Supplementary Material for Organic & Biomolecular Chemistry

Supplementary data

Functionalized Foldamers: Synthesis and Characterization of a Glycosylated β-Peptide 3_{14}-helix conveying the T_N-antigen

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I. General Information

Fmoc-protected α-amino acids, HOBt, HBTU, and Rink amide resin were purchased from Senn Chemicals (Switzerland). DIPEA, *N*-methyl morpholine (NMM) and piperidine were purchased from Acros Organics (Belgium), dimethylformamide (DMF) and trifluoroacetic acid (TFA) was obtained from Scharlau Chemie (Spain). All other reagents were purchased from Sigma-Aldrich. Solvents were dried and distilled according to standard methods; diethyl ether and THF were distilled from sodium and benzophenone, dichloromethane (DCM) was distilled from sodium hydride.

Flash column chromatography was performed employing silica gel (Matrex Silica 60, 35-70 μm). Thin-layer chromatography (TLC) was performed using silica-plates (Machery-Nagel Layer: 0.20 mm silica gel 60 with fluorescent indicator UV_{254}). The carbohydrates were not UV-active, and therefore visualised using 10% H_2SO_4 in EtOH. RP-HPLC purification and analysis were carried out on a Gilson system, equipped with UV absorbance detector, at a wavelength of 220 nm, and a Finnigan AQA ESI mass spectrometer. The products were analyzed and purified on C18 columns (analytical: Phenomenex 75x2 mm, particle size: 4μm; preparative: YMC 150x20 mm; particle size 5μm). Products were eluted in either 0.1 % TFA in acetonitrile/water (preparative) or in 0.05 % formic acid in acetonitrile/water (analytical) using a linear gradient, optimized for every compound at flow rates of 1.2 mL min^{-1} for analytical and 15 mL min^{-1} on the preparative column.

The β-peptides were synthesized using a semiautomatic Quest 210 from Argonaut. Loading levels of the resins were determined using a Cary 3Bio UV-Visible spectrophotometer.

II. β-Peptide preparation

A short description of the syntheses involved is given below. A full account will be included in a forthcoming full paper.

A. β-Peptide synthesis

Fmoc-protected β^3-amino acids were synthesized following procedures published by Seebach.[1] Fmoc-β^3- hSer-OBn, the β-amino acid to be glycosylated, was prepared in 2 steps, starting from commercially available benzyl-protected D-Asp.[2] The unnatural amino acid and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide, derived from 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose,[3] was combined to yield a mixture of α- and β-anomeric azido derivative.[4] After separation, the α-anomer was subjected to reductive acylation and finally debenzylation of the carboxylic acid.[5]

β^3-Peptides were synthesized using Fmoc-chemistry optimized for β-peptides[6] on a Rink amide resin. The synthesis started by leaving the resin to swell in DMF (2 x 15 min), followed by removal of the Fmoc-protecting group using a solution of 2% piperidine/2% DBU in DMF (5 x 5 min). After washing the resin (DMF, 5 x 5 min), a mixture of β^3-hVal (2 equiv.), HBTU (3.8 equiv.), HOBt (4 equiv.), and diisopropylethylamine (DIPEA) (8 equiv.) in 6 ml DMF was added and left under an N_2-atmosphere over night.

Completeness of the coupling was controlled by TNBS-test[7] and the resin was washed (5 x 5 min) with DMF. The loading level of the resin was calculated to 91%. This was made by measuring the UV-absorption from the Fmoc-group (cleaved off by treating the resin with 20% piperidine in DMF for 10 min) at 290 nm.[6]

After coupling of the first amino acid to the resin, unreacted amino groups were capped by acetylation. First, the resin was washed with DCM (2 x 5 min) followed by treatment for 3 h with a mixture of Ac_2O in DCM (1:1 ratio). The resin was then washed again (6 x 2 min with DCM, then 4 x 2 min with DMF). This capping procedure was repeated once more at the penta-peptide stage.

Thereafter, peptide elongation was done using the following cycles: The loaded resin was washed with DMF (6 x 3 min) and the terminal Fmoc-protecting group removed with 2% piperidine/2% DBU in DMF (5 x 5 min) (after the coupling of the glycosylated amino acid, Fmoc-deprotection was made using 20% piperidine in DMF). The resin was once again washed with DMF (6 x 3 min) and then treated for 5-18 h with a cocktail containing 2 equiv. of the desired β^3-amino acid, 3.8 equiv. HBTU, 4 equiv. HOBt and 8 equiv. of DIPEA. The coupled resin was then washed with DMF (5 x 5 min). These steps were repeated until the β-peptide sequence was complete. Final Fmoc-deprotection was performed with mixtures of 20% piperidine in DMF (5 x 5 min) and the resin was washed with DMF (5 x 5 min), DCM (5 x 5 min) and shrunk with MeOH (5 x 5 min). The peptides were cleaved from the resin by

treatment of TFA/H_2O/TIPS 95:2.5:2.5 (6 ml). The resin was filtered off and washed twice with TFA and the organic phase was concentration under reduced pressure. The oily residue was precipitated using cold diethyl ether; whereupon a white solid was obtained. The acetyl protecting groups on the carbohydrate residue was removed with saturated NH_3 in MeOH.[8]

B. β-Peptide purification and analysis

The success of each synthesis was first assessed by HPLC and ESI mass spectrometric analysis of the reaction mixtures. Preparative RP-HPLC was then used to purify the β-peptides, followed by concentration through freeze-drying. Another run of analytical HPLC and ESI mass spectrometry assessed the identities and purities of the products.

III. CD Spectroscopy

The measurements were performed using a Jasco J-810 spectropolarimeter, in methanol-solution and in an aqueous phosphate buffer (pH 6.9). Both samples had the concentration 0.1 mM. The length of the cuvette was 0.2 mm and numbers of scans were 10. Smoothing was made afterwards.

IV. NMR Spectroscopy of β-heptapeptide 1

The assignment of **1** in methanol was accomplished using TOCSY,[9] P.E.-COSY,[10] ROESY,[11] and gHMBC[12] experiments (Table S1).

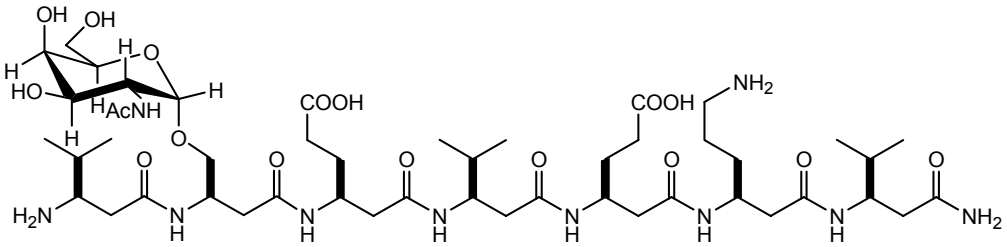

Table S1: Assignment in methanol

β-Amino acid	NH *J (Hz)*	2H-C(α) ax./ekv.	C=O	H-C(β)	H-C(γ) or 2H-C(γ)	2H-C(δ) or Me-C(δ)	2H-C(ε)
Val 1	-	2.88/2.66	*172.9*	3.55 *55.0*	2.06 *30.9*	1.10 *18.1/16.9*	
Ser 2	8.33 *9.31Hz*	2.62/2.76		4.71	3.64		
Glu 3	8.48 *9.16Hz*	2.48/2.77		4.35	1.87/1.77	2.31	
Val 4	8.25 *9.14Hz*	2.48/2.56	*171.4*	4.19 *51.5*	1.77 *33.2*	0.95 *18.7*	
Glu 5	7.99 *9.18Hz*	2.53/2.35		4.41	1.88/1.74	2.35	
Orn 6	7.62 *8.70Hz*	2.57/2.36		4.46	1.62/1.49	1.72	2.94
Val 7	7.67 *9.76Hz*	2.50/2.31	*174.7*	4.25 *51.2*	1.69 *33.1*	0.90 *18.0*	

All data were acquired on a Varian Unity 500 spectrometer. Sample: 4 mg dissolved in 0.7 ml of CH_3OH and CD_3OD (ratio 4:1). Solvent suppression were done using the WET pulse sequence.[9]

^{1}H-NMR (500 MHz): 30 K data points, 128 transients, acquisition time 3 s (Figure S1).

The ^{1}H-NMR spectrum shows good dispersion of the amide region, indicating the presence of a secondary structure. The J^3 (NH –C(β)H) coupling constants are all large (see Table S1), verifying the expected antiperiplanar orientation of these protons.

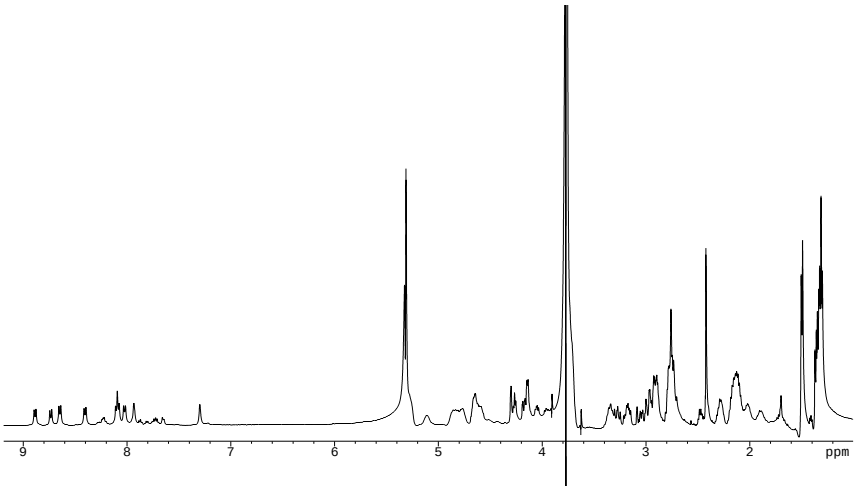

Figure S1. ^{1}H NMR of **1**in methanol

TOCSY(500 MHz, CH_3OH / CD_3OD) (Figure S2.) *Acquisition:* 2048 datapoints, 8 transients, acquisition time 0.205 s. The numbers of increments were 196 and the relaxation delay 1.3 s.

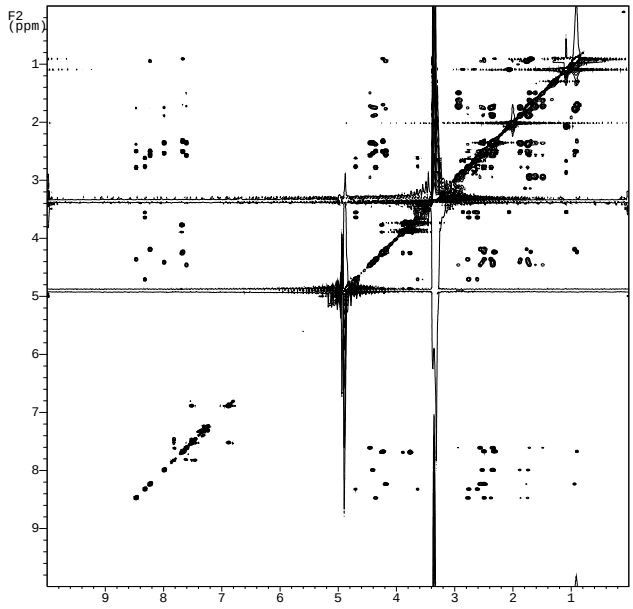

Figure S2. TOCSY spectra of **1** in methanol

P.E.-COSY (500 MHz, CH_3OH / CD_3OD) *Acquisition:* 3080 datapoints, 16 transients, acquisition time 0.205 s. The numbers of increments were 256 and the relaxation delay 1.5 s. The diastereotopic $CH_2(\alpha)$ protons could be assigned assuming that the axial protons exhibit a large, and the lateral protons a small, coupling with H-C(β), which is in agreement with the stronger NOEs observed from H-C(β) to the lateral H-C(α) protons than to the axial H-C(α) protons.

ROESY (500 MHz, CH_3OH / CD_3OD) (Table S2, Figure S3, Figure S4) *Acquisition:* 2 ROESY spectra with mixing times of 150 and 300 ms were acquired. 2048 datapoints, 16 transients, acqusition time 0.300 s. The numbers of increments were 196, the relaxation delay 1.3 s. Several non-sequential NOE-effects indicated that **1** posses a 3_{14}-helical conformation.

gHMBC (500 MHz, CH_3OH / CD_3OD) *Acquisition:* 2048 datapoints, 16 transients, acquisition time 0.205 s. The number of increments was set to 400 and the relaxation delay 1.2 s.

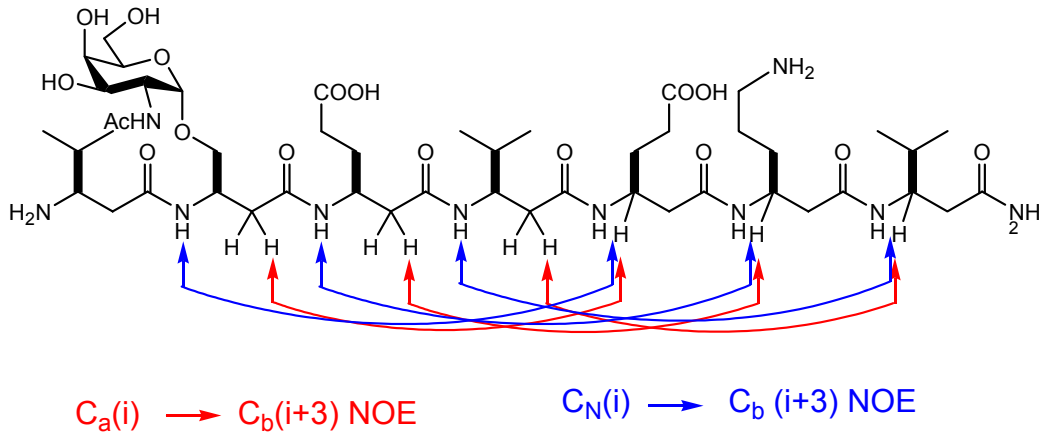

Figure S3. NOEs observed for heptapeptide 1 in methanol

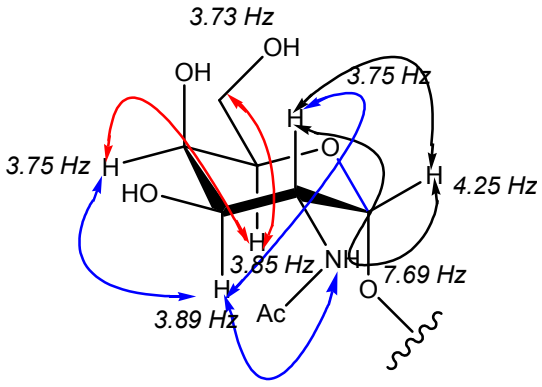

Figure S4. NOE-effects of carbohydrate moiety.

Table S2. NOEs observed in the ROESY NMR spectra of heptapeptide **1** in methanol.

Proton 1	Proton 2	NOE		Restraints included in the structure calc.
a1	d1	strong		
a1a	g1	medium		
a1a	*a1e*	*strong*	Cal.	
a1e	*a1a*	*strong*	Cal.	
b1	a1e	medium		X
b1	d1	medium		
a2a	a3e	strong		
a2a	*a2e*	*strong*	Cal.	
a2e	*a2a*	*strong*	Cal.	
b2	a2a	weak		X
nh2	b4	medium		
nh2	a1a	strong		X
nh2	a1e	weak		X
nh2	a2a	weak		X
nh2	b2	v. weak		
nh2	b5	weak		X
nh2	b4	weak		
a3a	a4a	strong		X
a3a	b6	medium		X
a3a	*a3e*	*strong*	Cal.	
a3e	*a3a*	*strong*	Cal.	
a3e	d3	medium		
b3	a3e	strong		X
b3	g3	medium		
nh3	b6	medium		X
nh3	b3	weak		X
nh3	a2e	weak		X
nh3	a2a	strong		X
nh3	a3a	strong		X
nh3	g3	weak		
a4a	d4	strong		
a4a	g4	medium		
a4a	b5	weak		
a4e	d4	medium		
a4e	b4	medium		X
a4e	d1	medium		
b4	a1a	medium		X
b4	a4a	medium		
b4	g4	medium		
b4	d4	medium		
nh4	b4	medium		
nh4	a3e	medium		X
nh4	a3a	strong		X
nh4	a4a	medium		X
nh4	a4e	weak		X
nh4	g4	weak		
nh4	d4	medium		
nh4	b7	weak		X
a5a	*a5e*	*strong*	Cal.	

a5e	*a5a*	*strong*	Cal.	
a5e	d5	medium		
b5	a5a	medium		X
b5	a5e	strong		X
b5	g5	medium		
b5	a2a	medium		X
nh5	b5	medium		X
nh5	b4	weak		
nh5	a5a	medium		X
nh5	a5e	weak		X
nh5	a4e	weak		X
nh5	a4a	strong		X
nh5	g5	weak		
a6a	d6	weak		
a6a	g6	medium		
a6a	*a6e*	*strong*	Cal.	
a6e	*a6a*	*strong*	Cal.	
a6e	d6	medium		
b6	a6e	strong		X
b6	a6a	medium		X
b6	d6	medium		
b6	g6	weak		
nh6	b6	weak		X
nh6	a6a	strong		X
nh6	a5a	strong		X
nh6	a5e	v. weak		
a7a	d7	strong		
a7a	g7	medium		
a7a	*a7e*	*strong*	Cal.	
a7e	*a7a*	*strong*	Cal.	
a7e	d7	strong		
b7	a7e	strong		X
b7	a7a	weak		X
b7	d7	strong		
b7	a4a	medium		X
b7	g7	medium		
nh7	b7	medium		X
nh7	a7a	strong		X
nh7	a7e	v. weak		
nh7	a6a	strong		X
nh7	g7	weak		

V. NOE restrained Monte-Carlo conformational sampling

Monte-Carlo conformational search (10000 steps), followed by PR Conjugate Gradient minimization (max 2000 iterations), was done in the program Macromodel 7.0.[13] The OPLS-AA atom force field with the dielectric constant for methanol (33.0) was used in the calculation. The ^{3}J (NH –C(β)H) coupling constants were used to restrain the dihedral angle to 170 ± 20° according to a modified Karplus curve.[14] Distance constraints derived from 40

ROESY cross peak volumes were introduced using the CDIS command (strong: 2.5 ± 1Å; medium: 3.0 ± 1Å; weak: 4.0 ± 1Å), Figure S5.

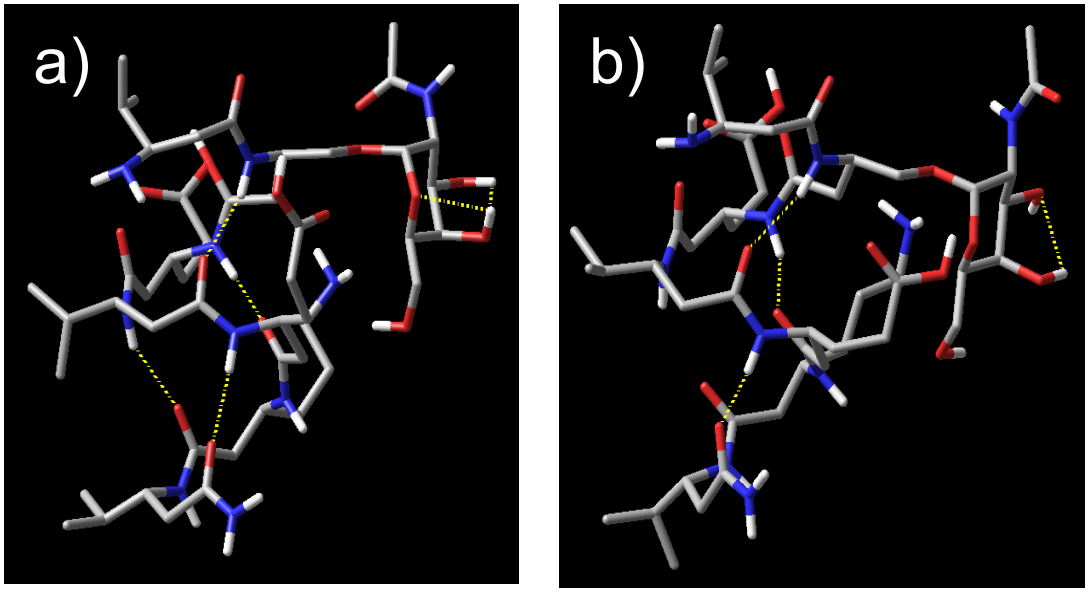

Figure S5. Molecular representation of the two (approx. isoenergetic) conformational families found in the restrained Monte-Carlo simulation: a) Close to an ideal 3_{14}-helical conformation (corresponding to the Blue representation in Fig. 2c); b) 3_{14}-helical conformation with fraying at the *C*-terminal side (corresponding to the Green representation in Fig. 2c).

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