Monatshefte für Chemie 135, 1539–1556 (2004)
DOI 10.1007/s00706-004-0218-2

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Allosteric Modulators of the Tertiary Alkanebisamino-Type. Variation of the Substitution of the Middle Chain Nitrogens

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Received April 16, 2004; accepted April 29, 2004
Published online July 23, 2004

Summary. Synthesis pathways of high flexibility for variously substituted alkanebisamine-type allosteric modulators of muscarinic receptors capable of passing the blood-brain barrier were developed starting either from *N*,*N*′-(hexane-1,6-diyl)bistosylamide or adipic acid chloride. Pharmacological evaluation of some representative compounds revealed the allosteric potency to fall in a submicromolar range.

Keywords. Alkanebisamine-type compounds; M_2-receptors; Structure-activity relationships; Drug research.

Introduction

Allosteric agents are capable of influencing the binding of agonists and antagonists to the orthosteric site of a receptor protein. The orthosteric site is the receptor domain where the endogenous messenger compound binds. Allosteric modulators are known for a variety of receptors, such as the muscarine, purine, histamine, and benzodiazepine receptors [1–4]. The treatment of *Alzheimer*'s disease, pain, or organophosphate poisoning may take advantage of the use of modulators of the muscarinic acetylcholine receptors enhancing the equilibrium binding of either agonists or antagonists.

Muscarinic allosteric modulators described so far [5] are structurally divergent and many of them are characterized by being positively charged, *e.g.* alcuronium-,

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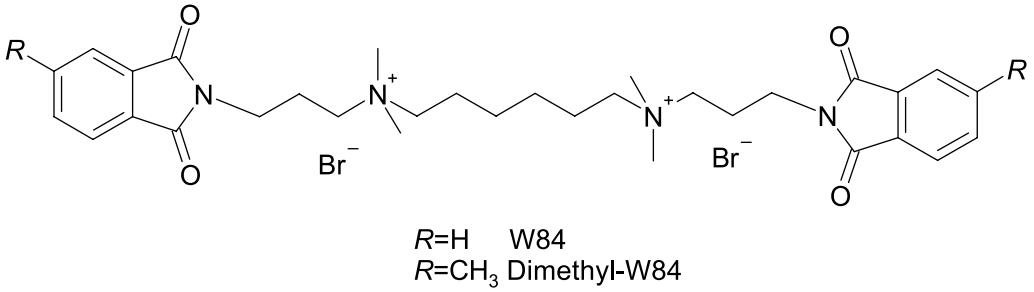

Fig. 1. Structural formulae of the hexamethonio-type lead compounds W84 and dimethyl-W84

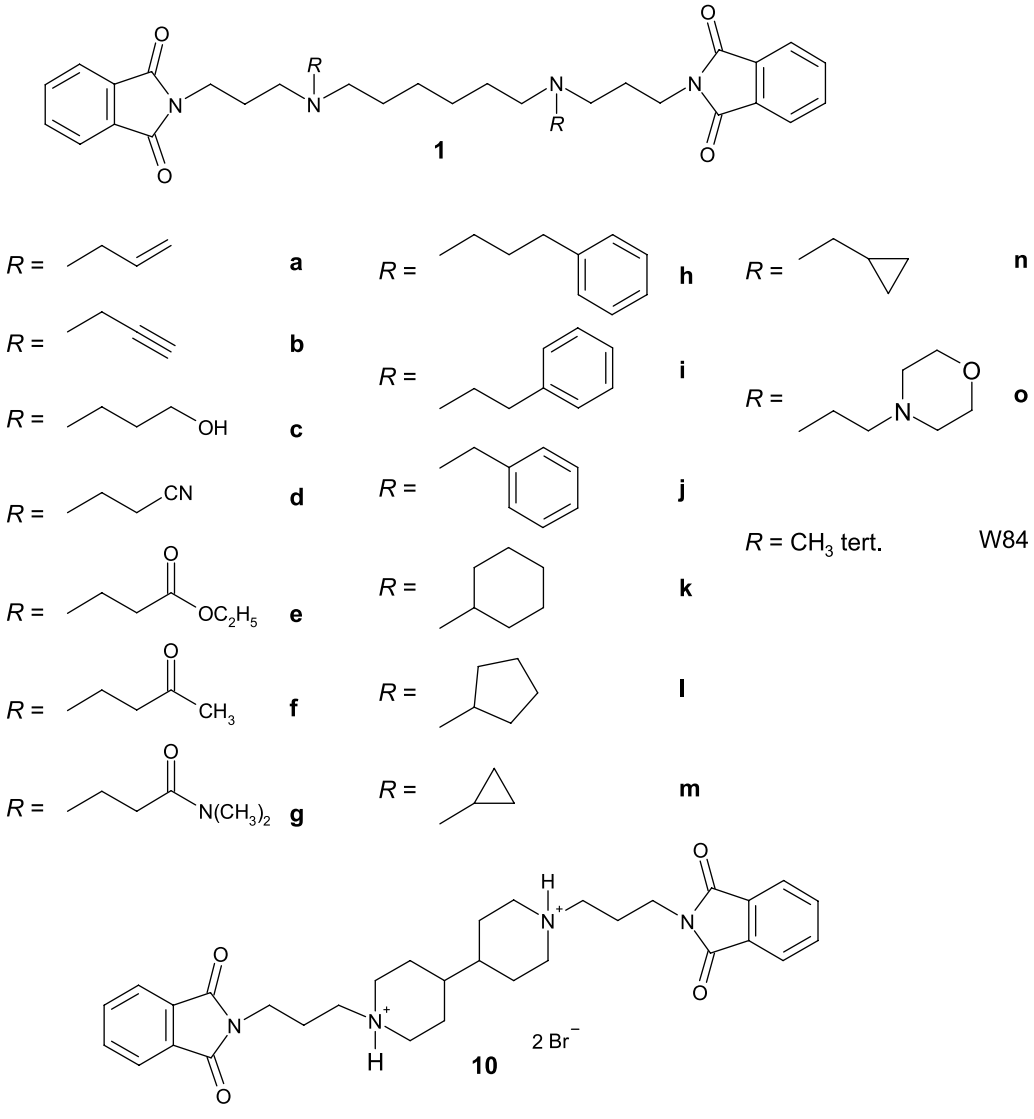

Fig. 2. Structural formulae of the alkanebisamino compounds

caracurine-, and strychnine-like compounds [6–10], tubocurarine [11], gallamine [12], alkane-bisammonium-type derivatives such as W84 (see Fig. 1) [13, 14], Chin3/6 [15], and bispyridinium-type derivatives, *e.g. WDUO* and *IWDUO* compounds [16]. The treatment of *Alzheimer*'s disease and pain requires drugs being able to pass the blood-brain barrier. Due to their permanent positive charge(s), the aforementioned compounds are not appropriate for these purposes. Thus, the aim of this study was to synthesize tertiary alkane-bisphthalimidopropylamine compounds with varying substituents attached to both nitrogens of the middle chain (see Fig. 2).

Results and Discussion

Synthesis

In a first approach it was tried to synthesize the compounds *via* an alkylation of the secondary analogue of W84. In order to obtain the secondary W84, bromopropylphthalimide was treated with hexanediamine in polar solvents. Despite

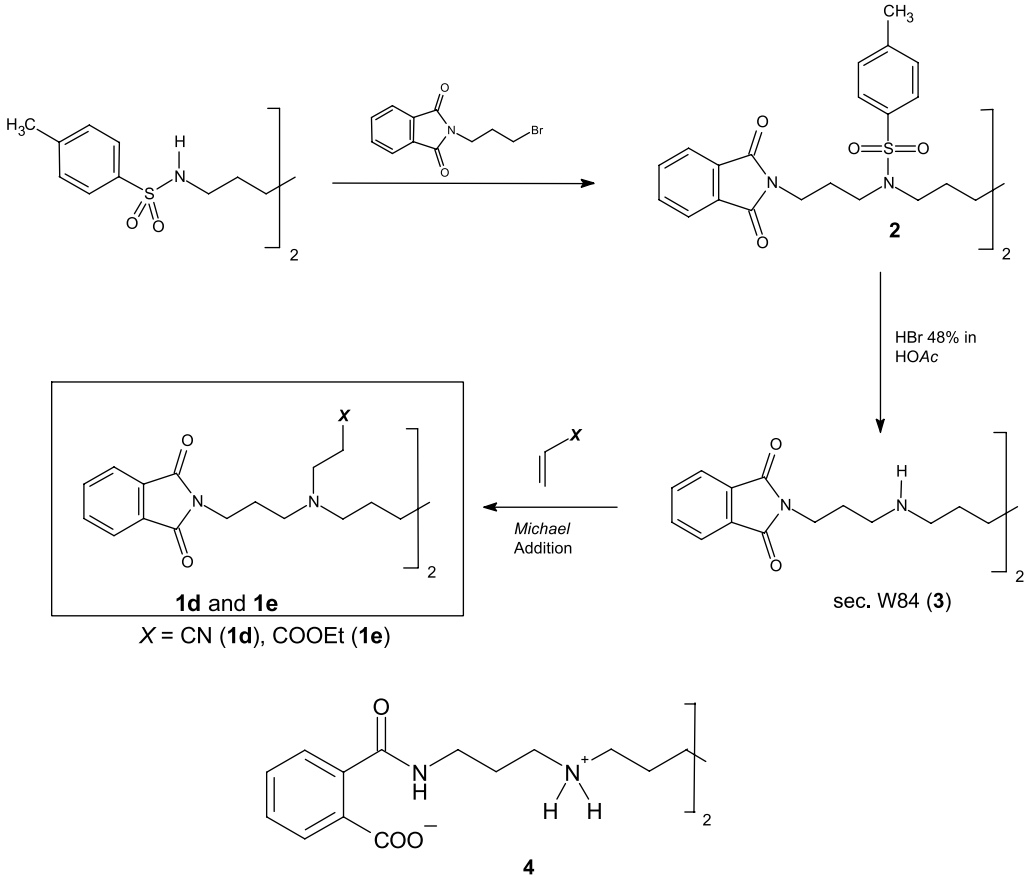

Fig. 3. Synthesis pathway of compounds **1d** and **1e**

many variations of the reaction solvent and time the phthalic acid **4** could be isolated only (see Fig. 3). Thus, the Hinsberg method was applied starting with the synthesis of the *N*,*N*′-hexane-1,6-diyl-bistosylamide (**2**) from hexane-1,6-diamine and tosylchloride in pyridine. The obtained diamide was alkylated by means of bromopropylphthalimide to give **2** and the tosyl group was eliminated using 48% HBr in glacial acid to obtain W84 **3**. In order to introduce varying alkyl groups, the hydrobromide had to be carefully converted into the base. It was impossible to alkylate the base with corresponding alkylhalogenides because again ring-opening resulting in the corresponding phthalic acid occurred in all cases. However, alkylation *via Michael* addition using acrylonitrile and ethyl acrylate gave the cyanoethyl and the ethyl propionate substituted compounds **1d** and **1e**.

Since the alkylation of the secondary W84 was not successful another synthesis strategy had to be applied. Reaction of adipoyl chloride with the corresponding amine in pyridine resulted in the amides **5h**–**5o** in a short reaction time and in very high yields. The bisamides **5** could be reduced using diborane-*THF* to give the hexanebisamines **6h**–**6o** in rather good yields. The hexanebisamines **6a** and **6c** were obtained by amination of dibromohexane with allylamine and aminopropanol in acetonitrile (see Fig. 4). The compounds **6b**, **6f**, and **6g** having a propine, a

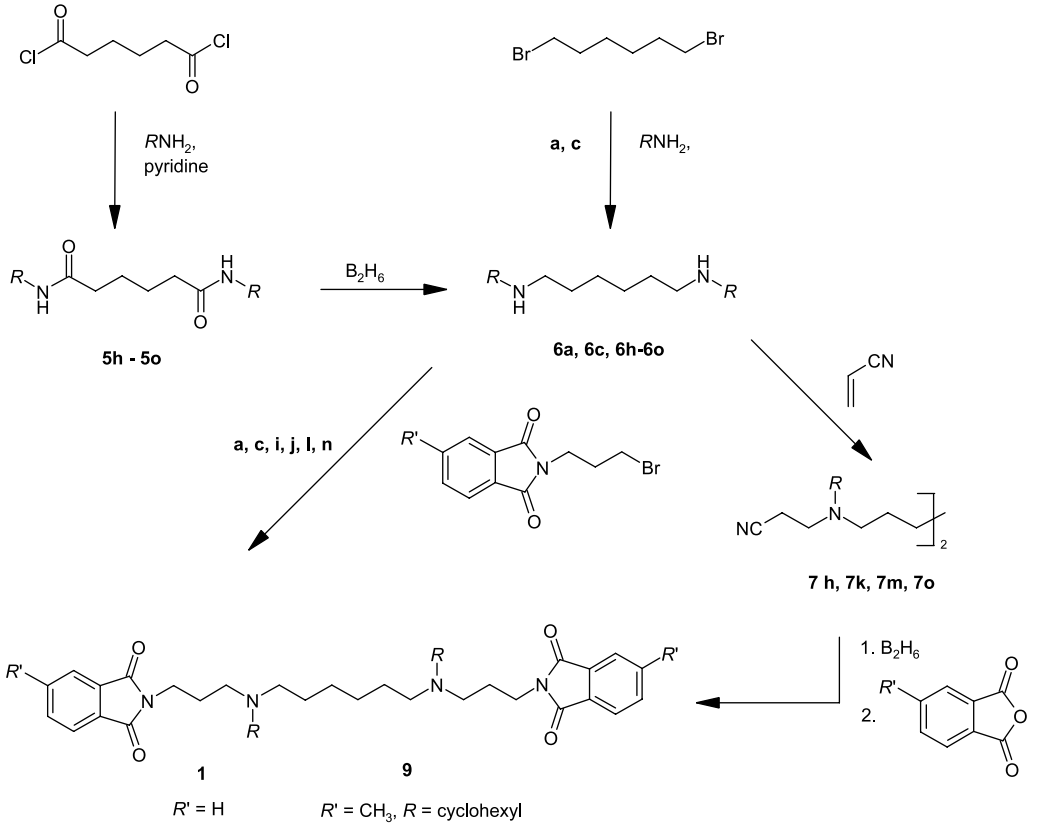

Fig. 4. Synthesis pathway of compounds **1a**, **1c**, and **1h**–**1n**

butanone, and a propionamide substituent could not be obtained on either synthesis pathway.

The tertiary bisamines **1a**, **1c**, **1i**, **1j**, **1l**, and **1m** were directly available by alkylation with bromopropylphthalimide in presence of an excess of anhydrous potassium carbonate in acetonitrile. In order to achieve high yields the reaction time had to be increased up to two weeks. For the synthesis of **1h**, **1k**, **1m**, and **1o** a circuitous route had to be used consisting of alkylation of the bisamine *via* a *Michael* addition with acrylonitrile to give **7h**, **7k**, **7m**, and **7o**, reduction of the nitrile by means of diborane-*THF* to yield the corresponding **8**, and forming the imide using phthalic acid anhydride. Using methylphthalic anhydride and cyclohexyl substituted **8k** compound **9** could be obtained (see Fig. 4). In addition, a compound was synthesized having the middle chain nitrogen incorporated in a piperidine ring. Therefore bipiperidine, which is characterized by an inter-nitrogen distance of six methylene groups, was alkylated by means of bromopropanephthalimide to give **10** in an acceptable yield (see Fig. 5).

Physicochemical Properties

Since the compounds have to be capable of passing the blood-brain barrier by passive diffusion they should be characterized by relatively high lipophilicity. Therefore, the lipophilicity was evaluated *via* the capacity factors in HPLC [17]. Using a RP-18 stationary phase and a mixture of methanol/phosphate buffer a calibration curve was established with references of similar lipophilicity. Thus, the $\log P$ values of **1a**, **1c**, **1d**, **1j**, **1k**, **1o**, and **10** were found to be in the range of 3 to 4.5 indicating an appropriate lipophilicity for passing the blood-brain barrier (see Table 2). The $\log P$ values of the other compounds were higher than 4.5.

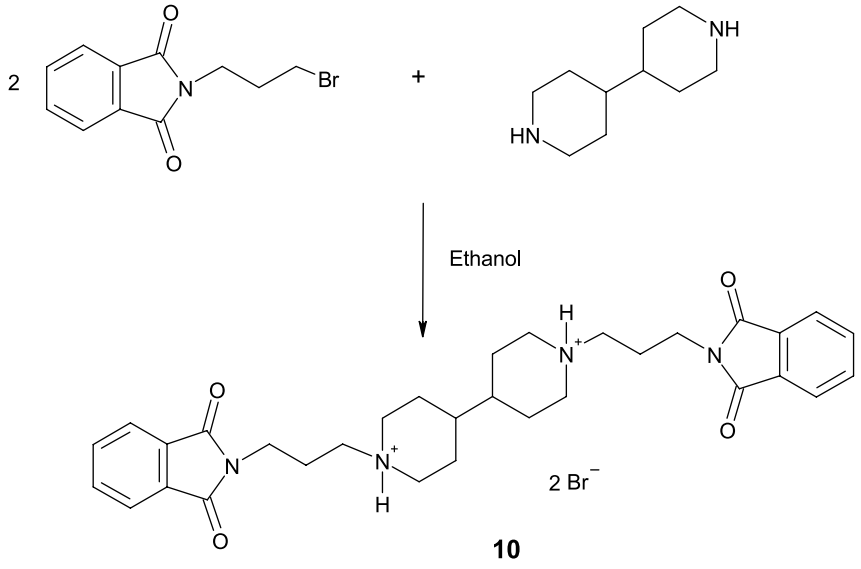

Fig. 5. Synthesis pathway of compound **10**

With respect to *Lipinski*'s rule of five [18], log *P* values higher than 5 will create problems in terms of solubility.

In order to estimate the pK_a values of the new compounds, the pK_a values of the model compounds dimethylaminopropylphthalimide and the secondary W84 were determined by means of a potentiometric method [25] using the microtitrator PCA 101. The pK_a values were found to be in the range of 9 to 10 indicating that 90% of the compounds will be protonated at *pH* 7.4 of the physiological media. The pK_a values of the other compounds could not be determined because they are not soluble over the entire pH range measured. However, the pK_a values are very likely to fall in the same range.

Taken together, it can be stated that the compounds having a log *P* lower than 4 will be soluble enough for pharmacological evaluation, especially due to the fact that they are protonated at *pH* 7.4. On the other hand they will be capable of passing the blood-brain barrier because a considerable fraction will be neutral and lipophilic.

Pharmacological Evaluation

The allosteric potency to inhibit the dissociation of the orthosteric agent [^{3}H]*N*-methylscopolamine ([^{3}H]*NMS*) was evaluated as described previously [19, 13]. In short, homogenates containing muscarinic M_2 receptors were prepared from porcine heart ventricles, incubated with [^{3}H]*NMS* in buffer (50 m*M Tris*-HCl, 3 m*M* $MgHPO_4$, *pH* 7.3, 37°C), and allosteric actions similar to those found under organ-bath conditions were observed. [^{3}H]*NMS*-dissociation was measured after addition of 1 μM atropine to displace the radioligand. Test compounds were added simultaneously with atropine. The concentration for a half-maximal inhibition of [^{3}H]*NMS*-dissociation, $EC_{50,diss}$, can be taken as a measure for the equilibrium dissociation constant of allosteric modulator binding to [^{3}H]*NMS*-occupied receptors [20]. Results of the binding experiments of compounds **1k** and **9** are displayed in Table 1 in comparison with W84, and the corresponding tertiary W84. In order

Table 1. Allosteric potencies of representative compounds; pEC_{50}: concentration for a half maximum inhibition of [^{3}H]*NMS* dissociation (mean values ± S.E.M from 3 to 7 independent experiments)

Entry	pEC_{50}
W84	6.08 ± 0.07
tert. W84	5.94 ± 0.04
Dimethyl-W84[a]	7.08 ± 0.10
1d	6.13 ± 0.18
1h	6.00 ± 0.09
1k	7.35 ± 0.04
2	4.56 ± 0.13[b]
9	7.21 ± 0.12
10	5.55 ± 0.08

[a] Data taken from Ref. [26]; [b] extrapolated value from a curve with the highest data point at 10^{-5} *M*

to speed up the pharmacological evaluation, compounds **1d**, **1h**, and **10** as well as the intermediate tosylamide **2** were checked at two concentrations (10^{-6} and 10^{-5} *M*) that allowed to estimate the concentration-effect-relationships.

Structure-Activity Relationships

The lead compounds W84 and dimethyl-W84 are characterized by pEC_{50} values of 6.08 and 7.08. Omitting one of the *N*-methyl groups at each of the two nitrogens in the middle chain of W84 yielding tertiary W84 resulted in almost the same allosteric potency. Thus, tertiary amino compounds appear to be as potent as the corresponding quaternary analogues. Even the secondary W84 exhibits an allosteric potency in the same range of concentration [21]. Thus, a permanent positive charge is not necessary for a high allosteric potency. However, **2**, whose nitrogens are not basic and, thus, unlikely to be protonated at *pH* 7.4, is more than tenfold less active than W84 suggesting that the secondary and tertiary compounds are protonated upon binding to the allosteric binding site.

Inclusion of the middle chain nitrogen in a piperidine ring (**10**) hardly influenced the allosteric potency. Similarly, attaching a cyanoethyl (**1d**) or a phenylpropyl group (**1h**) to the nitrogen did not affect the allosteric potency to inhibit [^{3}H]*NMS*-dissociation. In contrast, **1k** carrying a cyclohexyl ring was very potent with $pEC_{50} > 7$. In analogy to the pair W84/Dimethyl-W84 (see Fig. 1), methyl residues were attached to the lateral phthalimido moieties of **1k** yielding **9**. Remarkably, in this pair of compounds (**1k**/**9**) lateral ring methylation did not yield a further gain in activity. Due to low solubility not all of the compounds could be pharmacologically evaluated properly.

Conclusion

Synthesis pathways for bis(phthalimidopropyl)hexanebisamine compounds of varying substitution patterns on the middle chain nitrogens were opened. Thus, allosteric modulators are available, which are probably capable of passing the blood-brain barrier. A selection of compounds tested gave promising results indicating that changing the quaternary to tertiary nitrogens does not reduce the allosteric activity. Furthermore, the substituent attached to the tertiary nitrogen can enhance the allosteric potency.

Experimental

Melting points were determined with a Gallenkamp MPD350:BM3.5 melting point apparatus (Büchi, CH). ^{1}H (299.956 MHz) and ^{13}C (75.43 MHz) NMR spectra were recorded on a Varian XL 300 spectrometer (Varian Inc. Darmstadt, Germany). $CDCl_3$ was applied as the solvent and the center of the signals of $CDCl_3$ and *DMSO*-d_6 were used as internal references. IR spectra were obtained using a Perkin Elmer IR spectrometer 298. The pK_a values were recorded on a Sirius PCA 101 microtitrator (Sirius Analytical Instruments, Forest Row, East Sussex, UK). Elemental analyses were run on a microanalyzer (Leco 932, St. Josef, MI, USA). The results agreed favorable with the calculated values. Dry solvents were used throughout. Chemicals were of analytical grade and were purchased from Aldrich (Steinheim, FRG) or Acros (Schwerte, FRG).

N,N′-(Hexane-1,6-diyl)bistosylamide (**2**)

In analogy to Ref. [22] 5.8 g of 1,6-hexanediamine (50 mmol) were dissolved in 30 cm^3 of pyridine and 19.0 g of tosylchloride (100 mmol) were added under ice cooling. The solution was refluxed for 1 h, afterwards poured onto crushed ice, and acidified with conc. HCl. The obtained precipitate was filtered, washed several times with diluted HCl, and subsequently with H_2O. The product was recrystallized from ethanol to give 19.4 g of **2** (91%); ^{1}H NMR (*DMSO*-d_6): $\delta = 1.07$ (m, CH_2–C$\underline{H}_2$), 1.27 (m, CH_2–C$\underline{H}_2$), 2.36 (s, C$\underline{H}_3$), 2.68 (q, N–C$\underline{H}_2$), 7.35 (d, H_{ar}), 7.65 (d, H_{ar}) ppm.

N,N′-Bis-[(1,3-dioxo-1,3-dihydro-isoindol-2-yl)propyl]hexane-1,6-diammonium dihydrobromide (**3**, sec. W84, Ref. [23])

In analogy to Ref. [23] 4.0 g of **2** (5 mmol) and 2.8 g of phenol (30 mmol) were dissolved in 50 cm^3 of HBr (33% in glacial acid). The solution was stirred at 50°C under N_2 for 32 h and after 24 h a second portion of 10 cm^3 of HBr was added. In order to precipitate the product 200 cm^3 of diethyl ether were added under ice cooling. The obtained precipitate was filtered, and washed with H_2O, $Na_2S_2O_7$ solution (3%), H_2O, and hot methanol. Yield 2.68 g (82%); mp 276°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.30$ (m, N–CH_2–CH_2–C$\underline{H}_2$), 1.55 (m, N–CH_2–C$\underline{H}_2$), 1.96 (quin, N_{phthal}–CH_2–C$\underline{H}_2$), 2.86 (t), 2.95 (t, N–C$\underline{H}_2$), 3.64 (t, N_{phthal}–C$\underline{H}_2$), 7.86 (m, H_{ar}), 8.47 (s, N^+–$\underline{H}$) ppm; IR (KBr): $\bar{\nu} = 720$, 1040, 1380, 1700, 1770, 2780, 2930, 3460 cm^{-1}.

3-[[6-[(2-Cyanoethyl)[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]amino]hexyl][3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]amino]propionitril (**1d**, $C_{34}H_{40}N_6O_4$) *and Ethyl 3-[[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl][6-[[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl][2-(ethoxycarbonyl)ethyl]amino]hexyl]amino]propionate* (**1e**, $C_{38}H_{50}N_4O_8$)

In order to obtain the base 4 mmol of **3** dihydrobromide were suspended in $CHCl_3$ and stirred with anhydr. K_2CO_3 for 3 min under ice cooling. The solution was filtered and the solvent was evaporated *in vacuo*. The oil and 10 mmol of acrylonitrile or ethyl acryl carboxylate were dissolved in 30 cm^3 of ethanol and stirred for 24 h at room temperature. The solvent was evaporated *in vacuo* and the residue purified by means of column chromatography (silica gel, $CHCl_3$/*Me*OH/NH_3 = 132/12/1, $R_f = 0.9$). The obtained oil was dissolved in ethanol and perchloric acid (70%) was added till precipitation. The solution was heated for a few minutes and allowed to stand for 3 days at 5°C. The perchlorate was filtered off.

1d diperchlorate: Yield 64%; mp 191°C (dec.); ^{1}H NMR ($CDCl_3$): $\delta = 1.27$ (br, –C$\underline{H}_2$–), 1.39 (br, –C$\underline{H}_2$–), 2.43 (t, N–C$\underline{H}_2$–), 2.51 (t, N–C$\underline{H}_2$–), 1.79 (quin, –C$\underline{H}_2$–), 2.46 (t, N–C$\underline{H}_2$–), 2.77 (t, NC–C$\underline{H}_2$–), 3.72 (t, N_{pthth}–C$\underline{H}_2$–), 7.70 (m), 7.80 (m) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 16.29$, 26.48, 27.20, 27.20, 36.21, 49.59, 51.44, 53.50, 123.02, 118.91 (–$\underline{C}$≡N), 133.74, 132.00, 168.13 ppm; IR (KBr): $\bar{\nu} = 720$, 1040, 1395, 1610, 1705, 1770, 2250, 2860, 2940 cm^{-1}.

1e diperchlorate: Yield 51%; mp 132°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.21$ (t, O–CH_2–C$\underline{H}_3$), 1.22 (br, –C$\underline{H}_2$–), 1.36 (br, –C$\underline{H}_2$–), 1.78 (quin, –C$\underline{H}_2$–), 2.39 (t, N–C$\underline{H}_2$–), 2.46 (t, N–C$\underline{H}_2$–), 2.36 (t, N–C$\underline{H}_2$–), 2.74 (t, O=C–C$\underline{H}_2$–), 3.68 (t, N_{pthth}–C$\underline{H}_2$–), 4.07 (q, O–C$\underline{H}_2$–CH_3), 7.69 (m), 7.79 (m) ppm; ^{13}C NMR ($HClO_4$, *DMSO*-d_6): $\delta = 13.88$ (–$\underline{C}H_3$), 22.79, 25.35, 28.31, 34.66, 38.67, 47.58, 50.39, 52.22, 60.51, 122.76, 131.37, 134.11, 167.65, 169.62 ppm; IR (KBr): $\bar{\nu} = 720$, 1030, 1295, 1395, 1620, 1710, 1770, 2810, 2920 cm^{-1}.

2,2′[(1,6-Hexanediyl)bis(iminio-3,1-propanediyliminocarbonyl)]bis[benzoate] (**4**, $C_{28}H_{36}N_4O_6$)

Compound **4** is obtained as a by-product in the synthesis of **1d** and **1e** in varying yields. Mp 169°C (dec.); ^{1}H NMR (D_2O): $\delta = 1.44$ (br, N–CH_2–CH_2–C$\underline{H}_2$), 1.73 (m, N–CH_2–C$\underline{H}_2$), 1.98 (quin, O=C–

NH–CH_2–C$\underline{H}_2$), 3.06 (t, N–C$\underline{H}_2$), 3.15 (t, N–C$\underline{H}_2$), 3.45 (t, O=C–NH–C$\underline{H}_2$), 7.54 (m, ph–$\underline{H}$) ppm; ^{13}C NMR (D_2O): $\delta = 27.77, 27.97, 28.36, 39.19, 47.69, 50.18, 129.63, 130.56, 131.93, 132.97, 136.70, 140.32, 175.97, 177.89$ ppm; IR (KBr): $\bar{\nu} = 715, 770, 850, 1385, 1580, 2850, 3050, 3405\,cm^{-1}$.

Synthesis of Substituted Adipoyl Amides ***5h–5o***

The corresponding primary amine (0.1 mol) was dissolved in 30 cm^3 of pyridine, 7.3 cm^3 of adipoyl chloride (0.05 mol) were carefully added under ice cooling and the reaction mixture was stirred for 45 to 60 min. Three different work up procedures were applied.

Method A: The reaction mixture was poured into crushed ice and acidified with conc. HCl. The obtained precipitate was filtered off, washed several times with dil. aqueous HCl and H_2O, and recrystallised from a mixture of *Et*OH/H_2O.

Method B: The reaction mixture was poured into crushed ice, acidified with conc. HCl, and extracted three times with $CHCl_3$. The combined organic layers were dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The resulting oil was crystallized from *Et*OH/diethyl ether.

Method C: The reaction mixture was poured into crushed ice, alkalized with dil. NaOH, and extracted three times with $CHCl_3$. The combined organic layers were dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The white precipitate was suspended in hot diethyl ether and *Me*OH added until dissolution. The solution was allowed to stand for several hours at 5°C.

Hexanedioic acid bis[(3-phenylpropyl)amide] (**5h**, $C_{24}H_{32}N_2O_2$)

Method A. Yield 84%; mp 139°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.49$ (m, O=C–CH_2–C$\underline{H}_2$–), 2.07 (t, O=C–C$\underline{H}_2$–CH_2), 1.68 (quin, ph–CH_2–C$\underline{H}_2$), 2.55 (t, ph–C$\underline{H}_2$), 3.45 (q, HN–C$\underline{H}_2$), 7.18 (m, $\underline{H}$–ph), 7.74 (t, N–$\underline{H}$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 24.83, 30.83, 32.25, 35.26, 37.92, 125.58, 127.76, 127.92, 141.42, 171.50$ ppm; IR (KBr): $\bar{\nu} = 690, 1275, 1540, 1630, 2920, 3090, 3300\,cm^{-1}$.

Hexanedioic acid bis[(2-phenylethyl)amide] (**5i**, $C_{22}H_{28}N_2O_2$)

Method A. Yield 94%; mp 185°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.45$ (m, O=C–CH_2–C$\underline{H}_2$–), 1.98 (t, O=C–C$\underline{H}_2$–CH_2), 2.65 (t, ph–C$\underline{H}_2$), 3.21 (q, HN–C$\underline{H}_2$), 7.18 (m, $\underline{H}$–ph), 7.76 (t, N–$\underline{H}$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 25.28, 36.14, 40.78, 75.88, 126.43, 128.57, 128.70, 138.76, 165.64$ ppm; IR (KBr): $\bar{\nu} = 675, 725, 1180, 1245, 1530, 1615, 2920, 3050, 3290\,cm^{-1}$.

Hexanedioic acid bisbenzylamide (**5j**, $C_{20}H_{24}N_2O_2$)

Method A. Yield 90%; mp 190°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.55$ (m, O=C–CH_2–C$\underline{H}_2$–), 2.15 (t, O=C–C$\underline{H}_2$–CH_2), 4.26 (t, ph–C$\underline{H}_2$), 7.25 (m, $\underline{H}$–ph), 8.19 (t, N–$\underline{H}$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 24.91, 35.05, 41.90, 126.25, 126.81, 127.82, 139.35, 171.54$ ppm; IR (KBr): $\bar{\nu} = 670, 705, 1010, 1190, 1260, 1440, 1535, 1620, 2915, 3080, 3280\,cm^{-1}$.

Hexanedioic acid biscyclohexylamide (**5k**, $C_{18}H_{32}N_2O_2$)

Method A. Yield 89%; mp 244°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.13$ (m), 1.33 (m), 1.65 (m, O=C–CH_2–C$\underline{H}_2$–), 1.90 (m, C$\underline{H}_2$-ring), 2.16 (t, O=C–C$\underline{H}_2$–CH_2), 3.75 (m, HN–C$\underline{H}$-ring), 5.52 (t, N–$\underline{H}$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 24.97, 25.18, 25.61, 33.27, 36.54, 48.18, 171.61$ ppm; IR (KBr): $\bar{\nu} = 720, 1140, 1435, 1540, 1630, 2840, 2930, 3280\,cm^{-1}$.

Hexanedioic acid biscyclopentylamide (**5l**, $C_{16}H_{28}N_2O_2$)

Method A. Yield 90%; mp 228°C (dec.); ^{1}H NMR ($CDCl_3$): $\delta = 1.42$ (m), 1.65 (m, O=C–CH_2–C$\underline{H}_2$–), 1.96 (m), 2.21 (t, O=C–C$\underline{H}_2$–CH_2), 2.42 (m, C$\underline{H}_2$-ring), 4.18 (m, HN–C$\underline{H}$-ring), 6.02 (t, N–$\underline{H}$) ppm;

^{13}C NMR ($CDCl_3$): $\delta = 23.73$, 24.91, 33.03, 35.98, 51.56, 172.57 ppm; IR (KBr): $\bar{\nu} = 1200$, 1540, 1635, 2880, 2950, 3310 cm^{-1}.

Hexanedioic acid biscyclopropylamide (**5m**, $C_{12}H_{20}N_2O_2$)

Method B. Yield 72%; mp 181°C; ^{1}H NMR (*Me*OH-d_4): $\delta = 0.46$ (m), 0.68 (m, C$\underline{H}_2$-ring), 1.57 (m, O=C–CH_2–C$\underline{H}_2$–), 2.13 (m, O=C–C$\underline{H}_2$–CH_2), 2.63 (m, HN–C$\underline{H}$-ring) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 6.49$, 23.32, 26.52, 36.52, 23.32, 177.16 ppm; IR (KBr): $\bar{\nu} = 690$, 880, 1100, 1180, 1270, 1345, 1400, 1445, 1530, 1625, 2940, 3040, 3230 cm^{-1}.

Hexanedioic acid biscyclopropylmethylamide (**5n**, $C_{14}H_{24}N_2O_2$)

Method B. Yield 75%; mp 176°C (dec.); ^{1}H NMR ($CDCl_3$): $\delta = 0.17$ (m), 0.45 (m, C$\underline{H}_2$-ring), 0.91 (HN–C$\underline{H}$-ring) 1.66 (m, O=C–CH_2–C$\underline{H}_2$–), 2.21 (t, O=C–C$\underline{H}_2$–CH_2), 6.23 (t, N–$\underline{H}$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 3.45$, 10.75, 25.15, 36.14, 44.30, 172.62 ppm; IR (KBr): $\bar{\nu} = 1185$, 1240, 1355, 1525, 1610, 2920, 3290 cm^{-1}.

Hexanedioic acid bis[(2-morpholino-4-ylethyl)amide] (**5o**, $C_{18}H_{34}N_4O_4$)

Method C. Yield 71%; mp 160°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.61$ (m), 2.15 (br), 6.18 (br), 2.41 (m, N(C$\underline{H}_2$–)$_3$), 3.28 (q, HN–C$\underline{H}_2$), 3.64 (t, O(C$\underline{H}_2$–)$_2$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 25.04$, 35.58, 36.11, 53.25; 57.01, 66.77, 172.41 ppm; IR (KBr): $\bar{\nu} = 870$, 1110, 1260, 1545, 1635, 2810, 2940, 3295 cm^{-1}.

*Synthesis of Substituted Hexane-1,6-diamines **6h–6o***

The corresponding diamide **5h–5o** (2 mmol) was dissolved in 100 cm^3 of *THF* and B_2H_6 in *THF* (1 *M*, 20 mmol) added dropwise in 15 min under N_2 and ice cooling. The solution was stirred for 30 min at room temperature and for 1 h at 60°C. After cooling with ice 100 cm^3 of 2 *M* HCl were added, the resulting solution was refluxed for 30 min, after cooling with ice, 25 cm^3 of conc. NaOH solution were added and the aqueous solution was extracted with $CHCl_3$. The combined organic layers were washed with H_2O, dried (Na_2SO_4), and evaporated *in vacuo*. The colorless oil was dissolved in diethyl ether, the solution filtered, and evaporated *in vacuo* to give the oily **6** which was used without further purification.

N,N′-Bis(3-phenylpropyl)hexane-1,6-diamine (**6h**, $C_{24}H_{36}N_2$)

Yield 86%; ^{1}H NMR ($CDCl_3$): $\delta = 1.26$ (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.35 (m, N–CH_2–C$\underline{H}_2$–), 1.67 (quin, ph–CH_2–C$\underline{H}_2$), 2.45 (t, N–C$\underline{H}_2$–CH_2–), 2.05 (br, N–$\underline{H}$), 2.48 (t, N–C$\underline{H}_2$), 2.58 (t, ph–C$\underline{H}_2$), 7.20 (m, $\underline{H}$–ph) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 26.79$, 29.59, 31.31, 32.90, 48.73, 49.25, 125.21, 127.85, 127.91, 141.96 ppm; IR (KBr): $\bar{\nu} = 700$, 745, 1120, 1450, 1650, 2935, 3020, 3300 cm^{-1}.

N,N′-Bis(2-phenylethyl)hexane-1,6-diamine (**6i**, $C_{22}H_{32}N_2$)

Yield 83%; ^{1}H NMR ($CDCl_3$): $\delta = 1.32$ (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.48 (m, N–CH_2–C$\underline{H}_2$–), 1.89 (br, N–$\underline{H}$), 2.88 (m, N–C$\underline{H}_2$–C$\underline{H}_2$–ph), 2.64 (t, N–C$\underline{H}_2$), 7.31 (m, $\underline{H}$–ph) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 27.21$, 29.94, 36.31, 49.74, 51.14, 126.06, 128.40, 128.64, 140.04 ppm; IR (KBr): $\bar{\nu} = 700$, 745, 1120, 1455, 1605, 2860, 2920, 3030, 3250 cm^{-1}.

N,N′-Dibenzylhexane-1,6-diamine (**6j**, $C_{20}H_{28}N_2$)

Yield 79%; ^{1}H NMR ($CDCl_3$): $\delta = 1.33$ (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.51 (m, N–CH_2–C$\underline{H}_2$–), 1.51 (br, N–$\underline{H}$), 2.62 (t, N–C$\underline{H}_2$), 3.79 (s, –C$\underline{H}_2$–ph), 7.31 (m, $\underline{H}$–ph) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 27.32$, 30.10,

49.41, 54.08, 126.71, 127.96, 128.21, 140.40 ppm; IR (KBr): $\bar{\nu} = 700$, 745, 1170, 1380, 1450, 2920, 3300 cm^{-1}.

N,N′-Dicyclohexylhexane-1,6-diamine (**6k**, $C_{18}H_{36}N_2$)

Yield 91%; ^{1}H NMR ($CDCl_3$): $\delta = 1.13$ (m), 1.26 (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.43 (m, N–CH_2–C$\underline{H}_2$–), 1.57 (br, N–$\underline{H}$), 1.83 (m, C$\underline{H}_2$-ring), 2.34 (s, NH–C$\underline{H}_2$-ring), 2.55 (t, N–C$\underline{H}_2$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 25.06$, 26.10, 27.33, 30.21, 33.40, 46.80, 56.84 ppm; IR (KBr): $\bar{\nu} = 1170$, 1370, 1445, 1640, 2850, 2920, 3300 cm^{-1}.

N,N′-Dicyclopentylhexane-1,6-diamine (**6l**, $C_{16}H_{32}N_2$)

Yield 72%; ^{1}H NMR ($CDCl_3$): $\delta = 1.27$ (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.47 (m, N–CH_2–C$\underline{H}_2$–), 1.60 (m, C$\underline{H}_2$-ring), 2.14 (br, N–$\underline{H}$), 2.99 (m, NH–C$\underline{H}_2$-ring), 3.53 (t, C$\underline{H}_2$-ring) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 23.94$, 27.27, 30.09, 32.97, 48.56, 59.78 ppm; IR (KBr): $\bar{\nu} = 1070$, 1120, 1350, 1450, 1650, 2880, 2920, 3250 cm^{-1}.

N,N′-Dicyclopropylhexane-1,6-diamine (**6m**, $C_{12}H_{24}N_2$)

Yield 75%; ^{1}H NMR ($CDCl_3$): $\delta = 0.31$ (m), 0.41 (m, CH_2), 1.30 (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.46 (m, N–CH_2–C$\underline{H}_2$–), 1.76 (br, N–$\underline{H}$), 2.65 (m, NH–C$\underline{H}_2$-ring), 2.08 ppm (t, N–C$\underline{H}$-ring); ^{13}C NMR ($CDCl_3$): $\delta = 6.28$, 27.37, 30.07, 49.61, 30.41 ppm; IR (KBr): $\bar{\nu} = 890$, 1070, 1255, 1370, 1445, 1585, 2920, 3250 cm^{-1}.

N,N′-Biscyclopropylmethylhexane-1,6-diamine (**6n**, $C_{14}H_{28}N_2$)

Yield 76%; ^{1}H NMR ($CDCl_3$): $\delta = 0.42$ (m), 0.88 (m, CH_2), 1.30 (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.47 (m, N–CH_2–C$\underline{H}_2$–), 2.30 (br, N–$\underline{H}$), 2.41 (d, NH–C$\underline{H}_2$-ring), 2.58 (m, NH–C$\underline{H}_2$–CH_2), 3.56 (t, C$\underline{H}$-ring) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 3.41$, 11.17, 27.33, 30.00, 49.73, 55.00 ppm; IR (KBr): $\bar{\nu} = 890$, 1070, 1300, 1450, 1570, 2920, 3300 cm^{-1}.

N,N′-Bis(2-morpholino-4-ylethyl)hexane-1,6-diamine (**6o**, $C_{18}H_{38}N_4O_2$)

Yield 66%; ^{1}H NMR ($CDCl_3$): $\delta = 1.31$ (m, N–CH_2–CH_2–C$\underline{H}_2$–), 1.47 (m, N–CH_2–C$\underline{H}_2$–), 2.00 (br, N–$\underline{H}$), 2.41 (m, NH–C$\underline{H}_2$), 2.46 (m, $N_{morpholine}$–C$\underline{H}_2$), 2.57 (m, $N_{morpholine}$–C$\underline{H}_2$), 3.67 (t, O–CH_2) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 27.31$, 30.04, 46.10, 50.03, 53.73, 58.27, 66.96 ppm; IR (KBr): $\bar{\nu} = 1070$, 1110, 1450, 1650, 2920, 3300 cm^{-1}.

Synthesis of the Allylamine and Hydroxypropyl Substituted Hexanediamines **6a** *and* **6c**

Allylamine or 3-aminopropanol (20 mmol) was dissolved in 20 cm^3 of acetonitrile, 1.5 cm^3 of 1,6-dibromopropane (10 mmol) were added and it was stirred for 5 h at room temperature. The precipitate was filtered off and recrystallized from *Me*OH/diethyl ether.

N,N′-Diallylhexane-1,6-diamine (**6a** × **2HBr**, $C_{12}H_{26}Br_2N_2$)

Yield 1.5 g (21%); ^{1}H NMR (*DMSO*-d_6): $\delta = 1.29$ (br, N–CH_2–CH_2–C$\underline{H}_2$–), 1.60 (br, N–CH_2–C$\underline{H}_2$–), 2.84 (t, N–C$\underline{H}_2$–CH_2–), 3.56 (d, –C$\underline{H}_2$–CH=CH_2), 5.40 (dd, –CH_2–CH=C$\underline{H}_2$), 5.89 (m, –CH_2–C$\underline{H}$=CH_2), 8.77 ppm (br, N^+–$\underline{H}$); ^{13}C NMR (*DMSO*-d_6): $\delta = 25.24$, 25.60, 45.98, 48.75, 122.69 (–$\underline{C}$H=CH_2), 129.15 ppm (–$\underline{C}$H=CH_2); IR (KBr): $\bar{\nu} = 940$, 1430, 1630, 2800, 2940, 3400 cm^{-1}.

3-[6-(3-Hydroxypropylamino)hexylamino]propan-1-ol (**6c** × **2HBr**, $C_{12}H_{30}Br_2N_2O_2$)

Yield 3.0 g (38%); ^{1}H NMR (*DMSO*-d_6): $\delta = 1.29$ (br, N–CH_2–CH_2–C$\underline{H}_2$–), 1.59 (br, N–CH_2–C$\underline{H}_2$–), 2.87 (t, N–C$\underline{H}_2$–CH_2–), 1.74 q, –C$\underline{H}_2$–), 2.91 (t, N–CH_2), 3.44 (t, –CH_2–OH), 5.97 (br, N^+–$\underline{H}$) ppm; IR (KBr): $\bar{\nu} = 720$, 1250, 1430, 2800, 2930, 3400 cm^{-1}.

Cyanoethylation of the Substituted Hexanebisamine ***6*** *to the Bisnitriles* ***7h****,* ***7k****,* ***7m****, and* ***7o***

The corresponding hexanebisamines **6h**, **6k**, **6m**, and **6o** were dissolved in 30 cm^3 of ethanol and 0.66 cm^3 of acrylonitrile (10 mmol) dropwise added at 30°C. The reaction solution was allowed to stand overnight, afterwards the solvent was evaporated *in vacuo*, and the resulting oil purified by means of column chromatography (silica gel, mobile phase: $CHCl_3/MeOH/NH_3 = 132/12/1$).

3-[[6-[(2-Cyanoethyl)-(3-phenylpropyl)amino]hexyl]-(3-phenylpropyl)amino]propionitrile (**7h**, $C_{30}H_{42}N_4$)

Yield 89%; ^{1}H NMR ($CDCl_3$): $\delta = 1.28$ (br, N–CH_2–CH_2–C$\underline{H}_2$–), 1.32 (br, N–CH_2–C$\underline{H}_2$–), 1.68 (m, N–CH_2–C$\underline{H}_2$–), 2.38 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.40 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.48 (t, N–C$\underline{H}_2$–), 2.51 (t, ph–C$\underline{H}_2$–), 2.63 (t, NC–C$\underline{H}_2$), 7.19 (m, ph–$\underline{H}$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 16.23$, 27.24, 27.28, 28.95, 33.35, 49.51, 53.04, 53.57, 119.02, 125.61, 128.15, 128.22, 141.87 ppm; IR (KBr): $\bar{\nu} = 700$, 745, 1070, 1360, 1450, 1640, 2250, 2900 cm^{-1}.

3-[[6-[(2-Cyanoethyl)cyclohexylamino]hexyl]cyclohexylamino]propionitrile (**7k**, $C_{24}H_{42}N_4$)

Yield 68%; ^{1}H NMR ($CDCl_3$): $\delta = 1.00$–1.20 (m), 1.50–1.80 (m, –C$\underline{H}_2$-ring), 1.27 (br, N–CH_2–CH_2–C$\underline{H}_2$–), 1.38 (br, N–CH_2–C$\underline{H}_2$–), 2.36 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.41 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.43 (t, N–C$\underline{H}_2$-ring), 2.74 (t, NC–C$\underline{H}_2$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 18.93$, 26.11, 26.11, 26.27, 27.20, 29.32, 46.49, 50.75, 60.20, 119.08 ppm; IR (KBr): $\bar{\nu} = 890$, 1100, 1260, 1370, 1450, 2250, 2850, 2920 cm^{-1}.

3-[[6-[(2-Cyanoethyl)cyclopropylamino]hexyl]cyclopropylamino]propionitrile (**7m**, $C_{18}H_{30}N_4$)

Yield 58%; ^{1}H NMR ($CDCl_3$): $\delta = 0.43$ (m), 0.48 (m, –C$\underline{H}_2$-cycl.C_3H_5), 127 (br, N–CH_2–CH_2–C$\underline{H}_2$–), 147 (br, N–CH_2–C$\underline{H}_2$–), 1.75 (m, –CH-ring), 2.51 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.54 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.92 (t, NC–C$\underline{H}_2$) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 6.99$, 16.00, 27.06, 27.35, 35.92, 50.72, 55.07 ppm; IR (KBr): $\bar{\nu} = 750$, 1020, 1210, 1350, 1460, 2250, 2920 cm^{-1}.

3-[[6-[(2-Cyanoethyl)-(3-morpholin-4-ylpropxyl)amino]hexyl]-(3-morpholin-4-ylpropyl)amino]propionitrile (**7o**, $C_{24}H_{44}N_6O_2$)

Yield 68%; ^{1}H NMR ($CDCl_3$): $\delta = 1.27$ (br, N–CH_2–CH_2–C$\underline{H}_2$–), 1.40 (m, N–CH_2–C$\underline{H}_2$–), 2.42 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.42 (m, N–CH_2), 2.42 (m, N_{morph}–CH_2), 2.59 (t, –C$\underline{H}_2$–N–C$\underline{H}_2$), 2.79 (t, NC–C$\underline{H}_2$), 3.67 (t, O–CH_2) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 16.53$, 27.18, 27.41, 50.04, 51.04, 54.11, 54.31, 57.16, 66.86, 118.90 ppm; IR (KBr): $\bar{\nu} = 865$, 1070, 1215, 1300, 1450, 1650, 2250, 2920 cm^{-1}.

Reduction of Bisnitriles **7** *to Tertiary Spermine Analogues* **8**

The bisnitrils **7** (2 mmol) were dissolved in 100 cm^3 of *THF* and 20 cm^3 of B_2H_6 (1 *M*, *THF*) was added within 15 min under N_2 and cooling. The reaction mixture was stirred for 30 min at room temperature

and refluxed for 1 h. 100 cm^3 of 2 *M* HCl were added under ice cooling and the resulting solution was refluxed for 30 min. After cooling to room temperature 50 cm^3 of 3 *M* NaOH were added and the solution was extracted three times with $CHCl_3$. The combined organic layers were washed with H_2O, dried (Na_2SO_4), and the solvent was evaporated *in vacuo*. The resulting oil was dissolved in diethyl ether, filtered, and again evaporated *in vacuo* to give oily **8**, which was used for the next step without further purification.

N,N′-Bis(3-aminopropyl)-N,N′-bis(3-phenylpropyl)hexane-1,6-diamine (**8h**, $C_{30}H_{50}N_4$)

Yield 64%; ^{1}H NMR ($CDCl_3$): $\delta = 1.26$ (m), 1.40 (m), 1.59 (quin), 1.75 (quin), 2.41 (m), 2.59 (t), 2.75 (t), 2.90 (br, –N<u>H</u>$_2$), 7.18 (m, ph–<u>H</u>) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 26.25$, 26.45, 27.51, 28.86, 29.16, 32.12, 40.82, 48.38, 50.45, 59.55 ppm; IR (KBr): $\bar{\nu} = 700$, 745, 1155, 1450, 1600, 2860, 2940, 3360 cm^{-1}.

N,N′-Bis(3-aminopropyl)-N,N′-dicyclohexylhexane-1,6-diamine (**8k**, $C_{24}H_{50}N_4$)

Yield 84%; ^{1}H NMR ($CDCl_3$): $\delta = 1.11$ (m), 1.21 (m), 1.34 (m), 1.53 (m), 1.70 (br), 2.23 (br, –N<u>H</u>$_2$), 2.38 (m), 2.68 (t, 4H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 26.98$, 27.50, 28.63, 29.85, 33.75, 40.75, 52.12, 53.53, 53.87, 125.51, 128.09, 128.17, 142.12 ppm; IR (KBr): $\bar{\nu} = 895$, 1170, 1450, 1590, 2860, 2915, 3280 cm^{-1}.

N,N′-Bis(3-aminopropyl)-N,N′-dicyclopropylhexane-1,6-diamine (**8m**, $C_{18}H_{38}N_4$)

Yield 62%; ^{1}H NMR ($CDCl_3$): $\delta = 0.39$ (m), 0.45 (m), 1.23 (m), 1.44 (m), 1.65 (m), 2.48 (m), 2.88 (t), 3.17 (br, –N<u>H</u>$_2$), 3.55 (m) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 6.81$, 6.91, 15.91, 26.96, 27.27, 35.84, 50.63, 55.00 ppm; IR (KBr): $\bar{\nu} = 750$, 1020, 1210, 1350, 1460, 2920, 3320 cm^{-1}.

N,N′-Bis(3-aminopropyl)-N,N′-bis(3-morpholin-4-ylpropyl)hexane-1,6-diamine
(**8o**, $C_{24}H_{52}N_4O_2$)

Yield 57%; ^{1}H NMR ($CDCl_3$): $\delta = 1.28$ (br), 1.42 (br), 1.67 (m), 2.46 (m), 2.84 (m), 3.28 (br, –N<u>H</u>$_2$), 3.71 (m) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 27.51$, 27.53, 40.55, 54.07, 54.10, 54.21, 66.83, 66.87, 66.94, 67.04 ppm; IR (KBr): $\bar{\nu} = 865$, 1070, 1215, 1300, 1450, 1650, 2920, 3310 cm^{-1}.

Alkylation of Hexanebisamines **6** *with Bromopropylphthalimide to* ***1a***, ***1c***, ***1i***, ***1j***, ***1l***, *and* ***1n***

The corresponding hexanebisamine **6** (3.5 mmol) was dissolved in 20 cm^3 of acetonitrile and 1.5 g of K_2CO_3 were added. 1.88 g of Bromopropylphthalimide (7 mmol) were added and the suspension was stirred for 5 to 14 days at room temperature (TLC control on silica gel, mobile phase: $CHCl_3/MeOH/NH_3 = 132/12/1$, $R_f \approx 0.9$). After the reaction was completed the suspension was filtered, the solvent evaporated *in vacuo*, and the resulting oil purified by means of column chromatography (silica gel, mobile phase: $CHCl_3/MeOH/NH_3 = 132/12/1$). The oily bases were converted into salts using the following procedures.

A (*diperchlorate*): The corresponding base was dissolved in ethanol and conc. $HClO_4$ was added dropwise. When the first precipitate appeared, the solution was refluxed for a couple of min and the resulting clear solution was allowed to stand at 5°C for several days until crystallization.

B (*dihydrochloride*): The corresponding base was dissolved in acetone and eth. HCl solution was added dropwise. When the first precipitate appeared, the solution was refluxed for a couple of min and the resulting clear solution was allowed to stand at 5°C for several days until crystallization.

2-[3-[Allyl[6-[allyl[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1a**, $C_{34}H_{44}Cl_2N_4O_4$)

Crystallization method A or B. Yield 67%; mp 194°C (base); ^{1}H NMR (*DMSO*-d_6, dihydrochloride): $\delta = 1.11$ (br, –C$\underline{H}_2$–), 1.63 (br, –C$\underline{H}_2$–), 2.04 (m, –C$\underline{H}_2$–), 2.97 (m, N–C$\underline{H}_2$–), 3.09 (m, N–C$\underline{H}_2$–), 3.63 (t, N_{pthth}–C$\underline{H}_2$–), 3.73 (m, –C$\underline{H}_2$–CH=CH_2), 5.46 (m, –CH_2–CH=C$\underline{H}_2$), 5.94 (m, –CH_2–C$\underline{H}$=CH_2), 7.85 (m, CH–ph), 10.15 (br, N^+–$\underline{H}$) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 22.84$, 25.63, 35.00, 49.65, 51.61, 54.22, 123.23 (–CH=$\underline{C}H_2$), 125.02 (CH_{phth}), 127.39 (–$\underline{C}$H=CH_2), 131.88 (CH_{phth}), 134.61 (C_{phth}), 168.17 (C=O) ppm; IR (KBr): $\bar{\nu} = 680$, 910, 1030, 1110, 1300, 1370, 1680, 1740, 2670, 2870, 3420 cm^{-1}.

2-[3-[[[6-[[3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)propyl](3-hydroxypropyl)amino]hexyl](3-hydroxypropyl)]amino]propyl]isoindole-1,3-dione (**1c**, $C_{34}H_{48}Cl_2N_4O_6$)

Crystallization method A or B. Yield 69%; mp 210°C (dec. base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.25$ (br, –C$\underline{H}_2$–), 1.44 (br, –C$\underline{H}_2$–), 1.66 (quin, –C$\underline{H}_2$–), 1.85 (q, –C$\underline{H}_2$–), 2.41 (t, N–C$\underline{H}_2$–), 2.51 (t, N–C$\underline{H}_2$–), 2.62 (t, N–C$\underline{H}_2$), 3.69 (t, N_{pthth}–C$\underline{H}_2$–), 3.76 (t, HO–C$\underline{H}_2$), 4.84 (br, –O$\underline{H}$), 7.70 (m), 7.80 (m) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 25.82$, 26.46, 27.22, 27.95, 36.17, 51.45, 53.81, 54.12, 63.85, 123.10 (CH_{phth}), 131.98 (C_{phth}), 133.83 (CH_{phth}), 168.19 (C=O) ppm; IR (KBr, dihydrochloride): $\bar{\nu} = 700$, 1000, 1380, 1680, 1760, 2940, 3370 cm^{-1}.

2-[3-[[6-[[3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)propyl](3-phenylethyl)amino]hexyl](2-phenylethyl)amino]propyl]isoindole-1,3-dione (**1i**, $C_{44}H_{50}N_4O_4$)

Yield 55% oil (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.26$ (br, –C$\underline{H}_2$–), 1.43 (br, –C$\underline{H}_2$–), 1.66 (quin, –C$\underline{H}_2$–), 1.86 (q, –C$\underline{H}_2$–), 2.49 (t, N–C$\underline{H}_2$–), 2.59 (t, N–C$\underline{H}_2$–), 2.71 (br, N–C$\underline{H}_2$–C$\underline{H}_2$–ph), 3.72 (t, N_{pthth}–C$\underline{H}_2$–), 7.24 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 26.15$, 26.91, 27.42, 33.34, 36.46, 51.50, 53.74, 55.71, 123.06 (CH_{phth}), 125.79 (–$\underline{C}H_{phenyl}$), 128.67 (–$\underline{C}H_{phenyl}$), 128.21 (–$\underline{C}H_{phenyl}$), 132.12 (C_{phth}), 133.76 (CH_{phth}), 140.52 (C–$\underline{C}_{phenyl}$), 168.27 (C=O) ppm; IR (KBr): $\bar{\nu} = 715$, 1030, 1395, 1605, 1705, 1770, 2850, 2930, 3010 cm^{-1}.

2-[3-[Benzyl[6-[benzyl[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1j**, $C_{42}H_{48}Cl_2N_4O_4$)

Crystallization method B. Yield 34%; mp 246°C (dec. salt); ^{1}H NMR (*DMSO*-d_6 + D_2O, dihydrochloride): $\delta = 1.21$ (br, –C$\underline{H}_2$–), 1.59 (br, –C$\underline{H}_2$–), 1.92 (m, –C$\underline{H}_2$–), 2.98 (m, N–C$\underline{H}_2$–), 3.51 (br, N_{pthth}–C$\underline{H}_2$–), 4.35 (br, –CH_2–ph), 7.15 (m, CH–ph) ppm; ^{13}C NMR (*DMSO*-d_6 + D_2O, dihydrochloride): $\delta = 23.57$, 24.24, 26.60, 35.91, 49.98, 54.04, 57.95, 124.84 (CH_{phth}), 130.45, 130.64, 132.45 (–$\underline{C}H_{phenyl}$), 131.33 (C_{phth}), 131.96 (CH_{phth}), 136.40 (C–$\underline{C}_{phenyl}$), 170.56 (C=O) ppm; IR (KBr): $\bar{\nu} = 720$, 1030, 1395, 1450, 1610, 1705, 1770, 2930, 3020 cm^{-1}.

2-[3-[Cyclopentyl[6-[cyclopentyl[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1l**, $C_{38}H_{50}N_4O_4$)

Yield 51%; mp 60°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.22$ (br, –C$\underline{H}_2$–), 1.45 (m, C$\underline{H}_{2cycpent}$), 1.63 (br, –C$\underline{H}_2$–), 1.84 (quin, –C$\underline{H}_2$–), 2.51 (t, N–C$\underline{H}_2$–), 2.60 (t, N–C$\underline{H}_2$–), 2.96 (m, N–C$\underline{H}_{cyclpent}$), 3.68 (t, N_{pthth}–C$\underline{H}_2$–), 7.72 (m, CH–ph), 7.81 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 23.93$, 25.90, 26.67, 27.52, 29.73, 36.55, 49.07, 51.50, 63.72, 123.12 (CH_{phth}), 132.15 (C_{phth}), 133.82 (CH_{phth}), 168.30 (C=O) ppm; IR (KBr): $\bar{\nu} = 715$, 880, 1030, 1185, 1390, 1610, 1705, 1770, 2860, 2930 cm^{-1}.

2-[3-[Cyclopropylmethyl[6-[cyclopropylmethyl[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1n**, $C_{36}H_{48}Cl_2N_4O_{12}$)

Crystallization method A. Yield 52%; mp 68°C (dec. base); ^{1}H NMR ($CDCl_3$, base): $\delta = 0.08$ (m, C$\underline{H}_{2cycloprop}$), 0.45 (m, C$\underline{H}_{2cycloprop}$), 0.83 (m, –C$\underline{H}_{cycloprop}$), 1.26 (br, –C$\underline{H}_2$–), 1.42 (br, –C$\underline{H}_2$–), 1.84 (q, –C$\underline{H}_2$–), 2.34 (t, N–C$\underline{H}_2$), 2.50 (t, N–C$\underline{H}_2$–), 2.62 (t, N–C$\underline{H}_2$–), 3.72 (t, N_{pthth}–C$\underline{H}_2$–), 7.71 (m, CH–ph), 7.81 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 3.81$, 8.24, 25.77, 26.52, 27.37, 36.44, 51.15, 53.69; 58.45, 123.01 (CH_{phth}), 132.07 (C_{phth}), 133.71 (CH_{phth}), 168.25 (C=O) ppm; IR (KBr): $\bar{\nu} = 700$, 1380, 1705, 1760, 2930 cm^{-1}.

*Condensation of the Amines **7h**, **7k**, **7m**, and **7o** with Phthalic Anhydride to **1h**, **1k**, **1m**, **1o**, and **9***

The corresponding primary amine **7** (4 mmol) was dissolved in 60 cm^3 of toluene and 8 mmol of phthalic anhydride or methylphthalic anhydride were added. The solution was refluxed for 2 h using a water separator. Afterwards the solvent was evaporated *in vacuo*. The resulting oil was purified by means of column chromatography on silica gel (mobile phase: $CHCl_3/MeOH/NH_3 = 132/12/1$, $R_f \approx 0.9$).

2-[3-[[6-[[3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)propyl](3-phenylpropyl)amino]hexyl]-(2-phenylpropyl)amino]propyl]isoindole-1,3-dione (**1h**, $C_{46}H_{56}Cl_2N_4O_{12}$)

Crystallization method A. Yield 67%; mp 82°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.24$ (br, –C$\underline{H}_2$–), 1.73 (t, N–CH$_2$–C$\underline{H}_2$–), 1.81 (quin, –C$\underline{H}_2$–), 2.39 (t, N–C$\underline{H}_2$–), 2.44 (t, N–C$\underline{H}_2$–CH$_2$–), 2.50 (t, N–C$\underline{H}_2$–), 2.60 (t, ph–C$\underline{H}_2$–), 3.71 (t, N_{pthth}–C$\underline{H}_2$–), 7.70 (m, CH–ph), 7.81 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 26.19$, 26.97, 27.58, 28.76, 33.72, 36.58, 51.52, 53.40, 53.84, 122.99 (CH_{phth}), 125.47 (–C$\underline{H}_{phenyl}$), 128.09 (–C$\underline{H}_{phenyl}$), 128.21 (–C$\underline{H}_{phenyl}$), 132.03 (C_{phth}), 133.64 (CH_{phth}), 142.26 (C–C$\underline{}_{phenyl}$), 168.13 (C=O) ppm; IR (KBr): $\bar{\nu} = 720$, 1030, 1395, 1600, 1705, 1765, 2800, 2930, 3010 cm^{-1}.

2-[3-[Cyclohexyl[6-[cyclohexyl[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1k**, $C_{40}H_{56}Cl_2N_4O_4$)

Crystallization method B. Yield 72%; mp 104°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.24$ (br, –C$\underline{H}_2$–), 1.24 (m), 1.39 (br, –C$\underline{H}_2$–), 1.58 (d, –C$\underline{H}_{Cyclohexyl}$), 1.74 (m, –C$\underline{H}_{2Cyclohexyl}$), 1.80 (quin, –C$\underline{H}_2$–), 2.42 (t, N–C$\underline{H}_2$–), 2.54 (t, N–C$\underline{H}_2$–), 2.60 (t, ph–C$\underline{H}_2$–), 3.68 (t, N_{pthth}–C$\underline{H}_2$–), 7.69 (m, CH–ph), 7.79 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 26.23$, 26.23, 26.39, 27.46, 27.77, 28.92, 36.60, 48.10, 50.48, 60.08, 122.96 (CH_{phth}), 132.09 (C_{phth}), 133.64 (CH_{phth}), 168.14 (C=O) ppm; IR (KBr): $\bar{\nu} = 720$, 895, 1030, 1395, 1610, 1705, 1765, 2850, 2920 cm^{-1}.

2-[3-[Cyclopropyl[6-[cyclopropyl[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]amino]hexyl]amino]propyl]isoindole-1,3-dione (**1m**, $C_{34}H_{42}N_4O_4$)

Yield 53%; mp 100°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 0.42$ (m, C$\underline{H}_{2cycloprop}$), 1.22 (br, –C$\underline{H}_2$–), 1.45 (br, –C$\underline{H}_2$–), 1.65 (t, N–C$\underline{H}_{cycloprop}$), 1.88 (quin, –C$\underline{H}_2$–), 2.53 (t, N–C$\underline{H}_2$–), 2.65 (t, N–C$\underline{H}_2$–), 3.68 (t, N_{pthth}–C$\underline{H}_2$–), 7.72 (m, CH–ph), 7.81 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_3$, base): $\delta = 6.57$, 25.83, 26.55, 27.52, 29.82, 36.50, 36.62, 52.78, 55.39, 123.01 (CH_{phth}), 132.10 (C_{phth}), 133.71 (CH_{phth}), 168.25 (C=O) ppm; IR (KBr): $\bar{\nu} = 710$, 825, 890, 1010, 1180, 1375, 1705, 1770, 2910, 3005 cm^{-1}.

2-[3-[[6-[[3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)propyl]-(2-morpholin-4-ylethyl)amino]hexyl](2-morpholin-4-ylethyl)amino]propyl]isoindole-1,3-dione (**1o**, $C_{40}H_{58}Cl_2N_6O_{14}$)

Crystallization method A. Yield 56%; mp 93°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.23$ (br, –C$\underline{H}_2$–), 1.36 (br, –C$\underline{H}_2$–), 1.81 (quin, –C$\underline{H}_2$–), 2.45 (m, 3×N–C$\underline{H}_2$–), 3.69 (t, N_{pthth}–C$\underline{H}_2$–), 3.69 (m, O–C$\underline{H}_2$–), 7.70 (m, CH–ph), 7.80 (m, CH–ph) ppm; ^{13}C NMR ($CDCl_{3,\ base}$): $\delta = 26.22$, 27.02, 27.00, 36.52, 51.00, 52.08, 54.15, 54.50, 56.94, 66.89, 123.02 (CH_{phth}), 132.06 (C_{phth}), 133.72 (CH_{phth}), 168.13 (C=O) ppm; IR (KBr): $\bar{\nu} = 720$, 865, 1030, 1110, 1395, 1615, 1705, 1770, 2900, 2930 cm^{-1}.

2-[3-[Cyclohexyl[6-[cyclohexyl[3-(5-methyl-1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]amino]hexyl]amino]propyl]-5-methylisoindole-1,3-dione (**9**, $C_{42}H_{60}Cl_2N_4O_4$)

Crystallization method B. Yield 68%; mp 127°C (base); ^{1}H NMR ($CDCl_3$, base): $\delta = 1.18$ (m), 1.24 (br, –C$\underline{H}_2$–), 1.38 (br, –C$\underline{H}_2$–), 1.58 (d, $CH_{2cyclhex}$), 1.72 (m, $CH_{2cyclhex}$), 1.75 (quin, –C$\underline{H}_2$–), 2.40 (t, N–C$\underline{H}_2$–), 2.52 (t, N–C$\underline{H}_2$–), 3.66 (t, N_{pthth}–C$\underline{H}_2$–), 3.69 (m, O–C$\underline{H}_2$–), 7.46 (d, CH–ph), 7.61 (s, CH–ph), 7.68 (d, CH–ph) ppm; ^{13}C NMR ($CDCl_{3,\ base}$): $\delta = 22.00$ (–$\underline{C}H_3$), 26.27, 26.45, 27.51, 27.94, 29.00, 29.16, 36.56, 48.10, 50.51, 60.01, 122.86 (CH_{phth}), 123.52 (CH_{phth}), 134.13 (CH_{phth}), 129.52 (C_{phth}), 132.50 (C_{phth}), 144.80 (C_{phth}), 168.23 (C=O), 168.33 (C=O) ppm; IR (KBr, salt): $\bar{\nu} = 730$, 1030, 1385, 1435, 1705, 1760, 2860, 2940, 3420 cm^{-1}.

Synthesis of 1,1′-Bis[1,3-dioxo-1,3-dihydroisoindol-2-ylpropyl]-[4,4′]-bipiperidinyl-1,1′-diium dibromide (**10**, $C_{32}H_{40}Br_2N_4O_4$)

Bipiperidine dihydrochloride (1.5 g, 6.25 mmol) was suspended in 30 cm^3 of ethanol, potassium tert. butylate (1.48 g, 12.5 mmol) added and stirred for a couple of min. Afterwards the solution was filtered, *N*-(3-bromopropyl)phthalimide (3.35 g, 12.5 mmol) added and the solution refluxed for 15 h. After cooling the product crystallizes, the crystals were filtered off, washed with diethyl ether, and recrystallized from H_2O/ethanol. Yield 1.5 g (34% dihydrobromide); mp 280°C; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.33$ (m, C$\underline{H}_2$–C$\underline{H}_{bipiperidine}$), 1.85 (m, C$\underline{H}_{2bipiperidin}$), 2.81 (m, N^+–C$\underline{H}_2$), 3.09 (m, N^+–C$\underline{H}_2$), 3.48 (m, N^+–C$\underline{H}_2$), 3.64 (t, N_{phthal}–C$\underline{H}_2$), 7.87 (m, CH–ph), 9.10 (br, N^+–H) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 22.86$, 25.91, 34.72, 51.76, 53.47, 36.90, 122.75, 134.11 (C–$H_{aromat.}$), 131.45 ($\underline{C}q_{aromat.}$), 167.60 ($\underline{C}$=O) ppm; IR (KBr): $\bar{\nu} = 715$, 960, 1020, 1360, 1395, 1610, 1705, 1770, 2535, 2630, 2910, 3460 cm^{-1}.

Determination of the Lipophilicity

The test and reference compounds (2-phenylethylamine, 2-phenylethanol, benzene, *N*,*N*′-dimethylaniline, chlorobenzene, toluene, ethylbenzene, cumene, biphenyl, anthracene) were dissolved in methanol (4 µg/cm^3). Using a RP column (LiChroCart® 125-4 HPLC cartridge; LiChrospher® 100, RP 18, 5 µm, endcapped, Merck) and a mobile phase of methanol/phosphate buffer *pH* 7.4 = 70/30 (0.02% *N*,*N*-dimethylamine added) the retention times were determined and converted to k'-values according to Eq. (1) where T_R = retention time of the test compound and T_0 = hold-up time.

$$k' = (T_r - T_0)/T_0 \quad (1)$$

The log k'-values of the reference substances were correlated with the log *P* values reported in Ref. [24]. The calibration curve was established (see Fig. 6) and the log *P* values of the test compounds calculated. The log *P* values are summarized in Table 2.

Determination of the pK_a Values

The pK_a values were determined potentiometrically in water using a Sirius PCA 101 apparatus. Exactly 0.004 to 0.007 mg of the compounds were dissolved in 8–12 cm^3 of aqueous methanol

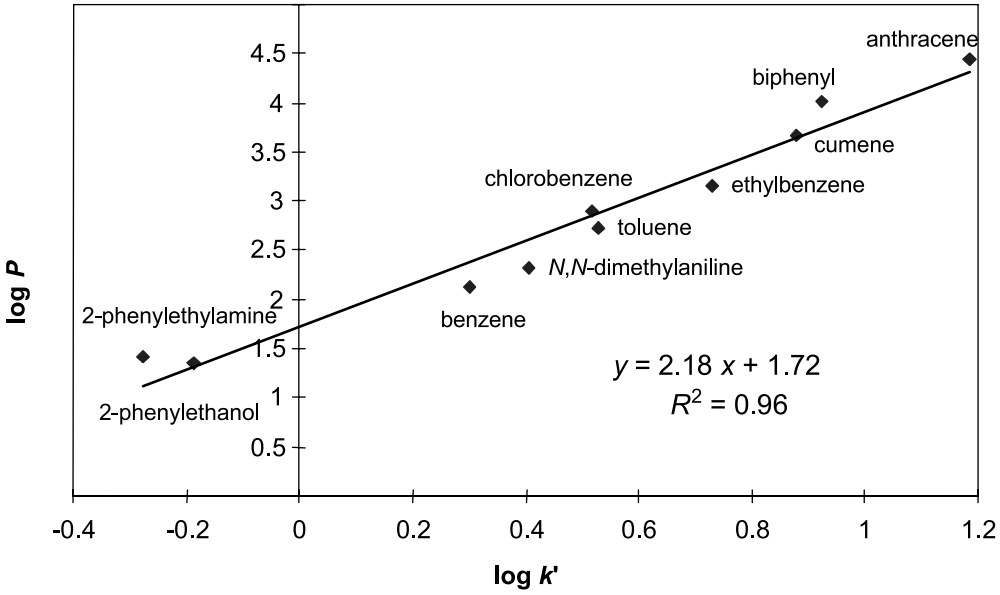

Fig. 6. Calibration curve for references' k' and log P values from Ref. [24]

Table 2. Lipophilicity values log P of representative compounds **1** and W84 derivatives

Entry	log k'	log P
sec. W84(3)	0.26	2.29
tert. W84	1.02	3.94
W84	−0.54	0.53
Dimethyl-W84	−0.21	1.25
1a	0.69	3.21
1c	0.64	3.11
1d	0.81	3.48
1j	1.15	4.22
1k	1.26	4.47
1l	2.08	6.25
1m	1.76	5.55
1n	1.76	5.56

(50%) and diluted to 20.0 cm^3 with a 0.15 *M* KCl. The titration was performed starting from p*H* 11. Using the *Yasuda-Shedlovsky* plot [25] the pK_a values were calculated and extrapolated to 0% methanol. The pK_a of dimethylaminopropylphthalimide was found to amount to 9.4 and that of tert. W84 to 9.0.

Acknowledgement

The skilful technical assistance of Ms. *I. Jusen* is gratefully acknowledged. Thanks are due to the DFG (HO 1368/7-1; MO 821/1-2) for financial support.

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