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Design and synthesis of a library of tertiary amides: Evaluation as mimetics of the melanocortins' active core

Felikss Mutulis, Jana Kreicberga,[†] Sviatlana Yahorava, Ilze Mutule,[‡] Larisa Borisova-Jan, Aleh Yahorau, Ruta Muceniece,[§] Sandra Azena, Santa Veiksina,[¶] Ramona Petrovska and Jarl E. S. Wikberg*

Department of Pharmaceutical Biosciences, Division of Pharmacology, Uppsala Biomedical center, Uppsala University, PO Box 591, S-751 24 Uppsala, Sweden

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Abstract—Two hundred and ten tertiary amides were prepared on solid phase. Diamines were coupled to activated carboxylated Wang polymer, and the polymeric substituted benzyloxycarbonyl protected diamines obtained were reacted with aldehydes or ketones in trimethyl orthoformate giving resin attached Schiff bases. Coupled resins were then reduced to secondary amines by sodium cyanoborohydride in 4% acetic acid/trimethyl orthoformate, followed by acylation with the carboxylic acid in the presence of PyBroP and diisopropylethylamine. Cleavage of tertiary amides from the resin was made by trifluoroacetic acid in the presence of scavengers (mainly 1,2-ethanedithiol). When indole derivatives were prepared, parallel alkylation with the linker fragment occurred, giving derivatives of 2-(4-hydroxybenzyl)-indole as side products. Solution synthesis or mixed liquid/solid phase preparation of title substances proved to be advantageous in cases when the above method did not give acceptable results. According to this approach an efficient formation of Schiff bases was achieved in the presence of $TiCl_4$. Substances were isolated by reversed phase chromatography; in some cases isomers were additionally separated by chiral chromatography on Chirobiotic T. When tested on human recombinant melanocortin receptors all the tertiary amides showed some binding affinities; for the highest affinity compounds the K_is reached 400 nM on MC_1, 2 μM on MC_3 and 1 μM on MC_4 and MC_5 receptors. cAMP assays of some of the title compounds showed that the tertiary amides are melanocortin receptor antagonists on the four MC receptor subtypes.

1. Introduction

Melanocortins are natural peptides formed by post-translational cleavage of pro-opiomelanocortin and include melanocyte stimulating hormones (α-, β-, γ-MSH) and adrenocorticotropin.[1] Melanocortins interact with five subtypes of melanocortin receptors $MC_{1-5}R$, identified by molecular cloning.[2–7] The MC receptors belong to the 7TM family of G-protein-coupled receptors[8,9] and their subtypes show both differing distributions and functions in the body. The MC_1R is present in melanocytes,[10] in the CNS[11] and in cells of the immune system.[12] This receptor is known to be involved in the regulation of skin and fur pigmentation,[13] and in the regulation of the immune system.[14] The MC_2R, which is responsive to ACTH only, but not the MSHs, controls the production of adrenal glucocorticoids.[3,15] The MC_3, MC_4 and MC_5Rs are expressed in the brain; the MC_3 and MC_5Rs also in the periphery.[6,16–18] These receptors have many multifaceted actions, e.g., the MC_4R regulates feeding behaviour[17] and is considered a prime novel target for drugs aimed to the control of body weight.[19] The MC_3 receptor may have a role in controlling sexual behaviours, while the MC_5 receptor may control exocrine gland functions.[1] Besides melanocortins the agouti and AgRP proteins are endogenous ligands for the melanocortin receptors. These small proteins are shown to act as inverse agonists.[20]

Keywords: Melanocortin receptor ligands; Tertiary amides; Solid phase organic synthesis; Indole derivatives.
* Corresponding author. Tel.: +46 18 4714238; fax: +46 18 559718; e-mail: Jarl.Wikberg@farmbio.uu.se
† Present address: Faculty of Material Science and Applied Chemistry, Riga Technical University, Azenes 14/24, LV-1048 Riga, Latvia.
‡ Present address: Department of Organic Chemistry Latvian Institute of Organic Synthesis, Aizkraukles iela 21, LV-1006 Riga, Latvia.
§ Present address: Department of Pharmacy, Faculty of Medicine, University of Latvia, Sarlotes iela la, LV-1001 Riga, Latvia.
¶ Present address: Institute of Organic and Bioorganic Chemistry, Tartu University, Jakobi 2, 51014 Tartu, Estonia.

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Melanocortins are linear peptides with about 11–27 amino acid residues that share a conserved core of between three and six amino acid residues [1] the most essential structure being Phe-Arg-Trp.[21] L-Phenylalanine residue of the active core can be exchanged to the D-isomer, or to D-2-naphthylalanine and still retaining or even increasing the peptides' activity on the MSH responsive MCRs.[21–23] By replacing the Phe[7] residue with its D-isomer and Met[4] with Nle in α-MSH, a very potent MC receptor agonist, NDP–MSH, was obtained.[22] A number of quite potent cyclic analogues of MSH (both of disulfide and lactam types) have also been prepared (see e.g., Refs. 23 and 24). Moreover, by combining sequences obtained from phage display library screening with parts of the sequence of α-MSH we found some highly active MC_1R selective peptides.[25]

To obtain substances suited for medical use there is an interest to non-peptides imitating melanocortins. Empiric search and lead optimization led in some cases to highly active and selective organic compounds, such as the MC_4R agonist THIQ[26] and derivatives thereof,[27,28] the MC_4R antagonist MCL0129,[29] and some MC_5R antagonistic phenylguanidines.[30] To obtain ligands for melanocortin receptors, an alternative possibility is the incorporation of the most important structural features of the melanocortins into non-peptide compounds.

Early studies led to the assumption that a feature of the 'biologically active' conformation of melanocortins is a β-turn of their core region.[31] Later investigators applied NMR and energy calculations on cyclic melanocortin-related peptides with reduced flexibility, and showed that turn structures (β-turn for L-phenylalanine derivatives and reversed turn for D-phenylalanine compounds) indeed predominate in the core region.[32–34] In one attempt the presumed bioactive β-turn conformation was mimicked in nine-membered cyclic non-peptides. The scaffolds were connected with three side-chains containing, respectively, an indole moiety, benzene or naphthalene structures and an amino or guanidine group.[35] Recently, also tri-substituted tetrahydropyrans were used as scaffolds to bring such pharmacophoric groups together.[36] Small peptoids are attractive as substituents of peptides as well. G. Heizmann et al.[37] synthesized a library of tripeptoids, which included side chains resembling those of the active core of α-MSH, Phe-Arg-Trp, and identified moderately active ligands. Our efforts in this direction concerning N-alkylated dipeptide amide peptoids and related structures led also to structures with moderate MC receptor activity.[38] Moreover, Thompson et al.[39] synthesized peptoids that mimicked the AgRP tripeptide and succeeded to obtain MC_4R antagonists. Urea derivatives, which included pharmacophoric groups from melanocortin tripeptide or their equivalents, were also synthesized on solid phase.[40] Our studies on the preparation of products of reductive amination[41] and *N*-alkylaminoacids and derivatives[42] resulted in substances showing moderate binding activity on melanocortin receptors.

According to one contemporary idea, the active core Phe-Arg-Trp of the melanocortins binds to the receptor solely through the pharmacophoric benzene, guanidinium and indole groups in the side chains of the phenylalanine, arginine and tryptophan residues, while the peptide backbone and the parts of the side-chains closer to the backbone do not participate in the binding. Such a view can be found, e.g., in a study devoted to docking of ligands to a hypothetical melanocortin-1 receptor structure.[43] If this view is true, a tempting perspective emerges to simplify a structure in an extreme way by removing the peptide backbone and connecting the remaining pharmacophoric groups through a minimal scaffold. Optimization would then merely be to place the pharmacophoric groups in a correct position by changing distances between them and introducing spatial constraints.

On the other hand, the most essential fragment of the agouti and AgRP is the tripeptide Arg-Phe-Phe,[44] which is closely related to the melanocortin sequence Phe-Arg-Trp. One may assume that the pharmacophoric groups in these fragments are spatially oriented similarly and determine the activity for both the melanocortins and agouti/AgRP, despite the other dissimilarities in sequence. Incorporation of the pharmacophoric groups of these tripeptides in compact organic structures might thus lead to MC receptor active compounds. We here developed this idea by trying to combine the three pharmacophoric groups with an extremely simple scaffold, based on tertiary amides.

2. Results

2.1. Structures

The general structure of the tertiary amides is shown in Figure 1A. Three parts of the molecule are connected through a centrally located amide linkage. This conjugated scaffold creates some rigidity in the centre of the molecule resulting in formation of two rotamers (Fig. 1B). The existence of such rotamers is well-known.[45] They were previously identified also by us in a study devoted to peptoids.[38] Here, rotamers were detected by NMR, where doubling of signals was observed in many cases (see Section 5).

It turned out that the tertiary amides were relatively easy to synthesize by a solid or liquid phase process allowing

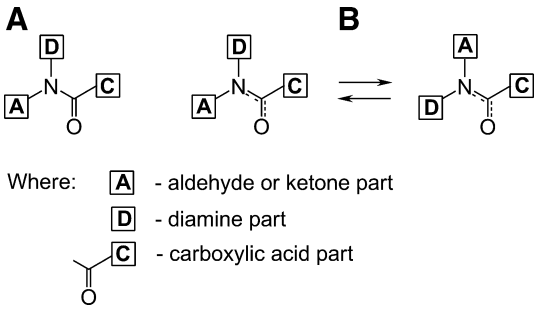


Figure 1. (A) General structure of tertiary amides. (B) Stabilized forms (rotamers).

us to apply a high diversity of reagents of three types, namely diamines, aldehydes or ketones and carboxylic acids. A large set of compounds was thereby possible to prepare. We thus introduced the three pharmacophoric groups of melanocortins, presumed to be the most essential, namely an amino or guanidino group, a benzene or naphthalene function, and an indole structure (or the equivalents thereof). An advantage of our approach was that the distances between the pharmacophores could be easily varied. Moreover, very compact structures, not covered by turn mimetics, could be prepared.

2.1.1. Coding of compounds. Compounds were coded by a three-number code referring to the structure **D**(**A**)═N─C(O)─**C** (Fig. 1). The numbers in the code denote, respectively, the diamine (**D**), aldehyde or ketone (**A**) and carboxylic acid [C(O)─**C**] parts listed below. Moreover, for the cases that the three-number-codes are followed by one or two numbers within parentheses the first of the numbers within parentheses denotes that the substance is obtained from the corresponding fraction of a reversed phase chromatography. The second number within the parentheses denotes the fraction number of a preparative chiral chromatography. Codes marked with letter 'a' at the end correspond to 4-hydroxybenzyl derivatives (Table 1).

The number codes for the diamine part (**D**) were as follows: **1**, pentyl; **2**, 2-aminoethyl; **3**, 3-aminopropyl; **4**, 4-aminobutyl; **5**, 5-aminopentyl; **6**, 6-aminohexyl; **7**, 7-aminoheptyl; **8**, 8-aminooctyl; **9**, 2-(3-aminopropyl)-2-ethyl-hexyl and/or 4-(aminomethyl)-4-ethyl-octyl; **10a**, 4-(4-amino-cyclohexylmethyl)-cyclohexyl, mixture of isomers from commercial diamine; **10b**, 4-(4-amino-cyclohexylmethyl)-cyclohexyl, mixture of isomers obtained from commercial diamine after partial separation of its trans, trans form; **10c**, *trans*,*trans*-4-(4-amino-cyclohexylmethyl)-cyclohexyl; **10d**, 4-(4-amino-cyclohexylmethyl)-cyclohexyl, mixture of isomers from commercial tritylpolymer attached diamine; **10e**, *cis*-4-(trans-4-amino-cyclohexylmethyl)-cyclohexyl; **11**, 2-guanidinoethyl; **12**, 3-guanidinopropyl; **13**, 4-guanidinobutyl; **14a**, 4-(4-guanidino-cyclohexylmethyl)-cyclohexyl, mixture of isomers obtained from commercial bis-(4-aminocyclohexyl)-methane after partial separation of its trans, trans form; **14b**, 4-(4-guanidino-cyclohexylmethyl)-cyclohexyl, mixture of isomers obtained from commercial tritylpolymer attached diamine; **15**, 3-aminomethyl-benzyl; **16**, 4-aminomethyl-benzyl; **17**, *trans*-2-aminocyclohexyl; **18**, 5-amino-2-methyl-pentyl and/or 5-amino-4-methyl-pentyl, mixture of isomers obtained from commercial diamine; **19**, 6-amino-2,4,4-trimethyl-hexyl and/or 6-amino-2,2,4-trimethyl-hexyl and/or 6-amino-3,3,5-trimethyl-hexyl and/or 6-amino-3,5,5-trimethylhexyl mixture of isomers obtained from commercial diamine; **20a**, 4-(4-amino-3-methyl-cyclohexylmethyl)-2-methyl-cyclohexyl, mixture of isomers obtained from commercial diamine; **20b**, 4-(4-amino-3-methyl-cyclohexylmethyl)-2-methyl-cyclohexyl, mixture of isomers obtained from fractionated commercial diamine; **21**, 3-[4-(3-aminopropyl)-piperazine-1-yl]-propyl; **22**, (*trans*-4-aminocyclohexyl)ureido-*trans*-cyclohexan-4-yl; **23**, (3-aminocyclohexyl)ureido-*trans*-cyclohexan-4-yl; **24**, *trans*-4-aminocyclohexyl; **25**, (4-aminomethyl-cyclohexyl) methyl; **26**, (trans-4-aminocyclohexyl)ureido-cyclohexan-3-yl; **27**, (3-aminocyclohexyl)ureido-cyclohexan-3-yl; **28**, 3-aminocyclohexyl; **29**, (3-aminomethyl-cyclohexyl) methyl.

Number codes for the aldehyde or ketone parts (**A**) were as follows: **0**, 2-naphthylmethyl; **1**, 3,4-dimethoxybenzyl; **2**, 3-ethoxy-4-methoxybenzyl; **3**, 2-fluorobenzyl; **4**, 4-carbmethoxybenzyl, **5**, 4-methylthiobenzyl; **6**, 3-methylbenzyl; **7**, 2-methylbenzyl; **8**, 4-methylbenzyl; **9**, 1-(benzothien-3-yl)ethyl; **10**, 2-bromobenzyl; **11**, 2-phenylethyl; **12**, 3-(2-methoxyphenyl)allyl; **13**, 2-pyridylmethyl; **14**, 3-bromo-4-methoxybenzyl; **15**, 3-phenylpropyl; **16**, 3-(2-methoxy-4-hydroxyphenyl)allyl; **17**, 1-(2-methyl-5-phenylthien-2-yl)ethyl; **18**, 2-bromo-4,5-dimethoxybenzyl; **19**, 4-pyridylmethyl; **20**, 2-chlorobenzyl; **21**, 2-chloro-4,5-dimethoxybenzyl; **22**, 2-fluoro-6-chlorobenzyl; **23**, 4-cyanobenzyl; **24**, 2,6-dichlorobenzyl; **25**, 2,6-difluorobenzyl; **26**, 2-adamantyl; **27**, 1-methylethyl; **28**, 2-bromoallyl; **29**, 1-(2-naphthyl)ethyl; **30**, (2-methylthien-6-yl)methyl; **31**, 1-(4-trifluoromethylphenyl)ethyl; **32**, cycloheptyl; **33**, 3,4-methylenedioxybenzyl; **34**, 3-(2-naphthyl)allyl; **35**, 3-phenylallyl; **36**, 2-chloro-3-phenylallyl; **37**, 2-methyl-3-phenylallyl; **38**, 1,2,3,4-tetrahydronaphthalene-2-yl; **39**, 4-diethylaminobenzyl; **40**, benzyl; **41**, 2-methyl-3-phenylpropyl; **42**, spiro[5.5]undecan-3-yl; **43**, spiro[5.5]undec-8-en-3-yl.

Number codes for the carboxylic acid parts (**C**─C═O) were as follows: **0**, indole-3-carbonyl; **1**, indole-3-acetyl; **2**, 3-(2-naphthyl)-D-alanyl; **3**, 3-(2-naphthyl)-L-alanyl; **4**, 4-nitro-D-phenylalanyl; **5**, indole-3-propanoyl; **6**, 3-(2-pyridyl)-D-alanyl; **7**, 3-(4-biphenyl)-D-alanyl; **8**, indole-3-butanoyl; **9**, 1-(4-chlorobenzoyl)-2-methyl-5-methoxyindole-3-acetyl; **10**, D-tryptophanyl; **11**, indole-3-glyoxylyl; **12**, L-histidyl; **13**, 2-naphthylacetyl; **14**, 2-naphthoxyacetyl; **15**, *trans*-3-indoleacryloyl; **16**, 2-thiophenebutanoyl; **17**, L-tryptophanyl; **18**, 5-benzyloxyindole-2-carbonyl; **19**, 5-hydroxyindole-2-carbonyl; **20**, 4-chloro-D-phenylalanyl.

A list of all end-products prepared herein is given in Table 1.

2.2. Chemistry

2.2.1. Preparation of reagents. We used in one case 3-(2-naphthyl)-2-propenal (**2**), which is not commercially available. We synthesized it in a simple and efficient way from 2-naphthaldehyde (**1**) and acetaldehyde (Scheme 1).

The use of commercial mixtures of diamines and/or unsymmetrical diamines led to the formation of mixtures of isomers already at the first step of the solid phase synthesis.

As we were interested to obtain information on the activity of individual compounds, diamine fractionation experiments were done before introducing them into the reactions, in some cases. Thus, in order to improve the

Table 1. Preparation procedures of tertiary amides and their binding affinities to melanocortin receptors[a]

Substance	P.p.[b] (yield, %)	K_i (μM)			
		MC_1R	MC_3R	MC_4R	MC_5R
1–39–8	H(16)	72	289	78	408
2–0–0	A(13)	3.6	134	66	27
2–0–1	A(24)	36	68	140	18
2–15–5	J(21)	21	127	nd[c]	20
2–17–8	C(23)	7.5	210	26	36
2–29–1	C(7)	4.4	11	11	19
2–30–9	C(27)	2.3	35	4.0	4.4
2–35–5	A(13)	17	51	106	13
2–36–8	A(8)	5.6	65	5.8	nd[c]
2–38–1	C(28)	4.0	16	42	29
2–38–13	C(33)	1.6	29	27	25
2–40–8	A(8)	22	49	21	nd[c]
2–42–8	J(22)	3.2	2.7	10	12
2–42–14	J(10)	6.0	2.1	4.9	3.4
2–42–20	J(25)	3.7	1.7	2.4	3.2
3–0–1	A(20)	20	63	161	12
3–0–8	A(26)	3.7	26	19	23
3–0–13	A(27)	5.9	12	16	267
3–9–8	C(23)	7.4	104	9.8	55
3–11–8	A(2)	21	30	164	33
3–15–1	A(31)	5.0	5.7	32	13
3–35–0	A(29)	17	172	23	12
3–35–1	A(19)	6.4	21	26	nb[d]
3–35–5	A(27)	21	65	81	11
3–35–11	A(6)	1.6	17	5.0	4.9
3–35–13	A(15)	2.5	33	6.4	10
3–36–8	A(28)	6.1	18	7.0	31
3–36–11	A(23)	1.0	7.4	6.8	4.3
3–37–0	A(24)	0.85	24	7.4	0.93
3–37–1	A(22)	9.3	44	67	89
3–37–11	A(29)	1.8	9.1	6.2	58
3–37–16	A(24)	6.8	63	14	91
3–38–0	C(27)	4.8	131	74	23
3–38–1	C(26)	2.4	37	33	25
3–38–5	I(14)	3.7	nd[c]	7.0	nd[c]
3–38–15	I(27)	6.0	48	11	12
3–38–18	I(38)	26	542	21	681
3–40–8	A(10)	17	45	16	25
4–0–0	A(28)	1.7	19	6.4	18
4–0–1	A(26)	15	63	111	16
4–15–5	A(15)	15	38	158	13
4–26–9	C(26)	3.1	19	10	35
4–27–0	C(4)	106	112	222	279
4–27–0a	C(0.2)	90	106	165	353
4–35–5	A(10)	8.3	67	18	10
4–36–8	A(17)	2.4	32	5.4	85
4–37–9	A(16)	1.8	30	7.1	56
4–40–8	A(9)	12	38	17	20
4–40–8a	A(1)	7.7	16	6.2	4.6
5–0–1	A(28)	1.5	50	13	5.2
5–0–5	A(23)	14	58	62	7.3
5–0–8	A(24)	5.1	20	12	34
5–15–1	A(6)	12	47	77	7.9
5–15–8	A(6)	18	61	nd[c]	19
5–26–8	C(14)	6.1	25	14	46
5–29–1	C(18)	4.5	13	9.4	8.0
5–31–1	C(25)	2.6	12	16	11
5–32–1	C(42)	6.0	14	16	35
5–33–8	A(26)	5.5	38	12	23
5–34–8	A(9)	2.5	5.4	8.4	nd
5–35–5	A(22)	3.7	27	8.1	8.0
5–35–5a	A(2)	3.3	27	7.1	6.7

Table 1 (*continued*)

Substance	P.p.[b] (yield, %)	K_i (μM)			
		MC_1R	MC_3R	MC_4R	MC_5R
5–35–8	A(20)	3.0	36	16	2.2
5–36–8	A(18)	0.77	5.4	2.3	28
5–36–15	A(26)	2.7	27	59	5.4
5–37–8	A(8)	3.5	22	4.7	8.7
5–37–15	A(5)	2.7	nd[c]	99	17
5–39–8	A(24)	6.8	33	25	34
5–40–2	D(23)	5.0	41	13	24
5–40–3	D(26)	10	39	21	34
5–40–4	D(27)	37	162	96	102
5–40–6	D(21)	nb[d]	nb[d]	nb[d]	nb[d].
5–40–7	D(24)	3.9	18	14	20
5–40–8	A(28)	3.2	15	6.4	4.6
5–40–8a	A(3)	2.0	47	5.0	5.1
5–40–10	D(23)	32	106	87	159
5–40–17	D(28)	34	43	44	54
5–43–14	J(22)	7.4	3.7	5.9	8.0
6–36–8	A(24)	2.4	26	3.8	21
6–37–19	A(10)	3.2	27	11	20
6–40–8	A(18)	13	43	3.4	3.8
6–40–8a	A(2)	2.3	36	3.7	2.1
6–41–19	A(12)	2.9	46	9.8	31
7–36–8	A(15)	5.0	42	7.4	29
7–40–8	A(13)	4.0	28	3.8	3.2
8–35–5	A(21)	1.2	24	17	40
9–36–8	A(14)	2.5	131	113	391
9–37–12	D(12)	5.7	23	15	7.5
9–40–8	A(17)	2.1	11	4.7	3.2
9–40–8a	A(2)	2.1	31	8.6	40
10a–36–8(1)	A, K(5)	0.51	7.1	4.1	47
10b–1–8(2)	A, K(13)	3.2	22	3.7	8.3
10b–1–8a	A(6)	1.9	23	2.4	11
10b–2–8(2)	A, K(10)	2.9	16	2.1	9.5
10b–2–8a	A(3)	2.1	26	2.1	12
10b–3–8(2)	A, K(10)	2.4	20	3.7	7.2
10b–3–8a	A(3)	1.0	55	1.5	36
10b–4–8(2)	A, K(10)	2.1	14	2.2	9.6
10b–4–8a	A(3)	4.3	46	2.7	6.7
10b–5–8(2)	A, K(13)	3.2	249	4.3	85
10b–5–8a	A(4)	17	201	4.5	8.9
10b–6–8(2)	A, K(9)	1.3	22	3.0	12
10b–6–8a	A(2)	2.9	78	3.4	16
10b–7–8(2)	A, K(4)	1.2	121	2.3	15
10b–7–8a	A(2)	4.7	349	4.1	4.4
10b–8–8(2)	A, K(10)	1.3	220	2.1	10
10b–8–8a	A(3)	4.7	147	1.9	2.2
10b–10–8(2)	A, K(8)	3.6	80	4.4	9.1
10b–12–8(1)	A, K(7)	1.5	17	4.9	7.5
10b–12–8(2)	A, K(10)	1.6	31	4.1	5.0
10b–13–8	A(29)	4.6	39	5.6	49
10b–14–8(2)	A, K(8)	2.9	9.3	4.8	278
10b–14–8a	A(2)	2.6	132	2.5	23
10b–16–8(1)	A, K(7)	2.9	37	6.4	7.6
10b–16–8(2)	A, K(9)	3.3	51	8.0	11
10b–18–8(1)	A, K(9)	2.0	14	3.2	266
10b–18–8a	A(4)	0.94	94	1.3	18
10b–19–8(1)	A, K(7)	2.5	28	7.0	nd[c]
10b–19–8(2)	A, K(9)	6.3	172	5.6	96
10b–20–8(2)	A, K(4)	3.2	22	2.8	5.2
10b–20–8a	A(3)	6.6	26	14	97
10b–21–8(2)	A, K(8)	1.3	17	1.9	4.3
10b–21–8(3)	A, K(9)	1.9	25	1.5	8.9
10b–21–8a	A(2)	0.63	8.3	1.9	11
10b–22–8(2)	A, K(4)	2.1	24	2.3	5.2

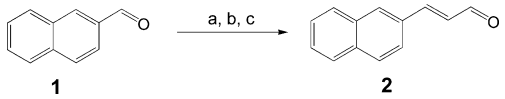
Table 1 (*continued*)

Substance	P.p.[b] (yield, %)	K_i (μM)			
		MC_1R	MC_3R	MC_4R	MC_5R
10b–22–8a	A(3)	1.1	23	1.9	15
10b–23–8(2)	A, K(10)	1.6	12	2.0	6.5
10b–23–8(3)	A, K(9)	1.5	20	1.6	3.9
10b–23–8a	A(2)	1.4	25	2.4	21
10b–24–8(2)	A, K(4)	2.5	25	2.6	7.2
10b–24–8a	A(1)	1.6	27	5.3	22
10b–25–8(2)	A, K(8)	1.8	52	3.0	83
10b–25–8a	A(1)	2.1	65	3.0	21
10b–28–8(1)	A, K(6)	1.2	19	3.2	4.9
10b–28–8(1,1)	A, K, L(2)	1.4	29	5.5	9.4
10b–28–8(1,2)	A, K, L(2)	1.0	21	3.3	7.8
10b–28–8(3)	A, K(7)	0.81	27	3.8	5.5
10b–36–8(1)	A, K(9)	0.96	17	2.0	8.7
10b–36–8(1,1)	A, K, L(2)	2.3	33	5.2	5.4
10b–36–8(1,2)	A, K, L(3)	1.4	31	5.6	4.9
10b–36–8(2)	A, K(8)	0.61	21	6.0	3.2
10b–36–14	A(17)	1.5	23	3.7	5.5
10b–36–14(1,1)	A, K, L(2)	1.9	nd[c]	4.7	nd[c]
10b–36–14(1,2)	A, K, L(3)	1.5	nd[c]	5.9	nd[c]
10b–36–14(2,1)	A, K, L(2)	0.41	nd[c]	3.4	nd[c]
10b–36–14(2,2)	A, K, L(2)	0.96	nd[c]	4.4	nd[c]
10b–36–14(3)	A, K(10)	1.9	22	3.5	6.7
10b–36–14(3,1)	A, K, L(3)	1.9	nd[c]	5.0	nd[c]
10b–36–14(3,2)	A, K, L(3)	1.3	nd[c]	4.0	nd[c]
10b-36-18	A(27)	4.2	12	4.8	12
10b-38–8	C(28)	4.5	278	12	44
10b–40–8(1)	A, K(7)	1.4	15	2.6	105
10c–36–8	A(25)	3.1	45	6.8	8.4
10d–35–8	B(8)	2.7	16	9.2	7.7
10d–40–8	B(12)	0.42	9.4	8.3	18
10e–18–8	F(6)	2.3	8.1	7.8	6.9
10e–18–8a	F(16)	2.9	5.4	7.4	9.1
11-0–1	A(24), G(50)	19	57	179	15
11–0–5	A(21), G(49)	14	65	149	17
11–0–8	A(29), G(55)	8.3	204	179	8.3
12–0–1	A(20), G(54)	35	228	150	28
12–0–5	A(27), G(52)	12	53	16	13
12–0–8	A(26), G(42)	1.9	7.5	6.0	6.1
13–0–1	A(26), G(60)	17	137	182	16
13–0–5	A(21), G(49)	12	50	116	21
13–0–8	A(24), G(40)	3.2	134	96	11
14a–36–8(1)	A(7), G(55), K(30)	2.7	13	3.4	27
14a–36–8(2)	A(10), G(43), K(45)	5.4	9.6	4.0	24
14a–40–8	A(25), G(46)	1.5	19	3.4	2.6
14b–35–8	A(20), G(49)	3.5	23	38	37
15–0–8	B(13)	1.5	17	7.7	6.4
15–40–8	B(22)	1.9	45	33	32
16–0–8	B(26)	4.0	40	11	17
16–35–8	B(27)	2.6	44	7.3	9.7
17–35–8	B(28)	12	62	116	555
18–36–8	A(24)	1.1	43	3.0	22
18–37–17	D(19)	8.0	17	14	4.4
18–40–8	A(22)	6.8	34	6.7	9.2
18–40–8(1)	A, K(8)	1.8	18	3.7	5.4
19–36–8	A(16)	3.9	62	6.6	64
19–37–10(1)	D, K(8)	2.8	19	14	2.1
19–37–10(2)	D, K(11)	2.2	10	4.8	1.2
19–40–8	A(26)	1.0	16	5.2	5.2
19–40–8(1)	A, K(7)	5.0	54	4.5	20
19–40–8(2)	A, K(13)	3.2	31	4.7	25
19–40–8(3)	A, K(6)	2.6	37	2.7	14
20a–36–8(1)	A, K(5)	5.6	64	7.7	9.1
20a–36–8(2)	A, K(12)	2.2	13	3.1	4.8

Table 1 (*continued*)

Substance	P.p.[b] (yield, %)	K_i (μM)			
		MC_1R	MC_3R	MC_4R	MC_5R
20a–40–8(1)	A, K(4)	0.69	4.3	1.2	1.7
20a–40–8(2)	A, K(6)	0.87	22	3.2	2.2
20b–40–8(1)	A, K(3)	1.6	62	3.7	42
20b–40–8(2)	A, K(10)	1.7	6.6	3.7	6.9
20b–40–8(3)	A, K(4)	1.3	12	3.8	5.6
20b–40–8(4)	A, K(2)	1.3	31	2.1	5.0
21–36–8	A(16)	4.6	78	9.2	6.6
21–40–8	A(14)	6.4	52	16	22
22–40–8	E(27)	11	99	25	176
23–40–8	E(23)	1.8	31	11	52
24–36–8	A(18)	2.8	10	3.2	10
24–40–8	A(15)	4.3	50	8.4	40
25–36–8	A(16)	1.6	33	2.5	11
25–40–8	A(17)	2.5	27	4.4	18
26–40–8	E(25)	2.7	59	7.3	37
27–36–8	E(19)	3.1	45	18	16
27–40–8	E(6)	2.2	57	5.3	49
27–40–8a	E(1)	2.9	25	6.5	23
28–36–8	A(16)	2.8	29	2.1	10
28–40–8	A(24)	3.3	30	5.7	18
29–36–8	A(13)	1.5	25	3.7	9.1
29–40–8	A(28)	2.7	24	3.6	8.7

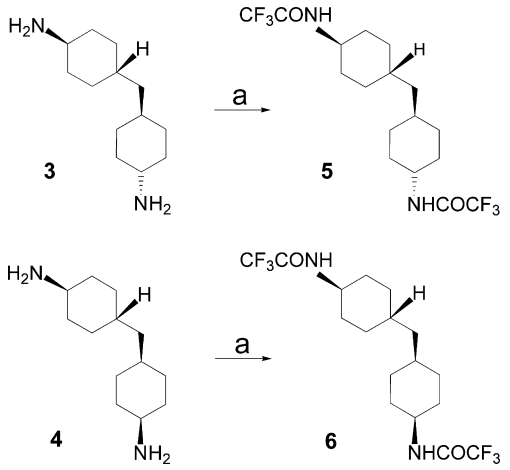
[a] All values represent means of at least two experiments, and difference between measurements for the same sample did not exceed 30%.
[b] Preparation procedure, see Section 5.
[c] Not determined.
[d] Not binding up to concentration 1 mM.



Scheme 1. Reagents and conditions: (a) CH_3CHO, KOH, MeOH, 0–5 °C, 2 h; (b) $(CH_3CO)_2O$, 100 °C, 30 min, (c) HCl, H_2O, 100 °C, 20 min.

homogeneity of 4,4′-diaminodicyclohexylmethane derivatives, the commercial product was crystallized from isooctane.[46] Most of the trans, trans isomer separated as crystals; after evaporation of solvent a mixture enriched with cis, trans and cis, cis forms was obtained from the mother liquor. Both pure trans, trans isomer and the enriched mixture were introduced in the tertiary amide synthesis.

More complicated operations were applied to introduce the pure cis, trans isomer of the above diamine (**4**, Scheme 2),[47] whose amino groups are not equivalent, in a selective way into the tripartide structure. In other words, our goal was that the central carbonyl group of the end product should be attached exclusively to, e.g., cis amino group of the diamine. First, prolonged reaction of *trans*,*trans*-4,4′-diaminodicyclohexylmethane (**3**) and its cis,trans isomer (**4**) with trifluoroacetic anhydride in trifluoroacetic acid solution gave the model substances *trans*,*trans*-bis-(4-trifluoroacetylamino-cyclohexyl)-methane (**5**) and its cis,trans isomer (**6**) (Scheme 2). The ^{1}H NMR spectra of both of these diacylated substances (Fig. 2A and B) in DMSO-d_6 solution



Scheme 2. Reagents and condition: (a) 50% $(CF_3CO)_2O$ in CF_3COOH, 40 °C, 7 days.

showed a doublet from the NHCO proton at 9.25 ppm, $J = 7.6$ or 7.9 Hz, and a multiplet from the neighbouring CH proton at 3.54 ppm. These almost identical signals could only be related to the trifluoroacetylated trans amino group present in both compounds. On the other hand, only the ^1H NMR spectrum of *cis*,*trans*-bis-(4-trifluoroacetylamino-cyclohexyl)-methane (**6**, Fig. 2B) displayed a doublet from the NHCO proton at 9.09 ppm, $J = 7.3$ Hz, and a multiplet from the neighbouring CH proton at 3.72 ppm. These signals could be related only to the trifluoroacetylated cis-amino group, uniquely present in **6**. The same signals could be observed also in the NMR spectra of monoacylated products (Fig. 2C and D), which allowed us to identify these substances, also in their mixtures.

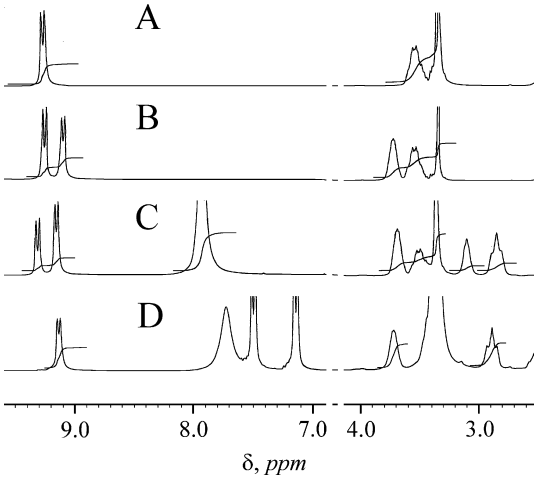
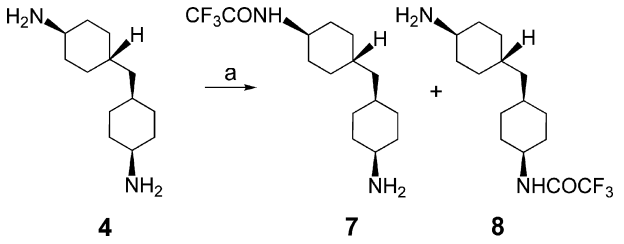


Figure 2. NMR spectra of *N*-trifluoroacetylated 4, 4′-diaminodicyclohexylmethane in DMSO-d_6. (A) *trans*,*trans*-Bis-(4-trifluoroacetylamino-cyclohexyl)-methane (**5**). (B) *cis*,*trans*-Bis-(4-trifluoroacetylamino-cyclohexyl)-methane (**6**). (C) Mixture of trifluoroacetate salts of *N*-[*trans*-4-(*cis*-4-amino-cyclohexylmethyl)-cyclohexyl] trifluoroacetamide (**7**) and *N*-[*cis*-4-(*trans*-4-amino-cyclohexylmethyl)-cyclohexyl] trifluoroacetamide (**8**). (D) *N*-[*cis*-4-(*trans*-4-Amino-cyclohexylmethyl)-cyclohexyl] trifluoroacetamide (**8**) 4-toluenesulfonate salt.



Scheme 3. Reagents and condition: (a) 40% $(CF_3CO)_2O$ in CF_3COOH, 40 °C, 48 h.

Partial trifluoroacetylation of **4** was performed using trifluoroacetic anhydride in trifluoroacetic acid solution using a short reaction time. All three expected products—two monosubstituted (**7** and **8**; Scheme 3) and one disubstituted (**6**, Scheme 2)—are formed. Reaction at the cis-amino group proceeded slightly easier, resulting in a mixture of trifluoroacetate salts of products **8** and **7** in the proportion 3:2. Further, the trifluoroacetate ion was exchanged with tosylate and several recrystallizations from methanol–acetonitrile allowed us to isolate the main isomer—*N*-[*cis*-4-(trans-4-amino-cyclohexylmethyl)-cyclohexyl]trifluoroacetamide (**8**) in pure form.

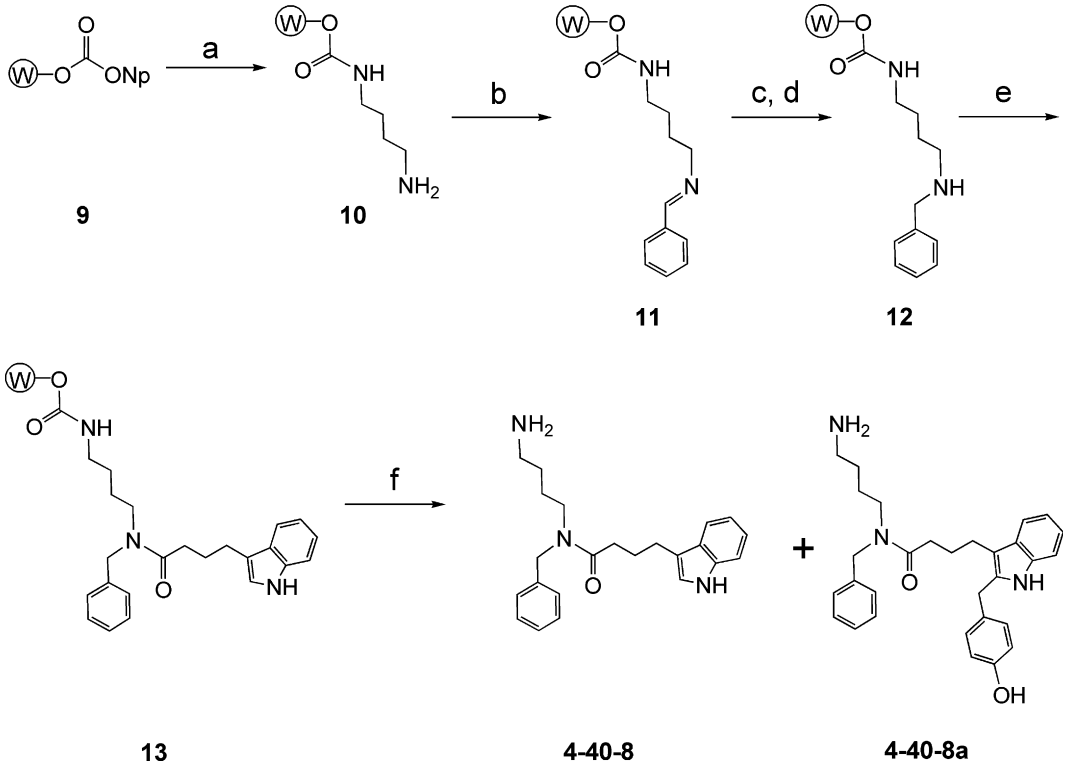
To fractionate 4,4′-methylene-bis-(2-methylcyclohexylamine), the commercial substance was kept at +5 °C causing crystallization. A small part of product remained liquid. This was used for synthesis of a tripartide (**20b**–**40**–**8**).

2.2.2. Solid phase synthesis. The main process for preparation of compounds on solid phase is illustrated in Scheme 4 for the preparation of compounds **4**–**40**–**8** and **4**–**40**–**8a**.

The process started with activated carboxylated polymer—4-nitrophenyl carbonate Wang resin (**9**) or its imidazolecarbonyl analogue. It was reacted with excess diamine in DMF yielding diamine, which was partially protected with the polymer attached substituted benzyloxycarbonyl group (**10**). In some cases we used commercially available diamines attached to trityl resin (Novabiochem).

The polymer attached partially protected diamine (**10**) was further introduced into reaction[48] with aldehydes or ketones dissolved in trimethyl orthoformate in order to obtain Schiff base (**11**). Aldehydes reacted better, whereas the reactions with ketones were generally far from complete. When diamines had been attached to the trityl resin, ketones did not react at all. In order to get better yields with ketones the reactions were carried out for several days at 30 °C in the presence of acetic acid (10%). Attempts to form Schiff bases from polymer attached derivatives of cyclohexylamine in reaction with ketones essentially failed, obviously due to steric hindrance. Use of trityl polymer and branched diamines also gave lower yields if sterically hindered structures were to be synthesized.

Schiff bases formed were then further reduced with sodium cyanoborohydride in 4% acetic acid/trimethyl



Scheme 4. Reagents and conditions: (a) tetramethylenediamine, DMF, rt, 24 h; (b) benzaldehyde, trimethyl orthoformate, rt, 20 h; (c) $NaCNBH_3$, 4% AcOH in trimethyl orthoformate, rt, 5 min; (d) diisopropylethylamine, CH_2Cl_2, rt, 10 min; (e) 3-indolebutyric acid, PyBroP, diisopropylethylamine, CH_2Cl_2, rt, 20 h; (f) trifluoroacetic acid–1,2-ethanedithiol–triisopropylsilane–water (10:2:0.25:0.25), rt, 1 h.

orthoformate,[49] which gave polymer attached secondary amines (**12**). We found that yields increased dramatically if we added the solvent–acetic acid mixture rapidly to a dry resin that had been carefully mixed with solid sodium cyanoborohydride using a spatula. Obviously the intensive and rapid reduction avoided the accumulation of intermediates that otherwise resulted in double alkylations. Slow reactions gave mainly side products.[50]

Acetate ions, which could have caused acetylation at the next step, were removed from **12** by treatment of resin with diisopropylethylamine. The polymeric secondary amine (**12**) was then introduced into reaction with carboxylic acid in methylene chloride (a small amount of DMF was added if necessary, in order to dissolve the carboxylic acid) in the presence of PyBroP[51] and diisopropylethylamine forming resin bound tertiary amide (**13**). The acylation usually proceeded well. Even the sterically hindered indomethacin could be introduced as the carboxylic component using this procedure.

Next, **13** was treated with trifluoroacetic acid in combination with 1,2-ethanedithiol (20%),[52] triisopropylsilane and water, which gave tertiary amides like **4**–**40**–**8**. Cleavage of the tertiary amides coupled to trityl resin was accomplished by using a mixture of TFA–1,2-ethanedithiol–methylene chloride (1:1:20).

Preliminary identification of substances present in the raw products was made by LC/MS. On preparing indole derivatives on Wang resin (a typical situation in this study), we observed formation of side products whose molecular masses were increased by 106 Da. As shown in our detailed NMR investigation,[53] these substances could be identified as 4-hydroxybenzyl derivatives, where an aralkyl group formed by fragmentation of the linker is introduced at position 2 of the indole residue (like in **4**–**40**–**8a**, Scheme 4). Yields of these side products reached up to 25% of the yield of the main product, even when the cleavage from the polymer was performed in presence of 1,2-ethanedithiol. Cleaving without this scavanger gave alkylated derivatives as the main products. 4-Hydroxybenzyl derivatives were investigated as potential melanocortin receptor ligands as well.

After cleavage raw end-products were isolated by filtration, partial evaporation of filtrates and precipitation with a mixture of dry ether and hexane. Along with abovementioned 4-hydroxybenzyl derivatives other side products were often present. Examples of such side products are shown in Figure 3. Thus, **14** was the result of an incomplete Schiff base formation during the synthesis of **4**–**26**–**9**; **6**–**37**–**19** formed by cleavage of benzyl ether in the carboxylic acid part during the final deprotection step in the attempt to synthesize **6**–**37**–**18**; **6**–**41**–**19** formed during the synthesis of the cinnamaldehyde derivative **6**–**37**–**18** due to double bond reduction. Fortunately, α-halogenated cinnamaldehyde derivatives were stable to double bond reduction in our process and were preferred for the purpose of the present investigations. One more side product, namely an unidentified dimer, turned out to be the main outcome in the preparation of tertiary amides derived from *trans*-3-indoleacrylic acid.

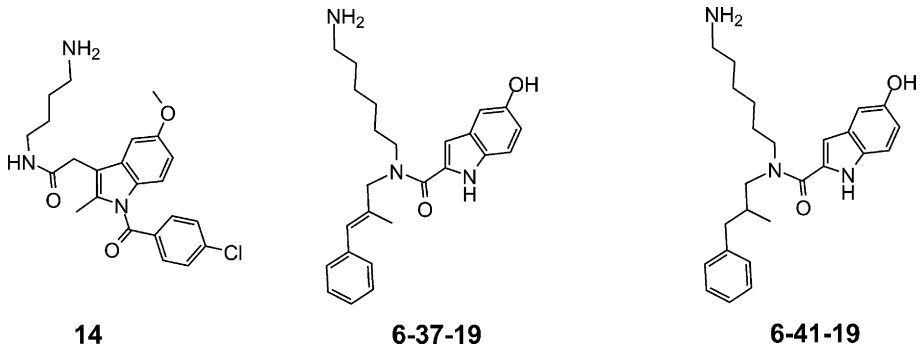


Figure 3. Side products of tertiary amide synthesis corresponding to the following processes: **14**, incomplete Schiff base formation; **6**–**37**–**19**, fragmentation at trifluoroacetic acid treatment, **3**–**15**–**1**, reduction of C=C bond.

When amino acids were brought in as carboxylic components, they were introduced as Fmoc-derivatives (preparation of **18**–**37**–**17** is shown on Scheme 5, as an example). Solid phase synthesis then included an additional step, namely elimination of the Fmoc group from polymeric intermediate **14** using piperidine solution in DMF, forming intermediate **15**.

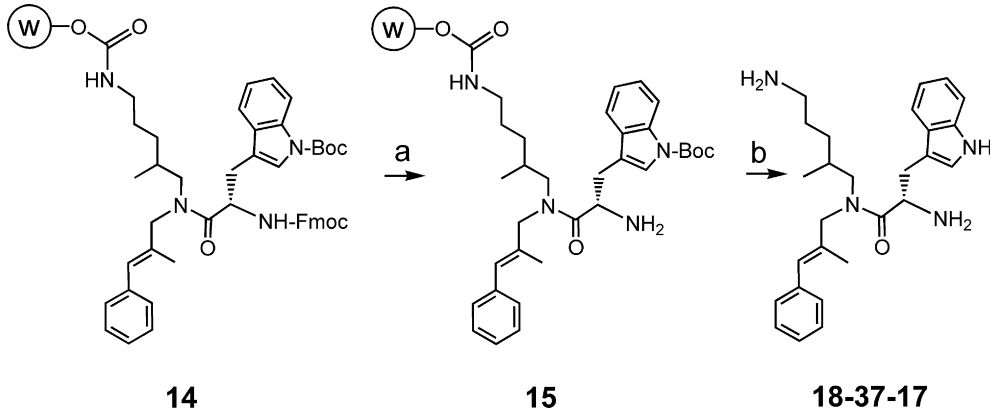
A particular case constituted the preparation of tertiary amides containing two diamine residues connected through a carbonyl group (Scheme 6 shows synthesis of one of them, **27**–**40**–**8**, as a typical example). First, activated carboxylated polymer reacted with one of the diamines. The polymeric product **16** formed was then introduced into reaction with 4-nitrophenylchloroformate yielding activated intermediate **17**. The latter reacted with the second diamine giving the polymeric urea derivative **18**, which was then introduced into reductive amination, acylation and cleavage steps in the usual way.

Another specific case was the introduction of a *cis*,trans-4,4′-diaminodicyclohexylmethane residue in a regioselective way (Scheme 7). *N*-[*cis*-4-(*trans*-4-aminocyclohexylmethyl)-cyclohexyl] trifluoroacetamide (**8**) was reacted with 4-nitrophenylcarbonate Wang resin (**9**). Polymeric diamine derivative **19** formed was deprotected using $NaBH_4$,[54] giving a product carrying a free amino group **20**. The following synthesis was performed in the usual way yielding tertiary amides **10e**–**18**–**8** and **10e**–**18**–**8a**.

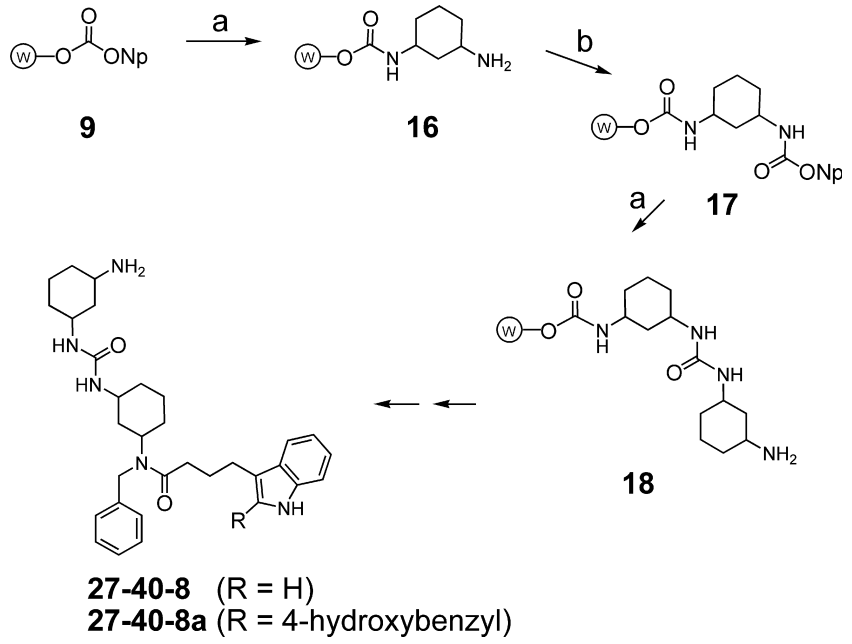
2.2.3. Solution synthesis. Some of the tertiary amides were subjected to guanidation in DMF using 1*H*-pyrazole 1-carboxamidine,[55] which gave guanidinoderivatives (like **12**–**0**–**8**, Scheme 8). The reaction was carried out in a concentrated DMF solution. This allowed us to apply the reaction mixture (after dilution with water) directly to the preparative reversed phase HPLC.

Tertiary amide **1**–**39**–**8** was prepared in solution (Scheme 9). The Schiff base from 4-diethylaminobenzaldehyde (**21**) and pentylamine was formed first. Subsequent reduction with $NaCNBH_3$ gave the corresponding secondary amine (**22**). Acylation with 3-indolebutyric acid and purification by HPLC finished the synthesis.

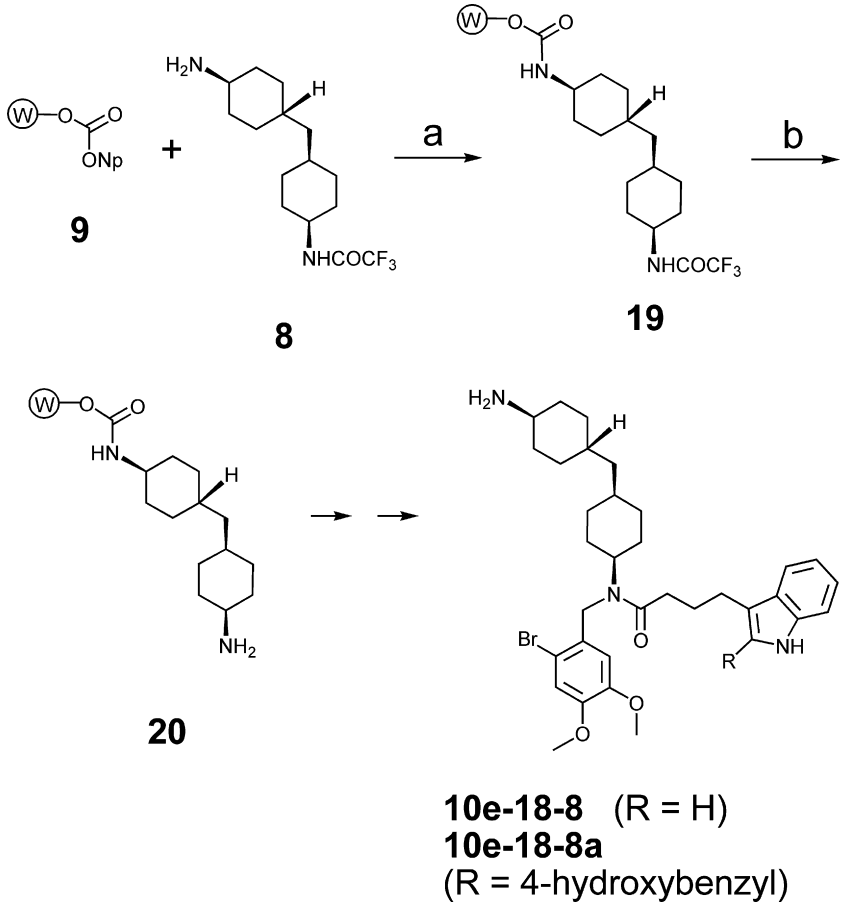
Solution synthesis proved to be very useful when ketones needed to be introduced into the preparation process of the tertiary amides. Thus, to prepare **3**–**38**–**5** (Scheme 10), β-tetralone (**23**) (dissolved in CH_2Cl_2) was reacted with 1,3-propylenediamine in the presence of $TiCl_4$.[56] Without isolating the Schiff base formed, $NaCNBH_3$ was added, and the diamine (**24**) formed was separated by extractions. The CH_2Cl_2 solution



Scheme 5. Reagents and conditions: (a) 20% piperidine in DMF, rt, 10 min; (b) trifluoroacetic acid–1,2-ethanedithiol–triisopropylsilane–water (10:2:0.25:0.25), rt, 1 h.

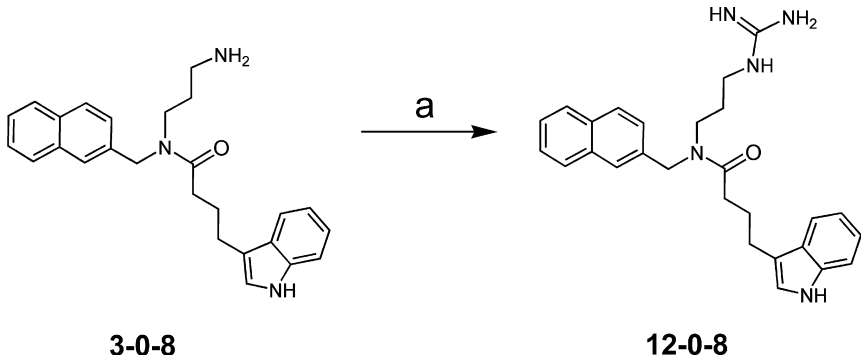


Scheme 6. Reagents and conditions: (a) 1,3-diaminocyclohexane, DMF, rt, 24 h; (b) 4-nitrophenylchloroformate, DIEA, DMF, rt, 24 h.

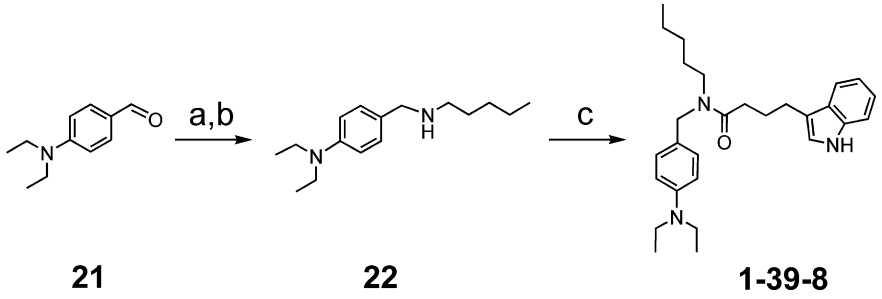


Scheme 7. Reagents and conditions: (a) DIEA, DMF, rt, 60 h; (b) $NaBH_4$, *n*-BuOH, rt, 49 h; 50 °C, 3 h.

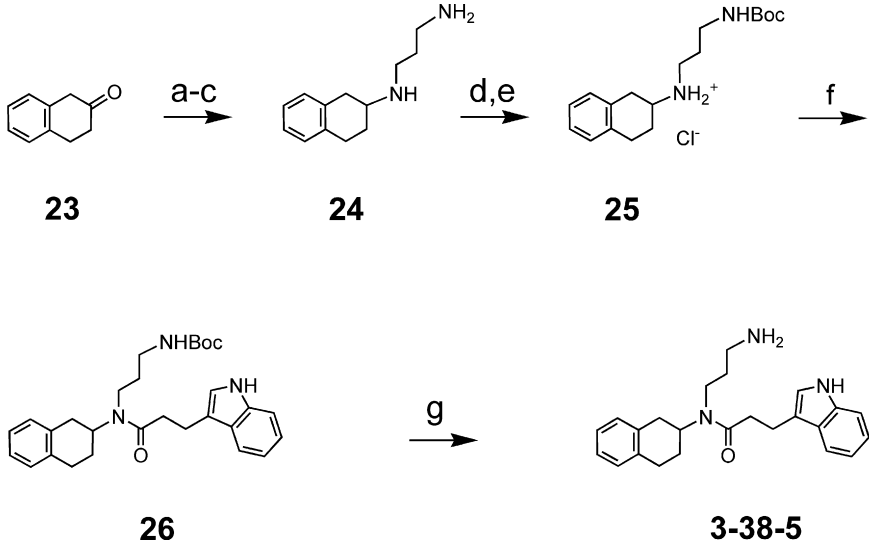
containing **24** was treated with di-*tert*-butyl dicarbonate, giving a solution containing the selectively protected diamine **25**. This compound was purified by silica gel chromatography and precipitated as hydrochloride. Further, acylation with 3-indolepropionic acid in the presence of PyBroP was carried out. The product,



Scheme 8. Reagents and condition: (a) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIEA, DMF, rt, 24 h.



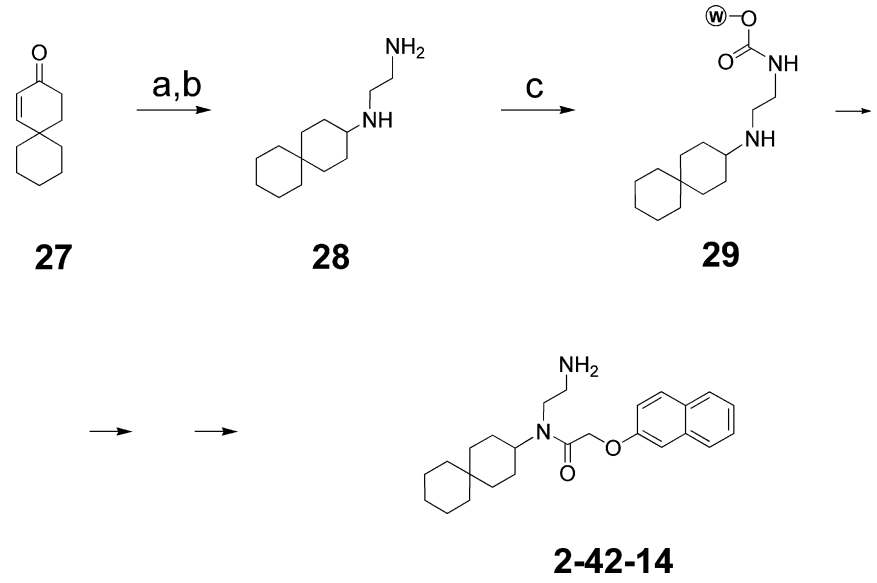
Scheme 9. Reagents and conditions: (a) pentylamine, trimethyl orthoformate, rt, 3 days; (b) $NaCNBH_3$, 0–5 °C, 2 h; (c) 3-indolebutyric acid, PyBroP, diisopropylethylamine, CH_2Cl_2, rt, 1 h.



Scheme 10. Reagents and conditions: (a) 1,3-propylenediamine, $TiCl_4$, CH_2Cl_2, Ar atmosphere, 0–20 °C, 20 h; (b) $NaCNBH_3$, MeOH, 0–20 °C, 45 min; (c) NaOH, water; (d) di-*tert*-butyl dicarbonate, CH_2Cl_2, rt, 24 h; (e) 4M HCl/dioxane, ether; (f) 3-indolepropionic acid, PyBroP, DIEA, CH_2Cl_2, rt, 24 h; (g) CF_3COOH—CH_2Cl_2 (1:8), rt, 15 min.

Boc-protected tertiary amide **26**, was cleaved with CF_3COOH—CH_2Cl_2 yielding the desired **3**–**38**–**5**.

A combination of solution and solid phase synthesis was applied to prepare **2**–**42**–**14**, a derivative of spiro[5.5]undecane (Scheme 11). Like in the previous example, spiro[5.5]undec-1-en-3-one[57] (**27**) was introduced into reaction with ethylenediamine in the presence of $TiCl_4$. During the following reduction of the Schiff base formed, hydrogenation of carbon—carbon double bond took also place, resulting in diamine **28**. This compound was further coupled with activated carboxylated Wang polymer. The reaction proceeded at the primary amino group forming intermediate **29**. Finally, acylation with 2-naphthoxyacetic acid and deprotection led to tertiary amide **2**–**42**–**14**. Similarly, using spiro[5.5]undec-1,8-diene-3-one[57] as a ketone, end-product **5**–**43**–**14** was obtained. Remarkably, double bond in position 8 (not conjugated with carbonyl group) was stable to $NaCNBH_3$ procedure.



Scheme 11. Reagents and conditions: (a) ethylenediamine, $TiCl_4$, CH_2Cl_2, Ar atmosphere, 0–20 °C, 20 h; (b) $NaCNBH_3$, MeOH, 0–20 °C, 45 min; (c) 4-nitrophenyl carbonate Wang resin, rt, 24 h.

2.2.4. HPLC procedures. All end-products were first purified by semipreparative HPLC on reversed phase in acetonitrile–water–trifluoroacetic acid (0.1%). Eluted fractions containing individual substances or their isomer mixtures were freeze-dried. In some cases, further separations were performed on chiral columns. (We tried both Chirobiotic T and Chirobiotic V and found that the first one separated tertiary amides slightly better.) An example for this is shown in Figure 4, for the separation of isomers of the 4,4′-methylene-bis-(2-methylcyclohexylamine) derivative **20b–40–8**. Thus, separation of isomers could be achieved already at the preparative reversed phase HPLC step by dividing the eluate corresponding to main product peak into several parts (Fig. 4A). The separation was verified by the Chirobiotic T column chromatography (Fig. 4B). Isomerism of substances was confirmed from their identical UV spectra of the chiral chromatography peaks [UV spectral data could be collected directly online using the HPLC photodiode array detector (Fig. 4C)], and from the LC/MS data showing the same molecular ions and fragmentation patterns. In selected cases Chirobiotic V preparative separations of the tertiary amides were performed in order to improve the isomeric homogeneity of the substances before investigating them for their activity on melanocortin receptors. The exact structures of tertiary amides obtained by the use of commercial mixtures of diamines or/and unsymmetrical diamines followed by separation by chromatography were not established.

Overall yield of purified tertiary amides was not high. However, quantitative determination of tertiary amides in the raw products using HPLC showed that the actual yields of them were about 1.5–2 times higher. Obviously, substance losses occurred due to irreversible adsorption on the reversed phase during semipreparative HPLC. Nevertheless, the amounts of compounds obtained were sufficient for their pharmacological characterizations.

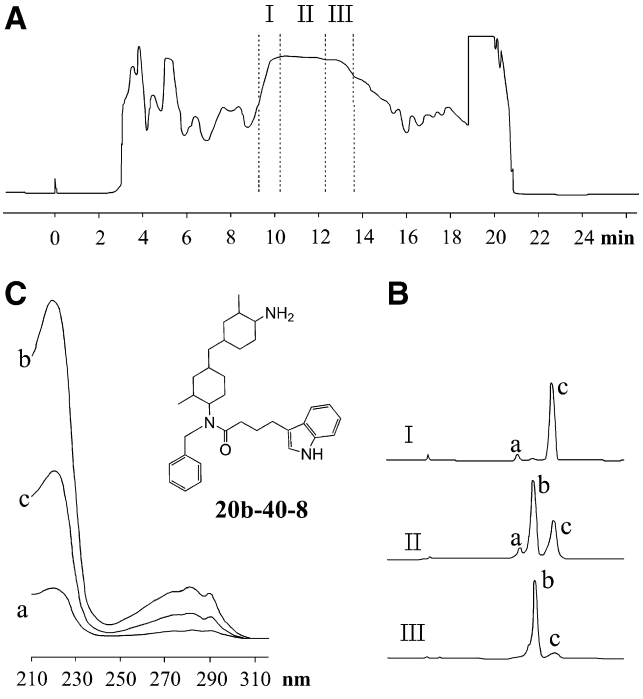


Figure 4. Chromatography of **20b–40–8**. (A) Preparative HPLC run of a raw product (25 mg). Vydac RP C_{18} column (10 × 250 mm, 90 Å, 201HS1010), eluent–30% MeCN in water + 0.1% TFA, flow rate 5 mL/min, detection at 280 nM. Eluate fractions I–III were lyophilized to give 1.8 mg **20b–40–8(1)**, 5.1 mg **20b–40–8(2)** and 2.2 mg **20b–40–8(3)** correspondingly. (B) Analytical chiral HPLC of **20b–40–8(1)** (I), **20b–40–8(2)** (II) and **20b–40–8(3)** (III) on a Chirobiotic T column (4.6 × 250 mm). Eluent–MeOH–AcOH–triethylamine (100:0.1:0.1), flow rate 1 mL/min, detection at 220 nm. Analysis time 20 min. (C) UV spectra of **20b–40–8(2)** components (obtained at chiral HPLC using a photodiode array detector).

2.3. Pharmacology

The 210 tertiary amide samples obtained were tested on recombinant human MC receptors in radioligand

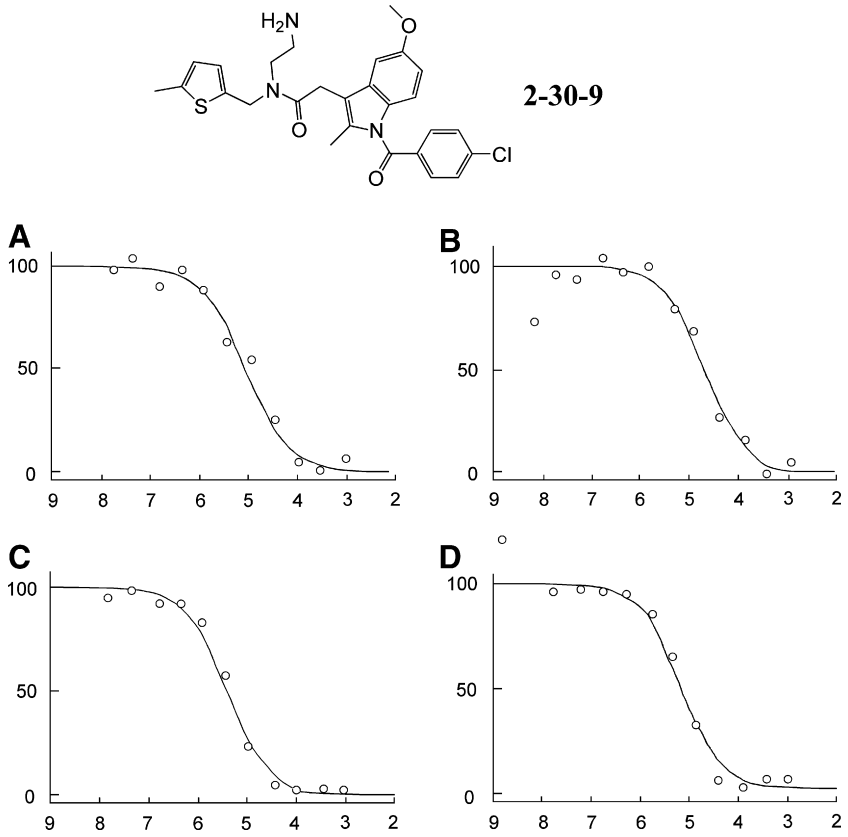


Figure 5. Competition binding curves for **2**–**30**–**9** (negative logarithm of ligand concentration versus percent of receptor bound [^{125}I]-NDP–MSH). (A) MC_1R, K_i 2575 nM. (B) MC_3R, K_i 12019 nM. (C) MC_4R, K_i 3443 nM. (D) MC_5R, K_i 5649 nM.

binding assays and most of them bound to all the MC_1, MC_3, MC_4 and MC_5 receptors evaluated in these tests (Fig. 5 and Table 1). For the most active compounds (structures shown in Fig. 6) the K_is reached 0.41 μM for **10b**–**36**–**14**(**2**,**1**) on the MC_1 receptor, 1.7 μM for **2**–**42**–**20** on the MC_3 receptor, 1.2 μM for **20a**–**40**–**8** (**1**) on the MC_4 receptor, and 0.93 μM for **3**–**37**–**0** on the MC_5 receptor. The compounds also showed varying selectivity patterns (Table 1). A few were uniquely selective for one of the receptor subtypes, namely the MC_1R. E.g., the binding affinities of **9**–**36**–**8** (Fig. 6), **2**–**38**–**13**, **3**–**38**–**1**, **8**–**35**–**5**, **10d**–**40**–**8** and **15**–**40**–**8** for the MC_1R exceeded those for the $MC_{3-5}Rs$ by 10-fold or more. The most MC_1R selective was **9**–**36**–**8** (Fig. 6). It showed 45-fold selectivity or better. The most non-selective was **5**–**40**–**17**, whose affinity was practically the same for all the evaluated MC receptor subtypes.

In order to determine if tertiary amides are agonists or antagonists on melanocortin receptors some of the title compounds (structures shown in Fig. 7) were tested for their ability to promote, respectively, block the accumulation of cAMP in cells expressing different MC receptor subtypes. The evaluated compounds had per se only negligible effects on cAMP in cells expressing either of the MC_1, MC_3, MC_4 and MC_5-receptor subtypes. [However, one of them (**12**–**0**–**5**) marginally stimulated cAMP in MC_1 and MC_4 receptor expressing cells at the highest concentration (100 μM) evaluated]. Control experiments where α-MSH was added showed the expected increase in cAMP for all the MC receptor assays.

In order to probe the possibility that the compounds are antagonists we investigated their ability to block the increase in the accumulation of cAMP caused by α-MSH. Results are shown graphically on Figure 8. Thus, at a concentration of 100 μM all the seven compounds caused clear inhibition of the increase in the cAMP levels caused by α-MSH; the latter being applied in concentrations up to 1 μM. The data are thus compatible with the notion that the tertiary amides are antagonists at melanocortin receptors.

3. Discussion

Our method for parallel synthesis of individual tertiary amides on solid phase made it convenient to prepare a large series of compounds. The raw product mixtures obtained could be well separated on reversed phase using acetonitrile–water–TFA as an eluent. The process allowed us to isolate title substances as well as side products. Freeze drying of eluates resulted mainly in amorphous powders that were convenient to handle. We found that the chiral phases Chirobiotic T and Chirobiotic V, with a standard methanol–acetic acid–triethylamine (100:0.1:0.1) eluent, were efficient to separate tertiary amide isomers. The chiral selector system was useful for preparative separations, as well.

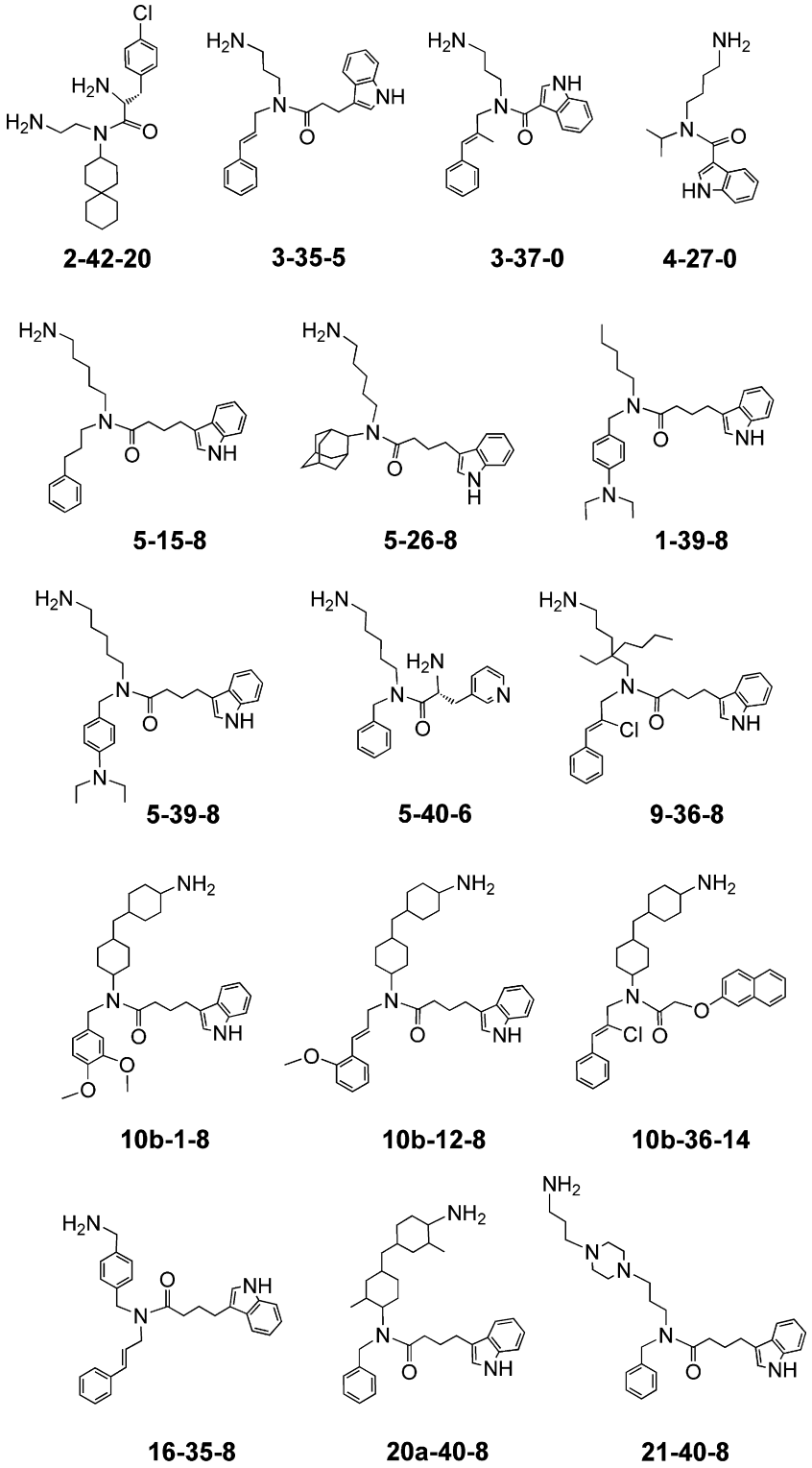


Figure 6. Selected tertiary amides' structures. Affinity (in μM, for MC_1R, MC_3R, MC_4R and MC_5R correspondingly): **2**–**42**–**20** (3.7, 1.7, 2.4, 3.2), **3**–**35**–**5** (21, 65, 81, 11), **3**–**37**–**0** (0.85, 24, 7.4, 0.93), **4**–**27**–**0** (106, 112, 222, 279), **5**–**15**–**8** (18, 61, not determined, 19), **5**–**26**–**8** (6.1, 25, 14, 46), **1**–**39**–**8** (72, 289, 78, 408), **5**–**39**–**8** (6.8, 33, 25, 34), **5**–**40**–**6** (not binding to all receptors), **9**–**36**–**8** (2.5, 131, 113, 391), **10b**–**1**–**8(2)** (3.2, 22, 3.7, 8.3), **10b**–**12**–**8**(2) (1.6, 31, 4.1, 5.0), **10b**–**36**–**14** (1.5, 23, 3.7, 5.5), **16**–**35**–**8** (2.6, 44, 7.3, 9.7), **20a**–**40**–**8(1)** (0.69, 4.3, 1.2, 1.7), **21**–**40**–**8** (6.4, 52, 16, 22).

From Table 1 it can be seen that despite considerable structural differences most of the tertiary amides show quite similar binding affinity for the MSH peptide binding MC receptors. Most of the compounds show a selectivity pattern: $MC_1R > MC_4R$, $MC_5R > MC_3R$. Looking at the structure–activity relationships we may see that the diamine part can be varied widely and still retaining MC receptor binding affinity. We thus introduced all oligomethylenediamines having from two to eight methylene groups, as well as diamines with

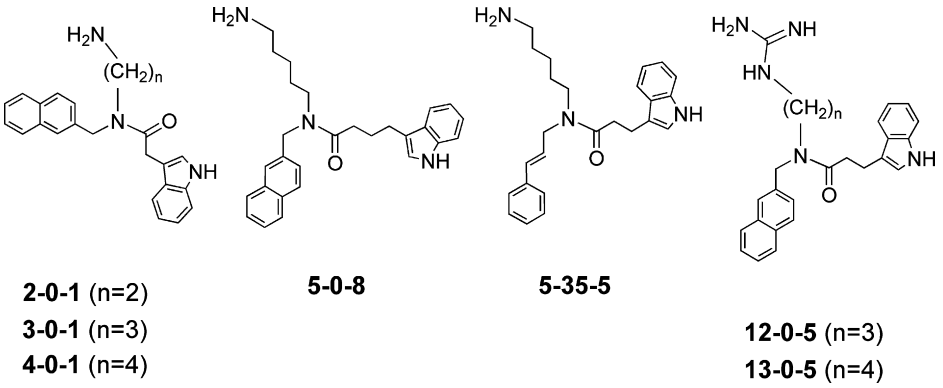


Figure 7. Structures of tertiary amides tested for functional activity.

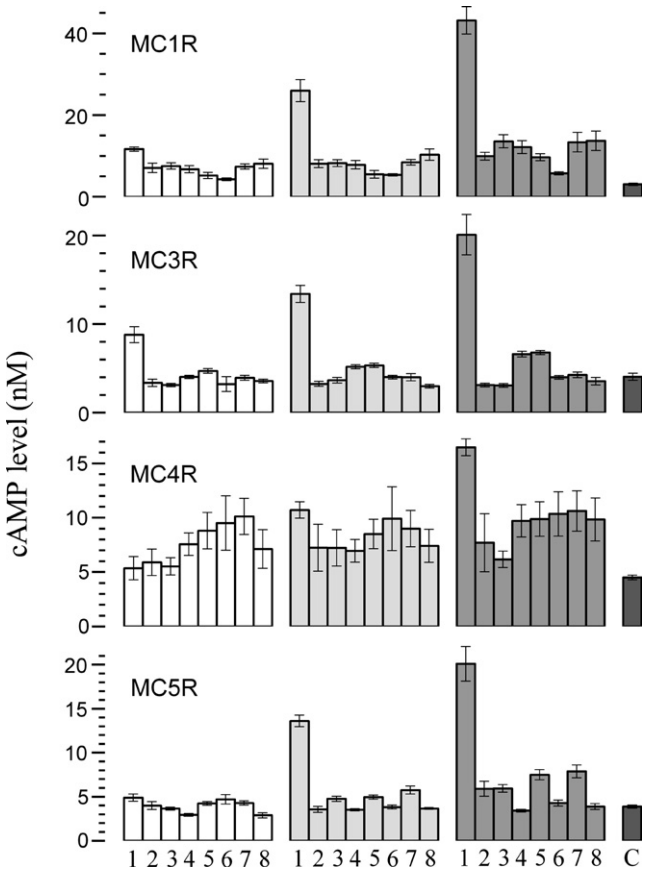


Figure 8. Effect of α-MSH on cAMP level in natural melanoma cells B16 (MC_1R) and COS-1 cells transfected with MC_3R, MC_4R, MC_5R DNA in absence and in presence of tertiary amides (100 μM). White bars, experiments performed at α-MSH concentration 0.1 nM. Light grey bars, experiments performed at α-MSH concentration 10 nM. Grey bars, experiments performed at α-MSH concentration 1 μM. 1, no tertiary amide; 2, **2–0–1**; 3, **3–0–1**; 4, **4–0–1**; 5, **5–0–8**; 6, **5–35–5**; 7, **12–0–5**; 8, **13–0–5**; C, cell control. Standard error of mean (SEM) is shown in each bar.

branched chains, like the ones in compounds **9–36–8** (Fig. 6), **18–36–8** and **19–40–8**. Besides, many derivatives of 4,4′-methylenebis(cyclohexylamine), like **10b–36–14** (Fig. 6), and derivatives of 4,4′-methylenebis(2-methylcyclohexylamine), like **20b–40–8** (Fig. 4), were prepared. Substances of this type showed relatively good affinities. We also prepared some substances where diamines containing a benzene ring were incorporated [i.e., substances **15–0–8**, **15–40–8**, **16–0–8** and **16–35–8** (Fig. 6)]. All of these compounds exhibited relatively good binding to the MC receptors. Only slightly reduced affinities were found for the triple charged derivatives of piperazine **21–36–8** and **21–40–8** (Fig. 6) obtained from 1,4-bis(3-aminopropyl)piperazine. Thus, even tertiary amides with two additional positive charges are tolerated by the MC receptors.

In order to determine the optimal number of methylene groups in oligomethylenediamine derivatives, we investigated series of compounds of the **X–40–8** type (Fig. 9), where the number of methylene groups was varied between two and seven. For benzaldehyde derivatives of the **X–40–8** type we found that, at least for the MC_1 and MC_4Rs a shorter chain of between two and four methylene groups was unfavourable. Moreover, in the range of 4–7 methylene groups an alternating pattern of affinities was observed as the compounds with odd numbers of methylene groups (5 and 7) were more active than compounds with even numbers (4 and 6). (This alternating pattern was documented in repeated parallel binding experiments, where the substances were applied on the same assay plates). The finding might be explained in terms of a stretched conformation of the

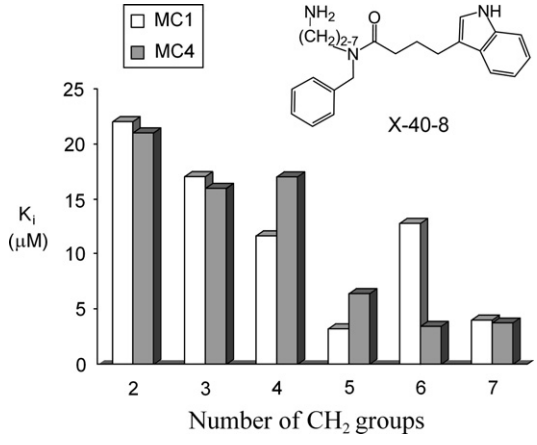


Figure 9. Affinity of compounds of type **X–40–8** on MC_1R and MC_4R as a function of the number of methylene groups.

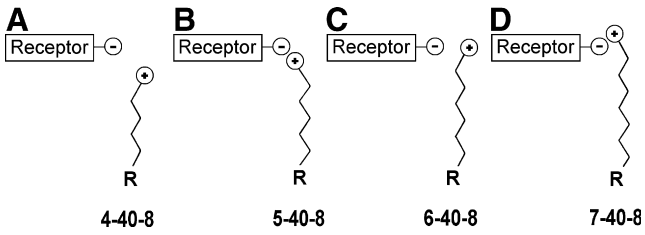


Figure 10. Geometry model explaining alternating affinity of compounds of type **X–40–8** on MC_1R. (A,C) Reduced complexation of positively charged ammonium group of tertiary amide with negatively charged receptor group for compounds whose diamine part contains four or six methylene groups (methylene chain turns ammonium group away from the receptor). (B,D) Improved complexation of compounds whose diamine part contains five or seven methylene groups. It is assumed that the remaining identical part of substances (**R**) is kept in position by other interactions with the receptor. Probably, methylene groups from diamine parts are also involved in such interactions.

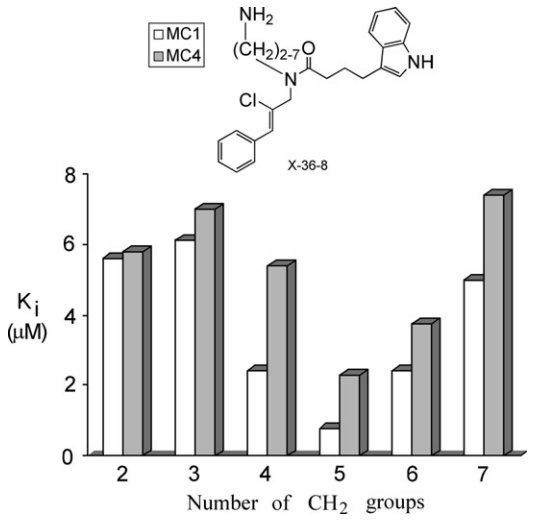


Figure 11. Affinity of compounds of type **X–36–8**, with varied number of methylene groups, on MC_1R and MC_4R.

methylene chain, which determines position of the amino group so that it is more favourable for binding to the MC_1R for compounds **5–40–8** and **7–40–8** (Fig. 10). However, binding of these substances to the MC_4R did not reveal the same alternating pattern. Instead, a higher activity was shown for substances containing between five and seven methylene groups in the diamine residue, while the ones including 2–4 groups showed lower activity (Fig. 9).

A similar study was made for derivatives of α-chlorocinnamaldehyde of the **X–36–8** type (Fig. 11). For this series the optimal number of methylene groups turned out to be five for both the MC_1R and MC_4R. Increasing the chain length further to six and seven methylene groups was unfavourable, which contrasted to the findings for the benzaldehyde derivatives (Fig. 9). The results can be explained on the basis that a too long distance between benzene ring and the amino group in **6–36–8** and **7–36–8** might not fit the receptor geometry well. On the other hand, α-chlorocinnamaldehyde derivatives **2–36–8**, **3–36–8** and **4–36–8**, which contain two to four methylene groups in the diamine part, were slightly (2- to 3-fold) better binders on the MC_1 and MC_4Rs than the corresponding benzaldehyde derivatives **2–40–8**, **3–40–8** and **4–40–8** (Figs. 9 and 10). Apparently a larger distance between the presumed pharmacophoric amino and phenyl groups (i.e., introduction of chloroethylene moiety) is preferred by these receptors.

These data supported the assumption that the benzene ring in the aldehyde part and the amino group from the diamine part are interacting with the receptors. In order to investigate this further we prepared two derivatives of 4-diethylaminobenzaldehyde: **5–39–8** and **1–39–8** (Fig. 6). These compounds differ only by the presence or absence of the primary amino group. Indeed, **1–39–8** showed a considerably reduced affinity for all the MC receptors, compared to **5–39–8**. Moreover, almost all other tertiary amides obtained in the present study showed higher activities than **1–39–8** (Table 1). Thus, the positively charged amino or guanidino group is a crucial function for the compounds' binding to MC receptors.

Guanidation of the amino group in tertiary amides did not generally improve the affinities. Thus, comparison of the affinity data for compounds **2–0–1**, **3–0–1**, **4–0–1** (structures shown in Fig. 7), **3–0–8**, **10d–35–8**, **10b–36–8** and **10b–40–8**, with the corresponding data for their guanidated analogues **11–0–1**, **12–0–1**, **13–0–1**, **12–0–8** (Scheme 8), **14b–35–8**, **14a–36–8** and **14a–40–8** (Table 1), revealed that guanidine derivatives exhibited better affinity in 10 cases, while in 17 cases they displayed lower affinity. This was quite unexpected if we assume that amino group of the tertiary amides mimics the guanidino group of arginine from the active core of the natural peptides.

The most common aldehyde components in our study were benzaldehyde, β-naphthaldehyde or α-chlorocinnamaldehyde. Changing the number of methylene groups between the benzene ring in the aldehyde part and the central amide nitrogen atom, as in compounds **3–40–8** (one methylene group), **3–11–8** (two methylene groups) and **5–15–8** (Fig. 6; three methylene groups), did not lead to any substantial change in binding affinities (Table 1). However, as pointed out above, introduction of a double bond in the aldehyde part improved the affinity slightly. For the series of substituted benzaldehyde derivatives **10b–1–8** (Fig. 6; 3,4-dimethoxy), **10b–2–8** (3-ethoxy-4-methoxy), **10b–3–8** (2-fluoro), **10b–4–8** (4-carbmethoxy), **10b–5–8** (4-methylthio), **10b–6–8** (3-methyl), **10b–7–8** (2-methyl), **10b–8–8** (4-methyl) and **10b–10–8** (2-bromo) no substantial differences in activity were seen.

Differently substituted cinnamaldehydes were also incorporated into tertiary amides as follows: 2-methoxycinnamaldehyde (**10b–12–8**, Figure 6), 2-methoxy-4-hydroxycinnamaldehyde (**10b–16–8**), α-methylcinnamaldehyde (**3–37–0**, and others), α-chlorocinnamaldehyde (**2–36–8**, and many others), α-bromocinnamaldehyde (**10b–28–8**) and 3-(2-naphthyl)-2-propenal (**5–34–8**). However, all the resulting tertiary amides exhibited

similar affinities. Heteroaromatic derivatives obtained from 2-pyridinaldehyde and 4-pyridinaldehyde **10b–13–8** and **10b–19–8** and 5-methyl-2-thiophenecarboxaldehyde **2–30–9** (Fig. 5) also showed about the same affinity (Table 1).

Ketone derivatives like **2–29–1** (product of 2-acetonaphthone), **3–9–8** (product of 2-acetylbenzothiophene), **5–26–8** (Fig. 6; product of 2-adamantanone), **3–38–5** (Scheme 10, product of β-tetralone), **2–17–8** (product of 2-methyl-3-acetyl-5-phenyl-thiophene), **5–31–1** (product of 4-trifluoromethyl-acetophenone) and **5–32–1** (product of cycloheptanone), which have reduced conformational freedom compared to aldehyde products, did not show any significant difference in binding affinity compared to aldehyde derivatives.

Derivatives of cyclic ketones, which contain bulky alicyclic residues instead of a benzene ring like in **5–32–1** (product of cycloheptanone) and **5–26–8** (Fig. 6, product of 2-adamantanone), displayed quite good activity in our tests with a preference for the MC_1R. Among the most active compounds, in particular for $MC_{3-5}Rs$, found were products of spiro[5.5]undec-1-en-3-one: **2–42–8**, **2–42–14** (Scheme 11) and **2–42–20** (Fig. 6), as well as spiro[5.5]undec-1,8-dien-3-one: **5–43–14**. The presence of an aromatic ring in the aldehyde or ketone part is thus not obligatory for the MC receptor binding as aliphatic groups interact with the receptor as well. However, it turned out that the acetone derivative **4–27–0** (Fig. 6) was considerably less active compared to most of the other investigated substances. Probably, this compact compound is not able to fill the receptor pocket efficiently and might therefore not interact well with receptor.

In order to imitate the tryptophan residue present in the natural MSH peptides' active core, we introduced, in most cases, indole derivatives in the tertiary amide tripartides' carboxylic acid part. For homologous 3-indole carboxylic acid derivatives changing the distance between the indole moiety and the central nitrogen atom [cf substances **2–0–0** and **4–0–0** (no methylene groups in carboxylic part), **2–0–1**, **3–0–1**, **4–0–1** (structures shown in Fig. 7), **5–0–1** (one methylene group in carboxylic acid part), **5–0–5** (two methylene groups in carboxylic acid part), **5–0–8** (three methylene groups in carboxylic acid part)] leads to compounds with similar affinities. Exceptions were compounds **2–0–1**, **3–0–1** and **5–0–5**, which showed a lower affinity on the MC_1R. Similar affinities were also found for the series **3–38–0**, **3–38–1**, **3–38–15** (Scheme 10) (β-tetralone derivatives) and **3–35–0**, **3–35–1**, **3–35–5** (Fig. 6) (cinnamaldehyde derivatives). Other indole derivatives investigated included 3-indolylglyoxalic acid, 3-indolylacrylic acid, 5-benzyloxyindol-2-carbonic acid, D- and L-tryptophan and indomethacine residues. Besides indole derivatives, other carboxylic acids such as 2-naphthoxyacetic acid, 4-(2-thienyl)butyric acid and L-histidine could be used to prepare MC receptor active compounds (Table 1).

Surprising data were obtained when we introduced unnatural amino acids. Compound **5–40–6** (Fig. 6), which contains a D-3-pyridinalanine residue, thus demonstrated a total absence of activity. In other words, introduction of a pyridine ring in the carboxylic acid part completely destroyed the tertiary amide binding. When other amino acids were used as the carboxylic component the resulting compounds did not exhibit any particular anomalies. E.g., naphthalene derivatives, like **5–40–2**, bound well to the MC receptors. The reduced affinity of derivatives of D- and L-tryptophan when introduced as the carboxylic part in tertiary amides (i.e., derivatives **5–40–10**, **5–40–17** and **18–37–17**, Scheme 5) was surprising also as this part could be considered as an imitation of the tryptophan in the natural melanocortin peptides. The highest activity from this 'amino acid' series was shown by the D-biphenylalanine derivative **5–40–7**.

Another observation was that affinities seem to increase with increasing size and hydrophobicity of the tertiary amide molecule. Thus, e.g., good MC receptor binding was found for the bulky adamantane derivative **5–26–8** (Fig. 6), as well as for the even more bulky and hydrophobic adamantane/indomethacine derivative **4–26–9**. On the other hand, e.g., the small molecule derivative of acetone and indole-3-carboxylic acid, **4–27–0** (Fig. 6), showed a markedly reduced binding for all the four MC receptor subtypes evaluated herein.

Comparison of binding affinities of solid phase synthesis side products, 4-hydroxybenzyl derivatives, with those of the corresponding main products (Table 1) gave results as follows: In 33 cases the affinity of the main products was better, but in 34 cases the affinity was better for the side product. For eight cases main and side products showed essentially the same affinities. However, the differences between the affinity values were quite small. Thus, in general the introduction of the 4-hydroxybenzyl group did not influence much the binding affinity of the tertiary amides; thus this group does not interact with the receptors and it also does not hinder the binding.

In the present study, we evaluated seven tertiary amides, **12–0–5**, **13–0–5**, **2–0–1**, **3–0–1**, **4–0–1**, **5–0–8** and **5–35–5** (structures shown in Fig. 7), for their agonist/antagonist properties by assaying cAMP in MC_1R, MC_3R, MC_4R and MC_5R expressing cells. As all of these compounds were capable of inhibiting the increase in the levels of cAMP induced by α-MSH (Fig. 8), it appears that our tertiary amides are antagonists at melanocortin responsive MC receptors [However, one of the compounds, **12–0–5**, induced a marginal activation of cAMP release at the highest concentration (100 μM) applied.].

4. Conclusions

The tertiary amides reported herein were conveniently prepared, allowing us to obtain a diverse library of peptidomimetics. Remarkable enough almost all of the 210 tertiary amides samples obtained showed moderate binding affinity to all of the MSH binding MC receptors (MC_1, MC_{3-5}). Seven title compounds were tested for

their agonistic/antagonistic properties and the data showed that they were antagonists on all the four MSH binding MC receptors. The structure–affinity relationships derived from the data indicate that hydrophobic structures are advantageous. We found also that bigger molecules are better binders than smaller ones. These data are in agreement with our earlier modelling results,[8] which indicated that hydrophobic interactions dominate the binding of ligands to melanocortin receptors. From our data, the participation of the amino or guanidino group in complexation of the peptidomimetics with the receptor is also evident. Some optimization of structures leading to increased affinity could be obtained by systematically changing the number of methylene groups in the compounds' diamine part. The data obtained for the present series of compounds may find use in the further development of melanocortin receptor subtype active compounds.

From our experience, it remains unclear whether really high affinity and selectivity compounds are attainable relying solely on the concept of the correct placement of three pharmacophoric groups from melanocortins' active core. However, taking into account the moderate affinity of large number high diversity compounds, which we obtained, it seems rather that the answer is negative. In other words, more attachment points between ligand and receptor are necessary. Essential elements of binding can be, e.g., neighbouring histidine residue and peptide bonds, present in the active core. Such idea is supported by the high affinity reported for slightly modified active core tetrapeptide sequences.[58]

Despite variable success in getting valuable properties, simulations of the features of the melanocortins' active core continue to emerge in the newest scientific and patent literature. In particular, diketopiperazine template was connected with 2-naphthylmethyl, biphenylmethyl and tetramethyleneamino groups, thus imitating Phe-Arg-Trp tripeptide.[59] Similarly, dipeptide from L-arginine and trans-3-benzyl-L-proline, capped with 1-naphthylacetyl and benzylamino groups, was prepared.[60] Remarkably, this substance exhibited a high and selective MC_4R affinity. Moreover, dipeptide from THIQ[26] was connected to a bicyclic template, the latter was coupled also with trimethylene-guanidine and methyleneoxy-2-naphthalene groups (associations with arginine and tryptophan from melanocortins' active core).[61] Analogous approach, using piperazine as the central template and functions resembling natural peptides and THIQ, was disclosed in another application,[62] and utilization of diphenyloxide structure in related case was investigated as well.[63]

5. Experimental

5.1. Chemistry

Reagents were used without purification. Unless otherwise noted, they were purchased from Aldrich, Fluka, Merck or Lancaster. DMF was purchased from Perkin-Elmer Biosystems. Melting points are uncorrected. Solid phase synthesis was performed on a Nautilus 2400 synthesiser (Argonaut Technologies). Exact molecular masses were determined on a Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. LC/MS was performed on a Perkin-Elmer PE SCIEX API 150EX instrument with a Turboionspray Ion Source (*m*/*z* obtained are labelled below as TISMS data) and Dr. Maisch Reprosil-Pur C18-AQ, 5 μ, 150 × 3 mm HPLC column using a gradient formed from water and acetonitrile with 5 mM ammonium acetate additive. ^{1}H NMR spectra were recorded on a Jeol JNM-EX270 spectrometer or a Varian Mercury-400 spectrometer, using DMSO-d_6 (2.50 ppm downfield from TMS) as an internal standard. Analytical and micro-preparative HPLC was performed on a Waters system equipped with Millenium-32 Workstation, 2690 Separation Module, 996 Photodiode Array Detector and Waters Fraction Collector. Semi-preparative HPLC was carried out on a LKB system consisting of a 2150 HPLC Pump, 2152 LC Controller and 2151 Variable Wavelength Monitor and Vydac RP C_{18} column (10 × 250 mm, 90 Å, 201HS1010), the eluent being an appropriate concentration of MeCN in water + 0.1% TFA, flow rate 5 mL/min, detection at 280 nM. Freeze-drying was performed at 0.1 bar on a Lyovac GT2 Freeze-Dryer (Finn-Aqua) equipped with a Busch 010-112 vacuum pump and a liquid nitrogen trap.

Tertiary amides were prepared according to type procedures A–L; see also Table 1.

5.1.1. 3-(2-Naphthyl)-2-propenal (2). To 2-naphthaldehyde (3.12 g, 19 mmol) in a flask cooled in a salt-ice bath acetaldehyde (24 mL) was added with stirring. A clear solution formed, to which potassium hydroxide (0.26 g, 4.6 mmol) dissolved in methanol (1.3 mL) was added dropwise, keeping the temperature of the reaction mixture at 0–5 °C. Stirring was continued for 2 h. At the end of the period the temperature was allowed to rise until it reached room temperature. Acetic anhydride (10 mL) was then added and the mixture was heated to 100 °C for 30 min. The reaction mixture was then poured into a flask containing water (72 mL) and concentrated hydrochloric acid (9.6 mL) was added, heated to 100 °C and stirred for 20 min. The reaction mixture was then cooled to 0 °C and the precipitate formed was filtered off, washed with water, dried under vacuum in the presence of P_2O_5 and KOH, and crystallized from heptane. Yield 2.2 g (64%). Mp 118–119 °C. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 9.75 and 9.72 (1H, 2s, CHO), 7.66–7.54 and 8.05–7.84 (8H, 2m, naphthyl), 6.99 (1H, dd, J = 16.0, 7.9 Hz, C*H*CHO). 2,4-Dinitrophenylhydrazone: red crystals, mp 254–255 °C. TISMS: *m*/*e* 363.1 ($M+H^+$).

5.1.2. *trans*,*trans*-Bis-(4-trifluoroacetylamino-cyclohexyl)-methane (5). *trans*,*trans*-Bis-(4-aminocyclohexyl)-methane[47] (1.50 g, 7.13 mmol) was dissolved in trifluoroacetic acid (10 mL) by stirring and cooling (0 °C). Trifluoroacetic anhydride (10 mL, 70 mmol) was added and the mixture stirred at 40 °C for 7 days. The volatiles were removed under vacuum, and the oily residue was dissolved in acetonitrile (20 mL) and water (100 mL) was added.

A crystalline precipitate formed, which was filtered off and recrystallized from acetonitrile–water. Yield 2.32 g (81%). Mp 216–218 °C. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 9.26 (2H, d, J = 7.6 Hz, 2CONH), 3.54 (2H, m, 2C*H*NH), 1.71 (8H, m), 1.28 (6H, m), 0.95 (6H, m). HRMS (M+H$^+$): 403.1823, $C_{17}H_{25}F_6N_2O_2$ requires 403.1820. Anal. Calcd for $C_{17}H_{24}F_6N_2O_2$: C, 50.75; H, 6.01; N, 6.96. Found: C, 50.82; H, 6.07; N, 6.83.

5.1.3. *cis*,*trans*-Bis-(4-trifluoroacetylamino-cyclohexyl)-methane (6). Compound (**6**) was prepared from *cis*,*trans*-bis-(4-aminocyclohexyl)-methane[47] as described for preparation of 5. Crystallization solvent was acetonitrile–water. Yield 83%. Mp 159–160 °C. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 9.25 (1H, d, J = 7.9 Hz, CONH, trans), 9.09 (1H, d, J = 7.3 Hz, CONH, *cis*), 3.72 (1H, m, C*H*NH, *cis*), 3.54 (1H, m, C*H*NH, *trans*), 1.72 (4H, m), 1.8–1.1 (14H, m), 0.93 (2H, m). HRMS (M+H$^+$): 403.1835, $C_{17}H_{25}F_6N_2O_2$ requires 403.1820. Anal. Calcd for $C_{17}H_{24}F_6N_2O_2$: C, 50.75; H, 6.01; N, 6.96. Found: C, 50.71; H, 6.15; N, 6.77.

5.1.4. *N*-[*cis*-4-(*trans*-4-Amino-cyclohexylmethyl)-cyclohexyl]trifluoroacetamide (8) 4-toluenesulfonate salt. *cis*,*trans*-Bis-(4-aminocyclohexyl)-methane[47] (**4**, 5.9 g, 28 mmol) was dissolved in trifluoroacetic acid (30 mL) at stirring and cooling (0 °C). Trifluoroacetic anhydride (20 mL, 140 mmol) was added and the mixture stirred at 40 °C for 48 h. The volatiles were removed under vacuum, and the oily residue formed triturated with anhydrous ether (200 mL). The precipitate formed was filtered off, washed with anhydrous ether, dried and water (300 mL) added. The mixture was stirred at room temperature for 1 h and then filtered. The filter cake was crystallized from acetonitrile–water giving side product—*cis*,*trans*-bis-(4-trifluoroacetyl-aminocyclohexyl)-methane (**6**, 0.75 g, 6.7%). The aqueous filtrate was evaporated to 100 mL volume, causing crystallization. The suspension obtained was heated to boiling, and the hot solution formed was slowly cooled to 4 °C. The crystals formed were filtered off and recrystallized from 60 mL water. Yield of mixture of trifluoroacetate salts of *N*-[*trans*-4-(*cis*-4-amino-cyclohexylmethyl)-cyclohexyl]trifluoroacetamide (**7**) and *N*-[*cis*-4-(*trans*-4-amino-cyclohexylmethyl)-cyclohexyl]trifluoroacetamide (**8**) was 2.55 g (22%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 9.30 (0.4H, d, J = 7.9 Hz, CONH, trans), 9.14 (0.6H, d, J = 6.9 Hz, CONH, *cis*), 7.93 (3H, br s, NH_3^+), 3.72 (0.6H, m, C*H*NHCO, *cis*), 3.52 (0.4H, m, C*H*NHCO, *trans*), 3.14 (0.4H, m, $CHNH_3^+$, *cis*), 2.89 (0.6H, m, $CHNH_3^+$ trans), 2.0–0.8 (m, 20H).

1.8 g (4.28 mmol) of the above mixture was dissolved in methanol (10 mL) and slowly passed through a column (10 × 195 mm) filled with a slurry of Dowex 1 × 4 in. OH^- form in methanol. The collected eluate was introduced into this column again, and then again (repeating four times). At the end the resin was washed with methanol. The united eluate was evaporated to 15 mL volume, 4-toluenesulfonic acid hydrate (0.9 g, 4.7 mmol) was added and the sample agitated until dissolution, acetonitrile (250 mL) was added and the mixture was placed into a refrigerator. After 20 h the crystals formed were filtered off and recrystallized (thrice) from methanol–acetonitrile. Yield 0.77 g (38%). Mp >234 °C. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 9.11 (1H, d, J = 6.9 Hz, CONH, *cis*), 7.7 (3H, br, NH_3^+), 7.47 and 7.11 (4H, AA′XX′ spin system, Tos), 3.73 (1H, m, C*H*NHCO, *cis*), 2.88 (1H, m, C*H*NH_3^+, *trans*), 2.28 (3H, s, CH_3, Tos), 2.0–0.8 (m, 20H). HRMS (M+H$^+$): 307.1984, $C_{15}H_{26}F_3N_2O$ requires 307.1997. Anal. Calcd for $C_{22}H_{33}F_3N_2O_4S$: C, 55.21; H, 6.95; N, 5.85. Found: C, 55.37; H, 7.00; N, 5.88.

5.1.5. Procedure A. *N*-(4-Amino-butyl)-*N*-benzyl-4-(1*H*-indol-3-yl)-butyramide (4–40–8) trifluoroacetate salt and *N*-(4-Amino-butyl)-*N*-benzyl-4-[2-(4-hydroxy-benzyl)-1*H*-indol-3-yl]-butyramide (4–40–8a) trifluoroacetate salt. Wang resin (Bapeks, 0.64 g, 0.7 mmol) was suspended in CH_2Cl_2 (5 mL), 4-nitrophenylchloroformate (0.42 g, 2.1 mmol) was added, and the mixture was cooled to 0 °C. A solution of *N*-methylmorpholine (0.5 mL, 4.0 mmol) in CH_2Cl_2 (2 mL) was added in small portions with shaking. The reaction mixture was allowed to warm to room temperature and gently agitated for 4 h. The resin was filtered off, washed successively with DMF (6× 4 mL), MeOH (3× 4 mL) and CH_2Cl_2 (2× 4 mL) and dried in a desiccator. The 4-nitrophenyl carbonate Wang resin obtained (0.84 g, 0.7 mmol) was placed in a reaction vessel (size 15 mL), a solution of 1,4-diaminobutane (0.31 g, 3.5 mmol) in DMF (5 mL) was added and agitated for 24 h at room temperature. The mixture was then filtered, the resin washed with DMF, MeOH and CH_2Cl_2 (each solvent 3× 5 mL) and dried in vacuum. To the 1,4-diaminobutane resin obtained a solution of benzaldehyde (0.37 g, 3.5 mmol) in trimethyl orthoformate (5 mL) was added and agitated for 20 h at room temperature. The mixture was then filtered, the resin washed with CH_2Cl_2 (3× 5 mL) and dried under vacuum. Sodium cyanoborohydride (220 mg, 3.5 mmol) was added, carefully mixed with the resin, 4% acetic acid in trimethyl orthoformate (6 mL) was added in one portion and immediately intensely shaken for 5 min. The mixture was thereafter filtered, the resin washed with MeOH, water, MeOH again and CH_2Cl_2 (each solvent 3× 5 mL), and a 10% solution of diisopropylethylamine in CH_2Cl_2 (6 mL) was added and agitated for 10 min at room temperature. The mixture was then filtered again and the resin washed with CH_2Cl_2 (6 mL). 3-Indolebutyric acid (213 mg, 1.05 mmol), PyBroP (489 mg, 1.05 mmol) and diisopropylethylamine (0.37 mL, 2.1 mmol) in CH_2Cl_2 (6 mL) were added followed by agitation for 20 h at room temperature. The mixture was filtered, the resin washed with DMF, MeOH and CH_2Cl_2 (each solvent 3× 5 mL) and dried in vacuum. The resin was then treated with 1,2-ethanedithiol (1.2 mL) followed by addition of a freshly prepared mixture of water (0.15 mL), triisopropylsilane (0.15 mL) and trifluoroacetic acid (6 mL). The reaction mixture was gently agitated at room temperature for 1 h, filtered and the resin on the filter was washed with trifluoroacetic acid (3 mL). The combined filtrate was evaporated under vacuum at room temperature and the oily residue treated with dry ether-hexane (1:1,

50 ml). The suspension formed was placed in a refrigerator for 15 h, and the supernatant liquid was thereafter removed by decantation. The material obtained was dried under vacuum in presence of KOH and P_2O_5. This raw product was further dissolved in 25% MeCN in water, centrifuged and the clear solution applied in several portions onto an HPLC semipreparative column; eluent — 30% MeCN in water + 0.1% TFA. Eluate fractions, containing pure putative **4–40–8** and **4–40–8**a trifluoroacetates (k′ 0.56 and 1.56 correspondingly) were separately pooled and lyophilized. White powders formed. Yield of **4–40–8** trifluoroacetate: 32 mg (9.4%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.81 and 10.78 (1H, 2s, NH indole), 7.70 (3H, br, NH_3^+), 7.46 (1H, m, indole), 7.35–6.86 (9H, m, indole and benzene CH), 4.53 and 4.51 (2H, 2s, CH_2-benzene), 3.32 (2H, m, CH_2CH_2NCO), 2.84-2.62 (4H, m, $CH_2NH_3^+$ and CH_2-indole),2.45 (2H, m, CH_2CO), 2.01–1.65 (6H, m, CH_2CH_2CO and $CH_2CH_2CH_2NCO$). HRMS (M+H$^+$): 364.2377, $C_{23}H_{30}N_3O$ requires 364.2389. Yield of **4–40–8**a trifluoroacetate: 4.4 mg (1.0%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.81 and 10.78 (1H, 2s, NH indole), 9.19 (1H, br s, OH), 7.70 (3H, br, NH_3^+), 7.48-6.86 (11H, m, indole and benzene CH), 6.65 (2H, m, phenol CH), 4.53 (2H, m, CH_2-benzene), 3.88 (2H, m, CH_2-phenol), 3.35 (2H, m, CH_2NCO), 2.87-2.63 (4H, m, $CH_2NH_3^+$ and CH_2-indole),2.48 (2H, m, CH_2CO), 2.05-1.66 (6H, m, CH_2CH_2CO and $CH_2CH_2CH_2NCO$). HRMS (M+H$^+$): 470.2823, $C_{30}H_{36}N_3O_2$ requires 470.2807.

5.1.6. Procedure B. *N*-[4-(4-Amino-cyclohexylmethyl)-cyclohexyl]-*N*-benzyl-4-(1*H*-indol-3-yl)-butyramide (10d-40-8) trifluoroacetate salt. Synthesis was made essentially as described under 5.1.5 with the exception that 4,4′-Diamino-dicyclohexylmethane trityl resin (Novabiochem) was used. For the cleavage of the synthesis product from the polymer, a mixture of TFA-1,2-ethane dithiol-methylene chloride (1:1:20) was applied for 30 min. Yield 12%. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.79 and 10.70 (1H, 2s, indole NH), 7.73 (3H, br, NH_3^+), 7.52 and 7.44 (2H, 2d, J = 7.7 and 7.6 Hz, indole CH), 7.36–6.87 (8H, m, benzene and indole CH), 4.47 and 4.44 (2H, 2s, CH_2N), 4.40-4.15 (1H, m, NCH), 3.2-0.5 (27H, m, aliphatic CH_2, CH). HRMS (M+H$^+$): 486.3481, $C_{32}H_{44}N_3O$ requires 486.3484.

5.1.7. Procedure C. *N*-(2-Amino-ethyl)-2-(1*H*-indol-3-yl)-*N*-(1-naphthalen-2-yl-ethyl)-acetamide (2-29-1) trifluoroacetate salt. Synthesis was made essentially as described in Section 5.1.5, with the exception that 2′-acetonaphthone was dissolved in 10% anhydrous AcOH in trimethyl orthoformate, added to ethylenediamine resin and agitated for 20 h at 30 °C. Yield 7.4%. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.97 (1H, s, indole NH), 7.90-6.90 (15H, m, naphthalene and indole CH, NH_3^+), 5.98 and 5.60 (1H, 2q, J = 6.9 and 6.4 Hz, CH-naphthyl), 4.07 and 4.05 (2H, 2s, $COCH_2$), 3.55 and 3.47 (2H, 2t, J = 6.7 Hz, NCH_2CH_2), 3.35-3.15 (2H, m, $CH_2NH_3^+$), 1.60 and 1.49 (3H, 2d, J = 7.0 and 6.5 Hz, CH_3). HRMS (M+H$^+$): 372.2074, $C_{24}H_{26}N_3O$ requires 372.2076.

5.1.8. Procedure D. *S*-2-Amino-*N*-(5-amino-2-methyl-pentyl)-3-(1*H*-indol-3-yl)-*N*-(2-methyl-3-phenyl-allyl)-propionamide and/or S-2-amino-*N*-(5-amino-4-methyl-pentyl)-3-(1*H*-indol-3-yl)-*N*-(2-methyl-3-phenyl-allyl)-propionamide (18-37-17, prepared as a mixture) ditrifluoracetate salts. Synthesis was made mainly as described in Section 5.1.5 with the exception that an α-Fmoc protected amino acid was applied. After addition of it the mixture was filtered, the resin washed with DMF (3× 5 mL), 20% piperidine in DMF (5 mL) was then added and the sample was agitated for 10 min. The mixture was filtered again, the resin washed with DMF (5× 5 mL), MeOH and CH_2Cl_2 (each solvent 3× 5 mL) and dried in vacuum. Cleavage from the polymer and the next procedures followed. Yield 19%. ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 11.12 and 11.08 (1H, 2s, NH indole), 8.28 (3H, br, $COCHNH_3^+$), 7.79 (3H, br, $CH_2NH_3^+$), 7.56 (1H, m, CH indole), 7.42–7.15 (7H, m, CH benzene and indole), 7.06 (2H, m, CH indole), 6.23 (1H, m, $CHCCH_3$), 4.35 (1H, m, $COCHNH_3^+$), 3.49 (2H, m, CH_3CCH_2), 3.20 (2H, m, CH_3CHCH_2), 2.72 (4H, m, CH_2-indole and $CH_2NH_3^+$), 1.64 (3H, s, CCH_3), 1.63, 1.27 and 1.07 (5H, 3m, $CH_2CH_2CHCH_3$), 0.77 (3H, m, $CHCH_3$). HRMS (M+H$^+$): 433.2965, $C_{27}H_{37}N_4O$ requires 433.2967.

5.1.9. Procedure E. *N*-{3-[3-(3-Amino-cyclohexyl)-ureido]-cyclohexyl}-*N*-benzyl-4-(1*H*-indol-3-yl)-butyramide (27-40-8) trifluoroacetate salt and *N*-{3-[3-(3-Amino-cyclohexyl)-ureido]-cyclohexyl}-*N*-benzyl-4-[2-(4-hydroxybenzyl)-1*H*-indol-3-yl]-butyramide (27-40-8a) trifluoroacetate salt. To a 4-nitrophenyl carbonate Wang resin (preparation described in Section 5.1.5., 0.84 g, 0.7 mmol) placed into a reaction vessel (size 15 mL), a solution of 1,3-diaminocyclohexane (0.40 g, 3.5 mmol) in DMF (5 mL) was added and the mixture agitated for 24 h at room temperature. The suspension was then filtered, and the resin washed with DMF (4× 5 mL). To the wet resin 4-nitrophenylchloroformate (0.42 g, 2.1 mmol) and *N*,*N*-diisopropylethylamine (0.37 mL, 2.1 mmol) in DMF (5 mL) were added and the mixture agitated for 24 h at room temperature. The mixture was then filtered and the resin washed with DMF (4× 5 mL). To the wet resin a solution of 1,3-diaminocyclohexane (0.40 g, 3.5 mmol) in DMF (5 mL) was added and the suspension agitated for 24 h at room temperature. The mixture was then filtered, the resin washed with DMF, MeOH and CH_2Cl_2 (each solvent 3× 5 mL) and dried in vacuum. The subsequent operations (starting with addition of benzaldehyde) were made as described under Section 5.1.5. Yield of **27–40–8** 26.8 mg (6.0%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.78 and 10.70 (1H, 2s, indole NH), 7.74 (3H, br, NH_3^+), 7.53 and 7.44(1H, 2d, J = 8.2 and 7.9 Hz, CH indole), 7.36–6.88 (9H, m, benzene and indole CH), 5.77 and 5.58 (2H, 2m, NHCONH), 4.46 (2H, s, CH_2-benzene), 3.84 (1H, m, CH_2N-CH cyclohexane-1), 3.37 (2H, m, 2CH-NH cyclohexanes-3, 1′), 3.01 (1H, m, $CHNH_3^+$ cyclohexane-3′), 2.74 and 2.58 (2H, 2t, J = 7.2 Hz, $COCH_2$), 2.20 (2H, m, $COCH_2CH_2CH_2$), 2.11-0.80 (18H, m, $COCH_2CH_2$ and cyclohexanes). HRMS (M+H$^+$): 530.3496, $C_{32}H_{44}N_5O_2$ requires 530.3495. Yield of **27–40–8a** 7.4 mg (1.4%). ^{1}H NMR (270 MHz,

DMSO-d_6, ppm): δ 10.66 and 10.61 (1H, 2s, indole NH), 9.18 (1H, br s, OH), 7.72 (3H, br, NH_3^+), 7.50–6.84 (11H, m, phenol, benzene and indole CH), 6.63 (2H, m, phenol CH), 5.74 and 5.56 (2H, 2m, NHCONH), 4.44 (2H, m, CH_2-benzene), 3.93 (2H, m, CH_2-phenol), 3.80 (1H, m, CH_2N-CH cyclohexane-1), 3.38 (2H, 2m, 2CH-NH cyclohexanes-3, 1′), 3.01 (1H, m, C$H$$NH_3^+$ cyclohexane-3′), 2.75 and 2.59 (2H, 2m, $COCH_2$), 2.15 (2H, m, $COCH_2CH_2CH_2$), 2.00-0.80 (18H, m, $COCH_2CH_2$ and cyclohexanes). HRMS ($M+H^+$): 636.3912, $C_{39}H_{50}N_5O_3$ requires 636.3913.

5.1.10. Procedure F. *N*-[cis-4-(trans-4-Amino-cyclohexylmethyl)-cyclohexyl]-*N*-(2-bromo-4,5-dimethoxy-benzyl)-4-(1*H*-indol-3-yl)-butyramide (10e–18–8) trifluoroacetate salt and *N*-[cis-4-(trans-4-Amino-cyclohexylmethyl)-cyclohexyl]-*N*-(2-bromo-4,5-dimethoxy-benzyl)-4-[2-(4-hydroxy-benzyl)-1*H*-indol-3-yl]-butyramide (10e–18–8a) trifluoroacetate salt. 4-Nitrophenyl carbonate Wang resin (preparation described in 5.1.5., 0.42 g, 0.35 mmol) was placed in a reaction vessel (size 8 mL), a solution of **8** (4-toluenesulfonate salt, 167 mg, 0.35 mmol) and *N*,*N*-diisopropylethylamine (61 μL, 0.35 mmol) in DMF (2 mL) was added and the mixture agitated for 60 h at room temperature. The resin was filtered off, washed successively with DMF (4× 3 mL), MeOH (3× 3 mL) and CH_2Cl_2 (2× 3 mL) and dried in a desiccator. To the dry resin, $NaBH_4$ (199 mg, 5.25 mmol) in 1-butanol (3 mL) was added and the mixture agitated, first for 49 h at room temperature, and then for 3 h at 50 °C. The resin was filtered off, washed successively with 1-butanol (4× 3 mL), DMF (4× 3 mL), MeOH (3× 3 mL) and CH_2Cl_2 (2× 3 mL) and dried in a desiccator. The subsequent operations (starting from the addition of 2-bromo-4,5-dimethoxy-benzaldehyde) were made as described for Procedure A, with the exception that 1,2-ethanedithiol was excluded from the cleavage mixture. Yield of **10e–18–8** 14.8 mg (5.8%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.74 and 10.65 (1H, 2s, indole NH), 7.68 (3H, br, NH_3^+), 7.45 (1H, m, indole CH), 7.26 (1H, m, indole CH), 7.14-6.83 (5H, m, benzene and indole CH), 4.31 (2H, m, CH_2N), 3.72 and 3.69 (3H, 2s, CH_3), 3.57 and 3.56 (3H, 2s, CH_3), 3.43 (1H, m, N-CH cyclohexane), 3.10 and 2.85 (1H, 2m, N-CH cyclohexane), 2.72 and 2.55 (2H, 2m, $COCH_2$), 2.19 (2H, m, $COCH_2CH_2CH_2$), 1.85 (2H, m, $COCH_2CH_2$), 1.73–0.70 (20H, m, CH_2-cyclohexane and CH and CH_2 cyclohexanes). HRMS ($M+H^+$): 624.2795, $C_{34}H_{47}BrN_3O_3$ requires 624.2801. Yield of **10e–18–8**a 48.2 mg (16%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.70 (1H, m, indole NH), 9.18 (1H, m, OH), 7.65 (3H, br, NH_3^+), 7.25 (1H, m, indole CH), 7.16 (1H, m, indole CH), 7.10-6.85 (6H, m, benzene, indole and phenol CH), 6.62 (2H, m, phenol CH), 4.31 (2H, m, CH_2N), 4.02 (2H, m, CH_2–phenol), 3.75 (3H, m, CH_3), 3.57 (3H, m, CH_3), 3.43 (1H, m, N-CH cyclohexane), 3.15 and 2.85 (1H, 2m, N-CH cyclohexane), 2.74 and 2.54 (2H, 2m, COCH_2), 2.23 (2H, m, $COCH_2CH_2CH_2$), 1.85 (2H, m, $COCH_2CH_2$), 1.76-0.80 (20H, m, CH_2-cyclohexane and CH and CH_2 cyclohexanes). HRMS ($M+H^+$): 730.3192, $C_{41}H_{53}BrN_3O_4$ requires 730.3219.

5.1.11. Procedure G. *N*-(3-Guanidino-propyl)-4-(1*H*-indol-3-yl)-*N*-naphthalen-2-yl-methyl-butyramide (12–0–8) trifluoroacetate salt. Compound **3–0–8** trifluoroacetate salt (56 mg, 0.11 mmol), 1H-pyrazole-1-carboxamidine hydrochloride (18 mg, 0.12 mmol) and *N*,*N*-diisopropylethylamine (40 μl, 0.23 mmol) were dissolved in DMF (0.2 mL) and allowed to stand at room temperature for 24 h. Then 0.1% trifluoroacetic acid in water (1 mL) was added, centrifuged and the clear solution applied onto an HPLC semipreparative (10× 250 mm) Vydac 219TP510 Diphenyl column, eluent—34% MeCN in water + 0.1% TFA. Eluate fractions, containing pure putative **12–0–8** trifluoroacetate, were pooled and lyophilized. A white powder formed. Yield 25.5 mg (42%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.78 and 10.73 (1H, 2s, indole NH), 8.0–6.9 (12H, m, indole, naphthalene CH), 7.60-7.40 (5H, br, guanidinium$^+$), 4.70 (2H, s, CH_2-naphthalene), 3.44–3.22 (2H, m, NCH_2CH_2), 3.16-3.01 (2H, m, CH_2-guanidinium$^+$), 2.8–2.6 (2H, m, CH_2-indole), 2.5–2.4 (2H, m, $COCH_2$), 2.05–1.80 (2H, m, $COCH_2CH_2$), 1.8–1.6 (2H, m, NCH_2CH_2). HRMS ($M+H^+$): 442.2622, $C_{27}H_{32}N_5O$ requires 442.2607.

5.1.12. Procedure H. *N*-Pentyl-*N*-(4-diethylamino-benzyl)-4-(1*H*-indol-3-yl)-butyramide (1-39-8) trifluoroacetate salt. 4-Diethylaminobenzaldehyde (1.77 g, 10 mmol) and 1-aminopentane (1.28 mL, 11 mmol) were dissolved in anhydrous trimethyl orthoformate (25 mL) and allowed to stand for 3 days at room temperature. The reaction mixture was then cooled to 0 °C and sodium cyanoborohydride (2.51 g, 40 mmol) was added during stirring. The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was then evaporated, the oily residue dissolved in CH_2Cl_2 (50 mL), washed with a saturated $NaHCO_3$ water solution (50 mL), and then with water (50 mL). The combined water phases were extracted with CH_2Cl_2 (50 mL) and discarded, the organic layer was then added to the previous organic phase, dried with Na_2SO_4 and filtered. The filtrate was evaporated, the residue dried under vacuum in presence of P_2O_5 and KOH. Light yellow oil formed. Yield of pentyl-(4-diethylamino-benzyl)-amine (**22**) 2.5 g (quantitative). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 7.18 (2H, d, J = 8.9 Hz, benzene CH), 6.65 (2H, d, J = 8.8 Hz, benzene CH), 6.60 (1H, m, NH), 3.81 (2H, s, benzene-CH_2), 3.36-3.23 [4H, m, N(CH_2)$_2$], 2.66 (2H, m, $NHCH_2CH_2$), 1.52 (2H, m, $NHCH_2CH_2$), 1.28 (4H, m, $CH_2CH_2CH_3$), 1.06 [6H, t, J = 6.9 Hz, N(CH_2CH_3)$_2$], 0.86 (3H, t, J = 6.8 Hz, $CH_2CH_2CH_3$). HRMS ($M+H^+$): 249.2340, $C_{16}H_{29}N_2$ requires 249.2330.

Compound **22** (100 mg, 0.40 mmol), 3-indolebutyric acid (98.5 mg, 0.485 mmol), PyBroP (226 mg, 0.485 mmol) and diisopropylethylamine (0.25 mL, 1.49 mmol) were dissolved in 1.0 mL CH_2Cl_2 and allowed to stand for 1 h at room temperature. The reaction mixture was then washed with a saturated aqueous solution of $NaHCO_3$ (2× 1 mL) followed by water (1 mL). The combined water phases were extracted with CH_2Cl_2 (2 mL), the organic layer was added to the previous organic phase and dried with Na_2SO_4. The mixture was then filtered, the filtrate evaporated and the residue dried under vacuum in the

presence of P_2O_5 and KOH. The oil formed was further dissolved in 30% acetonitrile in water + 0.1% TFA, centrifuged and the clear solution was divided in several portions. Each portion was applied onto a semipreparative HPLC column; the mobile phase consisting of 35% MeCN in water + 0.1% TFA. Fractions from repeated runs containing pure putative **1**–**39**–**8** were pooled and lyophilized. A white powder formed. Yield 34.4 mg (16%). ^{1}H NMR (270 MHz, DMSO-d_6, ppm): δ 10.77 and 10.72 (1H, 2s, indole NH), 7.60–6.85 (9H, m, benzene and indole CH), 7.39 (1H, br, NH^+), 4.52 (2H, s, CH_2-Ph), 3.52–3.36 [4H, m, $^+$NH(CH_2CH$_3$)$_2$], 3.28-3.08 (2H, m, CONCH_2CH$_2$), 2.76–2.57 (2H, m, CH_2-indole), 2.42 and 2.32 (2H, 2t, J = 7.2 Hz, COCH$_2$), 1.98-1-76 (2H, m, COCH$_2$CH_2), 1.51–1.28 (2H, m, NCH$_2$CH_2), 1.28–1.05 (4H, m, CH$_3$CH_2CH_2), 0.96 [6H, t, J = 6.9 Hz, $^+$NH(CH$_2$CH_3)$_2$], 0.86–0.70 (3H, m, CH$_2$CH$_2$CH_3). HRMS (M+H$^+$): 434.3156, $C_{28}H_{40}N_3O$ requires 434.3171.

5.1.13. Procedure I. *N*-(3-Aminopropyl)-*N*-(1,2,3,4-tetrahydronaphthalene-2-yl)-3-(1*H*-indol-3-yl)-propionamide (3–38–5) trifluoroacetate salt. To a dry 250-mL three-necked flask equipped with septum and argon inlet, CH_2Cl_2 (100 mL), 1,3-propylenediamine (4.37 mL, 51.7 mmol) and β-tetralone (2.52 g, 17.2 mmol) were added. The mixture was cooled to 0 °C, and $TiCl_4$ (0.95 mL, 8.6 mmol) was added using a syringe. The reaction mixture was allowed to warm to room temperature and stirred for 20 h, maintaining the argon atmosphere. The mixture was thereafter again cooled to 0 °C, and a solution of $NaCNBH_3$ (2.92 g, 46.5 mmol) in MeOH (35 mL) was slowly added. The stirring was continued for 15 min at 0 °C and 30 min at room temperature. Solution of NaOH (1.24 g, 31 mmol) in water (18 mL) was then added portionwise. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (50 mL). The combined organic solutions, which contained *N*-(1,2,3,4-tetrahydronaphthalene-2-yl)-1,3-propylenediamine [**24**, TISMS: *m*/*e* 205.1 (M+H$^+$)], were directly used for the following synthesis. Di-tert-butyl dicarbonate (3.76 g. 17.2 mmol) was added, and the solution allowed to stand for 24 h at room temperature. The mixture was then applied onto a silica gel column (3.5 × 35 cm) and eluted with a chromatographic system comprising $CHCl_3$–MeOH–NH_4OH–petrol ether, gradually changing its volume proportions from 30:2:1:90 to 9:1:1:0. Fractions, containing pure *N*-*tert*-butoxycarbonyl-*N*′-(1,2,3,4-tetrahydronaphthalene-2-yl)-1,3-propylenediamine [**25**, TISMS: *m*/*e* 305.2 (M+H$^+$)], were pooled, evaporated and dried in desiccator over H_2SO_4 until constant weight. The product was dissolved in anhydrous ether (50 mL), and a 4 M HCl solution in dioxane was added at stirring until pH reached 5 (wet indicator paper). A brownish grey precipitate of *N*-*tert*-butoxycarbonyl-*N*′-(1,2,3,4-tetrahydronaphthalene-2-yl)-1,3-propylenediamine (**25**) hydrochloride formed. It was filtered off, washed on filter with anhydrous ether and dried in desiccator over P_2O_5 and KOH. Yield 1.43 g (24%). ^{1}H NMR (270 MHz, DMSO-d_6): δ 9.08 (2H, m, NH_2^+), 7.04 (4H, m, β-tetralone), 6.93 (1H, m, NHCO), 3.43 (1H, m, CHNH$_2^+$), 3.02 (4H, m, β-tetralone), 2.85 (4H, m, CH_2CH$_2$CH_2), 1.79 (4H, m, β -tetralone and CH$_2$CH_2CH$_2$), 1.38 (9H, s, Boc). HRMS (M+H$^+$): 305.2227, $C_{18}H_{29}N_2O_2$ requires 305.2229. 100 mg (0.29 mmol) of this product, 3-indolepropionic acid (66 mg, 0.35 mmol), and PyBroP (163 mg, 0.35 mmol) were dissolved in CH_2Cl_2 (3 mL), *N*,*N*-diisopropylethylamine (0.17 mL, 1.0 mmol) added and allowed to stand for 24 h. CH_2Cl_2 (3 mL) was added and solution was extracted with 10% aqueous $KHSO_4$ (3× 5 mL), saturated aqueous $NaHCO_3$ (5× 5 mL) and water(3× 5 mL). The organic solution was dried over $MgSO_4$, filtered and the filtrate evaporated. An oily residue, containing *N*-(3-*tert*-butoxycarbonylaminopropyl)-*N*-(1,2,3,4-tetrahydronaphthalene-2-yl)-3-(1*H*-indol-3-yl)-propionamide (**26**) formed. ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.76 and 10.73 (1H, 2s, indole NH), 7.51 and 7.46 (1H, 2d, J = 7.8 Hz, indole CH), 7.29 (1H, m, indole CH), 7.11–6.93 (7H, m, β-tetralone and indole CH), 6.89 and 6.80 (1H, 2m, NHBoc), 3.98 (1H, m, NCH β-tetralone), 3.20 (2H, m, β-tetralone CH$_2$), 2.98-2.79 (6H, m, β-tetralone CH$_2$, COCH$_2$CH_2 and CH_2NH), 2.66 (4H, COCH_2CH_2), 1.76 and 1.63 (4H, 2m, β-tetralone CH$_2$ and CH$_2$CH_2CH$_2$), 1.34 and 1.29 (9H, 2s, Boc). TISMS: *m*/*e* 476.3 (M+H$^+$). This product was dissolved in a mixture CF_3COOH–water–triisopropylsilane-1,2-ethanedithiol (92.5:2.5:2.5:2.5; 2 mL), allowed to react for 45 min at room temperature, the reaction mixture was evaporated under vacuum at room temperature and the oily residue treated with mixture dry ether–hexane (1:1, 50 mL). A suspension formed, which was placed in a refrigerator for 15 h whereafter the liquid was removed by decantation. The material obtained was dried under vacuum in the presence of KOH and P_2O_5. This raw product was further dissolved in 20% MeCN in water, centrifuged and the clear solution applied onto an HPLC semipreparative column, eluent—22% MeCN in water + 0.1% TFA. Eluate fractions, containing pure putative (**3**–**38**–**5**) trifluoroacetate, were pooled and lyophilized. A white powder formed. Yield 71.5 mg (57% from **24**, 14% summary). ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.78 and 10.75 (1H, 2s, indole NH), 7.69 (3H, br s, NH_3^+), 7.53 and 7.51 (1H, 2d, J = 8.0 Hz and 7.8 Hz, indole CH), 7.31 (1H, m, indole CH), 7.16–6.87 (7H, m, β-tetralone and indole CH), 4.03 (1H, m, β-tetralone NCH), 3.27 (2H, m, β-tetralone CH$_2$), 2.93 (2H, m, β-tetralone CH$_2$), 2.89–2.62 (8H, m, COCH_2CH_2 and CH_2CH$_2$CH_2), 1.77 and 1.65 (4H, 2m, β-tetralone CH$_2$ and CH$_2$CH_2CH$_2$). HRMS (M+H$^+$): 376.2394, $C_{24}H_{30}N_3O$ requires 376.2389.

5.1.14. Procedure J. *N*-(2-Aminoethyl)-*N*-(spiro[5.5]undecan-3-yl)-2-(naphthalen-2-yloxy)-acetamide (2-42-14) trifluoroacetate salt. To a dry 100-mL three-necked flask equipped with septum and argon inlet, CH_2Cl_2 (50 mL), ethylenediamine (2.49 mL, 29.3 mmol) and spiro[5.5]undec-1-en-3-one[57] (1.6 g, 9.8 mmol) were added. The mixture was cooled to 0 °C, and at stirring $TiCl_4$ (0.54 mL, 4.9 mmol) added using a syringe. The reaction mixture was allowed to warm to room temperature and stirred for 20 h, maintaining the argon atmosphere. Then the mixture was cooled to 0 °C again, and a solution of $NaCNBH_3$ (1.23 g, 19.5 mmol) in MeOH (15 mL) was slowly added. The stirring was continued for 15 min at

0 °C, and 30 min at room temperature. A new portion of $NaCNBH_3$ in MeOH was added with stirring and the temperature was changed as before. Then a saturated aqueous solution of $NaHCO_3$ (50 mL) was added with stirring. The organic phase was separated, and the aqueous phase was extracted twice with CH_2Cl_2 (40 mL). The combined organic solutions were dried over Na_2SO_4, filtered, and the filtrate evaporated to afford *N*-(spiro[5.5]undecan-3-yl)-ethylenediamine (**27**) as yellow oil. Yield 1.8 g (88%). ^1H NMR (270 MHz, DMSO-d_6, ppm): δ 3.36 (1H, m, NHC*H* spiro[5.5]undecan-3-yl), 3.29 and 3.18 (2H, 2m, NHCH_2), 2.97 and 2.80 (2H, 2m, CH_2NH_2), 1.84–0.88 (18H, m, CH_2 spiro[5.5]undecan-3-yl). HRMS ($M+H^+$): 211.2179, $C_{13}H_{27}N_2$ requires 211.2174. 0.31 g (1.5 mmol) of this substance was dissolved in DMF (5 mL) and added to 4-nitrophenyl carbonate Wang resin (0.59 g, 0.50 mmol). The mixture was agitated at room temperature for 24 h, filtered, the resin washed with DMF (5× 5 mL), MeOH and CH_2Cl_2 (each solvent 3× 5 mL), and dried in vacuum. Following operations (starting with addition of carboxylic acid–2-naphthoxyacetic acid) were made as described in Procedure A. Eluent for the semipreparative HPLC contained 32% MeCN. Yield 87 mg (11% from **27**, 10% summary). ^1H NMR (270 MHz, $CDCl_3$): δ 7.97 (3H, br s, NH_3^+), 7.80–7.64 (3H, m, naphthalene), 7.47–7.27 (2H, m, naphthalene), 7.12 (2H, m, naphthalene), 4.85 (2H, m, $COCH_2$), 3.54 (1H, m, spiro[5.5]undecan-3-yl NCH), 3.41 (1H, m, NCH_2), 3.13 (2H, m, C$H_2NH_3^+$), 1.72–0.9 (18H, m, spiro[5.5]undecan-3-yl 9CH_2). HRMS ($M+H^+$): 395.2709, $C_{25}H_{35}N_2O_2$ requires 395.2698.

5.1.15. Procedure K. Fractionation of mixtures of tertiary amides on reversed phase. Raw syntheses product was purified as usual with the exception that eluate portion, which according to LC/MS analysis contained desired mixture of isomers, was collected as several fractions, which were numerated according to their order of coming. They were then separately freeze-dried and used for further investigations.

5.1.16. Procedure L. Fractionation of mixtures of tertiary amides on chiral phase. Product (3–5 mg), obtained according to Procedure K, was dissolved in MeOH–triethylamine–AcOH (100:0.1:0.1, 1.5 mL), and the solution divided in several portions. They were applied onto a Chirobiotic V HPLC semipreparative (Astec, 10 × 250 mm) column equlibrated with the same solution, serving also as eluent. Flow rate 5 mL/min, detection at 220 nM. Eluate fractions corresponding to observed UV peaks were separately pooled and repeatedly lyophilized (until a constant weight of the product was reached). White powders formed. Fractions were numerated according to their order of coming.

5.2. Pharmacology

5.2.1. Radioligand binding on recombinant human MC receptor subtypes. Assays of tertiary amides binding to human melanocortin receptors were performed using (^{125}I–Tyr^2, Nle^4, D-Phe^7)-α-MSH ([^{125}I]-NDP–MSH) radioligand binding essentially as described earlier.[64] Each of the radioligand binding assays comprised a dilution series of 12 concentrations with duplicates with a 5 1/2 order of magnitude of concentration range, and each compound was analyzed with at least two repeats. In brief, B16 mouse melanoma cells which express the MC_1 receptor naturally or Sf9 cells expressing either one of the recombinant human MC_3, MC_4 or MC_5 receptors were taken from frozen stocks, washed with binding buffer (minimum essential medium with Earle's salts, pH 7.0, 0.2% bovine serum albumin, 1 mM 1,10-phenanthroline, 0.5 mg per litre leupeptin and 200 mg per litre bacitracin). The assays were performed in 96-well binding plates, to each well 50,000 cells were dispensed, 0.5 nM (approximately 50,000 cpm) [^{125}I]-NDP–MSH and appropriate concentrations of tertiary amide in 50 μl binding buffer. The plates were then incubated for 1 h at 37 °C whereafter the free radioligand was separated from the bound radioligand by centrifugation (3500 rpm for 5 min at 5 °C). The cells were washed with ice-cold binding buffer and then detached from the plates with 0.1 N NaOH and the radioactivity counted in a Wallac, Wizard automatic gamma counter and data analyzed with the BindAid software, essentially as previously described.[65]

5.2.2. cAMP assays. The ability of tertiary amides to influence the levels of cAMP was, for the MC_1 receptor, assessed in B16 mouse melanoma cells which express the MC_1 receptor naturally, and for MC_3, MC_4 and MC_5 receptors in COS-1 cells that had transiently been transfected with the MC_3, MC_4 and MC_5 receptor DNA, using the procedures previously described.[65] In brief, cells were cultured in DMEM supplemented with heat-inactivated 10% FBS at 37 °C in humidified 5% CO_2 cell incubator. B16 cells were cultured until experiments were performed. COS-1 cells were transfected when they reached 50% confluence with the appropriate receptor DNA by using Lipofectine reagent (Gibco-BRL) according to manufacturers' instruction. After 48 h transfected COS-1 cells were used for cAMP determination. Cells were harvested and incubated for 20 min at 37 °C in 0.05 mL DMEM without FBS containing 0.5 mM 3-isobutyl-1-methylxanthine and appropriate concentrations of substances of interest. cAMP was then extracted from cells by adding 4.4 M perchloric acid to achieve a final concentration of 1.25 M. After 5 min the samples were neutralized with 5 M KOH/1 M Tris and centrifuged at 3000 rpm for 5 min at 20 °C. After centrifugation 50 μl of the supernatants or cAMP standards ranging from 0.2 to 250 nM were added to 96-well microtitre plates. The cAMP concentration was measured by a bovine adrenal cAMP binding protein competitive binding assay using [^{3}H]-cAMP (specific activity 24 Ci/mmol) as labelled ligand, essentially as described.[66] In brief, the sample, [^{3}H]-cAMP (0.14 pmol, approximately 11,000 cpm), cAMP standard and the binding protein were incubated at 4 °C for 150 min whereafter the incubates were harvested by filtration on Whatman GF/B filters using semiautomatic Brandel cell harvester, Gaithersburg, MD, USA. Each filter was rinsed with 10 mL ice-cold 50 mM Tris·HCl, pH 7.4, punched out and placed into scintillation vials with scintillation fluid and counted.

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Supplementary data

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