A Convenient Method for the Synthesis of N-Free 5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyribonucleosides via the 5′-O-Selective Tritylation of the Parent Substances

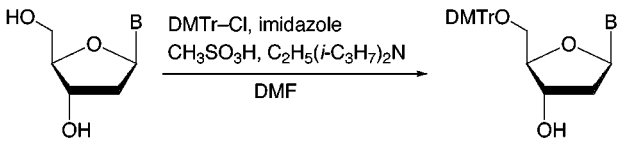
Masanori Kataoka and Yoshihiro Hayakawa*

Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Received January 11, 1999

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyribonucleosides without protection of the amino functions, **5**−**8**, are important substances for the synthesis of various kinds of nucleic acid-related derivatives. For example, these compounds are useful as building blocks for oligonucleotide synthesis via the hydroxyl-activation method.[1] They have also served as key intermediates of N-free nucleoside-3′-phosphoramidites[2,3] and -*H*-phosphonates,[4] which are monomer units for the synthesis of DNA oligomers via the N-unprotected approach. Among the N-free 5′-*O*-dimethoxytritylated nucleosides, the deoxyadenosine derivative **5**,[5] the deoxycytidine derivative **6**,[2,6] and the thymidine derivative **8**[7] can be prepared from the parent nucleosides in one step via 5′-O-selective tritylation. By contrast, there is no method for the direct preparation of the deoxyguanosine derivative **7** through the 5′-O-selective tritylation of 2′-deoxyguanosine, since tritylations of the 5′-hydroxyl and the amino function of the guanyl base occur at a similar rate. Thus, the formation of deoxyguanosine derivative **7** is currently performed via the following three steps:[2] (1) protection of the amino function of the guanyl base by a dimethylamidine group with (dimethoxy)(dimethylamino)methane in methanol,[8] (2) dimethoxytritylation of the 5′-hydroxyl with dimethoxytrityl chloride in pyridine, and (3) removal of the amidine protector with aqueous pyridine. This multistep process requires troublesome purification of the product in each step.[9] In addition, (dimethoxy)(dimethylamino)-methane employed in the first step is expensive. Therefore, development of a single-step preparation of **7** via 5′-O-selective tritylation of the parent nucleoside using an inexpensive reagent is desirable. This paper reports such a direct, economical method.

The 5′-O-selective dimethoxytritylation of 2′-deoxyguanosine (**3**) was conducted by the use of *p*,*p*′-dimethoxytrityl chloride (1.0 equiv) in the presence of a mixture of imidazolium mesylate (2.0 equiv) and diisopropylethylamine (2.0 equiv) (method A), a mixture of imidazole (2.0 equiv) and diisopropylethylammonium mesylate (2.0 equiv) (method B), or a 1:1:1 mixture of imidazole, diisopropylamine, and methanesulfonic acid (2.0 equiv each) (method C) in DMF (25 °C, 2 h). All methods showed similar results to give **7** in 74% to 78% isolated yield. This reaction yielded a small amount of the N-tritylated product (<5% yield), but this was easily removed by recrystallization during purification. The yield of **7** is higher than the 63% overall yield obtained by the above-mentioned multistep preparation.[2] This new regio- and chemoselective tritylation could be applied to other 2′-deoxyribonucleosides. For example, the reaction of 2′-deoxyadenosine (**1**) was carried out under similar conditions and gave the 5′-O-tritylated product **5** in a 79% to 86% yield, which was higher than that of the reported method (77%).[5] Similarly, the reaction of 2′-deoxycytidine (**2**) and thymidine (**4**) gave **6** and **8** in yields of 76% to 85% (versus 70% in the existing method[6]) and 82% to 89% (versus 85%[7]), respectively.



1, B = Ade
2, B = Cyt
3, B = Gua
4, B = Thy

5, B = Ade
6, B = Cyt
7, B = Gua
8, B = Thy

DMTr = $C_6H_5(p\text{-}CH_3OC_6H_4)_2C$

The reaction of deoxyguanosine and dimethoxytrityl chloride, using 1 equiv each of imidazole, methanesulfonic acid, and diisopropylethylamine, showed higher O-selectivity, giving no detectable N-tritylated product, while the desired product **7** was obtained in only 46% yield. The attempt using 2 equiv of imidazolium mesylate in the absence of diisopropylethylamine as the additive provided **7** in ca. 10% yield, and a considerable amount of unreacted deoxyguanosine was recovered after 24 h. The trial with imidazole (4 equiv) but without methanesulfonic acid and diisopropylethylamine gave a complex mixture, including the starting nucleoside, **7**, N-tritylated product, and a bis-N,O-tritylated product. No tritylation took place in the presence of only diisopropylethylammonium mesylate (2 equiv) as the additive. In method A, the use of imidazolium triflate,[2] imidazolium tosylate, benzimidazolium mesylate, or benzimidazolium triflate[10] in place of imidazolium mesylate was not effective. Further, triethylamine or pyridine in place of diisopropylethylamine could not be used in all cases. DMSO,

(1) (a) Hayakawa, Y.; Wakabayashi, S.; Nobori, T.; Noyori, R. *Tetrahedron Lett.* **1987**, *28*, 2259−2262. See also, (b) Hayakawa, Y.; Uchiyama, M., Noyori, R. *ibid.* **1984**, *25*, 4003−4006. (c) Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373−379 and references therein.

(2) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395−12401.

(3) (a) Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 1879−1882. (b) Gryaznov, S. M.; Letsinger, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 5846−5877. (c) Fourrey, J.-L.; Varenne, J. *Tetrahedron Lett.* **1985**, *26*, 2663−2666.

(4) (a) Wada, T.; Sato., Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710−12721. (b) Kung, P. P.; Jones, R. A. *Tetrahedron Lett.* **1992**, *33*, 5869−5872.

(5) Ti, G. S.; Gaffney, B. L.; Jones, A. R. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.

(6) Ishido, Y. *Jpn. Kokai Tokkyo Koho* Jp. Patent 01 303 294 [89 308 294], 1989; *Chem. Abstr.* **1990**, *112*, 700.

(7) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.

(8) (a) Vu, H.; McCollum, C.; Jacobson, K.; Theisen, P. *Tetrahedron Lett.* **1990**, *31*, 7269−7272. (b) McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040−2048.

(9) A multistep synthesis using an isobutyryl group in place of the amidine group for the protection of the nucleoside base has been also reported in ref 4, but this method gives the target product in a lower yield than does the amidine-protected method.

(10) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996−7997.

10.1021/jo990051i CCC: $18.00
Published on Web 07/14/1999

THF, dioxane, dichloromethane, and pyridine were not useful as the solvent. These results indicated that the use of imidazole is essential for the reaction, and thus it is conceivable that dimethoxytritylimidazolide is intermediately produced from dimethoxytrityl chloride and imidazole to act as the true tritylating agent. However, ^{1}H NMR analysis of the reaction of dimethoxytrityl chloride (1 equiv) and imidazole (2 equiv) in the presence of diisopropylethylammonium mesylate (2 equiv) in DMF-d_7 showed no signals supporting the intervention of dimethoxytritylimidazolide. This reaction formed a complex mixture, and the addition of a nucleoside to this mixture gave no desired tritylation product. These results also suggest that the presence of equimolar amounts of methanesulfonic acid and diisopropylethylamine is important to obtain good chemoselectivity. This mixture might create a certain buffering effect favorable for gaining the desirable chemoselectivity, but the effect has not been fully elucidated. Thus, although the individual roles of the additives have not yet been exactly defined, we know that the use of 2 equiv each of imidazole, methanesulfonic acid, and diisopropylethylamine toward a nucleoside and dimethoxytrityl chloride in DMF is crucial for obtaining good chemoselectivity and high product yield.[11]

In summary, we have developed a general, convenient method for 5′-O-selective dimethoxytritylation of 2′-deoxyribonucleosides to produce N-free 5′-O-protected derivatives. This approach is particularly useful for the preparation of the deoxyguanosine derivative **7**, whose production currently requires expensive reagents and a multistep process.

Experimental Section

Materials. E. Merck Kieselgel 60 (70−230 mesh) deactivated by adding 6% water was used for column chromatography. DMF was distilled from CaH_2 under reduced pressure. The solvents for chromatography were used after simple distillation of the commercially available solvents. Commercially supplied imidazole (Nacalai Tesque), methanesulfonic acid (Tokyo Chemical Industry), diisopropylethylamine (Tokyo Chemical Industry), 2′-deoxyribonucleosides (Yamasa), and *p*,*p*′-dimethoxytrityl chloride (Tokyo Chemical Industry) were used without further purification.

Imidazolium Mesylate. To a solution of imidazole (34.0 g, 0.499 mol) in dichloromethane (500 mL) was added dropwise methanesulfonic acid (48.0 g, 32.4 mL, 0.499 mol) at 0 °C. The resulting precipitate was collected by filtration and washed with dichloromethane (100 mL) to give imidazolium mesylate as a colorless powder (81.1 g, 99%), mp 184−186 °C. IR (KBr): 3140, 1589, 1460, 1437, 1339, 1194 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H), 7.62 (s, 2H), 9.03 (s, 1H). ^{13}C NMR 40.1, 119.7, 134.8. Anal. Calcd for $C_4H_8N_2O_3S$: C, 29.26; H, 4.91; N, 17.06. Found: C, 29.06; H, 4.90; N, 17.15.

Diisopropylethylammonium Mesylate. To a solution of diisopropylethylamine (64.4 g, 500 mmol) in dichloromethane (50 mL) was added methanesulfonic acid (48.1 g, 32.4 mL, 500 mmol) at room temperature. The mixture was concentrated and dried under reduced pressure to afford diisopropylethylammonium mesylate as a colorless solid (112.5 g, 100%). This material was hygroscopic and the melting point could not be measured: IR (KBr) 2668, 1649, 1427, 1395, 1318 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, J = 7.3 Hz), 1.37 (d, 12H, J = 6.3 Hz), 2.46 (s, 3H), 3.23 (q, 2H, J = 7.3 Hz), 3.70 (seven lines, 2H, J = 6.3 Hz), 8.88 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 12.7, 16.9, 18.3, 40.0, 42.8, 54.5. Anal. Calcd for $C_9H_{23}NO_3S$: C, 47.97; H, 10.29; N, 6.22. Found: C, 47.83; H, 10.44; N, 6.19.

A Typical Procedure for 5′-O-Selective Tritylation of Deoxyribonucleosides. Method A. To a suspension of a deoxyribonucleoside (40.0 mmol), imidazolium mesylate (13.1 g, 80.0 mmol), and diisopropylethylamine (10.3 g, 13.9 mL, 80.0 mmol) in dry DMF (200 mL) was added *p*,*p*′-dimethoxytrityl chloride (13.6 g, 40.0 mmol) in three portions at room temperature. The reaction mixture was stirred for 2 h. During this period, the mixture became homogeneous. The resulting solution was poured into water (3 L), and the resulting precipitate was collected by filtration. The crude product was recrystallized or subjected to silica gel column chromatography to give a 5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyribonucleoside as crystals or an amorphous solid.

Method B. To a suspension of a deoxyribonucleoside (40.0 mmol), imidazole (5.45 g, 80.0 mmol), and diisopropylethylammonium mesylate (18.0 g, 80.0 mmol) in dry DMF (200 mL) was added *p*,*p*′-dimethoxytrityl chloride (13.6 g, 40.0 mmol) in three portions at room temperature, and the resulting mixture was stirred for 2 h. The reaction mixture was poured into water (3 L). The resulting precipitate was purified as described above to give a 5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyribonucleoside as crystals or an amorphous solid.

Method C. To a stirred solution of imidazole (5.45 g, 80.0 mmol), methanesulfonic acid (5.19 mL, 7.69 g, 80.0 mmol), and diisopropylethylamine (13.9 mL, 10.3 g, 80.0 mmol) in dry DMF (100 mL) were successively added a deoxyribonucleoside (40.0 mmol) in three portions and *p*,*p*′-dimethoxytrityl chloride (13.6 g, 40.0 mmol) in three portions at room temperature. Stirring was continued for 2 h. The resulting homogeneous mixture was poured into water (3 L) to give a precipitate, which was recrystallized or subjected to silica gel column chromatography to give a 5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyribonucleoside.

The reaction on a 4.00 mmol scale was carried out in a similar manner. Purification conditions and yield of the product are as follows.

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyadenosine (5). Silica gel column chromatography of the crude product of a 40 mmol synthesis with a 9:1 mixture of ethyl acetate and hexane as the eluent afforded **5** (17.5 g, 79% yield) as a colorless amorphous solid. This product showed IR, UV, and ^{1}H and ^{13}C NMR spectral data identical to those of an authentic sample of **5**.[2,4] When the reaction was carried out on 4 mmol scale, **5** was obtained in an 86% yield (1.90 g).

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxycytidine (6). Column chromatography of the crude product obtained in a 40 mmol scale preparation on silica gel eluted with a 1:20 mixture of methanol and dichloromethane gave **6** (16.1 g, 76% yield) as a colorless powder. The IR, UV, and ^{1}H and ^{13}C NMR spectral data of this compound were identical with those of an authentic sample of **6**.[2] The 4 mmol scale synthesis gave **6** in an 85% yield (1.94 g).

5′-*O*-(*p*,*p*′-Dimethoxytrityl)-2′-deoxyguanosine (7). Recrystallization of the crude precipitate from a mixture of ethyl acetate and acetone gave **7** (17.8 g, 78% yield; 40 mmol synthesis) as colorless needles, mp 190−191 °C, which indicated IR, UV, and ^{1}H and ^{13}C NMR spectra superimposable on those of an authentic sample of **7**.[2] The yield of **7** in the synthesis on a 4 mmol scale was 74% (1.56 g).

5′-*O*-(*p*,*p*′-Dimethoxytrityl)thymidine (8). Silica gel column chromatography of the crude product of 40 mmol synthesis, eluting with a 3:1 mixture of ethyl acetate and hexane, afforded **8** (17.9 g, 82% yield) as a colorless amorphous solid. This product is identical in all respects with the commercially supplied material. The product **8** was obtained in an 89% yield (1.94 g) in the preparation on a 4.00 mmol scale.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 08454200, 10169225, and 10554042) from the Ministry of Education, Science, Sports and Culture, by a grant from the "Research for the Future" Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301), and

(11) Preparation of N-free 5′-*O*-monomethoxytrityl- and 5′-*O*-trityl-2′-deoxyribonucleosides was similarly achieved via the direct, chemoselective reaction of the parent substances using *p*-methoxytrityl chloride or trityl chloride, respectively.

by contributions from the Asahi Glass Foundation and the Daiko Foundation.

Supporting Information Available: Characterization data including IR, ^{1}H NMR, and ^{13}C NMR spectral charts for imidazolium mesylate and diisopropylethylammonium mesylate, which are new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO990051I