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ONE-STEP PROTECTION OF THE NUCLEOSIDE BASE IN THYMIDINE AND URIDINE

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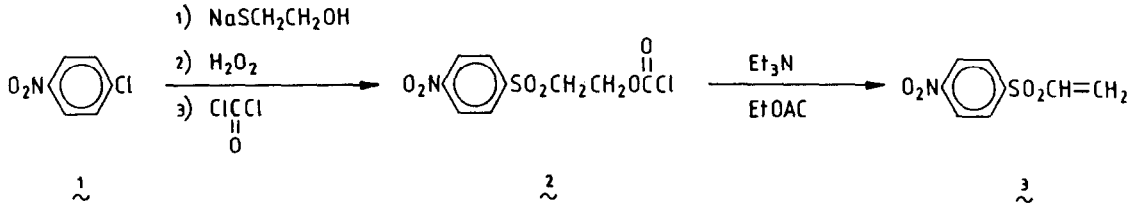
Abstract: Unprotected thymidine and uridine react with 4-nitrophenylsulfonyl ethene (3) in a base catalyzed Michael type addition to give O^4-(4-nitrophenylsulfonylethyl)thymidine (6) and -uridine (7), respectively. The 4-nitrophenylsulfonylethyl group is cleaved within 2.5 hours at 50-55°C by concentrated aqueous ammonia, *via* ß-elimination.

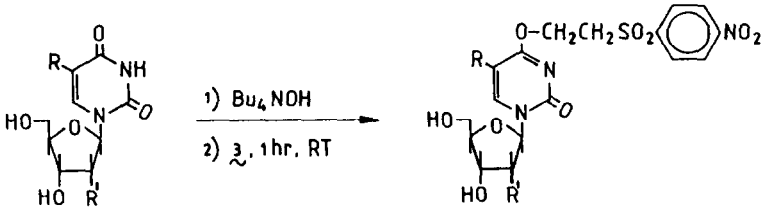
During the past eight years it has been found out, that the synthesis of oligonucleotides *via* the phosphotriester approach can give rise to unwanted side reactions on the imide moieties of guanine[1-3], uracil[4-8] and, to a somewhat lesser extent[3], thymine[9]. These side reactions were shown to arise from the electrophilic attack of condensing- and phosphorylating reagents commonly employed in the phosphotriester approach[1-11]. Since this difficulty can be solved by protection of the endangered positions Reese and coworkers recently investigated the 2,4-dimethylphenyl and the phenyl group for the semipermanent protection of O^4 in uracil and thymine, respectively[10,11]. These groups are cleaved during the deprotection of the aryl groups from the internucleotide phosphate residues, by oximate. Very recently, the 4-nitrophenylethyl group has been proposed by Pfleiderer and coworkers for the protection of the O^4-atom in the uracil and thymine moieties.[13] This protecting group is removed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry pyridine. Protection of the uracil imide moiety has been achieved at the N^3-position with (base labile) acyl functions[12,14-16] or by the 2,2,2-trichloro-1,1-dimethylethyloxycarbonyl group, which is cleaved under reductive conditions.[8]

The introduction on the imide functions of uracil and thymine of the protecting functions mentioned above, requires that the sugar moiety of the corresponding (deoxy)nucleoside be fully protected, which inevitably leads to multi-step procedures for the preparation of desired building blocks. We now wish to report that 4-nitrophenylsulfonylethene (3) can be used for the selective protection of O^4 in the uracil or thymine residue in the corresponding unprotected nucleosides. Compound 3 was prepared in the following way: Nucleophilic aromatic substitution of the chlorine atom in 1 by the sodium salt of 2-mercapto ethanol



1 2 3

(3 hrs, 70°C) gave the corresponding thioether which was subsequently oxidized with H_2O_2[21] to give crystalline 2-(4-nitrophenylsulfonyl)ethanol. Treatment of this alcohol with excess phosgene in THF[17] afforded the chloroformate 2 in quantitative yield. This compound gives upon β-elimination with triethylamine in ethyl acetate (10 min, 77°C), the desired compound 3 as a crystalline solid (65-70% overall yield, m.p. 112-113°C). A slight excess of compound 3, dissolved in THF, was carefully added to a pyridine solution of thymidine (4) or uridine (5) and a catalytic amount (0.01 eq.) of tetrabutylammonium hydroxide. After one hour at room temperature some Dowex cation exchanger (H^+-cycle) was added and the mixture was filtered and then evaporated. Flash chromatography afforded pure 6 or 7[22] in 90 and 80%, respectively. The O^4-(4-nitrophenylsulfonylethyl) group in 6 and 7 is stable towards concentrated aqueous or methanolic ammonia and 0.5 M DBU in dry pyridine for at least one hour at room temperature. Moreover, compound 7 was found completely unchanged under the conditions applied for the removal[18] of the 1,1,3,3-tetra-isopropyldisiloxane-1,3-di-yl (TIPS) protecting group. Complete deprotection of 6 and 7 to give back the starting nucleosides can be achieved within 2.5 hours by concentrated aqueous ammonia at 50-55°C. These conditions may seem quite severe as compared to those needed for the removal of the 2-methylsulfonylethyl group from phosphotriesters[19,20]. These results therefore clearly demonstrate that the lability of a β-functionalized protecting group not only depends on the acidity of the β-proton, but also on the leaving ability of the eliminated group in question (*i.e.* the difference in basicity of the $^{(-)}$O- on phosphate or on the pyrimidine ring system).



4 R=CH_3 , R'=H
5 R=H , R'=OH

6 R=CH_3 , R'=H
7 R=H , R'=OH

We inferred from infra-red spectra that alkylation occurred at the O^4-position, rather than at the N^3-position. The spectra of the two compounds 6 and 7 underwent a conspicuous change in their carbonyl region around 1700 cm^{-1}, when compared with the parent nucleosides 4 and 5. The broad and deep "trough" around 1690 cm^{-1}, (1600-1750) consisting of the symmetric and asymmetric stretching frequencies of the two carbonyl groups, the stretching frequency of the imido function (C=N) and the α,β-unsaturated site of the pyrimidinyl ring, were resolved into two bands (1710 cm^{-1} med. and 1660 cm^{-1} strong) following the "freezing" of the tautomeric form in the compounds 6 and 7. These two bands were assigned as ν(C=O) and ν(C=N), respectively.

As a final proof, compounds 6 and 7 were also synthesized *via* the recently published method of Reese and Skone[11]. Thus, 3',5'-O-diacetylthymidine and 2',3',5'-tri-O-acethyluridine were treated with tri-triazolyl phosphate/triazole/Et_3N to give the corresponding fully protected 4-triazolyl derivatives, which fluoresce beautifully on thin layer plates when exposed to long wave U.V. light (350 nm). Treatment of these compounds with 4 eq. of 2-(4-nitrophenylsulfonyl)-ethanol in CH_3CN/Et_3N for three days at 21°C or for 16 h at 80°C and subsequent hydrolysis of the acetyl groups (conc. NH_3/MeOH = 1:1) gave the compounds 6 and 7.

Probably due to the lower nucleophilicity of the hydroxylic function of the attacking β-functionalized ethanol which replaces the triazolyl group, the yields were rather modest. The obtained compounds proved however unambiguously to be identical (TLC, IR, ^1H-NMR) with 6 and 7 synthesized *via* the route proposed above.

REFERENCES AND NOTES

1. P.K. Bridson, W.T. Markiewicz and C.B. Reese, J. Chem. Soc. Chem. Commun. 1977, 447.
2. idem, J. Chem. Soc. Chem. Commun. 1977, 791.
3. C.B. Reese and A. Ubasawa, Tetrahedron Lett. 1980, 2265.
4. S.S. Jones, B. Rayner, C.B. Reese, A. Ubasawa and M. Ubasawa, Tetrahedron 36, 3075 (1980).
5. C.B. Reese, R.C. Titmas and L. Yau, Tetrahedron Lett. 1978, 2727.
6. J. Stawinski, T. Hozumi and S.A. Narang, Can. J. Chem. 54, 670 (1976).
7. H.P. Daskalow, M. Sekine and T. Hata, Bull. Chem. Soc. Jap. 54, 3076 (1981).
8. T. Kamimura, T. Masegi and T. Hata, Chem. Letters 1982, 965.
9. W.L. Sung, J. Org. Chem. 47, 3623 (1982).
10. S.S. Jones, C.B. Reese, S. Sibanda and A. Ubasawa, Tetrahedron Lett. 22, 4755 (1981).
11. C.B. Reese and P.A. Skone, J. Chem. Soc. Perk. Trans. I 6, 1263 (1984).
12. M. Sekine, K. Shinozaki, K-i Miura and T. Hata, J. Am. Chem. Soc. 106, 4552 (1984).
13. B.S. Schulz and W. Pfleiderer, Tetrahedron Lett. 24, 3587 (1983).
14. T. Kamimura, M. Tsuchiya, K. Urakami, S. Honda, M. Sekine and T. Hata, Chem. Letters 1983, 1051.

15. C.J. Welch and J. Chattopadhyaya, Act. Chem. Scand. B37, 147 (1983).
16. T. Kamimura, T. Masegi, M. Sekine and T. Hata, Tetrahedron Lett. 25, 4241 (1984).
17. G.I. Tesser and I.C. Balvert-Geers, Int. J. Pept. Prot. Res. 7, 295 (1975).
18. S. Honda, K. Terada, Y. Sato, M. Sekine and T. Hata, Chem. Letters 1982, 15.
19. C.A.A. Claesen, G.I. Tesser, C.E. Dreef, J.E. Marugg, G.A. van der Marel and J.H. van Boom, Tetrahedron Lett. 25, 1307 (1984).
20. A. Beld, C.A.A. Claesen, E.S. Roersma, W.J.M. Schippers, L.M. Keizer and G.I. Tesser, Recl. Trav. Chim. Pyas-Bas 103, 196 (1984).
21. H.S. Schultz, H.B. Freyermuth and S.R. Buc, J. Org. Chem. 28, 1140 (1963).
22. Compound 6: m.p. 140-142°C; mass spectrum (FAB^+): M^++1 = 456, M^++Na = 478; TLC (15% MeOH/CH_2Cl_2 v/v): Rf = 0.54; ^{1}H-NMR ($CDCl_3$/TMS): δ 1.89 (CH_3, d, ^4J = 0.9 Hz, 3H, coinciding with 2x OH), 2.32 (H_2', ^3J = 6.7 Hz, 2H), 3.62 (β-CH_2, t, ^3J = 6.4 Hz, 2H), 3.91 (H_5', t, ^3J = 1.5 Hz, 2H), 4.0 (H_4', m, 1H), 4.30 (α-CH_2, t, ^3J = 6.4 Hz, 2H), 4.55 (H_4', dt, ^3J = 3 Hz, 1H), 6.15 (H_1', t, ^3J = 6.7 Hz, 1H), 7.38 (H_6, d, ^4J = 0.9 Hz, 1H), 8.30 (aryl, AABB pattern, 4H).
Compound 7: m.p. 153-155°C; mass spectrum (FAB^+): M^++1 = 458; TLC (15% MeOH/CH_2Cl_2 v/v): Rf = 0.49; ^{1}H-NMR ($CDCl_3$/CD_3OD/TMS): δ 3.64 (β-CH_2, t, ^3J = 6.4 Hz, 2H), 3.80-4.15 (m, H_2', H_5', 3x OH, H_3', H_4', 8H), 4.23 (α-CH_2, t, ^3J = 6.4 Hz, 2H), 5.72 (H_5, d, ^3J = 8.2 Hz, 1H), 5.79 (H_1', d, ^3J = 4.1 Hz, 1H), 7.92 (H_6, d, ^3J = 8.2 Hz, 1H), 8.30 (aryl, AABB pattern, 4H).

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