Tetrahedron. Vol 25, pp 5989 to 5994 Pergamon Press 1968. Printed in Great Britain

UNCONVENTIONAL NUCLEOTIDE ANALOGUES—III[1]

4-(N_1-PYRIMIDYL)-2-AMINOBUTYRIC ACIDS

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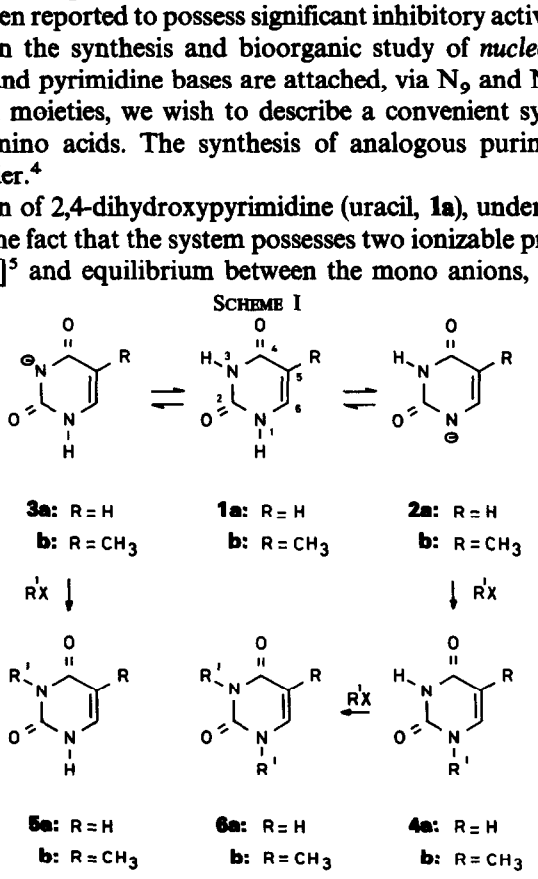
(Received in the UK 8 August 1969; Accepted for publication 28 August 1969)

Abstract—Alkylation of 4-ethoxy-2-hydroxypyrimidines (**10a, b**), via their anions, provides a convenient procedure for preparing N_1-substituted derivatives. Reaction of **10a, b** with t-butyl 4-bromo-2-benzyloxycarbamidobutyrate (**17**) gives good yields of the N_1-alkylated products **18a, b**. The latter have been converted to uracil- (**20a**), thymine- (**20b**) and cytosine- (**21**) substituted α-aminobutyric acids.

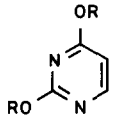
THE synthesis of pyrimidyl amino acids has attracted a great deal of attention in the search for a new class of potential antimetabolites. Several C-substituted pyrimidine derivatives have been reported to possess significant inhibitory activity. In connection with a program on the synthesis and bioorganic study of *nucleotide analogues* in which the purine and pyrimidine bases are attached, via N_9 and N_1 respectively, to suitable functional moieties, we wish to describe a convenient synthesis of certain N_1-pyrimidyl α-amino acids. The synthesis of analogous purine derivatives has been reported earlier.[4]

The N-alkylation of 2,4-dihydroxypyrimidine (uracil, **1a**), under basic conditions, is complicated by the fact that the system possesses two ionizable protons ($pKa_{N(1)}$ = 9·43 : $pKa_{N(3)} > 13$][5] and equilibrium between the mono anions, **2** and **3**, can lead

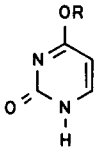
SCHEME I



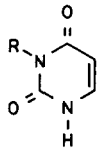
to the formation of a mixture of isomeric products (**4** and **5**). While, in principle, it should be possible to obtain N_1-substituted products, selectively, by carefully controlling the pH,[6] this objective is not attained since in the N_1-monosubstituted product the acidity of the N_3 proton is increased sufficiently (p*Ka* 9·71) to give a competitive dialkylation reaction; namely, formation of **6**. Several approaches have been devised to attain N_1-alkylation specifically and to circumvent the dialkylation process. Amongst these the (a) alkylation of 2,4-dialkoxy-[7] or disilyloxypyrimidines[8] (**7**); (b) reaction of mercury salts of 4-alkoxy-2-keto-1,2-dihydropyrimidines[9] (**8**) with active halides; (c) direct alkylation of 2,4-dihydroxypyrimidines in DMSO.[10] in presence of anhydrous K_2CO_3; (d) Michael addition of dihydroxypyrimidines[11] and (e) alkylation of N_3-substituted 2,4-dihydroxpyrimidines[12] (**9**) deserve special mention in view of their widespread application. It is noteworthy that none of the aforementioned methods are wholly satisfactory in view of the limitations from which they suffer. In (a), R = alkyl, for example, the alkylating reagent, usually a halide, has to be of greater reactivity than the reagent R-X, which is liberated during the reaction and can competitively cause alkylation of **7**.[10] The Michael addition (d) is restricted only to electrophilic olefins, while alkylation of N_3-substituted systems (e) rests solely upon the availability of suitable protecting groups which can be con-



7 R = alkyl or silyl



8 R = alkyl



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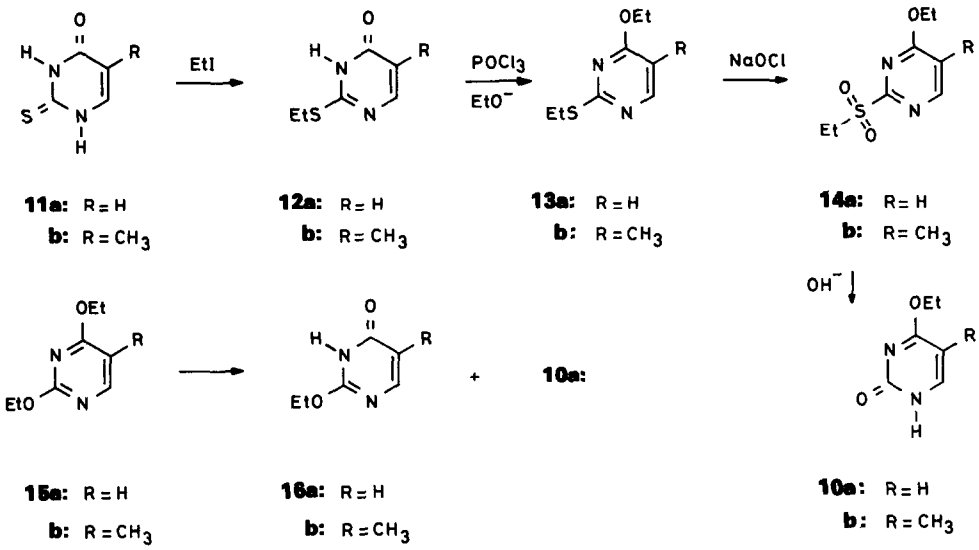
veniently removed later in the sequence. The other methods mentioned are also frequently limited in their application to simple alkylating agents which are devoid of functional complexities.[8b]

A form in which 2,4-dihydroxypyrimidines can, in principle, be most effectively utilized for exclusive substitution at N_1 and, what is more, with non-activated alkyl halides, is represented by the anion derived from 4-alkoxypyrimidines (**10**). Such monoethers can be readily prepared via the sequence **11** → **12** → **13** → **14** → **10**[13] (Scheme II). In an alternate approach **10a** was directly obtained by careful hydrolysis of **15a** and separation of the 4-alkoxy derivative from the accompanying 2-alkoxy isomer[14] (**16a**). Surprisingly, an analogous hydrolysis of the thymine system **15b** gave **16b** as the only monoalkoxy product. With the facile availability of **10a** and **10b** established, the approach involving selective substitution at N_1, with non-activated alkylating reagents, becomes a practically attainable objective.

For synthesis of the desired pyrimidine substituted amino acid derivatives, **10a** and **10b** were coupled with t-butyl 4-bromo-2-benzyloxycarbamidobutyrare[4] (**17**), in DMF, with sodium hydride as base. (Scheme III). The reaction products of this alkylation reaction constituted good yields of the N_1-substituted derivatives (**18a**, **18b**), with some amount of **19a** and **19b**, from which the former could be conveniently separated by fractional crystallization. The formation of **19a**, **b** obviously occurs via

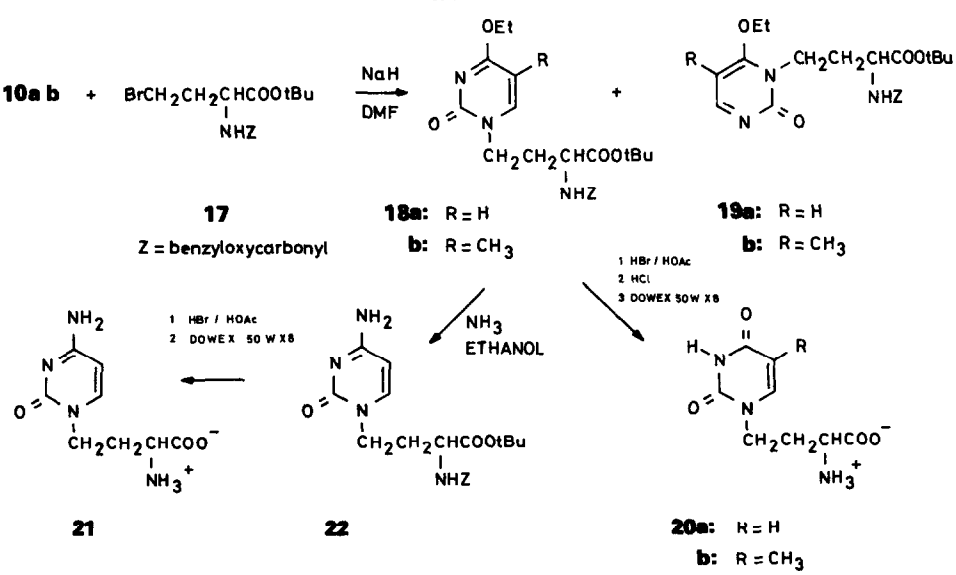
SCHEME II



the resonance form of the anion from **10a, b** in which the negative charge is located at N_3.

The conversion of **18a, b** to the uracil (**20a**) and thymine (**20b**) substituted α-amino-butyric acids (Scheme III) was achieved by unmasking of the amino acid functions with HBr/HOAc, followed by hydrolysis of the N_4-ethoxy group (dil HCl). Final isolation of the amino acid from its salt was carried out by passing a solution of the latter over a Dowex 50W-X 8 ion exchange column.

SCHEME III



For preparation of the cytosine derivative **21**, the ester **18a** was aminated by treatment with ethanol saturated with ammonia, at room temperature, and the resulting 4-amino-pyrimidine system **22** subjected to deblocking of the amine and carboxyl functions. Conventional separation of the amino acid was achieved over a Dowex column. The structure of the various products was attested by their spectroanalytical data. Distinction between the N_1-alkylation (**18a, b**) and N_3-alkylation (**19a, b**) derivatives was made on the basis of UV spectra of the compounds; comparison being made with spectra of appropriate model systems. The UV maxima of **18a, b, 19a, b** and that of the amino acids **20a, b** and **21** at different pH,[15] are described in Table 1. We have also observed that the NMR spectra of the N_1- and N_3-substituted derivatives of pyrimidines show marked differences which can be utilized for structural assignment of the positional isomers. A discussion on the application of NMR spectroscopy in distinguishing between N_1- and N_3-pyrimidine derivatives will be presented elsewhere.

TABLE 1

Compound	Solvent	λ_{max}(nm)	$\varepsilon \times 10^{-3}$
18a	ethanol	278	6·26
18b	ethanol	284	5·99
19a	ethanol	259	4·26
19b	ethanol	265	4·81
20a	0·1N HCl	265	9·58
20a	pH7*	265	10·26
20a	0·1N NaOH	264	7·32
20b	0·1N HCl	270	9·25
20b	pH7*	270	9·94
20b	0·1N NaOH	269	8·44
21	0·1N HCl	281	13·73
21	pH7*	272·5	9·53
21	0·1N NaOH	274	8·43
22	ethanol	274	10·00

* Sörensen-Phosphate-Buffer (0·0066 m)

EXPERIMENTAL

All m.ps are uncorrected. Analyses were carried out by Messrs. H. Pieters, W. J. Buis and W. van Duyl of the Microanalytical Department of this laboratory. IR, UV and mass spectra were recorded on Unicam SP 200, Cary-14 Recording and AE MS-9 Spectrometers respectively. NMR spectra (concentration 100 mg/0·5 ml, unless otherwise stated) were measured on a Varian Associates Model A-60 instrument.

2-O-*Ethylthymine* (**16b**). To a soln of 7·25 g of Na (0·315 mol) in 350 ml 90% EtOH were added 38·3 g (0·23 mol) 2,4-di-O-ethylthymine.[16] The mixture was refluxed for 170 hr and the solvent was evaporated. The residue was taken up in 150 ml water and the soln extracted with ether. From the ether layer 9·0 g starting 2,4-di-O-ethylthymine was recovered. The water layer, after evaporation to a small volume (80 ml), was acidified with HOAc at 60°. **16b** crystallized out upon standing overnight at 0°, yield 18·3 g (68%), m.p. 147–148°; IR (KBr) 1660, 1630, 1540 cm^{-1} (characteristic for 2-O-ethylthymine); UV (EtOH) 270·5 nm (6910); NMR ($CDCl_3$-Varian HA-100) 1·98, d (3H, C_5-CH_3 J = 1 c/s) 7·56, qu (1H, C_6-H, J = 1 c/s). Mol. wt. (MS) 154·07649; calc. 154·07422. (Found: C, 54·4; H, 6·5; N, 18·3. $C_7H_{10}N_2O_2$ requires: C, 54·53; H, 6·54; N, 18·17%).

1(*γ-Benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl*)-4-O-*ethyluracil* (**18a**). 2·719 g (19·4 mmol) of **10a** were dissolved in 50 ml DMF and 0·931 g (19·4 mmol) of a NaH dispersion (50% in oil) were added. The mixture was stirred for 1½ hr at 50°, after which a soln of 7·22 g (19·4 mmol) **17** in 60 ml DMF were added. The reaction mixture was brought to 80° and stirred at that temp for 64 hr. After evaporation of the solvent, the residual material was triturated with $CHCl_3$. The insoluble salts were filtered off by suction and the solvent was evaporated to give an oil which – after crystallization with ether – provided 5·0 g (60%) of pure white **18a**; TLC (silicagel–EtOAc) indicated the presence of some more **18a** (R_f 0·64) in the ether layer and a component with R_f 0·80. The ether was evaporated and the resulting oil passed over a silicagel column, which provided another 0·70 g of **18a** and 1·09 g of **19a** as an oil. After recrystallization from EtOH/water the total yield of **18a** was 5·60 g (67%): m.p. 130·5–132. IR (KBr) 1730 (ester C=O), 1660, 1630 and 1540 (characteristic for N_1 substituted 4-O-alkyl-uracil) and 1310 cm^{-1} (OCH_2CH_3); UV (EtOH) 278 nm (6260); NMR ($CDCl_3$): δ 1·43, s (9H, *t*-butyl), 5·81, d (1H, C_5–H, J = 7 c/s) and 7·51, d (1H, C_6–H, J = 7 c/s). Mol. wt. (MS) 431·20340; calc. 431·20562. (Found: C, 61·3; H, 7·0; N, 9·8. $C_{23}H_{31}N_3O_6$ requires: C, 61·24; H, 6·77; N, 9·74%).

3-(*γ-Benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl*)-4-O-*ethyluracil* (**19a**) yield 1·09 g (13%); IR ($CHCl_3$) 1580 and 1570 cm^{-1} (characteristic for N_3-alkylated 4-O-ethyluracil); UV (EtOH) 259 nm (4260); NMR ($CDCl_3$) δ 1·46, s (9H, t-butyl) 5·10, s (2H, benzylic CH_2) 6·32, d (1H, C_5-H, J = 5½ c/s) 7·32, s (5H, phenyl) 8·13, d (1H, C_6-H, J = 5½ c/s). Mol. wt. (MS) 431·20585; calc. 431·20562.

1-(*γ-Benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl*)-4-O-*ethylthymine* (**18b**). Following the procedure described for the conversion of **10a** to **18a**; compound **10b** resulted in the formation of **18b** and **19b**. Starting with 3·93 g (25·5 mmol) of **10b**, 0·612 g (25·5 mmol) of NaH (50% in oil), 9·49 g (25·5 mmol) of **17** in 150 ml DMF, 7·0 g (62%) of **18b** was obtained, m.p. 106–108°; IR (KBr) 1740 (ester C=O); 1665, 1640 and 1540 cm^{-1} (characteristic for N_1 substituted 4-O-ethylthymine[17]); UV (EtOH) 284 nm (5990); NMR (50 mg in 0·5 ml $CDCl_3$) δ 1·45, s (9H, t-butyl) 1·90, d (3H, C_5-CH_3) 7·23, m (1H, C_6-H). Mol.wt. (MS) 445·22472; calc. 445·22127. (Found: C, 62·1; H, 7·1; N, 9·4. $C_{23}H_{31}N_3O_6$ requires: C, 62·00; H, 7·01; N, 9·43%).

3-(*γ-Benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl*)-4-O-*ethylthymine* (**19b**) yield 2·0 g (18%); IR ($CHCl_3$) 1600 and 1575 cm^{-1} (characteristic for N_3-alkylated 4-O-ethylthymine); UV (EtOH) 265 nm (4810); NMR ($CDCl_3$) δ 1·46, s (9H, t-butyl) 2·03, broadened s (3H, C_5-CH_3), 5·09, s (2H, benzylic CH_2) 7·33, s (5H, phenyl), 7·93, m (1H, C_6-H). Mol. wt. (MS) 445·22497; calc. 445·22127.

1-(*γ-Benzyloxycarbonylamido-γ-t-butoxycarbonylpropyl*)*cytosine* (**22**). 2 g of **18a** were dissolved in 50 ml abs EtOH saturated with ammonia, and the soln was placed in a Carius tube. After 3 weeks at room temp the white ppt consisting of **22** was isolated by suction. The weight of the analytically pure compound was 1·30 g (70%), m.p. 215°; IR (KBr) 1660 and 1645 cm^{-1} (characteristic for N_1-substituted cytosine); UV (EtOH) 274 nm (10000); NMR 50 mg in 0·5 ml DMSO-d_6) δ 5·63, d (1H, C_5-H, J = 7 c/s) 7·05, s (2H, C_4-NH_2) 7·38, s (5½ H, phenyl) 7·47 part of a d (½H, C_6-H). Mol. wt. (MS) 402·19261; calc. 402·19261; calc. 402·19031. (Found: C, 59·5; H, 6·4; N, 14·1. $C_{20}H_{26}N_4O_5$ requires: C, 59·69; H, 6·51; N, 13·92%).

1-(*γ-Amino-γ-carboxypropyl*)*uracil* (**20a**). 2·00 g of **18a** were dissolved in 15 ml HOAc. To this soln, 15 ml HBr/HOAc (40%) were added. After stirring at room temp for 2·5 hr abs ether (60 ml) was added and the stirring continued for a further ½ hr. The precipitated 1-(γ-amino-γ-carboxypropyl)-4-O-ethyluracil dihydrobromide was isolated by suction and washed thoroughly with dry ether. The di-HBr salt was dissolved in 200 ml 1N HCl and refluxed for 2 hr. The soln was evaporated and the HCl salt of the amino acid so formed was dissolved in 30 ml water and put on a Dowex 50W-X 8 cation exchanger in the H^+ form. After washing with distilled water until the eluate showed a negative test for Cl^- (with $AgNO_3$), elution was carried out with 2N NH_4OH, until the eluate was basic. Upon further elution with 10% NH_4OH and evaporation amino acid **20a** was obtained as a colourless product. After recrystallization from water the weight amounted to 800 mg (81%), m.p. 258°. One ninhydrine positive spot on TLC; IR (KBr) 1710, 1680 and 1660 (characteristic for the diketo structure of uracil) 1623 (NH_3^+) and 1590 cm^{-1} (COO^-); UV (Table 1); NMR (50 mg **20a** and 50 mg K_2CO_3 dissolved in 0·75 ml D_2O dioxan as internal standard) δ 1·8–2·3, m (2H, CH_2) 3·75–4·2, m (N-CH_2 and HC—COO^-) 3·78 dioxan, 5·85, d (1H, C_5-H, J = 7½ c/s) 7·63, d (1H, C_6-H, J = 7½ c/s). Mol. wt. (MS) 213·07619; calc. 213·07495. (Found: C, 44·9; H, 5·3; N, 19·6. $C_8H_{11}N_3O_4$ requires: C, 45·07; H, 5·20; N, 19·71%).

1-(*γ-Amino-γ-carboxypropyl*)*thymine* (**20b**). 2·00 g of **18b** was converted into **20b** by the procedure described for the conversion of **18a** to **20a**, yield: 810 mg (81%), TLC: one ninhydrine positive spot, m.p. 252–258°; IR (KBr) 1685, 1670 (diketo structure of thymine) 1620 (NH_3^+); UV (Table 1); NMR (50 mg of **20b** and 50 mg

K_2CO_3 dissolved in 0·75 ml D_2O; dioxane as internal standard δ 1·8–2·3, m (CH_2) 1·92, d (C_5-CH_3, J = 1 c/s) 3·8–4·2 (N-CH_2 and HC—COO^-) 3·79 (dioxan). 7·48, qu (1H, C_6-H, J = 1 c/s). Mol. wt. (MS) 227·09334; calc. 227·09060. (Found: C, 47·3; H, 5·7; N, 18·5. $C_9H_{13}N_3O_4$ requires: C, 47·57; H, 5·77; N, 18·49%).

1-(γ-Amino-γ-carboxypropyl)cytosine (**21**). 687 mg of **22** were dissolved in 5 ml HOAc and 5 ml HBr/HOAc (40%). After stirring at room temp for 2·5 hr abs ether (40 ml) was added and the stirring continued for a further ½ hr. The precipitated 1-(γ-amino-γ-carboxypropyl)cytosine.dihydrobromide was isolated by suction and washed thoroughly with dry ether. This HBr salt was put on an ion exchange column and the free amino acid isolated in the usual way, yield 270 mg (75%), TLC (one ninhydrine positive spot). M.p. dec > 318; IR (KBr) 1680, 1630 and 1600 cm^{-1}; UV (Table 1); NMR (50 mg **21** and 50 mg K_2CO_3 dissolved in 0·75 ml D_2O; dioxan as internal standard) δ 3·69 (dioxan) 5·90, d (1H, C_5-H, J = 7 c/s) 7·53, d (1H, C_6-H, J = 7 c/s). (Found: C, 45·3; H, 5·6; N, 26·5; $C_8H_{12}N_4O_3$ requires: C, 45·28; H, 5·70; N, 26·40%).

Acknowledgement—We thank Professor Dr H. O. Huisman for his interest and encouragement.

REFERENCES

[1] Part II. A. J. H. Nollet and U. K. Pandit. *Tetrahedron* **25**, 5983 (1969).
[2] Taken in part from the forthcoming doctorate dissertation of A. J. H. Nollet.
[3] To whom all correspondence should be addressed.
[4] Part I. A. J. H. Nollet, C. M. Huting and U. K. Pandit, *Tetrahedron* **25**, 5971 (1969).
[5] J. Jonáš and J. Gut, *Coll. Czech. Chem. Comm.* **27**, 716 (1962).
[6] [a] K. Nakanishi, N. Suzuki, and F. Yamazaki, *Bull. Chem. Soc. Japan* **34**, 53 (1961);
[b] E. Wittenburg, *Chem. Ber.* **99**, 2391 (1966).
[7] G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.* **52**, 4489 (1930).
[8] [a] E. Wittenburg, *Chem. Ber.* **101**, 1095 (1968);
[b] D. T. Browne, J. Eisinger and N. J. Leonard, *J. Am. Chem. Soc.* **90**, 7302 (1968).
[9] T. Nishimura and B. Shimizo, *Chem. Pharm. Bull. Japan* **13**, 224 (1965).
[10] B. R. Baker and G. B. Chheda, *J. Pharm. Sci.* **54**, 25 (1965).
[11] [a] Yu. P. Shvachkin, N. T. Azarova and J. J. Rapanovich, *Vestnik. Mosk. Univ., Ser II Chim.* **18**, 68 (1963);
[b] A. Nováček and M. Lissnerová, *Coll. Czech. Chem. Comm.* **33**, 604 (1968).
[12] A. Nováček, D. Hesoun and J. Gut, *Ibid.* **30**, 1890 (1965).
[13] [a] H. Plaut, *Organic sulphur compounds* (Edited by N. Kharasch) Vol. 1; p. 512. Pergamon Press, Oxford (1961).
[b] T. Ueda and J. J. Fox, *J. Med. Chem.* **6**, 697 (1963);
[c] E. Wittenburg, *J. Prakt. Chem.* **33**, 165 (1966).
[14] G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.* **57**, 552 (1935).
[15] [a] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta* **9**, 199 (1952);
[b] J. J. Fox and I. Wempen, *Adv. Carbohydrate Chem.* **14**, 283 (1959).
[16] W. Schmidt-Nickels and T. B. Johnson, *J. Am. Chem. Soc.* **52**, 4511 (1930).
[17] E. Wittenburg, *Chem. Ber.* **99**, 2380 (1966).