Multicomponent diversity oriented synthesis of multivalent glycomimetics containing hexafluorovaline

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1. Introduction

The introduction of a fluorine atom or perfluorinated groups into chemical scaffolds is now a standard strategy for modulating the chemical and biophysical properties of molecular leads and for studying the biological aspects associated with the fluorine substitution/introduction, such as metabolism, excretion properties, and fluorine-containing ligand binding interactions.[1] This is due to the unique properties of the fluorine atom, its strongest inductive effect compared to other elements, its small size, and low polarizability. For instance, the highly fluorinated amino acids hexafluorovaline (hfVal) and hexafluoroleucine (hfLeu), being often well tolerated, have been used to modulate such properties of peptides and proteins like their hydrophobicity, acidity/basicity, folding and stability.[2] Moreover, ^{19}F is the most stable and abundant fluorine isotope, thus fluorinated peptides and proteins can be easily tracked with NMR and/or MRI spectroscopy, even in living cells.[3] Thus, since fluorine containing organic molecules are almost absent in nature,[4] there is great interest in developing new synthetic strategies to introduce selectively fluorinated moieties into chemical scaffolds.

Multivalent glycomimetics are a class of very intriguing compounds.[5] Indeed, there is a growing interest in glycosidase inhibition, and in elucidating fundamental biological pathways through the synthesis of glycomimetics. Multivalency can increase dramatically the binding potency of the corresponding monovalent ligands as already demonstrated.[6] However, despite such interest, compared to monovalent glycomimetics, there are relatively few example in the literature of multivalent glycosidase inhibitors, and no fluorinated multivalent glycosidase inhibitors, probably because they are not synthetically accessible in an easy way.[6] One strategy to overcome such a deficiency could be the development of straightforward methodologies for the diversity oriented synthesis (DOS) of these compounds.[7] Indeed, DOS, often combined with multicomponent reactions (MCRs), is an indispensable tool for modern pharmaceutical and drug discovery research programs because they offer the possibility to access molecular complexity with a minimum of effort, in addition to atom economy, operational simplicity, and bond-forming efficiency.[8] This is well evidenced by the growing application of such strategies for the synthesis of libraries of different collections of molecules. However, to the best of our knowledge, there are no examples of DOS of multivalent, selectively fluorinated glycomimetics.[9]

Very recently, in the frame of a research program dealing with the discovery of novel MCRs for the synthesis of heterocycles[10] and glycoconjugates,[11] we have reported for the first time the MC combinatorial synthesis of diversity oriented multifunctional glycomimetics **1**–**4**, where the sugar moieties were tethered through artificial linkers such as aspartic acid, a hydantoin ring, or urea

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frameworks (Fig. 1).[12] Herein we wish to report an extension of such process, using commercially available 4,4,4-trifluoro-3-trifluoromethyl(Tfm)-crotonic acid in order to prepare multifunctional glycomimetics where the Asp moiety of **1**–**4** is substituted by hfVal.

Since this process is high yielding, totally regioselective and operationally very simple (MC domino process which does not require high temperatures, dry solvents, difficult purification steps), we wished to exploit it for the synthesis of multivalent glycomimetics containing hfVal by using *N*-glycosyl amines as *N*-

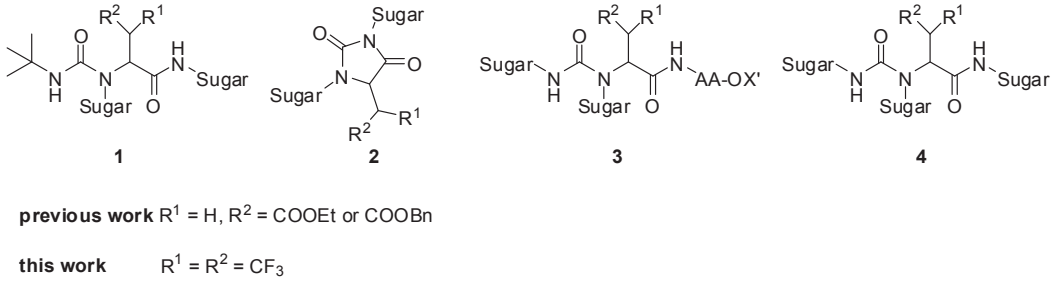


Fig. 1. Multivalent glycomimetics containing hfVal.

We propose that the introduction of hfVal in the multivalent glycomimetic scaffolds can impart interesting new properties to this intriguing class of compounds such as, for instance, higher metabolic stability and lipophilicity and would facilitate the investigation of target-ligand interactions through ^{19}F NMR spectroscopy.

2. Results and discussion

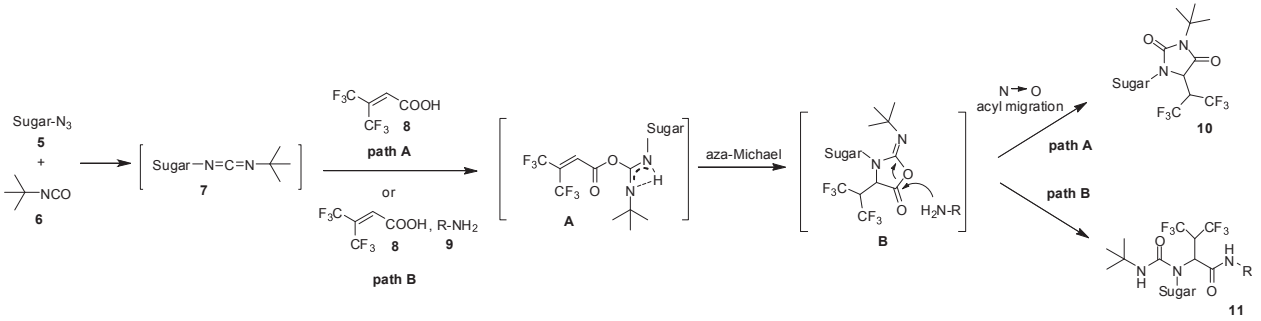
4,4,4-Trifluoro-3-Tfm-crotonic acid is a commercially available fluorinated building block that, due to the high electronegativity of the trifluoromethyl groups, has been used for the synthesis of hfVal amino acid derivatives through *anti*-Michael addition of *N*-nucleophiles.[13] Recently, our group has exploited the reactivity of 4,4,4-trifluoro-3-Tfm-crotonic acid **8** for the synthesis of glycoconjugates containing hfVal **10** and **11** through the MC sequential domino process depicted in Scheme 1.[10,11] The reaction of **8** with in situ generated *N*-glycosyl, *N*′-*tert*-butyl carbodiimides **7** pro-duces intermediate **A**, which undergoes intramolecular *anti*-aza-Michael reaction affording a second highly reactive intermediate **B**. The latter step is totally regioselective when the steric hin-drance of the two *N*-substituents in the carbodiimide framework is very different, namely a primary versus a tertiary alkyl group. In the absence of an *N*-nucleophile, **9** intermediate **B** rearranges through an O→N acyl migration process affording hydantoin scaffold **10** containing a hfVal moiety (path A, Scheme 1).[14] In-stead, in the presence of *N*-nucleophile **9**, such as amines, amino esters, or peptides, intermediate **B** undergoes nucleophilic attack leading to the formation of urea-hfVal-glycopeptides **11** (path B, Scheme 1).

nucleophiles, and/or by introducing a new sugar moiety in the carbodiimide framework.

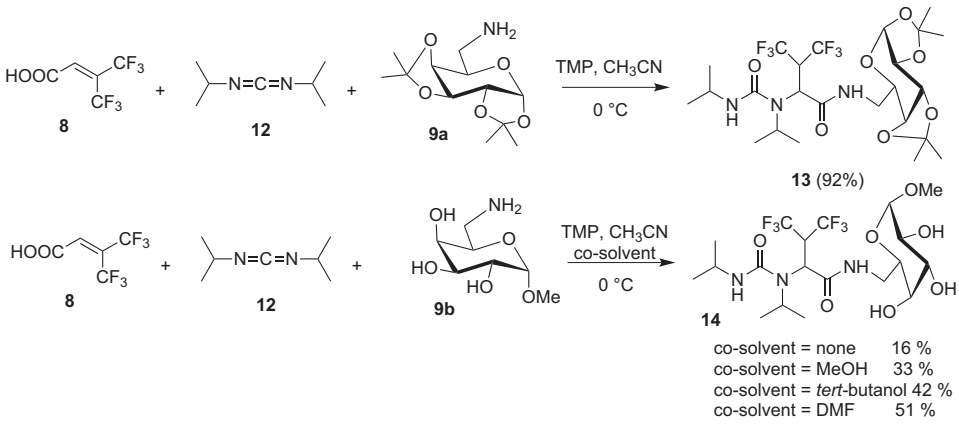
Firstly, in order to be able to prepare divalent glycomimetics **1**, we checked if the MC component process works efficiently also by using *N*-glycosyl amines as nucleophiles. Thus we reacted *O*-protected amino-galactose **9a** with acid **8** and DIC **12** under the standard conditions (CH_3CN as solvent, 0 °C) and we were delighted to recover glycomimetic **13** in very good yield as an equimolar mixture of epimers at the hexafluorovaline stereocentre (Scheme 2).[15]

We explored also the possibility of running the process with the corresponding deprotected amino-galactose **9b**. However, probably because of its low solubility in the reaction medium (CH_3CN, 0 °C), the reaction produced the desired product **14** in very low yield (16%) along with the formation of the undesired hydantoin in 76% yield (see Scheme 1, path A). We tried to raise the yield of the process by adding a co-solvent in order to increase the solubility of the nucleophile. Indeed, in the presence of 10% MeOH, the yields were raised only to a 33% due to the concomitant formation of the corresponding urea-hfVal-OMe derivative arising from the competitive nucleophilic attack of MeOH which is present in large excess (Scheme 1, path B). Thus we tried with less nucleophilic *tert*-BuOH but without a real improvement (42% yield). Disappointingly, even in the presence of highly polar DMF the yields were raised only to 51% (Scheme 2).

Once proved that the reaction works efficiently even with *O*-protected *N*-glycosyl amines as nucleophiles in the standard conditions, we applied the process for the four-component sequential synthesis of a library of divalent glycoconjugates containing hfVal **1** starting from different *N*-*tert*-butyl, *N*′-glycosyl carbodiimides **7**, which are formed in situ by Staudinger reaction between sugar azides **5** and *tert*-butyl isocyanate **6**, and *N*-glycosyl nucleophiles **9** (Table 1).

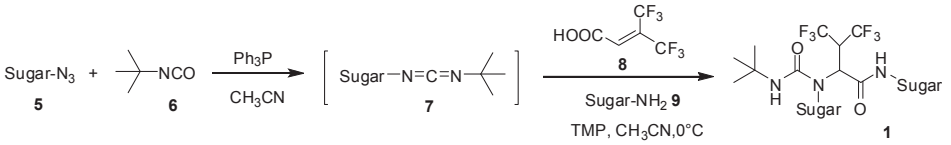


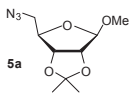
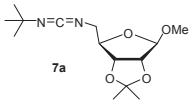
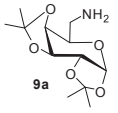
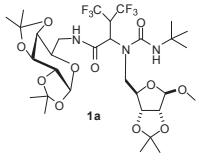
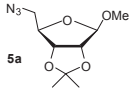
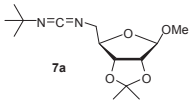
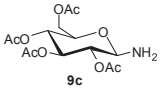
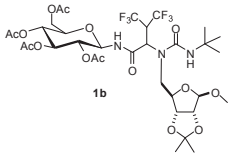
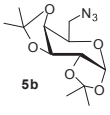
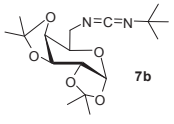
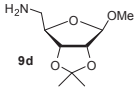
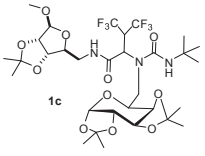
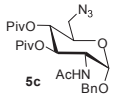
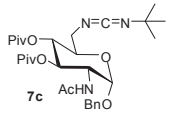
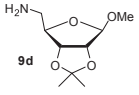
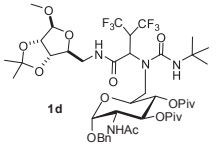
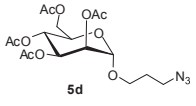
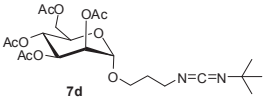
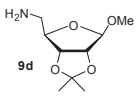
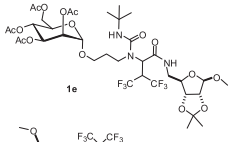
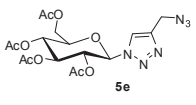
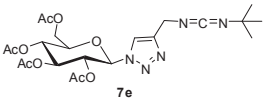
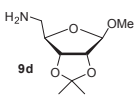
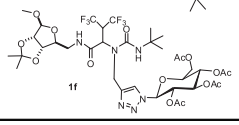
Scheme 1. Mechanism of the domino MC process.



Scheme 2. MC process with glycosylamine as nucleophile.

Table 1
MC combinatorial synthesis of multivalent glycomimetics **1**



Entry	Sugar-N_3	Carbodiimide	Sugar-NH_2	Product	Yield (%)[a]
1					81[b]
2					53[b,d]
3					78[c]
4					68[b]
5					83[b]
6					76[b]

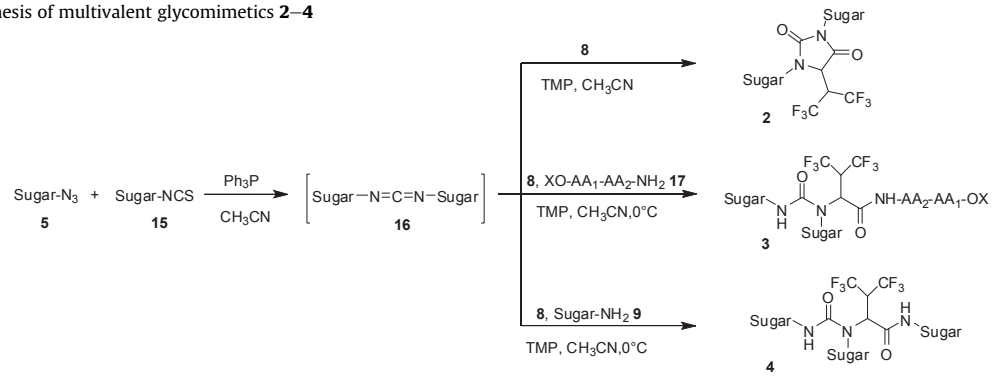
[a] Isolated yields.
[b] An almost 3:1 diastereoisomeric mixture.
[c] An almost 1.5:1 diastereoisomeric mixture.
[d] A 30% of the corresponding hydantoin was recovered.

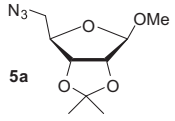
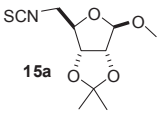
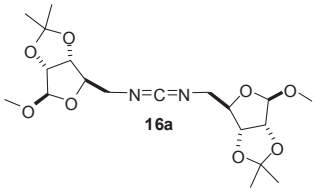
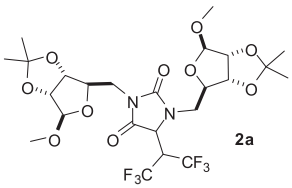
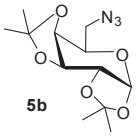
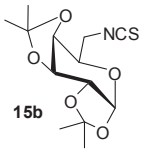
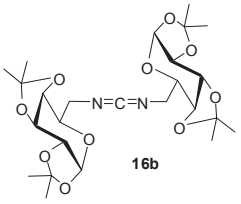
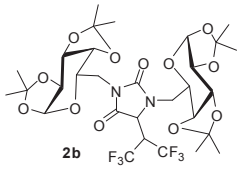
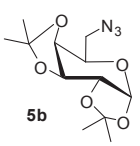
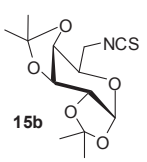
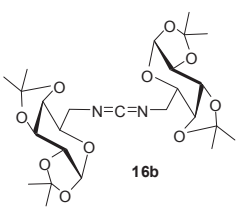
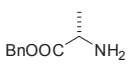
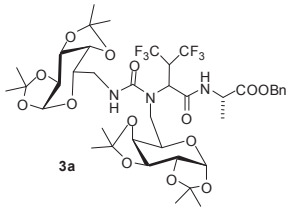
Indeed, the reaction between glycosyl azides **5a**–**e** and commercially available *tert*-butyl isocyanate **6** lead to the formation of the corresponding carbodiimides **7a**–**e**, respectively, upon treatment with Ph_3P in CH_3CN at rt (Staudinger reaction). The resulting carbodiimides **7a**–**e** can also be isolated by quick flash chromatography and characterized. However, compounds **7a**–**e** can be used in situ in order to achieve a four component sequential process. In this case, once the carbodiimide is formed (TLC monitoring), the temperature is cooled to 0 °C and 2,4,6-trimethylpyridine (TMP) followed by nucleophile **9** and, finally, acid **8** are added. The process was very general, working efficiently either starting from sugar azides (where the functional group is attached to pri-mary carbon 6 in hexoses or carbon 5 in pentoses) leading to the formation of multivalent glycomimetics having enzymatically sta-ble 'CH_2NH_2' backbone mimicking glycine (entries 1–4, Table 1),[16] or attached to the anomeric carbon through a suitable linker (entries 5 and 6, Table 1). In all cases the reaction was completely regioselective leading to the formation of regioisomers **1a**–**f** as a mixture of diastereoisomers, which arose from the nucleophilic attack of the less sterically congested primary *N*-glycosyl moiety in the intramolecular *anti*-aza-Michael step (Scheme 1, path B).[17] The yields were generally high except when glycosylamine **9c** was used as nucleophile (entry 2, Table 1). This result was due to the poor nucleophilicity of the hemiacetalic amino group and scarce solubility of **9c** as already stated in previous works.[11d] In this way we were able to obtain a small library of six divalent glycomimetics where the sugar moieties are connected through a urea-hfVal linker. The reaction is very general, and any kind of appropriately functionalized carbohydrates could be used, such as ribose, galactose, glucose, glucosamine, and mannose.

In order to obtain other multivalent glycomimetics containing hfVal, where the sugar moieties are connected with different scaffolds (DOS), we tested the same reaction starting with carbodiimides having two *N*-sugar substituents. In this way, considering the synthetic pathway depicted in Scheme 1, we would be able to obtain three new multivalent glycomimetics, i.e., divalent glycomimetics **2**, **3** where the carbohydrates are connected through a hydantoin scaffold or a urea linker, respectively, and trivalent glycomimetic **4** where the sugar moieties are tethered through a urea-hfVal scaffold. *N,N′*-Disugar carbodiimides **16** could be prepared starting from the corresponding sugar azides **5** and sugar isothiocyanates **15** (Table 2).

Accordingly, Staudinger reaction between ribose azide **5a** and isothiocyanate **15a** lead to the clean formation of carbodiimide **16a** along with Ph_3PS (TLC monitoring). By adding in situ TMP followed by acid **8**, we obtained the formation of divalent glycomimetic **2a**, as an almost 1.5:1 mixture of diastereoisomers, where the sugar moieties are connected through a hydantoin ring (entry 1, Table 2). Under the same conditions we obtained the formation of diglycohydantoin **2b** in very high yields starting with galactose azide **5b**

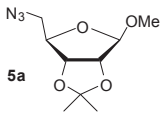
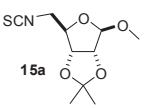
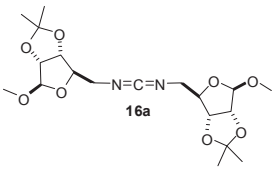
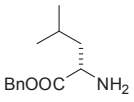
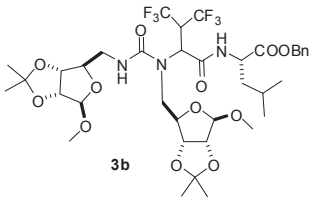
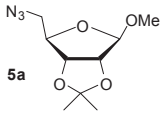
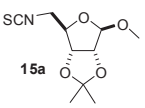
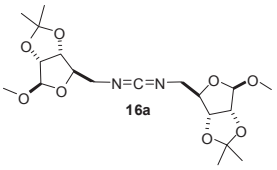
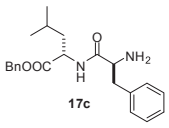
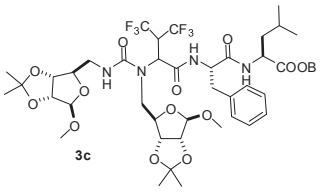
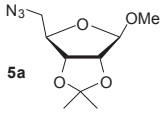
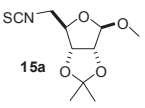
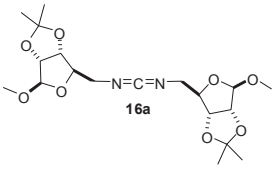
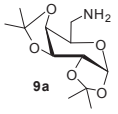
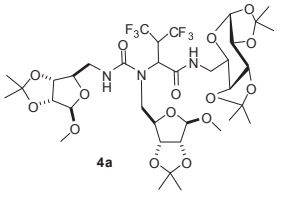
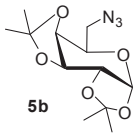
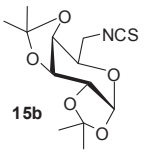
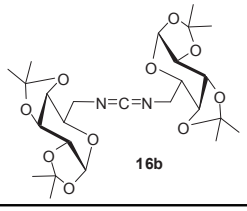
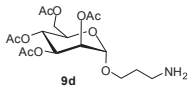
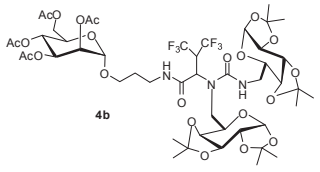
Table 2
MC combinatorial synthesis of multivalent glycomimetics **2**–**4**



Entry	Sugar-N_3	Sugar-NCS	Carbodiimide	Sugar-NH_2	Product	Yield (%)[a]
1	 **5a**	 **15a**	 **16a**	/	 **2a**	78[b]
2	 **5b**	 **15b**	 **16b**	/	 **2b**	82[c]
3	 **5b**	 **15b**	 **16b**	 **17a**	 **3a**	75[c]

(*continued on next page*)

Table 2 (*continued*)

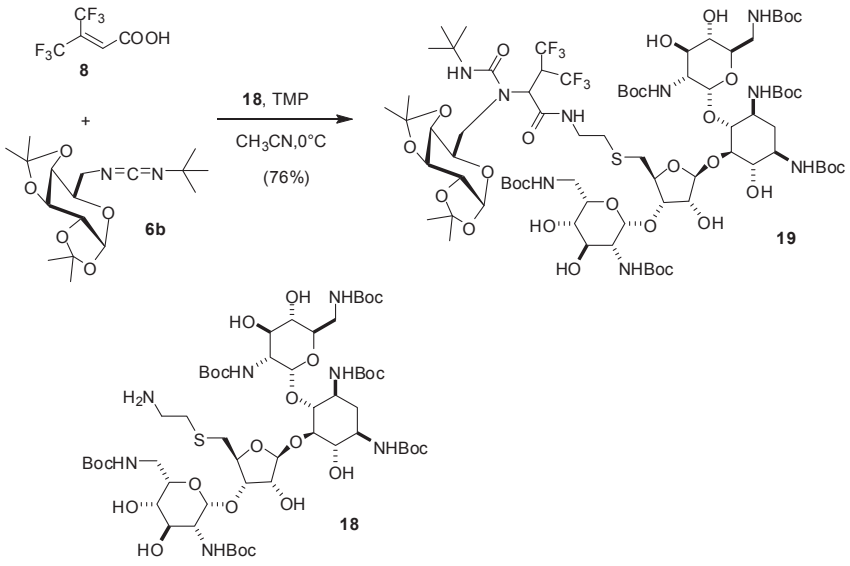
Entry	Sugar-N_3	Sugar-NCS	Carbodiimide	Sugar-NH_2	Product	Yield (%)[a]
4	 **5a**	 **15a**	 **16a**	 **17b**	 **3b**	72[b]
5	 **5a**	 **15a**	 **16a**	 **17c**	 **3c**	76[b]
6	 **5a**	 **15a**	 **16a**	 **9a**	 **4a**	62[b]
7	 **5b**	 **15b**	 **16b**	 **9d**	 **4b**	64[c]

[a] Isolated yields.
[b] An almost 1.5:1 diastereoisomeric mixture.
[c] An almost 3:1 diastereoisomeric mixture.

and isothiocyanate **15b** (entry 2, Table 2). Interestingly, the reaction carried out in the presence of α-amino esters nucleophiles such as H-Ala–OBn **17a** and H-Leu–OBn **17b** furnished divalent glycodipeptides **3a,b**, respectively (entry 3 and 4, Table 2). The latter reaction is particularly promising because it permits the unprecedented multicomponent synthesis of a class of very intriguing scaffolds, i.e., divalent glycopeptides incorporating the unnatural amino acid hfVal. Moreover, the process works very efficiently also starting from dipeptide nucleophiles such as H-Phe-Leu–OBn **17c** leading to the one pot formation of divalent glycotripeptides containing hfVal **3c** in high yields (entry 5, Table 2). Finally, in order to demonstrate that this process could also be exploited for the preparation of more complicated trivalent glycomimetics, we tested the reaction starting with in situ generated symmetric *N,N′*-diglyco carbodiimide **16a,b** and sugar nucleophile **9a,d**, respectively. We were delighted that in this case the reaction also works effectively giving rise to the formation of trivalent glycomimetics **4a,b** in good yields (entries 6 and 7, Table 2). It is worth noting that, in order to increase complexity, the synthesis of glycomimetics **2**–**4** could be also achieved starting with asymmetric *N,N′*-diglyco carbodiimides. However, even if high yielding, due to the similarity on the steric hindrance of the *N*-substituents, the process lead to the formation of mixtures of regioisomers that are difficult to separate (data not shown).

Beside the synthesis of multivalent glycomimetics **1**–**4**, this process could be exploited as a general procedure for the introduction of sugar-hfVal tags into biologically relevant molecules in a simple and convenient way. With this in mind, we sought to functionalize aminoglycoside antibiotics. Aminoglycosides are a class of polyaminosugars, which selectively bind RNA.[18] The selective functionalization of such scaffolds has been exploited for the synthesis of conjugates with better characteristics, such as, for instance, activity, selectivity, and ability to fight against aminoglycosides resistance.[19] In this sense, the introduction of fluorinated probes in the aminoglycoside scaffolds could facilitate the investigation of RNA-ligand interaction through ^{19}F NMR spectroscopy.[20] However, besides the interest in the development of new practical ways to selectively functionalize aminoglycosides, there are only a few examples that use a multicomponent one-pot procedure, and, to the best of our knowledge, none of them are used for tethering aminoglycosides with fluorinated tags.[21] Thus, the successful application of our process to aminoglycoside chemistry would provide a general way to selectively functionalize such molecules with other scaffolds (in this case carbohydrates) along with a fluorinated tag (hfVal) with a practical one pot multicomponent procedure. Accordingly, we decided to use neomycin derivative **18** as nucleophile in our multicomponent process. To our delight, reacting carbodiimide **6b** in the presence of acid **8** and neomycin derivative **18** under the same conditions described above, i.e., TMP, CH_3CN at 0 °C, we obtained the clean regiospecific formation of conjugate **19** in very good yield, as a mixture of diastereoisomers (Scheme 3).[22]

The application of this procedure for the combinatorial synthesis of different aminoglycoside conjugates bearing a hfVal probe will be studied more in detail and reported in a forthcoming paper.



Scheme 3. Synthesis of neomycin-hfVal-galactose conjugate **19**.

3. Conclusions

In conclusion, we have demonstrated that the MC sequential process recently developed by us can also be for the DOS of multivalent glycomimetics containing hfVal amino acid. Indeed, the reaction between in situ generated *N*-glycosyl, *N*′-*tert*-butyl carbodiimides or *N*, *N*′-diglycosyl carbodiimides with 4,4,4-trifluoro-3-Tfm-crotonic in the presence or absence of *N*-nucleophiles, such as α-aminoesters, peptides or glycosyl amines, gave rise to the clean formation of di- or trivalent glycomimetics containing hfVal where the glycosyl moieties are tethered trough different scaffolds, such as urea, hydantoin ring, and urea-peptide linkers. The MC sequential domino process is completely regioselective, high yielding, occurs under mild conditions, and is very general, working efficiently with different sugars and linkers. This process could also be exploited as a favourable tool to conjugate biological important compounds with a sugar-hfVal tag. For instance, the reaction performed using an appropriately functionalized neomycin derivative such as *N*-nucleophile allowed us to obtain the synthesis of a neomycin-hfVal conjugate in a very efficient way. The combinatorial synthesis of different aminoglycoside-hfVal-sugar conjugates and the determination of their antibacterial activity and RNA interaction trough ^{19}F NMR spectroscopy is ongoing in our laboratories and will be reported in a forthcoming paper.

4. Experimental section

4.1. General methods

Commercially available reagent-grade solvents were employed without purification. Primary glycosylazides **5** were prepared following reported procedures.[11] Glycosylisothiocyanate **15** were prepared by aza-Wittig reaction with CS_2.[23] Glycosylamines **9** were prepared by catalytic hydrogenation of the corresponding azides. Neomycin derivative **18** was obtained as reported in Ref. 19a. ^1H NMR spectra were run on spectrometers operating at 400 or 500 MHz. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^1H and ^{13}C nuclei (δ_H and δ_C=0.00). ESIMS was performed with an Esquire 3000 plus ion-trap mass spectrometer equipped with an ESI source. The IR spectra were obtained by a Varian 640 high-performance FTIR spectrometer. Elemental analysis were obtained on FlashEA 1112 NC Analyzers. TLC was run on silica gel 60 F254 Merck. Flash chromatography (FC) was performed with silica gel 60 (60–200 mm, Merck).

4.2. Synthesis of divalent glycomimetics 1: general procedure

To a solution of glycosylazide **5** (1 equiv) in CH_3CN (0.1 M) *tert*-butyl isocyanate (1.05 equiv) **6** followed by Ph_3P (1.05 equiv) were added at rt. The solution was stirred until complete formation of the corresponding carbodiimide **7** 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 4,4,4-trifluoro-3-Tfm-crotonic 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, ca. 3 h), was quenched with a 1M solution of HCl. The mixture was extracted with AcOEt, the organic phases collected, dried over Na_2SO_4, filtered, the solvent removed under reduced pressure and the crude purified by flash chromatography.

1a: *Major diastereoisomer*: R_f=0.29 (hexane/AcOEt 80:20); FTIR (neat) ν 1779, 1756, 1726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ: 8.44 (br s, 1H), 5.49–5.46 (m, 2H), 5.11 (s, 1H), 4.63 (d, J=5.6 Hz, 1H), 4.59 (dd, J=8.0 and 2.4 Hz, 2H), 4.53 (d, J=5.6 Hz, 1H), 4.38–4.36 (m, 1H), 4.29 (dd, J=5.2 and 2.4 Hz, 1H), 3.98–3.96 (m, 1H), 3.89–3.86 (m, 1H), 3.47 (s, 3H), 3.33–3.31 (m, 1H), 2.90–2.88 (m, 1H), 1.49 (s, 3H), 1.49 (s, 3H), 1.47 (s, 3H), 1.41 (s, 3H), 1.35 (s, 3H), 1.32 (s, 3H), 1.30 (s, 12H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 168.3, 154.9, 123.1 (q, J=281.7 Hz, CF_3), 112.6, 111.5, 109.4, 108.5, 96.2, 87.0, 84.4, 71.4, 70.8,70.5, 70.4, 56.0, 51.4, 45.9 (septet, J=25.8 Hz), 40.4, 29.0, 26.3, 25.9, 25.7, 24.9, 24.7; ESI (m/z) 774.7 [M^++Na, (100)]; Anal. calcd for $C_{31}H_{47}F_6N_3O_{11}$: C 49.53, H 6.30, N 5.59; found: C 49.53, H 6.32, N 5.60. *Minor diastereoisomer*: R_f=0.22 (hexane/AcOEt 80:20); FTIR (neat) ν 1781, 1754, 1722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ: 7.12 (br s, 1H), 5.77 (s, 1H), 5.49 (d, J=5.2 Hz, 2H), 5.11 (s, 1H), 4.98 (s, 1H), 4.60–4.56 (m, 2H), 4.41–4.39 (m, 1H), 4.32–4.28 (m, 2H), 4.15 (dd, J=8.0 and 2.0 Hz,1H), 3.97–3.94 (m, 1H), 3.47–3.44 (m, 3H), 3.41 (s, 3H), 3.24 (dd, J=16.4 and 5.2 Hz 1H), 1.49 (s, 3H), 1.49 (s, 3H), 1.47 (s, 3H), 1.45 (s, 3H), 1.35 (s, 9H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 168.0, 157.0, 123.3 (q, J=281.5 Hz, CF_3), 112.9, 110.3, 109.4, 108.7, 96.3, 85.8, 84.5, 82.1, 71.3, 70.9, 70.5, 65.4, 55.5, 51.5, 46.9 (s, J=26.1 Hz), 40.2, 29.0, 26.5, 26.0, 25.9, 25.1, 24.9. 24.4;

ESI (m/z) 774.6 [M^++Na, (100)]; Anal. calcd for $C_{31}H_{47}F_6N_3O_{11}$: C 49.53, H 6.30, N 5.59; found: C 49.55, H 6.31, N 5.58.

4.3. Synthesis of divalent glycomimetics 2: general procedure

To a solution of glycosylazide **5** (1 equiv) in CH_3CN (0.1 M) a solution of glycosylisothiocyanante **15** (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 **16** was achieved (TLC monitoring). A solution of 4,4,4-trifluoro-3-Tfm-crotonic acid **8** (1 equiv) in a minimum amount of CH_3CN was added at rt and the resulting solution stirred until the reaction was finished (TLC monitoring, ca. 3 h). The reaction was quenched with a 1M solution of HCl. The mixture was extracted with AcOEt, the organic phases collected, dried over Na_2SO_4, filtered, the solvent removed under reduced pressure and the crude purified by flash chromatography.

2a: *Mixture of two diastereoisomers*: R_f=0.24 (hexane/AcOEt 80:20); FTIR (neat) ν 1777, 1754, 1728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *major diastereoisomer* δ: 4.96 (s, 2H), 4.75 (s, 1H), 4.63–4.61 (m, 4H), 4.34–4.32 (m, 2H), 4.09 (dd, J=12.0 and 4.8 Hz, 1H), 3.95–3.92 (m, 1H), 3.71–3.68 (m, 2H), 3.36 (s, 3H), 3.35 (s, 3H), 3.12 (dd, J=12.0 and 6.4 Hz, 1H), 1.44 (s, 3H), 1.43 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); *minor diastereoisomer* δ: 4.95 (s, 2H), 4.75 (s, 1H), 4.63–4.61 (m, 4H), 4.34–4.32 (m, 1H), 4.31–4.29 (m, 1H), 3.95–3.92 (m, 2H), 3.71–3.68 (m, 2H), 3.40–3.37 (m, 1H), 3.37 (s, 3H), 3.32 (s, 3H), 1.48 (s, 3H), 1.47 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 169.0, 156.5, 156.0, 113.1, 112.5, 110.5, 110.2, 110.0, 109.8, 85.2, 85.0, 84.6, 83.8, 83.4, 83.3, 82.3, 82.2, 82.1, 81.9, 56.0, 55.5, 55.4, 55.3, 55.1, 48.8 (septet, J=28.2 Hz), 45.9, 44.7, 42.9, 42.7, 29.6, 26.6, 26.5, 26.4, 26.3, 25.1, 25.0, 24.9, 24.8; ESI (m/z) 645.2 [M^++Na, (100)]; Anal. calcd for $C_{24}H_{32}F_6N_2O_{10}$: C 46.31, H 5.18, N 4.50; found: C 46.33, H 5.20, N 4.53.

4.4. Synthesis of divalent glycomimetics 3: general procedure

To a solution of glycosylazide **5** (1 equiv) in CH_3CN (0.1 M) a solution of glycosylisothiocyanante **15** (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 **16** was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (2 equiv), solid α-aminoester hydrochloride **17** (1 equiv) followed by a solution of 4,4,4-trifluoro-3-Tfm-crotonic 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, ca. 3 h), was quenched with a 1M solution of HCl. The mixture was extracted with AcOEt, the organic phases collected, dried over Na_2SO_4, filtered, the solvent removed under reduced pressure and the crude purified by flash chromatography.

3a: *Major diastereoisomer*: R_f=0.34 (hexane/AcOEt 70:30); FTIR (neat) ν 1769, 1753, 1723 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ: 8.11 (br s, 1H), 7.38–7.35 (m, 5H), 6.57 (s, 1H), 5.48 (d, J=4.3 Hz, 1H), 5.44 (d, J=4.2 Hz, 1H), 5.18 (d, J=12.1 Hz, 1H), 5.11 (d, J=12.1 Hz, 1H), 4.78 (br s, 1H), 4.65–4.56 (m, 3H), 4.31–4.28 (m, 2H), 4.25–4.22 (m, 2H), 4.11 (q, J=7.0 Hz, 1H), 3.40–3.38 (m, 4H), 1.47 (s, 6H), 1.45 (s, 3H), 1.43 (s, 3H), 1.36 (s, 6H), 1.33 (s, 3H), 1.30–1.26 (m, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 172.5, 167.5, 160.0, 136.0, 128.9, 128.6, 128.4, 123.7 (q, J=282.2 Hz), 123.2 (q, J=282.2 Hz), 110.0, 109.6, 109.4, 109.0, 96.6, 71.8, 71.3, 71.2, 70.9, 70.7, 67.2, 66.4, 65.4, 48.8, 47.4 (septet, J=26.5 Hz), 41.6, 26.4, 26.3, 26.2, 26.0, 25.3, 25.2, 24.9, 24.6, 17.9; ESI (m/z) 936.3 [M^++Na, (100)]; Anal. calcd for $C_{40}H_{53}F_6N_3O_{14}$: C 52.57, H 5.85, N 4.60; found: C 52.58, H 5.82, N 4.62. *Minor diastereoisomer*: R_f=0.30 (hexane/AcOEt 70:30); FTIR (neat) ν 1770, 1761, 1721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ: 7.65 (br s, 1H), 7.38–7.35 (m, 5H), 6.34 (br s, 1H), 5.49 (d, J=5.08 Hz, 1H), 5.47 (d, J=5.03 Hz, 1H), 5.18 (d, J=12.1 Hz, 1H), 5.11 (d, J=12.1 Hz, 1H), 4.56–4.54 (m, 2H), 5.45 (septet, J=7.0 Hz, 1H), 4.30–4.26 (m, 2H), 4.22–4.19 (m, 2H), 3.97–3.94 (m, 1H), 3.85–3.83 (m, 1H), 3.55–3.54 (m, 1H), 3.23–3.21 (m, 2H), 1.44 (s, 6H), 1.42 (s, 3H), 1.36 (s, 6H), 1.33 (s, 3H), 1.13 (s, 6H), 1.29 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 171.9, 168.0, 135.9, 128.9, 128.7, 128.5, 123.5 (q, J=281.0 Hz), 123.3 (q, J=282.2 Hz), 109.8, 109.6, 109.5, 108.9, 96.7, 96.6, 96.5, 72.2, 71.9, 71.3, 70.94, 70.91, 69.2, 67.3, 67.2, 49.4, 46.6 (septet, J=26.7 Hz), 41.5, 26.4, 26.3, 26.1, 25.3, 24.8, 24.5; ESI (m/z) 936.2 [M^++Na, (100)]; Anal. calcd for $C_{40}H_{53}F_6N_3O_{14}$: C 52.57, H 5.85, N 4.60; found: C 52.59, H 5.86, N 4.58.

4.5. Synthesis of trivalent glycomimetics 4: general procedure

To a solution of glycosylazide **5** (1 equiv) in CH_3CN (0.1 M) a solution of glycosylisothiocyanante **15** (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 **16** was achieved (TLC monitoring). The temperature was lowered to 0 °C and TMP (2 equiv), a solution of glycosylamine **9** (1 equiv) followed by a solution of 4,4,4-trifluoro-3-Tfm-crotonic 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, ca. 3 h), was quenched with a 1M solution of HCl. The mixture was extracted with AcOEt, the organic phases collected, dried over Na_2SO_4, filtered, the solvent removed under reduced pressure and the crude purified by flash chromatography.

4a: *Mixture of two diastereoisomers*: R_f=0.21 (hexane/AcOEt 20:80); FTIR (neat) ν 1778, 1755, 1721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *major diastereoisomer* δ: 6.01 (br s, 1H), 5.46 (d, J=5.1 Hz, 1H), 5.14 (s, 1H), 4.97 (s, 1H), 4.62–4.54 (m, 6H), 4.18–4.15 (m, 3H), 4.14–4.11 (m, 2H), 3.91–3.90 (m, 1H), 3.84 (d, J=9.2 Hz, 1H), 3.78–3.76 (m, 1H), 3.53–3.50 (m, 2H), 3.42 (s, 3H), 3.37 (s, 3H), 3.25–3.22 (m, 2H), 3.08–3.05 (m, 1H), 1.48 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H); *minor diastereoisomer* δ: 6.38 (br s, 1H), 5.49 (d, J=5.2 Hz, 1H), 5.14 (s, 1H), 4.96 (s, 1H), 4.62–4.54 (m, 6H), 4.18–4.15 (m, 3H), 4.14–4.11 (m, 2H), 3.91–3.90 (m, 1H), 3.84 (d, J=9.2 Hz, 1H), 3.78–3.76 (m, 1H), 3.53–3.50 (m, 2H), 3.48 (s, 3H), 3.41 (s, 3H), 3.25–3.22 (m, 2H), 3.08–3.05 (m, 1H), 1.47 (s, 3H), 1.44 (s, 3H), 1.39 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 168.1, 167.6, 158.2, 156.0, 123.2 (q, J=280.7 Hz), 123.0 (q, J=281.4), 113.1, 112.8, 112.4, 112.3, 111.4, 110.3, 110.2, 110.0, 109.4, 109.3, 108.6, 108.5, 96.3, 96.2, 86.5, 86.2, 85.6, 85.4, 85.3, 84.5, 82.0, 81.9, 81.7, 71.5, 71.4, 70.9, 70.5, 66.8, 65.6, 60.3, 57.9, 56.8, 55.8, 55.2, 55.1, 53.8, 51.4, 47.0, 46.7, 46.5, 46.3, 46.0, 45.8, 44.2, 43.7, 40.6, 40.3, 26.5, 26.4, 26.35, 26.31, 25.9, 25.8, 25.7, 25.0, 24.9, 24.8, 24.7, 24.6, 24.4, 24.3; ESI (m/z) 904.7 [M^++Na, (100)]; Anal. calcd for $C_{36}H_{53}F_6N_3O_{15}$: C 49.03, H 6.06, N 4.77; found: C 49.00, H 6.08, N 4.76.

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Supplementary data

Supplementary data available: characterization of compounds **1b**–**f**, **2b**, **3b,c**, **4b**, **13** and **19**. Copies of ^1H, ^{13}C NMR, and ESI-MS spectra of all new compounds.

Supplementary data associated with this article can be found in the online version.

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