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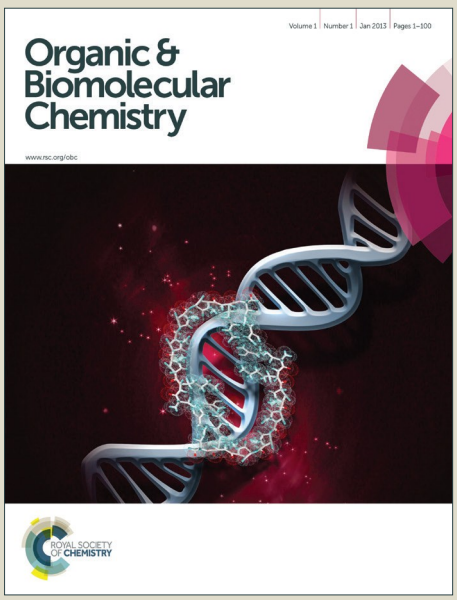

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ARTICLE

Direct synthesis of methyl phosphoramidates in carbohydrates†

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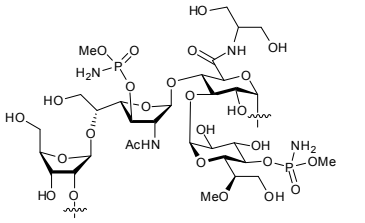
A direct installation of methyl phosphoramidate group by using methyl benzylphosphoramidochloridate into carbohydrates and amino acid is described. This one-step synthesis is efficient for both primary and secondary alcohols and exhibited excellent regioselectivity and funcational group compatibility. Formation of single diastereomer is observed in certain cases. The *N*-benzyl protecting group on methyl phosphoramidates is easily removed under mild conditions.

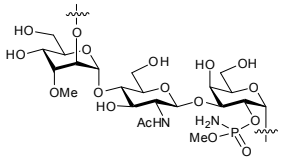
Introduction

Campylobacter jejuni (*C. jejuni*) is a food-borne bacterial pathogen that causes bacterial gastroenteritis worldwide.[1] The outer surface of this gram-negative bacterium is functionalized with various capsular polysaccharides (CPSs), which are responsible for its virulence.[2] *O*-Methyl phosphoramidate (MeOPN) moiety is commonly found as a modification in these CPSs (Figure 1).[3] However, the role of this *O*-MeOPN group is not clearly understood; though several reports have postulated that it is crucial for pathogenicity and serum resistance.[4] Although the MeOPN group appears as single diastereomer in CPSs, its exact stereochemistry is unknown.[4b]

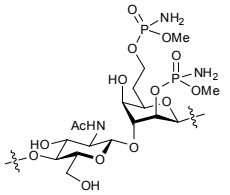
While diphenyl phosphate group can be directly introduced to carbohydrates by using various P(V) phosphorylating agents,[5] such as phosphoryl chloride,[6] incorporation of MeOPN group cannot be achieved in one step due to the poor electrophilicity of the corresponding P(V) agent. Currently, MeOPN is installed in two steps through a reaction with methyl pivolyl *H*-phosphonate, a P(III) phosphorylating agent, followed by Atherton–Todd oxidation.[7] This two-step protocol shows low diastereoselectivity and is not compatible with amide, a functional group often found in biologically potent carbohydrate molecules, and azide, which is an important amine protecting group in synthesis of glycoconjugates. Its regioselectivity is not investigated. In addition, to facilitate the synthesis of MeOPN-containing glycoconjugates and to understand their biochemistry, there is a need for more efficient methods to directly install MeOPN into carbohydrates, preferably in a highly diastereoselective manner. Herein, we report the use of methyl benzyl phosphoramidochloridate (**1**) as the phosphorylating reagent for the direct introduction of MeOPN moiety to carbohydrates.



repeating unit of CPS from *C. jejuni* 11168H

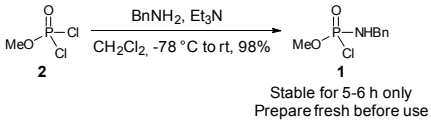


repeating unit of CPS from *C. jejuni* 81-176



repeating unit of CPS from *C. jejuni* CG8486

Figure 1 Repeating unit of CPSs from *C. jejuni*.



Scheme 1 Synthesis of methyl benzylphosphoramidochloridate **(1)**

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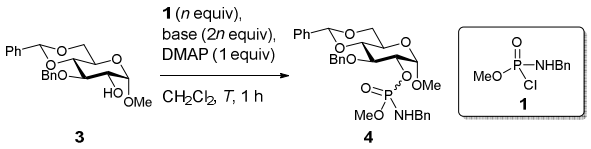
Electronic Supplementary Information (ESI) available: *experimental procedures, characterisation data on new compounds, and copies of NMR spectra.* See DOI: 10.1039/x0xx00000x

Results and discussion

Methyl benzylphosphoramidochloridate (**1**), the phosphorylating reagent, was readily prepared in one step by using the commercially available methyl dichlorophosphate (**2**) and benzylamine (Scheme 1). It is stable for 5-6 h only and has to be freshly prepared for phosphorylation. Its reaction was first examined with 2-alcohol **3** in the presence of 1 equiv of DMAP and a base. Compound **3** hardly reacted when pyridine or trimethylamine was used as the base (Table 1, entries 1 and 2). When 1-methylimidazole (NMI) was used as the base, the yield of **4** dramatically increased to 77% (Table 1, entry 3), and reducing the temperature to 0 °C did not improve the yield (Table 1, entry 4). The reaction was further optimized when a larger excess of **1** (4 equiv) and NMI (8 equiv) was used to produce a quantitative yield of **4** as an inseparable 1:1 mixture of both MeOPN diastereomers (Table 1, entry 5). Without DMAP, the reaction could still proceed, but in a lower yield (88%) (Table 1, entry 6). Since we were not able to determine the exact diastereochemistry of the two diastereomers, the diastereomeric ratio of **4** in each reaction was determined based on the integration ratio of the downfield to upfield signals in the ^{31}P NMR spectrum. As shown in Table 1, using trimethylamine as the base led to a 1:1 mixture of both diastereomers of **4**, but when pyridine or NMI was used, the diastereoselectivities improved to 2:1 and 2.7:1 respectively.

Table 1 Optimization of direct synthesis of methyl phosphoramidate **4**.



Entry	*n*	Base	*T* (°C)	Yield (%)[a] [d.r.][d]
1	3	pyridine	rt	33 [2:1]
2	3	Et_3N	rt	27[b] [1:1]
3	3	NMI	rt	77 [1.7:1]
4	3	NMI	0	78 [2.3:1]
5	4	NMI	-20	98 [2.5:1]
6	**4**	**NMI**	**rt**	**quant. [2.6:1]**
7[c]	4	NMI	rt	88 [2.7:1]

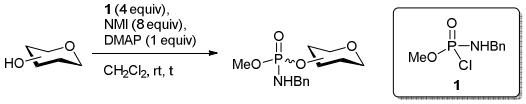
[a] Yield of isolated **4**. [b] 52% **3** recovered. [c] Without DMAP. [d] Based on the ^{31}P NMR integration of the downfield vs. upfield signals.

After obtaining the optimized reaction conditions, we explored the scope of the reaction by using different substrates (Table 2). The reactions with primary alcohols in protected glucoses and galactose were clean, and moderate to excellent yields of MeOPNs were obtained. Various functionalities, including benzyl ether, acetoxy, and acid-sensitive trimethylsilyoxy and isopropylidene, were well tolerated under this mild reaction condition (Table 2, entries 1–4). Similar to the reaction of **3** yielding **4** (Table 1), the reaction of secondary 3-OH in **14** (Table 2, entry 6) was successful, and a satisfactory yield of MeOPN was obtained. However, this phosphorylation was highly sensitive to the steric environment around the reaction centre. Sterically crowded 4-OH in **16** reacted slowly (Table 2, entry 7), and most of **12** remained unreacted even after the addition of excess reagent and a prolonged reaction time (Table 2, entry 5), probably because the β-1-STol group in **12** imposed severe steric hindrance around 2-OH; thus rendering the 2-OH less accessible for reaction with **1**. Although the reaction rates were greatly influenced by the steric hindrance, **13** (Table 2, entry 5) and **17** (Table 2, entry 7) were formed as single diastereomer as shown in their NMR spectra. The reaction was not limited to the pyranose form of carbohydrates. Furanose **18** and amino acid derivative **20** were also phosphorylated efficiently, but with moderate yields (Table 2, entries 8 and 9). The relatively poor reactivity of the amino acid derivative **20** compared with the other primary alcohols is due to the deactivation imposed by the unfavourable hydrogen bonding between the carbamate and hydroxyl group.[8]

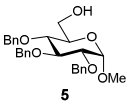
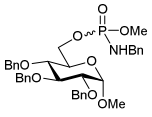
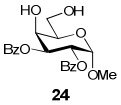
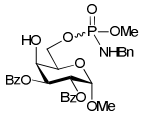
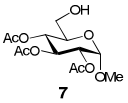
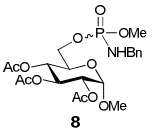
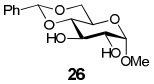
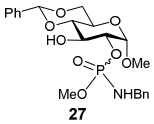
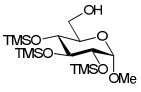
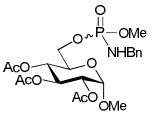
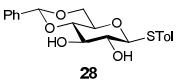
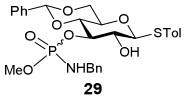
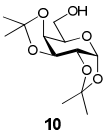
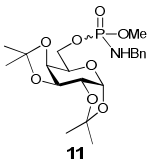
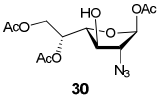
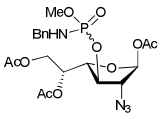
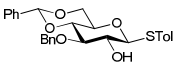
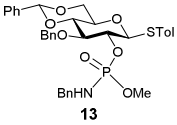
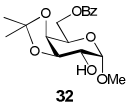
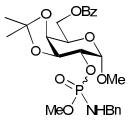
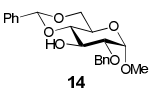
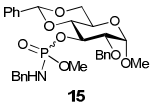
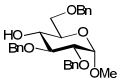
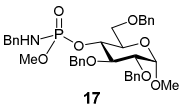
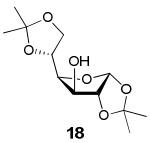
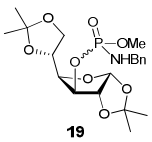
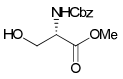
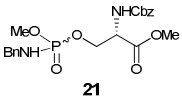
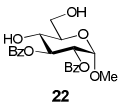
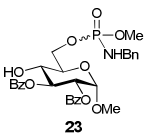
The regioselectivity of the phosphorylation of diols was investigated. Only mono-phosphoramidate was formed in all cases. The primary 6-OH was phosphorylated in preference to the secondary 4-OH in **22** and **24**[9] and a single regioisomer was afforded in a satisfactory yield in both cases (Table 2, entries 10 and 11). In addition, the regioselectivity depended on the relative steric hindrance at different reaction sites. Phosphorylation occurred exclusively at the *O*2 position in 2,3-diol **26** (Table 2, entry 12) but at the *O*3 position in **28** (Table 2, entry 13) to afford **27** and **29**, respectively, despite these substrates differ only at the anomeric positions that is remote from the reaction centre, in excellent yields and surprisingly with excellent diastereoselectivities.

Here, we obtained **13** and **17** as single diastereomer and **27** with excellent diastereoselecitiviy. However, it is difficult to unambiguously confirm their P stereochemistries using X-ray crystallography as none of these methyl phosphoramidates is solid. The nOe correlations between benzylic protons and *H*2, methoxy protons and *H*5 observed in the NOESY spectrum of **13** is shown in Figure 2. However, only the signals of an average rotameric conformation are shown in NMR studies.[10] Therefore, even with the NOESY spectra of **13**, **17** and **27** (See Supplementary Information), we still could not confirm their stereochemistries.

Table 2 Substrate scope of direct synthesis of methyl phosphoaramidates.



Entry	Substrate	Product	*t* (h)	Yield (%)[a] [d.r.][f]

1	 5	 6	1	91 [1:1.1]	11	 24	 25	1	76 [1.1:1]
2	 7	 8	5	65 [1:1.5]	12	 26	 27	2	90 [13:1]
3	 9	 8	12	75[c] [1:1.5]	13	 28	 29	2	85[d] [9:1]
4	 10	 11	1	quant. [1:1.1]	14	 30	 31	16	40[e] (58) [1.4:1]
5[b]	 12	 13	2	20 [1:0]	15	 32	 33	16	85 [1:0.8]
6[b]	 14	 15	6	65 (88) [1.6:1]					
7[b]	 16	 17	6	22 [1:0]					
8	 18	 19	2	56 (90) [1:1.3]					
9	 20	 21	2	45 [3.6:1]					
10	 22	 23	1	89 [1:1]					

[a] Yield of isolated products (In the brackets is the yield based on the recovered starting material). [b] Extra 3 equiv **1** and 6 equiv NMI added. [c] Product was directly derivatized into **8** by using Ac_2O for isolation. [d] Contained less than 10% of the other diastereomer. [e] Additional 4 equiv of **1** and 8 equiv of NMI were added after 2 h. [f] Based on the ^{31}P NMR integration of the downfield vs. upfield signals.

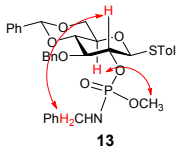
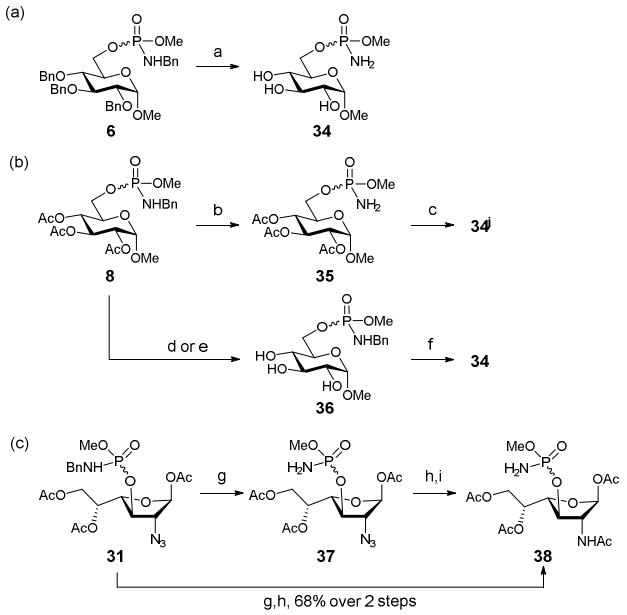

13

Figure 2 Selected nOe enhancements observed for **13**.

The utility of this one-step installation of MeOPN group was demonstrated with **30**[11], a galactofuranose moiety present in a CPS from *C. jejuni* 11168H and **32**, a protected galactose component in a CPS from *C. jejuni* 81-176 (Figure 1). Both **30** and **32** underwent phosphorylation efficiently, yielding methyl phosphoramidates **31** and **33** respectively in excellent yield (Table 2, entries 14 and 15). Of note, the reactive azide group at the C2-position of **30** was well tolerated and remained intact after phosphorylation. Thus, this mild phosphorylation condition enables late stage introduction of MeOPN in nitrogen-functionalized carbohydrate synthesis.

It was reported that the *N*-benzyl group on MeOPN is stable under standard hydrogenolytic conditions using hydrogen and palladium on carbon.[7] We found that when switching to palladium hydroxide on carbon, not only the *O*-benzyl groups in **6** were cleaved, the *N*-benzyl protected MeOPN was concomitantly deprotected and gave **34** quantitatively (Scheme 2a). The *N*-benzyl protecting group on MeOPN in different carbohydrates can be removed under biphasic conditions using sodium bromate and

sodium dithionite ($NaBrO_3/Na_2S_2O_4$).[7, 12] Under the same conditions, the MeOPN in **8** was deprotected to give **35** (76%) (d.r. 1:1.5) and all the acetyl groups that remained intact was subsequently removed by reaction using methanol-water-triethylamine to give **34**, but the product was contaminated with inseparable triethylamine salts (Scheme 2b). Instead, removing the acetyl groups of **8** using methanol-water-triethylamine first gave **36** in 85% or using K_2CO_3 in methanol-water condition[13] also produced **36** in 74% and the following hydrogenolysis using hydrogen and palladium hydroxide on carbon gave clean **34** quantitatively (Scheme 2b). On the other hand, the azide group in **31** was found to be compatible with the typical deprotection conditions using $NaBrO_3/Na_2S_2O_4$[14] to afford **37** in 81% (d.r. 1.6:1) and the unprotected MeOPN was stable in the ensuing



Scheme 2 Deprotection of methyl phosphoramidates. Reagents and conditions: [a] H_2, $Pd(OH)_2/C$, EtOH, rt, overnight, quant.; [b] $NaBrO_3$, $Na_2S_2O_4$, EtOAc, H_2O, rt, 1 h, 76%; [c] MeOH, H_2O, Et_3N, rt, 2h, 80%; [d] MeOH, H_2O, Et_3N, rt, 2h, 85%; [e] K_2CO_3, MeOH, H_2O, rt, 2 h, 74%; [f] H_2, $Pd(OH)_2/C$, EtOH, rt, 16 h, quant.; [g] $NaBrO_3$, $Na_2S_2O_4$, EtOAc, H_2O, rt, 1 h, 81%; [h] H_2, $Pd(OH)_2/C$, EtOH, rt, 5 h; [i] Ac_2O, py, rt, 71% over 2 steps; [j] contains inseparable Et_3N salts.

hydrogenolytic reduction of azide and *N*-acetylation to produce 71% of 38 over 2 steps (Scheme 2c). Alternatively, deprotection of *N*-benzyl on MeOPN and reduction of azide to amine of 31 can be achieved in one step using $Pd(OH)_2/C$ under hydrogen atmosphere, and the subsequent *N*-acetylation using Ac_2O and pyridine gave **38** in 68% over 2 steps (d.r. 1:1.6). Here, to retain the furanose structure, i.e. to prevent from transforming into pyranose, we had to keep the acetyl groups of **37** at this stage.

Conclusions

In conclusion, a one-step direct synthesis of MeOPN by using methyl benzyl phosphoramidochloridate was established. This approach was found to be efficient for both primary and secondary alcohols. Various functionalities were compatible with the mild reaction conditions. This reaction exhibited excellent regioselectivity, and the sterically less hindered hydroxyl group was preferentially phosphorylated. Moreover, in a sterically hindered environment, this direct MeOPN reaction can show excellent diastereoselectivity as this reaction is highly sensitive to the steric environment around the reaction centre. Also, removal of protecting groups in several *N*-benzyl protected methyl phosphoramidates was demonstrated. Continuing studies to control the diastereoselectivity and applications of this direct introduction of MeOPN to syntheses of oligosaccharides, such as the repeating unit of CPS from *C. jejuni* 11168H, are currently underway in our laboratory.

Experimental

Methyl benzylphosphoramidochloridate (1).

To the solution of benzylamine (710 mg, 740 μL, 6.71 mmol) in dry CH_2Cl_2 was added triethylamine (680 mg, 940 μL, 6.71 mmol) at rt. The mixture was cooled to -78 °C. Methyl dichlorophosphate (1.0 g, 670 μL, 6.71 mmol) was added dropwise to the mixture over 20 min. After complete addition, the temperature was raised to rt and stirred for additional 1 h, and the reaction was monitored by TLC using Nihydrin as the stain. Upon completion, the CH_2Cl_2 was evaporated under reduced pressure and a white solid was obtained, which was re-suspended with diethyl ether. The undesired solid was filtered off, and the diethyl ether filtrate was concentrated under a reduced pressure to afford **1** as a colorless oil (1.45 g, 98%); IR ($CHCl_3$) ν 3199, 1496, 1252, 1026, 732, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32-7.25 (m, 5H, ArH), 4.15 (d, *J* = 11.6 Hz, 2H, $PhCH_2$), 3.81 (d, *J* = 13.7 Hz, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8 (d, *J* = 8.5 Hz, C), 128.6 (CH x 2), 127.7 (CH), 127.4 (CH x 2), 54.0 (d, *J* = 6.1 Hz, CH_3), 45.7 (CH_2); ^{31}P NMR (161.97 MHz, $CDCl_3$) δ 16.8; HRMS (ESI) calcd for $C_8H_{12}NO_2ClP$ $[M+H]^+$ 220.0294, found 220.0290.

General procedure for direct synthesis of methyl phosphoramidates:

To a solution of an alcohol (100 mg) and DMAP (1.0 equiv) in dry CH_2Cl_2 (2 mL) was added NMI (*N*-methylimidazole) (8.0 equiv) at rt. Methyl benzylphosphoramidochloridate (**1**) (4.0 equiv) in CH_2Cl_2 (1 mL) was added dropwise over a period of 2-3 min at rt. The reaction mixture was allowed to stir at rt as the time indicated in Table 2. The reaction was monitored by TLC, and upon completion, the volatiles were removed *in vacuo*. The residue was purified by column chromatography to afford the desired product in good to excellent yield.

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