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New types of glycoconjugates: *O*-glycosylated, *N*-glycosylated and *O*-,*N*-diglycosylated isoserine derivatives☆

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Abstract—Starting from hexafluoroacetone-protected malic acid *O*-glycosylated, *N*-glycosylated and *O*-,*N*-diglycosylated (*S*)-isoserine derivatives have been synthesized. The new compounds represent glycosylated β-alanine surrogates, i.a. suitable for β-peptide modification.

Glycoproteins are ubiquitous in nature, playing a key role in various biological recognition processes on membranes.[2,3] The peptide and carbohydrate part are linked together by glycosidic bonds.[4] There are two modes for the attachment of glycosides to the peptide backbone involving either the oxygen atom in the side chain of serine and threonine, or the nitrogen atom in the side chain of asparagine.[5] These bonds are potentially acid-sensitive and in some cases labile even under moderate basic conditions.[3] Glycopeptides contain numerous functional groups. Therefore, the regio- and stereoselective synthesis of glycopeptides requires sophisticated, selective and mild protection and activation strategies.[2b,4]

In general, glycoconjugates isolated from biological sources or produced by gentechnological methods are microheterogeneous and can not be applied for biological studies. Therefore, the development of new methodology for the assembly of more robust, homogeneous glycopeptide mimetics as well as the construction of new binding motifs by chemoselective ligation is of current interest, i.a. glycoconjugates have been synthesized where *O*- and *N*-glycosidic linkages are replaced by non-natural linkages, like carbon–carbon,[6,7] carbon–sulfur[7,8] and carbon–aminooxy units.[9] Alternative concepts proposed by Kessler et al. are the replacement of the amide group of *N*-glycosylated species by a retroamide subunit[10] or an ethylene isoster,[11] and by Imperiali et al. are the incorporation of alanine-β-hydroxylamine[9] and alanine-β-hydrazide[9,12] as asparagine surrogates. Recently, a new approach to glycopeptide mimetics was disclosed, where *O*- and *N*-glycosidic linkages are replaced by urea–glycosyl bonds.[13] The urea–glycosyl bonds were constructed by coupling glycosyl isocyanates and amino acid derivatives.

Herein we report on the incorporation of isoserine directly at the interface carbohydrate/peptide and on an alternative synthetic approach for an efficient incorporation of an urea linkage between the carbohydrate backbone and a β-amino acid using hexafluoroacetone as protecting and activating reagent.

[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]-methyl isocyanate (**2**)[14] readily obtained from malic acid (**1**) and hexafluoroacetone is excellently suited for regioselective trifunctionalization.[14,15] Fluoren-9-yl-methanol adds exclusively across the isocyanate group of **2** to give the fully protected, α-carboxy-activated Fmoc-(*S*)-isoserine derivative, which on treatment with an excess of allyl alcohol reacts to give the corresponding allyl ester **3** (Scheme 1). Concomitantly, the hydroxy group is deblocked, and can be glycosylated in the next step, for example applying Schmidt's imidate strategy.[16] Compound **4** is an *O*-glycosylated orthogonally protected β-amino α-hydroxy acid. Deblocking of the carboxy moiety can be achieved by applying the protocol of Kunz and Waldmann[17] to obtain *O*-glycosylated Fmoc-Ise-OH (**5**), a new building block for glycopeptide modification.

Keywords: hexafluoroacetone; (*S*)-isoserine; β-amino acids; glycosylation; peptide modification.

☆ Neoglycoconjugates, Part 4. For Part 3, see Ref. 1.

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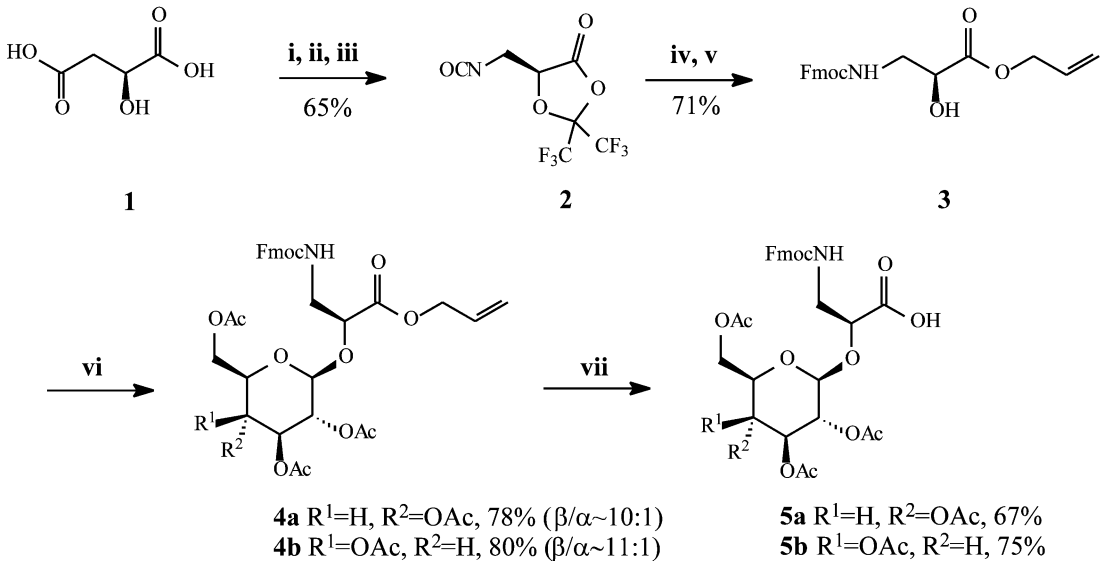


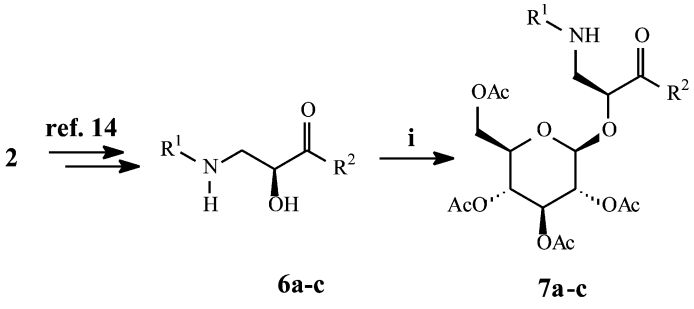
Scheme 1. *Reagents and conditions*: (i) $(CF_3)_2CO$, DMSO; (ii) $SOCl_2$, reflux; (iii) $TMSN_3$, toluene, 80°C; (iv) fluoren-9-yl-methanol, $CHCl_3$, reflux; (v) excess allyl alcohol, $CHCl_3$, reflux, (vi) 1.2 equiv. α-D-Ac_4-Glc{Gal}-O-C(=NH)CCl_3, cat. TMSOTf, MS 4 Å, CH_2Cl_2, (vii) $(PPh_3)_4Pd$, *N*-methylaniline, THF.

By a slightly modified procedure (Scheme 2) compound **2** can be transformed into *O*-glycosylated dipeptide derivatives **7b**,**c**. After introduction of the Fmoc group, the cleavage of the lactone ring is now performed with an α-amino acid ester (**2→6**). In a consecutive step the hydroxy group is glycosylated on treatment with the corresponding imidate-activated carbohydrate building block. *O*-glycosylated dipeptides of type Fmoc-Ise-Xaa-OMe (**7b**: Fmoc-Ise-Phe-OMe) are obtained in modest yields.

Dipeptides of type Fmoc-Xaa-Ise-OMe (**7c**: Fmoc-Phe-Ise-OMe) with *O*-glycosylated isoserine in C-terminal position are obtainable from **2** in a three step sequence, consisting of a Goldschmidt reaction,[14] alcoholysis, and finally *O*-glycosylation (Table 1).

N-Glycosylation with introduction of an urea linkage can be achieved in a preparatively simple way on reaction of **2** with the corresponding glycosylamine (**2→8**) (Scheme 3). Compounds **8** are obtained as anomeric mixtures, the β-anomers are the main products (Table 2). **8a**-β, **8b**-β, **8d**-β have been isolated analytically pure from the crude reaction mixtures by flash chromatography. However, experiments to separate **8c**-α and **8c**-β failed. Compounds **8** are α-carboxy-activated species. Therefore, they can be directly submitted to peptide synthesis. Dipeptide formation is coupled with a simultaneous deprotection of the hydroxyl group (**8→9**). Compounds **9** are *N*-glycosylated dipeptides with an unprotected α-hydroxy group which can be functionalized further. Therefore, they represent building blocks, i.e. suitable for the construction of glycosylated depsipeptides.

On heating a solution of **8** in chloroform in the presence of pyridine we expected an intramolecular cyclization to form a six-membered heterocyclic ring system,



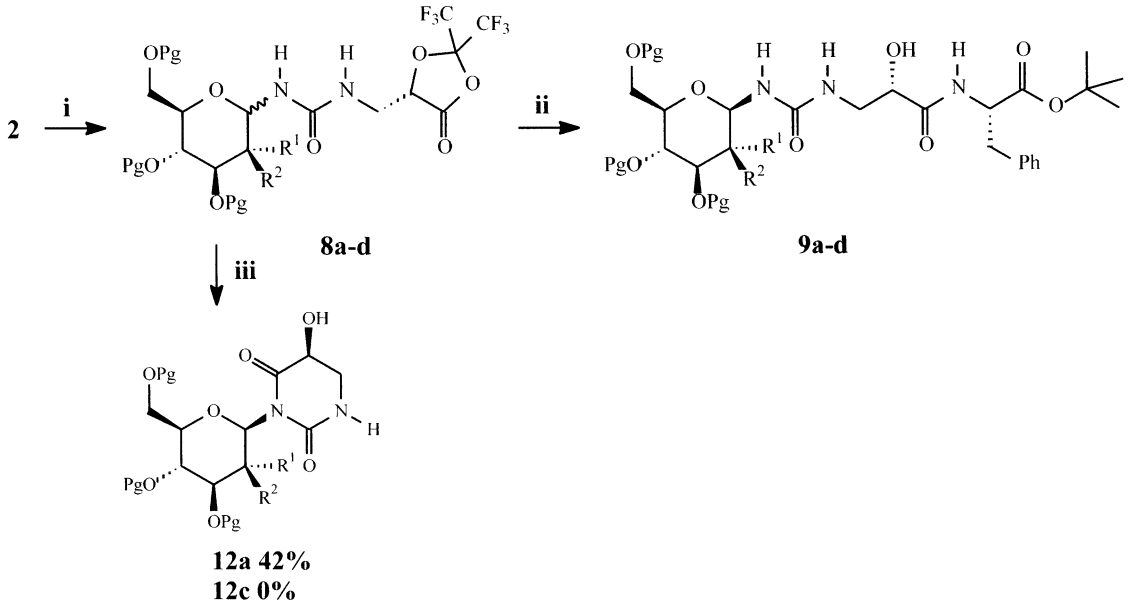
Scheme 2. *Reagents and conditions*: (i) 1.2 equiv. α-D-Ac_4-Glc-O-C(=NH)CCl_3, cat. TMSOTf, MS 4 Å.

Table 1.

No.	R^1	R^2	Solvent	Yield (%)
7a	Fmoc	OMe	CH_2Cl_2	78
7b	Fmoc	Phe-OMe	CH_2Cl_2	25
7c	Fmoc-Phe	OMe	CH_2Cl_2/THF 5:1	20

namely a 3-glycosylated (5*S*)-5-hydroxy-2,4-dioxo-5,6-dihydropyrimidine (**12**) (Scheme 3). While conversion of the glucose derivative proceeded with acceptable yields (**8a→12a**), the mannose derivative **8c** did no react to give an analoguous cyclocondensation product under identical reaction conditions. On prolonged heating **8c** decomposed slowly. An intramolecular ring closure of a similar type was observed when *N*-glycosylated HFA-protected malic acid derivatives were treated with pyridine in chloroform under reflux.[1]

When to a solution of **8**-β in dichloromethane two equivalents of TFA were added, a slow anomerization process was induced (Scheme 4). Equilibrium ratios

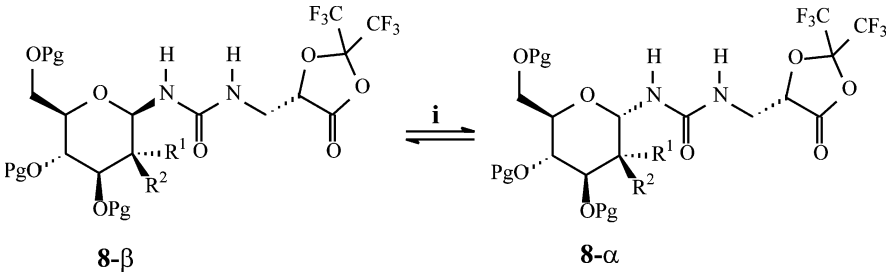


Scheme 3. *Reagents and conditions*: (i) 0.95 equiv. glycosylamine, CH_2Cl_2, 0°C; (ii) 1.2 equiv. HCl*H-Phe-O^{t}Bu, NEM, DMF; (iii) 25 equiv. pyridine, $CHCl_3$, reflux.

Table 2.

No.	R^1	R^2	Pg	Yield (%) **1**→**8**-β+α	Anomeric ratio[a] **8**-β/**8**-α	Yield (%) **8**-β→**9**
a	OAc	H	Ac	64	12:1	86
b	OBzl	H	Bzl	62	3:1	74
c	H	OAc	Ac	64	12:1	83
d	H	OBzl	Bzl	70	10:1	73

[a] Anomeric ratio of the crude product was determined by ^1H and ^{19}F NMR spectroscopy.



Scheme 4. *Reagents and conditions*: (i) 2 equiv. TFA, CH_2Cl_2.

were taken from the ^1H and ^{19}F NMR spectra after 36 h (Table 3).

Another new type of building block, namely *N*-,*O*-diglycosylated dipeptide allyl ester (**11**), was obtained on *O*-glycosylation of **10** (Scheme 5). Since cleavage of the lactone ring can be also performed with β-amino acid esters diglycosylated β-dipeptides become accessible via this reaction sequence.

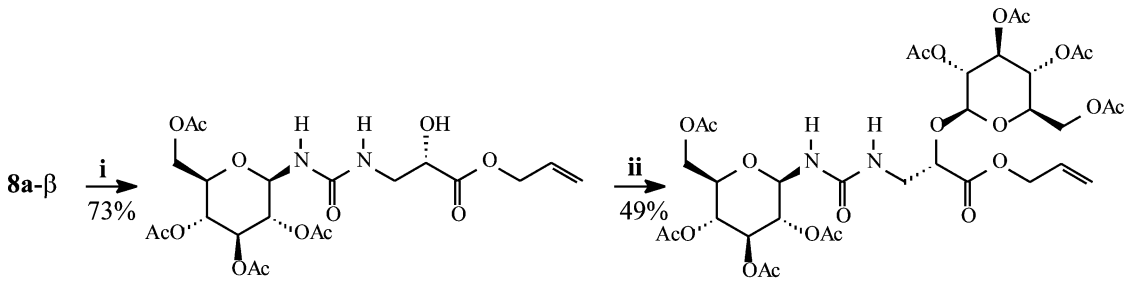
Multivalency of the interactions between carbohydrates and carbohydrate-binding proteins[18] or nucleic acids[19] has recently become a subject of considerable interest. To study the factors governing the strength of these cluster effects, methodology for the synthesis of an arsenal of new types of oligosaccharides and glycoconjugates has to be developed.[20]

Table 3.

No.	Ratio **8**-β/**8**-α[a]	$J_{12}{}^{\beta}$ (Hz)[b]	$J_{12}{}^{\alpha}$ (Hz)[b]
8a	4:1	9.6	4.8
8b	1:3	9.0	2.5
8c	4:1	~1	–
8d	3:2	~1	3.3

[a] Anomeric ratio of the equilibrated mixture was determined by ^1H and ^{19}F NMR spectroscopy.

[b] Coupling constants were measured in $CDCl_3$.



Scheme 5. *Reagents and conditions*: (i) excess allyl alcohol, $CHCl_3$, reflux; (ii) 1.2 equiv. α-D-Ac_4-Glc-O-C(=NH)CCl_3, cat. TMSOTf, MS 4 Å, CH_2Cl_2.

On *O*-,*N*-glycosylation and *O*-,*N*-diglycosylation of multifunctional β-amino acids (α-methylisoserine, isocysteine) and multifunctional γ-amino acids (homoisoserine, α-methylhomoisoserine, homoisocysteine) applying the 'hexafluoroacetone route' we report elsewhere.

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