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SYNTHESIS OF 1-(2′-O-METHYL-β-D-RIBOFURANOSYL)-URACIL (2′-O-METHYLURIDINE) AND 3-(2′-O-METHYL-β-D-RIBOFURANOSYL)URACIL

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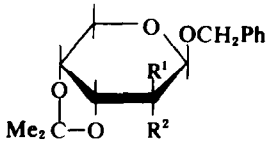
Abstract– 1-O-Acetyl-3,5-di-O-benzoyl-2-O-methyl-β-D-ribofuranose has been prepared in five steps from benzyl 2-O-methyl-β-D-ribopyranoside. Reaction of the furanose derivative with 2,4-bis-(trimethylsilyl)uracil in the presence of stannic chloride provides a new synthesis of 2′-O-methyl uridine and also yields the hitherto unknown 3-(2′-O-methyl-β-D-ribofuranosyl)uracil.

2′-O-Methyl-D-ribonucleosides are of particular interest since they are minor constituents of RNA from a variety of sources.[1] These nucleosides have been synthesised by two procedures. The first involves partial methylation of a nucleoside with diazomethane[2–7] followed generally by a chromatographic separation of the mixture obtained. In this way, the 2′-O-methyl ethers of adenosine, guanosine, cytidine (and indirectly uridine)† have been prepared.

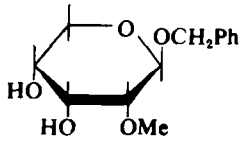
The second procedure involves the partial tritylation of uridine, chromatographic separation of the products, and subsequent methylation of the 3′,5′-di-O-trityl derivative.[8] In addition to reaction at the 2′-OH group, O- and N-methylation occurs in the pyrimidine moiety, and the preparation of the 2′-O-methyl ethers of uridine and cytidine requires removal of the N-alkylated isomer.

The most usual and generally useful method for nucleoside synthesis, that involving coupling of the carbohydrate and heterocyclic base components, requires the preparation of a suitably substituted carbohydrate, which is usually a furanose derivative. This paper describes the preparation of such a derivative of 2-O-methyl-D-ribose, which is readily available as a result of previous work in our laboratory,[9] and demonstrates its use in a pyrimidine nucleoside synthesis.

†By the deamination of 2′-O-methylcytidine.[4]



1: R^1 = OH, R^2 = H
2: R^1, R^2 = =O
3: R^1 = H, R^2 = OH
4: R^1 = H, R^2 = OMe



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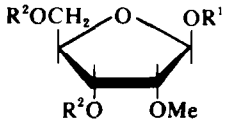
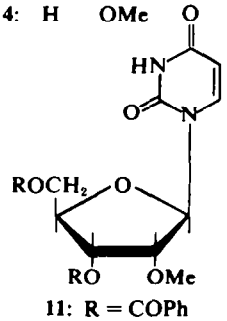
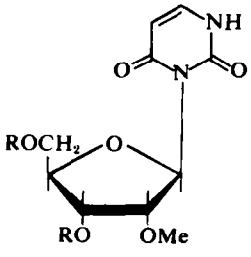


6: R^1 = Me, R^2 = H
7: R^1 = Me, R^2 = COPh
8: R^1 = H, R^2 = COPh
9: R^1 = Ac, R^2 = COPh
10: R^1 = Me, R^2 = Me



11: R = COPh
12: R = H



13: R = COPh
14: R = H

The synthetic sequence previously described in the L-series,[9] was used to convert benzyl 3,4-O-isopropylidene-β-D-arabinopyranoside (**1**), through compounds **2**, **3** and **4** into benzyl 2-O-methyl-β-D-ribopyranoside (**5**). It was previously observed that hydrogenolysis of the L-isomer of **5** in methanol gave, as well as the free sugar, methyl 2-O-methyl-L-ribofuranoside; glycoside formation was attributed to trace acid catalysis. After hydrogenolysis of glycoside (**5**) in methanol, the reaction mixture was treated with an acidic ion exchange resin in order to promote the formation of the furanoside (**6**), which was obtained in 60% yield sufficiently pure* for further transformation. Proof of the glycoside's structure was based on its methylation to yield a product (**10**) identical to that obtained by methylation of methyl β-D-ribofuranoside.[10]

Benzoylation of glycoside (**6**) yielded the dibenzoate (**7**), as a thick liquid which was best purified from trace contaminants by PLC. Treatment of the compound **7** under acetolysis conditions which convert methyl 2,3,5-tri-O-benzoyl-α,β-D-ribofuranoside into 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside,[11] gave several products, and an alternative procedure for preparation of the 1-O-acetyl derivative was sought. Compound **7** was hydrolysed with trifluoroacetic acid–water mixture[12] to yield 3,5-di-O-benzoyl-2-O-methyl-D-ribofuranose (**8**) which on acetylation gave syrupy 1-O-acetyl-3,5-di-O-benzoyl-2-O-methyl-D-ribofuranose. Its NMR spectrum showed a singlet absorption for the H-1 proton, showing it to be the β-D-anomer (**9**).[13] Reaction of this compound with 2,4-bis(trimethylsilyl)-uracil in 1,2-dichloroethane in the presence of stannic chloride[14] yielded two major products, readily separated by PLC, in the ratio of 1·3:1. The NMR spectra of these materials, which were not obtained analytically pure, were consistent with their being (3′,5′-di-O-benzoyl-2′-O-methyl-D-ribofuranosyl)uracils. The formation of two anomeric N-1 nucleosides in this reaction might reasonably be expected, in view of previous results using this procedure[14] and the poor participating ability of the C-2 substituent in **9**. Assignment of anomeric configuration to the two isomers was not possible on the basis of $J_{1',2'}$ values, since the latter were similar (4 Hz).[13] Debenzoylation of the product formed in greater amount gave 1-(2′-O-methyl-β-D-ribofuranosyl)uracil (**12**) whose physical constants were in good agreement with those reported,[4,8] and which gave a mass spectrum identical to that of an authentic sample. Debenzoylation of the second product yielded a crystalline compound melting at 126–127°, whose elemental analysis was identical to that of **12**. Its mass spectrum was similar to that of **12** and contained a significant peak at *m/e* 146, which is characteristic of 2′-O-methylribonucleosides.[15] It did not react with sodium metaperiodate, and although its UV spectrum at pH 7 was similar to that of **12**, a bathochromic shift, accompanied by a substantial increase in intensity, was observed in basic solution. Such spectral changes with increasing pH are characteristic of an N-3 substituted uracil; an N-1 substituted uracil shows an absorption near 265 nm, and the position of the band is virtually invariant with increase of pH.[16–19] Its NMR spectrum showed chemical shifts and coupling constants for H-5 and H-6 of the uracil residue and for H-1 of the furanose ring very similar to those reported for the analogous protons in 3-β-D-ribofuranosyluracil.[19] Methylation of the compound followed by acidic hydrolysis yielded material chromatographically indistinguishable from 1-methyluracil. On the foregoing evidence coupled with the fact that its CD spectrum showed a negative Cotton effect centred on 273 nm (*cf* reported ORD spectrum of 3-β-D-ribofuranosyluracil[20]) it was concluded that the new nucleoside was 3-(2′-O-methyl-β-D-ribofuranosyl)uracil (**14**) and not the expected 1-(2′-O-methyl-α-D-ribofuranosyl)uracil.

In view of the utility of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in nucleoside syntheses, intermediate (**9**) should also find general application in the preparation of 2′-O-methylribonucleosides.

EXPERIMENTAL

TLC was performed on Kieselgel GF_{254} and PLC on Kieselgel PF_{254}. Descending paper chromatography was performed on Whatman No. 1 paper with solvent systems (A) butan-1-ol–acetic acid–water (5:2:3 v/v), (B) propan-2-ol–ammonia–water (7:1:2 v/v), (C) butan-1-ol–water (86:14 v/v), and (D) ethyl acetate–water–formic acid (12:7:1 v/v). Optical rotations were determined on chloroform solns except where stated otherwise, with a Perkin–Elmer 141 polarimeter. NMR spectra were measured on solns in $CDCl_3$ at 100 MHz on a Varian HA-100 spectrometer with TMS as internal reference except where stated otherwise. CD measurements were made with a Cary 61 Spectropolarimeter and GLC was performed on a Perkin-Elmer F-11 instrument. Routine identifications were based on TLC, GLC, and IR and NMR spectra.

Benzyl 3,4-O-*isopropylidene*-β-D-*ribopyranoside* (**3**). The procedure followed was identical to that described previously for the preparation of the L-derivative.[9] Benzyl 3,4-O-isopropylidene-β-D-arabino-pyranoside[21] (5·1 g) gave the *product* (24·6 g, 48%), m.p. 95–96°, $[\alpha]_D^{21}$ −129° (*c* 0·3), $[\alpha]_D^{21}$ −148° (*c* 0·3 in EtOH) (Found: C, 64·7; H, 7·2. Calc. for $C_{15}H_{20}O_5$: C, 64·3; H, 7·2%). Lit,[22] m.p. 94–96°, $[\alpha]_D^{25}$ −105 ± 5° (*c* 3·1 in EtOH).

Benzyl 3,4-O-*isopropylidene*-2-O-*methyl*-β-*ribopyranoside* (**4**). Methylation of benzyl 3,4-O-isopropylidene-β-D-ribopyranoside (11·2 g) as described for the L-isomer,[9] gave the *methyl ether* **4** (10·4 g, 88%), b.p. 160–162°/0·5

*Several recrystallisations were generally necessary to obtain sharply melting material. However for further transformation, material of lower purity was suitable since purification by PLC was performed after the next (benzoylation) step, following which the material obtained (**7**) was identical to that obtained by benzoylation of glycoside (**6**) with m.p. 73–75°.

mm Hg, $[\alpha]_D^{20}$ −121° (*c* 1·4) (Found: C, 64·9; H, 7·7. $C_{16}H_{22}O_5$ requires: C, 65·3; H, 7·5%).

Benzyl 2-O-*methyl*-β-D-*ribopyranoside* (**5**). The foregoing ether (2 g) was hydrolysed as described for the L-isomer,[9] and gave the *product* (1·3 g, 76%), m.p. 92–94°, $[\alpha]_D^{20}$ −135° (*c* 0·4) (Found: C, 61·2; H, 7·2. $C_{13}H_{18}O_5$ requires: C, 61·4; H, 7·1%).

Methyl 2-O-*methyl*-β-D-*ribofuranoside* (**6**). A soln of **5** (3 g) in MeOH (60 ml) was stirred under a slight over pressure of H_2 in the presence of 10% Pd-C (0·5 g). Uptake of H_2 (330 ml, 1·16 mole) was complete in 5 hr. After removal of the catalyst, Amberlite IR-120 resin (H^+) (10 ml), which had previously been thoroughly washed with MeOH, was added; the mixture was then shaken overnight. The filtered soln was concentrated, and the residue crystallised from EtOAc–light petroleum to yield material (1·25 g), m.p. 69–74°. Two further crystallisations from the same solvent yielded the *product*, m.p. 73–75°, $[\alpha]_D^{18}$ −24·5° (*c* 0·55) (Found: C, 47·5; H, 7·9. $C_7H_{14}O_5$ requires: C, 47·2; H, 7·9%), τ (D_2O – int. ref. 3-(trimethylsilyl)-propanesulphonic acid) 5·0 (d, H-1), 5·7–6·4 (m, H-2,3,4,5,5'), 6·52 (s) and 6·6 (s) (2 × OMe).

Concentration of the mother liquors yielded a syrup which on retreatment in MeOH with Amberlite IR-120 resin yielded a second crop (0·36 g) of impure furanoside. Crude crystalline material was suitable for further transformation since purification was readily achieved after benzoylation (see below).

Methyl 2,3,5-*tri*-O-*methyl*-β-D-*ribofuranoside* (**10**). Methyl β-D-ribofuranoside[10] (0·66 g) in dimethoxyethane (15 ml) was treated, with stirring, with NaH (1·4 g) followed by MeI (4·1 ml) in dimethoxyethane. After 12 hr, MeOH (1 ml) was added and the soln was concentrated. Water (20 ml) was added and the aqueous suspension extracted with chloroform (4 × 25 ml). The combined extracts were dried and concentrated to an oil which was distilled to yield the *ether* (0·6 g, 71%), b.p. 126–130°/12 mm Hg, $[\alpha]_D^{20}$ −1·9° (*c* 0·4) (Found: C, 52·2; H, 8·8. $C_9H_{18}O_5$ requires: C, 52·4; H, 8·8%); τ 5·10 (s, H-1), 5·76–6·00 and 6·08–6·70 (m, 5H, H-2,3,4,5,5'), 6·53 (s), 6·61 (s), 6·62 (s) and 6·65 (s) (4 × OMe).

Methylation of **6** with MeI–Ag_2O yielded material having an NMR spectrum indistinguishable from authentic methyl 2,3,5-tri-O-methyl-β-D-ribofuranose prepared as described. On GLC the two samples showed identical retention times on two columns (15% Apiezon-L on Chromosorb P at 210° and 15% Carbowax-20M on Chromosorb W at 180°).

Methyl 3,5-*di*-O-*benzoyl*-2-O-*methyl*-β-D-*ribofuranoside* (**7**). The furanoside **6**, on treatment with benzoyl chloride in pyridine in the usual manner yielded a syrup which consisted (TLC in chloroform) of a major component (R_F 0·5) and a contaminant (R_F 0·66) shown to be benzoic acid. Isolation of the major component by PLC in chloroform yielded as a thick liquid the *dibenzoate* (**7**), $[\alpha]_D^{18}$ +21·9° (*c* 0·3) (Found: C, 65·2; H, 5·4. $C_{21}H_{22}O_7$ requires: C, 65·3; H, 5·7%); τ 1·94, 2·56 (both m, 10H, Ph), 4·50 (q, 1H, $J_{2,3}$ = 5 Hz, $J_{3,4}$ = 6 Hz, H-3), 5·02 (d, 1H, $J_{1,2}$ = 1 Hz, H-1), 5·30–5·80 (m, 3H, H-4,5,5'), 5·95 (q, 1H, H-2), 6·58 (s) and 6·64 (s) (2 × OMe).

In later preparations of **7**, impure furanoside, m.p. 65–74°, was benzoylated. Purification of the di-ester by PLC yielded material in 88% yield identical to material obtained by benzoylation of pure **6**.

De-esterification of **7** regenerated **6** (58%), m.p. 73–75·5°, IR spectrum identical to that of material obtained from **5**.

3,5-Di-O-benzoyl-2-O-methyl-D-ribofuranose (**8**). The dibenzoate **7** (2·6 g) was dissolved in trifluoroacetic acid–water (9 : 1 v/v) (30 ml) and the soln stored at room temp for 2 hr. Evaporation of the solvent yielded a syrup which contained {TLC in EtOAc–benzene (1 : 9 v/v)} starting furanoside (R_F 0·4) and a new component (R_F 0·15). PLC in the same solvent system gave unchanged **7** (0·44 g) and the *title compound* (1·72 g, 82% on utilized furanoside), $[\alpha]_D^{23}$ +60·1° (0·3h), +61·6° (48h) (*c* 0·3) (Found: C, 64·1; H, 5·5. $C_{20}H_{20}O_7$ requires: C, 64·5; H, 5·4%); ν_{max} 3430 cm^{-1} (OH).

1-O-*Acetyl*-3,5-*di*-O-*benzoyl*-2-O-*methyl*-β-D-*ribofuranose* (**9**). A soln of **8** (1·7 g) in pyridine (12 ml) containing Ac_2O (2 ml) was stored at room temp for 24 hr. The mixture was poured onto ice and the product isolated by chloroform extraction to give the *product* **9** (1·84 g, 96%), $[\alpha]_D^{20}$ +40·9° (*c* 0·5) (Found: C, 64·0; H, 5·3. $C_{22}H_{22}O_8$ requires: C, 63·8; H, 5·35%); τ 1·95, 2·5 (both m, 10H, Ph), 3·70 (s, 1H, H-1), 4·50 (q, 1H, $J_{2,3}$ = 5 Hz, $J_{3,4}$ = 7 Hz, H-3), 5·2–5·7 (m, 3H, H, 4,5,5'), 5·81 (d, 1H, H-2), 6·54 (s, 3H, OMe) and 8·02 (s, 3H, Ac).

1-(2'-O-*Methyl*-β-D-*ribofuranosyl*)*uracil* (2'-O-*methyluridine*) (**12**) *and* 3-(2'-O-*methyl*-β-D-*ribofuranosyl*)*uracil* (**14**). A mixture of **9** (1·84 g), 2,4-O-bis(trimethylsilyl)-uracil[23] (2·2 g) and stannic chloride (2·3 g) in 1,2-dichloroethane (40 ml) was stirred at room temp for 23 hr. TLC {$CHCl_3$–MeOH (19 : 1 v/v)} indicated the disappearance of **9** and the presence of two new components with R_F 0·23 and 0·11. Sat $NaHCO_3$ aq was added with vigorous stirring and the mixture was then filtered through a kieselguhr pad. The organic layer was separated, dried (Na_2SO_4), and concentrated to a foam (1·94 g). PLC in the $CHCl_3$–MeOH system yielded the fast and slow running components as foams (0·77 g and 0·6 g respectively). Component with R_F 0·23: τ 1·92, 2·50 (both m, 11 H, Ph and H-6 of uracil moiety), 3·96 (d, 1H, $J_{1,2}$ = 4 Hz, H-1) 4·46 (d, 1H, $J_{5,6}$ = 8 Hz, H-5 of uracil moiety), 4·6 (t, 1H, H-3), 5·20–5·50 (m, 3H, H4,5,5'), 5·78 (q, 1H, H-2) and 6·54 (s, 3H, OMe). Component with R_F 0·11: τ 1·94, 2·56 (both m, 11H, Ph and H-6 of uracil moiety), 3·46 (d, 1H, $J_{1,2}$ = 4 Hz, H-1), 4·1–4·3 (m, 2H, H-3, and H-5 of uracil moiety), 5·24–5·50 (m, 4H, H-2,4,5,5') and 6·67 (s, 3H, OMe).

A soln of the fast running component in MeOH (50 ml) to which a small quantity of Na had been added was heated under reflux for 24 hr. The soln was passed through a small column of IR 120 (H^-) ion exchange resin in MeOH and the eluent was concentrated to dryness. The syrup so obtained crystallised on trituration with light petroleum. Crystallisation of the residue from propan-2-ol: EtOAc gave **12** (0·28 g, 24%), m.p. 158–160°, $[\alpha]_D^{24}$ +41·8° (*c* 0·18 in H_2O), UV (H_2O) λ_{max}^{pH1} 262 nm (ε 10,900), λ_{max}^{pH7} 262 nm (10,700), λ_{max}^{pH12} 261 nm (ε 8,100) (Found: C, 46·7; H, 5·45; N, 10·75. Calc. for $C_{10}H_{14}N_2O_6$: C, 46·5; H, 5·5; N, 10·85%); τ (D_2O – int. ref. t-BuOH) 2·10 (d, 1H, $J_{5,6}$ = 8·0 Hz, H-6 of uracil moiety), 4·00 (d, 1H, $J_{1,2}$ = 4 Hz, H-1), 4·07 (d, 1-H, H-5 of uracil moiety), 5·4–6·3 (complex, 5H, H-2,3,4,5,5') and 6·46 (s, 3H, OMe). Lit,[8] m.p. 159°, $[\alpha]_D^{20}$ +41° (*c* 1·6 in H_2O); Lit,[4] m.p. 157–158·5°, UV (95% EtOH) λ_{max} 263 nm (ε 10,100). The mass spectrum of **12** and that of an authentic sample were identical. CD spectrum (H_2O), λ_{nm} (Δε): 292(0), 265 (+3·83), 248(0), 233(−1·21), 217(−1·21).

Debenzoylation of the slower running component gave, from propan-2-ol: EtOAc, 3-(2'-O-*methyl*-β-D-*ribofuranosyl*)*uracil* **14** (0·18 g, 16%, $[\alpha]_D^{24}$ +37·3° (*c* 0·2 in H_2O), UV (H_2O) λ_{max}^{pH1} 263 nm (ε 7,000), λ_{max}^{pH7} 263 nm (ε 7,000),

λ_{max}^{pH12} 292 nm (ϵ 9,500)* (Found: C, 46·2; H, 5·4; N, 10·8. $C_{10}H_{14}N_2O_6$ requires: C, 46·5; H, 5·5; N, 10·85%); τ (D_2O—int. ref. t-BuOH) 2·46 (d, 1H, $J_{5,6}$ = 7·5 Hz, H-6 of uracil moiety), 3·66 (d, 1H, $J_{1,2}$ = 3 Hz, H-1), 4·13 (d, 1H, H-5 of uracil moiety), 5·30–5·64 and 5·90–6·36 (complex, 5H, H-2,3,4,5,5′) and 6·52 (s, OMe).* Its mass spectrum had a base peak at *m/e* 113 and showed a significant peak at *m/e* 146 (50% relative abundance), diagnostic of 2′-O-methyl nucleosides.[15] The compound did not consume sodium metaperiodate. CD spectrum (H_2O), λ_{nm} ($\Delta\epsilon$): 280(0), 273(−1·1), 260(0), 250(+1·98), 239-(+1·76), 223(0).

Paper chromatography. Nucleosides **12** and **14** were subject to chromatography in system (A), (B) and (C) with uridine as a standard. Measured R_F values are given in the order uridine, (**12**), (**14**): (A) 0·39, 0·57, 0·58; (B) 0·31, 0·44, 0·58; (C) 0·12, 0·33, 0·35.

Methylation and hydrolysis of **14**. A mixture of **14** (9 mg), MeI (2 ml) and Ag_2O (0·12 g) was stirred vigorously at room temp for 12 hr. The mixture was filtered through kieselguhr and the filtrate concentrated to yield a residue which was dissolved in 4N HSO_4 (0·2 ml). The soln was maintained at 100° for 3 hr, and then neutralised by the addition of Amberlite IRA-400 resin (OH). The soln was investigated by paper chromatography in systems (A) and (D), and on viewing the chromatograms under UV radiation (254 nm) spots were observed at R_f 0·32 (solvent A) and R_F 0·35 (solvent D). 1-Methyluracil had R_F 0·32 and 0·35, and 3-methyluracil (prepared by methylation and hydrolysis of uridine) had R_F 0·72 and 0·86 in solvents (A) and (D) respectively.

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*Cf reported data for 3-β-D-ribofuranosyluracil:[19] UV (H_2O) λ_{max}^{pH1} 262 nm (ϵ 7,700), λ_{max}^{pH11} 292 nm (ϵ 10,600); τ (D_2O) 2·40 (d, 1H, $J_{5,6}$ = 8·0 Hz, H-6 of uracil moiety), 3·63 (d, 1H, $J_{1,2}$ = 3·5 Hz, H-1) and 4·10 (d, 1H, H-5 of uracil moiety).

REFERENCES

[1]R. H. Hall, *The Modified Nucleosides in Nucleic Acids*. Columbia University Press, London (1971)
[2]A. D. Broom and R. K. Robins, *J. Am. Chem. Soc.* **87**, 1145 (1965)
[3]T. A. Khwaja and R. K. Robins, *Ibid.* **88**, 3640 (1966)
[4]D. M. G. Martin, C. B. Reese and G. F. Stephenson, *Biochemistry* **7**, 1406 (1968)
[5]J. B. Gin and C. A. Dekker, *Ibid.* **7**, 1413 (1968)
[6]F. Rottman and K. Heinlein, *Ibid.* **7**, 2634 (1968)
[7]M. J. Robins and S. R. Naik, *Biochim. Biophys. Acta* **246**, 341 (1971)
[8]Y. Furukawa, K. Kobayashi, Y. Kanai and M. Honjo, *Chem. and Pharm. Bull. Japan* **13**, 1273 (1965)
[9]A. H. Haines and P. R. Lundie, *J. Chem. Soc.* (C) 1691 (1970)
[10]R. Barker and H. G. Fletcher, *J. Org. Chem.* **26**, 4605 (1961)
[11]E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta* **121**, 1171 (1959)
[12]J. E. Christensen and L. Goodman, *Carbohydrate Res.* **7**, 510 (1968)
[13]R. U. Lemieux and J. W. Lown, *Canad. J. Chem.* **41**, 889 (1963)
[14]U. Niedballa and H. Vorbruggen, *Angew. Chem. Internat. Edn.* **9**, 461 (1970)
[15]H. A. Howlett, M. W. Johnson, A. R. Trim, J. Eagles and R. Self, *Analyt. Biochem.* **39**, 429 (1971)
[16]D. Shugar and J. J. Fox, *Biochim. Biophys. Acta* **9**, 199 (1952)
[17]J. P. Scannel and F. W. Allen, *J. Org. Chem.* **25**, 2143 (1960)
[18]M. Sano, *Chem. Pharm. Bull. Japan* **10**, 320 (1962)
[19]M. W. Winkley and R. K. Robins, *J. Chem. Soc.* (C) 791 (1969)
[20]T. R. Emerson, R. J. Swan and T. L. V. Ulbricht, *Biochemistry* **6**, 843 (1967)
[21]C. E. Ballou, *J. Am. Chem. Soc.* **79**, 165 (1957)
[22]W. B. Gleason and R. Barker, *Canad. J. Chem.* **49**, 1433 (1971)
[23]E. Wittenburg, *Z. Chem.* **4**, 303 (1964)