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Inverse agonists at the polyamine-sensitive modulatory site of the NMDA receptor: 50-fold increase in potency by insertion of an aromatic ring into an alkanediamine chain

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Abstract – Polyamines like spermine and spermidine increase the opening frequency of the NMDA receptor associated ion channel and, as a consequence, specific binding of non-saturating concentrations of the channel radioligand [^{3}H]MK-801. Compounds exhibiting the contrary effect have been described as polyamine inverse agonists, with 1,12-dodecanediamine (N-12-N) being one of the most specific ones (IC_{50} 16.5 μM). Here we describe the synthesis of a series of long-chain alkanediamines, with a thiophene nucleus inserted at various positions, and report the discovery of 5-(4-aminobutyl)-2-thiopheneoctanamine (N-4-T-8-N), which inhibited specific binding of [^{3}H]MK-801 by 50% at 0.33 μM. In the presence of 100 μM of spermine, 4.0 μM N-4-T-8-N was necessary to achieve the same degree of inhibition. N-4-T-8-N is the most potent polyamine inverse agonist presently known and should be a useful tool to elucidate the physiological significance of the polyamine regulatory site of the NMDA receptor complex.

polyamine / [^{3}H]MK-801 binding / NMDA receptor / thiophenedialkanamine / polyamine inverse agonist

1. Introduction

The NMDA receptor complex mediates Na^+ and Ca^{2+} ion fluxes in neuronal membranes initiated by glutamic acid, the most important excitatory neurotransmitter in the mammalian central nervous system. The participating ion channel is selectively labelled by [^{3}H]dizocilpine ([^{3}H]MK-801) in a 'use-dependent' manner: only activated, conducting channels are accessible for the radioligand. Polyamines like spermine and spermidine increase the opening frequency of the channel and also the specific binding of [^{3}H]MK-801, if a non-saturating ligand concentration was used (for reviews, see [1, 2]). Although they are constituents of every living cell, their physiological significance for the NMDA receptor complex is unclear. Several synthetic compounds have been reported to block specific [^{3}H]MK-801 binding via the polyamine regulatory site: 1,10-decanediamine (N-10-N) [3], 1,12-dodecanediamine (N-12-N) [4], arcaine [5, 6], 1,10-bis(guanidino)decane (BG10) [7], and pentamidine [8]. In contrast to the polyamines, these compounds reduce the opening frequency of the NMDA receptor associated ion channel and specific binding of [^{3}H]MK-801, and have therefore been designated as *polyamine inverse agonists* [3]. However, their potency is low, and direct channel blockade at high concentrations is a common observation [9, 10, 11, 12]. Only ifenprodil and eliprodil (SL 82.0715), which bear no structural resemblance to the above-mentioned diamines, appear to act with high affinity at a polyamine site of the NMDA receptor [13, 14]. In vivo, however, ifenprodil seems to interact with σ-sites, rather than with the NMDA receptor complex [15, 16]. Some efforts to increase the potency of diamines by structural modifications have had only minor success [17, 18]. No systematic investigation has been devoted to the chain connecting the terminal amino groups, with exception of the insertion of a cyclohexyl moiety [17]. To provide a further element for ligand interaction, in addition to the effect of rigidization of the molecule by a ring system, we inserted an aromatic ring at various positions in the chain.

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2. Chemistry

All 2,5-thiophenealkanediamines were synthesized via their homologous dicarboxylic acids. 2-Thiophenealkanoic acids were prepared by thiophene acylation [19], introducing the acyl residue via the ω-alkanoic acid chloride into position 2. The second alkyl residue was introduced in position 5 by an analogous reaction sequence; for the synthesis of N-3-T-9-N, an alternative route was chosen. The dicarboxylic acids were converted to the corresponding diamines by reduction of their diamides.

2.1. 2-Thiophenealkanoic acids (methyl esters)

The diethyl esters of the 1, ω-dialkanoic acids (4–9 methylene groups between the carboxylic groups) were hydrolized using one equivalent of potassium hydroxide in ethanol (room temperature). The monoesters were isolated by vacuum distillation. The acid chlorides, obtained from the monoesters by reaction with thionyl chloride, were purified in the same manner. Acylation of thiophene in position 2 was carried out with these acid chlorides, using aluminum trichloride as catalyst and carbon disulfide as solvent [20]. The oxo group of the newly introduced chain was reduced with hydrazine and potassium hydroxide in ethylene glycol [21], with concomitant hydrolysis of the ester group at the chain end. For purification purposes, the 2-thiophenealkanoic acids obtained were converted into methylesters by a method described for amino acids [22].

2.2. 2,5-Thiophenedialkanoic acids

For the introduction of the second alkyl chain into position 5 of the thiophene ring, care had to be taken to use only highly purified mono acid chlorides, to avoid formation of side products (due to reactions with alkanedioic acid dichlorides) which were not only difficult to detect, but also difficult to remove. In one case (synthesis of **3e**), the anhydride was used instead of the acid chloride. For purification purposes it is advisable to transform the resulting diacids **4** into their dimethyl esters **10**, which can be subjected to chromatography and then hydrolyzed to yield the purified acids.

*2.3. Synthesis of N-3-T-9-N **6h***

Introduction of the C-3 chain into position 5 of the 2-thiophenealkanoic acid ester **2e** by the reaction sequence given above would have involved reduction of a keto-group in a β-keto acid, a reaction frequently resulting in concomitant decarboxylation. Therefore the ester **2e** was formylated with DMF and phosphoryl chloride, and the resulting aldehyde **7** subjected to condensation with malonic acid in pyridine/piperidine. After ester hydrolysis, the double bond of the newly introduced C-3 chain was reduced by treatment with sodium amalgam in methanol/acetic acid. A small amount of the unsaturated diacid **9** resisted this treatment. After the subsequent reduction of **5g** leading to the diamine **6h**, no more unsaturated product could be detected in the purified product **6h**, possibly due to complete reduction of the α,β-unsaturated carboxylic acid amide **5g**.

2.4. 2,5-Thiophenedialkanamines

The 2,5-thiophenedialkanoic acids were converted into the acid dichlorides with oxalyl chloride in benzene/pyridine [23]. (The use of thionyl chloride resulted in the formation of polymeric compounds.) Introduction of gaseous ammonia led to precipitation of the amides **5**, which were thoroughly washed and recrystallized. A suspension of amides **5** in dry THF was treated with lithium aluminum hydride, excess reagent was destroyed, and amines **6b–i** were extracted. Chlorohydrates were prepared from the amines by addition of HCl in ether.

*2.5. Synthesis of **6a***

The smallest diamine **6a** of this series could be obtained by 'Hofmann' rearrangement of the diamide **5a**, using KOBr in aqueous potassium hydroxide. The method was not applicable for the synthesis of other diamines due to insufficient solubility of the diamides in alkaline aqueous solution, or extensive side product formation.

3. Pharmacology

To evaluate the polyamine sensitivity of test compounds as inhibitors of the NMDA receptor complex, IC_{50}-values of [^{3}H]MK-801 binding were determined in the absence and in the presence of the polyamine agonist spermine (100 μM). A strong influence of spermine on the inhibitory potency of a test compound can be taken as indication for a mechanism of action involving the polyamine regulatory site. The Cheng–Prusoff equation in its simplest form [24] predicts that the IC_{50} of an inhibitor should increase linearly with the concentration of the competing agonist:

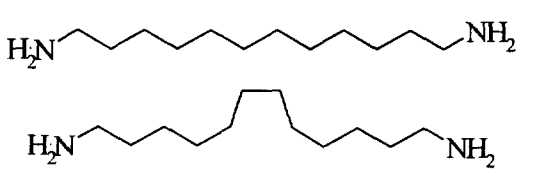
$$IC_{50}\,([\text{agonist}] = A) = IC_{50}\,([\text{agonist}] = 0) \times (1 + A/EC_{50}).$$

From experiments performed in parallel at various concentrations of spermine, we know that the EC_{50} of spermine stimulating the specific binding of [^{3}H]MK-801 was 4.2 ± 1.9 μM (mean ± S.D., 9 experiments).

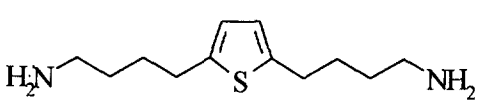
Thus, in the presence of 100 μM spermine (A = 100 μM), the IC_{50} of an inhibitor competing with spermine should increase 25-fold (1 + 100/4.2). None of the hitherto described polyamine inverse agonists complies with this prediction. For one of the most selective compounds, N-12-N, we reported a factor 10.4 [4].

4. Results and discussion

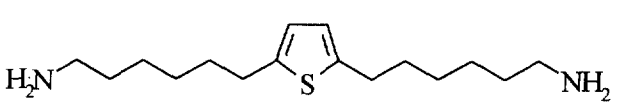
The polyamine inverse agonist N-12-N inhibits binding of [^{3}H]MK-801 with moderate potency (IC_{50} = 16.5 μM [4]). Formal connection of the four central methylene groups of this compound with a divalent sulfur atom and aromatization of the resulting ring (see *scheme 1*) results in 2,5-thiophenedibutanamine (N-4-T-4-N). This compound was two times weaker than N-12-N itself, with inhibitory properties largely unrelated to polyamines; the Hill coefficient greater than unity was most likely related to amphipathic properties of the compound at the relatively high concentrations applied (4–400 μM). Increasing the chain length on both sides of the thiophene nucleus by one methylene group results in 2,5-thiophenedipentanamine (N-5-T-5-N). This compound was considerably more potent than its shorter chain homologue, and inhibition of [^{3}H]MK-801 binding exhibited a moderate degree of spermine sensitivity, with 100 μM spermine increasing the IC_{50} by a factor 4.5 (*table I*). Further increasing the chain length results in 2,5-thiophenedihexanamine (N-6-T-6-N), which in the presence of 100 μM spermine was 9 times less potent than in its absence (*table I*). The compound was comparable in potency to BG10 [7] and pentamidine [8], the most potent polyamine inverse agonists described up to now. Hill coefficients were close to unity. Further increase in chain length on both sides of the thiophene ring did not increase potency any further, and inhibition of [^{3}H]MK-801 binding became more and more independent of spermine. Inhibition of [^{3}H]MK-801 binding by 2,5-thiophenedidecanamine (N-10-T-10-N), the largest compound studied, was completely insensitive to spermine, with extremely high Hill coefficients most likely reflecting the highly amphipathic character of this compound. The properties of the long-chain compounds (N-8-T-8-N and N-10-T-10-N) were reminiscent of the properties of N-16-N [4].



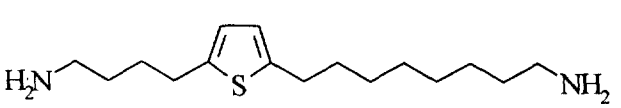
1,12-dodecanediamine (N-12-N)



2,5-thiophenedibutanamine (N-4-T-4-N)



2,5-thiophenedihexanamine (N-6-T-6-N)



5-(4-aminobutyl)-2-thiopheneoctanamine (N-4-T-8-N)

Scheme 1.

The overall length of the most active symmetrical thiophenedialkanamine, N-6-T-6-N, exceeds that of the most active alkanediamine N-12-N (see *scheme 1*) by the size of the thiophene nucleus, i.e. by –(=C)– S–(C=)–, sharing the same total number of methylene groups. The strong dependence of the inhibitory potency of the alkanediamines on their chain length suggests a rather extended conformation of these compounds at their site of interaction. The increased demand in chain length of the compounds containing a thiophene ring points to a specific interaction of this aromatic ring with a particular binding domain. This would also explain the significant increase in inhibitory potency of this group of compounds.

Most interestingly, the potency of N-6-T-6-N was further increased by moving the thiophene nucleus away from the centre of the molecule whilst maintaining the overall length of the compound. As a result of this optimization we obtained N-4-T-8-N, in which the aromatic ring is shifted away from the centre of the molecule by two CH_2 units. N-4-T-8-N was the most potent compound in this series, and it is the most potent polyamine inverse agonist described so far. Further dislocation of the ring from the centre was not favourable and yielded a compound weaker than the symmetrical one. In the presence of 100 μM spermine, N-4-T-8-N was 12 times weaker than in its absence. Similar ratios were only observed for N-12-N

Table I. Inhibition of [^{3}H]MK-801 binding by 2,5-thiophenedialkanamines, influence of the polyamine spermine (means ± S.D.; n_H: Hill coefficient; *i*: number of experiments).

Compound[a]	Without spermine		100 μM spermine		IC_{50} (100)	(*i*)
	IC_{50} (μM)	n_H	IC_{50} (μM)	n_H	IC_{50} (0)	
N-12-N	16.5 ± 1.90	0.95 ± 0.10	171 ± 12	1.12 ± 0.18	10.4 ± 0.7	(4)[b]
N-4-T-4-N (**6a**)	38.2 ± 3.0	1.51 ± 0.11	105 ± 19	2.40 ± 0.27	2.81 ± 0.44	(4)
N-5-T-5-N (**6b**)	7.83 ± 1.99	1.32 ± 0.20	34.3 ± 3.3	1.81 ± 1.02	4.50 ± 0.71	(3)
N-3-T-9-N (**6h**)	4.98	1.26	25.6	1.57	5.35	(2)
N-4-T-8-N (**6f**)	0.33 ± 0.03	1.17 ± 0.07	4.03 ± 0.22	1.11 ± 0.07	12.0 ± 0.59	(4)
N-5-T-7-N (**6c**)	1.51 ± 0.13	1.14 ± 0.03	11.3 ± 1.9	1.11 ± 0.10	7.44 ± 0.82	(3)
N-6-T-6-N (**6d**)	2.15 ± 0.36	1.20 ± 0.10	18.6 ± 1.5	1.40 ± 0.07	8.87 ± 1.81	(5)
N-7-T-7-N (**6e**)	2.49 ± 0.08	1.35 ± 0.16	12.7 ± 1.15	1.40 ± 0.08	5.04 ± 0.59	(3)
N-8-T-8-N (**6g**)	5.50 ± 0.72	2.10 ± 0.32	11.4 ± 0.7	2.10 ± 0.39	2.10 ± 0.34	(3)
N-10-T-10-N (**6i**)	4.83 ± 1.04	8.7 ± 4.3	5.37 ± 1.48	6.6 ± 2.1	1.10 ± 0.08	(3)

[a]N: primary amino group; T: thiophene; arabic number: unbranched aliphatic chain consisting of as many methylene groups as indicated by the number; [b]data taken from [4].

(*table I*) and for pentamidine [25]. N-4-T-8-N was 50 times more potent than N-12-N and should be expected to be more selective than this compound, with a lower incidence of non-specific effects. It remains unclear, however, why a potent compound such as N-4-T-8-N did not exhibit the full spermine sensitivity predicted for compounds interacting competitively with the polyamine agonist spermine (see above, Pharmacology section). The impossibility to reverse N-12-N's and N-4-T-8-N's inhibition of [^{3}H]MK-801 binding completely by spermine might be explained by nonspecific properties of the inhibitors, but also by inhibitory actions of spermine itself at higher concentrations [26].

5. Conclusions

In this study we present a new structural lead for the development of high-affinity ligands for the polyamine regulatory site of the NMDA receptor complex. Structural modifications of the aliphatic chain of alkanediamines by introduction of a thiophene nucleus at various positions resulted in N-4-T-8-N, the most potent polyamine inverse agonist presently known (IC_{50} = 0.33 μM). The pronounced increase in polyamine inverse agonist potency of aliphatic diamines achieved by insertion of a thiophene ring at a specific position of the chain may be explained by the specific conformational constraint imposed on the molecule, or may point to an interaction of this aromatic ring with a particular receptor domain.

6. Experimental protocols

6.1. General chemical procedures

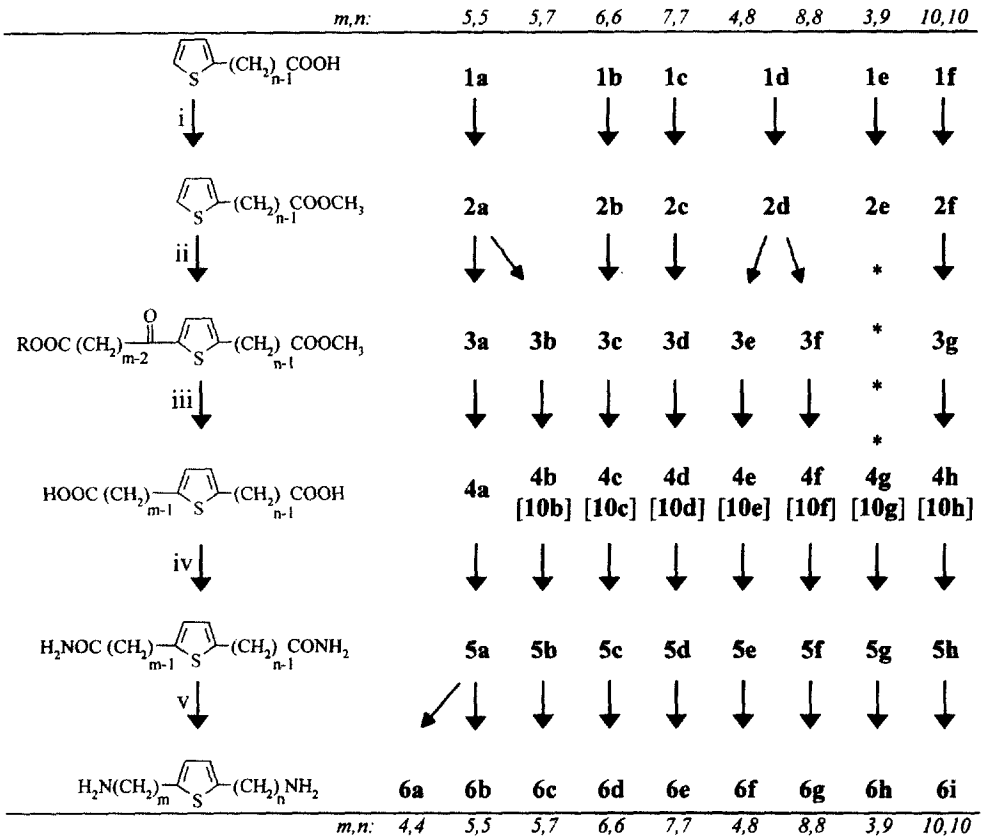
NMR spectra were obtained for all compounds synthesized using a Bruker 200 spectrometer at 200 MHz for ^{1}H nuclei and at 50 MHz for ^{13}C nuclei. Melting points were determined with a Kofler apparatus and are uncorrected. Elemental analyses were obtained for all new compounds; all samples were dried at 40 °C in vacuo for 12 h over diphosphorus pentoxide. Thin-layer chromatography was performed on TLC aluminum foils with SG 60 F_{254} (Merck, Darmstadt, Germany). Spots were visualized under UV (254 nm), directly or after spraying with 5% ethanolic molybdato phosphoric acid and subsequent heating. Preparative column chromatography (at least 200 g silicagel) was performed using an automatic MPLC machine (pressure 2–4 bar). Silicagel material of particle size 0.04–0.06 mm was used in vacuum flash chromatography (ratio silicagel/sample/fraction = [10–20]:1:[0.5–1.0]). Solvents and reagents were of commercial quality; diethyl ether, petroleum ether, and dichloromethane were distilled before use.

6.2. Abbrevations used

CC: column chromatography; DMF: dimethylformamide; E: diethylether; EA: ethyl acetate; PE: petroleum ether; SG: silicagel; THF: tetrahydrofuran; TLC: thin-layer chromatography; VFC: vacuum flash chromatography.

6.3. Chemical syntheses

In the following, a detailed description of the synthetic pathway leading to the thiophenedialkanamine **6d** is indicated, starting from the monosubstituted thiophenealkanoic acid **1b**. The pathways leading to the other diamines were almost identical and are not described in detail (for full details, see [27]); nevertheless, yields, melting and/or boiling points, and the results of elementary and NMR analyses of the other interme-



i, $SOCl_2/CH_3OH$; ii, $ClOC\text{-}(CH_2)_{m-2}COOR$, $AlCl_3$; iii, N_2H_4/KOH; iv, ClOC-COCl/benzene, NH_3; v, $LiAlH_4$/THF; R is ethyl, only in **3e** it is methyl; ******** indicates another synthetic pathway (see scheme 3). Compounds in brackets have been synthetized for purification reasons only (methylesters).

Scheme 2. Synthesis of 2,5-thiophenedialkanamines, $H_2N\text{-}(CH_2)_m\text{-T-}(CH_2)_n\text{-}NH_2$.

diates are given too. Yields are given in terms of the non-purified product, if no further purification was necessary for the next synthetic steps (in these cases, no NMR spectra were recorded). Some differing steps in the syntheses of **6a**, **6f**, and **6h** are described separately.

*6.3.1. 2-Thiophenehexanoic acid, methyl ester **2b***

13.50 g (113 mmol) $SOCl_2$ was dropped slowly into a stirred ice cold solution of 22.40 g (113 mmol) of **1b** [19] in 280 mL dry methanol (CH_3OH). The reaction mixture was stirred at room temperature overnight. The solution was concentrated, the residue taken up in CH_2Cl_2, washed three times with saturated $NaHCO_3$ solution and once with water. The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness in vacuo. A brown liquid was obtained. Yield: 20.30 g (85%) **2b** [19]. The product was used without further purification.

*6.3.2. 2-Thiophenepentanoic acid, methylester **2a** [19]*

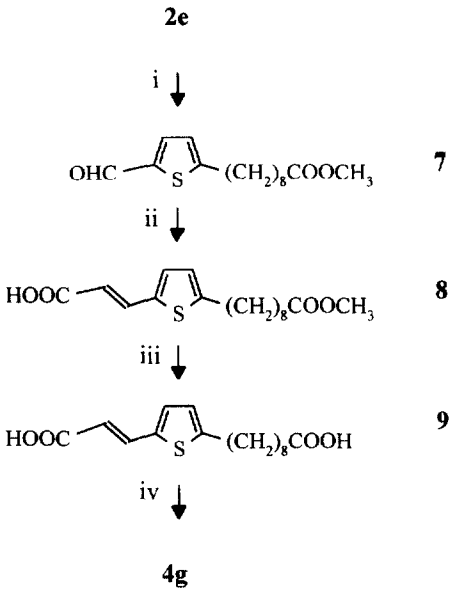
Colourless liquid. Yield 93% after VFC (PE/E = 5:1).

*6.3.3. 2-Thiopheneheptanoic acid, methylester **2c***

Colourless liquid. Yield 73%; bp 110 °C (0.02 mbar). Anal. $C_{12}H_{18}O_2S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.09 (dd, 1 H, H-5), 6.90 (m, 1 H, H-4), 6.77 (d, 1 H, H-3), 3.67 (s, 3 H, CH_3O), 2.81 (t, 2 H, H-ζ), 2.30 (t, 2 H, H-α), 1.75–1.57 (m, 4 H, H-β, -ε), 1.48–1.30 (m, 4 H, H-γ, -δ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.1 (s, 1 C, $COOCH_3$), 145.5 (s, 1 C, C-2), 126.6 (d, 1 C, C-4), 123.9 (d, 1 C, C-3), 122.7 (d, 1 C, C-5), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-α), 31.5 (t, 1 C, C-ζ), 29.7 (t, 1 C, C-ε), 28.8 and 28.6 (2 t, 2 C, C-γ, -δ), 24.8 (t, 1 C, C-β).

*6.3.4. 2-Thiopheneoctanoic acid, methylester **2d***

Colourless liquid. Yield 93%; bp 92–100 °C (0.02 mbar). Anal. $C_{13}H_{20}O_2S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.10 (dd,



i, $POCl_3$/DMF; ii, HOOC-CH_2-COOH/pyridine, piperidine; iii, 2N NaOH/CH_3OH; iv, 1. Na/Hg/CH_3OH/EA.

Scheme 3. Intermediates in the synthesis of N-3-T-9-N (**6h**) deviating from the steps in *scheme 2* (****).

1 H, H-5), 6.90 (dd, 1 H, H-4), 6.77 (dd, 1 H, H-3), 3.66 (s, 3 H, CH_3O), 2.81 (t, 2 H, CH_2-η), 2.30 (t, 2 H, CH_2-α), 1.75–1.55 (m, 4 H, CH_2-β, -ζ), 1.40–1.28 (m, 6 H, CH_2-γ, -δ, -ε). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 1 C, $COOCH_3$), 145.6 (s, 1 C, C-2), 126.6 (d, 1 C, C-4), 123.9 (d, 1 C, C-3), 122.7 (d, 1 C, C-5), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-α), 31.7 (t, 1 C, C-η), 29.8 (t, 1 C, C-ζ), 29.0, 28.9 and 28.8 (3 t, 3 C, C-γ, -δ, -ε), 24.9 (t, 1 C, C-β).

*6.3.5. 2-Thiophenenonanoic acid, methylester **2e***

Colourless liquid. Yield 76%; bp 115 °C (0.01 mbar). Anal. $C_{14}H_{22}O_2S$•0.21H_2O (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.10 (dd, 1 H, H-5), 6.91 (dd, 1 H, H-4), 6.76 (dd, 1 H, H-3), 3.67 (s, 3 H, CH_3O), 2.81 (t, 2 H, CH_2-θ), 2.30 (t, 2 H, CH_2-α), 1.75–1.54 (m, 4 H, CH_2-β, -η), 1.40–1.27 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 1 C, $COOCH_3$), 145.7 (s, 1 C, C-2), 126.6 (d, 1 C, C-4), 123.9 (d, 1 C, C-3), 122.7 (d, 1 C, C-5), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-α), 31.7 (t, 1 C, C-θ), 29.8 (t, 1 C, C-η), 29.1, 29.1 and 29.0 (3 t, 4 C, C-γ, -δ, -ε, -ζ), 24.9 (t, 1 C, C-β).

*6.3.6. 2-Thiophenedecanoic acid, methylester **2f***

Colourless liquid. Yield 84%; bp 118–122 °C (0.02 mbar). Anal. $C_{15}H_{24}O_2S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.10 (d, 1 H, H-5), 6.90 (dd, 1 H, H-4), 6.77 (d, 1 H, H-3), 3.66 (s, 3 H, CH_3O), 2.80 (t, 2 H, CH_2-ι), 2.30 (t, 2 H, CH_2-α), 1.75–1.53 (m, 4 H, CH_2-β, -θ), 1.40–1.20 (m, 10 H, CH_2-γ, -δ, -ε, -ζ, -η). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 1 C, $COOCH_3$), 145.7 (s, 1 C, C-2), 126.6 (d, 1 C, C-4), 123.8 (d, 1 C, C-3), 122.6 (d, 1 C, C-5), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-ι), 31.7 (t, 1 C, C-α), 29.8 (t, 1 C, C-θ), 29.3, 29.2, 29.2, 29.1 and 29.0 (5 t, 5 C, C-γ, -δ, -ε-ζ, -η), 24.9 (t, 1 C, C-β).

*6.3.7. ε-Oxo-2,5-thiophenedihexanoic acid, α-ethylester α'-methylester **3c***

Dry $AlCl_3$ (38.40 g, 288 mmol) was added to a cooled solution of 18.5 g (96.0 mmol) 6-chloro-6-oxohexanoic acid ethylester in 260 mL CS_2. A solution of 20.30 g (95.6 mmol) **2b** in 50 mL CS_2 was added slowly to that mixture. Cooling was discontinued and the reaction mixture stirred at room temperature till TLC showed full consumption of educt. The mixture was poured onto ice and acidified to pH 1 using 6 N HCl. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with 6 N HCl, saturated $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, filtered and evaporated to dryness. 34.0 g (91%) of pale brown crystals of **3c** [19] were obtained and used without further purification.

*6.3.8. δ-Oxo-2,5-thiophenedipentanoic acid, α-ethylester α'-methylester **3a***

Colourless liquid. Yield 52%. Anal. $C_{17}H_{24}O_5S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.55 (d, 1 H, H-3), 6.82 (d, 1 H, H-4), 4.14 (q, 2 H, CH_2O), 3.67 (s, 3 H, CH_3O), 2.96–2.82 (m, 4 H, CH_2-γ, -1-butyl), 2.45–2.30 (m, 4 H, CH_2-α, -4-butyl), 2.06 (m, 2 H, CH_2-β), 1.71 (m, 4 H, CH_2-2, -3-butyl). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 192.0 (s, 1 C, CO-δ), 173.6 and 173.0 (2 s, 2 C, $COOCH_3$ and COOEt), 154.6 (s, 1 C, C-2), 141.6 (s, 1 C, C-5), 132.2 (d, 1 C, C-3), 125.6 (d, 1 C, C-4), 60.2 (t, 1 C, CH_2O), 51.4 (q, 1 C, CH_3O), 37.5 (t, 1 C, C-γ), 33.4 and 33.2 (2 t, 2 C, C-α, -1-butyl), 30.5 and 30.1 (2 t, 2 C, C-β, -4-butyl), 24.0 and 19.7 (2 t, 2 C, C-2-, -3-butyl), 14.1 (q, 1 C, CH_3).

*6.3.9. 5-(5-Methoxy-5-oxopentyl)-ζ-oxo-2-thiopheneheptanoic acid, ethylester **3b***

Orange crystals. Yield 67%. After distillation colourless crystals, bp 150 °C (0.02 mbar), mp 35–35.5 °C. Anal. $C_{19}H_{28}O_5S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.54 (d, 1 H, H-3), 6.82 (d, 1 H, H-4), 4.13 (q, 2 H, OCH_2), 3.67 (s, 3 H, CH_3O), 2.80–2.70 (m, 4 H, CH_2-ε, -1-pentyl), 2.40–2.28 (m, 4 H, CH_2-α, -4-pentyl), 1.83–1.60 (m, 8 H, CH_2-β, -δ, -2-, -3-pentyl), 1.44 (m, 2 H, CH_2-γ), 1.26 (t, 3 H, CH_3). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 192.8 (s, 1 C, C-ζ), 173.7 and 173.6 (2 s, 2 C, COOMe and COOEt), 154.4 (s, 1 C, C-2), 142.0 (s, 1 C, C-5), 132.0 (d, 1 C, C-3), 125.6 (d, 1 C, C-4), 60.1 (t, 1 C, OCH_2), 51.5 (q, 1 C, CH_3O), 38.6 (t, 1 C, C-ε), 34.1, 33.6, 30.6, 30.2, 28.7 (5 t, 5 C, C-α, -δ, -1-, -2-, -4-pentyl), 24.6, 24.4 and 24.2 (2 t, 2 C, C-β, -γ, -3-pentyl), 14.2 (q, 1 C, CH_3).

*6.3.10. ζ-Oxo-2,5-thiophenediheptanoic acid, α-ethylester α'-methylester **3d***

Light brown crystals. Yield 96%. After CC colourless crystals, bp 190 °C (0.015 mbar), mp 50 °C. Anal. $C_{21}H_{32}O_5S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.54 (d, 1 H, H-3), 6.80 (d, 1 H, H-4), 4.12 (q, 2 H, CH_2O), 3.67 (s, 3 H, CH_3O), 2.89–2.80 (m, 4 H, CH_2-ε, -ζ'), 2.30 (t, 4 H, CH_2-α, -α'), 1.82–1.57 (m, 8 H, CH_2-β, -β', -δ, -ε'), 1.50–1.31 (m, 6 H, CH_2-γ, -γ', -δ'), 1.25 (t, 3 H, CH_3). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 192.6 (s, 1 C, CO), 173.8 and 173.4 (2 s, 2 C, COOMe and COOEt), 155.0 (s, 1 C, C-2), 141.7 (s, 1 C, C-5), 131.9 (d, 1 C, C-3), 125.3 (d, 1 C, C-4), 60.0 (t, 1 C, CH_2O), 51.2 (q, 1 C, CH_3O), 38.4 (t, 1 C, C-ε), 33.9, 33.7, 30.9, 30.3, 28.6, 28.5, 28.4 (7 t, 7 C, C-α, -α', -γ, -γ', -δ', -ε', -ζ'), 24.5 and 24.2 (2 t, 3 C, C-β, -β', -δ), 14.0 (q, 1 C, CH_3).

*6.3.11. η-Oxo-2,5-thiophenedioctanoic acid, α-ethylester α'-methylester **3f***

Colourless crystals. Yield 79%; mp 32–33 °C. Anal. $C_{23}H_{36}O_5S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.54 (d, 1 H, H-3), 6.80 (d, 1 H, H-4), 4.12 (q, 2 H, CH_2O), 3.68 (s, 3 H, CH_3O), 2.90–2.79 (m, 4 H, CH_2-ζ, -η'), 2.36–2.24 (m, 4 H, CH_2-α, -α'), 1.81–1.51 (m, 8 H, CH_2-β, -β', -ε, -ζ'), 1.48–1.22 (m, 13 H, CH_2-γ, -γ', -δ, -δ', -ε' and CH_3). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 193.0 (s, 1 C, CO), 174.1 and 173.7 (2 s, 2 C, COOMe and COOEt), 155.3 (s, 1 C, C-2), 141.8 (s, 1 C, C-5), 132.0 (d, 1 C, C-3), 125.4 (d, 1 C, C-4), 60.1 (t, 1 C, CH_2O), 51.4 (q, 1 C, CH_3O), 38.7 (t, 1 C, C-ζ), 34.2, 33.9, 31.2, 30.5, 28.9, 28.8, 28.7 (7 t, 9 C, C-α, -α', -γ, -γ', -δ, -δ', -ε', -ζ', -η'), 24.8, 24.7 and 24.6 (3 t, 3 C, C-β, -β', -ε), 14.2 (q, 1 C, CH_3).

*6.3.12. ι-Oxo-2,5-thiophenedidecanoic acid, α-ethylester α'-methylester **3g***

Light brown crystals. Yield 93%. After distillation colourless crystals, bp 190–230 °C (0.1 mbar), mp 49–50 °C. Anal. $C_{27}H_{44}O_5S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.53 (d, 1 H, H-3), 6.80 (d, 1 H, H-4), 4.13 (q, 2 H, CH_2O), 3.67 (s, 3 H, CH_3O), 2.82 (t, 4 H, CH_2-θ, -ι'), 2.36–2.25 (m, 4 H, CH_2-α, -α'), 1.80–1.55 (m, 8 H, CH_2-β, -β', -η, -θ'), 1.42–1.22 (m, 21 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η' and CH_3). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 193.0 (s, 1 C, CO), 174.1 and 173.7 (2 s, 2 C, COOEt and COOMe), 155.3 (s, 1 C, C-2), 141.8 (s, 1 C, C-5), 131.9 (d, 1 C, C-3), 125.3 (d, 1 C, C-4), 60.0 (t, 1 C, CH_2O), 51.3 (q, 1 C, CH_3O), 38.8 (t, 1 C, C-θ, 34.2, 34.0, 31.2, 30.5, 29.1, 29.0, 29.0, 28.8 (8 t, 13 C, C-α, -α', -γ, -γ', -δ, -δ', -ε, -ε', -ζ', -η, -η', -θ', -ι'), 24.8 (t, 3 C, C-β, -β', -ζ).

*6.3.13. 2,5-Thiophenedihexanoic acid **4c***

A solution of 22.00 g (392 mmol) KOH, 34.00 g (92.3 mmol) **3c**, and 15.70 g (307 mmol) 98% N_2H_4 hydrate in 250 mL ethylene glycol was refluxed for 2.5 h. Excess N_2H_4 hydrate was slowly distilled off, the residues heated further until flask temperature reached 180 °C, and the reaction mixture refluxed for further 3.5 h. The cold solution was poured onto ice and acidified with concentrated HCl to pH = 1. The organic layer was separated and the aqueous layer extracted with EA. The combined organic layers were washed with 2 N HCl and water, dried over Na_2SO_4, filtered and evaporated to dryness yielding 26.60 g (98%) of brown crystals **4c**, crude. For chromatographic purification 5.48 g (17.5 mmol) **4c** and 4.09 g (34.4 mmol) $SOCl_2$ in 250 mL dry CH_3OH were reacted to obtain **10c**, the dimethylester of **4c**, according to the method given for the preparation of **2b**. CC: eluent: toluene/E = 30:1, 532 g SG. Yield: 4.73 g (79%) colourless liquid **10c**. TLC: R_f 0.25 (toluene/EA = 30:1). Anal. $C_{18}H_{28}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.54 (s, 2 H, H-3, -4), 3.67 (s, 6H, 2 CH_3O), 2.75 (t, 4 H, CH_2-ε, -ε'), 2.31 (t, 4 H, CH_2-α, -α'), 1.77–1.59 (m, 8 H, CH_2-β, -β', -δ, -δ'), 1.40 (m, 4 H, CH_2-γ, -γ'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.1 (s, 2 C, $COOCH_3$), 142.8 (s, 2 C, C-2,5), 123.4 (d, 2 C, C-3, -4), 51.4 (q, 2 C, CH_3O), 33.9 (t, 2 C, C-α, -α'), 31.2 (t, 2 C, C-ε, -ε'), 29.8 (t, 2 C, C-δ, -δ'), 28.5 (t, 2 C, C-γ, -γ'), 24.6 (t, 2 C, C-β, -β'). After addition of 8 mL 2 N aqueous NaOH solution, a solution of 1.00 g (2.94 mmol) **10c** in 4 mL CH_3OH was refluxed until no di- and monoesters could be detected by TLC (3 h). The reaction mixture was filtered, washed with chloroform, and acidified with concentrated phosphoric acid; pH was adjusted to 1 by addition of 6 N HCl and the precipitate collected. Yield: 918 mg (77%) light brown crystals **4c**, mp 69–71 °C, lit. 60–68 °C [19]. Anal. $C_{16}H_{24}O_4S{\cdot}0.46H_2O$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.53 (s, 2 H, H-3, -4), 2.76 (t, 4 H, CH_2-ε, -ε'), 2.37 (t, 4 H, CH_2-α, -α'), 1.75–1.59 (m, 8 H, CH_2-β, -β', -δ, -δ'), 1.41 (m, 4 H, CH_2-γ, -γ'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 180.3 (s, 2 C, COOH), 142.8 (s, 2 C, C-2, -5), 123.6 (d, 2 C, C-3, -4), 34.0 (t, 2 C, C-ε, -ε'), 31.1 (t, 2 C, C-α, -α'), 29.8 (t, 2 C, C-δ, -δ'), 28.2 (t, 2 C, C-γ, -γ'), 24.4 (t, 2 C, C-β, -β').

*6.3.14. 2,5-Thiophenedipentanoic acid **4a***

Yellow crystals. Yield 61%. Purification by extraction and precipitation.

*6.3.15. 5-(4-Carboxybutyl)-2-thiopheneheptanoic acid **4b***

Brown crystals. Yield 86%. After purification at the stage of the dimethylester (**10b**, VFC): Colourless crystals. Yield 48%; mp 122 °C (diisopropyl ether). Anal. $C_{16}H_{24}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 10.05 (bs, 2 H, COOH), 6.55 (m, 2 H, H-3, -4), 2.80–2.69 (m, 4 H, CH_2-ζ, -1-butyl), 2.37–2.25 (m, 4 H, CH_2-α, -4-butyl), 1.77–1.55 (m, 8 H, CH_2-β, -ε, -2-, -3-butyl), 1.45–1.30 (m, 4 H, CH_2-γ, -δ). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 176.5 and 176.2 (2 s, 2 C, COOH), 142.9 and 142.2 (2 s, 2 C, C-2, -5), 123.3 and 123.2 (2 d, 2 C, C-3, -4), 33.8 and 33.6 (2 t, 2 C, C-ζ, -1-butyl), 31.2, 30.8, 29.8, 29.5, 28.6, 28.4 (6 t, 6 C, C-α, -γ, -δ, -ε, -2-, -4-butyl), 24.5 and 24.1 (2 t, 2 C, C-β, -3-butyl).

Dimethylester **10b** colourless liquid. Yield 75%; bp 130–140 °C (0.015 mbar). Anal. $C_{18}H_{28}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.55 (m, 2 H, H-3, -4), 3.67 (s, 6 H, 2 CH_3O), 2.80–2.69 (m, 4 H, CH_2-ζ, -1-pentyl), 2.38–2.27 (m, 4 H, CH_2-α, -4-pentyl), 1.72–1.54 (m, 8 H, CH_2-β, -ε, -2-pentyl, -3-pentyl), 1.42–1.30 (m, 4 H, CH_2-γ, -δ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 173.9 and 173.7 (2 s, 2 C, *C*OOCH$_3$), 143.0 and 142.2 (2 s, 2 C, C-2, -5), 123.4 and 123.3 (2 d, 2 C, C-3, -4), 51.2 and 51.2 (2 q, 2 C, CH_3O), 33.8 and 33.6 (2 t, 2 C, C-ζ, -1-pentyl), 31.3, 30.9, 29.8, 29.6, 28.7 and 28.5 (6 t, 6 C, C-α, -γ, -δ-, -ε, -2-, -4-pentyl), 24.6 and 24.2 (2 t, 2 C, C-β, -3-pentyl).

*6.3.16. 2,5-Thiophenediheptanoic acid **4d***

Brown crystals. Yield 81%. After purification at the stage of the dimethylester (**10d**, VFC): Light yellow crystals. Yield 51%; mp 123–124 °C. Anal. $C_{18}H_{28}O_4S{\cdot}0.14H_2O$ (C, H, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 6.52 (s, 2 H, H-3, -4), 5.65 (bs, 2 H, COOH), 2.71 (t, 4 H, CH_2-ζ, -ζ'), 2.26 (t, 4 H, CH_2-α, -α'), 1.74–1.50 (m, 8 H, CH_2-β, -β', -ε, -ε'), 1.50–1.26 (m, 8 H, CH_2-γ, -γ', -δ, -δ'). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 175.0 (s, 2 C, COOH), 142.1 (s, 2 C, C-2, -5), 122.6 (d, 2 C, C-3, -4), 33.3 (t, 2 C, C-ζ, -ζ'), 30.6 (t, 2 C, C-α, -α'), 29.2 (t, 2 C, C-ε, -ε'), 28.0 and 27.9 (2 t, 4 C, C-γ, -γ', -δ, -δ'), 24.0 (t, 2 C, C-β, -β').

Dimethylester **10d** colourless liquid. Yield 67%; bp 170 °C (0.015 mbar). Anal. $C_{20}H_{32}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.54 (s, 2 H, H-3, -4), 3.67 (s, 6 H, 2 CH_3O), 2.73 (t, 4 H, CH_2-ζ, -ζ'), 2.30 (t, 4 H, CH_2-α, -α'), 1.72–1.52 (m, 8 H, CH_2-β, -β', -ε, -ε'), 1.45–1.28 (m, 8 H, -γ, -γ', -δ, -δ'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 2 C, $COOCH_3$), 143.0 (s, 2 C, C-2, -5), 123.3 (d, 2 C, C-3, -4), 51.4 (q, 2 C, CH_3O), 34.0 (t, 2 C, C-α, -α'), 31.4 (t, 2 C, C-ζ, -ζ'), 30.0 (t, 2 C, C-ε, -ε'), 28.8 and 28.6 (2 t, 4 C, C-γ, -γ', -δ, -δ'), 24.8 (t, 2 C, C-β, -β').

*6.3.17. 5-(3-Carboxypropyl)-2-thiopheneoctanoic acid **4e***

Brown oil. Yield 95%. After purification at the stage of the dimethylester (**10e**, VFC): Colourless crystals. Yield 50%; mp 83–84 °C. Anal. $C_{16}H_{24}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 10.25 (bbs, 2 H, COOH), 6.57 (m, 2 H, H-3,-4), 2.82 and 2.74 (2 t, 4 H, CH_2-η, -1-propyl), 2.41 and 2.35 (2 t, 4 H, CH_2-α, -3-propyl), 1.98 (m, 2 H, CH_2-2-propyl), 1.70–1.56 (m, 4 H, CH_2-β, -ζ), 1.40–1.27 (m, 6 H, CH_2-γ, -δ, -ε). ^{13}C-NMR

($CDCl_3$): δ (ppm) = 180.4 and 179.9 (2 s, 2 C, COOH), 143.7 (s, 1 C, C-5), 141.2 (s, 1 C, C-2), 124.1 and 123.5 (2 d, 2 C, C-3, -4), 34.1 (t, 1 C, C-η), 33.1 (t, 1 C, C-1-propyl), 31.5 (t, 1 C, C-α), 30.1 (t, 1 C, C-ζ), 29.2 (t, 1 C, C-3-propyl), 28.9 and 28.8 (2 t, 3 C, C-γ, -δ, -ε), 26.2 (t, 1 C, C-2-propyl), 24.6 (t, 2 C, C-β).

Dimethylester **10e** colourless liquid. Yield 54%. Anal. $C_{18}H_{28}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.57 (m, 2 H, H-3, -4), 3.67 (s, 6 H, 2 CH_3O), 2.80 and 2.73 (2 t, 4 H, CH_2-η, -1-butyl), 2.37 and 2.30 (2 t, 4 H, CH_2-α, -3-butyl), 1.96 (m, 2 H, CH_2-2-butyl), 1.70– 1.55 (m, 4 H, CH_2-β, -ζ), 1.40–1.27 (m, 6 H, CH_2-γ, -δ, -ε). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 and 173.7 (2 s, 2 C, $COOCH_3$), 143.6 (s, 1 C, C-5), 141.4 (s, 1 C, C-2), 124.0 and 123.4 (2 d, 2 C, C-3, -4), 51.5 and 51.4 (2 q, 2 C, CH_3O), 34.0 (t, 1 C, C-α), 33.1 (t, 1 C, C-3-butyl), 31.5 (t, 1 C, C-η), 30.0 (t, 1 C, C-ζ), 29.3 (t, 1 C, C-1-butyl), 29.0, 28.9 and 28.8 (3 t, 3 C, C-γ, -δ, -ε), 26.7 (t, 1 C, C-2-butyl), 24.9 (t, 1 C, C-β).

6.3.18. 2,5-Thiophenedioctanoic acid ***4f***

Yellow crystals. Yield 100%. After purification at the stage of the dimethylester (**10f**, VFC): Colourless crystals. Yield 88%; mp 86–87 °C. Anal. $C_{20}H_{32}O_4S$•0.47H_2O (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.56 (s, 2 H, H-3, -4), 2.73 (t, 4 H, CH_2-η, -η'), 2.37 (t, 4 H, CH_2-α, -α'), 1.80–1.50 (m, 8 H, CH_2-β, -β', -ζ, -ζ'), 1.50–1.30 (m, 12 H, CH_2-γ, -γ', -δ, -δ' ,-ε, -ε'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 180.5 (s, 2 C, COOH), 143.1 (s, 2 C, C-2, -5), 123.3 (d, 2 C, C-3, -4), 34.1 (t, 2 C, C-η, -η'), 31.5 (t, 2 C, C-α, -α'), 30.1 (t, 2 C, C-ζ, -ζ'), 28.9, 28.9 and 28.8 (3 t, 6 C, C-γ, -γ', -δ, -δ', -ε, -ε'), 24.6 (t, 2 C, C-β, -β').

Dimethylester **10f** colourless liquid. Yield 92%. Anal. $C_{22}H_{36}O_2S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.54 (s, 2 H, H-3, -4), 3.67 (s, 6 H, 2 CH_3O), 2.73 (t, 4 H, CH_2-η, -η'), 2.30 (t, 4 H, CH_2-α, -α'), 1.72–1.51 (m, 8 H, CH_2-β, -β', -ζ, -ζ'), 1.45–1.26 (m, 12 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 2 C, $COOCH_3$), 143.1 (s, 2 C, C-2, -5), 123.3 (d, 2 C, C-3, -4), 51.4 (q, 2 C, CH_3O), 34.0 (t, 2 C, C-α, -α'), 31.5 (t, 2 C, C-η, -η'), 30.0 (t, 2 C, C-ζ, -ζ'), 29.0, 28.9 and 28.8 (3 t, 6 C, C-γ, -γ', -δ, -δ', -ε, -ε'), 24.8 (t, 2 C, C-β, -β').

6.3.19. 2,5-Thiophenedidecanoic acid ***4h***

Yellow crystals. Yield 100%. After purification at the stage of the dimethylester (**10h**, VFC): Colourless crystals. Yield 83%; mp 81.5–84 °C. Anal. $C_{24}H_{40}O_4S$•1.61H_2O (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.54 (s, 2 H, H-3, -4), 2.73 (t, 4 H, CH_2-ι, -ι'), 2.35 (t, 4 H, CH_2-α, -α'), 1.70–1.56 (m, 4 H, CH_2-β, -β', -θ, -θ'), 1.45–1.25 (m, 20 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 180.5 (s, 2 C, COOH), 143.1 (s, 2 C, C-2, -5), 123.2 (d, 2 C, C-3, -4), 34.1 (t, 2 C, C-ι, -ι'), 31.6 (t, 2 C, C-α, -α'), 30.1 (t, 2 C, C-θ, -θ'), 29.2, 29.1, 29.0 and 29.0 (4 t, 10 C, C-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'), 24.6 (t, 2 C, C-β, -β').

Dimethylester **10h** colourless crystals. Yield 83%; mp 28.5–29 °C; bp 180–190 °C (0.01 mbar). Anal. $C_{26}H_{44}O_4S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.54 (s, 2 H, H-3, -4), 3.68 (s, 6H, 2 CH_3O), 2.73 (t, 4 H, CH_2-ι, -ι'), 2.30 (t, 4 H, CH_2-α, -α'), 1.70–1.54 (m, 4 H, CH_2-β, -β', -θ, -θ'), 1.40–1.25 (m, 20 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.3 (s, 2 C, $COOCH_3$), 143.2 (s, 2 C, C-2, -5), 123.2 (d, 2 C, C-3, -4), 51.4 (q, 2 C, CH_3O), 34.1 (t, 2 C, C-ι, -ι'), 31.6 (t, 2 C, C-α, -α'), 30.1 (t, 2 C, C-θ, -θ'), 29.3, 29.2 and 29.1 (3 t, 10 C, C-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'), 24.9 (t, 2 C, C-β, -β').

6.3.20. 2,5-Thiophenedihexanamide ***5c***

600 mg (1.92 mmol) **4c** was dissolved in 10 mL dry benzene; after addition of one drop of dry pyridine, 0.35 mL (4.06 mmol) oxalyl chloride was added dropwise. The mixture was stirred at room temperatur until the evolution of gas had stopped (10–20 min) and refluxed for a further 45 min. After cooling down to 15 °C, dry NH_3 was bubbled in. After 10 min, cooling was discontinued, and NH_3 bubbled in for further 50 min. After a further 30 min, 20 mL diethyl ether was added, the precipitate was filtered off, washed three times with saturated aqueous $NaHCO_3$ and water, transferred into a flask, suspended in water and boiled for 10 min. The crystals were filtered off, recrystallized from CH_3OH and vacuum-dried at 50 °C, yielding 500 mg (84%) colourless crystals **5c**, mp 180 °C (CH_3OH). Anal. $C_{16}H_{26}N_2O_2S$ (C, H, N, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 7.12 and 6.51 (2 bs, 4 H, 2 $CONH_2$), 6.54 (s, 2 H, H-3, -4), 2.72 (t, 4 H, CH_2-ε, -ε'), 2.11 (t, 4 H, CH_2-α, -α'), 1.69–1.49 (m, 8 H, CH_2-β, -β', -δ, -δ'), 1.35 (m, 4 H, CH_2-γ, -γ'). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 173.0 (s, 2 C, $CONH_2$), 140.5 (s, 2 C, C-2, -5), 121.6 (d, 2 C, C-3, -4), 33.3 (t, 2 C, C-ε, -ε'), 29.3 (t, 2 C, C-α, -α'), 27.7 (t, 2 C, C-δ, -δ'), 26.5 (t, 2 C, C-γ, -γ'), 23.1 (t, 2 C, C-β, -β').

6.3.21. 2,5-Thiophenedipentanamide ***5a***

Colourless crystals. Yield 75%; mp 170–173 °C (ethanol). Anal. $C_{14}H_{22}N_2O_2S$ (C, H, N, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 7.20 and 6.64 (2 bs, 4 H, 2 NH_2), 6.57 (s, 2 H, H-3, -4), 2.70 (t, 4 H, CH_2-δ, -δ'), 2.08 (t, 4 H, CH_2-α, -α'), 1.56 (m, 8 H, CH_2-β, -β', -γ, -γ'). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 173.1 (s, 2 C, CO), 140.5 (s, 2 C, C-2, -5), 121.8 (d, 2 C, C-3, -4), 33.2 (t, 2 C, C-α, -α'), 29.2 (t, 2 C, C-δ, -δ'), 27.7 (t, 2 C, C-γ, -γ'), 23.0 (t, 2 C, C-β, -β').

6.3.22. 5-(5-Amino-5-oxopentyl)-2-thiopheneheptanamide ***5b***

Colourless crystals. Yield 88%; mp 171–172 °C. Anal. $C_{16}H_{26}N_2O_2S$ (C, H, N, S). ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.22 and 6.68 (2 bs, 4 H, $CONH_2$), 6.59 (s, 2 H, H-3, -4), 2.75–2.63 (m, 4 H, CH_2-ζ, -1-pentyl), 2.10–1.98 (m, 4 H, CH_2-α, -4-pentyl), 1.64–1.20 (m, 12 H, CH_2-β,-γ, -δ, -ε, -2-, -3-pentyl). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.3 and 174.1 (2 s, 2 C, $CONH_2$), 142.4 and 142.1 (2 s, 2 C, C-2, -5), 123.7 and 123.7 (2 d, 2 C, C-3, -4), 35.1 and 34.7 (2 t, 2 C, C-ζ, -1-pentyl), 31.1, 30.8, 29.3, 29.2, 28.4, 28.2 (6 t, 6 C, C-α, -γ, -δ, -ε, -2-, -4-pentyl), 25.0 and 24.5 (2 t, 2 C, C-β, -3-pentyl).

6.3.23. 2,5-Thiophenediheptanamide ***5d***

Colourless crystals. Yield 80%; mp 167–170 °C (CH_3OH). Anal. $C_{18}H_{30}N_2O_2S$•0.21H_2O (C, H, N, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 6.94 and 6.20 (2 bs, 4 H, 2 $CONH_2$), 6.52 (s, 2 H, H-3, -4), 2.71 (t, 4 H, CH_2-ζ, -ζ'), 2.15 (t, 4 H, CH_2-α, -α'), 1.70–1.46 (m, 8 H, CH_2-β, -β', -ε, -ε'), 1.46–1.20 (m, 8 H, CH_2-γ, -γ', -δ, -δ'). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.4 (s, 2 C, $CONH_2$), 142.3 (s, 2 C, C-2, -5), 123.7 (d, 2 C, C-3, -4), 35.1 (t, 2 C, C-ζ, -ζ'), 31.1 (t, 2 C, C-α, -α'), 29.4 (t, 2 C, C-ε, -ε'), 28.4 and 28.3 (2 t, 4 C, C-γ, -γ', -δ, -δ'), 25.1 (t, 2 C, C-β, -β').

6.3.24. 5-(4-Amino-4-oxobutyl)-2-thiopheneoctanamide ***5e***

Colourless crystals. Yield 84%; mp 165–169 °C. Anal. $C_{16}H_{26}N_2O_2S$•0.10H_2O (C, H, N, S). ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.24 and 6.70 (2 m, 4 H, $CONH_2$), 6.60 (s, 2 H, H-3, -4), 2.69 (t, 4 H, CH_2-η, -1-propyl), 2.13–1.99 (m, 4 H, CH_2-α, -3-propyl), 1.78 (m, 2 H, CH_2-2-propyl), 1.67– 1.20 (m, 10 H, CH_2-β, -γ, -δ, -ε, -ζ). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.4 and 173.9 (2 s, 2 C, $CONH_2$), 142.5 and 141.9 (2 s, 2 C, C-2,

-5), 123.9 and 123.7 (2 d, 2 C, C-3, -4), 35.1 (t, 1 C, C-η), 34.3 (t, 1 C, C-3-propyl), 31.2 (t, 1 C, C-α), 29.4 (t, 1 C, C-ζ), 29.0 (t, 1 C, C-1-propyl), 28.6, 28.5 and 28.4 (3 t, 3 C, C-γ, -δ, -ε), 27.1 (t, 1 C, C-2-propyl), 25.1 (t, 1 C, C-β).

6.3.25. 2,5-Thiophenedioctanamide 5f

Colourless crystals. Yield 74%; mp 155–156 °C (CH_3OH). Anal. $C_{20}H_{34}N_2O_2S \cdot 1.39H_2O$ (C, H, N, S). ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.21 and 6.67 (2 bs, 4 H, 2 $CONH_2$), 6.58 (s, 2 H, H-3, -4), 2.69 (t, 4 H, CH_2-η, -η'), 2.02 (t, 4 H, CH_2-α, -α'), 1.80–1.07 (m, 20 H, CH_2-β, -β', -γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ'). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.3 (s, 2 C, $CONH_2$), 142.3 (s, 2 C, C-2, -5), 123.6 (d, 2 C, C-3, -4), 35.1 (t, 2 C, C-η, -η'), 31.2 (t, 2 C, C-α, -α'), 29.4 (t, 2 C, C-ζ, -ζ'), 28.6, 28.5 and 28.4 (3 t, 6 C, C-γ, -γ', -δ, -δ', -ε, -ε'), 25.1 (t, 2 C, C-β, -β').

6.3.26. 5-(3-Amino-3-oxopropyl)-2-thiophenenonanamide 5g

Colourless crystals. Yield 59%; mp 160–170 °C. Contained 7% unsaturated diamide. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.34, 7.22, 6.81 and 6.68 (4 bs, 4 H, NH_2), 6.59 (s, 2 H, H-3, -4), 2.92 (t, 2 H, CH_2-θ), 2.69 and 2.38 (t, 2 H, CH_2-1- and -2-propyl), 2.02 (t, 2 H, CH_2-α), 1.63–1.20 (m, 12 H, CH_2-β, -γ, -δ, -ε, -ζ, -η). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.3 and 173.0 (2 s, 2 C, $CONH_2$), 142.6 and 141.3 (2 s, 2 C, C-2, -5), 123.8 and 123.6 (2 d, 2 C, C-3, -4), 36.7 and 35.1 (2 t, 2 C, C-α and C-2-propyl), 29.3, 28.7, 28.7 and 28.4 (4 t, 5 C, C-γ, -δ, -ε, -ζ, -η), 25.2 and 25.1 (2 t, 2 C, C-β, -1-propyl).

6.3.27. 2,5-Thiophenedidecanamide 5h

Colourless crystals. Yield 78%; mp 143–144 °C (CH_3OH). Anal. $C_{24}H_{42}N_2O_2S \cdot 0.6NaHCO_3 \cdot 0.7H_2O$ (C, H, N, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 7.09 and 6.45 (2 bs, 4 H, 2 $CONH_2$), 6.53 (s, 2 H, H-3, -4), 2.70 (t, 4 H, CH_2-ι, -ι'), 2.10 (t, 4 H, CH_2-α, -α'), 1.70–1.42 (m, 8 H, CH_2-β, -β', -θ, -θ'), 1.42–1.20 (m, 20 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'). ^{13}C-NMR (DMSO-d_6): δ (ppm) = 174.3 (s, 2 C, $CONH_2$), 142.3 (s, 2 C, C-2, -5), 123.6 (d, 2 C, C-3, -4), 35.1 (t, 2 C, C-ι, -ι'), 31.2 (t, 2 C, C-α, -α'), 29.3, 28.8, 28.7, 28.7 and 28.4 (5 t, 12 C, C-γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η', -θ, -θ'), 25.1 (t, 2 C, C-β, -β').

6.3.28. 2,5-Thiophenedihexanamine, dihydrochloride 6d

To a suspension of 400 mg (1.29 mmol) of powdered **5c** under N_2 in 50 mL dry THF was dropped 19 mL (19.0 mmol) of 1 N $LiAlH_4$ in THF. The reactants were stirred for 30 min at room temperature and then refluxed overnight under N_2. The cooled reaction mixture was hydrolized in an ice bath with 1.5 mL saturated aqueous potassium sodium tartrate solution, concentrated to 20% of its volume and filtered over celite (the flask was rinsed several times with CH_2Cl_2). The combined organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in dry CH_3OH, ethereal HCl was added, and the mixture was evaporated to dryness. This procedure was repeated and the dihydrochloride was precipitated with dry ether from dry CH_3OH. For purification, the product was heated in CH_3OH with charcoal for 10 min, filtered, evaporated, and dissolved in dry CH_3OH; the final product was precipitated with dry ether. Pale brown crystals **6d**. Yield 66%; mp > 250 °C (decomposition). Anal. $C_{16}H_{32}Cl_2N_2S \cdot 0.27C_4H_{10}O$ (C, H, N). ^{1}H-NMR (D_2O): δ (ppm) = 6.72 (s, 2 H, H-3, -4), 3.03 (t, 4 H, CH_2-α, -α'), 2.81 (t, 4 H, CH_2-ζ, -ζ'), 1.70 (m, 8 H, CH_2-β, -β', -ε, -ε'), 1.42 (m, 8 H, CH_2-γ, -γ', -δ, -δ'). ^{13}C-NMR (D_2O): δ (ppm) = 145.8 (s, 2 C, C-2, -5), 126.1 (d, 2 C, C-3, -4), 41.8 (t, 2 C, C-α, -α'), 33.4 (t, 2 C, C-ζ, -ζ'), 31.8 (t, 2 C, C-ε, -ε'), 30.3 (t, 2 C, C-β, -β'), 29.1 (t, 2 C, C-δ, -δ'), 27.9 (t, 2 C, C-γ, -γ').

6.4. An alternative acylation step in the preparation of 5-(4-methoxy-1,4-dioxobutyl)-2-thiopheneoctanoic acid, methylester 3e

A suspension of 1.201 g (12.0 mmol) succinic anhydride in 30 mL CH_2Cl_2 was cooled to 10 °C and 3.33 g (25 mmol) dry $AlCl_3$ was added. **2d** (2.5 g, 10.4 mmol) was added with stirring such that the temperature did not exceed 12 °C. Stirring was continued at room temperature, till TLC showed full consumption of the educt (3 h). The content of the flask was poured onto a mixture of 20 g crushed ice and 10 mL concentrated HCl. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined and washed three times with 2 N HCl and once with water and brine. The washed layers were re-extracted with CH_2Cl_2, the organic layers combined, dried over sodium sulphate, filtered, and evaporated to dryness. The residue was treated with $CH_3OH/SOCl_2$ according to the procedure given for the synthesis of **2b**. The crude reaction product was purified by CC (eluent: PE/E = 1:1, 300 g SG). Colourless crystals **3e**. Yield 58%; mp 39.5–41.5 °C. Anal. $C_{18}H_{26}O_5S \cdot 0.39H_2O$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.60 (d, 1 H, H-4), 6.82 (d, 1 H, H-3), 3.70 (s, 3 H, CH_3O-4-butyl), 3.67 (s, 3 H, CH_3O-octanoic acid meth.), 3.21 (t, 2 H, CH_2-2-butyl), 2.82 and 2.75 (2 t, 4 H, CH_2-η, -3-butyl), 2.31 (t, 2 H, CH_2-α), 1.76– 1.55 (m, 4 H, CH_2-β, -ζ), 1.40–1.26 (m, 6 H, CH_2-γ, -δ, -ε). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 190.6 (s, 1 C, CO), 174.2 and 173.2 (2 s, 2 C, $COOCH_3$), 155.7 (s, 1 C, C-5), 141.0 (s, 1 C, C-2), 132.3 (d, 1 C, C-4), 125.5 (d, 1 C, C-3), 51.8 and 51.4 (2 q, 2 C, CH_3O), 34.0 and 33.4 (2 t, 2 C, C-η, -2-butyl), 31.2 and 30.5 (2 t, 2 C, C-α, -3-butyl), 28.9, 28.8, 28.7 and 28.0 (4 t, 4 C, C-γ, -δ, -ε, -ζ), 24.8 (t, 1 C, C-β).

6.5. Alternative synthetic steps leading to 4g

6.5.1. 5-Formyl-2-thiophenenonanoic acid, methylester 7

2.34 mL (25.1 mmol) $POCl_3$ was added to 15 mL DMF in an ice bath, followed by dropwise addition of a solution of 5.00 g (19.7 mmol) **2e** in 15 mL DMF. After heating for 2.5 h, the mixture was cooled, a further 0.2 mL (2,1 mmol) $POCl_3$ was added dropwise, and refluxing was continued for another hour. The reaction mixture was cooled and poured onto ice, the pH adjusted to 6.0 with 2 N NaOH, and the aqueous layer was thoroughly extracted with ether. The organic layers were combined, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by VFC (eluent: gradient of PE/E = 4:1 to 1:1, 180 g SG) and dried at 50 °C in vacuo. Yellow crystals **7**. Yield 45%; mp 60–61 °C. Anal. $C_{15}H_{22}O_3S$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 9.81 (s, 1 H, CHO), 7.60 (d, 1 H, H-4), 6.90 (d, 1 H, H-3), 3.67 (s, 3 H, CH_3O), 2.87 (t, 2 H, CH_2-θ), 2.30 (t, 2 H, CH_2-α), 1.78–1.54 (m, 4 H, CH_2-β, -η), 1.42– 1.24 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 182.6 (d, 1 C, CHO), 174.2 (s, 1 C, *C*OOCH$_3$), 157.6 (s, 1 C, C-5), 141.5 (s, 1 C, C-2), 137.0 (d, 1 C, C-4), 125.8 (d, 1 C, C-3), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-α), 31.1 (t, 1 C, C-θ), 30.7 (t, 1 C, C-η), 29.0 and 28.8 (2 t, 4 C, C-γ, -δ, -ε, -ζ), 24.8 (t, 1 C, C-β).

6.5.2. (E)-5-(2-Carboxyethenyl)-2-thiophenenonanoic acid, methylester 8

2.08 g (7.37 mmol) **7**, 3.83 g (36.8 mmol) malonic acid, 25 mL pyridine and 1 mL piperidine were heated for three hours in an oil bath to 130 °C, poured onto a mixture of ice and 2 N HCl (pH 1), and collected by filtration. The residue was dissolved in ether, dried over Na_2SO_4, filtered, and the product was crystallized from the resulting clear solution by cooling.

Colourless crystals **8**. Yield 93%; mp 101–102 °C. Anal. $C_{17}H_{24}O_4S \cdot 0.22H_2O$ (C, H, S). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.80 (d, J = 15.5 Hz, 1 H, H-1-ethenyl), 7.11 (d, 1 H, H-4), 6.73 (d, 1 H, H-3), 6.10 (d, 1 H, H-2-ethenyl), 3.67 (s, 3 H, CH_3O), 2.81 (t, 2 H, CH_2-θ), 2.30 (t, 2 H, CH_2-α), 1.75–1.54 (m, 4 H, CH_2-β, -η), 1.40–1.25 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.3 (s, 1 C, $COOCH_3$), 172.2 (s, 1 C, COOH), 151.1 (s, 1 C, C-5), 139.7 (d, 1 C, C-1-ethenyl), 136.9 (s, 1 C, C-2), 132.2 (d, 1 C, C-4), 125.4 (d, 1 C, C-3), 114.3 (d, 1 C, C-2-ethenyl), 51.4 (q, 1 C, CH_3O), 34.0 (t, 1 C, C-α), 31.3 (t, 1 C, C-θ), 30.5 (t, 1 C, C-η), 29.0 and 28.9 (2 t, 4 C, C-γ, -δ, -ε, -ζ), 24.8 (t, 1 C, C-β).

6.5.3. (E)-5-(2-Carboxyethenyl)-2-thiophenenonanoic acid ***9***

2.00 g (6.16 mmol) **8** and 15 mL (30 mmol) 2 N NaOH in 25 mL CH_3OH were refluxed with stirring for 90 min, acidified with 2 N HCl to pH 1, and the precipitate collected. The product was dried under reduced pressure at 50 °C and, without further purification, used in the following reaction. Colourless crystals **9**. Yield 98%; mp 170–172.5 °C (CH_3OH). Anal. $C_{16}H_{22}O_4S \cdot 0.13H_2O$ (C, H, S). ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 8.00–7.40 (m, 3 H, incl: bs 2 H, COOH and 7.67, d, J = 15.5 Hz, 1 H, H-1-ethenyl), 7.06 (d, 1 H, H-4), 6.72 (d, 1 H, H-3), 6.07 (d, 1 H, H-2-ethenyl), 2.80 (t, 2 H, CH_2-θ), 2.26 (t, 2 H, CH_2-α), 1.74–1.52 (m, 4 H, CH_2-β, -η), 1.42–1.26 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 175.1 (s, 1 C, COOH), 167.9 (s, 1 C, HOOC-ethenyl), 149.2 (s, 1 C, C-5), 136.8 (d, 1 C, C-1-ethenyl), 136.5 (s, 1 C, C-2), 130.6 (d, 1 C, C-4), 124.7 (d, 1 C, C-3), 115.4 (d, 1 C, C-2-ethenyl), 33.5 (t, 1 C, C-α), 30.6 (t, 1 C, C-θ), 29.7 (t, 1 C, C-η), 28.4 and 28.2 (2 t, 4 C, C-γ, -δ, -ε, -ζ), 24.2 (t, 1 C, C-β).

6.5.4. 5-(3-Methoxy-3-oxopropyl)-2-thiophenenonanoic acid, methylester ***10g****, and 5-(2-Carboxyethyl)-2-thiophenenonanoic acid* ***4g***

A suspension of 1.54 g (4.96 mmol) **9** in 200 mL CH_3OH/EA was added dropwise to 100 g 3% Na/Hg. Glacial acetic acid was added to pH 5, and stirring continued to the end of H_2 evolution (2 h, educt consumption about 50%). Hg was separated using a separating funnel, the organic layer was evaporated, the residue taken up in 200 mL 50% aqueous CH_3OH and treated with Na/Hg for two further hours. After removal of Hg, the solution was acidified with concentrated phosphoric acid and thoroughly extracted with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated to dryness. Yield: 1.50 g (97%) colourless crystals **4g**, crude. Purification of **4g** via the dimethyl ester **10g** according to the procedure given for **2b**: 1.50 g (4.80 mmol) **4g**, crude, 1.14 g (9.60 mmol) $SOCl_2$, 30 mL dry CH_3OH, purification: distillation, CC: eluent: PE/EA = 5:1, 80 g SG. Colourless crystals **10g** (+ 7% unsaturated diester). Yield 71%; bp 130 °C (0.015 mbar). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.60 and 6.54 (2 d, 2 H, H-3, -4), 3.69 and 3.68 (2 s, 6 H, CH_3O), 3.09 (t, 2 H, CH_2-θ), 2.77–2.63 (m, 4 H, CH_2-1, -2-propyl), 2.30 (t, 2 H, CH_2-α), 1.70–1.53 (m, 4 H, CH_2-β, -η), 1.40–1.25 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 174.2 (s, 1 C, $COOCH_3$), 172.9 (s, 1 C, CH_3OOC-$(CH_2)_2$-thiophene, 144.0 (s, 1 C, C-2), 140.4 (s, 1 C, C-5), 124.0 and 123.5 (2 d, 2 C, C-3, -4), 51.6 and 51.4 (2 q, 2 C, CH_3O), 35.9 (t, 1 C, C-2-propyl), 34.0 (t, 1 C, C-α), 31.6 (t, 1 C, C-θ), 30.0 (t, 1 C, C-η), 29.1, 29.1 and 29.0 (3 t, 4 C, C-γ, -δ, -ε, -ζ), 25.4 (t, 1 C, C-1-propyl), 24.9 (t, 1 C, C-β). Hydrolysis: 1.00 g (2.94 mmol) **10g**, 15 mL 3 N aqueous NaOH, 15 mL CH_3OH.

Colourless crystals **4g** (+ 7% **9**). Yield 97%; mp 115–116.5 °C. ^{1}H-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 9.90 (bs, 2 H, COOH), 6.50 and 6.43 (2 d, 2 H, H-3, -4), 2.94 (t, 2 H, CH_2-θ), 2.60 and 2.50 (2 t, 4 H, CH_2-1- and 2-ethyl), 2.15 (t, 2 H, CH_2-α), 1.60–1.40 (m, 4 H, CH_2-β, -η), 1.30–1.12 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR ($CDCl_3$/DMSO-d_6): δ (ppm) = 175.6 (s, 1 C, COOH), 174.0 (s, 1 C, HOOC-ethyl), 143.2 (s, 1 C, C-2), 140.2 (s, 1 C, C-5), 123.4 and 122.9 (2 d, 2 C, C-3, -4), 35.4 (t, 1 C, C-2-ethyl), 33.6 (t, 1 C, C-α), 31.0 (t, 1 C, C-θ), 29.5 (t, 1 C, C-η), 28.6, 28.6, 28.5 and 28.4 (4 t, 4 C, C-γ, -δ, -ε, -ζ), 24.8 (t, 1 C, C-1-ethyl), 24.3 (t, 1 C, C-β).

6.6. Alternative preparation of 2,5-thiophenedibutanamine, dihydrochloride ***6a***

565 mg (3.54 mmol) bromine was added dropwise to an aqueous solution of 1061 mg (18.9 mmol) KOH, with constant stirring and cooling, followed by addition of 500 mg (1.77 mmol) **5a**. Stirring was continued for 30 min at room temperature, and the mixture was finally refluxed, until the reaction was complete. The aqueous layer was extracted with chloroform, the organic layers combined, washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in dry ether, the dihydrochloride precipitated with ethereal HCl, filtered, washed with dry ether, and dried in vacuum at 50 °C. Colourless crystals **6a**. Yield 30%; mp > 300 °C. Anal. $C_{12}H_{24}Cl_2N_2S$ (C, H, N, S). ^{1}H-NMR (D_2O): δ (ppm) = 6.62 (s, 2 H, H-3, -4), 2.91 (t, 4 H, CH_2-α, -α'), 2.72 (t, 4 H, CH_2-δ, -δ'), 1.60 (m, 8 H, CH_2-β, -β', -γ, -γ'). ^{13}C-NMR (D_2O): δ (ppm) = 145.5 (s, 2 C, C-2, -5), 126.6 (d, 2 C, C-3, -4), 41.6 (t, 2 C, C-α, -α'), 31.0 (t, 2 C, C-δ, -δ'), 30.2 (t, 2 C, C-β, -β'), 28.5 (t, 2 C, C-γ, -γ').

6.7. Data of characterization of the diamines ***6b,c,e,f,g,h,i*** *(for* ***6a*** *and* ***6d****, see above)*

6.7.1. 2,5-Thiophenedipentanamine, dihydrochloride ***6b***

Pale brown crystals. Yield 79%; mp > 230 °C (decomposition). Anal. $C_{14}H_{28}Cl_2N_2S \cdot 0.35C_4H_{10}O$: (C, H, N, S). ^{1}H-NMR (D_2O): δ (ppm) = 6.61 (s, 2 H, H-3, -4), 2.91 (t, 4 H, CH_2-α, -α'), 2.71 (t, 4 H, CH_2-ε, -ε'), 1.69–1.25 (m, 12 H, CH_2-β, -β', -γ, -γ', -δ, -δ'). ^{13}C-NMR (D_2O): δ (ppm) = 145.8 (s, 2 C, C-2, -5), 126.3 (d, 2 C, C-3, -4), 41.8 (t, 2 C, C-α, -α'), 32.8 (t, 2 C, C-ε, -ε'), 31.4 (t, 2 C, C-δ, -δ'), 28.9 (t, 2 C, C-β, -β'), 27.5 (t, 2 C, C-γ, -γ').

6.7.2. 5-(5-Aminopentyl)-2-thiopheneheptanamine, dihydrochloride ***6c***

Colourless crystals. Yield 63%; mp 198–215 °C (decomposition). Anal. $C_{16}H_{32}Cl_2N_2S$ (C, H, N, S). ^{1}H-NMR (D_2O/DMSO-d_6): δ (ppm) = 6.52 (s, 2 H, H-3, -4), 2.79 (t, 4 H, CH_2-α, -5-pentyl), 2.70–2.53 (m, 4 H, CH_2-η, -1-pentyl), 1.60–1.37 (m, 8 H, CH_2-β, -ζ, -2-, -3-pentyl), 1.35– 1.15 (m, 8 H, CH_2-γ, -δ, -ε, -4-pentyl). ^{13}C-NMR (D_2O/DMSO-d_6): δ (ppm) = 144.8 and 144.2 (2 s, 2 C, C-2, -5), 125.5 and 125.4 (2 d, 2 C, C-3, -4), 40.7 and 40.6 (2 t, 2 C, C-α, -5-pentyl), 32.4, 31.9, 30.7, 30.4, 29.4, 29.4, 28.1, 27.8, 27.0 and 26.5 (10 t, 10 C, C-β, -γ, -δ, -ε, -ζ, -η, -1-, -2-, -3-, -4-pentyl).

6.7.3. 2,5-Thiophenediheptanamine, dihydrochloride ***6e***

Colourless crystals. Yield 81%; mp > 300 °C (decomposition). Anal. $C_{18}H_{36}Cl_2N_2S$ (C, H, N, S). ^{1}H-NMR (D_2O): δ (ppm) = 6.60 (s, 2 H, H-3, -4), 2.92 (t, 4 H, CH_2-α, -α'), 2.70 (t, 4 H, CH_2-η, -η'), 1.70–1.45 (m, 8 H, CH_2-β, -β', -ζ, -ζ'), 1.30 (m, 12 H, CH_2-γ, -γ', -δ, -δ', -ε, -ε'). ^{13}C-NMR (D_2O/DMSO): δ (ppm) = 144.8 (s, 2 C, C-2, -5), 125.2 (d, 2 C, C-3, -4), 41.0 (t, 2 C, C-α, -α'), 32.8 (t, 2 C, C-η, -η'), 31.2 (t, 2 C, C-ζ, -ζ'), 30.0 and 29.9 (2 t, 4 C, C-β, -β', -δ, -δ'), 28.5 (t, 2 C, C-ε, -ε'), 27.5 (t, 2 C, C-γ, -γ').

6.7.4. 5-(4-Aminobutyl)-2-thiopheneoctanamine, dihydrochloride ***6f***

Colourless crystals. Yield 51%; mp 238–244 °C (decomposition). Anal. $C_{16}H_{32}Cl_2N_2S$ (C, H, N). ^{1}H-NMR (D_2O/DMSO-d_6): δ (ppm) = 6.66 (m, 2 H, H-3, -4), 3.02–2.86 (m, 4 H, CH_2-α, -4-butyl), 2.86–2.65 (m, 4 H, CH_2-θ, -1-butyl), 1.74–1.50 (m, 8 H, CH_2-β, -η, -2-butyl, -3-butyl), 1.43–1.20 (m, 8 H, CH_2-γ, -δ, -ε, -ζ). ^{13}C-NMR (D_2O/DMSO-d_6): δ (ppm) = 145.9 and 144.1 (2 s, 2 C, C-2, -5), 125.8 and 125.4 (2 d, 2 C, C-3, -4), 41.1 and 40.8 (2 t, 2 C, C-α, -4-butyl), 32.6 (t, 1 C, C-θ), 30.9 (t, 1 C, C-η), 30.3, 29.9, 29.8 and 29.4 (4 t, 5 C, C-β, -δ, -ε, -1-butyl, -3-butyl), 28.3, 27.7 and 27.2 (3 t, 3 C, C-γ, -ζ, -2-butyl).

6.7.5. 2,5-Thiophenedioctanamine, dihydrochloride ***6g***

Colourless crystals. Yield 77%; mp 188 °C. Anal. $C_{20}H_{40}Cl_2N_2S$ (C, H, N, S). ^{1}H-NMR (D_2O): δ (ppm) = 6.50 (s, 2 H, H-3, -4), 2.91 (t, 4 H, CH_2-α, -α'), 2.62 (t, 4 H, CH_2-θ, -θ'), 1.90–1.00 (m, 24 H, CH_2-β, -β', -γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η'). ^{13}C-NMR (D_2O/DMSO-d_6): δ (ppm) = 144.8 (s, 2 C, C-2, -5), 125.5 (d, 2 C, C-3, -4), 41.5 (t, 2 C, C-α, -α'), 33.5 (t, 2 C, C-θ, -θ'), 31.9 (t, 2 C, C-η, -η'), 31.0 and 30.8 (2 t, 3 C, C-β, -β', -δ, -δ', -ε, -ε'), 29.0 (t, 1 C, C-ζ, -ζ'), 28.2 (t, 2 C, C-γ, -γ').

6.7.6. 5-(3-Aminopropyl)-2-thiophenenonanamine, dihydrochloride ***6h***

Colourless crystals. Yield 65%; mp > 300 °C (decomposition beginning at 190 °C). Anal. $C_{16}H_{32}Cl_2N_2S$ (C, H, N, S). ^{1}H-NMR (D_2O/DMSO-d_6): δ (ppm) = 6.55 and 6.49 (2 d, 2 H, H-3, -4), 2.88–2.52 (m, 8 H, CH_2-α, -ι, -1-, -3-propyl), 1.81 (m, 2 H, CH_2-2-propyl), 1.53–1.35 (m, 4 H, CH_2-β, -θ), 1.20–1.05 (m, 10 H, CH_2-γ, -δ, -ε, -ζ, -η). ^{13}C-NMR (D_2O/DMSO-d_6): δ (ppm) = 146.3 and 142.5 (2 s, 2 C, C-2, -5), 126.2 and 125.5 (2 d, 2 C, C-3, -4), 41.1 and 40.3 (2 t, 2 C, C-α, -3-propyl), 32.6, 30.9, 30.3, 30.1, 30.0, 29.8, 28.3, 27.8 and 27.2 (9 t, 10 C, CH_2-β, -γ, -δ, -ε, -ζ, -η, -θ, -ι, -1-, -2-propyl).

6.7.7. 2,5-Thiophenedidecanamine, dihydrochloride ***6i***

Colourless crystals. Yield 73%; mp 186 °C (decomposition). Anal. $C_{24}H_{48}Cl_2N_2S{\cdot}0.80H_2O$ (C, H, N, S). ^{1}H-NMR (D_2O/DMSO-d_6): δ (ppm) = 6.38 (s, 2 H, H-3, -4), 2.80 (t, 2 H, CH_2-α, -α'), 2.56 (t, 2 H, CH_2-κ, -κ'), 1.65–1.00 (m, 32 H, CH_2-β, -β', -γ, -γ', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η', -θ, -θ', -ι, -ι'). ^{13}C-NMR (D_2O/DMSO-d_6): δ (ppm) = 144.1 (s, 2 C, C-2, -5), 125.0 (d, 2 C, C-3, -4), 40.5 (t, 2 C, C-α, -α'), 32.7 (t, 2 C, C-κ, -κ'), 30.9, 30.4, 30.3, 29.9, 28.2 (5 t, 14 C, C-β, -β', -δ, -δ', -ε, -ε', -ζ, -ζ', -η, -η', -θ, -θ', -ι, -ι'), 27.2 (t, 2 C, C-γ, -γ').

6.8. Pharmacological evaluation

6.8.1. Membrane preparation

The preparation of neuronal membranes and the [^{3}H]MK-801 binding assay have been described elsewhere [28]. In short, male Wistar rats (age 3–6 month) were stunned and killed by decapitation, the brains removed and chilled in an ice/water mixture (– 0.5 °C). The part of the hippocampus comprising the CA1 and the dentate gyrus were dissected on a cold plate [29] and homogenized in cold 50 mM Tris acetate (pH 7.0) in a glass/teflon Potter-type homogenizer. The homogenate was centrifuged (10 min, 35.000 *g*) and resuspended in fresh buffer 4 times, after the second resuspension, Triton X-100 was added to 0.02%, and membranes were incubated in a 37 °C water bath for 10 min. The final suspension was stored in aliquots at – 80 °C for at least 24 h. Before the assay, an aliquot was thawn, diluted, and centrifuged once more.

6.8.2. [^{3}H]MK-801 binding assay

The binding assay was conducted in polypropylene vials (duplicates) containing 5 nM [^{3}H]MK-801 (22–30 Ci/mMol, NEN), 1 μM glutamic acid, 1 μM glycine, and 50 mM Tris-acetate (pH 7.0), in a final volume of 1 mL at 23–24 °C (water bath) for 2 h, and terminated by dilution and filtration through glass fibre filters (GF-C). For assessment of non-specific binding, glutamic acid and glycine were replaced by their respective antagonists D-aminophosphonovaleric acid (10 μM) and 5,7-dichlorokynurenic acid (1 μM, both obtained from Tocris). Filters were rinsed 3 times with the buffer, shaken in warmed (40 °C) toluene based scintillation liquid for 1 h and radioactivity quantified in a liquid scintillation counter.

6.8.3. Data analysis

Concentration-dependent inhibition of [^{3}H]MK-801 binding was computer-fitted to the function $y = B_0 \times (IC_{50})^{n_H} / [x^{n_H} + (IC_{50})^{n_H}] + NB$, where y is the amount of specifically bound radioligand at various concentrations x of the (inhibitory) test compound, NB the amount of radioligand bound non-specifically, B_0 the specific binding at $x = 0$, and n_H is the Hill coefficient.

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