Supporting Information for:

A solid-phase approach to the phallotoxins: total synthesis of [Ala^7]-phalloidin

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Abbreviations:

Boc = *tert*-butoxycarbonyl

DHPP = dihydropyranyl polystyrene (free resin)

Fmoc = 9-fluorenemethoxycarbonyl

HOAt = 1-hydroxy-7-azabenzotriazole

HOBt = 1-hydroxybenzotriazole

Ns = Nosyl = 2-nitrobenzenesulfonyl

PyAOP = (7-azabenzotriazole-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate

PyBOP = benzotriazole-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate

Tmse = 2-trimethylsilylethyl

THPP = tetrahydropyranyl polystyrene (loaded resin)

General: Purification and Analysis. Flash column chromatography was performed using the general procedure described by Still et al.[1] Analytical RP-HPLC was carried out using C18 stationary phase (column: 3.5 μm; 4.6 x 50 mm) at 1 ml/min. One of three solvent gradients was used: (A) H_2O:CH_3CN (0.05% CF_3CO_2H) 90:10 to 0:100 over 12 min; (B) 90:10 to 70:30 over 22 min; or (C) 90:10 to 80:20 over 2 min, then 80:20 isocratic for 20 min. The following abbreviations are used to

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

describe NMR peak splitting when appropriate: s=singlet, d=doublet, t=triplet, q=quartet, bs=broad singlet, mult=multiplet. FT-IR spectra were recorded in $CDCl_3$ solution. MALDI-MS was carried out using α-cyano-4-hydroxycinammic acid matrix. Melting points are uncorrected.

General: Solution-phase synthesis methods. Solution phase reactions were carried out under an atmosphere of argon. Solvents specified as "anhydrous" were dried using the procedure recommended by Grubbs et al.[2] using the solvent purification system manufactured by Glass Contour, Inc. (Laguna Beach, CA). Reagents were of commercial quality unless otherwise indicated. The term "solvents were removed in vacuo" typically implies rotary evaporation followed by the use of a hi-vacuum pump. Sulfuryl chloride was used as the commercially available material, but was stored over potassium carbonate before use. Developed TLC plates were visualized using short wave UV light (254 nm), and were typically stained in an iodine/silica chamber and/or using a staining dip (e.g. *p*-anisaldehyde or ceric ammonium molybdate) followed by heating with a heat gun.

General: Solid-phase synthesis methods. Reactions were carried out in Biorad Biospin chromatography columns, attached to 3-way solvent-resistant stopcocks. Reaction vessels were agitated by 360^0 rotation. Progress of solid-phase reactions was followed by complementary methods. The presence of resin-bound primary amines was confirmed with the standard Kaiser test, while the presence of secondary amines was confirmed with the chloranil test. Micro-scale cleavage experiments were done to characterize certain key intermediates: a small amount of beads (~1-2 mg) is placed in a micro-scale reactor, and cleaved by the addition of CF_3CO_2H:H_2O:Et_3SiH (8:2:10) (0.4 ml), followed by agitation for 15 minutes. The cleavage mixture was then azeotropically concentrated in vacuo by the addition and evaporation of toluene (x 3). The crude material was analyzed by either ESI-MS or

[2] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen R. K.; Timmers, F. J. *Organometallics*, **1996**, *15*(5), 1518-1520.

MALDI-TOF-MS (α-cyano-4-hydroxycinammic acid matrix). The purity of the material was gauged by RP-HPLC.

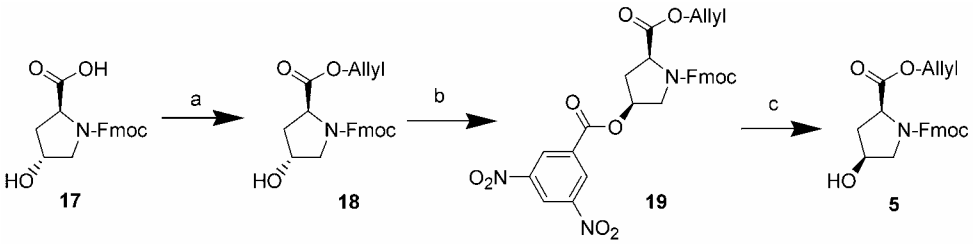

Figure S1. Synthesis of protected *cis*-Hyp fragment **5**. *Reagents and conditions:* (a) Cs_2CO_3, MeOH; then allyl bromide, DMF, 20 h, rt (87%); (b) 3,5-dinitrobenzoic acid, di-*tert*-butyldiazodicarboxylate (DBAD), PPh_3, THF, 16 h, rt (100%); (c) NaN_3, MeOH-Dioxane, 15-crown-5, reflux overnight (quantitative).

Fmoc-*trans*-Hyp-O-Allyl ester (18). To a solution of Fmoc-*trans*-Hyp-OH (**17**) (3.065 g, 8.67 mmol) in CH_3OH (90% aq., 30 ml) was added Cs_2CO_3 (1.413 g, 4.34 mmol, 0.5 eq). The solution was stirred for 5 min at rt and then concentrated *in vacuo* to a white residue, which was then taken up in DMF (30 ml) and treated with allyl bromide (1.101 g, 9.11 mmol, 1.05 eq). The solution was stirred at rt for 20 h, at which time TLC (2:3 hexanes:ethyl acetate, UV, product R_f = 0.3) showed complete conversion. The solution was taken up in diethyl ether (100 ml), washed with water (2 x 100 ml) and brine (100 ml), dried over $MgSO_4$, and concentrated *in vacuo* to a colorless oil (2.747 g, 81%): $[\alpha]_D^{25}$ -50.7 (*c*, CH_2Cl_2); IR ($CDCl_3$): 3608, 3154, 2984, 1745, 1701 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.06 – 2.10 (mult, 1H), 2.29 – 2.32 (mult, 1H), 3.30 (bs, 1H), 3.63 – 3.73 (mult, 2H), 4.12 (t, 1H, J = 7 Hz), 4.22 – 4.31 (mult, 2H), 4.37 – 4.63 (mult, 4H), 5.14 – 5.31 (mult, 2H), 5.77 – 5.88 (mult, 1H), 7.27 (t, 2H, J = 7 Hz), 7.36 (t, 2H, J = 8 Hz), 7.52 (d, 2H, J = 7), 7.72 (d, 2H, J = 8); ^{13}C-NMR (100 Hz, $CDCl_3$): two rotomers observed – some carbons are doubled. δ 38.6 and 39.5, 47.3 and 47.4, 54.8 and 55.5, 58.0 and 58.3, 66.1, 67.9 and 68.1, 69.3 and 70.1, 118.8 and 119.1, 120.2, 125.2 and 125.3, 127.3,

127.9, 131.7 and 131.9, 141.5, 143.8, 144.0, 144.2, 144.2, 155.2 and 155.3, 172.6. HRMS Calc. For $C_{23}H_{23}NO_5$ [M+H] = 394.1654, found [M+H] = 394.1656.

Fmoc-*cis*-Hyp(*O*-3,5-dinitrobenzoate)-O-Allyl ester (19). A solution of Fmoc-trans-Hyp-O-Allyl ester (**18**) (2.747 g, 6.98 mmol) in THF (35 ml) and toluene (35 ml) was cooled to 0 ^{0}C, and was then treated with 3,5-dinitrobenzoic acid (2.962 g, 13.96 mmol, 2.0 eq) and triphenylphosphine (3.663 g, 13.96 mmol, 2.0 eq). The mixture was stirred until it was completely homogenous, and was then treated with di-*tert*-butyldiazodicarboxylate (DBAD) (3.215 g, 13.96 mmol, 2.0 eq) in a single portion. The solution was stirred for 16 h at rt, after which the reaction was judged complete by TLC (3:2 hexanes:ethyl acetate, UV, product R_f = 0.2). The resulting solution was concentrated *in vacuo*, dissolved in diethyl ether (50 ml), washed with $NaHCO_3$ (10% aq., 2 x 50 ml) and brine (50 ml), dried over $MgSO_4$, and concentrated *in vacuo* on silica. The product was isolated by dry-loaded silica flash column chromatography (5:2 to 3:2 hexanes:ethyl acetate) as a slightly yellow foam (3.296 g, 80%): $[\alpha]_D^{25}$ -23.6 (*c*, CH_2Cl_2); IR ($CDCl_3$): 3154, 3101, 2984, 1735 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.49 – 2.61 (mult, 2H), 3.86 (bs, 2H), 4.05 – 4.19 (mult, 2H), 4.34 – 4.51 (mult, 2H), 4.62 – 4.72 (mult, 2H), 5.11 (t, 1H, *J* = 8 Hz), 5.26 (t, 1H, *J* = 16 Hz), 5.61 (d, 1H, *J* = 19 Hz), 5.82 – 5.88 (mult, 1H), 7.26 (t, 2H, *J* = 9 Hz), 7.32 – 7.36 (mult, 2H), 7.49 – 7.57 (mult, 2H), 7.69 (d, 2H, *J* = 7 Hz), 8.97 (s, 1H), 9.01 (s, 1H), 9.13 (d, 1H, *J* = 2 Hz); ^{13}C-NMR (100 Hz, $CDCl_3$): two rotomers observed – some carbons are doubled. δ 35.6 and 36.6, 47.3, 52.8 and 53.3, 57.8 and 58.2, 66.2, 67.8 and 67.9, 74.6 and 75.7, 118.5 and 118.8, 120.1, 122.8, 125.0 and 125.1, 127.3, 127.9, 129.7, 131.8 and 131.9, 133.3, 141.3 and 141.4, 143.7 and 144.0, 148.8, 154.3 and 154.6, 162.0 and 162.1, 171.2 and 171.3; HRMS Calc. For $C_{30}H_{25}N_3O_{10}$ [M+H] = 588.1618, found [M+H] = 588.1616.

Fmoc-*cis*-Hyp-O-Allyl ester (5). To a solution of Fmoc-*cis*-Hyp(*O*-3,5-dinitrobenzoate)-O-Allyl ester (**19**) (2.955 g, 5.03 mmol) in 1,4-dioxane (50 ml) and CH_3OH (50 ml) was added NaN_3 (2.616 g, 40.3 mmol, 8 eq) and 15-crown-5 (0.100 ml, 0.503 mmol, 0.1 eq). The solution was stirred at 40 ^{0}C for

20 h, after which the reaction was judged complete by TLC (3:2 hexanes:ethyl acetate, UV, product R_f = 0.3). The reaction mixture was then concentrated *in vacuo* on silica. The product was isolated by dry-loaded silica flash column chromatography (2:3 hexanes:ethyl acetate) as a slightly yellow foam (1.995 g, quantitative yield): $[\alpha]_D^{25}$ -26.6 (*c* 0.5, CH_2Cl_2); IR ($CDCl_3$): 3470, 2953, 2884, 1701 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): two rotomers observed – some protons are doubled. δ 2.18 (t, 1H, *J* = 12 Hz), 2.30 – 2.37 (mult, 1H), 2.99 (d, 1H, *J* = 9 Hz, OH_a), 3.34 (d, 1H, *J* = 10 Hz, OH_b), 3.59 – 3.76 (mult, 2H), 4.11 – 4.59 (mult, 5H), 4.68 (d, 2H, *J* = 6 Hz), 5.21 – 5.37 (mult, 2H), 5.86 – 5.92 (mult, 1H), 7.27 – 7.31 (mult, 2H), 7.36 (t, 2H, *J* = 8 Hz), 7.51 – 7.57 (mult, 2H), 7.74 (d, 2H, *J* = 8 Hz), 8.99 (bs, 1H); ^{13}C-NMR (100 Hz, $CDCl_3$): two rotomers observed – some carbons are doubled. δ 38.0 and 39.2, 47.4 and 47.5, 56.0 and 56.3, 58.0 and 58.5, 66.2 and 66.3, 66.6 and 68.0, 70.2 and 71.4, 119.2 and 119.4, 120.2, 125.2 and 125.3, 127.3 and 128.0, 131.6, 141.5, 143.8 and 143.9, 144.1 and 144.3, 154.8 and 155.2, 174.4; HRMS Calc. For $C_{23}H_{23}NO_5$ [M+H] = 394.1654, found [M] = 394.1656.

(*o*-NO_2Ph)SO_2-Trp-O-Allyl ester = Ns-Trp-O-Allyl ester (12). A suspension of L-tryptophan (**22**) (5.044 g, 24.7 mmol) in allyl alcohol (62 ml) and benzene (21 ml) was degassed by bubbling argon through the mixture for 20 min. *Para*-toluenesulfonic acid hydrate (9.392 g, 49.38 mmol, 2.0 eq) was added, a Dean-Stark apparatus was attached, and the suspension was heated to reflux for 4.5 h, at which point 1.4 ml H_2O had been collected, and TLC (5:3:1 $CHCl_3$:CH_3OH:CH_3CO_2H, product R_f = 0.8) showed mostly complete conversion. The suspension was concentrated *in vacuo*, re-suspended in $NaHCO_3$ (5% aq., 50 ml), the pH was adjusted to 9.0 with NaOH (1 M aq), and the product was extracted with 1:1 ether:ethyl acetate (2 x 30 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield H-Trp-O-Allyl ester as a viscous amber oil (5.889 g, quant). The material was slightly contaminated with allyl alcohol, but could be used in the next step without further purification.

A solution of H-Trp-O-Allyl ester (5.683 g) in CH_2Cl_2 (70 ml) was stirred in an ice bath for 10 min, and treated with Et_3N (2.8 ml, 19.7 mmol, 0.8 eq). Next, a solution of *o*-nitrobenzenesulfonyl chloride

(4.38 g, 19.7 mmol, 0.8 eq) in CH_2Cl_2 (2 x 10 ml) was added by dropping funnel over several minutes. The solution turned amber during the addition and was then stirred for 1 h at rt. The solution was then concentrated *in vacuo* on silica. The target compound (**12**) was isolated by dry-loaded silica flash column chromatography (2:1 to 3:2 hexanes:ethyl acetate) as an amber oil (6.215 g, 73%): $[\alpha]_D^{25}$ -69.8 (*c* $CHCl_3$); IR ($CDCl_3$): 3476, 3154, 2253, 1740 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.24 – 3.32 (mult, 2H), 4.35 – 4.51 (mult, 3H), 5.14 – 5.20 (mult, 2H), 5.65 – 5.69 (mult, 1H), 6.04 (d, 1H, *J* = 8 Hz), 6.97 (t, 1H, *J* = 8 Hz), 7.03 (d, 1H, *J* = 3 Hz), 7.10 (t, 1H, *J* = 8 Hz), 7.22 (d, 1H, *J* = 8 Hz), 7.36 (d, 1H, *J* = 8 Hz), 7.47 – 7.52 (mult, 2H), 7.65 (dd, 1H, *J* = 2, 8 Hz), 7.85 (dd, 1H, *J* = 2, 8 Hz), 8.05 (bs, 1H); ^{13}C-NMR (100 Hz, $CDCl_3$): δ 29.3, 57.1, 66.5, 108.9, 111.6, 118.5, 119.3, 119.9, 122.4, 124.0, 125.6, 126.9, 130.4, 131.3, 132.9, 133.5, 133.8, 136.4, 147.1, 170.9. HRMS Calc. For $C_{20}H_{19}N_3O_6S$ [M+H] = 430.1073, found [M+H] = 430.1072.

General: NMR-derived computational restraints. 2D NMR spectra were processed using the SPARKY program.[3] Values of the ^{3}J NH-H_α coupling constants in the final products were derived by measurement of the distance between crosspeaks in the DQF-COSY spectra. ROESY spectra were recorded at 200 and 400 msec mixing times. Distance restraints for computational modeling experiments were established qualitatively using the isolated spin-pair approximation (ISPA) on ROESY peak volumes measured at the 400 msec mixing time. Thus, distances were estimated to be 3, 4, 5, or 6 angstroms based on comparison of relative peak volumes.

General: Computational modeling. All calculations were performed within the AMBER 8.0 suite of programs.[4] Molecular coordinate and topology files were constructed using the *tleap* module.

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[3] Goddard, T. D.; Kneller, D. G. *SPARKY 3*, University of California, San Francisco.

[4] Case, D. A.; Darden, T. A., Cheatham, T. E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlman, D. A.; Crowley, M.; Brozell,, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. A. (2004), *AMBER 8*, University of California, San Francisco.

Energy minimizations were performed using the AMBER 99 force field with the modified Generalized Born implicit solvent model[5] (parameters *igb*=5, *gbsa*=1, *extdiel*=48.75) as implemented in the *sander* module. MOE 2002.03 was used to visually analyze and build structures.[6]

Torsional restraints were applied by refining directly to ^3J NH-H_α coupling constants using a Karplus relation with A=9.5, B=-1.4, and C=0.3. In order to account for experimental error, deviations less than or equal to ±10% of the experimental J-coupling were not penalized. Deviations greater than ±10% and less than or equal to ±20% of the experimental value were penalized by a harmonic potential with a force constant of 2 kcal/mol; deviations greater than ±20% were penalized by a linear potential with a force constant of 5 kcal/mol. The *makeDIST_RST* module was used to convert experimentally derived distance cutoffs to distance restraint limits appropriate for the *sander* module. To prevent C_α-proton epimerization during the minimization, the *makeCHIR_RST* module provided appropriate *sander* chirality restraints for all amino acids except Ala[5]. The chirality restraint for this residue was omitted to allow equilibration of L-Ala[5] to D-Ala[5]. Stereochemical assignment of the C_α-proton was not possible by NMR-data alone, and inversion is mechanistically possible in the carboxyl activation of Ala[5] preceding the second cyclization of the synthesis. As mentioned in the "Results and Discussion" section, the absence of this restraint had no effect, and did not lead to epimerization of Ala[5] in these simulations. The potential function for distance and chirality restraints followed a harmonic function with force constants of 2 kcal/mol and 100 kcal/mol for small deviations, followed by a linear function with force constants of 5 kcal/mol and 200 kcal/mol, respectively. The bounds for the penalty functions were specific to the atoms involved and were determined by *makeDIST_RST or makeCHIR_RST*. All restraints were imposed at 100% weight for the entire simulation.

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[5] Onufriev, A.; Bashford, D.; Case, D. A. *Proteins-Structure Function and Bioinformatics*. **2004**, *55*(2), 383-394.

[6] "Molecular Operating Environment", Chemical Computing Group (http://www.chemcomp.com)

Method I results (restrained minimization with "U-Type" structure): The solution structure of [Ala^7]-phalloidin established by Paolillo et al.[7] provided the starting conformation for species **1** and **1'**. These two structures were subjected to 7,500 steps of steepest descent minimization followed by 2,500 steps of conjugate gradient minimization using the *sander* module. As reported in Table S5, the final structure of atropisomer **1**, following restrained minimization using NMR-derived restraints, had minimal violations. Heavy-atom backbone RMSD to the previously reported NMR and X-ray structures of [Ala^7]-phalloidin[7] were 0.54 Å and 0.80 Å, respectively. The absence of the chirality restraint at residue Ala^5 had no effect in the simulation, namely epimerization from L-Ala^5 to D-Ala^5 was not observed. The final model had a U-type structure with a positive indole-thioether angle (57.3) between atoms Cys^{3}C$_\beta$-Cys^{3}S-Trp^{6}C'2-Trp^{6}N$_{ind}$. However, when method I was employed on species **1'**, significant restraint violations and relatively high total energy were observed in the resulting model (Table S7).

Method II results (restrained minimization with "D-Type" structure): The inverted starting conformation used for species **1'** was manually constructed from the solution structure of species **1** by breaking the thioether bridge and rotating the Cys^3 and Trp^6 backbones roughly 180 degrees in MOE. The thioether bridge was reformed and the resulting structure was minimized in MOE using the MMFF94 force field.[8] This structure was then subjected to 7,500 steps of steepest descent minimization followed by 2,500 steps of conjugate gradient minimization using the *sander* module. The final structure was consistent with the NMR-derived restraints, and the overall energy is significantly lower than the structure resulting from method I (Table S8). The absence of a chirality restraint at residue

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[7] Zanotti, G.; Falcigno, L.; Saviano, M.; D'Auria, G.; Bruno, B. M.; Campanile, T.; Paolillo, L. *Chem. Eur. J.* **2001**, *7*, 1479-1485.

[8] (a) Halgren, T. A. *J. Am. Chem. Soc.* **1992**, *114*, 7827-7843. (b) Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 490-519. (c) Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 520-552. (d) Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 553-586. (e) Halgren, T. A.; Nachbar, R. B. *J. Comp. Chem.* **1996**, *17*, 587-615. (f) Halgren, T. A. *J. Comp. Chem.* **1996**, *17*, 616-641.

Ala^5 did not affect the model, and epimerization from L-Ala^5 to D-Ala^5 was not observed in the simulation. The model had a D-type structure with a negative indole-thioether angle (-62.7) between atoms Cys^{3}C$_\beta$-Cys^{3}S-Trp^{6}C'2-Trp^{6}N$_{ind}$.

Table S1: [Ala^7]-phalloidin "natural" atropisomer (1): ^{1}H assignments (recorded in DMSO$_{d6}$ at 298 K)

Residue	NH	H_α	H_β	H_γ	others	^3J NH-H_α [Hz]
Ala^1	7.31	4.48	1.21			7.9
D-Thr^2	8.50	3.96	4.24	1.06	OH (4.79)	7.6
Cys^3	7.69	4.73	3.22, 3.52			7.5
Hyp^4		4.14	1.80, 2.28	4.33	H_δ (3.50, 3.75)	
Ala^5	7.70	3.90	0.79			7.0
Trp^6	7.25	4.81	3.12, 3.32		H'$_4$ (7.71), H'$_5$ (6.97), H'$_6$ (7.10), H'$_7$ (7.23), NH_{ind} (11.22)	9.9
Ala^7	8.49	3.89	1.16			5.1

Table S2: [Ala^7]-phalloidin "natural" atropisomer (1): ^{13}C assignments of protonated carbons via HMQC (recorded in DMSO$_{d6}$ at 298 K)

Residue	C_α	C_β	C_γ	others
Ala^1	48.6	18.4		
D-Thr^2	59.0	64.4	20.1	
Cys^3	49.5	38.1		
Hyp^4	60.8	36.5	68.4	C_δ (53.9)
Ala^5	50.5	16.1		
Trp^6	51.7	28.6		C'$_4$ (119.8), C'$_5$ (118.2), C'$_6$ (122.1) C'$_7$ (110.4)
Ala^7	48.3	16.0		

Table S3: [Ala^7]-phalloidin "non-natural" atropisomer (1'): ^{1}H assignments (recorded in DMSO$_{d6}$ at 298 K)

Residue	NH	H_α	H_β	H_γ	others	^3J NH-H_α [Hz]
Ala^1	8.20	4.46	1.25			8.7
D-Thr^2	6.96	4.34	4.18	1.06		9.5
Cys^3	7.49	3.02	3.43, 3.75			7.6
Hyp^4		3.50	1.67, 2.12	4.09	H_δ (3.29, 3.45)	
Ala^5	7.89	4.14	1.07			8.8
Trp^6	6.23	4.68	3.10,3.19		H'$_4$ (7.53), H'$_5$ (7.06), H'$_6$ (7.16), H'$_7$ (7.32), NH_{ind} (11.45)	4.9
Ala^7	8.6	4.07	1.36			5.3

Table S4: [Ala^7]-phalloidin "non-natural" atropisomer (1'): ^{13}C assignments of protonated carbons via HMQC (recorded in DMSO$_{d6}$ at 298 K)

Residue	C_α	C_β	C_γ	others
Ala^1	47.1	17.9		
D-Thr^2	57.8	65.7	19.8	
Cys^3	54.0	33.6		
Hyp^4	54.8	38.6	66.3	C_δ (54.6)
Ala^5	46.8	1.07		
Trp^6	56.9	24.4		C'$_4$ (117.0), C'$_5$ (119.0), C'$_6$ (122.5) C'$_7$ (110.7)
Ala^7	51.5	15.9		

Table S5: Calculated and experimental NMR-derived restraints for "natural" atropisomer 1 (method I): total restraint violation = 0.33 kcal/mol; total energy = -28.8 kcal/mol.

Atom	Residue	Atom	Residue	Experimental	Calculated	Limit	Violation
(J-Value restraints):				*(Hz)*	*(Hz)*	*(Hz)*	*(kcal/mol)*
HN	Ala[1]	HA	Ala[1]	7.9	8.718	8.7	0.002
HN	D-Thr[2]	HA	D-Thr[2]	7.6	8.403	8.4	0
HN	Cys[3]	HA	Cys[3]	7.5	6.685	6.7	0
HN	Ala[5]	HA	Ala[5]	7	6.221	6.3	0.013
HN	Trp[6]	HA	Trp[6]	9.9	10.653	10.9	0
HN	Ala[7]	HA	Ala[7]	5.1	5.658	5.6	0.017
(distance restraints):				*(Å)*	*(Å)*	*(Å)*	*(kcal/mol)*
QB	Ala[5]	HA	Ala[5]	4	2.605	4.8	0
QG	D-Thr[2]	HA	D-Thr[2]	4	2.933	4.8	0
QG	D-Thr[2]	HB	D-Thr[2]	4	2.592	4.8	0
QB	Ala[7]	HA	Ala[7]	4	2.539	4.8	0
QB	Ala[1]	HA	Ala[1]	4	2.595	4.8	0
QB	Hyp[4]	HA	Hyp[4]	4	2.544	4.49	0
QB	Hyp[4]	HO	Hyp[4]	4	2.793	4.49	0
QB	Hyp[4]	HG3	Hyp[4]	3	2.578	3.37	0
QB	Hyp[4]	QD	Hyp[4]	4	3.399	5.04	0
QB	Hyp[4]	OH	Hyp[4]	5	2.683	5.61	0
QB	Hyp[4]	HA	Hyp[4]	4	2.544	4.49	0
QB	Trp[6]	HA	Trp[6]	5	2.542	5.61	0
HD2	Hyp[4]	HD3	Hyp[4]	3	1.79	1.8	0.01
QD	Hyp[4]	OH	Hyp[4]	4	2.667	4.49	0
QD	Hyp[4]	HA	Cys[3]	5	2.258	5.61	0
QD	Hyp[4]	HG3	Hyp[4]	4	2.527	4.49	0
QD	Hyp[4]	HA	Cys[3]	5	2.258	5.61	0
QD	Hyp[4]	OH	Hyp[4]	5	2.667	5.61	0
HA	D-Thr[2]	HB	D-Thr[2]	4	3.04	4.0	0
HA	Hyp[4]	HA	Trp[6]	6	6.243	6.0	0.296
HA	Hyp[4]	HG	Hyp[4]	4	2.973	4.0	0
HB	D-Thr[2]	HG	D-Thr[2]	4	2.248	4.0	0
HG3	Hyp[4]	OH	Hyp[4]	4	2.077	4.0	0
HA	Trp[6]	HE3	Trp[6]	5	2.429	5.0	0
(chirality restraints):				*(deg)*	*(deg)*	*(deg)*	*(deg)*
C	Ala[1]	HA	Ala[1]	-	73.967	80	0
C	D-Thr[2]	HA	D-Thr[2]	-	283.326	310	0
C	Cys[3]	HA	Cys[3]	-	75.596	80	0
C	Hyp[4]	HA	Hyp[4]	-	72.459	80	0
C	Trp[6]	HA	Trp[6]	-	74.658	80	0
C	Ala[7]	HA	Ala[7]	-	76.923	80	0

Table S6: Calculated Cartesian coordinates for "natural" atropisomer 1 (method I).

#	Atom	Residue	X	Y	Z	Element
1	N	Ala[1]	-0.396	-3.467	0.198	N
2	H	Ala[1]	-0.261	-2.496	0.439	H
3	CA	Ala[1]	-1.733	-4.03	0.412	C
4	HA	Ala[1]	-1.919	-4.829	-0.31	H
5	CB	Ala[1]	-1.792	-4.628	1.828	C
6	HB1	Ala[1]	-1.583	-3.858	2.573	H
7	HB2	Ala[1]	-2.784	-5.042	2.013	H
8	HB3	Ala[1]	-1.053	-5.426	1.925	H
9	C	Ala[1]	-2.797	-2.943	0.174	C
10	O	Ala[1]	-3.091	-2.132	1.056	O
11	N	D-Thr[2]	-3.335	-2.89	-1.049	N
12	H	D-Thr[2]	-3.046	-3.584	-1.726	H
13	CA	D-Thr[2]	-4.22	-1.805	-1.515	C
14	HA	D-Thr[2]	-4.871	-1.502	-0.693	H
15	CB	D-Thr[2]	-5.137	-2.267	-2.665	C
16	HB	D-Thr[2]	-4.54	-2.48	-3.552	H
17	CG2	D-Thr[2]	-5.948	-3.516	-2.305	C
18	HG21	D-Thr[2]	-6.518	-3.343	-1.391	H
19	HG22	D-Thr[2]	-6.633	-3.761	-3.118	H
20	HG23	D-Thr[2]	-5.282	-4.366	-2.156	H
21	OG1	D-Thr[2]	-6.081	-1.257	-2.958	O
22	HG1	D-Thr[2]	-6.577	-1.548	-3.735	H
23	C	D-Thr[2]	-3.367	-0.59	-1.912	C
24	O	D-Thr[2]	-3.082	-0.35	-3.089	O
25	N	Cys[3]	-2.868	0.136	-0.906	N
26	H	Cys[3]	-3.072	-0.174	0.038	H
27	CA	Cys[3]	-1.888	1.219	-1.057	C
28	HA	Cys[3]	-2.254	1.905	-1.819	H
29	CB	Cys[3]	-1.805	2.023	0.251	C
30	HB2	Cys[3]	-0.924	2.666	0.21	H
31	HB3	Cys[3]	-2.68	2.671	0.312	H
32	SG	Cys[3]	-1.751	1.035	1.776	S
33	C	Cys[3]	-0.497	0.678	-1.471	C
34	O	Cys[3]	0.069	-0.147	-0.745	O
35	N	Hyp[4]	0.105	1.143	-2.592	N
36	CD	Hyp[4]	-0.507	1.975	-3.626	C
37	HD2	Hyp[4]	-0.391	3.03	-3.37	H
38	HD3	Hyp[4]	-1.559	1.734	-3.773	H
39	CG	Hyp[4]	0.243	1.663	-4.918	C
40	OH	Hyp[4]	0.233	2.768	-5.802	O
41	HO	Hyp[4]	0.683	2.503	-6.617	H
42	HG3	Hyp[4]	-0.192	0.778	-5.387	H
43	CB	Hyp[4]	1.648	1.345	-4.419	C
44	HB2	Hyp[4]	2.215	2.273	-4.308	H
45	HB3	Hyp[4]	2.167	0.668	-5.1	H
46	CA	Hyp[4]	1.428	0.689	-3.047	C
47	HA	Hyp[4]	1.392	-0.395	-3.162	H
48	C	Hyp[4]	2.594	1.021	-2.104	C
49	O	Hyp[4]	3.6	0.312	-2.11	O
50	N	Ala[5]	2.457	2.058	-1.268	N
51	H	Ala[5]	1.6	2.592	-1.302	H
52	CA	Ala[5]	3.44	2.4	-0.231	C
53	HA	Ala[5]	4.435	2.413	-0.683	H
54	CB	Ala[5]	3.101	3.799	0.302	C
55	HB1	Ala[5]	2.119	3.794	0.779	H
56	HB2	Ala[5]	3.848	4.099	1.04	H
57	HB3	Ala[5]	3.106	4.522	-0.515	H

58	C	Ala[5]	3.504	1.361	0.908	C
59	O	Ala[5]	4.517	1.288	1.611	O
60	N	Trp[6]	2.441	0.554	1.077	N
61	H	Trp[6]	1.643	0.703	0.474	H
62	CA	Trp[6]	2.307	-0.469	2.129	C
63	HA	Trp[6]	3.127	-0.357	2.835	H
64	CB	Trp[6]	0.986	-0.256	2.884	C
65	HB2	Trp[6]	0.194	-0.747	2.321	H
66	HB3	Trp[6]	1.054	-0.765	3.846	H
67	CG	Trp[6]	0.607	1.181	3.136	C
68	CD1	Trp[6]	-0.506	1.819	2.701	C
69	NE1	Trp[6]	-0.495	3.142	3.07	N
70	HE1	Trp[6]	-1.246	3.782	2.847	H
71	CE2	Trp[6]	0.652	3.449	3.766	C
72	CZ2	Trp[6]	1.136	4.637	4.332	C
73	HZ2	Trp[6]	0.559	5.549	4.258	H
74	CH2	Trp[6]	2.377	4.628	4.991	C
75	HH2	Trp[6]	2.766	5.538	5.432	H
76	CZ3	Trp[6]	3.113	3.432	5.074	C
77	HZ3	Trp[6]	4.07	3.425	5.581	H
78	CE3	Trp[6]	2.617	2.244	4.504	C
79	HE3	Trp[6]	3.201	1.336	4.576	H
80	CD2	Trp[6]	1.374	2.217	3.833	C
81	C	Trp[6]	2.383	-1.902	1.605	C
82	O	Trp[6]	2.733	-2.791	2.38	O
83	N	Ala[7]	2.035	-2.11	0.325	N
84	H	Ala[7]	1.635	-1.307	-0.143	H
85	CA	Ala[7]	1.998	-3.377	-0.438	C
86	HA	Ala[7]	2.123	-3.106	-1.489	H
87	CB	Ala[7]	3.203	-4.28	-0.111	C
88	HB1	Ala[7]	3.074	-4.763	0.858	H
89	HB2	Ala[7]	3.29	-5.061	-0.869	H
90	HB3	Ala[7]	4.124	-3.695	-0.112	H
91	C	Ala[7]	0.645	-4.125	-0.329	C
92	O	Ala[7]	0.547	-5.292	-0.719	O

Table S7: Calculated and experimental NMR-derived restraints for "non-natural" atropisomer 1' (method I): total restraint violation = 18.48 kcal/mol; total energy = -3.48 kcal/mol.

Atom	Residue	Atom	Residue	Experimental	Calculated	Limit	Violation
(J-Value restraints):				*(Hz)*	*(Hz)*	*(Hz)*	*(kcal/mol)*
HN	Ala[1]	HA	Ala[1]	8.7	9.635	9.6	0.006
HN	D-Thr[2]	HA	D-Thr[2]	9.5	10.583	10.5	0.034
HN	Cys[3]	HA	Cys[3]	7.6	6.746	6.8	0.006
HN	Ala[5]	HA	Ala[5]	8.8	7.711	7.9	0.071
HN	Trp[6]	HA	Trp[6]	4.9	5.438	5.4	0.007
HN	Ala[7]	HA	Ala[7]	5.3	5.869	5.8	0.024
(distance restraints):				*(Å)*	*(Å)*	*(Å)*	*(kcal/mol)*
QG	D-Thr[2]	HA	Ala[1]	5.0	4.167	6.0	0
QG	D-Thr[2]	HA	D-Thr[2]	4.0	2.932	4.8	0
QG	D-Thr[2]	HB	D-Thr[2]	3.0	2.593	3.6	0
QG	D-Thr[2]	HN	D-Thr[2]	4.0	3.086	4.8	0

QB	Ala^5	HN	Ala^5	4.0	2.607	4.8	0
QB	Ala^5	HA	Ala^5	3.0	2.642	3.6	0
QB	Ala^5	QB	Cys^3	6.0	3.62	8.09	0
QB	Ala^5	HA	D-Thr^2	5.0	9.072	6.0	14.112
QB	Ala^1	HN	Ala^1	4.0	3.14	4.8	0
QB	Ala^1	HA	Ala^1	3.0	2.599	3.6	0
QB	Ala^1	HN	D-Thr^2	5.0	4.498	6.0	0
QB	Ala^1	QB	Cys^3	6.0	7.111	8.09	0
QB	Ala^1	HA	Ala^7	5.0	5.491	6.0	0
QB	Ala^1	HA	Ala^1	4.0	5.198	4.8	0.793
QB	Ala^1	HA	Ala^7	3.0	2.544	3.6	0
QB	Hyp^4	HA	Hyp^4	4.0	2.537	4.49	0
QB	Hyp^4	HG3	Hyp^4	4.0	2.61	4.49	0
QB	Hyp^4	QD	Hyp^4	5.0	3.219	6.3	0
QB	Hyp^4	HA	Hyp^4	3.0	2.537	3.37	0
QB	Trp^6	HA	Trp^6	4.0	2.608	4.49	0
QB	Trp^6	HN	Trp^6	4.0	2.721	4.49	0
QB	Trp^6	HE3	Trp^6	4.0	3.55	4.49	0
QB	Trp^6	HG3	Hyp^4	6.0	7.537	6.73	2.786
QB	Trp^6	HA	Trp^6	4.0	2.608	4.49	0
QB	Trp^6	HE3	Trp^6	5.0	3.55	5.61	0
HD2	Hyp^4	HD3	Hyp^4	3.0	1.792	1.8	0
QD	Hyp^4	HG3	Hyp^4	4.0	2.55	4.49	0
QB	Cys^3	HN	Ala^1	6.0	5.192	6.73	0
HA	Ala^7	HN	Ala^1	5.0	3.076	5.0	0
HA	Ala^5	HN	Trp^6	4.0	3.549	4.0	0
HB	D-Thr^2	HN	Cys^3	5.0	4.47	5.0	0
HB	D-Thr^2	HN	D-Thr^2	4.0	2.662	4.0	0
HA	D-Thr^2	HN	D-Thr^2	3.0	2.996	3.0	0
HA	Ala^1	HN	D-Thr^2	4.0	2.479	4.0	0
HA	Ala^1	HN	Ala^1	3.0	2.957	3.0	0
HA	Trp^6	HN	D-Thr^2	7.0	7.356	7.0	0.633
(chirality restraints):				*(deg)*	*(deg)*	*(deg)*	*(deg)*
C	Ala^1	HA	Ala^1	-	73.801	80	0
C	D-Thr^2	HA	D-Thr^2	-	283.338	310	0
C	Cys^3	HA	Cys^3	-	74.887	80	0
C	Hyp^4	HA	Hyp^4	-	72.56	80	0
C	Trp^6	HA	Trp^6	-	73.753	80	0
C	Ala^7	HA	Ala^7	-	76.741	80	0

Table S8: Calculated and experimental NMR-derived restraints for "non-natural" atropisomer 1' (method II): total restraint violation = 3.86 kcal/mol; total energy = -24.84 kcal/mol.

Atom	Residue	Atom	Residue	Experimental	Calculated	Limit	Violation
(J-Value restraints):				*(Hz)*	*(Hz)*	*(Hz)*	*(kcal/mol)*
HN	Ala^1	HA	Ala^1	8.7	9.665	9.6	0.021
HN	D-Thr^2	HA	D-Thr^2	9.5	10.486	10.5	0
HN	Cys^3	HA	Cys^3	7.6	8.006	8.4	0
HN	Ala^5	HA	Ala^5	8.8	7.863	7.9	0.003
HN	Trp^6	HA	Trp^6	4.9	4.355	4.4	0.004
HN	Ala^7	HA	Ala^7	5.3	5.747	5.8	0

(distance restraints):				*(Å)*	*(Å)*	*(Å)*	*(kcal/mol)*
QG	D-Thr^2	HA	Ala^1	5.0	3.712	6.0	0
QG	D-Thr^2	HA	D-Thr^2	4.0	3.216	4.8	0
QG	D-Thr^2	HB	D-Thr^2	3.0	2.582	3.6	0
QG	D-Thr^2	HN	D-Thr^2	4.0	3.272	4.8	0
QB	Ala^5	HN	Ala^5	4.0	2.681	4.8	0
QB	Ala^5	HA	Ala^5	3.0	2.599	3.6	0
QB	Ala^5	QB	Cys^3	6.0	6.979	8.09	0
QB	Ala^5	HA	D-Thr^2	5.0	4.961	6.0	0
QB	Ala^1	H	Ala^1	4.0	3.069	4.8	0
QB	Ala^1	HA	Ala^1	3.0	2.622	3.6	0
QB	Ala^1	HN	D-Thr^2	5.0	4.399	6.0	0
QB	Ala^1	QB	Cys^3	6.0	8.803	8.09	2.315
QB	Ala^1	HA	Ala^7	5.0	5.241	6.0	0
QB	Ala^7	HN	Ala^1	5.0	3.711	6.0	0
QB	Ala^7	HA	Ala^1	4.0	5.031	4.8	0.268
QB	Ala^7	HA	Ala^7	3.0	2.593	3.6	0
QB	Hyp^4	HG3	Hyp^4	3.0	2.598	3.37	0
QB	Hyp^4	HA	Hyp^4	4.0	2.545	4.49	0
QB	Hyp^4	QD	Hyp^4	5.0	3.2	6.3	0
QB	Hyp^4	HA	Hyp^4	3.0	2.545	3.37	0
QB	Trp^6	HA	Trp^6	4.0	2.711	4.49	0
QB	Trp^6	HN	Trp^6	4.0	2.66	4.49	0
QB	Trp^6	HE3	Trp^6	4.0	2.774	4.49	0
QB	Trp^6	HG3	Hyp^4	6.0	7.229	6.73	1.245
QB	Trp^6	HA	Trp^6	4.0	2.711	4.49	0
QB	Trp^6	HE3	Trp^6	5.0	2.774	5.61	0
HD2	Hyp^4	HD3	Hyp^4	3.0	1.791	1.8	0
QD	Hyp^4	HG3	Hyp^4	4.0	2.597	4.49	0
QB	Cys^3	HN	Ala^1	6.0	6.359	6.73	0
HA	Ala^7	HN	Ala^1	5.0	3.474	5.0	0
HA	Ala^5	HN	Trp^6	4.0	2.463	4.0	0
HB	D-Thr^2	HN	Cys^3	5.0	4.069	5.0	0
HB	D-Thr^2	HN	D-Thr^2	4.0	3.065	4.0	0
HA	D-Thr^2	HN	D-Thr^2	3.0	2.992	3.0	0
HA	Ala^1	HN	D-Thr^2	4.0	3.052	4.0	0
HA	Ala^1	HN	Ala^1	3.0	2.959	3.0	0
HA	Trp^6	HN	D-Thr^2	7.0	3.418	7.0	0
(chirality restraints):				*(deg)*	*(deg)*	*(deg)*	*(deg)*
C	Ala^1	HA	Ala^1	-	71.963	80	0
C	D-Thr^2	HA	D-Thr^2	-	283.841	310	0
C	Cys^3	HA	Cys^3	-	76.155	80	0
C	Hyp^4	HA	Hyp^4	-	72.781	80	0
C	Trp^6	HA	Trp^6	-	74.492	80	0
C	Ala^7	HA	Ala^7	-	73.399	80	0

Table S9: Calculated Cartesian coordinates for "non-natural" atropisomer 1' using method II.

#	Atom	Residue	X	Y	Z	Element
1	N	Ala^1	-0.902	-3.252	3.001	N
2	H	Ala^1	-0.49	-2.566	2.38	H
3	CA	Ala^1	-2.1	-2.83	3.736	C
4	HA	Ala^1	-2.662	-3.72	4.026	H
5	CB	Ala^1	-1.593	-2.092	4.986	C

6	HB1	Ala[1]	-1.078	-1.181	4.674	H
7	HB2	Ala[1]	-2.431	-1.828	5.631	H
8	HB3	Ala[1]	-0.906	-2.725	5.546	H
9	C	Ala[1]	-3.087	-1.945	2.931	C
10	O	Ala[1]	-3.835	-1.168	3.532	O
11	N	D-Thr[2]	-3.108	-2.026	1.59	N
12	H	D-Thr[2]	-2.515	-2.714	1.148	H
13	CA	D-Thr[2]	-4.086	-1.289	0.744	C
14	HA	D-Thr[2]	-4.431	-0.422	1.308	H
15	CB	D-Thr[2]	-5.336	-2.153	0.426	C
16	HB	D-Thr[2]	-5.13	-2.768	-0.45	H
17	CG2	D-Thr[2]	-5.806	-3.077	1.555	C
18	HG21	D-Thr[2]	-5.996	-2.499	2.46	H
19	HG22	D-Thr[2]	-6.722	-3.59	1.262	H
20	HG23	D-Thr[2]	-5.049	-3.835	1.76	H
21	OG1	D-Thr[2]	-6.454	-1.326	0.167	O
22	HG1	D-Thr[2]	-7.174	-1.902	-0.123	H
23	C	D-Thr[2]	-3.477	-0.698	-0.551	C
24	O	D-Thr[2]	-4.208	-0.382	-1.493	O
25	N	Cys[3]	-2.136	-0.563	-0.615	N
26	H	Cys[3]	-1.633	-0.803	0.234	H
27	CA	Cys[3]	-1.332	0.042	-1.71	C
28	HA	Cys[3]	-0.287	-0.088	-1.453	H
29	CB	Cys[3]	-1.538	-0.732	-3.03	C
30	HB2	Cys[3]	-2.581	-1.028	-3.11	H
31	HB3	Cys[3]	-1.332	-0.06	-3.861	H
32	SG	Cys[3]	-0.534	-2.228	-3.275	S
33	C	Cys[3]	-1.552	1.566	-1.922	C
34	O	Cys[3]	-2.674	2.058	-1.778	O
35	N	Hyp[4]	-0.509	2.332	-2.331	N
36	CD	Hyp[4]	-0.719	3.546	-3.109	C
37	HD2	Hyp[4]	-0.81	4.405	-2.442	H
38	HD3	Hyp[4]	-1.602	3.464	-3.744	H
39	CG	Hyp[4]	0.529	3.683	-3.978	C
40	OH	Hyp[4]	0.712	5.007	-4.436	O
41	HO	Hyp[4]	1.507	5.029	-4.988	H
42	HG3	Hyp[4]	0.489	2.959	-4.797	H
43	CB	Hyp[4]	1.612	3.242	-3.006	C
44	HB2	Hyp[4]	1.816	4.036	-2.284	H
45	HB3	Hyp[4]	2.524	2.941	-3.525	H
46	CA	Hyp[4]	0.939	2.046	-2.319	C
47	HA	Hyp[4]	1.097	1.175	-2.947	H
48	C	Hyp[4]	1.605	1.75	-0.963	C
49	O	Hyp[4]	2.764	1.341	-0.939	O
50	N	Ala[5]	0.876	1.857	0.155	N
51	H	Ala[5]	-0.023	2.315	0.094	H
52	CA	Ala[5]	1.153	1.092	1.38	C
53	HA	Ala[5]	2.214	1.175	1.632	H
54	CB	Ala[5]	0.325	1.696	2.528	C
55	HB1	Ala[5]	-0.742	1.632	2.304	H
56	HB2	Ala[5]	0.523	1.154	3.454	H
57	HB3	Ala[5]	0.597	2.743	2.672	H
58	C	Ala[5]	0.853	-0.402	1.124	C
59	O	Ala[5]	-0.172	-0.922	1.565	O
60	N	Trp[6]	1.641	-1.066	0.265	N
61	H	Trp[6]	2.492	-0.609	-0.043	H
62	CA	Trp[6]	1.278	-2.335	-0.4	C
63	HA	Trp[6]	0.265	-2.237	-0.781	H
64	CB	Trp[6]	2.221	-2.608	-1.584	C
65	HB2	Trp[6]	3.247	-2.562	-1.224	H
66	HB3	Trp[6]	2.045	-3.62	-1.945	H
67	CG	Trp[6]	2.117	-1.715	-2.789	C

68	CD1	Trp^{6}	1.029	-1.528	-3.577	C
69	NE1	Trp^{6}	1.325	-0.715	-4.643	N
70	HE1	Trp^{6}	0.655	-0.495	-5.371	H
71	CE2	Trp^{6}	2.631	-0.289	-4.592	C
72	CZ2	Trp^{6}	3.387	0.556	-5.415	C
73	HZ2	Trp^{6}	2.937	1.004	-6.292	H
74	CH2	Trp^{6}	4.726	0.818	-5.084	C
75	HH2	Trp^{6}	5.323	1.472	-5.708	H
76	CZ3	Trp^{6}	5.288	0.226	-3.938	C
77	HZ3	Trp^{6}	6.321	0.429	-3.682	H
78	CE3	Trp^{6}	4.52	-0.627	-3.119	C
79	HE3	Trp^{6}	4.972	-1.067	-2.241	H
80	CD2	Trp^{6}	3.167	-0.908	-3.419	C
81	C	Trp^{6}	1.161	-3.552	0.516	C
82	O	Trp^{6}	0.552	-4.545	0.118	O
83	N	Ala^{7}	1.627	-3.464	1.761	N
84	H	Ala^{7}	2.186	-2.658	2.004	H
85	CA	Ala^{7}	1.286	-4.418	2.822	C
86	HA	Ala^{7}	1.47	-5.432	2.461	H
87	CB	Ala^{7}	2.216	-4.142	4.01	C
88	HB1	Ala^{7}	2.017	-3.147	4.412	H
89	HB2	Ala^{7}	2.028	-4.877	4.794	H
90	HB3	Ala^{7}	3.26	-4.212	3.698	H
91	C	Ala^{7}	-0.189	-4.352	3.291	C
92	O	Ala^{7}	-0.653	-5.276	3.965	O

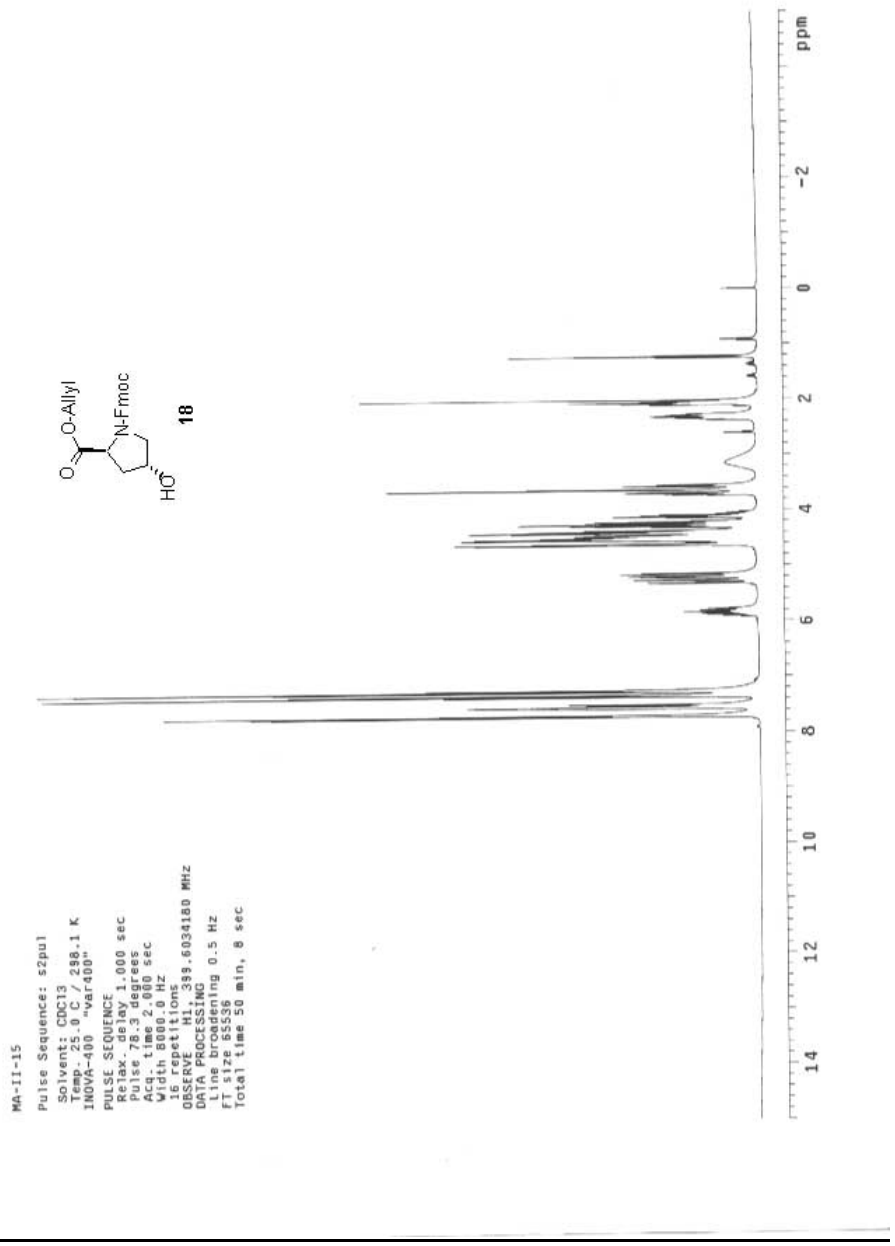

Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$): Fmoc-*trans*-Hyp-O-Allyl ester (**18**) (note presence of two rotomers)

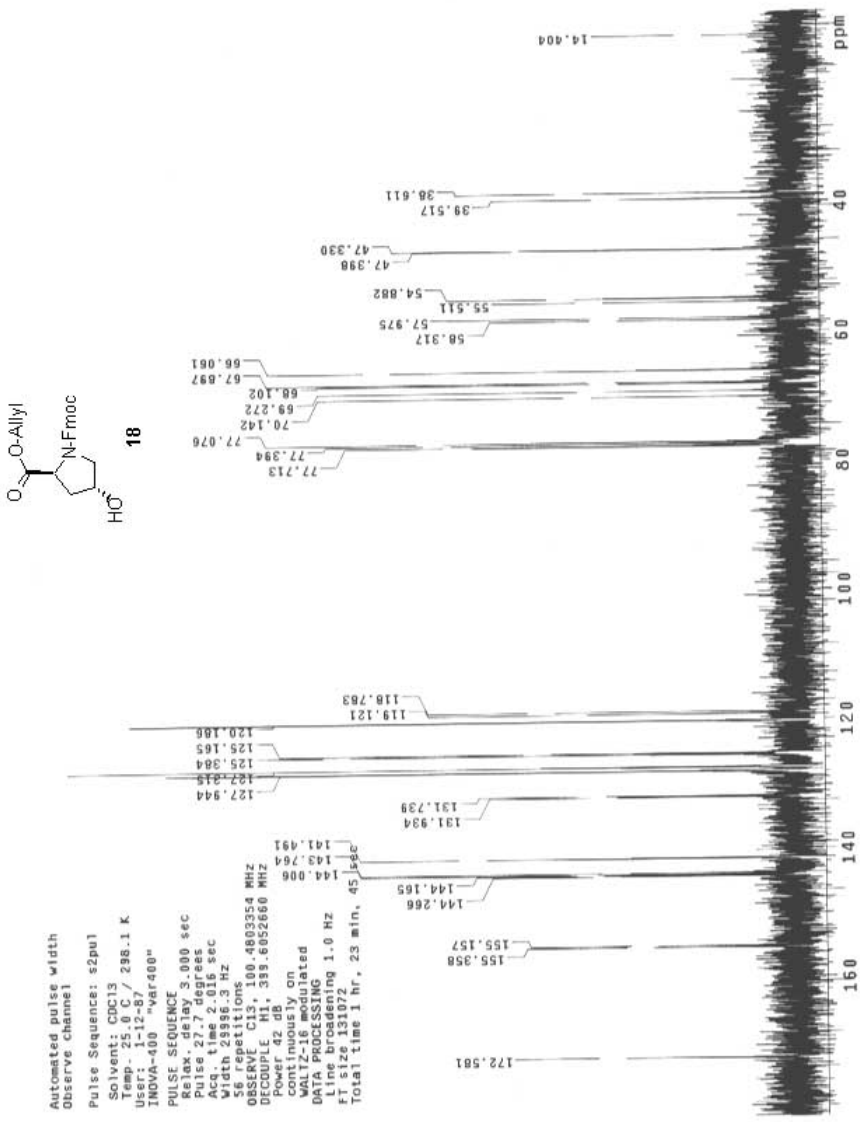

Figure S3. ^{13}C NMR (400 MHz, $CDCl_3$): Fmoc-*trans*-Hyp-O-Allyl ester (**18**) (note presence of two rotomers)

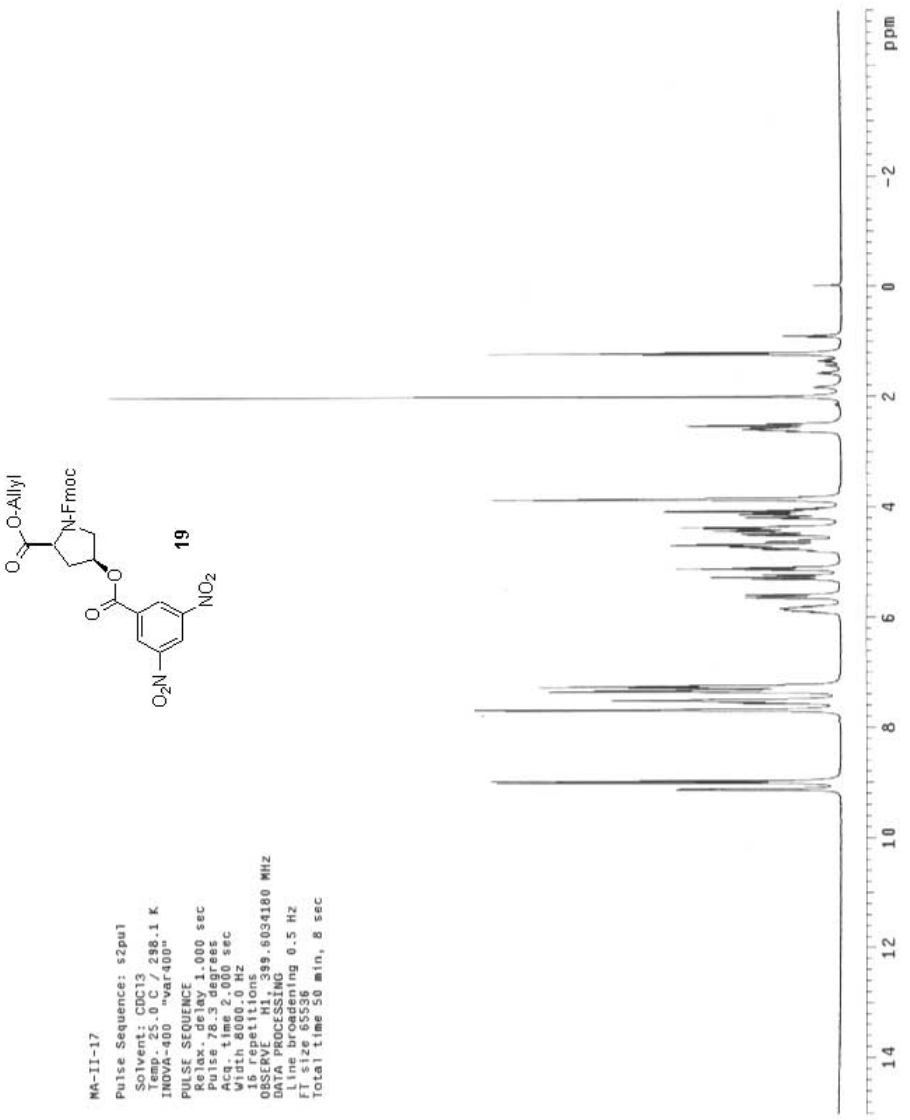

Figure S4. ^{1}H NMR (400 MHz, $CDCl_3$): Fmoc-*cis*-Hyp(*O*-3,5-dinitrobenzoate)-O-Allyl ester (**19**) (note presence of two rotomers)

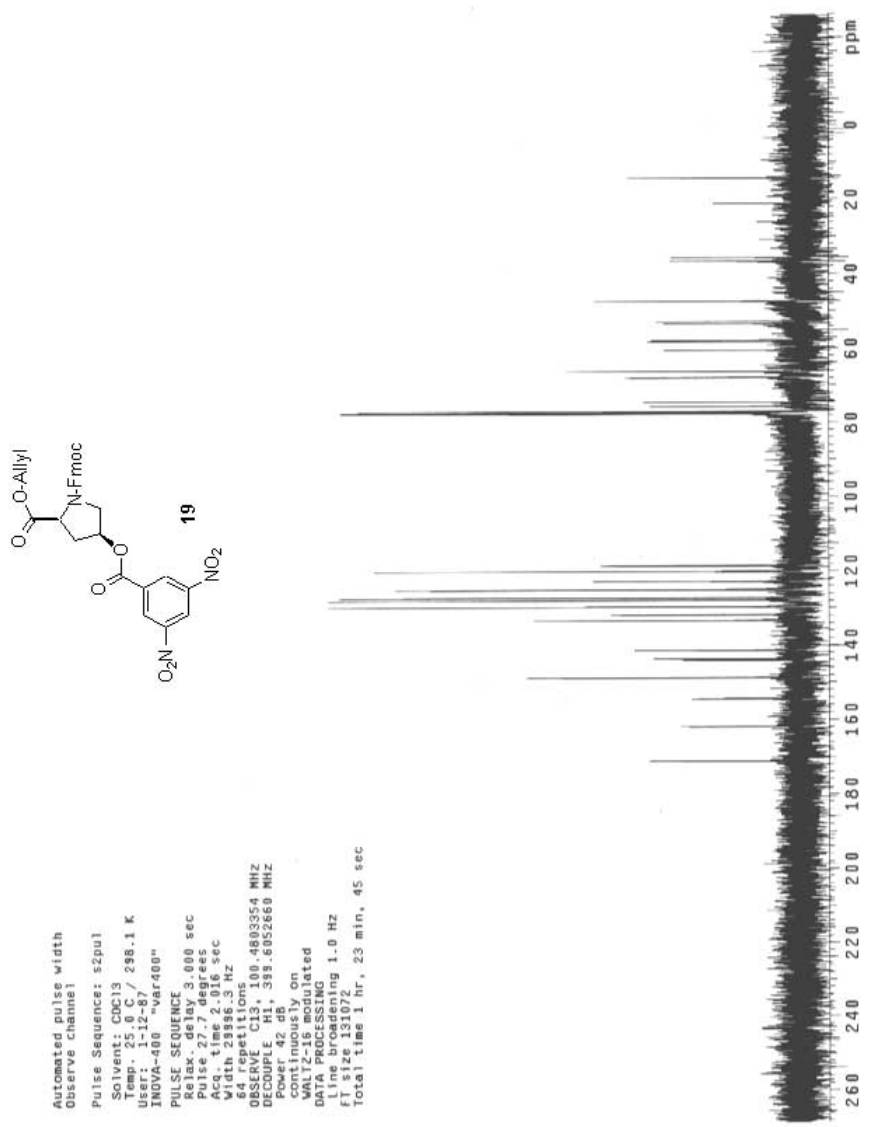

Figure S5. ^{13}C NMR (400 MHz, $CDCl_3$): Fmoc-*cis*-Hyp(*O*-3,5-dinitrobenzoate)-O-Allyl ester (**19**) (note presence of two rotomers)

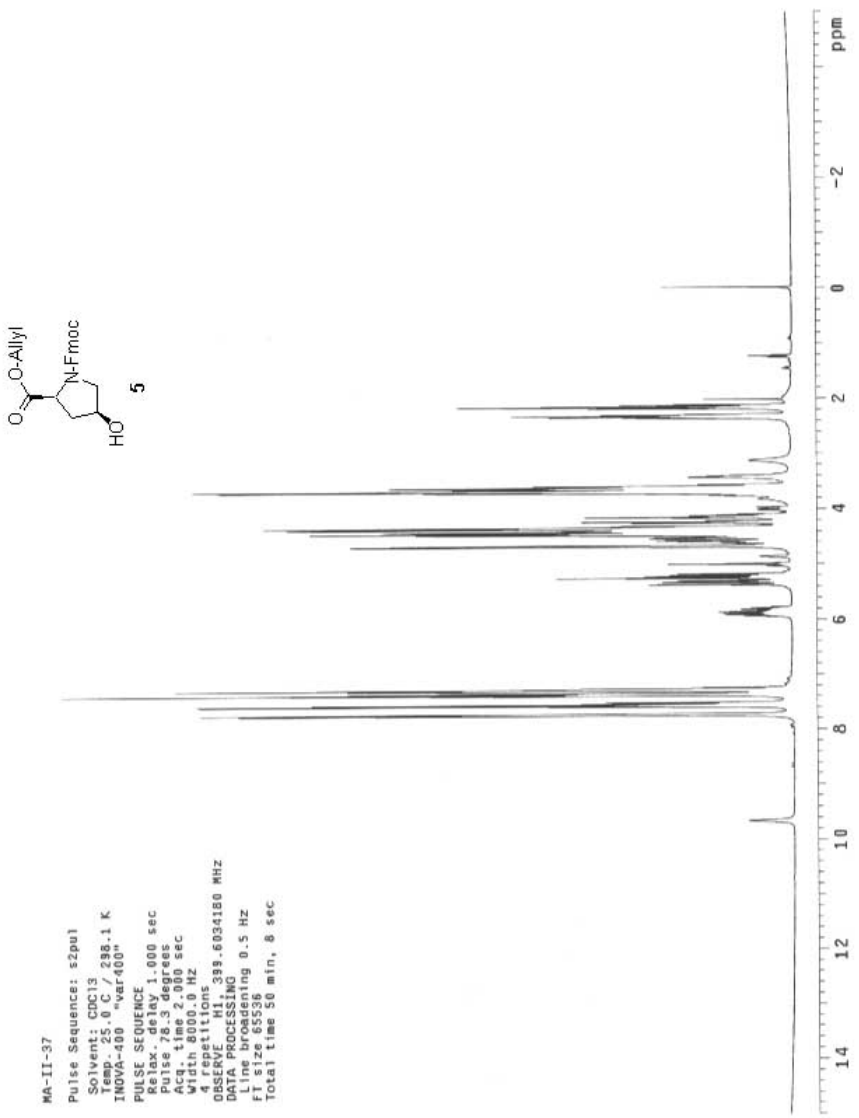

Figure S6. ^{1}H NMR (400 MHz, $CDCl_3$): Fmoc-*cis*-Hyp-O-Allyl ester (**5**) (note presence of two rotomers)

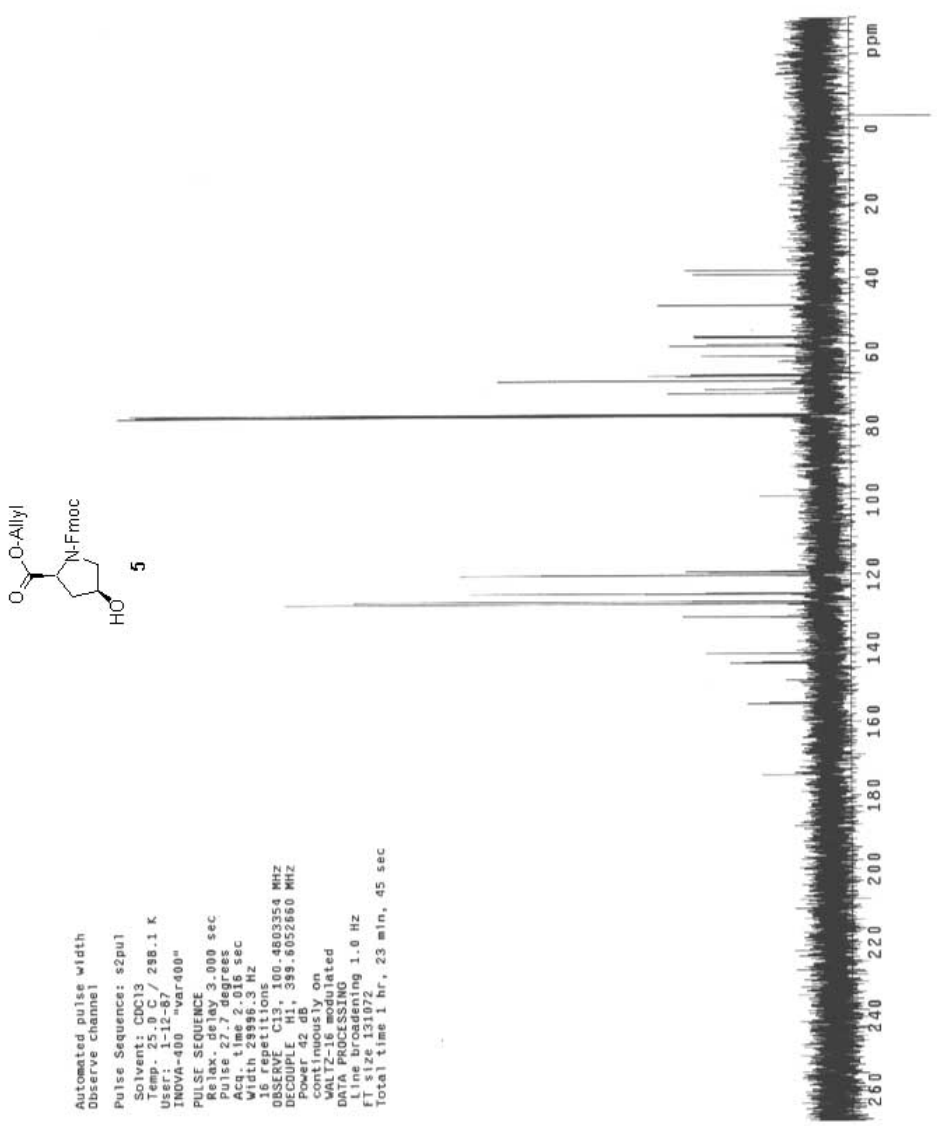

Figure S7. ^{13}C NMR (400 MHz, $CDCl_3$): Fmoc-*cis*-Hyp-O-Allyl ester (**5**) (note presence of two rotomers)

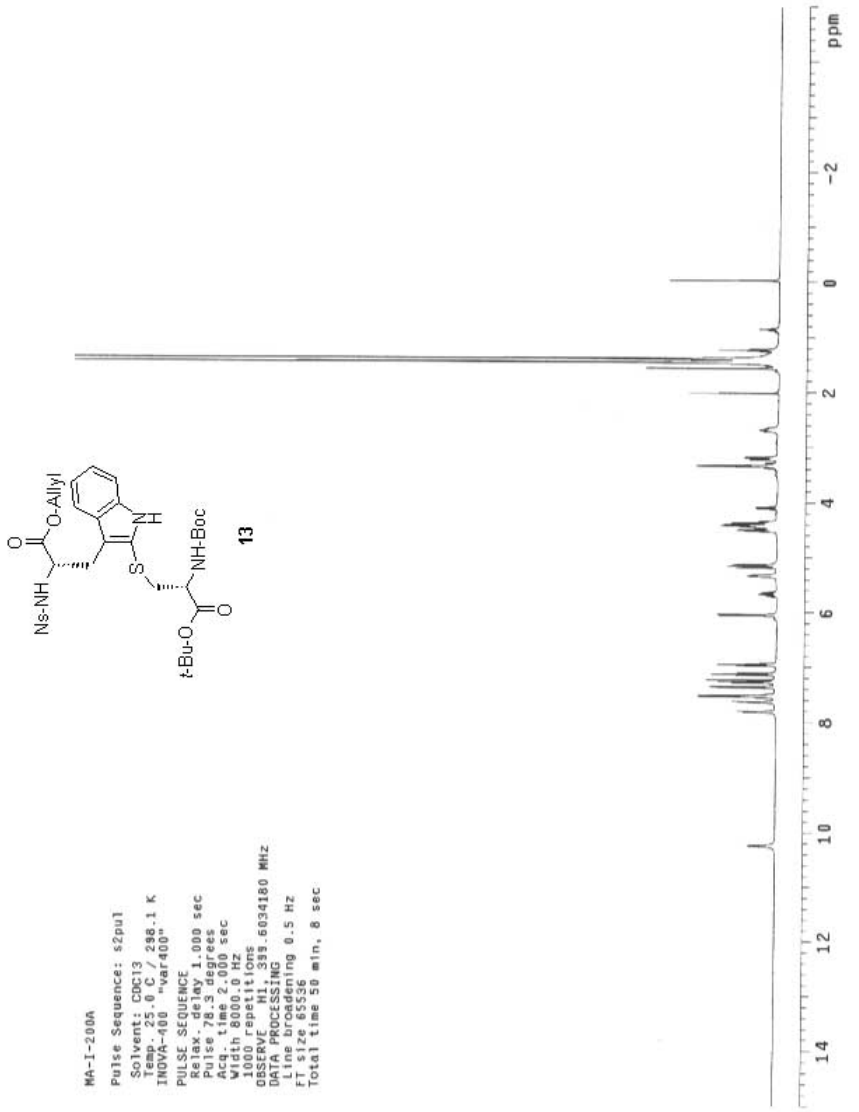

Figure S8. ^{1}H NMR (400 MHz, $CDCl_3$): Boc-Cys-[*S*-(2-((*o*-NO_2Ph)SO_2-Trp-O-Allyl))]-O-*t*-Bu ester (**13**)

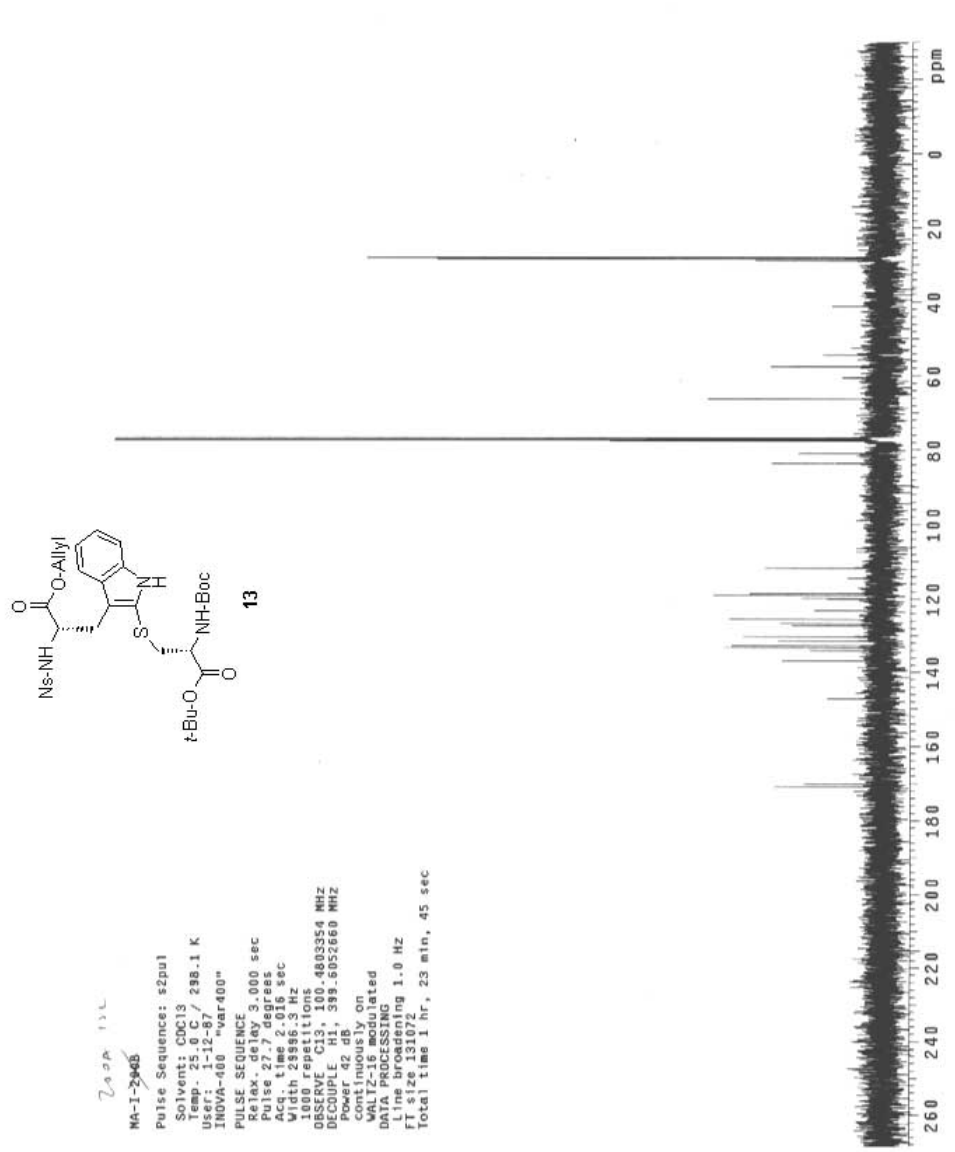

Figure S9. ^{13}C NMR (400 MHz, $CDCl_3$): Boc-Cys-[*S*-(2-((*o*-NO_2Ph)SO_2-Trp-O-Allyl))]-O-*t*-Bu ester (**13**)

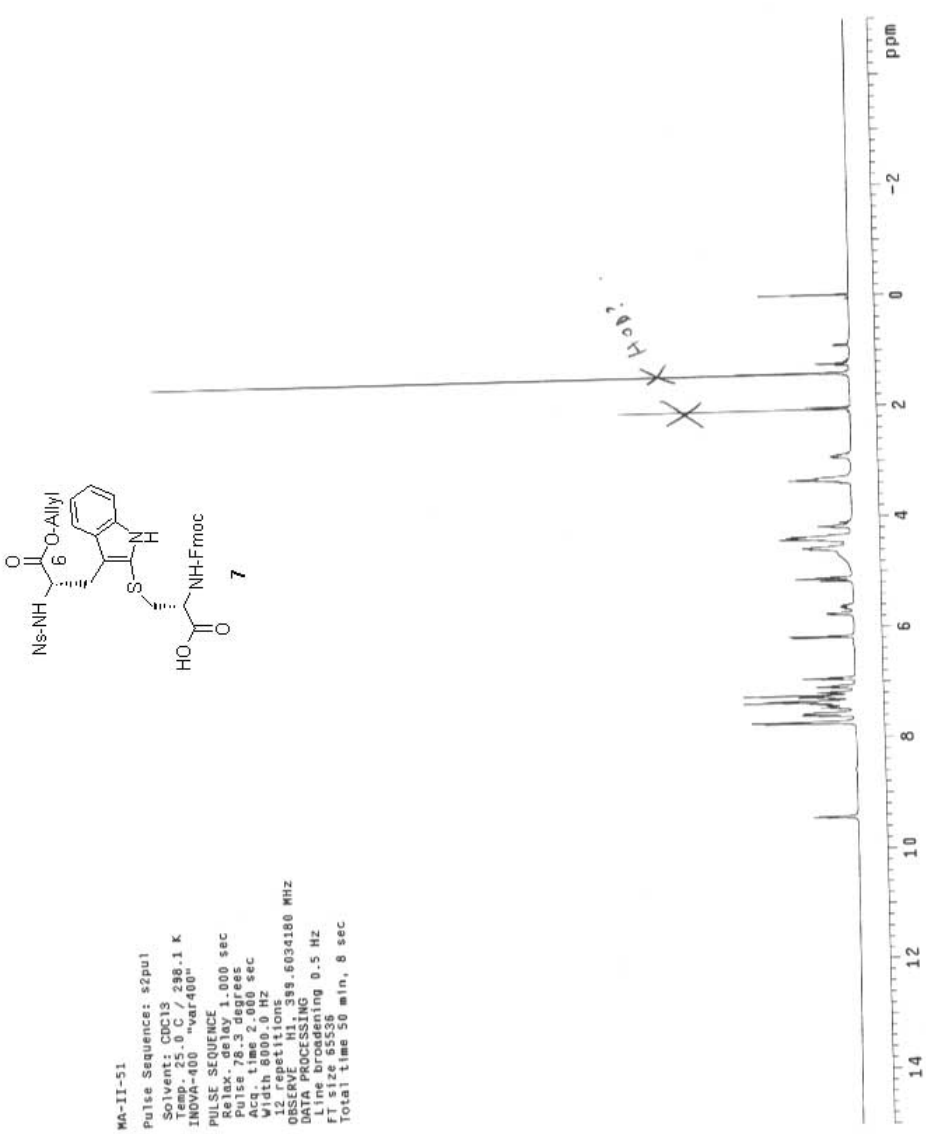

Figure S10. ^{1}H NMR (400 MHz, $CDCl_3$): Fmoc-Cys-[*S*-(2-((*o*-NO_2Ph)SO_2-Trp-O-Allyl))]-OH (**7**)

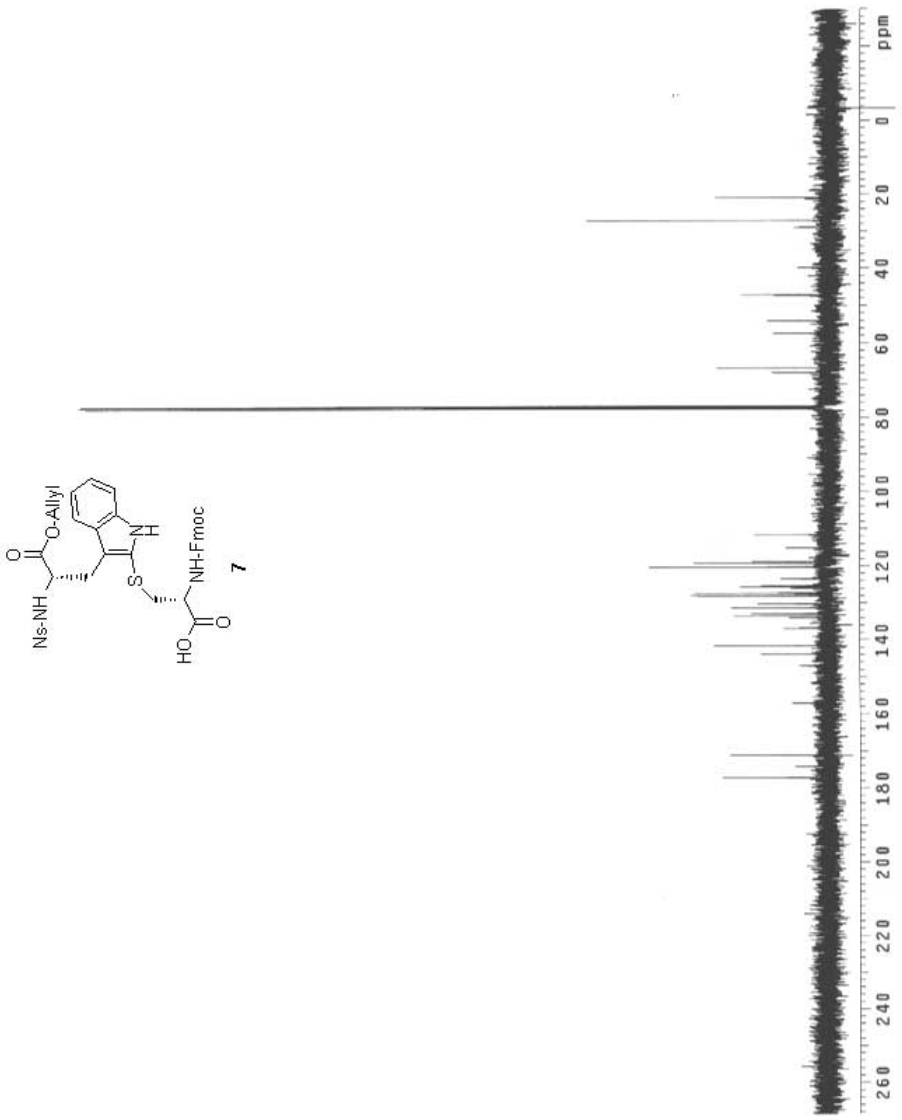

Figure S11. ^{13}C NMR (400 MHz, $CDCl_3$): Fmoc-Cys-[*S*-(2-((*o*-NO_2Ph)SO_2-Trp-O-Allyl))]-OH (**7**)

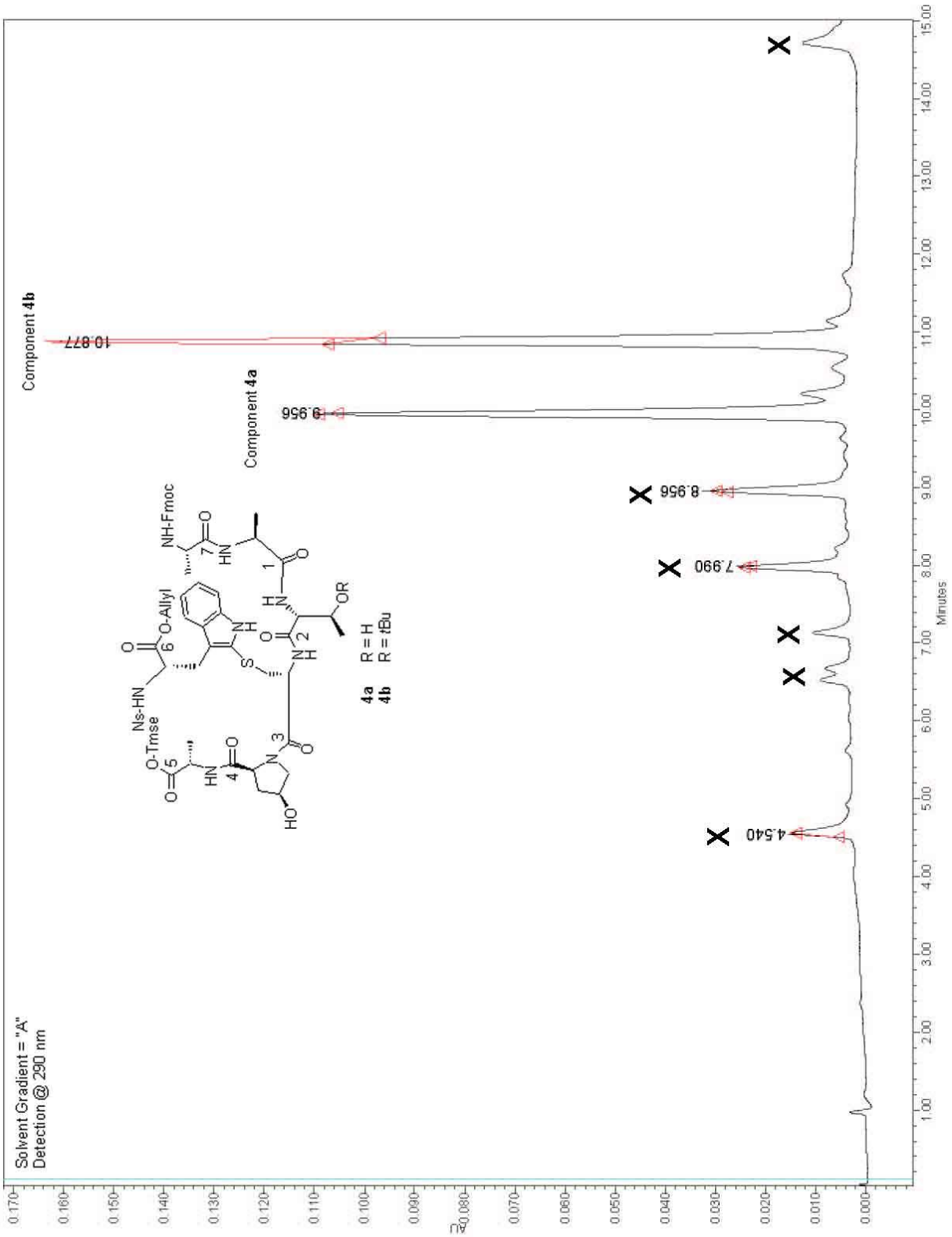

Figure S12. Analytical RP-HPLC: Non-cyclic heptapeptide **4**. Partially deprotected components **4a** (9.0 min) and **4b** (10.9 min) were collected and analyzed by MALDI-TOF-MS (Figures S3 and S4). Other peaks were not identified.

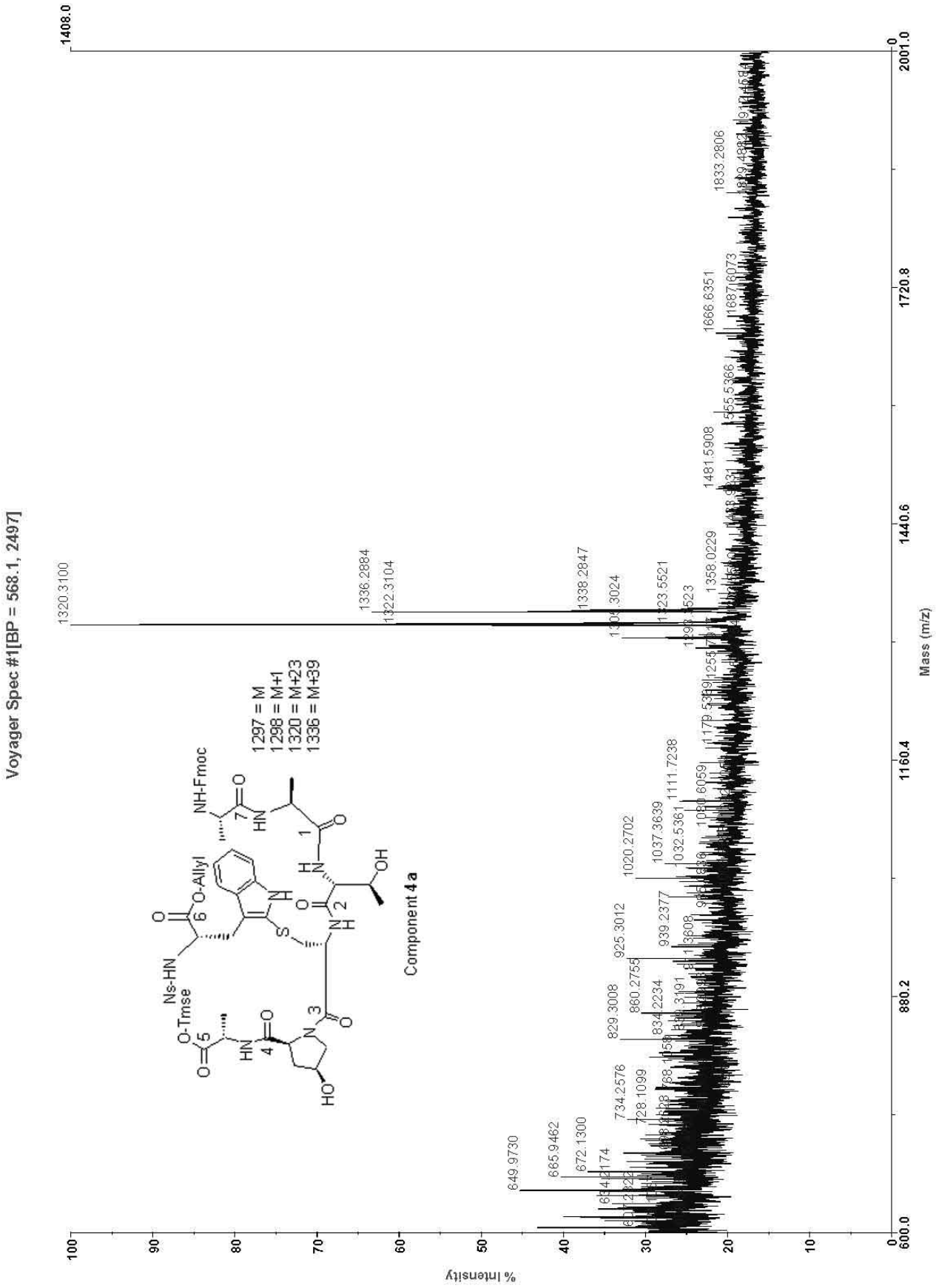

Figure S13. MALDI-TOF-MS: Partially deprotected component **4a**.

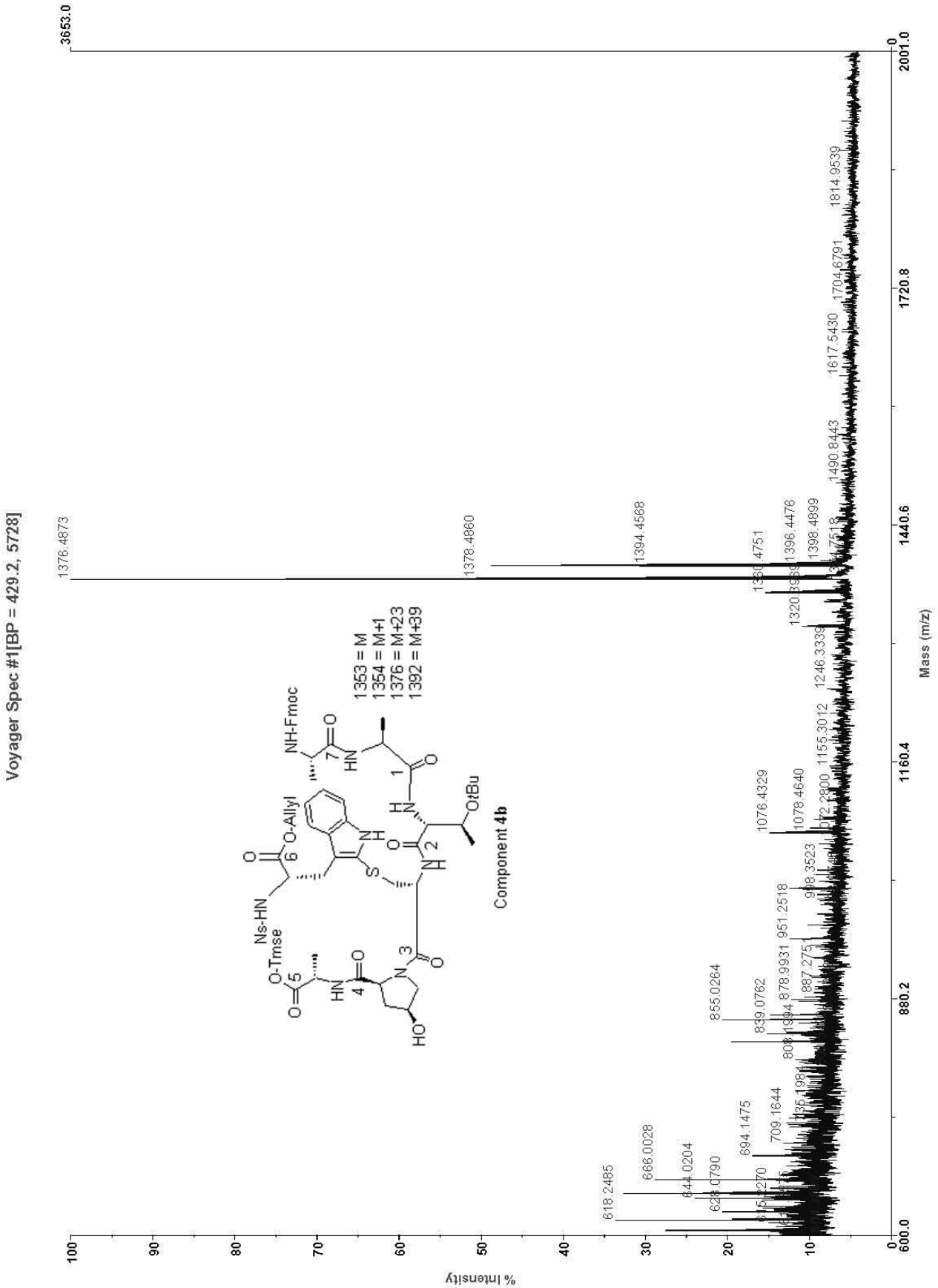

Figure S14. MALDI-TOF-MS: Partially deprotected component **4b**.

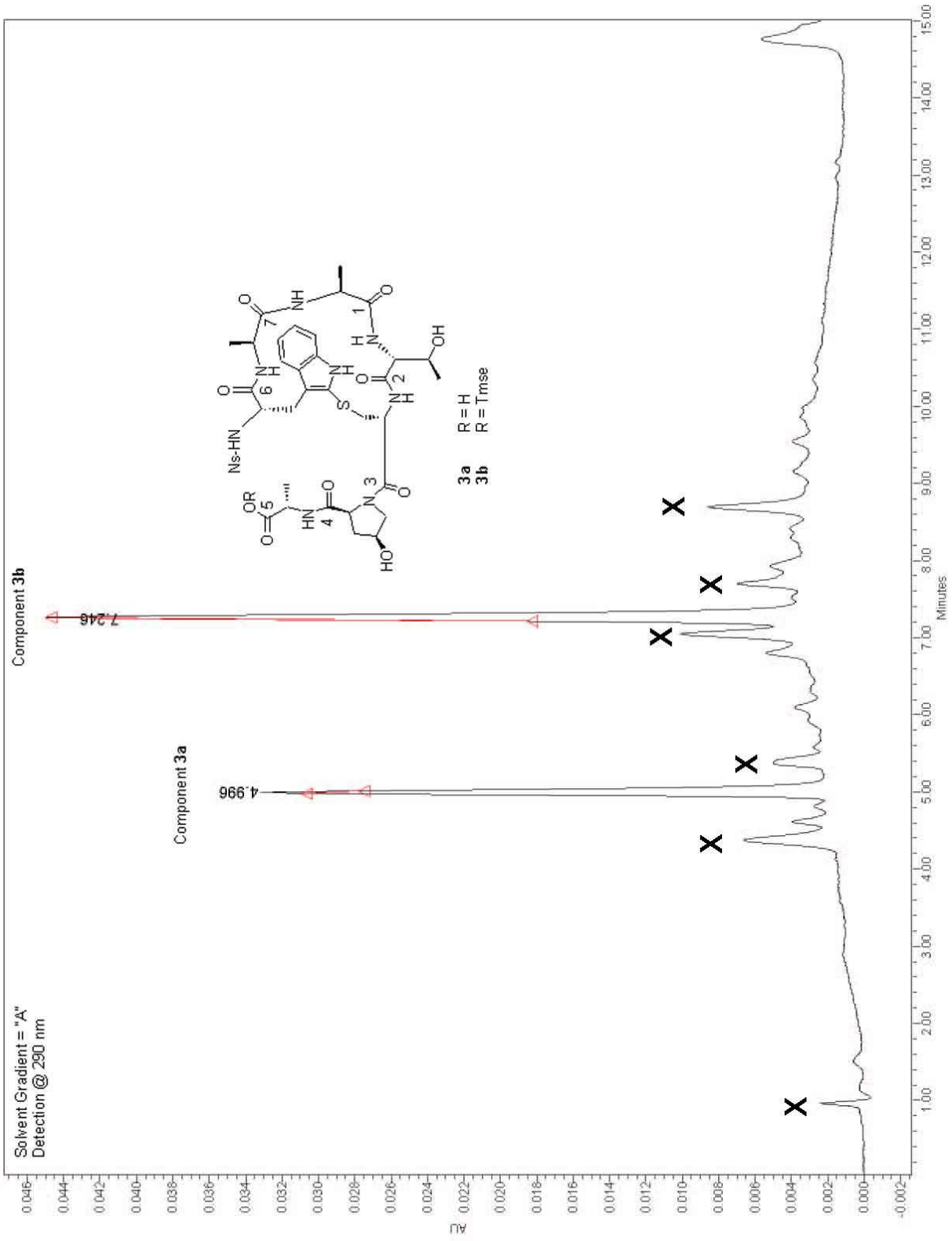

Figure S15. Analytical RP-HPLC: Monocyclic heptapeptide **3**: Partially deprotected components **3a** (5.0 min) and **3b** (7.2 min) were collected and analyzed by MALDI-TOF-MS (Figures S6 and S7).

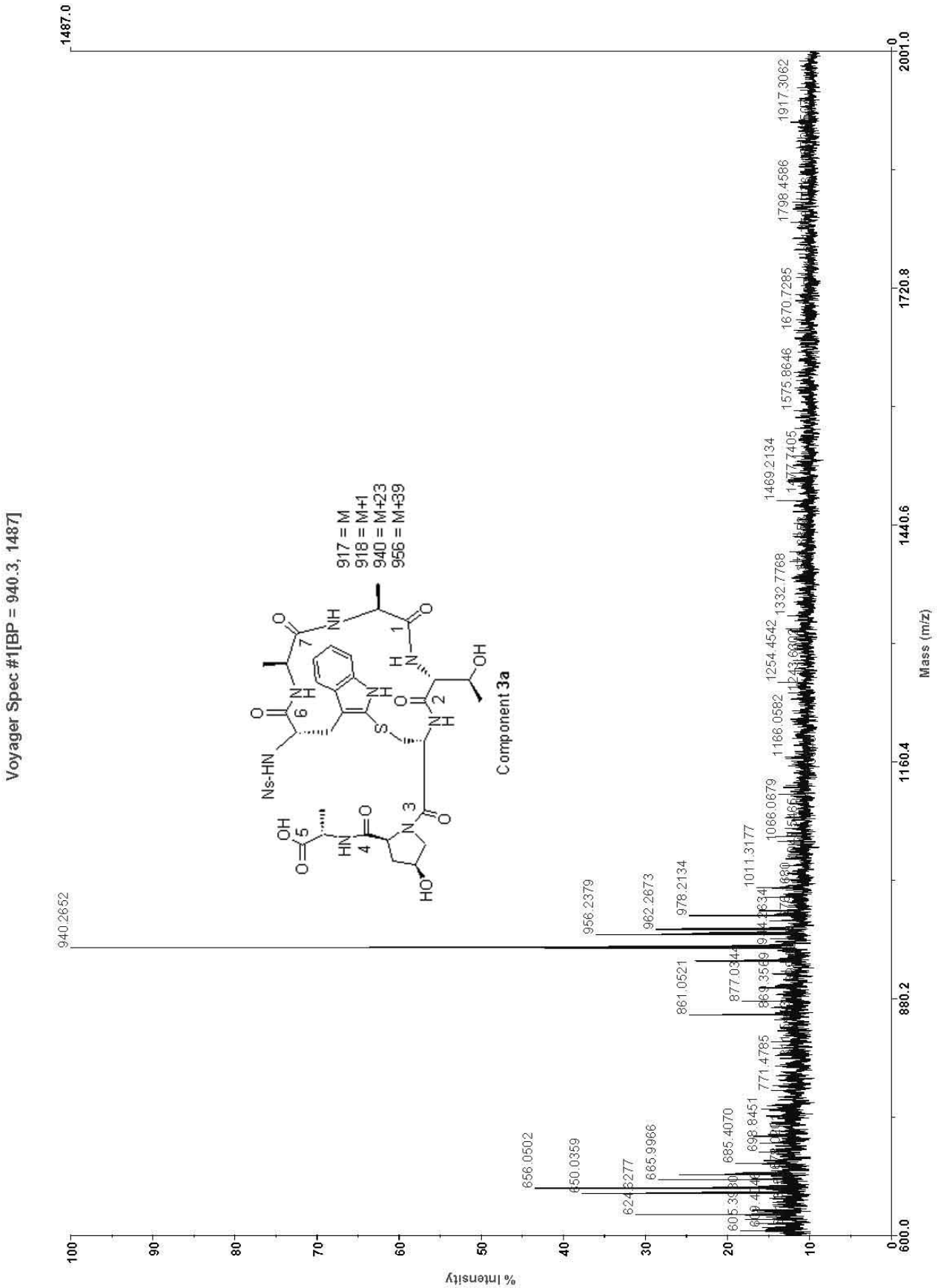

Figure S16. MALDI-TOF-MS: Partially deprotected component **3a**.

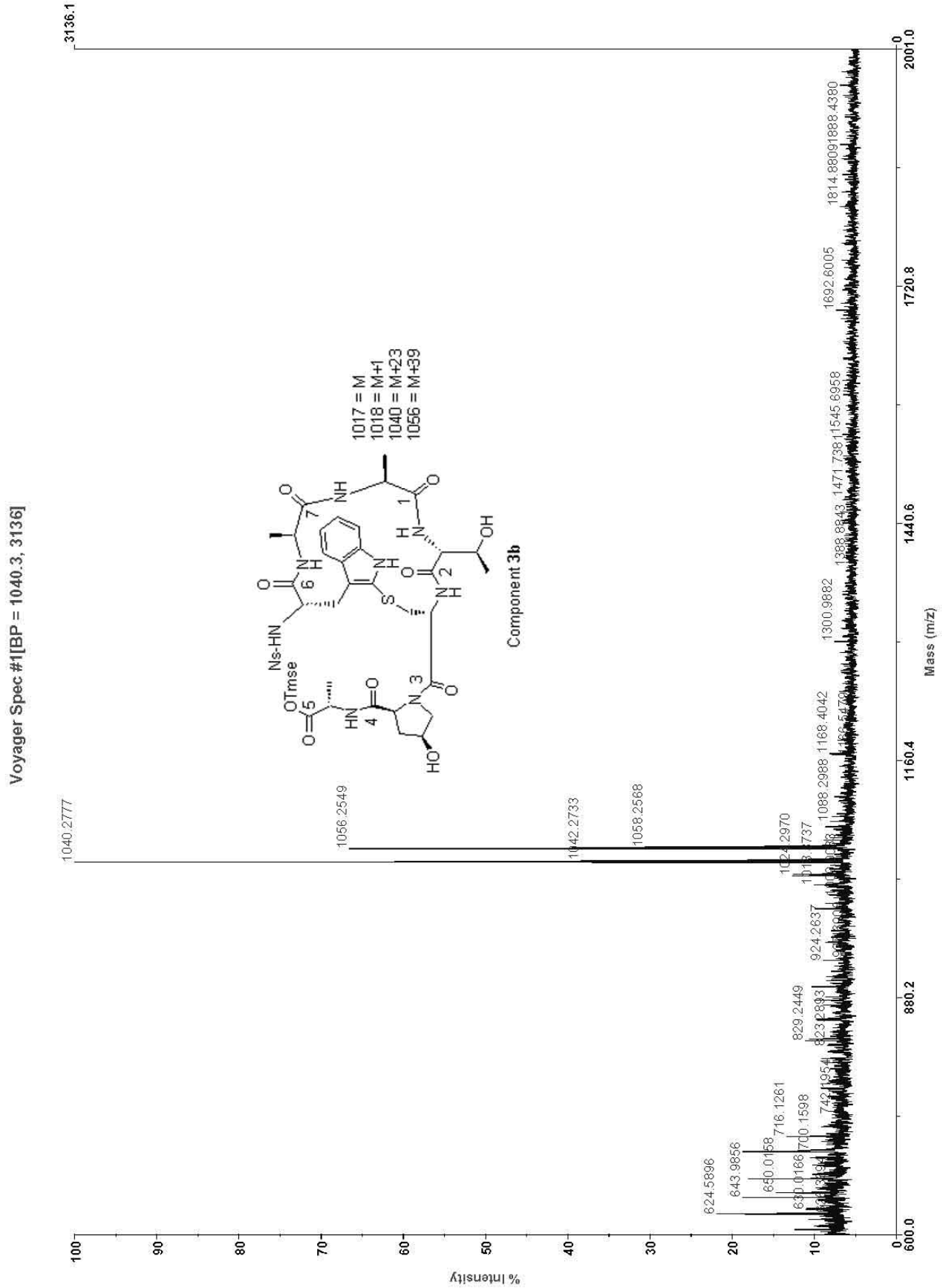

Figure S17. MALDI-TOF-MS: Partially deprotected component **3b**.

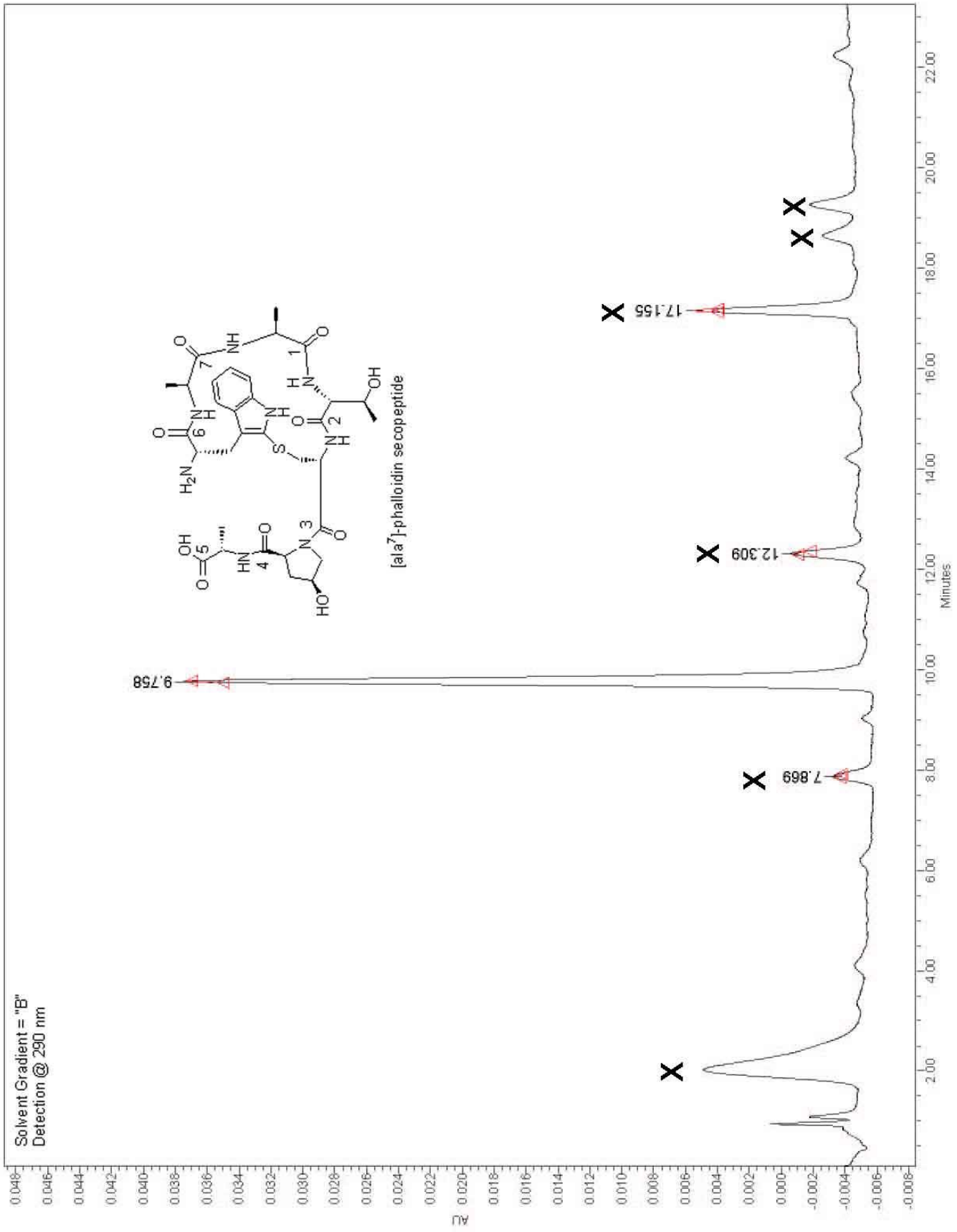

Figure S18. Analytical RP-HPLC: [Ala^7]-phalloidin secopeptide (9.8 min) was collected and analyzed by ESI-MS (Figure S9).

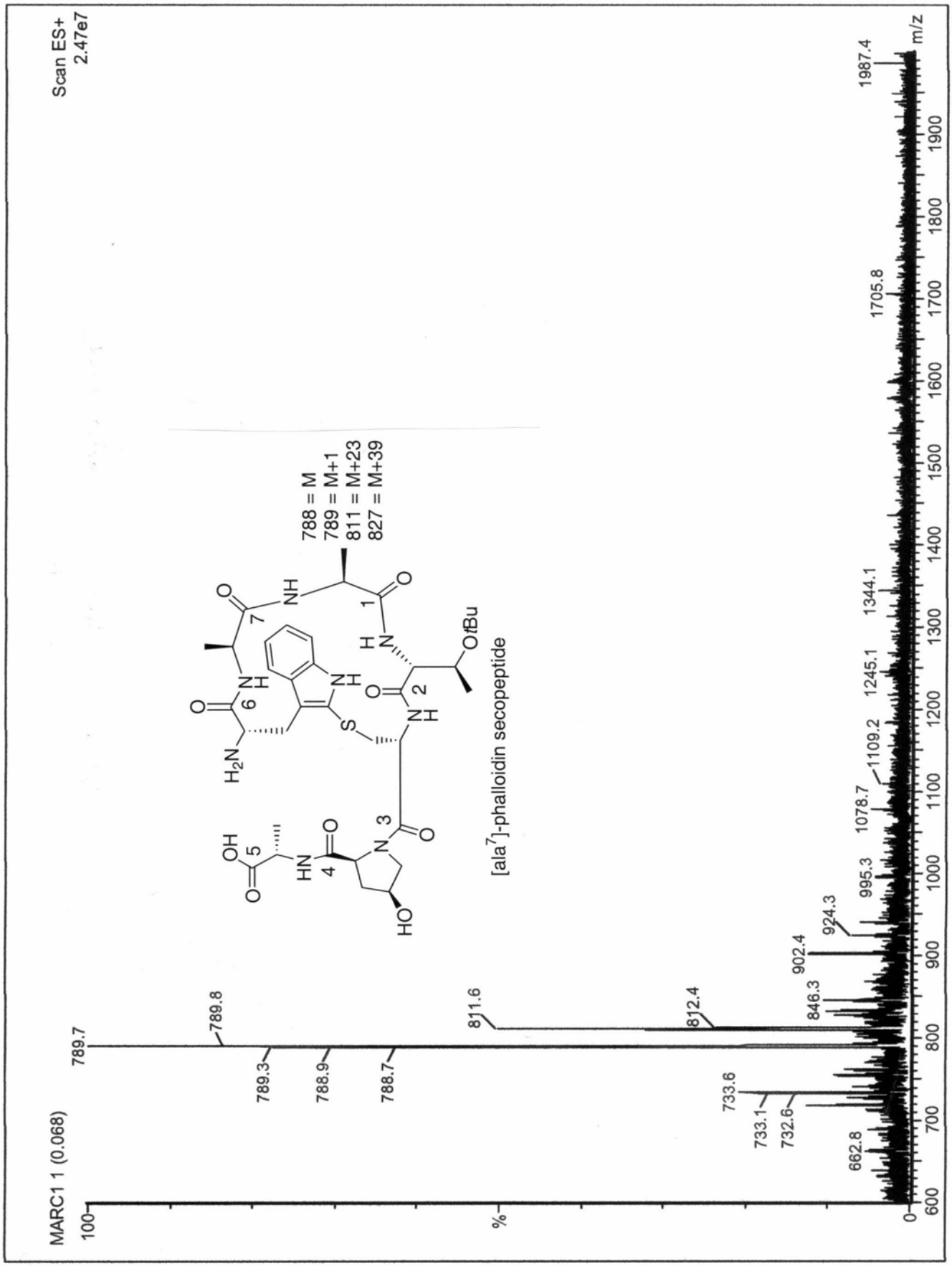

Figure S19. ESI-MS: [Ala^7]-phalloidin secopeptide intermediate.

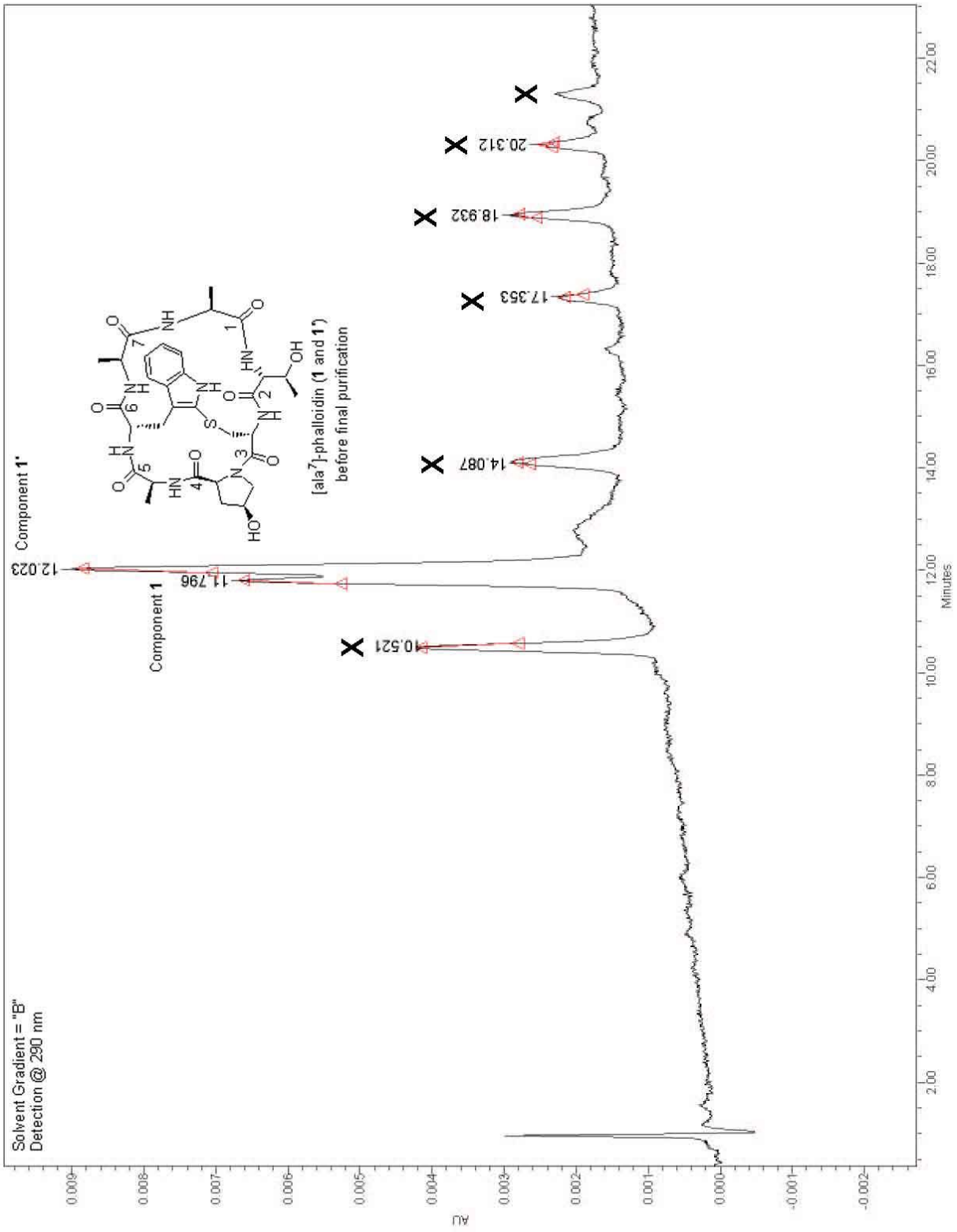

Figure S20. Analytical RP-HPLC: crude mixture before final purification: ~1:2 ratio of [Ala^7]-phalloidin natural atropisomer (**1**) (11.8 min) and non-natural atropisomer (**1'**) (12.0 min) and other un-identified impurities.

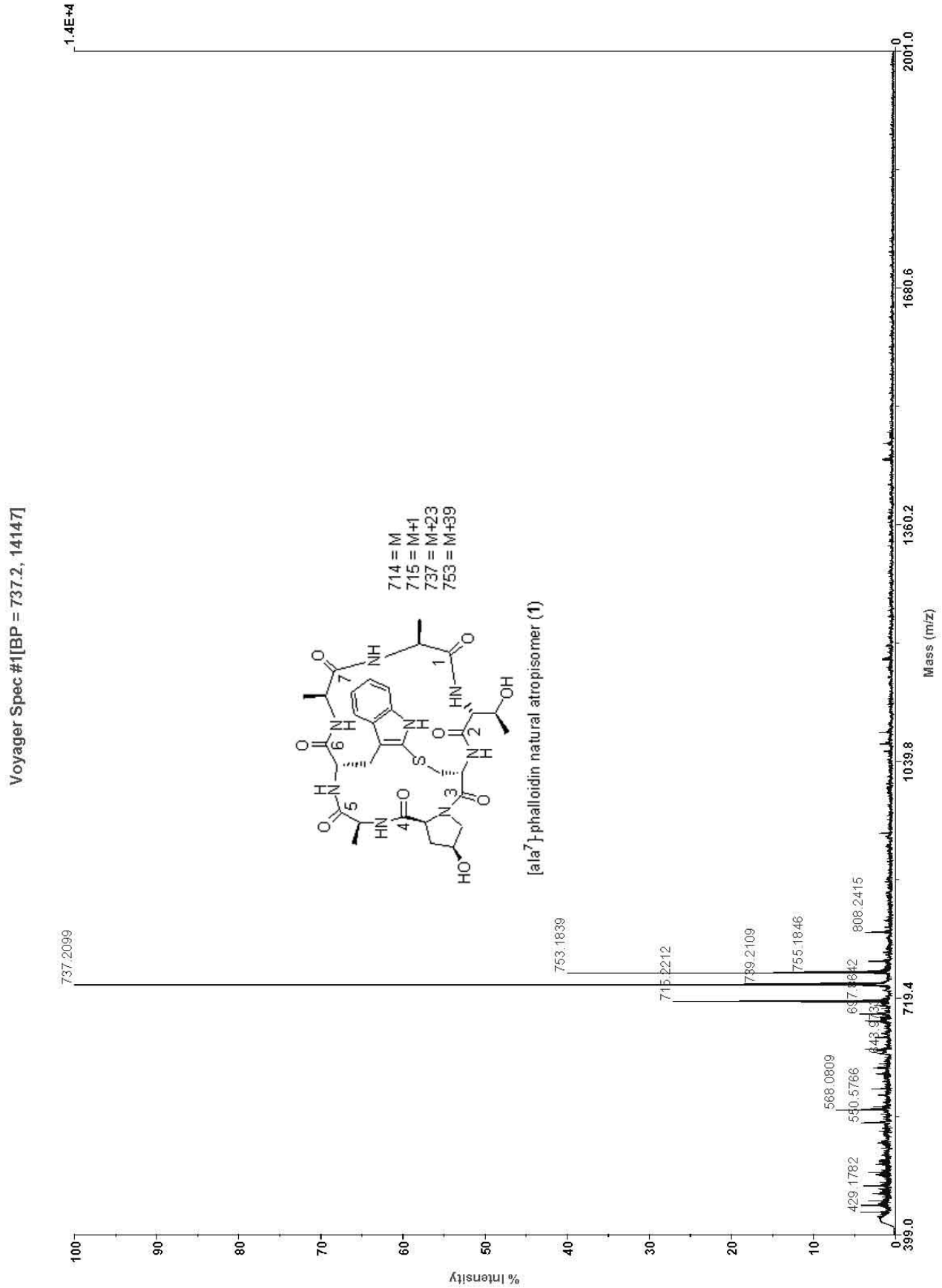

Figure S21. MALDI-TOF-MS: [Ala7]-phalloidin natural atropisomer (**1**) after purification.

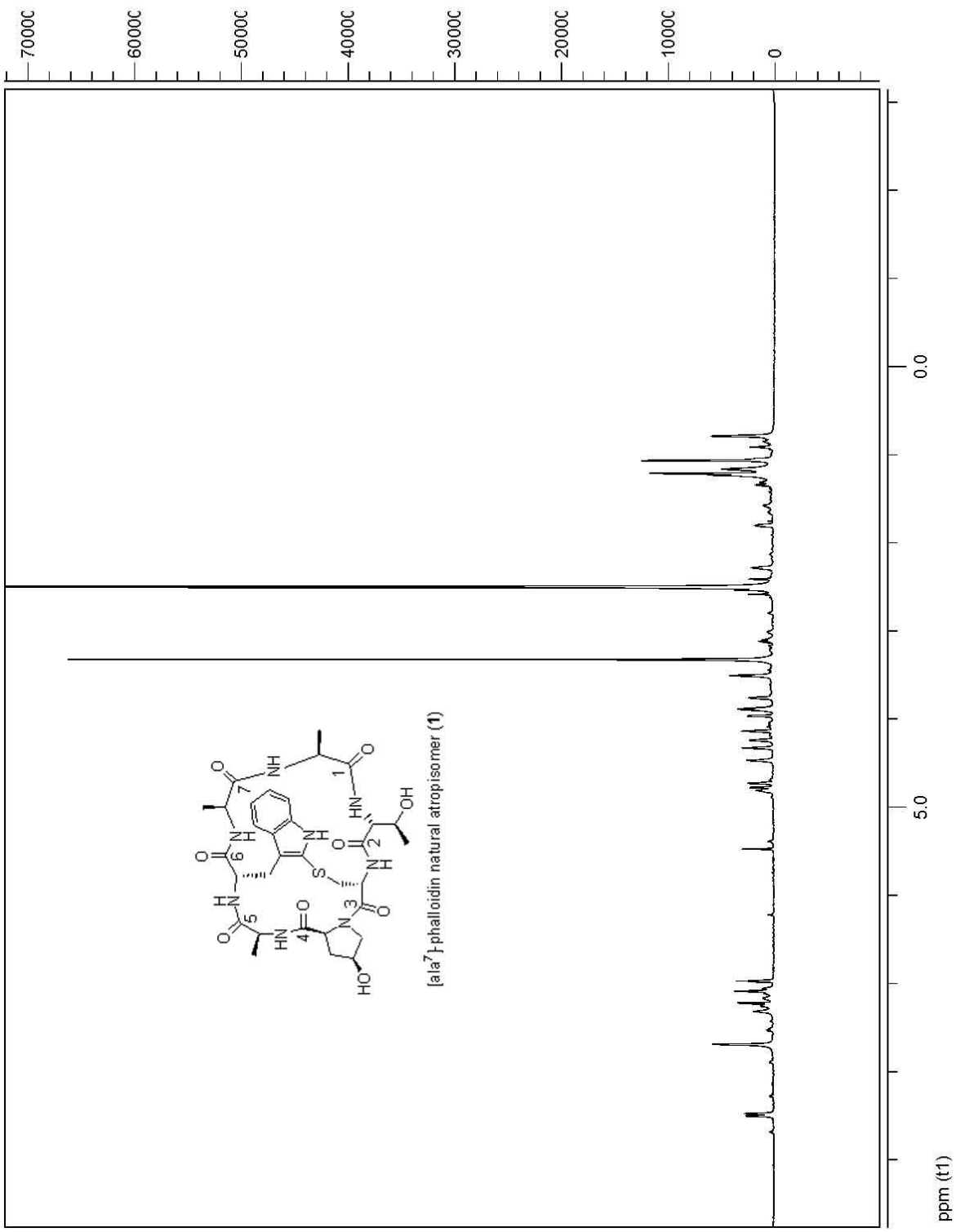

Figure S22. ^{1}H NMR (800 MHz, DMSO-d6): [Ala^{7}]-phalloidin natural atropisomer (**1**) after purification, contaminated with ~10% of non-natural atropisomer (**1'**).

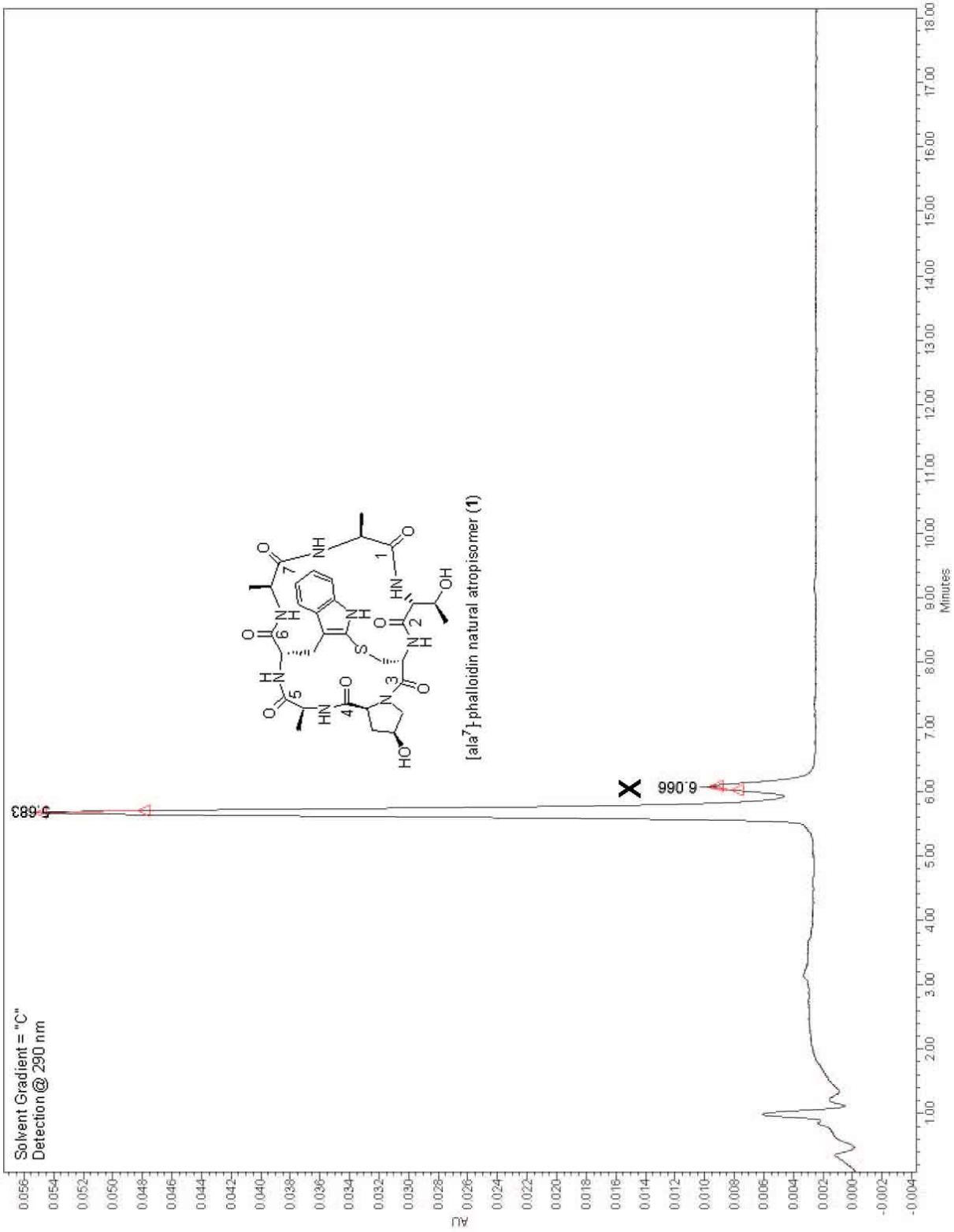

Figure S23. Analytical RP-HPLC: [Ala^7]-phalloidin natural atropisomer (**1**) (5.7 min) after purification, contaminated with ~10% of non-natural atropisomer (**1'**) (6.1 min).

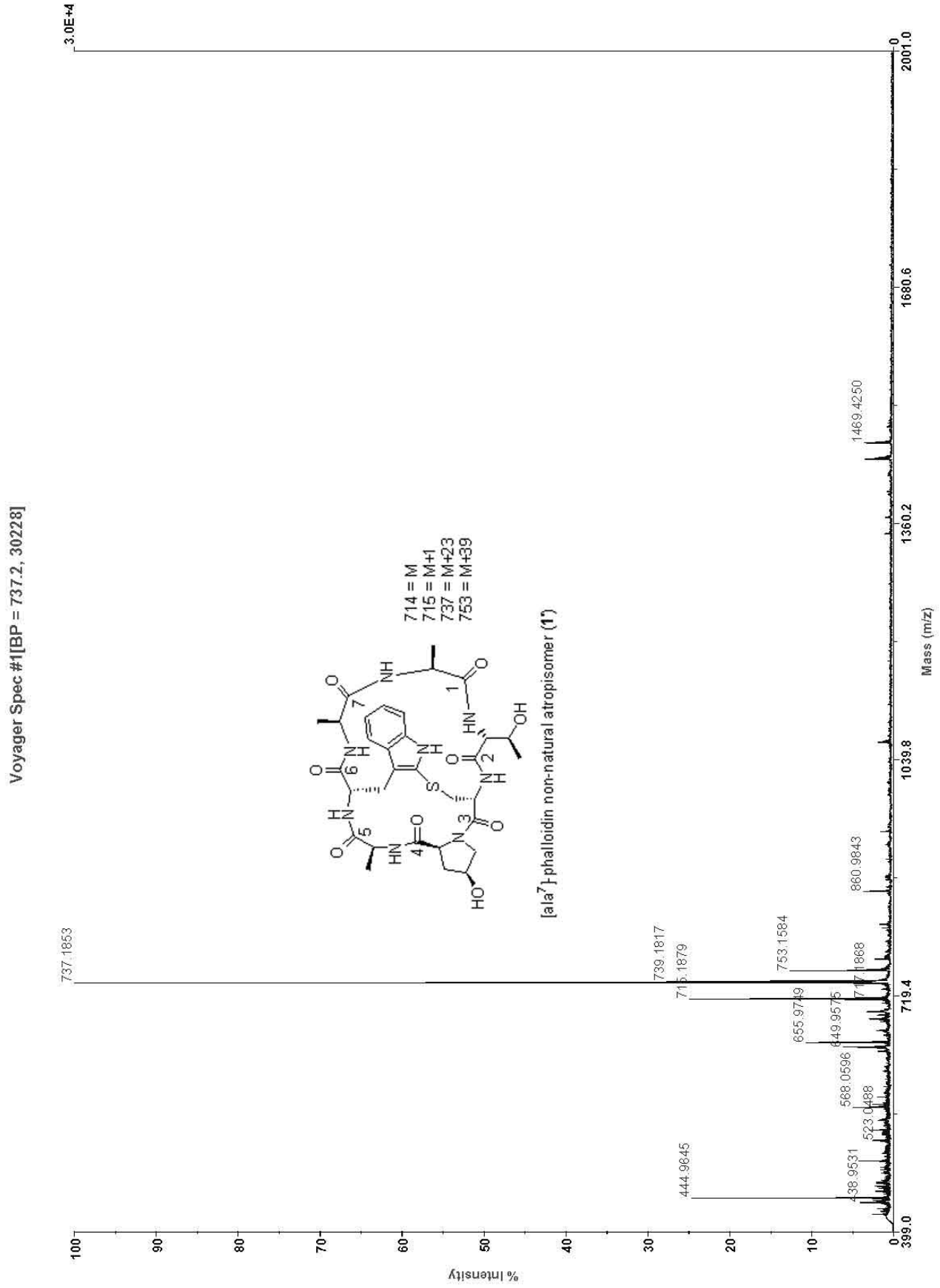

Figure S24. MALDI-TOF-MS: [Ala^7]-phalloidin non-natural atropisomer (**1'**).

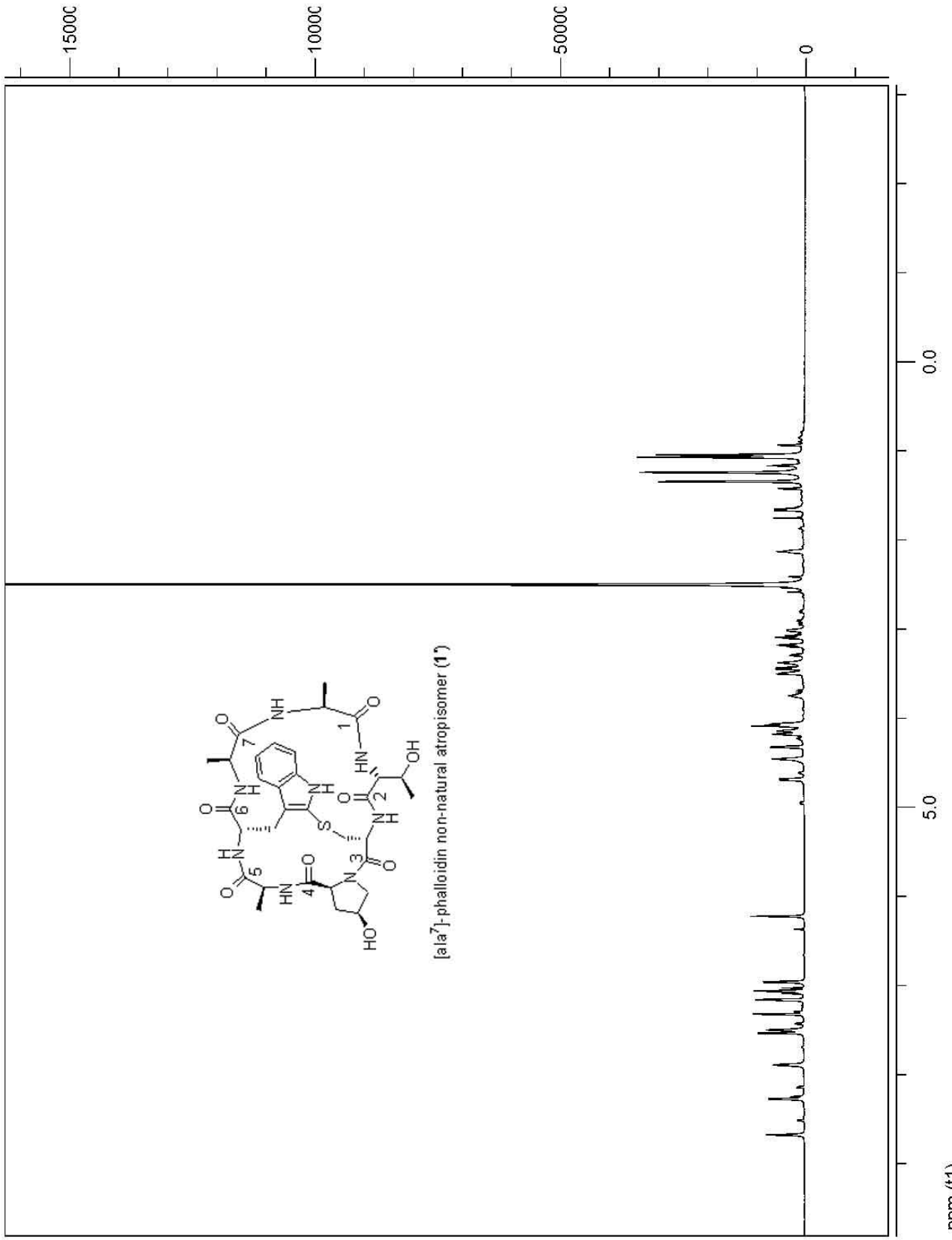

Figure S25. ^{1}H NMR (800 MHz, DMSO-d_6): [Ala^7]-phalloidin non-natural atropisomer (**1'**) after purification, contaminated with ~5% of natural atropisomer (**1**).

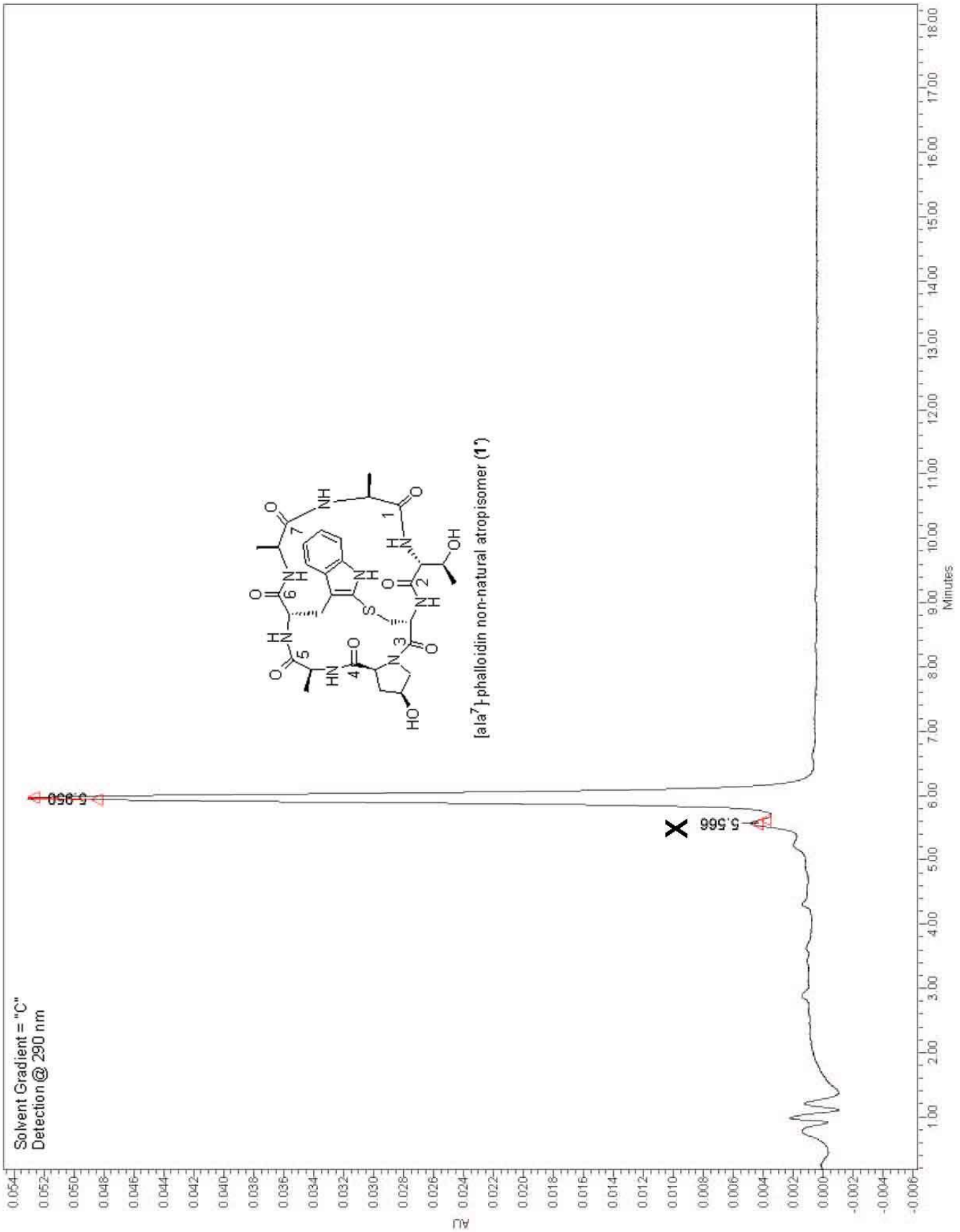

Figure S26. Analytical RP-HPLC: [Ala^7]-phalloidin non-natural atropisomer (1') (6.0 min) after purification, contaminated with ~5% of natural atropisomer (1) (5.6 min).