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Antiprotozoal activity of bicyclic diamines with a *N*-methylpiperazinyl group at the bridgehead atom

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ABSTRACT

ω-Aminoacyl and -alkyl derivatives of 4-(4-methylpiperazin-1-yl)bicyclo[2.2.2]octan-2-amines and of 5-(4-methylpiperazin-1-yl)-2-azabicyclo[3.2.2]nonanes were prepared and their activities were examined in vitro against the multiresistant K_1 strain of *Plasmodium falciparum* and against *Trypanosoma brucei rhodesiense* (STIB 900). Some of the newly synthesized compounds showed very promising antiprotozoal activity and selectivity. A few of the alkylamino-2-azabicyclo[3.2.2]nonanes exhibited high antiplasmodial activity, whereas a single bicyclo[2.2.2]octane derivative was the most potent antitrypanosomal compound. The results of the newly synthesized compounds were compared with the activities of already synthesized compounds and of drugs in use. Structure–activity relationships were discussed.

1. Introduction

Malaria and Human African Trypanosomiasis (HAT) are both one-celled parasitic tropical diseases transmitted by the bite of infected insects either.

Malaria is caused by the protozoan genus *Plasmodium*. According to the World malaria report 2012, there were about 219 million cases of malaria and estimated 660,000 deaths in 2010.[1] There are five types of human malaria parasites: *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi* and *Plasmodium falciparum*. The majority of infections are caused by *P. falciparum*, the most lethal causative organism of the various malaria parasites. *P. falciparum* has become resistant to conventional treatment, in some areas even to recommended artemisinin-based combination therapies.[2,3] Therefore, new drugs have to be developed for the fight against the most deathly drug-resistant strains of *P. falciparum*.

HAT, also known as sleeping sickness, is caused by the genus *Trypanosoma*. In 2009, after continued control efforts, the number of cases reported has dropped below 10,000 for the first time in 50 years. But the estimated number of actual cases is currently about 30,000 cases a year.[4] In the case of *Trypanosoma brucei rhodesiense* infections, the disease is acute, lasting from a few weeks to several months while in *Trypanosoma brucei gambiense* infections the affection is chronic, generally lasting several years without any major signs or symptoms.[5] Without treatment sleeping sickness ends fatal. There are only a handful of drugs for the therapy of HAT: pentamidine, suramin, melarsoprol and eflornithine. But all of them suffer from unacceptable toxicity, administration difficulties and increasing drug-resistance as main treatment failure.[6] For the treatment of the late stage of *T. b. rhodesiense*, melarsoprol is the only available effective drug.[5] Unfortunately melarsoprol may cause an encephalopathy, killing 5% of the patients.[7] Therefore, the development of new drugs against Human African Trypanomiasis is urgently required.

The antiprotozoal properties of several bicyclo[2.2.2]octane and 2-azabicyclo[3.2.2]nonane derivatives strongly depended on the amino substitution of the bridgehead atom and the terminal amino function of the side chain in ring position 2.[8–10] Recently, we reported the synthesis of the new 4-(4-methylpiperazin-1-yl)bicyclo[2.2.2]octan-2-one **1a** and the new 5-(4-methylpiperazin-1-yl)-2-azabicyclo[3.2.2]nonane **17a**.[10] The present study deals with the influence of varying terminal amino groups in the side-chain of several bicyclic compounds with a *N*-methylpiperazinyl group as amino component at the bridgehead atom. All newly synthesized compounds were characterized and tested in vitro for their activities against *T. brucei rhodesiense* and the multiresistant K_1 strain of *P. falciparum*. The results were compared to formerly prepared analogues and of drugs in use.

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2. Results

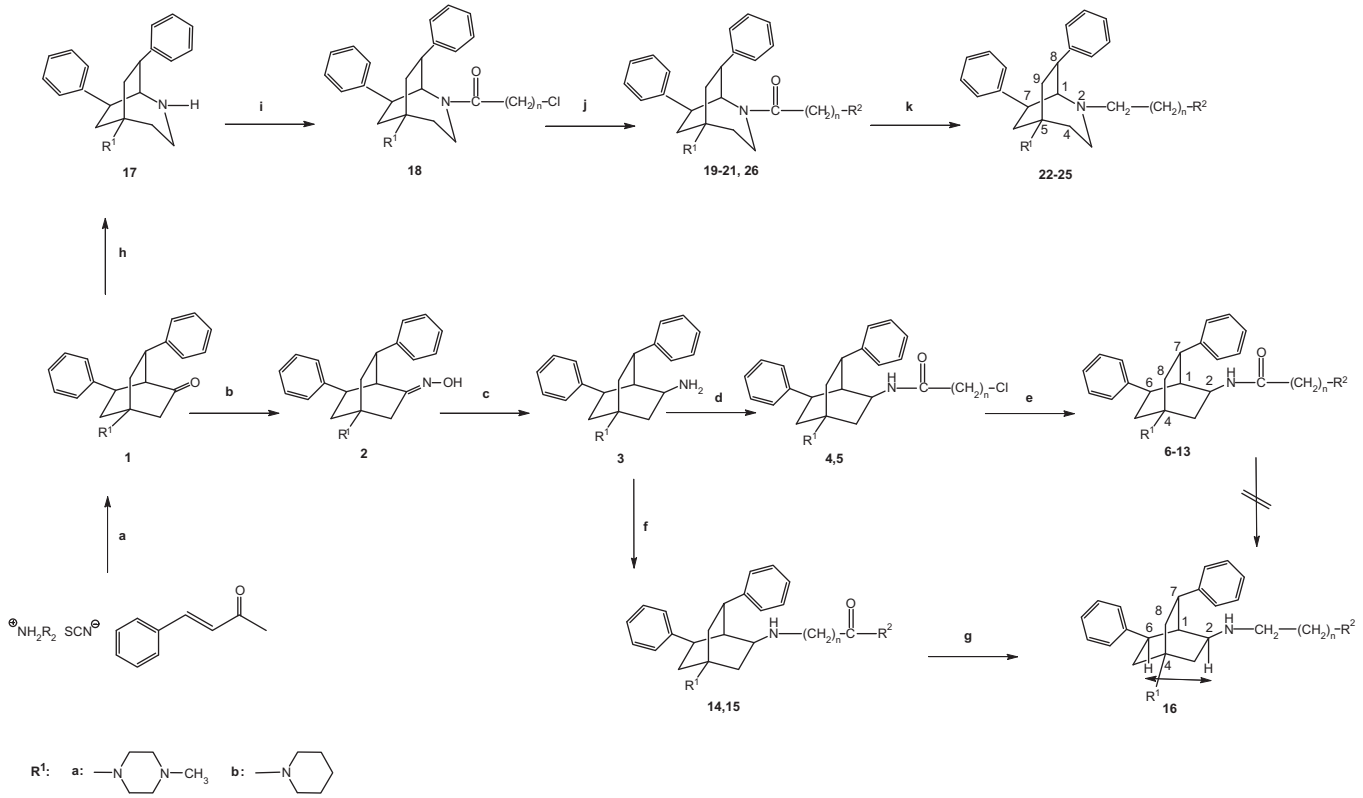
2.1. Chemistry

The syntheses of all new compounds ran out of a one-pot reaction using acyclic products to achieve 4-(4-methylpiperazin-1-yl)bicyclo[2.2.2]octan-2-one **1a** as starting material. The 2-(ω-aminoacyl) derivatives **6a**–**13a** of bicyclo[2.2.2]octan-2-amine **3a** were prepared from bicyclo[2.2.2]octan-2-one **1a** via the oxime **2a**. This was stereoselectively hydrogenated with Raney nickel giving the (2-*exo*)-amine **3a**.[10] The 2-(ω-chloroalkanoyl) amides **4a**, **5a** were afforded by reaction with the corresponding ω-chloroacyl chloride and triethylamine. In the next step the 2-(ω-chloroalkanoyl) derivatives **4a**, **5a** were substituted with different kinds of secondary amines yielding **6a**–**13a**. As recently reported it was not possible to hydrogenate 2-(ω-aminoalkanoyl)bicyclo[2.2.2]octane amides any way.[8] Therefore another method was developed to get 2-(ω-aminoalkyl) derivatives of bicyclo[2.2.2]octanes. Different ω-chloroamides were prepared which gave with the bicyclo[2.2.2]octan-2-amine **3a** several ω-(bicyclo[2.2.2]octan-2-yl) alkanamides **14a**, **15a**. Compound **15a** was converted into the corresponding *N*-(aminoalkyl) analogue **16a** using $LiAlH_4$.

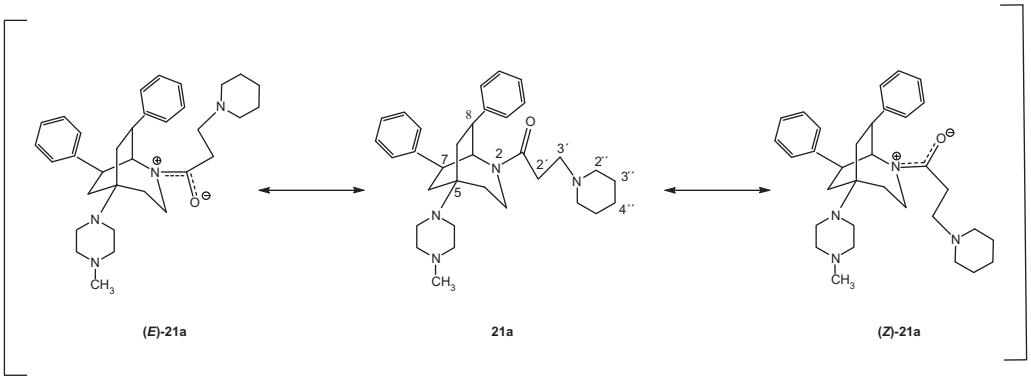
2-Azabicyclo[3.2.2]nonane **17a** was accessible by a Beckmann rearrangement of the bicyclo[2.2.2]octane-2-one **1a** yielding the 2-azabicyclo[3.2.2]nonan-2-one which was subsequently hydrogenated using $LiAlH_4$. The next step was the acylation of the bicyclic nonane **17a** with ω-chloropropionyl chloride in the presence of triethylamine, followed by the substitution of the ω-chloropropionylnonanes **18a** with secondary amines to form 2-(ω-aminoacyl)-2-azabicyclo[3.2.2]nonanes **19a**–**21a**. In addition these 2-(ω-aminoacyl)nonanes **19a**–**21a** were converted to 2-(ω-aminopropyl)-2-azabicyclo[3.2.2]nonanes **22a**–**24a** by hydrogenation using $LiAlH_4$ (Scheme 1). Compounds **25** have been prepared in a similar way from acylnonanes **26** [10].

Compounds	n	R^2
1a, 1b, 2a, 2b, 3a, 3b, 17a, 17b	-	-
4a, 4b	1	-
8a, 14a	1	Diethylamino
9a	1	Pyrrolidino
10a	1	Piperidino
6a, 6b	1	N-Methylpiperazinyl
5a, 5b, 18a, 18b	2	-
11a, 19a , 22a	2	Diethylamino
12a, 15a, 15b, 16a, 16b, 20a, 23a	2	Pyrrolidino
13a, 21a, 24a, 24b	2	Piperidino
7a, 7b, 25a, 25b, 26a, 26b	2	N-Methylpiperazinyl

The structure of all newly synthesized compounds was elucidated by one- and two-dimensional NMR spectroscopy. The relative configuration in ring position 2 of the bicyclo[2.2.2]octanes **8a**–**16a** was confirmed by through-space couplings in their NOE spectra from the 2-H to the 6-H (Scheme 1). The appearance of two sets of signals in the ^1H and ^{13}C NMR spectra of compounds **19a**–**21a** indicated the restricted rotation around the C(=O)–N bond which is due to their partial double-bond character (Scheme 2).The exact distinction between the signals of the corresponding (*E*)- and (*Z*)-diastereomers of compounds **19a**–**21a** succeeded with the aid of the typical upfield-shifts[11] for the C-1 or the C-3 in (*Z*)-relation to the carbonyl oxygen in their ^{13}C NMR spectra.



Scheme 1. Preparation of compounds **1**–**26**. Reagents and conditions: (a) toluene, 160 °C, 4–6 h; (b) $NH_2OH \times HCl$, NaOEt, 110 °C, 16 h; (c) Raney nickel, EtOH, 50 psi (H_2), rt, 16 h; (d) (1) ω-chloroacyl chloride, triethylamine, CH_2Cl_2, rt, 16 h; (e) *sec.* amine, KI, rt, 48–120 h; (f) *method A:* ω-chloroalkanoyl diethylamine, EtOH, $KHCO_3$, KI, rt, 48 h, 85 °C, 20 h; *method B:* ω-chloroalkanoyl pyrrolidine, EtOH, KI, 48 h, 110 °C; (g) $LiAlH_4$, diethylether, 55 °C, 16 h; (h) (1) NH_2OSO_3H, glacial acetic acid, conc. H_2SO_4, 145 °C, 16 h; (2) $LiAlH_4$, diethylether, 55 °C, 48 h; (i) ω-chloroacyl chloride, triethylamine, CH_2Cl_2, rt, 16 h; (j) *sec.* amine, KI, rt, 72 h; (k) $LiAlH_4$, diethylether, 55 °C, 20 h;



Scheme 2. (*E*/*Z*)-Character of compound **21a**.

Table 1
Activities of compounds **6**–**25** against *T. brucei rhodesiense*, *P. falciparum* K_1, and[12] L-6 cells, expressed as IC_{50} (μM)[a]

Compd	*T. brucei rhodesiense*	S.I. = IC_{50} (Cytotoxicity)/ IC_{50} (*T.b.r.*)	*P. falciparum* K_1[b]	S.I. = IC_{50} (Cytotoxicity)/ IC_{50} (*P. falciparum*)	Cytotoxicity L-6 cells
6a 10	2.06	20.43	0.62	67.87	42.08
6b 12	0.69	83.36	0.38	151.4	57.52
7a 10	3.30	20.57	0.55	123.4	67.88
7b 12	0.86	71.00	0.28	218.1	61.06
8a	0.67	39.40	0.37	71.35	26.40
9a	0.25	111.2	0.28	99.28	27.80
10a	0.094	235.4	0.35	63.23	22.13
11a	2.29	15.69	0.67	53.64	35.94
12a	3.10	13.60	0.55	76.65	42.16
13a	1.27	12.09	0.23	66.74	15.35
14a	0.50	75.88	1.16	32.71	37.94
15a	0.50	42.90	0.59	36.35	21.45
16a	1.61	14.05	0.67	33.76	22.62
16b 8	2.02	13.36	0.23	117.3	26.99
22a	2.56	26.60	0.06	1135	68.09
23a	2.59	16.81	0.06	725.7	43.54
24a	2.54	11.36	0.05	577.2	28.86
24b 8	18.43	7.50	0.12	1152	138.3
25a 10	6.67	7.16	0.25	191.0	47.75
25b 10	17.57	3.55	0.11	567.7	62.45
Art.			0.0064	70.39	450.5
Chl.			0.15	1257	188.5
Mel.	0.009	1945			7.78

Art. = artemisinin; chl. = chloroquine; mel. = melarsoprol.
[a]Values represent the average of four determinations (two determinations of two independent experiments).
[b]Resistant to chloroquine and pyrimethamine.

2.2. Antiplasmodial activity and cytotoxicity

Routinely all newly synthesized compounds were purified over small aluminum oxide columns. They were tested via microplate assays for their activity against the multiresistant K_1 strain of *P. falciparum* and *T. brucei rhodesiense*. Their cytotoxicity was determined as well. The IC_{50} values of all newly synthesized compounds, formerly prepared analogues and drugs in use are given in Table 1. Selected compounds were tested in vivo in mice against *T. brucei rhodesiense* (STIB 900) and *Plasmodium berghei*.

3. Discussion

In the 2-azabicyclo[3.2.2]nonane series compounds so far turned out to be quite active against *P. falciparum* K_1 but only moderate potent against *T. brucei rhodesiense*.

The formerly prepared 2-(3-aminopropyl)-5-piperidino-2-azabicyclononanes **24b** and **25b** showed good antiplasmodial activity (IC_{50} = 0.11–0.12 μM) and very promising selectivity indices (S.I.: 567.7–1152). Their new synthesized 5-(*N*-methylpiperazinyl) analogues **22a**–**24a** were more active (IC_{50} = 0.05–0.06 μM). Their selectivity indices covered a similar range (S.I.: 577.2–1135). When the positions of the piperidino and *N*-methylpiperazinyl substituent were swapped the antiplasmodial activity was changed (**24a:** IC_{50} = 0.05 μM; **25b**: IC_{50} = 0.11 μM). The formerly prepared compound **25a** which possesses two *N*-methylpiperazinyl groups exhibited lower antiplasmodial activity (IC_{50} = 0.25 μM) and decreased selectivity (S.I.: 191.0). The antitrypanosomal activity in this current 5-(*N*-methylpiperazinyl) series was slightly increased compared to formerly prepared 2-azabicyclononane analogues but nevertheless is not really worth mentioning (**22a**–**24a**: IC_{50} = 2.54–2.59 μM; S.I.: 11.36–26.60; **24b**, **25a**,**b**: IC_{50} = 6.67–18.43 μM; S.I.: 3.55–7.50).

In the 2-(ω-aminoacylamino)-4-dialkylaminobicyclo[2.2.2]octane series compounds showed moderate to good antitrypanosomal activity and quite good antiplasmodial activity. Representatives with a 4-piperidino substituent (**6b**, **7b**: IC_{50} (*P.f.*) = 0.28–0.38 μM; IC_{50} (*T.b.r.*) = 0.69–0.86 μM) were more active against *P. falciparum*

Table 2
In vivo activities of compounds **22a**, **23a**, **24a** against *Plasmodium berghei* expressed as IC_{50} (μM)

Comp.	Application	Dose (mg/kg)	MSD	Activity (%)
22a	I.p.	4 × 50	4	17
23a	I.p.	4 × 50	4	0
24a	I.p.	4 × 50	4	11
Chloroquine	I.p.	4 × 50	20	99.6
Control			6–7	

K_1 as well as against *T. brucei rhodesiense* than their 4-(*N*-methylpiperazinyl) analogues (**6a**, **7a**: IC_{50} (*P.f.*) = 0.55–0.62 μM; IC_{50} (*T.b.r.*) = 2.06–3.30 μM). However, particularly the antitrypanosomal activity of the latter compounds was noticeably increased by replacement of the terminal *N*-methylpiperazinyl group by amino substituents featuring only a single nitrogen atom as in compounds **8a**–**10a**. The antiplasmodial activity did not change significantly. The most promising representative in this series which possesses a terminal piperidino group was compound **10a** exhibiting superior antitrypanosomal activity (IC_{50} = 0.094 μM; S.I.: 235.4). The piperidino group turned out once more to be a potent substituent. Concerning the length of the aminoacyl chain the acetyl derivatives **8a**–**10a** (IC_{50} (*T.b.r.*) = 0.094–0.67 μM) were in general more active than their propionyl analogues **11a**–**13a** (IC_{50} (*T.b.r.*) = 1.27–3.10 μM).

In the 2-azabicyclo-nonane series the ω-aminopropyl derivatives **22a**–**24a** exhibited much better biological activity than their parent acyl analogues **19a**–**21a**. However, the first aminoalkyl representatives **16a** and **16b** in the bicyclo-octane series possessed only moderate activity (**16a**, **16b**: IC_{50} (*P.f.*) = 0.23–0.67 μM; IC_{50} (*T.b.r.*) = 1.61–2.02 μM). This indicates that antiprotozoal activity is positively influenced by the aminoacyl structure in the side-chain of 2-(ω-aminoacylamino)-4-dialkylaminobicyclo[2.2.2]octanes.

The in vivo activities of the most active compounds **10a**, **22a**, **23a**, **24a** were determined either against *P. berghei* or *T. brucei rhodesiense* in a mouse model. The in vitro test of compound **10a** was very promising but in the in vivo testing against *T. brucei rhodesiense* STIB900 with a dose of 4 × 50 mg/kg administered intraperitoneally it was inactive. Compounds **22a**, **23a**, **24a** were tested for their activity against *P. berghei*. But they showed only weak activity. The results are given in Table 2. The most promising compounds meet the requirements of Lipinski's rule of five. However, our calculations (http://www.chemaxon.com) have revealed that the problems could probably be attributed to the rather low log*D*-values of the compounds.[13] Therefore compounds with increased log*D*-values will be prepared in a future study.

4. Conclusion

This paper reports the syntheses and the antitrypanosomal and antiplasmodial activities of new bicyclic compounds with a *N*-methylpiperazinyl group as amino component at the bridgehead atom. Several bicyclo[2.2.2]octanes and 2-azabicyclo[3.2.2]nonanes with different terminal ω-amino substituents in the side chain as well as different chain length were prepared. The correlation between spacer-length and antiprotozoal activity was non-uniform. The influence of the terminal ω-amino substituent on the antiplasmodial activity and the cytotoxicity was obviously heterogeneous. It turned out that derivatives with a single *N*-methylpiperazinyl group at the bridgehead atom were among the more active compounds, whereas a second *N*-methylpiperazinyl group in a molecule generally decreased the antiprotozoal activity. 2-Azabicyclo[3.2.2]nonanes with an ω-aminopropyl substituent possessed high antiplasmodial activity, whereas in the bicyclo[2.2.2]octane series a compound exhibiting a ω-piperidinoacetamido group showed high antitrypanosomal activity. Compounds with a terminal piperidino group were among the most active against *T. brucei rhodesiense* and *P. falciparum*.

Therefore the emphasis for further studies will be placed on the piperidino group as amino substituent.

5. Experimental

5.1. Instrumentation and chemicals

The purity of compounds is generally checked with HPLC using UV detection. By default compounds are in addition purified prior to spectroscopic analysis over small aluminum oxide columns. IR spectra: infrared spectrometer system 2000 FT (Perkin Elmer) in KBr discs; frequencies are reported in cm^{-1}. UV/vis: Lambda 17 UV/vis spectrometer (Perkin Elmer), maxima reported in nm. NMR spectra: Varian Unity Inova 400 (298 K) 5 mm tubes, TMS as internal standard. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra are reported in ppm, ^1H and ^{13}C-resonances were assigned using $^1H,^1H$- and $^1H,^{13}C$-correlation spectra and are numbered as given in the formulas. Signal multiplicities are abbreviated as follows: br broad, d doublet, ddd double double doublet, dt double triplet, m multiplet, s singlet, t triplet, td triple doublet, q quartet, resonances marked with a single quote belong to the alkyl chain of the compounds, those with a double quote belong to their ω-dialkylamino group. HRMS: Micromass Tofspec spectrometer (MALDI), GCT-Premier, Waters (EI, 70 eV). Materials: column chromatography (CC): aluminum oxide (pH: 9.5, Fluka), thin-layer chromatography (TLC): TLC plates (Merck) aluminum oxide 60 F254 neutral.

5.2. Syntheses

The syntheses of compounds **1a**, **3a** and **17a** have already been reported[10].

5.2.1. General procedure for the synthesis of (2*SR*,6*RS*,7*RS*)-(±)-*N*-(4-(4-methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)-ω-aminoalkanamides (8a–13a)

To an ice-cooled solution of bicyclo-octanamine **3a** and triethylamine in dry CH_2Cl_2 the ω-chloroacyl chloride was added under stirring. After 30 min the ice-bath was removed and the reaction batch was stirred overnight at ambient temperature in an atmosphere of Ar. Subsequently the reaction batch was shaken with 1 N aq NaOH and the aqueous phase was exhaustively extracted with CH_2Cl_2. The organic phase was washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo yielding the ω-chloroalkanamide **4a**, **5a**. Compounds **4a**, **5a** and a catalytic amount of KI were dissolved in an excess of secondary amine. The mixture was stirred for 48–120 h at ambient temperature in an atmosphere of Ar. Subsequently benzene was added and the reaction batch was evaporated. The residue was dissolved in CH_2Cl_2, washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and finally the solvent was removed in vacuo giving **8a**–**13a**.

5.2.1.1. (2*SR*,6*RS*,7*RS*)-(±)-2-Diethylamino-*N*-(4-(4-methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)acetamide (8a). The reaction of bicyclo-octanamine **3a** (0.273 g, 0.728 mmol), triethylamine (0.110 g, 1.09 mmol) and chloroacetyl chloride (0.123 g, 1.09 mmol) in CH_2Cl_2 (15 mL) gave ω-chloroacetamide **4a** (0.303 g, 0.670 mmol, 92%). **4a**, diethylamine (2.5 mL) and a catalytic amount of KI yielded after 96 h **8a** (0.224 g, 0.445 mmol, 68%). IR = 3057, 3025, 2933, 2871, 2793, 1671, 1600, 1497, 1448,

1163, 1031, 1011, 745, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.513); ^{1}H NMR ($CDCl_3$) δ = 0.71 (t, J = 7.1 Hz, 6H, 2″-H), 1.42 (d, J = 13.3 Hz, 1H, 3-H), 1.82–1.91 (m, 2H, 5-H, 8-H), 2.07–2.12 (m, 1H, 5-H), 2.14 (q, J = 7.1 Hz, 4H, 1″-H), 2.26-2.33 (m, 1H, 3-H), 2.31 (s, 3H, NCH_3), 2.35 (ddd, J = 12.8, 9.8, 3.0 Hz, 1H, 8-H), 2.47 (d, J = 16.8 Hz, 1H, 2′-H), 2.47-2.58 (m, 4H, $N(CH_2)_2$), 2.66 (d, J = 16.8 Hz, 1H, 2′-H), 2.71 (d, J = 3.2 Hz, 1H, 1-H), 2.71–2.82 (m, 4H, $N(CH_2)_2$), 3.12 (t, J = 9.2 Hz, 1H, 6-H), 3.20 (t, J = 9.8 Hz, 1H, 7-H), 4.35–4.44 (m, 1H, 2-H), 7.09 (d, J = 8.2 Hz, 1H, NH), 7.10–7.39 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ = 11.48 (C-2″), 30.25 (C-5), 33.20 (C-8), 33.75 (C-7), 37.15 (C-3), 39.22 (C-1), 40.73 (C-6), 45.30 ($N(CH_2)_2$), 45.80 (NCH_3), 47.93 (C-1″), 48.43 (C-2), 55.66 ($N(CH_2)_2$), 56.37 (C-4), 56.52 (C-2′), 125.61, 126.15, 126.20, 127.21, 128.19, 128.37 (aromatic C), 143.19, 144.03 (aromatic C_q), 171.53 (CO); HRMS (EI+) calcd for $C_{31}H_{44}N_4O$: 488.3515; found: 488.3546.

5.2.1.2. (2*SR*,6*RS*,7*RS*)-(±)-*N*-(4-(4-Methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)-2-pyrrolidinoacetamide (9a). The reaction of bicyclo-octanamine **3a** (0.292 g, 0.779 mmol), triethylamine (0.118 g, 1.17 mmol) and chloroacetyl chloride (0.132 g, 1.17 mmol) in CH_2Cl_2 (15 mL) gave ω-chloroacetamide **4a** (0.324 g, 0.716 mmol, 92%). **4a**, pyrrolidine (2.5 mL) and a catalytic amount of KI yielded after 48 h **9a** (0.253 g, 0.520 mmol, 73%). IR = 3057, 3024, 2932, 2874, 2795, 1646, 1600, 1542, 1497, 1447, 1164, 1032, 1013, 747, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.255), 266 (3.077); ^{1}H NMR ($CDCl_3$) δ = 1.41 (br d, J = 13.3 Hz, 1H, 3-H), 1.53–1.60 (m, 4H, 3″-H, 4″-H), 1.82–1.92 (m, 2H, 5-H, 8-H), 2.04–2.13 (m, 3H, 2″-H, 5-H, 5″-H), 2.26–2.40 (m, 4H, 2″-H, 3-H, 5″-H, 8-H), 2.31 (s, 3H, NCH_3), 2.47–2.57 (m, 4H, $N(CH_2)_2$), 2.51 (d, J = 16.3 Hz, 1H, 2′-H), 2.64 (d, J = 16.3 Hz, 1H, 2′-H), 2.72 (d, J = 3.4 Hz, 1H, 1-H), 2.72–2.84 (m, 4H, $N(CH_2)_2$), 3.13 (t, J = 9.2 Hz, 1H, 6-H), 3.19 (t, J = 9.5 Hz, 1H, 7-H), 6.83 (d, J = 8.2 Hz, 1H, NH), 7.10–7.40 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ = 23.79 (C-3″, C-4″), 29.81 (C-5), 33.13 (C-8), 33.77 (C-7), 37.44 (C-3), 39.59 (C-1), 40.68 (C-6), 45.43 ($N(CH_2)_2$), 45.90 (NCH_3), 48.33 (C-2), 54.08 (C-2″, C-5″), 55.75 ($N(CH_2)_2$), 56.45 (C-4), 59.16 (C-2′), 125.65, 126.20, 126.29, 127.29, 128.36, 128.50 (aromatic C), 143.31, 144.14 (aromatic C_q), 170.46 (CO); HRMS (EI+) calcd for $C_{31}H_{42}N_4O$: 486.3359; found: 486.3358.

5.2.1.3. (2*SR*,6*RS*,7*RS*)-(±)-*N*-(4-(4-Methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)-2-piperidinoacetamide (10a). The reaction of bicyclo-octanamine **3a** (0.290 g, 0.772 mmol), triethylamine (0.117 g, 1.16 mmol) and chloroacetyl chloride (0.130 g, 1.16 mmol) in CH_2Cl_2 (15 mL) gave ω-chloroacetamide **4a** (0.321 g, 0.710 mmol, 92%). **4a**, piperidine (2.5 mL) and a catalytic amount of KI yielded after 48 h **10a** (0.223 g, 0.445 mmol, 62%). IR = 3057, 3025, 2934, 2869, 2793, 1676, 1600, 1497, 1451, 1162, 1129, 1039, 1011, 794, 746, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.487), 268 (3.061); ^{1}H NMR ($CDCl_3$) δ = 1.27–1.40 (m, 7H, 3-H, 3″-H, 4″-H, 5″-H), 1.82 (br t, J = 12.8 Hz, 1H, 8-H), 1.85–1.92 (m, 3H, 2″-H, 5-H, 6″-H), 2.10 (ddd, J = 13.5, 9.2, 1.9 Hz, 1H, 5-H), 2.13–2.20 (m, 2H, 2″-H, 6″-H), 2.18 (d, J = 16.2 Hz, 1H, 2′-H), 2.28 (td, J = 13.5, 3.0 Hz, 1H, 3-H), 2.31 (s, 3H, NCH_3), 2.38 (ddd, J = 12.8, 9.8, 3.0 Hz, 1H, 8-H), 2.47 (d, J = 16.2 Hz, 1H, 2′-H), 2.48–2.59 (m, 4H, $N(CH_2)_2$), 2.69–2.81 (m, 4H, $N(CH_2)_2$), 2.81 (d, J = 3.5 Hz, 1H, 1-H), 3.12 (t, J = 9.2 Hz, 1H, 6-H), 3.19 (t, J = 9.8 Hz, 1H, 7-H), 4.37–4.45 (m, 1H, 2-H), 6.83 (d, J = 8.2 Hz, 1H, NH), 7.08-7.39 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ = 23.51 (C-4″), 25.78 (C-3″, C-5″), 30.56 (C-5), 33.59 (C-8), 33.67 (C-7), 36.60 (C-3), 38.86 (C-1), 40.34 (C-6), 45.32 ($N(CH_2)_2$), 45.83 (NCH_3), 48.05 (C-2), 54.24 (C-2″, C-6″), 55.68 ($N(CH_2)_2$), 56.40 (C-4), 61.78 (C2′), 125.50, 125.99, 126.20, 127.24, 128.26, 128.39 (aromatic C), 143.22, 144.35 (aromatic C_q), 169.93 (CO); HRMS (EI+) calcd for $C_{32}H_{44}N_4O$: 500.3515; found: 500.3531.

5.2.1.4. (2*SR*,6*RS*,7*RS*)-(±)-3-Diethylamino-*N*-(4-(4-methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)propionamide (11a). The reaction of bicyclo-octanamine **3a** (0.307 g, 0.818 mmol), triethylamine (0.124 g, 1.23 mmol) and 3-chloropropionyl chloride (0.156 g, 1.23 mmol) in CH_2Cl_2 (15 mL) gave ω-chloropropionamide **5a** (0.332 g, 0.712 mmol, 87%). **5a**, diethylamine (2.5 mL), CH_2Cl_2 (1 mL) and a catalytic amount of KI yielded after 120 h **11a** (0.235 g, 0.358 mmol, 66%). IR = 3057, 3026, 2934, 2872, 2794, 1645, 1600, 1541, 1497, 1448, 1164, 1012, 746, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.510), 266 (2.857); ^{1}H NMR ($CDCl_3$) δ = 0.92 (t, J = 7.2 Hz, 6H, 2″-H), 1.43 (br d, J = 13.5 Hz, 1H, 3-H), 1.64–1.74 (m, 1H, 2′-H), 1.80–1.99 (m, 4H, 2′-H, 3′-H, 5-H, 8-H), 2.08–2.18 (m, 2H, 3-H, 5-H), 2.26–2.42 (m, 6H, 1″-H, 3′-H, 8-H), 2.33 (s, 3H, NCH_3), 2.46–2.60 (m, 4H, $N(CH_2)_2$), 2.70–2.84 (m, 4H, $N(CH_2)_2$, 2.99 (d, J = 3.5 Hz, 1H, 1-H), 3.11 (t, J = 9.5 Hz, 1H, 6-H), 3.20 (t, J = 9.9 Hz, 1H, 7-H), 4.31–4.38 (m, 1H, 2-H), 7.10–7.39 (m, 10H, aromatic H), 7.42–7.45 (br, 1H, NH); ^{13}C NMR ($CDCl_3$) δ = 10.62 (C-2″), 32.16 (C-2′), 32.53 (C-5), 33.72 (C-7), 33.98 (C-8), 34.77 (C-3), 37.28 (C-1), 40.00 (C-6), 45.09 (C-1″), 45.38 ($N(CH_2)_2$), 45.85 (NCH_3), 48.42 (C-3′), 48.60 (C-2), 55.78 ($N(CH_2)_2$), 56.49 (C-4), 125.54, 126.06, 126.20, 127.32, 128.17, 128.41 (aromatic C), 143.33, 144.99 (aromatic C_q), 171.61 (CO); HRMS (EI+) calcd for $C_{32}H_{46}N_4O$: 502.3672; found: 502.3698.

5.2.1.5. (2*SR*,6*RS*,7*RS*)-(±)-*N*-(4-(4-Methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)-3-pyrrolidinopropionamide (12a). The reaction of bicyclo-octanamine **3a** (0.229 g, 0.609 mmol), triethylamine (0.092 g, 0.913 mmol) and 3-chloropropionyl chloride (0.116 g, 0.913 mmol) in CH_2Cl_2 (10 mL) gave ω-chloropropionamide **5a** (0.247 g, 0.530 mmol, 87%). **5a**, pyrrolidine (2.5 mL), CH_2Cl_2 (1 mL) and a catalytic amount of KI yielded after 72 h **12a** (0.261 g, 0.521 mmol, 98%). IR = 3426, 3190, 3054, 2929, 2872, 2793, 1666, 1601, 1555, 1496, 1447, 1377, 1341, 1292, 1162, 1012, 767, 748, 699; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.940), 265 (3.720); ^{1}H NMR ($CDCl_3$) δ = 1.38 (br d, J = 13.7 Hz, 1H, 3-H), 1.64–1.71 (m, 4H, 3″-H, 4″-H), 1.67–1.79 (m, 2H, 2′-H, 8-H), 1.81–1.93 (m, 2H, 3′-H, 5-H), 1.98 (ddd, J = 16.2, 8.0, 4.4 Hz, 1H, 2′-H), 2.06–2.18 (m, 4H, 2″-H, 3-H, 5-H, 5″-H), 2.23–2.32 (m, 3H, 2″-H, 3′-H, 5″-H), 2.32 (s, 3H, NCH_3), 2.41 (ddd, J = 12.2, 9.8, 3.0 Hz, 1H, 8-H), 2.45–2.60 (m, 4H, $N(CH_2)_2$), 2.66–2.83 (m, 4H, $N(CH_2)_2$, 3.02 (d, J = 4.1 Hz, 1H, 1-H), 3.11 (t, J = 9.3 Hz, 1H, 6-H), 3.20 (t, J = 9.8 Hz, 1H, 7-H), 4.35–4.43 (m, 1H, 2-H), 7.10–7.37 (m, 10H, aromatic H), 8.18 (d, J = 6.7 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$) δ = 23.42 (C-3″, C-4″), 33.17 (C-5), 33.63 (C-2′), 33.64 (C-7), 34.42 (C-8), 34.86 (C-3), 36.32 (C-1), 39.82 (C-6), 45.27 ($N(CH_2)_2$), 45.86 (NCH_3), 47.91 (C-2), 50.69 (C-3′), 52.88 (C-2″, C-5″), 55.78 ($N(CH_2)_2$), 56.40 (C-4), 125.48, 125.86, 126.12, 127.26, 128.10, 128.35 (aromatic C), 143.28, 145.21 (aromatic C_q), 171.52 (CO); HRMS (EI+) calcd for $C_{32}H_{44}N_4O$: 500.3515; found: 500.3504.

5.2.1.6. (2*SR*,6*RS*,7*RS*)-(±)-*N*-(4-(4-Methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)-3-piperidinopropionamide (13a). The reaction of bicyclo-octanamine **3a** (0.220 g, 0.587 mmol), triethylamine (0.089 g, 0.880 mmol) and 3-chloropropionyl chloride (0.112 g, 0.880 mmol) in CH_2Cl_2 (10 mL) gave ω-chloropropionamide **5a** (0.238 g, 0.511 mmol, 87%). **5a**, piperidine (2.5 mL), CH_2Cl_2 (1 mL) and a catalytic amount of KI yielded after 72 h **13a** (0.197 g, 0.263 mmol, 75%). IR = 3423, 3222, 3053, 2928, 2816, 2792, 1665, 1601, 1542, 1496, 1446, 1377, 1350, 1162, 1011, 766, 748, 699; UV (CH_2Cl_2, ($\log\varepsilon$)): 230 (3.878), 262 (3.523); ^{1}H NMR ($CDCl_3$) δ = 1.37–1.54 (m, 7H, 3-H, 3″-H, 4″-H, 5″-H), 1.58–1.70 (m, 2H, 2′-H, 3′-H), 1.81 (ddd, J = 12.6, 9.8, 1.8 Hz, 1H, 8-H), 1.88 (ddd, J = 13.0, 9.6, 2.2 Hz, 1H, 5-H), 1.92–2.09 (m, 4H, 2′-H, 2″-H, 3′-H, 6″-H), 2.10–2.24 (m, 4H, 2″-H, 3-H, 5-H, 6″-H), 2.33 (s, 3H, NCH_3), 2.41 (ddd, J = 12.6, 9.8, 3.0 Hz, 1H, 8-H), 2.46–2.59 (m, 4H, $N(CH_2)_2$), 2.71–2.85 (m, 4H, $N(CH_2)_2$, 3.09 (d, J = 3.3 Hz, 1H,

1-H), 3.11 (t, J = 9.6 Hz, 1H, 6-H), 3.20 (t, J = 9.8 Hz, 1H, 7-H), 4.33–4.41 (m, 1H, 2-H), 7.09–7.38 (m, 10H, aromatic H), 8.20 (d, J = 5.9 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$) δ = 24.12 (C-4″), 26.06 (C-3″, C-5″), 31.39 (C-2′), 33.32 (C-5), 33.65 (C-7), 34.38 (C-3), 34.57 (C-8), 36.18 (C-1), 39.75 (C-6), 45.45 ($N(CH_2)_2$), 45.81 (NCH_3), 48.23 (C-2), 53.34 (C-2″, C-6″), 53.58 (C-3′), 55.79 ($N(CH_2)_2$), 56.53 (C-4), 125.39, 125.85, 126.12, 127.28, 128.06, 128.35 (aromatic C), 143.34, 145.22 (aromatic C_q), 171.67 (CO); HRMS (EI+) calcd for $C_{33}H_{46}N_4O$: 514.3672; found: 514.3666.

5.2.1.7. (2*SR*,6*RS*,7*RS*)-(±)-*N*, *N*-Diethyl-3-((4-(4-methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)amino)acetamide (14a). *Method A:* To an ice-cooled (−5 °C) solution of the diethylamine (0.213 g, 2.91 mmol) in diethylether (8 mL) a solution of chloroacetyl chloride (0.164 g, 1.45 mmol) in diethylether (1 mL) was added through a dropping funnel. The reaction mixture was stirred for 3 h at ambient temperature, then the hydrochloride sideproduct was filtered with suction and the solvent was removed in vacuo giving *N*, *N*-diethyl-2-chloroacetamide (0.183 g, 84%). To a solution of bicyclo-octanamine **3a** (0.460 g, 1.22 mmol), $KHCO_3$ (0.123 g, 1.22 mmol) and a catalytic amount of KI in EtOH (20 mL), *N*, *N*-diethyl-2-chloroacetamide (0.183 g, 1.22 mmol) dissolved in EtOH (10 mL) was added dropwise. The reaction batch was stirred for 48 h at ambient temperature in an atmosphere of Ar and afterwards refluxed for 20 h at 85 °C. Then it was extracted with water and diethylether. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo yielding **14a** (0.260 g, 0.532 mmol, 43%). IR = 3056, 3024, 2968, 2932, 2873, 2792, 1648, 1600, 1496, 1450, 1222, 1164, 1030, 1012, 793, 746, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 268 (2.967), 230 (3.712); ^{1}H NMR ($CDCl_3$): δ = 0.92 (t, J = 7.1 Hz, 3H, 2″-H), 1.03 (t, J = 7.1 Hz, 3H, 2″-H), 1.56 (br d, J = 12.8 Hz, 1H, 3-H), 1.92–2.02 (m, 3H, 3-H, 5-H, 8-H), 2.03–2.10 (m, 1H, 5-H), 2.24 (d, J = 3.7 Hz, 1H, 1-H), 2.32 (s, 3H, NCH_3), 2.35–2.42 (m, 1H, 8-H), 2.48-2.58 (m, 4H, $N(CH_2)_2$), 2.56 (d, J = 15.7 Hz, 1H, 1′-H), 2.73–2.84 (m, 4H, $N(CH_2)_2$), 2.81 (d, J = 15.7 Hz, 1H, 1′-H), 2.82–2.94 (m, 2H, 1″-H), 2.95 (t, J = 9.2 Hz, 1H, 6-H), 3.12–3.20 (m, 2H, 2-H, 7-H), 3.21–3.34 (m, 2H, 1″-H), 7.04 (t, J = 7.0 Hz, 1H, aromatic H), 7.15–7.44 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 12.89 (C-2″), 13.91 (C-2″), 30.73 (C-8), 32.30 (C-5), 35.51 (C-7), 35.95 (C-3), 40.06 (C-1″), 40.57 (C-1″), 41.17 (C-6), 42.72 (C-1), 45.43 ($N(CH_2)_2$), 45.81 (NCH_3), 47.53 (C-1′), 55.70 ($N(CH_2)_2$), 56.99 (C-4), 58.68 (C-2), 125.15, 126.15, 127.30, 127.49, 127.73, 128.41 (aromatic C), 143.98, 144.79 (aromatic C_q), 169.92 (CO); HRMS (EI+) calcd for $C_{31}H_{44}N_4O$: 488.3515; found: 488.3515.

5.2.1.8. (2*SR*,6*RS*,7*RS*)-(±)-3-*N*-((4-(4-Methylpiperazin-1-yl)-6,7-diphenylbicyclo[2.2.2]octan-2-yl)amino)-1-pyrrolidinopropan-1-one (15a). *Method B:* To an ice-cooled solution of 3-chloropropionyl chloride (0.995 g, 7.84 mmol) and triethylamine (0.793 g, 7.84 mmol) in dry CH_2Cl_2 (10 mL), pyrrolidine (0.372 g, 5.23 mmol) dissolved in dry CH_2Cl_2 (2 mL) was added under stirring. After 30 min the ice-bath was removed and the reaction batch was stirred overnight at ambient temperature in an atmosphere of Ar. Subsequently the reaction batch was shaken with 1 N aq NaOH and the aqueous phase was exhaustively extracted with CH_2Cl_2. The organic phase was washed with water until the aqueous phase reacted neutral, dried over sodium sulfate, filtered and the solvent was evaporated in vacuo yielding the 1-(3-chloropropionyl)pyrrolidine (0.296 g, 35%). To an iced solution of bicyclo-octanamine **3a** (0.687 g, 1.83 mmol) in EtOH (5 mL) and a catalytic amount of KI a solution of 1-(3-chloropropionyl)pyrrolidine (0.296 g, 1.83 mmol) in EtOH (5 mL) was added dropwise. The mixture was refluxed for 48 h at 110 °C. Subsequently the residue was acidified with 2 N aq HCl and extracted with diethylether. The aqueous phase was alkalified with 2 N aq NaOH and exhaustively extracted with diethylether. The organic layer was dried over anhydrous sodium sulfate, filtered and finally the solvent was removed in vacuo giving a residue, which was purified for analytical purposes by CC over aluminum oxide eluting with ethyl acetate/cyclohexane (4/1,v/v) giving compound **15a** (0.140 g, 0.280 mmol, 16%). IR = 3056, 3024, 2929, 2871, 2793, 1638, 1601, 1496, 1448, 1165, 1031, 1012, 746, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 231 (3.621), 268 (3.061); ^{1}H NMR ($CDCl_3$) δ = 1.46 (br d, J = 13.2 Hz, 1H, 3-H), 1.67–1.76 (m, 1H, 2′-H), 1.77–1.83 (m, 3H, 2′-H, 3″-H), 1.83–1.90 (m, 2H, 4″-H), 1.90–2.07 (m, 4H, 3-H, 5-H, 8-H), 2.17–2.27 (m, 2H, 1′-H, 8-H), 2.31 (s, 3H, NCH_3), 2.44 (d, J = 2.6 Hz, 1H, 1-H), 2.45–2.59 (m, 4H, $N(CH_2)_2$), 2.67 (dt, J = 11.1, 7.1 Hz, 1H, 1′-H), 2.70–2.85 (m, 4H, $N(CH_2)_2$), 2.97 (t, J = 9.3 Hz, 1H, 6-H), 3.06–3.19 (m, 4H, 2-H, 5″-H, 7-H), 3.36 (t, J = 6.8 Hz, 2H, 2″-H), 7.05-7.42 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ = 24.26 (C-3″), 25.91 (C-4″), 30.99 (C-8), 31.61 (C-5), 34.45 (C-2′), 34.79 (C-7), 36.71 (C-3), 41.28 (C-6), 41.45 (C-1), 42.65 (C-1′), 45.28 (C-2″), 45.42 ($N(CH_2)_2$), 45.86 (NCH_3), 46.32 (C-5″), 55.79 ($N(CH_2)_2$), 56.65 (C-4), 58.82 (C-2), 124.97, 126.06, 127.01, 127.39, 127.72, 128.34 (aromatic C), 144.05, 145.08 (aromatic C_q), 170.29 (CO); HRMS (EI+) calcd for $C_{32}H_{44}N_4O$: 500.3515; found: 500.3542.

5.2.1.9. (2*SR*,6*RS*,7*RS*)-(±)-4-(4-Methylpiperazin-1-yl)-6,7-diphenyl-N-(3-pyrrolidinopropyl)bicyclo[2.2.2]octan-2-amine (16a). The alkanone **15a** (0.094 g, 0.189 mmol) was suspended in dry diethylether (15 mL), cooled with an ice-bath and $LiAlH_4$ (0.029 g, 0.764 mmol) was added in portions. After 1 h the ice-bath was removed and the reaction batch was refluxed at 55 °C for 20 h. Subsequently the chemical reaction was quenched cautiously with ice-water. Then 2 N aq NaOH was added and the reaction mixture was exhaustively extracted with ether. The combined organic layers were washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo giving compound **16a** (0.062 g, 0.127 mmol, 67%). IR = 3057, 3024, 2928, 2872, 2792, 1600, 1496, 1453, 1164, 1144, 1031, 1012, 746, 698; UV (CH_2Cl_2, ($\log\varepsilon$)): 231 (3.689); ^{1}H NMR ($CDCl_3$) δ = 1.00–1.11 (m, 1H, 2′-H), 1.22-1.34 (m, 1H, 2′-H), 1.45 (br d, J = 13.2 Hz, 1H, 3-H), 1.71–1.77 (m, 4H, 3″-H, 4″-H), 1.91-1.98 (m, 1H, 5-H), 2.00–2.12 (m, 6H, 1′-H, 3-H, 3′-H, 5-H, 8-H), 2.13-2.20 (m, 1H, 8-H), 2.31 (s, 3H, NCH_3), 2.27–2.40 (m, 5H, 2″-H, 3′-H, 5″-H), 2.44 (br s, 1H, 1-H), 2.46–2.57 (m, 4H, $N(CH_2)_2$), 2.71–2.86 (m, 4H, $N(CH_2)_2$), 2.96 (t, J = 9.1 Hz, 1H, 6-H), 3.09–3.17 (m, 2H, 2-H, 7-H), 7.06–7.42 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ = 23.28 (C-3″, C-4″), 29.14 (C-2′), 31.23 (C-8), 31.37 (C-5), 34.69 (C-7), 36.95 (C-3), 41.47 (C-1), 41.59 (C-6), 45.43 ($N(CH_2)_2$), 45.74 (C-3′), 45.89 (NCH_3), 54.03 (C-2″, C-5″), 54.39 (C-1′), 55.81 ($N(CH_2)_2$), 56.66 (C-4), 59.19 (C-2), 125.44, 126.11, 126.85, 127.39, 128.00, 128.39 (aromatic C), 144.09, 144.76 (aromatic C_q); HRMS (EI+) calcd for $C_{32}H_{46}N_4$: 486.3723; found: 486.3728.

5.2.2. General procedure for the synthesis of (7*RS*,8*RS*)-(±)-3-amino-1-(5-(4-methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo-[3.2.2]nonan-2-yl)propan-1-ones (19a–21a)

To an ice-cooled solution of bicyclo-nonane **17a** and triethylamine in dry CH_2Cl_2 the 3-chloropropionyl chloride was added under stirring. After 30 min the ice-bath was removed and the reaction batch was stirred overnight at ambient temperature in an atmosphere of Ar. Subsequently the reaction batch was shaken with 1 N aq NaOH and the aqueous phase was exhaustively extracted with CH_2Cl_2. The organic phase was washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo yielding the 3-chloropropionyl derivative **18a**. Compound **18a** and a catalytic amount of KI were dissolved in an excess of secondary amine. The mixture was stirred for 72 h at ambient temperature in an atmosphere of Ar. Subsequently benzene was added and the reaction batch was evaporated. The residue was dissolved

in CH_2Cl_2, washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and finally the solvent was removed in vacuo giving **19a–21a**.

5.2.2.1. (7*RS*,8*RS*)-(±)-3-Diethylamino-1-(5-(4-methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-2-yl)propan-1-one (19a). Bicyclo-nonane **17a** (0.289 g, 0.769 mmol) and triethylamine (0.117 g, 1.15 mmol) in dry CH_2Cl_2 (10 mL) gave with chloropropionyl chloride (0.147 g, 1.15 mmol) compound **18a** (0.278 g, 78%). **18a** (0.278 g, 0.60 mmol), diethylamine (0.5 mL) and a catalytic amount of KI yielded after 72 h **19a** (0.220 g, 0.438 mmol, 73%). (***E***)-**19a**: ^{1}H NMR ($CDCl_3$): δ = 0.80 (t, *J* = 7.1 Hz, 6H, 2″-H), 1.69 (ddd, *J* = 15.4, 10.9, 4.8 Hz, 1H, 2′-H), 1.83–1.90 (m, 1H, 6-H), 1.91–2.16 (m, 5H, 2′-H, 4-H, 9-H), 2.20–2.28 (m, 2H, 3′-H, 6-H), 2.22 (q, *J* = 7.1 Hz, 4H, 1″-H), 2.27 (s, 3H, NCH_3), 2.32-2.58 (m, 5H, 3′-H, $N(CH_2)_2$), 2.58–2.72 (m, 4H, $N(CH_2)_2$), 3.20–3.36 (m, 2H, 3-H, 7-H), 3.45 (td, *J* = 10.1, 2.8 Hz, 1H, 8-H), 3.98 (d, *J* = 2.8 Hz, 1H, 1-H), 4.42–4.50 (m, 1H, 3-H), 7.13–7.37 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 11.45 (C-2″), 30.56 (C-4), 31.21 (C-2′), 33.52 (C-9), 37.18 (C-6), 40.74 (C-3), 40.94 (C-8), 45.02 ($N(CH_2)_2$), 45.81 (NCH_3), 46.66 (C-7), 46.74 (C-1″), 48.34 (C-3′), 55.68 ($N(CH_2)_2$), 57.33 (C-5), 61.27 (C-1), 126.72, 127.02, 127.05, 127.66, 128.86, 129.12 (aromatic C), 142.13, 144.19 (aromatic C_q), 171.41 (CO); (***Z***)-**19a**: ^{1}H NMR ($CDCl_3$): δ = 1.04 (t, *J* = 7.1 Hz, 6H, 2″-H), 1.80 (td, *J* = 13.6, 4.2 Hz, 1H, 4-H), 1.90–2.01 (m, 2H, 4-H, 6-H), 2.30 (s, 3H, NCH_3), 2.24–2.31 (m, 2H, 6-H, 9-H), 2.32–2.60 (m, 7H, 2′-H, 9-H, $N(CH_2)_2$), 2.57 (q, *J* = 7.1 Hz, 4H, 1″-H), 2.58–2.72 (m, 4H, $N(CH_2)_2$), 2.74–2.90 (m, 2H, 3′-H), 3.12 (td, *J* = 13.8, 3.1 Hz, 1H, 3-H), 3.16–3.23 (m, 1H, 7-H), 3.24–3.34 (m, 1H, 8-H), 3.77–3.87 (m, 1H, 3-H), 5.10 (d, *J* = 2.8 Hz, 1H, 1-H), 7.15–7.37 (m, 8H, aromatic H), 7.55 (d, *J* = 7.7 Hz, 2H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 11.56 (C-2″), 31.63 (C-4), 32.04 (C-2′), 35.13 (C-8), 35.22 (C-6), 36.21 (C-9), 42.65 (C-3), 44.88 ($N(CH_2)_2$), 45.15 (C-7), 45.84 (NCH_3), 46.83 (C-1″), 48.87 (C-3′), 55.39 (C-1), 55.75 ($N(CH_2)_2$), 57.81 (C-5), 126.20, 126.45, 126.47, 127.64, 128.39, 128.47 (aromatic C), 143.04, 143.48 (aromatic C_q), 170.60 (CO). HRMS (EI+) calcd for $C_{32}H_{46}N_4O$: 502.3672; found: 502.3714.

5.2.2.2. (7*RS*,8*RS*)-(±)-1-(5-(4-Methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-2-yl)-3-pyrrolidinopropan-1-one (20a). Bicyclo-nonane **17a** (0.221 g, 0.589 mmol) and triethylamine (0.089 g, 0.885 mmol) in dry CH_2Cl_2 (10 mL) gave with chloropropionyl chloride (0.112 g, 0.885 mmol) compound **18a** (0.216 g, 78%). **18a** (0.216 g, 0.460 mmol), pyrrolidine (0.5 mL) and a catalytic amount of KI yielded after 72 h **20a** (0.210 g, 0.419 mmol, 91%). (***E***)-**20a**: ^{1}H NMR ($CDCl_3$): δ = 1.58–1.66 (m, 4H, 3″-H, 4″-H), 1.72–1.80 (m, 1H, 2′-H), 1.87–1.92 (m, 1H, 6-H), 1.93–2.01 (m, 1H, 4-H), 2.11–2.17 (m, 4H, 2′-H, 4-H, 9-H), 2.17–2.29 (m, 6H, 2″-H, 3′-H, 5″-H, 6-H), 2.28 (s, 3H, NCH_3), 2.43–2.58 (m, 5H, 3′-H, $N(CH_2)_2$), 2.60–2.78 (m, 4H, $N(CH_2)_2$), 3.21–3.35 (m, 2H, 3-H, 7-H), 3.44 (td, *J* = 9.8, 2.7 Hz, 1H, 8-H), 4.00 (d, *J* = 2.7 Hz, 1H, 1-H), 4.44–4.51 (m, 1H, 3-H), 7.13–7.38 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 23.24 (C-3″, C-4″), 30.54 (C-4), 31.55 (C-2′), 33.42 (C-9), 37.17 (C-6), 40.75 (C-3), 40.90 (C-8), 45.02 ($N(CH_2)_2$), 45.81 (NCH_3), 46.60 (C-7), 51.72 (C-3′), 53.83 (C-2″, C-5″), 55.68 ($N(CH_2)_2$), 57.32 (C-5), 61.30 (C-1), 126.75, 127.00, 127.07, 127.65, 128.89, 129.14 (aromatic C), 142.12, 144.17 (aromatic C_q), 171.04 (CO); (***Z***)-**20a**: ^{1}H NMR ($CDCl_3$): δ = 1.77–1.86 (m, 5H, 3″-H, 4-H, 4″-H), 1.97–2.04 (m, 2H, 4-H, 6-H), 2.10–2.16 (m, 1H, 9-H), 2.30 (s, 3H, NCH_3), 2.31–2.41 (m, 2H, 6-H, 9-H), 2.43–2.60 (m, 11H, 2′-H, 2″-H, 3′-H, 5″-H, $N(CH_2)_2$), 2.60–2.78 (m, 4H, $N(CH_2)_2$), 2.80–2.87 (m, 1H, 3′-H), 3.13 (td, *J* = 13.6, 3.1 Hz, 1H, 3-H), 3.22 (t, *J* = 9.6 Hz, 1H, 7-H), 3.29–3.35 (m, 1H, 8-H), 3.77–3.85 (m, 1H, 3-H), 5.10 (d, *J* = 3.1 Hz, 1H, 1-H), 7.13–7.38 (m, 8H, aromatic H), 7.55 (d, *J* = 7.9 Hz, 2H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 23.43 (C-3″, C-4″), 32.86 (C-4), 33.73 (C-2′), 35.15 (C-6), 35.20 (C-8), 36.15 (C-9), 42.63 (C-3), 44.88 ($N(CH_2)_2$), 45.18 (C-7), 45.84 (NCH_3), 51.72 (C-3′), 54.07 (C-2″, C-5″), 55.47 (C-1), 55.74 ($N(CH_2)_2$), 57.81 (C-5), 126.21, 126.48, 127.65, 128.41, 128.48 (aromatic C), 143.00, 143.45 (aromatic C_q), 170.13 (CO). HRMS (EI+) calcd for $C_{32}H_{44}N_4O$: 500.3515; found: 500.3546.

5.2.2.3. (7*RS*,8*RS*)-(±)-1-(5-(4-Methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonan-2-yl)-3-piperidinopropan-1-one (21a). Bicyclo-nonane **17a** (0.240 g, 0.641 mmol) and triethylamine (0.097 g, 0.961 mmol) in dry CH_2Cl_2 (10 mL) gave with chloropropionyl chloride (0.122 g, 0.961 mmol) compound **18a** (0.234 g, 78%). **18a** (0.234 g, 0.50 mmol), piperidine (0.5 mL) and a catalytic amount of KI yielded after 72 h **21a** (0.234 g, 0.454 mmol, 91%). (***E***)-**21a**: ^{1}H NMR ($CDCl_3$): δ = 1.27–1.36 (m, 2H, 4″-H), 1.38–1.46 (m, 4H, 3″-H, 5″-H), 1.71–1.80 (m, 1H, 2′-H), 1.82–1.91 (m, 1H, 6-H), 1.92–1.98 (m, 2H, 2′-H, 4-H), 1.98–2.18 (m, 8H, 2″-H, 3′-H, 4-H, 6″-H, 9-H), 2.22–2.28 (m, 1H, 6-H), 2.27 (s, 3H, NCH_3), 2.40–2.54 (m, 5H, 3′-H, $N(CH_2)_2$), 2.64–2.78 (m, 4H, $N(CH_2)_2$), 3.23–3.37 (m, 2H, 3-H, 7-H), 3.44 (td, *J* = 10.0, 2.5 Hz, 1H, 8-H), 4.00 (d, *J* = 2.5 Hz, 1H, 1-H), 4.43–4.50 (m, 1H, 3-H), 7.13–7.36 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 24.04 (C-4″), 25.59 (C-3″, C-5″), 30.56 (C-4), 30.80 (C-2′), 33.52 (C-9), 37.21 (C-6), 40.75 (C-3), 40.89 (C-8), 44.99 ($N(CH_2)_2$), 45.78 (NCH_3), 46.66 (C-7), 54.09 (C-2″, C-6″), 54.43 (C-3′), 55.65 ($N(CH_2)_2$), 57.33 (C-5), 61.16 (C-1), 126.77, 127.00, 127.07, 127.65, 128.88, 129.12 (aromatic C), 142.11, 144.12 (aromatic C_q), 171.26 (CO); (***Z***)-**21a**: ^{1}H NMR ($CDCl_3$): δ = 1.40–1.48 (m, 2H, 4″-H), 1.57–1.62 (m, 4H, 3″-H, 5″-H), 1.71–1.82 (m, 1H, 4-H), 1.92–2.02 (m, 2H, 4-H, 6-H), 2.05–2.12 (m, 1H, 9-H), 2.30 (s, 3H, NCH_3), 2.30–2.40 (m, 2H, 6-H, 9-H), 2.40–2.54 (m, 9H, 2″-H, 3′-H, 6″-H, $N(CH_2)_2$), 2.57–2.78 (m, 7H, 2′-H, 3′-H, $N(CH_2)_2$), 3.12 (td, *J* = 13.5, 3.0 Hz, 1H, 3-H), 3.21 (t, *J* = 9.8 Hz, 1H, 7-H), 3.28–3.37 (m, 1H, 8-H), 3.77–3.86 (m, 1H, 3-H), 5.09 (d, *J* = 2.9 Hz, 1H, 1-H), 7.13–7.38 (m, 8H, aromatic H), 7.54 (d, *J* = 8.0 Hz, 2H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 24.04 (C-4″), 25.67 (C-3″, C-5″), 31.56 (C-4), 31.84 (C-2′), 35.12 (C-8), 35.21 (C-6), 36.17 (C-9), 42.58 (C-3), 44.85 ($N(CH_2)_2$), 45.17 (C-7), 45.81 (NCH_3), 54.43 (C-2″, C-6″), 54.66 (C-3′), 55.42 (C-1), 55.71 ($N(CH_2)_2$), 57.81 (C-5), 126.21, 126.45, 127.63, 128.41, 128.48 (aromatic C), 143.00, 143.45 (aromatic C_q), 170.35 (CO). HRMS (EI+) calcd for $C_{33}H_{46}N_4O$: 514.3672; found: 514.3688.

5.2.3. General procedure for the synthesis of (7*RS*,8*RS*)-(±)-2-(3-aminopropyl)-5-(4-methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonanes (22a–24a)

The alkanones **19a–21a** were suspended in dry diethylether, cooled with an ice-bath and $LiAlH_4$ was added in portions. After 1 h the ice-bath was removed and the reaction batch was refluxed at 55 °C for 20 h. Subsequently the chemical reaction was quenched cautiously with ice-water. Then 2 N aq NaOH was added and the reaction mixture was exhaustively extracted with diethylether. The combined organic layers were washed with water until the aqueous phase reacted neutral, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in vacuo giving compounds **22a–24a**.

5.2.3.1. (7*RS*,8*RS*)-(±)-2-(3-Diethylaminopropyl)-5-(4-methylpiperazin-1-yl)-7,8-diphenyl-2-azabicyclo[3.2.2]nonane (22a). The reaction of compound **19a** (0.155 g, 0.310 mmol) and $LiAlH_4$ (0.050 g, 1.24 mmol) in 20 mL dry diethylether gave after work-up **22a** (0.130 g, 0.266 mmol, 86%). IR = 3059, 3024, 2929, 2793, 1601, 1494, 1453, 1159, 1105, 1031, 1010, 757, 699; UV (CH_2Cl_2, ($\log\varepsilon$)): 233 (3.910); ^{1}H NMR ($CDCl_3$): δ = 0.92 (t, *J* = 7.1 Hz, 6H, 2″-H), 1.20–1.38 (m, 2H, 2′-H), 1.84-1.96 (m, 3H, 4-H, 6-H), 2.04–2.15 (m, 2H, 3′-H, 9-H), 2.20–2.26 (m, 3H, 3′-H, 6-H, 9-H), 2.27 (s, 3H, NCH_3), 2.29–2.41 (m, 6H, 1′-H, 1″-H), 2.41-2.52 (m, 4H, $N(CH_2)_2$), 2.64–2.74 (m, 4H, $N(CH_2)_2$), 2.77–2.84 (m,

1H, 3-H), 2.88 (d, J = 2.7 Hz, 1H, 1-H), 2.96 (dt, J = 12.5, 6.2 Hz, 1H, 3-H), 3.16 (td, J = 9.6, 2.7 Hz, 1H, 8-H), 3.44 (t, J = 9.2 Hz, 1H, 7-H), 7.12 (t, J = 7.3 Hz, 1H, aromatic H), 7.19–7.37 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 11.48 (C-2″), 25.52 (C-2′), 32.20 (C-4), 34.94 (C-6), 37.56 (C-9), 38.32 (C-8), 40.36 (C-7), 44.79 ($N(CH_2)_2$), 45.83 (NCH_3), 46.67 (C-1″), 47.60 (C-3), 50.63 (C-3′), 55.76 (C-1′), 55.86 ($N(CH_2)_2$), 57.58 (C-5), 68.07 (C-1), 125.73, 125.92, 127.41, 127.72, 128.47, 128.56 (aromatic C), 144.77, 145.95 (aromatic C_q); HRMS (EI+) calcd for $C_{32}H_{48}N_4$: 488.3879; found: 488.3881.

5.2.3.2. (7*RS*,8*RS*)-(±)-5-(4-Methylpiperazin-1-yl)-7,8-diphenyl-2-(3-pyrrolidinopropyl)-2-azabicyclo[3.2.2]nonane (23a). The reaction of compound **20a** (0.177 g, 0.350 mmol) and $LiAlH_4$ (0.050 g, 1.32 mmol) in 20 mL dry diethylether gave after work-up **23a** (0.163 g, 0.335 mmol, 96%). IR = 3024, 2928, 2792, 1600, 1453, 1158, 1106, 1031, 1009, 757, 699; UV (CH_2Cl_2, ($\log\varepsilon$)): 233 (3.896); ^{1}H NMR ($CDCl_3$): δ = 1.26–1.42 (m, 2H, 2′-H), 1.67–1.73 (m, 4H, 3″-H, 4″-H), 1.85–1.95 (m, 3H, 4-H, 6-H), 2.04–2.12 (m, 2H, 3′-H, 9-H), 2.20–2.36 (m, 3H, 3′-H, 6-H, 9-H), 2.27 (s, 3H, NCH_3), 2.30–2.36 (m, 4H, 2″-H, 5″-H), 2.37–2.52 (m, 6H, 1′-H, $N(CH_2)_2$), 2.64–2.74 (m, 4H, $N(CH_2)_2$), 2.75–2.82 (m, 1H, 3-H), 2.88 (d, J = 2.7 Hz, 1H, 1-H), 2.96 (dt, J = 12.8, 6.2 Hz, 1H, 3-H), 3.15 (td, J = 9.4, 2.7 Hz, 1H, 8-H), 3.44 (t, J = 9.2 Hz, 1H, 7-H), 7.13 (t, J = 7.1 Hz, 1H, aromatic H), 7.19–7.37 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 23.27 (C-3″, C-4″), 27.59 (C-2′), 32.29 (C-4), 34.82 (C-6), 37.73 (C-9), 38.21 (C-8), 40.20 (C-7), 44.79 ($N(CH_2)_2$), 45.84 (NCH_3), 47.56 (C-3), 54.01 (C-2″, C-5″), 54.25 (C-3′), 55.84 (C-1′), 55.86 ($N(CH_2)_2$), 57.56 (C-5), 68.07 (C-1), 125.70, 125.90, 127.43, 127.70, 128.45, 128.62 (aromatic C), 144.80, 145.94 (aromatic C_q); HRMS (EI+) calcd for $C_{32}H_{46}N_4$: 486.3723; found: 486.3737.

5.2.3.3. (7*RS*,8*RS*)-(±)-5-(4-Methylpiperazin-1-yl)-7,8-diphenyl-2-(3-piperidinopropyl)- 2-azabicyclo[3.2.2]nonane (24a). The reaction of compound **21a** (0.169 g, 0.330 mmol) and $LiAlH_4$ (0.050 g, 1.32 mmol) in 20 mL dry diethylether gave after work-up **24a** (0.138 g, 0.276 mmol, 83%). IR = 3058, 3024, 2931, 2851, 2793, 1635, 1601, 1494, 1452, 1377, 1295, 1159, 1114, 1032, 1009, 757, 744, 699; UV (CH_2Cl_2, ($\log\varepsilon$)): 233 (3.927); ^{1}H NMR ($CDCl_3$): δ = 1.25–1.41 (m, 4H, 2′-H, 4″-H), 1.48–1.54 (m, 4H, 3″-H, 5″-H), 1.85–1.92 (m, 3H, 4-H, 6-H), 1.93–1.99 (m, 1H, 3′-H), 2.04–2.14 (m, 2H, 3′-H, 9-H), 2.19–2.26 (m, 6H, 2″-H, 6-H, 6″-H, 9-H), 2.27 (s, 3H, NCH_3), 2.31–2.41 (m, 2H, 1′-H), 2.42–2.50 (m, 4H, $N(CH_2)_2$), 2.65–2.73 (m, 4H, $N(CH_2)_2$), 2.75–2.82 (m, 1H, 3-H), 2.87 (d, J = 2.6 Hz, 1H, 1-H), 2.95 (dt, J = 12.5, 6.2 Hz, 1H, 3-H), 3.15 (td, J = 9.7, 2.6 Hz, 1H, 8-H), 3.44 (t, J = 9.2 Hz, 1H, 7-H), 7.12 (t, J = 7.2 Hz, 1H, aromatic H), 7.19–7.36 (m, 9H, aromatic H); ^{13}C NMR ($CDCl_3$): δ = 24.37 (C-4″), 25.53 (C-2′), 25.83 (C-3″, C-5″), 32.27 (C-4), 34.88 (C-6), 37.66 (C-9), 38.26 (C-8), 40.27 (C-7), 44.79 ($N(CH_2)_2$), 45.84 (NCH_3), 47.54 (C-3), 54.42 (C-2″, C-6″), 55.87 (C-1′, $N(CH_2)_2$), 57.12 (C-3′), 57.58 (C-5), 68.12 (C-1), 125.71, 125.92, 127.44, 127.72, 128.47, 128.62 (aromatic C), 144.80, 145.94 (aromatic C_q); HRMS (EI+) calcd for $C_{33}H_{48}N_4$: 500.3879; found: 500.3885.

5.3. Biological tests

5.3.1. In vitro microplate assay against *P. falciparum* K_1

Antiplasmodial activity was tested using the chloroquine- and pyrimethamine-resistant K_1 strain *of P. falciparum*. Viability was determined by the incorporation of [^{3}H]-hypoxanthine into living protozoal cells by a modification of a reported assay.[14] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions ranging from 5 to 0.078 μg/mL in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi ^{3}H-hypoxanthine were added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. IC_{50} values were calculated from the sigmoidal inhibition curves. Assays were run in duplicate and repeated once. Standards were artemisinin and chloroquine.

5.3.2. In vitro microplate assay against *T. brucei rhodesiense*, cytotoxicity

Minimum essential medium (50 μL) supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate according to a known procedure.[15] Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then 10^4 bloodstream forms of *T. brucei rhodesiense* STIB 900 in 50 μl were added to each well and the plate was incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. 10 μL of Alamar Blue were then added to each well and incubation was continued for a further 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm.[16] Fluorescence development was expressed as percentage of the control. Melarsoprol served as standard.

Cytotoxicity was assessed using the same assay and rat skeletal myoblasts (L-6 cells) with mefloquine as standard.

5.3.3. In vivo assay against *P. berghei*

In vivo efficacy studies in mice were conducted at the Swiss Tropical and Public Health Institute (Basel) according to the rules and regulations for the protection of animal rights ('Tierschutzverordnung') of the Swiss 'Bundesamt für Veterinärwesen'. They were approved by the veterinary office of Canton Basel-Stadt, Switzerland. The animal experiment approval number for in vivo testing against *P. berghei* is 1731.

In vivo antimalarial activity was assessed basically as previously described.[17] Groups of three female NMRI mice (20–22 g) are intravenously infected with 2×10^7 with GFP-transfected *P. berghei* strain ANKA[18] parasitized erythrocytes on day 0. Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water and administered intraperitoneally in a volume of 10 mL/kg on four consecutive days (4, 24, 48 and 72 h post infection). Parasitemia was determined on day 4 post infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean per cent parasitemia for the control (n = 5 mice) and treated groups expressed as a per cent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites. Mice were euthanized by carbon dioxide inhalation.

5.3.4. In vivo assay against *T. brucei rhodesiense* STIB900

In vivo efficacy studies in mice were conducted at the Swiss Tropical and Public Health Institute (Basel) according to the rules and regulations for the protection of animal rights ('Tierschutzverordnung') of the Swiss 'Bundesamt für Veterinärwesen'. They were approved by the veterinary office of Canton Basel-Stadt, Switzerland. The animal experiment approval number for in vivo testing against *T. brucei rhodesiense* is 739.

The *T. brucei rhodesiense* STIB900 acute mouse model mimics the first stage of the disease and was assessed as previously described.[19] Four female NMRI mice were used per experimental group. Each mouse was inoculated i.p. with 2×10^4 bloodstream forms of STIB900, respectively. Heparinized blood from a donor mouse with approximately 5×10^6 /mL parasitemia was suspended in PSG to

obtain a trypanosome suspension of 1×10^5/mL. Each mouse was injected with 0.25 mL. Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water or as specified by the supplier. Compound treatment was initiated 3 days post-infection on four consecutive days for all administration routes (i.p., p.o.) in a volume of 0.1 mL/10 g. Three mice served as infected-untreated controls. Parasitemia was monitored using smears of tail-snip blood twice a week after treatment for two weeks followed by once a week until 60 days post-infection. Mice were considered cured when there was no parasitemia relapse detected in the tail blood over the 60-day observation period. Mean relapse days were determined as day of relapse post-infection of mice. When mice relapsed they were promptly euthanized by carbon dioxide inhalation.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.06.059.

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