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A New Scaffold for the Stereoselective Synthesis of α-*O*-Linked Glycopeptide Mimetics

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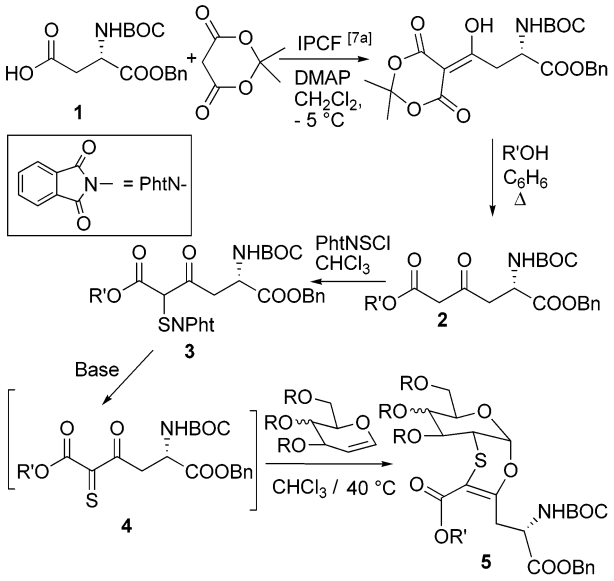
Abstract: α-O-Linked glycohomoglutamates are obtained as diastereomerically pure compounds by chemo-, regio-, and stereoselective cycloadditions between glycals and aspartic acid derivatives. The latter constitute orthogonally functionalized scaffolds for glycopeptide mimetics.

The remarkable structural complexity of glycoproteins, which arises from the different combination of amino acids and carbohydrates, is an ideal tool for coding cell's information.[1] As a matter of fact, in eukaryotic organisms most proteins are glycosylated and the consequences are evident in many physiological events such as cell−cell adhesion, cell growth, or cell−virus interaction.[2] The study of cell surface glycopeptides has recently been focused on glycopeptide mimetics[3] because the synthesis of native glycoproteins still remains an ambitious, time-consuming target. Thus, novel chemical approaches have been developed to introduce non-native linkages into large biomolecules to render complex glycopeptidic structures more easily available. In this context, although most of the glycoproteins present the peptide motif *β*-linked at the anomeric carbon of the terminal monosaccharide unit, the importance of naturally occurring α-*O*-glycopeptides such as mucins[4] or α-*N*-glycopeptides such as nephritogenoside[5] has stimulated many researchers to develop new selective methods for achieving α-*O*-glycosidation of amino acids.[6,7] We wish to describe here an easy and totally stereoselective method for the preparation of *O*-glycoamino acids that retain the α stereochemistry of native sugar−peptide linkage[8] and that can be used as multifunctional scaffolds for glycopeptide mimetics.

SCHEME 1. Synthesis of *β*-Ketoesters 2 and Glycohomoglutamates 5



In the present approach, *O*-glycohomoglutamates are obtained as diastereomerically pure α-isomers from glycals and aspartic esters. The issue of linking a carbohydrate domain to an amino acid under strict stereochemical control has been solved by the cycloaddition of appropriately protected glycals to *δ*-amino-α-thio-*β*-ketoesters[9] derived from *N*-BOC-aspartic acid benzyl ester (**1**) which occurs with complete chemo-, regio- and stereoselectivity. (Scheme 1)

The transformation of **1** into *β*-ketoesters **2** using Meldrum's acid[10,11] represents a valuable tool for the preparation of a variety of suitable phthalimidosulfenyl derivatives **3**, by reaction with phthalimidosulfenyl chloride (PhtNSCl). Highly reactive **4**, generated from **3** by base treatment, was trapped "in situ" by glycals to give α-*O*-glycohomoglutamates **5** as diastereomerically pure isomers,[12] in good yields (Table 1).

Selective transformation of multifunctional *O*-glycoamino acids **5** afforded new glycopeptide mimetics. For example, the *tert*-butoxycarbonyl group (BOC) on **5a** was

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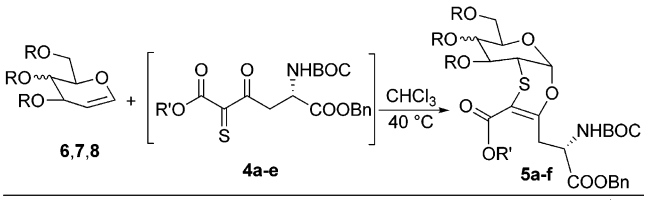
TABLE 1. *β*-Ketoesters 4a−e and Synthesis of Cycloadducts 5a−f

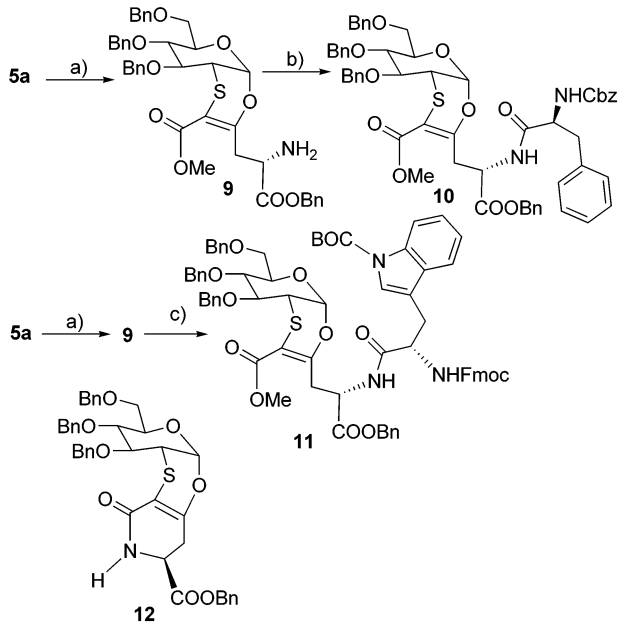


Entry	R'	Glycal	Cycloadduct	Yield(%)[a)]
1	Me **4a**	BnO, BnO, BnO, O **6**	**5a**	68
2	Et **4b**	TIPSO, HO, TIPSO, O **7**	**5b**	40
3	Bn **4c**	**6**	**5c**	50
4	^{t}Bu **4d**	**6**	**5d**[b)]	---
5	$Me_3Si(CH_2)_2$ **4e**	**6**	**5e**	35
6	**4a**	BnO, OBn, BnO, O **8**	**5f**	52

[a] Isolated. [b] Cycloadduct **5d** is not stable: if it is stored at rt for few hours retrocycloaddition is observed.

removed and the crude amino derivative **9** was reacted with *N*-benzyloxycarbonyl-L-Phe (Cbz-L-Phe-OH) and with *N*-fluorenyl-(*N*-*tert*-butoxycarbonyl)-L-Trp [Fmoc-L-Trp(BOC)-OH] under standard conditions[13] to give the desired α-*O*-glycodipeptides **10** and **11**, respectively (Scheme 2).

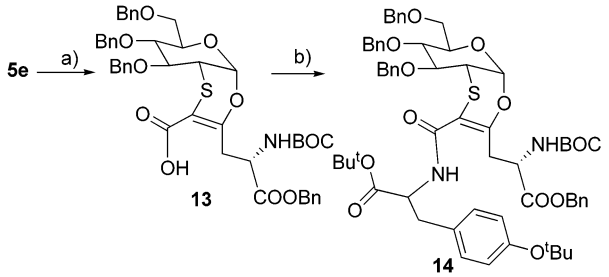
SCHEME 2. Synthesis of α-*O*-Glucodipeptides 10 and 11



[a] Key: (a) Me_3SiCl (4 M)−PhOH (4 M) CH_2Cl_2; (b) *N*-Cbz-L-Phe, HOBt, EDCl, DIPEA, DMF, rt, 62% (two steps); (c) *N*-Fmoc-(*N*-BOC)-L-Trp, HOBt, EDCI, DIPEA, DMF, rt, 66% (two steps).

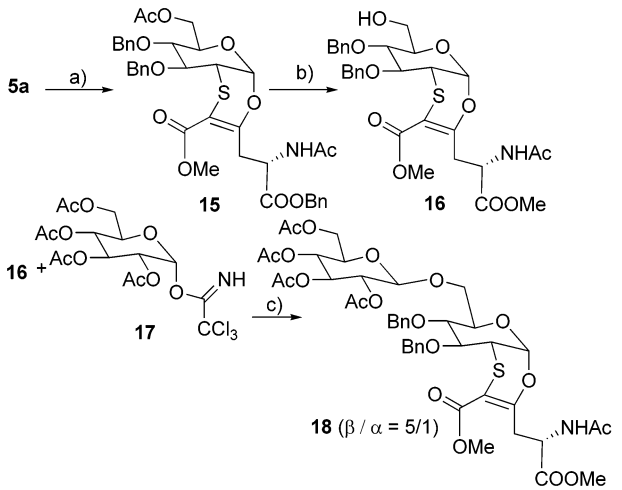
Removal of the BOC group was successfully achieved by the treatment of **5a** with solutions of Me_3SiCl (4 M in CH_2Cl_2) and PhOH (4 M in CH_2Cl_2);[14] instead, compound

SCHEME 3. Synthesis of Glucodipeptide 14[a]



[a] Key: (a) TBAF, THF, rt, 4 h, 65%; (b) H-L-Tyr(tBu)-O-tBu·HCl, HOBt, EDCI, DIPEA, DMF, 1.5 h, 84%.

SCHEME 4. Synthesis of Dibenzyl Derivative 16 and Disaccharide 18[a]



[a] Key: (a) TMSOTf, Ac_2O, −40 °C, 1.5 h, 77%; (b) MeONa, MeOH, CH_2Cl_2, rt, 86%; (c) TMSOTf, CH_2Cl_2−Et_2O, −5 °C to rt (65%).

12 was obtained as major or single product through a more common deprotection procedure using trifluoroacetic or hydrochloric acid[15] (see Scheme 2).

The selective deprotection of the α,*β*-unsaturated ester was initially attempted on cycloadduct **5a**. Alkaline treatment (NaOH, dioxane, rt or 60 °C) of the latter afforded the desired carboxylic acid **13**, though with unsatisfactory yields (10−30%). Treatment under different conditions [K_2CO_3, MeOH, rt (83%); DBU, LiBr, $Me_3SiCH_2CH_2OH$ THF, rt (15%)] gave mainly transesterification of the benzyl ester. More efficiently, cycloadduct **5e** was transformed into the corresponding monoester **13** by treatment with tetrabutylammonium fluoride (TBAF) in THF (65%) or CsF in DMF (65%) at room temperature. Reaction of **13** with *O*-*tert*-butyl-L-Tyr-*tert*-butyl ester

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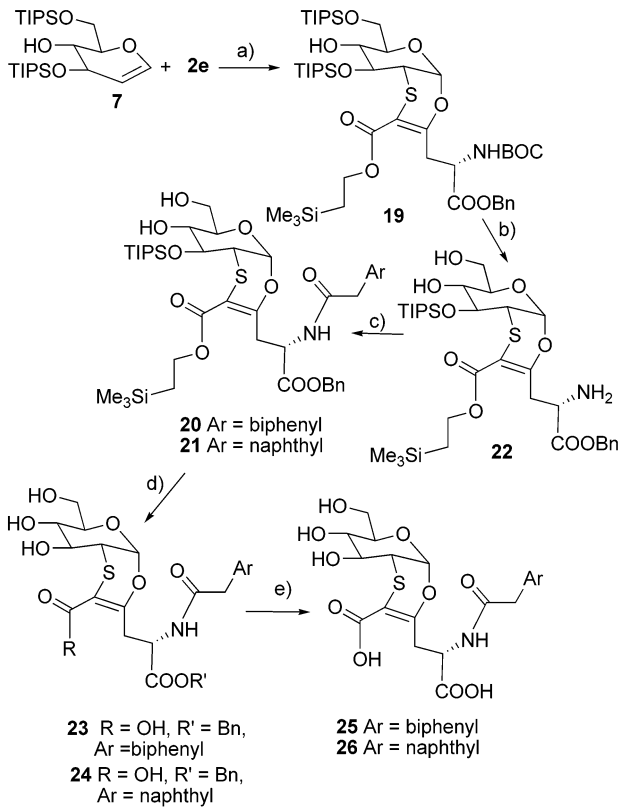
gave the non-natural α-*O*-glycodipeptide **14** (84%) as diastereomerically pure compound (Scheme 3).

Cycloadduct **5a** was converted into the monoacetyl glycoside **15** by trimethylsilyltriflate and acetic anhydride at low temperature;[16] the latter was deacetylated to give the dibenzyl derivative **16** (Scheme 4). The complete removal of benzyl groups on the carbohydrate moiety through standard methods (H_2−Pd/C or $Pd(OH)_2$/C in THF, MeOH, or EtOAc) was instead unsuccessful, consistently leading to recovery of unaltered starting material. Compound **16** exposed to a trichloroacetimidate donor **17** gave the expected disaccharide **18** in good yield (64%) (Scheme 4), thus proving the effective use of the new scaffold we propose for the assembly of oligosaccharide chains.

α-*O*-Glucohomoglutamates with two unprotected carboxylic acids and a fully unprotected glyco moiety were prepared by cycloaddition of the disilyl glucal **7** to **4e**. The *O*-glycoside **19** (Scheme 5), obtained as the pure α-isomer, was subsequently transformed into the corresponding amides **20** and **21** by reacting the crude aminoderivative **22** with biphenyl-4-yl- and naphthalene-2-ylacetyl chlorides, respectively. Treatment of **20** and **21** with CsF in DMF at room temperature allowed the simultaneous removal of the triisopropyl silyl group at C-3 and the deprotection of the trimethylethyl ester to afford monoesters **23** (65%) and **24** (65%). Hydrogenation of **23** and **24** gave the dicarboxylic acids **25** and **26** in quantitative yield (Scheme 5).

In conclusion, a highly efficient approach for the synthesis of α-O-linked glycohomoglutamates scaffolds **5** has been described. These scaffolds, characterized by two differentiated carboxyls, a protected α-amino function, and a selectively functionalized monosaccharidic unit, were successfully employed to give an array of diastereomerically pure building blocks for α-*O*-glycopeptide mimetics.

SCHEME 5. Synthesis of α-*O*-Glucohomoglutamate Derivatives 25 and 26[a]



[a] Key: (a) PhtNSCl, Py, $CHCl_3$, 60 °C, 30 h, 40%; (b) Me_3SiCl (4 M)−PhOH (4 M), CH_2Cl_2, conv > 90%; (c) $ArCH_2COCl$, Et_3N, rt, 1 h, **20** (50%), **21** (43%); (d) CsF, DMF, rt, 12 h, **23** (65%), **24** (65%); (e) H_2−$Pd(OH)_2$/C, MeOH, rt, 20 h, **25** (>90%), **26** (>90%).

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **2a**−**e**, **5a**−**c**,**e**,**f**, **9**, **10**−**16**, **18**−**21**, and **23**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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