JOURNAL OF combinatorial CHEMISTRY

Volume 9, Number 3 May/June 2007

Reports

Efficient Use of Ellman Safety-Catch Linker for Solid-Phase Assisted Synthesis of Multivalent Glycoconjugates

Alejandro Díaz-Moscoso,[†] Juan M. Benito,*[,†] Carmen Ortiz Mellet,[‡] and José M. García Fernández[†]

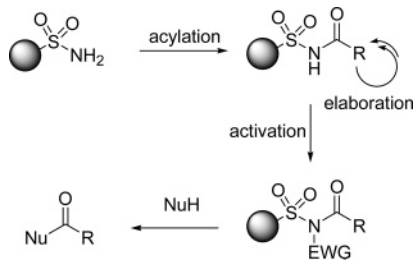
Instituto de Investigaciones Químicas, CSIC, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain, and Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 553, E-41071 Sevilla, Spain

Received February 16, 2007

The specific interaction between carbohydrates and carbohydrate-binding proteins (lectins) is involved in extensive glycobiological recognition processes including cell development, differentiation, morphogenesis, fertilization, the immune response, cell migration, or cancer metastasis.[1] As a common feature of lectin binding to oligosaccharides, the affinity of a single-ligand interaction is usually too weak to trigger a useful biological response. Multivalent ligand presentation on an appropriate scaffold can largely overcome this drawback. This concept, termed the multivalent or cluster effect,[2] has received considerable attention because of the possibility of interfering in fundamental cellular processes.[3] The design of third generation glycoligands that not only possess multivalent carbohydrate recognition sites but also incorporate additional biomedical relevant features, for instance, inclusion capabilities,[4] is particularly appealing in this context.

The attaining of optimal lectin−carbohydrate interactions in terms of binding energy and specificity is not merely dependent on the number of recognition motifs but also is influenced by the spatial arrangement imposed by the scaffold to which the ligands are attached.[5] The profiling of the lectin specificity or the fine-tuning of lectin−oligosaccharide binding requires a thorough evaluation of a structurally diverse series of well-defined multivalent glycoconjugates, for which parallel synthesis and purification often represent serious drawbacks.[6] Solid-phase-based techniques have become a useful alternative to overcome this bottle neck. They have contributed to the development of high-throughput chemical[7] and screening methods[8] to evaluate the lectin-binding properties of immobilized multivalent glycoligands. Further conjugation of the built-in glycocluster to a functional moiety (e.g., a receptor molecule,[4] a reporter tag,[9] or a reactive segment)[10] is, however, a far more complicated challenge.

Scheme 1. General Application of Ellman Sulfonamide Safety-Catch Linker

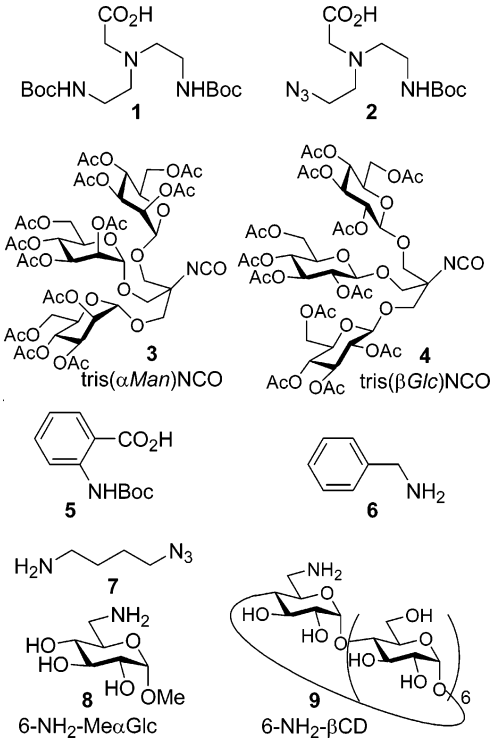


Herein, we report a strategy that ensures high-yielding release of the glycoligands from the solid support in mild and chemoselective conditions, minimizing purification steps of the final adducts, taking advantage of the safety-catch linker principle.[11] For this particular purpose, the *N*-acylsulfonamide linker developed by Ellman and co-workers[12] is an attractive choice because it is stable to most conditions used in carbohydrate and peptide chemistry.[13] Furthermore, the resin-bound compounds can be released under very mild conditions using a two-step strategy involving (i) selective N-alkylation of the *N*-acylsulfonamide group and (ii) attack of a mild nucleophile, for instance an amine, to the *N*-alkyl-*N*-acylsulfonamide intermediate (Scheme 1).

* To whom correspondence should be addressed. E-mail: juanmab@iiq.csic.es.
[†] Instituto de Investigaciones Químicas, CSIC.
[‡] Universidad de Sevilla.

10.1021/cc070025v CCC: $37.00
Published on Web 03/31/2007

Chart 1. Building Blocks Used in the Solid-Phase Assisted Synthesis of Multivalent Glycoconjugates



To assess the scope of the approach, a set of readily available building blocks that can be efficiently coupled through amide and urea bonds have been selected, including the AB_2- and ABC-type branching elements **1** and **2**, the trivalent α-D-manno- and β-D-glucopyranosyl dendrons **3** and **4**, and the fluorescent probe **5** (Chart 1). We first assayed benzylamine (**6**), 4-azidobutylamine (**7**),[14] and methyl 6-amino-6-deoxy-α-D-glucopyranoside (**8**) as catching elements. The strategy has been further implemented in the preparation of complex multivalent β-cyclodextrin (βCD) conjugates by using the corresponding monoamine **9**[15] as the releasing nucleophile. This type of compound has proven to be useful in site-specific drug delivery by forming lectin−multivalent conjugate−drug ternary complexes.[16]

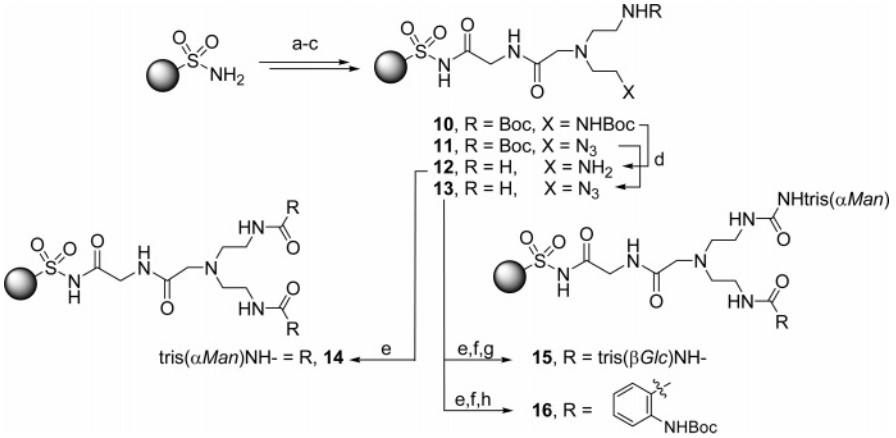
The developed synthetic scheme uses commercially available sulfamylbutyryl polystyrene beads (Scheme 2). To ensure optimum resin loading, DIC/DMAP-assisted coupling of Fmoc-protected glycine was first carried out. This choice avoids the risk of α-epimerization,[17] while providing a suitable spacer segment to prevent unfavorable steric interactions both at the branching element coupling step and at the nucleophilic-assisted release of the formed resin-supported glycodendron. Subsequent Fmoc-cleavage using 20% piperidine in DMF, followed by TBTU-mediated amide coupling of **1** or **2**, gave rise to the corresponding homo- or heterobifunctional resins **10** or **11**. Resin **10** can be employed to prepare homovalent glycodendrons, while resin **11**, with two orthogonal reactive nitrogen functions, would allow the design of heterovalent dendrimers.

Resin glycocoating is a crucial step in the synthetic scheme. To prevent incomplete resin functionalization, a highly efficient coupling reaction is required. Amine−isocyanate coupling, broadly used in dendrimer synthesis,[18] was considered. Although sugar isocyanates are rather unstable building blocks,[19] the tris(2-glycosyloxymethyl)methyl derivatives **3** and **4**, which have the isocyanate function on a quaternary carbon, are fairly stable trivalent glycodendrons.[20] Acid-mediated Boc-cleavage in **10** (→ **12**) and subsequent coupling with isocyanate **3** in dry dichloromethane furnished the solid-supported hexamannosylated conjugate **14**.

The heterobifunctional resin **13** is a particularly versatile starting material. It has recently been demonstrated that heteromultivalent model systems with controlled ligand distribution are valuable tools in the exploration of cell-surface binding events involving carbohydrates.[21] The coupling of resin **13** with isocyanate **3**, followed by $SnCl_2$-promoted azide reduction,[22,23] and the subsequent coupling of the resulting amine with triglucoside **4** afforded the solid-supported mixed-dendron **15**. Resin **13** additionally offers the possibility of incorporating non-carbohydrate elements in the construct, as demonstrated by the TBTU-mediated coupling of *o*-aminobenzoic acid (→ **16**), a useful fluorescent probe (Scheme 2).[24]

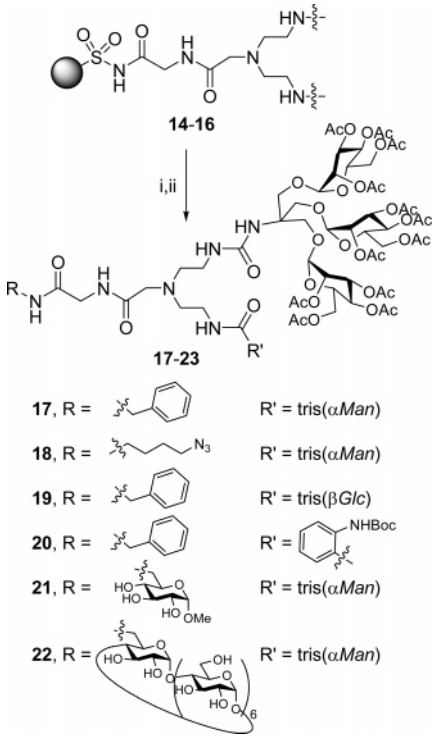
The release of multivalent conjugates from the solid support in resins **14**−**16** by tandem safety-catch linker activation−nucleophilic displacement was further investigated (Scheme 3). The *N*-acylsulfonamide functionality was

Scheme 2. Modular Solid-Phase Construction of Homo- and Heteromultivalent Glycoclusters[a]



[a] Reagents and conditions: (a) FmocGlyOH (3 equiv), DIC, DMAP; (b) 1:4 Pip/DMF; (c) **1** (3 equiv, → **10**), or **2** (3 equiv, → **11**), TBTU (3 equiv), DIPEA (4 equiv); (d) 1:1 TFA/CH_2Cl_2; (e) **3** (2 equiv), CH_2Cl_2, Et_3N; (f) $SnCl_2$ (3 equiv), 1,3-propanedithiol (4 equiv), Et_3N (6 equiv); (g) **4** (2 equiv), CH_2Cl_2, Et_3N; (h) **5** (3 equiv), TBTU (3 equiv), DIPEA (4 equiv).

Scheme 3. Nucleophilic Release of Homo- and Heteromultivalent Glycoclusters[a]



[a] Reagents and conditions: (a) I−CH_2CN (25 equiv), DIPEA (10 equiv), DMF; (b) **6**−**9**, DMF.

Table 1. Products and Yields for the Solid-Phase Assisted Synthesis of Mulivalent Glycoconjugates

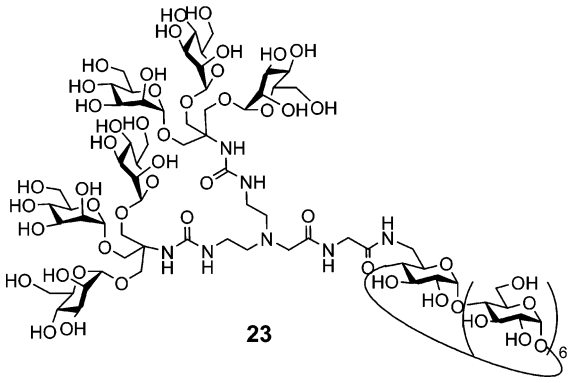
entry	resin	NuH (equiv)	product	yield (%)[a]
1	**14**	**6** (3.0)	**17**	77
2	**14**	**6** (0.9)	**17**	75
3	**14**	**7** (0.9)	**18**	70
4	**15**	**6** (0.9)	**19**	67
5	**16**	**6** (0.9)	**20**	75
6	**14**	**8** (0.9)	**21**	71
7	**14**	**9** (0.9)	**22**	68

[a] Estimated from initial resin loading.

selectively alkylated using iodoacetonitrile in DMF.[17] By using a 3-fold excess of benzylamine as the model releasing nucleophile, we achieved clean cleavage of the solid-supported glycodendrons with simultaneous formation of the corresponding benzyl conjugates (Table 1, entry 1). Interestingly, the yield was not significantly affected when just a stoichiometric amount of the amine was used (Table 1, entry 2), facilitating purification and broadening the range of acceptable nucleophiles to nonvolatile and more costly amines. Thus, when the azido-functionalized amine **7**[14] was used, the corresponding azide-armed glycodendron **18** was obtained in an equally satisfactory yield (Table 1, entry 3). We confirmed that when a slight defect of the nucleophile is used, complete consumption of amine is granted. In this way, the released conjugate is isolated in very high purity by just resin filtration and solvent evaporation.[25] Comparable results were obtained upon cleavage of heterobifunctional resins **15** and **16** with benzylamine to furnish **19** and **20**, respectively (Table 1, entries 4 and 5).

An additional advantage of the selected linker is its absence of reactivity toward primary or secondary hydroxyl

Chart 2



functions. Hydroxyl groups do not interfere even when present in large excess. Accordingly, the fully unprotected aminosugar **8**[26] afforded the corresponding amide-linked conjugate **21** with total chemoselectivity (Table 1, entry 6).

The strong potential of the methodology for the synthesis of complex multivalent architectures was further demonstrated by the preparation of the glycodendrimer−βCD conjugate **23**. Chemoselective cleavage of the solid-supported hexamannoside on resin **14** by βCD monoamine **9** furnished **22** in excellent yield and purity (Table 1, entry 7). Base-catalyzed removal of the acetyl protecting groups afforded the target fully unprotected βCD derivative **23** (Chart 2), showing mannose-specific lectin-binding properties and inclusion capabilities analogous to that previously reported for similar mannosyl dendrimer−βCD constructs.[27]

In summary, a preliminary assessment of the versatility of Ellman safety-catch sulfonamide linker for the solid-phase assisted synthesis of functional multivalent glycoarchitectures is reported. The methodology allows the rapid synthesis of homo- and heteromultivalent ligands and their chemoselective transfer/conjugation to an acceptor molecule, eventually, bearing additional functional units. The multistep synthetic scheme is significantly accelerated, while chromatographic purification is virtually unnecessary: two major issues concerning multivalent glycoconjugate synthesis. Application of the safety-catch principle to facilitate the development of functional multivalent systems based on protein−carbohydrate recognition events is currently been investigated in our laboratory.

Acknowledgment. This contribution was financially supported by the Spanish Ministerio de Educación y Ciencia (Contracts CTQ2004-05854/BQU and CTQ2006-15515-C02-−01/BQU). A.D.-M. is grateful to the I3P Program for a doctoral fellowship.

Supporting Information Available. Details of experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357−2364. (b) Koeller, K. M.; Wong, C.-H. *Nat. Biotechnol.* **2000**, *18*, 835−841. (c) Sears, P.; Wong, C.-H. *Angew. Chem.* **1999**, *111*, 2446−2471; *Angew. Chem., Int. Ed.* **1999**,

38, 1875−1917. (d) Singh, R. S.; Tiwary, A. K.; Kennedy, J. F. *Crit. Rev. Biotechnol.* **1999**, *19*, 145−178. (e) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674. (f) Dwek, R. *Chem. Rev.* **1996**, *96*, 683−720.

(2) (a) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578. (b) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2000**, *17*, 543−551. (c) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem.* **1998**, *110*, 2908−2953; *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794. (d) Lee, R. T.; Lee, Y. C. *Acc. Chem. Res.* **1995**, *28*, 321−327.

(3) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Angew. Chem.* **2006**, *118*, 2408−2429; *Angew. Chem., Int. Ed.* **2006**, *45*, 2348−2368. (b) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922−14933.

(4) (a) Benito, J. M.; Gómez-García, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355−10363. (b) Smiljanic, N.; Moreou, V.; Yockot, D.; Benito, J. M.; García Fernández, J. M.; Djedaïni-Pilard, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 5465−5468; *Angew. Chem.* **2006**, *118*, 5591−5594.

(5) (a) Fan, E.; Zhang, Z.; Minke, W. E.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J. *J. Am. Chem. Soc.* **2000**, *122*, 2663−2664. (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669−672. (c) Köhn, M.; Benito, J. M.; Ortiz Mellet, C.; Lindhorst, T. K.; García Fernández, J. M. *ChemBioChem* **2004**, *5*, 771−777. (d) Dubber, M.; Sperling, O.; Lindhorst, T. K. *Org. Biomol. Chem.* **2006**, *4*, 3901−3912.

(6) Turnbull, W. B.; Stoddart, J. F. *Rev. Mol. Biotechnol.* **2002**, *90*, 231−255.

(7) For pioneering work on the solid-phase synthesis of glycodendrimers, see: (a) Llinares, M.; Roy, R. *Chem. Commun.* **1997**, 2119−2120. (b) Zanini, D.; William, K. C.; Roy, R. *Tetrahedron Lett.* **1995**, *36*, 7383−7386.

(8) (a) Wittmann, V.; Seeberger, S. *Angew. Chem.* **2004**, *116*, 918−921; *Angew. Chem., Int. Ed.* **2004**, *43*, 900−903. (b) Renaudet, O.; Dumy, P. *Org. Biomol. Chem.* **2006**, *4*, 2628−2633.

(9) (a) Mechref, Y.; Novotny, M. V. *Chem. Rev.* **2002**, *102*, 321−369. (b) Shilova, N. V.; Bovin, N. V. *Russ. J. Bioorg. Chem.* **2003**, *29*, 309−324.

(10) Shin, I.; Park, S.; Lee, M.-r. *Chem.— Eur. J.* **2005**, *11*, 2894−2901.

(11) Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1971**, 636−637.

(12) Backes, R. J.; Virgilio, A. A.; Ellmann, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056.

(13) For a recent review on the applications of the Ellman sulfonamide safety-catch linker, see: Heidler, P.; Link, A. *Bioorg. Med. Chem.* **2005**, *13*, 585−599.

(14) Carboni, B.; Benalil, A.; Vaultier, M. *J. Org. Chem.* **1993**, *58*, 3736−3741.

(15) Baussanne, I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M.; Law, H.; Defaye, J. *Chem. Commun.* **2000**, 1489−1490.

(16) García Fernández, J. M.; Ortiz Mellet, C.; Defaye, J. *J. Inclusion Phenom. Macrocyclic Chem.* **2006**, *56*, 149−159.

(17) Backes, R. J.; Ellmann, J. A. *J. Org. Chem.* **1999**, *64*, 2322−2330.

(18) (a) Atkinson, S. J.; Ellis, V.-J.; Boyd, S. E.; Brown, C. L. *New J. Chem.* **2007**, *31*, 155−162. (b) Ambade, A. V.; Kumar, A. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5134−5145.

(19) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001**, *66*, 4200−4205.

(20) For a paper describing the preparation and purification of isocyanates from sterically hindered amines, see: Knölker, H.-J.; Braxmeier, T.; Schlechtingen, G. *Angew. Chem.* **1995**, *107*, 2746−2749; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2497−2500.

(21) For a recent paper, see: Gómez-García, M.; Benito, J. M.; Rodríguez-Lucena, D.; Yu, J.-X.; Chmurski, K.; Ortiz Mellet, C.; Guetiérrez Gallego, R.; Maestre, A.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 7970−7971.

(22) Bartra, M.; Romea, P.; Urpí, F.; Villarasa, J. *Tetrahedron* **1990**, *46*, 587−594.

(23) Completion of azide reduction was monitored by IR spectroscopy.

(24) (a) Duus, J. Ø.; Meldal, M.; Winkler, J. R. *J. Phys. Chem.* **1998**, *104*, 6413−6418. (b) Benito, J. M.; Meldal, M. *QSAR Comb. Sci.* **2004**, *23*, 117−129.

(25) Only minor gel permeation chromatography (Sephadex LH-20) has been conducted prior to complete characterization of the cleaved compounds **17**−**22**.

(26) García Fernández, J. M.; Ortiz Mellet, C.; Fuentes, J. *J. Org. Chem.* **1993**, *58*, 5192−5199.

(27) A 100-fold increase in binding affinity towards Concanavalin A as compared with methyl α-D-mannopyranoside was measured by the enzyme-linked lectin assay (ELLA). The water solubility of the anticancer drug docetaxel (Taxotere) was increased by a factor of ∼1000 in a 50 mM aqueous solution of **23**. See supporting information for experimental details.

CC070025V