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Multicomponent Synthesis of Peptide-Sugar Conjugates Incorporating Hexafluorovaline

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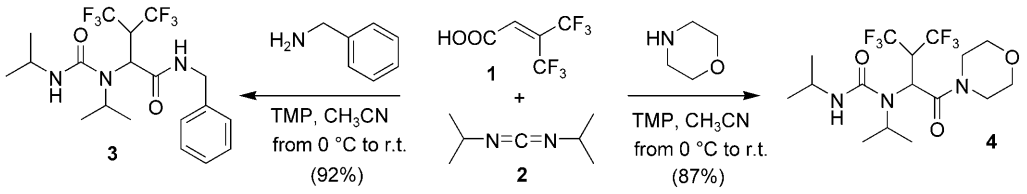
Abstract: The development of new methods for linking sugars to peptides or proteins is an active area of research because natural glycopeptides or neoglycoconjugates play important roles in biology and medicine and are indispensable tools for probing several biological processes. Herein we report a novel one-pot, three-component process for the synthesis of peptide-urea conjugates incorporating a hexafluorovaline residue under very mild conditions and high yields using commercially available starting materials such as carbodiimides, α-amino acid derivatives and 4,4,4-trifluoro-3-trifluoromethylcrotonic acid. The reaction has been exploited for the synthesis of a library of structurally diverse peptide-sugar conjugates incorporating hexafluorovaline through a four-component, one-pot sequential process by generating the carbodiimides *in situ* from easily accessible sugar containing azides and commercial available isocyanates through the Staudinger (aza-Wittig) reaction.

Keywords: fluorine; glycoconjugates; multicomponent reactions; peptides

Multicomponent reactions (MCRs)[1] are convergent transformations, in which three or more starting materials react to form a product, where basically all or most of the atoms contribute to the newly formed compound. Applications of MCRs in all areas of applied chemistry are very popular because they offer a wealth of products, while requiring only a minimum effort combining many elements of an ideal synthesis, such as operational simplicity, atom economy, bond-forming efficiency, the access to molecular complexity from simple starting materials. As such, MCRs have become the cornerstones of both combinatorial chemistry and diversity-oriented synthesis (DOS) and thus playing a central role in the development of modern synthetic methodology for pharmaceutical and drug discovery research.[2] In this context, the development of new methods for linking sugars to peptides or proteins is an active area of research because natural glycopeptides or neoglycoconjugates play important roles in biology and medicine and are indispensable tools for probing several biological processes.[3] A large number of elegant methods have been developed for the synthesis and assembly of native *N*- and *O*-linked as well as not-native *C*- and *S*-linked glycopeptides,[4] but these methods may be complicated by low glycosylation efficiencies and extensive protection regimes to ensure regioselectivity. Otherwise, conjugation at primary positions of 6-aminohexoses and 5-aminopentoses could be an easier way to accomplish such an achievement by providing peptide-sugar conjugates with enzymatically stable artificial linkages, also considering the fact that the $-CH_2NH_2$ moiety present in these sugars might mimic some elements of the glycine structure.[5] For instance, the 6-position of L-galactose has been conjugated to different amino acids in the synthesis of sialyl Lewis X mimetics[6] as well as the primary 6′-[7] and 5″-positions[8] of neomycine in the modification of aminoglycoside antibiotics targeting RNA. However, glycoconjugate synthesis involving sugars and amino acids or peptides remains a formidable task since the developed synthetic protocols are quite demanding and involve multiple reaction steps. The problem may be in part or completely obviated through the use of MCRs.

Recently, we demonstrated that carbodiimides, when treated with suitable carboxylic acids such as 4,4,4-trifluoro-3-trifluoromethyl(Tfm)-crotonic acid in the absence of a nucleophile, are useful reagents for the synthesis of diversely substituted hydantoins through a regiospecific domino condensation/aza-Mi-



Scheme 1. Three-component synthesis of urea-hfVal amides.

chael/N→O acyl migration process.[9] Herein we wish to report that the same reaction conducted in the presence of nucleophiles such as amines or α-amino acid derivatives give rise to a totally new one-pot, three-component process for the synthesis of peptide-urea conjugates incorporating a hexafluorovaline (hfVal) residue under very mild conditions and high yields. The reaction has been exploited for the synthesis of a library of structurally diverse peptide-sugar conjugates incorporating hfVal through a four-component, one-pot sequential process by generating the carbodiimides *in situ* from sugar-containing azides and isocyanates through the Staudinger (aza-Wittig) reaction.[10] It is noteworthy that highly fluorinated amino acids such as hfVal and hexafluoroleucine (hfLeu) have been used to stabilize proteins[11] for potential application in various protein-based biotechnologies, such as protein therapeutics, industrial-scale biotrasformations and biosensors. Moreover, the incorporation of fluorinated amino acids into peptides or proteins can significantly increase diffusion across membranes such as the blood-brain barrier,[12] improve the pharmacokinetical properties of the peptide-based drug[13] and allows monitoring of the chemical environment of the fluorine-containing residues by non-invasive ^{19}F NMR.[14]

4,4,4-Trifluoro-3-Tfm-crotonic acid **1** was treated with commercially available DIC **2** in the presence of primary and secondary amines, such as benzylamine and morpholine, in different conditions of solvents and temperature (data not shown). The best conditions found were those outlined in Scheme 1, namely the presence of 1 equivalent of a base (TMP) and acetonitrile as solvent at 0 °C. In both cases, we were able to recover the target urea-hfVal amides **3** and **4** in excellent yields.

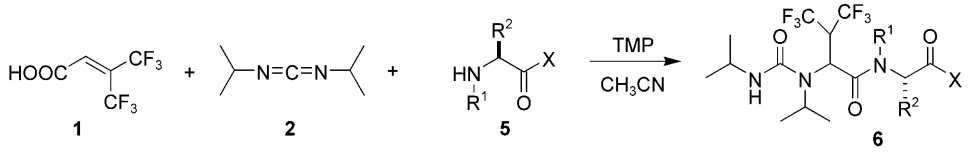
Thus, in order to demonstrate the feasibility of this methodology for the synthesis of urea-peptide conjugates, we explored the reaction with less nuclophilic α-amino acid esters and derivatives (Table 1). Under the same conditions as outlined before, the reaction worked well with α-amino acid esters **5a–c** giving rise to the formation of an equimolar mixture of two, easily separable, urea-hflVal-peptide conjugates **6a–c**, respectively, in very good yields (entries 1–3, Table 1).[15] Also *N*-alkylated α-amino acid esters **5d**, **e** reacted smoothly leading to *N*-alkylpeptides **6d**, **e** respectively. In these cases the yields turned out to be lower probably because of the steric hindrance of the nucleophiles.[16] The process proved to be highly versatile since also α-amino acid amides H-Gly-NH_2 **5f** and H-Phe-$NHCH_3$ **5g** reacted thoroughly producing urea-peptide conjugates **6f** and **6g**, respectively, in high yields (entries 6 and 7, Table 1). Finally we tried the reaction with dipeptide **5h** in order to prove that such a process could be used for the synthesis of longer urea-peptide conjugates. Also in this case, the reaction worked efficiently giving rise to the formation of the expected urea-tripeptide conjugate **6h** in excellent yields.

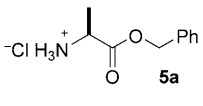
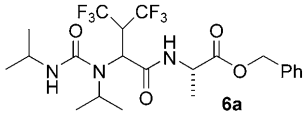
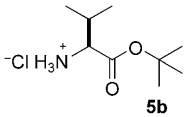
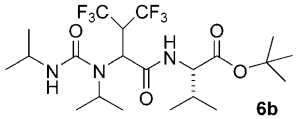
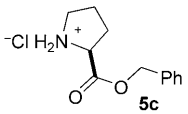
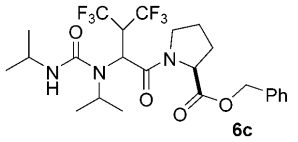
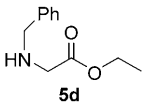
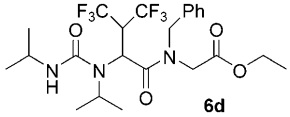
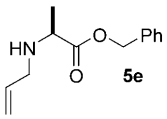
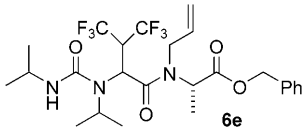
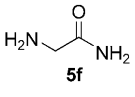
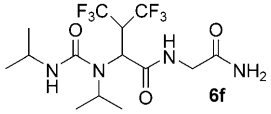
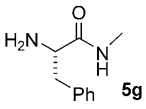
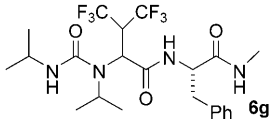
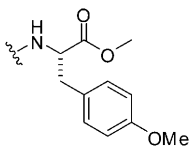
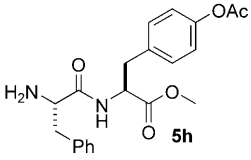
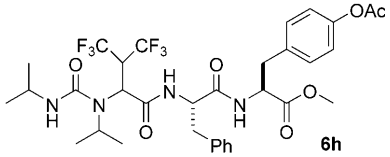
All the experimental data shown before suggest that the reaction arises through a domino process in which the acid **1** reacts with carbodiimide **2** forming an *O*-acylisourea intermediate **7** which readily cyclizes to intermediate **8** through an intramolecular aza-Michael reaction. The cyclic *O*-acylisourea **8** results to be more reactive toward nucleophiles than **7** and in the presence of amines or α-amino acid derivatives undergoes ring opening by nucleophilic attack to the more reactive carbonyl moiety leading to the desired urea-peptide conjugate **6** (Scheme 2).[17]

To support the proposed mechanism and to amplify the usefulness of this method for the synthesis of more complicated scaffolds by getting regioselectivity with asymmetric carbodiimides, we thought to perform the reaction with a carbodiimide bearing a primary alkyl substituent and a tertiary alkyl substituent at the two nitrogen atoms. Thus, *N*-allyl-*N'*-*tert*-butylcarbodiimide **2** was reacted with 4,4,4-trifluoro-3-Tfm-crotonic acid **1** in the presence of H-Ala-OBn **5a** giving rise to the formation, as expected, of only one out of the two possible regioisomers **9** which arose from the nucleophilic attack of the less congested nitrogen bearing the primary alkyl substituent in the intramolecular aza-Michael step (Scheme 3).[18]

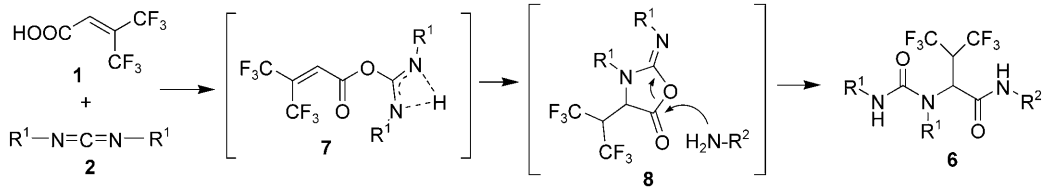
Finally, we investigated the possibility to use this process for the MC synthesis of sugar-urea-peptide conjugates by incorporating the glycosyl moiety in the carbodiimide framework. We considered that by using the Staudinger reaction starting from easily accessible primary glycosyl azide derivatives and commercially available *tert*-butyl isocyanate we would be able to generate *in situ* the desired carbodiimides. These, in turn, would react with 4,4,4-trifluoro-3-Tfm-crotonic acid in the presence of α-amino acid derivatives through a regioselective four-component one-pot se-

Table 1. Three-component synthesis of urea-peptide conjugates.

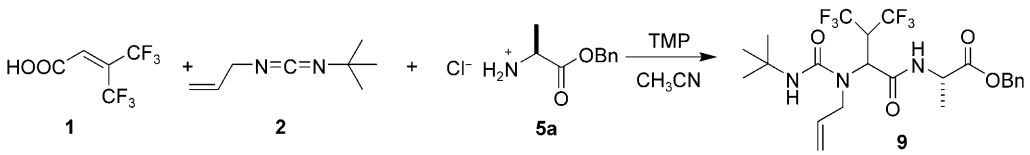


Entry	R^1	R^2	X	Nucleophile	Product[a]	Yield [%][b]
1	H	Me	OCH_2Ph	 5a	 6a	80
2	H	*i*-Pr	$OC(CH_3)_3$	 5b	 6b	85
3	$-(CH_2)_4-$		OCH_2Ph	 5c	 6c	81
4	CH_2Ph	H	OCH_2CH_3	 5d	 6d	60
5	allyl	Me	OCH_2Ph	 5e	 6e	53[c]
6	H	H	NH_2	 5f	 6f	67
7	H	CH_2Ph	$NHCH_3$	 5g	 6g	84
8	H	CH_2Ph		 5h	 6h	75

[a] Equimolecular ratio of two diastereoisomers.
[b] Isolated yields.
[c] Unseparable diastereoisomers.



Scheme 2. The proposed mechanism.



Scheme 3. Reaction with asymmetric carbodiimide.

quential process leading to the formation of the target conjugates (Table 2). Indeed, when azidogalactose derivative **10a** was reacted with *tert*-butyl isocyanate **11a** in CH_3CN in the presence of triphenylphosphine, carbodiimide **12a** and triphenylphosphine oxide were cleanly formed. By adding to the resulting solution TMP followed by the hydrochloride salts of H-Ala-OBn **5a** or H-Leu-OBn **5i** and the acid **1**, we were able to obtain the desired conjugates **13a** and **13b**, respectively, in high yields and complete regiocontrol, in a 3.5 to 1 diastereisomeric ratio (entries 1 and 2, Table 2).[19] Again, in order to prove the versatility of the process, we performed the reaction with α-amino amide **5j** and dipeptide **5k** recovering the corresponding hfVal-glyco-dipeptide **13c** and -tripeptide **13d** in very high yields (entries 3 and 4, Table 2).

It is worth noting that since the diasteroselectivity of the process depends on the nucleophilic attack of the chiral primary glycosyl-amino moiety, we obtained the same diasteroisomeric ratio when using the same carbodiimide, that is, 3.5 to 1 with carbodiimide **12a**. Importantly, the process also worked efficiently with the symmetrical diglycosylcarbodiimide generated *in situ* from azide **10a** and thioisocyanate **11b** leading to the formation of of an equimolar mixture of two diastereoisomers **13e**. This is very intriguing because the product could be seen as the precursor for a new class of glycomimetics in which two saccharides are tethered through a urea-hfVal-peptide moiety (entry 5, Table 2). Finally, we tried the reaction starting with another glycosyl azide, namely methyl 5-azido-5-deoxy-2,3-*O*-isopropylidene-β-ribofuranoside **10b**. Here again, we were able to obtain an almost equimolar ratio (1.5:1 *dr*) of the conjugates **13f**, **g**, respectively, in high yields and with total regiocontrol, after generating *in situ* the corresponding carbodiimide with isocyanate **11a** and performing the reaction with the hydrochloric salts of H-Ala-OBn **5a** and H-Val-O-*t*-Bu **5b** (entries 6 and 7, Table 2). More sterically congested *N*-alkyl-α-amino acid esters **5d**, **e** reacted with the same carbodiimide **12c** leading to the formation of the corresponding products **13h**, **i**, respectively, in lower, although acceptable, yields (entries 8 and 9, Table 2).[20] As expected, in this case we also obtained very good results on performing the reaction with dipeptide **5h** affording the hfVal-tetrapeptide conjugate **13j** in very good yields (entry 10, Table 2). It is noteworthy that β-ribofuranose is the central sugar of many aminoglycoside antibiotics, such as neomycin and paromomycin, and that their 5″ position (5 position of ribofuranose) has been often functionalizated in order to obtain more potent/selective antibiotics.[21] The functionalization of such a position by this process is currently being examined in our laboratories.

In conclusion, we have developed a novel and efficient process for the synthesis of libraries of urea-peptide and glycosyl-peptide conjugates containing hfVal amino acid through a multi-component reaction involving simple and readily accessible starting materials. The operational simplicity and the good chemical yields, combined with favourable atom-economy aspects and a small number of synthetic steps, render this new synthetic strategy attractive and promising for the preparation of novel classes of glycosyl-peptide conjugates and particularly suitable for solid-phase/combinatorial chemistry. The latter issues as well as the modification of aminoglycoside antibiotics are currently in progress.

Experimental Section

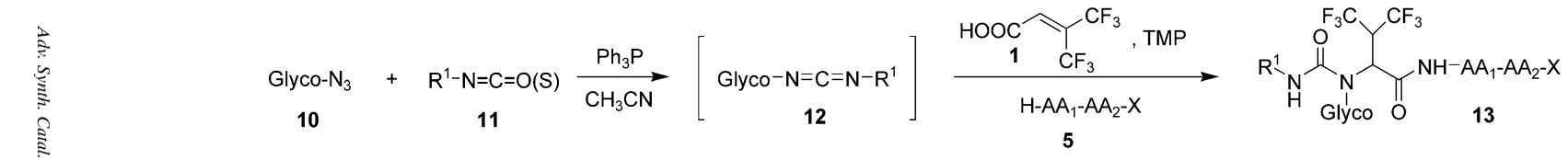
General Methods

Commercially available reagent grade solvents were employed without purification. TLC analyses were run on silica gel 60 F_{254} Merck. Flash chromatographies (FC) were performed with silica gel 60 (60–200 μm, Merck). ^{1}H NMR spectra were run on spectrometers at 250, 400 or 500 MHz. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^{1}H and ^{13}C nuclei (δ_H and $\delta_C = 0.00$), while C_6F_6 was used as external standard ($\delta_F = 162.90$) for ^{19}F. Glycosyl azides **10a**, **b** and glycosyl isothiocyanate **11b** were prepared following reported procedures.[22]

General Procedure for the Three-Component Synthesis of Urea-Peptide Conjugates

To a stirred solution of carbodiimide (1 equiv.) in CH_3CN (0.1 M) amino acid derivative (1 equiv.) followed by TMP (1 equiv. or 2 equiv. when the hydrochloride salts of the amino acid derivatives were used) and a solution of 4,4,4-trifluoro-3-Tfm-crotonic acid (1 equiv.) in a minimun amount of CH_3CN were added at 0 °C. The resulting solution was stirred until the reaction was complete (TLC monitoring) allowing the temperature to slowly reach room temperature. A 1 N HCl aqueous solution was added and the mixture extracted three times with AcOEt. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, concen-

Table 2. Four-component sequential synthesis of sugar-peptide conjugates.



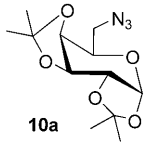
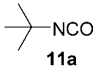
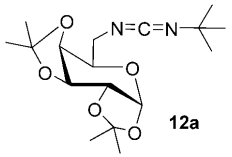
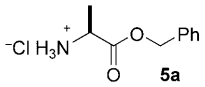
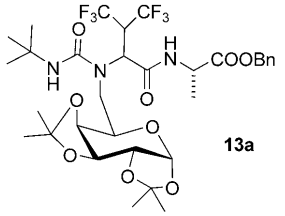
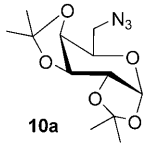
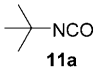
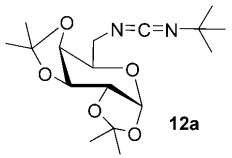
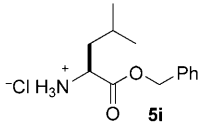
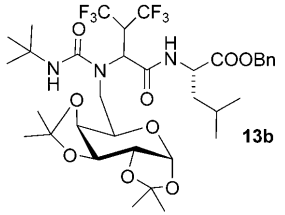
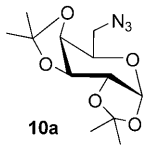
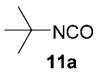
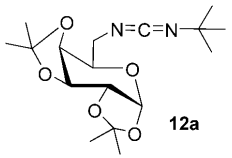
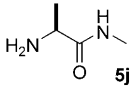
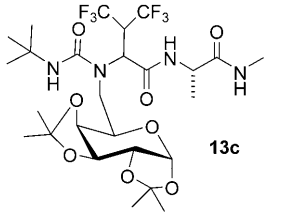
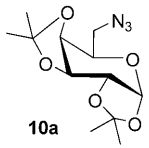
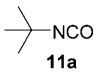
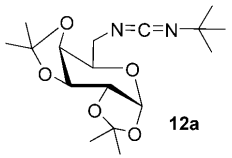
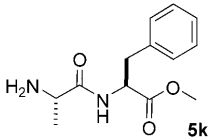
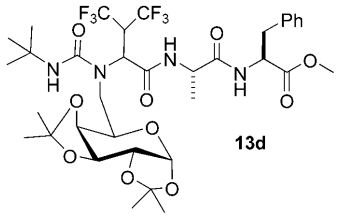
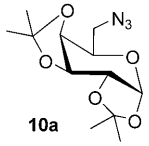
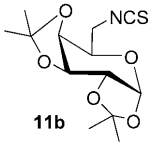
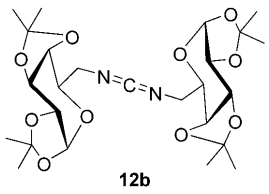
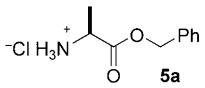
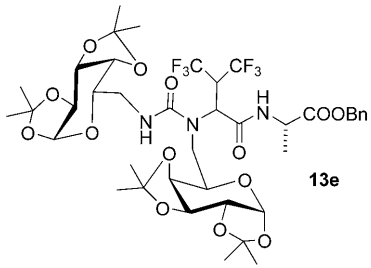
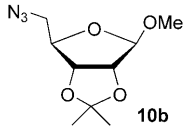
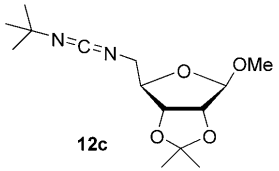
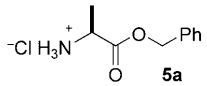
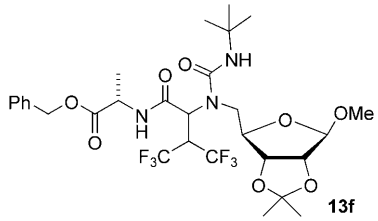
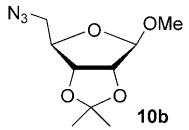
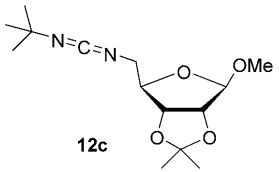
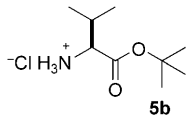
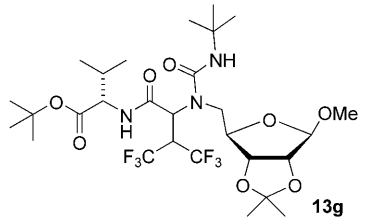
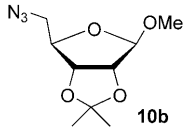
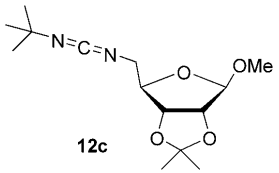
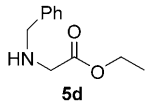
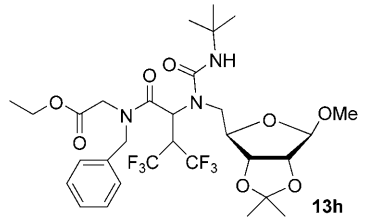
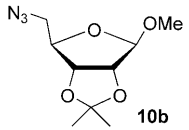
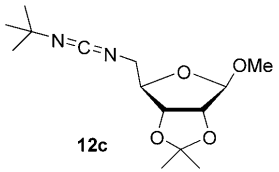
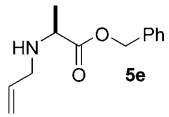
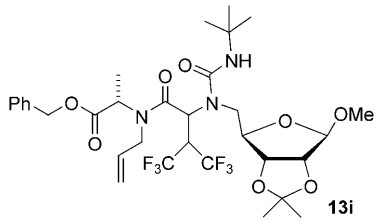
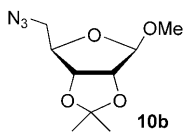
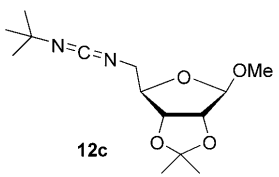
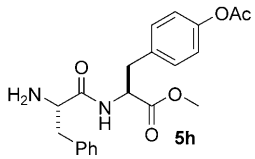
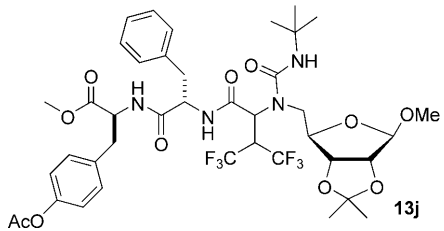
Entry	Glyco-N_3	$R^1NCO(S)$	Carbodiimide	H-AA_1-AA_2-X	Product	Yield [%][d]
1	 **10a**	 **11a**	 **12a**	 **5a**	 **13a**	82[a]
2	 **10a**	 **11a**	 **12a**	 **5i**	 **13b**	75[a]
3	 **10a**	 **11a**	 **12a**	 **5j**	 **13c**	73[a]
4	 **10a**	 **11a**	 **12a**	 **5k**	 **13d**	68[a]
5	 **10a**	 **11b**	 **12b**	 **5a**	 **13e**	70[b]

Table 2. (Continued)

Entry	Glyco-N_3	$R^1NCO(S)$	Carbodiimide	H-AA_1-AA_2-X	Product	Yield [%][d]
6	 **10b**	 **11a**	 **12c**	 **5a**	 **13f**	85[c]
7	 **10b**	 **11a**	 **12c**	 **5b**	 **13g**	77[c]
8	 **10b**	 **11a**	 **12c**	 **5d**	 **13h**	54[c,e]
9	 **10b**	 **11a**	 **12c**	 **5e**	 **13i**	48[c]
10	 **10b**	 **11a**	 **12c**	 **5h**	 **13j**	71[c]

[a] 3.5:1 ratio of two diastereoisomers.
[b] Equimolar mixture of two diasteroisomers.
[c] 1.5:1 ratio of two diastereoisomer.
[d] Isolated yields.
[e] Inseparable mixture of two diastereoisomers.

trated under vacuum and the crude material purified by flash chromatography.

3: R_f = 0.34 (hexane:AcOEt, 80:20); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.65 (br s, 1H), 7.25 (m, 5H), 4.71 (br s, 1H), 4.58 (br d, J = 7.0 Hz, 1H), 4.49 (dd, J = 15.0 and 6.0 Hz, 1H), 4.40 (br s, 1H), 4.28 (dd, J = 15.0 and 6.0 Hz, 1H), 3.91 (octet, J = 6.5 Hz, 1H), 3.77 (septet, J = 7.0 Hz, 1H), 1.26 (d, J = 6.5 Hz, 3H), 1.18 (d, J = 6.5 Hz, 3H), 1.15 (d, J = 6.5 Hz, 3H), 1.13 (d, J = 6.5 Hz, 3H); ^{19}F NMR (235.4 MHz, $CDCl_3$): δ = −64.6 (br s, 3F), −64.1 (br s, 3F); ^{13}C NMR (125.7 MHz, $CDCl_3$); δ = 169.1, 158.4, 137.9, 128.5, 127.6, 127.3, 123.0 (q, J = 281.8 Hz), 122.8 (q, J = 282.1 Hz), 56.4, 50.4, 47.0 (septet, J = 26.8 Hz), 43.6, 43.0, 23.0, 21.1, 20.5; ESI-MS: m/z = 480.0 [M^+ + K, (4)], 464.1 [M^+ + Na, (100)].

(*S*)-**6a**: R_f = 0.41 (hexane:AcOEt ,70:30); $[\alpha]_D^{20}$: +80.5° (*c* 0.48, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.05 (br d, J = 6.4 Hz, 1H), 7.33 (m, 5H), 5.17 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 12.0 Hz, 1H), 4.80 (m, 1H), 4.54 (quintet, J = 7.2 Hz, 1H), 4.40 (d, J = 7.2 Hz, 1H), 4.17 (d, J = 11.2 Hz, 1H), 3.94 (octet, J = 6.4 Hz, 1H), 3.76 (septet, J = 7.2 Hz, 1H), 1.40 (d, J = 7.2 Hz, 3H), 1.28–1.16 (m, 12H); ^{19}F NMR (235.6 MHz, $CDCl_3$): δ = −64.9 (quintet, J = 7.2 Hz, 3F), −63.9 (quintet, J = 7.2 Hz, 3F); ^{13}C NMR (62.9 MHz, $CDCl_3$); δ = 172.3, 169.0, 158.4, 135.5, 128.5, 128.3, 128.0, 123.0 (q, J = 281.2 Hz), 122.3 (q, J = 282.0 Hz), 66.8, 50.9, 48.2, 46.7 (septet, J = 25.9 Hz), 43.0, 23.0, 21.3, 20.0, 17.5; ESI-MS: m/z = 514 [M^+, (5)], 222 (89), 91 (100).

(*R*)-**6a**: R_f = 0.35 (hexane:AcOEt, 70:30); $[\alpha]_D^{20}$: −33.1° (*c* 0.64, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.83 (br s, 1H), 7.35 (m, 5H), 5.20 (d, J = 12.0 Hz, 1H), 5.14 (d, J = 12.0 Hz, 1H), 4.71 (m, 1H), 4.54 (m, 2H), 4.34 (br d, J = 9.6 Hz, 1H), 3.94 (octet, J = 6.4 Hz, 1H), 3.78 (septet, J = 7.2 Hz, 1H), 1.39 (d, J = 7.2 Hz, 3H), 1.28–1.16 (m, 12H); ^{19}F NMR (235.6 MHz, $CDCl_3$): δ = −64.6 (m, 3F), −64.1 (m, 3F); ^{13}C NMR (62.9 MHz, $CDCl_3$); δ = 171.9, 168.7, 158.3, 135.4, 128.5, 128.3, 67.0, 56.0, 50.3, 48.4, 46.8 (septet, J = 27.7 Hz), 42.9, 23.0, 21.2, 20.5, 18.0. The CF_3 signal was obscured due to its low intensity; ESI-MS: m/z = 514 [M^+, (9)], 222 (93), 91 (100).

General Procedure for the Four-Component Sequential Synthesis of Peptide-Sugar Conjugates

To a stirred solution of glycosyl azide (1 equiv.) in CH_3CN (0.1 M) neat iso(thio)cyanate (1 equiv.) followed by a solution of Ph_3P (1 equiv.) in a minimun amount of CH_3CN were added at room temperature. After the formation of the corresponding carbodiimide was complete (TLC monitoring, *ca.* 3 h.), the solution was cooled to 0 °C and amino acid derivative (1 equiv.) followed by TMP (1 equiv. or 2 equiv. when the hydrochloride salts of the amino acid derivatives were used) and a solution of 4,4,4-trifluoro-3-Tfm-crotonic acid (1 equiv.) in a minimun amount of CH_3CN were added. The resulting solution was stirred until the reaction was complete (TLC monitoring) allowing the temperature to slowly reach room temperature. A 1 N HCl aqueous solution was added and the mixture extracted three times with AcOEt. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated under vacuum and the crude material purified by flash chromatography.

(*R*)-**13a (major diasteroisomer):** R_f = 0.22 (hexane:AcOEt, 80:20); $[\alpha]_D^{20}$: −8.3° (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.96 (br s, 1H), 7.35 (m, 5H), 5.87 (br s, 1H), 5.49 (d, J = 4.7 Hz, 1H), 5.19 (d, J = 12.7 Hz, 1H), 5.12 (d, J = 12.7 Hz, 1H), 4.55 (br d, J = 6.6 Hz, 1H), 4.44 (septet, J = 7.0 Hz, 1H), 4.27 (dd, J = 4.7 and 2.3 Hz, 1H), 4.21 (dd, J = 8.0 and 2.3 Hz, 1H), 4.12 (q, J = 7.0 Hz, 1H), 3.97–3.73 (br m, 2H), 3.15 (br m, 1H), 1.45 (s, 6H), 1.33–1.30 (m, 15H), 1.25 (s, 3H); ^{19}F NMR (235.4 MHz, $CDCl_3$): δ = −65.1 (m, 3F), −64.2 (m, 3F); ^{13}C NMR (125.7 MHz, $CDCl_3$); δ = 171.3, 171.1, 167.9, 135.5, 128.6, 128.3, 128.2, 123.7 (q, J = 281.6 Hz), 123.0 (q, J = 282.5 Hz), 109.4, 96.2, 70.6, 70.5, 66.9, 60.3, 51.2, 51.1, 49.0, 46.4 (septet, J = 29.1 Hz), 28.7, 25.9, 25.8, 25.0, 24.2, 20.9, 14.2; ESI-MS: m/z = 750.1 [M^+ + Na, (100)].

(*S*)-**13a (minor diasteroisomer):** R_f = 0.15 (hexane:AcOEt, 80:20); $[\alpha]_D^{20}$: −25.2° (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10 (br s, 1H), 7.33 (m, 5H), 6.05 (br s, 1H), 5.51 (d, J = 4.5 Hz, 1H), 5.16 (d, J = 12.5 Hz, 1H), 5.13 (d, J = 12.5 Hz, 1H), 4.79 (br s, 2H), 4.64 (dd, J = 7.0 and 2.0 Hz, 1H), 4.57 (septet, J = 7.0 Hz, 1H), 4.32 (dd, J = 5.0 and 2.0 Hz, 1H), 4.18 (dd, J = 7.5 and 2.0 Hz, 1H), 3.96 (m, 1H), 3.47 (br m, 1H), 3.33 (dd, J = 16.5 and 6.0 Hz, 1H), 1.47 (s, 6H), 1.38–1.31 (m, 9H); ^{19}F NMR (235.4 MHz, $CDCl_3$): δ = −64.9 (m, 3F), −64.3 (m, 3F); ^{13}C NMR (62.9 MHz, $CDCl_3$); δ = 172.1, 167.3, 158.7, 135.6, 128.5, 128.3, 128.2, 109.6, 109.2, 96.4, 70.6, 70.2, 66.8, 51.2, 48.6, 28.9, 26.0, 25.9, 25.6, 24.8, 24.4, 17.5. The CF_3 and C-CF_3 signals were obscured due to their low intensity; ESI-MS: m/z = 750.1 [M^+ + Na, (100)].

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[15] With an achiral carbodiimide the reaction is not stereoselective because the stereogenic α-amino acid ester steps in the process once the new stereogenic center is already formed (see the proposed mechanism). However, from the perspective of combinatorial application, the formation of both stereoisomers is not necessarily a drawback. The stereochemistry of the two diastereoisomers was assessed by X-ray diffraction of (*S*)-**6b** (the reported stereochemistry is related to the new stereogenic center formed in the reaction, that is, the stereochemistry of hfVal) and on the basis of their spectroscopic and analytical features. The X-ray structure and relative full details will be published in a full paper.

[16] A *ca.* 20% yield of the corresponding hydantoin derivatives was recovered in both reactions. For the mechanistic insight of such transformation, see ref.[9a]

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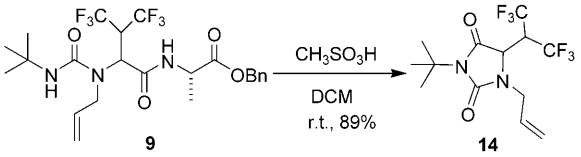

9 **14**

[19] The stereochemistry of the major diastereoisomer was assessed by X-ray diffraction of sugar-peptide conjugate (*R*)-**13f** (see Supporting Information) and on the basis of their spectroscopic and analytical features. Full details will be given in a full paper.

[20] Also in this case, the diasteroisomeric glycosyl-hydantoin conjugates were recovered as by-products. A detailed study of the synthesis of such compounds will be published in a forthcoming paper.

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