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Original article

New polyamine-sensitive inhibitors of the NMDA receptor: Syntheses and pharmacological evaluation

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Abstract

Derivatives of 5-(4-aminobutyl)-2-thiophene-octylamine, a potent polyamine-sensitive inhibitor of the NMDA receptor, were synthesized and evaluated as inhibitors of [^{3}H]MK-801 binding to rat brain membranes. Alkylations of the terminal amino groups reduced inhibitory potency; only incorporation of the amino group of the short 4-aminobutyl arm into a piperidine ring was tolerated. Substitution of the thiophene nucleus with methyl or ethyl, and its replacement by a benzene nucleus, was of minor influence. The corresponding diguanidines exhibited high potency independent of chain length, whereas their sensitivity to spermine was sharply dependent on chain length. Insertion of an amide bond into the long octylamine arm increased sensitivity to spermine and to Tris buffer. Our results indicate that spermine sensitivity of [^{3}H]MK-801 binding inhibition is responsive to subtle changes in inhibitor structure and represents a promising target for pharmaceutical research.

Keywords: Polyamine inverse agonist; NMDA receptor; 1,12-Diaminododecane; Spermine; Thiophene

1. Introduction

The glutamate receptor of the NMDA type is an ion channel with high Ca^{2+} permeability, its conductance critically depending on partial depolarization [1]. By virtue of these properties, NMDA receptors not only play a crucial role in synaptic plasticity, but also convey a dangerous threat to neurons, since prolonged NMDA receptor stimulation, as it may occur in stroke, epilepsy, or neurological conditions as Chorea Huntington, can result in Ca^{2+} overload. The endogenous polyamines spermidine and spermine increase frequency and burst length of NMDA-induced currents in rat hippocampal neurons, due to relief from a partial block by protons, even at physiological pH [2]. Compounds like 1,12-diaminododecane (**1**, Table 4) and arcaine (a diguanidine) appear to counteract this effect (inverse polyamine agonists) [3–5]; thus, they seem to block the NMDA receptor complex via the same mechanism as protons do. At higher concentrations, polyamines and inverse polyamine agonists additionally act as direct NMDA channel blockers [6–9]. Allosteric inhibitors, e.g. Ifenprodil, increase the sensitivity of the NMDA receptor complex to inhibition by protons [10]; they achieve this effect by interaction with a site which is not a direct target of polyamines. Our search for polyamine inverse agonists with increased potency started with a study of long chain diamines with various chain lengths [5]. To introduce an additional site for target interaction, we interrupted the carbon chain with a thiophene nucleus. By placing the nucleus specifically between the fourth and the fifth carbon atom of **1**, we arrived at 5-(4-aminobutyl)-2-thiophene-octylamine (**2**, Table 1) [11], one of the most potent compounds of this class ($IC_{50} = 0.3$ μM). Here we document in detail the syntheses

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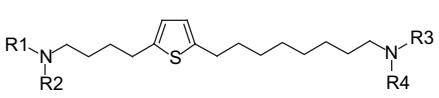
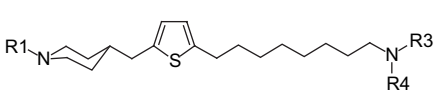
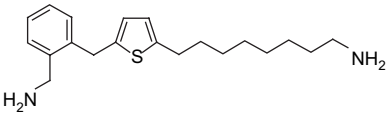
Table 1
Influence of *N*-alkylation on the potency of **2** as inhibitor of [^{3}H]MK-801 binding (50 mM Tris buffer)



2,5d,5e,5f,5g,5h



12,11a,11b



16

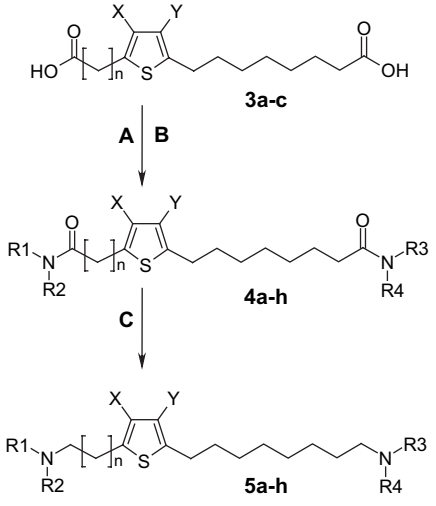
	R1	R2	R3	R4	IC_{50} (μM)	n_H[a]	100 spermine[b]
2	H	H	H	H	0.54 ± 0.18 (31)	1.15 ± 0.10 (28)	8.84 ± 3.94 (24)
5d	CH_3	H	CH_3	H	5.30 ± 0.94 (6)	1.17 ± 0.16 (5)	7.70 ± 4.96 (6)
5e	CH_3	CH_3	CH_3	CH_3	358 ± 86 (4)	1.43 ± 0.25 (3)	2.79 ± 0.67 (4)
5f	C_3H_7	H	C_3H_7	H	114, 147 (2)	0.90, 1.00 (2)	2.98, 3.81 (2)
5g	CH_2=$CHCH_2$	H	CH_2=$CHCH_2$	H	13.3 ± 1.7 (3)	1.20, 1.08 (2)	6.57, 7.75 (2)
5h	$PhCH_2$	H	$PhCH_2$	H	17.7, 8.67 (2)	2.21, 2.00 (2)	0.98, 2.12 (2)
12	H	–	H	H	0.63 ± 0.06 (3)	1.09 ± 0.09 (3)	9.07 ± 1.71 (3)
11a	H	–	CH_3	H	2.48, 2.87 (2)	1.03, 0.95 (2)	8.58, 10.9 (2)
11b	H	–	CH_3	CH_3	15.2 ± 3.7 (4)	1.19 ± 0.19 (3)	7.67 ± 5.94 (4)
16	H	H	H	H	1.13 ± 0.43 (3)	1.49 ± 0.24 (3)	2.96 ± 0.91 (3)

[a] Hill coefficient.
[b] Attenuation in the presence of 100 μM spermine.

of various structural analogues of **2** and their effect on spermine-stimulated [^{3}H]MK-801 binding. Studies on the structure/activity relationships (SARs) of this type of compounds are of high interest, since the polyamine modulatory site of the NMDA receptor may offer a means to influence this receptor (e.g. to provide neuroprotection) without the risks associated with full NMDA agonists (that may induce seizures) or direct blockers of the NMDA channel (like phencyclidine) that may induce psychosis. Our results allow delineation in more detail of the structural requirements for addressing the polyamine regulatory site of the NMDA receptor with pharmaceutical agents.

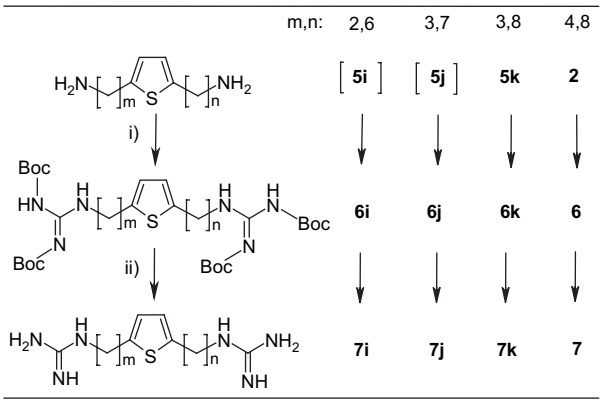
2. Chemistry

N,*N*-Substituted thiophene-dialkyldiamines **5a–h**, **5k** (Scheme 1) were synthesized from dicarboxylic acids as described [11]. To generate the corresponding diamides, gaseous amines were applied as such to the acid chlorides (method A), whereas liquid amines were coupled by method B (with the Mukaiyama reagent [12]). From the diamides, the desired diamines were obtained by $LiAlH_4$-reduction (method C) [11]. The terminal amine functions were extended to guanidine residues under mild conditions with a Boc-protected guanylating reagent [13], resulting in protected guanidines (Scheme 2). Purification of the



	n	X	Y	R1	R2	R3	R4
3a	3	Me	H	-	-	-	-
3b	3	H	Me	-	-	-	-
3c	3	H	Et	-	-	-	-
3k	2	H	H	-	-	-	-
4a	3	Me	H	H	H	H	H
4b	3	H	Me	H	H	H	H
4c	3	H	Et	H	H	H	H
4d	3	H	H	Me	H	Me	H
4e	3	H	H	Me	Me	Me	Me
4f	3	H	H	Pro	H	Pro	H
4g	3	H	H	All	H	All	H
4h	3	H	H	Bz	H	Bz	H
4k	2	H	H	H	H	H	H
5a	3	Me	H	H	H	H	H
5b	3	H	Me	H	H	H	H
5c	3	H	Et	H	H	H	H
5d	3	H	H	Me	H	Me	H
5e	3	H	H	Me	Me	Me	Me
5f	3	H	H	Pro	H	Pro	H
5g	3	H	H	All	H	All	H
5h	3	H	H	Bz	H	Bz	H
5k	2	H	H	H	H	H	H

Scheme 1. Minor modifications of 2,5-thiophene-dialkyldiamines. Reagents and conditions: method A: THF/oxalyl chloride/bubbling with gaseous amine. Method B: DCM/Mukaiyama reagent/TEA/liquid amine. Method C: THF/$LiAlH_4$ for **5a–h**, TFA/Et_3SiH for **5k**.



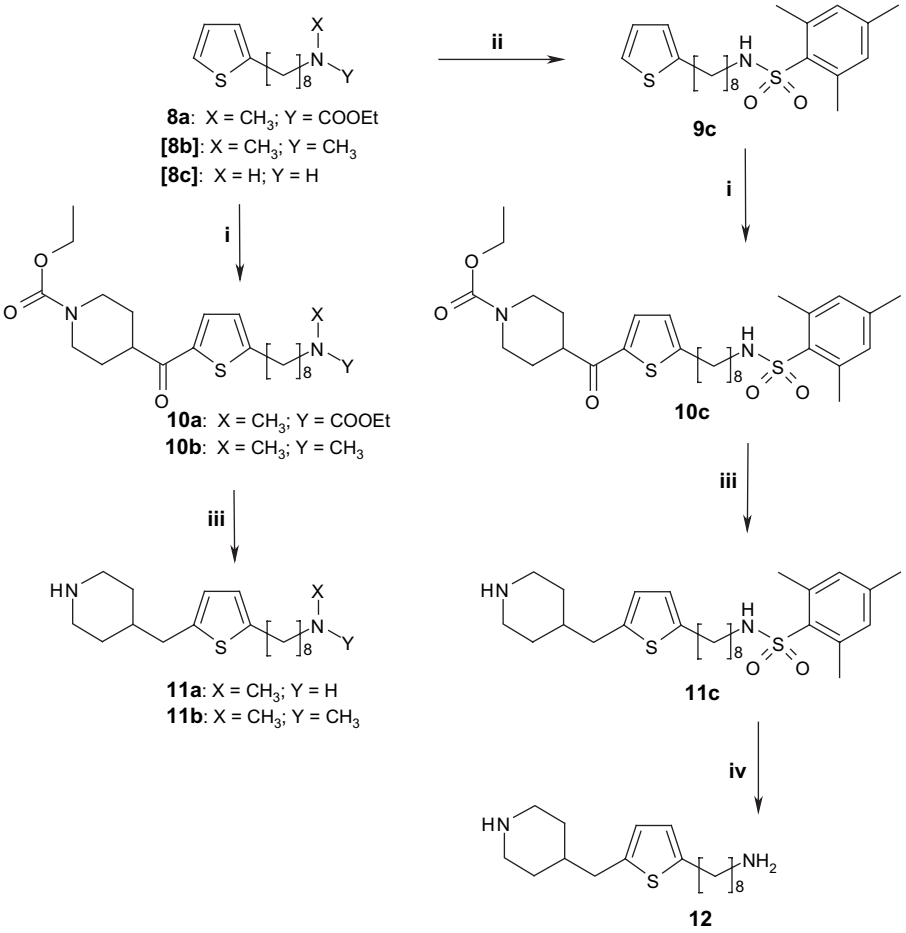
Scheme 2. 2,5-Thiophene-dialkyldiguanidines. Reagents and conditions: (i) MeCN/guanylating reagent; (ii) 3 M HCl/AcOEt. [Compound number]: intermediate of synthesis, not isolated.

protected tetra-Boc-guanidines **6** and **6i–k** by column chromatography and deprotection under acid conditions resulted in the HCl salts of the diguanidines **7** and **7i–k**.

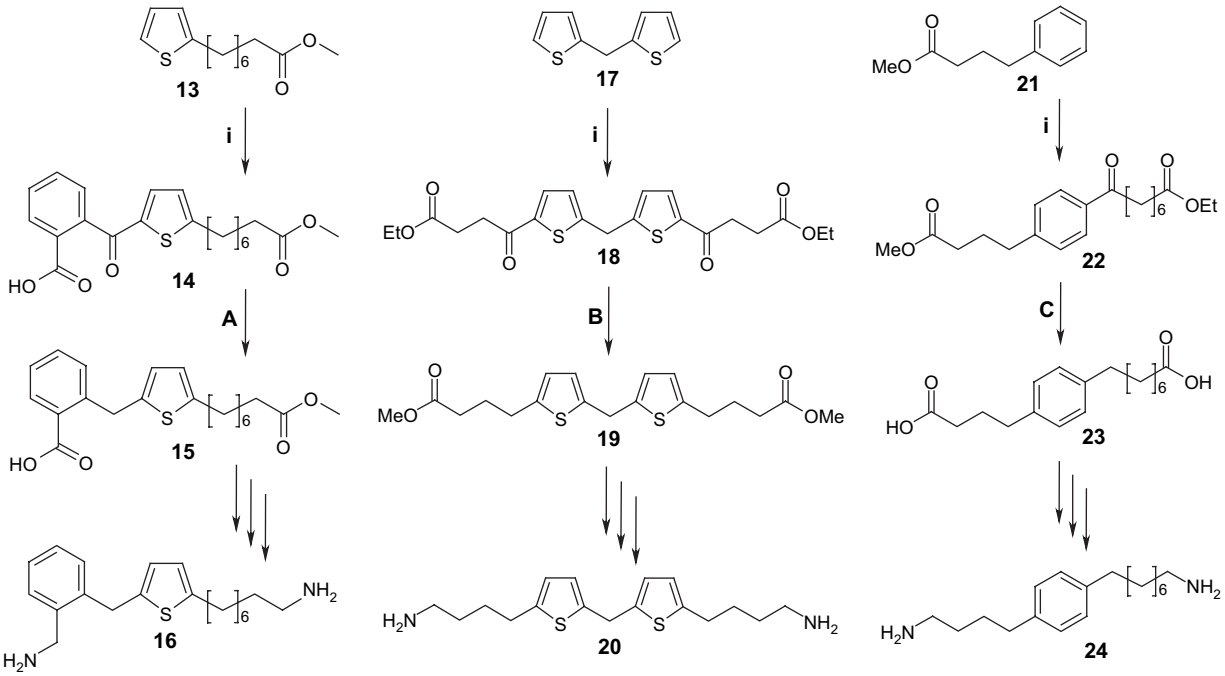
As a further side chain modification, we studied the effect of structural integration of the short arm amino group into a piperidine ring (Scheme 3). The *N*-methyl derivatives **11a** and **11b** were accessible as described for **2** [11], but the synthesis of the primary amine **12** required a specific protecting group strategy, reacting amine **8c** to sulfonamide **9c**, followed by Friedel–Crafts acylation and Wolff–Kishner reduction [11].

Scheme 4 gives an overview of the synthetic pathways leading to diamines with other aromatic rings inserted into the chain. The benzylamine derivative **16** was synthesized as described for **2** [11], but using Clemmensen instead of Wolff–Kishner reduction. The synthesis of the symmetric diamine **20** started with 2-fold Friedel–Crafts acylation of dithienylmethane **17** [14] with succinyl chloride monoethylester, and continued as described [11]. The synthesis of the phenyl analogue **24** started with the ester **21**. Here, the reduction (from **22** to **23**, see Scheme 4) was achieved by ionic deoxygenation in TFA/Et_3SiH [15,16].

The increased potency of **2** in comparison to aliphatic homologues could be due to the π-donor functionality in the chain. With **28** we synthesized a structurally related compound providing such a functionality without an aromatic ring. The synthetic route (Scheme 5) started with the alkyne **25** [17], accessible from 1,4-butandiol. Linkage with THP-protected 8-bromo-octanol by nucleophilic displacement under basic conditions in THF/hexamethyl phosphoric acid triamide resulted in **26**. THP-deprotection and mesylation were followed



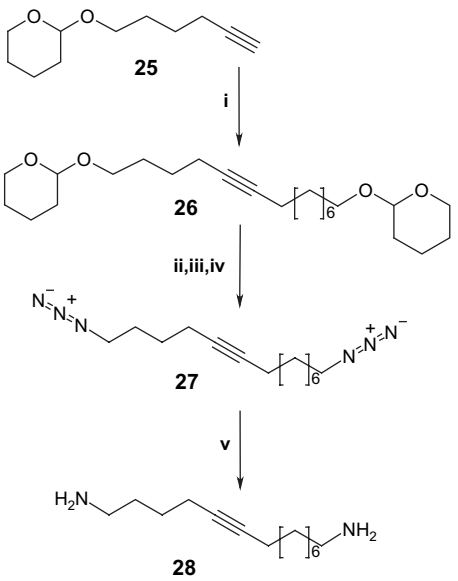
Scheme 3. Piperidine containing analogues **11a**, **11b** and **12**. Reagents and conditions: (i) 4-chlorocarbonyl-piperidine carboxylic acid ethyl ester/$AlCl_3$/DCE; (ii) 2,4,6-Me_3-benzene-SO_2Cl/NEt_3/DCM; (iii) KOH/$N_2H_4 \cdot H_2O$/ethylene glycol; (iv) Na/naphthaline/DME. [Compound number]: intermediate of synthesis, not isolated.



Scheme 4. Aromatic diamines **16**, **20** and **24**. Reagents and conditions: (i) acid chloride/anhydride/$AlCl_3$/DCE; (A) Zn/HCl_{conc}/toluene/H_2O; (B) $N_2H_4 \cdot H_2O$/KOH/ethylene glycol; (C) TFA/Et_3SiH. Arrow horde: steps have been described earlier [11].

by nucleophilic attack with sodium azide in MeOH. The resulting diazide **27** was finally subjected to tin chloride reduction.

Finally, we studied the effect of inserting heteroatoms into the carbon backbone of the long arm of thiophene-dialkyldiamines. Introduction of two oxygens, in addition to the increase in polarity, was expected to increase rigidity of the long arm (due to steric *gauche* alignment). The first ether bond was formed with 2-(2-thienyl)ethanol **29** [18] and THP-protected bromo-ethanol in dry DMF with NaH (Scheme 6). After



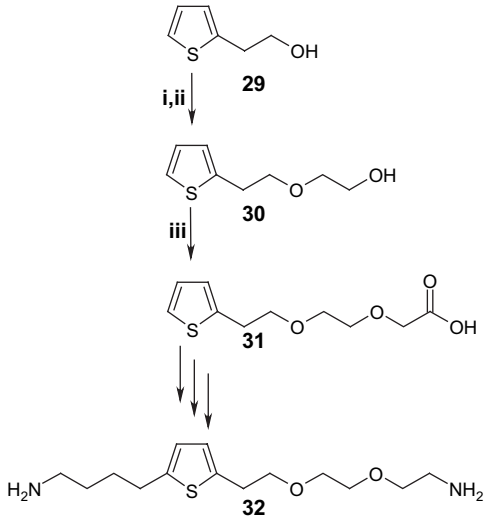
Scheme 5. The alkyne diamine **28**. Reagents and conditions: (i) 2-(8-Br-octyloxy)-THP/*n*-BuLi/THF/hexamethyl phosphoric acid triamide; (ii) toluene-4-sulfonic acid/MeOH; (iii) $MeSO_2Cl$/DCM/TEA; (iv) NaN_3/MeOH; (v) $SnCl_2$/PhSH/TEA.

THP-deprotection, the second ether oxygen was introduced with bromo-acetic acid and NaH in dry THF. After this critical step, the final diether **32** was accessible as described [11].

The two amides **35a** and **35b** made accessible the corresponding polyamines **36a** and **36b**, with nitrogen atoms inserted into the carbon backbone of the long arm of **2** (Scheme 7). The precursor mono-Boc-protected thiophene-dialkyldiamines **34a** and **34b** were coupled with the amino acid constructs *N*-α-Boc-alanine and *N*-α-Boc-glycine–glycine, respectively (active ester coupling). Boc-deprotection under acidic conditions yielded **35a** and **35b**, with one or two amide bonds in the long arm, and reduction with $LiAlH_4$ in THF yielded the corresponding polyamines **36a** and **36b**. Other compounds with amide insertion (**38a–d**, **39** and **40**) were synthesized by solid phase amino acid coupling, using nitrophenylcarbonate-activated Wang® resin [19] or trityl chloride resin. The resin-coupled amine was subjected to amino acid active ester coupling (Scheme 8), using HOBt as coupling reagent [20]. For synthesis of **42**, the amide-interrupted analogue of **1**, mono-Boc-protected 1,8-diaminooctane [21] was coupled with Boc-protected β-alanine (as described for **4f–h**), and deprotected (as described for **35a**).

3. Pharmacology

Specific binding of [^{3}H]MK-801 to rat brain membranes [22] allows the distinction from each other of positive and negative modulators of the ion channel associated with the NMDA receptor, data otherwise accessible in living cells only. Polyamines and polyamine agonists increase the opening frequency of the channel and thereby the accessibility of the binding site for [^{3}H]MK-801, located in the depth of the



Scheme 6. The diether **32**. Reagents and conditions: (i) 2-(4-Br-butoxy)-THP/NaH/DMF; (ii) toluene-4-sulfonic acid/MeOH; (iii) NaH/THF/$BrCH_2COOH$. Arrow horde: steps have been described earlier [11].

channel. If low nanomolar concentrations of the radioligand are used (we used 5 nM), specific binding of [^{3}H]MK-801 increases with the opening frequency of the channel. An inhibitor of [^{3}H]MK-801 binding is likely to act as an inverse polyamine agonist, if its potency is markedly reduced in the presence of 10–100 μM spermine, since it competes with spermine for the same site, and since 10–100 μM spermine are high concentrations relative to the EC_{50} of spermine (2–3 μM [3–5]). The attenuation factors achieved depend on buffer concentration and other factors, and meaningful information is only obtained if several compounds are compared to each other under identical conditions. Many compounds exhibit mixed inhibitory properties, acting at the polyamine site and – especially at higher concentrations – also directly at the channel [6–9]. Inhibition at the glutamate or at the glycine site would have been masked by the high excess of the co-agonists added (10 μM each; the EC_{50} for glycine is 40 nM and that for glutamate even lower [23]).

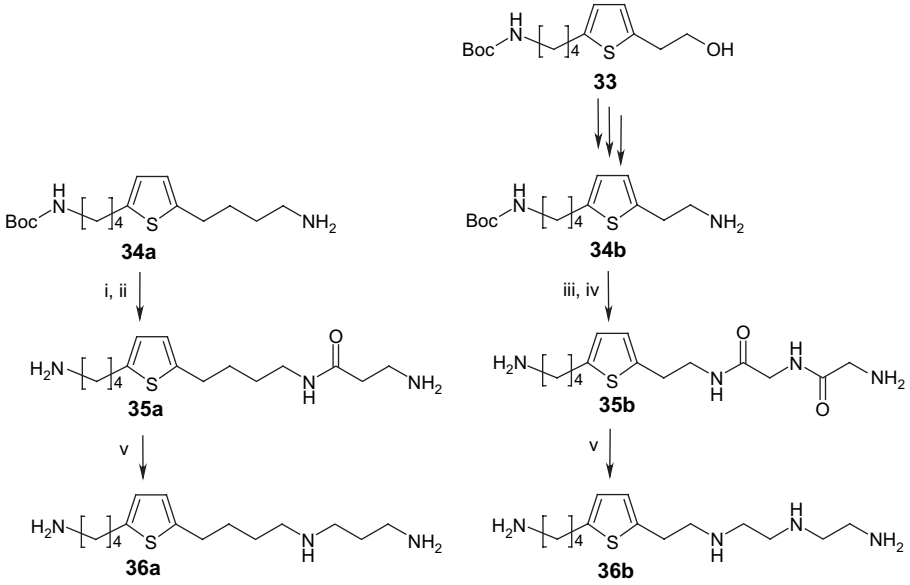
4. Results

4.1. Alkylation of the amino groups

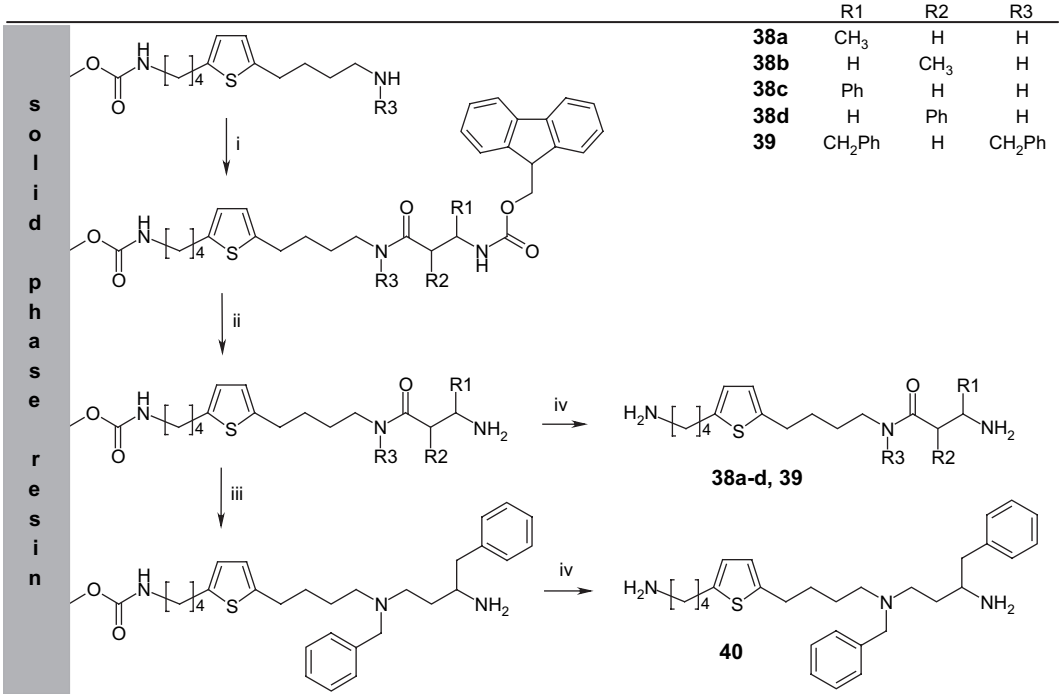
The potency of many pharmaceuticals is improved by increasing lipophilicity. Therefore, we were interested in *N*-alkylated derivatives of **2**. However, already **5d** was 10-fold weaker than the parent compound (Table 1), and **5e** was almost inactive. Also the *N*,*N*′-propyl derivative **5f** was a very weak inhibitor, whereas the analogous derivatives with allyl **5g** and with benzyl **5h** acted only slightly weaker than methylated **5d**. If the amino group of the long octylamine arm was free as in the piperidine analogue **12** and the benzylamine analogue **16**, potency was preserved. In the short aminobutyl arm, the secondary amino group resulting from formation of a piperidine ring was better tolerated than methylation (compare **11a** to **5d**). The feasibility of annulating rings to the short arm of **2** is demonstrated by the encouraging potency of **16**. However, inhibition by this benzylamine was only slightly influenced by spermine (Table 1, last column). Spermine sensitivity comparable to the parent compound was observed with the secondary diamine **5d**, with the piperidines **12**, **11a** and **11b** and, surprisingly, with the allyl derivative **5g**.

4.2. Modifications at the central nucleus

We decided for a thieno nucleus to be inserted into the carbon chain [11] due to the high expertise of one of us in thiophene chemistry [24]. There was no specific reason to prefer this



Scheme 7. Synthesis of peptide and polyamine derivatives. Reagents and conditions: (i) Mukaiyama reagent, AcOEt, TEA, Boc-β-alanine; (ii) TFA/DCM; (iii) DIC/HOBt/DMF/Boc-Gly-Gly; (iv) TFA/H_2O; (v) $LiAlH_4$/THF; Arrow horde: -OH converted to $-NH_2$ via -I and N_3 [31].



	R1	R2	R3
38a	CH_3	H	H
38b	H	CH_3	H
38c	Ph	H	H
38d	H	Ph	H
39	CH_2Ph	H	CH_2Ph

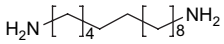
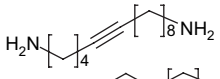
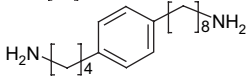
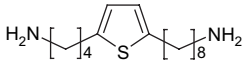
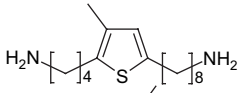
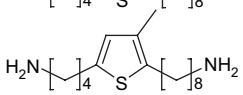
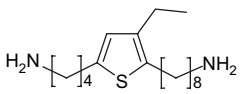
Scheme 8. Solid phase syntheses. Reagents and conditions: (i) protected amino acid in DMF/DIC/HOBt; (ii) piperidine/DMF; (iii) BH_3/THF, 1,8-diazabicyclo[5.4.0]undec-7-en in MeOH/*N*-methylpyrrolidinone; (iv) TFA/DCM.

nucleus to any other. Now we show that the benzene analogue **24** did almost as well as the parent compound **2** (Table 2). However, if the aminobutyl and the octylamine arms of **2** were joined together by two carbon atoms connected by a triple bond (also rich in π-electrons), the resulting alkyne **28** was not more potent than the corresponding saturated alkyldiamine (1,14-diaminotetradecane, **37** [5], Table 2). Substituting the parent compound **2** in position 3 or 4 of the thieno ring with methyl or ethyl resulted in the slightly less potent analogues **5a–c** (Table 2).

*4.3. Modifications in the octylamine arm of **2***

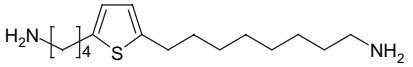
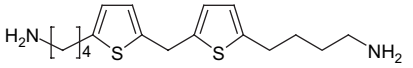
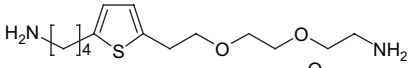
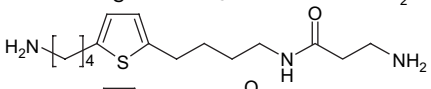
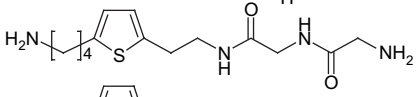
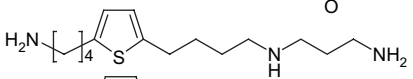
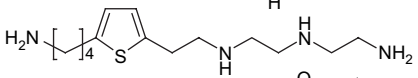
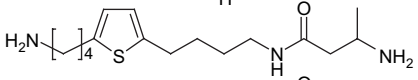
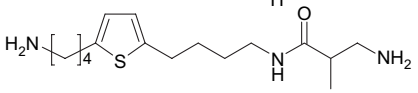
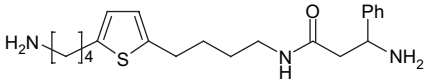
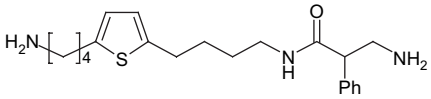
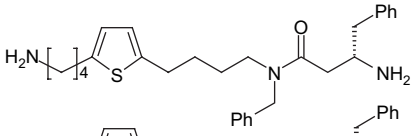
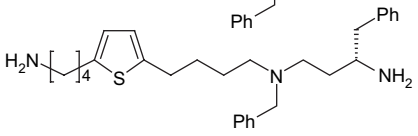
While insertion of a second thieno ring was tolerated (**20**, Table 3), all other attempts to insert heteroatoms into the

Table 2
Influence of modifications at the central nucleus on the potency of **2** as inhibitor of [^{3}H]MK-801 binding (50 mM Tris buffer)

	Structure	IC_{50} (μM)	n_H[a]	100 spermine[b]
37		5.28 ± 1.23 (6)	1.23 ± 0.05 (3)	6.94 ± 1.45 (4)
28		8.25, 6.73 (2)	1.14, 1.03 (2)	5.38, 5.90 (2)
24		0.87 ± 0.12 (3)	1.29 ± 0.11 (3)	7.95 ± 0.91 (3)
2		0.54 ± 0.18 (31)	1.15 ± 0.10 (28)	8.84 ± 3.94 (24)
5a		1.08, 1.97 (2)	1.19, 1.93 (2)	4.98, 4.07 (2)
5b		1.62, 2.66 (2)	1.25, 1.17 (2)	5.84, 3.90 (2)
5c		3.29, 3.23 (2)	1.12, 1.00 (2)	2.67, 3.11 (2)

[a] Hill coefficient.
[b] Attenuation in the presence of 100 μM spermine.

Table 3
Influence of modifications in the long arm on the potency of **2** as inhibitor of [^{3}H]MK-801 binding (10 mM Tris buffer)

	Structure	IC_{50} (μM)	n_H[a]	30 spermine[b]
2		0.52 ± 0.32 (46)	1.18 ± 0.19 (43)	15.1 ± 7.1 (24)
20		1.76 ± 0.62 (3)	1.10 ± 0.09 (3)	16.8 ± 8.2 (3)
32		4.20 ± 2.19 (4)	1.43 ± 0.36 (4)	16.8 ± 3.6 (4)
35a		1.19 ± 0.69 (19)	1.00 ± 0.12 (17)	51.4 ± 23.7 (8)
35b		111 ± 61 (6)	0.95 ± 0.09 (4)	5.48, 12.1 (2)
36a		5.2 ± 2.2 (4)[c]	n.d.	29.0 ± 13.9 (4)
36b		282 ± 66 (3)[d]	n.d.	n.d.
38a		2.21 ± 1.25 (9)	0.96 ± 0.11 (7)	65.4 ± 32.8 (5)
38b		24.7 ± 3.9 (3)	1.25 ± 0.09 (3)	10.3 ± 2.4 (3)
38c		6.81 ± 2.78 (5)	1.34 ± 0.10 (5)	15.0 ± 6.7 (5)
38d		11.7 ± 1.1 (3)	1.37 ± 0.17 (3)	10.1 ± 1.7 (3)
39		16.2, 13.4 (2)	1.56, 2.24 (2)	1.71, 2.44 (2)
40		9.64, 9.62 (2)	1.30, 2.09 (2)	1.66, 2.57 (2)

[a] Hill coefficient.
[b] Attenuation in the presence of 30 μM spermine.
[c] Extracted by computer analysis from biphasic data containing a stimulatory component (EC_{50} 35 μM).
[d] At lower concentrations, stimulation up to 125% was observed (EC_{50} 1.9 μM).

eight-carbon chain resulted in inhibitors at least one order of magnitude weaker than **2**, under our usual assay conditions (50 mM Tris buffer). Reducing the buffer concentration to 10 mM, however, revealed the considerable potency of **35a**, synthesized originally only as precursor for the triamine **36a**. The amide **35a** was almost as potent as the parent compound (in 10 mM Tris; in 50 mM Tris it was 10 times weaker than **2**, see Fig. 1), and inhibition of [^{3}H]MK-801 binding by **35a** was 3–4 times more sensitive to spermine than inhibition by **2** itself (Fig. 1). Introduction of a second amide bond resulted in the diglycyl derivative **35b**, lower in potency than **35a** by a factor of 100 and without impressive sensitivity to spermine (Table 3); **35b** served as precursor for the tetraamine **36b**. The polyamines **36a** and **36b** exhibited mixed properties (see the footnotes in Table 3), with agonistic stimulation hiding behind inhibition. If the two secondary amino groups in **36b** were replaced by oxygens, the resulting diether **32** was more potent than **36b**, but still considerably less potent than **2** (and also less potent than **35a**).

The peptidic nature of **35a** and **35b** opened avenues for more powerful parallel synthesis procedures for compounds **38a–d**, **39** and **40**. A methyl substituent at position 3 (“α-position”) of the propionamide part of **35a** was well tolerated, with excellent spermine sensitivity (**38a**), but not in position 2 (**38b**). Many amino groups are protected against oxidation by MAO by methyl substitution in α-position [25]. Phenyl substitution of **35a** was neither tolerated in position 3 (**38c**) nor in position 2 (**38d**), however, some

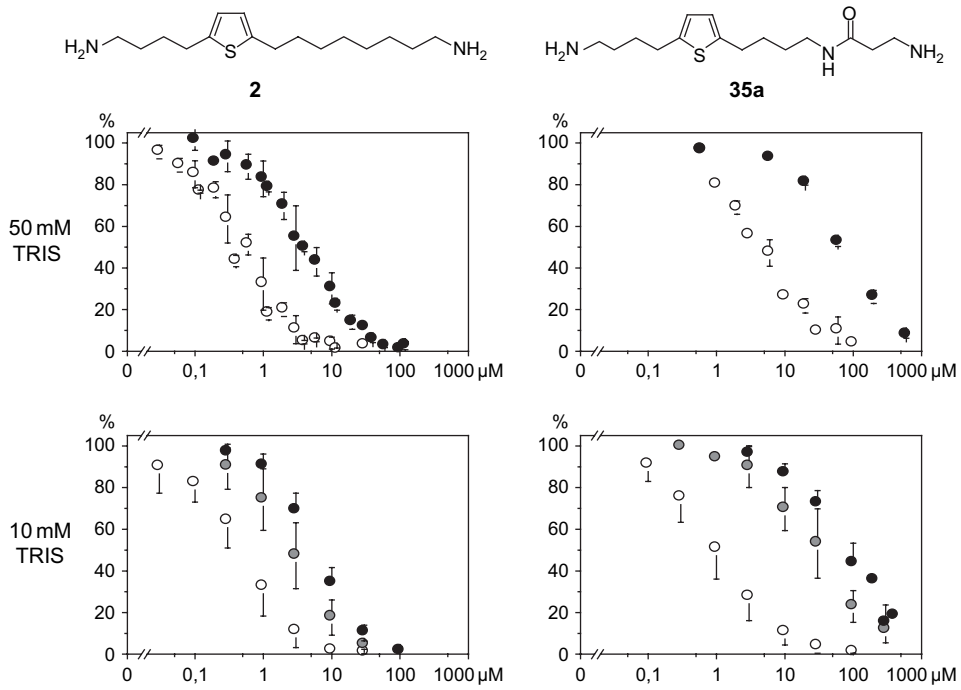


Fig. 1. Inhibition of [^{3}H]MK-801 binding by **2** (left) and by its amide analogue **35a** (right), in 50 mM (upper row, filled circles: +100 µM spermine) and in 10 mM Tris buffer (lower row, grey circles: +10 µM spermine, filled circles: +30 µM spermine). Note that spermine and Tris have a stronger influence on inhibition by **35a** than on inhibition by **2**. Data pooled from at least four experiments.

spermine sensitivity was maintained (factor 10–15) also in these latter compounds. Exploratory compounds with more substituents (**39** and **40**) exhibited moderate potencies with practically no influence of spermine.

Introduction of an amide bond into the standard polyamine inverse agonist **1** resulted in **42** (Table 4); spermine sensitivity of inhibition by **42** was higher than that of inhibition by **1**. Thus, also here a highly spermine-sensitive inhibitor emerged, however, with relatively poor potency.

4.4. Diguanidine analogues with various chain lengths

As the diguanidine arcaine is mentioned in the polyamine literature at least as often as the diaminoalkanes, we synthesized and evaluated several diguanidine analogues of **2**, and for comparison also **5k**, the shorter chain homologue of **2**. The potency of **5k** was 10 times lower than that of the parent compound **2** (Table 5); this critical dependence of diamine potency on chain length had already been demonstrated in our earlier publication [11]. The diguanidines **7** and **7i–k**, however, were all as potent as **2** (Table 5), although their chain length varied considerably. Variable was only their sensitivity to spermine: only **7i**, the shortest analogue, with the terminal nitrogens at the same distances from the thieno ring as in **2**, exhibited highly spermine-sensitive inhibition of [^{3}H]MK-801 binding, comparable to **2**.

5. Discussion

5.1. The terminal amino groups

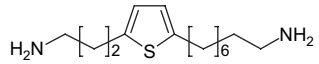
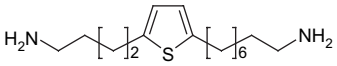
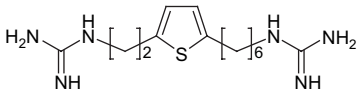
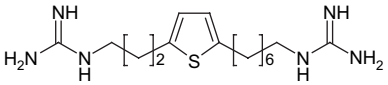
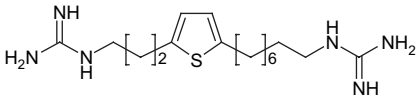
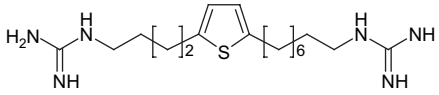
In this study, we explored the influence of several structural modifications on the potency of our parent compound **2** as polyamine-sensitive NMDA receptor blocker. Neither the modifications at the amino groups, nor the manoeuvres at the centre of the molecule resulted in any relevant improvement of potency. The relative impact of the various

Table 4
Inhibition of [^{3}H]MK-801 binding (10 mM Tris buffer) by **42**, corresponding structurally to **1** with an amide bond interrupting the carbon chain

	Structure	IC_{50} (µM)	n_H[a]	30 spermine[b]
1	H_2N–[8]–NH_2	7.05 ± 2.38 (11)	1.12 ± 0.21 (11)	34.6 ± 5.8 (4)
42	H_2N–[8]–N(H)–C(=O)– NH_2	53 ± 33 (4)	0.88 ± 0.46 (3)	75 ± 64 (4)

[a] Hill coefficient.
[b] Attenuation in the presence of 30 µM spermine.

Table 5
Diguanidine analogues of **2** as inhibitor of [^{3}H]MK-801 binding (10 mM Tris buffer)

	Structure	IC_{50} (μM)	n_H[a]	30 spermine[b]
5k		5.47 ± 1.62 (3)	1.27 ± 0.27 (3)	9.57 ± 2.24 (3)
2		0.54 ± 0.33 (42)	1.19 ± 0.20 (39)	15.1 ± 7.1 (24)
7i		1.04 ± 0.09 (3)	1.20 ± 0.11 (3)	23.4 ± 7.3 (3)
7j		0.74 ± 0.31 (3)	1.65 ± 0.53 (3)	7.47 ± 3.35 (3)
7k		0.31 ± 0.22 (3)	1.22 ± 0.54 (3)	6.29 ± 2.73 (3)
7		0.77 ± 0.19 (3)	1.76, 1.26 (2)	3.95 ± 1.66 (3)

[a] Hill coefficient.
[b] Attenuation in the presence of 30 μM spermine.

modifications, however, may shed some light on the mechanisms of interaction involved. It appears that the primary amino group at the end of the long octylamine arm was essential for high potency. For the short aminobutyl arm, a secondary amino group was tolerated, but only if integrated into the carbon chain. Since propyl residues were much less tolerated than methyl groups, a picture of size-limited targets for the positively charged amino groups emerges; that allyl and even benzyl residues were less disturbing than propyl ones was not necessarily in contradiction to that idea. The π-electrons in these latter substituents might have provided interaction sites for cationic targets [26] at some distance from the primary target sites, thereby attenuating the negative influence of size exclusion. The rather limited collection of compounds of our earlier paper [11] suggested that high potency was correlated with high spermine sensitivity. Our new, structurally more diverse collection does not support such a correlation (Fig. 2). For example compound **5d**, the analogue of **2** with secondary amino groups, had a 10 times weaker potency than **2**, but similar sensitivity to spermine (Table 1).

To our surprise, the sharp dependence on chain length demonstrated for the potencies of diamine homologues of **2**, an observation that had prompted us to postulate the interaction with a specific target [11], was not reproduced for the diguanidino homologues. They were all practically equipotent to the diamine **2**, but only **7i**, corresponding in chain length exactly to **2**, exhibited equivalent spermine sensitivity. Thus, also for the diguanidines absolute potency (IC_{50}) and sensitivity to spermine (attenuation by 30 μM spermine) differed in their SARs. Both amino and guanidino groups bear a positive charge at pH 7.0, however, guanidino groups are more basic (as e.g. in arginine, pK_a 12.5) than amino groups (as e.g. in lysine, pK_a 10.5) [27] and may more successfully compete for an acidic target site with any arginine residue intrinsic to the receptor protein.

5.2. The aromatic nucleus

The benzene analogue **24** was nearly equipotent to the thieno parent compound **2**, demonstrating that the orientation of the alkyl arms attached to the central nucleus was not of eminent importance. The aromatic nuclei in **2** and **24** might contribute to improved interaction with the target site either by their lipophilicity, or by interaction of their π-electrons with a cationic target [26]. Substitutions increasing the

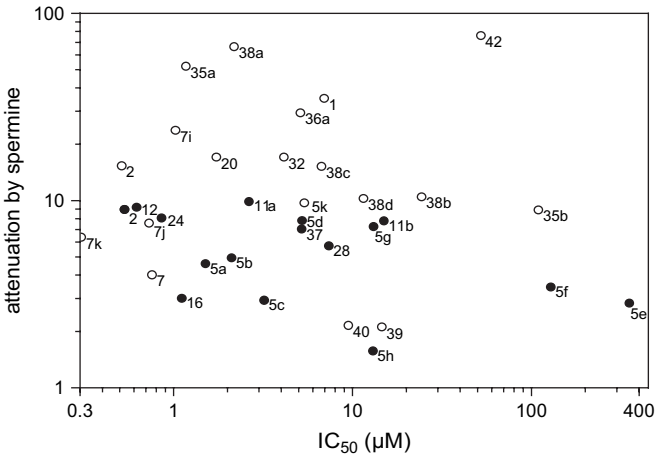


Fig. 2. Inhibitory potency of diamines at the NMDA receptor (IC_{50}) is no significant predictor of attenuation of inhibition by spermine; filled circles, compounds analysed in 50 mM Tris buffer (attenuation by 100 μM spermine); open circles, compounds analysed in 10 mM Tris buffer (attenuation by 30 μM spermine); all values are listed in Tables 1–5.

lipophilicity of the central portion of **2** (as in **5a–c**, Table 2) did not increase, but rather decrease potency. But also the alkyne **28** – providing π-electrons by a triple bond – was not more potent than its saturated analogue **37**. In both cases, however, alternative explanations might be given. The substitutions did not only increase lipophilicity, but may also have been incompatible with tight steric requirements. And the triple bond in **28** might not only offer π-electrons, but also result in a change in overall length (slight changes in overall length can result in huge differences in potency [11], compare **2** with **5k**, Table 5). While for a thiophene-interrupted alkyldiamine chain the optimum position of the π-system was with four methylenes on one side and eight methylenes on the other [11], this is not necessarily the case for a triple bond-interrupted chain. Further studies with additional structural analogues are needed to clarify these questions.

5.3. Modifications in the long arm

The tetraamine **36b** (and to some extent also the triamine **36a**) exhibited interesting stimulatory (polyamine agonistic) properties at low micromolar concentrations, in agreement with well known structural requirements for such a property (i.e. several positive charges at defined distances from each other) [3,4]. The weak inhibitory potency of the diether **32** could have been a consequence not only of reduced lipophilicity, but also of chain rigidization, extending the critical distance between the thiophene ring and the terminal amino group. It might be of interest to test shorter chain homologues. Introduction of an amide bond led to a new class of compounds with remarkable properties. Inhibition of [^{3}H]MK-801 binding by **35a** exhibited unprecedented sensitivity to spermine, suggesting that inhibition of the NMDA receptor by this amide was intimately related to polyamine regulation. Under low ionic strength conditions, this new compound was almost as potent as the parent compound **2**. Analogous introduction of an amide bond into the aliphatic diamine **1** returned the highly spermine- and Tris-sensitive compound **42**. The potencies of these amide inhibitors may depend on target interaction of their uncharged amide moiety, hindered in the presence of excess charged buffer molecules shielding non-specifically the target site. Exploiting the amenability of amide bond formation, we were able to introduce further structural variations by an efficient parallel synthesis approach. Our demonstration that methyl substitution as in **38a** was tolerated without loss in potency offers the prospect of stabilization against degradation by monoamine oxidase [25]. It remains to be investigated if also the short butylamine arm of **35a**, and both arms of the parent compound **2** tolerate such a modification, and whether compounds of this type can reach their target site in physiological media.

6. Conclusion

Synthesis and evaluation in vitro of various structural analogues of the high potency polyamine inverse agonist **2** resulted in divergent SARs (Fig. 2) for potency and polyamine-sensitivity, respectively, endorsing the latter as an independent structural lead. The new amide model compound **35a** provided inhibition of the NMDA receptor almost as potent as the parent compound **2**, with even higher sensitivity to spermine. The compound was amenable to pharmacokinetic stabilization by α-methylation (**38a**) without loss in inhibitory potency, inviting the in vivo evaluation of this new class of inverse polyamine agonists.

7. Experimental protocols

7.1. General chemical procedures

NMR spectra were recorded on a Bruker spectrometer (^{1}H at 200–500, ^{13}C at 50–125 MHz). The chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane ($\delta = 0$ ppm). The signal patterns are indicated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; qn, quintet; m, multiplet. The coupling constants (J) are given in hertz. Melting points were determined with a Büchi 530 capillary-tube melting-point apparatus and are not corrected. TLC's were performed on silica gel (Merck™ 60 F-254); reaction products were visualized by UV fluorescence (254 nm), by exposure to iodine vapour, or after spraying with 5% ethanolic molybdato phosphoric acid and subsequent heating. The products were purified by flash chromatography on silica gel 60 (70–200 mesh ASTM, 0.063–0.200 mm) purchased by Merck. Products from solid phase synthesis were obtained in low quantities, therefore characterization by mass spectroscopy replaced NMR analysis (electro spray ionization mass spectrometer); additionally, they were subjected to purity control by capillary electrophoresis (CE; in a 100 mM phosphate buffer at 25 °C, voltage 25 kV, 67/60 capillaries). Commercial grade reagents and solvents were used as supplied with the following exceptions: DE and THF were freshly distilled from sodium; dichlorethane was distilled from phosphorous pentoxide; MeOH was distilled from magnesium methoxide; PE and DCM were distilled before use. Abbreviations: AcCN, acetonitrile; AcOEt: ethyl acetate; AcOH: acetic acid; Boc: *tert*-butoxycarbonyl; CE: capillary electrophoresis; DCM: dichlormethane; DE, diethyl ether; DIC: diisopropylcarbodiimide; DIPEA: diisopropylethylamine; DME: dimethoxyethane; DMF: dimethylformamide; DMSO: dimethylsulfoxide; Fmoc-: 9*H*-fluoren-9-ylmethyloxycarbonyl-; HOBt: 1-hydroxybenzotriazol (peptide coupling reagent); MeOH: methanol; MS, mass spectrometry; PE: petroleum ether; rt: room temperature; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran; THP: tetrahydropyranyl.

7.2. Syntheses of N,N-substituted thiophene-dialkyldiamines

7.2.1. Dicarboxylic acid precursors

These precursors were synthesized as described [11]. In the following, we give the properties of four of them that have not been described before (Scheme 1).

*7.2.1.1. 8-[5-(3-Carboxy-propyl)-4-methyl-thien-2-yl]-octanoic acid (**3a**).* Yield: 97% colorless solid, m.p.: 68–69 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.21–1.31 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.44–1.56 (m, 4H, CH_2-(3), CH_2-(7)), 1.71 (m, $J = 7.4/7.5$ Hz, 2H, CH_2-(2″)), 2.01 (s, 3H, CH_3-(4′)), 2.18 (t, $J = 7.4$ Hz, 2H, CH_2-(3″)), 2.24 (t, $J = 7.3$ Hz, 2H, CH_2-(2)), 2.58–2.67 (m, 4H, 2H–C(8), 2H–C(1″)), 6.48 (s, 1H, H-(3′)), 12.05 (bs, 2H, COOH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 13.24 (1C, CH_3–C(4′)), 24.47/31.05 (2C, C(3), C(7)), 26.44 (1C, C(2″)), 26.47/29.23 (2C, C(8), C(1″)), 28.33/28.43/28.45 (3C, C(4), C(5), C(6)), 32.80/33.64 (2C, C(2), C(3″)), 127.08 (1C, C(3′)), 131.89/134.33/140.41 (3C, C(2′), C(4′), C(5′)), 174.14/174.48 (2C, COOH). Analysis: C, H, N for $C_{17}H_{26}O_4S$.

*7.2.1.2. 8-[5-(3-Carboxy-propyl)-3-methyl-thien-2-yl]-octanoic acid (**3b**).* Yield: 96% yellowish solid, m.p.: 69–70 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.27 (bs, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.46–1.51 (m, 4H, CH_2-(3), CH_2-(7)), 1.76 (m, 2H, CH_2-(2″)), 2.01 (s, 3H, CH_3-(3′)), 2.14–2.26 (m, 4H, CH_2-(2), CH_2-(3″), 2.56–2.69 (m, 4H, CH_2-(1″), CH_2-(8)), 6.49 (s, 1H, H-(4′)), 12.01 (bs, 2H, COOH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 13.28 (1C, CH_3), 24.45/31.00 (2C, C(3), C(7)), 26.43/27.08 (2C, C(2″), C(1″)), 28.39/28.45/28.55 (4C, C(4), C(5), C(6), C(8)), 32.81/33.62 (2C, C(2), C(3″)), 127.31 (1C, C(4′)), 131.50 (1C, C(3′)), 135.47 (1C, C(2′)), 139.19 (1C, C(5′)), 174.10/174.45 (2C, COOH). Analysis: C, H, N for $C_{17}H_{26}O_4S$.

*7.2.1.3. 8-[5-(3-Carboxy-propyl)-3-ethyl-thien-2-yl]-octanoic acid (**3c**).* Yield: 98% yellowish solid, m.p.: 66–68 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.14 (t, $J = 7.6$ Hz, 3H, CH_3–CH_2-(3′)), 1.31–1.39 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.56–1.67 (m, 4H, CH_2-(3), CH_2-C(7)), 1.90 (m, $J = 7.4/7.5$ Hz, 2H, CH_2-(2″)), 2.26–2.36 (m, 4H, CH_2-(2), CH_2-(3″)), 2.47 (q, $J = 7.6$ Hz, 2H, CH_3–CH_2-(3′)), 2.67 (t, $J = 7.5$ Hz, 2H, CH_2-(1″)), 2.76 (t, $J = 7.5$ Hz, 2H, CH_2-(8)), 6.53 (s, 1H, 1H-(4′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 15.81 (1C, CH_3–CH_2-(3′)), 22.34 (1C, CH_3–CH_2-(3′)), 26.04/33.05 (2C, C(3), C(7)), 28.04 (1C, C(2″)), 28.46/30.06/30.15/30.20 (5C, C(4), C(5), C(6), C(8), C(1″)), 33.97/34.92 (2C, C(2), C(3″)), 126.86 (1C, C(4′)), 136.91/139.93/141.03 (3C, C(2′), C(3′), C(5′)), 177.21/177.68 (2C, 2 × COOH). Analysis: C, H, N for $C_{18}H_{28}O_4S$.

*7.2.1.4. 8-[5-(2-Carboxyethyl)-thien-2-yl]-octanoic acid (**3k**).* This precursor for the synthesis of **5k**, a homologue of **2**, was obtained as described [11], with one exception: after Friedel–Crafts acylation of 3-(2-thienyl)-propanoic acid methylester with suberic acid monoethylester chloride, the obtained ketone was deoxygenated not as the diester, but after hydrolysis as the diacid by Et_3SiH (as described below for the reduction of **22** to **23**, see Section 7.5.3.2). Yield: 72% white powder, m.p.: 108 °C. ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.26 (s, 6H, CH_2-(4″), CH_2-(5″), CH_2-(6″)), 1.43–1.57 (m, 4H, CH_2-(3″), CH_2-(7″)), 2.17 (t, $J = 7.2$ Hz, 2H, CH_2-(2″)), 2.48–2.55 (m, 2H, CH_2-(2)), 2.67 (t, $J = 7.3$ Hz, 2H, CH_2-(8″)), 2.92 (t, $J = 7.3$ Hz, 2H, CH_2-(3)), 6.57–6.62 (m, 2H, 1H-C(3′), 1H-C(4′)), 12.06 (s, 2H, COOH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 24.45 (C(3″)), 24.84 (C(3)), 28.31/28.41/28.45 (C(4″), C(5″), C(6″)), 29.30 (C(8″)), 31.15 (C(7″)), 33.61 (C(2″)), 35.42 (C(2)), 123.75/124.08 (C(3′), C(4′)), 140.66 (C(5′)), 142.80 (C(2′)), 173.37/174.49 (C(1), C(1′)). Analysis: C, H, N for $C_{15}H_{22}O_4S$.

7.2.2. Substituted diamides from dicarboxylic acids, method A for gaseous amines

The respective dicarboxylic acid was dissolved under inert atmosphere in absolute THF. After adding a few drops of absolute pyridine, 1.2 eq. oxalyl chloride were added slowly at rt. The mixture was stirred at rt for 30 min and then refluxed until no further gas developed (ca. 1 h). After cooling to 15–20 °C, the gaseous amine was bubbled in for 30 min, and for another 30 min without cooling. The respective amide was precipitated by addition of DE, filtered, washed with aqueous bicarbonate and brine, dried, and purified by normal phase column chromatography.

*7.2.2.1. 8-[5-(3-Carbamoyl-propyl)-4-methyl-thien-2-yl]-octanoic acid amide (**4a**).* Yield: 67% colorless solid, m.p.: 154–156 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.29–1.39 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.55–1.66 (m, 4H, CH_2-(3), CH_2-(7)), 1.85 (m, $J_1 = 7.4/7.5$ Hz, 2H, CH_2-(2″)), 2.05 (s, 3H, CH_3-(4′)), 2.15 (t, $J = 7.4$ Hz, 2H, CH_2-(3″)), 2.23 (t, $J = 7.3$ Hz, 2H, CH_2-(2)), 2.64–2.71 (m, 4H, CH_2-(8), CH_2-(1″)), 6.42 (s, 1H, 1H-C(3′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 13.39 (s, 1C, CH_3–C(4′)), 25.19/31.19 (2C, C(3), C(7)), 26.84 (1C, C(1″)), 27.15 (1C, C(2″)), 28.48/28.59/28.71 (3C, C(4), C(5), C(6)), 29.35 (1C, C(8)), 34.38/35.19 (2C, C(2), C(3″)), 127.20 (1C, C(3′)), 131.89 (1C, C(4′)), 134.74 (1C, C(2′)), 140.43 (1C, C(5′)), 174.25/174.74 (2C, 2 × $CONH_2$). Analysis: C, H, N for $C_{17}H_{28}N_2O_2S$.

*7.2.2.2. 8-[5-(3-Carbamoyl-propyl)-3-methyl-thien-2-yl]-octanoic acid amide (**4b**).* Yield: 78% colorless solid, m.p.: 144–145 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.23 (bs, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.37–1.45 (m, 4H, CH_2-(3), CH_2-(7)), 1.71 (m, 2H, CH_2-(2″)), 1.97–2.07 (m, 7H, CH_2-(2), CH_2-(3″), CH_3-C(3′)), 2.53–2.62 (m, 4H, CH_2-(1″), CH_2-(8)), 6.45 (s, 1H, 1H-C(4′)), 6.64/6.70/7.18/7.22 (4 bs, 4H, 2 × $CONH_2$). ^{13}C NMR (50 MHz; DMSO-d_6) δ 13.29 (1C, CH_3–C(3′)), 25.05/31.03 (2C, C(3), C(7)), 26.96 (1C, C(2″)), 27.09 (1C, C(1″)), 28.44/28.50/28.62 (3C, C(4), C(5), C(6)), 28.83 (1C, C(8)), 34.24/35.06 (2C, C(2), C(3″)), 127.20 (1C, C(4′)), 131.44 (1C, C(3′)), 135.34 (1C, C(2′)), 139.50 (1C, C(5′)), 173.81/174.28 (2C, 2 × $CONH_2$). Analysis: C, H, N for $C_{17}H_{28}N_2O_2S$.

*7.2.2.3. 8-[5-(3-Carbamoyl-propyl)-3-ethyl-thien-2-yl]-octanoic acid amide (**4c**).* Yield: 59% colorless solid, m.p.: 135–136 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.18 (t, $J = 7.6$ Hz, 3H, CH_3–CH_2-(3′)), 1.36–1.46 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.59–1.70 (m, 4H, CH_2-(3), CH_2-(7)), 1.96 (m, $J = 7.4/7.5$ Hz, 2H, CH_2-(2″)), 2.21–2.34 (m,

4H, CH_2-(2), CH_2-(3″)), 2.51 (q, $J = 7.6$ Hz, 2H, CH_3–CH_2-(3′)), 2.71 (t, $J = 7.5$ Hz, 2H, CH_2-(1″)), 2.79 (t, $J = 7.5$ Hz, 2H, CH_2-(8)), 6.58 (s, 1H, 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 15.81 (1C, CH_3–CH_2-(3′)), 22.34 (1C, CH_3–CH_2-(3′)), 26.85/33.07 (2C, C(3), C(7)), 28.46 (1C, C(2″)), 28.80/30.09/30.21/30.41 (5C, C(4), C(5), C(6), C(8), C(1″)), 35.67/36.50 (2C, C(2), C(3″)), 126.81 (1C, C(4′)), 136.88/139.93/141.11 (3C, C(2′), C(3′), C(5′)), 178.70/179.29 (2C, 2 × $CONH_2$). Analysis: C, H, N for $C_{18}H_{30}N_2O_2S$.

*7.2.2.4. 8-[5-(3-Methylcarbamoyl-propyl)-thien-2-yl]-octanoic acid methylamide (**4d**).* Yield: 87% yellow crystals. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.33 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.62 (m, 4H, CH_2-(3), CH_2-(7)), 1.97–2.03 (m, 2H, CH_2-(2″)), 2.13–2.22 (m, 4H, CH_2-(2), CH_2-(3″)), 2.73 (t, 4H, CH_2-(8), CH_2-(1″)), 2.80 (2s, 6H, 2 × N–CH_3), 5.58 (bs, 2H, 2 × NH), 6.56 (2d, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.27/32.85 (2C, C(3), C(7)), 26.95 (2C, 2 × N–CH_3), 28.98 (1C, C(2″)), 29.91/30.10/30.19/30.46 (4C, C(4), C(5), C(6), C(1″)), 30.95 (1C, C(8)), 36.14/36.99 (2C, C(2), C(3″)), 124.71/125.06 (2C, C(3′), C(4′)), 142.95/144.57 (2C, C(2′), C(5′)), 176.28/176.89 (2C, 2 × $CONHCH_3$). Analysis: C, H, N for $C_{18}H_{30}N_2O_2S$.

*7.2.2.5. 8-[5-(3-Dimethylcarbamoyl-propyl)-thien-2-yl]-octanoic acid methylamide (**4e**).* Yield: 83% yellow solid, m.p.: 37–39 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.33–1.35 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.60–1.62 (m, 4H, CH_2-(3), CH_2-(7)), 1.96–2.01 (m, 2H, CH_2-(2″)), 2.27–2.37 (m, 4H, CH_2-(2), CH_2-(3″)), 2.72 (t, 2H, CH_2-(8)), 2.82 (t, 2H, CH_2-(1″)), 2.96 (s, 12H, 2N–$(CH_3)_2$), 6.55 (2d, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 25.05/26.74/28.86/29.08/29.30/29.50/30.02/31.54/32.16/33.27/35.27/37.13/37.20 (14C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(3″), C(2″), C(1″), 4 × N–CH_3), 123.34/123.76 (2C, C(3′), C(4′)), 141.91/143.47 (2C, C(2′), C(5′)), 172.53/173.12 (2C, 2 × $CON(CH_3)_2$). Analysis: C, H, N for $C_{20}H_{34}N_2O_2S$.

*7.2.2.6. 8-[5-(2-Carbamoyl-ethyl)-thien-2-yl]-octanoic acid amide (**4k**).* Yield: 52% white powder, m.p.: 165–166 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.24 (s, 6H, CH_2-(4″), CH_2-(5″), CH_2-(6″)), 1.39–1.55 (4H, CH_2-(3″), CH_2-(7″)), 1.99 (t, $J = 7.5$ Hz, 2H, CH_2-(2″)), 2.34 (t, $J = 7.2$ Hz, 2H, CH_2-(2)), 2.67 (t, $J = 7.5$ Hz, 2H, CH_2-(8″), 2.89 (t, $J = 7.5$ Hz, 2H, CH_2-(3)), 6.56–6.60 (m, 2H, 1H-C(3′), 1H-C(4′)), 6.67/6.80/7.21/7.32 (4s, 4H, 2 × NH_2). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.04/25.24 (C(3), C(3″)), 28.33/28.45/28.60 (C(4″), C(5″), C(6″)), 29.31 (C(8″)), 31.17 (C(7″)), 35.06 (C(2″)), 36.67 (C(2)), 123.66/123.83 (C(3′), C(4′)), 141.29/142.61 (C(2′), C(5′)), 172.95/174.33 (C(1), C(1″)). Analysis: C, H, N for $C_{15}H_{24}N_2O_2S$.

7.2.3. Substituted diamides from dicarboxylic acids, method B for liquid amines

In absolute DCM, 3 eq. dicarboxylic acid were combined with 3 eq. Mukaiyama reagent (2-chloro-1-methylpyridinium iodide) and 6 eq. TEA, and 1 eq. of the liquid amine dissolved in DCM was slowly added. The mixture was boiled until the reaction was complete. The solvent was evaporated and the residue dissolved in AcOEt and washed with 1 N HCl and 1 N NaOH. The organic phase was dried, evaporated, and the residue purified by normal phase chromatography.

*7.2.3.1. 8-[5-(3-Propylcarbamoyl-propyl)-thien-2-yl]-octanoic acid propylamide (**4f**).* Yield: 83% colorless solid, m.p.: 94 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 0.85 (t, 6H, 2 × CH_3–CH_2–CH_2–N), 1.26–1.30 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.34–1.54 (m, 8H, CH_2-(3), CH_2-(7), 2 × CH_3–CH_2–CH_2–N), 1.91 (m, 2H, CH_2-(2″)), 2.04–2.17 (m, 4H, CH_2-(2), CH_2-(3″)), 2.69–2.76 (m, 4H, CH_2-(1″), CH_2-(8)), 3.09–3.19 (m, 4H, 2 × CH_3–CH_2–CH_2–N), 6.53–6.61 (m, 2H, 1H-(3′), 1H-(4′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 11.31 (2C, 2 × CH_3–CH_2–CH_2–N), 22.83 (2C, 2 × CH_3–CH_2–CH_2–N)), 25.69 (1C, C(3)), 27.38 (1C, C(2″)), 28.77/28.96/29.09 (3C, C(4), C(5), C(6)), 29.36/30.00 (2C, C(1″), C(8)), 31.47 (1C, C(7)), 35.58/36.77 (2C, C(2), C(3″)), 41.10/41.13 (2C, 2 × CH_3–CH_2–CH_2–N)), 123.42/123.88 (2C, C(3′), C(4′)), 141.65/143.54 (2C, C(2′), C(5′)), 172.36/172.93 (2C, 2 × $CONH$–propyl). Analysis: C, H, N for $C_{22}H_{38}N_2O_2S$.

*7.2.3.2. 8-[5-(3-Allylcarbamoyl-propyl)-thien-2-yl]-octanoic acid allylamide (**4g**).* Yield: 82% colorless solid, m.p.: 87–89 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.33 (bs, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.54–1.72 (m, 4H, CH_2-(3), CH_2-(7)), 1.95–2.06 (m, 2H, CH_2-(2″)), 2.14–2.27 (m, 4H, CH_2-(2), CH_2-(3″)), 2.69–2.84 (m, 4H, CH_2-(1″), CH_2-(8)), 3.84–3.91 (m, 4H, 2 × H_2C=CH–CH_2), 5.09–5.22 (m, 4H, 2 × H_2C=CH–CH_2), 5.59 (bs, 2H, 2 × NH), 5.74–5.93 (m, 2H, 2 × H_2C=CH–CH_2), 7.53–7.57 (m, 2H, 1H-(3′), 1H-(4′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 25.61 (1C, C(3)), 27.31 (1C, C(2″)), 28.75/28.94/29.08 (3C, C(4), C(5), C(6)), 29.37/30.00 (2C, C(1″), C(8)), 31.46 (1C, C(7)), 35.45/36.62 (2C, C(2), 1C(3″)), 41.79/41.83 (2C, 2 × H_2C=CH–CH_2), 116.15/116.22 (2C, 2 × H_2C=CH–CH_2), 123.44/123.92 (2C, C(3′), C(4′)), 134.28/134.35 (2C, 2 × H_2C=CH–CH_2), 141.58/143.55 (2C, C(2′), C(5′)), 172.36/172.93 (2C, 2 × $CONH$–propyl). Analysis: C, H, N for $C_{22}H_{34}N_2O_2S$.

*7.2.3.3. 8-[5-(3-Benzylcarbamoyl-propyl)-thien-2-yl]-octanoic acid benzylamide (**4h**).* Yield: 74% colorless solid, m.p.: 118–119 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.21–1.32 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.58–1.69 (m, 4H, CH_2-(3), CH_2-(7)), 2.02 (m, 2H, CH_2-(2″)), 2.17–2.29 (m, 4H, CH_2-(2), CH_2-(3″)), 2.73 (t, $J = 7.5$ Hz, 2H, CH_2-(1″)), 2.81 (t, $J = 7.3$ Hz, 2H, CH_2-(8)), 4.43 (d, $J = 5.7$ Hz, 4H, 2 × CH_2–benzyl), 5.81 (bs, 2H, 2 × NH), 6.55 (s, 2H, 1H-C(3′), 1H-C(4′)), 7.25–7.38 (m, 10H, 2 × H–benzyl). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 25.62/31.48 (2C, C(3), C(7)), 27.32 (1C, C(2″)), 28.77/28.96/29.10/30.03 (5C, C(4), C(5), C(6), C(8), C(1″)), 35.48/36.68 (2C, C(2), C(3″)), 43.52 (2C, 2 × CH_2–benzyl), 123.48/123.99 (2C, C(3′), C(4′)), 127.44 (2C, 2 × (*para*-arom.)), 127.76/128.65 (8C, 4 × (*ortho*-arom.), 4 × (*meta*-arom.)), 138.34/138.41 (2C, 2 × arom.),

141.57/143.61 (2C, C(2′), C(5′)), 172.34/172.91 (2C, 2 × *C*ONH$_2$). Analysis: C, H, N for $C_{30}H_{38}N_2O_2S$.

7.2.4. Reduction of diamides to diamines (method C)

Under inert atmosphere, $LiAlH_4$ was dissolved in absolute, icy THF and the amide was slowly added. The mixture was refluxed until the reactant disappeared (15–30 h). After cooling to 0 °C, aqueous tartrate was added, the volume reduced by evaporation, DCM added, and filtered over celite. The residue was washed with DCM, the combined organic layers washed with water, dried, and the solvent evaporated. The obtained raw amine was dissolved in absolute DE, precipitated as the hydrochloride with gaseous HCl, and further purified by crystallization or by column chromatography.

*7.2.4.1. 8-[5-(4-Aminobutyl)-4-methyl-thien-2-yl]-octylamine dihydrochloride (**5a**).* Yield: 60% colorless solid, m.p.: 135–140 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.30–1.40 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.56–1.71 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(3″), CH_2-(2″)), 2.64–2.74/2.87–2.93 (2m, 8H, CH_2-(1), CH_2-(8), CH_2-(4″), CH_2-(1″)), 6.43 (s, 1H, 1H-C(3′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 13.67 (1C, *C*H$_3$–C(4′)), 27.41/28.00/28.09/28.51/29.46/29.96/30.10/30.19/30.81/32.80 (10C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(1″), C(2″), C(3″)), 40.62/40.75 (2C, C(1), C(4″)), 128.14 (1C, C(3′)), 133.44 (1C, C(4′)), 135.59 (1C, C(2′)), 142.32 (1C, C(5′)). Analysis: C, H, N for $C_{17}H_{34}N_2SCl_2$.

*7.2.4.2. 8-[5-(4-Aminobutyl)-3-methyl-thien-2-yl]-octylamine dihydrochloride (**5b**).* Yield: 68% brown solid, m.p. ≥160 °C (decomposition). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.26 (bs, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.43–1.58 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 2.01 (s, 3H, CH_3–C(3′)), 2.56–2.73 (m, 8H, CH_2-(1), CH_2-(1″), CH_2-(4″), CH_2-(8)), 6.51 (s, 1H, 1H-C(4′)), 8.17 (bs, 6H, 2 × NH_3). ^{13}C NMR (50 MHz; DMSO-d_6) δ 13.33 (s, *C*H$_3$–C(3′)), 25.85/28.45 (4C, C(3), C(4), C(5), C(6)), 26.3/26.82/27.96/31.03 (4C, C(2), C(7), C(2″), C(3″)), 27.10 (1C, C(1″)), 28.77 (1C, C(8)), 38.25/38.38 (2C, C(1), C(4″)), 127.32 (1C, C(4′)), 131.48 (1C, C(3′)), 135.38 (1C, C(2′)), 139.37 (1C, C(5′)). Molecular weight calculated for $C_{17}H_{32}N_2S$: 296.52; measured by MS (*m*/*z*): 297.3 (M + H).

*7.2.4.3. 8-[5-(4-Aminobutyl)-3-ethyl-thien-2-yl]-octylamine dihydrochloride (**5c**).* Yield: 71% colorless solid, m.p.: not accessible (too hygroscopic). ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.18 (t, $J = 7.6$ Hz, 3H, *CH*$_3$–CH_2-(3′)), 1.36–1.46 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.59–1.80 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 2.51 (q, $J = 7.6$ Hz, 2H, CH_3–*CH*$_2$-(3′)), 2.71 (t, $J = 7.4$ Hz, 2H, CH_2-(1″)), 2.83 (t, $J = 6.4$ Hz, 2H, CH_2-(8)), 2.93–3.00 (m, 4H, CH_2-(1), CH_2-(4″)), 6.61 (s, 1H, 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 15.82 (1C, *C*H$_3$–CH_2-(3′)), 22.32 (1C, CH_3–*CH*$_2$-(3′)), 27.43/30.12/30.26 (4C, C(3), C(4), C(5), C(6)), 27.92/28.46/29.50/33.13 (4C, C(2), C(7), C(2″), C(3″)), 28.52 (1C, C(8)), 30.34 (1C, C(1″)), 40.57/40.77 (2C, C(1), C(4″)), 126.91 (1C, C(4′)), 136.90/139.98/141.02 (3C, C(2′), 1C(3′), C(5′)). Analysis: C, H, N for $C_{18}H_{36}N_2SCl_2 \cdot 0.4H_2O$.

*7.2.4.4. Methyl-{8-[5-(4-methylaminobutyl)-thien-2-yl]-octyl}-amine dihydrochloride (**5d**).* Yield: 61% colorless solid, m.p.: 220–230 °C (with decomposition). ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.37 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.63–1.75 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 1.68 (s, 6H, 2 × N–CH_3), 2.71–2.82 (m, 4H, CH_2-(1), CH_2-(4″)), 2.98–3.00 (m, 4H, CH_2-(8), CH_2-(1″)), 6.57 (d, 1H, H-C(3′)), 6.61 (d, 1H, H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 25.73/26.20/26.58/28.76/29.12/29.23/29.68/30.21/32.01/33.40/33.80/44.88 (12C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(1″), C(2″), C(3″), 2 × N–*C*H$_3$), 49.13/49.45 (2C, C(1), C(4″)), 124.94/125.19 (2C, C(3′) C(4′)), 142.60/143.93 (2C, C(2′) C(5′)). Analysis: C, H, N for $C_{18}H_{36}N_2SCl_2 \cdot 0.5H_2O$.

*7.2.4.5. {8-[5-(4-Dimethylaminobutyl)-thien-2-yl]-octyl}-dimethyl-amine dihydrochloride (**5e**).* Yield: 58% colorless solid, m.p.: 218–219 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.38 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.62–1.76 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 2.72–2.87 (m, 16H, CH_2-(1), CH_2-(4″), 4 × N–CH_3), 3.08–3.14 (m, 4H, CH_2-(8), CH_2-(1″)), 6.58–6.64 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 24.95/25.59/27.40/29.51/29.91/30.07/30.15/30.28/30.93/32.84/43.39 (14C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(1″), C(2″), C(3″), 4 × N–*C*H$_3$), 58.69/59.00 (2C, C(1), C(4″)), 124.80/125.24 (2C, C(3′), C(4′)), 142.74/144.66 (2C, C(2′), C(5′)). Analysis: C, H, N for $C_{20}H_{40}N_2SCl_2 \cdot 0.5H_2O$.

*7.2.4.6. Propyl-{8-[5-(4-propyllaminobutyl)-thien-2-yl]-octyl}-amine dihydrochloride (**5f**).* Yield: 72% colorless solid, m.p. ≥240 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.01 (2t, $J_1 = 11.2$ Hz, $J_2 = 11.0$ Hz, 6H, 2 × *CH*$_3$–CH_2–CH_2–N), 1.36 (bs, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.59–1.79 (m, 12H, CH_2-(2), CH_2-(2″), CH_2-(7), CH_2-(3″), 2 × CH_3–*CH*$_2$–CH_2–N)), 2.69–3.03 (m, 12H, CH_2-(1), CH_2-(1″), CH_2-(8), CH_2-(4″), 2 × CH_3–CH_2–*CH*$_2$–N), 6.54–6.61 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 11.25 (2C, 2 × *C*H$_3$–CH_2–CH_2–N), 20.65 (2C, 2 × CH_3–*CH*$_2$–CH_2–N), 26.56 (1C, C(2″)), 27.22/29.64 (2C, C(2), C(3″)), 27.54/29.93/30.10/30.16 (4C, C(3), C(4), C(5), C(6)), 30.35/30.94 (2C, C(1″), C(8)), 32.87 (1C, C(7)), 48.58/48.67 (2C, C(1), C(4″)), 50.49 (2C, 2 × CH_3–CH_2–*CH*$_2$–N), 124.77/125.21 (2C, C(3′), C(4′)), 142.83/144.62 (2C, C(2′),C(5′)). Analysis: C, H, N for $C_{22}H_{44}N_2SCl_2$.

*7.2.4.7. Allyl-{8-[5-(4-allylaminobutyl)-thien-2-yl]-octyl}-amine dihydrochloride (**5g**).* Yield: 76% colorless solid, m.p.: 224–228 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.36 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.59–1.82 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 2.68–2.98 (m, 8H, CH_2-(1), CH_2-(8), CH_2-(1″), CH_2-(4″)), 3.62 (2d, $J = 8.4$ Hz, 4H, 2 × H_2C=CH–*CH*$_2$), 5.44–5.57 (m, 4H,

2 × H_2C=CH–CH_2), 5.86–5.97 (m, 2H, 2 × H_2C=CH–CH_2), 6.55–6.60 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 26.53 (1C, C(2″)), 27.19/29.65 (2C, C(2), C(3″)), 27.53/29.95/30.11/30.17 (4C, C(3), C(4), C(5), C(6)), 30.35/30.95 (2C, C(1″), C(8)), 32.89 (1C, C(7)), 47.96/48.24 (2C, C(1), C(4″)), 50.77 (2C, 2 × H_2C=CH–CH_2), 124.11/124.15 (2C, 2 × H_2C=CH–CH_2), 124.79/125.23 (2C, C(3′), C(4′)), 129.24/129.31 (2C, 2 × H_2C=CH–CH_2), 142.77/144.61 (2C, C(2′), C(5′)). Analysis: C, H, N for $C_{22}H_{40}N_2SCl_2 \cdot 0.25H_2O$.

*7.2.4.8. Benzyl-{8-[5-(4-benzylaminobutyl)-thien-2-yl]-octyl}-amine dihydrochloride (**5h**).* Yield: 74% colorless solid, m.p. ≥230 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.12–1.22 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.58–1.77 (m, 8H, CH_2-(2), CH_2-(7), CH_2-(2″), CH_2-(3″)), 2.74 (t, J = 7.3 Hz, 2H, CH_2-(8)), 2.82 (t, J = 6.5 Hz, 2H, CH_2-(1″)), 2.99–3.08 (m, 4H, CH_2-(1), CH_2-(4″)), 4.20 (d, J = 3.9 Hz, 4H, 2 × CH_2–benzyl), 6.55–6.63 (m, 2H, 1H-C(3′), 1H-C(4′)), 7.41–7.55 (m, 10H, 2 × H–benzyl). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 26.46/27.10/27.59/29.69/29.97/30.10/30.17/30.35/30.96/32.89 (10C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(1″), C(2″), C(3″)), 52.38 (2C, 2 × CH_2–benzyl), 124.79/125.24 (2C, C(3′), C(4′)), 130.29/130.67/131.01/132.53/132.60 (12C, 2 × arom.), 142.78/144.66 (2C, C(2′), C(5′)). Molecular weight calculated for $C_{30}H_{42}N_2S$: 462.75; measured by MS (*m*/*z*): 463.4 (M + H).

*7.2.4.9. 7-[5-(3-Amino-propyl)-thien-2-yl]-octylamine dihydrochloride (**5k**).* Yield: 65% brown solid, m.p. ≥180 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.25 (s, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.52–1.54 (m, 4H, CH_2-(2), CH_2-(7)), 1.85 (qn, J = 7.5 Hz, 2H, CH_2-(3″)), 2.66–2.80 (m, 8H, CH_2-(2″), CH_2-(4″), CH_2-(1), CH_2-(8)), 6.60–6.64 (m, 2H, 1H-C(3′), 1H-C(4′)), 7.98–8.09 (m, 6H, 2 × NH_3). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.79/28.28/28.41/28.46 (4C, C(3), C(4), C(5), C(6)), 26.35/38.04/38.64 (4C, C(2″), C(4″), C(1), C(8)), 26.85/31.13 (2C, C(2), C(7)), 28.87 (1C, C(3″)), 123.86/124.25 (2C, C(3′), C(4′)), 140.69/142.84 (2C, C(2′), C(5′)). Analysis: C, H, N for $C_{15}H_{30}Cl_2N_2S$.

7.3. Guanylation of thiophene-dialkyldiamines

The di-Boc-protected pyrazole amidine reagent 1*H*-pyrazole-1-[*N*,*N*′-bis(Boc)]carboxamidine was prepared from 1*H*-pyrazol-1-carboxamidine-HCl with NaH and Boc_2O in THF [13]. The respective diamines **5i–k** and **2** (Scheme 2) were obtained as described [11] (**5i** and **5j** were only intermediates and not isolated; **5k** was obtained via **3k** and **4k**, See Sections 7.2.1.4 and 7.2.2.6) and combined in AcCN at rt with the di-Boc-protected guanylating reagent. The mixture was stirred till completion of the reaction (2–5 h). The solvent was subsequently evaporated, the residue purified by column chromatography, and the products fully characterized (**6**, **6i–k**). The protecting Boc-group was cleaved off in AcOEt/HCl_{conc} (4/1) at rt (30 min). After evaporation, the diguanidine hydrochlorides **7**, **7i–k** were re-crystallized from MeOH/DE.

*7.3.1. tert-Butyl-N-({[8-(5-{4-[2,3-bis(Boc)-guanidino] butyl}-2-thienyl)octyl]amino}[(Boc)-amino]methylidene) carbamate (**6**)*

Yield: 53% yellowish resin. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.27–1.32 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.49–1.50 (m, 36H, 4 × ((CH_3)$_3$–O)), 1.55–1.70 (m, 8H, CH_2-(2″), CH_2-(3″), CH_2-(2), CH_2-(7)), 2.70–2.80 (m, 4H, CH_2-(1″), CH_2-(8)), 3.39–3.44 (m, 4H, CH_2-(4″), CH_2-(1)), 6.56 (s, 2H, 1H-C(3′), 1H-C(4′)), 8.30 (s, 2H, NH–C(4″), NH–C(1)), 11.50 (s, 2H, 2 × (CH_3)$_3$–OCO–NH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.13/27.56/27.72/28.38/28.49 (7C, C(1″), C(2″), C(2), C(3), C(4), C(5), C(6)), 27.95/28.21 (12C, 4 × (OC(CH_3)$_3$)), 28.95 (1C, C(8)), 29.38 (1C, C(3″)), 31.16 (1C, C(7)), 40.18 (2C, C(4″), C(1)), 77.98 (2C, 2 × NHCOOC(CH_3)$_3$), 82.80 (2C, 2 × C=NCOOC(CH_3)$_3$), 123.63/123.83 (2C, C(3′), C(4′)), 141.99/142.44 (2C, C(2′), C(5′)), 152.12 (2C, 2 × NHCOOC(CH_3)$_3$), 155.18/155.23 (2C, 2 × C=NCOOC(CH_3)$_3$), 163.10 (2C, 2 × C=NCOOC(CH_3)$_3$). Analysis: C, H, N for $C_{38}H_{66}N_6O_8S$.

*7.3.2. tert-Butyl-N-({[6-(5-{2-[2,3-bis(Boc)-guanidino] ethyl}-2-thienyl)hexyl]amino}[(Boc)-amino] methylidene)carbamate (**6i**)*

Yield: 9% colorless foam, m.p.: 40–43 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.36–1.38 (m, 4H, CH_2-(3), CH_2-(4)), 1.47–1.70 (m, 40H, CH_2-(2), CH_2-(5), 4 × (OC(CH_3)$_3$)), 2.73 (t, J = 7.5 Hz, 2H, CH_2-(6)), 2.99 (t, J = 6.8 Hz, 2H, CH_2-(3″)), 3.39 (q, J = 7.0 Hz, 2H, CH_2-(1)), 3.66 (q, J = 6.8 Hz, 2H, CH_2-(4″)), 6.56/6.63 (2d, J_1 = 3.2 Hz, J_2 = 3.2 Hz, 2H, 1H-C(3′), 1H-C(4′)), 8.29/8.43 (2s, 2H, NH–C(4″), NH–C(1)), 11.47–11.49 (m, 2H, 2 × (CH_3)$_3$–OCO–NH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.55 (1C, C(3)), 28.01/28.05 (12C, 4 × (OC(CH_3)$_3$)), 28.63 (1C, C(4)), 28.88 (1C, C(2)), 29.71 (1C, C(6)), 29.96 (1C, C(3″)), 31.44 (1C, C(5)), 40.88 (1C, C(1)), 42.19 (1C, C(4″)), 79.136/79.21 (2C, 2 × NHCOOC(CH_3)$_3$), 82.96 (2C, 2 × C=NCOOC(CH_3)$_3$), 123.78/124.93 (2C, C(3′), C(4′)), 138.21 (1C, C(2′)), 144.14 (1C, C(5′)), 153.09/153.31 (2C, 2 × NHCOOC(CH_3)$_3$), 156.10 (2C, 2 × C=NCOOC(CH_3)$_3$), 163.57/163.64 (2C, 2 × C=NCOOC(CH_3)$_3$). Analysis: C, H, N for $C_{34}H_{58}N_6O_8S$.

*7.3.3. tert-Butyl-N-({[7-(5-{3-[2,3-bis(Boc)-guanidino] propyl}-2-thienyl)heptyl]amino}[(Boc)-amino]methylidene) carbamate (**6j**)*

Yield: 51% white foam, m.p.: 37–39 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.35 (m, 6H, CH_2-(3), CH_2-(4), CH_2-(5)), 1.48–1.55 (m, 38H, CH_2-(2), 4 × (OC(CH_3)$_3$)), 1.60 (s, 2H, CH_2-(6)), 1.92 (q, J = 7.3 Hz, 2H, CH_2-(3″)), 2.72 (t, J = 7.7 Hz, 2H, CH_2-(7)), 2.81 (t, J = 7.6 Hz, 2H, CH_2-(2″)), 3.40 (q, J = 7.2 Hz, 2H, CH_2-(1)), 3.47 (q, J = 7.1 Hz, 2H, CH_2-(4″)), 6.54/6.59 (2d, J_1 = 3.3 Hz, J_2 = 3.3 Hz, 2H, 1H-C(3′), 1H-C(4′)), 8.29/8.36 (2s, 2H,

NH–C(4″), NH–C(1)), 11.50 (s, 2H, 2 × $(CH_3)_3$–OCO–N*H*). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.69 (1C, C(3)), 27.34 (1C, C(2″)), 28.01/28.25 (12C, 4 × (OC(*C*H_3)$_3$)), 28.85/28.90 (3C, C(4), C(5), C(7)), 30.01 (1C, C(2)), 30.85 (1C, C(6)), 31.50 (1C, C(3″)), 40.04 (1C, C(4″)), 40.87 (1C, C(1)), 79.09/79.13 (2C, 2 × NHCOO*C*$(CH_3)_3$), 82.89/82.98 (2C, 2 × C=NCOO*C*$(CH_3)_3$), 123.44/123.90 (2C, C(3′), C(4′)), 141.24/143.56 (2C, C(2′), C(5′)), 153.26 (2C, 2 × NH*C*OOC$(CH_3)_3$), 156.04/156.13 (2C, 2 × C=N*C*OOC$(CH_3)_3$), 163.57/163.60 (2C, 2 × *C*=NCOOC$(CH_3)_3$). Analysis: C, H, N for $C_{36}H_{62}N_6O_8S$.

7.3.4. tert-Butyl-N-({[8-(5-{3-[2,3-bis(Boc)-guanidino] propyl}-2-thienyl)heptyl]amino}[(Boc)-amino]methylidene) carbamate (**6k**)

Yield: 52% white foam, m.p.: 35–36 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.32 (s, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.49–1.50 (38H, CH_2-(2), 4 × (OC(CH_3)$_3$)), 1.58–1.61 (m, 2H, CH_2-(7)), 1.93 (q, J = 7.3 Hz, 2H, CH_2-(3″)), 2.73 (t, J = 7.6 Hz, 2H, CH_2-(8)), 2.82 (t, J = 7.6 Hz, 2H, CH_2-(2″)), 3.39–3.48 (m, 4H, CH_2-(4″), CH_2-(1)), 6.55/6.59 (2d, J_1 = 3.3 Hz, J_2 = 3.3 Hz, 2H, 1H-C(3′), 1H-C(4′)), 8.32–8.39 (m, 2H, NH–C(4″), NH–C(1)), 11.50 (s, 2H, 2 × $(CH_3)_3$–OCO–N*H*). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 26.77/28.89/28.97/29.11/29.12 (5C, C(2), C(3), C(4), C(5), C(6)), 27.35 (1C, C(2″)), 28.02/28.26 (12C, 4 × (OC(*C*H_3)$_3$)), 30.06 (1C, C(8)), 30.85 (1C, C(3″)), 31.59 (1C, C(7)), 40.05/40.91 (2C, C(4″), C(1)), 79.13/79.17 (2C, 2 × NHCOO*C*$(CH_3)_3$), 82.91/83.00 (2C, 2 × C=NCO O*C*$(CH_3)_3$), 123.41/123.91 (2C, C(3′), C(4′)), 141.21/143.67 (2C, C(2′), C(5′)), 153.27 (2C, 2 × NH*C*OOC$(CH_3)_3$), 156.04/156.14 (2C, 2 × C=N*C*OOC$(CH_3)_3$), 163.57/163.60 (2C, 2 × *C*=NCOOC$(CH_3)_3$). Analysis: C, H, N for $C_{37}H_{64}N_6O_4S$.

7.3.5. N-{8-[5-(4-Guanidino-butyl)-thien-2-yl]octyl}-guanidine dihydrochloride (**7**)

Yield: 88% dark yellow solid, m.p.: 105–106 °C. ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.25 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.43–1.58 (m, 8H, CH_2-(2″), CH_2-(3″), CH_2-(2), CH_2-(7)), 2.64–2.74 (m, 4H, CH_2-(1″), CH_2-(8)), 3.01–3.12 (m, 4H, CH_2-(4″), CH_2-(1)), 6.59/6.61 (s, 2H, 1H-C(3′), 1H-C(4′)), 7.20–7.33 (bs, 8H, 2 × ((H_2N(C=N*H*))·*H*Cl)), 7.79–7.88 (m, 2H, N*H*–C(4″), N*H*–C(1)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.99/28.38/28.44/28.52/28.62 (5C, C(2), C(3), C(4), C(5), C(6)), 27.97/28.19 (2C, C(1″), C(2″)), 28.92 (1C, C(8)), 29.35 (1C, C(3″)), 31.17 (1C, C(7)), 40.61 (2C, C(4″), C(1)), 123.75/123.89 (2C, C(3′), C(4′)), 141.84/142.50 (2C, C(2′), C(5′)), 157.03 (2C, 2 × *C*=NCOOC$(CH_3)_3$). Analysis: C, H, N for $C_{18}H_{36}Cl_2N_6S$.

7.3.6. N-{6-[5-(2-Guanidino-ethyl)-thien-2-yl]hexyl}-guanidine dihydrochloride (**7i**)

Yield: 91% colorless solid, m.p.: not accessible (too hygroscopic). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.30 (s, 4H, CH_2-(3), CH_2-(4)), 1.56 (m, 2H, CH_2-(2)), 1.65 (m, 2H, CH_2-(5)), 2.70 (t, J = 7.4 Hz, 2H, CH_2-(6)), 2.90 (t, J = 7.1 Hz, 2H, CH_2-(3″)), 3.06 (q, J = 6.1 Hz, 2H, CH_2-(1)), 3.30–3.33 (m, 2H, CH_2-(4″)), 6.64/6.72 (2d, J_1 = 3.3 Hz, J_2 = 3.2 Hz, 2H, 1H-C(3′), 1H-C(4′)), 7.12–7.60 (m, 8H, 2 × (NH–(C=N*H*)–NH_2·*H*Cl)), 7.74–7.81 (m, 2H, 2 × N*H*–(C=NH)–NH_2). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.66 (1C, C(3)), 27.94 (1C, C(4)), 28.34/28.98/29.20/31.04 (4C, C(3″), C(2), C(5), C(6)), 40.56 (1C, C(1)), 42.01 (1C, C(4″)), 124.00 (1C, C(4)), 125.27 (1C, C(3)), 137.66 (1C, C(2)), 143.27 (1C, C(5)), 157.04/157.10 (2C, 2 × NH–(*C*=NH)–NH_2). Analysis: C, H, N for $C_{14}H_{28}Cl_2N_6S\cdot 0.5H_2O$. Molecular weight calculated for $C_{14}H_{26}N_6S$: 310.47; measured by MS (*m*/*z*): 311.2 (M + 1).

7.3.7. N-{7-[5-(3-Guanidino-propyl)-thien-2-yl]heptyl}-guanidine dihydrochloride (**7j**)

Yield: 89% colorless solid, m.p.: not accessible (too hygroscopic). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.28 (s, 6H, CH_2-(3), CH_2-(4), CH_2-(5)), 1.40–1.42 (m, 2H, CH_2-(2)), 1.53–1.55 (m, 2H, CH_2-(6)), 1.75 (q, J = 7.4 Hz, 2H, CH_2-(2″)), 2.66–2.81 (m, 4H, CH_2-(1″), CH_2-(7)), 3.03–3.15 (m, 4H, CH_2-(3″), CH_2-(1)), 6.60–6.78 (m, 2H, 1H-C(3′), 1H-C(4′)), 6.85–7.46 (m, 8H, 2 × NH–(C=N*H*)–NH_2·*H*Cl), 7.77 (t, J = 5.3 Hz, 1H, N*H*–(C=NH)–NH_2), 7.94 (t, J = 5.2 Hz, 1H, N*H*–(C=NH)–NH_2). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.91/28.24/28.26 (3C, C(3), C(4), C(5)), 26.37/29.29 (2C, C(1″), C(7)), 28.41 (1C, C(2)), 30.49 (1C, C(2″)), 31.05 (1C, C(6)), 39.87/40.58 (2C, C(3″), C(1)), 123.82/124.10 (2C, C(3′), C(4′)), 140.97/142.65 (2C, C(2′), C(5′)), 157.10/157.19 (2C, 2 × NH–(*C*=NH)–NH_2). Analysis: C, H, N for $C_{16}H_{32}Cl_2N_6S\cdot 0.5H_2O$.

7.3.8. N-{8-[5-(3-Guanidino-propyl)-thien-2-yl]octyl}-guanidine dihydrochloride (**7k**)

Yield: 93%, white solid, m.p.: not accessible (too hygroscopic). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.25 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.39–1.42 (m, 2H, CH_2-(2)), 1.51–1.57 (m, 2H, CH_2-(7)), 1.74 (qn, J = 7.6 Hz, 2H, CH_2-(2″)), 2.64–2.79 (m, 4H, CH_2-(1″), CH_2-(8)), 3.05–3.11 (m, 4H, CH_2-(3″), CH_2-(1)), 6.58–6.63 (m, 2H, 1H-C(3′), 1H-C(4′)), 7.26 (bs, 8H, 2 × ((H_2N(C=N*H*))·*H*Cl)), 7.83 (t, J = 5.2 Hz, 1H, N*H*–C(1)), 8.01 (t, J = 5.3 Hz, 1H, N*H*–C(3″)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.02/28.39/28.46/28.54/28.65 (5C, C(2), C(3), C(4), C(5), C(6)), 26.41 (1C, C(1″)), 29.36 (1C, C(8)), 30.54 (1C, C(2″)), 31.18 (1C, C(7)), 40.64 (2C, C(3″), C(1)), 123.86/124.14 (2C, C(3′), C(4′)), 141.00/142.73 (2C, C(2′), C(5′)), 157.08/157.17 (2C, 2 × NH–(*C*=NH)–NH_2). Analysis: C, H, N for $C_{17}H_{34}Cl_2N_6S$.

7.4. The piperidine containing analogues **11a**, **11b**, and **12**

Compounds **11a** and **11b** were prepared from **8a** and **8b**, respectively, as described [11], via the protected intermediates **10a** and **10b** (Scheme 3); for the synthesis of **12**, an alternative route was chosen (described in detail Section 7.4.5). The

starting thiophene alkylamines **8a** [28], **8b**, and **8c** were synthesized from the respective carboxylic acids in two steps via the amides, as described above (Sections 7.2.2, 7.2.3, 7.2.4); **8b** and **8c** were used without characterization.

*7.4.1. 4-{5-[8-(Ethoxycarbonyl-methyl-amino)-octyl]-thieno-2-carbonyl}-piperidine-1-carboxylic acid ethyl ester (**10a**)*

Yield: 27% yellow liquid. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.22–1.34 (m, 14H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6), $2 \times CH_3CH_2COON$), 1.50–1.91 (m, 8H, 2H–C(2), 2H–C(7), 1H-C(3″), 1H-C(3″), 1H-C(5″), 1H-C(5″)), 2.86–3.28 (m, 9H, CH_2-(1), CH_2–C(8), 1H-C(2″), 1H-C(6″), N–CH_3), 3.39–3.51 (m, 1H, 1H-C(4″)), 4.09–4.18 (m, 6H, 1H-C(2″), 1H-C(6″), $2 \times CH_3CH_2COON$), 6.95 (d, $J = 3.8$ Hz, 1H, 1H-C(4′)), 7.81 (d, $J = 3.8$ Hz, 1H, 1H-C(3′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 14.65/14.72 (2C, $2 \times CH_3CH_2COON$), 26.55/28.90/29.06 (4C, C(3), C(4), C(5), C(6)), 27.62/27.85 (2C, C(3″), C(5″)), 28.56/31.26 (2C, C(2), C(7)), 30.63 (1C, C(1)), 34.25 (1C, N–CH_3), 43.25 (2C, C(2″), C(6″)), 44.57 (1C, C(4″)), 61.02/61.30 (2C, $2 \times CH_3CH_2COON$), 125.61 (1C, C(4′)), 132.06 (1C, C(3′)), 140.58 (1C, C(5′)), 155.45 (1C, 1C(2′)), 156.12 (1C, NCOOEt)), 156.56 (1C, NCOOEt), 194.42 (1C, C=O). Analysis: C, H, N for $C_{25}H_{40}N_2O_5S$.

*7.4.2. 4-[5-(8-Dimethylamino-octyl)-thieno-2-carbonyl]-piperidine-1-carboxylic acid ethyl ester (**10b**)*

Yield: 67% pale yellow solid, m.p.: 126–128 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.26 (t, $J = 7.1$ Hz, 3H, CH_3CH_2COON), 1.29–1.36 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.65–1.91 (m, 8H, CH_2-(2), CH_2-(7), 1H-C(3″), 1H-C(3″), 1H-C(5″), 1H-C(5″)), 2.71 (s, 6H, $2 \times$ N–CH_3), 2.80–2.91 (m, 6H, CH_2-(1), CH_2-(8), 1H-C(2″), 1H-C(6″)), 4.09–4.23 (m, 4H, 1H-C(2″), 1H-C(6″), CH_3CH_2COON), 6.82 (d, $J = 3.8$ Hz, 1H, 1H-C(4′)), 7.57 (d, $J = 3.8$ Hz, 1H, 1H-C(3′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 14.55 (1C, CH_3CH_2COON), 24.52/26.49/28.47/28.59/28.75/28.79/30.45/31.02 (9C, C(1), C(2), C(3), C(4), C(5), C(6), C(7), C(3″), C(5″)), 42.98/43.15 (4C, C(2″), C(6″), $2 \times$ N–CH_3), 44.42 (1C, C(4″)), 58.05 (1C, C(8)), 61.18 (1C, CH_3CH_2COON), 125.64 (1C, C(4′)), 132.05 (1C, C(3′)), 140.50 (1C, C(5′)), 155.33/155.86 (2C, C(2′), NCOOEt), 194.37 (1C, C=O). Analysis: C, H, N for $C_{23}H_{38}N_2O_3S$.

*7.4.3. Methyl-[8-(5-piperidin-4-ylmethyl-thien-2-yl)-octyl]-amine dihydrochloride (**11a**)*

Yield: 33% yellow solid, m.p.: 214–216 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.35–1.42 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.63–1.97 (m, 9H, CH_2-(2), CH_2-(7), CH_2-(3″), CH_2-(5″), 1H-C(4″)), 2.70 (s, 3H, N–CH_3), 2.73–2.79/2.94–3.02 (2m, 8H, CH_2-(1), CH_2-(8), CH_2-(2″), CH_2-(6″)), 3.39 (m, 2H, CH_2–C(4″)), 6.61–6.63 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.14/27.43/29.60/29.96/30.10/30.15/30.93/32.86/33.59/37.13/37.17 (12C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(3″), C(4″), C(5″), C(7″), N–CH_3), 45.20 (2C, C(2″), C(6″)), 50.44 (1C, C(1)), 124.89/126.42 (2C, C(3′), C(4′)), 140.12/145.19 (2C, C(2′), C(5′)). Molecular weight calculated for $C_{19}H_{34}N_2S$: 322.56, measured by MS (*m*/*z*): 323.4 (M + 1).

*7.4.4. Dimethyl-[8-(5-piperidin-4-ylmethyl-thien-2-yl)-octyl]-amine dihydrochloride (**11b**)*

Yield: 37% colorless solid, m.p.: 196–198 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.35–1.95 (m, 17H, CH_2-(2), CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6), CH_2-(7), CH_2-(3″), CH_2-(5″), 1H-C(4″)), 2.70–3.40 (m, 16H, CH_2-(1), CH_2-C(4″), CH_2-(8), CH_2-(2″), CH_2-(6″), $2 \times$ N–CH_3), 6.56–6.61 (m, 2H, 1H-C(3″), 1H-C(4″)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 25.61/27.42/29.59/29.94/30.08/30.16/30.93/32.85/37.14/37.17 (11C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(3″), C(4″), C(5″), CH_2–C(4″)), 43.40 (2C, $2 \times$ N–CH_3), 45.20 (2C, C(2″), C(6″)), 59.03 (1C, C(1)), 124.91/126.42 (2C, C(3′), C(4′)), 140.14/145.19 (2C, C(2′), C(5′)). Analysis: C, H, N for $C_{20}H_{38}N_2SCl_2 \cdot 0.5H_2O$.

*7.4.5. Synthetic route to the primary amine **12***

*7.4.5.1. 2,4,6-Trimethyl-N-(8-thien-2-yl-octyl)-benzenesulfonamide (**9c**).* The amino group of (8-thien-2-yl-octyl)-amine **8c** (Scheme 3) was sulphonamide-protected under inert atmosphere in absolute DCM. After addition of 1 eq. TEA and cooling to 0 °C, 1 eq. 2,4,6-trimethylbenzene sulfonyl chloride dissolved in DCM was slowly (20 min) added and the mixture stirred overnight at rt without cooling. After washing with aqueous $KHSO_4$ and drying, the product **9c** was purified by column chromatography. Yield: 56% colorless oil. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.18–1.33 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.36–1.45 (m, 2H, CH_2-(7)), 1.56–1.63 (m, 2H, CH_2-(2)) 2.29 (s, 3H, CH_3-*para*), 2.62 (s, 6H, $2 \times CH_3$-*ortho*), 2.76–2.88 (m, 4H, CH_2-(1), CH_2-(8)), 4.39 (m, 1H, NH)), 6.75 (m, 1H, 1H-C(3′)), 6.90 (m, 1H, 1H-C(4′)), 6.94 (s, 2H, $2 \times$ 1H-C_{meta}), 7.09 (m, 1H, 1H-C(5′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 20.87 (1C, CH_3–C_{para}), 22.91 (2C, $2 \times CH_3$–C_{ortho}), 26.48/28.86/29.06 (4C, C(3), C(4), C(5), C(6)), 29.47 (1C, C(7)), 29.82 (1C, C(8)), 31.64 (1C, C(2)), 42.53 (1C, C(1)), 122.70 (1C, C(5′)), 123.89 (1C, C(3′)). 126.61 (1C, C(4′)), 131.90 (2C, $2 \times C_{meta}$), 133.64/139.03/142.03 (4C, C_{ipso}, $2 \times C_{ortho}$, $2 \times C_{meta}$), 145.64 (1C, C(2′)). Analysis: C, H, N for $C_{21}H_{31}NO_2S_2$.

*7.4.5.2. 4-{5-[8-(2,4,6-Trimethyl-benzenesulfonylamino)-octyl]-thieno-2-carbonyl}-piperidine-1-carboxylic acid ethyl ester (**10c**).* Compound **9c** was (as described in [11]) subjected to Friedel–Crafts acylation by 4-chlorocarbonyl piperidine carboxylic acid ethyl ester to yield 58% **10c** as a dark oil. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.18–1.29 (m, 11H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6), CH_3CH_2COON), 1.42 (m, 2H, CH_2-(2)), 1.60–1.82 (m, 6H, CH_2-(7), 1H-C(3″), 1H-C(3″), 1H-C(5″), 1H-C(5″)), 2.28 (s, 3H, CH_3-*para*), 2.62 (s, 6H, $2 \times CH_3$-*ortho*), 2.77–2.89 (m, 6H, CH_2-(1), CH_2-(8), 1H-C(2″), 1H-C(6″)), 3.16–3.26 (m, 1H, 1H-C(4″), 4.09–4.25 (m, 4H, 1H-C(2″), 1H-C(6″), CH_3CH_2COON), 5.29 (s, 1H, NH), 6.81 (d, 1H, $J = 3.8$ Hz,

1H-C(4′)), 6.94 (s, 2H, 2 × 1H-C_{meta}), 7.56 (d, 1H, $J = 3.8$ Hz, 1H-C(3′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 14.65 (1C, CH_3CH_2COON), 20.86 (1C, CH_3–C_{para}), 22.89 (2C, 2 × CH_3–C_{ortho}), 26.46/28.56/28.79/28.82/28.99/29.49/30.60/31.20 (9C, C(1), C(2), C(3), C(4), C(5), C(6), C(7), C(3″), C(5″)), 42.49 (1C, C(4″)), 43.26 (2C, C(2″), C(6″)), 44.56 (1C, C(8)), 61.32 (1C, CH_3CH_2COON), 125.62 (1C, C(4′)), 131.88 (2C, 2 × C_{meta}), 132.06 (1C, C(3′)), 133.72 (1C, C_{ipso}), 139.00 (2C, 2 × C_{ortho}), 140.62 (1C, C_{para}), 142.02 (1C, C(5′)), 155.47/156.01 (2C, C(2′), CH_3CH_2COON), 194.43 (1C, C=O). Analysis: C, H, N for $C_{30}H_{44}N_2O_5S_2$.

*7.4.5.3. 2,4,6-Trimethyl-N-[8-(5-piperidin-4-ylmethyl-thien-2-yl)-octyl]-benzenesulfonamide (**11c**).* Compound **10c** was subjected to Wolff–Kishner reduction (as described in [11]) to yield 64% **11c** as a pale yellow solid, m.p.: 80–82 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.10–1.40 (m, 10H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6), CH_2-(7)), 1.45–1.72 (m, 6H, CH_2-(2), CH_2-(3″), CH_2-(5″)), 2.27 (s, 3H, CH_3-*para*), 2.59 (s, 6H, 2 × CH_3-*ortho*), 2.52–2.70 (m, 4H, 1H-C(2″), 1H-C(6″), CH_2–C(4″)), 2.81 (t, $J = 6.9$ Hz, 2H, CH_2-(8)), 2.96–3.01 (m, 2H, 1H-C(2″), 1H-C(6″)), 3.28–3.30 (m, 2H, CH_2-(1)), 6.53 (s, 2H, 1H-C(3′), 1H-C(4′)), 6.99 (s, 2H, 2 × 1H-C_{meta}). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 20.96 (1C, 1C, CH_3–C_{para}), 23.14 (2C, 2 × CH_3–C_{ortho}), 27.50/29.92/30.15/30.37/30.97/32.82/33.37/39.59 (10C, C(2), C(3), C(4), C(5), C(6), C(7), C(8), C(3″), C(5″), CH_2–C(4″)), 43.14 (1C, C(1)), 46.88 (2C, C(2″), C(6″)), 124.66/125.84 (2C, C(3′), C(4′)), 132.89 (2C, 2 × C_{meta}), 135.89 (1C, C_{ipso}), 140.18 (2C, 2 × C_{ortho}), 141.37/144.68 (2C, C(2′), C(5′)), 143.30 (1C, C_{para}). Analysis: C, H, N for $C_{27}H_{42}N_2O_2S_2$.

*7.4.5.4. 8-(5-Piperidin-4-ylmethyl-thien-2-yl)-octylamine dihydrochloride (**12**).* To remove the protecting group from **11c**, 6 eq. metallic sodium was added under inert atmosphere at −40 °C to 6 eq. naphthalene dissolved in DME. After the solution had turned green (60 min), 1 eq. of the sulphonamide **11c** (dissolved in DME) was slowly added (with preservation of the green color). After stirring without cooling for 2 h, excess naphthalide was hydrolysed and the mixture distributed between 2 N NaOH and AcOEt. The organic layer was dried and evaporated, the residue was precipitated as hydrochloride and crystallized from MeOH/DE. Yield: 46% colorless solid, m.p.: 198–201 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.34–1.44 (m, 10H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6), CH_2-(7)), 1.61–1.66 (m, 4H, CH_2-(2), 1H-C(3″), 1H-C(5″)), 1.79–1.89 (m, 3H, 1H-C(3″), 1H-C(4″) 1H-C(5″)), 2.70–2.76 (m, 4H, CH_2–C(4″), CH_2-(8)), 2.86–3.00 (m, 4H, CH_2-(1), 1H-C(2″), 1H-C(6″)), 3.33–3.39 (m, 2H, 1H-C(2″), 1H-C(6″)), 6.57–6.61 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.40/29.95/30.09/30.15 (4C, C(3), C(4), C(5), C(6)), 28.51/32.85 (2C, C(2), C(7)), 29.57 (2C, C(3″), C(5″)), 30.92/37.16 (2C, 1C(8), CH_2–C(4″)), 37.12 (1C, C(4″)), 40.76 (1C, C(1)), 45.18 (2C, C(2″), C(6″)), 124.88/126.40 (2C, C(3′), C(4′)), 140.13/145.18 (2C, C(2′), C(5′)). Analysis: C, H, N for $C_{18}H_{34}N_2SCl_2$.

7.5. Various aromatic diamines

7.5.1. Synthetic route to the benzylamine **16**

*7.5.1.1. 2-[5-(7-Methoxycarbonyl-heptyl)-thieno-2-carbonyl]-benzoic acid (**14**).* 8-(2-Thienyl)-octanoic acid methylester (**13**, Scheme 4), characterized earlier [11], was Friedel–Crafts acylated by phthalic acid anhydride as described [11]. Yield: 79% colorless solid, m.p.: 91–93 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.26–1.40 (m, 6H, CH_2-(3), CH_2-(4), CH_2-(5)), 1.59–1.74 (m, 4H, CH_2-(2), CH_2-(6)), 2.30 (t, $J = 7.5$ Hz, 2H, CH_2-(7)), 2.86 (t, $J = 7.6$ Hz, 2H, CH_2–C(1)), 3.65 (s, 3H, $COOCH_3$), 6.85 (d, $J = 3.5$ Hz, 1H, 1H-C(4′)), 7.26 (d, $J = 3.5$ Hz, 1H, 1H-C(3′)), 7.81–7.86 (m, 2H, 1H-C(3″), 1H-C(4″)), 8.18 (d, $J = 8.1$ Hz, 1H, 1H-C(2″)), 8.51 (d, $J = 7.5$ Hz, 1H, 1H-C(5″)), 10.42 (s, 1H, COOH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 24.33 (1C, C(6)), 28.23/28.37/29.18 (3C, C(3), C(4), C(5)), 30.94/33.17 (3C, C(1), C(2), C(7)), 51.05 (1C, $OCOCH_3$), 125.03/126.05/126.19/127.75/128.23 (5C, C(3′), C(4′), C(5′), C(3″), C(6″)), 131.68 (1C, C(1″)), 133.80/134.31 (2C, C(4″), C(5″)), 140.94/146.94 (2C, C(2′), C(2″)), 158.81/173.25 (3C, C=O, *C*OOH, *C*$OOCH_3$). Analysis: C, H, N for $C_{21}H_{24}O_5S \cdot 0.1H_2O$.

*7.5.1.2. 2-[5-(7-Methoxycarbonyl-heptyl)-thien-2-ylmethyl]-benzoic acid (**15**).* Compound **14** was subjected to Clemmensen reduction (A in Scheme 4). The amalgam was prepared by shaking 7.1 eq. Zn powder and 0.14 eq. $HgCl_2$ in aqueous HCl. After discarding the supernatant, the amalgam was stirred with 1 eq. of the ketone **14** in toluene/HCl_{conc} for 30 min at rt and refluxed for another 3 h. After cooling and dilution with 2 N HCl, the aqueous phase was extracted with AcOEt. After washing with brine and drying, the solvent was evaporated and the obtained product **15** purified by column chromatography. Yield: 66% colorless solid, m.p.: 60–62 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.29–1.34 (m, 6H, CH_2-(3), CH_2-(4), CH_2-(5)), 1.54–1.61 (m, 4H, CH_2-(2), CH_2-(6)), 2.28 (t, $J = 7.4$ Hz, 2H, CH_2-(7)), 2.69 (t, $J = 7.5$ Hz, 2H, CH_2-(1)), 3.63 (s, 3H, $COOCH_3$), 4.48 (s, 2H, CH_2–C(6″)), 6.52 (m, 2H, 1H-C(3′), 1H-C(4′)), 7.26–7.32 (m, 2H, 1H-C(3″), 1H-C(4″)), 7.41–7.47 (m, 1H, 1H-C(2″)), 7.87 (d, $J = 7.3$ Hz, 1H, 1H-C(5″)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 25.93 (1C, C(6)), 29.84/29.98/30.01 (3C, C(3), C(4), C(5)), 30.93/32.73/34.73/34.89 (4C, C(1), C(2), C(7), CH_2-C(6″)), 51.94 (1C, OCH_3), 124.50/125.71/127.49 (3C, C(3″), C(3′), C(4′)), 131.21 (1C, C(1″)), 131.86/132.17/133.08 (3C, C(2″), C(4″), C(5″)), 142.67/143.43/145.17 (3C, C(2′), C(5′), C(6″)), 170.91/176.01 (2C, *C*OO*H*, *C*$OOCH_3$). Analysis: C, H, N for $C_{21}H_{26}O_4S$.

*7.5.1.3. 8-[5-(2-Aminomethyl-benzyl)-thien-2-yl]-octylamine dihydrochloride (**16**).* The further steps to **16** were as described [11], and the intermediates are not described here. Yield: 45% (last step) brownish solid, m.p.: 178–182 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.34–1.40 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.58–1.70 (m, 4H, CH_2-(2), CH_2-(7)), 2.75 (t, $J = 7.4$ Hz, 2H, CH_2-(1)), 2.93 (t,

$J = 7.4$ Hz, 2H, CH_2-(8)), 4.18 (s, 2H, CH_2-C(1″)), 4.24 (s, 2H, CH_2-C(6″)), 6.54–6.60 (m, 2H, 1H-C(3′), 1H-C(4′)), 7.35–7.48 (m, 4H, 1H-C(2″),1H-C(3″), 1H-C(4″), 1H-C(5″)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.39/28.51/29.93/30.07/30.12/30.93 (6C, C(2), C(3), C(4), C(5), C(6), C(7)), 32.78 (1C, C(8)), 34.28 (1C, CH_2-C(6″)), 40.79/41.01 (2C, C(1), CH_2–C(1″)), 124.90/125.97/128.76/130.33/130.53/131.80 (6C, C(3′), C(4′), C(2″), C(3″), C(4″), C(5″)), 132.66 (1C, C(1″)), 140.56/141.61/145.92 (3C, C(2′), C(5′), C(6″)). Analysis: C, H, N for $C_{20}H_{32}N_2SCl_2 \cdot 0.6H_2O$.

*7.5.2. The synthetic route to the dithienylmethane derivative **20***

*7.5.2.1. 4-{5-[5-(3-Ethoxycarbonyl-propionyl)-thien-2-ylmethyl]-thien-2-yl}-4-oxo-butyric acid ethyl ester (**18**).* 2,2′-Dithienylmethane (**17**, Scheme 4 [14]) was Friedel–Crafts acylated with succinyl chloride monoethylester as described [11] to yield 32% **18** as a brownish solid, m.p.: 110–112 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.25 (t, $J = 7.1$ Hz, 6H, $2 \times CH_3CH_2OCO$), 2.72 (t, $J = 6.8$ Hz, 4H, CH_2-(2), CH_2-(3‴)), 3.19 (t, $J = 6.8$ Hz, 4H, CH_2-(3), CH_2-(2‴)), 4.14 (q, $J = 7.1$ Hz, 4H, $2 \times CH_3CH_2OCO$), 4.36 (s, 2H, CH_2-(α-thiophene)), 6.92 (d, $J = 3.8$ Hz, 2H, 1H-C(3″), 1H-C(4′)), 7.61 (d, $J = 3.8$ Hz, 2H, 1H-C(3), 1H-C(4″)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 14.13 (2C, $2 \times CH_3CH_2OCO$), 28.25 (2C, C(2), C(3‴)), 31.28 (1C, CH_2-α-thiophene), 33.55 (2C, C(3), C(2‴)), 126.95 (2C, C(3″), C(4′)), 132.16 (2C, C(3), C(4″)), 142.70 (2C, C(5′), C(2″)), 150.47 (2C, C(2′), C(5″)), 172.59 (2C, $2 \times COOEt$), 190.71 (2C, $2 \times C{=}O$). Analysis: C, H, N for $C_{21}H_{24}O_6S_2$.

*7.5.2.2. 4-{5-[5-(4-Aminobutyl)-thien-2-ylmethyl]-thien-2-yl}-butylamine dihydrochloride (**20**).* From **18** we obtained **19** ([29], Scheme 4) by Wolff–Kishner reduction as described [11]. The steps for the formation of **20** from **19** have been described as well [11], and the intermediates are not characterized here. Yield: 21% (last step) colorless solid, m.p.: not accessible (too hygroscopic). ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.66–1.80 (m, 8H, CH_2-(2), CH_2-(3), CH_2-(2‴), CH_2-(3‴)), 2.83/2.94 (2t, $J_1 = 6.5$ Hz, $J_2 = 6.7$ Hz, 8H, CH_2-(1), CH_2-(4), CH_2-(1‴), CH_2-(4‴)), 4.20 (s, 2H, CH_2-(α-thiophene)), 6.64–6.70 (m, 4H, 1H-C(3′), 1H-C(4′), 1H-C(3″), 1H-C(4″)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.89 (2C, C(3), C(2‴)), 29.51/30.39 (4C, C(2), C(4), C(1‴), C(3‴)), 31.34 (1C, CH_2-α-thiophene), 40.53 (2C, C(1), C(4‴)), 125.24/125.83 (4C, C(3′), C(4′), C(3″), C(4″)), 142.72/144.29 (4C, C(2′), C(5′), C(2″), 1C(5″)). Molecular weight calculated for $C_{17}H_{26}N_2S_2$: 322.54; measured by MS (*m*/*z*): 323.2 (M + 1).

*7.5.3. Synthetic route to **24**, the benzene analogue of **2***

*7.5.3.1. 8-[4-(3-Methoxycarbonyl-propyl)-phenyl]-8-oxo-octanoic acid ethyl ester (**22**).* 4-Phenylbutanoic acid methylester **21** (Scheme 4, commercially available) was Friedel–Crafts acylated [11] with suberic acid monoethylester chloride to yield **22** (54%) as a white solid, m.p.: 41–42 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.26 (t, $J = 7.1$ Hz, 3H, CH_3CH_2OCO), 1.38–1.40 (m, 4H, CH_2-(4), CH_2-(5)), 1.63–1.76 (m, 4H, CH_2-(3), CH_2-(6)), 1.98 (qn, $J = 7.5$ Hz, 2H, CH_2-(2″)), 2.28–2.37 (m, 4H, CH_2-(3″), CH_2-(2)), 2.71 (t, $J = 7.6$ Hz, 2H, CH_2-(1″)), 2.94 (t, $J = 7.3$ Hz, 2H, CH_2-(7)), 3.68 (s, 3H, CH_3OCO), 4.13 (q, $J = 7.1$ Hz, 2H, CH_3CH_2OCO), 7.27 (d, $J = 8.1$ Hz, 2H, 1H-C(3′), 1H-C(5′)), 7.89 (d, $J = 8.2$ Hz, 2H, 1H-C(2′), 1H-C(6′)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 14.20 (CH_3CH_2OCO), 24.16/24.77 (2C, C(3), C(6)), 26.04 (1C, C(2″)), 28.94 (2C, C(4), C(5)), 33.21/34.24 (2C, C(2), C(7)), 35.02 (1C, C(1″)), 38.35 (1C, C(3″)), 51.51 (CH_3OCO), 60.13 (CH_3CH_2OCO), 128.27/128.63 (4C, C(2′), C(3′), C(5′), C(6′)), 135.11 (1C, C(4′)), 146.88 (1C, C(1′)), 173.62/173.72 ($COOCH_3$, $COOCH_2CH_3$), 199.97 (C=O). Analysis: C, H, N for $C_{21}H_{30}O_5$.

*7.5.3.2. 8-[4-(3-Carboxy-propyl)-phenyl]-octanoic acid (**23**).* After saponification of **22** in MeOH/H_2O (50/1) with 1.5 eq. LiOH, **23** was obtained by deoxygenation, stirring 1 eq. of the free acid (m.p. 118–120 °C) for 15 h at 55–60 °C with 3 eq. Et_3SiH and 5 eq. TFA. After addition of H_2O and acidification with $KHSO_4$, **23** was extracted with AcOEt. After drying, the solvent was evaporated and the residue purified by chromatography. Yield: 76%, white solid, m.p.: 121–122 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.28–1.32 (m, 6H, CH_2-(4), CH_2-(5), CH_2-(6)), 1.54–1.60 (4H, CH_2-(3), CH_2-(7)), 1.86 (qn, $J = 7.5$ Hz, 2H, CH_2-(2″)), 2.23–2.29 (m, 4H, CH_2-(3″), CH_2-(2)), 2.52–2.61 (m, 4H, CH_2-(1″), CH_2-(8)), 7.07 (s, 4H, arom-H). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 26.04/32.68 (2C, C(3), C(7)), 27.96 (1C, C(2″)), 30.14/30.22 (3C, C(4), C(5), C(6)), 34.23/34.92 (2C, C(3″), C(2)), 35.68/36.47 (2C, C(1″), C(8)), 129.36/129.41 (4C, C(2′), C(3′), C(5′), C(6′)), 140.05/141.50 (2C, C(1′), C(4′)), 177.44/177.71 (2C, $2 \times COOH$). Analysis: C, H, N for $C_{18}H_{24}O_5$.

*7.5.3.3. 8-[4-(4-Aminobutyl)-phenyl]-octylamine dihydrochloride (**24**).* The steps for the formation of diamine **24** from **23** were as described [11], and the intermediates are not characterized here. Yield: 47% (last step) pale greenish powder, m.p. ≥300 °C (decomposition). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.35 (s, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.58–1.68 (m, 8H, CH_2-(2″), CH_2-(3″), CH_2-(2), CH_2-(7)), 2.52–2.65 (m, 4H, CH_2-(1″), CH_2-(8)), 2.87–2.91 (m, 4H, CH_2-(4″), CH_2-(1)), 7.05–7.11 (m, 4H, arom-H). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.42/30.14/30.18/30.32 (4C, C(3), C(4), C(5), C(6)), 28.07/28.52/29.32/32.74 (4C, C(2″), C(3″), C(2), C(7)), 35.77/36.45 (2C, C(1″), C(8)), 40.66/40.76 (2C, C(4″), C(1)), 129.35/129.42 (4C, C(2′), C(3′), C(5′), C(6′)), 140.04/141.52 (2C, C(1′), C(4′)). Analysis: C, H, N for $C_{18}H_{34}N_2Cl_2$.

*7.6. The synthetic route to the alkyne diamine **28***

*7.6.1. 2-{[14-Tetrahydro-2H-2-pyranyloxy)-5-tetradecinyl]-oxy}tetrahydro-2H-pyran (**26**)*

At −78 °C under inert atmosphere, 1 eq. of *n*-butyllithium (1.6 M in THF) was added slowly to a solution of 1 eq. of the

THP-protected 2,5-hexinol **25** [17] in absolute THF. The mixture was allowed to warm to 0 °C and was cooled again to −78 °C. The addition of 1 eq. of hexamethyl phosphoric acid triamide (in absolute THF) was followed slowly (30 min) by 1 eq. of THP-protected 2,8-bromo-octanol (in absolute THF). The mixture was stirred at rt overnight and slowly hydrolysed at 0 °C. The organic layer was isolated, dried and evaporated. Yield: 54% colorless liquid. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.31−1.74 (m, 28H, CH_2-(3′), CH_2-(4′), CH_2-(5′), CH_2-(2), CH_2-(3), CH_2-(8), CH_2-(9), CH_2-(10), CH_2-(11), CH_2-(12), CH_2-(13), CH_2-(3″), CH_2-(4″), CH_2-(5″)), 2.10−2.18 (m, 4H, CH_2-(4), CH_2-(7)), 3.36−3.39/3.42−3.51/3.71−3.76/3.86−3.89 (4m, 8H, CH_2-(6′), CH_2-(1), CH_2-(14), CH_2-(6″)), 4.57 (s, 2H, 1H-C(2′), 1H-C(2″)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 18.58/18.71/19.60/19.67 (4C, C(4′), C(4), C(7), C(4″)), 25.48/25.91/26.18/28.79/28.90/29.08/29.34/29.71/30.72/30.76 (12C, C(3′), C(5′), C(2), C(3), C(8), C(9), C(10), C(11), C(12), C(13), C(3″), C(5″)), 62.24/62.29 (2C, C(6′), C(6)), 67.04 (1C, C(14)), 67.63 (1C, C(1)), 79.80/80.45 (2C, C(5), C(6)), 98.75/98.81 (2C, C(2′), C(2″)). Analysis: C, H, N for $C_{24}H_{42}O_4$.

7.6.2. 1,14-Diazido-tetradec-5-yne (27)

The THP-protected alkynediol **26** was deprotected by stirring in MeOH with a catalytic amount of 0.005 eq. toluene-4-sulfonic acid at rt. After adding 0.84 eq. K_2CO_3 and stirring for 1 h at rt, the precipitate was removed by filtration, the solvent evaporated and the alkynediol purified by column chromatography. The diol was characterized by NMR analysis (not shown) and converted via the dimesylate to the diazide **27** (Scheme 5): to a solution of 1 eq. of the diol in absolute DCM, 2 eq. of TEA and 2.2 eq. methane sulfonic acid chloride were added at 0 °C within 5 min. The mixture was stirred for 15 min at 0 °C, and overnight at rt. After hydrolysis, the organic layer was isolated, dried, and evaporated. The obtained dimesylate was characterized by NMR analysis (not shown), dissolved in MeOH, and mixed with 2.6 eq. sodium azide (in MeOH). After stirring overnight at rt, the solvent was evaporated, the residue was dispersed in water and extracted with DE. The organic layer was dried and evaporated. Yield (last step): 66% yellow liquid. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.32−1.71 (m, 14H, CH_2-(2), CH_2-(3), CH_2-(8), CH_2-(9), CH_2-(10), CH_2-(11), CH_2-(12), CH_2-(13)), 2.11−2.22 (m, 4H, CH_2-(4), CH_2-(7)), 3.23−3.31 (m, 4H, CH_2-(1), CH_2-(14)). ^{13}C NMR (50 MHz; $CDCl_3$-d_1) δ 18.49/18.63 (2C, C(4), C(7)), 26.08 (1C, C(3)), 26.65 (1C, C(12)), 27.91/28.71/28.80/28.97/29.02 (6C, C(2), C(8), C(9), C(10), C(11), C(13)), 51.02/51.44 (2C, C(1), C(14)), 79.20/80.92 (2C, C(5), C(6)). Analysis: C, H, N for $C_{14}H_{24}N_6$.

7.6.3. Tetradec-5-yne-1,14-diamine dihydrochloride (28)

The alkyne diazide **27** was dissolved in AcCN and slowly dropped into a solution of 3 eq. $SnCl_2$, 12 eq. thiophenol and 9 eq. TEA in AcCN. After stirring for 1 h at rt, 2 N NaOH was added and the diamine extracted into DCM. The organic layer was dried and evaporated. The obtained alkyne diamine **28** was dissolved in HCl-saturated MeOH and precipitated as the hydrochloride by adding DE. The residue was finally vacuum-dried. Yield: 83% white powder, m.p.: 146−148 °C. ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.27−1.69 (m, 16H, CH_2-(2), CH_2-(3), CH_2-(8), CH_2-(9), CH_2-(10), CH_2-(11), CH_2-(12), CH_2-(13)), 2.11−2.14 (m, 4H, CH_2-(4), CH_2-(7)), 2.70−2.77 (m, 4H, CH_1-(1), CH_2-(14)), 8.18 (s, 6H, $2 \times NH_3$). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 18.95/19.35 (2C, C(4), C(7)), 26.99/27.41 (2C, C(3),C(12)), 27.67/28.46/29.77/30.08/30.13 (6C, C(2), C(8), C(9), C(10), C(11), C(13)), 40.39/40.74 (2C, C(1), C(14)), 79.87/81.66 (2C, C(5), C(6)). Analysis: C, H, N for $C_{14}H_{30}Cl_2N_2$.

7.7. The synthetic route to the diether 32

7.7.1. 2-(2-Thien-2-yl-ethoxy)-ethanol (30)

Under inert atmosphere at 0 °C in dry DMF, 2-(2-thienyl) ethanol **29** (Scheme 6) [18] was mixed with 1.5 eq. NaH and stirred for 1.5 h at rt. THP-protected bromo-ethanol (1.1 eq. in dry DMF) was added slowly, keeping the mixture at rt. After stirring overnight at 30−40 °C and hydrolysis at 0 °C, solvent was evaporated and the residue purified by column chromatography. Deprotection in MeOH with a catalytic amount of toluene-4-sulfonic acid resulted in the alcohol **30**. Yield: 70% colorless liquid. ^{1}H NMR (200 MHz; DMSO-d_6) δ 2.30 (t, $J = 6.70$ Hz, 2H, CH_2-(2)), 3.42−3.50 (m, 4H, CH_2-(1), CH_2-(2′)), 3.60 (t, $J = 6.68$ Hz, 2H, CH_2-(1′)), 4.58 (t, $J = 5.18$ Hz, 1H, OH), 6.87−6.94 (m, 2H, 1H-C(3″), 1H-C(4″)), 7.28/7.30 (q, $J = 1.28/1.26$ Hz, 1H, 1H-C(5″)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 29.77 (1C, C(2)), 60.18 (1C, C(1′)), 70.99 (1C, C(1′)), 72.05 (1C, C(2′)), 123.94 (1C, C(5″)), 125.25 (1C, C(3″)), 126.72 (1C, C(4″)), 141.14 (1C, C(2″)). Analysis: C, H, N for $C_8H_{12}O_2S$.

7.7.2. [2-(2-Thien-2-yl-ethoxy)-ethoxy]-acetic acid (31)

The alcohol **30** was dissolved in dry THF and added slowly (1 h) at rt to 7 eq. NaH (dissolved in dry THF) under inert atmosphere. After stirring for one more hour at rt, 2 eq. 2-bromo-acetic acid (dissolved in dry THF) were added slowly (4 h) and the mixture refluxed overnight. Excess NaH was destroyed by addition of water. After evaporation, the residue was dissolved in water and excess alcohol extracted with DE. The aqueous layer was acidified with HCl and extracted with AcOEt. The organic layer was washed, dried, and evaporated. Yield: 80% colorless liquid. ^{1}H NMR (200 MHz; DMSO-d_6) δ 3.01 (t, $J = 6.56$, 2H, CH_2-(2)), 3.56−3.65 (m, 6H, CH_2-(1), −O−CH_2−CH_2−O−), 4.02 (s, 2H, −OCH_2COOH), 6.89−6.95 (m, 2H, 1H-C(4′), 1H-C(3′)), 7.29/7.32 (q, $J = 1.26/1.38$ Hz, 1H-C(5′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 29.33 (1C, C(2)), 67.19 (1C, −O−CH_2−CH_2−O), 69.11 (1C, −O−CH_2−CH_2−O−), 69.39 (1C, C(1)), 70.57 (1C, −OCH_2COOH), 123.57 (1C, C(5′)), 124.87 (1C, C(3′)), 126.33 (1C, C(4′)), 140.69 (1C, C(2′)), 171.26 (COOH). Analysis: C, H, N for $C_{10}H_{14}O_4S \cdot 0.25H_2O$.

7.7.3. 4-(5-{2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-thien-2-yl)-butylamine dihydrochloride (**32**)

The carboxylic acid **31** was further processed to the diamine **32**. In short, the methylester was Friedel–Crafts acylated with succinic anhydride [11], the obtained ketone deoxygenated with Et_3SiH as described for the reduction of **22** to **23** (see Section 7.5.3.2), and the final diamine **32** prepared as the dihydrochloride via the dimethylester and the diamide [11] (intermediates characterized by NMR analysis, not shown). Yield: 52% (last step), colorless oil. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.61 (m, 4H, CH_2-(3‴), CH_2-(2‴)), 2.67–2.82 (m, 4H, CH_2-(4‴), CH_2-(1‴)), 2.91–2.96 (m, 4H, CH_2-(1), $-O-CH_2-CH_2-NH_3$)), 3.56–3.62 (m, 4H, $-O-CH_2-CH_2-O-$), 3.64–3.74 (m, 4H, CH_2-(2), $-O-CH_2-CH_2-NH_3$), 6.64/6.68 (d, $J_1 = 4.45$ Hz, $J_2 = 5.70$ Hz, 2H, 1H-C(3′), 1H-C(4′)), 8.12 (s, 6H, $2 \times NH_3$). ^{13}C NMR (125 MHz; DMSO-d_6) δ 26.38 (1C, C(3‴)), 28.00 (1C, C(4‴)), 28.82 (1C, C(1)), 29.94 (1C, C(2‴)), 38.40 (2C, $-O-CH_2-CH_2-NH_3$, C(1‴)), 66.56 (1C, C(2)), 69.35 (1C, $-O-CH_2-CH_2-O$), 69.58 (1C, $-O-CH_2-CH_2-O$), 70.97 (1C, $-O-CH_2-CH_2-NH_3$), 123.97 (1C, C(3′)), 124.84 (arom. CH-(4′)), 138.66 (1C, C(5′)), 142.33 (1C, C(2′)). Molecular weight calculated for $C_{14}H_{26}N_2O_2S$: 286.44; measured by MS (*m/z*): 287.18 (M + H).

7.8. Peptidic and polyaminic derivatives

The mono-Boc-protected diamines **34a** and **34b** used as starting material for the synthesis of **35a** and **35b**, respectively (Scheme 7), were prepared via different routes: **34a** was derived from 4-[5-(4-aminobutyl)-thien-2-yl]-butylamine [11], **34b** in a nine-step process from 2-(2-thienyl)ethanol **29** [18], that will be outlined only briefly here (Section 7.8.4), since most reactions have been described elsewhere.

7.8.1. {4-[5-(4-Aminobutyl)-thiophen-2-yl]-butyl}-carbamic acid tert-butyl ester (**34a**)

A solution of Boc_2O in dioxane was added slowly (20 min) to a solution of the symmetric diamine 4-[5-(4-aminobutyl)-thien-2-yl]-butylamine [11] in 2 N NaOH/dioxane. The mixture was stirred overnight at rt, the solvent evaporated, and the residue distributed between saturated aqueous $NaHCO_3$ and AcOEt. The organic layer was washed with brine and dried, and the solvent evaporated. The residue was purified by chromatography. Yield: 23% (for two steps from the diamide) yellow solid, m.p.: 74–77 °C. ^{1}H NMR (300 MHz; $CDCl_3$-d_1) δ 1.45 (s, 9H, $((CH_3)_3CO)$), 1.48–1.73 (m, 8H, CH_2-(2), CH_2-(3), CH_2-(2″), CH_2-(3″)), 2.71–2.80 (m, 6H, CH_2-(4), CH_2-(1″), CH_2-(4″)), 3.13–3.18 (m, 2H, CH_2-(1)), 4.56 (bs, 1H, OCONH), 6.56 (s, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 28.40 (3C, $(CH_3)_3CO$), 28.79/28.91 (2C, C(3), C(3″)), 29.46 (1C, C(2)), 29.71/29.90 (2C, C(4), C(4″)), 32.74 (1C, C(2″)), 40.32 (1C, C(1)), 41.76 (1C, C(1″)), 79.10 (1C, $(CH_3)_3CO$), 123.57/123.64 (2C, C(3′), C(4′)), 142.60/142.89 (2C, C(2′), C(5′)), 155.97 (1C, OCON–). Molecular weight calculated for $C_{17}H_{30}N_2O_2S$: 326.51; measured by MS (*m/z*): 327.0 (M + H).

7.8.2. 3-Amino-N-{4-[5-(4-aminobutyl)-thien-2-yl]-butyl}-propionamide dihydrochloride (**35a**)

N-Boc-protected β-alanine was mixed in absolute DCM with 1.5 eq. 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent [12]) and 2 eq. TEA. The mono-Boc-protected diamine **34a** dissolved in DCM was added slowly. The mixture was refluxed until the reaction was complete. After evaporation, the residue was dissolved in AcOEt and washed twice with 1 N HCl and 1 N NaOH. After drying, evaporation, and purification by chromatography, the Boc-groups were removed in DCM with 20 eq. TFA at rt (ca. 2 h). The solution was washed with 2 N NaOH, dried, and evaporated. The resulting diamine **35a** was isolated as the hydrochloride from methanolic HCl. Yield: 87% colorless solid, m.p.: 253–256 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.55/1.74 (m, 8H, CH_2–C(2), CH_2-(3), CH_2-(2″), CH_2-(3″)), 2.61 (t, $J = 6.6$ Hz, 2H, $-COCH_2CH_2NH_3$), 2.76–2.83 (m, 4H, CH_2-(4), CH_2-(1″)), 2.94 (m, 2H, CH_2-(1)), 3.16–3.24 (m, 4H, CH_2-(4″), $-COCH_2CH_2NH_3$), 6.60–6.63 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 27.87/29.54/29.65/30.35/30.56/32.85 (7C, $-COCH_2CH_2NH_3$, C(2), C(3), C(4), C(2″), C(3″), C(4″)), 37.42 (1C, C(4″)), 40.09/40.56 (2C, C(1), C(1″)), 125.00/125.22 (2C, C(3′), C(4′)), 143.06/144.08 (2C, C(2′), C(5′)), 172.01 (1C, CONH). Analysis: C, H, N for $C_{15}H_{29}N_3OSCl_2 \cdot 1.25H_2O$.

7.8.3. N^1-{4-[5-(4-Aminobutyl)-thien-2-yl]-butyl}-propane-1,3-diamine trihydrochloride (**36a**)

Under inert atmosphere, 10 eq. $LiAlH_4$ were dissolved in absolute THF, followed by the amide **35a** (in THF). The mixture was refluxed to the exhaustion of the reactant (15–30 h), cooled to 0 °C, and hydrolysed by adding aqueous tartrate solution. After volume reduction, DCM was added and precipitate removed by filtration over celite. The organic layer was washed, dried and evaporated. The hydrochloride of the triamine **36a** was obtained from methanolic HCl and purified by crystallization and/or chromatography. Yield: 59%, colorless solid, m.p. ≥230 °C. ^{1}H NMR (300 MHz; CD_3OD-d_4) δ 1.69–1.80 (m, 8H, CH_2–C(2), CH_2-(3), CH_2-(2″), CH_2-(3″)), 2.08–2.19 (m, 2H, $-NHCH_2CH_2CH_2NH_2$), 2.78–3.22 (m, 12H, $-NHCH_2CH_2CH_2NH_2$, CH_2-(1), CH_2-(4), CH_2-(4″), CH_2-(1″)), 6.64 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 25.28/26.55/27.83/29.53/29.63/30.33 (7C, $-NHCH_2CH_2CH_2NH_2$, C(2), C(3), C(4), C(1″), C(2″), C(3″)), 37.93/40.55/45.90 (4C, $-NHCH_2CH_2CH_2NH_2$, C(1), C(4″)), 125.33/125.37 (2C, C(3′), C(4′)), 143.35 (2C, C(2′), C(5′)). Molecular weight calculated for $C_{15}H_{29}N_3S$: 283.48; measured by MS (*m/z*): 284.3 (M + H).

7.8.4. {4-[5-(2-Hydroxyethyl)-thien-2-yl]-butyl}carbamic acid tert-butyl ester (**33**)

This intermediate was prepared as the precursor for the asymmetric, short diamine **34b** and was obtained from the acetic acid ester of 2-(2-thienyl)ethanol **29** (providing the ethyl arm) and succinic anhydride (providing the butyl

arm) as described [11]. Deoxygenation of the butyl arm keto group was achieved with Et_3SiH as described for the reduction of **22** to **23** (see Section 7.5.3.2). The carboxyl was converted into an amide group (by oxalyl dichloride/NH_3 [11]), and the amide group in turn into an amino group (by BH_3 [30]); the latter was Boc-protected (as described above in Section 7.8.1) to yield 39% (last two steps) **33** as an oil (solid at <4 °C). ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.35–1.57 (m, 13H, Boc–CH_3, CH_2-(2), CH_2-(3)), 2.68 (t, $J = 6.94$ Hz, 2H, CH_2-(4)), 2.79–2.95 (m, 4H, CH_2-(1), CH_2-(1″)), 3.55 (q, $J = 6.31$ Hz, 2H, $-CH_2O$ (2″)), 4.73 (t, $J = 5.30$ Hz, 1H, –OH), 6.59/6.62 (2d, $J = 3.40/3.40$ Hz, 2H, arom. CH-(4′), arom. CH-(3′)), 6.78 (s, 1H, –NHCO). ^{13}C NMR (50 MHz; DMSO-d_6) δ 28.26 (Boc–CH_3), 33.32 (CH_2-(1″)), 62.03 ($-CH_2OH$ (2″)), 77.32 ($-OCCH_3$), 123.69 (arom. CH-(3′)), 124.52 (arom. CH-(4′)), 138.99 (arom. C(5′)), 142.64 (arom. C(2′)), 155.58 (–NHCO). Analysis: C, H, N for $C_{15}H_{25}NO_3S \cdot 0.1H_2O$; Molecular weight calculated 299.44; measured by MS (*m/z*): 322.91 (M + Na), 339.04 (M + K).

7.8.5. {4-[5-(2-Amino-ethyl)-thiophen-2-yl]-butyl}-carbamic acid tert-butyl ester (**34b**)

The OH group of the 2-hydroxyethyl substituent of **33** was transformed into an amino group via –I and $-N_3$ [31] as follows: 2 eq. Ph_3P, 2 eq. imidazole, and 2 eq. I_2 were dissolved under argon in water-free DCM. After formation of a white precipitate, **33** was slowly added at rt and stirred overnight. The solvent was evaporated, the obtained iodide purified by column chromatography (yield: 64%) and characterized by NMR (not shown). For conversion to the azide, the iodide was mixed in water-free acetone with 2 eq. NaN_3 and refluxed for 48 h. After removal of the solvent, the residue was distributed between EtOAc and water. From the dried organic layer the crude azide was obtained as yellow oil and immediately converted to the amine by adding 1.65 eq. Ph_3P in THF, followed by 2.5 eq. water. After stirring for 50 h at rt, solvents were evaporated and the residue purified by flash chromatography (DCM/MeOH/TEA, 30/1/1). Compound **34b** was finally obtained as a yellow oil (yield 69%). ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.35–1.53 (m, 13H, ((CH_3)$_3$CO)), CH_2-(2), CH_2-(3)), 2.65–2.75 (m, 4H, CH_2-(1), CH_2-(4)), 2.85–2.96 (m, 4H, CH_2-(1″), CH_2-(2″)), 6.60 (m, 2H, 1H-C(4′), 1H-C(3′)), 6.80 (s, 2H, NH_2). ^{13}C NMR (50 MHz; DMSO-d_6) δ 28.25 (3C, (CH_3)$_3$CO)), 28.50 (1C, C(3)), 28.96 (1C, C(4)), 29.03 (2C, C(2), C(1″)), 31.18 (1C, C(1)), 43.52 (1C, C(2″)), 77.31 (1C, (CH_3)$_3CO$)), 123.82 (1C, C(4′)), 124.33 (1C, C(3′)), 139.92 (1C, C(5′)), 142.59 (1C, C(2′)), 155.58 (1C, –OCON–). Molecular weight calculated for $C_{15}H_{24}N_2O_2S$: 296.45; measured by MS (*m/z*): 297.44 (M + H).

7.8.6. 2-Amino-N-({2-[5-(4-aminobutyl)-thien-2-yl]-ethyl-carbamoyl}-methyl)-acetamide trifluoroacetate (**35b**)

One equivalent of Boc-protected glycine–glycine was first stirred for 10 min in DMF with 1 eq. of each HOBt and DIC, before being added to the mono-Boc-protected diamine **34b** (10% in DMF) and stirred for 2 h at rt. The solvent was evaporated and the residue purified by column chromatography. Deprotection was achieved in TFA containing 5% water (for no particular reason slightly different from deprotection of **34a** described above), to yield the TFA salt **35b** quantitatively as a brown oil. ^{1}H NMR (200 MHz; CD_3OD-d_4) δ 1.40–1.44 (m, 4H, CH_2-(2″), CH_2-(3″)), 2.53 (m, 2H, CH_2-(1″)), 2.64 (m, 4H, CH_2-(4″), CH_2-(2)), 3.13 (t, $J = 7.20$ Hz, 2H, CH_2-(1)), 3.46 (s, 2H, $-COCH_2NH_3$), 3.61 (s, 2H, $-COCH_2NH-$), 6.35 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; CD_3OD-d_4) δ 27.87 (1C, C(3″)), 29.49 (1C, C(4″)), 30.31 (1C, C(2)), 30.75 (1C, C(2″)), 40.50 (1C, C(4″)), 41.53 (1C, C(1)), 42.22 (1C, $-COCH_2NH_3$), 43.25 (1C, $-COCH_2NH-$), 125.49 (1C, C(4′)), 126.05 (1C, C(3′)), 140.40 (1C, C(2′)), 143.90 (1C, C(5′)), 167.99/171.19 (2C, 2 × C=O). Molecular weight calculated for $C_{14}H_{24}N_4O_2S$: 312.44; measured by MS (*m/z*): 313.19 (M + H).

7.8.7. 4-(5-{2-[2-(2-Amino-ethylamino)-ethylamino]-ethyl}-thien-2-yl)-butylamine tetratrifluoroacetate (**36b**)

Compound **36b** was obtained from **35b** as described above (Section 7.8.3). For purification, the obtained raw tetraamine was dissolved in dioxane/2 N NaOH (2/1) and reacted with 6 eq. Boc_2O (overnight at rt). After removal of dioxane, the residue was partitioned between water and AcOEt. The organic layer was dried and evaporated, and the residue purified by column chromatography. Deprotection in 95% TFA for 2 h resulted in the tetraamine **36b**. Yield: quantitative as an orange oil. ^{1}H NMR (200 MHz; CD_3OD-d_4) δ 1.57–1.60 (m, 4H, CH_2-(2″), CH_2-(3″)), 2.74–2.86 (m, 6H, CH_2-(4″), CH_2-(1″), CH_2-(2)), 3.06–3.33 (m, 10H, CH_2-(1), $-NHCH_2CH_2NH-$, $-NHCH_2CH_2NH_3^+$), 6.65–6.76 (m, 2H, 1H-C(3′), 1H-C(4′)). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.08 (1C, C(3″)), 26.47 (1C, C(4″)), 27.90 (1C, C(1)), 28.79 (1C, C(2″)), 35.13 (1C, C(1″)), 38.54 (1C, $-CH_2NH_3^+$), 42.63/42.75 (2C, $-NHCH_2CH_2NH-$, $-NHCH_2CH_2NH-$), 44.12 (1C, C(2)), 47.81 (1C, $-CH_2CH_2NH_3^+$), 116.43 ($-CF_3$, $J = 292.94$ Hz), 124.52 (1C, C(4′)), 125.69 (1C, C(3′)), 136.03 (1C, C(5′)), 143.31 (1C, C(2′)), 158.77 ($-COCF_3$, $J = 33.91$ Hz). Molecular weight calculated for $C_{14}H_{28}N_4S$: 284.47; measured by MS (*m/z*): 285.22 (M + H).

7.9. Compounds obtained by solid phase synthesis

All solid phase synthesis steps were carried out in cellpor filter fitted polypropylene syringes and in silylated glass vessels, using 4-[5-(4-aminobutyl)-thien-2-yl]-butylamine [11] as the first building block. Since the amounts obtained from solid phase synthesis were insufficient for NMR analysis, the identity of the end products was verified by CE and by MS analysis.

7.9.1. [3-(4-[5-(4-Ammoniobutyl)-2-thienyl]butylamino)-1-methyl-3-oxopropyl]ammonium ditrifluoroacetate (**38a**)

4-[5-(4-Aminobutyl)-thien-2-yl]-butylamine (5 eq.) was coupled in DCM to trityl chloride resin over 14 h. After three

washing cycles with DCM/MeOH/DIPEA, Fmoc-protected 3-amino-3-methylpropionic acid [32] was coupled to the free amine function (3 h) in a mixture of DIC (3 eq.) and HOBt (3 eq.) in DMF. After Fmoc-deprotection with a solution of 40% piperidine in DMF over two cycles for 20 min, the resin was prepared for product release by washing three times with each of the following solvents: DMF, THF, DCM, MeOH, DE. Product-splitting in 5% TFA in DCM for 3 h yielded 88% **38a** as a brown oil. Molecular weight calculated for $C_{16}H_{29}N_3O_2S$: 311.49; measured by MS (*m/z*): 312.3 (M + H).

7.9.2. [3-(4-[5-(4-Ammoniobutyl)-2-thienyl]butylamino)-2-methyl-3-oxopropyl]ammonium ditrifluoroacetate (**38b**)

Same procedure as for **38a**. Fmoc-protected 3-amino-2-methylpropionic acid was used as amino acid component. Yield: 75% as a brown oil. Molecular weight calculated for $C_{16}H_{29}N_3O_2S$: 311.49; measured by MS (*m/z*): 312.3 (M + H).

7.9.3. [3-(4-[5-(4-Ammoniobutyl)-2-thienyl]butylamino)-3-oxo-1-phenylpropyl]ammonium ditrifluoroacetate (**38c**)

Same procedure as for **38a**. Fmoc-protected 3-amino-3-phenylpropionic acid was used as amino acid component. Yield: 73% as a brown oil. Molecular weight calculated for $C_{21}H_{31}N_3OS$: 373.56; measured by MS (*m/z*): 374.3 (M + H).

7.9.4. [3-(4-[5-(4-Ammoniobutyl)-2-thienyl]butylamino)-3-oxo-2-phenylpropyl]ammonium ditrifluoroacetate (**38d**)

Same procedure as for **38a**. Fmoc-protected 3-amino-2-phenylpropionic acid [33] was used as amino acid component. Yield: 78% as a brown oil. Molecular weight calculated for $C_{21}H_{31}N_3OS$: 373.56; measured by MS (*m/z*): 374.3 (M + H).

7.9.5. (1*S*)-3-[4-[5-(4-Ammoniobutyl)-2-thienyl]butyl(benzyl)amino]-1-benzyl-3-oxopropylammonium ditrifluoroacetate (**39**)

4-[5-(4-Aminobutyl)-thien-2-yl]-butylamine [15] (5 eq.) was coupled in DMF to 4-nitrophenylcarbonate-activated Wang® resin over 12 h. After three washing cycles with DMF/DCM/DMF, reductive amination [34] was achieved by addition of benzaldehyde (3 eq.) and $NaCNBH_3$ in DMF/AcOH (99/1) over two cycles (2 × 18 h) at 25 °C. After repeated washing cycles (DMF, THF, DCM and DMF), Fmoc-protected (*S*)-3-amino-4-phenylbutyric acid was coupled for 3 h in a solution of DIC (3 eq.), HOBt (3 eq.) and DIPEA (10 eq.) in DMF. After Fmoc-deprotection in 40% piperidine in DMF (2 × 20 min), the resin was prepared for product release by washing three times with each of the following solvents: DMF, THF, DCM, MeOH, DE. Product-splitting in TFA/DCM (1/1) for 3 h yielded 42% **39** as a brown oil. Molecular weight calculated for $C_{29}H_{39}N_3OS$: 477.71; measured by MS (*m/z*): 478.2 (M + H).

7.9.6. (1*S*)-3-[4-[5-(4-Ammoniobutyl)-2-thienyl]butyl(benzyl)amino]-1-benzylpropylammonium ditrifluoroacetate (**40**)

Procedure as described for **39**. One step before product release, the amide function was reduced [30] by addition of 1 M BH_3 in THF (50 eq.) at 50 °C (two cycles over a period of 18 h). Borane complexes were removed by addition of a mixture of 1,8-diazabicyclo[5.4.0]undec-7-en (0.6 mol) in MeOH/*N*-methylpyrrolidinone (10/90). Product release as described for **39**. Yield: 65% as a brown oil. Molecular weight calculated for $C_{29}H_{41}N_3S$: 463.72; measured by MS (*m/z*): 464.3 (M + H).

7.10. Diaminododecane (**1**) with amide insertion

7.10.1. [8-(3-Boc-amino-propionylamino)-octyl]-carbamic acid tert-butyl ester (**41**)

Mono-Boc-protected 1,8-diaminooctane [21] was coupled with Boc-protected β-alanine as described for **4f–h** (7.2.3). Yield: 71% colorless solid, m.p.: 113–115 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.14–1.21 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.21–1.35 (m, 22H, CH_2-(2), CH_2-(4), 4 × (OC(CH_3)$_3$)), 2.17 (t, J = 7.2 Hz, 2H, NHCO$CH_2$$CH_2$NH), 2.83–2.89 (m, 2H, CH_2-(1)), 2.95–3.01 (m, 2H, CH_2-(8)), 3.04–3.11 (m, 2H, NHCOCH$_2$CH_2NH), 6.69–6.73 (m, 1H, NHCOCH$_2$CH$_2$N*H*), 7.76 (s, 1H, N*H*COCH$_2$CH$_2$NH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 26.20/26.30 (2C, C(3), C(6)), 28.19 (6C, 2 × (OC(CH_3)$_3$)), 28.64/28.68 (2C, C(4), C(5)), 29.02/29.43 (2C, C(2), C(7)), 35.72 (1C, NHCOCH_2CH$_2$NH), 36.75 (1C, NHCOCH$_2$$CH_2$NH), 38.35 (1C, C(8)), 39.77 (1C, C(1)), 77.21/77.48 (2C, 2 × NHCOOC(CH$_3$)$_3$), 155.39/155.52 (2C, (CH$_3$)$_3$OCONHC(1), NHCOCH$_2$CH$_2$NHCO), 170.00 (1C, NHCOCH$_2$CH$_2$NH). Analysis: C, H, N for $C_{21}H_{41}N_3O_5$.

7.10.2. 8-(3-Ammonium-propionylamino)-octyl-ammonium dichloride (**42**)

Compound **41** was deprotected as described for the synthesis of **35a** (7.8.2). Yield: (99%) white powder, m.p.: 218–219 °C. ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.24–1.28 (m, 8H, CH_2-(3), CH_2-(4), CH_2-(5), CH_2-(6)), 1.35–1.38 (m,2H, CH_2-(7)), 1.49–1.54 (m, 2H, CH_2-(2)), 2.46 (t, J = 7.1 Hz, 2H, NHCOCH_2CH$_2$NH$_3$), 2.71 (t, J = 7.5 Hz, 2H, CH_2-(1)), 2.92 (t, J = 7.5 Hz, 2H, NHCOCH$_2$$CH_2NH_3$), 2.98–3.05 (m, 2H, CH_2-(8)), 8.01 (s, 6H, 2 × NH$_3$), 8.12–8.16 (m, 1H, NH). ^{13}C NMR (50 MHz; DMSO-d_6) δ 25.79/26.25/26.79 (3C, C(4), C(5), C(6)), 28.43/28.87 (3C, C(2), C(3), C(7)), 31.77 (1C, NHCOCH_2CH$_2$NH$_3$), 35.04 (1C, NHCOCH$_2$$CH_2NH_3$), 38.24/38.42 (2C, C(1), C(8)), 169.10 (1C, CO). Analysis: C, H, N for $C_{11}H_{27}N_3OCl_2 \cdot 0.25H_2O$.

7.11. Pharmacological evaluation

Specific binding of [^{3}H]MK-801 (17–25 Ci/mmol, ART-661, ARC Inc., St. Louis USA; NET-972, Perkin Elmer Life Sciences Inc., Boston, USA) to rat brain membranes (prepared from hippocampus or from cerebral cortex) was performed as described [5,11]. Against widespread habits (non-equilibrium conditions, i.e. incubation time 1 h or shorter, some authors even prefer the nominal absence of glutamate and glycine), we prefer conditions close to equilibrium (2 h incubation

time in 50 mM Tris acetate pH 7.0, saturation with 10 μM glutamate and glycine). Nonspecific binding was defined by binding of [^{3}H]MK-801 to closed channels, i.e. without addition of the channel opening co-agonists glutamic acid and glycine, but with addition of their respective antagonists D-APV (10 μM) and 5,7-DCKA (1 μM; both from Tocris–Cookson). When it turned out that inhibition of [^{3}H]MK-801 binding by some of our test compounds was extremely sensitive to Tris buffer concentration, we switched from 50 to 10 mM Tris; to maintain equilibrium conditions, incubation time was increased from 2 to 3 h. IC_{50} values were obtained by computer-fitting the inhibition data to the function $B = B_0 \times {IC_{50}}^{nH}/(x^{nH} + {IC_{50}}^{nH}) + NB$, where B was the amount of radioligand bound at various concentrations x of the (inhibitory) test compound, B_0 the specific binding at $x = 0$, nH the Hill coefficient, and NB the nonspecific binding. At two occasions, data had to be fitted to an inhibition function superimposed to stimulation, $B = [B_0 + B_m \times x^{nHs}/(x^{nHs} + {EC_{50}}^{nHs})] \times {IC_{50}}^{nHi}/(x^{nHi} + {IC_{50}}^{nHi}) + NB$, where B_m is the extent of maximum stimulation, and nHs and nHi the Hill coefficients of the stimulatory and the inhibitory components, respectively.

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