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Diversity Oriented Combinatorial Synthesis of Multivalent Glycomimetics Through a Multicomponent Domino Process

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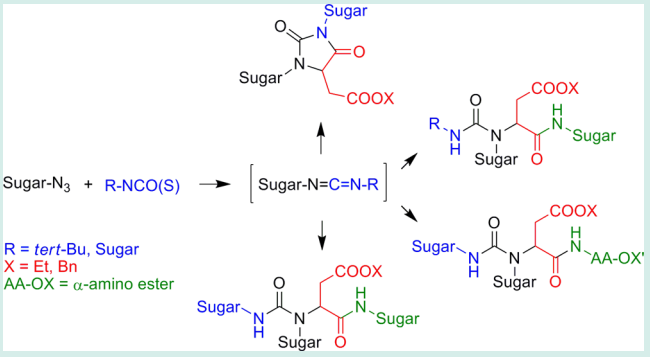
Supporting Information

ABSTRACT: Both multicomponent reactions and diversity oriented synthesis are indispensable tools for the modern medicinal chemist. However, their employment for the synthesis of multivalent glycomimetics has not been exploited so far although the importance that such compounds play in exploring multivalency on glycoside inhibition. Herein, we report the combinatorial synthesis of diversity oriented hetero di- and trivalent glycomimetics through a multicomponent domino process. The process is high yielding and very general, working efficiently with easily accessible sugar starting materials such as glycosylamines, glycosylazides, and glycosylisothiocyanates, having the reactive functional groups tethered either directly to the anomeric carbon, through a suitable linker, or to the primary 6 position of hexoses (or 5 position of pentoses), leading, in the latter case, to glycomimetics with artificial enzymatically stable backbone. The process has been also exploited for the multicomponent synthesis of aminoglycoside (neomycin) conjugates.



KEYWORDS: *combinatorial chemistry, multicomponent reaction, domino reaction, diversity oriented synthesis, multivalent glycomimetics*

INTRODUCTION

Carbohydrates are key modulators of different biological process, such as cell−cell communication and protein−carbohydrate interactions.[1] As such, it is of paramount importance to better understand the key interactions between carbohydrates and their ligands to be able to drive important cellular recognition events and to treat diseases. From a drug design perspective, it is noteworthy to consider that carbohydrates on cell surfaces are displayed in multivalent arrays and that complementary receptors recognize the individual arrays of functional groups present on the different monosaccharides, leading to the so-called "glycoside clustering effect".[2] This means that the different sugars that compose the polysaccharides do not have to be tethered necessary through glycosidic linkages, but also through other moieties that do not restrict too much the network of the essential functional groups for recognition.[3] This characteristic leads to different advantages. Indeed, in addition to avoid the stereospecific construction of the glycosidic linkage, the "artificial" linkage may be more stable toward glycosyl hydrolase activity and may be fine-tuned to obtain better activity/selectivity, eventually also after further selective functionalization. Thus, the synthesis of oligosaccharide mimetics (multivalent glycomimetics) by attaching two or more sugars to a synthetic scaffold has become a fundamental tool to investigate multivalency.[4]

Another advantage of the synthesis of multivalent glycomimetics compared to the synthesis of natural oligosaccharides connected via glycosidic linkages lies on the possibility to use a combinatorial approach. Indeed, rational design of functional mimics of carbohydrates is difficult because of the flexible and branched nature of oligosaccharides and combinatorial approaches seem the best choice to obtain lead glycomimetics to be used for better understand the weak carbohydrate−protein interactions.[5] Combinatorial chemistry, both in solution and in solid phase, has contributed in a fundamental way to elucidate the structure−function relationships of biologically important molecules, such as peptides, proteins, and nucleic acids.[6] However, because of the synthetic challenges for the stereospecific synthesis of the glycosidic linkage and the chemical complexity of these biomolecules, the combinatorial synthesis of oligosaccharides have been precluded and only few example have been reported in literature.[7] The possibility to link different sugar moieties to a synthetic scaffold renders the

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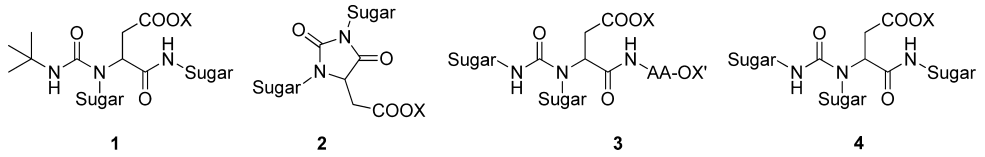
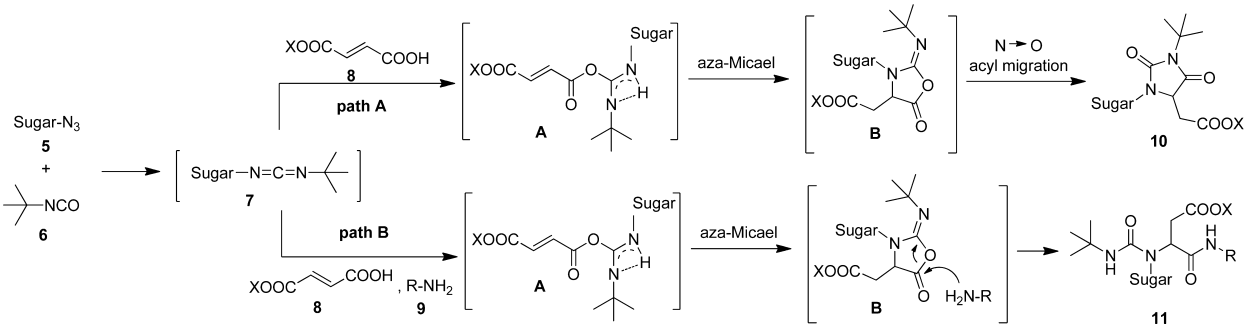


Figure 1. Structure of the multivalent glycomimetics **1**−**4**.

Scheme 1. Mechanism of the MC Sequential Domino Process



synthesis of oligosaccharide mimetics more automation-amenable and different approaches have been developed in the past decade. Moreover, in principle the combinatorial synthetic plan could be selected, with a forward synthetic strategy, in order to be able to synthesize diverse glycomimetics tethered trough different scaffolds starting from the same materials, namely, to obtain the diversity oriented synthesis (DOS) of multivalent glycomimetics. This is very important because DOS has an indispensable role to access molecular frameworks both in terms of scaffolds and stereochemistry, leading to a wider exploration of the chemical space.[8]

A method of choice to generate combinatorial libraries of small molecule relies on the exploitation of multicomponent (MC) reactions, where starting from three or more reactants it is possible to obtain the product one-pot, with a procedure which saves time and minimizes waste production.[9] Indeed, MC reactions are often the basis for DOS of small molecules in medicinal chemistry. However, to the best of our knowledge, MC reactions have not been exploited for DOS of multivalent glycomimetics. Indeed, the only examples appeared in literature describes the use of the well-known Ugi-MC reaction for the combinatorial synthesis of multivalent glycomimetics[10] and aminoglycoside mimics.[11] Thus, the invention of new MC reactions allowing for the combinatorial synthesis of diversity oriented libraries of glycomimetics could open the possibility to identify new lead compounds and to study key multivalent interactions in a fast and greener way.

Recently, we have been engaged in the development of a new MC process for the combinatorial synthesis of glycoconjugates starting from carbodiimides bearing a sugar N-linked carbohydrate residue.[12] The efficiency and the versatility of this process could be exploited for the synthesis of combinatorial libraries of diversity oriented multivalent glycomimetics. Herein, we would like to report the application of this process for the combinatorial DOS of di- and trivalent glycoconjugates **1**−**4** where the sugar moieties are tethered by artificial scaffolds such as aspartic acid, hydantoin ring, or urea linker (Figure 1).

RESULT AND DISCUSSION

In the previous works,[12] we have demonstrated that in situ formed *N*-sugar, *N*′-*tert*-butyl carbodiimides **7** react straightforwardly with fumaric acid monoesters **8** to give rise the formation of glyco-hydantoin conjugates **10** through a regiospecific sequential MC domino condensation/aza-Michael/N → O acyl migration one-pot process (path A, Scheme 1).[13] The same reaction carried out in the presence of nucleophiles such as amines, aminoesters, or peptides **9** leads to the MC synthesis of glyco-peptide conjugates **11** by reaction of the nucleophile with the cyclic *O*-acylisourea intermediate **B** which occurs after the intramolecular aza-Michael step and before the N → O acyl migration process (path B, Scheme 1).

To exploit such process for the combinatorial synthesis of multivalent glycomimetics **1**−**4**, we conceived to introduce the sugar moiety not only in the azide component but also in the iso(thio)cyanate and/or in the N-nucleophile components. Thus, we have synthesized seven sugar azides **5**{*1*−*7*}, four sugar isothiocyanates **6**{*1*−*4*} and seven sugar amines **9**{*1*−*7*}, starting from different carbohydrates such as ribose (compounds **5**{*1*}, **6**{*1*}, and **9**{*1*}), galactose (compounds **5**{*2*}, **6**{*2*}, and **9**{*2*}), glucosamine (compounds **5**{*3*} and **9**{*7*}), glucose (compounds **5**{*4,6*}, **6**{*3*}, and **9**{*3,4*}), mannose (compounds **5**{*5*}, and **9**{*6*}), and disaccharide lactose (compounds **5**{*7*}, **6**{*4*}, and **9**{*5*}), as depicted in Figure 2. The reactive functional groups of these derivatives, i.e. the azido group in chemset **5**, the isothiocyanate group in chemset **6** and the amine group in chemset **9**, were introduced either at the glycosylic carbon of the sugar, directly or through a linker, and at the primary 5 or 6 carbon of pentoses or hexoses, respectively, in order to synthesize glycomimetics having an enzymatically stable artificial "CH_2NH" glycine moiety. Moreover, for the synthesis of divalent glycopeptides mimetics **3** we used three different α-amino esters as nucleophilies (Figure 2). Such compounds were tested in the reaction with fumaric acid monoesters **8**{*1*−*2*} having different protecting group at the ester moiety to be able for a selective hydrolysis for a further functionalization, as already demonstrated in the previous works.[12]

First, we investigated the reactivity of *N*-sugar, *N*′-*tert*-butyl carbodiimides **7** with fumaric acid monoesters **8** in the presence

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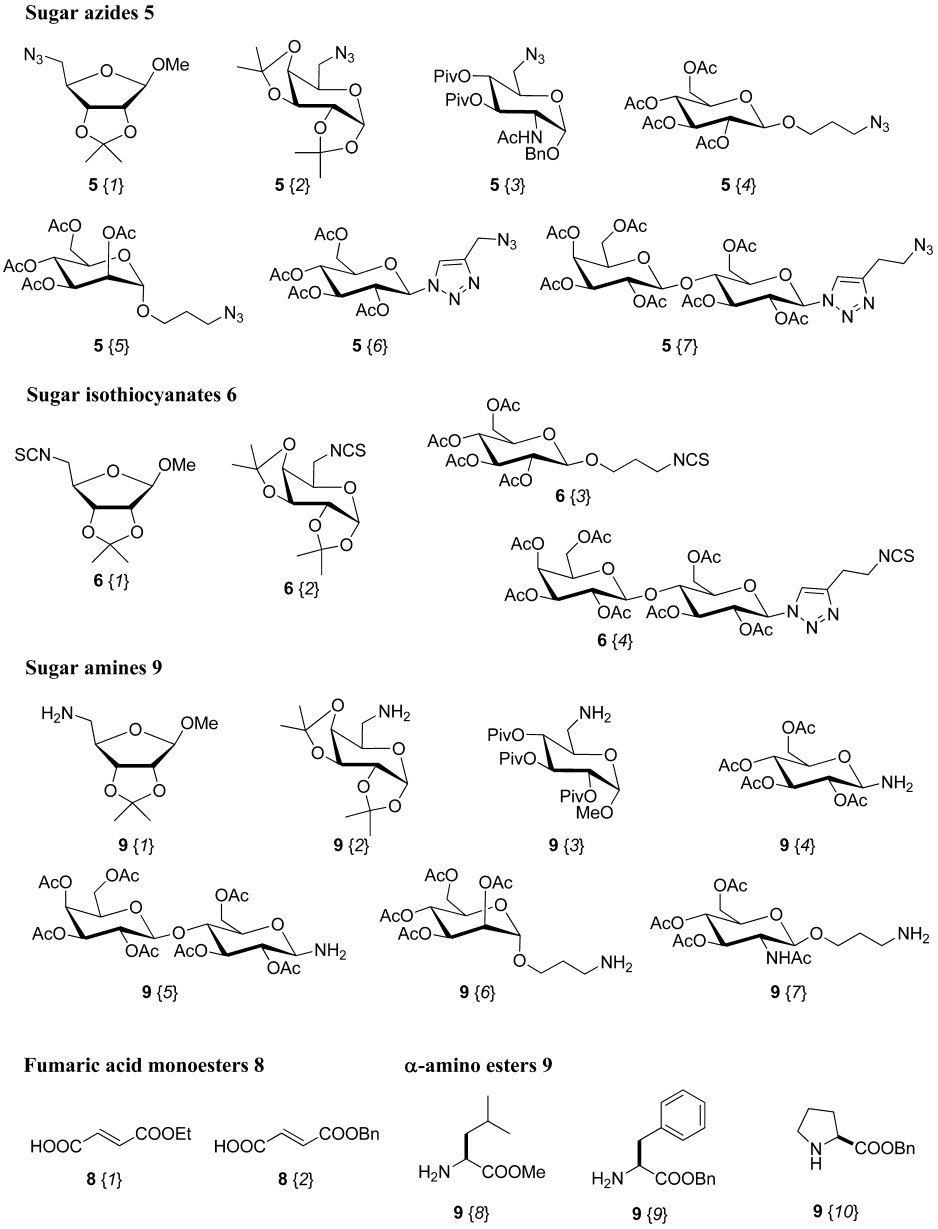

Figure 2. Reaction chemsets.

of sugar N-nucleophiles **9**. Indeed, following the mechanism depicted above (Scheme 1, path B), the reaction could lead to the regiospecific formation of divalent glycomimetics **1** with the sugar moieties tethered by an aspartic acid linker (Table 1). Accordingly, chemset **5** was reacted, at room temperature in CH_3CN, with *tert*-butylisocyanate **6** in the presence of triphenylphosphine until complete formation of carbodiimides **7** were achieved (TLC monitoring). Thus, the temperature was lowered to 0 °C and 2,4,6-trimethylpyridine (TMP), chemset **9** following by chemset **8** were added, and the reaction was run for 3 h. when formation of the final chemset **1** was obtained in good yields.[14] The exclusive formation of regioisomers **1** arose from the nucleophilic attack of the less sterically congested primary *N*-sugar moiety of intermediate **A** compared to the bulky tertiary *N*-*tert*-butyl amino group in the intramolecular aza-Michael step (see mechanism in Scheme 1).[15]

The reaction was very general and worked well with all the sugar azides and all the sugar amines tested. The yields were lower when the reactive amino group of glucosamines **9** is directly linked to the anomeric carbon, such as in **9**{*4*} and **9**{*5*}, because less nucleophilic, thus less reactive (entries 3, 4, 10, and 15, Table 1).[16] Indeed, we were able to obtain a library of 16 heterodivalent glycomimetics **1** tethered by an Asp unit linker (Figure 3) where the sugar moieties are linked directly through a glycosyl linkage, when glucosamines **9**{*4*–*5*} were used as nucleophiles (entries 3, 4, 10, and 15, Table 1), and/or through a glycosyl linker when glycosyl azides **5**{5–7} (entries 11–17, Table 1) and/or glycosyl amines **9**{*6*–*7*} were used (entries 8, 9, and 12, Table 1), and/or through a methyleneamino moiety, leading to conjugates with enzymatically stable artificial linkages, when azides **5**{*1*–*3*} (entries 1–10, Table 1) and/or amines **9**{*1*–*3*} were used (entries 1, 2, 5, 6, 9, 11, 13, 14, and 16, Table 1). Moreover we were able to obtain the Asp linker either protected as ethyl ester, when the reaction was performed with fumaric acid monoethyl ester **8**{*1*}, and as benzyl ester, when acid **8**{*2*} was used instead.

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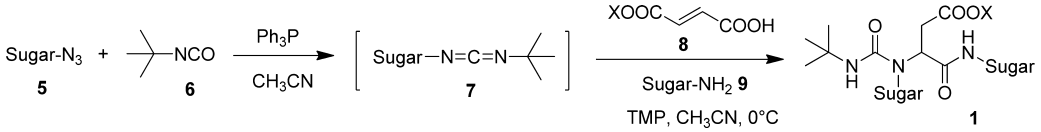
Table 1. Synthesis of Chemset 1



entry	sugar-N_3	fumaric acid	sugar-NH_2	product	yield (%)[a]
1	5{*1*}	8{*1*}	9{*1*}	1{*1,1,1*}	77
2	5{*1*}	8{*2*}	9{*1*}	1{*1,2,1*}	79
3	5{*1*}	8{*2*}	9{*4*}	1{*1,2,4*}	56[b]
4	5{*1*}	8{*2*}	9{*5*}	1{*1,2,5*}	58[b,c]
5	5{*2*}	8{*2*}	9{*1*}	1{*2,2,1*}	82
6	5{*2*}	8{*1*}	9{*3*}	1{*2,1,3*}	75
7	5{*2*}	8{*2*}	9{*6*}	1{*2,2,6*}	77
8	5{*2*}	8{*2*}	9{*7*}	1{*2,2,7*}	81
9	5{*3*}	8{*2*}	9{*1*}	1{*3,2,1*}	72
10	5{*3*}	8{*1*}	9{*4*}	1{*3,1,4*}	56[b,c]
11	5{*5*}	8{*2*}	9{*1*}	1{*5,2,1*}	81
12	5{*5*}	8{*1*}	9{*6*}	1{*5,1,6*}	69
13	5{*6*}	8{*1*}	9{*1*}	1{*6,1,1*}	71
14	5{*6*}	8{*2*}	9{*3*}	1{*6,2,3*}	74
15	5{*6*}	8{*2*}	9{*5*}	1{*6,2,5*}	54[b,c]
16	5{*7*}	8{*2*}	9{*3*}	1{*7,2,3*}	68

[a]Overall yields. [b]An ∼20% of the corresponding hydantoin was recovered. [c]The reaction was performed with a 20% in volume of DMF (see ref 12d).

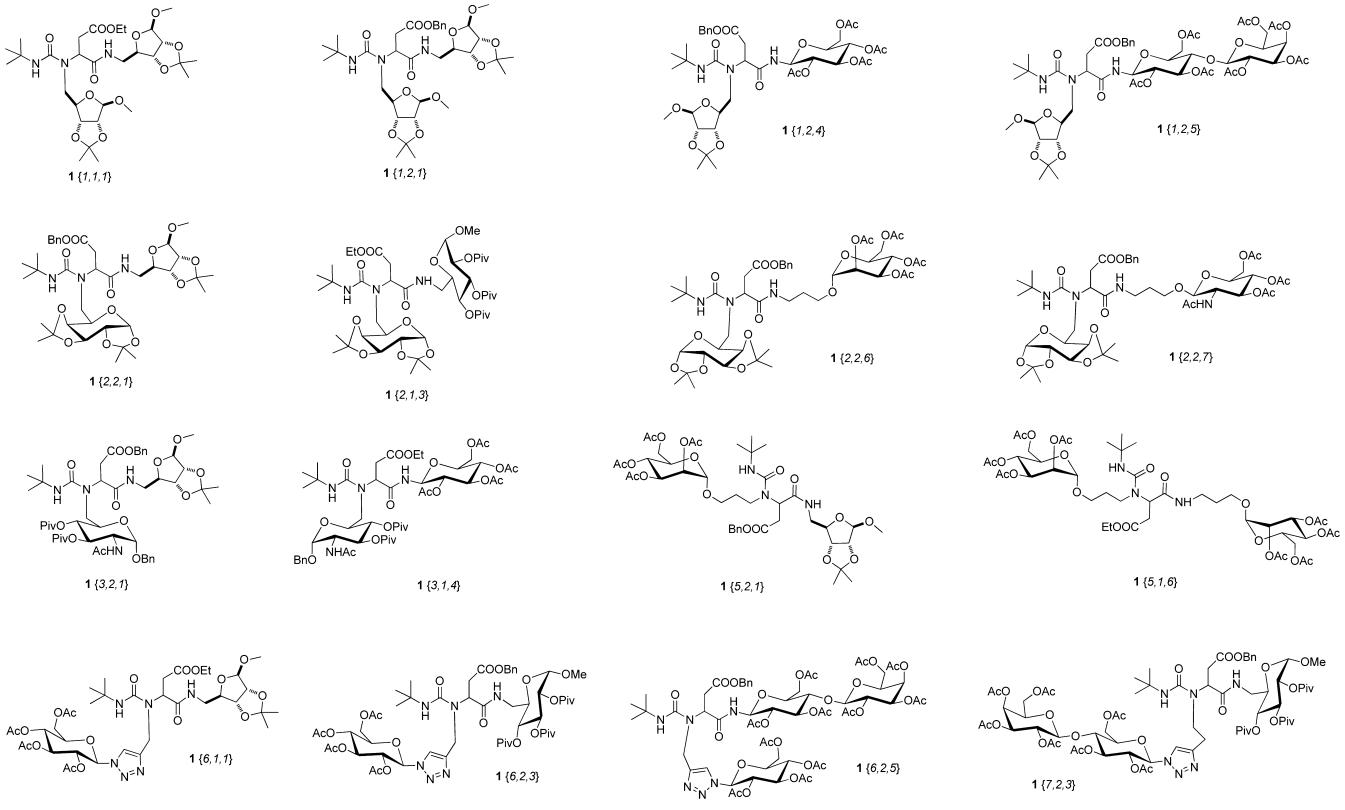


Figure 3. Divalent glycomimetics chemset **1**.

This is very important for a further functionalization because we can always project the synthesis of glycomimetics orthogonally protected at the glyco- and at the Asp-linkage moieties.[12]

Then, we turned our attention in the synthesis of carbodiimide intermediates having two *N*-sugar substituents which, in a forward planning strategy typical of DOS chemistry, could have been the starting materials for the synthesis of multivalent glycomimetics **2**−**4**. Indeed, starting from *N*−*N*′-diglycocarbodiimides, which can be prepared in situ by Staudinger reaction between glycosylazides and glycosylisothiocyanates, and performing the reaction in absence of a

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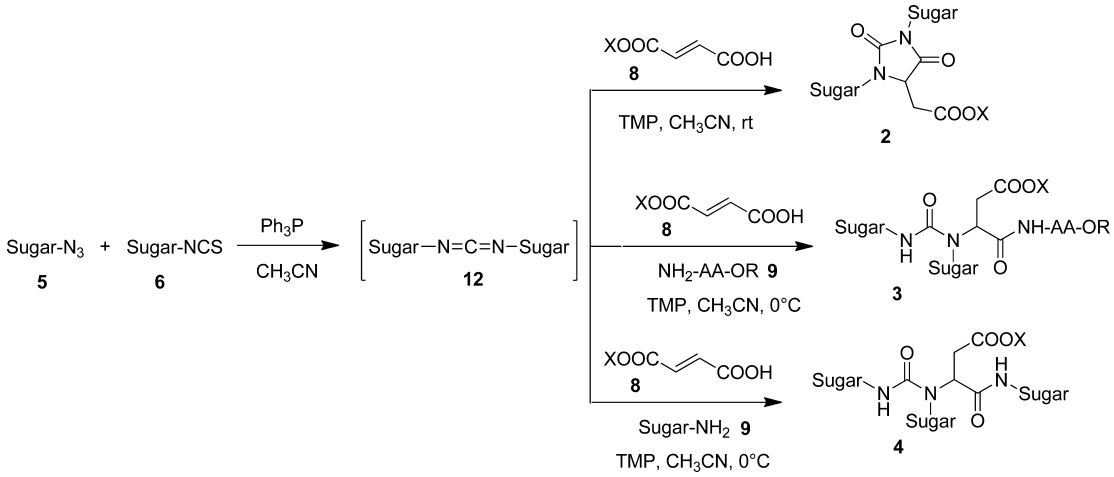
Table 2. Synthesis of Chemsets 2−4



entry	sugar-N_3	sugar-NCS	acid	sugar-NH_2	product	yield (%)[a]
1	5{*1*}	6{*1*}	8{*1*}		2{*1,1,1*}	81
2	5{*1*}	6{*1*}	8{*2*}		2{*1,1,2*}	85
3	5{*2*}	6{*2*}	8{*1*}		2{*2,2,1*}	78
4	5{*2*}	6{*2*}	8{*2*}		2{*2,2,2*}	82
5	5{*4*}	6{*3*}	8{*1*}		2{*4,3,1*}	77
6	5{*4*}	6{*3*}	8{*2*}		2{*4,3,2*}	80
7	5{*7*}	6{*4*}	8{*2*}		2{*7,4,2*}	69
8	5{*1*}	6{*1*}	8{*1*}	9{*9*}	3{*1,1,1,9*}	78
9	5{*1*}	6{*1*}	8{*2*}	9{*8*}	3{*1,1,2,8*}	81
10	5{*2*}	6{*2*}	8{*2*}	9{*8*}	3{*2,2,2,8*}	83
11	5{*2*}	6{*2*}	8{*1*}	9{*10*}	3{*2,2,1,10*}	67
12	5{*4*}	6{*3*}	8{*2*}	9{*8*}	3{*4,3,2,8*}	70
13	5{*1*}	6{*1*}	8{*2*}	9{*1*}	4{*1,1,2,1*}	55[b]
14	5{*1*}	6{*1*}	8{*1*}	9{*6*}	4{*1,1,1,6*}	58[b]
15	5{*2*}	6{*2*}	8{*2*}	9{*1*}	4{*2,2,2,1*}	60[b]
16	5{*2*}	6{*2*}	8{*2*}	9{*3*}	4{*2,2,2,3*}	56[b]
17	5{*4*}	6{*3*}	8{*1*}	9{*1*}	4{*4,3,1,1*}	52[b]
18	5{*4*}	6{*3*}	8{*2*}	9{*6*}	4{*4,3,2,6*}	53[b]

[a]Overall yields. [b]An ∼20% of the corresponding hydantoin was recovered.

nucleophiles we can synthesize divalent glycomimetics tethered through an hydatoin ring **2**, while by performing the reaction in the presence of *α*-aminoester nucleophiles we can prepare divalent glycopeptide mimetics **3**, or trivalent glycomimetics **4** if in the presence of *N*-sugar nucleophiles instead. Thus, isothiocyanates **6** depicted in Figure 2 were synthesized starting from the corresponding azides **5** by aza-Wittig reaction with carbon disulfide CS_2. The resulting chemset **6** was reacted with chemset **5** in the same condition described above for the synthesis of carbodiimides, that is, Ph_3P in CH_3CN at rt, leading to the formation of *N,N′*-disugar carbodiimides **12** which were reacted in situ with chemset **8** in absence or in the presence of chemset **9** to obtain bivalent glycomimetics **2** and **3** and trivalent glycomimetics **4** (Table 2).

Also in these cases the reaction was very general and worked efficiently with all the sugar components tested. In particular, homodivalent glycomimetics **2** (entries 1−7, Table 2) and glycopeptide mimetics **3** (entries 8−12, Table 2) were achieved with high yields either starting from "non-glycosylic" azide compounds **5**{*1*−*3*} and isothicyanates **6**{*1,2*}, or starting from the corresponding glycosylic compounds (compounds **5**{*1,2*}, **6**{*3,4*}). The same reactions carried out starting from asymmetric carbodiimides, i.e. starting from different azide and isothiocyanate, lead to the efficient, in terms of yields, formation of the corresponding heterodivalent derivatives, but as a mixture of regioisomers which were difficult to separate (data not shown). This was due to the fact that the primary azides **5** and the primary isothiocyanate **6** are not very different in terms of steric hindrance, resulting in a missing regioselectivity during the intramolecular aza-Michael step (see Scheme 1, path A). Concerning the synthesis of heterotrivalent glycomimetics **4** (entries 13−18, Table 2), although they were obtained in a satisfactory fashion, the yields were lower compared to those obtained in the precedent cases, probably because of steric factors, and the corresponding hydantoin were recovered in ∼ 20% yield along with the desired compounds.

A possible interesting application of this methodology could be the synthesis of libraries of conjugated aminoglycosides, a class of selective RNAs binders.[17] Indeed, to improve the activity of aminoglycosides and to better understand the structural basis for recognition of diverse RNA targets, the development of a practical and quick strategy for the combinatorial synthesis of libraries of aminoglycoside conjugates by functionalization of known aminoglycoside scaffolds is very appealing since this strategy has already produced results and shows enormous promise.[18] Thus, we tried to use this procedure to tether sugars to aminoglycosides. Actually, starting

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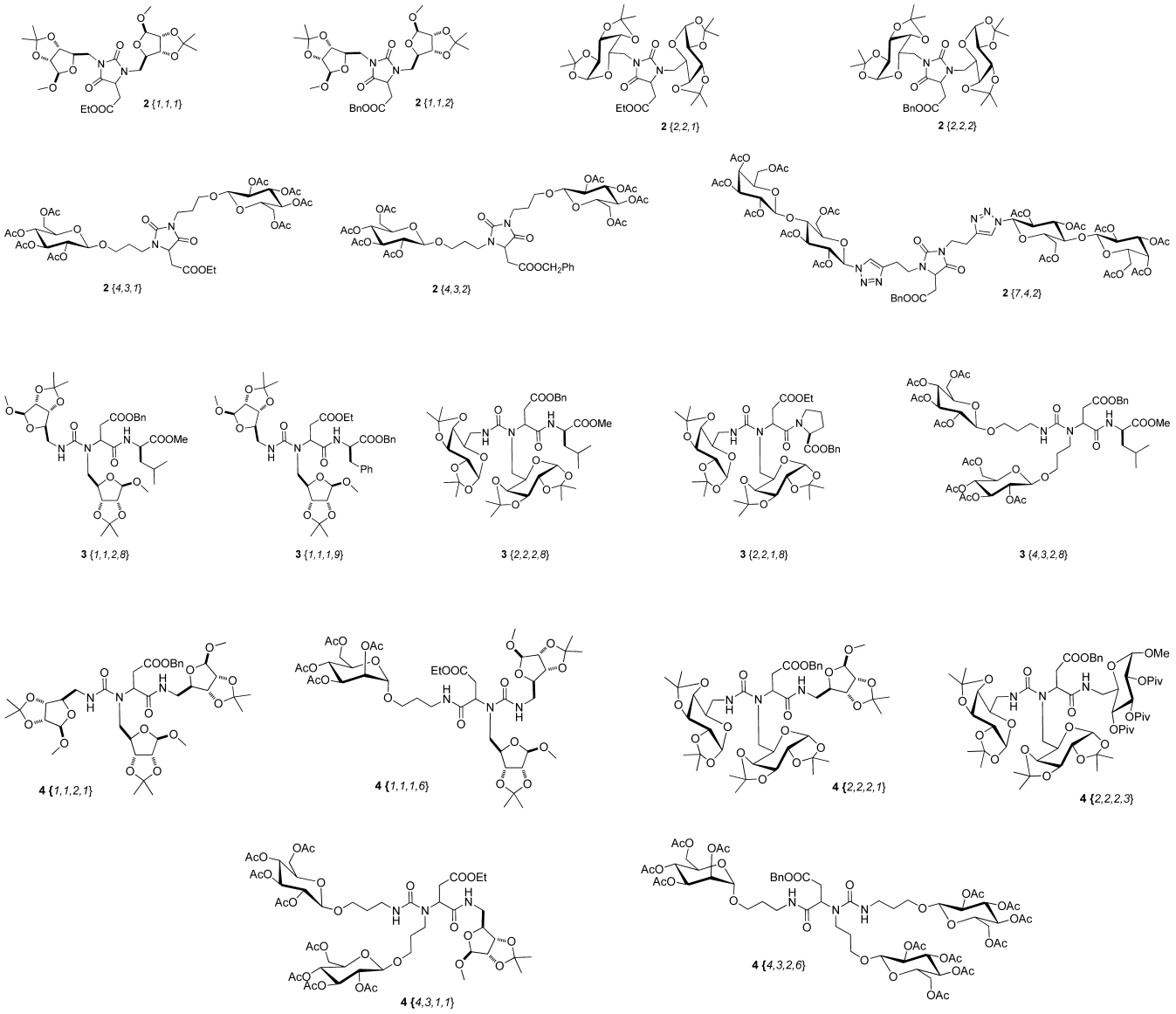

Figure 4. Di- and trivalent glycomimetic chemsets **2**−**4**.

from glycoazide **5**{*4*}, *tert*-butyl isocyanate **6**, fumaric acid benzyl ester **8**{*2*}, and neomycin derivative **13**, we were able to synthesize through this MC process the neomycin conjugate **14** in good yields (Scheme 2).[19]

Interestingly, by treatment of **14** with $MeNH_2$ in EtOH at rt, along with deacetylation of glucose, we obtained the formation of a dihydrouracil ring by cyclization of the *N*-*tert*-butylurea moiety with the benzyl ester, producing neomycin conjugate **15** in almost quantitative yield.[20] The exploitation of such procedure for the MC combinatorial synthesis of aminoglycoside conjugates and the study of their activity will be reported in a forthcoming paper.

CONCLUSION

In conclusion, we have reported, for the first time, the combinatorial synthesis of diversity oriented heteromultivalent glycomimetics through a domino MC process starting from easily accessible glyco-compounds. Through this process, we have synthesized 34 different multivalent glycomimetics, where the sugar moieties are tethered through an aspartic acid, a hydantoin ring or an urea linker, demonstrating that the process is very general and works efficiently with different glycosylazides, glycosylisothiocyanates and glycosylamines. Moreover, we have demonstrated that such procedure could be exploited for the combinatorial synthesis of aminoglycoside conjugates. The latter issue is in progress and will be reported in due course.

EXPERIMENTAL PROCEDURES

General Methods. Commercially available reagent-grade solvents were employed without purification. TLC were run on silica gel 60 F_{254} Merck. Flash chromatography (FC) was performed with silica gel 60 (60−200 μm, Merck). ^{1}H NMR spectra were recorded on 400 MHz spectrometers. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^{1}H and ^{13}C nuclei (δ_H and δ_C = 0.00). Glycosylazides **5** were prepared as described in ref 12. Glycosylisothiocyanates **6** were prepared by aza-Wittig reaction with CS_2.[21] Glycosylamines **9** were prepared by catalytic hydrogenation of the corresponding azides obtained as reported in ref 12. Neomycin derivative 13 was obtained as reported in ref 18a.

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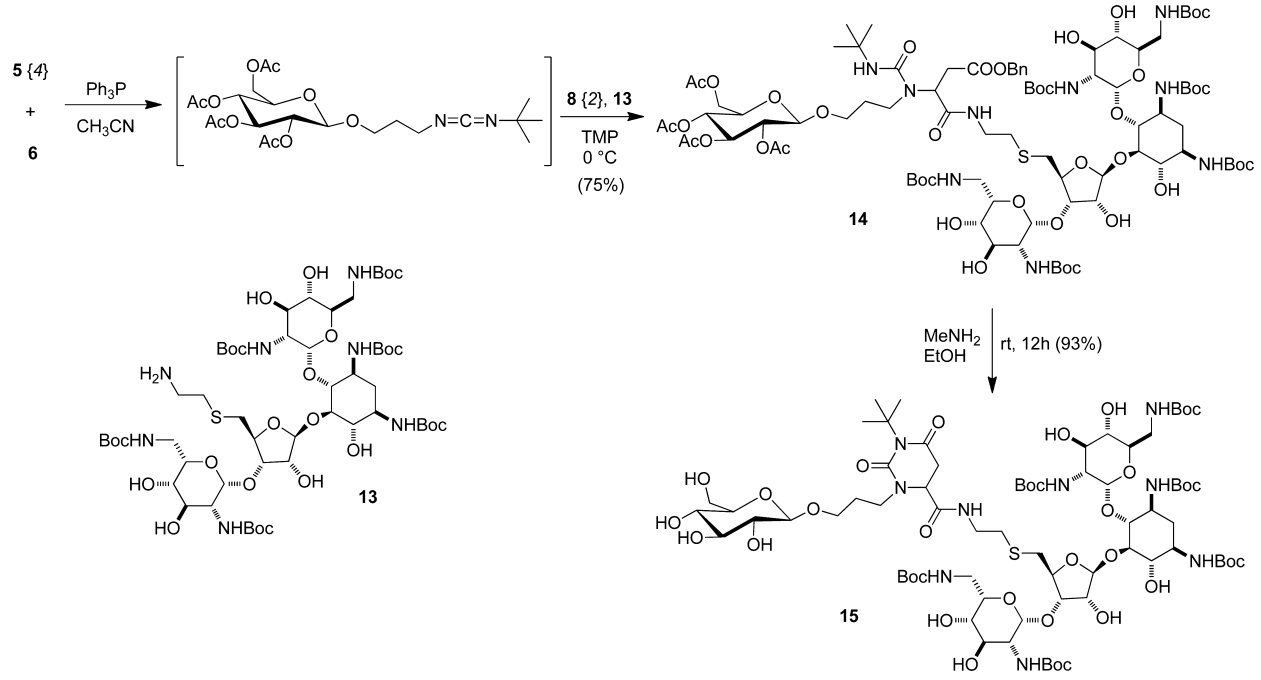
Scheme 2. MC Synthesis of Neomycin Derivative 14



Synthesis of Divalent Glycomimetics 1: General Procedure. To a stirred solution of glycosylazide (1 equiv) **5** in CH_3CN (0.1 M) *tert*-butyl isocyanate **6** (1.05 equiv) followed by Ph_3P (1.05 equiv) were added at rt. The solution was stirred until complete formation of the corresponding carbodiimide was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (1 equiv), a solution of glycosylamine **9** (1 equiv) in a minimum amount of CH_3CN, followed by a solution of fumaric acid **8** (1 equiv) in a minimum amount of CH_3CN were added. The temperature was slowly left to reach rt and the reaction, when finished (TLC monitoring, ~3 h), was quenched with an aqueous 1 N solution of HCl. The mixture was extracted with AcOEt, the organic phases collected and anhydrified over Na_2SO_4, the solvent removed under pressure and the crude purified by flash chromatography.

***1*{2,1,3}.** *Major diastereoisomer*: R_f = 0.48 (hexane/AcOEt 70:30); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.59 (br s, 1H, amidic NH), 5.56 (t, J = 10.0 Hz, 1H), 5.49 (d, J = 4.8 Hz, 1H, anomeric), 5.35 (br s, 1H, urea NH), 4.91 (m, 2H), 4.80 (dd, J = 10.0 and 3.6 Hz, 1H), 4.61 (m, 1H), 4.59 (dd, J = 8.0 and 2.4 Hz, 1H), 4.31 (dd, J = 4.8 and 2.4 Hz, 1H), 4.23 (dd, J = 8.0 and 1.6 Hz, 1H), 4.14 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$), 4.11 (m, 1H), 3.88 (m, 1H), 3.80 (m, 1H), 3.67 (m, 1H), 3.38 (s, 3H, OCH_3), 3.16 (dd, J = 16.8 and 7.6 Hz, 1H, CH*H*COOEt), 3.08 (dd, J = 15.6 and 8.8 Hz, 1H), 2.95 (m, 1H), 2.66 (dd, J = 16.8 and 6.8 Hz, 1H, CH*H*COOEt), 1.48 (s, 3H, CH_3 acetonide), 1.44 (s, 3H, CH_3 acetonide), 1.34 (s, 6H, CH_3 acetonide), 1.29 (s, 9H, $C(CH_3)_3$), 1.26 (t, J = 7.2 Hz, $COOCH_2CH_3$), 1.21 (s, 9H, $C(CH_3)_3$), 1.18 (s, 9H, $C(CH_3)_3$), 1.13 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 177.7, 177.3, 176.7, 171.2, 170.9, 156.7, 109.4, 109.2, 96.4, 96.3, 71.6, 71.4, 70.7, 69.9, 69.4, 68.1, 68.0, 60.7, 55.3, 51.0, 40.1, 38.8, 38.7, 38.6, 34.1, 29.2, 27.1, 27.0, 26.9, 26.0, 25.9, 25.1, 24.3, 14.1; ESI (*m/z*) 952.9 [M^++Na, (100)]; Anal. Calcd for $C_{45}H_{75}N_3O_{17}$ C 58.11, H 8.13, N 4.52; found C 58.11, H 8.15, N 4.51. *Minor diastereoisomer*: R_f = 0.33 (hexane/AcOEt 70:30); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.48 (br s, 1H, amidic NH), 6.09 (br s, 1H, urea NH), 5.55 (t, J = 9.6 Hz, 1H), 5.52 (d, J = 4.8 Hz, 1H, anomeric), 4.97−89 (m, 3H), 4.82 (dd, J = 10.4 and 4.0 Hz, 1H), 4.60 (dd, J = 8.0 and 2.4 Hz, 1H), 4.32 (dd, J = 5.2 and 2.4 Hz, 1H), 4.22 (dd, J = 8.0 and 2.0 Hz, 1H), 4.14 (m, $COOCH_2CH_3$), 4.04 (m, 1H), 3.88 (m, 1H), 3.47 (m, 1H), 3.40−3.25 (m, 3H), 3.38 (s, 3H, OCH_3), 3.08 (dd, J = 10.0 and 8.0 Hz, 1H), 2.63 (dd, J = 17.2 and 6.8 Hz, 1H, CH*H*COOEt), 1.54 (s, 3H, CH_3 acetonide), 1.43 (s, 3H, CH_3 acetonide), 1.35 (s, 6H, CH_3 acetonide), 1.24 (t, J = 7.2 Hz, $COOCH_2CH_3$), 1.18 (s, 9H, $C(CH_3)_3$), 1.17 (s, 9H, $C(CH_3)_3$), 1.12 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 177.4, 177.0, 176.7, 171.2, 170.8, 159.2, 109.4, 109.2, 96.6, 96.4, 96.2, 71.7, 71.4, 70.8, 70.6, 69.6, 69.1, 68.0, 67.9, 60.6, 55.6, 50.7, 47.1, 39.5, 38.8, 38.7, 35.2, 29.2, 27.2, 27.1, 26.9, 26.0, 25.9, 24.8, 24.2, 14.1; ESI (*m/z*) 952.6 [M^+ + Na, (100)]; Anal. Calcd for $C_{45}H_{75}N_3O_{17}$ C 58.11, H 8.13, N 4.52; found C 58.13, H 8.14, N 4.53.

Synthesis of Divalent Glycomimetics 2: General Procedure. To a stirred solution of glycosylazide (1 equiv) **5** in CH_3CN (0.1 M) a solution of glycosylisothiocyanate **6** (1.05 equiv) in a minimum amount of CH_3CN followed by Ph_3P (1.05 equiv) were added at rt. The solution was stirred until complete formation of the corresponding carbodiimide was achieved (TLC monitoring). A solution of fumaric acid **8** (1 equiv) in a minimum amount of CH_3CN was added at rt and the resulting solution stirred until was complete (~3 h). The reaction, was quenched with an aqueous 1 N solution of HCl. The mixture was extracted with AcOEt, the organic phases collected and anhydrified over Na_2SO_4, the solvent removed under pressure and the crude purified by flash chromatography.

***2*{2,2,2}.** *Major diastereoisomer*: R_f = 0.32 (hexane:AcOEt 60:40); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.35 (m, 5H, aromatics), 5.45 (d, J = 5.2 Hz, 1H, anomeric), 5.40 (d, J = 4.8 Hz, 1H, anomeric), 5.14 (s, 2H, $COOCH_2Ph$), 4.60 (m, 1H), 4.59 (m, 1H), 4.35 (dd, J = 6.0 and 4.4 Hz, 1H), 4.27 (m, 2H),

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4.22−4.15 (m, 3H), 4.08 (m, 1H), 3.95 (dd, J = 14.4 and 10.0 Hz, 1H), 3.62 (dd, J = 14.4 and 8.8 Hz, 1H), 3.44 (t, J = 3.6 Hz, 1H), 3.40 (t, J = 2.8 Hz, 1H), 3.01 (dd, J = 17.2 and 4.0 Hz, 1H, CH*H*COOBn), 2.93 (dd, J = 17.2 and 6.0 Hz, 1H, CH*H*COOBn), 1.49 (s, 6H, CH_3 acetonide), 1.47 (s, 3H, CH_3 acetonide), 1.45 (s, 3H, CH_3 acetonide), 1.35 (s, 3H, CH_3 acetonide), 1.33 (s, 3H, CH_3 acetonide), 1.30 (s, 3H, CH_3 acetonide), 1.29 (s, 3H, CH_3 acetonide); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 172.4, 169.6, 156.9, 135.5, 128.6, 128.4, 128.3, 109.8, 109.5, 108.8, 108.7, 96.3, 96.2, 71.5, 71.4, 71.05, 71.01, 70.6, 70.5, 66.9, 65.5, 64.5, 57.3, 42.9, 39.3, 35.4, 26.0, 25.9, 25.8, 25.1, 25.0, 24.7, 24.4; ESI (*m/z*) 755.3 [M^+ + Na, (100)], 733.3 [M^+ + 1, (11)]; Anal. Calcd for $C_{36}H_{48}N_2O_{14}$ C 59,01, H 6,60, N 3,82; found C 59,03, H 6,61, N 3,83. *Minor diastereoisomer*: R_f = 0.26 (hexane/AcOEt 60:40); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.35 (m, 5H, aromatics), 5.51 (d, J = 4.8 Hz, 2H, anomerics), 5.44 (t, J = 5.6 Hz, 1H), 5.12 (s, 2H, $COOCH_2Ph$), 4.63−4.59 (m, 2H), 4.32−4.25 (m, 5H), 4.06 (m, 1H), 3.86 (m, 1H), 3.55−3.46 (m, 2H), 3.20 (dd, J = 15.2 and 9.2 Hz, 1H, CH*H*COOBn), 3.02 (dd, J = 15.2 and 4.8 Hz, 1H, CH*H*COOBn), 1.52 (s, 6H, CH_3 acetonide), 1.47 (s, 3H, CH_3 acetonide), 1.46 (s, 3H, CH_3 acetonide), 1.36 (s, 3H, CH_3 acetonide), 1.35 (s, 3H, CH_3 acetonide), 1.33 (s, 3H, CH_3 acetonide), 1.32 (s, 3H, CH_3 acetonide).

Synthesis of Divalent Glycomimetics 3: General Procedure. To a stirred solution of glycosylazide (1 equiv) **5** in CH_3CN (0.1 M) a solution of glycosylisothiocyanate **6** (1.05 equiv) followed by Ph_3P (1.05 equiv) was added at rt. The solution was stirred until complete formation of the corresponding carbodiimide was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (2 equiv), solid α-aminoester hydrochloride **9** (1 equiv), followed by a solution of fumaric acid **8** (1 equiv) in a minimum amount of CH_3CN were added. The temperature was slowly left to reach rt and the reaction, when finished (TLC monitoring, ∼3 h), was quenched with an aqueous 1 N solution of HCl. The mixture was extracted with AcOEt, the organic phases collected and anhydrified over Na_2SO_4, the solvent removed under pressure and the crude purified by flash chromatography.

***3*{1,1,2,8}**. *Major diastereoisomer*: R_f = 0.50 (hexane/AcOEt 40:60); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.35 (m, 5H, aromatics), 7.12 (d, J = 8.0 Hz, 1H, amidic NH), 6.01 (br t, J = 5.6 Hz, 1H, urea NH), 5.16 (d, J = 12.0 Hz, 1H, COOC*H*HPh), 5.12 (d, J = 12.0 Hz, 1H, COOC*H*HPh), 5.00 (s, 1H, anomeric), 4.96 (s, 1H, anomeric), 4.73 (m, 1H), 4.61 (m, 3H), 4.55 (m, 2H), 4.35 (m, 1H), 4.22 (t, J = 6.8 Hz, 1H), 3.70 (s, 3H, $COOCH_3$), 3.50 (m, 1H), 3.42 (s, 3H, OCH_3), 3.40 (m, 2H), 3.36 (s, 3H, OCH_3), 3.30 (dd, J = 16.8 and 6.4 Hz, 1H, C*H*HCOOBn), 3.22 (m, 1H), 2.95 (dd, J = 16.8 and 7.6 Hz, 1H, CHHCOOBn), 1.61 (m, 3H), 1.48 (s, 3H, CH_3 acetonide), 1.46 (s, 3H, CH_3 acetonide), 1.33 (s, 3H, CH_3 acetonide), 1.30 (s, 3H, CH_3 acetonide), 0.93 (d, J = 3.6 Hz, 3H, CH_3 leucine), 0.91 (d, J = 3.6 Hz, 3H, CH_3 leucine); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 172.7, 171.3, 170.2, 158.4, 135.8, 128.5, 128.2, 128.1, 113.3, 112.2, 110.5, 109.8, 86.2, 86.1, 85.4, 84.5, 82.2, 81.9, 66.6, 57.0, 55.9, 55.1, 52.0, 51.1, 50.9, 44.2, 41.0, 34.3, 26.6, 26.5, 25.1, 25.0, 24.8, 22.7, 21.8; ESI (*m/z*) 788.4 [M^+ + Na, (100)]; [M^+ + Na, (100)]; Anal. Calcd for $C_{37}H_{55}N_3O_{14}$: C 58.03, H 7.24, N 5.49; found: C 58.05, H 7.25, N 5.50. *Minor diastereoisomer*: R_f = 0.42 (hexane/AcOEt 40:60); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.68 (br s, 1H, amidic NH), 7.35 (m, 5H, aromatics), 5.71 (br t, J = 5.6 Hz, 1H, urea NH), 5.16 (d, J = 12.0 Hz, 1H, COOC*H*HPh), 5.09 (d, J = 12.0 Hz, 1H, COOC*H*HPh), 4.99 (s, 1H, anomeric), 4.96 (s, 1H, anomeric), 4.59 (m, 3H), 4.54 (m, 1H), 4.43 (m, 1H), 4.28 (t, J = 6.4 Hz, 1H), 3.89 (m, 1H), 3.70 (s, 3H, $COOCH_3$), 3.51 (m, 1H), 3.40 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3), 3.28 (m, 2H), 3.05 (dd, J = 15.2 and 10.0 Hz, 1H, C*H*HCOOBn), 2.86 (dd, J = 15.2 and 7.6 Hz, 1H, CH*H*COOBn), 1.67 (m, 3H), 1.46 (s, 6H, CH_3 acetonide), 1.32 (s, 3H, CH_3 acetonide), 1.29 (s, 3H, CH_3 acetonide), 0.94 (d, J = 5.6 Hz, 6H, CH_3 leucine), 0.91 (d, J = 3.6 Hz, 3H, CH_3 leucine); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 172.6, 171.0, 170.8, 157.1, 135.7, 128.5, 128.25, 128.21, 128.1, 112.7, 112.2, 110.8, 109.9, 86.9, 86.1, 85.5, 84.6, 82.1, 81.8, 66.6, 58.7, 56.0, 55.1, 52.5, 52.1, 51.3, 44.0, 41.0, 33.8, 26.6, 26.5, 26.4, 25.1, 25.0, 24.9, 24.89, 24.85, 24.80, 22.8, 21.8; ESI (*m/z*) 788.7 [M^+ + Na, (100)]; Anal. Calcd for $C_{37}H_{55}N_3O_{14}$ C 58.03, H 7.24, N 5.49; found C 58.04, H 7.26, N 5.49.

Synthesis of Trivalent Glycomimetics 4: General Procedure. To a stirred solution of glycosylazide (1 equiv) **5** in CH_3CN (0.1 M) a solution of glycosylisothiocyanate **6** (1.05 equiv) followed by Ph_3P (1.05 equiv) were added at rt. The solution was stirred until complete formation of the corresponding carbodiimide was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (1 equiv), a solution of glycosylamine **9** (1 equiv) in a minimum amount of CH_3CN, followed by a solution of fumaric acid **8** (1 equiv) in a minimum amount of CH_3CN were added. The temperature was slowly left to reach rt and the reaction, when finished (TLC monitoring, ∼3 h), was quenched with an aqueous 1 N solution of HCl. The mixture was extracted with AcOEt, the organic phases collected and anhydrified over Na_2SO_4, the solvent removed under pressure and the crude purified by flash chromatography.

***4*{1,1,2,1}**. *Mixture of two diastereoisomers:* R_f = 0.31 (hexane/AcOEt 10:90); ^{1}H NMR (500 MHz, $CDCl_3$), *major diastereoisomer* δ = 7.35 (m, 5H, aromatics), 7.26 (br s, 1H, amidic NH), 6.08 (t, J = 5.6 Hz, 1H, urea NH), 5.15 (d, J = 14.6 Hz, 1H, COOCH*H*Ph), 5.09 (d, J = 14.6 Hz, 1H, COOCH*H*Ph), 5.00 (s, 1H, anomeric), 4.96 (s, 1H, anomeric), 4.95 (s, 1H, anomeric), 4.82 (m, 1H), 4.67−4.57 (m, 5H), 4.48 (m, 1H), 4.41 (m, 1H), 4.31 (m, 1H), 4.24 (m, 1H), 3.77 (m, 1H), 3.52 (m, 1H), 3.42 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.40−3.30 (m, 3H), 3.24 (m, 1H), 3.05 (dd, J = 15.2 and 10.4 Hz, 1H, CH*H*COOBn), 2.87 (dd, J = 15.2 and 7.6 Hz, 1H, CH*H*COOBn), 1.47−1.44 (three s, 9H, CH_3 acetonide), 1.32−1.30 (three s, 9H, CH_3 acetonide); *minor diastereoisomer* δ = 7.75 (br s, 1H, amidic NH), 7.35 (m, 5H, aromatics), 5.66 (t, J = 5.6 Hz, 1H, urea NH), 5.16 (d, J = 12.0 Hz, 1H, COOCH*H*Ph), 5.08 (d, J = 12.0 Hz, 1H, COOCH*H*Ph), 5.05 (s, 1H, anomeric), 4.97 (s, 1H, anomeric), 4.96 (s, 1H, anomeric), 4.67−4.57 (m, 6H), 4.48 (m, 1H), 4.31 (m, 2H), 4.24 (m, 1H), 3.52 (m, 1H), 3.42 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 3.40−3.30 (m, 4H), 3.24 (m, 2H), 2.80 (dd, J = 16.8 and 6.4 Hz, 1H, CH*H*COOBn), 1.47−1.44 (three s, 9H, CH_3 acetonide), 1.32−1.30 (three s, 9H, CH_3 acetonide); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 171.2, 171.1, 170.8, 170.5, 158.5, 156.5, 135.7, 128.5, 128.3, 128.2, 113.1, 112.7, 112.3, 112.2, 110.9, 110.6, 110.1, 109.9, 109.86, 109.80, 86.6, 86.5, 86.2, 85.9, 85.6, 85.56, 85.52, 85.4, 85.3, 84.7, 84.4, 82.2, 82.1, 82.08, 82.04, 82.02, 81.6, 66.6, 66.5, 59.8, 56.4, 55.9, 55.2, 55.1, 55.0, 53.0, 50.3, 44.2, 43.8, 42.9, 42.6, 34.2, 34.1, 29.6, 26.5, 26.46, 26.41, 26.3, 25.0, 24.98, 24.92, 24.89, 24.86, 24.7; ESI (*m/z*) 846.4

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[M^+ + Na, (100)]; Anal. Calcd for $C_{39}H_{57}N_3O_{16}$ C 56.86, H 6.97, N 5.10; found C 56.87, H 6.97, N 5.12.

■ ASSOCIATED CONTENT

Supporting Information

Characterization of all the new compounds not present in the Experimental Section and copies of ^{1}H NMR, ^{13}C NMR, and ESI-MS spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etzler, M. E. *Essentials of Glycobiology*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 2009. (b) Gabius, H. J.; Andre, S.; Jimenez-Barbero, J.; Romero, A.; Solis, D. From Lectin Structure to Functional Glycomics: Principles of the Sugar Code. *Trends Biochem. Sci.* **2012**, *36*, 298−313. (c) Lis, H.; Sharon, N. Lectins: Carbohydrate-Specific Proteins that Mediate Cellular Recognition. *Chem. Rev.* **1998**, *98*, 637−674.

(2) (a) Lee, Y. C.; Lee, R. T. Carbohydrate−Protein Interactions: Basis of Glycobiology. *Acc. Chem. Res.* **1995**, *28*, 321−327. (b) Lundquist, J. J.; Toone, E. J. The Cluster Glycoside Effect. *Chem. Rev.* **2002**, *102*, 555−578. (c) Bernardi, A.; Jimenez-Barbero, J.; Casnati, A.; De Castro, C.; Darbre, T.; Fieschi, F.; Finne, J.; Funken, H.; Jaeger, K.-E.; Lahmann, M.; Lindhorst, T. K.; Marradi, M.; Messner, P.; Molinaro, A.; Murphy, P. V.; Nativi, C.; Oscarson, S.; Penadés, S.; Peri, F.; Pieters, R. J.; Renaudet, O.; Reymond, J.-L.; Richichi, B.; Rojo, J.; Sansone, F.; Schaffer, C.; Turnbull, W. B.; Velasco-Torrijos, T.; Vidal, S.; Vincent, S.; Wennekes, T.; Zuilhofxy, H.; Imberty, A. Multivalent Glycoconjugates as Anti-Pathogenic Agents. *Chem. Soc. Rev.* **2013**, *42*, 4709−4727.

(3) Giannis, A. The Sialyl Lewis^x Group and its Analogue as Ligands for Selectins: Chemoenzymatic Syntheses and Biological Functions. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 178−180. (b) Musser, J. H.; Anderson, M. B. Fügedi. Glycomimetics: An approach to Discovery Leads for Novel Therapeutics. *Pharm. News* **1996**, *3*, 11−17. (c) Kolb, H. C.; Ernst, B. Recent Progresses in the Glycodrug Area. *Pure Appl. Chem.* **1997**, *69*, 1879−1884.

(4) (a) Kiessling, L. L.; Pohl, N. L. Strength in Number: Polyvalent Carbohydrate Derivatives. *Chem. Biol.* **1996**, *3*, 71−77. (b) Fasting, C.; Schalley, C. A.; Weber, M.; Seitz, O.; Hecht, S.; Koksch, B.; Dernedde, J.; Graf, C.; Knapp, E.-W.; Haag, R. Multivalency as a Chemical Organization and Action Principle. *Angew. Chem., Int. Ed.* **2012**, *51*, 10472−10498. (c) Pieters, R. J. Maximising Multivalency Effect in Protein−Carbohydrate Interaction. *Org. Biomol. Chem.* **2009**, *7*, 2013−2025.

(5) Mammen, M.; Choi, S.-K.; Whitesides, G. M. Polyvalent Interactions in Biological Systems: Implication for Design and Use of Multivalent Ligands and Inhibitors. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794.

(6) See for example: (a) Babine, R. E.; Bender, S. L. Molecular Recognition of Protein−Ligand Complexes: Application to Drug Design. *Chem. Rev.* **1997**, *97*, 1359−1472. (b) Chow, C. S.; Bogdan, F. M. A Structural Basis for RNA−Ligand Interactions. *Chem. Rev.* **1997**, *97*, 1489−1514.

(7) Barkley, A.; Arya, P. Combinatorial Chemistry toward Understanding the Function(s) of Carbohydrates and Carbohydrate Conjugates. *Chem.—Eur. J.* **2001**, *7*, 555−563.

(8) (a) Schreiber, S. L. Target-Oriented and Diversity-Oriented Organic Synthesis in Drug Discovery. *Science* **2000**, *287*, 1964−1969. (b) Spring, D. R. Diversity-Oriented Synthesis: A Challenge for Synthetic Chemists. *Org. Biomol. Chem.* **2003**, *1*, 3867−3870. (c) Burke, M. D.; Schreiber, S. L. A Planning Strategy for Diversity-Oriented Synthesis. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58. (d) Tan, D. S. Diversity-Oriented Synthesis: Exploring the Intersections between Chemistry and Biology. *Nat. Chem. Biol.* **2005**, *1*, 74−84. (e) Galloway, W. R. J. D.; Isidro-Llobet, A.; Spring, D. R. Diversity-Oriented Synthesis as a Tool for the Discovery of Novel Biologically Active Small Molecules. *Nat. Commun.* **2008**, *80*, 1−13.

(9) (a) Dömling, A.; Ugi, I. Multicomponent Reactions with Isocyanides. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168−3210. (b) Dömling, A. Recent Developments in Isocyanide Based Multicomponent Reactions in Applied Chemistry. *Chem. Rev.* **2006**, *106*, 17−89. (c) Touré, B. B.; Hall, D. G. Natural Product Synthesis Using Multicomponent Strategies. *Chem. Rev.* **2009**, *109*, 4439−4486. (d) Ganem, B. Strategies for Innovation in Multicomponent Reaction Design. *Acc. Chem. Res.* **2009**, *42*, 463−472. (e) Biggs-Houck, J. E.; Younai, A.; Shaw, J. T. Recent Advances in Multicomponent Reactions for Diversity-Oriented Synthesis. *Curr. Opin. Chem. Biol.* **2010**, *14*, 371−382.

(10) Lockhoff, O. An Access to Glycoconjugate Libraries through Multicomponent Reactions. *Angew. Chem., Int. Ed.* **1998**, *37*, 3436−3438.

(11) Westermann, B.; Dörner, S. Synthesis of Multivalent Aminoglycoside Mimics via the Ugi Multicomponent Reaction. *Chem. Commun.* **2005**, 2116−2118.

(12) (a) Bellucci, M. C.; Volonterio, A. Multicomponent Synthesis of Peptide−Sugar Conjugates Incorporating Hexafluorovaline. *Adv. Synth. Catal.* **2010**, *252*, 2791−2798. (b) Bellucci, M. C.; Ghilardi, A.; Volonterio, A. Three-Component, One-Pot Sequential Synthesis of Glyco-Hydantoin Conjugates. *Org. Biomol. Chem.* **2011**, *9*, 8379−8392. (c) Bellucci, M. C.; Terraneo, G.; Volonterio, A. Multi-Component Synthesis of Peptide−Sugar Conjugates. *Org. Biomol. Chem.* **2013**, *11*, 2421−2444. (d) Bellucci, M. C.; Volonterio, A. Synthesis of *N*-Glycosyl Conjugates through a Multicomponent Domino Process. *Eur. J. Org. Chem.* **2014**, 2386−2397.

(13) For more detail on the mechanism of the process, see also: (a) Volonterio, A.; Ramirez de Arellano, C.; Zanda, M. Synthesis of 1,3,5-Trisubstituted Hydantoins by Regiospecific Domino Condensation/Aza-Michael/O → N Acyl Migration of Carbodiimides with Activated α,β-Unsaturated Carboxylic Acids. *J. Org. Chem.* **2005**, *70*, 2161−2170. (b) Marcelli, T.; Olimpieri, F.; Volonterio, A. Domino Synthesis of 1,3,5-Trisubstituted Hydantoins: A DFT Study. *Org. Biomol. Chem.* **2011**, *9*, 5156−5161. (c) Olimpieri, F.; Bellucci, M. C.; Marcelli, T.; Volonterio, A. Regioselective Multicomponent Sequential Synthesis of Hydantoins. *Org. Biomol. Chem.* **2012**, *10*, 9538−9555.

(14) The process works also starting from not protected glycosylamines. However, since these compounds are not very soluble in CH_3CN, the yield are low and the process leading to the formation of the corresponding hydantoin rings (Scheme 1, path A) becomes predominant.

(15) We always obtained a mixture of diastereoisomers with diastereoselectivities varying between 3.0:1.0 to 1.5:1.0 depending on the sugar present in the carbodiimide framework. See also ref 12.

(16) When *N*-glycosylamines were used as nucleophiles, because of their low solubility in CH_3CN, a 4:1 mixture of CH_3CN/DMF was used as solvent, and ∼20% of the corresponding hydantoin ring (Scheme 1, path (A) was recovered (see also ref 12d).

(17) (a) Tor, Y.; Hermann, T.; Westhof, E. Deciphering RNA Recognition: Aminoglycoside Binding to the Hammerhead Ribozyme. *Chem. Biol.* **1998**, *5*, R277−R283. (b) Tor, Y. Targeting RNA with Small Molecules. *ChemBioChem.* **2003**, *4*, 998−1007. (c) Houghton, J. L.; Green, K. D.; Chen, W.; Garneau-Tsodikova, S. The Future of Aminoglycosides: The End or Renaissance? *ChemBioChem.* **2010**, *11*,

880−902. (d) McCoy, L. S.; Xie, Y.; Tor, Y. Antibiotics that Target Protein Synthesis. *Wiley Interdiscip. Rev. RNA* **2011**, *2*, 209−232.
(18) (a) Luedtke, N. W.; Liu, Q.; Tor, Y. RNA−Ligand Interactions: Affinity and Specificity of Aminoglycoside Dimers and Acridine Conjugates to the HIV-1 Rev Response Element. *Biochemistry* **2003**, *42*, 11391−11403. (b) Fridman, M.; Belakhov, V.; Yaron, S.; Baasov, T. A New Class of Branched Aminoglycosides: Pseudo-Pentasaccharide Derivatives of Neomycin B. *Org. Lett.* **2003**, *5*, 3575−3578. (c) Blount, K. F.; Tor, Y. A Tale of Two Targets: Differential RNA Selectivity of Nucleobase−Aminoglycoside Conjugates. *ChemBioChem.* **2006**, *7*, 1612−1621. (d) Jeon, J.; Lee, K. H.; Rao, J. Enhance the Binding Affinity of Fluorophore−Aptamer Pairs for RNA Tagging with Neomycin Conjugation. *Chem. Commun.* **2012**, *48*, 10034−10036. (e) Kumar, S.; Xue, L.; Arya, D. P. Neomycin−Neomycin Dimer: An All-Carbohydrate Scaffold with High Affinity for AT-Rich DNA Duplexes. *J. Am. Chem. Soc.* **2010**, *133*, 7361−7375.
(19) Neomycin derivative **13** is very soluble in the reaction condition, namely CH_3CN at 0 °C, thus the acetylation of the hydroxyl groups was not necessary to obtain high yield.
(20) Any attempt to obtain deacetylation of glucose with MeONa resulted in the formation of a mixture of products.
(21) Garcia-Fernandez, J. M.; Ortiz Mellet, C.; Fuentes, J. Chiral 2-Thioxotetrahydro-1,3-O,N-Heterocycles from Carbohydrates. 2. Stereocontrolled Synthesis of Oxazolidine Pseudo-C-Nucleosides and Bicyclic Oxazine-2-thiones. *J. Org. Chem.* **1993**, *58*, 5192−5199.