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A Convenient Preparation of *N*-Protected Nucleosides with the 2,2,2-Trichloro-*t*-butyloxycarbonyl (TCBOC) Group. Structural Assignment of *N,N-bis*-TCBOC Guanoside and Its 2′-Deoxy Analogue

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TCBOC protected derivatives of nucleosides have been prepared using a "one-pot" procedure involving protection of the hydroxyl functions with trimethylsilyl chloride. Spectroscopic studies showed that the TCBOC groups of the guanine moiety of *bis*-TCBOC-guanosine and its 2′-deoxy analogue are at N^1 and at the N^2 positions respectively; while in the mono-TCBOC derivatives, the TCBOC group is, as expected, at the N^2 position.

The 2,2,2-trichloro-*t*-butyloxycarbonyl- (TCBOC) group was proposed[1] for the protection of the exocyclic amino function of adenosine and for the protection of the urethane function of uridine.[2] Subsequently, the TCBOC-protected derivatives of cytidine and guanosine and their 2′-deoxy analogues have also been prepared.[3] Both groups employed a two-step procedure for the preparation of these base-protected nucleosides which made the employment of TCBOC group in oligoribo- and deoxyribonucleotide synthesis time consuming. It may be noted that uridine, cytidine and adenosine, both in deoxyribo and ribo series, gave only *mono*-TCBOC derivatives; while guanosine and 2′-deoxyguanosine[3] gave *bis*-TCBOC derivatives. The structures of these *bis*-TCBOC derivatives of guanosine and its 2′-deoxy analogue were not clearly elucidated; nevertheless, evidence was advanced to support that the second TCBOC group could be at the N^1 and *not* at the O^6 position. We herein describe a simple and general procedure for the "one-step" preparation of TCBOC protected nucleosides with free sugar hydroxyl groups and subsequently, we present direct spectroscopic arguments for the chemical structure of *bis*-TCBOC-guanosine and its 2′-deoxy analogue.

The general procedure for the "one-pot" synthesis of the TCBOC-protected nucleosides, *1* to *10*, involved trimethylsilylation of a nucleoside in dry pyridine solution at 20 °C, which is followed by the addition of TCBOC-Cl *in situ* and then hydrolysis. Standard work-up and crystallization from aqueous ethanol gave compound *1* to *10* in 60–95 % yield respectively (experimental section). Element analysis and spectroscopic properties of these newly synthesized compounds were identical to the authentic ones.[1–3] We believe that such a

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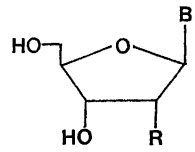


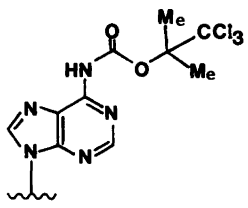
1, R=OH; B=A^{TCBOC}
2, R=H; B=A^{TCBOC}
3, R=OH; B=C^{TCBOC}
4, R=H; B=C^{TCBOC}
5, R=OH; B=U^{TCBOC}
6, R=H; B=T^{TCBOC}
7, R=OH; B=G^{TCBOC}
8, R=H; B=G^{TCBOC}
9, R=OH; B=$G^{(TCBOC)_2}$
10, R=H; B=$G^{(TCBOC)_2}$

"one-step" preparation of TCBOC-protected nucleosides, *1* to *10*, would promote their applications in the areas of DNA and tRNA synthesis.

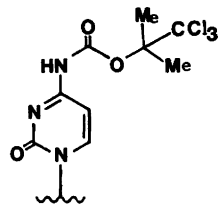
We then elucidated the position of the second TCBOC group in *9* and *10*, in comparison with the corresponding *mono*-TCBOC derivatives *7* and *8*, respectively. A perusal of their ultraviolet absorption spectra at pH 2, 7 and 13 show that the compounds *7* and *8* absorb at 257 and 278 nm(sh). While the former band has a bathochromic shift (*ca.* 12 nm) in the alkaline pH, the latter undergoes a hypsochromic shift. Compounds *9* and *10*, on the other hand, have absorptions at 255 and 245 nm(sh) at pH 2 and 7. These bands undergo a much smaller bathochromic shift in alkaline pH (4 to 5 nm). A comparison of infrared spectra of compounds *9* and *10* with *7* and *8* respectively, shows that the stretching frequencies of the conjugated carbonyl groups originate, in the latter, in the region of 1710–1690 cm^{-1}, while in the former, one observes a non-conjugated carbonyl stretching frequency at 1820 cm^{-1} beside other conjugated carbonyl frequencies (1720–1680 cm^{-1}). It may be added that such a small high frequency shift of carbonyl frequency has been also observed[5] upon the protonation of the N^7-nitrogen of the guanine residue. A comparison of ^{13}C chemical shifts of compounds *7* to *10* with guanosine and deoxyguanosine (Table 1) show that the chemical shifts of the C^6-carbon atoms were very similar, suggesting that the nature of the sp^2 hybridized carbon in these compounds have remained unchanged. However, a comparison of the chemical shifts of the C^2-carbons in guanosine and its *mono*- and *bis*-TCBOC derivatives has revealed that the C^2-carbon is more shielded by 1.9 and 10.5 ppm in *7* and *9*, respectively, in comparison with its chemical shift in guanosine. A similar correlation is also observed for the chemical shift of the C^2-carbon in comparison with the ^{13}C spectra of 2′-deoxyguanosine and its *mono*- and *bis*-TCBOC derivatives. It is clear that the attachment of an acyl group to the *ring* nitrogen would appear to favour localization of *ring* π-electrons in the various double bonds within the ring which is very similar to a situation that exists in the case of a protonation. Such a localization effect, due to N^1-acylation, would be expected, in an analogy with the "α-protonation effect"[6], to be most pronounced on the C^2-carbon as seen in its shielding (ca. 10.5 ppm) in the derivatives *9* and *10* as compared to *7* and *8* respectively; while the small change of the chemical shifts of the C^6-carbon can be attributed to its overall non-conjugated nature from the rest of ring π-electrons.

These spectroscopic properties, along with the observed stability towards alkali, of the *bis*-TCBOC derivatives of guanosine and deoxyguanosine, *9* and *10*, are consistent with their 1,2-*bis*-*N*-TCBOC structures, *11* and *12*, respectively.

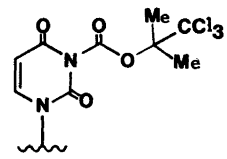
Further indirect evidence for the bis-TCBOC structures, *11* and *12*, has emerged by the ^1H NMR study of the exchangeable protons of the aglycones. Such a study has shown that the N^1 and N^2 protons of *7* and *8*, in anhydrous deuterated dimethylsulfoxide absorb at δ 11.48 and 11.38 respectively. These protons are shifted to δ 11.22 and 10.5 respectively upon warming the solutions to 70 °C; these chemical shifts remained unchanged upon returning to



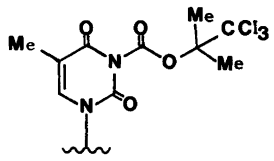
$_{A}$TCBOC



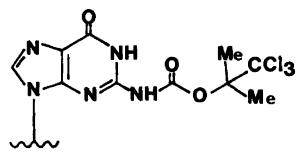
$_{C}$TCBOC



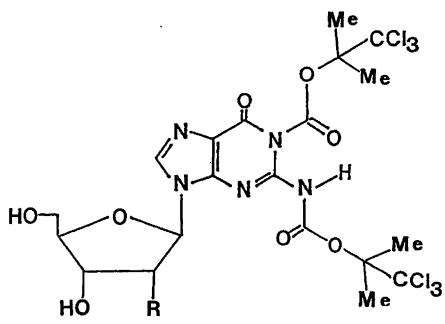
$_{U}$TCBOC



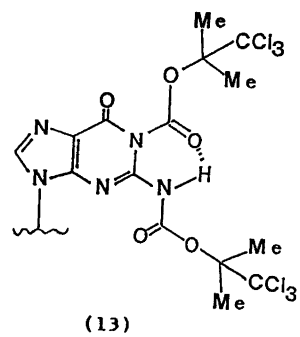
$_{T}$TCBOC



$_{G}$TCBOC



(11) R = OH
(12) R = H



(13)

the ambient temperature. In contrast, the N–H proton of the *bis*-TCBOC derivatives, *9* and *10*, absorbed at δ 13.0 which, upon warming at 70 °C, shifted to the high field region where all other exchangeable sugar protons absorb (above δ 6.0): Upon cooling to the ambient temperature, the N–H proton reverted to the original chemical shift. This suggests that there is very little hydrogen bonding between N^1H and N^2-acyl function in *7* and *8*; on the other hand, a strong hydrogen bonded structure for the *bis*-TCBOC derivatives, like *13*, seems to be possible.

EXPERIMENTAL

^{1}H NMR spectra were measured (δ scale) at 60 MHz with a Perkin-Elmer R 600 and at 90 MHz with a Jeol FX 90Q spectrometer using tetramethylsilane as an internal standard. ^{13}C NMR spectra were recorded at 23.7 MHz in the same solvent mixture. UV absorption spectra were recorded with a Cary 2200 spectrophotometer in pure methanol. Reactions were monitored by using Merck pre-coated silica gel 60 F_{254} plates. 2,2,2-Trichloro-*tert.*-butyloxychloroformate (TCBOC–Cl) was prepared using a literature procedure.[1,3]

Table 1. ^{13}C-absorptions from the base residues (δ scale).

	C-6	C-2	C-4	C-8	C-5
Guanosine	157.12	153.7	151.4 (4.5 Hz)	136.0 (214 Hz)	116.6 (11.2 Hz)
(*7*)	155.2	151.8	148.9 (2 Hz)	137.9 (215.6 Hz)	120.0 (11.2 Hz)
(*9*)	156.7	143.2	147.1 (1.5 Hz)	139.6 (212.3 Hz)	123.5 (11.2 Hz)
2'-Deoxyguanosine	157.4	153.8	151.1 (1 Hz)	136.0 (214.6 Hz)	116.6 (11.2 Hz)
(*8*)	155.2	151.8	148.4 (1.5 Hz)	137.7 (211.2 Hz)	120.0 (12.3 Hz)
(*10*)	156.8	143.0	146.8 (2.5 Hz)	139.5 (213.0 Hz)	123.7 11.2 Hz)

Preparation of 6-N-(2,2,2-trichloro-t-butyloxycarbonyl)adenosine (1) A general procedure: Adenosine (1.34g, 5 mmol) was dried by co-evaporation with dry pyridine (2 × *ca.* 10 ml) and was then re-dissolved in the same solvent (50 ml). To this solution was added trimethylsilyl chloride (10 eq. per mmol of Nucleoside) under an atmosphere of argon at 20 °C. The reaction was monitored by TLC using 30 % ethanol–chloroform mixture as an eluent. When TLC showed the formation of a single higher R_f product, TCBOC–Cl (1.25 g, 5.2 mmol) was added. After 3h at 20 °C, TLC (5 % ethanol–chloroform mixture) showed a complete conversion to a higher R_f product. Water (2.0 ml) was added. After about 30 min, a TLC analysis (20 % ethanol–chloroform mixture) revealed a mixture of two compounds in the reaction mixture. Aqueous ammonia (d 0.9) was added to adjust the pH to 9–10. Water (20 ml) was added after 30 min stirring; the reaction mixture was subsequently extracted with chloroform (3 × 20 ml). The organic layers were pooled, dried ($MgSO_4$) and concentrated on a rotavapor to a gum. The residue was first dissolved in a volume of pyridine (*ca.* 0.2 ml) and then taken up in chloroform (*ca.* 1.5 ml). This solution was subsequently precipitated from light petroleum. The precipitate was subsequently crystallized from 20 % ethanol–water mixture. Yield 2.14 g (91.2 %); mp. 162 °C; UV: λ_{max} 267 nm (ε 17000) (pH 7); 268 nm (ε 13500) (pH 2); 294 nm (ε 17500) (pH 13). IR (nujol): 1750 cm^{-1}; ^{1}H NMR ($CDCl_3$): 8.67 (*s*, 1H); 8.2 (*s*, 1H); 5.92 (*d*, 7 Hz, 1H); 4.85 (*m*, 1H); 4.38 (*m*, 2H); 3.88 (*m*, 2H), 2.03 (*s*, 6H).

Compound (2). This was prepared using essentially similar procedures described for the compound *1*. A precipitate of *2* was crystallized from *ca.* 15 % aqueous ethanol in 82.3 % yield. mp. 168 °C.

UV: λ_{max} 267 nm (ε 17500) (pH 7); 268 nm (ε 14000) (pH 2); 294 nm (ε 18000) (pH 13); IR (nujol): 1755 cm^{-1}; ^{1}H NMR ($CDCl_3$): 8.73 (s, 1H); 8.20 (s, 1H); 6.42 (*dd*, 6 Hz, 1H); 4.7 (*m*, 1H); 4.19 (*m*, 1H); 3.88 (*m*, 2H); 2.68 (*m*, 2H); 2.05 (*s*, 6H).

Compound (3). Essentially, a similar reaction condition as for *1* was used for the preparation of *3*; however the solubility of *3* allowed it to be dissolved in a small volume of chloroform for precipitation in light petroleum. The precipitate was crystallized from *ca.* 5 % ethanol–chloroform mixture in 95.5 % yield. mp. 125 °C.

UV: λ_{max} 295 nm (ε 8000), 241 nm (ε 15500) (pH 7); 295 nm (ε 7200), 241 nm (ε 12000) (pH 2); 293 nm (ε 17000) (pH 13). IR (nujol): 1755 cm^{-1}. ^{1}H NMR ($CDCl_3$): 8.49 (*d*, 8 Hz, 1H); 7.25 (*d*, 8 Hz, 1H); 5.75 (*s*, 1H); 4.21 (*bs*, 3H); 3.91 (*m*, 2H); 1.98 (*s*, 6H).

Compound: (4). This was also prepared using a reaction condition as for compound *1*; however, the solubility properties permitted it to be dissolved in a small volume of chloroform for precipitation in light petroleum. The precipitate was subsequently crystallized from *ca.* 5 % ethanol–chloroform mixture in 93 % yield. mp. 155 °C.

UV: λ_{max} 294 nm (ε 7000), 240 nm (ε 13500) (pH 7); 295 nm (ε 6300), 240 nm (ε 10500) (pH 2); 293 nm (ε 14500) (pH 13). IR (nujol): 1750 cm^{-1}. ^1H NMR ($CDCl_3$): 8.29 (*d*, 8.2 Hz, 1H); 7.16 (*d*, 8.2 Hz, 1H); 6.12 (*dd*, 6 Hz, 1H); 4.36 (*m*, 1H); 4.30 (*m*, 1H); 3.84 (*m*, 2H); 2.50 (m, 2H); 1.94 (s, 6H).

Compound: (5). This has been prepared using a very similar condition as reported for the preparation of *1* except that the TCBOC–Cl reaction time is around 24 h. The precipitate has been crystallized from *ca.* 5 % ethanol–chloroform mixture in 89 % yield. mp. 136 °C. UV: λ_{max} 263 nm (ε 8500), (pH 7), 262 nm (ε 8000) (pH 2); 265 nm (ε 5000) (pH 13). IR (nujol): 1785, 1720 and 1680 cm^{-1}. ^1H NMR ($CDCl_3$): 8.17 (*d*, 8.2 Hz, 1H); 6.0 (*d*, 8.2 Hz, 1H); 6.0 (*d*, 4Hz, 1H); 4.44 (*m*, 3H); 4.09 (*m*, 2H); 2.31 (*s*, 6H).

Compound: (6). This has been prepared using a very similar condition as reported for the preparation of *1* except that TCBOC–Cl reaction time is around 24 h. The precipitate has been crystallized from *ca.* 5 % ethanol–chloroform mixture in 88 % yield, mp. 132 °C. UV: λ_{max} 270 nm (ε 9500), (pH 7); 270 nm (ε 8000) (pH 2): 270 nm (ε 8000) (pH 13). IR (nujol): 1795, 1715 and 1660 cm^{-1}. ^1H NMR ($CDCl_3$): 7.61 (*bs*, 1H); 6.17 (*dd*, 6.9 Hz, 1H); 4.37 (*m*, 1H); 3.80 (*m*, 3H); 2.25 (*m*, 2H); 2.07 (*s*, 6H); 1.93 (*bs*, 3H).

Compound (7). Same procedure as for the preparation of *1* except that 1.05 equiv. of TCBOC–Cl was used for the reaction for 3 h. The precipitate was crystallized from *ca.* 10 % ethanol–water mixture in 72 % yield. mp. 224 °C. UV: λ_{max} 257 nm (ε 15000), 278 nm (ε 10300) (pH 7); 257 nm (ε 1400), 278 nm (ε 9600) (pH 2); 268 nm (12800) (pH 13). IR (nujol): 1710 and 1695 cm^{-1}. ^1H NMR (DMSO-d_6): 8.26 (*s*, 1H); 5.83 (*d*, 6.1 Hz, 1H); 4.50 (*m*, 1H); 4.05 (*m*, 4H); 1.95 (*s*, 6H).

Compound (8). Same procedure as for compound *7* was used. The precipitate was crystallized from *ca.* 10 % ethanol–water mixture in 60 % yield. mp. 214 °C. UV: λ_{max} 257 nm (ε 16000), 278 nm (ε 11300) (pH 7); 257 nm (ε 12300), 278 nm (ε 9800) (pH 2); 268 nm (ε 14600) (pH 13). IR (nujol): 1705 and 1690 cm^{-1}. ^1H NMR (DMSO-d_6): 8.22 (*s*, 1H); 6.22 (*dd*, 6.2 Hz, 1H); 4.46 (*m*, 1H); 4.0 (*m*, 3H); 2.52 (*m*, 2H); 1.94 (*s*, 6H).

Compound (9). Same procedure as for the preparation of *1* was used except that 2.5 equiv. of TCBOC–Cl was used for reaction for *ca.* 16 h and then a hydrolysis step with water. The precipitate was crystallized from *ca.* 25 % ethanol–water mixture in 90 % yield. mp. 252 °C. UV: λ_{max} 252 nm (ε 7700), 244 nm (sh) (pH 7); 252 nm (ε 6500), 244 nm (sh) (pH 2); 256 nm (ε 7500) (pH 13). IR (nujol): 1820, 1705 and 1695 cm^{-1}. ^1H NMR (DMSO-d_6): 8.37 (*s*, 1H); 5.78 (*d*, 6 Hz, 1H); 4.44 (*dd*, 6 Hz, 1H); 3.96 (*m*, 2H); 3.66 (*m*, 2H); 1,90 (*s*, 12H).

Compound (10). Same procedure as for compound *9* was used. The precipitate was crystallized from *ca.* 25 % ethanol–water mixture in 88 % yield. mp. 234 °C.

UV: λ_{max} 252 nm (ε 7600), 245 nm (sh) (pH 7); 252 nm (ε 6400), 244 nm (sh) (pH 2); 257 nm (ε 7400) (pH 13). IR (nujol): 1820, 1720 and 1705 cm^{-1}. ^1H NMR (DMSO-d_6): 8.30 (*s*, 1H); 6.22 (*dd*, 7.2 Hz, 1H); 4.36 (*m*, 1H); 3.78 (*m*, 3H); 2.52 (*m*, 2H); 1.88 (*s*, 12H).

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