Monatshefte für Chemie 135, 1225–1242 (2004)
DOI 10.1007/s00706-004-0187-5

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Hexafluoroacetone as Protecting and Activating Reagent. Glycosylated Malic, Citramalic, and Thiomalic Acid Derivatives, New Glycosylated Building Blocks for Drug Design [1]

Christoph Böttcher, Jan Spengler, and **Klaus Burger**[*,#]

Department of Organic Chemistry, University of Leipzig, D-04103 Leipzig, Germany

Received January 7, 2004; accepted February 2, 2004
Published online July 23, 2004

Summary. Glycosylated α-hydroxy and α-mercapto acids have been synthesized starting from malic/citramalic/thiomalic acid and *Ac*$_4$-β-*D-Glc*-NH_2/*Bzl*$_4$-β-*D-Glc*-NH_2 using hexafluoroacetone as protecting and activating reagent.

Keywords. α-Hydroxy acids; α-Mercapto acids; Dielectrophiles; Glycosylamines; Maleimido sugars; 3-Hydroxysuccinimido sugars; *N*-Glycosylated depsipeptides; α-Hydroxyacylamino acids; α-Mercaptoacylamino acids.

Introduction

Glycoproteins and glycopeptides are ubiquitous in nature. They play a key role in various biological processes both within cells and at cell surfaces. These include protein transport, cell adhesion, and signal transduction [2]. Furthermore, glycosylation influences properties of peptides and proteins such as solubility, thermal and proteolytical stability [3] as well as conformation and folding [4]. Aberrant glycosylation of cellular proteins and glycolipids are associated with various diseases, including cancer [5]. Another remarkable feature are the enhanced binding properties of glycoclusters [6]. Therefore, glycoconjugates are promising candidates for drug design [7].

Deciphering the roles of glycoproteins in controlled studies requires convenient synthetic access to a large number of glycoconjugates with different binding motifs. Glycoconjugates isolated from biological sources are microheterogeneous

* Corresponding author. E-mail: burger@organik.chemie.uni-leipzig.de
# Dedicated to Prof. Dr. *Horst Wilde* on the occasion of his 65th birthday

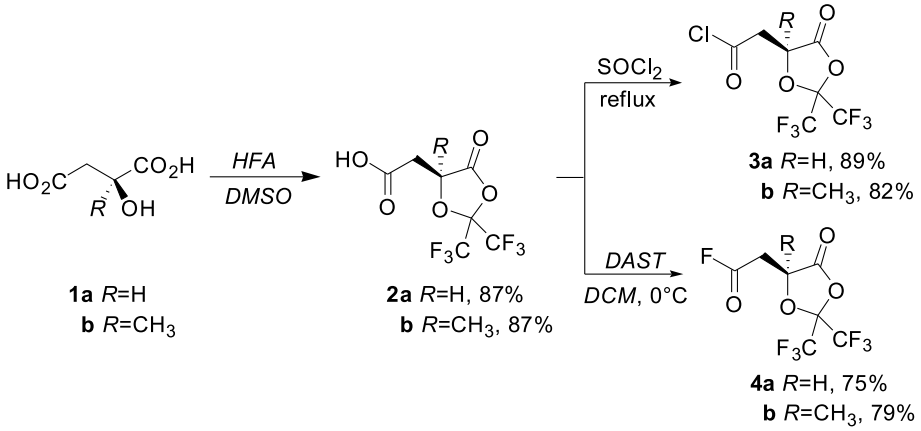
and cannot be applied for mechanistic studies. Therefore, the development of new methodologies for the assembly of homogeneous glycopeptidomimetics by chemoselective ligation is of current interest. Although glycosylated α-hydroxy acids are known for some time, they received little attention as building blocks for peptide and depsipeptide modification. *Fisher et al*. [8] reported on a considerable improvement of the pharmacokinetic properties of the peptidic *Renin* inhibitor *Ditekiren* on *N*-terminal incorporation of *N*-glycosylated malic and tartaric acid. *Risley et al*. [9] synthesized a series of β-*N*-glycosylasparagine analogues, including *N*-glycosylated malic acid derivatives. As protective groups for site-selective β-functionalization of malic acid acetone [8, 10] and chloral have been used [9, 11].

As part of an ongoing program we synthesized new types of building blocks for glycopeptide modification, including *N*-glycosylated α-hydroxy acid derivatives [12] testing new protection/activation concepts, like the ''hexafluoroacetone route'' [13], since the choice of the protecting group is crucial for the success in the synthesis of glycopeptides [14].

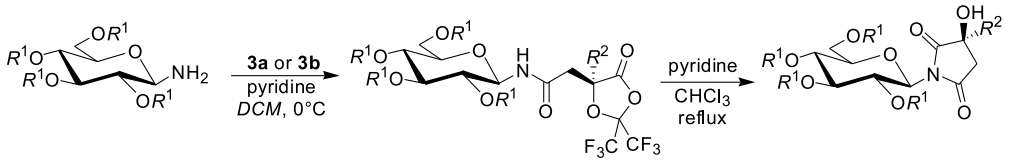
Results and Discussion

α-Hydroxy and α-mercapto acids, including multifunctional species like malic (**1a**), citramalic (**1b**), and thiomalic acid (**1c**), react with hexafluoroacetone in *DMSO* or *DMF* at room temperature to give five-membered lactones **2** (Scheme 1) [15–17]. The reaction proceeds quantitatively, no six-membered lactone could be identified by NMR spectroscopy in the crude material. However, 10–15% were lost during the work-up procedure, because of the water solubility of compounds **2**.

In one step, protection of both the α-hydroxy and the adjacent carboxy group is achieved. Concomitantly, the α-carboxy group is selectively activated toward nucleophiles. The β-carboxy groups of malic, citramalic, and thiomalic acid remain unaffected and can be derivatized in a consecutive step [18]. 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 as well as 2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-ones can be stored at -30°C for months without decomposition.



Scheme 1



5a R^1=*Ac*
b R^1=*Bzl*

6a R^1=*Ac*, R^2=H, 89%
b R^1=*Ac*, R^2=CH_3, 91%
c R^1=*Bzl*, R^2=H, 79%
d R^1=*Bzl*, R^2=CH_3, 73%

7a R^1=*Ac*, R^2=H, 79%
b R^1=*Ac*, R^2=CH_3, 79%
c R^1=*Bzl*, R^2=H, 84%
d R^1=*Bzl*, R^2=CH_3, 88%

Scheme 2

Upon treatment with thionyl chloride or *DAST* (diethylaminosulfur trifluoride), compounds **2** can be transformed into β-acid chlorides **3** [19] and β-acid fluorides **4** [20], respectively (Scheme 1). Compounds of type **3** and **4** represent a new class of dielectrophiles having two centers of different reactivity. Recently, *N*-protected amino acid chlorides and amino acid fluorides have received growing attention as acylating agents in *N*-glycopeptide synthesis [21]. Therefore, we became interested in studying the efficiency of compounds **3** and **4** as double acyl transfer reagents. As models for acyl acceptors we chose glycosyl amines *Ac*$_4$-β-*D*-*Glc*-NH_2 (**5a**) [22] and *Bzl*$_4$-β-*D*-*Glc*-NH_2 (**5b**) [23].

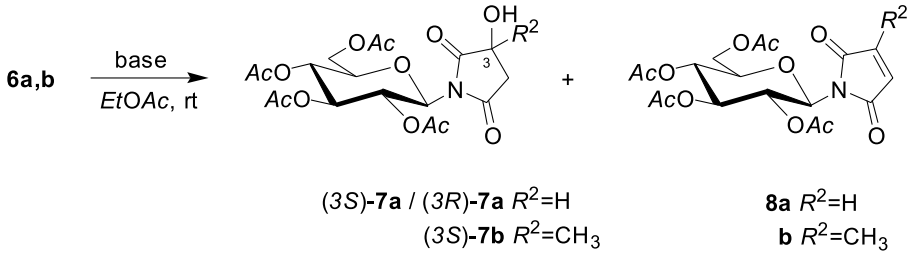
We found that β-acid chlorides **3a**, **3b** and glycosylamines **5a**, **5b**, which are weak nucleophiles, react site-selectively at 0°C in *DCM* (dichloromethane) in the presence of one equivalent of pyridine or 2,6-lutidine to give the *N*-acylated products **6a**–**6d** within minutes in high yields (73–91%), while the lactone moiety remains unaffected, acting as protecting group (Scheme 2).

^{1}H NMR analysis (300 MHz) of the crude products reveals that the β-anomer ($^3J_{\text{H-1/H-2}} = 9.0$–$9.3$ Hz) is formed exclusively in the case of **6a**–**6c**. Whereas for **6d**, after isolation of the β-anomer by crystallization, the α-anomer can be detected by ^{1}H NMR ($^3J_{\text{H-1/H-2}} = 5.7$ Hz) and TLC in the concentrated mother liquor. Purification of compounds **6** by crystallization is the method of choice. On work-up by column chromatography the yields are lower. When stronger bases like *NEM* (*N*-ethylmorpholine) and *DIEA* (diisopropylethylamine) are used a competing retro Michael addition is the reason for decreasing yields (40–60%) [24].

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

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

However, when a solution of **6a** was treated in ethyl acetate with stronger bases like *NEM*, *DIEA*, or *DBU* (1,8-diazabicyclo[5.4.0]undec-7-ene) a diastereomeric mixture of **7a**, together with maleimido sugar **8a** was obtained (Scheme 3, Table 1) [25]. **6b** reacts analogously. Since there is no α-proton ($R = CH_3$) present, no epimerization can take place.



Scheme 3

Table 1. Product ratio **7a**/**8a,** *(3S)*-**7a**/*(3R)*-**7a**, and **7b**/**8b** on treatment of **6a**, **6b** with different bases

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

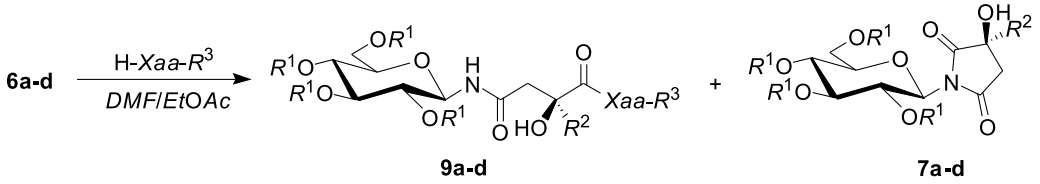
[a] Determined by integration of the ^{1}H NMR spectra of the crude products; [b] determined by integration of the ^{1}H NMR spectra of an isolated diastereomeric mixture **7a**

Finally, we demonstrated that glycosylated hexafluoroacetone-protected α-hydroxy acid derivatives **6a**–**6d** are excellent acyl transfer reagents. With amino acid and dipeptide esters or amides, they react to give *N*-glycoconjugates **9** (Scheme 4) which can be used as building blocks for the construction of libraries of glycosylated depsipeptides which we suggest to be an interesting new class of drug candidates. To the best of our knowledge glycosylated depsipeptides are unknown.

As by-products of the aminolytic cleavage **7a**–**7d** are formed in variable yields. In all cases studied so far, chromatographic separation of by-products **7** from compounds **9** was achieved without problems. As expected, the sterically more demanding compounds **6b** and **6d** ($R^2 = CH_3$) exhibit lower reactivity toward *N*-nucleophiles. Consequently, an increasing tendency to undergo the intramolecular ring closure is observed. Furthermore, the amount of by-products is increasing with the steric demand of the side-chain of the amino acid derivatives which act as nucleophiles (Table 2).

The product ratio of the aminolytic ring opening can be influenced by the solvent used. So far *DMF* was the best solvent for the synthesis of *N*-glycosylated dipeptide amides from **6b** and H-*Phe*-NH_2.

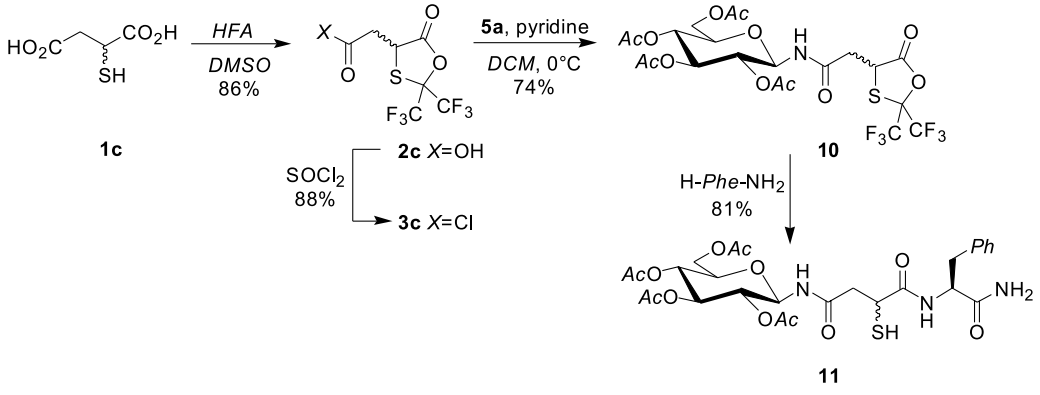
Mercapto carboxylic acids have been isolated from biologically active naturally occurring peptides [26]. Furthermore, mercapto acids as well as acylmercapto acids have been introduced as a new class of monomers for the construction of new types of peptidomimetics only recently [17, 27]. A β-mercapto acid is a subunit of *Captopril*, a classical drug for treatment of hypertension [28]. Likewise, an α-mercapto acid subunit is present in *Omaprilat* and *Gemopatrilat*. Both are vasopeptidase inhibitors, currently under clinical evaluation [29]. Compounds containing mercapto or acylmercapto subunits exhibit strong inhibitor activities on metal-containing enzymes [30].



Scheme 4

Table 2. Product ratio **9**/**7** obtained on treatment of compounds **6a**–**6d** with amino acid esters and dipeptide esters

Educt	Nucleophile H-*Xaa*-R^3	Product	Product ratio **9**:**7**	Yield/% **9** + **7**
6a	H-*Gly*-O^t*Bu*	**9a**/**1**	**7a** n.d.	76
6a	H-*Ala*-O^t*Bu*	**9a**/**2**	9.3:1	72
6a	H-*Val*-O^t*Bu*	**9a**/**3**	6.5:1	82
6a	H-*Phe*-O^t*Bu*	**9a**/**4**	7.0:1	80
6a	H-*Pro*-O^t*Bu*	**9a**/**5**	**7a** n.d.	72
6a	H-*Phe*-NH_2	**9a**/**6**	**7a** n.d.	80
6b	H-*Gly*-O^t*Bu*	**9b**/**1**	3.9:1	78
6b	H-*Ala*-O^t*Bu*	**9b**/**2**	1.8:1	72
6b	H-*Val*-O^t*Bu*	**9b**/**3**	1.4:1	81
6b	H-*Phe*-O^t*Bu*	**9b**/**4**	1.1:1	82
6b	H-*Pro*-O^t*Bu*	–	<1:20	76
6b	H-*Phe*-NH_2	**9b**/**5**	1.4:1	80
6c	H-*Phe*-O^t*Bu*	**9c**/**1**	**7c** n.d.	78
6c	H-*Pro*-O^t*Bu*	**9c**/**2**	**7c** n.d.	74
6c	H-*Phe-Ala*-O^t*Bu*	**9c**/**3**	**7c** n.d.	67
6d	H-*Phe*-O^t*Bu*	**9d**/**1**	5.2:1	69
6d	H-*Pro*-O^t*Bu*	**9d**/**2**	1:1.4	82
6d	H-*Phe-Ala*-O^t*Bu*	**9d**/**3**	4.2:1	88



Scheme 5

The hexafluoroacetone route can also be applied for the synthesis of the so far undescribed *N*-glycosylated thioanalogues. Glycosylated 2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-ones **10** are slightly less reactive than 2,2-bis(trifluoromethyl)-1,3-dioxolan-4-ones **6**. Thiomalic acid was introduced as a racemate, consequently the products obtained were diastereomeric mixtures (Scheme 5).

Since a growing number of reports focus on peptidomimetics as potential drug candidates, built from two or more different types of monomers [31], the development of new types of building blocks, like α-mercapto acids, adds a new facette to peptide and depsipeptide modification [17].

Conclusion

We described a preparatively simple access to *N*-glycosylated α-hydroxy and α-mercapto acids as well as to *N*-glycosylated α-hydroxyacyl and α-mercaptoacyl amino acid derivatives. They represent new types of building blocks for the synthesis of depsipeptide surrogates and their thioanalogues in solution and on solid phase [32].

Experimental

General

Solvents were purified and dried prior to use. Reagents were used as purchased. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light or phosphomolybdic acid/ceric sulphate in 5% aqueous sulfuric acid followed by heating. Flash column chromatography was performed using silica gel (32–63 μm) with solvent systems given in the text. Melting points (uncorrected) were determined on a *Boetius* heating table. Optical rotation indices were measured using a Schmidt & Haensch Polartronic-D polarimeter in a 5 cm cell. ^{1}H (200 MHz, 300 MHz, 400 MHz, 600 MHz), ^{13}C (50 MHz, 75 MHz, 100 MHz), and ^{19}F (188 MHz, 282 MHz) NMR spectra were recorded on a Varian Gemini 2000, Gemini 300, Bruker DRX-400 and Bruker AVANCE DRX-600 spectrometer, respectively. *TMS* was used as reference for ^{1}H and ^{13}C NMR spectra (internal), and CF_3COOH for ^{19}F NMR spectra (external). IR spectra were obtained with a FTIR spectrometer (Genesis ATI Mattson/Unicam). Mass spectra were recorded on a Finnigan ZAB-HSQ spectrometer (FAB-matrix: 3-*NBA*).

Synthesis of N-Glycosylated Malic and Citramalic Acid Derivatives

Protocol 1. A solution of **3** (1 equiv.) in dry *DCM* (5 cm^3 per 1 mmol) was cooled to 0°C. Then a freshly prepared solution of the corresponding β-*D*-glycosylamine **5** (1 equiv.) and pyridine (1 equiv.) in *DCM* (5 cm^3 per 1 mmol) was added over a period of 30 min. After stirring the mixture for 1 h at 0°C *DCM* was added (20 cm^3 per 1 mmol). The organic layer was washed with ice-cold citric acid (10% solution) and ice water (10 cm^3 per 1 mmol). After drying with $MgSO_4$, the solvent was removed and the crude product recrystallized.

Protocol 2. A solution of **6** (1 equiv.) in dry $CHCl_3$ (20 cm^3 per 1 mmol) was heated after addition of pyridine (5 equiv.) under reflux for 16 h. Then $CHCl_3$ (40 cm^3 per 1 mmol) was added and the organic layer was washed with citric acid (10% solution) and water (10 cm^3 per 1 mmol). After drying the organic layer with $MgSO_4$ the solvent was removed and the crude product purified by crystallization or flash chromatography.

Protocol 3. A solution of **6** (1 equiv.) in dry ethyl acetate (10 cm^3 per 1 mmol) was treated with a base (1 equiv.) at room temperature until the starting material was consumed (TLC analysis). Then

ethyl acetate was added (20 cm^3 per 1 mmol) and the organic layer was extracted with citric acid (10% solution) and water (10 cm^3 per 1 mmol). After drying with $MgSO_4$ the solvent was evaporated and the residue purified by flash chromatography.

Protocol 4. To a solution of the amino acid *tert*-butylester hydrochloride (1.2 equiv.) and *NEM* (1.2 equiv.) in dimethylformamide (*DMF*) (5 cm^3 per 1 mmol) a solution of **6** (1 equiv.) in ethyl acetate (5 cm^3 per 1 mmol) was added. The mixture was stirred at room temperature until the starting material was consumed (TLC analysis). Then the solvent was evaporated *in vacuo* and the residue dissolved in $CHCl_3$ (50 cm^3 per 1 mmol). The organic layer was washed with citric acid (10% solution), water, and brine (10 cm^3 per 1 mmol). After drying with $MgSO_4$ the organic solvent was removed and the residue purified by column chromatography.

N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-[(5S)-4-oxo-2,2-bis(trifluoromethyl)-1,3-dioxolan-5-yl]acetamide (**6a**, $C_{21}H_{23}F_6NO_{13}$)

3a (1.85 g, 6.16 mmol) and **5a** (2.14 g, 6.16 mmol) were reacted due to protocol 1. Purification by crystallization from $CHCl_3$/petroleum ether. Yield 3.35 g (89%), colorless needles, mp 165–166°C, $[\alpha]_D = -1^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.5$, *DCM*); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 2.02$ (s, 6H), 2.03 (s, 3H), 2.07 (s, 3H) (4OAc), 2.61 (dd, 1H, $J = 8.5$, 16.2 Hz, CH_2^{Mal}), 2.93 (dd, 1H, $J = 2.8$, 16.2 Hz, CH_2^{Mal}), 3.84 (m, 1H, 5-H), 4.08 (dd, 1H, $J = 2.1$, 12.3 Hz, 6-H_a), 4.28 (dd, 1H, $J = 4.8$, 12.3 Hz, 6-H_b), 4.90 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.04 (dd, 1H, $J = 9.6$, 9.9 Hz, 4-H), 5.16 (dd, 1H, $J = 2.8$, 8.5 Hz, CH^{Mal}), 5.27 (dd, 1H, $J = 9.3$, 9.6 Hz, 1-H), 5.31 (dd, 1H, $J = 9.6$, 9.6 Hz, 3-H), 6.06 (d, 1H, $J = 9.3$ Hz, 1-NH) ppm; ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -3.14$ (q, 3F, $J = 9.0$ Hz), -2.86 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HETCOR): $\delta = 20.76$, 20.97, 21.00, 21.11 (4OAc), 38.48 (CH_2^{Mal}), 61.16 (6-CH_2), 68.66 (4-CH), 71.12 (2-CH), 72.26 (CH^{Mal}), 72.92 (3-CH), 74.31 (5-CH), 78.79 (1-CH), 98.19 (sept, $J = 36$ Hz), 119.23 (q, $J = 286$ Hz), 120.03 (q, $J = 289$ Hz), 167.04, 167.45, 170.06, 170.25, 171.06, 171.58 ppm; IR (KBr): $\bar{\nu} = 1854$, 1753, 1706, 1543 cm^{-1}; MS (FAB): $m/z = 612.1$ $[M + H]^+$.

N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-[(5S)-5-methyl-4-oxo-2,2-bis(trifluoromethyl)-1,3-dioxolan-5-yl]acetamide (**6b**, $C_{22}H_{25}F_6NO_{13}$)

3b (2.10 g, 6.68 mmol) and **5a** (2.32 g, 6.68 mmol) were reacted due to protocol 1. Purification by crystallization from ethyl acetate/petroleum ether. Yield 3.80 g (91%), colorless needles, mp 106–108°C, $[\alpha]_D = -8^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.72$ (s, 3H, CH_3^{Citr}), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H) (4OAc), 2.67 (d, 1H, $J = 15.0$ Hz, CH_2^{Citr}), 2.77 (d, 1H, $J = 15.0$ Hz, CH_2^{Citr}), 3.79 (ddd, 1H, $J = 1.8$, 4.8, 9.9 Hz, 5-H), 4.01 (dd, 1H, $J = 1.8$, 12.3 Hz, 6-H_a), 4.28 (dd, 1H, $J = 4.8$, 12.3 Hz, 6-H_b), 4.87 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 4.97 (dd, 1H, $J = 9.6$, 9.9 Hz, 4-H), 5.22 (dd, 1H, $J = 9.3$, 9.3 Hz), 5.24 (dd, 1H, $J = 9.6$, 9.3 Hz) (1,3-H), 6.81 (d, 1H, $J = 9.3$ Hz, 1-NH) ppm; ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -2.63$ (q, 3F, $J = 9.0$ Hz), -2.08 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.28$, 20.47, 20.52, 20.59 (4OAc), 22.54 (CH_3^{Citr}), 43.53 (CH_2^{Citr}), 61.88 (6-CH_2), 68.20, 70.25, 72.83, 73.75 (2,3,4,5-CH), 77.93 (1-CH), 79.96 (C^{Citr}), 97.14 (sept, $J = 36$ Hz), 119.0 (q, $J = 290$ Hz), 119.1 (q, $J = 287$ Hz), 166.51, 169.10, 169.68, 169.93, 170.49, 170.72 ppm; IR (KBr): $\bar{\nu} = 1843$, 1755, 1710, 1541 cm^{-1}; MS (FAB): $m/z = 626.1$ $[M + H]^+$.

N-(2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl)-2-[(5S)-4-oxo-2,2-bis(trifluoromethyl)-1,3-dioxolan-5-yl]acetamide (**6c**, $C_{41}H_{39}F_6NO_9$)

3a (802 mg, 2.67 mmol) and **5b** (1.44 g, 2.67 mmol) were reacted due to protocol 1. Purification by crystallization from $CHCl_3$/petroleum ether. Yield 1.70 g (79%), colorless crystals, mp 161–162°C,

$[\alpha]_D = -11^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 2.0$, CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 2.41$ (dd, 1H, $J = 7.9$, 16.6 Hz, CH_2^{Mal}), 2.67 (dd, 1H, $J = 3.0$, 16.6 Hz, CH_2^{Mal}), 3.37 (dd, 1H, $J = 9.0$, 9.0 Hz, 2-H), 3.53 (m, 1H, 5-H), 3.69 (dd, 1H, $J = 1.8$, 10.7 Hz, 6-H_a), 3.72–3.80 (m, 3H, 3-H, 4-H, 6-H_b), 4.46 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 4.54 (d, 1H, $J = 10.8$ Hz, CH_2Ph), 4.61 (d, 1H, $J = 12.1$ Hz, CH_2Ph), 4.65 (d, 1H, $J = 11.7$ Hz, CH_2Ph), 4.83 (d, 1H, $J = 11.7$ Hz, CH_2Ph), 4.84 (d, 1H, $J = 10.8$ Hz, CH_2Ph), 4.93 (s, 2H, CH_2Ph), 5.09 (dd, 1H, $J = 9.0$, 9.0 Hz, 1-H), 5.16 (dd, 1H, $J = 3.0$, 7.9 Hz, CH^{Mal}), 5.58 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.15–7.38 (m, 20H, H^{arom}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -3.01$ (q, 3F, $J = 9.0$ Hz), -2.69 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HMQC): $\delta = 37.87$ (CH_2^{Mal}), 68.16 (6-CH_2), 71.78 (CH^{Mal}), 73.73, 74.68, 75.09, 75.97 (4CH_2Ph), 76.53 (5-CH), 77.58 (4-CH), 79.10 (1-CH), 79.49 (2-CH), 86.09 (3-CH), 97.77 (sept, $J = 36$ Hz), 118.94 (q, $J = 288$ Hz), 119.66 (q, $J = 290$ Hz), 127.9–129.1, 137.76, 137.97, 138.11, 138.35 (C^{arom}), 166.30, 167.47 ppm; IR (KBr): $\bar{\nu} = 1851$, 1673, 1547 cm^{-1}; MS (FAB): $m/z = 826.2$ $[M + Na]^+$.

N-(2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl)-2-[(5S)-5-methyl-4-oxo-2,2-bis(trifluoromethyl)-1,3-dioxolan-5-yl]acetamide (**6d**, $C_{42}H_{41}F_6NO_9$)

3b (1.18 g, 3.75 mmol) and **5b** (2.02 g, 3.74 mmol) were reacted due to protocol 1. Purification by crystallization from ethyl acetate/petroleum ether. Yield 2.23 g (73%), colorless crystals, mp 129–131°C, $[\alpha]_D = -17^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 2.0$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.68$ (s, 3H, CH_3^{Citr}), 2.57 (d, 1H, $J = 15.0$ Hz, CH_2^{Citr}), 2.66 (d, 1H, $J = 15.0$ Hz, CH_2^{Citr}), 3.41 (m, 1H, 2-H), 3.55 (m, 1H, 5-H), 3.73 (dd, 1H, $J = 1.8$, 10.5 Hz, 6-H_a), 3.77–3.82 (m, 3H, 3-H, 4-H, 6-H_b), 4.52 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.57 (d, 1H, $J = 10.8$ Hz, CH_2Ph), 4.67 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.69 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 4.86 (d, 1H, $J = 10.8$ Hz, CH_2Ph), 4.88 (d, 1H, $J = 11.7$ Hz, CH_2Ph), 4.91 (s, 2H, CH_2Ph), 5.17 (dd, 1H, $J = 9.3$, 9.0 Hz, 1-H), 5.80 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.16–7.38 (m, 20H, H^{arom}); ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -2.68$ (q, 3F, $J = 9.0$ Hz), -2.29 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HETCOR, APT): $\delta = 22.48$ (CH_3^{Citr}), 44.43 (CH_2^{Citr}), 68.32 (6-CH_2), 73.90, 74.91, 75.25, 76.06 (4CH_2Ph), 76.76 (5-CH), 77.76 (4-CH), 79.10 (1-CH), 80.25 (C^{Citr}), 80.79 (2-CH), 86.19 (3-CH), 97.30 (sept, $J = 36$ Hz), 119.35 (q, $J = 289$ Hz), 128.0–128.8, 137.97, 138.18, 138.25, 138.50 (C^{arom}), 166.04, 169.35 ppm; IR (KBr): $\bar{\nu} = 1846$, 1668, 1558 cm^{-1}: MS (FAB): $m/z = 840.3$ $[M + Na]^+$, 818.3 $[M + H]^+$.

From the mother liquor 20 mg of the α-anomer were isolated and spectroscopically fully characterized. Separation by flash chromatography (ethyl acetate/petroleum ether = 1/4, R_f (**6d-α**) = 0.35, R_f (**6d-β** = 0.30); ^1H NMR ($CDCl_3$, 600 MHz, COSY): $\delta = 1.63$ (s, 3H, CH_3^{Citr}), 2.69 (s, 2H, CH_2^{Citr}), 3.49–3.52 (m, 2H, 3,5-H), 3.56 (dd, 1H, $J = 1.5$, 11.1 Hz, 6-H_a), 3.62 (dd, 1H, $J = 9.9$, 9.6 Hz, 4-H), 3.65 (dd, 1H, $J = 3.6$, 11.1 Hz, 6-H_b), 3.74 (dd, 1H, $J = 5.7$, 9.6 Hz, 2-H), 4.38 (d, 1H, $J = 12.5$ Hz, CH_2Ph), 4.45 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 4.49–4.56 (m, 3H, CH_2Ph), 4.69 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 4.71 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 4.80 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 5.66 (dd, 1H, $J = 5.7$, 6.6 Hz, 1-H), 6.41 (d, 1H, $J = 6.6$ Hz, 1-NH), 7.06–7.34 (m, 20H, H^{arom}) ppm; ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = -2.76$ (q, 3F, $J = 9.0$ Hz), -2.34 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HMQC, APT): $\delta = 22.46$ (CH_3^{Citr}), 44.37 (CH_2^{Citr}), 68.34 (6-CH_2), 71.77 (5-CH), 72.96, 73.65, 74.93 (3CH_2Ph), 75.28 (1-CH), 75.67 (CH_2Ph), 76.96 (4-CH), 77.41 (2-CH), 79.99 (C^{Citr}), 82.02 (3-CH), 97.26 (sept, $J = 36$ Hz), 119.21 (q, $J = 289$ Hz), 127.7–128.7, 137.20, 138.03, 138.27, 138.34 (C^{arom}), 166.52, 169.45 ppm.

(3S)-3-Hydroxy-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)pyrrolidin-2,5-dione (**7a**, $C_{18}H_{23}NO_{12}$)

6a (611 mg, 1.00 mmol) was heated with pyridine (396 mg, 5.00 mmol) in dry $CHCl_3$ under reflux for 16 h due to protocol 2. Purification by flash chromatography (ethyl acetate/petroleum ether = 4/1, $R_f = 0.38$). Yield 350 mg (79%), needles from ethyl acetate/petroleum ether, mp 174–176°C, $[\alpha]_D = -33^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.0$, acetone); ^1H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.95$ (s,

3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H) (4OAc), 2.63 (dd, 1H, $J = 4.5$, 18.3 Hz, CH_2^{Mal}), 3.06 (dd, 1H, $J = 8.4$, 18.3 Hz, CH_2^{Mal}), 3.80 (m, 1H, 5-H), 4.14–4.18 (m, 2H, 6-H_2), 4.56 (dd, 1H, $J = 4.5$, 8.4 Hz, CH^{Mal}), 5.18 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.26 (dd, 1H, $J = 9.6$, 9.0 Hz, 3-H), 5.27 (d, 1H, $J = 9.3$ Hz, 1-H), 5.86 (dd, 1H, $J = 9.3$, 9.3 Hz, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.55$, 20.66 (2c), 20.81 (4OAc), 37.14 (CH_2^{Mal}), 61.77 (6-CH_2), 66.49, 67.82, 67.90, 73.33, 74.83 (2,3,4,5-CH, CH^{Mal}), 78.29 (1-CH), 169.55, 169.86, 170.24, 170.87, 172.61, 176.53 ppm; IR (KBr): $\bar{\nu} = 3430$, 1751, 1628 cm^{-1}; MS (FAB): $m/z = 446.1$ $[M + H]^+$.

(3S)-3-Hydroxy-3-methyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)pyrrolidin-2,5-dione (**7b**, $C_{19}H_{25}NO_{12}$)

6b (625 mg, 1.00 mmol) was heated with pyridine (396 mg, 5.00 mmol) in dry $CHCl_3$ under reflux for 16 h due to protocol 2. Purification by crystallization from ethyl acetate/petroleum ether. Yield 361 mg (79%), mp 182–184°C, $[\alpha]_D = -16°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 2.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.53$ (s, 3H, CH_3^{Citr}), 1.97 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H) (4OAc), 2.75 (d, 1H, $J = 18.0$ Hz, CH_2^{Citr}), 2.86 (d, 1H, $J = 18.0$ Hz, CH_2^{Citr}), 3.43 (s, 1H, OH), 3.82 (m, 1H, 5-H), 4.18–4.21 (m, 2H, 6-H_2), 5.22 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.28 (d, 1H, $J = 9.3$ Hz, 1-H), 5.30 (dd, 1H, $J = 9.3$, 9.6 Hz, 3-H), 5.88 (dd, 1H, $J = 9.3$, 9.3 Hz, 2-H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.48$, 20.65 (2c), 20.81 (4OAc), 24.74 (CH_3^{Citr}), 43.34 (CH_2^{Citr}), 61.80 (6-CH_2), 67.89 (2c), 71.98, 73.26, 74.79 (2,3,4,5-CH, C^{Citr}), 78.35 (1-CH), 169.54, 169.77, 170.21, 170.86, 172.35, 178.28 ppm; IR (KBr): $\bar{\nu} = 3450$, 1735, 1730 cm^{-1}; MS (FAB): $m/z = 460.1$ $[M + H]^+$.

(3S)-3-Hydroxy-1-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)pyrrolidin-2,5-dione (**7c**, $C_{38}H_{39}NO_8$)

6c (402 mg, 0.50 mmol) was heated with pyridine (198 mg, 2.50 mmol) in dry $CHCl_3$ under reflux for 16 h due to protocol 2. Purification by flash chromatography (ethyl acetate/petroleum ether = 1/1, $R_f = 0.39$). Yield 268 mg (84%), needles from ethyl acetate/petroleum ether, mp 118–119°C; $[\alpha]_D = -30°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.9$, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 2.40$ (dd, 1H, $J = 5.1$, 18.0 Hz, CH_2^{Mal}), 2.92 (br.s, 1H, CH_2^{Mal}), 3.58 (m, 1H, 5-H), 3.64–3.75 (m, 4H, 3,4-H, 6-H_2), 4.46–4.60 (m, 6H, 2-H, CH^{Mal}, 4CH_2Ph), 4.83 (d, 1H, $J = 10.5$ Hz, CH_2Ph), 4.85 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.91 (s, 2H, CH_2Ph), 5.05 (d, 1H, $J = 9.3$ Hz, 1-H), 7.17–7.35 (m, 20H, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 37.29$ (CH_2^{Mal}), 66.65 (CH^{Mal}), 68.94 (6-CH_2), 73.72, 75.27, 75.44, 75.97 (4CH_2Ph), 77.43, 77.91, 78.24 (2,4,5-CH), 80.03 (1-CH), 86.82 (3-CH), 127.9–128.8, 138.18, 138.27, 138.63 (C^{arom}), 173.10, 177.83 ppm; IR (KBr): $\bar{\nu} = 3500$, 1760 cm^{-1}; MS (FAB): $m/z = 638.2$ $[M + H]^+$.

(3S)-3-Hydroxy-3-methyl-1-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)pyrrolidin-2,5-dione (**7d**, $C_{39}H_{41}NO_8$)

6d (409 mg, 0.50 mmol) was heated with pyridine (198 mg, 2.50 mmol) in dry $CHCl_3$ under reflux for 16 h due to protocol 2. Purification by flash chromatography (ethyl acetate/petroleum ether = 1/2, $R_f = 0.26$). Yield 287 mg (88%), oil which crystallizes within a few days, mp 151–152°C, $[\alpha]_D = -9°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.4$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.43$ (br.s, 3H, CH_3^{Citr}), 2.56 (br.d, 1H, $J = 18.0$ Hz, CH_2^{Citr}), 2.72 (br.d, 1H, $J = 18.0$ Hz, CH_2^{Citr}), 3.65 (m, 1H, 5-H), 3.72–3.83 (m, 4H, 3,4-H, 6-H_2), 4.55 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.61–4.69 (m, 4H, 2-H, 3CH_2Ph), 4.89 (d, 1H, $J = 10.8$ Hz, CH_2Ph), 4.90 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 4.93 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 4.98 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 5.15 (br.d, 1H, $J = 9.0$ Hz, 1-H), 7.22–7.39 (m, 20H, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 25.44$ (br., CH_3^{Citr}), 43.28 (br., CH_2^{Citr}), 68.65 (6-CH_2), 72.00, 73.52, 75.07, 75.26, 75.74 (4CH_2Ph, C^{Citr}), 76.99, 77.84, 78.32, 79.88 (br.), 86.65 (1,2,3,4,5-CH), 127.9–128.8, 138.19, 138.39, 138.52, 138.57 (C^{arom}), 173.13, 179.62 ppm; IR (KBr): $\bar{\nu} = 3500$, 1728 cm^{-1}. MS (FAB): $m/z = 652.3$ $[M + H]^+$.

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-pyrrol-2,5-dione (**8a**, $C_{18}H_{21}NO_{11}$)

A solution of **6a** (611 mg, 1.00 mmol) in dry ethyl acetate was stirred at room temperature with *DIEA* (129 mg, 1.00 mmol) due to protocol 3. Reaction time: 2 d. Purificaton by flash chromatography (ethyl acetate/petroleum ether = 3/1). Yield 72 mg (17%) **8a** ($R_f = 0.44$), mp 139–141°C, $[\alpha]_D = -5°$ cm^3 g^{-1} dm^{-1} ($c = 1.2$, $CHCl_3$) and 226 mg (51%) of a diastereomeric mixture of (3S)-**7a** and (3R)-**7a** (ratio 7:3, $R_f = 0.25$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.94$ (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H) (4OAc), 3.79 (m, 1H, 5-H), 4.17–4.20 (m, 2H, 6-H_2), 5.20 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.22 (d, 1H, $J = 9.3$ Hz, 1-H), 5.27 (dd, 1H, $J = 9.6$, 9.0 Hz, 3-H), 5.89 (dd, 1H, $J = 9.3$, 9.3 Hz, 2-H), 6.75 (s, 2H, H^{olef}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.55$, 20.69, 20.71, 20.84 (4OAc), 61.80 (6-CH_2), 67.91, 68.11, 73.63, 74.66 (2,3,4,5-CH), 77.62 (1-CH), 134.78, 168.71, 169.34, 169.48, 170.23, 170.77 ppm; IR (KBr): $\bar{\nu} = 1751$, 1728, 1628 cm^{-1}; MS (FAB): $m/z = 450.1$ $[M + Na]^+$, 428.1 $[M + H]^+$.

3-Methyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-pyrrol-2,5-dione (**8b**, $C_{19}H_{23}NO_{11}$)

A solution of **6b** (625 mg, 1.00 mmol) in dry ethyl acetate was stirred at room temperature with *DBU* (152 mg, 1.00 mmol) due to protocol 3. Reaction time: 1 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 3/2). Yield 52 mg (12%) **8b** ($R_f = 0.40$), mp 143–145°C, $[\alpha]_D = -8°$ cm^3 g^{-1} dm^{-1} ($c = 0.6$, $CHCl_3$) and 242 mg (53%) **7b** ($R_f = 0.22$); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 1.92$ (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H) (4OAc), 2.07 (d, 3H, $J = 2.0$ Hz, CH_3), 3.78 (ddd, 1H, $J = 2.3$, 4.7, 9.9 Hz, 5-H), 4.13 (dd, 1H, $J = 2.3$, 12.6 Hz, 6-H_a), 4.17 (dd, 1H, $J = 4.7$, 12.6 Hz, 6-H_b), 5.18 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.19 (d, 1H, $J = 9.3$ Hz, 1-H), 5.25 (dd, 1H, $J = 9.3$, 9.3 Hz, 3-H), 5.89 (dd, 1H, $J = 9.3$, 9.3 Hz, 2-H), 6.36 (m, 1H, H^{olef}) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta = 11.19$ (CH_3), 20.57, 20.68, 20.70, 20.82 (4OAc), 61.82 (6-CH_2), 67.90, 68.07, 73.71, 74.53 (2,3,4,5-CH), 77.59 (1-CH), 128.13, 146.46, 168.71, 169.29, 169.48, 169.82, 170.27, 170.77 ppm; IR (KBr): $\bar{\nu} = 1753$, 1728 cm^{-1}; MS (FAB): $m/z = 442.1$ $[M + H]^{-1}$.

tert-Butyl N-{(2S)-2-hydroxy-1,4-dioxo-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}glycinate (**9a**/**1**, $C_{24}H_{36}N_2O_{14}$)

6a (306 mg, 0.50 mmol) was reacted with HCl*H-*Gly*-O*t*Bu* (101 mg, 0.60 mmol) in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 1 d. Purification by flash chromatography (ethyl acetate, $R_f = 0.36$). Yield 220 mg (76%), foam, $[\alpha]_D = -6°$ cm^3 g^{-1} dm^{-1} ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.51$ (s, 9H, CH_3^{tBu}), 2.06 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H) (4OAc), 2.63 (dd, 1H, $J = 7.8$, 15.9 Hz, CH_2^{Mal}), 2.88 (dd, 1H, $J = 3.6$, 15.9 Hz, CH_2^{Mal}), 3.88 (m, 1H, 5-H), 3.97 (d, 2H, $J = 5.7$ Hz, CH_2^{Gly}), 4.13 (dd, 1H, $J = 1.8$, 12.3 Hz, 6-H_a), 4.33 (dd, 1H, $J = 4.5$, 12.3 Hz, 6-H_b), 4.49 (m, 1H, CH^{Mal}), 4.57 (br.s, 1H, OH), 5.00 (dd, 1H, $J = 9.6$, 9.6 Hz), 5.11 (dd, 1H, $J = 9.9$, 9.6 Hz), 5.34 (dd, 1H, $J = 9.6$, 9.0 Hz), 5.35 (dd, 1H, $J = 9.6$, 9.3 Hz) (1,2,3,4-H), 7.12 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.42 (t, 1H, $J = 5.7$ Hz, NH^{Gly}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 20.86$ (2c), 20.92, 21.01 (4OAc), 28.31 (CH_3^{tBu}), 39.77 (CH_2^{Mal}), 41.89 (CH_2^{Gly}), 61.93 (6-CH_2), 68.33, 69.09, 70.76, 73.02, 73.92 (2,3,4,5-CH, CH^{Mal}), 78.10 (1-CH), 82.75 (C^{tBu}), 169.15, 169.82, 170.24, 170.94, 171.34, 172.57, 174.75 ppm; IR (KBr): $\bar{\nu} = 3400$, 1751, 1676, 1535 cm^{-1}; MS (FAB): $m/z = 577.2$ $[M + H]^+$.

tert-Butyl N-{(2S)-2-hydroxy-1,4-dioxo-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}alaninate (**9a**/**2**, $C_{25}H_{38}N_2O_{14}$)

6a (611 mg, 1.00 mmol) was reacted with HCl*H-*Ala*-O*t*Bu* (218 mg, 1.20 mmol) in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 5/1). Yield 30 mg (7%) **7a** ($R_f = 0.30$) and 382 mg (65%) **9a**/**2** ($R_f = 0.23$), foam, $[\alpha]_D = -4°$ cm^3 g^{-1} dm^{-1} ($c = 1.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.40$ (d, 3H,

$J = 7.2$ Hz, CH_3^{Ala}), 1.48 (s, 9H, CH_3^{tBu}), 2.03 (s, 3H), 2.05 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H) (4OAc), 2.55 (dd, 1H, $J = 8.1$, 15.9 Hz, CH_2^{Mal}), 2.85 (dd, 1H, $J = 3.3$, 15.9 Hz, CH_2^{Mal}), 3.88 (m, 1H, 5-H), 4.11 (dd, 1H, $J = 2.1$, 12.6 Hz, 6-H_a), 4.31 (dd, 1H, $J = 4.5$, 12.3 Hz, 6-H_b), 4.41–4.46 (m, 2H, α-CH^{Ala}, CH^{Mal}), 4.51 (d, 1H, $J = 5.7$ Hz, OH), 4.97 (dd, 1H, $J = 9.6$, 9.6 Hz), 5.09 (dd, 1H, $J = 9.9$, 9.6 Hz), 5.32 (dd, 1H, $J = 9.3$, 9.3 Hz), 5.34 (dd, 1H, $J = 9.6$, 9.6 Hz) (1,2,3,4-H), 7.07 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.39 (d, 1H, $J = 7.8$ Hz, NH^{Ala}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 15.51$ (CH_3^{Ala}), 20.67 (2c), 20.72, 20.82 (4OAc), 28.04 (CH_3^{tBu}), 39.57 (CH_2^{Mal}), 48.56 (CH^{Ala}), 61.77 (6-CH_2), 68.20, 68.87, 70.60, 72.86, 73.73 (2,3,4,5-CH, CH^{Mal}), 77.92 (1-CH), 82.16 (C^{tBu}), 169.64, 170.09, 170.76, 171.10, 171.88, 171.91, 172.50 ppm; IR (KBr): $\bar{\nu} = 3400$, 1753, 1668, 1533 cm^{-1}; MS (FAB): $m/z = 591.2$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}valinate (**9a**/**3**, $C_{27}H_{42}N_2O_{14}$)

6a (611 mg, 1.00 mmol) and HCl*H-*Val*-O*t*-*Bu* (252 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/petroleum ether/*Me*OH = 10/5/1). Yield 48 mg (11%) **7a** ($R_f = 0.33$) and 440 mg (71%) **9a**/**3** ($R_f = 0.26$), foam, $[\alpha]_D = +5^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.93$ (d, 3H, $J = 6.9$ Hz, γ-CH_3^{Val}), 0.95 (d, 3H, $J = 6.6$ Hz, γ'-CH_3^{Val}), 1.49 (s, 9H, CH_3^{tBu}), 2.03 (s, 3H), 2.05 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H) (4OAc), 2.20 (m, 1H, β-CH^{Val}), 2.58 (dd, 1H, $J = 7.8$, 15.9 Hz, CH_2^{Mal}), 2.86 (dd, 1H, $J = 3.3$, 15.9 Hz, CH_2^{Mal}), 3.87 (m, 1H, 5-H), 4.10 (dd, 1H, $J = 1.8$, 12.3 Hz, 6-H_a), 4.31 (dd, 1H, $J = 4.2$, 12.3 Hz, 6-H_b), 4.39–4.43 (m, 2H, α-CH^{Val}, CH^{Mal}), 4.55 (br.s, 1H, OH), 4.96 (dd, 1H, $J = 9.6$, 9.6 Hz), 5.08 (dd, 1H, $J = 9.6$, 9.9 Hz), 5.29 (dd, 1H, $J = 9.0$, 9.3 Hz), 5.37 (dd, 1H, $J = 9.6$, 9.6 Hz) (1,2,3,4-H), 6.89 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.29 (d, 1H, $J = 9.0$ Hz, NH^{Val}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 17.60$ (γ-CH_3^{Val}), 19.03 (γ'-CH_3^{Val}), 20.66 (2c), 20.74, 20.81 (4OAc), 28.12 (CH_3^{tBu}), 31.51 (β-CH^{Val}), 39.39 (CH_2^{Mal}), 57.30 (α-CH^{Val}), 61.72 (6-CH_2), 68.18, 69.13, 70.52, 72.80, 73.70 (2,3,4,5-CH, CH^{Mal}), 77.93 (1-CH), 82.13 (C^{tBu}), 169.62, 170.07, 170.68, 170.75, 171.19, 172.22, 172.71 ppm; IR (KBr): $\bar{\nu} = 3400$, 1753, 1672, 1526 cm^{-1}; MS (FAB): $m/z = 619.3$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}phenylalaninate (**9a**/**4**, $C_{31}H_{42}N_2O_{14}$)

6a (306 mg, 0.50 mmol) and HCl*H-*Phe*-O*t*-*Bu* (155 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.6 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/*Me*OH = 10/1). Yield 22 mg (10%) **7a** ($R_f = 0.30$) and 233 mg (70%) **9a**/**4** ($R_f = 0.21$), foam, $[\alpha]_D = +23^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.49$ (s, 9H, CH_3^{tBu}), 1.99 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H) (4OAc), 2.39 (dd, 1H, $J = 8.3$, 15.9 Hz, CH_2^{Mal}), 2.74 (dd, 1H, $J = 3.3$, 15.9 Hz, CH_2^{Mal}), 3.03–3.07 (m, 2H, β-CH_2^{Phe}), 3.84 (ddd, 1H, $J = 2.1$, 4.2, 9.9 Hz, 5-H), 4.05 (dd, 1H, $J = 2.1$, 12.3 Hz, 6-H_a), 4.27 (dd, 1H, $J = 4.2$, 12.3 Hz, 6-H_b), 4.35 (m, 1H, CH^{Mal}), 4.45 (d, 1H, $J = 6.0$ Hz, OH), 4.69 (m, 1H, α-CH^{Phe}), 4.93 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.05 (dd, 1H, $J = 9.9$, 9.3 Hz, 4-H), 5.25 (dd, 1H, $J = 9.3$, 9.3 Hz, 1-H), 5.30 (dd, 1H, $J = 9.6$, 9.6 Hz, 3-H), 6.97 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.13–7.30 (m, 6H, H^{arom}, NH^{Phe}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HET-COR): $\delta = 20.83$ (2c), 20.91, 20.99 (4OAc), 28.18 (CH_3^{tBu}), 38.46 (β-CH_2^{Phe}), 39.58 (CH_2^{Mal}), 53.57 (α-CH^{Phe}), 61.90 (6-CH_2), 68.34 (4-CH), 69.10 (CH^{Mal}), 70.74 (2-CH), 72.97 (3-CH), 73.88 (5-CH), 78.10 (1-CH), 82.69 (C^{tBu}), 127.28, 128.68, 129.76, 136.28 (C^{arom}), 169.81, 170.23, 170.51, 170.93, 171.34, 172.02, 172.65 ppm; IR (KBr): $\bar{\nu} = 3400$, 1749, 1670, 1525 cm^{-1}; MS (FAB): $m/z = 667.3$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}prolinate (**9a**/**5**, $C_{27}H_{40}N_2O_{14}$)

6a (611 mg, 1.00 mmol) and HCl*H-*Pro*-O*t*-*Bu* (249 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromato-

graphy (ethyl acetate, $R_f = 0.29$). Yield 442 mg (72%), foam, $[\alpha]_D = -40^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.1$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz, 298 K, COSY): rotamers, ratio 5:1, δ (major rotamer) = 1.41 (s, 9H, CH_3^{tBu}), 1.99 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H) (4OAc), 1.85–2.25 (m, 4H, γ,β-CH_2^{Pro}), 2.35 (dd, 1H, $J = 9.3$, 14.4 Hz, CH_2^{Mal}), 2.54 (dd, 1H, $J = 3.0$, 14.4 Hz, CH_2^{Mal}), 3.48–3.63 (m, 2H, δ-CH_2^{Pro}), 3.82 (m, 1H, 5-H), 3.97 (d, 1H, $J = 7.8$ Hz, OH), 4.07 (dd, 1H, $J = 2.4$, 12.6 Hz, 6-H_a), 4.25 (dd, 1H, $J = 4.2$, 12.6 Hz, 6-H_b), 4.42 (m, 1H, α-CH^{Pro}), 4.58 (m, 1H, CH^{Mal}), 4.99 (dd, 1H, $J = 9.0$, 9.3 Hz, 2-H), 5.06 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.27 (dd, 1H, $J = 9.3$, 9.0 Hz, 1-H), 5.31 (dd, 1H, $J = 9.6$, 9.6 Hz, 3-H), 7.38 (d, 1H, $J = 9.0$ Hz, 1-NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HETCOR): δ (major rotamer) = 20.85 (2c), 20.93, 20.98 (4OAc), 25.08 (γ-CH_2^{Pro}), 28.18 (CH_3^{tBu}), 29.05 (β-CH_2^{Pro}), 41.82 (CH_2^{Mal}), 46.92 (δ-CH_2^{Pro}), 60.07 (α-CH^{Pro}), 62.15 (6-CH_2), 67.41 (CH^{Mal}), 68.48 (4-CH), 70.43 (2-CH), 73.26 (3-CH), 73.83 (5-CH), 78.23 (1-CH), 81.94 (C^{tBu}), 169.83, 170.21, 170.83, 170.88, 171.12, 171.20, 171.71 ppm; IR (KBr): $\bar{\nu} = 3400$, 1749, 1691, 1643, 1541 cm^{-1}; MS (FAB): $m/z = 617.1$ $[M + H]^+$.

N-{(2S)-1,4-Dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}-phenylalanine amide (**9a**/**6**, $C_{27}H_{35}N_3O_{13}$)

6a (306 mg, 0.50 mmol) and HCl*H-*Phe*-NH_2 (121 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/*Me*OH = 20/3, $R_f = 0.30$). Yield: 244 mg (80%), foam, $[\alpha]_D = +17^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.2$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 2.04$ (s, 3H), 2.05 (s, 3H), 2.06 (s, 6H) (4OAc), 2.22 (dd, 1H, $J = 9.6$, 15.9 Hz, CH_2^{Mal}), 2.65 (dd, 1H, $J = 3.0$, 15.9 Hz, CH_2^{Mal}), 3.04 (dd, 1H, $J = 7.5$, 13.8 Hz, β-CH_2^{Phe}), 3.15 (dd, 1H, $J = 6.0$, 13.8 Hz, β-CH_2^{Phe}), 3.86 (m, 1H, 5-H), 4.12 (dd, 1H, $J = 1.8$, 12.6 Hz, 6-H_a), 4.30 (dd, 1H, $J = 4.5$, 12.6 Hz, 6-H_b), 4.38 (m, 1H, CH^{Mal}), 4.66 (m, 1H, α-CH^{Phe}), 5.00 (dd, 1H, $J = 9.6$, 9.6 Hz), 5.11 (dd, 1H, $J = 9.9$, 9.6 Hz), 5.30 (dd, 1H, $J = 9.3$, 9.3 Hz), 5.34 (dd, 1H, $J = 9.6$, 9.6 Hz) (4,3,2,1-H), 6.46 (s, 1H, $CONH_2$), 6.60 (s, 1H, $CONH_2$), 7.23–7.35 (m, 5H, H^{arom}), 7.53 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.61 (d, 1H, $J = 8.4$ Hz, NH^{Phe}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.84$ (2c), 20.95 (2c) (4OAc), 38.22 (β-CH_2^{Phe}), 40.09 (CH_2^{Mal}), 54.26 (α-CH^{Phe}), 61.95 (6-CH_2), 68.35, 68.88, 70.75, 73.17, 73.89 (CH^{Mal}, 5,4,3,2-CH), 78.04 (1-CH), 127.43, 128.97, 129.54, 136.50 (C^{arom}), 169.91, 170.27, 171.05, 171.32, 172.49, 173.50, 172.25 ppm; IR (KBr): $\bar{\nu} = 3400$, 1749, 1668, 1527 cm^{-1}; MS (FAB): $m/z = 610.3$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}glycinate (**9b**/**1**, $C_{25}H_{38}N_2O_{14}$)

6b (625 mg, 1.00 mmol) and HCl*H-*Gly*-O^t*Bu* (201 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 1 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 5/1). Yield 74 mg (16%) **7b** ($R_f = 0.30$) and 369 mg (62%) **9b**/**1** ($R_f = 0.19$), foam, $[\alpha]_D = +25^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 2.0$, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.36$ (s, 3H, CH_3^{Citr}), 1.45 (s, 9H, CH_3^{tBu}), 1.98 (s, 3H), 2.00 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H) (4OAc), 2.43 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 2.85 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 3.78 (ddd, 1H, $J = 1.8$, 4.2, 9.9 Hz, 5-H), 3.79 (dd, $J = 5.4$, 18.0 Hz, CH_2^{Gly}), 3.89 (dd, 1H, $J = 5.4$, 18.0 Hz, CH_2^{Gly}), 4.05 (dd, 1H, $J = 1.8$, 12.3 Hz, 6-H_a), 4.26 (dd, 1H, $J = 4.2$, 12.3 Hz, 6-H_b), 4.92 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.03 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.19–5.28 (m, 3H, 1,3-H, OH), 7.08 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.34 (t, 1H, $J = 5.4$ Hz, NH^{Gly}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HETCOR): $\delta = 20.83$ (2c), 20.90, 20.99 (4OAc), 26.76 (CH_3^{Citr}), 28.30 (CH_3^{tBu}), 42.10 (CH_2^{Gly}), 43.54 (CH_2^{Citr}), 61.92 (6-CH_2), 68.29 (4-CH), 70.43 (2-CH), 73.00 (3-CH), 73.92 (5-CH), 74.81 (C^{Citr}), 77.91 (1-CH), 82.55 (C^{tBu}), 168.81, 169.76, 170.20, 170.90, 171.42, 172.42, 175.85 ppm; IR (KBr): $\bar{\nu} = 3400$, 1751, 1670, 1533 cm^{-1}; MS (FAB): $m/z = 591.2$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}alaninate (**9b**/**2**, $C_{26}H_{40}N_2O_{14}$)

6b (625 mg, 1.00 mmol) and HCl*H-*Ala*-O^t*Bu* (218 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/petroleum ether/*Me*OH = 10/5/1). Yield 276 mg (46%) **9b**/**2** ($R_f = 0.36$), foam, $[\alpha]_D = +22^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.5$, $CHCl_3$) and 119 mg (26%) **7b** ($R_f = 0.26$).

9b/**2**:^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.33$ (d, 3H, $J = 7.2$ Hz, CH_3^{Ala}), 1.35 (s, 3H, CH_3^{Citr}), 1.43 (s, 9H, CH_3^{tBu}), 1.99 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.12 (s, 3H) (4OAc), 2.42 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 2.81 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 3.77 (ddd, 1H, $J = 2.1$, 4.2, 9.9 Hz, 5-H), 4.05 (dd, 1H, $J = 2.1$, 12.3 Hz, 6-H_a), 4.24–4.30 (m, 2H, α-CH^{Ala}, 6-H_b), 4.92 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.04 (dd, 1H, $J = 9.6$, 9.6 Hz, 4-H), 5.17 (s, 1H, OH), 5.18 (dd, 1H, $J = 9.0$, 9.3 Hz, 1-H), 5.28 (dd, 1H, $J = 9.6$, 9.3 Hz, 3-H), 6.81 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.23 (d, 1H, $J = 7.5$ Hz, NH^{Ala}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 18.39$ (CH_3^{Ala}), 20.68 (2c), 20.73, 20.83 (4OAc), 26.23 (CH_3^{Citr}), 28.05 (CH_3^{tBu}), 43.18 (CH_2^{Citr}), 48.66 (α-CH^{Ala}), 61.69 (6-CH_2), 68.15, 70.20, 72.65, 73.78 (2,3,4,5-CH), 74.68 (C^{Citr}), 77.84 (1-CH), 81.92 (C^{tBu}), 169.61, 170.05, 170.72, 171.53, 171.71, 173.52, 174.94 ppm; IR (KBr): $\bar{\nu} = 3400$, 1751, 1666, 1533 cm^{-1}; MS (FAB): $m/z = 605.2$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}valinate (**9b**/**3**, $C_{28}H_{44}N_2O_{14}$)

6b (312 mg, 0.50 mmol) and HCl*H-*Val*-O^t*Bu* (126 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/petroleum ether/*Me*OH = 10/3/1). Yield 149 mg (47%) **9b**/**3** ($R_f = 0.38$), foamy solid, $[\alpha]_D = +23^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.6$, $CHCl_3$) and 80 mg (34%) **7b** ($R_f = 0.24$).

9b/**3**: ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.92$ (d, 3H, $J = 6.9$ Hz, γ-CH_3^{Val}), 0.93 (d, 3H, $J = 6.9$ Hz, γ′-CH_3^{Val}), 1.39 (s, 3H, CH_3^{Citr}), 1.48 (s, 9H, CH_3^{tBu}), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.17 (s, 3H) (4OAc), 2.18 (m, 1H, β-CH^{Val}), 2.45 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 2.86 (d, 1H, $J = 15.3$ Hz, CH_2^{Citr}), 3.82 (m, 1H, 5-H), 4.09 (dd, 1H, $J = 2.1$, 12.3 Hz, 6-H_a), 4.27–4.35 (m, 2H, α-CH^{Val}, 6-H_b), 4.96 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.08 (dd, 1H, $J = 9.6$, 9.9 Hz, 4-H), 5.16 (dd, 1H, $J = 9.0$, 9.3 Hz, 1-H), 5.31 (dd, 1H, $J = 9.6$, 9.3 Hz, 3-H), 5.35 (s, 1H, OH), 6.73 (d, 1H, $J = 8.7$ Hz, NH^{Val}), 7.25 (d, 1H, $J = 9.3$ Hz, 1-NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 17.47$ (γ-CH_3^{Val}), 19.07 (γ′-CH_3^{Val}), 20.70 (2c), 20.80, 20.84 (4OAc), 26.26 (CH_3^{Citr}), 28.14 (CH_3^{tBu}), 31.42 (β-CH^{Val}), 43.17 (CH_2^{Citr}), 57.42 (α-CH^{Val}), 61.66 (6-CH_2), 68.19, 70.09, 72.58, 73.72 (2,3,4,5-CH), 75.07 (C^{Citr}), 77.97 (1-CH), 81.95 (C^{tBu}), 169.63, 170.08, 170.56, 170.73, 171.83, 173.77, 175.19 ppm; IR (KBr): $\bar{\nu} = 3400$, 1751, 1668, 1524 cm^{-1}; MS (FAB): $m/z = 633.3$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}phenylalaninate (**9b**/**4**, $C_{32}H_{44}N_2O_{14}$)

6b (625 mg, 1.00 mmol) and HCl*H-*Phe*-O^t*Bu* (309 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/petroleum ether/*Me*OH = 10/5/1). Yield 290 mg (43%) **9b**/**4** ($R_f = 0.36$), foamy solid, $[\alpha]_D = +43^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.6$, $CHCl_3$) and 180 mg (39%) **7b** ($R_f = 0.25$).

9b/**4**: ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.35$ (s, 3H, CH_3^{Citr}), 1.39 (s, 9H, CH_3^{tBu}), 2.02 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H) (4OAc), 2.44 (d, 1H, $J = 15.6$ Hz, CH_2^{Citr}), 2.79 (d, 1H, $J = 15.6$ Hz, CH_2^{Citr}), 2.99–3.15 (m, 2H, β-CH_2^{Phe}), 3.82 (m, 1H, 5-H), 4.08 (dd, 1H, $J = 1.5$, 12.6 Hz, 6-H_a), 4.32 (dd, 1H, $J = 4.2$, 12.6 Hz, 6-H_b), 4.62 (m, 1H, α-CH^{Phe}), 4.96 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.08 (dd, 1H, $J = 9.6$, 9.9 Hz, 4-H), 5.19 (dd, 1H, $J = 9.3$, 9.3 Hz, 1-H), 5.29 (s, 1H, OH), 5.31 (dd, 1H, $J = 9.6$, 9.3 Hz, 3-H), 6.64 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.21–7.36 (m, 6H, NH^{Phe}, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 20.62$, 20.64, 20.69, 20.78 (4OAc), 26.19 (CH_3^{Citr}), 27.98

(CH_3^{tBu}), 38.18 (β-CH_2^{Phe}), 42.93 (CH_2^{Citr}), 53.51 (α-CH^{Phe}), 61.68 (6-CH_2), 68.11, 70.13, 72.67, 73.71 (2,3,4,5-CH), 74.66 (C^{Citr}), 77.72 (1-CH), 82.25 (C^{tBu}), 127.07, 128.55, 129.58, 136.13 (C^{arom}), 169.57, 169.98, 170.05, 170.69, 171.38, 173.45, 174.85 ppm; IR (KBr): $\bar{\nu} = 3400$, 1755, 1672, 1521 cm^{-1}; MS (FAB): $m/z = 681.3$ $[M + H]^+$.

N-{(2S)-1,4-Dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-amino]butyl}phenylalanine amide (**9b**/**5**, $C_{28}H_{37}N_3O_{13}$)

6b (625 mg, 1.00 mmol) and HCl*H-*Phe*-NH_2 (241 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography (gradient elution, $CHCl_3/MeOH = 15/1 \rightarrow 10/1$). Yield 150 mg (33%) **7b** ($R_f = 0.60$) and 290 mg (47%) **9b**/**5** ($R_f = 0.15$), foamy solid, $[\alpha]_D = +3^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.30$ (s, 3H, CH_3^{Citr}), 2.00 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H) (4OAc), 2.44 (d, 1H, $J = 15.6$ Hz, CH_2^{Citr}), 2.71 (d, 1H, $J = 15.6$ Hz, CH_2^{Citr}), 3.11–3.16 (m, 2H, β-CH_2^{Phe}), 3.81 (m, 1H, 5-H), 4.06 (dd, 1H, $J = 1.8$, 12.6 Hz, 6-H_a), 4.29 (dd, 1H, $J = 4.2$, 12.6 Hz, 6-H_b), 4.46 (m, 1H, α-CH^{Phe}), 4.92 (dd, 1H, $J = 9.6$, 9.6 Hz, 2-H), 5.05 (dd, 1H, $J = 9.6$, 9.9 Hz, 4-H), 5.16 (dd, 1H, $J = 9.3$, 9.3 Hz, 1-H), 5.21 (s, 1H, OH), 5.29 (dd, 1H, $J = 9.6$, 9.6 Hz, 3-H), 5.84 (s, 1H, NH_2^{Phe}), 6.06 (s, 1H, NH_2^{Phe}), 7.15 (d, 1H, $J = 9.0$ Hz, 1-NH), 7.21–7.34 (m, 5H, H^{arom}), 7.42 (d, 1H, $J = 8.4$ Hz, NH^{Phe}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 20.86$ (2c), 20.93, 20.97 (4OAc), 26.04 (CH_3^{Citr}), 37.51 (β-CH_2^{Phe}), 43.40 (CH_2^{Citr}), 54.76 (α-CH^{Phe}), 61.89 (6-CH_2), 68.31, 70.50, 73.08, 73.89 (2,3,4,5-CH), 74.62 (C^{Citr}), 77.78 (1-CH), 127.30, 128.99, 129.57, 137.05 (C^{arom}), 169.85, 170.24, 170.91, 171.41, 173.33 (2c), 175.95 ppm; IR (KBr): $\bar{\nu} = 3400$, 1751, 1670, 1522 cm^{-1}; MS (FAB): $m/z = 624.2$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-amino]butyl}phenylalaninate (**9c**/**1**, $C_{51}H_{58}N_2O_{10}$)

6c (804 mg, 1.00 mmol) and HCl*H-*Phe*-O^t*Bu* (309 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 3/2, $R_f = 0.29$). Yield 669 mg (78%), thin needles, mp 128–130°C, $[\alpha]_D = +1^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.4$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.40$ (s, 9H, CH_3^{tBu}), 2.30 (dd, 1H, $J = 8.2$, 16.1 Hz, CH_2^{Mal}), 2.66 (dd, 1H, $J = 3.6$, 16.1 Hz, CH_2^{Mal}), 3.08–3.12 (m, 2H, β-CH_2^{Phe}), 3.40 (m, 1H, 2-H), 3.54 (m, 1H, 5-H), 3.68–3.75 (m, 4H, 3,4-H, 6-H_2), 4.38 (m, 1H, CH^{Mal}), 4.44 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.50 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 4.60 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.61 (br.s, 1H, OH), 4.68 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.70 (m, 1H, α-CH^{Phe}), 4.81 (d, 1H, $J = 11.1$ Hz, CH_2Ph), 4.83 (d, 1H, $J = 12.0$ Hz, CH_2Ph), 4.90 (s, 2H, CH_2Ph), 5.08 (dd, 1H, $J = 9.3$, 9.3 Hz, 1-H), 5.96 (d, 1H, $J = 9.3$ Hz, 1-NH), 7.17–7.42 (m, 26H, NH^{Phe}, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, HETCOR, APT): $\delta = 28.22$ (CH_3^{tBu}), 38.57 (β-CH_2^{Phe}), 39.17 (CH_2^{Mal}), 53.62 (α-CH^{Phe}), 68.32 (6-CH_2), 68.98 (CH^{Mal}), 73.86, 74.87, 75.25, 76.04 (4CH_2Ph), 76.70 (5-CH), 77.77 (4-CH), 78.91 (1-CH), 79.71 (2-CH), 82.58 (C^{tBu}), 86.34 (3-CH), 127.2–129.9, 136.43, 137.98, 138.09, 138.28, 138.59 (C^{arom}), 170.47, 172.03, 172.54 ppm; IR (KBr): $\bar{\nu} = 3400$, 1730, 1662, 1525 cm^{-1}; MS (FAB): $m/z = 859.4$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-amino]butyl}prolinate (**9c**/**2**, $C_{47}H_{56}N_2O_{10}$)

6c (402 mg, 0.50 mmol) and HCl*H-*Pro*-O^t*Bu* (125 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 2/1, $R_f = 0.23$). Yield 298 mg (74%), foamy solid, $[\alpha]_D = -21^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K, COSY): rotamers,

ratio 5:1, δ (major rotamer) = 1.49 (s, 9H, CH_3^{tBu}), 1.82–2.24 (m, 4H, β,γ-CH_2^{Pro}), 2.45 (dd, 1H, J = 8.7, 14.8 Hz, CH_2^{Mal}), 2.57 (dd, 1H, J = 3.6, 14.8 Hz, CH_2^{Mal}), 3.38–3.51 (m, 3H, 2-H, δ-CH_2^{Pro}), 3.55 (m, 1H, 5-H), 3.68–3.75 (m, 4H, 3,4-H, 6-H_2), 4.08 (d, 1H, J = 7.4 Hz, OH), 4.43 (dd, 1H, J = 5.1, 8.4 Hz, α-CH^{Pro}), 4.50 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.52 (m, 1H, CH^{Mal}), 4.58 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.64 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.84–4.89 (m, 3H, CH_2Ph), 4.91 (d, 1H, J = 11.1 Hz, CH_2Ph), 4.96 (d, 1H, J = 11.1 Hz, CH_2Ph), 5.25 (dd, 1H, J = 9.3, 9.3 Hz, 1-H), 7.10 (d, 1H, J = 9.3 Hz, 1-NH), 7.29–7.41 (m, 20H, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): δ (major rotamer) = 25.13 (γ-CH_2^{Pro}), 28.28 (CH_3^{tBu}), 29.11 (β-CH_2^{Pro}), 42.26 (CH_2^{Mal}), 46.95 (δ-CH_2^{Pro}), 60.24 (α-CH^{Pro}), 67.00 (CH^{Mal}), 68.66 (6-CH_2), 73.77, 75.05, 75.24, 75.95 (4CH_2Ph), 76.62, 77.86, 79.33, 81.26 (5,4,2,1-CH), 82.05 (C^{tBu}), 86.15 (3-CH), 127.97–128.84, 138.12, 138.41, 138.57, 138.75 (C^{arom}), 170.88, 171.05, 171.60 ppm; IR (KBr): $\bar{\nu}$ = 3400, 1737, 1647, 1542 cm^{-1}; MS (FAB): m/z = 809.4 $[M+H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-amino]butyl}phenylalanylalaninate (**9c**/**3**, $C_{54}H_{63}N_3O_{11}$)

A solution of *Z-Phe-Ala-O^{t}Bu* (277 mg, 0.65 mmol) in ethyl acetate (40 cm^3) was vigorously stirred for 4 h in an atmosphere of hydrogen in the presence of 100 mg of 10% Pd/C catalyst. After filtration and removal of the solvent *in vacuo* the remaining residue was reacted with **6c** (434 mg, 0.54 mmol) in the presence of *NEM* (75 mg, 0.65 mmol) due to protocol 4. Reaction time: 3 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 5/2, R_f = 0.27). Yield 336 mg (67%), needles, mp 171–173°C, $[\alpha]_D = -21°$ cm^3 g^{-1} dm^{-1} (c = 2.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): δ = 1.31 (d, 3H, J = 7.2 Hz, CH_3^{Ala}), 1.44 (s, 9H, CH_3^{tBu}), 2.17 (dd, 1H, J = 8.7, 15.9 Hz, CH_2^{Mal}), 2.59 (dd, 1H, J = 3.6, 15.9 Hz, CH_2^{Mal}), 3.01–3.15 (m, 2H, β-CH_2^{Phe}), 3.36 (dd, 1H, J = 9.0, 9.0 Hz, 2-H), 3.51 (m, 1H, 5-H), 3.64–3.77 (m, 4H, 3,4-H, 6-H_2), 4.32–4.37 (m, 2H, CH^{Mal}, α-CH^{Ala}), 4.43 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.49 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.57 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.60 (m, 1H, α-CH^{Phe}), 4.65 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.79 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.80 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.89 (s, 2H, CH_2Ph), 5.03 (dd, 1H, J = 9.0, 9.3 Hz, 1-H), 5.90 (d, 1H, J = 9.0 Hz, 1-NH), 6.49 (d, 1H, J = 6.9 Hz, NH^{Ala}), 7.10–7.36 (m, 26H, NH^{Phe}, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): δ = 18.64 (CH_3^{Ala}), 28.23 (CH_3^{tBu}), 38.43 (β-CH_2^{Phe}), 39.46 (CH_2^{Mal}), 49.10 (α-CH^{Ala}), 54.31 (α-CH^{Phe}), 68.37 (6-CH_2), 68.97 (CH^{Mal}), 73.78, 74.93, 75.24, 76.03 (4CH_2Ph), 76.68, 77.80, 79.00, 80.09 (5,4,2,1-CH), 82.28 (C^{tBu}), 86.24 (3-CH), 127.2–129.7, 136.69, 137.91, 138.14, 138.27, 138.59 (C^{arom}), 170.25, 171.99, 172.34, 172.81 ppm; IR (KBr): $\bar{\nu}$ = 3400, 1736, 1645, 1544 cm^{-1}; MS (FAB): m/z = 952.4 $[M+Na]^+$, 930.4 $[M+H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)amino]butyl}phenylalaninate (**9d**/**1**, $C_{52}H_{60}N_2O_{10}$)

6d (409 mg, 0.50 mmol) and HCl*H-*Phe*-O^{t}Bu* (155 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 3 d. Purification by flash chromatography (gradient elution, ethyl acetate/petroleum ether = 1/2 → 1/1). Yield: 35 mg (11%) **7d** (R_f = 0.19) and 251 mg (58%) **9d**/**1** (R_f = 0.10), crystalline solid, mp 121–122°C, $[\alpha]_D = +24°$ cm^3 g^{-1} dm^{-1} (c = 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): δ = 1.42 (s, 3H, CH_3^{Citr}), 1.44 (s, 9H, CH_3^{tBu}), 2.37 (d, 1H, J = 15.6 Hz, CH_2^{Citr}), 2.75 (d, 1H, J = 15.6 Hz, CH_2^{Citr}), 2.99–3.14 (m, 2H, β-CH_2^{Phe}), 3.41 (m, 1H, 2-H), 3.56 (m, 1H, 5-H), 3.74–3.80 (m, 4H, 3,4-H, 6-H_2), 4.50 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.56 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.66 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.70 (m, 1H, α-CH^{Phe}), 4.77–4.88 (m, 3H, CH_2Ph), 4.95 (s, 2H, CH_2Ph), 5.12 (dd, 1H, J = 9.3, 9.0 Hz, 1-H), 5.64 (s, 1H, OH), 6.04 (d, 1H, J = 9.0 Hz, 1-NH), 7.16–7.45 (m, 26H, NH^{Phe}, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): δ = 26.38 (CH_3^{Citr}), 28.05 (CH_3^{tBu}), 38.22 (β-CH_2^{Phe}), 42.79 (CH_2^{Citr}), 53.38 (α-CH^{Phe}), 68.36 (6-CH_2), 73.86, 74.58, 74.79, 75.24, 76.04 (4CH_2Ph, C^{Citr}), 76.70, 77.78, 78.87, 79.64 (5,4,2,1-H), 82.30

(C^{tBu}), 86.27 (3-CH), 127.2–129.6, 136.39, 138.02, 138.08, 138.31, 138.60 (C^{arom}), 170.37, 173.20, 175.14 ppm; IR (KBr): $\bar{\nu} = 3400$, 1732, 1659, 1518 cm^{-1}; MS (FAB): $m/z = 873.4$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)amino]butyl}prolinate (**9d**/**2**, $C_{48}H_{58}N_2O_{10}$)

6d (409 mg, 0.50 mmol) and HCl*H-*Pro*-O^t*Bu* (125 mg, 0.60 mmol) were reacted in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 4 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 3/4). Yield: 155 mg (48%) **7d** ($R_f = 0.32$) and 141 mg (34%) **9d**/**2** ($R_f = 0.10$), foamy solid, $[\alpha]_D = +10°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.4$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, 300 K, COSY): rotamers, ratio 3:1, $\delta = 1.32$ (s, $CH_3^{Citr}{}_{min}$), 1.38 (s, $CH_3^{tBu}{}_{maj}$), 1.40 (s, $CH_3^{tBu}{}_{min}$), 1.44 (s, $CH_3^{Citr}{}_{maj}$), 1.70–1.92 (m, β,γ-$CH_2^{Pro}{}_{maj}$, γ-$CH_2^{Pro}{}_{min}$), 2.02–2.09 (m, β-$CH_2^{Pro}{}_{min}$), 2.24 (d, $J = 15.2$ Hz, $CH_2^{Citr}{}_{min}$), 2.28 (d, $J = 14.8$ Hz, $CH_2^{Citr}{}_{maj}$), 2.86 (d, $J = 15.2$ Hz, $CH_2^{Citr}{}_{min}$), 2.93 (d, $J = 14.8$ Hz, $CH_2^{Citr}{}_{maj}$), 3.32–3.36 (m, 2-H), 3.39–3.46 (m, 5-H, δ-$CH_2^{Pro}{}_{min}$), 3.63–3.70 (m, 3,4-H, 6-H_2), 3.77 (m, δ-$CH_2^{Pro}{}_{maj}$), 3.91 (m, δ-$CH_2^{Pro}{}_{maj}$), 4.10 (dd, $J = 3.9$, 8.2 Hz, α-$CH^{Pro}{}_{maj}$), 4.41 (d, $J = 12.0$ Hz, CH_2Ph_{min}), 4.42 (d, $J = 12.0$ Hz, CH_2Ph_{maj}), 4.46 (d, $J = 11.0$ Hz, $CH_2Ph_{min+maj}$), 4.55 (d, $J = 12.0$ Hz, CH_2Ph_{min}), 4.56 (d, $J = 12.0$ Hz, CH_2Ph_{maj}), 4.67 (d, $J = 10.4$ Hz, $CH_2Ph_{min+maj}$), 4.73 (α-$CH^{Pro}{}_{min}$), 4.74–4.86 (4 $CH_2Ph_{min+maj}$), 5.11 (dd, $J = 9.3$, 9.3 Hz, 1-H), 5.65 (br.s, OH_{maj}), 5.79 (br.s, OH_{min}), 5.99 (d, $J = 9.3$ Hz, 1-NH_{min}), 6.21 (d, $J = 9.3$ Hz, 1-NH_{maj}), 7.07–7.30 (H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, 300 K, HETCOR, APT): $\delta = 21.17$ (γ-$CH_2^{Pro}{}_{min}$), 25.41 ($CH_3^{Citr}{}_{maj}$), 25.62 (γ-$CH_2^{Pro}{}_{maj}$), 26.16 ($CH_3^{Citr}{}_{min}$), 28.18 (β-$CH_2^{Pro}{}_{maj}$), 28.30 (CH_3^{tBu}), 32.12 (β-$CH_2^{Pro}{}_{min}$), 44.71 ($CH_2^{Citr}{}_{min}$), 45.30 ($CH_2^{Citr}{}_{maj}$), 48.36 (δ-$CH_2^{Pro}{}_{min}$), 48.62 (δ-$CH_2^{Pro}{}_{maj}$), 61.33 (α-$CH^{Pro}{}_{min}$), 61.46 (α-$CH^{Pro}{}_{maj}$), 68.51 (6-CH_2), 73.84 (CH_2Ph), 74.74 (C^{Citr}), 75.00, 75.25, 76.00 (3CH_2Ph), 76.58 (5-CH_{min}), 76.74 (5-CH_{maj}), 77.69 (4-CH), 78.89 (1-CH_{min}), 79.08 (1-CH_{maj}), 80.53 (2-CH_{min}), 80.90 (2-CH_{maj}), 81.10 ($C^{tBu}{}_{maj}$), 81.35 ($C^{tBu}{}_{min}$), 86.06 (3-CH), 127.94–129.24, 138.06, 138.34 (2c), 138.72 (C^{arom}), 171.86, 172.41, 173.22, 173.63, 173.93 ppm; IR (KBr): $\bar{\nu} = 3400$, 1732, 1628, 1552 cm^{-1}; MS (FAB): $m/z = 823.4$ $[M + H]^+$.

tert-Butyl N-{(2S)-1,4-dioxo-2-hydroxy-2-methyl-4-[(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)amino]butyl}phenylalanylalaninate (**9d**/**3**, $C_{55}H_{65}N_3O_{11}$)

A solution of *Z-Phe-Ala-*O^t*Bu* (256 mg, 0.60 mmol) in ethyl acetate (40 cm^3) was vigorously stirred for 4 h in an atmosphere of hydrogen in the presence of 100 mg of 10% Pd/C. After filtration and removal of the organic solvent *in vacuo* the remaining residue was reacted with **6d** (409 mg, 0.50 mmol) in the presence of *NEM* (69 mg, 0.60 mmol) due to protocol 4. Reaction time: 5 d. Purification by flash chromatography (ethyl acetate/petroleum ether = 1/1). Yield: 54 mg (17%) **7d** ($R_f = 0.46$) and 337 mg (71%) **9d**/**3** ($R_f = 0.27$), crystalline solid, mp 154–156°C; $[\alpha]_D = -3°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 2.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, COSY): $\delta = 1.34$ (d, 3H, $J = 7.2$ Hz, CH_3^{Ala}), 1.40 (s, 3H, CH_3^{Citr}), 1.49 (s, 9H, CH_3^{tBu}), 2.31 (d, $J = 15.6$ Hz, CH_2^{Citr}), 2.69 (d, $J = 15.6$ Hz, CH_2^{Citr}), 3.04 (dd, 1H, $J = 6.3$, 13.8 Hz, β-CH_2^{Phe}), 3.20 (dd, 1H, $J = 6.3$, 13.8 Hz, β-CH_2^{Phe}), 3.38 (m, 1H, 2-H), 3.54 (m, 1H, 5-H), 3.71–3.81 (m, 4H, 3,4-H, 6-H_2), 4.39 (m, 1H, α-CH^{Ala}), 4.49 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.55 (d, 1H, $J = 10.5$ Hz, CH_2Ph), 4.56 (m, 1H, α-CH^{Phe}), 4.64 (d, 1H, $J = 12.3$ Hz, CH_2Ph), 4.76 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 4.83 (d, 1H, $J = 11.4$ Hz, CH_2Ph), 4.85 (d, 1H, $J = 10.5$ Hz, CH_2Ph), 4.94 (s, 2H, CH_2Ph), 5.08 (dd, 1H, $J = 9.3$, 9.0 Hz, 1-H), 5.55 (s, 1H, OH), 5.94 (d, 1H, $J = 9.0$ Hz, 1-NH), 6.54 (d, 1H, $J = 6.9$ Hz, NH^{Ala}), 7.15–7.43 (m, 26H, NH^{Phe}, H^{arom}) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz, APT): $\delta = 18.76$ (CH_3^{Ala}), 26.55 (CH_3^{Citr}), 28.21 (CH_3^{tBu}), 37.72 (β-CH_2^{Phe}), 42.92 (CH_2^{Citr}), 49.01 (α-CH^{Ala}), 54.15 (α-CH^{Phe}), 68.30 (6-CH_2), 73.84, 74.58, 74.76, 75.24, 76.06 (4 CH_2Ph, C^{Citr}), 76.67, 77.76, 78.81, 79.56 (5,4,2,1-CH), 82.19 (C^{tBu}), 86.24 (3-CH), 127.2–129.8, 136.56, 137.94, 138.03, 138.25, 138.52 (C^{arom}), 170.02, 171.97, 173.02, 175.85 ppm; IR (KBr): $\bar{\nu} = 3400$, 1732, 1653, 1528 cm^{-1}; MS (FAB): $m/z = 944.5$ $[M + H]^+$.

Synthesis of N-Glycosylated Derivatives of Thiomalic Acid

N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-[(4RS)-5-oxo-2,2-bis(trifluoromethyl)-1,3-oxathiolan-4-yl]acetamide (**10**, $C_{21}H_{23}F_6NO_{12}S$)

3c (2.66 g, 8.40 mmol) and **5a** (2.92 g, 8.40 mmol) were reacted due to protocol 1. Purification by flash chromatography (ethyl acetate/petroleum ether = 1/1, $R_f = 0.25$, both diastereomers). Yield 3.90 g (74%), recrystallization from toluene, mp 77–78°C; ^{1}H NMR ($CDCl_3$, 300 MHz): mixture of diastereomers, ratio 1:1, $\delta = 2.03$ (s, 6H), 2.04 (s, 6H), 2.07 (s, 6H), 2.09 (s, 6H) (OAc), 2.68 (dd, 1H, $J = 11.4$, 16.8 Hz), 2.81 (dd, 1H, $J = 9.9$, 16.8 Hz), 3.08 (dd, 1H, $J = 4.2$, 16.8 Hz), 3.25 (dd, 1H, $J = 3.3$, 16.8 Hz) ($CH_2^{Thiomal}$), 3.81 (ddd, 2H, $J = 2.1$, 4.5, 9.9 Hz, 5-H), 4.06–4.11 (m, 2H, 6-H_a), 4.29 (dd, 1H, $J = 4.5$, 12.3 Hz, 6-H_b), 4.33 (dd, 1H, $J = 4.5$, 12.3 Hz, 6-H_b), 4.49–4.54 (m, 2H, $CH^{Thiomal}$), 4.90 (dd, 2H, $J = 9.6$, 9.3 Hz), 5.06 (dd, 1H, $J = 9.9$, 9.6 Hz), 5.07 (dd, 1H, $J = 9.9$, 9.6 Hz), 5.20 (dd, 1H, $J = 9.3$, 9.3 Hz), 5.22 (dd, 1H, $J = 9.3$, 9.3 Hz), 5.31 (dd, 1H, $J = 9.6$, 9.3 Hz), 5.32 (dd, 1H, $J = 9.6$, 9.3 Hz) (1,2,3,4-H), 6.43 (d, 2H, $J = 9.0$ Hz, 1-NH) ppm; ^{19}F NMR ($CDCl_3$, 282 MHz): $\delta = 0.81$–0.97 (m, 6F), 1.54 (q, 3F, $J = 9.0$ Hz), 1.73 (q, 3F, $J = 9.0$ Hz) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 20.71$, 20.75, 20.84, 20.86 (4OAc), 39.76, 40.05 ($CH_2^{Thiomal}$), 41.80, 41.85 ($CH^{Thiomal}$), 61.94 (6-CH_2), 68.36 (4-CH), 70.90 (2-CH), 72.78, 72.84 (3-CH), 73.97, 74.04 (5-CH), 78.47, 78.55 (1-CH), 83.87 (sept, $J = 35.0$ Hz), 121.00 (q, $J = 283.0$ Hz), 121.10 (q, $J = 283.0$ Hz), 168.93, 169.14, 169.91 (2c), 170.11 (2c), 170.61, 170.69, 170.93, 170.96, 171.21, 171.35 ppm; IR (KBr): $\bar{\nu} = 1817$, 1754, 1799, 1543 cm^{-1}; MS (FAB): $m/z = 628.1$ $[M + H]^+$.

N-{(2RS)-1,4-Dioxo-2-mercapto-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino]butyl}-phenylalanine amide (**11**, $C_{27}H_{35}N_3O_{12}S$)

10 (627 mg, 1.00 mmol) and HCl*H-*Phe*-NH_2 (241 mg, 1.20 mmol) were reacted in the presence of *NEM* (138 mg, 1.20 mmol) due to protocol 4. Reaction time: 2 d. Purification by flash chromatography ($CHCl_3$/*Me*OH = 10/1, $R_f = 0.25$, both diastereomers). Yield 506 mg (81%), foamy solid; ^{1}H NMR (CD_3OD, 300 MHz): mixture of diastereomers, ratio 1:1, $\delta = 2.00$–2.05 (several s, 24H, OAc), 2.59 (dd, 1H, $J = 7.2$, 15.9 Hz, $CH_2^{Thiomal}$), 2.69 (dd, 1H, $J = 6.9$, 12.6 Hz, $CH_2^{Thiomal}$), 2.85–3.24 (m, 6H, $CH_2^{Thiomal}$, β-CH_2^{Phe}), 3.73–3.81 (m, 2H, $CH^{Thiomal}$), 3.93–3.40 (m, 2H, 5-H), 4.08–4.15 (m, 2H, 6-H_a), 4.25–4.32 (m, 2H, 6-H_b), 4.56 (dd, 1H, $J = 5.7$, 8.7 Hz, α-CH^{Phe}), 4.64 (dd, 1H, $J = 4.8$, 9.3 Hz, α-CH^{Phe}), 4.97–5.11 (m, 4H, 2,4-H), 5.33–5.42 (m, 4H, 1,3-H), 7.23–7.35 (m, 10H, H^{arom}) ppm; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 19.50$, 19.57, 19.60 (OAc), 37.15, 37.33 (β-CH_2^{Phe}, $CH_2^{Thiomal}$), 41.13 ($CH^{Thiomal}$), 54.63, 55.03 (α-CH^{Phe}), 62.04 (6-CH_2), 68.47, 70.86, 70.96, 73.51, 73.60, 73.65 (2,3,4,5-CH), 77.59, 77.68 (1-CH), 126.63, 126.69, 128.35, 128.40, 129.27, 129.35, 137.46 (C^{arom}), 170.23, 170.38, 170.51, 171.23, 171.74, 171.98, 173.04, 173.50, 174.65, 174.96 ppm; IR (KBr): $\bar{\nu} = 1749$, 1664, 1525 cm^{-1}; MS (FAB): $m/z = 626.2$ $[M + H]^+$.

Acknowledgements

We thank Deutsche Forschungsgemeinschaft (Bu 277/21-1) and Stiftung Volkswagenwerk, Hannover, for financial support.

References

[1] Burger K, Kluge M, Fehn S, Koksch B, Hennig L, Müller G (1999) Angew Chem Int Ed **38**: 1414
[2] (a) Varki A (1993) Glycobiology **3**: 97; (b) Dwek RA (1996) Chem Rev **96**: 683; (c) Kihlberg J, Ahman J, Walse B, Drakenberg T, Nilsson A, Söderberg-Ahlm C, Bengtsson B, Ohlson HJ (1995) Med Chem **38**: 161
[3] Opdenakker G, Rudd PM, Ponting CP, Dwek RA (1993) FASEB J **7**: 1330

[4] (a) Helenius A (1994) Mol Biol Cell **5**: 253; (b) Trombetta ES, Helenius A (1998) Curr Opin Struct Biol **8**: 587; (c) Helenius A, Aebi M (2001) Science **291**: 2364
[5] Hakomori SI (1989) Adv Cancer Res **52**: 257
[6] Roy R (1997) Top Curr Chem **187**: 241
[7] (a) Miller JS, Dudkin VJ, Lyon GJ, Muir TW, Danishefsky SJ (2003) Angew Chem Int Ed **42**: 431; (b) Danishefsky SJ, Allen J (2000) Angew Chem Int Ed **39**: 836; Lohof E, Planker E, Mang C, Burkhart F, Dechantsreiter MA, Haubner R, Wester H-J, Schwaiger M, Hölzemann G, Goodman SL, Kessler H (2000) Angew Chem Int Ed **39**: 2761; (d) Herpin TF, Motherwell WB, Weibel J-M (1997) Chem Commun 923; (e) O'Connor SE, Imperiali B (1997) J Am Chem Soc **119**: 2295; (f) Fisher JF, Harrison AW, Bundy GL, Wilkinson KF, Rush BD, Ruwart MJ (1991) J Med Chem **34**: 3141
[8] Harrison AW, Fisher JF, Guido DM, Couch SJ, Lawson JA, Sutter DM, Williams MV, DeGraaf GL, Rogers JE, Pals DT, DuCharme DW (1994) Biorg Med Chem **2**: 1339
[9] Risley JM, Huang DH, Kaylor JJ, Malik JJ, Xia Y-Q, York WM (2001) Arch Biochem Biophys **391**: 165
[10] (a) Simmons HE, Wiley DW (1960) J Am Chem Soc **82**: 2288; (b) Weygand F, Burger K, Engelhardt K (1966) Chem Ber **99**: 1461
[11] Coppola GM, Schuster HF (1997) α-Hydroxy Acids in Enantioselective Syntheses. VCH, Weinheim, p 191
[12] Böttcher C, Burger K (2002) Tetrahedron Lett **43**: 9711
[13] Burger K, Windeisen E, Pires R (1995) J Org Chem **60**: 7641
[14] Sjölin P, Kihlberg J (2001) J Org Chem **66**: 2957 and references cited therein
[15] Weygand F, Burger K (1996) Chem Ber **99**: 2880
[16] Burger K, Windeisen E, Heistracher E, Lange T, Abdel Aleem AAH (2002) Monatsh Chem **133**: 41
[17] Pumpor K, Windeisen E, Burger KJ (2003) Heterocycl Chem **40**: 435
[18] Burger K, Windeisen E, Pires R (1995) J Org Chem **60**: 7641
[19] (a) Pires R, Burger K (1996) Synthesis 1277; (b) Pires R, Burger K (1997) Tetrahedron **53**: 9213
[20] Kaduk C, Wenschuh H, Beyermann M, Forner K, Carpino LA, Bienert M (1995) Lett Pept Sci **2**: 285
[21] (a) Christiansen-Brams I, Meldal M, Bock K (1993) J Chem Soc Perkin Trans 1, 1461; (b) Ito Y, Gerz M, Nakahara Y (2000) Tetrahedron Lett **41**: 1039
[22] Ziegler T, Kaisers HJ, Schloemer R, Kocj C (1999) Tetrahedron **55**: 8397
[23] Ratcliff AJ, Fraser-Reid B (1990) J Chem Soc Perkin Trans 1, 747
[24] Böttcher C (2003) PhD Thesis, University of Leipzig
[25] Shin I, Jung H-J, Lee M-R (2001) Tetrahedron Lett **42**: 1325
[26] (a) Cohen DS, Fink CA, Trapani AJ, Webb RL, Zane PA, Chatelain RE (1999) Cardiovasc Drug Rev **17**: 16; (b) Gaucher JF, Selkti M, Tiraboschi G, Prangé T, Roques P, Thomas A, Fournié-Zaluski (1999) Bio-Chemistry **38**: 12569; (c) Bohacek R, Lombaert SD, McMartin C, Priestle J, Grütter M (1996) J Am Chem Soc **118**: 8231
[27] Kraus GA, Bae J, Choudhury PK (2003) Synthesis 19
[28] Kleemann A, Engel J (1987) Pharmazeutische Chemie. Synthesen – Patente – Anwendungen. Thieme, Stuttgart, p 1104
[29] (a) Robl JA (April 16, 1996) Bristol-Meyers-Squibb, Patent No US-5,508,272; Chem Abstr (1996) **125**: 33695h; (b) Karanewsky DS, Robl JA (September 3, 1996) Bristol-Meyers-Squibb, Patent No US-5,552,397; Chem Abstr (1966) **125**: 328738a
[30] Fray MJ, Ellis D (1998) Tetrahedron **54**: 13825
[31] (a) Soth MJ, Nowick JS (1999) Curr Opin Chem Biol **64**: 276; (b) Aubé J (ed) (2000) Recent Advances in Peptidomimetics, Tetrahedron Symposium in Print No 83 **56**: 9725
[32] Spengler J (unpublished results)