Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
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Synthesis, biological and structural characterization of novel glycopeptide analogues of nociceptin N/OFQ

Supplementary Information

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Data for nociceptin (N/OFQ) and the following glycopeptides:

#	Glycopeptide sequence	Name
1	H-Phe-Gly-Gly-Phe-*Thr*^{5}*-(α-GalNAc)*-Gly-Ala-Arg-Lys-Ser^{10}-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Thr^{5}-*O*-α-D-GalNAc]-N/OFQ
2	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}*-(α-GalNAc)*-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-α-D-GalNAc]-N/OFQ
3	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}*-(β-GlcNAc*)-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-β-D-GlcNAc]-N/OFQ

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UPLC-TOF/MS

NOCICEPTIN (N/OFQ)

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NC-SerGalNAc-OH3

2	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}-*(α-GalNAc)*-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-α-D-GalNAc]-N/OFQ

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NC-SerGlcNAc-OH3

3	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}-*(β-GlcNAc*)-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-β-D-GlcNAc]-N/OFQ

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NC-SerGlcNAc-OH3 (expanded)

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NC-ThrGalNAc-OH3

1	H-Phe-Gly-Gly-Phe-*Thr*^{5}-*(α-GalNAc)*-Gly-Ala-Arg-Lys-Ser^{10}-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Thr^{5}-*O*-α-D-GalNAc]-N/OFQ

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UPLC-TOF-MS

NC-ThrGalNAc-OH3

1	H-Phe-Gly-Gly-Phe-*Thr*^{5}*-(α-GalNAc)*-Gly-Ala-Arg-Lys-Ser^{10}-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Thr^{5}-*O*-α-D-GalNAc]-N/OFQ

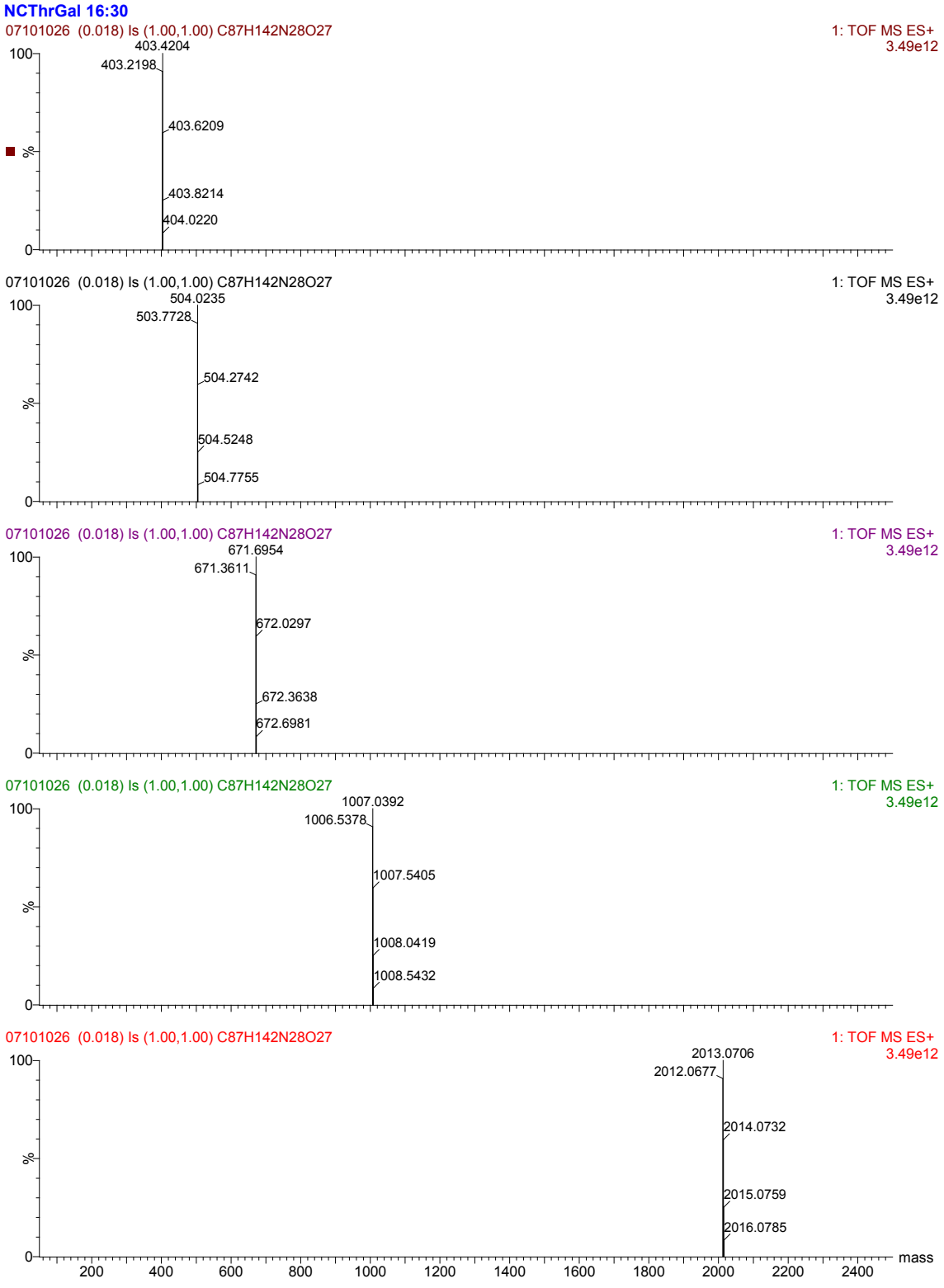

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Table S1. ^{1}H Chemical shift (p.p.m.) for nociceptin **(1)** in water solution at 278K.

Nociceptin				
a.a.	NH	Hα	Hβ	misc.
Phe^{1}				
Gly^{2}				
Gly^{3}	7,887	3,663		
Phe^{4}	8,205	4,513	2,853 ; 2,917	*
Thr^{5}	8,198	4,110	0,966	1,029(Hγ)
Gly^{6}	7,659	3,661		
Ala^{7}	8,109	4,093	1,181	
Arg^{8}	8,188	4,024	1,555	1,412(Hγ); 2,974(Hδ); 7,056(Hε)
Lys^{9}	8,384	4,071	1,579	1,224(Hγ); 1,465(Hδ); 2,783(Hε)
Ser^{10}	8,278	4,195	3,647	
Ala^{11}	8,343	4,099	1,203	
Arg^{12}	8,307	4,075	1,561	1,417(Hγ); 2,961(Hδ); 7,019(Hε)
Lys^{13}	8,347	4,056	1,579	1,224(Hγ); 1,465(Hδ); 2,780(Hε)
Leu^{14}	8,276	4,128	1,412 ; 1,431	1,373(Hγ); 0,676(Hδ)
Ala^{15}	8,225	4,079	1,196	
Asn^{16}	8,338	4,423	2,589 ; 2,659	7,553(Hδ21); 6,784(Hδ22)
Gln^{17}	7,743	3,951	1,917 ; 1,756	2,068(Hγ); 7,460(Hε21); 6,745(Hε22)
*overlapped				

Table S2. ^{1}H Chemical shift (p.p.m.) for (Ser^{10}-*O*-α-D-GalNAc)-N/OFQ **(2)** in water solution at T=278K.

(Ser^{10}-O-α–D-GalNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc.
Phe^{1}				
Gly^{2}				
Gly^{3}	7,747	2,530		
Phe^{4}	8,067	4,384	2,783 ; 2,727	*
Thr^{5}	8,058	3,972	0,838	1,042(Hγ)
Gly^{6}	7,539	3,531		
Ala^{7}	7,961	3,948	1,036	
Arg^{8}	8,092	3,948	1,453	1,298(Hγ); 2,824(Hδ); 6,939(Hε)
Lys^{9}	8,193	3,967	1,424	1,099(Hγ); 1,348(Hδ); 2,637(Hε)
Ser^{10}	8,362	4,243	3,584 ; 3,419	
Ala^{11}	8,298	3,996	1,046	
Arg^{12}	8,163	3,889	1,430	1,292(Hγ); 2,851(Hδ); 6,989(Hε)
Lys^{13}	8,272	3,924	1,348	1,095(Hγ); 1,348(Hδ); 2,647(Hε)
Leu^{14}	8,173	3,993	1,298	1,237(Hγ); 0,568(Hδ)
Ala^{15}	8,112	3,939	1,052	
Asn^{16}	8,216	4,295	2,512 ; 2,475	7,325(Hδ21); 6,621 (Hδ22)
Gln^{17}	7,620	3,825	1,786 ; 1,599	1,942(Hγ); 7,417(Hε21); 6,662(Hε22)
*overlapped				

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	$NH_{acetamide}$	$CH_{3\,acetamide}$
α-D-GacNAc	4,529	3,811	3,539	3,604		3,532	3,390	7,781	1,682

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Table S3. ^{1}H Chemical shift for (Ser^{10}-*O*-β-D-GlcNAc)-N/OFQ **(3)** in water solution at 278K.

(Ser^{10}-O-β–D-GlcNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc.
Phe^{1}				
Gly^{2}	8,252	3,680		
Gly^{3}	7,872	3,649		
Phe^{4}	8,332	4,507	2,844 ; 2,898	*
Thr^{5}	8,171	4,099	3,997	0,960(Hγ)
Gly^{6}	7,616	3,656		
Ala^{7}	8,069	4,082	1,156	
Arg^{8}	8,155	4,026	1,554	1,417(Hγ); 2,965(Hδ); 7,039(Hε)
Lys^{9}	8,287	4,069	1,556	1,210(Hγ); 1,459(Hδ); 2,773(Hε)
Ser^{10}	8,201	4,285	3,761	
Ala^{11}	8,232	4,065	1,166	
Arg^{12}	8,219	4,066	1,554	1,419(Hγ); 2,974(Hδ); 7,053(Hε)
Lys^{13}	8,348	4,067	1,559	1,179(Hγ); 1,465(Hδ); 2,764(Hε)
Leu^{14}	8,276	4,086	1,543	1,414(Hγ); 0,677(Hδ)
Ala^{15}	8,225	4,060	1,192	
Asn^{16}	8,332	4,422	2,570	7,527(Hδ21); 6,785(Hδ22)
Gln^{17}	7,736	3,941	1,911 ; 1,712	2,059(Hγ); 7,434(Hε21); 6,751(Hε22)
*overlapped				

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	NH acetamide	CH_3 acetamide
β-D-GlcNAc	4,321	3,497	3,316	3,237	3,715	3,521	3,208	8,136	1,804

Table S4. ^{1}H chemical shift (p.p.m.) for (Thr^{5}-*O*-α-D-GalNAc)-N/OFQ **(4)** in water solution at 278K.

(Thr^{5}-O-α–D-GalNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc,
Phe^{1}				
Gly^{2}				
Gly^{3}	7,794	3,649		
Phe^{4}	8,194	4,597	2,895	*
Thr^{5}	8,446	4,326	4,135	0,978(Hγ)
Gly^{6}	8,224	3,684		
Ala^{7}	8,231	4,068	1,163	
Arg^{8}	8,196	4,044	1,544	1,406(Hγ); 2,964(Hδ); 7,058(Hε)
Lys^{9}	8,336	4,068	1,560	1,220(Hγ); 1,464(Hδ); 2,772(Hε)
Ser^{10}	8,308	4,184	3,637	
Ala^{11}	8,372	4,080	1,173	
Arg^{12}	8,368	4,024	1,407	1,407(Hγ); 2,953(Hδ); 7,099(Hε)
Lys^{13}	8,432	4,087	1,565	1,203(Hγ); 1,461(Hδ); 2,765(Hε)
Leu^{14}	8,259	4,105	1,415	1,344(Hγ); 0,676(Hδ)
Ala^{15}	8,210	4,052	1,167	
Asn^{16}	8,321	4,417	2,640 ; 2,577	7,535(Hδ21); 6,778(Hδ22)
Gln^{17}	7,737	2,934	1,902 ; 1,732	2,060(Hγ); 7,441(Hε21); 6,731(Hε22)
*overlapped				

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	NH acetamide	CH_3 acetamide
α–D-GalNAc	4,688	3,815	3,672	3,686	3,541	3,507	3,792	7,717	1,748

Table S5. 1H Chemical shift (p.p.m.) for nociceptin **(1)** in SDS micelles at 288K.

Nociceptin				
a.a.	NH	Hα	Hβ	misc.
Phe[1]	7,909	4,512	3,091 ; 2,937	7,114 (Hδ); 7,006 (Hε); 6,706 (Hζ)
Gly[2]	8,232	3,507 ; 3,604		
Gly[3]	8,186	3,981 ; 3,742		
Phe[4]	7,864	4,564	3,236 ; 2,846	7,241 (Hδ); 7,090 (Hε); 6,952(Hζ)
Thr[5]	8,320	4,169	4,101	1,029(Hγ)
Gly[6]	8,341	4,162		
Ala[7]	7,836	3,928	1,220	
Arg[8]	8,176	4,147	1,683	1,536(Hγ); 2,965(Hδ); 7,049(Hε)
Lys[9]	7,769	3,991	1,651	1,261(Hγ); 1,459(Hδ); 2,797(Hε)
Ser[10]	7,930	4,065	3,681	
Ala[11]	7,745	4,003	1,258	
Arg[12]	7,704	3,944	1,675	1,508(Hγ); 2,976(Hδ); 7,031(Hε)
Lys[13]	7,783	3,809	1,683	1,242(Hγ); 1,506(Hδ); 2,799(Hε)
Leu[14]	7,744	3,999	1,499	1,378(Hγ); 0,687(Hδ)
Ala[15]	7,665	4,052	1,222	
Asn[16]	7,851	4,545	2,681 ; 2,523	7,428(Hδ21); 6,727(Hδ22)
Gln[17]	7,561	3,929	1,934 ; 1,731	2,129(Hγ); 7,357(Hε21); 6,658(Hε22)

Table S6. [1]H Chemical shift (p.p.m.) for (Ser[10]-*O*-α-D-GalNAc)-N/OFQ **(2)** in SDS micelles at 288K.

(Ser[10]-O-α-D-GalNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc.
Phe[1]		4,164	2,968 ; 3,104	7,124(Hδ); 7,021(Hε); 6,708(Hζ)
Gly[2]	8,232	3,511 ; 3,603		
Gly[3]	8,241	4,004 ; 3,747		
Phe[4]	7,851	4,595	2,846 ; 3,275	7,269(Hδ); 7,111(Hε); 6,955 (Hζ)
Thr[5]	8,414	4,201		1,042(Hγ)
Gly[6]	8,390	4,202		
Ala[7]	7,786	3,965	1,227	
Arg[8]	8,198	3,958	1,692	1,514(Hγ); 2,969(Hδ); 7,074(Hε)
Lys[9]	7,767	3,789	1,650	1,256(Hγ); 1,520(Hδ); 2,801(Hε)
Ser[10]	7,859	4,271	3,837	
Ala[11]	7,910	3,939	1,287	
Arg[12]	7,894	3,977	1,700	1,492(Hγ); 2,989(Hδ); 7,029(Hε)
Lys[13]	7,775	4,002	1,669	1,292(Hγ); 1,536(Hδ); 2,804(Hε)
Leu[14]	7,600	4,015	1,491	1,391(Hγ); 0,708(Hδ)
Ala[15]	7,661	4,053	1,242	
Asn[16]	7,823	4,562	2,699 ; 2,532	7,411(Hδ21); 6,736 (Hδ22)
Gln[17]	7,543	3,930	1,947 ; 1,756	2,158(Hγ); 7,368(Hε21); 6,669(Hε22)

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	$NH_{acetamide}$	$CH_{3\ acetamide}$
α–D-GalNAc	4,662	3,982	3,632	3,774		3,691	3,426	8,049	1,823

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Table S7. ^{1}H Chemical shift (p.p.m.) for (Ser^{10}-*O*-β-D-GlcNAc)-N/OFQ **(3)** in SDS micelles at 288K.

(Ser^{10}-O-β-D-GlcNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc.
Phe^{1}		4,159	2,956 ; 3,088	7,124(Hδ); 7,023(Hε); 6,734(Hζ)
Gly^{2}	8,225	3,577		
Gly^{3}	8,141	3,971 ; 3,701		
Phe^{4}	7,862	4,571	3,226 ; 2,858	7,242(Hδ); 7,100(Hε); 6,957(Hζ)
Thr^{5}	8,265	4,159	4,070	1,035(Hγ)
Gly^{6}	8,264	4,149		
Ala^{7}	7,805	4,083	1,233	
Arg^{8}	8,141	3,984	1,676	1,486(Hγ); 2,995(Hδ); 7,030(Hε)
Lys^{9}	7,792	4,060	1,637	1,304(Hγ); 1,518(Hδ); 2,808 (Hε)
Ser^{10}	8,004	4,187	3,925 ; 3,711	
Ala^{11}	7,788	3,988	1,227	
Arg^{12}	7,761	4,021	1,653	1,501(Hγ); 2,990(Hδ); 6,974(Hε)
Lys^{13}	7,750	4,016	1,671	1,268(Hγ); 1,530(Hδ); 2,795(Hε)
Leu^{14}	7,740	4,008	1,516	1,390(Hγ); 0,701(Hδ)
Ala^{15}	7,675	4,073	1,218	
Asn^{16}	7,905	4,534	2,664 ; 2,524	7,415(Hδ21); 6,724(Hδ22)
Gln^{17}	7,600	3,923	1,939 ; 1,746	2,117(Hγ); 7,350(Hε21); 6,653(Hε22)

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	$NH_{acetamide}$	$CH_{3\,acetamide}$
β–D-GlcNAc	4,356	3,483	3,348	3,241	3,232	3,726	3,404	7,955	1,805

Table S8. ^{1}H Chemical shift (p.p.m.) for (Thr^{5}-*O*-α-D-GalNAc)-N/OFQ **(4)** in SDS micelles at 288K.

(Thr^{5}-O-α-DGalNAc)-N/OFQ				
a.a.	NH	Hα	Hβ	misc,
Phe^{1}		4,153	2,949 ; 3,091	7,129(Hδ); 6,759(Hε); 7,039(Hζ)
Gly^{2}	8,212	3,590		
Gly^{3}				
Phe^{4}	7,909	4,606	2,927 ; 3,188	7,249(Hδ); 7,112(Hε); 6,977(Hζ)
Thr^{5}	8,240	4,401	4,269	0,969(Hγ)
Gly^{6}	8,240*	4,401*		
Ala^{7}	8,163	4,121	1,216	
Arg^{8}	8,085	4,002	1,682	1,491(Hγ); 2,983(Hδ); 7,008(Hε)
Lys^{9}	7,879	3,972	1,630	1,241(Hγ); 1,508(Hδ); 2,803(Hε)
Ser^{10}	7,839	4,163	3,740 ; 3,656	
Ala^{11}	7,932	4,025	1,241	
Arg^{12}	7,783	3,975	1,655	1,500(Hγ); 3,015(Hδ); 7,000(Hε)
Lys^{13}	7,800	4,065	1,642	1,255(Hγ); 1,504(Hδ); 2,801(Hε)
Leu^{14}	7,770	4,010	1,513	1,376(Hγ); 0,699(Hδ)
Ala^{15}	7,704	4,057	1,214	
Asn^{16}	7,921	4,534	2,677 ; 2,523	7,430(Hδ21); 6,725(Hδ22)
Gln^{17}	7,623	3,923	1,942 ; 1,736	2,122(Hγ); 6,655(Hε21); 7,350(Hε22)
*overlapped				

sugar residue	H1	H2	H3	H4	H5	$H6_1$	$H6_2$	NH acetamide	CH_3 acetamide
α–D-GalNAc	4,686	3,791	3,528			3,694	3,451	7,717	1,835

Table S9. Statistics for the NMR solution structure of the peptides **1-4** in SDS micelles at 288K. Average values over the 20 conformers obtained by CYANA program.

PARAMETER	PEPTIDE			
	Nociceptin	(Ser10-*O*-α–D-GalNAc)-N/OFQ	(Ser10-O-β–D-GlcNAc)-N/OFQ	(Thr^{5}-O-α–D-GalNAc)-N/OFQ
	1	**2**	**3**	**4**
NOE cross-peaks				
Assigned (%)	95,1			
NOE upper distant limits				
Short-range, |i–j|≤1	162	143	139	157
Medium-range, 1<|i–j|<5	36	45	34	20
Long-range, |i–j|≥5	6	0	8	7
CYANA target function (Å^{2})	0,01	0,01	0,03	0,01
Rmsd to mean co-ordinates (Å)				
Backbone	1.69	2.68	2.03	2.76
All heavy atoms	2.11	3.59	2.87	3.47
Ramachandran plot statistics (%)				
Residues in the most favoured	59,6	70	57,9	44,6
Residues in the additionally	40	30	42,1	55
Residues in the generously	0,4	0	0	0,4
Residues in the disallowed	0	0	0	0

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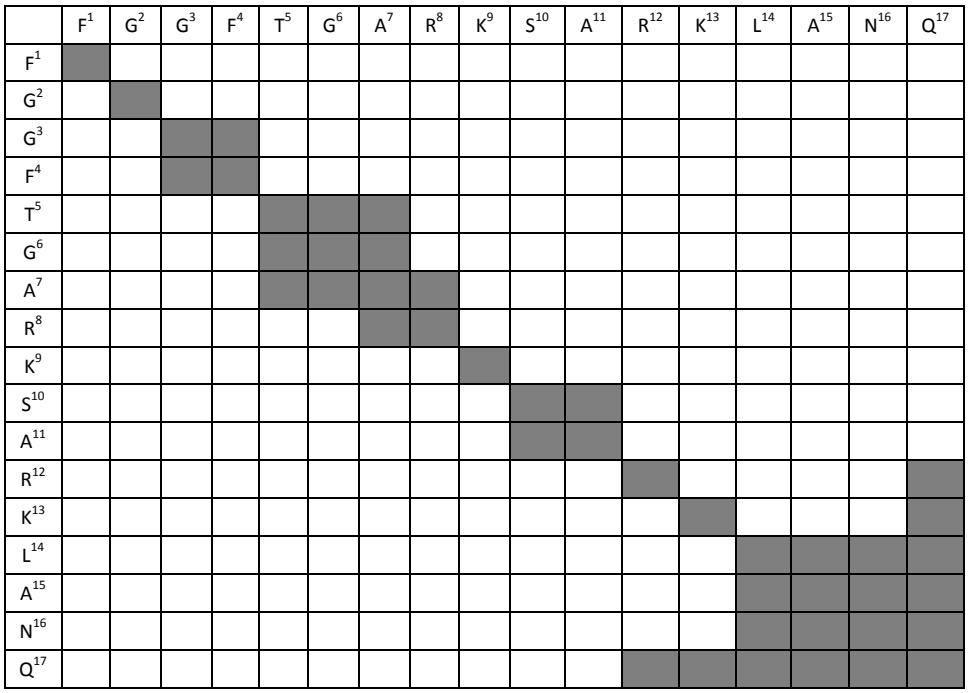

Fig. S1. Inter-residual NOEs observed on NH region from NOESY spectrum (T=278K, 300ms) for nociceptin in water solution.

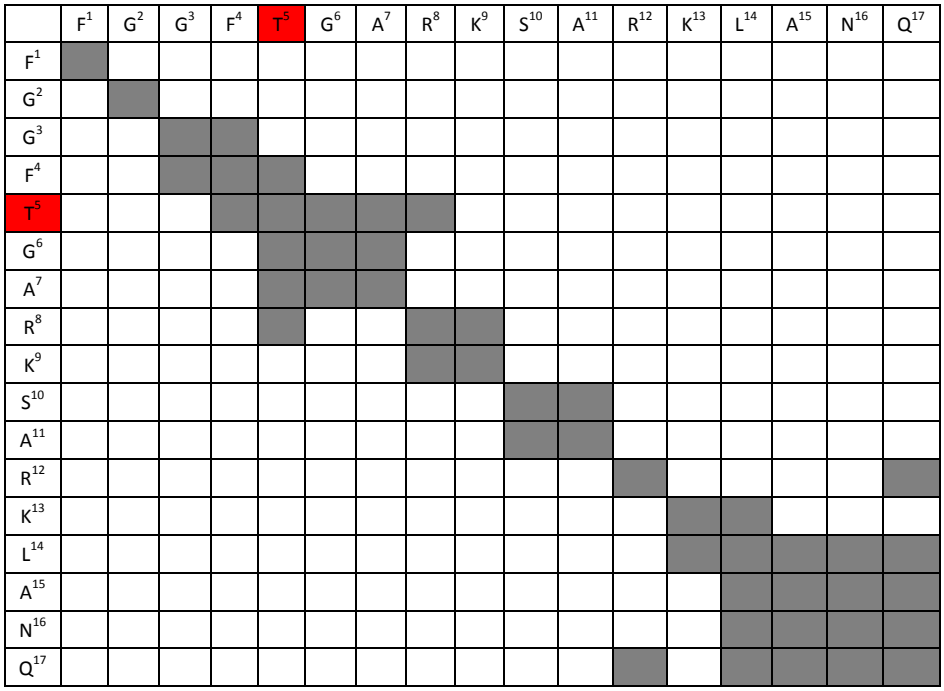

Fig. S2. Inter-residual NOEs observed on NH region from NOESY spectrum (T=278K, 300ms) for (Thr^5-*O*-α-D-GalNAc)-N/OFQ **(1)** in water solution.

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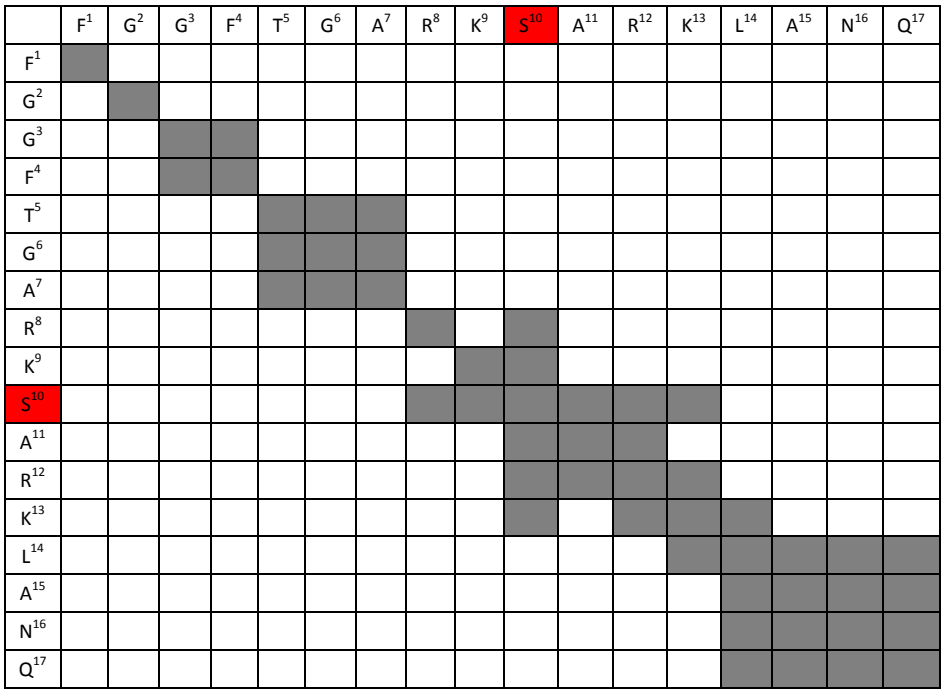

Fig. S3. Inter-residual NOEs observed on NH region from NOESY spectrum (T=278K, 300ms) for (Ser^{10}-*O*-α-D-GalNAc)-N/OFQ **(2)** in water solution.

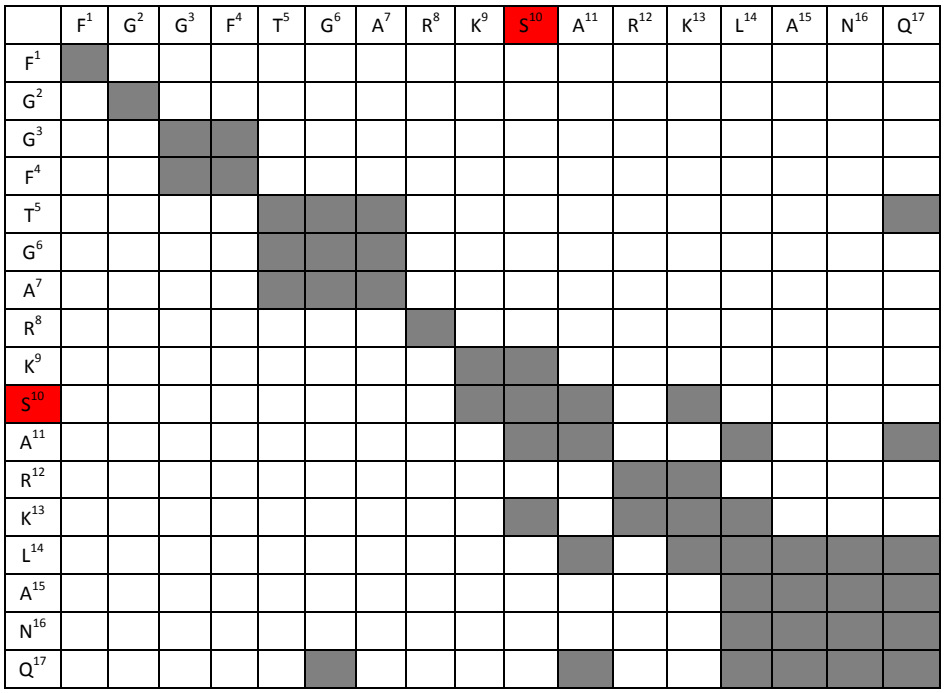

Fig. S4. Inter-residual NOEs observed on NH region from NOESY spectrum (T=278K, 300ms) for (Ser^{10}-*O*-β-D-GlcNAc)-N/OFQ **(3)** in water solution.

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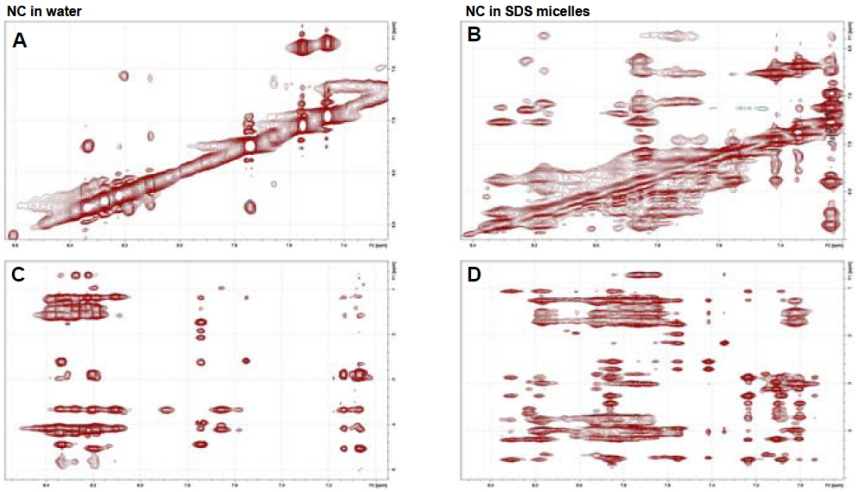

Fig. S5. NH-NH (A and B) and NH-αH (C and D) regions from NOESY spectra for nociceptin **(1)** in water and in presence of SDS.

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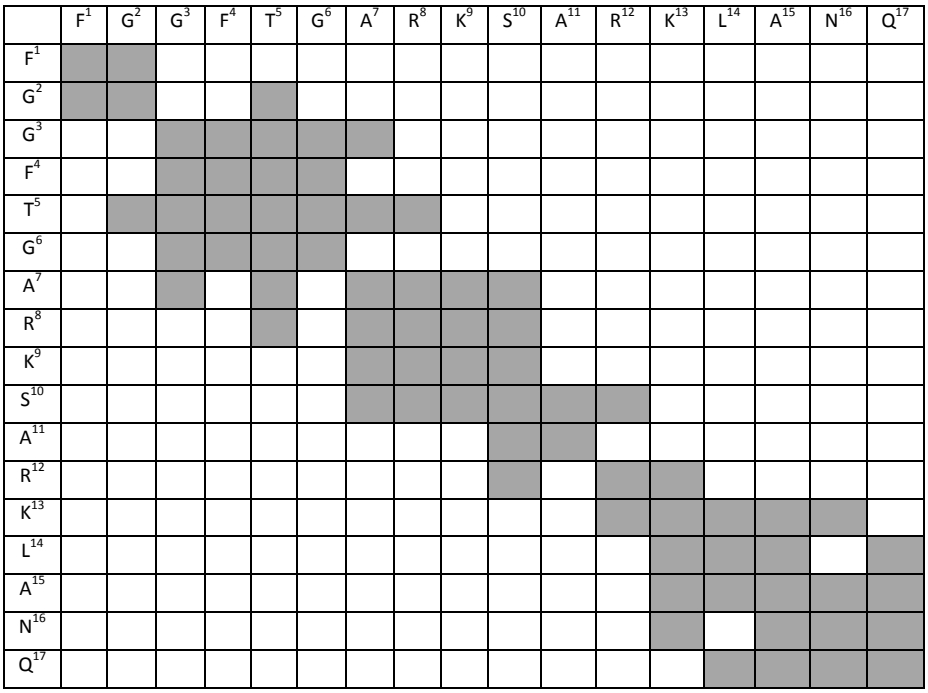

Fig. S6. Inter-residual NOEs observed on NH region from NOESY spectrum (T=288K, 300ms) for nociceptin in SDS micelles.

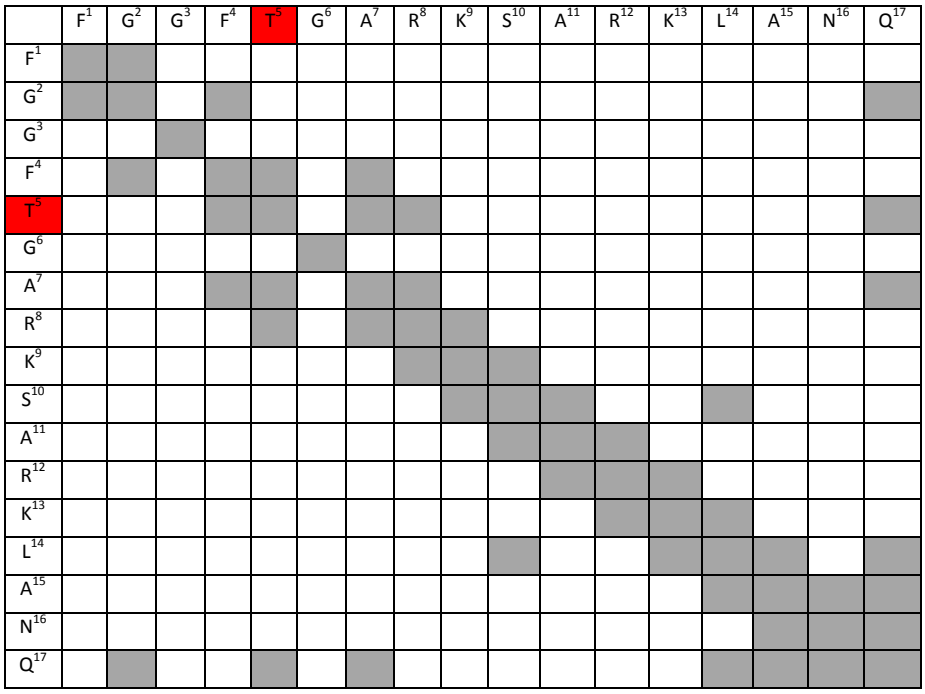

Fig. S7. Inter-residual NOEs observed on NH region from NOESY spectrum (T=278K, 300ms) for (Thr^5-*O*-α-D-GalNAc)-N/OFQ **(1)** in water solution.

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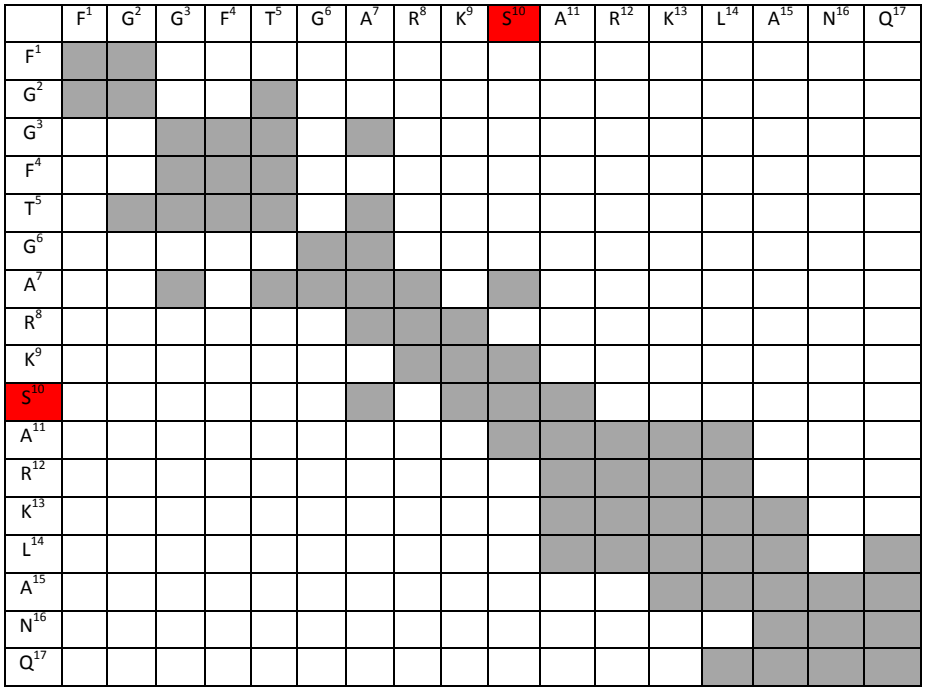

Fig. S8. Inter-residual NOEs observed on NH region from NOESY spectrum (T=288K, 300ms) for (Ser^{10}-*O*-α-D-GalNAc)-N/OFQ **(2)** in SDS micelles.

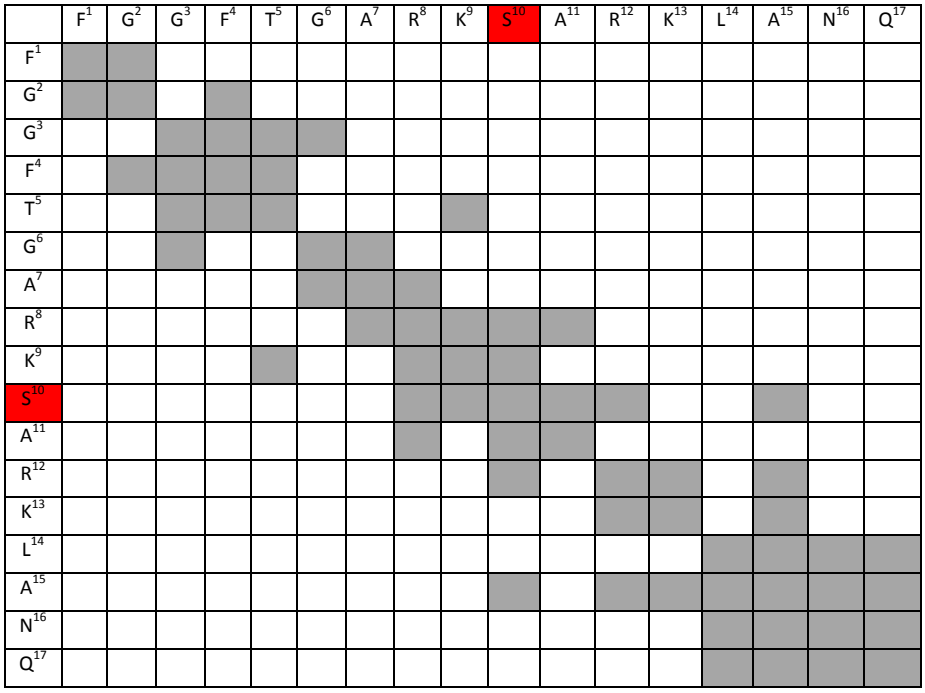

Fig. S9. Inter-residual NOEs observed on NH region from NOESY spectrum (T=288K, 300ms) for (Ser^{10}-*O*-β-D-GlcNAc)-N/OFQ **(3)** in SDS micelles.

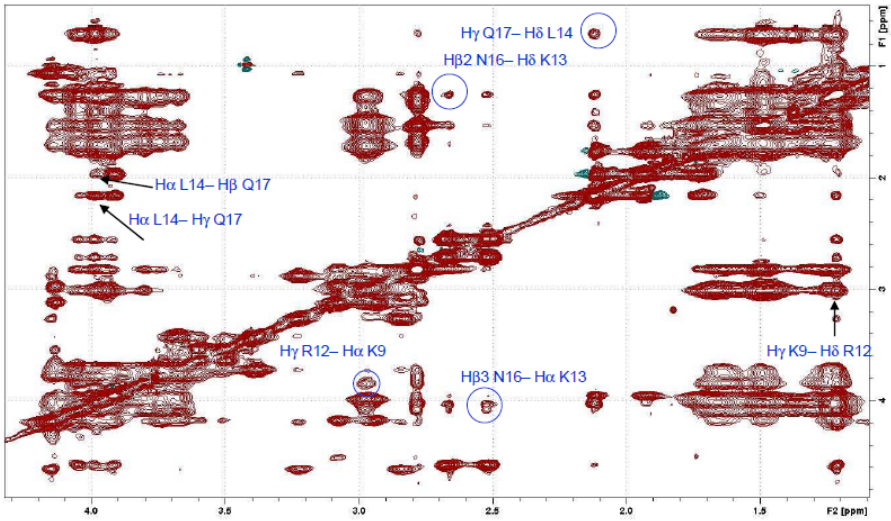

Fig S10. Aliphatic region from 600MHz NOESY (T=288K, 300 ms) spectrum for nociceptin. The NOEs (in blue) support the presence of a helical structure. Nociceptin **(1)** at 2mM with SDS solution at 132mM.

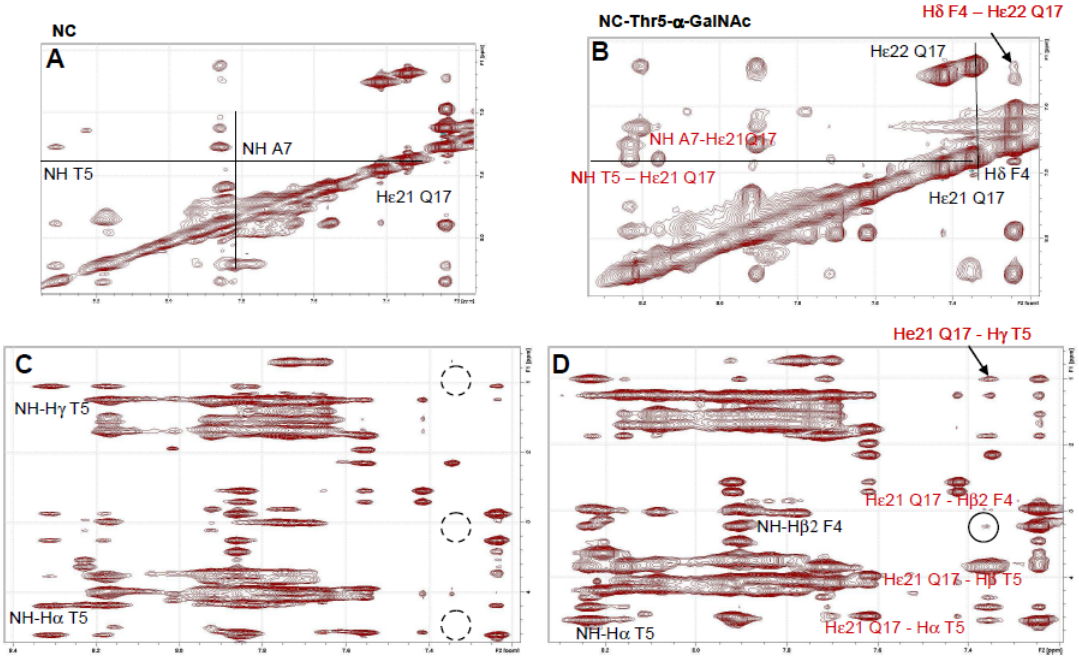

Fig S11. Selected regions from 600MHz NOESY spectra (T=288K, 300ms) for nociceptin (A and C) and (Thr^5-*O*-α-D-GalNAc)-N/OFQ (B and D). Remote NOEs contacts displayed in red (B and D) were observed in the case of (Thr^5-*O*-α-D-GalNAc)-N/OFQ. However, these remote contacts were not detected either in the case of nociceptin (A and C) and the others glycopeptides (data not shown).

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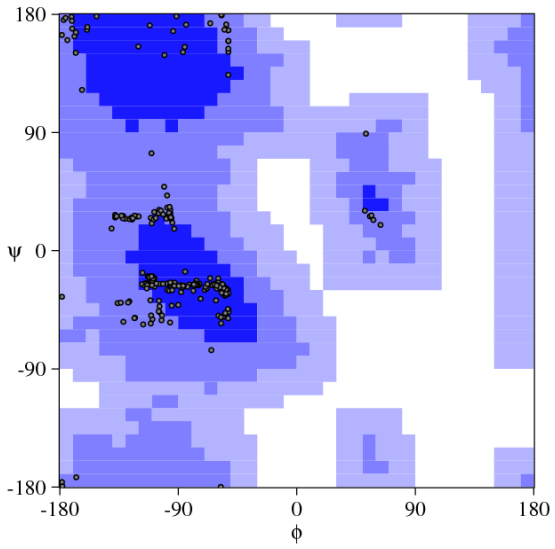

Fig S12. Ramachandran plot obtained by CYANA program for nociceptin **(1)** in SDS micelles.

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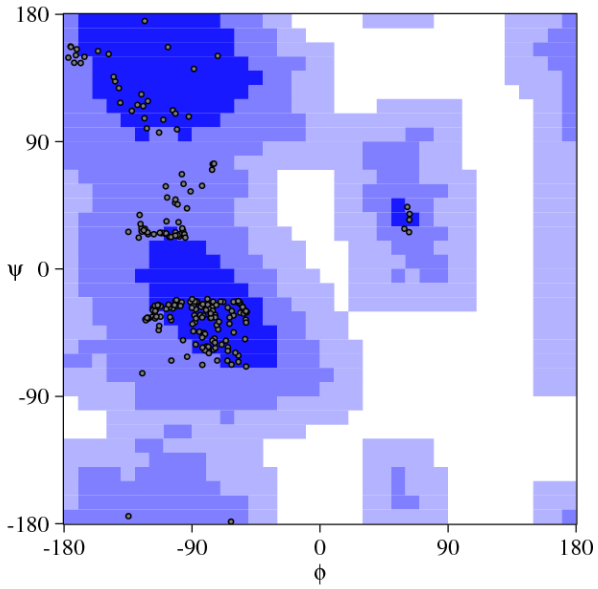

Fig S13. Ramachandran plot obtained by CYANA program for (Ser[10]-*O*-α-D-GalNAc)-N/OFQ **(2)** in SDS micelles.

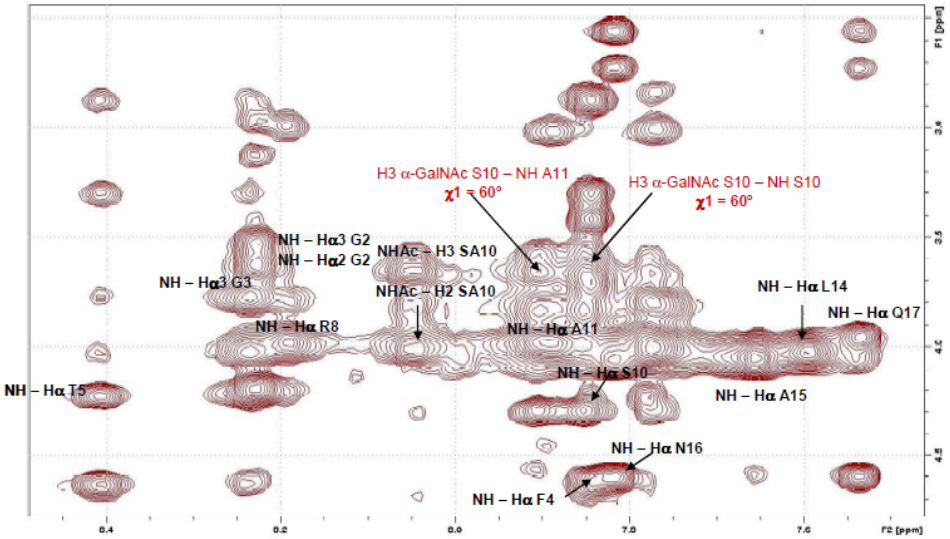

Fig S14. Hα-NH region from 600MHz NOESY (T=288K, 300 ms) spectrum for (Ser^{10}-*O*-α-D-GalNAc)-N/OFQ **(2)**. The assignment for some residues are shown, as well as the key NOEs (in red) that indicates the presence of the structure with χ1 = 60º (χ1 = N2-Cα-Cβ-O1). Glycopeptide at 2mM with SDS solution at 132mM.

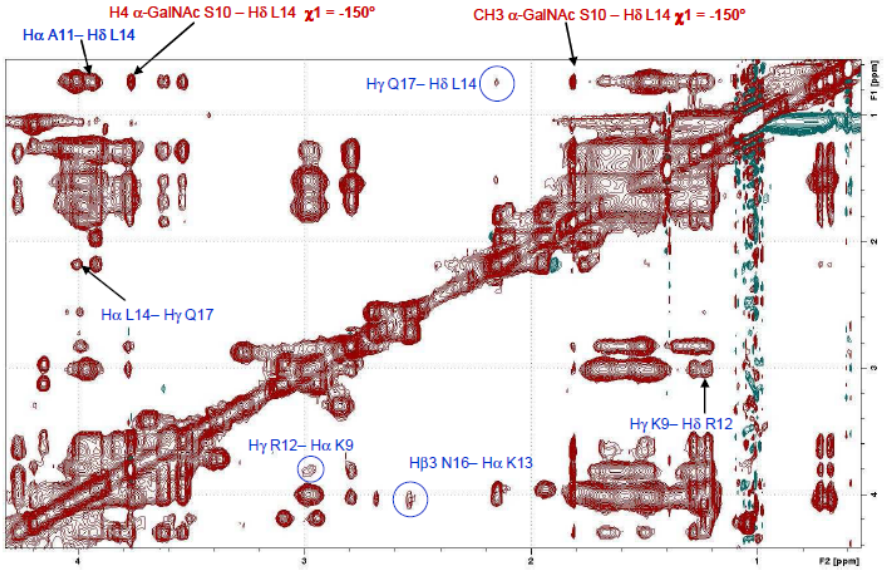

Fig S15. Aliphatic region from 600MHz NOESY (T=288K, 300 ms) spectrum for (Ser^{10}-*O*-α-D-GalNAc)-N/OFQ **(2)**. The key NOEs (in red) indicate the presence of the structure with χ1 = -150º (χ1 = N2-Cα-Cβ-O1), as well as, some of the NOEs (in blue) that support the presence of a helical structure. Glycopeptide at 2mM with SDS solution at 132mM.

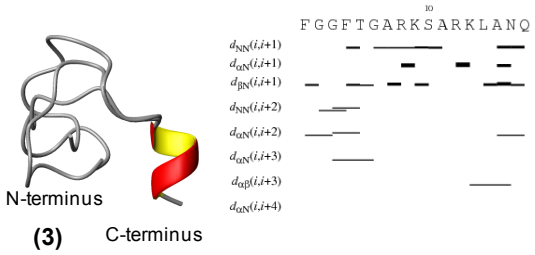

Fig S16. Superposition of 3 selected structures, NOE connectivities and Hα chemical shift index (CSI) for (Ser[10]-*O*-β-D-GlcNAc)-N/OFQ **(3)**.

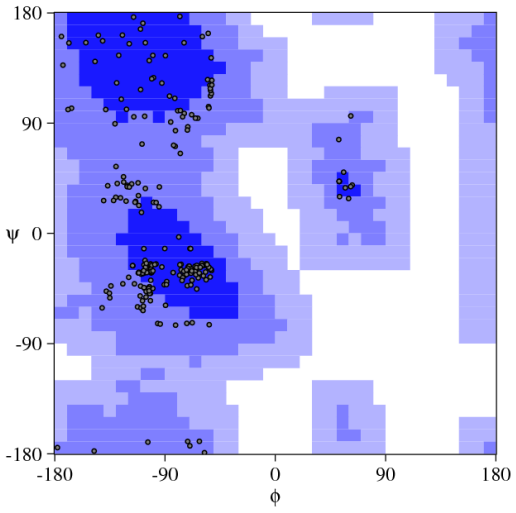

Fig S17. Ramachandran plot obtained by CYANA program for (Ser[10]-*O*-β-D-GlcNAc)-N/OFQ **(3)** in SDS micelles.

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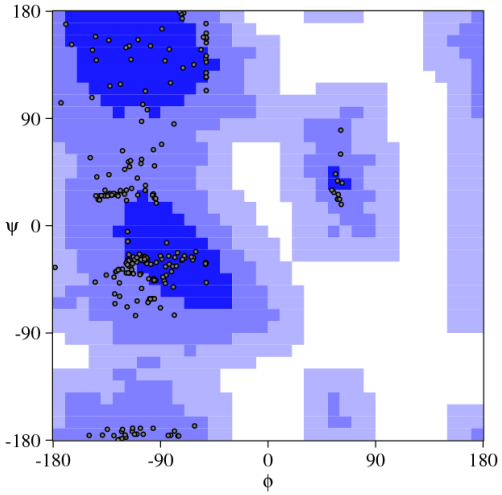

Fig S18. Ramachandran plot obtained by CYANA program for (Thr^5-*O*-α-D-GalNAc)-N/OFQ **(4)** in SDS micelles.

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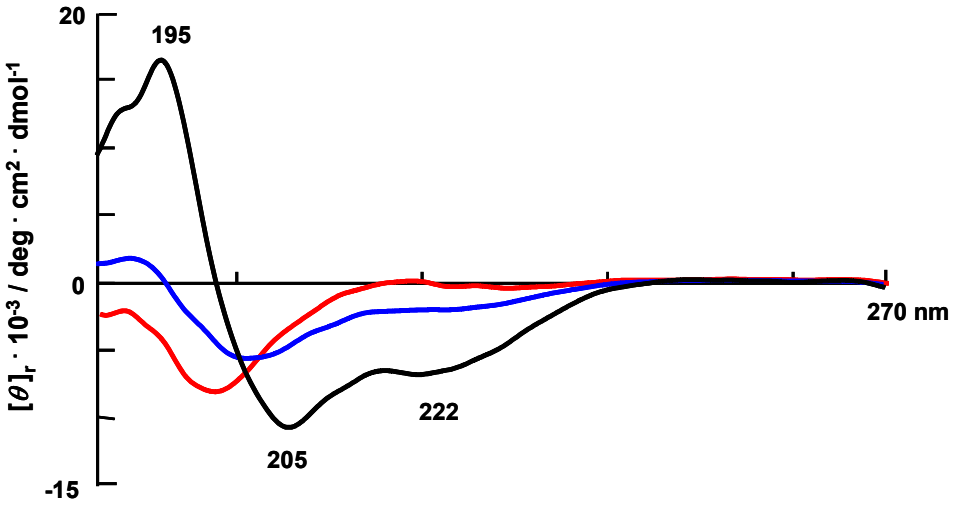

Figure S19. CD spectra of glycopeptide **1** ([Thr^{5}-*O*-α-D-GalNAc]-N/OFQ) in TFE (black color), TFE-water (1:1) (blue), and water (red).

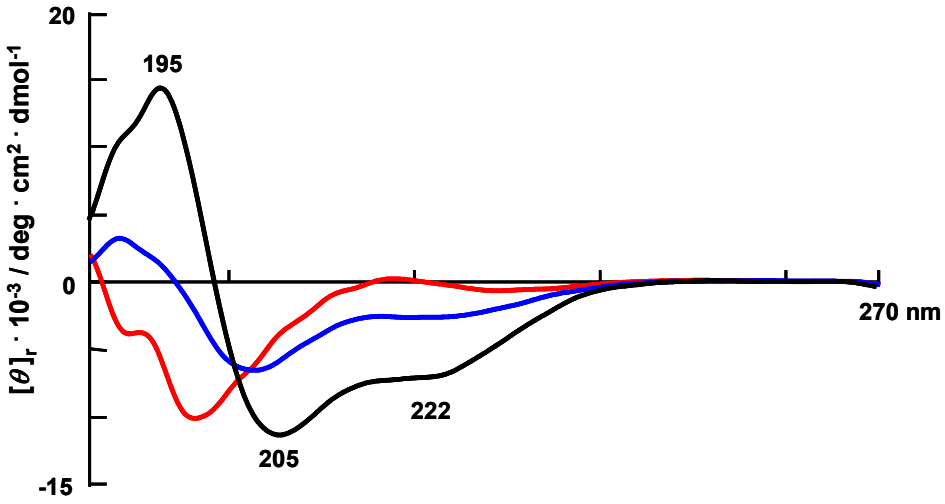

Figure S20. CD spectra of glycopeptide **2** ([Ser^{10}-*O*-α-D-GalNAc]-N/OFQ) in TFE (black color), TFE-water (1:1) (blue), and water (red).

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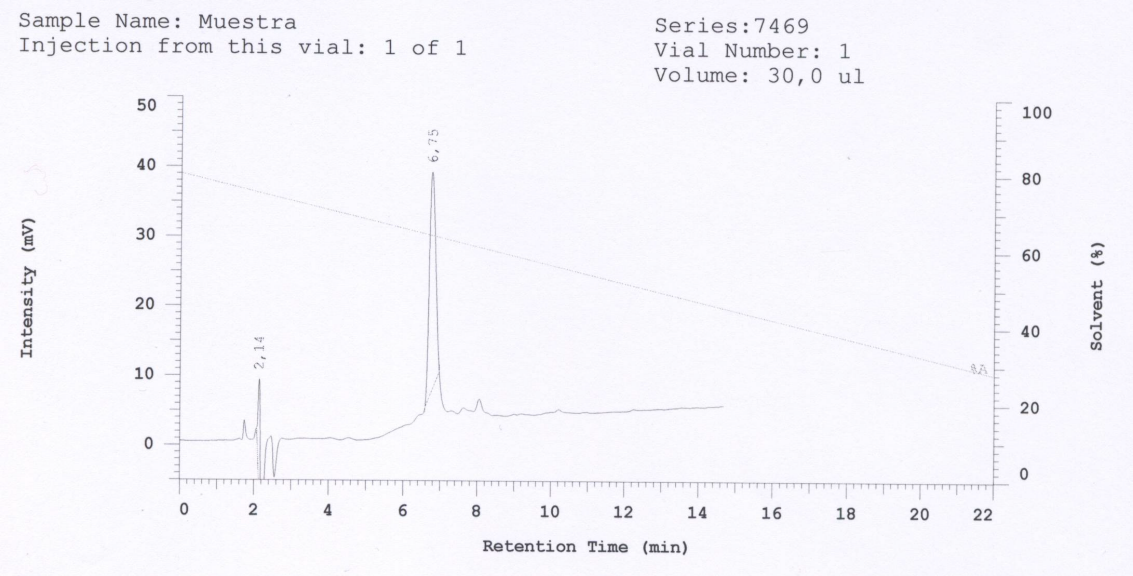
HPLC

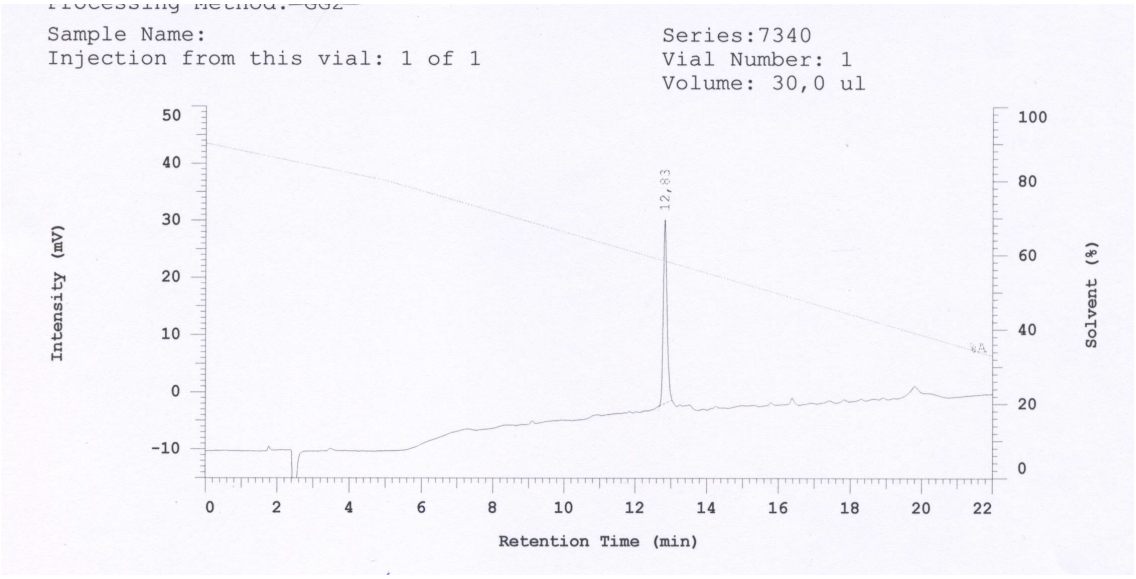
1	H-Phe-Gly-Gly-Phe-*Thr*^{5}-*(α-GalNAc)*-Gly-Ala-Arg-Lys-Ser^{10}-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Thr^{5}-*O*-α-D-GalNAc]-N/OFQ

Solvents: A: water + 0.1% TFA; B: acetonitrile + 0.1% TFA; From A:B (80:20) to A:B (20:80) in 30 min.



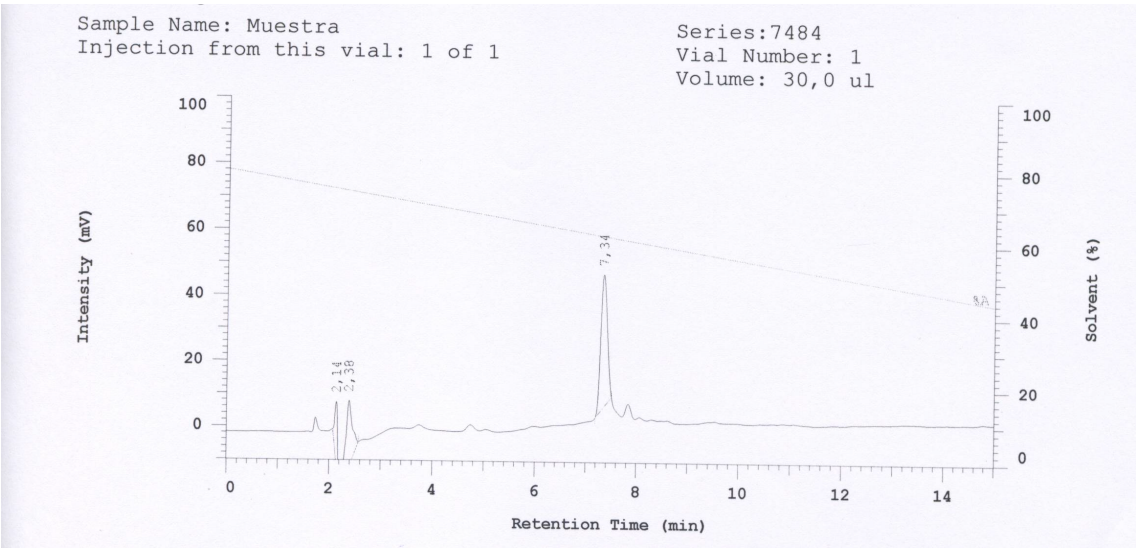
2	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}-*(α-GalNAc)*-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-α-D-GalNAc]-N/OFQ

Solvents: A: water + 0.1% TFA; B: acetonitrile + 0.1% TFA. From A:B (90:10) to A:B (80:20) in 5 min, then to A:B (10:90) in 30 min



3	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-*Ser*^{10}-*(β-GlcNAc*)-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	[Ser^{10}-*O*-β-D-GlcNAc]-N/OFQ

Solvents: A: water + 0.1% TFA; B: acetonitrile + 0.1% TFA; From A:B (80:20) to A:B (20:80) in 30 min.



	H-Phe-Gly-Gly-Phe-Thr^{5}-Gly-Ala-Arg-Lys-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH	N/OFQ

Solvents: A: water + 0.1% TFA; B: acetonitrile + 0.1% TFA; From A:B (80:20) to A:B (20:80) in 30 min.

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