A Convenient Preparation of 3'-Deoxyadenosine (Cordycepin) and 9-[3'(*R*)-Deuterio-β-D-2'(*R*)-pentofuranosyl]-adenine

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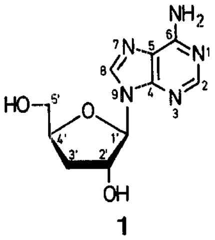
A new procedure is described for the synthesis of 3'-deoxyadenosine and 9-[3'-deoxy-3'(*R*)-deuterio-β-D-2'(*R*)-pentofuranosyl]-adenine by lithium aluminium hydride or deuteride reduction of appropriately protected 2',3'-anhydro-*ribo*-adenosine.

Cordycepin (3'-deoxyadenosine; **1**) was the first naturally occurring, potent nucleoside antibiotic to be isolated from the fungus *Cordyceps militaris*[1]. its cytostatic activity is derived from the chain terminating ability at the 3'-terminus during RNA synthesis. This is due to the lack of a 3'-hydroxy group on the sugar moiety. Several groups have described the synthesis [2–17] of **1**; however, large scale preparative procedures are based upon a strategy which involves the reduction of the *pure* 3'-halosugar **2**[7–14]. It is evident from the literature[7–14] that, in preparations of **2** involving a carboxonium ion intermediate **4**, the product is invariably contaminated with its 2'-isomer **3** (~ 5–12%). Thus a careful chromatographic separation procedure has to be used to obtain pure 3'-isomer **2** for a regioselective synthesis of **1** or 2'-deoxyadenosine has to be separated from 3'-deoxyadenosine, after reduction has taken place, by chromatography on Dowex ($OH^{\ominus}$) resin.



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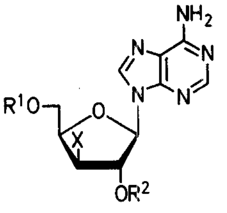
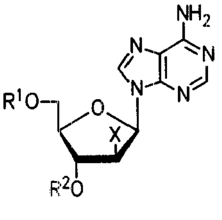
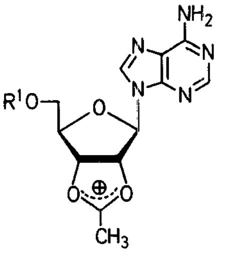


2 (X = halogen)



3 (X = halogen)



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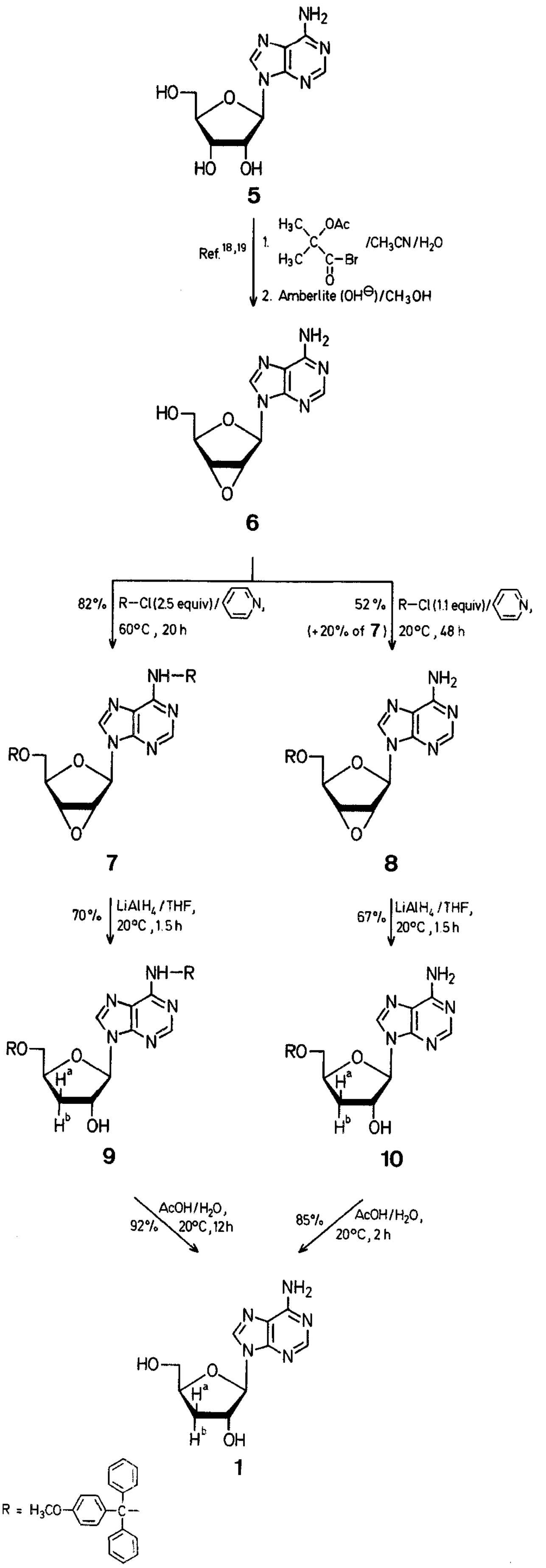
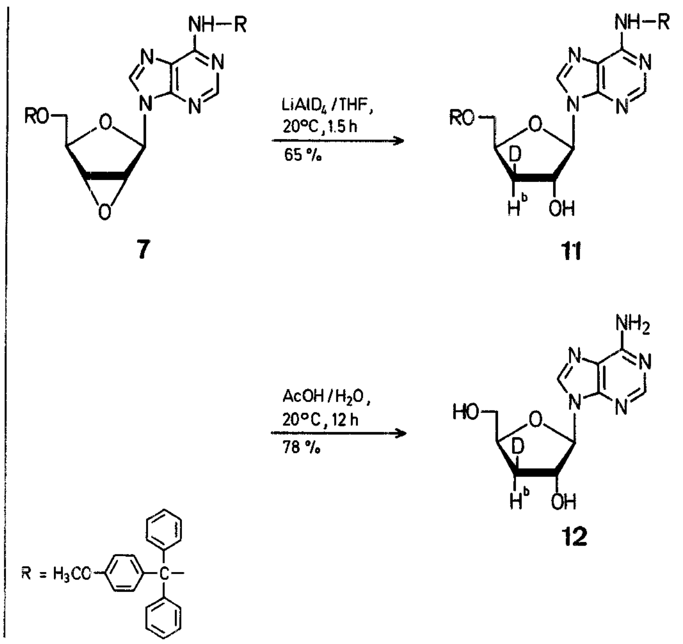
Our procedure involves a regioselective reduction at the 3'-carbon of an appropriately protected *ribo*-epoxide (**7** or **8**) with an excess of lithium aluminium hydride to cordycepin derivatives **9** or **10**. The 2',3'-anhydro-nucleoside **6** was obtained from adenosine **5** (5 g scale) in almost quantitative



yield using a previously described one-pot procedure[18,19]. After converting **6** to the 6-*N*-5′-*O*-bis[4-methoxytriphenylmethyl] derivative **7** in 82% yield and then the lithium aluminium hydride reduction furnishes **9** in 70% yield. Alternatively, **6** was converted to its 5′-*O*-4-methoxytriphenylmethyl derivative **8** in 52% yield and the lithium aluminium hydride reduction product **10** was obtained in 69% yield. The latter sequence (**6** → **8** → **10**) opens the possibility to protect the exocylic amino function of either **8** or **10** for further nucleotide synthesis. Finally, the 4-methoxytriphenylmethyl protecting group was cleaved from **9** and **10** by using 80% acetic acid at 20°C to give crude **1** (homogenous on T.L.C.) in a yield of 92 and 85%, respectively.

Since the lithium aluminium hydride reduction takes place in an S_N^2 fashion, the epoxide **7** will be opened specifically from the *β*-face. Using lithium aluminium deuteride, the isotopically labelled 3′(*R*)-deuterio derivative of cordycepin (**12**) was obtained, which enabled us for the first time to assign all coupling constants of the sugar protons unambiguously using ^{1}H-N.M.R. spectroscopy. It may be added that an attempt to synthesise **12** through a 3′-halo derivative **3** with tri-*n*-butyltin deuteride[20] would presumably lead to an intractable mixture of *threo* and *erythro* deuterio epimers.



Reactions were monitored by T.L.C. using Merck pre-coated silica gel 60 F_{254} plates using following solvent systems: (A) 5% methanol/chloroform (v/v); (B) 10% methanol/chloroform (v/v); (C) 30% methanol/chloroform (v/v).

6-*N*-5′-*O*-Bis[4-methoxytriphenylmethyl]-2′,3′-anhydroadenosine (7):

Dry 2′,3′-anhydroadenosine[18,19] (**6**; 3.4 g, 13.6 mmol) is dissolved in dry pyridine (150 ml), 4-methoxytriphenylmethyl chloride (10.5 g, 34 mmol) is added, and the mixture is stirred in the dark at 60°C for 20 h. T.L.C. (2% methanol/dichloromethane, v/v) shows one major spot (R_f: 0.58). Methanol (10 ml) is added, the mixture is stirred for 2 h, then poured into saturated aqueous sodium hydrogen carbonate (150 ml), and extracted with dichloromethane (2 × 100 ml). Evaporation in vacuo leaves a light brown foam which is dissolved in a mixture of dichloromethane/petroleum ether (6 : 4, v/v) and applied to a short silica gel column. Elution is carried out with dichloromethane followed by 1% ethanol/dichloromethane mixture. Appropriate fractions are pooled and evaporated leaving a white foam which is dissolved in diethyl ether/tetrahydrofuran (90 ml, 9 : 1 v/v) and precipitated from petroleum ether (300 ml). The supernatant liquid is evaporated and precipitated again using the same solvents to give chromatographically pure **7** [R_f: 0.66 in solvent (A)]; yield: 8.9 g (82%). Crystallization of **7** was unsuccessful in our hands; $[\alpha]_D^{20}$: + 17° (*c* 0.5, chloroform).

M.S. (chemical ionization, NH_3): *m/e* = 794.9 (29.8%, M^+).

U.V. (95% ethanol): λ_{max} = 275 nm (ε = 21,400).

^{1}H-N.M.R. ($CDCl_3$): δ = 7.83 (s, 1 H, H-8); 7.73 (s, 1 H, H-2); 7.24 (m, 24 H_{arom}); 6.87 (br s, 1 H, NH); 6.76 (m, 4 H_{arom}); 6.08 (s, 1 H, H-1′); 4.47 (dd, 1 H, H-4′); 4.31 (d, 1 H, $J_{2',3}$ = 2.6 Hz, H-2′); 4.00 (d, 1 H, H-3′); 3.76 (s, 3 H, O—CH_3); 3.71 (s, 3 H, O—CH_3); 3.35 (dd, 1 H, $J_{4',5'\text{-}H^a}$ = 6 Hz, 5′-H^a); 3.12 ppm (dd, 1 H, $J_{4',5'\text{-}H^b}$ = 5.6 Hz, $J_{5'\text{-}H^a,H^b}$ = 10 Hz, 5′-H^b).

^{13}C-N.M.R. ($CDCl_3$): δ = 83.95 (d, J_{CH} = 175 Hz, C-1′); 79.81 (d, J_{CH} = 158 Hz, C-4′); 63.36 (t, J_{CH} = 140 Hz, C-5′); 58.87 (d, J_{CH} = 195 Hz, C-2′); 57.73 ppm (d, J_{CH} = 195 Hz, C-3′).

5′-*O*-(4-Methoxytriphenylmethyl)-2′,3′-anhydroadenosine (8):

Compound **6** (3.34 g, 13.4 mmol) is coevaporated with dry pyridine, then dissolved in dry pyridine (150 ml), and 4-methoxytriphenylmethyl chloride (4.54 g, 14.74 mmol) is added. The mixture is stirred at 20 °C for two days and then worked up as described above for **7**. Chromatography on silica gel gives chromatographically pure [R_f: 0.43 in solvent (A)] **7**; yield: 2.1 g (20%), followed by **8**; yield: 3.6 g (52%), and unreacted **6** (140 mg, 4%). Crystallization of **8** was unsuccessful; $[\alpha]_D^{20}$: + 23.6° (*c* 0.5, chloroform).

M.S. (chemical ionization, NH_3): *m/e* = 522.6 (41%, M^+).

U.V. (95% ethanol): λ_{max} = 259 (ε = 13,700); 233 nm (ε = 17,000).

^{1}H-N.M.R. ($CDCl_3$): δ = 8.18 (s, 1 H, H-8); 7.79 (s, 1 H, H-2); 7.26 (m, 12 H_{arom}); 6.7 (m, 2 H_{arom}); 6.12 (s, 1 H, H-1′); 5.69 (br.s, 2 H, NH_2); 4.5 (m, 1 H, H-4′); 4.44 (d, 1 H, $J_{2',3'}$ = 2.3 Hz, H-2′); 4.09 (d, 1 H, H-3′); 3.76 (s, 3 H, O—CH_3); 3.28 ppm (m, 2 H, H-5′).

^{13}C-N.M.R. ($CDCl_3$): δ = 83.8 (d, J_{CH} = 170 Hz, C-1′); 79.7 (d, J_{CH} = 153 Hz, C-4′); 63.1 (t, J_{CH} = 142 Hz, C-5′); 58.9 (d, J_{CH} = 192 Hz, C-2′); 57.8 ppm (d, J_{CH} = 196 Hz, C-3′).

6-*N*-5′-*O*-Bis-[4-methoxytriphenylmethyl]-3′-deoxyadenosine (9):

A solution of **7** (3.82 g, 4.8 mmol) in tetrahydrofuran dry (50 ml) is added dropwise, under argon at 0 °C, over a period of 30 min to a stirred suspension of lithium aluminium hydride (1.15 g, 30.3 mmol) in dry tetrahydrofuran (150 ml, distilled over lithium aluminium hydride before use). After 90 min at 20 °C, the mixture is cooled in a ice bath, and a mixture of water (10 ml) and tetrahydrofuran (40 ml) added slowly. Further addition of 20% aqueous sodium dihydrogen phosphate solution (30 ml) is followed by addition of water (100 ml). The reaction mixture is extracted with ethyl acetate (3 × 100 ml). The organic phase is washed with aqueous sodium hydrogen carbonate solution (150 ml) and evaporated in vacuo. The residue is dissolved in dichloromethane (~ 15 ml) and coevaporated with hexane (3 × 50 ml). The resulting white foam is dissolved in dichloromethane/petroleum ether (5 ml; 6 : 4 v/v) and chromatographed on a short silica gel column which is eluted first with the latter solvent mixture followed by dichloromethane and chloroform. Evaporation of the appropriate fractions gives chromatographically pure [R_f: 0.55 in solvent (A)] **9**; yield: 2.66 g (70%). Crystallization of **9** was unsuccessful; $[\alpha]_D^{20}$: − 14.4° (*c* 0.5, chloroform).

M.S. (chemical ionization, NH_3): *m/e* = 796.9 (26.2%, M^+).

U.V. (95% ethanol): λ_{max} = 276 nm (ε = 19,400).

^{1}H-N.M.R. ($CDCl_3$): δ = 8.02 (s, 1 H, H-8); 7.99 (s, 1 H, H-2); 7.29 (m, 24 H_{arom}); 7.00 (br. s, 1 H, NH); 6.78 (m, 4 H_{arom}); 5.82 (d, 1 H, $J_{1',2'}$ = 3.4 Hz, H-1′); 5.72 (br. s, 1 H, 2′—OH); 4.72 (m, 1 H, $J_{2',3'}$ = 6 Hz, H-2′); 4.53 (m, 1 H, H-4′); 3.77 (s, 6 H, O—CH_3); 3.3 (ddd, 2 H, $J_{5'H^a,H^b}$ = 10 Hz, $J_{4',5'\text{-}H^a}$ = 2.8 Hz, $J_{4',5'H^b}$ = 4.5 Hz, 5′-H^aH^b); 2.19 ppm (m, 2 H, H-3′).

^{13}C-N.M.R. ($CDCl_3$): δ = 92.7 (d, J_{CH} = 171 Hz, C-1′); 80.0 (d, J_{CH} = 146 Hz, C-4′); 75.62 (d, J_{CH} = 156 Hz, C-2′); 64.6 (t, J_{CH} = 147 Hz, C-5′); 33.9 ppm (t, J_{CH} = 132 Hz, C-3′).

5′-*O*-(Methoxytriphenylmethyl)-3′-deoxyadenosine (10):

On treatment of **8** (595 mg, 1.1 mmol) with lithium aluminium hydride (266 mg) as described above, chromatographically pure **10** [R_f: 0.35 in solvent (B)] is obtained which could not be crystallized; yield: 400 mg (67%); $[\alpha]_D^{20}$: − 18.6° (*c* 0.5, chloroform).

M.S. (chemical ionization, NH_3): *m/e* = 524.6 (37.3%, M^+).

U.V. (95% ethanol): λ_{max} = 259 (ε = 14,000), 232 nm (ε = 15,700).

^{1}H-N.M.R. ($CDCl_3$): δ = 8.33 (s, 1 H, H-8); 8.27 (s, 1 H, H-2); 7.3 (m, 12 H_{arom}); 6.8 (m, 2 H_{arom}); 6.26 (br. s, 2 H, NH_2); 6.06 (s, 1 H, H-1′); 4.72 (m, 1 H, H-4′); 4.51 (m, 1 H, H-2′); 3.8 (s, 3 H, O—CH_3); 3.62 (dd, 1 H, $J_{4',5'\text{-}H^a}$ = 2.6 Hz, 5′-H^a); 3.40 (dd, 1 H, $J_{4',5'\text{-}H^b}$ = 4.3 Hz, $J_{5'\text{-}H^a,H^b}$ = 10.7 Hz, 5′-H^b); 2.14 ppm (m, 2 H, H-3′).

^{13}C-N.M.R. ($CDCl_3$: δ = 92.7 (d, J_{CH} = 169 Hz, C-1′); 80.4 (d, J_{CH} = 152 Hz, C-4′); 76.0 (d, J_{CH} = 151 Hz, C-2′); 64.7 (t, J_{CH} = 143 Hz, C-5′); 33.6 ppm (t,J_{CH} = 135 Hz, C-3′).

3′-Deoxyadenosine (1):

A stirred suspension of **9** (2.3 g, 2.89 mmol) in a mixture of acetic acid/water (100 ml, 8 : 2 v/v) is kept at 20 °C for 12 h. After evaporation in vacuo the residue is coevaporated several times with toluene (3 × 50 ml). The residue is taken up in water (150 ml) and washed with dichloromethane (3 × 50 ml). During concentration of the aqueous phase in vacuo, the compound starts to crystallize. The crystals are filtered and dried to give 473 mg of the title compound. The supernatant liquid is lyophylized to give another crop of white amorphous title compound; both crops are chromatographically pure [R_f: 0.52 in solvent (C)]; total crude yield: 671 mg (92%); $[\alpha]_D^{20}$: − 44° (*c* 0.5; water); Lit.[5], $[\alpha]_D^{20}$: − 44°; m.p. 224 °C (from water); Lit.[13], m.p. 224–225 °C.

Deprotection of **10** (198 mg, 0.37 mmol) for 2 h at 20 °C and a usual work-up gives **1**; yield: 80 mg (85%).

$C_{10}H_{13}N_5O_3$	calc.	C 47.80	H 5.22	N 27.88
(251.2)	found	47.91	5.07	27.91

U.V. (95% ethanol): λ_{max} = 259 nm (ε = 13,700).

^{1}H-N.M.R. (DMSO-d_6): δ = 8.36 (s, 1 H, H-8); 8.15 (s, 1 H, H-2); 7.28 (br. s, 2 H, NH_2); 5.88 (d, 1 H, $J_{1',2'}$ = 2.2 Hz, H-1′); 5.68 (d, 1 H, $J_{2',2'\text{-}OH}$ = 4.4 Hz, 2′-OH); 5.19 (t, 1 H, $J_{5',5'\text{-}OH}$ = 5.4 Hz, 5′-OH); 4.58 (m, 1 H, H-2′); 4.35 (ddd, 1 H, $J_{3',4'\text{-}H^b}$ = 6.2 Hz, $J_{4',3'\text{-}H^a}$ = 7.5 Hz, H-4′); 3.58 (m, 2 H, 5′-H^a, -H^b); 2.26 (ddd, 1 H, $J_{2',3'\text{-}H^a}$ = 5.6 Hz, $J_{3'\text{-}H^a,3'\text{-}H^b}$ = 13 Hz, 3′-H^a); 1.90 ppm (ddd, 1 H, $J_{2',3'\text{-}H^b}$ = 3.5 Hz, 3′-H^b).

^{13}C-N.M.R. (DMSO-d_6): δ = 155.9; 152.5; 148.8; 139.3; 119.2; 91.0 (d, J_{CH} = 167 Hz, C-1′); 80.7 (d, J_{CH} = 148 Hz, C-4′); 74.7 (d, J_{CH} = 149 Hz, C-2′); 62.7 (t, J_{CH} = 140 Hz, C-5′); 34.0 ppm (t, J_{CH} = 132 Hz, C-3′).

6-*N*-5′-*O*-Bis-[4-methoxytriphenylmethyl]-3′-deoxy-3′(*R*)-deuterio-adenosine (11):

By treating **7** (1.55 g, 1.95 mmol) with lithium aluminium deuteride (400 mg, 98 atom % D), under conditions described above, chromatographically pure [R_f: 0.55 in solvent (A)] **11** is obtained which could not be crystallized; yield: 1.02 g (65%); $[\alpha]_D^{20}$: − 14° (*c* 0.5, chloroform).

M.S. (chemical ionization, NH_3): *m/e* = 797.9 (17%, M^+); 796.9 (0,35%).

U.V. (95% ethanol): λ_{max} = 276 (ε = 21,200).

^{1}H-N.M.R. ($CDCl_3$): δ = 8.02 (s, 1 H, H-8); 7.99 (s, 1 H, H-2); 7.29 (m, 24 H_{arom}); 6.99 (br. s, 1 H, NH); 6.78 (m, 4 H_{arom}); 5.81 (d, 1 H, $J_{1',2'}$ = 3.7 Hz, H-1′); 5.54 (br. s, 1 H, 2′-OH); 4.76 (dd, 1 H, $J_{2',3'}$ = 6 Hz, H-2′); 4.62 (ddd, 1 H, $J_{3',4'}$ = 3.5 Hz, H-4′); 3.76 (s, 6 H, O—CH_3); 3.41 (dd, 1 H, $J_{4',5'\text{-}H^a}$ = 3.4 Hz, 5′-H^a); 3.20 (dd, 1 H, $J_{4',5'\text{-}H^b}$ = 4.5 Hz, $J_{5'\text{-}H^a,H^b}$ = 10.7 Hz, 5′-H^b); 2.06 ppm (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, H-3′).

^{13}C-N.M.R. ($CDCl_3$): δ = 92.9 (C-1′); 80.2 (C-4′); 75.7 (C-2′); 64.9 (C-5′); 33.8 ppm (C-3′):

9-[3′-Deoxy-3′(*R*)-deuterio-*β*-D-2′(*R*)-pentofuranosyl]-adenine (12):

Treatment of **11** (1.02 g, 1.28 mmol) under the conditions described for **9** and evaporation gives 189 mg of crystalline compound; the mother liquor is subsequently freeze-dried to yield another crop of amorphous, chromatographically pure [R_f: 0.52 in solvent (C)] title compound; total yield: 252 mg (78%); m.p. 219 °C (water); $[\alpha]_D^{20}$: − 43° (*c* 0.5, water).

$C_{10}DH_{13}N_5O_3$	calc.	C 47.62	H /D 5.59	N 27.88
(252.25)	found	47.38	5.82	27.71

M.S. (chemical ionization, NH_3): $m/e = 253.2$ (21%); 252.2 (0.4%).

U.V. (95% ethanol): $\lambda_{max} = 260$ nm ($\varepsilon = 14{,}100$).

^{1}H-N.M.R. (DMSO-d_6): $\delta = 8.36$ (s, 1 H, H-8); 8.15 (s, 1 H, H-2); 7.28 (br. s, 2 H, NH_2); 5.87 (d, 1 H, $J_{1',2'} = 2.7$ Hz, H-1'); 5.69 (d, 1 H, $J_{2',OH} = 4.1$ Hz, 2'-OH); 5.18 (t, $J_{5',OH} = 4.6$ Hz, 5'-OH); 4.56 (ddd, $J_{2',3'} = 3.2$ Hz, H-2'); 4.35 (ddd, 1 H, $J_{4',5'} = 3.3$ Hz, H-4'); 3.62 (m, 2 H, H-5'); 1.91 ppm (dd, $J_{3',4'} = 6.2$ Hz, H-3').

^{13}C-N.M.R. (DMSO-d_6): $\delta = 155.9$; 152.4; 148.8; 139.2; 119.2; 90.9 (d, $J_{CH} = 166$ Hz, C-1'); 80.7 (d, $J_{CH} = 149$ Hz, C-4'); 74.6 (d, $J_{CH} = 148$ Hz, C-2'); 62.6 (t, $J_{CH} = 141$ Hz, C-5'); 33.6 ppm (dt, $J_{CH} = 132$ Hz, $J_{CD} = \sim 15$ Hz, C-3').

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