Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supporting Information

Photocontrolled chignolin-derived β-hairpin peptidomimetics

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1 General Remarks

Thin-layer chromatography and flash chromatography on silica gel

Thin-layer chromatography for reaction control was performed on aluminium foils (silicagel 60 F_{254}, *Merck KGaA, Grafen/Germany*). Detection of compounds was achieved by either fluorescence quenching at λ = 254 nm or by dyeing with ninhydrin solution (1.5 g ninhydrin, 15 mL glacial acetic acid and 500 mL methanol). Flash chromatography was performed on silicagel (grain size 35-70 µm, *Acros Organics, Geel/Belgium*) under nitrogen pressure (0.2-1.0 bar).

Peptide synthesis

Peptide synthesis was performed on a *CEM Liberty 1 Peptide Synthesizer* with a *CEM Discovery Microwave* (*CEM GmbH, Kamp-Lintfort/*Germany) using standard Fmoc-protected solid phase peptide synthesis protocols. Detailed information to peptide synthesis, resin, coupling reagents and conditions can be found under chapter 2.2.

Reversed-phase HPLC

Analytical RP-HPLC was performed on *Jasco* (*Jasco Germany GmbH, Groß-Umstadt/Germany*) devices (PU-2080 Plus, LG-2080-02-S, DG-2080-53 and MD-2010 Plus) with a *Phenomenex* (*Aschaffenburg/Germany*) *Luna* column (C18, 5 µm, 250x4.6 mm). As eluent a water/acetonitrile gradient with 0.1% TFA with a 1 mL/min flow rate was used. Semi-preparative RP-HPLC was performed on *Jasco* (*Groß-Umstadt/Germany*) devices (PU-2087 Plus, LG-2080-02-S and UV-2075 Plus) with a *Phenomenex* (*Torrance/USA*) *Luna* column (C18, 5 µm, 250x20 mm). As eluent a water/acetonitrile gradient with 0.1% TFA with a 20 mL/min flow rate was used.

NMR spectroscopy

NMR spectra were recorded on *Varian* (*Darmstadt/Germany*) *AC 300* (300 MHz), *WH 400* (400 MHz) and *AMX 600* (600 MHz), as well as on *Bruker* (*Billerica/USA*) *AV-III* (400 MHz) devices. The spectra were recorded, unless otherwise indicated, at room temperature. Chemical shifts δ are denoted in ppm based on TMS as external standard. For the measurements the deuterated solvents $CDCl_3$, CD_3OD and DMSO-d_6 were used. The resonance of the remaining protons in these solvents was used as internal standard [δ ($CDCl_3$) = 7.24, δ (CD_3OD) = 3.31 and δ (DMSO-d_6) = 2.50 ppm]. *J*-coupling constants are given in Hz, multiplicity is abbreviated as s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. NMR spectra were analyzed with the software *MestReC V8.1.4-12489* (*Mestrelab Research, Santiago de Compostela/Spain*).

Mass spectrometry

Electron ionization (EI) measurements were performed with a *Thermo Finnigan MAT 95* (*Thermo Fisher Scientific Inc., Waltham/USA*). Noted are the ionization method, the mass-to-charge ratio value (*m/z*) and related fragmentation. The resolution of EI-MS is 1000 u and of HR-EI-MS is 5000 u. Electron spray ionization (ESI) measurements were recorded on a *Thermo Finnigan LTQFT* (*Thermo Fisher Scientific Inc., Waltham/USA*).

CD spectroscopy

CD measurements were recorded on a *Jasco 810* with a *Jasco CDF-4265* Peltier-Element (*Jasco Germany GmbH, Groß-Umstadt/Germany*) and MeOH as solvent. The used cuvettes were of 1 mm thickness. For baseline correction a pure MeOH spectrum was recorded. Sample concentrations were calculated *via* the specific absorption at 323 nm, with $\varepsilon_{azobenzene}$ = 25000 L $mol^{-1}cm^{-1}$. The recorded spectra were processed with the software *Origin 8.0* (*OriginLab Corporation, Northampton/USA*) and were smoothed with the *Savitzky-Golay-Filter*, using 35 number of points for the CD spectra of **AzoChig1-3**. The temperature and solvent dependent CD spectra were smoothed using 23 number of points.

FT-IR spectroscopy

FT-IR spectroscopy was performed with a *Bruker IFS 66* (*Bruker Optik GmbH, Ettlingen/Germany*). All spectra were recorded with c = 5.0 mM solutions in CD_3OD.

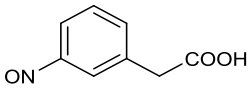
UV/Vis spectroscopy

UV spectra were recorded on a *Jacso V-650* with a *Jasco PAC-743* Peltier-Element (*Jasco Germany GmbH, Groß-Umstadt/Germany*) and MeOH as solvent. The used cuvettes were of 1 mm thickness.

2 Synthesis

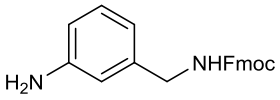
2.1 Synthesis of Building Blocks 1, 5 and 6

Synthesis of 2-(3-nitrosophenyl)acetic acid (4)



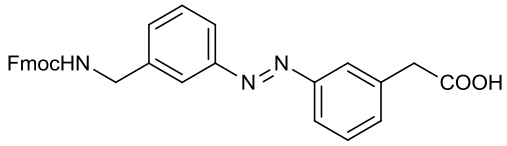
A solution of 2.50 g (13.2 mmol, 1.0 eq.) 2-(3-nitrophenyl)acetic acid in 100 mL 2-methoxyethanol in an argon atmosphere was stirred for 10 min at room temperature, before a solution of 1.09 g (20.3 mmol, 1.5 eq.) NH_4Cl in 25 mL H_2O was added. The reaction solution was cooled to 0 °C and 2.10 g (32.3 mmol, 2.5 eq.) zinc was added portionwise within 30 min. After 1 h, the reaction solution was filtered off and the filtrate was added within 15 min to a 0 °C cold solution of 11.2 g (41.4 mmol, 3.0 eq.) $FeCl_3 \cdot 6\ H_2O$ in 120 mL EtOH/H_2O (2:1). After stirring for 1.5 h, the solution was diluted with 200 mL water and extracted 5 times with 100 mL Et_2O. The combined organic layers were dried with $MgSO_4$ and after removal of the solvent, the crude product was purified *via* column chromatography on silica gel (eluent EtOAc/*c*Hex/AcOH 10:1:0.5). The product was obtained as green-turquoise oil in 95% (2.18 g, 13.2 mmol) yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.16 (d, $J_{H4,H5}$ = 7.5 Hz, 1H), 7.88 (d, $J_{H5,H6}$ = 7.3 Hz, 1H), 7.76 (s, 1H), 7.63 (t, $J_{H4,H5,H6}$ = 7.5 Hz, 1H), 3.82 (s, 2H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 176.6 , 165.5, 136.2, 136.0, 129.6, 121.4, 120.5, 40.3 ppm. EI-MS (positive), m/z: found: 165.09 **[**$C_8H_7O_3N]^+$, calc.: 165.04.

Synthesis of (9*H*-fluoren-9-yl)methyl (3-aminobenzyl)carbamate (2)



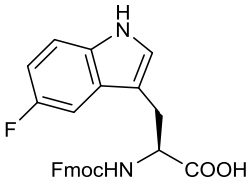
A solution of 14.1 g (44.0 mmol, 1 eq.) Fmoc-*O*Su in 100 mL MeCN was added slowly to a solution of 4.42 mL (44.0 mmol, 1 eq.) 3-(aminobenzyl)amine in 6.1 mL (44.0 mmol, 1 eq.) Et_3N and 55 mL of a MeCN/DMF (10:1) mixture. The resulting solution was stirred for 4 h at room temperature and was subsequently quenched with 50 mL H_2O. The obtained precipitate was filtered off, washed with 50 mL *tert*-butyl methyl ether/trifluoroethanol (1:1) and dried *in vacuo*. The product was obtained as colorless solid (7.85 g, 52%) and used in the next reaction without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, J = 7.2 Hz, 2H), 7.60 (d, $J_{H1,H2}$ = J = 7.4 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.31 (t, J = 7.3 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.65 (d, J = 7.6 Hz, 1H), 6.62-6.58 (m, 3H), 5.03 (bs, 1H), 4.45 (d, J = 6.9 Hz, 2H), 4.30 (d, J = 5.8 Hz, 2H), 4.24 (t, J = 7.0 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 156.4, 146.6, 143.9, 141.3, 139.6, 129.7, 128.7, 127.6, 127.0, 125.0, 120.1, 114.2, 114.0, 66.7, 47.3, 45.0 ppm. HR EI-MS (positive), m/z: found: 344.1596 **[**$C_{22}H_{20}O_2N_2]^+$, calc.: 344.1525.

Synthesis of [3-(3-aminomethyl)phenylazo]acetic acid (Fmoc-AMPP-OH, 1)



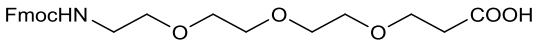
To a solution of 0.27 g (1.65 mmol, 1 eq.) 2-(3-nitrosophenyl)acetic acid (**4**) in 8 mL glacial acetic acid were added portionwise 0.57 g (1.65 mmol, 1 eq.) (9*H*-fluoren-9-yl)methyl (3-aminobenzyl)carbamate. The reaction solution was stirred for 24 h at room temperature, before the solvent was evaporated and the crude product was purified through flash chromatography on silica gel (EtOAc/*c*Hex/AcO 1:1:0.01). The product was obtained as orange solid (3.83 g, 59%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.85-7.81 (m, 4H), 7.75 (d, J = 7.4 Hz, 2H), 7.60 (d, J = 7.1 Hz, 2H), 7.48 (t, J = 8.3 Hz, 2H), 7.42-7.36 (m, 4H), 7.30 (t, J = 7.6 Hz, 2H) 5.22 (bs, 1H), 4.51-4.47 (m, 4H), 4.24 (t, $J_{H9,CH2}$ = 6.9 Hz, 1H), 3.75 (s, 2H) ppm. ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 175.8, 156.5, 152.8, 152.7, 143.9, 141.3, 139.6, 134.7, 134.5, 132.0, 130.1, 129.5, 129.4, 127.7, 127.1, 125.0, 123.6, 122.4, 122.3, 121.4, 120.0, 66.8, 47.3, 44.8, 40.7 ppm. HR EI-MS (positive), m/z: found: 491.1845 **[**$C_{30}H_{25}O_4N_3]^+$, calc.: 491.1845.

Synthesis of (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-fluoro-1*H*-indol-3-yl)-propanoic acid (Fmoc-5FTrp, 5)



125 mg (0.56 mmol, 1 eq.) (*S*)-2-Amino-3-(5-fluoro-1*H*-indol-3-yl)propanoic acid was dissolved in a acetone/10% $NaHCO_3$ (1:1) solution and cooled to 0 °C. 154 mg (0.60 mmol, 1.06 eq.) *N*-Fluorenyl-methoxycarbonyl chloride (Fmoc-Cl) in 2 mL acetone were added and the reaction solution was stirred 1.5 h at 0 °C and subsequently warmed to room temperature and stirred for additional 4 h. Afterwards the solution was triturated with 100 mL H_2O and extracted 3 times with 100 mL Et_2O. The aqueous phase was acidified with 1 M HCl to pH 2 and stirred for 1.5 h at room temperature. The formed precipitate was filtered off, washed with 300 mL cold water and dried *in vacuo*. The product was obtained as colorless solid (178 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.70 (bs, 1H), 10.96 (s, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.72-7.60 (m, 2H), 7.45-7.22 (m, 6H), 6.91 (td, J = 9.2, 2.6 Hz, 1H), 4.25-4.18 (m, 1H), 4.19 (s, 1H), 3.32 (s, 3H), 3.16 (dd, J = 14.7, 4.6 Hz, 1H), 3.00 (dd, J = 14.7, 9.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 173.6, 157.9, 155.9, 155.6, 143.8, 143.7, 140.6, 132.7, 127.6, 127.0, 125.8, 125.3, 125.2, 120.1, 112.3, 112.2, 110.6, 110.6, 109.1, 108.8, 103.1, 102.8, 65.7, 55.0, 46.6, 26.8 ppm. HR EI-MS (positive), m/z: found: 467.1377 **[**$C_{26}H_{21}O_4N_2FNa]^+$, calc.: 467.1383.

Synthesis of *tert*-butyl 1-(9*H*-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-oic acid (6)



The synthesis of Fmoc-TEG-OH (**6**) was achieved by a known synthetic strategy starting with triethylene glycol. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 9.36 (bs, 1H), 7.73 (d, J = 7.5 Hz, 2H), 7.57-7.54 (m, 4H), 7.39-7.27 (m, 4H), 6.45 (bs, 1H), 4.47-4.35 (m, 2H), 4.24-4.16 (m, 1H), 3.75-3.51 (m, 12H), 3.40-3.32 (m, 2H), 2.57 (t, J = 6.0 Hz) ppm. ^{13}C-NMR (150 MHz, $CDCl_3$): δ = 176.1, 144.0, 141.8, 127.9, 127.3, 125.5, 120.2, 70.7, 70.4, 70.2, 66.5, 47.4, 34.9 ppm. HR ESI-MS (positive), m/z: found 444.2014 $[C_{24}H_{29}O_7N+H]^+$, calc.: 444.2017.

2.2 General peptide synthesis

As solid phase pre-loaded Fmoc-Glycine-Wang LL resin (*Novabiochem®, Merck KGaA, Darmstadt Germany*) with 0.36 mmol/g amino acid loading was used. The peptides were synthesized in 0.25 mmol scale with the standard coupling reagents HBTU/HOBt·H_2O 0.5 M in DMF, activated with DIEA 2 M in DMF. Amino acids were coupled in a tenfold excess (2 M solutions) as Fmoc-protected compounds with standard residual protecting groups (*Orpegen Peptide Chemicals GmbH, Heidelberg Germany* and *Sigma-Aldrich, Taufkirchen Germany*). Fmoc deprotection was achieved by treatment with 20% piperidine in DMF. Special building blocks **1** and **5** were coupled in a 1.5 fold excess with the coupling reagents HATU/HOAt·H_2O (1.5 eq.) 0.5 M in DMF activated with NMM (5 eq.) in DMF. Compound **6** was coupled in a fourfold excess with HBTU/HOBt·H_2O/DIEA. All coupling conditions are summarized in table 1. After coupling of all compounds the resin-bound peptide was transferred into a *Merrifield* reactor followed by global deprotection with TFA/phenol/triisopropylsilane/H_2O (88:5:5:2) solution within 2 h. The solvent was then precipitated in 180 mL chilled diethyl ether and stored overnight at -38 °C. The precipitated peptide was centrifugated and after decantation of the solution, the residue was dried and purified with RP-HPLC to yield the desired peptides **AzoChig1-3**.

Table 1: Coupling conditions of the three **AzoChig1-3** peptides. The coupling cycles consist of an initial deprotection step, followed by washing with DMF, addition of amino acid, coupling reagents and base and subsequent microwave-assisted coupling.

	Step	**AzoChig1**			**AzoChig2**			**AzoChig3**		
		t [s]	p [w]	T [°C]	t [s]	p [w]	T [°C]	t [s]	p [w]	T [°C]
Fmoc-Gly-solid phase	1	480	23	75	480	23	75	480	23	75
Fmoc-Trp(Boc)-OH	1	480	23	75				480	23	75
Fmoc-5FTrp-OH (**5**)	1				300	0	60			
	2				1800	23	60			
Fmoc-Thr(O*t*Bu)-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-Gly-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-AMPP-OH (**1**)	1	300	0	60	300	0	60	300	0	60
	2	1800	23	60	1800	23	60	1800	23	60
Fmoc-Pro-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-Asp(O*t*Bu)-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-Tyr-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-Gly-OH	1	480	23	75	480	23	75	480	23	75
Fmoc-TEG-OH (**6**)	1							480	23	75

3 Analysis of peptides AzoChig1-3

3.1 AzoChig1 peptide

The peptide **AzoChig1** was synthesized according to the previously stated peptide synthesis strategy. After global deprotection and cleavage from the resin and subsequent diethyl ether precipitation, the peptide was purified by RP-HPLC with a water/acetonitrile (80:10 → 40:60) gradient. The product was obtained upon lyophilisation with a water/acetonitrile (60:40) mixture. HR ESI-MS (positive), m/z: found 1103.4572 $[C_{54}H_{63}O_{14}N_{12}+H]^+$, calc.: 1103.4508. HR ESI-MS (negative), m/z: found 1101.4420 $[C_{54}H_{61}O_{14}N_{12}-2H]^{2-}$, calc.: 1103.4508.

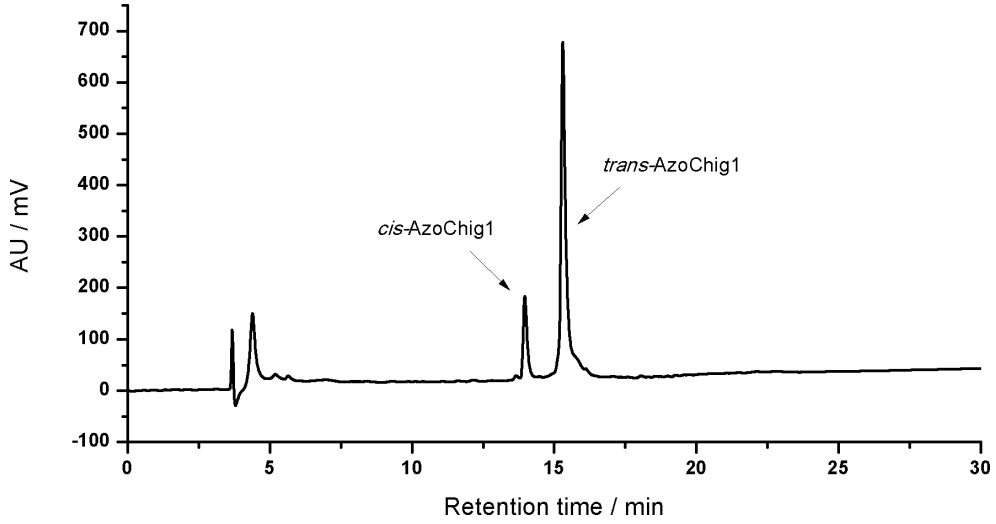


Figure 1: Analytical RP-HPLC spectrum of **AzoChig1** peptide. Retention time *cis* isomer = 13.9 min, *trans* isomer = 15.5 min.

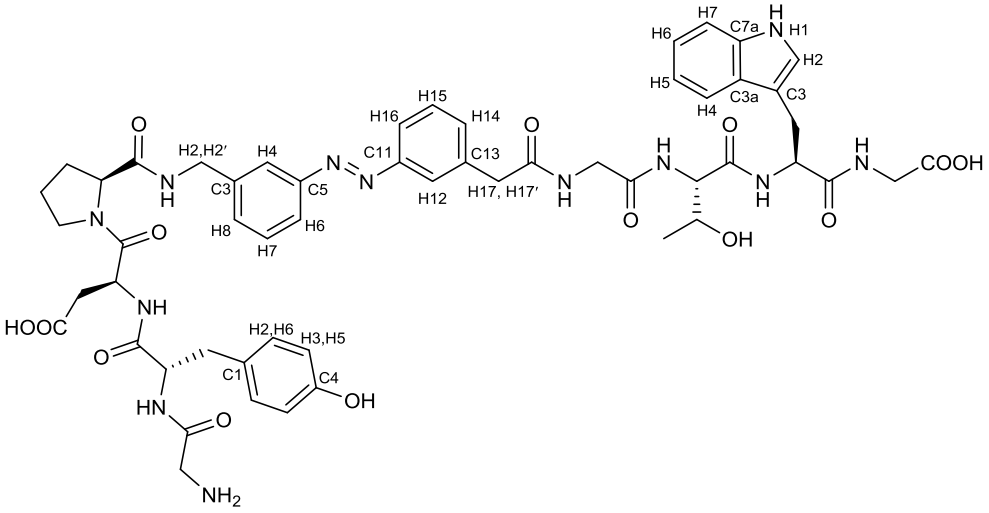


Figure 2: Numbering of certain H and C atoms in the **AzoChig1** peptide.

Table 2: ^{1}H- and ^{13}C-NMR chemical shifts of *trans*-**AzoChig1** peptide. All NMR spectra where recorded in CD_3OD, thus no NH and OH signals were recorded.

trans AzoChig1	H [ppm]	m	*J* [Hz]	C [ppm]	*trans* AzoChig1	H [ppm]	m	*J* [Hz]	C [ppm]
G01/NH2	-	-	-	-	**AMPP56/C11**	-	-	-	154.1
G01/Hα,Hα'	3.63	dd	16.0, 5.0	41.4	**AMPP56/H12**	7.96	s	-	125.1
G01/CO	-	-	-	167.7	**AMPP56/C13**	-	-	-	138.0
Y02/NH	-	-	-	-	**AMPP56/H14**	7.46	d	7.8	133.2
Y02/Hα	4.65	t	7.5	56.2	**AMPP56/H15**	7.47	t	7.8	133.4
Y02/Hβ,Hβ'	2.98, 2.83	dd	13.9, 7.9	38.3	**AMPP56/H16**	7.79	d	7.8	122.2
Y02/C1	-	-	-	128.3	**AMPP56/H17,H17'**	3.73	s	-	43.2
Y02/H2,H6	7.01	d	8.3	131.4	**AMPP56/CO**	-	-	-	174.4
Y02/H3,H5	6.68	d	8.3	116.2	**G07/NH**	-	-	-	-
Y02/C4	-	-	-	157.5	**G07/Hα,Hα'**	3.85	dd	16.5, 10.4	44.0
Y02/OH	-	-	-	-	**G07/CO**	-	-	-	172.1
Y02/CO	-	-	-	172.8	**T08/NH**	-	-	-	-
D03/NH	-	-	-	-	**T08/Hα**	4.29	d	4.1	60.2
D03/Hα	4.93	dd	8.7, 5.5	49.1	**T08/Hβ**	4.08	dq	6.2, 5.3	68.2
D03/Hβ,Hβ'	3.01, 2.61	dd	17.3, 4.7	37.0	**T08/Hγ**	1.05	d	6.5	19.9
D03/COOH	-	-	-	174.9	**T08/OH**	-	-	-	-
D03/CO	-	-	-	171.6	**T08/CO**	-	-	-	172.3
P04/N	-	-	-	-	**W09/NH**	-	-	-	-
P04/Hα	4.46	dd	8.9, 3.5	62.2	**W09/Hα**	4.70	dd	8.0, 5.5	55.6
P04/Hβ,Hβ'	2.20, 2.07	d	8.3	30.8	**W09/Hβ,Hβ'**	3.32, 3.13	m	-	28.6
P04/Hγ,Hγ'	1.97	bs	-	25.4	**W09/NH**	-	-	-	-
P04/Hδ,Hδ'	3.75, 3.60	m	-	48.6	**W09/H2**	7.13	s	-	124.8
P04/CO	-	-	-	174.2	**W09/C3**	-	-	-	110.9
AMPP56/NH	8.31	t	6.1	-	**W09/C3a**	-	-	-	128.8
AMPP56/H2,H2'	4.52, 4.41	d	15.5	43.5	**W09/H4**	7.29	d	8.4	112.3
AMPP56/C3	-	-	-	141.3	**W09/H5**	7.00	t	7.9	119.8
AMPP56/H4	7.78	s	-	121.6	**W09/H6**	7.06	t	7.9	122.4
AMPP56/C5	-	-	-	154.2	**W09/H7**	7.56	d	7.9	119.3
AMPP56/H6	7.76	d	7.8	123.2	**W09/C7a**	-	-	-	137.9
AMPP56/H7	7.47	t	7.8	130.5	**W09/CO**	-	-	-	174.3
AMPP56/H8	7.40	d	7.4	131.0	**G10/NH**	-	-	-	-
AMPP56/N9	-	-	-	-	**G10/Hα,Hα'**	3.92	d	16.5	41.9
AMPP56/N10	-	-	-	-	**G10/COOH**	-	-	-	171.7

Table 3: ^{1}H- and ^{13}C-NMR chemical shifts of *cis*-**AzoChig1** peptide. All NMR spectra where recorded in CD_3OD, thus no NH and OH signals were recorded.

cis AzoChig1	H [ppm]	m	*J* [Hz]	C [ppm]	*cis* AzoChig1	H [ppm]	m	*J* [Hz]	C [ppm]
G01/NH2	-	-	-	-	**AMPP56/C11**	-	-	-	154.8
G01/Hα,Hα'	3.61	dd	16.0, 5.0	41.4	**AMPP56/H12**	6.69	s	-	121.6
G01/CO	-	-	-	167.0	**AMPP56/C13**	-	-	-	137.6
Y02/NH	-	-	-	-	**AMPP56/H14**	6.88	d	7.8	121.1
Y02/Hα	4.62	m	7.5	56.3	**AMPP56/H15**	7.27	t	7.8	130.2
Y02/Hβ,Hβ'	2.88, 2.74	dd	13.6, 8.1	38.3	**AMPP56/H16**	7.14	m	-	130.0
Y02/C1	-	-	-	128.2	**AMPP56/H17,H17'**	3.47	s	-	43.1
Y02/H2,H6	6.90	d	8.3	131.3	**AMPP56/CO**	-	-	-	174.3
Y02/H3,H5	6.62	d	8.3	116.1	**G07/NH**	-	-	-	-
Y02/C4	-	-	-	157.4	**G07/Hα,Hα'**	3.90	dd	16.5, 10.4	44.0
Y02/OH	-	-	-	-	**G07/CO**	-	-	-	172.1
Y02/CO	-	-	-	172.5	**T08/NH**	-	-	-	-
D03/NH	-	-	-	-	**T08/Hα**	4.39	d	4.4	60.0
D03/Hα	4.97	m	-	48.5	**T08/Hβ**	4.09	m	-	68.5
D03/Hβ,Hβ'	2.92, 2.60	dd	17.3, 4.7	37.1	**T08/Hγ**	1.10	d	6.5	19.9
D03/COOH	-	-	-	174.6	**T08/OH**	-	-	-	-
D03/CO	-	-	-	171.3	**T08/CO**	-	-	-	172.1
P04/N	-	-	-	-	**W09/NH**	-	-	-	-
P04/Hα	4.33	dd	8.9, 3.5	62.0	**W09/Hα**	4.80	dd	8.0, 5.5	55.5
P04/Hβ,Hβ'	2.19, 2.07	m	-	30.8	**W09/Hβ,Hβ'**	3.35, 3.15	m	-	28.8
P04/Hγ,Hγ'	1.93	m	-	25.4	**W09/NH**	-	-	-	-
P04/Hδ,Hδ'	3.71, 3.54	m	-	48.6	**W09/H2**	7.13	m	-	124.8
P04/CO	-	-	-	174.1	**W09/C3**	-	-	-	111.0
AMPP56/NH	-	-	-	-	**W09/C3a**	-	-	-	128.8
AMPP56/H2,H2'	4.24, 4.20	d	15.5	43.5	**W09/H4**	7.27	d	8.4	112.3
AMPP56/C3	-	-	-	141.0	**W09/H5**	6.98	m	-	119.8
AMPP56/H4	6.80	s	-	120.7	**W09/H6**	7.06	m	-	122.3
AMPP56/C5	-	-	-	155.1	**W09/H7**	7.57	m	-	119.3
AMPP56/H6	6.59	d	7.8	119.6	**W09/C7a**	-	-	-	137.7
AMPP56/H7	7.13	m	-	124.7	**W09/CO**	-	-	-	174.3
AMPP56/H8	7.06	m	-	127.6	**G10/NH**	-	-	-	-
AMPP56/N9	-	-	-	-	**G10/Hα,Hα'**	3.94	d	16.5	42.0
AMPP56/N10	-	-	-	-	**G10/COOH**	-	-	-	172.9

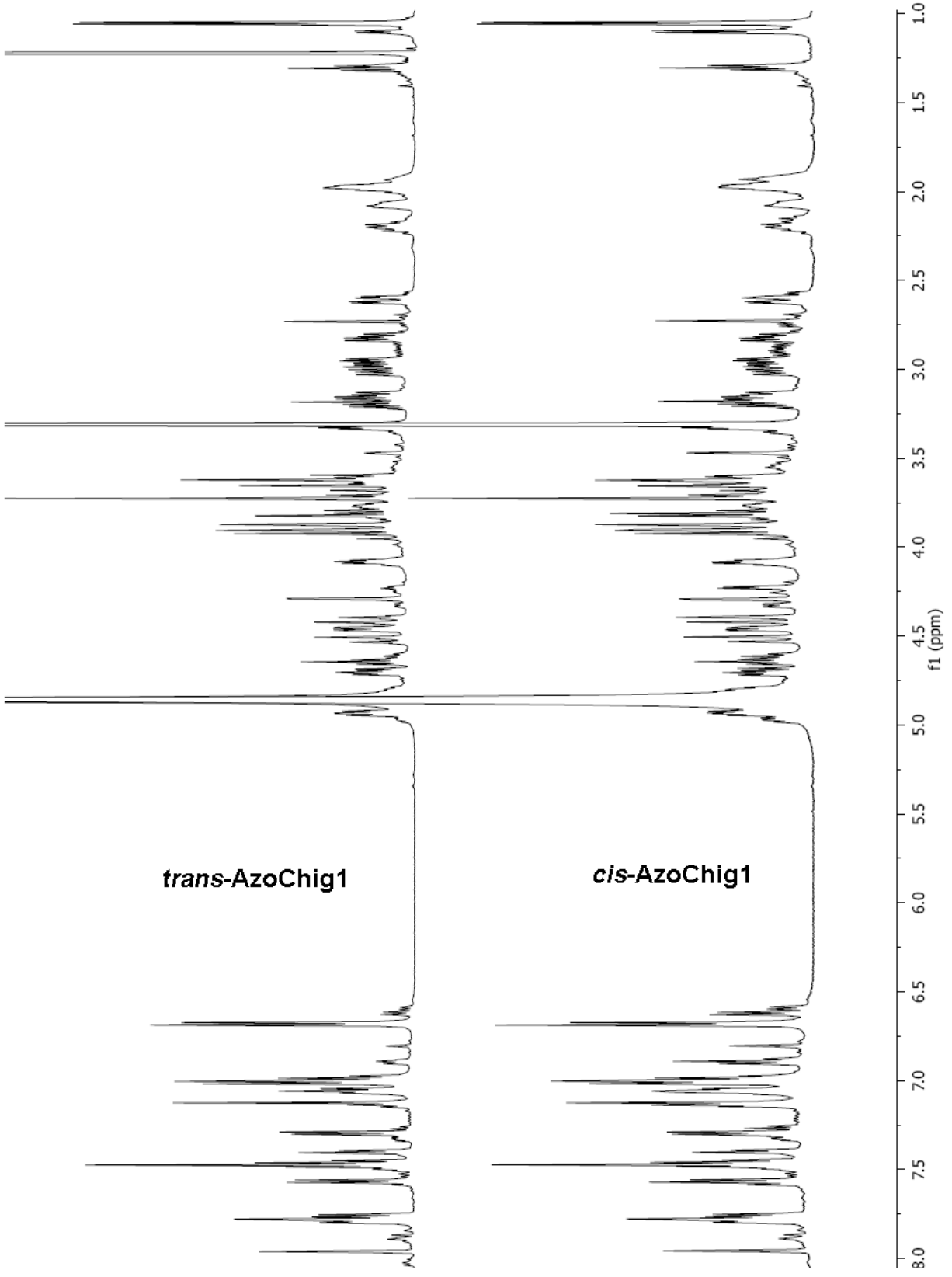


Figure 3: Top: ^{1}H-NMR spectrum of *trans*-**AzoChig1** peptide in MeOD-d_4. Bottom: ^{1}H-NMR spectrum of *cis*-**AzoChig1** peptide in MeOD-d_4.

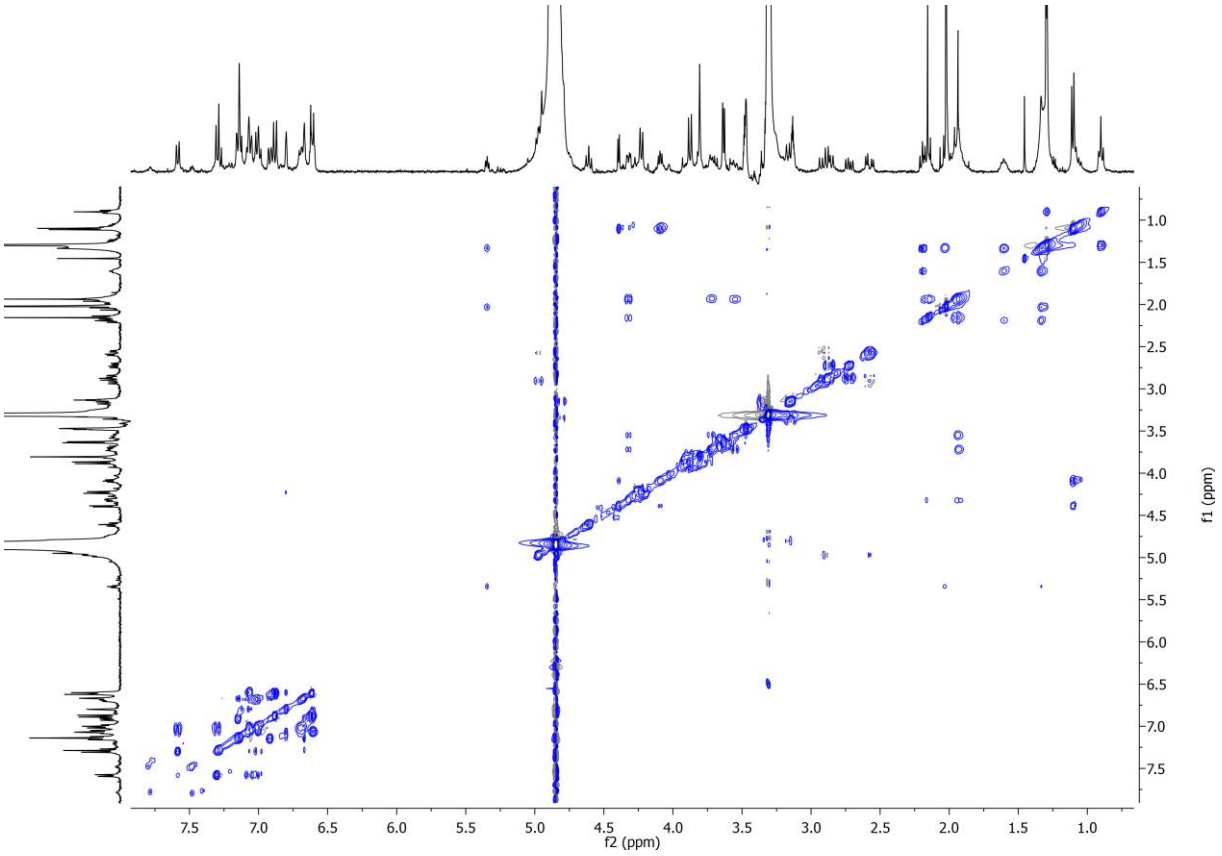


Figure 4: ^{1}H,^{1}H-TOCSY NMR spectrum of *cis*-**AzoChig1** peptide in MeOD-d_4, *c* = 2 mM.

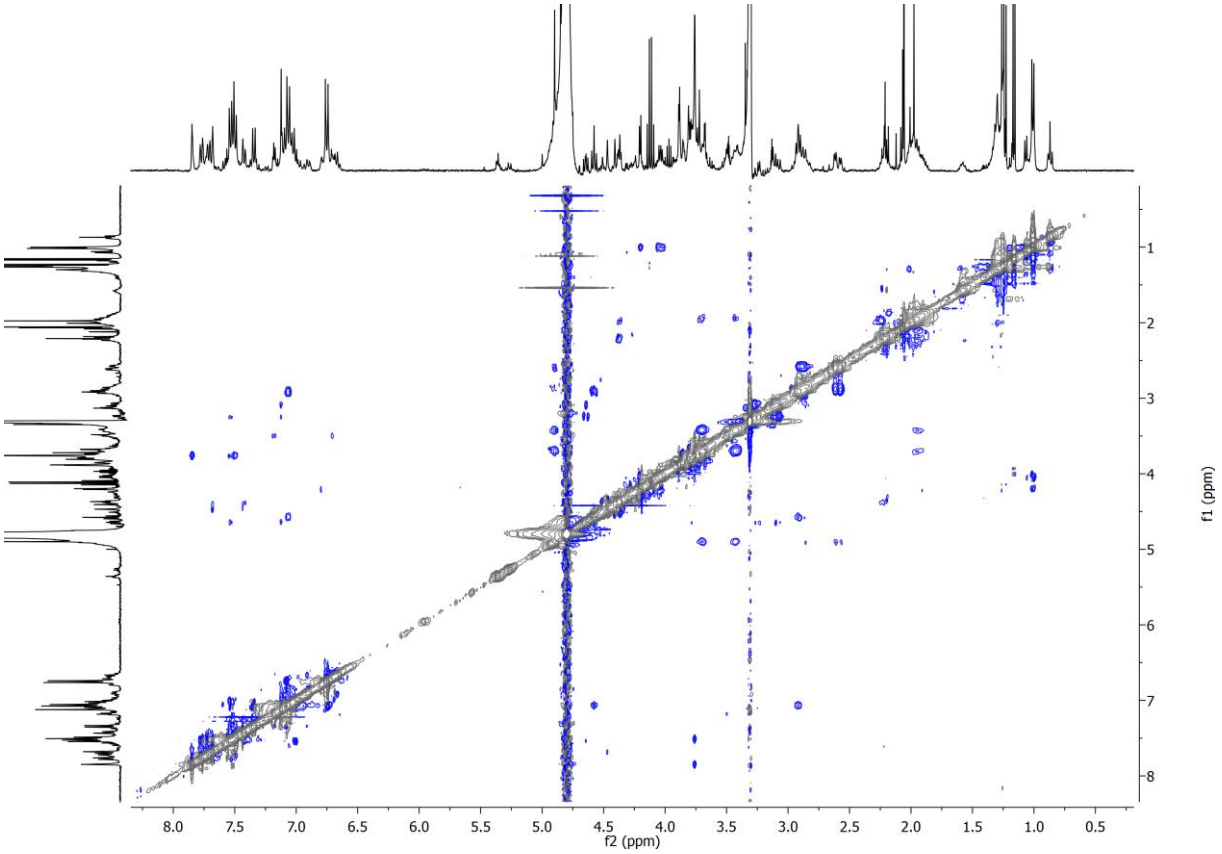


Figure 5: ^{1}H,^{1}H-ROESY NMR spectrum of *trans*-**AzoChig1** peptide in MeOD-d_4, *c* = 2 mM.

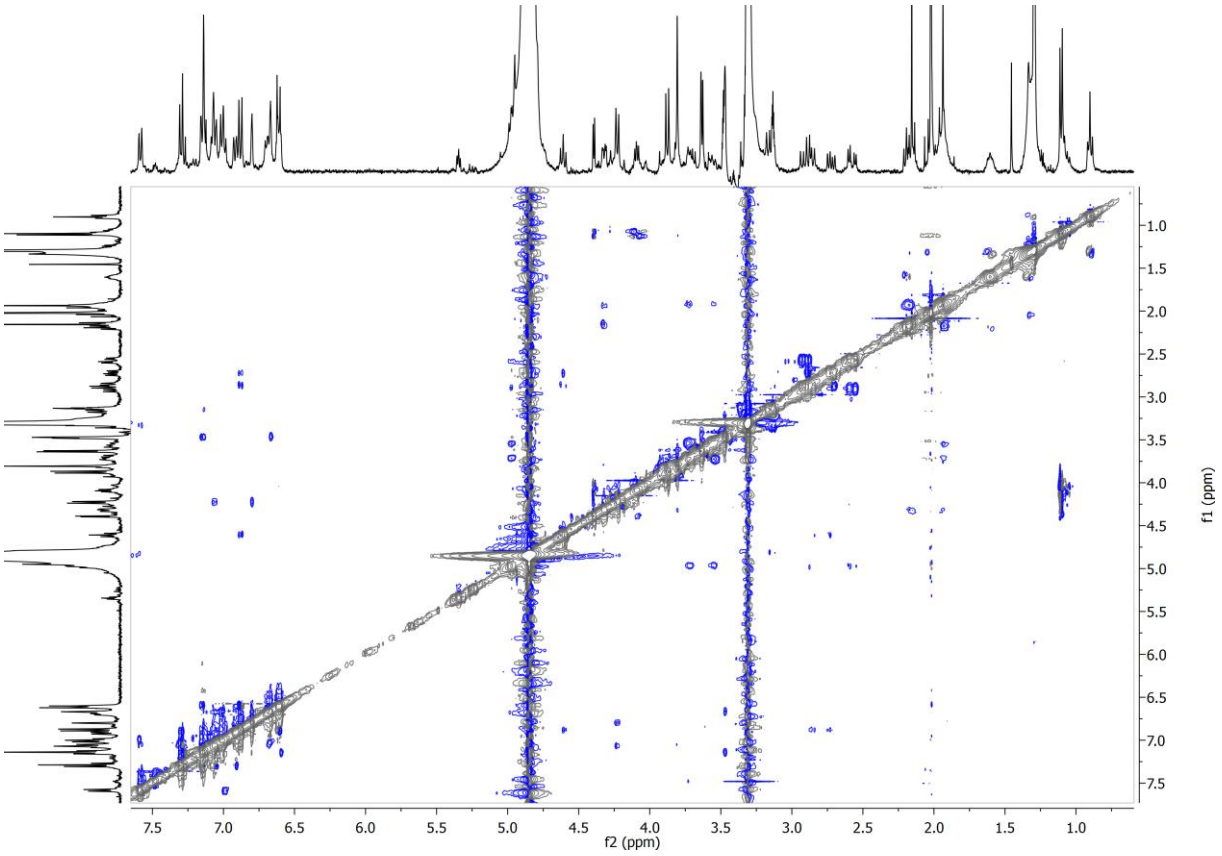


Figure 6: ^{1}H,^{1}H-ROESY NMR spectrum of *cis*-**AzoChig1** peptide in MeOD-d_4, c = 2 mM.

3.2 AzoChig2 peptide

The peptide **AzoChig2** was synthesized according to the previously stated peptide synthesis strategy. After global deprotection and cleavage from the resin and subsequent diethyl ether precipitation, the peptide was purified by RP-HPLC with a water/acetonitrile (80:10 → 40:60) gradient. The product was obtained upon lyophilisation with a water/acetonitrile (60:40) mixture. HR ESI-MS (positive), m/z: found 1227.4891 $[C_{54}H_{61}FO_{14}N_{12}+iPrOH+2Na+H]^{1+}$, calc.: 1227.4858. HR ESI-MS (negative), m/z: found 1225.4738 $[C_{54}H_{60}FO_{14}N_{12}+iPrOH+2Na-H]^{1-}$, calc.: 1225.4712.

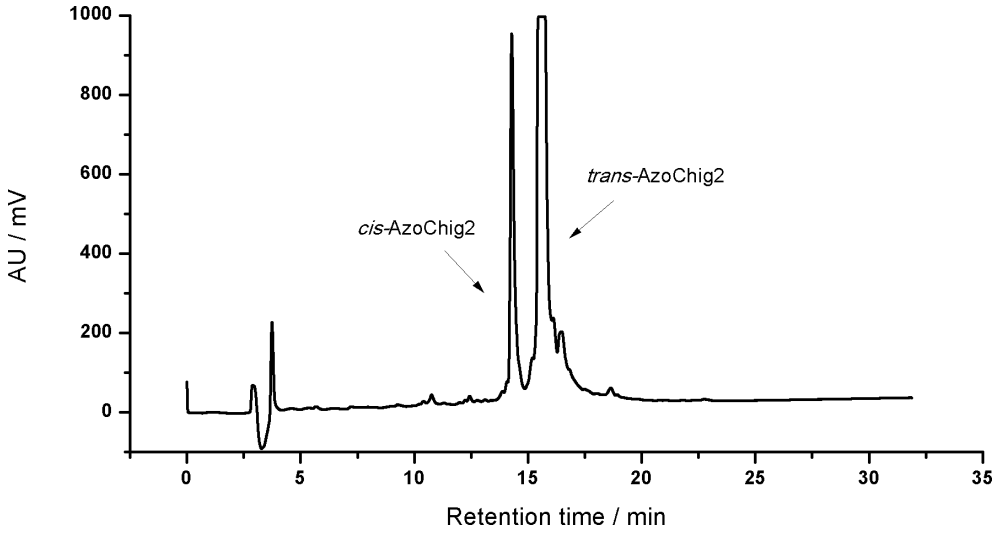


Figure 7: Analytical RP-HPLC spectrum of **AzoChig2** peptide. Retention time *cis* isomer = 14.3 min, *trans* isomer = 15.6 min.

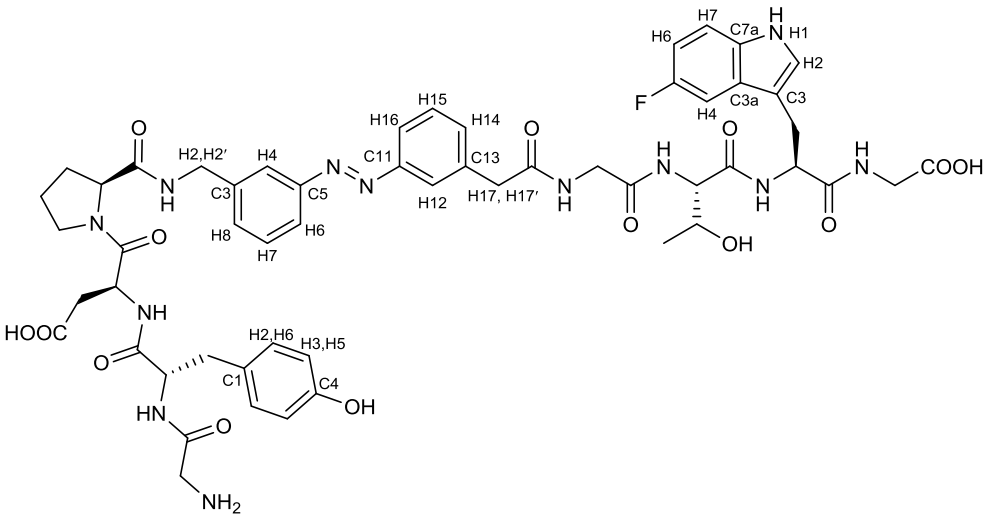


Figure 8: Numbering of certain H and C atoms in **AzoChig2** peptide.

Table 4: ^{1}H- and ^{13}C-NMR chemical shifts of *trans*-**AzoChig2** peptide. All NMR spectra where recorded in CD_3OD, thus no NH and OH signals were recorded.

trans AzoChig2	H [ppm]	m	*J* [Hz]	C [ppm]	*trans* AzoChig2	H [ppm]	m	*J* [Hz]	C [ppm]
G01/NH2	-	-	-	-	**AMPP56/C11**	-	-	-	154.1
G01/Hα,Hα'	3.65	dd	21.3, 16.5	41.4	**AMPP56/H12**	7.97	s	-	125.1
G01/CO	-	-	-	167.1	**AMPP56/C13**	-	-	-	138.0
Y02/NH	-	-	-	-	**AMPP56/H14**	7.46	d	7.8	131.0
Y02/Hα	4.63	t	7.5	56.2	**AMPP56/H15**	7.47	t	7.8	133.2
Y02/Hβ,Hβ'	2.97, 2.83	dd	13.5, 7.7	38.3	**AMPP56/H16**	7.77	d	8.0	122.3
Y02/C1	-	-	-	128.3	**AMPP56/H17,H17'**	3.74	s	-	43.2
Y02/H2,H6	7.01	d	8.3	131.4	**AMPP56/CO**	-	-	-	174.4
Y02/H3,H5	6.68	d	8.3	116.3	**G07/NH**	-	-	-	-
Y02/C4	-	-	-	157.5	**G07/Hα,Hα'**	3.89	dd	18.3, 16.0	44.1
Y02/OH	-	-	-	-	**G07/CO**	-	-	-	172.1
Y02/CO	-	-	-	172.8	**T08/NH**	-	-	-	-
D03/NH	-	-	-	-	**T08/Hα**	4.26	d	4.1	60.2
D03/Hα	4.93	dd	8.7, 5.5	48.7	**T08/Hβ**	4.04	m	-	68.2
D03/Hβ,Hβ'	3.01, 2.61	dd	17.3, 4.7	37.0	**T08/Hγ**	1.04	d	6.3	19.9
D03/COOH	-	-	-	174.9	**T08/OH**	-	-	-	-
D03/CO	-	-	-	171.6	**T08/CO**	-	-	-	172.3
P04/N	-	-	-	-	**W09/NH**	-	-	-	-
P04/Hα	4.46	dd	8.9, 3.5	62.2	**W09/Hα**	4.65	t	7.3	56.2
P04/Hβ,Hβ'	2.20, 2.07	d	8.3	30.8	**W09/Hβ,Hβ'**	3.27, 3.11	dd	14.6, 7.7	28.0
P04/Hγ,Hγ'	1.97	bs	-	25.4	**W09/NH**	-	-	-	-
P04/Hδ,Hδ'	3.75, 3.60	m	-	48.6	**W09/H2**	7.18	s	-	125.0
P04/CO	-	-	-	174.2	**W09/C3**	-	-	-	107.7
AMPP56/NH	-	-	-	-	**W09/C3a**	-	-	-	130.3
AMPP56/H2,H2'	4.52, 4.41	d	15.5	43.5	**W09/H4**	7.24	dd	10.0, 2.1	103.7
AMPP56/C3	-	-	-	141.3	**W09/C5**	-	-	-	157.7
AMPP56/H4	7.75	s	-	121.6	**W09/H6**	6.75	dt	8.7, 1.8	109.5
AMPP56/C5	-	-	-	154.2	**W09/H7**	7.14	dd	8.5, 4.2	112.3
AMPP56/H6	7.73	d	8.0	123.2	**W09/C7a**	-	-	-	134.2
AMPP56/H7	7.47	t	7.8	133.2	**W09/CO**	-	-	-	174.2
AMPP56/H8	7.39	d	7.4	130.6	**G10/NH**	-	-	-	-
AMPP56/N9	-	-	-	-	**G10/Hα,Hα'**	3.92	dd	25.0, 18.0	41.9
AMPP56/N10	-	-	-	-	**G10/COOH**	-	-	-	172.1

Table 5: ^{1}H- and ^{13}C-NMR chemical shifts of *cis*-**AzoChig2** peptide. All NMR spectra where recorded in CD_3OD, thus no NH and OH signals were recorded.

cis AzoChig2	H [ppm]	m	*J* [Hz]	C [ppm]	*cis*-AzoChig2	H [ppm]	m	*J* [Hz]	C [ppm]
G01/NH2	-	-	-	-	**AMPP56/C11**	-	-	-	154.8
G01/Hα,Hα'	3.65	dd	21.3, 16.5	41.4	**AMPP56/H12**	6.69	s	-	121.4
G01/CO	-	-	-	167.1	**AMPP56/C13**	-	-	-	137.6
Y02/NH	-	-	-	-	**AMPP56/H14**	7.14	d	7.8	129.8
Y02/Hα	4.64	t	7.5	56.2	**AMPP56/H15**	7.28	t	8.1	130.3
Y02/Hβ,Hβ'	2.98, 2.83	m	-	38.3	**AMPP56/H16**	6.91	d	7.8	121.2
Y02/C1	-	-	-	128.3	**AMPP56/H17,H17'**	3.47	dd	15.2, 8.3	43.1
Y02/H2,H6	6.83	d	8.3	131.4	**AMPP56/CO**	-	-	-	173.9
Y02/H3,H5	6.59	d	8.3	116.1	**G07/NH**	-	-	-	-
Y02/C4	-	-	-	157.4	**G07/Hα,Hα'**	3.86	dd	18.3, 16.0	44.2
Y02/OH	-	-	-	-	**G07/CO**	-	-	-	172.1
Y02/CO	-	-	-	172.5	**T08/NH**	-	-	-	-
D03/NH	-	-	-	-	**T08/Hα**	4.41	d	4.1	59.9
D03/Hα	4.98	m	8.7, 5.5	48.5	**T08/Hβ**	4.09	m	-	68.5
D03/Hβ,Hβ'	2.91, 2.58	dd	17.3, 4.7	37.1	**T08/Hγ**	1.11	d	6.3	19.8
D03/COOH	-	-	-	174.6	**T08/OH**	-	-	-	-
D03/CO	-	-	-	171.2	**T08/CO**	-	-	-	172.0
P04/N	-	-	-	-	**W09/NH**	-	-	-	-
P04/Hα	4.46	dd	8.9, 3.5	62.0	**W09/Hα**	4.59	t	7.5	56.2
P04/Hβ,Hβ'	2.20, 2.07	d	8.3	30.8	**W09/Hβ,Hβ'**	3.27, 3.11	dd	14.6, 7.7	28.1
P04/Hγ,Hγ'	1.97	bs	-	25.5	**W09/NH**	-	-	-	-
P04/Hδ,Hδ'	3.75, 3.60	m	-	48.6	**W09/H2**	7.21	s	-	125.0
P04/CO	-	-	-	174.2	**W09/C3**	-	-	-	111.3
AMPP56/NH	-	-	-	-	**W09/C3a**	-	-	-	129.1
AMPP56/H2,H2'	4.26, 4.19	d	15.5	43.5	**W09/H4**	7.27	m	-	104.1
AMPP56/C3	-	-	-	141.0	**W09/C5**	-	-	-	159.7
AMPP56/H4	6.78	s	7.8	120.7	**W09/H6**	6.83	m	-	110.5
AMPP56/C5	-	-	-	155.1	**W09/H7**	7.24	m	-	113.0
AMPP56/H6	6.61	d	8.0	119.8	**W09/C7a**	-	-	-	134.5
AMPP56/H7	7.13	t	8.5	129.8	**W09/CO**	-	-	-	172.5
AMPP56/H8	7.05	d	7.8	127.6	**G10/NH**	-	-	-	-
AMPP56/N9	-	-	-	-	**G10/Hα,Hα'**	3.92	dd	25.0, 18.0	41.9
AMPP56/N10	-	-	-	-	**G10/COOH**	-	-	-	172.1

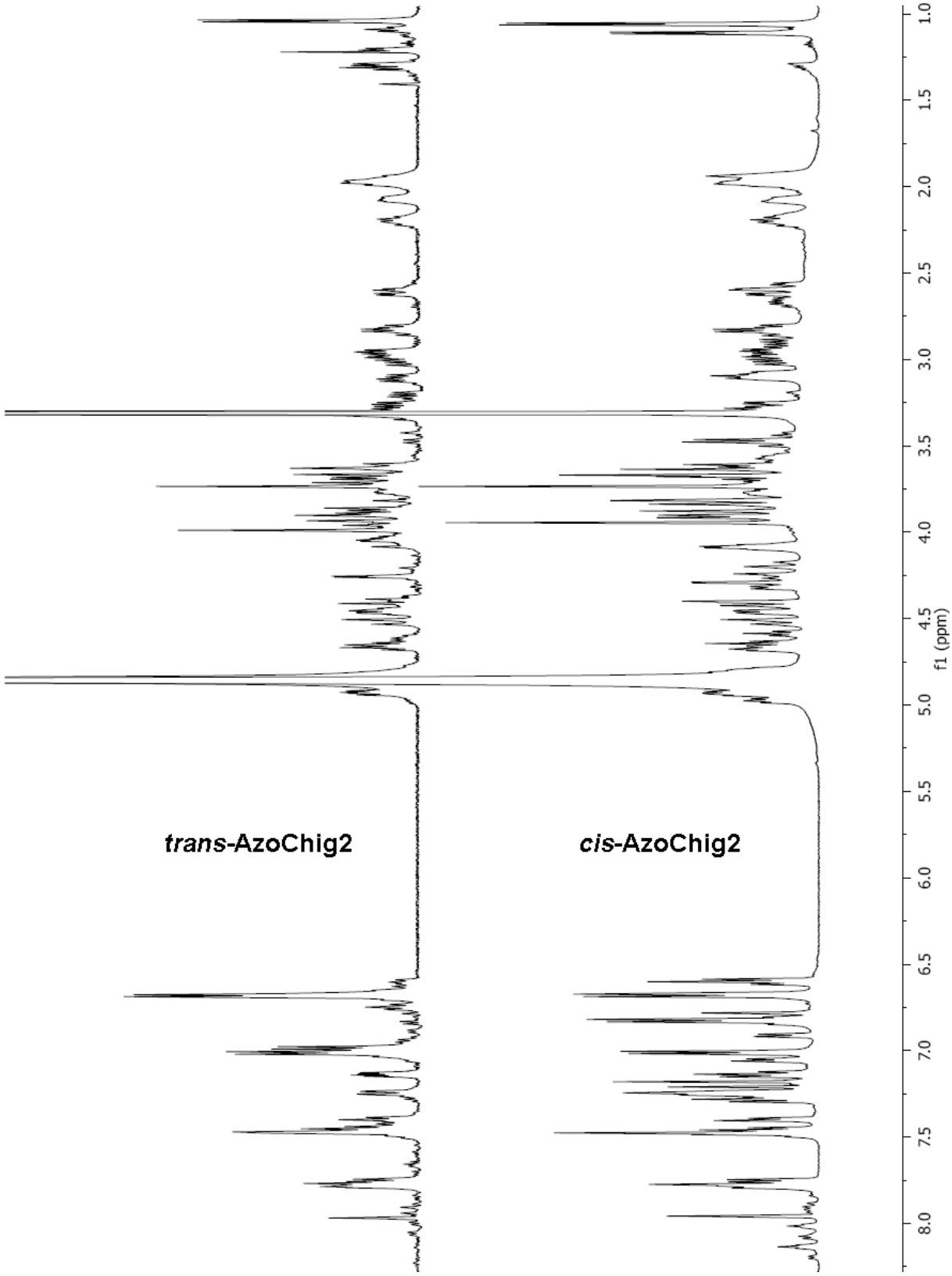


Figure 9: Left: ^{1}H-NMR spectrum of *trans*-**AzoChig2** (2) peptide in MeOD-d_4, *c* = 5 mM; Right: ^{1}H-NMR spectrum of *cis*-**AzoChig2** (2) peptide in MeOD-d_4, *c* = 5 mM.

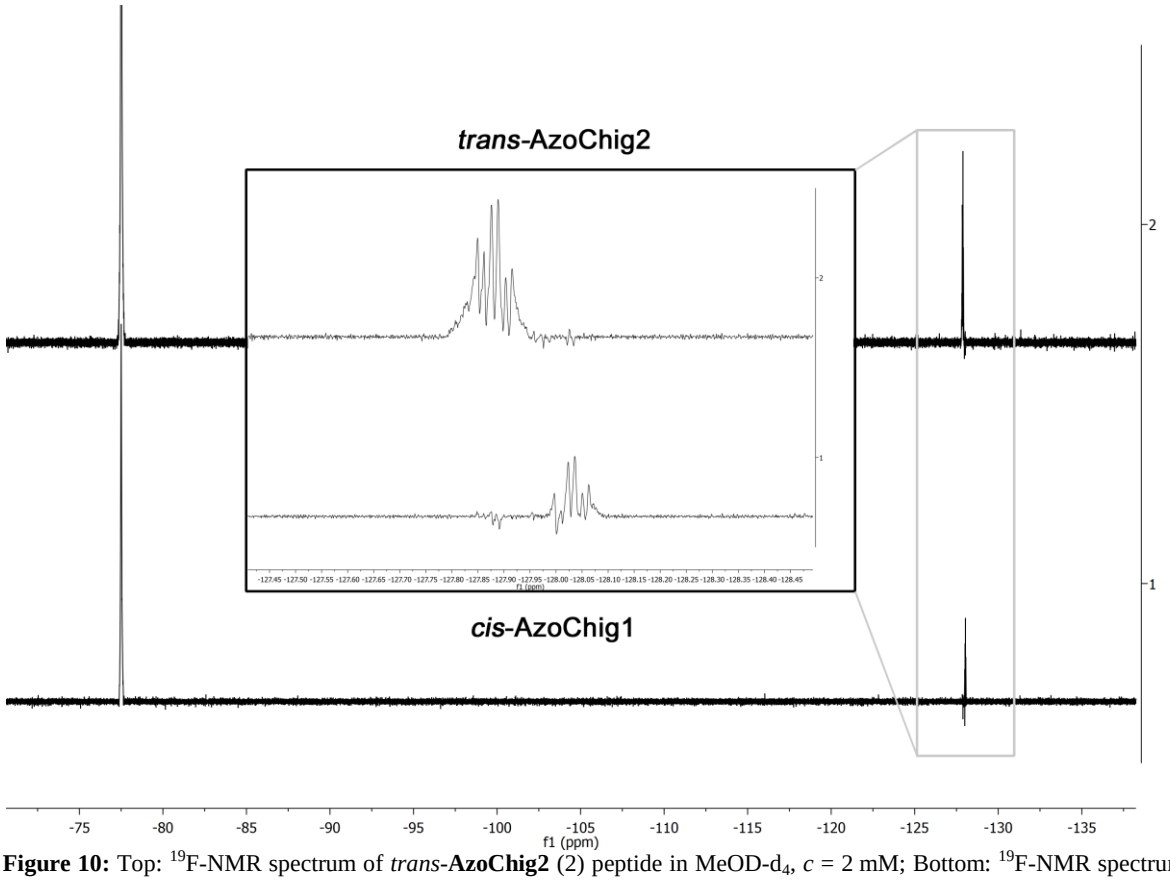


Figure 10: Top: ^{19}F-NMR spectrum of *trans*-**AzoChig2** (2) peptide in MeOD-d_4, *c* = 2 mM; Bottom: ^{19}F-NMR spectrum of *cis*-**AzoChig2** (1) peptide in MeOD-d_4, *c* = 5 mM. Peak at -76 ppm originates from trifluoroacetic acid.

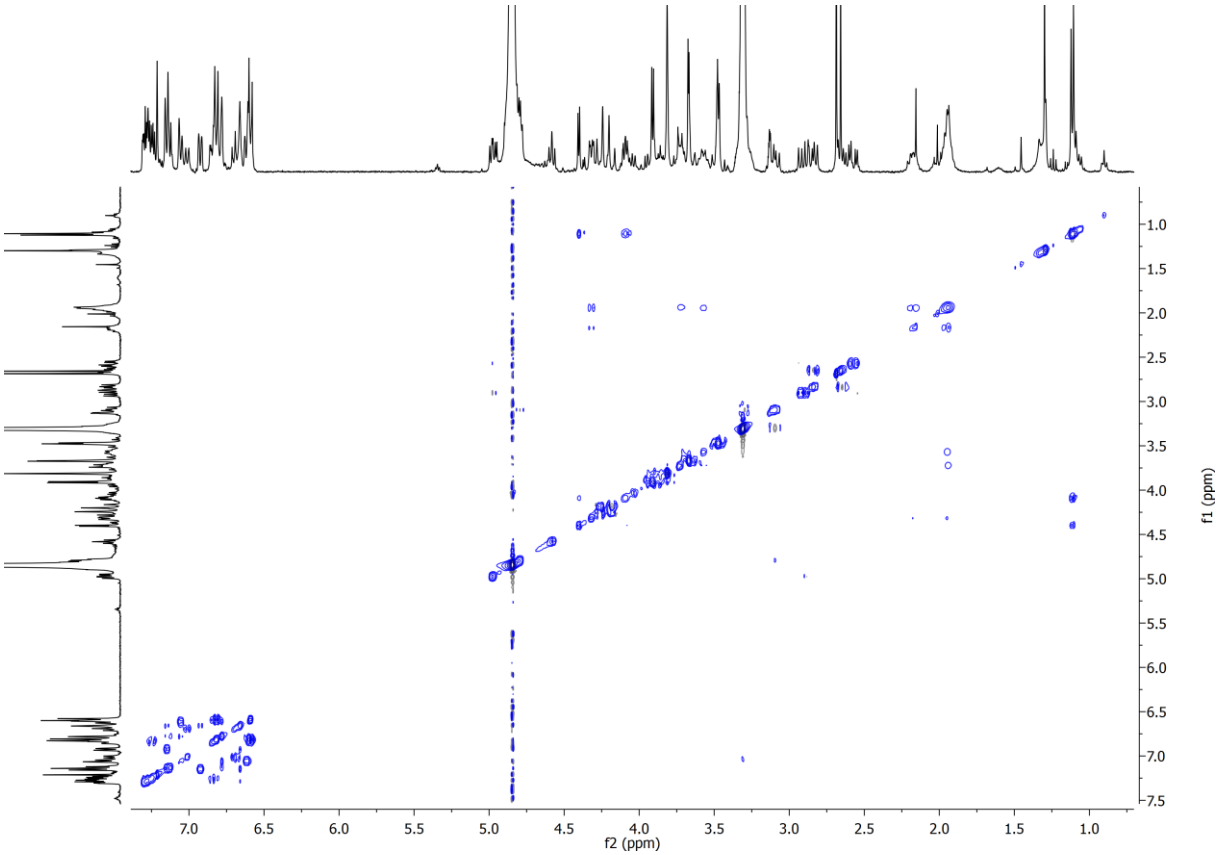


Figure 11: ^{1}H,^{1}H-TOCSY NMR spectrum of *cis*-**AzoChig2** peptide in MeOD-d_4, *c* = 2 mM.

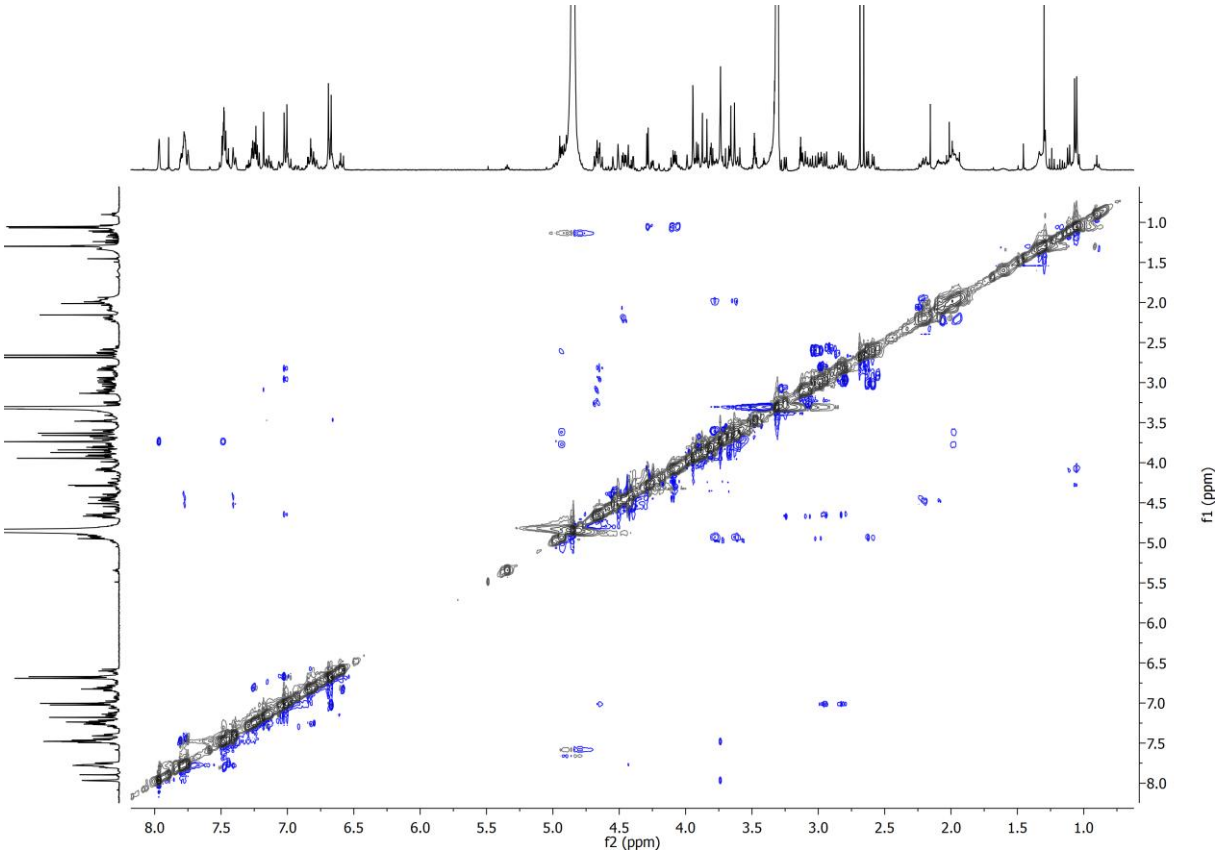


Figure 12: ^{1}H,^{1}H-ROESY NMR spectrum of *trans*-**AzoChig2** peptide in MeOD-d_4, *c* = 2 mM.

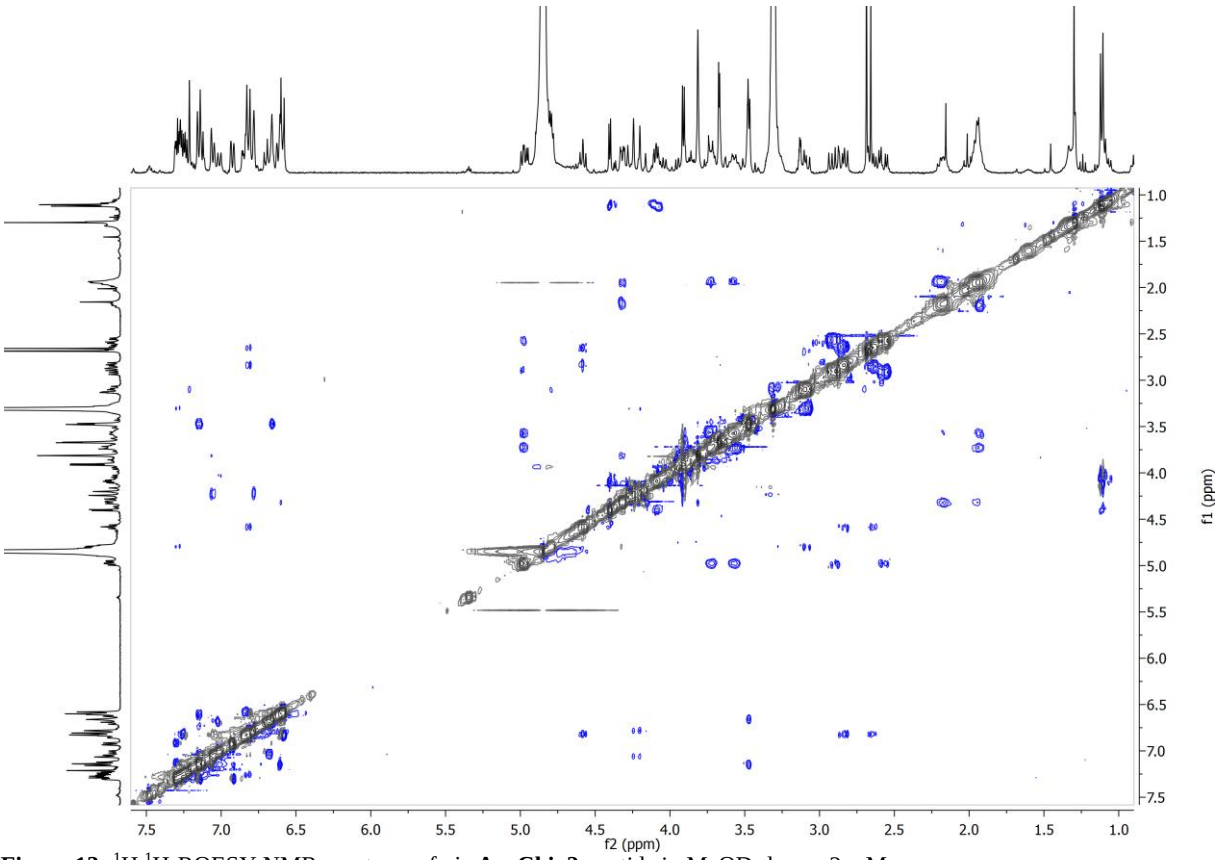


Figure 13: ^{1}H,^{1}H-ROESY NMR spectrum of *cis*-**AzoChig2** peptide in MeOD-d_4, *c* = 2 mM.

3.3 AzoChig3 peptide

The peptide **AzoChig3** was synthesized according to the previously stated peptide synthesis strategy. After global deprotection and cleavage from the resin and diethyl ether precipitation, the peptide was purified by RP-HPLC with a water/acetonitrile (80:10 → 40:60) gradient. The product was obtained upon lyophilisation with a water/acetonitrile (60:40) mixture. HR ESI-MS (positive), m/z: found 1306.5706 $[C_{63}H_{80}O_{18}N_{13}+H]^+$, calc.: 1306.5739. The signals of the recorded NMR spectra of the *cis/trans*-**AzoChig3** could not be assigned due to excessive signal broadening.

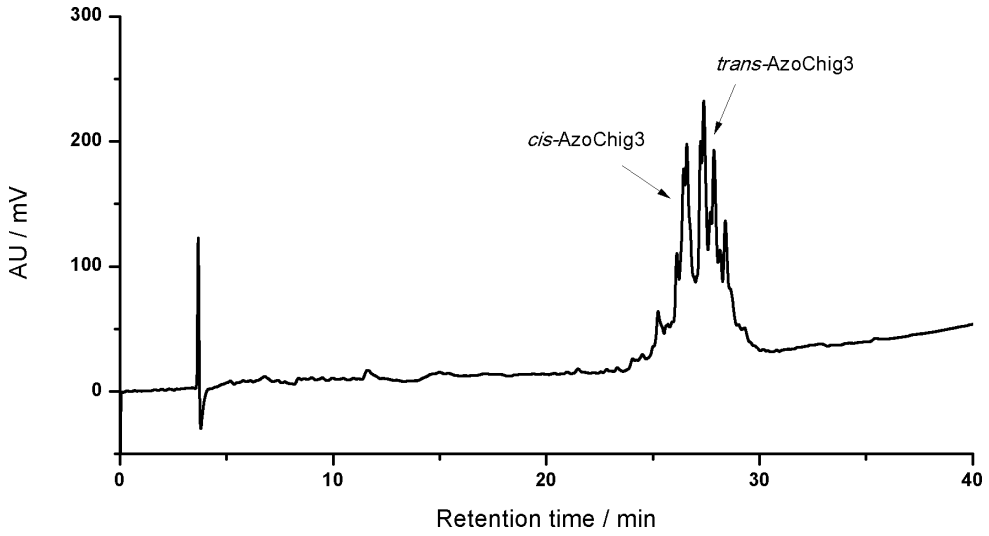


Figure 14: Analytical RP-HPLC spectrum of **AzoChig3** peptide. Retention time *cis* isomer = 27.4 min, *trans* isomer = 27.9 min.

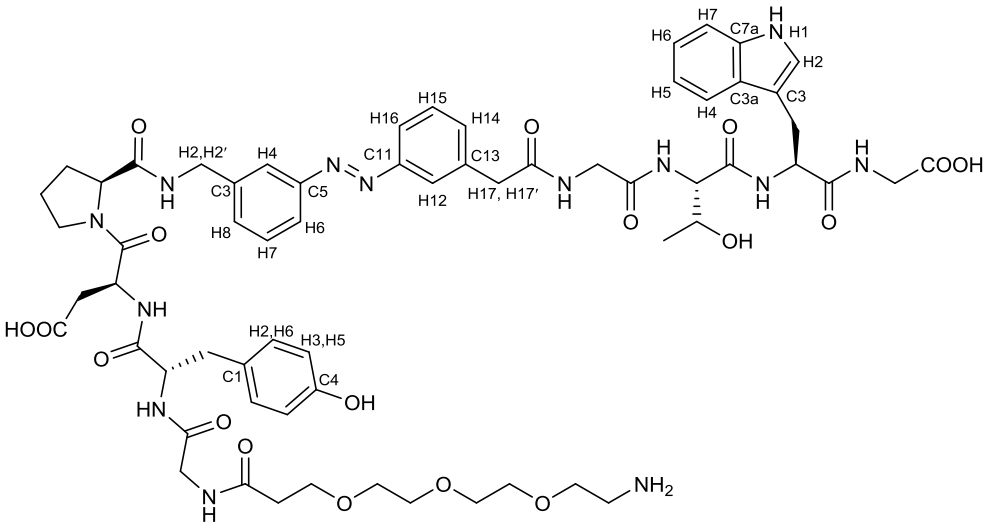


Figure 15: Numbering of certain H and C atoms in **AzoChig3** peptide.

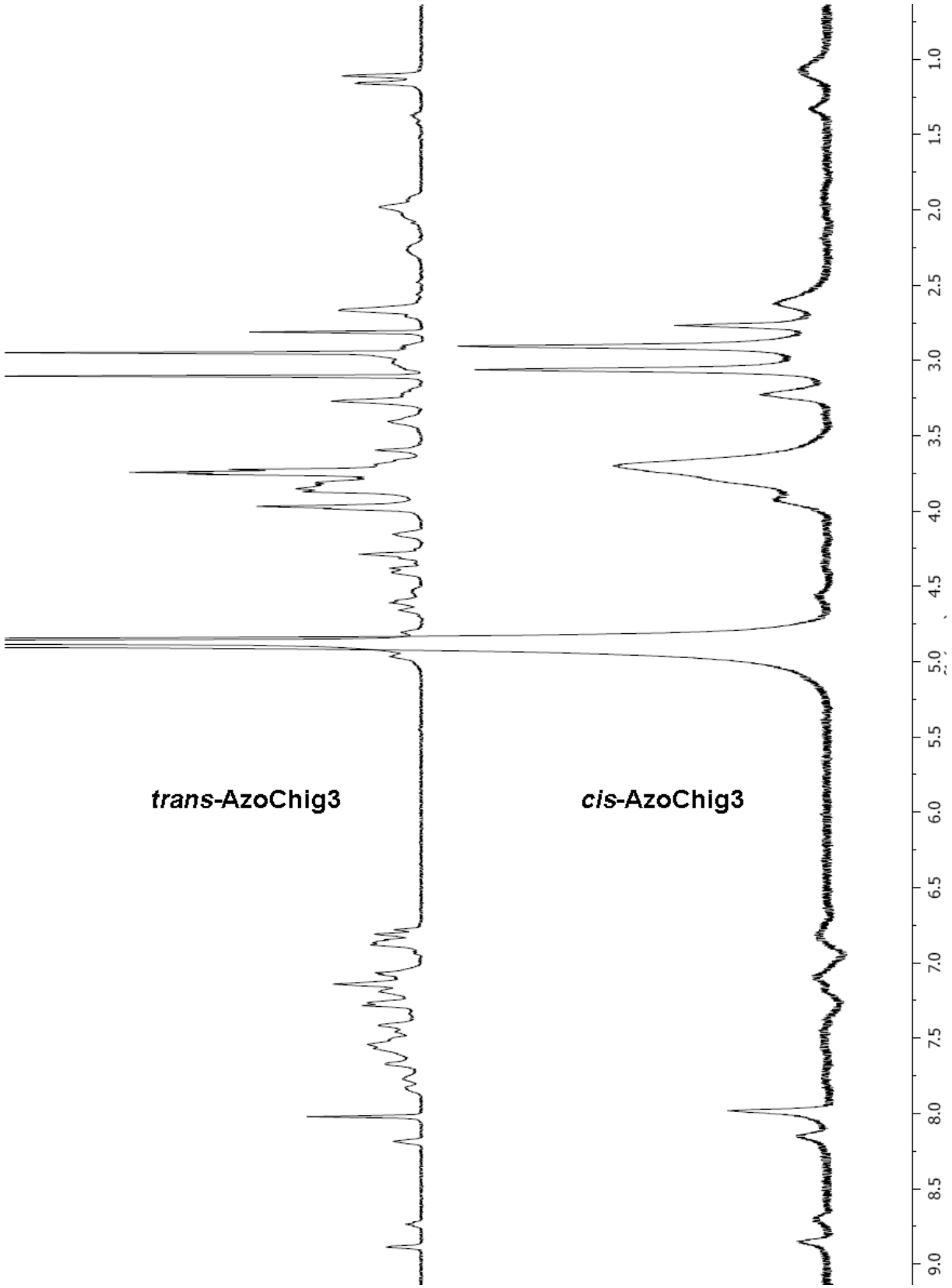


Figure 16: ^{1}H-NMR spectrum of *trans*-**AzoChig3** (2) peptide in MeOD-d_4. Bottom: ^{1}H-NMR spectrum of *cis*-**AzoChig3** (2) peptide in MeOD-d_4.

4 UV/Vis, CD and FT-IR spectra of AzoChig1-3 peptides

4.1 UV/Vis spectra of AzoChig1-3 peptides

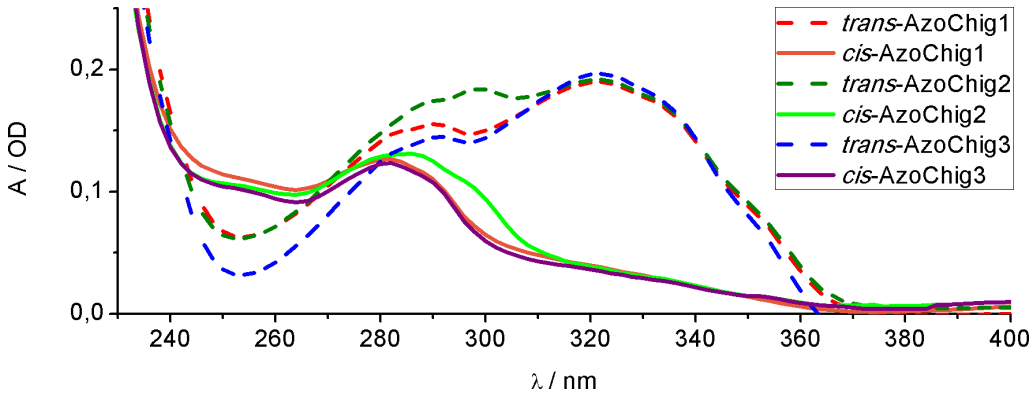


Figure 17: UV/Vis spectra of *cis/trans*-**AzoChig1-3** peptides in methanol, *c* = 77 µM **AzoChig1**, *c* = 77 µM **AzoChig2** and *c* = 78 µM **AzoChig3**.

4.2 CD spectra of AzoChig1-3 peptides

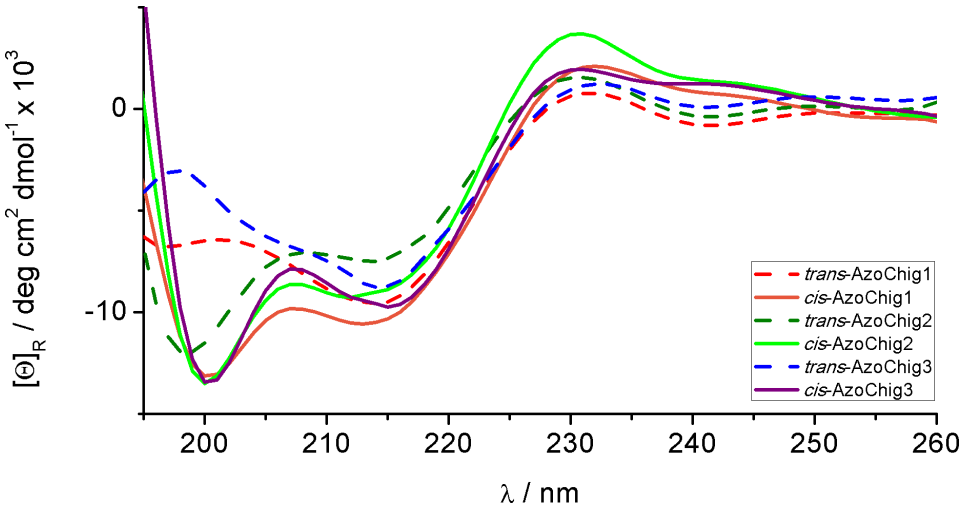


Figure 18: CD spectra of *cis/trans*-**AzoChig1-3** peptides in methanol, *c* = 77 µM **AzoChig1**, *c* = 77 µM **AzoChig2** and *c* = 78 µM **AzoChig3**.

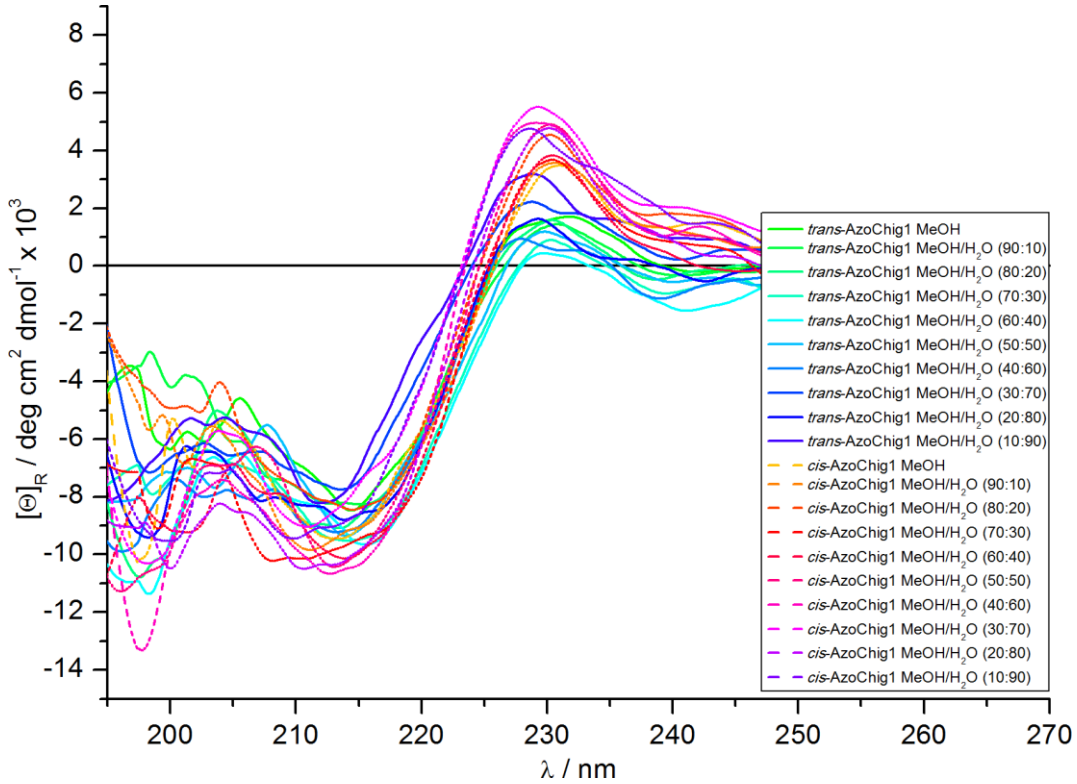


Figure 12: Solvent dependent CD spectra of *cis*-**AzoChig1** (dashed lines, red to purple) and *trans*-**AzoChig1** (solid lines, green to blue) peptides in methanol/water ratios from 100% methanol to 10:90% methanol/water. The CD spectra were measured at 5 °C with concentrations of c = 82-112 µM.

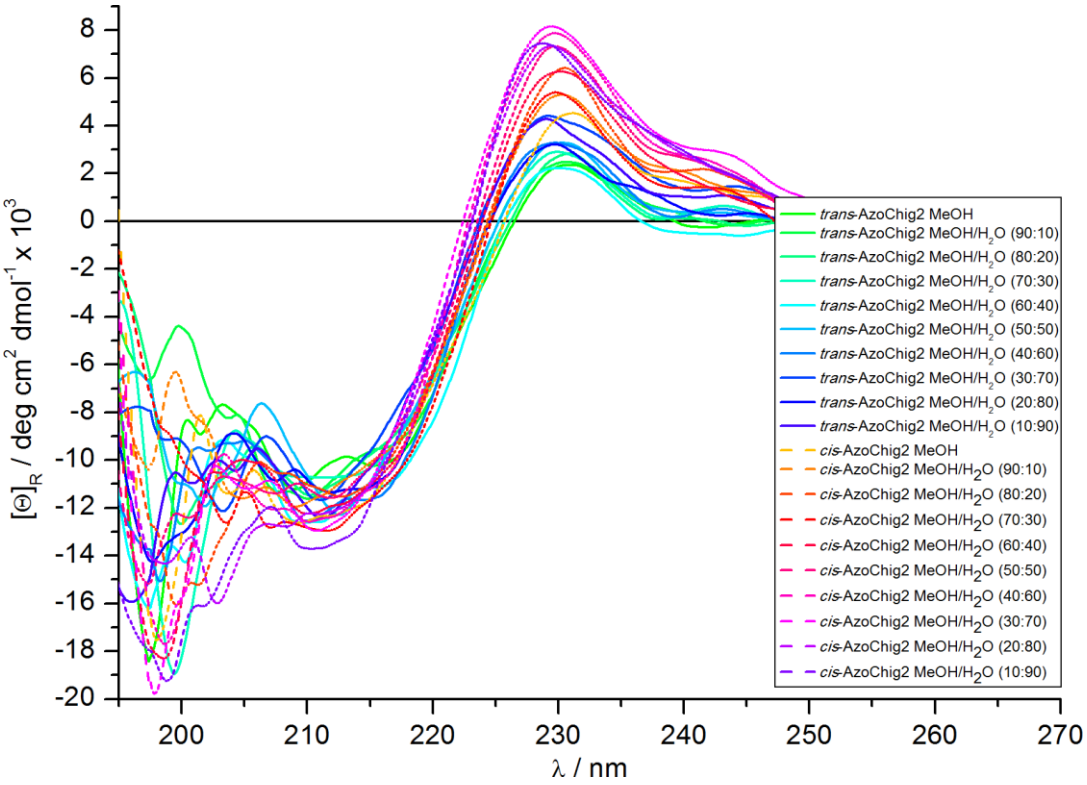


Figure 13: Solvent dependent CD spectra of *cis*-**AzoChig2** (dashed lines, red to purple) and *trans*-**AzoChig2** (solid lines, green to blue) peptides in methanol/water ratios from 100% methanol to 10:90% methanol/water. The CD spectra were measured at 5 °C with concentrations of c = 84-102 µM.

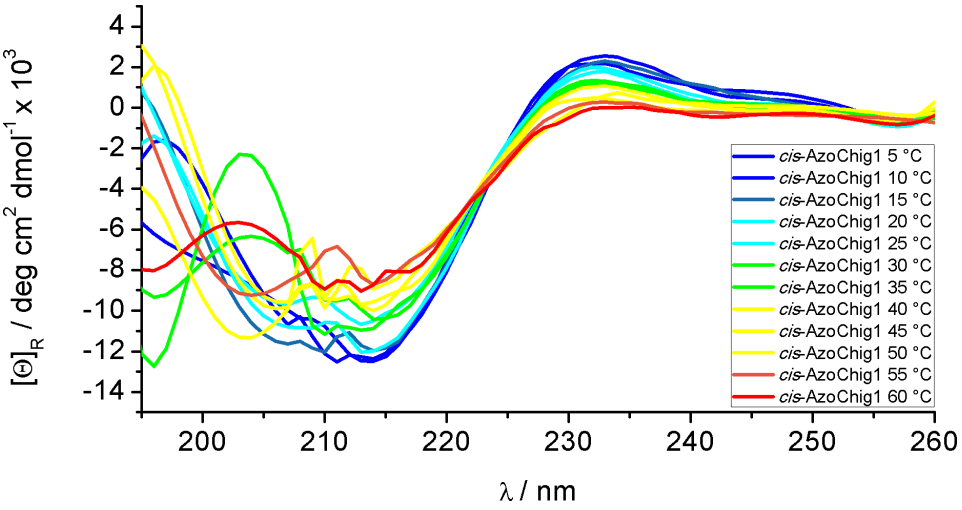


Figure 14: Temperature dependent CD spectra of the *cis*-**AzoChig1** peptide in methanol, *c* = 76 µM.

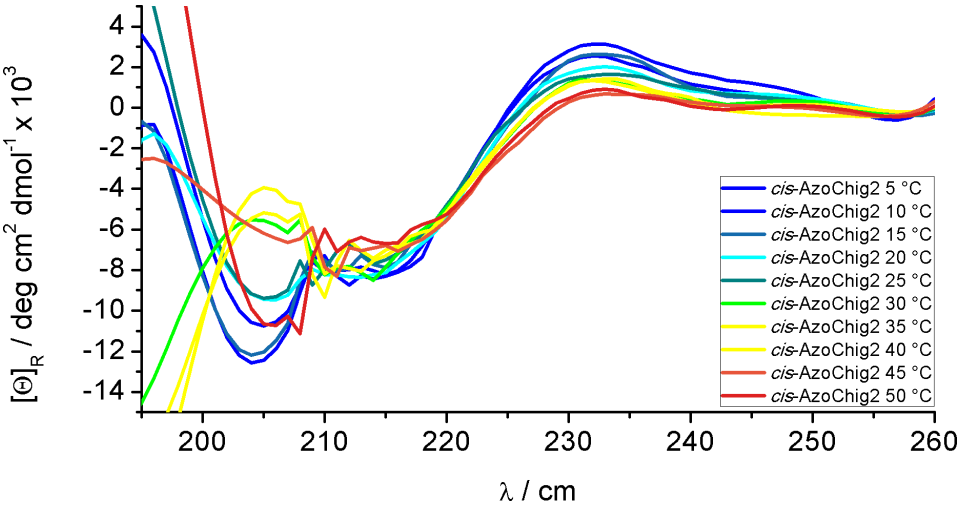


Figure 15: Temperature dependent CD spectra of *cis*-**AzoChig2** peptide in methanol, *c* = 77 µM.

4.3 FT-IR spectra of AzoChig1-3 peptides

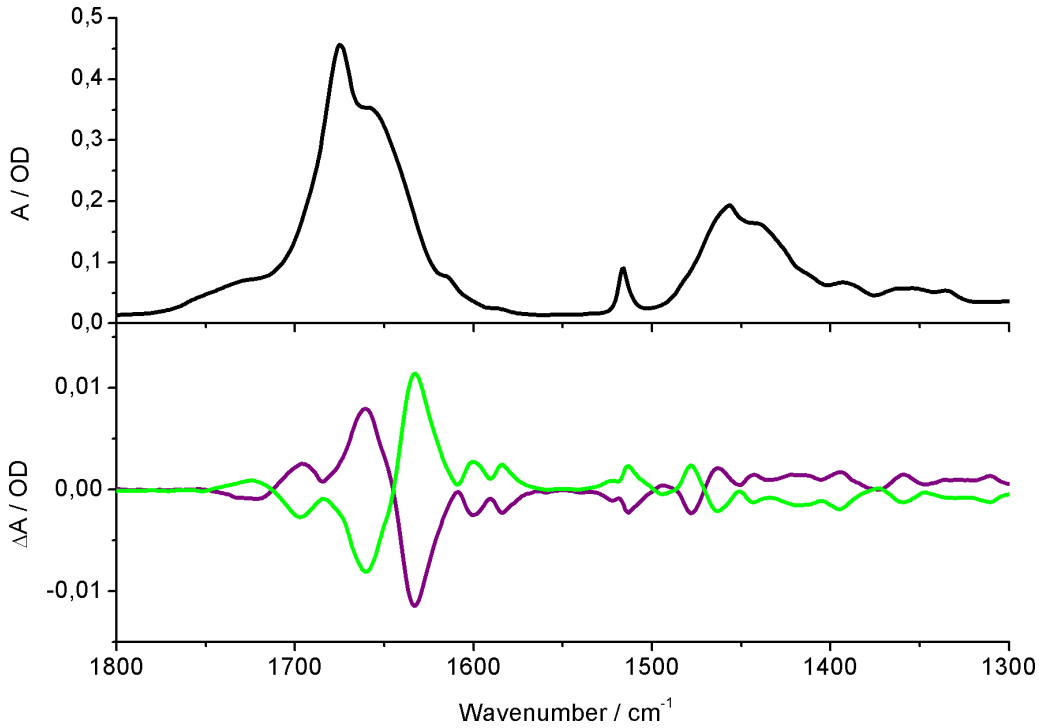


Figure 16: Top: FT-IR spectrum of **AzoChig1** peptide in methanol-d_4, c = 5.0 mM; Bottom: IR differential spectra of the *cis* → *trans* (purple) and *trans* → *cis* (green) isomerization of **AzoChig1** peptide in methanol-d_4, c = 5.0 mM.

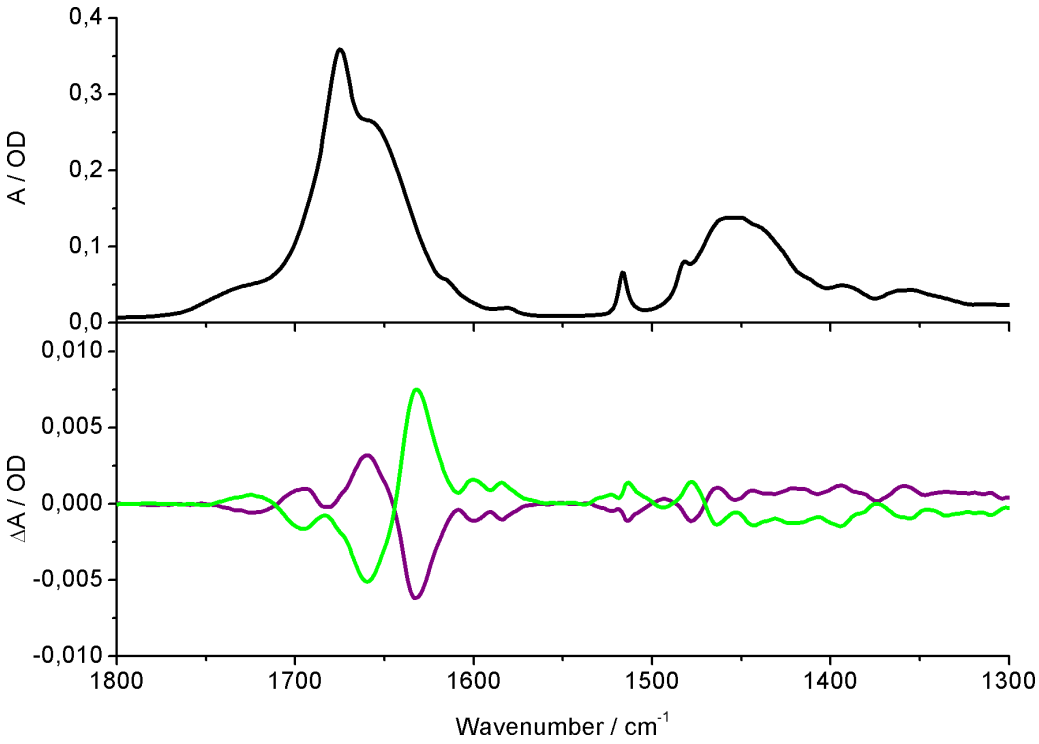


Figure 17: Top: FT-IR spectrum of **AzoChig2** peptide in methanol-d_4, c = 5.0 mM; Bottom: IR differential spectra of the *cis* → *trans* (purple) and *trans* → *cis* (green) isomerization of **AzoChig2** peptide in methanol-d_4, c = 5.0 mM.

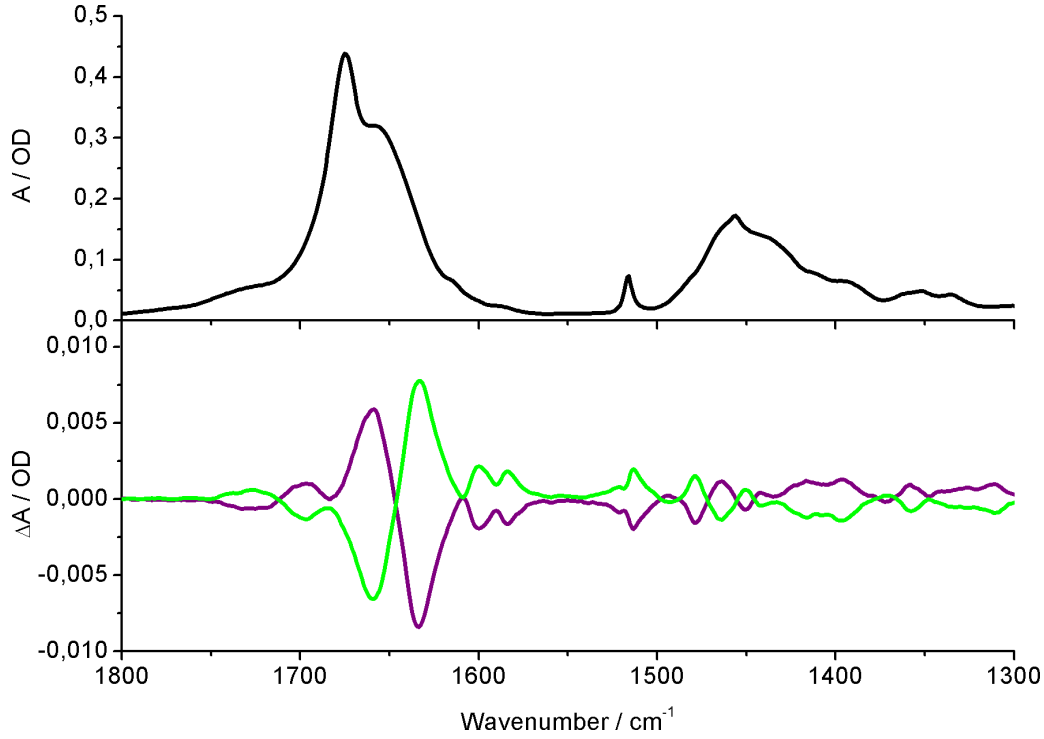


Figure 18: Top: FT-IR spectrum of **AzoChig3** peptide in methanol-d_4, c = 5.0 mM; Bottom: IR differential spectra of the *cis* → *trans* (purple) and *trans* → *cis* (green) isomerization of **AzoChig3** peptide in methanol-d_4, c = 5.0 mM.