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Short Communications

The 2-Nitrophenylsulfenyl (Nps) Group for the Protection of Amino Functions of Cytidine, Adenosine, Guanosine and Their 2′-Deoxysugar Derivatives

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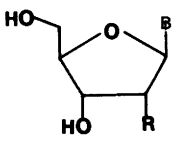
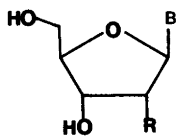
It is clear from the reports in the literature that the exocyclic amino groups of cytosine, adenine and guanine residues should be blocked for the chemical synthesis of DNA and RNA fragments.[1] It is a usual practice that such exocyclic amino groups are protected as amide derivatives[1,2]; the removal of these amides are time-consuming and is dependent upon the nature of the base residues.[1] Thus, the relative rates of the hydrolysis of a particular *N*-acyl group from the nucleoside base residues follow the order: cytosine>adenine>guanine. There are a few examples of carbamates[3–7] and one example each of (dimethylamino)methylene[8a] and amidine[8b] groups in the literature which have been employed for this purpose. Except for two examples,[6,7] all these blocking groups are removed under alkaline hydrolytic conditions. Recently we have reported on the 9-fluorenylmethoxycarbonyl (Fmoc) group[9] for the protection of amino functions of cytidine, adenosine, guanosine and their 2′-deoxysugar derivatives. The Fmoc groups are removable from these protected nucleosides by a tertiary, non-nucleophilic base like triethylamine as well as by alkaline hydrolytic conditions within 3 h at room temperature. Here we report on the preparation, properties and applications of 2-nitrophenylsulfenyl (Nps) as a new exocyclic amino protective group of cytosine, guanine and adenine residues in both the DNA and RNA series as shown in the derivatives, *2* to *7*, which have been prepared from common ribonucleosides and their 2′-deoxysugar derivatives of the general formula *1*. The application of the Nps group has its origin in the work of Goerdeler and Holst who synthesized the first Nps-amino acids.[10] Subsequently, several groups of workers[11] have employed the Nps-amino acids in the peptide synthesis. However, it was Fontana *et. al.*[12] and Tun-kyi[13] who first demonstrated that the Nps group could indeed be successfully cleaved from Nps-protected peptides using a thiolysis procedure. We reasoned that if the Nps group from Nps-protected nucleosides and nucleotides could be similarly removed under such mild conditions, one should be able to reduce the time of deprotection which is the most time-consuming part in the chemical synthesis of DNA fragments on solid support. It also occurred to us that such a method of removal of the Nps group from the Nps protected nucleic acid residues, prepared by the phosphite-triester approach,[14] should constitute an easy access to fully deprotected DNA fragments since the removal of the methyl groups from internucleotide phosphates and the Nps groups from the base residues might be accomplished in a single chemical operation. These considerations have led us to prepare the Nps derivatives of cytidine, adenosine and guanosine and their corresponding 2′-deoxyribose derivatives, as in *2* to *7*, from their respective parent nucleosides through a "one-





GENERAL FORMULA: (1)

R=H or OH	*2*; R=OH; B=(a)
B=1-Cytosinyl	*3*; R=OH; B=(b)
9-Adeninyl	*4*; R=OH; B=(c)
9-Guaninyl	*5*; R=H; B=(a)
	6; R=H; B=(b)
	7; R=H; B=(c)

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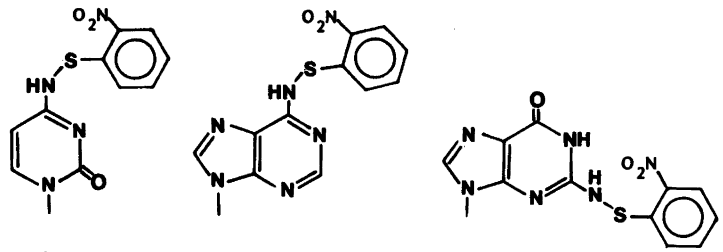
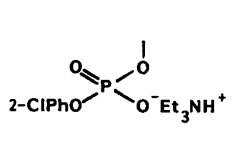
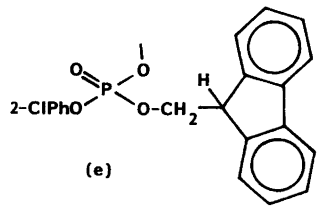
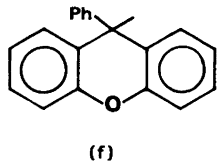
Table 1. The physical and the spectroscopic properties of Nps protected ribonucleosides and 2'-deoxyribonucleosides.

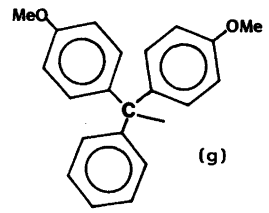
Compound	M.p.(°C) (crystallization media)	Yield %	R_f MeOH–$CHCl_3$(3:7v/v)	UV absorption properties in Methanol (nm) λ_{max}	^1H NMR absorptions in DMSO-d_6+D_{20} (δ scale)
2	156 (Toluene)	88	0.51	(pH 2):288 (pH 7):282 (pH 13)282(sh.),327	8.37–7.20(m,5H),6.16(d,7.2Hz,1H) 5.79(d,2.4Hz,1H);3.97(m,2H); 3.64(m,2H)
3	145 (EtOH–H_2O 1:1v/v)	84	0.63	(pH 2): 240(sh.),270; (pH 47):240(sh.),270; (pH 13):240(sh.),270	8.53(s,1H);8.32(s,1H);8.20–7.20(m,4H; 6.00(d,5.4Hz,1Hz);4.62(m,1H);4.13–366(m,4H)
4	190 (EtOH–H_2O 1:1v/v)	80	0.28	(pH 2):250,260,276(sh.) (pH 7):250,276(sh.) (pH 13):250(sh.),260(sh.)	8.39–7.44(m,5H);5.67(q,7.2Hz,1H); 4.44(m,1H);4.00–3.40(m,4H)
5	193 (EtOH–H_2O 1:4v/v)	82	0.56	(pH 2):284; (pH 7)282 (pH 13):284(sh.), 327	8.83–7.17(m,6H);6.16(t,1H); 4.32(m,1H);4.1–3.3(m,5H)
6	175 (EtOH–H_2O 1:1v/v)	90	0.65	(pH 2):242(sh.)270 (pH 7):242,272 (pH 13):242,270	8.55–7.40(m,6H);6.45(t,1H); 4.48(m,1H);3.93–3.40(m,5H)
7	216 (EtOH–H_2O 1:1v/v)	88	0.37	(pH 2):248,256,276(sh.) (pH 7):246,262(sh.),276(sh.) (pH 13):250(sh.),260(sh.),266(sh.)	8.73–7.20(m,5H);6.07(t,1H) 4.19(m,1H);3.83–3.19(m,5H)

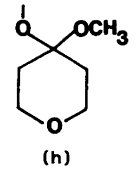












pot" synthesis in 88, 84, 80, 82, 90 and 88 % yields, respectively, as crystalline compounds. The general procedure for such a synthesis involves trimethylsilylation[15] of the nucleoside in dry pyridine solution which is followed by the addition of 2-nitrophenylsulfenyl chloride (Nps-Cl) (1.2 equiv., with respect to the nucleoside) and then hydrolysis. Table 1 records some physical and spectroscopic properties of compounds *2* to *7* in support of their structures. The Nps groups in *2* to *7* are completely stable under the following conditions which illustrates its compatibility with the commonly available protective groups that are employed in the phosphotriester[1] or the phosphitetriester[14] approaches in the chemical synthesis of DNA or RNA fragments: (1) stable for 12 h in the presence of tetrabutylammonium fluoride (10 equiv.) in dry tetrahydrofuran [$(F^-) \simeq 0.1$ *M*] at room temperature; thus it is compatible with t-butyldimethylsilyl-[16] and 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl-[17]; (2) stable for 24h in triethylamine (25 equiv.) in dry pyridine solution (10 ml/mmol); thus it is compatible with 2-phenylsulfonylethyl-[18], 2-phenylsulfonylethoxycarbonyl-[19], 2-(4-chlorophenyl)-sulfonylethoxycarbonyl-,[20] 9-fluorenylmethoxycarbonyl-[21] and 9-fluorenylmethyl-[22]; (3) stable in presence of 1*M* hydrazine hydrate in acetic acid: pyridine::3:4 (v/v) at room temperature for over 2 h; thus it is compatible with the levulinyl group[23]; (4) finally, the Nps group is also completely stable in presence of 5 % 4-toluenesulfonic acid. H_2O in 2 % ethanol-chloroform mixture for over 8 h. at room temperature, which makes it compatible with the acid-labile 5'-protective groups like 9-phenylxanthen-9-yl (pixyl)[24] and 4,4'-dimethoxytrityl (DMTr)[25] groups.

The Nps group may be smoothly cleaved, from *2* to *7* within an hour at room temperature, as shown in Table 2, using a dry pyridine solution (1 ml/mmol) of triethylammonium thiocresolate (3 equiv.) under an atmosphere of argon.

We then examined the effect of the Nps, as the 6-amino protective group of 2'-deoxyadenosine (*6*), on the cleavage of the glycosidic bond (depurination)[1] in view of the recent reports in the literature.[26] Thus, the half-lives of depurination reaction of 6-*N*-benzoyl-2'-deoxyadenosine,[26] 6-*N*-phthaloyl-2'-deoxyadenosine[26] and 6-*N*-Fmoc-2'-deoxyadenosine[9] are respectively 30, 120 and 180 min. in 80 % aqueous acetic acid at 30 °C. Under a similar acidic condition, the glycosidic bond on 6-*N*-Nps-2'-deoxyadenosine

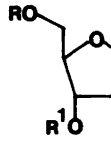
Table 2. Removal of the Nps group from Nps protected nucleoside derivatives, (2) to (7), using triethylammonium thiocresolate (3 equiv.) in dry pyridine (1 ml/mmol) under argon at 20 °C.

Substrates	$t_{\frac{1}{2}}$ (min)	t_{∞} (min)
2	—	10
3	6	60
4	—	20
5	—	10
6	6	60
7	—	20

(*6*) remained completely intact for 24 h. Thus it is clear that the employment of a building block like *6* should provide an acceptable solution to the problem of depurination of 2'-deoxyadenosine residues in the chemical synthesis of DNA segments.

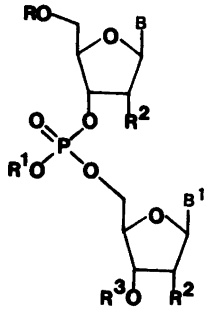
Finally the applicability of the Nps group in the chemical synthesis of DNA and RNA fragments have been demonstrated by the preparation of two dimeric RNA units: cytidylyl-(3'→5')-adenosine-3'-*O*-2-chlorophenylphosphate (*8*) and guanylyl-(3'→5')-cytidine-3'-*O*-2-chlorophenylphosphate (*9*) and a tetrameric DNA segment: $^{5'}$d(TpGpCpA)$^{3'}$ (*25*), starting with the corresponding Nps protected ribonucleosides and 2'-deoribonucleoside derivatives. The building blocks for the dimeric RNA units, *10* to *13*, have been prepared in high yields as powders using a procedure which has already been reported by us in the literature.[27] Two condensation reactions in dry pyridine solutions with the appropriate building blocks, *11*+*13* and *10*+*12* respectively in presence of an excess of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT)[29], gave the fully protected derivatives *14* and *15* in 72 and 69 % yields respectively. For the synthesis of the fully protected tetrameric DNA segment, $^{5'}$d(TpGpCpA)$^{3'}$ (*24*), the building blocks *16* to *19* were also prepared in high overall yields using our literature procedure.[28] They were then coupled, in dry pyridine solutions using MS-NT, to give the fully protected dimers, *20* and *21* in 74 and 68 % yields respectively. The dimer blocks were then converted to 5'-hydroxy-(*22*) and 5'-protected-blocks (*23*) in the usual manner[28] in 80 and 91 % yields respectively. Finally the fully protected tetramer (24) was obtained, upon the condensation of *22* and *23* under an usual condition,[28] in 70 % yield as powder. The fully protected oligonucleotides, *14*, *15* and *24* were then subsequently deprotected with (1) 4-nitrobenzaldoximate ions[29]; (2) then with triethylammonium thiocresolate (3 equiv.) in dry pyridine (1 ml/mmol) under argon (compound *24* was then subjected to a treatment of aq. NH_3 (d 0.9) for 24 h. at 22 °C to remove the 3'-*O*-benzoyl group) and (3) with 80 % aq. acetic acid at 20 °C (incubation times for *14* and *15* were 6 h; and for *24*, it was 20 min). The reaction mixture was worked up using standard procedures[27,28] and purified by a DEAE sephadex A25 column ($Et_3NH^+HCO_3^-$ buffer; pH 7.4; linear gradient 0.001 *M* to 0.3 *M*).

The compounds 8, 9 and 25 were thus obtained in 60, 57 and 73 % yields, in terms of A_{260} o.d. units, respectively. They were completely



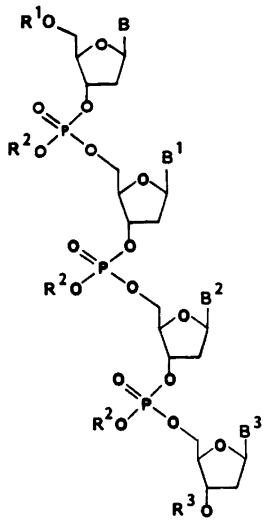
10; B=(c); R=(f); R^1=(d); R^2=(h);
11; B=(a); R=(f); R^1=(d); R^2=(h);
12; B=(a); R=H; R^1=(e); R^2=(h);
13; B=(b); R=H; R^1=(e); R^2=(h);

16; B=1-Thyminyl-; R=(g); R^1=(d); R^2=H;
17; B=(c); R=H; R^1=(e); R^2=H;
18; B=(a); R=(g); R^1=(d); R^2=H;
19; B=(b); R=H; R^1=Bz; R^2=H;



8; B=1-Cytosinyl-; B^1=9-Adeninyl-; R=R^1=H; R^2=OH; R^3=(d);
9; B=9-Guaninyl-; B^1=1-Cytosinyl-; R=R^1=H; R^2=OH; R^3=(d);
14; B=(a); B^1=(b); R=(f); R^1=2-ClPh; R^2=(h); R^3=(e);
15; B=(c); B^1=(a); R=(f); R^1=2-ClPh; R^2=(h); R^3=(e)

20; B=(a); B^1=(b); R=(g); R^1=2-ClPh; R^2=H, R^3= −OBz;
21; B=1-Thyminyl-; B^1=(c); R=(g); R^1=2-ClPh; R^2=H; R^3=(e);
22; B=(a); B^1=(b); R=H; R^1=2-ClPh; R^2=H; R^3= −OBz;
23; B=1-Thyminyl-; B^1=(c); R=H; R^1=2-ClPh; R^2=H; R^3=(d);



24; B=1-Thyminyl-; B^1=(c); B^2=(a); B^3=(b); R^1=(9); R^2=2-ClPh; R^3=Bz

25; B=1-Thyminyl-; B^1=9-Guaninyl-; B^2=1-Cytosinyl-; B^3=9-Adeninyl-; R^1=R^2=R^3=H;

digested to monomeric components by spleen phosphodiesterase and were subsequently quantitiated by HPLC to give the desired ratios of the monomeric components confirming the structure of the oligonucleotides. The chemical syntheses of these oligonucleotides have thus clearly established the stability of the Nps, as an exocyclic amino protective group both in DNA and RNA series, through the actual multistep chemical operations that are required in the synthesis nucleic acids using the phosphotriester approach. Further work is in progress in this laboratory to establish the application of the Nps group in the solid phase chemical synthesis of DNA and RNA fragments using the phosphite-triester approach.

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1. Review: Reese, C. B. *Tetrahedron 34* (1978) 3143.
2. Khorana, H. G., *Pure Appl. Chem. 17* (1968) 349; Agarwal, K. L., Yamazaki, A., Cashion, P. J. and Khorana, H. G. *Angew, Chem. Int. Ed. Eng. 11* (1972) 451.
3. Letsinger, R. L., Miller, P. S., *J. Am. Chem. Soc. 91* (1969) 3356.
4. Ched. C. B., Hong, C. I., *J. Med. Chem. 14* (1971) 748. Lyon, P. A., Reese, C. B.; *J. Chem. Soc., Perkin Trans. 1* (1978) 131.
5. Adamiak, R. W., Stawinsky, J., *Tetrahedron Lett.* (1977) 1935.
6. Scheiderwind, R. G. K., Ugi, I. *Z. Naturforsch., B: Anorg. Chem., Org. Chem. 36 B* (1981) 1173.
7. Watkins, B. E. and Rapoport, H. *J. Org. Chem. 47* (1982) 4471.
8. (a) Zemlicka, J., Chladek, S., Holy, A., Smrt, J., *Collect. Czech. Chem. Commun. 31* (1966) 3198. (b) McBride, L. J. and Caruthers, M. H., *Tetrahedron Lett. 24* (1983) 2953.
9. Heikkilä, J. and Chattopadhyaya, J. *Acta Chem. Scand. B 37* (1983) 263.
10. Goerdeler, J., and Holst, A., *Angew. Chem. 71* (1959) 775.
11. (a) Zervas, L., Borovas, D., and Gazis, E., *J. Am. Chem. Soc. 85* (1963) 3660; (b) Schröder, E. and Lübke, K., *The peptides* Vol. 1, Academic, New York (1965); (c) Wünsch, E., Deffner, M., Deimer, K-H., Jaeger, E., Stelzel, P., Thamm, P., and Wendelberger, G. in *Methoden der organischen Chemie (Houben-Weyl)* (E. Mueller *et. al.* Eds.), 1974, Vol. XV, part 2. Thieme, Stuttgart; (d) Kessler, W., and Iselin, B., *Helv. Chim. Acta 49* (1966) 1330.
12. Fontana, A., Marchiori, F., Moroder, L. and Scoffone, E., *Tetrahedron Lett.* (1966) 2985.
13. Tun-kyi, A., *Helv. Chim. Acta, 61* (1978) 1086.
14. (a) Letsinger, R. L., and Lunsford, W. B., *J. Am. Chem. Soc., 98* (1976) 3655. (b) Matteucci, M. D. and Caruthers, M. H., *J. Am. Chem. Soc. 103* (1981) 3185; (c) Beaucage, S. L. and Caruthers, M. H. *Tetrahdron Lett. 22* (1981) 1859.
15. Ti, G. S., Gaffney, B. L. and Jones, R. A. *J. Am. Chem. Soc. 104* (1982) 1316.
16. Corey, E. J. and Venkateswarlu, A., *J. Am. Chem. Soc. 94* (1972) 6190.
17. Markiewicz, W. T., *J. Chem. Res. (S),* (1979) 24.
18. Balgobin, N., Josephson, S. and Chattopadhyaya, J. *Tetrahedron Lett. 22* (1981) 1915.
19. Balgobin, N., Josephson, S. and Chattopadhyaya, J. *Tetrahedron Lett. 22* (1981) 3667.
20. Josephson, S., Balgobin, N. and Chattopadhyaya, J. *Tetrahedron Letts. 22* (1981) 4537.

21. Gioeli, C. and Chattopadhyaya, J. *J. Chem. Soc. Chem. Comm.* (1982) 672.
22. Gioeli, C. and Chattopadhyaya, J. *Chem. Scr. 19* (1982) 235.
23. Van Boom, J. H. and Burgers, P. M. J. *Tetrahedron Lett.* (1976) 4875.
24. Chattopadhyaya, J. and Reese, C. B. *J.C.S. Chem. Comm.* 639 (1978).
25. Schaller, H., Weimann, G., Lerch, B. and Khorana, H. G. *J. Am. Chem. Soc. 85* (1963) 3821.
26. Kume, A., Sekine, M. and Hata, T. *Tetrahedron Lett. 23* (1982) 4365.
27. Kwiatkowski, M., Heikkilä, J., Björkman, S., Chattopadhyaya, J. and Seliger, H. *Chem. Scr. 22* (1983) 30.
28. Balgobin, N., Josephson, S. and Chattopadhyaya, J. *Acta Chem. Scand. B 35* (1981) 201.
29. Reese, C. B., Titmus, R. C. and Yau, L. *Tetrahedron Lett.* (1978) 2727.

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