ORGANIC LETTERS

2013
Vol. 15, No. 23
5948–5951

Stereoselective Synthesis of *P*-Modified α-Glycosyl Phosphates by the Oxazaphospholidine Approach

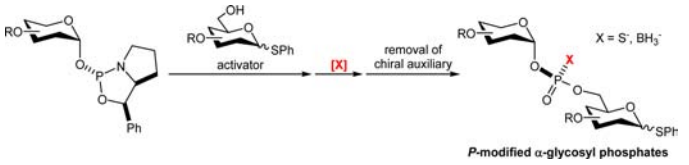
Mihoko Noro,[†] Shoichi Fujita,[†] and Takeshi Wada*[,†,‡]

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan, and Department of Medicinal and Life Schience, Faculty of Pharmaceutical Sciences, Tokyo University of Science 2641, Yamazaki, Noda, Chiba 278-8510, Japan

twada@rs.tus.ac.jp

Received September 26, 2013

ABSTRACT



α-Glycosyl phosphate derivatives are widely known as constituents of biomolecules. To date, several types of non-natural α-glycosyl phosphates including "*P*-modified analogs" have been synthesized to investigate their characteristics. Herein a new approach to the stereoselective modification of the intersugar phosphorus atom in α-glycosyl phosphates by use of the oxazaphospholidine method is presented. Via this approach, the dimers of α-glycosyl phosphorothioates and α-glycosyl boranophosphates were obtained efficiently and stereoselectively.

α-Glycosyl phosphate derivatives are widely known as constituents of capsular polysaccharides in pathogenic bacteria such as *Neisseria meningitidis* and *Streptococcus pneumoniae*,[1,3] or the glycocalyx of parasitic protozoans such as *Leishmania* and *Trypanosoma*.[2,3]They have repeating units of phosphoglycans, many of which are considered to be important factors in biophenomena such as immunological responses and infection. Therefore, investigation of their roles as biomolecules is being carried out for the purpose of elucidating their biomechanisms and applying them in drug development.[1−3]

With such aims, several types of non-natural α-glycosyl phosphate analogs have been synthesized in previous studies.[4] Among them, we focused on the "*P*-modified analogs," where one of the nonbridging oxygen atoms is replaced by a different atom or a substituent. The intersugar phosphodiester linkage is considered to be an important factor affecting several biophenomena. Thus, *P*-modified analogs could become useful tools to clarify their biological modes of action. Moreover, the phosphodiester moiety is known to make a large contribution to chemical stability, and proper modification of the phosphorus atom is reported to further improve chemical stability.[5] For these reasons, *P*-modified glycosyl phosphate derivatives are considered promising analogs.

These molecules have a chiral center at the intersugar phosphorus atom. Because biological activity generally differs between stereoisomers, stereochemically controlled

[†] The University of Tokyo.
[‡] Tokyo University of Science.

(1) (a) Trotter, C. L.; McVernon, J.; Ramsay, M. E.; Whitney, C. G.; Mulholland, E. K.; Goldblatt, D.; Hombach, J.; Kieny, M.-P. *Vaccine* **2008**, *26*, 4434–4445. (b) Joshi, V. S.; Bajaj, I. B.; Survase, S. A.; Singhal, R. S.; Kennedy, J. F. *Carbohydr. Polym.* **2009**, *75*, 553–565.

(2) (a) Mendonça-Previato, L.; Todeschini, A. R.; Heise, N.; Agrellos, O. A.; Dias, W. B.; Previato, J. O. *Curr. Org. Chem.* **2008**, *12*, 926–939. (b) Mendonça-Previato, L.; Todeschini, A. R.; Heise, N.; Previato, J. O. *Curr. Org. Struct. Biol.* **2005**, *15*, 499–505. (c) Hansson, J.; Oscarson, S. *Curr. Org. Chem.* **2000**, *4*, 535–564.

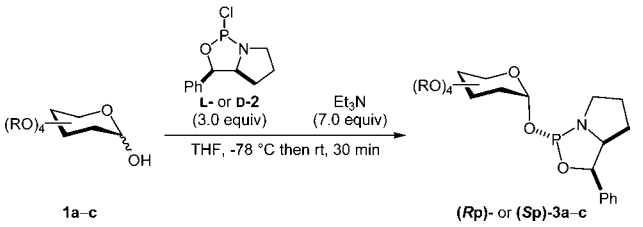
(3) Nikolaev, A. V.; Botvinko, I. V.; Ross, A. J. *Carbohydr. Res.* **2007**, *342*, 297–344.

(4) (a) Routier, F. H.; Higson, A. P.; Ivanova, I. A.; Ross, A. J.; Tsvetkov, Y. E.; Yashunsky, D. V.; Bates, P. A.; Nikilaev, A. V.; Ferguson, M. A. J. *Biochemistry* **2000**, *39*, 8017–8025. (b) Singh, A. H.; Newborn, J. S.; Raushel, F. M. *Bioorg. Chem.* **1988**, *16*, 206–214. (c) Cohen, S. B.; Halcomb, R. L. *J. Org. Chem.* **2000**, *65*, 6145–6152. (d) Klinger, M. M.; McCarthy, D. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 197–200. (e) Sheu, F. R.; Frey, P. A. *J. Biol. Chem.* **1978**, *253*, 3378–3380. (f) Yamazaki, Y.; Nagatsuka, Y.; Oshima, E.; Suzuki, Y.; Hirabayashi, Y.; Hashikawa, T. *J. Immunol. Methods* **2006**, *311*, 106.

(5) (a) Prosperi, D.; Panza, L.; Poletti, L.; Lay, L. *Tetrahedron* **2000**, *56*, 4811–4815. (b) Matsumura, F.; Oka, N.; Wada, T. *Org. Lett.* **2008**, *8*, 1557–1560.

10.1021/ol402785h
Published on Web 11/13/2013

Table 1. Preparation of Monomer Units



entry	2	product	R	Man/ Glc	yield (%)	diastereo ratio[a] *trans*:*cis*	diastereo ratio[a] α:β[b]
1	**L**	**(*R*p)-3a**	Bn	Man	quant	>99:1	>99:1
2	**D**	**(*S*p)-3a**	Bn	Man	74	>99:1	>99:1
3	**L**	**(*R*p)-3b**	Bz	Man	90	>99:1	>99:1
4	**D**	**(*S*p)-3b**	Bz	Man	83	>99:1	>99:1
5	**L**	**(*R*p)-3c**	Bz	Glc	82	>99:1	95:5
6	**D**	**(*S*p)-3c**	Bz	Glc	65	>99:1	>99:1

[a] Estimated by ^{31}P NMR. [b] For isolated product.

derivatives are required for assessment of their biological properties. However, stereoselective synthesis of *P*-modified glycosyl phosphates has not been accomplished yet.

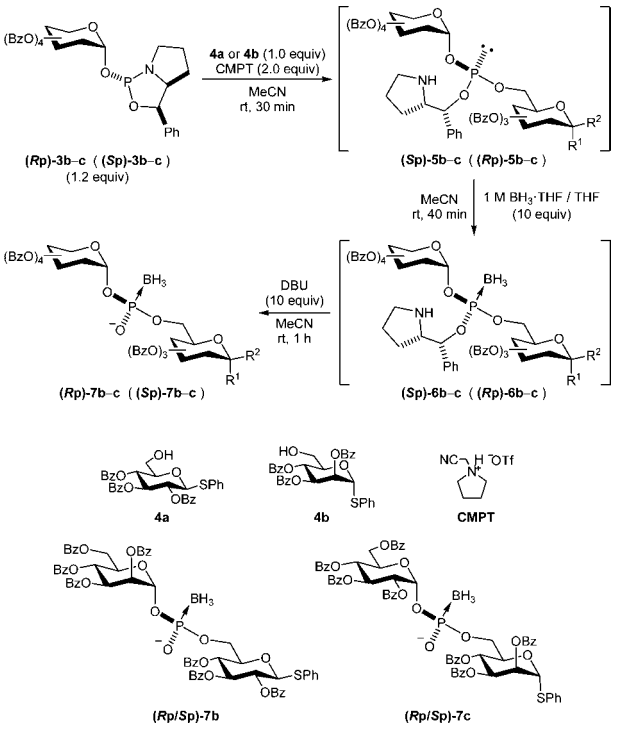
Against such a background, we herein present a new approach to the stereoselective modification of the intersugar phosphorus atom with the "oxazaphospholidine method." This method has been used for the stereocontrolled synthesis of phosphorothioate DNA,[6a] RNA,[6b] and boranophosphate DNA[6c] for nucleic acid drugs. We applied this method to the synthesis of glycosyl phosphate analogs and first accomplished the stereocontrol of the intersugar phosphorus atom.

We prepared monomer units **(*R*p)-** and **(*S*p)-3a**–**c** as shown in Table 1. They were synthesized by the stereocontrolled phosphitylation of sugar derivatives bearing free anomeric hydroxyl groups with (4*S*,5*R*)- or (4*R*,5*S*)-2-chloro-1,3,2-oxazaphospholidine derivatives (L- or D-**2**), which were obtained by the reaction between PCl_3 and the corresponding 1,2-amino alcohols as previously reported.[4a] Mannopyranosyl monomers **(*R*p)-** and **(*S*p)-3a**–**b** were obtained in a highly α-selective manner because of the stereoelectronic effect of their axial 2-OH. As for glucopyranosyl derivatives, precursor **1c**, which was a mixture of α and β isomers, was recrystallized several times from AcOEt or CH_2Cl_2/hexane to obtain α-rich isomers. The isomers almost retained their stereochemical purity through the following phosphitylation step even though the reaction conditions could induce anomerization. This experimental fact suggests that phosphitylation of **1** was substantially more rapid than its anomerization.[7]

Using the synthesized monomer units, we attempted to synthesize stereoregulated *P*-modified dimers. In this study, as representative *P*-modified analogs, we selected boranophosphates and phosphorothioates to be the synthetic targets.

First, we describe the synthesis of glycosyl boranophosphates. We employed *O*-benzoyl-protected monomer units **(*R*p)-** and **(*S*p)-3b**–**c** because the benzyl groups could not be removed without associated deboronation of boranophosphodiester derivatives.[8]

Scheme 1. Synthesis of Glycosyl Boranophosphates



The *P*-modified dimers were synthesized via a three-step reaction in one pot (Scheme 1). The monomer units **(*R*p)-** or **(*S*p)-3b**–**c** were condensed with 1-*O*-thiophenyl-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside **4a** or 1-*O*-thiophenyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside **4b**, in the presence of *N*-(cyanomethyl)pyrrolidinium triflate (CMPT), which is an acid activator we developed for the stereospecific condensation of oxazaphospholidine monomers.[9] Then, the resultant diastereopure glycosyl phosphite intermediates **(*S*p)-** or **(*R*p)-5b**–**c** were boronated by treatment with 1 M BH_3·THF in THF to give the glycosyl boranophosphotriesters **(*S*p)-** or **(*R*p)-6b**–**c**.

In the last step, the removal of the chiral auxiliary was attempted in the presence of several basic reagents (Table 2). Each reaction was monitored by ^{31}P NMR, and it appeared that pyridine, 2,6-lutidine, and triethylamine (TEA) were not sufficiently basic to activate the nitrogen atom of the

(6) (a) Oka, N.; Yamamoto, M.; Sato, T.; Wada, T. *J. Am. Chem. Soc.* **2008**, *130*, 16031–16037. (b) Oka, N.; Kondo, T.; Fujiwara, S.; Maizuru, Y.; Wada, T. *Org. Lett.* **2009**, *11*, 967–970. (c) Wada, T.; Maizuru, Y.; Shimizu, M.; Oka, N.; Saigo, K. *Bioorg. Mol. Chem. Lett.* **2006**, *16*, 3111–3114.

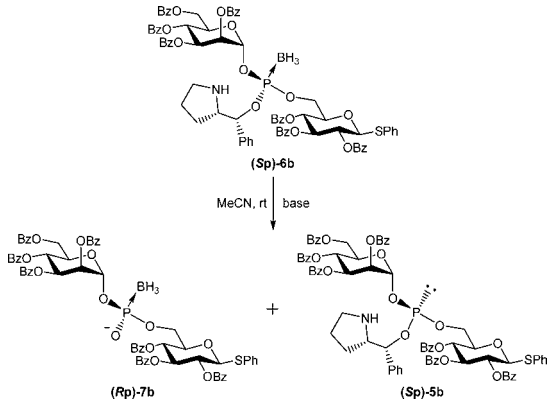
(7) We observed that glucopyranosyl derivative **1** (α:β = 79:21) gave monomer unit **3** (α:β = 80:20) through this phosphitylation step.

(8) Unpublished data.

(9) (a) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2002**, *124*, 4962–4967. (b) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2003**, *125*, 8307–8317.

pyrrolidine ring of the chiral auxiliary and caused deboronation as a competitive side reaction. Although such a side reaction was not observed when using *N*,*N*-diisopropylethylamine (DIPEA), the intended reaction was not complete. In contrast, the chiral auxiliary was successfully removed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 5 min and glycosyl boranophosphodiester **7b** was quantitatively produced as a DBU salt. These results suggest that the main reaction should be rapid enough compared to deboronation in this case.

Table 2. Reaction Conditions for the Removal of Chiral Auxiliaries



	reaction conditions			
entry	base	equiv	time	**6b**:**7b**:**5b**[a]
1	pyridine	10	overnight	–[b]
2	2,6-lutidine	20	overnight	85:0:15
3	TEA	30	overnight	8:77:15
4	DIPEA	10	overnight	29:71:0
5	DBU	10	1 h	0:100:0

[a] Estimated by ^{31}P NMR. [b] Not monitored.

Next, we describe the synthesis of glycosyl phosphorothioates (Scheme 2). It was performed by condensation of monomer units and sugar derivatives bearing free 6-OH, and the chiral auxiliaries were removed by the previously reported procedure.

In the sulfurization reaction of benzyl-protected glycosyl phosphites using *N*,*N*′-dimethylthiuram disulfide (DTD)[10] as a sulfurizing reagent, the formation of undefined byproducts was observed. Because it seemed to be caused by the decomposition of the *P*-activated intermediate, we changed the protecting group of the sugar hydroxyl moiety from a benzyl group to a benzoyl group, which destabilized the oxocarbenium cation. In addition, we tested other types of sulfurizing reagents such as POS[11] and S_8 whose sulfurizing reactions are expected to be more rapid than that of DTD.

The results are shown in Table 3. As we expected, the benzoyl-protected glycosyl phosphites were more efficiently

Scheme 2. Synthesis of Glycosyl Phosphorothioates

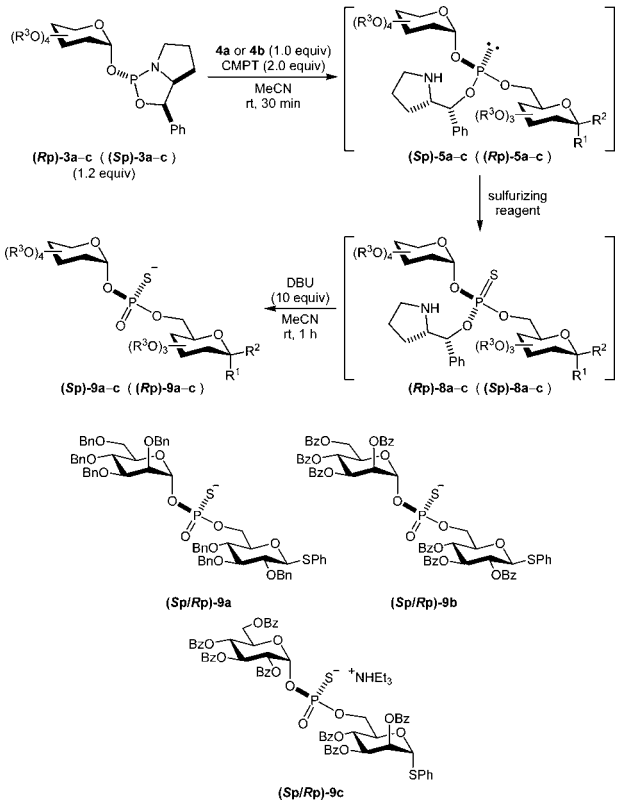
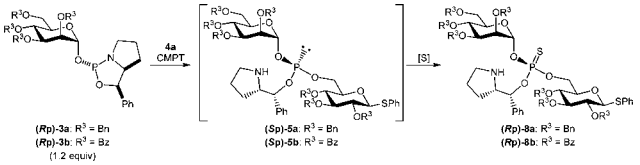
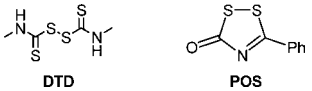


Table 3. Reaction Conditions for Sulfurization



entry	R^3	sulfurizing reagents	product ratio of **8a**–**b**[a]	diastereo ratio[a]
1	Bn	DTD	46%	–[b]
2	Bn	POS	80%	–[b]
3	Bn	S_8	quant	>99:1
4	Bz	DTD	87%	–[b]
5	Bz	POS	quant	>99:1
6	Bz	S_8	quant	>99:1

[a] Estimated by ^{31}P NMR. [b] Not determined.



transformed into the corresponding glycosyl phosphorothioate triesters than the benzyl-protected compounds. In addition, both of the alternative sulfurizing reagents improved

(10) Song, Q.; Wang, Z.; Sanghvi, Y. S. *Nucleosides, Nucleotides, Nucleic Acids* **2003**, *22*, 629–633.
(11) Roy, S. K.; Tang, J. Y. U.S. Patent 6, 500, 944, 2002.

Table 4. Stereocontrolled Synthesis of Dimers of Glycosyl Boranophosphate and Phosphorothioate Derivatives

entry	target compounds		yield	diastereo ratio[a] (*R*p:*S*p)
1	(*R*p)-Man-PB-Glc	**(*R*p)-7b**	79%	>99:1
2	(*S*p)-Man-PB-Glc	**(*S*p)-7b**	76%	>1:99
3	(*R*p)-Glc-PB-Man	**(*R*p)-7c**	77%	>99:1
4	(*S*p)-Glc-PB-Man	**(*S*p)-7c**	96%	>1:99
5	(*R*p)-Man-PS-Glc	**(*R*p)-9b**	82%	>99:1
6	(*S*p)-Man-PS-Glc	**(*S*p)-9b**	80%	>1:99
7	(*R*p)-Glc-PS-Man	**(*R*p)-9c**	77%	>99:1
8	(*S*p)-Glc-PS-Man	**(*S*p)- 9c**	73%	>1:99

[a] Estimated by ^{1}H or ^{31}P NMR.

this step and the formation of byproducts was reduced compared to the reaction using DTD.

On the basis of these results, we synthesized eight types of *P*-modified glycosyl phosphate dimers. The structure of α-D-Glc-(1-*P*-6)-D-Man is a fragment of glycocalyx lipophosphoglycans of *Leishmania*. The results are shown in Table 4.

In conclusion, we successfully accomplished an efficient and highly stereoselective synthesis of glycosyl boranophosphates and glycosyl phosphorothioates in different combinations of sugar moieties. These results suggest that this method could be a versatile approach to the synthesis of a wide variety of *P*-modified glycosyl phosphate analogs. In addition, this method is expected to be applicable to solid-phase synthesis, which can enable the synthesis of oligoglycosyl phosphate analogs.

Supporting Information Available. Experimental details and characterization data, including ^{1}H, ^{13}C, and ^{31}P spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.