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M_2 Subtype preferring dibenzodiazepinone-type muscarinic receptor ligands: Effect of chemical homo-dimerization on orthosteric (and allosteric?) binding

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ABSTRACT

A series of new dibenzodiazepinone-type muscarinic receptor ligands, including two homo-dimeric compounds, was prepared. Sixteen representative compounds were characterized in equilibrium binding studies with [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) at the muscarinic receptor subtype M_2, and seven selected compounds were additionally investigated at M_1, M_3, M_4 and M_5 with respect to receptor subtype selectivity. The side chain of the known M_2 preferring muscarinic receptor antagonist DIBA was widely varied with respect to chain length and type of the basic group (amine, imidazole, guanidine and piperazine). Most of the structural changes were well tolerated with respect to muscarinic receptor binding, determined by displacement of [^{3}H]NMS. Compounds investigated at all subtypes shared a similar selectivity profile, which can be summarized as $M_2 > M_1 \approx M_4 > M_3 \approx M_5$ (**46**, **50**, **57**, **62**–**64**) and $M_2 > M_1 \approx M_4 > M_3 > M_5$ (**1**, **58**). The homo-dimeric dibenzodiazepinone derivatives UNSW-MK250 (**63**) and UNSW-MK262 (**64**) exhibited the highest M_2 receptor affinities (pIC_{50} = 9.0 and 9.2, respectively). At the M_2 receptor a steep curve slope of −2 was found for the dimeric ligand **63**, which cannot be described according to the law of mass action, suggesting a more complex mechanism of binding. In addition to equilibrium binding studies, for selected ligands, we determined $pEC_{50,diss}$, an estimate of affinity to the allosteric site of M_2 receptors occupied with [^{3}H]NMS. Compounds **58** and **62**–**64** were capable of retarding [^{3}H]NMS dissociation by a factor >10 ($E_{max,diss}$ >92%), with highest potency ($pEC_{50,diss}$ = 5.56) residing in the dimeric compound **64**. As the monomeric counterpart of **64** was 100 times less potent (**62**: $pEC_{50,diss}$ = 3.59), these data suggest that chemical dimerization of dibenzodiazepinone-type M receptor ligands can enhance allosteric binding.

1. Introduction

Muscarinic acetylcholine receptors (M receptors) are members of the GPCR superfamily type A and comprise five receptor subtypes (M_1–M_5), which mediate the action of the neurotransmitter acetylcholine in the peripheral and central nervous system. As the orthosteric (i.e., neurotransmitter) site within the binding pocket is highly conserved among M receptors, the development of selective M receptor ligands has been very challenging. Over the last two decades, M receptors were repeatedly suggested to exhibit distinct allosteric binding sites,[1–9] and there has been an increasing number of reports on ligands that address the less conserved allosteric sites of M receptors.[10] This approach harbors a potential with regard to the development of new types of selective M receptor targeting drugs, for example, for the treatment of Alzheimer's disease.[11–13] To date, there is a lack of highly subtype-selective M receptor ligands.[14,15] The M_2 preferring pyridobenzodiazepinone-type M receptor antagonists AF-DX 384 (**1**), AF-DX 116 (**2**) and AQ-RA 741 (**3**), first described in 1989, were developed from the M_1 preferring M receptor antagonist pirenzepine[16] by modifying the side chain (Fig. 1A).[17] Reported binding data of the tricyclic M receptor antagonist DIBA, a molecule that represents the dibenzodiazepinone congener of **3** (Fig. 1A), and binding data

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of the dibenzodiazepinone analog of pirenzepine (data not shown), revealed that replacement of the pyridobenzodiazepinone scaffold by a dibenzodiazepinone moiety leads to a considerable increase in M_1, M_2 and M_3 receptor affinity.[18,19] Shortening of the linear alkyl chain in DIBA from 4 to 2 carbon atoms, was shown to result in a marked decrease in M_1 and M_2 affinity.[20] In Figure 1A, compounds **4** and **5** are shown as examples of DIBA derivatives in which one of the terminal *N*-ethyl residues was replaced by an acyl substituent (2,2-dimethylpentanoyl and 4-fluorobenzoyl, respectively). These structural changes caused a decrease in M receptor affinity, and, in case of **5**, impaired M_2 selectivity (Fig. 1A). Replacement of the terminal basic nitrogen in DIBA by an imidazole moiety and shortening of the alkyl chain to three carbon atoms resulted in compound **6** (Fig. 1A). The synthesis and crystal structure of **6** were reported recently,[21] and the determination of M_2 receptor binding data of **6** is a subject of the present study.

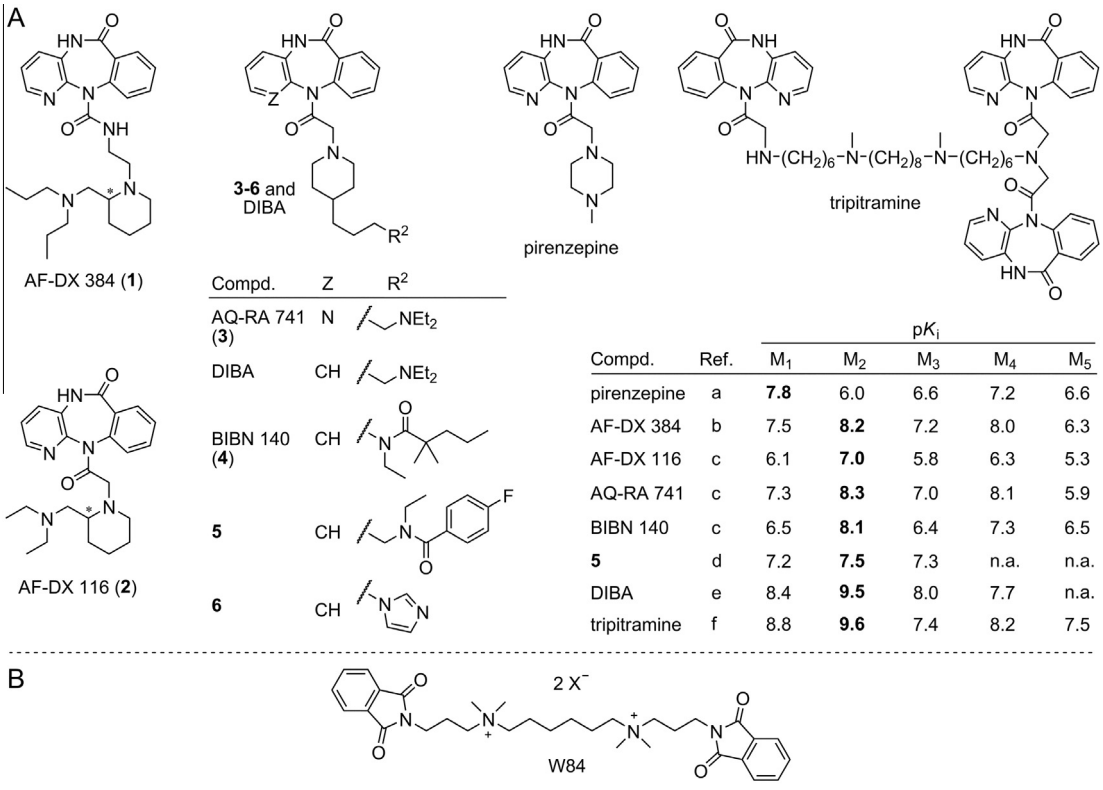
Compound **1** belongs to the most intensely investigated tricyclic diazepine derivatives. Its tritium-labeled analog was used by several groups for the pharmacological characterization of various M receptor ligands and autoradiographic studies.[27–31] Compound **1** was suggested to bind to both the orthosteric binding site and the so-called common allosteric site, at M_2 receptors occupied by the orthosteric antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]NMS). The M_2 receptor subtype preference of **1** was explained by partial occupation of both binding sites.[31] Moreover, hybrid molecules, containing the scaffold of **1** and parts of the allosteric M receptor modulator W84 (cf. Fig. 1B), were reported to exhibit higher affinity towards the allosteric site of [^{3}H]NMS occupied M_2 receptors compared to W84 alone.[30,32] Linkage of two or three pyridobenzodiazepinone moieties via a highly flexible linker yielded a dimeric and a trimeric M receptor antagonist, respectively, both of which showed high M_2 affinity (pA_2 values >9).[33] The trimeric pyridobenzodiazepinone derivative, referred to as tripitramine (Fig. 1A), was reported to exhibit considerably higher M_2 selectivity over monomeric analogs such as **3**.[26,33] Tripitramine has been primarily described as a competitive M receptor antagonist in both binding studies and functional investigations.[26,34,35] However, binding of tripitramine to an allosteric site was suggested.[36]

The present study was directed towards bioisosteric replacement of the terminal diethylamino group in DIBA by basic heterocycles (imidazole, piperazine) and a guanidine group to further investigate M receptor affinity and subtype selectivity (Fig. 2). In addition, in some derivatives the side chain attached to the dibenzodiazepinone moiety was considerably extended. Ultimately, two symmetrical homo-dimeric (in the following referred to as 'dimeric') dibenzodiazepinone derivatives, considerably varying in linker length, were synthesized and pharmacologically investigated. The binding mode of these compounds is still unknown. Therefore, we prefer the term dimeric ligand over bivalent ligand, as the latter usually implies a specific interaction with a receptor dimer. Binding studies at muscarinic receptors M_1–M_5 were performed to assess the affinity and selectivity of the new ligands (Fig. 2).

2. Results and discussion

2.1. Synthesis of the DIBA derived M receptor ligands

The preparation of piperidine derivatives **15**, **23**,[37] **30**, **31**, **36**, and **39**, which were later linked to the dibenzodiazepinone scaffold, is outlined in Schemes 1 and 2. For the synthesis of compound **15**, the phosphonium salt **7**,[38] prepared in-house by the literature method as a 6:1 mixture of **7a** and **7b** from 1-bromo-4-chlorobutane, and the commercially available piperidinone **8** were converted to intermediate **11** under Wittig conditions (Scheme 1). Correct temperature, reaction time and quantity of base were



Compd.	Ref.	pK_i M_1	M_2	M_3	M_4	M_5
pirenzepine	a	**7.8**	6.0	6.6	7.2	6.6
AF-DX 384	b	7.5	**8.2**	7.2	8.0	6.3
AF-DX 116	c	6.1	**7.0**	5.8	6.3	5.3
AQ-RA 741	c	7.3	**8.3**	7.0	8.1	5.9
BIBN 140	c	6.5	**8.1**	6.4	7.3	6.5
5	d	7.2	**7.5**	7.3	n.a.	n.a.
DIBA	e	8.4	**9.5**	8.0	7.7	n.a.
tripitramine	f	8.8	**9.6**	7.4	8.2	7.5

Figure 1. (A) Structures and binding data of selected tricyclic M receptor antagonists described in literature. (B) Structure of the M receptor allosteric ligand W84. n.a.: no data available. References: (a) Esqueda et al.,[22] (b) Dörje et al.,[23] (c) Doods et al.,[24] (d) Kassiou et al.,[25] (e) Gitler et al.,[19] (f) Maggio et al.[26]

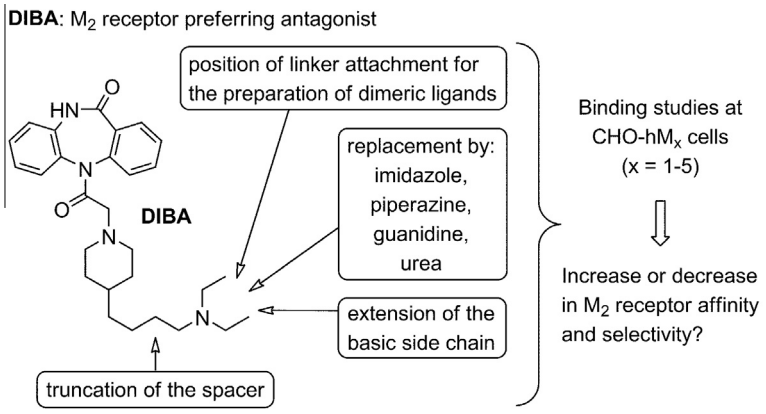
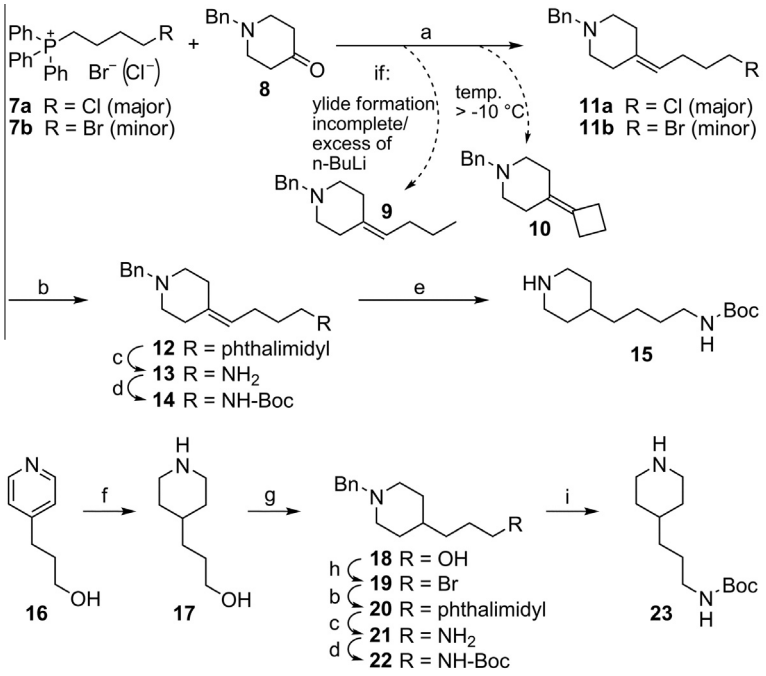


Figure 2. Schematic presentation of the concept and aim of the present study.



Scheme 1. Synthesis of the piperidine derivatives **15** and **23**. Reagents and conditions: (a) *n*-BuLi, THF, 36%; (b) potassium phthalimide, DMF, **12**: 78%, **20**: 70%; (c) hydrazine monohydrate, EtOH, **13**: 87%, **21**: 80%; (d) di-*tert*-butyldicarbonate, triethylamine, CH_2Cl_2, **14**: 95%, **22**: 96%; (e) 20% Pd/C, hydrogen, MeOH, 75%; (f) Pt_2O, hydrogen, 32% HCl, MeOH, 98%; (g) benzyl bromide, K_2CO_3, acetonitrile, 96%; (h) 48% HBr, 78%; (i) 10% Pd/C, hydrogen, MeOH, 90%.

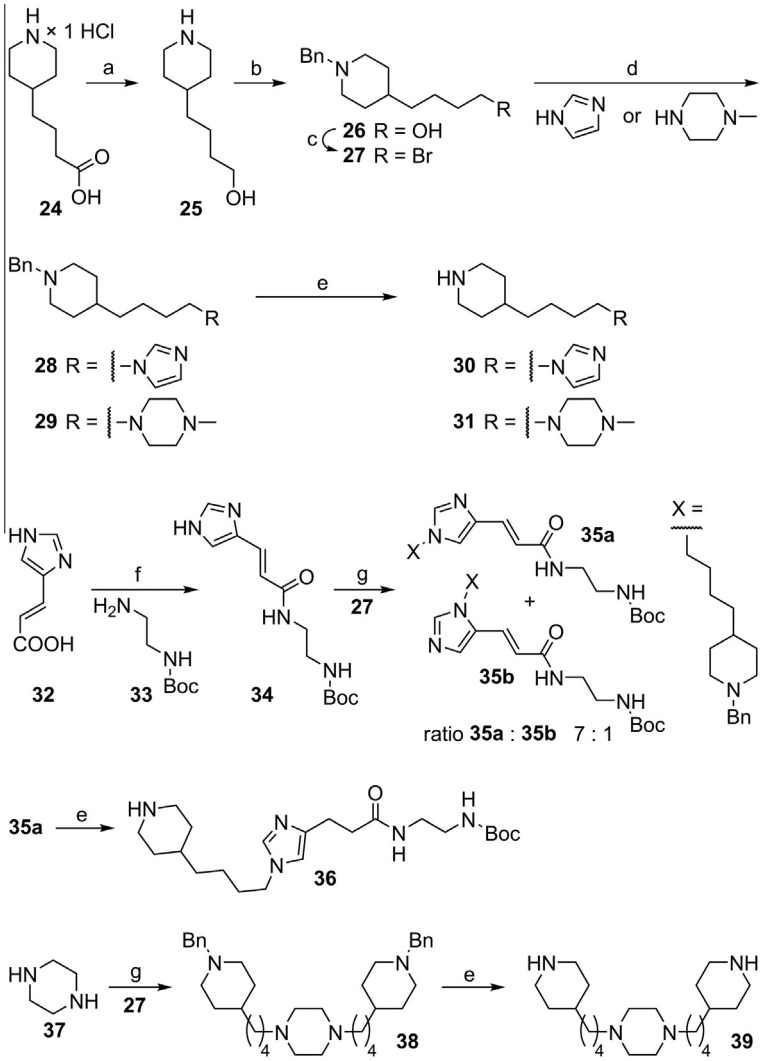
essential in preventing the formation of side products: excess *n*-butyllithium as well as too short periods for ylide formation (resulting in unreacted *n*-butyllithium) probably led to the formation of by-product **9**, and temperatures above −10 °C during ylide formation resulted in the cyclobutylidene derivative **10**. Neither **9** nor **10** (identified by NMR spectroscopy and mass spectrometry; data not shown) could be separated from the product (**11**) by column chromatography. However, using a stoichiometric amount of base and reaction temperatures below −10 °C, the formation of **9** and **10** could be avoided. Treatment of **11**, which comprised a ~40:1 mixture of halides **11a** and **11b**, with potassium phthalimide gave derivative **12**, which in turn was converted to amine **13** by hydrazinolysis. Boc-protection of amine **13** and debenzylation of product **14**, using palladium catalyzed hydrogenolysis, gave piperidine derivative **15** (Scheme 1).

Synthesis of the shorter homolog of **15**, namely compound **23**,[37] began with the pyridinyl alcohol **16**, which was initially reduced by platinum catalyzed hydrogenation to its piperidine analog **17**.[39] Benzyl protection of amino alcohol **17**[39] and treatment of the resulting tertiary amino alcohol **18**[39] with 48% hydrobromic acid gave bromide **19**.[40] Conversion of bromide **19**[40] via phthalimide **20**,[41] amine **21**,[42] and *N*-Boc derivative **22**, into piperidine derivative **23**[37] proceeded using the conditions described for the preparation of **15** from bromide **11** (via **12**, **13**, **14**) (Scheme 1).

Reduction of commercially available carboxylic acid **24** to the alcohol **25**[43] using lithium aluminium hydride, followed by *N*-benzyl protection, gave amino alcohol **26**[44] (Scheme 2). Treatment with 48% hydrobromic acid gave bromide **27**,[45] a key intermediate that was used for the preparation of the remaining piperidine derivatives **30**, **31**, **36**, and **39**.

Nucleophilic displacement of the bromide from compound **27**[45] with imidazole and with 4-methylpiperazine gave intermediates **28** and **29**, respectively, which were subjected to hydrogenolysis to afford **30** and **31** (Scheme 2).

Amidation of urocanic acid (**32**) with amine **33** gave imidazole **34**, which was N-alkylated using bromide **27** to afford a 7:1 mix-



Scheme 2. Synthesis of the piperidine derivatives **30**, **31**, **36** and **39**. Reagents and conditions: (a) $LiAlH_4$, THF, 75%; (b) benzyl bromide, diisopropylethylamine, acetonitrile, 65%; (c) 48% HBr, 78%; (d) K_2CO_3, acetonitrile, **28**: 56%, **29**: 86%; (e) 10% Pd/C, hydrogen, MeOH, **30**: 80%, **31**: 93%, **36**: 89%, **39**: 66%; (f) *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxybenzotriazole (HOBt), DMF, 86%; (g) K_2CO_3, acetonitrile, **35a**/**35b**: 91%, **38**: 48%.

ture of the 1,4- and 1,5-substituted derivative (**35a** and **35b**). A separation of the isomers **35a** and **35b** could be achieved by repeated column chromatography. Simultaneous benzyl deprotection and reduction of the major isomer **35a** by hydrogenolysis gave the piperidine derivative **36**. Meanwhile, twofold alkylation of piperazine (**37**) using bromide **27** yielded intermediate **38**, which was subjected to hydrogenolysis to give compound **39** (Scheme 2).

For the preparation of the dibenzodiazepinone portion of the target molecules, 2-chlorobenzoic acid (**40**) and *o*-phenylenediamine (**41**) were cyclocondensed as previously reported (Scheme 3).[46] Acylation of the resulting dibenzodiazepinone **42** with chloroacetyl chloride gave the chloroacetamide **43**, an essential building block, to which was introduced by N-alkylation, piperidines **15**, **23**, **30**, **31**, **36**, and **39** (cf. Schemes 1 and 2).

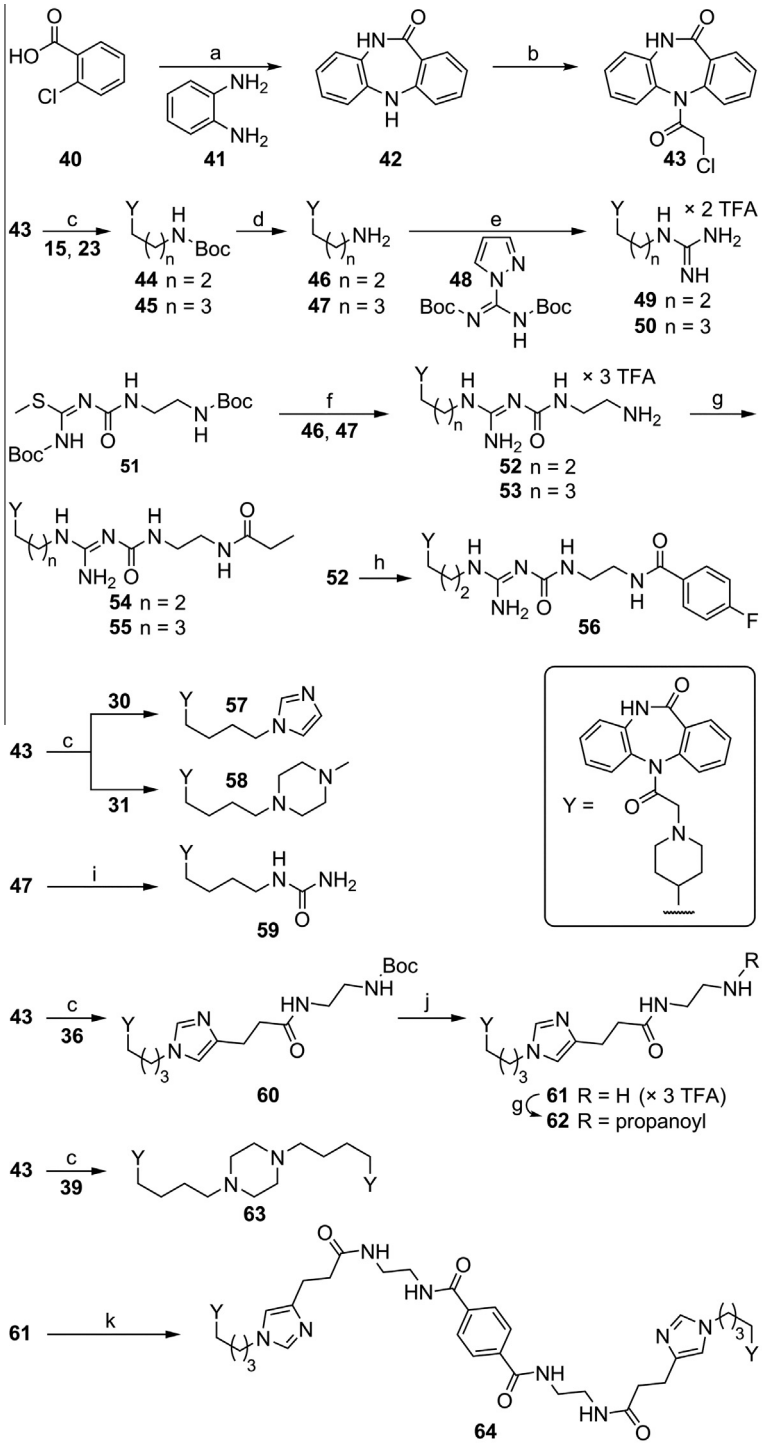
Reaction of chloroacetamide **43** with piperidines **15** and **23** yielded the Boc-protected intermediates **44** and **45**, respectively. These were converted to the primary amines **46** and **47** by treatment with hydrochloric acid. Guanidinylation of amines **46** and **47** using the pyrazole derived guanidinylating reagent **48** and subsequent deprotection afforded the guanidinylated congeners **49** and **50** (Scheme 3). Guanidinylation of **46** and **47** with the *S*-methylisothiourea derivative **51** followed by treatment with trifluoroacetic acid afforded compounds **52** and **53**, which represent N^G-carbamoylated congeners of compounds **49** and **50**. Propanoylation of **52** and **53** with succinimidyl propionate resulted in compounds **54** and **55**, and 4-fluorobenzoylation of **52** using succinimidyl 4-fluorobenzoate gave derivative **56**.

Reaction of chloroacetamide **43** with piperidines **30** and **31** resulted directly in the target compounds **57** and **58**, bearing a terminal imidazolyl and *N*-methylpiperazinyl moiety, respectively. The ureido derivative **59** was obtained by treatment of amine **47** with isocyanic acid liberated from potassium cyanate.

Reaction of building block **43** with piperidine **36** gave the Boc-protected intermediate **60**, which was treated with TFA to obtain amine **61**. Propanoylation of the latter afforded compound **62**. The dimeric ligand **63** was obtained by treatment of compound **39** with **43**, and the twin compound **64** was prepared by acylation of amine **61** using terephthalic acid chloride (Scheme 3).

2.2. Binding studies at muscarinic receptors

The dibenzodiazepinone-type M receptor ligands were investigated in equilibrium binding experiments applying intact CHO cells stably expressing the human muscarinic receptor subtypes M_1–M_5. The muscarinic antagonist [^{3}H]NMS was used as radioligand. Receptor saturation binding experiments with [^{3}H]NMS were



Scheme 3. Synthesis of building block **43** and dibenzodiazepinone derivatives **46**, **47**, **49**, **50**, **52**–**59**, **61**–**64**. Reagents and conditions: (a) copper bronze, chlorobenzene, 27%; (b) *N*,*N*-dimethylaniline, chloroacetyl chloride, THF, 79%; (c) K_2CO_3, acetonitrile, **44**: 60%, **45**: 93%, **57**: 73%, **58**: 81%, **60**: 70%, **63**: 37%; (d) **46**: 5 M HCl, 85%, **47**: HCl (gas), CH_2Cl_2/MeOH 10:1, 93%; (e) (1) triethylamine, CH_2Cl_2; (2) TFA, H_2O, CH_2Cl_2, **49**: 93%, **50**: 67%; (f) (1) $HgCl_2$, DMF or DMSO; (2) TFA, H_2O, CH_2Cl_2 or MeOH, **46**: 82%, **47**: 66%; (g) succinimidyl propionate, triethylamine or diisopropylethylamine, DMF, **54**: 70%, **55**: 87%, **62**: 91%; (h) succinimidyl 4-fluorobenzoate, diisopropylethylamine, DMF, 56%; (i) KOCN, 1 M HCl, EtOH, H_2O, 59%; (j) TFA, H_2O, MeOH, 96%; (k) terephthalic acid chloride, diisopropylethylamine, DMF, CH_2Cl_2, 64%.

performed to determine the equilibrium dissociation constant ($pK_d = -\log K_D$ value) of [^{3}H]NMS at the five subtypes M_1–M_5. The pK_d values amounted to 9.85 (M_1), 10.1 (M_2), 10.1 (M_3), 10.5 (M_4) and 9.63 (M_5) and were in good agreement with previously reported data[23] obtained from saturation binding at membranes of CHO cells expressing the receptor of interest (CHO-hM_x cells (x = 1–5); see Supplementary material). Competition binding experiments with four reference ligands (atropine, pirenzepine, 4-DAMP and propantheline) at the five M receptor subtypes revealed pK_i values that were in good accordance with reported data (see Supplementary Table S2).

The new DIBA derivatives **46**, **47**, **49**, **50**, **52**, **54**–**59**, **61**–**64** (Fig. 3) as well as AF-DX 384 (**1**) and **6** were studied in equilibrium binding experiments at M_2 receptors applying the orthosteric

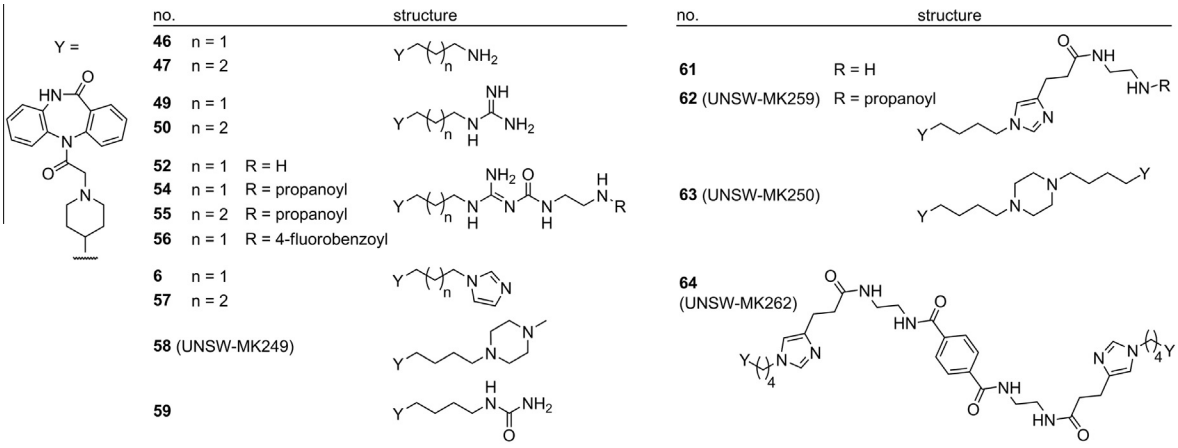


Figure 3. Structures of the investigated dibenzodiazepinone derivatives.

muscarinic antagonist radioligand [^{3}H]NMS (Fig. 4). Since the mode(s) of potential orthosteric/allosteric receptor interactions of the compounds upon inhibition of radioligand binding were unknown, data from equilibrium binding studies were analyzed by simple logistic curve fitting, and not according to the ternary complex model.[47] The resulting pIC_{50} values and slope factors are summarized in Table 1. Figure 4A shows the curves of **1** and a subset of six dibenzodiazepinone derivatives (**6**, **46**, **49**, **52**, **54**, **56**), which all contain a three-membered carbon chain (instead of a tetramethylene chain as in DIBA) connecting the piperidine ring and the terminal basic group (cf. Figs. 1 and 2).

Within the designated subset of compounds (Fig. 4A), **1** and the imidazole derivative **6** exhibited the highest M_2 receptor affinity (pIC_{50} = 8.3 and 8.29, respectively), two orders of magnitude higher compared to the primary amine **46** (pIC_{50} = 6.21) (Table 1), showing that the replacement of the terminal amine group in **46** by an imidazole moiety (**6**) considerably favors the interaction with the M_2 receptor. The compounds **49**, **52**, **54**, **56**, all containing a guanidine, revealed pIC_{50} values between 7.0 and 7.5, that is, substitution of the guanidine group, resulting in markedly longer side chains as in **54** and **56**, had only little impact on M_2 affinity (pIC_{50} of **49**: 7.50 ± 0.05 *vs.* pIC_{50} of **54**: 7.21 ± 0.05 (P <0.05) and **56**: 7.16 ± 0.04 (P <0.05) (Fig. 4A, Table 1).

The displacement curves of the second subset of compounds, comprising **47**, **50**, **55**, and **57**, the higher homologues of **46**, **49**, **54**, **6**, as well as the ureido derivative **59** (cf. Fig. 3), are depicted in Figure 4B. A markedly higher M_2 affinity resided in the higher homolog compared to the lower homolog in case of the primary amines **46** and **47** (pIC_{50} = 6.21 ± 0.14 and 7.40 ± 0.07, respectively, P <0.01) and the guanidines **49** and **50** (pIC_{50} = 7.50 ± 0.05 and 8.33 ± 0.08, respectively, P <0.01) (Table 1). By contrast, the difference in M_2 affinity was low for the N^G-substituted compounds **54** and **55** (pIC_{50} = 7.21 ± 0.05 and 7.69 ± 0.08, respectively, P <0.02) and the imidazole derivatives **6** and **57** (pIC_{50} = 8.29 ± 0.06 and 8.01 ± 0.05, respectively, P <0.05) (cf. Table 1). Compound **59** represents the non-basic ureido congener of the guanidine derivative **50** (Fig. 3). The lower M_2 affinity of **59** compared to **50** (pIC_{50} = 6.92 ± 0.03 and 8.33 ± 0.08, respectively, P <0.01) suggested that a basic group is indeed favorable with respect to M_2 receptor affinity.

The effect on [^{3}H]NMS equilibrium binding of the third subset of compounds, comprising the piperazine derivatives **58** and **63** and the di-substituted imidazole derivatives **61**, **62** and **64** (Fig. 3), is depicted in Figure 4C. These compounds exhibited the highest M_2 affinities within the herein presented series of dibenzodiazepinone derivatives with pIC_{50} values in the range of 8.6–9.2 (Table 1). Regarding the two pairs of monomeric/dimeric ligands **58**/**63** and **62**/**64**, the linkage of two dibenzodiazepinone moieties as in the dimeric ligands **63** and **64**, resulted in slightly increased affinities compared to the monomeric counterparts **58** and **62** (**58**/**63**: pIC_{50} = 8.66 ± 0.06 and 8.98 ± 0.06, respectively, P <0.02; **62**/**64**: pIC_{50} = 8.60 ± 0.05 and 9.20 ± 0.05, respectively, P <0.001). Interestingly, although the linkers in **63** and **64** differ in the chemical nature and by 22 atoms in length, both compounds showed comparable M_2 affinities (pIC_{50} = 9.0 and 9.2, respectively). Whereas the slope of the displacement curve was significantly increased for the dimeric ligand **63** (slope = −2.01, P <0.001, Table 1), the slope obtained for the dimeric compound **64** with the longer spacer was not significantly different from unity (P >0.05). The steepness of the curve, as indicated by a slope of −2.01 in case of the dimeric ligand **63**, suggests an unusual binding mode, which remains to be resolved in future studies. At M_2 receptors the lower plateaus of the curves for the inhibition of [^{3}H]NMS equilibrium binding were not different from zero percent specific [^{3}H]NMS binding (P >0.05) for all compounds (Fig. 4).

The propionamide **62**, exhibiting high M_2 affinity (pIC_{50} = 8.60), represents the non-labeled ('cold') form of a potential radioligand, which is available by treatment of amine precursor **61** with commercially available succinimidyl [^{3}H]propionate. The radiolabeled analog of **62** might be an interesting alternative to radioligands such as [^{3}H]AF-DX 384 or [^{3}H]AF-DX 116.

Reference compound **1** and a selection of the new dibenzodiazepinone derivatives, comprising primary amine **46**, guanidine derivative **50**, imidazole derivative **57**, and the two pairs of monomeric/dimeric ligands **58**/**63** and **62**/**64**, were also studied in equilibrium binding experiments with [^{3}H]NMS at the M receptor subtypes M_1, M_3, M_4 and M_5. The respective [^{3}H]NMS displacement curves are shown in Figure 5, the pIC_{50} values and slopes are included in Table 1. The receptor subtype selectivity profile was similar for these compounds: all compounds showed a preference for the M_2 receptor. Lower affinities were obtained for the subtypes M_1 and M_4, and the lowest affinities were found at subtypes M_3 and M_5, that is, the selectivity pattern can be summarized as $M_2 > M_1 \approx M_4 > M_3 \approx M_5$ (**46**, **50**, **57**, **62**–**64**) and $M_2 > M_1 \approx M_4 > M_3 > M_5$ (**1**, **58**).

It should be noted that, due to comparable fractional receptor occupancies ($frac_{occ} = L/L + K_D$) of [^{3}H]NMS at each subtype (ranging from $frac_{occ[^3H]NMS}$ = 0.55 at M_5 to 0.74 at M_4, cf. footnote Table 1), the selectivity profile would not change after conversion of the pIC_{50} values to pK_i values via the Cheng–Prusoff equation (assuming a competitive interaction throughout), which was not done for reasons given below. Moderately increased slopes, significantly different from −1 (t-test) were obtained at the M_1 receptor for the dimeric ligands **63** (P <0.01) and **64** (P <0.01), and at the M_3

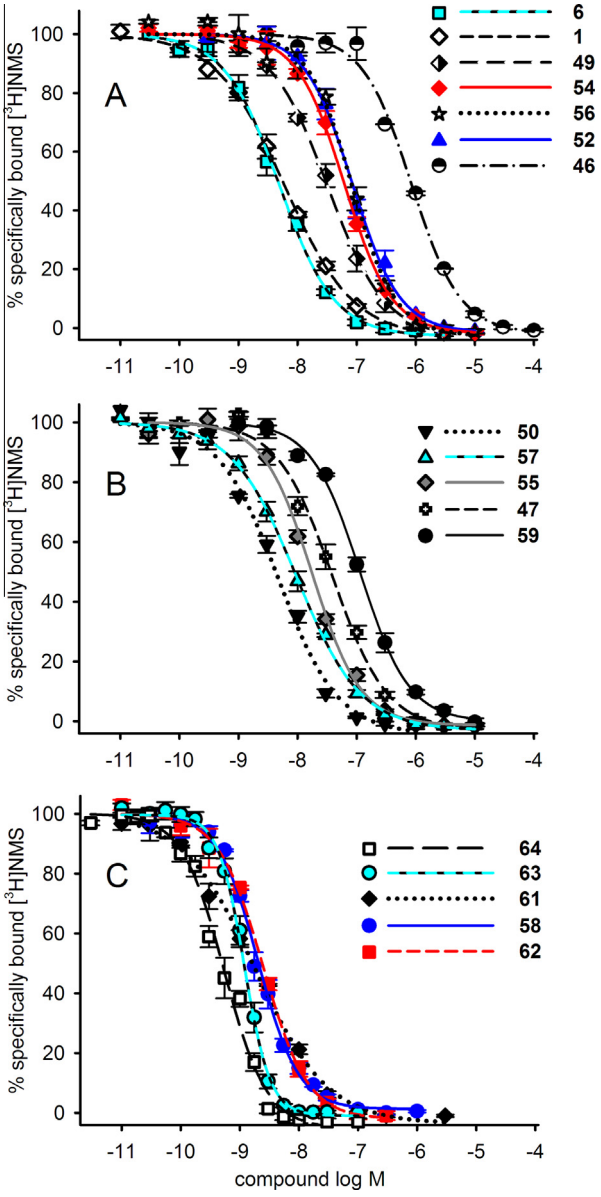


Figure 4. Concentration-dependent effects of **(A)** the tricyclic M receptor ligands AF-DX-384 (**1**), **6**, **46**, **49**, **52**, **54**, **56**, **(B) 47**, **50**, **55**, **57**, **59** and **(C) 58**, **61**–**64** on [^{3}H]NMS equilibrium binding in muscarinic M_2 receptors. Live CHO-hM_2 cells were incubated with 0.2 nM [^{3}H]NMS (K_d = 0.090 nM) and the compound of interest at 23 °C for 3 h. Data points represent mean values ± SEM from 2 (**49**), 3 (**1**, **6**, **46**, **50**, **54**–**59**, **61**, **62**), 4 (**47**, **52**), 5 (**64**) and 9 (**63**) independent experiments (each performed in triplicate). (For interpretation to colors in this figure, the reader is referred to the web version of this paper.)

subtype for **1** (P <0.05) and **64** (P <0.05) (Table 1). In the equilibrium binding experiments performed at subtypes M_1, M_3, M_4 and M_5 the lower plateaus of the four-parameter logistic curves were not significantly different from zero percent specific [^{3}H]NMS binding throughout for all investigated compounds at all subtypes (P >0.05).

Since an interaction with allosteric sites at the M_2 receptor was proposed for **1**, **2**, the dimeric M receptor antagonist methoctramine, as well as the trimeric pyridodibenzodiazepinone tripitramine,[31,36,48–50] the reference compound **1** and the two pairs of monomeric/dimeric ligands **58**/**63** and **62**/**64**, were studied with respect to a potential contribution of allosteric receptor binding. For this purpose these compounds were also studied at [^{3}H]NMS occupied M_2 receptors, that is, their effect on the time course of [^{3}H]NMS dissociation was determined. In these experiments the dissociation of [^{3}H]NMS is determined in the absence and in the presence of the allosteric ligand. The ratio of the rate constants of [^{3}H]NMS dissociation determined in the presence of allosteric modulator (k_{-1} values) to the rate constant of [^{3}H]NMS dissociation determined in the absence of modulator ($k_{-1(0)}$) was plotted against the log concentration of test compound and the data were fitted by a four parameter logistic equation (Fig. 6).

All compounds were capable of inhibiting [^{3}H]NMS dissociation almost completely (cf. $E_{max,diss}$ values in Table 2). The $pEC_{50,diss}$ values summarized in Table 2 can be interpreted as estimates of affinities to the allosteric site, assuming that an occupation of the allosteric site becomes obvious by a retardation of NMS dissociation. The $pEC_{50,diss}$ value of 4.27 obtained for **1** was in good agreement with a previously reported $pEC_{50,diss}$ value of 4.65 determined at porcine heart membranes using the same type of experiment with an incubation temperature of 37 °C.[30] The strongest effect was observed for the dimeric ligand **64** ($pEC_{50,diss}$ = 5.56, Table 2), which proved to be almost as potent as the pure allosteric M receptor modulator W84 ($pEC_{50,diss}$ = 5.87; porcine heart M_2 receptor, phosphate/TrisHCl buffer, 37 °C).[30] Remarkably, compound **64** was about 100 times more potent than its monomeric counterpart **62** ($pEC_{50,diss}$ values of **64** and **62**: 5.56 ± 0.04 vs 3.59 ± 0.07, P <0.001) (Table 2). Likewise, the dimeric ligand **63** was more potent compared to its monomer **58** by one order of magnitude ($pEC_{50,diss}$ values: 5.10 ± 0.01 and 4.02 ± 0.02, respectively, P <0.01) (Table 2).

These results suggest that chemical dimerization of dibenzodiazepinone-type M receptor ligands favors allosteric binding, a phenomenon observed with tacrine and a tacrine dimer containing a hexamethylene spacer.[51] However, the ability of the compounds to inhibit [^{3}H]NMS equilibrium binding was much stronger than their capability to retard the [^{3}H]NMS dissociation ($pIC_{50} \gg pEC_{50,diss}$, cf. Figs. 3 and 5, respectively). Therefore, the contribution of allosteric interactions to binding of the new compounds to 'free' muscarinic receptors seems to be rather small. Assuming that the displacement of [^{3}H]NMS is exclusively attributed to competitive binding to the orthosteric site, the compounds could be formally described as competitive antagonists and IC_{50} values from equilibrium binding experiments converted to K_i values via the Cheng–Prusoff equation. However, as this is uncertain, in particular in the case of the dimeric ligands **63** and **64**, the authors decided to present pIC_{50} values (Table 1). Aiming at unmasking a potential allosteric receptor binding by an incomplete inhibition of [^{3}H]NMS equilibrium binding at high concentrations, compounds **58**, **62**–**64** were also studied at a tenfold higher [^{3}H]NMS concentration of 2 nM. However, this approach in neither case elevated the bottom level of the respective curve above zero percent specific [^{3}H]NMS binding (Fig. 7), suggesting either a formally competitive, or a very strong negatively cooperative interaction as previously described for **1**.[31]

3. Summary and conclusion

In this study the synthesis and pharmacological characterization of various new dibenzodiazepinone-type muscarinic receptor ligands, including two homo-dimeric compounds, was presented. For 16 compounds M_2 receptor binding data were determined by equilibrium binding studies with [^{3}H]NMS, a muscarinic receptor antagonist binding orthosterically to M receptors, and for seven selected ligands, additionally, on the subtypes M_1, M_3, M_4 and M_5. The terminal basic group of the previously described M_2 preferring muscarinic receptor antagonist DIBA was replaced by imidazole, guanidine, and piperazine, which all proved to be bioisosteric moieties. In some compounds the basic side chain was considerably extended by introducing additional substituents

Table 1
pIC_{50} values and curve slopes obtained from nonlinear logistic curve analyses of AF-DX 384 (**1**) and the dibenzodiazepinone-type M receptor ligands **46**, **47**, **49**, **50**, **52**, **54**–**59**, **61**–**64** characterizing the inhibition of [^{3}H]NMS equilibrium binding to the indicated M receptor subtypes in live CHO-hM_x cells (x = 1–5)

Compound	M_1[a]		M_2[b]		M_3[c]		M_4[d]		M_5[e]	
	pIC_{50}	Slope	pIC_{50}	Slope	pIC_{50}	Slope	pIC_{50}	Slope	pIC_{50}	Slope
1	7.36 ± 0.18	−0.93 ± 0.09	8.23 ± 0.03	−0.74 ± 0.09	6.24 ± 0.09	−1.26 ± 0.04[#]	7.72 ± 0.11	−0.69 ± 0.05[#]	5.68 ± 0.06	−1.05 ± 0.06
6	n.d.	n.d.	8.29 ± 0.06	−1.03 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
46	5.80 ± 0.05	−0.94 ± 0.10	6.21 ± 0.14	−0.83 ± 0.13	5.14 ± 0.05	−1.05 ± 0.11	5.93 ± 0.04	−1.04 ± 0.12	5.46 ± 0.05	−0.83 ± 0.10
47	n.d.	n.d.	7.40 ± 0.07	−0.98 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
49	n.d.	n.d.	7.50 ± 0.05	−0.89 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
50	7.63 ± 0.07	−0.99 ± 0.07	8.33 ± 0.08	−0.92 ± 0.10	6.92 ± 0.06	−0.86 ± 0.06	7.72 ± 0.01	−0.82 ± 0.10	6.83 ± 0.07	−0.92 ± 0.14
52	n.d.	n.d.	7.04 ± 0.13	−1.10 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
54	n.d.	n.d.	7.21 ± 0.05	−1.12 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
55	n.d.	n.d.	7.69 ± 0.08	−1.14 ± 0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
56	n.d.	n.d.	7.16 ± 0.04	−0.99 ± 0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
57	7.34 ± 0.03	−1.05 ± 0.03	8.01 ± 0.05	−0.81 ± 0.02[#]	6.65 ± 0.01	−0.86 ± 0.07	7.61 ± 0.08	−0.81 ± 0.18	6.65 ± 0.06	−1.03 ± 0.13
58	7.57 ± 0.08	−1.13 ± 0.10	8.66 ± 0.06	−1.25 ± 0.08	6.60 ± 0.08	−0.92 ± 0.09	7.88 ± 0.11	−0.99 ± 0.09	6.01 ± 0.09	−0.97 ± 0.14
59	n.d.	n.d.	6.92 ± 0.03	−0.98 ± 0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
61	n.d.	n.d.	8.72 ± 0.04	−0.82 ± 0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
62	7.70 ± 0.06	−1.11 ± 0.11	8.60 ± 0.05	−1.19 ± 0.06	6.68 ± 0.08	−1.09 ± 0.08	8.05 ± 0.02	−0.86 ± 0.03	6.40 ± 0.08	−0.68 ± 0.11
63	8.63 ± 0.04	−1.32 ± 0.09[#]	9.00 ± 0.19	−2.01 ± 0.07[#]	7.63 ± 0.03	−1.05 ± 0.14	8.38 ± 0.08	−1.32 ± 0.17	7.29 ± 0.08	−1.09 ± 0.09
64	8.55 ± 0.05	−1.56 ± 0.08[#]	9.20 ± 0.05	−1.27 ± 0.12	7.34 ± 0.03	−1.30 ± 0.09[#]	8.60 ± 0.08	−1.17 ± 0.14	7.09 ± 0.10	−1.46 ± 0.18

Presented are mean values ± SEM from 2–9 independent experiments (performed in triplicate). K_d values/applied concentrations of [^{3}H]NMS:
[a] 0.15/0.2 nM ($frac_{occ}$ = 0.57).
[b] 0.090/0.2 nM ($frac_{occ}$ = 0.69).
[c] 0.089/0.2 nM ($frac_{occ}$ = 0.69).
[d] 0.035/0.1 nM ($frac_{occ}$ = 0.74).
[e] 0.24/0.3 nM ($frac_{occ}$ = 0.55).
[#] Significantly different (P <0.05) from −1.

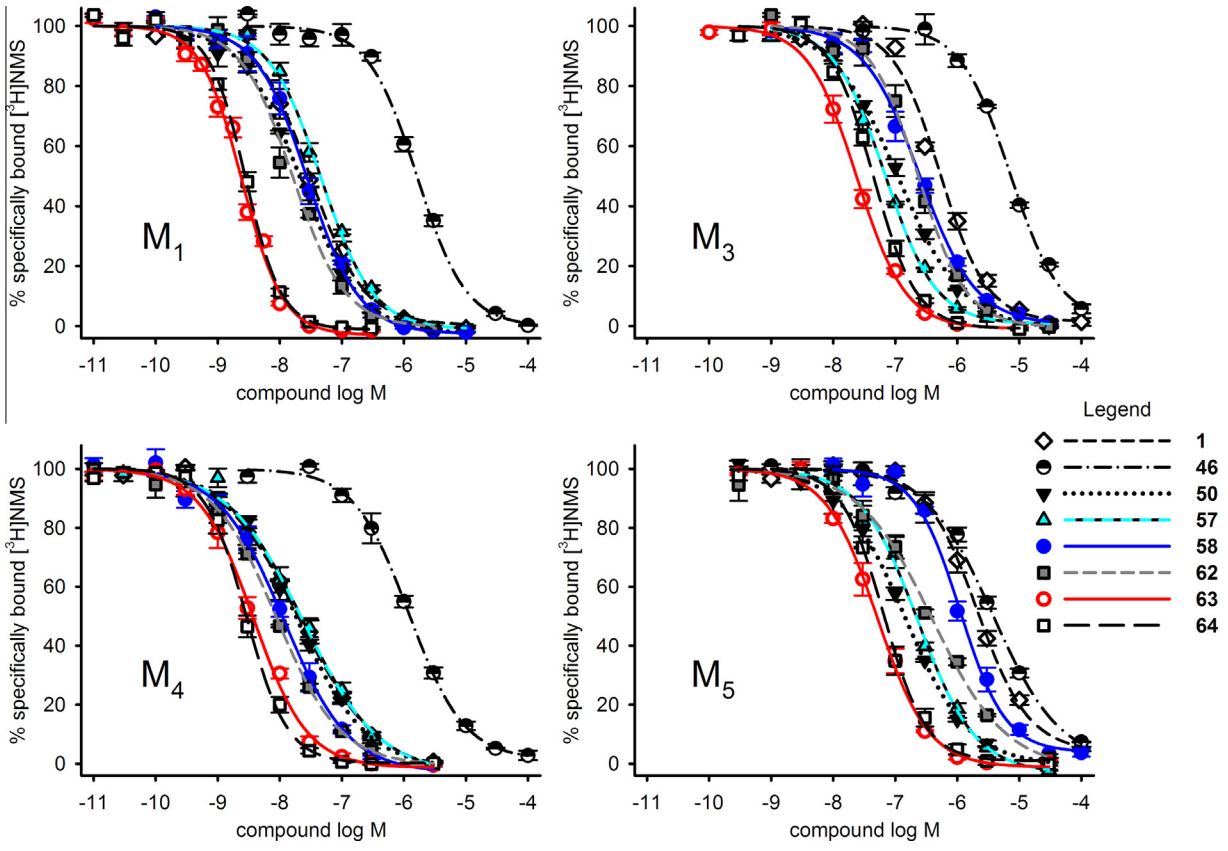


Figure 5. Concentration-dependent effects of the tricyclic M receptor ligands AF-DX-384 (**1**), **46**, **50**, **57**, **58**, **62**–**64** on [^{3}H]NMS equilibrium binding at muscarinic receptors M_1, M_3, M_4, M_5. Intact CHO-hM_x cells (x = 1, 3, 4, 5) were incubated with [^{3}H]NMS (c = 0.2 nM (M_1, M_3), 0.1 nM (M_4) or 0.3 nM (M_5)) and the compound of interest at 23 °C for 3 h. Data points represent mean values ± SEM from at least 3 independent experiments (each performed in triplicate). (For interpretation to colors in this figure, the reader is referred to the web version of this paper.)

at the imidazole or guanidine moiety. Interestingly, the extension of the side chain retained and could even enhance receptor binding properties compared to the reference compound AF-DX 384 (**1**), as became obvious from displacement of [^{3}H]NMS from M receptors (Figs. 4 and 5, Table 1). The dimeric ligands UNSW-MK250 (**63**) and UNSW-MK262 (**64**) exhibited the highest M_2 receptor affinities

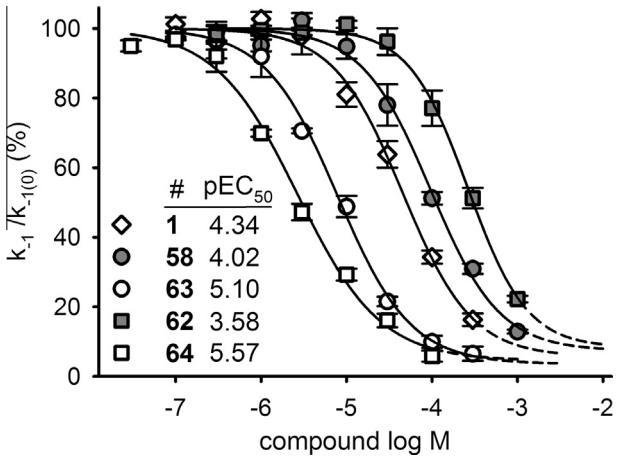


Figure 6. Concentration-dependent effect of AF-DX 384 (**1**), the monomeric dibenzodiazepinone derivatives **58**, **62** and the dimeric ligands **63**, **64** on the rate constant of [^{3}H]NMS dissociation at muscarinic M_2 receptors. The dissociation of [^{3}H]NMS (c = 1 nM) was studied in the presence of atropine (c = 500 nM) alone (determination of $k_{-1(0)}$), and in the presence of atropine (500 nM) plus the compound of interest at increasing individual concentrations (determination of k_{-1}) over a period of 2 h. Data points represent mean values ± SEM from 2 (**1**, **58**) or 3 (**62**–**64**) independent experiments (each performed in triplicate). The dashed lines mean extrapolation of the curves.

Table 2
Parameters characterizing the inhibition of the dissociation of [^{3}H]NMS from muscarinic M_2 receptors by AF-DX 384 (**1**), **58**, **62**–**64**

Compound	$pEC_{50,diss}$	Slope	$E_{max,diss}$ (%)
1	4.27 ± 0.08	−0.99 ± 0.10	99 ± 6.0
58	4.02 ± 0.02	−1.09 ± 0.09	93 ± 1.6
62	3.59 ± 0.07	− 1.09 ± 0.03	92 ± 5.3
63	5.10 ± 0.01	−1.01 ± 0.06	97 ± 2.5
64	5.56 ± 0.04	−0.97 ± 0.12	95 ± 3.1

Experiments were performed with live CHO-hM_2 cells at 23 °C. Presented are mean values ± SEM from 2 (**1**, **58**) or 3 (**62**–**64**) independent experiments (performed in triplicate). All values obtained for the slope and $E_{max,diss}$ were not significantly different from −1 and 100%, respectively (P >0.05).

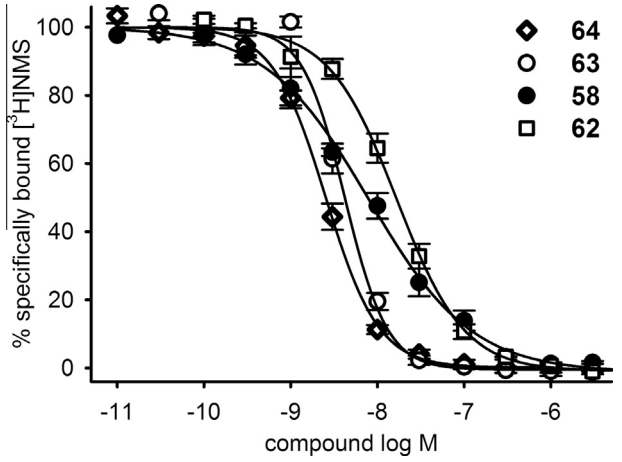


Figure 7. Concentration-dependent effects of the dibenzodiazepinones **58**, **62**–**64** on [^{3}H]NMS equilibrium binding at a concentration of 2 nM. Note, that elevating the concentration of [^{3}H]NMS did *not* unmask the formation of putative allosteric ternary complexes by an elevated lower plateau of the inhibition curve. Intact CHO-hM_2 cells were incubated with 2 nM [^{3}H]NMS (K_d = 0.090 nM) and the compound of interest at 23 °C for 3 h. Data points represent mean values ± SEM from 3 (**58**), 4 (**62**, **63**) and 7 (**64**) independent experiments (each performed in triplicate), respectively. pIC_{50} values/curve slopes amounted to: 8.60 ± 0.04/−1.49 ± 0.08 (**64**) 8.42 ± 0.05/−1.72 ± 0.09 (**63**), 8.08 ± 0.11/−0.81 ± 0.11(**58**) and 7.77 ± 0.07/−1.14 ± 0.08 (**62**).

(pIC_{50} = 9.0 and 9.2, respectively). All compounds, which were investigated at all subtypes in equilibrium binding experiments applying [^{3}H]NMS, shared a similar selectivity profile, which can be summarized as $M_2 > M_1 \approx M_4 > M_3 \approx M_5$ (**46**, **50**, **57**, **62**–**64**) and $M_2 > M_1 \approx M_4 > M_3 > M_5$ (**1**, **58**). Steep curve slopes were observed for the dimeric ligand **63** at the M_2 receptor (slope = −2.0) and for the dimeric ligand **64** at the M_1 receptor (slope = −1.56), suggesting a complex mechanism of binding. In addition to the equilibrium binding experiments applying [^{3}H]NMS, the retarding effect of the homo-dimeric ligands **63** and **64** as well as their monomeric counterparts UNSW-MK249 (**58**) and UNSW-MK259 (**62**) on the dissociation of [^{3}H]NMS from M_2 receptors was determined. In these investigations, with a $pEC_{50,diss}$ of 5.56, the dimeric dibenzodiazepinone derivative **64** showed the strongest effect. As the monomeric counterpart of **64** was 100 times less potent (**62**, $pEC_{50,diss}$ = 3.59), these data are compatible with the assumption that chemical dimerization of dibenzodiazepinone-type M receptor ligands favors allosteric binding, but cannot be considered a proof. Interestingly, although the increase in putative allosteric binding of **64** compared to **62** was 100-fold, and the increase in orthosteric affinity amounted only to a factor of 4, this was not reflected by positive cooperativity between **64** and NMS. Principally, the role and the extent of orthosteric and allosteric interactions involved in the binding of such compounds in the sense of a dualsteric (bitopic) interaction, as well as the potential impact on functional selectivity remain unresolved challenging questions.[52] Aiming at an elucidation of the topology of the binding domains of the DIBA derivatives presented in this study, apart from crystal structures of receptor-ligand complexes, pharmacological tools such as a radiolabeled **64**, the study of ligands with capped linkers, and orthosterical/allosterical loss of function M receptor mutants might be included in future studies.

4. Experimental section

4.1. Chemistry: Experimental protocols and analytical data

4.1.1. General experimental conditions

Compounds **8**, **16**, **24**, **32**, **40**, **41**, benzyl bromide, 1-bromo-2-chlorobutane, *n*-butyllithium, chloroacetyl chloride, copper bronze, di-*tert*-butyldicarbonate, *N*,*N*-dimethylaniline, imidazole, lithium aluminiumhydride and platinum(IV)oxide were purchased from Sigma–Aldrich. Potassium phthalimide was obtained from Merck–Schuchardt and succinimidyl [2-(Boc-amino)ethyl]carbamate was from Fluka. EDC, HOBt and *N*-methylpiperazine were purchased from Alfa Aesar. Piperazine was obtained from Columbia Organic Chemicals, and terephthaloyl chloride was from Lancaster. The syntheses of *N*-Boc-*S*-methylisothiourea,[53] guanidinylating reagent **48**,[54] succinimidyl propionate,[55] and succinimidyl 4-fluorobenzoate[54] were described elsewhere. Solvents were obtained from commercial suppliers and used without further purification. A Biotage Initiator microwave synthesizer (Biotage, Uppsala, Sweden) was used for microwave driven reactions. Thin layer chromatography was performed on Merck silica gel 60 F_{254} TLC aluminum plates. For column chromatography silica gel DAVISIL (0.040–0.063 mm; GRACE Davison, Worms, Germany) was used. NMR spectra were recorded on Bruker Avance 300 (7.05 T, ^{1}H: 300 MHz, ^{13}C: 75 MHz), Bruker Avance 400 (9.40 T, ^{1}H: 400 MHz, ^{13}C: 100 MHz), Bruker Avance 500 (11.75 T, ^{1}H: 500 MHz, ^{13}C: 125 MHz), Bruker Avance 600 (14.1 T, ^{1}H: 600 MHz, ^{13}C: 150 MHz) and Bruker Avance 700 (16.4 T, ^{1}H: 700 MHz) instruments (Bruker, Karlsruhe, Germany). Low-resolution mass spectrometry (MS) was performed on a Waters Micromass ZQ™ Detector (Waters, Milford, MA, US). High-resolution mass spectrometry (HRMS) was performed on an Orbitrap LTQ XL ion trap mass spectrometer (Thermo Fisher Scientific, San Jose,

CA, US) using an electrospray ionization (ESI) source. Elemental analysis was carried out by the Microanalytical Unit, Research School of Chemistry, The Australian National University, Canberra, on a Carlo Erba 1106 instrument. IR spectra were measured on a Nicolet™ 380 spectrophotometer (Thermo Electron Corporation). Melting points were determined with a MEL-TEMP II apparatus (Laboratory Devices Inc., US) and are uncorrected.

Annotation concerning the NMR spectra (^{1}H, ^{13}C) of the dibenzodiazepinone derivatives (compounds **44**–**47**, **49**, **50**, **52**–**64**): Due to a slow rotation about the exocyclic amide group on the NMR time scale, two isomers (ratios provided in the experimental protocols) were evident in the NMR spectra. Provided that adjacent signals in the ^{13}C NMR spectra could be unambiguously clarified (using ^{1}H COSY and HSQC data) to arise from one carbon nucleus, these signals were depicted as a set of signals (e.g., 123.7/123.9 ppm).

4.1.2. 4-Chlorobutyltriphenylphosphonium bromide (7a)/4-bromobutyltriphenylphosphonium chloride (7b)[38]

Triphenylphosphine (160.6 g, 0.61 mol) and 1-bromo-4-chlorobutane (105 g, 0.61 mol) were dissolved in anhydrous toluene (500 mL) and the mixture was refluxed for 16 h (after 10 min of reflux a white solid began to precipitate). The reaction mixture was cooled to rt and the liquid was removed by filtration. The white solid was washed twice with toluene and hexane and dried in vacuo at 70 °C to give the salt **7a**/**7b** as 6:1 mixture (198.3 g, 75%). IR (Nujol) 1460, 1375, 1110 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) (**7a**) 1.72 (m, 2H), 1.96 (p, 2H, *J* 6.8 Hz), 3.63–3.73 (m, 2H), 3.76 (t, 2H, *J* 6.4 Hz), 7.88–7.90 (m, 12H), 7.91–7.98 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) (**7a**) 20.0, 20.7, 33.2, 33.5, 45.1, 118.8, 119.9, 131.1, 131.3, 134.5, 134.6, 135.9. MS (ESI, MeOH) *m*/*z* (%) 397/399 (10/10) [*M*+H]$^+$ (**7b**), 353/355 (100/34) [*M*+H]$^+$ (**7a**). $C_{22}H_{23}BrClP$ (433.7).

4.1.3. 1-Benzyl-4-(4-chlorobutylidene)piperidine (11a)/1-benzyl-4-(4-bromobutylidene) piperidine (11b)

Under an atmosphere of argon **7a**/**7b** (2 g, 4.61 mmol) was suspended in anhydrous THF (15 mL) and the mixture was cooled to −72 °C. *n*-Butyllithium (2.5 M in *n*-hexane, 1.68 mL, 4.19 mmol) was added to the stirred suspension and the mixture was allowed to warm up to −10 °C over a period of 45 min. During that period the color of the mixture changed from yellow via orange to red. The temperature was kept at −10 °C for 1.5 h and then cooled again to −72 °C. *N*-Benzylpiperidin-4-one (**8**) (0.79 g, 4.19 mmol) was added, the mixture was allowed to warm up to rt over a period of 60 min and stirring was continued for 16 h. Solid material was removed by filtration and the filtrate column chromatographed using mixtures of CH_2Cl_2 and EtOAc as eluent (R_f = 0.5 for *n*-hexane/EtOAc 1:1) to give the product **11a**/**11b** (ratio ca 40:1) as a yellowish oil (0.393 g, 36%). IR (neat) 2935, 2900, 2795, 2360, 2340 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) (**11a**) 1.80 (m, 2H), 2.15–2.35 (m, 4H), 2.33 (t, 2H, *J* 5.5 Hz), 2.47 (m, 4H), 3.55 (s, 2H), 3.56 (t, 2H, *J* 6.5 Hz), 5.17 (t, 1H, *J* 7.4 Hz), 7.25–7.41 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) (**11a**) 25.9, 29.5, 34.7, 37.4, 45.9, 56.4, 57.2, 64.9, 123.1, 129.3, 130.1, 131.6, 139.1, 139.3. MS (ESI, MeOH) *m*/*z* (%) 308/310 (2/2) [*M*+H]$^+$ (**11b**), 264/266 (100/34) [*M*+H]$^+$ (**11a**). HRMS (ESI, MeOH) *m*/*z* calcd for [$C_{16}H_{23}ClN$]$^+$ 264.1519 (**11a**), found: 264.1508. $C_{16}H_{22}ClN$ (263.8) (**11a**).

4.1.4. 1-Benzyl-4-(4-phthalimidobutylidene)piperidine (12)

Potassium phthalimide (183 mg, 0.986 mmol) was added to a solution of **11a**/**11b** (200 mg, 0.758 mmol) in DMF (1 mL) and the mixture was stirred in a bath at 100 °C for 16 h. Water (10 mL), 10% aq NaOH (1 mL) and brine (1 mL) were added and the product was extracted with diethyl ether (3 × 10 mL). The combined extracts were washed twice with 10% aq NaOH (2 mL) and brine (5 mL) and dried over Na_2SO_4. Evaporation of the volatiles yielded a yellow oil, which was subjected to column chromatography (eluent: *n*-hexane/Et_2O 3:1 to 1:1). Removal of the solvent from the eluate under reduced pressure and drying in vacuo afforded product **12** as a yellow oil (220 mg, 78%). R_f = 0.3 (*n*-hexane/EtOAc 1:1). Anal. calcd for $C_{24}H_{26}N_2O_2$: C, 76.98; H, 7.00; N, 7.48; found: C, 77.31; H, 6.93; N, 7.81. IR (neat) 2940, 2900, 2795, 1770, 1715, 1615 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 1.74 (p, 2H, *J* 7.1 Hz), 2.05–2.16 (m, 4H), 2.27 (t, 2H, *J* 5.5 Hz), 2.42 (m, 4H), 3.52 (s, 2H), 3.68 (t, 2H, *J* 7.1 Hz), 5.17 (t, 1H, *J* 7.2 Hz), 7.25–7.39 (m, 5H), 7.78–7.89 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 26.4, 29.5, 30.4, 37.2, 39.5, 56.2, 57.0, 64.8, 123.9, 124.9, 129.3, 130.1, 131.7, 134.3, 136.2, 138.3, 139.3, 170.7. MS (ESI, MeOH) *m*/*z* (%) 407 (13), 397 (7) [*M*+Na]$^+$, 375 (100) [*M*+H]$^+$. $C_{24}H_{26}N_2O_2$ (374.5).

4.1.5. 4-(4-Aminobutylidene)-1-benzylpiperidine (13)

Hydrazine monohydrate (3.54 g, 70.8 mmol) was added to a solution of **12** (5.3 g, 14.15 mmol) in ethanol (200 mL) and the mixture was stirred at 80 °C bath temperature for 2 h. The white precipitate was removed by filtration and the volatiles were removed from the filtrate under reduced pressure. Purification by column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 100:10:1 to 50:10:1) yielded product **13** as a yellowish oil (3.0 g, 87%). R_f = 0.6 (CH_2Cl_2/MeOH/28% aq NH_3 50:10:1). Anal. calcd for $C_{16}H_{24}N_2$: C, 78.64; H, 9.90; N, 11.46; found: C, 78.34; H, 10.18; N, 11.48. IR (neat) 3370, 3290, 3060, 3025, 2930, 2850, 2795 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 1.52 (p, 2H, *J* 7.4 Hz), 2.06 (q, 2H, *J* 7.4 Hz), 2.23 (t, 2H, *J* 5.5 Hz), 2.31 (t, 2H, *J* 5.5 Hz), 2.46 (m, 4H), 2.64 (t, 2H, *J* 7.2 Hz), 3.55 (s, 2H), 5.20 (t, 1H, *J* 7.4 Hz), 7.25–7.39 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 26.4, 29.5, 35.1, 37.3, 43.0, 56.4, 57.2, 64.9, 124.4, 129.3, 130.1, 131.7, 137.8, 139.3. MS (ESI, MeOH) *m*/*z* (%) 245 (100) [*M*+H]$^+$. $C_{16}H_{24}N_2$ (244.4).

4.1.6. 1-Benzyl-4-(4-*N*-*tert*-butoxycarbonyl-aminobutylidene)-piperidine (14)

A solution of di-*tert*-butyldicarbonate (2.7 g, 12.2 mmol) in anhydrous CH_2Cl_2 (25 mL) was added dropwise to an ice cold solution of **13** (2.85 g, 11.66 mmol) and triethylamine (0.24 g, 2.33 mmol) in CH_2Cl_2 (60 mL). The ice bath was removed and stirring was continued at rt overnight. The volatiles were removed under reduced pressure and the residue column chromatographed (eluent: CH_2Cl_2 to CH_2Cl_2/MeOH 20:1) to yield product **14** as a yellowish oil (3.8 g, 95%). R_f = 0.4 (CH_2Cl_2/MeOH 20:1). Anal. calcd for $C_{21}H_{32}N_2O_2$: C, 73.22; H, 9.36; N, 8.13; found: C, 73.56; H, 9.66; N, 8.20. IR (neat) 3350 br, 3030, 2935, 2800, 1690 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.44 (s, 9H), 1.50 (p, 2H, *J* 7.3 Hz), 2.01 (q, 2H, *J* 7.3 Hz), 2.22 (t, 2H, *J* 5.3 Hz), 2.27 (t, 2H, *J* 5.5 Hz), 2.45 (m, 4H), 3.09 (m, 2H), 3.55 (s, 2H), 5.11 (t, 1H, *J* 7.3 Hz), 7.22–7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.7, 28.3, 28.8, 30.6, 36.0, 40.5, 54.7, 55.5, 63.2, 79.4, 122.1, 127.5, 128.6, 129.7, 136.7, 138.1, 156.3. MS (ESI, MeOH) *m*/*z* (%) 367 (6) [*M*+Na]$^+$, 345 (100) [*M*+H]$^+$, 289 (46) [*M*−C_4H_8+H]$^+$. $C_{21}H_{32}N_2O_2$ (344.5).

4.1.7. 4-(4-*N*-*tert*-Butoxycarbonyl-aminobutyl)piperidine (15)

Under an atmosphere of argon a 20% palladium-on-charcoal catalyst (0.4 g) was suspended in MeOH (10 mL) and a solution of **14** (3.24 g, 9.4 mmol) in MeOH (60 mL) was added. A slow stream of hydrogen was passed through a glass tube into the vigorously stirred suspension for 20 h. The catalyst was filtered off and the solvent was removed under reduced pressure. Purification by column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 200:20:1 to 100:20:1) afforded amine **15** as a yellowish oil, which solidified in the refrigerator to give a pale yellow solid (1.82 g, 75%)

mp 49–50 °C. R_f = 0.2 (CH_2Cl_2/MeOH/28% aq NH_3 50:10:1). Anal. calcd for $C_{14}H_{28}N_2O_2$: C, 65.59; H, 11.01; N, 10.93; found: C, 65.67; H, 11.09; N, 10.77. IR (Nujol) 3375, 3300, 3195, 1695 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 0.98 (dq, 2H, *J* 11.8, 3.6 Hz), 1.11–1.31 (m, 5H), 1.35 (m, 2H), 1.38 (s, 9H), 1.56 (d, 2H, *J* 12.2 Hz), 2.42 (dt, 2H, *J* 12.1, 2.3 Hz), 2.91 (m, 4H), 6.76 (t, 1H, *J* 5.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 24.2, 29.3, 30.7, 34.0, 36.8, 37.5, 40.8, 47.2, 78.4, 156.7. MS (ESI, MeOH) *m*/*z* (%) 257 (45) $[M+H]^+$, 201 (100) $[M-C_4H_8+H]^+$, 157 (5) $[M-C_4H_8-CO_2+H]^+$. $C_{14}H_{28}N_2O_2$ (256.4).

4.1.8. 4-(3-Hydroxypropyl)piperidine (17)[39]

Under an atmosphere of argon platinum(IV)oxide (1.45 g, 6.4 mmol) was added to a solution of **16** (10.0 g, 72.89 mmol) in MeOH (110 mL) and 32% hydrochloric acid (18 mL). The mixture was vigorously stirred under a low pressure of hydrogen (8 kPa) for 46 h. The major part of the catalyst was removed by filtration and the volatiles were removed under reduced pressure. The oily residue was taken up in 15% aq NaOH (80 mL) and the product was extracted with CH_2Cl_2 (150 and 3 × 100 mL). The pooled extracts were washed with water (20 mL) and dried over Na_2SO_4. Evaporation of the volatiles and drying in vacuo yielded product **17** as a white crystalline, compact solid (10.3 g, 98%) mp 58–60 °C. R_f = 0.2 (CH_2Cl_2/MeOH/28% aq NH_3 50:10:1). IR (Nujol) 3290, 1320 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.08–1.23 (m, 2H), 1.27–1.36 (m, 2H), 1.36–1.49 (m, 1H), 1.53–1.63 (m, 2H), 1.74 (br d, 2H, *J* ca 13.4 Hz), 2.59 (dt, 2H, *J* 12.4 2.6 Hz), 3.04 (td, 2H, *J* 12.4 2.9 Hz), 3.56 (t, 2H, *J* 6.6 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 31.5, 34.8, 35.2, 38.0, 47.9, 64.0. MS (ESI, MeOH) *m*/*z* (%) 287 (36) $[2M+H]^+$, 144 (100) $[M+H]^+$. $C_8H_{17}NO$ (143.2).

4.1.9. 1-Benzyl-4-(3-hydroxypropyl)piperidine (18)[39]

Compound **18** was prepared from piperidine **17** (9.0 g, 62.85 mmol) and benzyl bromide (11.82 g, 69.13 mmol) as previously described,[39] but anhydrous acetonitrile (100 mL) was used instead of absolute EtOH. For purification by column chromatography CH_2Cl_2 and MeOH were used as solvents (eluent: CH_2Cl_2 to CH_2Cl_2/MeOH 10:1). Product **18** was obtained as a yellowish oil (14.1 g, 96%). R_f = 0.7 (CH_2Cl_2/MeOH/28% aq NH_3 200:40:1). IR (neat) 3335 br, 2925, 1495, 1455, 1365, 1340 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.19–1.37 (m, 5H), 1.51–1.62 (m, 2H), 1.72 (br d, 2H, *J* ca 9.6 Hz), 2.02 (br t, 2H, *J* 11.3 Hz), 2.92 (br d, 2H, *J* 11.8 Hz), 3.52 (s, 2H), 3.55 (t, 2H, *J* 6.7 Hz), 7.25–7.38 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 31.7, 33.8, 34.6, 37.6, 55.7, 64.0, 65.3, 129.3, 130.1, 131.8, 139.2. MS (ESI, MeOH) *m*/*z* (%) 256 (9) $[M+Na]^+$, 234 (100) $[M+H]^+$. $C_{15}H_{23}NO$ (233.35).

4.1.10. 1-Benzyl-4-(3-bromopropyl)piperidine (19)[40]

Compound **18** (13.6 g, 58.29 mmol) was dissolved in 48% aq HBr (33 mL, 291.5 mmol) and the mixture was heated under reflux in a bath at 120 °C for 3 h. The mixture was cooled to rt, and water (150 mL) and 28% aq NH_3 (30 mL) were added under cooling in a water bath. The product was extracted with *n*-hexane/Et_2O (1:1 v/v, 2 × 150 mL). The extracts were pooled, washed with water (30 mL) and brine (30 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure and purification by column chromatography (eluent: *n*-hexane/Et_2O 3:1 to 1:1) afforded **19** as a pale yellow oil (13.45 g, 78%). R_f = 0.4 (*n*-hexane/Et_2O 1:1). Anal. calcd for $C_{15}H_{22}BrN$: C, 60.81; H, 7.49; N, 4.73; found: C, 61.18; H, 7.12; N, 4.88. IR (neat) 3025, 2920, 2850, 2800, 2755, 1495, 1450, 1365, 1340 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.19–1.35 (m, 3H), 1.36–1.45 (m, 2H), 1.71 (br d, 2H, *J* ca 9.6 Hz), 1.82–1.93 (m, 2H), 2.03 (br t, 2H, *J* 11.3 Hz), 2.92 (br d, 2H, *J* ca 11.9 Hz), 3.44 (t, 2H, *J* 6.7 Hz), 3.53 (s, 2H), 7.25–7.37 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 32.2, 33.7, 35.4, 36.9, 37.1, 55.6, 65.3, 129.3, 130.1, 131.8, 139.2. MS (ESI, MeOH) *m*/*z* (%) 298/296 (97/100) $[M+H]^+$. $C_{15}H_{22}BrN$ (296.25)

4.1.11. 1-Benzyl-4-(3-phthalimidopropyl)piperidine (20)[41]

Potassium phthalimide (5.85 g, 31.60 mmol) was added to a solution of **19** (7.2 g, 24.31 mmol) in DMF (25 mL), the mixture was stirred in a bath at 100 °C for 5 h, and then cooled to rt. Water (200 mL), 10% aq NaOH (25 mL) and brine (25 mL) were added and the product was extracted with Et_2O (200 mL and 2 × 100 mL). The combined extracts were washed twice with 10% aq NaOH (50 mL), once with water (30 mL) and brine (30 mL), and were dried over Na_2SO_4. Evaporation of the volatiles yielded a yellow oil, which was subjected to column chromatography (eluent: *n*-hexane/Et_2O 3:1 to 1:2). Removal of the solvent from the eluate under reduced pressure gave a yellowish oil, which solidified during drying in vacuo to afford product **20** as a white crystalline solid (6.21 g, 70%) mp 77–78 °C. R_f = 0.4 (*n*-hexane/Et_2O 1:2). IR (Nujol) 1710, 1400 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.14–1.33 (m, 5H), 1.62–1.74 (m, 4H), 2.02 (br t, 2H, *J* ca 12.1 Hz), 2.88 (br d, 2H, *J* 12.1 Hz), 3.50 (s, 2H), 3.64 (t, 2H, *J* 7.2 Hz), 7.23–7.36 (m, 5H), 7.77–7.87 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 27.6, 33.7, 35.5, 37.2, 39.8, 55.6, 65.2, 124.9, 129.2, 130.1, 131.8, 134.2, 136.2, 139.1, 170.6. MS (ESI, MeOH) *m*/*z* (%) 395 (13) $[M+Na]^+$, 363 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{23}H_{27}N_2O_2]^+$ 363.2073, found: 363.2058. $C_{23}H_{26}N_2O_2$ (362.5).

4.1.12. 4-(3-Aminopropyl)-1-benzylpiperidine (21)[42]

Hydrazine monohydrate (4.14 g, 82.77 mmol) was added to a solution of **20** (6.0 g, 16.55 mmol) in ethanol (250 mL), the mixture was stirred in a bath at 80 °C for 2 h, and then cooled to rt. The white precipitate was removed by vacuum filtration and the volatiles were removed from the filtrate under reduced pressure. Purification by column chromatography (eluent: CH_2Cl_2/MeOH 20:1 to CH_2Cl_2/MeOH/28% aq NH_3 100:20:1) yielded **20** as a yellowish oil (3.08 g, 80%). R_f = 0.6 (CH_2Cl_2/MeOH/28% aq NH_3 50:10:1). IR (neat) 3365 br, 2925, 2850, 2800, 2755, 1495, 1455, 1370 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.18–1.34 (m, 5H), 1.44–1.56 (m, 2H), 1.67–1.78 (m, 2H), 2.02 (br t, 2H, *J* 11.2 Hz), 2.62 (t, 2H, *J* 7.2 Hz), 2.92 (br d, 2H, *J* ca 11.5 Hz), 3.53 (s, 2H), 7.25–7.38 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 31.9, 33.8, 35.7, 37.6, 43.6, 55.7, 65.3, 129.3, 130.1, 131.8, 139.2. MS (ESI, MeOH) *m*/*z* (%) 233 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{15}H_{25}N_2]^+$ 233.2018, found: 233.2005. $C_{15}H_{24}N_2$ (232.4).

4.1.13. 1-Benzyl-4-(3-*N*-(*tert*-butoxycarbonyl)aminopropyl)-piperidine (22)

A solution of di-*tert*-butyldicarbonate (2.76 g, 12.65 mmol) in anhydrous CH_2Cl_2 (25 mL) was added dropwise to an ice cold solution of **21** (2.94 g, 12.65 mmol) in CH_2Cl_2 (50 mL) over a period of 30 min. The ice bath was removed and stirring was continued at rt overnight. The volatiles were removed under reduced pressure and the product was purified by column chromatography (eluent: *n*-hexane/Et_2O 2:1 to 1:2). Removal of the solvent from the eluate under reduced pressure yielded a yellowish oil, which solidified during drying in vacuo to afford **22** as a white crystalline solid (4.03 g, 96%) mp 81–82 °C. R_f = 0.3 (Et_2O). Anal. calcd for $C_{20}H_{32}N_2O_2$: C, 72.25; H, 9.70; N, 8.43; found: C, 72.19; H, 9.74; N, 8.27. IR (Nujol) 3360, 1680, 1530 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.17–1.34 (m, 5H), 1.42–1.56 (m, 11H), 1.71 (br d, 2H, *J* ca 9.5 Hz), 2.02 (br t, 2H, *J* 11.2 Hz), 2.92 (br d, 2H, *J* ca 11.7 Hz), 3.03 (t, 2H, *J* 7.0 Hz), 3.53 (s, 2H), 7.25–7.38 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 29.0, 29.6, 33.8, 35.6, 37.4, 42.4, 55.6, 65.3, 80.6, 129.3, 130.1, 131.8, 139.2, 159.4. MS (ESI, MeOH) *m*/*z* (%) 665 (5) $[2M+H]^+$, 355 (8) $[M+Na]^+$, 333 (100) $[M+H]^+$. $C_{20}H_{32}N_2O_2$ (332.5).

4.1.14. 4-(3-*N*-*tert*-Butoxycarbonyl-aminopropyl)piperidine (23)[37]

Under an atmosphere of argon, 10% Pd/C catalyst (600 mg) was added to a solution of compound **22** (3.92 g, 11.79 mmol) in MeOH (35 mL). The mixture was vigorously stirred under a low pressure of hydrogen (8 kPa) for 22 h (after 15 h more catalyst (400 mg) was added). The catalyst was filtered off and the solvent was removed under reduced pressure. The oily residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:40:1 to 100:25:1), which afforded amine **23** as a pale yellow oil (2.56 g, 90%). R_f = 0.15 (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1). IR (neat) 3355 br, 2925 br, 1685, 1530, 1455, 1390, 1365 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.11–1.33 (m, 4H), 1.38–1.58 (m, 12H), 1.77 (br d, 2H, *J* ca 13.6 Hz), 2.65 (dt, 2H, *J* 12.4 2.7 Hz), 3.04 (t, 2H, *J* 7.1 Hz), 3.10 (td, 2H, *J* 12.3 2.9 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 28.7, 29.7, 34.1, 35.9, 37.4, 42.3, 47.6, 80.6, 159.4. MS (ESI, MeOH) *m*/*z* (%) 485 (17) $[2M+H]^+$, 243 (100) $[M+H]^+$, 187 (15) $[M-C_4H_8+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{13}H_{27}N_2O_2]^+$ 243.2073, found: 243.2062. $C_{13}H_{26}N_2O_2$ (242.4).

4.1.15. 4-(Piperidin-4-yl)butanol (25)[43]

Under an atmosphere of argon 4-(piperidin-4-yl)butanoic acid hydrochloride (**24**) (15 g, 72.2 mmol) was suspended in anhydrous THF (200 mL). The suspension was immersed in an ice bath and lithium aluminiumhydride (6.85 g, 180.5 mmol) was added in portions under stirring. The mixture was slowly warmed to rt and then kept under reflux overnight. The mixture was cooled in an ice bath and water (7 mL), 15% aq NaOH (7 mL) and water (20 mL) was added dropwise under stirring. The white precipitate was separated by vacuum filtration and the volatiles were removed from the filtrate under reduced pressure yielding a colorless oil. The white solid was washed with chloroform (3 × 50 mL) and the washing filtrates (product **25** was evident by TLC analysis) were combined with the colorless oil. 0.3% aq NaOH (150 mL) was added, the mixture was vigorously shaken, the phases were separated and the aqueous phase repeatedly treated with chloroform (100 mL, 80 mL and 2 × 50 mL). The chloroform extracts were combined, washed with water (50 mL) and dried over Na_2SO_4. Evaporation of the solvent yielded crude **25** as a colorless oil (15 g). A portion (5.2 g, 35%) of this material was subjected to column chromatography (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1 to 25:50:1) to afford pure product **25** as pale yellow oil, which crystallized during storage at −20 °C to give a hard white solid (2.94 g, 75% (referred to 15 g crude)) mp 49–51 °C. R_f = 0.2 (CH_2Cl_2/MeOH/28% aq NH_3 50:25:1). IR (Nujol) 3255, 1320 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.07–1.21 (m, 2H), 1.24–1.34 (m, 2H), 1.35–1.48 (m, 3H), 1.49–1.60 (m, 2H), 1.73 (br d, 2H, *J* 13.1 Hz), 2.58 (dt, 2H, *J* 12.4 2.6 Hz), 3.04 (td, 2H, *J* 12.2 2.8 Hz), 3.57 (t, 2H, *J* 6.5 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 24.7, 34.7, 34.8, 38.1, 39.0, 48.0, 63.8. MS (ESI, MeOH) *m*/*z* (%) 315 (36) $[2M+H]^+$, 158 (100) $[M+H]^+$. $C_9H_{19}NO$ (157.25).

4.1.16. 1-Benzyl-4-(4-hydroxybutyl)piperidine (26)[44]

In two 20-mL pressure vials (20 bar) amine **25** (2 × 4.0 g, 2 × 25.45 mmol) was dissolved in acetonitrile (2 × 10 mL) under warming. Diisopropylethylamine (2 × 3.62 g, 2 × 28.0 mmol) and benzyl bromide (2 × 4.78 g, 2 × 28.0 mmol) were added under stirring and the mixture was heated in a bath at 105 °C for 60 min. The solvent was removed under reduced pressure and the residue taken up in CH_2Cl_2 (250 mL) and 5% aq NaOH (150 mL). The mixture was vigorously shaken, the phases were separated, and the aqueous phase treated with CH_2Cl_2 (100 mL). The CH_2Cl_2 extracts were combined, washed with water (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by column chromatography (eluent: CH_2Cl_2 to CH_2Cl_2/MeOH 10:1). Amino alcohol **26** was obtained as an orange oil (8.2 g, 65%). R_f = 0.5 (CH_2Cl_2/MeOH 5:1). IR (neat) 3330 br, 2925, 1495, 1455 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.17–1.33 (m, 5H), 1.34–1.46 (m, 2H), 1.48–1.59 (m, 2H), 1.72 (br d, 2H, *J* 9.6 Hz), 2.03 (br t, 2H, *J* 11.3 Hz), 2.92 (br d, 2H, *J* 11.8 Hz), 3.54 (s, 2H), 3.56 (t, 2H, *J* 6.5 Hz), 7.26–7.37 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 24.9, 33.8, 34.7, 37.6, 38.3, 55.7, 63.8, 65.3, 129.3, 130.1, 131.8, 139.0. MS (ESI, MeOH) *m*/*z* (%) 270 (7) $[M+Na]^+$, 248 (100) $[M+H]^+$. $C_{16}H_{25}NO$ (247.4).

4.1.17. 1-Benzyl-4-(4-bromobutpyl)piperidine (27)[45]

Bromide **27** was prepared from **26** (8.2 g, 33.95 mmol) using the procedure for the preparation of the lower homologue **19**, and was obtained as a pale yellow oil, which solidified during storage below 0 °C. (13.45 g, 78%). R_f = 0.4 (*n*-hexane/Et_2O 1:1). Anal. calcd for $C_{16}H_{24}BrN$: C, 61.94; H, 7.80; N, 4.51; found: C, 61.71; H, 7.66; N, 4.56. IR (Nujol) 1260, 1125, 735, 695 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.17–1.35 (m, 5H), 1.42–1.55 (m, 2H), 1.67–1.76 (m, 2H), 1.79–1.90 (m, 2H), 2.02 (br t, 2H, *J* 11.2 Hz), 2.92 (br d, 2H, *J* 11.8 Hz), 3.46 (t, 2H, *J* 6.7 Hz), 3.53 (s, 2H), 7.23–7.38 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 27.2, 33.8, 35.0, 35.3, 37.6 (two carbons), 55.6, 65.3, 129.3, 130.1, 131.8, 139.1. MS (ESI, MeOH) *m*/*z* (%) 312/310 (98/100) $[M+H]^+$. $C_{16}H_{24}BrN$ (310.3).

4.1.18. 1-Benzyl-4-(4-(imidazol-1-yl)butyl)piperidine (28)

In a 5-mL pressure vial imidazole (0.15 g, 2.2 mmol) and finely ground potassium carbonate (0.35 g, 2.53 mmol) were suspended in anhydrous acetonitrile (4 mL). Bromide **27** (0.52 g, 1.69 mmol) was added and the mixture was kept under vigorous stirring at 70 °C for 16 h. Insoluble material was filtered off and washed with CH_2Cl_2 (2 × 15 mL). The combined filtrates were evaporated to dryness and the residue was subjected to column chromatography (eluent: CH_2Cl_2 to CH_2Cl_2/MeOH 20:1). The fractions containing the product were combined and the solvent was removed under reduced pressure. Uptake of the oily residue in CH_2Cl_2 (10 mL) and removal of the volatiles in vacuo yielded **28** as a yellowish oil, which crystallized during storage at −20 °C to a give white compact solid (0.28 g, 56%). R_f = 0.7 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{19}H_{27}N_3$: C, 76.72; H, 9.15; N, 14.13; found: C, 76.92; H, 9.01; N, 13.90. IR (Nujol) 1230, 1110, 1075 cm^{-1} cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.15–1.37 (m, 7H), 1.67 (br d, 2H, *J* ca 9.8 Hz), 1.73–1.84 (m, 2H), 2.01 (br t, 2H, *J* 11.3 Hz), 2.90 (br d, 2H, *J* 11.8 Hz), 3.52 (s, 2H), 4.03 (t, 2H, *J* 7.0 Hz), 6.98 (t, 1H, *J* 1.2 Hz), 7.13 (t, 1H, *J* 1.2 Hz), 7.25–7.36 (m, 5H), 7.65 (br s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 25.5, 33.1, 33.7, 37.5, 37.8, 48.8, 55.6, 65.2, 121.4, 129.3, 129.8, 130.1, 131.8, 139.1, 139.2. MS (ESI, MeOH) *m*/*z* (%) 595 (100) $[2M+H]^+$, 298 (72) $[M+H]^+$. $C_{19}H_{27}N_3$ (297.4).

4.1.19. 1-Benzyl-4-(4-(4-methylpiperazin-1-yl)butyl)piperidine (29)

In a 20-mL pressure vial *N*-methylpiperazine (0.30 g, 3.02 mmol) was dissolved in anhydrous acetonitrile (3 mL). Finely ground potassium carbonate (1.28 g, 9.28 mmol) and bromide **27** (0.72 g, 2.32 mmol) (dissolved in 5 mL of acetonitrile) were added and the mixture was kept under vigorous stirring at 100 °C for 2 h. The suspension was transferred to a round bottom flask and the solvent was evaporated. The residue was taken up in saturated aq K_2CO_3 (10 mL) and water (10 mL). The product was extracted with CH_2Cl_2 (40 mL and 2 × 20 mL), the combined extracts were washed with water (10 mL) and dried over Na_2SO_4. The volatiles were evaporated and the product was purified by column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:40:1 to 200:50:1. Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake of the oily residue in CH_2Cl_2 (6 mL), filtration of the solution with a cotton wool packed Pasteur pipette followed by removal of the solvent in vacuo yielded a yellowish oil, which crystallized at −20 °C to afford product **29** as a white crystalline compact solid (0.66 g, 86%) mp 36–37 °C. R_f = 0.5 (CH_2-

Cl_2/MeOH/28% aq NH_3 200:50:1). IR (Nujol) 2785, 2765, 1285, 1165 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.18–1.42 (m, 7H), 1.46–1.58 (m, 2H), 1.71 (br d, 2H, *J* ca 9.7 Hz), 2.03 (br t, 2H, *J* ca 11.2 Hz), 2.31 (s, 3H), 2.32–2.80 (br m, 10H), 2.92 (br d, 2H, *J* ca 11.6 Hz), 3.54 (s, 2H), 7.25–7.37 (m, 5H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 26.6, 28.5, 33.8, 37.6, 38.4, 46.8, 54.6, 55.7, 56.4, 60.5, 65.3, 129.3, 130.1, 131.8, 139.1. MS (ESI, MeOH) *m*/*z* (%) 352 (5) $[M+Na]^+$, 330 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{21}H_{36}N_3]^+$ 330.2909, found: 330.2900. $C_{21}H_{35}N_3$ (329.5).

4.1.20. 4-(4-(Imidazol-1-yl)butyl)piperidine (30)

Under an atmosphere of argon, 10% Pd/C catalyst (100 mg) was added to a solution of compound **28** (574 mg, 1.93 mmol) in MeOH (12 mL). The mixture was vigorously stirred under a low pressure of hydrogen (12 kPa) for 24 h. After the first 2 h and then 6 h, more catalyst was added (50 mg each). The catalyst was filtered off and the solvent was removed under reduced pressure. The yellow oily residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 500:25:1 to 75:75:1), which afforded **30** as a yellowish oil (319 mg, 80%). R_f = 0.15 (CH_2Cl_2/MeOH/28% aq NH_3 200:40:1). IR (neat) 3390 br, 3285 br, 3105, 2925, 2850, 1510, 1450, 1370 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.05–1.21 (m, 2H), 1.26–1.46 (m, 5H), 1.70 (br d, 2H, *J* ca 13.3 Hz), 1.76–1.86 (m, 2H), 2.59 (dt, 2H, *J* 12.4 2.7 Hz), 3.04 (td, 2H, *J* 12.2 2.8 Hz), 4.05 (t, 2H, *J* 7.0 Hz), 6.99 (t, 1H, *J* ca 1.0 Hz), 7.15 (t, 1H, *J* ca 1.1 Hz), 7.67 (br s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 25.2, 33.1, 34.4, 37.8, 38.4, 47.8, 48.8, 121.4, 129.8, 139.2. MS (ESI, MeOH) *m*/*z* (%) 415 (90) $[2M+H]^+$, 208 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{12}H_{22}N_3]^+$ 208.1814, found: 208.1802. $C_{12}H_{21}N_3$ (207.3).

4.1.21. *N*-Methyl-*N*′-(4-(piperidin-4-yl)butyl)piperazine (31)

Under an atmosphere of argon, 10% Pd/C catalyst (60 mg) was added to a solution of compound **29** (0.46 g, 1.39 mmol) in MeOH (12 mL). A slow stream of hydrogen was passed through a glass tube into the vigorously stirred suspension for 24 h (after 5 h more 10% Pd/C catalyst (60 mg) was added). The catalyst was filtered off, the solvent was removed under reduced pressure and the oily residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:40:1 to 50:50:1). Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake of the oil in CH_2Cl_2 (6 mL), filtration of the solution with a cotton wool packed Pasteur pipette followed by removal of the solvent in vacuo yielded **31** as a yellowish oil, which solidified during storage at 4 °C (0.31 g, 93%). R_f = 0.15 (CH_2Cl_2/MeOH/28% aq NH_3 100:25:1). IR (neat) 3280 br, 2930, 2795, 1445, 1370 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ (ppm) 1.14 (dq, 2H, *J* 12.2 3.6 Hz), 1.25–1.46 (m, 5H), 1.47–1.59 (m, 2H), 1.73 (br d, 2H, *J* ca 12.6 Hz), 2.31 (s, 3H), 2.32–2.90 (br m, 12H), 3.05 (br d, 2H, *J* ca 12.3 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 26.4, 28.6, 34.7, 38.0, 39.0, 46.9, 47.9, 54.6, 56.4, 60.5. MS (ESI, MeOH) *m*/*z* (%) 240 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{14}H_{30}N_3]^+$ 240.2440, found: 240.2428. $C_{14}H_{29}N_3$ (239.4).

4.1.22. *tert*-Butyl 2-aminoethylcarbamate (33)[56]

Compound **33** was prepared from ethane-1,2-diamine (24 g, 0.4 mol) and di-*tert*-butyl dicarbonate (8.72 g, 40 mmol) using a described procedure,[57] and was obtained as a yellowish oil (6.21 g, 97%). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.39 (s, 9H), 1.43 (s, 2H), 2.74 (t, 2H, *J* 5.9 Hz), 3.12 (m, 2H), 5.09 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 28.5, 41.9, 43.4, 79.2, 156.3. $C_7H_{16}N_2O_2$ (160.2).

4.1.23. 4-((*N*-(*N*-*tert*-Butoxycarbonyl-2-aminoethyl)-3-amino-3-oxo)propenyl)1*H*-imidazole (34)

Urocanic acid (**32**) (4.74 g, 34.33 mmol), amine **33** (5.5 g, 34.33 mmol) and HOBt (5.26 g, 34.33 mmol) were suspended/dissolved in DMF (50 mL) and the mixture was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (6.91 g, 36.05 mmol) was added and the mixture was slowly warmed to rt and stirred overnight (The suspension turned to a clear orange solution after 2 h). The mixture was diluted with 2.5% aq NaOH (500 mL) and brine (100 mL). Repeated treatment with EtOAc (12 × 200 mL) afforded an extraction of product **34** only in low amounts and extraction with $CHCl_3$ (2 × 150 mL) failed as well. Therefore, the aqueous phase was concentrated under reduced pressure at 40 °C to a volume of 300 mL and then treated twice with $CHCl_3$/MeOH 5:1 (500 and 400 mL). The organic phases were combined with the earlier EtOAc and $CHCl_3$ extracts and removal of the volatiles under reduced pressure yielded a yellow liquid (ca 50 mL). DMF was removed under reduced pressure (50 °C, 10 mbar) and the residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 50:1 to 10:1). Removal of the solvent from the eluate under reduced pressure, uptake in CH_2Cl_2 (80 mL), evaporation, uptake in CH_2Cl_2 (50 mL) followed by removal of the solvent in vacuo afforded **34** as a white powder (8.27 g, 86%), mp 159–161 °C. A minor fraction was recrystallized from acetone/EtOAc to yield colorless needles, mp 165–167 °C. R_f = 0.6 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{13}H_{20}N_4O_3$: C, 55.70; H, 7.19; N, 19.99; found: C, 55.65; H, 7.29; N, 19.86. IR (Nujol) 3355, 3300, 1685, 1665, 1630, 1530 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ (ppm) 1.46 (s, 9H), 3.23 (t, 2H, *J* 6.2 Hz), 3.39 (t, 2H, *J* 6.2 Hz), 6.52 (d, 1H, *J* 15.6 Hz), 7.36 (s, 1H), 7.46 (d, 1H, *J* 15.6 Hz), 7.77 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 29.6, 41.5, 42.0, 81.0, 120.1, 123.2 (br), 133.1, 137.5 (br), 139.2, 159.4, 170.2. MS (ESI, MeOH) *m*/*z* (%) 583 (76) $[2M+Na]^+$, 319 (38) $[M+K]^+$, 303 (100) $[M+Na]^+$, 281 (17) $[M+H]^+$, 203 (26) $[M-C_4H_8-CO_2+Na]^+$, 181 (22) $[M-C_4H_8-CO_2+H]^+$, $C_{13}H_{20}N_4O_3$ (280.32).

4.1.24. 1-(4-(1-Benzylpiperidin-4-yl)butyl)-4-((*N*-(*N*-*tert*-butoxycarbonyl-2-aminoethyl)-3-amino-3-oxo)propenyl)1*H*-imidazole (35a)/1-(4-(1-benzylpiperidin-4-yl)butyl)-5-((*N*-(*N*-*tert*-butoxycarbonyl-2-aminoethyl)-3-amino-3-oxo)propenyl)1*H*-imidazole (35b)

Compound **34** (3.35 g, 11.95 mmol) and finely ground potassium carbonate (8.26 g, 59.76 mmol) were suspended in anhydrous acetonitrile (40 mL). Bromide **27** (3.89 g, 12.55 mmol) was added and the mixture was vigorously stirred under reflux for 4 h. CH_2Cl_2 (250 mL) and saturated aq K_2CO_3 (100 mL) were added, the mixture was vigorously shaken, the phases were separated, and the aqueous phase treated with CH_2Cl_2 (150 mL). The organic phases were combined, washed with water (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the pale yellow, solid residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 40:1 to 10:1), which afforded a mixture of product **35a** and the by-product **35b** (ratio: 7:1) as a white powder (5.56 g, 91%). A portion (1.16 g) of this material was re-chromatographed (eluent: CH_2Cl_2/MeOH 30:1 to 5:1) to yield pure product **35a** (0.76 g) mp 184–186 °C from the first fraction of the eluate, a 3:1 mixture of **35a** and **35b** (0.32 g) from the medium fraction, and a 1:10 mixture of **35a** and **35b** (16 mg) from the last fraction. **35a**: R_f = 0.6 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{29}H_{43}N_5O_3$: C, 68.34; H, 8.50; N, 13.74; found: C, 68.32; H, 8.75; N, 13.62. IR (Nujol) 3255, 1705, 1670, 1630 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ (ppm) 0.99–1.15 (m, 3H), 1.19 (br s, 4H), 1.37 (s, 9H), 1.55 (br d, 2H, *J* ca 10.9 Hz), 1.66 (br s, 2H), 1.85 (t, 2H, *J* 10.5 Hz), 2.74 (br d, 2H, *J* ca 11.5 Hz), 3.01 (q, 2H, *J* ca 6.1 Hz), 3.17 (q, 2H, *J* 6.2 Hz), 3.40 (s, 2H), 3.92 (t, 2H, *J* 7.0 Hz), 6.48 (d, 1H, *J* 15.3 Hz), 6.82 (t, 1H, *J* 5.5 Hz), 7.18–7.33 (m, 6H), 7.43 (d, 1H, *J* 0.9 Hz), 7.66 (s, 1H), 8.01 (t, 1H, *J* 5.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 23.1, 28.2, 30.6, 31.9, 35.1, 35.5, 38.8, 39.9, 46.1, 53.3, 62.5, 77.6, 118.3, 121.5, 126.7, 128.1, 128.7, 131.5, 137.6, 138.7 (two carbons), 155.6, 165.9. MS (ESI, MeOH) *m*/*z* (%) 1041 (10) $[2M+Na]^+$,

1019 (39) $[2M+H]^+$, 532 (19) $[M+Na]^+$, 510 (100) $[M+H]^+$, 266.5 (25) $[M+H+Na]^{2+}$. HRMS (ESI, MeOH) *m/z* calcd for $[C_{29}H_{44}N_5O_3]^+$ 510.3444, found: 510.3437. $C_{29}H_{43}N_5O_3$ (509.68).

Compound **35b**: R_f = 0.55 (CH_2Cl_2/MeOH 5:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 0.98–1.14 (m, 3H), 1.18 (br s, 4H), 1.37 (s, 9H), 1.49–1.67 (m, 4H), 1.84 (br t, 2H, *J* ca 11.1 Hz), 2.74 (br d, 2H, *J* ca 11.5 Hz), 3.01 (q, 2H, *J* ca 6.0 Hz), 3.18 (q, 2H, *J* 6.2 Hz), 3.39 (s, 2H), 4.05 (t, 2H, *J* 6.9 Hz), 6.40 (d, 1H, *J* 15.8 Hz), 6.85 (t, 1H, *J* 5.6 Hz), 7.18–7.33 (m, 7H), 7.75 (d, 1H, *J* 0.8 Hz), 8.18 (t, 1H, *J* 5.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 23.0, 28.2, 30.6, 31.9, 35.1, 35.4, 39.0, 39.8, 44.3, 53.3, 62.5, 77.6, 120.0, 124.5, 126.7, 128.1 (two carbons), 128.7, 129.9, 138.7, 140.3, 155.6, 165.1. HRMS (ESI, MeOH) *m/z* calcd for $[C_{29}H_{44}N_5O_3]^+$ 510.3444, found: 510.3432. $C_{29}H_{43}N_5O_3$ (509.68).

4.1.25. 1-(4-Piperidin-4-yl-butyl)-4-((*N*-(*N*-*tert*-butoxycarbonyl-2-aminoethyl)-3-amino-3-oxo)propyl)1*H*-imidazole (36)

Under an atmosphere of argon, 10% Pd/C catalyst (500 mg) was added to a solution of compound **35a**/**35b** (7:1) (4.11 g, 8.06 mmol) in MeOH (25 mL). A slow stream of hydrogen was passed through a glass tube into the vigorously stirred suspension for 29 h (after 5 h and 21 h more catalyst was added, 200 mg each). The catalyst was filtered off, the solvent was removed under reduced pressure and the colorless oily residue, which showed only one component by TLC, was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 800:40:1 to 100:50:1). The forerun of the eluate contained a single product **36** by NMR spectroscopy. The residual eluate contained an 85:15 mixture of product **36** and what was presumed to be the equivalent 1,5-disubstitued imidazole derivative, respectively. The solvent was removed from the eluates under reduced pressure, the residues were dried in vacuo and then redissolved in CH_2Cl_2 (6 and 12 mL, respectively). The solutions were filtered through a cotton wool packed Pasteur pipette and the solvent was removed in vacuo. The first fraction afforded pure product **36** as a pale yellow oil, which solidified to a white crystalline solid (0.36 g, 11%) mp 110–113 °C. The second fraction afforded a mixture of product **36** and the above mentioned by-product (ratio ca 86:14) as a yellowish resin (2.64 g, 78%). R_f = 0.3 (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1). Anal. calcd for $C_{22}H_{39}N_5O_3$ 0.5H_2O: C, 61.36; H, 9.13; N, 16.27; found: C, 61.56; H, 9.48; N, 16.10. IR (Nujol) 1700, 1645, 1540 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 1.05–1.21 (m, 2H), 1.23–141 (m, 5H), 1.46 (s, 9H), 1.70 (br d, 2H, *J* ca 14 Hz), 1.74–1.83 (m, 2H), 2.50 (t, 2H, *J* 7.6 Hz), 2.59 (dt, 2H, *J* 12.3 2.5 Hz), 2.84 (t, 2H, *J* 7.6 Hz), 3.05 (br d, 2H, *J* 12.4 Hz), 3.14 (t, 2H, *J* 6.0 Hz), 3.25 (t, 2H, *J* 6.1 Hz), 3.98 (t, 2H, *J* 7.0 Hz), 6.88 (s, 1H), 7.56 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 25.3, 26.0, 29.6, 33.1, 34.4, 37.76, 37.83, 38.4, 41.3, 41.8, 47.8, 48.8, 80.9, 117.8, 138.7, 142.6, 159.3, 176.4. MS (ESI, MeOH) *m/z* (%) 843 (6) $[2M+H]^+$, 444 (4) $[M+Na]^+$, 422 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m/z* calcd for $[C_{22}H_{40}N_5O_3]^+$ 422.3131, found: 422.3124. $C_{22}H_{39}N_5O_3$ (421.58).

4.1.26. *N,N′*-Bis(4-(1-benzylpiperidin-4-yl)butyl)piperazine (38)

In a 20-mL pressure vial piperazine (0.18 g, 2.09 mmol) was dissolved in anhydrous acetonitrile (8 mL). Finely ground potassium carbonate (2.31 g, 16.72 mmol) and bromide **27** (1.33 g, 4.28 mmol) were added and the mixture was kept under vigorous stirring at 100 °C for 2 h. The suspension was transferred to a round bottom flask, the solvent was evaporated and the residue was taken up in saturated aq K_2CO_3 (10 mL). The product was extracted with CH_2Cl_2 (40, 30 and 20 mL), the combined extracts were washed with water (10 mL) and dried over Na_2SO_4. The volatiles were evaporated and the product was column chromatographed (eluent: CH_2Cl_2/MeOH 20:1 to 5:1). Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake of the white solid in CH_2Cl_2 (7 mL), filtration of the solution with a cotton wool packed Pasteur pipette followed by removal of the solvent in vacuo yielded product **38** as a white solid (0.55 g, 48%) mp 104–105 °C (soluble in chloroform, but insoluble in MeOH and DMSO). R_f = 0.4 (CH_2Cl_2/MeOH/28% aq NH_3 200:20:1). Anal. calcd for $C_{36}H_{56}N_4$: C, 79.36; H, 10.36; N, 10.28; found: C, 79.52; H, 10.55; N, 10.14. IR (Nujol) 2805, 2765, 1350 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.13–1.34 (m, 14H), 1.39–1.51 (m, 4H), 1.57–1.67 (m, 4H), 1.90 (br t, 4H, *J* ca 10.9 Hz), 2.27–2.34 (m, 4H), 2.46 (br s, 8H), 2.85 (br d, 4H, *J* ca 10.9 Hz), 3.47 (s, 4H), 7.20–7.34 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 25.0, 27.3, 32.5, 35.8, 36.7, 53.4, 54.1, 59.0, 63.7, 127.0, 128.2, 129.4, 138.8. MS (ESI, MeOH/10 mM HCl 10:1) *m/z* (%) 581 (27) $[M+2H+Cl]^+$, 545 (2) $[M+H]^+$, 273 (100) $[M+2H]^{2+}$. $C_{36}H_{56}N_4$ (544.86).

4.1.27. *N,N′*-Bis(4-(piperidin-4-yl)butyl)piperazine (39)

Under an atmosphere of argon, 10% Pd/C catalyst (100 mg) was added to a solution of compound **38** (0.49 g, 0.90 mmol) in CH_2Cl_2 (1 mL) and MeOH (5 mL). A slow stream of hydrogen was passed through a glass tube into the vigorously stirred suspension for 48 h (after 7 h and 23 h more catalyst was added, 50 and 80 mg, respectively). The catalyst was filtered off, the solvent was removed under reduced pressure and the oily residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:20:1 to 75:75:1). Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake in MeOH (6 mL) and CH_2Cl_2 (2 mL), filtration of the solution with a cotton wool packed Pasteur pipette followed by removal of the solvent in vacuo yielded **39** as a white crystalline solid (0.22 g, 66%) mp >240 °C (decomp.). R_f = 0.1 (CH_2Cl_2/MeOH/28% aq NH_3 100:25:1). IR (Nujol) 3460, 1400 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 1.33–1.53 (m, 12H), 1.60–1.80 (m, 6H), 1.98 (br d, 4H, *J* ca 12.7 Hz), 2.87–3.07 (m, 8H), 3.10–3.48 (br m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm) 25.5, 26.7, 30.8, 35.4, 37.3, 46.1, 52.2, 58.9. MS (ESI, MeOH) *m/z* (%) 365 (3) $[M+H]^+$, 190 (27) $[M+2NH_4]^{2+}$, 183 (100) $[M+2H]^{2+}$. HRMS (ESI, MeOH) *m/z* calcd for $[C_{22}H_{46}N_4]^{2+}$ 183.1861, found: 183.1853. $C_{22}H_{44}N_4$ (364.61).

4.1.28. 5-((4-(3-*N*-(*tert*-Butoxycarbonyl)aminopropyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*]-[1,4]diazepin-11(10*H*)-one·0.5 EtO_2 (44)

Compound **44** was prepared from amine **23** (1.88 g, 7.75 mmol) and compound **43** (2.11 g, 7.35 mmol) using the procedure for the preparation of **29**. Eluent for column chromatography: Et_2O to CH_2Cl_2/Et_2O/MeOH 100:100:1 to CH_2Cl_2/MeOH 50:1. Removal of the volatiles from the eluate under reduced pressure and drying in vacuo afforded **44** as a slightly tan colored glass (2.16 g, 60%) mp >120 °C (turned to a resin). R_f = 0.5 (CH_2Cl_2/MeOH 10:1). Anal. calcd for $C_{28}H_{36}N_4O_4$·0.5 $C_4H_{10}O$: C, 68.03; H, 7.80; N, 10.58; found: 68.25; H, 7.73; N, 10.51. IR (Nujol) 1710, 1680, 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 0.99–1.30 (m, 8H), 1.38–1.52 (m, 11.5H), 1.56 (br d, 1H, *J* ca 10.3 Hz), 1.63 (d, 0.5H, *J* 11.6 Hz), 1.87–2.04 (m, 2H), 2.52 (br d, 0.45H, *J* ca 8.5 Hz), 2.65 (br d, 0.55H, *J* ca 8.2 Hz), 2.78–2.88 (m, 1H), 3.00 (t, 2H, *J* 7.1 Hz), 3.03–3.25 (m, 2H), 3.51 (q, 2H, *J* 7.0 Hz), 7.23–7.31 (m, 2H), 7.34 (t, 0.45H, *J* 7.0 Hz), 7.41 (t, 0.55H, *J* 7.2 Hz), 7.45–7.56 (m, 2H), 7.56–7.61 (m, 1H), 7.64–7.70 (m, 1H), 7.86–7.94 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 16.3 (Et_2O), 29.0, 29.7, 33.6/33.8/33.9, 35.4, 37.0, 42.4, 55.6/55.8, 61.9/62.2, 67.7 (Et_2O), 80.6, 123.8, 127.4, 127.8, 128.6, 129.76, 129.84, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1/135.5, 136.9, 137.8, 144.6/144.7, 159.4, 170.0/170.2, 172.1/172.4. MS (ESI, MeOH) *m/z* (%) 1023 (12) $[2M+K]^+$, 1007 (35) $[2M+Na]^+$, 985 (41) $[2M+H]^+$, 531 (15) $[M+K]^+$, 515 (40) $[M+Na]^+$, 493 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m/z* calcd for $[C_{28}H_{37}N_4O_4]^+$ 493.2815, found: 493.2805. $C_{28}H_{36}N_4O_4$ (492.61).

4.1.29. 5-((4-(4-*N*-*tert*-Butoxycarbonyl-aminobutyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*]-[1,4]diazepin-11(10*H*)-one (45)

In a 20-mL pressure vial compounds **43** (2.16 g, 7.54 mmol) and **15** (1.93 g, 7.54 mmol) were dissolved in anhydrous acetonitrile (18 mL). Finely ground potassium carbonate (1.03 g, 7.39 mmol) was added and the mixture was kept under stirring in a microwave reactor at 100 °C (pressure: ca 2 bar) for 100 min. Solid material was removed by filtration, the filtrate evaporated to dryness, and the residue subjected to column chromatography (eluent 1. column: CH_2Cl_2/MeOH 100:1 to 5:1, eluent 2. column: Et_2O/MeOH 100:1 to 5:1). Removal of the solvent under reduced pressure, re-uptake in Et_2O and removal of the solvent in vacuo afforded product **45** as a pale tan colored glass (3.67 g, 93%) mp 94–96 °C. R_f = 0.3 (CH_2Cl_2/MeOH 20:1). IR (Nujol) 3325, 3185, 1685, 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (400 MHz, 293 K, CD_3OD) δ (ppm) 0.97–1.18 (m, 2H), 1.18–1.37 (m, 5H), 1.39–1.50 (m, 11H), 1.50–1.68 (m, 2H), 1.87–2.05 (m, 2H), 2.54 (br d, 0.45H, *J* 8.2 Hz), 2.66 (br d, 0.55H, *J* 8.3 Hz), 2.85 (t, 1H, *J* 11.8 Hz), 3.03 (t, 2H, *J* 6.9 Hz), 3.05–3.30 (m, 2H), 7.23–7.32 (m, 2H), 7.36 (t, 0.45H, *J* 7.3 Hz), 7.42 (t, 0.55H, *J* 7.6 Hz), 7.46–7.56 (m, 2H), 7.56–7.62 (m, 1H), 7.65–7.72 (m, 1H), 7.87–7.95 (m, 1H). ^{13}C NMR (100 MHz, 293 K, CD_3OD) δ (ppm) 25.8, 29.7, 32.0, 33.6/33.8, 37.2, 38.2, 42.1, 55.7/55.8, 61.9/62.2, 80.6, 123.8/123.9, 127.4, 127.8, 128.6, 129.8, 130.3, 130.8, 131.4, 131.9, 132.9/133.0, 135.1/135.6, 136.8, 137.8, 144.6/144.7, 159.4, 170.0/170.2, 172.1/172.3. MS (ESI, MeOH) *m*/*z* (%) 545 (10) $[M+K]^+$, 529 (55) $[M+Na]^+$, 507 (100) $[M+H]^+$, 451 (14) $[M-C_4H_8+H]^+$. $C_{29}H_{38}N_4O_4$ (506.6).

4.1.30. 5-((4-(3-Aminopropyl)piperidin-1-yl)acetyl)-5*H*-dibenzo-[*b*,*e*][1,4]diazepin-11(10*H*)-one·H_2O (46)

Compound **44** (1.99 g, 4.04 mmol) was suspended in 5 M HCl (12 mL) and the mixture was stirred at rt for 90 min (turned to a clear solution within 15 min). Under ice cooling 28% aq NH_3 (6 mL) was added and the product was extracted with CH_2Cl_2 (50, 30 and 20 mL). The pooled extracts were washed with water (10 mL) and dried over Na_2SO_4. The volatiles were evaporated and the product was column chromatographed (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:20:1 to 100:25:2). Removal of the volatiles from the eluate under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 and removal of the solvent in vacuo afforded product **46** as a white glass (1.35 g, 85%) mp 84–86 °C (turned to a resin). R_f = 0.3 (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1). Anal. calcd for $C_{23}H_{28}N_4O_2 \cdot H_2O$: C, 67.29; H, 7.37; N, 13.65; found: C, 67.43; H, 7.13; N, 13.51. IR (Nujol) 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (600 MHz, CD_3OD) δ (ppm) 1.00–1.19 (m, 2H), 1.20–1.30 (m, 3H), 1.42–1.53 (m, 2.5H), 1.57 (d, 1H, *J* 11.5 Hz), 1.64 (d, 0.5H, *J* 11.9 Hz), 1.87–2.04 (m, 2H), 2.52 (br d, 0.45H, *J* 8.6 Hz), 2.60 (t, 2H, *J* 7.3 Hz), 2.66 (br d, 0.55H, *J* 8.0 Hz), 2.79–2.89 (m, 1H), 3.03–3.25 (m, 2H), 7.22–7.31 (m, 2H), 7.34 (t, 0.45H, *J* 7.0 Hz), 7.41 (t, 0.55H, *J* 7.2 Hz), 7.46–7.56 (m, 2H), 7.56–7.61 (m, 1H), 7.64–7.70 (m, 1H), 7.87–7.94 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 31.7, 33.6/33.8/33.9, 35.6, 37.2, 43.6, 55.6/55.8, 61.9/62.2, 123.8, 127.4, 127.8, 128.6, 129.8, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1/135.5, 136.9, 137.8, 144.6/144.7, 170.0/170.2, 172.1/172.4. MS (ESI, MeOH) *m*/*z* (%) 785 (22) $[2M+H]^+$, 393 (100) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{23}H_{29}N_4O_2]^+$ 393.2291, found: 393.2277. $C_{23}H_{28}N_4O_2$ (392.5).

4.1.31. 5-((4-(4-Aminobutyl)piperidin-1-yl)acetyl)-5*H*-dibenzo-[*b*,*e*][1,4]diazepin-11(10*H*)-one (47)

Compound **45** (3.53 g, 6.97 mmol) was dissolved in 60 mL of a mixture of CH_2Cl_2 and methanol (10:1 v/v) and gaseous hydrochloric acid (generated from ammonium chloride and sulphuric acid) was passed through a glass tube into the stirred solution for 60 min. The solvent was removed under reduced pressure and the residue was taken up in chloroform (200 mL), 28% aq NH_3 (10 mL) and 1% aq NaOH (70 mL). After extraction the aqueous phase was separated from the organic phase followed by two more extractions with chloroform (100 mL each). The combined extracts were washed with water (40 mL) and dried over Na_2SO_4. The volatiles were evaporated and the product was purified by column chromatography using mixtures of CH_2Cl_2, MeOH and 28% aq NH_3 as eluent (R_f = 0.4 for CH_2Cl_2/MeOH/28% aq NH_3 80:18:2). Removal of the solvent under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 and removal of the solvent in vacuo afforded product **47** as a white glass (2.63 g, 93%) mp 65–68 °C. Anal. calcd for $C_{24}H_{30}N_4O_2$: C, 70.91; H, 7.44; N, 13.78; found: C, 70.75; H, 7.60; N, 13.80. IR (Nujol) 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (400 MHz, CD_3OD) δ (ppm) 0.97–1.18 (m, 2H), 1.18–1.38 (m, 5H), 1.45 (p, 2H, *J* 7.2 Hz), 1.50–1.68 (m, 2H), 1.86–2.05 (m, 2H), 2.48–2.73 (m, 3H), 2.85 (t, 1H, *J* 12.1 Hz), 3.00–3.30 (m, 2H), 7.22–7.32 (m, 2H), 7.36 (t, 0.45H, *J* 7.6 Hz), 7.42 (t, 0.55H, *J* 7.6 Hz), 7.46–7.56 (m, 2H), 7.56–7.62 (m, 1H), 7.64–7.72 (m, 1H), 7.86–7.95 (m, 1H). ^{13}C NMR (100 MHz, 293 K, CD_3OD) δ (ppm) 26.0, 33.6/33.8, 34.8, 37.3, 38.3, 43.4, 55.7/55.8, 61.8/62.2, 123.8/123.9, 127.5, 127.8, 128.6, 129.8, 130.3, 130.8, 131.4, 131.9, 132.9/133.0, 135.1/135.6, 136.8, 137.8, 144.6/144.7, 170.0/170.2, 172.1/172.3. MS (ESI, MeOH) *m*/*z* (%) 469 (25), 447 (93) $[M+K]^+$, 407 (100) $[M+H]^+$. $C_{24}H_{30}N_4O_2$ (406.5).

4.1.32. 5-((4-(3-Guanidinopropyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one bis(hydrotrifluoroacetate) (49)

Guanidinylating reagent **48** (146 mg, 0.47 mmol), amine **46** (176 mg, 0.45 mmol) and triethylamine (23 mg, 0.22 mmol) were dissolved in CH_2Cl_2 (3 mL). The mixture was stirred at rt for 16 h and then subjected to column chromatography (eluent: CH_2Cl_2/MeOH 100:1 to 20:1) for purification of the Boc-protected intermediate (R_f = 0.3 for CH_2Cl_2/MeOH 20:1). The solvent was removed from the eluate under reduced pressure and the residue was dissolved in CH_2Cl_2 (2 mL). Water (100 μL) and trifluoroacetic acid (TFA) (1 mL) were added and the solution was stirred at rt for 4 h. CH_2Cl_2 (20 mL) was added, the solvent was evaporated, and the process repeated. The residue was column chromatographed (eluent: CH_2Cl_2/MeOH/TFA 400:40:1 to 300:100:1). The volatiles were removed from the eluate under reduced pressure, the oily residue was dissolved in 0.1% aq TFA (40 mL) and the solution was filtered through a 0.45 μm filter (Minisart RC 25, cellulose membrane, polypropylene housing, Sartorius Stedium Biotech GmbH, Goettingen, Germany). Lyophilisation afforded product **49** as a white hygroscopic solid (276.4 mg, 93%). R_f = 0.2–0.3 (CH_2Cl_2/MeOH/TFA 200:100:1). Ratio of configurational isomers evident in the NMR spectra: ca 1.7:1. ^1H NMR (600 MHz, CD_3OD) δ (ppm) 1.38 (br s, 2H), 1.47–1.66 (m, 5H), 1.91–2.02 (m, 2H), 2.98 (m, 1H), 3.09 (m, 1H), 3.19 (t, 2H, *J* 7.1 Hz), 3.50 (br t, 1H, *J* ca 10.6 Hz), 3.72–3.85 (m, 2H), 4.44 (d, 0.6H, *J* 16.7 Hz), 4.80 (d, 0.35H, *J* 16.6 Hz), 7.28–7.33 (m, 0.8H), 7.34–7.43 (m, 1.6H), 7.48–7.57 (m, 2.2H), 7.63–7.68 (m, 1H), 7.68–7.73 (m, 1H), 7.78 (t, 0.35H, *J* 7.3 Hz), 7.93 (d, 0.6H, *J* 7.7 Hz), 8.00 (d, 0.35H, *J* 7.7 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 27.7, 31.1, 34.5, 35.1, 43.2, 55.8/56.1, 58.9, 123.9, 124.5, 127.7, 128.4, 128.8, 129.4, 129.7, 130.3, 130.9, 131.4, 131.8, 132.1, 132.6, 132.8, 133.2/133.8, 134.3, 135.4, 135.8, 136.3, 136.6, 137.9, 141.9, 143.6, 159.6, 165.8/166.3, 169.4/169.7. MS (ESI, MeOH) *m*/*z* (%) 435 (36) $[M+H]^+$, 218 (100) $[M+2H]^{2+}$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{24}H_{31}N_6O_2]^+$ 435.2508, found: 435.2493, *m*/*z* calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9853. $C_{24}H_{30}N_6O_2 \cdot 2 \times C_2HF_3O_2$ (434.5 + 2 × 114.025).

4.1.33. 5-((4-(4-Guanidinobutyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one bis(hydrotrifluoroacetate) (50)

Compound **48** (124 mg, 0.4 mmol), amine **47** (148 mg, 0.36 mmol) and triethylamine (18 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (3 mL). The mixture was heated to 60 °C in a microwave reactor for 50 min and then subjected to column chromatography. The Boc-protected intermediate was isolated using mixtures of CH_2Cl_2 and MeOH as eluent (R_f = 0.4 for CH_2Cl_2/MeOH 20:1). The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (2 mL). Water (50 μL) and TFA (1 mL) were added and the mixture was stirred at rt for 7 h. CH_2Cl_2 (20 mL) was added twice followed by evaporation. The product was purified by column chromatography using mixtures of CH_2Cl_2 and MeOH as eluent (R_f = 0.2–0.4 for CH_2Cl_2/MeOH/TFA 200:40:1). The eluent was supplemented with TFA (0.2%). After elution from column the solvent was evaporated and the oily residue was dissolved in 0.1% aq TFA (10 mL). Lyophilisation afforded the product as a white, hygroscopic solid (167 mg, 67%). IR (KBr) 3385, 1675, 1600 cm^{-1}. Ratio of isomers evident in the NMR spectra: ca 1.8:1. ^1H NMR (400 MHz, CD_3OD) δ (ppm) 1.32–1.48 (m, 4.5H), 1.48–1.66 (m, 4.5H), 1.90–2.05 (m, 2H), 2.90–3.02 (m, 1H), 3.08 (t, 1H, *J* 11.3 Hz), 3.19 (t, 2H, *J* 7.1 Hz), 3.48 (br s, 1H), 3.71–3.87 (m, 2H), 4.40–4.50 (m, 1H), 7.27–7.39 (m, 2H), 7.42 (dt, 0.35H, *J* 7.7 1.3 Hz), 7.48–7.59 (m, 2.2H), 7.64–7.70 (m, 1.4H), 7.73 (dt, 0.6H, *J* 7.7 1.5 Hz), 7.79 (dt, 0.35H, *J* 7.7 1.4 Hz), 7.96 (dd, 0.6H, *J* 8.1 1.5 Hz), 8.00 (dd, 0.35H, *J* 7.7 1.2 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 25.4, 30.7, 31.3, 35.2, 37.1, 43.2, 55.9/56.2, 58.8/58.9, 116.0 (TFA), 117.8 (TFA), 123.9, 124.5, 127.7, 128.4, 128.8, 129.4, 129.8, 130.3, 131.0, 131.4, 131.8, 132.1, 132.6, 132.8, 133.2/133.9, 134.3, 135.4/136.3, 135.8, 136.6, 137.9, 141.9, 143.6, 159.6, 165.8/166.3, 169.5/169.7. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{25}H_{33}N_6O_2]^+$ 449.2665, found: 449.2647, *m*/*z* calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9845. $C_{25}H_{32}N_6O_2{\cdot}2{\times}C_2HF_3O_2$ (448.6 + 2 × 114.025).

4.1.34. *N*-*tert*-Butoxycarbonyl-*N*′-(*N*-(2-*tert*-butoxycarbonylaminoethyl)aminocarbonyl)-*S*-methylisothiourea (51)[58]

N-Boc-*S*-methylisothiourea (0.73 g, 3.84 mmol) and succinimidyl [2-(Boc-amino)ethyl]carbamate (1.05 g, 3.49 mmol) were suspended in anhydrous CH_2Cl_2 (25 mL). Triethylamine (0.18 g, 1.74 mmol) was added and the mixture was stirred at rt overnight. The volume was reduced by evaporation to about 8 mL and the solution was subjected to column chromatography (eluent: CH_2Cl_2/Et_2O 50:1 to CH_2Cl_2/MeOH 20:1). The volatiles were removed from the eluate under reduced pressure, the residue (colorless resin) was re-dissolved in CH_2Cl_2 (25 mL), the solvent evaporated, and the residue taken up in Et_2O (1 mL) and *n*-hexane (5 mL). The volume was reduced by evaporation to about 2 mL resulting in the formation of two phases. Storage at −20 °C afforded **51** as white needles (0.83 g, 63%) mp 141–142 °C. R_f = 0.5 (CH_2Cl_2/Et_2O 5:1). Anal. calcd for $C_{15}H_{28}N_4O_5S$: C, 47.86; H, 7.50; N, 14.88; found: C, 47.71; H, 7.50; N, 14.69. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.43 (s, 9H), 1.48 (s, 9H), 2.40 (br s, 3 H), 3.22–3.38 (m, 4H), 4.91 (br s, 1H), 6.53 (br s, 1H), 12.43 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 15.0, 28.3, 28.7, 40.5, 41.1, 80.0, 83.8, 151.0, 156.7. MS (ESI, MeOH) *m*/*z* (%) 399 (100) $[M+Na]^+$, 299 (27) $[M-C_4H_8-CO_2+H]^+$. $C_{15}H_{28}N_4O_5S$ (376.47).

4.1.35. 5-((4-(3-(2-(2-Aminoethylcarbamoyl)guanidin-1-yl)-propyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one tris(hydrotrifluoroacetate) (52)

Compound **51** (397 mg, 1.05 mmol) and amine **46** (455 mg, 1.16 mmol) were dissolved in DMSO (8 mL). Mercury(II)chloride (429 mg, 1.58 mmol) was added and the mixture was stirred at rt overnight. Water (100 mL) and 28% aq NH_3 (2 mL) were added and the Boc-protected intermediate was extracted with EtOAc/Et_2O (1:1 v/v, 2 × 100 mL and 2 × 80 mL). The pooled extracts were washed with brine (30 mL) and dried over Na_2SO_4. The volatiles were evaporated and the Boc-protected intermediate was isolated by column chromatography (eluent: CH_2Cl_2 to CH_2Cl_2/MeOH 30:1; R_f = 0.6 for CH_2Cl_2/MeOH 20:1). Removal of the solvent under reduced pressure and drying in vacuo yielded a glass (585 mg), which was dissolved in MeOH (10 mL) and water (10 mL). TFA (1 mL) was added and the mixture was stirred for 72 h at rt, then concentrated under reduced pressure at 50 °C to a volume of about 8 mL and filtered through a 0.45 μm filter (for filter type cf. exp. protocol of **49**). The filtrate was diluted with water (80 mL) and lyophilisation afforded product **52** as a white fluffy, highly hygroscopic solid (0.75 g, 82%). Ratio of configurational isomers evident in the NMR spectra: ca 1.8:1. ^1H NMR (600 MHz, CD_3OD) δ (ppm) 1.40 (br s, 2H), 1.47–1.63 (m, 3H), 1.69 (p, 2H, *J* 7.5 Hz), 1.90–2.04 (m, 2H), 2.92–3.02 (m, 1H), 3.05–3.15 (m, 3H), 3.30 (t, 2H, *J* 7.1 Hz), 3.45–3.51 (m, 1H), 3.53 (t, 2H, *J* 5.7 Hz), 3.72–3.85 (m, 2H), 4.44 (d, 0.65H, *J* 16.7 Hz), 4.49 (d, 0.35H, *J* 16.6 Hz), 7.27–7.33 (m, 0.8H), 7.34–7.39 (m, 1.2H), 7.41 (t, 0.35H, *J* 7.3 Hz), 7.48–7.57 (m, 2.3H), 7.63–7.73 (m, 2H), 7.78 (t, 0.35H, *J* 7.2 Hz), 7.93 (d, 0.65H, *J* 8.0 Hz), 8.00 (d, 0.35H, *J* 7.7 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 27.2, 31.2, 34.4, 35.0, 39.4, 41.6, 43.2, 55.8/56.1, 58.9, 115.8 (TFA), 117.7 (TFA), 119.6 (TFA), 121.6 (TFA), 123.9/124.5, 127.7, 128.4, 128.8, 129.4, 129.7, 130.3, 130.9, 131.4, 131.7, 132.1, 132.6, 132.8, 133.2/133.8, 134.3, 135.4, 135.8, 136.3, 136.6, 137.8, 141.8, 143.6, 156.6, 157.3, 162.9 (TFA), 163.2 (TFA), 163.4 (TFA), 163.6 (TFA), 165.8/166.3, 169.4/169.7. MS (ESI, MeOH) *m*/*z* (%) 521 (6) $[M+H]^+$, 261 (100) $[M+2H]^{2+}$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{27}H_{37}N_8O_3]^+$ 521.2989, found: 521.2978, *m*/*z* calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9868. $C_{27}H_{36}N_8O_3{\cdot}3{\times}C_2HF_3O_2$ (520.63 + 3 × 114.025).

4.1.36. 5-((4-(4-(2-(2-Aminoethylcarbamoyl)guanidin-1-yl)-butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one tris(hydrotrifluoroacetate) (53)

Compound **51** (400 mg, 1.06 mmol) and amine **47** (432 mg, 1.06 mmol) were dissolved in DMF (10 mL). Mercury(II)chloride (432 mg, 1.59 mmol) was added and the mixture was stirred at rt overnight. CH_2Cl_2 (80 mL) was added and insoluble material was removed by filtration. The volatiles were removed under reduced pressure, the residue was taken up in Et_2O (100 mL), water (100 mL) and 28% aq NH_3 (1 mL) and the mixture was vigorously shaken. The aqueous phase was separated from the organic phase followed by two more extractions with Et_2O (50 mL each). The pooled extracts were washed with brine (30 mL) and dried over Na_2SO_4. The volatiles were evaporated and the Boc-protected intermediate was isolated by column chromatography using mixtures of CH_2Cl_2 and MeOH as eluent (R_f = 0.6 for CH_2Cl_2/MeOH 20:1). Removal of the solvent under reduced pressure and drying in vacuo yielded a glass, which was dissolved in CH_2Cl_2 (3 mL). Water (100 μL) and trifluoroacetic acid (TFA) (1.5 mL) was added and the mixture was stirred for 4 h at rt. CH_2Cl_2 (25 mL) was added twice followed by evaporation. The product was purified by column chromatography using mixtures of CH_2Cl_2 and MeOH as eluent. The eluent was supplemented with TFA (0.2%). As thin layer chromatography (TLC) analysis could not properly be performed using acidic conditions the product was analyzed using basic conditions (R_f = 0.3 for CH_2Cl_2/MeOH/28% aq NH_3 80:18:2). The solvent was evaporated from the eluate and the oily residue was dissolved in 0.1% aq TFA (8 mL). Lyophilisation afforded product **53** as a pale brown, hygroscopic resin (610 mg, 66%), which contained a small amount

of by-product (HRMS: found: 531.3182 [M+H$^+$]). Using semi-preparative reversed-phase HPLC,[†] a portion (40 mg) of this material was chromatographed to give pure product **53** (24 mg) as a white, highly hygroscopic solid (after lyophilisation of the eluate). Ratio of configurational isomers evident in the NMR spectra: ca 1.7:1. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 1.33–1.47 (m, 4H), 1.47–1.61 (m, 3H), 1.66 (p, 2H, J 7.1 Hz), 1.90–2.04 (m, 2H), 2.90–3.02 (m, 1H), 3.02–3.11 (m, 1H), 3.12 (t, 2H, J 5.7 Hz), 3.31 (t, 2H, J 7.1 Hz), 3.48 (br s, 1H), 3.53 (t, 2H, J 5.7 Hz), 3.71–3.86 (m, 2H), 4.40–4.50 (m, 1H), 7.30 (t, 0.6H, J 7.4 Hz), 7.33–7.44 (m, 1.7H), 7.48–7.59 (m, 2.3H), 7.63–7.81 (m, 2.4H), 7.93 (dd, 0.65H, J 8.0 1.3 Hz), 8.00 (d, 0.35H, J 7.7 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 25.4, 30.1, 31.3, 35.2, 37.1, 39.4, 41.6, 43.2, 55.8/56.2, 58.9, 123.9/124.5, 127.7, 128.4, 128.7, 129.3, 129.7, 130.3, 131.0, 131.4, 131.7, 132.1, 132.6, 132.8, 133.2/133.9, 134.3, 135.4/136.3, 135.8, 136.6, 137.9, 141.9, 143.5, 156.6, 157.4, 163.3 (TFA), 163.6 (TFA), 164.0 (TFA), 164.4 (TFA), 165.8/166.3, 169.5/169.7. HRMS (ESI, MeOH) m/z calcd for $[C_{28}H_{39}N_8O_3]^+$ 535.3145, found: 535.3132, m/z calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9849. $C_{28}H_{38}N_8O_3{\cdot}3\times C_2HF_3O_2$ (534.7 + 3 × 114.025).

4.1.37. 5-((4-(3-(2-(2-Propionamidoethylcarbamoyl)guanidin-1-yl)propyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one (54)

Compound **52** (0.27 g, 0.31 mmol) and diisopropylethylamine (0.15 g, 1.19 mmol) were dissolved in DMF (2 mL). Under stirring a solution of *N*-succinimidyl propionate (51 mg, 0.30 mmol) in DMF (0.3 mL) was added dropwise and the mixture was stirred at rt for 2.5 h. The solvent was removed under reduced pressure at 60 °C, the residue was dried in vacuo, then taken up in CH_2Cl_2 (30 mL) and washed with 5% aq NaOH (10 mL). As the product was evident in the aq phase by TLC analysis, it was extracted from the aq phase with CH_2Cl_2 (3 × 20 mL). The organic layers were combined, washed with brine/water (2:1 v/v, 8 mL) and dried over Na_2SO_4. The volatiles ware evaporated and the residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH/28% aq NH_3 400:20:1 to 200:40:1). Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 (5 mL) and filtration of the solution with a cotton wool packed Pasteur pipette followed by removal of the solvent in vacuo afforded **54** as a white glass (121 mg, 70%) mp >93 °C (turned to a resin). R_f = 0.4 (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1). IR (Nujol) 3305 br, 1655, 1600, 1500 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (700 MHz, CD_3OD) δ (ppm) 1.00–1.23 (m, 5H), 1.27 (br s, 3H), 1.47–1.60 (m, 3.5H), 1.64 (br d, 0.5H, J ca 12 Hz), 1.89–2.05 (m, 2H), 2.22 (q, 2H, J 7.6 Hz), 2.51 (br s, 0.45H), 2.65 (br s, 0.55H), 2.79–2.89 (m, 1H), 3.02–3.09 (m, 0.5H), 3.16 (t, 2H, J 6.6 Hz), 3.17–3.32 (m, 5.5H), 7.24–7.32 (m, 2H), 7.35 (t, 0.45H, J 7.3 Hz), 7.42 (t, 0.55H, J 7.4 Hz), 7.46–7.52 (m, 2.45H), 7.54 (t, 0.55H, J 7.2 Hz), 7.56–7.61 (m, 1H), 7.65–7.71 (m, 1H), 7.89 (d, 0.55H, J 7.5 Hz), 7.93 (d, 0.45H, J 7.4 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 11.3, 28.3, 31.1, 33.5/33.7/33.8, 35.4, 37.0, 41.2, 41.6, 43.0, 55.7, 61.8/62.2, 123.9, 127.4/127.8, 128.6, 129.76, 129.81, 130.2, 130.7, 131.4, 131.9, 132.8, 132.9/133.0, 135.1/135.5, 136.8, 137.7, 144.62/144.69, 161.5, 170.0/170.2, 172.1/172.3, 178.2. MS (ESI, MeOH) m/z (%) 1153 (20) [2M+H]$^+$, 599 (12) [M+Na]$^+$, 577 (100) [M+H]$^+$. HRMS (ESI, MeOH) m/z calcd for $[C_{30}H_{41}N_8O_4]^+$ 577.3251, found: 577.3243. $C_{30}H_{40}N_8O_4$ (576.32).

[†] Column: RESTEK Pinnacle BD C18 (150 × 10 mm, 5 μm) (RESTEK, Bellefonte, PA); column temperature: ambient; mobile phase: mixtures of acetonitrile (A) and 0.1% aqueous TFA (B), linear gradient: 0–2 min: A/B 15:85, 2–6 min: 15:85 to 28:72, 6–12 min: 28:72 to 33:67, 12–14 min: 33:67 to 70:30, 14–16 min: 70:30; flow rate: 3 mL/min; detection: 190–800 nm; retention times: **53**: 10.5 min, by-component: 11.2 min.

4.1.38. 5-((4-(4-(2-(2-Propionamidoethylcarbamoyl)guanidin-1-yl)butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one (55)

Amine **53** (265 mg, 0.3 mmol) and triethylamine (153 mg, 1.51 mmol) were dissolved in a mixture of acetonitrile (4 mL) and DMF (0.8 mL). Two portions of *N*-succinimidyl propionate were added with a time lag of 15 min (1. 20.7 mg, 0.12 mmol in 0.4 mL of acetontrile; 2. 10.3 mg, 0.06 mmol in 0.2 mL of acetonitrile) and the mixture was stirred at rt for 45 min. The volatiles were removed under reduced pressure, the residue was taken up in a mixture of Et_2O (10 mL), 1% aq NaOH (10 mL) and 28% aq NH_3 (0.5 mL), and the mixture was vigorously shaken. The phases were separated and three more extractions with Et_2O followed (10 mL each). The product could not be extracted using Et_2O, but an impurity which appeared nearby the product spot in TLC analysis. The product was extracted with EtOAc (3 × 10 mL, 8 mL and 6 mL). The EtOAc extracts were pooled, washed with water (5 mL) and dried over Na_2SO_4. The solvent was evaporated and the product was column chromatographed using mixtures of CH_2Cl_2, MeOH and 28% aq NH_3 as eluent (R_f = 0.5 for CH_2Cl_2/MeOH/28% aq NH_3 80:19:1). Removal of the solvent under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 and removal of the solvent in vacuo afforded the product as a pale yellow solid (93 mg, 87%) mp >80 °C (turned to a resin). A portion (53 mg) of the product was dissolved in 0.2% aq TFA (28 mL) and the slightly cloudy solution was filtered through a 0.45 μm filter (for filter type cf. exp. protocol of **49**). Lyophilisation yielded a white fluffy, hygroscopic solid (**55**·2 TFA, 63 mg). IR (Nujol) (free base of **55**) 3290, 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.7:1. ^{1}H NMR (600 MHz, CD_3OD, **55**·2 TFA) δ (ppm) 1.14 (t, 3H, J 7.7 Hz), 1.34–1.30 (m, 2H), 1.41–1.47 (m, 2H), 1.47–1.61 (m, 3H), 1.66 (p, 2H, J 7.2 Hz), 1.95 (d, 1H, J 13.9 Hz), 2.01 (d, 1H, J 11.9 Hz), 2.23 (q, 2H, J 7.6 Hz), 2.92–3.01 (m, 1H), 3.08 (t, 1H, J 11.5 Hz), 3.30 (t, 2H, J 7.0 Hz), 3.33 (br s, 4H), 3.49 (t, 1H, J 11.5 Hz), 3.72–3.85 (m, 2H), 4.43 (d, 0.65H, J 16.7 Hz), 4.48 (d, 0.35H, J 16.7 Hz), 7.28–7.39 (m, 2H), 7.42 (dt, 0.4H, J 7.7 1.1 Hz), 7.49–7.58 (m, 2.2H), 7.64–7.70 (m, 1.4H), 7.72 (dt, 0.6H, J 7.7 1.4 Hz), 7.79 (t, 0.4H, J 7.4 Hz), 7.93 (dd, 0.6H, J 8.0 1.3 Hz), 8.00 (dd, 0.4H, J 7.8 1.0 Hz). ^{13}C NMR (150 MHz, CD_3OD, **55**·2 TFA) δ (ppm) 11.2, 25.4, 30.1, 31.0, 31.3, 35.2, 37.1, 40.7, 41.3, 43.1, 55.9/56.2, 58.8/58.9, 115.6 (TFA), 117.5 (TFA), 119.4 (TFA), 121.3 (TFA), 124.0/124.5, 127.7, 128.4, 128.7, 129.3, 129.7, 130.3, 131.0, 131.4, 131.7, 132.1, 132.6, 132.8, 133.2/133.9, 134.3, 135.4/136.3, 135.8, 136.6, 137.9, 141.9, 143.5, 156.7, 162.4 (TFA), 162.7 (TFA), 162.9 (TFA), 163.2 (TFA), 165.8/166.3, 169.4/169.7, 178.4. MS (ESI, MeOH) m/z (%) 613 (11) [M+Na]$^+$, 591 [M+H]$^+$ (100), 475 (13), 432 (27). HRMS (ESI, MeOH, **55**·2 TFA) m/z calcd for $[C_{31}H_{43}N_8O_4]^+$ 591.3407, found: 591.3396, m/z calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9849. $C_{31}H_{42}N_8O_4$ (590.7).

4.1.39. 5-((4-(3-(2-(2-(4-Fluorobenzamido)ethylcarbamoyl)guanidin-1-yl)propyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]-diazepin-11(10*H*)-one (56)

Compound **56** was prepared from amine **52** (0.26 g, 0.30 mmol) and *N*-succinimidyl 4-fluorobenzoate (68 mg, 0.29 mmol) using the procedure for the synthesis of **54**. The product **56** was obtained as a white glass (103 mg, 56%) mp >97 °C (turned to a resin). R_f = 0.6 (CH_2Cl_2/MeOH/28% aq NH_3 100:20:1). Anal. calcd for $C_{34}H_{39}FN_8O_4{\cdot}H_2O$: C, 61.80; H, 5.95; N, 16.96; found: C, 61.82; H, 6.29; N, 16.56. IR (Nujol) 3325 br, 1655, 1600, 1500 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (700 MHz, CD_3OD) δ (ppm) 0.98–1.20 (m, 2H), 1.25 (br s, 3H), 1.43–1.58 (m, 3.5H), 1.62 (br d, 0.5H, J ca 11 Hz), 1.87–2.03 (m, 2H), 2.50 (br s, 0.45H), 2.64 (br s, 0.55H), 2.78–2.87 (m, 1H), 3.02–3.08 (m, 0.5H), 3.14 (t, 2.5H, J 6.5 Hz), 3.16–3.26 (m, 1H), 3.38 (br s, 2H), 3.50 (t, 2H, J 5.8 Hz), 7.15–7.21 (m, 2H),

7.24–7.31 (m, 2H), 7.34 (t, 0.45H, *J* 7.2 Hz), 7.41 (t, 0.55H, *J* 7.4 Hz), 7.45–7.51 (m, 1.45H), 7.53 (t, 0.55H, *J* 7.3 Hz), 7.56–7.60 (m, 1H), 7.64–7.70 (m, 1H), 7.83–7.94 (m, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 28.4, 33.5/33.7/33.8, 35.6, 37.0, 41.2, 42.8, 42.9, 55.6, 61.8/62.2, 117.1, 117.2, 123.9, 127.4/127.8, 128.6, 129.75, 129.81, 130.3, 130.7, 131.4, 131.7, 131.8, 131.9, 132.9/133.0, 135.1/135.5, 136.8, 137.8, 144.6/144.7, 162.2, 166.2, 167.4 (br), 167.8, 170.1 (two carbons), 170.2, 172.1/172.4. MS (ESI, MeOH) *m*/*z* (%) 1307 (27) [2*M*+Na]$^+$, 1285 (21) [2*M*+H]$^+$, 665 (88) [*M*+Na]$^+$, 643 (100) [*M*+H]$^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{34}H_{40}FN_8O_4]^+$ 643.3157, found: 643.3150. $C_{34}H_{39}FN_8O_4$ (642.72).

4.1.40. 5-((4-(4-(1*H*-Imidazol-1-yl)butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one·1H$_2$O (57)

Compound **57** was prepared from piperidine **30** (0.35 g, 1.22 mmol) and compound **43** (254 mg, 1.22 mmol) using the procedure for the preparation of **29** (instead of a 20-mL vial a 5-mL vial was used). Eluent for column chromatography: CH_2Cl_2/MeOH 100:1 to 10:1. Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake of the resin-like residue in CH_2Cl_2 (5 mL), filtration of the solution with a cotton wool packed Pasteur pipette, evaporation, re-uptake in CH_2Cl_2 (2 mL) and *n*-pentane (3 mL) followed by removal of the solvent in vacuo afforded product **57** as a white glass (409 mg, 73%) mp >83 °C (turned to a resin). R_f = 0.6 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{27}H_{31}N_5O_2\cdot H_2O$: C, 68.19; H, 6.99; N, 14.73; found: C, 68.67; H, 6.82; N, 14.64. IR (Nujol) 1665, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (600 MHz, CD_3OD) δ (ppm) 0.97–1.18 (m, 2H), 1.25 (br s, 5H), 1.44 (d, 0.45H, *J* 11.6 Hz), 1.52 (d, 1H, *J* 12.4 Hz), 1.60 (d, 0.55H, *J* 11.9 Hz), 1.76 (p, 2H, *J* 6.9 Hz), 1.87–2.02 (m, 2H), 2.48 (br s, 0.45H), 2.64 (br s, 0.55H), 2.82 (m, 1H), 3.03–3.24 (m, 2H), 4.02 (t, 2H, *J* 7.1 Hz), 6.98 (s, 1H), 7.12 (s, 1H), 7.23–7.31 (m, 2H), 7.34 (t, 0.45H, *J* 6.6 Hz), 7.41 (t, 0.55H, *J* 7.1 Hz), 7.45–7.59 (m, 3H), 7.65 (s, 1H), 7.66 (br s, 1H), 7.87–7.94 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 25.5, 33.1, 33.5/33.7/33.8, 37.1, 37.7, 48.8, 55.5/55.8, 61.8/62.2, 121.4, 123.8, 127.4, 127.8, 128.6, 129.8, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1, 135.5, 136.8, 137.8, 139.2, 144.7, 170.0/170.2, 172.2/172.3. MS (ESI, MeOH) *m*/*z* (%) 937 (18) [2*M*+Na]$^+$, 915 (42) [2*M*+H]$^+$, 480 (32) [*M*+Na]$^+$, 458 (100) [*M*+H]$^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{27}H_{32}N_5O_2]^+$ 458.2556, found: 458.2547. $C_{27}H_{31}N_5O_2$ (457.6).

4.1.41. 5-((4-(4-(4-Methylpiperazin-1-yl)butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one·0.5H$_2$O (58)

Compound **58** was prepared from **31** (213 mg, 0.89 mmol) and compound **43** (255 mg, 0.89 mmol) using the procedure for the preparation of **29**. Eluent for column chromatography: CH_2Cl_2/MeOH 100:1 to 10:1. Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 (10 mL), filtration of the solution with a cotton wool packed Pasteur pipette, evaporation, re-uptake in CH_2Cl_2 (1.5 mL) and *n*-pentane (6 mL) followed by removal of the solvent in vacuo afforded **58** as a white glass (355 mg, 81%) mp >75 °C (turned to a resin). R_f = 0.35 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{29}H_{39}N_5O_2\cdot 0.5H_2O$: C, 69.85; H, 7.88; N, 14.05; found: C, 69.87; H, 8.22; N, 13.93. IR (Nujol) 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 0.97–1.20 (m, 2H), 1.20–1.36 (m, 5H), 1.44–1.53 (m, 2.5H), 1.56 (d, 1H, *J* 11.3 Hz), 1.63 (d, 0.5H, *J* 11.8 Hz), 1.87–2.04 (m, 2H), 2.30 (s, 3H), 2.31–3.00 (br m, 12H), 3.02–3.36 (m, 2H), 7.22–7.32 (m, 2H), 7.35 (t, 0.45H, *J* 6.9 Hz), 7.41 (t, 0.55H, *J* 7.2 Hz), 7.45–7.56 (m, 2H), 7.56–7.61 (m, 1H), 7.64–7.71 (m, 1H), 7.86–7.95 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 26.6, 28.5, 33.6/33.8/33.9, 37.3, 38.2, 46.8, 54.5, 55.8, 56.4, 60.5, 61.9/62.2, 123.8, 127.4, 127.8, 128.6, 129.77, 129.84, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1/135.5, 136.8, 137.8, 144.6/144.7, 170.0/170.2, 172.1/172.3. MS (ESI, MeOH) *m*/*z* (%) 979 (47) [2*M*+H]$^+$, 512 (12) [*M*+Na]$^+$, 490 (100) [*M*+H]$^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{29}H_{40}N_5O_2]^+$ 490.3182, found: 490.3169. $C_{29}H_{39}N_5O_2$ (489.65).

4.1.42. 5-((4-(Ureidobutyl)piperidin-1-yl)acetyl)-5*H*-dibenzo-[*b*,*e*][1,4]diazepin-11(10*H*)-one (59)

Amine **47** (0.34 g, 0.84 mmol) was dissolved in ethanol (4 mL) and water (5 mL). 1 M HCl (0.84 mL) and potassium cyanate (88 mg, 1.09 mmol) were added and the mixture was stirred in a bath at 80 °C. 1 M HCl (0.84 mL) was added (pH of the mixture: 7–8) and the formation of the product was traced by TLC analysis. As a considerable amount of amine **47** could still be detected after 6 h, more potassium cyanate (380 mg, 4.69 mmol) was added and the pH was adjusted to ca 8 through addition of 1 M HCl (ca 1.5 mL). After continued stirring at 80 °C for 2 h the heater was turned off and stirring was continued overnight. 10% aq K_2CO_3 was added and the product was extracted with CH_2Cl_2 (30 and 25 mL). The extracts were combined, washed with brine/water (5:1 v/v, 5 mL) and dried over Na_2SO_4. The volatiles ware evaporated and the glassy residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 100:1 to 10:1). Removal of the solvent from the eluate under reduced pressure and drying in vacuo yielded product **59** as a white glass (0.22 g, 59%) mp >113 °C (turned to a resin). R_f = 0.5 (CH_2Cl_2/MeOH 5:1), 0.3 (Et_2O/MeOH 2:1). Anal. calcd for $C_{25}H_{31}N_5O_3\cdot 0.2H_2O$: C, 66.26; H, 6.90; N, 15.46; found: C, 66.22; H, 7.20; N, 15.08. IR (Nujol) 3345 br, 1660, 1600, 1500 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 0.68–0.83 (m, 1H), 0.99 (br s, 1H), 1.04–1.15 (m, 3H), 1.19 (br s, 2H), 1.25–1.34 (m, 2.5H), 1.39 (br t, 1H, *J* ca 14 Hz), 1.49 (br d, 0.5H, *J* ca 12 Hz), 1.75–1.87 (m, 2H), 2.17 (br d, 0.45H, *J* ca 10 Hz), 2.30 (br d, 0.55H, *J* ca 10 Hz), 2.60 (br t, 1H, *J* ca 11 Hz), 2.84–2.97 (m, 3H), 3.14 (d, 0.55H, *J* 14.4 Hz), 3.30 (d, 0.45H, *J* 14.3 Hz), 5.38 (m, 2H), 5.96 (br s, 1H), 7.10–7.22 (m, 2H), 7.24–7.32 (m, 1H), 7.36–7.47 (m, 2H), 7.54–7.63 (m, 2H), 7.74 (d, 0.55H, *J* 7.5 Hz), 7.78 (d, 0.45H, *J* 7.5 Hz), 10.6 (br s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 23.5, 30.2, 31.3/31.6/31.9, 34.6, 35.7, 39.1, 52.6/52.9/53.2, 60.2/60.6, 121.7, 124.1, 124.2, 126.1, 127.1, 127.8, 127.9, 128.0, 128.4, 128.9, 130.5, 130.8, 132.2, 132.6, 134.3, 134.7, 135.5, 136.7, 142.3/142.7, 158.8, 166.5/166.6, 168.6/169.0. MS (ESI, MeOH) *m*/*z* (%) 937 (7) [2*M*+K]$^+$, 921 (52) [2*M*+Na]$^+$, 899 (13) [2*M*+H]$^+$, 488 (11) [*M*+K]$^+$, 472 (53) [*M*+Na]$^+$, 450 (100) [*M*+H]$^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{25}H_{32}N_5O_3]^+$ 450.2505, found: 450.2496. $C_{25}H_{31}N_5O_3$ (449.55).

4.1.43. 5-((4-(4-(4-(((*N*-(*N*-*tert*-Butoxycarbonyl-2-aminoethyl))-3-amino-3-oxo)propyl)1*H*-imidazol-1-yl)butyl)piperidin-1-yl)-acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one·0.5H$_2$O (60)

Compound **36** (2.64 g, 6.26 mmol, included a minor amount of the respective 1,5-disubstitued imidazole derivative (ratio ca 7:1), cf. prep. of **36**) and finely ground potassium carbonate (3.46 g, 25.05 mmol) were suspended in anhydrous acetonitrile (20 mL). Chloride **43** (1.63 g, 5.69 mmol) was added and the mixture was vigorously stirred under reflux for 4 h. CH_2Cl_2 (150 mL) and 2% aq NaOH (80 mL) were added, the mixture was vigorously shaken, the phases were separated, and the aqueous phase treated with CH_2Cl_2 (100 mL). The organic phases were combined, washed with water (40 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the brown resin-like residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 100:1 to 10:1), which afforded a mixture of product **60** and the by-product, namely the respective 1,5-disubstitued 1*H*-imidazole

derivative (ratio ca 7:1) as a white glass-like solid (2.67 g, 70%). A portion (0.97 g) of this material was re-chromatographed (eluent: as above) to yield pure product **60** as a white glass-like solid (0.50 g) mp 69–73 °C. R_f = 0.4 (CH_2Cl_2/MeOH 10:1). Anal. calcd for $C_{37}H_{49}N_7O_5 \cdot 0.5H_2O$: C, 65.27; H, 7.25; N, 14.40; found: C, 64.97; H, 7.57; N, 14.11. IR (Nujol) 1660, 1600, 1500 cm^{-1}. Ratio of isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (600 MHz, CD_3OD) δ (ppm) 0.95–1.17 (m, 2H), 1.24 (br s, 5H), 1.45 (s, 9H), 1.48–1.55 (m, 1.5H), 1.60 (d, 0.5H, *J* 12.1 Hz), 1.73 (p, 2H, *J* 6.8 Hz), 1.87–2.03 (m, 2H), 2.45–2.53 (m, 2.45H), 2.65 (br d, 0.55H, *J* ca 9.5 Hz), 2.78–2.88 (m, 3H), 3.05 (d, 0.55H, *J* 15.5 Hz), 3.10–3.18 (m, 2.45H), 3.20–3.27 (m, 3H), 3.94 (t, 2H, *J* 7.0 Hz), 6.86 (s, 1H), 7.23–7.31 (m, 2H), 7.35 (t, 0.45H, *J* 7.0 Hz), 7.41 (t, 0.55H, *J* 7.3 Hz), 7.45–7.56 (m, 3H), 7.56–7.61 (m, 1H), 7.64–7.70 (m, 1H), 7.87–7.95 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 25.5, 26.0, 29.6, 33.0, 33.5/33.7/33.8, 37.1, 37.7, 37.8, 41.3, 41.8, 48.7, 55.5/55.8, 61.8/62.2, 81.0, 117.8, 123.9, 127.4, 127.8, 128.6, 129.77, 129.83, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1/135.5, 136.8, 137.8, 138.7, 142.57/142.60, 144.7, 159.3, 170.0/170.2, 172.1/172.3, 176.4. MS (ESI, MeOH) *m*/*z* (%) 1365 (10) $[2M+Na]^+$, 694 (64) $[M+Na]^+$, 672 (100) $[M+H]^+$, 364 (20). HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{37}H_{50}N_7O_5]^+$ 672.3873, found: 672.3871. $C_{37}H_{49}N_7O_5$ (671.83).

There followed a 5:1 mixture of **60** and the respective 1,5-disubstitued 1*H*-imidazole derivative as a white glass (0.38 g).

4.1.44. 5-((4-(4-(4-(((*N*-2-Aminoethyl)-3-amino-3-oxo)propyl)-1*H*-imidazol-1-yl)butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one tris(hydrotrifluoroacetate) (61)

Compound **60** (1.70 g, 2.52 mmol, included a minor amount of the respective 1,5-disubstitued imidazole derivative (ratio ca 7:1), cf. prep. of **60**) was dissolved in MeOH (8 mL) and water (2 mL). TFA (1 mL) was added, the mixture was stirred for 2 min and then water (8 mL) and TFA (1.5 mL) were added. Stirring was continued at rt for 24 h, the mixture was concentrated under reduced pressure at 50 °C to a volume of about 8 mL and filtered through a 0.45 μm filter (for filter type cf. exp. protocol of **49**). The filtrate was diluted with water (200 mL) and lyophilisation afforded a mixture of product **61** and the respective 1,5-disubstitued 1*H*-imidazole derivative (ratio ca 7:1) as a white fluffy, highly hygroscopic solid (2.37 g, 94%). Applying the same procedure another batch of compound **61** was prepared from isomerically pure carbamate **60** (434 mg, 0.65 mmol) to yield pure product **61** as a white fluffy, highly hygroscopic solid (570 mg, 96%). Ratio of configurational isomers evident in the NMR spectra: ca 1.8:1. ^1H NMR (700 MHz, CD_3OD) δ (ppm) 1.38 (br s, 4H), 1.44–1.52 (m, 1H), 1.52–1.60 (m, 2H), 1.86–1.95 (m, 3H), 1.98 (br d, 1H, *J* ca 10.8 Hz), 2.67 (t, 2H, *J* 7.4 Hz), 2.91–3.00 (m, 1H), 3.02 (t, 2H, *J* 7.1 Hz), 3.05–3.11 (m, 3H), 3.45–3.52 (m, 3H), 3.72–3.84 (m, 2H), 4.20 (t, 2H, *J* 7.4 Hz), 4.44 (d, 0.65H, *J* 16.7 Hz), 4.48 (d, 0.35H, *J* 16.7 Hz), 7.27–7.33 (m, 0.8H), 7.34–7.39 (m, 1.2H), 7.39–7.44 (m, 1.35H), 7.51 (dt, 0.65H, *J* 7.7 1.3 Hz), 7.52–7.57 (m, 1.65H), 7.63–7.68 (m, 1H), 7.68–7.74 (m, 1H), 7.78 (dt, 0.35H, *J* 7.7 1.3 Hz), 7.93 (dd, 0.65H, *J* 8.2 1.4 Hz), 8.00 (dd, 0.35H, *J* 7.8 1.3 Hz), 8.85 (d, 1H, *J* 1.5 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 22.1, 25.0, 31.3, 31.9, 35.1, 35.6, 36.9, 39.1, 41.6, 51.2, 55.8/56.2, 58.9, 118.0 (TFA), 120.0 (TFA), 120.7, 123.9, 124.5, 127.7, 128.4, 128.8, 129.4, 129.7, 130.3, 131.0, 131.4, 131.8, 132.1, 132.6, 132.8, 133.2/133.8, 134.3, 135.4, 135.8, 136.2, 136.3, 136.6, 137.9, 141.9, 143.6, 162.9 (TFA), 163.1 (TFA), 163.4 (TFA), 163.6 (TFA), 165.8/166.3, 169.4/169.7, 175.7. MS (ESI, MeOH) *m*/*z* (%) 572 (7) $[M+H]^+$, 286.5 (100) $[M+2H]^{2+}$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{32}H_{42}N_7O_3]^+$ 572.3349, found: 572.3338, *m*/*z* calcd for $[C_2F_3O_2]^-$ 112.9850, found 112.9868. $C_{32}H_{41}N_7O_3 \cdot 3 \times C_2HF_3O_2$ (571.71 + 3 × 114.025).

4.1.45. 5-((4-(4-(4-(((*N*-(2-Propionamidoethyl))-3-amino-3-oxo)propyl)1*H*-imidazol-1-yl)butyl)piperidin-1-yl)acetyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one (62)

Amine **61** (0.31 g, 0.34 mmol, included a minor amount of the respective 1,5-disubstitued imidazole derivative (ratio 7:1), cf. prep. of **61**) and diisopropylethylamine (0.17 g, 1.29 mmol) were dissolved in DMF (2 mL). *N*-Succinimidyl propionate (51 mg, 0.30 mmol) dissolved in DMF (0.3 mL) was added and the mixture was stirred at rt for 2.5 h. The solvent was removed under reduced pressure at 60 °C, the residue was dried in vacuo, then taken up in CH_2Cl_2 (35 mL) and washed with 5% aq NaOH (10 mL). As the product was evident in the aq phase by TLC analysis, it was extracted from the aq phase with CH_2Cl_2 (20 and 15 mL). The organic layers were combined, washed with brine/water (2:1 v/v, 8 mL) and dried over Na_2SO_4. The volatiles ware evaporated and the residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 40:1 to 7.5:1). Pure product **62** was obtained from the first fraction of the eluate. The residual eluate afforded **62** as major component and the respective 1,5-disubstitued imidazole derivative as minor component (ca 30%). The solvent was removed from the eluates under reduced pressure, the residues were dried in vacuo and redissolved in CH_2Cl_2 (4 and 5 mL, respectively). The solutions were filtered through a cotton wool packed Pasteur pipette, the solvent was removed in vacuo and the residue taken up in CH_2Cl_2/*n*-pentane (1:1 v/v, 2 mL) and CH_2Cl_2 (5 mL), respectively. Removal of the solvent in vacuo afforded pure **62** (103 mg, 55%) mp >80 °C (turned to a resin) as well as a mixture of **62** and the above mentioned by-product (ratio 2.5:1) (68 mg, 36%), each as a white glass. R_f = 0.5 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{35}H_{45}N_7O_4 \cdot 0.2H_2O$: C, 66.58; H, 7.18; N, 15.53; found: C, 66.34; H, 7.67; N, 15.08. IR (Nujol) 3295 br, 1655, 1600, 1545, 1500 cm^{-1}. Ratio of isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (600 MHz, CD_3OD) δ (ppm) 0.96–1.12 (m, 2H), 1.14 (t, 3H, *J* 7.6 Hz), 1.24 (br s, 5H), 1.45 (br d, 0.5H, *J* ca 11 Hz), 1.53 (br d, 1H, *J* ca 12 Hz), 1.60 (br d, 0.5H, *J* ca 12 Hz), 1.69–1.76 (m, 2H), 1.87–2.05 (m, 2H), 2.21 (q, 2H, *J* 7.6 Hz), 2.46–2.53 (m, 2.45H), 2.66 (br d, 0.55H, *J* ca 10 Hz), 2.79–2.89 (m, 3H), 3.07 (br d, 0.55H, *J* ca 15 Hz), 3.17 (br d, 0.45H, *J* ca 15 Hz), 3.21–3.31 (m, 5H), 3.94 (t, 2H, *J* 6.9 Hz), 6.87 (s, 1H), 7.23–7.32 (m, 2H), 7.35 (t, 0.45H, *J* 7.1 Hz), 7.42 (t, 0.55H, *J* 7.2 Hz), 7.45–7.56 (m, 3H), 7.56–7.61 (m, 1H), 7.64–7.70 (m, 1H), 7.89 (d, 0.55H, *J* 7.4 Hz), 7.92 (d, 0.45H, *J* 7.3 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm) 11.6, 25.4/25.5, 26.0, 31.0, 33.0, 33.4/33.65/33.74, 37.1, 37.7, 37.8, 40.8, 40.9, 48.7, 55.5/55.8, 61.8/62.2, 117.8, 123.8/123.9, 127.4, 127.8, 128.6, 129.77, 129.82, 130.3, 130.7, 131.4, 132.0, 132.8/133.0, 135.1/135.5, 136.76, 136.83, 137.8, 138.7, 142.6, 144.6/144.7, 170.0/170.2, 172.0/172.2, 176.4, 178.1. MS (ESI, MeOH) *m*/*z* (%) 1277 (22) $[2M+Na]^+$, 650 (100) $[M+Na]^+$, 628 (44) $[M+H]^+$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{35}H_{46}N_7O_4]^+$ 628.3611, found: 628.3602. $C_{35}H_{45}N_7O_4$ (627.78).

4.1.46. *N*,*N*′-Bis(4-((1-(2-(5*H*-dibenzo[*b*,*e*][1,4]diazepin-11(10*H*)-one-5-yl)-2-oxo)ethyl)piperidin-4-yl)butyl)piperazine·0.5H_2O (63)

Compound **63** was prepared from amine **39** (186 mg, 0.51 mmol) and chloride **43** (307 mg, 1.07 mmol) using the procedure for the preparation of **29**. Potassium carbonate: 0.56 g, 4.08 mmol. Eluent for column chromatography: CH_2Cl_2/MeOH 100:1 to 10:1. Removal of the solvent from the eluate under reduced pressure, drying in vacuo, re-uptake in CH_2Cl_2 (5 mL), filtration of the solution with a cotton wool packed Pasteur pipette, evaporation, re-uptake in CH_2Cl_2 (1 mL) and *n*-pentane (2 mL) followed by removal of the solvent in vacuo afforded product **63** as a pale tan colored glass (165 mg, 37%) mp >170 °C (turned to a resin). R_f = 0.5 (CH_2Cl_2/MeOH 5:1). Anal. calcd for $C_{52}H_{64}N_8O_4 \cdot 0.5H_2O$: C, 71.45; H, 7.38; N, 12.82; found: C, 71.40; H, 7.49; N, 12.78. IR (Nujol) 1660, 1600 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.2:1. ^1H NMR (400 MHz, CD_3OD) δ (ppm)

0.97–1.20 (m, 4H), 1.20–1.36 (m, 10H), 1.44–1.53 (m, 5H), 1.56 (d, 2H, *J* 11.8 Hz), 1.63 (d, 1H, *J* 11.5 Hz), 1.86–2.04 (m, 4H), 2.20–2.95 (br m, 16H), 3.02–3.36 (m, 4H), 7.22–7.31 (m, 4H), 7.32–7.44 (m, 2H), 7.46–7.56 (m, 4H), 7.56–7.61 (m, 2H), 7.64–7.70 (m, 2H), 7.89 (d, 1.1H, *J* 7.3 Hz), 7.92 (d, 0.9H, *J* 7.3 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 26.6, 28.5, 33.6/33.8/33.9, 37.2, 38.2, 54.6, 55.6/55.8, 60.5, 61.9/62.2, 123.9, 127.4, 127.8, 128.6, 129.77, 129.83, 130.3, 130.7, 131.4, 132.0, 132.9/133.0, 135.1/135.5, 136.8, 137.8, 144.6/144.7, 170.0/170.2, 172.1/172.4. MS (ESI, MeOH) *m*/*z* (%) 865 (100) $[M+H]^+$, 444 (76) $[M+H+Na]^{2+}$, 433 (29) $[M+2H]^{2+}$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{52}H_{65}N_8O_4]^+$ 865.5129, found: 865.5119. $C_{52}H_{64}N_8O_4$ (865.12).

4.1.47. *N*,*N*′-Bis(*N*-((1-(4-((1-(2-(5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-(10*H*)-one-5-yl)-2-oxo)ethyl)piperidin-4-yl)butyl))1*H*-imidazol-4-yl)propanoyl-2-aminoethyl)terephthalic acid diamide·2H$_2$O (64)

Amine **61** (1.05 g, 1.15 mmol, included a minor amount of the respective 1,5-disubstitued imidazole derivative (ratio ca 7:1), cf. prep. of **61**) and diisopropylethylamine (0.57 g, 4.38 mmol) were dissolved in anhydrous DMF (1.5 mL) and the mixture was cooled at 0 °C. Terephthaloyl choride (0.11 g, 0.55 mmol) dissolved in anhydrous CH_2Cl_2 (3 mL) was added dropwise over a period of 10 min, the ice bath was removed and the mixture was stirred at rt for 1 h. The solvent was removed under reduced pressure at 50 °C, the residue was dried in vacuo, and taken up in $CHCl_3$/MeOH (50:1 v/v, 50 mL) and 5% aq K_2CO_3 (30 mL). The mixture was vigorously shaken, the phases were separated, and the aqueous phase treated with $CHCl_3$/MeOH (20:1 v/v, 4 × 40 mL). The extracts were combined, washed with water (30 mL) and dried over Na_2SO_4. The volatiles ware evaporated and the residue was subjected to column chromatography (eluent: CH_2Cl_2/MeOH 30:1 to CH_2Cl_2/MeOH/28% aq NH_3 200:30:1). The first major fraction of the eluate was re-chromatographed (eluent: Et_2O/MeOH 3:1 to Et_2O/MeOH/28% aq NH_3 100:100:1) to give pure product **64**. The solvent was removed from the eluate under reduced pressure, the residue was dried in vacuo and then redissolved in CH_2Cl_2 (5 mL). The solution was filtered through a cotton wool packed Pasteur pipette and removal of the volatiles in vacuo afforded product **64** as a white glass (183 mg, 27%) mp >145 °C (turned to a resin). R_f = 0.25 (CH_2Cl_2/MeOH/28% aq NH_3 200:20:1). Anal. calcd for $C_{72}H_{84}N_{14}O_8{\cdot}2H_2O$: C, 66.03; H, 6.47; N, 14.97; found: C, 65.91; H, 6.83; N, 14.79. IR (Nujol) 1655, 1600, 1540 cm^{-1}. Ratio of configurational isomers evident in the NMR spectra: ca 1.1:1. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 0.64–0.79 (m, 2H), 0.95 (br s, 2H), 1.00–1.18 (m, 10H), 1.26 (br d, 1H, *J* ca 11 Hz), 1.30–1.38 (m, 2H), 1.45 (br d, 1H, *J* ca 11 Hz), 1.57 (br s, 4H), 1.73–1.85 (m, 4H), 2.12 (br d, 0.95H, *J* ca 9 Hz), 2.27 (br d, 1.05H, *J* ca 9 Hz), 2.34 (t, 4H, *J* 7.8 Hz), 2.58 (br t, 2H, *J* ca 12 Hz), 2.66 (t, 4H, *J* 7.7 Hz), 2.85 (d, 0.95H, *J* 14.2 Hz), 2.92 (d, 1.05H, *J* 14.4 Hz), 3.12 (d, 0.95H, *J* 14.3 Hz), 3.20–3.26 (m, 4H), 3.27–3.34 (m, 5.05H), 3.80 (t, 4H, *J* 6.6 Hz), 6.81 (s, 2H), 7.13–7.24 (m, 4H), 7.25–7.33 (m, 2H), 7.37–7.47 (m, 6H), 7.55–7.64 (m, 4H), 7.74–7.80 (m, 2H), 7.90 (s, 4H), 8.01 (t, 2H, *J* 5.7 Hz), 8.65 (t, 2H, *J* 5.5 Hz), 10.54 (s, 0.95H), 10.70 (s, 1.05H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 23.1, 24.2, 30.7, 31.2/31.5/31.7, 34.46/34.52, 35.3, 35.5, 38.2, 39.4, 45.8, 52.5/52.8/53.2, 60.2/60.6, 115.0, 121.4/121.5, 124.4/124.7, 126.1/127.2, 127.1, 127.8, 127.9, 128.1, 128.5, 128.9, 130.0, 130.5, 130.6, 132.5/132.8, 134.3, 134.7, 135.0, 135.7, 136.3, 136.7, 140.7, 142.3/142.8, 165.7, 166.4, 168.6/169.0, 172.1. MS (ESI, MeOH/0.1% aq TFA 8:1 v/v) *m*/*z* (%) 1273 (4) $[M+H]^+$, 637 (58) $[M+2H]^{2+}$, 425 (100) $[M+3H]^{3+}$, 319 (29) $[M+4H]^{4+}$, 260 (32) $[M+4H+Na]^{5+}$. HRMS (ESI, MeOH) *m*/*z* calcd for $[C_{72}H_{85}N_{14}O_8]^+$ 1273.6675, found: 1273.6701. $C_{72}H_{84}N_{14}O_8$ (1273.53).

There followed a mixture of product **64** and the hetero bivalent compound with the respective 1,5-disubstitued imidazole moiety at one side of the molecule (ratio ca 2.5:1) as a white glass (261 mg, 37%).

4.2. Radioligand binding studies at muscarinic receptors M$_1$–M$_5$

CHO-K9 cells, stably transfected with the human muscarinic receptors M$_1$–M$_5$, were purchased from Missouri S&T cDNA Resource Centre and were cultured in HAM's F12 medium supplemented with fetal calf serum (Biochrom, Berlin, Germany) (10%) and geneticin (750 μg/mL). The M receptor antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) (specific activity = 80 Ci/mmol), purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO) via Hartman Analytics GmbH (Braunschweig, Germany)), was used as radioligand.

Cells were seeded in tissue culture treated white 96-well plates with clear bottom (Corning Incorporated Life Sciences, Tewksbury, MA; Corning cat. no. 3610) one or two days prior to the experiment. Depending on the level of receptor expression (receptor expression by the cells decreased with passaging) the confluency of the cells was 10–20% (high receptor density, approx. 500,000 sites per cell), 40–60% (medium receptor density, 100,000–300,000 sites per cell) or 100% (low receptor level, <100,000 sites per cell) on the day of the experiment. All experiments were performed at 23 ± 1 °C. The culture medium was removed by suction, the cells were washed with phosphate buffered saline (PBS) (200 μL) and covered with 150 μL of Leibovitz's L15 culture medium (Gibco, Life Technologies GmbH, Darmstadt, Germany) supplemented with 1% bovine serum albumin (Serva, Heidelberg, Germany), in the following referred to as L15 medium. For total binding L15 medium (18.8 μL) and L15 medium (18.8 μL) containing the radioligand (10-fold concentrated) were added. For non-specific binding and displacement of [^{3}H]NMS, L15 medium (18.8 μL) containing the competitor (10-fold concentrated) and L15 medium (18.8 μL) containing the radioligand (10-fold concentrated) were added. During incubation (3 h in case of saturation and equilibrium competition binding studies) the plates were gently shaken. After incubation the liquid was removed by suction, the cells were washed twice with ice-cold PBS (200 μL; washing period ⩽2 min) and lysis solution (urea (8 M), acetic acid (3 M) and Triton-X-100 (1%) in water) (25 μL) was added. The plates were shaken for 20 min, liquid scintillator (Optiphase Supermix, PerkinElmer, Überlingen, Germany) (200 μL) was added, and the plates were sealed with a transparent sealing tape (permanent seal for microplates, PerkinElmer, prod. no. 1450–461). The plates were turned up-side down several times in order to achieve complete mixing of scintillator and lysis solution. The samples were kept in the dark for at least 1 h prior to the measurement of radioactivity (dpm) with a MicroBeta2 plate counter (PerkinElmer, Rodgau, Germany). The radioligand concentration was 0.2 nM for equilibrium binding studies on CHO-M$_1$/M$_2$/M$_3$ cells, 0.1 nM for experiments on CHO-M$_4$ cells and 0.3 nM in case of CHO-M$_5$ cells. Nonspecific binding was determined in the presence of atropine (1000-fold excess) and amounted to <10% of total binding.

To investigate the effect of compounds **1**, **58**, **62**–**64** on the dissociation of [^{3}H]NMS from the M$_2$ receptor, CHO-M$_2$ cells were incubated with 1 nM [^{3}H]NMS for 60 min before removal of the liquid by suction and addition of L-15 medium (200 μL) containing 500 μM atropine alone or in combination with the compound of interest. Radioligand dissociation was monitored for a time period of 120 min (0, 0.5, 2, 5, 10, 20, 35, 60, 90, 120 min). In the absence of test compound, the half-life of [^{3}H]NMS dissociation amounted to $t_{1/2}$ = 7.5 ± 0.4 min (mean ± SEM, *n* = 14).

4.3. Data analysis

Specific binding data (dpm) from saturation binding experiments were analyzed by an equation describing hyperbolic radioligand binding (one site saturation) using SigmaPlot Software version 11.0 (Systat Software Inc., Chicago, IL) to obtain K_d and B_{max}

values. Data of total radioligand binding (%) from equilibrium binding studies were plotted over log concentration of test compound and analyzed by a four-parameter logistic equation, followed by normalization of the data (100% Y defined as upper plateau of the initial fit, 0% Y defined as 0) to yield % specific radioligand binding. (GraphPad Prism Software version 5.0, GraphPad Software, San Diego, CA).

For the determination of dissociation rate constants (k_{-1} values), data from radioligand dissociation experiments were plotted as $\ln B$ (B = specifically bound radioligand) over time and analyzed by linear regression. To obtain concentration effect curves for the deceleration of radioligand dissociation, the ratios $k_{-1}/k_{-1(0)}$ ($k_{-1(0)}$ = rate constant in the absence of test compound) were plotted in % over log concentration of test compound and analyzed by a four parameter logistic function. Statistical significance was assessed by a one-sample t-test (deviation of the curve slope and bottom level of concentration–effect curves from −1 and 0, respectively) or a Welch two-sample t-test for unpaired samples (comparison of two given pIC_{50} or $pEC_{50,diss}$ values).

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Supplementary data

Supplementary data (characterization of [^{3}H]NMS in saturation binding experiments at muscarinic receptors M_1–M_5; M receptor binding data of atropine, pirenzepine, 4-DAMP and propantheline; ^{1}H NMR and ^{13}C NMR spectra of compounds **46**, **47**, **49**, **50**, **52**–**59**, **61**–**64**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.015. These data include MOL files and InChiKeys of the most important compounds described in this article.

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