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A PROGRAMMED FIVE-MEMBERED CYCLIC PHOSPHORYLATING REAGENT FOR THE SYNTHESIS OF OLIGONUCLEOTIDES AND ITS USE

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Dedicated to the 80th birthday of Professor Wang Yu.

Abstract: 2-Chloro-2,4-dioxo-3-methyl-tetrahydro-1,3,2λ^5-thiazaphosphole **1** is introduced as a programmed phosphorylating reagent for oligonucleotide syntheses. The homogeneous phase one-pot synthesis of a crystalline dithymidine derivative is described as an illustration of its application.

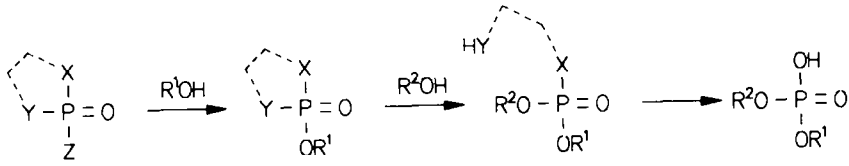
Introduction

Presently, the phosphite amidite based solid phase methods[1] dominate in oligonucleotide synthesis. The hydrogen phosphonate approach has been presented as an alternative to the aforementioned methods[2]. In the early 1970ies the research groups of F. Ramirez and I. Ugi started a joint effort to develop a new class of five-membered cyclic phosphorylating reagents, because their high relative phosphorylation rates[3] provide a distinct advantage over acyclic phosphorylating reagents. An attractive feature of the aforementioned cyclic P(V) reagents vs. the currently most popular P(III) reagents is that no oxidation step is needed after formation of the phosphite esters.

The early respective work of Ramirez, Ugi et al.[4] was confined to the synthesis and exploration of some cyclic O-phosphates of the CAP (cyclic acyl phosphate) and CEP (cyclic endiol phosphate) type. However, such cyclic O-phosphates do not have the required rate differences between the first and the second phosphorylation step (see below).

A cyclic phosphorylating reagent **1** would be ideally suited for the synthesis of unsymmetrical phosphodiesters **4**, including the nucleotide derivatives, if it met the following criteria:

Scheme 1



1 2 3 4

(a) The first phosphorylation step must completely preserve the five-membered ring of **1**, and the reaction **1** → **2** must proceed very rapidly.

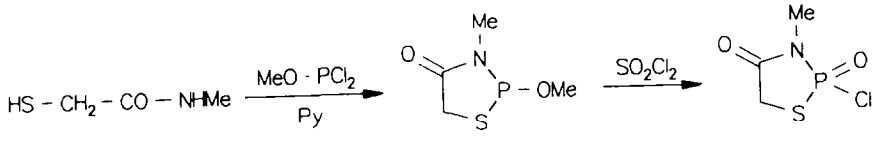
(b) The second phosphorylation step **2** → **3** must not take place while **1** → **2** is carried out. However, it must occur rapidly, when the reaction **2** → **3** is required.

(c) When the five-membered ring of **2** is opened by the reaction **2** → **3** it must be converted into a suitably cleavable (**3** → **4**) protective group of the P(V) system.

A cyclic five-membered phosphorylating reagent that is suitable for oligonucleotide syntheses

Some years ago it was recognized that none of the conceivable cyclic O-phosphates can fulfil the requirements. A broad computer-assisted search for an ideal cyclic phosphorylating reagent led to a set of candidates[5], including the S- and N-phosphates from which a few were picked for closer examination[6]. Among these, only **7** had all of the properties and behaviour needed.

Scheme 2



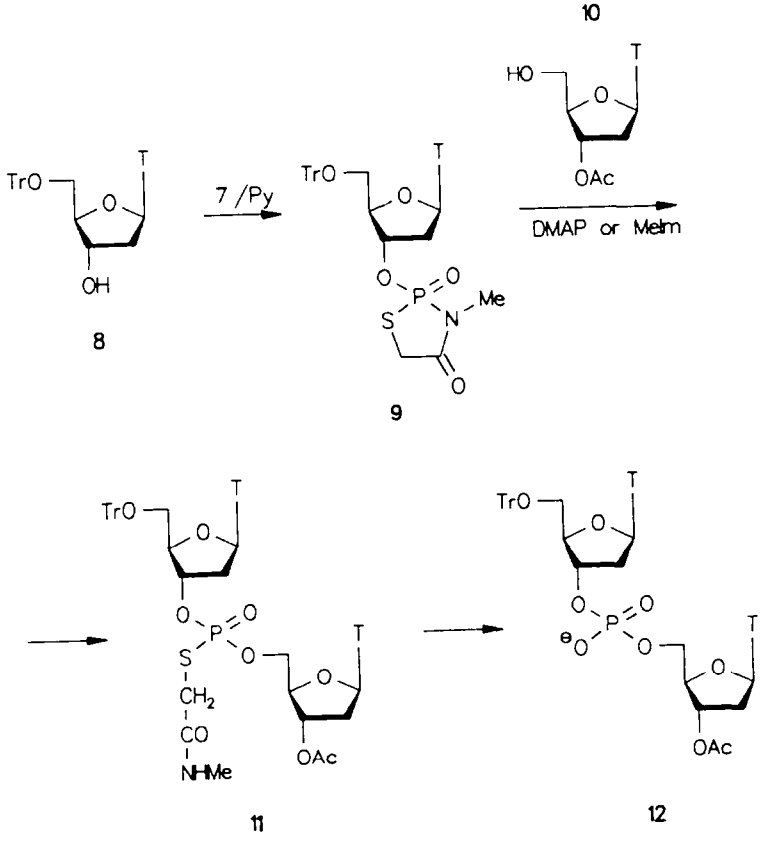
5 6 7

The reagent 7 is readily available from 5[6,7]. It reacts rapidly with alcohols R^1-OH in the sense of 1 → 2. In the absence of catalysts the reaction proceeds no further. However, in the presence of catalysts, such as 4-dimethylamino pyridine (DMAP)[8] or N-methyl imidazole (MeIm), compounds of type 2 react rapidly with alcohols R^2-OH to form the phosphorothioates corresponding to 3. These are conveniently deprotected by various methods (see below) according to 3 → 4, and is still under further investigation. Due to its constitutional features, the reagent 7 is programmed to perform according to the sequence of reactions in scheme 1.

The one-pot synthesis of a dinucleotide derivative, an application of the programmed cyclic phosphorylating reagent

The homogeneous-phase one-pot synthesis of 11 from 8 and 10 illustrates the use of 7 as a reagent for the synthesis of oligonucleotides.

Scheme 3



T = Thymine

Tr = Trityl-

5'-O-Tritylthymidine **8** reacts rapidly with **7** to yield **9**. When **10** and DMAP, or MeIm, are added, **11** is formed immediately. Treatment with aqueous iodine[10] or bis(tri-n-butyltin) oxide[11] leads to **12**[12].

Experimental section:

The NMR data were measured on a Bruker AM 360 (360.13 MHz for ^{1}H, 90.56 MHz for ^{13}C, with TMS as internal standard and 145.78 MHz for ^{31}P, with 85% H_3PO_4 as external standard). Values of coupling constants are given in hertz and chemical shifts (δ) in ppm. Melting points were determined with Büchi SMP-20 apparatus in capillaries and are uncorrected. Flash chromatography was done on TLC silica gel 60, 20-45 µm (Amicon) and chloroform/ethanol as eluents (93:7 v/v → 9:1 v/v) with increasing polarity. Reactions were monitored by analytical TLC using 2x5 cm TLC plates: silica gel F_{254}, 0.25 mm layer. All reactions were performed under a blanket of argon.

Synthesis of the thymidyl(3'→5')thymidine derivative (11):

At 0°C 5'-O-Tritylthymidine (**8**)[9] (1 g, 2.06 mmol) is dissolved in anhydrous methylene chloride (10 ml). A suspension of 2-chloro-2,4-dioxo-3-methyl-tetrahydro-1,3,2λ^5-thiazaphosphole (**1**) (390 mg, 2.10 mmol) in anhydrous pyridine (0.25 ml) is added, and 15 min later 3'-O-acetylthymidine (**10**)[9] (585 mg, 2.06 mmol) with N-methylimidazole (0.1 ml); stirring is continued for 2 h. The reaction mixture is extracted with water (3 x 20 ml), the combined organic layers are dried over magnesium sulphate and evaporated to dryness in vacuo. The residue is purified by flash chromatography in $CHCl_3$/EtOH (93:7 → 9:1 v/v). The resulting product is dissolved in $CHCl_3$ and precipitated by dropping into hexane, yielding an amorphous solid (1.3 g, 64 %); mp 94°C (shrinking), 206°C (dec.); R_f ($CHCl_3$/EtOH 9:1 v/v) = 0.30. Anal. calcd for $C_{44}H_{48}N_5O_{13}PS$ M.W. 917.9: C, 57.57; H, 5.27; N, 7.63. Found: C, 57.58; H, 5.34; N, 7.52. **^{31}P-NMR** ($CDCl_3$): δ 27.70, 27.79 (two diastereomers; ratio 1:1). **^{1}H-NMR** ($CDCl_3$): δ 9.77, 9.71, 9.61 (br s, 4H, NH); 7.55, 7.51 (2 x s, 2H, H-6); 7.39-7.24 (m, 32H, Tr-H, H-6); 6.90 (quar, J = 4.7 Hz, 1H, NH-CH_3); 6.80 (quar, J = 4.7 Hz, 1H, NH-CH_3); 6.39 (2 x tr, J = 4.5 Hz, 2H, H-1'); 6.27 (2 x tr, J = 8.2, 8.3 Hz, 2H, H-1'); 5.30 (m, 4H, H-3'); 4.41-4.34 (m, 4H, H-4'); 4.25, 4.16 (2 x m, 4H, CH_2-S); 3.48 (m, 8H, H-5'); 2.75, 2.74 (2 x d, J = 4.5 Hz, 6H, CH_3NH); 2.54-2.29 (m, 8H, H-2'); 2.09, 2.08 (2 x s, 6H, CH_3CO-); 1.89, 1.87 (2 x s, 6H, T-CH_3); 1.44, 1.42 (2 x s, 6H, T-CH_3). **^{13}C-NMR** ($CDCl_3$): δ 170.5, 170.4, 167.7, 163.9, 150.7, 150.6, 150.5 (C=O); 142.9, 128.6, 128.0, 127.5 (Tr); 135.4, 135.0 (C-5); 111.7, 111.6 (C-6); 87.8, 87.7 (C-Tr); 85.2, 85.1, 84.4, 84.2 (4 x s, C-1'); [84.6, 84.1 (2 x d, $J_{C,P}$ = 5.6, 6.2 Hz); 82.5,

82.4 (2 x d, $J_{C,P}$ = 6.1, 5.7 Hz); 79.7, 79.4 (2 x d; $J_{C,P}$ = 5.3, 5.6 Hz), C-3',C-4']; 67.3, 67.2 (2 x d, $J_{C,P}$ = 10.0, 10.9 Hz, C-5'); 63.4, 63.3 (C-5'); 36.7, 33.6 (CH_2-S); 31.5 (CH_3-NH); 22.6, 20.8 (CH_3CO); 14.0, 12.4 (CH_3-T).

Positive- and negative-ions FAB mass spectra (DMF + glycerol-matrix) were taken; here we only report the diagnostically more useful negative-ions spectra: m/z: 917 (4%, $M^{\ominus}$); 845 (100%, $M^{\ominus}$-$CH_2CONHCH_3$), 719 (6%, 845-thymine); 650 (22%, $M^{\ominus}$- 5'-deoxythymidine-OAc); 579 (6%, 650-C_3H_5NO); 450 (42%, $M^{\ominus}$-3'-deoxy-5'-Tr-thymidine); 379 (15%, 450 - C_3H_5NO).

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