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Synthesis of enantiopure 12 and PZM21

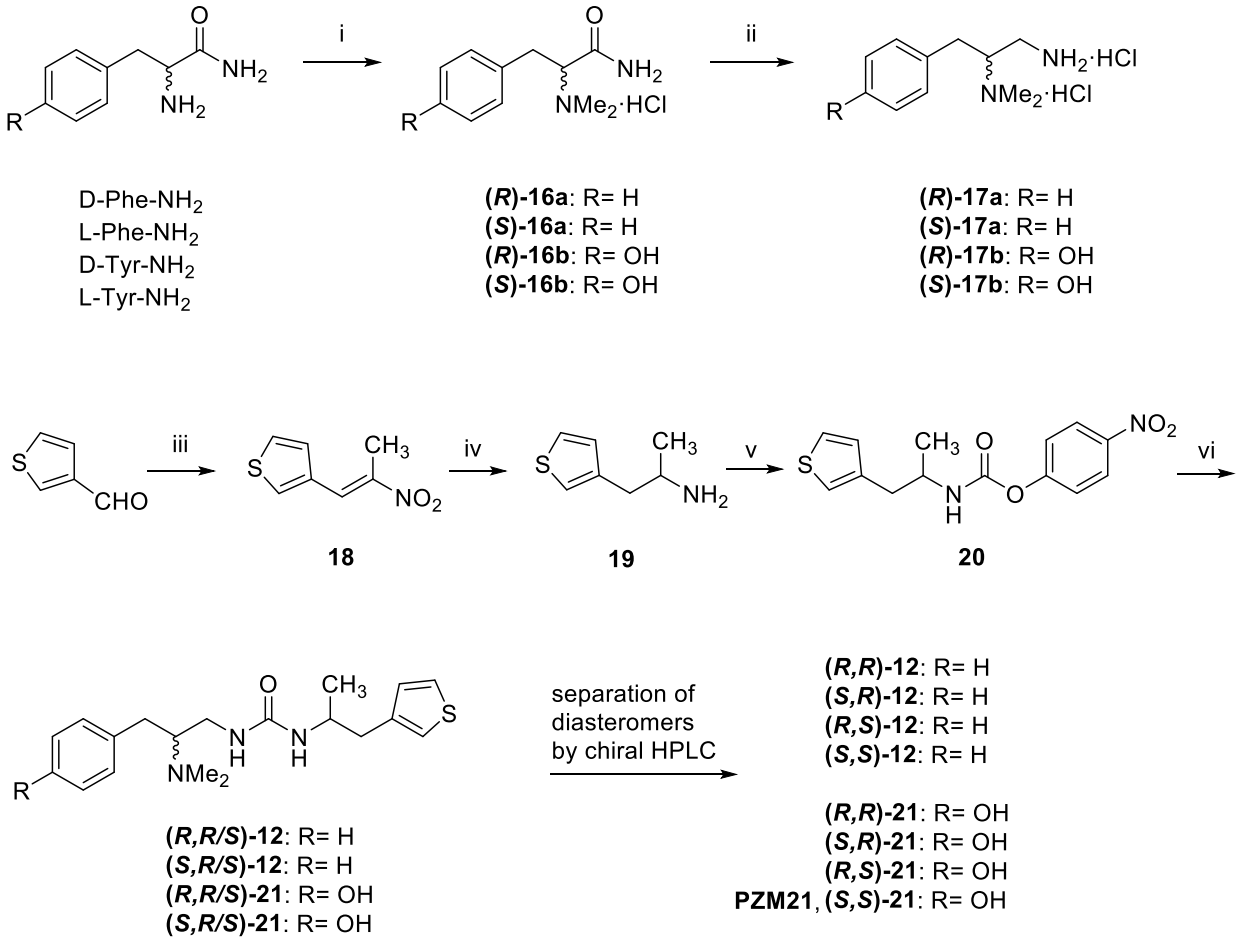
The stereochemically pure isomers of **12** and PZM21 were synthesized from corresponding (*R*)- and (*S*)-amino acid amides, which were either commercially available or readily prepared from the corresponding acid or ester. The primary amino group was dimethylated using an excess of aqueous formaldehyde and sodium triacetoxyborohydride in aqueous acetonitrile. The carboxamides **16a,b** were converted to primary amines by treatment with borane-tetrahydrofurane complex under reflux yielding the diamines **17a,b**. *Henry* reaction of thiophene-3-carbaldehyde with nitroethane afforded the nitropropene derivative **18**, which was converted into the racemic alkylamine **19**. Activation with 4-nitrophenyl chloroformate yielded the carbamates **20**, which were coupled with the enantiopure primary amines **17a,b** to achieve diastereomeric mixtures of the corresponding ureas **12** and **21**. HPLC separation using a semi-preparative Chiralpak AS-H column gave the overall eight pure stereoisomers of **12** and **21** including PZM21.

To determine the absolute configuration of the final products and efficiently prepare PZM21, we synthesized enantiomerically enriched carbamate **20**, coupled it with the corresponding primary amines. For enantiomeric enrichment, we performed chiral resolution of the racemic primary amine **19** via repetitive crystallization with di-p-anisoyl-(S)-tartaric acid. After triple crystallization, we obtained **19** enriched in dextrorotatory enantiomer ($[\alpha]_D25$ = +20.5 °). The corresponding (*R*)-acetamide has been previously characterized as dextrorotatory ($[\alpha]_D20$ = +49.8°), so enantiomerically enriched **19** was treated with acetic anhydride and triethylamine, and the specific rotation of the product was measured. Based on the value of specific rotation of the resulting acetamide ($[\alpha]_D21$ = −46.6 °), we assigned the absolute configuration of the major isomer to be (*S*). (*S*)-enriched **20** was used for synthesis of the final urea derivatives and absolute configuration of diastereomers in pairs was assigned based on the equality of retention time in chiral HPLC.

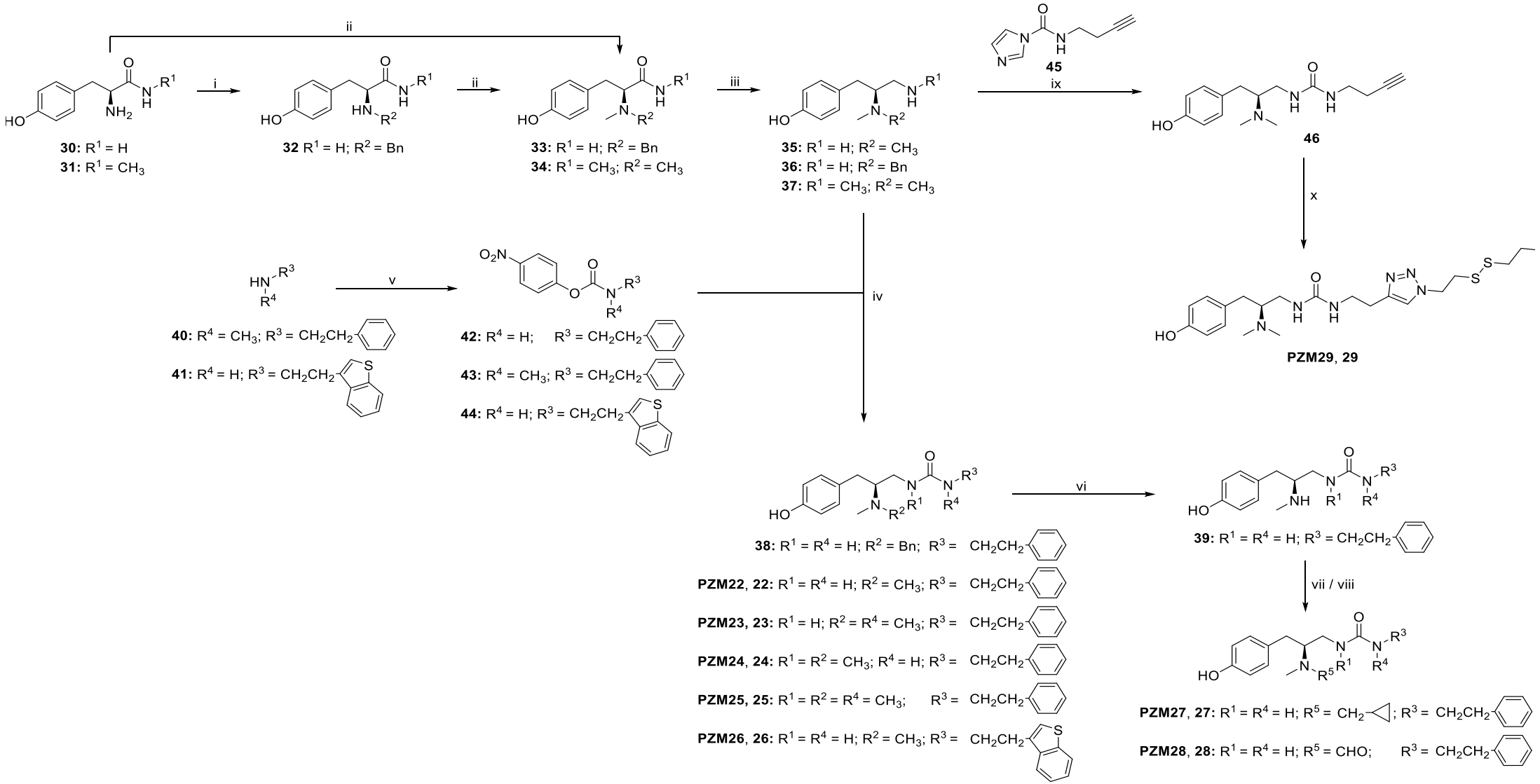
Synthesis of structural analogs PZM22-29:

Structural analogs of PZM21 were synthesized starting from stereochemically pure L-tyrosine amide (**30**) or L-tyrosine methylamide (**31**) (see scheme 2). *N*-methylation or *N*-benzylation and subsequent *N*-methylation followed by amide reduction led to the respective diamines **35-37**. Coupling with the nitrophenyl carbamates **42-44** resulted in the phenethyl ureas **22-25** (PZM22-25) and compound **38**, and the benzothiophene analog **26** (PZM26). The *N*-benzyl protected derivative **38** was converted into the respective secondary amine **39** by catalytic hydrogenation. This intermediate was used to introduce additional substituents leading to the *N*-cyclopropylmethyl derivative **27** (PZM27) and the *N*-formyl derivative **28** (PZM28).

The disulfide functionalized PZM21 analog PZM29 could be prepared starting from the dimethylamine **35**. Coupling with the *N*-butynylimidazole-1-carboxamide **45** led to the alkynyl functionalized urea **46**. Copper catalyzed cycloaddition with bis(2-azidoethyl) disulfide resulted in the desired triazole derivative **29** (PZM29).



Scheme 1. Syntheses of stereochemically pure **12** and **21**. Reactants and conditions: i) CH_2O, $NaBH(OAc)_3$, acetonitrile/water, rt, 15−30 min, 77−98%; ii) 1M BH_3·THF, reflux, 20 h, 48−95%; iii) nitroethane, HCOOH/ethanolamine, 90 °C, 7 h, 82%; iv) 1M $LiAlH_4$, reflux, 30 min, 56%; v) 4-nitrophenyl chloroformate, triethylamine, THF, 0 °C to rt, 6 h, 75%; vi) corresponding primary amine, DMF, rt, 20 h, 70−98%.



Scheme 2: Syntheses of target compounds **22-29** (PZM22-29): i) benzaldehyde, $NaBH(OAc)_3$, acetonitrile/ water, rt, 2 h, 75 %; ii) CH_2O, $NaBH(OAc)_3$, acetonitrile/water, rt, 12 min, 88–90 %; iii) 1M BH_3 in THF, reflux, 20 h, 11–95 %; iv) TEA, DMF, rt (to 120 °C), 3–22 h, 9–93 %; v) 4-nitrophenyl chloroformate, Et_3N, THF, 0 °C to rt, 8 h, 49–85 %; vi) H_2, Pd/C, 1,1,2-trichloroethane, MeOH, rt, 5 h, 87 %; vii) cyclopropylcarbaldehyde, $NaBH(OAc)_3$, acetonitrile/ water, rt, 12 h, 86 %; viii) ammonium formate, acetonitrile, reflux, 24 h, 26 %; ix) DMF, 50 °C, 24 h, 80 %; x) bis(2-azidoethyl) disulfide, $CuSO_4$ x $5H_2O$, sodium ascorbate, DMF/water, rt, 6 h, 44 %.

Experimental part:

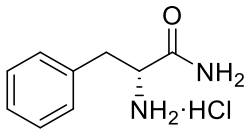
All chemicals and solvents were purchased from Sigma Aldrich, Acros, or Alfa Aesar and were used without additional purification. Anhydrous solvents were of the highest commercially available grade and were stored over molecular sieves under a nitrogen atmosphere.

Flash chromatography was performed on Merck silica gel 60 (40-63 μm) as stationary phase under positive pressure of dry nitrogen gas. Dry column vacuum chromatography was performed on Alfa Aesar TLC high purity grade silica gel without binder (12 μm) as a stationary phase and Florisil® (100-200 mesh) or celite for sample preparation. Elution was performed under negative pressure from water aspirator. Preparative chiral HPLC was performed on Agilent 1100 HPLC system with UV detection (λ = 254 nm and λ = 210 nm) using Chiralpak® AS-H semi-preparative column (250 × 10 mm, 5 μm) with eluent specified for each particular compound. Purification by preparative RP-HPLC was performed on Agilent 1100 preparative series, column: Zorbax Eclipse XDB – C8 PrepHT (21.2 x 150mm, 5μm [C8]), flow rate: 10 mL/min, employing solvent system as specified below.

HR-ESIMS analyses were conducted on a Bruker Daltonik microTOF II or a Bruker maXis MS in the laboratory of the Chair of Bioinorganic Chemistry, Friedrich Alexander Universität.

The purity of all test compounds and key intermediates was determined by reversed phase HPLC or HPLC-MS. HPLC-MS purity analyses were performed with an Agilent binary gradient system using UV detection (λ = 254 nm) in combination with ChemStation software. The Zorbax Eclipse XDB-C8 (4.6mm × 150 mm, 5 μm) column was used with a flow rate of 0.5 mL/min in reversed phase mode (eluent: MeOH/H_2O + 0.1% HCOOH, 10% to 100% in 21 min, 100% 3 min). Mass detection was conducted with a Bruker Esquire 2000 ion-trap mass spectrometer using APCI or ESI ionization source or with Bruker amaZon SL mass spectrometer in combination with a Agilent 1100 or Dionex Ultimate 3000 UHPLC system; respectively. HPLC purity analyses were performed on analytical systems (Agilent 1100 analytical series, VWD detector, Zorbax Eclipse XDB-C8 analytical column (4.6mm × 150 mm, 5 μm), flow rate: 0.5 mL/min). System A: MeOH in H_2O + 0.1% HCO_2H (0 – 3 min 10 %, 3 – 18 min 10 – 100%, 18 – 24 min 100%), System B: acetonitrile in H_2O + 0.1% HCO_2H (0 – 3 min 5 %, 3 – 18 min 5 – 95%, 18 – 24 min 95%), System C: MeOH in H_2O + 0.1% CF_3COOH (0 – 3 min 10 %, 3 – 18 min 10 – 100%, 18 – 24 min 100%), System D: acetonitrile in H_2O + 0.1% CF_3COOH (0 – 3 min 5 %, 3 – 18 min 5 – 95%, 18 – 24 min 95%). ^1H, and ^{13}C spectra were recorded on a Bruker Avance 360 or a Bruker Avance 600 FT-NMR-Spectrometer. Chemical shifts were calculated as ppm relative to TMS (^1H) or solvent signal (^{13}C) as internal standards.

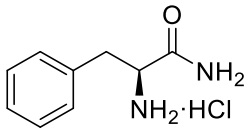
(*R*)-2-Amino-3-phenylpropanamide hydrochloride D-Phe-NH_2:



Dry ammonia gas was bubbled through the ice-cold solution of D-phenylalanine methyl ester hydrochloride (1.01 g, 4.7 mmol) in anhydrous methanol (12 mL) for 25 min. Then the flask was sealed and the mixture was allowed to warm up to the ambient temperature and stirred for 20 h (progress of the conversion was monitored by TLC). After the consumption of the starting material, the reaction mixture was purged with dry nitrogen gas (10 min) and the solvent was removed under reduced pressure. The white solid residue was suspended in boiling ethanol (30 mL), filtered and the precipitate was washed with ethanol (5 mL). Combined filtrate and washing were concentrated under reduced pressure. The white solid residue was dried under high vacuum. Yield: 0.89 g (95%). LCMS (ESI): t_R = 9.0 min, purity: 99%; m/z: no molecular ion. ^1H NMR (360 MHz, DMSO-d_6) δ 8.27 (br. s., 3 H), 7.99 (s, 1 H), 7.49 (s, 1 H), 7.18 − 7.41 (m, 5 H), 3.97 (t, *J*=6.8 Hz, 1 H), 3.12 (dd, *J*=14.0, 6.5 Hz, 1 H), 3.05 (dd, *J*=14.0, 7.0 Hz, 1 H). ^1H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.21 − 7.40 (m, 5 H), 3.97 (t, *J*=6.8 Hz, 1 H), 3.11 (dd, *J*=14.1, 6.3 Hz, 1

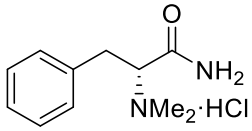
H), 3.03 (dd, *J*=14.1, 7.2 Hz, 1 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 169.6, 135.2, 129.5 (2 C), 128.4 (2 C), 127.0, 53.4, 36.6. HR-EIMS: found 164.0950; calcd. 164.0950 for $C_9H_{12}N_2O$ ([M–HCl]$^+$). $[\alpha]_D^{24}$ = −17.4 ° (c 5, H_2O).

(*S*)-2-Amino-3-phenylpropanamide hydrochloride D-Phe-NH$_2$:



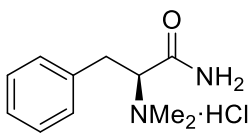
(*S*)-2-Amino-3-phenylpropanamide hydrochloride was synthesized following the synthetic protocol for (*R*)-2-amino-3-phenylpropanamide hydrochloride. From L-phenylalanine methyl ester hydrochloride (1.02 g, 4.7 mmol) the desired product was obtained as a white solid (0.89 g, 94%). LCMS (ESI): t_R = 9.0 min, purity: 99%; m/z: no molecular ion. ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.24 (br. s., 3 H), 8.00 (br. s., 1 H), 7.48 (br. s., 1 H), 7.18 − 7.37 (m, 5 H), 3.97 (t, *J*=6.7 Hz, 1 H), 3.11 (dd, *J*=14.0, 6.5 Hz, 1 H), 3.05 (dd, *J*=13.9, 7.1 Hz, 1 H). ^{1}H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.21 − 7.40 (m, 5 H), 3.96 (dd, *J*=7.3, 6.4 Hz, 1 H), 3.10 (dd, *J*=14.0, 6.4 Hz, 1 H), 3.01 (dd, *J*=14.0, 7.3 Hz, 1 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 169.7, 135.2, 129.5 (2 C), 128.4 (2 C), 127.0, 53.4, 36.7. $[\alpha]_D^{22}$ = +17.5 ° (c 0.9, H_2O).

(*R*)-2-(Dimethylamino)-3-phenylpropanamide (*R*)-16a:



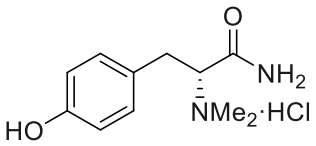
37% Aqueous formaldehyde (2 mL, 27 mmol) was added to a suspension of (*R*)-2-amino-3-phenylpropanamide hydrochloride (0.39 g, 1.94 mmol) in acetonitrile (10 mL), followed by addition of sodium triacetoxyborohydride (1.65 g, 7.8 mmol). After 30 min of vigorous stirring the reaction was quenched with 1N NaOH, basified to pH > 10, and extracted with ethyl acetate (3 × 20 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude free base was dissolved in isopropanol (1.5 mL) and hydrochloride salt was precipitated via addition of 2N HCl in diethyl ether (1.5 mL, 3 mmol) and dilution with diethyl ether (10 mL). The slurry was filtered and the white solid was washed with diethyl ether (2 × 5 mL). The product was obtained as a white solid (0.34 g, 77%) after drying under high vacuum. LCMS (ESI): t_R = 10.7 min; m/z found: 193.4; calcd. 193.2 ([M–Cl]$^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.13 (br. s., 1 H), 7.92 (s, 1 H), 7.61 (s, 1 H), 7.19 − 7.38 (m, 5 H), 4.05 (dd, *J*=10.9, 4.0 Hz, 1 H), 3.31 (dd, *J*=12.9, 3.9 Hz, 1 H), 3.07 (dd, *J*=12.9, 11.0 Hz, 1 H), 2.82 (s, 6 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 167.0, 135.1, 129.2 (2 C), 128.4 (2 C), 127.0, 66.9, 33.5. ($NHMe_2$ signals are overlapping with DMSO signal). $[\alpha]_D^{23}$ = −71.0 ° (c 0.7, H_2O). HR-EIMS: found 192.1261; calcd. 192.1263 for $C_{11}H_{16}N_2O$ (M^+).

(*S*)-2-(Dimethylamino)-3-phenylpropanamide (*S*)-16a:



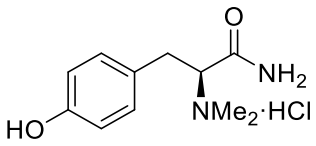
(*S*)-16a was synthesized following the protocol described for **(*R*)-16a**. From (*S*)-2-amino-3-phenylpropanamide hydrochloride (0.39 g, 1.94 mmol), 37% formaldehyde (1.44 mL, 19.4 mmol), and sodium triacetoxyborohydride (1.64 g, 7.8 mmol) the desired product was obtained as a white solid (0.36 g, 80%). LCMS (ESI): t_R = 10.7 min; m/z found: 193.4; calcd. 193.2 ($[M–Cl]^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.08 (br. s., 1 H), 7.89 (br. s., 1 H), 7.58 (br. s., 1 H), 7.15 − 7.43 (m, 5 H), 4.02 (dd, *J*=10.7, 3.9 Hz, 1 H), 3.22 − 3.29 (m, 1 H), 3.06 (dd, *J*=12.8, 10.8 Hz, 1 H), 2.80 (s, 6 H). ^{1}H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.19 − 7.39 (m, 5 H), 4.02 (dd, *J*=10.6, 4.3 Hz, 1 H), 3.28 (dd, *J*=12.9, 4.3 Hz, 1 H), 3.05 (dd, *J*=13.0, 10.6 Hz, 1 H), 2.82 (s, 6 H). $[\alpha]_D^{23}$ = +71.4 ° (c 0.7, H_2O).

(*R*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propanamide hydrochloride (*R*)-16b:



To a suspension of D-tyrosinamide (0.50 g, 2.3 mmol) in acetonitrile/water (9:1 v/v, 7 mL), 37% aqueous formaldehyde (2.0 mL, 27 mmol) was added followed by sodium triacetoxyborohydride (2.35 g, 11.1 mmol). The mixture was stirred for 15 min at an ambient temperature and then quenched by saturated $NaHCO_3$ (8 mL). The pH was adjusted to 8 by addition of 5% aqueous Na_2CO_3, and the mixture was extracted with isopropanol/ethyl acetate (1:3 v/v, 4 × 25 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in isopropanol (7 mL) and treated with 37% aqueous HCl (0.25 mL). The solution was concentrated to 2-3 mL, the product was precipitated by diethyl ether (15 mL) and filtered. White solid was washed with diethyl ether (2 × 10 mL) and dried under high vacuum. Yield: 0.54 g (96%). TLC (NH_4OH/MeOH/$CHCl_3$ 2.5:22.5:75): R_f = 0.62 ($KMnO_4$ stain). LCMS (ESI): t_R = 6.1−7.1 min, purity: 97% (254 nm); m/z found: 209.3, calcd.: 209.3 ($[M−Cl]^+$). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.10 (br. s., 1 H), 9.43 (s, 1 H), 7.91 (s, 1 H), 7.59 (s, 1 H), 7.02 (d, *J*=8.3 Hz, 2 H), 6.71 (d, *J*=8.3 Hz, 2 H), 3.96 (dd, *J*=10.9, 4.1 Hz, 1 H), 3.17 (dd, *J*=13.0, 4.0 Hz, 1 H), 2.95 (dd, *J*=12.9, 10.9 Hz, 1 H), 2.79 (s, 6 H). ^{1}H NMR (600 MHz, D_2O) δ 7.20 (d, *J*=8.5 Hz, 2 H), 6.91 (d, *J*=8.3 Hz, 2 H), 4.04 (dd, *J*=10.8, 5.1 Hz, 1 H), 3.42 (dd, *J*=13.0, 5.1 Hz, 1 H), 2.87 − 3.17 (m, 7 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 167.3, 156.4, 130.1 (2 C), 124.9, 115.3 (2 C), 67.1, 32.7. ^{13}C NMR (91 MHz, D_2O, acetone as internal standard) δ 170.5, 155.7, 131.4 (2 C), 126.0, 116.4 (2 C), 70.1, 42.4 (2 C), 34.0. HR-EIMS: m/z found: 209.1291, calcd.: 209.1290 for $C_{11}H_{17}N_2O_2$ ($[M−Cl]^+$). $[\alpha]_D^{26}$ = −55.7 ° (c 0.5, MeOH).

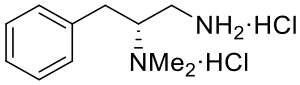
(*S*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propanamide hydrochloride (*S*)-16b:



(*S*)-16b was synthesized following the synthetic protocol for **(*R*)-16b**. From L-tyrosinamide (0.54 g, 3.0 mmol), 37% aqueous formaldehyde (2.5 mL, 33 mmol), and sodium triacetoxyborohydride (2.9 g, 13.5 mmol) the desired product was obtained (0.57 g, 78%) as a white solid. LCMS (ESI): t_R = 6.1−7.1 min, purity: 98% (254 nm); m/z found: 209.3, calcd.: 209.3 ($[M−Cl]^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.99 (br. s., 1 H), 9.39 (br. s., 1 H), 7.88 (s, 1 H), 7.58 (s, 1 H), 7.02 (d, *J*=8.6 Hz, 2 H), 6.71 (d, *J*=8.6 Hz, 2 H),

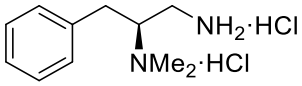
3.95 (dd, *J*=10.8, 3.9 Hz, 1 H), 3.17 (dd, *J*=13.0, 3.9 Hz, 1 H), 2.95 (dd, *J*=13.0, 10.8 Hz, 1 H), 2.79 (s, 6 H). ^{1}H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.04 (d, *J*=8.4 Hz, 2 H), 6.71 (d, *J*=8.5 Hz, 2 H), 3.95 (dd, *J*=10.4, 4.5 Hz, 1 H), 3.17 (dd, *J*=13.2, 4.5 Hz, 1 H), 2.93 (dd, *J*=13.1, 10.6 Hz, 1 H), 2.82 (d, *J*=7.7 Hz, 6 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 167.2, 156.3, 130.1 (2 C), 124.8, 115.2 (2 C), 67.1, 32.6. HR-EIMS: found 208.1214; calcd. 208.1212 for $C_{11}H_{16}N_2O_2$ ([M–HCl]$^+$). $[\alpha]_D^{26}$ = +58.6 ° (c 0.59, MeOH).

(*R*)-N^2,N^2-dimethyl-3-phenylpropane-1,2-diamine dihydrochloride (*R*)-17a:



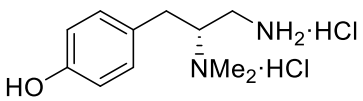
1M Borane-tetrahydrofurane complex (8.5 mL, 8.5 mmol) was slowly added to a suspension of **(*R*)-16a** (0.32 g, 1.40 mmol) in anhydrous THF (5 mL) under cooling with ice bath under nitrogen atmosphere. The mixture was refluxed for 20 h and then quenched by slow addition of anhydrous methanol (15 mL). The solvent was removed under reduced pressure and the dilution/evaporation sequence was repeated twice. The obtained oily residue was dissolved in isopropanol (5 mL) and 2N HCl in diethyl ether (1.4 mL, 2.8 mmol) was added, followed by dilution with diethyl ether (50 mL). The slurry was sonicated and filtered. Free-flowing white solid was obtained (0.25 g, 72%) after drying under high vacuum. TLC (NH_4OH/MeOH/$CHCl_3$ 1.5:13.5:85): R_f (prod) = 0.22. LCMS (ESI): t_R = 9.0 min; m/z found: 179.4; calcd. 179.3 ([M–H–2Cl]$^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.15 (br. s., 1 H), 8.56 (br. s., 3 H), 7.18 − 7.44 (m, 5 H), 3.78 − 4.00 (m, 1 H), 3.32 − 3.48 (m, 1 H), 3.27 (dd, *J*=13.9, 3.9 Hz, 1 H), 2.69 − 2.99 (m, 8 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 135.7, 129.4 (2 C), 128.8 (2 C), 127.2, 64.1, 36.8, 30.8. $[\alpha]_D^{23}$ = +12.5 ° (c 1.1, H_2O). HR-ESIMS: found 179.1540; calcd. 179.1543 for $C_{11}H_{19}N_2$ ([M–Cl–HCl]$^+$).

(*S*)-N^2,N^2-dimethyl-3-phenylpropane-1,2-diamine dihydrochloride (*S*)-17a:



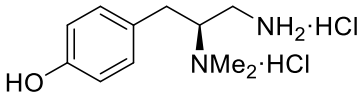
(*S*)-17a was synthesized following the protocol for **(*R*)-17a**. From **(*S*)-16a** (0.32 g, 1.40 mmol) and 1M borane-tetrahydrofurane complex (8.5 mL, 8.5 mmol) the desired product was obtained as a free-flowing white solid (0.143 g, 48%) after additional purification by dry-column vacuum chromatography (gradient elution with NH_4OH/MeOH/$CHCl_3$ from 1:9:90 to 2:18:80). LCMS (ESI): t_R = 9.0 min; m/z found: 179.4; calcd. 179.3 ([M–H–2Cl]$^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.16 (br. s., 1 H), 8.58 (br. s., 3 H), 7.11 − 7.49 (m, 5 H), 3.82 − 3.98 (m, 1 H), 3.37 − 3.48 (m, 1 H), 3.28 (dd, *J*=14.1, 3.9 Hz, 1 H), 2.73 − 2.96 (m, 8 H). ^{1}H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.25 − 7.47 (m, 5 H), 3.79 − 3.95 (m, 1 H), 3.43 (dd, *J*=14.6, 8.8 Hz, 1 H), 3.26 (dd, *J*=14.6, 4.3 Hz, 1 H), 2.77 − 2.98 (m, 8 H). $[\alpha]_D^{23}$ = −13.7 ° (c 0.9, H_2O).

(*R*)-4-[3-Amino-2-(dimethylamino)propyl]phenol dihydrochloride (*R*)-17b:



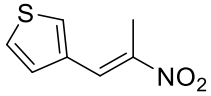
1M Borane-tetrahydrofurane complex (12.0 mL, 12.0 mmol) was slowly added to suspension of **(*R*)-16b** (0.45 mg, 1.85 mmol) in anhydrous THF (5 mL) under cooling with ice bath. The mixture was refluxed for 15 h under nitrogen atmosphere and then quenched with anhydrous methanol (10 mL dropwise, gas release). The solvent was removed under reduced pressure and dilution-evaporation sequence was repeated with anhydrous methanol (2 × 10 mL). The residue was resuspended in methanol (10 mL) with addition of 37% aqueous HCl (0.2 mL), concentrated under reduced pressure, diluted with ethanol and evaporated again (repeated twice). The residue was suspended in diethyl ether (ca. 5 mL), sonicated, and filtered. The product was washed with acetone (3 × 10 mL). Yield: 0.46 g (93%) of a white solid after drying under high vacuum. TLC (NH_4OH/MeOH/$CHCl_3$ 2.5:22.5:75): R_f = 0.23 ($KMnO_4$ stain). LCMS (ESI): t_R = 5.0 min, purity: 95%; m/z found: 195.4, calcd.: 195.3 ($[M-2Cl-H]^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.10 (br. s., 1 H), 9.49 (br. s., 1 H), 8.58 (br. s., 3 H), 7.13 (d, *J*=8.4 Hz, 2 H), 6.77 (d, *J*=8.4 Hz, 2 H), 3.63 − 3.92 (m, 1 H), 3.21 − 3.50 (m, 1 H), 3.07 − 3.20 (m, 1 H), 2.80 (br. m., 6 H), 2.57 − 2.74 (m, 2 H). ^{1}H NMR (360 MHz, DMSO-d_6, exchange with D_2O) δ 7.15 (d, *J*=8.4 Hz, 2 H), 6.78 (d, *J*=8.4 Hz, 2 H), 3.72 (br. s., 1 H), 3.36 (dd, *J*=14.0, 7.6 Hz, 1 H), 3.11 (dd, *J*=14.0, 4.1 Hz, 1 H), 2.58 − 2.96 (m, 8 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 156.5, 130.2 (2 C), 125.3, 115.5 (2 C), 64.4, 36.6, 30.1. $[\alpha]_D^{25}$ = +5.1 ° (c 0.96, H_2O).

(*S*)-4-[3-Amino-2-(dimethylamino)propyl]phenol dihydrochloride (*S*)-17b:



(*S*)-17b was synthesized following the synthetic protocol for **(*R*)-17b**. From **(*S*)-16b** (0.46 g, 1.89 mmol) and 1M borane-tetrahydrofurane complex (11.5 mL, 11.5 mmol) the desired product was obtained as a white solid (0.23 g, 46%) after additional purification by washing with acetonitrile (5 mL). LCMS (ESI): t_R = 4.3−4.9 min, purity: 95%; m/z found: 195.4, calcd.: 195.3 ($[M-2Cl-H]^+$). ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.13 (br. s., 1 H), 9.52 (br. s., 1 H), 8.63 (br. s., 3 H), 7.14 (d, *J*=8.5 Hz, 2 H), 6.78 (d, *J*=8.5 Hz, 2 H), 3.68 − 4.00 (m, 1 H), 3.24 − 3.56 (m, 1 H), 3.16 (dd, *J*=14.3, 3.7 Hz, 1 H), 2.80 − 2.94 (m, 6 H), 2.67 − 2.79 (m, 2 H). ^{1}H NMR (600 MHz, DMSO-d_6, exchange with D_2O) δ 7.14 (d, *J*=8.5 Hz, 2 H), 6.77 (d, *J*=8.5 Hz, 2 H), 3.80 (br. s., 1 H), 3.34 − 3.39 (m, 1 H), 3.14 (dd, *J*=14.0, 3.5 Hz, 1 H), 2.76 − 2.95 (m, 7 H), 2.66 − 2.75 (m, 1 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 156.5, 130.2 (2 C), 125.3, 115.5 (2 C), 64.3, 36.6, 30.1. ^{13}C NMR (91 MHz, D_2O, MeOH as external standard) δ 155.4, 130.7 (2 C), 125.0, 116.3 (2 C), 64.4, 39.9 (2 C), 37.2, 31.8. $[\alpha]_D^{24}$ = −10.1 ° (c 0.47, H_2O). HR-ESIMS: m/z found 195.1498; calcd. 195.1492 for $C_{11}H_{19}N_2O$ ($[M-Cl-HCl]^+$).

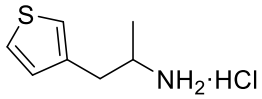
(*E*)-3-(2-Nitroprop-1-en-1-yl)thiophene 18:



Nitroethane (13 ml, 0.18 mmol) and thiophene-3-carbaldehyde (3.9 ml, 45 mmol) were added to an ice-cold mixture of formic acid (7.5 mL, 0.20 mol) and ethanolamine (8.5 mL, 0.14 mol). The reaction mixture was heated to 85−90°C and stirred for 7 h. The resulting solution was poured into cold water (300 mL), and the slurry was filtered. The precipitated product was washed with water (3 × 50 mL) yielding yellow solid (7.4 g, 98%). From two batches, 8.4 g of the crude product was recrystallized from ethanol/water (4:1 v/v) yielding yellow crystalline solid (7.0 g, 83 %). LCMS (ESI): t_R = 19.5 min, purity: 98% (254 nm); no molecular ion. ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.08 (br. s, 1 H), 7.60 (m, *J*=3.0, 1.3 Hz, 1 H), 7.44 (ddd, *J*=5.1, 3.0, 0.5 Hz, 1 H), 7.28 (ddd, *J*=5.1, 1.3, 0.4 Hz, 1 H), 2.50 (d, *J*=0.9 Hz, 3 H). ^{13}C NMR (151 MHz,

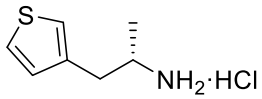
$CDCl_3$) δ 146.2, 133.7, 129.9, 128.2, 127.5, 127.0, 14.2. HR-EIMS: m/z found: 169.0197, calcd.: 169.0197 for $C_7H_7NO_2S$ ($[M]^+$).

(*RS*)-1-(Thiophen-3-yl)propan-2-amine 19a:



The solution of **18** (6.9 g, 41 mmol) in anhydrous THF (30 mL) was added dropwise to 1M $LiAlH_4$ in THF (200 mL, 200 mmol) at a rate adjusted to keep the mixture under a gentle reflux. After complete addition of the starting material, the reaction mixture was refluxed for 30 min, cooled to 0°C, and $Na_2SO_4 \cdot 10H_2O$ was slowly added until the mixture solidified. The resulting slurry was diluted with water, followed by additional $Na_2SO_4 \cdot 10H_2O$ (2 × 0.5 g). The suspension was stirred for 15 min at ice bath and then filtered through Celite; the filter cake was washed thoroughly with ethyl acetate (total volume 500 mL). The yellow filtrate was concentrated under reduced pressure and the brown residue was dissolved in diethyl ether (100 mL). Ether solution was extracted with 1N HCl (3 × 100 mL). Combined aqueous layers were basified with 25% aqueous NH_3, and back-extracted with ethyl acetate (3 × 100 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The brown residue was dissolved in diethyl ether (50 mL), cooled to 0°C and the product was precipitated by 2N HCl in diethyl ether (15 mL, 30 mmol) and filtered off. The precipitate was recrystallized from acetonitrile (ca. 100 mL), washed with cold acetone, and dried under high vacuum. Yield: 4.02 g (56%) as gray crystalline solid. LCMS (ESI): t_R = 12.3 min, purity: 98% (254 nm); m/z found: 142.3, calcd.: 142.2 ($[M-Cl]^+$). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (br. s., 3 H), 7.52 (dd, *J*=4.9, 2.8 Hz, 1 H), 7.32 (dddd, *J*=2.8, 1.3, 1.0, 0.6 Hz, 1 H), 7.04 (dd, *J*=4.9, 1.3 Hz, 1 H), 3.38 − 3.47 (m, 1 H), 3.01 (ddd, *J*=14.0, 4.9, 1.0 Hz, 1 H), 2.76 (ddd, *J*=14.0, 9.1, 0.6 Hz, 1 H), 1.13 (d, *J*=6.4 Hz, 3 H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 136.8, 128.5, 126.4, 122.9, 47.3, 34.6, 17.7. HR-EIMS: m/z found: 142.0689, calcd.: 142.0690 for $C_7H_{12}NS$ ($[M-Cl]^+$).

(*S*)-1-(Thiophen-3-yl)propan-2-amine (*S*)-19a (Chiral resolution).

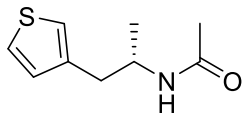


Racemic **19** (1.05 g, 5.9 mmol) was dissolved in water (40 mL), the solution was basified by 25% aq. NH_3, and extracted with chloroform (3 × 40 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The liquid residue was dissolved in ethanol (10 mL) and added to a hot solution of di-*p*-anisoyl-D-tartaric acid (2.5 g, 5.9 mmol) in acetonitrile (20 mL). White precipitate formed immediately. The slurry was diluted with water (10 mL) and heated to the boiling point. Ethanol was added in small portions until all the solids dissolved. Seeding crystals (~5 mg) were added and the solution was allowed to cool down to ambient temperature. Crystalline precipitate was filtered off and washed with acetonitrile (2 × 6 mL). Washings were combined with mother liquor, heated to boiling and the solution was allowed to cool down to ambient temperature in an open beaker.

The crystals from the first crop were dissolved in 1N NaOH (25 mL) and the solution was extracted by chloroform (3 × 15 mL). Combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in diethyl ether (30 mL) and hydrochloride salt was precipitated by adding excess of 2M HCl in diethyl ether. The solvent was removed under reduced pressure and the resulting white solid was dried under high vacuum, yielding a white solid (0.45 g).Specific optical rotation of the first crop (converted to hydrochloride) $[\alpha]^{21}_D$ = +15.5° (c 1.25, H_2O).The 2nd crop of crystals

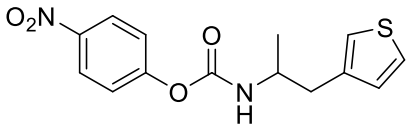
formed as a voluminous precipitate after two days of standing in the open beaker. It was collected by filtration and washed with acetonitrile (3 × 3 mL). Then the diastereomeric salt was converted to hydrochloride as described above. White crystalline solid (0.32 g) was obtained.Specific optical rotation $[\alpha]^{21}_{D}$= −13.0° (H_2O, 20.4°C, c 0.92).Crystals from the first crop (0.45 g, 2.5 mmol) were converted to free amine as described above, and dissolved in ethanol (10 mL). The solution was mixed with di-*p*-anisoyl-D-tartaric acid (1.05 g, 2.5 mmol) and acetonitrile (10 mL). Total amount of 120 mL of water-ethanol (3:1 v/v) was added to completely dissolve the solids under heating to boiling point. The hot solution was left in an open Erlenmeyer flask for crystallization at 4°C. After 20 h precipitated crystals were collected by filtration, washed with acetonitrile (2 × 4 mL) and dried by suction. Di-*p*-anisoyl-D-tartrate salt was converted to hydrochloride as described above. Yield: 0.32 g (60% after double crystallization).
$[a]_D^{22}$ = +19.2 ° (c 0.94, H_2O).Triple crystallization delivers the product with $[a]_D^{25}$ = +20.5 ° (c 0.52, H_2O). Absolute configuration was established by acylation of enantiomerically enriched amine with acetic anhydride and comparing measured optical rotation to published data[1].

(*S*)-*N*-[1-(Thiophen-3-yl)propan-2-yl]acetamide (*S*)-*N*-Ac-19a



Acetic anhydride (30 µL, 0.32 mmol) was slowly added to a solution of **(*S*)-19** (50 mg, 0.28 mmol) and triethylamine (80 µL, 0.57 mmol) in chloroform (1.5 mL). The mixture was stirred for 3 h at ambient temperature. Then the mixture was diluted with chloroform (3 mL), washed with saturated $NaHCO_3$ (3 × 3 mL) and brine (3 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under high vacuum yielding white crystalline product (49 mg, 95%). LCMS (ESI): t_R = 20.0 min, purity: 97%; m/z found: 184.3; calcd. 184.3 ($[M+H]^+$). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.27 (dd, *J*=4.9, 3.0 Hz, 1 H), 6.96 − 7.02 (m, 1 H), 6.94 (dd, *J*=4.9, 1.2 Hz, 1 H), 5.26 (br. s., 1 H), 4.26 (dquind, *J*=8.3, 6.6, 5.9 Hz, 1 H), 2.83 (dd, *J*=14.3, 5.9 Hz, 1 H), 2.79 (dd, *J*=14.2, 6.6 Hz, 1 H), 1.93 (s, 3 H), 1.12 (d, *J*=6.7 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 169.3, 138.1, 128.8, 125.6, 122.0, 45.5, 36.7, 23.5, 20.2. HR-EIMS: found 183.0718; calcd. 183.0718 for $C_9H_{13}NOS$ (M^+). $[\alpha]_D^{21}$=−46.6 ° (c 1.0, $CHCl_3$). Based on literature data ($[\alpha]_D^{20}$=+49.8° for (*R*)-enantiomer; c 0.5, $CHCl_3$),[1] the absolute configuration was assigned to be (*S*) for the product and the amine precursor.

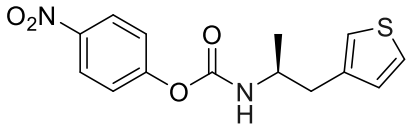
4-Nitrophenyl (*RS*)-[1-(thiophen-3-yl)propan-2-yl]carbamate 20:



A 25 mL Schlenk flask was charged with racemic **19** (0.20 g, 1.13 mmol) and triethylamine (0.32 mL, 2.3 mmol) in anhydrous THF (5 mL) under nitrogen atmosphere and cooling with ice bath. A solution of 4-nitrophenyl chloroformate (0.23 g, 1.13 mmol) in anhydrous THF (2 mL) was added dropwise followed by a rinse with anhydrous THF (1 mL). The reaction mixture was allowed to warm up to an ambient temperature and stirred for 6 h. Then the slurry was diluted with dichloromethane (20 mL) and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ (3 × 15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (100% dichloromethane) yielding desired product as a white foam (0.26 g, 75%). TLC:

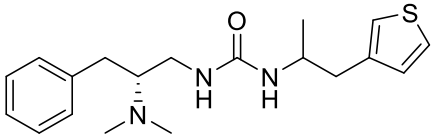
(ethyl acetate/hexane 4:6): R_f = 0.64. LCMS (ESI): t_R = 20.4 min, purity: 96%; m/z found: 329.3, calcd.: 329.3 ([M+Na]$^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 − 8.26 (m, 2 H), 7.31 (dd, *J*=4.7, 3.0 Hz, 1 H), 7.24 − 7.29 (m, 2 H), 7.05 (ddt, *J*=2.9, 1.4, 0.8, Hz, 1 H), 6.98 (dd, *J*=4.9, 1.1 Hz, 1 H), 4.98 (d, *J*=7.7 Hz, 1 H), 4.07 (dqt, *J*=7.6, 6.7, 6.4 Hz, 1 H), 2.90 (d, *J*=6.4 Hz, 2 H), 1.25 (d, *J*=6.7 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 155.9, 152.3, 144.7, 137.6, 128.6, 125.9, 125.1 (2 C), 122.3, 121.9 (2 C), 48.0, 37.0, 20.3. HR-EIMS: m/z found: 306.0674, calcd.: 306.0674 for $C_{14}H_{14}N_2O_4S$ (M^+).

4-Nitrophenyl (*S*)-[1-(thiophen-3-yl)propan-2-yl]carbamate (*S*)-19:



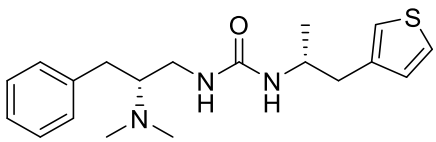
(*S*)-20 was synthesized following the protocol for racemic **20**, using enantiomerically enriched **(*S*)-19** as starting material. Spectral characteristics were the same as for the racemic compound. LCMS (ESI): t_R = 20.4 min, purity: 98%; m/z found: 329.3, calcd.: 329.3 ([M+Na]$^+$). ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.20 − 8.26 (m, 2 H), 7.31 (dd, *J*=4.9, 3.0 Hz, 1 H), 7.24 − 7.29 (m, 2 H), 7.05 (ddt, *J*=2.9, 1.4, 0.8, Hz, 1 H), 6.98 (dd, *J*=4.9, 1.3 Hz, 1 H), 4.96 (d, *J*=7.6 Hz, 1 H), 4.07 (dqt, *J*=7.6, 6.7, 6.4 Hz, 1 H), 2.90 (d, *J*=6.4 Hz, 2 H), 1.26 (d, *J*=6.7 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 155.9, 152.3, 144.7, 137.6, 128.6, 125.9, 125.1 (2 C), 122.3, 121.9 (2 C), 48.0, 37.0, 20.3. $[\alpha]_D^{22}$ = −44.9 ° (c 0.5, $CHCl_3$).

1-[(*R*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*RS*)-1-(thiophen-3-yl)propan-2-yl]urea (*R,RS*)-12:



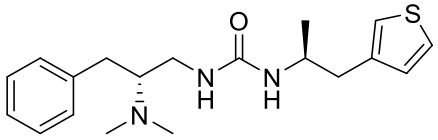
Triethylamine (50 µL, 0.36 mmol) was added to a suspension of **(*R*)-17a** (85 mg, 0.34 mmol) and racemic **20** (104 mg, 0.34 mmol) in anhydrous DMF (1 mL) under nitrogen atmosphere. The mixture was stirred at ambient temperature for 20 h and then diluted with isopropanol/ethyl acetate (1:3 v/v, 20 mL), washed with 1N NaOH (5 × 15 mL), and extracted with 0.1N HCl (3 × 20 mL). Combined aqueous extracts were basified with carbonate buffer to pH 9 and back-extracted with ethyl acetate (3 × 25 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. No further purification was required. The product was obtained as a colorless oil (107 mg, 92%). TLC (NH_4OH/MeOH/$CHCl_3$ 1:9:90): R_f = 0.50. Diastereomers were separated by semi-preparative HPLC (AS-H chiral column, 0.1% diethyl amine in isopropanol/hexane 50:50, 6 mL/min, 6 min).

1-[(*R*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*R*)-1-(thiophen-3-yl)propan-2-yl]urea (*R,R*)-12:



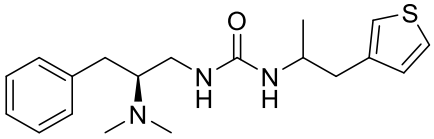
Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 50:50, 6 mL/min, 6 min: t_R = 4.3 min. LCMS (ESI): t_R = 21.3 min, purity: 99%; m/z found: 346.6; calcd. 346.5 ($[M+H]^+$). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.24 (dd, *J*=4.9, 3.0 Hz, 1 H), 7.07 − 7.32 (m, 5 H), 6.94 − 6.99 (m, 1 H), 6.92 (dd, *J*=4.9, 1.2 Hz, 1 H), 4.88 (d, *J*=5.8 Hz, 1 H), 4.37 (d, *J*=7.7 Hz, 1 H), 3.96 (dquind, *J*=8.1, 6.7, 5.4 Hz, 1 H), 3.20 (ddd, *J*=12.8, 6.9, 4.2 Hz, 1 H), 2.95 (dd, *J*=13.3, 3.6 Hz, 1 H), 2.91 (ddd, *J*=12.8, 10.0, 2.0 Hz, 1 H), 2.80 (dd, *J*=14.0, 5.3 Hz, 1 H), 2.76 (tt, *J*=10.0, 4.0 Hz, 1 H), 2.72 (dd, *J*=14.0, 7.0 Hz, 1 H), 2.31 (s, 6 H), 2.31 (dd, *J*=13.3, 10.0 Hz, 1 H), 1.07 (d, *J*=6.6 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 157.7, 139.4, 138.6, 129.0 (2 C), 129.0, 128.6 (2 C), 126.2, 125.3, 121.9, 65.4, 46.4, 40.7, 40.1 (2 C), 37.5, 31.5, 20.7. $[\alpha]_D^{25}$ = –1.5 ° (c 0.42, $CHCl_3$). HR-ESIMS: found 346.1952, calcd. 346.1948 for $C_{19}H_{28}N_3OS$ ($[M+H]^+$).

1-[(*R*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*S*)-1-(thiophen-3-yl)propan-2-yl]urea (*R,S*)-12:



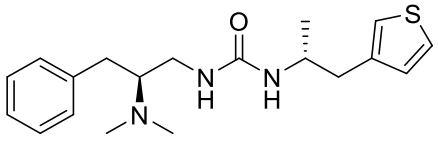
Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 50:50, 6 mL/min, 6 min: t_R = 5.3 min. LCMS (ESI): t_R = 20.6 min, purity: 99%; m/z found: 346.7; calcd. 346.5 ($[M+H]^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 − 7.30 (m, 2 H), 7.23 (dd, *J*=4.9, 3.0 Hz, 1 H), 7.17 − 7.21 (m, 1 H), 7.12 − 7.15 (m, 2 H), 6.94 − 6.96 (m, 1 H), 6.92 (dd, *J*=4.9, 1.3 Hz, 1 H), 4.90 (br. d, *J*=5.8 Hz, 1 H), 4.46 (br. s., 1 H), 3.98 (dquind, *J*=8.1, 6.6, 5.4 Hz, 1 H), 3.18 (ddd, *J*=13.0, 6.8, 4.3 Hz, 1 H), 2.94 (dd, *J*=13.3, 3.6 Hz, 1 H), 2.91 (ddd, *J*=13.3, 10.0, 1.4 Hz, 1 H), 2.79 (dd, *J*=14.0, 5.5 Hz, 1 H), 2.74 (tt, *J*=10.0, 4.0 Hz, 1 H), 2.73 (dd, *J*=14.0, 6.8 Hz, 1 H), 2.31 (s, 6 H), 2.29 (dd, *J*=13.4, 10.3 Hz, 1 H), 1.07 (d, *J*=6.6 Hz, 3 H). $[\alpha]_D^{23}$ = −16.2 ° (c 0.46, $CHCl_3$). The absolute configuration at 1-(thiophen-3-yl)propan-2-yl substituent was established by synthesizing the final product with enantiomerically enriched **(*S*)-20** of known configuration, and comparing the retention time of the resulting product with the retention time of diastereomers in the chiral HPLC.

1-[(*S*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*RS*)-1-(thiophen-3-yl)propan-2-yl]urea (*S,RS*)-12:



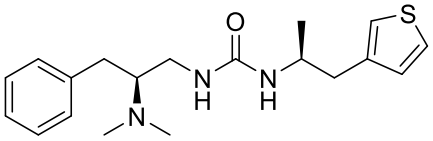
(*S,RS*)-12 was synthesized following the protocol for **(*R,RS*)-12**. From **(*S*)-17a** (85 mg, 0.34 mmol), racemic **20** (109 mg, 0.36 mmol), and triethylamine (55 µL, 0.40 mmol) the desired product was obtained as a colorless oil (109 mg, 93%). Diastereomers were separated by semi-preparative HPLC (AS-H chiral column, 0.1% diethyl amine in isopropanol/hexane 10:90, 11 mL/min, 8.5 min).

1-[(*S*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*R*)-1-(thiophen-3-yl)propan-2-yl]urea (*S,R*)-12:



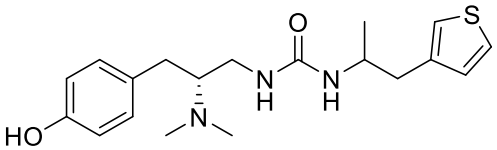
Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 10:90, 11 mL/min, 108 bar, 8.5 min: t_R = 4.9 min. LCMS (ESI): t_R = 20.6 min, purity: 99%; m/z found: 346.7; calcd. 346.5 ($[M+H]^+$). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.22 (dd, *J*=4.9, 3.0 Hz, 1 H), 7.08 − 7.32 (m, 5 H), 6.93 − 6.96 (m, 1 H), 6.92 (dd, *J*=4.9, 1.3 Hz, 1 H), 4.89 (br. d, *J*=5.6 Hz, 1 H), 4.46 (br. d, *J*=6.2 Hz, 1 H), 3.97 (dquind, *J*=8.1, 6.6, 5.4 Hz, 1 H), 3.18 (ddd, *J*=13.0, 6.8, 4.3 Hz, 1 H), 2.94 (dd, *J*=13.3, 3.6 Hz, 1 H), 2.91 (ddd, *J*=13.3, 10.0, 1.4 Hz, 1 H), 2.80 (dd, *J*=14.3, 5.7 Hz, 1 H), 2.74 (tt, *J*=10.0, 4.0 Hz, 1 H), 2.72 (dd, *J*=14.0, 6.8 Hz, 1 H), 2.31 (s, 6 H), 2.29 (dd, *J*=13.3, 10.0 Hz, 1 H), 1.07 (d, *J*=6.6 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 157.7, 139.3, 138.6, 129.0 (2 C), 129.0, 128.6 (2 C), 126.2, 125.2, 121.9, 65.7, 46.3, 40.6, 40.1 (2 C), 37.5, 31.4, 20.7. $[\alpha]_D^{29}$ = +16.7 ° (c 0.44, $CHCl_3$). HR-ESIMS: found 346.1950, calcd. 346.1948 for $C_{19}H_{28}N_3OS$ ($[M+H]^+$).

1-[(*S*)-2-(Dimethylamino)-3-phenylpropyl]-3-[(*S*)-1-(thiophen-3-yl)propan-2-yl]urea (*S*,*S*)-12:



Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 10:90, 11 mL/min, 8.5 min: t_R = 6.5 min. LCMS (ESI): t_R = 21.3 min, purity: 99%; m/z found: 346.6; calcd. 346.5 ($[M+H]^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 − 7.29 (m, 2 H), 7.23 (dd, *J*=4.9, 2.9 Hz, 1 H), 7.17 − 7.21 (m, 1 H), 7.12 − 7.16 (m, 2 H), 6.94 − 6.97 (m, 1 H), 6.92 (dd, *J*=4.9, 1.3 Hz, 1 H), 4.91 (br. d, *J*=5.9 Hz, 1 H), 4.42 (br. s., 1 H), 3.96 (dquind, *J*=8.1, 6.6, 6.6, 6.6, 6.6, 5.4 Hz, 1 H), 3.19 (ddd, *J*=12.8, 6.9, 4.2 Hz, 1 H), 2.94 (dd, *J*=13.3, 3.6 Hz, 1 H), 2.91 (ddd, *J*=12.8, 10.0, 2.0 Hz, 1 H), 2.80 (dd, *J*=14.0, 5.4 Hz, 1 H), 2.75 (tt, *J*=10.0, 4.1 Hz, 1 H), 2.72 (dd, *J*=14.1, 6.9 Hz, 1 H), 2.30 (s, 6 H), 2.30 (dd, *J*=13.3, 10.0 Hz, 1 H), 1.07 (d, *J*=6.7 Hz, 3 H). $[\alpha]_D^{23}$ = +3.5 ° (c 0.70, $CHCl_3$).

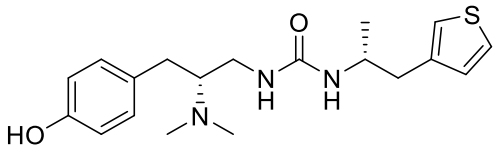
1-[(*R*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*RS*)-1-(thiophen-3-yl)propan-2-yl)urea (*R*,*RS*)-21:



To a suspension of **(*R*)-17b** (102 mg, 0.382 mmol) in acetonitrile (3 mL), triethylamine (0.16 mL, 1.15 mmol) was added. The flask was sealed and heated to 60°C. Solution of racemic **20** (117 mg, 0.382 mmol) in acetonitrile (3 mL) was added. The mixture immediately turned yellow. The temperature was increased to 80°C. The mixture was stirred for 2 h and then filtered through a cotton pad and concentrated under reduced pressure. The residue was suspended in 33% isopropanol/ethyl acetate (9 mL) and washed with carbonate buffer (pH 9, 5 × 5 mL). Organic layer was extracted with 0.1N HCl (3 × 5 mL). Combined acidic aqueous layers were basified with carbonate buffer to pH 9 and back-extracted with ethyl acetate (3 × 10 mL). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by dry-column vacuum chromatography[2] (gradient elution with NH_4OH/MeOH/$CHCl_3$ from 0.5:4.5:95 to 1.5:13.5:85). Yield: 94 mg (68%) as a white solid after trituration with hexane. LCMS (ESI): t_R = 19.0 min, purity: 98%; m/z found: 362.6, calcd.: 362.5 ($[M+H]^+$). ^{1}H NMR (·HCl) (360 MHz, DMSO-d_6) δ 10.27 (br. s., 1 H), 9.31 (br. s., 1 H), 7.42 (ddd, *J*=4.9, 3.0, 1.9 Hz, 1 H),

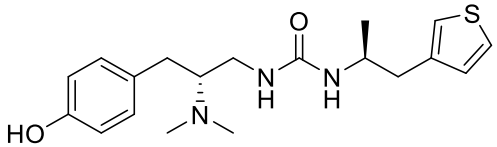
7.15 (d, *J*=2.8 Hz, 1 H), 7.08 (d, *J*=7.9 Hz, 2 H), 6.96 (dd, *J*=4.9, 1.2 Hz, 1 H), 6.72 (d, *J*=8.4 Hz, 2 H), 5.99 − 6.37 (m, 2 H), 3.79 (dt, *J*=13.3, 6.7 Hz, 1 H), 2.87 − 3.26 (m, 4 H), 2.53 − 2.85 (m, 9 H), 0.97 (d, *J*=6.6 Hz, 3 H). ^{13}C NMR (91 MHz, DMSO-d_6) δ 157.7, 155.9, 139.2, 130.0 (2 C), 128.8, 125.3, 121.6, 115.2 (2 C), 79.1, 66.5 (br. s, 2 C), 45.8, 37.7, 36.9, 30.8, 20.7. Diastereomers were separated by semi-preparative HPLC (AS-H chiral column, 0.1% diethyl amine in isopropanol/hexane 50:50, 8 mL/min, 8.5 min).

1-[(*R*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*R*)-1-(thiophen-3-yl)propan-2-yl)urea (*R,R*)-21:



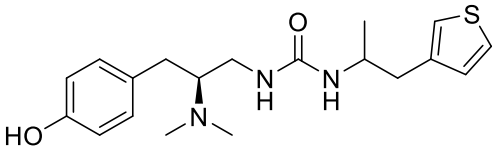
Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 50:50, 8 mL/min: t_R = 4.2 min. LCMS (ESI): t_R = 19.0 min, purity: 98%; m/z found: 362.7, calcd.: 362.5 ($[M+H]^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (dd, *J*=4.9, 3.0 Hz, 1 H), 6.97 − 7.00 (m, 1 H), 6.96 (d, *J*=8.3 Hz, 2 H), 6.94 (dd, *J*=4.9, 1.0 Hz, 1 H), 6.76 (d, *J*=8.4 Hz, 2 H), 5.13 (br. s., 1 H), 4.53 (br. s., 1 H), 3.97 (spt, *J*=6.7 Hz, 1 H), 3.31 (m, *J*=12.7 Hz, 1 H), 2.91 (dd, *J*=12.7, 10.2 Hz, 1 H), 2.87 (dd, *J*=13.4, 3.2 Hz, 1 H), 2.80 (dd, *J*=13.9, 5.4 Hz, 1 H), 2.72 (dd, *J*=14.1, 6.9 Hz, 1 H), 2.64 − 2.72 (m, 1 H), 2.33 (s, 6 H), 2.25 (dd, *J*=13.2, 10.7 Hz, 1 H), 1.08 (d, *J*=6.6 Hz, 3 H). $[\alpha]_D^{23}$ = −30.4 ° (c 0.39, $CHCl_3$).

1-[(*R*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*S*)-1-(thiophen-3-yl)propan-2-yl)urea (*R,S*)-21:



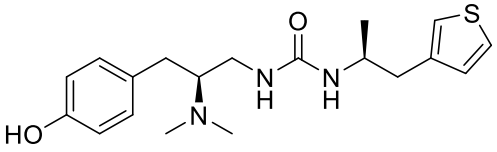
Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 50:50, 8 mL/min: t_R = 6.3 min. LCMS (ESI): t_R = 19.0 min, purity: 98%; m/z found: 362.7, calcd.: 362.5 ($[M+H]^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (dd, *J*=4.9, 2.9 Hz, 1 H), 6.96 − 6.99 (m, 1 H), 6.95 (d, *J*=8.4 Hz, 2 H), 6.93 (dd, *J*=4.9, 1.0 Hz, 1 H), 6.76 (d, *J*=8.4 Hz, 2 H), 5.12 (br. s., 1 H), 4.59 (br. s., 1 H), 3.97 (spt, *J*=6.7 Hz, 1 H), 3.29 (s, 1 H), 2.91 (dd, *J*=12.9, 10.2 Hz, 1 H), 2.86 (dd, *J*=13.4, 3.3 Hz, 1 H), 2.80 (dd, *J*=14.0, 5.7 Hz, 1 H), 2.73 (dd, *J*=14.0, 6.9 Hz, 1 H), 2.68 (tt, *J*=10.2, 3.8 Hz, 1 H), 2.33 (s, 6 H), 2.24 (dd, *J*=13.3, 10.7 Hz, 1 H), 1.08 (d, *J*=6.5 Hz, 3 H). $[\alpha]_D^{30}$ = −34.1 ° (c 0.28, $CHCl_3$). The absolute configuration at 1-(thiophen-3-yl)propan-2-yl substituent was established by synthesizing the final product with enantiomerically enriched **(*S*)-20** of known configuration, and comparing the retention time of the resulting product with the retention time of diastereomers in the chiral HPLC.

1-[(*S*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*RS*)-1-(thiophen-3-yl)propan-2-yl)urea (*S,RS*)-21:



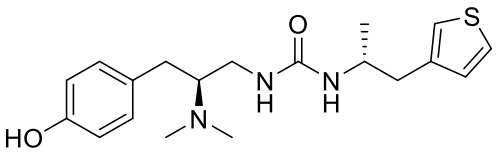
Triethylamine (0.16 mL, 1.15 mmol) was slowly added to a suspension of **(*S*)-17b** (106 mg, 0.40 mmol) and racemic **20** (121 mg, 0.40 mmol) in anhydrous DMF (2.5 mL) under nitrogen atmosphere. The mixture was stirred for 20 h at ambient temperature, and then diluted with isopropanol/ethyl acetate (1:3 v/v, 12 mL). The organic layer was washed with carbonate buffer (pH 9, 5 × 5 mL) and extracted with 0.1N HCl (3 × 5 mL). Combined acidic aqueous layers were basified with carbonate buffer to pH 9 and back-extracted with ethyl acetate (3 × 10 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Further purification of the crude yellow oil was accomplished by dry-column vacuum chromatography (gradient elution with NH_4OH/MeOH/$CHCl_3$ from 0.7:6.3:93 to 1:9:90), yielding the desired product (100 mg, 70%) as a white solid after trituration with hexane. Diastereomers were separated by semi-preparative HPLC (AS-H chiral column, 0.1% diethyl amine in isopropanol/hexane 35:65, 7 mL/min, 9 min).

1-[(*S*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*S*)-1-(thiophen-3-yl)propan-2-yl)urea 21 (PZM21):



Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 35:65, 7 mL/min, 9 min: t_R = 7.1 min. The stereoisomeric purity was determined to be > 99%. LCMS (ESI): t_R = 19.0 min, purity: 99%; m/z found: 362.7, calcd.: 362.5 ($[M+H]^+$). ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.22 (dd, *J*=4.9, 3.0 Hz, 1 H), 6.95 − 6.97 (m, 1 H), 6.94 (d, *J*=8.5 Hz, 2 H), 6.92 (dd, *J*=4.9, 1.2 Hz, 1 H), 6.76 (d, *J*=8.4 Hz, 2 H), 5.18 (br. d, *J*=5.2 Hz, 1 H), 4.70 (br. s., 1 H), 3.95 (spt, *J*=6.8 Hz, 1 H), 3.29 (ddd, *J*=13.2, 6.6, 4.0 Hz, 1 H), 2.91 (ddd, *J*=13.3, 9.7, 2.2 Hz, 1 H), 2.85 (dd, *J*=13.3, 3.3 Hz, 1 H), 2.79 (dd, *J*=14.1, 5.4 Hz, 1 H), 2.70 (dd, *J*=14.1, 6.6 Hz, 1 H), 2.66 (m, *J*=9.7, 6.6, 3.3 Hz, 1 H), 2.31 (s, 6 H), 2.23 (dd, *J*=13.3, 10.6 Hz, 1 H), 1.07 (d, *J*=6.8 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 158.2, 155.6, 138.5, 129.9 (2 C), 129.4, 128.9, 125.3, 122.0, 115.7 (2 C), 65.7, 46.6, 40.4, 40.1 (2 C), 37.5, 30.4, 20.6. $[\alpha]_D^{23}$ = +40.3 ° (c 1.0, $CHCl_3$). HR-ESIMS: m/z found 362.1901; calcd. 362.1897 for $C_{19}H_{28}N_3O_2S$ ($[M+H]^+$).

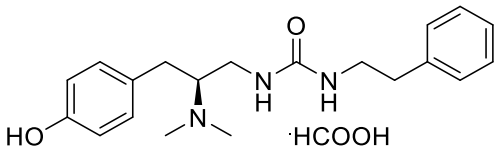
1-[(*S*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl]-3-((*R*)-1-(thiophen-3-yl)propan-2-yl)urea (*S,R*)-21:



Chiral HPLC: AS-H column, 0.1% diethyl amine in isopropanol/hexane 35:65, 7 mL/min, 9 min: t_R = 5.4 min. LCMS (ESI): t_R = 19.0 min, purity: 99%; m/z found: 362.7, calcd.: 362.5 ($[M+H]^+$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 (dd, *J*=4.8, 2.9 Hz, 1 H), 6.96 − 6.98 (m, 1 H), 6.94 (d, *J*=8.1 Hz, 2 H), 6.93 (dd, *J*=4.8,

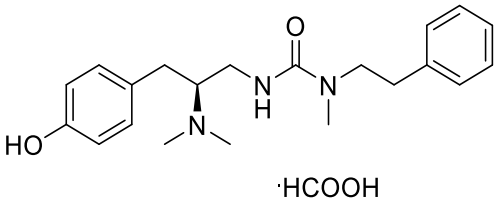
1.0 Hz, 1 H), 6.76 (d, *J*=8.1 Hz, 2 H), 5.13 (br. d, *J*=6.1 Hz, 1 H), 4.67 (br. s, 1 H), 3.96 (spt, *J*=6.7 Hz, 1 H), 3.23 − 3.33 (m, 1 H), 2.91 (ddd, *J*=13.3, 9.8, 2.2 Hz, 1 H), 2.85 (dd, *J*=13.2, 3.2 Hz, 1 H), 2.80 (dd, *J*=14.2, 5.5 Hz, 1 H), 2.72 (dd, *J*=14.2, 7.0 Hz, 1 H), 2.66 (tt, *J*=10.2, 3.8 Hz, 1 H), 2.31 (s, 6 H), 2.23 (dd, *J*=13.3, 10.7 Hz, 1 H), 1.07 (d, *J*=6.6 Hz, 3 H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 158.1, 155.4, 138.5, 129.9 (2 C), 129.6, 129.0, 125.3, 122.0, 115.7 (2 C), 65.9, 46.6, 40.4, 40.1 (2 C), 37.5, 30.4, 20.7. $[\alpha]_D^{25}$ = +32.2 ° (c 0.58, $CHCl_3$). HR-ESIMS: m/z found 362.1901; calcd. 362.1897 for $C_{19}H_{28}N_3O_2S$ ($[M+H]^+$). The absolute configuration at 1-(thiophen-3-yl)propan-2-yl substituent was established by synthesizing the final product with enantiomerically enriched **(*S*)-20** of known configuration, and comparing the retention time of the resulting product with the retention time of diastereomers by HPLC on a chiral column.

(*S*)-1-(2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl)-3-phenethylurea x HCOOH 22 (PZM22):



Compound **35** (72 mg, 0.27 mmol) was suspended in dry DMF (2.5 mL) in a microwave tube. Triethylamine (20 µL, 0.14 mmol)) and 4-nitrophenyl phenethylcarbamate (**42**) (70 mg, 0.24 mmol)) were added and the mixture was stirred at room temperature under argon atmosphere for 3 hours. Then, the solution was diluted with isopropanol/EtOAc (1:3) and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was extracted with 0.1N HCl-solution and the aqueous extracts were adjusted to pH 9 with saturated aqueous $NaHCO_3/Na_2CO_3$ solution and finally extracted with EtOAc. The organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (acetonitrile in 0.1 % and concentrated aqueous HCOOH, 5 % to 30 % acetonitrile in 13 min) to give pure **22** as a white powder in 37% yield (34.6 mg) after lyophilisation. LCMS (ESI) t_R = 4.2 - 4.8 min, *m/z* found 342.2, calcd 342.2 $[M+H]^+$. HR EIMS *m/z* found 342.2186, calcd 342.2176 for $C_{20}H_{27}N_3O_2$ $[M+H]^+$. HPLC System A: 14.4 min, purity 96 %, System B: 12.0 min, purity 95%. ^{1}H NMR (600 MHz, DMSO) δ 8.29 (s, 1H), 7.29 – 7.24 (m, 2H), 7.20 – 7.15 (m, 3H), 6.99 – 6.93 (m, 2H), 6.70 – 6.65 (m, 2H), 6.11 (t, *J* = 5.6 Hz, 1H), 5.71 – 5.63 (m, 1H), 3.16 (dd, *J* = 13.4, 6.0 Hz, 2H), 3.09 – 3.04 (m, 1H), 2.84 – 2.76 (m, 1H), 2.71 (dd, *J* = 13.5, 4.2 Hz, 1H), 2.63 (t, *J* = 7.3 Hz, 2H), 2.59 – 2.54 (m, 1H), 2.25 (s, 6H), 2.19 (dd, *J* = 13.5, 9.3 Hz, 1H). ^{13}C NMR (91 MHz, DMSO) δ 157.75, 155.32, 139.72, 129.97, 129.70 (2C), 128.54 (2C), 128.17 (2C), 125.84, 115.01 (2C), 65.38, 40.79, 39.96 (2C), 39.14, 36.12, 30.30.

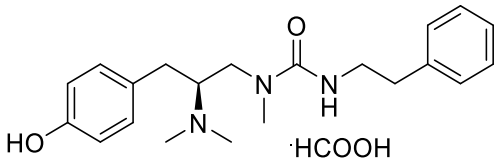
(*S*)-3-(2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl)-1-methyl-1-phenethylurea x HCOOH 23 (PZM23):



23 was prepared as described for **22** using (*S*)-4-[3-amino-2-(dimethylamino)propyl]phenol (**35**) (50.9 mg, 0.26 mmol) and 4-nitrophenyl methyl(phenethyl)carbamate (**43**) (90.0 mg, 0.30 mmol) as starting materials. The reaction mixture was stirred 8 h at 120 °C. Purification by preparative HPLC (acetonitrile in 0.1 % aqueous HCOOH, 5 % to 45 % acetonitrile in 13 min) gave the pure product in 9 % yield (9.1 mg). LCMS

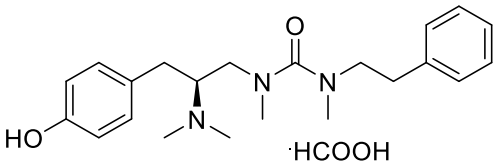
(ESI) t_R = 4.4 – 4.9 min, *m/z* found 356.0, calcd 356.2 $[M+H]^+$. HR EIMS *m/z* found 356.2344, calcd 356.2333 for $C_{21}H_{29}N_3O_2$ $[M+H]^+$. HPLC System A: 14.4 min, purity 99 %, System B: 13.8 min, purity 99 %. ^{1}H NMR (600 MHz, DMSO) δ 8.45 (s, 1H), 7.30 – 7.24 (m, 2H), 7.23 – 7.15 (m, 3H), 7.00 – 6.92 (m, 2H), 6.69 – 6.63 (m, 2H), 5.75 – 5.65 (m, 1H), 3.34 – 3.30 (m, 2H), 3.00 – 2.95 (m, 2H), 2.74 – 2.63 (m, 7H), 2.27 (dd, *J* = 9.2, 4.1 Hz, 1H), 2.24 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 157.66, 155.78, 139.86, 130.83, 130.17 (2C), 129.15 (2C), 128.74 (2C), 126.46, 115.44 (2C), 65.35, 50.36, 40.53, 40.13 (2C), 34.58, 34.13, 31.68.

(*S*)-1-(2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl)-1-methyl-3-phenethylurea x HCOOH 24 (PZM24):



24 was prepared as described for **22** using (*S*)-4-(2-(dimethylamino)-3-(methylamino)propyl)phenol (**37**) (15.0 mg, 72.0 µmol) and 4-nitrophenyl phenethylcarbamate (**42**) (18.7 mg, 66.0 µmol) as starting materials. After purification by preparative HPLC (acetonitrile in 0.1 % aqueous HCOOH, 5 % to 47 % acetonitrile in 13 min), the pure compound (10.3 mg, 39 %) was obtained. LCMS (ESI) t_R = 3.3 – 4.2 min, *m/z* found 356.0, calcd 356.2 $[M+H]^+$. HR EIMS *m/z* found 356.2338, calcd 356.2333 for $C_{21}H_{29}N_3O_2$ $[M+H]^+$. HPLC System A: 14.8 min, purity 100 %, System B: 14.3 min, purity 99 %. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (s, 1H), 7.30 – 7.25 (m, 2H), 7.21 – 7.15 (m, 3H), 6.99 – 6.92 (m, 2H), 6.69 – 6.62 (m, 2H), 6.48 – 6.37 (m, 1H), 3.32 (dd, *J* = 14.5, 8.1 Hz, 1H), 3.20 (ddd, *J* = 9.2, 7.0, 1.6 Hz, 2H), 2.94 (dd, *J* = 14.5, 4.7 Hz, 1H), 2.83 – 2.77 (m, 1H), 2.72 – 2.63 (m, 3H), 2.55 (s, 3H), 2.25 – 2.15 (m, 7H). ^{13}C NMR (91 MHz, DMSO) δ 157.97, 155.27, 139.85, 130.19, 129.66 (2C), 128.55 (2C), 128.18 (2C), 125.82, 115.01 (2C), 64.60, 48.12, 41.77, 40.04 (2C), 36.01, 34.06, 31.00, 30.62.

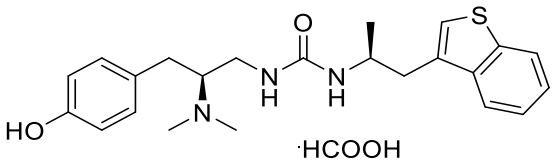
(*S*)-1-(2-(Dimethylamino)-3-(4-hydroxyphenyl)propyl)-1,3-dimethyl-3-phenethylurea x HCOOH 25 (PZM25):



25 was prepared as described for **22** using (*S*)-4-(2-(dimethylamino)-3-(methylamino)propyl)phenol (**37**) (20.0 mg, 96.0 µmol) and 4-nitrophenyl methyl(phenethyl)carbamate (**43**) (52.5 mg, 0.18 mmol) as starting materials. The reaction mixture was stirred 4 h at 120 °C after it had been stirred for 18 h at ambient temperature. Purification by preparative HPLC (acetonitrile in 0.1 % aqueous HCOOH, 5 % to 48 % acetonitrile in 14 min) gave the pure product in 20 % yield (7.8 mg). LCMS (ESI) t_R = 4.2 – 4.8 min, *m/z* found 370.0, calcd 370.2 $[M+H]^+$. HR EIMS *m/z* found 370.2495, calcd 370.2489 for $C_{22}H_{31}N_3O_2$ $[M+H]^+$. HPLC System A: 15.5 min, purity 100 %, System B: 15.3 min, purity 100 %. ^{1}H NMR (600 MHz, DMSO) δ 8.39 (s, 1H), 7.29 – 7.25 (m, 2H), 7.21 – 7.16 (m, 3H), 6.98 – 6.93 (m, 2H), 6.67 – 6.63 (m, 2H), 3.26 (dd, *J* = 13.7, 8.5 Hz, 1H), 3.23 – 3.14 (m, 2H), 2.88 (dd, *J* = 13.8, 5.3 Hz, 1H), 2.86 – 2.80 (m, 1H), 2.78 – 2.70 (m, 2H), 2.66 – 2.60 (m, 4H), 2.60 (s, 3H), 2.19 (s, 6H), 2.18 – 2.14 (m, 1H). ^{13}C NMR (151 MHz,

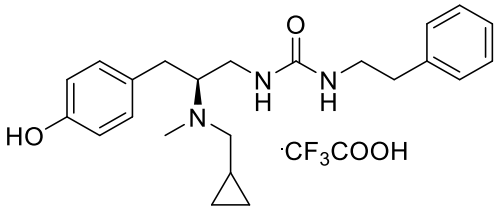
DMSO) δ 163.97, 155.29, 139.52, 130.27, 129.63 (2C), 128.59 (2C), 128.24 (2C), 125.96, 114.98 (2C), 63.10, 51.58, 49.30, 40.07, 40.04, 36.88, 36.32, 33.16, 31.36.

1-((*S*)-1-(Benzo[*b*]thiophen-3-yl)propan-2-yl)-3-((*S*)-2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea x HCOOH 26 (PZM26):



26 was prepared as a mixture of diastereomers as described for **22** using (*S*)-4-[3-amino-2-(dimethylamino)propyl]phenol (**35**) (25.0 mg, 0.13 mmol) and 4-nitrophenyl (1-(benzo[*b*]thiophen-3-yl)propan-2-yl)carbamate (**44**) (43.3 mg, 0.12 mmol) as starting materials. Yield: 31.7 mg (57 %). Diastereomers were separated by semi-preparative HPLC (AS-H chiral column, 0.1 % diethyl amine in isopropanol/hexane 15:85, 12 mL/min, 25 min, t_R (1-((*R*)-1-(benzo[*b*]thiophen-3-yl)propan-2-yl)-3-((*S*)-2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea): 12.5 min, t_R (1-((*S*)-1-(benzo[*b*]thiophen-3-yl)propan-2-yl)-3-((*S*)-2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea (**26**)): 18.5 min). Pure **26** was obtained after additional purification by preparative HPLC (acetonitrile in 0.1 % aqueous HCOOH, 5 % to 74 % acetonitrile in 10 min). LCMS (ESI) t_R = 5.3 – 6.4 min, *m/z* found 412.2, calcd 412.2 $[M+H]^+$, HR EIMS *m/z* found 412.2062, calcd 412.2053 for $C_{23}H_{29}N_3O_2S$ $[M+H]^+$. HPLC System A: 16.8 min, purity 98 %, System B: 14.6 min, purity 95%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.90 – 7.86 (m, 1H), 7.85 – 7.81 (m, 1H), 7.37 – 7.29 (m, 2H), 7.16 (s, 1H), 6.97 – 6.92 (m, 2H), 6.78 – 6.74 (m, 2H), 5.72 (s, 1H), 4.85 (s, 1H), 4.17 (dp, *J* = 13.5, 6.7 Hz, 1H), 3.34 (d, *J* = 13.2 Hz, 1H), 3.12 (dd, *J* = 14.2, 5.4 Hz, 1H), 2.98 (dd, *J* = 13.3, 9.6 Hz, 1H), 2.93 – 2.80 (m, 3H), 2.40 (s, 6H), 2.38 – 2.31 (m, 1H), 1.12 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (91 MHz, DMSO) δ 158.17, 156.17, 139.48, 138.92, 133.52, 130.08 (2C), 124.06, 123.86, 123.23, 122.73, 121.90, 115.35 (2C), 66.86, 45.19, 40.51, 39. 92 (2C), 37.69, 35.54, 30.65, 20.41.

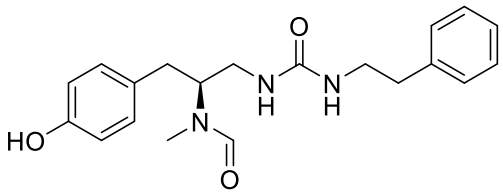
(*S*)-1-(2-((Cyclopropylmethyl)(methyl)amino)-3-(4-hydroxyphenyl)propyl)-3-phenethylurea x CF_3COOH 27 (PZM27):



To compound **39** (23.9 mg, 0.07 mmol) in 3 mL of a 9:1 (v/v) mixture of acetonitrile/ H_2O (3 mL), AcOH (10 µL), cyclopropylcarboxaldehyde (55 µL, 0.73 mmol) and sodium triacetoxyborohydride (155 mg, 0.73 mmol) were added consecutively. After stirring for 12 h at ambient temperature the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and adjusted to pH 8-9 with saturated aqueous $NaHCO_3$/ Na_2CO_3 solution. The mixture was extracted with EtOAc, organic layers were dried (Na_2SO_4), filtered and evaporated. The residue was purified by preparative HPLC (acetonitrile in 0.1 % aqueous CF_3COOH, 10 % to 65 % acetonitrile in 10 min) to give the product as a white powder (31.1 mg, 86 %) after lyophilisation. LCMS (ESI) t_R = 4.8 – 5.5 min, *m/z* found 382.25, calcd 382.25 $[M+H]^+$. HR EIMS *m/z* found 382.2496, calcd 382.2489 for $C_{23}H_{31}N_3O_2$ $[M+H]^+$. HPLC System A: 15.7 min, purity 100 %, System B: 14.1 min,

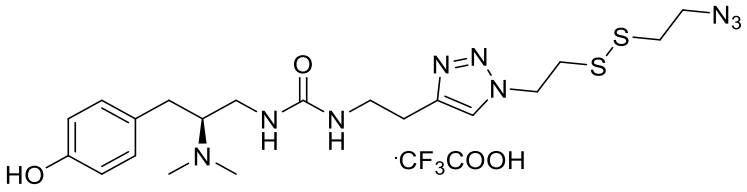
purity 100 %. ^{1}H NMR (3:2 isomer ratio, 600 MHz, D_2O) δ 7.39 – 7.31 (m, 2H), 7.31 – 7.23 (m, 3H), 7.22 – 7.15 (m, 2H), 6.96 – 6.86 (m, 2H), 3.82 – 3.71 (m, 1H), 3.49 (dd, *J* = 15.3, 7.1 Hz), 3.40 (dd, *J* = 15.5, 8.1 Hz, 1H), 3.32 (t, *J* = 6.5 Hz, 2H), 3.27 – 3.15 (m, 2H), 3.09 – 2.97 (m, 2H), 2.95 (dd, *J* = 13.2, 7.6 Hz), 2.90 (s, 1H), 2.87 (s, 2H), 2.82 (dd, *J* = 14.3, 9.1 Hz), 2.78 – 2.69 (m, 3H), 1.12 – 1.00 (m, 1H), 0.79 – 0.66 (m, 2H), 0.44 – 0.30 (m, 2H). ^{13}C NMR (3:2 isomer ratio, 151 MHz, DMSO) δ 159.88, 158.81, 156.30, 156.28, 139.46, 139.36, 130.23, 130.14, 128.58, 128.57, 128.27, 128.26, 126.17, 126.06, 126.03, 126.01, 115.49, 115.44, 66.81, 66.75, 58.19, 56.08, 41.16, 41.03, 38.34, 37.56, 35.92, 35.85, 37.47, 34.92, 31.19, 29.89, 6.34, 6.11, 4.70, 4.14, 3.97, 3.44.

(*S*)-*N*-(1-(4-Hydroxyphenyl)-3-(3-phenethylureido)propan-2-yl)-*N*-methylformamide 28 (PZM28):



(*S*)-1-(3-(4-Hydroxyphenyl)-2-(methylamino)propyl)-3-phenethylurea (**39**) (14.7 mg, 45.0 µmol) was dissolved in a microwave tube in dry acetonitrile (1 mL). Ammonium formate (14.2 mg, 0.23 mmol) was added and the reaction mixture was stirred at reflux temperature for 24 h. Then the solvent was evaporated and the obtained residue was purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to give the compound as a mixture of cis- /trans- isomers in 26 % yield (4.2 mg). LCMS (ESI) t_R = 5.7 – 6.4 min, *m/z* found 356.21, calcd 356.20 $[M+H]^+$. HR EIMS *m/z* found 356.1968 and 378.1793, calcd 356.1969 and 378.1788 for $C_{20}H_{25}N_3O_3$ $[M+H]^+$and $[M+Na]^+$, respectively. HPLC System A: 17.3 min, purity 95 %, System B: 14.9 min, purity 95%. ^{1}H NMR (2:1 isomer ratio, 500 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.47 (s, 1H), 7.41 (s, 1H), 7.29 - 7.10 (m, 5H), 6.99 - 6.98(m, 1H), 6.92 – 6.90 (m, 1H), 6.75 – 6.71 (m, 2H), 5.14 – 4.54 (m, 2H), 3.67 – 3.58 (m, 2H), 3.42 – 3.38 (m, 2H), 3.22 – 3.05 (m, 1H), 2.82 – 2.55 (m, 7H). ^{13}C NMR (2:1 isomer ratio, 125MHz, $CDCl_3$) δ 164.39, 163.85, 158.29, 158.24, 155.57, 155,33, 139.06, 129.74, 128.81, 128.55, 128.20, 127.88, 126.38, 115.78, 115.58, 61.13, 41.57, 41.40, 40.67, 40.49, 36.33, 36.29, 35.38, 34.76, 30.82, 29.67.

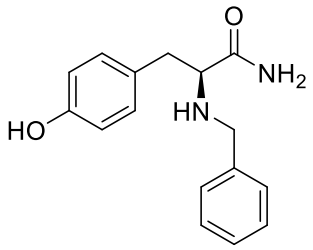
(*S*)-1-(2-(1-(2-((2-Azidoethyl)disulfanyl)ethyl)-1*H*-1,2,3-triazol-4-yl)ethyl)-3-(2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea x CF_3COOH 29 (PZM29):



29 was synthesized via CuAAc reaction: (*S*)-1-(But-3-yn-1-yl)-3-(2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea (**46**) (31.5 mg, 0.11 mmol), $CuSO_4$ x $5H_2O$ (7.10 mg, 28.4 µmol), sodium ascorbate (8.10 mg, 41.0 µmol) and bis(2-azidoethyl) disulfide (46.3 mg, 0.23 mmol) were dissolved in of DMF/H_2O (1.2 mL, 2:1 (v/v)) and stirred at room temperature for 6 h. The reaction mixture was quenched with 0.1M EDTA-solution, adjusted to pH 8-9 with saturated aqueous $NaHCO_3$/ Na_2CO_3 solution and extracted with EtOAc. Combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (acetonitrile in 0.1 % aqueous CF_3COOH,

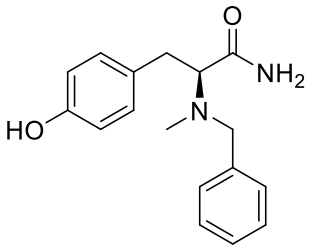
5 % to 50 % acetonitrile in 12 min) to give the pure product in 44 % (29 mg) yield. LCMS (ESI) t_R = 3.5 – 4.5 min, *m/z* found 494.1, calcd 494.2 $[M+H]^+$. HR EIMS *m/z* found 494.2110, calcd 494.2115 for $C_{20}H_{31}N_9O_2S_2$ $[M+H]^+$. HPLC System C: 16.1 min, purity 98 %, System D: 14.4 min, purity 98 %. ^{1}H NMR (600 MHz, DMSO) δ 9.64 (s, 1H), 9.37 (s, 1H), 7.92 (s, 1H), 7.18 – 7.01 (m, 2H), 6.80 – 6.67 (m, 2H), 6.38 (s, 2H), 4.61 (t, *J* = 6.6 Hz, 2H), 3.59 (t, *J* = 6.4 Hz, 2H), 3.49 – 3.40 (m, 1H), 3.32 – 3.25 (m, 3H), 3.23 (t, *J* = 6.6 Hz, 2H), 3.21 – 3.12 (m, 1H), 3.00 (dd, *J* = 13.8, 3.5 Hz, 1H), 2.95 (t, *J* = 6.4 Hz, 2H), 2.88 (d, *J* = 4.9 Hz, 3H), 2.81 (d, *J* = 4.9 Hz, 3H), 2.74 (t, *J* = 7.3 Hz, 2H), 2.60 (dd, *J* = 13.7, 10.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 158.76, 156.28, 144.50, 130.19 (2C), 126.14, 122.50, 115.44 (2C), 67.14, 49.11, 47.90, 40.90, 40.06, 38.32, 37.85, 37.20, 36.70, 30.56, 26.22.

(*S*)-2-(Benzylamino)-3-(4-hydroxyphenyl)propanamide 32:



To a suspension of L-tyrosinamide (**30**) (0.80 g, 4.47 mmol) in a 9:1 (v/v) mixture of acetonitrile/ H_2O (19 mL), AcOH (0.40 mL), benzaldehyde (0.47 g, 4.44 mmol) and sodium triacetoxyborohydride (2.82 g, 13.3 mmol) were added. The reaction mixture was stirred for 2 hours and quenched with a saturated aqueous solution of $NaHCO_3$. The pH was adjusted to pH 8-9 by saturated aqueous $NaHCO_3$/ Na_2CO_3 solution and extracted with isopropanol/EtOAc (1:3). The organic layers were dried (Na_2SO_4), filtered and evaporated. The product (901 mg, 75 %) was obtained as a white solid after purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 15:1:0.1). LCMS (ESI) *m/z* found 271.0, calcd 271.1 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 9.14 (s, 1H), 7.33 (d, *J* = 1.9 Hz, 1H), 7.27 – 7.22 (m, 2H), 7.21 – 7.17 (m, 3H), 7.03 – 6.96 (m, 3H), 6.66 – 6.62 (m, 2H), 3.68 (d, *J* = 13.6 Hz, 1H), 3.47 (d, *J* = 13.6 Hz, 1H), 3.12 (dd, *J* = 7.6, 6.0 Hz, 1H), 2.75 (dd, *J* = 13.6, 5.8 Hz, 1H), 2.61 (dd, *J* = 13.6, 7.8 Hz, 1H), 2.10 (s, 1H). ^{13}C NMR (91 MHz, DMSO) δ 175.60, 155.57, 140.37, 130.05 (2C), 128.57, 128.00 (2C), 127.66 (2C), 126.49, 114.76 (2C), 62.83, 50.99, 38.40.

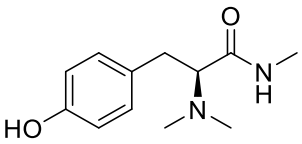
(*S*)-2-(Benzyl(methyl)amino)-3-(4-hydroxyphenyl)propanamide 33:



Compound **33** was prepared as described for compound **(*R*)-16b**. Starting from (*S*)-2-(benzylamino)-3-(4-hydroxyphenyl)propanamide (**32**) (0.80 g, 2.96 mmol), 37% aqueous formaldehyde (1.20 mL, 14.8 mmol) and sodium triacetoxyborohydride (1.88 g, 8.88 mmol), the product was obtained (0.76 g, 90 %) as a white solid after purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 20:1:0.1). LCMS (ESI) *m/z* found 285.0, calcd 285.2 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 9.12 (s, 1H), 7.31 – 7.18 (m, 6H), 7.05 – 6.98

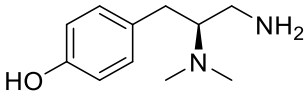
(m, 2H), 6.93 (d, *J* = 1.6 Hz, 1H), 6.69 – 6.62 (m, 2H), 3.67 (d, *J* = 13.7 Hz, 1H), 3.54 (d, *J* = 13.7 Hz, 1H), 3.37 – 3.30 (m, 1H), 2.92 (dd, *J* = 13.6, 8.5 Hz, 1H), 2.73 (dd, *J* = 13.6, 5.9 Hz, 1H), 2.14 (s, 3H). ^{13}C NMR (91 MHz, DMSO) δ 172.40, 155.36, 139.84, 129.93 (2C), 129.44, 128.28 (2C), 128.00 (2C), 126.61, 114.79 (2C), 68.16, 57.72, 37.92, 33.13.

(*S*)-2-(Dimethylamino)-3-(4-hydroxyphenyl)-*N*-methylpropanamide 34:



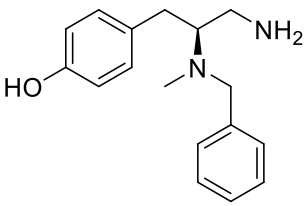
Compound **34** was prepared as described for compound **(*R*)-16b**. Starting from (*S*)-2-amino-3-(4-hydroxyphenyl)-*N*-methylpropanamide (**31**) (0.40 g, 2.06 mmol), 37% aqueous formaldehyde (1.54 mL, 20.6 mmol) and sodium triacetoxyborohydride (2.20 g, 10.3 mmol) the pure product was obtained (0.40 g, 88%) as a white solid after purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 9:1:0.1). LCMS (ESI) *m/z* found 222.92, calcd 223.14 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 9.09 (s, 1H), 7.61 (d, *J* = 4.5 Hz, 1H), 7.00 – 6.88 (m, *J* = 8.4 Hz, 2H), 6.67 – 6.55 (m, 2H), 3.04 (dd, *J* = 9.0, 5.4 Hz, 1H), 2.81 (dd, *J* = 13.4, 9.0 Hz, 1H), 2.63 (dd, *J* = 13.4, 5.3 Hz, 1H), 2.53 – 2.46 (m, 3H), 2.21 (s, 6H). ^{13}C NMR (91 MHz, DMSO) δ 170.61, 155.34, 129.76 (2C), 129.32, 114.79 (2C), 69.53, 41.62 (2C), 33.33, 25.00.

(*S*)-4-[3-Amino-2-(dimethylamino)propyl]phenol 35:



Compound **35** was synthesized following the synthetic protocol for **(*S*)-17b**. Starting from **(*S*)-16b** (4.10 g, 16.8 mmol) and 1M borane-tetrahydrofurane complex (102 mL, 103 mmol), the product was obtained as a yellowish white foam (3.10g, 95%) after flash chromatography (CH_2Cl_2/MeOH/NH_3 9:1:0.1). LCMS (ESI) *m/z* found 194.90, calcd 195.15 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 9.11 (s, 1H), 6.98 – 6.92 (m, 2H), 6.69 – 6.63 (m, 2H), 2.68 (dd, *J* = 13.4, 3.9 Hz, 1H), 2.48 – 2.40 (m, 2H), 2.34 (d, *J* = 8.9 Hz, 1H), 2.26 – 2.14 (m, 7H). ^{13}C NMR (91 MHz, DMSO) δ 155.28, 130.36, 129.77 (2C), 115.02 (2C), 67.51, 40.63, 40.08 (2C), 30.18.

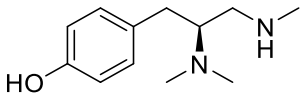
(*S*)-4-(3-Amino-2-(benzyl(methyl)amino)propyl)phenol 36:



Compound **36** was synthesized as described for compound **35** using (*S*)-2-(benzyl(methyl)amino)-3-(4-hydroxyphenyl)propanamide (0.70 g, 2.46 mmol) as starting material. **36** (0.52 g, 78 %) was obtained after purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 9:1:0.1). LCMS (ESI) *m/z* found 271.03, calcd

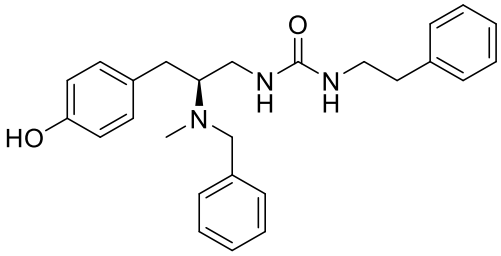
271.18 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 7.37 – 7.24 (m, *J* = 8.4, 4.6 Hz, 4H), 7.24 – 7.17 (m, 1H), 7.02 – 6.89 (m, *J* = 8.4 Hz, 2H), 6.72 – 6.61 (m, 2H), 3.70 (d, *J* = 13.5 Hz, 1H), 3.55 (d, *J* = 13.5 Hz, 1H), 2.78 (dd, *J* = 13.4, 3.2 Hz, 1H), 2.63 (s, 2H), 2.38 (dd, *J* = 4.7, 2.7 Hz, 1H), 2.29 (dd, *J* = 13.3, 8.5 Hz, 1H), 2.16 (s, 3H).

(*S*)-4-(2-(Dimethylamino)-3-(methylamino)propyl)phenol 37:



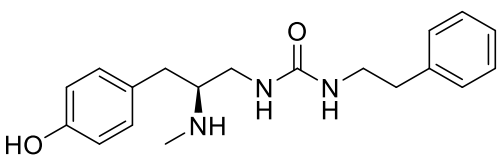
Compound **37** was prepared as described for compound **35** using (*S*)-2-(dimethylamino)-3-(4-hydroxyphenyl)-*N*-methylpropanamide (**34**) (0.38 g, 1.71 mmol) as starting material. The product was obtained after purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 9:1:0.1) in 11 % (38.2 mg) yield. LCMS (ESI) *m/z* found 208.89, calcd 209.16 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 9.13 (s, 1H), 6.98 – 6.90 (m, *J* = 5.6 Hz, 2H), 6.71 – 6.58 (m, 2H), 2.70 (dd, *J* = 13.3, 4.5 Hz, 1H), 2.67 – 2.59 (m, 1H), 2.48 – 2.43 (m, 1H), 2.26 (dd, *J* = 12.0, 4.2 Hz, 1H), 2.24 – 2.15 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.25, 130.34, 129.75, 129.73, 115.05, 114.96, 64.70, 50.92, 40.04 (2C), 35.86, 30.50.

(*S*)-1-(2-(Benzyl(methyl)amino)-3-(4-hydroxyphenyl)propyl)-3-phenethylurea 38:



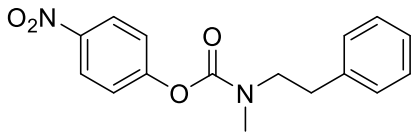
Compound **38** was prepared as described for **22** using (*S*)-4-(3-amino-2-(benzyl(methyl)amino)propyl)phenol (**36**) (0.11g, 0.42 mmol) and 4-nitrophenyl phenethylcarbamate (**42**) (0.10 g, 0.35 mmol) as starting materials. Pure product was obtained after flash chromatography (CH_2Cl_2/MeOH/NH_3 50:1:0.1 → 40:1:0.1 → 30:1:0.1) in 93 % (0.14 g) yield. LCMS (ESI) *m/z* found 418.22, calcd 418.25 $[M+H]^+$. ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.43 – 7.07 (m, 10H), 7.00 – 6.85 (m, 2H), 6.85 – 6.65 (m, 2H), 5.10 (d, *J* = 5.6 Hz, 1H), 4.54 – 4.31 (m, 1H), 3.74 (d, *J* = 13.1 Hz, 1H), 3.53 (d, *J* = 13.2 Hz, 1H), 3.43 – 3.26 (m, 3H), 3.02 – 2.88 (m, 2H), 2.87 – 2.78 (m, 1H), 2.76 (t, *J* = 7.0 Hz, 2H), 2.32 (dd, *J* = 13.2, 10.4 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 157.35, 154.32, 138.25, 137.92, 128.86, 128.83 (2C), 127.80, 127.78, 127.76 (2C), 127.56 (2C), 127.39 (2C), 126.15, 125.41, 114.60 (2C), 62.91, 56.85, 40.87, 39.49, 35.23, 35.15, 30.01.

(*S*)-1-(3-(4-Hydroxyphenyl)-2-(methylamino)propyl)-3-phenethylurea 39:



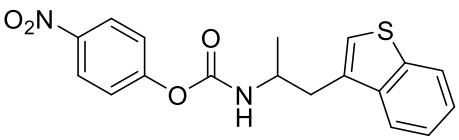
Compound **38** (0.12 g, 0.30 mmol) was dissolved in MeOH (10 mL) in a Schlenk flask and stirred under N_2 atmosphere. 1,1,2-Trichloroethane (27 µL, 0.30 mmol) and 10% Pd-C were added and the mixture was stirred for 5 h under H_2 atmosphere at ambient temperature. The suspension was filtered through celite, the filtrate was evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH/NH_3 15:1:0.1) to give pure **39** in 87 % (81.3 mg) yield. LCMS (ESI) *m/z* found 328.15, calcd 328.20 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 7.36 – 7.24 (m, 2H), 7.23 – 7.14 (m, 3H), 7.09 – 6.93 (m, 2H), 6.75 – 6.61 (m, 2H), 6.16 (s, 2H), 3.30 – 3.15 (m, 2H), 3.17 – 3.02 (m, 1H), 3.02 – 2.72 (m, 2H), 2.72 – 2.50 (m, 4H), 2.29 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 158.93, 156.30, 140.21, 130.57 (2C), 129.13 (2C), 128.76 (3C), 126.45, 115.64 (2C), 61.01, 41.48, 40.55, 40.43, 39.59, 36.64.

4-Nitrophenyl methyl(phenethyl)carbamate 43:



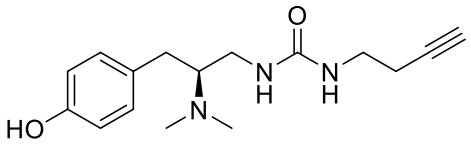
4-Nitrophenyl chloroformate (0.32 g, 1.56 mmol) in a Schlenk flask was solved in dry THF (4 mL) and stirred under N_2 atmosphere in an ice-water-bath. A mixture of *N*-methyl-(*β*-phenylethyl) amine (**40**) (0.20g, 1.48 mmol) and triethylamine (0.41 mL, 2.96 mmol) in dry THF (5 mL) was added dropwise. The reaction mixture was allowed to warm up to room temperature and was stirred for 8 h. The mixture was diluted with dichloromethane, filtered and the filtrate was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (100% dichloromethane) to give the product as a white solid (85 %, 0.38 g) after trituration with hexane. LCMS (ESI) *m/z* found 300.84, calcd 301.12 $[M+H]^+$. ^{1}H NMR (400K, 600 MHz, DMSO) δ 8.20 – 8.15 (m, 2H), 7.34 – 7.22 (m, 6H), 7.22 – 7.17 (m, 1H), 3.63 (t, *J* = 7.1 Hz, 2H), 2.96 (s, 3H), 2.92 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (mixture of rotamers, 300K, 151 MHz, DMSO) δ 156.24, 156.10, 152.55, 144.22, 138.81, 138.67, 128.91, 128.73, 128.35, 126.26, 125.01, 124.87, 122.66, 122.53, 50.34, 50.17, 34.58, 34.45, 33.53, 32.88.

4-Nitrophenyl (1-(benzo[*b*]thiophen-3-yl)propan-2-yl)carbamate 44:



Compound **44** was prepared in racemic form as described for compound **43** using 1-(1-benzothiophen-3-yl)propan-2-amine hydrochloride (0.11 g, 0.49 mmol), 4-nitrophenyl chloroformate (97.5 mg, 0.49 mmol) and triethylamine (0.14 mL, 0.97 mmol) as starting materials. Pure **44** was obtained after purification by flash chromatography (100% dichloromethane). Yield: 84.7 mg (49 %) as a white solid after trituration with hexane. LCMS (ESI) *m/z* found 357.13, calcd 357.09 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 – 8.18 (m, 2H), 7.93 – 7.82 (m, 2H), 7.39 (dt, *J* = 23.2, 7.6 Hz, 2H), 7.30 – 7.26 (m, 2H), 7.22 (s, 1H), 5.05 (d, *J* = 7.7 Hz, 1H), 4.26 – 4.15 (m, 1H), 3.25 (dd, *J* = 14.2, 5.7 Hz, 1H), 3.02 (dd, *J* = 14.3, 7.4 Hz, 1H), 1.30 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (91 MHz, $CDCl_3$) δ 155.76, 152.34, 144.73, 140.38, 138.91, 132.18, 125.03 (2C), 124.36, 124.14, 123.46, 122.86, 121.89 (2C), 121.75, 47.36, 35.54, 20.30.

(*S*)-1-(But-3-yn-1-yl)-3-(2-(dimethylamino)-3-(4-hydroxyphenyl)propyl)urea 46:



N-3-Butyn-1-yl-1*H*-imidazole-1-carboxamide (**45**) (0.33 g, 2.01 mmol) and (*S*)-4-[3-amino-2-(dimethylamino)propyl]phenol (**35**) (0.50 g, 2.57 mmol) were solved in dry DMF (10 mL) and stirred for 24 h under Ar atmosphere at 50 °C. H_2O was added and the mixture was lyophilisated. After purification by flash chromatography (CH_2Cl_2/MeOH/NH_3 9:1:0.1) the product was obtained as a white solid (0.47 g, 80 %). LCMS (ESI) *m/z* found 290.05, calcd 290.19 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97 – 6.91 (m, 2H), 6.75 – 6.70 (m, 2H), 5.28 (d, *J* = 5.4 Hz, 1H), 5.14 (s, 1H), 3.35 – 3.24 (m, 3H), 2.96 – 2.90 (m, 1H), 2.86 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.69 (tt, *J* = 10.2, 3.8 Hz, 1H), 2.37 – 2.34 (m, 2H), 2.33 (s, 6H), 2.24 (dd, *J* = 13.4, 10.6 Hz, 1H), 1.98 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.53, 155.33, 129.89 (2C), 129.58, 115.69 (2C), 82.02, 69.82, 65.77, 40.32, 40.16 (2C), 39.09, 30.35, 20.11.

References

(1) Liu, G.; Liu, X.; Cai, Z.; Jiao, G.; Xu, G.; Tang, W. Design of Phosphorus Ligands with Deep Chiral Pockets: Practical Synthesis of Chiral B-Arylamines by Asymmetric Hydrogenation. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 4235–4238.

(2) Pedersen, D. S.; Rosenbohm, C. Dry Column Vacuum Chromatography. *Synthesis (Stuttg)*. **2001**, *2001*, 2431–2434.

Supplementary Table. Overview of molecular dynamics simulations

μOR structure	His297$^{6.52}$ protonation state	Simulation size (# total atoms)	Length of each simulation	Total simulation time
Inactive	δN (HID)	101,161	350 ns; 450 ns; 350 ns	2.0 μs
Inactive	εN (HIE)	101,167	400 ns; 450 ns	
Active	δN (HID)	101,473	950 ns; 900 ns; 950 ns	3.6 μs
Active	εN (HIE)	101,476	1000 ns; 950 ns; 950 ns	