

## Recent Progress in the Synthesis of Glycosyl Phosphate Derivatives

## 糖-1-リン酸誘導体の合成化学の最近の進展

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**Key Words:** glycosyl phosphate, phosphoglycan, H-phosphonate, boranophosphate, phosphoramidite**Abstract**

Phosphoglycans consisting of glycosyl phosphate repeating units have received much attention as synthetic targets mainly for their potential use as vaccines against pathogenic bacteria and protozoa. This review describes recent progress in the synthesis of glycosyl phosphates, especially of phosphoglycans, focusing on methods of synthesizing intersaccharide phosphodiester linkages.

**要 約**

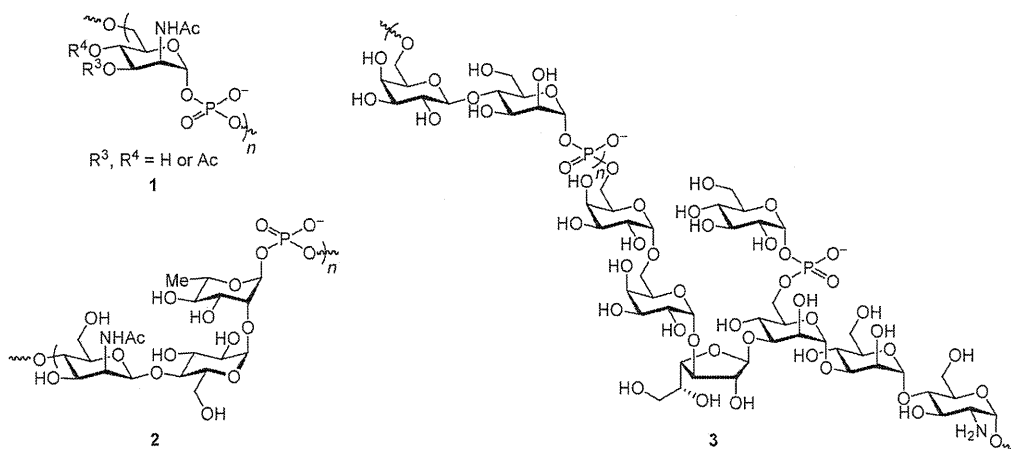
糖-1-リン酸繰り返し構造からなるホスホグリカンは、病原性細菌や寄生性原虫に対するワクチンなどとしての需要から、化学合成のターゲットとして注目を集めている。本総説では、このホスホグリカンを中心とする糖-1-リン酸誘導体の化学合成に関する最近の研究について、リン酸ジエステル結合の合成法に焦点をあてて述べる。

**A. Introduction**

Phosphoglycans consisting of glycosyl phosphate repeating units are found in capsular polysaccharides (CPSs) and cell-wall lipopolysaccharides (LPSs) of bacteria, cell-wall and extracellular polysaccharides of yeasts, and surface glycoalyx and secreted glycoproteins of protozoan parasites (1). Because these polysaccharides and glycoconjugates define the immunological specificity of the parent microorganism, the primary constituent phosphoglycans have been studied as potential vaccines, particularly against virulent species such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Leishmania* (Fig. 1) (2–5). In fact, the CPSs isolated from *N. meningitidis* and *S. pneumoniae* as well as their conjugates with proteins are used as vaccines (2, 3). Furthermore, the CPSs of bacteria as well as the glycoalyx and secreted glycoproteins of parasites play important roles in infection and the evasion of the host innate defense, contributing to the virulence of these microorganisms. For this reason, the structure–activity relationship and biosynthesis of these polysaccharides and glycoconjugates have also been important subjects of research (1, 6–8). In addition, glycosyl phosphates are the primary constituents of glycosyl donors in the biosynthesis of carbohydrates, such as sugar nucleotides (9, 10) and dolichol phosphate sugars (11, 12). Sugar nucleotides are useful for the enzymatic synthesis of oligosaccharides and glycosides (13, 14). Chemically modified analogs of these

**A. はじめに**

糖-1-リン酸繰り返し構造からなるホスホグリカンは、細菌の莢膜多糖や細胞壁リポ多糖、酵母の細胞壁多糖や細胞外多糖、寄生性原虫の糖衣や分泌糖タンパク質などに含まれる(1)。これらの多糖や複合糖質は、多くの場合抗原決定基として働くことから、その中心構造であるホスホグリカンは、特に感染被害の大きい髄膜炎菌、肺炎球菌、リーシュマニアなどに由来するもの(図1)を対象に、ワクチンとしての応用を指向した研究が行われてきた(2–5)。実際に、髄膜炎菌や肺炎球菌から抽出精製した莢膜多糖やそのタンパク質との複合体はワクチンとして用いられている(2,3)。また、細菌の莢膜多糖や寄生虫の糖衣、及び分泌糖タンパク質は、これらの病原体の宿主への感染や宿主内での免疫機構の回避などにおいて重要な役割を担い、病原性の一因となっている。そのため、これらの主構成要素であるホスホグリカンの分子構造と機能の関係や、生合成経路に関しても重要な研究対象となっている(1,6–8)。加えて、糖-1-リン酸は、糖ヌクレオチド(9,10)やドリコールリン酸糖(11,12)など、生体内で働く糖供与体の基本構造でもある。糖ヌクレオチドは、酵素を用いた糖鎖や配糖体などの合成原料として用いられる(13,14)。また、これらの生体内糖供与体の化学修飾アナログは、糖鎖の生合成経路をターゲットとするプローブや阻害剤としても有用である



**Fig. 1. Glycosyl phosphate repeating units of *Neisseria meningitidis* and *Streptococcus pneumoniae* type 19F capsular polysaccharides (1, 2);  $\beta$ -D-Galp-(1  $\rightarrow$  4)- $\alpha$ -D-Manp-(1-P-6) repeating units and heptasaccharide core of *Leishmania donovani* lipophosphoglycan (3).**

glycosyl donors are used as probes and inhibitors against the biosynthetic pathways of the oligosaccharides (15, 16).

Chemically synthesized glycosyl phosphate derivatives have become indispensable for all of these studies (1, 4, 5, 8, 10). Chemical synthesis of biomolecules is generally advantageous over methods using isolation of those from living organisms in terms of producing sufficient quantities of pure compounds at low costs. The availability of chemically modified analogs is another advantage of the chemical synthesis. Such chemical modifications can also be used to develop the aforementioned probes and inhibitors as well as to improve molecular properties such as physicochemical and biological stabilities (1, 5).

The synthesis of phosphoglycans essentially requires efficient formation of phosphodiester bonds for intersaccharide linkages, which is the most distinct difference from the synthesis of usual oligosaccharides. Thus, this review describes recent progress in the synthesis of glycosyl phosphate derivatives, especially phosphoglycans, focusing on methods to synthesize intersaccharide phosphodiester linkages. Methods to synthesize oligonucleotides can be applied for this purpose in many cases; however, the lability of glycosyl phosphate units must always be considered. Glycosyl phosphate derivatives, particularly electrically neutral triesters, are prone to undergo elimination reactions, generating oxocarbenium ions. These elimination reactions proceed rapidly, especially under acidic conditions. Owing to this property, glycosyl phosphate triesters can be used as glycosyl donors for the synthesis of oligosaccharides (17, 18), whereas the lability of glycosyl phosphate derivatives can be a major factor in reducing the yield of products in the synthesis of phosphoglycans.

(15, 16)。

化学合成によって得られる糖-1-リン酸誘導体は、今やこの様な研究に欠かせないものとなっている(1, 4, 5, 8, 10)。化学合成は多くの場合、生体試料からの分離精製を経る手法に比べ、純粋な化合物をより低コストで大量に供給できる。また、化学合成のもう一つのメリットとして、生体分子の化学修飾アナログが入手できることが挙げられる。この様な化学修飾は、前述のプロープや阻害剤の開発に加え、生体分子の化学的、生物学的安定性を高めるなどの性質の強化改善にも用いることができる(1, 5)。

ホスホグリカンなどの糖-1-リン酸誘導体の化学合成と通常の糖鎖合成との一番の相違点は、リン酸ジエステル結合による糖の連結であり、リン酸ジエステル結合の効率的な合成が鍵となる。そこで本総説では、ホスホグリカンを中心とする糖-1-リン酸誘導体の化学合成について、最近の研究成果を中心に、リン酸ジエステル結合の合成法に焦点をあてて述べる。ホスホグリカンの化学合成では、同じく糖がリン酸ジエステル結合によって連なった核酸の合成法を参考にすることも多い。しかしながら、前者では糖-1-リン酸構造の不安定さに常に留意する必要がある。糖-1-リン酸誘導体、特にリン酸部位に負電荷を持たないリン酸トリエステル中間体では、リン酸基がアノマー位から容易に脱離してオキソカルベニウムイオンを生成する。この脱離反応は、特に酸性条件下迅速に進行する。この様な性質から、糖-1-リン酸トリエステルは、糖鎖合成における糖供与体として利用される(17, 18)が、逆にホスホグリカンの合成では、糖-1-リン酸トリエステルを始めとする不安定な中間体の分解が収率低下の原因となることも多い。

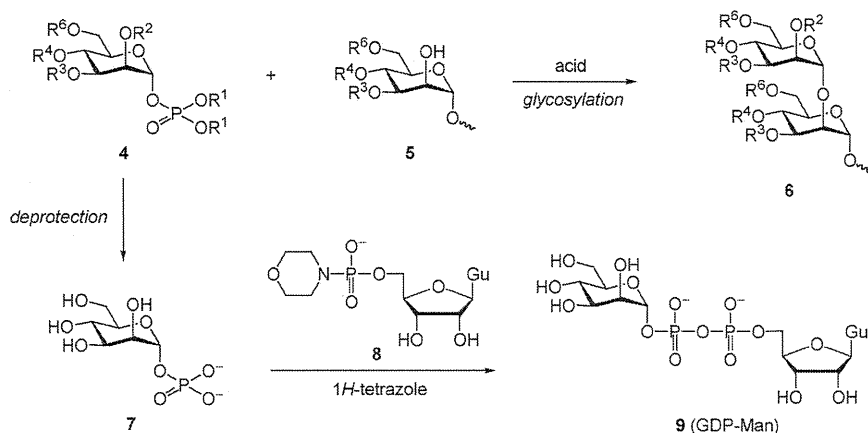


Fig. 2. Application of  $\alpha$ -D-mannosyl phosphate triester **4** to the syntheses of oligomannoside **6** and GDP-mannose **9**.

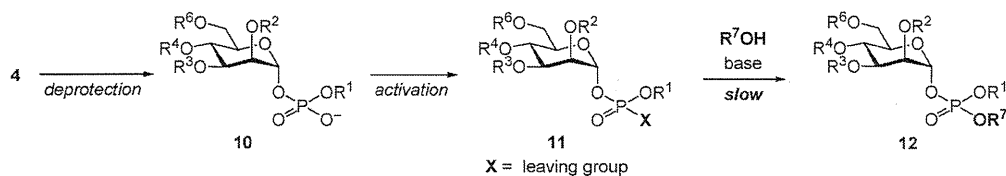


Fig. 3. Synthesis of glycosyl phosphate derivatives **12** by condensation of glycosyl phosphate diester derivatives **10** with alcohols ( $R^7OH$ ).

### B. Synthesis of Glycosyl Phosphate Derivatives Using Glycosyl Phosphate Triesters as Starting Materials

As mentioned above, glycosyl phosphate triesters are widely used as glycosyl donors to synthesize oligosaccharides because they can be easily activated by Brønsted and Lewis acids (Fig. 2, **4**  $\rightarrow$  **6**) (17, 18). For this reason, the synthesis of glycosyl phosphate triesters has been well studied, and both the  $\alpha$ - and  $\beta$ -isomers are available from various types of protected sugars (19–23). In addition, these triesters can be used to synthesize other glycosyl phosphate derivatives, especially sugar nucleotides. For example, GDP-mannose **9** (Fig. 2) can be synthesized by condensation of mannosyl phosphate monoester **7** that is derived from the triester derivative **4** with guanosine 5'-monophosphate derivative **8** (10, 24, 25).

However, it is difficult to apply these glycosyl phosphate triesters to the synthesis of more complex glycosyl phosphate derivatives such as phosphoglycans. Figure 3 shows a synthetic route to convert the glycosyl phosphate triester derivative **4** into other glycosyl phosphate derivatives **12** via a glycosyl phosphate diester intermediate **10** (phosphotriester method). The diester intermediate **10** can be easily synthesized by partial deprotection of the triester **4**, while subsequent activation with a condensing

B. 糖-1-リン酸トリエステルを出発物質とする糖-1-リン酸誘導体の合成

前述の通り、糖-1-リン酸トリエステルはBrønsted酸やLewis酸によって容易に活性化されるため、糖供与体として糖鎖合成に多用されている(図2、**4**  $\rightarrow$  **6**) (17, 18)。そのため、その化学合成に関する研究は進んでおり、現在では糖骨格、保護基、アノマー位の立体配置を問わず様々な誘導体の合成が可能である(19–23)。一方、これらは糖供与体としての利用だけでなく、他の糖-1-リン酸誘導体、特に糖ヌクレオチドの合成にも用いられる。例えば、GDP-マンノース**9**(図2)は、糖-1-リン酸トリエステル**4**を脱保護して得られるモノエステル**7**とグアノシン-リン酸誘導体**8**との間の縮合反応によって得られる(10, 24, 25)。

この様に、糖-1-リン酸トリエステルの応用例は多いものの、ホスホグリカンなどの複雑な糖-1-リン酸誘導体の合成に用いることは難しい。図3に示す通り、糖-1-リン酸トリエステル**4**のリン酸部位を部分的に脱保護することによって糖-1-リン酸ジエステル**10**を合成し、脱水縮合剤による活性化を経て糖水酸基などのアルコールと縮合すれば、ホスホグリカンの伸長など、より複雑な糖-1-リン酸誘導体への誘導が可能

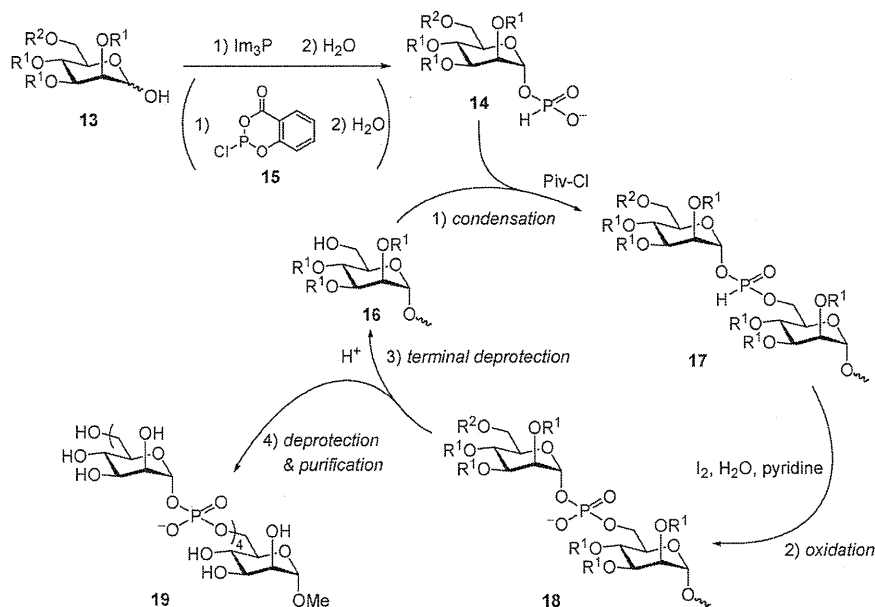


Fig. 4. Synthesis of *Hansenula capsulata* Y-1842 exophosphomannan fragment 19 by *H*-phosphonate method.

agent and condensation with alcohols would give the desired products 12. However, it has been reported that the yields of the products obtained by such reactions are generally low owing to the slowness of the reaction and the presence of side reactions such as those between the hydroxy group of sugars and the condensing agent (1, 26). Moreover, the active intermediate 11 is prone to decomposition *via* an elimination and/or nucleophilic substitution at the anomeric position owing to the leaving ability of the anomeric phosphate group enhanced by the activation. The phosphodiester method using glycosyl phosphate monoesters gives the desired products in better yields than the phosphotriester method; however, the yields drop significantly where more sterically hindered secondary hydroxy groups are involved in the condensation reactions (27, 28).

### C. Synthesis of Glycosyl Phosphate Derivatives Using the *H*-Phosphonate Method

As described in the previous section, it is difficult to synthesize phosphoglycans and other complex glycosyl phosphate derivatives from glycosyl phosphate triesters. Currently, such complex molecules are generally synthesized by the *H*-phosphonate method (1, 4, 8). Figure 4 shows as an example the reaction cycle for the synthesis of a fragment of the extracellular phosphomannan from *Hansenula capsulata* Y-1842 19 (29). This method uses a glycosyl *H*-phosphonate monoester 14 as a monomer, which can be synthesized by anomeric phosphorylation of the corresponding reducing

である (4 → 12) (リン酸トリエステル法)。しかしながら、この脱水縮合反応は遅く、また縮合剤と糖水酸基との副反応などが競合するため、目的物の収率は一般に低い (1, 26)。加えて、化合物 11 の様な活性中間体は、元々不安定な糖-1-リン酸誘導体のグリコシド結合が、リン酸ジエステルの活性化に伴ってさらに不安定化されているため、リン酸基の脱離や求核置換反応を経て容易に分解すると考えられる。同じく糖-1-リン酸トリエステルから合成可能な糖-1-リン酸モノエステルと糖水酸基との脱水縮合を用いる手法 (リン酸ジエステル法) では、リン酸トリエステル法より良好な収率で脱水縮合生成物が得られるものの、反応性が低い第二級水酸基との縮合反応では目的物の収率が著しく低下する (27, 28)。

### C. *H*-ホスホネート法による糖-1-リン酸誘導体の合成

前節で述べた様に、糖-1-リン酸トリエステルを出発物質としてホスホグリカンなどの分子を合成することは難しい。現在、このような分子は一般に *H*-ホスホネート法を用いて合成される (1, 4, 8)。例として、酵母 *Hansenula capsulata* Y-1842 の細胞外ホスホマンナの部分構造 19 の合成サイクルを図 4 に示す (29)。この方法では、まず還元糖 13 のアノマー水酸基のホスホニル化によって、糖-1-*H*-ホスホネートモノエステル 14 を合成する。このホスホニル化には、三塩化リンとイミダゾールから調製したトリイミダゾリルホスフィンや化合物 15

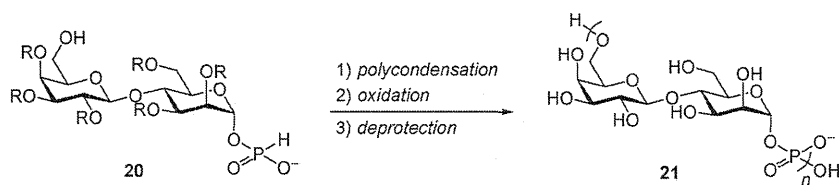


Fig. 5. Synthesis of *Leishmania* phosphoglycan repeating units 21 by polycondensation of 20.

sugar **13**. Triimidazolylphosphine, which is prepared from phosphorus trichloride and imidazole, or the compound **15** are generally used for phosphorylation. Such anomeric phosphorylation preferentially gives  $\alpha$ -isomers from common aldohexoses, and only  $\alpha$ -isomers are generated in many cases (e.g., mannose and mannosamine derivatives or aldohexoses carrying a 2-*O*-acyl protecting group) (1). Furthermore, even if a diastereomixture is obtained by phosphorylation, it can be equilibrated to the more thermodynamically stable  $\alpha$ -isomer by prolonged treatment with phosphonic acid under anhydrous conditions, although the yield is generally lowered owing to partial decomposition of the product (30). For this reason, the glycosyl *H*-phosphonate monoesters are useful as monomers to synthesize phosphoglycans because the glycosyl phosphate units found in natural phosphoglycans are mostly  $\alpha$ -isomers. Using the compound **14** as a monomer, a phosphoglycan chain is synthesized according to the reaction cycle shown in Fig. 4. First, the monomer **14** is condensed with the terminal hydroxy group of a sugar or oligo(glycosyl phosphate) **16** in the presence of a condensing agent, such as pivaloyl chloride, to give an *H*-phosphonate diester intermediate **17**. Because this intermediate is unstable, it is oxidized to a phosphate diester intermediate **18** without isolation. Finally, the terminal hydroxy group is deprotected for the next cycle. This cycle is repeated to form a phosphoglycan chain, and the final product **19** is obtained after deprotection and purification.

To date, various phosphoglycans consisting of 2–4 glycosyl phosphate units have been synthesized, such as those from *Leishmania* glycoalyx (31–33), CPSs of *E. coli* K51 (29), *N. meningitidis* (34, 35), *S. pneumoniae* 19F (36), and *Haemophilus influenzae* c and f (37). In addition, phosphoglycans containing 10–20 glycosyl phosphate units **21** (Fig. 5) have been synthesized by polycondensation of glycosyl *H*-phosphonate monoesters having a free hydroxy group **20** (31, 38). As described above, CPSs isolated from *N. meningitidis* and *S. pneumoniae* as well as their conjugates with proteins have already been used as vaccines (2, 3). Similarly, it has been reported that phosphoglycan fragments of *Leishmania* glycoalyx synthesized by the *H*-phosphonate method and their conjugates with proteins are effective as vaccines in mice (33). Further progress in this subject is

が用いられ、一般的なアルドヘキソースからは  $\alpha$ -ホスホネートが優先して得られる。特に、マンノース、マンノサミン誘導体や2位がアシル基で保護されたアルドヘキソースからは、 $\alpha$ 体のみが立体選択的に得られることも多い(1)。さらに、 $\alpha$ 体、 $\beta$ 体の混合物が生成する場合でも、無水条件下ホスホン酸と長時間反応させることによって、より熱力学的に安定な  $\alpha$ 体へ異性化させることができる。但し、この場合は分解反応も同時に起こり、収率は低下する(30)。なお、天然に存在するホスホグリカン中の糖-1-リン酸ユニットのほとんどは  $\alpha$ 体である(1)。そのため、 $\alpha$ 体が入りやすい糖-1-*H*-ホスホネートモノエステルは、ホスホグリカン合成のモノマーとして用いやすい。次に、この化合物 **14** をモノマーとし、図4に示す合成サイクルに従ってホスホグリカン鎖を伸長する。まず、モノマー **14** を塩化ジバロイルなどの脱水縮合剤によって糖 **16** の水酸基と縮合し、*H*-ホスホネートジエステル中間体 **17** を得る。この中間体は不安定なため、単離せずに酸化してリン酸ジエステル **18** へと変換する。最後に末端水酸基を脱保護し、次のサイクルへと備える。このサイクルを繰り返すことによって、複数の糖-1-リン酸ユニットからなる分子骨格を構築し、脱保護と精製を行って目的物 **19** を得る。

これまでに、リーシュマニアの糖衣ホスホグリカン(31–33)、大腸菌 K51(29)、髄膜炎菌(34,35)、肺炎球菌(36)、インフルエンザ菌 c 型及び f 型の莢膜多糖(37)など、糖-1-リン酸構造を2–4個含むホスホグリカンの部分構造がこの方法によって合成されている。また、図5に示す様に、遊離の水酸基を有する糖-1-*H*-ホスホネートモノエステル **20** を重縮合させることによって、混合物ではあるが10から20以上の糖-1-リン酸繰り返し構造からなるリーシュマニアの糖衣ホスホグリカン **21** も合成されている(31,38)。前述の通り、細菌から採取精製した莢膜多糖やそのタンパク質との複合体は、既に髄膜炎菌や肺炎球菌などに対するワクチンとして用いられており(2,3)、*H*-ホスホネート法によって化学合成したリーシュマニアの糖衣ホスホグリカンの部分構造とタンパク質との複合体も、マウスによる実験でワクチンとして働くことが示された

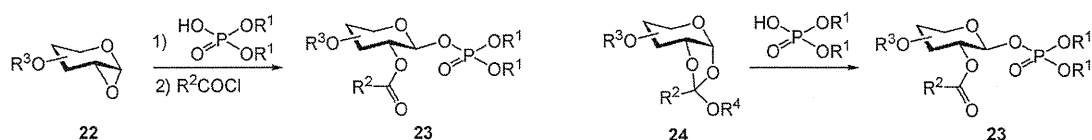


Fig. 6. Synthesis of 1,2-*trans*-glycosyl phosphate triesters **23** from 1,2-anhydrosugars **22** and 1,2-orthoesters **24**.

expected because vaccines against *Leishmania* have not yet been developed. Furthermore, because chemical synthesis would be advantageous in view of the increased production and improved quality control compared with the purification of biological materials, the synthesis of phosphoglycans of other pathogens including *N. meningitidis* and *S. pneumoniae* and their application as vaccines would also be an important subject in the future.

Thus, the *H*-phosphonate method is advantageous for the synthesis of phosphoglycan fragments, although a number of challenges still remain. For example, the glycosyl *H*-phosphonate diester intermediates are susceptible to hydrolysis. Therefore, they are oxidized into the glycosyl phosphate diesters without isolation. However, the resultant glycosyl phosphate diesters are highly polar, causing difficulties in purification. The phosphate diester linkages are also potentially reactive to condensing agents. It may be for these reasons that the efficiency of the synthesis of phosphoglycan fragments has been reported to decrease upon chain elongation in some cases (29, 34, 35, 37). To overcome these problems, the *H*-phosphonate method has been applied to solid-phase syntheses in order to exclude the need of purifying intermediates in every synthetic cycle, although the synthesis of phosphoglycan fragments longer than those obtained by conventional synthesis in solution has not yet been reported (31). Furthermore, the *H*-phosphonate method is not suitable for the synthesis of thermodynamically less stable  $\beta$ -glycosyl phosphate moieties. In the next three sections, we describe the results of recent studies to complement this method and our new trials to overcome these problems.

#### D. Synthesis Using Phosphate Derivatives as Glycosyl Acceptors

Nucleophilic substitution of sugar derivatives having an anomeric leaving group (*e.g.*, glycosyl halides) with phosphates is one of the conventional methods used for synthesizing glycosyl phosphates (39, 40). In addition to glycosyl halides, glycosyl imidates (41), 1,2-anhydrosugars **22** (Fig. 6) (21, 42, 43), and 1,2-orthoesters **24** (Fig. 6) (23, 44) have recently been used to synthesize glycosyl phosphate triesters. The triesters thus obtained can be used as glycosyl donors in the synthesis of oligosaccharides and also as the precursors of sugar nucleotides, as described above.

(33). リーシュマニアに対するワクチンは未だ開発されていないことから、今後の研究の発展が期待される。また、化学合成によるワクチンの製造は、病原性微生物から単離する方法に比べ、大量合成や品質管理の観点からも有利と考えられるため、髄膜炎菌や肺炎球菌など様々な病原性微生物に対する合成ワクチンの開発も今後の重要な課題になると考えられる。

この様に、*H*-ホスホネート法はホスホグリカンの部分構造の化学合成法として優れているが、改善すべき点も残っている。例えば、*H*-ホスホネートジエステル中間体は不安定であり、加水分解などの副反応を受けやすい。また、この中間体は単離が困難なため、酸化してリン酸ジエステルとした後単離精製を行うが、複数のリン酸ジエステルを含む分子は極性が極めて高く、精製上の困難を伴う。加えて、このリン酸ジエステルは反応性も高く、縮合剤との反応などの副反応を受ける可能性がある。このような理由からか、*H*-ホスホネート法を用いたホスホグリカンの合成では、鎖長が長くなるにつれて収率が低下することが報告されている(29,34,35,37)。このような問題点を克服するため、サイクル毎の中間体の精製の必要が無い固相合成法の開発が進められているが、現在までに液相法を上回る鎖長の分子の合成は報告されていない(31)。さらに、この方法は熱力学的により不安定な  $\beta$ -糖-1-リン酸構造の合成には適していない。次節以降では、このような *H*-ホスホネート法の問題点を補う手法、あるいは克服するための筆者らの新たな試みについて述べる。

#### D. リン酸誘導体をグリコシル受容体として用いる糖-1-リン酸誘導体の合成

アノマー位にハロゲンなどの脱離基を有する糖誘導体とリン酸塩との求核置換反応は、古くから糖-1-リン酸の合成に用いられてきた(39,40)。近年ではこのハロゲン化糖に加え、イミデート糖(41)や1,2-アンヒドロ糖**22**(図6)(21,42,43)、1,2-オルトエステル**24**(図6)(23,44)などとリン酸ジエステルとの求核置換反応が糖-1-リン酸トリエステルの合成に用いられている。得られた糖-1-リン酸トリエステルは、先に述べた通り糖鎖合成における糖供与体や糖ヌクレオチドの合成に用いら

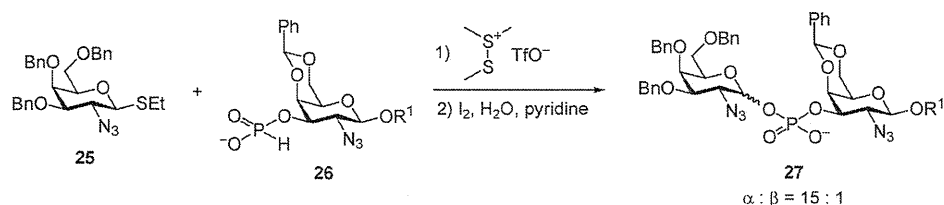


Fig. 7. Synthesis of disaccharide monophosphate derivative **27** by glycosylation of *H*-phosphonate monoester **26**.

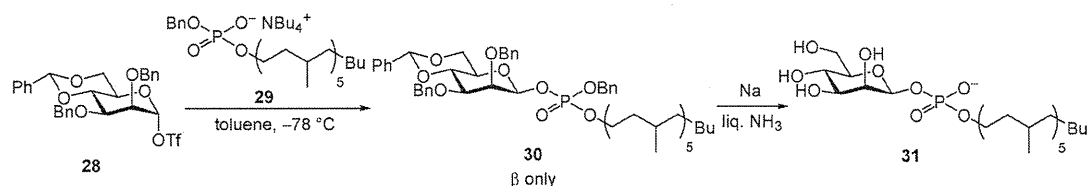


Fig. 8. Synthesis of  $\beta$ -D-mannosyl phosphoisoprenoid **31** isolated from *Mycobacterium avium* by glycosylation of phosphate diester **29**.

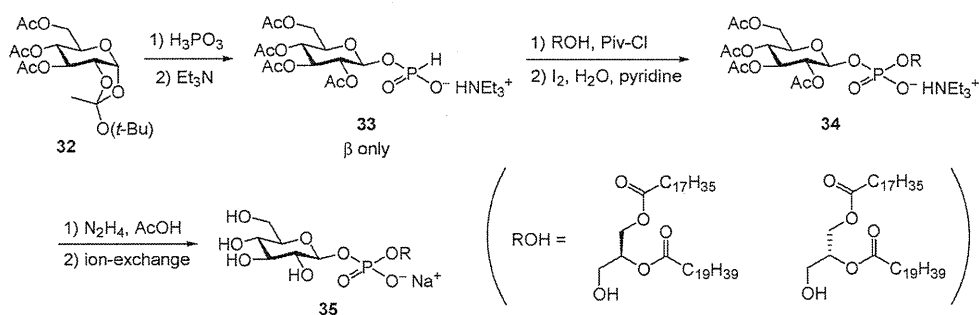


Fig. 9. Synthesis of phosphatidyl- $\beta$ -D-glucosides **35** isolated from rat embryonic brain.

The nucleophilic substitutions of the glycosyl halides and imidates with phosphates generally afford 1,2-*trans*-glycosyl phosphates with neighboring-group participation, or proceed with inversion of configuration without a participating group. On the other hand, the reaction of compounds **22** and **24** with phosphate diesters proceeds with inversion of configuration to give 1,2-*trans*-isomers.

Applications of these anomeric nucleophilic substitutions to the synthesis of phosphoglycans are limited to a few examples (45, 46), such as the reaction given in Fig. 7, in which the thioglycoside **25** and the sugar 3-*H*-phosphonate monoester **26** were used as the glycosyl donor and acceptor, respectively (45). However, these methods are advantageous because both the  $\alpha$ - and  $\beta$ -isomers of various glycosyl phosphate derivatives are available, and are therefore particularly useful for the synthesis of  $\beta$ -glycosyl phosphate derivatives that are difficult to synthesize using the *H*-phosphonate method. The stereocontrolled syntheses of a  $\beta$ -D-mannosyl phosphoisoprenoid **31** isolated from

れる。なお、ハロゲン化糖やイミデート糖とリン酸との反応は、隣接基関与を伴う場合には1,2-トランス体が得られ、そうでない場合は立体反転を伴うことが多い。また、化合物 **22** や **24** の反応は立体反転を伴って1,2-トランス体を与える。

この方法を用いたホスホグリカンの合成例(45,46)は少なく、図7に示すチオ糖 **25** を用いた糖-3-*H*-ホスホネートモノエステル **26** のグリコシル化などに限られる(45)。しかしながら、リン酸誘導体のグリコシル化には、様々な糖-1-リン酸の $\alpha$ 、 $\beta$ 両立体異性体が入手しやすいという利点がある。そのため、*H*-ホスホネート法で合成が困難な $\beta$ -糖-1-リン酸誘導体の合成で威力を発揮しやすい。その例として、マイコバクテリウム属から単離された $\beta$ -マンノース-1-リン酸イソプレノイド **31** (図8) (22) や、ラットから単離されたホスファチジル $\beta$ -グル

*Mycobacterium avium* (Fig. 8) (22) and rat phosphatidyl  $\beta$ -glucosides **35** (Fig. 9) (47, 48) illustrate the usefulness of these methods. Compounds **31** and **35**, both of which are difficult to synthesize by other methods such as the anomeric phosphorylation, have been successfully synthesized in a stereocontrolled manner.

### E. Synthesis of Glycosyl Phosphate Derivatives Using the Boranophosphotriester Method

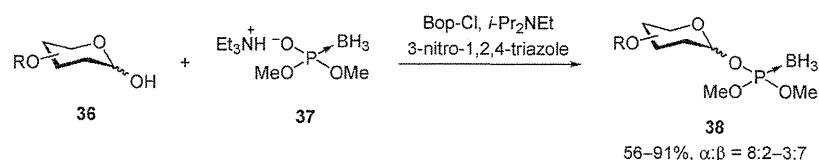
As described above, the synthesis of phosphoglycans using the *H*-phosphonate method has associated problems, *i.e.*, the lability of the glycosyl *H*-phosphonate diester intermediates as well as the reactivity and polarity of the glycosyl phosphate diesters. In an attempt to solve these problems, our group has been working on the development of a new method for the synthesis of glycosyl phosphate derivatives using glycosyl boranophosphates as intermediates. Boranophosphates are phosphate analogs in which one of the two non-bridging oxygen atoms of the phosphodiester linkage is replaced with a  $\text{BH}_3$  group. These compounds were originally developed as nucleic acid analogs with improved lipophilicity and stability to nucleases (49). Glycosyl boranophosphates have also been synthesized from glycosyl *H*-phosphonates (50–52) and by nucleophilic substitution of a glycosyl bromide with dimethyl boranophosphate (53). However, the glycosyl boranophosphates have only been used as probes to investigate *Leishmania* mannosyl phosphate transferase (52). The properties of glycosyl boranophosphates are not well-known, but they have been reported to be more stable than the glycosyl phosphate diester counterparts under acidic conditions (51). We focused on the stability of glycosyl boranophosphates. Previously, we developed a method to convert nucleoside boranophosphate triesters into the corresponding phosphate diester counterparts *via* boranophosphate diester and *H*-phosphonate diester intermediates (54). If glycosyl boranophosphate triesters had comparable stability to glycosyl boranophosphate diesters, it would be possible to synthesize phosphoglycan fragments by chain extension with a stable boranophosphate triester backbone, with conversion of the resultant oligo(glycosyl boranophosphate)s to the desired phosphoglycans having a phosphate diester backbone at the final stage of the synthesis. We expected that this strategy would avoid the problems resulting from the generation of unstable *H*-phosphonate intermediates in every synthetic cycle. Furthermore, because the boranophosphate triester backbone is electrically neutral, purification would be easier than for highly polar glycosyl phosphate diester intermediates. On the basis of this hypothesis, we studied the synthesis of glycosyl boranophosphate derivatives and their application to the synthesis of phosphoglycan fragments.

コシド **35** (図9) (47,48) の合成が挙げられる。これらの報告例では、糖アノマー水酸基のホスホニル化などでは合成が困難な  $\beta$ -マンノース-1-リン酸や  $\beta$ -グルコース-1-リン酸誘導体の立体選択的な合成に成功している。

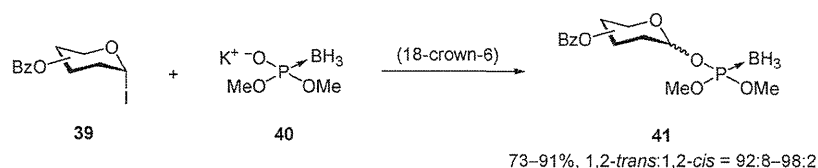
### E. ボラノホスホトリエステル法による糖-1-リン酸誘導体の合成

先に述べた通り、*H*-ホスホネート法によるホスホグリカンの化学合成では、*H*-ホスホネートジエステル中間体の不安定性や、その酸化体であるリン酸ジエステルの反応性、極性が問題となる。これらの問題点を解決すべく、筆者らは糖-1-ボラノホスフェートを中間体とする糖-1-リン酸誘導体の合成法の開発を行っている。ボラノホスフェートは、リン酸エステルの非架橋酸素原子の一つを  $\text{BH}_3$  基に置換したリン酸アナログで、元々は脂溶性や酵素耐性などに優れた核酸アナログ (49) として開発されたものであり、糖-1-リン酸アナログとしても、糖-1-*H*-ホスホネートからの誘導 (50–52) や、糖-1-プロミドとボラノホスフェートジエステルの求核置換反応 (53) による合成が報告されている。しかしながら、糖-1-リン酸アナログとしての応用例は、リーシュマニア外膜におけるマンノース-1-リン酸転移反応の解析にプローブとして用いられたのみである (52)。また、その性質についてもほとんど知られていないが、糖-1-ボラノホスフェートジエステルが酸性条件下、糖-1-リン酸ジエステルより安定であると報告されている (51)。ここで筆者らは、この安定性に着目した。筆者らは、核酸アナログの研究において、ボラノホスフェートトリエステルから、ボラノホスフェートジエステルと *H*-ホスホネートジエステルを経てリン酸ジエステルへと変換する手法を開発している (54)。もし、糖-1-ボラノホスフェートトリエステルが糖-1-ボラノホスフェートジエステルと同様に安定であれば、ホスホグリカン鎖をボラノホスフェートトリエステル体として伸長し、合成の最終段階でリン酸ジエステルへと変換することによって、不安定な *H*-ホスホネートジエステル中間体がサイクル毎に生じる問題点を解決できると考えた。また、ボラノホスフェートトリエステルは負電荷を持たないため、リン酸ジエステルの高い反応性や極性に起因する問題点も解決できる。筆者らはこの仮説に基づいて、糖-1-ボラノホスフェートの合成とホスホグリカン合成への応用についての検討を行った。

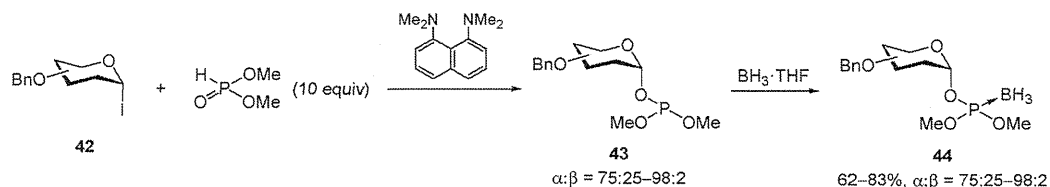




**Fig. 10. Synthesis of glycosyl boranophosphate triesters 38 by boranophosphorylation of reducing sugars 36.** Bop-Cl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride.



**Fig. 11. 1,2-Trans-selective synthesis of glycosyl boranophosphate triesters 41 via nucleophilic substitution of per-*O*-Bz-glycosyl iodides 39 with boranophosphate diester 40.**



**Fig. 12.  $\alpha$ -Selective synthesis of glycosyl boranophosphate triesters 44 by nucleophilic substitution of per-*O*-Bn-glycosyl iodides 42 with dimethyl *H*-phosphonate.**

Initially, we developed several methods to synthesize glycosyl boranophosphate monomers (Figs. 10–12). As shown in Fig. 10, we first carried out the synthesis by condensation of reducing sugars **36** with triethylammonium dimethyl boranophosphate **37** (55, 56). The desired glycosyl boranophosphate triesters were obtained in good yields, although mixtures of  $\alpha$ - and  $\beta$ -diastereomers were obtained (57). It should be noted that the synthesis of glycosyl phosphate triesters by such condensation reactions is generally difficult (1, 58). We also confirmed this by conducting a few condensation reactions between reducing sugars and phosphate diesters. On the other hand, per-*O*-Bz-glycosyl boranophosphate triesters **41** were obtained with high 1,2-*trans*-selectivity by the neighboring-group-assisted nucleophilic substitution of glycosyl iodides **39** with potassium dimethyl boranophosphate **40** (Fig. 11) (59). Furthermore, we found that per-*O*-Bn-glycosyl boranophosphate triesters **44** were obtained with good to excellent  $\alpha$ -selectivity by nucleophilic substitution of glycosyl iodides **42** with dimethyl *H*-phosphonate and subsequent boronation (Fig. 12) (60). Although this method still has some drawbacks, such as the use of an excess amount of the nucleophile and a narrow range of applicable sugars, it allows the synthesis of 1,2-*cis*-glycosyl

初めに、糖-1-ボラノホスフェートモノマーの合成法の開発を行った(図10–12)。まず、図10に示す様に、還元糖**36**とボラノホスフェートジエステル**37**の脱水縮合反応(55,56)を試みたところ、 $\alpha$ 、 $\beta$ 混合物ではあるものの、糖-1-ボラノホスフェートトリエステル**38**が収率良く得られた(57)。なお、この様な脱水縮合による糖-1-リン酸トリエステルの合成は一般的に困難であり(1,58)、生成したトリエステルが系内で分解することを筆者らも確認している。一方、ヨウ化糖**39**とボラノホスフェートジエステル**40**の求核置換反応では、隣接基関与によって1,2-トランス体**41**が高い選択性で得られた(図11)(59)。さらに、図12に示す様に、ヨウ化糖**42**と*H*-ホスホネートジエステルの求核置換反応とボラノ化によって、糖-1-ボラノホスフェートトリエステル**44**が高い $\alpha$ 選択性で得られることがわかった(60)。この反応は、過剰量の求核剤を必要とすることや、基質の適用範囲が狭いことが難点であるが、図11の手法では得られない1,2-シス体の合成が可能である。なお、これらの糖-1-ボラノホスフェートトリエステルは、対応する糖-1-

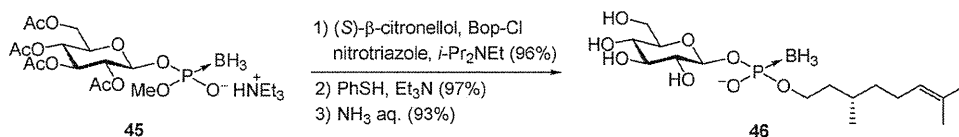


Fig. 13. Synthesis of  $\beta$ -D-Glc-PB-Cit 46 by boranophorylation of (S)- $\beta$ -citronellol.

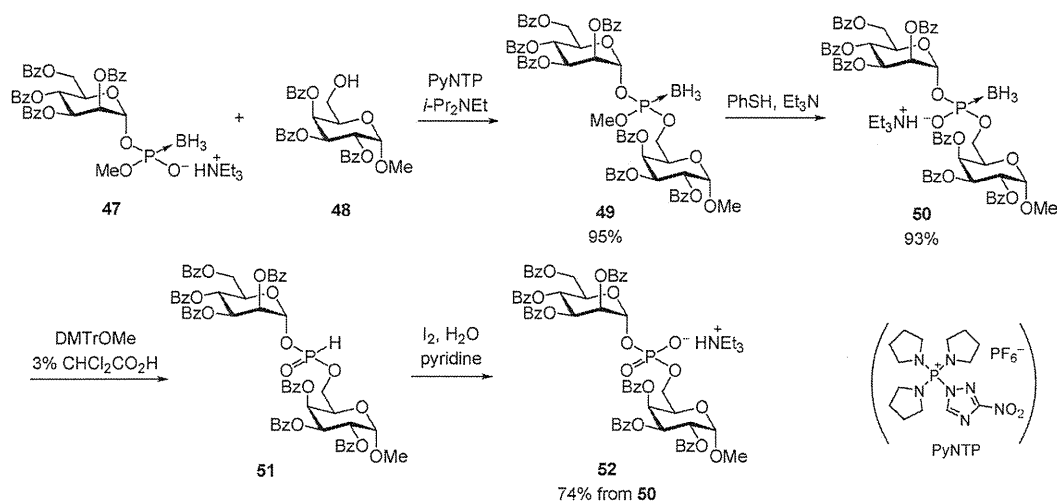


Fig. 14. Synthesis of disaccharide phosphate 52 via disaccharide boranophosphate (49, 50) and H-phosphonate (51) intermediates.

boranophosphates, which are unavailable using the method in Fig. 11. It is notable that all of these glycosyl boranophosphate triesters are chemically more stable than their glycosyl phosphate counterparts, and thus should be useful as synthetic intermediates.

Next, we applied these glycosyl boranophosphate triesters to the synthesis of more complex glycosyl boranophosphate derivatives. These triesters can be converted into diesters by partial deprotection and subsequent condensation reactions with alcohols in order to generate new molecules. For example, as shown in Fig. 13, we synthesized a model compound of dolichol phosphate sugars 46 in excellent yield by the reaction of a glycosyl boranophosphate diester 45 and citronellol (57). Such a compound would be useful as a probe to study enzymatic reactions. It is noteworthy that the equivalent compound containing a C=C bond, which would be reduced by boronating agents, cannot be synthesized using the previous method of silylation and boronation of a glycosyl H-phosphonate intermediate (50–52).

Thus, the method used to synthesize glycosyl boranophosphate derivatives using the condensation of glycosyl boranophosphate diesters with alcohols (boranophosphotriester method) afforded the desired product in excellent yield via the highly efficient condensation and deprotection, which was in sharp contrast with the

リン酸トリエステルより化学的に安定であり、合成中間体として取り扱いやすいことがわかった。

そこで次に、より複雑な糖-1-ボラノホスフェート誘導体の合成について検討した。これらの糖-1-ボラノホスフェートトリエステルは脱保護によってジエステルへと変換でき、アルコールとの脱水縮合反応を経て分子骨格の拡張が可能である。例えば、図13に示す様に、糖-1-ボラノホスフェートジエステル45とシトロネロールの反応を試みたところ、ドリコールリン酸糖のモデル化合物46を収率良く得ることができた(57)。このような化合物は、酵素反応のメカニズムを調べるためのプローブとしての利用が考えられる。なお、H-ホスホネートジエステルのシリル化とボラノ化を経る既存の糖-1-ボラノホスフェートの手法(50–52)では、ボラノ化剤と反応するC=C結合などの官能基を含む化合物の合成はできない。

この様に、糖-1-ボラノホスフェートジエステルとアルコールとの縮合反応を用いる糖-1-リン酸誘導体の合成法(ボラノホスホトリエステル法)は、種々の副反応を伴うリン酸トリエステル法とは対照的に、縮合反応、脱保護共に効率良く進行し、収率良く目的物を与えることがわかった。そこで次に、この

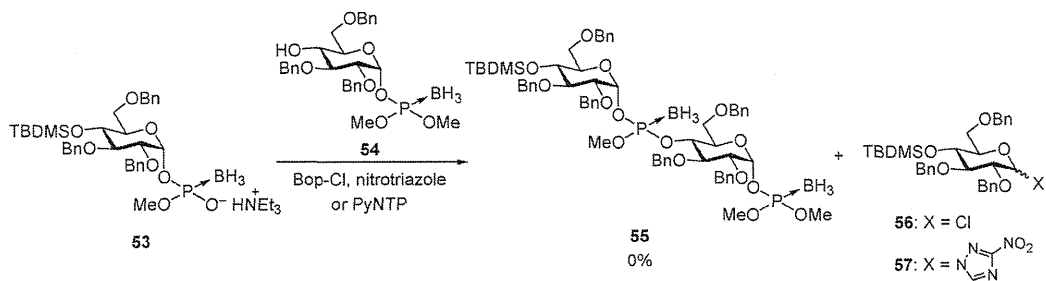


Fig. 15. Attempted synthesis of disaccharide boranophosphate **55** by boranophosphotriester method.

phosphotriester method that is generally accompanied by different side reactions. Therefore, we applied this method to the synthesis of phosphoglycan fragments. As shown in Fig. 14, a mannosyl boranophosphate diester **47** was allowed to condense with a protected galactose **48** in order to give a disaccharide boranophosphate triester **49**, which was subsequently deprotected to afford the desired disaccharide boranophosphate diester **50**. Both the condensation and the deprotection of the boranophosphate linkage proceeded almost quantitatively. Compound **50** was then deboronated by treatment with a dimethoxytrityl cation generated *in situ* according to the procedure we developed previously for the synthesis of nucleic acid analogs (54). Finally, the resultant *H*-phosphonate diester intermediate **51** was oxidized to give the desired protected phosphoglycan fragment **52** (59). This method also involves an unstable *H*-phosphonate intermediate **51**; however, the problems derived from the lability of *H*-phosphonate diester intermediates would be expected to be alleviated by synthesizing the phosphoglycan chains with the boranophosphate triester backbone such as compound **49** and converting them to the phosphodiester-linked phosphoglycans at the final stage.

However, we found that this method was not applicable to the synthesis of some phosphoglycan fragments. Thus, as shown in Fig. 15, the condensation of a glycosyl boranophosphate diester **53** with the 4-hydroxy group of another glycoside **54** did not give the desired compound **55** but afforded a glycosyl chloride **56** and azolide **57** (61). These side reactions can be attributed to the decomposition of compound **53** upon activation with the condensing agent. As a result, an oxocarbenium ion was generated and then reacted with nucleophiles in the reaction mixture as in the phosphotriester method, generating side products **56** and **57**. This is probably because the compound **54** has low reactivity owing to the steric hindrance and also because the compound **53** is more likely to generate an oxocarbenium ion upon activation than the compound **47** (Fig. 14) that was protected by electron-withdrawing benzoyl groups.

Thus, these results show that the glycosyl

方法を用いてホスホグリカンの部分構造の合成を試みた。図 14 に示す通り、マンノース-1-ボラノホスフェート **47** とガラクトース誘導体 **48** との縮合反応を行ったところ、目的とするボラノホスフェートトリエステル誘導体 **49** が得られた。さらに、リン酸部位の脱保護を行い、目的物 **50** を得た。縮合反応、及びボラノホスフェートの脱保護共にほぼ定量的に進行した。次に、筆者らが核酸アナログの合成で開発した手法(54)に従い、系中で発生させたジメトキシトリチルカチオンによって化合物 **50** を脱ボラノ化し、生成した *H*-ホスホネートジエステル中間体 **51** を酸化することによって、目的とするホスホグリカンの部分構造 **52** の合成に成功した(59)。この手法では、*H*-ホスホネート法と同様に不安定な *H*-ホスホネートジエステル中間体 **51** が生じるが、化合物 **49** の様に、安定なボラノホスフェートトリエステル体として鎖延長し、最後に脱ボラノ化、酸化を行うことによって、*H*-ホスホネートジエステルの不安定さに起因する問題は克服できると考えられる。

しかしながら、このボラノホスホトリエステル法はホスホグリカンの分子骨格によっては適用できないことがわかった。すなわち、図 15 に示す様に、糖-1-ボラノホスフェート **53** と化合物 **54** から目的とする縮合生成物 **55** は得られず、糖-1-クロリド **56** や糖-1-アゾリド **57** が副生成物として得られた(61)。この副反応は、リン酸トリエステル法と同様に、縮合剤によって活性化された化合物 **53** からオキソカルベニウムイオンが生成し、系中の求核剤と反応したことによると考えられる。これは、化合物 **54** の水酸基の反応性が立体障害のために低いことや、電子求引性のベンゾイル基によって保護された化合物 **47** (図 14) に比べ、化合物 **53** は活性化によってオキソカルベニウムイオンを生じやすいことが理由と考えられる。

以上の検討から、糖-1-ボラノホスフェート誘導体は安定

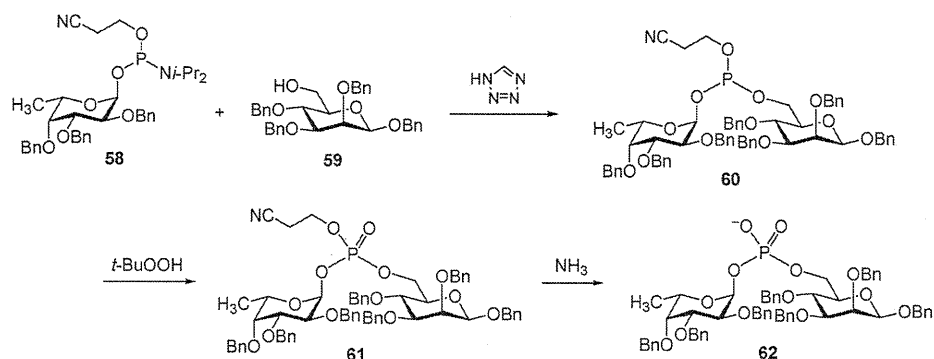


Fig. 16. Synthesis of disaccharide phosphate 62 by phosphoramidite method.

boranophosphate intermediates are stable, easy to handle, and suitable as precursors of glycosyl phosphate diesters. However, the condensation efficiency of the glycosyl boranophosphate diesters is highly dependent on their molecular structures. Therefore, the scope of their application should be further investigated.

#### F. Synthesis of Glycosyl Phosphate Derivatives Using the Phosphoramidite Method

The phosphoramidite method is currently the most efficient method for the synthesis of oligonucleotides, and can produce long oligomers using an automatic synthesizer (62, 63). However, the application of this method to the synthesis of glycosyl phosphate derivatives has not been well explored (64–69). Figure 16 shows an example of the synthesis of a phosphoglycan fragment by this method (64). In this method, a glycosyl phosphoramidite **58** is activated by an acidic activator such as 1*H*-tetrazole and allowed to condense with another saccharide **59** to give a phosphite intermediate **60**. Because this intermediate is unstable, it is oxidized to a phosphate triester intermediate **61** without isolation. In the case of the synthesis of an oligonucleotide, the chain elongation is conducted along the phosphate triester backbone. However, glycosyl phosphate triester intermediates are unstable and may be partially decomposed during synthesis and purification. Chain extension with a more stable phosphate diester backbone could also be accompanied by side reactions on the nucleophilic phosphate diester groups. These may be the reasons that the method has not been used to synthesize molecules consisting of more than one glycosyl phosphate unit.

To overcome this limitation, we have been developing a new phosphoramidite method using glycosyl boranophosphate intermediates (Fig. 17) (**61**). In this method, phosphite intermediates **65**, which are obtained by condensation of a glycosyl phosphoramidite monomer **63** with the terminal hydroxy group of an (oligo)saccharide **64**, are

で取り扱いやすい中間体であり、糖-1-リン酸ジエステルへの変換も可能である。しかしながら、糖-1-ボラノホスフェートジエステルの脱水縮合の反応効率は分子骨格によって大きく変化するため、さらなる適用範囲の検討が必要である。

#### F. ホスホロアミダイト法による糖-1-リン酸誘導体の合成

ホスホロアミダイト法は、DNA や RNA の化学合成法としては現在最も効率的な方法であり、自動合成機を用いた長鎖のオリゴマー合成も可能である (62,63)。しかしながら、この方法による糖-1-リン酸誘導体の合成例は数少ない (64–69)。この方法でホスホグリカンの合成を行う場合、図 16 (64) に示す通り、糖-1-ホスホロアミダイト **58** を 1*H*-テトラゾールなどの酸性活性化剤の存在下、糖水酸基 **59** と縮合し、ホスファイト中間体 **60** を得る。この中間体は不安定であるため、単離せずに酸化してリン酸トリエステル中間体 **61** へと誘導する。核酸合成の場合、このリン酸トリエステル中間体の状態で末端を脱保護し、鎖長延長を行うが、ホスホグリカン合成の場合、このような糖-1-リン酸トリエステルは不安定であるため、合成サイクルを繰り返す際や精製時に一部分解することが考えられる。また、リン酸ジエステル **62** の状態で鎖長延長を行うと、リン酸ジエステルに対する副反応が懸念される。このような理由からか、現在までに複数の糖-1-リン酸ユニットからなる分子の合成は報告されていない。

この問題点を克服すべく、筆者らは、糖-1-ボラノホスフェートを合成中間体とする新しいホスホロアミダイト法の開発を試みている (図 17) (**61**)。この手法では、ホスホロアミダイトモノマー **63** と糖 **64** の水酸基との縮合によって得られたホスファイト中間体 **65** を、酸化ではなくボラノ化によって安

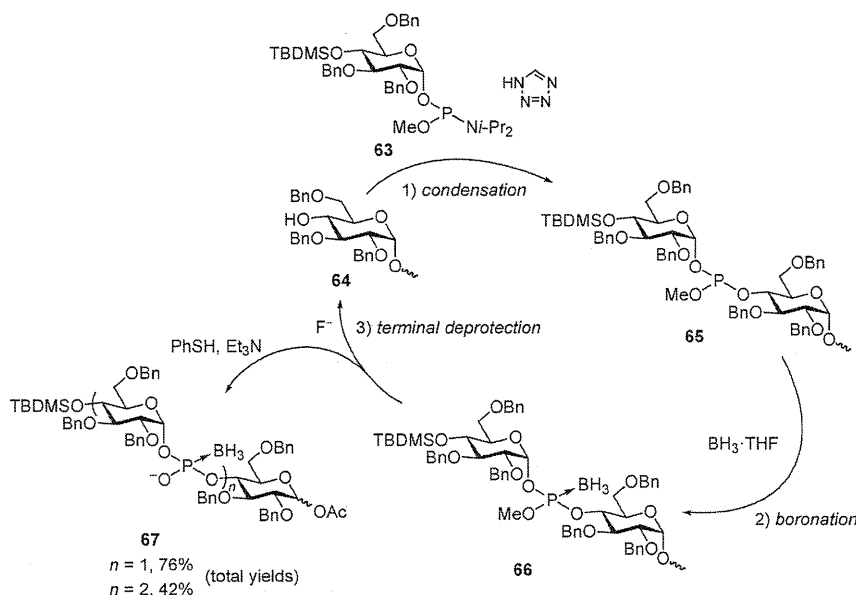


Fig. 17. Synthesis of di- and tri( $\alpha$ -D-glucosyl boranophosphate) derivatives **67** by phosphoramidite method.

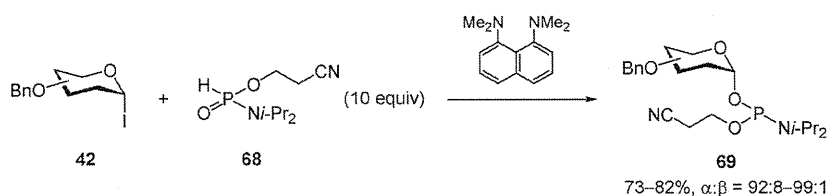


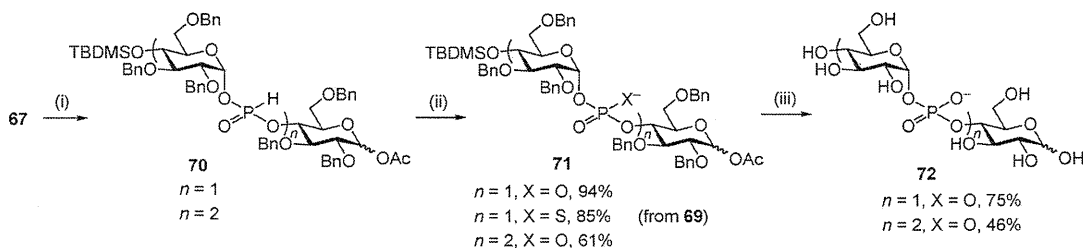
Fig. 18.  $\alpha$ -Selective synthesis of glycosyl phosphoramidites **69** by nucleophilic substitution of per-*O*-Bn-glycosyl iodides **42** with *H*-phosphonamidate **68**.

subjected to boronation instead of oxidation for synthesizing stable boranophosphate triester intermediates **66**. The terminal silylated hydroxy group is then deprotected for the next cycle. Effectiveness of this new phosphoramidite method was verified by the synthesis of molecules consisting of 1–2 glycosyl boranophosphate units **67**, although the yields were still moderate. It should be noted that the products **67** were structurally analogous to the compound **55** (Fig. 15) that was difficult to synthesize using the boranophosphotriester method. In addition, because there are very limited reports available on the stereoselective synthesis of glycosyl phosphoramidites, we developed a method to synthesize glycosyl phosphoramidites by applying the reaction shown in Fig. 12 (Fig. 18) (60). The phosphoramidite monomer **63** was synthesized using this method with a diastereoselectivity of 98 : 2 ( $\alpha$  :  $\beta$ ) (61).

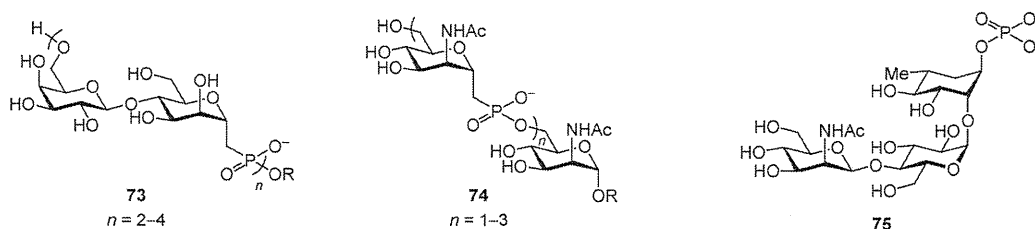
Next, we attempted to convert compounds **67** into phosphoglycan fragments incorporating phosphate diester linkage(s) **72** as shown in Fig. 19. Similar to Fig. 14, the compounds **67** were treated with a dimethoxytrityl cation to give *H*-phosphonate diester intermediates **70**, and then

定なボラノホスフェートトリエステル中間体 **66** へと導き、末端水酸基を脱保護して次の鎖長延長を行う。実際に、ボラノホスホトリエステル法では合成が困難であった分子骨格 (図 15, **55**) を持つ化合物 **67** の合成を行ったところ、合成収率に改善の余地はあるものの、糖-1-ボラノホスフェートユニット 1-2 個からなる分子を得ることができた。なお、糖-1-ホスホロアミダイト誘導体の立体選択的の合成に関する報告例は限られていたため、筆者らは図 12 で示した方法を応用し、図 18 に示す様に糖-1-ホスホロアミダイト誘導体を高い  $\alpha$  選択性で合成する手法を開発した (60)。化合物 **63** はこの手法によって  $\alpha:\beta = 98:2$  の選択性で合成した (61)。

次に、図 19 に示す様に、化合物 **67** からリン酸ジエステル骨格を有する目的化合物 **72** への変換を試みた。図 14 と同様に、化合物 **67** をまずジメトキシトリチルカチオンによって *H*-ホスホネートジエステル骨格を持つ中間体 **70** へと変換し、さら



**Fig. 19. Synthesis of di- and tri( $\alpha$ -D-glucosyl phosphate) derivatives 71, 72 from boranophosphate precursors 67.** Reagents: (i) DMTrOMe,  $\text{CHCl}_2\text{CO}_2\text{H}$  (3–3.5 vol%); (ii)  $\text{I}_2$ , pyridine– $\text{H}_2\text{O}$  (95:5, v/v) ( $X = \text{O}$ ) or  $\text{S}_8$ , pyridine ( $X = \text{S}$ ); (iii) (a)  $\text{Et}_3\text{N}\cdot 3\text{HF}$ , (b)  $\text{Me}_2\text{NH}$ , (c)  $\text{H}_2$ , Pd/C.



**Fig. 20. Stable analogs of phosphoglycan fragments of *Leishmania donovani* (73), *Neisseria meningitidis* A (74) and *Streptococcus pneumoniae* 19F (75).**

subjected to oxidation and deprotection to afford the desired products 72. The intermediate 70 was also useful to synthesize a disaccharide phosphorothioate derivative 71 ( $X = \text{S}$ ) by replacing the oxidation with sulfurization. Thus, the new phosphoramidite method using glycosyl boranophosphate derivatives as intermediates proved to be useful for the synthesis of short phosphoglycan fragments, although the yields must be further improved.

### G. Future Perspectives

The *H*-phosphonate method is currently the method of choice for the synthesis of phosphoglycan fragments, while other methods such as the glycosylation of phosphates are used to synthesize other glycosyl phosphate derivatives that are difficult to synthesize by the *H*-phosphonate method (e.g., sugar nucleotides and  $\beta$ -glycosyl phosphate derivatives). The present *H*-phosphonate method can afford phosphoglycan fragments consisting of 2–4 glycosyl phosphate units, while their conjugates with proteins have shown potential as vaccines (34). However, data regarding the potential of synthetic phosphoglycan fragments as vaccines are very limited, and the relationship between the chain lengths of fragments and the efficacy as vaccines has not yet been clarified. Therefore, a more efficient synthetic method is required for further progress in the application research of synthetic phosphoglycans as vaccines. Resultant phosphoglycan fragments with a range of chain lengths would be useful not only in research to develop synthetic vaccines but also for studies on the relationship

に酸化と脱保護を行うことによって目的化合物 72 を得た。なお、中間体 70 の硫化によって、チオリン酸骨格を有する化合物 71 ( $X = \text{S}$ ) の合成も可能である。以上述べた様に、現時点では収率に改善の余地があるものの、ボラノホスフェートを中間体とすることで、ホスホロアミダイト法を用いた糖-1-リン酸繰り返し構造の合成が可能であることがわかった。

### G. 今後の展望

現在、糖-1-リン酸誘導体、特に複数の糖-1-リン酸ユニットからなるホスホグリカンの合成法としては *H*-ホスホネート法が最も優れており、この方法では合成が困難な一部の糖-1-リン酸(糖ヌクレオチドや $\beta$ -糖-1-リン酸ユニット等)の合成に、リン酸誘導体を求核剤とするグリコシル化などの手法が用いられている。先に述べた通り、現在の *H*-ホスホネート法でも糖-1-リン酸ユニット 2–4 個からなるホスホグリカンの部分構造の合成が可能であり、この合成ホスホグリカンとタンパク質との複合体がワクチンとして働き得ることも示されている(34)。しかしながら、合成ホスホグリカンのワクチンとしての効果に関するデータは極めて限られたものであり、どの程度の鎖長のホスホグリカンがワクチンとして最も効果があるのかについては判明していない。合成ホスホグリカンのワクチンへの応用研究をさらに進めるためには、長鎖のホスホグリカンの合成が可能で、より効率的な化学合成法が必要と

between the molecular structures of phosphoglycans and the virulence of pathogenic microorganisms. Further progress in the solid-phase synthesis of phosphoglycan fragments by the *H*-phosphonate method, which can reduce the difficulties of purification, and the development of our method using stable boranophosphate intermediates are expected, so that phosphoglycan fragments of desired length become available.

In addition to application as synthetic intermediates, phosphoglycans composed of glycosyl boranophosphate units may have potential as vaccines with improved stability. Recently, some stable phosphoglycan analogs such as phosphonates **73** and **74** (Fig. 20) (70–73) and carbasugar analogs (*e.g.*, **75**) (74) have been developed as vaccine candidates to overcome the current vaccine problems derived from the inherent lability of the glycosyl phosphate units. However, the syntheses of these non-natural sugars are nontrivial. In contrast, the boranophosphate-based phosphoglycans can be synthesized from natural sugars in fewer steps. It has also been shown with a variety of nucleic acid analogs (49) and glycosyl phosphates (52) that boranophosphate analogs are very similar both sterically and electronically to the natural phosphate diester linkages. For these reasons, application of the glycosyl boranophosphate derivatives to vaccine research would also be interesting.

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#### References

1. Nikolaev, A. V., Botvinko, I. V., and Ross, A. J. (2007) *Carbohydr. Res.* **342**, 297–344.
2. Joshi, V. S., Bajaj, I. B., Survase, S. A., Singhal, R. S., and Kennedy, J. F. (2009) *Carbohydr. Polym.* **75**, 553–565.
3. Trotter, C. L., McVernon, J., Ramsay, M. E., Whitney, C. G., Mulholland, E. K., Goldblatt, D., Hombach, J., and Kieny, M.-P. (2008) *Vaccine* **26**, 4434–4445.
4. Nikolaev, A. V., and Sizova, O. V. (2011) *Biochemistry (Moscow)* **76**, 761–773.
5. Morelli, L., Poletti, L., and Lay, L. (2011) *Eur. J. Org. Chem.*, 5723–5777.
6. Mendonça-Previato, L., Todeschini, A. R., Heise, N., Agrellos, O. A., Dias, W. B., and Previato, J. O. (2008) *Curr. Org. Chem.* **12**, 926–939.
7. Novozhilova, N. M., and Bovin, N. V. (2010) *Biochemistry (Moscow)* **75**, 686–694.
8. Ruhela, D., Banerjee, P., and Vishwakarma, R. A. (2012) *Curr. Sci.* **102**, 194–211.
9. Leloir, L. F. (1971) *Science* **172**, 1299–1303.
10. Wagner, G. K., Pesnot, T., and Field, R. A. (2009) *Nat. Prod. Rep.* **26**, 1172–1194.
11. Kornfeld, R., and Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664.
12. Hirschberg, C. B., and Snider, M. D. (1987) *Annu. Rev. Biochem.* **56**, 63–87.
13. Schmaltz, R. M., Hanson, S. R., and Wong, C.-H. (2011) *Chem. Rev.* **111**, 4259–4307.
14. Gantt, R. W., Peltier-Pain, P., and Thorson, J. S. (2011) *Nat. Prod. Rep.* **28**, 1811–1853.
15. Wagner, G. K., and Pesnot, T. (2010) *ChemBioChem* **11**, 1939–1949.
16. Kajimoto, T., and Node, M. (2009) *Synthesis*, 3179–3210.

なる。様々な鎖長の合成ホスホグリカンが得られるようになれば、ワクチンとしての利用だけでなく、ホスホグリカンの分子構造と病原体の病原性の関係に関する研究の進展なども期待される。*H*-ホスホネート法については、合成サイクル毎の中間体の精製の必要が無い固相合成の研究が行われており、液相法を上回る鎖長のホスホグリカンの合成に期待がかかる。また、*H*-ホスホネート法の問題点を克服すべく、より安定な糖-1-ボラノホスフェート骨格を合成中間体とする手法の開発も筆者らによって行われている。

なお、糖-1-ボラノホスフェート繰り返し構造を持つ分子は、それ自身が安定なホスホグリカンアナログとして働くことも考えられる。近年、ホスホグリカンからなるワクチンの安定性を改善すべく、ホスホネートアナログ **73**, **74** (図20) (70–73) やカルバ糖アナログ **75** (74) が開発されているが、糖骨格の合成に多段階を要する。糖-1-ボラノホスフェートは天然型の糖をそのまま用いており、より短い工程で合成が可能である。また、ボラノホスフェートジエステルとリン酸ジエステルが立体的、電子的に極めて類似していることは、核酸アナログにおける研究 (49) や、数少ないが糖-1-リン酸を用いた研究 (52) でも示されている。糖-1-ボラノホスフェートアナログのワクチンへの応用に関する研究の進展にも期待したい。

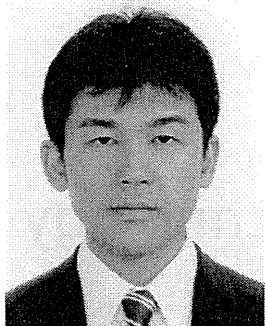
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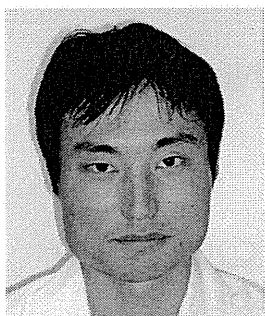
17. Hashimoto, S., Honda, T., and Ikegami, S. (1989) *J. Chem. Soc., Chem. Commun.*, 685–687.
18. Seeberger, P. H. (2003) *Chem. Commun.*, 1115–1121.
19. Sim, M. M., Kondo, H., and Wong, C.-H. (1993) *J. Am. Chem. Soc.* **115**, 2260–2267.
20. Garcia, B. A., and Gin, D. Y. (2000) *Org. Lett.* **2**, 2135–2138.
21. Plante, O. J., Palmacci, E. R., Andrade, R. B., and Seeberger, P. H. (2001) *J. Am. Chem. Soc.* **123**, 9545–9554.
22. Crich, D., and Dudkin, V. (2002) *J. Am. Chem. Soc.* **124**, 2263–2266.
23. Ravidà, A., Liu, X., Kovacs, L., and Seeberger, P. H. (2006) *Org. Lett.* **8**, 1815–1818.
24. Chambers, R. W., and Moffatt, J. G. (1958) *J. Am. Chem. Soc.* **80**, 3752–3756.
25. Wittmann, V., and Wong, C.-H. (1997) *J. Org. Chem.* **62**, 2144–2147.
26. Van Boeckel, C. A. A., Visser, G. M., Hermans, J. P. G., and van Boom, J. H. (1983) *Recl. Trav. Chim. Pays-Bas* **102**, 526–537.
27. Cawley, T. N., and Letters, R. (1971) *Carbohydr. Res.* **19**, 373–382.
28. Srivastava, O. P., and Hindsgaul, O. (1985) *Carbohydr. Res.* **143**, 77–84.
29. Nikolaev, A. V., Ivanova, I. A., and Shibaev, V. N. (1993) *Carbohydr. Res.* **242**, 91–107.
30. Ross, A. J., Ivanova, I. A., Ferguson, M. A. J., and Nikolaev, A. V. (2001) *J. Chem. Soc., Perkin Trans. 1*, 72–81.
31. Ruhela, D., and Vishwakarma, R. A. (2003) *J. Org. Chem.* **68**, 4446–4456.
32. Higson, A. P., Ross, A. J., Tsvetkov, Y. E., Routier, F. H., Sizova, O. V., Ferguson, M. A. J., and Nikolaev, A. V. (2005) *Chem. Eur. J.* **11**, 2019–2030.
33. Rogers, M. E., Sizova, O. V., Ferguson, M. A. J., Nikolaev, A. V., and Bates, P. A. (2006) *J. Infect. Dis.* **194**, 512–518.
34. Berkin, A., Coxon, B., and Pozsgay, V. (2002) *Chem. Eur. J.* **8**, 4424–4433.
35. Slättegård, R., Teodorovic, P., Kinfe, H. H., Ravenscroft, N., Gammon, D. W., and Oscarson, S. (2005) *Org. Biomol. Chem.* **3**, 3782–3787.
36. Nilsson, M., and Norberg, T. (1998) *J. Chem. Soc., Perkin Trans. 1*, 1699–1704.
37. Hansson, J., Garegg, P. J., and Oscarson, S. (2001) *J. Org. Chem.* **66**, 6234–6243.
38. Nikolaev, A. V., Chudek, J. A., and Ferguson, M. A. J. (1995) *Carbohydr. Res.* **272**, 179–189.
39. Cori, C. F., Colowick, S. P., and Cori, G. T. (1937) *J. Biol. Chem.* **121**, 465–477.
40. Wolfrom, M. L., Smith, C. S., Pletcher, D. E., and Brown, A. E. (1942) *J. Am. Chem. Soc.* **64**, 23–26.
41. Schmidt, R. R., Stumpp, M., and Michel, J. (1982) *Tetrahedron Lett.* **23**, 405–408.
42. Stevens, C. L., and Harmon, R. E. (1969) *Carbohydr. Res.* **11**, 99–102.
43. Timmers, C. M., van Straten, N. C. R., van der Marel, G. A., and van Boom, J. H. (1998) *J. Carbohydr. Chem.* **17**, 471–487.
44. Volkova, L. V., Danilov, L. L., and Evstigneeva, R. P. (1974) *Carbohydr. Res.* **32**, 165–166.
45. Garegg, P. J., Hansson, J., Helland, A.-C., and Oscarson, S. (1999) *Tetrahedron Lett.* **40**, 3049–3052.
46. Crich, D., and Picard, S. (2009) *J. Org. Chem.* **74**, 9576–9579.
47. Greimel, P., and Ito, Y. (2008) *Tetrahedron Lett.* **49**, 3562–3566.
48. Greimel, P., Lapeyre, M., Nagatsuka, Y., Hirabayashi, Y., and Ito, Y. (2008) *Bioorg. Med. Chem.* **16**, 7210–7217.
49. Li, P., Sergueeva, Z. A., Dobrikov, M., and Shaw, B. R. (2007) *Chem. Rev.* **107**, 4746–4796.
50. Ross, A. J., Higson, A. P., Ferguson, M. A. J., and Nikolaev, A. V. (1999) *Tetrahedron Lett.* **40**, 6695–6698.
51. Prospero, D., Panza, L., Poletti, L., and Lay, L. (2000) *Tetrahedron* **56**, 4811–4815.
52. Routier, F. H., Higson, A. P., Ivanova, I. A., Ross, A. J., Tsvetkov, Y. E., Yashunsky, D. V., Bates, P. A., Nikolaev, A. V., and Ferguson, M. A. J. (2000) *Biochemistry* **39**, 8017–8025.
53. Imamoto, T., Nagato, E., Wada, Y., Masuda, H., Yamaguchi, K., and Uchimaru, T. (1997) *J. Am. Chem. Soc.* **119**, 9925–9926.
54. Shimizu, M., Tamura, K., Wada, T., and Saigo, K. (2004) *Tetrahedron Lett.* **45**, 371–374.
55. Wada, T., Shimizu, M., Oka, N., and Saigo, K. (2002) *Tetrahedron Lett.* **43**, 4137–4140.
56. Shimizu, M., Wada, T., Oka, N., and Saigo, K. (2004) *J. Org. Chem.* **69**, 5261–5268.
57. Matsumura, F., Oka, N., and Wada, T. (2008) *Org. Lett.* **10**, 1557–1560.
58. Hung, S.-C., and Wong, C.-H. (1996) *Tetrahedron Lett.* **37**, 4903–4906.
59. Sato, K., Oka, N., Fujita, S., Matsumura, F., and Wada, T. (2010) *J. Org. Chem.* **75**, 2147–2156.
60. Matsumura, F., Oka, N., and Wada, T. (2008) *Org. Lett.* **10**, 5297–5300.
61. Fujita, S., Oka, N., Matsumura, F., and Wada, T. (2011) *J. Org. Chem.* **76**, 2648–2659.
62. Beaucage, S. L., and Iyer, R. P. (1992) *Tetrahedron* **48**, 2223–2311.
63. Shiba, Y., Masuda, H., Watanabe, N., Ego, T., Takagaki, K., Ishiyama, K., Ohgi, T., and Yano, J. (2007) *Nucleic Acids Res.* **35**, 3287–3296.
64. Westerduin, P., Veeneman, G. H., Marugg, J. E., van der Marel, G. A., and van Boom, J. H. (1986) *Tetrahedron Lett.* **27**, 1211–1214.
65. Westerduin, P., Veeneman, G. H., van der Marel, G. A., and van Boom, J. H. (1986) *Tetrahedron Lett.* **27**, 6271–6274.
66. Hecker, S. J., Minich, M. L., and Lackey, K. (1990) *J. Org. Chem.* **55**, 4904–4911.
67. Makino, S., Ueno, Y., Ishikawa, M., Hayakawa, Y., and Hata, T. (1993) *Tetrahedron Lett.* **34**, 2775–2778.
68. Kajihara, Y., Ebata, T., Koseki, K., Kodama, H., Matsushita, H., and Hashimoto, H. (1995) *J. Org. Chem.* **60**, 5732–5735.
69. Majumdar, D., Elsayed, G. A., Buskas, T., and Boons, G.-J. (2005) *J. Org. Chem.* **70**, 1691–1697.
70. Borodkin, V. S., Milne, F. C., Ferguson, M. A. J., and Nikolaev, A. V. (2002) *Tetrahedron Lett.* **43**, 7821–7825.
71. Teodorović, P., Slättegård, R., and Oscarson, S. (2006) *Org. Biomol. Chem.* **4**, 4485–4490.
72. Torres-Sanchez, M. I., Zaccaria, C., Buzzi, B., Miglio, G., Lombardi, G., Polito, L., Russo, G., and Lay, L. (2007) *Chem. Eur. J.* **13**, 6623–6635.
73. Manca, F., Bindoli, C., Fallarini, S., Lombardi, G., Polito, L., Lay, L., Bonomi, R., Mancin, F., and Scrimin, P. (2008) *Adv. Mater.* **20**, 4348–4352.
74. Legnani, L., Ronchi, S., Fallarini, S., Lombardi, G., Campo, F., Panza, L., Lay, L., Poletti, L., Toma, L., Ronchetti, F., and Compostella, F. (2009) *Org. Biomol. Chem.* **7**, 4428–4436.



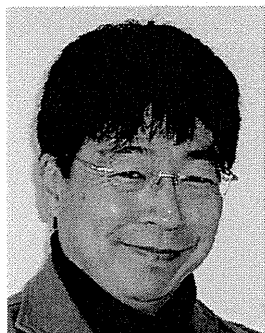
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**Natsuhisa Oka** received his Ph.D. degree from the University of Tokyo in 2003 under the supervision of Prof. Kazuhiko Saigo and Prof. Takeshi Wada. After working as a postdoctoral fellow at the University of California, San Diego with Prof. Emmanuel A. Theodorakis and at the Johns Hopkins University with Prof. Marc M. Greenberg from 2003 to 2005, he moved to the Graduate School of Frontier Sciences, the University of Tokyo, where he worked as an Assistant Professor with Prof. Saigo and Prof. Wada from 2005 to 2009. In 2009, he joined the Department of Chemistry at the Gifu University as an Associate Professor. His research interests are centered on the chemistry of biomolecules.



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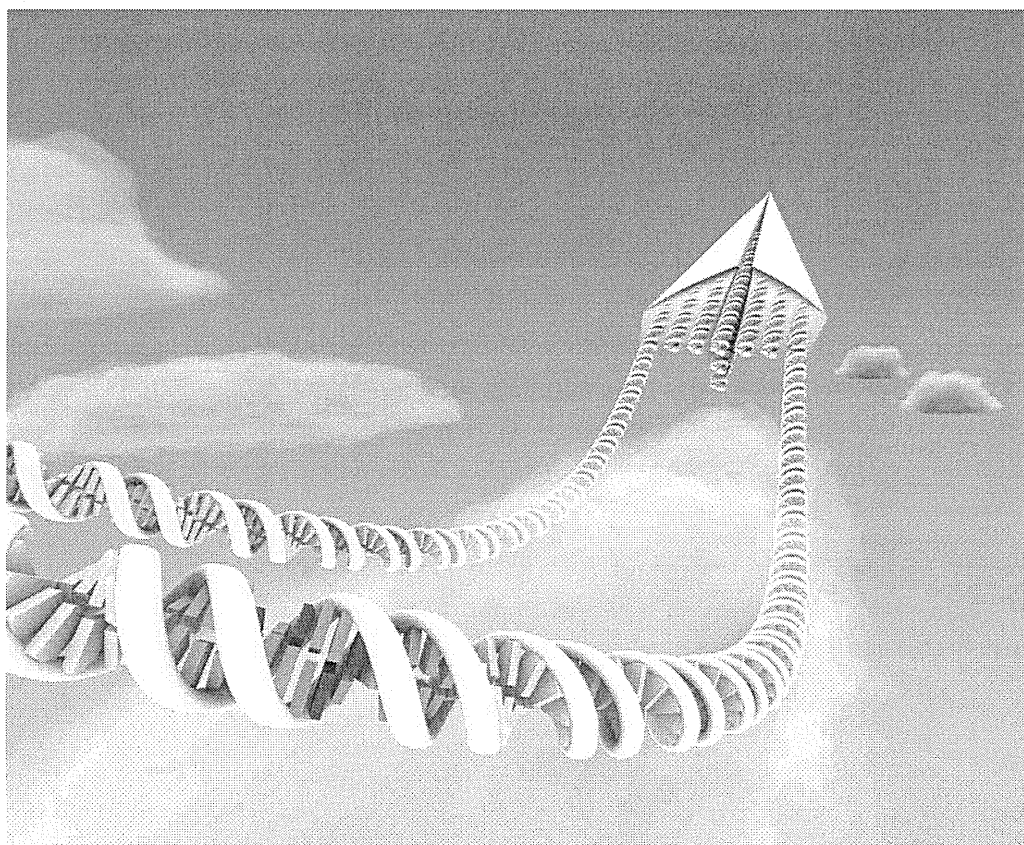
**Takeshi Wada** received his Ph.D. degree from Tokyo Institute of Technology in 1991 under the supervision of Prof. Tsujiaki Hata. He worked as an Assistant Professor with Prof. Mitsuo Sekine from 1991 to 1999 at Tokyo Institute of Technology. Then he moved to the University of Tokyo as an Associate Professor. His research interests are centered on nucleic acid chemistry, carbohydrate chemistry and peptide chemistry.

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## CRITICAL REVIEW

## Stereocontrolled synthesis of oligonucleotide analogs containing chiral internucleotidic phosphorus atoms†

Natsuhisa Oka<sup>a</sup> and Takeshi Wada<sup>\*b</sup>

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Oligonucleotides, in which one of the two nonbridging oxygen atoms of internucleotidic phosphates is replaced by a different type of atom or a substituent, are useful as therapeutic agents and probes to elucidate mechanisms of enzymatic reactions. The internucleotidic phosphorus atoms of these oligonucleotides are chiral, and the properties of these oligonucleotides are affected by the absolute configuration of the chiral phosphorus atoms. In order to address the issue of chirality, various methods have been developed to synthesize these *P*-chiral oligonucleotide analogs in a stereocontrolled manner. This *critical review* focuses on the recent progress in this field (123 references).

## 1. Introduction

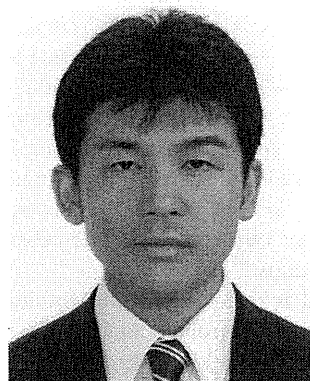
Oligonucleotides form rigid double helices in a predictable manner through A–T(U) and G–C base pairings. Because of this capability, oligonucleotides have found numerous applications, such as in gene function analysis<sup>1</sup> and gene-targeting therapy,<sup>2,3</sup> both of which are based on the specific knockdown

of target gene expression, genetic diagnosis,<sup>4,5</sup> and assembly of nanostructures.<sup>6–9</sup> However, because of some undesirable properties, such as susceptibility to nucleases and poor cell membrane permeability, natural oligonucleotides are not suitable for many of these applications. Therefore, various types of chemical modifications have been developed to improve their properties and/or integrate new functions, which are desirable or indispensable for the above applications.<sup>4,10–14</sup> Improvements in property, including stability to nuclease digestion and cell membrane permeability, are needed for therapeutic applications,<sup>13,14</sup> and higher hybridization affinity to complementary oligonucleotides is also desirable. Chemical labeling of oligonucleotides is a functional development that has become indispensable for diagnostic applications.<sup>4</sup>

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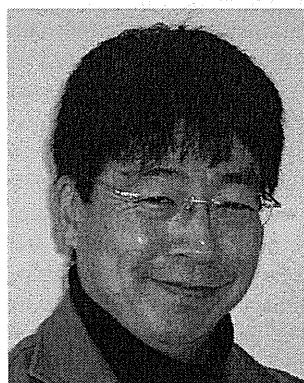
† Part of a themed issue on the advances in DNA-based nanotechnology.



Natsuhisa Oka

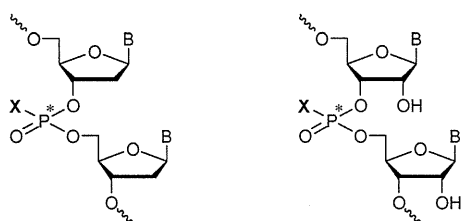
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Takeshi Wada

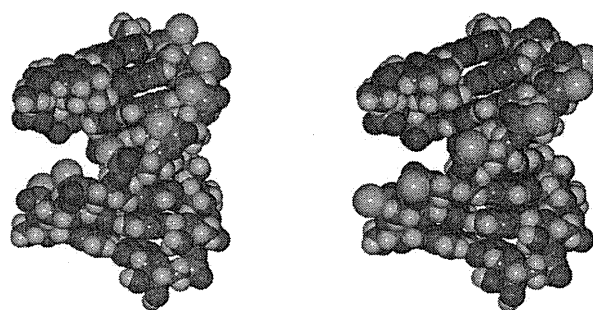
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**Fig. 1** Oligodeoxyribonucleotide and oligoribonucleotide analogs containing chiral internucleotidic phosphorus atoms. B = nucleobase, X = Me, S<sup>-</sup>, Se<sup>-</sup>, NR<sup>1</sup>R<sup>2</sup>, BH<sub>3</sub><sup>-</sup>, H, *etc.*

Because some of the properties of natural oligonucleotides, such as good aqueous solubility and the ability to form sequence-specific duplexes, are necessary for their applications, an ideal modification should improve their properties or expand their functional capabilities without compromising their inherent advantages. To achieve this objective, one approach is to minimize structural changes to the molecule. Replacement of one of the two nonbridging oxygen atoms of internucleotidic phosphate diesters<sup>15–22</sup> is suitable in this regard. Moreover, such modifications greatly improve the stability of the resulting derivatives to nuclease digestion.

Various oligonucleotide analogs with such modified phosphates have been developed to date. Representative analogs are shown in Fig. 1. Methylphosphonate (X = Me)<sup>15,19</sup> and phosphorothioate (X = S<sup>-</sup>)<sup>17,20,22</sup> are among the oldest examples and have been extensively studied for gene-targeting therapeutic applications. In particular, the latter has significantly improved cellular uptake and stability to nucleases while maintaining water solubility and the ability to form duplexes with strict base sequence specificity. Therefore, phosphorothioates have been incorporated into many of the therapeutic oligonucleotides in clinical trials, including the one which has been approved for marketing.<sup>3,13,23</sup> In addition to their therapeutic applications, phosphorothioate analogs as well as methylphosphonates have been used as probes for exploring the functions of internucleotidic phosphates in enzymatic reactions.<sup>10,21</sup> Moreover, the P–S<sup>-</sup> group is potentially useful for further modifications of oligonucleotides through S-alkylation.<sup>10</sup> Phosphoroselenoates (X = Se<sup>-</sup>) have attracted attention with respect to their applicability to multiwavelength anomalous diffraction phasing for X-ray structural studies.<sup>24,25</sup> Phosphoramidates (X = NR<sup>1</sup>R<sup>2</sup>), which can be synthesized from an *H*-phosphonate derivative (X = H) *via* the Atherton–Todd reaction,<sup>26–28</sup> are useful for the preparation of a variety of oligonucleotide analogs by applying various primary and secondary amines to this reaction. Boranophosphates (X = BH<sub>3</sub><sup>-</sup>)<sup>29</sup> have been developed relatively recently and have been reported to show exceptional stability to nucleases compared to the corresponding phosphorothioates, and short interfering RNAs having this type of modification have shown a promising RNA interference activity. Boranophosphate is also expected to be less cytotoxic than the corresponding phosphorothioate.<sup>29</sup> *H*-Phosphonates (X = H) function as precursors of a variety of phosphate-modified oligonucleotide analogs, including those shown in Fig. 1, although the *H*-phosphonate itself is not suitable for the applications mentioned above because of its instability.<sup>26</sup>



**Fig. 2** NMR structure of all-(*Rp*)-PS-5'-d[CGTCAGG]-3'-3'-r[CGCAGUCC]-5'-5'<sup>30</sup> (left)<sup>30</sup> and model structure of all-(*Sp*)-PS-5'-d[CGTCAGG]-3'-3'-r[CGCAGUCC]-5'-5' in which internucleotidic nonbridging oxygen and sulfur atoms are exchanged (right). Black, carbon; blue, nitrogen; orange, phosphorus; red, oxygen; white, hydrogen; yellow, sulfur.

The oligonucleotide analogs represented in Fig. 1 have chiral centers on their phosphorus atoms. When they form double helices with complementary oligonucleotides, the substituents on the chiral phosphorus atoms are oriented either “inward” toward the major groove or “outward” from the duplex depending on the absolute configuration of each phosphorus atom<sup>16</sup> (Fig. 2).<sup>30</sup> As a result, the properties of their duplexes with complementary oligonucleotides, such as thermal stability, are affected by the configuration of the phosphorus atoms. For this reason, their stereocontrolled synthesis has been a subject of great importance over the past few decades.<sup>16–21</sup> This *critical review* covers the recent progress on this subject.

## 2. Diastereomeric separation by chromatography

The problems introduced by the chirality of phosphorus atoms have been recognized from the early stages of research on *P*-chiral nucleotide analogs.<sup>15</sup> However, because diastereopure isomers of these analogs were unobtainable by stereocontrolled chemical synthesis, chromatographic separation has played an important role in obtaining stereodefined *P*-chiral nucleotide analogs. It has often been used since the 1970s to separate *P*-chiral dinucleoside phosphate analogs into (*Rp*)- and (*Sp*)-diastereomers, which in turn enabled studies such as their configurational assignment, comparative studies on their stability to nucleases, and affinity to single- or double-stranded DNA or RNA.<sup>31–33</sup> The method has also been used to separate the diastereomers of oligonucleotides containing a single chiral phosphorus atom. Purified diastereomers are used to probe the roles of pro-*Rp* and *Sp* oxygen atoms of specific phosphates in the stabilization of duplexes or higher-order structures and in enzymatic reactions.<sup>34–36</sup>

The advantage of this method is that the oligonucleotides can be synthesized in a nonstereocontrolled manner. The disadvantage is that the degree of separation is dependent on the target structure (length, base sequence, and position of the chiral phosphorus atom) as well as the conditions used for chromatography, and satisfactory separation is not always guaranteed.<sup>37</sup> Furthermore, separation of diastereomers becomes increasingly difficult as the number of chiral phosphorus atoms increases, because the number of diastereomers increases