[5] Crystal data for BTMA$^+$**2**$^-\cdot 2CH_2Cl_2$: $C_{31}H_{44}B_9Cl_4MoNO_2$, $M_r = 797.7$, crystal size $0.2\times0.3\times0.8$ mm, monoclinic, $P2_1/n$, $a = 11.003(2)$, $b = 26.100(2)$, $c = 14.2707(14)$ Å, $\beta = 108.646(10)°$, $V = 3883.3(8)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.364$ g cm^{-3}, $F(000) = 1632$, $\mu = 0.64$ mm^{-1}, of 6771 unique reflections 3490 were observed ($F_o > 4\sigma(F_o)$), 464 parameters, $R_1 = 0.0660$, $wR_2 = 0.1371$ (for observed data), $S = 1.014$, max. and min. residual electron density: 0.55 and -0.56 e Å^{-3}. See ref. [4].

[6] Crystal data for Et_4N^+**3**$^-$: $C_{27}H_{44}B_9MoNO_2$, $M_r = 607.9$, crystal size $0.2\times0.3\times0.7$ mm, monoclinic, Cc, $a = 24.403(10)$, $b = 10.1090(10)$, $c = 17.612(3)$ Å, $\beta = 133.060(10)°$, $V = 3174.4(14)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.272$ g cm^{-3}, $F(000) = 1264$, $\mu = 0.44$ mm^{-1}, of 2963 unique reflections 2240 were observed ($F_o > 4\sigma(F_o)$). 362 parameters, $R_1 = 0.0536$, $wR_2 = 0.1343$ (for observed data), $S = 1.039$, Flack parameter $= -0.3(2)$, max and min. residual electron density: 0.58 and -0.50 e Å^{-3}. See ref. [4]

The Phenylacetyl Group—The First Amino Protecting Group That Can Be Removed Enzymatically from Oligonucleotides in Solution and on a Solid Support**

Herbert Waldmann* and Armin Reidel

A central problem in the chemical synthesis of oligodeoxynucleotides on solid supports is the protection and deprotection of the amino groups of the different nucleobases.[1] Even under the strongly basic conditions required for the removal of the established blocking groups deprotection may remain incomplete,[2] and unwanted side reactions like the formation of 2,6-diaminopurines from 6-O-alkylated guanine moieties may occur.[3] In addition, new protecting groups are needed, for example, for the construction of complex and sensitive nucleopeptides,[4] nonradioactive DNA probes,[5] and aminoacyl-modified tRNAs[6] and for the preparation of matrix-bound deprotected DNA fragments.[7a] Therefore, the development of new methods for the selective protection and deprotection of the amino functions of nucleobases under alternative and mild conditions in solution and on solid supports is of particular *interest in natural product synthesis*.[1,7]

Enzymatic protecting group techniques[8] have proven their efficiency (completely selective removal under mild conditions, for example pH 7, room temperature) in particular, in the construction of sensitive multifunctional acid- and base-labile peptide conjugates like lipo-,[9] glyco-,[10,11] and phosphopeptides,[11,12] and might also open up viable alternatives to established classical chemical methods for solution- and solid-phase oligonucleotide chemistry. Furthermore, in the light of the intense recent interest in combinatorial chemistry,[13] the successful implementation of enzymatic transformations on solid supports[14] is of general relevance. The introduction of biocatalysts for this challenging task would greatly expand the toolbox of methods available for combinatorial synthesis. We now report that the enzyme-labile phenylacetyl (PhAc) group[15] can be removed from oligonucleotides with the enzyme penicillin G acylase under very mild conditions (pH 7, room temperature) both in solution and on a solid support.

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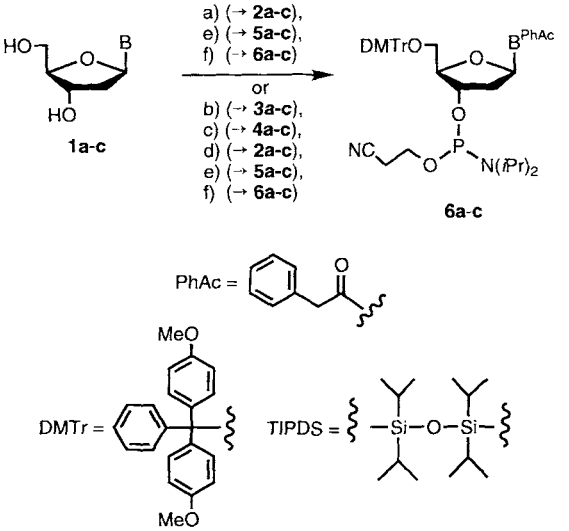
The synthesis of the selectively protected phosphoramidite building blocks **6**, which are needed for the solid-phase synthesis of the PhAc-protected oligonucleotides, is detailed in Scheme 1. The unprotected 2′-deoxynucleosides **1** were N-acylated directly by means of the "transient protection" method:[16] simultaneous O- and N-silylation was followed by treatment of the



Scheme 1. Synthesis of the PhAc-protected nucleoside phosphoramidites **6**. a) Trimethylsilyl chloride (TMSCl, 5 equiv), pyridine, room temperature, 30 min; $PhCH_2C(O)Cl$ (1.5 equiv), *N*-hydroxybenzotriazole (HOBt, 1.5 equiv), CH_3CN/pyridine 2:1, 0 °C → room temperature; yields: **2a**: 71%, **2b**: 51%; **2c**: 63%; b) 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl chloride (TIPDSCl, 1.1 equiv), pyridine, room temperature; yields: **3a**; 96%, **3b**: 95%, **3c**: 97%; c) $(PhAc)_2O$ (4 equiv), pyridine, 120 °C, 30 min; yields: **4a**: 87%, **4b**: 77%, **4c**: 85%; d) $(nBu)_4NF$, (5 equiv), THF, room temperature, 1 h; yields: **2a**: 60%, **2b**: 88%; **2c**: 86%; e) 4,4′-dimethoxytriphenylmethyl chloride (DMTrCl, 1.4 equiv), 4-dimethylaminopyridine (DMAP, cat.), pyridine, room temperature, 3–5 h; yields: **5a**: 83%, **5b**: 82%, **5c**: 81%; f) $NC(CH_2)_2OP(Cl)N(iPr)_2$ (2 equiv), $(iPr)_2NEt$ (3 equiv), THF, room temperature, 20 min; yields: **6a**: 90%, **6b**: 80%, **6c**: 55%. B = adenine (**a**), guanine (**b**), cytosine (**c**).

silylated nucleosides with phenylacetyl chloride and *N*-hydroxybenzotriazole (HOBt) to deliver the desired N-PhAc nucleosides **2** in yields of 51–71%. Alternatively, the OH groups of **1a–c** were simultaneously masked with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group.[17] The resulting TIPDS ethers **3** (obtained in 95–97% yield) were then N-acylated with phenylacetic anhydride to give the amides **4**, which were subsequently desilylated to deliver the desired N-protected nucleosides **2** in overall yields of 52–73%. After the primary OH groups of **2a–c** had been masked as DMTr ethers by alkylation with DMTrCl in the presence of DMAP, the secondary OH groups were treated with chloro(β-cyanoethoxy)-*N*,*N*-diisopropylaminophosphane to provide the desired phosphoramidites **6**. The crude products were precipitated with cold hexane and used without further purification for solid-phase synthesis of the PhAc-masked oligonucleotides **7–11** (Table 1).[18]

Table 1. Yields of oligonucleotides **7–11** produced by solid-phase synthesis [a].

pentanucleotide	d(5′-AAA AT-3′)	**7** (77)
	d(5′-GGG GT-3′)	**8** (95)
	d(5′-CCC CT-3′)	**9** (91)
dodecanucleotide	d(5′-AAT TCC GGA ATT-3′)	**10** (86)
hexadecanucleotide	d(5′-GTC ATA GCT GTT TCCT-3′)	**11** (95)

[a] All A, G, and C bases are PhAc-protected; the average yields of the couplings are given in brackets (%).

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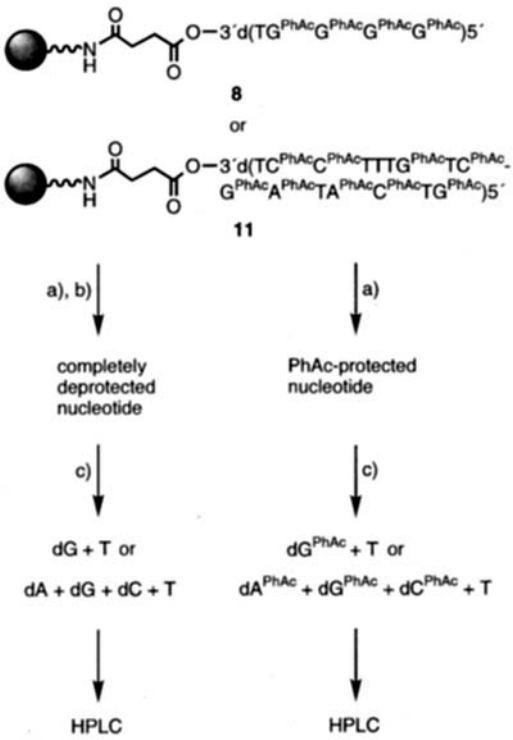
To determine whether the PhAc group can be cleaved enzymatically from oligonucleotides under mild conditions pentanucleotide **8** was removed from the solid support and simultaneously deprotected at the internucleotide linkage by treatment with concentrated ammonia for 45 min at room temperature. The PhAc-protected sample obtained was then incubated with penicillin G acylase at pH 7 for 2 h at room temperature (Scheme 2). The deprotected oligonucleotide was next digested



Scheme 2. Penicillin G acylase mediated removal of the PhAc group from the oligonucleotides **8** and **11** in solution. a) Conc. NH_3 (**8**: room temperature, 45 min; **11**: 10 °C, 10 min); b) penicillin G acylase, pH 7, room temperature, 2 h; c) phosphodiesterase from *Crotalus duriscus*, alkaline phosphatase from *E. coli*, tris(hydroxymethyl)aminomethane (TRIS) buffer, $MgCl_2$, 37 °C, 12 h; the spheres represent the CPG beads.

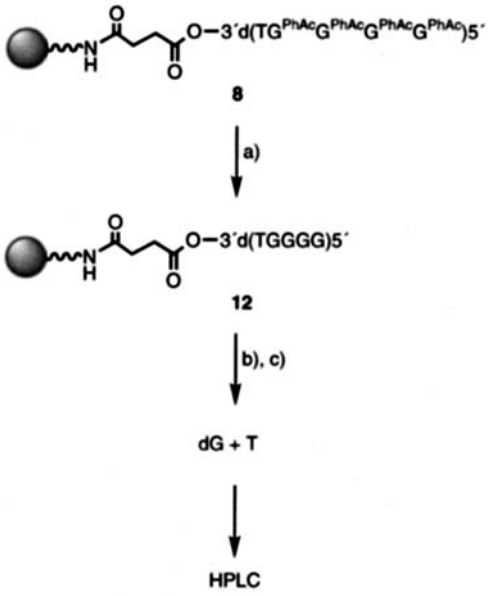
by successive treatment with phosphodiesterase from *Crotalus duriscus* and alkaline phosphatase from *E. coli*, and the resulting nucleoside mixture was analyzed by HPLC (Spherisorb ODSII RP18 column, 250 × 4 mm, 5 μm; methanol/water 30:70 v/v, flow rate 0.6 mL min^{-1}; detection at 260 nm). Parallel to these experiments a sample of **8** was subjected to the same procedure but the nucleobases were not deprotected with penicillin G acylase.[19]

When **8** was treated with penicillin acylase, only deoxyguanidine (retention time $R_t = 4.75$ min) and thymidine ($R_t = 5.01$ min) but no PhAc-protected nucleoside could be detected by chromatography. In contrast, when **8** was not enzymatically deprotected, phenylacetylated deoxyguanidine ($R_t = 56.07$ min) and thymidine were found. This result demonstrates that penicillin acylase removed the four PhAc protecting groups present in **8** quantitatively.

To determine if penicillin G acylase is also capable of removing all PhAc groups from larger nucleotides, the hexadecanucleotide **11** was cleaved from controlled pore glass (CPG) beads by treatment with ammonia at room temperature for 10 min and then subjected to the enzyme-mediated deprotection reaction. The outcome of the biocatalyzed transformation was analyzed as detailed above by enzymatic digestion and HPLC analysis; the results were compared to those obtained for a reference sample from which the PhAc groups had not been cleaved with penicillin acylase. After treatment of **11** with the acylase only the completely deprotected deoxynucleosides dA, dG, dC, and T could be detected, whereas in the control experiment the PhAc-masked nucleosides were obtained. Thus, the biocatalyst removed all nine amino protecting groups in the hexadecanucleotide **11**. For **7**, **9**, and **10** the results were analogous.

These results demonstrate that the phenylacetyl group can be applied advantageously in the construction and for the deprotection of oligonucleotides under mild conditions. Its enzymatic removal offers a viable alternative to classical deprotection by heating with concentrated ammonia for several hours.

Finally, in addition to the enzymatic unmasking of oligonucleotides in solution we investigated whether penicillin G acylase is capable of removing the amino protecting groups from a polymer-bound oligonucleotide. To this end, CPG beads with bound **8** were treated with the enzyme first to give the immobilized but deprotected pentanucleotide **12** (Scheme 3). After the



Scheme 3. Penicillin G acylase mediated removal of the PhAc group from the oligonucleotide **8** on solid support. a) Penicillin G acylase, pH 7, room temperature, 72 h; b) conc. NH_3, room temperature, 45 min; c) phosphodiesterase from *Crotalus duriscus*, alkaline phosphatase from *E. coli*, TRIS buffer, $MgCl_2$, 37 °C, 12 h; the spheres represent the CPG beads.

beads had been washed, the oligonucleotide was released from the solid support by treatment with ammonia and analyzed as described previously by enzymatic digestion and HPLC. In analogy to the enzymatic deprotection of **8** in solution, only deblocked deoxyguanosine and thymidine had formed. Thus, penicillin acylase is a biocatalyst that performs well not only in solution but also on suitable solid supports.

Penicillin G acylase is a very robust, readily available enzyme with a broad substrate tolerance. It accepts, for instance, esters and amides of various chiral alcohols and amines, α- and β-amino acids, aminophosphonic acids, and β-lactams.[20] Thus our results are not only relevant to oligonucleotide synthesis in particular but to solid-phase chemistry in general. Our findings

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might open a new and advantageous route to the development of enzymatic transformations for solid-phase and combinatorial chemistry.

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[18] Oligonucleotides were synthesized by established protocols with a DNA synthesizer (Applied Biosystems, model 394) on controlled pore glass (CPG) beads prederivatized with a DMTr-protected thymidine, which was linked to the solid support by a succinyl linker (see Scheme 2). The phosphoramidites were employed as 0.08 M solutions in CH_3CN; coupling was carried out in the presence of tetrazole (10 min); 3% trichloroacetic acid in CH_2Cl_2 (75 min) was used for detritylation; 3% iodine in water/pyridine/THF (2/20/75) (10 min) was employed to oxidize the phosphites to the phosphates. Average yields of coupling were determined by measuring the absorption of the DMTr cation at 498 nm released upon deprotection of the corresponding 5′-OH group.
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A Tetrakis(imido) Phosphate Anion Isoelectronic with PO_4^{3-}

Paul R. Raithby, Christopher A. Russell,* Alexander Steiner, and Dominic S. Wright

The oxoanions of phosphorus are very numerous and exhibit great structural diversity. It is perhaps surprising then that very few analogous anions with imido groups in place of oxygen have been unequivocally identified. Recent interest in this area has been focused on cyclic PN "multianions". Lithiation of hexakis(cyclohexylamino)cyclotriphosphazene, for example, gives a complex containing $[\{NP(NCy)_2\}_3]^{6-}$.[1] In addition, recent studies on Group 16 elements have shown that the $[E(NtBu)_3]^{2-}$ dianions (E = S, Te), which are isoelectronic with the sulfite ion SO_3^{2-}, can be prepared.[2, 3] With this in mind, we wondered whether small imido phosphorus anions that are analogous to the simple oxoanions could be synthesized.

We report here the synthesis and structure of **1** (R = naphthyl, $C_{10}H_7$), which contains a $[P(NR)_4]^{3-}$ trianion that is isoelectronic with PO_4^{3-}. Complex **1** is the product of the reaction of P_2I_4 with 1-aminonaphthalene in THF/NEt_3 followed by lithiation with *n*BuLi (see Experimental Section).

$[Li(thf)_4][(thf)_2Li(\mu\text{-}NR)_2P(\mu\text{-}NR)_2Li(thf)_2]$ **1**

A low-temperature X-ray crystallographic study of **1** shows an ion-separated complex containing $[Li(thf)_4]^+$ and $[(thf)_2Li(\mu\text{-}NR)_2P(\mu\text{-}NR)_2Li(thf)_2]^-$, both of D_2 symmetry.[4] The N atoms of the anion are in a distorted tetrahedral geometry about the central P atom, and both Li cations bridge two of the N centers (Figure 1). Each Li atom has a distorted tetrahedral geometry, in which the coordination sphere is completed by two

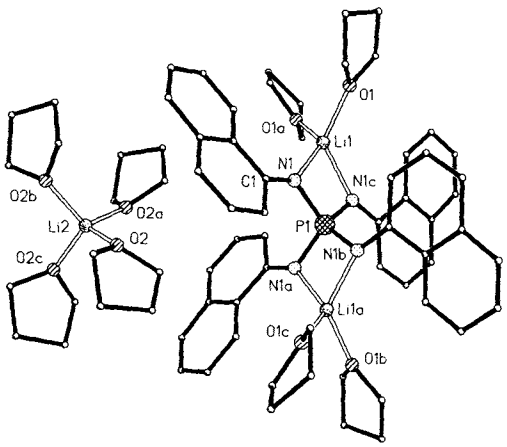

Figure 1. The crystal structure of **1**. Hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and angles [°]: P1–N1 1.645(4), N1–C1 1.369(6), N1–Li1 1.995(10), Li1–O1 1.99(2); N1a-P1-N1b 97.3(3), N1c-P1-N1b 113.1(3), N1c-P1-N1a 118.7(3), C1-N1-P1 123.8(3), N1-Li1-N1a 76.5(5).

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