Molecular Recognition of Carbohydrates: Strong Binding of Alkyl Glycosides by Phosphonate Derivatives

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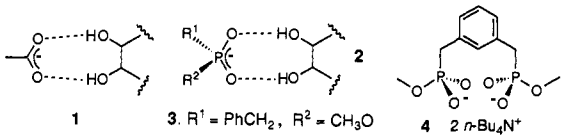
The design of artificial receptors that bind strongly and selectively to sugars in aqueous or nonaqueous media is an important current goal in bioorganic chemistry.[1] Recently receptors containing neutral hydrogen bonding sites, such as amide NH and alcohol OH groups to interact with the carbohydrate, have been shown to have modest binding affinity in nonpolar chlorocarbon solvents.[2,3] This is in sharp contrast to sugar binding proteins which have high substrate affinities even in aqueous solution and which often employ many charged residues in their binding pockets.[4] A good example is seen in the maltose binding protein (shown schematically in Figure 1)[5] which forms 11 hydrogen bonds to the disaccharide, of which nine are between charged residues and the substrate hydroxyls.[6] Four carboxylate groups act as hydrogen bond acceptors with one (Asp65) forming a bidentate interaction to a vicinal diol. This bidentate motif (**1**) is seen in other sugar binding proteins[7] and offers a potentially general approach to the design of strong binding, synthetic receptors for polyol substrates. Phosphate or phosphonate can form a similar complex with 1,2-diols (**2**), and indeed such interactions have been observed in the solid state[8] and have been proposed in the binding of fructose to DNA.[9]



Our interest lay in incorporating this anionic, bidentate motif into synthetic receptors[10] that might bind sugars in relatively polar solvents. We chose phosphonate derivatives as the basis for our design because they remain anionic over a wider range of pH than carboxylates ($pK_a \approx 1.8$ compared to ≈ 4.8) and, also, the tetrahedral phosphorus with its additional substituent allows for more facile structural modification. In this communication we report the development of anionic mono and bis phosphonate derivatives that bind alkyl glycosides strongly in polar organic solvents.

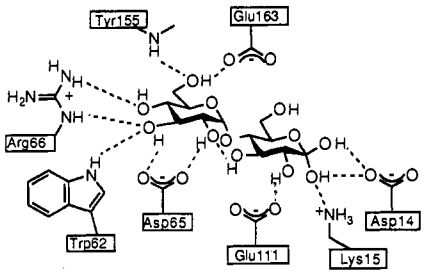


Figure 1. Substrate recognition pocket of maltose binding protein.[5]

To test the strategy outlined in **2** we studied the interaction of the tetrabutylammonium salt of methyl benzylphosphonate (**3**)[11] with *cis*- and *trans*-cyclopentane-1,2-diols and cyclohexane-1,2-diols. Titrations were carried out in CD_3CN by following changes in the ^1H NMR resonances of the alcohol or ^{31}P NMR resonance of the anion.[12] In particular, large (1.8−4.5 ppm) downfield shifts of the O*H* resonance and small (0.1−0.2 ppm) upfield shifts of the C*H*(OH) resonances of the alcohol were seen. Additionally, the ^{31}P resonance of phosphonate **3** showed a downfield shift on diol binding. The binding curves from the different NMR experiments were analyzed by nonlinear regression methods,[13] and the calculated association constants are collected in Table 1. A stoichiometry of 1:1 was confirmed for each complex either by Job plots or by the method of Ramirez et al.[14] The results show that phosphonate **3** forms weak complexes with simple alcohols, but shows appreciable binding to cyclic vicinal diols. The more than 10-fold difference in K_a values between *trans*-cyclopentane-1,2-diol and *trans*-2-methoxycyclopentanol is strongly indicative of a diol complex of type **2**. While the diol and monool have similar pK_a values, only the diol can bind in a bidentate fashion.[15]

Methyl benzylphosphonate (**3**) binds even more strongly to alkyl glycoside derivatives. For example, titration of **3** into a CD_3CN solution of 1-*O*-octyl β-D-glucopyranoside[16] gave large downfield shifts of the sugar O*H* resonances from which a K_a of 4.4×10^3 M^{-1} was measured (Figure 2a). A strong preference for 1:1 complexation was confirmed by Job analysis and reflects the electrostatic repulsion that would result from associating two anionic phosphonates with the carbohydrate. Similar results were seen with the other octyl glycosides (Table 1), and in all cases **3** binds ≈10-fold more strongly than to the

(1) For recent reviews, see: Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347−374. Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319−324.
(2) (a) Kikuchi, Y.; Tanaka, Y.; Sutarto, S.; Kobayashi, K.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10302−10306. (b) Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *102*, 1407−1408. (c) Bhattarai, K. M.; Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *J. Chem. Soc., Chem. Commun.* **1992**, 752−754. (d) Kikuchi, Y.; Kobayashi, K.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1351−1358. (e) Liu, R.; Still, W. C. *Tetrahedron Lett.* **1993**, *34*, 2573−2576. (f) Savage, P. B.; Holmgren, S. K.; Desper, J. M.; Gellman, S. H. *Pure Appl. Chem.* **1993**, *65*, 461−466. (g) Savage, P. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 10448−10449. (h) Huang, C.-Y.; Cabell, L. A.; Lynch, V.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778−2792.
(3) Tsukagoshi, K.; Shinkai, S. *J. Org. Chem.* **1991**, *56*, 4089−4091.
(4) Quiocho, F. A. *Pure Appl. Chem.* **1989**, *61*, 1293−1306.
(5) Spurlino, S. P.; Lu, G.-Y.; Quiocho, F. A. *J. Biol. Chem.* **1991**, *266*, 5202−5219.
(6) Lemieux, R. U. In *Carbohydrate Antigens*; Garegg, P. J., Lindberg, A. A., Eds.; ACS Symposium Series 519; American Chemical Society: Washington, DC, 1993; Chapter 2, pp 5−18.
(7) For other examples of 1,2- or 1,3-diol binding to carboxylate, see the following references. D-Glucose bound to galactose binding protein: Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Science (Washington, D.C.)* **1988**, *242*, 1290−1295. The Lewis b human blood group determinant bound to GS-IV lectin: Nikrad, P. V.; Beierbeck, H.; Lemieux, R. U. *Can. J. Chem.* **1992**, *70*, 241−253. Methyl α-D-mannopyranoside bound to concanavalin A: Derewenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb, A. J.; Dodson, E. J.; Papiz, M. Z.; Wan, T.; Cambell, J. *EMBO J.* **1989**, *8*, 2189. 1-Deoxynojirimycin bound to glucoamylase: Harris, E. M. S.; Aleshin, A. E.; Firsov, L. M.; Honzatko, R. B. *Biochemistry* **1993**, *32*, 1618−1626. Trimannoside bound to pea lectin: Rini, J. M.; Hardman, K. D.; Einspahr, H.; Suddath, F. L.; Carver, J. P. *J. Biol. Chem.* **1993**, *268*, 10126−10132.
(8) Rao, S. T.; Sundaralingam, M. *J. Am. Chem. Soc.* **1969**, *91*, 1210−1217.
(9) Pelmore, H.; Eaton, G.; Symons, M. C. *J. Chem. Soc., Perkin Trans. 2* **1992**, 149−150.
(10) Carboxylate sites have been employed in amphiphiles that extract glucose into organic solvents. Greenspoon, N.; Wachtel, E. *J. Am. Chem. Soc.* **1991**, *113*, 7233−7236.
(11) (a) Collins, D. J.; Drygala, P. F.; Swan, J. M. *Aust. J. Chem.* **1983**, *36*, 2095−2110. (b) Worms, K. H.; Schmidt-dunker, M. In *Organic Phosphorus Compounds*; Kosolapoff, G. M., Maier, L., Eds.; John-Wiley & Sons: New York, 1975; Vol. 7, pp 1−486.
(12) Negligible self-association of receptors **3** and **4** in CD_3CN was confirmed by ^1H NMR dilution experiments and VPO studies. Cf.: Reetz, M. T.; Hütte, S.; Goddard, R. *J. Am. Chem. Soc.* **1993**, *115*, 9339−9340.
(13) Using different versions of Hostest programs: Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H. J., Durr, H., Eds.; VCH: Weinheim, 1990; p 123.
(14) Beltrán-Porter, A.; Beltrán-Porter, D.; Cervilla, A.; Ramirez, J. A. *Talanta* **1983**, *30*, 2, 124−126.
(15) pK_a values: CH_2OHCH_2OH = 15.4; $EtOCH_2CH_2OH$ = 14.98; EtOH = 16; Source: Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795−798. Takahashi, S.; Cohen, L. A.; Miller, H. K.; Peake, E. G. *J. Org. Chem.* **1971**, *36*, 1205−1209.
(16) The octyl D-glycosides show strong self-association in $CDCl_3$, as observed by the concentration dependence of the chemical shift of the OH peaks.[2a] In CD_3CN, this effect was judged to be negligible by both ^1H NMR dilution experiments and VPO studies at concentrations of 6−20 mM.

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Table 1. Association Constants ($K_{1:1}$)[a,b] (M^{-1}) of Tetrabutylammonium salts of Methyl Benzylphosphonate (**3**) and *m*-Xylene Bis(methyl phosphonate) (**4**) with Representative Substrates in CD_3CN at 20 °C

substrate	3	substrate	3	4
n-octanol	18	*cis*-cyclohexane-1,2-diol	2.1×10^2	
cyclohexanol	11	*trans*-cyclohexane-1,2-diol	3.3×10^2	
cyclopentanol	15			
3-hydroxytetrahydrofuran	42	1-*O*-octyl β-D-glucopyranoside	4.4×10^3	2.6×10^4
trans-2-methoxycyclopentanol	25	1-*O*-octyl α-D-glucopyranoside	4.2×10^3	1.8×10^4
cis-cyclopentane-1,2-diol	2.0×10^2	1-*O*-octyl β-D-galactopyranoside	3.9×10^3	2.5×10^4
trans-cyclopentane-1,2-diol	3.9×10^2	1-*O*-octyl α-D-mannopyranoside	4.0×10^3	3.6×10^4

[a] Results of ^1H NMR titrations performed by keeping the substrate concentration constant and varying the receptor concentration. All K_a's are the mean of at least two determinations. [b] Titration data analyzed using versions of the Hostest program. Errors for K_a's less than 10^4 were estimated at ±10%; for K_a's above 10^4, errors were estimated at ±20%.

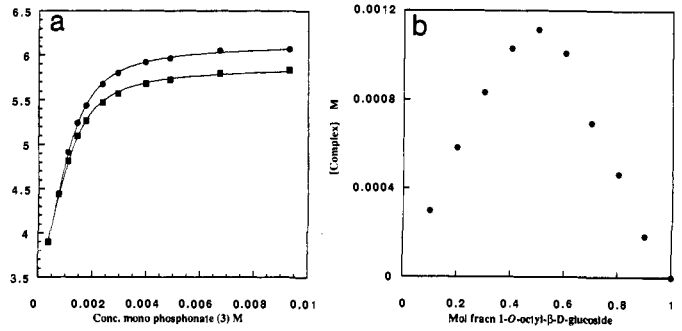

Figure 2. (a) Plot of the chemical shifts of two O*H* resonances of 1-*O*-octyl β-D-glucopyranoside (c = 1.70 mM) vs concentration of phosphonate (**3**) in CD_3CN at 20 °C. (b) Job plot of **4** + glucoside at a total concentration of 3 mM in CD_3CN at 20 °C.

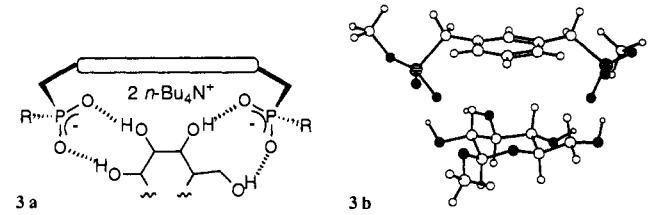

Figure 3. (a) Schematic and (b) calculated structure for the complex between bis phosphonate receptor **4** and 1-*O*-methyl β-D-glucopyranoside.[19]

simple cyclohexane-1,2-diols. This increase in affinity is presumably due to the higher acidity of the carbohydrate hydroxyls[17] as well as the statistical advantage that four hydroxyl groups (four potential 1,2- or 1,3-diol binding sites) affords.

The above results suggested that two phosphonates linked by a suitable spacer should bind to all four hydroxyls of an alkyl glycoside, as shown in Figure 3a. Receptor **4**, with two phosphonate groups separated by a *m*-xylyl spacer, was prepared in 33% overall yield by Arbuzov reaction of 1,3-bis(bromomethyl)benzene with trimethyl phosphite[18] followed by monodemethylation of the bis phosphonate diester with *N*-methylmorpholine[11b] and subsequent conversion to the bistetrabutylammonium salt. A calculated structure for the complex between **4** and 1-*O*-methyl β-D-glucoside (Figure 3b)[19] showed the phosphonate groups on either side of the glucoside in good position to form four H-bonds to the four free hydroxyl groups.

Binding of bis phosphonate **4** to the various octyl glycosides, particularly at higher concentrations, is more complex than with the mono phosphonate **3**. Initial dilution studies with a 1:1 mixture of 1-*O*-octyl β-D-glucoside and **4** in CD_3CN showed essentially no change in the ^1H NMR spectrum of either host or guest over a concentration range of 0.4−10 mM, reflecting very strong association with $K_a > 10^4$ M^{-1}. In all the octyl glycosides studied so far (Table 1), the four hydroxyl resonances broaden during the titration with **4** but become distinct near saturation (shifted downfield by ~2−2.9 ppm). However, binding could be followed by monitoring the upfield shifts of their 1- and 2-C*H* resonances (~0.1−0.2 ppm, respectively, at saturation). While titrations performed at glycoside concentrations greater than 1 mM gave evidence of multiple binding modes, at concentrations lower than 0.5 mM, the binding isotherms fitted well to a 1:1 binding scheme[13] with K_a values in the range $1.8-3.6 \times 10^4$ M^{-1}.[20] For example, 1-*O*-octyl α-D-mannopyranoside[21] gives a $K_a \approx 3.6 \times 10^4$ M^{-1} corresponding to a nearly 100-fold increase in affinity over the simple *trans*-cyclohexane-1,2-diol/**3** complex and 9-fold compared to mono phosphonate **3**. Although the modest increase in binding could be explained by only one phosphonate binding to the glycoside,[22] the sizable downfield shift of the phenyl 2-C*H* of **4** (~0.2 ppm) is strongly suggestive of a conformational change in the receptor resulting in enforced proximity of the glycoside due to binding by both phosphonates (the analogous protons in **3** do not shift under equivalent conditions). The results are therefore most consistent with both phosphonates binding to the glycoside but presumably with some mutual repulsion. This model was further supported by Job plots (Figure 2b)[23] using resonances from both the pyranosides (1- and 2-C*H*) and **4** (^{31}P), which gave maxima in the range 0.45−0.5. That some higher order aggregation is seen is not surprising since both **4** and 1-*O*-octyl D-glycosides contain several potential hydrogen bonding sites. Similar results were observed for 1-*O*-octyl β-D-glucopyranoside and 1-*O*-octyl β-D-galactopyranoside[24] (Table 1). Association constants of receptor **4** and α- and β-anomers of 1-octyl D-glucoside were similar, indicating that steric interactions between the xylene spacer and the glycosidic substituent are negligible.

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Supplementary Material Available: Data for titration of selected glycosides with **3** and **4** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(17) The pK_a's of 1-*O*-alkyl monosaccharides were unavailable. The pK_a's of glucose, mannose, and galactose are ~12.4, 12.1, and 12.4, respectively; however, these values reflect the pK_a of the most acidic 1-O*H*. From the relationship of pK_a and $\Sigma\sigma_I$ (ref. 15) and the pK_a of glycerol (14.4) we estimate that the pK_a's of 1-*O*-alkyl monosaccharide hydroxyls are ≤14. The pK_a of cyclohexanol was not available; a good approximation is the pK_a of 2-propanol: calcd 16.6, lit. 17.1. Thus, there is a difference of at least 2 units between the pK_a's of cyclohexanol and alkyl monosaccharides.

(18) Tewari, R. S.; Kumari, N.; Kendurkar, P. S. *Indian J. Chem., Sect. B* **1977**, *15* (8), 753−755.

(19) Calculated (excluding counterions) using the Monte Carlo global minimum search routine and the MM2 force field in Macromodel v. 3.5, Still, C., Columbia University.

(20) The reverse titration of glycoside into a fixed concentration of 4 gave more complex behavior. Analysis of the binding curve indicated that at higher glycoside concentrations (relative to **4**) some formation of larger aggregates (2:1, 3:1, etc.) occurs; however, the 1:1 complex remains the dominant species.

(21) Vill, V.; Böcker, T.; Thiem, J.; Fischer, F. *Liq. Cryst.* **1989**, *6* (3), 349−356.

(22) Considering the statistical factor of 2 and slightly increased basicity due to the other phosphonate.

(23) Connors, K. A. *Binding Constants*; John Wiley & Sons: New York, 1987; p 24.

(24) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *59*, 261−267.