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Published in:
ACS Medicinal Chemistry Letters

DOI:
10.1021/acsmedchemlett.8b00105

Publication date:
2018

License:
Unspecified

Document Version:
Accepted author manuscript

ACS Medicinal Chemistry Letters

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.8b00105 • Publication Date (Web): 23 Apr 2018

ACS Publications

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Synthesis and *in vitro* evaluation of stabilized and selective Neuromedin U-1 receptor agonists

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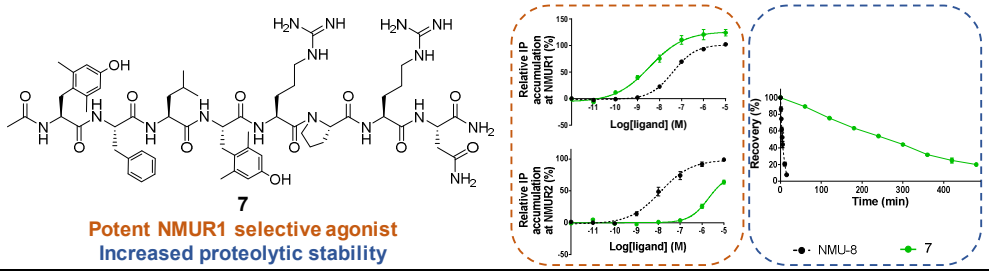
Neuromedin U (NMU), NMU-8, Neuromedin U receptor agonist, NMUR1, in vitro plasma stability



ABSTRACT: Neuromedin U (NMU) is a multifunctional neuropeptide which is characterized by a high conservation through all species. Herein, we describe the synthesis of a novel set of NMU-analogs based on the truncated NMU-8. Through combination of previously reported modifications, an elaborate structure-activity relationship study was performed aiming for the development of peptides with an increased selectivity towards NMU receptor 1 (NMUR1). Compound **7** possessed the highest NMUR1 selectivity (IC_{50} = 0.54 nM, selectivity ratio = 5313) together with an increased potency (EC_{50} = 3.7 nM), an 18% increase of the maximal effect at NMUR1 and a higher resistance against enzymatic degradation as compared to the native NMU-8. The development of a potent NMUR1 agonist with extended half-life could represent an attractive tool to further unveil the role of NMUR1 in NMU signaling.

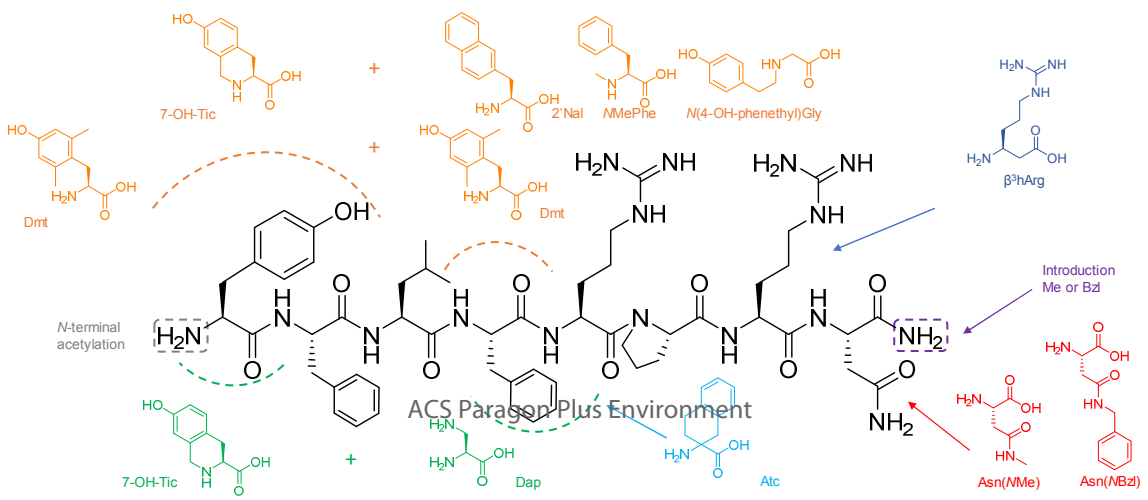
Neuromedin U (NMU) is a highly conserved neuropeptide which occurs in two main isoforms, a 23 to 25 amino acid long peptide, and in certain species a truncated version of 8 or 9 amino acids is present and considered to be a degradation product from the larger peptide. The highest homology between variants in different species is found at the *C*-terminus of the NMU peptide with the *C*-terminal heptapeptide (-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2) being entirely conserved in mammalian species.[1] NMU exerts its biological effects through two G protein-coupled receptors (GPCRs), more precisely neuromedin U receptor 1 (NMUR1) and NMUR2. These receptors have a complementary tissue distribution

since NMUR1 is most abundant in the periphery whereas NMUR2 is predominantly found in the central nervous system.[1,2] NMU is involved in various physiological processes including smooth muscle contraction, blood pressure control, regulation of the stress response, nociception, immune regulation and suppression of feeding behavior.[1] In the search for novel treatments in the field of obesity and diabetes, NMU is gaining interest since it is reported to exert anorexigenic effects and possess beneficial effects on glucose tolerance,[3] which resulted in extensive structure-activity relationship (SAR) studies and the development of different potent and long acting NMU-analogs.[4-10] To date, several promising agonists for the NMURs are described, such as PEGylated NMU-25[8] and NMU-8[10,11] analogs, a NMU-human serum albumin conjugate,[9] lipidated NMU-analogs,[7,6] an alkylated NMU-analog,[12] and all are reported to have potent and long-lasting effects on food intake. Selective agonists towards NMUR1 and NMUR2 were lately synthesized as well (e.g., 2-thienylacetyl-Trp-(αMe)Trp-Arg-Pro-Arg-Asn-NH_2[5] and 3-cyclohexylpropionyl-Leu-Leu-Dap-Pro-Arg-Asn-NH_2[4], resp.). Recently our group performed a SAR study, with the native NMU-8 sequence (H-Tyr[1]-Phe[2]-Leu[3]-Phe[4]-Arg[5]-Pro[6]-Arg[7]-Asn[8]-NH_2) as starting point and using human embryonic kidney 293 (HEK293) cells expressing the NMURs for screening. Our study revealed that acetylation of the *N*-terminus results in peptides with a higher potency and plasma stability, as compared to the non-acetylated analog. Secondly, replacement of the Tyr residue in position 1 by 7-hydroxy-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (7-OH-Tic), 2'-naphtylalanine (2'Nal) or 2',6'-dimethyltyrosine (Dmt) resulted in potent NMU-8 analogs. NMUR1 selectivity could be obtained by modification of the Phe[4] residue whereas selectivity towards NMUR2 was observed when Pro[6] was modified. Finally, an increased resistance against proteolytic degradation was found for all molecules tested, as compared to NMU-8.[13]

In this letter we report the synthesis and *in vitro* biological evaluation of a novel set of NMU-8 analogs, in which novel modifications were introduced, but also promising modifications of our previous findings were combined, with the aim to develop molecules with improved pharmacological profiles (Figure 1). The novel NMU-analogs were synthesized manually as reported before via conventional Fmoc-based solid phase peptide synthesis using Rink Amide AM resin as a solid support and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) / *N,N*-diisoprpylethylamine (DIPEA) as coupling mixture (see Supporting Information).[13] *N*-substituted glycines (the so-called 'peptoid' residues) were synthesized following the solid phase submonomer method.[14] After complete deprotection and cleavage from the resin with trifluoroacetic acid (TFA) / triethylsilane (TES) / water (95/2.5/2.5 v/v/v), purification of the peptides was performed by preparative high-performance liquid chromatography (HPLC), using a water - acetonitrile gradient system containing 0.1 % TFA. All NMU-analogs had a purity greater than 95 % as assessed by HPLC analysis and their structure was confirmed by high resolution mass spectrometry (HRMS) (see Supporting Information, Table S1). Tritiated NMU-8, which was used for the binding studies, was obtained as described before (see Supporting Information for experimental details).[13] Evaluation of the affinities and agonistic activities of the NMU-analogs at the NMURs was performed in the present study on HEK293 cells transiently expressing human (h)NMUR1 and hNMUR2 as reported before.[13] Evaluation of the affinity of the novel NMU-analogs for the NMURs was performed with a competitive binding assay using [^{3}H]-NMU-8 as radioligand. An inositol triphosphate (IP_3) accumulation assay was carried out to study the functional activity of the NMU-analogs. All analogs were tested in at least 3 independent experiments using triplicates in a concentration range of 10^{-5} to 10^{-11} M. Table 1 shows the results for the novel NMU-analogs.

Since previous SAR data revealed that *N*-terminal acetylation of NMU results in peptides with an increased potency and proteolytic stability, the current letter reports only acetylated NMU-analogs. Several studies state the *C*-terminal part of the sequence, more precisely -Pro-Arg-Asn-NH_2, to be the core structure necessary for activation of the NMURs.[4] Interestingly, we successfully modified this critical region by modification of the Pro[6] residue and found that modification of this residue could lead to selectivity towards NMUR2.[13] In the current study, this critical and conserved region was further explored. In a first step, Arg[7] was replaced by the homologated β^{3}homoArg (β^{3}hArg) (to give compound **1**, Ac-Tyr-Phe-Leu-Phe-Arg-Pro-**β^{3}hArg**-Asn-NH_2). This NMU-analog displayed decreased affinity for both NMURs (IC_{50}= 28.1 nM and 116.5 nM for NMUR1 and NMUR2, resp.). Additionally, it proved to be a full agonist at NMUR1 with a 4-fold reduction in potency in comparison with NMU-8. Compound **1** only partially activated NMUR2 with an EC_{50} value (i.e. 55.6 nM) in the range of the potency reported for NMU-8. When analog **1** was compared with Ac-NMU-8, lower affinities and potencies for both NMURs were found. By insertion of β-homo-amino acids, the backbone is elongated by one carbon atom, in this case between the Arg[7] and Asn[8] residues. This shift might have caused an unfavorable projection of the side chains of these residues,

Figure 1. Structures of the NMU-8 peptide (black) and the modifications introduced into the sequence.



Compound	hNMUR1			hNMUR2			Selectivity[d]
	Affinity	Potency		Affinity	Potency		
	IC_{50} (nM)[a]	EC_{50} (nM)[b]	E_{max} (%)[c]	IC_{50} (nM)	EC_{50} (nM)	E_{max} (%)	IC_{50} NMUR2/ IC_{50} NMUR1
NMU-8	0.78	38.9	100.0	1.7	30.8	100.0	2
Ac-NMU-8	0.29	0.51	111.3	2.0	0.71	100.0	7
1	28.1	155.7	113.2	116.5	55.6	81.4	4
2	8.2	57.2	118.1	29.1	51.2	85.1	4
3	0.75	10.9	93.8	18.8	28.6	65.8	25
4	21.3	843.0	118.8	327.5	440.8	106.8	15
5	37.0	1314	114.8	28.8	354.2	103.9	0.8
6	8.8	28.5	106.9	40.4	3.8	91.4	5
7	**0.54**	**3.7**	**118.1**	**2869**	**1993**	**83.2**	**5313**
8	**0.092**	**2.9**	**111.8**	**301.9**	**459.3**	**100.3**	**3282**
9	350.1	1028	64.0	598.9	2205	91.1	2
10	3.0	18.9	127.7	465.5	925.2	97.2	155
11	2.4	53.3	99.0	72.4	59.7	97.6	30

1 Ac-Tyr-Phe-Leu-Phe-Arg-Pro-**β^{3}hArg**-Asn-NH_2
2 Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Me)**-NH_2
3 Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Bzl)**-NH_2
4 Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-**NHMe**
5 Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-**NHBzl**
6 Ac-Tyr-Phe-Leu-**Atc**-Arg-Pro-Arg-Asn-NH_2
7 Ac-**Dmt**-Phe-Leu-**Dmt**-Arg-Pro-Arg-Asn-NH_2
8 Ac-**7-OH-Tic**-Phe-Leu-**2'Nal**-Arg-Pro-Arg-Asn-NH_2
9 Ac-**7-OH-Tic**-Phe-Leu-***N*(4-OH-phenethyl)Gly**-Arg-Pro-Arg-Asn-NH_2
10 Ac-**7-OH-Tic**-Phe-Leu-***N*MePhe**-Arg-Pro-Arg-Asn-NH_2
11 Ac-**7-OH-Tic**-Phe-Leu-Phe-**Dap**-Pro-Arg-Asn-NH_2

Table 1. *In vitro* affinity and activity of the NMU-analogs at hNMUR1 and hNMUR2.

[a] The affinity (IC_{50} value) is calculated based on the competitive binding assay with [^{3}H]-NMU-8 as radioligand (Supporting Information, Table S2, for K_i values). [b] The potency (EC_{50} value) is calculated based on the IP_3 accumulation assay. [c] E_{max} is the percentage of the maximum response at 10^{-5} M compared with the NMU-8 response at the same concentration. [d] Receptor selectivity is expressed as the ratio of the IC_{50} value for NMUR2 over the IC_{50} value for NMUR1 of each analog. Sequences are shown below and modifications are marked in bold.

eventually leading to a loss in binding to the NMURs. Although it was reported that the *C*-terminal Asn[8] is essential for NMUR activation, we modified this residue in two different manners: i) by the introduction of a methyl or benzyl group in the side chain amide of Asn, and ii) by modification of the *C*-terminal amide. The addition of a methyl group in the side chain amide of Asn[8] (to present compound **2**, Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Me)**-NH_2), caused a more than 10-fold decreased affinity for the NMURs, when compared to NMU-8 and Ac-NMU-8, but a preserved potency as compared to the native NMU-8 peptide was found. Analog **3**, in which a benzyl amide was present in the side chain of Asn[8] (i.e, Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Bzl)**-NH_2), possessed a NMU-8-like potency at NMUR1 in combination with a subnanomolar affinity. At NMUR2, this peptide was a partial agonist (E_{max} = 65.8 %) with a decreased receptor affinity. Next, we also modified the *C*-terminal amide. When a methyl or a benzyl group was inserted, to present compounds **4** (Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-**NHMe**) and **5** (Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-**NHBzl**), respectively, weak agonists with a decreased affinity at both receptors were found. The loss in receptor binding and activation indicates that the *C*-terminal amidation is not only a natural protection mechanism against carboxypeptidase driven degradation,[15] but plays as well an important role in the NMUR binding and activation. Hence, we demonstrated that it is possible to modify the *C*-terminal region of the NMU-8. The substitution of Arg[7] by β^{3}hArg resulted in a peptide which was still able to bind to the NMURs, although with a lower affinity as compared to NMU-8, yet substantial receptor activation was found. Modification of the Asn residue also gave rise to full agonists for NMUR1 which were able to activate the NMUR2 as well, though only partially. Our previous SAR study revealed that substitution of Phe[4] by unnatural Phe- and Tyr-analogs served as a tool to obtain potent NMU-peptides with an increased NMUR1 selectivity and improved plasma stability.[13] Here, we report the synthesis of a NMU-analog in which Phe[4] was replaced by (D,L)-2-aminotretralin-2-carboxylic acid (Atc), resulting in compound **6** (Ac-Tyr-Phe-Leu-**Atc**-Arg-Pro-Arg-Asn-NH_2). Due to peak overlay in the HPLC analysis, the resulting diastereomers were inseparable and tested as a mixture. The introduction of Atc induced only a small selectivity shift towards NMUR1, with a selectivity ratio (IC_{50} NMUR2 / IC_{50} NMUR1) of 4.6. Analog **6** possessed similar potencies at the

NMURs as compared to NMU-8 with a slightly better NMUR2 potency. However, it did not reach the levels reported for the Ac-NMU-8 potency. Neither selectivity towards NMUR1 nor the subnanomolar affinity for this receptor was observed for compound **6**. A possible explanation can be the negative influence of the introduced Atc on the backbone folding, since α,α-dialkylated amino acids are known to induce turn/helix conformations. Moreover, the aromatic moiety of the Atc residue could be positioned/stabilized differently, as compared to the one of 7-OH-Tic, 1'Nal, 2'Nal or Dmt. The Atc residue stabilizes the gauche (-) or trans with dihedral χ_1 angles of -60° and 180°, respectively, over the C_α-C_β bond (in case of *(S)*-Atc).[16,17] Next, we synthesized a series of peptides in which promising modifications were combined. With the aim to develop a NMUR1 selective agonist with increased potency and resistance against biodegradation, compound **7** was synthesized in which both Tyr[1] and Phe[4] were replaced by Dmt (Ac-**Dmt**-Phe-Leu-**Dmt**-Arg-Pro-Arg-Asn-NH_2). An increased selectivity towards NMUR1 was found for **7**, which was of the same magnitude as reported for Ac-[Dmt[4]]-NMU-8 (selectivity ratio of 5313 and 5158 for compound **7** and Ac-[Dmt[4]]-NMU-8 respectively).[13] Moreover, NMUR1 affinity and potency were in line with the ones found for the non-selective Ac-[Dmt[1]]-NMU-8, more precisely a subnanomolar affinity in combination with an approximately 10-fold increased activity at NMUR1, compared to NMU-8. Gratifyingly, the combination of the previously reported modifications to the NMU sequence (i.e. potency of Ac-[Dmt[1]]-NMU-8 and selectivity of Ac-[Dmt[4]]-NMU-8) culminated to the *in vitro* characteristics of the potent and selective analog **7**. With the same goal in mind, Tyr in position 1 was replaced by the conformationally constrained 7-OH-Tic together with the introduction of the bulky 2'Nal in position 4 to present compound **8** (Ac-**7-OH-Tic**-Phe-Leu-**2'Nal**-Arg-Pro-Arg-Asn-NH_2). Again, a cumulative effect of the modifications was found, resulting in a NMU-analog with a similar affinity and potency at NMUR1 as compared to Ac-[7-OH-Tic[1]]-NMU-8 (i.e. a subnanomolar affinity together with an increased potency compared to NMU-8), in combination with an elevated NMUR1 selectivity. An even 5-fold higher NMUR1 selectivity was observed compared to Ac-[2'Nal]-NMU-8 (selectivity ratio = 3282 and 610 for compound **8** and Ac-[2'Nal[4]]-NMU-8, resp.),[13] although the selectivity level of compound **7** was not exceeded. Our previous work revealed as well that the introduction of *N*-substituted glycines in position 4 of NMU-8 resulted in weak and partial NMURs agonists. Nonetheless, the introduction of *N*(4-OH-phenethyl)Gly was able to extend plasma half-life up to 18 h (for NMU-8 only 4 min was found).[13] With the aim to develop potent and proteolytically stable NMU-8 analogs, the use of a peptoid residue in position 4 was combined with the replacement of Tyr[1] by 7-OH-Tic, which was reported to give rise to potent NMURs agonists. Surprisingly compound **9** (Ac-**7-OH-Tic**-Phe-Leu-***N*****(4-OH-phenethyl)Gly**-Arg-Pro-Arg-Asn-NH_2) resulted in an even bigger loss in potency on both NMURs, as compared to Ac-[*N*(4-OH-phenethyl)Gly[4]]-NMU-8 (EC_{50} = 216.6 nM and 339.2 nM for NMUR1 and NMUR2 respectively).[13] To verify whether the side chain is placed in an unfavorable position when peptoid residues are used or the backbone amide proton in that position is necessary for receptor interaction, an analog in which Phe[4] was replaced by *N*MePhe, together with the 7-OH-Tic substitution in position 1, was synthesized, to give compound **10** (Ac-**7-OH-Tic**-Phe-Leu-***N*****MePhe**-Arg-Pro-Arg-Asn-NH_2). A loss in affinity was observed at both NMURs, though more pronounced at NMUR2. Analog **10** was a weak agonist at NMUR2. At NMUR1, it exerted full and potent agonistic properties with a 27.7 % increase in the maximal effect at this receptor. These findings indicate that the side chain of the peptoid residues was in an unfavorable position for receptor binding and activation rather than the need of the backbone amide proton for interaction with the NMURs. Of note, compound **10**, encompassing the *N*MePhe residue in position 4, gave rise to the highest E_{max} values, tested to date (i.e. 127.7%), indicating that other *N*-alkylations might be worthwhile to investigate in search of selective hNMUR1 agonists. Takayama *et al* described that selectivity towards NMUR2 could be obtained by substitution of Arg[5] by α,β-diaminopropionic acid (Dap).[4] In the present study, the substitution of Arg[5] by Dap was combined with the introduction of 7-OH-Tic in position 1, aiming for the development of a potent NMUR2 receptor agonist with an elevated resistance against proteolytic degradation (Ac-**7-OH-Tic**-Phe-Leu-Phe-**Dap**-Pro-Arg-Asn-NH_2, compound **11**). Surprisingly, analog **11** possessed a 30-fold higher selectivity for NMUR1, although it was equipotent at both NMURs with EC_{50} values of similar magnitude as compared to NMU-8.

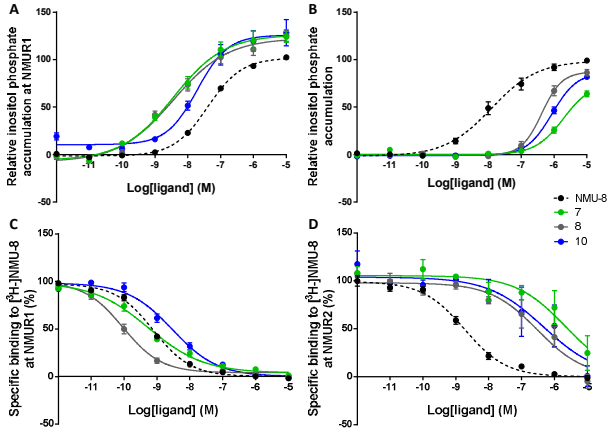
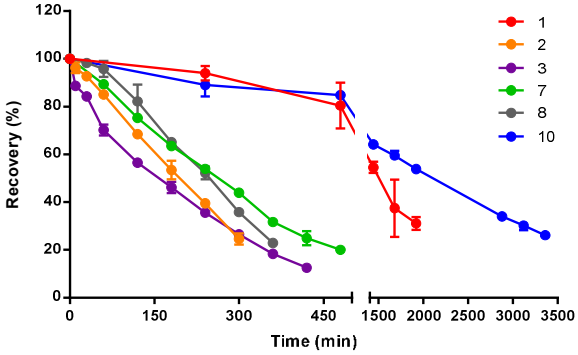


Figure 2. *In vitro* evaluation of NMU-analogs with increased NMUR1 selectivity. IP_3 assay performed on HEK293 cells, transiently expressing A) NMUR1 or B) NMUR2. Affinity for NMUR1 (C) or NMUR2 (D) was evaluated in a competitive binding assay with [^{3}H]-NMU-8. For clarity, the selective compounds **3** and **11** were not shown. Data are shown as mean ± SEM (n=3).

Overall, this set of novel NMU-analogs contains several peptides with an elevated selectivity towards NMUR1, whereas none of the compounds possessed NMUR2 selectivity. Figure 2 gives an overview of the NMU-analogs with an improved selectivity profile. Especially compound **7** is a high-affinity, potent NMUR1 agonist (Figure 2A and 2C) with no significant NMUR2 activation up to 10^{-6} M (relative inositol phosphate accumulation of 25.7 % at a concentration of 1 μM) due to a loss in receptor affinity (Figure 2B and 2D).

An *in vitro* degradation study in human plasma was performed as described before[13] (see Supporting Information) to investigate the effect of the introduced modifications on the proteolytic stability of a selection of the novel NMU-analogs. The

percentage of intact peptide was measured in function of time via HPLC-UV analysis (Figure 3). To evaluate the degradation profile, samples were subsequently analyzed by LC-MS (Figure 4). NMU is characterized by a short half-life of less than 5 min after subcutaneous injection.[3] Moreover, the plasma half-life of NMU-8 was found to be 4.3 ± 0.2 min, with the neuropeptide being rapidly cleaved at its *N*-terminus. *N*-terminal acetylation of NMU-8 was able to increase the resistance against proteolytic degradation, resulting in a 25-fold longer half-life. The cleavage site after Tyr[1] was protected by acetylation of the *N*-terminus and degradation of Ac-NMU-8 occurred by cleavage between Phe[2]-Leu[3].[13]



	Peptide	Sequence	$t_{1/2}$ (min)
●	1	Ac-Tyr-Phe-Leu-Phe-Arg-Pro-**β³hArg**-Asn-NH_2	1170.5 ± 128.4
●	2	Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Me)**-NH_2	156.4 ± 10.5
●	3	Ac-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-**Asn(*N*Bzl)**-NH_2	166.8 ± 5.4
●	7	Ac-**Dmt**-Phe-Leu-**Dmt**-Arg-Pro-Arg-Asn-NH_2	201.5 ± 6.3
●	8	Ac-**7-OH-Tic**-Phe-Leu-**2'Nal**-Arg-Pro-Arg-Asn-NH_2	142.3 ± 1.3
●	10	Ac-**7-OH-Tic**-Phe-Leu-***N*MePhe**-Arg-Pro-Arg-Asn-NH_2	1804.2 ± 77.5

Figure 3. Relative recovery over time of the NMU-analogs in human plasma at 37°C and calculated half-lifes. Experiments were performed in triplicate and data are presented as mean ± SD.

As can intuitively be expected, modification of the Arg[7] (β[3]hArg in **1**) or Asn[8] (Asn(*N*Me) in **2**, Asn(*N*Bzl) in **3**) residue, together with *N*-terminal acetylation, did not stabilize the Phe[2]-Leu[3] cleavage site, and calculated half-lifes of 156.4 ± 10.5 min and 166.8 ± 5.4 min were found for compounds **2** and **3** respectively. Although replacement of Arg[7] by β[3]hArg did not alter the major cleavage site of the peptide, an extraordinary resistance against biodegradation was observed resulting in a half-life of 1170.5 ± 128.4 min (or 19.5 ± 2.1 h) in human plasma for analog **1**. We previously reported that the major cleavage site of the NMU-analogs was shifted to the middle of the sequence, between Phe[4] and Arg[5], when Tyr[1] was substituted by 7-OH-Tic or 2'Nal, or upon replacement of Phe[4] by Dmt.[13] When both Tyr[1] and Phe[4] were substituted, as for compounds, **7** (Ac-[Dmt[1],Dmt[4]]-NMU-8), **8** (Ac-[7-OH-Tic[1],2'Nal[4]]-NMU-8) and **10** (Ac-[7-OH-Tic[1],Dap[4]]-NMU-8), two major cleavage sites were found more precisely, in the middle of the sequence and between Arg[7]-Asn[8] (Figure 4). For NMU-analogs **7** and **8**, the major degradation site was found to be in the middle of the NMU-sequence, between the modified Phe[4] residue and Arg[5], resulting in plasma half-lifes of 201.5 ± 6.3 min and 142.3 ± 1.3 min for **7** and **8** respectively. In a smaller extent, the peptides were cleaved between Arg[7]-Asn[8]. These degradation profiles indicate that unnatural amino acids such as Dmt and 2'Nal are able to suppress, but not eliminate, hydrolysis of the adjacent amide bonds. When the backbone amide of the Phe residue in position 4 was modified, as in the case of compound **10** where Phe[4] was replaced by *N*MePhe, the priority of the biodegradation sites switched to Arg[7]-Asn[8] as the major cleavage site (see Supporting Information, Table S4 and Figure S5). The scissile Phe[4]-Arg[5] amide bond was protected by the introduction of a methyl group on the backbone amide and was only cleaved in a minor extent, resulting in a prolonged half-life of 1804.2 ± 77.5 min (or 30.1 ± 1.3 h). Again, this observation indicates that *N*-alkylation of the amide bond between Phe[4] and Arg[5] seems a promising avenue towards extremely stable hNMUR1 agonists.

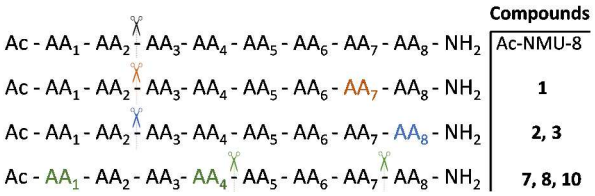


Figure 4. Schematic representation of the biodegradation profiles of the novel NMU-analogs. Colored amino acid positions (AA) indicate where modifications are introduced (cfr. Figure 1). The major cleavage sites are indicated with dotted lines.

In conclusion, the present study proves that modifications in the *C*-terminal region, previously reported as critical segment, of the NMU-8 sequence are tolerated. Moreover, the substitution of the Arg[7] by β[3]hArg increased the resistance against proteolytic degradation resulting in a half-life of more than 19 h in human plasma. Importantly, we synthesized a series of NMU-analogs with an increased selectivity for NMUR1. The novel NMU-derivative **7** possesses subnanomolar affinity for the receptor and a 10-fold increased potency as compared to NMU-8, together with an 18 % higher E_{max}. No significant NMUR2 receptor activation was found up to 10^{-6} M. Moreover, a more than 50-fold extension of the plasma half-life was observed for compound **7**. When modifications were introduced in the backbone, as for analog **10**, a plasma half-life up to 30 h was found. In general, stabilization of the Phe[4]-Arg[5] bond is necessary for obtaining NMU-8 analogs with prolonged plasma stability. We are convinced that potent and proteolytical stable NMUR1 agonists, such as the ones reported in this report, could serve as useful tools for further elucidating to role of this receptor in NMU signaling, which seems to be involved in feeding behavior and glucose tolerance.[3, 18] Moreover, NMUR1 is recently gaining a lot of attention since it seems to play a role in type 2 lymphoid driven inflammation and allergic lung inflammation.[19-21]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information, including experimental procedures along with peptide characterization data (PDF), is available free of charge on the ACS Publications website.

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Author Contributions

ADP synthesized the ligands, performed the experiments and wrote the paper. CM assisted with the identification of the metabolites and YVW with the plasma stability assay. CT provided the [^{3}H]-NMU-8 peptide. VC helped revising the manuscript. DT contributed to confine the synthesis strategy. BH and MMR supervised the *in vitro* activity and binding assays. AVE supervised the stability study. IS and SB supervised and designed the research study. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENTS

ADP was supported by the Flanders Innovation & Entrepreneurship Agency (VLAIO) and the Research Foundation Flanders (FWO). The authors thank the Research Council (OZR) of the Vrije Universiteit Brussel for funding through the Strategic Research Program and the Queen Elisabeth Medical Foundation (G.S.K.E – 2017-2019) for the financial support.

ABBREVIATIONS

2'Nal, 2'-naphtylalanine; 7-OH-Tic, 7-hydroxy-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Atc, (D,L)-2-aminotetraline-2-carboxylic acid; Dap, *α,β*-diaminoropionic acid; DIPEA, diethyldothiocarbamate; Dmt, 7-hydroxy-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Fmoc, 9-fluorenylmethyloxycarbonyl; HEK293, human embryonic kidney 293; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; IP_3, inositol triphosphate; NMU, Neuromedin U; NMUR, Neuromedin U receptor; TES, triethylsilane; TFA, trifluoroacetic acid; TBTU, 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; β^3hArg, β^3homo-arginine

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