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Synthesis of Carbohydrate Methyl Phosphoramidates

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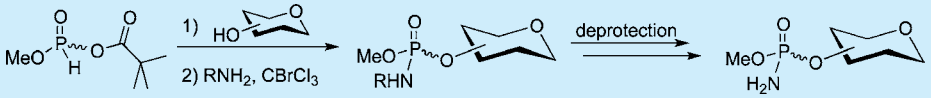
Supporting Information



ABSTRACT: A two-step route for introducing methyl phosphoramidate moieties onto carbohydrates is reported. The approach uses methyl pivolyl *H*-phosphonate as the phosphorylating reagent to produce an isolable carbohydrate *H*-phosphonate intermediate that is then oxidized by a Todd−Atherton reaction. The stability of the product methyl phosphoramidates was subsequently evaluated using various deprotection strategies.

Campylobacter jejuni is the leading cause of bacterial gastroenteritis worldwide.[1] The outer surface of campylobacters is functionalized with species-specific capsular polysaccharides (CPS), the production of which is required for virulence.[2] The CPS produced by different *C. jejuni* strains express a broad diversity of structures, due in part to phase-variable modifications. Among these motifs are unique *O*-methyl phosphoramidate (MeOPN) groups, which are found in over 70% of all *C. jejuni* strains.[3] As examples, Figure 1 shows the

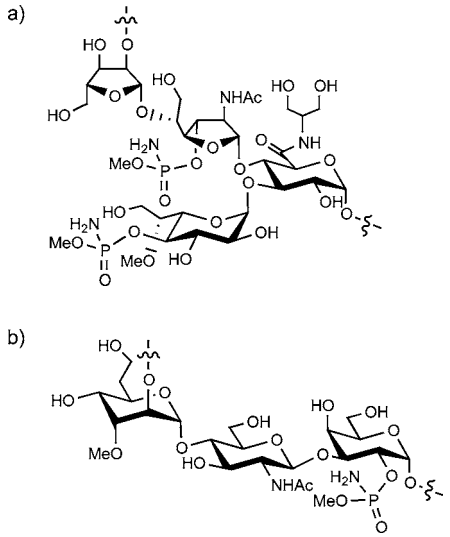


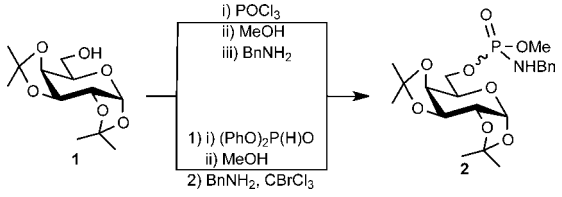
Figure 1. Repeating structures of CPS from (a) *C. jejuni* 11168H and (b) *C. jejuni* 81−176.

repeating core structures of two *C. jejuni* strains that possess MeOPN motifs in their CPS. The role of these MeOPN moieties remains unclear. However, recent studies suggest they play roles in serum resistance and colonization[3] and they have also been shown to have insecticidal activity, although this latter activity has been questioned.[4]

Due to the rarity of these MeOPN motifs, which exist as a single although currently unknown stereochemistry on phosphorus,[3] their antigenicity could be explored for the development of vaccines.[5] In addition, access to MeOPN-functionalized carbohydrates could help unravel the biosynthetic pathway by which these groups are assembled and lead to the future development of new therapeutic agents against *C. jejuni*. However, such studies require a method for the synthesis of MeOPN groups.

Previous reports have described the preparation of *N*-protected alkyl/aryl phosphoramidate diesters[6] and aryl phosphoramidates on nucleotide analogues.[7] However, to date, the synthesis of these moieties possessing an unprotected nitrogen has not been reported. Syntheses of phosphoramidates often involve the use of phosphoryl chloride as the phosphorylating reagent.[8] We therefore initially investigated the use of $POCl_3$ with a model substrate, 1,2:3,4-di-*O*-isopropylidene galactopyranose (**1**, Scheme 1). However,

Scheme 1. Initial Approaches for Introducing MeOPN Motifs onto Carbohydrates



several products were formed in this reaction, and purification of the desired product was difficult. As an alternative, we explored the use of diphenyl phosphite by double displacement of the *O*-phenyl groups, first with **1** and then methanol, followed by a Todd−Atherton reaction with a primary amine (Scheme 1). Although the product is formed and can be

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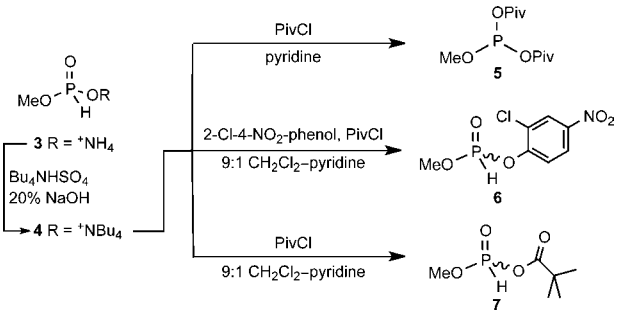
isolated with relative ease, the yields were low. We also explored other commercially available phosphorylating reagents including 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and methyl dichlorophosphate; however, the results obtained were inferior to those obtained using diphenyl phosphite.

Given the modest, albeit positive, results obtained using diphenyl phosphite, we explored other *H*-phosphonate-based reagents (**5**–**7**, Scheme 2) to affect this transformation. The

Scheme 2. Synthesis of Phosphorylating Reagents 5–7



preparation of **5**–**7** involved first the conversion of methyl *H*-phosphonate ammonium salt **3**[9] into its tetra-*N*-butylammonium counterpart (**4**).[10] Reagent **5** can be synthesized by the addition of pivaloyl chloride (PivCl) to **4** in pyridine.[11] Treatment of a solution of **4** in 9:1 CH_2Cl_2–pyridine with PivCl either in the presence or absence of 2-chloro-4-nitrophenol[12] led to **6** and **7**, respectively.[7c,8]

In the protocol developed, reagents **5**–**7** were generated in situ, **1** was added and the reaction was monitored by thin-layer chromatography. Of the three reagents, we found **7** to be the most successful in producing the desired methyl sugar *H*-phosphonate **8** (Figure 2). The compound can easily be converted to the benzyl-protected MeOPN-containing monosaccharide **2** in a subsequent Todd–Atherton reaction with benzylamine.

Figure 2 shows a representative reaction sequence with monitoring by ^{31}P NMR spectroscopy. *H*-Phosphonate salt **4** was consumed within 20 min after addition of PivCl to generate a new product with a ^{31}P signal consistent with **7**.[7c,13] After the formation of **7** was complete, **1** was added, and after being stirred for 1 h, the mixture was worked up. ^{31}P NMR spectroscopy of the product showed two new signals in an ~1:1 ratio, indicative of the two diastereomers present in the methyl sugar *H*-phosphonate **8**.[14] Without further purification, **8** was converted to **2** via a Todd–Atherton reaction with benzylamine and bromotrichloromethane in the presence of triethylamine for 4 h. Following workup, ^{31}P NMR spectroscopy of the product revealed two new signals for **2**, corresponding to an ~1:1 ratio of diastereomers.

With these optimized conditions developed, a panel of substrates was evaluated (Table 1). Entries 1 and 2 show that the method can be used with benzylamine, *p*-methoxybenzylamine, or *o*-nitrobenzylamine as the oxidant in the Todd–Atherton reaction. In addition, the method is successful with both primary and secondary hydroxyl groups on glucose and galactose analogues in either in the pyranose or furanose ring configurations. Notably, the method was capable of producing protected precursors (**28**–**30**) of the motifs found in the CPS of *C. jejuni* strains 11168H and 81–176 (see Figure 1).

The examples in Table 1 reveal that the conditions are sufficiently mild that a variety of protecting groups can be used

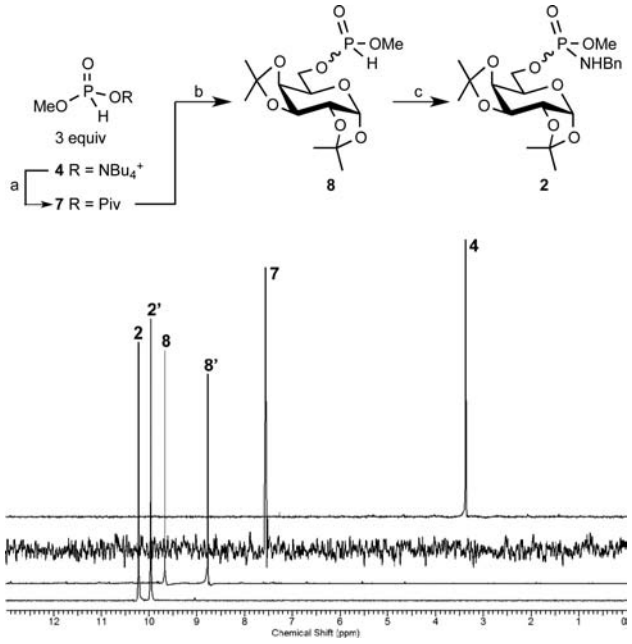


Figure 2. (a) PivCl (3.6 equiv), 9:1 CH_2Cl_2–pyridine, 20 min, (b) sugar substrate (1 equiv), 1 h followed by basic workup, (c) Bn-NH_2 (1.5 equiv), TEA (3 equiv), $CBrCl_3$ (10 equiv), DCM, 4 h followed by basic workup. ^{31}P NMR of two-step method for synthesizing the MeOPN-containing saccharide.

in the substrates. These groups include benzylidene acetals, benzyl ethers, benzoates and acetate esters, isopropylidene ketals, and silyl ethers. In each case, the product was produced as an ~1:1 mixture of diastereomers, which in most cases is inseparable. Two substrates, **49** and **50** (Figure 3), were, however, found to be incompatible. In the reaction of *N*-acetate **49** with **7**, a product of unknown structure was formed that was resistant to the Todd–Atherton reaction. In the case of the azide containing substrate **50**, treatment with **7** presumably leads to a Staudinger-type reaction leading to a methyl sugar phosphate derivative.

With these protected MeOPN-containing saccharides in hand, we studied the stability of this motif using various deprotection strategies (Table 1). We first studied removal of the nitrogen protecting group on the MeOPN motif. We found that *N*-benzyl and *N*-*p*-methoxybenzyl groups on the MeOPN can be removed oxidatively using sodium bromate/sodium thiosulfite[15] and ceric ammonium nitrate, respectively. We found these groups could not be removed using standard hydrogenolytic conditions (H_2, Pd–C); no consumption of the starting material was observed. The *o*-nitrobenzyl groups (**18** and **21**) could be removed by photolysis in apolar protic solvents.

With regard to the protecting groups on oxygen, benzylidene acetals, and benzyl ethers can be reductively cleaved by hydrogenation in the presence of Raney nickel (entries 4 and 5). Hydrogenation in the presence of a palladium catalyst led to incomplete cleavage of the benzylidene acetals even after long reaction times and the use of high pressure. In addition, benzylidene acetals can be opened oxidatively to produce a benzoate ester.[15] Entry 8 demonstrates the simultaneous removal of *N*-benzyl group and regioselective oxidative ring-opening of the benzylidene acetal to produce **44** in good yield. Benzoyl and acetyl esters can also be removed using a 7:2:1 mixture of methanol–water–triethylamine (entries 3, 6, 8, and

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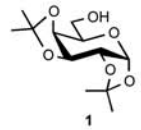
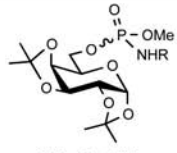
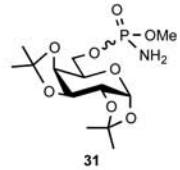
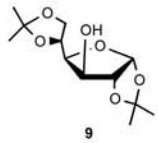
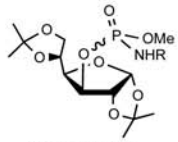
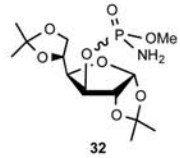
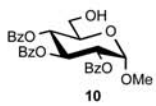
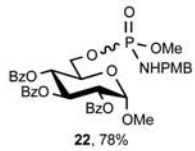
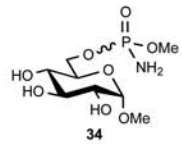
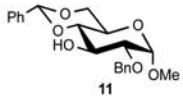
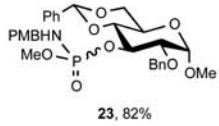
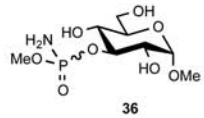
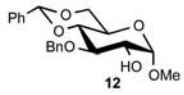
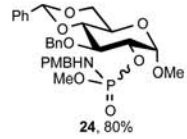
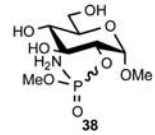
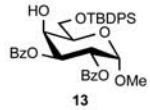
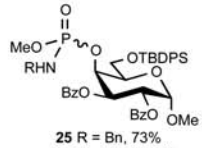
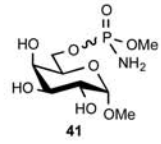
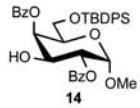
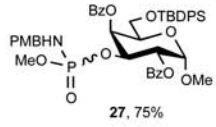
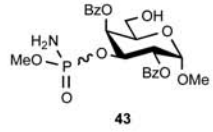
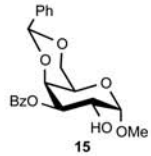
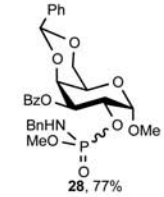
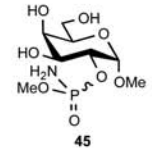
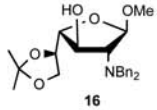
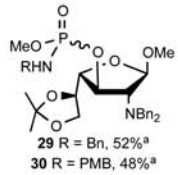
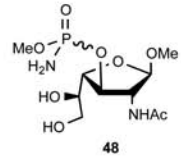
Table 1. Introduction of MeOPN Moieties onto Carbohydrates Followed by Deprotection

entry	MeOPN acceptor	product 1	product 2	deprotection conditions
1	 **1**	 **2** R = Bn, 79% **17** R = PMB, 81% **18** R = ONB, 73%	 **31**	R = Bn, $NaBrO_3$–$Na_2S_2O_4$ 91% R = PMB, CAN, 81% R = ONB*, *hν*, 83%
2	 **9**	 **19** R = Bn, 75% **20** R = PMB, 71% **21** R = ONB, 71%	 **32**	R = Bn, $NaBrO_3$–$Na_2S_2O_4$ 88% R = ONB*, *hν*, 74%
3	 **10**	 **22**, 78%	 **34**	1) CAN, 83% (**33**)** 2) methanol–water–triethylamine, 79% (**34**)
4	 **11**	 **23**, 82%	 **36**	1) CAN, 76% (**35**)** 2) H_2, Raney Ni, 61% (**36**)
5	 **12**	 **24**, 80%	 **38**	1) CAN, 73% (**37**)** 2) H_2, Raney Ni, 65% (**38**)
6	 **13**	 **25** R = Bn, 73% **26** R = PMB, 77%	 **41**	R = Bn 1) HF•py, 85% (**39**)** 2) $NaBrO_3$–$Na_2S_2O_4$, 74% (**40**)** 3) methanol–water–triethylamine, 44% (**41**)
7	 **14**	 **27**, 75%	 **43**	1) HF•py, 80% (**42**)** 2) CAN, 74% (**43**)
8	 **15**	 **28**, 77%	 **45**	1) $NaBrO_3$–$Na_2S_2O_4$, 89% (**44**)** 2) methanol–water–triethylamine, 81% (**45**)
9	 **16**	 **29** R = Bn, 52%[a] **30** R = PMB, 48%[a]	 **48**	R = Bn 1) 7.5% I_2 in methanol, 66% (**46**)** 2) H_2, Pd–C 3) Ac_2O 74% (**47**)** 5) $NaBrO_3$–$Na_2S_2O_4$ 6) methanol–water–triethylamine, 45% (**48**)

*ONB = *o*-nitrobenzyl. [a]Used 5 and 6 equiv of phosphorylating reagent and PivCl, respectively. **Products without structures can be found in the Supporting Information.

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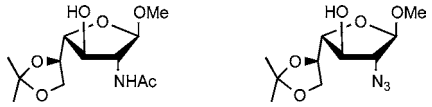

Figure 3. Methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (**49**) and methyl 2-azido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (**50**).

9). However, the use of sodium methoxide results in significant (often complete) cleavage of the phosphoramidate from the carbohydrate. Silyl ethers can be removed using HF·pyridine (entries 6 and 7) and isopropylidene acetals can be removed with a 7.5% iodine in methanol mixture (entry 9). Use of standard acid hydrolysis to cleave isopropylidene ketals or benzylidene acetals led to loss of the phosphoramidate motif.

In conclusion, an efficient route for synthesizing MeOPN-containing carbohydrates was developed using a two-step protocol involving phosphorylation with methyl pivaloyl *H*-phosphonate (**7**) followed by a Todd–Atherton reaction. The method is compatible with secondary and primary hydroxyl groups in the presence of a variety of protecting groups. In addition, various deprotection strategies could be used without affecting the MeOPN moiety. This method should be readily applied to more complex glycans containing this unusual motif. Currently under investigation is the development of methods for installing these groups as a single diastereomer on phosphorus as are explorations of the pathway by which the MeOPN group is biosynthesized by campylobacters.

■ ASSOCIATED CONTENT

Supporting Information

Experimental data and ^{1}H, ^{13}C, and ^{31}P NMR spectra for all previously unreported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

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