Supplementary Information

Covalent agonists for studying G protein-coupled receptor activation

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**SI Materials and Methods:**

**β₂-adrenergic receptor expression and purification**

Human β₂-adrenergic receptor (β₂AR) bearing an amino-terminal FLAG epitope tag, truncated after residue 365 and with or without H93C mutation was expressed in Sf9 cells using the FastBac baculovirus system (Expression Systems; Davis, CA). Cells were infected at a density of $4 \times 10^6$ cells/mL and then were incubated for two days at 27 °C. Receptor was extracted as described previously (1). Receptor was first purified by FLAG affinity chromatography, then purified by alprenolol sepharose chromatography to isolate only functional receptor. Alprenolol sepharose eluate was concentrated on FLAG affinity resin and washed with ligand-free buffer for 30 minutes at room temperature to eliminate bound alprenolol. Detergent was gradually exchanged from dodecyl maltoside (DDM) to lauryl maltose neopentyl glycol (MNG) by washing in buffer containing decreasing amounts of DDM and MNG at a fixed concentration of 0.1% (w/w). Receptor was eluted, aliquoted, and frozen in 20% glycerol.

**G protein activation assay with ICI-118,551 reversal**

Wild-type β₂AR or β₂AR<sup>H93C</sup> were purified in unliganded form as described above. Samples of these receptors were reconstituted into rHDL (recombinant high density lipoprotein) particles as described (2). For the [³⁵S]GTP<sub>γ</sub>S binding assay, receptor-rHDL particles were preincubated with 5 µM of 2 or 5 µM alprenolol for 4 h at 4 °C. Samples were diluted 20-fold into binding buffer and split. Half of the samples were used in [³⁵S]GTP<sub>γ</sub>S binding assays without ICI-118,551 competition, while the other half was incubated with 20 µM ICI-118,551 at room temperature for 1 h. Control samples of receptor-rHDL particles with no ligand, 20 µM isoproterenol or 20 µM ICI-118,551 were also prepared. Purified G<sub>s</sub> heterotrimer (3) was added to each sample and incubated for 10 min at 23 °C. The final concentrations of reconstituted receptor and G<sub>s</sub> were 100 nM and 600 nM, respectively. [³⁵S]GTP<sub>γ</sub>S binding reactions were
initiated by the addition of 0.4 nM [$^{35}$S]GTP$\gamma$S. Free [$^{35}$S]GTP$\gamma$S was removed by rapid filtration of the particles using glass fibre filters. Filter-bound radioactivity was determined by liquid scintillation counting using a Beckman LS6000 scintillation counter. The data shown in Fig. 2D are from three independent experiments each performed in triplicate.

**Radioligand depletion assay for the $\beta_2$AR ligands**

Purified and unliganded $\beta_2$AR$^{H93C}$ was obtained as described earlier. Samples of these receptors were reconstituted into rHDL (recombinant high density lipoprotein) particles as described (2). For the depletion assay receptor-rHDL particles were preincubated at 25 °C with either 10 nM of the covalent ligands or 10 nM alprenolol in binding buffer (75 mM Tris pH 7.4, 12.5 mM MgCl$_2$, 1 mM EDTA, 1 mM ascorbic acid, 1 mg/ml BSA). At different times, aliquots were removed, and diluted 10-fold into fresh binding buffer and [$^3$H]dihydroalprenolol (DHA, Perkin Elmer) was added to a final concentration of 10 nM. These competition reactions were incubated for 60 minutes, and membranes were filtered and counted for bound radioactivity using a Beckman LS6000 scintillation counter. Non-specific binding was measured using identical reactions with 10 μM cold alprenolol added. The data in Figure 2A correspond to residual specific binding after different times of preincubation and was obtained from three independent experiments each performed in triplicate.

**Expression of nanobody in E. coli**

Nanobodies were cloned into the periplasmic expression vector pET26b, containing an amino terminal signal sequence and a carboxy terminal 8x Histidine tag, and were transformed into BL21(DE3) Rosetta2 E. coli (Novagen). Cells were induced in Terrific Broth at an OD$_{600nm}$ of 0.8 with 1 mM IPTG and incubated with shaking at 22°C for 24 hours. Periplasmic protein was obtained by osmotic shock and the nanobodies were purified using nickel-nitrilotriacetic acid (Ni-NTA) chromatography (4). Eluted nanobodies were digested with carboxypeptidase A (sigma) to
remove His tag, then purified by size-exclusion chromatography over a Sephadex S200 size-exclusion column (GE Healthcare).

**Purification and crystallization of $\beta_2$AR$^{H93C}$-Nb6B9 complex.**

Human $\beta_2$AR$^{H93C}$ fused to an N-terminal T4lysozyme (5) was expressed in Sf9 insect cells and purified as described earlier. After purification by alprenolol sepharose, the receptor was washed extensively with 30 $\mu$M of the low-affinity antagonist atenolol while bound to Flag affinity resin to fully displace alprenolol, then washed and eluted in buffer devoid of ligand to produce a homogeneously unliganded preparation. The receptor was then incubated for 60 min. at room temperature with a stoichiometric excess of compound 2. A 1.3-fold molar excess of Nb6B9 was then added and incubated for additional 30 min. The sample was then concentrated using a 50kDa spin concentrator and purified over a Sephadex S200 size-exclusion column (GE Healthcare) in a buffer that consisted of 100 mM sodium chloride, 20 mM HEPES pH 7.4, 0.01% lauryl maltose neopentyl glycol detergent, and 0.001% cholesterylhemisuccinate and the ligand 2, included at a concentration of 10 $\mu$M. The $\beta_2$AR-Nb6B9-2 ternary complex was isolated.

After purification, samples were concentrated to $A_{280nm} = 48$ using a 50kDa concentrator to minimize the detergent concentration in the final sample, then aliquoted into thin-walled PCR tubes at 8 $\mu$L per aliquot. Aliquots were flash frozen in liquid nitrogen and stored at -80°C for crystallization trials. For crystallization, samples were thawed and reconstituted into lipidic cubic phase with a 1:1 mass:-mass ratio of lipid. The lipid stock consisted of a 10:1 mix by mass of 7.7 monoacylglycerol (provided by M. Caffrey) with cholesterol (Sigma). Samples were reconstituted by the two-syringe mixing method (6) and then dispensed into glass sandwich plates using a GryphonLCP robot (Art Robbins Instruments). Crystals were grown using 35 nL protein/lipid drops with 600 nL overlay solution, which consisted of 24–27 % PEG400, 100 mM
Tris pH 7.8 to pH 8.4, and 5-10 mM Sodium formate. Crystals grew in 1-2 days, and were harvested and frozen in liquid nitrogen for data collection.

**Crystallographic data collection and refinement.**

X-ray diffraction data were collected at Advanced Photon Source GM/CA beamline 23ID-B. The best diffracting crystals were identified by rastering, and wedges of 1–10° were collected using a 10 μm beam with typically 2 s exposure, 0.6° oscillation, and no beam attenuation. Data collection statistics are summarized in Supplementary Table 1. Diffraction data were processed in HKL 2000 (7) (HKL Research, Inc.), and the structure was solved using molecular replacement with the structures of active β2AR bound to Nb6B9 (PDB accession 4LDO (8)) as search models in Phaser (9). The resulting structure was iteratively refined in Phenix (10) and manually rebuilt in Coot (11). Final refinement statistics are summarized in Table S1. Figures were prepared in PyMol.

**Radioligand depletion assay for the D₂R and 5-HT₂A receptor ligands.**

Membranes from human embryonic kidney (HEK 293) cells transiently expressing the human D₂RL94C were preincubated in binding buffer (50 mM Tris, 1 mM EDTA, 5 mM MgCl₂, 100 μg/mL bacitracin, 5 μg/mL soybean trypsin inhibitor at pH 7.4) at a protein concentration of 210 µg/mL and the covalent ligand 3 (at 0.1 μM) or the reference quinpirole (at 5 μM) for different times. Tests with the human 5-HT₂AS131G-T134C were done at 280 μg/mL protein with 4 (at 1 μM) or serotonin (at 1 μM). Incubation was stopped by centrifugation and the amount of reversibly bound ligand was washed out for three times (resuspension in buffer for 30 min and centrifugation). Membranes were then used for radioligand binding experiments with [³H]spiperone (specific activity = 81 Ci/mmol) or [³H]ketanserin (specific activity = 53 Ci/mmol) (both: PerkinElmer, Rodgau, Germany) to determine specific binding at the D₂R and the 5-HT₂A
receptor, respectively as described (12). Non-specific binding was determined in the presence of 10 μM haloperidol (for D2R) or ketanserin (for 5-HT2A receptor). Data in Figure 2A and C correspond to an average specific binding of three independent experiments each performed in triplicate.

**Determination of Covalent D2 and 5-HT2A and H1 receptor activation via inositol phosphate assays.**

Agonist-induced activation of the human D2<sub>L94C</sub>, 5-HT<sub>2A</sub><sup>S131G-T134C</sup> and H1<sup>Y87C</sup> receptors was studied employing inositol phosphate (IP) accumulation assays as described (13, 14). For activation studies with G<sub>i</sub> coupled GPCRs, HEK 293 cells were transiently co-transfected with cDNA encoding for D<sub>2</sub>R<sup>L94C</sup> and the hybrid G protein Gα<sub>qi5</sub> (Gα protein with the last five amino acids at the C terminus replaced by the corresponding sequence of Gα<sub>i</sub>; gift from The J. David Gladstone Institutes) (15). Twenty-four hours after transfection, cells were transferred into 24-well plates. After adding myo-[^3]H]inositol (specific activity = 22.5 Ci mmol<sup>-1</sup>, PerkinElmer) and incubation for 15 h, medium was aspirated, the cells were washed with serum-free medium supplemented with 10 mM LiCl, and incubated with 3 (at 300 nM) or quinpirole (at 30 nM) at 37 °C. After incubation for 30 min, the antagonist haloperidol (10 μM) was added to one-half of the samples (buffer to the other half) and incubation was continued for additional 150 min. Then, cells were lysed by adding 0.1 M NaOH. After neutralization with formic acid, the cell extract was separated by anion-exchange chromatography using an AG1-X8 resin (Bio-Rad, Munich, Germany) by washing and finally eluting total IP directly into scintillation counting vials. Radioactivity was determined by scintillation counting in a Beckman LS 6500 (Beckman, Krefeld, Germany). Data were analyzed by normalizing disintegrations per minute (d.p.m.) values n-fold to the data for basal stimulation by only buffer.
Activation studies with a $G_\alpha_\text{q}$ coupled GPCR were done with HEK 293 cells, which were transiently transfected with cDNA encoding for the $5-HT_2^\text{A}_{\text{S131G-T134C}}$ or $H_1^{\text{Y87C}}$ receptor, similar to the method described above. After incubation of $5-HT_2^\text{A}_{\text{S131G-T134C}}$ with compound 4 and serotonin at concentrations of 1 $\mu$M for 90 min, respectively, inhibition of further receptor activation was initiated by adding 10 $\mu$M of ketanserin to half of the samples. After incubation for further 150 min, total IP was determined as described. Likewise, the $H_1^{\text{Y87C}}$ receptor was incubated with compound 5 and histamine for 90 min at concentrations of 10 $\mu$M and 1 $\mu$M, respectively, then diphenhydramine (10 $\mu$M) was added to half of the samples. After further incubation for 150 min, total IP was determined as described.
**SI Figures and Tables:**

**Figure S1:** Synthesis of the covalent neurotransmitter analogs 1-7. The key step comprises chemoselective N-alkylation of the respective biogenic amines using the prepared electrophilic building blocks 22-25. Adjustment of base and temperature and the use of the solvent DMSO guaranteed a successful conversion of the starting material and a low degradation of the corresponding products. After the alkylation reaction, an excess of degassed water was added directly to the mixture, which was subsequently frozen and lyophilized to remove DMSO. The remaining material was dissolved in methanol and cysteamine HCl was added. Immediate purification by preparative HPLC yields the pure (nor)adrenaline-derived products 1, 2, 6 and 7, the covalent dopamine derivative 3 and the histamine analog 5. All steps had been carried out under inert atmosphere, since the catechol and 5-hydroxyindole-derived ligands are inherently unstable.

**L-noradrenaline:**

nhg = 1,2-dihydroxy-4-phenyl, X = OH

**dopamine:**

nhg = 1,2-dihydroxy-4-phenyl, X = H

**histamine:**

nhg = 1H-imidazol-4-yl, X = H

nhg: native head group

1: X = OH, Y = OMe, n = 2
2: X = OH, Y = OMe, n = 1
3: X = H, Y = OMe, n = 2
6: X = OH, Y = H, n = 2,
7: X = OH, Y = H, n = 1

**Notes:**
a, 1) DMSO, 70°C, argon, 7-9 h, 2) cysteamine HCl, MeOH, rt, argon, 5-22 %. 
Figure S2: Synthesis of the serotonin derivative 4. After N-alkylation of unprotected serotonin according to the procedure in Fig. S1, the product showed substantial impurities even after preparative HPLC. Protection of the hydroxyl group of serotonin with a tert-butyldimethylsilyl (TBS) group yielded intermediate 28. Using building block 24, N-alkylation of 28 gave the stable precursor 29, which could be isolated by flash chromatography. Starting from purified compound 29, final disulfide-exchange and deprotection reaction gave access to pure product 4.
Figure S3: Synthesis of the key intermediates 22-25. Alkylation of the phenylethylalcohols 8, 9, followed by a nucleophilic substitution with thioacetate, yielded the stable precursors 14-17 via the intermediates 10-13. Subsequent methanolysis of the thioester function, in situ formation of the disulfide moiety and O-activation of the aliphatic alcohol moiety with methanesulfonyl chloride gave the required electrophilic building blocks 22-25.
Figure S4: Structure of the $\beta_2$AR$^\text{H93C}$ (green) bound to FAUC50 (blue), aligned with covalent (nor)adrenaline analog 2 (magenta). The notion that the linker in FAUC50 appeared to be longer than necessary led to the development of the short chain derivative 2, which displayed a considerably higher efficiency in the formation of the covalent bond than its predecessor 1 (Fig. S5).
Figure S5: Radioligand depletion assay of compounds 1, 2, 6 and 7 with $\beta_2$AR$_{H93C}$. A small series of covalent (nor)adrenaline-derived ligands 1 (green), 2 (magenta), 6 (red) and 7 (blue) was synthesized to probe the structure of the linker moiety. Their efficiency in the formation of the covalent bond was assessed, using purified $\beta_2$AR$_{H93C}$ reconstituted into HDL particles. [$^3$H]Dihydroalprenolol binding after different time frames of incubation revealed various fractions of covalent ligand-receptor complexes compared to the reversible reference alprenolol (black). The ligand without a methoxy group and with a longer linker (6) displays the lowest efficiency in the formation of the covalent bond. Compound 1 has a long linker, together with the methoxy substituent, which resulted in a higher fraction of covalent ligand-receptor complexes of 1 compared to 6. The short linker in 7 compensates for the lack of the methoxy group, making it equally effective as 1. Compound 2 combines a short linker and a methoxy moiety, leading to the highest efficiency in the formation of covalent ligand-receptor complexes.
Figure S6: IP-accumulation assay for activation of D₂R<sup>wt</sup> and D₂R<sup>L94C</sup>. The investigation of activation of the wild-type dopamine receptor D₂R<sup>wt</sup> (black) and the mutant D₂R<sup>L94C</sup> (blue) by compound 3 reveals full agonist activity at both receptors relative to the effect of the reference agonist quinpirole. The representative curves of two individual experiments result in potencies of 92 nM and 19 nM for the wild-type and the mutant, respectively. Experiments were carried out as described in literature (13).
Figure S7: Radioligand depletion assay of compound 4 with the serotonin receptor mutant 5-HT_{2A}^{T134C}. The covalent ligand 4 (green) and the reversible reference serotonin (black) were pretreated with membrane-bound 5-HT_{2A}^{T134C} for different time frames. After washing, the membranes were incubated with [^3H]ketanserin and specific binding was determined yielding a moderate fraction of covalent ligand-receptor complexes compared to the reversible neurotransmitter serotonin.
**Figure S8:** IP-accumulation assay for activation of 5-HT$_{2A}^{wt}$, 5-HT$_{2A}^{T134C}$ and 5-HT$_{2A}^{S131G-T134C}$.

The determination of activation of the wild-type serotonin receptor 5-HT$_{2A}^{wt}$ (black) and the mutant receptors 5-HT$_{2A}^{T134C}$ (blue) and 5-HT$_{2A}^{S131G-T134C}$ (green) by compound 4 reveals full agonist activity at all receptors relative to the effect of the neurotransmitter serotonin. The representative curves of two individual experiments result in potencies of 2.6 nM, 14 nM and 6.5 nM for the wild-type and the mutant receptors, respectively. Experiments were carried out as described in literature (13).
Figure S9: IP-accumulation assay for activation of wild-type H₁R. The investigation of activation of the wild-type histamine receptor H₁R<sup>wt</sup> by histamine (grey) and compound 5 (black) reveals agonist activity for 5 of 90% relative to the full effect of histamine. The representative curves of one individual experiment result in potencies of 50 nM and 740 nM for histamine and 5, respectively. Experiments were carried out as described in literature (13).
Figure S10: An IP-accumulation assay for the covalent histamine analog 5 was conducted to measure activation at H$_1$R$^{Y87C}$ in comparison to the reference histamine. While the effect of histamine is considerably reduced by an excess of the antagonist diphenhydramine, the 5-H$_1$R$^{Y87C}$ complex still induces a substantial non-reversible IP formation (blue hatched).
Figure S11: $F_0 - F_c$ electron density map for 2 (contoured at 2.4 $\sigma$ within a 3 Å radius of ligand atoms)
**Figure S12**: Superimposition of the active $\beta_2$AR complex (blue, ligand in magenta) and the BI167107-bound active state structure of the $\beta_2$AR (grey, ligand in orange), both stabilized by Nb6B9. In order to maintain a hydrogen bond with the smaller catechol group, N293$^{6.55}$ tilts towards the $m$-hydroxyl group by 0.8 Å, contributing to an overall inward movement of TM6. This rearrangement results in more contracted binding pocket in case of catechol-derived ligands compared to the binding pocket of ligands with a larger heterocyclic head group.
Figure S13: Schematic depiction of the interactions between 2 and the active $\beta_2$AR. Polar contacts indicated as red dotted lines and hydrophobic contacts shown as green solid lines.
Table S1: Crystallography statistics

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<th><strong>Data collection</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><strong>β&lt;sub&gt;2&lt;/sub&gt;AR-Nb6B9-2 complex</strong></th>
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<td>α, β, γ (°)</td>
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<sup>a</sup>Values in parentheses are for highest-resolution shell  
<sup>b</sup>As calculated by Molprobity
Table S2: Receptor binding data of the covalent ligands 1-4, 6 and 7 to their corresponding wild-type receptors\(^a\) (\(K_i\) values\(^b\) in [nM ± SEM])

<table>
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<tr>
<th>compound</th>
<th>(\beta_2)AR(^{wt})</th>
<th>(D_2)R(^{wt})</th>
<th>5-HT(2\alpha)(^{wt})</th>
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<td></td>
<td>([^3]H)CGP12177</td>
<td>([^3]H)spiperone</td>
<td>([^3]H)ketanserin</td>
</tr>
<tr>
<td>1</td>
<td>17 ± 3.1</td>
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<tr>
<td>2</td>
<td>85 ± 29</td>
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</tr>
<tr>
<td>3</td>
<td>-</td>
<td>41 ± 13</td>
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</tr>
<tr>
<td>4</td>
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<td>21 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>3.6 ± 2.0</td>
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</tr>
<tr>
<td>7</td>
<td>2.2 ± 0.77</td>
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\(^a\) Determined with membranes from HEK 293 cells transiently expressing the corresponding human receptors as described in literature (12). \(^b\) \(K_i\) values are the mean of 3-4 individual experiments each done in triplicate.
**SI Note S1:**

**Materials and methods for organic synthesis.**

Dry solvents and reagents were of commercial quality and were used as purchased. MS were run on a Finnigan MAT TSQ 700 spectrometer by EI (70 eV) with solid inlet or a Bruker Esquire 2000 by APC ionization. HRMS were run on a JEOL JMS-GC Mate II using peak-matching (M/ΔM>5000) and on a Bruker Daltonics maXis instrument employing an ESI source. NMR spectra were obtained on a Bruker Avance 360 (1H at 360 MHz, 13C at 90 MHz) or a Bruker Avance 600 (1H at 600 MHz, 13C at 150 MHz) spectrometer in the solvents indicated. Chemical shifts are reported relative to TMS or to the residual solvent peak. Melting points were determined with a MEL-TEMP II melting point apparatus (Laboratory Devices, USA) in open capillaries and given uncorrected. IR spectra were performed on a Jasco FT/IR 410 spectrometer (film of substance on a NaCl crystal) or on a Perkin Elmer Spectrum BX FT-IR (neat). Purification by flash chromatography was performed using Silica Gel 60; TLC analyses were performed using Merck 60 F254 aluminum sheets and the spots were visualized using UV light (254 nm) and reagents such as Ninhydrin. Analytical HPLC/MS was performed on Agilent 1100 HPLC systems employing a VWL detector using (254 nm), or (220nm) connected to a Bruker Esquire 2000. The purity of all test compounds and key intermediates was determined by analytical HPLC on Agilent 1100 HPLC systems employing a VWL detector, using (254 nm), or (220nm) as indicated, to be > 95% and an Agilent Zorbax SB-C8 (4.6 mm x 150 mm, 5 μm) with a flow rate of 0.5 mL/min **(System 1):** eluent, methanol/0.1% aq. formic acid, 10% methanol for 3 min to 100% methanol in 15 min, 100% for 6 min to 10% in 3 min, 10% for 3 min, **System 2:** eluent, CH₃CN/ 0.1% aq. Formic acid, 10 % CH₃CN to 70% in 15 min., to 95% in 1 min., 95% for 3 min., to 10% in 1 min. and 10% for 3 min., **System 3:** eluent, CH₃CN/ 0.1% aq. Formic acid, 5 % CH₃CN to 60% in 15 min., to 95% in 1 min., 95% for 3 min., to 5% in 1 min. and 5% for 3 min., **System 4:** eluent, CH₃CN/ 0.1% aq. trifluoroacetic acid, 5 % CH₃CN to 60% in 15 min., to 95% in 1 min., 95% for 3 min., to 5% in 1 min. and 5% for 3 min., **System 5:** eluent, methanol/0.1% aq. trifluoroacetic acid, 10% methanol for 3 min to 100% methanol in 15 min, 100% for 6 min to 10% in 3 min, 10% for 3 min. Preparative HPLC was conducted on an Agilent (1100 Preparative Series), using a MACHEREY-NAGEL NUCLEODUR C18 HTec (32 mm x 250 mm x 5 μm) column, at a flow of 32 ml/min, with the solvent system indicated.

A suspension of L-noradrenaline (177.0 mg, 1.05 mmol) and 24 (150.0 mg, 0.35 mmol) in anhydrous DMSO (5 mL) was heated to 70°C and was stirred for 9 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (50 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (3 mL) and a solution of cysteamine hydrochloride (40.0 mg, 0.35 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (8-25% CH₃CN/0.1% aq. trifluoroacetic acid) to give 1 as a yellow-brownish solid (17.0 mg, 7% yield). [α]₀ = -9.5° (c = 2.99 g/100mL in water); ¹H-NMR: (D₂O, 600 MHz, δ from acetone, ppm): 2.08-2.12 (m, 2H), 2.84 (t, J = 7.1, 2H), 2.88-2.93 (m, 4H), 3.13-3.19 (m, 2H), 3.26-3.30 (m, 4H), 3.77 (s, 3H), 4.10 (t, J = 6.1 Hz, 2H), 4.79-4.81 (m, 1H), 6.70 (dd, J = 8.2, 1.7 Hz, 1H), 6.78-6.83 (m, 3H), 6.89 (d, J = 1.7 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H); ¹³C-NMR: (D₂O, 150 MHz, δ from acetone, ppm): 28.45, 31.65, 34.23, 34.48, 38.42, 49.15, 53.48, 56.40, 68.02, 68.99, 113.30, 114.35, 114.63, 116.95, 119.10, 122.10, 130.41, 132.61, 144.93, 144.97, 147.09, 149.49, 163.22, 163.45, 163.68, 163.92, 216.07; HPLC (220nm): System 1: tᵣ = 13.6 min, purity: 100%; System 3: tᵣ = 8.9 min purity: 98.2%; APCI-MS (m/z): 469.2 [M+1]⁺; HRMS (m/z): [M+1]⁺ calcd. for C₂₂H₃₂N₂O₅S₂, 469.1825; found: 469.1838;

A suspension of L-noradrenaline (133.0 mg, 0.79 mmol) and 25 (109.0 mg, 0.26 mmol) in anhydrous DMSO (4 mL) was heated to 70°C and was stirred for 8 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (40 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (3 mL) and a solution of cysteamine hydrochloride (30.0 mg, 0.26 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (3-28% CH$_3$CN/0.1% aq. trifluoroacetic acid) to give 2 as a yellow-brownish solid (15.2 mg, 9% yield). $[\alpha]_D = -7.6$ (c = 0.68 g/100mL in water); $^1$H-NMR: (D$_2$O, 600 MHz, $\delta$ from acetone, ppm): 2.88-2.94 (m, 4H), 3.06 (t, $J = 5.9$ Hz, 2H), 3.14-3.18 (m, 2H), 3.25-3.31 (m, 4H), 3.78 (s, 3H), 4.28 (t, $J = 5.9$ Hz, 2H), 4.79-4.82 (m, 1H), 6.71 (dd, $J = 8.3, 2.1$ Hz, 1H), 6.79-6.84 (m, 3H), 6.91 (d, $J = 2.0$ Hz, 1H), 6.97 (d, $J = 8.3$ Hz, 1H); $^{13}$C-NMR: (D$_2$O, 150 MHz, $\delta$ from acetone, ppm): 31.65, 34.45, 37.12, 38.28, 49.12, 53.45, 57.44, 68.98, 113.41, 114.35, 114.91, 116.96, 119.12, 122.07, 130.86, 132.60, 144.92, 144.96, 146.74, 149.57, 163.26, 163.49, 163.72, 163.96, 216.08; HPLC (220nm): System 1: $t_R = 10.1$ min, purity: 100%; System 3: $t_R = 9.9$ min purity: 100%; APCI-MS (m/z): 455.4 [M+1]$^+$; HRMS (m/z): [M+1]$^+$ calcd. for C$_{21}$H$_{30}$N$_2$O$_5$S$_2$, 455.1669; found: 455.1677;

![chemical structure]

A suspension of dopamine (138.0 mg, 0.90 mmol) and 24 (77.0 mg, 0.18 mmol) in anhydrous DMSO (5 mL) was heated to 70°C for 7 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (50 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (1 mL) and a solution of cysteamine hydrochloride (21.0 mg, 0.18 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (5-30% CH$_3$CN/ 0.1% aq. trifluoroacetic acid) to give 3 as a yellow-brownish solid (11.9 mg, 10% yield). $^1$H-NMR: (D$_2$O, 600 MHz, $\delta$ from acetone, ppm): 2.17-2.21 (m, 2H), 2.86 (t, $J = 7.2$ Hz, 2H), 2.89-2.95 (m, 4H), 3.24-3.30 (m, 4H), 3.38 (t, $J = 6.4$ Hz, 2H), 3.48 (s, 3H), 4.18 (t, $J = 6.2$ Hz, 2H), 6.65 (dd, $J = 8.1$, 2.1 Hz, 1H), 6.77 (d, $J = 2.1$ Hz, 1H), 6.81 (dd, $J = 8.2$, 2.0 Hz, 1H), 6.83 (d, $J = 8.1$ Hz, 1H), 6.92 (d, $J = 2.0$ Hz, 1H), 7.00 (d, $J = 8.2$ Hz, 1H); $^{13}$C-NMR: (D$_2$O, 150 MHz, $\delta$ from acetone, ppm): 27.79, 30.73, 30.97, 33.55, 33.82, 37.73, 48.18, 48.27, 55.71, 67.36, 112.54, 113.89, 116.34, 116.45, 120.98, 121.42, 128.66, 129.56, 143.15, 144.30, 146.44, 148.82, 215.41; HPLC (220nm): System 1: $t_R = 11.3$ min, purity: 100%; System 3: $t_R = 10.2$ min purity: 100%; APCI-MS (m/z): 453.2 [M+1]$^+$; HRMS (m/z): [M+1]$^+$ calcd. for C$_{21}$H$_{30}$N$_2$O$_5$S$_2$, 453.1876; found: 453.1885;
To a solution of 29 (21.0 mg, 0.03 mmol) in anhydrous MeOH (0.2 mL), a solution of cysteamine hydrochloride (3.4 mg, 0.03 mmol) in anhydrous MeOH (0.2 mL) was added. The mixture was stirred for 1 h at room temperature under argon atmosphere. Then, a solution of ammonium fluoride (17.0 mg, 0.45 mmol) in anhydrous MeOH (1.3 mL) was added and the reaction mixture was stirred for 3 h at room temperature under Argon atmosphere. The solution was subsequently purified by preparative HPLC (3-35% CH$_3$CN/0.1% aq. trifluoroacetic acid) to give 4 as a white solid (11.4 mg, 54% yield). $^1$H-NMR: (D$_2$O, 600 MHz, $\delta$ from acetone, ppm): 2.17-2.21 (m, 2H), 2.88 (t, $J$ = 7.0 Hz, 2H) 2.93 (t, $J$ = 7.1 Hz, 2H), 2.98 (t, $J$ = 6.5 Hz, 2H), 3.07 (t, $J$ = 6.9 Hz, 2H), 3.30 (t, $J$ = 7.0 Hz, 2H), 3.33 (t, $J$ = 6.9 Hz, 2H), 3.38 (t, $J$ = 6.5 Hz, 2H), 3.75 (s, 3H), 4.12 (t, $J$ = 6.2 Hz, 2H), 6.65 (dd, $J$ = 8.2, 2.0 Hz, 1H), 6.77 (d, $J$ = 2.0 Hz, 1H), 6.81 (d, $J$ = 8.2 Hz, 1H), 6.84 (dd, $J$ = 8.7, 2.3 Hz, 1H), 6.97 (d, $J$ = 2.3 Hz, 1H), 7.16 (s, 1H), 7.35 (d, $J$ = 8.7 Hz, 1H); $^{13}$C-NMR: (D$_2$O, 150 MHz, $\delta$ from acetone, ppm): 21.38, 27.75, 30.80, 33.54, 33.79, 37.73, 47.14, 47.93, 55.60, 67.15, 102.13, 107.46, 111.95, 112.05, 112.86, 113.53, 121.20, 125.27, 126.74, 128.96, 131.56, 146.31, 148.67, 148.89, 215.41; HPLC (220nm): System 1: $t_R$ = 12.7 min, purity: 100%; System 3: $t_R$ = 10.6 min purity: 100%; APCI-MS (m/z): 476.6 [M+1]$^+$; HRMS (m/z): [M+1]$^+$ calcd. for C$_{24}$H$_{33}$N$_3$O$_3$S$_2$, 476.2036; found: 476.2042;
N-[2-(1H-imidazol-4-yl)ethyl]-2-[4-[3-[(2-aminoethyl)disulfanyl]propoxy]-3-methoxyphenyl] ethanamine 3xTFA (5):

A suspension of histamine (78.0 mg, 0.70 mmol) and 24 (60.0 mg, 0.14 mmol) in anhydrous DMSO (2 mL) was heated to 70°C for 6 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (20 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (1 mL) and a solution of cysteamine hydrochloride (16.0 mg, 0.14 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (3-30% CH₃CN/ 0.2% aq. trifluoroacetic acid) to give 5 as a colorless oil (25.0 mg, 24% yield). ¹H-NMR:(C₅D₅N, 600 MHz, δ from TMS, ppm): 2.13-2.17 (m, 2H), 2.96 (t, J = 7.1, 2H), 3.25-3.30 (m, 4H), 3.33 (t, J = 6.9 Hz), 3.54-3.56 (m, 2H), 3.61-3.66 (m, 2H) 3.73-3.75 (m, 5H), 4.04 (t, J = 6.0 Hz, 2H), 6.89-6.94 (m, 2H), 7.04 (d, J = 1.2 Hz, 1H), 7.17 (s, 1H), 8.00 (s, 1H); ¹³C-NMR:(C₅D₅N, 150 MHz, δ from TMS, ppm): 21.17, 28.35, 31.05, 33.94, 37.85, 44.96, 47.81, 55.52, 66.72, 112.79, 113.84, 116.73, 120.61, 129.17, 129.80, 134.32, 146.77, 149.19, 158.29, 158.49; HPLC (220nm): System 1: tᵣ = 8.1 min, purity: 100%; System 3: tᵣ = 18.1 min purity: 100%; ESI-MS (m/z): 411.4 [M+1]⁺; HRMS (m/z): [M+1]⁺ calcd. for C₁₉H₂₉N₄O₂S₂, 411.1883; found: 411.1886;

A suspension of L-noradrenaline (203.0 mg, 1.05 mmol) and 22 (160.0 mg, 0.40 mmol) in anhydrous DMSO (5 mL) was heated to 70°C and was stirred for 9 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (50 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (3 mL) and a solution of cysteamine hydrochloride (45.0 mg, 0.40 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (6-36% CH₃CN/ 0.1% aq. trifluoroacetic acid) to give 6 as a yellow-brownish solid (59.2 mg, 22% yield). [α]₀ = -9.7° (c = 1.8 g/100mL in water); ¹H-NMR: (D₂O, 600 MHz, δ from acetone, ppm): 2.13-2.17 (m, 2H), 2.93 (t, J = 7.1 Hz, 2H), 2.97-3.00 (m, 4H), 3.24-3.26 (m, 2H), 3.33-3.39 (m, 4H), 4.17 (t, J = 6.1 Hz, 2H), 4.88-4.90 (m, 1H), 6.81 (dd, J = 8.1, 2.0 Hz, 1H), 6.90-6.92 (m, 2H), 6.97-7.00 (m, 2H), 7.24-7.26 (m, 2H), 8.47 (s, 2H); ¹³C-NMR: (D₂O, 150 MHz, δ from acetone, ppm): 28.52, 31.20, 34.25, 34.54, 38.41, 49.24, 53.44, 67.38, 69.00, 114.41, 116.08, 116.98, 119.17, 129.77, 130.72, 132.66, 144.91, 144.96, 157.86, 171.61; HPLC (220nm): System 3: t_R = 9.8 min, purity: 100%; System 5: t_R = 14.4 min, purity: 100%; APCI-MS (m/z): 439.4 [M+1]⁺; HRMS (m/z): [M+1]⁺ calcd. for C₂₁H₃₀N₂O₆S₂, 439.1720; found: 439.1725;

A suspension of L-norepinephrine (211.0 mg, 1.25 mmol) and 23 (160.0 mg, 0.42 mmol) in anhydrous DMSO (7 mL) was heated to 70°C and was stirred for 7 h under argon atmosphere. The reaction mixture was allowed to cool to room temperature. Degassed water (70 mL) was added and the resulting mixture was frozen and lyophilized. The residue was dissolved in MeOH (4 mL) and a solution of cysteamine hydrochloride (47.0 mg, 0.42 mmol) in MeOH (1 mL) was added under argon atmosphere. The reaction was stirred for 1 h at room temperature and subsequently purified by preparative HPLC (3-28% CH₃CN/0.1% aq. trifluoroacetic acid) to give 7 as a yellow-brownish solid (36.0 mg, 13% yield). [α]D = -9.2° (c = 1.72 g/100mL in water); ¹H-NMR: (D₂O, 600 MHz, δ from acetone, ppm): 2.97-3.01 (m, 4H), 3.14 (t, J = 5.8 Hz, 2H), 3.24-3.27 (m, 2H), 3.33-3.39 (m, 4H), 4.16 (t, J = 5.9 Hz, 2H), 4.88-4.90 (m, 1H), 6.81 (dd, J = 8.2, 2.0 Hz, 1H), 6.90-6.92 (m, 2H), 7.01-7.03 (m, 2H), 7.25-7.27 (m, 2H); ¹³C-NMR: (D₂O, 150 MHz, δ from acetone, ppm): 30.47, 31.20, 34.51, 37.21, 49.22, 53.44, 66.70, 68.99, 114.41, 116.17, 116.98, 119.17, 130.10, 130.79, 132.66, 144.90, 144.95, 157.53, 163.50, 163.74; HPLC (220nm): System 4: tR = 18.8 min, purity: 96.2%; System 5: tR = 12.9 min, purity: 97.2%; APCI-MS (m/z): 425.2 [M+1]+; HRMS (m/z): [M+1]+ calcd. for C₂₀H₂₈N₂O₄S₂, 425.1563; found: 425.1575;
2-[4-(3-Bromopropoxy)phenyl]ethanol (10):

![structure](image)

A suspension of 2-(4-hydroxyphenyl)ethanol (0.5 g, 3.6 mmol), 1,3-dibromopropane (1.8 mL, 18.1 mmol) and K₂CO₃ (1.0 g, 7.2 mmol) in anhydrous CH₃CN (15.0 mL) was stirred at reflux temperature for 6 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H₂O and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 4:1) to give 10 as a colorless oil (0.6 g, 68% yield).

**¹H-NMR:** (CDCl₃, 600 MHz, δ from TMS, ppm): 2.23-2.33 (m, 2H), 2.82 (t, J = 6.5 Hz, 2H), 3.61 (t, J = 6.5 Hz, 2H), 3.83 (t, J = 6.5 Hz, 2H) 4.09 (t, J = 5.6 Hz, 2H). 6.85-6.88 (m, 2H), 7.14-7.16 (m, 2H).

**¹³C-NMR:** (CDCl₃, 90 MHz, δ from TMS, ppm): 29.99, 32.41, 38.28, 63.80, 65.37, 114.72, 130.01, 130.74, 157.42; IR: (NaCl) v (cm⁻¹): 3351, 2975, 2932, 2878, 1610, 1582, 1513, 1469, 1417, 1388, 1284, 1246, 1178, 1027, 930, 823; HPLC (220nm): System 1: tᵣ = 17.6 min, purity: 100%; APCI-MS (m/z): 258.9 [M-1]⁺; 260.8 [M+1]⁺; HRMS (m/z): calcd. for C₁₁H₁₅BrO₂, 258.0255; found: 258.0255;
2-[4-(2-Bromoethoxy)phenyl]ethanol (11):

![Chemical Structure](image)

A suspension of 2-(4-hydroxyphenyl)ethanol (1.0 g, 7.2 mmol), 1,2-dibromoethane (3.1 mL, 36.2 mmol) and K$_2$CO$_3$ (2.0 g, 14.5 mmol) in anhydrous CH$_3$CN (15.0 mL) was stirred at reflux temperature for 48 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H$_2$O and extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 4:1) to give 11 as a colorless oil (1.1 g, 62% yield).

$^1$H-NMR: (CDCl$_3$, 600 MHz, δ from TMS, ppm): 2.82 (t, J = 6.5 Hz, 2H), 3.63 (t, J = 6.3 Hz, 2H), 3.81–3.85 (m, 2H), 4.28 (t, J = 6.3 Hz, 2H), 6.85-6.88 (m, 2H), 7.14–7.17 (m, 2H); $^{13}$C-NMR: (CDCl$_3$, 90 MHz, δ from TMS, ppm): 29.10, 38.28, 63.76, 68.02, 130.10, 115.02, 131.37, 156.84; IR: (NaCl) v (cm$^{-1}$): 3376, 3045, 2936, 2908, 2858, 1610, 1510, 1422, 1299, 1271, 1246, 1177, 1145, 1075, 1045, 1016, 835, 813; HPLC (220nm): System 1: t$_R$ = 18.7 min, purity: 96.6%; APCI-MS (m/z): 244.8 [M-H]$^+$, 246.9 [M+H]$^+$; HRMS (m/z): calcd. for C$_{10}$H$_{13}$BrO$_2$, 244.0099; found: 244.0100;
2-[4-(2-Bromopropoxy)-3-methoxyphenyl]ethanol (12):

A suspension of homovanillyl alcohol (1.0 g, 6.0 mmol), 1,3-dibromopropane (3.0 mL, 30.0 mmol) and K₂CO₃ (1.7 g, 12.0 mmol) in anhydrous CH₃CN (15.0 mL) was stirred at reflux temperature for 24 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H₂O and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 12 as a white solid (1.4 g, 81% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 2.33-2.38 (m, 2H), 2.82 (t, J = 6.4 Hz, 2H), 3.63 (t, J = 6.4 Hz, 2H), 3.83-3.86 (m, 5H), 4.13-4.15 (m, 2H), 6.75-6.77 (m, 2H), 6.86-6.88 (m, 1H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 30.16, 32.46, 38.77, 55.98, 63.76, 66.97, 112.95, 114.20, 121.07, 131.37, 146.91, 149.77; IR: (NaCl) v (cm⁻¹): 3398, 3031, 2937, 2874, 1590, 1513, 1466, 1418, 1261, 1230, 1140, 1034, 930, 850, 805; HPLC (220nm): System 1: tᵣ = 16.6 min, purity: 100.0%; ESI-MS (m/z): 289.0 [M-H]⁺, 290.1 [M+H]⁺; HRMS (m/z): calcd. for C₁₂H₁₇BrO₃, 288.0361; found: 288.0363;
2-[4-(2-Bromoethoxy)-3-methoxyphenyl]ethanol (13):

A suspension of homovanillyl alcohol (1.0 g, 6.0 mmol), 1,2-dibromoethane (2.6 mL, 30.0 mmol) and K₂CO₃ (1.7 g, 12.0 mmol) in anhydrous CH₃CN (15.0 mL) was stirred at reflux temperature for 48 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H₂O and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 13 as a colorless oil (0.8mg, 49% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 2.82 (t, J = 6.5 Hz, 2H), 3.64 (t, J = 13.4 Hz, 2H), 3.83-3.88 (m, 5H), 4.31 (t, J = 13.4 Hz, 2H), 6.76-6.75 (m, 1H), 6.77-6.78 (m, 1H), 6.86-6.88 (m, 1H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 28.96, 38.76, 56.02, 63.62, 69.53, 113.25, 115.42, 121.09, 132.82, 146.13, 150.11; IR: (NaCl) v (cm⁻¹): 3330, 3031, 2936, 2871, 1590, 1514, 1452, 1417, 1326, 1261, 1227, 1138, 1021, 848; HPLC (220nm): System 1: tᵣ = 19.5 min, purity: 97.0%; ESI-MS (m/z): 297.2 [M-1+Na]⁺, 299.2 [M+1+Na]⁺; HRMS (m/z): calcd. for C₁₁H₁₅BrO₃, 274.0205; found: 274.0205
S-[3-[4-(2-Hydroxyethyl)phenoxy]propyl] ethanethioate (14):

A solution of 10 (506 mg, 2.0 mmol) and KSAc (457 mg, 4.0 mmol) in acetone (15 mL) was stirred at reflux temperature for 30 min. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H₂O and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 7:3) to give 14 as a brown oil (357 mg, 70% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 1.60 (s, 3H), 2.30-2.35 (m, 2H), 2.80 (t, J = 6.5 Hz, 2H), 3.62 (t, J = 6.5 Hz, 2H), 3.84 (t, J = 6.5 Hz, 2H), 4.10 (t, J = 5.8 Hz, 2H), 6.87-6.89 (m, 2H), 7.12-7.17 (m, 2H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 25.90, 29.32, 30.62, 38.29, 63.82, 66.25, 114.71, 129.97, 130.59, 157.52, 195.74 IR: (NaCl) v (cm⁻¹): 3369, 3032, 2933, 2871, 1690, 1611, 1511, 1470, 1430, 1243, 1177, 1134, 1112, 1044, 957, 824; HPLC (254nm): System 1: tᵣ = 19.4 min, purity: 98.7%; ESI-MS (m/z): 277.0 [M+Na]⁺; HRMS (m/z): calcd. for C₁₃H₁₆O₅S, 254.0977; found: 254.0976;
S-[2-[4-(2-Hydroxyethyl)phenoxy]ethyl] ethanethioate (15):

A solution of 11 (600 mg, 2.5 mmol) and KSAc (570 mg, 5.0 mmol) in acetone (20 mL) was stirred at reflux temperature for 30 min. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H₂O and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 3:2) to give 15 as a brown oil (548 mg, 91% yield). 

$^1$H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 2.33 (s, 3H), 2.81 (t, $J = 6.5$ Hz, 2H), 3.26 (t, $J = 6.4$ Hz, 2H), 3.81-3.84 (m, 2H), 4.08 (t, $J = 6.5$ Hz, 2H), 6.83-6.87 (m, 2H), 7.12-7.15 (m, 2H); 13C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 28.52, 30.54, 38.28, 63.78, 114.82, 66.59, 114.82, 130.02, 130.95, 157.09, 195.38; IR: (NaCl) v (cm⁻¹): 3371, 3033, 2935, 2872, 1691, 1611, 1511, 1466, 1419, 1241, 1299, 1134, 1113, 1043, 955, 824; HPLC (254nm): System 1: $t_R = 18.6$ min, purity: 100.0%; APCI-MS (m/z): 241.2 [M+H]⁺; HRMS (m/z): calcd. for C₁₂H₁₆NO₂S, 240.0820; found: 240.0820;
A solution of 12 (1.3 g, 4.5 mmol) and KSAc (1.0 g, 9.0 mmol) in acetone (36 mL) was stirred at reflux temperature for 30 min. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H$_2$O and extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 16 as a brown oil (1.1 g, 85% yield). $^1$H-NMR: (CDCl$_3$, 360 MHz, 6 from TMS, ppm): 2.07-2.12 (m, 2H), 2.33 (s, 3H), 2.81 (t, $J$ = 6.5 Hz, 2H), 3.07 (t, $J$ = 7.1 Hz, 2H), 3.83-3.86 (m, 5H), 4.03-4.05 (m, 2H), 6.73-6.76 (m, 2H), 6.82-6.85 (m, 1H); $^{13}$C-NMR: (CDCl$_3$, 90 MHz, 6 from TMS, ppm): 25.88, 29.33, 30.59, 38.76, 55.99, 63.69, 67.70, 112.96, 114.03, 121.03, 131.63, 146.96, 149.73, 195.76; IR: (NaCl) $\nu$ (cm$^{-1}$): 3415, 3070, 3052, 3004, 2959, 1689, 1591, 1512, 1466, 1428, 1260, 1231, 1127, 1100, 1030, 959, 845; HPLC (254 nm): System 1: $t_R$ = 16.6 min, purity: 99.0%; ESI-MS (m/z): 285.0 [M+H]$^+$; HRMS (m/z): calcd. for C$_{14}$H$_{20}$O$_4$S, 284.1082; found: 284.1081;
S-[3-[4-(2-Hydroxyethyl)-2-methoxyphenoxy]ethyl] ethanethioate (17):

A solution of 13 (800 mg, 2.9 mmol) and KSAc (662 mg, 5.8 mmol) in acetone (20 mL) was stirred at reflux temperature for 30 min. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. The residue was dissolved in H$_2$O and extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 3:2) to give 17 as a brown oil (686 mg, 87% yield). $^1$H-NMR: (CDCl$_3$, 360 MHz, $\delta$ from TMS, ppm): 2.36 (s, 3H), 2.81 (t, $J$ = 6.5 Hz, 2H), 3.28 (t, $J$ = 6.7 Hz, 2H), 3.79-3.86 (m, 5H), 4.13 (t, $J$ = 6.7 Hz, 2H), 6.74-6.76 (m, 2H), 6.88-6.89 (m, 1H); $^{13}$C-NMR: (CDCl$_3$, 90 MHz, $\delta$ from TMS, ppm): 28.37, 35.53, 38.77, 56.02, 63.68, 67.85, 113.06, 114.41, 121.08, 132.08, 146.46, 149.71, 195.76; IR: (NaCl) v (cm$^{-1}$): 3415, 3070, 3052, 3004, 2959, 1689, 1591, 1512, 1466, 1428, 1260, 1231, 1127, 1100, 1030, 959, 845; HPLC (254nm): System 1: $t_R$ = 17.9 min, purity: 96.4%; ESI-MS (m/z): 253.0 [M+H-H$_2$O]$^+$; HRMS (m/z): calcd. for C$_{13}$H$_{18}$O$_4$S, 270.0926; found: 270.0926;
2-[4-(3-(Pyridine-2-yl)sulfanyl)propoxy]phenyl]ethanol (18):

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To a solution of 14 (200 mg, 0.79 mmol) and 2,2′-dithiodipyridine (209 mg, 0.95 mmol) in anhydrous MeOH (6.0 mL), 2.4 mL of a 0.5 N solution of NaOMe in MeOH was added. The resulting mixture was stirred at room temperature for 17 h under argon atmosphere. The solvent was evaporated and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 18 as a yellow oil (74 mg, 29% yield). \(^1\)H-NMR: (CDCl\(_3\), 600 MHz, \(\delta\) from TMS, ppm): 2.16-2.21 (m, 2H), 2.81 (t, \(J = 6.5\) Hz, 2H), 3.00 (t, \(J = 7.1\) Hz, 2H), 3.83 (t, \(J = 6.5\) Hz, 2H), 4.06 (t, \(J = 5.9\) Hz, 2H), 6.82-6.85 (m, 2H), 7.08-7.10 (m, 1H), 7.12-7.14 (m, 2H), 7.62-7.65 (m, 1H), 7.71-7.73 (m, 1H), 8.45-8.47 (m, 1H); \(^{13}\)C-NMR: (CDCl\(_3\), 90 MHz, \(\delta\) from TMS, ppm): 28.57, 35.29, 38.27, 63.79, 65.83, 114.71, 119.71, 120.63, 129.97, 130.62, 136.99, 149.64, 157.45, 160.18; IR: (NaCl) \(v\) (cm\(^{-1}\)): 3341, 3044, 2930, 2869, 1611, 1575, 1561, 1511, 1447, 1417, 1241, 1176, 1117, 1044, 824; HPLC (254nm): System 1: \(t_R = 20.3\) min, purity: 96.0%; APCI-MS (m/z): 322.1 [M+H]\(^+\); HRMS (m/z): calcd. for C\(_{16}\)H\(_{19}\)NO\(_2\)S\(_2\), 321.0857; found: 321.0856;
2-[4-{2-(Pyridine-2-yldisulfanyl)ethoxy}phenyl]ethanol (19):

To a solution of 15 (200 mg, 0.83 mmol) and 2,2'-dithiodipyridine (220 mg, 1.00 mmol) in anhydrous MeOH (6.0 mL), 2.5 mL of a 0.5N solution of NaOMe in MeOH was added. The resulting mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give 19 as a yellow oil (154 mg, 58% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 2.80 (t, J = 6.5 Hz, 2H), 3.17 (t, J = 6.3 Hz, 2H), 3.82 (t, J = 6.3 Hz, 2H), 4.21 (t, J = 6.3 Hz, 2H), 6.80-6.82 (m, 2H), 7.04-7.06 (m, 1H), 7.10-7.14 (m, 2H), 7.53-7.55 (m, 1H), 7.71-7.73 (m, 1H), 8.43-8.44 (m, 1H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 38.02, 38.27, 63.79, 65.72, 114.75, 119.80, 120.71, 129.99, 130.91, 137.01, 149.58, 157.04, 159.98; IR: (NaCl) ν (cm⁻¹): 3366, 3078, 2918, 2867, 1610, 1576, 1561, 1509, 1448, 1417, 1382, 1298, 1239, 1176, 1115, 1043, 820; HPLC (254nm): System 1: tᵣ = 18.6 min, purity: 100.0%; ESI-MS (m/z): 322.2 [M+H]+; HRMS (m/z): calcd. for C₁₅H₁₇NO₂S₂, 321.0857; found: 321.0856;
2-[3-Methoxy-4-[3-(pyridine-2-yl-disulfanyl)propoxy]phenyl]ethanol (20):

![Chemical structure](image)

To a solution of 16 (350 mg, 1.23 mmol) and 2,2’-dithiodipyridine (327 mg, 1.48 mmol) in anhydrous MeOH (10.0 mL), 3.7 mL of a 0.5N solution of NaOMe in MeOH was added. The resulting mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/isopropyl alcohol 4:1) to give 20 as a yellow oil (276 mg, 64% yield).

$^1$H-NMR: (CDCl$_3$, 600 MHz, δ from TMS, ppm): 2.19-2.23 (m, 2H), 2.81 (t, J = 6.5 Hz, 2H), 3.01 (t, J = 7.1 Hz, 2H), 3.82-3.85 (m, 5H), 4.09-4.11 (m, 2H), 6.75-6.77 (m, 2H), 6.81-6.83 (m, 1H), 7.07-7.09 (m, 1H), 7.61-7.64 (m, 1H), 7.71-7.72 (m, 1H), 8.44-8.46 (m, 1H);

$^{13}$C-NMR: (CDCl$_3$, 90 MHz, δ from TMS, ppm): 25.50, 28.50, 35.20, 55.85, 63.48, 67.28, 112.89, 114.07, 119.62, 120.55, 120.94, 131.92, 136.93, 146.86, 149.45, 149.56, 160.09;

IR: (NaCl) ν (cm$^{-1}$): 3366, 3021, 2976, 2942, 1573, 1512, 1471, 1447, 1417, 1399, 1260, 1230, 1140, 1036, 916, 850, 806;

HPLC (254nm): System 1: $t_R = 19.5$ min, purity: 97.0%;

ESI-MS (m/z): 352.2 [M+H]$^+$;

HRMS (m/z): calcd. for C$_{14}$H$_{20}$O$_4$S, 351.0963; found: 351.0964;

![Structure Diagram]

To a solution of 17 (300 mg, 1.11 mmol) and 2,2'-dithiodipyridine (293 mg, 1.33 mmol) in anhydrous MeOH (8.0 mL), 3.33 mL of a 0.5N solution of NaOMe in MeOH was added. The resulting mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue was purified by flash chromatography (cyclohexane/isopropyl alcohol 4:1) to give 21 as a yellow oil (118 mg, 31% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 2.81 (t, J = 6.7 Hz, 2H), 3.20 (t, J = 6.7 Hz, 2H), 3.81-3.86 (m, 5H), 4.27 (t, J = 6.7 Hz, 2H), 6.70-6.79 (m, 3H), 7.05-7.07 (m, 1H), 7.56-7.59 (m, 1H), 7.74-7.75 (m, 1H), 8.43-8.44 (m, 1H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): 37.51, 37.71, 55.93, 63.60, 67.11, 112.88, 114.16, 119.76, 120.68, 120.97, 132.07, 137.03, 146.33, 149.48, 149.59, 159.91; IR: (NaCl) v (cm⁻¹): 3338, 3046, 2994, 2934, 1574, 1513, 1448, 1417, 1382, 1326, 1262, 1228, 1190, 1141, 1120, 1039, 852, 807; HPLC (254nm): System 1: tᵣ = 16.9 min, purity: 97.2%; APCI-MS (m/z): 338.1 [M+H]⁺; HRMS (m/z): calcd. for C₁₆H₁₉NO₅S₂, 337.0806; found: 337.0804;
4-[3-(Pyridine-2-yldisulfanyl)propoxy]phenylethyl methanesulfonate (22):

A solution of 18 (145 mg, 0.45 mmol) and triethylamine (69 µL, 0.50 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) was cooled to 0°C under argon atmosphere. Methanesulfonyl chloride (35 µL, 0.45 mmol) was added dropwise and the mixture was stirred for 1 h at 0°C. Water was added and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated to give 22 as a yellow oil (165 mg, 92%). The crude material was used for the next reaction.
4-[3-(Pyridine-2-ylsulfanyl)ethoxy]phenylethyl methanesulfonate (23):

A solution of 19 (148 mg, 0.46 mmol) and triethylamine (70 µL, 0.51 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) was cooled to 0°C under argon atmosphere. Methanesulfonyl chloride (36 µL, 0.46 mmol) was added dropwise and the mixture was stirred for 1 h at 0°C. Water was added and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated to give 24 as a yellow oil (160 mg, 90%). The crude material was used for the next reaction.
3-Methoxy-4-[3-(pyridine-2-yldisulfanyl)propoxy]phenylethyl methanesulfonate (24):

A solution of 20 (270 mg, 0.77 mmol) and triethylamine (117 µL, 0.84 mmol) in anhydrous CH₂Cl₂ (6 mL) was cooled to 0°C under argon atmosphere. Methanesulfonyl chloride (60 µL, 0.77 mmol) was added dropwise and the mixture was stirred for 1h at 0°C. Water was added and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated to give 24 as a yellow oil (307 mg, 93%). The crude material was used for the next reaction.
3-Methoxy-4-[3-(pyridine-2-yldisulfanyl)ethoxy]phenylethyl methanesulfonate (25):

A solution of 21 (71 mg, 0.21 mmol) and triethylamine (32 µL, 0.23 mmol) in anhydrous CH$_2$Cl$_2$ (3 mL) was cooled to 0°C under argon atmosphere. Methanesulfonyl chloride (16 µL, 0.17 mmol) was added dropwise and the mixture was stirred for 1h at 0°C. Water was added and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated to give 25 as a yellow oil (72 mg, 83%). The crude material was used for the next reaction.
Benzyl[2-(5-hydroxy-1H-indol-3-yl)ethyl]carbamate (26):

A suspension of serotonin hydrochloride (300 mg, 1.4 mmol) and NaHCO₃ (391 mg, 4.7 mmol) in 20 mL water/THF (1:1) was cooled to 0°C. Then, N-(benzyloxy carbonyloxy)succinimide (366 mg, 1.47 mmol) was added and the reaction mixture was stirred for 15 min at 0°C and for 3 h at room temperature under argon atmosphere. Water was added and the resulting mixture was extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄), filtered and evaporated to give 26 as a yellowish oil (350 mg, 80% yield). Analytical data described in literature (16).
Benzyl[2-[5-[(tert-butyldimethylsilyl)oxy]-1H-indol-3-yl]ethyl] carbamate (27):

A solution of 26 (155 mg, 0.50 mmol) and imidazole (68 mg, 1.00 mmol) in anhydrous DMF (4.00 mL) was cooled to 0°C under argon atmosphere. tert-butyldimethylsilyl triflate (0.23 mL, 1.00 mmol) was added dropwise, the reaction mixture was allowed to warm to room temperature and was stirred for 45 min. Water was added and the resulting mixture was extracted with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$), filtered and evaporated. The residue was purified by flash chromatography (hexane/EtOAc 4:1) to give 27 as a colorless oil (186 mg, 87% yield). $^1$H-NMR: (CDCl$_3$, 600 MHz, $\delta$ from TMS, ppm): 0.18-0.19 (m, 6H), 0.99-1.02 (m, 9H), 2.90-2.92 (m, 2H), 3.50-3.53 (m, 2H), 4.81 (s, 1H), 5.09 (s, 1H) 6.76 (dd, $J = 6.8$, 2.3 Hz, 1H), 6.97 (m, 2H), 7.18 (d, $J = 8.6$ Hz, 1H), 7.28-7.35 (m, 5H), 7.88 (s, 1H); $^{13}$C-NMR: (CDCl$_3$, 90 MHz, $\delta$ from TMS, ppm): -4.40, 18.22, 28.68, 28.82, 41.10, 66.59, 108.16, 111.45, 112.43, 116.44, 122.83, 127.96, 128.04, 128.10, 128.48, 131.98, 136.66, 149.23, 156.35; HPLC (254nm): System 1: $t_R = 22.3$ min, purity: 100.0%; APCI-MS (m/z): 425.5 [M+H]$^+$; HRMS (m/z): calcd. for C$_{24}$H$_{32}$N$_2$O$_3$Si, 424.2182; found: 424.2184;
Benzyl[2-[5-[(tert-butyldimethylsilyl)oxy]-1H-indol-3-yl]ethyl]carbamate (28):

A suspension of 27 (150 mg, 0.35 mmol) and 10% Pd/C (15 mg) in anhydrous MeOH (3 mL) was stirred for 5 h under H₂ atmosphere (balloon) at room temperature. The suspension was filtered through Celite and the filtrate was concentrated in vacuo. The residue gave 28 as a colorless oil (88 mg, 86% yield). ¹H-NMR: (CDCl₃, 600 MHz, δ from TMS, ppm): 0.18-0.19 (m, 6H), 1.00-1.02 (m, 9H), 2.86-2.88 (m, 2H), 2.94-2.96 (m, 2H), 6.67 (dd, J = 8.7, 2.3 Hz, 1H), 6.96 (d, J = 2.3 Hz, 1H), 7.05 (s, 1H), 7.20 (d, J = 8.6 Hz, 1H), 8.51 (s, 1H); ¹³C-NMR: (CDCl₃, 90 MHz, δ from TMS, ppm): -4.24, 19.02, 26.31, 28.83, 42.76, 108.79, 112.50, 112.63, 116.58, 124.66, 129.22, 134.05, 149.74 HPLC (254nm): System 1: tᵣ = 18.3 min, purity: 100.0%; ESIMS (m/z): 291.5 [M+H]+; HRMS (m/z): calcd. for C₂₄H₃₂N₂O₃Si, 290.1815; found: 290.1815;
2-[[[(tert-Butyldimethylsilyl)oxy]-1H-indol-3-yl]-N-[3-methoxy-4-[3-(pyridin-2-yl disulfanyl)propoxy] phenethyl]ethanamine (29):

A solution of 28 (150 mg, 0.52 mmol) and 24 (45 mg, 0.10 mmol) in anhydrous DMSO (5 mL) was stirred for 7 h at 70°C under argon atmosphere. Degassed water (50 mL) was added and the resulting mixture was frozen and lyophilized. The residue was purified by flash chromatography (CH$_2$Cl$_2$/MeOH 19:1 + 0.2% NH$_4$OH) give 29 as a brown oil (22 mg, 35% yield).$^1$H-NMR: (CD$_3$OD, 600 MHz, $\delta$ from TMS, ppm): 0.18-0.19 (m, 6H), 1.00-1.02 (m, 9H), 2.11-2.15 (m, 2H), 2.81-2.84 (m, 2H), 3.01-3.04 (m, 4H), 3.07-3.08 (m, 2H), 3.13-3.17 (m, 2H), 3.75 (s, 3H) 4.05 (d, $J$ = 5.9 Hz, 2H), 6.65 (dd, $J$ = 8.1, 1.6 Hz, 1H), 6.70 (dd, $J$ = 8.7, 2.2 Hz, 1H), 6.77 (d, $J$ = 2.3 Hz, 1H), 6.79 (d, $J$ = 8.1 Hz, 1H), 6.97 (d, $J$ = 2.2 Hz, 1H), 7.07 (s, 1H), 7.19-7.20 (m, 1H), 7.22 (d, $J$ = 8.7 Hz, 1H), 7.74-7.77 (m, 1H), 7.84-7.85 (m, 1H), 8.37-8.38 (m, 1H); $^{13}$C-NMR: (CD$_3$OD, 151 MHz, $\delta$ from TMS, ppm): -4.21, 19.02, 24.35, 26.32, 29.80, 34.01, 35.86, 46.73, 50.63, 56.52, 68.49, 108.59, 110.80, 112.87, 113.93, 115.66, 116.85, 121.21, 122.07, 122.30, 124.97, 128.90, 132.17, 134.03, 139.10, 148.57, 149.98, 150.34, 151.31, 161.47; HPLC (254nm): System 1: $t_R$ = 19.6 min, purity: 95.6%; ESIMS (m/z): 625.4 [M+H]$^+$;
SI Note S2:

$^1$H and $^{13}$C NMR spectra of key compounds 1-5.

1:

$^1$H spectrum of 1 (600 MHz, D$_2$O):

$^{13}$C spectrum of 1 (150 MHz, D$_2$O):
2:

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\text{H spectrum of 2 (600 MHz, D}_2\text{O):}
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\[
\text{\[^{13}\text{C spectrum of 2 (150 MHz, D}_2\text{O):}}
\]
3:

\[ \text{HO-} \quad \text{H} \quad \text{NH} \quad \text{O-} \quad \text{S-S-} \quad \text{NH}_2 \]

\(^1\)H spectrum of 3 (600 MHz, D\(_2\)O):

\(^{13}\)C spectrum of 3 (150 MHz, D\(_2\)O):
4:

$^1$H spectrum of 4 (600 MHz, D$_2$O):

$^{13}$C spectrum of 4 (150 MHz, D$_2$O):
5:

$^1$H spectrum of 5 (600 MHz, C$_5$H$_5$N):

$^{13}$C spectrum of 5 (150 MHz, C$_5$H$_5$N):
Literature Cited: