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Supporting Information

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Probing Integrin Selectivity: Rational Design of Highly Active and Selective Ligands for the •5•1 and •v•3 Integrin Receptor••

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1. Synthesis

1.1 Materials and methods

All technical solvents were distilled prior to use. Dry solvents were purchased from *Aldrich*, *Fluka* or *Merck*. Reactions sensible to oxygen or water were performed in flame-dried reaction vessels under an argon atmosphere (99.996%). All chemicals used in synthesis were purchased from *Aldrich*, *Acros*, *Fluka* or *Lancaster* and used without further purification. Apart from the Mitsunobu reaction, yields are not optimized. Column chromatography was performed using silica gel purchased from *Merck* at 0.8 – 1.5 atm pressure. Analytical and preparative **HPLC** was performed on A) *Amersham Pharmacia Biotech*: Äkta Basic 10F; Pump system P-900; Detector UV-900; Driver software Unicorn, vers. 3.00; Column material: ODS-A C_{18} (120 Å, 5 µm, 250 mm x 4.6 mm); B) *Amersham Pharmacia Biotech:* Äkta Basic 100F; Pump system P-900; Detector UV-900, Driver software Unicorn vers. 3.00; Column material ODS-A C_{18} (120 Å, 10 µm, 250 mm x 20 mm), C) *Beckman*: System Gold, High pressure pump module 125; UV-Detector 166; Column material: ODS-A C_{18} (120 Å, 5 µm, 250 mm x 20 mm), D) *Waters*: System Breeze; Pump System 1525, UV-Detector 2487 Dual; Driver Software Breeze vers. 3.20; Column material ODS-A C_{18} (120 Å, 10 µm, 250 mm x 20 mm). **ESI mass spectra** were recorded on a *Finnigan* LCQ combined with an HPLC-system *Hewlett Packard* HP1100 (Column material: Omnicrom YMC ODS-A C_{18} (120 Å, 3 µm, 125 mm x 2 mm). **NMR** spectra were recorded on *Bruker* AC250 and DMX500 using $CDCl_3$ or DMSO-d_6 as solvent and internal standard. Assignment was performed using different 2D-experiments such as TOCSY, HMQC-COSY.

1.2 General procedures

1.2.1 Preparation of methyl 2-[(*tert*.butyloxcarbonyl)amino]-{4-[3-(pyridin-2-ylamino)propyl]oxyphenyl} propylate (general procedure A)

In a dried flask, *N*-Boc-tyrosine methyl ester (1 eq.), the aminoalcohol (1.1 eq) and tributylphosphine (1.3 eq.) were dissolved in dry THF and stirred at 0°C under argon. Azodicarboxydipiperidid (ADDP, 1.3 eq.) was dissolved in dry THF and added dropwise to the reaction mixture in 4 h time. The resulting light yellow suspension was allowed to warm to room temperature overnight. After addition of silica gel and evaporation of the THF, the mixture was subjected to column chromatography (DCM/ ethyl acetate 2:1) to give the desired compound as a colorless foam.

1.2.2 Preparation of (2-arylcarbamido)-3-{4-[3-(pyridin-2-ylamino)propyl]oxyphenyl}propanoic acids (general procedure B1)

The protected starting material (1 eq.) was dissolved in 3 mL dioxane. After addition of 1 mL concentrated hydrochloric acid, the mixture was stirred for 1 h at room temperature. After evaporation of the solvents, the resulting amine hydrochloride was re-dissolved in 3 mL of DMF. On addition of 1.2 eq. of the corresponding aromatic acid, HATU (1.2 eq.) and DIPEA (5 eq.) the resulting yellow solution was allowed to stir over night. After total consumption of the starting material (HPLC monitoring), the DMF was evaporated and the solid dissolved in 4 mL of methanol/water 3:1. 5 eq. LiOH were added and the reaction monitored by HPLC until total consumption of the starting material. After evaporation of the solvent, the crude product was purified by preparative reverse phase HPLC to afford the final compound as TFA salt (colorless solid).

1.2.3 Preparation of (2-benzamido)-3-{4-[3-(pyridin-2-ylamino) propyl]oxyphenyl} propanoic acids (general procedure B2)

The protected starting material (1 eq.) was dissolved in 3 mL of dioxane. After addition of 1 mL concentrated hydrochloric acid, the mixture was stirred for 1 h at room temperature. After evaporation of the solvents, the resulting amine hydrochloride was re-dissolved in 3 mL of dioxane/water (1:1). On addition of 3 eq. of $NaHCO_3$ and 1.1 eq. of benzoyl chloride, the resulting solution was stirred for 30 min. After evaporation of the solvent the resulting solid was dissolved in 4 mL of methanol/water 3:1. 5eq. LiOH were added and the reaction monitored by HPLC until total consumption of the starting material. After evaporation of the solvent, the crude product was purified by preparative reverse phase HPLC to afford the final compound as TFA salt.

1.2.4 Preparation of (2-arylsulphonamido)-{4-[3-(pyridin-2-ylamino)propyl]oxyphenyl}propanoic acids (general procedure B3)

The protected starting material (1 eq.) was dissolved in 3 mL of dioxane. After addition of 1 mL concentrated hydrochloric acid, the mixture was stirred for 1 h at room temperature. After evaporation of the solvents, the resulting amine hydrochloride was re-dissolved in 4 mL of DMF. On addition of 3 eq. of the corresponding aryl sulphonyl chloride and 5 eq. of DIPEA, the resulting solution was stirred over night, until all starting material was consumed (HPLC monitoring). After evaporation of the solvent the resulting solid was dissolved in 4 mL of methanol/water 3:1. 5eq. LiOH were added and the reaction monitored by HPLC until total consumption of the starting material. After evaporation of the solvent, the crude product was purified by preparative reverse phase HPLC to afford the final compound as TFA salt.

1.2.5 Preparation of methyl-2-chloropyridin-*N*-oxides (general procedure C)

A solution of 2-chloromethylpyridine (1 eq.) and *m*-chloroperbenzoic acid (70%, 1.2 eq.) in chloroform (~1 M) was stirred at 50°C for 12 h. After cooling to -10°C, most of the acid was removed by filtration and the filtrate concentrated under reduced

pressure. Column chromatography on silica gel (DCM/methanol/TEA 95/5/0.1) gave the desired compound as a light yellow solid.

1.2.6 Preparation of 3-[(methylpyridin-*N*-oxid-2-yl)amino]propan-1-ol (general procedure D)

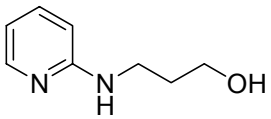
A solution of the 2-chloro-methylpyridine-*N*-oxid (1 eq.) in 3-aminopropanol (20 eq.) was heated to 150°C in a sealed glass tube for 2 h. After cooling, the mixture was directly applied onto a silica gel column (DCM/methanol/TEA 10:1:0.1) to give the desired compound as yellow solid.

1.2.7 Preparation of 3-(methylpyridin-2-ylamino)propan-1-ol (general procedure E)

The pyridine-*N*-oxid (5 mmol) was dissolved in methanol. After addition of 80 mg of catalyst (5 % Pd/C), the mixture was hydrogenated (1 atm H_2) at ambient temperature. The progress of the reaction was monitored by TLC until all starting material was consumed. The catalyst was removed by filtration over Celite®, the solvent was removed and the residue purified by flash chromatography on silica gel (DCM/methanol/TEA 9:1:1) to give the desired compound as pale brown oil.

1.3 Synthesis and compound characterization

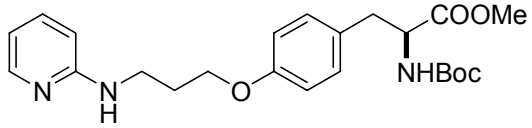
1.3.1 Preparation of 3-(pyridin-2-ylamino)propanol



2-Bromopyridine (5.2 g, 33 mmol) was dissolved in 6 mL of the 3-aminopropanol in a sealed glass tube and heated to 150°C over night. The resulting light brown mixture was directly applied on a silica gel column (DCM/methanol 95:5) to give the desired compound (4.8 g, 31.5 mmol, 95%) as a light yellow oil.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.99 (dd, *J* = 1.04 Hz, *J* = 5.14 Hz, 1H, Ar-*H*6); 7.34 (ddd, *J* = 1.90 Hz, *J* = 7.12 Hz, *J* = 8.56 Hz, 1H, Ar-*H*5); 6.51 (ddd, *J* = 0.80 Hz, *J* = 5.20 Hz, *J* = 7.0 Hz, 1H, Ar-*H*4); 6.37 (d, 8.4 Hz, 1H, Ar-*H*3); 4.70 (bs, 1H); 4.60 (bs, 1H); 3.63 (m, 2H, CH_2OH); 3.49 (dd, *J* = 6.20 Hz, *J* = 12.20 Hz, 2H, -NHCH_2-); 1.73 (m, 2H, -CH_2-). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 159.0, 147.4, 136.5, 111.2, 107.8, 58.7, 37.9, 32.4. **HPLC** (10-50%, 30 min): t_R = 8.75 min. **MS** (ESI): m/z = 153.0 [m+H^+].

1.3.2 Methyl-2-(*S*)-[(*tert.*butyloxycarbonyl)amino]-3-{4-[3-(pyridine-2-yl)-aminopropoxy]phenyl}propylate (2)



Prepared from **1** (100 mg, 657 µmol) and Boc-tyrosine-methyl ester (176 mg, 597 mmol) according to general procedure **A**. Yield: 84 mg (195 mmol, 33%), colorless foam.

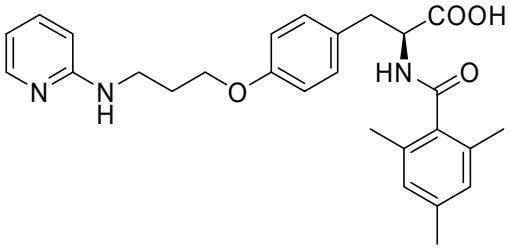
^{1}H-NMR (250 MHz, $CDCl_3$):δ = 8.05 (d, *J* = 4.3 Hz, 1H, Py-*H*6); 7.37 (m, 1H, Py-*H*4); 7.01 (d, *J* = 8.6 Hz, 2H, Tyr-*H*3,3'); 6.81 (d, *J* = 8.6 Hz, 2H, Tyr-*H*2,2'); 6.53 (dd, *J* = 5.4 Hz, *J* = 6.8 Hz, Py-*H*4); 6.38 (d, *J* = 8.5 Hz, 1H, Py-*H*3); 4.99 (bs, 1H, -N*H*); 4.81 (bs, 1H, -N*H*); 4.51 (m, 1H, -C*H*NHBoc-); 4.04 (t, *J* = 5.9 Hz, 2H, -OCH_2-); 3.69 (s, 3H, -COOCH_3) 3.48 (m, 2H, -NH-CH_2-CH_2-); 3.00 (m, 2H, -CH_2-NHBoc-); 2.07 (m, 2H, -CH_2C$H_2$$CH_2$-); 1.40 (s, 9H, ^{t}Bu). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 172.4, 158.7, 157.9, 155.1, 148.1, 137.4, 130.3, 128.1, 114.5, 112.8, 106.7, 79.8, 65.8, 54.5, 52.1, 39.4, 37.5, 29.2, 28.3. **HPLC** (10-100%, 30 min): t_R = 17.01 min. **MS** (ESI): m/z = 452.2 ($M+Na^+$), 430.3 ($M+H^+$), 374.5 ($M+H^+$-^{t}Bu), 330.6 ($M+H^+$-Boc).

1.3.3 2-(*S*)-Benzamido-3-{4-[3-(pyridin-2-yl)aminopropoxy]phenyl} propionic acid (3a)

Prepared from **2** (94 mg, 219 µmol), benzoyl chloride (33 µL, 285 µmol) and $NaHCO_3$ (92 mg, 1.1 mmol) according to general procedure **B2**. Yield after purification : 10 mg (24.1 µmol, 11%), TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 13.32 (bs, 1H), 12.77 (bs, 1H), 8.71 (bs, 1H, Py-N*H*), 8.66 (d, ^3J = 8.2 Hz, 1H, -N*H*COPh), 7.89 (d, ^3J = 6.1 Hz, 1H, Py-*H*6), 7.83 (t, ^3J = 7.9 Hz, 1H, Py-*H*4), 7.80 (d, ^3J = 7.5 Hz, 2H, Ph-*H*2,2'), 7.52 (t, ^3J = 7.3 Hz, 1H, Ph-*H*4), 7.45 (t, ^3J = 7.6 Hz, 2H, Ph-*H*3,3'), 7.23 (d, ^3J = 8.5 Hz, 2H, Tyr-*H*3,3'), 7.00 (d, ^3J = 9.0 Hz, 1H, Py-*H*3), 6.83 (d, ^3J = 8.6 Hz, 2H, Tyr-*H*2,2'), 6.80 (t, *J* = 6.8 Hz, 1H, Py-*H*5), 4.57 (m, 1H, -C*H*COOH), 4.01 (t, ^3J = 6.0 Hz, 2H, -CH_2OAr), 3.45 (t, ^3J = 6.2 Hz, 2H, Py-NHCH_2-), 3.12 (dd, ^2J = 13.8 Hz, ^3J = 4.3 Hz, 1H, Ar-C*H*(H')-), 3.00 (dd, 1H, ^2J = 13.7 Hz, ^3J = 10.9 Hz, 1H, Ar-CH(*H*')-), 2.01 (m, 2H, -CH_2C$H_2$$CH_2$-).**^{13}C-NMR** (125 MHz, DMSO): δ = 173.1, 166.2, 156.8, 152.9, 142.4, 136.5, 133.8, 131.2, 130.1, 129.9, 128.1, 127.2, 114.0, 112.7, 111.7, 64.6, 54.3, 39.4, 38.5, 35.4, 27.6. **HPLC** (10-50%, 30 min): t_R = 20.96 min. **MS** (ESI): m/z = 420.4 $[M+H]^+$.

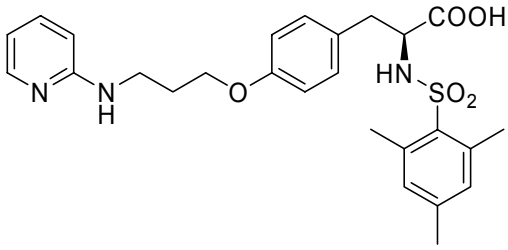
1.3.4 2-(*S*)-(2,4,6-trimethylbenzamido)-3-{4-[3-(pyridin-2-yl)aminopropoxy]phenyl} propionic acid (3b)



Prepared from **2** (100 mg, 233 µmol) and 2,4,6-trimethyl benzoic acid (46 mg, 279 µmol) according to general procedure **B1**. Yield after purification: 32 mg (55 µmol, 24%), TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 15-12 (bs, 1H, -COO*H*), 8.86 (bs, 1H, Py-N*H*), 8.47 (d, *J* = 7.6 Hz, 1H, NHCOAr), 7.93 (m, 1H, Py-*H*6), 7.89 (m, 1H, Py-*H*4), 7.21 (d, *J* = 7.0 Hz, 2H, Ar-*H*3/3'), 7.06 (d, *J* = 8.1 Hz, 1H, Py-*H*3), 6.85 (d, *J* = 6.6 Hz, 3H, Tyr-*H*2/2' + Py-*H*5), 6.75 (s, 2H, Ar-*H*3/3'), 4.62 (m, 1H, -C*H*COOH-), 4.05 (m, 2H, -CH_2-OAr), 3.48 (m, 2H, Py-NH-CH_2-), 3.10 (d, *J* = 13.3 Hz, 1H, Ar-C*H*(H')-), 2.79 (t, *J* = 12.2 Hz, 1H, Ar-CH(*H'*)-), 2.20 (s, 3H, Ar(CH_3)), 2.05 (m, 2H, -CH_2-CH_2-CH_2-), 1.93 (s, 6H, Ar(CH_3)$_2$). **^{13}C-NMR** (125 MHz, DMSO): δ = 173.1, 169.1, 156.9, 152.8, 142.7, 136.9, 136.1, 135.3, 133.7, 130.0, 127.3, 114.1, 113.2, 111.8, 64.7, 53.4, 38.6, 35.4, 27.5, 20.5, 18.4. **HPLC** (10-50%, 30 min): t_R = 24.98 min. **MS** (ESI): m/z = 961.4 (2M+K^+), 945.4 (2M+Na^+), 923.1 (2M+H^+), 462.4 (M+H^+). **HRMS** (ESI) ($C_{27}H_{32}N_3O_4^+$): Calc.: 462.2387, found: 462.2382.

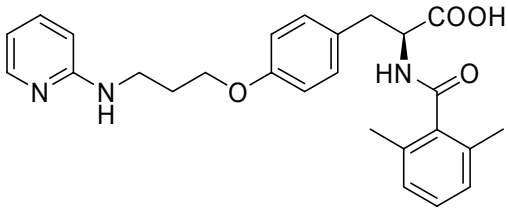
1.3.5 2-(*S*)-[(2,4,6-trimethylphenyl)sulphonamido]-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl} propionic acid (3c)



Prepared from **2** (60 mg, 140 µmol) according to general procedure **B3**. Yield after purification: 14 mg (23 µmol, 15%), TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 8.86 (bs, 1H, Py-N*H*), 8.00 (d, *J* = 9.5 Hz, 1H, -N*H*SO_2Ar), 7.93 (d, *J* = 6.1 Hz, 1H, Py-*H*6), 7.88 (t, *J* = 8.0 Hz, 1H, Py-*H*4), 7.06 (d, *J* = 9.0 Hz, 1H, Py-*H*3), 6.96 (d, *J* = 8.4 Hz, 2H, Tyr-*H*3/3'), 6.85 (s, 2H, Ar-*H*3/3') + (m, 1H, Py-*H*5), 6.65 (d, *J* = 8.4 Hz, 2H, Tyr-*H*2/2'), 4.02 (t, *J* = 6.1 Hz, 2H, -CH_2-OAr), 3.70 (dt, *J* = 5.3 Hz, *J* = 9.4 Hz, 1H, -C*H*COOH-), 3.49 (t, *J* = 6.4 Hz, 2H, Py-NH-CH_2-), 2.85 (dd, *J* = 5.2 Hz, *J* = 13.8 Hz, 1H, Ar-C*H*(H')-), 2.66 (dd, *J* = 9.6 Hz, *J* = 13.8 Hz, 1H, Ar-CH(*H'*)-), 2.41 (s, 6H, Ar(CH_3)$_2$), 2.21 (s, 3H, Ar(CH_3)), 2.05 (m, 2H, -CH_2-CH_2-CH_2-).**^{13}C-NMR** (125 MHz, DMSO): δ = 172.4, 156.9, 152.8, 142.7, 140.8 , 138.0, 136.1 134.4, 131.2, 129.7, 128.7, 113.8, 113.0, 111.8, 64.5, 57.0, 38.6, 36.7, 27.6, 22.4, 20.2. **HPLC** (10-50%, 30 min): t_R = 28.24 min. **MS** (ESI): m/z = 498.5 (M+H^+). **HRMS** (ESI) ($C_{26}H_{32}N_3O_5S^+$): Calc.: 498.2057, found: 498.2049.

1.3.6 2-(*S*)-(2,6-dimethylbenzamido)-3-{4-[3-(pyridin-2-ylamino) propoxy]phenyl} propionic acid (3d)

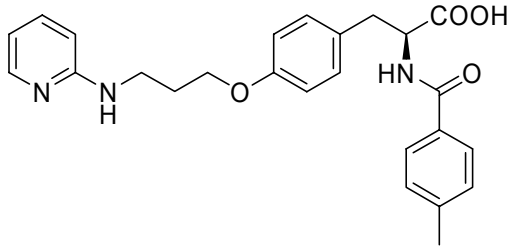


Prepared from **2** (75 mg, 175 µmol) and 2,6-dimethylbenzoic acid (32 mg, 210 µmol) according to general procedure **B1**. Yield after purification: 17 mg (30 µmol, 17%) as TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 13.56,12.71 (bs, 1H, COO*H*), 8.65 (bs, 1H, Py-N*H*), 8.57 (d, *J* = 8.3 Hz, 1H, -N*H*COAr), 7.92 (d, *J* = 6.0 Hz, 1H, Py-*H*6), 7.85 (t, *J* = 7.7 Hz, 1H, Py-*H*4), 7.21 (d, *J* = 8.5 Hz, Tyr-*H*3,3'), 7.11 (t, *J* = 7.6 Hz, 1H, Ar-*H*4), 7.01 (d, *J* = 8.9 Hz, 2H, Ar-*H*3,3'), 6.95 (d, *J* = 7.6 Hz, 1H, Py-*H*3), 6.86 (d, *J* = 8.5 Hz, 2H, Tyr-*H*2,2'), 6.82 (t, *J* = 6.6 Hz, 1H, Py-*H*5), 4.63 (m, 1H, -C*H*COOH), 4.05 (t, *J* = 6.0 Hz, 2H, Py-NHCH_2-), 3.47 (m, 2H, -CH_2OAr), 3.11 (dd, *J* = 13.9

Hz, J = 4.0 Hz, 1H, Ar-C*H*(H')-), 2.79 (dd, J = 13.7 Hz, J = 11.5 Hz, 1H, Ar-CH(*H'*)-), 2.04 (m, 2H, -CH_2-CH_2-CH_2-), 1.96 (s, 6H, Ar(CH_3)$_2$). **^{13}C-NMR** (125 MHz, DMSO): δ = 173.0, 168.9, 156.9, 153.1, 142.2, 137.9, 136.9, 133.7, 130.0, 127.8, 126.7, 114.1, 112.9, 111.7, 64.8, 53.3, 39.4, 38.5, 35.4, 27.6, 18.4. **HPLC** (10-50%, 30 min): t_R = 22.34 min. **MS** (ESI): m/z = 448.4 $[m+H]^+$. **HRMS** ($C_{26}H_{30}N_3O_4^+$): Calc.: 448.2231, found: 448.2227.

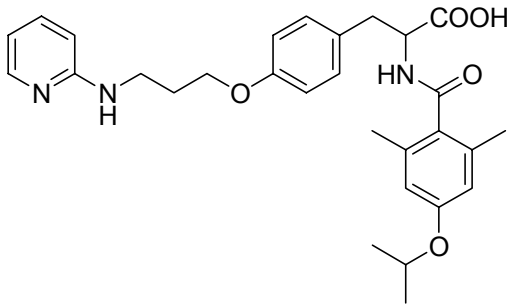
1.3.7 2-(*S*)-(4-Methylbenzamido)-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl}-propionic acid (3e)



Prepared from **2** (100 mg, 233 µmol) and 4-methylbenzoic acid (38 mg, 280 µmol) according to general procedure **B1**. Yield: 28 mg (51 µmol, 22%) as TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 14.50 (bs, -COO*H*), 8.82 (bs, 1H, Py-N*H*), 8.54 (d, J = 8.2 Hz, 1H, -N*H*Ar), 7.85 (d, J = 6.1 Hz, 1H, Py-*H*6), 7.82 (m, 1H, Py-*H*4), 7.68 (d, J = 7.8 Hz, 2H, Ar-*H*2), 7.21 (d, J = 8.1 Hz, 2H, Tyr-*H*3/3'), 7.19 (d, J = 8.3 Hz, 2H, Ar-*H*3/3'), 7.00 (d, J = 9.0 Hz, 1H, Py-*H*3), 6.79 (d, J = 8.0 Hz, 2H, Tyr-*H*2/2', m, 1H, Py-*H*5), 4.52 (m, 1H, -C*H*COOH-), 3.98 (t, J = 5.9 Hz, 2H, -CH_2OAr-), 3.42 (m, 1H, Py-NHCH_2-), 3.07 (dd, J = 13.8 Hz, J = 4.0 Hz, 1H, ArC*H*(H')-), 2.96 (m, 1H, Ar-CH(*H'*)-), 2.30 (s, 3H, ArCH_3), 1.98 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 173.2, 166.1, 156.1, 152.7, 142.7, 141.2, 135.9, 131.0, 130.2, 130.0, 128.7, 127.3, 114.0, 113.2, 111.8, 64.5, 54.3, 38.6, 35.4, 27.5, 20.8. **HPLC** (10-50%, 30 min): t_R = 23.21 min. **MS** (ESI): m/z = 434.5 $[M+H]^+$. **HRMS** (ESI) ($C_{25}H_{28}N_3O_4^+$) Calc.: 434.2074, found: 434.2070.

1.3.8 2-(*S*)-(4-Isopropoxy-2,6-dimethylbenzamido)-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl} propionic acid (3f)

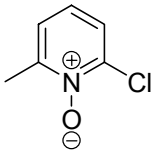


Prepared from **2** (67 mg, 155 µmol) and 4-isopropoxy-2,6-dimethylbenzoic acid (34 mg, 186 µmol) according to general procedure **B1**. Yield: 31 mg (50 µmol, 32%), Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 13.40, 12.70 (bs, 1H, -COO*H*), 8.74 (bs, 1H, Py-N*H*), 8.45 (d, J = 8.3 Hz, 1H, -CON*H*Ar), 7.92 (d, J = 5.8 Hz, 1H, Py-*H*6), 7.86 (t, J = 7.7 Hz, 1H, Py-*H*4), 7.20 (d, J = 8.3 Hz, 2H, Tyr-*H*3,3'), 7.03 (d, J = 8.9 Hz, 1H, Py-*H*3), 6.85 (d, J = 8.3 Hz, 2H, Tyr-*H*2,2'), 6.83 (t, J = 6.7 Hz, 1H, Py-*H*5), 6.50 (s, 2H, Ar-*H*2,2'), 4.60 (m, 1H, Ar-OC*H*(CH_3)$_2$), 4.55 (m, 1H, -C*H*COOH), 4.05 (t, J = 5.1 Hz, 2H, -CH_2OAr), 3.47 (m, 2H, PyNHCH_2-), 3.09 (dd, J = 13.8 Hz, J = 3.5 Hz, 1H, ArC*H*(H')-), 2.79 (dd, J = 13.4 Hz, J = 11.7 Hz, 1H, ArCH(*H'*)-), 2.04 (m, 2H, -CH_2-CH_2-CH_2-), 1.94 (s, 6H, -CH(CH_3)$_2$). **^{13}C-NMR** (125 MHz, DMSO): δ = 173.1, 169.0, 156.9, 156.7, 152.9, 142.5, 136.6, 135.5, 130.7, 130.0,

130.0, 114.1, 113.8, 113.1, 111.8, 68.7, 64.7, 53.4, 39.4, 38.5, 27.5, 21.7, 18.7. **HPLC** (10-100%, 30 min): t_R = 16.18 min. **MS** (ESI): m/z = 506.5 $[m+H]^+$, 191.2 $[COC_6H_2(CH_3)_2OCH(CH_3)_2]^+$, 149.2 $[C_6H_2(CH_3)_2OCH(CH_3)_2]^+$, **HRMS** (ESI) ($C_{27}H_{32}N_3O_5^+$): Calc.: 506.2649, found: 506.2645.

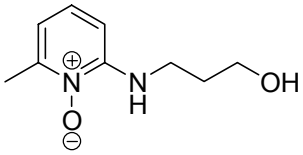
1.3.9 2-Chloro-6-methylpyridin-*N*-oxid



Prepared from 2-Chloro-6-methylpyridine (1 g, 7.84 mmol) according to general procedure **C**.Yield: 993 mg (6.94 mmol, 89%) of a yellow solid.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.37 (dd, *J* = 8.0 Hz, *J* = 1.5 Hz, 1H, Py-*H*3), 7.19 (dd, *J* = 7.2 Hz, *J* = 1.1 Hz, 1H, Py-*H*5), 7.10 (t, *J* = 8.0 Hz, 1H, Py-*H*4), 2.53 (s, 3H, -C*H*$_3$). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 151.1, 142.2, 125.3, 124.6, 124.2, 18.5. **HPLC** (5-20%, 30 min):t_R = 10.75 min. **MS** (ESI): m/z = 144.0 $[m+H^+]$.

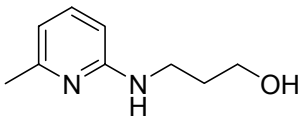
1.3.10 3-(6-Methylpyridin-*N*-oxid-2-ylamino)propan-1-ol



Prepared from 2-Chloro-6-methylpyridin-*N*-oxid (744 mg, 5.18 mmol) according to general procedure **D**. Yield: 934 mg (5.13 mmol, 99%) of a brown solid.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.19 (bt, *J* = 5.4 Hz, 1H, Py-N*H*), 7.07 (t, *J* = 8.1 Hz, 1H, Py-*H*4), 6.46 (m, 2H, Py-*H*3,5), 3.70 (t, *J* = 5.9 Hz, 2H, -C*H*$_2$OH), 3.39 (m, 2H, Py-NHC*H*$_2$-), 3.38 (m, 1H, -O*H*), 2.47 (s, 3H, -C*H*$_3$), 1.84 (m, 2H, -CH_2-C*H*$_2$-CH_2-). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 150.5, 147.3, 128.5, 111.6, 103.2, 59.3, 39.3, 31.2, 18.0. **HPLC** (5-20%, 30 min): t_R = 14.08 min. **MS** (ESI): m/z = 183.1 $[m+H^+]$.

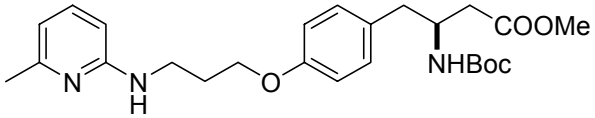
1.3.11 3-(6-Methylpyridin-2-ylamino)propan-1-ol (4a)



Prepared from 3-(6-Methylpyridin-*N*-oxid-2-ylamino)-propan-1-ol (714 mg, 3.92 mmol) according to general procedure **E**. Yield: 469 mg (2.82 mmol, 72%), light yellow oil.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.26 (t, *J* = 7.6 Hz, 1H, Py-*H*4), 6.38 (d, *J* = 7.2 Hz, 1H, Py-*H*5), 6.19 (d, *J* = 8.4 Hz, 1H, Py-*H*3), 4.79 (bs, 1H, -N*H*), 4.64 (bs, 1H, -O*H*), 3.62 (t, *J* = 5.6 Hz, 2H, -C*H*$_2$OH), 3.51 (dd, *J* = 12.1 Hz, *J* = 6.3 Hz, 2H, Py-NH-C*H*$_2$-), 2.35 (s, 3H, Py-C*H*$_3$), 1.72 (m, 2H, -CH_2-C*H*$_2$-CH_2-). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 158.7, 156.2, 137.8, 111.7, 104.9, 58.4, 37.9, 33.5, 23.8. **HPLC** (5-20%, 30 min): t_R = 11.52 min. **MS** (ESI): m/z = 167.1 $[m+H^+]$.

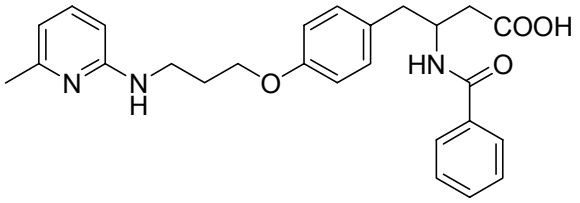
1.3.12 Methyl 3-(*S*)-[(*tert.*Butyloxycarbonyl)amino]-4-{4-[3-(6-methylpyridin-2-yl)aminopropoxy]phenyl}-butanoate (5a)



Prepared from **4a** (130 mg, 782 µmol) and Boc-β-tyrosine-methyl ester (160 mg, 517 µmol) according to general procedure **A**. Yield: 161 mg (350 µmol, 68%) as colorless foam.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.29 (t, *J* = 7.5 Hz, 1H, Py-*H*4), 7.05 (d, *J* = 8.2 Hz, 2H, Tyr-*H*3/3'), 6.79 (d, *J* = 8.2 Hz, 2H, Tyr-*H*2/2'), 6.40 (d, *J* = 7.2 Hz, 1H, Py-*H*5), 6.20 (d, *J* = 8.3 Hz, 1H, Py-*H*3), 5.01 (bs, 1H, N*H*-Boc), 4.77 (bs, 1H, Py-N*H*-), 4.08 (m, 1H, -C*H*NHBoc-); 4.01 (t, *J* = 5.9 Hz, 2H, -CH_2OAr-); 3.64 (s, 3H, -COOCH_3); 3.41 (q, *J* = 5.9 Hz, 2H, -NH-CH_2-); 2.83 (dd, *J* = 13.4 Hz, *J* = 6.7 Hz, 1H, -C*H*(H')COOMe); 2.71 (dd, *J* = 13.9 Hz, *J* = 7.3 Hz, 1H, -CH(*H'*)COOMe); 2.43 (m, 2H, Ar-CH_2-); 2.33 (s, 3H, Py-CH_3); 2.07 (m, 2H, -$CH_2CH_2CH_2$-); 1.39 (s, 9H, ^{t}*Bu*). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 171.9, 158.8, 157.7, 156.6, 155.1, 138.0, 130.2, 129.8, 115.4, 114.5, 102.7, 79.2, 65.7, 51.5, 49.0, 39.5, 39.4, 37.5, 29.2, 28.2, 23.9. **HPLC** (10-100%, 30 min): t_R = 17.83 min. **MS** (ESI): m/z = 458.3 $[m+H]^+$, 402.2 $[m+H-^tBu]^+$, 358.2 $[m+H-Boc]^+$.

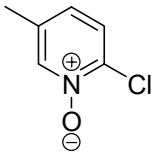
1.3.13 3-(*S*)-(Benzamido)-4-{4-[3-(6-methylpyridin-2-yl)-aminoprooxy]phenyl} butanoic acid (6a)



Prepared from **5a** (150 mg, 328 µmol) according to general procedure **B2**. Yield after purification: 49 mg (87 µmol, 27%) as TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 8.73 (bs, 1H, Py-N*H*), 8.34 (d, *J* = 8.4 Hz, 1H, -CON*H*-), 7.76 (d+m, *J* = 7.2 Hz, 2+1H, Ph-*H*2/2'+ Py-*H*4), 7.50 (t, 1H, *J* = 7.3 Hz, Ph-*H*4), 7.44 (t, *J* = 7.4 Hz, 2H, Ph-*H*3/3'), 7.14 (d, *J* = 8.6 Hz, 2H, Tyr-*H*3/3'), 6.88 (d, *J* = 9.0 Hz, 1H, Py-*H*3), 6.83 (d, *J* = 8.6 Hz, 2H, Tyr-*H*2/2'), 6.65 (d, 1H, *J* = 7.2 Hz, Py-*H*5), 4.44 (m, 1H, -C*H*NHBoc-), 4.01 (t, *J* = 6.0 Hz, 2H, -CH_2OAr), 3.49 (t, *J* = 6.0 Hz, 2H, Py-NHCH_2-), 2.81 (dd, *J* = 13.6 Hz, *J* = 8.0 Hz, 1H, Ar-C*H*(H')-), 2.75 (dd, *J* = 13.6 Hz, *J* = 5.9 Hz, 1H, Ar-CH(*H'*)-), 2.52 (dd, *J* = 15.5 Hz, *J* = 7.7 Hz, 1H, -C*H*(H')COOH), 2.44 (dd, *J* = 15.5 Hz, *J* = 6.2 Hz, 1H, -CH(*H'*)COOH), 2.40 (s, 3H, Py-CH_3), 2.01 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 172.4, 165.6, 156.7, 153.3, 147.4, 143.5, 134.6, 130.9, 130.8, 130.0, 128.1, 127.1, 114.1, 111.4, 108.8, 64.6, 48.4, 38.8, 38.7, 28.7, 18.7. **HPLC** (10-50%, 30 min): t_R = 23.04 min. **MS** (ESI): m/z = 448.4 $[m+H]^+$. **HRMS** (ESI) ($C_{26}H_{30}N_3O_4^+$): Calc.: 448.2231, found: 448.2232.

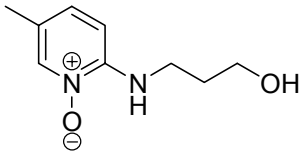
1.3.14 2-Chloro-5-methylpyridin-*N*-oxid



Prepared from 2-Chloro-5-methylpyridine (1 g, 7.84 mmol) according to general procedure **C**. Yield: 915 mg (6.40 mmol, 82%) of a yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.24 (s, 1H, Py-*H*6), 7.38 (d, *J* = 8.41 Hz, 1H, Py-*H*3), 7.06 (dd, *J* = 8.0 Hz *J* = 0.4 Hz, 1H, Py-*H*4), 2.31 (s, 3H, -C*H*$_3$). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 140.5, 134.9, 128.0, 126.4, 17.9. **HPLC** (5-20%, 30 min): t_R = 11.10 min. **MS** (ESI): m/z = 144.0 [m+H$^+$].

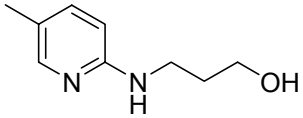
1.3.15 3-(5-Methylpyridin-*N*-oxid-2-yl)aminopropan-1-ol



Prepared from 2-Chloro-5-methylpyridin-*N*-oxid (670 mg, 4.67 mmol) according to general procedure **D**. Yield: 840 mg (4.61 mmol, 98%) of a brown solid.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.90 (s, 1H, Py-*H*6), 7.06 (m, 1H, Py-N*H*), 7.03 (d, *J* = 8.7 Hz, 1H, Py-*H*3), 6.55 (d, *J* = 8.6 Hz, 1H, Py-*H*4), 3.74 (t, *J* = 5.6 Hz, 2H, -C*H*$_2$OH), 3.45 (q, 2H, *J* = 6.2 Hz, Py-NH-C*H*$_2$-), 2.17 (s, 3H, Py-C*H*$_3$), 1.85 (m, 2H, -CH$_2$-C*H*$_2$-CH$_2$-). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 148.4, 136.9, 130.8, 120.9, 105.8, 59.0, 38.8, 31.3, 17.2. **HPLC** (5-20%, 30 min): t_R = 14.69 min. **MS** (ESI): m/z = 183.0 [m+H$^+$], 205.0 [m+Na$^+$].

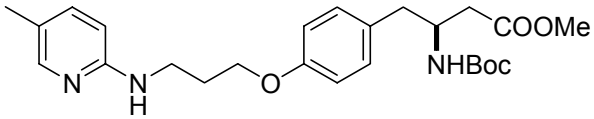
1.3.16 3-(5-Methylpyridin-2-yl)aminopropan-1-ol (4b)



Prepared from 3-(5-Methylpyridin-*N*-oxid-2-ylamino)propan-1-ol (815 mg, 4.47 mmol) according to general procedure **E**. Yield: 446 mg (2.68 mmol, 60%) yellow solid.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.81 (s, 1H, Py-*H*6), 7.18 (dd, *J* = 8.5 Hz, *J* = 2.2 Hz, 1H, Py-*H*4), 6.32 (d, *J* = 8.5 Hz, 1H, Py-*H*3), 4.99 (bs, 1H, -N*H*), 4.68 (bs, 1H, -O*H*), 3.62 (t, *J* = 5.6 Hz, 2H, -C*H*$_2$OH), 3.51 (m, 2H, Py-NH-C*H*$_2$-), 2.12 (s, 3H, Py-C*H*$_3$), 1.71 (m, 2H, -CH$_2$-C*H*$_2$-CH$_2$-). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 157.2, 146.4, 138.6, 121.2, 107.9, 58.7, 38.2, 33.3, 17.2. **HPLC** (5-20%, 30 min): t_R = 12.65 min. **MS** (ESI): m/z = 167.1 [m+H$^+$].

1.3.17 Methyl 3-(*S*)-[(*tert.*Butyloxycarbonyl)amino]-4-{4-[3-(5-methylpyridin-2-yl)aminopropoxy]phenyl} butanoate (5b)

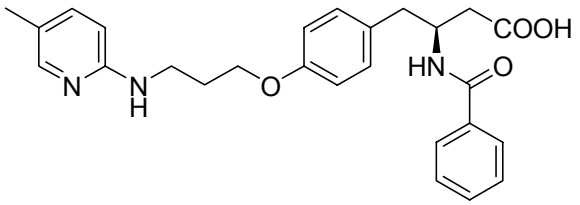


Prepared from **4b** (130 mg, 970 µmol) and Boc-β-tyrosine methyl ester (150 mg, 485 µmol) according to general procedure **A**. Yield: 60 mg (131 µmol, 27%) of a colorless foam.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.88 (m, 1H, Py-*H*6); 7.24 (m, 1H, Py-*H*4), 7.07 (d, *J* = 8.5 Hz, 2H, Tyr-*H*3,3'); 6.82 (d, *J* = 8.5 Hz, 2H, Tyr-*H*2,2'), 6.34 (d, *J* = 8.4 Hz, 1H, Py-*H*3); 4.98 (bs, 1H, -N*H*); 4.69 (bs, 1H, -N*H*); 4.10 (m, 1H, -C*H*NHBoc-); 4.04 (t, *J* = 5.92 Hz, 2H, -C*H*$_2$OAr-); 3.67 (s, 3H, -COOC*H*$_3$); 3.46 (q, *J* = 6.4 Hz, 2H, -NHC*H*$_2$-); 2.85 (dd, *J* = 13.8 Hz, *J* = 6.8 Hz, 1H, -C*H*(H')COOMe); 2.72 (dd, *J* = 13.8 Hz, *J* = 7.6 Hz, 1H, -CH(*H'*)COOMe); 2.45 (m, 2H, Ar-C*H*$_2$-); 2.15 (s, 3H, Py-C*H*$_3$); 2.07 (m, 2H, -CH$_2$C*H*$_2$CH$_2$-); 1.41 (s, 9H, ^{t}*Bu*). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 172.1, 157.5, 156.5, 155.1, 146.2,

139.0, 130.2, 129.8, 121.4, 114.5, 106.6, 79.2, 65.6, 51.6, 48.9, 39.5, 39.4, 37.4, 29.1, 28.3, 17.2. **HPLC** (10-100%, 30 min): t_R = 17.78 min, **MS** (ESI): m/z = 458.2 $[m+H]^+$, 402.3 $[m+H-{}^t Bu]^+$, 358.3 $[m+H-Boc]^+$.

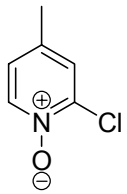
1.3.18 3-(*S*)-(Benzamido)-4-{4-[3-(5-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6b)



Prepared from **5b** (59 mg, 131 µmol) according to general procedure **B2**. Yield after purification: 35 mg (62 µmol, 48%), TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 8.67 (bs, 1H, Py-N*H*), 8.33 (d, *J* = 8.4 Hz, 1H, -N*H*COPh), 7.77-7.72 (s, 1H, Py-*H*6 + d, 2H, Ph-*H*2/2' + d, 1H, Py-*H*4), 7.51 (t, *J* = 7.3 Hz, 1H, Ph-*H*4), 7.44 (t, *J* = 7.4 Hz, 2H, Ph-*H*3/3'), 7.14 (d, *J* = 8.5 Hz, 2H, Tyr-*H*3/3'), 6.98 (d, *J* = 9.1 Hz, 1H, Py-*H*3), 6.84 (d, *J* = 8.5 Hz, 1H, Tyr-*H*2/2'), 4.43 (m, 1H, -C*H*(NHCOPh)), 4.02 (t, *J* = 6.0 Hz, 2H, -CH_2OAr), 3.44 (t, *J* = 6.3 Hz, Py-NH-CH_2-), 2.81 (dd, *J* = 13.6 Hz, *J* = 8.0 Hz, 1H, Ar-C*H*(H')-), 2.75 (dd, *J* = 13.6 Hz, *J* = 5.9 Hz, 1H, Ar-CH(*H'*)-), 2.52 (dd, *J* = 15.7 Hz, *J* = 7.7 Hz, 1H, -C*H*(H')COOH), 2.44 (dd, *J* = 15.4 Hz, *J* = 6.2 Hz, -CH(*H'*)COOH), 2.16 (s, 3H, Py-CH_3), 2.01 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 172.4, 165.6, 156.7, 151.2, 144.8, 134.6, 133.3, 131.0, 130.8, 128.1, 127.1, 121.2, 114.1, 113.0, 65.5, 49.3, 39.8, 39.7, 39.6, 28.6, 17.2. **HPLC** (10-50%, 30 min): t_R = 23.08 min. **MS** (ESI): m/z = 448.4 $[m+H]^+$. **HRMS** (ESI) ($C_{26}H_{30}N_3O_4^+$): Calc.: 448.2231, found: 448.2228.

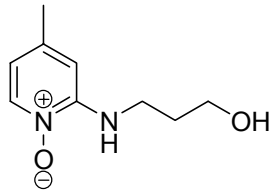
1.3.19 2-Chloro-4-methylpyridin-*N*-oxide



Prepared from 2-Chloro-4-methylpyridine (1 g, 7.84 mmol) according to general procedure **C**. Yield: 870 mg (6.08 mmol, 78%) of a yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.25 (d, *J* = 6.8 Hz, 1H, Py-*H*6), 7.29 (d, *J* = 1.9 Hz, 1H, Py-*H*3), 7.00 (dd, *J* = 6.6 Hz, *J* = 2.2 Hz, 1H, Py-*H*5), 2.32 (s, 3H, -CH_3). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 139.9, 138.7, 137.3, 127.4, 124.8, 20.0. **HPLC** (5-20%, 30 min): t_R = 11.08 min. **MS** (ESI): m/z = 144.0 $[m+H^+]$.

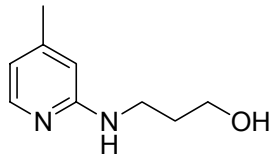
1.3.20 3-(4-Methylpyridin-*N*-oxid-2-ylamino)propan-1-ol



Prepared from 2-Chloro-4-methylpyridin-*N*-oxide (633 mg, 4.41 mmol) according to general procedure **D**. Yield: 802 mg (4.40 mmol, 99%) of a light brown solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.90 (d, *J* = 6.5 Hz, 1H, Py-*H*6), 7.24 (bs, 1H, Py-N*H*), 6.44 (s, 1H, Py-H3), 6.34 (dd, *J* = 6.7 Hz, *J* = 2.1 Hz, 1H, Py-*H*5), 3.74 (t, *J* = 5.7 Hz, 2H, -C*H*$_2$OH), 3.45 (q, *J* = 6.3 Hz, 2H, NHC*H*$_2$), 2.28 (s, 3H, -C*H*$_3$), 1.86 (m, 2H, -CH_2-C*H*$_2$-CH_2-). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 149.6, 141.8, 136.6, 112.4, 106.4, 59.1, 38.9, 31.2, 21.1. **HPLC** (5-20%, 30 min): t_R = 13.89 min. **MS** (ESI): m/z = 183.1 [m+H^+].

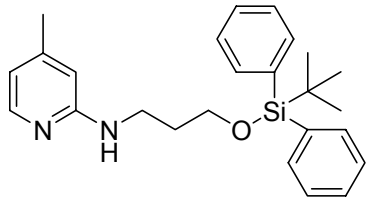
1.3.21 3-(4-Methylpyridin-2-ylamino)propan-1-ol



Prepared from 3-(4-Methylpyridin-*N*-oxid-2-ylamino)propan-1-ol (685 mg, 3.74 mmol) according to general procedure **E**. Yield: 404 mg (2.43 mmol, 65%) of a light brown oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.82 (d, *J* = 5.4 Hz, 1H, Py-*H*6), 6.37 (dd, *J* = 5.5 Hz, *J* = 0.8 Hz, 1H, Py-*H*5), 6.26 (s, 1H, Py-*H*3), 5.10 (bs, 1H, Py-N*H*), 3.63 (t, *J* = 5.7 Hz, 2H, -C*H*$_2$OH), 3.48 (bs, 1H, -O*H*), 3.08 (q, *J* = 7.3 Hz, 2H, Py-NHC*H*$_2$-), 2.20 (s, 3H, -C*H*$_3$), 1.74 (m, 2H, -CH_2-C*H*$_2$-CH_2-). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 149.7, 141.8, 136.6, 112.4, 106.4, 59.1, 38.9, 31.2, 21.1. **HPLC** (5-20%, 30 min): t_R = 13.19 min. **MS** (ESI): m/z = 167.1 [m+H^+].

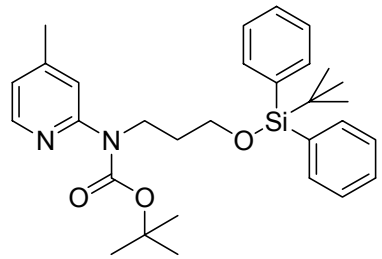
1.3.22 1-(Dimethylphenylsilyloxy)-3-(4-methylpyridin-2-ylamino)propane



3-(4-Methylpyridin-2-ylamino)propanol (554 mg, 3.33 mmol, 1 eq.) and imidazole (453 mg, 6.66 mmol, 2 eq.) were dissolved in 20 mL of dry DCM and cooled to 0°C. TBDPSCl (1.3 mL, 4.99 mmol, 1.3 eq.) was added dropwise, then the reaction was allowed to warm up to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel (DCM, ethyl acetate 8:2) to give the desired compound (1.02 g, 2.52 mmol, 76%) as a colorless oil.

^{1}H-NMR (250 MHz, $CDCl_3$):δ = 7.95 (d, *J* = 7.3 Hz, 1H, Py-*H*6), 7.70 (m, 4H, Ph-*H*), 7.48-7.35 (m, 6H, Ph-*H*), 6.40 (dd, *J* = 5.2 Hz, *J* = 0.8 Hz, 1H, Py-*H*5), 6.15 (s, 1H, Py-*H*3), 4.68 (bt, 1H, Py-N*H*-), 3.82 (t, *J* = 5.8 Hz, 2H, -C*H*$_2$OSi), 3.44 (m, 2H, Py-NHC*H*$_2$-), 2.22 (s, 3H, -C*H*$_3$), 1.87 (m, 2H, -CH_2-C*H*$_2$-CH_2-), 1.11 (s, 9H, Si-^{t}Bu). **^{13}C-NMR** (62.9 MHz, $CDCl_3$): δ = 159.1, 148.1, 147.7, 135.5, 133.6, 129.7, 127.7, 114.1, 106.9, 62.1, 32.0, 26.9, 21.1, 19.2. **HPLC** (10-100%, 30 min): t_R = 18.03 min. **MS** (ESI): m/z = 405.3 [m+H^+].

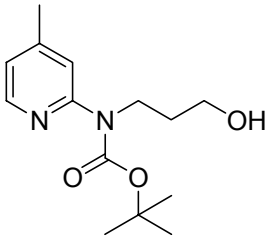
1.3.23 1-(Dimethylphenylsilyloxy)-3-(4-methylpyridin-2-yl)-3-(tert. butyloxycarbonyl)aminopropane



To a solution of 1-(Dimethylphenylsilyloxy)-3-(4-methylpyridin-2-ylamino)propane (1.02 g, 2.52 mmol, 1 eq.) in 20 mL dry THF was added triethyl amine (700 µL, 5.04 mmol, 2 eq.), DMAP (31 mg, 0.25 mmol, 0.1 eq.) and di(*tert.*butyl)carbonate (660 mg, 3.02 mmol, 1.2 eq.). After 18 h, the solvent was removed *in vaccuo* and the product purified by flash chromatography on silica gel (hexanes, ethyl acetate 8:2) to give the desired compound (1.05 g, 2.08 mmol, 83%) as a colorless oil.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 8.26 (d, *J* = 5.2 Hz, 1H, Py-*H*6), 7.70 (m, 4H, Ph-*H*), 7.47-7.35 (m, 6H, Ph-*H*), 6.84 (d, *J* = 4.9 Hz, 1H, Py-H5), 4.14 (t, *J* = 7.1 Hz, 2H, CH_2OSi), 3.76 (t, *J* = 6.2 Hz, 2H, Py-NCH_2), 2.34 (-CH_3), 1.97 (m, 2H, -CH_2-CH_2-CH_2-), 1.52 (s, 9H, NCO^{t}Bu), 1.08 (s, 9H, Si-^{t}Bu). **^{13}C-NMR** (62.9 MHz, $CDCl_3$): δ = 154.7, 154.2, 147.8, 147.2, 135.4, 133.7, 129.4, 127.5, 120.7, 120.5, 80.5, 61.8, 44.2, 32.0, 28.2, 26.7, 21.0, 19.0. **HPLC** (10-100%, 30 min): t_R = 29.66 min. **MS** (ESI): m/z = 505.3 [m+H^+], 449.0 [m+H^+-^{t}Bu], 405.3 [m+H^+-Boc].

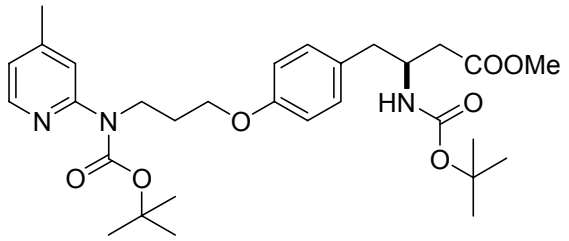
1.3.24 3-[*N*-(4-Methylpyridin-2-yl)]-3-*N*-(*tert.* butyloxycarbonyl)aminopropan-1-ol (4c)



A solution of 1-(Dimethylphenylsilyloxy)-3-(4-methylpyridin-2-yl)-3-(tert. butyloxycarbonyl)aminopropane (1.05 g, 2.08 mmol, 1 eq.) and TBAF (788 mg, 2.49 mmol, 1.1 eq.) in 20 mL of THF was stirred at ambient temperature for 18 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel (DCM, ethyl acetate 8:2) to give the desired compound (532 mg, 1.99 mmol, 96%) as a colorless oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.82 (d, *J* = 5.4 Hz, 1H, Py-*H*6), 6.37 (dd, *J* = 5.5 Hz, *J* = 0.8 Hz, 1H, Py-*H*5), 6.26 (s, 1H, Py-*H*3), 5.10 (bs, 1H, Py-N*H*), 3.63 (t, *J* = 5.7 Hz, 2H, -CH_2OH), 3.48 (bs, 1H, -O*H*), 3.08 (q, *J* = 7.3 Hz, 2H, Py-NHCH_2-), 2.20 (s, 3H, -CH_3), 1.74 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 149.7, 141.8, 136.6, 112.4, 106.4, 59.1, 38.9, 31.2, 21.1. **HPLC** (10-100%, 30 min): t_R = 12.62 min. **MS** (ESI): m/z = 267.2 [m+H^+], 211.1 [m+H^+-^{t}Bu], 167.1 [m+H^+-Boc].

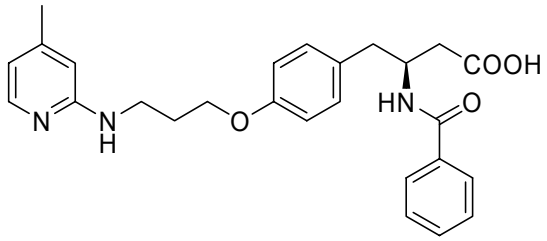
1.3.25 Methyl 3-(*S*)-[(*tert.*-butyloxycarbonyl)amino]-4-{4-[3-(*N*-(4-methylpyridin-2-yl)-*N*-(*tert.*butyloxycarbonyl))aminopropoxy] phenyl} butanoate (5c)



Prepared from **4c** (223 mg, 838 µmol) and Boc-β-tyrosine methyl ester (216 mg, 698 µmol) according to general procedure **A**. Yield: 90 mg (161 µmol, 23%) of a colorless foam.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.17 (d, *J* = 4.9 Hz, 1H, Py-*H*6), 7.40 (s, 1H, Py-*H*3), 7.03 (d, 2H, *J* = 8.1 Hz Tyr-*H*3,3'), 6.79 (d, *J* = 4.4 Hz, 1H, Py-*H*5), 6.74 (d, *J* = 8.2 Hz, 2H, Tyr-*H*2,2'), 5.01 (d, *J* = 5.7 Hz, 1H, -N*H*Boc), 4.10 (t, *J* = 6.8 Hz, 2H, -CH_2O-; m, 1H, -C*H*CH_2COOMe), 3.95 (t, 2H, *J* = 6.1 Hz, Py-NBoc-CH_2-), 3.65 (s, 3H, -COOCH_3), 2.83 (m, 1H, -C*H*(H')COOMe), 2.70 (dd, *J* = 13.3 Hz, *J* = 7.9 Hz, 1H, -CH(*H'*)COOMe), 2.28 (s, 3H, Py-CH_3), 2.46 (dd, *J* = 15.6 Hz, *J* = 5.2 Hz, 1H, Ar-C*H*(H')-), 2.39 (dd, *J* = 15.7 Hz, *J* = 5.5 Hz, 1H, Ar-CH(*H'*)-), 2.07 (m, 2H, -CH_2-CH_2-CH_2-), 1.47 (s, 9H, ^{t}Bu), 1.37 (s, 9H, ^{t}Bu). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 172.0, 157.7, 155.0, 154.5, 154.1, 148.0, 147.2, 130.0, 129.4, 120.7, 120.4, 114.4, 80.8, 79.2, 65.6, 51.5, 48.8, 40.0, 39.3, 37.3, 28.8, 28.3, 28.2, 21.0. **HPLC** (10-100%, 30 min): t_R = 22.17 min. **MS** (ESI): m/z = 580.2 $[m+Na]^+$, 558.1 $[m+H]^+$, 502.1 $[m+H-^tBu]^+$, 458.3 $[m+H-Boc]^+$, 402.4 $[m+H-Boc-^tBu]^+$, 358.6 $[m+H-2Boc]^+$.

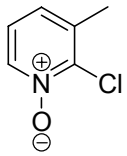
1.3.26 3-(*S*)-(Benzamido)-4-{4-[3-(4-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6c)



Prepared from **5c** (90 mg, 161 µmol) according to general procedure **B2**. Yield after purification: 17 mg (30 µmol, 19%) as TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 13.44, 12.22 (bs, 1H), 8.71 (bs, 1H, Py-N*H*), 8.34 (d, *J* = 8.4 Hz, -N*H*COPh), 7.82 (d, *J* = 6.5 Hz, 1H, Py-*H*6), 7.77 (d, *J* = 7.4 Hz, 2H, Ph-*H*2,2'), 7.51 (t, *J* = 7.3 Hz, 1H, Ph-*H*4), 7.44 (t, *J* = 7.5 Hz, 2H, Ph-*H*3,3'), 7.15 (d, *J* = 8.5 Hz, 2H, Tyr-*H*3,3'), 6.85 (s, 1H, Py-*H*3), 6.84 (d, *J* = 8.4 Hz, 2H, Tyr-*H*2,2'), 6.69 (d, *J* = 6.8 Hz, 1H, Py-*H*5), 4.44 (m, 1H, -C*H*CH_2COOH), 4.01 (t, *J* = 5.9 Hz, 2H, -CH_2-OAr), 3.45 (m, 2H, PyNHCH_2-), 2.82 (dd, *J* = 13.6 Hz, *J* = 8.1 Hz, 1H, -C*H*(H')COOH), 2.76 (dd, *J* = 13.6 Hz, *J* = 5.8 Hz, 1H, -CH(H')COOH), 2.53 (dd, *J* = 15.8 Hz, *J* = 8.0 Hz, 1H, ArC*H*(H')-), 2.44 (dd, *J* = 15.4 Hz, *J* = 6.1 Hz, 1H, Ar-CH(*H'*)-), 2.30 (s, 3H, Py-CH_3), 2.01 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 172.4, 165.5, 156.7, 155.1, 152.4, 135.3, 134.6, 130.9, 130.8, 130.0, 128.0, 127.1, 114.1, 113.8, 111.2, 64.5, 48.3, 38.8, 38.7, 38.4, 27.7, 21.3. **HPLC** (10-50%, 30 min): t_R = 23.36 min. **MS** (ESI): m/z = 448.6 $[m+H]^+$. HR-ESI ($C_{26}H_{30}N_3O_4^+$): Calc.: 448.2231, found: 448.2230.

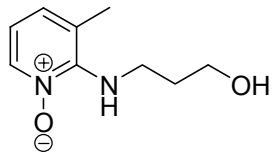
1.3.27 2-Chloro-3-methylpyridin-*N*-oxide



Prepared from 2-Chloro-3-methylpyridine (1 g, 7.84 mmol) according to general procedure **C**. Yield: 881 mg (6.16 mmol, 79%) of a yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.22 (d, *J* = 6.1 Hz, 1H, Py-*H*6), 7.12-7.06 (m, 2H, Py-*H*4,5), 2.40 (s, 3H, -C*H*$_3$). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 142.4, 138.1, 136.1, 127.1, 122.4, 20.4. **HPLC** (5-20%, 30 min): t_R = 12.08 min. **MS** (ESI): m/z = 143.9 [m+H$^+$].

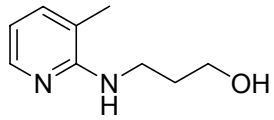
1.3.28 3-(3-Methylpyridin-*N*-oxid-2-yl)aminopropan-1-ol



Prepared from 2-Chloro-3-methylpyridine (683 mg, 4.78 mmol) according to general procedure **C**. Yield: 862 mg (4.71 mmol, 99%) of a yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.97 (d, *J* = 6.2 Hz, 1H, Py-*H*6), 7.14 (bs, 1H, Py-N*H*), 6.95 (t, *J* = 7.7 Hz, 1H, Py-*H*4), 6.51 (t, *J* = 6.9 Hz, 1H, Py-*H*5), 3.72 (t, *J* = 5.8 Hz, 2H, -C*H*$_2$OH), 3.60 (m, 2H, Py-NHC*H*$_2$), 2.34 (s, 3H, -C*H*$_3$), 1.80 (m, 2H, -CH$_2$C*H*$_2$CH$_2$-). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ = 151.2, 135.2, 131.6, 120.6, 112.6, 59.3, 41.9, 33.4, 19.1. **HPLC** (5-20%, 30 min): t_R = 13.89 min. **MS** (ESI): m/z = 183.2 [m+H$^+$].

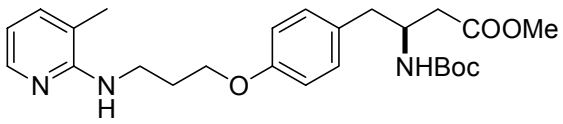
1.3.29 3-(3-Methylpyridin-2-yl)aminopropan-1-ol (4d)



Prepared from 3-(3-Methylpyridin-*N*-oxid-2-ylamino)propan-1-ol (846 mg, 4.61 mmol) according to general procedure **E**. Yield: 645 mg (3.88 mmol, 84%) of a light brown oil.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.89 (d, *J* = 4.7 Hz, 1H, Py-*H*6), 7.19 (dt, *J* = 7.1 Hz, *J* = 0.7 Hz, 1H, Py-*H*4), 6.47 (dd, *J* = 7.0 Hz, *J* = 5.2 Hz, 1H, Py-*H*5), 5.45 (bs, 1H, -N*H*), 4.50 (bs, 1H, -O*H*), 3.67-3.56 (m, 4H, -C*H*$_2$OH, Py-NH-C*H*$_2$-), 2.06 (s, 3H, Py-C*H*$_3$), 1.73 (m, 2H, -CH$_2$-C*H*$_2$-CH$_2$-). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 157.4, 144.7, 137.1, 116.2, 112.4, 58.3, 42.5, 33.9, 17.0. **HPLC** (5-20%, 30 min): t_R = 10.93 min. **MS** (ESI): m/z = 167.1 [m+H$^+$].

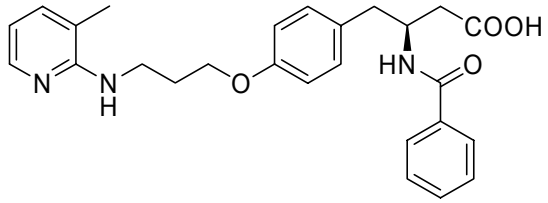
1.3.30 Methyl 3-(*S*)-[(*tert.*Butyloxycarbonyl)amino]-4-{4-[3-(4-methylpyridin-2-yl)aminopropoxy]phenyl} butanoate (5d)



Prepared from **4d** (117 mg, 702 µmol) and Boc-β–tyrosine methyl ester (181 mg, 585 µmol) according to general procedure **A**. Yield: 55 mg (120 µmol, 21%) of a colorless foam.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 5.0 Hz, 1H, Py-*H*6), 7.19 (d, *J* = 7.1 Hz, 1H, Py-*H*4), 7.08 (d, *J* = 8.5 Hz, 2H, Tyr-*H*3/3'), 6.82 (d, *J* = 8.6 Hz, 2H, Tyr-*H*2/2'), 6.49 (dd, *J* = 5.2 Hz, *J* = 7.0 Hz, 1H, Py-*H*5), 5.04, 5.02 (2 bs, 2H, Py-N*H*-, -N*H*Boc), 4.66 (m, 1H, -C*H*NHBoc-), 4.08 (t, *J* = 5.7 Hz, 2H, ArOCH_2-), 3.67 (s+m, 5H, -COOCH_3 + -NHCH_2-CH_2-), 2.85 (dd, *J* = 13.5 Hz, *J* = 6.3 Hz, 1H, -C*H*(H')COOMe), 2.72 (dd, *J* = 13.6 Hz, *J* = 7.7 Hz, 1H, -CH(*H'*)COOMe), 2.45 (m, 2H, Ar-CH_2CHNHBoc-), 2.14 (m, 2H, -CH_2C$H_2$$CH_2$-), 2.07 (s, 3H, Py-C$H_3$), 1.40 (s, 9H, -^{t}Bu). **^{13}C-NMR** (62 MHz, $CDCl_3$): δ = 172.1, 157.5, 156.7, 155.8, 146.1, 136.7, 130.3, 129.9, 116.7, 114.4, 112.9, 79.4, 67.0, 51.7, 39.9, 39.4, 37.5, 29.0, 28.3, 17.0. **HPLC** (10-100%, 30 min): t_R = 17.52 min. **MS** (ESI): m/z = 480.2 $[m+Na]^+$, 458.2 $[m+H]^+$, 402.4 $[m+H-^tBu]^+$, 358.4 $[m+H-Boc]^+$.

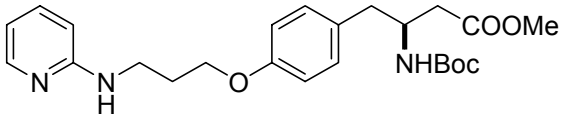
1.3.31 3-(*S*)-(Benzamido)-4-{4-[3-(3-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6d)



Prepared from **5d** (55 mg, 120 µmol) according to general procedure **B2**. Yield after purification: 24 mg (42 µmol, 36%) as TFA salt. Colorless solid.

^{1}H-NMR (500 MHz, DMSO): δ = 13.32, 12.25 (bs, -COO*H*), 8.32 (d, *J* = 8.3 Hz, 1H, -N*H*COPh), 8.03 (bs, 1H, Py-N*H*), 7.80 (d, *J* = 6.2 Hz, 1H, Py-*H*6), 7.76 (d, *J* = 7.4 Hz, 2H, Ph-*H*2,2'), 7.74 (d, *J* = 7.2 Hz, 1H, Py-*H*4), 7.50 (t, *J* = 7.2 Hz, 1H, Ph-*H*4), 7.44 (t, *J* = 7.5 Hz, 2H, Ph-H3,3'), 7.14 (d, *J* = 8.3 Hz, 2H, Tyr-*H*3,3'), 6.81 (d, *J* = 8.7 Hz, 2H, Tyr-*H*2,2'), 6.80 (m, 1H, Py-*H*5), 4.43 (m, 1H, -C*H*CH_2COOH), 4.03 (t, *J* = 5.8 Hz, 2H, -CH_2-OAr), 3.56 (m, 2H, Py-NHCH_2-), 2.81 (dd, *J* = 13.5 Hz, *J* = 8.1 Hz, 1H, -C*H*(H')COOH), 2.75 (dd, *J* = 13.6 Hz, *J* = 5.8 Hz, 1H, -CH(*H'*)COOH), 2.52 (dd, *J* = 15.8 Hz, *J* = 7.7 Hz, 1H, Ar-C*H*(H')-), 2.43 (dd, *J* = 15.4 Hz, *J* = 6.1 Hz, 1H, Ar-CH(*H'*)-), 2.17 (s, 3H, Py-(CH_3)), 2.05 (m, 1H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 172.4, 165.5, 156.7, 151.6, 141.1, 134.6, 133.5, 130.9, 130.7, 130.0, 128.1, 127.0, 122.6, 114.0, 111.8, 64.8, 48.3, 38.9, 38.8, 38.7, 27.5, 16.6. **HPLC** (10-100%, 30 min): t_R = 22.26 min. **MS** (ESI): m/z = 448.4 $[m+H]^+$. **HRMS** (ESI) ($C_{26}H_{30}N_3O_4^+$): Calc.: 448.2231, found: 448.2227.

1.3.32 Methyl 3-(*S*)-[(*tert.*Butyloxycarbonyl)amino]-4-{4-[3-(pyridin-2-yl)aminopropoxy]phenyl} butanoate (5e)

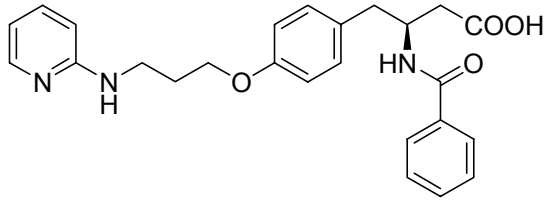


Prepared from **1** (152 mg, 1.0 mmol) and Boc-β-tyrosine methyl ester (261 mg, 825 µmol) according to general procedure **A**. Yield: 106 mg (244 µmol, 30%) of a colorless foam.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 7.6 Hz, 1H, Py-*H*6); 7.36 (m, 1H, Py-*H*5); 7.06 (d, *J* = 8.4 Hz, 2H, Tyr-*H*3,3'); 6.80 (d, *J* = 8.4 Hz, 2H, Tyr-*H*2,2'); 6.52 (dd, *J* = 5.2 Hz, *J* = 6.9 Hz, 1H, Py-*H*4); 6.37 (d, *J* = 8.4 Hz, 1H, Py-*H*3); 5.05 (bs, 1H, -N*H*), 4.95 (bs, 1H, -N*H*); 4.08 (m, 1H, -C*H*NHBoc-); 4.05 (t, *J* = 5.9 Hz, 2H, ArOCH_2-); 3.65 (s, 3H, -COOCH_3); 3.46 (m, 2H, -NH-CH_2-CH_2-); 2.84 (m, 2H, -C*H*(H')COOMe); 2.71 (dd, *J* = 7.7 Hz, *J* = 13.6 Hz, 1H, -CH(*H'*)COOMe), 2.44 (m,

2H, Ar-CH_2CHNHBoc-); 2.07 (m, 2H, -CH_2-CH_2-CH_2-); 1.39 (s, 9H, ^{t}Bu). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 178.0, 158.6, 157.5, 155.0, 147.8, 137.4, 130.2, 129.8, 114.4, 112.6, 106.6, 79.2, 65.7, 51.6, 48.9, 39.4, 39.3, 37.3, 29.0, 28.3. **HPLC** (10-100%, 30 min): t_R = 17.19 min. **MS** (ESI): m/z = 466.2 $[m+Na]^+$, 444.2 $[m+H]^+$, 388.2 $[m+H-^tBu]^+$, 344.3 $[m+H-Boc]^+$.

1.3.33 3-(*S*)-(Benzamido)-4-{4-[3-(pyridine-2-ylamino)propoxy]phenyl} butanoic acid (6e)

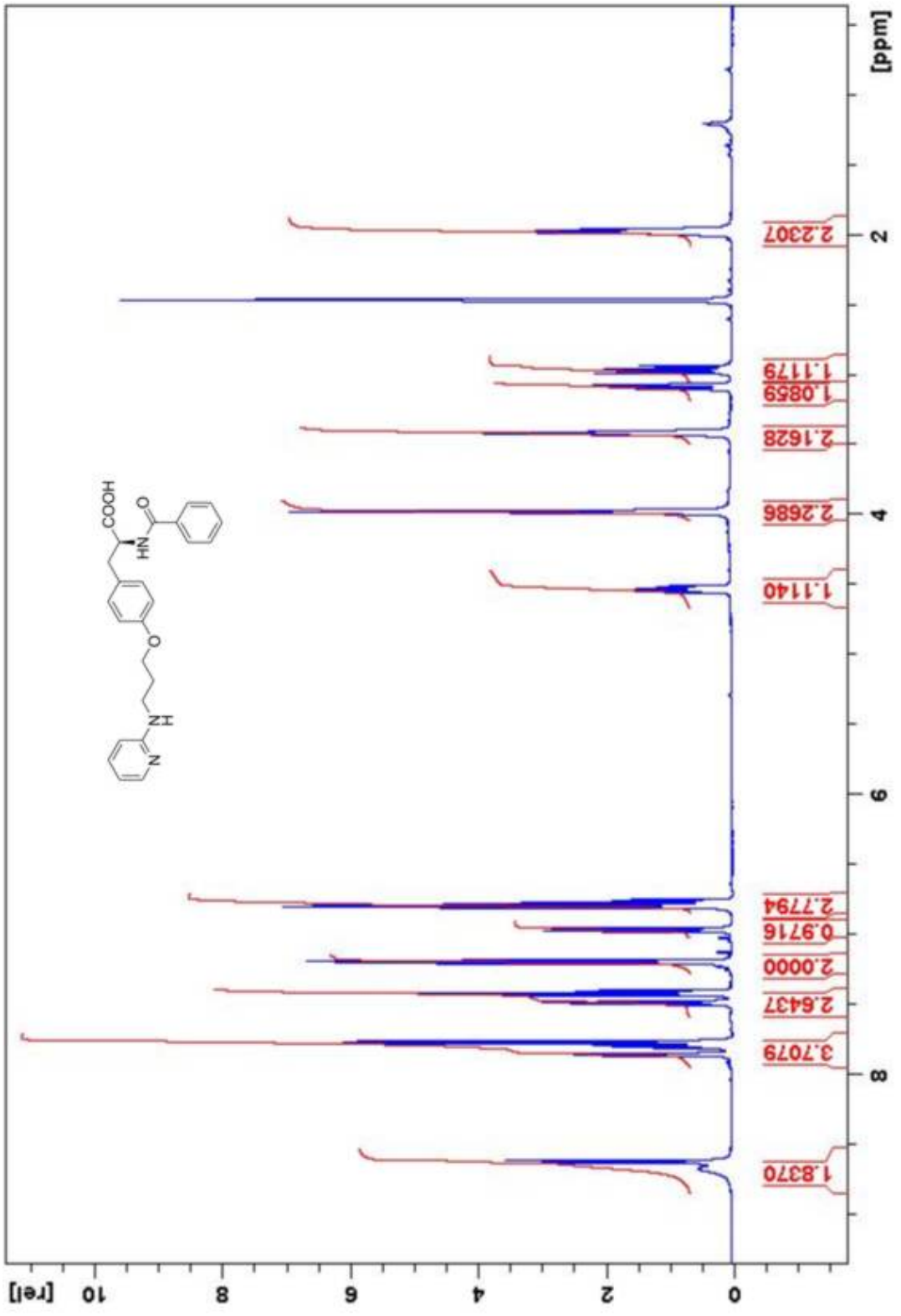


Prepared from **5e** (50 mg, 112 µmol) according to general procedure **B2**. Yield after purification: 11 mg (20 µmol, 18%) as TFA salt. Colorless solid.

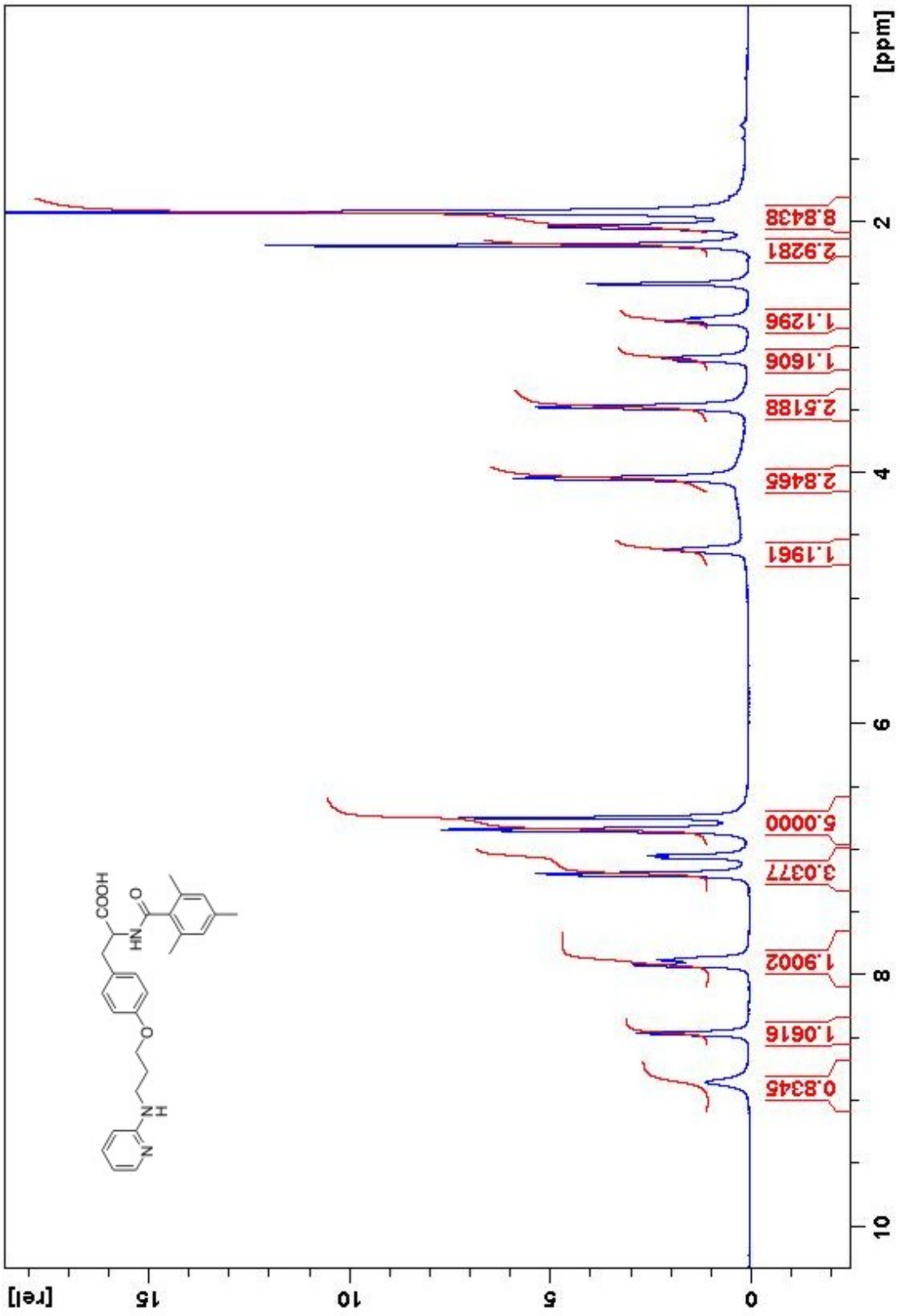
^{1}H-NMR (500 MHz, DMSO): δ = 13.51, 12.26 (bs, 1H, -COO*H*), 8.82 (bs, 1H, Py-N*H*-), 8.32 (d, 1H, *J* = 8.4 Hz, 1H, -CON*H*Ph), 7.91 (d, 1H, *J* = 6.1 Hz, 1H, Py-*H*6), 7.85 (t, *J* = 7.9 Hz, 1H, Py-*H*4), 7.76 (d, *J* = 7.2 Hz, 2H, Ph-*H*2,2'), 7.50 (t, *J* = 7.3 Hz, 1H, Ph-*H*4), 7.44 (t, *J* = 7.5 Hz, 2H, Ph-*H*3,3'), 7.14 (d, *J* = 8.5 Hz, 2H, Tyr-*H*3,3'), 7.03 (d, *J* = 9.0 Hz, Py-*H*3), 6.83 (d, *J* = 8.5 Hz, 2H, Tyr-*H*2,2'), 6.81 (t, *J* = 6.5 Hz, 1H, Py-*H*5), 4.44 (m, 1H, -C*H*CH_2COOH), 4.02 (t, *J* = 6.0 Hz, 2H, -CH_2OAr), 3.47 (t, *J* = 6.5 Hz, 2H, PyNHCH_2-), 2.82 (dd, *J* = 13.6 Hz, *J* = 8.0 Hz, 1H, -C*H*(H')COOH), 2.76 (dd, *J* = 13.6 Hz, *J* = 5.9 Hz, 1H, -CH(*H'*)COOH), 2.53 (dd, *J* = 15.5 Hz, *J* = 7.7 Hz, 1H, ArC*H*(H')-), 2.44 (dd, *J* = 15.4 Hz, *J* = 6.2 Hz, 1H, Ar-CH(*H'*)-), 2.02 (m, 2H, -CH_2-CH_2-CH_2-). **^{13}C-NMR** (125 MHz, DMSO): δ = 172.3, 165.5, 156.7, 152.9, 142.6, 136.3, 134.6, 130.9, 130.8, 130.0, 128.0, 127.0, 114.1, 112.9, 111.7, 64.6, 48.3, 38.8, 38.7, 38.5, 27.6. **HPLC** (10-50%, 30 min): t_R = 21.68 min. **MS** (ESI): m/z = 434.3 $[m+H]^+$. **HRMS** (ESI) ($C_{25}H_{28}N_3O_4^+$): Calc.: 434.2074, found: 434.2076.

1.4 ^{1}H-NMR spectra of target compounds

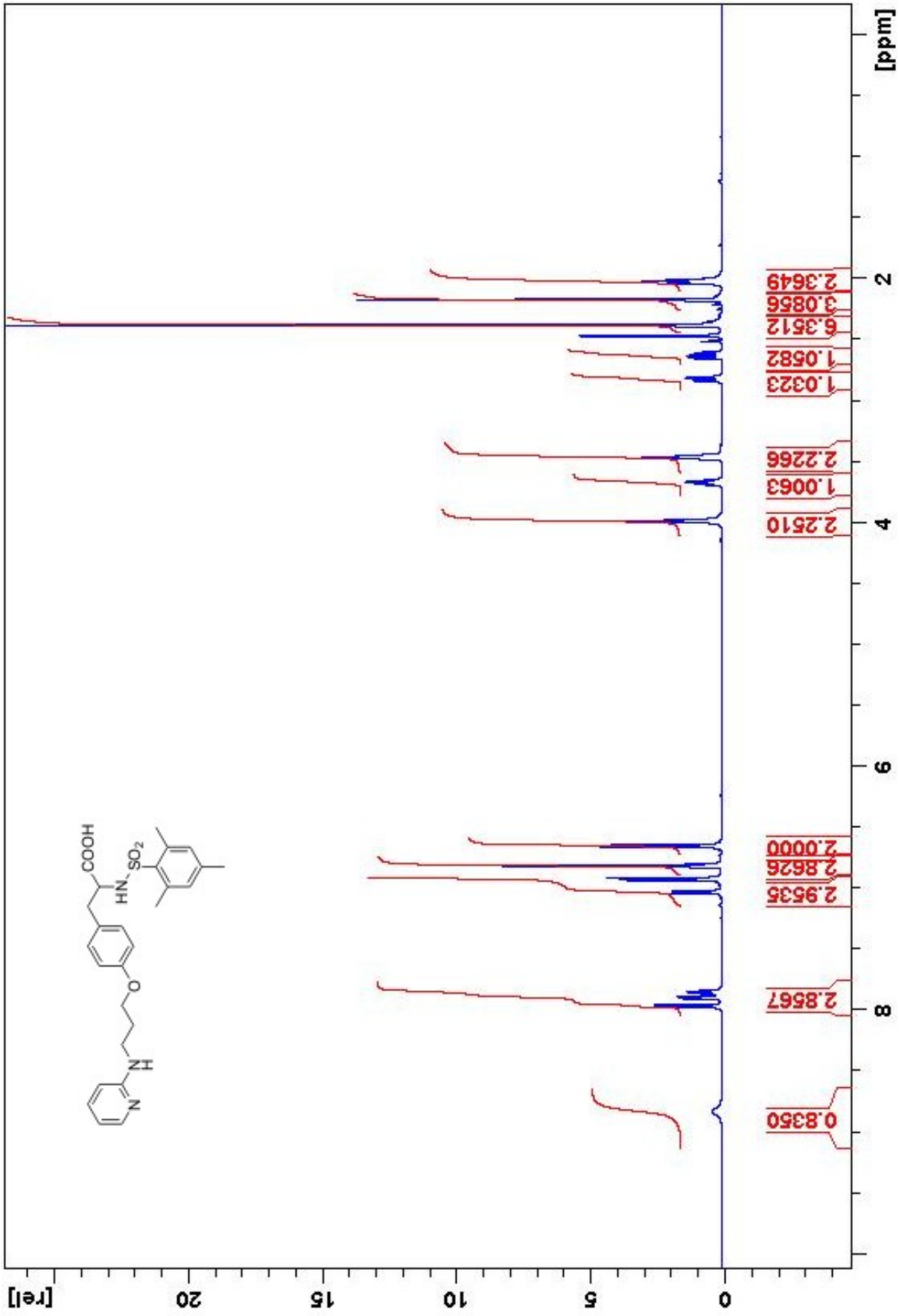
1.4.1 2-(S)-Benzamido)-3-{4-[3-(pyridin-2-yl)aminopropoxy]phenyl} propionic acid (3a)



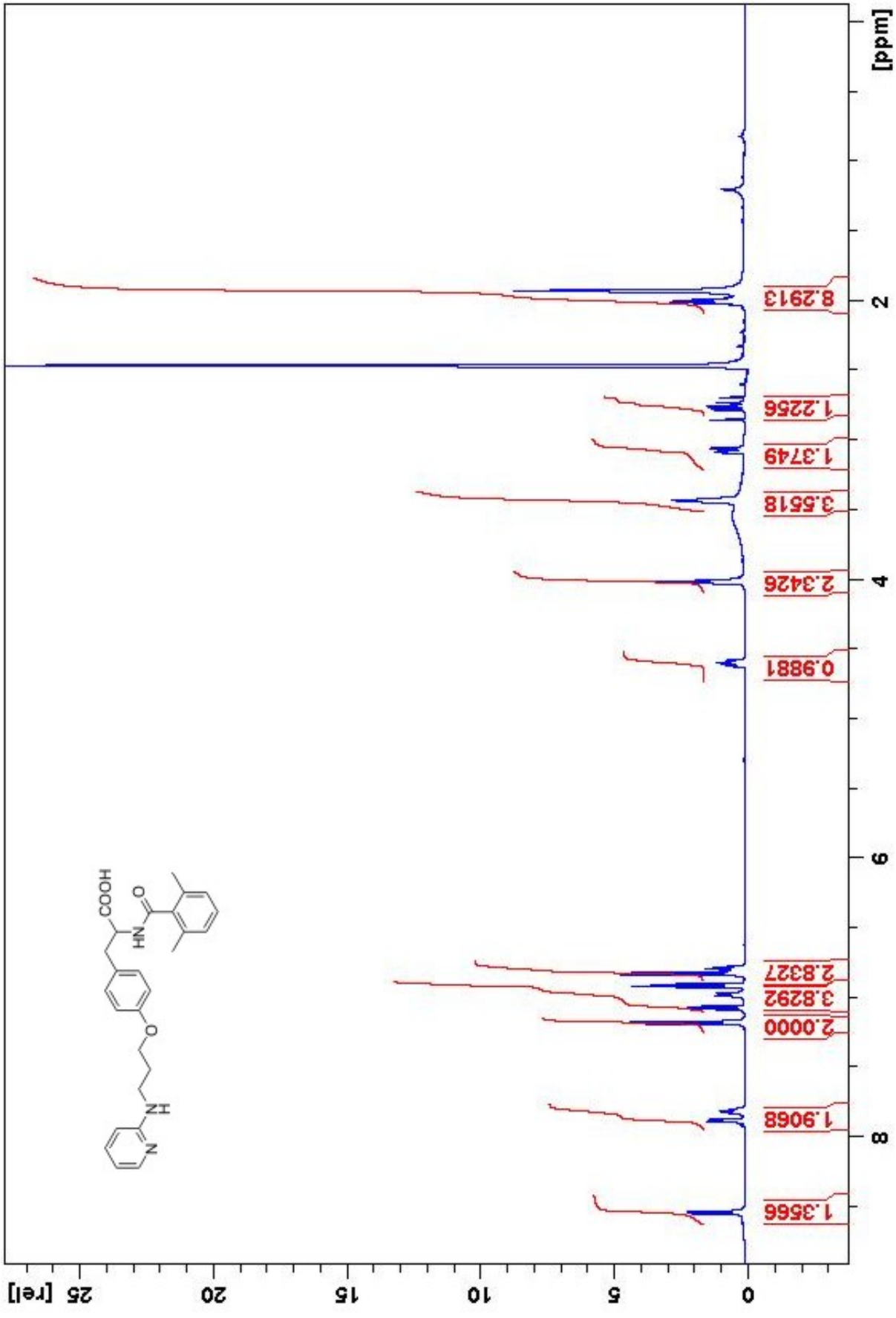
1.4.2 2-(*S*)-(2,4,6-trimethylbenzamido)-3-{4-[3-(pyridin-2-yl)aminopropoxy]phenyl} propionic acid (3b)



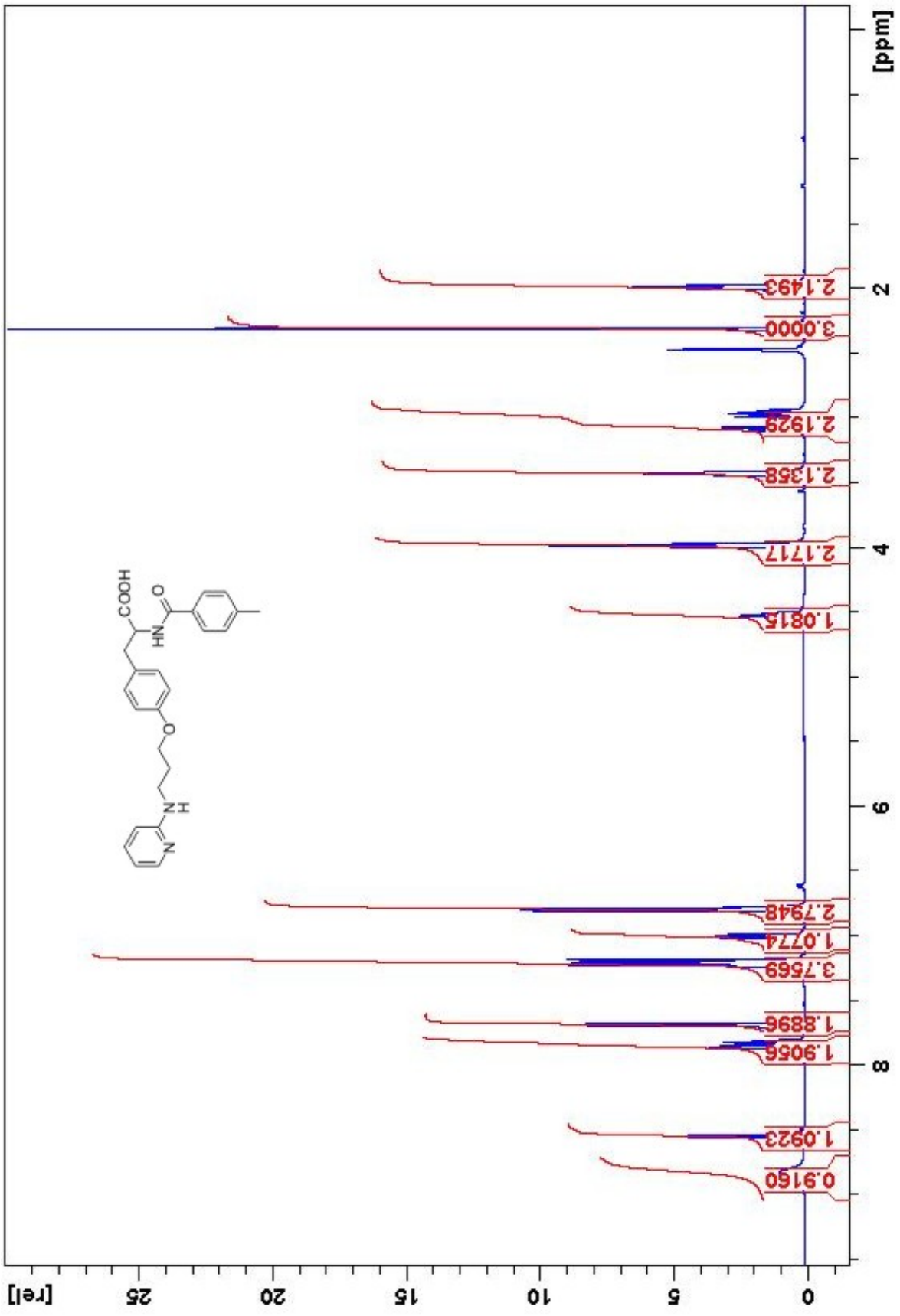
1.4.3 2-(*S*)-[(2,4,6-trimethylphenyl)sulphonamido]-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl} propionic acid (3c)



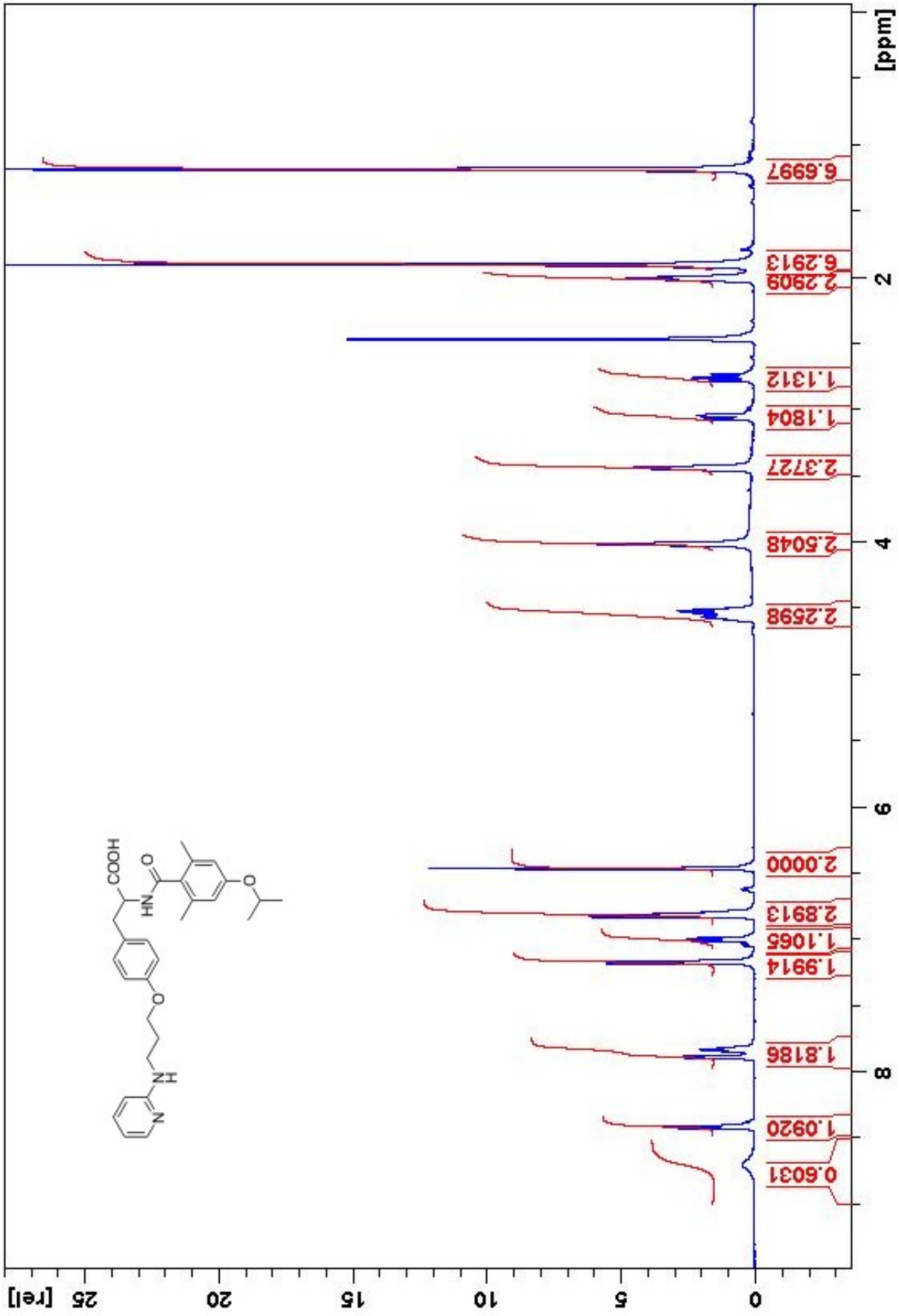
1.4.4 2-(*S*)-(2,6-dimethylbenzamido)-3-{4-[3-(pyridin-2-ylamino) propoxy]phenyl} propionic acid (3d)



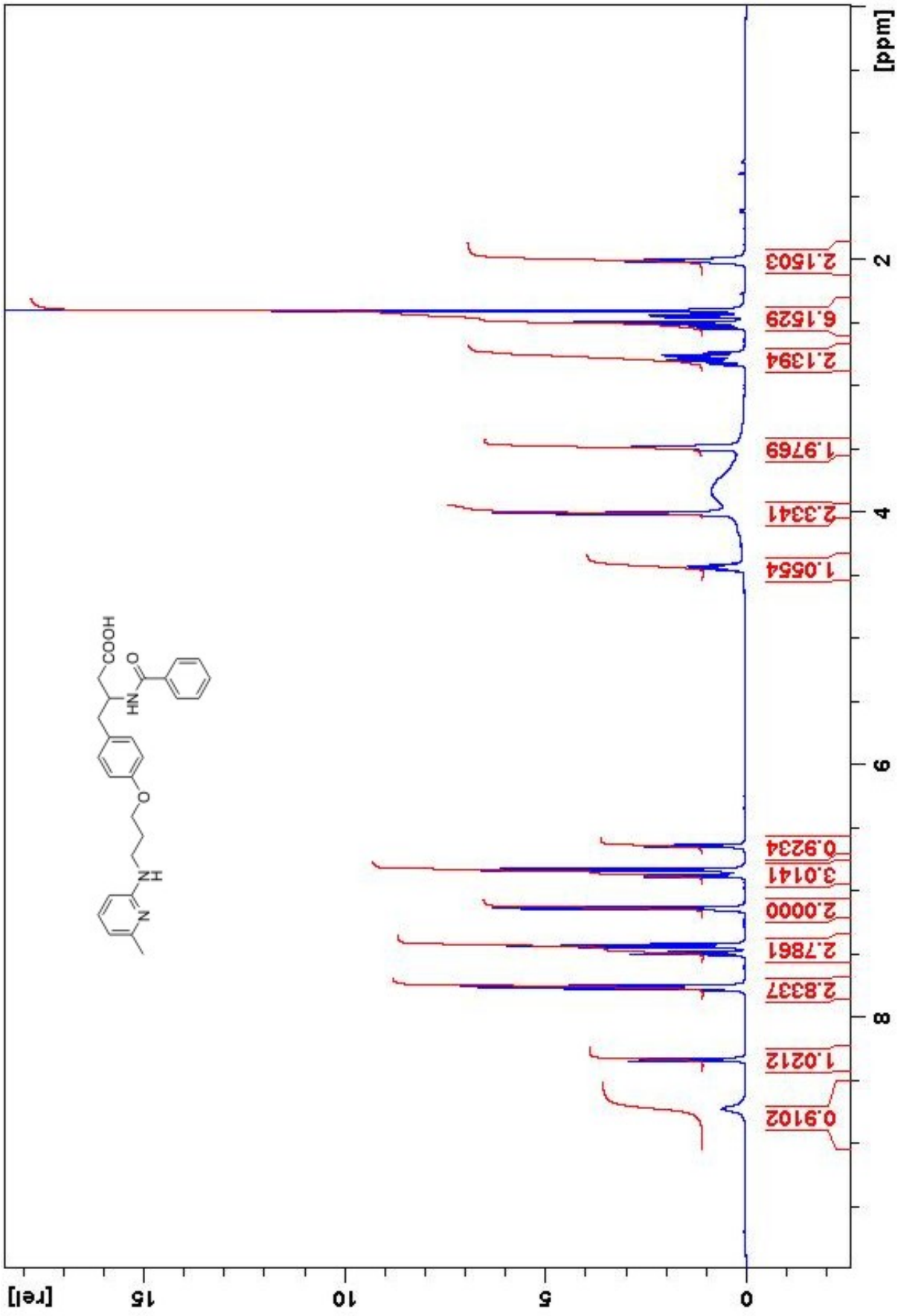
1.4.5 2-(*S*)-(4-Methylbenzamido)-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl}-propionic acid (3e)



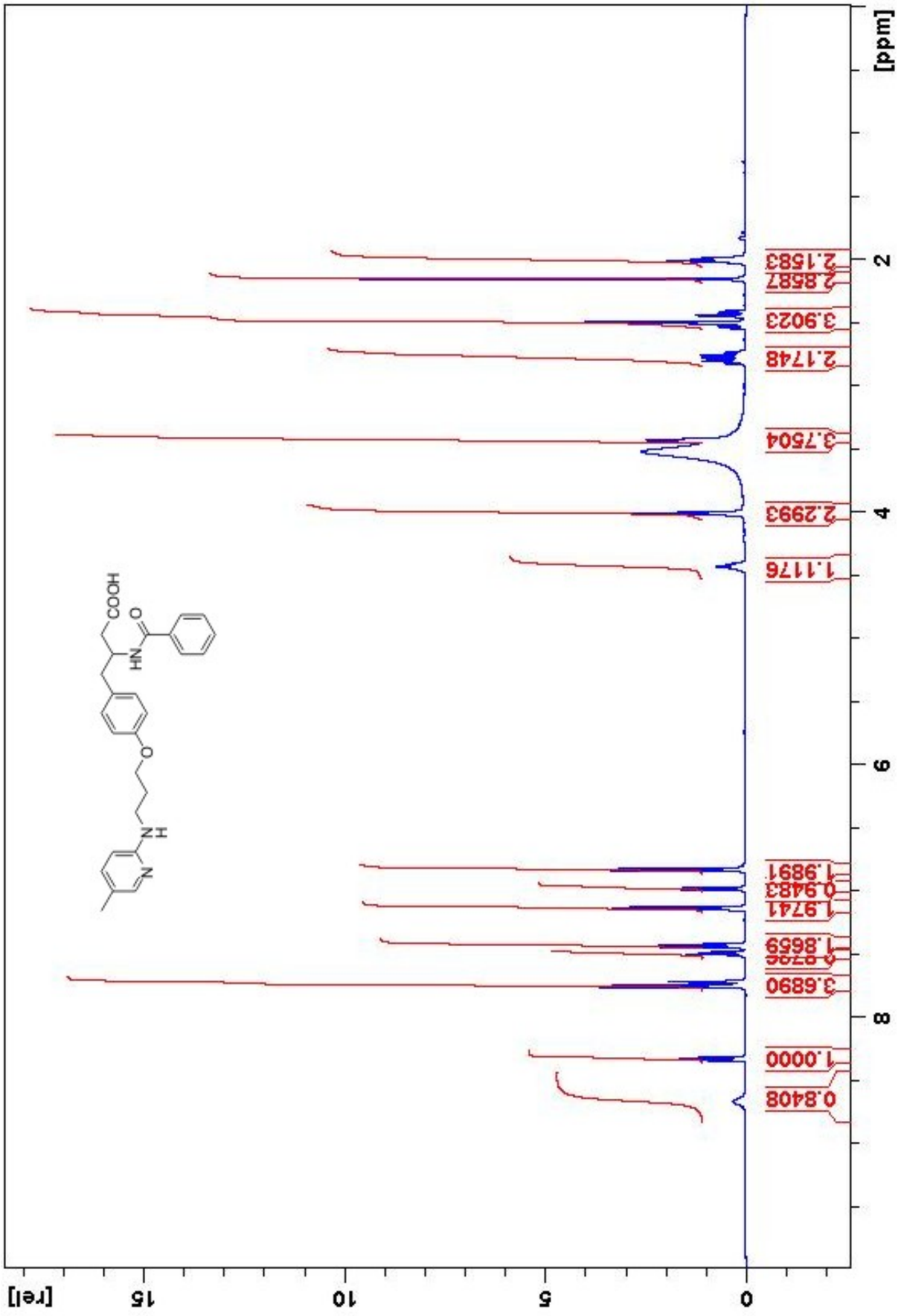
1.4.6 2-(*S*)-(4-Isopropoxy-2,6-dimethylbenzamido)-3-{4-[3-(pyridin-2-ylamino)propoxy]phenyl} propionic acid (3f)



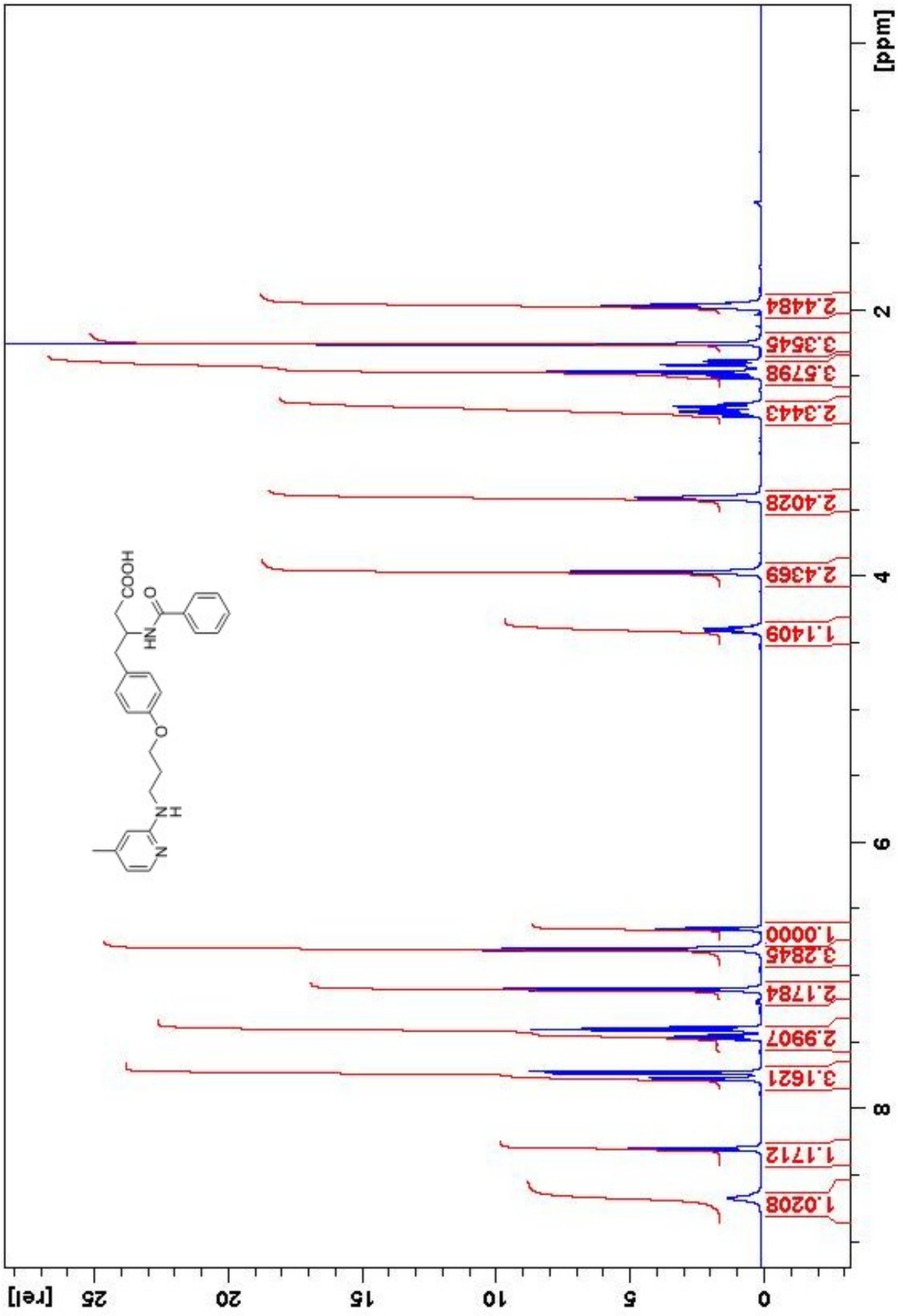
1.4.7 3-(*S*)-(Benzamido)-4-{4-[3-(6-methylpyridin-2-yl)-aminoprooxy]phenyl} butanoic acid (6a)



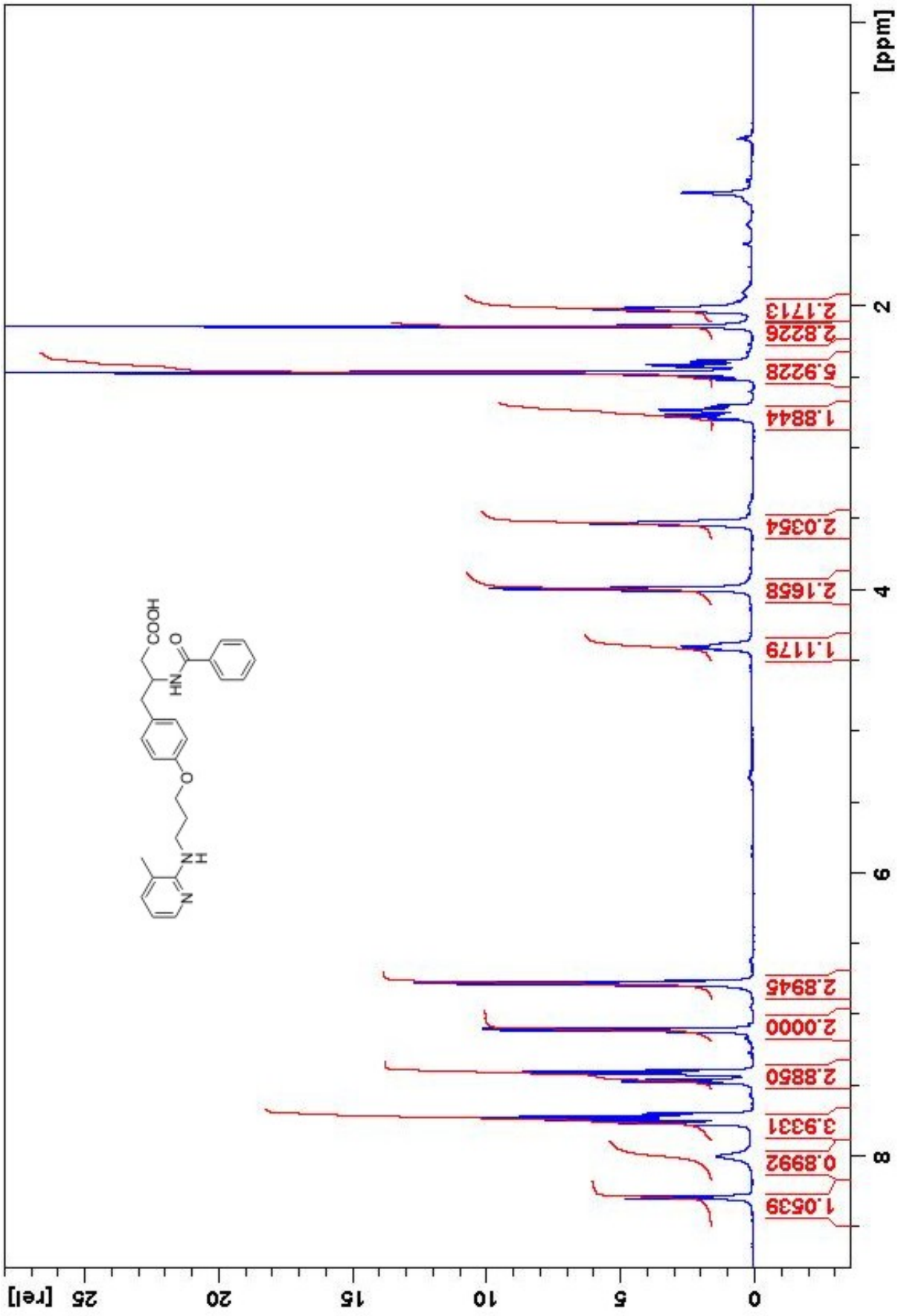
1.4.8 3-(*S*)-(Benzamido)-4-{4-[3-(5-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6b)



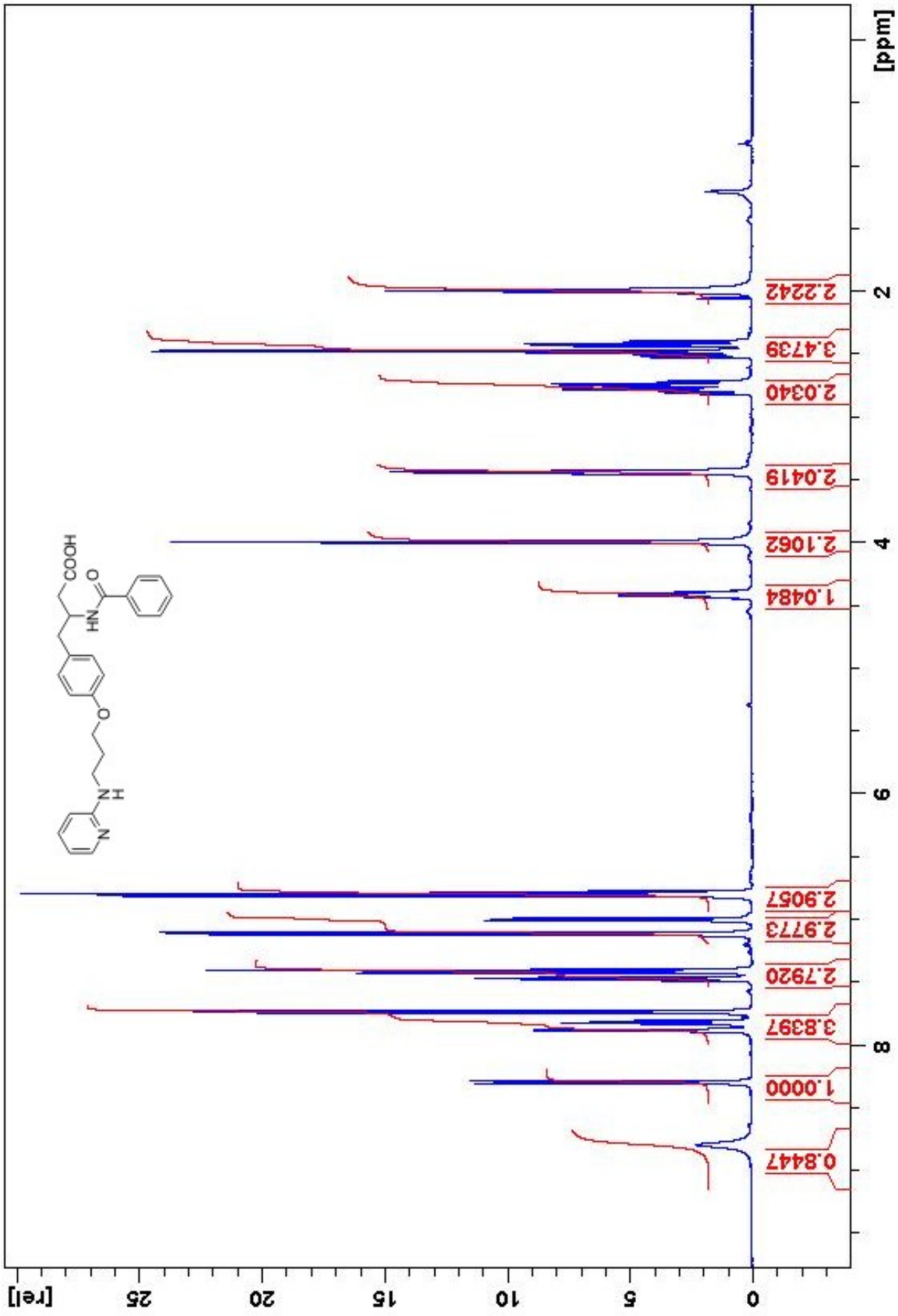
1.4.9 3-(*S*)-(Benzamido)-4-{4-[3-(4-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6c)



1.4.10 3-(*S*)-(Benzamido)-4-{4-[3-(3-methylpyridin-2-yl)aminopropoxy]phenyl} butanoic acid (6d)



1.4.11 3-(*S*)-(Benzamido)-4-{4-[3-(pyridine-2-ylamino)propoxy]phenyl} butanoic acid (6e)



2 Biological testings

Solid phase binding assay

The inhibiting activity and integrin selectivity of the integrin inhibitors were determined in a solid phase binding assay using soluble integrins and coated extracellular matrix protein. Binding of integrins was than detected by specific antibodies in an enzyme-linked immunosorbant assay. Fibronectin and vitronectin were purchased from Sigma (St Louis, MO) and fibrinogen from Calbiochem (EMD Biosciences, Darmstadt, Germany). The integrin $\alpha_5\beta_1$ extracellular domain Fc-fusion protein was a generous gift from M. Humphries (University of Manchester), $\alpha_V\beta_3$ was purchased from Chemicon (Chemicon Europe, Germany) and αIIbβ3 from Kordia (Kordia Life Science, Leiden, Netherlands). The integrin antibodies were purchased from Pharmingen, BD Bioscience Europe ($\alpha_V\beta_3$, and αIIbβ3) and Sigma (anti-human-Fc-HRP antibody conjugate and anti-mouse-HRP conjugate). The detection of HRP was performed using HRP substrate solution 3.3.5.5'-tetramethylethylenediamine (TMB, Seramun, Germany) and 1M H_2SO_4 for stopping the reaction. The developed color was measured at 450nm with SpectraMax Plus reader (Molecular Devices). The resulting inhibition curves were analyzed using SoftMaxPro 4.0 software, the turning point describes the IC50 value.

$\alpha_5\beta_1$:Nunc-Immuno maxisorp plates (Nalge Nunc Europe Ltd) were coated over night at 4°C with fibronectin (0.25 µg/ml) in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH9.6. All subsequent washing and binding were performed in 25 mM Tris, pH7.6, 150 mM NaCl, 1 mM $MnCl_2$, 1 mg/ml BSA. The plates were blocked with 3 % BSA in PBS 0.1% Tween20 for 1 hour at room temperature. Soluble integrin $\alpha_5\beta_1$ (0.5 µg/ml) and a serial dilution of integrin inhibitor were incubated in the coated wells for one hour at room temperature. The detection antibody (anti-human-Fc-HRP antibody conjugate) was then applied for 1 hour at room temperature and the binding visualized as described above. For the $\alpha_V\beta_3$ assay, plates were coated with vitronectin (1 µg/ml) and blocked as described for $\alpha_5\beta_1$. Soluble $\alpha_V\beta_3$ (1 µg/ml) was incubated with a serial dilution of integrin inhibitor for one hour at room temperature. Primary (anti-$\alpha_V\beta_3$) and secondary antibody (anti-mouse-HRP conjugate) were applied for 1 hour at room temperature and the binding visualized as described above.

For the αIIbβ3 assay, plates were coated with fibrinogen (10 µg/ml) and blocked as described for α5β1. Soluble αIIbβ3 (5 µg/ml) was incubated with a serial dilution of integrin inhibitor (25 mM Tris, pH7,6, 150 mM NaCl, 1 mM $MnCl_2$, 1 mg/mL BSA 1 mM $MgCl_2$, 1 mM $CaCl_2$) for one hour at room temperature. Primary (anti-CD41b) and secondary antibody (anti-mouse-HRP conjugate) were applied for 1 hour at room temperature and the binding visualized as described above.

HRP – horse radish peroxidase

3 Molecular docking

Automated docking studies were performed using the AutoDock 3.05 program package on the basis of the α5β1 homology model published before. The protein structure was set up for docking experiments as follows: Unpolar hydrogens were removed and Kollman united-atom partial charges were assigned. Solvation parameters were added to the energy-minimized protein file using the ADDSOL utility of the AutoDock program. The grid maps were calculated with AutoGrid. The grids were chosen to be large enough to include a significant part of the protein around the binding site using maps with 61×61×61

points with a grid-point spacing of 0.375 Å. The structures of the ligands were generated from the standard fragment library of the SYBYL software version 7.1 (Tripos). Geometry optimizations were achieved with the SYBYL/MAXIMIN2 minimizer by applying the BFGS (Broyden, Fletcher, Goldfarb and Shannon) algorithm with a convergence criterion of 0.001 kcal/mol and employing the TRIPOS force field. Partial atomic charges were assigned using Gasteiger and Marsili formalism as implemented in the SYBYL package. Rotable bonds were defined by the Autotors module of AutoDock .

Docking itself was performed by LGA algorithm as implemented in AutoDock applying a protocol with a maximum number of 1.5×10^6 energy evaluations, a mutation rate of 0.01, a crossover rate of 0.80 and an elitism value of 1. For the local search the pseudo-Solis and Wets algorithm was applied using a maximum of 300 interactions per local search and a search_freq of 0.06. 50 independent docking runs were carried out for each ligand and results differing by less than 1.5 Å in positional root-mean-square deviation (rmsd) were clustered together and represented by the binding mode with most favorable free energy of binding. Pictures were generated using the PyMol program version 0.97.