Biolog. J. Armenia, *Special issue: Cyclodextrins*, 2001

CYCLODEXTRIN NEOGLYCOCONJUGATES

Carmen Ortiz Mellet[*], Jacques Defaye[**], José M. García Fernández[***]

[*]*Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla Aptdo. 553, E-41071, Sevilla, Spain*

[**]*CNRS and Université Joseph Fourier-Grenoble I (UMR 5063), Département de Pharmacochimie Moléculaire-Glucides, BP 138, F-38243, Meylan, France*

[***]*Instituto de Investigaciones Químicas, CSIC, Américo Vespucio s/n, Isla de la Cartuja, E-41092, Sevilla, Spain.*

Progress towards the use of cyclodextrins as drug targetting devices is summarized.

Обобщено развитие работ, ведущих к использованию циклодекстринов в качестве целевых мишеней лекарственных веществ.

Ամփոփված են այն աշխատանքները, որոնք նպաստում են ցիկլոդեքստրինների որպես դեղամիջոցների նպատակային թիրախի օգտագործմանը:

Introduction

Because of their unique structure, cyclodextrins (cyclomaltooligosaccharides, CDs) [1-4] have found applications as molecular encapsulating agents in various fields, including drug delivery [5-8]. Their truncated cone-shaped hydrophobic cavity can accommodate other organic "guest" molecules which, eventually, can be solubilised and stabilised in water. Yet, the anomalous low water solubility of the most interesting representative at least from the industrial point of view (and of its inclusion complexes), namely β-cyclodextrin (cyclomaltoheptaose, β-CD), and its relatively high haemolytic character are important drawbacks to pharmacological uses. To overcome these limitations, several chemically modified CDs, such as hydroxypropyl and methyl ethers, have been proposed [9]. Albeit these so-called "second generation CDs" exhibit a much higher water solubility while still keeping reasonable inclusion ability, they are generally obtained as mixtures of compounds differing in their substitution pattern. In any case, drug transport using either the native (first generation) or the second generation CDs is, essentially, site-unspecific, since CDs do not possess the capability of molecular recognition within the organism.

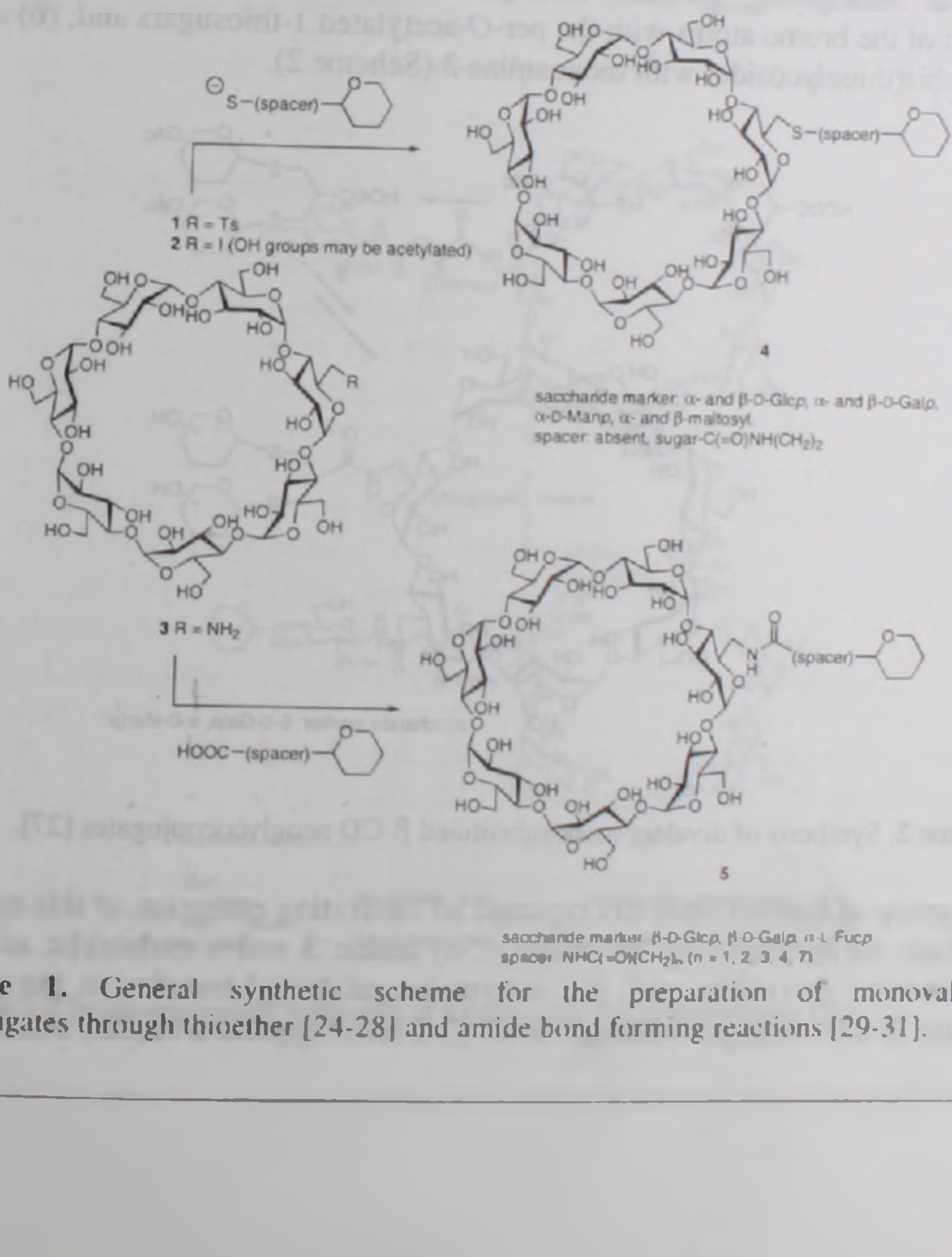
In the last few years an intense effort has been directed towards the design of drug delivery systems able to target pharmacologically active molecules to specific cells, tissues or organs. Since interactions between carbohydrates and carbohydrate-binding proteins (lectins) play important roles in numerous cell recognition processes [10-13], grafting biorecognisable saccharide epitopes onto suitable molecular carriers appears as an attractive approach for the design of new systems for site-specific delivery of therapeutics. Several examples of oligosaccharide-branched CDs have been reported for this purpose. In a first approach, enzymatic transglycosylation of industrially produced glucosyl- or maltosyl-branched CDs was put forward for the preparation of heterogeneously branched CDs incorporating galactose [14-17] or mannose ligands [18]. Although specific interactions of these conjugates with complementary lectins was demonstrated, the heterogeneity of the reaction mixtures precludes a systematic study of the inclusion and recognition properties.

On the other hand, the chemical glycosylation of the native CDs requires long protection/deprotection sequences and is unpractical [19]. As an alternative, substitution of the *O*-glycosidic linkages at the branching points by other atoms or group of atoms has been proposed for the preparation of CD-carbohydrate conjugates (CD neoglycoconjugates), a field in which our laboratories have been actively involved. The development of such strategies runs parallel to the development of efficient methodologies for the chemical functionalisation of CDs [20].

In this article, we summarize the progress that we and others have made to the design of CD-neoglycoconjugates as drug carriers. For reasons of applicability, these works concentrate on β-CD derivatives. Emphasis is placed in the versatility of the synthetic approaches and the lectin-binding and drug inclusion capabilities of the resulting conjugates.

Monovalent Cyclodextrin Neoglycoconjugates

The key precursor for the preparation of monobranched β-CD neoglycoconjugates is the 6^I-deoxy-6^I-tosylcyclomaltoheptaose **1** [21], for which we have recently reported an improved synthesis [22, 23]. This compound can experience S_N2 displacement reactions by suitable nucleophiles in high yield. This reactivity was first exploited by the group of Defaye in the preparation 6^I-*S*-glycosyl-6^I-thiocyclomaltoheptaose derivatives (**4**) by using the sodium salt of 1-thio-α- and β-D-glucopyranose as nucleophile [24] and was further extended to the synthesis of the analogous monovalent α- and β-thiomaltosyl β-CD conjugates [25] (Scheme 1).

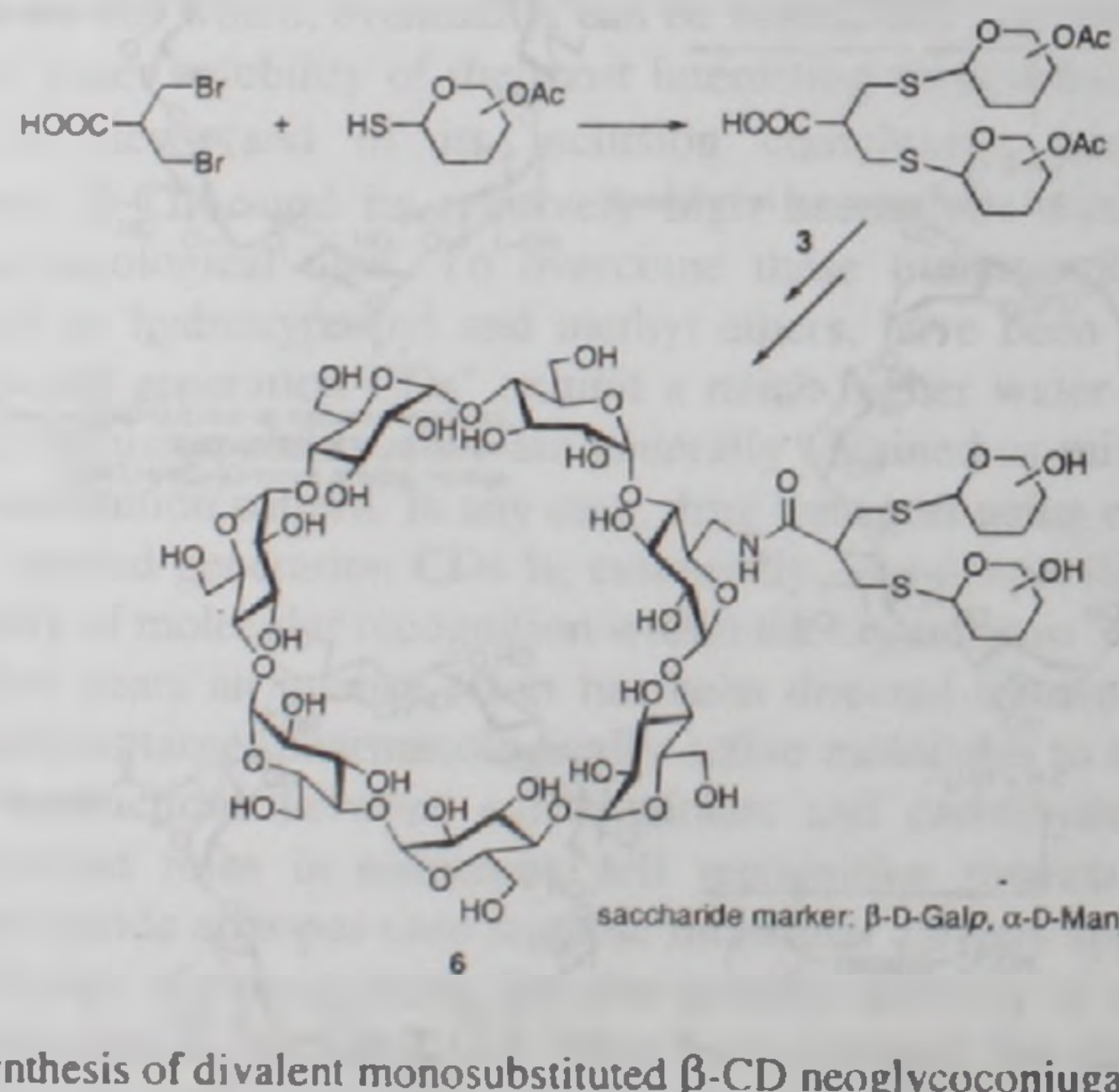


Scheme 1. General synthetic scheme for the preparation of monovalent β-CD neoglycoconjugates through thioether [24-28] and amide bond forming reactions [29-31].

Driguez et al. have used a similar approach that employs the peracetylated mono-(C-6)-iodo-β-CD derivative **2**, obtained from the monotosylate **1**, as precursor [26, 27]. The peracetylated 1-thioglycose (β-D-glucopyranose, β-D-galactopyranose, α-D-mannopyranose) is then used as the nucleophile. A final deacetylation step afforded the target fully unprotected neoglycoconjugates. More recently, Hattori et al. [28] have reported the incorporation of a spacer arm between the CD core and the saccharide marker by: (i) reacting lactonolactone and aminoethanol and; (ii) introducing the resulting galactosyl-gluconoamide-ethanethiol fragment at the 6^1 position of β-CD following the above general synthetic scheme (Scheme 1).

A second general approach for the preparation of monovalent β-CD-neoglycoconjugates consists in the amidation reaction between 6^1-amino-6^1-deoxycyclomaltoheptaose (**3**), readily available from **1** via the corresponding azide, and saccharide markers bearing a carboxylic acid functional group. This strategy has been widely developed by the group of Parrot-Lopez [29-31]. First, a monoester of a dicarboxylic acid was coupled to a glycosyl isothiocyanate to give a glycosylamide. After hydrolysis of the ester group, the resulting carboxylic acid was allowed to react with amine **3** to give adducts **5** (Scheme 1). The length of the spacer can be easily modified just by changing the starting dicarboxylic acid. Alternatively, aldonolactones can be used as amidating agents [32].

Driguez et al. [27] have applied the amidation reaction to the synthesis of divalent monosubstituted β-CD-neoglycoconjugates bearing β-D-galactopyranosyl and α-D-mannopyranosyl ligands (**6**). Commercially available 3-bromo-2-(bromoethyl)propionic acid was used as multiplying element through a sequence involving: (a) nucleophilic displacement of the bromo atoms with the per-*O*-acetylated 1-thiosugars and; (b) coupling of the resulting bis(thioglycoside) with monoamine **3** (Scheme 2).



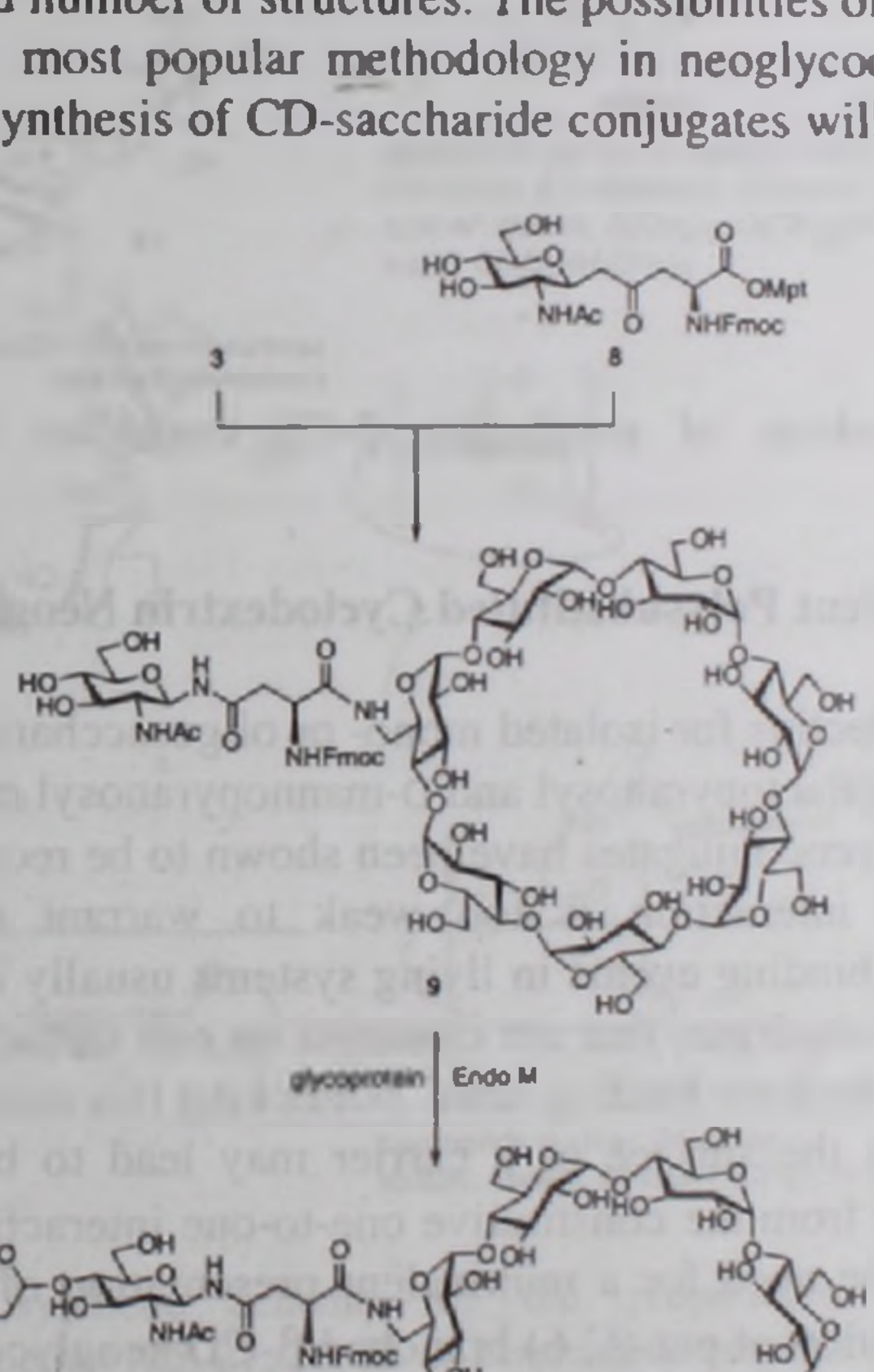
6

Scheme 2. Synthesis of divalent monosubstituted β-CD neoglycoconjugates [27].

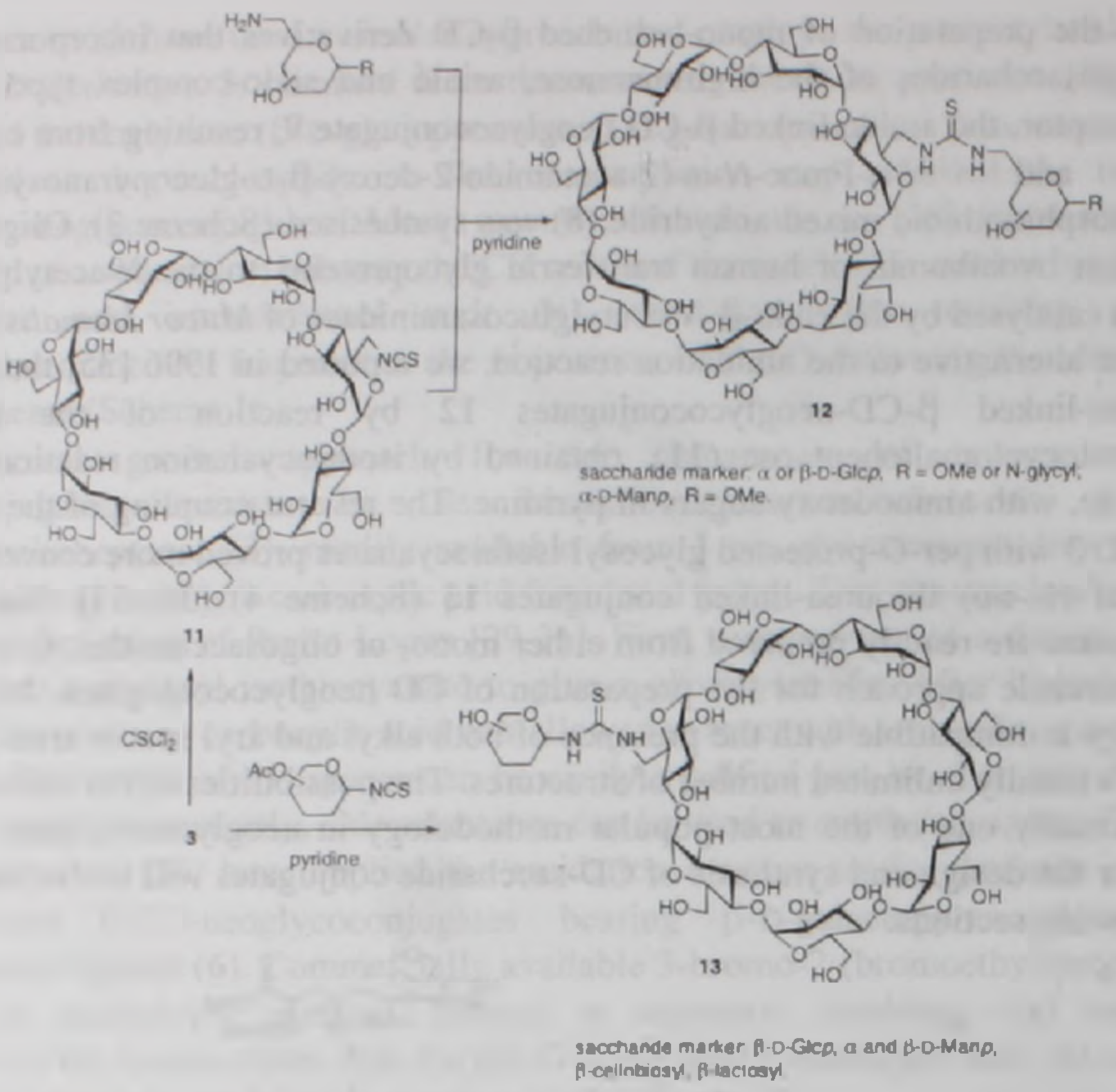
The group of Parrot-Lopez has reported an interesting extension of this approach [33] that combines: (i) amidation of the mono-(C-6) amine **3** and a carboxylic acid-armed *N*-acetylglucosamine derivative and; (ii) enzymatic galactosyl-transfer to the glucosamine moiety in the β-CD-conjugate. Hattori et al. [34] have applied a similar chemo-enzymatic

strategy to the preparation of mono-branched β-CD derivatives that incorporate complex natural oligosaccharides of the high-mannose, asialo and sialo-complex type (**10**). As a suitable acceptor, the amide-linked β-CD neoglycoconjugate **9**, resulting from condensation of **3** and *N*-α-Fmoc-*N*-ω-(2-acetamido-2-deoxy-β-D-glucopyranosyl)asparagine dimethylphosphinothioic mixed anhydride (**8**) was synthesised (Scheme 3). Oligosaccharide transfer from ovoalbumin or human transferrin glycoproteins to the *N*-acetylglucosamine moiety was catalysed by the endo-β-*N*-acetylglucosaminidase of *Mucor hiemalis* (Endo M).

As an alternative to the amidation reaction, we reported in 1996 [35] the preparation of thiourea-linked β-CD-neoglycoconjugates **12** by reaction of the 6^{I}-deoxy-6^{I}-isothiocyanatocyclomaltoheptaose (**11**), obtained by isothiocyanation reaction of **3** with thiophosgene, with aminodeoxy sugars in pyridine. The reverse coupling of the mono-(C-6) amine β-CD **3** with per-*O*-protected glycosyl isothiocyanates proved more convenient for the synthesis of (1-->6) thiourea-linked conjugates **13** (Scheme 4) [36, 37]. Since glycosyl isothiocyanates are readily prepared from either mono- or oligosaccharides, this is probably the most versatile approach for the preparation of CD neoglycoconjugates. Moreover, the methodology is compatible with the presence of both alkyl and aryl spacer arms [36], giving access to a virtually unlimited number of structures. The possibilities of the thiourea-forming strategy, actually one of the most popular methodology in neoglycoconjugate preparation [38, 39], for the design and synthesis of CD-saccharide conjugates will be further illustrated in the following sections.



Scheme 3. Chemo-enzymatic synthesis of monovalent β-CD conjugates [34].



Scheme 4. Synthesis of monovalent β-CD conjugates through the thiourea-forming reaction [35-37].

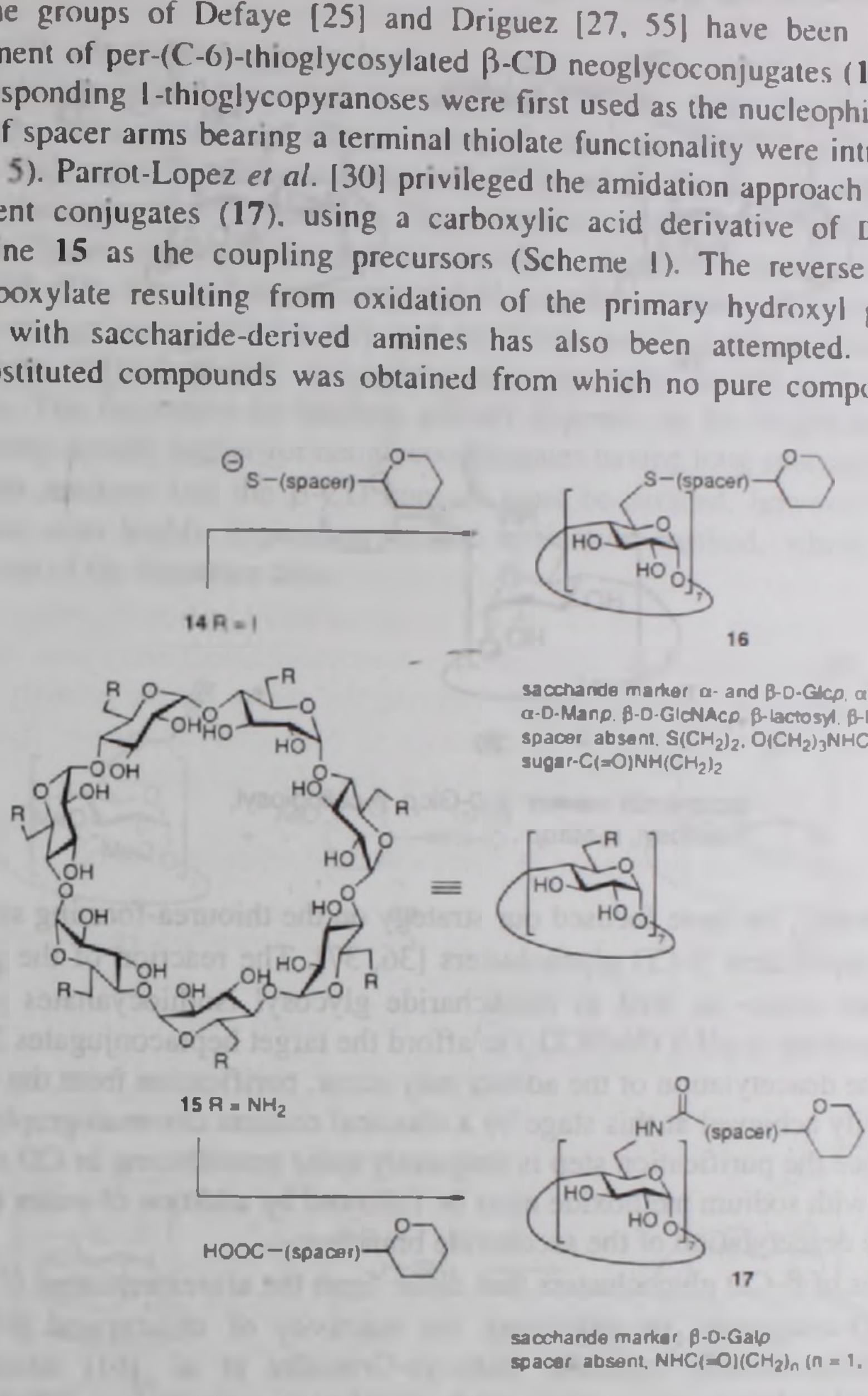
Multivalent Polysubstituted Cyclodextrin Neoglycoconjugates

The affinity of lectins for isolated mono- or oligosaccharide ligands is, generally, low. Actually although D-galactopyranosyl and D-mannopyranosyl moieties in some of the above monovalent CD neoglycoconjugates have been shown to be recognised by specific lectins in in vitro assays, the interaction is too weak to warrant efficient targeting in vivo. Carbohydrate-protein binding events in living systems usually involve several simultaneous contacts between carbohydrates that are clustered on cell surfaces and protein receptors that contain multiple carbohydrate binding-sites. Mimicking this scenario by multiplication of the saccharide epitope on the surface of a carrier may lead to biologically useful affinities, greater than predicted from the constitutive one-to-one interactions [40-49] — the so-called cluster effect [50]. The need for a multivalent presentation of the saccharide markers has stimulated the preparation of per-(C-6) branched β-CD neoglycoconjugates. Highly efficient coupling reactions are a prerequisite for such prospect, since partial substitution would lead to a mixture of undersubstituted regioisomers from which purification of the desired persubstituted compound becomes a difficult task.

Basically, the preparation of per-(C-6) substituted β-CD derivatives bearing carbohydrate appendages relies on the same synthetic methodologies previously commented for the synthesis of monovalent conjugates, i.e. thiol displacement of a good leaving group (I or Br; **14**), amidation of the per-(C-6)-amino β-CD (**15**), or reaction of the latter with sugar isothiocyanates. The development of very efficient procedures for the preparation of the key

per-(C-6)-halo β-CD precursors by the group of Defaye has been, not surprisingly, a turning point in the chemistry of these derivatives [51-54].

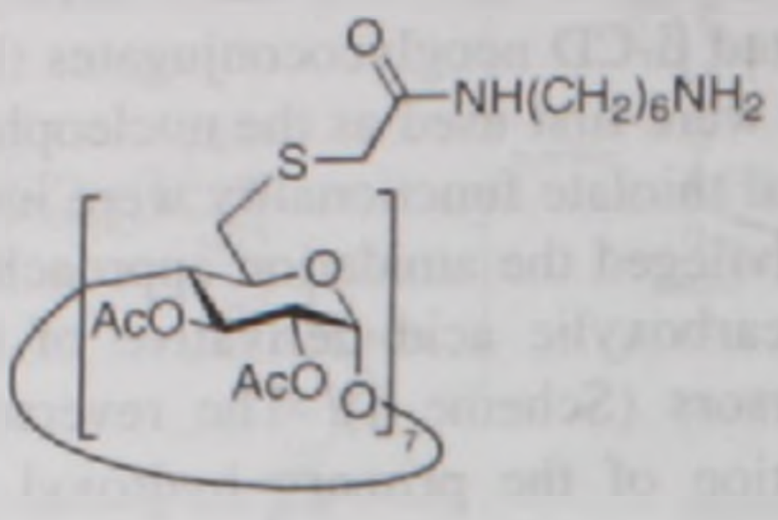
The groups of Defaye [25] and Driguez [27, 55] have been instrumental in the development of per-(C-6)-thioglycosylated β-CD neoglycoconjugates (**16**). The thiolates of the corresponding 1-thioglycopyranoses were first used as the nucleophiles. Alternatively, a variety of spacer arms bearing a terminal thiolate functionality were introduced [28, 55-57] (Scheme 5). Parrot-Lopez *et al.* [30] privileged the amidation approach for the synthesis of heptavalent conjugates (**17**), using a carboxylic acid derivative of D-galactose and the heptaamine **15** as the coupling precursors (Scheme 1). The reverse strategy using the heptacarboxylate resulting from oxidation of the primary hydroxyl groups of β-CD in reaction with saccharide-derived amines has also been attempted. Yet, a mixture of undersubstituted compounds was obtained from which no pure compounds were isolated [58].



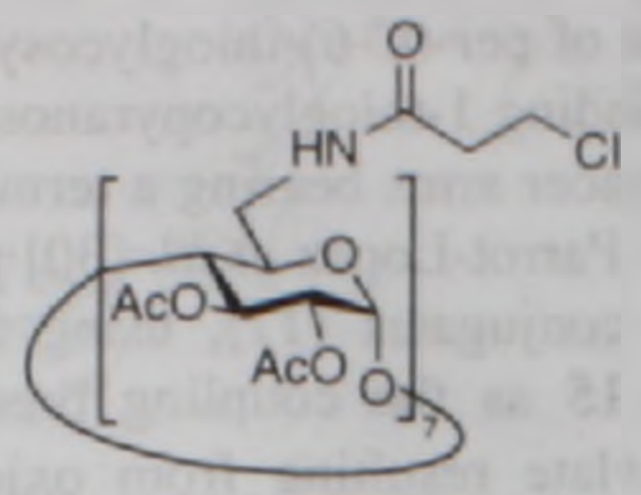
Scheme 5. General synthetic scheme for the preparation of heptavalent β-CD neoglycoconjugates through thioether [25, 27, 28, 55-57] and amide bond forming reactions [30].

Recently, the groups of Santoyo-González and Vargas-Berenguel [59, 60] have reported some modifications of this general scheme aimed at diversifying the nature of the linkers while keeping the coupling efficiency, including: (i) the use of sugar derived thiouronium salts as nucleophilic agents instead of the corresponding thiolates, (ii) the preparation of the heptakis(6-chloroacetamido-6-deoxy)cyclomaltoheptaose **18** and its use as precursor in coupling reactions with the aforementioned nucleophiles, and (iii) the synthesis of the per-(C-6)-thioether β-CD derivative **19** bearing a terminal amino group that can be

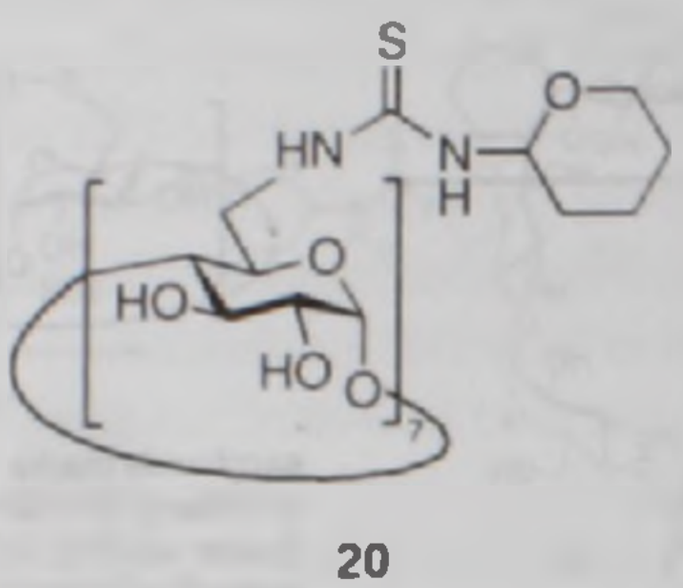
used in coupling reactions with sugar isothiocyanates. Yet, all these approaches need the protection of the hydroxyl groups both in the β-CD precursor and in the functionalised saccharide marker.



18



19



20

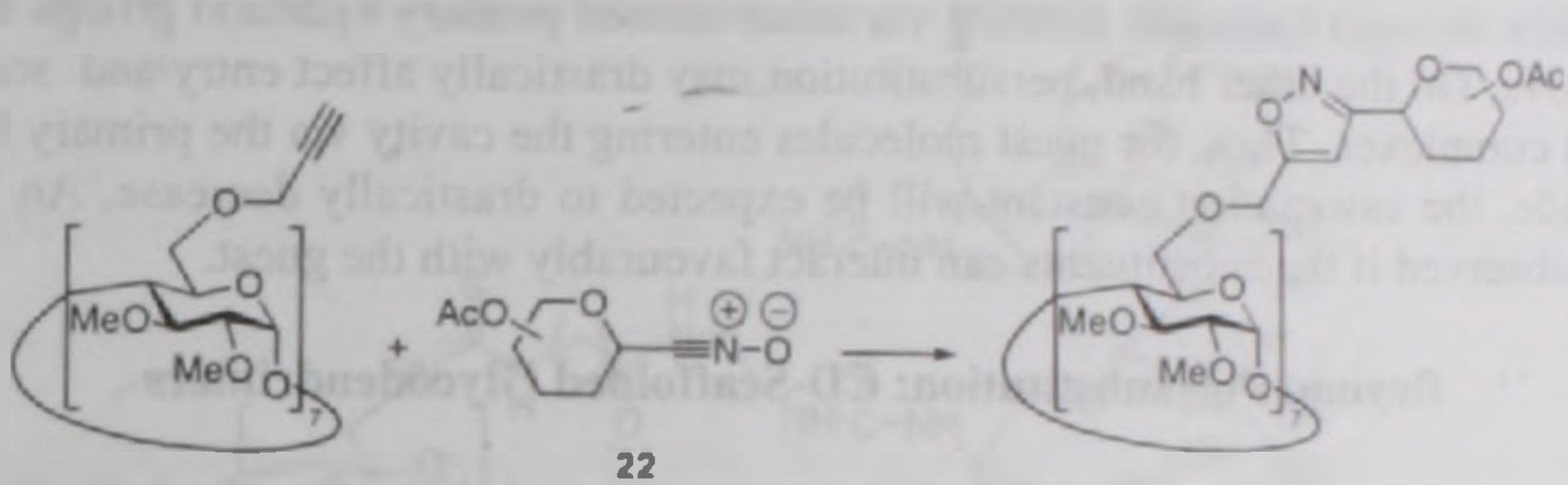
saccharide marker: β-D-Glc*p*, β-cellobiosyl, β-lactosyl, D-Man*p* .

In our laboratories, we have focused our strategy on the thiourea-forming strategy for the preparation of heptavalent β-CD glycoclusters [36, 37]. The reaction of the per-(C-6)-amine β-CD **15** with mono- as well as disaccharide glycosyl isothiocyanates proceeded smoothly in water-acetone at pH 8 ($NaHCO_3$) to afford the target heptaconjugates **20** in high yield. Although some deacetylation of the adduct may occur, purification from the unreacted precursors was readily achieved at this stage by a classical column chromatography step, an important aspect since the purification step is frequently quite troublesome in CD chemistry. The final treatment with sodium methoxide must be followed by addition of water to warrant full and quantitative deacetylation of the saccharide branches.

Two examples of β-CD glycoclusters that differ from the aforementioned (C-6)-*S* or -*N* substituted β-CD conjugates by exploiting the reactivity of unsaturated β-CD ether derivatives have been recently reported. Santoyo-Gonzalez et al. [61] described the preparation of an heptavalent neoglycoconjugate having heterocyclic linkers (**23**) by the 1,3-dipolar cycloaddition of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl nitrile oxide **22** and the per-(2,3-di-*O*-methyl-6-*O*-propargyl)-β-CD **21** (Scheme 6). Stoddart and Fulton [62] prepared the per-(6-*O*-allyl-2,3-di-*O*-methyl-)-β-CD derivative and effected the photochemical (anti-Markovnikov) addition of 2,3,4,6- tetra-*O*-acetyl-1-thio-β-D-glucopyranose to the double-bonds. Interestingly, this strategy was also extended to β-CD derivatives per-*O*-allylated at O-2 and per-(di-*O*-allylated) at O-2 and O-6 positions (**24**), giving rise to the first examples of an heptavalent β-CD neoglycoconjugate branched at the secondary face and of a 14-valent (**25**) β-CD conjugate (Scheme 6).

Lectin-Binding and inclusion ability of Monovalent *versus* Multivalent Polysubstituted Cyclodextrin Neoglycoconjugates

β-CD neoglycoconjugates bearing biorecognisable saccharide markers have been shown to be recognised by complementary lectins in vitro. Thus, D-galactopyranosyl ligands binds to the cell wall lectin of *Kluyveromyces bulgaricus* (KbCWL) [29-31, 33, 63], *Arachis hypogea* (peanut) [60], *Ricinus communis* [27] and *Griffonia simplicifolia* I (GSI) lectins [58]; D-glucopyranosyl ligands to *Pisum sativum* (pea) lectin [60], D-mannopyranosyl ligands to *Pisum sativum* [60] and *Concanavalia ensiformis* (concanavalin A, Con A) lectins [32, 34, 58, 60]; *N*-acetylglucosamine and N-acetyllactosamine to *Triticum vulgaris* (WGA, wheat germ agglutinin) [56-58, 60], and *Erythrina corallodendron* (EcorL) lectins [57, 58]. Multivalent polysubstituted conjugates are generally bound with higher association constants. The increment on binding affinity depends on the length and the nature of the linker, being usually higher for neoglycoconjugates having long spacers between the external saccharide markers and the β-CD core. It must be noticed, however, that lectin binding results are also highly depending on the evaluation method, which hampers a reliable comparison of the literature data.



Scheme 6. Synthesis of multivalent b-CD neoglycoconjugates from unsaturated ether derivatives [61, 62].

Concanavalin A is one of the most popular lectin for the study of carbohydrate-protein interactions. Its commercial availability and extensive structural knowledge make it particularly attractive for preliminary evaluation of targeting devices. Moreover, several reports on Con A-mannosyl clusters associations have recently been reported using the enzyme-linked lectin assay (ELLA) protocol [64-68]. For these reasons, we have undertaken a systematic evaluation of thiourea-linked mannose-β-CD conjugates using this technique, including both monovalent and heptavalent adducts, in comparison with model compounds

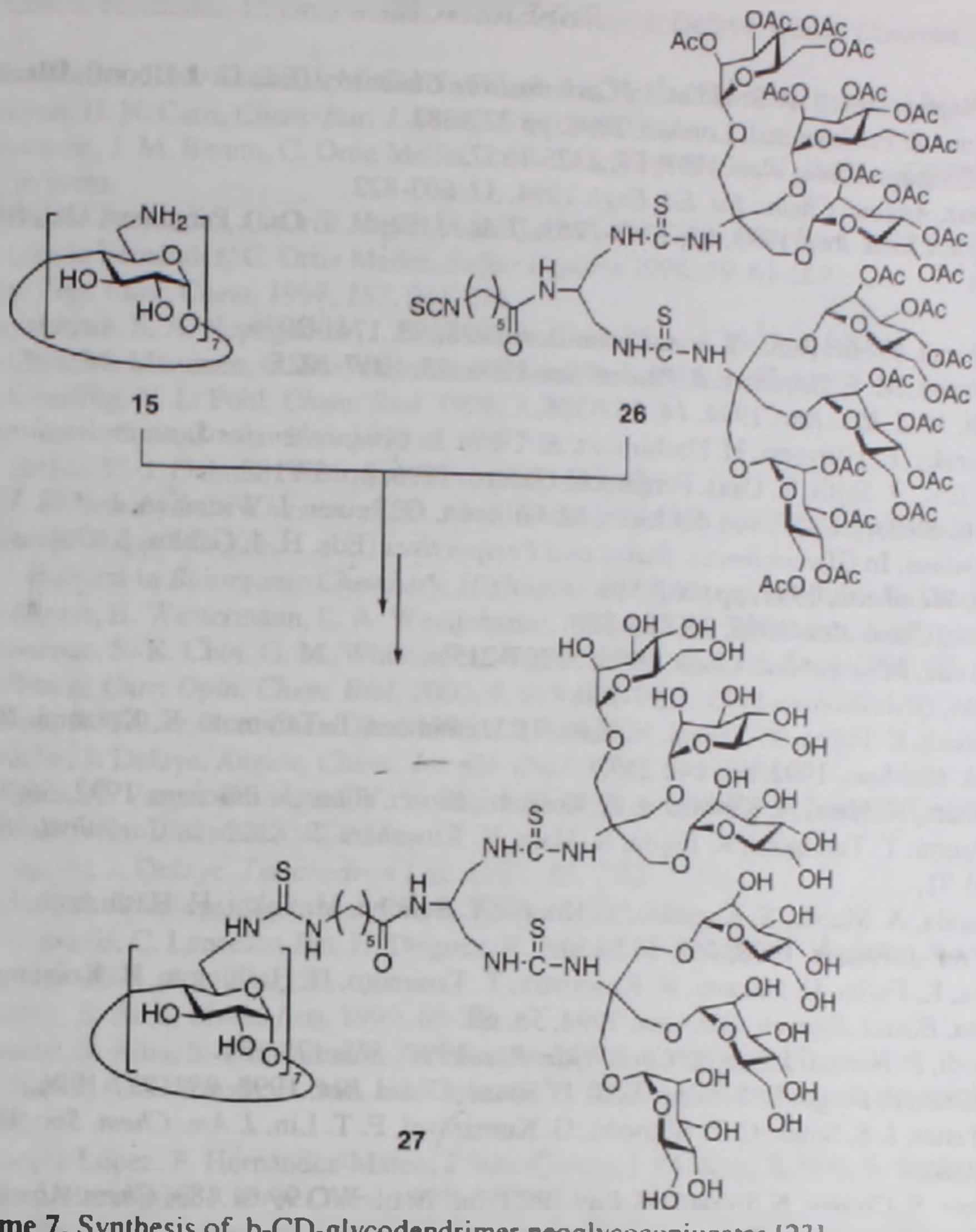
lacking the CD moiety [37]. Several interesting conclusions have been drawn: (i) The thiourea linker slightly decreases the binding affinity in comparison with classical *O*-glycosidic linkages and totally abolishes anomeric specificity (in contrast with the known 10-fold higher affinity for the α-anomer in the *O*-mannoside series); (ii) the β-CD aglycon in monovalent conjugates interacts with the protein stabilising the complex by about -0.35 kcal; (iii) surprisingly, heptasubstitution at the primary hydroxyls rim with mannopyranosylthioureido ligands fully abolishes Con A binding, probably due to an unfavorable steric effect. The expected increase in binding affinity was observed after intercalation of a C_5 spacer.

Only a few data on the inclusion ability of β-CD neoglycoconjugates is available in the literature. Defaye and coworkers [25] have shown that the stability of inclusion complexes of monovalent (C-6)-*S*-linked β-CD neoglycoconjugates is strongly dependent on the inclusion dynamic and complex stabilisation mechanism. Complexes with guest molecules entering the cavity through the narrower rim may experience some decrease in the association constant as compared with the corresponding native β-CD due to the steric hindrance imposed by the substituent. No significant difference was observed, however, for molecules entering the cavity through the wider secondary hydroxyls rim and complex stabilisation through hydrogen bonding via unsubstituted primary hydroxyl groups may then be operative. On the other hand, persubstitution may drastically affect entry and stability of inclusion complexes. Thus, for guest molecules entering the cavity via the primary hydroxyl groups side, the association constant will be expected to drastically decrease. An opposite effect is observed if the substituents can interact favourably with the guest.

Beyond Polysubstitution: CD-Scaffolded Glycodendrimers

From all the above reported data, it can be concluded that heptasubstitution at the primary hydroxyls rim of β-CD with incorporation of saccharides markers has beneficial effects from the biological standpoint as compared with monovalent conjugates, provided that longer enough spacer arms are used to warrant the accessibility of the glycocluster structure to carbohydrate-lectin recognition events. Yet, heptasubstitution may also impair inclusion and stabilisation of potential guests.

Ideal CD-carriers for use in drug targeting should combine both the advantages of monosubstitution regarding efficient inclusion capabilities and a multivalent display of the required saccharide epitope to comply with the need of high biological receptor binding affinity. Towards this aim, we have recently reported the preparation of β-CD-scaffolded glycodendrimers (e.g. **27**) as a new class of multivalent monosubstituted β-CD neoglycoconjugates [23]. A modular strategy for the preparation of isothiocyanate-armed mannosyl-coated dendritic wedges (e.g. **26**) was devised and these structures were attached to the β-CD monoamine **3** in a final step (Scheme 7). Evaluation of the Con A binding ability by ELLA tests for a series of derivatives going from mono to hexavalent indicated a dramatic increase in binding efficiency for the higher-valent conjugates. Moreover, the solubilisation experiments using the anticancer drug Taxotère® as model guest indicated solubility values similar to those previously encountered for monovalent monobranched β-CD conjugates, about 20% higher as compared with per-(C-6) substituted analogues.



Scheme 7. Synthesis of b-CD-glycodendrimer neoglycoconjugates [23].

Concluding remarks

From the present body of results, it becomes clear that combination of the ability to form host-guest inclusion complexes characteristic of CDs and the biorecognition capabilities of oligosaccharide appendage makes CD neoglycoconjugates promising candidates as site-specific drug delivery systems. Efficient and high yielding synthetic methodologies involving a limited number of protection/deprotection sequences have been settled, opening the way to unlimited possibilities of biorecognition structures which could result from the combination of chemical and enzymatic methods as well.

Acknowledgements. Part of the results from the authors laboratories herein reported have been obtained with the support of the European Commission DG XII, under the FAIR programme CT95-0300.

REFERENCES

1. S. A. Nepogodiev, J. F. Stoddart, in *Carbohydrate Chemistry* (Ed.: G.-J. Boons), Blackie Academic & Professional, London, 1998, pp 322-383.
2. K. A. Connors, *Chem. Rev.* 1997, *97*, 1325-1357.
3. G. Wenz, *Angew. Chem., Int. Ed. Engl.* 1994, *33*, 803-822
4. J. Szejtli, *Chem. Rev.* 1998, *98*, 1743-1753. (Eds.: J. Sejtli, T. Osa), Pergamon, Oxford, 1996, pp. 57-188.
5.
6. K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* 1998, *98*, 1741-2076.
7. T. Loftsson, M. E. Brewster, *J. Pharm. Sci.* 1996, *85*, 1017-1025
8. J. Sejtli, *Med. Res. Rev.* 1994, *14*, 353-386.
9. L. Jicsinsky, E. Fenyvesi, H. Hashimoto, A. Ueno. In *Comprehensive Supramolecular Chemistry*, vol. 3 (Eds.: J. Sejtli, T. Osa), Pergamon, Oxford, 1996, pp. 57-188.
10. (a) H.-C. Siebert, C.-W. von der Lieth, M. Gilleron, G. Reuter, J. Wittmann, J. F. G. Vliegenthart, H.-J. Gabius. In *Glycosciences, Status and Perspectives* (Eds. H.-J. Gabius, S. Gabius), Chapman & Hall, Weinheim, 1997, pp. 291-310.
11. R. Dwek, *Chem. Rev.* 1996, *96*, 683-720.
12. M. Fukuda, *Bioorg. Med. Chem.* 1995, *3*, 207-215.
13. A. Varki, *Glycobiology* 1993, *3*, 97-130.
14. S. Kitahata, K. Fujita, Y. Takagi, K. Hara, H. Hashimoto, T. Tanimoto, K. Koizumi, *Biosci. Biotech. Biochem.* 1992, *56*, 242-245.
15. S. Kitahata, K. Hara, N. Kuwahara, K. Koizumi, *Biosci. Biotech. Biochem.* 1992, *56*, 1518-.
16. K. Koizumi, T. Tanimoto, K. Fujita, K. Hara, N. Kuwahara, S. Kitahata, *Carbohydr. Res.* 1993, *238*, 75-91.
17. T. Shimoda, A. Maeda, S. Kagatani, Y. Konno, T. Sonobe, M. Fukui, H. Hashimoto, K. Hara, K. Fujita, *Int. J. Pharm.* 1998, *167*, 147-154.
18. K. Hara, K. Fujita, H. Nakano, N. Kuwahara, T. Tanimoto, H. Hashimoto, K. Koizumi, S. Kitahata, *Biosci. Biotech. Biochem.* 1994, *58*, 60.
19. P. Fügedi, P. Nánási, J. Szejtli, *Carbohydr. Res.* 1998, *175*, 173-181.
20. A. R. Khan, P. Forgo, K. J. Stine, V. T. D'Souza, *Chem. Rev.* 1998, *98*, 1977-1996.
21. R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, F. T. Lin, *J. Am. Chem. Soc.* 1990, *112*, 3860-3861.
22. J. Defaye, S. Crouzy, N. Evrard, H. Law, PCT Int. Appl. WO 99 61,483; *Chem. Abstr.* 2000, *132*, 24007a.
23. I. Baussanne, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, H. Law, J. Defaye, *Chem. Commun.* 2000, 1489-1490.
24. J. Defaye, A. Gadelle, A. Guiller, R. Darcy, T. O'Sullivan, *Carbohydr. Res.* 1989, *192*, 251-258.
25. V. Lainé, A. Coste-Sarguet, A. Gadelle, J. Defaye, B. Perly, F. Djedaini-Pilard, *J. Chem. Soc., Perkin Trans. 2*, 1995, 1479-1487.
26. H. Driguez, S. Cottaz, *Synthesis* 1989, 755-758.
27. H. Driguez, C. Lancelon-Pin, *Tetrahedron Lett.* 1992, *33*, 3125-3128.
28. N. Yasuda, N. Aoki, H. Abe, K. Hattori, *Chem. Lett.* 2000, 706-707.
29. H. Parrot-Lopez, H. Galons, A. W. Coleman, J. Mahuteau, M. Miocque, *Tetrahedron Lett.* 1992, *33*, 209-212.
30. H. Parrot-Lopez, E. Leray, A. W. Coleman, *Supramol. Chem.* 1993, *3*, 37-42.
31. R. Kasab, C. Félix, H. Parrot-Lopez, R. Bonaly, *Tetrahedron Lett.* 1997, *38*, 7555-7558.
32. H. Imata, K. Kubota, K. Hattori, M. Aoyagi, C. Indoh, *Bioorg. Med. Chem. Lett.* 1997, *7*, 109-112.
33. E. Leray, H. Parrot-Lopez, C. Augé, A. W. Coleman, C. Finance, R. Bonaly, *J. Chem. Soc., Chem. Commun.* 1995, 1019-1020.
34. K. Matsuda, T. Inazu, K. Haneda, M. Mizuno, T. Yamamoi, K. Hattori, K. Yamamoto, H. Kumagai, *Bioorg. Med. Chem. Lett.* 1997, *7*, 2353-2356.

35. J. M. García Fernández, C. Ortiz Mellet, S. Maciejewski, J. Defaye, *Chem. Commun.* 1996, 2741-2742.
36. C. Ortiz Mellet, J. M. Benito, J. M. García Fernández, H. Law, K. Chmurski, J. Defaye, M. L. O'Sullivan, H. N. Caro, *Chem. Eur. J.* 1998, *4*, 2523-2531.
37. I. Baussanne, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, J. Defaye, *CHEMBIOCHEM* 2001, in press.
38. J. M. García Fernández, C. Ortiz Mellet, *Adv. Carbohydr. Chem. Biochem.* 1999 55, 35-135.
39. J. M. García Fernández, C. Ortiz Mellet, *Sulfur Reports* 1996, *19*, 61-169.
40. R. Roy, *Top. Curr. Chem.* 1997, *187*, 241-274.
41. N. Nayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* 1997, *4*, 97-104
42. S.-K. Choi, M. Mammen, G. M. Whitesides, *Chem. Biol.* 1996, *3*, 97-104.
43. L. L. Kiessling, N. L. Pohl, *Chem. Biol.* 1996, *3*, 71-77.
44. R. Roy, *Curr. Opin. Struct. Biol.* 1996, *6*, 692-702.
45. N. V. Bovin, H.-J. Gabius, *Chem. Soc. Rev.* 1995, 413-42.
46. R. Roy. n *Carbohydrate Chemistry* (Ed.: G.-J. Boons), Blackie Academic & Professional, London, 1998, pp 243-321.
47. T. K. Lindhorst in *Bioorganic Chemistry. Highligths and Perspectives* (Eds.: U. Diederichsen, T. K. Lindhorst, B. Westermann, L. A. Wessjohann), Wiley-VCH, Weinheim, 1999, pp.133-150
48. M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem., Int. Ed. Engl.* 1998, *37*, 2754-2794.
49. S. L. Flitsch, *Curr. Opin. Chem. Biol.* 2000, *4*, 619-625.
50. Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* 1995, *28*, 321-327.
51. A, Gadelle, J. Defaye, *Angew, Chem., Int. Ed. Engl.* 1991, *30*, 78-80.
52. H. H. Baer, A. Vargas Berenguel, Y. Y. Shu, J. Defaye, A. Gadelle, F. Santoyo González, *Carbohydr. Res.* 1992, *228*, 307-314.
53. K. Chmurski, J. Defaye, *Tetrahedron Lett.* 1997, *38*, 7365-7368.
54. K. Chmurski, J. Defaye, *Supramol. Chem.* 2000, *12*, 221-224.
55. L. De Robertis, C. Lancelon-Pin, H. Driguez, F. Attiom, R. Bonaly, A. Marsura, *Bioorg. Med. Chem. Lett.* 1994, *4*, 1127-1130.
56. T. Furuike, S. Aiba, *Chem. Lett.* 1999, 69-70.
57. T. Furuike, S. Aiba, S.-I. Nishimura, *Tetrahedron* 2000, *56*, 9909-9915.
58. M. Ichikawa, A. S. Woods, H. Mo, I. J. Golstein, Y. Ichikawa, *Tetrahedron: Asymmetry* 2000, *11*, 389-392.
59. J. J. García-López, F. Hernández-Mateo, J. Isac-García, J. M. Kim, R. Roy, F. Santoyo-González, A. Vargas-Berenguel, *J. Org. Chem.* 1999, *64*, 522-531.
60. J. J. García-López, F. Santoyo-González, A. Vargas-Berenguel, J. J. Giménez Martínez, *Chem. Eur. J.* 1999, *5*, 1775-1784.
61. F. G. Calvo-Flores, J. Isac-García, F. Hernández-Mateo, F. Pérez-Balderas, J. A. Calvo-Asín, E. Sánchez-Vaquero, F. Santoyo-González, *Org. Lett.* 2000, *2*, 2499-2502.
62. D. A. Fulton, J. F. Stoddart, *Org. Lett.* 2000, *2*, 1113-1116.
63. F. Attioni, A. Al-Omar, E. Leray, H. Parrot-Lopez, C. Finance, R. Bonaly, *Biol. Cell* 1994, *82*, 161-167.
64. R. Roy, D. Pagé, S. Figueroa Pérez, V. Vérez-Bencomo, *Glycoconjugate J.* 1998, *15*, 251-263.
65. D. Pagé, D. Zanini, R. Roy, *Bioorg. Med. Chem.* 1996, *4*, 1949-1961.
66. D. Pagé, R. Roy, *Bioconjugate Chem.* 1997, *8*, 714-72.
67. P. R. Ashton, E. F. Hounsell, N. Jayaraman, T. M. Nilsen, N. Spencer, J. F. Stoddart, M. Young, *J. Org. Chem.* 1998, *63*, 3429-3437.
68. J. B. Corbell, J. J. Lundquist, E. Toone, *Tetrahedron: Asymmetry* 2000, *11*, 95-111.