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SELECTIVE ACYLATION OF N-(2-PHOSPHONOETHYL)ETHYLENEDIAMINE

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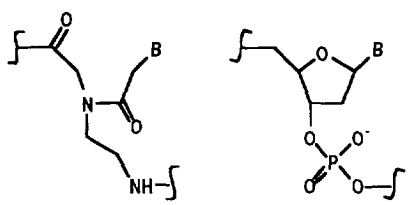
Abstract: *Selective acylation of the primary amino group in phosphonoethyl substituted ethylenediamine* ***1*** *with a variety of acylating agents has been achieved in good yield. These derivatives are potential precursors of backbone modified, "hybrid" synthetic oligonucleotides.*

Regulation of transcription and translation at the *m*RNA and DNA level by antisense and triplex approaches, respectively, have gained considerable attention.[1-3] While formation of antisense duplexes and triple helices with specific target sequences can be achieved with oligonucleotides (oligos) of 12 residues or longer, the *in vivo* susceptibility of these molecules to enzymatic degradation is problematic. There has been extensive effort in developing methods of chemical capping and modification of natural oligos. The ultimate goal is to be able to provide gene-specific agents which exhibit desirable cell permeability and intracellular stability. To meet these requirements, synthetic oligos containing achiral, neutral backbones employing formacetal,[4] thioformacetal,[5] amide[6] and peptide-nucleic acid (PNA)[7] linkers, are particularly promising. These oligonucleotide analogues are resistant to nucleases and bind to DNA and RNA with moderate to high affinity. However, chemical modifications involving charge-reduction on the backbone of oligos often aggravate cell permeability problems. Additionally, some modifications render oligo analogues less soluble in aqueous solution. For instance, in the absence of a positively charged terminal lysine residue, PNA oligos exhibit low solubility in physiological buffers.

We are interested in synthesizing chimeric PNA-DNA oligos that contain peptide[7] and phosphodiester backbone linkages (Fig. 1) in alternating positions. To enhance water solubility and to increase binding affinity to target gene sequences, polar phosphodiester moieties are to be alternated with neutral amide moieties. We hope that the hybrid peptide-phosphodiester backbone will facilitate cell uptake of modified oligos.

Figure 1.

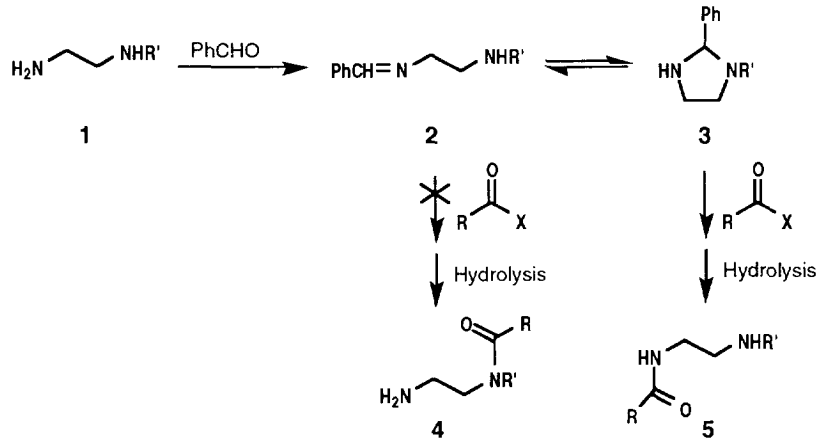


PNA Phosphodiester

To construct a hybrid PNA-DNA backbone that matches the natural phosphodiester backbone in chain length, the peptide and the phosphodiester moieties (Fig 1) must be linked by phosphonoethyl ethylenediamine **1**. The secondary amine of **1** must react with a nucleotide base derivative to give a nucleotide phosphonate monomer. Thus, it is necessary to temporarily protect the primary amine in **1** with a group which can be removed at a later stage under conditions that are stable to phosphonate nucleotide monomers.

A number of methods have been described for achieving regiospecific substitution of amino groups in polyamine chemistry.[8] Direct acylation of N-(2-aminoethyl)glycinate with benzyl *p*-nitrophenyl carbonate yields primary amine-substituted product[9] in 44% yield in the synthesis of PNA.[7] In this reaction, the selectivity may be reationalized by the geater basicity and lesser steric hinderence of the primary amino group. However, this approach in general does not give high degree of regioselectivity. Improvement has been made in the syntheses of spermidine derivatives using multi-step acylation-deacylation reactions. Although time-consuming, these reactions discriminate primary and secondary amines by their different reactivities.[10] Similar reactions permit differentiation of the two terminal primary amine groups in spermidine *via* a cyclohexaminal intermediate, which converts one of the amino group into a secondary amine moiety.[11]

We have developed a simple and efficient approach for introducing acyl protecting groups to the N-(2-phosphonoethyl)ethylenediamine **1** to give primary amino-substituted ethylenediamine derivatives **5** (Scheme 1 and Table 1). Specifically, benzyloxycarbonyl (Z) chloride acylation of **1** was carried out in a two step manner to give **5a** (Scheme 1), without isolation of the amino-aldehyde condensation reaction intermediates. We have extended the reaction to a variety of commonly used amino acid protecting groups, such as tert-butyloxycarbonyl (Boc) and 9-fluorenylmethoxycarbonyl (Fmoc) groups (**5b-e**, Table 1). These reactions were carried out under mild conditions to give primary amine-substituted products in good yield (65 - 85 %) as compared to the 44% yield of the direct acylating reaction of the N-(2-aminoethyl)glycinate in PNA synthesis.[7] The acylated derivatives **5a-e** are important precursors for preparation of various backbone modified nucleotide analogs. Furthermore, the versatility of the reaction is demonstrated in the preparation of **5f** and **5g**, which contain a nucleoside base or an amino acid moiety at the primary amino position (Table 1).



Scheme 1. Acylation reaction of the ethylenediamine derivative. R'=$CH_2CH_2PO(OEt)_2$
RCOX = acylation agents listed in Table 1.

Table 1. Regiospecific Acylation Reactions of the N-(2-phosphonoethyl)ethylenediamine[a]

	Product[12]	Reagent	eq. Reagent	Reaction Conditions Temp.(°C)	Time(h)	Yield (%)
5a	Z-NHR	Z-Cl	1.2	-20	0.5	65
				r.t.	2.5	
5b	Boc-NHR	Boc_2O	1.2	r.t.	3.0	80
5c	Fmoc-NHR	Fmoc-Su	1.2/2mL $CHCl_3$	r.t.	4.0	65
5d	Ac-NHR	Ac_2O	1.1	r.t.	3.0	85
5e	PhCONHR	$(PhCO)_2O$	1.2/1mL $CHCl_3$	r.t.	3.0	65
5f	R'CONHR[b]	R'COOH[c]	1.4/1mL DMF	r.t.	3.0	65
5g	Z-Ala-NHR	Z-Alanine[c]	1.4/1mL CHCN	r.t.	3.0	65

a. R: $-CH_2CH_2NHCH_2CH_2PO(OC_2H_5)_2$; Z: $PhCH_2OCO-$; Boc: t-BuOCO- ; Su: succinimdyl- ; Fmoc: 9-fluorenylmethoxycarbonyl-; Ac: CH_3CO-,

b. R': thymine-CH_2-.

c. 1.6 equivalent of DCC was added in the reaction.

For preparation of **5a** the reaction was carried out by adding 1.5 eq. of benzaldehyde to a suspension of **1** (10 mmol) and excess anhydrous K_2CO_3 and $MgSO_4$ in $CHCl_3$. The mixture was stirred for 1 h at room temperature and 1.2 eq. of benzyl chloroformate was added dropwise at -20°C. Reaction was terminated after stirred at room temperature for 2.5 h and then, the mixture was filtered to remove solid materials and the filtrate was evaporated on a rotary evaporator. The residue was treated with dilute HCl (pH 2.5) and allowed to stand ~2 h at room temperature to yield final products. The acidic solution was washed with diethylether to remove excess benzaldehyde and then lyophilized to give crude product **5a**, which was purified on a silica gel column eluted with 5-10% MeOH in $CHCl_3$ (65% yield, Table 1). Several common acylating reagents were also exployed in this reaction and all produced satisfactory results (Table 1).

In contrast to the formation of a stable cyclohexaminal in the reaction of spermidine and formaldehyde,[11] we observed the formation of a cyclopentaminal and a Schiff base species when **1** was treated with benzaldehyde.[13] ^1H and ^{13}C NMR analysis of the reaction mixture revealed that the Schiff base **2** and the cyclic aminal **3** (Scheme 1) were produced in about 1 to 1 ratio. This ratio was unchanged when the reaction mixture was allowed to stand at room temperature for several days. The exchange cross peaks correlating proton resonances of the Schiff base with those of the cyclic aminal were detected in a NOESY spectrum (500 ms mixing time), indicating that the interconvesion of the two intermediates is on ms time scale. As shown in Table 1, addition of various acylating reagents to the mixture of **2** and **3** proceeded in a selective manner, although priori it appeared that selective acylation of the two amino groups might be difficult to achieve (Scheme 1). The Schiff base **2** blocks the primary amine group but leaves the secondary amine free to react to yield **4**. On the other hand, formation of the cyclic aminal **3** converts the primary and the secondary amines into secondary and tertiary amines, respectively. Consequently, the secondary amine in **1** is transiently protected, whereas the primary amine in **1** can still react with acylating agents to give **5**. Nonetheless, except for the reaction of **5a** (15% acylation of the secondary amine), no secondary amine substituted product is found in all other reactions. The results shown in Table 1 suggests that acylation of the cycloaminal amine and conversion of the Schiff base to the cyclic aminal species must proceed significantly faster than acylation at the

secondary amine of the Schiff base species. Thus, as the cyclic aminal **3** reacts, Schiff base **2** is rapidly converted to **3** to re-establish the equilibrium.

In summary, *in situ* formation of the cyclopentaminal by phosphonoethyl ethylenediamine **1** followed by acylation provides regiospecific primary amine-acylated products in the presence of a secondary amino group. One can also take the advantage of the described reaction to achieve regiospecific substitution at the secondary amine site by temporary protection of the primary amine using proper acylation agents (Table 1). The protected compounds can then be treated with the desired reagents to react with the secondary amine. Removal of the primary protecting group finally yields products which contain secondary amine-substitutions. The acylated derivatives listed in Table 1 are important potential precursors of various N-substituted nucleotide phosphonate analogues. The synthesis of the PNA-DNA chimeric oligos will be reported in due course.

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References and Footnotes:

1. Gene Regulation. Biology of antisense RNA and DNA'. Eds. Erickson, R.P.; Izant, J. G. **1992**, Raven Press: New York.
2. Uhlmann, E.; Peyman, A. *Chemical Reviews* **1990**, *90*, 544-584.
3. Crooke, S.T. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 329-340.
4. Matteucci, M.D. *Tetrahedron Lett.* **1990**, *31*, 2385-2388.
5. Jones, R.J.; Lin, K.-Y.; Milligan, J.F.; Wadwani, S.; Matteucci, M.D. *J. Org. Chem.* **1993**, *58*, 2983-2991.
6. Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Mesmaeker, A. D. *Tetrahedron Lett.* **1994**, *35*, 5225-5228.
7. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
8. Bergeron, R. J. *Acc. Chem. Res.* **1986**, *19*, 105-113.
9. Heimer, E.P.; Gallo-Torres, H.E.; Felix, A.M.; Ahmad, M.; Lambros, T.J.; Scheidl, F.; Meienhofer, J. *Intl. J. Pept. Res.* **1984**, *23*, 203-211. Note: Acylation at the primary amino group with AcONp proceeds in 76% yield.
10. Lurdes, M.; Almeida, S.; Grehn, L; Ragnarsson, U. *Acta Chemica Scandinavica* **1989**, *43*, 990-994.
11. Chantrapromma, K.; McManis, J. S.; Ganem, B. *Tetrahedron Lett.* **1980**, *21*, 2475-2476.
12. **5a**: δ_H ($CDCl_3$) 1.29 (t, 6H), 2.19-2.33 (m, 2H), 3.16-3.36 (m, 4H), 3.45-3.55(m, 2H), 4.03-4.16(m, 4H), 5.06 (s, 2H), 7.32 (s, 5H), Mass calc. 358, found 358 (EI+); **5b**: δ_H ($CDCl_3$) 1.32 (t, 6H), 1.40 (s, 9H), 2.20-2.32 (m, 2H), 3.14-3.30(m, 4H), 3.40-3.52 (m, 2H), 4.06-4.19 (m, 4H), Mass calc. 324, found 324 (EI+); **5c**: δ_H ($CDCl_3$) 1.30 (t, 6H), 2.18-2.34 (m, 2H), 3.10-3.32 (m, 4H), 3.44-3.60 (m, 2H), 4.00-4.36 (m, 7H), 7.20-7.80 (m, 8H); **5d**: δ_H ($CDCl_3$) 1.32 (t, 6H), 2.00 (s, 3H), 2.20-2.33 (m, 2H), 3.14-3.33 (m, 4H), 3.43-3.57 (m, 2H), 4.05-4.20 (m, 4H), Mass calc. 266, found 266 (EI+); **5e**: δ_H ($CDCl_3$) 1.32 (t, 6H), 2.18-2.35 (m, 2H), 3.18-3.36 (m, 4H), 3.67-3.77 (m, 2H), 4.00-4.14 (m, 4H), 7.34-7.90 (m, 5H), Mass calc. 328, found 328 (EI+); **5f**: δ_H ($CDCl_3$) 1.30 (t, 6H), 1.84 (s, 3H), 2.20-2.40 (m, 2H), 3.14-3.35 (m, 4h), 3.50-3.63 (m, 2H), 4.05-4.20 (m, 4H), 4.35 (s, 2H), 7.11 (s, 1H), Mass calc. 390, found 389 (EI+) 391 (FAB); **5g**: δ_H ($CDCl_3$) 1.16-1.40 (m, 9H), 2.16-2.34 (m, 2H), 3.07-3.30 (m, 4H), 3.38-3.68 (m, 2H), 3.95-4.23 (m, 5H), 4.90-4.16 (m, 2H), 7.32 (s, 5H).
13. ^1H-^1H and ^1H-^{13}C bond correlations were assigned in DQF-COSY and HMQC spectra (data not shown). Exchange cross peaks between **2** and **3** were observed in a NOESY (0.5 s mixing time) experiment. Specifically, **2** gives a cross peak in ^1H-^{13}C HMQC, which is unambiguously identified from the scalar coupled imine carbon at 159 ppm and the proton at 8.55 ppm. The diagnostic NMR signal for the presence of **3** is the ^{13}C resonance at 80.5 ppm, which is scalar coupled to a methine ^1H singlet at 4.30 ppm. The chemical shifts of methylene proton resonances of **3** are non-equivalent, consistent with the formation of a relatively rigid ring structure. In contrast, the methylene protons of **2** resonate at a narrow spectral region of < 0.03 ppm.

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