A fluorescent glucose sensor binding covalently to all five hydroxy groups of α-D-glucofuranose. A reinvestigation

Mia Bielecki, Hanne Eggert and Jens Chr. Norrild *

Department of Chemistry, University of Copenhagen, Symbion, Fruebjergvej 3, DK-2100 Copenhagen, Denmark

Received (in Cambridge) 13th November 1998, Accepted 21st January 1999

The structures of the complexes between a fluorescent bisboronic acid **7** and glucose have been determined. Shinkai *et al.*[1] previously studied the complex between **7** and glucose and they deduced a 1,2 : 4,6-α-D-glucopyranose bisboronate structure. We have shown that this structure is only valid as an initial complex formed under completely nonaqueous conditions. In the presence of water the pyranose complex rearranges rapidly into an α-D-glucofuranose-1,2 : 3,5,6-bisboronate in which all five free hydroxy groups of glucose are covalently bound by the sensor molecule. A favourable B–N interaction around the 1,2-binding site and the effect of an *o*-ammoniomethyl group on the pK_a value of the second boronic acid group allow for the observed binding at neutral pH. The structure evaluations are based on ^{1}H and ^{13}C NMR data as well as information obtained from $^1J_{CC}$ coupling constants. The fluorescence spectra of both complexes were measured and discussed. MALDI TOF-MS experiments showed competitive formation of 1 : 2 (boronic acid : glucose) complexes under conditions of physiological glucose levels.

Introduction

There is today a strong demand for the development of new efficient, selective and cheap carbohydrate sensors and in this sense especially new glucose sensors. Recently boronic acid based carbohydrate sensors have been suggested as a new and very promising alternative to glucose oxidase based sensor systems.[2–4] For more than 50 years studies on the interactions between boronic acids and carbohydrates have been performed and complexes between boronic acids and especially glucose and fructose are well recognised although their structures only just recently have been determined.[5,6]

To be able to distinguish between various carbohydrates, a concept of having a bisboronic acid capable of binding simultaneously to two specific sites in the carbohydrate was investigated.[3,7] This concept has now, through numerous interesting investigations by S. Shinkai's group,[2,8] been further developed into sensor molecules of which some are presently reported capable of giving a larger response to glucose relative to fructose[1,3,9] and others of distinction between enantiomeric forms of certain carbohydrates.[10] The detection methods demonstrated include *e.g.* circular dichroism,[3,9] UV–vis absorbance,[11,12] electrochemistry,[13] and fluorescence.[4,8,14] For most sensors suggested the working range however has been limited to pH values between 9 and 12.

We have recently studied the structures of the boronic acid complexes under both aqueous and non-aqueous conditions as this information to us seems essential for the future design of new custom-built sensor molecules. Our results show that for the binding of glucose there is a strong preference for binding of the α-glucofuranose form.[6] When we read a recent paper by Shinkai and co-workers[1] concerning a new fluorescent anthracene based bisboronic acid we became very curious. In the design of this new bisboronic acid (**7**, Scheme 1) they ingeniously used a concept originally developed by Wulff,[15,16] where an intramolecular boron–nitrogen interaction lowers the pseudo pK_a-value (pK_{a1}, Fig. 1) of the boronic acid. As it is well known that significant complex formation between boronic acids and carbohydrates only occurs at pH ≥ pK_a of the boronic acid (*i.e.* with tetrahedral boronates),[17] the low pK_{a1} value thus obtained expands the pH-window for strong binding of carbohydrates to include neutral pH values.

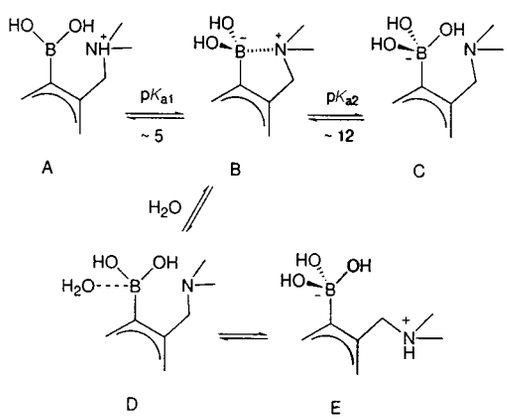
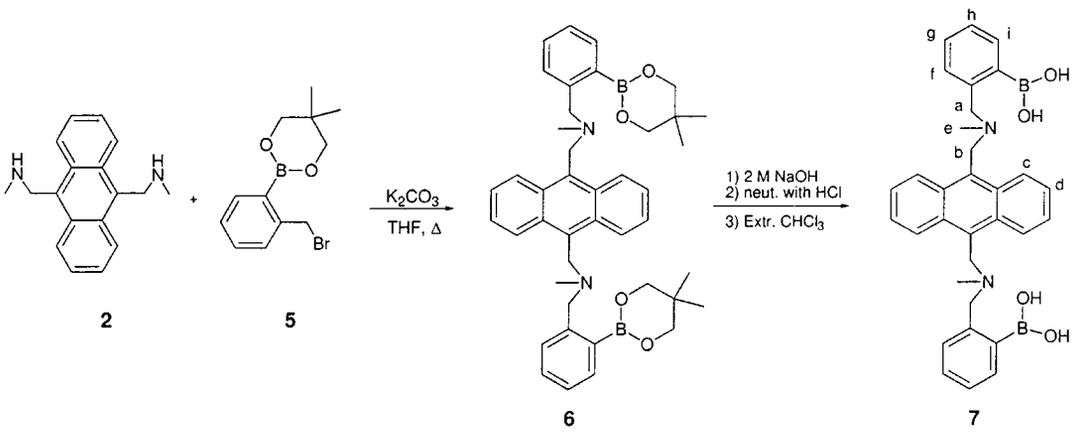


Fig. 1 Boron–nitrogen interactions at varying pH in *o*-(*N*,*N*-dialkylaminomethyl)arylboronic acids. Approximate p*K* values refer to aryl = phenyl (ref. 16). Only structures with tetrahedral boron (structures B, C and E) form strong complexes with diols in aqueous solution.

From a ^{1}H NMR study Shinkai and co-workers deduce the complex between **7** and glucose to be a 1,2 : 4,6-α-glucopyranose complex which in our eyes was quite surprising with regard to our previous studies[6] and a very recent investigation by our lab of a water soluble bisboronic acid capable of binding at neutral pH in the furanose form.[18] The ^{1}H NMR evidence in the article was very convincing; however, experiments in our laboratory revealed that the ^{1}H NMR spectrum, as presented without solvent specification, was obtained from pure methanol-d_6 and not in the water–methanol mixture which was used in the fluorescence experiments. This was not clear from the article and it made us wonder whether the suggested pyranose complex would be representative also for the conditions under which the fluorescence experiments were performed. On this basis we synthesised **6** and **7** and reinvestigated the complexes with glucose as described in the following section. The results of this study are a reassignment of the ^{1}H NMR spectrum previously reported[1] and a determination of the structure of the complex present under the conditions of the fluorescence measurements different from the one reported in methanol. Furthermore the differences in



Scheme 1

fluorescence intensities of the two complexes have been measured and discussed.

Results and discussion

9,10-Bis-[[*N*-[*o*-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzyl]-*N*-methylamino]methyl]anthracene (**6**) was synthesised and purified as described in the Experimental section. This procedure is slightly different from the one used by Shinkai and co-workers and includes a final crystallisation of the compound. Compound **6** was deprotected under alkaline aqueous conditions followed by extraction of the liberated bisboronic acid into chloroform with the aid of a little DMSO followed by evaporation and drying. This gave a sample of the free bisboronic acid **7** sufficiently pure for NMR studies.

To reproduce the ^{1}H NMR spectrum previously published, we freshly dissolved a 1 : 1 mixture of **6** and *anhydrous* α-D-glucose in methanol-d_6 and recorded the ^{1}H NMR spectrum instantaneously. The spectrum is shown in Fig. 2(a) and is in full agreement with the one previously reported.[1] However, re-recording the spectrum after a few hours revealed several new peaks in the spectrum. After 20 h we observed a considerable change as seen by the appearance of a totally new set of signals (Fig. 2(b)). These signals are most obvious in the regions from

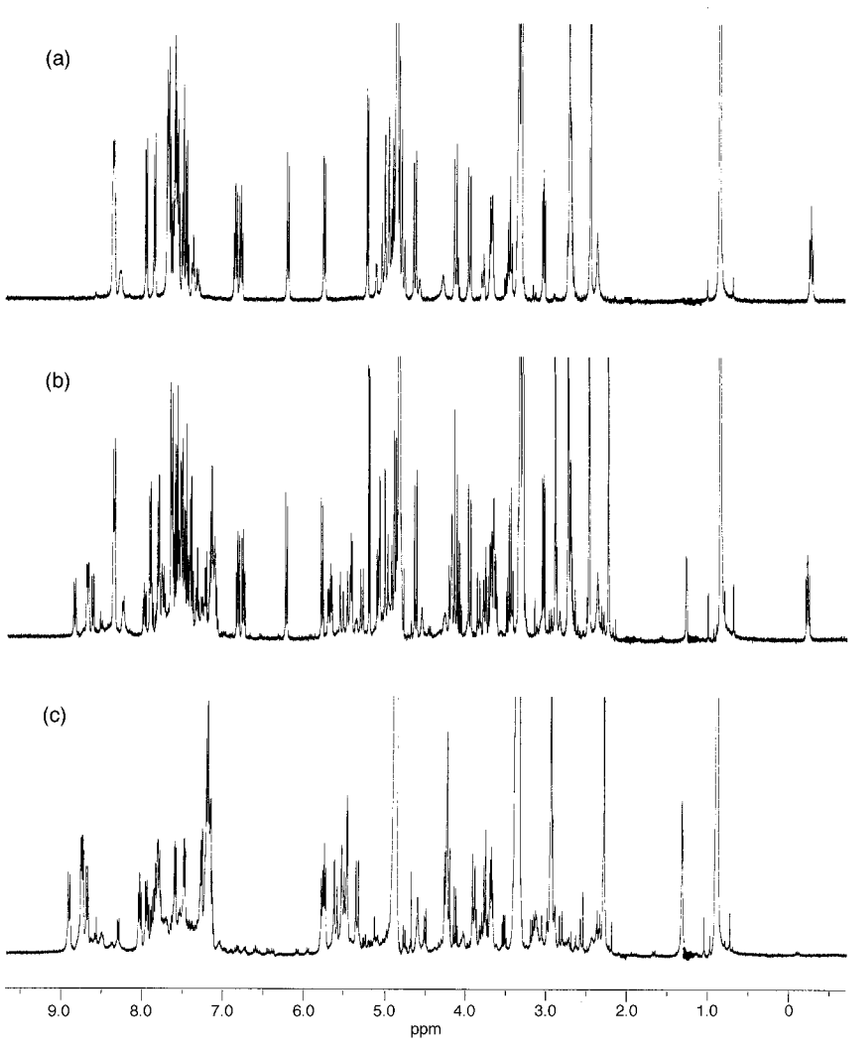
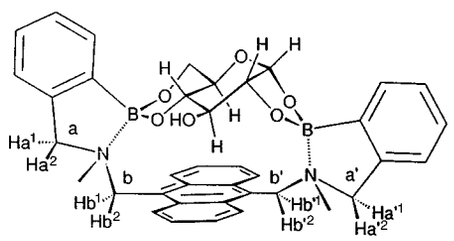


Fig. 2 The spectrum of **6** and anhydrous α-D-glucopyranose (1 : 1) in methanol-d_6 recorded (a) instantly, (b) after 20 h and (c) after 8 days.

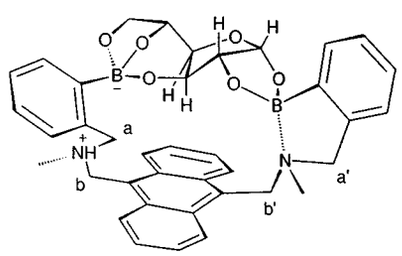
Table 1 ^{13}C Chemical shifts (ppm) for the aliphatic part of the boronic acid complexes and reference compounds[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	N–CH_3	N–CH_3′	Ca	Ca′	Cb	Cb′
Complex-1[b]	98.3	79.0	77.1	74.1	69.1	62.3	42.9	42.8	63.4[c]	64.2[c]	47.6[c]	47.8[c]
Complex-2[b]	104.6	82.2	76.3	80.1	73.2	67.0	38.5	40.5	60.5[c]	63.8[c]	51.5[c]	53.7[c]
8[d]	104.0	85.8	73.6	74.7	70.8	62.0						
9[e]	107.6	85.7	79.5	81.1	73.9	67.0						
10[e]	105.7	86.1	79.2	83.3	72.2	67.3						
α-D-Glucopyranose[e]	94.9	74.3	75.4	72.3	74.0	63.3						
α-D-Glucopyranose[d]	92.4	72.5	73.2	70.7	72.1	61.4						

[a] The data are given relative to TMS. The assignments are in agreement with the information obtained from ^1H–^{13}C-heterocorrelated and ^{13}C–^{13}C-correlated spectra and with 1D selective decoupling experiments. [b] In CD_3OD. [c] As the sugar's orientation related to the ligand has not been determined the chemical shifts of the ligand CH_2 groups marked respectively a/a′ and b/b′ can be interchanged. [d] In DMSO-d_6. [e] In D_2O at pD = 11–12.



Complex-1



Complex-2

Fig. 3 Assigned structures of **Complex-1** and **Complex-2**.

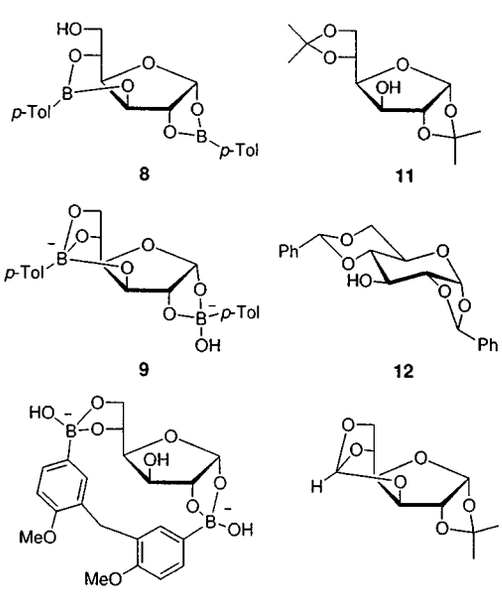
5.3 to 5.7 ppm and from 8.5 to 8.9 ppm. After 8 days the signals from the original complex had disappeared and only the additional signals were left (Fig. 2(c)). In the following we denote the initially formed complex as **Complex-1** and the complex formed later as **Complex-2**.

Repeating the above experiment using α-D-glucopyranose *monohydrate* it was impossible to reproduce the spectrum of Fig. 2(a). After only 20 min the signals from **Complex-2** were dominant and after 3 days **Complex-1** could not be observed. The final mixture contained signals from **Complex-2** (>90%) in mixture with other unidentified products.

Our results show that the initially formed α-D-glucopyranose complex under the conditions is converted slowly to a thermodynamically more stable α-D-glucofuranose complex. This transformation has to comprise a mutarotation of the initial bound α-D-glucopyranose and here the solvent has a central role. The observed enhanced transformation rate in methanol of **Complex-1** to **Complex-2** by using the monohydrate of glucose compared to anhydrous glucose agrees with the well known effect of water compared to methanol on the mutarotation of α-D-glucopyranose. The ~30 times faster mutarotation of glucose in water as compared to methanol[19] thus suggests a very fast transformation of **Complex-1** to **Complex-2** in the presence of water. Therefore under the conditions for fluorescence measurements, as applied by S. Shinkai and co-workers (water–methanol (2:1), buffer pH 7.8), we would expect only the presence of **Complex-2** and not **Complex-1**. In order to prove this assumption we recorded the ^1H and ^{13}C NMR spectra of a 1:1 mixture of **6** and α-D-glucose in water–methanol (1:2). Due to the low solubility of the ligand, the latter solvent mixture was the closest we could get to the above solvent conditions while still being able to obtain NMR spectra. The ^{13}C NMR spectrum was obtained with uniformly $^{13}C_6$ labelled α-D-glucose. Due to the still very low solubility of **6** under these solvent conditions, acceptable spectra were obtained only after very long data accumulation. However, in accordance with our expectations we were able to conclude the presence of **Complex-2** only 10 min after mixing and longer accumulation did not unveil the presence of other complexes.

Structure evaluation: Complex-1

We assign the structure of **Complex-1** as shown in Fig. 3. This structure is in agreement with the one earlier deduced by Shinkai *et al.* based on ^1H NMR data. However, our reinvestigation of the complex affords several corrections to the assignments and provides new significant and consistent evidence for the structure. The α-D-glucopyranose structure of the glucose part of **Complex-1** is substantiated by the relatively low ^{13}C-chemical shift values for the glucose part compared to those of similar α-furanose complexes[6] (Scheme 2 and Table 1). The



Scheme 2

measured $^3J_{H-H}$ coupling constants (Table 4) show, except for J_{12}, high values in agreement with an approximate *trans* axial arrangement of these protons as expected for an α-glucopyranose ring. Our assignment of the ^1H NMR data of the complex differs considerably from the one previously published. The H-5,6a/b protons have been reassigned and the chemical shifts of 5.78, 6.23, 6.81 and 6.88 ppm assigned by Shinkai *et al.* to the methylene protons (Hb and Hb′, Fig. 3) proved by the

Table 2 ^{1}H Chemical shifts (ppm) in CD_3OD for the aliphatic part of **Complex-1** and **Complex-2**[a]

Complex-1	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	$N–CH_3$	$N–CH_3'$
	5.25	3.08	−0.21	2.75	2.75	3.48	3.73	2.49	2.75
	Hal[b]	Ha2[b]	Ha′1[b]	Ha′2[b]	Hbl[b]	Hb2[b]	Hb′1[b]	Hb′2[b]	
	3.98	4.93[c]	4.17	4.66	4.85[c]	4.92[c]	5.01	5.11	
$^2J_{HH}$/Hz	11.9		11.4		13.9		14.2		
Complex-1-(3-*O*-Me)	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	$N–CH_3$	$N–CH_3'$
	5.33	3.67	0.11	3.20	2.67	3.52	3.78		
	Hal[b]	Ha2[b]	Ha′1[b]	Ha′2[b]	Hbl[b]	Hb2[b]	Hb′1[b]	Hb′2[b]	
	4.04	4.94	4.21	4.72	—[d]	—[d]	5.04	5.09	
$^2J_{HH}$/Hz	11.7		11.9		—[d]		14.2		
Complex-2	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	$N–CH_3$	$N–CH_3'$
	5.49	2.95	1.32	3.40	3.35[e]	3.76	3.65	2.30	2.95
	Hal[b]	Ha2[b]	Ha′1[b]	Ha′2[b]	Hbl[b]	Hb2[b]	Hb′1[b]	Hb′2[b]	
	3.87	4.24	4.24	5.36	5.54	5.77	5.65	5.79	
$^2J_{HH}$/Hz	13.8		12.1		14.4		14.5		

[a] The data are given relative to TMS. The assignments are in agreement with the information obtained from ^{1}H–^{1}H-COSY, ^{1}H–^{13}C-heterocorrelated and ^{13}Cβ^{13}C-correlated spectra and with 1D selective decoupling experiments. The chemical shifts for the free 2,2-dimethylpropane-1,3-diol in CD_3OD are 3.36 and 0.89 ppm. [b] As the sugar's orientation related to the ligand has not been determined the chemical shifts of the ligand CH_2 groups marked respectively a/a′ and b/b′ can be interchanged. Within each methylene group 1 and 2 can be interchanged. [c] The signals are partly hidden under the water signal and exact values were obtained by solvent inverse recovery decoupling. [d] Not determined. [e] The chemical shift of H-5 was disclosed by decoupling of the methanol signal giving an effect at H-4 and H-6b.

Table 3 $^1J_{CC}$ Coupling constants (Hz)

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5}$
Complex-1[a]	36	37	38	38	43
Complex-2[a]	35	44	33	40	35
8[b]	34.4	44.0	34.4	40.5	40.9
9[c]	35.7	43.6	34.3	40.0	34.5
11[b]	34.0	42.3	38.2	48.1	34.2
13[b]	33.9	46.6	31.8	40.0	33.4
α-D-Glucopyranose[a]	44.4	38.1	35.9	39.6	42.7
β-D-Glucopyranose[a]	45.6	38.9	39.6	40.5	43.4

[a] In CD_3OD. [b] In DMSO-d_6. [c] In D_2O at pD = 11–12.

method of ^{1}H–^{13}C-heterocorrelated spectra and 1D selective decoupling experiments to be aromatic signals while the methylene protons had chemical shifts close to that of water and were consequently hidden under the broad water signal. An inverse recovery decoupling of the water signal unveiled the chemical shifts and coupling constants of these protons (See Table 2). A reassignment of the approximately triplet signals 6.81 and 6.88 ppm to neighbouring aromatic protons of one of the boron substituted phenyl rings explains their multiplicity in a consistent manner. To exclude hidden signals under those of the protecting group, an experiment with the free boronic acid **7** was performed and new data for **Complex-1** did not appear.

To determine the binding sites of the pyranose ring we prepared a 1:1 solution of **6** and uniformly $^{13}C_6$ labelled α-D-glucose and obtained the one bond C–C coupling constants within the sugar part of the complex (Table 3). The one bond C–C coupling constant, within a RO–C–C–OR′ fragment containing sp^3 hybridised carbon atoms, depends a) on the O–C–C–O dihedral angle and b) on the R–O–C–C torsions, the latter showing the greater variations. According to calculations by Serianni *et al.*[20] a minimum value of $^1J_{C-C}$ should be expected for an approximately all eclipsed geometry within such a fragment. In agreement with this we showed earlier that exceptionally low $^1J_{C-C}$ values are found when the two carbons are contained in five membered 1,3-dioxolane or 1,3,2-dioxaborinane rings relative to values for the free sugar.[5,6] The measured value of $^1J_{C1–C2}$ = 36 Hz compared to 44 Hz of the free α-D-glucopyranose (Table 3) thus strongly indicates a 1,2-boronate. The $^1J_{C5–C6}$ of 43 Hz on the other hand excludes C-5 and C-6 from being members of such a five membered ring and thus eliminating a 5,6-bound furanose. This leaves three imaginable secondary binding sites, namely i) a *trans* vicinal pyranose 3,4-boronate, ii) a seven membered 3,6-boronate, and iii) a six membered pyranose 4,6-boronate. *trans* Vicinal boronic acid complexation of pyranose hydroxy groups was reported earlier but only under aprotic conditions and always comprising 1,3,5,2,4-trioxadiborepane-2,4-diyl structures which are not possible here.[21,22] Seven membered boronate rings have been isolated from aprotic media but always under constrained conditions where five or six membered rings were not possible.[23–25] We therefore, in accordance with the proposal of Shinkai and co-workers, conclude the secondary binding site to be 4,6 giving an α-D-glucopyranose 1,2:4,6 bisboronate structure for **Complex-1**. This conclusion is substantiated by the close resemblance of the measured $^3J_{HH}$ coupling constants to those of 1,2:4,6-di-*O*-benzylidene-α-D-glucopyranose (**12**) (Table 4) and by the fact that no complex formation was observed in an experiment with 6-deoxy-L-glucose.[26] On the other hand 3-*O*-methyl-α-D-glucopyranose did indeed give the corresponding *O*-3 methylated **Complex-1** (see Fig. 4 and Tables 2 and 4).

Structure evaluation: Complex-2

Complex-2, which is the thermodynamically more stable under the conditions applied, is assigned the structure shown in Fig. 3. The ^{1}H NMR data together with data from model compounds are compiled in Tables 2 and 4. The chemical shift of H-4 was best determined from an experiment with the free boronic acid **7** as the signal is partly hidden under the protection group signal at 3.36 ppm. The $^3J_{H4–H5}$ coupling constant was estimated to be 2–3 Hz by selective decoupling of H-3 which transformed the H-4 triplet to a distorted doublet. In the proton spectrum H-2 and H-3 are both doublets indicating $^3J_{23} \sim 0$ as also found in similar boronic acid complexes of α-D-glucofuranose[6] (Table 4). In agreement with a furanose ring no large vicinal coupling constants are found. Furthermore, the glucose part of the complex shows relative high ^{13}C-chemical shift values (Table 1) with *e.g.* the anomeric carbon signal less shielded by 6 ppm as compared to **Complex-1**.

Table 4 J_{H-H} Coupling constants (Hz) for the glucose part of boronic complexes and selected model compounds

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
Complex-1 [a]	5.5	7.0	9.6	—[b]	9.6	4.4	9.6
Complex-1-(3-*O*-Me) [a]	6.1	6.8	10.2	9.2	9.8	4.2	9.8
Complex-2 [a]	4.2	~0	3.1	2–3[c]	~0	4.8	8.0
8 [d]	4.1	~0	2.4	~0	2.4	2.4	m.
9 [e]	3.6	~0	2.8	2.6	~0	5.1	8.8
10 [e]	4.0	~0	2.4	9.5	6.0	3.5	9.0
11 [e]	3.6	~0	2.8	6.8	6.4	5.5	8.8
12 [f]	4.9	6.4	9.4	9.2	10.0	4.9	10.3
13 [d]	3.6	~0	3.0	1.3	~0	5.2	7.9
α-D-Glucopyranose [g]	3.8	9.9	9.6	9.6	2.2	5.5	12.3

[a] In CD_3OD. [b] Could not be determined. [c] Could not be precisely determined. [d] In DMSO-d_6. [e] In D_2O at pD = 11–12. [f] In $CDCl_3$ according to Liptak *et al.*[34] [g] In D_2O according to Curatolo *et al.*[35]

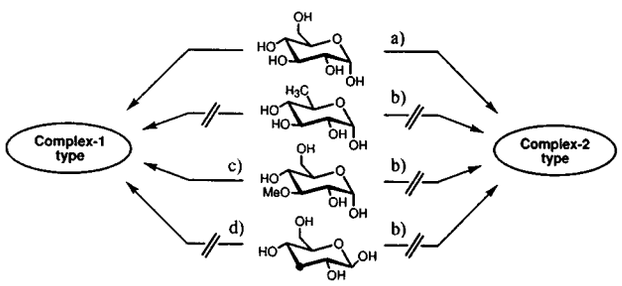


Fig. 4 Complex formation between bisboronic acid **6** and various glucose derivatives in methanol. (a) Formation of **Complex-2** *via* **Complex-1**. In dry MeOH rearrangement takes 7–8 days. Traces of water dramatically enhance the rate of transformation. (b) Multiple complexes form. No NMR evidence of **Complex-2** type structures within the mixtures. (c) Initial formation of **Complex-1** (with 3-*O*-methyl group). Rearranges with time to a complicated mixture. (d) Due to use of 3-deoxy-D-glucose as the crystalline β-D-*ribo*-hexopyranose initial formation of the 1,2 bound pyranose is prevented. During the slow mutarotation in dry MeOH multiple complexes form and neither **Complex-1** nor **Complex-2** type structures can be identified.

From experiments with 1:1 mixtures of **6** and ^{13}C labelled glucose it was possible to obtain the $^1J_{C-C}$ coupling constants as listed in Table 3. For comparison we have included $^1J_{C-C}$ values for the *p*-tolylboronates **8** and **9** obtained from ^{13}C NMR spectra of samples prepared with uniformly $^{13}C_6$ labelled glucose together with data for the model compounds **11** and **13**. The $^1J_{C-C}$ coupling constants of **11** and **13** were measured in unlabeled samples using the INADEQUATE technique. For the reasons discussed above the values of $^1J_{C1-C2}$ and $^1J_{C5-C6}$ being relatively low strongly indicate a 1,2:5,6 or a 1,2:3,5,6 bound α-furanose complex. Comparing the measured $^1J_{C-C}$ coupling constants of **Complex-2** with those of α-D-glucofuranose 1,2:3,5,6-bis(*p*-tolylboronate)[6] (**9**) one observes two almost identical data sets (see Table 3) whereas large differences are seen for the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**11**). This does, on the basis of our earlier studies,[5,6] provide very strong evidence of the 1,2:3,5,6 bound structure. Further evidence for 3,5,6 binding is the measured $^3J_{HH}$ coupling constants which correlate well with those of **9** and of the 3,5,6-orthoester **13** (Table 4). Therefore we conclude **Complex-2** to be the 1,2:3,5,6 bound α-D-glucofuranose as shown in Fig. 3.

Our assignment of **Complex-2** is further substantiated by experiments substituting α-D-glucopyranose with respectively 6-deoxy-L-glucose, 3-*O*-methyl-α-D-glucopyranose[26] and 3-deoxy-β-D-*ribo*-hexopyranose (3-deoxy-D-glucose). These compounds do not form **Complex-2** analogs in accordance with their lack of the appropriate hydroxy groups as summarized in Fig. 4. Instead, after a few days, non-resolvable mixtures of multiple components are found in all three experiments.

As we observe no differences between the NMR data of **Complex-2** in methanol and aqueous buffer solution, we deduce the structure of **Complex-2** to be the *N*-protonated form as shown in Fig. 3 under both conditions. In MeOH this zwitterionic form is a prerequisite whereas in aqueous buffer other factors may favour this structure. Drawing from earlier work by Yurkevich *et al.*[27] it can be anticipated that ammoniomethyl substituents generally have a notable lowering effect on the pK_a values of boronic acids, consequently allowing for structure E (Fig. 1) to be involved in binding at neutral pH. This effect, together with a superior stabilisation of tridentate boronates, enables the one boronic acid group to bind in the observed 3,5,6 fashion at neutral pH. We stress that our conclusion is limited to a 1:1 mixture at pH 7.8.

B–N interactions

The B–N interactions in complexation between **6** and glucose under aqueous conditions were deduced by Shinkai and co-workers as a result of fluorescence based pH titrations. In accordance with the work of Wulff[15,16] they observed a low pK_{a1} value of the free boronic acid as a result of the formation of the tetrahedral N-bonded boronic acid (see B, Fig. 1). As the observed decrease of the fluorescence (*i.e.* N-lone pair PET quenching) at pH around pK_{a1} was absent when glucose was added they concluded an even stronger B–N interaction upon complexation. In the latter case only a decrease of the fluorescence corresponding to pK_{a2} was observed (see Fig. 1). As ^{11}B NMR experiments, for reasons including low solubility and extensive line broadening, did not provide unambiguous information on the boron atom geometries, we have authenticated the B–N interactions with regard to our assigned structures of **Complex-1** and **-2** by using the available information from our ^1H and ^{13}C NMR experiments.

The ^1H NMR spectrum in CD_3OD of the free ligand **7** shows broad lines ($\Delta\nu_{1/2}$ = 13 and 15 Hz) at room temperature for the methylene groups CH_2(a) and CH_2(b), respectively, in accordance with an intermediate fast exchange between the B–N bonded form (B, Fig. 1) and the non-bonded structure (D, Fig. 1). However, for all signals of **Complex-1** and **-2** only sharp lines are observed. Regarding the ^1H NMR data of **Complex-1** one observes large chemical shift differences (0.95 and 0.59 ppm) for the geminal protons Ha1 and Ha2 and for Ha′1 and Ha′2 in agreement with these protons being fixed in a five membered B–N containing ring. For comparison small chemical shift differences of 0.1 ppm are observed for geminal protons of the CH_2(b) groups. A difference is also observed between the geminal coupling constants of the two types of methylene groups. The two CH_2(a) and the two CH_2(b) groups each have very similar $^2J_{H-H}$ values, however the "b-type" is (numerically) larger by ~2 Hz.

The structure deduced for **Complex-2** (Fig. 3) allows for only one B–N interaction, making the two alkylamine substituents on the anthracene ring clearly different. The NMR data of the alkylamine groups reflect this difference as follows. In **Complex-2** the chemical shift differences between non-equivalent geminal protons are increased relative to **Complex-1** except for one of

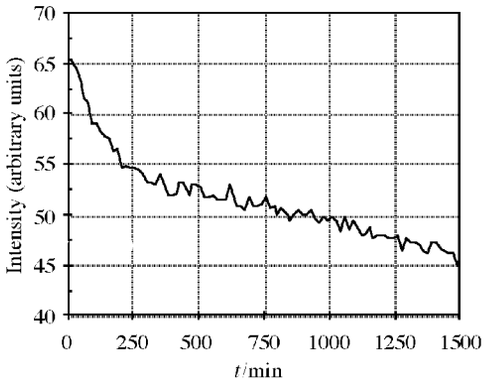


Fig. 5 Relative fluorescence of a 1:1 mixture of **6** and anhydrous α-D-glucopyranose (10^{-5} M) in pure methanol as function of time. (λ_{ex} = 370 nm; λ_{em} = 425 nm).

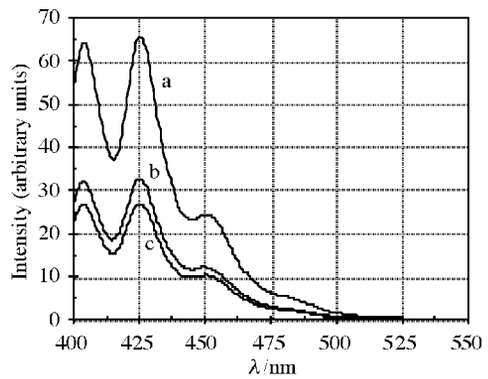


Fig. 6 Emission spectra of a 1:1 mixture of **6** and anhydrous α-D-glucopyranose (10^{-5} M) in pure methanol to (a) t = 0 h, (b) t = 72 h and (c) t = 5 days. (λ_{ex} = 370 nm).

the CH_2(a) groups, where it has decreased by 0.37 ppm as a consequence of the group not being part of a five membered ring. Furthermore this CH_2 group shows a geminal coupling constant 1.7 Hz larger than that of the other CH_2(a) group and 1.9 and 2.4 Hz larger than those of the two CH_2(a) groups in **Complex-1**. For **Complex-2** the ^{13}C chemical shift differences between N–CH_3, CH_2(a) and CH_2(b) groups at each side of the anthracene ring are 2.0, 3.3 and 2.2 ppm respectively. In **Complex-1** the corresponding values are much smaller (0.1, 0.8 and 0.2 ppm).

Fluorescence

To confirm the results obtained from NMR spectroscopy, a series of fluorescence measurements were made under the same conditions with regard to solvent and reactants. The fluorescence of a 10^{-5} M 1:1-solution of **6** and anhydrous glucose in methanol was measured as a function of time (Fig. 5). A clear drop in intensity from t = 0 to t = 25 h was observed and after 120 h the intensity was only 41% of the initial one (Fig. 6); however no significant shifts in the emission maxima were observed.

The observed time dependence of the fluorescence is in full agreement with the time span of the transformation of **Complex-1** to **Complex-2** under similar conditions as seen from Fig. 2. Therefore the observed decrease certainly reflects the differences in the fluorescence of the two complexes. It is interesting to note that in methanol solution the transformation of **Complex-1** to **Complex-2** implements the breaking of one B–N bond leaving a protonated nitrogen atom (see Fig. 3).

In an experiment using a 33% methanol–water buffered solution of pH = 7.80 (which are the conditions applied by Shinkai and co-workers for their fluorescence measurements) no time dependent intensity changes could be observed within 8 h. In agreement with the evidence obtained from the NMR experiments under closely related conditions (see above) we suggest that under the fluorescence conditions the thermodynamically more stable furanose complex (**Complex-2**) is instantly formed even though extrapolating to the very dilute conditions of the fluorescence experiment could be troublesome.

In order to obtain further knowledge of the species present under the conditions of the fluorescence experiments we have performed MALDI TOF-MS experiments of bisboronic acid **6** (10^{-5} M) and glucose in 33% methanol–water. For a 1:1 boronic acid:glucose ratio we observe the peak from the 1:1 complex but also significant amounts of the 1:2 (boronic acid:glucose) complex. The latter was not mentioned previously by Shinkai and co-workers.[1] Going to ratios of 1:400 and 1:4000 the 1:2 peak increases to around 1/3 of the 1:1 peak. This suggests that the expected strong fluorescence of the 1:2 complex might interfere in the interpretation of the fluorescence titrations at the 1:4000 ratio in the original paper.[1] From this follows that the titration curve for a **6**:glucose ratio of 1:4000 will be a sum of fluorescence of at least two species but also that formation of a 1:2 complex cannot be neglected for this system under conditions of physiological glucose levels (*i.e.* 3–20 mM).

Conclusions

We have shown that the earlier proposed structure of **Complex-1** is only valid as an initially formed complex between α-D-glucopyranose and **6** or **7** under completely nonaqueous conditions. We found that **Complex-1** rearranges to the thermodynamically more stable 1,2:3,5,6 bound α-D-glucofuranose complex **Complex-2** as a function of time and water content of the medium. Under conditions related to those of the fluorescence measurements in the original work by Shinkai and co-workers[1] (water–methanol, 1:2) we have shown that **Complex-2** is instantly formed. The structure of **Complex-2** is to our knowledge the first example of a bisdentate 1,2:3,5,6 bound α-D-glucofuranose complex and it strongly indicates the superior stability of this type of complex over 1,2:3,5 or 1,2:5,6 bound complexes in agreement with our findings on the corresponding bis-(*p*-tolylboronates).[6] In this manner **Complex-2** represents the first example of a sensor molecule which binds covalently to all five hydroxy groups of glucose. The binding to all five hydroxy groups is obtained by a delicate balance of the equilibria considered in Fig. 1, where the superior stabilisation of a trisdentate boronate clearly suppresses one of the otherwise favoured B–N interactions.

We have shown that the rearrangement of **Complex-1** to **Complex-2** in methanol causes a decrease in the observed fluorescence and that a 1:2 ligand to sugar complex is present in substantial amounts under conditions of physiological glucose levels.

We believe that further efforts can be made to optimise sensor structures and properties towards this superior type of α-D-glucofuranose binding and hence low pK_a values and increased water solubility should certainly be implemented.

Experimental

NMR spectra were recorded at 25 °C, on a Varian Unity 400 NMR spectrometer ^{13}C (100 MHz) and ^{1}H (400 MHz). Chemical shifts are reported in ppm and referenced to CD_3OD, 49.03 ppm (^{13}C NMR) and CHD_2OD, 3.35 ppm (^{1}H NMR). All coupling constants are given as numerical values. Column chromatography was performed on silica gel 60 $PF_{254+366}$. Evaporations were performed *in vacuo* on a rotary evaporator. Melting points are uncorrected. Fluorescence spectroscopy was performed on a Perkin-Elmer LS50B instrument. Mass spectrometry was performed on a Jeol JMS-HX/HX 110A instrument. Microanalyses were performed by Micro Analytical Laboratory, The Örsted Institute, University of Copenhagen, Denmark.

Materials

All chemicals used were of reagent grade and all solvents were of HPLC grade. 3-Deoxy-D-glucose[28,29] was crystallised as the 3-deoxy-β-D-*ribo*-hexopyranose according to Anet.[30]

9,10-Bis(chloromethyl)anthracene (1). Prepared according to Miller *et al.*[31] **CAUTION:** This compound is highly allergenic and should be handled with care. ^{1}H NMR (acetone-d_6) δ 8.40 (4H, anthracene-H1,4,5,8), 7.60 (4H, anthracene-H2,3,6,7), 5.70 (4H, s, CH_2). Mp 254–256 °C (decomposes). MS: M^+: 274 *m*/*z*. Anal. Calcd. for $C_{16}H_{12}Cl_2$: C, 69.84; H, 4.40. Found: C, 69.66; H, 4.26%.

9,10-Bis[(methylamino)methyl]anthracene (2). Prepared according to T. D. James *et al.*[1] and further purified by chromatography (silica gel; toluene–MeOH–NEt_3, 94 : 5 : 1). Mp 149–150 °C. ^{1}H NMR (benzene-d_6) δ 8.32 (4H, anthracene-H1,4,5,8), 7.35 (4H, anthracene-H2,3,6,7), 4.44 (4H, s, anthr–CH_2–N), 2.39 (6H, s, N–CH_3), 0.90 (2H, bs, –NH–). MS: M^+: 264 *m*/*z*. Mp 149–150 °C. Anal. Calcd. for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60. Found: C, 80.74; H, 7.36; N, 9.18%.

2-Methylphenylboronic acid (*o*-tolylboronic acid) (3). Prepared according to König and Scharrnbeck.[32]

***o*-(Bromomethyl)phenylboronic acid (4).** Prepared according to Takeuchi *et al.*[33]

2,2-Dimethylpropane-1,3-diyl [*o*-(bromomethyl)phenyl]-boronate (5).[1] (*o*-Bromomethyl)phenylboronic acid (**4**, 4.79 g, 22 mmol) was dissolved in toluene (50 mL) upon heating. 2,2-Dimethylpropane-1,3-diol (4.30 g, 41 mmol, 1.9 equiv) was added and the orange solution refluxed for 2 h and left at room temperature overnight with stirring. The orange organic phase was washed with water (3 × 25 mL) and dried over $MgSO_4$. Evaporation afforded **5** as an orange oil. The yield was 5.91 g (94%). The crude **5** was >98% pure by ^{1}H NMR and was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, ArH), 7.34–7.37 (2H, ArH), 7.24–7.30 (1H, ArH), 4.93 (2H, s, Ar–CH_2–Br), 3.81 (4H, s, O–CH_2–C–CH_2–O), 1.05 (6H, s, $(CH_3)_2$C).

9,10-Bis[[*N*-[*o*-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzyl]-*N*-methylamino]methyl]anthracene (6). Prepared according to T. D. James *et al.*[1] but the orange powder thus obtained was recrystallised from ethyl acetate to afford **6** as light yellow crystals. ^{1}H NMR (CD_3OD) δ 8.33 (4H, ArH), 7.27–7.80 (12H, ArH), 5.00 (4H, bs, b: anthr–CH_2–N), 4.29 (4H, bs, a: phen–CH_2–N), 3.35 (8H, bs, O–CH_2–C–CH_2–O), 2.40 (6H, bs, e: N–CH_3), 0.85 (12H, bs, $(CH_3)_2$C). ^{13}C NMR (CD_3OD, only proton bearing carbon atoms) δ 135.0 (i: phenyl), 132.6 (f, g or h: phenyl), 131.3 (g, h or f: phenyl), 128.8 (h, f or g: phenyl), 127.9 (d: anthracene-C2,3,6,7), 126.2 (c: anthracene-C1,4,5,8), 69.7 (protecting group –CH_2–), 64.3 (a: phen–CH_2–N), 50.8 (b: anthr–CH_2–N), 40.8 (e: N–CH_3), 21.7 ($(CH_3)_2$C). MS: 668 *m*/*z*. Anal. Calcd. for $C_{42}H_{50}N_2B_2O_4$: C, 75.46; H, 7.54; N, 4.19. Found: C, 75.14; H, 7.24; N, 4.03%.

9,10-Bis({*N*-methyl-*N*-[*o*-(dihydroxyboryl)benzyl]amino}-methyl)anthracene (7). 9,10-Bis[[*N*-[*o*-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzyl]-*N*-methylamino]methyl]anthracene was suspended in 2 M NaOH and vigorously stirred for 20 h. HCl (1 M) was added to pH 6.5 and the resulting clear yellow solution was extracted with $CHCl_3$ (2×) and $CHCl_3$–DMSO (5 : 1, 1×). The organic phase was washed with water and dried over $MgSO_4$. Evaporation of solvents and drying *in vacuo* afforded **7** as a yellow powder. ^{1}H NMR (CD_3OD) δ 8.31 (4H, c: anthracene-H1,4,5,8), 7.71 (2H, i: phenyl-H), 7.60 (4H, d: anthracene-H2,3,6,7), 7.31–7.41 (6H, f, g, h: phenyl-H), 4.99 (4H, s, b: anthr–CH_2–N), 4.30 (4H, s, a: phen–CH_2–N), 2.41 (6H, s, e: N–CH_3). ^{13}C NMR (CD_3OD, only proton bearing carbon atoms) δ 137.1 (i: phenyl), 134.4 (f, g or h: phenyl), 133.4 (g, h or f: phenyl), 130.7 (h, f or g: phenyl), 129.7 (d: anthracene-C2,3,6,7), 128.0 (c: anthracene-C1,4,5,8), 66.4 (a: phen–CH_2–N), 52.6 (b: anthr–CH_2–N), 40.2 (e: N–CH_3). Anal. Calcd. for $C_{32}H_{34}N_2B_2O_4$: C, 72.11; H, 6.43; N, 5.26. Found: C, 71.79; H, 6.18; N, 4.93%.

Acknowledgements

Dr Mikkel Jørgensen and Risoe National Laboratory, Denmark are acknowledged for providing equipment and help for MALDI TOF-MS experiments. The Carlsberg Foundation, Denmark is gratefully acknowledged for financing Dr J. C. Norrild through post doctoral grant #960298/20.

References

1 T. D. James, K. R. A. S. Sandanayake, R. Iguchi and S. Shinkai, *J. Am. Chem. Soc.*, 1995, **117**, 8982.
2 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1910, and refs. therein.
3 K. Tsukagoshi and S. Shinkai, *J. Org. Chem.*, 1991, **56**, 4089.
4 J. Yoon and A. W. Czarnik, *J. Am. Chem. Soc.*, 1992, **114**, 5874.
5 J. C. Norrild and H. Eggert, *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, 2583.
6 J. C. Norrild and H. Eggert, *J. Am. Chem. Soc.*, 1995, **117**, 1479.
7 A. P. Russell. Patent. In *WO 91/04488, PCT/US90/05401*, 1991.
8 T. D. James, P. Linnane and S. Shinkai, *J. Chem. Soc.*, *Chem. Commun.*, 1996, 281, and refs. therein.
9 M. Takeuchi, T. Imada and S. Shinkai, *J. Am. Chem. Soc.*, 1996, **118**, 10658.
10 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Nature*, 1995, **374**, 345.
11 M. Takeuchi, M. Taguchi, H. Shinmori and S. Shinkai, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2613.
12 H. Shinmori, M. Takeuchi and S. Shinkai, *Tetrahedron*, 1995, **51**, 1893.
13 A. Ori and S. Shinkai, *J. Chem. Soc.*, *Chem. Commun.*, 1995, 1771.
14 M. Yamamoto, M. Takeuchi and S. Shinkai, *Tetrahedron*, 1998, **54**, 3125.
15 T. Burgemeister, R. Grobe-Einsler, R. Grotstollen, A. Mannschreck and G. Wullf, *Chem. Ber.*, 1981, **114**, 3403.
16 G. Wulff, *Pure Appl. Chem.*, 1982, **54**, 2093.
17 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769.
18 We found the formation of a strong 1,2 : 3,5-α-D-glucofuranose complex between glucose and a water soluble bis(pyridineboronic) acid at physiological pH. Submitted for publication elsewhere.
19 F. P. Worley and J. C. Andrews, *J. Phys. Chem.*, 1927, 742.
20 I. Carmichael, D. M. Chipman, C. A. Podlasek and A. S. Serianni, *J. Am. Chem. Soc.*, 1993, **115**, 10863.
21 R. J. Ferrier, A. J. Hannaford, W. G. Overend and B. C. Smith, *Carbohydr. Res.*, 1965, **1**, 38.
22 R. J. Ferrier, *J. Chem. Soc.*, 1961, 2325.
23 J. M. Sugihara and C. M. Bowman, *J. Am. Chem. Soc.*, 1958, **80**, 2443.
24 R. A. Bowie and O. C. Musgrave, *J. Chem. Soc.*, 1963, 3945.
25 E. J. Bourne, I. R. McKinley and H. Weigel, *Carbohydr. Res.*, 1974, **35**, 141.
26 6-Deoxy-L-glucose and 3-*O*-methyl-α-D-glucopyranose were kindly provided by Prof. Christian Pedersen, DTU, Denmark.
27 A. M. Yurkevich, I. I. Kolodkina, E. A. Ivanova and E. I. Pichuzhkina, *Carbohydr. Res.*, 1975, **43**, 215.
28 E. J. Hedgley, W. G. Overend and R. A. C. Rennie, *J. Chem. Soc.*, 1963, 4701.
29 S. Iacono and J. R. Rasmussen, *Org. Synth.*, 1990, **Coll. Vol. 7**, 139.
30 E. F. L. J. Anet, *Chem. Ind.* (*London*), 1960, 345.
31 M. W. Miller, R. W. Amidon and P. O. Tawney, *J. Am. Chem. Soc.*, 1955, **77**, 2845.
32 W. König and W. Scharrnbeck, *J. Prakt. Chem.*, 1930, **128**, 153.
33 M. Takeuchi, T. Mizuno, H. Shinmori, M. Nakashima and S. Shinkai, *Tetrahedron*, 1996, **52**, 1195.
34 A. Liptak, J. Imre, J. Harangi and P. Nanasi, *Carbohydr. Res.*, 1983, **116**, 217.
35 W. Curatolo, L. J. Neuringer, D. Ruben and R. Haberkorn, *Carbohydr. Res.*, 1983, **112**, 297.

Paper 8/08896I