Carbohydrate-Based Receptors with Multiple Thiourea Binding Sites. Multipoint Hydrogen Bond Recognition of Dicarboxylates and Monosaccharides†

Juan M. Benito,‡ Marta Gómez-García,‡ José L. Jiménez Blanco,‡ Carmen Ortiz Mellet,*,‡ and José M. García Fernández*,§

Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Aptdo. 553, E-41071 Sevilla, Spain, and Instituto de Investigaciones Químicas, CSIC, Américo Vespucio s/n, Isla de la Cartuja, E-41092 Sevilla, Spain

jogarcia@cica.es

Received October 23, 2000

The binding properties of multitopic sugar thiourea receptors toward dicarboxylate and monosaccharide guests have been examined taking glutarate and octyl β-D-glucopyranoside as model ligands. For the anionic hydrogen bond acceptor, both the complex stoichiometry and the association constants (K_{as}) were found to be strongly dependent on the relative disposition of recognition elements in the host. In contrast, for the glucoside guest a 1:1 stoichiometry was observed in all cases, the K_{as} values being largely independent of the unbound state provided that geometrically equivalent supramolecular topologies can be achieved.

Introduction

The design and synthesis of model receptors to recognize polar molecules of biochemical significance is receiving much increased attention.[1] The intermolecular interactions involved in these processes are of direct relevance to many biological events and may also lead to new biosensors, therapeutics, and transport systems.[2] Considerable effort has recently been directed toward the development of synthetic receptors that rely solely on hydrogen bond arrays for molecular recognition of carboxylic acid[3] and carbohydrate derivatives.[4] The former are simpler model compounds for amino acids and peptides. Although hydrogen bonds are in general significantly weaker than electrostatic interactions when the corresponding carboxylate anions are involved, they may become a dominating force in the association process if several act concertedly.[5] On the other hand, the recognition and subsequent complementary binding between an oligosaccharide epitope and its cognate receptor is the first step in many vital supramolecular processes in living systems, such as cell−cell recognition and adhesion.[6] A common feature of the protein-sugar complexes is the existence of extensive hydrogen bonding between host and guest.[7] Mimicking these strategies by designing hydrogen bonding abiotic receptors should help to unravel the principles of saccharide recognition by biomolecules.

Thiourea derivatives have proven particularly useful in the construction of neutral hydrogen bonding receptors.[3a,b,8] The relatively acidic thiourea NH protons,[9] with a strong hydrogen-bond donor capability, can establish

† Dedicated to Professor Gérard Descotes on the occasion of his retirement.

* To whom correspondence should be addressed. Tel: + 34 954489559. Fax: + 34 954460565. E-mail: jogarcia@cica.es.

‡ Universidad de Sevilla.

§ Instituto de Investigaciones Químicas, CSIC.

(1) For leading references, see: (a) Lehn, J.-M. *Supramolecular Chemistry. Concepts and Perspectives*; VCH: Weinheim: 1995. (b) Dugas, H. *Bioorganic Chemistry*; Springer-Verlag: New York, 1996. (c) Aoyama, Y. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, 1996; Vol. 2, pp 279−307. (d) Seel, C.; de Mendoza, J. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: Oxford, 1996; Vol. 2, pp 519−552. (e) Chen, H.; Weiner, W. S.; Hamilton, A. D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 458−466. (f) Beer, P. D.; Schmitt, P. *Curr. Opin. Chem. Biol.* **1997**, *1*, 475−482.

(2) (a) Bell, T. W. *Curr. Opin. Chem. Biol.* **1998**, *2*, 711−716. (b) Snowden, T. S.; Anslyn, E. V. *Curr. Opin. Chem. Biol.* **1999**, *3*, 740−746.

(3) For a complete account on carboxylate receptors, see ref 1d,f. For recent reports on hydrogen-bond receptors for carboxylates see: (a) Jullian, V.; Shephard, E.; Hursthouse, M. B.; Kilburn, J. D. *Tetrahedron Lett.* **2000**, *41*, 3963−3966. (b) Sasaki, S,; Mizuno, M.; Naemura, K.; Tobe, Y. *J. Am. Chem. Soc.* **2000**, *65*, 275−283. (c) Kubo, Y.; Tsukahara, M.; Ishihara, S.; Tokita, S. *Chem. Commun.* **2000**, 653−654. (d) Snellink-Ruël, B. H. M.; Antonisse, M. M. G.; Engbersen, J. F. J.; Reinhoudt, D. N. *Eur. J. Org. Chem.* **2000**, 165−170. (e) Al-Sayah, M. H.; Branda, N. R. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 945−947.

(4) For reviews on artificial hosts for sugars, see: (a) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2978−2996. (b) Haseltine, J.; Doyle, T. J. In *Organic Synthesis: Theory and Applications*; Hudlicky, T., Ed.; JAI Press: Greenwich, 1996; Vol. 3, 85−107. For recent reports on hydrogen-bond receptors for carbohydrates, see: (c) Budka, J.; Tkadleková, M.; Lhoták, P.; Stibor, I. *Tetrahedron* **2000**, *56*, 1883−1887. (d) Inouye, M.; Takahashi, K.; Nakazumi, H. *J. Am. Chem. Soc.* **1999**, *121*, 341−345. (e) Inouye, M.; Chiba, J.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 8170−8176. (f) Rusin, O.; Král, V. *Chem. Commun.* **1999**, 2367−2368. (g) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2270−2273.

(5) A paradigmatic example from nature, that has inspired much work on artificial neutral receptors for carboxylate anions, is the carboxylate binding pocket of the vancomycin group antibiotics. See: Loll, P. J.; Bevivino, B. D.; Korty, B. D.; Axelsen, P. H. *J. Am. Chem. Soc.* **1997**, *119*, 1516−1522. (b) Sharman, G. J.; Williams, D. H. *Chem. Commun.* **1997**, 723−724. (c) Bardsley, B.; Williams, D. H. *Chem. Commun.* **1997**, 1049−1050. (d) Albert, J. S.; Hamilton, A. D. *Tetrahedron Lett.* **1993**, *34*, 7363−7366, and references therein.

(6) For leading references see: (a) Siebert, H.-C.; von der Lieth, C.-W.; Renter, G.; Wittmann, J.; Vliegenthart, J. F. G.; Gabius, H.-J. In *Glycosciences, Status and Perspectives*; Gabius, H.-J., Gabius, S., Eds.; Chapman & Hall: Weinheim, 1997; pp 291−310. (b) Dwek, R. *Chem. Rev.* **1996**, *96*, 683−720. (c) Fukuda, M. *Bioorg. Med. Chem.* **1995**, *3*, 207−215. (d) Varki, A. *Glycobiology* **1993**, *3*, 97−130.

(7) (a) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2300−2334. (b) Poveda, A.; Jiménez-Barbero, J. *Chem. Soc. Rev.* **1998**, *27*, 133−143. (c) Weiss, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441−473. (d) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719−728. (e) Jeffrey, G. A. *Adv. Enzymol.* **1992**, *65*, 217−254. (f) Vyas, N. K. *Curr. Opin. Struct. Biol.* **1991**, *1*, 732−740.

10.1021/jo001508n CCC: $20.00
Published on Web 02/01/2001

multipoint hydrogen bonded patterns with complementary acceptor groups in a specific and predictable manner. Moreover, the diffusiveness of the electronic charge in the lone pairs of sulfur leads to the thiocarbonyl group being a weak hydrogen-bonding acceptor, unlikely to interfere in conformational or complexing studies involving other stronger acceptor centers.[10] Our own results have shown that thiourea functionalities embedded into an oligosaccharide structure provide efficient anchoring points for hydrogen bonding of carboxylates.[11] The sugar moieties allow conformational control at the pseudoamide bonds and act as intramolecular probes during NMR titration experiments. In the frame of a program aimed at the understanding of molecular recognition processes involving carbohydrates, we now report on the binding properties of the multitopic sugar−thiourea conjugates **1**−**3**. The complex stoichiometry and association constants (K_{as}) for glutarate (**4**) and octyl β-D-glucopyranoside (**5**) have been determined by ^{1}H NMR spectroscopy in order to evaluate the effect of the number of hydrogen bond donating sites and their relative disposition on the binding efficiency for each type of ligand.

Results and Discussion

Design Criteria. In the present work, we have purposely chosen systems having C_{2v} symmetry to promote cooperative interactions on complexation while facilitating NMR analysis. The *p*-xylylene bis(thiourea) framework was selected as an appropriate motif for receptor design because it has been shown to be complementary of dicarboxylate **4**.[8a,12] Since **4** has a chain length close to that of a hexose, it was also expected to expand properly through the pyranoid ring of the glucopyranoside **5**.[13] By incorporating additional thiourea functionalities and *m*-xylylene linkers we intended to create a deeper cavity, favoring ligand desolvation and maximizing hydrogen bond donating interactions.[14] The relative disposition of the thiourea groups was chose to allow an optimal contact between the corresponding binding sites without building up of substantial strain. In contrast to the tetradentated receptor **1**, the hydrogen-bonding centers of **2** and **3** can converge to complement all acceptor centers of ligand **4**, in its most stable transoid conformation, by rotation about a limited number of single bonds; this was checked by computer-aid molecular modeling. The goal of considering both alternating combinations of *p*- and *m*-xylylene spacers was to examine binding characteristics as the arrangement of recognition elements in the receptor changed with the overall architecture of the 1:1 complex remaining the same. The terminal glucopyranosyl subunits were expected to fix the *Z*-configuration at the contiguous NH—C(=S) bonds, as previously observed for other sugar thioureas, promoting the "active" 1,3-parallel disposition of the thiourea NH protons.[15]

Synthesis and Structure of Receptors 1−3. The tetradentated host **1** was efficiently prepared by direct

(8) (a) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369−370. (b) Scheerder, J.; Fochi, M.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1994**, *59*, 7815−7820. (c) Pernía, G. J.; Kilburn, J. D.; Rowley, M. *J. Chem. Soc., Chem. Commun.* **1995**, 305−306. (d) Goodman, M. S.; Jubian, V.; Linton, B.; Hamilton, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 11610−11611. (e) Nishizawa, S.; Bühlmann, P.; Iwao, M.; Umezawa, Y. *Tetrahedron Lett.* **1995**, *36*, 6483−6486. (f) Scheerder, J.; Engbersen, J. F. J.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 307−320. (g) Scheerder, J.; Engbersen, J. F. J.; Verboom, W.; van Duynhoven, J. P. M.; Reinhoudt, D. N. *J. Org. Chem.* **1996**, *61*, 3476−3481. (h) Pernía, G. J.; Kilburn, J. D.; Essex, J. W.; Mortishire-Smith, R. J.; Rowley, M. *J. Am. Chem. Soc.* **1996**, *118*, 10220−10227. (i) Bühlmann, P.; Nishizawa, S.; Xiao, K. P.; Umezawa, Y. *Tetrahedron* **1997**, *53*, 1647−1654. (j) Tobe, Y.; Sasaki, S.; Mizuno, M.; Naemura, K *Chem. Lett.* **1998**, 835−836. (k) Linton, B. R.; Goodman, M. S.; Hamilton, A. D. *Chem. Eur. J.* **2000**, *6*, 2449−2455.

(9) Bordwell, F. G.; Algrim, D. J.; Harrelson, J. A., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 5903−5904.

(10) (a) Molina, M. T.; Yáñez, M.; Mó, O.; Notario, R.; Abboud, J.-L. M. In *Supplement A3, The Chemistry of Double-Bonded Functional Groups, Part 2*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1997; pp 1355−1496. (b) Rienäcker, C. M.; Klapötke, T. In *Supplement A3, The Chemistry of Double-Bonded Functional Groups, Part 1*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1997; pp 341−365.

(11) Jiménez Blanco, J. L.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Org. Lett.* **1999**, *1*, 1217−1220.

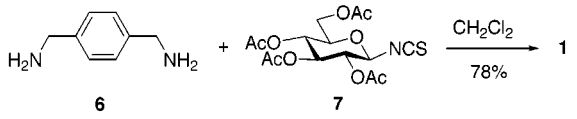
(12) For leading references on artificial receptors for dicarboxylic acids and their salts, see: (a) Goswami, S.; Ghosh, K.; Dasgupta, S.; *J. Org. Chem.* **2000**, *65*, 1907−1914. (b) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3666−3669. (c) Prohens, R.; Tomàs, S.; Deyà, P. M.; Ballester, P.; Costa, A. *Tetrahedron Lett.* **1998**, *39*, 1063−1066. (d) Takeuchi, M.; Imada, T.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2096−2099. (e) Eblinger, F.; Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 826−829.

(13) Rigid receptors for five-carbon chain dicarboxylic acids have been previously shown to be efficient for hexopyranoside binding: Cuntze, J.; Owens, L.; Alcázar, V.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 367−390.

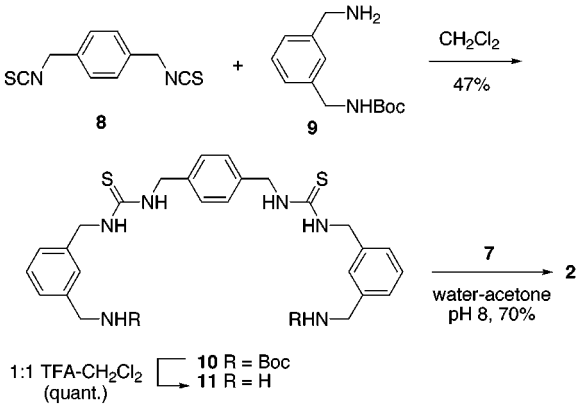
(14) Most reported artificial receptors for carbohydrates relying solely on hydrogen bonds for binding encompass simultaneously donor and acceptor centers (cf. ref 4). A few examples of hosts having exclusively hydrogen bond acceptor centers have been reported: (a) Das, G.; Hamilton, A. D. *J. Am. Chem. Soc.* **1994**, *116*, 11139−11140. (b) Neidlein, U.; Diederich, F. *Chem. Commun.* **1996**, 1493−1494. To the best of our knwoledge, there are no reports on sugar binding by receptors involving exclusively hydrogen bond donor centres.

(15) For recent reviews on the synthesis and conformational properties of sugar thioureas, see: (a) García Fernández, J. M.; Ortiz Mellet, C. *Sulfur Reports* **1996**, *19*, 61−169. (b) García Fernández, J. M.; Ortiz Mellet, C. *Adv. Carbohydr. Chem. Biochem.* **2000**, *55*, 35−135.

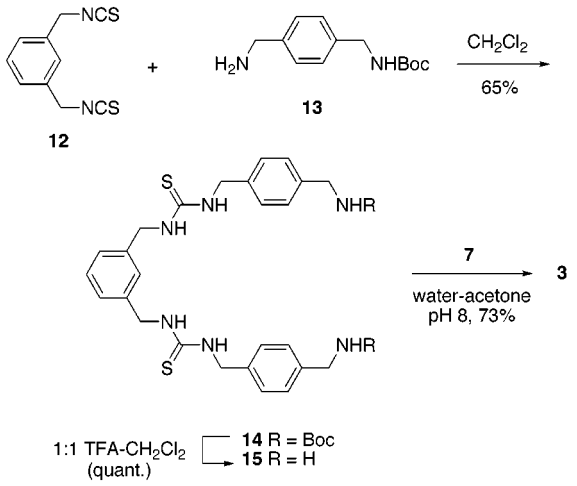
Scheme 1



Scheme 2



Scheme 3



coupling of commercial *p*-xylylene diamine (**6**) and 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate[16] (**7**) (Scheme 1). The tetrathiourea derivatives **2** and **3** were obtained in three steps by reaction of 1,4- (**8**)[17] or 1,3-bis(isothiocyanatomethyl)benzene[18] (**12**), respectively, with the corresponding mono-Boc-protected diamine **9**[18,19] or **13**,[19] subsequent TFA-catalyzed hydrolysis of the carbamate groups in the adducts (**10** or **14**), and final coupling of the resulting diamine (**11** or **15**) with the glucosyl isothiocyanate **7** (Schemes 2 and 3).

The ^{1}H and ^{13}C NMR spectra of receptors **1**−**3** recorded at room temperature in $CDCl_3$ and in DMSO-d_6 showed the typical line broadening associated with restricted rotation at the pseudoamide NH−C(=S) bonds.[20] At 313 K, a single set of signals was observed, thus confirming

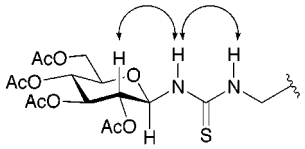


Figure 1. Relative disposition of the H-2, H-1, and NH protons in the anti-*Z*,*Z* conformation of glucosylthioureido segments. The diagnostic ROE contacts are shown.

the expected C_{2v} symmetry. In all cases, ROESY experiments exhibited intense cross-peaks between the resonances of the sugar-NH proton and H-2 in the pyranose ring, supporting the anti conformation at the anomeric bond (Figure 1). The low-field chemical shift for the H-1 resonance is in agreement with a relative 1,3-parallel disposition with respect to the thiocarbonyl sulfur atom, implying the *Z*-configuration at the corresponding pseudoamide bond.[21] The presence of intense ROE contacts between NH protons located at the same thiourea group indicated a significant proportion of *Z*,*Z* conformers in the rotameric equilibria. Nevertheless, the low-temperature range ^{1}H NMR spectra of **1**−**3** in $CDCl_3$ evidenced the presence of several rotamers in the solution. The relatively low coalescence temperatures (255−260 K), the chemical shifts of the NH resonances (7.2−6.5 ppm) and the complexity of the spectra discarded the presence of intramolecular hydrogen-bonded stabilized folding patterns that should be disrupt upon complexation.[22]

Binding Studies. Evaluation of complex stoichiometry and binding strength was effected by continuous variation plots (Job plots) and ^{1}H NMR titration experiments. Addition of aliquots of tetrabutylammonium (TBA) glutarate **4** (in $CDCl_3$ or DMSO-d_6) or octyl *β*-D-glucopyranoside **5** (in $CDCl_3$) to 5 mM solutions of receptors **1**−**3** at 303 K caused all NH proton signals to shift downfield, presumably due to their involvement on hydrogen bonding. In all cases, the ^{1}H chemical shifts were found to be independent of the concentration of host molecule in the range 1.0−10.0 mM at this temperature, discarding significant self-association under the conditions used for association constant (K_{as}) measurements. Dilution experiments in $CDCl_3$ showed weak self-association of the glucopyranoside **5** above 5 mM. To confirm that the effect of self-association on the host−guest binding is negligible, additional inverse titrations were carried out in which the concentration of **5** was held constant and that of receptors **1**−**3** varied. The K_{as} values obtained by this procedure were virtually identical to those obtained by the direct protocole.

As anticipated, receptor **1** displayed a binding isotherm against **4** in DMSO-d_6 compatible with structure **16**

(16) (a) Camarasa, M. J.; Fernández-Resa, P.; García-López, M. T.; de las Heras, F. G., Méndez-Castrillón, P. P.; San Félix, A. *Synthesis* **1984**, 509−510. (b) Lindhorst, T. K.; Kieburg, C. *Synthesis* **1995**, 1228−1230.

(17) (a) Shibanuma, T.; Shiono, M.; Mukaiyama, T. *Chem. Lett.* **1977**, 573−574. (b) Kritián, P.; Sprinzl, M., Antos, K. *Collect. Czech. Chem. Commun.* **1965**, 3658−3663.

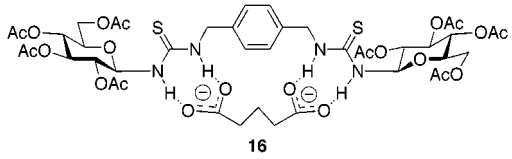
(18) Gross, R.; Dürner, G.; Göbel, M. W. *Liebigs Ann. Chem.* **1994**, 49−58.

(19) Smith, J.; Lira, J. L.; Schneider, S. E.; Anslyn, E. V. *J. Org. Chem.* **1996**, *61*, 8811−8818.

(20) Duus, F. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Pergamon Press: London, 1979; Vol. 3, pp 373−487.

(21) Díaz Pérez, V. M.; Ortiz Mellet, C.; Fuentes, J.; García Fernández, J. M. *Carbohydr. Res.* **2000**, *326*, 161−175.

(22) Typically, the presence of intramolecular hydrogen-bonding stabilized folding patterns in sugar thioureas results in increased caolescence temperatures (285−295 K), lowfield shifted NH resonances (11−7.3 ppm) and a simplification of the rotameric equilibrium. See cf. ref 11 and: (a) García-Moreno, M. I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Tetrahedron: Asymmetry* **2000**, *11*, 1331−1341. (b) García Fernández, J. M.; Ortiz Mellet, C.; Jiménez Blanco, J. L.; Fuentes, J.; Diánez, M. J.; Estrada, M. D.; López-Castro, A.; Pérez-Garrido, S. *Carbohydr. Res.* **1996**, *286*, 55−65. (c) García Fernández, J. M.; Jiménez Blanco, J. L.; Ortiz Mellet, C.; Fuentes, J. *J. Chem. Soc., Chem. Commun.* **1995**, 57−58. (d) Ortiz Mellet, C.; Moreno Marín, A.; Jiménez Blanco, J. L.; García Fernández, J. M.; Fuentes, J. *Tetrahedron*: *Asymmetry* **1994**, *5*, 2325−2334.



16

Figure 2. Probable mode of complexation of receptor **1** with ligand **4** in DMSO-d_6.

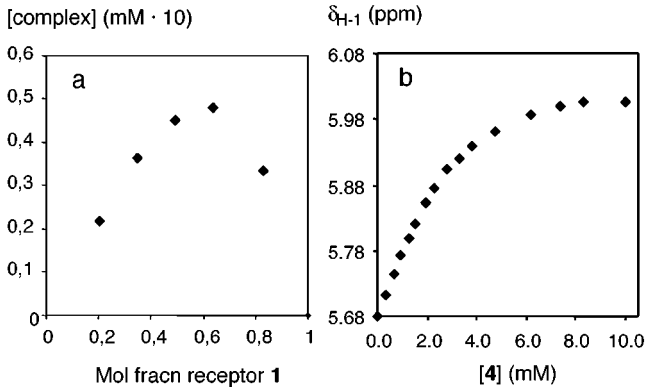
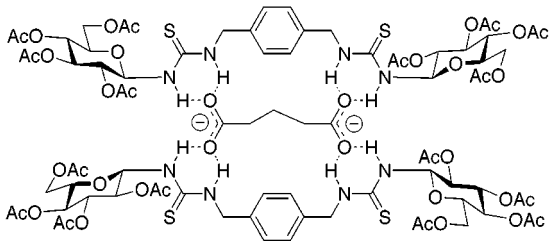


Figure 3. (a) Job plot of **1** + **4** in $CDCl_3$ at a total concentration of 5 mM (H-1). (b) Plot of the chemical shifts of the H-1 resonance of receptor **1** (c = 5 mM) vs concentration of ligand **4** in $CDCl_3$ at 303 K.

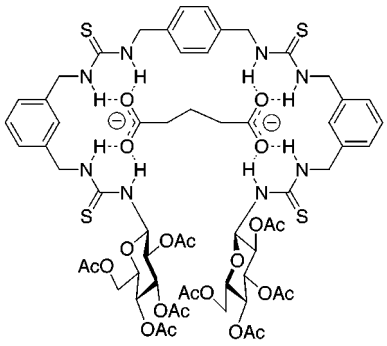
(Figure 2). Furthermore, the resolution of the spectra significantly improved in the presence of ligand, which is consistent with the postulated formation of transbidentated hydrogen bonds that anchors the *Z,Z* configuration at the thiourea segments upon complex formation.[23] Nonlinear regression analysis[24] of the resulting curve indicated relatively weak binding (K_{as} 391 M^{-1}),[25] which is in contrast with the much higher association constant reported for a similar bis(thiourea) structure bearing butyl substituents at the N-atoms.[8a] Possibly, the higher lipophilicity of the alkyl chains at the vicinity of the hydrogen bond donor centers in the latter facilitate the dessolvation processes of the incoming carboxylate guest. In $CDCl_3$, however, where interactions other that hydrogen bonds are suppressed, the titration data and continuous variation plots (Figure 3) were consistent with an equilibrium between **16** and a 2:1 (host/guest) complex, probably having structure **17** (Figure 4). Since the isotherm begins with host **1** in excess, the 2:1 complex **17** is first formed, which exists in highest concentration near 0.5 equiv of ligand. As the concentration of **4** increases, the equilibrium shifts to predominantly the 1:1 complex **16**. Fitting the curve to a two-equilibria expression[26] gave microscopic binding constants K_{as1} and K_{as2} of 235 and 247 M^{-1}, respectively.[25]

The podant-like tetrathiourea receptors **2** and **3** both incorporate the potential to form bimolecular octaden-



17

Figure 4. Probable struture of the 2:1 (host/guest) complex of receptor **1** with ligand **4** in $CDCl_3$.



18

Figure 5. Probable mode of complexation of receptor **2** with ligand **4** in both DMSO-d_6 and $CDCl_3$.

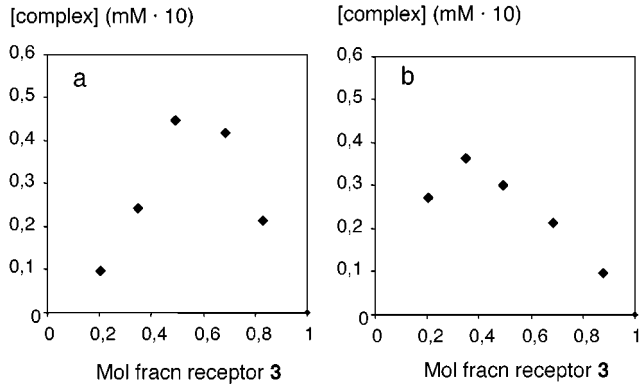


Figure 6. (a) Job plot of **3** + **4** in $CDCl_3$ at a total concentration of 5 mM (H-3). (b) Job plot of **3** + **4** in DMSO-d_6 at a total concentration of 5 mM (H-1).

tated complexes with **4**. Notwithstanding, the corresponding binding isotherms and Job's plots evidenced significant differences in their binding behavior. Receptor **2** formed the expected 1:1 complex **18** either in $CDCl_3$ or in DMSO-d_6 solution (Figure 5), with K_{as} values that exceeded those that can be measured by NMR titration experiments (>10^6 M^{-1}). Binding was still clearly observable at 10% D_2O/DMSO-d_6 (K_{as} 10^3 M^{-1}). In the case of **3**, the NMR data did not fit for the 1:1 host:guest binding model (Figure 6). In $CDCl_3$ the binding data suggested an equilibrium between the 1:1 (**19**) and a 2:1 (host:guest) complex (K_{as1} 10^4 M^{-1}, K_{as2} 10^3 M^{-1}),[25] probably having structure **20**. This is in agreement with the high tendency of *m*-xylylene bis(thiourea) fragments to form tetradentated hydrogen bond patterns with carboxylate groups.[8e] In DMSO-d_6 the titration curves and Job's plots (Figure 6) were consistent with a 1:2 stoichiometry (**21**) at high

(23) Although a fast equilibrium between monodentate complexes is also possible, strong binding necessarily involves the *Z,Z*-rotameric form of the thiourea group. See: Haushalter, K. A.; Lan, J.; Roberts, J. D. *J. Am. Chem. Soc.* **1996**, *118*, 8891−8896.

(24) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H. J., Durr, H., Eds.; VCH: Weinheim, 1991; p 123.

(25) Binding constants were obtained in triplicate at 303 K. Estimated errors are ±10% for the host:guest fitting and ±20% for the host−host−guest and host−guest−guest fittings. We thank Dr. C. A. Hunter for kindly providing the fitting program.

(26) Kneeland D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042−10055.

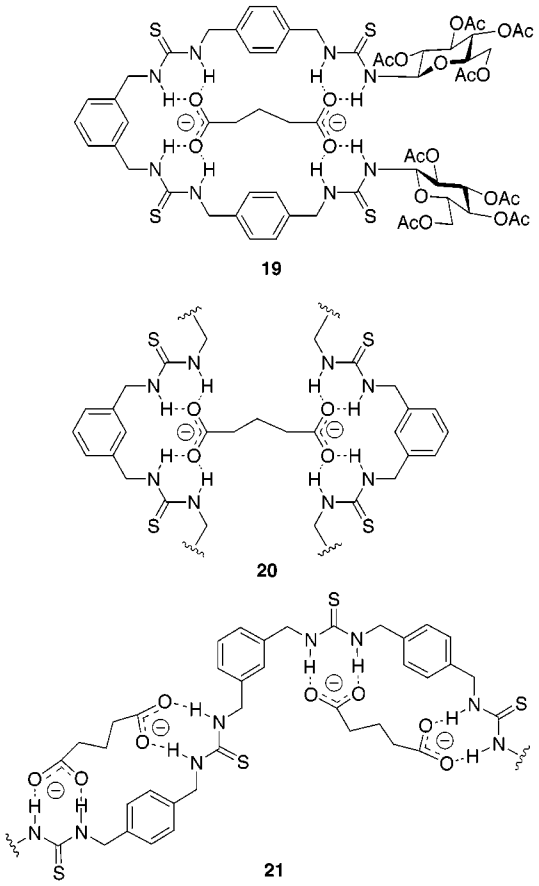


Figure 7. Probable struture of the 1:1 (**19**), 2:1 (**20**), and 1:2 (host/guest) complex (**21**) of receptor **3** with ligand **4** in $CDCl_3$ or DMSO-d_6.

ligand concentrations (K_{as1} 10^5 M^{-1}, K_{as2} 10^2 M^{-1})[25] (Figure 7).

The above ensemble of thermodynamic data illustrates the dramatic influence that subtle changes in the relative disposition of recognition elements may have in the binding properties of flexible hydrogen-bonding receptors. Structures **17**, **18**, and **19** represent geometrically equivalent supramolecular arrangements, with the glutarate ligand, in the most stable transoid conformation, fully hydrogen-bonded solvated by the four surrounding thiourea groups. The marked differences in their relative stability (**18** > **19** ≫ **17**) underline the advantages of positioning hydrogen-bonding sites close together in the host, where they are less effectively solvated than when widely spaced. Moreover, the cooperativity of multipoint interactions at both ends of the receptor facilitates an optimal fitting between the host and the dicarboxylate ligand.

The binding behavior of **1**−**3** toward glucoside **5** in $CDCl_3$ revealed a totally different scenario. First, the titration experiments and Job plots were indicative of the 1:1 stoichiometry in all cases. Second, the K_{as} value for the **1**:**5** complex (900 M^{-1}) was almost three times higher than K_{as} values for the **2**:**5** (K_{as} 314 M^{-1}) and **3**:**5** (K_{as} 304 M^{-1}) complexes.[25] No significant difference in binding efficiency was found between the two latter. Most probably, compounds **2** and **3** act solely as hydrogen bond donating receptors, whereas additional hydrogen-bonding interactions between the OH groups of **5** and the ester functionalities of **1** operate in the case of the **1**:**5** complex. The observation of weak intermolecular ROE contacts

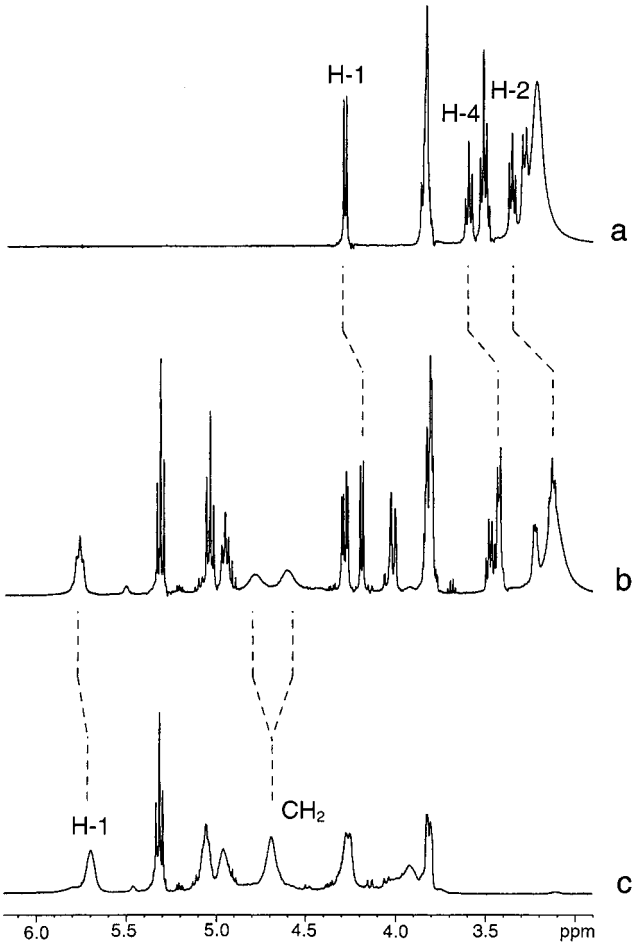
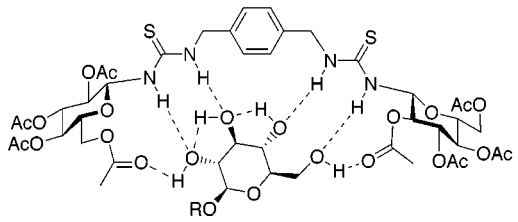


Figure 8. Comparative ^1H NMR spectra (sugar proton region, 500 MHz, $CDCl_3$) of octyl β-D-glucopyranoside **5** (a), an equimolecular mixture of **1** and **5** (b), and receptor **1** (c).

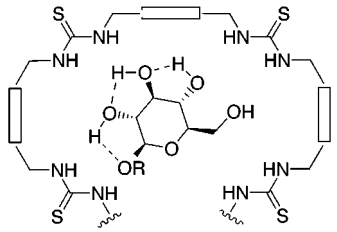
between the sugar units of the host and the ligand in the latter, which were absent in the case of the **2**:**5** and **3**:**5** complexes, supports this hypothesis.

The dramatic improvement of spectral resolution in the **1**:**5** complex as compared with the unbound host **1** (Figure 8) is consistent with anchoring of the receptor conformation and formation of a relatively rigid supramolecular architecture. The highfield complexation-induced chemical shifts of the ligand protons clearly shows that the pyranose ring lies over the ring current of the benzene π-system in the binding site. This effect is much stronger for the H-2 and H-4 protons, suggesting that the β-face of the glycoside is closer to the center of the aromatic ring. This relative disposition allows the formation of four NH···O hydrogen bonds involving the ligand O-2, O-3, O-4, and O-6 atoms, with both thiourea segments in the *Z,Z* configuration as shown in Figure 9 (**22**). Additionally, at least two acetyl groups of the host (located at C-2 and C-6) can act as acceptor centers in intermolecular OH···O hydrogen-bonding interactions, which probably accounts for the unexpectedly high association constant. Interestingly, this binding mode do not compete with the intramolecular hydrogen-bonding network around the adjacent OH-2/OH-3/OH-4 triol system of the glucoside.[27] The presence of H-4/benzylic NH, H-4/Ar and H-2/Ar intermolecular cross-peaks in the ROESY spectra further supports this possible mode of complexation.



22

Figure 9. Probable mode of complexation of receptor **1** with ligand **5** in $CDCl_3$.



23

Figure 10. Schematic representation of the complexes of the tetrathiourea receptors **2** and **3** with ligand **5** in $CDCl_3$.

Significant complexation-induced highfield chemical shifts were also observed for the ligand protons in the complexes of the tetrathiourea hosts **2** and **3** with octyl β-D-glucopyranoside **5** in $CDCl_3$, suggesting its location in an aromatic ring surrounded cavity. In contrast, changes on line shape and chemical shifts in the ^{1}H NMR spectra of the tetrathiourea hosts **2** and **3** upon addition of ligand **5** were much less pronounced. Line broadening was still evident in the presence of high proportions of ligand, indicating that slow chemical exchange processes, probably associated to restricted rotations about NH−C(=S) bonds, occur in the complexes. These observations can be rationalized assuming that the recognition pattern above-discussed, involving two xylylene-bridged thiourea groups and four O-atoms of the pyranose ring, is the main binding mode that operates in these systems.[28] Although supramolecular arrangements with a higher degree of hydrogen-bonding solvation of the sugar ligand are accessible, the additional intermolecular hydrogen bonds would be formed either at the expenses of the intramolecular OH···OH interactions[29] or would imply single two centers NH···O interactions. Such situations will contribute only marginally to the complexing enthalpy, while the entropy penalty will be presumably high. Only one of the three xylylene bis(thiourea) fragments present in the structutre of hosts **2** and **3** is efficiently participating in binding at a time, the rest of the bound receptor molecule keeping a high mobility degree. The ligand molecule is probably in fast exchange between the cluster of binding sites (Figure 10), the average result being equivalent to an aromatic-surrounded cavity (**23**).

It is noteworthy that the much weaker hydrogen bond acceptor ligand **5**, as compared with **4**, exhibits a higher efficiency in inducing an optimal 1:1 host−guest fitting. This suggests that the requirements for multipoint recognition and binding through hydrogen bonds depend strongly on the nature of the individual interactions between complementary recognition elements in the host and guest. Preorganization of hydrogen bond donor centers in a flexible host is crucial to achieve a well defined supramolecular organization if individual interactions are strong (e.g., thiourea−carboxylate associations). In contrast, the need for a maximum effective surface contact between host and guest in the bound state for recognition processes characterized by weak affinities (e.g., thiourea−hydroxyl associations) largely overcomes the contributions to the binding energy arising from differences in the arrangement of recognition elements in the unbound state, a situation already encountered in natural systems.[30] Identification of recognition elements and quantification of individual interactions should be, then, of particular relevance in the design of artificial sugar-based receptors for hydrogen bond recognition of molecules possessing simultaneously strong and weak hydrogen-bonding domains, such as carboxylate (uronic acids, ulosonic acids) and phosphate-containing carbohydrate derivatives (nucleotides, sugar phosphates). Work in this sense is currently under progress in our group.

Experimental Section

General Methods. Optical rotations were measured at room temperature in 1-dm tubes. ^{1}H (^{13}C) NMR spectra were recorded at 500 (125.7) or 300 (75.5) MHz. 2D ROESY experiments were obtained at 500 MHz in the phase-sensitive mode with time proportional phase incrementation (TPPI) and at two different mixing times (200 and 400 ms). In the FABMS spectra, the primary beam consisted of Xe atoms with a maximum energy of 8 keV. The samples were dissolved in *m*-nitrobezyl alcohol or thioglycerol and the positive ions were separated and accelerated over a potential of 7 keV. NaI was added as cationizing agent. TLC was performed with precoated TLC plates, silica gel 30F-245, with visualization by UV and by charring with 10% sulfuric acid. Microanalyses were performed by the Instituto de Investigaciones Químicas (Sevilla, Spain).

Materials. Tetra-*n*-butylammonium (TBA) glutarate (**4**) was prepared from glutaric acid by addition of 2 equiv of TBA hydroxide and freeze-drying of the resulting dicarboxylate salt. Octyl β-D-glucopyranoside **5** was a commercial compound. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate **7** was synthesized from acetobromoglucose following the methodology of Camarasa et al.[16] 1,4- and 1,3-bis(isothiocyanatomethyl)-benzene **8** and **12** were obtained by isothiocyanation of the corresponding commercially available diamines with thiophosgene as reported.[17,18] The *N*-*tert*-butyloxycarbonyl monoprotected diamine derivatives **9** and **13** were prepared by the reaction of bis(*tert*-butyloxycarbonyl) carbonate with an excess of the corresponding diamine.[18,19]

Binding Titrations. $CDCl_3$ used for binding experiments was deacidified and partially dried by storage over basic alumina. DMSO-d_6 was stored over 4 Å molecular sieve and

(27) Docking studies were performed by using the MACROMODEL v6.0 package and the GB/SA continuum solvent model for chloroform. Initially, the host molecule was extensively minimized using the MM2* program, with the thiourea groups in the *Z*,*Z* configuration and the anti conformation at the glycosidic bonds. The global minimum was then used to dock the ligand, with the octyl chain in the transoid conformation and oriented to satisfy the *exo*-anomeric effect (Φ angle H-1−C-1−O-1-octyl of ca. 60°). The ligand was manually docked into the binding site and extensively minimized. The three-dimensional geometries obtained were in agreement with the discussed NMR data.

(28) An analogous binding mode has been proposed for a *m*-xylylene-derived receptor incorporating phosphonate groups as bidentated hydrogen bond acceptor centres. See cf. ref 18a.

(29) Huang C.-Y. Cabell, L. A., Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778−2792.

(30) (a) Navarre, N.; Amiot, N.; van Oijen, A.; Imberty, A.; Poveda, A.; Jiménez-Barbero, J.; Cooper, A.; Nutley, M. A.; Boons, G.-J. *Chem. Eur. J.* **1999**, *5*, 2281−2294. (b) Wacowich-Sgarbi, Bundle, D. R. *J. Org. Chem.* **1999**, *64*, 9080−9089. (c) Alibés, R.; Bundle, D. R. *J. Org. Chem.* **1998**, *63*, 6288−6301.

contained 0.2% water as detected by ^{1}H NMR. The association constants at 303 K were experimentally determined by measuring the proton chemical shift changes of 5 mM solutions of the thiourea receptor **1**−**3** upon increased amounts of the corresponding guest **4** or **5**. In a typical titration experiment, a 5 mM solution of host in $CDCl_3$ or DMSO-d_6 was prepared, a 500 μL aliquot was transferred to a 5 mm NMR tube, and the initial NMR spectrum was recorded. A solution (20−75 mM) of the guest in the previous host solution was prepared and then added via microsyringe initially in 10 μL portions. These amounts were increased until complete complexation of the host. The ^{1}H NMR spectrum of each solution was recorded and the chemical shift of the diagnostic sugar protons obtained at 12−15 different host−guest concentration ratios were used in an iterative least-squares fitting procedure. Inverse titration experiments were also carried out for the complexes between receptors **1**−**3** and ligand **5**.

Job Plots. Stock solutions of host and guest were prepared (5 mM each) and separated into 5-mm NMR tubes to give the following host:guest volume ratio: 5:0, 4:1, 3:3, 2:3, 1:4. ^{1}H NMR spectra of all samples were obtained and the concentration of complex ([C]) for each solution was determined from the equation

$$[C] = [H]_0(\delta_{obs} - \delta_0)/(\delta_{max} - \delta_0)$$

where $[H]_0$ is the preequilibrium host concentration, δ_{obs} is the observed chemical shift, δ_0 is the chemical shift of the free host, and δ_{max} is the chemical shift of the complex. The conventional Job plot ($[C]_{eq}$ vs $[H]_0/[H]_0 + [G]_0$) was then determined.

1,4-Bis[3-[3-(*tert*-butoxycarbonylaminomethyl)benzyl]-thioureidomethyl]benzene (10). A solution of 1,4-bis-(isothiocyanatomethyl)benzene **8** (125 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) was added to a solution of the mono-Boc-protected diamine **9** (280 mg, 1.2 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 3 h, evaporated and purified by column chromatography eluting first with 1:1 EtOAc-petroleum ether and then with MeOH, to give **10** (181 mg, 47%) as an amorphous solid: R_f 0.15 (1:1 EtOAc−petroleum ether); FABMS *m*/*z* 715 (100, $[M + Na]^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (s, 18 H, CMe_3), 4.09 (d, 4 H, J = 6.2 Hz, CH_2NHCO), 4.64 (bs, 8 H, CH_2NHCS), 7.09−7.28 (m, 12 H, Ph), 7.36 (t, 2 H, J = 6.2 Hz, N*H*CO), 7.91 (bs, 4 H, N*H*CS); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 28.3, 43.4, 46.8, 77.8, 125.4−140.2, 155.1, 182.9. Anal. Calcd for $C_{36}H_{48}N_6O_4S_2$: C, 62.40; H, 6.98; N, 12.13. Found: C, 62.40; H, 6.90; N, 12.12.

1,4-Bis[3-[3-(aminomethyl)benzyl]thioureidomethyl]-benzene (11). Compound **10** (205 mg, 0.30 mmol) was treated with 50% TFA−CH_2Cl_2 (5 mL) for 1 h and evaporated under reduced pressure to give **11** as a hygroscopic solid which was used in the next step without further purification: FABMS *m*/*z* 493 (100, $[M + H]^+$); ^{1}H NMR (500 MHz, CD_3OD, 313 K) δ 4.06 (s, 4 H, CH_2NH$_2$), 4.69, 4.76 (2 s, each 4 H, CH_2NHCS), 7.24−7.37 (m, 12 H, Ph); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K) δ 43.0, 47.2, 127.2−140.0, 183.1.

1,3-Bis[3-[4-(*tert*-butoxycarbonylaminomethyl)benzyl]-thioureidomethyl]benzene (14). Coupling reaction of **12** (125 mg, 0.3 mmol) and **13** (280 mg, 1.2 mmol) and purification as above-described for the preparation of **10** afforded **14** (250 mg, 65%) as an amorphous solid: R_f 0.15 (1:1 EtOAc−petroleum ether); FABMS: *m*/*z* 715 (40, $[M + Na]^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (s, 18 H, CMe_3), 4.07 (d, 4 H, J = 6.1 Hz, CH_2NHCO), 4.64 (bs, 8 H, CH_2NHCS), 7.15−7.28 (m, 12 H, Ph), 7.36 (t, 2 H, J = 6.1 Hz, N*H*CO), 7.92 (bs, 4 H, N*H*CS); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 28.6, 43.1, 46.9, 77.8, 125.8−139.3, 155.8, 183.0. Anal. Calcd for $C_{36}H_{48}N_6O_4S_2$: C, 62.40; H, 6.98; N, 12.13. Found: C, 62.41; H, 6.87; N, 11.96.

1,3-Bis[3-[4-(aminomethyl)benzyl]thioureidomethyl]-benzene (15). Removal of the Boc groups of **14** (205 mg, 0.30 mmol) with 1:1 TFA−CH_2Cl_2, as described above for the preparation of **11**, gave **15** as a hygroscopic solid which was used in the next step without further purification: FABMS *m*/*z* 515 (10%, $[M + Na]^+$); ^{1}H NMR (500 MHz, CD_3OD) δ 4.08 (s, 4 H, CH_2NH$_2$), 4.70, 4.77 (2 s, each 4 H, CH_2NHCS), 7.20−7.40 (m, 12 H, Ph); ^{13}C NMR (125.7 MHz, CD_3OD) δ 43.8, 48.5, 126.9−135.5, 183.1.

1,4-Bis[3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thioureidomethyl]benzene (1). To a solution of 1,4-bis-(aminomethyl)benzene (**6**, 68 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**7**, 389 mg, 1 mmol). The reaction mixture was stirred for 30 min at room temperature and concentrated. Further purification of the residue by column chromatography (20:1 CH_2Cl_2−MeOH) yielded **1** (355 mg, 78%) as an amorphous solid: R_f 0.56 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D$ +1.0 (*c* 1.0, CH_2Cl_2); FABMS *m*/*z* 937 (40, $[M + Na]^+$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.09, 2.06, 2.03, 2.01 (4 s, each 6 H, Ac), 3.82 (ddd, 2 H, J = 2.2, 4.7, 9.7 Hz, H-5), 4.00 (bd, 2 H, J = 4.7, 12.6 Hz, H-6b), 4.27 (dd, 2 H, J = 2.2, 12.6 Hz, H-6a), 4.67 (bs, 4 H, CH_2), 4.94 (t, 2 H, J = 9.7 Hz, H-2), 5.06 (t, 2 H, J = 9.7 Hz, H-4), 5.34 (t, 2 H, J = 9.7 Hz, H-3), 5.68 (bt, 2 H, J = 9.7 Hz, H-1), 6.56, 6.62 (2 bs, each 2 H, NH), 7.26 (s, 4 H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 20.7, 20.6, 20.5, 48.6, 61.7, 68.3, 69.5, 72.8, 73.4, 82.7, 128.4, 136.9, 169.1−171.2, 183.6. Anal. Calcd for $C_{38}H_{50}N_4O_{18}S_2$: C, 49.88; H, 5.51; N, 6.12. Found: C, 49.88; H, 5.55; N, 6.13.

1,4-Bis[3-[3-[3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thioureidomethyl]benzyl]thioureidomethyl]-benzene (2). A solution of the diamine **11** (73 mg, 0.15 mmol) in acetone−water (1:1, 3 mL) was adjusted to pH 8 by addition of saturated aqueous $NaHCO_3$. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**7**, 129 mg, 0.33 mmol) in acetone (1.5 mL) was then added, and the reaction mixture was stirred for 2 h at room temperature. Acetone was removed by distillation and the aqueous residue was extracted with CH_2Cl_2 (3 × 5 mL). The organic solution was concentrated, and the resulting residue was purified by column chromatography (5:1 EtOAc−petroleum ether) to afford **2** (134 mg, 70%) as an amorphous solid: R_f 0.52 (5:1 EtOAc−petroleum ether); $[\alpha]_D$ 0.0 (*c* 1.0, CH_2Cl_2); FABMS *m*/*z* 1293 (20, $[M + Na]^+$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02, 2.01, 2.00, 1.99 (4 s, each 6 H, Ac), 3.79 (m, 1 H, H-5), 3.89 (bd, 2 H, J = 12.2 Hz, H-6b), 4.27 (dd, 2 H, J = 4.0, 12.2 Hz, H-6a), 4.62 (bs, 12 H, CH_2), 4.95 (t, 2 H, J = 9.1 Hz, H-2), 5.01 (t, 2 H, J = 9.1 Hz, H-4), 5.29 (t, 2 H, J = 9.1 Hz, H-3), 5.70 (bt, 2 H, J = 9.1 Hz, H-1), 6.77, 7.01 (2 bs, each 4 H, NH), 7.16−7.18 (m, 12 H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 21.2, 20.8, 20.5, 20.3, 47.7, 47.9, 60.1, 68.3, 70.6, 72.9, 73.3, 82.6, 125.1−138.0, 169.5−170.8, 181.9, 183.5. Anal. Calcd for $C_{56}H_{70}N_8O_{18}S_4$: C, 52.90; H, 5.55; N, 8.81. Found: C, 52.89; H, 5.53; N, 8.80.

1,3-Bis[3-[4-[3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thioureidomethyl]benzyl]thioureidomethyl]-benzene (3). The coupling reaction of **15** (73 mg, 0.15 mmol) and **7** (129 mg, 0.33 mmol) in water−acetone at pH 8, following the procedure described above for the preparation of **2** and purification by column chromatography (3:1 EtOAc−petroleum ether) afforded **3** (140 mg, 73%) as an amorphous solid: R_f 0.25 (3:1 EtOAc−petroleum ether); $[\alpha]_D$ + 84.0 (*c* 1.0, CH_2Cl_2); FABMS *m*/*z* 1293 (10, $[M + Na]^+$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02, 1.99, 1.98, 1.97 (4 s, each 6 H, 8 Ac), 3.78 (m, 2 H, H-5), 3.95 (bd, 2 H, J = 12.0 Hz, H-6b), 4.17 (bd, 1 H, J = 12.0 Hz, 2 H-6a), 4.48 (bs, 12 H, CH_2), 4.93 (t, 2 H, J = 9.4 Hz, H-2), 4.98 (t, 2 H, J = 9.4 Hz, H-4), 5.28 (t, 2 H, J = 9.4 Hz, H-3), 5.75 (bt, 2 H, J = 9.4 Hz, H-1), 6.75, 6.88 (2 bs, each 4 H, NH), 7.10−7.28 (m, 12 H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 20.6, 20.4 (*Me*CO), 47.9, 48.0, 61.7, 68.3, 70.6, 72.9, 73.2, 82.5, 125.1−137.8, 169.6−170.7, 181.5, 183.0. Anal. Calcd for $C_{56}H_{70}N_8O_{18}S_4$: C, 52.90; H, 5.55; N, 8.81. Found: C, 52.65; H, 5.43; N, 8.75.

Acknowledgment. We thank the Dirección General de Investigación Científica y Técnica (Grant No. PPQ 2000-1341) for financial support.

JO001508N