* = 12 Hz, 3Hz, 1H), 4.47 (m, 1H), 5.05 (d, *J* = 6 Hz, 1H), 6.58 (dd, *J* = 9.0 Hz, 5.2 Hz, 1H), 7.32-7.65 (m, 11H), 7.76-7.89 (m, 4H), 9.19 (br s, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 12.00, 19.20, 26.85, 37.18, 64.08, 76.36, 76.99, 77.63, 83.15, 84.58, 88.02, 111.17, 123.75, 127.91, 128.70, 130.08, 132.01, 132.56, 134.72, 135.03, 135.17, 135.39, 150.11, 163.7; HRMS (FAB, MH^+) calcd for $C_{34}H_{36}N_3O_7Si$: 626.2322; found: 626.2328.

3′-O-Amino-5′-O-tert-butyldiphenylsilyl-thymidine (9)

To a solution of **8** (2.0 g, 3.20 mmol) in CH_2Cl_2 (30 mL) at -10 °C was added methyl hydrazine (0.18 g, 3.84 mmol). The reaction mixture was stirred at -10 °C for 30 min. and at 0 °C for 1.5 hours. Saturated brine (30 mL) was then added to wash the reaction mixture. The organic layer was collected, dried ($MgSO_4$), and concentrated to give a white solid. The crude product was recrystallized (CH_2Cl_2/cyclohexane) to provide **9** (1.38 g, 87%). $[\alpha]_D^{25}$ +5.1 (c 0.73, $CHCl_3$), mp 85-86 °C; IR: 3387 (br), 2937, 1690, 1466, 1276, 1109, 1080 cm^{-1}; R_f 0.53 (2:1, EtOAc/n-hexane); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.10 (s, 9H), 1.57 (s, 3H), 2.06 (ddd, *J* = 13.8 Hz, 9.2 Hz, 6.0 Hz, 1H), 2.58 (dd, *J* = 13.8 Hz, 5.2 Hz, 1H), 3.85 (dd, *J* = 11.4 Hz, 2.2 Hz, 1H), 4.04 (dd, *J* = 11.4 Hz, 2.2 Hz, 1H), 4.16 (m, 1H), 4.46 (m, 1H), 6.36 (dd, *J* = 9.2 Hz, 5.2 Hz, 1H), 7.36-7.69 (m, 11H), 9.08 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 11.93, 19.36, 26.90, 38.67, 64.84, 83.68, 84.22, 84.68, 111.20, 127.95, 130.01, 132.27, 135.22, 135.54, 150.42, 163.80; HRMS (FAB, MH^+) calcd for $C_{26}H_{34}N_3O_5Si$: 496.2267; found: 496.2272.

Compound 11

To a solution of **9** (0.45 g, 0.91 mmol) in acetic acid (15 mL) was added **10** (0.45 g, 0.95 mmol). After being stirred for 3 hours at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (30 mL), dried ($MgSO_4$), and concentrated. The crude product was then purified with silica gel column chromatography (24:1, CH_2Cl_2/MeOH) to give **11** (0.73 g, 85%) as a white solid. $[\alpha]_D^{25}$ +47.8 (c 0.46, $CHCl_3$), mp 98-99 °C; IR: 3377 (br), 2937, 1690, 1471, 1109 cm^{-1}; R_f 0.57 (24:1, CH_2Cl_2/MeOH); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.10 (s, 18H), 1.86 (s, 6H), 1.96 (m, 1H), 2.08-2.25 (m, 2H), 2.48 (m, 1H), 3.20-4.08 (m, 4H), 4.44-4.88 (m, 2H), 6.20-6.41 (m, 2H), 7.19 (s, 1H), 7.30 (s, 1H), 7.34-7.68 (m, 21H), 8.82 (bs, 1H), 8.91 (bs, 1H); Anal. Calcd for $C_{52}H_{61}N_5O_9Si_2 \cdot 0.5\,H_2O$: C, 64.71; H, 7.30; N, 7.26; Found: C, 64.71; H, 7.05; N, 7.17.

Compound 12

Dimethylphenylsilane (0.13 g, 0.95 mmol) was added dropwise to a solution of **11** (0.60 g, 0.52 mmol) in trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred for 3 hours, then concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (20 mL). The organic layer was washed with saturated $NaHCO_3$ solution (50 mL), dried ($MgSO_4$), and concentrated. The crude product was purified with silica gel column chromatography (3:2:1, EtOAc/n-hexane/CH_2Cl_2) to give **12** as a white solid (0.35 g, 55%). $[\alpha]_D^{25}$ +84.5 (c 0.47, $CHCl_3$), mp 93-94 °C; IR: 3377 (br), 2928, 1690, 1466, 1428, 1109 cm^{-1}; R_f 0.64 (3:2:1, EtOAc/n-hexane/CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.09 (s, 18H), 1.56 (s, 3H), 1.86 (s, 3H), 2.02-2.13 (m, 2H), 2.33 (ddd, *J* = 13.6 Hz, 6.0 Hz, 3.0 Hz, 1H), 2.50 (dd, *J* = 13.6 Hz, 5.0 Hz, 1H), 2.64-2.83 (m, 2H), 3.75 (dd, *J* = 12 Hz, 3 Hz, 1H), 3.95-4.0 (m, 2H), 4.08 (m, 1H), 4.50 (m, 1H), 6.17-6.34 (m, 2H), 6.99 (bs, 1H), 7.34-7.67 (m, 22H), 9.05 (bs, 1H), 9.07 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 11.94, 12.52, 19.00, 19.36, 26.85, 26.99, 37.33, 39.39, 53.39, 64.76, 74.32, 83.20, 83.71, 84.63, 111.28, 127.95, 127.99, 130.06, 130.18, 132.18, 132.84, 132.88, 132.97, 135.20, 135.54, 135.66, 135.72, 136.11, 150.27, 150.36, 163.52, 163.76; HRMS (FAB, MH^+) calcd for $C_{52}H_{63}N_5O_9Si_2$: 957.4164, found: 957.4181; Anal. Calcd for $C_{52}H_{63}N_5O_9Si_2 \cdot 1.5\,H_2O$: C, 63.40;

H, 6.71; N, 7.11. Found: C, 63.17; H, 6.31; N, 6.99.

Compound 13

To a solution of **12** (0.33 g, 0.35 mmol) in pyridine (5 mL) was added acetic anhydride (0.1 g, 1.05 mmol). The reaction mixture was stirred at room temperature for 2 hours and then concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ solution (20 mL), dried ($MgSO_4$, and concentrated. The crude product was purified with silica gel column chromatography (3:2:1, EtOAc/n-hexane/CH_2Cl_2) to give **13** (0.28 g, 81%) as a white solid. $[\alpha]_D^{25}$ +54.8 (c 0.98, $CHCl_3$), mp 89-90 °C; IR: 3377, 2937, 1690, 1466, 1109, 1071 cm^{-1}; R_f 0.65 (3:2:1, EtOAc/n-hexane/CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.08 (s, 18H), 1.67 (s, 3H), 1.90 (s, 3H), 1.98 (s, 3H), 1.95-2.55 (m, 4H), 3.23 (m, 1H), 3.53-3.72 (m, 2H), 3.90-4.48 (m, 5H), 6.31 (m, 2H), 7.38-7.63 (m, 21H), 9.33 (bs, 1H), 9.55 (bs, 1H). ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 12.08, 12.19, 18.93, 19.22, 20.91, 26.74, 26.91, 37.03, 39.25, 64.40, 74.39, 82.59, 83.07, 84.26, 84.87, 85.64, 112.90, 127.92, 127.98, 128.05, 130.13, 130.20, 130.34, 131.94, 132.35, 132.59, 132.88, 134.70, 135.20, 135.39, 135.61, 150.21, 150.27, 163.85. Anal. Calcd for $C_{54}H_{65}N_5O_{10}Si_2$: C, 64.84; H, 6.55; N, 7.00; Found: C, 64.73; H, 6.89; N, 6.73.

Compound 5

A mixture of **13** (0.20 g, 0.20 mmol) and tetrabutylammonium fluoride (78 mg, 0.30 mmol) in THF (10 mL) was stirred at room temperature for 6 hours. The reaction mixture was then concentrated under reduced pressure. The residue was purified with silica gel column chromatography (14:1, CH_2Cl_2/MeOH) to give **5** (86 mg, 82%) as a white solid. $[\alpha]_D^{25}$ +65.0 (c 0.26, MeOH), mp 128-129 °C; IR: 3396 (br), 2930, 1718, 1473, 1276, 1200, 1132 cm^{-1}; R_f 0.64 (14:1, CH_2Cl_2/MeOH); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 1.76 (s, 3H), 1.77 (s, 3H), 2.04 (s, 3H), 2.10-2.34 (m, 4H), 3.59 (m, 2H), 3.82-4.16 (m, 5H), 4.70 (m, 1H), 5.17 (t, J = 5 Hz, 1H, 5′-OH), 5.35 (d, J = 4 Hz, 1H, 3′-OH), 6.15 (m, 2H), 7.46 (s, 1H), 7.69 (s, 1H), 11.26 (bs, NH), 11.30 (bs, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 12.17, 12.45, 20.99, 35.47, 48.79, 55.02, 61.67, 71.64, 82.51, 82.87, 83.53, 84.18, 84.28, 109.88, 109.95, 136.08, 136.40, 150.63, 150.66, 163.98, 164.01, 172.33; HRMS (FAB, MH^+) calcd for $C_{22}H_{29}N_5O_{10}$: 523.1914; found: 523.1922.

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Thymidine dimers; Nucleotides; Antisense agents; Acetylimino linkage.

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15. Unfortunately, neither $NaBH_4$ nor $LiAlH_4$ was a good reagent for the reduction of the oxime linkage.