Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 11, pp. 2255-2260, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $7.00+0.00

0957-4166(94)00305-X

TWO CATIONIC ANALOGUES OF DISACCHARIDE HETEROLYSIS. POTENTIAL EPITOPES FOR PREPARING ANTIBODIES WITH SACCHARIDASE ACTIVITY

Jochen Lehmann* and Beatrice Rob,

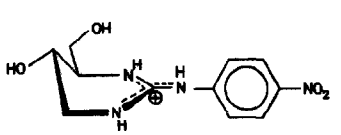
Institut für Organische Chemie und Biochemie
Universität Freiburg, Albertstrasse 21
D 79104 Freiburg, Germany

Abstract The preparation of two analogues of disaccharides containing the structural element of a guanidinium ion is described. By mimicking the hypothetical transition-state of the heterolysis of a disaccharide they stereospecifically inhibit glycosidases.

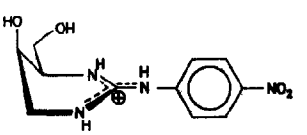
"Custom tailored" antibodies with glycanase activity may be of interest for the selective cleavage of glycosidic bonds within oligo- or polysaccharides. Catalytic antibodies have been prepared before for non carbohydrate organic substrates as reaction catalysts[1]. Recently regio- and stereospecific reactions, otherwise almost impossible to perform, were catalysed by antibodies[2]. So far, the only antibody assisted cleavage of a very sensitive gyclosidic bond was published by J. L. Reymond et al.[3]. For the preparation of such antibodies, protein linked piperidinium derivatives were used as epitopes.

The principle of catalysis by proteins, be it an enzyme or an antibody, is stabilisation of the transition-state by tight, noncovalent binding[4]. We assume that the transition-state of the heterolysis of a given disaccharide resembles a halfchair or envelope conformation of the glyconic unit with a developing positive charge around its anomeric center. The properly placed hydroxy groups in the glyconic, but also in the aglyconic glycosyl unit would supply by hydrogen bonding a large part of the stabilisation energy and at the same time warrant stereospecificity.

Since sixmembered cyclic N-phenyl-guanidinium ions carrying properly placed hydroxymethyl and hydroxy groups like compounds 1 and 2 were shown to be good competitive inhibitors for the corresponding glycosidases[5], we felt encouraged to synthesise analogous compounds as transition-state mimics of an enzymic disaccharide cleavage.



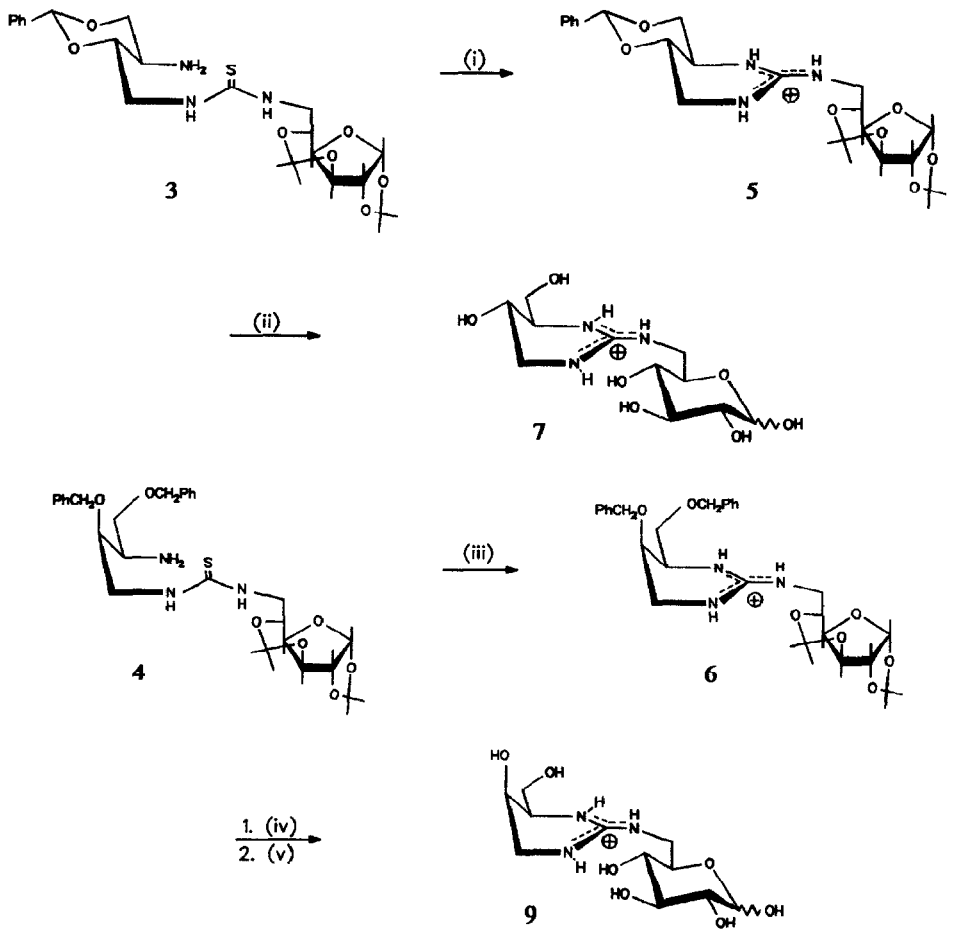
1



2

For reasons of practicability 1,6-linked structures mimicking gentiobiose and allolactose were chosen.

Analogous to the synthesis of the cyclic guanidines **1** and **2** the synthesis of the disaccharides started from the corresponding diamines 1,3-diamino-2,4-*O*-benzylidene-1,3-dideoxy-D-erythritol[6] and 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol[6]. Treatment of the two diamines with 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-isothiocyanato-α-D-glucofuranose[7] gave the corresponding thiourea derivatives **3** and **4**, which were cyclisised in the presence of PbO[8] to the blocked cyclic guanidines **5** and **6**.



(i) PbO, EtOH (ii) 50% trifluoroacetic acid (iii) PbO, EtOH (iv) 80% trifluoroacetic acid (v) Pd/C, H_2, acetic acid/MeOH

Compound **5** was deprotected using 50% aqueous trifluoroacetic acid at 40°C to give **7** with 98% yield. Compound **6** was partly deprotected with 80% aqueous trifluoroacetic acid at 60°C to give **8**. The benzyl groups in compound **8** were removed by hydrogenolysis using Pd/C in acetic acid, to give compound **9** with 100% yield.

Compounds **7** - **9** were obtained as anomeric mixtures, but could be characterised by their H-H COSY spectra and FAB-MS.

Compounds **7** and **9** were shown to be stereospecific competitive inhibitors of ß- and α-glycosidases. In analogy with the 1,6-linked compounds **7** and **9** the 1,4-linked isomeres will be prepared and results reported elsewhere.

Acknowledgement. We thank the Deutsche Forschungsgemeinschaft for financial support.

Experimental

General Methods. Thin layer chromatography was performed on Silica Gel 60 F_{254} (Merck) with detection by quenching of fluorescence and/or charring with 2% H_2SO_4/MeOH. Column chromatography was performed on Silica Gel 32-63 A (ICN).

Optical rotations were obtained with a Schmidt & Haensch Polartronic I polarimeter, IR spectra with a Perkin-Elmer 1320 spectrophotometer. ^{1}H-NMR spectra were recorded at 400 MHz (Bruker AM 400) for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal DSS). Mass spectra were recorded on Finnigan MAT 312.

*N[3-Amino-2,4-*O*-benzylidene-1,3-dideoxy-D-erythrityl]-N'[6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranosyl]-thiourea* **3**. To a solution of 1,3-diamino-2,4-*O*-benzylidene-1,3-dideoxy-D-erythritol[6] (2.3 g, 11.05 mmol) in dichloromethane (150 ml) was added dropwise at room temperature a solution of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-isothiocyanato-α-D-glucofuranose[7] (3.66 g, 12.15 mmol) in dichloromethane (50 ml). After 2h, t.l.c. (ethyl acetate/methanol/water 27:2:1) revealed a major product (R_f 0.45). The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (ethyl acetate) to afford **3** as a colourless foam, which was used without further characterisation to prepare compound **5**.

*(4R,5S)-2-[N-(6-Deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranosyl)amino]-benzylidene-5-oxy-4-oxymethyl-1,4,5,6-tetrahydropyrimidine* **5**. The crude thiourea **3** (4.2 g, 8.28 mmol) was dissolved in ethanol (250 ml) and yellow PbO (9.3 g, 41 mmol) was added. The mixture was heated under reflux for 9h when t.l.c. (ethyl acetate/methanol/ammonia (25%) 5:4:1) revealed a single product (R_f 0.50). After filtration of the mixture the solvent was evaporated *in vacuo*. Purification by column

chromatography (ethyl acetate/methanol/ammonia(25%) 5:4:1) yielded the guanidine **5** (3.18 g, 81%) as a colourless foam; $[\alpha]_D^{23}$ +74.0 (*c*, 1.0 in MeOH); ν_{max}(KBr): 3400 (br,NH) 1650 (C=N) 1540 (NH) cm^{-1}; δ_H (400 MHz; $CDCl_3$): 1.32, 1.36, 1.37, 1.49 (4 x 3H, 4 x s, 4 x Me) 3.23 (1 H, dd, $J_{6'a,6'b}$ 14.25, $J_{6'a,5'}$ 6.75, H-6'a) 3.29 (1 H, t, $J_{6a,6b}$ 10.5, $J_{6a,5}$ 10.5, H-6a) 3.38 (1 H, ddd, $J_{4,\alpha a}$ 10.5, $J_{4,\alpha b}$ 4.8, $J_{4,5}$ 8.0, H-4) 3.44 (1 H, dd, $J_{6'b,5'}$ 3.0, H-6'b) 3.49 (1 H, dd, $J_{6b,5}$ 6.0, H-6b) 3.64 (1 H, ddd, $J_{5',4'}$ 7.2, H-5') 3.70 (1 H, t, $J_{\alpha a,\alpha b}$ 10.5, H-αa) 3.77 (1 H, ddd, H-5) 4.20 (1 H, d, $J_{3',4'}$ 3.75, H-3') 4.34 (1 H, dd, H-4') 4.42 (1 H, dd, H-αb) 4.57 (1H, d, $J_{2',1'}$ 3.75, H-2') 5.62 (1 H, s, C<u>H</u>Ph) 5.98 (1 H, d, H-1') 7.31-7.53 (5 H, m, ArC<u>H</u>). (Found: C, 60.81; H, 7.02; N, 8.90. $C_{24}H_{33}N_3O_7$ requires: C, 60.62; H, 6.99; N, 8.84%).

(4R,5S)-2-[N-[6-Deoxy-α/β-D-glucopyranosyl)amino]-5-hydroxy-4-hydroxymethyl-1,4,5,6-tetrahydropyrimidine, trifluoroacetate **7**. The guanidine **5** (0.25 g, 0.526 mmol) was dissolved in 50% aqueous trifluoroacetic acid (10 ml) and stirred for 2h at 40°C. T.l.c. (ethyl acetate/methanol/ammonia(25%) 2:2:1) indicated complete conversion to a single product (R_f 0.23). The solvent was removed *in vacuo*, and the residue was coevaporated with water (4 x 10 ml) to afford **7** as a colourless oil (0.217 g, 98%); $[\alpha]_D^{23}$ +38.7 (*c*, 1.60 in H_2O); δ_H (400MHz; D_2O): 3.24 (1 H, dd, $J_{1'(\beta)/2'(\beta)}$ 7.8, $J_{2'(\beta)/3'(\beta)}$ 9.3, H-2'(β)) 3.33 (1 H, t, $J_{4'(\alpha)/3'(\alpha)} = J_{4'(\alpha)/5'(\alpha)}$ 9.75, H-4'(α)) 3.34 (1 H, dd, $J_{4'(\beta)/3'(\beta)}$ 9.3, $J_{4'(\beta)/5'(\beta)}$ 4.2, H-4'(β)) 3.31-3.39 (2 H, m, H-6a(α), H-6a(β)) 3.43 (1 H, dd, $J_{6'a(\beta)/6'b(\beta)}$ 14.25, $J_{6'a(\beta)/5'(\beta)}$ 8.25, H-6'a(β)) 3.44 (1H, dd, $J_{6'a(\alpha)/6'b(\alpha)}$ 14.7, $J_{6'a(\alpha)/5'(\alpha)}$ 6.0, H-6'a(α)) 3.48 (1 H, t, $J_{3'(\beta)/4'(\beta)} = J_{3'(\beta)/2'(\beta)}$ 9.3, H-3'(β)) 3.53 (1 H, dd, $J_{2'(\alpha)/3'(\alpha)}$ 9.75 $J_{2'(\alpha)/1'(\alpha)}$ 3.75, H-2'(α)) 3.51-3.58 (7 H, m, H-6b(α), H-6b(β), H-4)α), H-4(β), H-5'(β), H-6'b(α), H-6'b(β)) 3.57 (1 H, dd, $J_{\alpha a(\alpha)/\alpha b(\alpha)}$ 9.75 $J_{\alpha a(\alpha)/4(\alpha)}$ 3.75, H-αa(α)) 3.64 (1 H, dd, $J_{\alpha a(\beta)/\alpha b(\beta)}$ 16.5, $J_{\alpha a(\beta)/4(\beta)}$ 6.0, H-αa(β)) 3.65 (1 H, dd, $J_{\alpha b(\beta)/4(\beta)}$ 5.25, H-αb(β)) 3.67 (1 H, dd, $J_{\alpha b(\alpha)/4(\alpha)}$ 5.7, H-αb(α)) 3.71 (1 H, t, $J_{3'(\alpha)/4'(\alpha)}$ 9.75, H-3'(α)) 3.91 (1 H, ddd, $J_{5'(\alpha)/4'(\alpha)}$ 2.7, $J_{5'(\alpha)/6'b(\alpha)}$ 9.0, H-5'(α)) 4.17 (2 H, m, H-5(α), H-5(β)) 4.64 (1 H, d, H-1'(β)) 5.23 (1 H, d, H-1'(α)); FAB-MS *m/z* 308 $(M-H)^+$.

*N[3-Amino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threityl]-N'-[6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranosyl]-thiourea* **4**. To a solution of 1,3-diamino-2,4-di-*O*-benzyl-1,3-dideoxy-D-threitol[6] (1.2 g, 3.99 mmol) in dichloromethane (75 ml) was added at room temperature a solution of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-isothiocyanato-α-D-glucofuranose[7] (1.32 g, 4.38 mmol) in dichloromethane (25 ml). After 3h t.l.c. (ethyl acetate/methanol/ammonia(25%) 10:2:1) indicated a major product (R_f 0.58), and the solution was evaporated *in vacuo*. The residue was purified by column chromatography (ethyl acetate) to afford **4** as a slightly yellow oil (1.92 g, 80%). The crude thiourea was used whithout characterisation for the preparation of the guanidine **6**.

(4R,5R)-2[N-(6-Deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranosyl)amino]-5-O-benzyl-4-O-benzylmethyl-1,4,5,6-tetrahydropyrimidine **6**. The crude thiourea **4** (1.92 g, 3.19 mmol) was dissolved in ethanol (100 ml) and heated under reflux in the presence of yellow PbO (3.6 g, 6 mmol). After 14h t.l.c. (ethyl acetate/methanol/ammonia(25%) 5:4:1) revealed a single product (R_f 0.49). After filtration the solution was evaporated *in vacuo* and the residue was purified by column chromatography (ethyl acetate/methanol/ammonia(25%) 5:4:1) to give the guanidine **6** as a colourless oil (1.35 g, 75%); $[\alpha]_D^{23}$ +9.8 (*c*, 1.43 in EtOH); ν_{max} (KBr): 3300 (br,NH) 1650 (C=N) 1540 (NH) cm^{-1}; δ_H (400MHz; $CDCl_3$): 1.20, 1.25, 1.32, 1.50 (4 x 3H, 4 x s, 4 x Me) 3.19 (1 H, dd, $J_{6'a,6'b}$ 15.75, $J_{6'a,5'}$ 8.25, H-6'a) 3.21 (1 H, dd, $J_{6a,6b}$ 13.8, $J_{6a,5}$ 1.5, H-6a) 3.47 (1 H, dd, $J_{6'b,5'}$ 2.25, H-6'b) 3.54-3.68 (5 H, m, H-6b, H-4, H-αa, H-αb, H-5') 3.79 (1 H, m, H-5) 4.13 (1 H, d, $J_{3',4'}$ 4.2, H-3') 4.22 (1 H, dd, $J_{4',5'}$ 7.5, H-4') 4.40-4.68 (4 H, m, 2 x C<u>H</u>$_2$Ph) 4.53 (1 H, d, $J_{1',2'}$ 3.75, H-2') 5.95 (1 H, d, H-1') 7.21-7.38 (10 H, m, ArC<u>H</u>). (Found: C, 65.78; H, 7.29; N, 7.47. $C_{31}H_{41}N_3O_7$ requires: C, 65.59; H, 7.28; N, 7.40%).

(4R,5R)-2[N-(6-Deoxy-α/β-D-glucopyranosyl)amino]-5-O-benzyl-4-O-benzylmethyl-1,4,5,6-tetrahydropyrimidine, acetate **8**. Compound **6** (0.8 g, 1.41 mmol) was dissolved in 80% trifluoroacetic acid (30 ml) and stirred at 60°C. After 3h t.l.c. (ethyl acetate/methanol/ammonia(25%) 5:4:1) indicated a major product (R_f 0.34). The solvent was removed *in vacuo* and the residue was coevaporated with water (4 x 15 ml). Column chromatography (acetonitrile/methanol/acetic acid 10:2:1) (R_f 0.23) afforded **8** as a colourless oil (0.57 g, 75%); $[\alpha]_D^{23}$ -5.0 (*c*, 2.0 in EtOH); δ_H (400 MHz; D_2O): 3.25 (1H, dd, $J_{2'(\beta)/1'(\beta)}$ 7.8 $J_{2'(\beta)/3'(\beta)}$ 9.0, H-2'(β)) 3.30 (1 H, dd, $J_{4'(\alpha)/3'(\alpha)}$ 9.75, $J_{4'(\alpha)/5'(\alpha)}$ 9.0, H-4'(α)) 3.35 (1 H, t, $J_{4'(\beta)/3'(\beta)}$ = $J_{4'(\beta)/5'(\beta)}$ 9.0, H-4'(β)) 3.32-3.40 (4 H, m, H-6a(α), H-6a(β), H-6b(α), H-6b(β)) 3.45 (1 H, dd, $J_{2'(\alpha)/3'(\alpha)}$ 9.75, $J_{2'(\alpha)/1'(\alpha)}$ 3.75, H-2'(α)) 3.50 (1 H, t, H-3'(β)) 3.50-3.70 (7 H, m, H-4(α), H-4(β), H-5'(β), H-6'a(α), H-6'a(β), H-6'b(α), H-6'b(β)) 3.70 (1 H, t, H-3'(α)) 3.75-3.82 (4 H, m, H-αa(α), H-αa(β), H-αb(α), H-αb(β)) 3.88 (1 H, ddd, $J_{5'(\alpha)/6'a(\alpha)}$ 2.25, $J_{5'(\alpha)/6'b(\alpha)}$ 6.75, H-5'(α)) 4.04-4.07 (2 H, m, H-5(α), H-5(β)) 4.48-4.72 (8 H, m, 2 x C<u>H</u>$_2$Ph (α), 2 x C<u>H</u>$_2$Ph (β)) 4.65 (1 H, d H-1'(β)) 5.10 (1 H, d, H-1'(α)) 7.35-7.49 (20 H, m, ArC<u>H</u> (α), ArC<u>H</u> (β)); FAB-MS *m/z* 488 $(M-H)^+$.

(4R,5R)-2[N-6-Deoxy-α/β-D-glucopyranosyl)amino]-5-hydroxy-4-hydroxymethyl-1,4,5,6-tetrahydropyrimidine, acetate **9**. The guanidine **8** (0.48 g, 0.876 mmol) was dissolved in a mixture of acetic acid (15 ml) and methanol (5 ml) and hydrogenated over Pd/C. After 3d t.l.c (ethyl acetate/methanol/ammonia(25%) 2:2:1) indicated a single product (R_f 0.10) .The mixture was filtered and the solvent evaporated *in vacuo*. Coevaporation with water (3 x 10 ml) afforded **9** as a colourless oil (0.321 g, 100%); $[\alpha]_D^{23}$ +7.9 (*c*, 2.28 in H_2O); δ_H (400 MHz; D_2O): 3.24 (1 H, dd, $J_{2'(\beta)/1'(\beta)}$ 7.8, $J_{2'(\beta)/3'(\beta)}$ 9.75, H-2'(β)) 3.34 (1 H, dd, $J_{4'(\alpha)/3'(\alpha)}$ 9.75, $J_{4'(\alpha)/5'(\alpha)}$ 9.0, H-4'(α)) 3.36 (1 H, dd, $J_{4'(\beta)/3'(\beta)}$ 9.0,

$J_{4'(\beta)/5'(\beta)}$ 9.75, H-4'(ß)) 3.37-3.43 (4 H, m, H-6'a(ß), H-6'b(ß), H-6a(α), H-6a(ß)) 3.43-3.51 (3 H, m, H-6'b(α), H-6b(α), H-6b(ß)) 3.49 (1 H, t, $J_{3'(\beta)/2'(\beta)} = J_{3'(\beta)/4'(\beta)}$ 9.0, H-3'(ß)) 3.52 (1 H, dd, $J_{2'(\alpha)/1'(\alpha)}$ 3.75, $J_{2'(\alpha)/3'(\alpha)}$ 9.75, H-2'(α)) 3.53 (1 H, dd, $J_{6'a(\alpha)/6'b(\alpha)}$ 12.0, $J_{6'a(\alpha)/5'(\alpha)}$ 6.0, H-6'a(α)) 3.54-3.58 (1 H, m, H-5'(ß)) 3.62-3.66 (3 H, m, H-αa(α), H-4(α), H-4(ß)) 3.69 (1 H, dd, $J_{\alpha a(\beta)/4(\beta)}$ 7.8, H-αa(ß)) 3.70 (1 H, t, H-3'(α)) 3.84 (1 H, dd, $J_{\alpha b(\beta)/\alpha a(\beta)}$ 10.5, $J_{\alpha b(\beta)/4(\beta)}$ 4.5, H-αb(ß)) 3.86 (1 H, dd, $J_{\alpha b(\alpha)/\alpha a(\alpha)}$ 9.75, $J_{\alpha b(\alpha)/4(\alpha)}$ 4.2, H-αb(α)) 3.91 (1 H, ddd, $J_{5'(\alpha)/6'a(\alpha)}$ 6.0, $J_{5'(\alpha)/6'b(\alpha)}$ 2.7, H-5'(α)) 4.26-4.30 (2 H, m, H-5(α), H-5(ß)) 4.64 (1 H, d, H-1'(ß)) 5.23 (1 H, d, H-1'(α)); FAB-MS *m/z* 308 $(M-H)^+$.

References

1. Lerner, R. A., Benkovic, S. J., Schultz, P. G., *Science*, 1991, **252**, 659.

2. Janda, K. D., Shevlin, C. G., Lerner, R. A., *Science*, 1993, **259**, 490; Hsieh, L. C., Yonkovich, S., Kochersperger, L., Schultz, P. G., *Science*, 1993, **260**, 337.

3. Reymond, J. L., Janda, K. D., Lerner, R. A., *Angew. Chem.*, 1991, **103**, 1690.

4. Jencks, W. P., *Catalysis in Chemistry and Enzymology* (Mac Graw-Hill, New York), 1969, 288; Pauling, L., *Chem. Eng. News*, 1946, **24**, 1375.

5. Lehmann, J., Rob, B., *Liebigs Ann. Chem.*, 1994, in press.

6. Jiricek, R., Lehmann, J., Rob, B., Scheuring, M., *Carbohydr. Res.*, 1993, **250**, 31.

7. Ramjeesing, M., Kahlenberg, A., *Can. J. Chem.*, 1977, **55**, 3717.

8. Hünig, S., Lehmann, H., Grimmer, G., *Liebigs Ann. Chem.*, 1953, **579**, 77.

(*Received* 10 *August* 1994)