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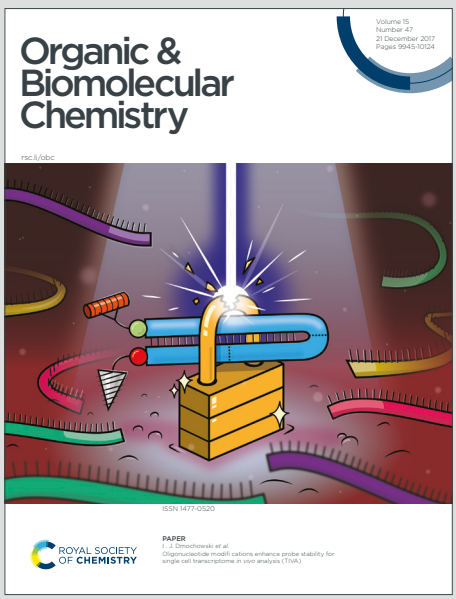

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An Effective Reagent to Functionalize Alcohols with Phosphocholine

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ABSTRACT. Phosphocholine is a small haptenic molecule that is both a precursor and degradation product of choline. Phosphocholine decorates a number of biologics such as lipids and oligosaccharides. In this study, an air and bench stable phosphocholine donor has been developed and evaluated with a number of alcohol acceptors. Using a one-pot, three-step sequence, (phosphitylation, oxidation, and phosphate deprotection) phosphocholine derivatives are synthesized in high yields. Of particular interest is the synthesis of miltefosine, the lone oral drug approved to treat leishmaniasis. Due to its prohibitive expense ($1500/g), miltefosine is not accesable for the majority of the world's patients. Based on the described reaction sequence, this drug can be produced for $25/g.

Carbohydrate modification is ubiquitous in nature. Nature's alterations to carbon (epimerization), oxygen (acylation, sulfation, methylation), and nitrogen (deacylation, sulfationn) occur post-translation (Figure 1A).[1] These changes increase both the structural complexity of the oligosaccharide and the biological information transmitted. While a number of carbohydrate modifications are well-studied, phosphocholine **2** (ChoP) modification is ambiguous (Figure 1B). In eukaryotes, ChoP is both a precursor and degradation product of phosphatidylcholine.[2] In microbial systems, ChoP modified glycans (particularly those belonging to pathogens) engage host proteins and are a virulence factor.[3] In contrast to eukaryotes, bacteria do not produce choline. Instead, choline is acquired in a three-step process.[4-5] First, a phosphodiesterase retrieves choline from the host. Next, a second enzyme converts choline to CDP-choline (citicoline, or cytidine 5'-diphosphocholine). Finally, a transferase adds ChoP to the target glycan. In order to characterize the role of ChoP modifications, there is a need for novel methods to install ChoP functionality.

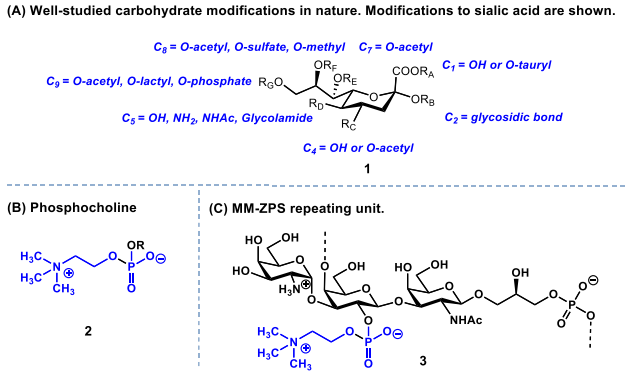

Figure 1. (a) Well-studied carbohydrate modifications in nature; (b) Structure of phosphocholine; (c) Structure of MM-ZPS.

We took an interest in ChoP over the course of a recent total synthesis of the *Morganella morganii* zwitterionic polysaccharide **3** (MM-ZPS) repeating unit (Figure 1C).[6] The structure is zwitterionic due to the presence of an alternating charge motif, driven, in part, by a ChoP residue. The synthesis hinged on installing both the ChoP residue and an additional site of phosphorylation (phosphoglycerol). A variety of methods to install these motifs are known – derived primarily from the synthetic oligonucleoside literature.[7] There are three major approaches to installing a ChoP residue, each riddled with its own challenges (Figure 2).

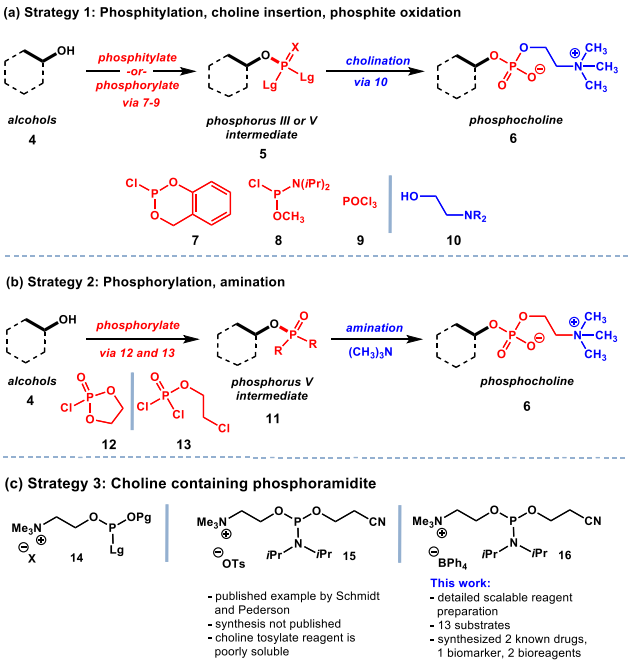

Figure 2. Strategies to install phosphocholine residues.

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The first approach involves coupling an alcohol acceptor **4** to a phosphorus donor, **7** - **9**, leveraging either the more electrophilic phosphorus (III) or more stable phosphorus (V) oxidation state, to generate a phosphorus III or V intermediate (Figure 2A). After the initial O-P bond formation, a wide variety of reagents (e.g. **10**) can be used to insert choline. The final step in the synthesis is to correct the oxidation state of the phosphorus atom, if necessary, to produce **6**. Due to the instability of phosphorus (III) donors, ethylene glycol derivatives of phosphoryl chloride (**12** and **13**) became the most widely used reagents to install the ChoP residue (Figure 2B).[8-12] This approach, however, is not without its limitations. Reagents **12** and **13** are air sensitive, water sensitive, and prone to decomposition on silica gel. Additionally, the final amination step requires the use of either gaseous trimethylamine or ammonia and methyl iodide.

It was after encountering these difficulties that we became interested in developing phosphorus III regents of type **14**, containing a pre-installed choline unit, a phosphorus protecting group, and a leaving group that would be displaced by the alcohol acceptor. It was in this vein that we recalled an obscure reagent, **15**, used by Pedersen and Schmidt during their elegant total synthesis of the lipoteichoic acid from *Streptococcus pneumoniae*.[13-14] In these studies, a tosylate salt of a phosphocholine-loaded phosphoramidite was used to install phosphocholine on a primary alcohol. Unfortunately, the reagent was only used on a single substrate. Moreover, the preparation of the choline donor is difficult, primarily due to the poor solubility of choline tosylate. Finally, the synthesis of the reagent is not reported in the literature. In our hands, all attempts at preparing the reagent were low yielding. Moreover, we observed oxidation of the phosphorus center, during the synthesis, which complicated purification.

The goal of the chemistry described herein was to synthesize and evaluate an improved phosphocholine donor, **16** (Figure 3). At the planning stage, we hypothesized that using a choline salt bearing a non-coordinating anion, such as tetraphenyl borate (BPh_4), (reagent **20**) would improve the solubility of all intermediates *en route* to **16**. Indeed, the use of tosylate salts provided insoluble intermediates over the course of the synthesis. Moreover, we anticipated the stability of the **16** would be greatly enhanced. In the forward direction, the synthesis of **16** started from 2-cyanoethanol **17**, which was reacted with phosphorus trichloride to provide phosphorus dichloride **18**. At this stage, monitoring the rate of addition of phosphorus trichloride and the temperature of the reaction is critical to avoiding disubstituition of cyanoethanol onto the electrophilic phosphorus atom. Following the first substitution, reaction with diisopropylamine gave diamine **19** with ^{31}P NMR indicating formation of the phosphordiamidite. The final step in the synthesis is reaction with choline tetraphenylborate **20** to obtain donor **16** in 71% yield over the entire synthesis.

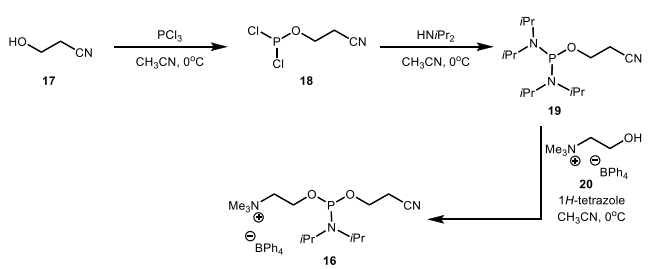

Figure 3. Synthesis of phosphoramidite **16**.

While subtle, we observed that the new version of the reagent could be synthesized in high yield with excellent purity. Importantly, the synthesis does not require a purification step. We hypothesize that the ease of synthesis is due primarily to the enhanced solubility of the tetraphenyl borate-choline salt and careful monitoring of each step of the reaction using a combination of ^{31}P NMR and LCMS. Moreover, phosphoramidite **16** is surprisingly stable to oxidation. Thus far, we have observed that **16** can be handled in air with minimal oxidation (as monitored by ^{31}P NMR) for several weeks. Finally, **16** is stable to aqueous work up.

Before revealing the utility of reagent **16**, we briefly digress into a mechanistic discussion of how the reagent works. The accepted mechanism for phosphotidylation of alcohols, using phosphoramidite reagents, is shown in Figure 4. 1*H*-tetrazole (pKa = 4.9) **21** is used as a promoter in the reaction. Based on its acidity, the first step in the coupling reaction should involve protonation of phosphoramidite **16** and nucleophilic displacement of diisopropyl amine by tetrazolide **22**. The key intermediate in the coupling is tetrazolylphosphoramidite **22**. At this stage, coupling with alcohol acceptor **4** will provide a phosphite ester **23**. Subsequent oxidation, and β-elimination of the cyano ethanol protecting group will provide phosphocholine **6**. Based on this mechanism, optimization of reaction conditions should involve an evaluation of different tetrazole based promoters, amine bases, and oxidants.

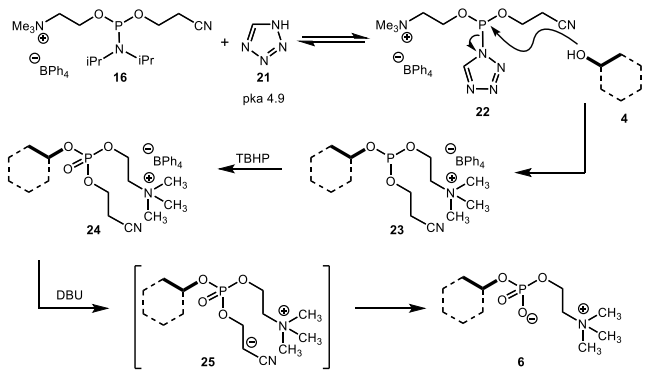

Figure 4. Proposed mechanism for the reaction between an alcohol **4** and phosphoramidite **16**.

The investigation began with the reaction of cetyl alcohol **26** and **16** to produce miltefosine **27**. Miltefosine is the only oral drug approved for the treatment of parasitic disease leishmaniasis. Unfortunately, it is also unavailable to most patients due to its prohibitive expense.[15] Upon extensive investigation, we realized that using slight excess (1.2 equiv.) of the phosphoramidite, 1*H*-tetrazole, and TBHP enabled access to the protected drug. Ultimately, 5.0 equiv. of DBU gave miltefosine in 90% yield.

Deviating from the standard procedure, we first investigated additional activators. We hypothesized that a superior proton donor and/or a stronger nucleophile would enable increased generation of the active intermediate - thereby increasing the rate of reaction. Interestingly, each tetrazole derivative performed with the same efficiency (entrys 1-3 and 5) likely due to two of the reagents (ethylthiotetrazole and benzyl thiotetrazole) being more acidic and another reagent (DCI) functioning as a superior nucleophile. Acetic acid, while of equal

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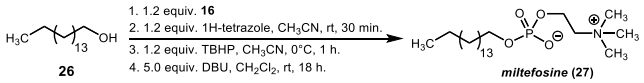
acidity to 1*H*-tetrazole, is not compatible with the reaction (entry 4) and produced miltefosine in just 20% yield. Excess 1*H*-tetrazole (entry 6) did not affect the reaction, whereas decreasing the amount of tetrazole (entry 7) decreased the yield over the same time period. While evaluating oxidants, we observed that mCPBA and H_2O_2 are comparable to TBHP (entry's 8 and 9). Finally, we assessed the base used in the elimination reaction. Decreasing the amount of DBU provided a reduced conversion to miltefosine (entry 10), whereas complete substitution to Et_3N provided a lower conversion overall (entry 11).

Table 1. Deviations from reaction conditions.

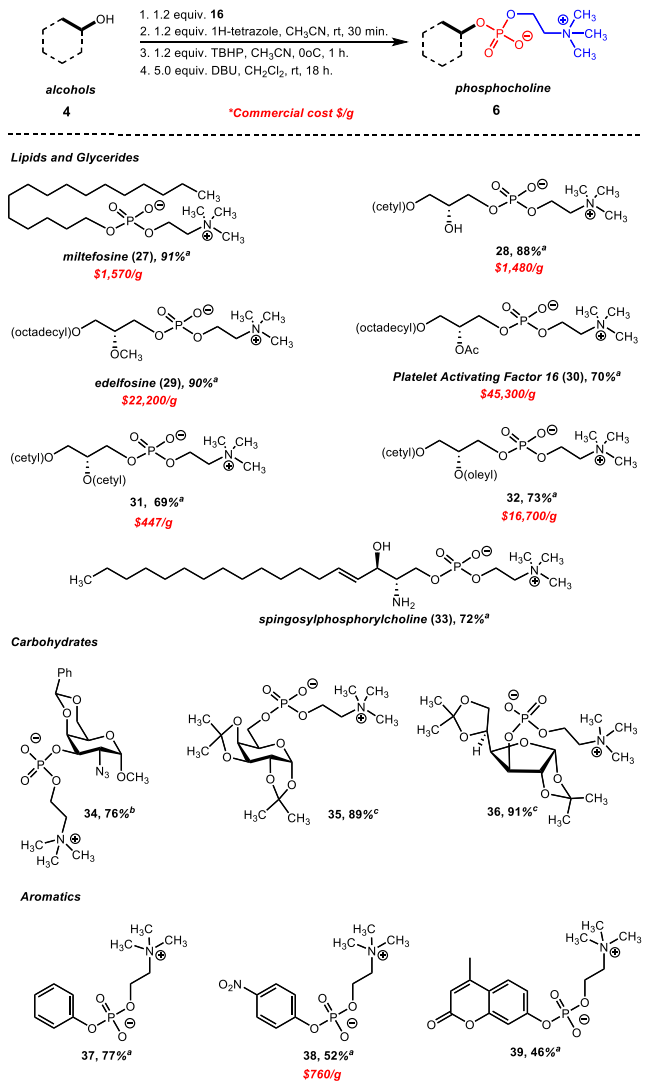


Entry	Deviation from standard condition	Yield (%)[a]
1	1.2 equiv. 4,5-Dicyanoimidazole (pka 5.2)	88
2	1.2 equiv. 5-(Ethylthio)-1*H*-tetrazole (pka 4.3)	82
3	1.2 equiv. 5-Benzylthio-1H-Tetrazole (pka 4.1)	81
4	1.2 equiv. Acetic Acid (pka 4.8)	20
5	1.2 equiv. 5-(4-nitrophenyl)-1H-tetrazole (pka 3.7)	77
6	3.0 equiv. tetrazole (pka 4.9)	90
7	0.1 equiv. tetrazole	55
8	1.2 equiv. *m*CPBA	90
9	1.2 equiv. H_2O_2	85
10	1.0 equiv. DBU	80
11	5.0 equiv. Et_3N	71

[a]isolated yields at 1.0 mmol scale.

With optimized conditions established, we examined the scope of the phosphocholine installation on a panel of alcohols. Test substrates were selected with an eye toward molecules that are of use in a biological setting and are otherwise prohibitively expensive. We first studied the reaction on a set of glycerol-based acceptors (**27**-**32**). Reaction on a monoalkylated derivative gave **28** in 88% yield. Methylation of the secondary alcohol did not affect the efficiency of the phosphocholine installation as is evidenced by the synthesis of edelfosine **29**. Edelfosine is an antineoplastic phospholipid, in 90% yield. To contrast, acylation at the secondary alcohol resulted in a decreased yield of platelet-activating factor **30**. Similarly, the addition of longer acyl chains resulted in decreased efficiency in the coupling reaction - **31** and **32** were produced in 69 and 73 % yields, respectively. Finally, sphingosylphosphorylcholine (SPC) **33** was synthesized in 72% yield with complete regio-control at the primary alcohol over the internal allylic alcohol. This result was interesting for several reasons. First, it is well-established in the lipid literature that it is difficult to selectively functionalize the primary alcohol of sphingosine over the neighboring allylic secondary alcohol.[16] Secondly, the reaction proceeds smoothly in the presence of the central amine. In theory, one could imagine the amine serving as the initial nucleophile in the reaction, followed by phosphorus transfer to the least hindered alcohol.

After studying lipids and glycerides, we examined the reaction in the context of carbohydrate modification at primary and secondary alcohols. Fully substituted galactoside **34** was synthesized in 76% yield at 10 mmol scale. Galactose diacetonide underwent functionalization to produce **35** in 89% yield. Similarly, glucose diacetonide produced **36** in 91% yield. We concluded the study by installing phosphocholine on three aromatic substrates. Whereas phenol gave **37** in 77% yield, nitrophenol (presumably due to its decreased nucleophilicity) provided **38** in a modest 52% yield. Similarly, methyl coumarin underwent reaction with **16** to give **39** in 46% yield. Overall, we found that purification of the lipids by flash column was more facile than the carbohydrate substrates, likely due to the difference in polarity between the lipids and phosphorus by-products.



*Reaction Scale. a: 1 mmol, b: 10 mmol, c: 0.1 mmol scale

Figure 4. Alcohol scope for phosphocholine installation

In closing, we have developed a choline donor that can be prepared in three steps with no intermediate purification. The reagent is moisture stable and not susceptible to oxidation over several weeks of indiscriminate storage. The reagent is able to serve as a choline donor under general conditions for a range of primary and secondary alcohols.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The supporting information is available free of charge on the ACS Publications website at DOI:

Experimental procedures, characterization data, and NMR spectra (PDF)

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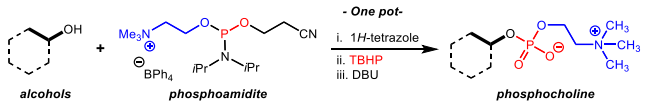

A one-pot, three-step sequence has been developed to synthesize phosphocholine derivatives. The method has been applied to the synthesis of several biologics such as the anti-parasitic drug miltefosine.