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N-Alkylaminoacids and Their Derivatives Interact with Melanocortin Receptors

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Abstract—Thirty four *N*-alkylaminoacids (Arg, Trp, Nal, Pro, Hyp, L and D) and derivatives were prepared by a process that included reductive alkylation of the amino function. Both solid phase and solution synthesis was used. Title substances displayed binding activity on melanocortin receptors $MC_{1,3-5}$ reaching the low micromolar range.

Melanocortin receptors exist in subtypes MC_{1-5}, for which drugs are desired for the treatment of inflammation, body weight disorders and sexual dysfunctions. Yet there do not exist any high affinity low molecular weight organic compounds for these receptors. Some peptoids,[1] nine-member heterocycles[2] and isoquinolines[3] related to the structure of the melanocortin peptides' active core, Phe-Arg-Trp, were reported to show moderate binding affinity for MC-receptors. However, no subtype selective or high-affinity, low-molecular-weight MC-receptor active compounds have up until now been reported. Here, we describe the synthesis of *N*-alkylated amino acids and derivatives, and demonstrate their activity on subtypes of the MC receptors.

N-Alkylarginines (**2**) (Scheme 1) were obtained from commercially available Fmoc-L-Arg(Pmc)-Wang resin or its D-form (**1**). The Fmoc group was cleaved off by piperidine in DMF, and the Schiff base was formed using 2-naphthaldehyde, *N*-Boc-3-indolylaldehyde, 3,4-dimethoxybenzaldehyde or 2-chloro-3,4-dimethoxybenzaldehyde, followed by reduction of the C=N double bond (i.e., 4% AcOH in trimethylorthoformate was added to polymer attached Schiff base and 5-fold excess $NaCNBH_3$, shaken for 10 min, filtered off, washed and dried in vacuo). The synthesis was completed by TFA cleavage, detaching the *N*-alkylarginine from the polymer and removing the Pmc protective group. Raw products were purified by reversed-phase HPLC using acetonitrile–water–0.1% TFA as eluent. Freeze drying provided pure end products DKP-1, DKP-2, MK-21, MK-31, MK-32, MK-33 and MK-34 (Table 1).

N-Alkylaminoacid amides (**4**) (Scheme 1) were obtained using Fmoc-PAL-PEG-PS support (**3**) (PE Biosystems). After removal of the Fmoc group, the support was coupled (1 h) with Fmoc-L-Arg(Pbf)-OH or its D-isomer in the presence of diisopropylethylamine and HATU in DMF. [For some compounds N^{α}-Fmoc derivatives of Trp(Boc) or Nal was used instead.] After the coupling the support was DMF-washed, treated with 20% piperidine in DMF and washed again. (For dipeptide derivatives this cycle was performed twice.) At the end the support was washed and dried. Several aldehydes and β-tetralones were then applied to form Schiff bases followed by reduction. TFA cleavage, purification and isolation of pure products gave substances MK-17, MK-19, MK-18, MK-20, MK-25, MK-26, MK-27 and MK-28 (Table 1).

The proline derivatives MK-50, MK-51 and MK-52 were prepared by solution synthesis. Boc-L-proline or its D-isomer (**5**) (Scheme 1) were reacted with amines in presence of dicyclohexylcarbodiimide and pentafluorophenol complex[4] (molar proportions 1:3, 'complex F') in methylene chloride. Schiff base formation using 2-naphthaldehyde or 3-indolylaldehyde and $NaCNBH_3$ reduction completed the process yielding (**6**).

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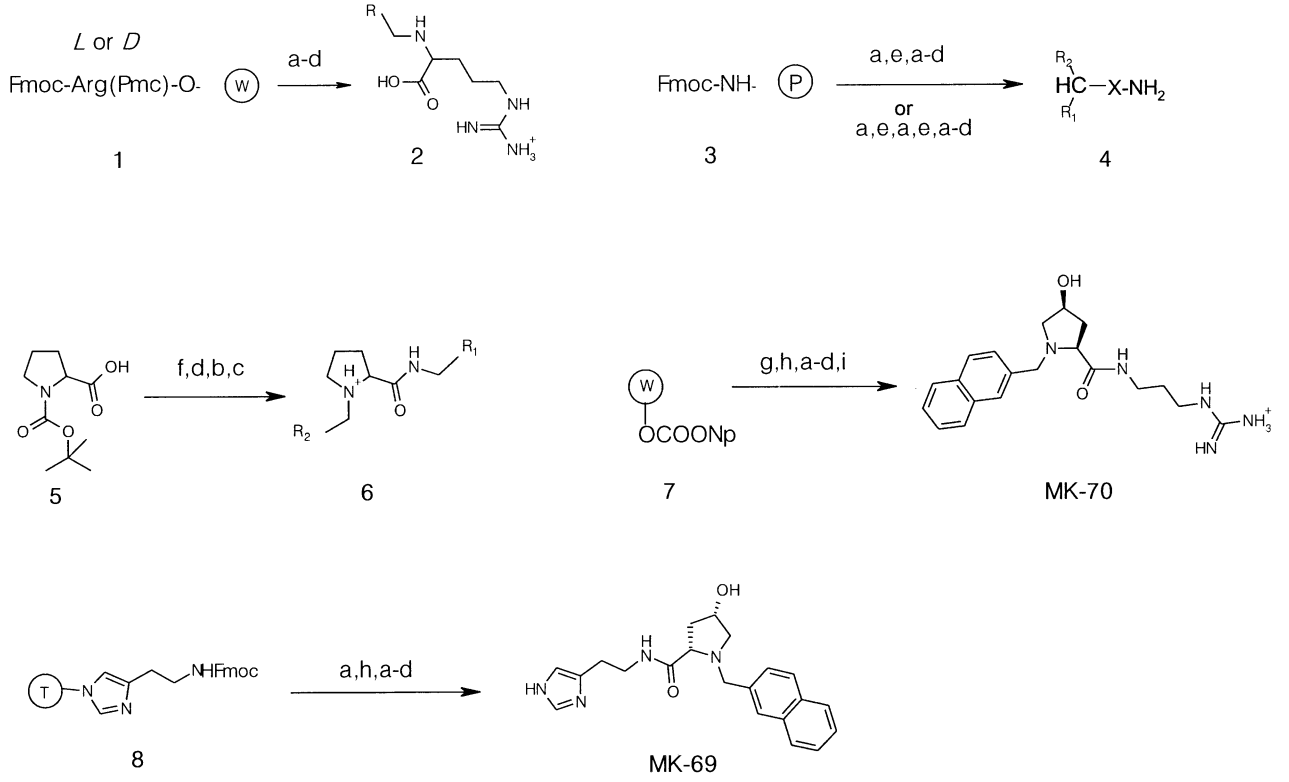


Scheme 1. Reagents and conditions: (a) 20% piperidine/DMF, 30 min; (b) R-CHO, trimethylorthoformate, 20 h or α-tetralone, 10% AcOH/trimethylorthoformate, 30 °C, 40 h; (c) $NaCNBH_3$, AcOH, trimethylorthoformate, 30 min; (d) TFA–1,2-ethandithiol–triisopropylsilane–water (925:25:25:25), 1 h; (e) Fmoc-Arg(Pbf)-OH or Fmoc-Trp(Boc)-OH or Fmoc-Nal-OH, DIEA, HATU, DMF, 1 h; (f) $R_1CH_2NH_2$, complex F, methylene chloride, 20 h; (g) trimethylenediamine, DMF, 20 h; (h) Fmoc-Hyp(*t*Bu)-OH, DIEA, HATU, DMF; (i) 1H-pyrazole 1-carboxamidine hydrochloride, DIEA, DMF, 20 h.

Table 1. Structures of compounds synthesized herein[a]

Code	General structure	R or R_1	R_2	X	Configuration
DKP-1	**2**	3-Indolyl	—	—	*S*
DKP-2	**2**	2-Naphthyl	—	—	*S*
MK-11	**4**	2-Naphthyl	H	L-Trp	*S*
MK-14	**4**	2-Naphthyl	H	D-Trp	*R*
MK-15	**4**	3-Indolyl	H	D-(2)Nal	*R*
MK-16	**4**	3-Indolyl	H	L-(2)Nal	*S*
MK-17	**4**	3-Indolyl	H	L-Arg	*S*
MK-18	**4**	2-Naphthyl	H	L-Arg	*S*
MK-19	**4**	3-Indolyl	H	D-Arg	*R*
MK-20	**4**	2-Naphthyl	H	D-Arg	*R*
MK-21	**2**	3,4-Dimethoxyphenyl	—	—	*S*
MK-25	**4**	3,4-Dimethoxyphenyl	H	L-Arg	*S*
MK-26	**4**	2-Chloro-3,4-dimethoxyphenyl	H	L-Arg	*S*
MK-27	**4**	Benzyl	H	L-Arg	*S*
MK-28	**4**	1,2,3,4-Tetrahydronaphthalene-2-yl		L-Arg	*S*
MK-29A	**4**	2-Naphthyl	H	L-Arg-L-Trp	*S*/*S*
MK-29B	**4**	3-Indolyl	H	L-Arg-L-Trp	*S*/*S*
MK-30A	**4**	2-Naphthyl	H	D-Arg-L-Trp	*R*/*S*
MK-30B	**4**	3-Indolyl	H	D-Arg-L-Trp	*R*/*S*
MK-31	**2**	2-Naphthyl	—	—	*R*
MK-32	**2**	3-Indolyl	—	—	*R*
MK-33	**2**	3,4-Dimethoxyphenyl	—	—	*R*
MK-34	**2**	2-Chloro-3,4-dimethoxyphenyl	—	—	*R*
MK-50	**6**	2-Naphthyl	(3-Indolyl)methyl	—	*R*
MK-51	**6**	3-Indolyl	1-Naphthyl	—	*S*
MK-52	**6**	3-Indolyl	1-Naphthyl	—	*R*
MK-53	**12**	(3-Indolyl)methyl	—	—	*S*
MK-54	**12**	1-Naphthyl	—	—	*S*
MK-55	**12**	(3-Guanidino)propyl	—	—	*S*
MK-56	**12**	2-Naphthyl	—	—	*S*

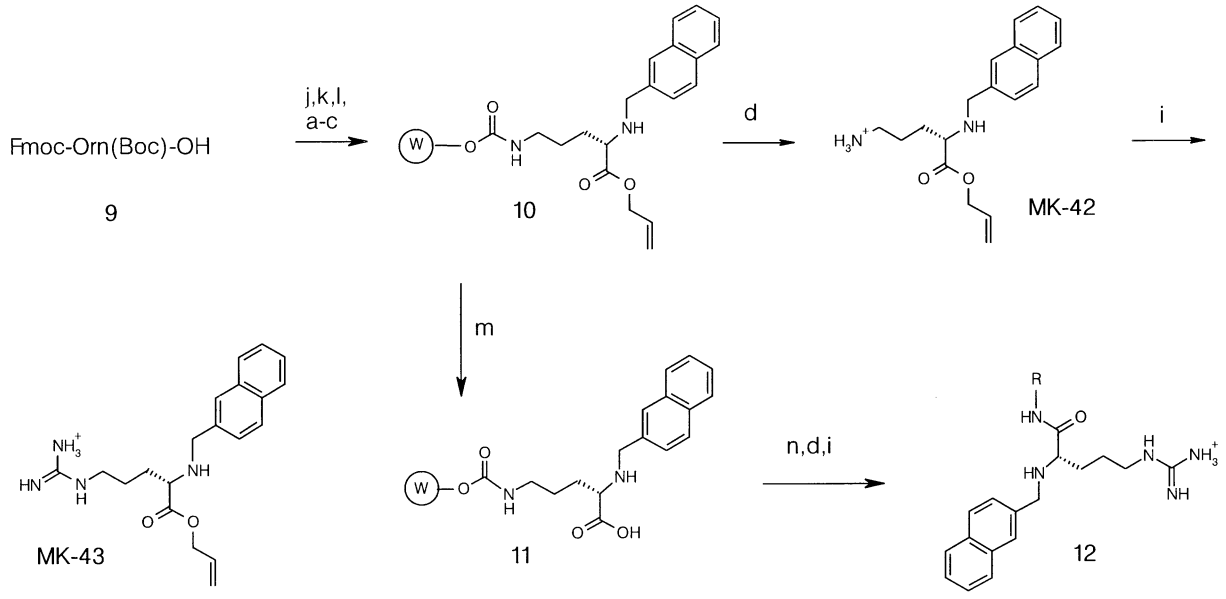
[a]Structures of compounds MK-42, MK-43, MK-69 and MK-70 are shown in Schemes 1 and 2.

MK-70 was prepared by treating *p*-nitrophenyl carbonate Wang resin (**7**) (Scheme 1) with trimethylenediamine in DMF. The product was coupled with Boc-L-Hyp (Bu^t)-OH in the presence of HATU and DIEA in DMF. Fmoc deprotection, addition of 2-naphthaldehyde, reduction and simultaneous cleavage of the *t*-butyl group and polymeric benzyloxycarbonyl structure then followed. The product was reacted with 1H-pyrazole 1-carboxamidine hydrochloride[6] in DMF in the presence of DIEA. The reaction mixture was diluted with water and applied directly to preparative HPLC, yielding MK-70.

MK-69 was obtained from *N*-Fmoc-histamine 4-methoxytrityl resin (Novabiochem) (**8**) (Scheme 1). The resin was Fmoc deblocked, coupled with Fmoc-Hyp(But)-OH followed by Fmoc removal. Subsequent steps were made exactly as described above for MK-70. Allylesters MK-42 and MK-43 (Scheme 2) were obtained by boiling commercial Fmoc-Orn(Boc)-OH (**9**) in neat allylbromide in the presence of DIEA.[5] The allylester formed was deblocked (i.e., removal of Boc) by TFA treatment. To obtain a pure crystalline substance, Fmoc-Orn-OAll·TFA was passed through a column of Dowex 1×4 in Cl^- form in methanol. The hydrochloride thus obtained was attached to polymer by reaction with p-nitrophenyl carbonate Wang resin (Novabiochem) in DMF in presence of DIEA. Removal of the Fmoc group, formation of Schiff base with 2-naphthaldehyde, reduction, TFA treatment and purification led to MK-42. Part of the product was guanidated as described above for MK-70 giving the arginine derivative MK-43.

Several *N*-alkylated substituted argininamides (**12**) were also prepared (Scheme 2). Polymeric allylester (**10**) from the previous synthesis was treated with tetrakis(triphenylphosphine)-palladium(0) dissolved in 5% AcOH + 2.5% NMM in chloroform for 2 h. The support was washed with 0.5% DIEA + 0.5% Na diethyldithiocarbamate in DMF, followed by a DMF wash. Part of the product (**11**) obtained was reacted with *N*-*t*-Boc-trimethylenediamine in the presence of HATU and DIEA. Deprotection with TFA and guanidation gave the diguanidated product, MK-55. Similarly, (**11**) was reacted with tryptamine, 1-naphthylmethylamine or 2-naphthylamine followed by deprotection and guanidation yielding the monoguanidated derivatives MK-53, MK-54 and MK-56 (Table 1). All end products of synthesis were identified using turboionspray MS and ^{1}H NMR. Yields ranged over 5–20%. Compound activities, K_i, were estimated using ^{125}I-NDP-MSH radioligand binding on recombinant human MC receptor subtypes $MC_{1,3-5}$, as described earlier.[7] Results are given in Table 2 as the averages of 2–3 independent measurements.

Substances herein show considerable diversity. They contain at least two of four presumed melanocortin pharmacophoric groups (i.e., the guanidine group, naphthalene or benzene, indole and imidazole rings). With regard to binding activity the compounds can be divided into three groups: (1) compounds with higher affinity (i.e., K_i below 10 μM for at least one MC receptor type), (2) compounds of intermediate affinity and (3) practically inactive substances (i.e., $K_i > 1$ mM for all receptor types). A further inspection of the data reveals that all the substances of the first group (i.e., nine compounds) are all in the *S* configuration. This would be expected, at least for Arg and Trp derivatives, in consistence with the known structure–activity relationship of melanocortin peptides.[8] It is quite unexpected, however, that already the presence of two of the above pharmacophoric groups (i.e., in DKP-2, MK-11, MK-43, MK-51 and MK-70) provide compounds with



Scheme 2. Reagents and conditions: (a) 20% piperidine/DMF, 30 min; (b) 2-naphthaldehyde, trimethylorthoformate, 20 h; (c) $NaCNBH_3$, AcOH, trimethylorthoformate, 30 min; (d) TFA–1,2-ethandithiol–triisopropylsilane–water (925:25:25:25), 1 h; (i) 1H-pyrazole 1-carboxamidine hydrochloride, DIEA, DMF, 20 h; (j) allylbromide, DIEA, reflux, 1 h; (k) TFA, 1 h; (l) *p*-nitrophenyl carbonate Wang resin, DIEA, DMF, 20 h; (m) tetrakis(triphenylphosphine)-palladium(0), chloroform, 2 h; (n) RNH_2, DIEA, HATU, DMF, 1 h.

Table 2. Binding activities K_i (in μM) determined in competition with ^{125}I-NDP-MSH on recombinant human MC receptor subtypes. nb, not binding[a]

Code	MC_1	MC_3	MC_4	MC_5
DKP-2	4.5	nb	66	nb
MK-11	8.7	56	139	42
MK-14	15	53	64	37
MK-15	74	>1 mM	>1 mM	95
MK-16	26	>1 mM	>1 mM	60
MK-20	>1 mM	>1 mM	>1 mM	125
MK-28	>1 mM	155	134	130
MK-29A	29	107	105	74
MK-29B	>1 mM	>1 mM	205	184
MK-30A	32	147	56	56
MK-42	22	161	127	200
MK-43	5.9	28	15	15
MK-50	nb	752	166	nb
MK-51	8.9	68	26	nb
MK-52	45	104	36	nb
MK-53	2.2	37	9.9	15
MK-54	1.3	6.4	6.8	8.7
MK-55	4.5	4.8	30	nb
MK-56	2.9	14	14	6.6
MK-69	nb	161	150	50
MK-70	2.9	82	279	221

[a]Compounds DKP-1, MK-17, MK-18, MK-19, MK-21, MK-25, MK-26, MK-27, MK-31, MK-32, MK-33, MK-34 showed k_i >1mM for all receptor types.

micromolar affinity. It is possible that these substances bind to the MC receptors via additional functions, such as via carboxylate and allyl groups. Addition of the second naphthalene in MK-54 and MK-56 was also favourable. By contrast combinations of indole and guanidine pharmacophoric groups (i.e., with naphthalene omitted) was unfavourable (MK-17, MK-19, MK-32), while combinations of naphthalene and guanidine (without the indole) were more favourable. Besides, it seems that the naphthalene derivative (DKP-2) is a better binder than the corresponding derivative of substituted benzene (MK-21). Derivatives of the Arg-Trp dipeptide (MK-29A, MK-29B, MK-30A, MK-30B) were only slightly active. The β-tetralone derivative (MK-28) showed intermediate activity. It was also interesting that the compounds showed quite differing patterns of selectivity for the four MC receptor subtypes evaluated.

In conclusion, we have elaborated chemistry allowing us to prepare a series of MC receptor active *N*-alkylaminoacids and derivatives thereof. Some binding was observed for 21 compounds, and several compounds reached low micromolar affinity. The structure–activity information obtained will be useful for further synthesis of MC receptor active low molecular weight compounds.

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