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Stereoselective β-*N*-Glycosylation of 2,3-Dideoxyribofuranose Derivatives Controlled by a Methylenephosphonothioate Functional Group at the 3-Position

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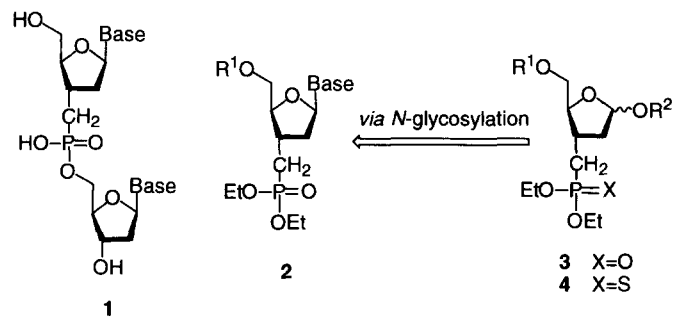
Abstract: *N*-glycolylation of 3-(diethoxyphosphorothioyl)methyl-5-*O*-benzoyl-1-*O*-ethyl-2,3-dideoxyriboses **9b** and **10b** with silylated thymine in the presence of $TiCl_4$ proceeded highly diastereoselectively (92% *de*) to give the corresponding β-nucleotide analogues in good yield. A remarkable neighboring group participation of the methylenephosphonothioate functionality was observed in the course of the β-*N*-glycosylation.

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 antisense oligonucleotides derived from naturally occurring nuclosides led to development of nucleotide analogues modified by replacement of the phosphodiester backbone with metabolically stable isosteres.[1] One tactic for the modification comprises replacement of one of the oxygen atoms in the phosphate bridge by a methylene group. Thus, the methylenephosphonate analogues **2** (R^1=Bz) of 2'-deoxyribonucleoside 3'-phosphates have been synthesized and incorporated to dinucleotide analogues **1**.[2,3] However, the reported synthesis of **2** is lengthy and non-convergent, and does not lend itself to the preparation of a variety of oligonucleotides.[2,3]

To obtain nucleotide analogues **2** of a variety of nucleobases, β-*N*-glycolylation reaction of 3-(diethoxyphosphoryl)methyl-2,3-dideoxyribofuranose derivatives **3** with nucleobases would be the most straightforward and convergent.[4,5] In this context, we have pursued *N*-glycosylation reactions of glycosyl donors **3** and **4** possessing either a methylenephosphonate or a methylenephosphonothioate functional group at the 3-position. In these reactions, we have observed a remarkable neighboring group participation of the methylenephosphonothioate functionality in favor of β-*N*-glycosyl formation. In this paper, we disclose stereoselective synthesis of glycosyl donors **3** (R_1= Bz, R_2 = Et) and **4** (R_1= Bz, R_2 = Et) as well as the results of the *N*-glycosylation reactions with bis-trimethylsilylthymine under the Vorbrüggen conditions.[6]



The radical mediated cyclyzation[4a] of conjugated phosphonate **8a** and phosphonothioate **8b** was applied to stereoselective synthesis of the required glycosyl donors **9a,b** and **10a,b** (Scheme 1). Horner-Emmons-Wadsworth reaction of 1,2-*O,O*-isopropylidene-(*R*)-glyceraldehyde **5** with either methylenebisphosphonates **6a**

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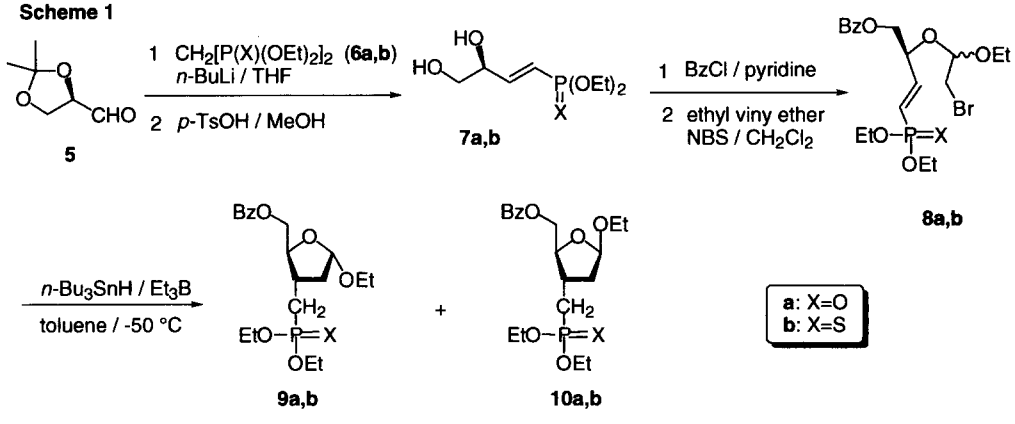
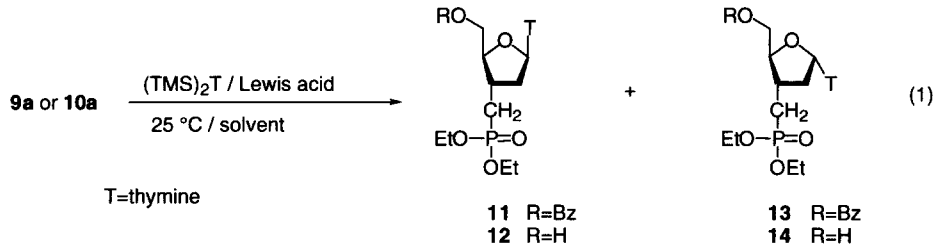
or the corresponding bisphosphonothioate **6b**, followed by acid hydrolysis, gave diols **7a,b** in good overall yields.[4a,7] Selective 4-*O*-benzoylation of **7a,b** and subsequent bromoacetalization as usual gave **8a** and **8b** as a 1:1 mixture of diastereoisomers in 54% and 41% yields for the 2 steps, respectively. Treatment of **8a,b** with *n*-Bu_3SnH in toluene in the presence of Et_3B at –50 °C according to our procedures described previously[4a] gave a 1:1 mixture of **9a,b** and **10a,b** (89% yield for a mixure of **9a** and **10a**; 95% yield for a mixture of **9b** and **10b**), individual anomers of which were readily separated by column chromatography on silica gel.[8] It is worth noting that both radical cyclyzations of **8a** and **8b** proceed with excellent diastereoselectivity (>99% *de*) with respect to the 3,4-stereogenic centers under the conditions.[9]

Scheme 1



Having established an efficient method for stereoselective synthesis of the required glycosyl donors **9a,b** and **10a,b**, in the first place, *N*-glycosylation reaction of the phosphonate analogues **9a** and **10a** with bis-trimethylsilylthymine was examined using several Lewis acids as an activator (Eq.1). The representative results are summarized in Table 1.



(1)

Table 1. *N*-glycosylation reaction of 2,3-dideoxyribofuranose derivatives **9a** and **10a** with bis-trimethylsilylthymine in the presence of Lewis acid

Entry	Glycosyl donor	Conditions[a]	Yield %[b]	Ratio (**11**:**13**)[c]
1	**9a**	$SnCl_4$ / CH_2Cl_2	59	42:58
2	**9a** and **10a** (1:1)	$TiCl_4$ / CH_2Cl_2	35	44:56
3	**9a**	TMSOTf / CH_3CN	52	61:39
4	**10a**	TMSOTf / CH_3CN	65	66:34

[a] All reactions were carried out at 25 °C for 15 h in the presence of bis-trimethylsilylthymine, prepared *in situ* from bis-trimethylsilylacetamide and thymine (2.0 eq.) in the solvent. [b] Combined yield of **11** and **13**. [c] The ratios were determined by ^{31}P NMR (162 MHz, $CDCl_3$) analysis of the crude materials.

When the reaction was carried out in the presence of either $SnCl_4$ or $TiCl_4$ in CH_2Cl_2 at 25 °C, non-stereoselective *N*-glycosylation was observed (entries 1 and 2). On the contrary, TMSOTf in CH_3CN induced β-

selective *N*-glycosylation of low diastereoselectivity at 25 °C (entries 3 and 4). The results suggest that the phosphonate functionality does not strongly participate in the formation of the *N*-glycosyl bond. An inseparable mixture of **11** and **13** thus obtained was hydrolyzed with *aq.* NH_3 in MeOH to isolate nucleotide analogues **12** and **14** in diastereomerically pure state. The spectral data of the benzoate **11** derived from the pure **12** are in good agreement with those of an authentic sample reported by Morr.[3d] On the basis of these results, the diastereoselection of *N*-glycosylation reactions of **9a** and **10a** under the conditions was unambiguously confirmed.

In an effort to develop the β-selective *N*-glycosylation with our glycosyl donors, Lewis acid-catalyzed coupling reactions of phosphonothioates **9b** and **10b** with bis-trimethylsilylthymine were explored (Eq. 2). In these reactions, it was anticipated that the phosphonothioate functional group would effectively participate in forming the bicyclic cationic intermediate **15**, a favorable intermediate for β-selective *N*-glycosylation reactions, because the phosphorus-to-sulfur double bond (1.886 Å) is much longer than the phosphorus-to-oxygen double bond (1.580 Å).[10,11] The results of the *N*-glycosylation reactions are summarized in Table 2.

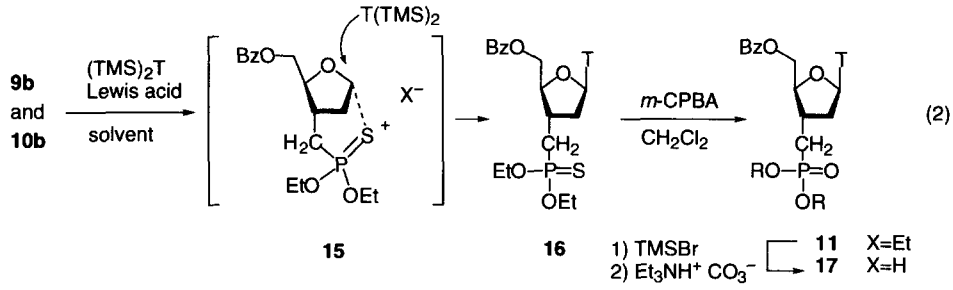


Table 2. *N*-glycosylation reaction of 2,3-dideoxyribofuranose derivatives **9b** and **10b** with bis-trimethylsilylthymine in the presence of Lewis acid

Entry	Glycosyl donor	Conditions[a,b]	Reaction time (h)	Yield of **16**	Ratio (β:α)[c]
1	**9b**	$SnCl_4$ / CH_2Cl_2	15	56	90:10
2	**9b**	$TiCl_4$ / CH_2Cl_2	8	84	90:10
3	**9b**	TMSOTf / CH_3CN	40	97	65:35
4	**10b**	$SnCl_4$ / CH_2Cl_2	15	65	92:8
5	**10b**	$TiCl_4$ / CH_2Cl_2	1	94	96:4
6	**10b**	TMSOTf / CH_3CN	40	99	65:35
7	**9b** and **10b** (1:1)	$TiCl_4$ / CH_2Cl_2	3	82	96:4

[a] Thymine(2.0 eq.) were silylated *in situ* with bis-trimethylsilylacetamide (4.0 eq.) at 40 °C in the solvent, before the *N*-glycosylation. [b] All reactions were carried out at 25 °C in the presence of 5.0 equiv. of the Lewis acid. [c] The ratios were determined by either ^{31}P NMR (162 MHz, $CDCl_3$) or HPLC analysis (Inertsil, GL-science, hexane:EtOH=90:10) of the crude materials.

When α-glycoside **9b** was treated with bis-trimethylsilylthymine in the presence of $SnCl_4$ in CH_2Cl_2 at 25 °C, β-selective *N*-glycosylation with high diastereoselectivity (80% *de*) proceeded to give **16** in 56% yield (entry 1). The yield significantly increased without a loss of the diastereoselectivity upon using $TiCl_4$ as a Lewis acid under similar conditions (entry 2). While yields of *N*-glycosylation of **9b** and **10b** induced by TMSOTf in CH_3CN were excellent, the diastereoselectivity was determined to be very low (entries 3 and 6). The β-glycoside **10b** was found to be a better substrate than **9b** for the *N*-glcosylation reactions with respect to yield and diastereoselectivity (entries 1,2 *vs* 4,5). Upon using $TiCl_4$ as a Lewis acid, the *N*-glycosylation reaction of **10b** proceeded more rapidly than that of **9b** to give **16**[12] of high diastereomeric purity (92% *de*) in 94% yield (entry

5). The reaction time and diastereoselectivity associated with the *N*-glycosylation reactions of **9b** and **10b** under the conditions suggest that the neighboring group participation of the phosphonothioate functionality of **10b** works more efficiently than that of **9b**. The results are consistent with the anomeric stereochemistry of the glycosyl donors; β-glycoside **10b** are expected to form the bicyclic cationic intermediate **15** more efficiently on the grounds of *anti*-periplanar arrangement between the 1-ethoxy and the methylenephosphonothioate functional groups. The differences in reactivity between **9b** and **10b** are tolerable for the practical synthesis of **16**, since *N*-glycosylation reaction of a mixture (1:1) of **9b** and **10b** proceeded with 92% *de* to give **16** in 82% yield (entry 7).

The thymidine analogue **16** thus obtained was converted to the corresponding methylenephosphonate analogue **11** in 81% yield by the oxidation with *m*-CPBA (5.0 equiv) in CH_2Cl_2 at room temperature, followed by aqueous work-up (Eq. 2). The ethyl protecting group of **11** was removed to give the triethylammonium salt of the phosphonic acid **17** by the literature procedure[3d] (Eq. 2). This class of phosphonic acids was previously manipulated to dinucleotide analogues **1** by Morr.[2] Thus, we have developed a highly stereoselective method for the synthesis of methylenephosphonate analogues **11** of thymidine 3'-phosphate, a useful component of dinucleotide analogue **1**, by a combination of β-*N*-glycosylation reactions controlled by the methylenephosphonothioate functionality and the oxidation of the phosphonothioate to a phosphonate functionality. Application of the methodology to stereoselective synthesis of methylenephosphonate analogues of other pyrimidine nucleotides as well as purine nucleotides is in progress and will be reported in due time.

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8. α-Anomers **9a,b** elute faster than β-anomers **10a,b** on silica gel column chromatography (hexane:EtOAc). The structural assignments of **9a,b** and **10a,b** are based on NOESY correlations (500 MHz, $CDCl_3$); relatively strong correlations between the protons on benzoyl and the 1-ethoxy functional groups were observed with **10a,b**.
9. The benzoyl protecting group of **8a,b** was found to be a good directing functionality to induce complete diastereoselectivity (>99% *de*) under the conditions. See ref. 4a for a comparison.
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11. The bond lengths are based on MM 2 calculation with CAChe Worksystem (SONY/Tektronix Corporation).
12. Selected characterization data of **16**: obtained as amorphous powder; $[\alpha]_D^{20}$+4.75 (c 1.01, MeOH) for a sample of 92% *de*; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (1H, broad s), 8.05 (2H, d with small split, J = 7.2 Hz), 7.62-7.58 (1H, m), 7.46 (2H, t, J = 7.2 Hz), 7.29 (1H, d, J = 1.0 Hz), 6.13 (1H, dd, J = 3.9, 7.0 Hz), 4.71 (1H, dd, J = 2.4, 12.5 Hz), 4.51 (1H, dd, J = 1.0, 12.5 Hz), 4.22-4.02 (5H, m), 2.83-2.73 (1H, m), 2.43 (1H, ddd, J = 3.9, 8.2, 14.1 Hz), 2.35-2.22 (2H, m), 2.09-1.99 (1H, m), 1.65 (3H, s), 1.31-1.24 (6H, m); ^{31}P NMR (162 MHz, $CDCl_3$) δ 95.61; MS *m/z* 497 (M^++1), 371 (M^+-thy).