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S-4-METHYLPHENYL-O,O-BIS[1-BENZOTRIAZOLYL]PHOSPHOROTHIOATE: A VERSATILE PHOSPHORYLATING AGENT

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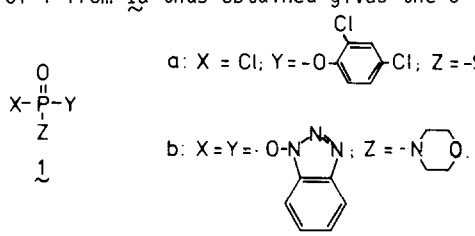
Abstract: The phosphorylating agent obtained by treatment of S-4-methylphenyl phosphorodichloridothioate with 1-hydroxybenzotriazole can not only be applied for the introduction of polyphosphate functions at the terminal ends of nucleic acids, but also for the formation of 3'-5'-phosphotriester linkages.

Nucleic acids which carry terminal polyphosphate groups are from a biological point of view important naturally occurring compounds[1].

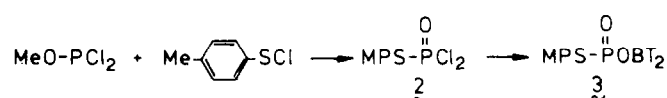
Up to now, two types of phosphorylating agents (i.e. the monofunctional and the bifunctional agents 1a[2] and 1b[3], respectively) have been applied successfully for the introduction of di- and triphosphates at the 5'-end of nucleic acids by a phosphotriester approach. For instance, phosphorylation[4] of the 5'-OH of a partially protected nucleic acid phosphotriester intermediate (ROH) with 1a affords a 5'-phosphotriester derivative 1a (X=OR; Y=2,4-$Cl_2C_6H_3$; Z=SMe). Selective removal of Y from 1a thus obtained gives the 5'-phosphorothioate 1a (X=OR;



Y=OH; Z=SMe), which can be converted, according to Nussbaum et al.[5], into the corresponding 5'-mono, di- or triphosphate derivatives. Recently, we reported[6] that the phosphorylating agent 1b could serve the same purpose. In this particular case, phosphorylation of a similar 5'-OH function gives the phosphotriester 1b [X=OR; Y=OBT; Z=-$N(C_2H_4)_2O$] from which the benzotriazolyl (OBT) group can be removed selectively to afford the phosphoromorpholidate 1b [X=OR; Y=OH; Z=-$N(C_2H_4)_2O$]. The latter can now be converted, according to the original procedure of Khorana et al.[7], into the required 5'-phosphorylated nucleic acids derivatives.

We now report that S-4-methylphenyl-O,O-bis[1-benzotriazolyl]phosphorothioate (3) is in several aspects more convenient than agents 1a,b and, further, that it can also be used for the formation of 3'-5'-internucleotidic linkages.

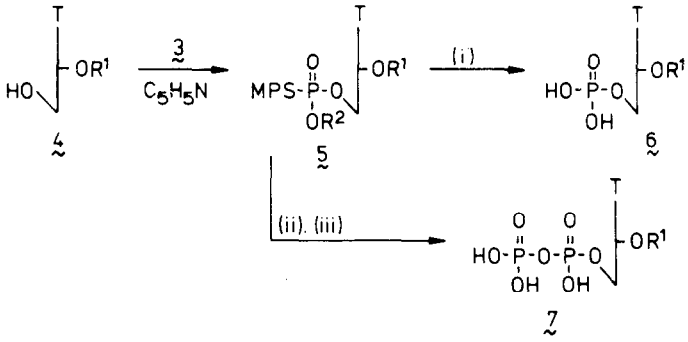
The phosphorylating agent 3 is readily attainable as follows. Treatment[8] of methoxydichlorophosphine with 4-methylphenylsulfenyl chloride gives, after distillation, 2 as a colourless



solid [Yield: 84%; b.p. 125°C/2 mm Hg; ^{31}P-NMR ($CDCl_3$) δ 33.36 p.p.m.]. A solution of the

latter in dioxan was converted into 3 by the addition of equimolar amounts of pyridine and 1-hydroxybenzotriazole. After 1 h at 20°C, the pyridinium HCl salt was filtered off to give a stock solution (0.2 M) of 3 in dioxan.

The synthesis of the 5'-phosphates 6 and 7 of thymidine was realized as follows. A solu-

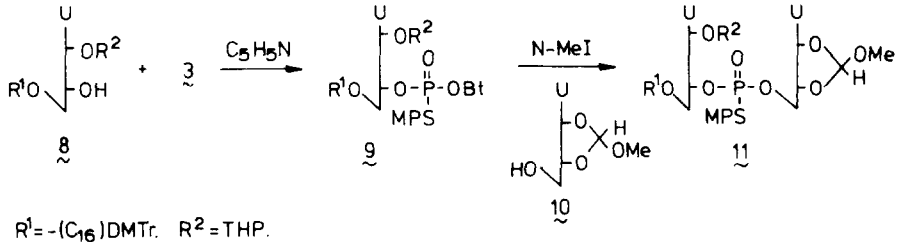


4 5 6 7

(i): H_2O/I_2. (ii): H_2O/C_5H_5N. (iii): H_3PO_4/I_2.

tion of 3'-O-diphenylacetyl-thymidine[9] 4 (1 mmol), which was previously dried by coevaporation with pyridine, in dioxan was added to a stirred solution of 3 (1.1 mmol). TLC-analysis, after 15 min at 20°C, showed the absence of 4. Intermediate 5 (R^1=DPA; R^2=BT) thus obtained was hydrolyzed with water-pyridine. Monitoring of this step by ^{31}P-NMR spectroscopy revealed instanteneous conversion of 5 (R^1=DPA; R^2=BT: δ_p 32.08 and 31.67) into 5 (R^1=DPA; R^2=H: δ_p 15.19). Oxidative removal of the MPS-group from 5 (R^2=H) to afford the 5'-phosphate 6 (R^1=DPA) was effected by the addition of iodine (10 equiv.) to the reaction mixture. Also in this case, ^{31}P-NMR spectroscopy showed a rapid (within 5 min) conversion of 5 (R^1=DPA) into 6 (R^1=DPA: δ_p -0.56). Crude 6 thus obtained gave, after basic hydrolysis of the DPA-group, and consecutive purification by anion-exchange chromatography (DEAE-Sephadex A25) pure 6 (R^1=H: δ_p 0.24) in 80% overall yield. For the preparation of 7, intermediate 5 (1 mmol; R^1=DPA; R^2=BT) was firstly hydrolyzed with water-pyridine and, after 5 min, concentrated to a small volume. The mixture was then repeatedly coevaporated with dry pyridine containing tri-n-butylammonium phosphate (15 equiv.). The formation of the pyrophosphate function was effected by adding iodine (10 equiv.) to the above solution of 5 (R^1=DPA; R^2=H). ^{31}P-NMR analysis of the reaction mixture showed rapid (5 min) formation of 7 (R^1=DPA: δ_p 8.70, 10.58, J 23.2 Hz). Further processing of crude 7 (R^1=DPA), as described for the preparation of 6 (R^1=H), gave homogeneous 7 (R^1=H) in 75% overall yield.

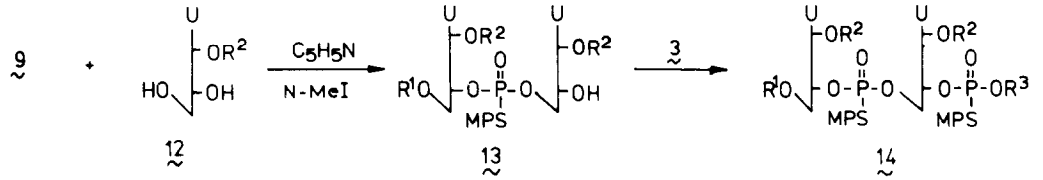
The introduction of a 3'-5'-phosphotriester linkage using 3 was firstly exemplified by the synthesis of the fully-protected RNA dimer 11. The 2',5'-protected uridine derivative 8[10]



R^1=-(C_{16})DMTr. R^2=THP.

(1 mmol) in dry pyridine was treated with 3 (1.15 mmol). TLC-analysis, after 20 min at 20°C, showed complete conversion of 8 into 9 having zero mobility. A solution of 2',3'-protected uridine 10[11] (1.3 mmol) in pyridine containing N-methylimidazole (5 equiv.) was added to 9. TLC-analysis, after 20 min at 20°C, showed the formation of dimer 11. Work-up of the crude product, followed by short column chromatography, afforded homogeneous 11 (δ_p 23.69 and 24.34) in a yield of 71% (based on 8). A small amount of dimer 11 was completely deblocked by removing[12] firstly the MPS-group with syn-pyridine-2-carboxaldoxime and N^1,N^1,N^3,N^3-tetramethylguanidine[13]. The different acid-labile [R^1=2-hexadecyloxy-4',4"-dimethoxytrityl; R^2=tetrahydropyranyl and the 2',3'-O-methoxymethylidene (MM)] groups were removed in the following order. Group R^1 was deblocked by a short treatment with aq. HOAc. The acetic acid was removed *in vacuo* and the solution was adjusted to pH 2 (0.1 N HCl: removal of the THP and ring opening of the MM group) and left for 24 h at 20°C. The solution was basified (pH 8) with aq. ammonia and further processed, to give, after purification, homogeneous UpU (δ_p -0.20).

The versatility of agent 3 was further illustrated by the regioselective introduction of a



3'-5'-phosphotriester bond (i.e. 13) and, also, by the synthesis of dimers 14. Thus addition of 12[14] (1.3 mmol) to 9, under the same conditions as used for the preparation of 11, gave dimer 13 (δ_p 24.69 and 25.19) in 73% yield. The conversion of 13 into dimers 14 (R^3=H and R^3=$-CH_2CH_2CN$) was performed in two different ways. In the first approach, a solution of 13 (1 mmol) in pyridine was phosphorylated with 3 (1.15 mmol) to give, after 20 min at 20°C, intermediate 14 (R^3=BT). The latter was hydrolyzed with water to afford 14 (R^3=OH), which could be isolated by extraction, followed by precipitation, as the triethylammonium salt in 85% yield. In the second approach, intermediate 14 (R^3=BT) was converted *in situ* by the addition of an excess of β-cyanoethanol into 14 (R^3=$-CH_2CH_2CN$), which could be isolated, after column chromatography, in a yield of 81%. Complete deblocking of 14 thus obtained was as follows. Treatment of 14 with N^1,N^1,N^3,N^3-tetramethylguanidine (9 equiv.) resulted in the instanteneous elimination, as followed from ^{31}P-NMR spectroscopy, of the β-cyanoethyl group. Addition of syn-pyridine-2-carboxaldoxime (10 equiv.) to the above mixture released the internucleotidic MPS-protecting group. The MPS-group at the 3'-phosphodiester was now removed by oxidation with iodine, and the R^1 and R^2 groups by acid. Purification of the crude product afforded UpUp, which was identical, ^{31}P-NMR (δ_p 1.30 and -0.18) and TLC-analysis[15] (R_f 0.51), with the product obtained after complete deblocking of 14 (R^3=OH). Furthermore, enzymatic digestion of the UpUp compounds, prepared by the two different approaches, were completely digested, as followed by TLC-analysis and monitoring of the enzymatic process by ^{31}P-NMR spectroscopy, by RNase to give solely uridine-3'-phosphate (δ_p 1.72; R_f 0.63).

In conclusion, we believe that the easily accessible agent 3 promises to be very convenient for the introduction of polyphosphate functions at the terminal ends of nucleic acids. It also seems to be an alternative for the existing procedure[12] in which the cyclohexylammo-

nium salt of S,S-diphenylphosphorodithioate has been used for the preparation of ArS-protected intermediate internucleotidic phosphotriester linkages. At present, we are studying in detail[16] the scope of the new agent 3 in nucleic acids and sugar chemistry.

ACKNOWLEDGEMENT

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15. Eluens: aq. ammonium acetate (1 M): ethanol, 3:7, v/v. Solid phase: DC-Alufolien Cellulose F (Merck).
16. Experimental evidence so far obtained indicated that agent 3 has the same favourable phosphorylating properties as 2-chlorophenyl-O,O-bis[1-benzotriazolyl]phosphate [C.T.J. Wreesmann et al., Nucleic Acids Res., 11, 8389 (1983) and references cited therein] and, further, that the pyridine-2-carboxaldehyde oximate ion-promoted unblocking of the MPS-group from dimer 11 proceeds four times more slowly than the removal, under the same conditions, of the 2-chlorophenyl group from a similarly protected UpU dimer.

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