8,5′-*O*-Cyclonucleosides of Tubercidin

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On treatment with NBS, 2′,3′-*O*-benzylidenetubercidin (**1**) and its isopropylidene analogue (**6**) afforded the 8,5′-*O*-cyclonucleosides **2** and **7** respectively. Catalytic hydrogenation of **2** and **7** afforded the dehalogenated compounds, **9** and **12**.

Conversion of tubercidin[1] to 5′-deoxytubercidin[2] has already been accomplished by the present authors, and 3′-deoxytubercidin has also been synthesized by Robins and his coworkers.[3] We wish to report here the reaction of NBS with 2′,3′-*O*-benzylidenetubercidin (**1**). This work was initially attempted to obtain the 2′(or 3′)-bromo derivative of 3′(or 2′)-*O*-benzoyltubercidin, which might be used as an intermediate in the synthesis of 2′(or 3′)-deoxytubercidin. Ponpipom and Hanessian have already succeeded[4] in the conversion of 2′,3′-*O*-benzylideneuridine into 3′-*O*-benzoyl-5, 2′-dibromo-2′-deoxyuridine; analogously, we carried out the reaction on **1**.

2′,3′-*O*-Benzylidenetubercidin (**1**), shown to be a diastereomeric mixture by a study of its NMR spectrum, was treated with 1.5 equivalent mol of NBS in a mixture of carbon tetrachloride and tetrachloroethane (1 : 1) under reflux. The reaction seemed to proceed with serious decomposition, and from the reaction mixture two products were isolated in low yields. The minor one was identified as 7-bromo-2′,3′-*O*-benzylidenetubercidin (**5**), obtained as a mixture of diastereomers.

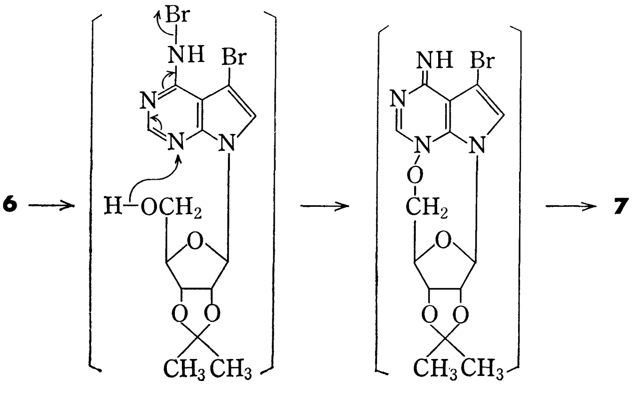
The main product, which was obtained in a 36% yield, was identified as 8,5′-*O*-anhydro-7-bromo-8-hydroxy-2′,3′-*O*-benzylidenetubercidin (**2**). Though the isolated sample showed a single spot on tlc, it was found to be a diastereomeric mixture as shown by the NMR spectrum; we succeeded in obtaining one diastereomer by careful crystallization.

The molecular formula, $C_{18}H_{15}N_4O_4Br$, suggested that the substitution with two bromine atoms, followed by the loss of one mole of hydrogen bromide, afforded the product. In the IR spectrum no absorption was observed in the range of 1660—2900 cm^{-1}, showing that it was not a benzoyl ester as had first been expected. Moreover, it was stable in *n*-propanol–1 M NaOH (4 : 1) on prolonged heating; thus, the benzoyl-ester structure is also unlikely. The NMR spectrum exhibited signals of the ABX type assignable to two hydrogens at C-5′ (δ 4.04 and δ 4.54, $J_{\text{gem}}=12$ Hz, $J_{4',5'\text{a}}=1$ Hz, $J_{4',5'\text{b}}=2$ Hz), showing that it was a cyclonucleoside. A signal corresponding to a hydrogen at C–8 in the starting material was lacking. The mass spectrum showed the parent peaks at *m/e* 432 ($C_{18}H_{15}N_4O_4{}^{81}Br$) and *m/e* 430 ($C_{18}\ H_{15}N_4O_4{}^{79}Br$) as well as four intense peaks corresponding to the ion species, **3b** (*m/e* 230), **4b** (*m/e* 229), **3a** (*m/e* 228) and **4a** (*m/e* 227).

Catalytic reduction of **2** afforded the dehalogenated compound **9**. The mass spectrum of **9** showed the parent peak at *m/e* 352 as well as two intense peaks at *m/e* 150 and *m/e* 149 corresponding to the ion species **10** and **11**.

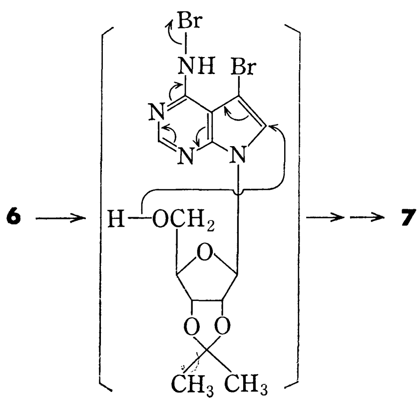
2′,3′-*O*-Isopropylidenetubercidin (**6**) similarly reacted with NBS to give 8,5′-*O*-anhydro-7-bromo-8-hydroxy-2′,3′-*O*-isopropylidenetubercidin (**7**). When the reaction was carried out at room temperature in a mixture of acetone and methylene chloride (1 : 1), with the molar ratio of NBS to **6** being 1.1, the product was a gummy material, from which **7** was isolated in a 7% yield. The formation of 7-bromo–2′,3′-*O*-isopropylidenetubercidin was also observed, though it was difficult to purify (See Exp). A few further attempts to improve the yield of **7** were made (See Exp.), but we now feel that it is a fruitless work.

The reaction path to give **7** may involve the dibromide **14** and the 3,5′-*O*-cyclonucleoside **15** as the intermediates (Scheme 1). In connection with this mechanism, conversion of adenine 3*N*-oxide and their xanthine and guanine analogues into the 8-oxygenated purines in acetic anhydride has been reported.[5] However, another possible path, which does not involve **15**, can not be excluded (Scheme 2).



14   **15**

Scheme 1



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Scheme 2

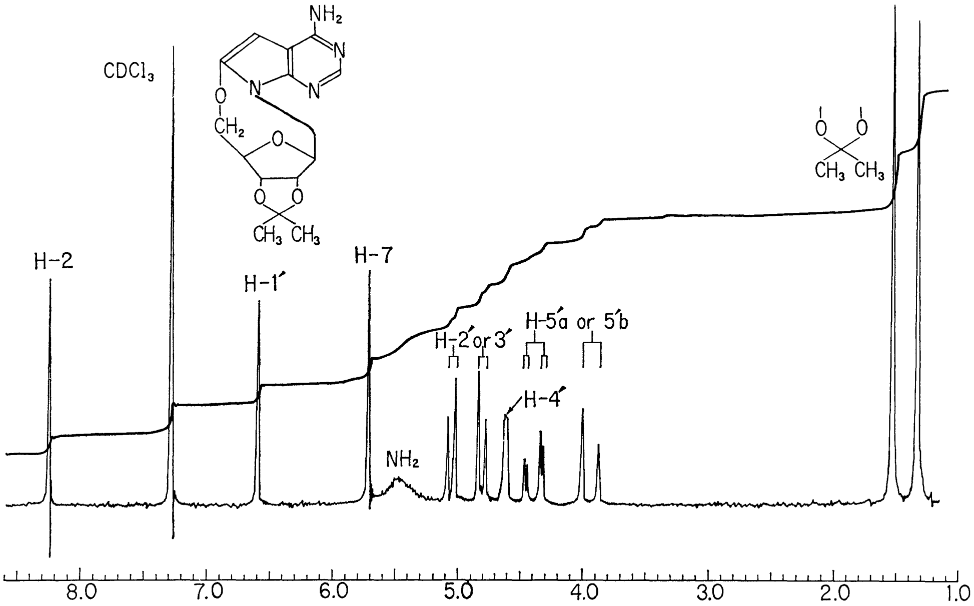
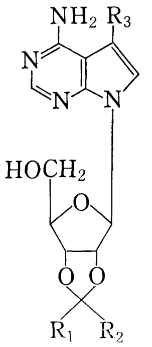


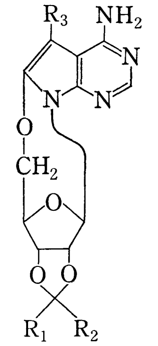
Fig. 1. The NMR spectrum of 8,5'-O-anhydro-2',3'-O-isopropylidenetubercidin (**12**): 100 MHz, CDCl$_3$.

Catalytic reduction of **7** afforded the dehalogenated compound **12**, the structure of which was also supported by its NMR spectrum (Fig. 1).

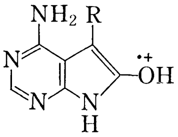
Treatment of **12** with excess *p*-toluoyl chloride afforded the di-*p*-toluoyl derivative, **13**, the NMR spectrum of which exhibited a six-proton singlet at δ 2.32 corresponding to two methyls of *p*-toluoyl groups and a pair of four-proton doublets at δ 7.24 and δ 7.67 (J=8 Hz), supporting the N^6,N^6-diacyl structure[6] in **13**.



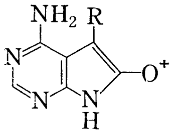
1, $R_1=H$, $R_2=C_6H_5$, $R_3=H$
5, $R_1=H$, $R_2=C_6H_5$, $R_3=Br$
6, R_1, $R_2=CH_3$, $R_3=H$
8, R_1, $R_2=CH_3$, $R_3=Br$



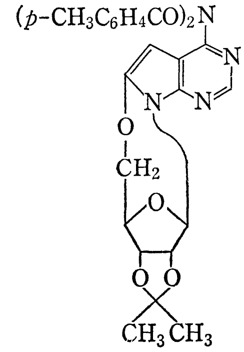
2, $R_1=H$, $R_2=C_6H_5$, $R_3=Br$
7, $R_1=CH_3$, $R_2=CH_3$, $R_3=Br$
9, $R_1=H$, $R_2=C_6H_5$, $R_3=H$
12, $R_1=CH_3$, $R_2=CH_3$, $R_3=H$



3a, $R={}^{79}Br$
3b, $R={}^{81}Br$
10, $R=H$



4a, $R={}^{79}Br$
4b, $R={}^{81}Br$
11, $R=H$



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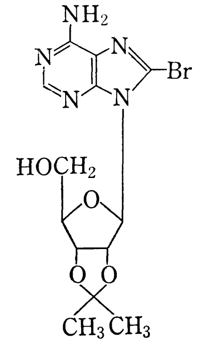

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When 2',3'-*O*-isopropylideneadenosine[7] (**16**) was treated with NBS under analogous conditions, the only product, which was isolated in a low yield, was 8-bromo-2',3'-*O*-isopropylideneadenosine (**17**).

Experimental

*Reaction of NBS with 2',3'-O-Benzylidenetubercidin (**1**).* NBS (700 mg, 3.94 mmol) and 2',3'-*O*-benzylidenetubercidin (1) (700 mg, 2.68 mmol) were dissolved in a mixture of carbon tetrachloride and tetrachloroethane (1 : 1) (100 ml), and then BaCO$_3$ (500 mg) was added. The solution was refluxed for 2 hr. After cooling, $(NH_4)_2SO_4$ (1 g) was added, and the solution was stirred for 1 hr. It was then washed with an aqueous solution of sodium bicarbonate and concentrated to dryness. Tlc showed that the residue contained three UV-absorbing compounds; they were separated by silica gel chromatography. The least polar compound was eluted with a mixture of benzene and ethyl acetate (1 : 1); it was thus obtained as a crystalline powder from ethyl acetate and *n*-hexane; yield, 412 mg (36%); mp 201—203 °C. NMR (100 MHz, CDCl$_3$) suggested that it was a 1 : 1 mixture of diastereomers of 8,5'-*O*-anhydro-7-bromo-8-hydroxy-

2′,3′-*O*-benzylidenetubercidin (**2**); δ 5.84 and 6.22 (two s, $C_6H_5CH<$), 6.79 and 6.82 (two s, H-1′), 8.23 and 8.25 (two s, H-2). Mass *m/e*: 432.0245 (M^+, $C_{18}H_{15}N_4O_4$ ^{81}Br gives 432.0256), 430 (M^+, $C_{18}H_{15}N_4O_4$ ^{79}Br), 360 and 358 (M^+–OCH_2CHO), 326 and 324 (M^+ –C_6H_5CHO), 283 and 281 (M^+ –C_6H_5CHO–CH_2CHO), 230 (**3b**) (observed 229.9648, $C_6H_5N_4O^{81}Br$ gives 229.9626), 229 (**4b**), 228 (**3a**), 227 (**4a**), *202* (**4b**-*HCN*), *200* (**4a**-HCN).

Found: C, 50.24; H, 3.50; N, 13.22; Br, 18.41%. Calcd for $C_{18}H_{15}N_4O_4Br$: C, 50.13; H, 3.51; N, 12.99; Br, 18.53%.

One of the diastereomers was separated as crystals from benzene at room temperature; mp 197—199 °C(dec), $\lambda_{max}^{H_2O}$ 280 nm (ε 12300), $[\alpha]_D^{24}$ −1.5 (*c* 1.5, $CHCl_3$); NMR (100 MHz, $CDCl_3$) δ: 4.04 (dd, 1H, H-5′$_a$, $J_{gem}=12$ Hz, $J_{4',5'a}=$ 1 Hz), 4.54 (dd, 1H, H-5′$_b$, $J_{gem}=12$ Hz, $J_{4',5'b}=2$ Hz), 4.81 (broad, 1H, H-4′), 4.90 and 5.15 (two d, 2H, H-2′ and H-3′, $J=_{2',3'}=5.5$ Hz), 5.84 (s, 1H, $C_6H_5CH<$), 5.95 (broad s, 2H, NH_2, disappeared on the addition of D_2O), 6.79 (s, 1H, H-1′), 8.23 (s, 1H, H-2).

Found: C, 53.94; H, 3.86; N, 12.06; Br, 17.10%. Calcd for $C_{18}H_{15}N_4O_4Br$ 1/2 C_6H_6: C, 53.63; H, 3.86; N, 11.92; Br 16.99%.

Next, a compound, which was identified as 7-bromo-2′,3′-*O*-benzylidenetubercidin (**5**), was eluted from the column using ethyl acetate as the developing solvent; it was obtained as a syrup by partial precipitation from ethyl acetate and *n*-hexane; yield, 226 mg; λ_{max}^{MeOH} 282 nm (ε 11000), M^+ 434 ($C_{18}H_{17}N_4O_4{}^{81}Br$) and 432($C_{18}H_{17}N_4O_4{}^{79}Br$).

Found: C, 49.19; H, 3.82; N, 12.53%. Calcd for $C_{18}H_{17}O_4N_4Br \cdot 1/2H_2O$: C, 48.88; H, 4.10; N, 12.67%.

The most polar compound eluted from the column with a mixture of ethyl acetate and methanol (9 : 1) was found to be the starting material.

*Reaction of NBS with 2′,3′-*O*-Isopropylidenetubercidin (**6**).* NBS (3.1 g, 17.4 mmol) and 2′,3′-*O*-isopropylidenetubercidin (**6**) (4.8 g, 15.6 mmol) were dissolved in a mixture of acetone and methylene dichloride (1 : 1) (200 ml); after the solution had been stirred at room temperature overnight, it was poured into a cold solution of sodium bicarbonate. The products were extracted with chloroform, and the organic phase was washed with an aqueous solution of sodium thiosulfate and then with water. Two main UV absorbing spots were shown on tlc, and each corresponding compound was separated on a silica gel column. 8,5′-*O*-anhydro-7-bromo-8-hydroxy-2′,3′-*O*-isopropylidenetubercidin (**7**) was first eluted from the column, using ethyl acetate as the developing solvent; yield, 389 mg. An analytical sample was obtained on crystallizing it first from benzene and then from ethanol and water; mp 215—218 °C (dec), λ_{max}^{MeOH} 280 nm (ε 12000), $[\alpha]_D^{23}$ +87.0 (*c* 1 : 1 $CHCl_3$); NMR (100 MHz, $CDCl_3$) δ: 1.37 and 1.57 (two s, 6H, two isopropylidene methyls), 4.03 (dd, 1H, H-5′$_a$, $J_{gem}=13$ Hz, $J_{3',5'a}=1$ Hz), 4.52 (dd, 1H, H-5′$_b$, $J_{gem}=13$ Hz, $J_{4',5'b}=2$ Hz), 4.66 (broad, 1H, H-4′), 4.82 and 5.19 (two d, 1H, H-2′ and H-3′, $H_{2',3'}=$ 5 Hz), 5.76 (broad s, 1H, NH_2), 6.63 (s, 1H, H-1′), 8.24 (s, 1H, H-2); Mass *m/e*: 384 ($C_{14}H_{15}N_4O_4{}^{81}Br$) and 382 ($C_{14}H_{15}N_4O_4{}^{79}Br$) ($M^+$), 230 (**3b**), 229 (**4b**), 228 (**3a**), 227 (**3b**).

Found: C, 43.84; H, 3.79; N, 14.66; Br, 20.85%. Calcd for $C_{14}H_{15}N_4O_4Br$: C, 43.88; H, 3.95; N, 14.62; Br, 20.85%.

The more polar compound was eluted from the column with a mixture of ethyl acetate and methanol (9 : 1); the subsequent evaporation of the solvent afforded 712 mg of a syrup. The mass spectrum suggested that the product was 7-bromo-2′,3′-*O*-isopropylidenetubercidin (**8**), showing the parent peaks at 386 ($C_{14}H_{17}N_4O_4{}^{81}Br$) and 384 ($C_{14}H_{17}N_4O_4{}^{70}Br$). However, attempts to obtain an analytically pure sample by repeated precipitation from benzene and *n*-hexane were unsuccessful, possibly because of the contamination of the unreacted starting material, which showed an R_f value close to that of **8** on tlc.

When a 4.28 g portion of **6** (14.0 mmol) was treated with 2.0 g of NBS (10.6 mmol) under comparable conditions, 550 mg of **7** and 1.37 g of the starting material were obtained.

The molar ratio of NBS to **6** was then increased to 2.5, and the reaction was carried out in a mixture of carbon tetrachloride and tetrachloroethane (1 : 1) under reflux. In this case, a serious decomposition seemed to proceed and the reaction mixture darkened markedly. No UV absorbing spot could be detected on tlc.

*8,5′-O-Anhydro-8-hydroxy-2′,3′-O-benzylidenetubercidin (**9**).* One diastereomer of **2** (350 mg), obtained in the crystalline state, was catalytically hydrogenated over 10% Pd-C(50 mg) in a mixture of ethanol (50 ml) and 10% CH_3COONa (3 ml) for 3 hr. After the catalyst had been removed by filtration through a Celite pad the ethanol was evaporated. The product was extracted with ethyl acetate and was crystallized from ethyl acetate and carbon tetrachloride; yield, 270 mg, mp 227—228 °C (dec) λ_{max}^{MeOH} 272 nm (ε 16400), $[\alpha]_D^{24}$ −48.7 (*c*, 1.3 $CHCl_3$); Mass *m/e*: 352 (M^+), 150 (**10**), 149 (**11**); ($CDCl_3$, 100 MHz) δ: 4.02 (dd, 1H, H-5′$_a$, $J_{gem}=12$ Hz, $J_{4',5'a}=1$ Hz), 4.48 (dd, 1H, H-5′$_b$, $J_{gem}=$ 12 Hz, $J_{4',5'b}=2$ Hz), 4.81 (broad, 1H, H-4′), 4.94 and 5.15 (two d, 2H, H-2′ and H-3′, $J_{2',3'}=6$ Hz), 5.86 (s, 2H, C_6H_5-$CH<$ and H-7), 6.80 (s, 1H, H-1′), 8.26 (s, 1H, H-2). The assignement of a two-proton singlet at δ 5.86 to a benzylidene methine proton and H-7 was confirmed by the spectrum in CD_3COCD_3, which exhibited two one-proton singlets at δ 5.92 and δ 6.06.

Found: C, 61.37; H, 4.58; N, 15.57%. Calcd for $C_{18}H_{16}N_4O_4$: C, 61.36; H, 4.58; N, 15.90%.

*8-5′-*O*-Anhydro-8-hydroxy-2′,3′-*O*-isopropylidenetubercidin (**12**).* Compound **7** (500 mg) was catalytically hydrogenated over 10% Pd-C (100 mg) for 4 hr in a mixture of ethanol (50 ml) and 10% CH_3COONa (2 ml). The reaction mixture was then filtered through a Celite pad, and the ethanol was evaporated. The product was extracted with chloroform and was thus obtained as a powder from benzene and *n*-hexane; yield, 350 mg; mp 107—113 °C, λ_{max}^{MeOH} 272 nm (ε 13900); $[\alpha]_D^{23}$ +86.0 (*c* 0.6 $CHCl_3$); Mass m/e: 304 (M^+), 150 (**10**), 149 (**11**); NMR (100 MHz, $CDCl_3$) δ: 1.36 and 1.56 (two s, 6H, two isopropylidene methyls), 3.96 (broad d, 1H, H-5′$_a$, $J_{gem}=13$ Hz), 4.40 (dd, 1H, H-5′$_b$, $J=_{gem}$ 13 Hz, $J_{4',5'b}=2$ Hz), 4.62(broad, 1H, H-4′), 4.82 and 5.04 (two d, 2H, H-2′ and H-3′, $J_{2',3'}=5$ Hz), 5.46 (broad s 2H, –NH_2, disappeared on the addition of D_2O), 5.72 (s, 1H, H-7), 6.60(s, 1H, H-1′), 8.26 (s, 1H, H-2).

Found: C, 54.45; H, 5.26; N, 17.91%. Calcd for $C_{14}H_{16}N_4O_4 \cdot 1/2H_2O$: C, 53.84; H, 5.17; N, 17.94%.

*8-5′-*O*-Anhydro-8-hydroxy-*N^6,N^6-p-*toluoyl-2′,3′-*O*-isopropylidenetubercidin (**13**).* Into a stirred solution of **12** (152 mg) in pyridine (20 ml), *p*-toluoyl chloride (0.5 ml) was added at 0 °C, after which the solution was left in a refrigerator overnight. The reaction mixture was then poured into an ice-cooled solution of sodium bicarbonate, and the product was extracted with chloroform. After evaporation of the solvent to dryness, the residue was washed with a small amount of hot ethyl acetate; it was obtained as a powder; yield, 190 mg; mp 273—273.5 °C (dec); λ_{max}^{MeOH} nm (ε): 305 (12500) 253 (28000); Mass *m/e*: 540 (M^+), 512 (M^+-CO), 484 (M^+-2CO), 421 (M^+-$CH_3C_6H_4CO$), 393 (M^+-$CH_3C_6H_4CO$-CO); NMR (100 MHz, DMSO-*d*) δ: 1.31 and 1.50 (two s, 6H, two isopropylidene methyls), 2.32 (s, 6H, 2*p*-$CH_3C_6H_4$-CO), 5.80 (s, 1H, H-7), 6.42 (s, 1H, H-1′), 7.24 and 7.67 (two d, 8H, 2*p*-$CH_3C_6H_4CO$, $J=8$ Hz), 8.48 (s, 1H, H-2).

Found: C, 66.34; H, 5.19; N, 9.98%. Calcd for $C_{30}H_{28}N_4O_6$: C, 66.65; H, 5.22; N, 10.37%.

8-Bromo-2′,3′-O-isopropylideneadenosine (17). A solution of 2′,3′-O-isopropylideneadenosine (**16**) (1.6 g, 5.2 mmol), NBS (2 g, 11.2 mmol), and $CaCO_3$ (1 g) in acetone (50 ml) was stirred overnight at room temperature and then poured into a cold solution of sodium bicarbonate. The chloroform extract was washed with an aqueous solution of sodium thiosulfate and then with water.

Silica gel chromatography on developing with a mixture of ethyl acetate and methanol (9 : 1) afforded two compounds, the more polar one being the starting material (720 mg). The less polar one, identified as **17**, was crystallized from ethyl acetate; yield, 267 mg; mp 229 °C; λ_{max}^{MeOH} 264 nm (ε 18900); $[\alpha]_D^{23}$ -37.0 (c 1.1 DMSO); Mass m/e: 387 (M^+, $C_{13}H_{14}N_5O_4{}^{81}Br$), 385 ($M^+$, $C_{13}H_{14}N_4O_5{}^{79}Br$), 214 and 212 (base peak, base moiety of **17**): NMR (100 MHz, DMSO-d) δ: 1.34 and 1.56 (two s, 6H, two isopropylidene methyls), 3.50 (m, 2H, H-5′), 4.18 (m, 1H, H-4′), 5.04 (q, 1H, H-3′, $J_{3',4'}=3$ Hz, $J_{2',3'}=6$ Hz), 5.67 (q, 1H, H-2′, $J_{2,'3'}=6$ Hz, $J_{1',2'}=3$ Hz), 6.04 (d, 1H, H-1′, $J_{1',2'}=3$ Hz), 7.55 (broad s, 2H, NH_2) 8.16 (s, 1H, H-2).

Found: C, 40.44; H, 4.18; N, 18.57; Br, 20.61%. Calcd for $C_{13}H_{14}N_5O_4Br$: C, 40.43; H, 4.18; N, 18.14; Br, 20.69%.

Under altered conditions, the yield of **17** was not improved; *e.g.*, when **16** (5.47 g) was treated with NBS (3.5 g) in a mixture of tetrachloroethane (75 ml) and carbon tetrachloride (25 ml) in the presence of $CaCO_3$ (5 g) under reflux for 4 hr, the yield of **17** was 257 mg, and 1.13 g of **16** was recovered. An extended reaction time or the use of an increased amount of NBS resulted in a darkening of the reaction mixture and a decreased yield of **17**.

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