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New building blocks for peptide and depsipeptide modification *N*-glycosylated L-malic and L-citramalic acid derivatives†,‡

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Abstract—Neoglycoconjugates have been synthesized from L-malic acid/L-citramalic acid and β-D-Ac_4Glc-NH_2/β-D-Bzl_4Glc-NH_2 using hexafluoroacetone as protecting and activating agent.

Peptide drugs are a fast growing class of therapeutics. However, they show a series of severe disadvantages, like proteolytic instability and low lipophilicity. Furthermore, the lack of specific transport systems to direct peptide drugs into cells or across the blood brain barrier is the reason why cell membranes generally resist passage of most peptides. Therefore, peptide drugs are rapidly degraded and excreted.[3,4]

The high conformational flexibility of peptides causes another problem. Bioactive peptides often bind to different receptor sites, causing undesired side effects.[5,6] Different strategies have been developed to overcome these drawbacks. The most versatile approach is the rational design of peptidomimetics[6–8] by backbone-modification,[6,9–11] incorporation of α-*C*-alkyl and α-*N*-alkyl amino acids[6,12–14] and incorporation of glycosylated peptide fragments[15,16] into key positions of the peptide chain.

Since glycopeptides are ubiquitous in nature, playing a key role in various biological recognition processes[17,18] and glycoclusters exhibiting enhanced binding properties,[19] the synthesis of glycopeptide mimetics has received significant attention. Deciphering these roles in controlled studies requires convenient synthetic access to a large number of different binding motifs. Glycoconjugates isolated from biological sources are often microheterogeneous and cannot be applied for mechanistic studies. Therefore, the development of new methodology for the assembly of glycopeptide mimetics by chemoselective ligation is of current interest. As part of an ongoing program we synthesized a series of glycosylated α-hydroxy acids[20] via the new 'hexafluoroacetone route'.[1,2]

α-Hydroxy acids, including multifunctional species like L-malic (**1a**) and L-citramalic acid (**1b**), react with hexafluoroacetone in DMSO at room temperature to give regiospecifically five-membered lactones (**2a**,**b**) in excellent yields.[21] In only one step, protection of both the α-hydroxy and the adjacent carboxy group can be achieved. Concomitantly, the α-carboxy group is selectively activated toward nucleophiles. The β-carboxy groups of malic and citramalic acid remain unaffected and can be derivatized in a consecutive step.[22] Compounds **2** can be easily prepared in a 50–100 g scale. On exclusion of moisture 2,2-bis-(trifluoromethyl)-1,3-dioxolan-4-ones (**2**) can be stored in a fridge, at −30°C, for months without decomposition.

Upon treatment with thionyl chloride or DAST, compounds **2** can be transformed into acid chlorides **3**[23] and acid fluorides **4**,[24] respectively. Compounds of type **3** and **4** represent double activated malic and citramalic acid derivatives with two electrophilic centers of different reactivity. Consequently, a new efficient, preparatively simple method for regioselective derivatization of α-functionalized α,β-dicarboxylic acids is now available. Recently, *N*-protected amino acid chlorides and amino acid fluorides have received significant attention as acylating agents in *N*-glycopeptide synthesis.[25] Therefore, we decided to study scope and limitation of com-

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‡ Dedicated to Professor Dr. P. Welzel on the occasion of his 65th birthday.

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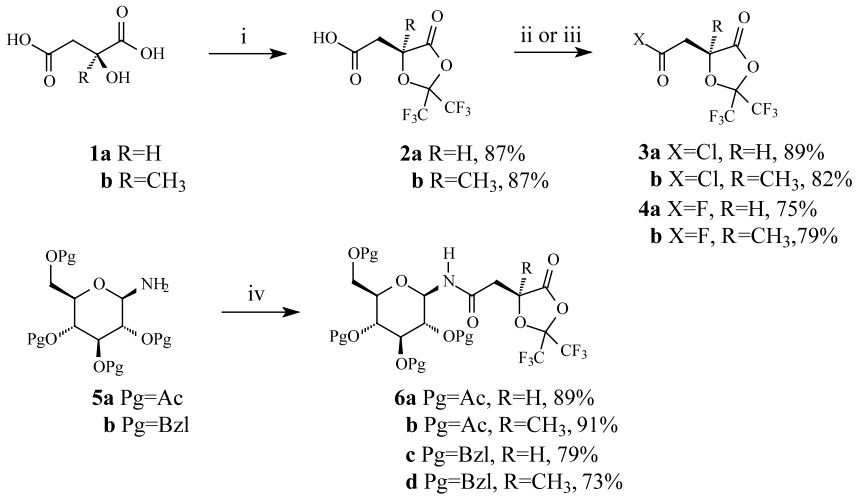


Scheme 1. *Reagents and conditions*: (i) hexafluoroacetone, DMSO, rt; (ii) $SOCl_2$, reflux; (iii) DAST, CH_2Cl_2, 0°C; (iv) **3a** or **3b**, pyridine, CH_2Cl_2, 0°C.

pounds **3** and **4** as acyl transfer reagents. As model acyl acceptors we choose glycosyl amines β-D-Ac_4Glc-NH_2 (**5a**)[26] and β-D-Bzl_4Glc-NH_2 (**5b**).[27]

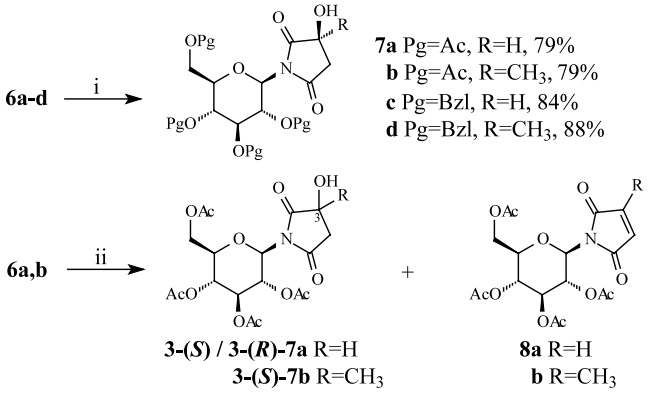
We found that acid chlorides **3a**,**b** and glycosyl amines **5a**,**b** react readily at 0°C in DCM in the presence of 1 equiv. of pyridine or 2,6-lutidine to give the acylated products **6a**–**d** within minutes in high yields (73–91%) (Scheme 1).

^{1}H NMR analysis (300 MHz) of the crude products reveals that the β-anomer ($^3J_{12}$ = 9.0–9.3 Hz) is formed exclusively in the case of **6a**–**c**. While for **6d**, after isolation of the β-anomer by crystallization, the α-anomer could be detected by ^{1}H NMR ($^3J_{12}$ = 5.8 Hz) and TLC in the concentrated mother liquor. It was isolated and fully characterized. Purification of compounds **6** by crystallization is the method of choice. On work-up by column chromatography the yields are notoriously lower. When stronger bases like NEM and DIEA are used in the acylation process, a competing reverse Michael addition is the reason for decreasing yields (40–60%).

The acid fluorides **4a**,**b** are noticeable less reactive than the corresponding acid chlorides **3a**,**b**. Nevertheless, with **4a**,**b** clean and regioselective acylation of **5a**,**b** can be achieved at 0°C in the presence of pyridine. With stronger bases like NEM and DIEA, the yields are lower.

On treatment with bases compounds **6a**–**d** undergo an intramolecular cyclocondensation. When the cyclization process is performed in the presence of pyridine in boiling chloroform **6a**–**d** are transformed to give stereoselectively 3-hydroxysuccinimido sugars **7a**–**d** in acceptable yields, which were purified by column chromatography (Scheme 2).

Treatment of a solution of **6a** in ethyl acetate with bases like NEM, DIEA or DBU gives rise to a



Scheme 2. *Reagents and conditions*: (i) 5 equiv. pyridine, $CHCl_3$, reflux, 16 h; (ii) 1 equiv. base, EtOAc, rt, 1–4 days.

diastereomeric mixture of **7a** and maleimido sugar **8a**[28] as elimination product (Table 1).

Compound **6b** reacts analogously, but since there is no α-proton (R = CH_3) present epimerization cannot be observed.

Finally, we demonstrated that glycosylated α-hydroxy acid derivatives **6a**–**d** are excellent acyl transfer reagents. With amino acid and dipeptide esters or amides, they react to give *N*-glycoconjugates **9a**–**d** which can be applied as new building blocks for peptide and depsipeptide modification (Scheme 3, Table 2).

As by-products of the aminolytic cleavage, compounds **7a**–**d** are formed with variable yields. In all cases studied so far, chromatographic separation of compounds **9** and **7** was achieved without problems. As expected, the sterically more demanding compounds **6b** and **6d** exhibit lower reactivity toward *N*-nucleophiles. Consequently, an increasing tendency to undergo the intramolecular cyclization process is observed. Furthermore, the amount of by-products is increasing with the sterical demand of the amino acid side-chain (H-Gly-O^{t}Bu<H-Ala-O^{t}Bu<H-Val-O^{t}Bu).

Table 1.

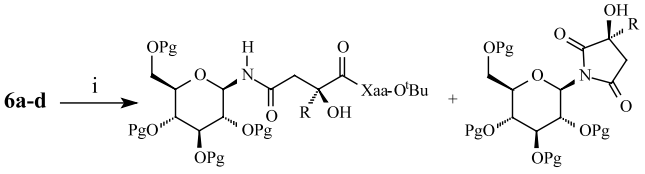
Base	Product ratio[a] **7a**:**8a**	Yield (%)[b]	Ratio[c] **3**-(*S*):**3**-(*R*)-**7a**	Product ratio **7b**:**8b**	Yield (%)[b]
DBU	5:1	57	5:3	4:1	65
DIEA	3:1	63	7:3	15:1	68
NEM	25:1	73	7:3	**8b** n.d.	72

[a] Determined by integration of the ^{1}H NMR spectra of the crude products.
[b] Isolated yields after chromatography [**7**+**8**].
[c] Determined by integration of the ^{1}H NMR spectra of an isolated diastereomeric mixture of **7a**.

Table 2.

No.	Xaa	Product ratio[a] **9**:**7**	Yield (%)[b]	No.	Xaa	Product ratio **9**:**7**	Yield (%)[b]
9a	Gly	>95:5	76	**9c**	Phe	>95:5	71
9a	Ala	8:1	72	**9c**	Phe-Ala	>95:5	74
9a	Val	7:1	82	**9c**	Pro	>95:5	67
9a	Phe	7:1	80				
9a	Pro	>95:5	72				
9b	Gly	5:1	78	**9d**	Phe	5:1	69
9b	Ala	2:1	72	**9d**	Phe-Ala	5:1	88
9b	Val	1.3:1	82	**9d**	Pro	1.4:1	80
9b	Phe	1.2:1	82				
9b	Pro	<5:95	76				

[a] Determined by integration of the ^{1}H NMR spectra of the crude products.
[b] Isolated yields after chromatography [**9**+**7**].



9a Pg=Ac, R=H
b Pg=Ac, R=CH_3
c Pg=Bzl, R=H
d Pg=Bzl, R=CH_3

Scheme 3. *Reagents and conditions*: (i) 1.2 equiv. HCl*H-Xaa-O′Bu, 1.2 equiv. NEM, EtOAc/DMF = 1:1.

On neoglycoconjugates obtainable from coupling reactions of glycosyl amines with homologues of aspartic, malic, thiomalic acid and α-, β-, γ-amino acid derivatives using the 'hexafluoroacetone route' we report elsewhere.

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