# STEREOSELECTIVE SYNTHESIS OF METHYLENE PHOSPHONATE ANALOGUES OF THYMIDINE 3'-PHOSPHATE AND 2'-DEOXYURIDINE 3'-PHOSPHATE *VIA* INTRAMOLECULAR *N*-GLYCOSYLATION REACTION OF THIOGLYCOSIDES[¶]

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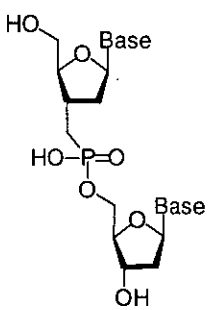
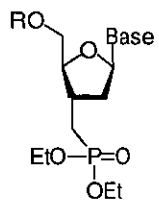
**Abstract**–Methylene phosphonate analogues of thymidine 3'-phosphate and 2'-deoxyuridine 3'-phosphate were prepared in a stereocontrolled manner through intramolecular *N*-glycosylation of phenyl 2,3-dideoxy-3-diethylphosphonomethyl-5-*O*-(2-pyrimidinyl)-1-thioglycosides, followed by acid hydrolysis.

The inhibition of protein synthesis by the antisense oligonucleotides is an area of considerable interest in medicinal chemistry.[1] It offers a highly specific chemical strategy for the disruption of diseases such as viral infection and cancer.[1] In recent years, significant advances have been made in chemical modification of antisense oligonucleotides. The relative metabolic instability of the phosphodiester function of natural oligonucleotides itself has focussed attention on the development of a variety of nucleotide analogues modified by replacement of the phosphodiester backbone with metabolically stable isosteres.[2] Replacement of the phosphodiester linkages by a methylene phosphonate functional group leading to dinucleoside monophosphate analogues (**1**) is one modification.[3] However, antisense properties of the oligomers containing **1** have not been extensively studied owing to lack of a facile method for the preparation of modified nucleotides (**2**).[3]




**1**

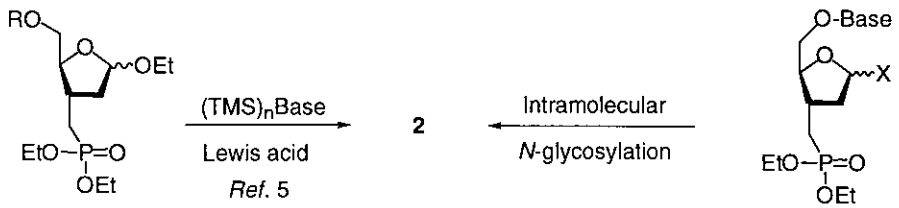



**2**

To obtain nucleotide analogues (**2**) efficiently, we and others have recently developed a facile method for the preparation of the sugar moiety (**3**)[4] of nucleotide analogues (**2**), and examined their *N*-glycosylation reactions[5] (Figure 1). However, as anticipated, the intermolecular *N*-glycosylation with **3** under the modified Vorbrüggen conditions was less useful for preparing diastereomerically pure nucleotide analogues (**2**), because of the lack of a 2α-acyloxy group for the neighboring participation. We reasoned that intramolecular *N*-glycosylation of glycosides of type (**4**) possessing nucleobases on its 5-hydroxyl would be a useful method for overcoming the problematic results associated with the intermolecular *N*-glycosylation reaction of **3** (Figure 1). Several research groups recently disclosed the usefulness of the

intramolecular *N*-glycosylation strategy for the stereocontrolled synthesis of 2'-deoxyribonucleosides.[6] In this paper, we disclose the methylene phosphonate analogues of thymidine 3'-phosphate and 2'-deoxyuridine 3'-phosphate can be prepared in a highly diastereoselective manner by application of Sugimura's intramolecular *N*-glycosylation methodology.[6]

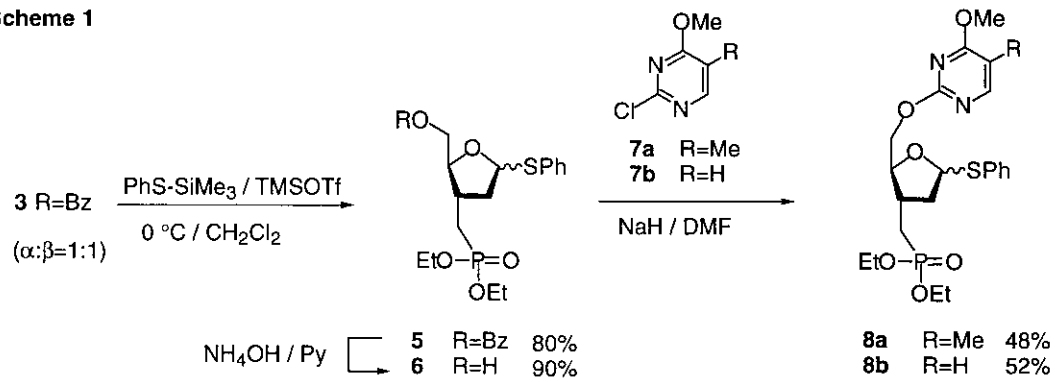
**Figure 1**




**3** R=Bz or Bn **4** X=leaving group

Requisite thioglycosides (**8a,b**) for the intramolecular *N*-glycosylation reaction were prepared from **3** (R=Bz; α:β = 1:1)[4a] as shown in Scheme 1. Treatment of **3** (R=Bz) with phenylthiotrimethylsilane in $CH_2Cl_2$ in the presence of TMSOTf, followed by removal of the benzoyl with aq. ammonia in pyridine, gave the thioglycoside (**6**) as a mixture (1:1) of anomeric isomers in 72% yield for 2 steps. The introduction of the 2-pyrimidinyl functionalities was achieved as follows: after treatment of **6** with sodium hydride in DMF at –20 °C for 60 min, the resulting sodium salts were allowed to react with **7a,b**[7] to afford **8a** and **8b** in 48% and 52% yield,[8] respectively.

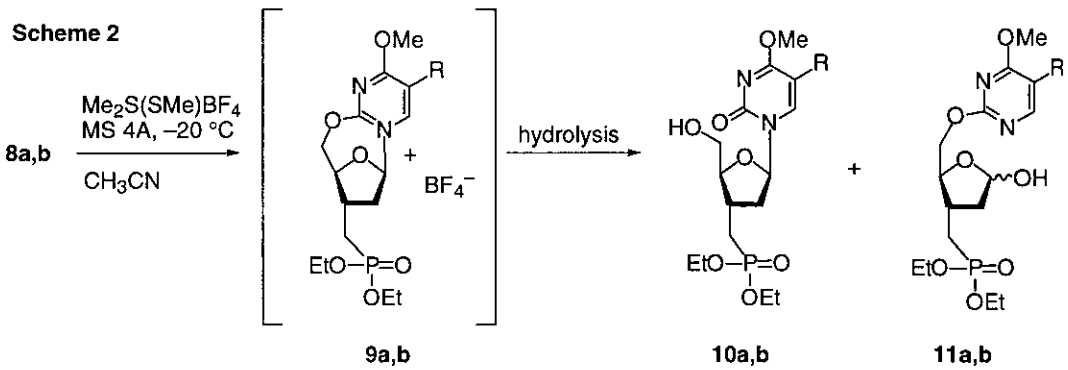
**Scheme 1**




Intramolecular *N*-glycosylation of **8a,b** was carried out according to the protocols described by Sugimura[6b,c] (Scheme 2). Upon treatment of **8a,b** with dimethyl(methylthio)sulfonium tetrafluoroborate ($Me_2S(SMe)BF_4$) in acetonitrile (0.004 M) in the presence of MS 4A at –20 °C, the starting thioglycoside (**8a,b**) disappeared within 5 h. Then, the resulting precipitates (**9a,b**) were hydrolyzed with either sat. $Na_2CO_3$ or 1 M NaOH in one-pot to give nucleotide analogues (**10a,b**). The hydrolysis conditions were critical to the selective formation of the desired **10a,b** (Table 1). When the hydrolysis of **9a** was carried out with sat. $Na_2CO_3$, the reaction proceeded slowly at room temperature to give a 1:1 mixture of **10a** and undesired **11a** in modest yield (Entry 1). However, when the hydrolysis was conducted with 1 M NaOH at 0 °C, the formation of **11a** was suppressed and the desired nucleotide analogue (**10a**) was produced highly selectively in 69% yield (Entry 2). Using the same conditions, diastereomerically pure **10b** was obtained in

80% yield (Entry 3). Careful analysis of $^1$H NMR (300 MHz) spectra and TLC of the crude materials reveals that detectable amounts of the corresponding α-glycosides are not formed in these *N*-glycosylation reactions.

**Scheme 2**



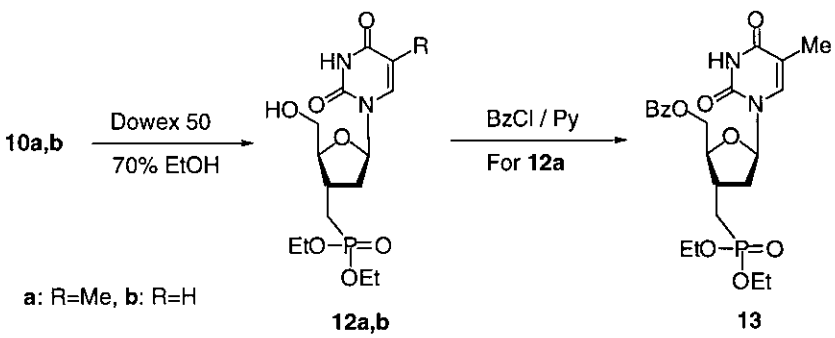

**9a,b** **10a,b** **11a,b**

Table 1. Yield of **10a,b** from thioglycosides (**8a,b**) *via* **9a,b**

| Entry | Pyrimidinium salt[a] | Hydrolysis conditions | Yield (%) of **10** | **10**:**11**[b] |
|---|---|---|---|---|
| 1 | **9a** (R=Me) | Sat. $Na_2CO_3$ / 25 °C / 12 h | 56[c] | 1:1 |
| 2 | **9a** | 1 M NaOH / 0 °C / 2.5 h | 69 | 12:1 |
| 3 | **9b** (R=H) | 1 M NaOH / 0 °C / 2.5 h | 80 | 50:1 |

[a] Prepared from **8a,b** under the conditions described in the text. [b] Determined by NMR ($^1$H and $^{31}$P) analysis of the crude material. [c] Combined yield of **10a** and **11a**.

Nucleotide analogues (**10a**) and (**10b**) were converted to methylene phosphonate analogues (**12a**) and (**12b**) of thymidine 3'-phosphate and 2'-deoxyuridine 3'-phosphate through acidic hydrolysis using an ion-exchange resin in 66% and 92% yield, respectively.[9] The spectral data of the benzoate (**13**) derived from **12a** are in good agreement with those of the authentic sample reported by Morr.[3f] At this stage, it was confirmed that the intramolecular *N*-glycosylations work well in the desired fashion.




**12a,b** **13**

In conclusion, we have developed a highly stereocontrolled method for preparation of the methylene phosphonate analogues (**12a,b**) of thymidine 3'-phosphate and 2'-deoxyuridine 3'-phosphate, which

would be valuable components for examining antisense properties of oligomers incorporated by modified dinucleotide analoges **1**.

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## REFERENCES AND NOTES

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8. Yields were not optimized. The modest yield of **8a,b** might be due to undesired de-esterification of the phosphonate functionality.

9. Spectroscopic data of **10a,b** and **12a,b**. **10a**: obtained as amorphous powder, $[\alpha]_D^{25}$ +64.7° (c 0.3, MeOH); $^1$H NMR (300 MHz, $CDCl_3$) δ 8.08 (1H, m), 6.07 (1H, dd, $J$ = 1.8, 6.7 Hz), 4.18-4.06 (4H, m), 3.97 (3H, s), 3.79-3.69 (3H, m), 2.61-2.50 (1H, m), 2.48-2.37 (1H, m), 2.35-2.26 (1H, m), 1.94 (3H, s), 1.92-1.81 (2H, m), 1.39-1.27 (6H, m); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 168.34, 157.80, 131.35, 116.77, 86.78 (d, $J_{CP}$ = 27.6 Hz), 86.49, 61.75 (d, $J_{CP}$ = 6.8 Hz), 61.51, 54.53, 39.86 (d, $J_{CP}$ = 5.2 Hz), 34.86 (d, $J_{CP}$ = 4.3 Hz), 28.63 (d, $J_{CP}$ = 142.2 Hz), 16.38 (d, $J_{CP}$ = 5.8 Hz), 12.03; $^{31}$P NMR (162 MHz, $CDCl_3$) δ 29.78; MS *m/z* 390 ($M^+$); High resolution MS *m/z* calcd for $C_{16}H_{27}N_2O_7P$ ($M^+$): 390.1556. Found: 390.1557. **10b**: obtained as amorphous powder, $[\alpha]_D^{25}$ +90.7° (c 1.5, MeOH), $^1$H NMR (300 MHz, $CDCl_3$) δ 8.30 (1H, d, $J$ = 7.4 Hz), 6.01 (1H, dd, $J$ = 1.2, 6.4 Hz), 5.84 (1H, d, $J$ = 7.4 Hz), 4.12-3.91 (5H, m), 3.89 (3H, s), 3.86-3.78 (1H, m), 3.76-3.72 (1H, m), 2.53-2.33 (2H, m), 2.24 (1H, ddd, $J$ = 6.6, 11.3, 13.5 Hz), 1.93-1.68 (2H, m), 1.28-1.21 (6H, m); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 171.74, 155.90, 143.15, 94.79, 87.62 (d, $J_{CP}$ = 13.3 Hz), 86.16, 61.95 (d, $J_{CP}$ = 6.1 Hz), 60.32, 54.17, 40.72 (d, $J_{CP}$ = 8.6 Hz), 30.32 (d, $J_{CP}$ = 3.7 Hz), 26.59 (d, $J_{CP}$ = 141.9 Hz), 16.25 (d, $J_{CP}$ = 5.8 Hz); $^{31}$P NMR (162 MHz, $CDCl_3$) δ 29.69; MS *m/z* 376 ($M^+$); High resolution MS *m/z* calcd for $C_{15}H_{25}N_2O_7P$ ($M^+$): 376.1399. Found: 376.1416. **12a**: obtained as amorphous powder, $[\alpha]_D^{25}$ +30.2° (c 0.9, MeOH); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.58 (1H broad s), 7.73 (1H, d, $J$ = 0.9 Hz), 6.07 (1H, dd, $J$ = 2.7, 7.0 Hz), 4.19-4.07 (4H, m), 3.97 (1H, dd, $J$ = 2.3, 12.8 Hz), 3.84 (1H, dd, $J$ = 2.8, 12.8 Hz), 3.74 (1H, ddd, $J$ = 2.3, 2.8, 9.0 Hz), 2.72-2.59 (1H, m), 2.38 (1H, ddd, $J$ = 2.7, 7.9, 13.8 Hz), 2.24 (1H, ddd, $J$ = 7.0, 10.7, 13.8 Hz), 1.98-1.90 (1H, m), 1.88 (3H, d, $J$ = 0.9 Hz), 1.82-1.74 (1H, m), 1.38-1.30 (6H, m); $^{13}$C NMR (75 MHz, $CD_3OD$) δ 166.31, 152.20, 138.34, 110.76, 88.19 (d, $J_{CP}$ = 20.1 Hz), 86.30, 63.48 (d, $J_{CP}$ = 6.6 Hz), 61.16, 40.64 (d, $J_{CP}$ = 3.5 Hz), 32.68 (d, $J_{CP}$ = 4.3 Hz), 27.69 (d, $J_{CP}$ = 141.6 Hz), 16.71 (d, $J_{CP}$ = 5.9 Hz), 12.50; $^{31}$P NMR (162 MHz, $CDCl_3$) δ 29.35; MS *m/z* 376 ($M^+$); High resolution MS *m/z* calcd for $C_{15}H_{25}N_2O_7P$ ($M^+$): 376.1399. Found: 376.1408. **12b**: obtained as amorphous powder, $[\alpha]_D^{25}$ +60.3° (c 0.5, MeOH); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, d, $J$ = 8.1 Hz), 6.04 (1H, dd, $J$ = 1.7, 6.9 Hz), 5.67 (1H, d, $J$ = 8.1 Hz), 4.13-4.02 (4H, m), 3.96 (1H, d, $J$ = 12.6 Hz), 3.81 (1H, d, $J$ = 12.6 Hz), 3.75-3.72 (1H, m), 2.68-2.55 (1H, m), 2.38 (1H, ddd, $J$ = 1.7, 7.6, 13.8 Hz), 2.22 (1H, ddd, $J$ = 6.9, 10.8, 13.8 Hz), 1.93 (1H, ddd, $J$ = 5.5, 15.4, 19.0 Hz), 1.77 (1H, ddd, $J$ = 8.0, 15.4, 18.8 Hz), 1.35-1.28 (6H, m); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 163.87, 150.47, 140.56, 101.65, 87.34 (d, $J_{CP}$ = 15.1 Hz), 85.16, 62.14 (d, $J_{CP}$ = 6.3 Hz), 60.55, 40.30 (d, $J_{CP}$ = 7.1 Hz), 31.09 (d, $J_{CP}$ = 2.6 Hz), 27.30 (d, $J_{CP}$ = 142.2 Hz), 16.35 (d, $J_{CP}$ = 5.6 Hz), $^{31}$P NMR (162 MHz, $CDCl_3$) δ 29.33; MS *m/z* 362 ($M^+$); High resolution MS *m/z* calcd for $C_{14}H_{23}N_2O_7P$ ($M^+$): 362.1243. Found: 362.1249.

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