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A Versatile One-Pot Procedure to Phosphate Monoesters and Pyrophosphates Using Di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite

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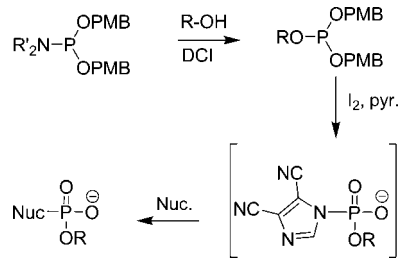
ABSTRACT



A one-pot procedure for the preparation of phosphoramidates, phosphorothioates, pyrophosphates, phosphodiesters, and phosphofluoridates has been devised using di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite as the common phosphitylating reagent.

Phosphorus-containing functional groups not only are essential elements of nucleic acids but also are present in other naturally occurring compounds such as proteins,[1] carbohydrates,[2] and lipids.[3] Among these biomolecules phosphoromono- and diesters,[1,2] pyro- and triphosphates,[4,5] phosphoramidates,[6] and C-phosphonates[7] can be discerned. The pivotal role that phosphorus-containing molecules play in a broad palette of biological processes has stimulated research into the synthesis of these classes of compounds.[8,9] In addition, the pharmacological potential of phosphorus compounds[10−12] led to the development of artificial phosphate derivatives such as phosphorothioates[13] and phosphofluoridates.[14] Initially developed to meet the needs of automated nucleic acid synthesis, the phosphoramidite approach is the synthetic methodology most applied toward phosphodiester-containing molecules.[15−17] Introduction of phosphate monoesters is generally attained with the aid of amidites provided with two protecting groups, which are often base-

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labile.[18,19] Upon activation with weakly acidic azole the amidite reagent reacts with an alcohol to give a phosphite triester. Subsequent oxidation to the phosphate triester and final deprotection furnishes the target phosphate.[20] As part of our efforts[21−23] aimed at the synthesis of naturally occurring phosphates and derivatives thereof via the phosphoramidite approach, we embarked on the exploration of the phosphitylation properties of methoxybenzyl-*N*,*N*-diisopropylphosphoramidites **1** and **2** (Figure 1). At the outset

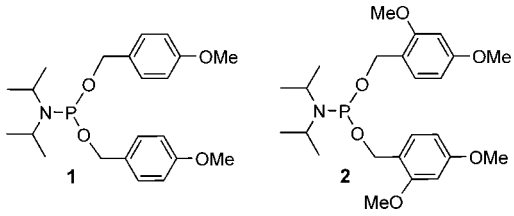


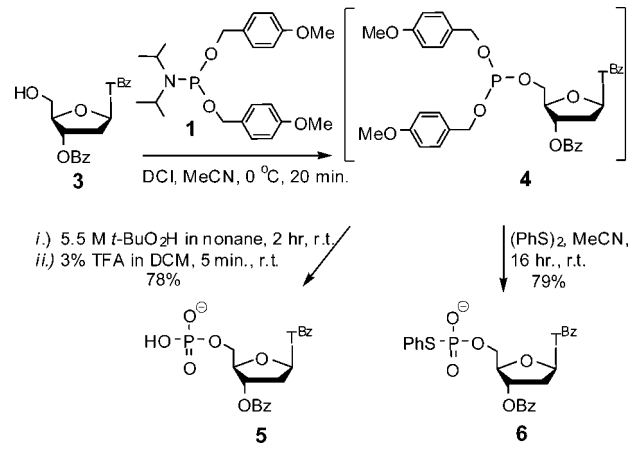
Figure 1. Methoxybenzyl phosphoramidites.

we deemed these reagents to provide attractive entries to phosphomonoesters, since we envisaged that phosphitylation of an alcohol of choice with di(p-methoxybenzyl)-*N*,*N*-diisopropyl phosphoramidite (**1**) followed by oxidation and mild acidic removal of both *p*-methoxybenzyl groups would readily provide the corresponding phosphomonoester. The reagent **2** (Figure 1) equipped with 2,4-dimethoxybenzyl groups would conceivably lead to a phosphotriester moiety convertible to a phosphomonoester under even milder conditions.

An additional useful feature of reagents **1** and **2** is the potential of methoxybenzyl substituents to engage in Arbuzov-type reactions.[24] This would open the way to the synthesis of modified phosphates. Phosphoramidites **1** and **2** were synthesized in good yields via a procedure reported for a related compound,[25] comprising treatment of *p*-methoxybenzylalcohol and 2,4-dimethoxybenzylalcohol, respectively, with *N*,*N*-diisopropylaminophosphorodichloridite in the presence of triethylamine.

Reaction of reagent **1** (1.2 equiv) with thymidine **3** (1.0 equiv) using dicyanoimidazole (DCI) (1.2 equiv) as activator (Scheme 1) gave, as monitored by ^{31}P NMR spectroscopy

Scheme 1. Synthesis of Phosphate **5** and Phosphorothioate **6**



(162 MHz, δ 141.8, acetone-d_6 capillary in MeCN), the formation of phosphite triester **4** as the major product together with some minor unidentified phosphonates (δ 14.1, 9.1). In the subsequent steps **4** was used without isolation, since silica gel column chromatography yielded only 29% of **4**, presumably due to its instability. Addition of *t*-BuO_2H to oxidize phosphite **4**, followed by *p*-methoxybenzyl deprotection using 3% TFA in DCM and chromatographic purification yielded phosphate **5** (^{31}P NMR, 162 MHz, δ 1.6, $CDCl_3$/MeOD-d_4) in 78% yield. In contrast, phosphitylation of **3** using agent **2** under identical conditions failed to yield the intended phosphite triester but instead produced an unidentified C- and H-phosphonate in a 1:1 ratio (^{31}P NMR, 162 MHz, δ 31.9, 14.5, acetone-d_6 capillary in MeCN).

Having demonstrated the effectiveness of phosphitylating agent **1** to introduce phosphate monoesters under mild acidic (deprotection) conditions, the susceptibility of di(*p*-methoxybenzyl) protected phosphite triester **4** to undergo an Arbuzov reaction was explored. Thymidine **3** was converted into phosphite triester **4** as described above, but now the reaction mixture was treated with diphenyldisulfide at the time the transformation of **3** to **4** was complete (as revealed by NMR), leading to phosphorothioate diester **6** in 79% yield (Scheme 1). To our surprise we observed complete in situ cleavage of both *p*-methoxybenzyl protecting groups when the reaction was monitored by ^{31}P NMR spectroscopy, where we expected to see the corresponding triester still carrying one *p*-methoxybenzyl group. This one-pot procedure thus allows an immediate access to *S*-phenyl phosphorothioates, known precursors[26] in the synthesis of pyrophosphates.

Guided by these results we were eager to find out whether we could extend the one-pot procedure we discovered to other Arbuzov-type reactions. It occurred to us that using iodine as the oxidation reagent for **4** would allow us to introduce a range of nucleophilic atoms at the phosphorus center via the putative phosphoryliodide.[27] We first focused

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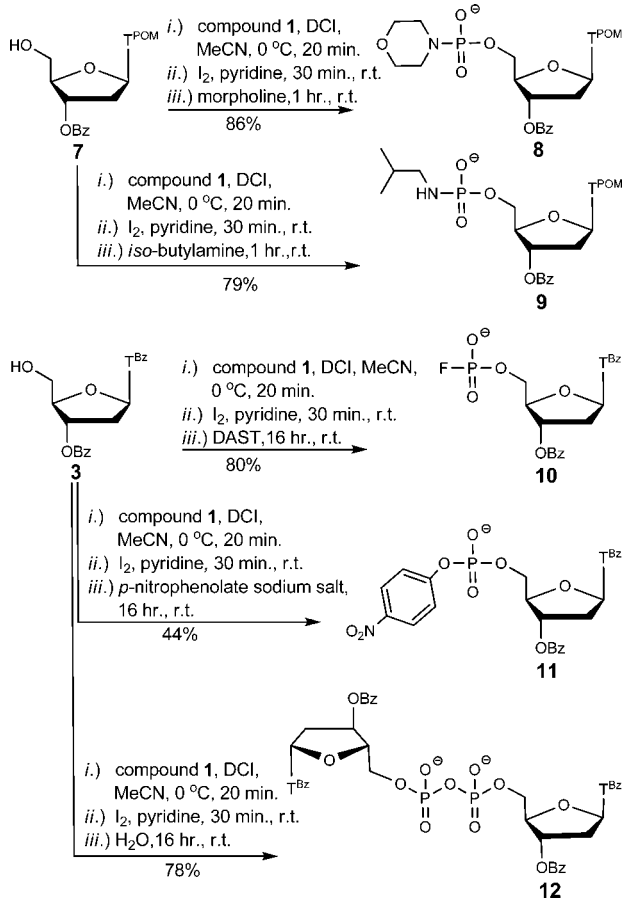
on phosphoromorpholidates, the most well-known intermediates en route to pyrophosphates.[28]

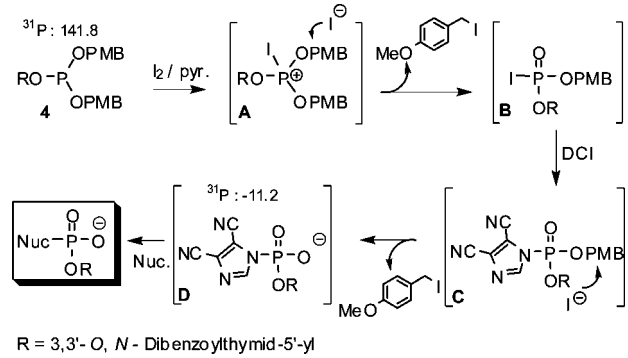
The solution-phase synthesis of thymidine derivative **8** was undertaken by adaptation of an iodine-mediated procedure to create phosphoromorpholidates on a solid support.[29] Thymidine **7** (Scheme 2) was phosphitylated with **1** in the presence of DCI under the same conditions as described above, and the obtained di(*p*-methoxybenzyl)phosphite triester was oxidized with iodine in pyridine and subsequently treated with morpholine for 1 h. After silica gel column chromatography morpholidate **8** was isolated in 86% yield.[30] Following an analogous procedure using *iso*-butylamine instead of morpholine as nucleophile led to the clean formation of the protected phosphoramidate **9** (79%).[30]

Scheme 2. Synthesis of Phosphate Monoesters **8**−**11** and Symmetric Pyrophosphate **12**



To broaden the scope of the one-pot procedure, the use of non-nitrogen nucleophiles was investigated. Addition of DAST as fluoride source to the reaction mixture after iodine-mediated oxidation resulted in the formation of phosphofluoridate **10** (80%). Similarly, addition of the sodium salt of *p*-nitrophenol to the oxidized reaction mixture resulted in formation of the corresponding phosphodiester **11** (44%). Surprisingly, addition of water did not result in the formation of the expected phosphate monoester but furnished the symmetric pyrophosphate **12** (78%). All reactions described above were conducted under the same conditions, and the formation of the pyrophosphate **12** upon addition of water as well as the fact that no special treatment is needed to remove the second *p*-methoxybenzyl group in the remaining reactions (Scheme 2) put forward the question of the identity of the reactive intermediates in the reaction sequence accompanying our one-pot procedure. On the basis of the data that resulted from the analysis of selected reactions by ^{31}P NMR spectroscopy, we propose the following mechanism (Scheme 3).

Scheme 3. Proposed Reaction Mechanism



Activation of amidite **1** (δ: 148.5) with DCI and subsequent coupling with alcohol **3** gave the phosphite triester **4** (^{31}P NMR, 162 MHz, δ 141.8, acetone-d_6 capillary in MeCN, see Scheme 1). Treatment of the phosphite triester **4** with iodine in pyridine (Scheme 3) would lead to phosponium species **A** and the subsequent phosphoryliodide **B**, having resonances around δ −44, as described.[27] However, these resonances could not be detected in our experiments, but instead a signal at δ −11.2 was observed. This indicates that the putative phosphoryliodide **B** was transformed immediately into another intermediate with concomitant cleavage of the second *p*-methoxybenzyl group (PMB). This loss of the protective group can be explained by nucleophilic attack of iodide generated in step **C** on the benzylic position of the PMB group. On the basis of literature data that comparable phosphorazolides have ^{31}P NMR resonances at δ −12.2[28] (162 MHz, pyridine/DMSO-d_6) and −11.24[31] (40 MHz, pyridine-d_5) we tentatively assign the resonance at δ −11.24 to the dicyanoimidazolide species **D**. Subsequent addition of a nucleophile leads to displacement of dicyanoimidazole to give the corresponding phosphate derivative. The proposed dicyanoimidazolide species **D** as ultimate reactive intermediate explains not only the removal of both

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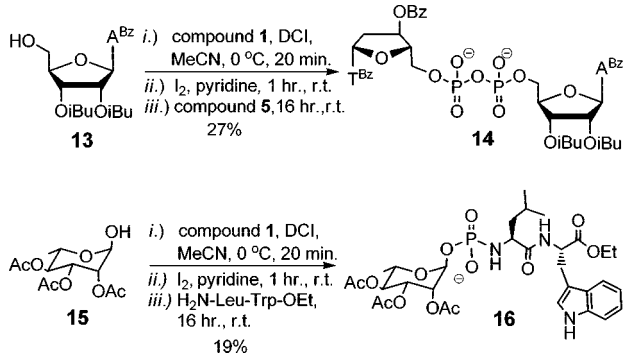
(30) It became apparent that under the influence of morpholine or isobutylamine the benzoyl protecting group on the nucleobase was removed. This problem was nonexistent when the more stable POM group was used for protection of the thymine base.

p-methoxybenzyl groups but also the formation of the pyrophosphate **12**. Addition of water will partly and slowly hydrolyze dicyanoimidazolide species **D** to give the corresponding monophosphate, which in turn will react more quickly than water with **D** to give pyrophosphate (for example **12**).

To further establish the applicability of our one-pot procedure using di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite **1**, the synthesis of asymmetric pyrophosphate[32] **14** and the phosphoramidon precursor[33] **16** (Scheme 4) were undertaken. Crude pyrophosphate **14** was obtained by phosphitylation of protected adenosine **13** with **1** under the influence of DCI, subsequent iodine/pyridine oxidation, and finally addition of thymidine phosphate **5**. RP-HPLC and gel filtration purification yielded homogeneous pyrophosphate **14** in moderate yield (27%). Similarly phosphoramidon precursor **16** was synthesized by phosphitylating β-rhamnose-triacetate **15** with **1**, iodine oxidation and addition of the *N*-terminal unprotected Leu-Trp ethyl ester in a moderate yield (19%). Such modest yields, compared to synthesis of compounds **8**−**12** are probably caused by the losses in the purification process, as no major side reactions have been observed.

Scheme 4. Asymmetric Pyrophosphate **14** and Phosphoramidon Precursor **16**



In conclusion, we have presented an efficient one-pot procedure to phosphoramidates, phosphorothioates, pyrophosphates, and phosphofluoridates by a sequence of reactions comprising phosphitylation of an alcohol with di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite **1**, oxidation with iodine, and treatment with the nucleophile of choice. The nature of the *p*-methoxybenzyl groups allows the use of reagent **1** to introduce phosphate monoesters under relatively mild acidic conditions.

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Supporting Information Available: Spectroscopic data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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