Electronic supplementary materials *Mendeleev Commun.*, 2023, **33**, 77–79

Annulated bicyclic isothioureas: identification of active and selective butyrylcholinesterase inhibitors

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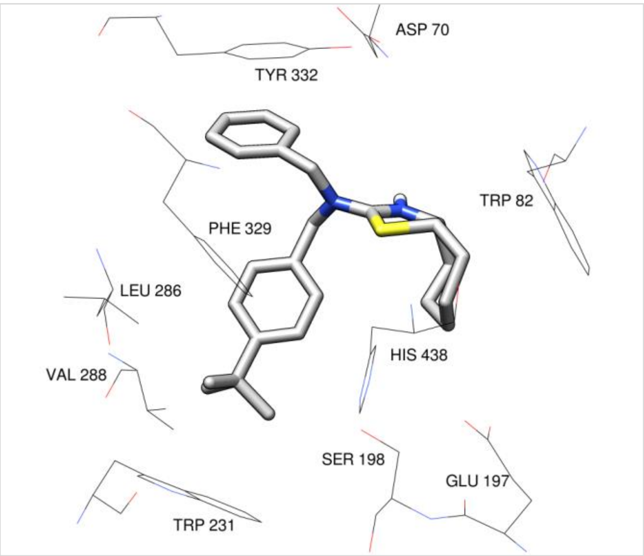
1. Molecular modeling

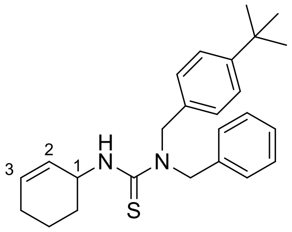
Computer molecular modeling was performed using 3D model of the active site in butyrylcholinesterase (PDB ID: 6R6W). All compounds used for X-ray of the protein and all water molecules were previously excluded from the model (other molecules and ions at the interface of α- and β-subunits of the protein were maintained). Atomic charges of protein amino acids were assigned by standard Kollman method using AutoDock Tools 1.5.6. 2D structures of the ligands were converted to the 3D structures and were submitted to a conformational MMFF Amber ff14SB optimization using Gasteiger charges in USCF Chimera 1.15 program [**S1**]. Docking procedure was performed with AutoDock Vina 1.1.2 software [**S2**] (grid box 16.5Å×16.5Å ×16.5Å, grid center size x=135.446 Å, y=110.001 Å, z=39.873 Å, energy range=4, exhaustiveness=20). Complexes with the best values of scoring functions were selected and visualized using UCSF Chimera 1.15 software. The position of $N^+_{exocyclic}$ protonated form of (3a*S*,8a*R*)-isomer of compound **7c** in the complex with hBChE is shown at the picture.



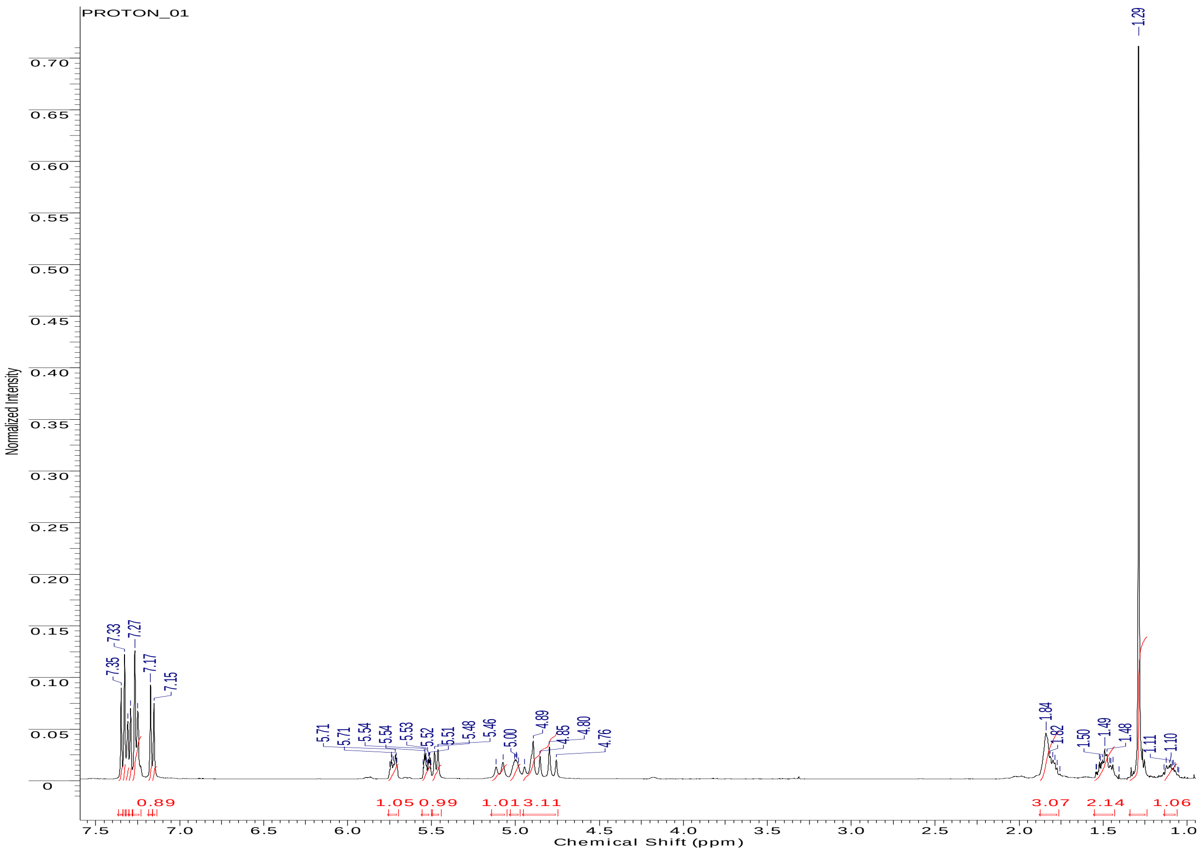
2. Chemistry

All starting materials, reagents and solvents were purchased as high-grade commercial products and used without further purification. 3-Isothiocyanatocyclohex-1-ene was prepared from 3-bromocyclohex-1-ene by literature procedure [**S3**]. *N*-Benzyl-*N*-(4-*tert*-butylbenzyl)amine was kindly provided by Alexey N. Proshin (IPAC RAS). Reactions sensitive to moisture and/or oxygen were carried out under an inert atmosphere of anhydrous argon. Liquid column chromatography was performed using silica gel Acros (40–60 μm). Thin-layer chromatography (TLC) was performed on Silufol-UV254 silica gel sheets, and spots were visualized with UV light (λ = 254 nm) or stained with iodine vapor or aqueous potassium permanganate solution. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400/Agilent 400-MR spectrometer at 27 °C in $CDCl_3$ at 400 and 100 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) referenced to residual protonated solvent peak ($CDCl_3$, $\delta = 7.27$ for ^{1}H NMR) or to carbon resonances in the NMR solvent ($CDCl_3$, $\delta = 77.0$ for ^{13}C NMR). Melting points were determined using a capillary melting point apparatus and were uncorrected. MS spectra were recorded on a system consisting of a liquid chromatograph Agilent 1100 Series, mass spectrometer Agilent Technologies LC/MSD VL (electrospray ionization), detector Sedex 75 ELSD (positive ion mode).

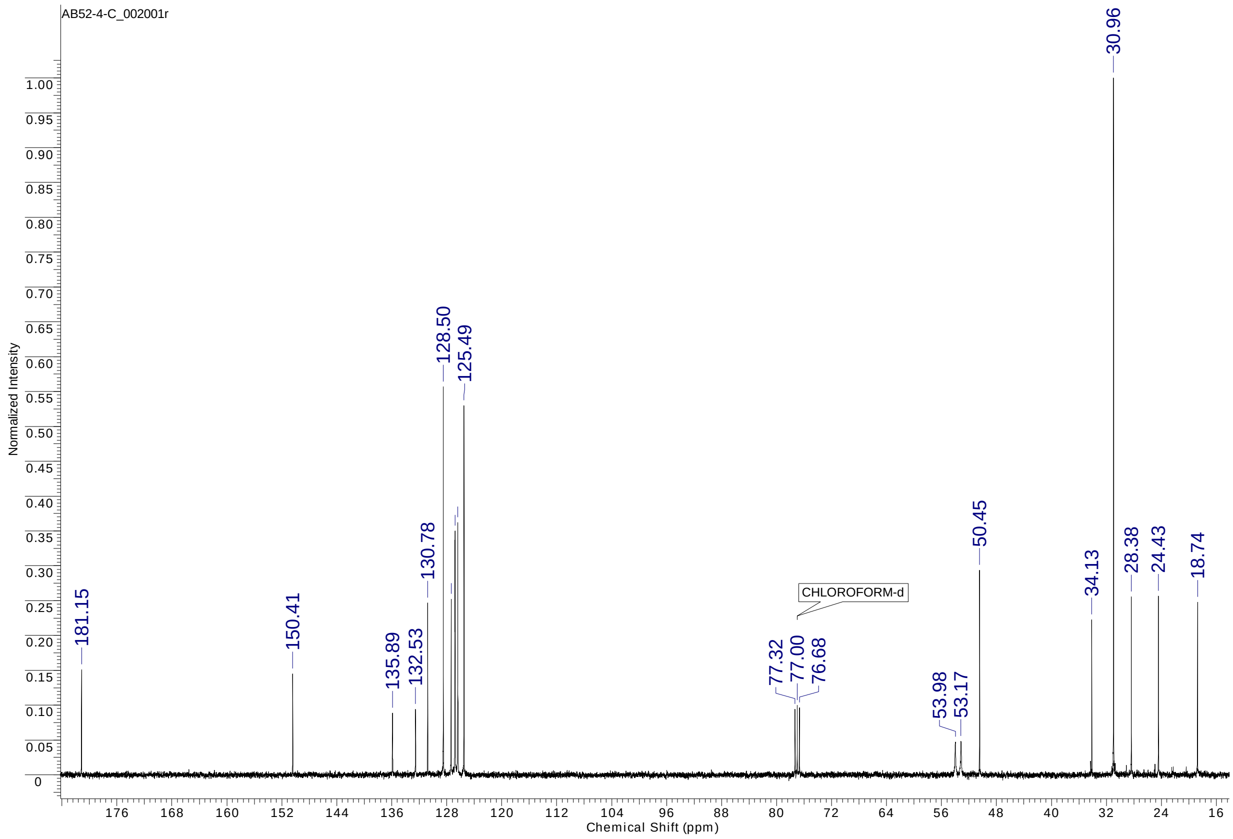
1-Benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohex-2-en-1-yl)thiourea (4b). 3-Isothiocyanatocyclohex-1-ene [S3] (0.565 ml, 4.34 mmol, 1 equiv.) dissolved in CH_2Cl_2 (10 ml) was treated with *N*,*N*-diisopropyl-*N*-ethylamine (DIPEA) (1.52 ml, 8.74 mmol, 2 equiv.) and ***N*-benzyl-*N*-(4-*tert*-butylbenzyl)amine** (1.1 g, 4.35 mmol, 1 equiv.). The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 1:10 ethyl acetate : hexane by v/v) to give **4b** as colorless oil (1.7 g, yield 99%).



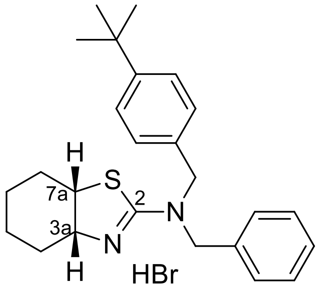
^{1}H NMR (δ, $CDCl_3$): 1.05 – 1.15 (m, 1H), 1.29 (s, 9H, *t*-Bu), 1.41 – 1.54 (m, 2H), 1.76 – 1.88 (m, 3H), 4.78 (d, 1H, J = 16.3 Hz, N$\underline{CH_2}$Ph), 4.87 (d, 1H, J = 16.3 Hz, N$\underline{CH_2}$Ph), 4.92 (d, 1H, J = 16,1 Hz, N$\underline{CH_2}$Ar), 5.00 (m, 1H, H^1), 5.09 (d, 1H, J = 16.1 Hz, N$\underline{CH_2}$Ar), 5.47 (d, 1H, J = 7.9 Hz, NH), 5.53 (ddt, 1H, J = 9.9, 3.9, 2.0 Hz, H^2), 5.72 (m, 1H, J = 9.9, 3.6, 1.7 Hz, H^3), 7.16 (d, J = 8.3 Hz, 2H, H^{Ar}), 7,23 – 7.27 (m, 3H, H^{Ar}), 7.30 (d, J = 7.0 Hz, 2H, H^{Ar}), 7.34 (d, J = 8.3 Hz, 2H, H^{Ar}).



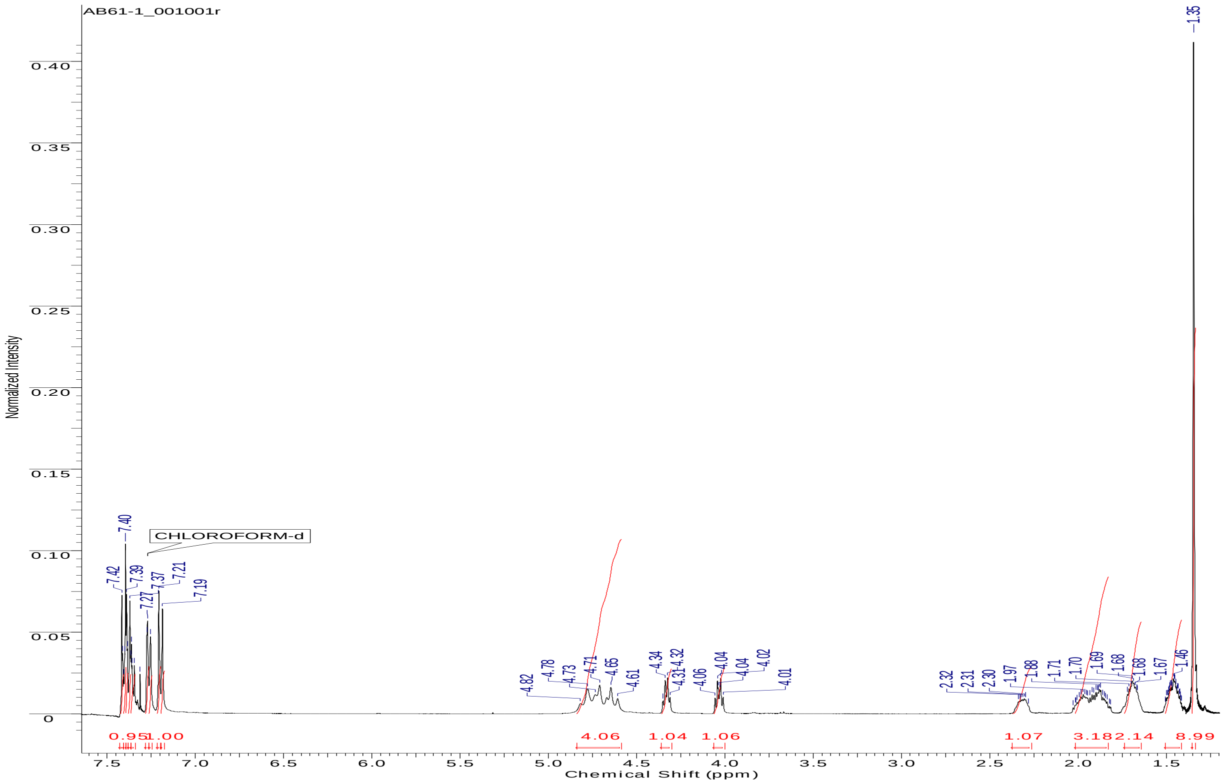
^{13}C NMR (δ, $CDCl_3$): 18.7 (C^5), 24.4 (C^4), 28.4 (C^6), 31.0 ($C(\underline{C}H_3)_3$), 34.1 ($\underline{C}(CH_3)_3$), 50.5 (C^1), 53.2 ($N\underline{CH_2}$), 54.0 ($N\underline{CH_2}$), 125.5, 126.4, 126.8, 126.8, 127.4, 128.5, 130.8, 132.5, 135.9, 150.4, 181.1 (C=S).



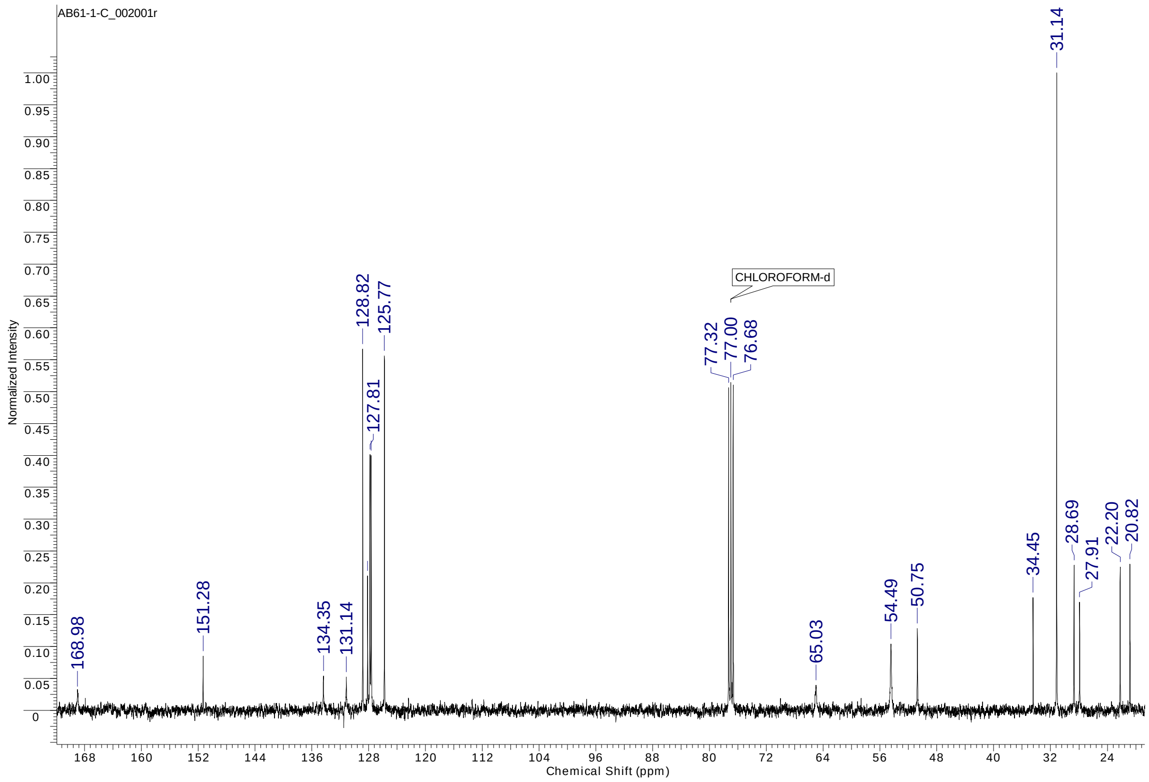
(±)-(3a*R*,7a*S*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine hydrobromide (7b). A solution of acetyl bromide (37 µl, 0.5 mmol, 3 equiv.) in CH_2Cl_2 (5 ml) was treated with methanol (20 µl, 0.5 mmol, 3 equiv.) and stirred at room temperature in darkness. In 10 min, a solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohex-2-en-1-yl)thiourea (4b)** (65 mg, 0.165 mmol, 3 equiv.) in CH_2Cl_2 (5 ml) was added, and stirring was continued for 12 h. The reaction mixture was concentrated and the residue purified by column chromatography (eluent: CH_2Cl_2, then 3% methanol in CH_2Cl_2) to yield **(±)-(3a*R*,7a*S*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine hydrobromide (7b)** as white solid (77 mg, 99 %). M.p. 169–171 ^{0}C.



^{1}H NMR (δ, $CDCl_3$): 1.35 (s, 9H, *t*-Bu), 1.41 – 1.51 (m, 2H), 1.64 – 1.74 (m, 2H), 1.83-2.03 (m, 3H), 2.26 – 2.37 (m, 1H), 4.04 (dt, 1H, *J* = 7.4, 5.4 Hz, H^{7a}), 4.33 (dt, 1H, *J* = 5.6, 5.4 Hz, H^{3a}), 4.63 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ar), 4.69 (d, 1H, *J* = 16.9 Hz, N$\underline{CH_2}$Ph), 4.75 (d, 1H, *J* = 16,9 Hz, N$\underline{CH_2}$Ph), 4.80 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ar), 7.20 (d, *J* = 8.2 Hz, 2H, H^{Ar}), 7.27 (d, *J* = 8.0 Hz, 2H, H^{Ar}), 7.35 – 7.42 (m, 5H, H^{Ar}).

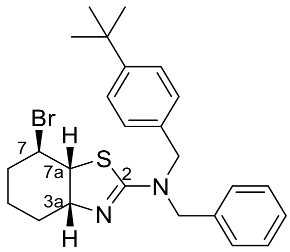


^{13}C NMR (δ, $CDCl_3$): 20.8, 22.2, 27.9, 28.7, 31.1 (C($\underline{C}H_3)_3$), 34.5 ($\underline{C}(CH_3)_3$), 50.7 (C^{7a}), 54.5 (N$\underline{CH_2}$Ar+ N$\underline{CH_2}$Ph), 65.0 (C^{3a}), 125.8 ($C^{3,5\text{-}Ar}$), 127.6, 127.8, 128.1, 128.8 ($C^{3,5\text{-}Ph}$), 131.1, 134.4, 151.3, 169.0 (C^2).

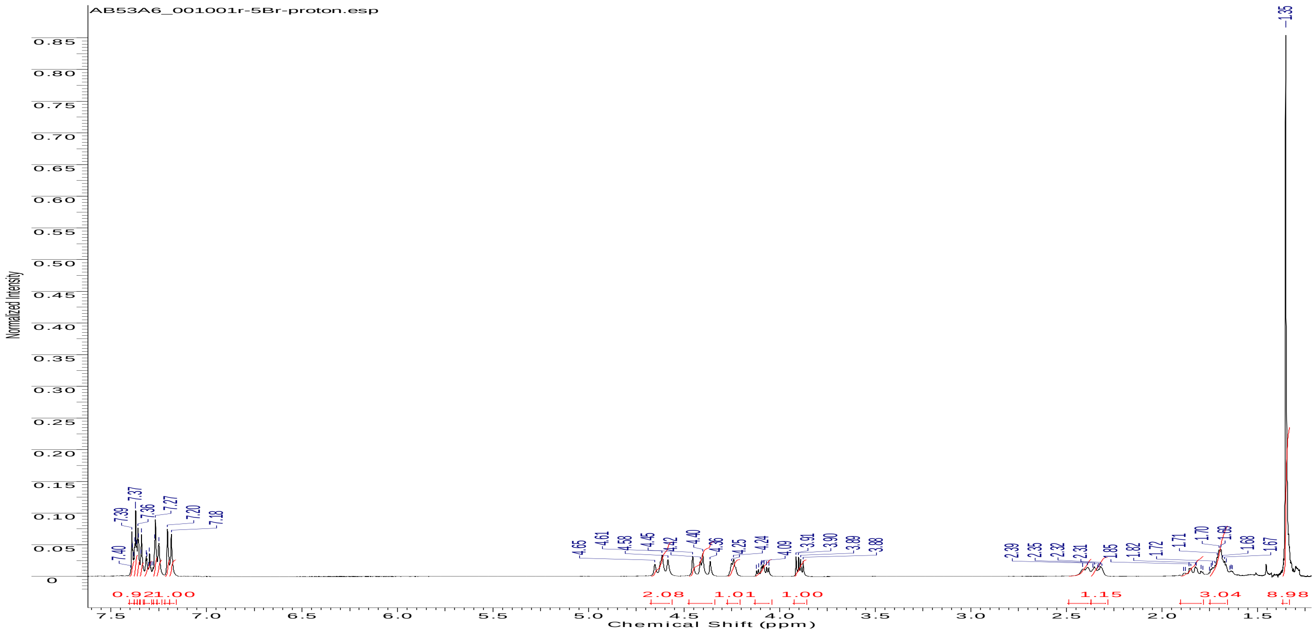


MS (ESI), m/z : 393 [M+H]$^+$.

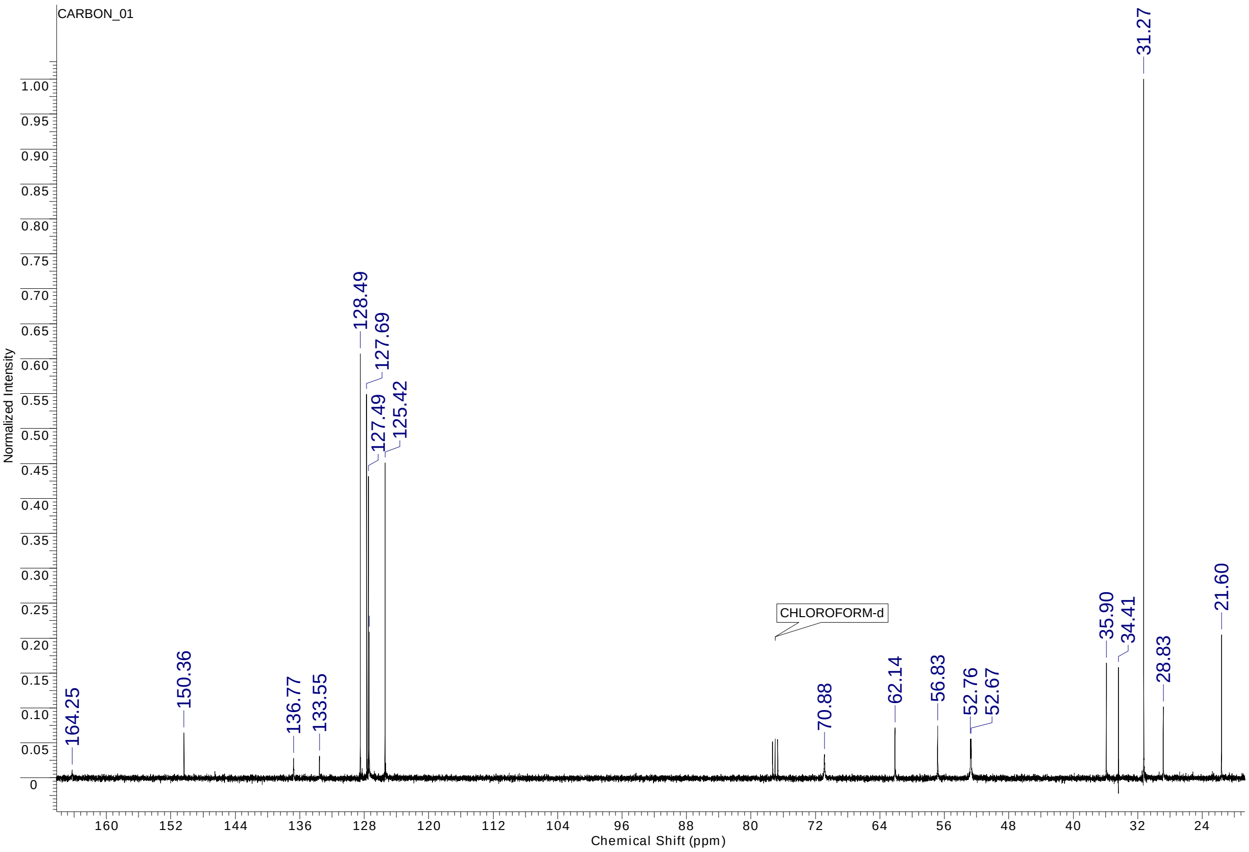
(±)-(3a*R*,7*R*,7a*R*)-*N*-Benzyl-7-bromo-*N*-(4-*tert*-butylbenzyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]-thiazol-2-amine (5b). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohex-2-en-1-yl)thiourea (4b)** (416 mg, 1.06 mmol, 1 equiv.) in CH_2Cl_2 (10 ml) was treated with Br_2 (55 µl, 1.06 mmol, 1 equiv.) and stirred in the dark at room temperature for 12 h. The reaction mixture was washed with aqueous saturated solution of Na_2SO_3 (3x20ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (eluent: 2% methanol in CH_2Cl_2) to yield **(±)-(3a*R*,7*R*,7a*R*)-*N*-benzyl-7-bromo-*N*-(4-*tert*-butylbenzyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine (5b)** as colorless oil (386 mg, 77%).



^{1}H NMR (δ, $CDCl_3$): 1.35 (s, 9H, *t*-Bu), 1.66 – 1.74 (m, 3H), 1.79-1.89 (m, 1H), 2.30 – 2.36 (m, 1H), 2.37 – 2.45 (m, 1H), 3.89 (dd, 1H, *J* = 9.8, 5.4 Hz, H^{7a}), 4.09 (ddd, 1H, *J* = 11.5, 9.8, 4.1 Hz, H^{7}), 4.24 (m, 1H, *J* = 4.1 Hz, H^{3a}), 4.38 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ph), 4.43 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ph), 4.59 (d, 1H, *J* = 11,7 Hz, N$\underline{CH_2}$Ar), 4.63 (d, 1H, *J* = 11.7 Hz, N$\underline{CH_2}$Ar), 7.19 (d, *J* = 8.4 Hz, 2H, H^{Ar}), 7.26 (d, *J* = 7.8 Hz, 2H, H^{Ar}), 7.30 (d, *J* = 7.1 Hz, 1H, H^{Ar}), 7.34 – 7.39 (m, 4H, H^{Ar}).

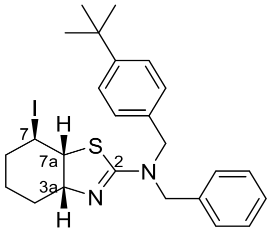


^{13}C NMR (δ, $CDCl_3$): 21.6 (C^{5}), 28.8 (C^{4}), 31.3 ($C(\underline{C}H_3)_3$), 34.4 ($\underline{C}(CH_3)_3$), 35.9 (C^{6}), 52.7 (N$\underline{CH_2}$Ar^{1}), 52.8 (N$\underline{CH_2}$Ar^{2}), 56.8 (C^{7a}), 62.1 (C^{7}), 70.9 (C^{3a}), 125.4 (C$^{3,5\text{-}Ar}$), 127.4, 127.5, 127.7, 128.5 (C$^{3,5\text{-}Ph}$), 133.6, 136.8, 150.4, 164.3(C^{2}).

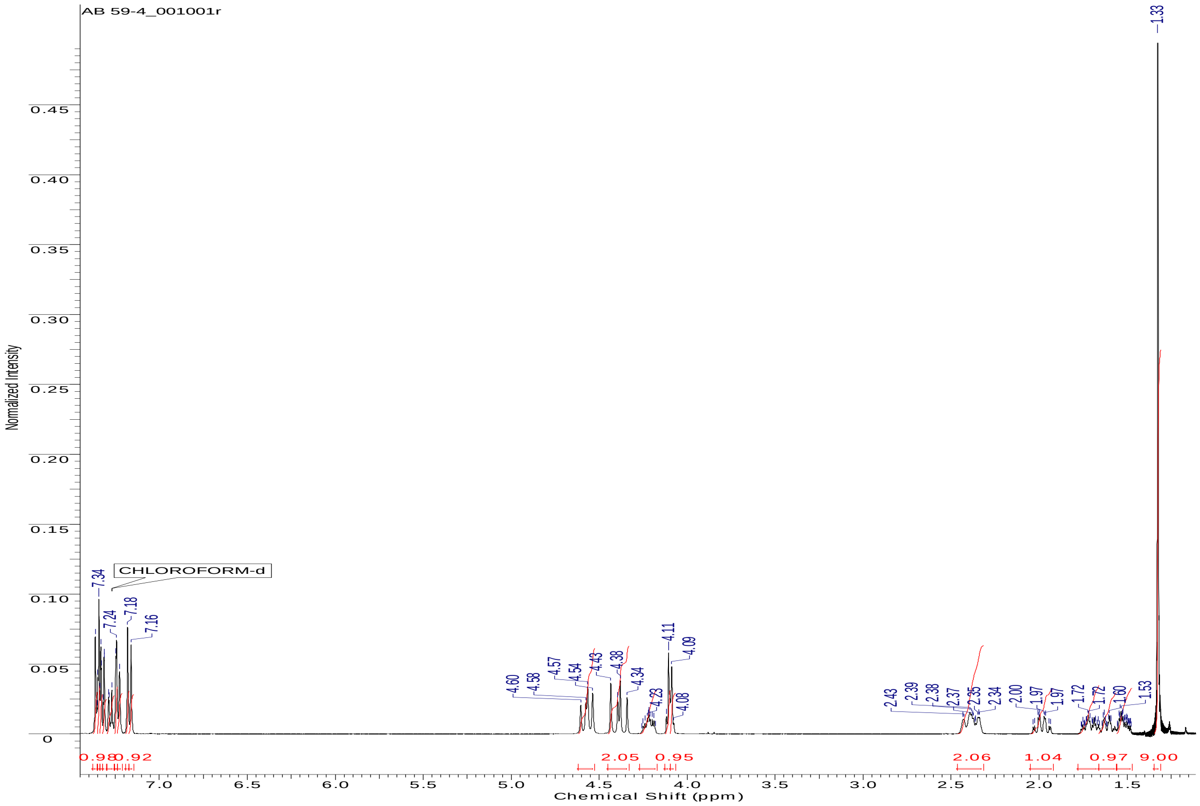


MS (ESI), m/z: 471 $[M+H]^+$ / 473 $[M+2+H]^+$.

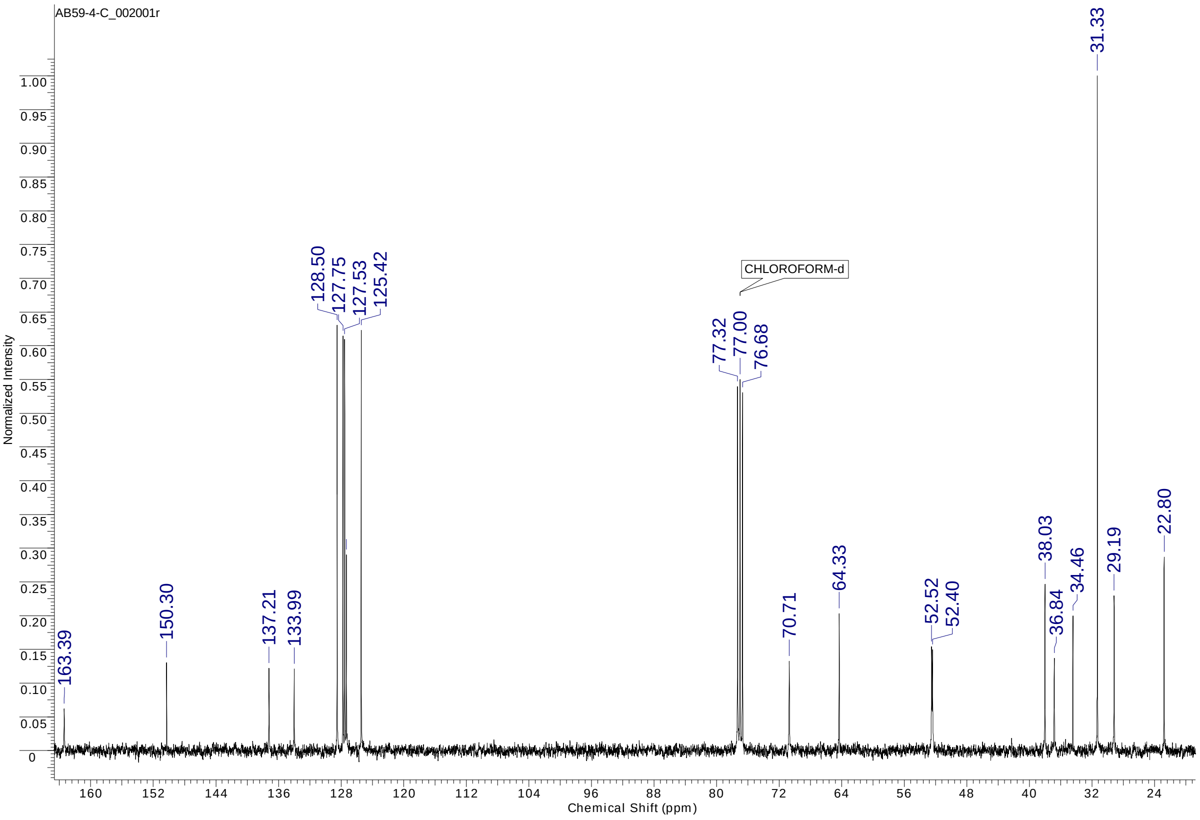
(±)-(3a*R*,7*R*,7a*R*)-*N*-Benzyl-*N*-(4-*tert*-butylbenzyl)-7-iodo-3a,4,5,6,7,7a-hexahydrobenzo[*d*]-thiazol-2-amine (6b). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohex-2-en-1-yl)thiourea (4b)** (96 mg, 0.244 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was treated with I_2 (62 mg, 0.244 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 12 h, then was washed with aqueous saturated solution of Na_2SO_3 (3x10ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (eluent: CH_2Cl_2) to yield **(±)-(3a*R*,7*R*,7a*R*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-7-iodo-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine (6b)** as colorless oil (125 mg, 99%).



^{1}H NMR (δ, $CDCl_3$): 1.33 (s, 9H, *t*-Bu), 1.48 – 1.58 (m, 1H), 1.60 – 1.65 (m, 1H), 1.66-1.77 (m, 1H), 1.94 – 2.04 (m, 1H), 2.34 – 2.44 (m, 2H), 4.08 – 4.12 (m, 2H, H^{7a}+ H^{3a}), 4.22 (m, 1H, *J* = 11.9, 4.6, 2.7 Hz, H^7), 4.36 (d, 1H, *J* = 15.8 Hz, $N\underline{CH_2}Ph$), 4.41 (d, 1H, *J* = 15.8 Hz, $N\underline{CH_2}Ph$), 4.55 (d, 1H, *J* = 11,3 Hz, $N\underline{CH_2}Ar$), 4.59 (d, 1H, *J* = 11.3 Hz, $N\underline{CH_2}Ar$), 7.17 (d, *J* = 8.3 Hz, 2H, H^{Ar}), 7.24 (m, *J* = 8.3, 1.3 Hz, 2H, H^{Ar}), 7.28 (m, *J* = 7.3 Hz, 1H, H^{Ar}), 7.33 (m, 1H, *J* = 7.3 Hz, 2H, H^{Ar}), 7.36 (m, 1H, *J* = 8.3 Hz, 2H, H^{Ar}).

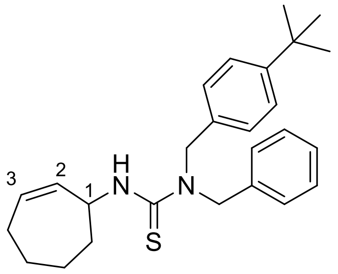


^{13}C NMR (δ, $CDCl_3$): 22.8 (C^5), 29.2 (C^4), 31.3 ($C(\underline{C}H_3)_3$), 34.5 ($\underline{C}(CH_3)_3$), 36.8 (C^7), 38.0, 52.4 ($N\underline{CH_2}Ar^1$), 52.5 ($N\underline{CH_2}Ar^2$), 64.3 (C^{7a}), 70.7 (C^{3a}), 125.4 ($C^{3,5\text{-}Ar}$), 127.3, 127.5, 127.8, 128.5, 134.0, 137.2, 150.3, 163.4.



MS (ESI), m/z: 519 $[M+H]^+$.

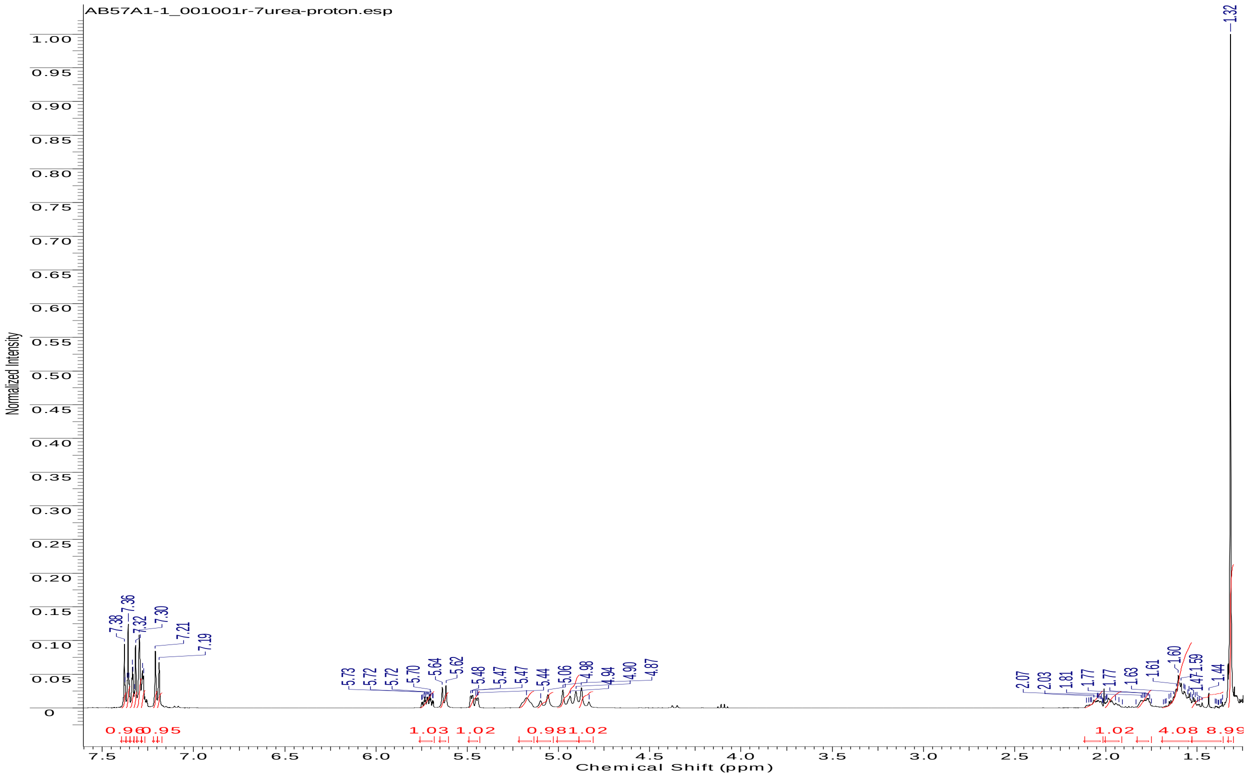
1-Benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohept-2-en-1-yl)thiourea (4c). Cycloheptene (2.43 ml, 20.8 mmol, 2 equiv.) dissolved in CCl$_4$ (10 ml) was treated at stirring with ***N*-bromosuccinimide** (NBS) (1.85 g, 10.4 mmol, 1 equiv.) and **azobisisobutyronitrile** (AIBN) (2 mg, 10.4 mmol, cat. equiv.). The reaction mixture was refluxed for 1.5 h, then cooled to 0°C, after that was filtered, the filtrate was washed with CCl$_4$ (5 ml). The control of reaction was carried out by ^{1}H NMR of the reaction mass. ^{1}H NMR (δ, CDCl$_3$): 1.78 – 1.92 (m, 3H), 1.99–2.08 (m, 2H), 2.16–2.22 (m, 1H), 2.23 – 2.28 (m, 2H), 4.93 (td, 1H, J = 6.5, 2.3 Hz, H^3), 5.83 (dt, 1H, J = 11.4, 5.7 Hz, H^1), 5.92 (m, 1H, J = 11.4, 6.3 Hz, H^2).



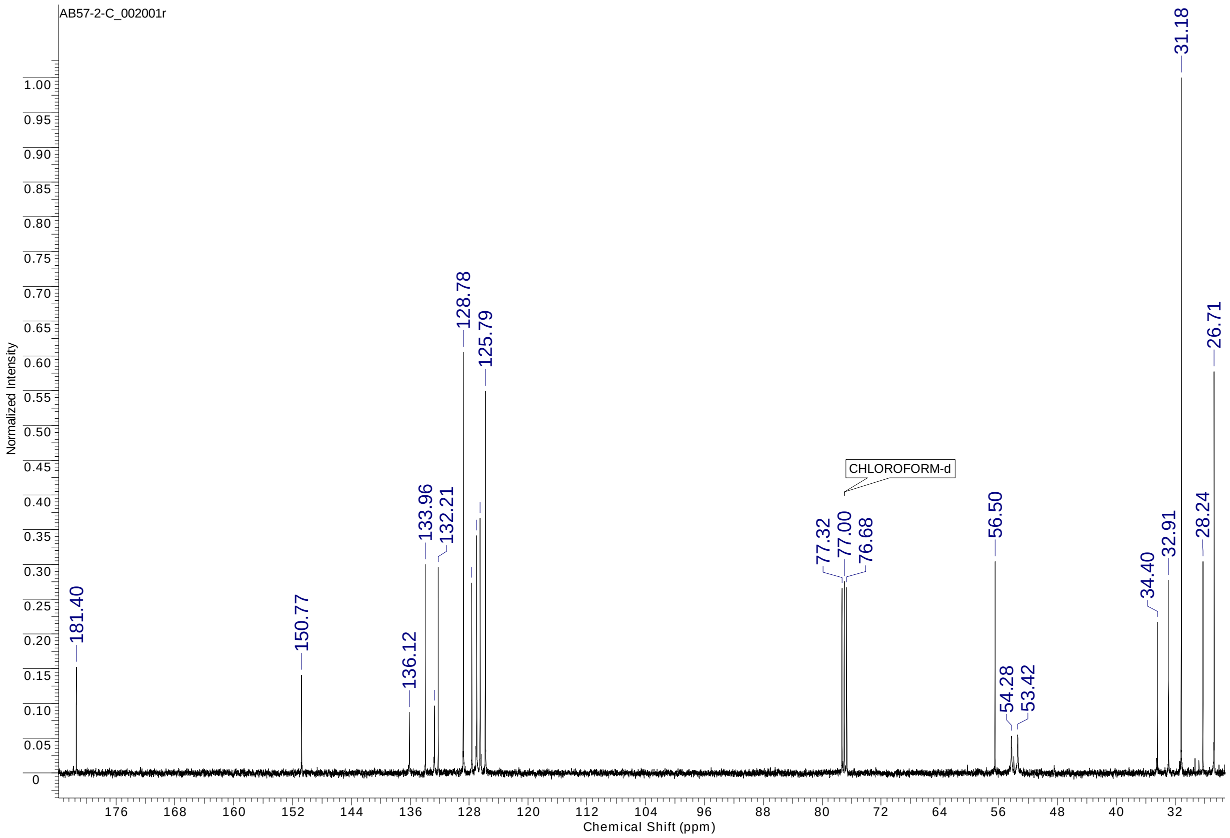
A solution of **3-bromocyclohep-1-ene** in CCl$_4$ was treated dropwise with solution of **potassium thiocyanate** (1.0 g, 10.4 mmol, 1 equiv.) in absolute MeOH (5 ml) at ice cooling, and was stirred at room temperature for 2 h, followed by filtration thought SiO$_2$ and washing with hexane (20 ml). The solution of **3-isothiocyanatocyclohep-1-ene** was used directly for the next step without evaporation and further purification. The control of reaction was carried out by ^{1}H NMR analysis of the reaction solution. The presence of resonance signal at 4.40 ppm (m, 1H, J = 9.5, 1.9 Hz) instead of 4.93 ppm confirms the conversion of bromide into isothiocyanate.

3-Isothiocyanatocyclohept-1-ene solution obtained from the previous step was treated with DIPEA (3.6 ml, 20.8 mmol, 2 equiv.) and ***N*-benzyl-*N*-(4-*tert*-butylbenzyl)amine** (2.6 g, 10.4 mmol, 1 equiv). The reaction mixture was stirred overnight at room temperature, concentrated under reduced pressure and purified by column chromatography on SiO$_2$ (eluent: CHCl$_3$, then 2% methanol in CHCl$_3$) to give **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohept-2-en-1-yl)thiourea (4c)** as colorless amorphous substance (2.3 g, summary yield from 3 steps 54%). M.p. 87-89 ^{0}C.

^{1}H NMR (δ, CDCl$_3$): 1.32 (s, 9H, *t*-Bu), 1.45 – 1.54 (m, 1H), 1.54 – 1.69 (m, 4H), 1.75 – 1.83 (m, 1H), 1.91 – 2.00 (m, 1H), 2.02 – 2.12 (m, 1H), 4.85 (d, 1H, J = 16.3 Hz, N$\underline{CH_2}$Ph), 4.92 (d, 1H, J = 12.5 Hz, N$\underline{CH_2}$Ar), 4.96 (d, 1H, J = 12.5 Hz, N$\underline{CH_2}$Ar), 5.08 (d, 1H, J = 16.3 Hz, N$\underline{CH_2}$Ph), 5.18 (m, 1H, J =9.7 Hz, H^1), 5.46 (m, J = 11.5, 3.7 Hz , H^2), 5.63 (d, 1H, NH), 5.72 (m, 1H, J = 11.5, 7.1, 5.3, 2.0 Hz, H^3), 7.20 (d, J = 8.3 Hz, 2H, H^{Ar}), 7.28 – 7.31 (m, 3H, H^{Ar}), 7.33-7.38 (m, 4H, H^{Ar}).

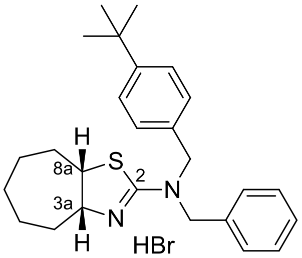


^{13}C NMR (δ, $CDCl_3$): 26.7, 28.2, 31.2 (C(<u>C</u>H_3)$_3$), 32.9, 34.4 (<u>C</u>(CH_3)$_3$), 53.4 (N<u>CH_2</u>Ar), 54.3 (N<u>CH_2</u>Ph), 56.5 (C^1), 125.8 ($C^{3,5\text{-}Ar}$), 126.5, 127.0, 127.6, 128.8, 132.2 , 132.7, 134.0, 136.1, 150.8, 181.4 (C=S).

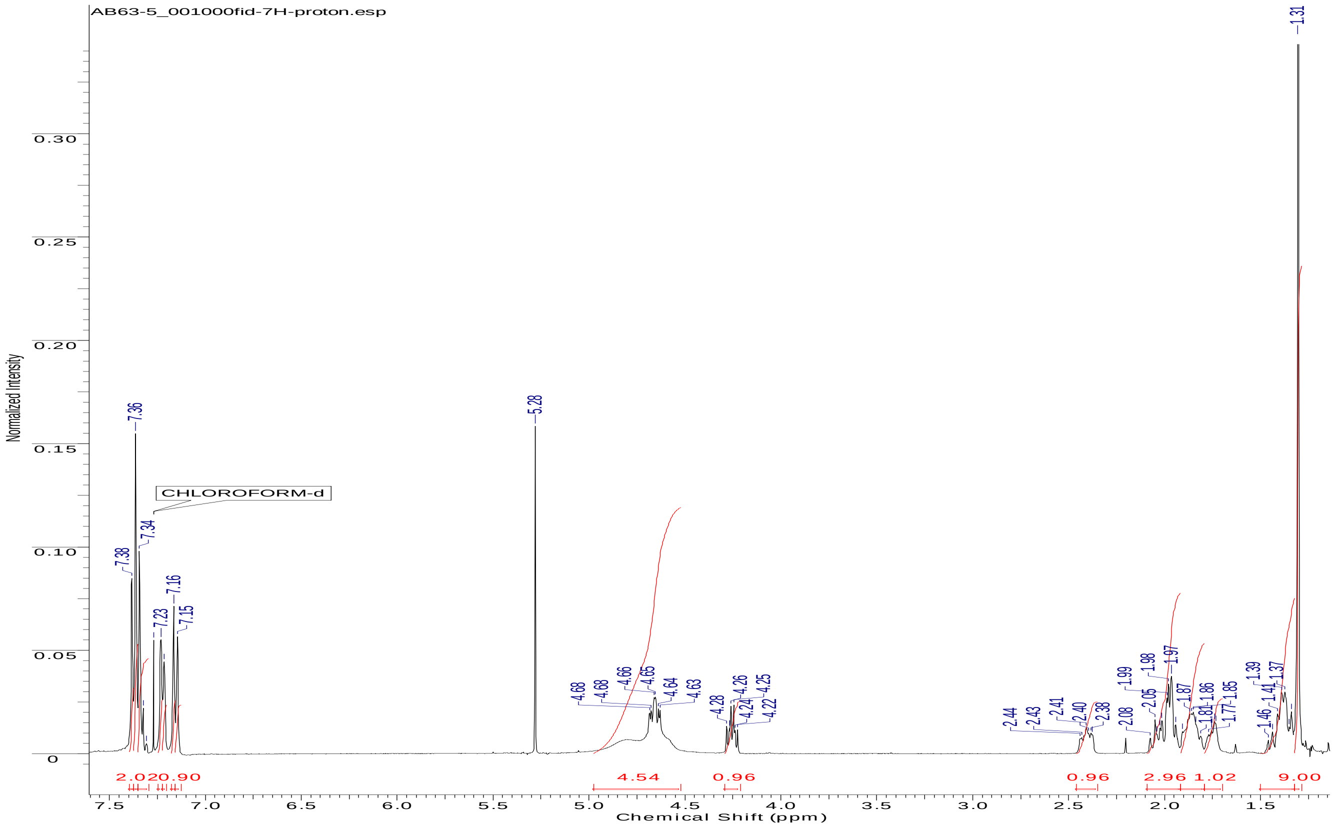


MS ESI, m/z: 407 $[M+H]^+$.

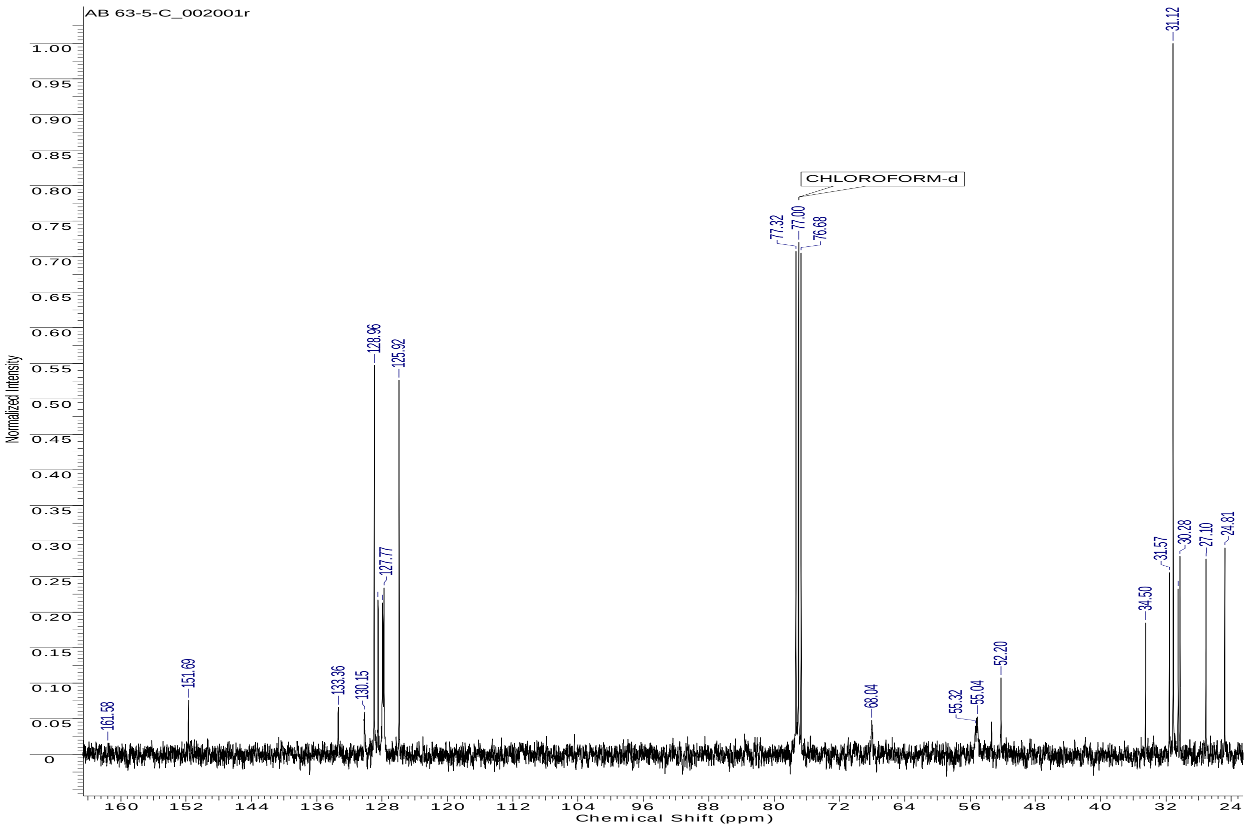
(±)-(3a*R*,8a*S*)-*N*-Benzyl-*N*-(4-*tert*-butylbenzyl)-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine hydrobromide (7c). A solution of acetyl bromide (82 µl, 1.1 mmol, 3 equiv.) in CH_2Cl_2 (5 ml) was treated with methanol (45 µl, 1.1 mmol, 3 equiv.) and stirred at room temperature in the dark. In 10 min a solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohept-2-en-1-yl)thiourea (4c)** (150 mg, 0.37 mmol) in CH_2Cl_2 (5 ml) was added and stirring was continued for 12 h. The reaction mixture was concentrated and the residue purified by column chromatography (eluent: ethyl acetate, then CH_2Cl_2) to yield **(±)-(3a*R*,8a*S*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine hydrobromide** as light yellow oil (150 mg, yield 83%).



^{1}H NMR (δ, $CDCl_3$): 1.31 (s, 9H, *t*-Bu), 1.32 – 1.46 (m, 3H), 1.70 – 1.78 (m, 1H), 1.81 – 1.91 (m, 2H), 1.94 – 2.08 (m, 3H), 2.38 – 2.44 (m, 1H), 4.25 (td, 1H, *J* = 8.8, 5.9 Hz, H^{8a}), 4.55 – 4.91 (br m, 4H, NCH_2Ar+ NCH_2Ph), 4.65 (m, 1H, *J* = 12.3, 8.8, 2.3 Hz, H^{3a}), 7.15 (d, *J* = 8.1.Hz, 2H, H^{Ar}), 7.22 (d, *J* = 6.4Hz, 2H, H^{Ar}), 7.29 – 7.38 (m, 5H, H^{Ar}).

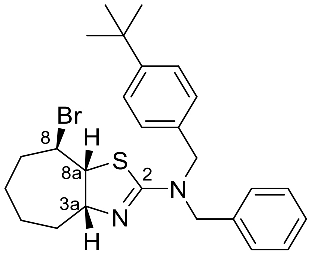


^{13}C NMR (δ, $CDCl_3$): 24.8, 27.1, 30.3, 30.5, 31.1 ($C(\underline{C}H_3)_3$), 31.6, 34.5 ($\underline{C}(CH_3)_3$), 52.2 (C^{8a}), 55.0 ($N\underline{CH_2}Ar$), 55.3 ($N\underline{CH_2}Ph$), 68.0 (C^{3a}), 125.9 ($C^{3,5\text{-}Ar}$), 127.8, 127.9, 128.5, 129.0 ($C^{3,5\text{-}Ph}$), 130.2 , 133.4 , 151.7 , 161.6 (C^2) .

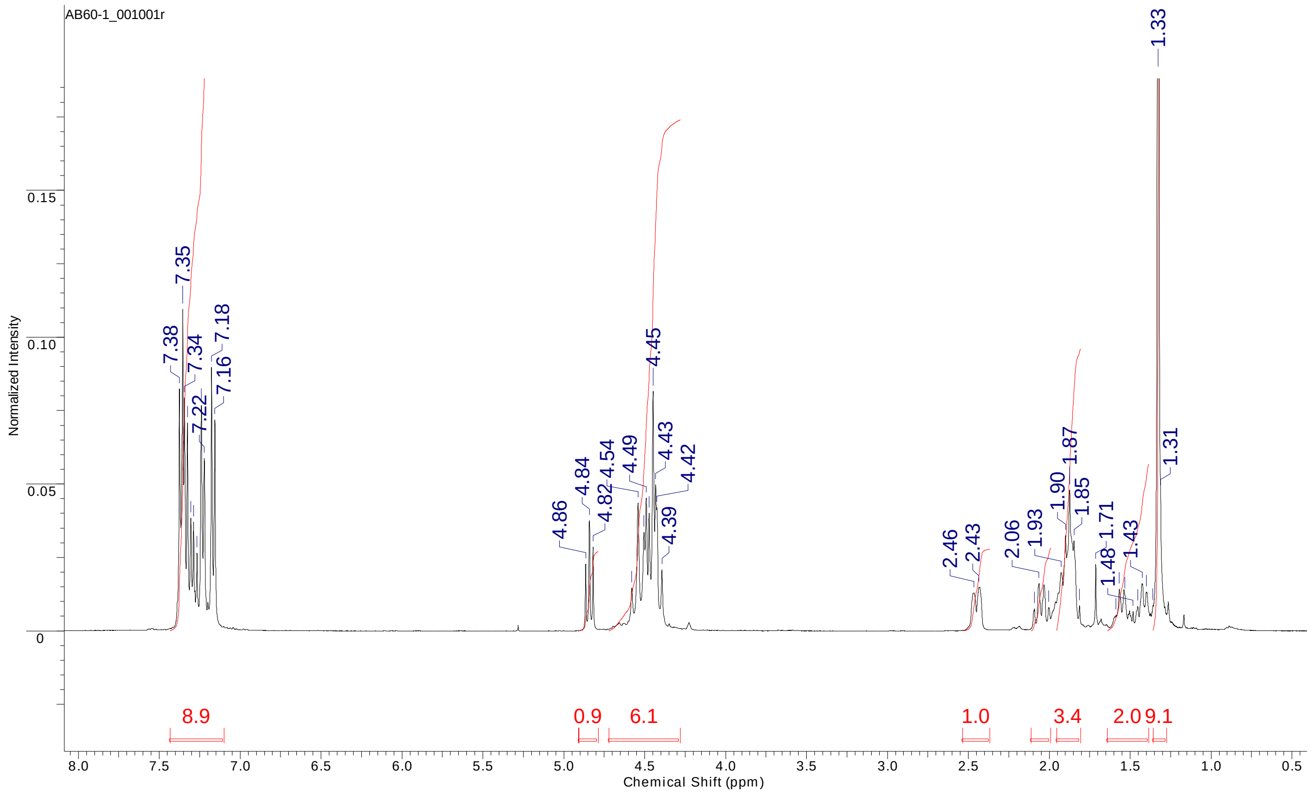


MS ESI, m/z: 407 $[M+H]^+$.

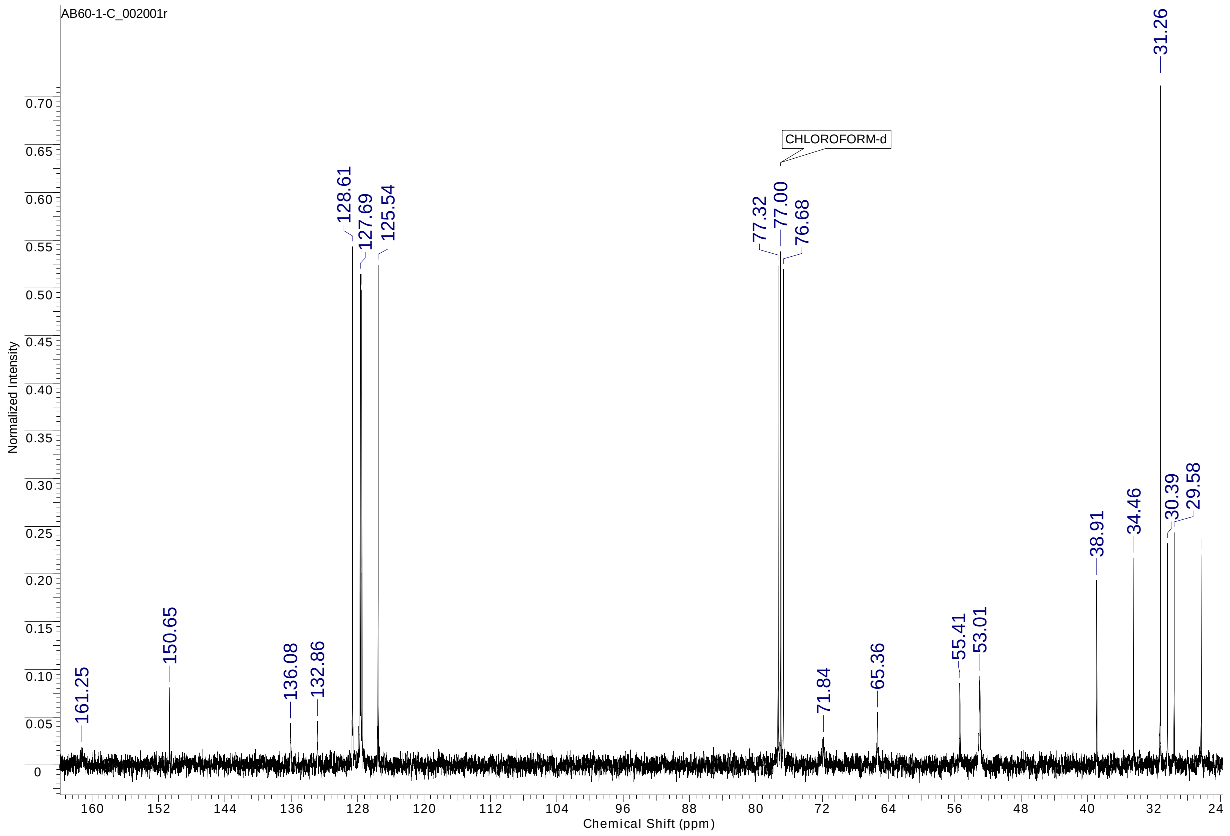
(±)-(3a*R*,8*R*,8a*R*)-*N*-Benzyl-8-bromo-*N*-(4-*tert*-butylbenzyl)-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine (5c). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohept-2-en-1-yl)thiourea (4c)** (77 mg, 0.19 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was treated with Br_2 (10 µl, 0.19 mmol, 1 equiv.) and stirred at room temperature for 12 h, then was washed with aqueous saturated solution of Na_2SO_3 (3x10ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4, concentrated and the residue purified by column chromatography (eluent: gradient of 0-2% methanol in CH_2Cl_2) to yield **(±)-(3a*R*,8*R*,8a*R*)-*N*-benzyl-8-bromo-*N*-(4-*tert*-butylbenzyl)-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine (5c)** as light yellow oil (80 mg, 87%).



^{1}H NMR (δ, $CDCl_3$): 1.33 (s, 9H, *t*-Bu), 1.40 – 1.61 (m, 2H), 1.81-1.93 (m, 3H), 1.92 – 2.00 (m, 1H), 2.02-2.10 (m, 1H), 2.41 – 2.49 (m, 1H), 4.39 – 4.58 (m, 6H, H^{8a+8}+ $N\underline{CH_2}Ph$+ $N\underline{CH_2}Ar$), 4.84 (dd, 1H, *J* = 9.8, 8.6 Hz, H^{3a}), 7.17 (d, *J* = 8.3 Hz, 2H, H^{Ar}), 7.23 (d, *J* = 7.0 Hz, 2H, H^{Ar}), 7.30 (m, *J* = 7.0 Hz, 1H, H^{Ar}), 7.33 – 7.38 (m, 4H, H^{Ar}).

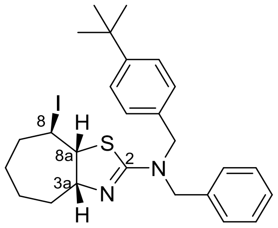


^{13}C NMR (δ, CDCl$_3$): 26.3, 29.6, 30.4, 31.3 ($C(\underline{C}H_3)_3$), 34.5 ($\underline{C}(CH_3)_3$), 38.9, 53.0 (NCH_2Ph+ $N\underline{CH_2}Ar$), 55.4 (C^{8a}), 65.4 (C^{8}), 71.8 (C^{3a}), 125.5 ($C^{3,5\text{-Ar}}$), 127.5, 127.6, 127.7, 128.6 ($C^{3,5\text{-Ph}}$), 132.9, 136.1, 150.7, 161.2 (C^{2}).

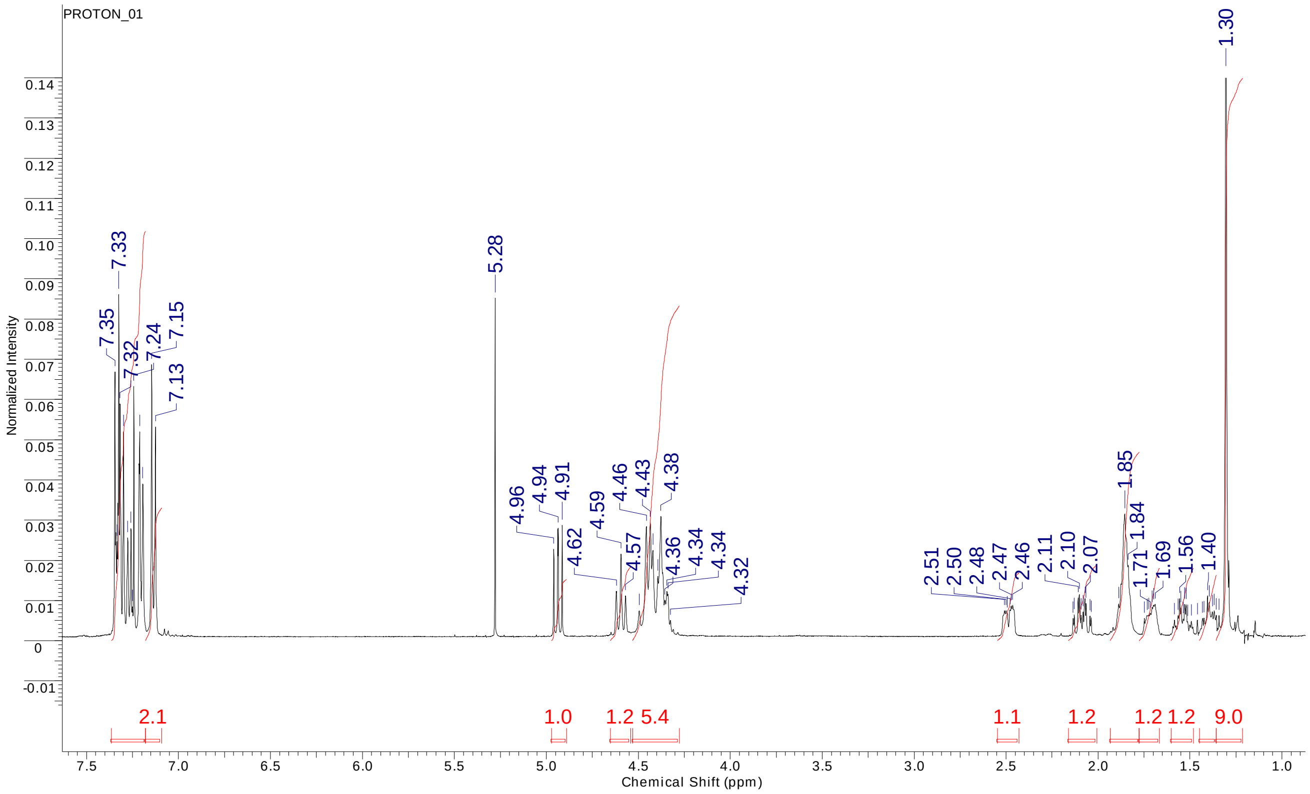


MS ESI, m/z : 485 $[M+H]^+$ / 487 $[M+2+H]^+$.

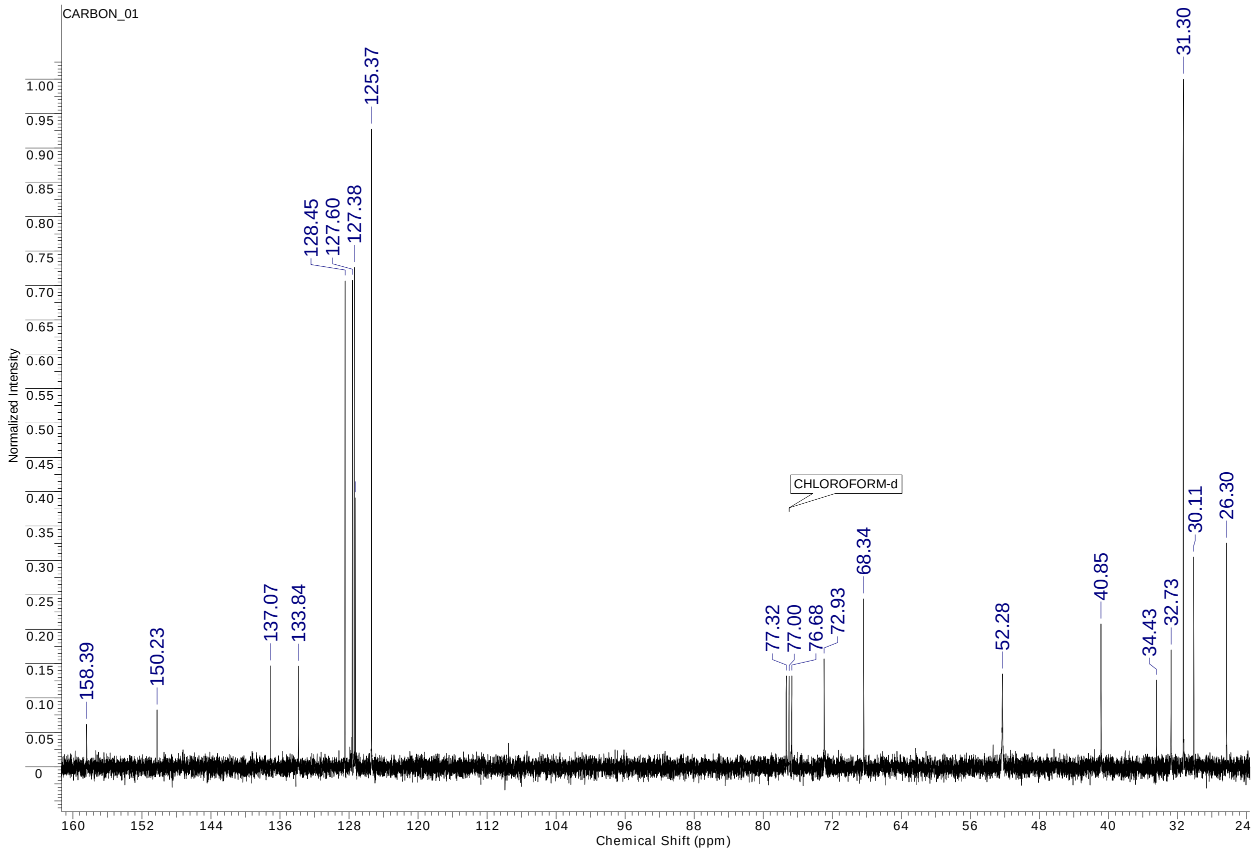
(±)-(3a*R*,8*R*,8a*R*)-*N*-Benzyl-*N*-(4-*tert*-butylbenzyl)-8-iodo-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine (6c). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclohept-2-en-1-yl)thiourea (4c)** (150 mg, 0.37 mmol, 1 equiv.) in CH_2Cl_2 (10 ml) was treated with I_2 (94 mg, 0.37 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 24 h, then was washed with aqueous saturated solution of Na_2SO_3 (3x10 ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4,. The organic layer was dried over Na_2SO_4, concentrated to yield **(±)-(3a*R*,8*R*,8a*R*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-8-iodo-4,5,6,7,8,8a-hexahydro-3a*H*-cyclohepta[*d*]thiazol-2-amine (6c)** as yellowish oil (195 mg, 99%).



^{1}H NMR (δ, $CDCl_3$): 1.30 (s, 9H, *t*-Bu), 1.34 – 1.43 (m, 1H), 1.49 – 1.59 (m, 1H), 1.69 – 1.75 (m, 1H), 1.84 – 1.94 (m, 3H), 2.04 – 2.14 (m, 1H), 2.45 – 2.52 (m, 1H), 4.32 – 4.50 (m, 5H, N<u>CH$_2$</u>Ar+N<u>CH$_2$</u>Ph+H^8), 4.59 (m, 1H, *J* = 10.5, 9.8 Hz, H^{8a}), 4.96 (dd, 1H, *J* = 9.8, 8.6 Hz, H^{3a}), 7.14 (d, *J* = 8.2 Hz, 2H, H^{Ar}), 7.20 (d, *J* = 7.1 Hz, 2H, H^{Ar}), 7.27 (m, *J* = 7.0 Hz, 1H, H^{Ar}), 7.30 – 7.35 (m, 4H, H^{Ar}).

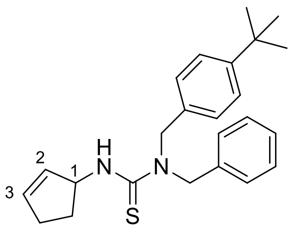


^{13}C NMR (δ, $CDCl_3$): 26.3, 30.1, 31.3 (C(<u>C</u>H$_3$)$_3$), 32.7, 34.4 (<u>C</u>(CH$_3$)$_3$), 40.8 (C^8), 52.3 (NCH$_2$Ph+ N<u>CH$_2$</u>Ar), 68.3 (C^{8a}), 72.9 (C^{3a}), 125.4 (C$^{3,5\text{-}Ar}$), 127.2 , 127.4, 127.6, 128.5, 133.8, 137.1, 150.2, 158.4 (C^2).



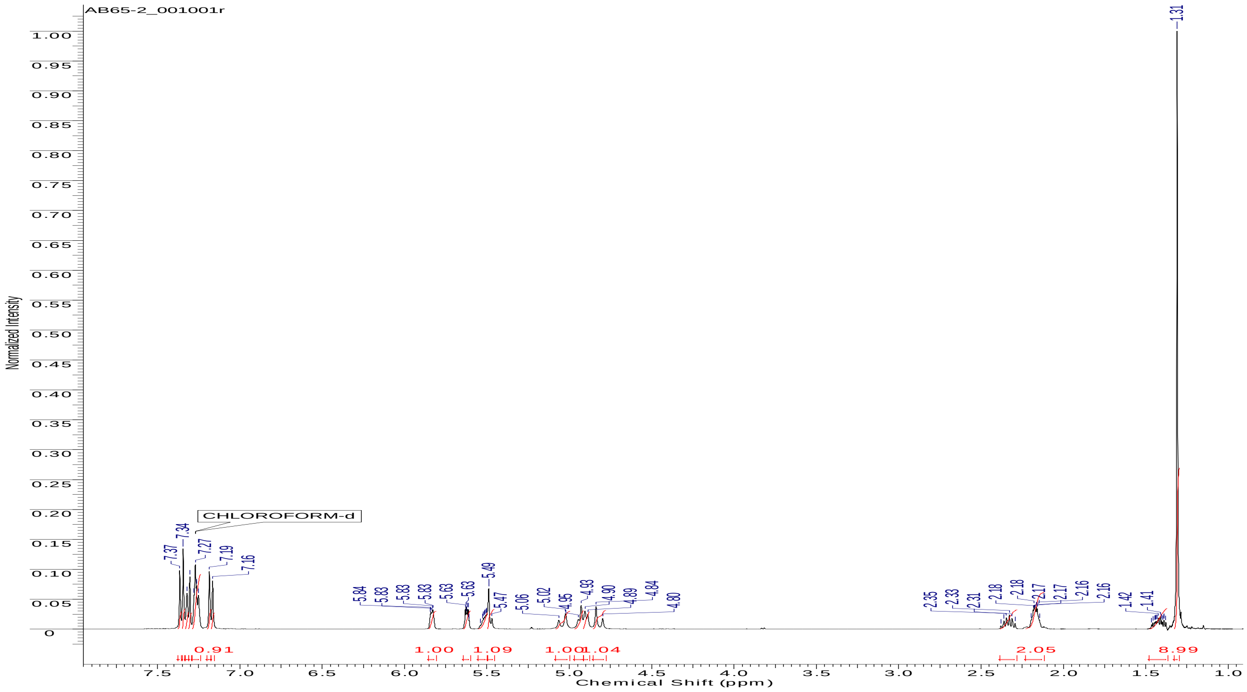
MS ESI ,m/z: 533 [M+H]$^+$.

1-Benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclopent-2-en-1-yl)thiourea (4a). Cyclopentene (1.3 ml, 18.1 mmol, 2 equiv.) dissolved in CCl_4 (10 ml) was treated at stirring with ***N*-bromosuccinimide** (NBS) (1.6 g, 9.0 mmol, 1 equiv.) and **azobisisobutyronitrile** (AIBN) (2 mg, 0.012 mmol, cat. equiv.). Reaction mass was refluxed for 1.5 h, then cooled to 0°C, after that was filtered and the precipitate was washed with CCl_4 (5 ml). The control of reaction was carried out by ^{1}H NMR. ^{1}H NMR (δ, $CDCl_3$): 2.37 – 2.42 (m, 3H), 2.60–2.68 (m, 1H), 5.17 (m, 1H, J =2.8 Hz, H^3), 6.00 – 6.04 (m, 1H, J = 2.1 Hz, H^2), 6.05 – 6.08 (m, 1H, J = 5.1, 2.2 Hz, H^1). A solution of **3-bromocyclopent-1-ene** in CCl_4 from the previous step was treated dropwise with the solution of **potassium thiocyanate** (0.88 g, 9.0 mmol, 1 equiv.) in absolute MeOH (5 ml) at ice cooling and was stirred for 2h at room temperature, after that was filtered through SiO_2, washed with hexane (20 ml). The solution of **3-isothiocyanatocyclopent-1-ene** was used directly for the next step without evaporation and further purification. The control of reaction was carried out by ^{1}H NMR analysis of the reaction solution. The presence of resonance signal at 4.66 – 4.72 ppm (m, 1H, H^3) instead of 5.17 ppm confirms the conversion of bromide into isothiocyanate.

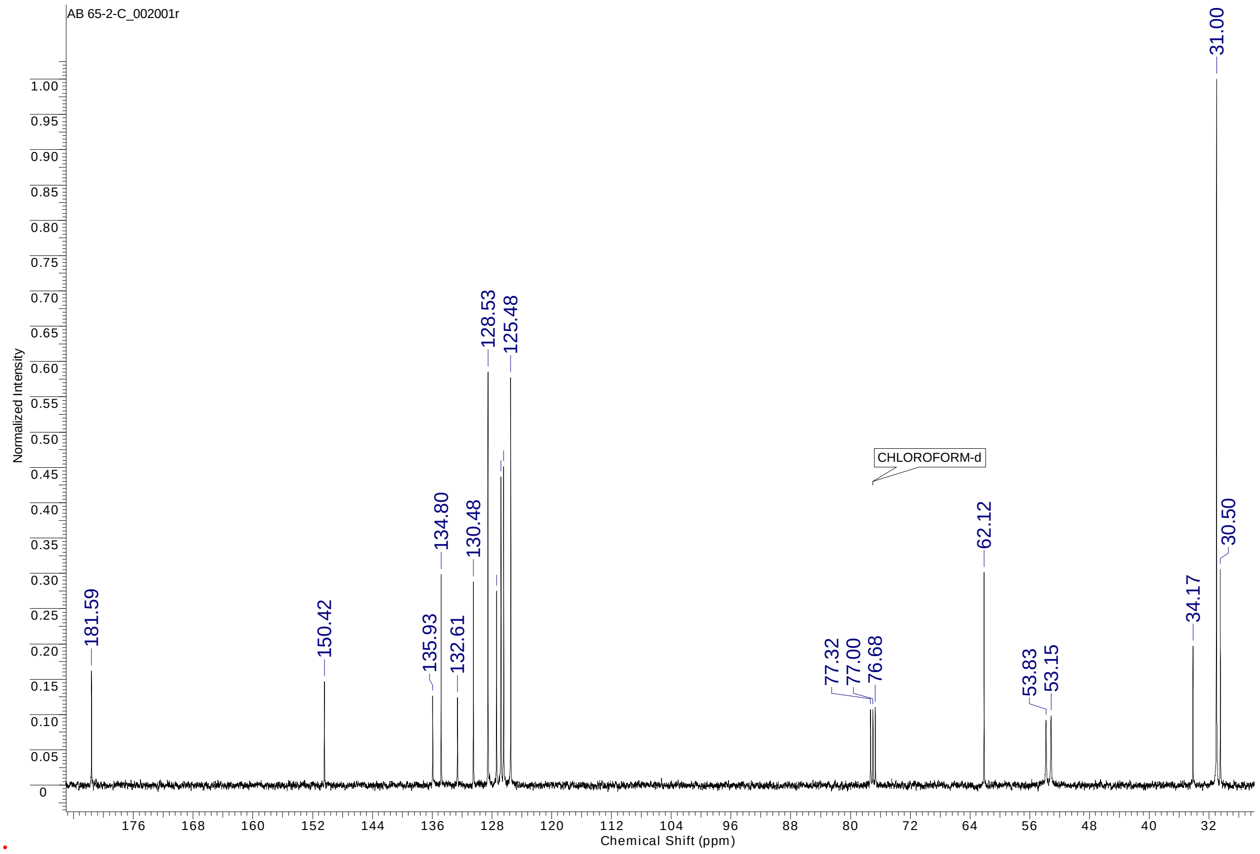


3-Isothiocyanatocyclopent-1-ene filtrate solution from the previous step was treated with DIPEA (3.1 ml, 18.1 mmol, 2equiv.) and ***N*-benzyl-*N*-(4-*tert*-butylbenzyl)amine** (2.3 g, 9.0 mmol, 1equiv). The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: ethyl acetate : hexane gradient v/v from 1:10 to 1:7) to give **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclopent-2-en-1-yl)thiourea (4a)** as a colorless oil (1.7 g, overall yield from 3 steps 50%).

^{1}H NMR (δ, CDCl$_3$): 1.31 (s, 9H, *t*-Bu), 1.38 – 1.46 (m, 1H), 2.13 – 2.21 (m, 2H), 2.29 – 2.38 (m, 1H), 4.82 (d, 1H, *J* = 16.2 Hz, N$\underline{\mathrm{CH_2}}$Ph), 4.91 (d, 1H, *J* = 16.1 Hz, N$\underline{\mathrm{CH_2}}$Ar), 4.93 (d, 1H, *J* = 16.1 Hz, N$\underline{\mathrm{CH_2}}$Ar), 5.04 (d, 1H, *J* = 16.2 Hz, N$\underline{\mathrm{CH_2}}$Ph), 5.48 – 5.55 (m, 2H, NH+H^3), 5.62 (m, 1H, *J* =5.6, 1.9 Hz, H^1), 5.83 (m, 1H, *J* =5.6, 1.4 Hz, H^2), 7.17 (d, *J* = 8.2 Hz, 2H, H^{Ar}), 7.23 – 7.32 (m, 5H, H^{Ar}), 7.35 (d, *J* = 8.2 Hz, 1H, H^{Ar}).

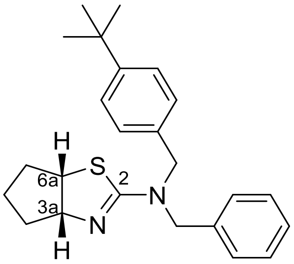


^{13}C NMR (δ, CDCl$_3$): 30.5, 31.0 (C($\underline{\mathrm{C}}$H$_3$)$_3$), 34.2 ($\underline{\mathrm{C}}$(CH$_3$)$_3$), 53.2 (N$\underline{\mathrm{CH_2}}$Ar), 53.8 (N$\underline{\mathrm{CH_2}}$Ph), 62.1 (C^1), 125.5 (C$^{3,5\text{-}Ar}$), 126.4, 126.8, 127.4 (C$^{4\text{-}Ph}$), 128.5, 130.5 (C^3), 132.6 (C^2), 134.8 (C$^{1\text{-}Ar}$), 135.9 (C$^{1\text{-}Ph}$), 150.4 (C$^{4\text{-}Ar}$), 181.6 (C^2).

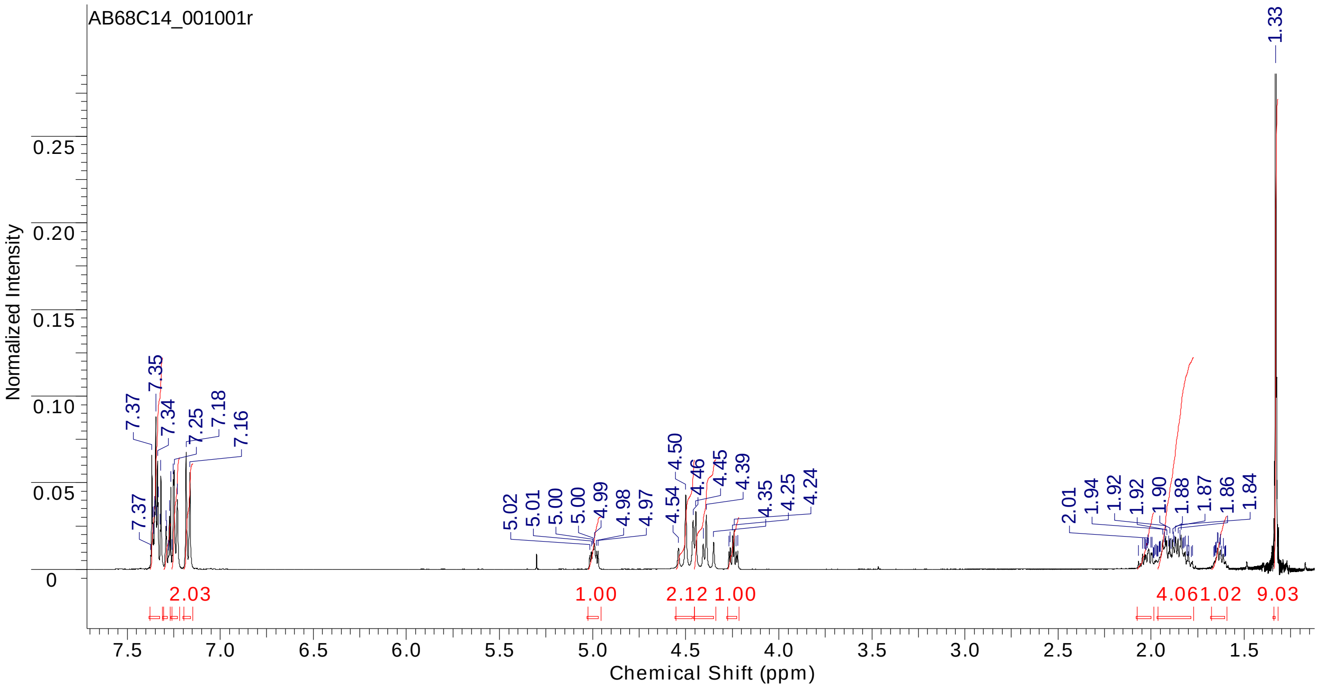


(±)-(3a*R*,6a*S*)-*N*-Benzyl-*N*-(4-*tert*-butylbenzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (7a).

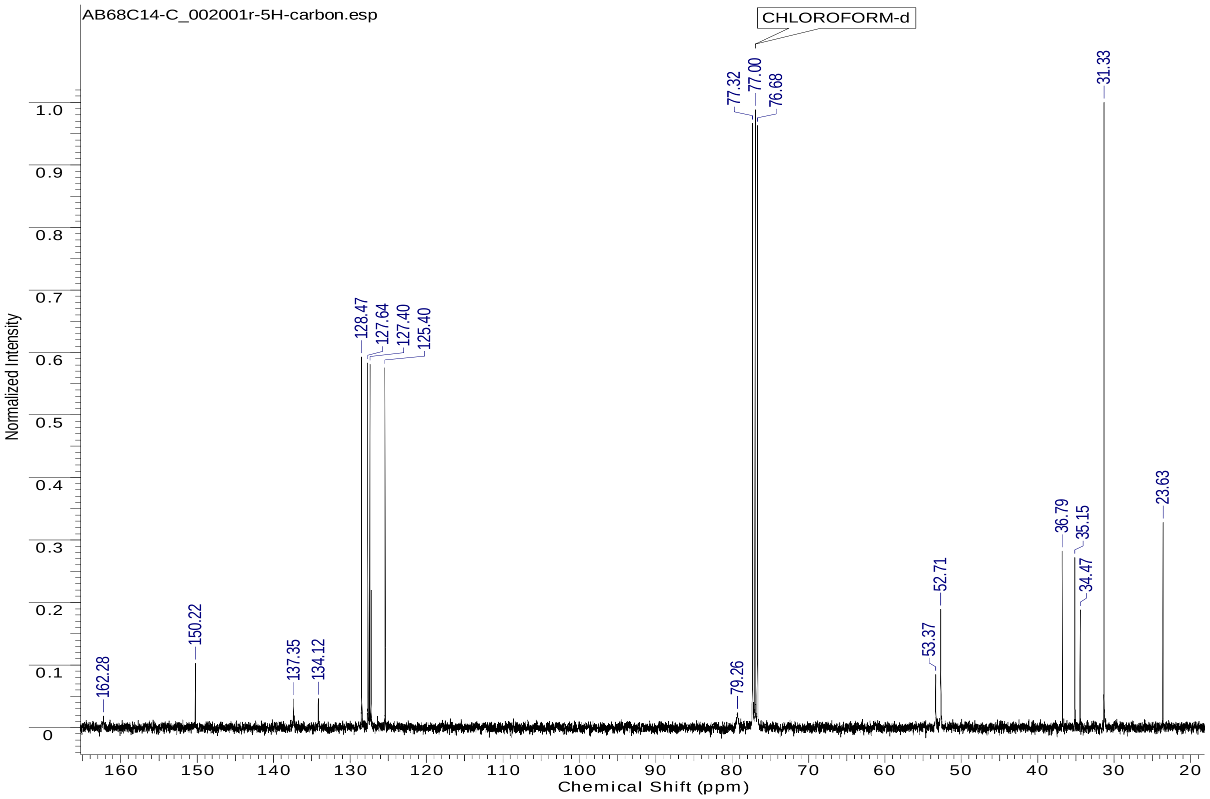
A solution of **(±)-(3aR,6R,6aR)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-6-iodo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (6a)** (131 mg, 0.21 mmol, 1 equiv.) in toluene (10 ml) was treated with tri-*n*-butyltin hydride (67 µl, 0.25 mmol, 1.2 equiv) and azobisisobutyronitrile (AIBN) (2 mg, 0.012 mmol, catalytic amount). The mixture was heated at 100 ^{0}C under argon atmosphere during 5 h, then concentrated and purified by column chromatography (eluent: 2% ethyl acetate and 2% Et$_3$N in hexane) to yield **(±)-(3a*R*,6a*S*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine** as colorless oil (63 mg, 64%).



^{1}H NMR (δ, CDCl$_3$): 1.33 (s, 9H, *t*-Bu), 1.59-1.67 (m, 1H), 1.77-1.97 (m, 4H), 1.99-2.07 (m, 1H), 4.24 (dt, 1H, J = 7.8, 3.3 Hz, H^{6a}), 4.37 (d, 1H, J = 15.8 Hz, N$\underline{\mathrm{CH_2}}$Ph), 4.43 (d, 1H, J = 16.0 Hz, N$\underline{\mathrm{CH_2}}$Ar), 4.48 (d, 1H, J = 16.0 Hz, N$\underline{\mathrm{CH_2}}$Ar), 4.52 (d, 1H, J = 15.8 Hz, N$\underline{\mathrm{CH_2}}$Ph), 5.00 (ddd, 1H, J = 7.8, 6.3, 3.3 Hz, H^{3a}), 7.17 (d, J = 8.3 Hz, 2H, H^{Ar}), 7.24 (m, J = 8.3 Hz, 2H, H^{Ar}), 7.28 (m, J = 7.2 Hz, 1H, H^{Ar}), 7.32 – 7.37 (m, 4H, H^{Ar}).

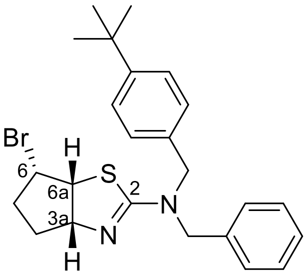


^{13}C NMR (δ, CDCl$_3$): 23.6 (C^5), 31.3 (C($\underline{C}$H$_3$)$_3$), 34.5 ($\underline{C}$(CH$_3$)$_3$), 35.1, 36.8, 52.7 (N$\underline{CH_2}$Ar + N$\underline{CH_2}$Ph), 53.4 (C^{6a}), 79.3 (C^{3a}), 125.4 (C$^{3,5\text{-}Ar}$), 127.2 (C$^{4\text{-}Ph}$), 127.4 (C), 127.6 (C), 128.5 (C), 134.1 (C$^{1\text{-}Ar}$), 137.3 (C$^{1\text{-}Ph}$), 150.2 (C$^{4\text{-}Ar}$), 162.3 (C^2).

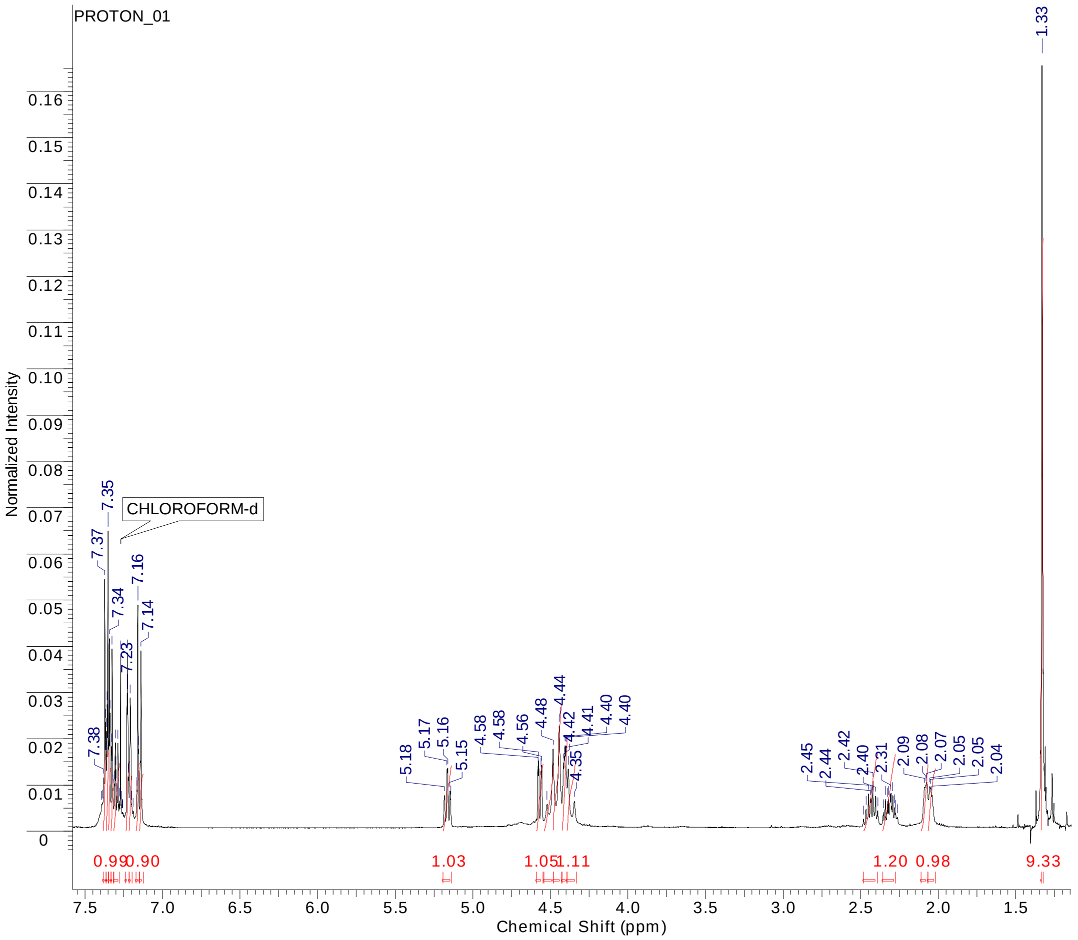


MS (ESI), m/z : 379 [M+H]$^+$.

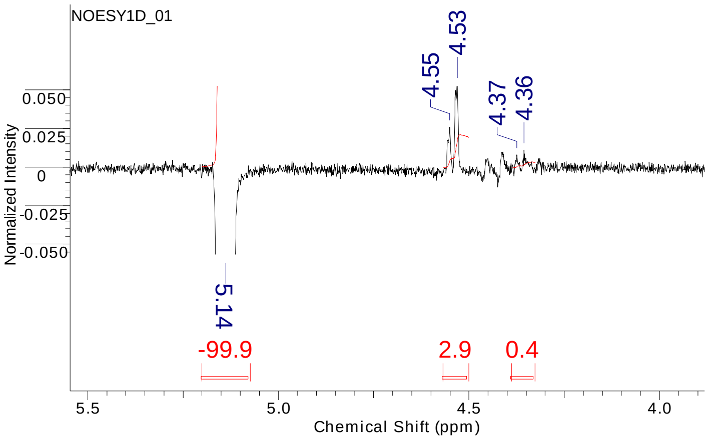
(±)-(3a*R*,6*S*,6a*R*)-*N*-Benzyl-6-bromo-*N*-(4-*tert*-butylbenzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta-[*d*]thiazol-2-amine (5'a). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclopent-2-en-1-yl)-thiourea** (128 mg, 0.34 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was treated with Br_2 (18 µl, 0.34 mmol, 1 equiv.) and stirred at room temperature for 12 h. Then washed with aqueous saturated solution of Na_2SO_3 (3x10ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4, concentrated and the residue purified by column chromatography (eluent: CH_2Cl_2) to yield **(±)-(3a*R*,6*S*,6a*R*)-*N*-benzyl-6-bromo-*N*-(4-*tert*-butylbenzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (5'a)** as slightly yellow oil (63 mg, 41%) and **(±)-(3a*R*,6*R*,6a*R*)-*N*-benzyl-6-bromo-*N*-(4-*tert*-butylbenzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (5a)** as slightly yellow oil (90 mg, 58%).



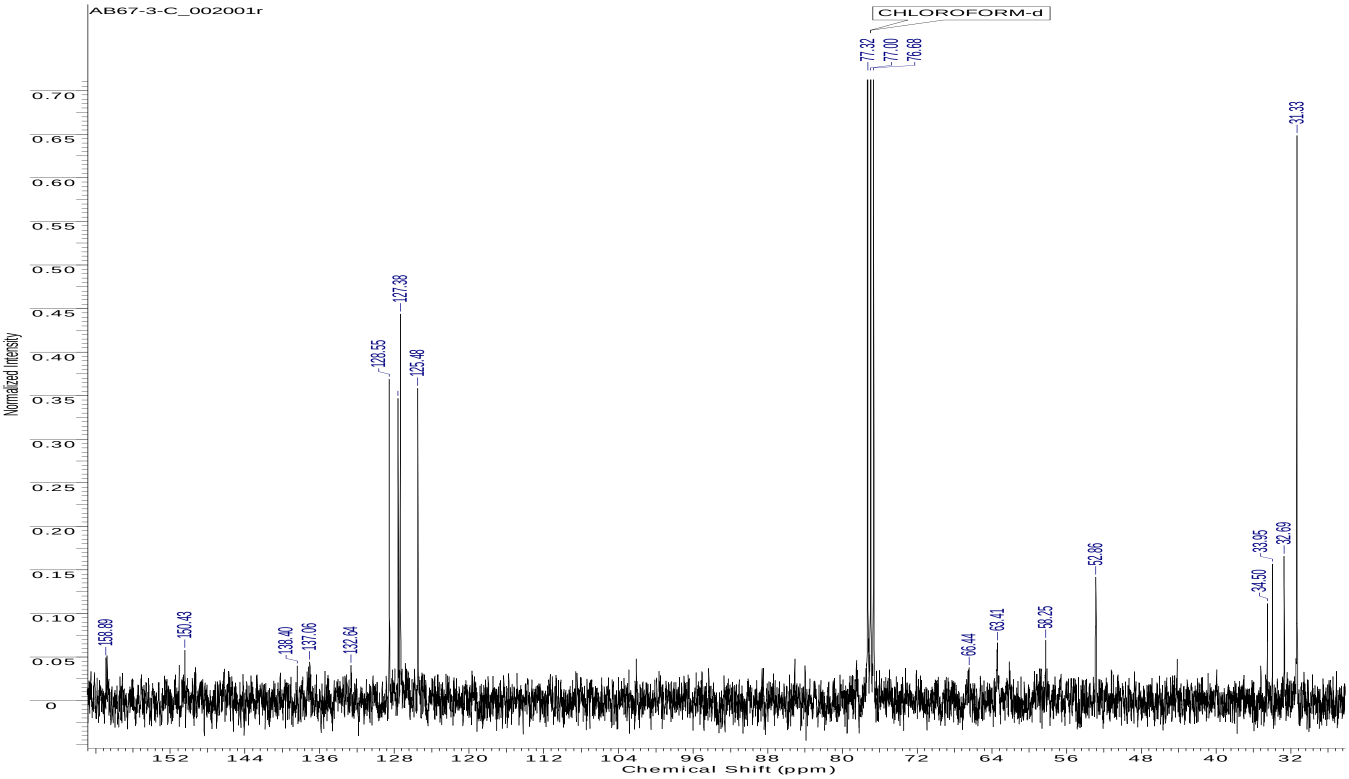
^{1}H NMR (δ, $CDCl_3$): ^{1}H NMR (δ, $CDCl_3$): 1.33 (s, 9H, *t*-Bu), 2.02-2.06 (m, 1H), 2.07-2.11 (m, 1H), 2.26-2.35 (m, 1H), 2.39-2.48 (m, 1H), 4.37 (d, 1H, J = 15.6 Hz, N$\underline{CH_2}$Ar), 4.41 (m, 1H, J = 4.0, 2.2, 1.8 Hz, H^{6}), 4.42 (d, 1H, J = 15.9 Hz, N$\underline{CH_2}$Ph), 4.46 (d, 1H, J = 15,9 Hz, N$\underline{CH_2}$Ph), 4.50 (d, 1H, J = 15.6 Hz, N$\underline{CH_2}$Ar), 4.57 (dd, 1H, J = 8.1, 1.8 Hz, H^{6a}), 5.16 (dt, 1H, J = 8.1, 1.5 Hz, H^{3a}), 7.15 (d, J = 8.4 Hz, 2H, H^{Ar}), 7.22 (m, J = 8.3 Hz, 2H, H^{Ar}), 7.29 (m, J = 7.0 Hz, 1H, H^{Ar}), 7.32 – 7.38 (m, 4H, H^{Ar}).



NOESY 1D: a) H^{3a} –H^{6a} (2.9 %), H^{3a} –H^{6} (0.4%)

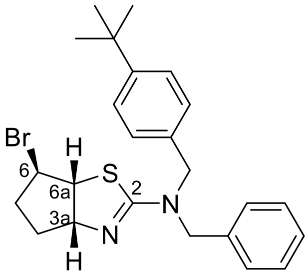


^{13}C NMR (δ, $CDCl_3$): 31.33 ($C(\underline{C}H_3)_3$) 32.69, 33.95, 34.50 ($\underline{C}(CH_3)_3$) 52.86 ($N\underline{CH_2}Ar$ + $N\underline{CH_2}Ph$), 58.25 (C^{6a}) 63.41 (C^{6}), 66.44(C^{3a}), 125.48 ($C^{3,5\text{-}Ar}$), 127.38, 127.60, 128.55 ($C^{3,5\text{-}Ph}$), 132.64, 137.20, 138.4, 150.43, 158.89 (C^{2}).



MS (ESI), m/z: 457 $[M+H]^+$ / 459 $[M+2+H]^+$.

(±)-(3a*R*,6*R*,6a*R*)-*N*-Benzyl-6-bromo-*N*-(4-(*tert*-butyl)benzyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (5a). ^{1}H NMR (δ, CDCl$_3$): 1.33 (s, 9H, *t*-Bu), 1.94-2.02 (m, 1H), 2.04-2.11 (m, 1H), 2.12-2.21 (m, 1H), 2.24-2.32 (m, 1H), 4.37 (ddd, 1H, *J* = 9.7, 6.2, 5.5 Hz, H^6), 4.49 (d, 1H, *J* = 16.0 Hz, N$\underline{\mathrm{CH}_2}$Ph), 4.44 (d, 1H, *J* = 16.0 Hz, N$\underline{\mathrm{CH}_2}$Ph), 4.55 (d, 1H, *J* = 14,7 Hz, N$\underline{\mathrm{CH}_2}$Ar), 4.58 (d, 1H, *J* = 14.7 Hz, N$\underline{\mathrm{CH}_2}$Ar), 4.63 (dd, 1H, *J* = 7.8, 6.2 Hz, H^{6a}), 4.96 (dt, 1H, *J* = 7.8, 2.6 Hz, H^{3a}), 7.18 (d, *J* = 8.4 Hz, 2H, H^{Ar}), 7.25 (m, *J* = 7.5 Hz, 2H, H^{Ar}), 7.29 (m, *J* = 7.2 Hz, 1H, H^{Ar}), 7.31 – 7.37 (m, 4H, H^{Ar}).



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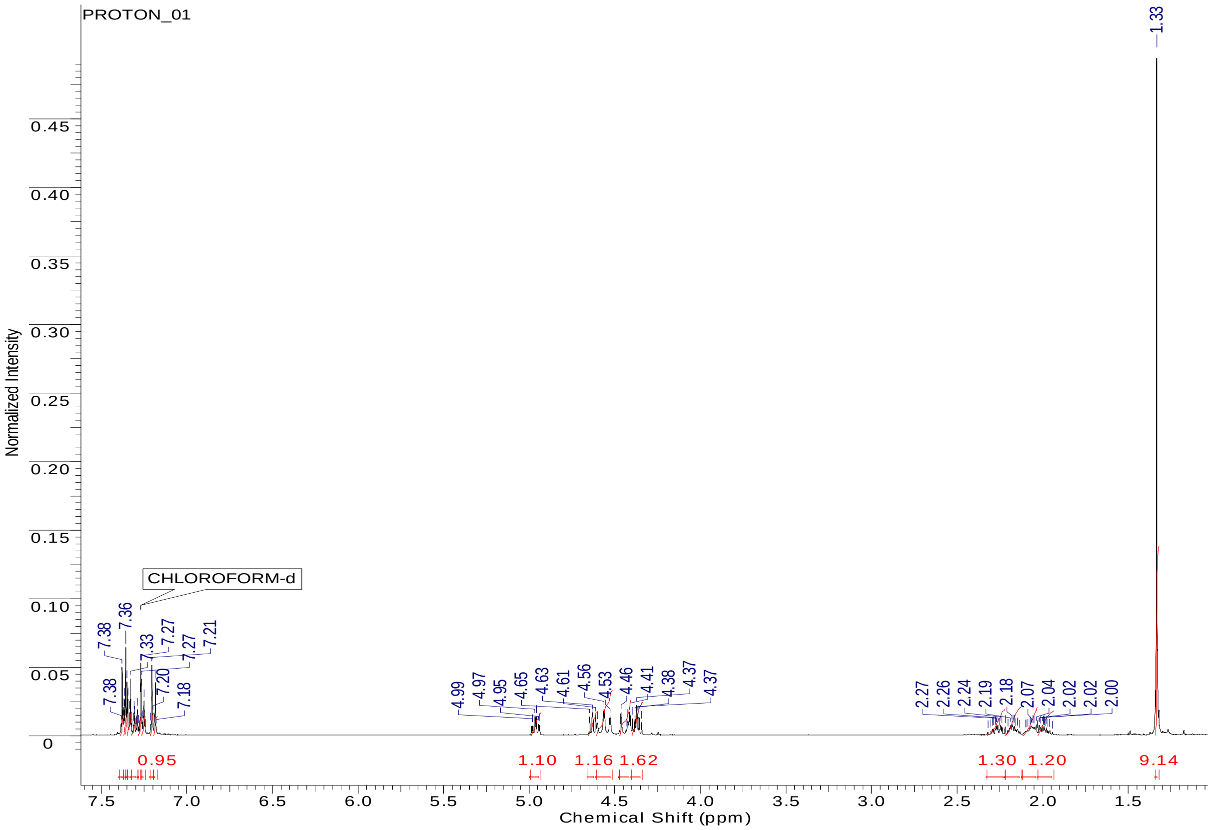
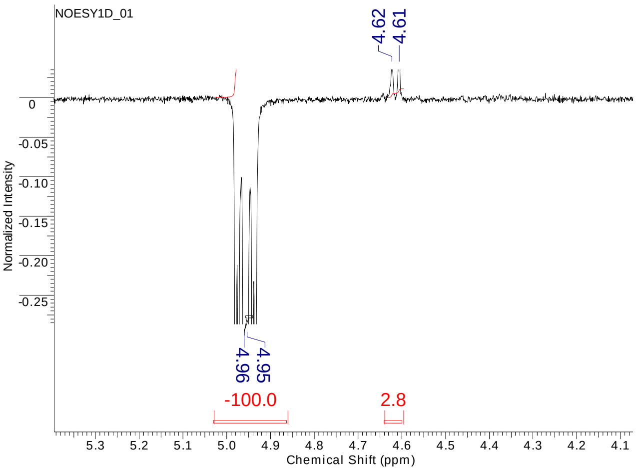
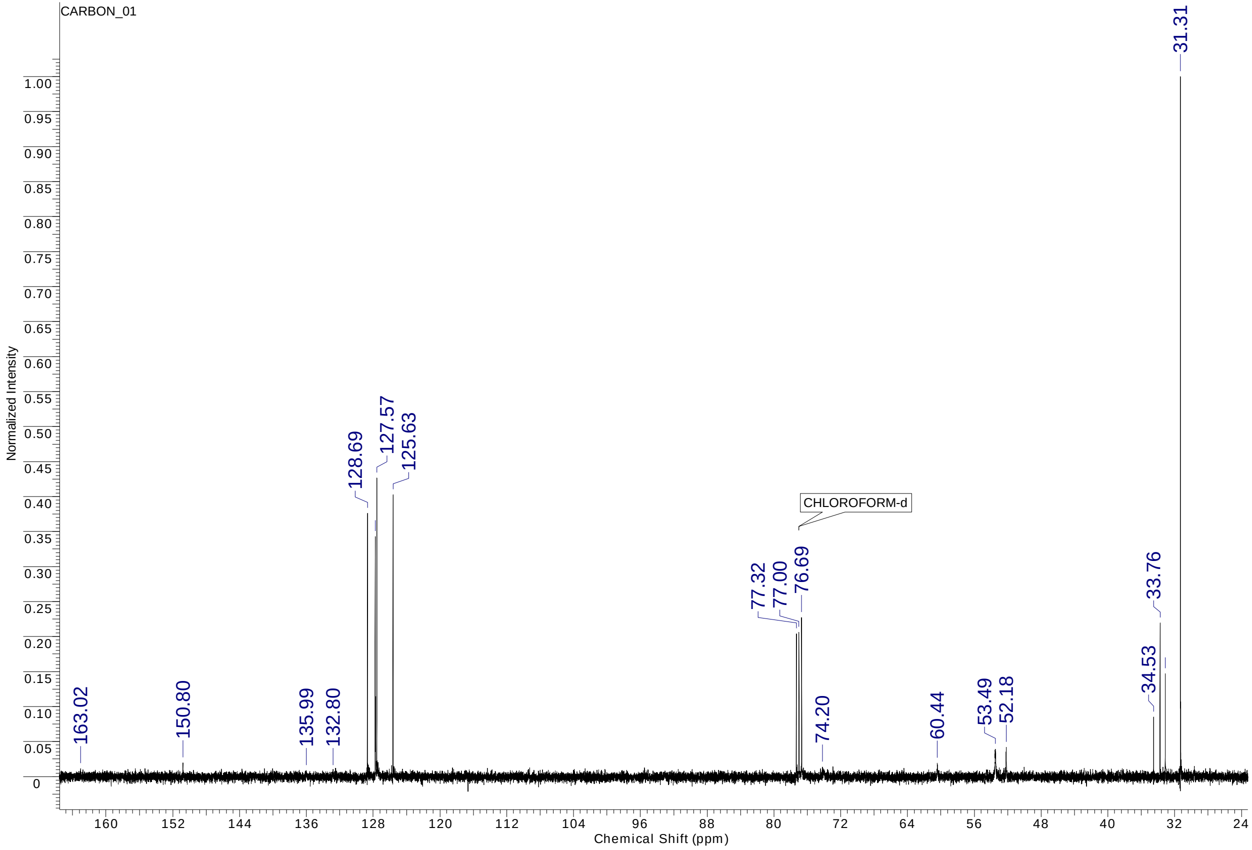


NOESY 1D: a) H^{3a} –H^{6a} (2.8 %)

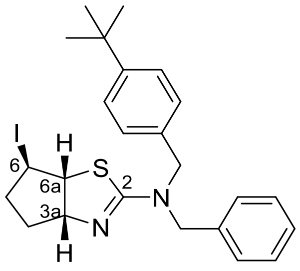


^{13}C NMR (δ, $CDCl_3$): 31.3 (C($\underline{C}H_3$)$_3$), 33.1, 33.8, 34.5 ($\underline{C}(CH_3)_3$), 52.2 (C^{6a}), 53.5 (N$\underline{CH_2}$Ar + N$\underline{CH_2}$Ph), 60.4 (C^{6}), 74.2 (C^{3a}), 125.6 (C$^{3,5\text{-}Ar}$), 127.6, 127.7(C$^{4\text{-}Ph}$), 127.8, 128.7, 132.8 (C$^{1\text{-}Ar}$), 136.0 (C$^{1\text{-}Ph}$), 150.8 (C$^{4\text{-}Ar}$), 163.0 (C^{2}).

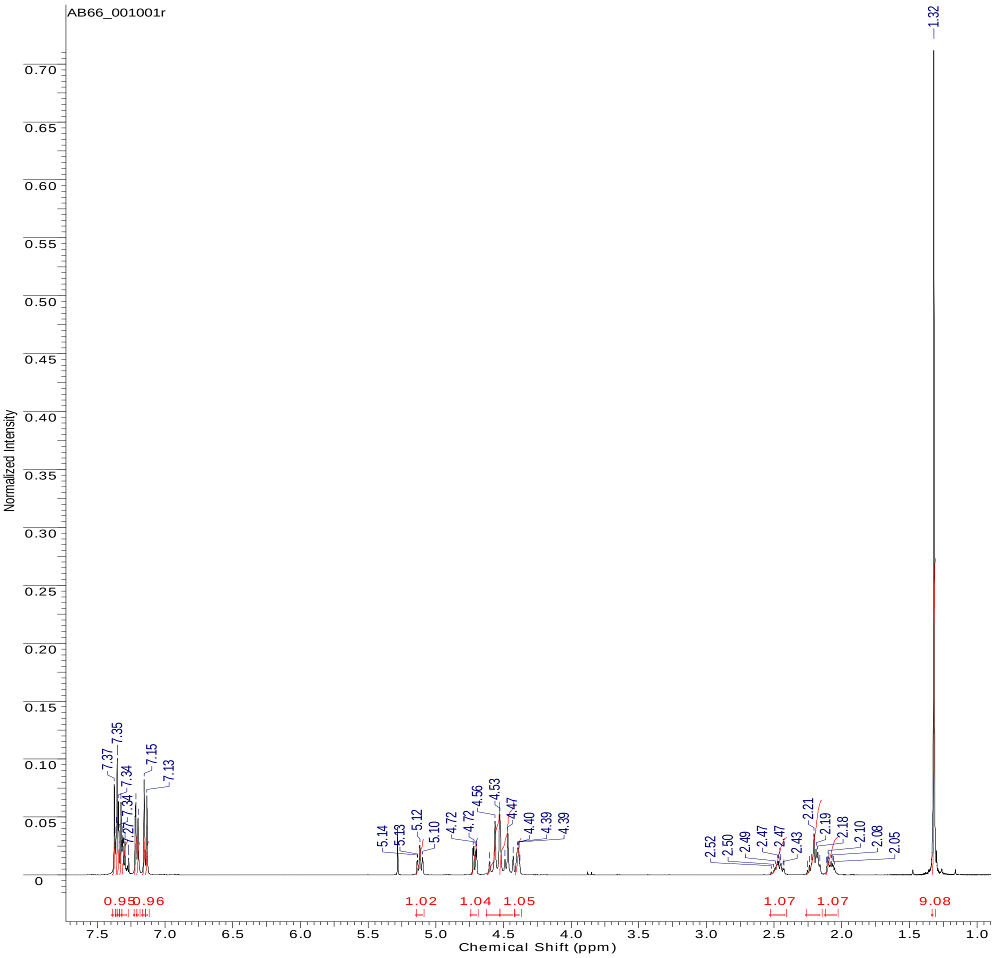


MS (ESI), m/z: 457 $[M+H]^+$ / 459 $[M+2+H]^+$.

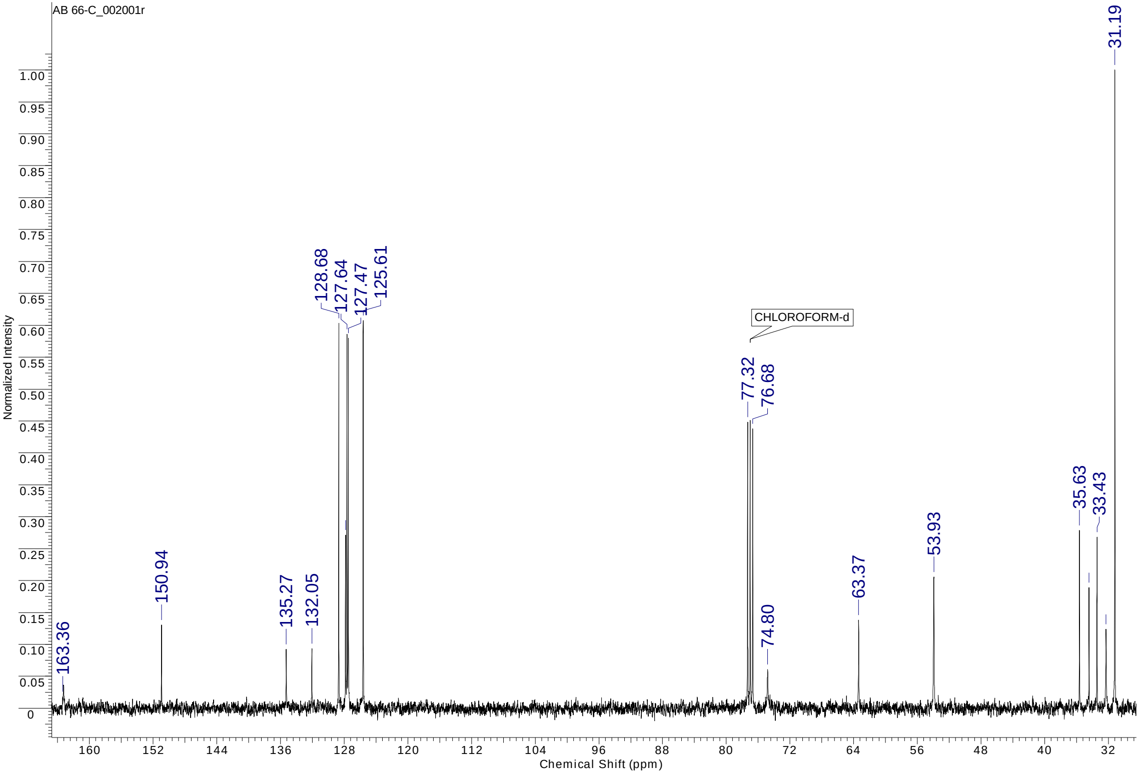
(±)-(3a*R*,6*R*,6a*R*)-*N*-Benzyl-*N*-(4-*tert*-butylbenzyl)-6-iodo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta-[*d*]thiazol-2-amine (6a). A solution of **1-benzyl-1-(4-*tert*-butylbenzyl)-3-(cyclopent-2-en-1-yl)thiourea (4a)** (130 mg, 0.34 mmol, 1 equiv.) in CH_2Cl_2 (10 ml) was treated with I_2 (87 mg, 0.34 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 12 h, then washed with aqueous saturated solution of Na_2SO_3 (3x10ml) and $NaHCO_3$ (3x10ml). The organic layer was dried over Na_2SO_4, concentrated to yield **(±)-(3a*R*,6*R*,6a*R*)-*N*-benzyl-*N*-(4-*tert*-butylbenzyl)-6-iodo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]thiazol-2-amine (6a)** as colorless oil (171 mg, 99%).



^{1}H NMR (δ, $CDCl_3$): 1.33 (s, 9H, *t*-Bu), 2.04-2.12 (m, 1H), 2.15-2.25 (m, 2H), 2.41-2.52 (m, 1H), 4.39 (m, 1H, *J* = 5.8, 2.6 Hz, H^{6a}), 4.45 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ph), 4.51 (d, 1H, *J* = 14.8 Hz, N$\underline{CH_2}$Ar), 4.55 (d, 1H, *J* = 14,8 Hz, N$\underline{CH_2}$Ar), 4.58 (d, 1H, *J* = 15.8 Hz, N$\underline{CH_2}$Ph), 4.71 (dd, 1H, *J* = 8.1, 2.6 Hz, H^{6}), 5.12 (dt, 1H, *J* = 8.1, 1.6 Hz, H^{3a}), 7.14 (d, 2H, *J* = 8.3 Hz, H^{Ar}), 7.21 (d, 2H, *J* = 8.1 Hz, H^{Ar}), 7.28-7.37 (m, 5H, H^{Ar}).



^{13}C NMR (δ, CDCl$_3$): 31.2 (C($\underline{C}$H$_3$)$_3$), 32.3, 33.4, 34.4 ($\underline{C}$(CH$_3$)$_3$), 35.6, 53.9 (N$\underline{CH_2}$Ar + N$\underline{CH_2}$Ph), 63.4 (C^{6a}), 74.8 (C^{3a}), 125.6 (C$^{3,5\text{-Ar}}$), 127.5, 127.6, 127.8, 128.7 (C$^{3,5\text{-Ph}}$), 132.1(C$^{1\text{-Ar}}$), 135.3 (C$^{1\text{-Ph}}$), 150.9 (C$^{4\text{-Ar}}$), 163.4 (C^{2})

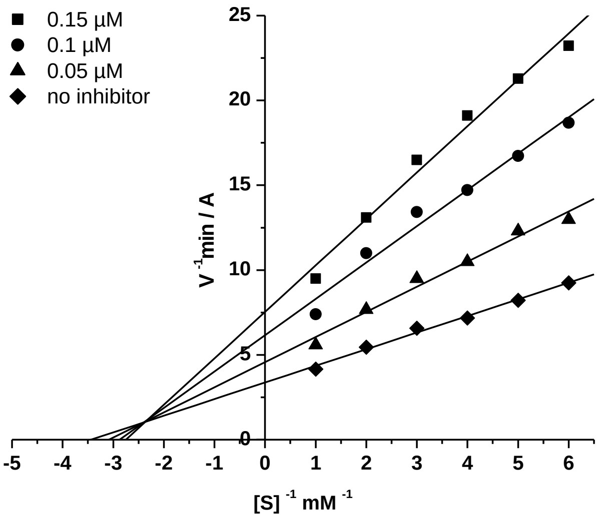


MS (ESI), m/z: 505 [M+H]$^+$.

3. Biological Assays

***In vitro* AChE, BChE Inhibition**. All experiments were carried out in accordance with the standard protocols approved by IPAC RAS. The following items were purchased from Sigma-Aldrich (St. Louis, MO, USA): human erythrocyte AChE, equine serum BChE, acetylthiocholine iodide (ATCh), butyrylthiocholine iodide (BTCh), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), tacrine. We measured the activity of AChE and BChE according to the colorimetric Ellman procedure (λ = 412 nm), as described in detail in [**S4**]. Measurements were carried out in 0.1M K/Na phosphate buffer at pH 7.5, 25 °C. Final concentrations of reactants were: 0.33 mM DTNB, 0.02 unit ml^{-1} of AChE or BChE and 1 mM of substrate (ATCh or BTCh, respectively). Freshly prepared solutions of the enzymes were used, which retained a constant activity during the experiment (2–2.5 h). Chromophore absorbances were measured with a FLUOStar Optima microplate reader (BMG Labtech, Ortenberg, Germany). DMSO (2% v/v) was employed as the solvent; the concentration used did not alter the activities of the enzymes. For inhibition assay, initially, we used a single concentration of 20 μM for all compounds. The test compounds were preincubated with the enzymes at 25 °C for 5 min, followed by the addition of the substrates. Subsequently, IC_{50} values (μM) were determined for the most active compounds against AChE, BChE.

Kinetic Study of AChE and BChE Inhibition. Determination of Steady-State Inhibition Constants. We assessed the mechanisms of AChE and BChE inhibition by performing a thorough analysis of enzyme kinetics. After a 5 min incubation at 25°C (for temperature equilibration) with three increasing concentrations of inhibitor and six decreasing substrate concentrations, the residual enzyme activity was measured as described above for enzymatic assays. Linear regression of 1/V versus 1/[S] double-reciprocal (Lineweaver–Burk) plots was used to determine the inhibition constants for the competitive component (*K*i) and noncompetitive component (α*K*i).



Steady state inhibition of BChE by compound **7c**. Lineweaver–Burk reciprocal plots of initial velocity and substrate concentrations in the presence of inhibitor (three concs.) and its absence are presented.

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