SUPPORTING INFORMATION

Probing Non Peptide Agonists Binding at Human Nociceptin Receptor: A Molecular Modelling Study

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Figure S1. Per residue N/OFQ(1-13)-NH_2 Cα atoms RMSF profile calculated for the three MD replicas (A) run1, blue box; B) run2, green box; C) run3, orange box). N/OFQ(1-13)-NH_2 functional division in different domains is highlighted in the graphs with different colours as follows: magenta, message; green, hinge; purple: address. domain).

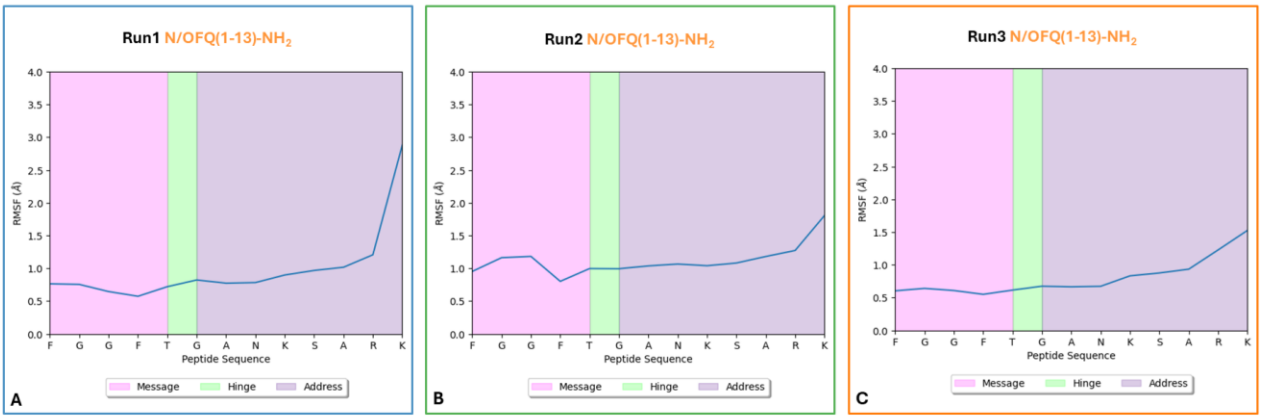


Table S1. Median hydrophobic contact (shades of red), hydrogen bond (shades of blue), and salt bridge (shades of green) scores computed for each N/OFQ(1-13)-NH_2 trajectory with PyContact. Only median scores exceeding 1 were reported, as they indicate a stable interaction over time. For the interaction between positively charge peptide N-terminus and D130 side chain (N-ter/D130) only the hydrogen bond contribution was considered, as PyContact is unable to detect salt-bridges involving groups other than standard protein charged side chains.

Table S2. MM/PBSA values and standard deviations of each run of the N/OFQ(1-13)-NH_2, RBM01, RBM03, MBM04, MBM05 systems, calculated using the MMPBSA.py script implemented in the AmberTools24 package. Lowest MM/PBSA values detected among the three runs of each system are in bold.

	MM/PBSA ± σ [kcal/mol]				
	N/OFQ(1-13)-NH_2	RBM01	RBM03	MBM04	MBM05
RUN1	-63.09 ± 20.51	-26.85 ± 8.59	-27.21 ± 5.76	-18.46 ± 12.79	-43.55 ± 15.62
RUN2	-75.67 ± 18.08	**-33.95 ± 4.72**	-31.92 ± 5.56	**-41.68 ± 17.83**	**-57.72 ± 15.24**
RUN3	**-78.65 ± 18.86**	-27.88 ± 7.14	**-34.20 ± 7.63**	-18.05 ± 16.13	-36.04 ± 12.04

Figure S2. Binding modes obtained by docking the ligands (*R*)-Ro65-6570 (A: RBM01, cyan sticks; and B: RBM02, tan sticks) and MCOPPB (C: MBM01, yellow sticks; and D: MBM02, red sticks) in the "8F7X_mod" structure (green ribbons). The residues surrounding the ligands are depicted with green sticks.

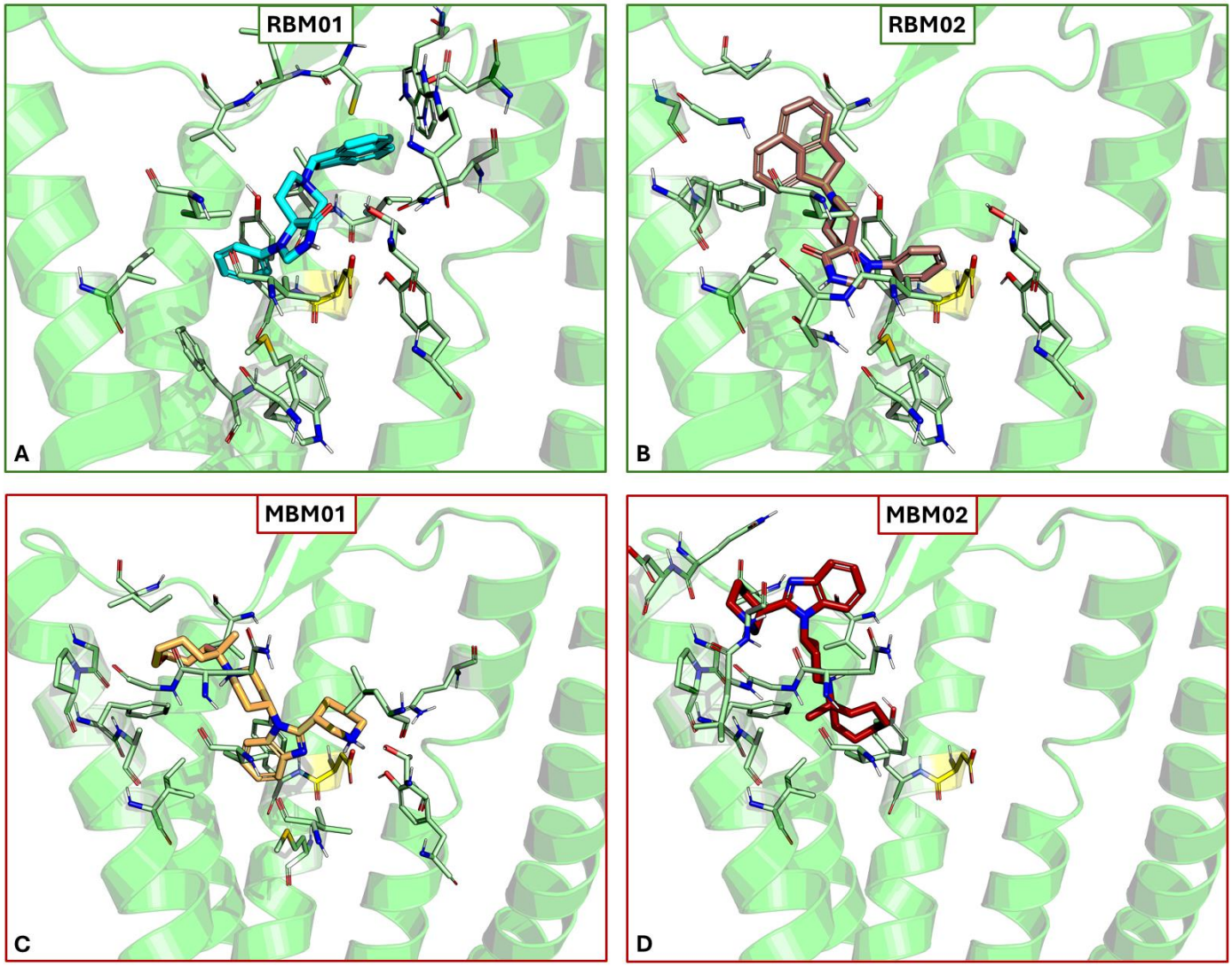
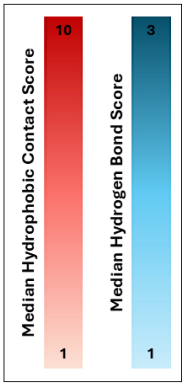


Table S3. Tanimoto similarity coefficients calculated between the reference ligands ((*R*)-Ro65-6570 and MCOPPB) and the three small molecule NOP antagonists C-24, C-35 and SB-612111 (PDB IDs of the antagonist-NOP complex: 4EA3, 5DHG and 5DHH, respectively). The Tanimoto coefficients were computed using the similarity search Node available in Knime and are based on MACCS Fingerprint. The highest value for each ligand, corresponding to the closest chemical similarity, is highlighted in green.

	C-24	C-35	SB-612111
MCOPPB	0.63	0.59	0.50
(*R*)-Ro65-6570	0.74	0.68	0.44

Table S4. Median hydrophobic contact (shades of red) and hydrogen bond (shades of blue) scores computed for each RBM01 and RBM03 trajectory with PyContact. Only median scores exceeding 1 were reported, as they indicate a stable interaction over time. For each run, the corresponding scores were calculated separately for the ligand functional groups, namely: the acenaphthenyl group, the phenyl ring, and the positively charged nitrogen of the 1,4-disubstituted piperidine (N+ moiety). For the interaction between the positively charged ligand nitrogen (N+) and the receptor D130 side-chain, only the hydrogen bond contribution is considered, as PyContact is unable to detect salt-bridges involving groups other than standard protein charged side chains.



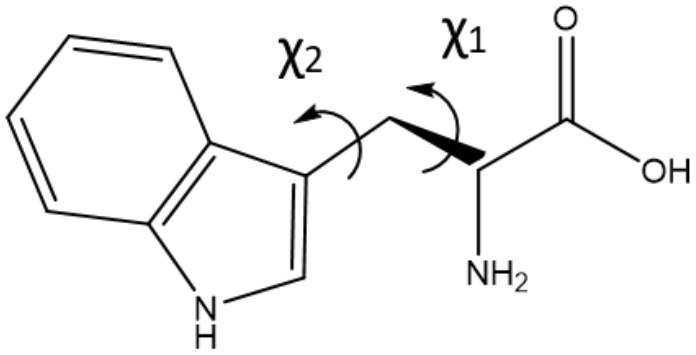
RBM01

		NOP residues										
	Ligand	T103	Q107	V126	I127	D130	M134	C200	W276	V279	Q280	Y309
RUN1	Acenaphthenyl Group		3.35	2.28	4.65	4.85		4.56				
	Phenyl Ring								2.04	1.72	1.66	
	N+ Moiety					1.99						
RUN2	Acenaphthenyl Group	1.32	3.53	2.48	8.42	5.54		2.56				
	Phenyl Ring						2.91		1.97	5.01		1.27
	N+ Moiety					2.91						
RUN3	Acenaphthenyl Group		9.56		1.53	3.20		3.34				
	Phenyl Ring						2.56		1.22		2.18	
	N+ Moiety					2.03						

RBM03

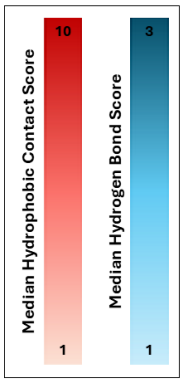
		NOP residues													
	Ligand	Q107	I127	D130	Y131	M134	F135	C200	I219	S223	W276	V279	Q280	V283	Y309
RUN1	Acenaphthenyl Group			5.39	7.97	4.61			1.55	2.31	1.43	2.71	6.13	2.50	
	Phenyl Ring	2.03	1.90					1.88							
	N+ Moiety			2.82											
RUN2	Acenaphthenyl Group			5.85	8.36	5.13	1.08		1.53	1.98	1.89	2.01	5.09	3.23	1.71
	Phenyl Ring	2.27	1.84					2.15							
	N+ Moiety			2.91											
RUN3	Acenaphthenyl Group			5.41	7.94	6.69	1.52		1.36	1.39	1.62	2.00	4.71	2.85	
	Phenyl Ring	2.37	1.81					2.69							
	N+ Moiety			2.90											

Figure S3. χ_1 and χ_2 dihedral angle definitions for the W276^{6x48} residue with corresponding angle values and conformer definitions.



χ_1			χ_2		
0 to 120	120 to -120	-120 to 0	60 to 180	180 to -60	-60 to 60
gauche (+)	*trans*	*gauche (-)*	*gauche (-)*	*gauche (+)*	*trans*

Table S5. Median hydrophobic contact (shades of red) and hydrogen bond (shades of blue) scores computed for each MBM04 and MBM05 trajectory with PyContact. Only median scores exceeding 1 were reported, as they indicate a stable interaction over time. For each run, the corresponding scores were calculated separately for the ligand functional groups, namely: the methyl-cyclo-octyl group, the phenyl ring, the positively charged nitrogen of the 3-monosubstituted piperidine (N+ Side Moiety), and the positively charged nitrogen of the 1,4-disubstituted piperidine (N+ Moiety). For the interactions between the positively charged ligand nitrogen atoms and the receptor D130 side-chain, indicated as N+/D130 and N+ Side/E199, respectively, only the hydrogen bond contribution is considered, as PyContact is unable to detect salt-bridges involving groups other than standard protein charged side chains.



MBM04

		NOP residues													
	Ligand	V100	Q107	D110	D130	Y131	M134	E199	C200	W276	V279	V283	L301	R302	T305	Y309
RUN1	Methyl-cyclo-octyl Group	1.50			3.54		1.54			1.31	1.11					1.84
	Phenyl Ring														1.49	
	N+ Side Moiety								1.63							
	N+ Moiety															
RUN2	Methyl-cyclo-octyl Group				3.15	4.96	1.79									
	Phenyl Ring		3.78	1.47									2.07	7.50	2.34	
	N+ Side Moiety			2.25				2.94								
	N+ Moiety															
RUN3	Methyl-cyclo-octyl Group				5.15		1.29			1.13		1.08				2.15
	Phenyl Ring													1.39		
	N+ Side Moiety								1.69							
	N+ Moiety															

MBM05

		NOP residues											
	Ligand	Q107	W116	V126	I127	D130	Y131	M134	E199	C200	I219	Q280	V283	Y309
RUN1	Methyl-cyclo-octyl Group					5.52	4.28	1.71						1.20
	Phenyl Group	7.69	1.50	2.26	5.48	2.32				1.36				
	N+ Side Moiety								3.24	1.16				
	N+ Moiety					2.11								
RUN2	Methyl-cyclo-octyl Group					4.76	4.39	1.94				1.85	1.01	1.28
	Phenyl Group	7.75	1.76	2.48	6.40	3.02				1.65				
	N+ Side Moiety								3.25	1.13				
	N+ Moiety					1.96								
RUN3	Methyl-cyclo-octyl Group					5.11	5.29	1.91			1.12			1.70
	Phenyl Group	7.37	1.86	2.24	4.78	2.70				1.57				
	N+ Side Moiety								3.18	1.06				
	N+ Moiety					1.97								

Figure S4. Final binding modes proposed for (*R*)-Ro65-6570 (RBM03; panels A and C, green sticks) and MCOPPB (MBM05; panels B and D; orange sticks). The receptor residues are coloured according to the type of interaction established with the ligand (yellow: salt bridges; blue: hydrogen bonds; salmon: hydrophobic interactions). Charged groups of both receptor and ligand are highlighted with a yellow sphere, salt-bridges and hydrogen bonds are represented with green and blue dashed lines, respectively. In panel C and D, TM7 was omitted for a better ligand visualization.

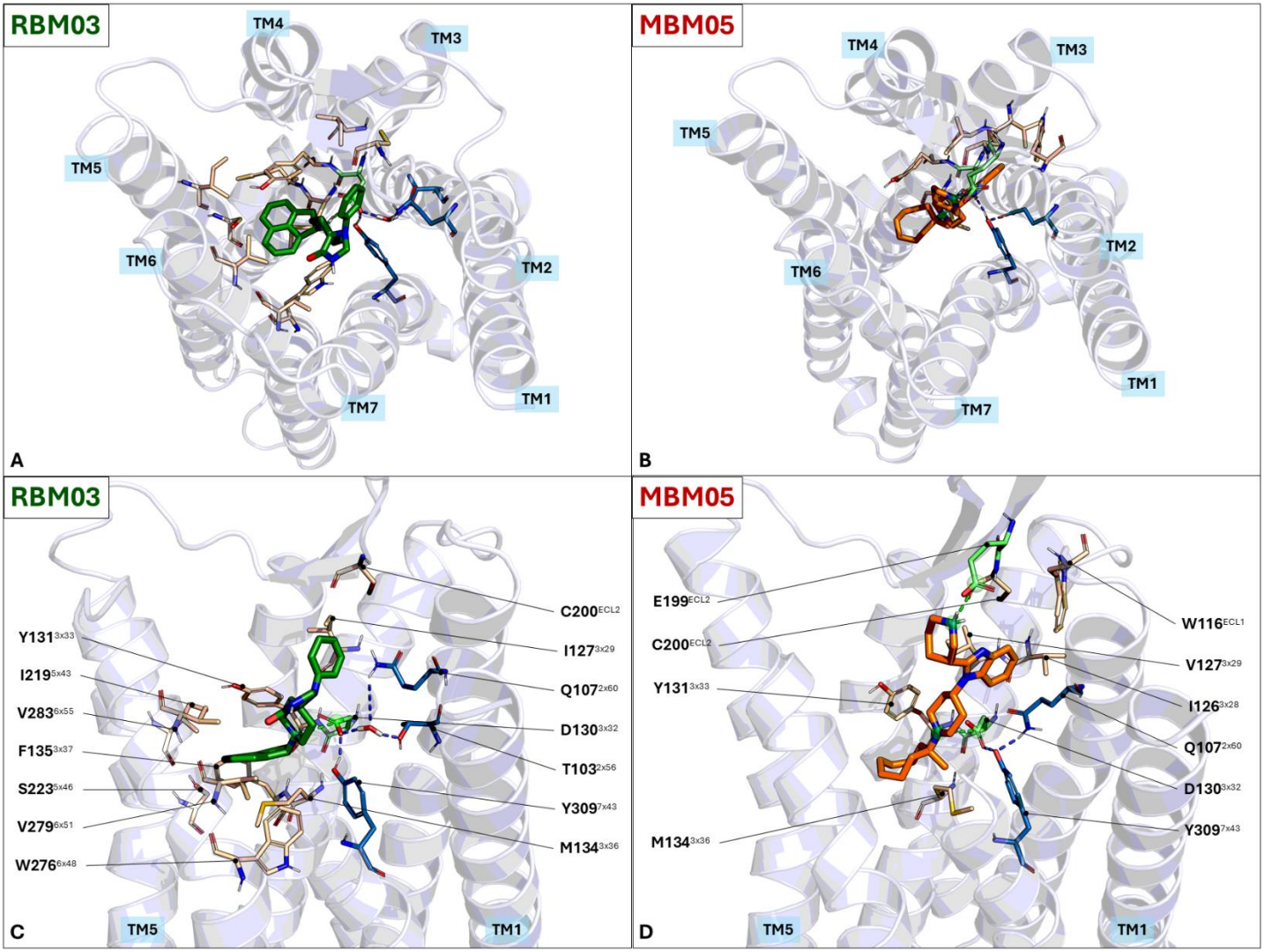
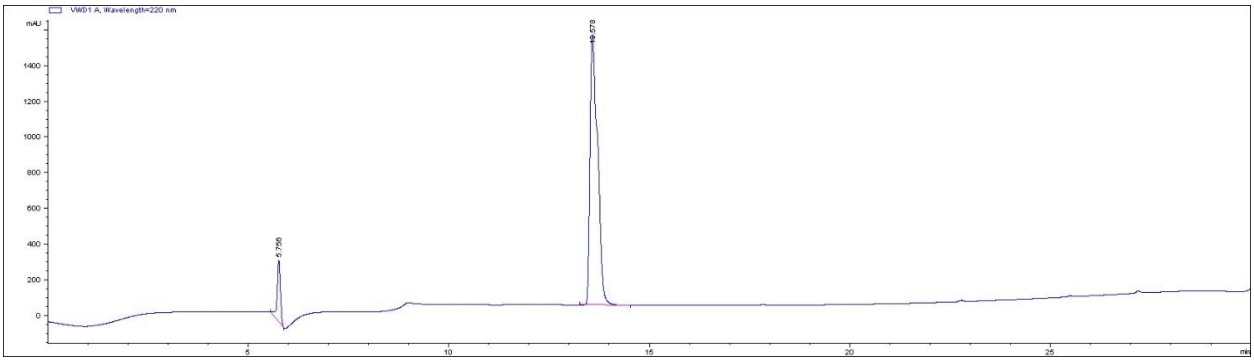


Table S6. Details of the N/OFQ(1-13)-NH_2, MBM04, MBM05, RBM01, and RBM03 Molecular Dynamics systems. For each system, the following parameters are specified: termini capping (blue and red for the N- and C-terminus, respectively), residue number on which disulfide patches were applicated (mustard), simulation cell dimensions (light-blue), number of lipids in the upper and lower leaflets (orange), ions (green), and water molecules and total atoms count (purple).

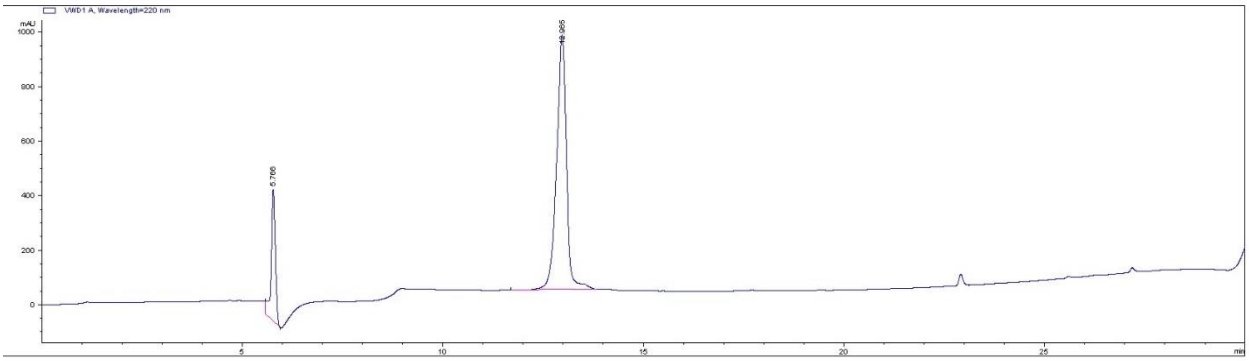
	Temini						Disufide bonds		Size			N° Lipids		N° Ions		N° atoms	
	LIG(N)	LIG(C)	PROT(N)	PROT(C)	Gαi(N)	Gαi(C)	PROT	PROT	X	Y	Z	Top	Bottom	Na+	Cl-	Water	Total
N/OFQ(1-13)-NH2	NTER	CT2	ACE	CT3	ACE	CTER	123	200	80.2402373	80.2402373	131.626134	77	77	47	56	17574	78644
MBM04			ACE	CT3			123	200	80.2079707	80.2079707	119.629941	77	78	40	50	15257	71235
MBM05			ACE	CT3			123	200	80.0750704	80.0750704	119.611586	77	77	40	50	15271	71143
RBM01			ACE	CT3			123	200	80.3239657	80.3239657	118.753916	77	78	40	47	15257	71213
RBM03			ACE	CT3			123	200	80.3004745	80.3004745	118.753826	77	78	40	47	15260	71222

HPLC chromatograms of in house synthesized NOP agonists

N/OFQ



N/OFQ(1-13)-NH_2



Ro65-6570