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Synthesis of Glycosyl Boranophosphates and Their Applications as Precursors of Glycosyl Phosphate Analogues

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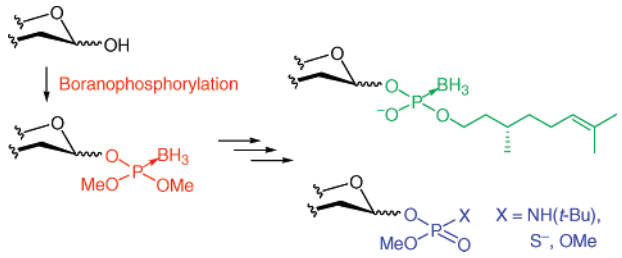
ABSTRACT



Glycosyl boranophosphate triesters were synthesized via a boranophosphorylation of reducing sugars. The usefulness of the resultant glycosyl boranophosphates as versatile chemically stable precursors of various glycosyl phosphate derivatives is demonstrated.

Glycosyl phosphates are the constituents of bacterial cell-wall lipopolysaccharide and capsular polysaccharide antigens.[1] They also exist in the glycocalyx lipophosphoglycans and secreted proteophosphoglycans of the protozoan parasite *Leishmania* and work as important factors for the infectivity and virulence of the parasite.[2] Since such polysaccharides and phosphoglycans are mostly unique to each organism, their structural and functional elucidation would lead to the development of antibacterial and anti-*Leishmania* vaccines and other drugs with high specificity.[2,3] Chemically synthesized glycosyl phosphates and their analogues have been used as indispensable tools for the structural and functional studies of the above-mentioned biomolecules and have also been studied as vaccine and drug candidates.[4,5]

However, the synthesis of the glycosyl phosphates is still a challenging task due to the lability of the anomeric phosphate moiety and the need to control the anomeric stereochemistry.[3,4] Currently, the glycosyl phosphate derivatives, especially complicated fragments of biomolecules, are usually synthesized by the *H*-phosphonate method[6] due to the good reaction efficiency.

The method is also advantageous because it can afford not only the natural phosphoglycans having phosphate diester linkages but also those having phosphorus-substituted linkages via the *H*-phosphonate diester intermediates.[7,8] However, the method has some drawbacks, such as the instability of

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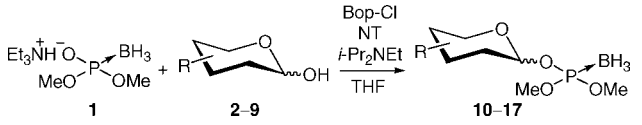
the glycosyl *H*-phosphonate diester intermediates, which often causes reduction in yield.[3,6a,b]

With this background, we sought to develop an efficient method to synthesize glycosyl phosphate derivatives and focused on glycosyl boranophosphates[8,9] as key compounds. The boranophosphate is one of the *P*-substituted phosphate analogues in which one of the nonbridging oxygen atoms of the phosphate diester linkage is replaced with a BH_3 group. It was originally developed as an isoelectronic, hydrophobic modification of oligonucleotides with high chemical and enzymatic stability[10] and also applied to the glycosyl phosphate derivatives to probe the phosphate functions[8a,b] and to improve the stability of the glycosyl phosphate moiety.[8c] In addition to these properties, the boranophosphate has another advantage that we focused on: it can be quantitatively converted into the corresponding *H*-phosphonate diesters by treatment with a trityl (Tr) cation,[11] indicating that the above-mentioned unstable glycosyl *H*-phosphonate diester intermediates can be protected as the stable boranophosphates throughout the synthesis. In this paper, we describe the synthesis of the glycosyl boranophosphates and their preliminary applications as precursors to glycosyl phosphate analogues.

Several glycosyl boranophosphate derivatives have been synthesized by a nucleophilic substitution[9] and the *H*-phosphonate method,[8] but the former method has been used for the synthesis of few monophosphate derivatives, and further applications have not been reported. The latter one suffers from some problems, such as the necessity of strictly anhydrous conditions, undesired reduction of substrates by boronating reagents, and insufficient boronation of sterically hindered intermediates. All of these problems had also been imposed on the synthesis of nucleoside boranophosphates by the *H*-phosphonate method.[10,12] Recently, we have demonstrated that these problems could be solved by a "boranophosphorylation", in which a simple boranophosphate diester (e.g., dimethyl boranophosphate) was used as a phosphorylating reagent of alcohols, so that the problematic boronation of reducible, sterically hindered molecules can be avoided.[13]

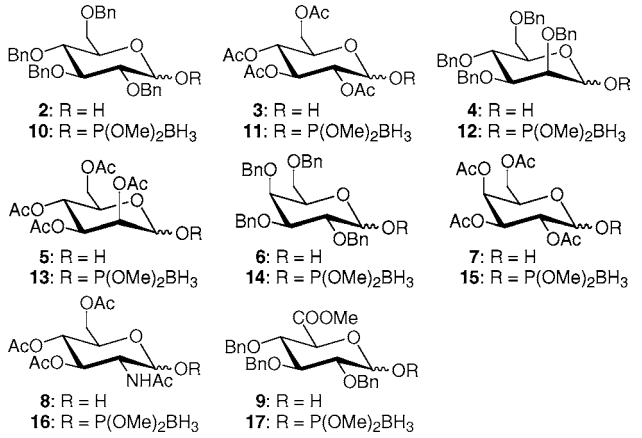
Boranophosphorylations of reducing sugars **2**−**9** with triethylammonium dimethyl boranophosphate[13a] (**1**) were performed in the presence of bis(2-oxo-3-oxazolidinyl)-phophinic chloride (Bop-Cl) as a condensing reagent, 3-nitro-1,2,4-triazole (NT) as a nucleophilic catalyst, and *i*-Pr_2NEt (Table 1). The reactions were completed within 1 h at rt for all of the substrates used, and the desired glycosyl boranophosphate triesters **10**−**17** were isolated as mixtures of α- and β-isomers in modest to good yields (56−91%, entries 1, 5−11). Any traces of decomposition were not observed during aqueous workup and silica gel column chromatography except for the *N*-acetylglucosamine derivative **16**, which was partly lost during the chromatography. Lower temperature reduced the reaction rate but did not significantly affect the anomeric ratio (entries 2−4).

Table 1. Boranophosphorylation of Reducing Sugars **2**−**9**



entry	reducing sugars (α:β)[b]	T (°C)	time (h)	yield[a] (%) (α:β)[b]
1	**2**[c] (4:1)	rt	1	90 (2:3)
2	**2**[c] (4:1)	0	1	91 (3:2)
3	**2**[d] (4:1)	0	2	88 (1:1)
4	**2**[d] (4:1)	−20	24	87 (1:1)
5	**3**[c] (1:1)	rt	1	88 (1:1)
6	**4**[c] (4:1)	rt	1	85 (4:1)
7	**5**[c] (4:1)	rt	1	78 (7:3)
8	**6**[c] (4:1)	rt	1	88 (2:3)
9	**7**[c] (3:7)	rt	1	91 (1:1)
10	**8**[c] (>9:1)	rt	1	56 (4:1)
11	**9**[c] (7:3)	rt	1	91 (3:7)

[a] Isolated yield. [b] Anomeric ratios were determined by ^{1}H NMR. [c] **2**−**9**/**1**/Bop-Cl/NT/*i*-Pr_2NEt molar ratio = 1/3/5/5/10. [d] **2**−**9**/**1**/Bop-Cl/NT/*i*-Pr_2NEt molar ratio = 1/2/2.5/2.5/10.



The study showed that the glycosyl boranophosphate triesters were chemically more stable than the phosphate triester counterparts. It has been reported that the attempts to synthesize glycosyl phosphate triesters by phosphorylation of reducing sugars resulted in the decomposition of the generated triesters by the attacks of nucleophilic species, such

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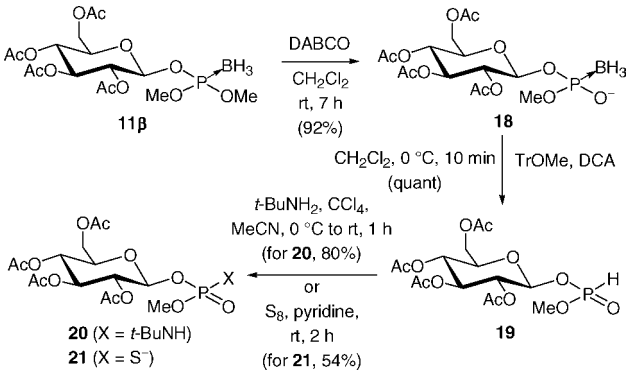
as Cl^-, in the reaction mixtures,[14] or via the neighboring group participation of *N*-acyl groups for glucosamine derivatives.[15] Decomposition of glycosyl phosphate triesters by the aqueous workup or silica gel column chromatography has also been reported.[16] In contrast, noticeable decomposition was not observed for the glycosyl boranophosphate triesters in the reaction mixtures containing highly nucleophilic species, such as Cl^- and 3-nitro-1,2,4-triazolide, or during the workup and chromatography except for the glucosamine derivative.

As mentioned above, we have reported that the boranophosphate diester linkages can be transformed into the corresponding *H*-phosphonate diesters upon addition of a Tr cation.[11a] If this method is applicable to the glycosyl boranophosphates, these compounds could be used as the chemically stable precursors to the unstable *H*-phosphonate diester counterparts. Applicability of this strategy to the glycosyl boranophosphates was investigated with the β-anomer of **11** (**11**β),[9] which was separated from the α-anomer by column chromatography, as a model compound. First, **11**β was converted into a diester **18** by treatment with 1,4-diazabicyclo[2.2.2]-octane (DABCO) (Scheme 1). Though this

Scheme 1. Synthesis of Glycosyl Phosphate Analogues **20** and **21** via *H*-Phosphonate Diester Intermediate **19**

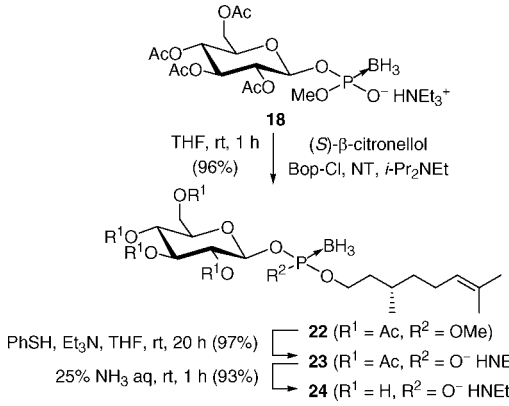


reagent is generally used to deboronate phosphine−borane complexes,[17] almost exclusive demethylation of the boranophosphate triester occurred in this case (ca. 5% deboronation was observed by ^{31}P NMR). The diester **18** was then treated with a Tr cation generated in situ from TrOMe and dichloroacetic acid (DCA).[11a] ^{31}P NMR analysis of the reaction mixture clearly showed that the desired glycosyl *H*-phosphonate diester **19** was quantitatively generated as a pair of *P*-diastereomers by the appearance of two singlets at 8.14 and 8.04 ppm with a J_{PH} value of ca. 725 Hz,[18] which is characteristic of *H*-phosphonate diesters.

The *H*-phosphonate **19** thus obtained was successfully converted into glycosyl phosphoramidate and phosphorothioate derivatives (**20** and **21**, respectively). As is widely used for oligonucleotides, *H*-phosphonate diesters are versatile precursors to a variety of *P*-substituted phosphate derivatives, such as phosphorothioates, phosphoramidates, alkylphosphonates, phosphorofluoridates, phosphoroselenoates, etc., as well as the natural phosphate.[7] Therefore, the strategy that uses chemically stable glycosyl boranophosphates as precursors to the *H*-phosphonate counterparts would significantly expand the availability of compounds containing *P*-substituted glycosyl phosphate moieties.

The results shown above indicate that the synthesis of glycosyl phosphate-containing biomolecules and a variety of *P*-substituted analogues would be feasible by using the glycosyl boranophosphates as stable precursors. In addition, the glycosyl boranophosphate itself is interesting as an isoelectronic, hydrophobic modification for these biomolecules. However, two factors still remain for the synthesis, i.e., condensation of the glycosyl boranophosphate with alcohols to form molecules and deprotection of the sugar and phosphate moieties in the final step. To examine these factors, citronellyl glucosyl boranophosphate (Glc-PB-Cit) (Scheme 2, **24**), a model compound of a glycosyl donor in the eukaryotic *N*-glycosylation pathway,[19] was synthesized.

Scheme 2. Synthesis of Glc-PB-Cit **24**



Condensation of (*S*)-β-citronellol with **18** proceeded under similar conditions for the boranophosphorylation of reducing sugars to afford a triester **22** in an excellent yield. The triester **22** was also a stable compound, and any decomposition was not observed during the workup and purification by silica gel column chromatography. Demethylation of the boranophosphate linkage and deacetylation of the sugar moiety were carried out by treatment with PhS^- and aqueous ammonia, respectively, and the fully deprotected **24** was isolated as a triethylammonium salt in an excellent yield. Thus, both the condensation and the deprotection steps proceeded nearly quantitatively; this would enable the synthesis of rather

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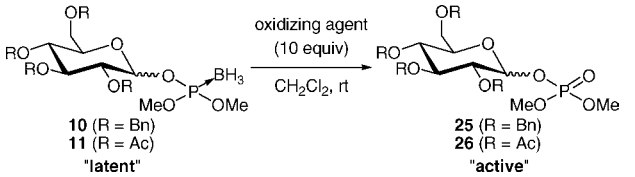
complicated biomolecules and their analogues with various *P*-substitutions.

Next, we describe another potential application of the unique stability of the glycosyl boranophosphates. Since glycosyl phosphate triesters have been widely used as highly reactive glycosyl donors[16a,20] and their reactivity can be tuned by changing the phosphorus substituents,[21] the reactivity of the glycosyl boranophosphate triesters as glycosyl donors is also intriguing. To examine the issue of reactivity, boranophosphate triesters **10** and **11**β were treated with TMSOTf (1 equiv) and 2-propanol (10 equiv) in CH_2Cl_2 at rt. Surprisingly, both of them were intact for at least 24 h. We considered that the exceptional stability of the glycosyl boranophosphate triesters under Lewis acid-mediated glycosylation conditions would be useful for an "active-latent" glycosylation strategy[22] if they could be converted into the "active" phosphate triester counterparts. It is well-known that BH_3 complexes of tricoordinate organophosphorus compounds are stable toward oxidizing agents,[23] but certain reagents, such as *m*-chloroperbenzoic acid (*m*-CPBA), are reported to oxidize them into oxides.[24]

Attempts to convert a boranophosphate triester **11**β into the phosphate triester counterpart (**26**)[25] directly by using *m*-CPBA, *tert*-butyl hydroperoxide (TBHP), or (+)-[(8,8-dichlorocamphoryl)sulfonyl]oxaziridine (DCSO) showed that *m*-CPBA promoted a virtually quantitative conversion within 10 min (Table 2, entry 1), whereas the substrate was resistant to TBHP and DCSO (entries 2, 3).

Benzyl-protected substrate **10** was also converted into the corresponding phosphate triester (**25**) by *m*-CPBA (entry 4). The anomeric ratios of the substrates were not changed during the oxidation process (entries 1, 4). The results indicate that the glycosyl boranophosphate triesters would be applicable as "latent" glycosyl donors that could be converted into the "active" glycosyl phosphate triesters. In addition, the direct oxidation of the boranophosphates into the phosphates may be applicable to the synthesis of biomolecules having glycosyl phosphate moieties.

Table 2. Oxidative Conversion of Glycosyl Boranophosphate Triesters **10**,**11**β to Phosphate Triesters **25** and **26**



entry	boranophosphate triester (α:β)	oxidizing agent	time	yield (%) (α:β)
1	**11**β (0:1)	*m*-CPBA	10 min	>90[a] (0:1)
2	**11**β (0:1)	TBHP	48 h	<10[b] (n.d.[c])
3	**11**β (0:1)	DCSO	48 h	<10[b] (n.d.[c])
4	**10** (43:57)	*m*-CPBA	10 min	>91[a] (43:57)

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

In conclusion, the study demonstrated that glycosyl boranophosphate triesters were obtained by boranophosphorylation of reducing sugars and worked as stable precursors of the corresponding *H*-phosphonate diesters and phosphate triesters. The unique properties of glycosyl boranophosphates would be applicable to the synthesis of biomolecule analogues as well as to an "active-latent" glycosylation strategy. Further study on these subjects is currently underway.

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Supporting Information Available: Experimental details and characterizion 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.

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