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Versatile reagents to introduce caged phosphates

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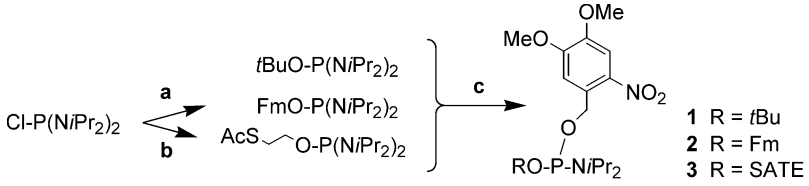
Abstract—Three novel reagents have been prepared to introduce photoactivatable *o*-nitrobenzyl phosphate esters. The use of fluorenylmethyl and *t*-butyl protecting groups allowed for a wide range of chemical transformations after phosphorylation. In addition, the use of *S*-acetylthioethyl and acyloxymethyl groups resulted in photo- and bioactivatable phosphate triesters of phosphatidic acid. © 2003 Elsevier Science Ltd. All rights reserved.

Photoactivatable derivatives of biologically active substances, usually referred to as caged compounds, are important tools in biomedical research.[1,2] The major advantage is that the inactive compound can be delivered to living cells without significantly effecting intracellular processes. Only after flash photolysis liberates the active compound within a few seconds, effects are monitored on a physiologically relevant time scale. Therefore, caged compounds allow for intracellular manipulations with a maximum of temporal resolution.[3] Nitrobenzyl esters are by far the most commonly used caging groups for phosphates, although coumarinylmethyl esters were also successfully attached.[4] Very recently, it was shown that an amido-substituted nitrobenzyl groups could be introduced by direct alkylation of cyclic nucleotides.[5] In the past, however, caging groups were usually linked to phosphates via diazomethane or diazoethane reagents. In case of sphingosine 1-phosphate, Qiao and co-workers turned to the phosphite reagent bis(2-nitrophenylethyl) *N*,*N*-diisopropylphosphoramidite to introduce a phosphate moiety with two photosensitive groups.[6] Another method involving P(III) reagents was used for the preparation of caged *myo*-inositol hexakisphosphate derivatives that relied on an *o*-nitrobenzyl allyl phosphoramidite.[7] However, the authors reported harsh conditions for the removal of the allyl ester, which might not be generally accepted by biomolecules. Finally, 1-(2-nitrophenyl)-ethyl-caged phosphopeptides were prepared with the help of a phosphoramidite reagent that proved suitable for solid phase synthesis.[8]

Herein, we report several novel phosphoramidite reagents that are suitable to prepare caged derivatives of AMP, mannose 1-phosphate, and phosphatidic acid (PA). The nitroveratryl phosphoramidites[9] containing fluorenylmethyl (Fm) or *t*-butyl protecting groups or the bioactivatable *S*-acetylthioethyl (SATE) group (**1**–**3**) were prepared in 64–78% total yield from the chloro- or the *t*-butyloxy phosphorbis(amidite), respectively, as shown in Scheme 1. The reagents were stable for many months when kept cold and at least for weeks when kept at room temperature. First targets to test the feasibility of the reagents were AMP and phosphatidic



Scheme 1. *Reagents and conditions*: (a) 9-Fluorenylmethyl (Fm) alcohol (1 equiv.), TEA, *n*-pentane, 0°C to rt, 3 h; (b) $AcSCH_2CH_2OH$ (1 equiv.), TEA, Et_2O, 0°C, 3 h; (c) DMNBnOH (1 equiv.), diisopropylamine tetrazolide (0.5 equiv.), DCM, 0°C to rt, 16 h.

Keywords: photoactivatable; caged compounds; membrane-permeant; bioactivatable; phosphorylation; phosphatidic acid; AMP; mannose 1-phosphate.

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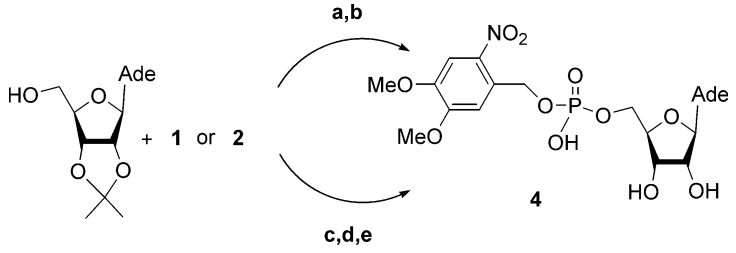
acid. A caged AMP (trinitrophenyl-AMP) was a valuable tool to investigate myosin movement in the past[10] and PA was shown to mediate vesicle budding from the Golgi, among other functions.[11] The classical starting material for the preparation of AMP derivatives is 2′,3′-*O*-isopropylidene adenosine. For a two-step reaction to caged-AMP (**4**) the use of reagent **1** was desirable, because both acid-labile protecting groups could be removed in a single step (Scheme 2). The Fm group of reagent **2** allowed phosphorylation followed by deprotection of the phosphate under mild basic conditions and subsequent removal of the ketale. This permitted additional modification of the phosphate diester, as was demonstrated by the reaction with propionoxymethyl iodide (PM-I), that yielded an uncharged, potentially bioactivatable caged AMP derivative after cleavage of the ketale (not shown). Acyloxymethyl esters or *S*-acetylthioethyl groups of biologically active phosphates were shown to facilitate cellular entry of usually impermeant compounds.[12–14] It should be mentioned that the quantitative nature of the Fm cleavage reaction combined with the products' large differences in polarity allowed for very fast preparations.

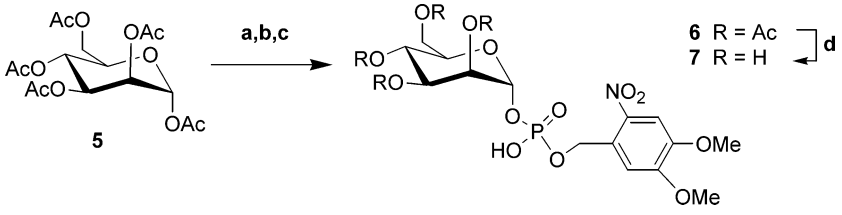
The next task was supposed to show the stability of the caged phosphate derivatives under stronger basic conditions. For this purpose, we prepared the caged 2,3,4,6-tetra-*O*-acetyl-mannose 1-phosphate **6** in three steps from penta-*O*-acetyl-mannose **5** (Scheme 3).[15] Deacetylation at pH 13 in the presence of the nitroveratryl group proceeded smoothly within 36 h to give caged mannose-1-phosphate **7** in 95% yield.

In order to provide caged tools to study PA activity,[16] we then prepared caged derivatives of di-*O*-octanoylglycerol (**8**)[17] and a fluorescent, nile red-tagged S_N1-ether derivative **9** thereof (Scheme 4). We considered the latter a challenge, because the nile red group does not comply with aggressive conditions. On the other hand, nile red exhibits little absorbance at 365 nm and is likely to be fairly resistant to bleaching under the conditions needed to removed the cage. Compounds **8** and **9** were readily phosphitylated with 1.5 equiv. of reagent **2** in the presence of tetrazole in 43% and 55% isolated yield after oxidation to the phosphate, respectively. Removal of the Fm group was quantitative and gave the caged-PA derivatives **10** and **11**. Both compounds were alkylated with acetoxymethyl bromide (AM-Br) to give the potentially bioactivatable triesters **12** and **13**, respectively. Alternatively, **9** was treated with reagent **3** to introduce cage and bioactivatable *S*-acetylthioethyl group in a single reaction. The diastereomeric phosphate triester **14** was isolated in 52% yield.

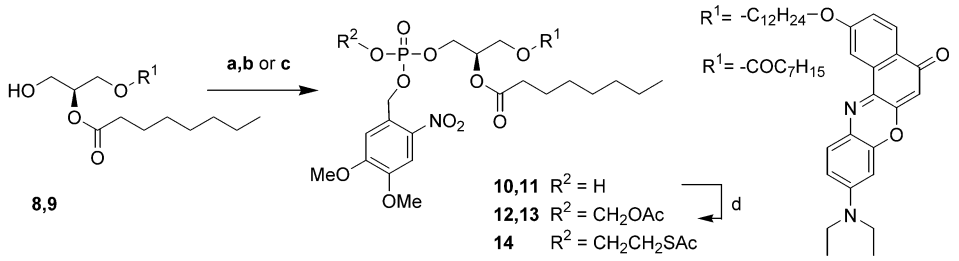
The reagents presented here combine the possibility to introduce phosphate and cage at the same time. In addition, it was shown, that bioactivatable protecting groups could be introduced in the same procedure or after removal of the phosphate protecting groups. The method might be generally suitable to replace diazo compounds when total synthesis of caged phosphate derivatives is required. An application in a more laborious synthetic pathway is described in an additional paper.[18] The biological potential of the final products described will be published elsewhere.



Scheme 2. *Reagents and conditions*: (a) **1**, tetrazole, MeCN, rt, 16 h, then *t*-BuOOH, rt; (b) TFA (95%), rt, 45% (two steps); (c) **2**, tetrazole, MeCN, rt, 16 h, then *t*-BuOOH, rt, 50%; (d) pyrrolidine, DCM, rt, 5 min, quant.; (e) TFA (95%), rt, 95%.



Scheme 3. *Reagents and conditions*: (a) Porcine liver esterase, 10% DMF in phosphate buffer (0.2 M), pH 7, rt, 8 h;[15] (b) **2**, tetrazole, MeCN, rt, 16 h, then *t*-BuOOH, rt, 61%; (c) pyrrolidine, DCM, rt, 5 min, quant.; (d) aq. KOH, pH 13, rt, 36 h, 95%.



Scheme 4. *Reagents and conditions*: (a) **2**, tetrazole, 5 h, then *t*-BuOOH, rt, 43–55%; (b) pyrrolidine, DCM, rt, 5 min, quant.; (c) **3**, 4,5-dicyanoimidazole, rt, 0.5 h, then *t*-BuOOH, rt; (d) acetoxymethyl bromide, DIPEA, MeCN, rt, 3 h, 46–52%.

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