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Journal of Organometallic Chemistry 686 (2003) 75–83
Journal of Organometallic Chemistry
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Synthesis and pharmacological characterization of new silicon-based W84-type allosteric modulators for ligand binding to muscarinic M_2 receptors

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Received 25 March 2003; received in revised form 23 May 2003; accepted 23 May 2003

Abstract

The silicon-based allosteric modulators of ligand binding to muscarinic acetylcholine receptors [R^1–$(CH_2)_3$–$SiMe_2$–$(CH_2)_5$–NMe_2–$(CH_2)_3$–R^1]Br (**3**), [R^2–$(CH_2)_3$–$SiMe_2$–$(CH_2)_5$–NMe_2–$(CH_2)_3$–R^2]Br (**4**), [R^1–$(CH_2)_3$–$SiMe_2$–$(CH_2)_5$–NMe_2–$(CH_2)_3$–R^2]Br (**5**), and [R^2–$(CH_2)_3$–$SiMe_2$–$(CH_2)_5$–NMe_2–$(CH_2)_3$–R^1]Br (**6**) (R^1 = phthalimido; R^2 = 1,8-naphthalimido) were synthesized, starting from chlorodimethylsilane. Compounds **3**–**6** were studied for their allosteric interaction at porcine heart muscarinic M_2 receptors. They inhibited the dissociation of the orthosteric ligand [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) with similar potency; compounds **4** and **6** yielded steep concentration–effect curves. All compounds enhanced [^{3}H]NMS equilibrium binding, but with different efficacies. The effect of **4** on [^{3}H]NMS binding was studied at cloned M_1–M_5 receptor subtypes. Compound **4** did not affect [^{3}H]NMS equilibrium binding at M_1, M_3, M_4, and M_5 receptors, thus representing an M_2-selective allosteric enhancer of [^{3}H]NMS binding.

Keywords: Silicon; Silicon-based drugs; Allosteric modulators; Muscarinic receptors

1. Introduction

W84 (**1**) is an allosteric agent for the "common allosteric site" of muscarinic M_2 receptors, its allosteric action being characterized by an inhibition of the dissociation of the orthosteric ligand [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) [1]. Compared with drugs that bind to the orthosteric neurotransmitter binding site of the receptor protein, allosteric modulators offer novel therapeutic perspectives [1]. These include a subtype-selective enhancement of ligand–receptor interactions, which could be exploited therapeutically, for instance, in states of synaptic neurotransmitter deficiency. Recently, we have synthesized a series of silicon-based derivatives of **1**, such as compounds **2** and **3**, and have studied their allosteric interaction with porcine heart muscarinic M_2 receptors [2]. The parent drug W84 (**1**) decreases [^{3}H]NMS equilibrium binding (negative cooperativity), whereas the silicon compound **2** has been found to enhance [^{3}H]NMS equilibrium binding (positive cooperativity); i.e., exchange of one ammonium nitrogen atom in **1** by a silicon atom switches the allosteric action from negative to positive cooperativity [2]. The silicon compound **3** ($Si(CH_2)_5N$ moiety) behaves similar to **2** ($Si(CH_2)_6N$ moiety) and also enhances [^{3}H]NMS binding, the degree of cooperativity even being more pronounced than that observed for **2**. To further optimize the pharmacological profile of the lead compound **3**, we have replaced one or two of its phthalimido groups by 1,8-naphthalimido substituents (→ compounds **4**–**6**). This particular derivatization of (silicon-free) W84-type α,ω-bis(ammonio)alkanes has been found to increase the affinity for the [^{3}H]NMS-occupied muscarinic M_2 receptors [1c,3]. We report here on the syntheses of **4**–**6** and the pharmacological characterization of **3**–**6** [4]. Preliminary results of these investiga-

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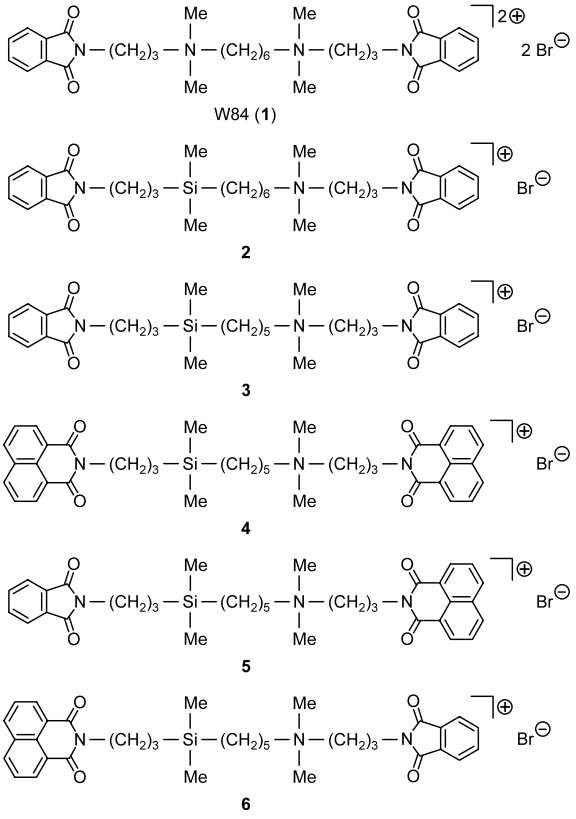
doi:10.1016/S0022-328X(03)00553-9

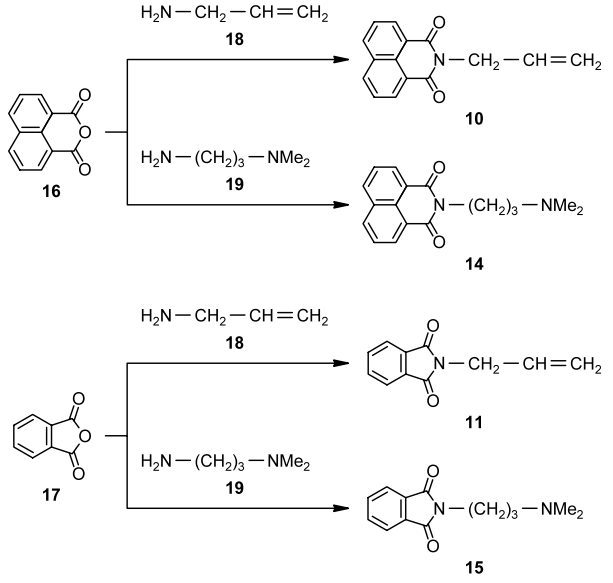
tions have already been reported elsewhere [5].



2. Results and discussion

2.1. Syntheses

Compounds **3**–**6** were prepared in four-step syntheses, starting from chlorodimethylsilane (**7**) (Scheme 1). Platinum-catalyzed (H_2PtCl_6) hydrosilylation of 5-bromo-1-pentene with **7** gave (5-bromopentyl)chlorodimethylsilane (**8**), which upon treatment with lithium aluminum hydride afforded (5-bromopentyl)dimethylsilane (**9**). Subsequent platinum-catalyzed (H_2PtCl_6) hydrosilylation of *N*-allyl-1,8-naphthalimide (**10**) or *N*-allylphthalimide (**11**) with **9** gave (5-bromopentyl)dimethyl(3-(1,8-naphthalimido)propyl)silane (**12**) and (5-bromopentyl)dimethyl(3-phthalimidopropyl)silane (**13**), respectively. Treatment of **12** or **13** with dimethyl(3-(1,8-naphthalimido)propyl)amine (**14**) or dimethyl(3-phthalimidopropyl)amine (**15**) finally gave the title compounds **3**–**6**.

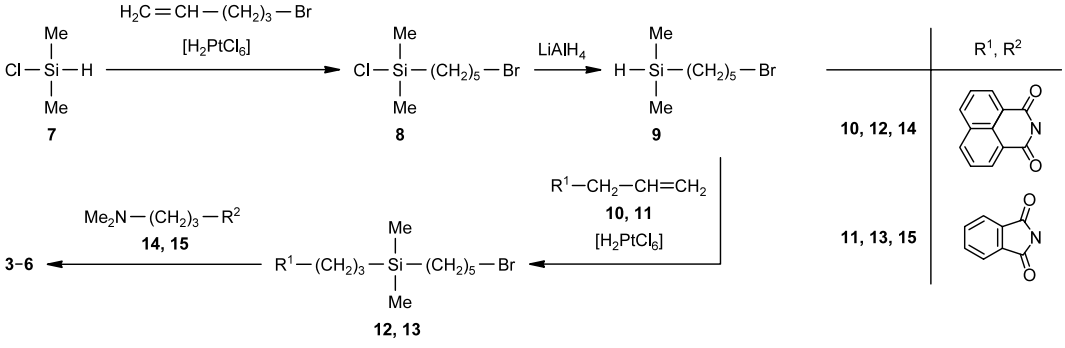


Scheme 2.

Compounds **10** and **11** were prepared by reaction of 1,8-naphthalenedicarboxylic acid anhydride (**16**) or phthalic acid anhydride (**17**) with allylamine (**18**), and compounds **14** and **15** were prepared analogously by reaction of **16** and **17** with *N*,*N*-dimethyl-1,3-propanediamine (**19**) (Scheme 2).

2.2. Pharmacological studies

The allosteric effects of the test compounds **3**–**6** on [^{3}H]NMS dissociation (Fig. 1) and [^{3}H]NMS equilibrium binding (Fig. 2) were tested at muscarinic M_2 receptors of porcine heart homogenates. The time course of [^{3}H]NMS dissociation from muscarinic receptors was monophasic under all conditions (data not



Scheme 1.

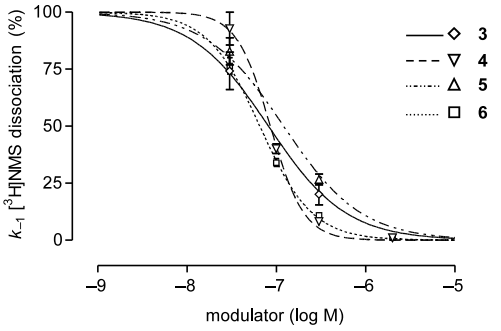


Fig. 1. Effect of the test compounds **3**–**6** on the dissociation of [^{3}H]NMS from porcine heart M_2 receptors: (ordinate) apparent rate constant k_{-1} of [^{3}H]NMS dissociation as percentage of the value under control conditions; (abscissa) log concentration of test compound. Indicated are mean values ± S.E.M. of two to four independent experiments; sigmoidal curve fitting fixing the top and bottom values at 10% and 0%, respectively; the Hill slope was constrained to $n_H = -1$ for compounds **3** and **5**. For details, see text.

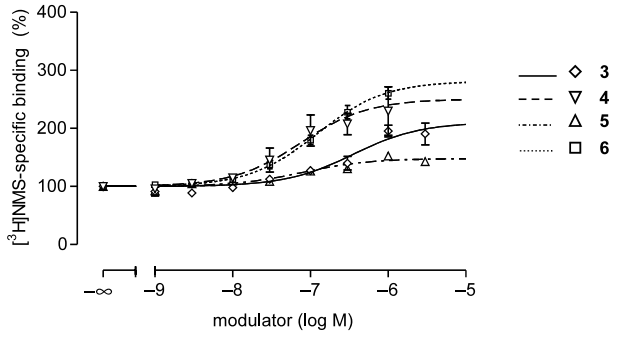


Fig. 2. Effect of the test compounds **3**–**6** on the equilibrium binding of [^{3}H]NMS: (ordinate) data expressed as percentage of specific [^{3}H]NMS binding in the absence of modulator; (abscissa) log concentration of test compound. Indicated are mean values ± S.E.M. of three to six independent experiments carried out as triplicate determinations; sigmoidal curve fitting.

shown). The apparent rate constant k_{-1} of [^{3}H]NMS dissociation was reduced concentration-dependently. Interestingly, the Hill coefficient n_H, characterizing the slope of a concentration–effect curve, was significantly different from unity for compounds **4** and **6**, whereas it did not differ from unity for **3** and **5** (Table 1). The inflection points ($pEC_{50,diss}$) of the curves did not differ from each other (Table 1). The $pEC_{50,diss}$ values can be taken as a measure for the binding affinity of the allosteric test compounds to [^{3}H]NMS-occupied receptors [6]. Thus, the exchange of phthalimido moieties by 1,8-naphthalimido groups in the silicon-containing modulators studied does not affect the affinity, whereas this kind of modification in related allosteric agents of the α,ω-bis(ammonio)alkane type [1c,3h] considerably increases the affinity for [^{3}H]NMS-occupied M_2 receptors. The equilibrium binding of [^{3}H]NMS was elevated by the test compounds **3**–**6** (Fig. 2), but to a different extent. Because of the atypically steep concentration–effect curves seen in the dissociation experiments, we preferred to apply a descriptive approach for curve fitting and used a four-parameter logistic function instead of the cooperativity model. Compared with compound **3**, the elevation of [^{3}H]NMS binding was significantly smaller with **5** and significantly higher with **6** (Table 1). The slope factor of the concentration–effect curves did not differ from unity in any case.

Compound **4** was studied for the subtype-selectivity of action at all five human muscarinic receptors. Fig. 3A shows the concentration–effect curves from [^{3}H]NMS dissociation experiments. The rank order of the affinities of **4** for [^{3}H]NMS-occupied muscarinic receptor subtypes was as follows: $M_4 > M_2 \geq M_1 > M_3 > M_5$. Except for the M_1 receptor, the curve slopes were remarkably steep for all subtypes studied (Table 2). In Fig. 3B, the effect of **4** on [^{3}H]NMS equilibrium binding is depicted. Compound **4** enhanced [^{3}H]NMS equilibrium binding at M_2 receptors, whereas equilibrium binding remained nearly unchanged at M_1 and M_3–M_5 receptors. At M_1 and M_4 receptors, the lack of effect on [^{3}H]NMS binding was accompanied by an inhibition of [^{3}H]NMS dissociation. Thus, it can be concluded that **4** interacts with [^{3}H]NMS binding at M_1 and M_4 receptors in a neutral cooperative fashion. At M_3 and M_5 receptors, compound **4** hardly had an effect on [^{3}H]NMS dissociation in the range of concentrations that were applied in the equilibrium-binding assay. In this case, the lack of effect is due to the low affinity of **4** for these subtypes. These results demonstrate that compound **4** is an M_2-selective allosteric enhancer of [^{3}H]NMS binding.

In conclusion, the silicon compounds studied here appear to be promising new leads for the development of highly potent and subtype-selective muscarinic allosteric enhancers. These new compounds are atypical of allosteric modulators of the W84-type, in that structure–activity relationships are divergent and concentration–effect curves are steep in some cases. This may point to a different way of interaction of these silicon compounds with muscarinic acetylcholine receptors compared with the modulators of the W84-type. Further studies will reveal whether binding of the physiological neurotransmitter acetylcholine can also be allosterically increased in a subtype-selective fashion by these silicon-containing modulators.

3. Experimental

3.1. Syntheses

3.1.1. General procedures

All syntheses were carried out under dry nitrogen. The organic solvents used were dried and purified according to standard procedures and stored under dry nitrogen. A Büchi GKR 50 apparatus was used

Table 1
Parameters describing the concentration–effect curves for the interaction of the allosteric test compounds **3**–**6** with [^{3}H]NMS-occupied and -unoccupied muscarinic M_2 receptors [a]

Compound	$pEC_{50,diss}$	$n_{H,diss}$	N	pEC_{50}	E_{max}	n_H	N
3	7.09±0.10	−1.1±0.2	3	6.45±0.06	210.5±18.3	3.0±1.8	4
4	7.07±0.05	−2.3±0.5 [b]	4	7.12±0.24	250.6±22.3	2.0±0.3	4
5	6.92±0.07	−1.1±0.2	2	7.10±0.24	148.0±5.6[c]	1.1±0.2	3
6	7.18±0.02	−1.5±0.1 [b]	5	6.91±0.07 [c]	281.2±10.0[c]	1.1±0.1	6

[a] $pEC_{50,diss}$ denotes the −log concentration of the test compound at which radioligand dissociation was reduced to 50% of the control value; n_H denotes the slope factor of the curve; N is the number of experiments; pEC_{50} reflects the inflection point of the concentration–effect curves for the elevation of radioligand equilibrium binding; E_{max} is the maximum enhancing effect expressed as percent of [^{3}H]NMS equilibrium binding under control conditions (100%). Mean values±S.E.M.

[b] Significantly different from $n_{H,diss} = -1$ ($P < 0.05$).

[c] Significantly different from the parameter value of the parent compound **3** ($P < 0.05$; for details, see text).

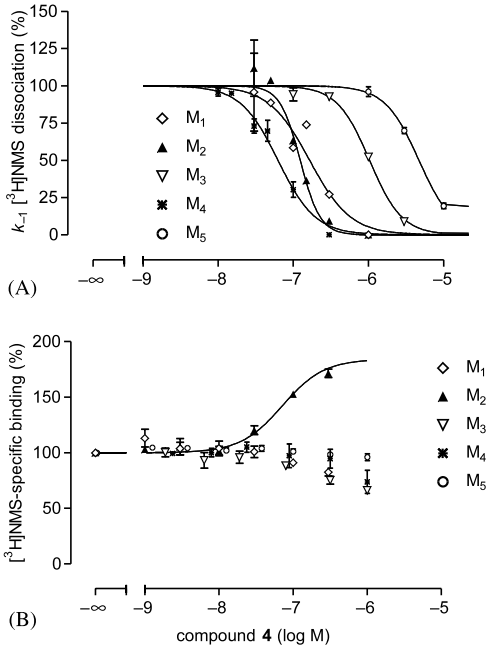


Fig. 3. Muscarinic receptor subtype dependent effects of compound **4** on [^{3}H]NMS dissociation (A) and [^{3}H]NMS equilibrium binding (B): (A, ordinate) apparent rate constant k_{-1} of [^{3}H]NMS dissociation as percentage of the value under control conditions; (B, ordinate) data expressed as percentage of specific [^{3}H]NMS binding in the absence of test compound; (A, B, abscissa) log concentration of test compound. Indicated are mean values±S.E.M. of (A) four to nine or (B) three to five independent experiments; sigmoidal curve fitting; (A) top and bottom values were fixed at 10% and 0%, respectively. For details, see text.

for the bulb-to-bulb distillations. Melting points were determined with a Büchi B540 apparatus using samples in open glass capillaries. The ^{1}H-, ^{13}C-, ^{15}N- and ^{29}Si-NMR spectra were recorded on a Bruker DRX-300 NMR spectrometer (^{1}H, 300.1 MHz; ^{13}C, 75.5 MHz; ^{15}N, 30.4 MHz; ^{29}Si, 59.6 MHz). $CDCl_3$ or [D_6]DMSO were used as the solvents. Spectra were recorded at 22 °C ($CDCl_3$) or at 30 °C ([D_6]DMSO). Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^{1}H, $\delta = 7.24$; solvent $CDCl_3$), $CDCl_3$ (^{13}C, $\delta = 77.0$; solvent $CDCl_3$), [D_5]DMSO (^{1}H, $\delta = 2.49$; solvent [D_6]DMSO), [D_6]DMSO (^{13}C, $\delta = 39.5$; solvent [D_6]DMSO), external formamide (^{15}N, $\delta = -268$; solvent [D_6]DMSO), or external TMS (^{29}Si, $\delta = 0$; solvent $CDCl_3$ or [D_6]DMSO). Assignment of the ^{1}H-NMR data was supported by ^{1}H,^{1}H and ^{13}C,^{1}H correlation experiments, and assignment of the ^{13}C-NMR data was supported by DEPT 135 and ^{13}C,^{1}H correlation experiments. Mass spectra (EI MS, 70 eV; CI MS, reactant gas methane) were recorded with a ThermoQuest Trio 1000 mass spectrometer. The selected m/z values given refer to the isotopes ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{28}Si, ^{35}Cl and ^{79}Br. IR spectra were obtained with a Bruker Equinox 55 IR spectrometer.

*3.1.2. (6-(Dimethyl(3-phthalimidopropyl)silyl)hexyl)dimethyl(3-phthalimidopropyl)ammonium bromide (**2**)*

This compound was synthesized according to Ref. [2].

Table 2
Parameters describing the concentration–effect curves for the interaction of the allosteric test compound **4** with [^{3}H]NMS-occupied human muscarinic M_1–M_5 receptor subtypes [a]

	M_1	M_2	M_3	M_4	M_5
$pEC_{50,diss}$	6.78±0.09	6.91±0.04	5.98±0.03	7.20±0.04	5.33±0.02
n_H	−1.6±0.5	−3.2±1.1 [b]	−2.0±0.4 [b]	−1.7±0.2 [b]	−1.9±0.1 [b]
N	7	7	4	9	5

[a] $pEC_{50,diss}$ denotes the −log concentration of the test compound at which radioligand dissociation was reduced to 50% of the control value; n_H denotes the slope factor of the curve; N is the number of experiments. Mean values±S.E.M.

[b] Significantly different from $n_{H,diss} = -1$ ($P < 0.05$) (for details, see text).

*3.1.3. (5-(Dimethyl(3-phthalimidopropyl)silyl)pentyl)-dimethyl(3-phthalimidopropyl)ammonium bromide (**3**)*

This compound was synthesized according to Ref. [2].

*3.1.4. (5-(Dimethyl(3-(1,8-naphthalimido)propyl)-silyl)pentyl)dimethyl(3-(1,8-naphthalimido)propyl)-ammonium bromide (**4**)*

A solution of **12** (1.36 g, 3.05 mmol) and **14** (968 mg, 3.43 mmol) in ethanol (12 ml) was heated under reflux for 48 h. After the mixture was cooled to room temperature, the solvent was removed at 30 mbar, and ethyl acetate (50 ml) was added. The solution was concentrated in vacuo to a volume of ca. 10 ml by lowering the pressure slowly from 200 to 30 mbar. The addition of ethyl acetate and subsequent evaporation of the solvent were repeated until the product precipitated almost quantitatively as a white amorphous solid. This product was isolated by centrifugation ($1100 \times g$, 5 min), washed with *n*-pentane (2×20 ml), and dried in vacuo (2 h, 0.001 mbar, 20 °C) to give **4** as a white amorphous solid. To purify the product, a boiling saturated solution of the solid in acetone was concentrated by distillation at atmospheric pressure until the product precipitated significantly. The precipitation was completed by storing the suspension for 1 day at room temperature and for a further 3 days at −25 °C. The product was isolated by centrifugation and washed with *n*-pentane (2×20 ml), the purification procedure was repeated twice, and the product was then dried in vacuo (2 days, 0.001 mbar, 60 °C) to give **4** in 56% yield as an amorphous white solid (1.25 g, 1.72 mmol); m.p.: 193–194 °C. ^{1}H-NMR ([D$_6$]DMSO): δ −0.10 (s, 6H, SiCH_3), 0.32–0.55 (m, 4H, CCH_2SiCH_2C), 1.09–1.32 (m, 4H, SiCH$_2$(CH_2)$_2$(CH$_2$)$_2$N$^+$), 1.41–1.71 (m, 4H, NCH$_2$CH_2CH$_2$Si, Si(CH$_2$)$_3$CH_2CH$_2$N$^+$), 1.98–2.18 (m, 2H, N$^+$CH$_2$CH_2CH$_2$N), 3.05 (s, 6H, N$^+$CH_3), 3.21–3.34 (m, 2H, Si(CH$_2$)$_4$CH_2N$^+$), 3.34–3.49 (m, 2H, N$^+$CH_2(CH$_2$)$_2$N), 3.77–3.88 (m, 2H, NCH_2(CH$_2$)$_2$Si), 3.97–4.08 (m, 2H, N$^+$(CH$_2$)$_2$CH_2N), 7.66–7.75 (m, 4H, *H*-3/*H*-6, 1,8-naphthalimido moiety (≡naphth)), 8.23–8.32 (m, 8H, *H*-2/*H*-7 and *H*-4/*H*-5, naphth). ^{13}C-NMR ([D$_6$]DMSO): δ −3.5 (SiCH$_3$), 11.8 (N(CH$_2$)$_2$CH$_2$Si), 14.2 (SiCH$_2$(CH$_2$)$_4$N$^+$), 21.2 (N$^+$CH$_2$CH$_2$CH$_2$N), 21.3 (Si(CH$_2$)$_3$CH$_2$CH$_2$N$^+$), 21.9 (NCH$_2$CH$_2$CH$_2$Si), 22.8 (SiCH$_2$CH$_2$(CH$_2$)$_3$N$^+$), 29.4 (Si(CH$_2$)$_2$CH$_2$(CH$_2$)$_2$N$^+$), 36.7 (N$^+$(CH$_2$)$_2$CH$_2$N), 42.4 (NCH$_2$(CH$_2$)$_2$Si), 50.0 (N$^+$CH$_3$), 60.7 (N$^+$CH$_2$(CH$_2$)$_2$N), 62.8 (Si(CH$_2$)$_4$CH$_2$N$^+$), 121.56 (*C*-1/*C*-8, naphth), 121.65 (*C*-1/*C*-8, naphth), 126.90 (*C*-8a, naphth), 126.92 (4C) (*C*-3/*C*-6, naphth), 127.1 (*C*-8a, naphth), 130.40 (*C*-2/*C*-7, naphth), 130.44 (*C*-2/*C*-7, naphth), 130.94 (*C*-4a, naphth), 130.99 (*C*-4a, naphth), 134.1 (*C*-4/*C*-5, naphth), 134.2 (*C*-4/*C*-5, naphth), 163.0 ((*C*=O)$_2$N(CH$_2$)$_3$Si), 163.4 (N$^+$(CH$_2$)$_3$N(*C*=O)$_2$). ^{15}N-NMR ([D$_6$]DMSO): δ −327 (*N*$^+$), −205 and −206 (*N*CH$_2$)$_3$Si, N$^+$(CH$_2$)$_3$*N*). ^{29}Si-NMR ([D$_6$]DMSO): δ 2.9. CI MS: *m*/*z* 634 (17) [M$_{Cation}$ − CH$_2$]$^+$, 633 (2) [M$_{Cation}$ − CH$_3$]$^+$, 95 (100) [BrCH$_4^+$]. Anal. found: C, 63.56; H, 6.36; N, 5.77. Calc. for $C_{39}H_{46}BrN_3O_4Si$ (M_r = 728.80): C, 64.27; H, 6.36; N, 5.77%.

*3.1.5. (5-(Dimethyl(3-phthalimidopropyl)silyl)pentyl)-dimethyl(3-(1,8-naphthalimido)propyl)ammonium bromide (**5**)*

This compound was prepared from **13** (1.24 g, 3.13 mmol) and **14** (971 mg, 3.44 mmol) analogous to the method reported for the synthesis of **4**; 53% yield (1.13 g, 1.66 mmol) of an amorphous white solid; m.p.: 147–148 °C. ^{1}H-NMR ([D$_6$]DMSO): δ −0.12 (s, 6H, SiCH_3), 0.33–0.48 (m, 4H, CCH_2SiCH_2C), 1.12–1.30 (m, 4H, SiCH$_2$(CH_2)$_2$(CH$_2$)$_2$N$^+$), 1.42–1.69 (m, 4H, NCH$_2$CH_2CH$_2$Si, Si(CH$_2$)$_3$CH_2CH$_2$N$^+$), 2.00–2.19 (m, 2H, N$^+$CH$_2$CH_2CH$_2$N), 3.04 (s, 6H, N$^+$CH_3), 3.21–3.33 (m, 2H, Si(CH$_2$)$_4$CH_2N$^+$), 3.36–3.50 (m, 2H, N$^+$CH_2(CH$_2$)$_2$N, NCH_2(CH$_2$)$_2$Si), 4.01–4.13 (m, 2H, N$^+$(CH$_2$)$_2$CH_2N), 7.73–7.83 (m, 6H, C(O)C$_6$H$_4$C(O), and *H*-3/*H*-6, naphth), 8.34–8.43 (m, 4H, *H*-2/*H*-7 and *H*-4/*H*-5, naphth). ^{13}C-NMR ([D$_6$]DMSO): δ −3.6 (SiCH$_3$), 11.6 (N(CH$_2$)$_2$CH$_2$Si), 14.2 (SiCH$_2$(CH$_2$)$_4$N$^+$), 21.2 and 21.4 (N$^+$CH$_2$CH$_2$CH$_2$N, Si(CH$_2$)$_3$CH$_2$CH$_2$N$^+$), 22.5 (NCH$_2$CH$_2$CH$_2$Si), 22.9 (SiCH$_2$CH$_2$(CH$_2$)$_3$N$^+$), 29.5 (Si(CH$_2$)$_2$CH$_2$(CH)$_2$N$^+$), 36.9 (N$^+$(CH$_2$)$_2$CH$_2$N), 40.3 (NCH$_2$(CH$_2$)$_2$Si), 49.9 (N$^+$CH$_3$), 60.7 (N$^+$CH$_2$(CH$_2$)$_2$N), 62.8 (Si(CH$_2$)$_4$CH$_2$N$^+$), 121.8 (*C*-1/*C*-8, naphth), 122.8 (*C*-3/*C*-6, C(O)C$_6$H$_4$C(O)), 127.1 (*C*-3/*C*-6, naphth), 127.2 (*C*-8a, naphth), 130.6 (*C*-2/*C*-7, naphth), 131.1 (*C*-4a, naphth), 131.3 (*C*-1/*C*-2, C(O)C$_6$H$_4$C(O)), 134.3 (4C) (*C*-4/*C*-5, naphth, and *C*-4/*C*-5, C(O)C$_6$H$_4$C(O)), 163.5 (*C*=O, naphth), 167.8 (*C*=O, C(O)C$_6$H$_4$C(O)). ^{15}N-NMR ([D$_6$]DMSO): δ −327 (*N*$^+$), −218 (*N*(CH$_2$)$_3$Si), −208 (N$^+$(CH$_2$)$_3$*N*). ^{29}Si-NMR ([D$_6$]DMSO): δ 2.9. CI MS: *m*/*z* 584 (27) [M$_{Cation}$ − CH$_2$]$^+$, 583 (3) [M$_{Cation}$ − CH$_3$]$^+$, 95 (100) [BrCH$_4^+$]. Anal. found: C, 61.79; H, 6.45; N, 6.27. Calc. for $C_{35}H_{44}BrN_3O_4Si$ (M_r = 678.74): C, 61.94; H, 6.53; N, 6.19%.

*3.1.6. (5-(Dimethyl(3-(1,8-naphthalimido)propyl)-silyl)pentyl)dimethyl(3-phthalimidopropyl)ammonium bromide (**6**)*

This compound was prepared from **12** (2.38 g, 5.33 mmol) and **15** (1.49 g, 6.41 mmol) analogous to the method reported for the synthesis of **4**; 73% yield (2.63 g, 3.87 mmol) of an amorphous white solid; m.p.: 201–202 °C (dec.) ^{1}H-NMR ([D$_6$]DMSO): δ −0.07 (s, 6H, SiCH_3), 0.36–0.58 (m, 4H, CCH_2SiCH_2C), 1.13–1.34 (m, 4H, SiCH$_2$(CH_2)$_2$(CH$_2$)$_2$N$^+$), 1.47–1.70 (m, 4H, NCH$_2$CH_2CH$_2$Si, Si(CH$_2$)$_3$CH_2CH$_2$N$^+$), 1.93–2.11 (m, 2H, N$^+$CH$_2$CH_2CH$_2$N), 3.01 (s, 6H, N$^+$CH_3), 3.20–3.30 (m, 2H, Si(CH$_2$)$_4$CH_2N$^+$), 3.30–3.41 (m, 2H, N$^+$CH_2(CH$_2$)$_2$N), 3.61 (t, $^3J_{HH}$ = 6.1 Hz, 2H,

$N^+(CH_2)_2CH_2N$), 3.86–3.97 (m, 2H, $NCH_2(CH_2)_2Si$), 7.74–7.83 (m, 6H, $C(O)C_6H_4C(O)$, and *H*-3/*H*-6, naphth), 8.33–8.41 (m, 4H, *H*-2/*H*-7 and *H*-4/*H*-5, naphth). ^{13}C-NMR ([D$_6$]DMSO): δ −3.5 ($SiCH_3$), 11.9 ($N(CH_2)_2CH_2Si$), 14.3 ($SiCH_2(CH_2)_4N^+$), 21.4 and 21.6 ($N^+CH_2CH_2CH_2N$, $Si(CH_2)_3CH_2CH_2N^+$), 21.9 ($NCH_2CH_2CH_2Si$), 22.9 ($SiCH_2CH_2(CH_2)_3N^+$), 29.5 ($Si(CH_2)_2CH_2(CH_2)_2N^+$], 34.6 ($N^+(CH_2)_2CH_2N$), 42.5 ($NCH_2(CH_2)_2Si$), 50.0 ($N^+CH_3$), 60.5 ($N^+CH_2(CH_2)_2N$), 63.0 ($Si(CH_2)_4CH_2N^+$), 121.8 (*C*-1/*C*-8, naphth), 122.9 (*C*-3/*C*-6, $C(O)C_6H_4C(O)$), 127.07 (*C*-3/*C*-6, naphth), 127.11 (*C*-8a, naphth), 130.6 (*C*-2/*C*-7, naphth), 131.1 (*C*-4a, naphth), 131.6 (*C*-1/*C*-2, $C(O)C_6H_4C(O)$), 134.20 and 134.29 (*C*-4/*C*-5, naphth, and *C*-4/*C*-5, $C(O)C_6H_4C(O)$), 163.2 (*C*=O, naphth), 167.8 (*C*=O, $C(O)C_6H_4C(O)$). ^{15}N-NMR ([D$_6$]DMSO): δ −326 (N^+), −221 ($N^+(CH_2)_3N$), $N(CH_2)_3Si$ not detected. ^{29}Si-NMR ([D$_6$]DMSO): δ 3.0. CI MS: *m*/*z* 584 (25) $[M_{Cation} - CH_2]^+$, 583 (3) $[M_{Cation} - CH_3]^+$, 95 (100) $[BrCH_4^+]$. Anal. found: C, 61.46; H, 6.59; N, 6.27. Calc. for $C_{35}H_{44}BrN_3O_4Si$ (M_r = 678.74): C, 61.94; H, 6.53; N, 6.19%.

3.1.7. Chlorodimethylsilane (7)

This compound was commercially available (Acros, 16284).

3.1.8. (5-Bromopentyl)chlorodimethylsilane (8)

Hexachloroplatinic acid hexahydrate (5 mg, 9.7 μmol) was added at room temperature to a solution of **7** (18.8 g, 199 mmol) and 5-bromo-1-pentene (25.1 g, 168 mmol) in toluene (200 ml), and the mixture was heated immediately in a preheated oil bath (140 °C). After the reaction has started, the oil bath was removed. As soon as the reaction started to become less vigorous, the mixture was heated under reflux (no drop in temperature below reflux temperature at any time), and further portions of $H_2PtCl_6 \cdot 6H_2O$ and **7** were added sequentially: first portion (after 30 min), $H_2PtCl_6 \cdot 6H_2O$ (5 mg, 9.7 μmol; dissolved in 2-propanol (50 μl)) and **7** (5.00 g, 52.8 mmol); second to fifth portion (after 40, 50, 60, and 70 min), $H_2PtCl_6 \cdot 6H_2O$ (5 mg, 9.7 μmol; dissolved in 2-propanol (50 μl)) and **7** (1.00 g, 10.6 mmol). After addition of the last portion, the mixture was heated under reflux for a further 50 min. The solvent was removed by distillation at ambient pressure and the residue distilled in vacuo to give **8** in 77% yield (related to 5-bromo-1-pentene) as a colorless liquid (31.5 g, 129 mmol); b.p.: 92–93 °C at 2 mbar. ^{1}H-NMR ($CDCl_3$): δ 0.38 (s, 6H, $SiCH_3$), 0.75–0.86 (m, 2H, $SiCH_2(CH_2)_4Br$), 1.34–1.53 (m, 4H, $SiCH_2(CH_2)_2(CH_2)_2Br$), 1.78–1.90 (m, 2H, $Si(CH_2)_3CH_2CH_2Br$), 3.37 (t, $^3J_{HH}$ = 6.8 Hz, 2H, $Si(CH_2)_4CH_2Br$). ^{13}C-NMR ($CDCl_3$): δ 1.6 ($SiCH_3$), 18.7 ($SiCH_2(CH_2)_4Br$), 22.2 ($SiCH_2CH_2(CH_2)_3Br$), 31.3 ($Si(CH_2)_2CH_2(CH_2)_2Br$), 32.3 ($Si(CH_2)_3CH_2CH_2Br$), 33.7 ($Si(CH_2)_4CH_2Br$). ^{29}Si-NMR ($CDCl_3$): δ 32.0. EI MS: *m*/*z* 227 (2) $[M^+ - CH_3]$, 93 (100) $[M^+ - (CH_2)_5Br]$. Anal. found: C, 34.11; H, 6.41. Calc. for $C_7H_{16}BrClSi$ (M_r = 243.64): C, 34.51; H, 6.62%.

3.1.9. (5-Bromopentyl)dimethylsilane (9)

Compound **8** (30.3 g, 124 mmol) was added at room temperature within a period of 5 min to a stirred suspension of lithium aluminum hydride (2.72 g, 71.7 mmol) in diethyl ether (250 ml). The resulting mixture was heated under reflux for 30 min, allowed to cool to room temperature, and was then added dropwise to a stirred mixture of concentrated hydrochloric acid (100 ml), diethyl ether (200 ml) and ice (200 g) (to avoid an ignition, this step was also performed under a nitrogen atmosphere). The organic layer was separated and the aqueous phase extracted with diethyl ether (3 × 100 ml), and the combined organic extracts were dried over anhydrous magnesium sulfate in an ice bath, followed by an additional thorough dynamic drying over anhydrous magnesium sulfate using a standard chromatographic column densely packed with anhydrous magnesium sulfate (column diameter, 3.5 cm; column length, 15 cm). The magnesium sulfate was finally eluted with diethyl ether (300 ml), and the organic solutions were combined. The solvent was removed at 800–900 mbar and the residue distilled in vacuo to give **9** in 87% yield (22.6 g, 108 mmol) as a colorless liquid; b.p.: 74–75 °C at 6 mbar. IR (film): *v* 2110 cm^{-1} (SiH). ^{1}H-NMR ($CDCl_3$): δ 0.04 (d, $^3J_{HH}$ = 3.6 Hz, 6H, $SiCH_3$), 0.52–0.61 (m, 2H, $SiCH_2(CH_2)_4Br$), 1.28–1.51 (m, 4H, $SiCH_2(CH_2)_2(CH_2)_2Br$), 1.84 (“quint”, $^3J_{HH}$ = 7.0 Hz, 2H, $Si(CH_2)_3CH_2CH_2Br$), 3.38 (t, $^3J_{HH}$ = 7.0 Hz, 2H, $Si(CH_2)_4CH_2Br$), 3.82 (“nonett”, $^3J_{HH}$ = 3.6 Hz, 1H, Si*H*). ^{13}C-NMR ($CDCl_3$): δ −4.5 ($SiCH_3$), 14.0 ($SiCH_2(CH_2)_4Br$), 23.6 ($SiCH_2CH_2(CH_2)_3Br$), 31.6 ($Si(CH_2)_2CH_2(CH_2)_2Br$), 32.5 ($Si(CH_2)_3CH_2CH_2Br$), 33.8 ($Si(CH_2)_4CH_2Br$). ^{29}Si-NMR ($CDCl_3$): δ −12.9. EI MS: *m*/*z* 207 (< 1) $[M^+ - H]$, 193 (2) $[M^+ - CH_3]$, 87 (11) $[M^+ - (CH_2)_3Br]$, 59 (100) $[M^+ - (CH_2)_5Br]$. Anal. found: C, 40.08; H, 7.86. Calc. for $C_7H_{17}BrSi$ (M_r = 209.20): C, 40.19; H, 8.19%.

3.1.10. N-Allyl-1,8-naphthalimide (10)

Compound **18** (14.4 g, 252 mmol) was added at room temperature in one portion to a suspension of **16** (50.0 g, 252 mmol) in toluene (250 ml), and the mixture was then stirred for 16 h at room temperature and heated under reflux for a further 14 h (removal of the resulting water using a water separator). The solvent was removed under reduced pressure, and the solid residue was purified by twofold crystallization from boiling methanol (700 ml for each crystallization; 1 day at room temperature, then 1 day at −26 °C). The product was dried at 0.0001 mbar for 8 h at room temperature; yield 74% of a yellowish crystalline solid (44.2 g, 186 mmol);

m.p.: 135 °C. ^{1}H-NMR ($CDCl_3$): δ 4.74 (δ_M), 5.17 (δ_A), 5.29 (δ_B), and 5.96 (δ_G) ($^2J_{AB} = 1.3$ Hz, $^3J_{AG,cis} = 10.2$ Hz, $^4J_{AM} = 1.3$ Hz, $^3J_{BG,trans} = 17.2$ Hz, $^4J_{BM} = 1.5$ Hz, $^3J_{GM} = 5.9$ Hz, 5H, $NC(H_M)_2CH_G = CH_AH_B$), 7.66 (dd, $^3J_{HH} = 7.4$ Hz, $^3J_{HH} = 8.1$ Hz, 2H, *H*-3/*H*-6, naphth), 8.11 (dd, $^3J_{HH} = 8.1$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-4/*H*-5, naphth), 8.49 (dd, $^3J_{HH} = 7.4$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-2/*H*-7, naphth). ^{13}C-NMR ($CDCl_3$): δ 42.3 (NCH_2C), 117.5 ($NCH_2CH=CH_2$), 122.3 (*C*-1/*C*-8, naphth), 126.8 (*C*-3/*C*-6, naphth), 127.9 (*C*-8a, naphth), 131.1 (*C*-2/*C*-7, naphth), 131.4 (*C*-4a, naphth), 132.1 ($NCH_2CH=CH_2$), 133.8 (*C*-4/*C*-5, naphth), 163.7 (*C*=O). EI MS: *m*/*z* 237 (20) [M^+], 222 (100) [$M^+ - CH_3$]. Anal. found: C, 75.79; H, 4.83; N, 5.91. Calc. for $C_{15}H_{11}NO_2$ ($M_r = 237.26$): C, 75.94; H, 4.67; N, 5.90%.

3.1.11. *N*-Allylphthalimide (**11**)

Preparation analogous to the synthesis of compound **10** from **17** (27.0 g, 182 mmol) and **18** (10.4 g, 182 mmol); recrystallization from boiling methanol (250 ml); yield 86% (29.5 g, 158 mmol) of a colorless crystalline solid, m.p.: 69 °C. See Ref. [2] for NMR data (^{1}H, ^{13}C). Anal. found: C, 70.19; H, 5.07; N, 7.41. Calc. for $C_{11}H_9NO_2$ ($M_r = 187.20$): C, 70.58; H, 4.85; N, 7.48%.

3.1.12. (5-Bromopentyl)dimethyl(3-(1,8-naphthalimido)propyl)silane (**12**)

A solution of hexachloroplatinic acid hexahydrate (10 mg, 19.3 μmol) in 2-propanol (50 μl) was added at room temperature to a solution of **9** (1.01 g, 4.83 mmol) and **10** (1.14 g, 4.80 mmol) in toluene (20 ml), and the mixture was heated immediately in a preheated oil bath (140 °C) for 1 h (complete disappearance of the Si–H IR absorption band at 2111 cm^{-1}; measured as film of the reaction mixture). The reaction mixture was filtered over silica gel (30 g, 0.063–0.200 mm; Fluka, 60741) packed in a chromatographic column (diameter, 3.5 cm), the product was then eluted with ethyl acetate (500 ml), and the organic solutions were combined. The solvent was removed under reduced pressure (200 mbar) and the product finally purified by column (diameter, 4.5 cm) chromatography on silica gel (300 g, 0.063–0.200 mm; Fluka, 60741) using diethyl ether/*n*-hexane (1:1 (v/v)) as the eluent. After bulb-to-bulb distillation (210–240 °C/0.001 mbar), compound **12** was obtained in 72% yield (1.54 g, 3.45 mmol) as a colorless viscous liquid. ^{1}H-NMR ($CDCl_3$): δ −0.05 (s, 6H, $SiCH_3$), 0.43–0.53 (m, 2H, $SiCH_2(CH_2)_4Br$), 0.54–0.65 (m, 2H, $N(CH_2)_2CH_2Si$), 1.23–1.34 (m, 2H, $SiCH_2CH_2(CH_2)_3Br$), 1.34–1.46 (m, 2H, $Si(CH_2)_2CH_2(CH_2)_2Br$), 1.61–1.75 (m, 2H, $NCH_2CH_2CH_2Si$), 1.81 ("quint", $^3J_{HH} = 7.0$ Hz, 2H, $Si(CH_2)_3CH_2CH_2Br$), 3.36 (t, $^3J_{HH} = 7.0$ Hz, 2H, $Si(CH_2)_4CH_2Br$), 4.08–4.16 (m, 2H, $NCH_2(CH_2)_2Si$), 7.72 (dd, $^3J_{HH} = 7.3$ Hz, $^3J_{HH} = 8.3$ Hz, 2H, *H*-3/*H*-6, naphth), 8.17 (dd, $^3J_{HH} = 8.3$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-4/*H*-5, naphth), 8.57 (dd, $^3J_{HH} = 7.3$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-2/*H*-7, naphth). ^{13}C-NMR ($CDCl_3$): δ −3.5 ($SiCH_3$), 12.5 ($N(CH_2)_2CH_2Si$), 15.0 ($SiCH_2(CH_2)_4Br$), 22.6 ($NCH_2CH_2CH_2Si$), 23.1 ($SiCH_2CH_2(CH_2)_3Br$), 32.0 ($Si(CH_2)_2CH_2(CH_2)_2Br$), 32.5 ($Si(CH_2)_3CH_2CH_2Br$), 34.0 ($Si(CH_2)_4CH_2Br$), 43.3 ($NCH_2(CH_2)_2Si$), 122.7 (*C*-1/*C*-8, naphth), 126.9 (*C*-3/*C*-6, naphth), 128.1 (*C*-8a, naphth), 131.1 (*C*-2/*C*-7, naphth), 131.5 (*C*-4a, naphth), 133.8 (*C*-4/*C*-5, naphth), 164.1 (*C*=O). ^{29}Si-NMR ($CDCl_3$): δ 3.0. EI MS: *m*/*z* 296 (100) [$M^+ - (CH_2)_5Br$]. Anal. found: C, 59.23; H, 6.45; N, 3.23. Calc. for $C_{22}H_{28}BrNO_2Si$ ($M_r = 446.46$): C, 59.19; H, 6.32; N, 3.14%.

3.1.13. (5-Bromopentyl)dimethyl(3-phthalimidopropyl)silane (**13**)

Hexachloroplatinic acid hexahydrate (5 mg, 9.7 μmol) was added at room temperature to a solution of **9** (1.62 g, 7.74 mmol) and **11** (1.29 g, 6.89 mmol) in toluene (20 ml), and the mixture was heated immediately in a preheated oil bath (140 °C). After the reaction has started, the oil bath was removed. As soon as the reaction started to become less vigorous, the mixture was heated under reflux (no drop in temperature below reflux temperature at any time) for 30 min, and another portion of $H_2PtCl_6 \cdot 6H_2O$ (5 mg, 9.7 μmol), dissolved in 2-propanol (50 μl), was added and the mixture heated under reflux for a further 1 h (complete disappearance of the Si–H IR absorption band at 2111 cm^{-1}; measured as film of the reaction mixture). The reaction mixture was filtered over silica gel (30 g, 0.063–0.200 mm; Fluka, 60741) packed in a chromatographic column (diameter, 3.5 cm), the product was then eluted with ethyl acetate (500 ml), and the organic solutions were combined. The solvent was removed under reduced pressure (200 mbar) and the product finally purified by column (diameter, 5.5 cm) chromatography on silica gel (615 g, 0.015–0.045 mm; Merck, 1.15111) using diethyl ether/*n*-hexane (1:1 (v/v)) as the eluent. After bulb-to-bulb distillation (200–220 °C/0.001 mbar), compound **13** was obtained in 76% yield (related to **9**) (2.34 g, 5.90 mmol) as a colorless viscous liquid. The NMR data (^{1}H, ^{13}C, ^{15}N, ^{29}Si) and EI MS data of the product were identical with those reported in Ref. [2]. Anal. found: C, 54.51; H, 6.61; N, 3.61. Calc. for $C_{18}H_{26}BrNO_2Si$ ($M_r = 396.40$): C, 54.54; H, 6.61; N, 3.53%.

3.1.14. Dimethyl(3-(1,8-naphthalimido)propyl)amine (**14**)

A solution of **16** (30.0 g, 151 mmol) and **19** (15.5 g, 152 mmol) in toluene (300 ml) was heated under reflux for 14 h, and the resulting water was removed using a water separator. The solvent was removed in vacuo (0.001 mbar) at room temperature and the residue distilled quickly (solidification of the distillate upon cooling to 0 °C). The solid brown distillate was purified

by twofold crystallization from boiling ethanol (500 ml for each crystallization; 1 week at −26 °C; crystallization was initiated by addition of a tiny amount of the solid distillate to the solution at −26 °C). The product was isolated in 75% yield (32.1 g, 114 mmol) as a yellowish crystalline solid; m.p.: 113–115 °C. ^{1}H-NMR ($CDCl_3$): δ 1.80–1.93 (m, 2H, $NCH_2CH_2CH_2N$), 2.20 (s, 6H, NCH_3), 2.38 (t, $^3J_{HH} = 7.3$ Hz, 2H, $N(CH_2)_2CH_2N(CH_3)_2$), 4.11–4.21 (m, 2H, $NCH_2(CH_2)_2N(CH_3)_2$), 7.66 (dd, $^3J_{HH} = 7.3$ Hz, $^3J_{HH} = 8.3$ Hz, 2H, *H*-3/*H*-6, naphth), 8.11 (dd, $^3J_{HH} = 8.3$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-4/*H*-5, naphth), 8.49 (dd, $^3J_{HH} = 7.3$ Hz, $^4J_{HH} = 1.1$ Hz, 2H, *H*-2/*H*-7, naphth). ^{13}C-NMR ($CDCl_3$): δ 26.0 ($NCH_2CH_2CH_2N$), 38.7 ($NCH_2(CH_2)_2N(CH_3)_2$), 45.3 (NCH_3), 57.2 ($N(CH_2)_2CH_2N(CH_3)_2$), 122.5 (*C*-1/*C*-8, naphth), 126.8 (*C*-3/*C*-6, naphth), 127.9 (*C*-8a, naphth), 131.0 (*C*-2/*C*-7, naphth), 131.4 (*C*-4a, naphth), 133.7 (*C*-4/*C*-5, naphth), 164.0 (*C*=O). EI MS: *m*/*z* 282 (< 1) [M^+], 58 (100) [$H_2C{=}N(CH_3)_2^+$]. Anal. found: C, 72.08; H, 6.43; N, 9.77. Calc. for $C_{17}H_{18}N_2O_2$ ($M_r = 282.34$): C, 72.32; H, 6.43; N, 9.92%.

3.1.15. Dimethyl(3-phthalimidopropyl)amine (**15**)

This compound was synthesized according to Ref. [2].

3.1.16. 1,8-Naphthalic acid anhydride (**16**)

This compound was commercially available (Acros, 12814).

3.1.17. Phthalic acid anhydride (**17**)

This compound was commercially available (Acros, 15496).

3.1.18. Allylamine (**18**)

This compound was commercially available (Fluka, 05810).

3.1.19. N,N-Dimethyl-1,3-propanediamine (**19**)

This compound was commercially available (Lancaster, 3539).

3.2. Pharmacology

3.2.1. Cell cultures and membrane preparations

Chinese hamster ovary (CHO) cells stably expressing cDNA encoding human muscarinic M_1–M_5 receptors were kindly provided by Prof. Dr. G. Lambrecht (Department of Pharmacology, Biocenter Niederursel, University of Frankfurt/Main, Germany). These cells were grown at 37 °C under humidified air supplemented with 5% CO_2 in Ham's F12 medium containing 10% (v/v) newborn calf serum, 100 IU ml^{-1} penicillin G, 100 μg ml^{-1} streptomycin, 1 mM glutamine, and 0.2 mg ml^{-1} geneticin (G 418). For membrane preparation, cells were grown to 80–90% confluence, treated for 24 h with a cell culture medium supplemented with 5 mM sodium butyrate, subsequently harvested by scraping in a hypotonic medium (20 mM HEPES and 10 mM disodium EDTA, pH 7.4, 4 °C), and then kept at 0 °C. After homogenization with a Polytron homogenizer (Kinematica, Lucerne, Switzerland) at 0 °C, the membranes were centrifuged (40,000 × *g*, 10 min, 4 °C). The supernatant was discarded, and the pellets were resuspended in storage buffer (20 mM HEPES and 0.1 mM disodium EDTA, pH 7.4, 4 °C). The centrifugation and resuspending processes were repeated twice. The membrane suspensions were stored at −80 °C.

3.2.2. [^{3}H]NMS binding experiments

The allosteric effects of the test compounds were measured using the orthosteric ligand [^{3}H]NMS (specific activity 70.0 or 83.5 Ci $mmol^{-1}$; Perkin–Elmer Life Science, Inc., Boston, MA) in binding experiments with porcine heart homogenates and homogenates of CHO cells stably expressing cDNA encoding human muscarinic M_1–M_5 receptors (3 mM $MgHPO_4$, 50 mM Tris–HCl, pH 7.3, 37 °C). Homogenates of porcine heart were prepared as described previously [3d]. To examine the effect of the test compounds on [^{3}H]NMS dissociation of porcine M_2 receptors, membranes were preincubated with 0.2 nM [^{3}H]NMS for 30 min in an assay volume of 24 ml. Dissociation of [^{3}H]NMS receptor complexes was made visible by addition of 1 μM atropine, alone or in combination with the respective test compound, and aliquots of 1 ml each were removed from the assay over a period of 120 min. In the absence of any test compound, i.e., under control conditions, the half-life of dissociation amounted to $t_{1/2,control} = 2.70 \pm 0.09$ min, mean ± S.E.M., $n = 11$. Membranes were collected by manual vacuum filtration over glass fiber filters (Schleicher & Schüll, Dassel, Germany). Radioactivity was determined by liquid scintillation counting. The effect of the test compounds on [^{3}H]NMS equilibrium binding ($pK_{D,control} = 9.40 \pm 0.02$, mean ± S.E.M., $n = 3$) was measured after an incubation of 3.5–5 h depending on the extent to which the test compounds slowed the dissociation kinetics of [^{3}H]NMS [7]. Nonspecific binding of [^{3}H]NMS was determined in the presence of 1 μM atropine. To determine the effect of the exemplarily selected compound **4** on [^{3}H]NMS binding to M_1–M_5 receptor subtypes, kinetic and equilibrium binding experiments were carried out using homogenates of CHO cells stably expressing human muscarinic M_1–M_5 receptors. To quantify the effect of **4** on [^{3}H]NMS dissociation, membranes were preincubated with [^{3}H]NMS (0.2 nM for experiments with M_2; 0.8 nM for experiments with M_1, M_3, M_4, and M_5) over periods of about 60 min (M_1, M_2, M_3) or 180 min (M_5) in the incubation buffer. To measure [^{3}H]NMS dissociation, aliquots (300 μl) were distributed to tubes containing 10 μl atropine in an aqueous solution (30 μM) and

either 10 μl of water (control) or 10 μl of test compound in aqueous solution. The dissociation measurement was allowed to proceed over eight fixed-time periods of up to 120 min. In case of the M_4 receptor, the allosteric agent could not be added together with atropine at the start of the dissociation measurement, because the effect of the agent was not instantaneously present. Therefore, the allosteric test compound was preincubated together with [^{3}H]NMS and the receptor suspension for about 180 min before distributing to the tubes. The dissociation experiments were terminated by filtering the samples over glass fiber filtermats (Wallac, Turku, Finland) presoaked in 0.2% polyethylenimine for 1 h, using a 96-well Tomtech cell harvester (Tomtech, Hamden, USA). Filter-bound radioactivity was measured by solid scintillation counting. Under control conditions, half-life times of dissociation amounted to: for M_1: $t_{1/2} = 12.5 \pm 1.4$ min, $n = 7$; for M_2: $t_{1/2} = 3.7 \pm 0.2$ min, $n = 17$; for M_3: $t_{1/2} = 13.5 \pm 1.5$ min, $n = 4$; for M_4: $t_{1/2} = 14.4 \pm 1.1$ min, $n = 15$; for M_5: $t_{1/2} = 25.3 \pm 2.5$ min, $n = 5$ (mean values ± S.E.M.). The effects of compound **4** on [^{3}H]NMS (0.2 nM) equilibrium binding at M_1–M_5 receptors was measured after 3 h of incubation. The affinity of NMS as determined by homologous competition experiments amounted to: for M_1: $pK_{D,control} = 9.20 \pm 0.03$, $n = 3$; for M_2: $pK_{D,control} = 9.10 \pm 0.08$, $n = 14$; for M_3: $pK_{D,control} = 9.19 \pm 0.08$, $n = 3$; for M_4: $pK_{D,control} = 9.34 \pm 0.15$, $n = 3$; for M_5: $pK_{D,control} = 8.96 \pm 0.02$, $n = 3$ (mean values ± S.E.M.). Nonspecific binding of [^{3}H]NMS was determined in the presence of 1 μM atropine.

Acknowledgements

Financial support of this work by the Deutsche Forschungsgemeinschaft (K.M.) and the Fonds der Chemischen Industrie (R.T.) is gratefully acknowledged. J.O.D. thanks the Fonds der Chemischen Industrie for a postgraduate fellowship.

References

[1] Reviews dealing with allosteric modulation at muscarinic receptors:
(a) S. Tucek, J. Proška, Trends Pharmacol. Sci. 16 (1995) 205;
(b) U. Holzgrabe, K. Mohr, Drug Discov. Today 3 (1998) 214;
(c) U. Holzgrabe, W. Bender, H.M. Botero Cid, M. Staudt, R. Pick, C. Pfletschinger, E. Balatková, C. Tränkle, K. Mohr, Pharm. Acta Helv. 74 (2000) 149;
(d) A. Christopoulos, Nature Rev. Drug Discov. 1 (2002) 198.
[2] J.O. Daiss, S. Duda-Johner, C. Burschka, U. Holzgrabe, K. Mohr, R. Tacke, Organometallics 21 (2002) 803.
[3] Publications dealing with structure–activity relationships of W84-type allosteric modulators:
(a) E. Kostenis, U. Holzgrabe, K. Mohr, Eur. J. Med. Chem. 29 (1994) 947;
(b) U. Holzgrabe, M. Wagener, J. Gasteiger, J. Mol. Graph. 14 (1996) 185, 217.;
(c) U. Holzgrabe, A.J. Hopfinger, J. Chem. Inf. Comput. Sci. 36 (1996) 1018;
(d) C. Tränkle, E. Kostenis, U. Burgmer, K. Mohr, J. Pharmacol. Exp. Ther. 279 (1996) 926;
(e) M. Staudt, C. Tränkle, K. Mohr, U. Holzgrabe, Life Sci. 62 (1998) 423;
(f) T. Nassif-Makki, C. Tränkle, D. Zlotos, G. Bejeuhr, A. Cambareri, C. Pfletschinger, E. Kostenis, K. Mohr, U. Holzgrabe, J. Med. Chem. 42 (1999) 849;
(g) H.M. Botero Cid, C. Tränkle, K. Baumann, R. Pick, E. Mies-Klomfass, E. Kostenis, K. Mohr, U. Holzgrabe, J. Med. Chem. 43 (2000) 2155;
(h) W. Bender, M. Staudt, C. Tränkle, K. Mohr, U. Holzgrabe, Life Sci. 66 (2000) 1675.
[4] Recent publications dealing with other silicon-based drugs:
(a) R. Tacke, M. Merget, R. Bertermann, M. Bernd, T. Beckers, T. Reissmann, Organometallics 19 (2000) 3486;
(b) M. Merget, K. Günther, M. Bernd, E. Günther, R. Tacke, J. Organomet. Chem. 628 (2001) 183;
(c) R. Tacke, T. Kornek, T. Heinrich, C. Burschka, M. Penka, M. Pülm, C. Keim, E. Mutschler, G. Lambrecht, J. Organomet. Chem. 640 (2001) 140;
(d) C.A. Chen, S.M.cN. Sieburth, A. Glekas, G.W. Hewitt, G.L. Trainor, S. Erickson-Viitanen, S.S. Garber, B. Cordova, S. Jeffry, R.M. Klabe, Chem. Biol. 8 (2001) 1161;
(e) J. Kim, A. Glekas, S.McN. Sieburth, Bioorg. Med. Chem. Lett. 12 (2002) 3625;
(f) M. wa Mutahi, T. Nittoli, L. Guo, S.McN. Sieburth, J. Am. Chem. Soc. 124 (2002) 7363;
(g) R. Tacke, V.I. Handmann, K. Kreutzmann, C. Keim, E. Mutschler, G. Lambrecht, Organometallics 21 (2002) 3727;
(h) R. Tacke, T. Heinrich, Silicon Chem. 1 (2002) 35;
(i) R. Tacke, V.I. Handmann, R. Bertermann, C. Burschka, M. Penka, C. Seyfried, Organometallics 22 (2003) 916.
[5] S. Duda-Johner, J. Daiß, U. Holzgrabe, R. Tacke, K. Mohr, Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl. 365 (2002) R25.
[6] C. Tränkle, E. Mies-Klomfass, M.H. Botero Cid, U. Holzgrabe, K. Mohr, Mol. Pharmacol. 54 (1998) 139.
[7] S. Lazareno, N.J.M. Birdsall, Mol. Pharmacol. 48 (1995) 362.