Supporting Information

Conformational determinants of the activity of Antiproliferative factor glycopeptide.

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Table S1: ^{3}J coupling constants and associated sampling of the H–N–C2–H2 dihedrals in the glycopeptides with the Acetylamino (–NHCOCH3) Side Chain

Comp. No.	Sequence	$^{3}J_{NH-H_2}$ Expt	HREX	Population distribution Anti[a]	Int[a]	Ecl[a]
1	Galβ1-3GalNAcα-O-TVPAAVVVA	10.0	10.1	97.3	2.7	0.0
2	Galβ1-3GalNAcα-O-**S**VPAAVVVA	10.1	9.3	89.8	6.4	3.8
3	Galβ1-3GalNAcα-O-**SL**PAAVVVA	10.0	10.0	96.5	3.0	0.4
4	Galβ1-3GalNAcα-O-T**L**PAAVVVA	10.2	10.0	97.5	2.5	0.0
5	Galβ1-3GalNAcα-O-TVPA**V**VVVA		10.2	97.6	2.2	0.2
6	Galβ1-3GalNAcα-O-TVPAA**A**VVA		10.5	98.9	1.1	0.0
7	Galβ1-3GalNAcα-O-TVPAAV**A**VA		10.3	97.2	1.6	1.2
8	Galβ1-3GalNAcα-O-TVPAAVV**A**A		10.0	80.9	2.0	17.1
9	Galβ1-3GalNAcα-O-TVPAA**AAA**A		10.3	97.1	2.6	0.3
10	Galβ1-3GalNAcα-O-TVPAAVVV	10.2	10.2	92.8	2.3	5.0
11	Galβ1-3GalNAcα-O-TVPAA**AAA**	10.0	10.1	97.6	2.4	0.0
14	Galβ1-3GalNAcα-O-TV**(dP)**AAVVVA		8.6	91.7	8.3	0.0
	RMS diff[b]		0.3			

[a]anti distributions binned from −120 to 120°; intermediate (int) distributions binned from −120 to −60° and from 60 to 120°; eclipsed (ecl) distributions binned from −60 to 60°.
[b]Root-mean-square (RMS) difference over the six systems for which experimental ^{3}J coupling constants are available.

Table S2: ^{3}J coupling constants and the associated sampling of the N–Cα–Cβ–O1 dihedral for the 14 glycopeptides.[a]

Comp. No.	Sequence	$^{3}J_{H\alpha-H\beta}$	Population distribution		
			+60°[b]	-60°[b]	±180°[b]
1	Gal*β*1-3GalNAcα-O-TVPAAVVVA	3.2 (0.8)	100.0	0.0	0.0
2	Gal*β*1-3GalNAcα-O-**S**VPAAVVVA[c]	3.8 (1.2), 5.0 (3.8)	80.5	19.5	0.0
3	Gal*β*1-3GalNAcα-O-**SL**PAAVVVA[c]	4.0 (1.0), 3.0 (0.8)	100.0	0.0	0.0
4	Gal*β*1-3GalNAcα-O-T**L**PAAVVVA	3.2 (0.8)	100.0	0.0	0.0
5	Gal*β*1-3GalNAcα-O-TVPA**V**VVVA	3.1 (0.8)	100.0	0.0	0.0
6	Gal*β*1-3GalNAcα-O-TVPAA**A**VVA	3.1 (0.8)	100.0	0.0	0.0
7	Gal*β*1-3GalNAcα-O-TVPAAV**A**VA	3.1 (0.8)	100.0	0.0	0.0
8	Gal*β*1-3GalNAcα-O-TVPAAVV**A**A	3.0 (0.8)	100.0	0.0	0.0
9	Gal*β*1-3GalNAcα-O-TVPAA**AAA**A	3.1 (0.8)	100.0	0.0	0.0
10	Gal*β*1-3GalNAcα-O-TVPAAVVV	3.1 (0.8)	100.0	0.0	0.0
11	Gal*β*1-3GalNAcα-O-TVPAA**AAA**	3.2 (0.8)	100.0	0.0	0.0
12	TVPAAVVVA	3.1 (0.9)	100.0	0.0	0.0
13	TVPAAVVV	4.0 (3.0)	89.8	10.2	0.0
14	Gal*β*1-3GalNAcα-O-TV**(dP)**AAVVVA	3.3 (0.9)	100.0	0.0	0.0
	Average	3.2 (1.0)			

[a]The standard deviation of the calculated coupling constant values is presented in parentheses. J coupling constants in Hz.

[b]+60° (g+) distributions binned from 0 to 120°; −60° (g−) distributions binned from −120 to −0°; ±180° (anti) distributions binned from −180 to −120° and from 120 to 180°.

[c]For the Ser glycopeptides the first value corresponds to $^{3}J_{H\alpha,H\beta proR}$ and the second value corresponds to $^{3}J_{H\alpha,H\beta proS}$.

Table S3: ^3J coupling constants for the C–N–C$_\alpha$–C dihedral.[a]

Comp. No.		Amino Acids													
		T/S		**V/L**		**A**		**A**		**V**		**V**		**V**	
1	Exp	8.2		5.0		5.8		7.9		8.2		8.4		6.6	
	Hrex (SD)	7.1	2.1	6.2	2.1	7.1	2.1	6.9	2.4	7.7	2.1	7.6	2.2	8.2	1.5
	Diff[b]	**1.1**		**-1.2**		**-1.3**		**1.0**		**0.5**		**0.8**		**-1.6**	
2	Exp	8.0		5.5		5.8		8.2		8.3		7.8		6.9	
	Hrex (SD)	6.7	2.2	7.1	2.1	7.0	2.0	6.9	2.4	7.3	2.1	7.6	2.3	8.2	1.5
	Diff[b]	**1.3**		**-1.6**		**-1.2**		**1.3**		**1.0**		**0.2**		**-1.3**	
3	Exp	7.1		5.7		5.8		7.8		8.1		8.8		4.6	
	Hrex (SD)	7.2	2.0	6.0	1.8	6.8	1.8	7.3	2.2	7.4	1.9	7.5	2.3	8.2	1.5
	Diff[b]	**-0.1**		**-0.3**		**-1.0**		**0.5**		**0.7**		**1.3**		**-3.6**	
4	Exp	7.5		5.2		5.9		7.8		8.1		8.3		6.8	
	Hrex (SD)	7.5	2.0	6.0	2.0	6.7	2.1	7.1	2.3	7.7	1.9	8.1	1.9	8.1	1.5
	Diff[b]	**0.0**		**-0.8**		**-0.8**		**0.7**		**0.4**		**0.2**		**-1.3**	
5	Hrex (SD)	7.2	2.1	6.4	2.0	7.0	2.3	7.6	2.2	7.4	2.2	7.2	2.3	8.3	1.4
6	Hrex (SD)	6.8	2.0	6.0	1.5	6.7	2.1	7.2	2.0	7.0	2.1	7.2	2.3	8.3	1.5
7	Hrex (SD)	6.8	2.1	6.4	2.1	7.0	2.0	7.0	2.4	6.8	1.9	7.3	2.3	8.3	1.4
8	Hrex (SD)	7.3	2.0	6.1	2.0	6.5	2.0	6.7	2.3	6.6	2.0	7.2	2.1	8.1	1.5
9	Hrex (SD)	7.1	2.0	5.0	1.6	5.9	1.9	6.5	2.0	6.7	1.9	6.5	2.2	8.0	1.6
10	Exp	8.2		5.3		5.8		8.1		8.5		8.7			
	Hrex (SD)	7.1	2.1	6.2	2.0	6.3	1.8	6.8	2.3	7.3	2.3	8.6	1.3		
	Diff[b]	**1.1**		**-0.9**		**-0.5**		**1.3**		**1.2**		**0.1**			
11	Exp	7.9		5.2		5.9		5.9		5.3		6.7			
	Hrex (SD)	7.1	2.1	5.3	1.9	6.4	2.0	7.0	1.9	7.1	2.1	7.9	1.6		
	Diff[b]	**0.8**		**-0.1**		**-0.5**		**-1.1**		**-1.8**		**-1.2**			
12	Hrex (SD)	6.4	2.4	6.5	2.1	6.5	1.7	7.1	2.2	7.1	2.1	7.4	2.3	8.3	1.4
13	Exp	7.5		5.9		5.8		7.8		8.9		8.7			
	Hrex (SD)	6.6	2.3	6.5	1.9	6.8	1.9	7.2	2.2	7.4	2.3	8.6	1.3		
	Diff[b]	**0.9**		**-0.6**		**-1.0**		**0.6**		**1.5**		**0.1**			
	RMS Diff[c]	**0.9**		**0.9**		**1.0**		**1.0**		**1.1**		**0.7**		**2.2**	
	RMSD		**2.1**		**2.0**		**2.0**		**2.2**		**2.1**		**1.9**		**1.5**

[a] ^3J coupling constants in Hz.

[b]Diff = $^3J_{Exp}$ - $^3J_{Hrex}$

[c]Root-mean-square (RMS) difference with respect to the experimental ^{3}J coupling constants.

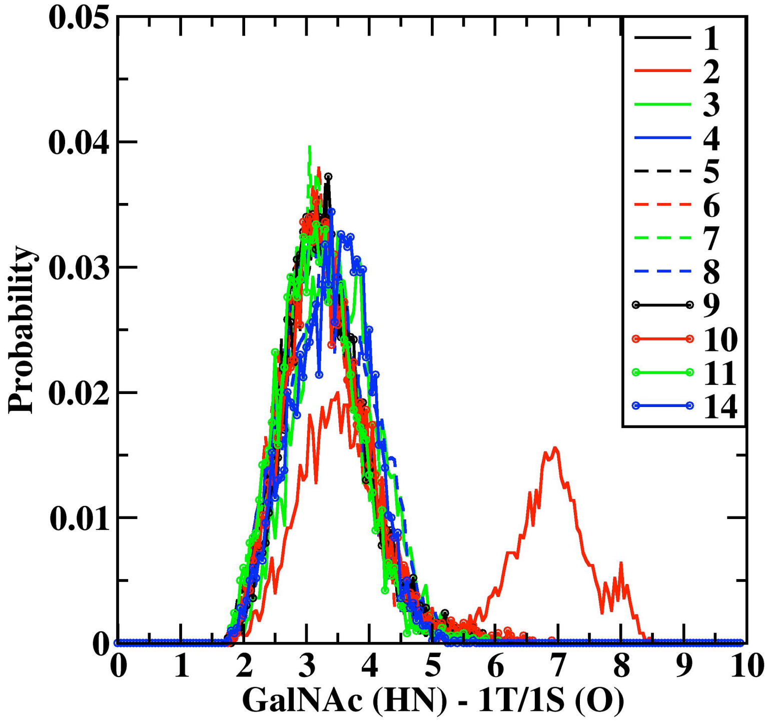

Figure S1: GalNAc (HN) – 1T/1S (O) H-bond distribution for all systems.

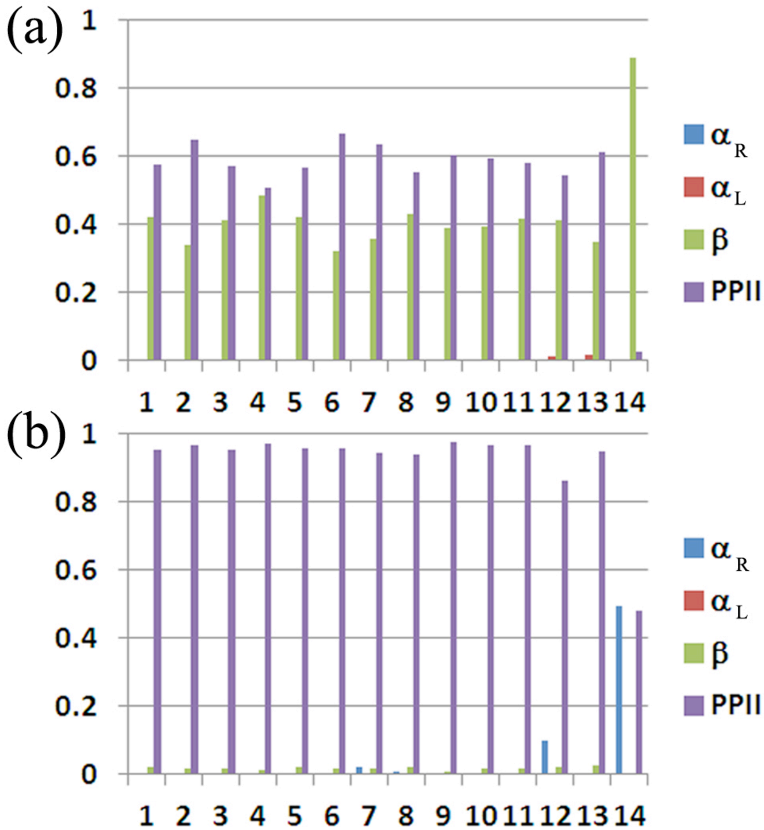

Figure S2: Population distribution of the ϕ/ψ dihedrals in the four commonly observed backbone conformational regions, α_R, α_L, β and PPII, as defined in the Ramachandran surface for amino acids (a) 2**V/L** to (b) 3**P**. All the regions in the Ramachandran surface are inverted in D-proline (3**P** in **14**).

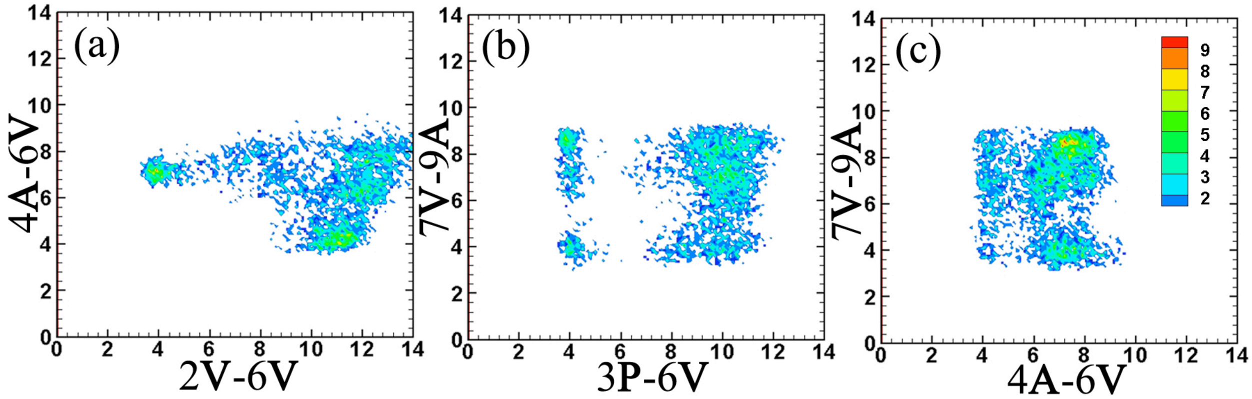

Figure S3: 2D distributions of the close contact probabilities (a) 2**V**-6**V**/4**A**-6**V**, (b) 3**P**-6**V**/7**V**-9**A**, and (c) 4**A**-6**V**/7**V**-9**A**. Distances are presented in Å.

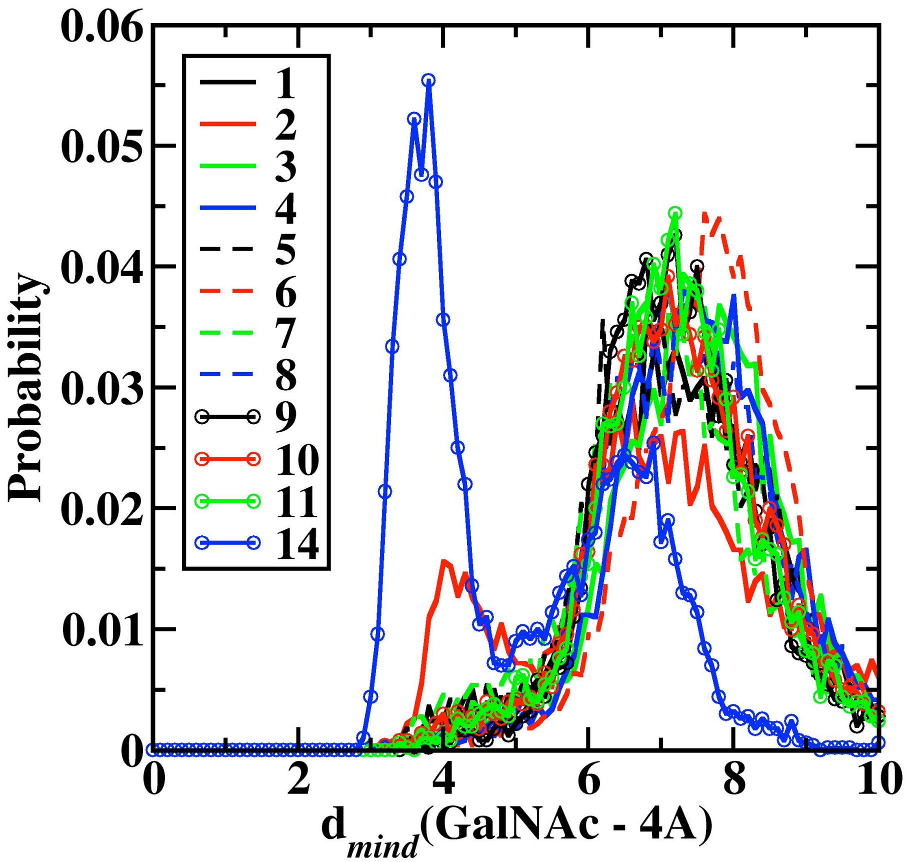

Figure S4: Close contact probabilities between GalNAc and 4**A** for all systems. Distances are presented in Å.