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# Synthesis of 2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine – bridged nitric oxide synthase inhibitor with enhanced lipophilicity

**Olga N. Zefirova, Evgeniya D. Plotnikova, Evgeniya V. Nurieva, Danil I. Peregud, Mikhail V. Onufriev and Natalia V. Gulyaeva**

*General information*

All reaction temperatures correspond to internal temperatures unless otherwise noted. Solvents for extraction and chromatography were technical grade and distilled from indicated drying agents: petroleum ether 40–60 °C ($P_2O_5$); ethyl acetate ($K_2CO_3$); dichloromethane and chloroform ($P_2O_5$); toluene and benzene (sodium); tetrahydrofuran and diethyl ether (sodium, benzophenone). Flash and column chromatography were performed on silica gel Acros (40–60 μm). Reaction control was carried out by thin-layer chromatography on "Silufol-UV254" plates. $^1$H NMR and $^{13}$C NMR spectra were recorded at room temperature on spectrometer Avance Bruker Avance 400 (Varian) in $CDCl_3$, $D_2O$ or $CD_3OD$ at 400 and 100 MHz, respectively. Spectra are referenced to the residual solvent signals. Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were obtained on quadrupole mass spectrometer Finnigan MAT Incos 50 with typical electron ionization voltage of 70 eV at 200 °C.

Elemental analysis of synthesized compounds was performed on CHN analyser "Vario Micro Cube". Infrared spectra (IR) were registered on Fourier Transform IR spectrometer IR-200 (Thermo Nicolet) in KBr and reported in $cm^{-1}$. Melting points were measured in block with sealed capillaries and are uncorrected.

***tert*-Butyl (+/-)-3-hydroxycyclohexylcarbamate (5).** *Method 1*. The stirred solution of azide **4** (8.96 g, 64.4 mmol) in methanol (100 ml) was treated with $NaBH_4$ (2.88 g, 77.8 mmol) portionwise at 5–10°C. After 16 h at room temperature the mixture was diluted with ethyl acetate (200 ml) and washed with brine (2×50 ml). The organic layer was dried with anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was redissolved in ethyl acetate (50 ml) and $Boc_2O$ (16.04 g, 73.6 mmol) was added. The above mixture was hydrogenated over 5% Pd/C (1.21 g) at room temperature under 1 atm for 17 h. Filtration of the suspension, evaporation and column chromatography (ethyl acetate–light petroleum 1:3, then 1:2) afforded compound **5** (4.30

g, 31%) as white solid. Mp 121–123 °C. $R_f$ = 0.1 (ethyl acetate–light petroleum, 1:1). Product **5** was isolated as a mixture of *cis*- and *trans*-isomers in the ratio of ~ 4:1.

$^1$H NMR ($CDCl_3$, δ): 1.10 (m, 1H); 1.19–1.35 (m, 2H); 1.45 (s, 9H, $9H^{t\text{-}Bu}$); 1.56–1.66 (m, 2H); 1.78–1.88 (m, 3.2H); 2.19 (m, 0.8H); 3.54 (m, 0.8H, *cis*-$H^3$); 3.74 (m, 0.8H, *cis*-$H^1$); 3.89 (m, 0.2H, *trans*-$H^3$); 4.06 (m, 0.2H, *trans*-$H^1$); 4.65 (br s, 0.2H, *trans*-NH); 4.71 (br s, 0.8H, *cis*-NH).

$^{13}$C NMR ($CDCl_3$, δ) *cis*-isomer: 20.8 ($C^5$); 28.4 ($C(\underline{C}H_3)_3$); 32.0 ($C^6$); 32.2 ($C^4$); 41.7 ($C^2$); 47.8 ($C^3$); 69.0 ($C^1$); 79.2 ($\underline{C}(CH_3)_3$); 155.2 (C=O).

IR ($\nu_{max}$): 1020, 1050, 1085, 1180, 1250, 1313, 1370, 1375, 1467, 1545, 1680 (C=O), 2830-3020 (C-H), 3150-3500 (O-H), 3320 (N-H).

Anal. calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.10; H, 9.75; N, 6.42.

*Method 2.* 3-Aminocyclohexanol (0.9 g, 7.8 mmol, *cis : trans* = 7:3) in the mixture of $CH_2Cl_2$ (30 ml) and DMF (3 ml) was treated with $Boc_2O$ (2.8 g, 12.8 mmol). After 20 h of stirring at room temperature the solution was evaporated under reduced pressure at 60 °C and the residue was purified by column chromatography ($CH_2Cl_2$, then $CH_2Cl_2$–methanol, 10:1). Product **5** (0.68 g, 41%) was isolated as a mixture of *cis*- and *trans*-isomers in the ratio of ~ 4:1 and displayed the same physico-chemical characteristics as the sample described above.

**The attempt of OH-substitution in 5 with $CBr_4$.** To the solution of compound **5** (0.320 g, 1.49 mmol) in $CH_2Cl_2$, $PPh_3$ (0.770 g, 2.94 mmol) and $CBr_4$ (0.648 g, 1.95 mmol) were added at 0–5°C. After 15 h at this temperature the mixture was washed with saturated solution of $NaHCO_3$ (2×30 ml), then with brine (2×20 ml), dried with anhydrous $Na_2SO_4$ and evaporated. Column chromatography of the residue failed to isolate desired product in the individual form.

***(+/-)-cis*-3-[(*tert*-Butoxycarbonyl)amino]cyclohexyl methanesulfonate (6a).** Compound **5** (0.963 g, 4.5 mmol) in $CH_2Cl_2$ (30 ml) was mixed with methanesulfonyl chloride (0.62 g, 5.4 mmol) and $Et_3N$ (0.75 ml, 5.4 mmol) at 0–5 °C. After 24 h of stirring at room temperature the solution was washed with brine (2×50 ml). The organic layer was dried with anhydrous $Na_2SO_4$ and evaporated. The residue was subjected to column chromatography (ethyl acetate–light petroleum 1:3, then 1:1) which resulted the product **6a** (1.050 g, 80%) as white solid, mp 102–105 °C. $R_f$ = 0.60 (ethyl acetate–light petroleum 1:1).

$^1$H NMR ($CDCl_3$, δ): 1.11 (m, 1H); 1.36–1.54 (m, 3H, $H^{Cy}$); 1.46 (s, 9H, *t*-Bu); 1.89 (m, 2H); 2.14 (m, $J_{gem}$=10.5 Hz, 1H, $H^6$); 2.42 (m, $J_{gem}$=10.5 Hz, 1H, $H^6$); 3.03 (s, 3H, $CH_3$); 3.58 (m, 1H, $H^3$); 4.53 (br s, 1H, NH); 4.65 (m, 1H, $H^1$).

In $^1$H NMR spectrum of crude reaction mixture the protons of *trans*-isomer were observed as the following signals with the intensity of ~15% towards *cis*-isomer: 2.27 (m, 1H); 3.08 (s, 3H, $CH_3$); 3.82 (m, 1H, $H^3$); 4.42 (br s, 1H, NH); 5.08 (m, 1H, $H^1$).

$^{13}$C NMR ($CDCl_3$, δ): 20.9 ($C^5$); 28.4 ($C(\underline{C}H_3)_3$); 31.8 ($C^4$); 32.1 ($C^6$); 38.8 ($C^2$); 39.4 ($SO_2CH_3$); 47.6 ($C^3$); 79.3 ($\underline{C}(CH_3)_3$); 79.6 ($C^1$); 155.0 (C=O).

IR ($\nu_{max}$): 956, 1173, 1238, 1336, 1454, 1533, 1684 (C=O), 2852–3055 (C-H), 3348 (N-H).

Anal. calcd for $C_{12}H_{23}NO_5S$: C, 49.13; H, 7.90; N, 4.77; S, 10.93. Found: C, 49.05; H, 7.73; N, 4.79; S, 10.79.

***(+/-)-cis*-3-[(*tert*-Butoxycarbonyl)amino]cyclohexyl *p*-toluenesulfonate (6b).** Compound **5** (1.13 g, 5.26 mmol) in pyridine (3 ml) was mixed with tosyl chloride (1.12 g, 5.84 mmol). After 12 h of stirring at room temperature the solution was diluted with saturated solution of $NaHCO_3$ (20 ml) and extracted with ethyl acetate (3×30 ml). The combined organic layers were washed with brine (2x50 ml), dried with anhydrous $Na_2SO_4$ and evaporated. The residue was subjected to column chromatography (ethyl acetate–light petroleum 1:5) which resulted in the product **6b** (1.54 g, 79%) as white solid, mp 116–118 °C. $R_f$ = 0.6 (ethyl acetate–light petroleum 1:5).

$^1$H NMR ($CDCl_3$, δ): 1.05 (m, 1H); 1.24–1.46 (m, $J_{gem}$=13.9 Hz, 3H, $H^{Cy}$); 1.42 (s, 9H, *t*-Bu); 1.77 (m, $J_{gem}$=13.9 Hz, 1H); 1.86 (m, 1H); 1.93 (m, $J_{gem}$=12.1 Hz, 1H, $H^6$); 2.16 (m, $J_{gem}$=12.1 Hz, 1H, $H^6$); 2.46 (s, 3H, Ar-$CH_3$); 3.47 (m, 1H, $H^3$); 4.42 (m, 1H, $H^1$); 4.51 (br s, 1H, NH); 7.35 (d, *J*=8.2 Hz, 2H, *2*$H^{3,5Ar}$); 7.79 (d, *J*=8,2 Hz, 2H, *2*$H^{2,6Ar}$).

$^{13}$C NMR ($CDCl_3$, δ): 20.7 ($C^5$); 21.7 (Ar-$CH_3$); 28.4 ($C(\underline{C}H_3)_3$); 31.7 ($C^4$); 31.9 ($C^6$); 38.9 ($C^2$); 47.5 ($C^3$); 79.4 ($\underline{C}(CH_3)_3$); 79.5 ($C^1$); 127.7 ($C^{3,5Ar}$); 129.9 ($C^{2,6Ar}$); 134.2 ($C^{4Ar}$); 144.7 ($C^{1Ar}$); 154.9 (C=O).

IR ($\nu_{max}$): 935, 1047, 1099, 1179, 1349 (O-S), 1457, 1534, 1602 (Ar), 1683 (C=O), 2863-3088 (C-H), 3345 (N-H).

Anal. calcd for $C_{18}H_{27}NO_5S$: C, 58.51; H, 7.37; N, 3.79, S, 8.68. Found: C, 58.50; H, 7.20; N, 3.67; S, 8.59.

***tert*-Butyl *(+/-)*-(*trans*-3-bromocyclohexyl)carbamate (7).** To the solution of mesylate **6a** (3.79 g, 12.9 mmol) (or tosylate **6b** (4.76 g, 12.9 mmol) in THF (100 ml) the anhydrous LiBr (2.78 g, 31.9 mmol) was added under inert atmosphere and the mixture was refluxed within 20 h. Evaporation of the solvent and column chromatography (light petroleum, then ethyl acetate–light petroleum 1:7) gave product **7** (0.76 g, 21% from **6a**; 0.65 g, 18% from **6b**) as white solid $R_f$ = 0.25 (ethyl acetate–light petroleum 1:5).

$^1$H NMR ($CDCl_3$, δ): 1.28 (m, 2H, $H^{Cy}$); 1.45 (s, 9H, *t*-Bu); 1.62 (m, 1H, $H^{Cy}$); 1.73-1.97 (m, 4H, $H^{Cy}$); 2.27 (m, 1H, $J_{gem}$=13.5 Hz, $H^5$); 3.96 (m, 1H, $H^1$); 4.44 (br s, 1H, *NH*); 4.55 (m, 1H, $H^3$).

$^{13}$C NMR ($CDCl_3$, δ): 20.8 ($C^5$); 28.4 ($C(\underline{C}H_3)_3$); 32.1 ($C^4$); 34.8 ($C^6$); 41.4 ($C^2$); 46.1 ($C^1$); 51.0 ($C^3$); 79.4 ($\underline{C}(CH_3)_3$); 155.9 (C=O).

MS (m/z, (%)): 279 ($(M+H)^+$, 4); 198 ($(M-Br)^+$; 1); 173 ($(M-Boc)^+$; 61); 140 ($(M-Br-Bu^t)^+$; 6); 81 ($C_6H_9^+$;64); 71 (1); 60 (56); 41 ($C_3H_5^+$; 100).

Anal. calcd for $C_{11}H_{20}BrNO_2$: C, 47.49; H, 7.25; N, 5.04. Found C, 47.65; H, 7.10; N, 7.05.

Together with compound **7** the products of elimination were isolated as an oily mixture of isomeric *tert*-butyl cyclohexenylcarbamates (0.760 g, 30%). $^1$H NMR ($CDCl_3$, δ): 1.24–1.28 (m, 0.6H); 1.43 (s, 9H, *t*-Bu); 1.46–1.63 (m, 1.4H); 1.82–2.10 (m, 3.4H); 2.36 (m, 0.6 H); 3.79 (br s, 0.6H, C<u>H</u>-NH); 4.16 (br s, 0.4H, C<u>H</u>-NH); 4.57 (br s, 1H, NH); 5.62–5.82 (m, 2H, CH=CH).

**(+/-)-*trans*-(3-Bromocyclohexyl)amine hydrobromide (8).** Compound **7** (0.79 g, 2.84 mmol) was treated with saturated solution of anhydrous HBr in diethyl ether (10 ml). After 10 h of stirring at room temperature the resulting precipitate was filtered off, washed with diethyl ether and dried to give product **8** as white crystals (0.70 g, 95%), mp 195–197 °C.

$^1$H NMR ($CD_3OD$, δ): 1.50 (m, 1H); 1.80 (m, 1H); 1.87-2.06 (m, 4H); 2.10 (m, 1H, $J_{gem}$=13.9 Hz, $H^5$); 2.39 (m, 1H, $J_{gem}$=13.9 Hz, $H^5$); 3.62 (m, 1H, $H^1$); 4.82 (m, 1H, $H^3$).

$^{13}$C NMR ($CD_3OD$, δ): 19.3 ($C^5$); 29.7 ($C^4$); 32.9 ($C^6$); 38.1 ($C^2$); 46.6 ($C^1$); 50.2 ($C^3$).

IR ($\nu_{max}$): 665 (C-Br), 896, 1201, 1255, 1481, 1602, 1992, 2480–3210 (C-H, N-H).

Anal. calcd for. $C_6H_{13}Br_2N$: C, 27.83; H, 5.06; N, 5.41. Found: C, 27.85; H, 5.10; N, 5.39.

***rac*-2-Thia-4-azabicyclo[3.3.1]nonane-3-thione (9).** Bromide **8** (0.55 g, 2.12 mmol) in DMF (5 ml) was mixed with $CS_2$ (1.11 ml, 18.40 mmol) and $K_2CO_3$ (0.61 g, 4.42 mmol). After 30 h of stirring at room temperature the suspension was diluted with water (30 ml) and extracted with ethyl acetate (3×25 ml). The combined organic layers were washed with brine (2×10 ml), dried with anhydrous $Na_2SO_4$ and evaporated. Column chromatography (ethyl acetate–light petroleum 1:2, then $CH_2Cl_2$) of the residue resulted in the product **9** (0.25 g, 69%) as white solid, mp 238–240 °C. $R_f$ = 0.3 (ethyl acetate–light petroleum, 1:2).

$^1$H NMR ($CDCl_3$, δ): 1.66 (m, $J_{gem}$=9,7 Hz, $J_{gem}$=13,8 Hz, 2H, $H^7$+$H^9$); 1.84–2.04 (m, $J_{gem}$=9,7 Hz, $J_{gem}$=13,8 Hz, 4H, $H^7$+$H^9$+$H^{Cy}$); 2.19 (m, 2H); 3.36 (m, 1H, $H^1$); 3.86 (m, 1H, $H^5$); 8.36 (br s, 1H, NH).

$^1$H NMR ($CDCl_3$+ $CD_3OD$, δ): 1.37 (m, 2H); 1.55-1.74 (m, 4H); 1.90 (m, 2H); 3.10 (m, 1H, $H^1$); 3.58 (m, 1H, $H^5$).

$^{13}$C NMR ($CD_3OD$+$CDCl_3$, δ): 16.1 ($C^7$); 28.0; 31.6; 32.5; 40.1 ($C^1$); 48.7 ($C^5$); 198.4 ($C^3$).

MS (m/z, (%)): 173 ($M^+$, 86); 140 ($(M-S)^+$, 10); 82 ($C_6H_{10}^+$, 16); 81 ($C_6H_9^+$, 100); 80 ($C_6H_8^+$, 61); 60 (40); 58 ($CNS^+$, 15); 41 (51).

Anal. calcd for $C_7H_{11}NS_2$: C, 48.51; H, 6.40; N, 8.08, S, 37.01. Found: C, 48.75; H, 6.57; N, 7.78, S, 36.90.

Zefirov−Palyulin (ZP) and Cremer−Pople (CP) puckering parameters for 2-thia-4-azabicyclo[3.3.1]nonane-3-thione **9** were calculated by RICON program [A. Yu. Zotov, V. A. Palyulin and N. S. Zefirov, *J. Chem. Inf. Comput. Sci*., 1997, **37**, 766].

Cycle C1-C9-C5-C6-C7-C8 (*chair*): **ZP** S=1.144, θ=2.3, $\psi_2$=1.7, σ=0.21; **CP** Q=0.573, θ=4.4, $\varphi_2$=2.3; cycle C1-S2-C3-N4-C5-C9 (*distorted envelope*): **ZP** S=0.859, θ=36.2, $\psi_2$=1.7, σ=0.08; **CP** Q=0.557, θ=50.7, $\varphi_2$=6.8.

***rac*-N-(*tert*-Butyl)-2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine hydrochloride (10).** Thione **9** (0.20 g, 1.15 mmol) was dissolved in DMF (5 ml) on heating, then cooled to room temperature and mixed with $Et_3N$ (0.55 ml, 4.0 mmol), *tert*-butylamine (0.27 ml, 2.55 mmol) and $HgCl_2$ (0.35 g, 1.29 mmol). During heating at 80 °C for 20 h the formation of bright orange precipitate was observed. The reaction mass was diluted with water (20 ml) and extracted with $CH_2Cl_2$ (3×20 ml). The combined organic layers were dried with anhydrous $Na_2SO_4$ and evaporated. Column chromatography (consecutively ethyl acetate–light petroleum 1:2, then $CH_2Cl_2$, then $CH_2Cl_2$–methanol 10:1) of the residue afforded the product **10** (0.13 g, 46%) as white solid. $R_f$ = 0.3 (4% methanol in $CH_2Cl_2$).

$^1$H NMR ($CDCl_3$, δ): 1.49 (s, 9H, $H^{t\text{-}Bu}$); 1.57 (m, 1H); 1.72 (m, 2H); 1.88 (m, 1H); 2.01 (m, 2H); 2.13 (m, 2H); 3.69 (m, 1H, $H^1$); 3.96 (m, 1H, $H^5$); 9.83 (s, 1H, NH); 10.78 (s, 1H, NH).

$^{13}$C NMR ($CDCl_3$, δ): 15.9; 28.7; 29.3 ($C(\underline{C}H_3)_3$); 31.5; 32.7; 38.3 ($C^1$); 45.4 ($C^5$); 54.8 ($\underline{C}(CH_3)_3$); 166.6 ($C^3$).

MS (m/z, (%)): 212 ($M^+$, 50); 197 ($(M-CH_3)^+$, 11); 156 ($(M-Bu^t)^+$, 30); 113 ($C_6H_9S^+$, 58); 84 ($C_5H_{10}N^+$, 78); 82 ($C_6H_{10}^+$, 9); 81 ($C_6H_9^+$, 25); 80 ($C_6H_8^+$, 11); 57 (*m*-$Bu^+$, 75); 41 ($CHN_2^+$, 89).

Anal. calcd for $C_{11}H_{21}N_2ClS$: C, 53.10; H, 8.51; N, 11.26; S, 12.89. Found: C, 53.01; H, 8.24; N, 10.99; S, 12.59.

**Data for the free base**: $^{1}$H NMR ($CDCl_3$, δ): 1.20–1.47 (m, 5H); 1.30 (s, 9H, $H^{t\text{-}Bu}$); 1.86 (m, 1H); 2.00 (m, 1H, $J_{gem}$=12.5 Hz); 2.11 (m, 1H); 2.41 (m, 1H, $J_{gem}$=12.5 Hz); 3.18 (m, 1H, $H^1$); 3.35 (m, 1H, $H^5$). $^{13}$C NMR ($CDCl_3$, δ): 25.1; 29.6; 31.4 ($C(\underline{C}H_3)_3$); 33.7; 40.9; 41.0; 49.5 ($\underline{C}(CH_3)_3$); 55.7 ($C^5$); 159.3 ($C^3$).

***rac*-2-Thia-4-azabicyclo[3.3.1]non-3-en-3-amine hydrochloride (3).**[i] Compound **10** (0.13 g, 0.52 mmol) in conc. HCl (10 ml) was refluxed for 15 h. The reaction mixture was evaporated under reduced pressure and the precipitate was washed with diethyl ether to give product **3** (0.094 g, 94%) as white solid, mp 210–212 °C.

$^{1}$H NMR ($CDCl_3$, δ): 1.60 (m, 1H); 1.76–1.93 (m, 3H); 2.04 (m, 2H); 2.17 (m, 2H); 3.72 (m, 1H, $H^1$); 4.01 (m, 1H, $H^5$); 8.0–9.0 (br s, 2H, $NH_2^+$); 10.77 (s, 1H, NH).

$^{1}$H NMR ($CD_3OD$, δ): 1.73 (m, 3H); 1.90 (m, 1H); 1.99 (m, 2H); 2.12 (m, 1H, $J_{gem}$=14.0 Hz, $H^9$); 2.33 (m, 1H, $J_{gem}$=14.0 Hz, $H^9$); 3.81 (s, 1H, $H^1$); 3.95 (s, 1H, $H^5$).

NMR $^{13}$C (δ, М.д., $CDCl_3$): 15.7 ($C^7$); 29.1 ($C^6$); 31.3 ($C^8$); 32.6 ($C^9$); 38.0 ($C^1$); 45.6 ($C^5$); 168.8 ($C^3$).

$^{13}$C NMR ($CD_3OD$, δ): 15.4 ($C^7$); 28.3 ($C^6$); 30.8 ($C^8$); 32.1 ($C^9$); 37.8 ($C^1$); 46.1 ($C^5$); 169.0 ($C^3$).

IR ($\nu_{max}$): 1070, 1090, 1270, 1290, 1325, 1350, 1380, 1468, 1610, 1635 (C=N), 2600–3150 ($N^+$-H), 2830–3020 (C-H), 3190–3340 (N-H).

MS (m/z, (%)): 156 ($M^+$, 100); 141 ($(M\text{-}NH)^+$, 7); 123 ($(M\text{-}SH)^+$, 20); 113 ($C_6H_9S^+$, 85); 86 (57); 81 ($C_6H_9S^+$, 22); 58 ($CNS^+$, 49); 44 (48); 42 (36).

Anal. calcd for $C_7H_{13}ClN_2S$: C, 43.63; H, 6.80; N, 14.54; S, 16.64. Found: C, 43.60; H, 6, 82; N, 14.19; S, 16.24.

[i] Spectral data indicate the presence of single tautomer of compound **3** and do not allow assigning it to the specific form. Here we give the most expected biologically active tautomer.