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Supporting Information

Enzymatic Late-Stage Halogenation of Peptides

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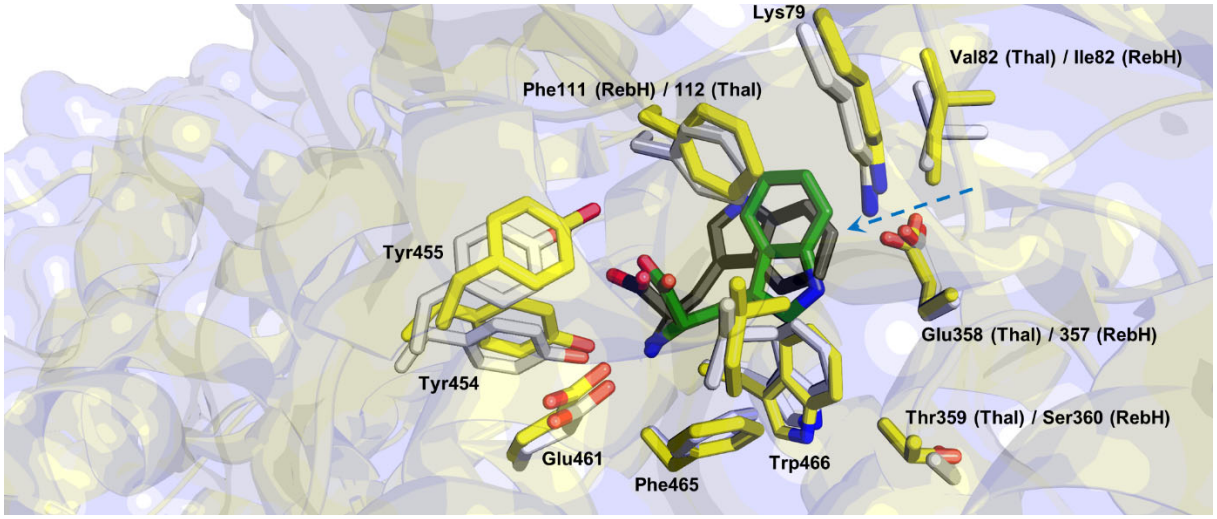
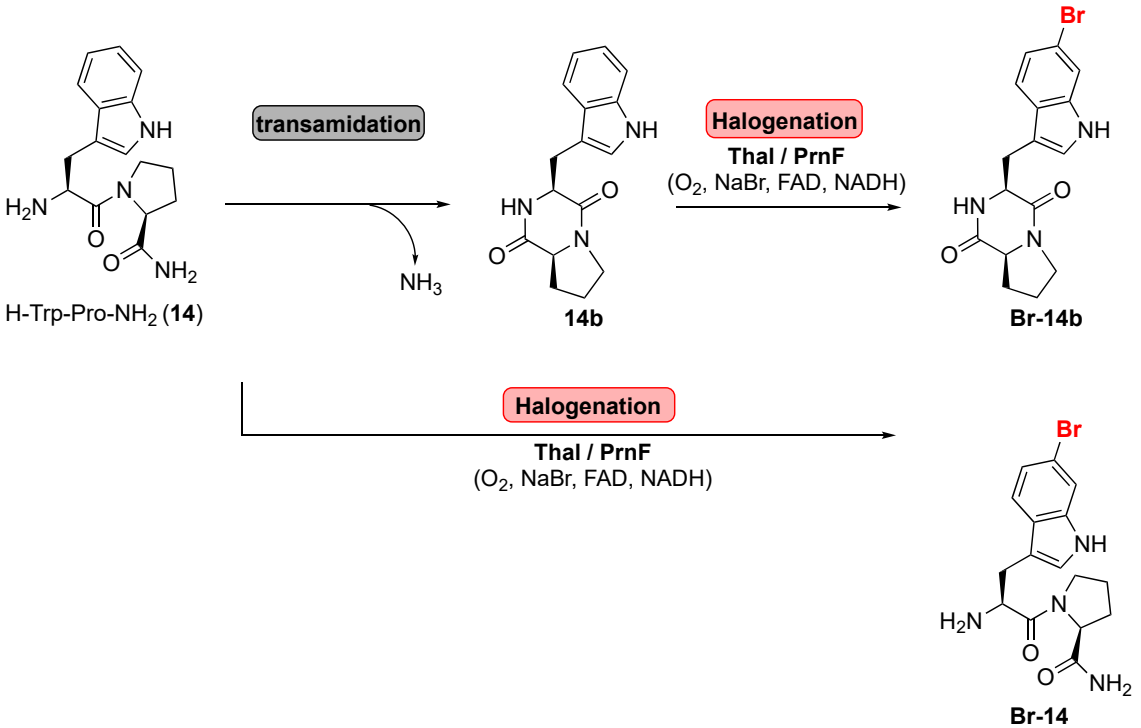


Figure S1. Overlay of tryptophan binding sites of RebH (PDB: 2OA1) and Thal (PDB: 6H44) highlighting crucial interactions of the amino acid residues of the enzyme with the substrate. Glu461 undergoes crucial hydrogen bonds to α-amino group of substrate required for binding the substrate.



Scheme S1. Proposed side reaction of peptide substrate **14** that occurs by incubation in cell-free extracts containing overexpressed halogenase. According to LC-MS analysis deamination of the substrate potentially resulted in a diketopiperazine species that is being brominated by the halogenase.

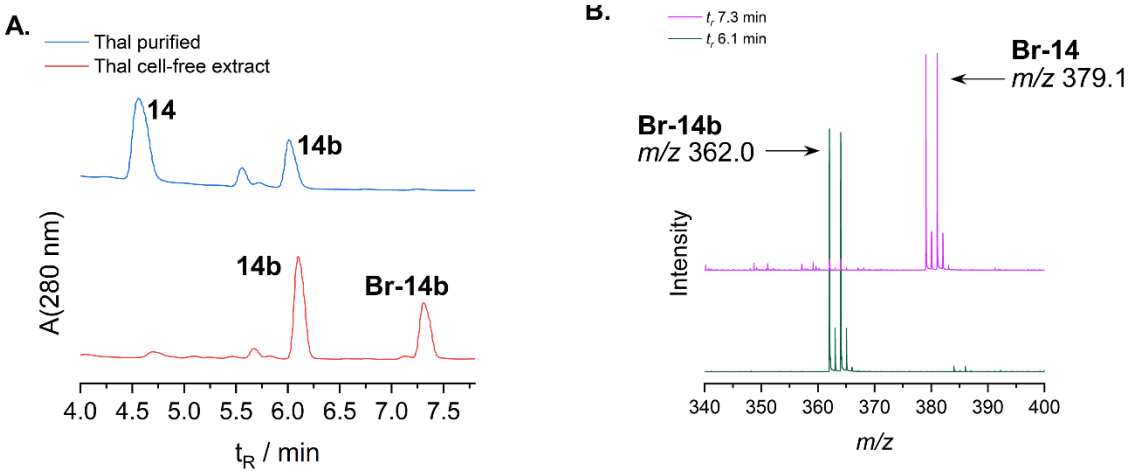


Figure S2. Side reaction of substrate **14**. (a) HPLC-UV traces (280 nm) of the reaction mixtures. (B) ESI-MS spectra (positive ionization) corresponding to the signals for **14b** (6.1 min) as well as **Br-14b** (7.3 min). By incubating the substrate with both purified Thal and cell-free extract it became evident that the observed deamination is a result of other contaminations in lysate that promote the formation of the diketopiperazine (14b) that is also accepted as a substrate of Thal. Thus, the brominated species 14b was detected.

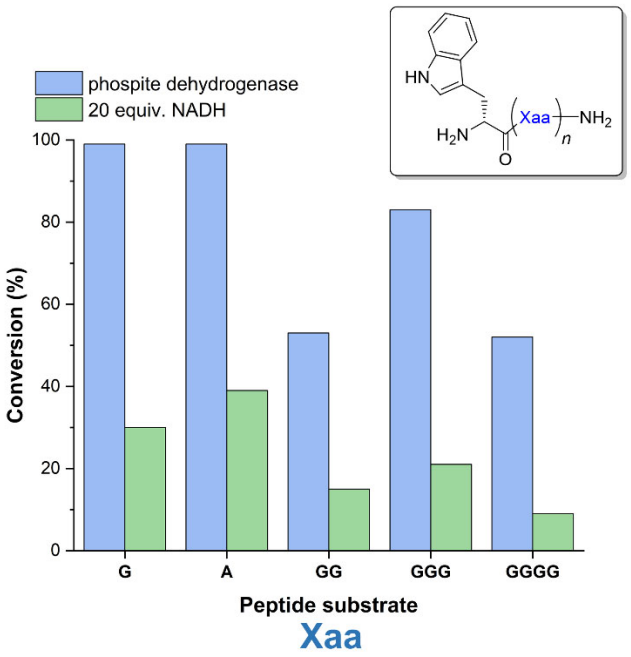


Figure S3. Impact of *in-situ* NADH regeneration on total conversion. Implementing phosphite dehydrogenase along with 1 mM NAD^+ and 50 mM Na_2HPO_3 considerably increased the efficiency of the halogenation system in comparison to using NADH in excess. Reaction conditions: 0.5 mM substrate, 0.01 mM FAD, 30 mM NaBr, 25°C.

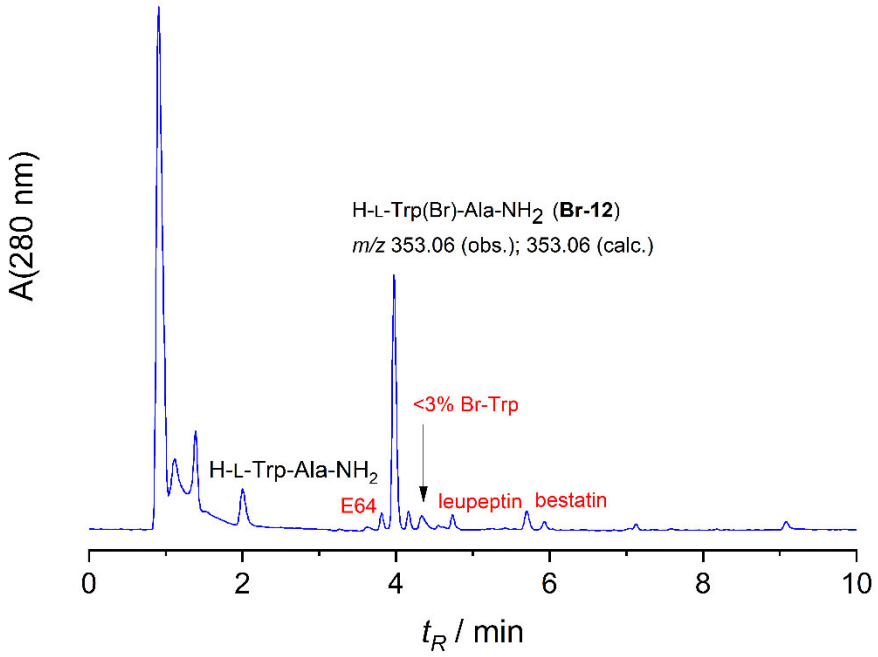
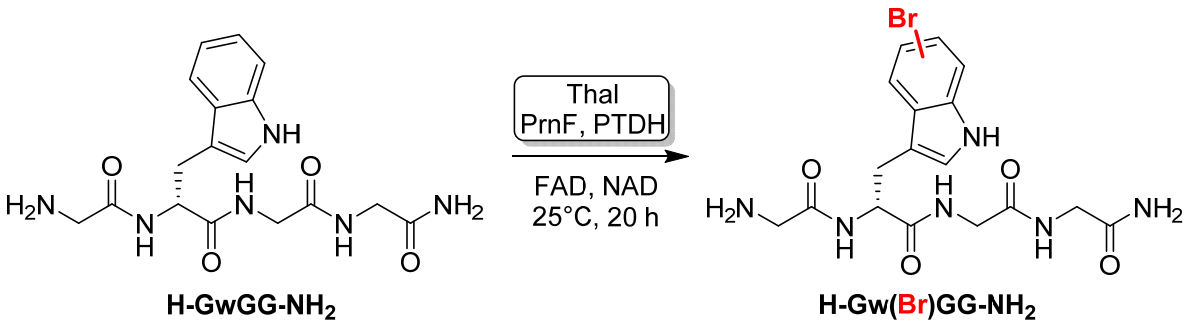


Figure S4. RP-HPLC diagram of Thal-catalysed bromination of a peptide containing an L-configured tryptophan residue in presence of a protease inhibitor mix (Halt™ Protease Inhibitor Cocktail, Thermo Scientific). While the brominated peptide resulted as the major species, protease inhibitors were also detected in the reaction mixture (marked in red) in minor intensities.



Scheme S2. Bromination of a peptide substrate containing an internal tryptophan residue in position 2.

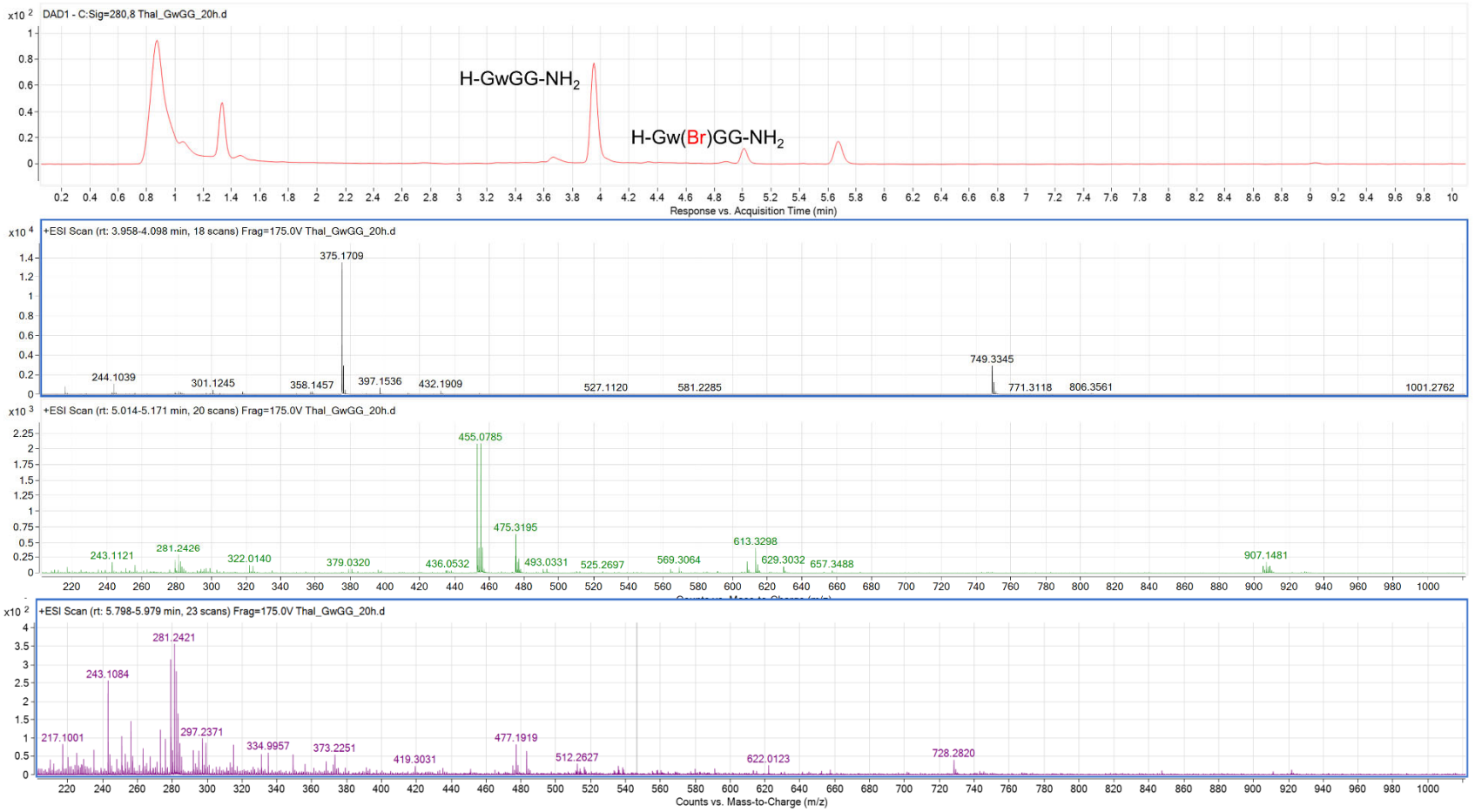


Figure S5. Impact of the position of tryptophan on bromination. HPLC-UV and -MS spectra after incubating peptide G-GwGG-NH2 with Thal (20 h, 25°C) are shown. Positioning Trp in position 2 of the substrate peptide only gave bromination in a conversion of <5% corroborating the preference for peptides carrying an *N*-terminal Trp residue. H-GwGG-NH_2 *m/z* 375.17 (found); 375.18 (calc.) / H-Gw(Br)GG-NH_2 *m/z* 453.09 (calc.); 453.08 (found).

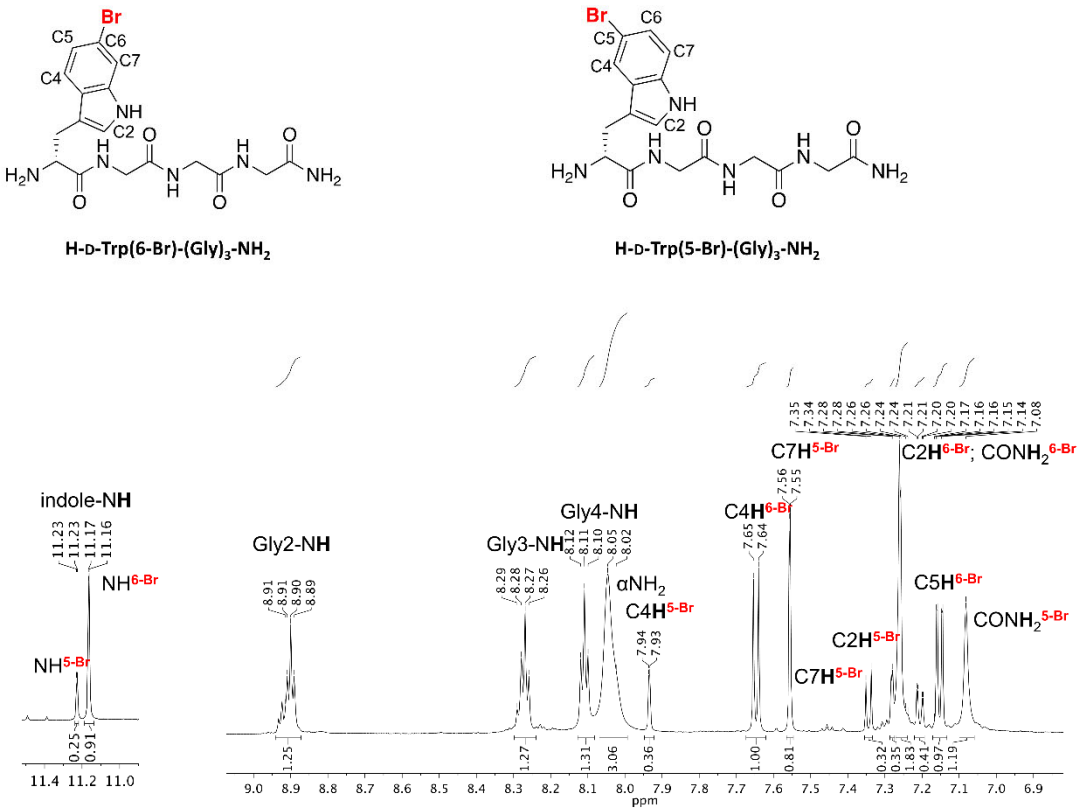


Figure S6. Representative ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of **Br-25** highlighting the region from 6.9-9.0 ppm. Resonance signals corresponding to protons of the indole moiety for both regioisomers were mentioned. The data show that a mixture of both C5- and C6-brominated peptide were isolated indicating a more relaxed regioselectivity of Thal caused by the substrate.

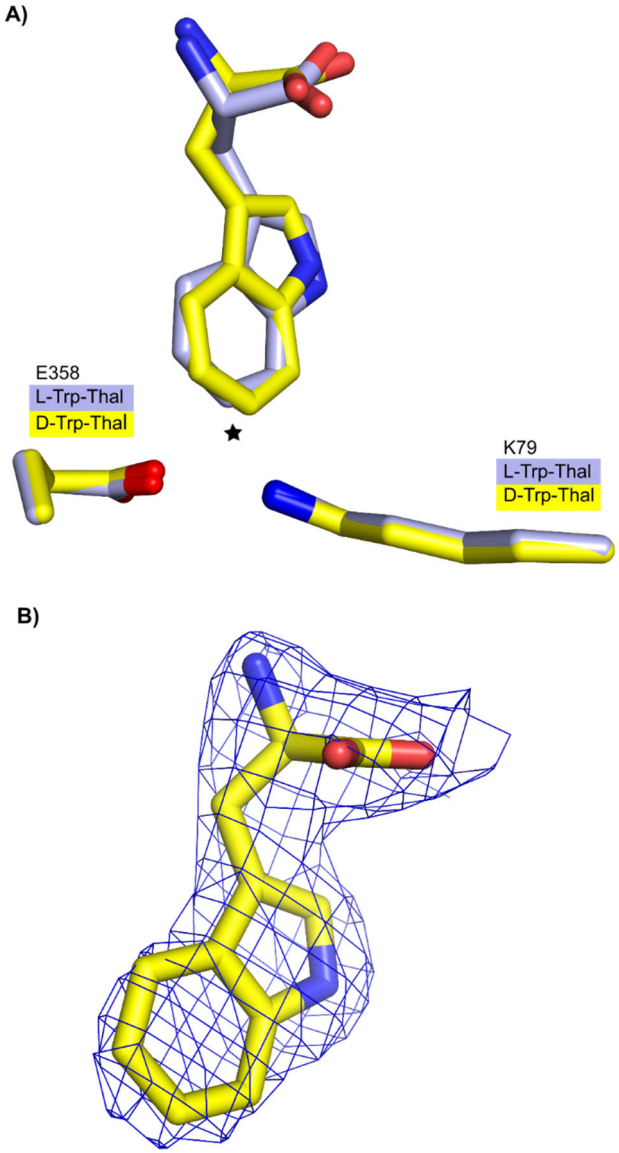


Figure S7. Orientation of D-Trp in Thal. A) Superposition of the bound D-Trp (PDB ID 8AD7) and bound L-Trp (PDB ID 6H44) within the active site. The halogenated C6 atoms of the indole moiety (highlighted with a star) superpose well and the distances towards the ε-amino group of the catalytic lysine are the same. B) Final 2mFo-DFc electron density of D-Trp in Thal (PDB ID 8AD7 contoured at 1 σ (blue).

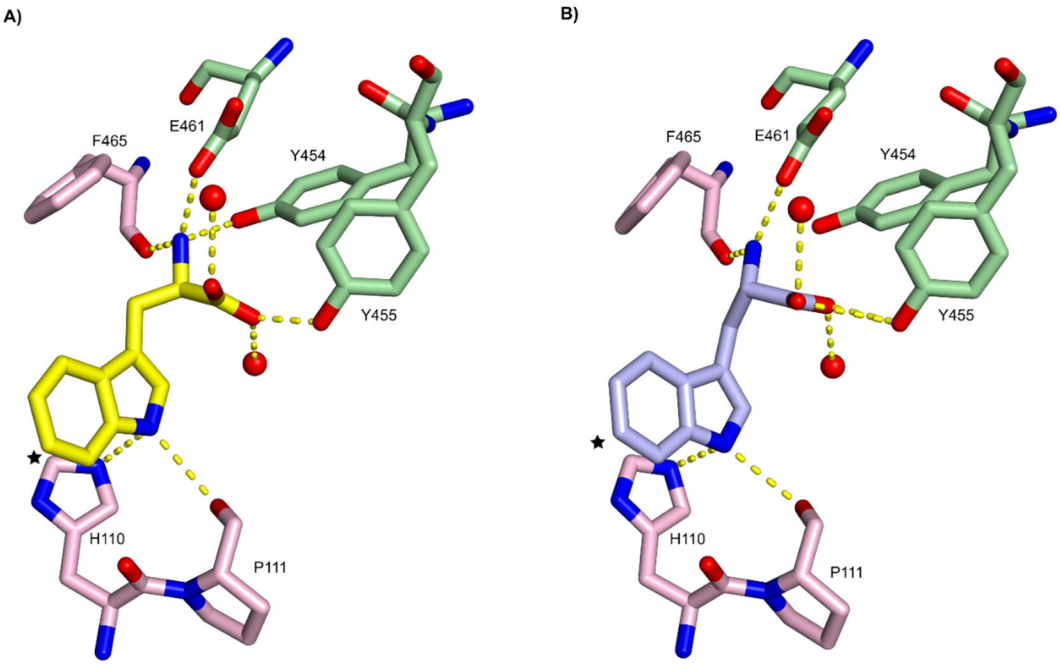


Figure S8. Polar contacts of D-Trp and L-Trp bound to Thal. A) All polar contacts of bound D-Trp from D-Trp-Thal (PDB ID 8AD7) to surrounding active site residues of the π-stacking region (light pink) and backbone-binding region (light green) as well as to conserved waters (red spheres). Direct hydrogen bonds are shown as dotted yellow lines and the halogenation position C6 is highlighted with a star. The substrate D-Trp is shown with yellow carbon atoms. B) All polar contacts of L-Trp from Trp-Thal (PDB 6H44) for comparison. The substrate L-Trp is shown with light blue carbon atoms.

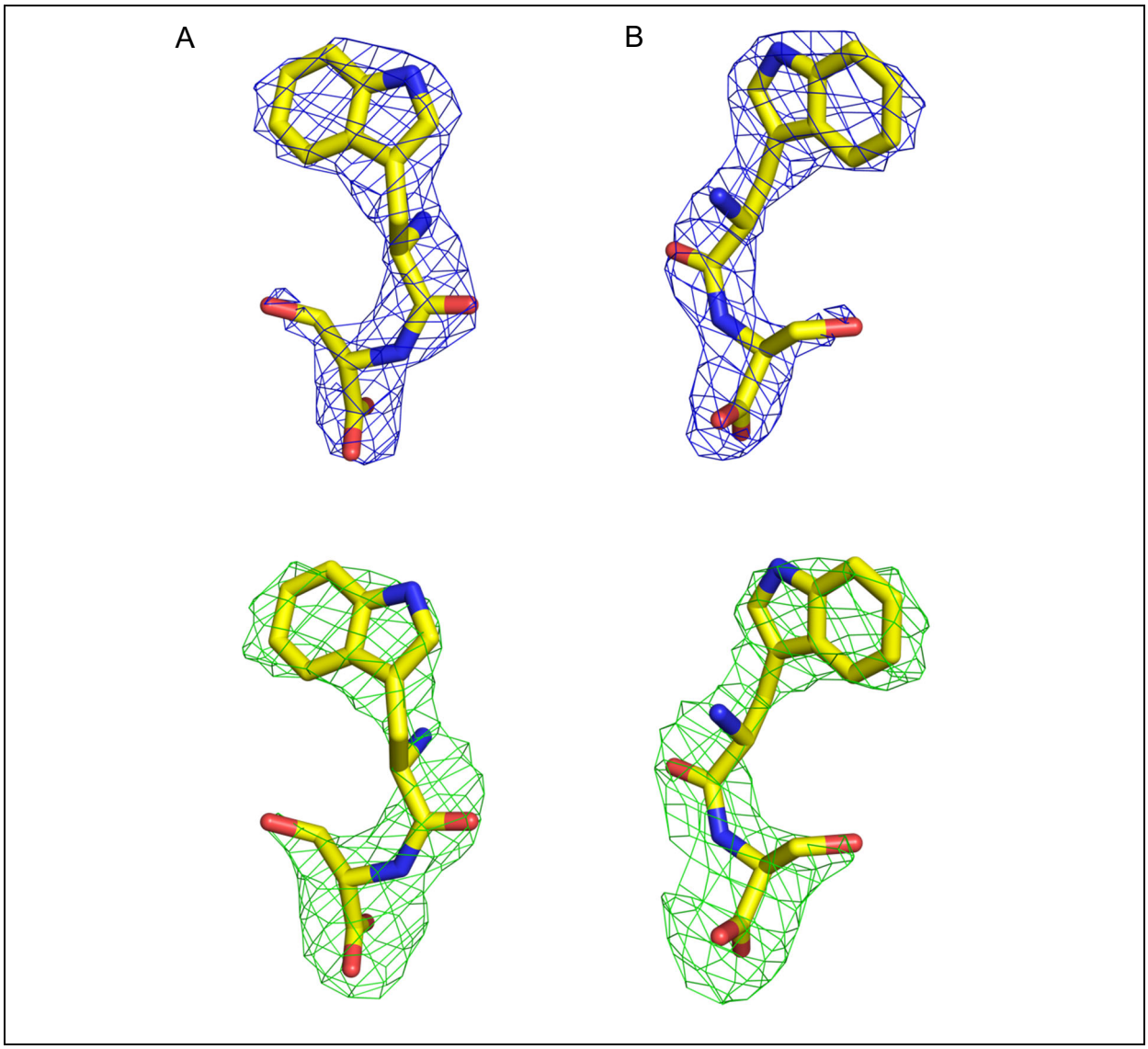


Figure S9. Electron density of D-Trp-Ser bound to Thal. The final 2mFo-DFc electron density contoured at 1 σ (blue, top) and the F_o-F_c electron density before building D-Trp-Ser contoured at +3 σ (green, bottom) are shown. The views A and B are rotated 180° to each other.

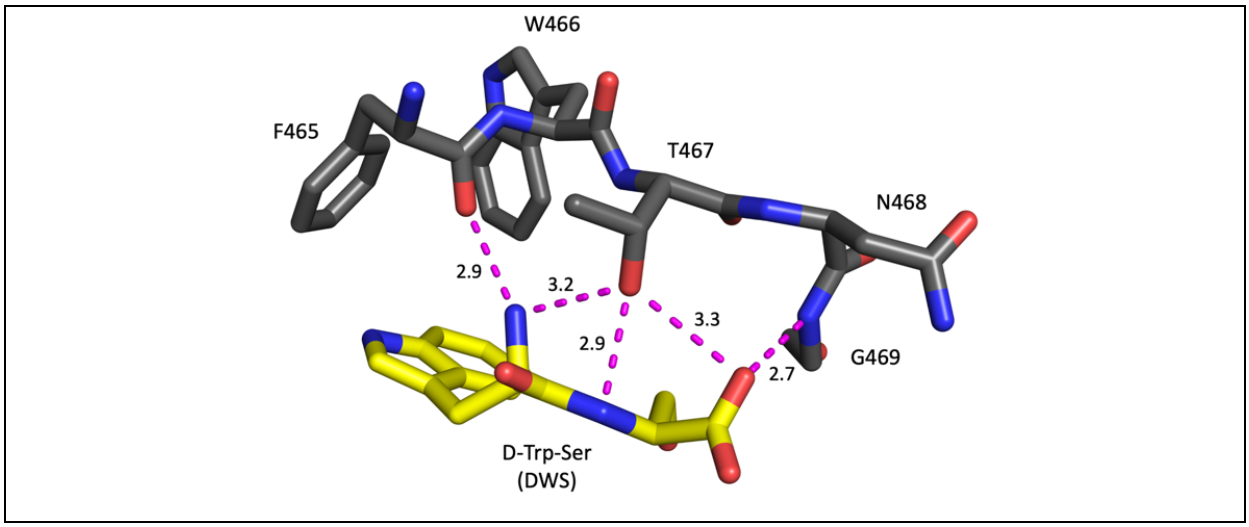


Figure S10. Polar contacts of D-Trp-Ser bound to Thal. All direct hydrogen bonds of bound D-Trp-Ser (yellow) to surrounding amino acids (grey) in Thal (PDB: 8AD8) are shown as dotted purple lines with their bond lengths in Å.

Table S1. Ligand geometry analysis of D-Trp-Thal (8AD7) and Trp-Thal (6H44). The original D-Trp-Thal (8AD7) structure containing D-Trp and D-Trp-Thal containing L-Trp after five rounds of refinement with refmac5 consisting of ten cycles each including occupancy refinement for Trp as well as the original Trp-Thal (6H44) structure with L-Trp and Trp-Thal containing D-Trp after five rounds of refinement were analysed with validation tools implemented in the program Coot with respect to overall ligand geometry, density fit, clashes to nearby residues and distortions of bond lengths and bond angles. This analysis delivers an objective measurement, whether the correct chirality of tryptophan was placed.

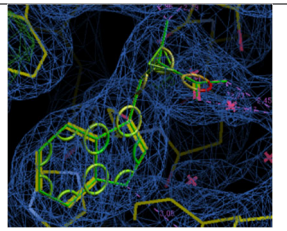
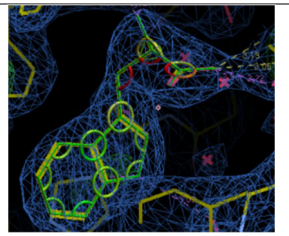
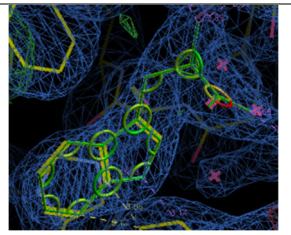
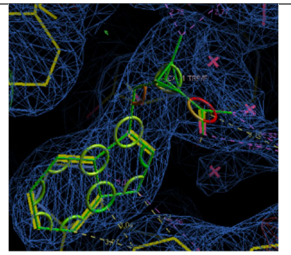
	Geometry Ligand	**Density fit Analysis**	**Clashes**	**Ligand Distortions**	
				average […] penalty	Displayed
original Trp-Thal (6H44)	light green z-score: 3.37	green 0.58	• none	• bond distortion: 0.0900052 • angle distortion: 1.24056 • overall distortion: 0.374677	
Trp-Thal (6H44) with D-Trp after 5. refinement	orange z-score: 4.34	green 0.54	• carboxy group to Y455: 3.05 Å and 3.19 Å	• bond distortion: 0.233408 • angle distortion: 3.04423 • overall distortion: 0.822342	
original D-Trp-Thal (8AD7)	light green z-score: 3.95	light green 0.27	• indole motif to F112: 3.08 Å and 3.17 Å	• bond distortion: 0.0620688 • angle distortion: 1.16588 • overall distortion: 0.293344	
D-Trp-Thal (8AD7) with L-Trp after 5. refinement	orange z-score: 6.32	light green 0.28	• indole motif to F112: 3.04 Å; 3.07 Å; 3.20 Å • carboxy group to P53: 3.16 Å • carboxy group to Y455: 3.16 Å	• bond distortion: 0.128764 • angle distortion: 4.10371 • overall distortion: 1.11226	

Table S2. Data collection and refinement statistics. Values in parentheses are for the highest resolution shell.

	D-Trp-Thal	D-Trp-Ser-Thal
PDB ID	8AD7	8AD8
Space group	$P6_4$	$P6_4$
Unit-cell constants		
***a*= *b*, *c* (Å)**	138.9, 142.6	137.2, 143.8
***α*= *β*, *γ* (°)**	90.0, 120.0	90.0, 120.0
Data collection statistics		
Beamline	EMBL Hamburg @PETRA III, DESY, P13	BESSY II, BL14.2
Wavelength (Å)	0.9164	0.9184
Resolution range (Å)	50.0-2.33 (2.39-2.33)	50.0-2.95 (3.03-2.95)
No. of reflections (measured/unique)	1387796 (103334)/66588 (4908)	341314 (26320)/32396 (2386)
Completeness (%)	100 (100)	100 (100)
R_{meas} (%)	13.0 (171.0)	15.4 (154.0)
Multiplicity	20.84 (21.05)	10.54 (11.03)
Mean *I*/σ(*I*)	14.09 (1.96)	16.71 (1.99)
CC ½ (%)	99.8 (74.6)	99.8 (67.6)
Wilson *B* factor(Å^2)	70	71
Refinement and model statistics		
Resolution range	49.80-2.33 (2.39-2.33)	47.98-2.95 (3.03-2.95)
No. of reflections (work/test)	63207(4649)/ 3342 (250)	30787 (2263) / 1577 (119)
R_{work}	0.1919 (0.314)	0.1952 (0.326)
R_{free}	0.2363 (0.362)	0.2499 (0.402)
r.m.s.d. bonds (Å)	0.007	0.007
r.m.s.d. angles (°)	1.475	1.465
substrate occupancy	0.85	1.00
No. of atoms	8695	8347
Protein	8420	8303
Ligands	51	38
Solvent	224	6
Average *B* factor (Å^2)	75	77
Protein	75	77
Ligands	90	85
Solvent	63	47
Ramachandran		
Favored (%)	95.70	91.65
Allowed (%)	4.20	8.35
Outliers (%)	0.1	0

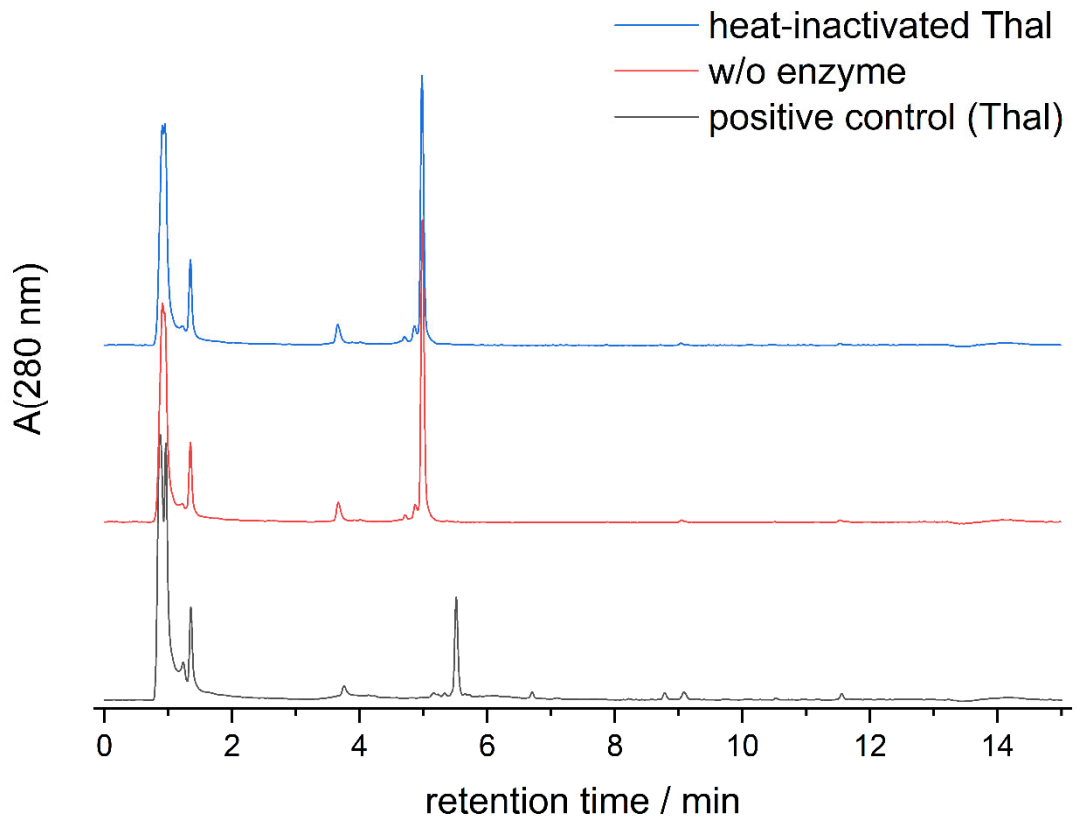


Figure S11. Control reactions for the bromination of RGD peptide **31**. Clearly, the positive control containing Thal (purified) shows quantitative conversion into the brominate peptide **Br-31** (retention time: 5.7 min) as proved by LC-MS. In absence of enzyme or upon adding a heat-inactivated biocatalyst (10 min at 95°C) only **31** (retention time: 5.3 min) is detected.

Biocatalysts used in this study

Genes were inserted into pET28a using standard cloning procedures with an *N*-terminal His_6 tag encoded except for the PTDH-construct, which has a C-terminally appended His_6-tag. Subcloning of halogenases was described previously.[1–3] Cloning of phosphite dehydrogenase is described below.

Enzyme name (function)	**Origin**	**Uniprot ID**
PyrH (Trp 5-halogenase)	*Streptomyces rugosporus*	A4D0H5
Thal (Trp 6-halogenase)	*Streptomyces albogriseolus*	A1E280
RebH (Trp 7-halogenase)	*Lechevalieria aerocolonigenes*	Q8HKZ8
PrnF (flavin reductase)	*Pseudomonas fluorescens*	GeneBank: AAY92875.1
PTDH (phosphite dehydrogenase), thermostable variant[4,5]	*Pseudomonas stutzeri*	O69054 + mutations

Preparation of auxiliary enzymes

Construction of pET28-PTDH was previously described by us.

Flavin reductase PrnF

Expression of PrnF and alcohol dehydrogenase was conducted according to a previously reported procedure.[2]

Phosphite dehydrogenase (PTDH)Cloning of pET28-PTDH was previously described us. A main culture of 1.5 L LB medium was inoculated from a preculture of *E. coli* BL21 (DE3) pET28-PTDH grown overnight at 37°C. The main culture was cultivated at 37°C until an $OD_{600\ nm}$ of 0.5-0.6 was reached The expression culture was incubated at 25 °C for 20 h after induction by addition of 0.2 mM IPTG. Cells were harvested and washed as mentioned before. The cell pellet was resuspended in 30 mL 50 mM Na_2HPO_4 (pH7.4) / 300 mM NaCl and lysed by French-Press (3 passages @ 1100 bar cell pressure). Cell debris was removed by centrifugation (12000 × g, 30 min, 4 °C) and the resulting lysate stored in 1 mL aliquots at -20 °C for direct use in biotransformations. The volumetric activity of PTDH was determined photometrically by measurement of NADH absorbance at 340 nm. Assay conditions in a total volume of 1 mL: 50 mM Na_2HPO_3 (adj. to pH = 7.4), 5 mM NAD^+, 10 µL of 10-fold dilution of PTDH lysate.

Synthesis procedures

L- and D-*N*-Boc-Tryptophan

L- tryptophan (**48**, 1.0 equiv., 10 mmol, 2.0 g) was suspended in 40 mL MeCN / DCM (1:1, v/v), di-tert-butyl dicarbonate (Boc_2O, 1.1 equiv., 11 mmol) as well as *N,*N'-diisopropylethylamine (DIPEA) (1.1 equiv., 11 mmol) were added and the suspension was stirred for 3 h at room temperature. Additional DIPEA (1.0 equiv., 10 mmol) was added and incubation was continued overnight. The solvent was evaporated in vacuo, re-suspended in 40 mL DCM and stirred with imidazole (1.0 equiv., 10 mmol, 0.68 g) for one hour at rt to perform Boc_2O hydrolysis. Subsequently, the organic layer was washed three times with 0.5% aq. HCl, two times with water and dried over Na_2SO_4. Solvent removal and freeze-drying gave a colorless powder in a yield of 92.0% (9.2 mmol, 2.8 g).

The synthesis of D-Boc tryptophan was carried out analogously using D-trytophan on a scale of 5.0 mmol yielding 93.6% (4.68 mmol, 1.4 g) of final product.

Coupling Strategy A via Mixed Anhydride

To a solution of Boc-tryptophan (**53**, 1.0 equiv., 0.5 mmol, 152 mg) in 10 mL DCM the primary alkylamine-hydrochloride (RNH_2)Cl (3.0 equiv., 1.5 mmol) was added. The coupling agent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (1.2 equiv., 0.6 mmol, 148 mg) was dissolved in 2 mL DCM and added to the reaction mixture. The suspension was stirred for 24 h at rt, while the reaction progress was regularly controlled by LC-MS. Upon conversion of **53** the mixture was diluted with 15 mL DCM, washed three times with 1% aq. HCl and with water. The organic layer was dried over Na_2SO_4, evaporated in vacuo and the crude extract was directly employed for N^{α}-deprotection.

Cleavage of Boc- / tBu-Protection Groups

5 mL of cleavage cocktail was prepared freshly containing 60% TFA (3 mL), 5% thioanisole (250 µL), 3% ethanedithiol (150 µL), 3% triisopropylsilane (150 µL), 3% water (150 µL) and 26% DCM (1.3 mL). The solution was added to the crude extract received from the previous coupling reaction and stirred for 1-2 hours at rt. Afterwards, 25 mL toluene was added for co-evaporation of TFA and dried completely in vacuo.

The residuum was dissolved in a mixture of MeCN, freeze-dried and purified by RP-HPLC using a gradient from 0→75% MeCN over 55 min to obtain the final amides as colorless to pale yellow powder.

Coupling Strategy B via Acyl Chloride

Boc-L-tryptophan (1.0 equiv., 0.5 mmol, 152 mg) dissolved in 10 mL DCM was mixed with amine component (1.1 equiv., 0.55 mmol) and triethylamine (3.0 equiv., 1.5 mmol, 209 µL). Afterwards thionylchloride (1.1 equiv., 0.55 mmol, 40 µL) was pipetted to the solution and the reaction was performed overnight at rt. The next day again amine component (3.0 equiv., 1.5 mmol), triethylamine (3.0 equiv., 1.5 mmol, 209 µL) and thionylchloride (3.0 equiv., 1.5 mmol, 109 µL) were spiked to the reaction mixture to improve total conversion. After stirring for additional 24 h, the reaction mixture was washed with 3% aq. $KHSO_4$ and afterwards with 1 M aq. NaOH. Solvent was removed under reduced pressure and the crude extract was employed for deprotection according as described in section 0.

Coupling Strategy C via carbodiimides using EDC and HOAt

Boc-tryptophan (1.0 equiv., 0.5 mmol, 152 mg) dissolved in 10 mL DCM was mixed with triethylamine (5.0 equiv., 2.5 mmol, 349 µL). The amino acid tert-butyl ester, EDC-HCl (1.5 equiv., 0.75 mmol, 144 mg) and HOBt (1.1 equiv., 0.55 mmol, 74 mg) were added stepwise to the solution. Stirring was performed at rt overnight until the starting material was gone that was monitored by LC-MS analysis. The solution was diluted with 15 mL DCM, washed three times with 5% aq. $KHSO_4$ solution and afterwards three times with water. The organic phase was dried and evaporated in vacuo to complete dryness. Final deprotection was carried out according to the general working procedure described in 0. Purification was performed using RP-HPLC with a linear gradient from 0→60% MeCN in 55 min.

Coupling Strategy D via Solid-Phase Synthesis

Loading of Rink amide resin

Rink amide resin (1.0 g) was swollen in 10 mL DMF for 20 min, solvent was removed and the Fmoc group was cleaved with 5 mL 20% piperidine in DMF by incubation for 15 min. Afterwards, the solution was removed, and the resin was washed with DMF (5x) and cleavage was repeated. The C-terminal amino acid, Fmoc-Gly-OH

(1.5 equiv., 1.04 mmol, 308 mg), was mixed for preactivation with HATU (1.5 equiv., 1.04 mmol, 395 mg) and DIPEA (3.0 equiv., 2.07 mmol, 353 µL). After stirring for 5 min the mixture was added to the resin and incubated for 3 h. Afterwards, the resin was washed with DMF (5x) and capped using acetic anhydride (10 equiv., 6.9 mmol, 651 µL) and pyridine (10 equiv., 6.9 mmol, 556 µL) for 20 min. After additional washing with DMF (5x) the capping step was repeated, again washed with DMF and finally with DCM. The resin was dried in vacuo and loading was determined spectrophotometrically by quantification of dibenzofulvene.

Loading of 2-Chlorotrityl-chloride-Polystyrene resin

2-Chlorotrityl chloride resin (1.5 g, 1 mmol/g labelling) was treated with a solution of Fmoc-Amino acid (3 mmol, 2 eq.) and DIPEA (22.5 mmol, 15 eq.) in DCM (10 mL) under an argon atmosphere and the slurry gently shaken at room temperature for 3 h. Methanol was added (1 mL) and shaking continued for another 45 min. The loaded resin was washed with DMF (5x), DCM (5x), DMF (5x) and *i*-PrOH (5x). The loaded resin was dried in vacuo and loading determined spectrophotometrically.

Fmoc deprotection and amino acid coupling

A solution of 20% piperidine in DMF (5 mL) was added to the resin (0.5 g, 0.375 mmol), incubated for 15 min. Afterwards this step was repeated for additional 30 min. The resin was washed sequentially with DMF (5x), DCM (5x) and DMF (5x). Fmoc-protected amino acid (3.0 equiv., 1.125 mmol, 334 mg), DIPEA (6.0 equiv., 2.25 mmol, 384 µL) and TBTU (3.0 equiv., 1.125 mmol, 361 mg) were dissolved in 5 mL DMF for 5 min and then added to the resin. For coupling the solution was left for 2 h on the resin by occasional shaking and afterwards washed with DMF (5x), DCM (5x) and DMF (5x). To couple the following amino acid Fmoc deprotection was carried out as described previously and the N^{α}-protected amino acid was coupled as described before.

Deprotection and cleavage from resin

Upon coupling of the *N*-terminal amino acid N^{α}-deprotection was again carried out using 20% piperidine as described. 5 mL of cleavage cocktail was added containing the following components: 86% TFA (4.3 mL), 5% thioanisole (250 µL), 3% ethanedithiol (150 µL), 3% triisopropylsilane (150 µL) and 3% water (150 µL). After

incubation for 2 h the cleavage solution containing the final peptide was added to toluene (50 mL) and the previous step was repeated. Collected soluble fractions were evaporated in vacuo, the residuum was redissolved in MeCN / water and freeze-dried. Purification was performed by RP-HPLC using a linear gradient form 0→60% MeCN in 55 min.

Note: During the synthesis of peptide **76** the crude extract was treated with 2 M HCl in a water / MeCN-mixture to cleave an intermediary CO_2 adduct from the resin. This resulted in partial hydrolysis of the terminal carboxamide to carboxylic acid. As a consequence, both peptides, acid **75** as well as amide **76**, were isolated. For the synthesis of tetrapeptide **77** the acidic treatment was omitted that finally gave the desired amide.

Synthesis of c(RGDfK(wVGG)) (31)

The linear precursor was assembled on 2-Chlorotrityl-resin following the general Fmoc/*t*Bu-strategy and protocols for SPPS. For resin-cleavage with fully retained side-chain protection, the resin was subjected to a mild cleavage cocktail of 1% TFA in DCM. RP-HPLC purification followed by lyophilisation gave H-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-OH as a colourless powder (251 mg, 0.22 mmol, 73.15%).

Head-to-Tail Cyclisation Protocol

H-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-OH (251 mg, 0.22 mmol) was dissolved in DMF (20 mL) and HATU (1.5 equiv., 125 mg, 0.33 mmol) and HOAt (1.5 equiv., 45 mg, 0.33 mmol) were separately dissolved in DMF (20 mL). Both solutions were loaded into Syringes and added to a stirred solution of DIPEA (3.0 equiv., 113 µL, 0.66 mmol) using a syringe pump at a rate of 1.25 mL/h. After complete addition, more DIPEA (1.5 equiv., 56 µL, 0.33 mmol) was added and the reaction mixture stirred at room temperature for another hour. The reaction mixture was concentrated under reduced pressure, the residue dissolved in MeCN/H_2O and lyphilised. The crude protected cyclopeptide was redissolved in 4N HCl in 1,4-Dioxane and stirred for 1.5 h at room temperature. Volatiles were removed in vacuo and the residue lyophilised. Purification by RP-HPLC followed by lyophilisation yielded c-(-R(Pbf)GDfK-) (140.4 mg, 0.14 mmol, 66%) as a colourless powder.

Fragment condensation procedure

A solution of c-(-R(Pbf)GDfK-) (40 mg, 0.042 mmol) in DMF (4 mL) was treated with a solution of Boc-wVGG-OH (0.9 equiv., 19.6 mg, 0.038 mmol), HATU (0.9 equiv., 14 mg, 0.038 mmol), HOAt (0.9 equiv., 5.2 mg, 0.038 mmol) and DIPEA (3 equiv., 22 µL, 0.126 mmol). The resulting mixture was stirred at room temperature for 30 min and the reaction progress monitored via LC-MS. After complete conversion, the reaction mixture was concentrated under reduced pressure, the residue dissolved in 50% MeCN/H_2O and lyophilised. Deprotection of the *N*-terminal Boc as well as the Pbf-group was achieved by treating with the general TFA/Thioanisole/EDT/TIS/H_2O cleavage cocktail (5 mL) for 2 h at room temperature. RP-HPLC-purification and lyophilization gave the final peptide **31** as a TFA-salt (30.6 mg, 0.024 mmol, 62.5%)

References

[1] J. F. Sambrook, D. W. Russel, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, **2001**.

[2] M. Frese, P. H. Guzowska, H. Voß, N. Sewald, *ChemCatChem* **2014**, *6*, 1270–1276.

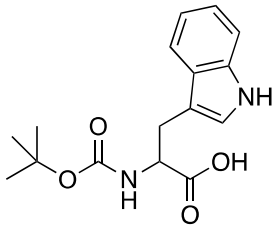
[3] M. Frese, C. Schnepel, H. Minges, H. Voß, R. Feiner, N. Sewald, *ChemCatChem* **2016**, *8*, 1799–1803.

[4] T. W. Johannes, R. D. Woodyer, H. Zhao, *Appl. Environ. Microbiol.* **2005**, *71*, 5728–5734.

[5] T. W. Johannes, R. D. Woodyer, H. Zhao, *Biotechnol. Bioeng.* **2007**, *96*, 18–26.

Analytical data

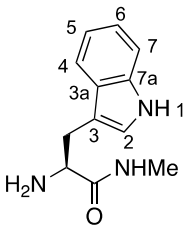
N-Boc L- or D-Tryptophan



Formula: $C_{16}H_{20}N_2O_4$

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.53 (s, 1H, CO_2**H**), 10.83 (s, 1H, indole-N**H**), 7.52 (d, ^3J = 7.8 Hz, 1H, indole-C^4**H**), 7.33 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.14 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.06 (dd, ^3J = 8.1 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 6.99 (dd, ^3J = 8.1 Hz, ^3J = 7.9 Hz, 2H, indole-C^5**H**), 6.96 (d, ^3J = 7.9 Hz, 1H, amide-N**H**), 4.14 (ddd, ^3J = 9.4 Hz, 7.9 Hz, 4.7 Hz, 1H, C$^\alpha$**H**), 3.12 (dd, ^2J = 14.6 Hz, ^3J = 4.8 Hz, 1H, C$^\beta$**H^a**), 2.97 (dd, ^2J = 14.6, ^3J = 9.4 Hz, 1H, C$^\beta$**H^b**), 1.33 (s, 9H, (C**H$_3$**)$_3$C^{Boc}).

L-Tryptophan *N*-methylamide (3)



Formula: $C_{12}H_{15}N_3O \times CF_3CO_2H$

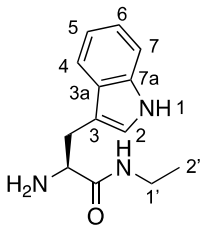
Yield: 101 mg, 0.3 mmol, 61.0%

HR-ESI-MS: m/z = $[M+H]^+$ 218.1289 (found); 218.1288 (calc.), Δ = 0.50 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (d, ^3J = 2.7 Hz, 1H, indole-N**H**), 8.45 (q, ^3J = 4.6 Hz, 1H, amide-N**H**), 8.12 (s, N$^\alpha$H, 3**H**), 7.60 (d, ^3J = 7.8 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, 1H, ^3J = 8.1 Hz, 6.9 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.0 Hz, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.89 (t, ^3J = 7.1 Hz, 1H, C$^\alpha$**H**), 3.19 (dd, ^2J = 14.6 Hz, ^3J = 6.2 Hz, 1H, C$^\beta$**H**), 3.09 (dd, ^2J = 14.6 Hz, ^3J = 7.5 Hz, 1H, C$^\beta$**H**), 2.61 (d, ^3J = 4.5 Hz, 3H, NHC**H$_3$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 168.8 (**C**=O), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 124.8 (indole-**C**^{2}H), 121.1 (indole-**C**^{6}), 118.4 (indole-**C^5**), 118.3 (indole-**C**^{4}), 111.5 (indole-**C^7**), 106.7 (indole-**C**^{3}), 52.9 (**C**$^\alpha$H), 27.4 (NH**C**H$_3$), 25.6 (**C**$^\beta$H).

L-Tryptophan *N*-ethylamide (4)



Formula: $C_{13}H_{17}N_3O \times CF_3CO_2H$

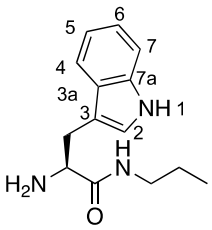
Yield: 109 mg, 0.32 mmol, 63.4%

HR-ESI-MS: m/z = $[M+H]^+$ 232.1452 (found); 232.1444 (calc.), Δ = 3.27 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (s, 1H, indole-N**H**), 8.42 (t, ^3J = 5.5 Hz, 1H, N**H**Et), 8.11 (s, 3H, N**H**$^{\alpha}$Et), 7.62 (dd, ^3J = 8.1 Hz, ^4J = 1.1 Hz, 1H, indole-C^4**H**), 7.37 (dt, ^3J = 8.2 Hz, ^4J = 0.9 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1 Hz, 7.0 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.0, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.87 (t, ^3J = 7.1 Hz, 1H, C$^{\alpha}$**H**), 3.18 (dd, ^2J = 14.6 Hz, ^3J = 6.3 Hz, 1H, C$^{\beta}$**H**), 3.11 (dd, ^2J = 14.2 Hz, ^3J = 7.2 Hz, 1H, C$^{\beta}$**H**), 3.10 – 3.04 (m, 2H, C1'**H$_2$**), 0.95 (t, ^3J = 7.2 Hz, 3H, C2'**H$_3$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 168.1 (**C**=O), 136.4 (indole-**C**7a), 127.2 (indole-**C^{3a}**), 125.0 (indole-**C**^{2}), 121.3 (indole-**C**^{6}), 118.6 (indole-**C**^{4}), 118.5 (indole-**C**^{5}), 111.6 (indole-**C**^{7}), 107.1 (indole-**C**^{3}), 53.1 (**C**$^{\alpha}$), 33.8 (**C**1'), 27.6 (**C**$^{\beta}$), 14.4 (**C**2').

L-Tryptophan *N*-propylamide (5)



Formula: $C_{14}H_{19}N_3O \times CF_3CO_2H$

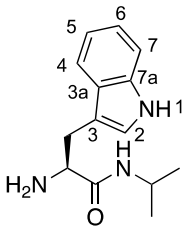
Yield: 128 mg, 0.36 mmol, 71.2%

HR-ESI-MS: m/z = $[M+H]^+$ 246.1610 (found); 246.1601 (calc.), Δ = 3.70 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (d, ^3J = 2.8 Hz, 1H, indole-N**H**), 8.42 (t, ^3J = 5.6 Hz, 1H, N**H**Pr), 8.12 (s, 3H, N$^\alpha$**H$_3^+$**), 7.63 (dd, ^3J = 8.0 Hz, ^4J = 1.1 Hz, 1H, indole-C^4**H**), 7.37 (dt, ^3J = 8.1 Hz, ^4J = 0.9 Hz, 1H, indole-C^7**H**), 7.19 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1, 7.0, ^4J = 1.1 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.1 Hz, 7.0 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.91 (t, ^3J = 7.1 Hz, 1H, C$^\alpha$**H**), 3.19 (dd, ^2J = 14.5 Hz, ^3J = 6.4 Hz, 1H, C$^\beta$**H**), 3.09 (dd, ^2J = 14.6 Hz, ^3J = 7.7 Hz, 1H, C$^\beta$**H**), 3.02 (td, ^3J = 7.1, 5.7 Hz, 2H, C$^{1'}$**H$_2$**), 1.35 (h, ^3J = 7.4 Hz, 2H, C$^{2'}$**H$_2$**), 0.77 (t, ^3J = 7.4 Hz, 3H, C$^{3'}$**H$_3$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 168.4 (**C**=O), 136.4 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 124.9 (indole-**C**^{2}), 121.3 (indole-**C**^{6}), 118.6 (indole-**C**^{4}, indole-**C**^{5}), 111.6 (indole-**C**^{7}), 107.1 (indole-**C**^{3}), 53.1 (**C**$^\alpha$), 40.7 (**C**$^{1'}$H), 27.6 (**C**$^\beta$), 22.1 (**C**$^{2'}$H$_2$), 11.4 (**C**$^{3'}$H$_3$).

L-Tryptophan *N*-isopropylamide (6)



Formula: $C_{14}H_{19}N_3O \times CF_3CO_2H$

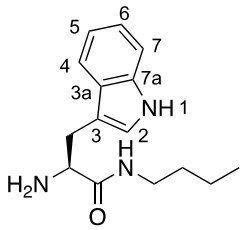
Yield: 120 mg, 0.33 mmol, 66.8%

HR-ESI-MS: m/z = $[M+H]^+$ 246.1613 (found); 246.1601 (calc.), Δ = 4.92 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.25 (d, ^3J = 7.5 Hz, 1H, N**H**iPr), 8.10 (s, 3H, N$^\alpha$ **H$_3^+$**), 7.63 (dd, ^3J = 8.0, ^4J = 1.1 Hz, 1H, indole-C^4**H**), 7.36 (dt, ^3J = 8.1 Hz, ^4J = 0.9 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1, 7.0 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.0, 7.0 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.89 – 3.76 (m, 2H, C$^\alpha$**H**, C**H**(CH$_3$)$_2$), 3.16 (dd, ^2J = 14.5 Hz, ^3J = 6.6 Hz, 1H, C$^\beta$**H^a**), 3.08 (dd, ^2J = 14.5 Hz, ^3J = 7.6 Hz, 1H, C$^\beta$**H^b**), 1.06 (d, ^3J = 6.6 Hz, 3H, CH(**CH$_3^a$**)$_2$), 0.90 (d, ^3J = 6.5 Hz, 3H, CH(C**H$_3^b$**)$_2$).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 167.2 (**C**=O), 136.2 (**C**^{7a}), 127.1 (**C**^{3a}), 124.8 (indole-**C**^{2}), 121.1 (indole-**C**^{6}), 118.4 (indole-**C**^{4}), 118.4 (indole-**C**^{5}), 111.4 (indole-**C**^{7}), 107.0 (indole-**C**^{3}), 52.9 (**C**$^\alpha$), 40.9 (**C**H(CH$_3$)$_2$), 27.4 (**C$^\beta$**), 22.2 (CH(**C^a**H$_3$)$_2$), 21.9 (CH(**C^b**H$_3$)$_2$).

L-Tryptophan *N*-butylamide (7)



Formula: $C_{15}H_{21}N_3O \times CF_3CO_2H$

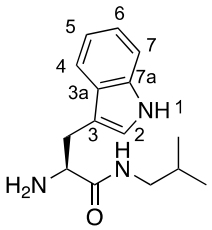
Yield: 136 mg, 0.37 mmol, 73.1%

HR-ESI-MS: m/z = $[M+H]^+$ 260.1770 (found); 260.1757 (calc.), Δ = 4.84 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (d, ^3J = 2.6 Hz, 1H, indole-N**H**), 8.37 (t, ^3J = 5.6 Hz, 1H, N**H**nBu), 8.11 (d, ^3J = 4.9 Hz, 3H, N$^{\alpha}$**H$_3^+$**), 7.62 (dd, ^3J = 8.0 Hz, ^4J = 1.1 Hz, 1H, indole-C^4**H**), 7.36 (dt, ^3J = 8.1 Hz, ^4J = 0.9 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1, 7.0 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 7.9, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.90 (dd, ^3J = 7.2, 6.3 Hz, 1H, C$^{\alpha}$**H**), 3.18 (dd, ^2J = 14.5 Hz, ^3J = 6.5 Hz, 1H, C$^{\beta}$**H**), 3.11 (dd, ^2J = 14.6 Hz, ^3J = 7.1 Hz, 1H, C$^{\beta}$**H**), 3.09 – 2.98 (m, 2H, C$^{1'}$**H$_2$**), 1.29 (p, ^3J = 7.0 Hz, 2H, C$^{2'}$**H$_2$**), 1.17 (h, ^3J = 7.3 Hz, 2H, C$^{3'}$**H$_2$**), 0.82 (t, ^3J = 7.3 Hz, 3H, C$^{4'}$**H$_3$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 168.1 (**C**=O), 136.2 (indole-**C**^{7a}), 127.0 (indole- **C**^{3a}), 124.7 (indole-**C**^{2}), 121.1 (indole-**C^6**), 118.3 (indole-**C^4**), 118.4 (indole-**C^5**), 111.5 (indole-**C^7**), 107.0 (indole-**C**^{3}), 53.0 (**C**$^{\alpha}$), 38.4 (**C**$^{1'}$H$_2$), 30.7 (**C**$^{2'}$H$_2$), 27.4 (**C**$^{\beta}$), 19.4 (**C**$^{3'}$H$_2$), 13.6 (**C**$^{4'}$H$_3$).

L-Tryptophan *N*-isobutylamide (8)



Formula: $C_{15}H_{21}N_3O \times CF_3CO_2H$

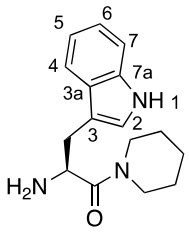
Yield: 146 mg, 0.39 mmol, 78.2%

HR-ESI-MS: m/z = $[M+H]^+$ 260.1679 (found); 260.1757 (calc.), Δ = 4.46 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (d, ^3J = 2.2 Hz, 1H, indole-N**H**), 8.41 (t, ^3J = 5.8 Hz, 1H, amide-N**H**), 8.10 (d, ^3J = 4.9 Hz, 3H, N$^\alpha$**H$_3^+$**), 7.63 (d, 1H, ^3J = 7.9 Hz, indole-C^4**H**), 7.36 (dt, ^3J = 8.1 Hz, ^4J = 0.9 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.2, 7.0 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 7.9, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 3.94 (dd, ^3J = 7.3, 6.5 Hz, 1H, C$^\alpha$**H**), 3.19 (dd, ^2J = 14.5 Hz, ^3J = 6.4 Hz, 1H, C$^\beta$**H^a**$_2$), 3.08 (dd, ^2J = 14.5 Hz, ^3J = 7.8 Hz, 1H, C$^\beta$**H^b**), 2.95 (ddd, ^2J = 12.9 Hz, ^3J = 6.3 Hz, 1H, C$^{1'}$**H^a**), 2.83 (ddd, ^2J = 12.9 Hz, ^3J = 7.0, 5.4 Hz, 1H C$^{1'}$**H^b**), 1.61 (hept, ^3J = 6.7 Hz, 1H, C$^{2'}$**H**), 0.78 (d, ^3J = 6.6 Hz, 3H, C$^{2'}$H(C**H$_3^a$**)$_2$), 0.75 (d, ^3J = 6.6 Hz, 3H, C$^{2'}$H(C**H$_3^b$**)$_2$).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 168.3 (**C**=O), 136.3 (**C**^{7a}), 127.0 (indole-**C**^{3a}), 124.5 (indole-**C**^{2}), 121.1 (indole-**C**^{6}), 118.4 (indole-**C^4**, indole-**C^5**), 111.5 (indole-**C**^{7}), 106.9 (indole-**C**^{3}), 52.9 (**C**$^\alpha$), 46.3 (**C**$^{1'}$H$_2$), 27.8 (**C**$^{2'}$H(CH$_3$)$_2$), 27.6 (**C**$^\beta$), 20.0 (C$^{2'}$H(**C**H$_3$)$_2$)**.**

L-Tryptophan piperidinylamide (9), strategy B



Formula: $C_{16}H_{21}N_3O \times CF_3CO_2H$

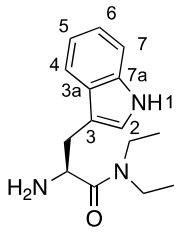
Yield: 70 mg, 0.18 mmol, 36.3%

HR-ESI-MS: m/z = $[M+H]^+$ 272.1765 (found); 272.1757 (calc.), Δ = 2.79 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.06 (s, 1H, indole-N**H**), 8.15 (s, 3H, N$^{\alpha}$**H$_3^+$**), 7.46 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 8.0 Hz, 1H, indole-C^7**H**), 7.24 (d, ^3J = 2.5 Hz, 1H, indole-C^2**H**), 7.09 (dd, ^3J = 7.6 Hz, 7.6 Hz, 1H, indole-C^6**H**), 7.01 (dd, ^3J = 7.5 Hz, 7.5 Hz, 1H, indole-C^5**H**), 4.58 (dt, ^3J = 9.7, 5.4 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.38–3.26 (m, 2H, C$^{2'}$/6'**H$_2$**), 3.21–3.06 (m, 3H, C$^{\beta,Trp}$**H$_2$,** C$^{2'}$/6'**H$_2$**), 2.94 (dt, ^2J = 12.7 Hz, ^3J = 7.0 Hz, 1H, C$^{2'}$/6'**H$_2$**), 1.45-1.37 (m, 1H, C$^{2'}$/5'**H$_2$**), 1.36-1.27 (m, 2H, C$^{3'}$/5'**H$_2$**), 1.23-1.16 (m, 1H, C$^{3'}$/5'**H$_2$**), 1.15-1.08 (m, 1H, C$^{4'}$**H$_2$**), 0.77-0.67 (m, 1H, C$^{4'}$**H$_2$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 167.3 (**C**ONH), 136.6 (**C**^{7a}), 127.6 (indole-indole-**C**^{3a}), 125.3 (indole-**C**^{2}), 121.7 (indole-**C**^{6}), 119.1 (indole-**C**^{5}), 118.4 (indole-**C^4**), 112.0 (indole-**C^7**), 107.2 (indole-**C**^{3}), 49.9 (**C**$^{\alpha}$), 46.3 (**C**$^{2'}$/6'H$_2$), 42.9 (**C**$^{2'}$/6'H), 27.6 (**C**$^{\beta}$), 25.5 (C$^{3'}$/4'H$_2$), 25.2 (C$^{3'}$/4'H$_2$), 24.0 (**C**$^{3'}$H$_2$).

L-Tryptophan *N,N*-diethylamide (10), strategy B



Formula: $C_{15}H_{21}N_3O \times CF_3CO_2H$

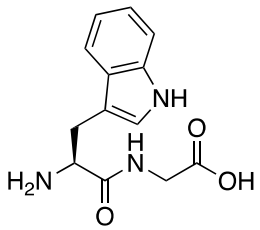
Yield: 24.4 mg, 0.065 mmol, 13.1%

HR-ESI-MS: m/z = $[M+H]^+$ 260.1757 (found); 260.1757 (calc.), Δ = 0.15 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.05 (d, *J* = 2.9 Hz, 1H, indole-N**H**), 8.20 (s, 3H, N$^{\alpha}$**H$_3^+$**), 7.50 (d, ^3J = 7.8 Hz, 1H, indole-C^{4}H), 7.36 (d, ^3J = 8.1 Hz, 1H, indole-C^{7}H), 7.21 (d, ^3J = 2.4 Hz, 1H, indole-C^{2}H), 7.09 (t, ^3J = 7.6 Hz, 1H, indole-C^{6}H), 7.01 (t, ^3J = 7.4 Hz, 1H, indole-C^{5}H), 4.37 (q, ^3J = 5.7 Hz, 1H, C$^{\alpha}$**H**), 3.34 (dq, ^2J = 13.8 Hz, ^3J = 6.9 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.15 (dq, ^2J = 15.1 Hz, ^3J = 7.9 Hz, 2H, NC**H$_2^a$**CH$_3$), 3.05 (dq, ^2J = 15.1 Hz, ^3J = 7.3 Hz, 2H, NC**H$_2^b$**CH$_3$), 2.87 (dq, ^2J = 14.4 Hz, ^3J = 7.0 Hz, 1H, C$^{\beta,Trp}$**H^b**), 0.88 (t, ^3J = 7.1 Hz, 3H, NCH$_2$C**H$_3^a$**), 0.84 (t, *J* = 7.1 Hz, 3H, NCH$_2$C**H$_3^b$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 167.9 (**C**ONEt$_2$), 136.3 (**C**^{7}a), 127.2 (indole-indole-**C**^{3a}), 125.0 (indole-**C**^{2}), 121.4 (indole-**C^6**), 118.8 (indole-**C**^{5}), 118.1 (indole-**C^4**), 111.7 (indole-**C**^{7}), 106.8 (indole-**C**^{3}), 49.9, 41.1 (N**C**H$_2$CH$_3$) (**C**$^{\alpha,Trp}$), 27.5 (**C**$^{\beta,Trp}$), 13.9 (NCH$_2$**C**H$_3^a$), 12.6 (NCH$_2$**C**H$_3^b$).

L-Trp-Gly-OH (11), strategy C



Formula: $C_{13}H_{15}N_3O_3 \times CF_3CO_2H$

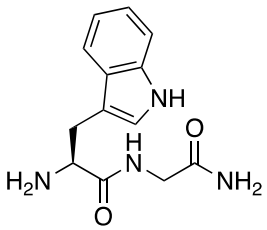
Yield: 127 mg, 0.34 mmol, 67.7%

HR-ESI-MS: m/z = $[M+H]^+$ 262.1190 (found); 262.1186 (calc.), Δ = 1.45 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.84 ppm (s, 1H, CO_2**H**), 11.03 (d, ^3J = 2.5 Hz, 1H, indole-C^{2}H), 8.97 (t, ^3J = 5.8 Hz, 1H, N**H**^{Gly}), 8.06 (s, 3H, C$^{\alpha}$**H**), 7.70 (dd, ^3J = 7.9 Hz, ^4J = 1.1 Hz, 1H, indole-C^{4}H), 7.37 (dt, ^3J = 8.2, 0.9 Hz, 1H, indole-C^{7}H), 7.23 (d, ^3J = 2.4 Hz, 1H, indole-C^{2}H), 7.10 (ddd, ^3J = 8.2, 6.9 Hz, ^4J = 1.2 Hz, 1H), 7.02 (ddd, ^3J = 8.0, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^{5}H), 4.05 (d, ^3J = 7.4 Hz, 1H, C$^{Trp,\alpha}$**H**), 3.92 (dd, ^2J = 15.1 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly}$**H$_2^a$**), 3.87 (dd, ^2J = 15.1 Hz, ^3J = 5.9 Hz, 1H, C$^{\alpha,Gly}$**H$_2^b$**), 3.26 (dd, ^2J = 14.8 Hz, ^3J = 5.0 Hz, 1H, C$^{\beta,Trp}$**H$_2^a$**), 3.09 (dd, ^2J = 14.9 Hz, ^3J = 8.5 Hz, 1H, C$^{\beta,Trp}$**H$_2^b$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 170.7 (**C**ONH^{Gly}), 168.9 (**C**ONH^{Trp}), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 125.1 (indole-**C**^{2}), 121.2 (indole-**C**^{6}), 118.5 (indole-**C**^{5}), 118.4 (indole-**C**^{4}), 106.7 (indole-**C**^{3}), 52.5 (**C**$^{\alpha,Trp}$), 40.8 (**C**$^{\alpha,Gly}$), 27.5 (**C**$^{\beta,Trp}$).

L-Trp-Gly-NH_2 (11), strategy C



Formula: $C_{13}H_{16}N_4O_2 \times CF_3CO_2H$

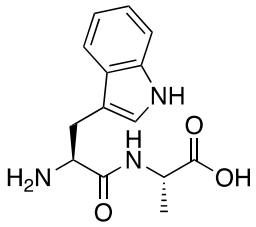
Yield: 47 mg, 0.125 mmol, 25.1%

HR-ESI-MS: m/z = $[M+H]^+$ 261.1349 (found); 261.1346 (calc.), Δ = 1.15 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.01 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.81 (t, ^3J = 5.6 Hz, 1H, CON**H**), 8.03 (s, 3H, amide-N**H^{Trp}**), 7.69 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.38 (s, 1H, CON**H^a**), 7.36 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.22 (d, J = 2.4 Hz, 1H, indole-C^2**H**), 7.16 (s, 1H, CON**H^b**), 7.10 (ddd, ^3J = 8.1, 6.9 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.0, 6.9 Hz, ^4J = 1.0 Hz, 1H, indole-C^5**H**), 4.07 (dd, J = 8.8, 5.3 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.80 (dd, ^2J = 16.8 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly}$**H^a**), 3.66 (dd, ^2J = 16.8 Hz, ^3J = 5.2 Hz, 1H, C$^{\alpha,Gly}$**H^b**), 3.25 (dd, ^2J = 14.8, ^3J = 5.3 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.08 (dd, J = 14.8, 8.3 Hz, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 170.1 (**C**ONH^{Trp}), 168.8 (**C**ONH^{Gly}), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 125.0 (indole-**C^2**), 121.1 (indole-**C^6**), 118.5 (indole-**C^4**), 118.4 (indole-**C^5**), 111.5 (indole-**C^7**), 106.8 (indole-**C^2**), 52.7 (**C**$^{\alpha,Trp}$), 41.9 (**C**$^{\alpha,Gly}$), 27.4 (**C**$^{\beta,Trp}$).

H-Trp-Ala-OH (12a), strategy C



Formula: $C_{14}H_{17}N_3O_3 \times CF_3CO_2H$

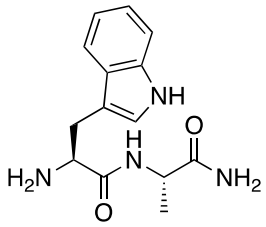
Yield: 138 mg, 0.35 mmol, 70.7%

HR-ESI-MS: m/z = $[M+H]^+$ 276.1345 (found); 276.1343 (calc.), Δ = 0.83 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.85 (s, 1H, CO_2**H**), 11.03 (d, ^3J = 2.9 Hz, 1H, indole-N**H**), 8.93 (d, ^3J = 7.3 Hz, 1H, amide-N**H**), 8.03 (s, 2H, N$^{\alpha}$**H$_2$**), 7.74 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.24 (d, ^3J = 2.6 Hz, 1H, indole-C^2**H**), 7.10 (t, ^3J = 7.5 Hz, 1H, indole-C^6**H**), 7.02 (t, ^3J = 7.4 Hz, 1H, indole-C^{5}H), 4.32 (dq, ^3J = 7.2, 7.4 Hz, 1H, C$^{\alpha,Ala}$**H**), 4.02 (dd, ^3J = 7.4 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.27 (dd, ^2J = 15.0 Hz, ^3J = 4.7 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.04 (dd, ^2J = 15.0 Hz, ^3J = 9.0 Hz, 1H, C$^{\beta,Trp}$**H^b**), 1.35 (d, ^3J = 7.5, 2.3 Hz, 3H, C$^{\beta,Ala}$**H$_3$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 173.5 (**C**O_2H^{Ala}), 168.3 (**C**O_2H^{Trp}), 136.4 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 125.1 (indole-**C^2**), 121.2 (indole-**C^6**), 118.5 (indole-**C^5**), 118.4 (indole-**C^4**), 111.5 (indole-**C^7**), 106.7 (indole-**C**^{3}), 52.5 (**C**$^{\alpha,Gly}$), 47.8 (**C**$^{\alpha,Trp}$), 27.4 (**C**$^{\beta,Trp}$), 17.2 (**C**^{Ala}).

H-Trp-Ala-NH_2 (12), strategy C



Formula: $C_{14}H_{18}N_4O_2 \times CF_3CO_2H$

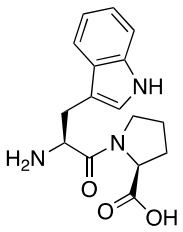
Yield: 75 mg, 0.19 mmol, 38.6%

HR-ESI-MS: m/z = $[M+H]^+$ 275.1511 (found); 275.1502 (calc.); Δ = 3.09 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (d, ^3J = 2.7 Hz, 1H, indole-N**H**), 8.76 (d, ^3J = 7.5 Hz, 1H, $N^{\alpha,Ala}$**H**), 8.00 (d, ^3J = 5.1 Hz, 3H, $N^{\alpha,Trp}$**H$_2$**), 7.70 (d, ^3J = 7.9 Hz, 1H, indole-C^4H), 7.43 (s, 1H, CON**H$_2^a$**), 7.36 (d, ^3J = 8.1 Hz, 1H, indole-C^7H), 7.22 (d, ^3J = 2.4 Hz, 1H, indole-C^2H), 7.13 – 7.06 (m, 2H, indole-C^6H, CON**H$_2^a$**), 7.01 (t, ^3J = 7.4 Hz, 1H, indole-C^5H), 4.31 (dq, ^3J = 7.1 Hz, 7.1 Hz, 1H, $C^{\alpha,Ala}$**H**), 4.06 (dt, ^3J = 9.3, 4.7 Hz, 1H, $C^{\alpha,Trp}$**H**), 3.26 (dd, ^2J = 14.9 Hz, ^3J = 5.1 Hz, 1H, $C^{\beta,Trp}$**H^a**), 3.04 (dd, ^2J = 14.9, 8.5 Hz, 1H, $C^{\beta,Trp}$**H^b**), 1.27 (d, ^3J = 7.0 Hz, 3H, $C^{\beta,Ala}H_3$).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 173.5 (**C**ONH_2^{Ala}), 168.0 (**C**ONH_2^{Trp}), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 125.0 (indole-**C**^{2}), 121.1 (indole-**C**^{6}), 118.5 (indole-**C**^{4}), 118.4 (indole-**C**^{5}), 111.5 (indole-**C**^{7}), 106.8 (indole-**C**^{3}), 52.4 (**C**$^{\alpha,Ala}$), 48.2 (**C**$^{\alpha,Trp}$), 27.4 (**C**$^{\beta,Trp}$), 18.7 (**C**$^{\beta,Ala}$).

H-Trp-Pro-OH (13), strategy C



Formula: $C_{16}H_{19}N_3O_3 \times CF_3CO_2H$

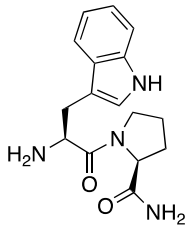
Yield: 160 mg, 0.38 mmol, 77.0% as a mixture of isomers

HR-ESI-MS: m/z = $[M+H]^+$ 302.1508 (found); 302.1499 (calc.), Δ = 2.91 ppm

^{1}H NMR (500 MHz, major isomer, DMSO-*d*$_6$) δ = 12.81 (s, 1H, COO**H**), 11.10 (dd, *J*=12.5, 2.5 Hz, 1H, indole-N**H**), 8.14 (s, 3H, N^{α}**H**$_3^+$), 7.62 (d, *J*=7.8, 1H, indole-C^4**H**), 7.44 – 7.34 (m, 1H, indole-C^7**H**), 7.31 (dd, *J*=18.0, 2.5, 1H, indole-C^2**H**), 7.15 – 6.98 (m, 1H, indole-C^6**H**), 7.07 – 6.95 (m, 1H, indole-C^5**H**), 4.36 – 4.28 (m, 1H, $C^{\alpha,Trp}$**H**), 3.94 (dd, *J*=8.5, 6.4, 1H), 3.71 – 3.63 (m, 1H, $C^{\alpha,Pro}$**H**), 3.33 – 3.00 (m, 4H, $C^{\beta,Trp}$**H**, $C^{\beta,Trp}$**H**), 2.26 – 2.14 (m, 1H, $C^{\delta,Pro}$**H**^{b}), 1.92 – 1.79 (m, 3H, $C^{\gamma,Pro}$**H**, $C^{\delta,Pro}$**H**^{b}).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 173.1 (**C**ONH_2^{Pro}), 167.8 (**C**ONH_2^{Trp}), 127.4 (indole-**C**^{3a}), 125.8 (indole-**C**^{2}), 121.7 (indole-**C**^{6}), 119.2 (indole-**C**^{4}), 118.5 (indole-**C**^{5}), 112.0 (indole-**C**^{7}), 107.0 (indole-**C**^{3}), 59.4 (**C**H^{al}), 51.9 (**C**H^{al}), 47.0 (**C**H^{al}), 46.7 (**C**H^{al}), 30.0 (**C**H^{al}), 29.1 (**C**H^{al}), 28.0 (**C**H^{al}), 26.7 (**C**H^{al}), 25.2 (**C**H^{al}), 22.0 (**C**H^{al}).

H-Trp-Pro-NH_2 (14), strategy C



Formula: $C^1{}_6H_{20}N_4O_2 \times CF_3CO_2H$

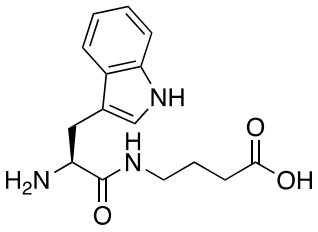
Yield: 154 mg, 0.37 mmol, 74.3%

HR-ESI-MS: m/z = $[M+H]^+$ 301.1669 (found); 301.1659 (calc.), Δ = 3.32 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.09 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 11.08 (d, ^3J = 2.6 Hz, 1H, indole-N**H**), 11.07 (d, ^3J = 2.4 Hz, 1H, indole-N**H**), 8.27 (s, 2H, CON**H$_2^{Pro}$**), 8.06 (s, 1H, N$^{\alpha,Trp}$**H$_2$**), 7.62 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.56 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.44 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.40–7.30 (m, 2H, indole-C^7**H**), 7.27 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.22 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.13–7.09 (m, 1H, indole-C^{6}H), 7.04 (t, ^3J = 7.7 Hz, 1H, indole-C^{5}H), 7.01 (t, ^3J = 8.0, 7.5 Hz, 1H, indole-C^{5}H), 4.32 (dd, ^3J = 8.5, 4.6 Hz, 1H, C$^{\alpha,Pro}$**H**), 4.26 (q, ^3J = 8.4, 6.1 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.66 (ddd, ^2J = 12.7 Hz, ^3J = 8.9, 6.6 Hz, 1H, C$^{\delta,Pro}$**H**), 3.37 – 3.29 (m, 1H, C$^{\delta,Pro}$H, C$^{\beta,Trp}$**H**), 3.25 (dd, ^3J = 6.2, 3.2 Hz, 1H), 3.22 – 3.10 (m, 1H), 3.04 (dd, ^2J = 15.0 Hz, 8.2 Hz, 1H, C$^{\beta,Trp}$**H**), 2.14–2.05 (m, 1H, C$^{\beta,Pro}$**H**), 1.97–1.78 (m, 1H, C$^{\beta,Pro}$**H**), 1.77–1.71 (m, 1H, C$^{\gamma,Pro}$**H**), 1.57–1.46 (m, 1H, C$^{\gamma,Pro}$**H**), 1.42 – 1.29 (m, 1H, C$^{\gamma,Pro}$**H**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 172.9 (**C**ONH_2^{Pro}), 167.3 (**C**ONH_2^{Trp}), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 125.4 (indole-**C**^{2}), 121.3 (indole-**C^6**), 118.7(indole-**C^5**), 111.6 (indole-**C**^{7}), 106.6 (indole-**C**^{3}), 59.8 (**C**$^{\alpha,Pro}$), 52.6, 51.5, 46.9, 30.8 (**C**H^{al}), 29.3 (**C**H^{al}), 28.5 (**C**H^{al}), 27.2 (**C**H^{al}), 26.1 (**C**H^{al}), 24.6 (**C**H^{al}), 21.7 (**C**H^{al}).

H-L-Trp-γAba-OH (15), strategy C



Formula: $C^{1}_{5}H_{19}N_3O_3 \times CF_3CO_2H$

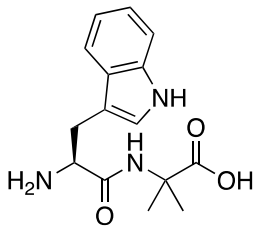
Yield: 128 mg, 0.32 mmol, 63.5%

HR-ESI-MS: m/z = $[M+H]^+$ 290.1514 (found); 290.1499 (calc.), Δ = 5.10 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (d, ^3J = 2.5 Hz, 1H, indole-NH), 8.47 (t, J = 5.7 Hz, 1H, amide-N**H**), 8.08 (d, J = 5.1 Hz, 3H, N$^{\alpha}$**H$_3^+$**), 7.63 (d, ^3J = 7.8 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 7.8 Hz, 1H, indole-C^7**H**), 7.18 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1, 7.0 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 8.0, 7.0 Hz, ^4J =1.1 Hz, 1H, indole-C^5**H**), 3.88 (dd, ^3J = 6.5, 6.7 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.19 (dd, ^2J = 14.6 Hz, ^3J = 6.2 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.13-3.04 (m, 3H, C$^{\beta,Trp}$**H^b**, C$^{\alpha,\gamma Aba}$**H$_2$**), 2.17 (t, ^3J = 7.7 Hz, 2H, C$^{\gamma,\gamma Aba}$**H$_2$**), 1.66-1.52 (m, 2H, C$^{\beta,\gamma Aba}$**H$_2$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 174.6 (**C**O$_2$H$^{\gamma Aba}$), 168.8 (**C**O$_2$H^{Trp}), 136.7 (indole-**C**^{7a}), 127.5 (indole-**C**^{3a}), 125.2 (indole-**C^2**), 121.6 (indole-**C^6**), 118.9 (indole-**C**^{5}), 118.8 (indole-**C^4**), 112.0 (indole-**C^7**), 107.4 (indole-**C**^{3}), 53.4 (C$^{\alpha,Trp}$), 38.6 (C$^{\alpha,\gamma Aba}$**H$_2$**), 31.3 (C$^{\gamma,\gamma Aba}$), 27.9 (C$^{\alpha,\gamma Aba}$).

H-Trp-Aib-OH (16), strategy C



Formula: $C^{1}{}_{5}H_{19}N_3O_3 \times CF_3CO_2H$

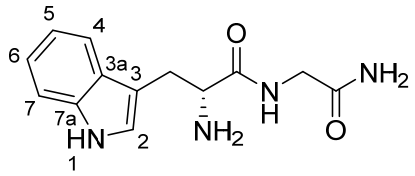
Yield: 171 mg, 0.42 mmol, 81.6%

HR-ESI-MS: m/z = $[M+H]^+$ 290.1506 (found); 290.1499 (calc.); Δ = 2.34 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.50 (s, 1H, CO_2**H**), 11.03 (d, ^{3}J = 2.5 Hz, 1H, indole-N**H**), 8.67 (s, 1H, amide-N**H**), 8.04 (s, 3H, **N**$^{\alpha}H_3^+$), 7.74 (d, ^{3}J = 7.9 Hz, 1H, indole-C^{4}**H**), 7.37 (d, ^{3}J = 8.1 Hz, 1H, indole-C^{7}**H**), 7.23 (d, ^{3}J = 2.4 Hz, 1H, indole-C^{2}**H**), 7.10 (ddd, ^{3}J = 8.2, 6.9 Hz, ^{4}J = 1.2 Hz, 1H, indole-C^{6}**H**), 7.03 (ddd, ^{3}J = 7.9, 6.9 Hz, ^{4}J = 1.1 Hz, 1H, indole-C^{5}**H**), 3.98 (dt, ^{3}J = 9.1, 5.1 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.24 (dd, ^{2}J = 14.8 Hz, ^{3}J = 5.4 Hz, 1H, C$^{\beta,Trp}$**H$_2^a$**), 3.04 (dd, ^{2}J = 14.8 Hz, ^{3}J = 8.6 Hz, 1H, C$^{\beta,Trp}$**H$_2^b$**), 1.40 (s, 3H C$^{\beta,Aib}$**H$_3^a$**), 1.35 (s, 3H, C$^{\beta,Aib}$**H$_3^b$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 175.5 (**C**ONH), 168.2 (**C**O_2H), 136.7 (indole-**C**^{7a}), 127.5 (indole-**C**^{3a}), 125.5 (indole-**C**^{2}), 121.6 (indole-**C**^{6}), 119.0 (indole-**C**^{5}), 118.8 (indole-**C**^{4}), 111.9 (indole-**C**^{7}), 107.3 (indole-**C**^{3}), 55.9 (**C**$^{\alpha Aib}$), 52.9 (**C**$^{\alpha,Trp}$), 27.8 (**C**$^{\beta,Trp}$), 25.2 (**C**$^{\beta,Aib}$), 25.2 (**C**$^{\beta,Aib}$).

H-D-Trp-Gly-NH_2 (17)



Formula: $C_{13}H_{16}N_4O_2 \times CF_3CO_2H$

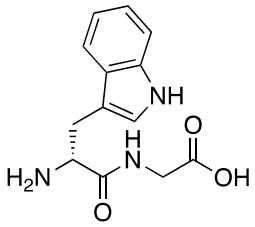
Yield: 13.3 mg, 35 µmol, 7.1%

ESI-MS: m/z = $[M+H]^+$ 261.1349 (found); 261.1346 (calc.); Δ = 1.15 ppm

^{1}H NMR (500 MHz, DMSO-d_6) δ [ppm] = 11.03 (d, *J*=2.5 Hz, 1H, indole-N**H**), 8.83 (t, *J*=5.6 Hz, 1H, amide-N$\mathbf{H}^{Gly}$), 8.06 (s, 3H, $N^{\alpha}\mathbf{H_3^+}$), 7.68 (d, *J*=7.8 Hz, 1H, indole-C^4**H**), 7.38 (m, 1H, CON$\mathbf{H_2}^a$), 7.37 (d, *J*=8.0 Hz, 1H, indole-C^7**H**), 7.23 (d, *J*=2.3, 1H, indole-C^2**H**), 7.15 (s, 1H, CON$\mathbf{H_2}^b$), 7.09 (dd, *J*=8.3 Hz, 0.9, 1H, indole-C^6**H**), 7.01 (dd, *J*=9.1, 0.5 Hz, 1H, indole-C^5**H**), 4.10 – 4.05 (m, 1H, $C^{\alpha,Trp}$**H**), 3.80 (dd, *J*=16.9, 5.9, 1H, $C^{\alpha,Gly}\mathbf{H_2}^a$), 3.66 (dd, *J*=16.8, 5.3, 1H, $C^{\alpha,Gly}\mathbf{H_2}^b$), 3.25 (dd, *J*=14.8, 5.4, 1H, $C^{\beta,Trp}\mathbf{H}^a$), 3.09 (dd, *J*=14.8, 8.3, 1H, $C^{\beta,Trp}\mathbf{H}^b$).

^{13}C NMR (126 MHz, DMSO) δ = 170.58 (CON$\mathbf{H_2}^{Gly}$), 169.23 (CON$\mathbf{H}^{Trp}$), 136.76 (indole-$\mathbf{C}^{7a}$), 127.49 (indole-$\mathbf{C}^{3a}$), 125.46 (indole-$\mathbf{C}^2$), 121.60 (indole-$\mathbf{C}^6$), 118.94 (indole-$\mathbf{C}^5$), 118.88 (indole-$\mathbf{C}^4$), 111.94 (indole-$\mathbf{C}^7$), 107.29 (indole-$\mathbf{C}^3$), 53.12 ($\mathbf{C}^{\alpha,Trp}$), 42.40 ($\mathbf{C}^{\alpha,Gly}$), 27.82 ($\mathbf{C}^{\beta,Trp}$).

H-D-Trp-Gly-OH (18), strategy C



Formula: $C_{14}H_{17}N_3O_3 \times CF_3CO_2H$

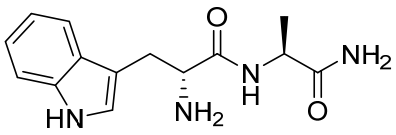
Yield: 116 mg, 0.31 mmol, 62.0%

HR-ESI-MS: m/z = $[M+H]^+$ 262.1189 (found); 262.1186 (calc.), Δ = 1.07 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.04 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.95 (t, ^3J = 5.8 Hz, 1H, CON**H**), 7.70 (d, ^3J = 7.9 Hz, 1H, C4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, C7**H**), 7.24 (d, ^3J = 2.4 Hz, 1H, C2**H**), 7.10 (ddd, ^3J = 8.0, 6.9, ^4J = 1.2 Hz, 1H, C6**H**), 7.01 (dd, ^3J = 7.9, 7.0 Hz, ^4J = 1.0 Hz, 1H, C5**H**), 4.05 (dd, ^3J = 8.5, 5.1 Hz, 1H, $C^{\alpha,Trp}$**H**), 3.87 (d, ^3J = 5.6 Hz, 2H, C^{Gly}**H$_2$**), 3.26 (dd, ^2J = 14.9 Hz, ^3J = 5.0 Hz, 1H, $C^{\beta,Trp}$**H^a**), 3.08 (dd, ^2J = 14.8 Hz, ^3J = 8.5 Hz, 1H, $C^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 170.7 (**C**O_2H^{Gly}), 169.0 (**C**O_2H^{Trp}), 136.3 (**C**7a), 127.1 (**C**3a), 125.1 (**C**2H), 121.1 (**C**6H), 118.5 (**C**5H), 118.4 (**C**4H), 111.5 (**C**7H), 106.7 (C**3**), 52.6 ($\mathbf{C}^{\alpha,Trp}$H), 40.8 ($\mathbf{C}^{\alpha,Gly}$H), 27.5 ($\mathbf{C}^{\beta,Trp}H_2$).

H-D-Trp-Ala-NH$_2$ (19), strategy C



Formula: $C_{14}H_{18}N_4O_2 \times CF_3CO_2H$

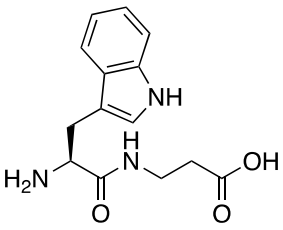
Yield: 38.4 mg, 0.14 mmol, 27.7% .

HR-ESI-MS: m/z = $[M+H]^+$ 275.1505 (found); 275.1503 (calc.), Δ = 0.91 ppm

^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ = 11.03 (d, *J*=2.4, 1H, indole-N**H**), 8.61 (d, *J*=7.7, 1H, amide-N**H**^{Ala}), 8.10 (s, 2H, N$^{\alpha,Trp}$**H**), 7.66 (d, *J*=7.8, 1H, indole-C^4**H**), 7.39 – 7.34 (m, 1H, indole-C^7**H**, CON**H$_2$**^{a}), 7.20 (d, *J*=2.4, 1H, indole-C^2**H**), 7.12 – 7.06 (m, 1H, indole-C^6**H**, CON**H$_2$**^{b}), 7.04 – 6.97 (m, 1H, indole-C^5**H**), 4.20 (m, *J*=7.2, 1H, C$^{\alpha,Ala}$**H**), 4.04 (s, 1H, C$^{\alpha,Trp}$**H**), 3.19 (dd, *J*=14.5, 6.6, 1H, C$^{\beta,Trp}$**H**^{a}), 3.09 (dd, *J*=14.5, 7.7, 1H, C$^{\beta,Trp}$**Hb**), 1.03 (d, *J*=7.1, 3H, C$^{\beta,Ala}$**H$_3$**).

^{13}C NMR (126 MHz, DMSO) δ = 173.60 (**C**ONH^{Ala}), 168.01 (**C**ONH^{Trp}), 136.22 (indole-**C**^{7a}), 127.10 (indole-**C**^{3a}), 124.90 (indole-**C**^{2}), 121.13 (indole-**C**^{6}), 118.49 (indole-**C**^{5}), 118.36 (indole-**C**^{4}), 111.44 (indole-**C**^{7}), 106.93 (indole-**C**^{3}), 52.78 (**C**$^{\alpha,Trp}$), 48.14 (**C**$^{\alpha,Ala}$), 27.31 (**C**$^{\beta,Trp}$), 18.10 (**C**$^{\beta,Ala}$).

H-Trp-βAla-OH (20a), strategy C



Formula: $C^{1}{}_{4}H_{17}N_3O_3 \times CF_3CO_2H$

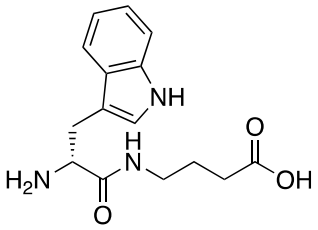
Yield: 132 mg, 0.34 mmol, 68.1%

HR-ESI-MS: m/z = $[M+H]^+$ 276.1352 (found); 276.1343 (calc.), Δ = 3.37 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (s, 1H, CO_2**H**), 8.59 (t, ^3J = 5.6 Hz, 1H, indole-NH), 8.08 (s, 3H, N^{α}**H$_3^+$**), 7.64 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.19 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.09 (ddd, ^3J = 8.1, 6.9 Hz, ^4J = 1.2 Hz, 1H, indole-C^6**H**), 7.01 (ddd, ^3J = 7.9, 6.9 Hz, ^4J = 1.1 Hz, 1H, indole-C^5**H**), 3.92 (t, ^3J = 6.8 Hz, 1H, $C^{\alpha,Trp}$**H**), 3.34–3.22 (m, 2H, $C^{\alpha,\beta Ala}$**H$_2$**), 3.17 (dd, ^2J = 14.7 Hz, ^3J = 5.9 Hz, 1H, $C^{\beta,Trp}$**H^a**), 3.07 (dd, ^2J = 14.7 Hz, ^3J = 8.0 Hz, 1H, $C^{\beta,Trp}$**H^b**), 2.34 (t, J = 6.8 Hz, 2H, $C^{\beta,\beta Ala}$**H$_2$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 172.7 (**C**O_2H), 168.5 (**C**ONH), 136.3 (indole-**C**^{7a}), 127.0 (indole-**C**^{3a}), 124.9 (indole-**C^2**), 121.2 (indole-**C^6**), 118.4 (indole-**C^4**), 118.4 (indole-**C^5**), 111.5, (indole-**C^7**), 106.9 (indole-**C^3**), 52.7 ($C^{\alpha,Trp}$), 34.9 (**C**$^{\beta,\beta Ala}$), 33.4 (**C**$^{\alpha,\beta Ala}$), 27.4 (**C**$^{\beta,Trp}$).

H-D-Trp-γAba-OH (21), strategy C



Formula: $C_{15}H_{19}N_3O_3 \times CF_3CO_2H$

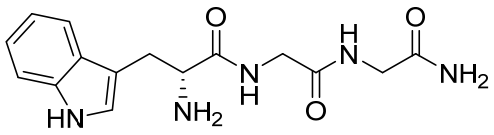
Yield: 125 mg, 0.31 mmol, 62.0%

HR-ESI-MS: m/z = $[M+H]^+$ 290.1506 (found); 290.1499 (calc.), Δ = 2.34 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.11 (s, 1H, CO_2**H**), 11.02 (s, 1H, indole-N**H**), 8.47 (t, ^{3}J = 5.6 Hz, 1H, amide-N**H**), 8.10 (s, 3H, N^{α}**H$_3^+$**), 7.63 (dd, ^{3}J = 7.9 Hz, ^{2}J = 1.1 Hz, 1H, C4**H**), 7.36 (dd, ^{3}J = 8.1 Hz, ^{4}J = 0.9 Hz, 1H, C7**H**), 7.18 (d, ^{3}J = 2.4 Hz, 1H, C2**H**), 7.09 (ddd, ^{3}J = 8.2, 7.0 Hz, ^{4}J = 1.2 Hz, 1H, C6**H**), 7.01 (ddd, ^{3}J = 8.0, 7.0 Hz, ^{4}J = 1.1 Hz, 1H, C5**H**), 3.89 (q, ^{3}J = 5.8, 5.2 Hz, 1H, Cα**H**), 3.19 (dd, ^{2}J = 14.6 Hz, ^{3}J = 6.2 Hz, 1H, $C^{\beta,Trp}$**H^a**), 3.15-3.0 (m, $C^{\beta,Trp}$**H^b**, $C^{\alpha,\gamma Aba}$**H$_2$**), 2.17 (t, ^{3}J = 7.0 Hz, 2H, $C^{\gamma,\gamma Aba}$**H$_2$**), 1.74 (s, 1H), 1.65 – 1.52 (m, 2H, $C^{\alpha,\gamma Aba}$**H$_2$**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 174.6 (**C**$O_2H^{\gamma Aba}$), 168.8 (**C**O_2H^{Trp}), 136.7 (**C**7a), 127.5 (**C**3a), 125.2 (**C**2H), 121.6 (**C**6H), 118.9 (**C**5H), 118.8 (**C**4H), 112.0 (**C**7H), 107.4 (**C**3), 53.4 ($C^{\alpha,Trp}$**H**), 38.6 ($C^{\alpha,\gamma Aba}$**H$_2$**), 31.3 ($C^{\gamma,\gamma Aba}$**H$_2$**), 27.9 ($C^{\alpha,\gamma Aba}$**H$_2$**).

H-D-Trp-Gly-Gly-NH_2 (22), strategy D



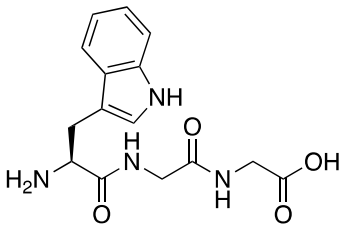
Formula: $C_{15}H_{19}N_5O_3 \times CF_3CO_2H$

Yield: 42 mg, 0.13 mmol, 49.6%

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.90 (t, ^3J = 5.6 Hz, 1H, amide-N**H**^{Gly2}), 8.17 (t, J = 5.9 Hz, 1H, amide-N**H**^{Gly3}), 8.07 (s, 3H, N$^{\alpha}$**H$_3^+$**), 7.70 (d, ^3J = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, indole-C^7**H**), 7.32 (s, 1H, CON**H^a**), 7.24 (d, ^3J = 2.4 Hz, 1H, indole-C^2**H**), 7.12–7.08 (m, 2H, CON**H^b**, indole-C^6**H**), 7.02 (ddd, ^3J = 7.5, 6.9 Hz, ^4J = 1.1 Hz, 1H, indole-C^5**H**), 4.07 (m, 1H, C$^{\alpha,Trp}$**H**), 3.90 (dd, ^2J = 16.6 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly2}$**H^a**), 3.81 (dd, ^2J = 16.7 Hz, ^3J = 5.4 Hz, 1H, C$^{\alpha,Gly2}$**H^b**), 3.70 (dd, ^2J = 16.8 Hz, ^3J = 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H^a**), 3.65 (dd, ^2J = 16.8 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly3}$**H^b**), 3.27 (dd, ^2J = 14.8 Hz, ^3J = 5.3 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.09 (dd, ^2J = 14.8 Hz, ^3J = 8.3 Hz, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 170.73 (**C**ONH$_2^{Gly3}$), 169.4 (**C**ONH^{Trp}), 168.9 (**C**ONH^{Gly2}), 136.8 (indole-**C**^{7a}), 127.5 (indole-**C**^{3a}), 125.5 (indole-**C**^{2}), 121.6 (indole-**C**^{6}), 118.9 (indole-**C**^{5}), 118.9 (indole-**C**^{4}), 111.9 (indole-**C7**), 107.3 (indole-**C**^{3}), 53.1 (**C**$^{\alpha,Trp}$), 42.13 (**C**$^{\alpha,Gly3}$), 41.78 (**C**^{Gly2}), 27.4 (**C**$^{\beta,Trp}$).

H-Trp-Gly-Gly-OH (23a), strategy D



Formula: $C_{15}H_{18}N_4O_4 \times CF_3CO_2H$

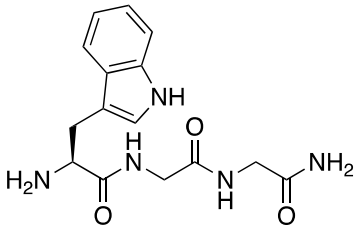
Yield: 52.2 mg, 0.121 mmol, 32.2%

HR-ESI-MS: m/z = $[M+H]^+$ 319.1414 (found); 319.1401 (calc.), Δ = 4.07 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 12.66 (s, 1H, CO_2**H**), 11.02 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.92 (t, ^3J = 5.8 Hz, 1H, amide-N**H**^{Gly2}), 8.29 (t, ^3J = 5.9 Hz, 1H, amide-N**H**^{Gly3}), 8.06 (s, 3H, N$^{\alpha}$**H$_3^+$**), 7.69 (d, ^3J = 7.9 Hz, 1H, C4**H**), 7.36 (d, ^3J = 8.4 Hz, 1H), 7.23 (d, ^3J = 2.4 Hz, 1H, C2**H**), 7.10 (ddd, ^3J = 8.1, 7.0 Hz, ^4J = 1.2 Hz, 1H, C6**H**), 7.02 (ddd, ^3J = 7.9, 7.0 Hz, ^4J = 1.0 Hz, 1H, C5**H**), 4.07 (t, ^3J = 7.5, 6.7 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.90 (dd, ^2J = 16.7, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly2}$**H^a**), 3.85–3.74 (m, 3H, C$^{\alpha,Gly2}$**H^b**, C$^{\alpha,Gly3}$**H$_2$**), 3.26 (dd, ^2J = 14.8 Hz, ^3J = 5.2 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.09 (dd, ^2J = 14.8 Hz, ^3J = 8.4 Hz, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 171.1 (**C**ONH^{Gly3}), 168.9 (**C**O_2H), 168.5 (**C**ONH ^{Gly2}), 136.3 (**C**7a), 127.0 (**C**3a), 125.0 (**C**2H), 121.2 (**C**6H), 118.5 (**C**5H), 118.4 (**C**4H), 111.5 (**C**7H), 106.8 (**C**3), 52.6 (**C**$^{\alpha}$H), 41.8 (**C**$^{Gly3}H_2$), 40.6 (**C**$^{Gly2}H_2$), 27.4 (**C**$^{\beta}H_2$).

H-Trp-Gly-Gly-NH_2 (23), strategy D



Formula: $C_{15}H_{19}N_5O_3 \times CF_3CO_2H$

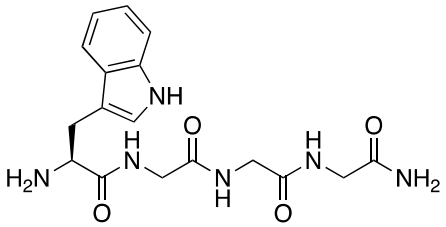
Yield: 44.8 mg, 0.10 mmol, 27.7%

HR-ESI-MS: m/z = $[M+H]^+$ 318.1575 (found); 318.1561 (calc.), Δ = 4.49 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.03 (d, ^3J = 2.5 Hz, 1H, indole-N**H**), 8.90 (t, ^3J = 5.6 Hz, 1H, amide-N**H**^{Gly2}), 8.17 (t, J = 5.9 Hz, 1H, amide-N**H**^{Gly3}), 8.07 (s, 3H, N$^{\alpha}$**H$_3^+$**), 7.69 (d, ^3J = 7.9 Hz, 1H, C4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, C7**H**), 7.31 (s, 1H, CON**H^a**), 7.24 (d, ^3J = 2.4 Hz, 1H, C2**H**), 7.12–7.08 (m, 2H, CON**H^b**, C6**H**), 7.02 (ddd, ^3J = 7.5, 6.9 Hz, ^4J = 1.1 Hz, 1H, C5**H**), 4.07 (t, ^3J = 6.8 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.89 (dd, ^2J = 16.6 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly2}$**H^a**), 3.80 (dd, ^2J = 16.7 Hz, ^3J = 5.4 Hz, 1H, C$^{\alpha,Gly2}$**H^b**), 3.70 (dd, ^2J = 16.8 Hz, ^3J = 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H^a**), 3.64 (dd, ^2J = 16.8 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly3}$**H^b**), 3.26 (dd, ^2J = 14.8 Hz, ^3J = 5.3 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.09 (dd, ^2J = 14.8 Hz, ^3J = 8.3 Hz, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 171.2 (**C**ONH^{Gly3}), 169.4 (**C**ONH$_2$), 168.9 (**C**ONH^{Gly2}), 136.8 (**C**7a), 127.5 (**C**3a), 125.5 (**C**2H), 121.6 (**C**6H), 118.9 (**C**5H), 118.9 (**C**4H), 111.9 (**C**7H), 107.3 (**C**3), 53.1 (**C**$^{\alpha}$H), 42.6 (**C**^{Gly3}H$_2$), 42.2 (**C**^{Gly2}H$_2$), 27.9 (**C**$^{\beta}$H$_2$).

H-Trp-Gly-Gly-Gly-NH_2 (24), strategy D



Formula: $C_{17}H_{22}N_6O_4 \times CF_3CO_2H$

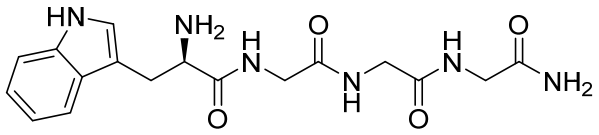
Yield: 135.6 mg, 0.278 mmol, 74.0%

HR-ESI-MS: m/z = $[M+H]^+$ 375.1782 (found); 375.1775 (calc.), Δ 1.87 ppm

^{1}H-NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 11.02 (d, ^3J = 2.5 Hz, 1H, amide-N**H**^{Gly2}), 8.92 (t, ^3J = 5.6 Hz, 1H, amide-N**H** ^{Gly3}), 8.26 (t, ^3J = 5.8 Hz, 1H, amide-N**H**^{Gly4}), 8.11 (t, ^3J = 5.8 Hz, 1H, amide-N**H**), 8.06 (d, ^3J = 5.2 Hz, 3H, N$^{\alpha}$**H$_3^+$**), 7.69 (d, ^3J = 7.9 Hz, 1H, C4**H**), 7.37 (d, ^3J = 8.1 Hz, 1H, C7**H**), 7.26 (d, ^3J = 2.4 Hz, 1H, CON**H^a**), 7.23 (d, ^3J = 2.4 Hz, 1H, C2**H**), 7.12–7.06 (m, 2H, CON**H^b**, C6**H**), 7.02 (ddd, ^3J = 8.0, 6.9 Hz, ^2J = 1.0 Hz, 1H, C5**H**), 4.07 (td, ^3J = 8.2, 4.8 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.90 (dd, ^2J = 16.6 Hz, ^3J = 5.8 Hz, 1H, C$^{\alpha,Gly2}$**H^a**), 3.82 (dd, ^2J = 12.1 Hz, ^3J = 4.5 Hz, 1H, C$^{\alpha,Gly2}$**H^b**), 3.78 (dd, ^2J = 13.2 Hz, ^2J = 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H^a**), 3.75 (dd, J = 16.5, 5.8 Hz, 1H, C$^{\alpha,Gly4}$**H^b**), 3.62 (dd, ^3J = 5.9 Hz, ^2J = 13.8 Hz, 2H, C$^{\alpha,Gly4}$**H$_2$**), 3.26 (dd, ^2J = 14.7 Hz, ^3J = 5.3 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.09 (dd, J = 14.8, 8.4 Hz, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 170.9 (**C**ONH^{Trp}), 168.9 (**C**ONH$_2^{Gly4}$), 168.9 (**C**ONH^{Gly3}), 168.6 (**C**ONH^{Gly2}), 136.3 (**C**7a), 127.0 (**C**3a), 125.0 (**C**2H), 121.2 (**C**6H), 118.5 (**C**5H), 118.4 (**C**4H), 111.5 (**C**7H), 106.8 (**C**3), 52.6 (**C**$^{\alpha,Trp}$H), 42.1 (**C**$^{\alpha,Gly}$H), 42.0 (**C**$^{\alpha,Gly}$H), 41.8 (**C**$^{\alpha,Gly}$H), 27.4 (**C**$^{\beta,Trp}$H$_2$).

H-D-Trp-Gly-Gly-Gly-NH_2 (25)



Formula: $C_{17}H_{21}BrN_6O_4 \times CF_3CO_2H$

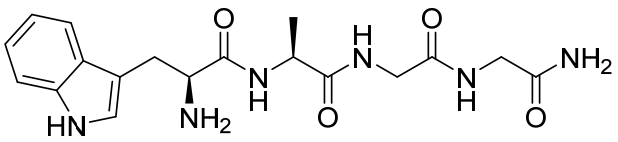
Yield: 65 mg, 0.13 mmol, 50%

ESI-MS: m/z = $[M+H]^+$ 375.1765 (found); 375.1775 (calc.); Δ = 2.69 ppm

^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.03 (d, *J* = 2.5 Hz, 1H, indole-N**H**), 8.93 (t, *J* = 5.6 Hz, 1H, amide-N**H**^{Gly2}), 8.26 (t, *J* = 5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.12 (t, *J* = 5.9 Hz, 1H, amide-N**H**^{Gly4}), 8.07 (d, *J* = 4.5 Hz, 2H, -N$^{\alpha,Trp}$**H**), 7.70 (d, *J* = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, *J* = 8.1 Hz, 1H, indole-C^7**H**), 7.27 (s, 1H, CON**H**^{a}), 7.24 (d, *J* = 2.4 Hz, 1H, indole-C^2**H**), 7.14 – 7.07 (m, 1H, indole-C^6**H**, CON**H**^{b}), 7.03 (m, 1H, indole-C^5**H**), 4.07 (m, 1H, C$^{\alpha,Trp}$**H**), 3.91 (dd, *J* = 16.6, 5.8 Hz, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.86 – 3.78 (m, 2H, C$^{\alpha,Gly2}$**H**^{a}, C$^{\alpha,Gly3}$**H**^{b}), 3.75 (dd, *J* = 16.6, 5.7 Hz, 1H, C$^{\alpha,Gly2}$**H**^{b}), 3.65 (d, *J* = 5.9 Hz, 1H, C$^{\alpha,Gly4}$**H**$_2$), 3.26 (dd, *J* = 14.7, 5.3 Hz, 1H, C$^{\beta,Trp}$**H**^{a}), 3.09 (dd, *J* = 14.8, 8.4 Hz, 1H, C$^{\beta,Trp}$**H**^{b}).

^{13}C NMR (126 MHz, DMSO) δ = 170.88 (**C**ONH_2^{Gly4}), 168.96 (**C**ONH^{Gly2}), 168.91 (**C**ONH^{Trp}), 168.68 (**C**ONH^{Gly3}), 136.32 (indole-**C**^{7a}), 127.05 (indole-**C**^{3a}), 125.05 (indole-**C**^{2}), 121.17 (indole-**C**^{6}), 118.49 (indole-**C**^{5}), 118.46 (indole-**C**^{4}), 111.50 (indole-**C**^{7}), 106.82 (indole-**C**^{3}), 52.67 (**C**$^{\alpha,Trp}$), 42.13 (**C**$^{\alpha,Gly2}$), 42.06 (**C**$^{\alpha,Gly3}$), 41.80 (**C**$^{\alpha,Gly4}$), 27.40 (**C**$^{\beta,Trp}$).

H-L-Trp-Ala-Gly-Gly-NH_2 (26)



Formula: $C_{18}H_{24}N_6O_4 \times CF_3CO_2H$

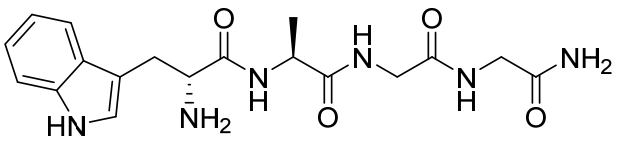
Yield: 60 mg, 0.12 mmol, 50%

ESI-MS: m/z = $[M+H]^+$ 389.1939 (found); 389.1932 (calc.), Δ = 1.75 ppm

^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ = 11.00 (d, *J*=2.5 Hz, 1H, indole-N**H**), 8.85 (d, *J*=7.5 Hz, 1H, amide-N**H**^{Ala}), 8.22 (t, *J*=5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.05 (t, *J*=5.8 Hz, 1H, amide-N**H**^{Gly4}), 8.03 (d, *J*=4.8 Hz, 3H, N$^{\alpha,Trp}$**H$_3^+$**), 7.70 (d, *J*=7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, *J*=8.1 Hz, 1H, indole-C^7**H**), 7.29 (s, 1H, CON**H$_2$**^{a}), 7.21 (d, *J*=2.3 Hz, 1H, indole-C^2**H**), 7.13 – 7.06 (m, 1H, indole-C^6**H**, CO**NH$_2$**^{b}), 7.02 (ddd, *J*=8.0, 6.9, 1.0 Hz, 1H, indole-C^5**H**), 4.42 (dq, *J*=14.4, 7.2 Hz, 1H, C$^{\alpha,Ala}$**H**), 4.03 (m, 1H, C$^{\alpha,Trp}$**H**), 3.78 (dd, *J*=16.6, 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.75 (dd, *J*=16.5, 5.6 Hz, 1H, C$^{\alpha,Gly3}$**H**^{b}), 3.68 (d, *J*=16.8, 5.8 Hz, 1H, C$^{\alpha,Gly4}$**H$_2$**), 3.65 (dd, *J* = 16.8, 5.8 Hz, 1H), 3.25 (dd, *J*=14.9, 5.3 Hz, 1H, C$^{\beta,Trp}$**H**^{a}), 3.04 (dd, *J*=14.8, 8.6 Hz, 1H, C$^{\beta,Trp}$**H**^{b}), 1.07 (d, *J*=7.0 Hz, 3H, C$^{\beta,Ala}$**H$_3$**).

^{13}C NMR (126 MHz, DMSO) δ = 172.48 (**C**ONH^{Ala}), 170.32 (**C**ONH$_2^{Gly4}$), 169.29 (**C**ONH^{Trp}), 168.71 (**C**ONH^{Gly3}), 136.78 (indole-**C**^{7a}), 127.48 (indole-**C**^{3a}), 124.53 (indole-**C**^{2}), 121.61 (indole-**C**^{6}), 118.97 (indole-**C**^{5}), 118.89 (indole-**C**^{4}), 111.94 (indole-**C**^{7}), 107.27 (indole-**C**^{3}), 52.98 (**C**$^{\alpha,Trp}$), 48.95 (**C**$^{\alpha,Ala}$), 42.51 (**C**$^{\alpha,Gly3}$), 42.26 (**C**$^{\alpha,Gly4}$), 27.85 (**C**$^{\beta,Trp}$), 18.78 (**C**$^{\beta,Ala}$).

H-D-Trp-Ala-Gly-Gly-NH_2 (27)



Formula: $C_{18}H_{24}N_6O_4 \times CF_3CO_2H$

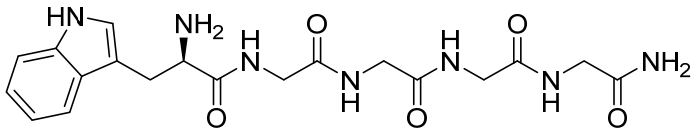
Yield: 77 mg, 0.15 mmol, 64%

ESI-MS: m/z = $[M+H]^+$ 389.1928 (found); 389.1932 (calc.); Δ = 1.75 ppm

^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ = 11.02 (d, *J*=2.5, 1H, indole-N**H**), 8.74 (d, *J*=7.5, 1H, amide-N**H**^{Ala}), 8.29 (t, *J*=5.8, 1H, amide-N**H**^{Gly3}), 8.08 (d, *J*=4.8, 3H, N$^{\alpha,Trp}$**H$_3^+$**), 8.00 (t, *J*=5.8, 1H, amide-N**H**^{Gly4}), 7.67 (d, *J*=7.9, 1H, indole-C^4**H**), 7.37 (d, *J*=8.1, 1H, indole-C^7**H**), 7.29 (s, 1H, CON**H$_2$**^{a}), 7.21 (d, *J*=2.3, 1H, indole-C^2**H**), 7.13 – 7.06 (m, 1H, indole-C^6**H**, CO**NH$_2$**^{b}), 7.02 (t, *J*=7.4, 1H, indole-C^5**H**), 4.32 (dq, *J*=14.4, 7.2 Hz, 1H, C$^{\alpha,Ala}$**H**), 4.04 (m, 1H, C$^{\alpha,Trp}$**H**), 3.77 (dd, *J*=16.6, 5.9, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.70 (dd, *J*=16.5, 5.6, 1H, C$^{\alpha,Gly3}$**H**^{b}), 3.64 (d, *J*=5.8, 1H, C$^{\alpha,Gly4}$**H$_2$**), 3.20 (dd, *J*=14.5, 6.3, 1H, C$^{\beta,Trp}$**H**^{a}), 3.09 (dd, *J*=14.5, 7.9, 1H, C$^{\beta,Trp}$**H**^{b}), 1.07 (d, *J*=7.0, 3H, C$^{\beta,Ala}$**H$_3$**).

^{13}C NMR (126 MHz, DMSO) δ = 172.06 (**C**ONH^{Ala}), 170.80 (**C**ONH$_2^{Gly4}$), 168.79 (**C**ONH^{Trp}), 168.20 (**C**ONH^{Gly3}), 136.24 (indole-**C**^{7a}), 127.08 (indole-**C**^{3a}), 124.96 (indole-**C**^{2}), 121.15 (indole-**C**^{6}), 118.49 (indole-**C**^{5}), 118.38 (indole-**C**^{4}), 111.46 (indole-**C**^{7}), 106.85 (indole-**C**^{3}), 52.70 (**C**$^{\alpha,Trp}$), 48.42 (**C**$^{\alpha,Ala}$), 41.91 (**C**$^{\alpha,Gly3}$), 41.74 (**C**$^{\alpha,Gly4}$), 27.36 (**C**$^{\beta,Trp}$), 18.10 (**C**$^{\beta,Ala}$).

H-D-Trp-Gly-Gly-Gly-Gly-NH_2 (28)



Formula: $C_{17}H_{21}BrN_6O_4 \times CF_3CO_2H$

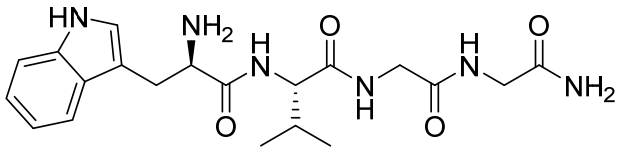
Yield: 91 mg, 0.17 mmol, 70%

ESI-MS: m/z = $[M+H]^+$ 375.1765 (found); 375.1775 (calc.); Δ = 2.69 ppm

^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ = 11.02 (d, *J* = 2.5 Hz, 1H, indole-N**H**), 8.90 (t, *J* = 5.6 Hz, 1H, amide-N**H**^{Gly2}), 8.26 (t, *J* = 5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.20 (t, *J* = 5.9 Hz, 1H, amide-N**H**^{Gly4}), 8.09 (t, *J* = 5.9 Hz, 1H, amide-N**H**^{Gly5}), 8.06 (s, 3H, -N$^{\alpha,Trp}$**H**), 7.69 (d, *J* = 7.9 Hz, 1H, indole-C^4**H**), 7.37 (d, *J* = 8.1 Hz, 1H, indole-C^7**H**), 7.26 – 7.21 (m, 2H, indole-C^2**H**, CON**H**^{a}), 7.10 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, indole-C^6**H**), 7.07 (s, 1H, CON**H**^{b}), 7.03 (dd, 1H, indole-C^5**H**), 4.07 (m, 1H, C$^{\alpha,Trp}$**H**), 3.90 (dd, *J* = 16.6, 5.8 Hz, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.86 – 3.78 (m, 2H, C$^{\alpha,Gly2}$**H**^{a}, C$^{\alpha,Gly3}$**H**^{b}), 3.75 (dd, *J* = 16.6, 5.7 Hz, 1H, C$^{\alpha,Gly2}$**H**^{b}), 3.63 (d, *J* = 5.9 Hz, 2H, C$^{\alpha,Gly5}$**H**$_2$), 3.26 (dd, *J* = 14.7, 5.3 Hz, 1H, C$^{\beta,Trp}$**H**^{a}), 3.09 (dd, *J* = 14.8, 8.4 Hz, 1H, C$^{\beta,Trp}$**H**^{b}).

^{13}C NMR (126 MHz, DMSO) δ = 170.85 (**C**ONH^{Gly4}), 168.18 (**C**ONH^{Trp}), 168.97 (**C**ONH^{Gly3}), 168.92 (**C**ONH$_2$^{Gly5}), 168.60 (**C**ONH^{Gly2}), 136.31 (indole-**C**^{7a}), 127.02 (indole-**C**^{3a}), 125.03 (indole-**C**^{2}), 121.16 (indole-**C**^{6}), 118.47 (indole-**C**^{5}), 118.44 (indole-**C**^{4}), 111.49 (indole-**C**^{7}), 106.79 (indole-**C**^{3}), 52.65 (**C**$^{\alpha,Trp}$), 42.08 (**C**$^{\alpha,Gly3}$), 41.99 (**C**$^{\alpha,Gly2}$), 41.80 (**C**$^{\alpha,Gly4}$), 70.06 (**C**$^{\alpha,Gly5}$), 27.40 (**C**$^{\beta,Trp}$).

H-D-Trp-Val-Gly-Gly-NH_2 (29)



Formula: $C_{20}H_{27}BrN_6O_4 \times CF_3CO_2H$

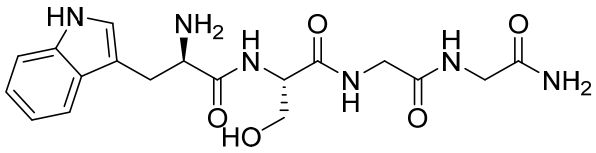
Yield: 74 mg, 0.14 mmol, 58%

ESI-MS: m/z = $[M+H]^+$ 417.2247 (found); 417.2245 (calc.); Δ = 0.62 ppm

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ = 11.01 (d, *J*=2.5 Hz, 1H, indole-N**H**), 8.74 (d, *J*=8.7 Hz, 1H, amide-N**H**^{Val}), 8.39 (t, *J*=5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.07-8.01 (m, *J*=5.8 Hz, 4H, Trp-C$^{\alpha}$N**H$_3$**$^+$, amide-N**H**^{Gly4}), 7.76 (d, *J*=8.4 Hz, 1H, indole-C^4**H**), 7.36 (d, *J*=1.7 Hz, 1H, indole-C^7**H**), 7.30 (s, 1H, CON**H**^{a}), 7.23 (d, *J*=2.4 Hz, 1H, indole-C^2**H),** 7.10 (ddd, *J* = 14.5, 6.3, 0.9 Hz, 1H, indole-C^6**H**), 7.08 (s, 1H, CON**H**^{b}), 7.02 (ddd, *J* = 13.9, 7.4, 0.8 Hz, 1H, indole-C^5**H**), 4.26 (dd, *J*=8.6, 6.5 Hz, 1H, C$^{\alpha,Val}$**H**), 4.17 (m, 1H, C$^{\alpha,Trp}$**H**), 3.80 (dd, *J*=16.4, 6.1 Hz, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.73 (dd, *J*=16.4, 5.5 Hz, 1H, , C$^{\alpha,Gly3}$**H**^{a}), 3.65 (d, *J*=5.8 Hz, 2H, C$^{\alpha,Gly4}$**H**$_2$), 3.22 (dd, *J*=14.5, 5.9 Hz, 1H, , C$^{\beta,Trp}$**H**^{a}), 3.01 (dd, *J*=14.5, 9.1 Hz, 1H, C$^{\beta,Trp}$**H**^{b}), 1.90 (m, 1H, C$^{\beta,Val}$**H**), 0.76 (d, *J*=2.2 Hz, 3H, C$^{\gamma,Val}$**H$_3$**), 0.75 (d, *J*=2.2 Hz, 3H, C$^{\gamma',Val}$**H$_3$**).

^{13}C-NMR (151 MHz, DMSO-d$_6$) δ = 170.81 (**C**ONH$_2^{Gly4}$), 170.75 (**C**ONH^{Val}), 168.80 (**C**ONH^{Gly3}), 168.74 (**C**ONH^{Trp}), 136.34 (indole-**C**^{7a}), 126.99 (indole-**C**^{3a}), 124.99 (indole-**C^2**), 121.16 (indole-**C^5**), 118.67 (indole-**C^7**), 118.33 (indole-**C^6**), 106.88 (indole-**C**^{3}), 57.84 (**C**$^{\alpha,Val}$), 52.59 (**C**$^{\alpha,Trp}$), 41.80 (**C**$^{\alpha,Gly3}$), 41.77 (**C**$^{\alpha,Gly4}$), 30.69 (**C**$^{\beta,Val}$), 27.88 (**C**$^{\beta,Trp}$), 18.95 (**C**$^{\gamma,Val}$), 17.91 **(C**$^{\gamma',Val}$)

H-D-Trp-Ser-Gly-Gly-NH_2 (30)



Formula: $C_{18}H_{23}BrN_6O_5 \times CF_3CO_2H$

Yield: 99 mg, 0.19 mmol, 79%

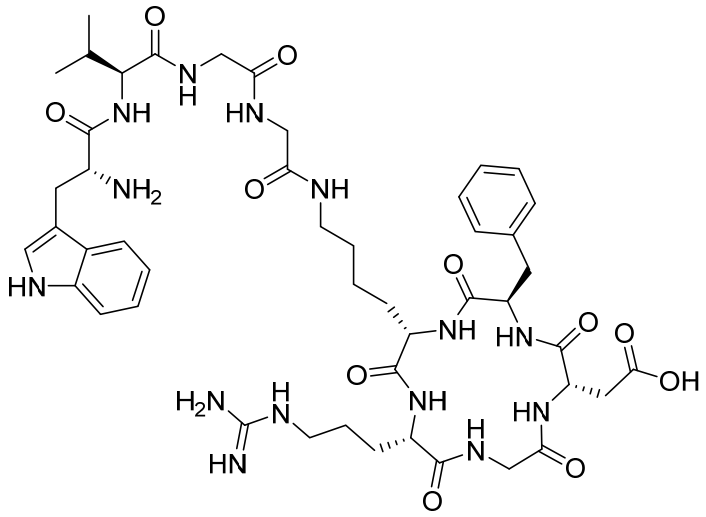
ESI-MS: m/z = $[M+H]^+$ 483.0981 (found); 483.0986 (calc.); Δ = 2.94 ppm

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ = 11.01 (d, *J*=2.6 Hz, 1H, indole-N**H**), 8.84 (d, *J*=7.7, 1H, amide-N**H**^{Ser}), 8.33 (t, *J* = 5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.05 (s, 3H, Trp-C$^{\alpha}$N**H$_3$**$^+$), 8.00 (t, *J*=5.9 Hz, 1H, amide-N**H**^{Gly4}), 7.74 (d, *J*=7.9, 1H, indole-C^4**H**), 7.37 (d, *J*=8.1 Hz, 1H, indole-C^7**H**), 7.26 (s, 1H, CON**H**^{a}), 7.22 (d, *J*=2.4 Hz, 1H, indole-C^2**H**), 7.13 – 7.06 (m, 2H, indole-C^6**H**, CON**H**^{b}), 7.02 (t, *J*=7.4, 1H, indole-C^5**H**), 5.14 (s, 1H, Ser-C$^{\beta}$-O**H**), 4.36 (m, 1H, C$^{\alpha,Ser}$**H**), 4.15 (m, 1H, C$^{\alpha,Trp}$**H**), 3.79 (dd, *J*=16.6, 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H^a**), 3.74 (dd, *J*=16.6, 5.8 Hz, 1H, C$^{\alpha,Gly3}$**H^b**), 3.66 (dd, *J*=16.9, 5.7 Hz, 1H, C$^{\alpha,Gly4}$**H^a**), 3.62 (dd, *J*=16.9, 5.5 Hz, 1H, C$^{\alpha,Gly4}$**H**^{b}), 3.55 (dd, *J*=10.7, 5.8 Hz, 1H, C$^{\beta,Ser}$**H^a**), 3.46 (dd, *J*=10.7, 5.7 Hz, 1H, C$^{\beta,Ser}$**H^b**), 3.25 (dd, *J*=14.6, 5.6 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.07 (dd, *J*=14.7, 8.4 Hz, 1H, C$^{\beta,Trp}$**H^a**).

^{13}C-NMR (151 MHz, DMSO-d$_6$) δ = 170.76 (**C**ONH$_2^{Gly4}$), 170.06 (**C**ONH^{Ser}), 168.87 (**C**ONH^{Gly3}), 168.85 (**C**ONH^{Trp}), 136.29 (indole-**C**^{7a}), 127.07 (indole-**C**^{3a}), 125.02 (indole-**C**^{2}), 121.15 (indole-**C**^{6}), 118.62 (indole-**C**^{4}), 118.36 (indole-**C^5**), 111.44 (indole-**C**^{7}) 106.86 (indole-**C**^{3}), 61.54 (**C$^{\beta}$**^{,Ser}), 55.30 (**C**$^{\alpha,Ser}$), 52.63 (**C**$^{\alpha,Trp}$), 42.18 (**C**$^{\alpha,Gly3}$), 41.76 (**C**$^{\alpha,Gly4}$), 27.45 (**C**$^{\beta,Trp}$).

H-D-Trp-Val-Gly-Gly
Arg—Gly-Asp-D-Phe-Lys

(31)



Formula: $C_{47}H_{66}N_{14}O_{11} \times CF_3CO_2H$

Yield: 15.9 mg, 67%

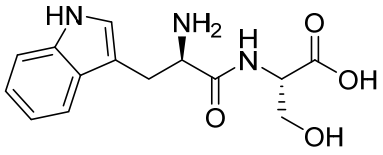
ESI-MS: m/z = $[M+H]^+$ 1003.511 (found); 1003.511 (calc.)

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ = 12.26 (s, 1H, $C^{\beta,Asp}$-COO**H**), 11.01 (d, *J*=2.6, 1H, indole-NH), 8.73 (d, *J*=8.6, 1H, isopeptidyl-amide-N**H**^{Val2}), 8.41 (m, 1H, amide-N**H**^{Gly2}), 8.38 (t, *J*=5.9, 1H, isopeptityl-amide-N**H**^{Gly3}), 8.13 – 8.03 (m, 5H, amide-N**H**^{Asp3}, amide-N**H**^{Lys5}, Arg^1-Guanidine-N**H**, isopeptidyl-amide-N**H**^{Gly4}), 8.01 (d, *J*=7.2, 1H, amide-N**H**^{Phe4}), 7.86 (t, *J*=5.7, 1H, amide-N$^{\varepsilon,Lys5}$**H**), 7.76 (d, *J*=7.9, 1H, indole-C^4**H**), 7.65 (d, *J*=8.0, 1H, amide-N**H**^{Arg1}), 7.59 (m, 1H, Arg^1-N$^{\delta}$**H**), 7.36 (d, *J*=8.1, 1H, indole-C^7**H**), 7.28 – 7.21 (m, 3H, Phe^4-C^{m}**H**, indole-C^2**H**), 7.18 (t, *J*=7.3, 1H, Phe^4-C^{p}**H**), 7.15 (d, *J*=7.5, 1H, Phe^4-C^{o}**H**), 7.10 (t, *J*=7.5, 1H, indole-C^6**H**), 7.02 (t, *J*=7.4, 1H, indole-C^5**H**), 4.63 (ddd, *J*=8.4, 8.4, 5.8, 1H, $C^{\alpha,Asp3}$**H**), 4.43 (ddd, *J*=7.1, 1H, $C^{\alpha,Phe4}$**H**), 4.26 (dd, *J*=7.8, 6.4, 1H, isopeptidyl-$C^{\alpha,Val2}$**H**), 4.21 – 4.12 (m, 2H, $C^{\alpha,Arg1}$**H**, isopeptidyl-$C^{\alpha,Trp1}$**H**), 4.04 (dd, *J*=15.0, 7.6, 1H, $C^{\alpha,Gly2}$**H**^{a}), 3.93 (ddd, *J*=11.3, 7.4, 4.2, 1H, $C^{\alpha,Lys5}$**H**), 3.79 (m, 1H, isopeptidyl-$C^{\alpha,Gly3}$**H**), 3.69 (d, *J*=5.8, 2H, isopeptidyl-$C^{\alpha,Gly4}$**H**), 3.27 – 3.22 (m, 2H, $C^{\alpha,Gly2}$**H**^{b}, isopeptidyl-$C^{\beta,Trp1}$**H**^{a}), 3.12 – 3.00 (m, 2H, C$^{\delta}$**H**$_2$, isopeptidyl-$C^{\beta,Trp1}$**H**^{b}), 2.96 (m, 2H, $C^{\varepsilon,Lys5}$**H**$_2$), 2.91 (dd, *J*=13.4, 8.4 Hz, 1H, $C^{\beta,Phe5}$**H**^{a}), 2.81 (dd, *J*=13.0, 5.5, 1H, $C^{\beta,Phe5}$**H**^{b}), 2.71 (dd, *J*=16.3, 8.6, 1H, $C^{\beta,Asp3}$**H**^{a}), 2.37 (dd, *J*=16.3, 5.7, 1H, $C^{\beta,Asp3}$**H**^{b}), 1.91 (m, 1H, isopeptidyl-$C^{\beta,Val2}$**H**), 1.71 (m, 1H, isopeptidyl-$C^{\beta,Trp1}$**H**^{a}), 1.54 (m, 1H, $C^{\beta,Arg1}$**H**^{a}), 1.54 (m, 1H, $C^{\beta,Lys5}$**H**^{a}), 1.47 (m, 1H, , $C^{\beta,Arg1}$**H**^{b})

1.4 – 1.31 (m, 3H, $C^{\gamma,Arg1}\mathbf{H_2}$, , $C^{\beta,Lys5}\mathbf{H}^{b}$), 1.32 – 1.21 (m, 2H, , $C^{\delta,Lys1}\mathbf{H_2}$), 1.07 – 0.95 (m, 2H, , $C^{\gamma,Lys1}\mathbf{H_2}$), 0.76 (d, *J*=6.7, 3H, isopeptidyl-$C^{\gamma,Val2}\mathbf{H_3}$, isopeptidyl-$C^{\gamma',Val2}\mathbf{H_3}$).

^{13}C NMR (151 MHz, DMSO) δ = 172.09, 171.68, 171.16, 170.77, 170.56, 169.95, 169.55, 168.89, 168.76, 168.47, 156.69, 137.28, 136.35, 129.11, 128.14, 126.99, 126.30, 125.01, 121.19, 118.69, 118.35, 111.45, 106.88, 57.87, 54.47, 54.44, 52.61, 51.83, 48.87, 43.25, 41.95, 41.76, 40.28, 40.06, 38.35, 37.43, 35.05, 30.89, 30.68, 28.52, 28.43, 27.89, 25.20, 22.80, 18.95, 17.90.

H-D-Trp-Ser-OH (32)



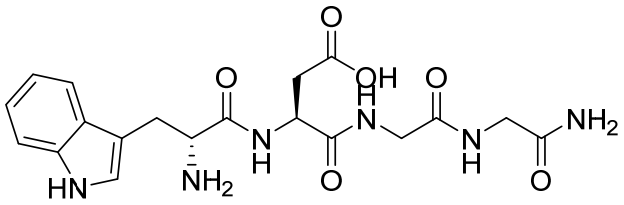
Formula: $C_{14}H_{17}N_3O_4 \times CF_3CO_2H$

Yield: 44.3 mg, 0.15 mmol ,73%

ESI-MS: m/z = $[M+H]^+$ 292.14 (found); 292.12 (calc.)

^{1}H NMR (600 MHz, DMSO-d_6) δ = 1H NMR (600 MHz, DMSO-d6) δ = 11.00 (d, *J* = 2.4 Hz, 1H, indole-N**H**), 8.87 (d, *J*=8.0 Hz, 1H, amide-N$\mathbf{H}^{Ser}$), 7.75 (d, *J*=7.9 Hz, 1H, indole-$C^4\mathbf{H}$), 7.37 (d, *J*=8.1 Hz, 1H, indole-$C^7\mathbf{H}$), 7.23 (d, *J* = 2.4 Hz, 1H, indole-$C^2\mathbf{H}$), 7.10 (ddd, *J* = 15.1, 7.1, 1.0 Hz, 1H, indole-$C^5\mathbf{H}$), 7.02 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, indole-$C^6\mathbf{H}$), 4.33 (m, 1H, $C^{\alpha,Ser}\mathbf{H}$), 4.13 (dd, *J* = 8.6, 5.2 Hz, 1H, $C^{\alpha,Trp}\mathbf{H}$), 3.70 (dd, *J* = 10.8, 5.0 Hz, 1H, $C^{\beta,Ser}\mathbf{H^a}$), 3.54 (dd, *J* = 10.6, 5.0 Hz, 2H, , $C^{\beta,Ser}\mathbf{H^b}$), 3.26 (dd, *J* = 14.7, 5.1 Hz, 1H, $C^{\beta,Trp}\mathbf{H^a}$), 3.07 (dd, *J*=14.8, 8.6 Hz, 1H, $C^{\beta,Trp}\mathbf{H^b}$).

H-D-Trp-Asp-Gly-Gly-NH_2 (33)



Formula: $C_{19}H_{24}N_6O_6 \times CF_3CO_2H$

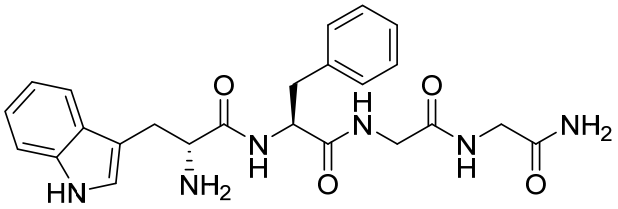
Yield: 87 mg, 0.16 mmol, 66%

ESI-MS: m/z = $[M+H]^+$ 433.1820 (found); 433.1830 (calc.); Δ = 2.42 ppm

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ = 12.51 (s, 1H, $C^{\beta,Asp}COO\mathbf{H}$), 11.02 (d, *J*=2.5, 1H, indole-N**H**), 8.99 (d, *J*=8.0, 1H, amide-$N\mathbf{H}^{Asp}$), 8.32 (t, *J*=5.8, 1H, amide-$N\mathbf{H}^{Gly4}$), 8.04 (d, *J*=4.6, 3H, $N^{\alpha,Trp}\mathbf{H_3}^+$), 7.97 (t, *J*=5.9, 1H, amide-$N\mathbf{H}^{Gly3}$), 7.75 (d, *J*=7.9, 1H, indole-$C^4\mathbf{H}$), 7.38 (d, *J*=8.1, 1H, indole-$C^7\mathbf{H}$), 7.28 – 7.25 (m, 1H, $CON\mathbf{H_2}^a$), 7.22 (d, *J*=2.4, 1H, indole-$C^2\mathbf{H}$), 7.14 – 7.08 (m, 2H, indole-$C^6\mathbf{H}$, $CON\mathbf{H_2}^b$), 7.03 (ddd, *J* = 6.6, 6.6, 0.9 Hz, 1H, indole-$C^5\mathbf{H}$), 4.69 (td, *J*=8.2, 5.3, 1H, $C^{\alpha,Asp}\mathbf{H}$), 4.04 (m, 1H, $C^{\alpha,Trp}\mathbf{H}$), 3.75 (d, *J*=5.8, 2H, $C^{\alpha,Gly4}\mathbf{H}$), 3.67 (dd, *J*=15.8, 4.9, 1H, $C^{\alpha,Gly3}\mathbf{H}^a$), 3.63 (dd, *J*=15.8, 4.9, 1H, $C^{\alpha,Gly3}\mathbf{H}^b$), 3.23 (dd, *J*=14.7, 5.1, 1H, $C^{\beta,Trp}\mathbf{H}^a$), 3.03 (dd, *J*=14.8, 9.1, 1H, $C^{\beta,Trp}\mathbf{H}^b$), 2.68 (dd, *J*=16.6, 5.3, 1H, $C^{\beta,Asp}\mathbf{H}^a$), 2.45 (dd, *J*=16.6, 8.4, 1H, $C^{\beta,Asp}\mathbf{H}^b$).

^{13}C NMR (151 MHz, DMSO) δ = 171.64 ($\mathbf{C}^{\gamma,Asp}OOH$), 170.81 ($\mathbf{C}ONH_2^{Gly4}$), 170.46 ($\mathbf{C}ONH^{Asp}$), 168.69 ($\mathbf{C}ONH^{Trp}$), 168.68 ($\mathbf{C}ONH^{Gly3}$), 136.34 (indole-$\mathbf{C}^{7a}$), 126.98 (indole-$\mathbf{C}^{3a}$), 125.06 (indole-$\mathbf{C}^2$), 121.19 (indole-$\mathbf{C}^6$), 118.55 (indole-$\mathbf{C}^5$), 118.40 (indole-$\mathbf{C}^4$), 111.51 (indole-$\mathbf{C}^7$), 106.81 (indole-$\mathbf{C}^3$), 52.79 ($\mathbf{C}^{\alpha,Trp}$), 49.63 ($\mathbf{C}^{\alpha,Asp}$), 42.26 ($\mathbf{C}^{\alpha,Gly3}$), 41.78 ($\mathbf{C}^{\alpha,Gly4}$), 36.29 ($\mathbf{C}^{\beta,Asp}$), 27.44 ($\mathbf{C}^{\beta,Trp}$).

H-D-Trp-Phe-Gly-Gly-NH_2 (34)



Formula: $C_{20}H_{27}BrN_6O_4 \times CF_3CO_2H$

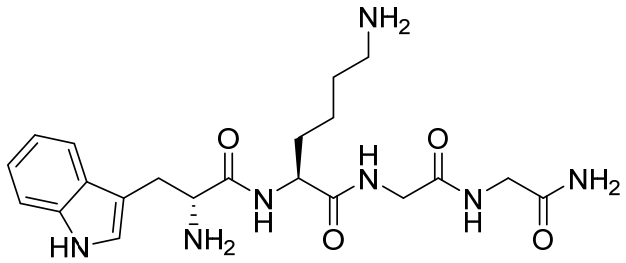
Yield: 90 mg, 0.16 mmol, 65%

ESI-MS: m/z = $[M+H]^+$ 465.2233 (found); 465.2245 (calc.); Δ = 2.51 ppm

^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.96 (d, *J*=2.5, 1H, indole-N**H**), 9.04 (d, *J*=8.7, 1H, amide-N**H**^{Phe}), 8.56 (t, *J*=5.8, 1H, amide-N**H**^{Gly3}), 8.06 (t, *J*=5.8, 1H, amide-N**H**^{Gly4}), 7.88 (d, *J*=5.3, 3H, $N^{\alpha,Trp}$**H$_3^+$**), 7.66 (d, *J*=7.8, 1H, indole-C^4**H**), 7.35 (d, *J*=8.1, 1H, indole-C^7**H**), 7.31 (s, 1H, CON**H**^{a}), 7.29 – 7.27 (m, 2H, Phe-C^o**H**), 7.23 (t, *J*=7.6, 2H, , Phe-C^m**H**), 7.16 (m, 1H, Phe-C^p**H**), 7.12 – 7.06 (m, 2H, indole-C^6, CON**H**^{b}), 7.04 – 6.97 (m, 2H, indole-C^2**H**, indole-C^5**H**), 4.77 (ddd, *J*=10.5, 8.7, 4.3, 1H, $C^{\alpha,Phe}$**H**), 3.96 (m, *J*=10.5, 5.3, 1H, $C^{\alpha,Trp}$**H**), 3.86 (dd, *J*=16.5, 6.0, 1H, $C^{\alpha,Gly3}$**H**^{a}), 3.75 (dd, *J*=16.5, 5.4, 1H, $C^{\alpha,Gly3}$**H**^{b}), 3.66 (d, *J*=5.8, 2H, $C^{\alpha,Gly4}$**H**$_2$), 3.09 (dd, *J*=13.7, 4.3, 1H, $C^{\beta,Phe}$**H**^{a}), 2.88 (dd, *J*=14.8, 4.4, 1H, $C^{\beta,Trp}$**H**^{a}), 2.75 (dd, *J*=13.7, 10.5, 1H, $C^{\beta,Phe}$**H**^{b}), 2.59 (dd, *J*=14.8, 9.7, 1H, $C^{\beta,Trp}$**H**^{a}).

^{13}C NMR (151 MHz, DMSO) δ = 171.10 (**C**ONH^{Phe}), 170.79 (**C**ONH^{Gly4}), 168.76 (**C**ONH^{Gly3}), 168.39 (**C**ONH^{Trp}), 137.49 (Phe-**C**^{i}), 136.33 (indole-**C**^{7a}), 129.35 (Phe-**C**^{o}), 128.04 (Phe-**C**^{m}), 126.92 (indole-**C**^{3a}), 126.42 (Phe-**C**^{p}), 124.99 (indole-**C**^{2}), 121.15 (indole-**C**^{6}), 118.63 (indole-**C**^{4}), 118.39 (indole-**C**^{5}), 111.41 (indole-**C**^{7}), 106.74 (indole-**C**^{3}), 53.85 (**C**$^{\alpha,Phe}$), 52.51 (**C**$^{\alpha,Trp}$), 41.95 (**C**$^{\alpha,Gly3}$), 41.77 (**C**$^{\alpha,Gly4}$), 38.18 (**C**$^{\beta,Phe}$), 27.40 (**C**$^{\beta,Trp}$).

H-D-Trp-Lys-Gly-Gly-NH_2 (35)



Formula: $C_{20}H_{27}BrN_6O_4 \times CF_3CO_2H$

Yield: 92 mg, 0.11 mmol, 45%

ESI-MS: m/z = $[M+H]^+$ 446.2504 (found); 446.2510 (calc.); Δ = 1.5 ppm

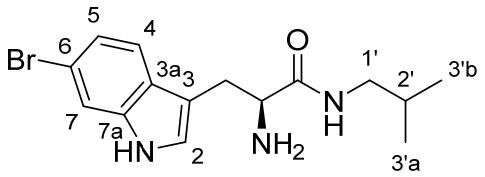
^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.03 (d, *J*=2.5, 1H, indole-N**H**), 8.75 (d, *J*=7.9, 1H, amide-N**H**^{Lys}), 8.34 (t, *J*=5.8, 1H, amide-N**H**^{Gly3}), 8.12 (s, 3H, N$^{\varepsilon,Lys}$**H$_3^+$**), 8.03 (t, *J*=5.8, 1H, amide-N**H**^{Gly4}), 7.75 (s, 3H, N$^{\alpha,Trp}$**H$_3^+$**), 7.68 (d, *J*=7.9, 1H, indole-C^4**H**), 7.37 (d, *J*=8.1, 1H, indole-C^7**H**), 7.31 (s, 1H, CON**H**^{a}), 7.22 (d, *J*=2.4, 1H, indole-C^2**H**), 7.09 (d, *J*=8.1, 1H, indole-C^6**H**), 7.08 (s, 1H, CON**H**^{b}), 7.02 (t, *J*=7.4, 1H, indole-C^5**H**), 4.26 (ddd, *J*=8.1, 8.1, 5.5, 1H, C$^{\alpha,Lys}$**H**), 4.09 (dd, *J*=7.4, 7.4, 1H, C$^{\alpha,Trp}$**H**), 3.77 (dd, *J*=16.5, 5.9, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.72 (dd, *J*=16.6, 5.8, 1H, C$^{\alpha,Gly3}$**H**^{b}), 3.65 (d, *J*=5.8, 1H, C$^{\alpha,Gly4}$**H$_2$**), 3.21 (dd, *J*=14.4, 6.5, 1H, C$^{\beta,Trp}$**H**^{a}), 3.08 (dd, *J*=14.5, 8.1, 1H, C$^{\beta,Trp}$**H**^{b}), 2.67 (t, *J*=7.8, 2H, C$^{\varepsilon,Lys}$**H$_2$**), 1.58 – 1.48 (m, 1H, C$^{\beta,Lys}$**H**^{a}), 1.46 – 1.38 (m, *J*=7.8, 2H, C$^{\delta,Lys}$**H$_2$**), 1.36 (m, 1H, C$^{\beta,Lys}$**H**^{b}), 1.11 – 1.00 (m, 2H, C$^{\gamma,Lys}$**H$_2$**).

^{13}C NMR (151 MHz, DMSO-d$_6$) δ = 171.30 (**C**ONH^{Lys}), 170.80 (**C**ONH^{Gly4}), 168.81 (**C**ONH^{Gly3}), 168.57 (**C**ONH^{Trp}), 136.25 (indole-**C**^{7a}), 127.04 (indole-**C**^{3a}), 124.91 (indole-**C**^{2}), 121.15 (indole-**C**^{6}), 118.52 (indole-**C**^{4}), 118.38 (indole-**C**^{5}), 111.45 (indole-**C**^{7}), 106.93 (indole-**C**^{3}), 52.76 (**C**$^{\alpha,Trp}$), 52.56 (**C**$^{\alpha,Lys}$), 41.86 (**C**$^{\alpha,Gly3}$), 41.74 (**C**$^{\alpha,Gly4}$), 38.60 (**C**$^{\varepsilon,Lys}$), 31.43 (**C**$^{\beta,Lys}$), 27.56 (**C**$^{\beta,Trp}$), 26.67 (**C**$^{\delta,Trp}$), 21.89 (**C**$^{\gamma,Trp}$).

1.1 Preparative-scale Thal-catalysed bromination of peptides

For the preparation of crosslinked enzyme aggregates, a 0.75 L batch of E. coli BL21 (DE3) pGro7 pET28-Thal were resuspended in 30 mL 0.1 M Na_2HPO_4 buffer (pH 7.4) and cells were lysed by French Press and centrifuged for 30 min (12000 × g, 4 °C). The supernatant was mixed with PrnF (2.5 U mL^{-1}), PTDH (2 U mL^{-1}) and 16.2 g ammonium sulfate were added. The suspension was inverted for 60 min at 4 °C and glutaraldehyde (1.0%, v/v) was added. Incubation was continued for additional 2 h at 4 °C. Afterwards, the CLEA suspension was centrifuged (10000 × g, 30 min, 4 °C), the supernatant was discarded and the CLEAs were washed three times by addition of 40 mL 0.1 M Na_2HPO_4 buffer (pH 7.4), subsequent resuspension and centrifugation (10000 × g, 30 min, 4 °C). CLEAs were ground thoroughly in reaction buffer containing 0.1 mmol substrate (L-tryptphan iso-butyl amide), 0.1 mM NAD^+, 1 µM FAD, 50 mM sodium phosphite, 30 mM NaBr and 15 mM Na_2HPO_4 at pH 7.4 (adjusted with phosphoric acid) in a total volume of 100 mL. The mixture was shaken at 25 °C (150 rpm) until no further conversion was observed. CLEAs were removed by filtration, solvent was evaporated in vacuo and the crude product was purified by RP-HPLC using a linear gradient from 0 → 100% MeCN in 100 min (Hypersil Gold C_{18} column, 8 µm, 250 × 21.2 mm).

L-6-bromotryptophan *iso*-butylamide



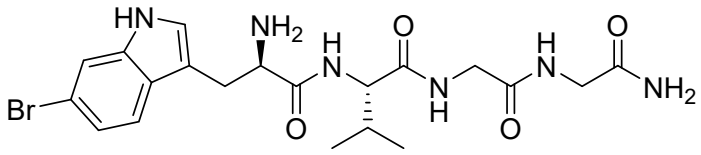
Formula: $C_{15}H_{20}BrN_3O \times CF_3CO_2H$

Yield: 1.5 mg, 3.3 µmol, 3.3%

ESI-MS: *m/z* = $[M+H]^+$ 338.09 (found); 338.08 (calc.)

^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ [ppm] = 1.15 (s, 1H, indole-N**H**), 8.38 (t, ^3J = 5.8 Hz, 1H, CON**H**), 8.06 (s, 3H, $N^{\alpha}H_3^+$), 7.59 (d, ^3J = 8.5 Hz, 1H, C4**H**), 7.56 (d, ^2J = 1.7 Hz, 1H, C7**H**), 7.21 (d, ^3J = 2.4 Hz, 1H, C2**H**), 7.16 (dd, ^3J = 8.5, ^4J = 1.7 Hz, 1H, C5**H**), 3.91 (dd, ^3J = 6.3, 7.8 Hz Hz, 1H, C^{α}**H**), 3.17 (dd, ^2J = 14.7 Hz, ^3J = 6.3 Hz, 1H, C^{β}**H^a**), 3.06 (dd, J = 14.5 Hz, ^3J = 7.8 Hz, 1H, C^{β}**H^b**), 2.95 (dd, ^2J = 13.0 Hz, ^3J = 6.4 Hz, 1H, $C^{1'}$**H^a**), 2.83 (dd, ^2J = 12.9 Hz, 6.2 Hz, 1H, $C^{1'}$**H^b**), 1.60 (qqdd, ^3J = 6.3 Hz, 7.3 Hz, 1H, C2'**H**), 0.78 (d, ^3J = 6.7 Hz, 3H, $C^{3'a}$**H$_3$**), 0.75 (d, J = 6.7 Hz, 3H, $C^{3'b}$**H$_3$**).

H-D-6-BrTrp-Val-Gly-Gly-NH_2 (Br-29)



Formula: $C_{20}H_{27}BrN_6O_4 \times CF_3CO_2H$

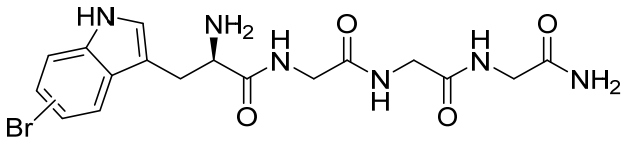
Yield:

ESI-MS: m/z = $[M+H]^+$ (found); 496.13 (calc.)

^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.15 (d, *J*=2.5 Hz, 1H, indole-N**H**), 8.74 (d, *J*=8.7 Hz, 1H, amide-N$\mathbf{H}^{Val}$), 8.40 (t, *J*=5.8 Hz, 1H, amide-N$\mathbf{H}^{Gly3}$), 8.06-8.00 (m, *J*=5.8 Hz, 4H, Trp-C$^{\alpha}$N$\mathbf{H_3}^+$, amide-N$\mathbf{H}^{Gly4}$), 7.73 (d, *J*=8.4 Hz, 1H, indole-C^4**H**), 7.55 (d, *J*=1.7 Hz, 1H, indole-C^7**H**), 7.30 (s, 1H, CON**H**^{a}), 7.25 (d, *J*=2.4 Hz, 1H, indole-C^2**H**), 7.16 (dd, *J*=8.5, 1.8 Hz, 1H, indole-C^5**H**), 7.08 (s, 1H, CON**H**^{b}), 4.26 (dd, *J*=8.7, 6.6 Hz, 1H, C$^{\alpha,Val}$**H**), 4.14 (m, 1H, C$^{\alpha,Trp}$**H**), 3.80 (dd, *J*=16.5, 6.0 Hz, 1H, C$^{\alpha,Gly3}$**H**^{a}), 3.73 (dd, *J*=16.5, 6.0 Hz, 1H, , C$^{\alpha,Gly3}$**H**^{a}), 3.65 (d, *J*=5.8 Hz, 2H, C$^{\alpha,Gly4}\mathbf{H_2}$), 3.22 (dd, *J*=14.5, 5.9 Hz, 1H, , C$^{\beta,Trp}$**H**^{a}), 3.01 (dd, *J*=14.5, 9.1 Hz, 1H, C$^{\beta,Trp}$**H**^{b}), 1.90 (m, 1H, C$^{\beta,Val}$**H**), 0.76 (d, *J*=2.2 Hz, 3H, C$^{\gamma,Val}\mathbf{H_3}$), 0.75 (d, *J*=2.2 Hz, 3H, C$^{\gamma',Val}\mathbf{H_3}$).

^{13}C-NMR (151 MHz, DMSO-d_6) δ = 171.41 (**C**ONH^{Val}), 171.17 (**C**ONH^{Gly3}), 169.35 (**C**ONH_2^{Gly4}), 169.12 (**C**ONH^{Trp}), 137.74 (indole-**C**^{7a}), 126.77 (indole-**C**^{3a}), 121.07 (indole-**C**^{4}), 114.49 (indole-**C**^{7}), 126.63 (indole-**C**^{2}), 121.70 (indole-**C**^{5}), 107.99 (indole-**C**^{3}), 58.22 (**C**$^{\alpha,Val}$), 52.97 (**C**$^{\alpha,Trp}$), 42.24 (**C**$^{\alpha,Gly3}$), 42.20 (**C**$^{\alpha,Gly4}$), 31.17 (**C**$^{\beta,Val}$), 28.16 (**C**$^{\beta,Trp}$), 18.78 (**C**$^{\gamma,Val}$), 17.89 (**C**$^{\gamma',Val}$)

H-D-BrTrp-Gly-Gly-Gly-NH_2 (Br-25)



Formula: $C_{17}H_{21}BrN_6O_4 \times CF_3CO_2H$

ESI-MS: m/z = $[M+H]^+$ (found); 453.09 (calc.)

^{1}H NMR (6-BrTrp-Regioisomer (major isomer), 600 MHz, DMSO-*d*$_6$) ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ = 11.17 (d, *J*=2.5 Hz, 1H, indole-N**H**), 8.90 (t, *J*=5.7 Hz, 1H, amide-N**H**^{Gly2}), 8.27 (t, *J*=5.8 Hz, 1H,), 8.11 (t, *J*=5.9 Hz, 1H, amide-N**H**^{Gly4}), 8.05 (s, 3H, Trp-C$^{\alpha}$N**H$_3$**$^+$), 7.65 (d, *J*=8.5 Hz, 1H, indole-C^4**H**), 7.56 (d, *J*=1.7 Hz, 1H, indole-C^7**H**), 7.34-7.17 (m, 2H, indole-C^2**H**, CON**H**^{a}), 7.16 (dd, *J*=8.4, 1.8 Hz, 1H, indole-C^5**H**), 7.08 (s, 1H, CON**H**^{b}), 4.06 (m, 1H, C$^{\alpha,Trp}$**H**), 3.96 – 3.72 (m, 4H, C$^{\alpha,Gly2}$**H$_2$**, C$^{\alpha,Gly3}$**H$_2$**), 3.65 (d, *J*=5.9 Hz, 2H, C$^{\alpha,Gly4}$**H$_2$**), 3.24 (dd, *J*=14.9, 5.2 Hz, 1H, C$^{\beta,Trp}$**H**^{a}), 3.07 (dd, *J*=14.9, 5.2 Hz, 1H, C$^{\beta,Trp}$**H**^{b}).

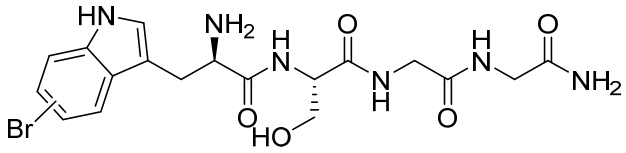
^{13}C-NMR (6-BrTrp-Regioisomer (major isomer), 151 MHz, DMSO-d$_6$)) δ = 170.85 (**C**ONH$_2^{Gly4}$), 168.88 (**C**ONH^{Trp}), 168.79 (**C**ONH^{Gly3}), 168.62 (**C**ONH^{Gly2}), 137.21 (indole-**C**^{7a}), 126.18 (indole-**C**^{2}), 126.14 (indole-**C**^{3a}), 121.31 (indole-**C**^{5}), 120.32 (indole-**C**^{4}), 114.02 (indole-**C**^{6}), 113.98 (indole-**C**^{7}), 107.23 (indole-**C**^{3}), 52.58 (**C**$^{\alpha,Trp}$), 42.07 (**C**$^{\alpha,Gly3}$), 42.03 (**C**$^{\alpha,Gly2}$), 41.78 (**C**$^{\alpha,Gly4}$), 27.20 (**C**$^{\beta,Trp}$).

^{1}H NMR (5-BrTrp-Regioisomer (minor isomer), 600 MHz, DMSO-*d*$_6$) δ = 11.23 (d, *J*=1.8 Hz, 1H, indole-N**H**), 8.93 (t, *J*=5.5, 1H, amide-N**H**^{Gly2}), 8.29 (t, *J*=5.9 Hz, 1H, amide-N**H**^{Gly2}), 8.11 (t, *J*=5.9 Hz, 1H, amide-N**H**^{Gly4}), 8.05 (s, 3H, Trp-C$^{\alpha}$N**H$_3$**$^+$), 7.94 (d, *J*=1.8 Hz, 1H, indole-C^4**H**), 7.35 (d, *J*=8.7 Hz, 1H, indole-C^7**H**), 7.29 (d, *J*=2.5 Hz, 1H, indole-C^2**H**), 7.34-7.17 (m, 1H, CON**H**^{a}), 7.21 (dd, *J*=8.6, 1.9 Hz, 1H, indole-C^6**H**), 7.08 (s, 1H, CON**H**^{b}), 4.06 (m, 1H, C$^{\alpha,Trp}$**H**), 3.96 – 3.72 (m, 4H, C$^{\alpha,Gly2}$**H$_2$**, C$^{\alpha,Gly3}$**H$_2$**), 3.65 (d, *J*=5.9 Hz, 2H, C$^{\alpha,Gly4}$**H$_2$**), 3.24 (dd, *J*=14.9, 5.2, 1H, C$^{\beta,Trp}$**H**^{a}), 3.05 (dd, *J*=14.8, 5.2 Hz, 1H, C$^{\beta,Trp}$**H**^{b}).

^{13}C-NMR (5-BrTrp-Regioisomer (minor isomer), 151 MHz, DMSO-d$_6$) δ = ^{13}C NMR (151 MHz, DMSO) δ = 170.85 (**C**ONH$_2^{Gly4}$), 168.88 (**C**ONH^{Trp}), 168.79 (**C**ONH^{Gly3}), 168.64 (**C**ONH^{Gly2}), 135.09 (indole-**C**^{7a}), 129.36 (indole-**C**^{3a}), 126.90 (indole-**C**^{2}),

123.62 (indole-**C**^{6}), 120.86 (indole-**C**^{4}), 113.51 (indole-**C**^{7}), 111.36 (indole-**C**^{5}), 106.72 (indole-**C**^{3}), 52.54 (**C**$^{\alpha,Trp}$), 42.07 (**C**$^{\alpha,Gly3}$), 42.03 (**C**$^{\alpha,Gly2}$), 41.78 (**C**$^{\alpha,Gly4}$), 27.29 (**C**$^{\beta,Trp}$).

H-D-BrTrp-Trp-Ser-Gly-Gly-NH$_2$ (Br-30)



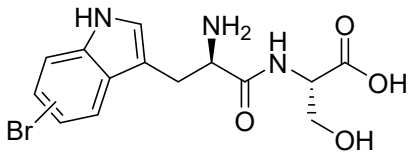
Formula:	$C_{18}H_{23}BrN_6O_5 \times CF_3CO_2H$
Yield:	6.05 mg, 13 µmol, 33%
ESI-MS:	m/z = $[M+H]^+$ 483.0981 (found); 483.0986 (calc.)

^{1}H NMR (6-BrTrp-Regioisomer (major isomer), 600 MHz, DMSO-*d*$_6$) δ = 11.17 (d, *J*=2.4 Hz, 1H, indole-N**H**), 8.86 (d, *J*=7.7, 1H, amide-N**H**^{Ser}), 8.35 (t, *J* = 5.8 Hz, 1H, amide-N**H**^{Gly3}), 8.03 (s, 3H, Trp-C$^{\alpha}$N**H$_3$**$^{+}$), 8.01 (t, *J*=5.8 Hz, 1H, amide-N**H**^{Gly4}), 7.71 (d, *J*=8.4, 1H, indole-C^{4}**H**), 7.56 (d, *J*=1.7 Hz, 1H, indole-C^{7}**H**), 7.27 (s, 1H, CON**H**^{a}), 7.25 (d, *J*=2.4 Hz, 1H, indole-C^{2}**H**), 7.16 (dd, *J*=8.5, 1.8 Hz, 1H, indole-C^{5}**H**), 7.10 (s, 1H, CON**H**^{b}), 4.06 (m, 1H, C$^{\alpha,Trp}$**H**), 3.79 (dd, J = 16.7, 5.9 Hz, 1H, C$^{\alpha,Gly3}$**H^{a}**), 3.74 (dd, J = 16.6, 5.8 Hz, 1fH, C$^{\alpha,Gly3}$**H^{b}**), 3.66 (dd, J = 17.0, 5.8 Hz, 3H, C$^{\alpha,Gly4}$**H^{a}**), 3.62 (dd, J = 16.9, 5.5 Hz, 1H, C$^{\alpha,Gly4}$**H^{a}**), 3.56 (dd, J = 10.7, 5.9 Hz, 1H, C$^{\beta,Ser}$**H^{a}**),), 3.48 (dd, J = 10.7, 5.6 Hz, 1H, C$^{\beta,Ser}$**H^{b}**), 3.23 (dd, J = 14.7, 5.5 Hz, 1H, C$^{\beta,Trp}$**H^{a}**), 3.06 (dd, J = 14.7, 8.5 Hz, 1H, C$^{\beta,Trp}$**H^{a}**).

^{13}C-NMR (6-BrTrp-Regioisomer (major isomer), 151 MHz, DMSO-d$_6$) δ = 170.77 (**C**ONH$_2$^{Gly4}), 170.02 (**C**ONH^{Ser}), 168.87 (**C**ONH^{Gly3}), 168.71 (**C**ONH^{Trp}), 137.20 (indole-**C**^{7a}), 126.19 (indole-**C**^{2}), 126.17 (indole-**C**^{3a}), 121.23 (indole-**C**^{5}), 120.51 (indole-**C**^{4}), 113.98 (indole-**C^{7}**), 107.30 (indole-**C**^{3}), 61.58 (**C$^{\beta}$**^{,Ser}), 55.27 (**C**$^{\alpha,Ser}$), 52.53 (**C**$^{\alpha,Trp}$), 42.16 (**C**$^{\alpha,Gly3}$), 41.75 (**C**$^{\alpha,Gly4}$), 27.28 (**C**$^{\beta,Trp}$).

^{1}H NMR (5-BrTrp-Regioisomer (minor isomer), 600 MHz, DMSO-*d*$_6$) δ = 11.23 (d, *J*=2.40 Hz, 1H, indole-N**H**), 7.96 (d, *J*=1.9 Hz, 1H, indole-C^{4}**H**), 7.35 (d, *J*=8.6 Hz, 1H, indole-C^{7}**H**), 7.21 (dd, *J*=8.6, 1.9 Hz, 1H, indole-C^{6}**H**)

H-D-BrTrp-Ser-OH (Br-32)



Formula: $C_{14}H_{17}N_3O_4 \times CF_3CO_2H$

Yield: 4.94 mg, 17 µmol, 13%

ESI-MS: m/z = $[M+H]^+$ 370.042 (found); 370.040 (calc.)

^{1}H NMR (6-BrTrp-Regioisomer (major isomer), 600 MHz, DMSO-*d*$_6$) δ = 1H NMR (600 MHz, DMSO-d6) δ = 11.12 (d, J = 2.4 Hz, 2H, indole-N**H**), 8.56 (d, J = 8.3 Hz, 2H, amide-N**H**^{Ser}), 7.65 (d, J = 8.4 Hz, 1H, indole-C^4**H**), 7.54 (d, J = 1.8 Hz, 2H, indole-C^7**H**), 7.24 (d, J = 2.4 Hz, 2H, indole-C^2**H**), 7.13 (dd, J = 8.5, 1.8 Hz, 1H, indole-C^5**H**), 4.19 (dd, J = 11.3, 6.1 Hz, 1H, , C$^{\alpha,Ser}$**H**), 3.95 (dd, J = 8.4, 5.2 Hz, 1H, C$^{\alpha,Trp}$**H**), 3.65 (dd, J = 10.6, 5.0 Hz, 2H, , C$^{\beta,Ser}$**H^a**), 3.47 (dd, J = 10.6, 5.1 Hz, 2H, C$^{\beta,Ser}$**H^b**), 3.18 (dd, J = 14.7, 5.1 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.00-2.90 (m, 1H, C$^{\beta,Trp}$**H^b**).

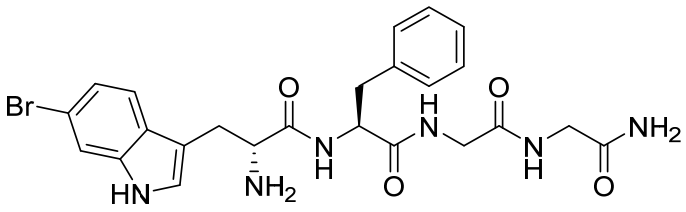
^{13}C-NMR (6-BrTrp-Regioisomer (major isomer), 151 MHz, DMSO-d$_6$) δ = 171.89 (**C**OOH^{Ser}), 170.06 (**C**ONH^{Trp}), 137.15 (indole-**C**^{3a}), 126.29 (indole-**C**^{7a}), 125.84 (indole-**C**^{2}), 121.16 (indole-**C**^{5}), 120.45 (indole-**C**^{4}), 113.90 (indole-**C**^{7}), 113.85 (indole-**C**^{6}), 108.42 (indole-**C**^{3}), 61.51 (**C$^{\beta}$**^{,Ser}), 54.60 (**C$^{\alpha}$**^{,Ser}), 53.18 (**C$^{\alpha}$**^{,Trp}), 28.31 ((**C$^{\beta}$**^{,Trp}).

^{1}H NMR (5-BrTrp-Regioisomer (minor isomer), 600 MHz, DMSO-*d*$_6$) δ = 11.19 (d, J = 2.4 Hz, 1H, d, *J*=2.4 Hz, 1H, indole-N**H**), 8.56 (d, J = 8.3 Hz, 2H, amide-N**H**^{Ser}), 7.89 (d, J = 1.8 Hz, 1H, indole-C^4**H**), 7.33 (d, J = 8.6 Hz, 1H, indole-C^7**H**), 7.27 (d, J = 2.4 Hz, 1H, indole-C^2**H**), 7.19 (dd, J = 8.6, 2.0 Hz, 1H, indole-C^6**H**), 4.22 (dd, J = 11.9, 5.4 Hz, 1H, C$^{\alpha,Ser}$**H**), 3.98 (dd, J = 8.6, 5.2 Hz, 1H, , C$^{\alpha,Trp}$**H**), 3.68 (dd, J = 10.7, 5.0 Hz, 1H, , C$^{\beta,Ser}$**H^a**), 3.50 (dd, J = 10.6, 5.1 Hz, 1H, C$^{\beta,Ser}$**H^b**), 3.18 (dd, J = 14.7, 5.1 Hz, 1H, C$^{\beta,Trp}$**H^a**), 3.00-2.90 (m, 1H, C$^{\beta,Trp}$**H^b**).

^{13}C-NMR (5-BrTrp-Regioisomer (minor isomer), 151 MHz, DMSO-d$_6$) δ = 171.89 (**C**OOH^{Ser}), 170.06 (**C**ONH^{Trp}), 135.01 (indole-**C**^{5}), 129.06 (indole-**C**^{7a}), 126.60 (indole-**C**^{2}), 123.49 (indole-**C**^{6}), 120.90 (indole-**C**^{4}), 113.41 (indole-**C**^{7}), 111.21

(indole-$\mathbf{C}^{3a}$), 107.74 (indole-$\mathbf{C}^{3}$), 61.54 ($\mathbf{C}^{\boldsymbol{\beta},Ser}$), 54.57 ($\mathbf{C}^{\boldsymbol{\alpha},Ser}$), 53.01 ($\mathbf{C}^{\boldsymbol{\alpha},Trp}$), 28.31 (($\mathbf{C}^{\boldsymbol{\beta},Trp}$).

H-D-6-BrTrp-Phe-Gly-Gly-NH_2 (Br-34)



Formula: $C_{24}H_{27}BrN_6O_4 \times CF_3CO_2H$

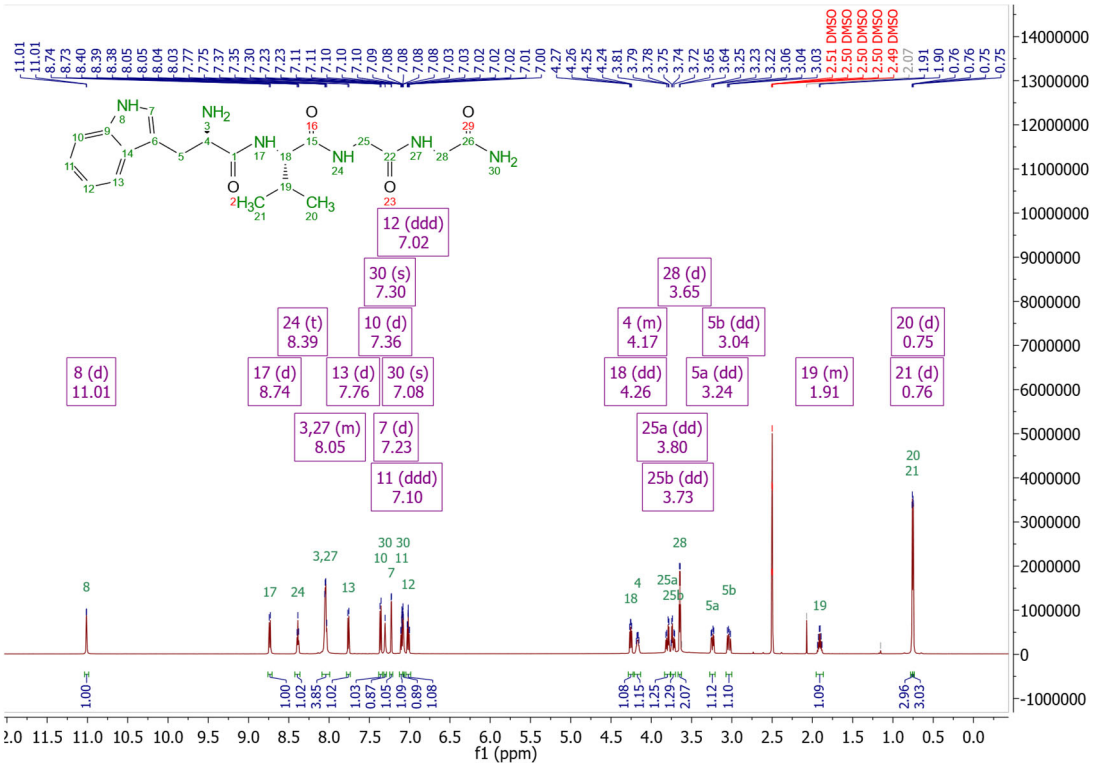
Yield: 9.6 mg, 14.5 µmol, 80%

ESI-MS: m/z = $[M+H]^+$ 543.14 (found); 543.14 (calc.);

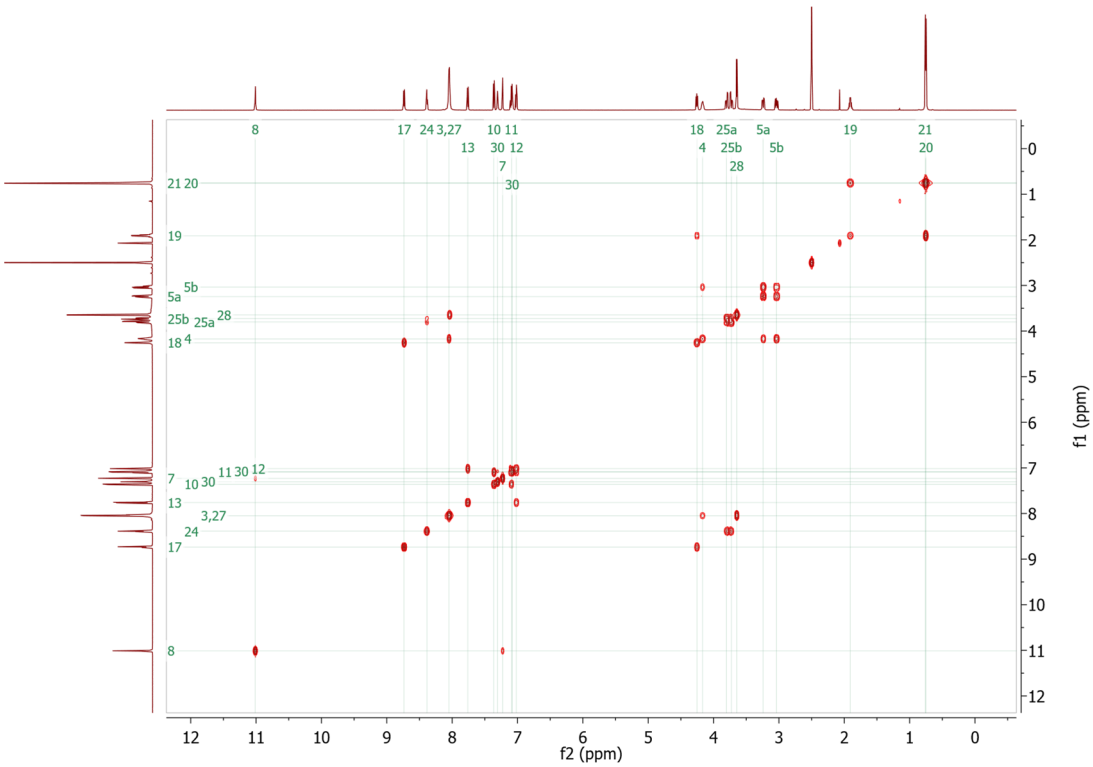
^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.08 (d, *J*=1.7 Hz, 1H, indole-N**H**), 9.03 (d, *J*=8.8 Hz, 1H, amide-N$\mathbf{H}^{Phe}$), 8.57 (t, *J*=5.8 Hz, 1H, amide-N$\mathbf{H}^{Gly3}$), 8.06 (t, *J*=5.8 Hz, 1H, amide-N$\mathbf{H}^{Gly4}$), 7.85 (s, 3H, $N^{\alpha,Trp}\mathbf{H_3^+}$), 7.58 (d, *J*=8.5 Hz, 1H, indole-C^4**H**), 7.54 (d, *J*=1.8 Hz, 1H, indole-C^7**H**), 7.31 (s, 1H, CON$\mathbf{H}^a$), 7.30 – 7.26 (m, 2H, Phe-C^o**H**), 7.24 (dd, *J*=7.5 Hz, 2H, Phe-C^m**H**), 7.16 (m, 1H, Phe-C^p**H**), 7.13 (dd, *J* = 8.4, 1.8 Hz, 1H, indole-C^5H), 7.09 (s, 1H, CON$\mathbf{H}^b$), 6.99 (d, *J*=2.4 Hz, 1H, indole-C^2**H**), 4.77 (ddd, *J* = 9.9, 4.3, 4.3, 1H, $C^{\alpha,Phe}$**H**), 3.86 (m, 1H, $C^{\alpha,Trp}$**H**), 3.86 (dd, *J*=16.5, 6.0, 1H, $C^{\alpha,Gly3}\mathbf{H}^a$), 3.75 (dd, *J*=16.5, 5.4, 1H, $C^{\alpha,Gly3}\mathbf{H}^b$), 3.66 (d, *J*=5.8, 2H, $C^{\alpha,Gly4}\mathbf{H}_2$), 3.09 (dd, *J*=13.7, 4.3, 1H, $C^{\beta,Phe}\mathbf{H}^a$), 2.85 (dd, *J*=14.8, 4.4, 1H, $C^{\beta,Trp}\mathbf{H}^a$), 2.73 (dd, *J*=13.6, 10.6, 1H, $C^{\beta,Phe}\mathbf{H}^b$), 2.55 (dd, *J*=14.7, 9.7, 1H, $C^{\beta,Trp}\mathbf{H}^a$).

NMR-Spectra

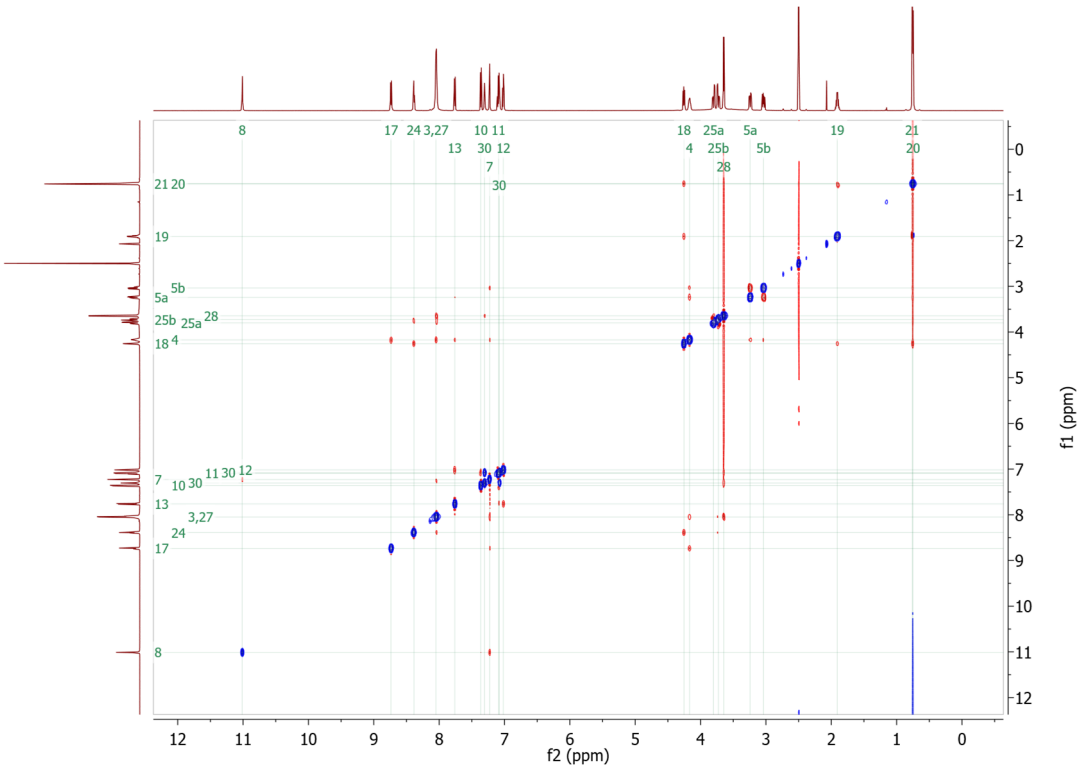
H-D-Trp-Val-Gly-Gly-NH_2



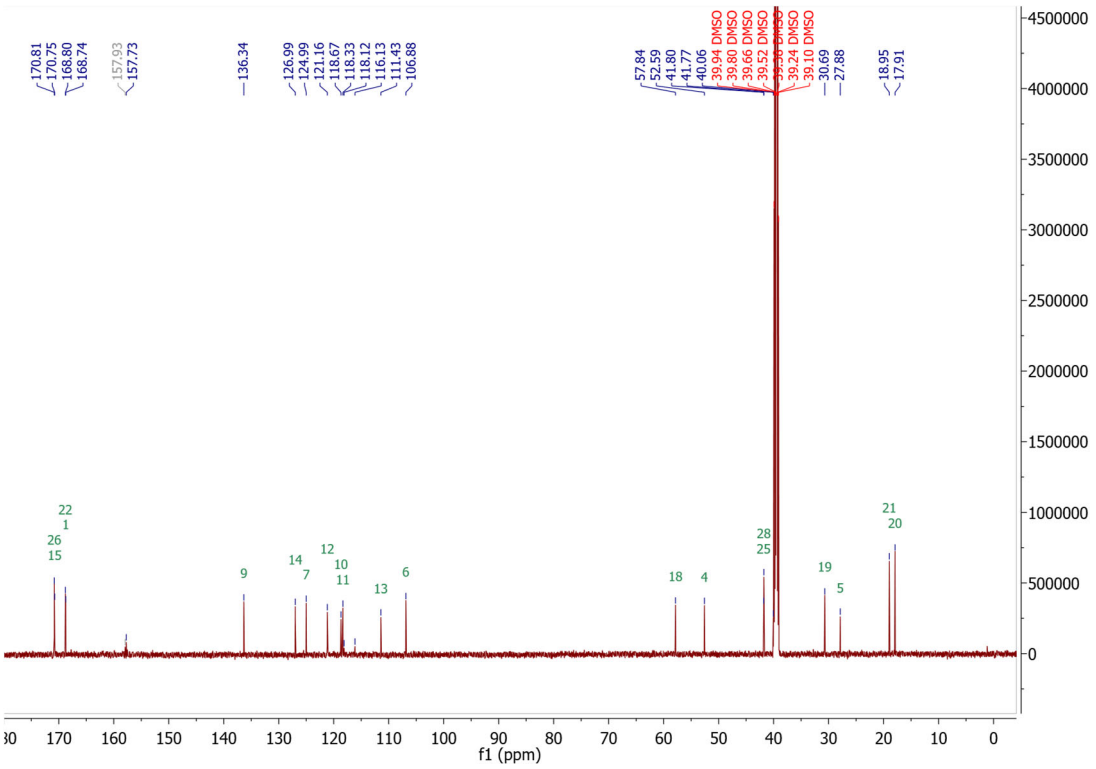
1H-NMR-Spectrum 1



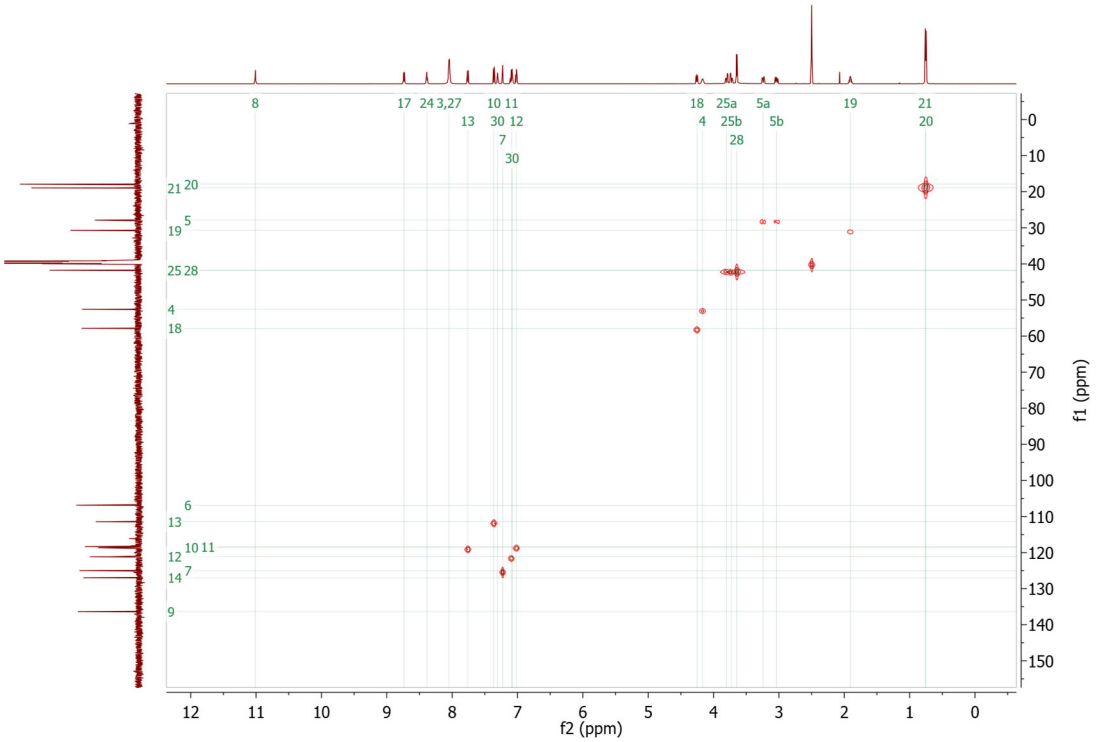
H-H-COSY-Spectrum 1



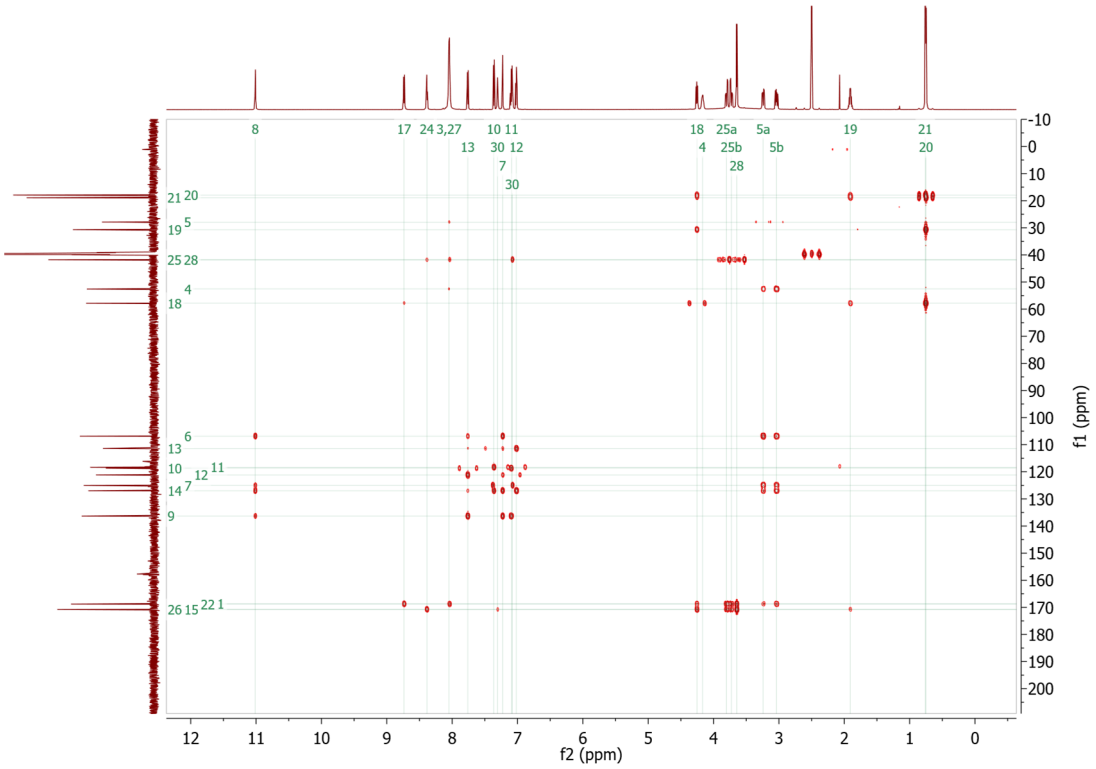
NOESY-Spectrum 1



13C-NMR-Spectrum 1

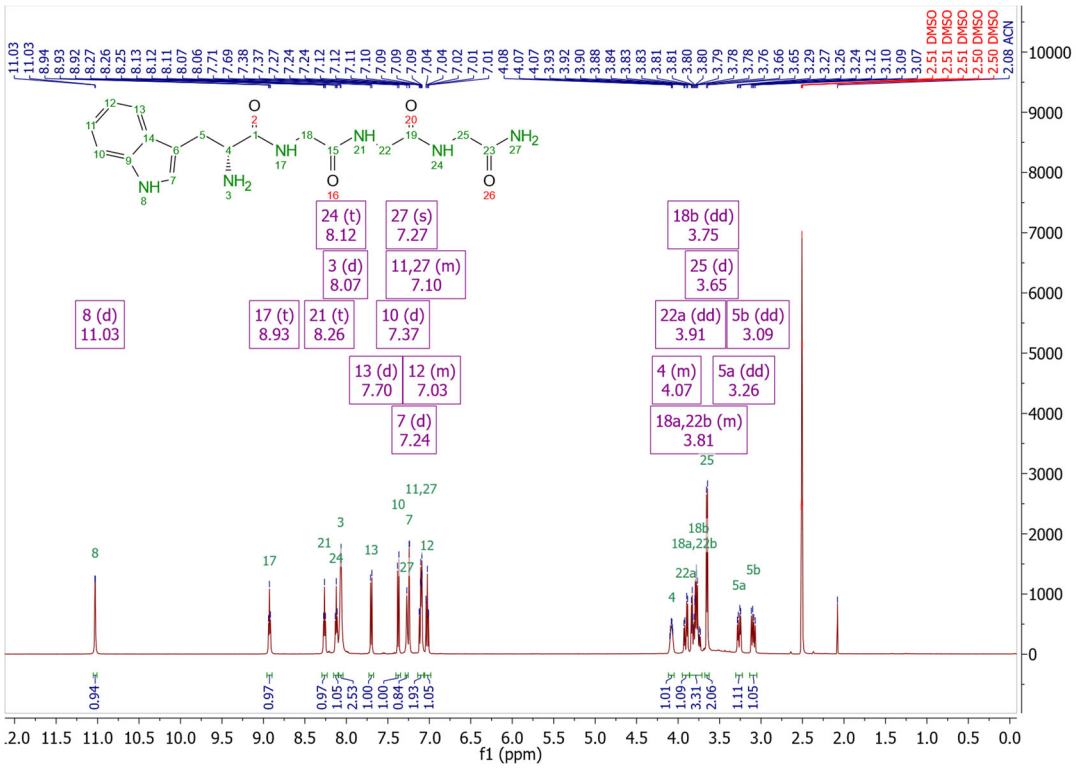


HSQC-Spectrum 1

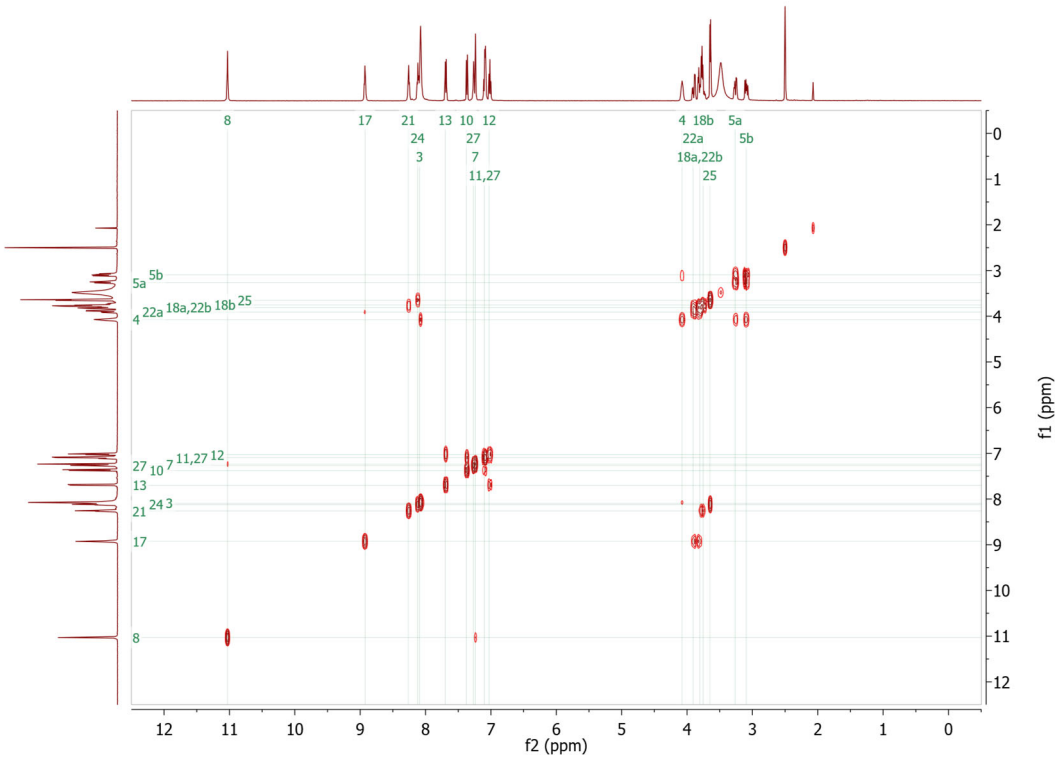


HMBC-Spectrum 1

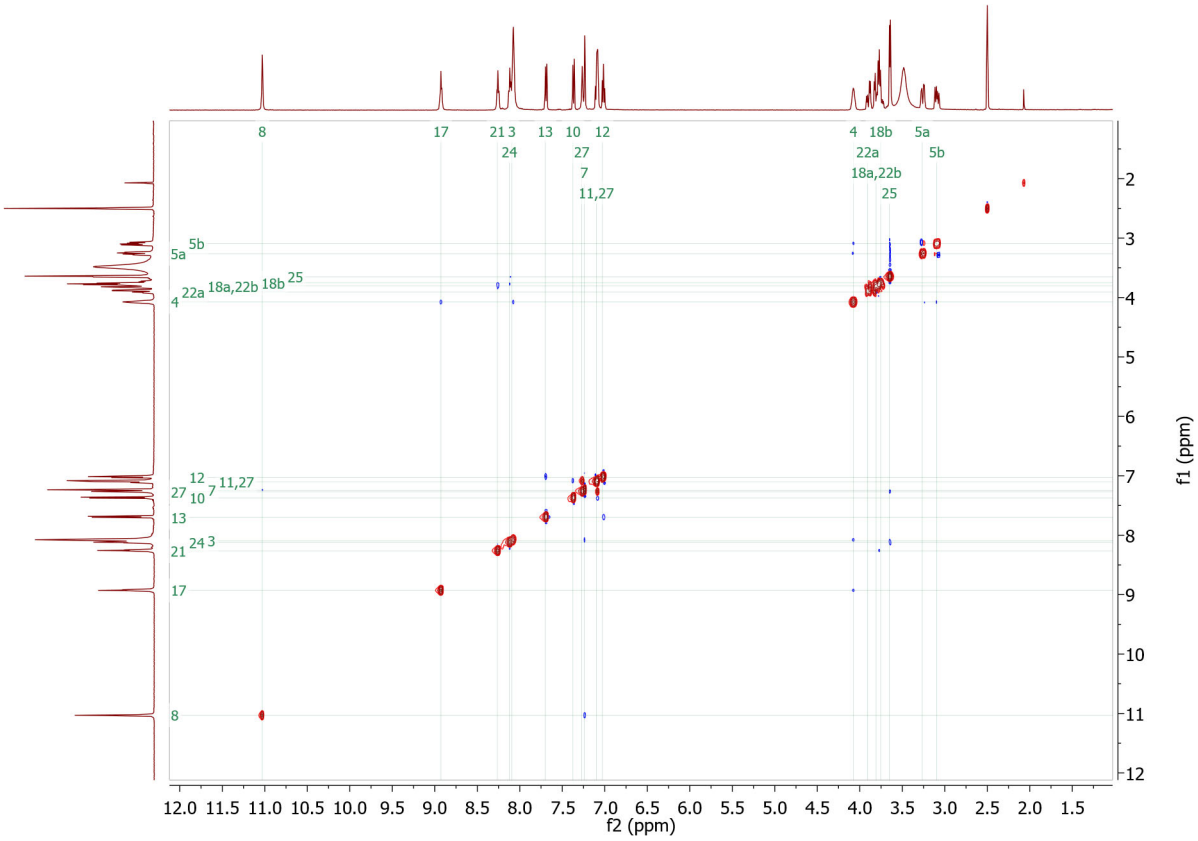
H-D-Trp-Gly-Gly-Gly-NH_2



1H-NMR-Spectrum 2



H-H-COSY-Spectrum 2



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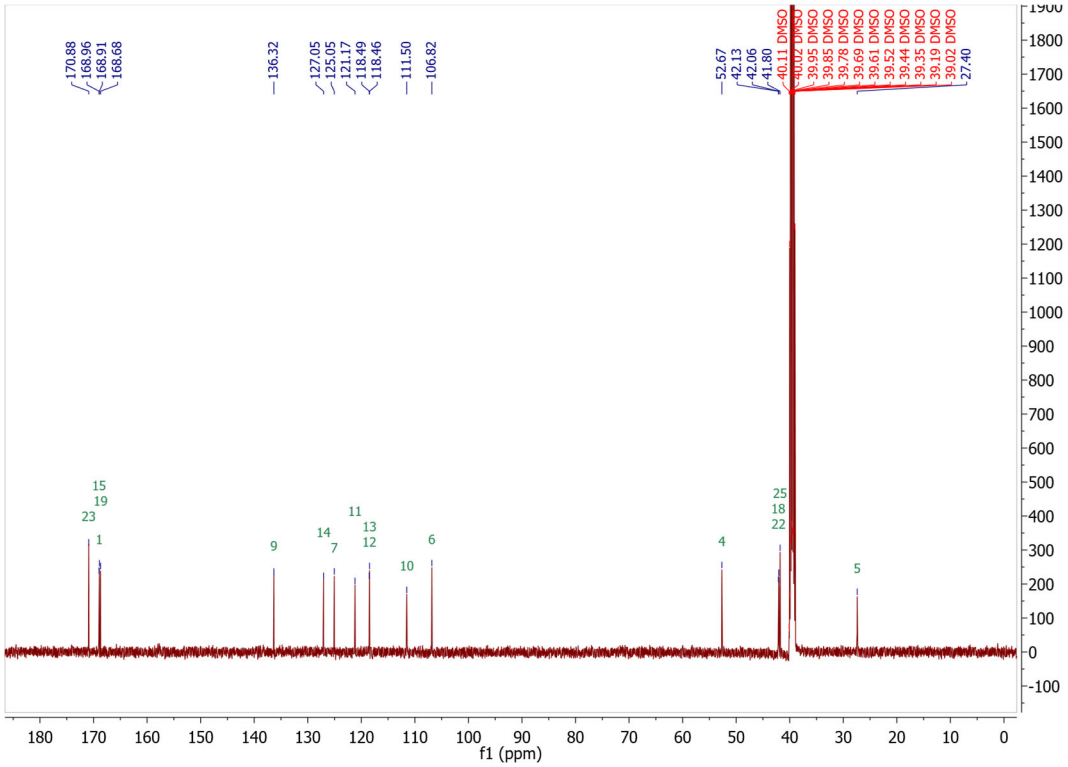
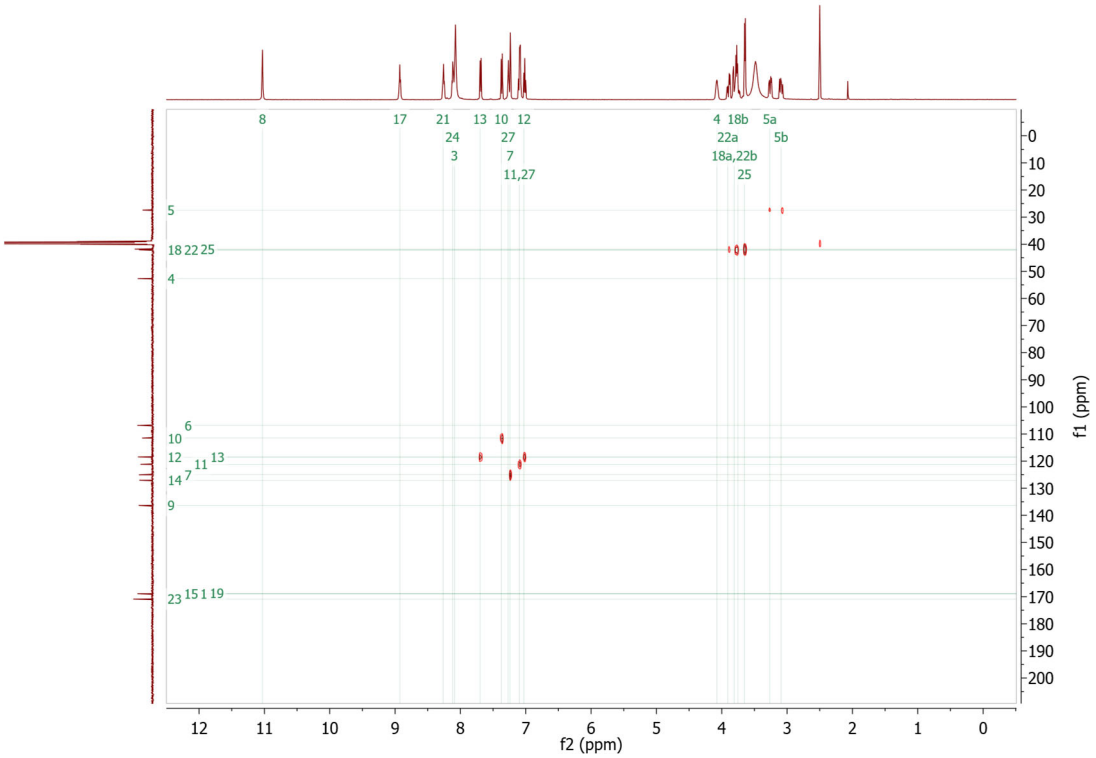
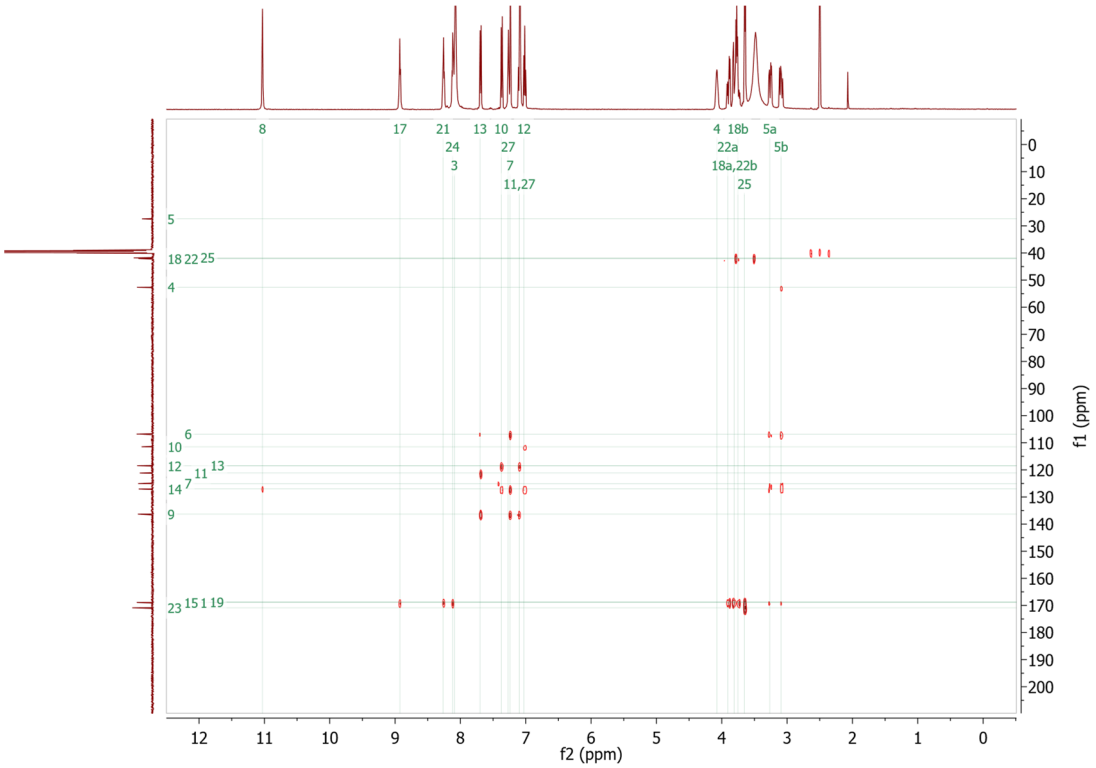


13C-NMR-Spectrum 2

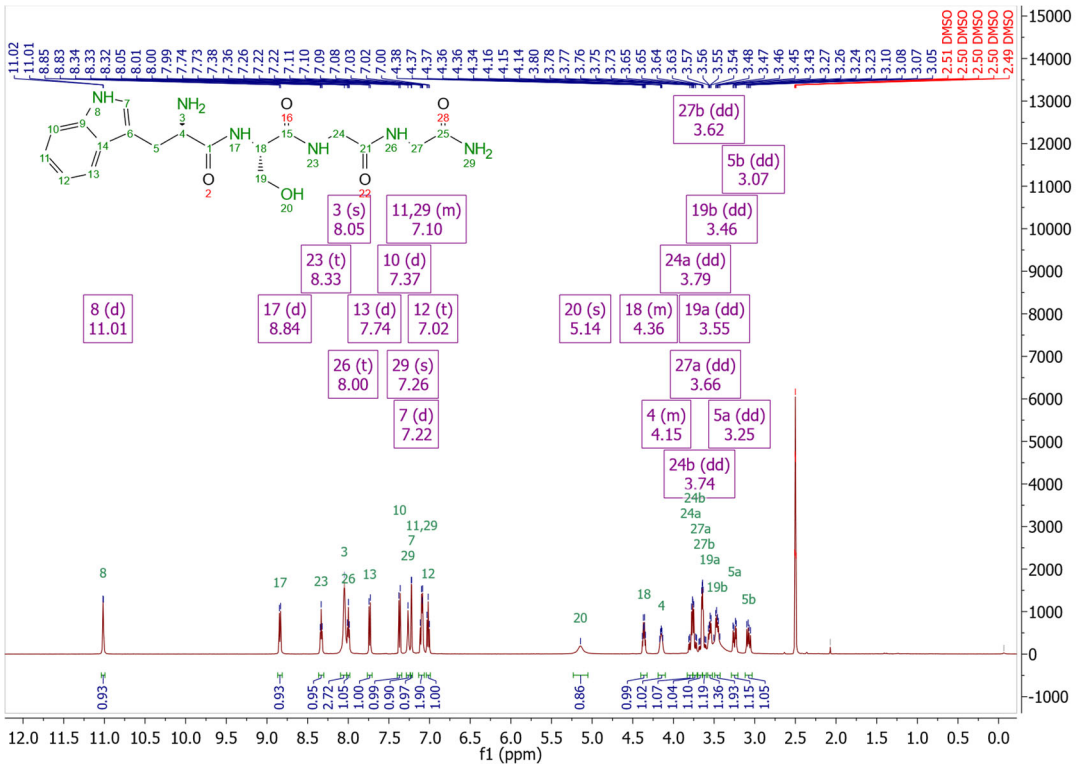


HSQC-Spectrum 2

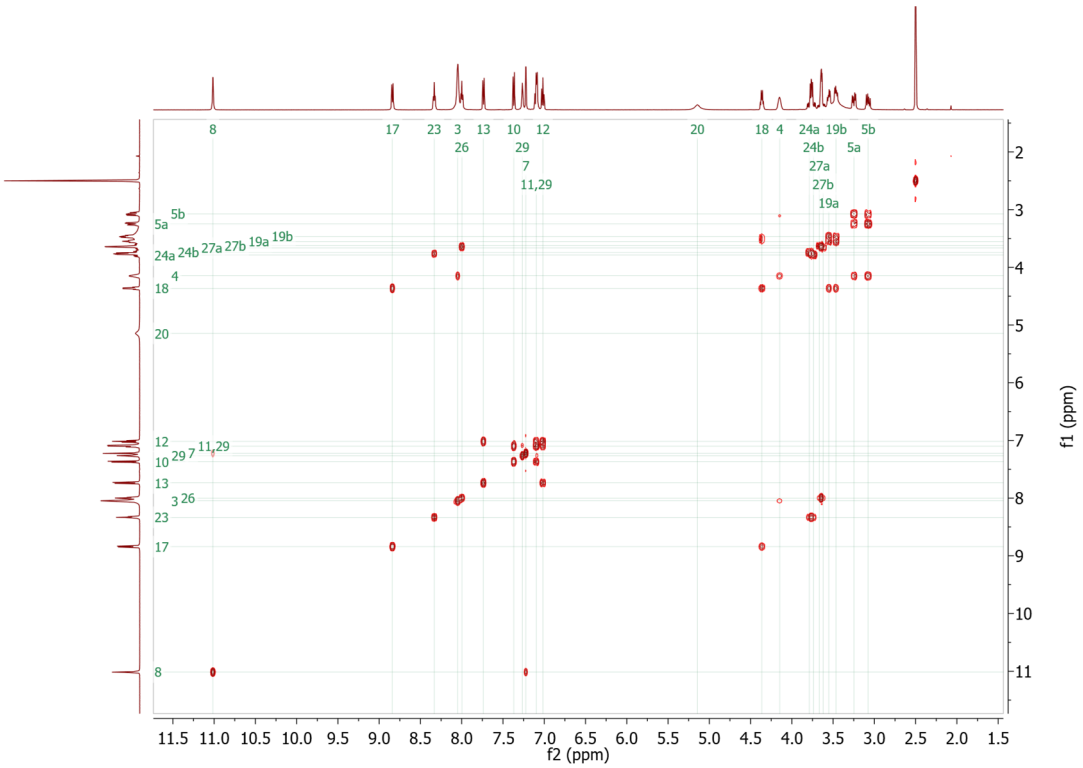


HMBC-Spectrum 2

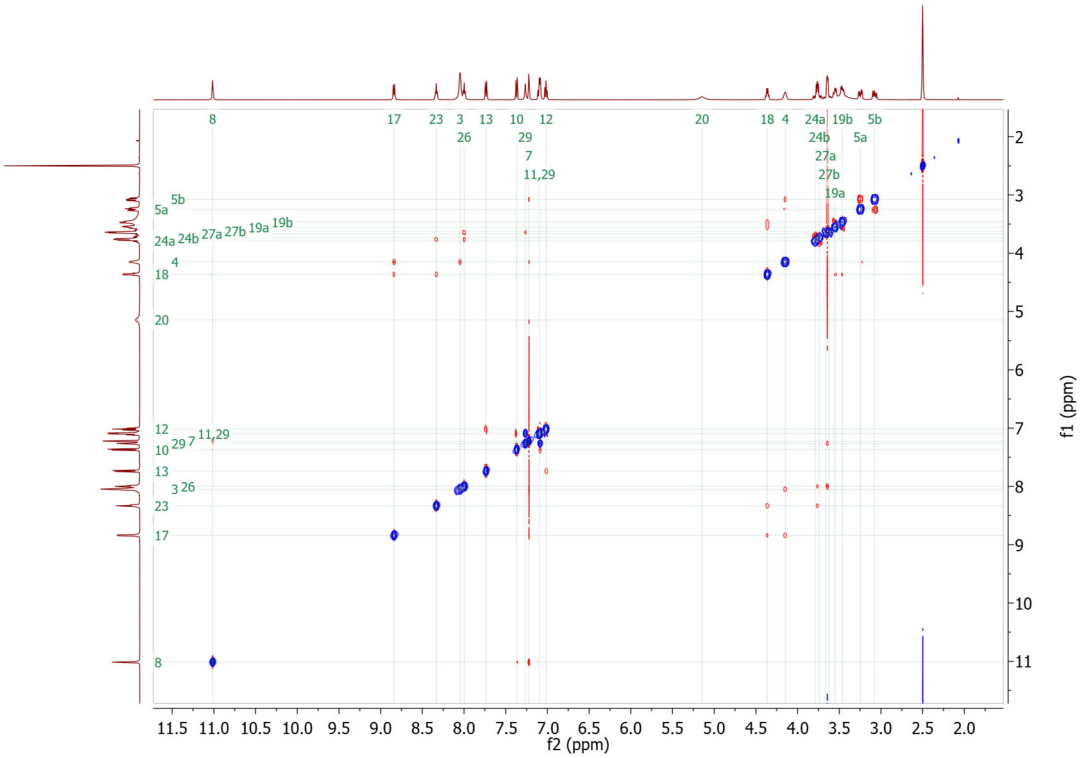
H-D-Trp-Ser-Gly-Gly-NH_2



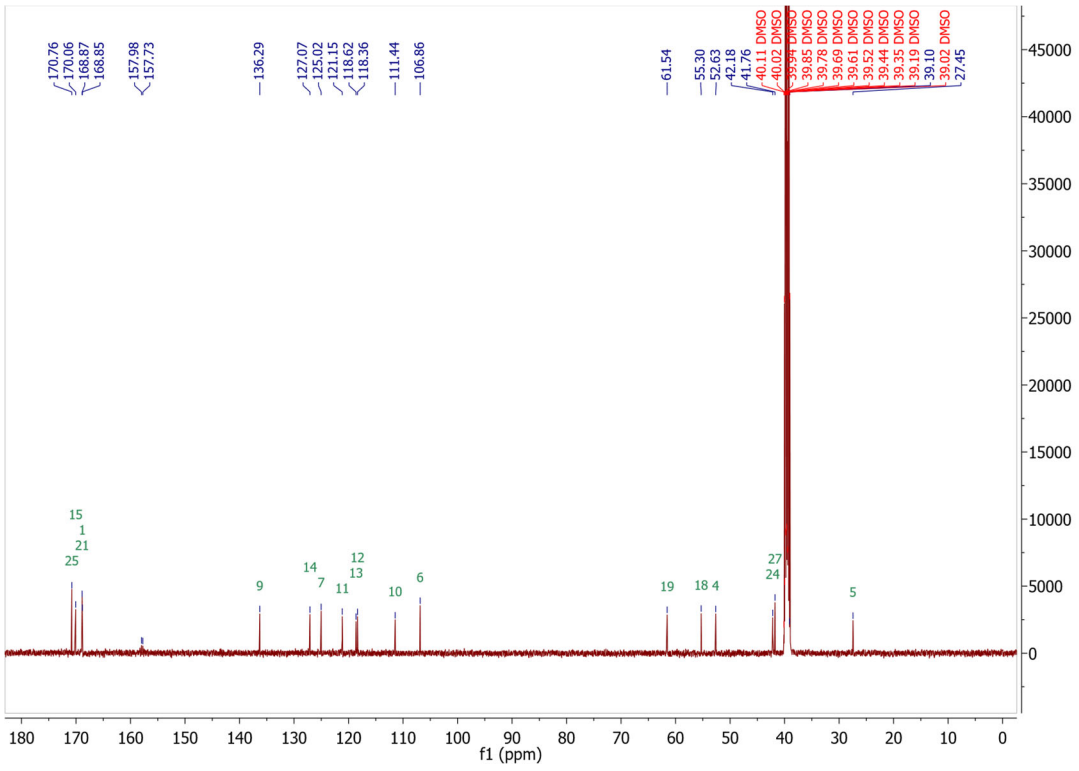
1H-NMR-Spectrum 3



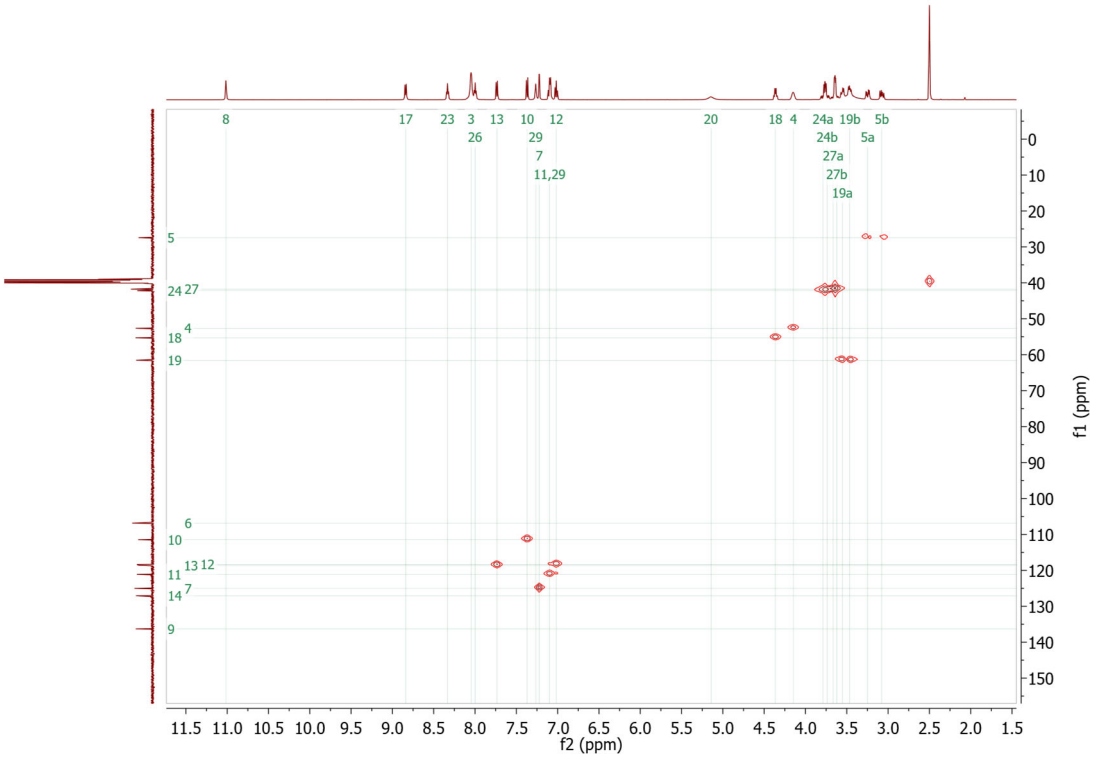
H-H-COSY-Spectrum 3



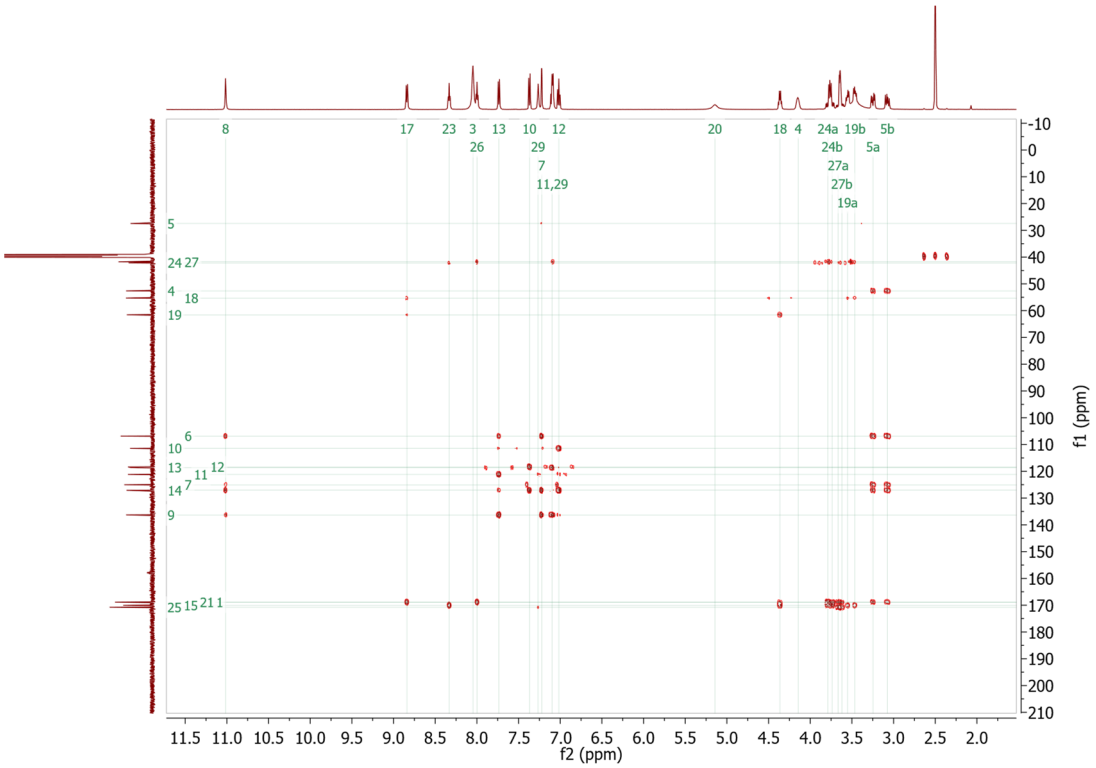
NOESY-Spectrum 3



13C-NMR-Spectrum 3

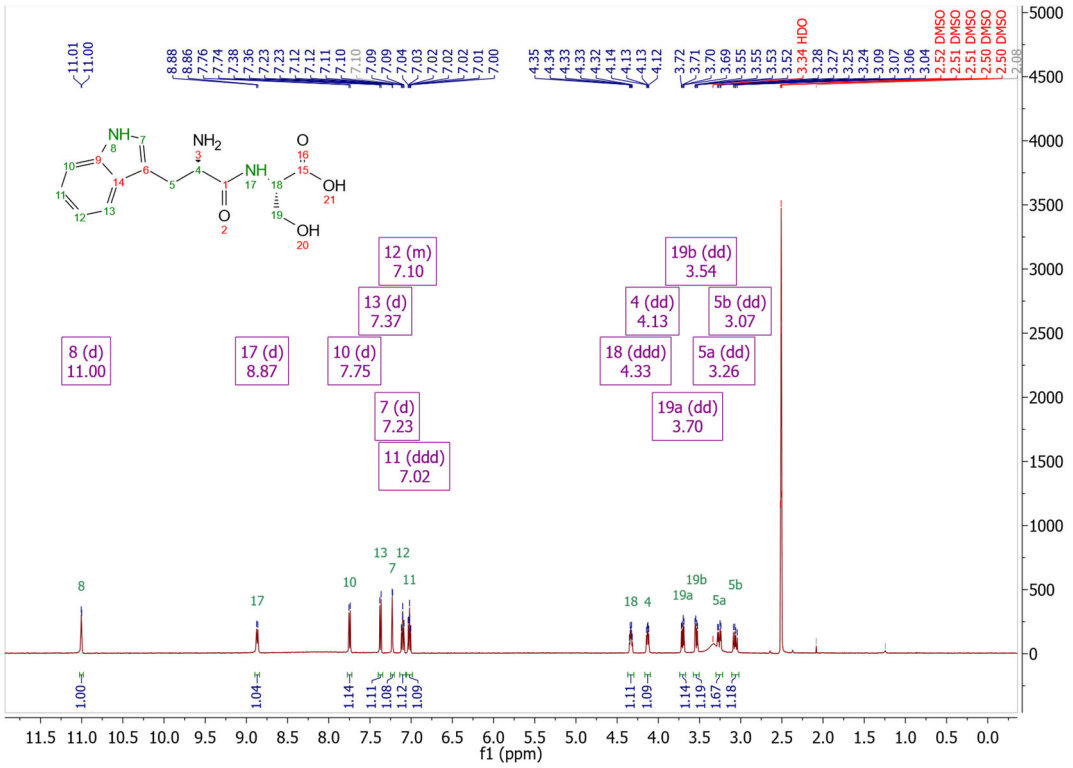


HSQC-Spectrum 3

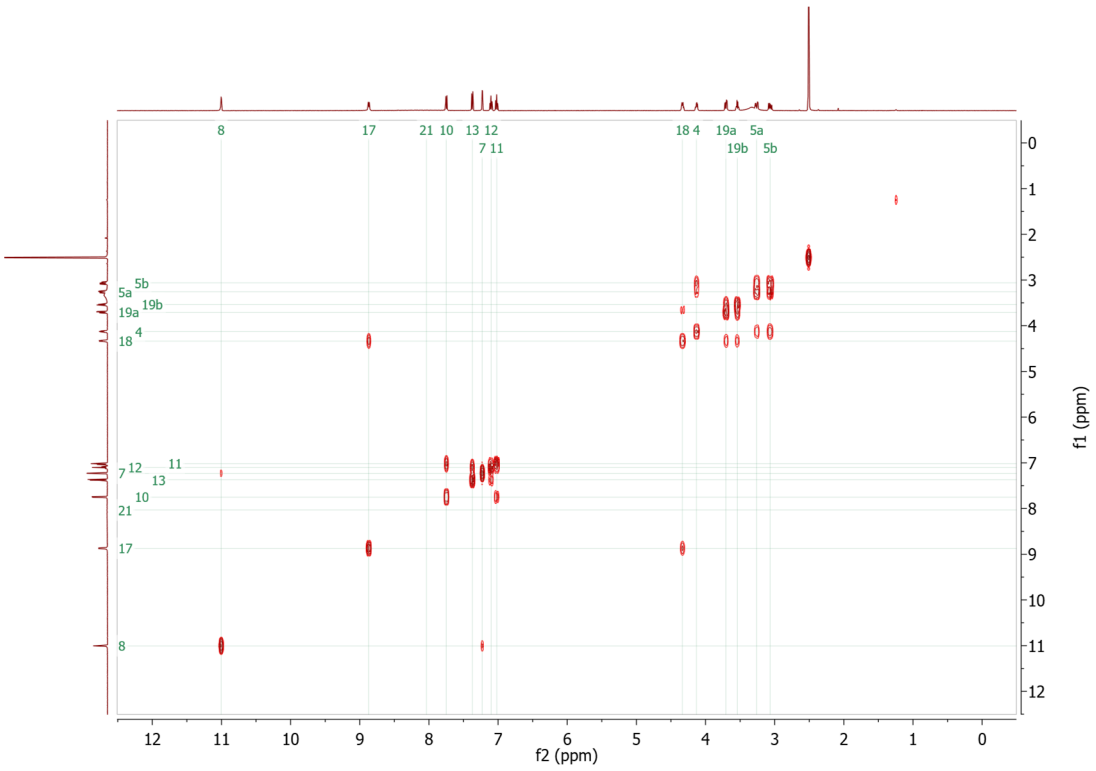


HMBC-Spectrum 3

H-D-Trp-Ser-OH

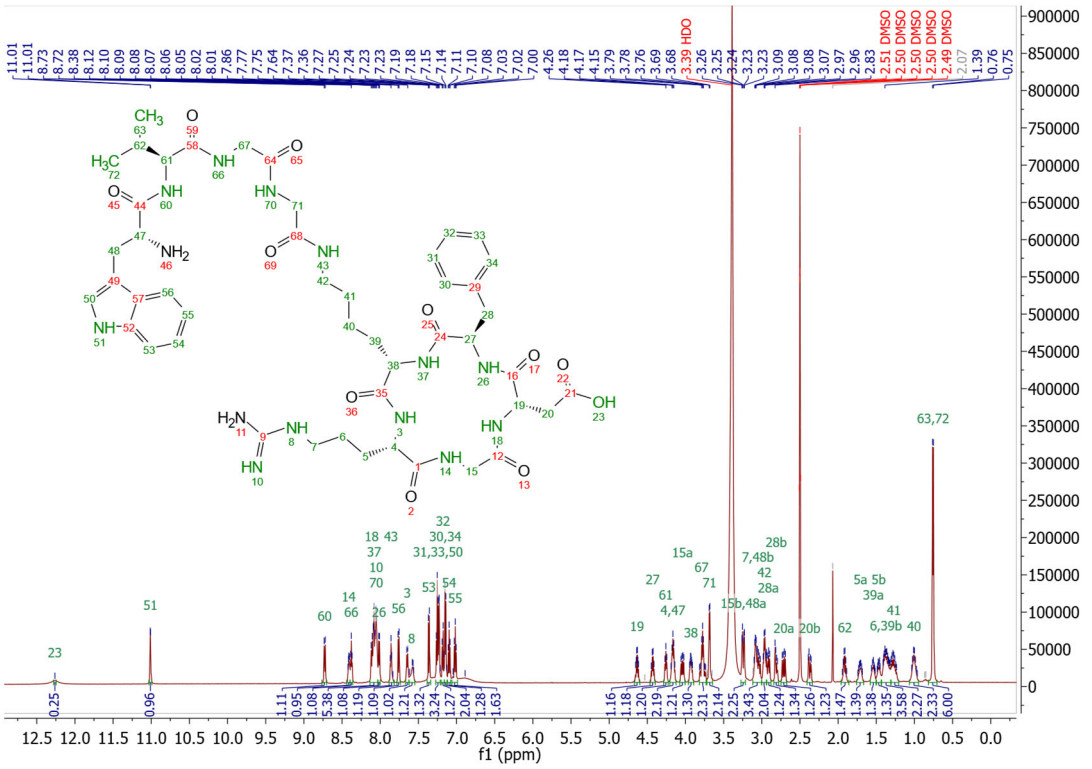


1H-NMR-Spectrum 4

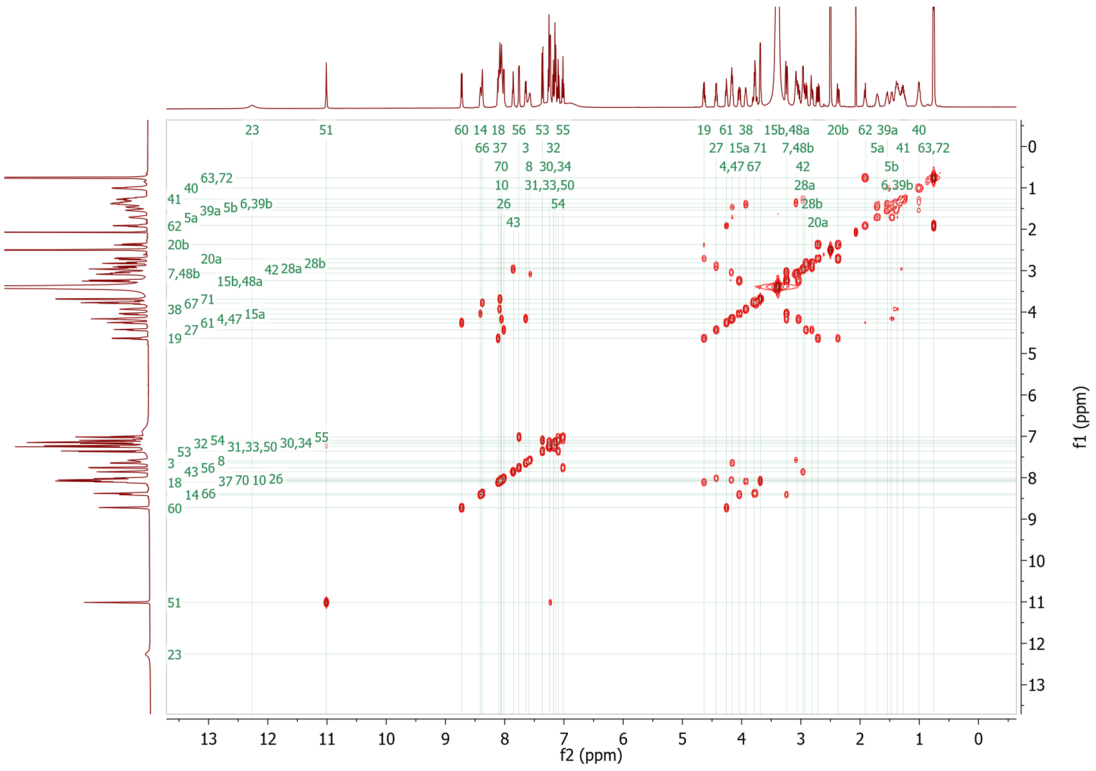


H-H-COSY-Spectrum 4

H-D-Trp-Val-Gly-Gly
Arg—Gly-Asp-D-Phe-Lys

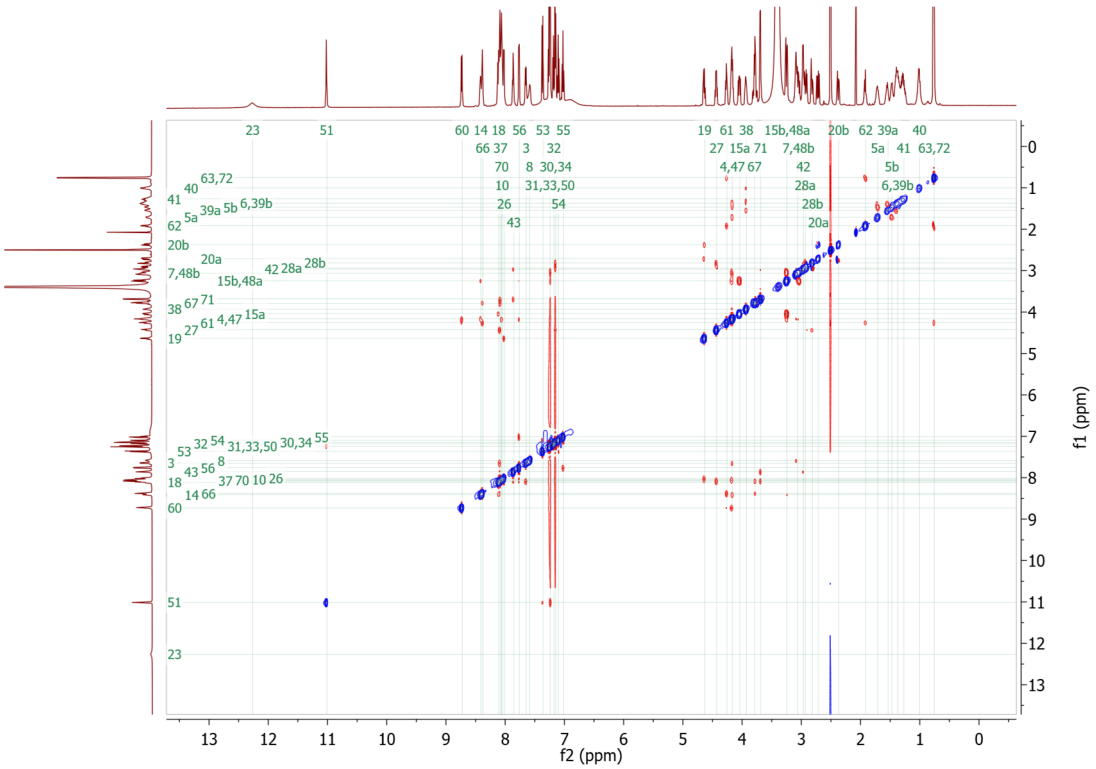


1H-NMR-Spectrum 5

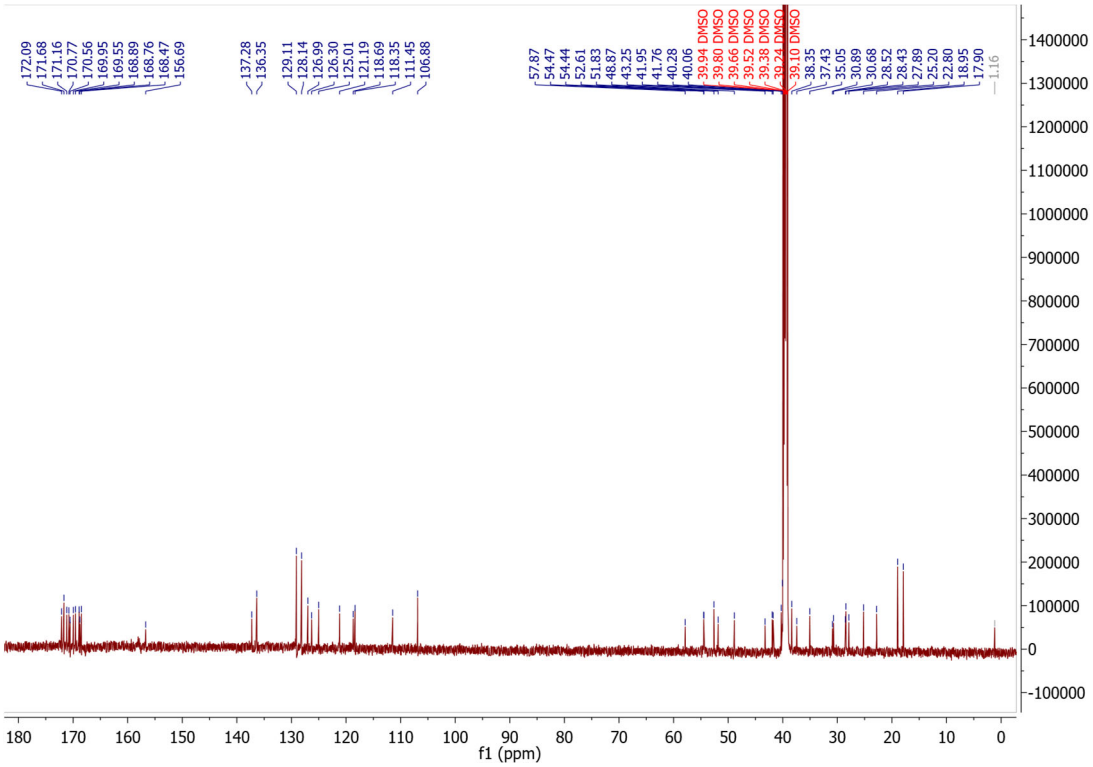


H-H-COSY-Spectrum 5

TOCSY-Spectrum 1

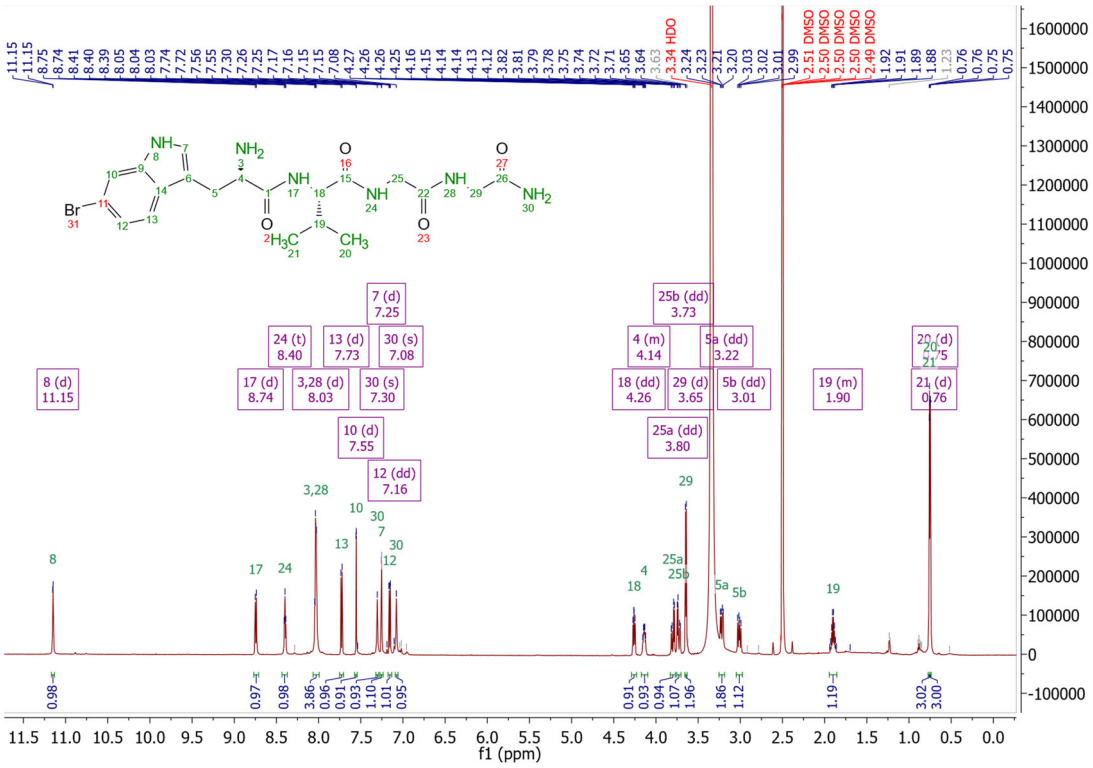


NOESY-Spectrum 4

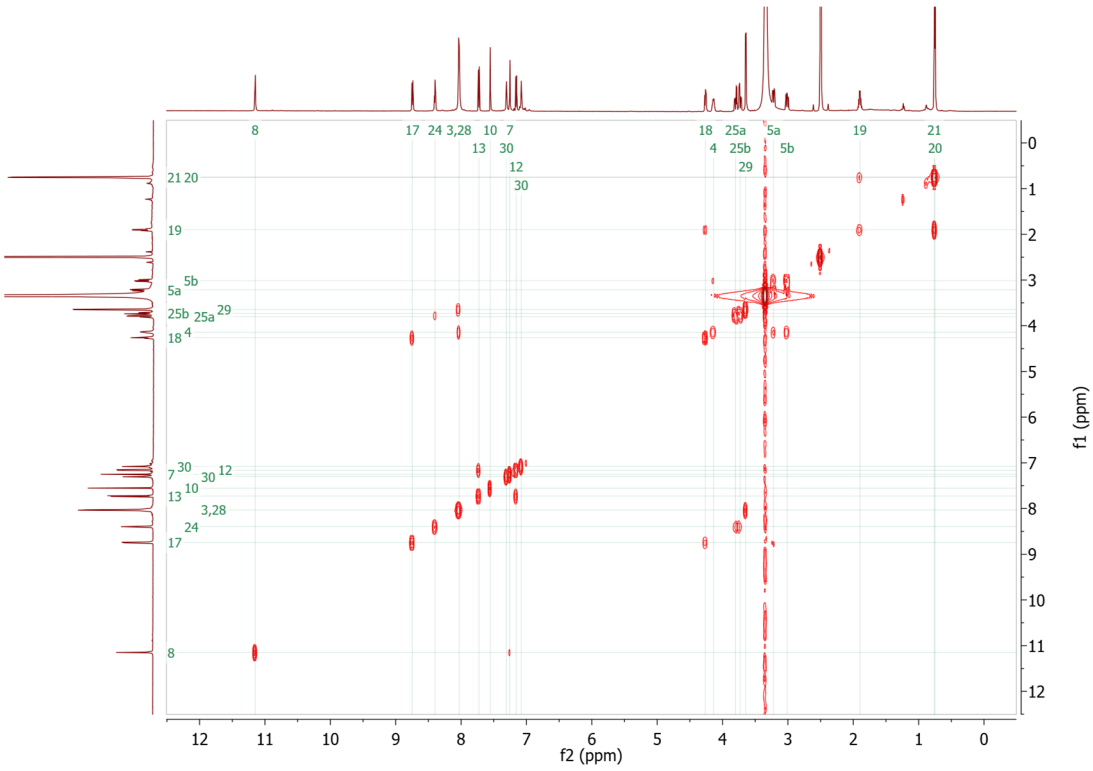


13C-NMR-Spectrum 4

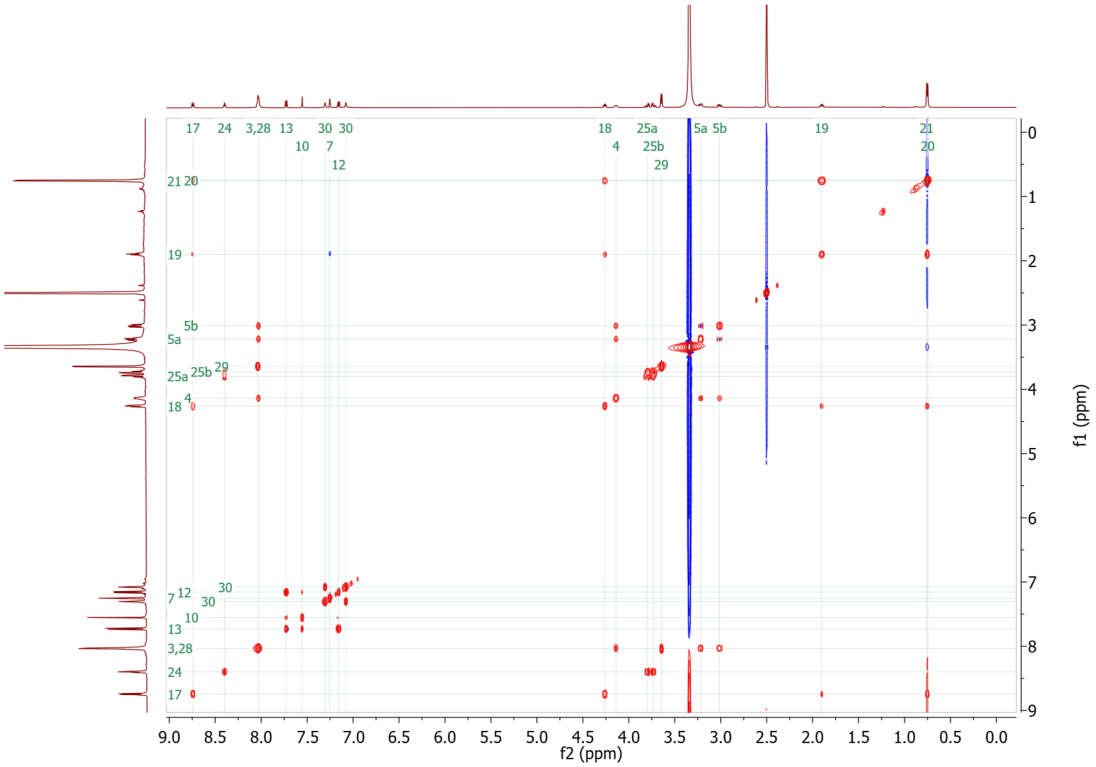
H-6-D-BrTrp-Val-Gly-Gly-NH_2



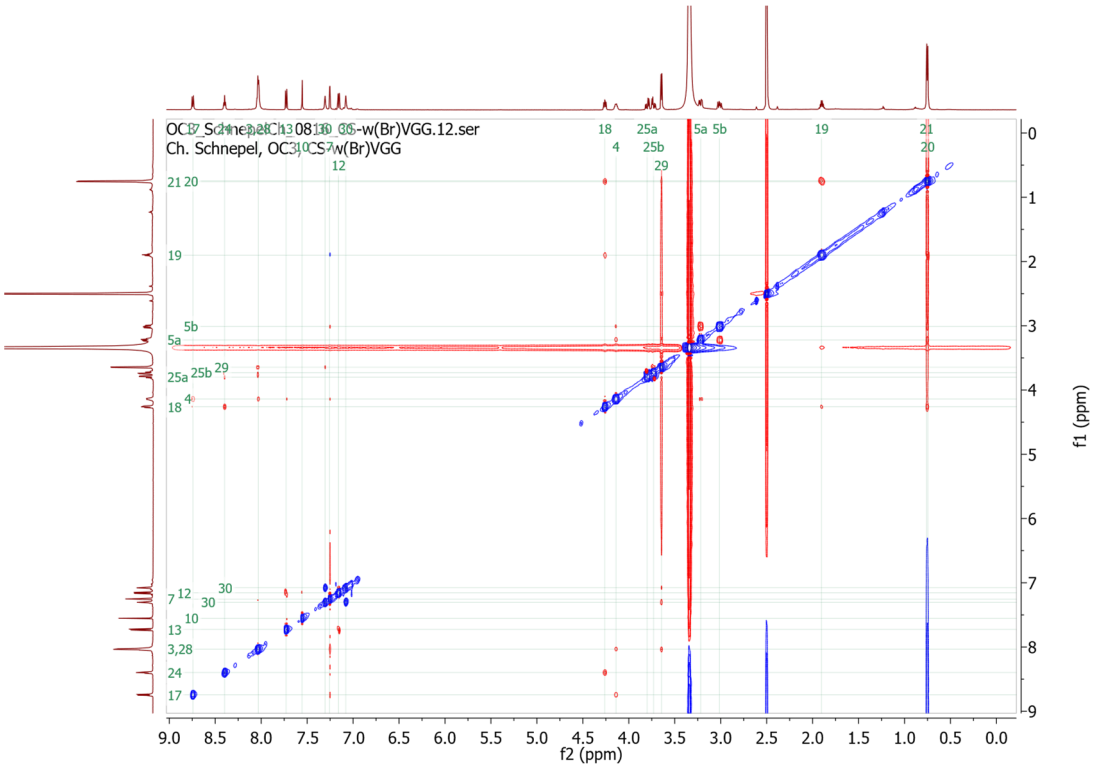
1H-NMR-Spectrum 6



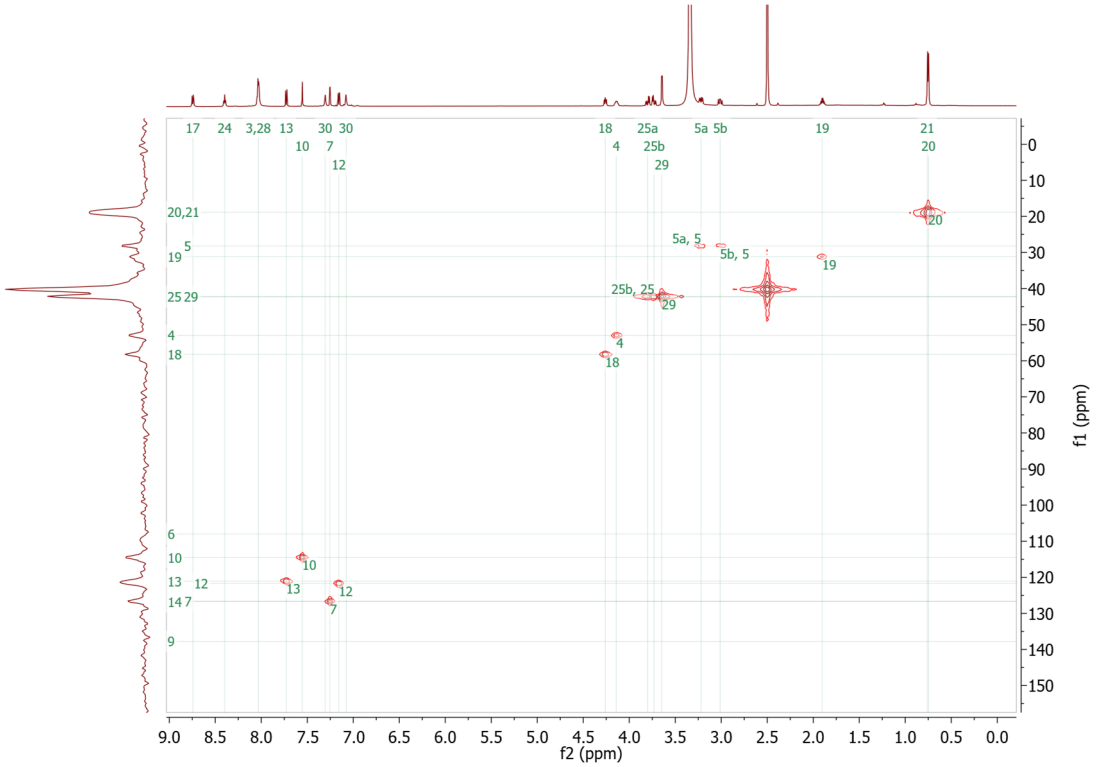
H-H-COSY-Spectrum 6



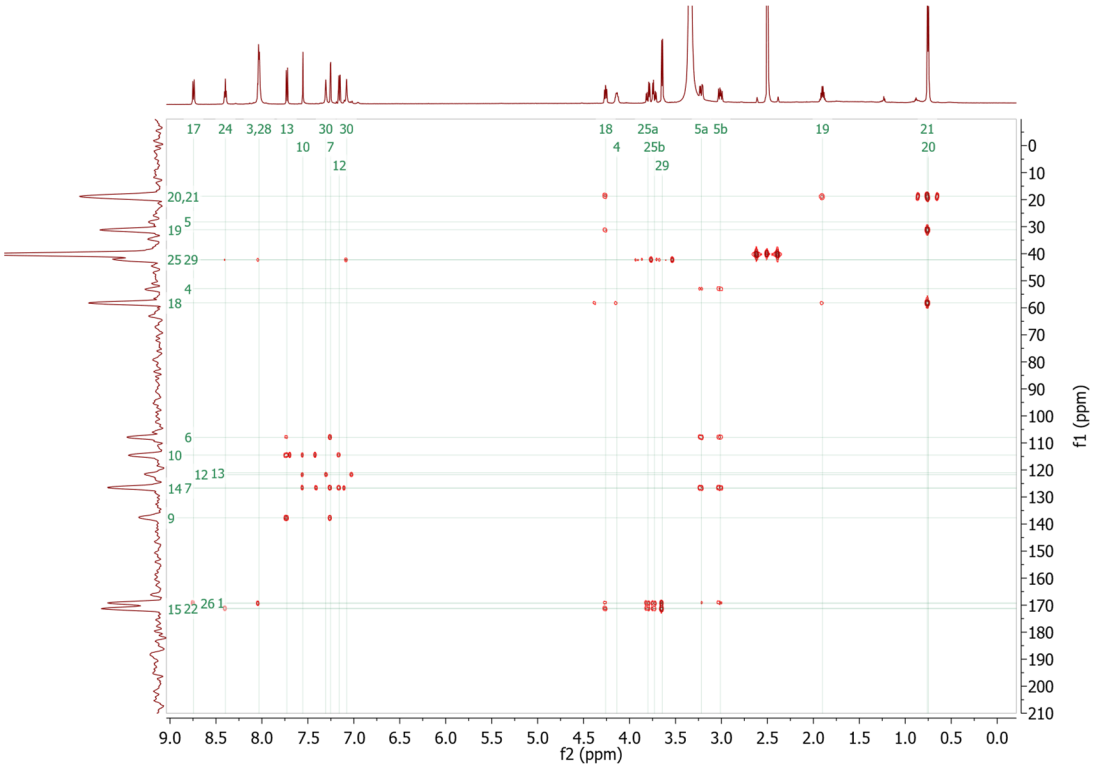
TOCSY-Spectrum 2



NOESY-Spectrum 5



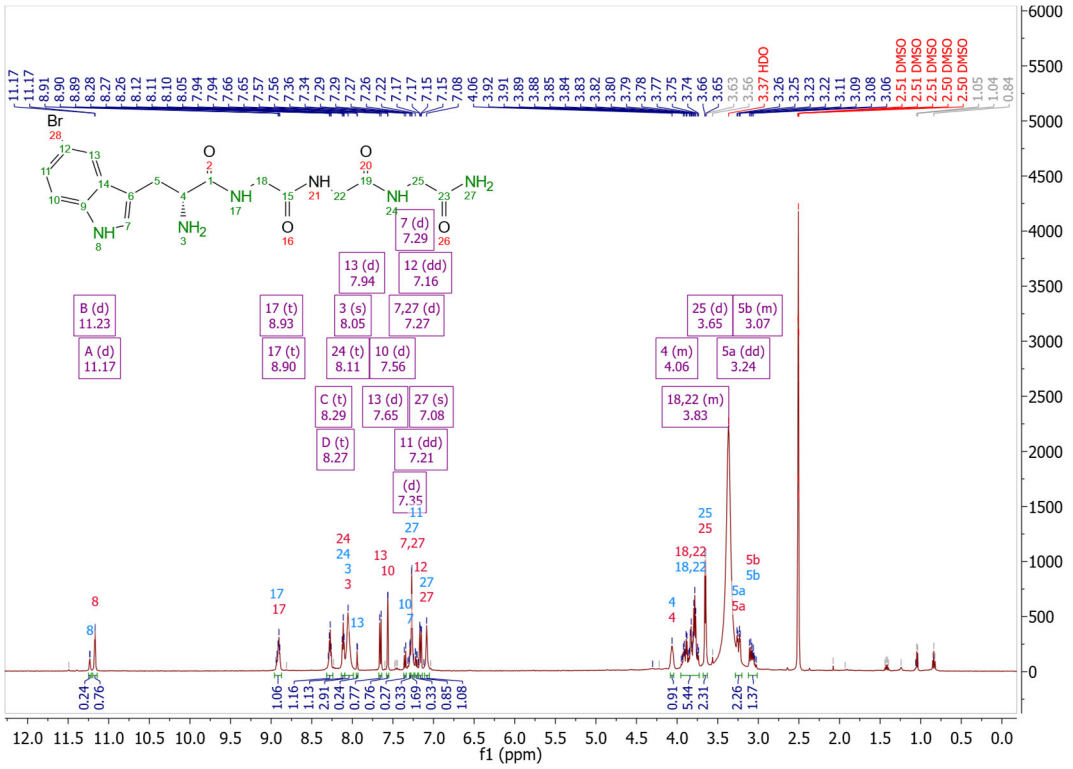
HSQC-Spectrum 4



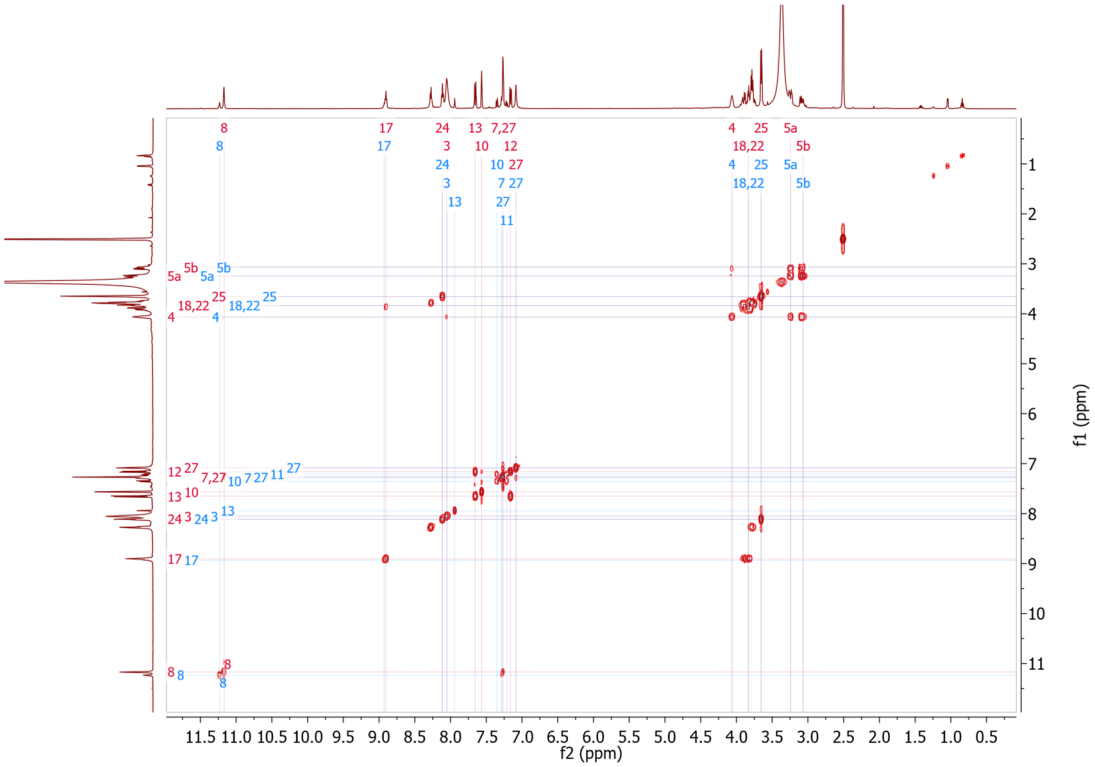
HMBC-Spectrum 4

H-D-BrTrp-Gly-Gly-Gly-NH_2

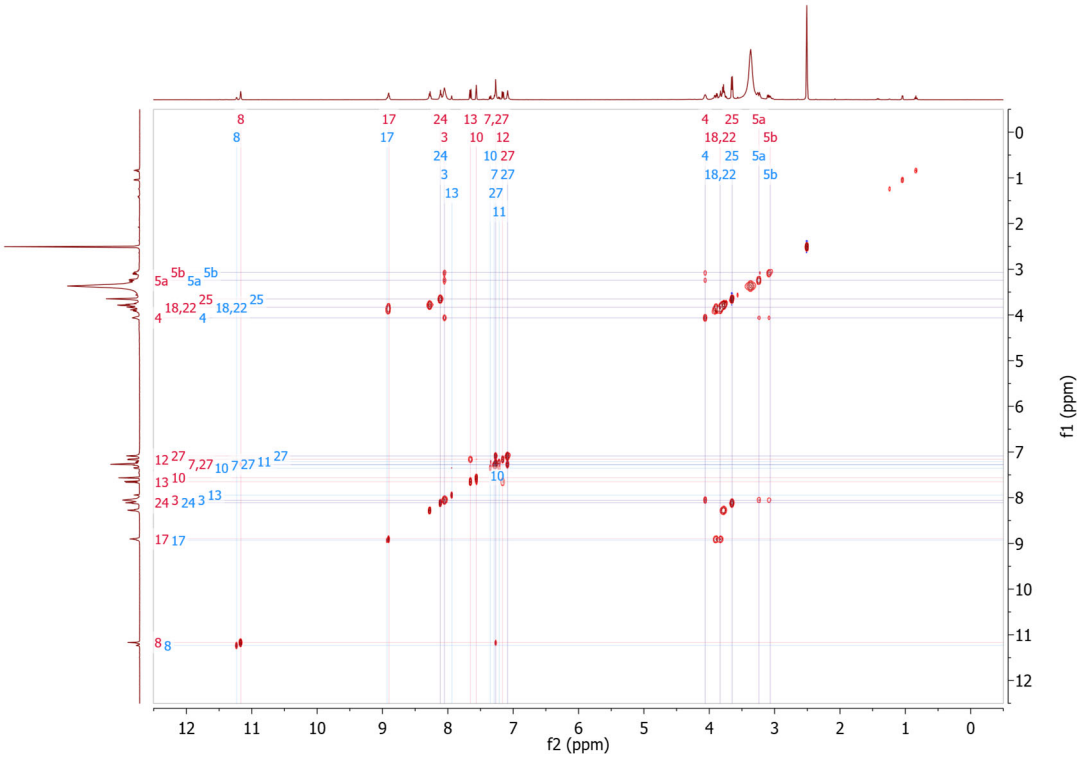
Signals assigned to the 6-Br-isomer are labelled in red; Signals assigned to the 5-Br-isomer are labelled in blue.



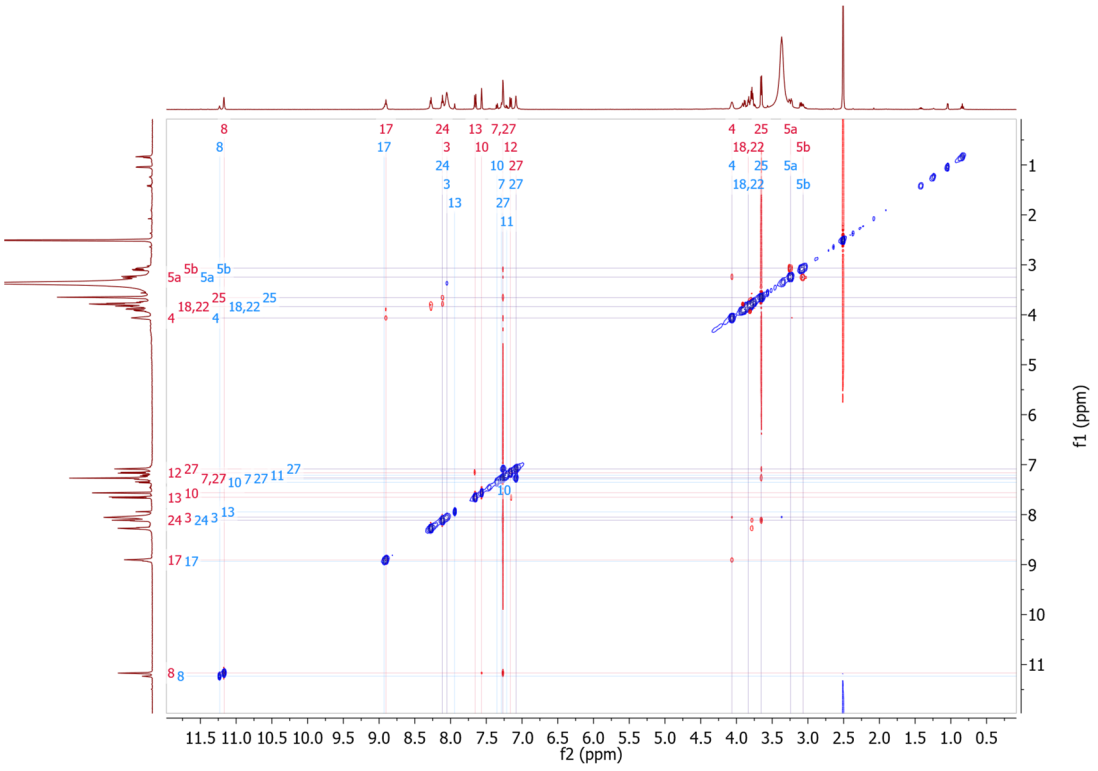
1H-NMR-Spectrum 7



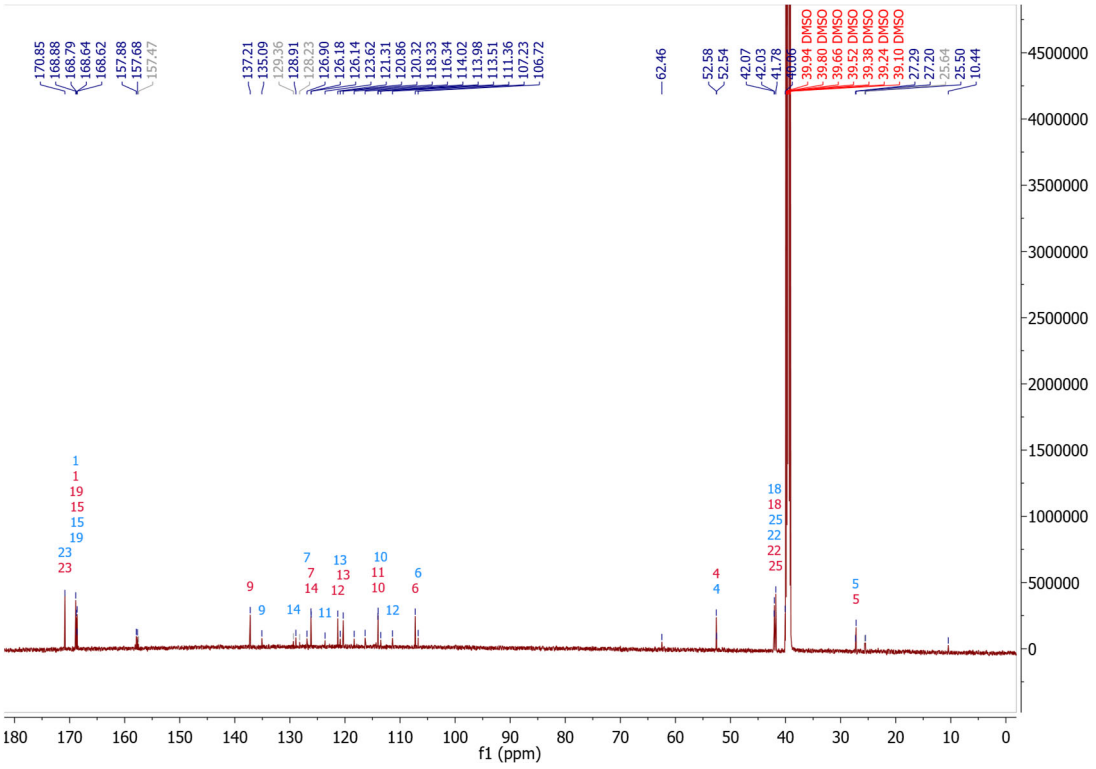
H-H-COSY-Spectrum 7



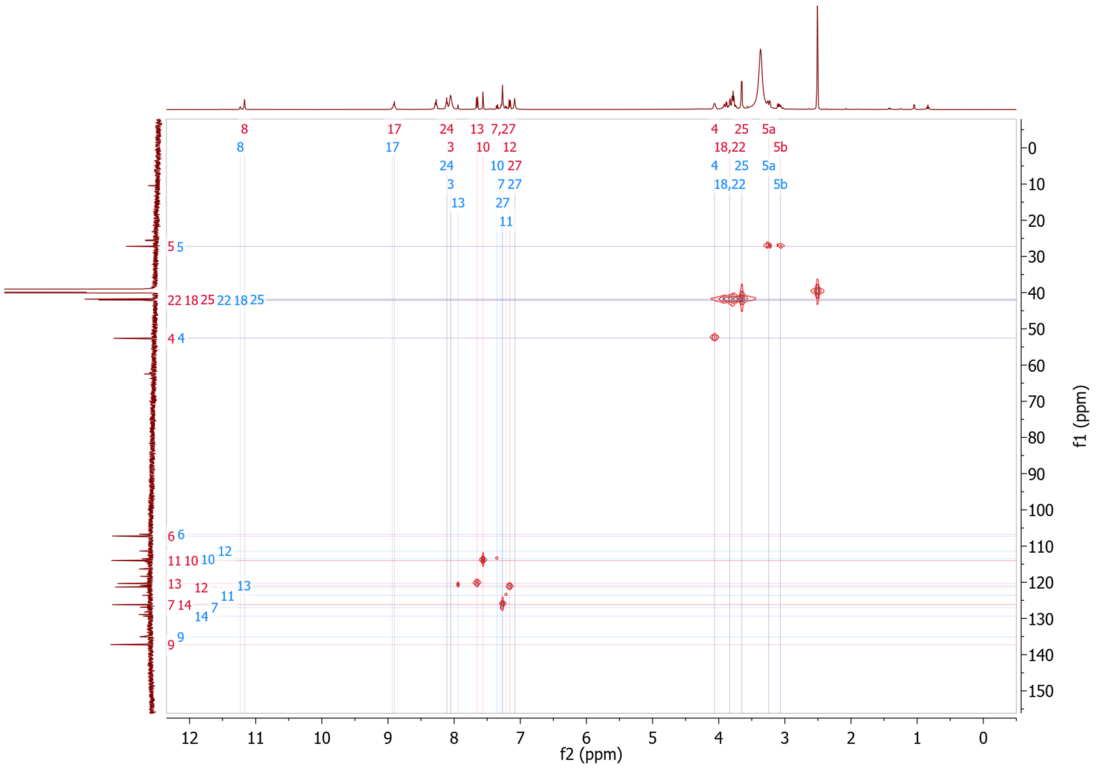
TOCSY-Spectrum 3



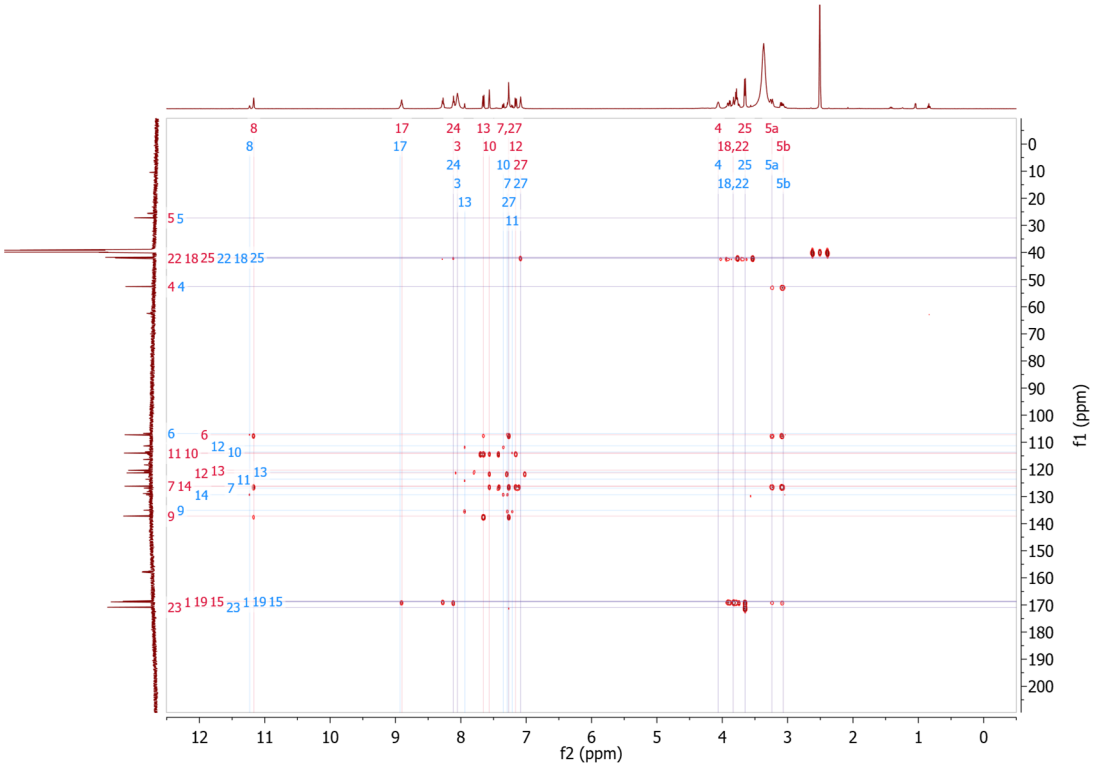
NOESY-Spectrum 6



13C-NMR-Spectrum 5



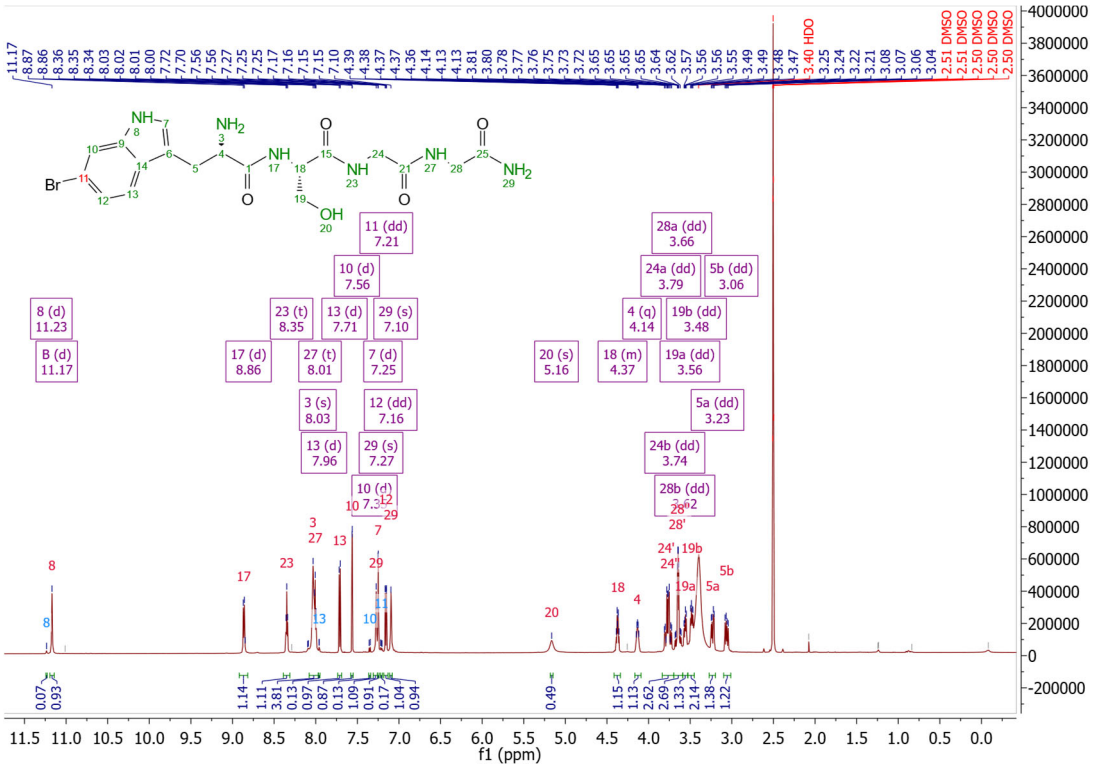
HSQC-Spectrum 5



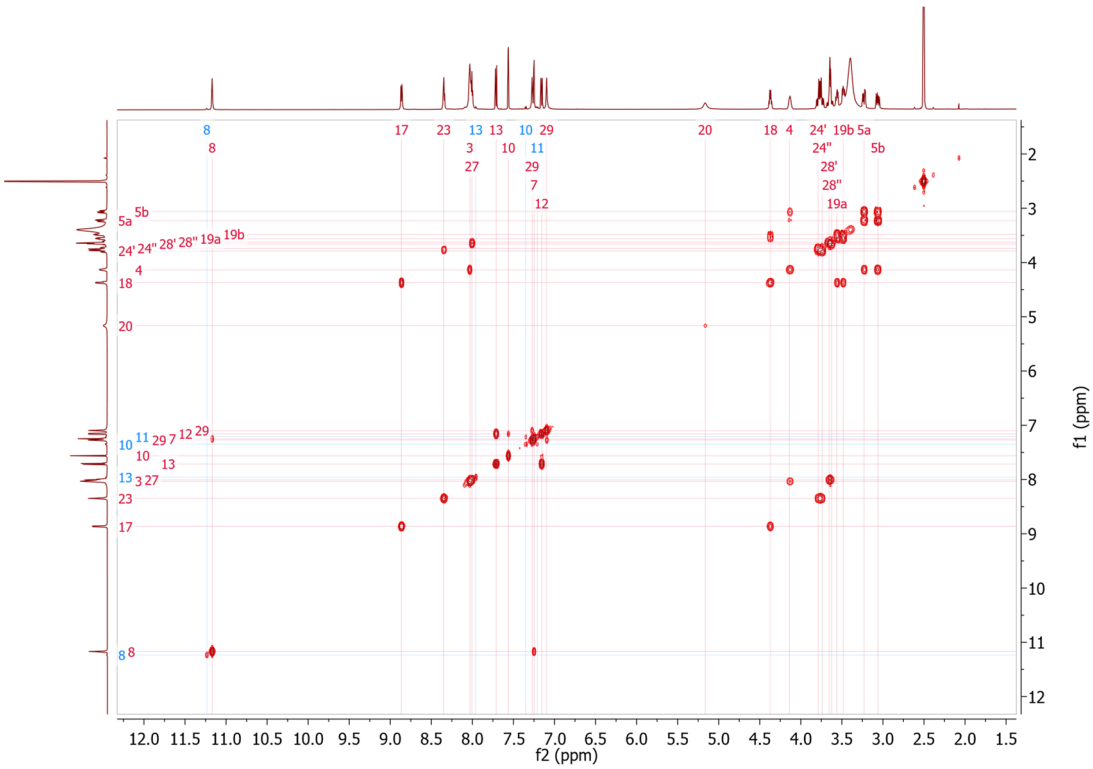
HMBC-Spectrum 5

H-BrTrp-D-Ser-Gly-Gly-NH_2

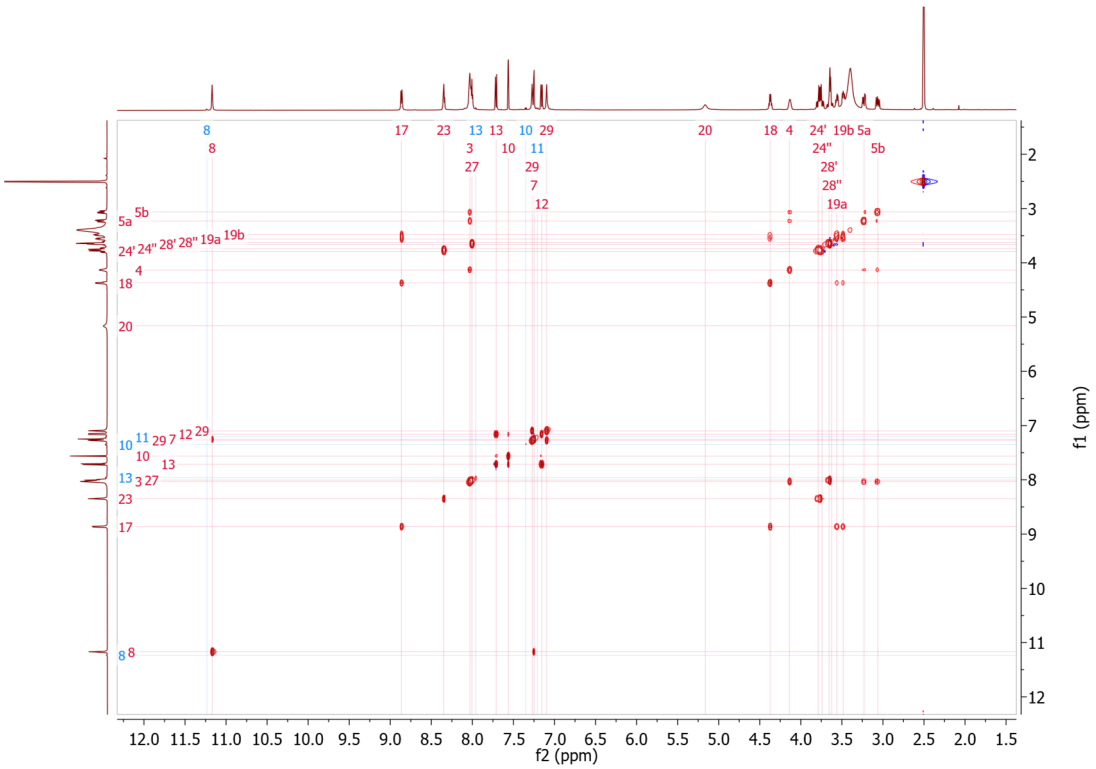
Signals assigned to the 6-Br-isomer are labelled in red; Signals assigned to the 5-Br-isomer are labelled in blue.



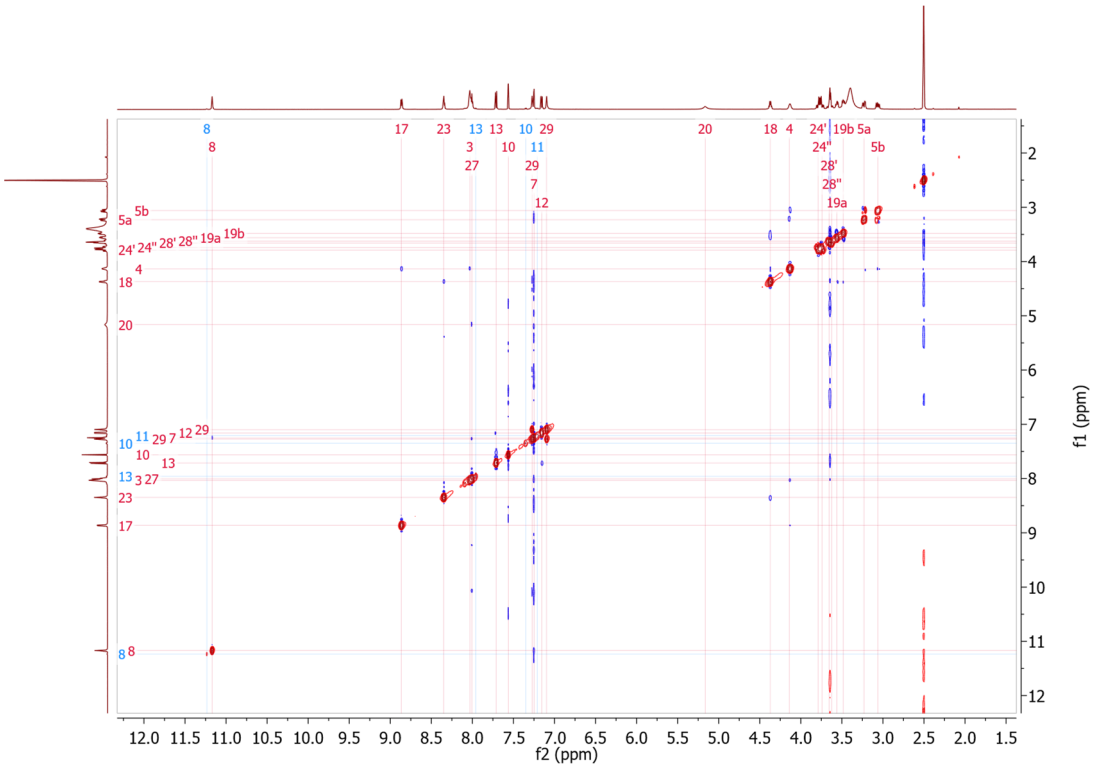
1H-NMR-Spectrum 8



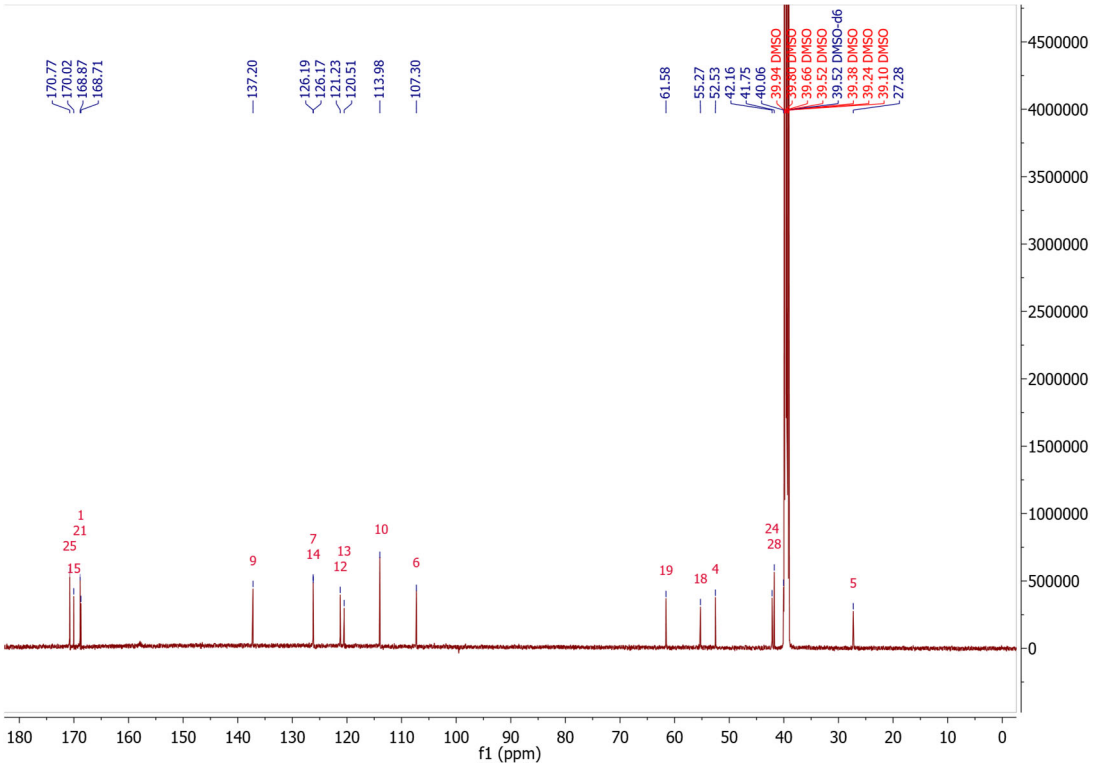
H-H-COSY-Spectrum 8



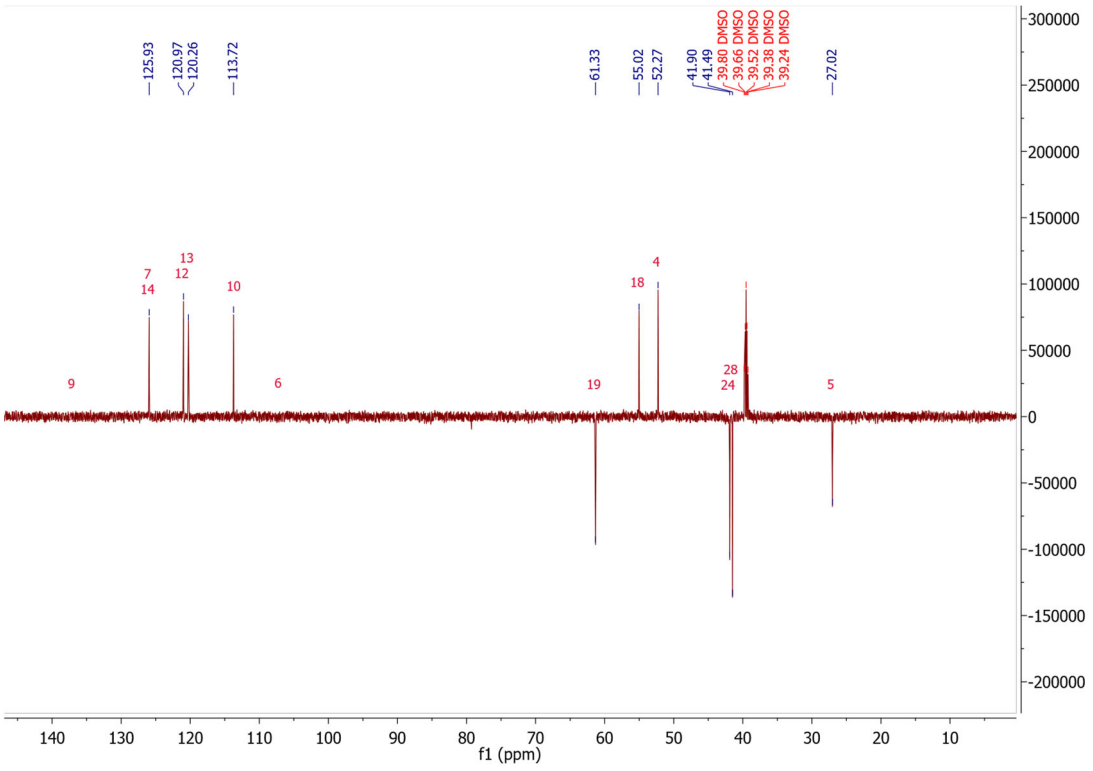
TOCSY-Spectrum 4



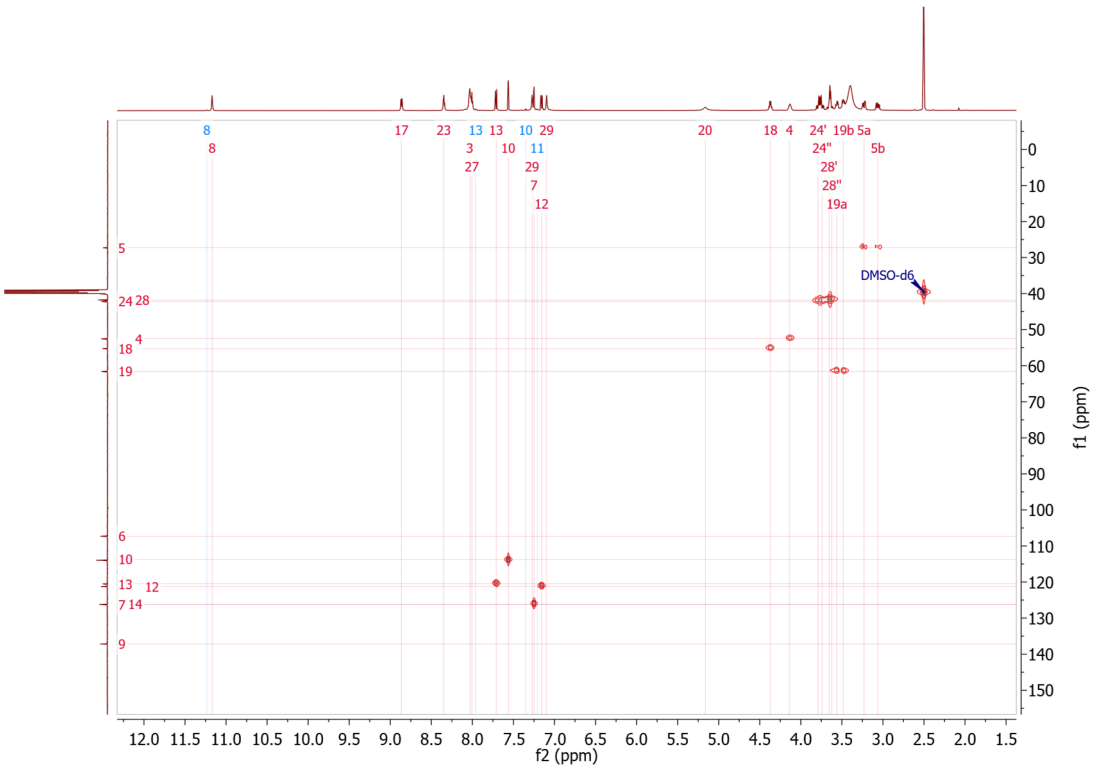
NOESY-Spectrum 7



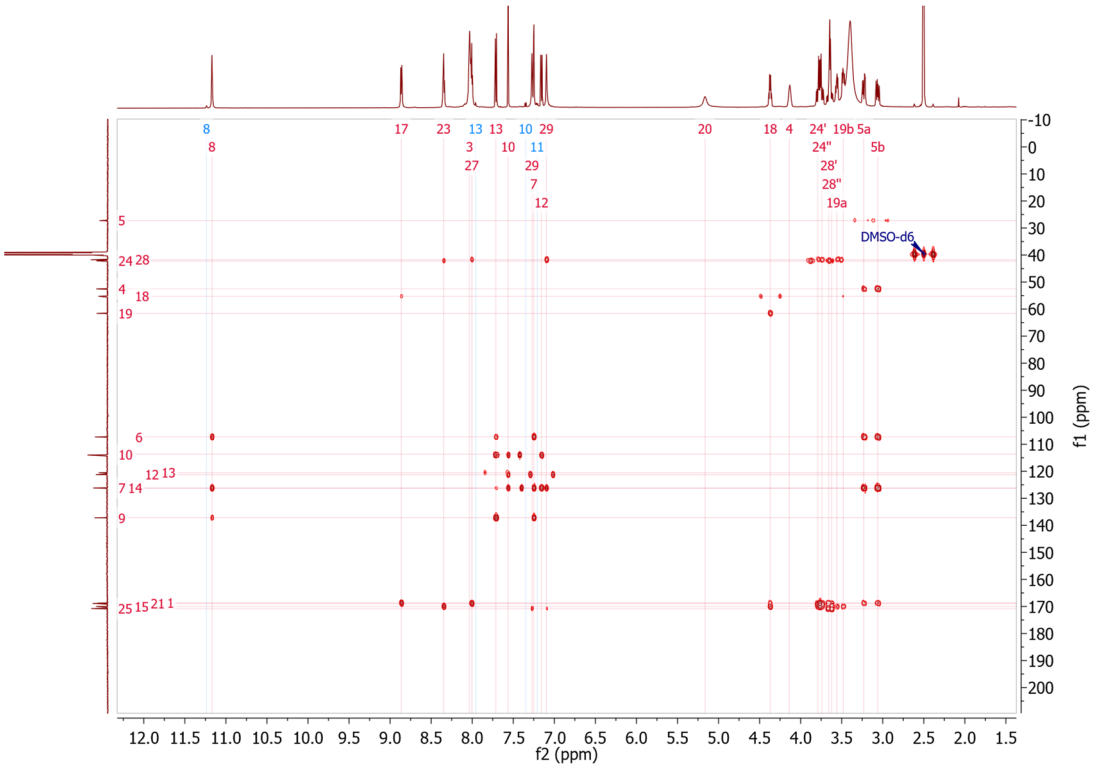
13C-NMR-Spectrum 6



DEPT-Spectrum 1



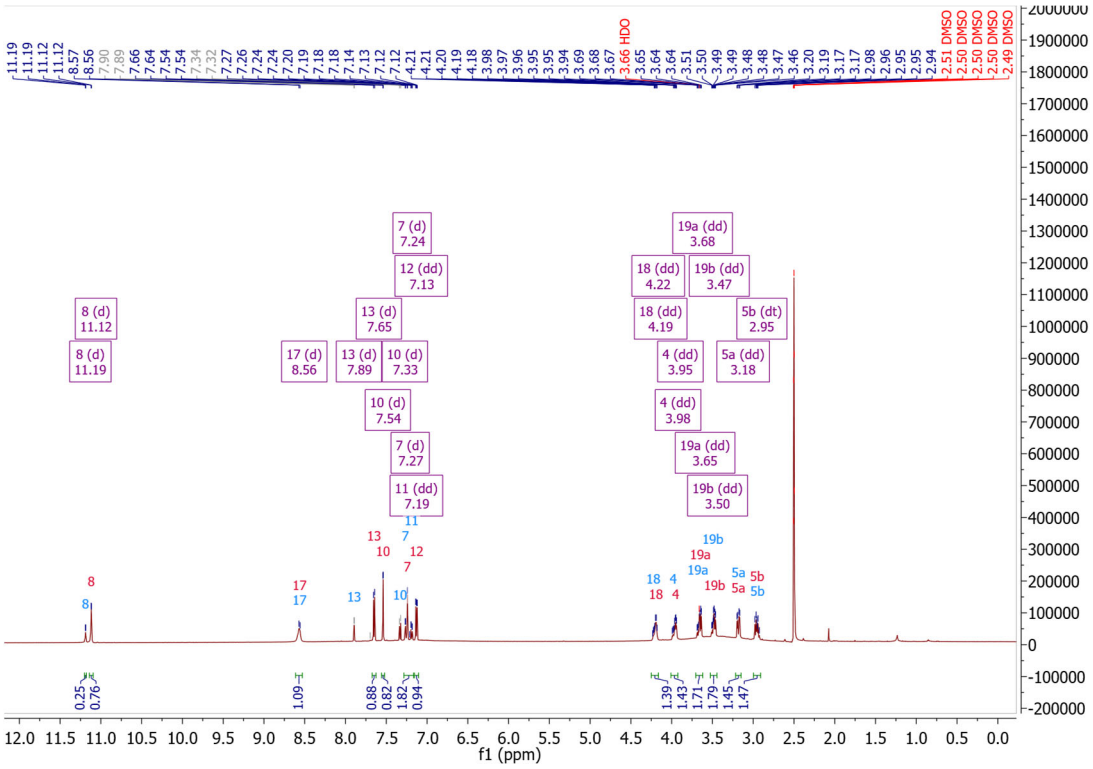
HSQC-Spectrum 6



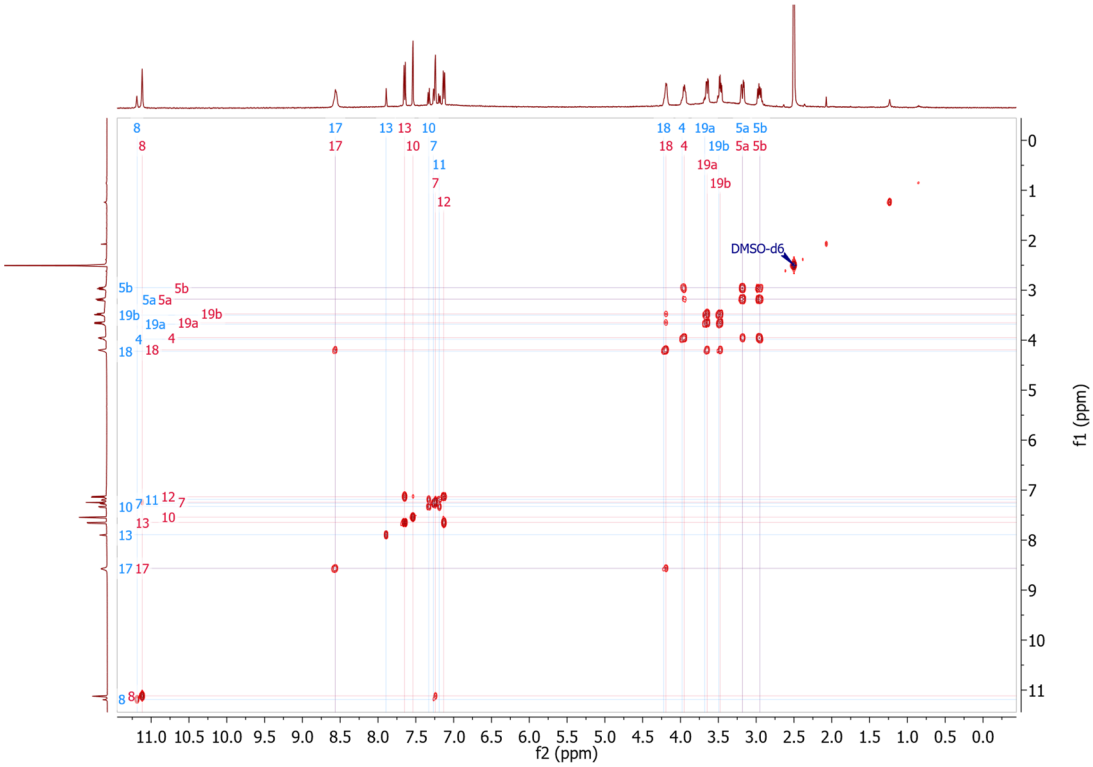
HMBC-Spectrum 6

H-BrTrp-D-Ser-OH

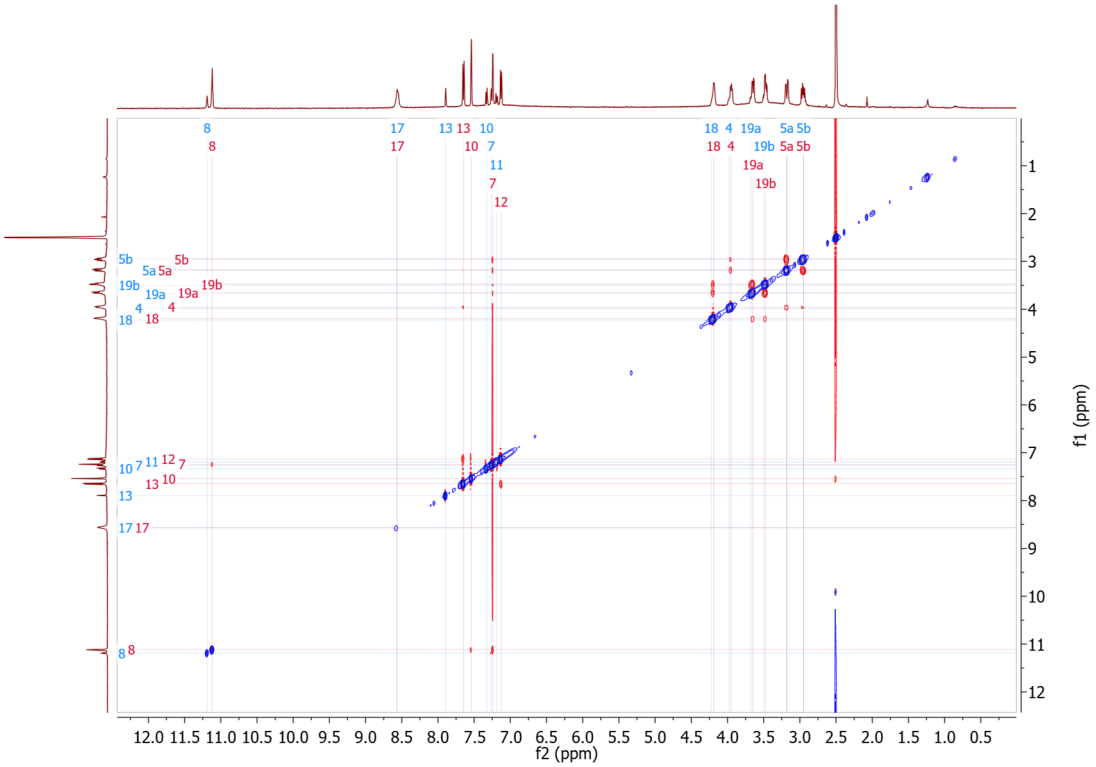
Signals assigned to the 6-Br-isomer are labelled in red; Signals assigned to the 5-Br-isomer are labelled in blue.



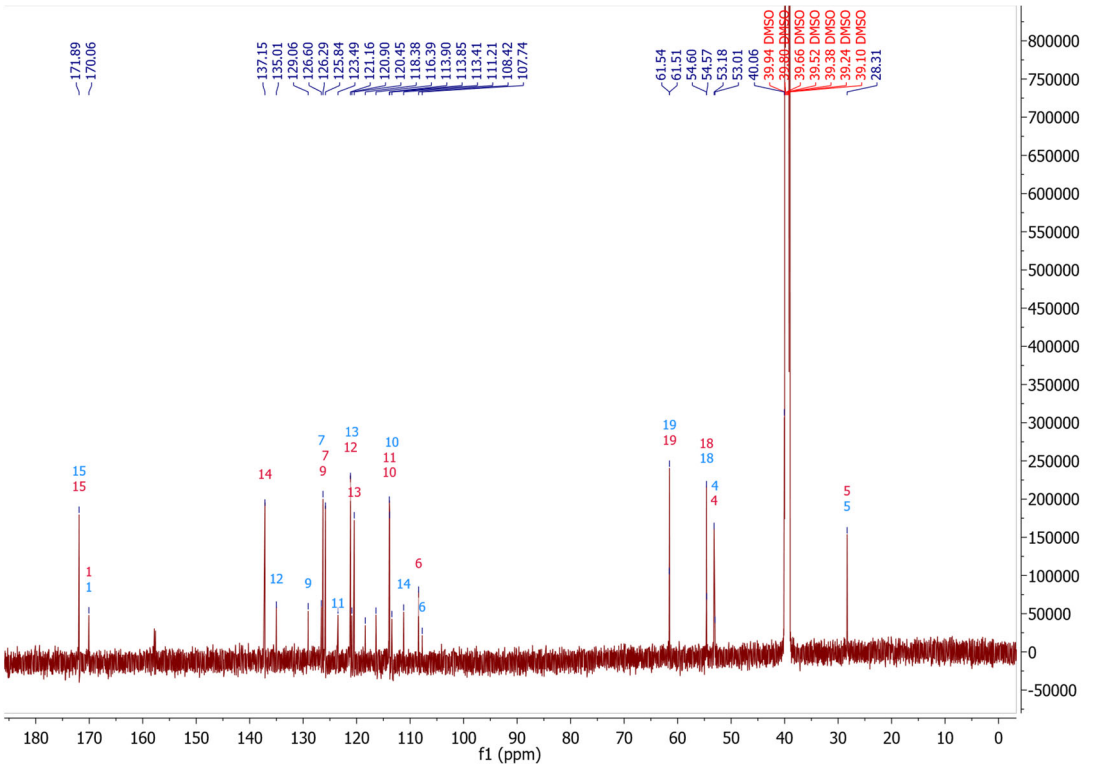
1H-NMR-Spectrum 9



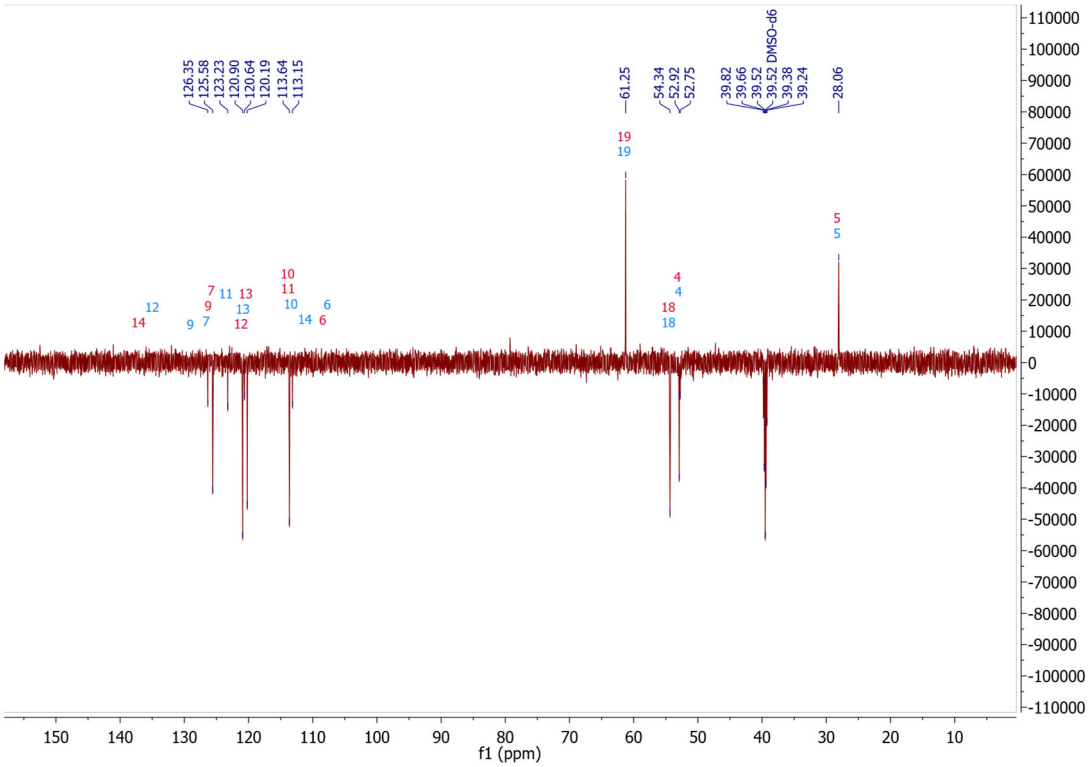
H-H-COSY-Spectrum 9



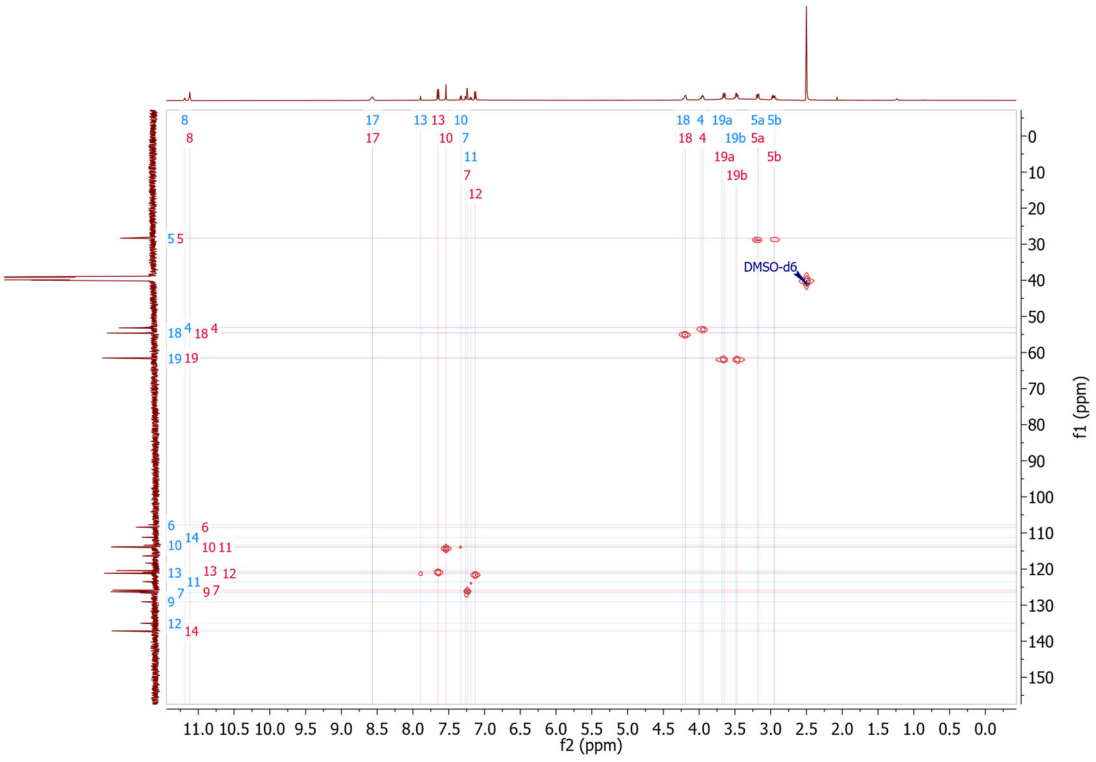
NOESY-Spectrum 8



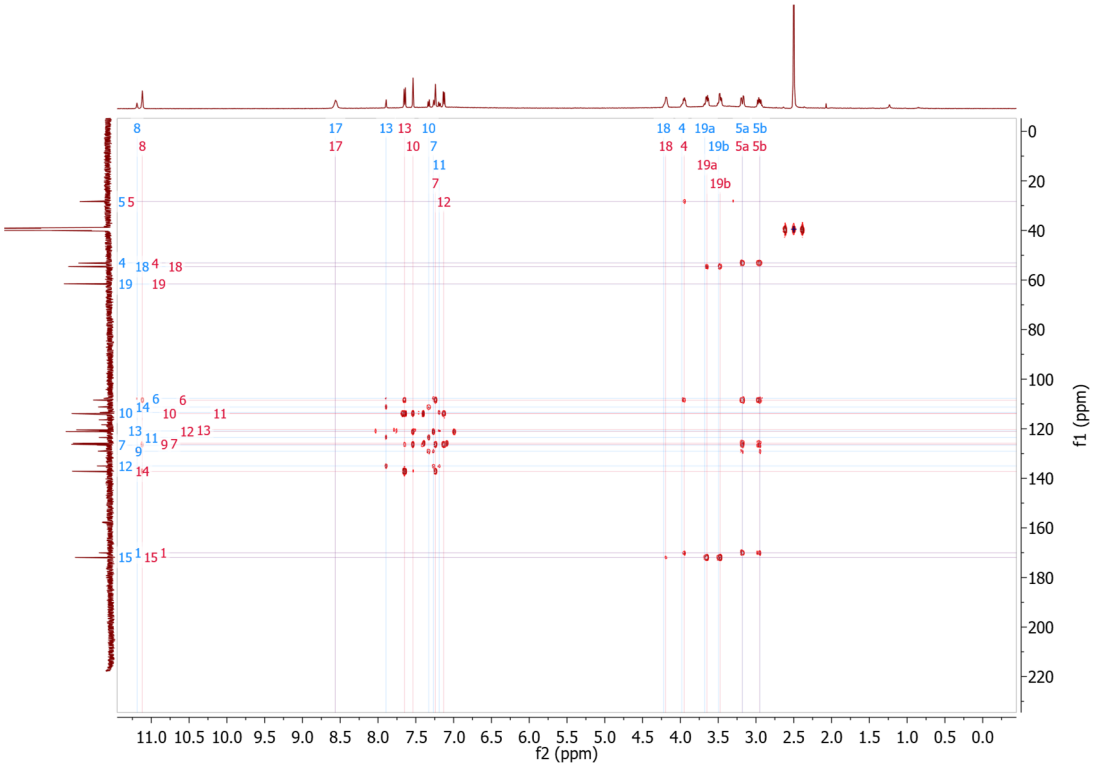
13C-NMR-Spectrum 7



DEPT-Spectrum 2

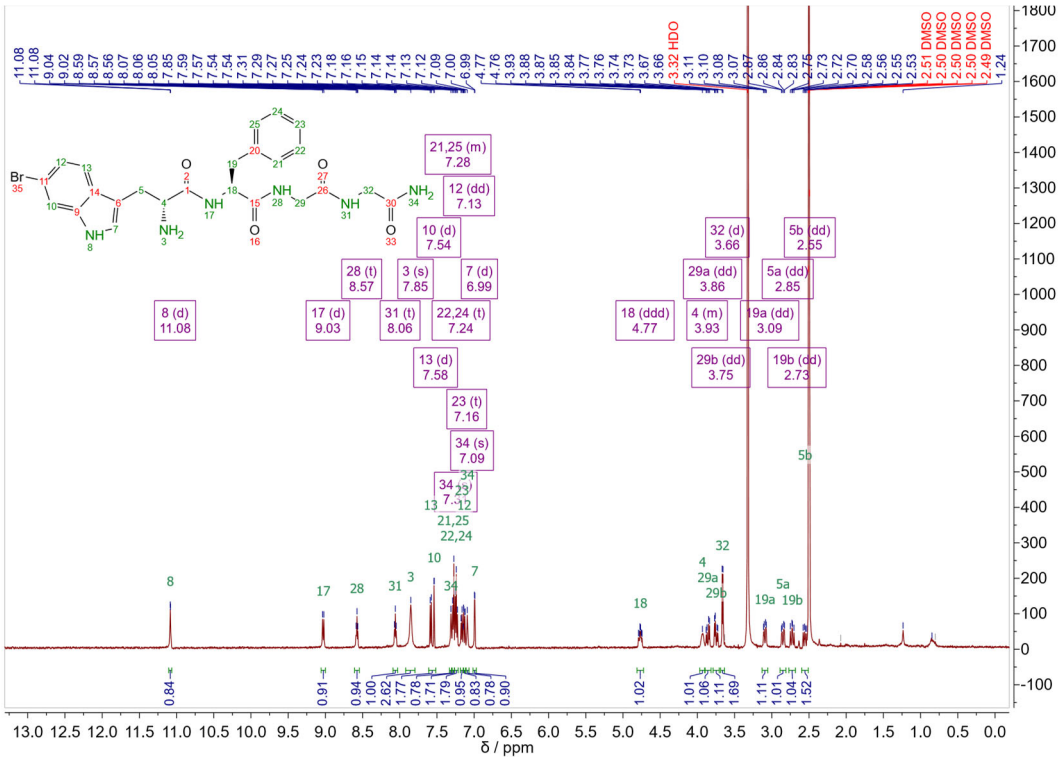


HSQC-Spectrum 7

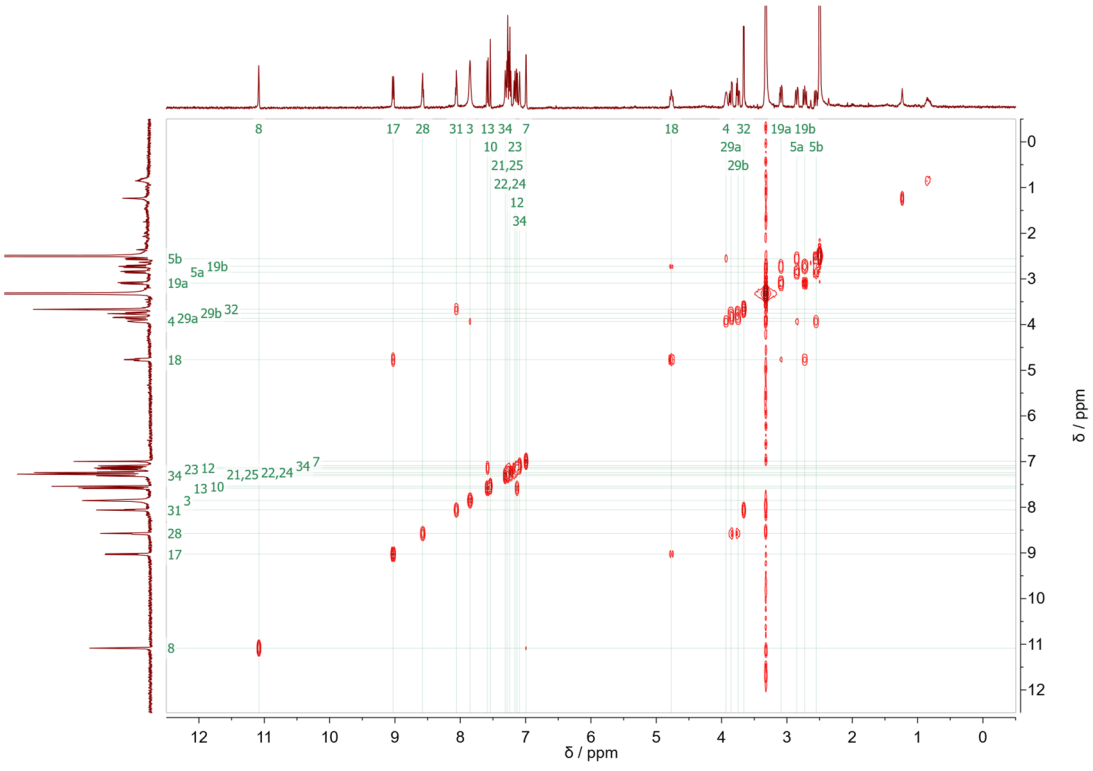


HMBC-Spectrum 7

H-D-6-BrTrp-Phe-Gly-Gly-NH_2 (Br-34)



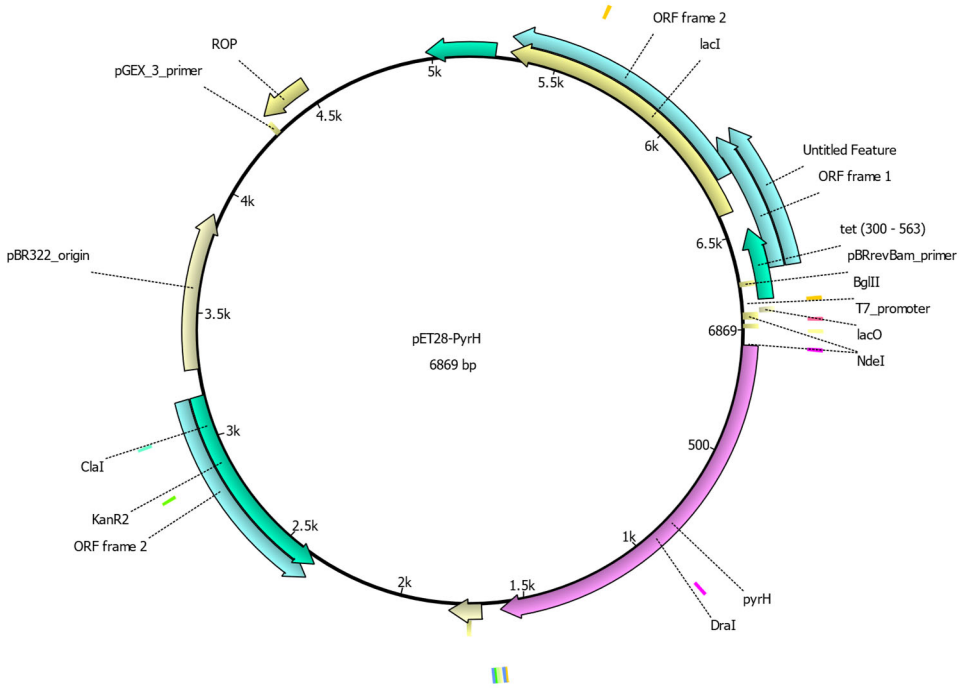
1H-NMR-Spectrum 10



H-H-COSY-Spectrum 10

Plasmids & Protein Sequences

pET28-pyrh



PyrH-Sequence:

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RSTVAPGRDDLEANHIQMRIGRNERTWINNCVAVGLSAAFVEPLESTGIFFIQHAIE
QLVKHFPGERWDPVLISAYNERMAHMVDGVKEFLVLHYKGAQREDTPYWKAAKT
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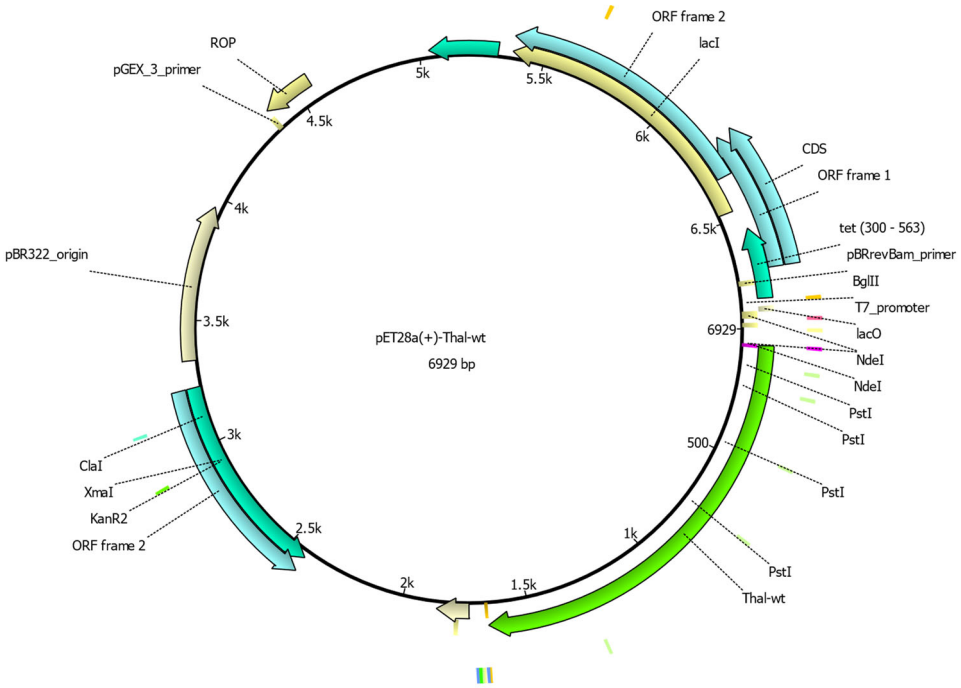
DPAPALAEFERLRREGDELIAALPSCYEYLASIQ*

pET28-thal



Thal-Sequence:

MGSSHHHHHHSSGLVPRGSHMDNRIKTVVILGGGTAGWMTAAYLGKALQNTVKIV
VLEAPTIPRIGVGEATVPNLQRAFFDYLGIPEEEWMRECNASYKMAVKFINWRTPG
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DTAIMNAKKAPRFLDMRRATNYAWHFDASKVAAFLRNFAVTKQAVEHVEDEMTEV
LTDERGFITALRTKSGRILQGDLFVDCSGFRGLLINKAMEEPFIDMSDHLLCNSAVAT
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KLWGLDPDNTEFNHVRFRVGRNRRAWVRNCVSVGLASCFVEPLESSGIYFIYAAIH
MLAKHFPDKTFDKVLVDRFNREIEEMFDDTRDFLQAHYYFSPRVDTPFWRANKELK
LADSIKDKVETYRAGLPVNLPVTDEGTYYGNFEAEFRNFWTNGSYYCIFAGLGLMP
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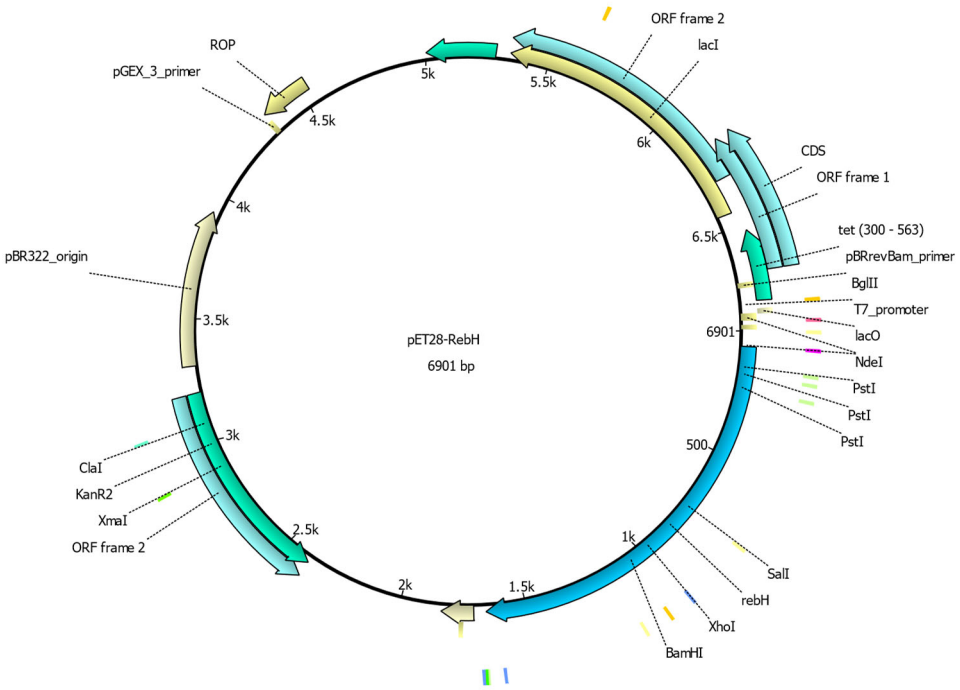
pET28-rebh



RebH-Sequence:

MGSSHHHHHHSSGLVPRGSHMSGKIDKILIVGGGTAGWMAASYLGKALQGTADIT
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VILDANRSPRRLDGSKVTNYAWHFDAHLVADFLRRFATEKLGVRHVEDRVEHVQR
DANGNIESVRTATGRVFDADLFVDCSGFRGLLINKAMEEPFLDMSDHLLNDSAVAT
QVPHDDDANGVEPFTSAIAMKSGWTWKIPMLGRFGTGYVYSSRFATEDEAVREFC
EMWHLDPETQPLNRIRFRVGRNRRAWVGNCVSIGTSSCFVEPLESTGIYFVYAALY
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ADGMQEKIDMYRAGMAINAPASDDAQLYYGNFEEEFRNFWNNSNYYCVLAGLGLV
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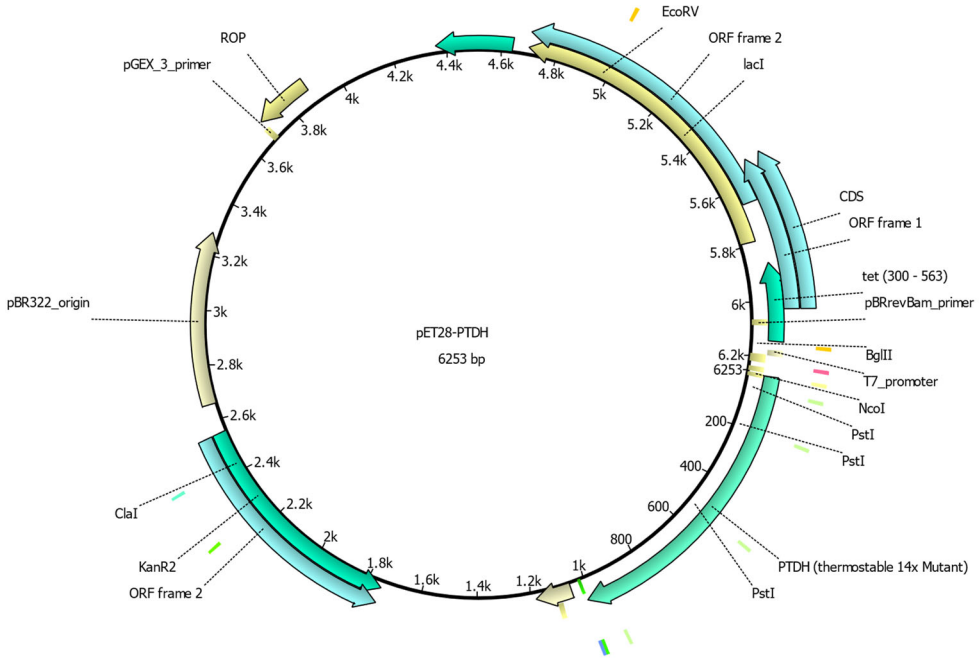
pET28-ptdh



PTDH-Sequence:

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LVRPGALLVNPCRGSVVDEAAVLAALERGQLGGYAADVFEMEDWARADRPQQIDP
ALLAHPNTLFTPHIGSAVRAVRLEIERCAAQNILQALAGERPINAVNRLPKANPAADA
AALEHHHHHH*