127. C-Glycoside Analogues of N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine: Synthesis and Conformational Analysis of a Cyclic C-Glycopeptide

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Dedicated to *Michael Hanack* on the occasion of his 65th birthday

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The synthesis of C-glycosidic analogues **15–22** of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine (Asn(N^4GlcNAc)) possessing a reversed amide bond as an isosteric replacement of the N-glycosidic linkage is presented. The peptide cyclo(-D-Pro-Phe-Ala-CGaa-Phe-Phe-) (CGaa = C-glycosylated amino acid; **24**) was prepared to demonstrate that 3-[(3-acetamido-2,6-anhydro-4,5,7-tri-O-benzyl-3-deoxy-β-D-glycero-D-guloheptonoyl)amino]-2-[(9H-fluoren-9-yloxycarbonyl)amino]propanoic acid (**22**) can be used in solid-phase peptide synthesis. The conformation of **24** was determined by NMR and molecular-dynamics (MD) techniques. Evidence is provided that the CGaa side chain interacts with the peptide backbone. The different C-glycosylated amino acids **15–21** were prepared by coupling 3-acetamido-2,6-anhydro-4,5,7-tri-O-benzyl-3-deoxy-β-D-*glycero*-D-*gulo*-heptonic acid (**4**) with diamino-acid derivatives **8–14** in 83–96% yield. The synthesis of **4** was performed from 2-(acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)tributylstannane (**2**) by treatment with BuLi and CO_2 in 83% yield. Similarly, propyl isocyanat yielded the glycoheptonamide **7** in 52% from **2**. Compound **2** was obtained from 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-D-glucopyranose (**1**) by chlorination and addition of tributyltinlithium in 74% yield. A procedure for a multigram-scale synthesis of **1** is given.

1. Introduction. – During the last decade, the interest in the synthesis of glycopeptides [1] steadily increased. They constitute partial structures of naturally occurring glycoproteins [2] and serve as model compounds which contribute to elucidate the function of oligosaccharides on proteins in biochemical and immunochemical studies [3].

On the other hand, the effect of glycosylation on bioactive peptides may be exploited to overcome shortcomings of potential peptide drugs. Recent investigations revealed that the attachment of saccharides to peptides improve bioavailability [4], increase resistance to proteases [5], improve water solubility [6], or enable them to penetrate the blood-brain barrier [7]. However, O- and N-linked glycopeptides are also subject to both chemical and enzymatic degradation *in vivo* that limitate their use as potential drugs. C-Glycosidic analogues possessing a C–C bond instead of a C–N or a C–O bond are thus valuable target molecules. Some examples of C-glycosylated amino acids are reported (for a naturally occurring C-glycosylated amino acid, see [8]) [9–11], but the linker length between the sugar and the amino-acid moiety is only in two cases comparable to naturally occurring glycosylated amino acids. Isosteric replacements with the glycosidic O-atom being substituted by a methylene group are known for O-(β-D-xylopyranosyl)-L-serine [10] and O-(β-D-galactopyranosyl)-L-serine [11]. The latter amino acid was incorporated into a helical model peptide to address the effect of C-glycosylation on the secondary

structure. None of the examples above represent a *C*-glycosidic analogue of an *N*-glycosylated amino acid. In a recent communication, we reported a novel unnatural linkage between glucose and asparagine with an inverted amide bond relative to the natural *N*-glycoside [12].

Herein, we present the synthesis of *C*-glycosidic analogues of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine having a reversed amide bond as an isosteric replacement of the *N*-glycosidic linkage (*Fig. 1*) (for replacements of peptide bonds, see [13]). The heptonamides were prepared with different linker lengths and amino-acid protecting groups. A model *C*-glycopeptide [14] was synthesized by Fmoc chemistry on the solid support (Fmoc = (9*H*-fluoren-9-ylmethoxycarbonyl)), cyclized, and its conformation determined by NMR and molecular-dynamics (MD) methods.

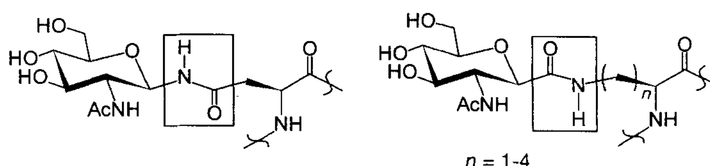
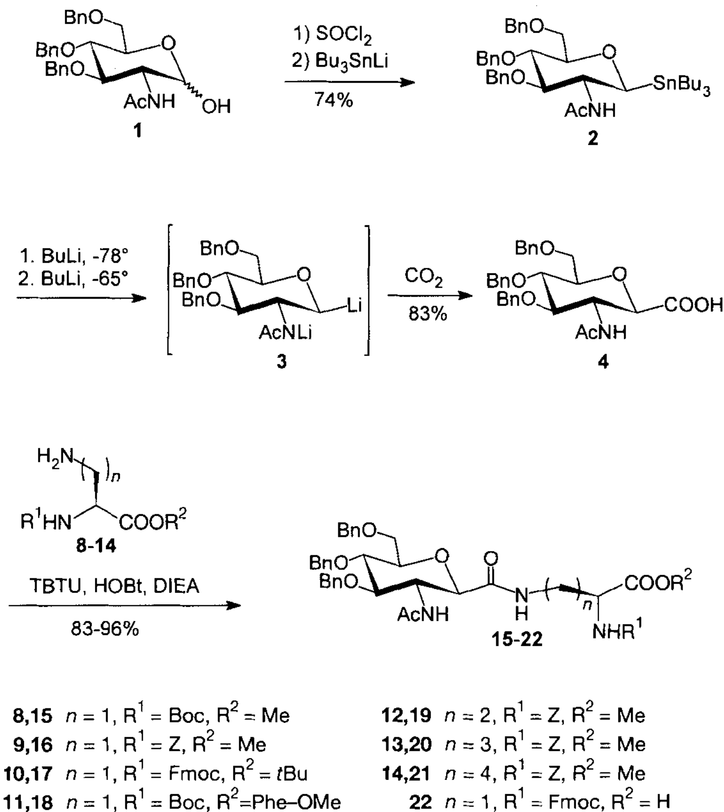


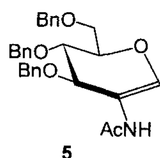
Fig. 1. N^4-*(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine and* C*-glycosidic analogues having an inverted amide bond*

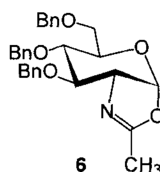
2. Results and Discussion. – 2.1. *Synthesis.* The *C*-glycosylated amino acids **15**–**21** were synthesized as outlined in *Scheme 1* from heptonic acid **4** and diamino-acid derivatives **8**–**14**. We obtained **4** from **1** according to a recently described procedure which allows the stereoselective synthesis of either α- or β-*C*-glycosides without neighbouring-group participation of the corresponding acetamide [15]. Alternatively, we tried to build up the *C*-glycosylated amino acids by the direct addition of an appropriate isocyanate to **3**. To test this route, propyl isocyanate was transformed to the heptonamide **7** in one step [16]. Due to the simple accessibility of the diamino-acid derivatives **8**–**14**, coupling of the heptonic acid **4** with **8**–**14** seemed more practical and was, therefore, exclusively pursued for the synthesis of the *C*-glycosylated amino acids.

Thus, 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucose (**1**) was synthesized in four steps from 2-acetamido-2-deoxy-D-glucose by a modified procedure of *Warren* and coworkers [17]. Purification in this batch process was achieved in the last step simply by crystallization (overall yield 38%). The improved synthesis [15] of **2** was accomplished by addition of the α-configurated sugar chloride obtained from **1** to a solution of Bu_3SnLi [18] at −78° (inverse addition) in 74% overall yield. Upscaling of the earlier reported synthesis which was performed by deprotonation of the sugar chloride by BuLi and addition of Bu_3SnLi gave the desired compound **2** in *ca.* 50% yield, besides glycal **5** in 6% and oxazoline **6** [17] in up to 23% yield. Substitution of BuLi by lithium diisopropylamide (LDA) prevented the elimination to **5**, but had, as expected, no influence on the amount of **6**. Since deprotonation of the amide favoured the formation of **6**, conditions where the sugar chloride is rapidly transformed to **2** should reduce the amount of **6**. Therefore, the sugar chloride was added to an excess of Bu_3SnLi. Using this inverse approach, only small amounts of **6** were observed by TLC.

Scheme 1. *Synthetic Route for the Preparation of the C-Glycosylated Amino Acids* **15–22**



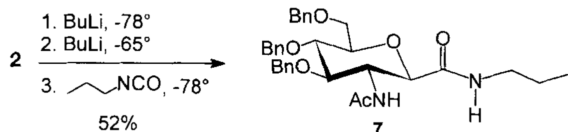




To form the *C*-glycosides, **2** was deprotonated at $-78°$ and transmetallated at $-65°$ by BuLi to give the intermediate **3**, indicated by a deep red colour of the solution. Experiments at lower temperatures than $-65°$ for the second step showed slow transmetallation. Subsequent addition of carbon dioxide provided **4** in 83% yield, besides 16% of 1-deoxysugar[1]). Similarly, propyl isocyanate yielded the desired glycoheptonamide **7** in 52% yield from **2** (*Scheme 2*).

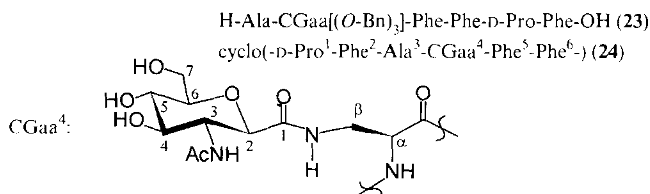
[1]) The by-product was formed during the preparation of **3**. However, its amount was reduced to *ca.* 1% by using MeLi for deprotonation at $-78°$ and BuLi at $-65°$ for transmetallation (details in [19]).

Scheme 2. *Synthesis of the Glycoheptonamide* **7**



Derivatives **8–11** of diaminopropionic acids and **12** of diaminobutyric acid were easily prepared from *C*- and *N*-protected asparagines (Asn)[2]) and glutamine using *I,I*-bis(trifluoroacetoxy)iodobenzene[3]) as a mild oxidant. The reagent is known to react only with primary amides. This allows to perform the reaction on intact peptides, as exemplified on Boc-Asn-Phe-OMe [20b] which was transformed to **11**. Thin-layer chromatography and ^{1}H-NMR of the crude amide-degradation products showed quantitative conversion; these products were used without further purification for the next step. Z-Orn-OMe · HCl (**13**) was prepared from Z-Orn-OH, whereas Z-Lys-OMe (**14**) was obtained by deprotection of Z-Lys(Boc)-OMe (Z = (benzyloxy)carbonyl, Boc = (*tert*-butoxy)carbonyl). Coupling of the amines **8–11** with **4** using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxybenzotriazol (HOBt) as coupling reagents [22] gave **15–18** in excellent yields. Since the isolation of **4** proved difficult, the synthesis of the *C*-glycosylated amino acids **20** and **21** were performed using crude **4** in a comparable overall yield starting from **2**.

The model peptide cyclo(-D-Pro-Phe-Ala-CGaa-Phe-Phe-) (**24**) was prepared using the *C*-glycosylated amino acid **22** for peptide synthesis. The latter was liberated from **17** by CF_3COOH[4]). Cyclic hexapeptides of the type cyclo(-D-Pro-Phe-Ala-Gaa-Phe-Phe-) (Gaa = glycosylated amino acid) have been prepared before determining the conformational influence of the sugar moiety on the peptide backbone [14]. Thus, first the linear hexapeptide H-Ala-CGaa[(*O*-Bn)$_3$]-Phe-Phe-D-Pro-Phe-OH (**23**) was prepared on the 2-chlorotrityl resin [23] using Fmoc-protected amino acids (1.1 equiv. of **22**, in all other cases 2.5 equiv.) and TBTU/HOBt for activation [22]. The linear peptide **23** was then cyclized by diphenylphosphoryl azide [24] in the presence of $NaHCO_3$ in DMF (quantitative) and the product purified by reversed-phase HPLC. The benzyl-ether groups were removed by hydrogenolysis in the presence of Pd/C in H_2O/AcOH 1:1 to provide the cyclic hexapeptide **24**.



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[2]) Z-Asn-OMe was obtained (in analogy to Boc-Asn-OMe) according to [20a] and Boc-Asn-Phe-OMe according to [20b]. Fmoc-Asn-O^{t}Bu was prepared from H-Asn-O^{t}Bu · HCl using $NaHCO_3$ and FmocONSu in THF/H_2O 5:3 in 93% yield after crystallization from MeCN (m.p. 136°).

[3]) For the preparation of diaminopropionic-acid analogs, see [21a], for the scope and mechanism of the reaction, see [21b], and for transpeptidation, see [21c].

[4]) To assure that also the *C*-glycosylated amino-acid derivatives **16** and **19–21** can be used in peptide chemistry, their Z protecting group was selectively cleaved by hydrogenolysis with H_2/Pd in MeOH/AcOH 10:1. This procedure had no influence on the benzyl-ether protecting groups.

2.2. *Conformational Analysis.* The conformation of cyclo(-D-Pro-Phe-Ala-CGaa-Phe-Phe-) (**24**) in (D_6)DMSO was investigated by NMR spectroscopy (assignment, see *Table 1*) and computer calculations as described previously [25] [26].

Table 1. *^{1}H- and ^{13}C-NMR Chemical Shifts* [ppm] *for* **24**

Residue	Group	δ(^{1}H)	δ(^{13}C)	Residue	Group	δ(^{1}H)	δ(^{13}C)
D-Pro[1]	CO		171.1	CGaa[4]	NH–C(β)	7.99	
	H–C(α)	4.08	59.4		C(1)O		171.0
	$H_{(proS)}$–C(β)	1.48	27.6		H–C(2)	3.61	77.2
	$H_{(proR)}$–C(β)	1.69			H–C(3)	3.74	53.6
	H–C(γ)	1.48	23.8		H–C(4)	3.38	74.3
	H′–C(γ)	1.84			H–C(5)	3.20	69.3
	H–C(δ)	3.03	46.5		H–C(6)	3.15	79.5
	H′–C(δ)	3.41			H–C(7)	3.52	60.2
Phe[2]	NH	8.50			H′–C(7)	3.66	
	CO		170.1		NH–C(3)	7.92	
	H–C(α)	4.34	53.3		CO(Ac)		170.1
	$H_{(proS)}$–C(β)	3.30	35.3		Me(Ac)	1.90	22.4
	$H_{(proR)}$–C(β)	2.74		Phe[5]	NH	7.84	
Ala[3]	NH	7.79			CO		169.4
	CO		171.1		H–C(α)	4.42	54.7
	H–C(α)	4.51	46.9		H–C(β)	2.95	37.9
	H–C(β)	1.49	16.6		H′–C(β)	3.08	
CGaa[4]	NH	8.15		Phe[6]	NH	7.69	
	CO		168.9		CO		169.1
	H–C(α)	3.87	56.8		H–C(α)	4.72	51.7
	H–C(β)	3.30	38.9		H–C(β)	2.91	37.8

To reduce overlap of NH signals, which was observed at 300 K, the measurements were performed at 325 K. One set of signals was observed in the NMR spectrum. All amide bonds in the backbone are *trans*-configurated since no strong H–C(α)/H–C(α) ROE's were observed. The two amide bonds in the CGaa[4] side chain are assumed to be *trans*-configurated which was confirmed later on in the detailed conformational analysis. H,H-Coupling constants (*Table 2*) within the sugar residue prove $^4C_1$-chair conformation.

A broad conformational search was performed for cyclopeptide **24** by the distance geometry calculation (DG) approach with a modified [27] version of the DISGEO [28] program using both ROE and coupling constants as experimental input. The lowest-error structure was used for restraint molecular dynamics (rMD) simulation in the explicit solvent DMSO [29]. The cyclic peptide shows a βII′ turn with D-Pro[1] in the $i + 1$ position and a distorted βI turn with CGaa[4] in the $i + 1$ position (*Table 3*, *Fig. 2*). All carbonyl groups of the non-prolinic amides show the preferred *cis*-orientation relative to the H–C(α) vector of the preceding amino acid. The only exception of the rule of the 1,3-allylic strain minimization [30] is the peptide bond for Phe[5]CO/Phe[6]NH which is twisted to orient the Phe[6]NH towards CGaa[4]CO(Ac). This can be taken as an indication of interaction between the sugar-containing side chain with the cyclic peptide backbone (see below). The βII′ turn region is well defined by the experimental data, whereas for the βI turn, data are less conclusive. The short distances from Phe[5]N*H* to Phe[5]*H*–C(α)

Table 2. *Experimental and Calculated Coupling Constants J(a,b) of* **24**. Calculated coupling constants were averaged over the rMD trajectories. Only those used during the DG calculation are presented.

Proton a	Proton b	$^3J_{exp}$ [Hz]	$^3J_{calc}$[a]) [Hz]	$^3J_{calc}$[b]) [Hz]
Phe²N*H*	Phe²*H*–C(α)	8.0	9.1	9.1
Ala³N*H*	Ala³*H*–C(α)	8.9	8.6	8.8
CGaa⁴N*H*	CGaa⁴*H*–C(α)	4.3	3.4	5.2
Phe⁵N*H*	Phe⁵*H*–C(α)	9.3	8.6	9.0
Phe⁶N*H*	Phe⁶*H*–C(α)	7.6	9.2	9.1
Pro¹*H*–C(α)	Pro¹$H_{(pro\text{-}R)}$–C(β)	9.3		
Pro¹*H*–C(α)	Pro¹$H_{(pro\text{-}S)}$–C(β)	4.0		
Phe²*H*–C(α)	Phe²$H_{(pro\text{-}R)}$–C(β)	10.4	12.2	12.2
Phe²*H*–C(α)	Phe²$H_{(pro\text{-}S)}$–C(β)	3.0	2.6	2.5
Phe⁵*H*–C(α)	Phe⁵*H*–C(β)	8.3		
Phe⁵*H*–C(α)	Phe⁵*H*–C(β)	7.0		
CGaa⁴*H*–C(2)	CGaa⁴*H*–C(1)	10.2		
CGaa⁴*H*–C(3)	CGaa⁴*H*–C(4)	10.2		
CGaa⁴*H*–C(4)	CGaa⁴*H*–C(5)	10.7		
CGaa⁴*H*–C(5)	CGaa⁴*H*–C(6)	7.6		
CGaa⁴*H*N–C(3)	CGaa⁴*H*–C(3)	9.0		

[a]) Obtained from MD1. [b]) Obtained from MD1 and MD2 in a ratio of 60:40.

Table 3. *Backbone Angles of the Turn Residues of* **24**. Angles were obtained from the averaged structure of the rMD trajectories (MD1 and MD2).

	MD1		MD2	
	ϕ	ψ	ϕ	ψ
D-Pro¹	60°	−129°	81°	−92°
Phe²	−100°	54°	−103°	−36°
CGaa⁴	−131°	−50°	−84°	−44°
Phe⁵	−106°	−51°	−135°	−60°

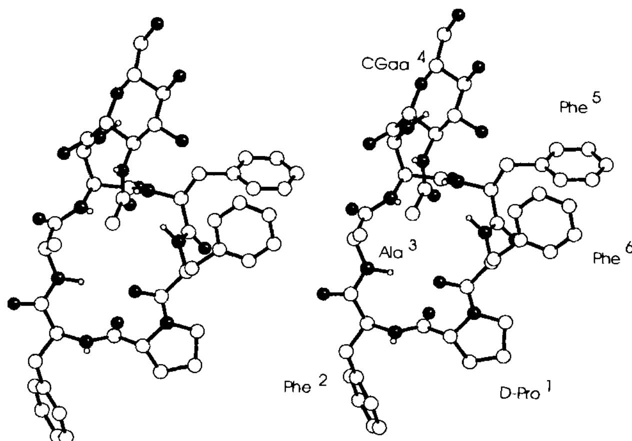

Fig. 2. *Conformation obtained for* **24**, *by averaging over the last 30 ps of the rMD trajectory* (MDI) *and subsequent energy minimization with 300 steps steepest descent.* According to the *Pachler* equation [30], the Phe² side chain populates a χ_1 angle of −60° to *ca.* 60%, whereas Phe⁵ is flexible according to the ^3J(H–C(α),H–C(β)) coupling constants. The 2 H–C(β) of Phe⁶ have identical chemical shifts, so parameters which determine the side-chain orientation cannot be obtained. Therefore, its depicted orientation is arbitrary.

and to CGaa[4]H–C(α) suggest the participation of a βII turn in an equilibrium with the predominant βI turn indicated by the DG and rMD (MD1) calculation. Such a βI/βII turn equilibrium was previously found in a number of related compounds [14]. However, in **24**, the distances between Phe[5]NH and Phe[5]H–C(α) and Gaa[4]H–C(α) are significantly longer than in the above discussed examples in the literature. This indicates a higher population of a βI turn in **24** which can be attributed to the H-bond between Phe[6]NH to the sugar side chain (see below).

An interesting feature of **24** is the preferred χ_1 angle of +60° for the side chain of CGaa[4] which is normally sterically less favoured. It results in a backfolding of this side chain over the peptide backbone with H-bonds occurring between CGaa[4]CO(Ac) and Phe[5]NH and Phe[6]NH (*Table 4*). This orientation was derived from the sum of the ^3J(H–C(α),H–C(β)) coupling constants of CGaa[4] of 10 Hz. According to the usual analysis [31], this value suggests a population of *ca.* 60% for the χ_1 angle of 60°. Further on, ROE's between the sugar moiety and especially Phe[5] and Phe[6]NH prove the proximity of the CGaa[4] side chain to the peptide backbone. To evaluate the possibility of the participation of another side-chain rotamer for CGaa[4], a second DG and rMD calculation (MD2) was performed omitting the ROE's which are responsible for the backfolding of the CGaa[4] side chain. It results in a χ_1 angle of −60° for the CGaa[4] side chain. ROE data averaged over both trajectories in a ratio of 60:40 (MD1/MD2) agree better with the experimental distances than those averaged over MD1 alone (*Table 5*), showing that both rotamers contribute to the observed ROE's. It should be mentioned that at all measuring conditions, the two H–C(β) signals are totally overlapped. Hence, individual ^3J(H–C(α),H–C(β)) coupling constants could not be obtained, and stereochemical assignment is impossible to obtain. Therefore, an experimental discrimination between the conformation χ_1 = +180° and χ_1 = −60° is not possible.

Table 4. *Occurrence of H-Bonding for* **24** *during rMD Trajectories and Temperature Coefficients.* Radial distribution functions calculated over MD1 agree with the observed temperature coefficients.

Donor	Acceptor	Population [%][a])	Population [%][b])	Temp. coeff. [ppb/K]
Ala[3]NH	Phe[6]CO	36.8	37.7	−2.0
Phe[2]NH	Phe[6]CO	5.0	10.2	−6.8
CGaa[4]NH	CGaa[4]C(1)–O	23.1	38.2	−2.7
Phe[5]NH	CGaa[4]CO(Ac)	97.2	48.6	−0.6
Phe[6]NH	CGaa[4]CO(Ac)	98.2	49.1	−4.8
CGaa[4]NH	CGaa[4]CO(Ac)	63.0	33.9	−2.7

[a]) Obtained from MD1. [b]) Obtained from MD1 and MD2 in a ratio of 60:40.

The conformational analysis exhibits distinct interaction of the sugar side chain with the peptide backbone. Evidence is provided that the side-chain orientation as well as the backbone conformation is mutually influenced by these interactions. According to the MD calculations, an interaction of the carbonyl O-atom of Ac with amide H-atoms of the cyclic backbone could be the origin of this effect.

3. Conclusion. – We devised a versatile synthetic route to a number of *C*-glycosidic analogues of Asn(GlcNAc). The synthesis allows the use of different amino-acid protecting groups and the variation of the linker length by employment of diamino acids. The

Table 5. *Experimental and Calculated Distances in* **24**. Experimental distances are calibrated on the distance between Phe^{2}$H_{(proR)}$–C(β) and Phe^{2}$H_{(proS)}$–C(β) being 178 ppm. Calculated distances are obtained by $< r^{-3} >$ averaging. Distance violations > 20% are given in bold figures. The ROE's indicating the backfolding of the CGaa^{4} side chain are printed in italic.

Atoms		d_{exp} [Å]	d_{calc}[a]) [Å]	d_{calc}[b]) [Å]
Phe^{2}NH	Ala^{3}NH	2.59	2.81	2.80
Phe^{5}NH	Phe^{6}NH	2.49	2.36	2.34
Phe^{2}NH	Pro^{1}H–C(α)	2.08	2.14	2.14
Phe^{2}NH	Phe^{2}H–C(α)	2.87	3.05	3.05
Phe^{2}NH	Phe^{2}$H_{(pro-R)}$–C(β)	2.48	2.55	2.53
Phe^{2}NH	Phe^{2}$H_{(pro-S)}$–C(β)	4.30	3.75	3.72
Ala^{3}NH	Pro^{1}H–C(α)	3.55	4.02	3.55
Ala^{3}NH	Phe^{2}H–C(α)	2.85	2.94	2.98
Ala^{3}NH	Ala^{3}H–C(α)	3.03	2.80	3.04
Ala^{3}NH	Ala^{3}H–C(β)	3.08	2.74[c])	2.76[c])
CGaa^{4}NH	Ala^{3}H–C(β)	2.73	2.57[c])	2.55[c])
CGaa^{4}NH	Ala^{3}H–C(α)	2.66	**3.34**	**3.25**
CGaa^{4}NH	CGaa^{4}H–C(α)	2.67	3.03	2.99
CGaa^{4}NH	CGaa^{4}H–C(3)	3.69	4.24	4.23
CGaa^{4}NH	CGaa^{4}H–C(2)	4.26	4.87	4.92
Phe^{5}NH	CGaa^{4}H–C(α)	2.88	**3.54**[d])	**3.55**[d])
Phe^{5}NH	Phe^{5}H–C(α)	2.66	3.06	3.06
Phe^{5}NH	Phe^{5}H–C(β)	3.20	2.81[c])	2.83[c])
Phe^{5}NH	Phe^{5}H′–C(β)	2.90		
Phe^{6}NH	Phe^{5}H–C(α)	3.24	3.59	3.59
Phe^{6}NH	Phe^{6}H–C(α)	2.87	3.03	3.03
Phe^{6}NH	*CGaa^{4}Me(Ac)*	4.76	**3.76**[c])	4.33[c])
Pro^{1}H–C(α)	Pro^{1}$H_{(pro-R)}$–C(β)	2.33	2.32	2.31
Pro^{1}H–C(α)	Pro^{1}$H_{(pro-S)}$–C(β)	2.64	2.84	2.81
Pro^{1}H–C(α)	Phe^{6}H–C(β)	4.39	4.83[c])	4.80[c])
Phe^{2}H–C(α)	Phe^{2}$H_{(pro-R)}$–C(β)	3.07	3.06	3.07
Phe^{2}H–C(α)	Phe^{2}$H_{(pro-S)}$–C(β)	2.45	2.57	2.58
Phe^{6}H–C(α)	Pro^{1}H–C(δ)	2.30	2.61[c])	2.60[c])
Phe^{6}H–C(α)	Pro^{1}H–C(δ)	2.29		
CGaa^{4}NH–C(β)	CGaa^{4}H–C(α)	3.15	**4.06**	3.34
Phe^{5}NH	*CGaa^{4}H–C(3)*	3.34	2.77	3.20
Phe^{6}NH	Phe^{6}H–C(β)	2.90	3.12[c])	3.11[c])
CGaa^{4}NH–C(β)	CGaa^{4}H–C(3)	2.88	2.66	2.95
CGaa^{4}NH–C(β)	CGaa^{4}H–C(2)	2.81	**3.49**	2.88
CGaa^{4}NH–C(β)	*Phe^{5}H–C(β)*	4.00	4.15[c])	4.66[c])
CGaa^{4}NH–C(3)	CGaa^{4}H–C(2)	2.69	2.84	2.77
CGaa^{4}NH–C(3)	CGaa^{4}H–C(3)	2.84	3.05	3.06
CGaa^{4}NH–C(3)	CGaa^{4}H–C(4)	2.42	2.41	2.49
Phe^{5}H–C(β)	*CGaa^{4}H–C(3)*	3.03	3.36[c])	**3.90**[c])
Phe^{5}H′–C(β)	*CGaa^{4}H–C(3)*	2.78	3.36[c])	
CGaa^{4}NH	CGaa^{4}NH–C(β)	3.87	4.01	3.93
CGaa^{4}Me(Ac)	*Phe^{6}H–C(β)*	4.03	4.29[c])	**4.96**[c])
Phe^{6}H–C(β)	Pro^{1}H–C(α)	4.39	4.83	4.80
Phe^{5}NH	CGaa^{4}H–C(β)	3.37	3.11[c])	3.08[c])
CGaa^{4}NH	CGaa^{4}H–C(β)	2.84	2.93[c])	2.82
Phe^{5}NH	*CGaa^{4}H–C(3)*	3.33	2.77	3.20
Phe^{5}NH	*CGaa^{4}H–C(2)*	4.20	4.89	**5.36**
Phe^{6}NH	*CGaa^{4}H–C(3)*	4.66	**3.72**	4.28

[a]) Obtained from MD1.

[b]) Obtained from MD1 and MD2 in a ratio of 60:40.

[c]) Distances are given to the corresponding C-atom. For those distance restraints, a pseudoatom corrrection was used during the calculation.

[d]) Violated due to βI/βII equilibrium.

glycosylated amino acid **22** was successfully utilized in standard solid-phase peptide synthesis. Alternatively, subsequent glycosylation of peptides is possible *via* amide degradation of asparagine-containing peptides as shown for Boc-Asn-Phe-OMe. NMR, DG, and MD investigations for the glycosylated hexapeptide cyclo(-D-Pro-Phe-Ala-CGaa-Phe-Phe-) (**24**) revealed a βII'-turn with D-Pro in the $i + 1$ position and a βI/βII-turn equilibrium with $CGaa^4$ in the $i + 1$ position. For the $CGaa^4$ side chain, a χ_1 angle of 60° is significantly populated which results in H-bonding between $CGaa^4$C*O*(Ac) and Phe^5N*H* and Phe^6N*H*. Comparing this result with previously studied similar cases gives evidence that in **24** the conformation of the peptide and the sugar-bearing side chain are influenced mutually.

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Experimental Part

1. *General.* Lithium (99.9%) was purchased from *Aldrich* and Z-Gln-OMe from *Bachem*. Moisture-sensitive reactions were performed under Ar. THF was distilled over sodium benzophenon ketyl, toluene distilled over Na and stored over 4 Å molecular sieves, and $CHCl_3$ and allyl alcohol dried over 4 Å molecular sieves. DMF and DMSO were distilled over CaH_2. Dioxane was dried over powdered KOH. Flash column chromatography (FC): silica gel *60* (40–60 µm) from *E. Merck*. TLC: silica gel plates (*$^{60}F_{254}$, E. Merck*); detection by UV light followed by charring with 5% H_2SO_4 in MeOH and 0.3% ninhydrin in BuOH/AcOH 79:3. M.p.: *Büchi-Tottoli* melting-point apparatus; uncorrected. Optical rotations: 1-dm cell; *Perkin-Elmer-241* polarimeter. IR Spectra: *Perkin-Elmer* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker-AC-250* or *-AMX-500*; δ(H) in ppm rel. to residual $CHCl_3$ (δ 7.24) or (D_5)DMSO (δ 2.49); δ(C) rel. to $CDCl_3$ (δ 77.0) and (D_6)DMSO (δ 39.5), assignment by HMQC [32] and HMQC-COSY [33] experiments; analysis of **24** in (D_6)DMSO at 325 K using ROESY [34] with a pulsed spinlock [35], PE-COSY [36], TOCSY [37], HMQC-COSY, and HMBC [38] experiments; for C-atom numbering of **15**–**22**, see **24**. FAB-MS: *Varian MAT 311 A* using 3-nitrobenzyl alcohol (NBA) or glycol matrix. Elemental analysis were performed at the Institute of Organic Chemistry and Biochemistry, TU München.

2. *2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**1**). A suspension of 2-acetamido-2-deoxy-D-glucose (50 g, 226 mmol), allyl alcohol (700 ml), and $BF_3 \cdot Et_2O$ (6.5 ml) was refluxed for 2 h (TLC ($MeCN/H_2O$ 4:1): R_f 0.68). The hot soln. was filtrated to remove impurities which might be present in the starting material, the filtrate was evaporated, and the solid residue dried *in vacuo* overnight. The crude product was suspended in dioxane (1 l), benzyl chloride (200 ml) and powdered KOH (150 g) were added, and the mixture was heated under reflux for 4 h (TLC (hexane/AcOEt 1:1): R_f 0.26). After evaporation, the orange residue was extracted with $CHCl_3/H_2O$, the org. phase dried (Na_2SO_4) and evaporated, and the residue co-evaporated with *p*-xylene (2 ×) and dried *in vacuo* overnight. The crude product was suspended in anh. DMSO (300 ml) and treated with KO^tBu (30 g). While the mixture was stirred for 3 h at 100° (TLC (hexane/AcOEt 1:1): R_f 0.34), the soln. turned dark brown. After being cooled, the soln. was diluted with H_2O (1 l) and the brown precipitate filtered off, dissolved in acetone (600 ml), and treated with 2M HCl (200 ml) under reflux for 30 min. H_2O (1 l) was added and the pale brown precipitate filtered off. Recrystallization (2 ×) from MeOH afforded **1** (42 g, 38%). Colourless needles. M.p. 212–214° ([17]: 216–219°). Anal. calc. for $C_{29}H_{33}NO_6$: C 70.86, H 6.77, N 2.85; found: C 70.85, H 6.69, N 2.83.

3. *(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)tributylstannane* (**2**). (Tributylstannyl) lithium [18] was prepared from Bu_3SnCl (34 ml, 125 mmol) which was added to pieces of Li (2.17 g, 313 mmol) in THF (70 ml). Within 10–30 min, heat was evolved and LiCl precipitated. The suspension was kept twice for *ca.* 30 min in the ultrasonic bath and stirred for *ca.* 4–6 h. Prior to use, the darkgreen reagent was removed from the residual Li.

At 20° and under Ar, **1** (20 g, 40.8 mmol) was suspended in toluene (50 ml) and $CHCl_3$ (50 ml) and treated with freshly distilled $SOCl_2$ (150 ml) for 30 min. The soln. was evaporated and co-evaporated with $CHCl_3$ (50 ml) to give the corresponding α-D-glucopyranosyl chloride as yellow crystalline moisture-sensitive solid. This solid was dissolved in THF (300 ml) and added within 15 min to a soln. of Bu_3SnLi at −78°. The mixture was stirred for 1.5 h at −78°, quenched with sat. aq. NH_4Cl soln. (10 ml), warmed to 20°, diluted with H_2O, and extracted with CH_2Cl_2

(3 × 500 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the yellow oil purified by FC (1 kg of SiO_2, hexane/AcOEt 1:0, 5:1, 3:1) to afford **2** (23.2 g, 74%) as a slightly yellow oil which solidified on standing. The product was stored at −30°, but a sample showed no decomposition at r.t. within 1 year. However, at elevated temp. and in the presence of silica gel, **2** decomposed. TLC (hexane/AcOEt 3:1): R_f 0.50. M.p. 59°. $[\alpha]_D^{20} = +2.4$ (c = 1.1, $CHCl_3$). IR (KBr): 3270, 3093, 2959, 2934, 2860, 1644, 1563, 1048, 732, 695. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.31–7.21 (*m*, 15 arom. H); 4.92–4.52 (*m*, 3 $PhCH_2$, NH); 4.14 (*dt*, *J* = 9.5, 11.6, H−C(2)); 3.64 (*m*, 2 H−C(6)); 3.61 ('*t*', *J* = 9.3, H−C(4)); 3.45 (*d*, *J* = 11.6, H−C(1)); 3.38 ('*t*', *J* = 9.2, H−C(3)); 3.25 (*m*, H−C(5)); 1.78 (*s*, Me); 1.53–1.44 (*m*, 6 H, CH_2); 1.37–1.22 (*m*, 6 H, CH_2); 1.02–0.78 (*m*, 15 H, CH_2, Me). ^{13}C-NMR (67.8 MHz, $CDCl_3$): 169.4; 138.6; 138.5; 138.3; 128.4–127.3; 85.5; 83.3; 79.2; 74.8; 74.5; 73.3; 69.4; 54.6; 29.1; 27.3; 13.6; 9.1. FAB-MS: 708 ($[M - Bu + 1]^+$). Anal. calc. for $C_{41}H_{59}NO_7Sn$: C 64.40, H 7.78, N 1.83; found: C 64.24, H 7.74, N 2.03.

4. *General Procedure for the Synthesis of* C-*Glycosides*[1]). Under Ar at −78°, BuLi (1 equiv. of a 1.6M soln. in hexanes) was added within 10 min to a soln. of **2** in THF (*ca.* 0.1 mol/l). The mixture was warmed to −65° and BuLi (1.3 equiv.) added within 5 min (→ deep red). The subsequent addition of electrophiles changed the colour of the soln. to pale yellow. After 1 h, the reaction was stopped with sat. aq. NH_4Cl or $KHSO_4$ soln. and the mixture warmed in the ultrasonic bath to 20°. The mixture was diluted with H_2O, extracted with AcOEt (2 ×), dried ($MgSO_4$), and evaporated.

*3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo-*heptonic Acid* (**4**). Purification was achieved by FC (hexane/AcOEt 1:0, 1:1, 1:4; AcOEt/MeOH/AcOH 45:5:3, 2:2:1) to furnish 4.60 g (83%) of **4**. Pale yellow solid. The compound forms gels in solvents like $CHCl_3$ or AcOEt. TLC ($CHCl_3$/MeOH 3:1 +0.1% CF_3COOH): R_f 0.34. M.p. 230° (AcOEt/AcOH, dec.). $[\alpha]_D^{20} = +29.4$ (c = 1.1, THF). IR (KBr): 3342, 3093, 3068, 3034, 2872, 1724, 1621, 1573, 1226, 1092, 741, 698. ^{1}H-NMR (500 MHz, (D_6)DMSO): 8.05 (*d*, *J* = 9.2, NH); 7.35–7.13 (*m*, 15 arom. H); 4.70 (*m*, 1 H, $PhCH_2$); 4.67 (*s*, 2 H, $PhCH_2$); 4.54 (*d*, *J* = 12.1, 1 H, $PhCH_2$); 4.50 (*d*, *J* = 10.5, 1 H, $PhCH_2$); 4.47 (*d*, *J* = 12.1, 1 H, $PhCH_2$); 3.94 (*m*, H−C(4)); 3.79 (*d*, *J* = 10.4, 1 H, H−C(3)); 3.70–3.56 (*m*, 3 H); 3.53–3.37 (*m*, 2 H); 1.76 (*s*, Me). ^{13}C-NMR (125 MHz, (D_6)DMSO): 169.7; 169.1; 138.6; 138.2; 128.2–127.5; 83.2; 78.1; 77.7; 77.5; 74.0; 72.3; 68.8; 52.3; 22.8. FAB-MS: 520 ($[M + 1]^+$). Anal. calc. for $C_{30}H_{33}NO_7$: C 69.35, H 6.40, N 2.70; found: C 69.11, H 6.36, N 2.85.

*3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-*N^1*-propyl-β-*D-glycero-D-gulo-*heptonamide* (**7**). Propyl isocyanate was added at −78°. The crude product was recrystallized from acetone: 137 mg (52%) of **7**. Colourless needles. TLC (hexane/acetone 1:1): R_f 0.24. M.p. 217–218°. $[\alpha]_D^{20} = +17.5$ (c = 1.0, THF). IR (KBr): 3271, 3092, 3036, 2969, 2933, 2876, 1661, 1562, 1270, 1097, 738, 697. ^{1}H-NMR (250 MHz, $CDCl_3$): 7.88 (*d*, *J* = 8.8, NH); 7.69 ('*t*', *J* = 5.8, NH); 7.33–7.14 (*m*, 15 arom. H); 4.72–4.61 (*m*, 3 H, $PhCH_2$); 4.56–4.44 (*m*, 3 H, $PhCH_2$); 3.87 (*m*, H−C(3)); 3.76 (*d*, *J* = 10.0, H−C(2)); 3.71–3.56 (*m*, 3 H); 3.48–3.37 (*m*, 2 H); 3.07–2.89 (*m*, CH_2); 1.73 (*s*, Me); 1.44–1.29 (*m*, CH_2); 0.80 (*t*, *J* = 7.3, Me). ^{13}C-NMR (76 MHz, (D_6)DMSO): 169.0; 167.4; 138.6; 138.2; 128.3–127.4; 83.2; 78.5; 78.4; 77.9; 74.0; 72.3; 69.0; 52.7; 40.3; 22.9; 22.2; 11.3. FAB-MS: 561 ($[M + 1]^+$). Anal. calc. for $C_{33}H_{40}N_2O_6$: C 70.69, H 7.19, N 5.00; found: C 70.69, H 7.11, N 4.98.

5. *Diaminopropanoic-Acid and Diaminobutanoic-Acid Derivatives* **8–12**. To a soln. of the Asn and Gln derivatives in H_2O/MeCN 1:1 at r.t., *I,I*-bis(trifluoroacetoxy)iodobenzene (1.3 equiv.) and pyridine (2–4 equiv.) were added. The resulting mixtures were stirred for 2–4 h, then extracted by AcOEt or CH_2Cl_2. The crude products were either obtained by lyophilization of the aq. phase (**8**, **9**, and **12**) or by evaporation of the org. phase (**10** and **11**) and used without further purification for the next step.

6. *General Procedure for the Synthesis of* C-*Glycosylated Amino Acids*. TBTU (1.2 equiv.) and HOBt (1.5 equiv.) were added to a soln. of **4** (1 equiv.) in 1-methylpyrrolidin-2-one at r.t. The pH of the soln. was adjusted to 6–7 by $(i\text{-}Pr)_2EtN$. The diamino acids (**8–14**, *ca.* 1.5 equiv.) was added and the pH maintained at 6–7 by addition of $(i\text{-}Pr)_2EtN$. After 1 h, the mixture was quenched with H_2O, concentrated, and partitioned between H_2O and AcOEt. The org. phase was washed by $NaHCO_3$ and $KHSO_4$ soln. and brine, dried (Na_2SO_4), and evaporated and the residue purified by FC (acetone/hexane).

*Methyl 3-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo-*heptonoyl)amino]-2-{[(*tert*-butoxy)carbonyl]amino}propanoate* (**15**): 655 mg (91%). White solid. TLC (hexane/acetone 1:1): R_f 0.45. $[\alpha]_D^{20} = +2.9$ (c = 0.5, $CHCl_3$). IR (KBr): 3342, 3288, 2933, 2873, 1743, 1715, 1670, 1097, 735, 697. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.34–7.15 (*m*, 15 arom. H); 6.76 ('*t*', NH−C(β)); 6.15 (*d*, *J* = 8.3, NH−C(α)); 5.88 (br., NH−C(3)); 4.81 (*d*, *J* = 10.7, 1 H, $PhCH_2$); 4.80 (*d*, *J* = 11.8, 1 H, $PhCH_2$); 4.67 (*d*, *J* = 11.4, 1 H, $PhCH_2$); 4.55 (*d*, *J* = 10.9, 1 H, $PhCH_2$); 4.54 (*m*, 1 H, $PhCH_2$); 4.50 (*d*, *J* = 12.1, 1 H, $PhCH_2$); 4.39 (*m*, H−C(α)); 3.82 (*m*, H−C(4)); 3.81 (*m*, H−C(2)); 3.70 (*m*, 2 H−C(7), MeO); 3.65 (*m*, H−C(5)); 3.52 (*m*, H−C(3)); 3.49 (*m*, H−C(6)); 3.39 (*m*, 2 H−C(β));

1.83 (*s*, Me); 1.41 (*s*, *t*Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.3; 169.3; 155.9; 138.2; 137.8; 137.7; 128.6–127.6; 82.0 (C(4)); 79.7 (C(6)); 78.7 (C(5)); 78.2 (C(2)); 75.0; 74.8; 73.5; 68.6 (C(7)); 54.3 (C(3)); 53.4 (C(α)); 52.4 (MeO); 40.5 (C(β)); 28.3 (*Me*$_3$C); 23.3 (Me). FAB-MS: 720 ([*M* + 1]$^+$). Anal. calc. for $C_{39}H_{49}N_3O_{10}$: C 65.07, H 6.86, N 5.84; found: C 64.98, H 6.81, N 5.90.

*Methyl 3-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo*-heptonoyl)amino]-2-[(benzyloxycarbonyl)amino]propanoate* (**16**): 723 mg (96%). White solid. TLC (hexane/acetone 1:1): R_f 0.39. M.p. 191–194° (acetone/hexane). $[\alpha]_D^{20} = -11.0$ (c = 1.0, $CHCl_3$). IR (KBr): 3289, 3094, 3069, 3036, 2956, 2873, 1735, 1682, 1667, 1214, 1093, 748, 696. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.34–7.16 (*m*, 20 arom. H); 6.73 ('*t*', *J* = 6.5, NH–C(β)); 6.64 (*d*, *J* = 8.3, NH); 5.37 (*d*, *J* = 8.8, NH); 5.11 (*d*, *J* = 12.3, 1 H, PhC*H*$_2$); 5.07 (*d*, *J* = 12.4, 1 H, PhC*H*$_2$); 4.80 (*d*, *J* = 10.9, 1 H, PhC*H*$_2$); 4.80 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$); 4.63 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$); 4.55 (*d*, *J* = 10.9, 1 H, PhC*H*$_2$); 4.54 (*d*, *J* = 12.0, 1 H, PhC*H*$_2$); 4.50 (*d*, *J* = 12.0, 1 H, PhC*H*$_2$); 4.46 (*m*, H–C(α)); 3.86 (*m*, H–C(3), 1 H–C(β)); 3.71 (*s*, MeO); 3.67 (*m*, 2 H–C(7)); 3.64 (*m*, H–C(5)); 3.63 (*m*, H–C(2)); 3.62 (*m*, H–C(4)); 3.46 (*m*, H–C(6)); 3.42 (*m*, 1 H–C(β)); 1.69 (*s*, Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.5; 171.0; 169.3; 156.5; 138.0; 137.7; 136.5; 128.8–127.7; 81.9 (C(4)); 78.8 (C(6)); 78.5 (C(2)); 78.2 (C(5)); 75.1; 74.7; 73.4; 68.6 (C(7)); 66.8 (PhCH$_2$(Z)); 53.9 (C(α)); 53.8 (C(3)); 52.6 (MeO); 40.2 (C(β)); 23.1 (Me). FAB-MS: 776 ([*M* + Na]$^+$). Anal. calc. for $C_{42}H_{47}N_3O_{10}$: C 66.92, H 6.28, N 5.57; found: C 66.65, H 6.31, N 5.67.

tert*-Butyl 3-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo*-heptonoyl)amino]-2-[(9*H*-fluoren-9-yloxycarbonyl)amino]propanoate* (**17**): 795 mg (90%). White solid. TLC (hexane/acetone 1:1): R_f 0.64. $[\alpha]_D^{20} = -16.3$ (c = 0.8, $CHCl_3$). IR (KBr): 3307, 3069, 3036, 2938, 1728, 1669, 1370, 1157, 1092, 740, 697. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.73 (*d*, *J* = 7.8, 2 arom. H); 7.69 (*d*, *J* = 7.8, 1 arom. H); 7.67 (*d*, *J* = 7.3, 1 arom. H); 7.36–7.15 (*m*, 19 arom. H); 6.74 (*d*, *J* = 8.4, NH–C(α)); 6.70 (*dd*, *J* = 5.5, 7.1, NH–C(β)); 5.40 (*d*, *J* = 8.8, NH–C(3)); 4.83 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$); 4.80 (*d*, *J* = 10.7, 1 H, PhC*H*$_2$); 4.66 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$); 4.54 (*d*, *J* = 10.8, 1 H, PhC*H*$_2$); 4.52 (*d*, *J* = 12.0, 1 H, PhC*H*$_2$); 4.47 (*d*, *J* = 12.0, 1 H, PhC*H*$_2$); 4.41 (*m*, H–C(α)); 4.33 (*ddd*, *J* = 7.9, 7.8, 10.4, 1 H, CH$_2$(Fmoc)); 4.28 (*ddd*, *J* = 7.4, 7.5, 10.4, 1 H, CH$_2$(Fmoc)); 4.21 ('*t*', *J* = 7.6, CH(Fmoc)); 3.91 (*m*, H–C(3)); 3.88 (*m*, H–C(β)); 3.66 (*m*, 2 H–C(7), H–C(2)); 3.65 (*m*, H–C(4), H–C(6)); 3.63 (*m*, H–C(5)); 3.43 (*m*, H–C(β)); 1.81 (*s*, Me); 1.46 (*s*, *t*Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.2; 169.6; 169.0; 156.5; 144.0; 141.2; 138.1; 137.8; 137.7; 128.6–127.0; 125.5; 125.4; 119.8; 82.5 (Me$_3$*C*); 81.9 (C(4)); 78.8 (C(6)); 78.4 (C(2)); 78.3 (C(5)); 75.0; 74.7; 73.4; 68.6 (C(7)); 67.1 (CH$_2$(Fmoc)); 54.5 (C(α)); 54.0 (C(3)); 47.2 (CH(Fmoc)); 40.5 (C(β)); 28.0 (*Me*$_3$C); 23.3. FAB-MS: 884 ([*M* + 1]$^+$). Anal. calc. for $C_{52}H_{57}N_3O_{10}$: C 70.64, H 6.49, N 4.75; found: C 70.56, H 6.72, N 4.59.

*Methyl N^2-{3-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo*-heptonoyl)amino]-2-{[(*tert*-butoxy)carbonyl]amino}propanoyl}-*L*-phenylalaninate* (**18**): 719 mg (83%). Pale yellow solid. TLC (hexane/acetone 1:1): R_f 0.47. $[\alpha]_D^{20} = -20.0$ (c = 0.4, $CHCl_3$). IR (KBr): 3320, 3067, 3035, 2932, 2875, 1737, 1714, 1671, 748, 698. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.44 (*d*, *J* = 8.4, NH–C(α)); 7.29–7.07 (*m*, 20 arom. H); 6.93 (br., NH–C(β)); 6.37 (*d*, *J* = 8.4, NH–C(α')); 5.50 (*d*, *J* = 10.3, NH–C(3)); 4.74 (*d*, *J* = 11.8, 1 H, PhC*H*$_2$); 4.71 (*d*, *J* = 10.9, 1 H, PhC*H*$_2$); 4.61 (*m*, H–C(α)); 4.58 (*d*, *J* = 11.8, 1 H, PhC*H*$_2$); 4.47 (*m*, 3 H, PhC*H*$_2$); 4.23 (*m*, H–C(α')); 3.90 (*m*, H–C(3)); 3.78 (*m*, H–C(β')); 3.60 (*s*, 2 H–C(7)); 3.54 (*s*, MeO); 3.52 (*m*, H–C(5)); 3.49 (*d*, *J* = 10.3, H–C(2)); 3.39 (*m*, H–C(6)); 3.33 (*m*, H–C(4)); 3.32 (*m*, 1 H–C(β')); 3.11 (*s*, 2 H–C(β)); 1.76 (*s*, Me); 1.34 (*s*, *t*Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.6; 171.3; 170.8; 170.0; 156.2; 138.1; 137.7; 136.7; 129.4; 126.7; 82.0 (C(4)); 79.7 (Me$_3$*C*); 79.0 (C(6)); 78.9 (C(2)); 78.3 (C(5)); 75.0; 74.6; 73.5; 68.6 (C(7)); 55.7 (C(α')); 54.1 (C(α)); 53.6 (C(3)); 52.1 (MeO); 40.7 (C(β')); 37.6 (C(β)); 28.3 (*Me*$_3$C); 23.2 (Me). FAB-MS: 867 ([*M* + 1]$^+$). Anal. calc. for $C_{48}H_{58}N_4O_{11}$: C 66.50, H 6.74, N 6.46; found: C 66.22, H 6.97, N 6.29.

*Methyl 4-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β-*D-glycero-D-gulo*-heptonoyl)amino]-2-[(benzyloxycarbonyl)amino]butanoate* (**19**): 707 mg (92%). White solid which was recrystallized from AcOEt. TLC (hexane/acetone 1:1): R_f 0.33. M.p. 194–196°. $[\alpha]_D^{20} = +12.2$ (c = 1.0, $CHCl_3$). IR (KBr): 3298, 3092, 3068, 3035, 2957, 2876, 1742, 1687, 1660, 1543, 1498, 1454, 1374, 1356, 1262, 1097, 748, 697. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.33–7.14 (*m*, 20 arom. H); 6.80 ('*t*', *J* = 5.9, NH–C(γ)); 5.97 (*d*, *J* = 7.9, NH–C(α)); 5.60 (*d*, *J* = 6.9, NH–C(3)); 5.04 (*s*, PhC*H*$_2$); 4.78 (*d*, *J* = 11.5, 1 H, PhC*H*$_2$); 4.77 (*d*, *J* = 10.8, 1 H, PhC*H*$_2$); 4.66 (*d*, *J* = 11.5, 1 H, PhC*H*$_2$); 4.55 (*d*, *J* = 12.6, 1 H, PhC*H*$_2$); 4.54 (*d*, *J* = 10.8, 1 H, PhC*H*$_2$); 4.51 (*d*, *J* = 12.6, 1 H, PhC*H*$_2$); 4.37 (*m*, H–C(α)); 3.85 (*m*, H–C(2)); 3.82 (*m*, H–C(3)); 3.81 (*m*, H–C(4)); 3.70 (*s*, MeO, 2 H–C(7)); 3.61 (*m*, H–C(5)); 3.52 (*m*, H–C(6), 1 H–C(γ)); 3.04 (*m*, 1 H–C(γ)); 2.05 (*m*, 1 H–C(β)); 1.86 (*m*, H–C(β)); 1.81 (*s*, Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 172.6; 170.8; 168.6; 156.2; 138.1; 137.8; 136.2; 128.3–127.6; 82.2 (C(4)); 78.7 (C(6)); 78.1 (C(5)); 77.3 (C(2)); 74.8; 74.6; 73.3; 68.8 (C(7)); 66.8 (CH$_2$(Z)); 54.1 (C(3)); 52.4 (MeO); 51.7 (C(α)); 35.0 (C(γ)); 31.5 (C(β)); 23.3 (Me). FAB-MS: 768 ([*M* + 1]$^+$). Anal. calc. for $C_{43}H_{49}N_3O_{10}$: C 67.26, H 6.43, N 5.47; found: C 67.21, H 6.39, N 5.54.

Methyl N^5*-(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β*-D-glycero-D-gulo-*heptonoyl)*-N^2*-(benzyloxycarbonyl)ornithinate* (**20**): 378 mg (80% from **2**). Pale yellow solid. The crude product was recrystallized from AcOEt: colourless crystalline solid. TLC (hexane/acetone 1:1): R_f 0.36. M.p. 210–212°. $[\alpha]_D^{20} = -20.9$ (c = 1.0, $CHCl_3$). IR (KBr): 3304, 3092, 3068, 3036, 2930, 2876, 1738, 1692, 1665, 1550, 1498, 1454, 1353, 1249, 1098, 739, 697. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.35–7.13 (*m*, 20 arom. H); 6.87 (*d*, J = 7.9, NH); 6.42 (*m*, NH–C(δ)); 5.26 (*d*, J = 9.3, NH); 5.08 (*d*, J = 12.2, 1 H, PhCH_2); 5.06 (*d*, J = 12.2, 1 H, PhCH_2); 4.80 (*d*, J = 11.8, 1 H, PhCH_2); 4.78 (*d*, J = 11.6, 1 H, PhCH_2); 4.62 (*d*, J = 11.6, 1 H, PhCH_2); 4.54 (*d*, J = 11.8, 1 H, PhCH_2); 4.53 (*d*, J = 12.5, 1 H, PhCH_2); 4.51 (*d*, J = 12.5, 1 H, PhCH_2); 4.23 (*m*, H–C(α)); 3.96 ('*q*', J = 9.8, H–C(3)); 3.72 (*s*, MeO); 3.67 (*m*, 2 H–C(7)); 3.64 (*m*, H–C(5)); 3.56 (*m*, H–C(2)); 3.54 (*m*, H–C(4)); 3.52 (*m*, 1 H–C(δ)); 3.46 (*m*, H–C(6)); 2.87 (*m*, 1 H–C(δ)); 1.83–1.67 (*m*, 2 H–C(β)); 1.72 (*m*, H–C(γ)); 1.68 (*s*, Me); 1.38 (*m*, H–C(γ)). ^{13}C-NMR (125 MHz, $CDCl_3$): 173.1; 171.0; 168.3; 156.5; 137.9; 137.6; 136.5; 128.4–127.7; 82.2 (C(4)); 78.7 (C(6)); 78.7 C(2)); 78.1 (C(5)); 74.9; 74.6; 73.3; 68.6 (C(7)); 66.5 (CH_2(Z)); 54.1 (C(α)); 53.4 (C(3)); 52.1 (MeO); 38.8 (C(δ)); 28.9 (C(β)); 25.3 (C(γ)); 22.8 (Me). FAB-MS: 782 ([M + 1]$^+$). Anal. calc. for $C_{44}H_{51}N_3O_{10}$: C 67.58, H 6.57, N 5.37; found: C 67.39, H 6.52, N 5.40.

Methyl N^6*-(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β*-D-glycero-D-gulo-*heptonoyl)*-N^2*-(benzyloxycarbonyl)lysinate* (**21**): 1.60 g (75% from **2**). TLC (hexane/acetone 1:1): R_f 0.29. $[\alpha]_D^{20} = +12.0$ (c = 1.1, $CHCl_3$). IR (KBR): 3292, 3093, 3069, 3036, 2930, 2872, 1728, 1691, 1662, 1097, 741, 697. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.34–7.15 (*m*, 20 arom. H); 6.40 (br., NH–C(ε)); 5.80 (*d*, J = 8.4, NH); 5.76 (br., NH); 5.07 (*s*, PhCH_2); 4.80 (*d*, J = 10.8, 1 H, PhCH_2); 4.78 (*d*, J = 11.5, 1 H, PhCH_2); 4.67 (*d*, J = 11.4, 1 H, PhCH_2); 4.54–4.47 (*m*, 3 H, PhCH_2); 4.30 (*m*, H–C(α)); 3.89 (*m*, H–C(3)); 3.79 (*d*, J = 9.9, H–C(2)); 3.72 (*m*, H–C(4)); 3.69 (*s*, MeO); 3.65 (*m*, 2 H–C(7)); 3.62 (*m*, H–C(5)); 3.49 (*m*, H–C(6)); 3.25 (*m*, 1 H–C(ε)); 3.09 (*m*, 1 H–C(ε)); 1.80 (*s*, Me); 1.78 (*m*, H–C(β)); 1.67 (*m*, 1 H–C(β)); 1.48 (*m*, 1 H–C(δ)); 1.43 (*m*, H–C(δ)); 1.31 (*m*, 2 H–C(γ)). ^{13}C-NMR (125 MHz, $CDCl_3$): 172.9; 170.7; 168.5; 156.1; 138.2; 137.9; 137.8; 136.4; 128.5–127.7; 82.7 (C(4)); 78.6 (C(6)); 78.0 (C(5)); 77.6 (C(2)); 74.9; 74.8; 73.3; 68.8 (C(7)); 66.8 (CH_2(Z)); 53.9 (C(3)); 53.8 (C(α)); 52.3 (MeO); 38.4 (C(ε)); 31.8 (C(γ)); 28.9 (C(δ)); 23.3 (Me); 22.3 (C(β)). FAB-MS: 796 ([M + 1]$^+$). Anal. calc. for $C_{45}H_{53}N_3O_{10}$: C 67.91, H 6.71, N 5.28; found: C 67.59, H 6.68, N 5.38.

7. *3-[(3-Acetamido-2,6-anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-β*-D-glycero-D-gulo-*heptonoyl)amino]-2-[(9*H*-fluoren-9-yloxycarbonyl)amino]propanoic Acid* (**22**). Compound **17** (440 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (15 ml), and CF_3COOH (2.5 ml) was added. After stirring the mixture for 2 h, the solvent was evaporated and the crude product dried *in vacuo* and recrystallized from MeOH: 312 mg (75%) of **22**. Colourless crystalline solid. TLC ($CHCl_3$/MeOH 3:1): R_f 0.42. M.p. 208–210°. $[\alpha]_D^{20} = -8.8$ (c = 0.8, $CHCl_3$). IR (KBR): 3312, 3069, 3036, 2879, 1716, 1682, 1535, 1452, 1091, 740, 698. ^{1}H-NMR (500 MHz, (D_6)DMSO): 7.95 (*d*, J = 9.4, NH); 7.87 (*d*, J = 7.6, 2 arom. H); 7.76–7.62 (*m*, 2 arom. H, NH); 7.52 (*d*, J = 7.2, NH); 7.42–7.11 (*m*, 19 arom. H); 4.71–4.63 (*m*, 3 H); 4.53–4.42 (*m*, 3 H); 4.31–4.18 (*m*, 3 H); 4.07 (*m*, H–C(α)); 3.87 (*m*, H–C(3)); 3.78–3.40 (*m*, 8 H); 1.77 (*s*, Me). ^{13}C-NMR (125 MHz, (D_6)DMSO): 169.5; 168.1; 155.8; 143.8; 143.7; 140.7; 138.6; 138.1; 128.2–127.3; 127.1; 125.3; 125.2; 120.0; 83.0; 78.2; 77.7; 77.2; 74.0; 73.9; 72.3; 68.9; 65.7; 53.4; 53.0; 46.6; 40.1–39.0; 22.8. FAB-MS: 828 ([M + 1]$^+$).

8. *Cyclo (-*D*-Pro-Phe-Ala-CGaa-Phe-Phe-)* (**24**). $[\alpha]_D^{20} = -49.5$ (c = 0.6, MeOH). IR (KBr): 3314, 3065, 2931, 1649, 1527, 1454, 1384, 1097, 1003, 703. FAB-MS: 927 ([M + 1]$^+$).

9. *Computational Methods.* Distance data were derived from a ROESY with 200 ms mixing time at 325 K. The integrals were converted to distance constraints using the isolated two spin approximation (ISPA) taking the offset correction into account [39]. Homonuclear coupling constants were obtained from the one-dimensional ^{1}H-NMR spectrum. ^3J(H–C(α),H–C(β))'s were obtained from the P.E. COSY spectrum. Temperature coefficients were determined between 315 und 340 K in steps of 5°.

Starting geometries for subsequent MD simulations were obtained by a modified version [27] of the DISGEO [28] program with H,H distances and ^3J(NH,H–C(α))'s and ^3J(H–C(α),H–C(β))'s as restraints using the *Karplus* equation [40]. In the DG calculation, 45 distance restraints, 5 ^3J(NH,H–C(α))'s and the two ^3J(H–C(α),H–C(β))'s for Phe^2 were used. The structures (100) were embedded and refined with distance-driven dynamics [41] and distance- and angle-driven dynamics [42] in the four- and three-dimensional space. During the DGF calculation, the geometry of the sugar moiety was fixed since the coupling constants clearly indicate a $^4C_1$-chair conformation.

For the following MD simulations, the DISCOVER [43] program with the CVFF [44] forcefield was used. The lowest-error structure of the DG calculation was placed in a cubic cell with box lengths of 35 Å and soaked with explicit DMSO molecules [29]. For the following calculation, a cutoff of 12 Å for the nonbonded interaction was

used with a switching function being applied for the last 1 Å. The solvent was relaxed with the solute fixed by 3000 steps steepest descent minimization followed by another 3000 steps with the conjugated gradient algorithm. Then the whole system was energy minimized by the same procedure applying NMR-based distance restraints with a force constant of 1 kcal mol^{-1} $Å^{-2}$. The system was gradually heated up to 325 K in 1-ps steps of 50 K and equilibrated at 325 K for 30 ps. Then 100 ps restraint MD followed, while every 0.1 ps the actual conformation was stored to obtain the trajectory.

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