Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION

Design, synthesis and binding properties of a fluorescent $\alpha_9\beta_1/\alpha_4\beta_1$ integrin antagonist and its application as an *in vivo* probe for bone marrow haemopoietic stem cells

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[#]Australian Regenerative Medicine Institute, Monash University, Wellington Rd, Clayton, VIC 3800, Australia

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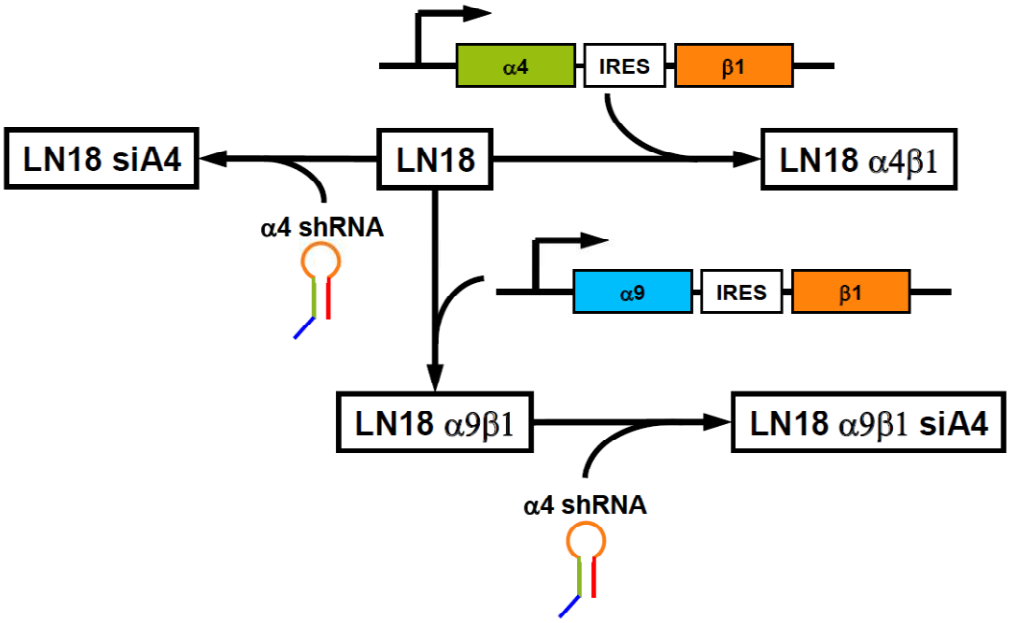
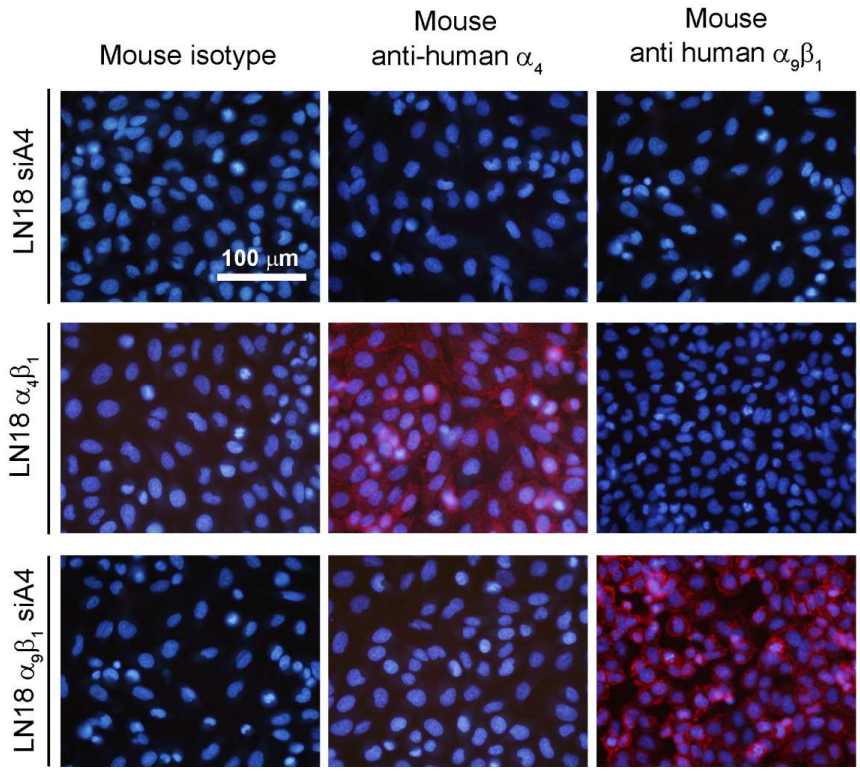
SUPPLEMENTARY FIGURES



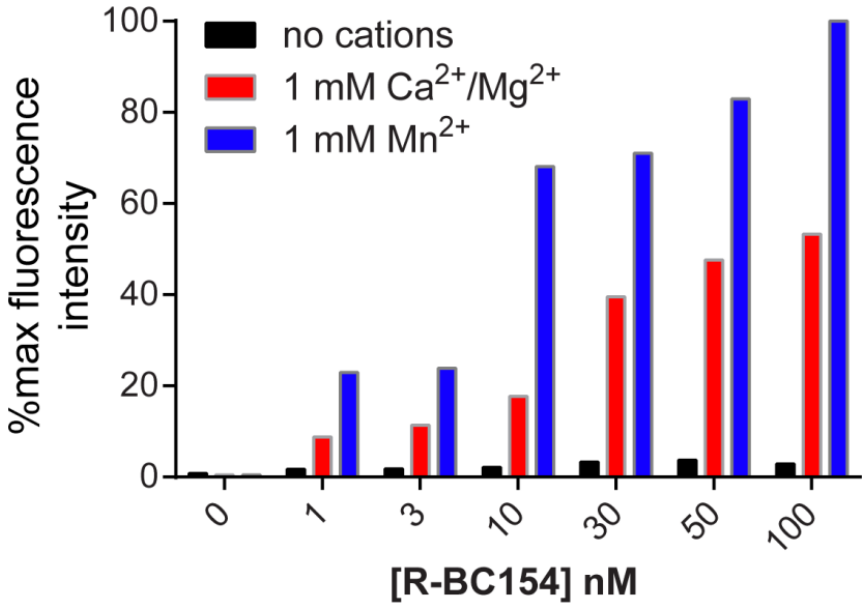
Supplementary Figure S1. Generation of LN18-derived cell lines.

Stable LN18 cells over-expressing integrin $\alpha_4\beta_1$ and $\alpha_9\beta_1$ were generated via retroviral transduction of human glioblastoma LN18 cell lines. Silencing of background α_4 expression in parental and $\alpha_9\beta_1$ transduced LN18 cells was achieved by retroviral vector delivery of α_4 shRNA.



Supplementary Figure S2. Antibody staining of $\alpha_4\beta_1$ and $\alpha_9\beta_1$ LN18 cells

Control LN18 SiA4, LN18 $\alpha_4\beta_1$, and LN18 $\alpha_9\beta_1$ cells were stained with mouse isotype control, mouse-anti-human α_4 antibody or mouse-anti-human $\alpha_9\beta_1$ antibody and then secondary labelled with Alexa Fluor 594 conjugated goat-anti-mouse IgG_1. Cells counterstained with DAPI (blue)



Supplementary Figure S3. Cation dependent binding of R-BC154

LN18 $\alpha_9\beta_1$ cells were treated with R-BC154 at the given concentrations in TBS buffer only (black bars), 1 mM Ca^{2+}/Mg^{2+} (red bars) or 1 mM Mn^{2+} (blue bars). Data obtained is from a single experiment and is expressed as %max fluorescence.

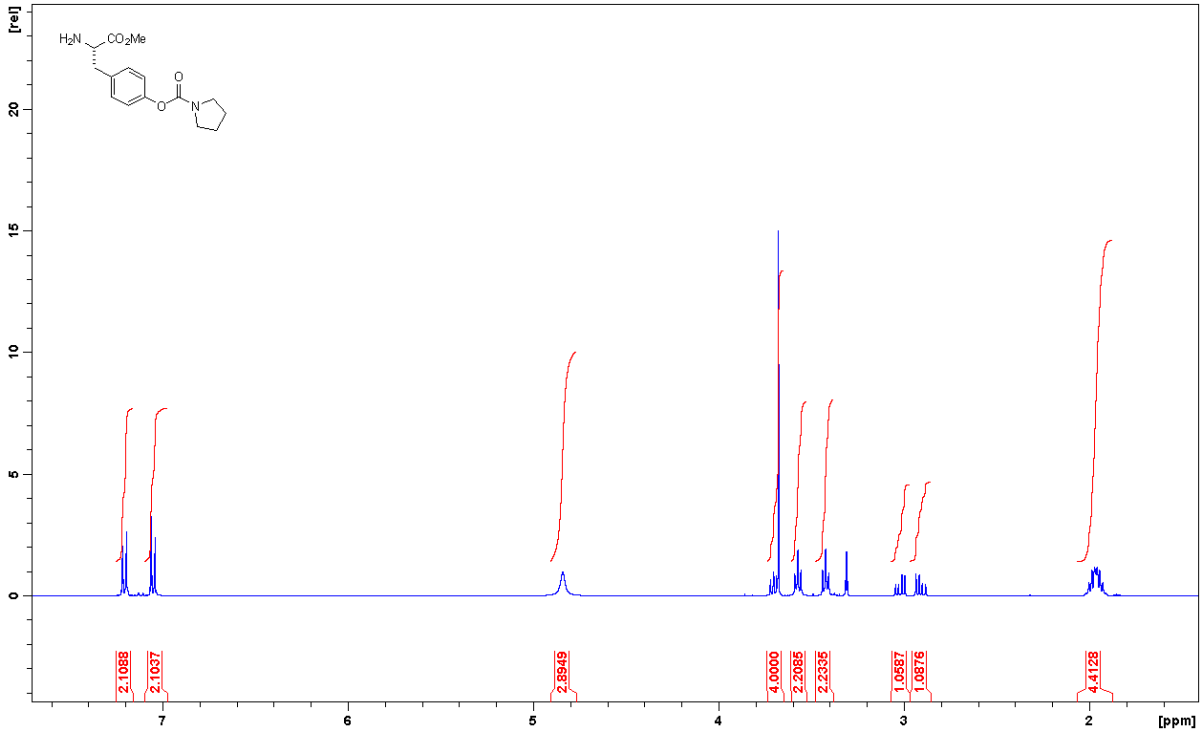
Synthesis of *N*-propynyl sulforhodamine B (24)

The synthesis of **24** was based on a slightly modified literature procedures.[1,2] A freshly prepared solution containing Et_3N (300 µl, 2.17 mmol), propargyl amine HCl salt (66 mg, 0.722 mmol) and DMAP (9 mg, 0.072 mmol) in dry CH_2Cl_2 (10 ml) was slowly cannulated to a suspension of lissamine rhodamine B sulfonyl chloride[3] (500 mg, 0.866 mmol) in dry CH_2Cl_2 (50 ml) at 0 °C under N_2. The mixture was slowly warmed to rt and stirred for 2 h, concentrated and the crude residue purified by flash chromatography chromatography (1%, 2%, 3% then 4% MeOH/CH_2Cl_2) to give alkyne **24** (235 mg, 55%) as a dark purple solid, δ_H (400 MHz, d_6-DMSO) 1.21 (12 H, t, *J* = 7.0 Hz), 3.08 (1 H, t, *J* = 2.5 Hz), 3.64 (8 H, m), 3.82 (2 H, d, *J* = 2.4 Hz), 6.92 (2 H, d, *J* = 2.4 Hz), 6.97 (2 H, d, *J* = 9.6 Hz), 7.06 (2 H, dd, *J* = 2.3, 9.6 Hz), 7.45 (1 H, d, *J* = 8.0 Hz), 7.95 (1 H, dd, *J* = 1.9, 8.0 Hz), 8.43 (1 H, d, *J* = 1.9 Hz); δ_C (100 MHz, d_6-DMSO) 12.44 (4 C), 31.93 (1 C), 45.20 (4 C), 74.77 (1 C), 79.15 (1 C), 95.32, 113.43, 113.67, 125.97, 126.85, 130.43, 132.62, 133.18, 141.36, 147.87, 155.00, 157.08, 157.49 (19 C); HRMS (ESI^+) *m/z* 596.1882 ($C_{30}H_{33}N_3O_6S_2H$ $[M+H]^+$ requires 596.1884).

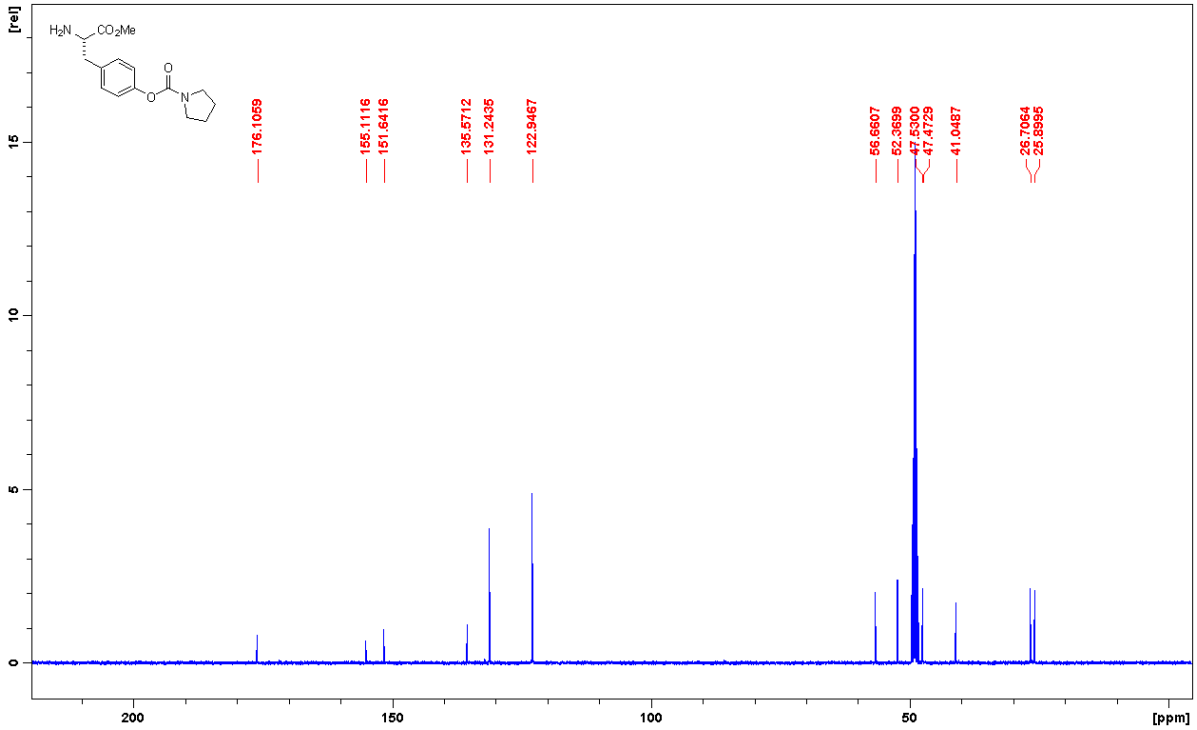
NMR data

(*S*)-4-(2-amino-3-methoxy-3-oxopropyl)phenyl pyrrolidine-1-carboxylate (7)

^{1}H NMR of 7

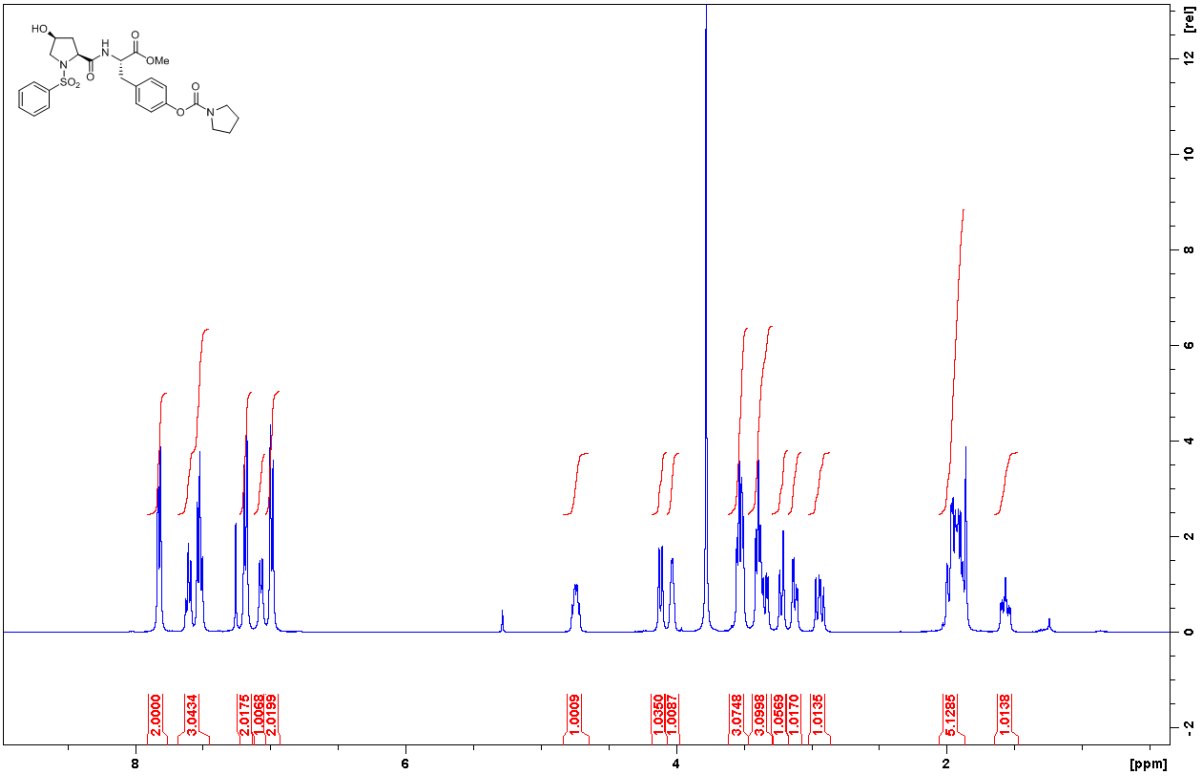


^{13}C NMR of 7

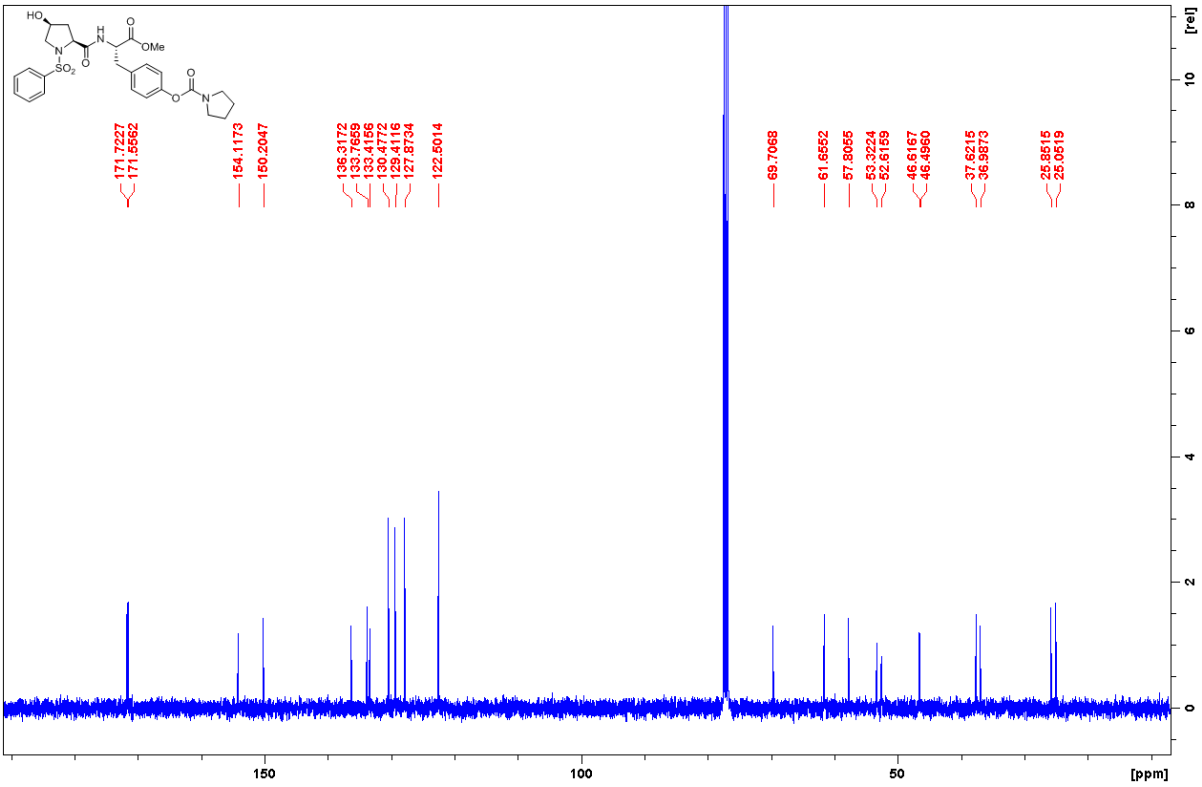


4-((*S*)-2-((2*S*,4*S*)-4-hydroxy-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)-3-methoxy-3-oxopropyl)phenyl pyrrolidine-1-carboxylate (8)

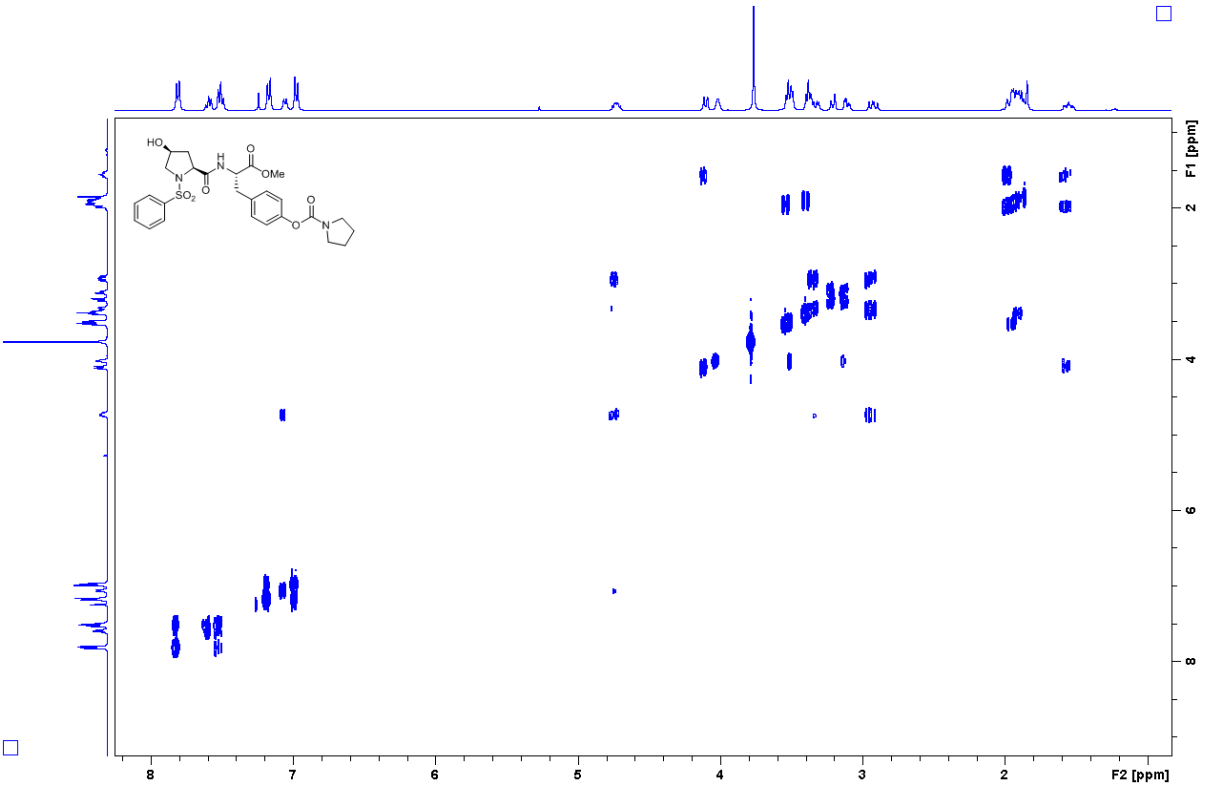
^{1}H NMR of 8



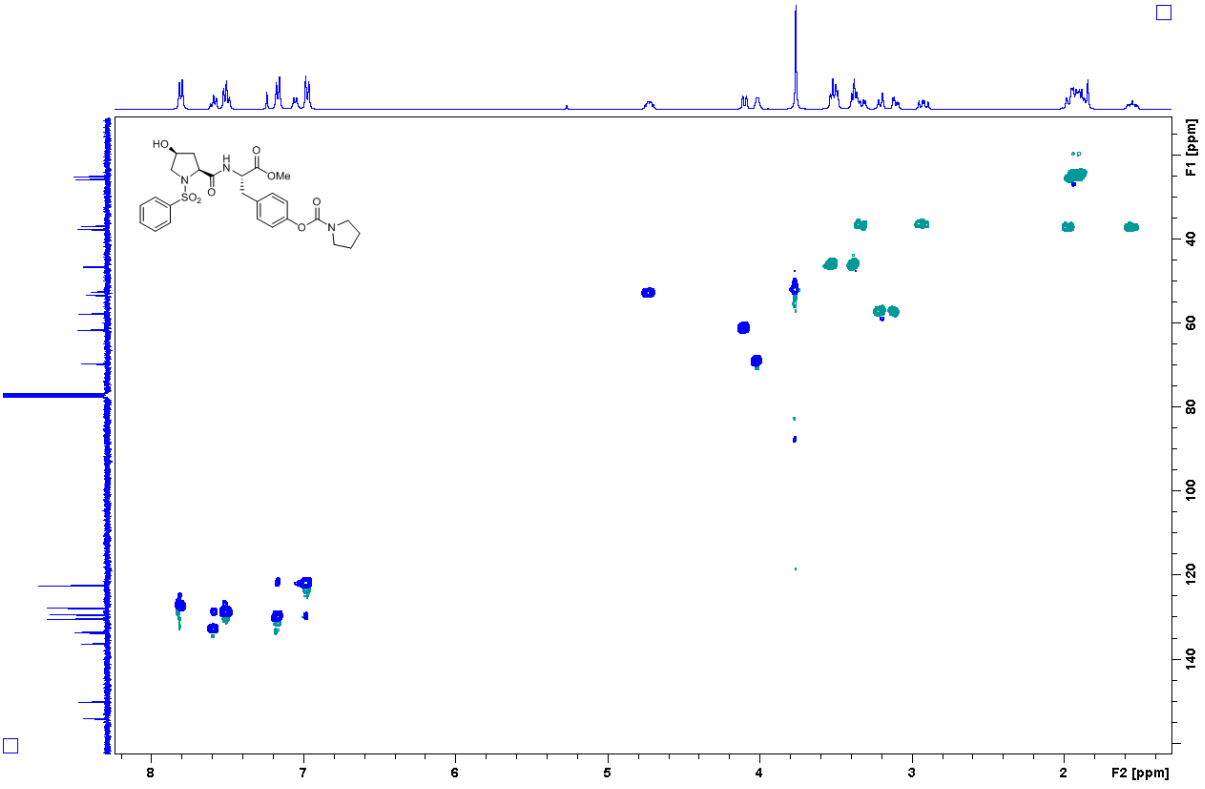
^{13}C NMR of 8



^{1}H-^{1}H COSY NMR of 8

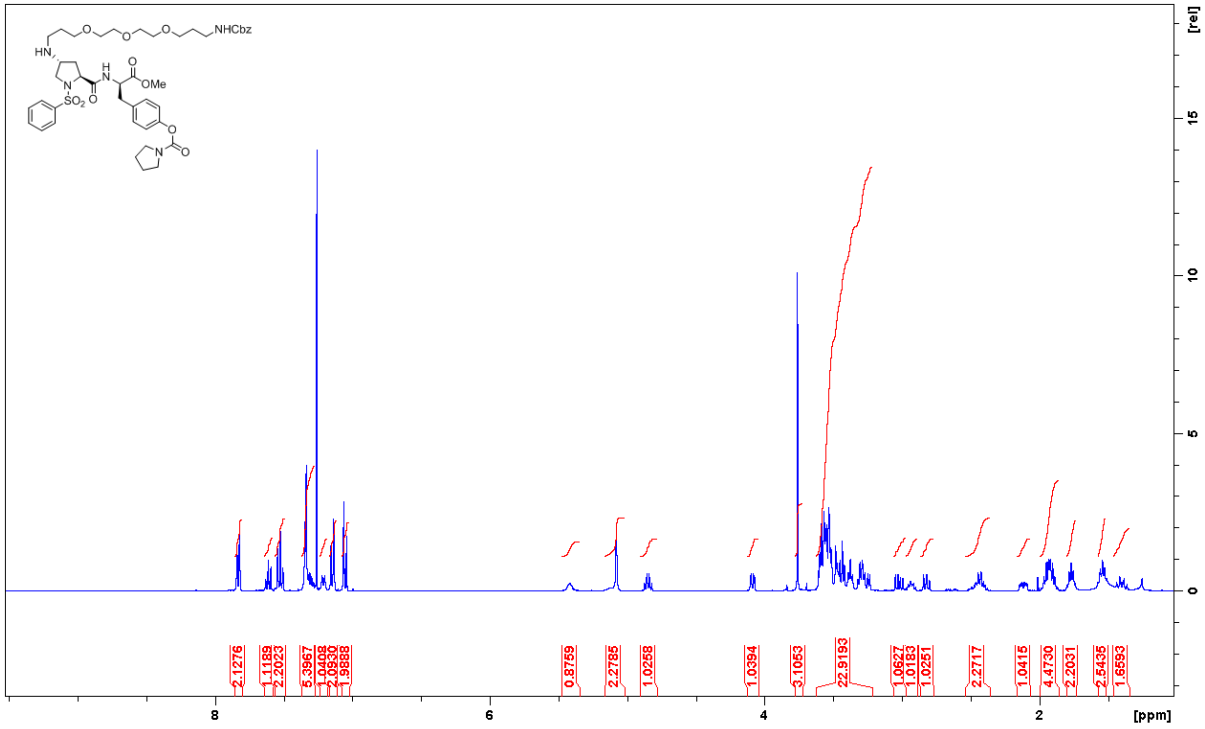


HSQC NMR of 8

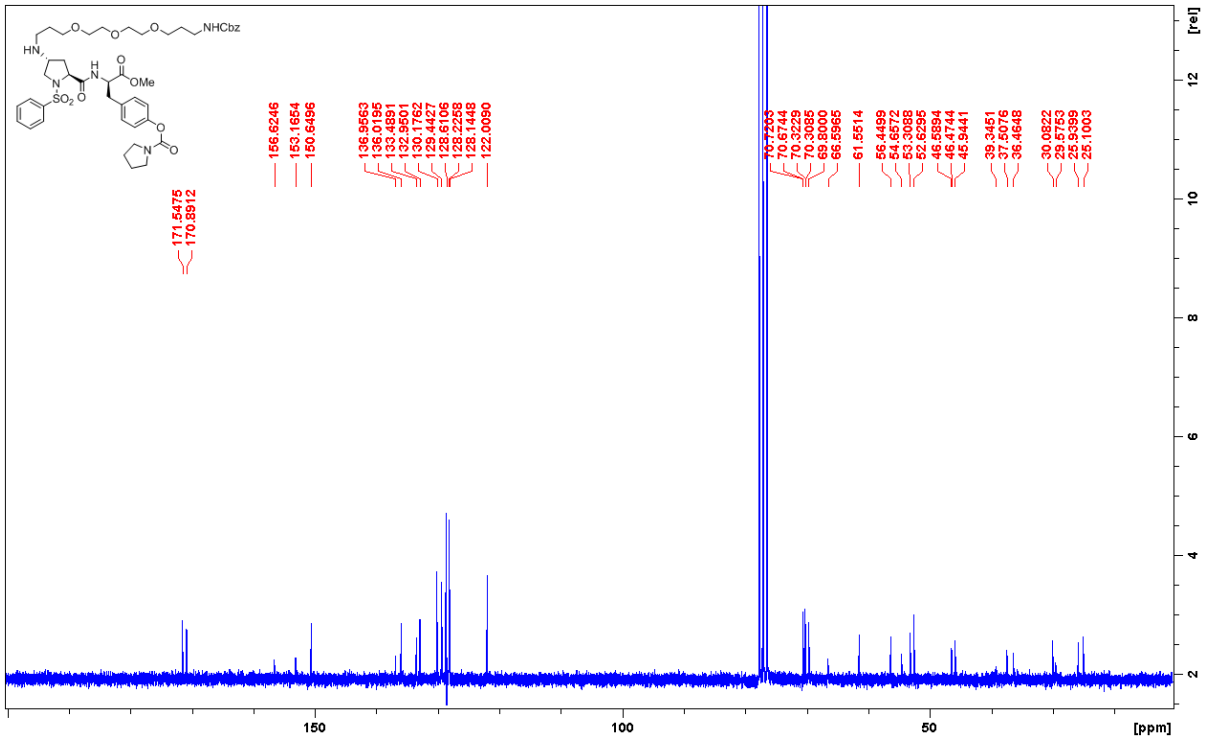


4-((R)-3-methoxy-3-oxo-2-((2S,4R)-4-((3-oxo-1-phenyl-2,8,11,14-tetraoxa-4-azaheptadecan-17-yl)amino)-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)propyl)phenyl pyrrolidine-1-carboxylate **(11)**

^{1}H NMR of 11

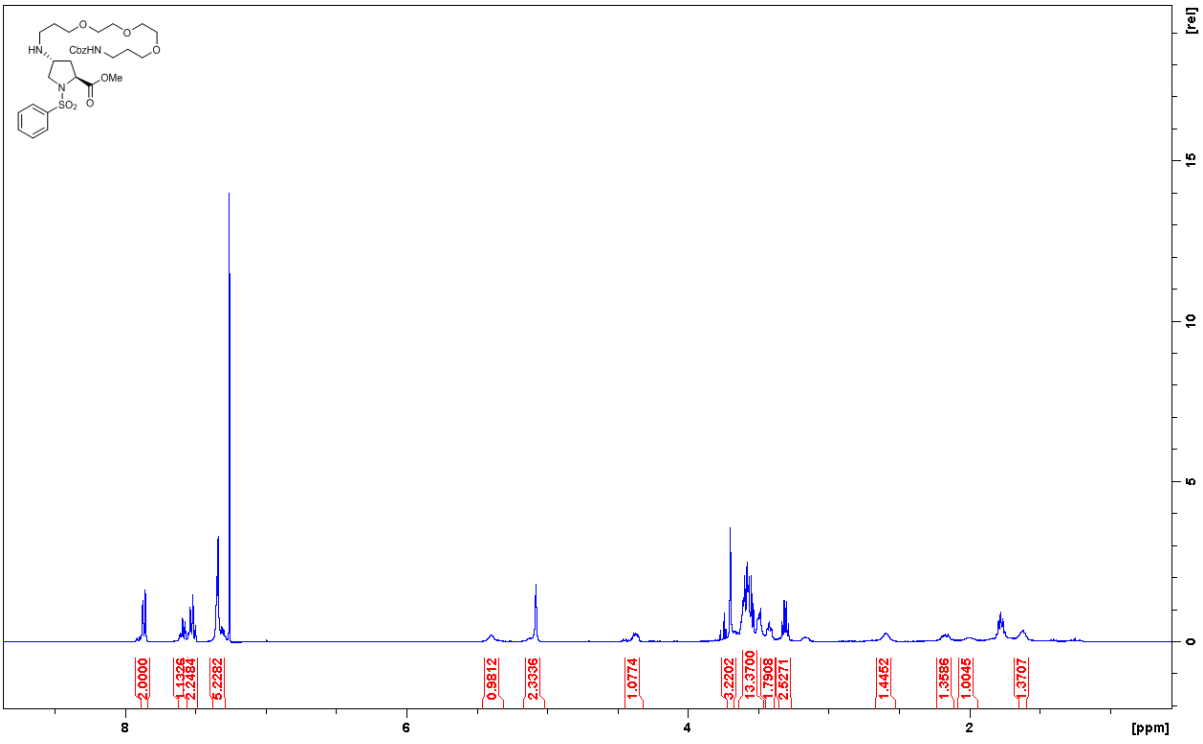


^{13}C NMR of 11

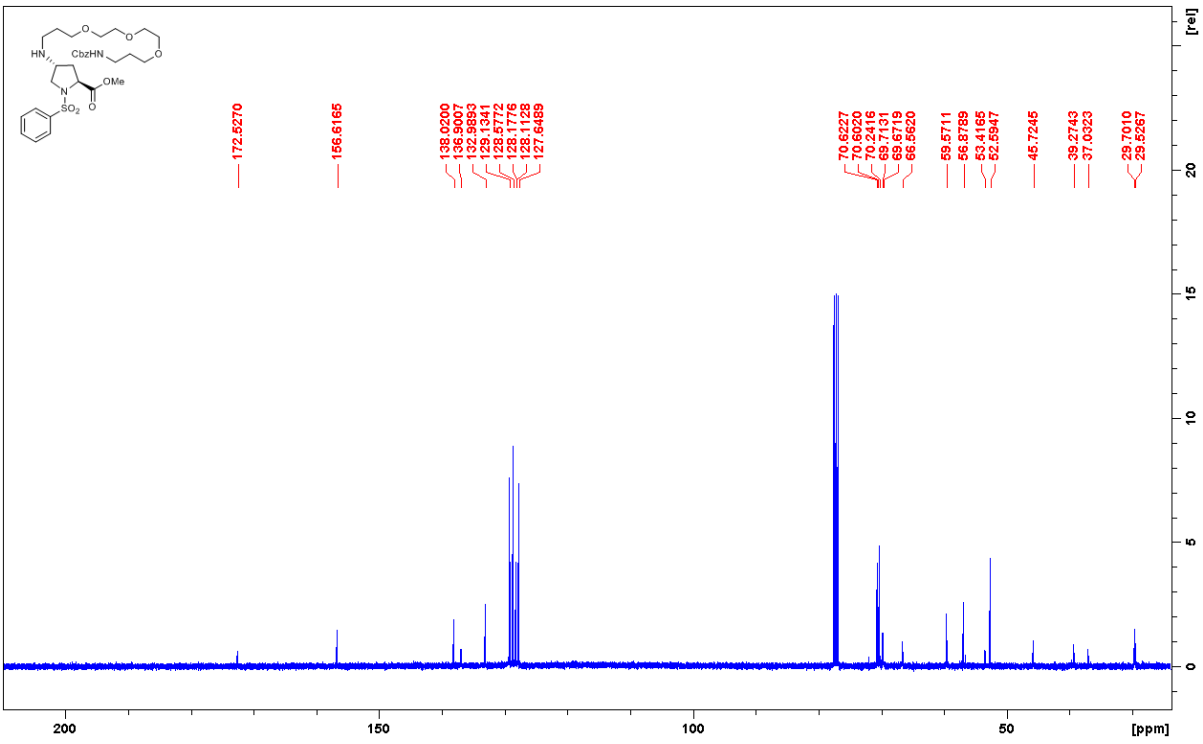


(2*S*,4*R*)-methyl-4-((3-oxo-1-phenyl-2,8,11,14-tetraoxa-4-azaheptadecan-17-yl)amino)-1-(phenylsulfo-nyl)-pyrrolidine-2-carboxylate (13)

^{1}H NMR of 13

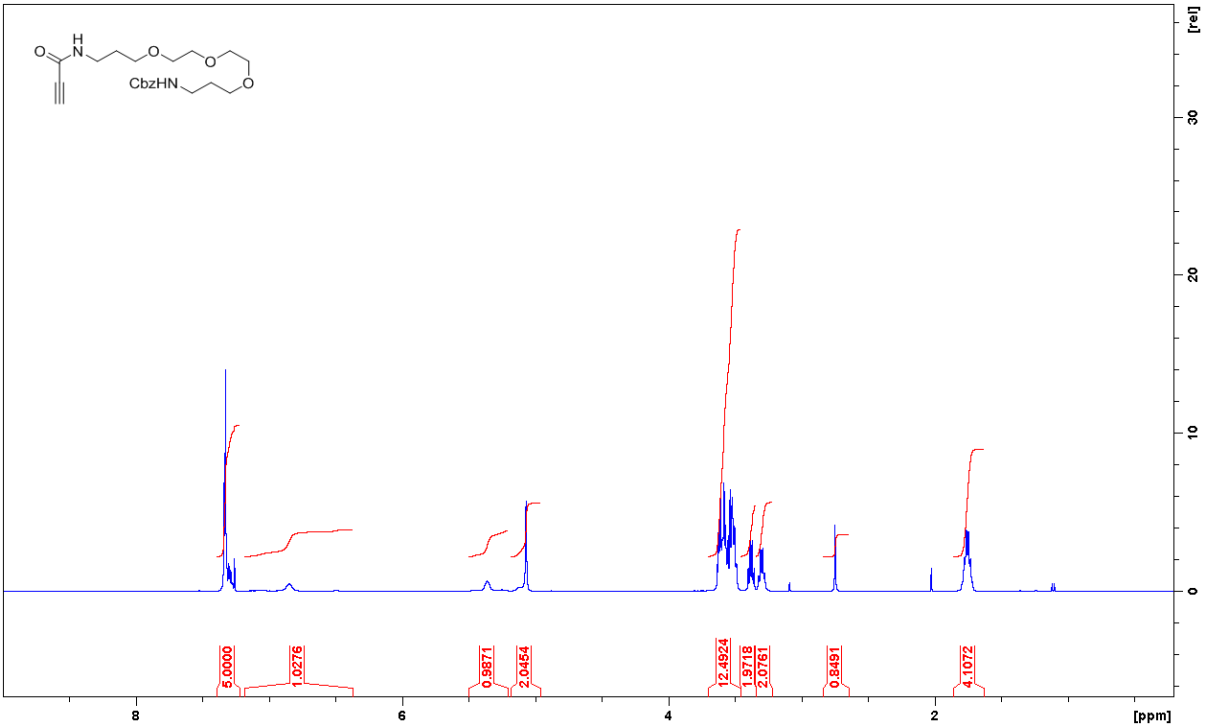


^{13}C NMR of 13

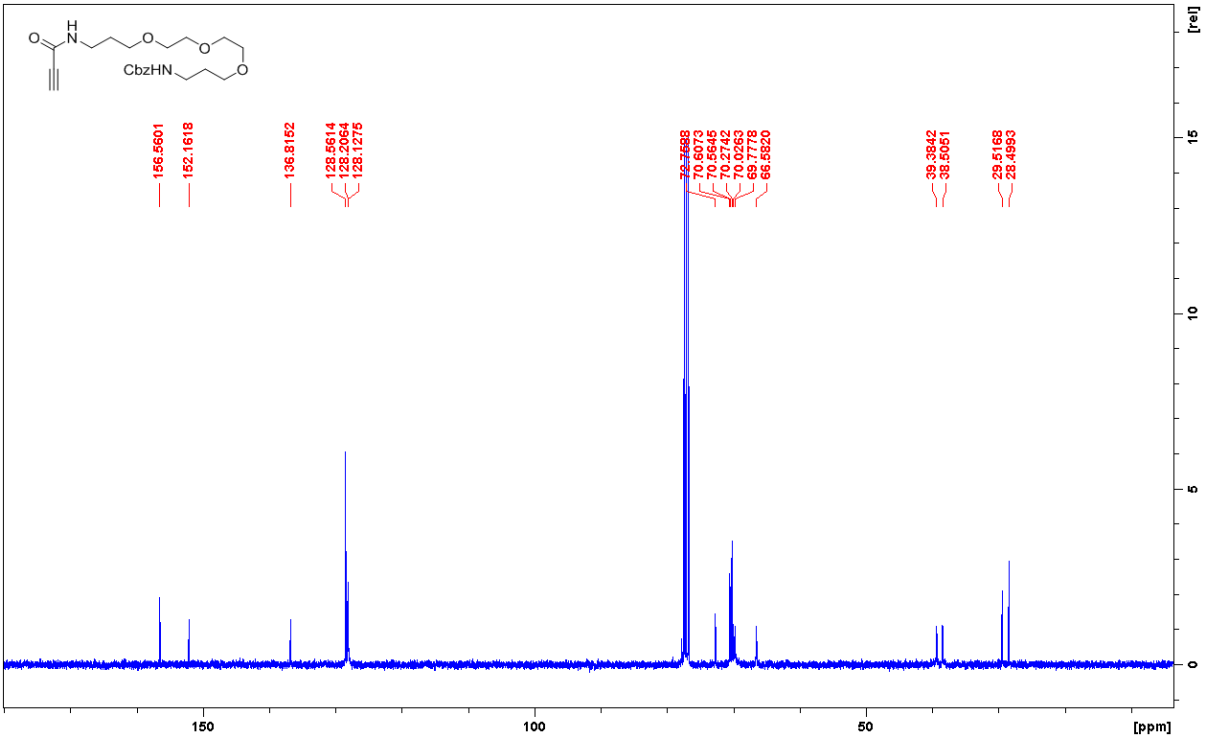


Benzyl (15-oxo-4,7,10-trioxa-14-azaheptadec-16-yn-1-yl)carbamate (15)

^{1}H NMR of 15

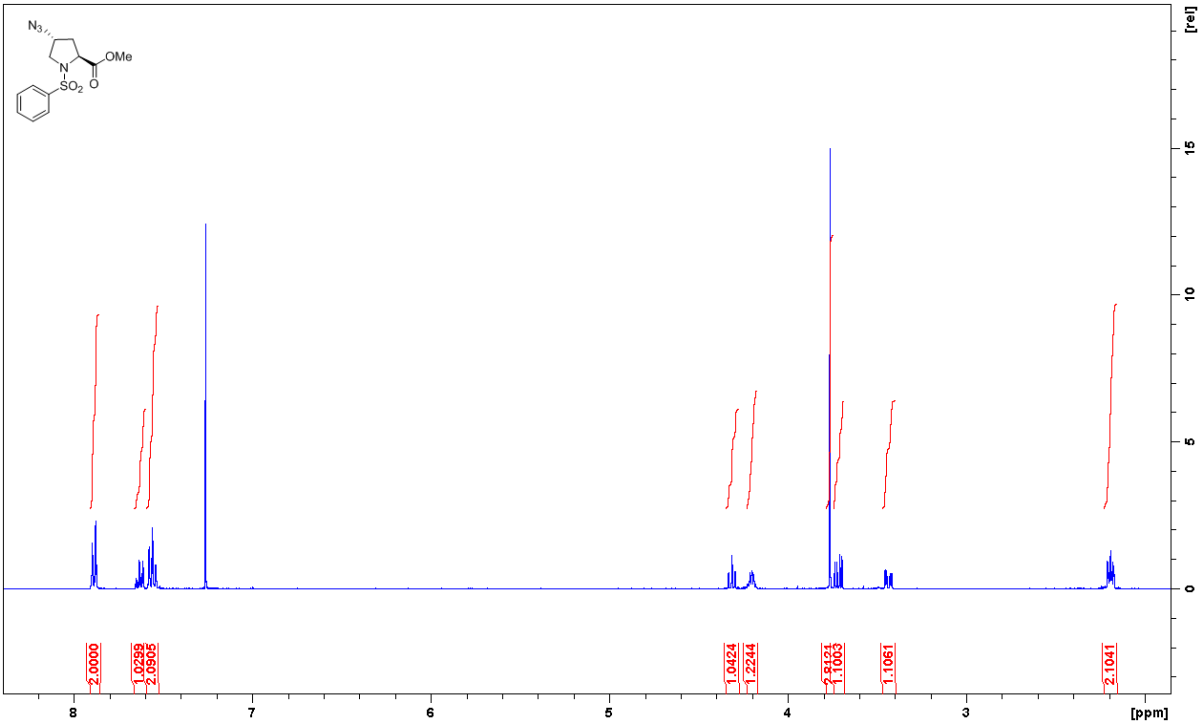


^{13}C NMR of 15

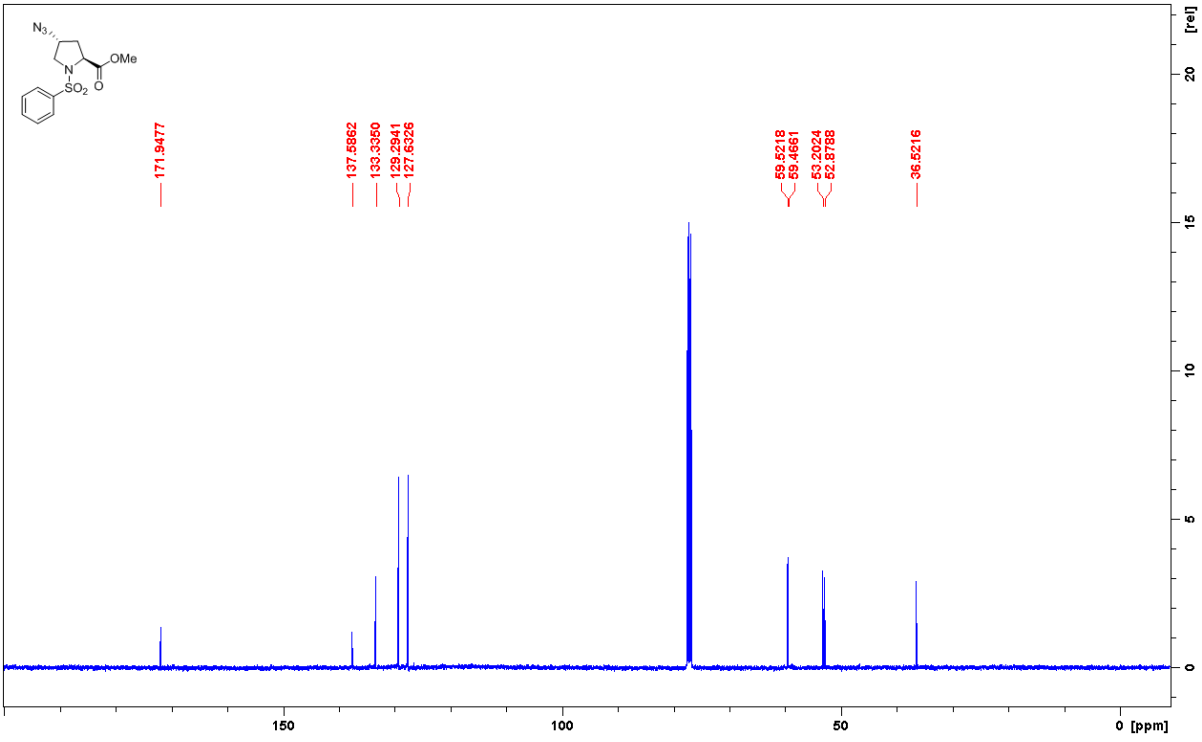


Methyl (2*S*,4*R*)-4-azido-1-(phenylsulfonyl)pyrrolidine-2-carboxylate (16)

^{1}H NMR of 16

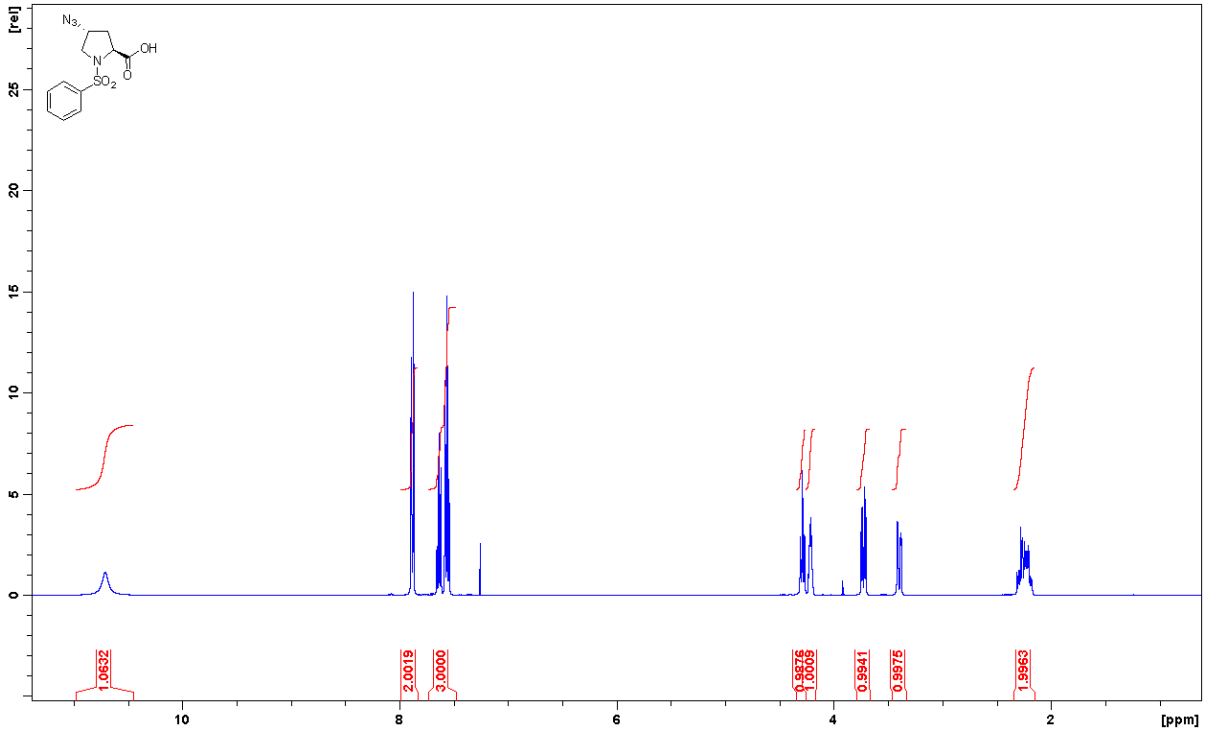


^{13}C NMR of 16

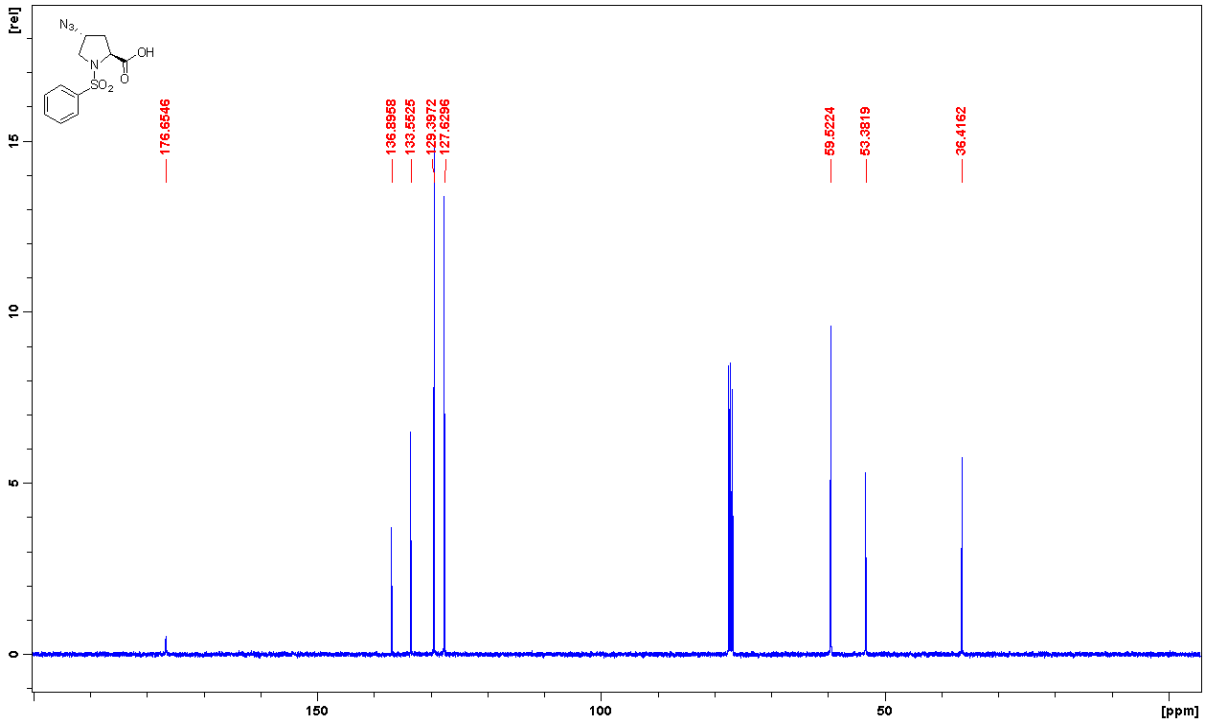


(2*S*,4*R*)-4-azido-1-(phenylsulfonyl)pyrrolidine-2-carboxylic acid (17)

^{1}H NMR of 17

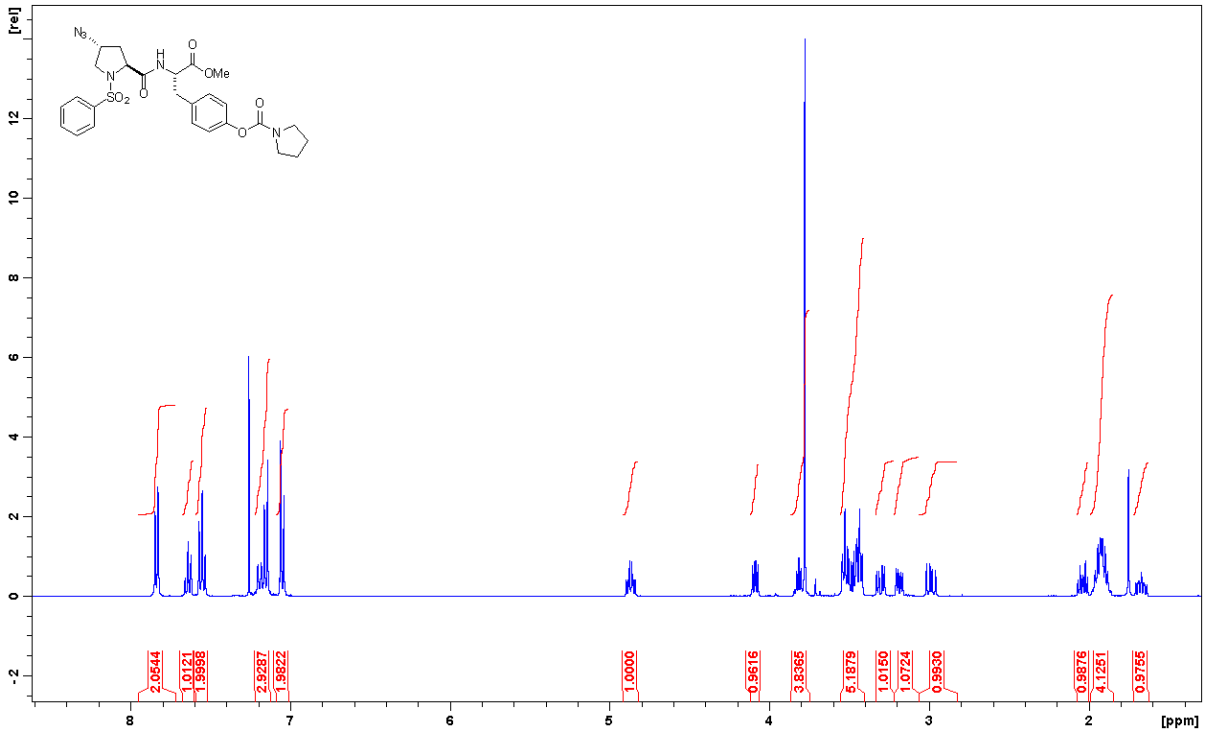


^{13}C NMR of 17

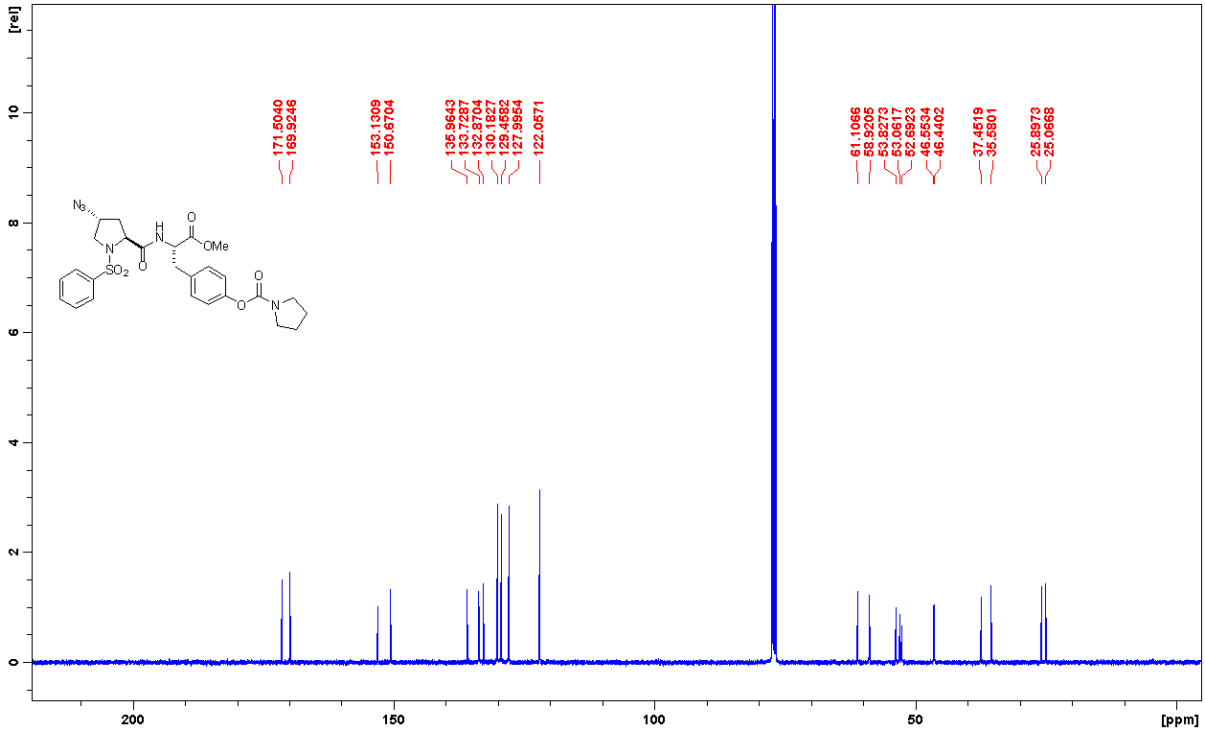


4-((*S*)-2-((2*S*,4*R*)-4-azido-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)-3-methoxy-3-oxopropyl)phenyl pyrrolidine-1-carboxylate (18)

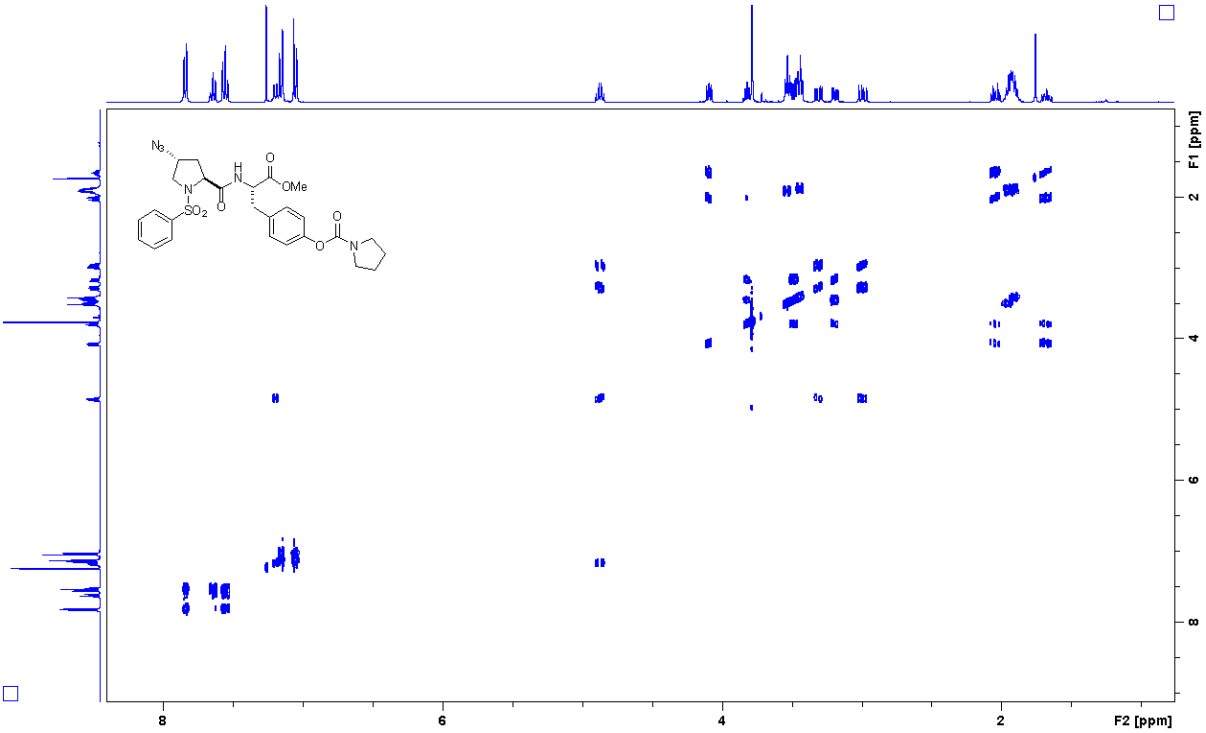
^{1}H NMR of 18



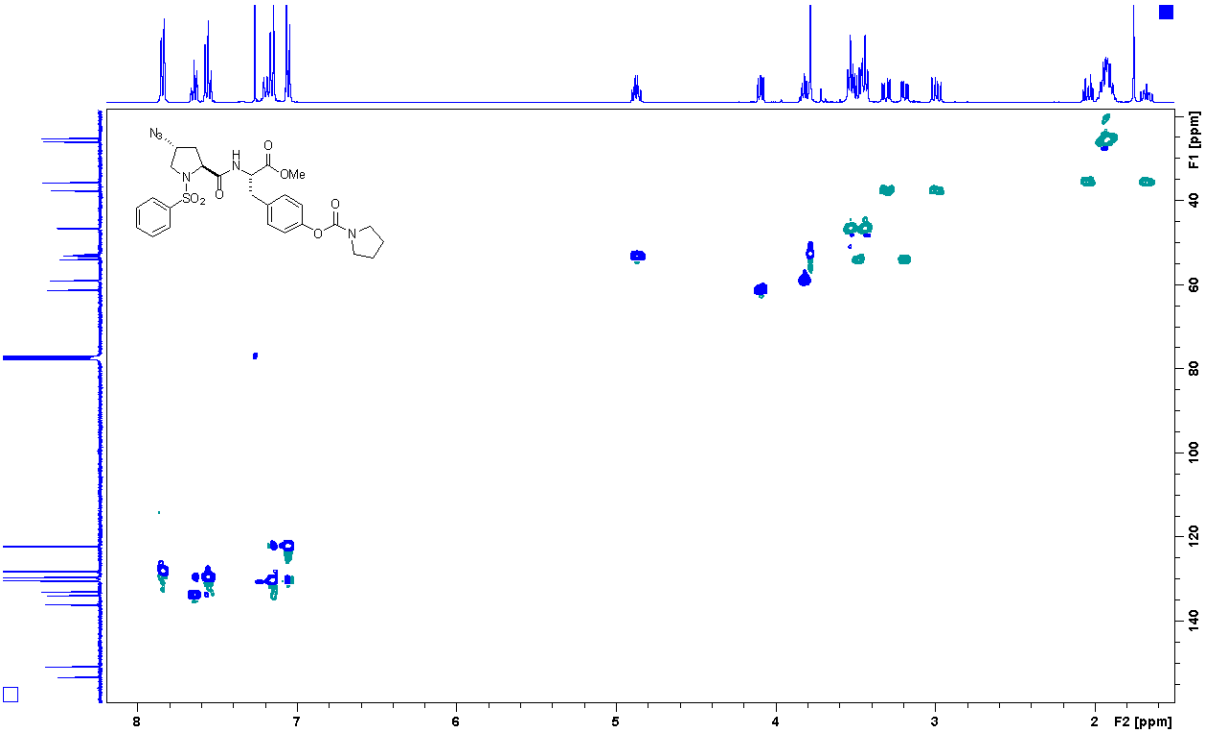
^{13}C NMR of 18



^{1}H-^{1}H COSY of 18

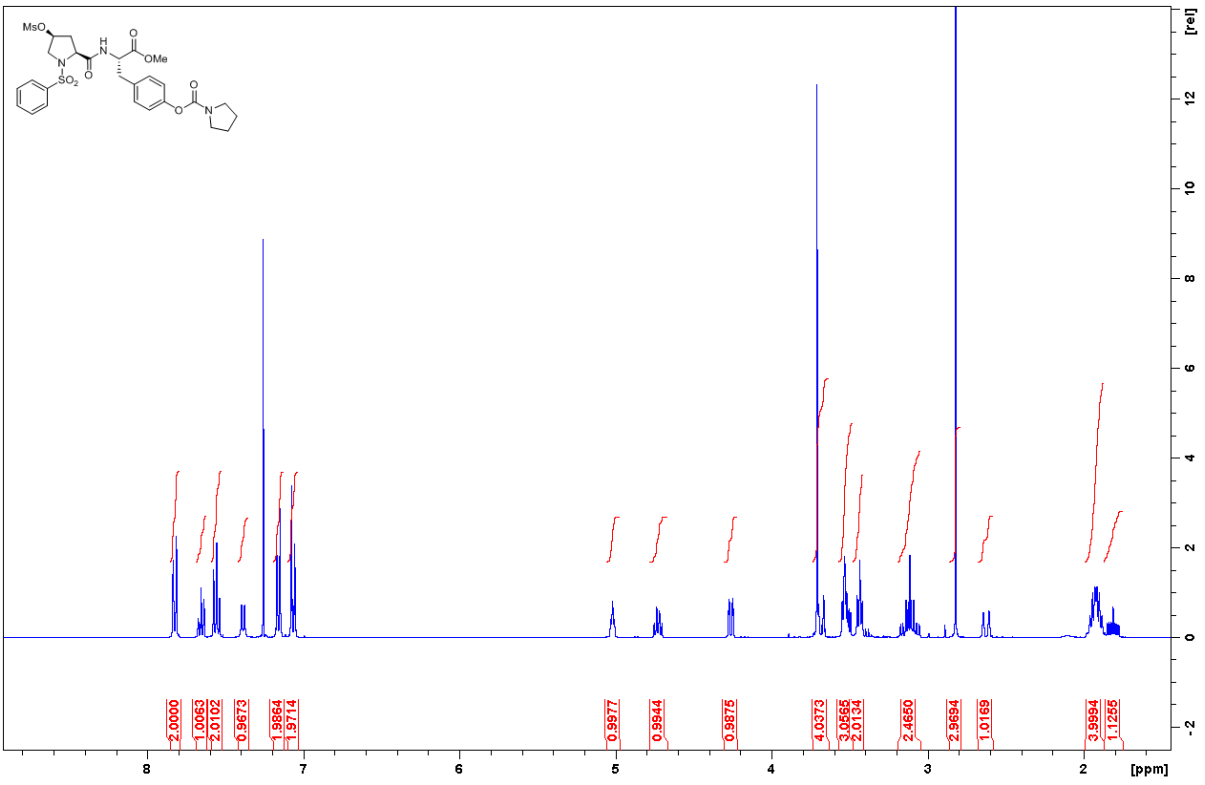


HSQC NMR of 18

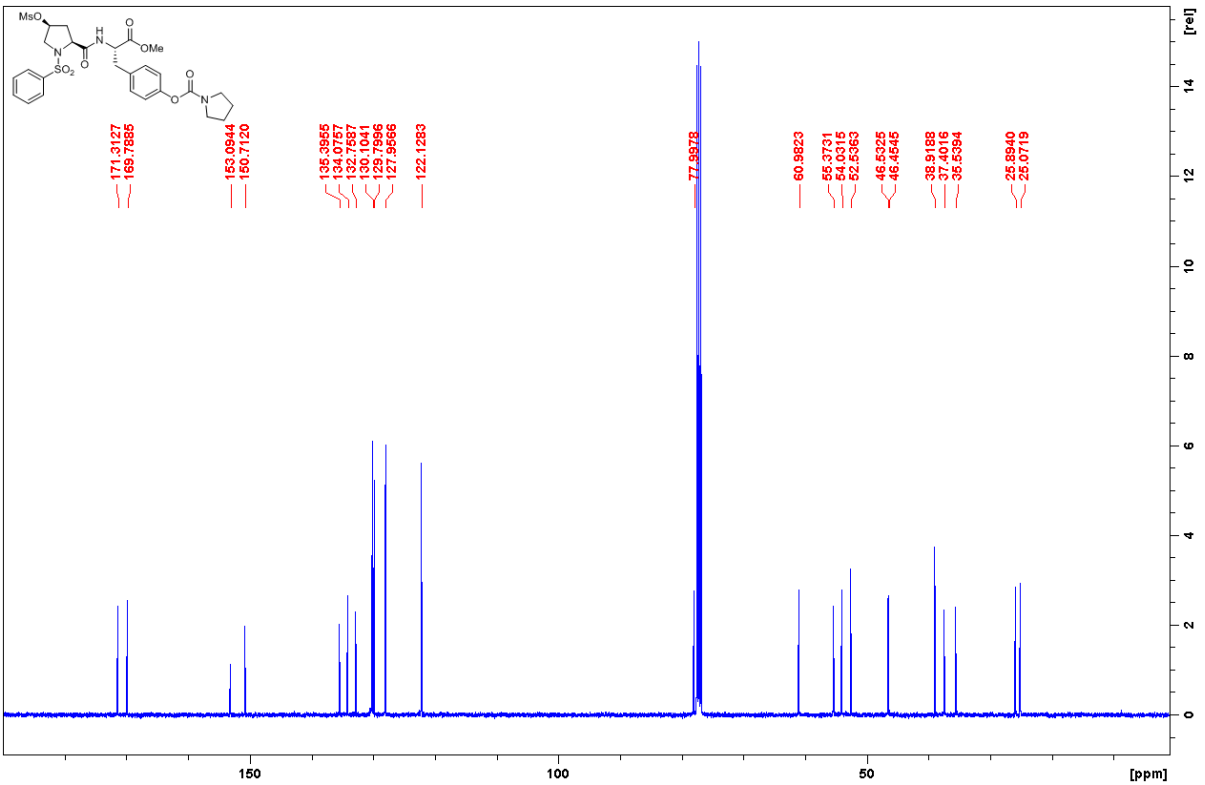


4-((*S*)-3-methoxy-2-((2*S*,4*S*)-4-((methylsulfonyl)oxy)-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)-3-oxopropyl)phenyl pyrrolidine-1-carboxylate

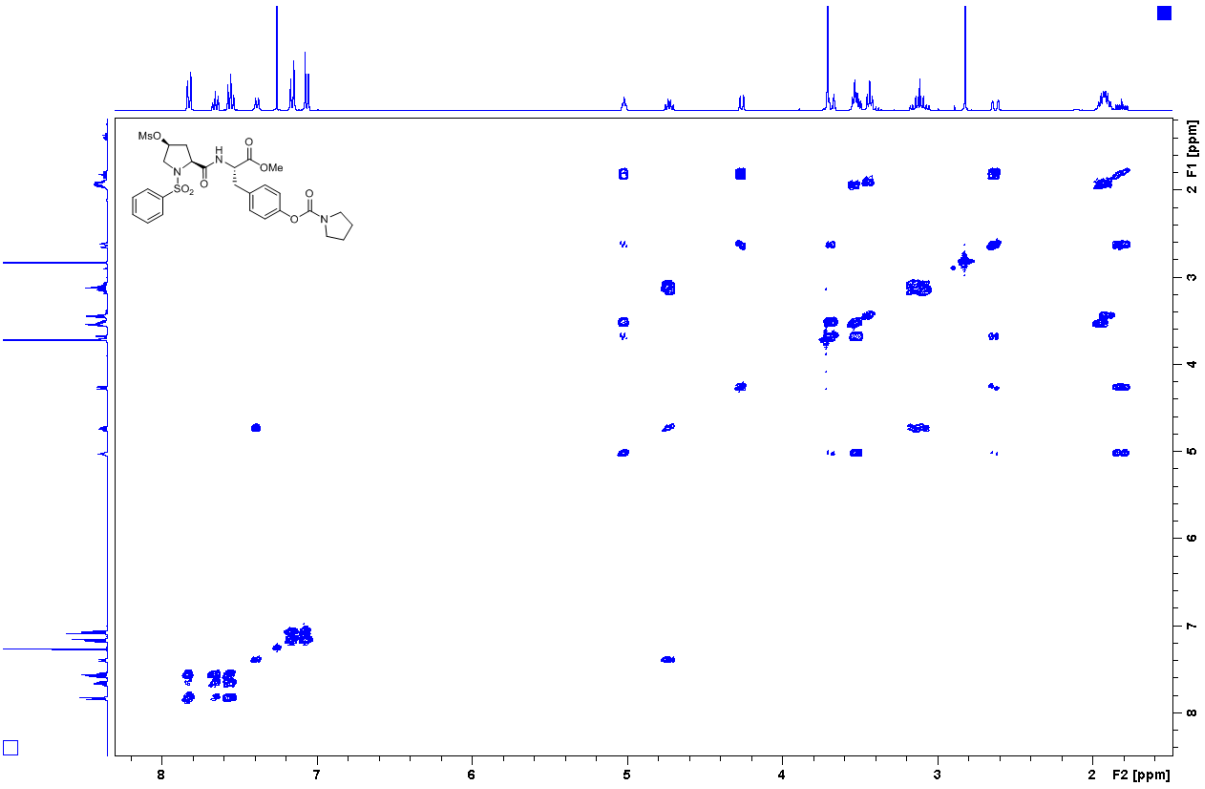
^{1}H NMR



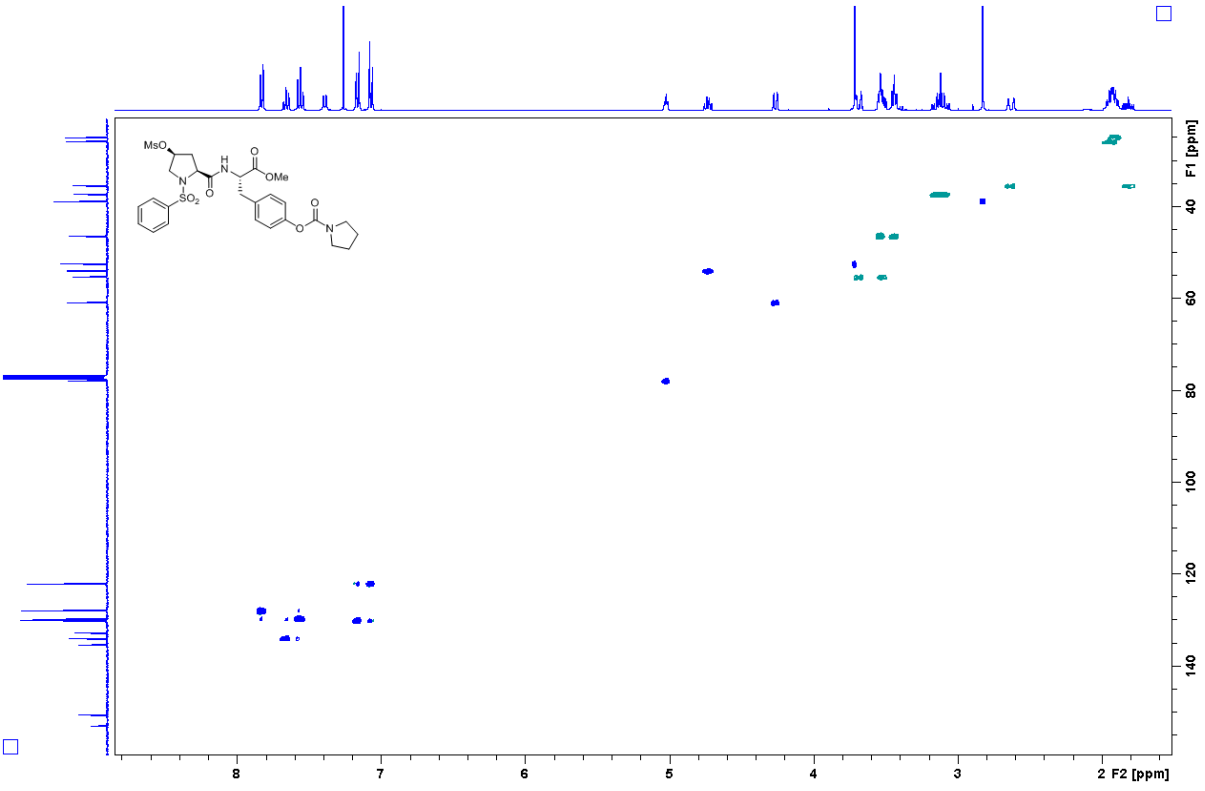
^{13}C NMR



^{1}H-^{1}H COSY NMR

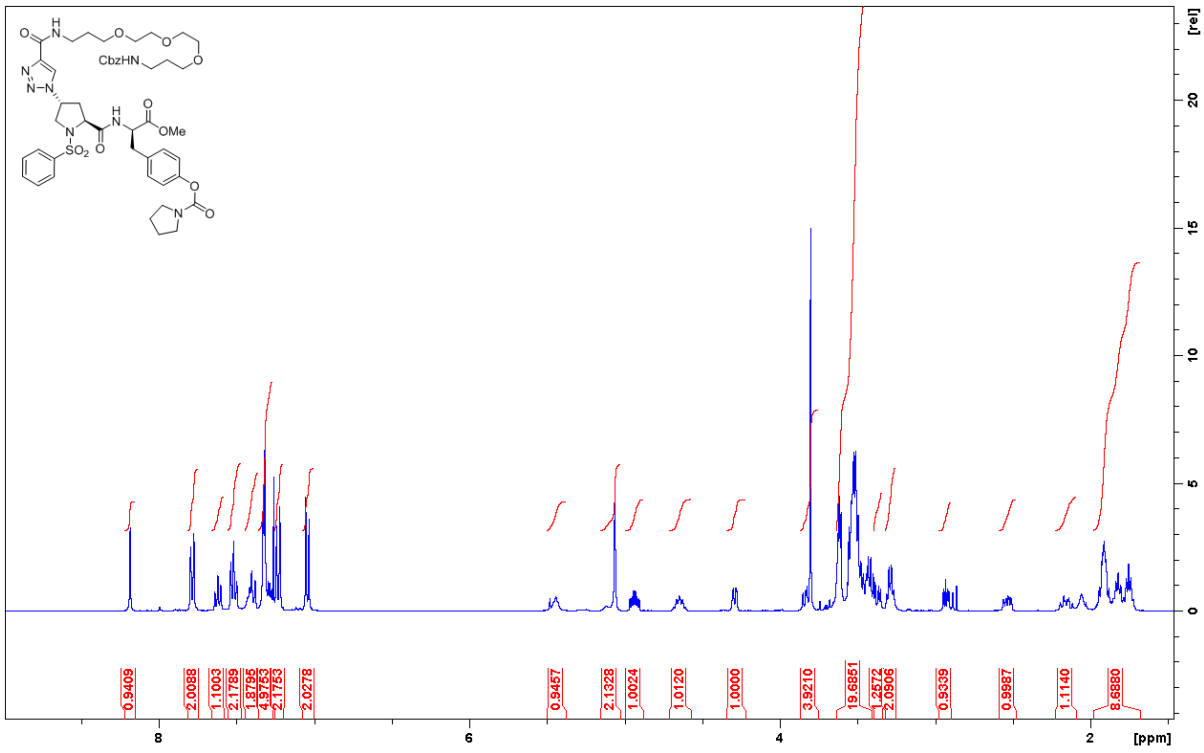


HSQC NMR



4-((*R*)-3-methoxy-3-oxo-2-((2*S*,4*R*)-4-(4-((3-oxo-1-phenyl-2,8,11,14-tetraoxa-4-azaheptadecan-17-yl)carbamoyl)-1*H*-1,2,3-triazol-1-yl)-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)propyl)phenyl pyrrolidine-1-carboxylate (19)

^{1}H NMR of 19



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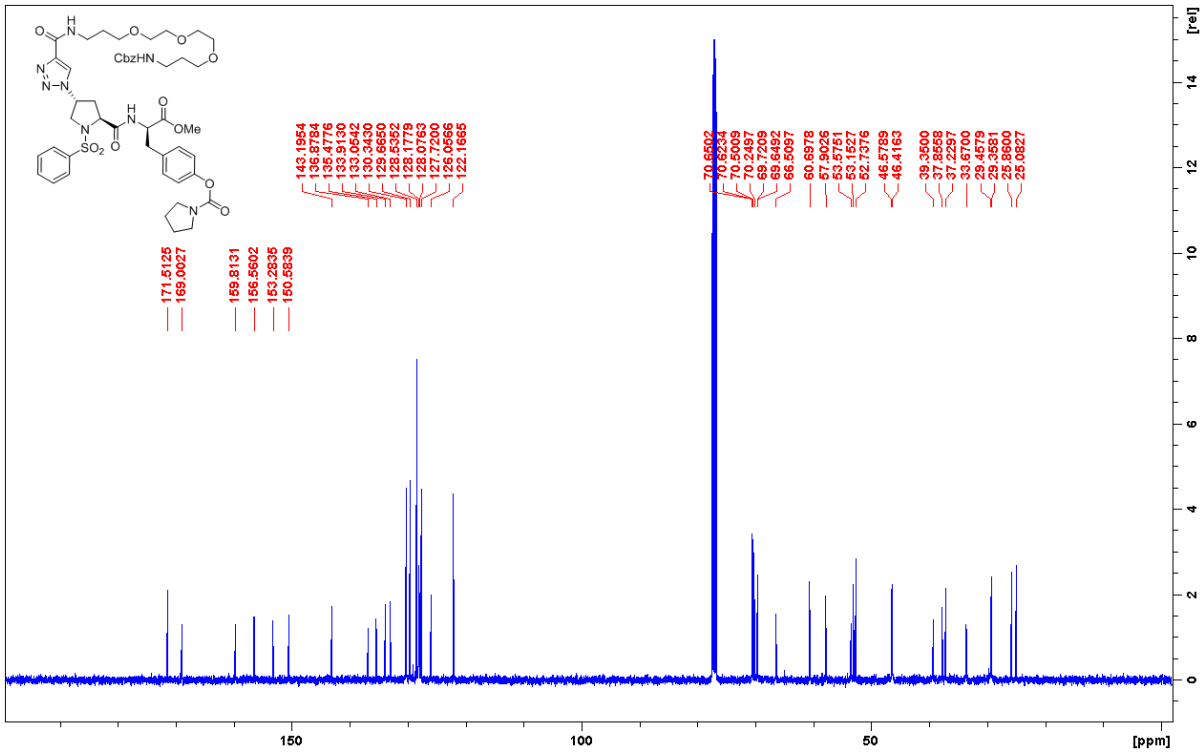
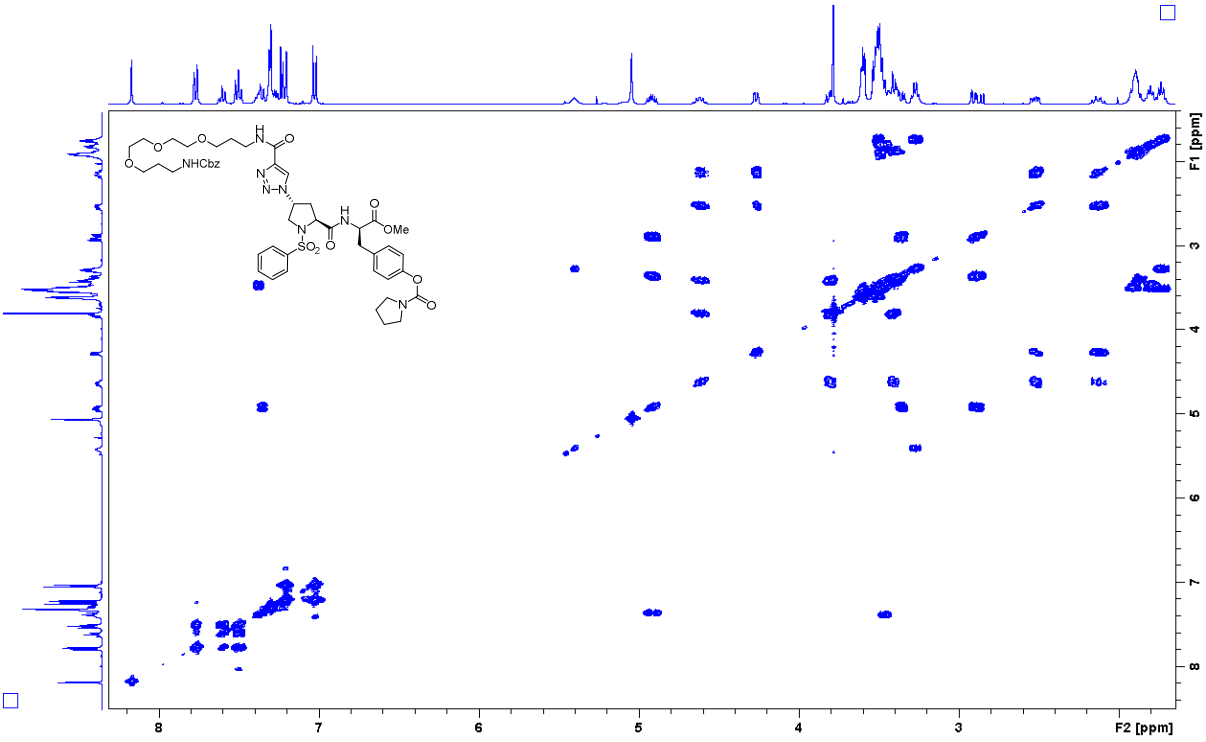
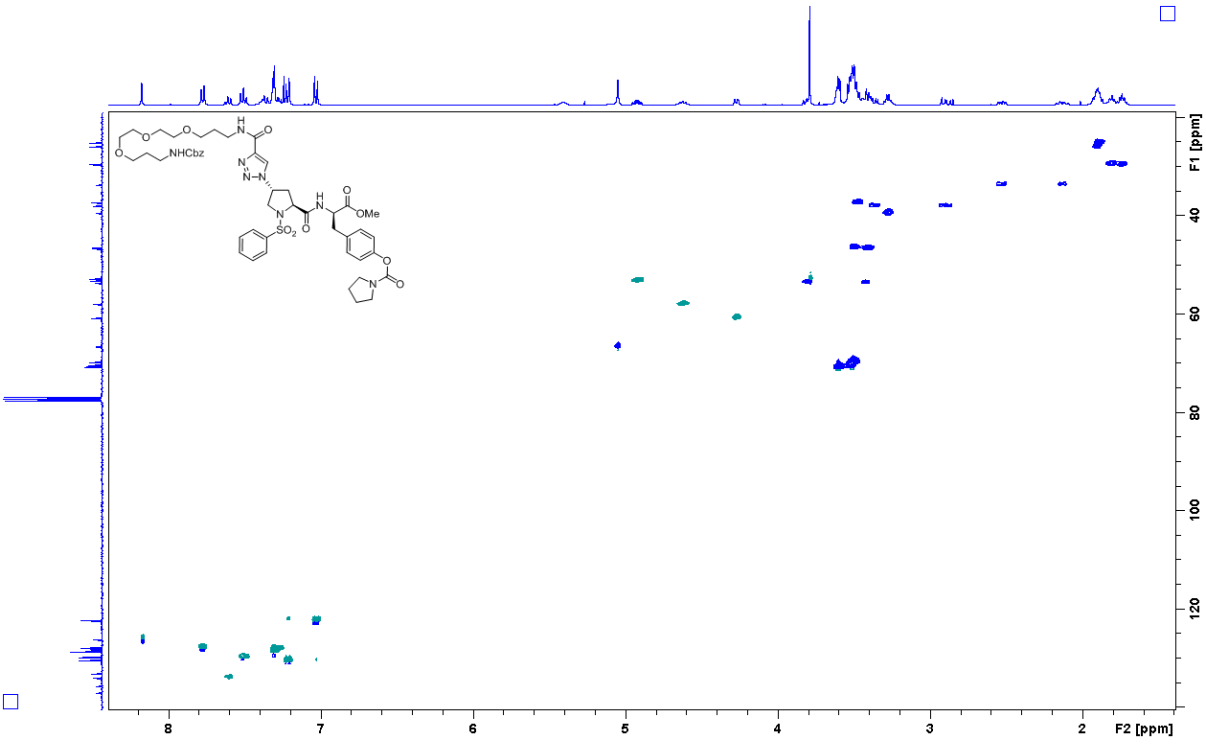


^{1}H-^{1}H COSY NMR of 19

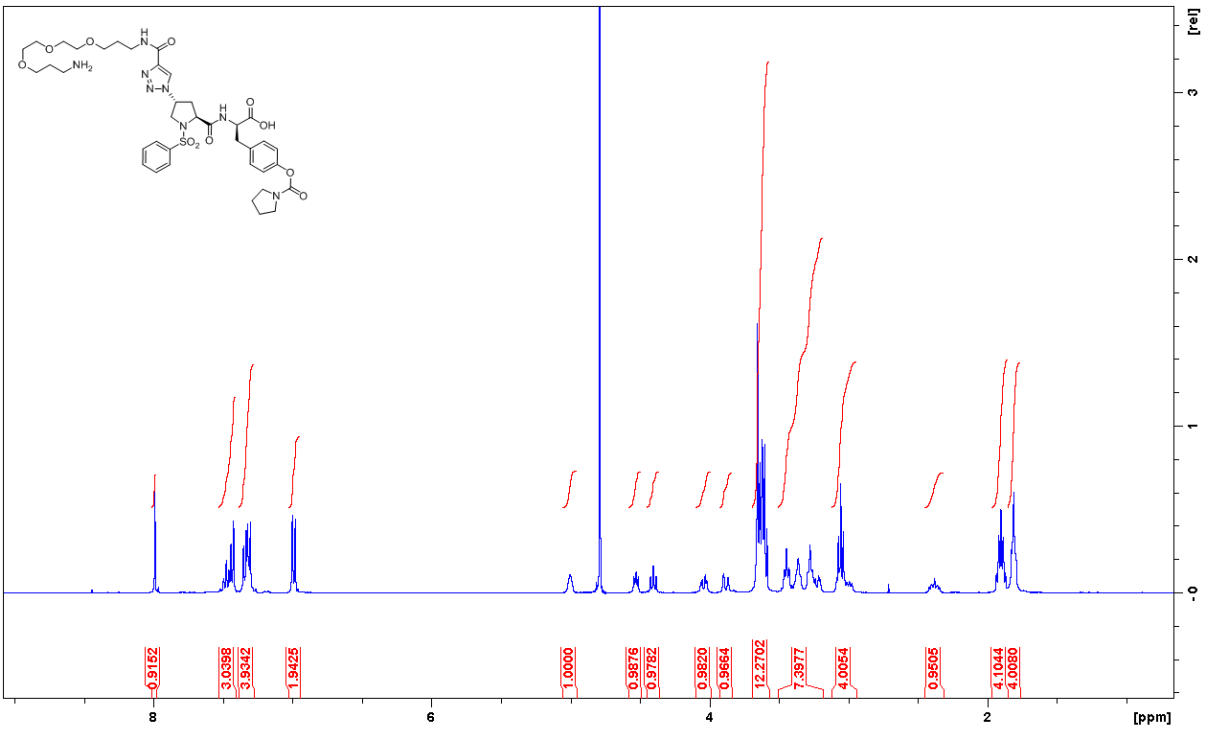


HSQC NMR of 19

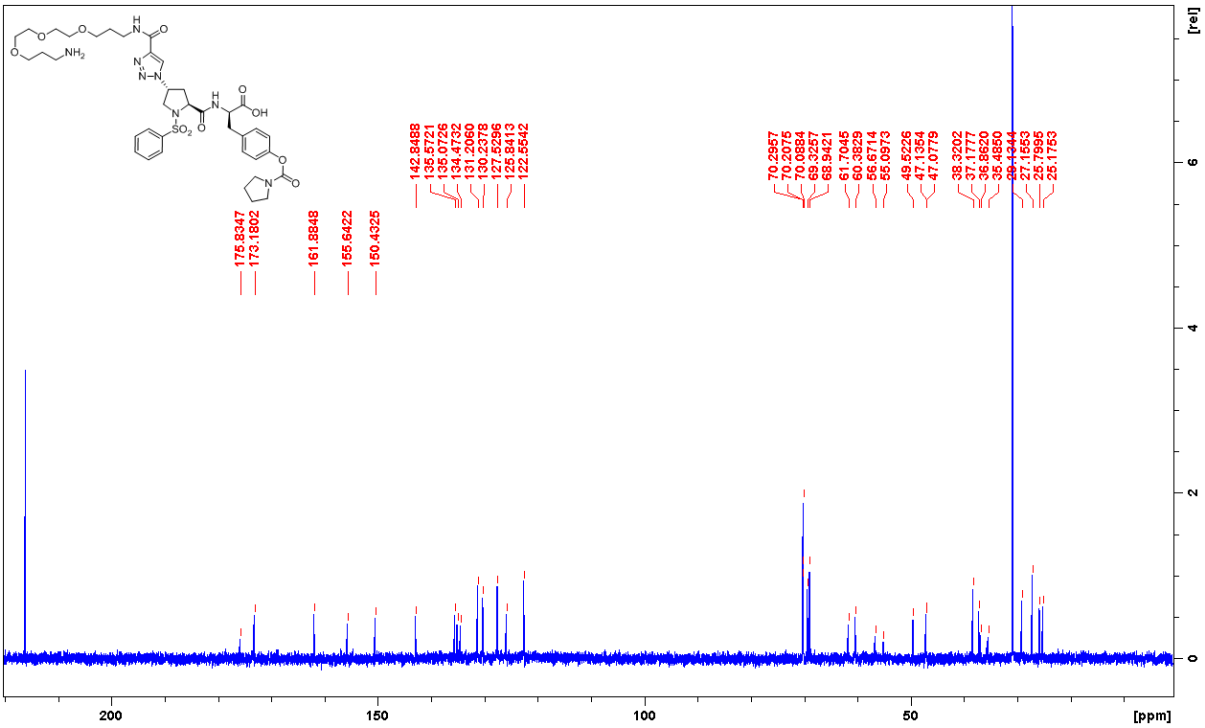


(*R*)-2-((2*S*,4*R*)-4-(4-((3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamoyl)-1*H*-1,2,3-triazol-1-yl)-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)-3-(4-((pyrrolidine-1-carbonyl)oxy)phenyl)propanoic acid

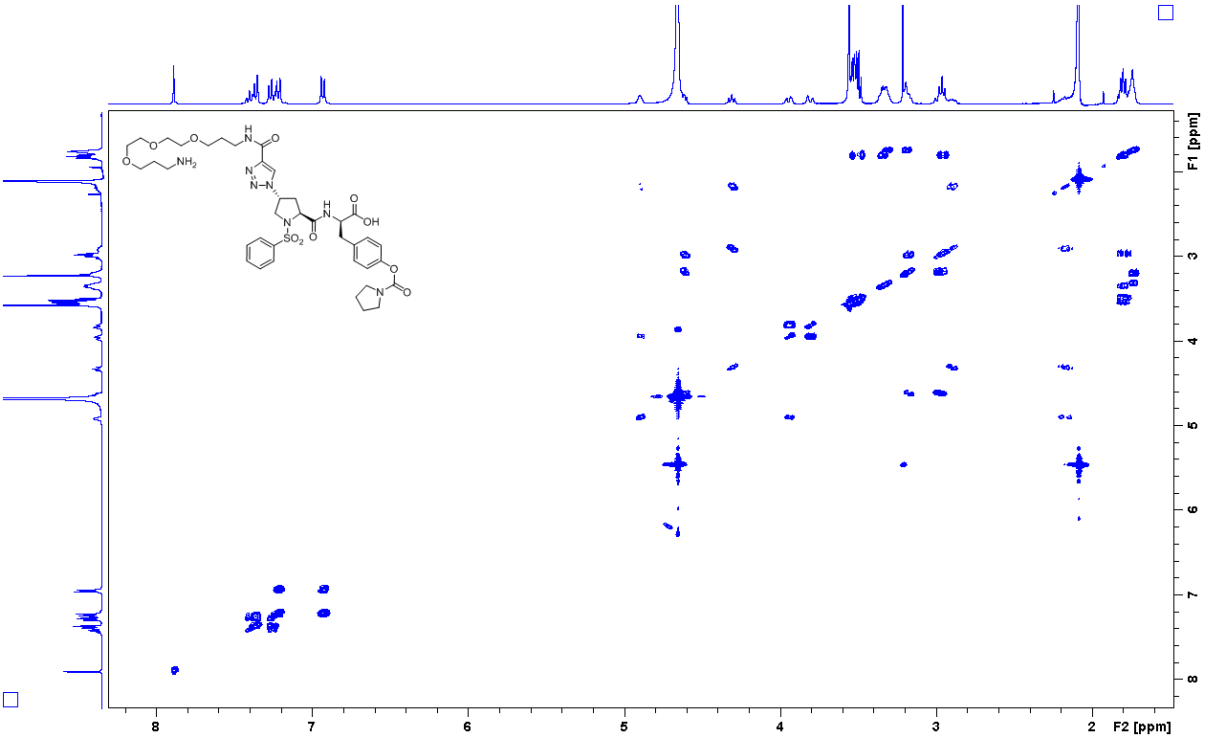
^{1}H NMR of 21 (no acetone)



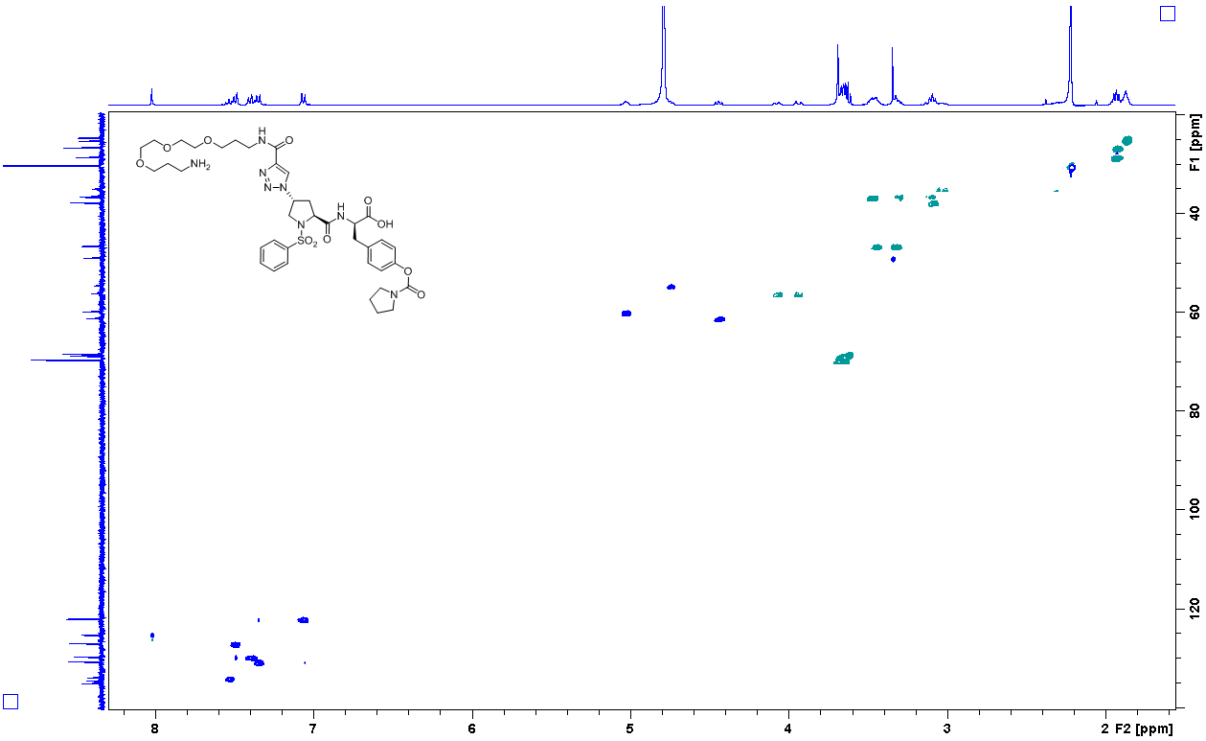
^{13}C NMR of 21 (referenced to acetone)



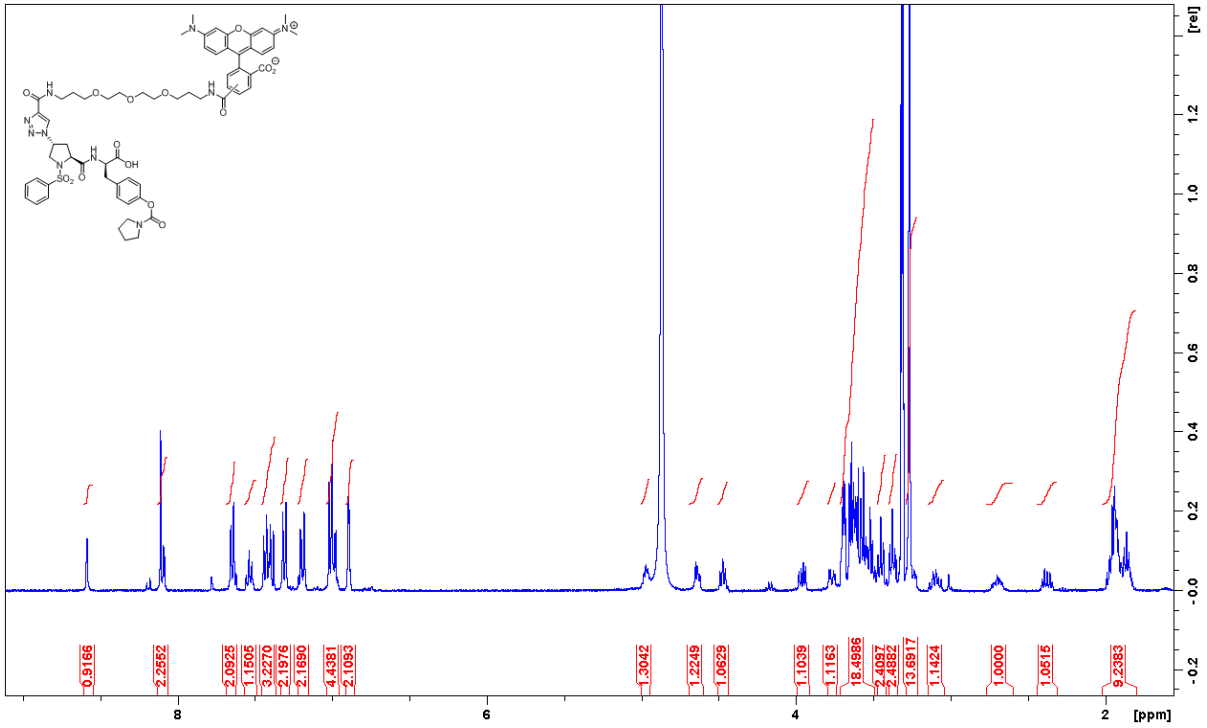
^{1}H-^{1}H COSY NMR (with acetone)



HSQC NMR of 19 (with acetone)



^{1}H NMR of 22



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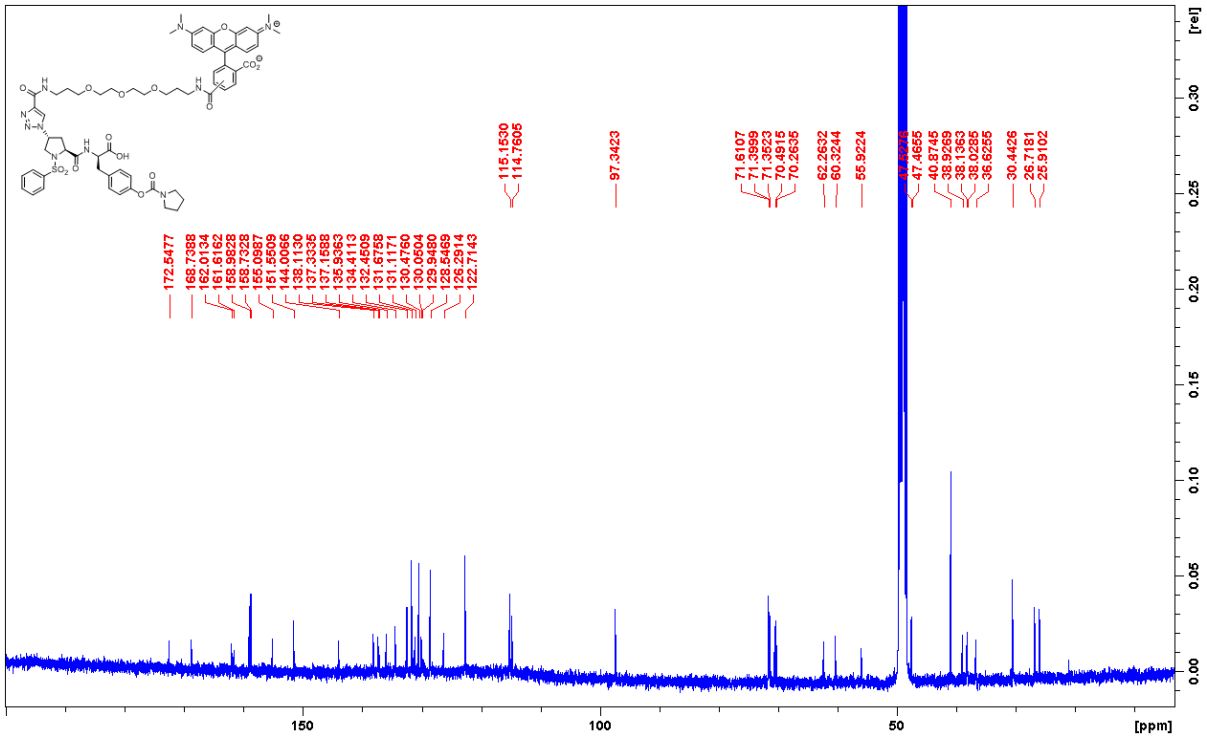

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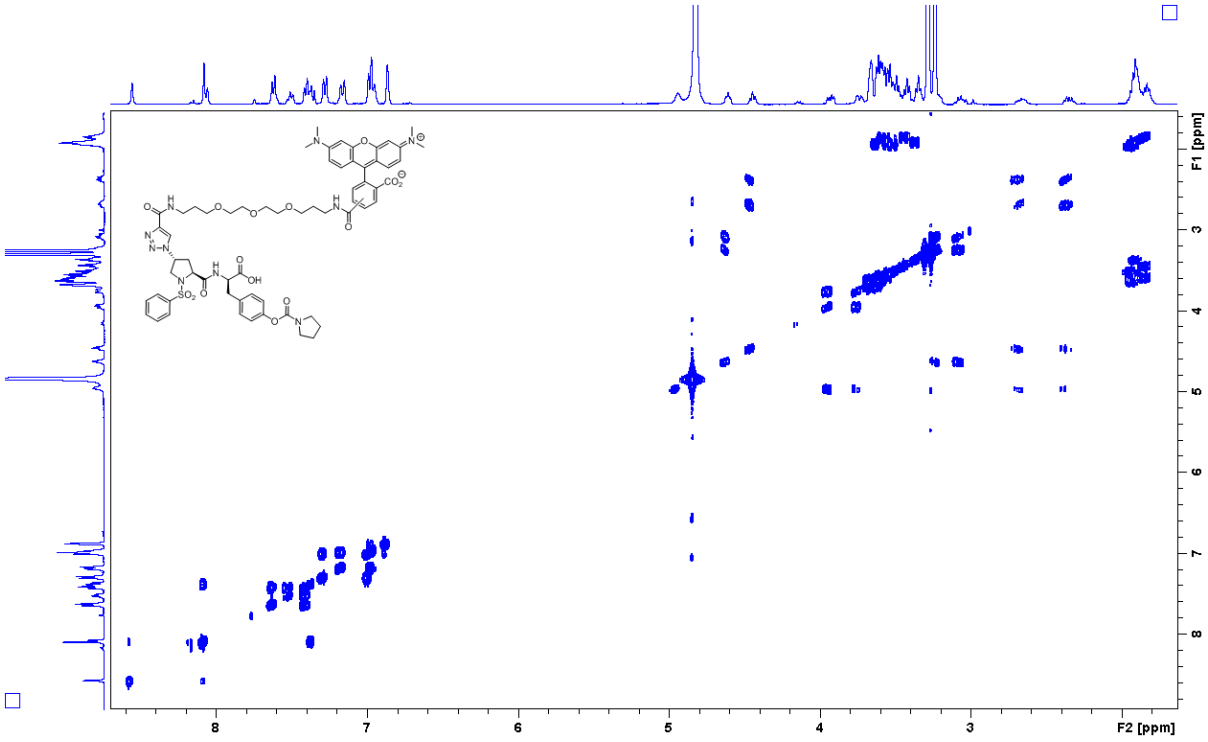
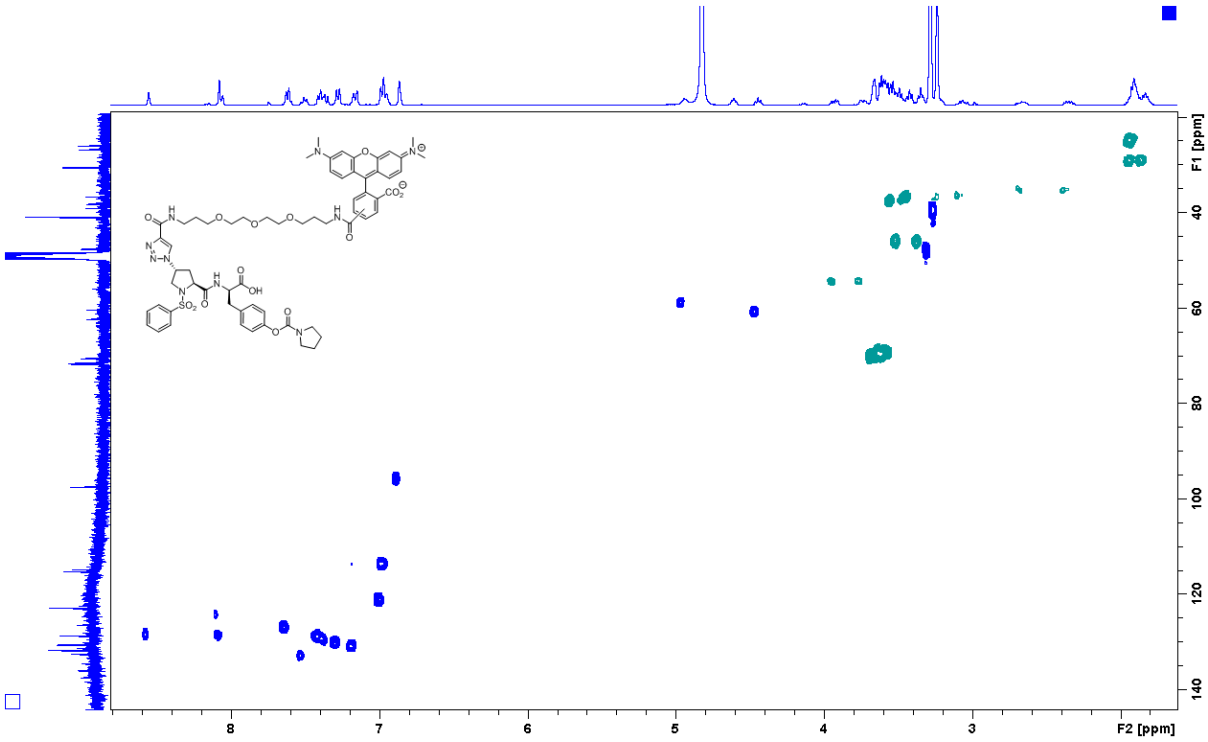
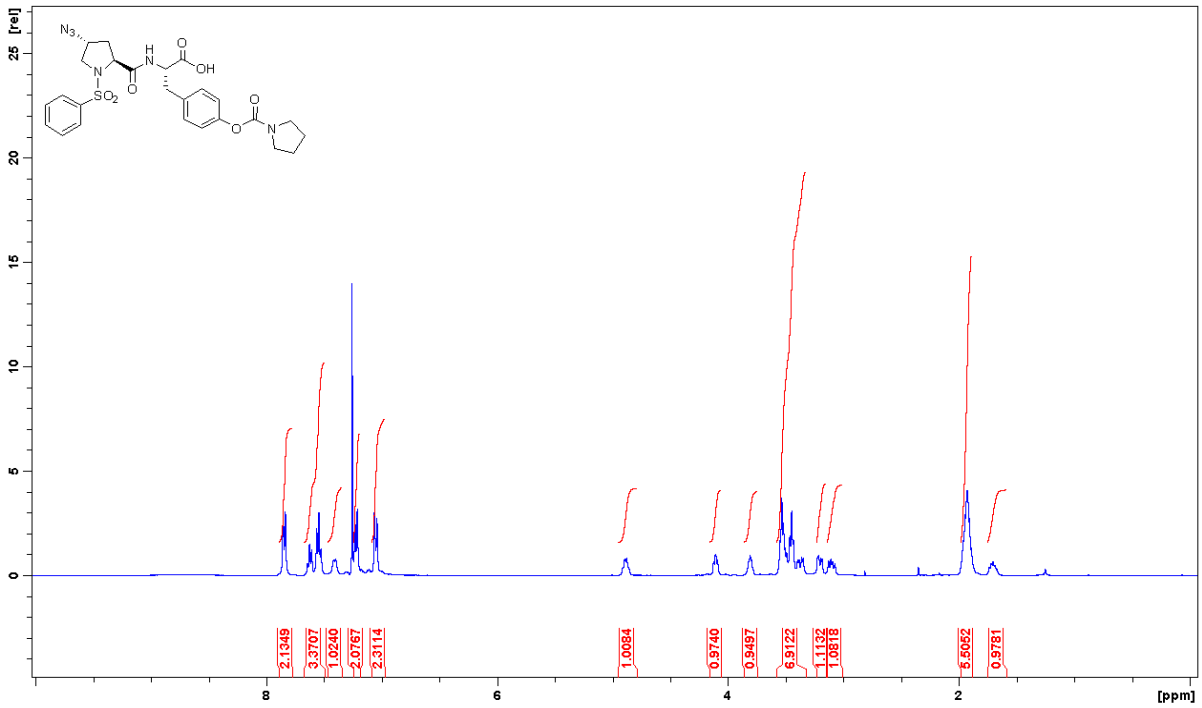


HSQC NMR of 22

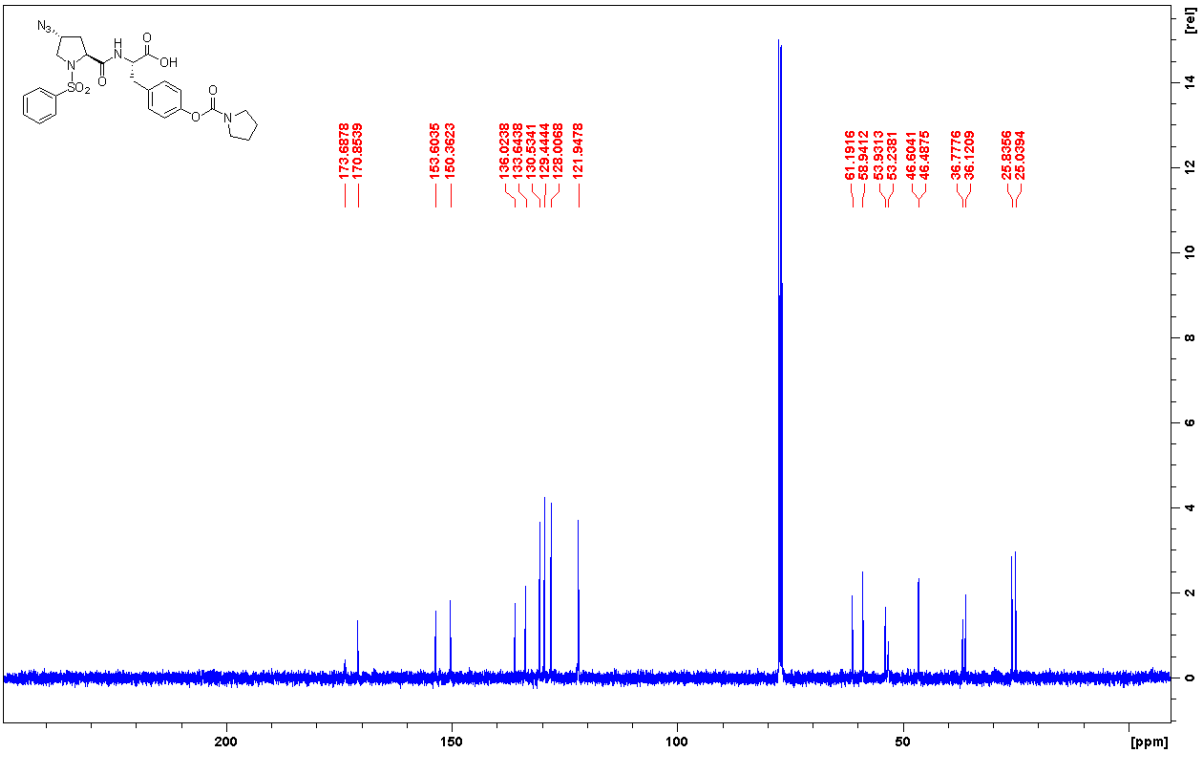


(*S*)-2-((2*S*,4*R*)-4-azido-1-(phenylsulfonyl)pyrrolidine-2-carboxamido)-3-(4-((pyrrolidine-1-carbonyl)oxy)phenyl)propanoic acid (23)

^{1}H NMR of 23

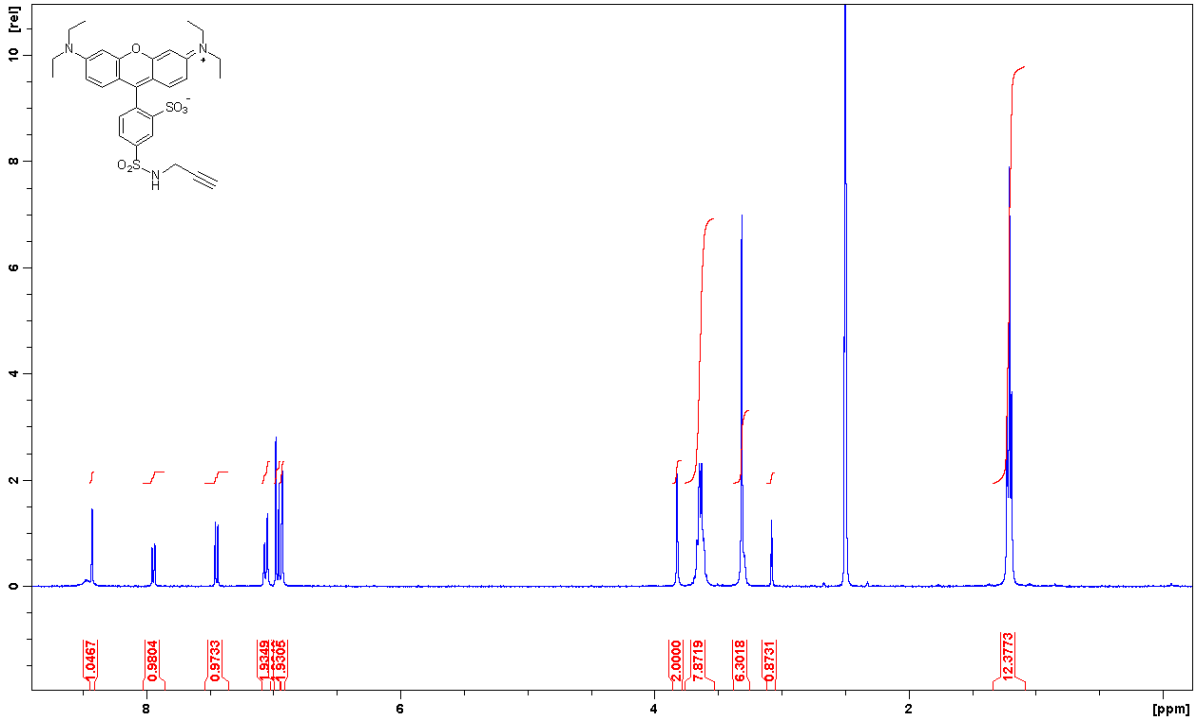


^{13}C NMR of 23

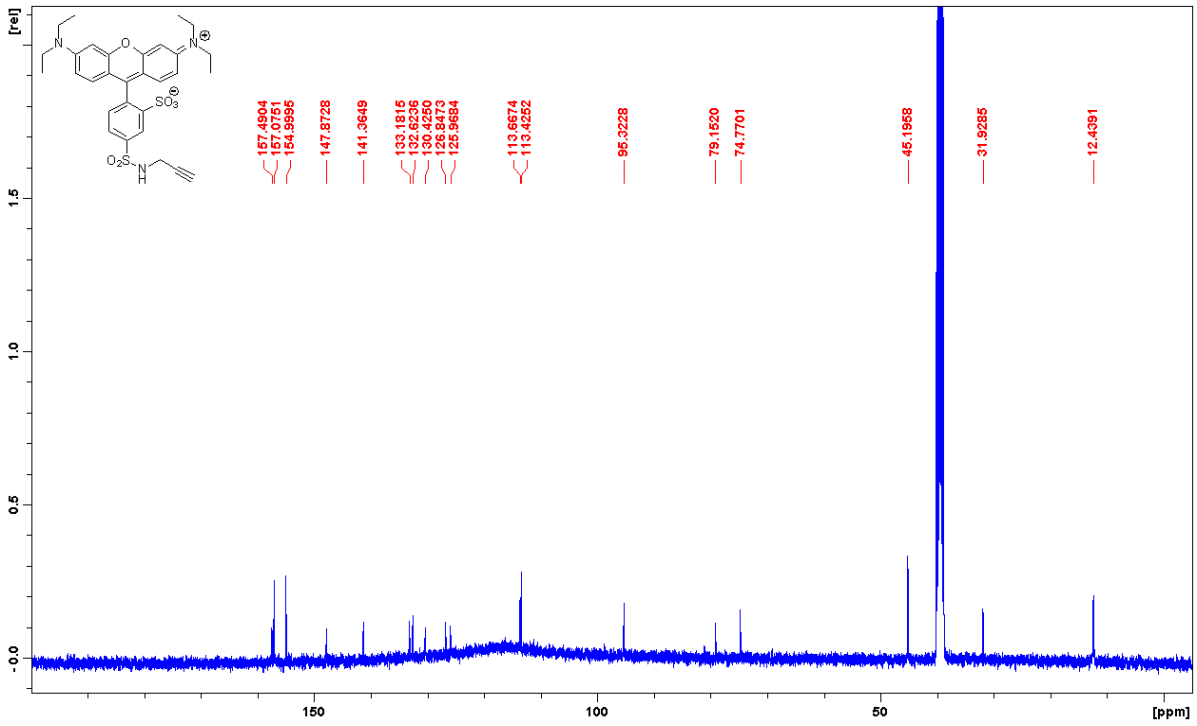


N-propynyl sulforhodamine B (24)

^{1}H NMR of 24

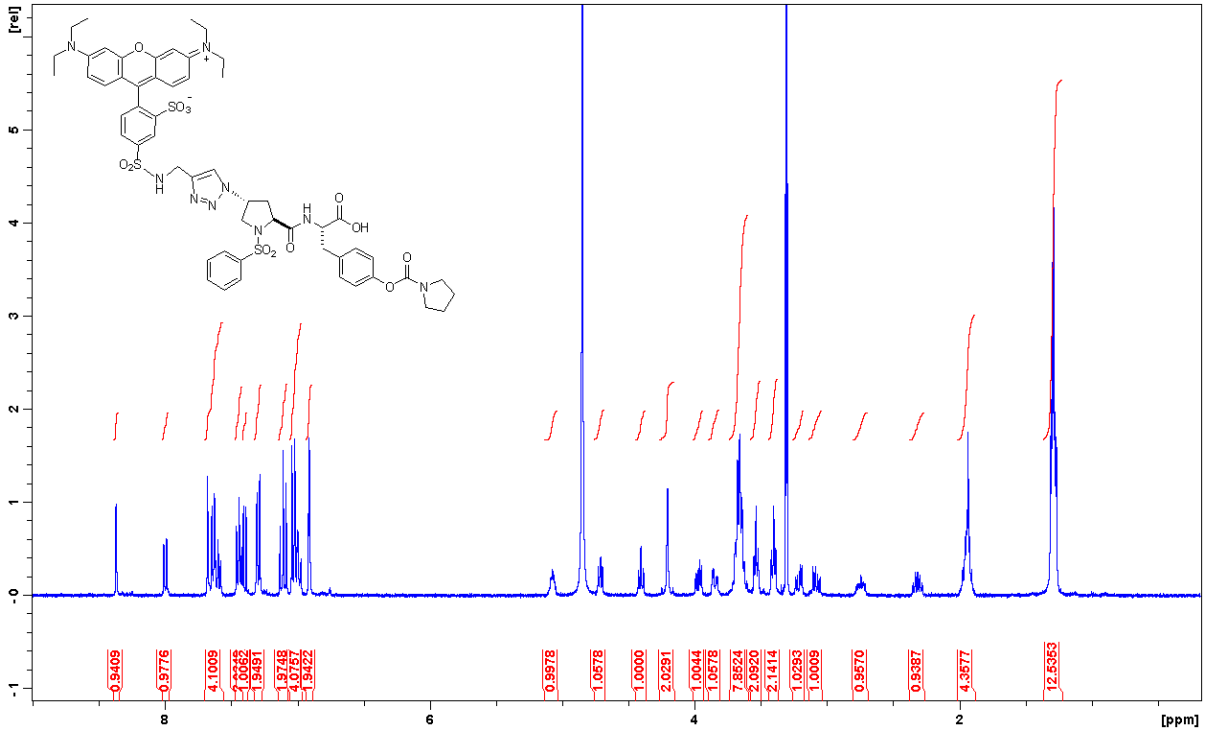


^{13}C NMR of 24

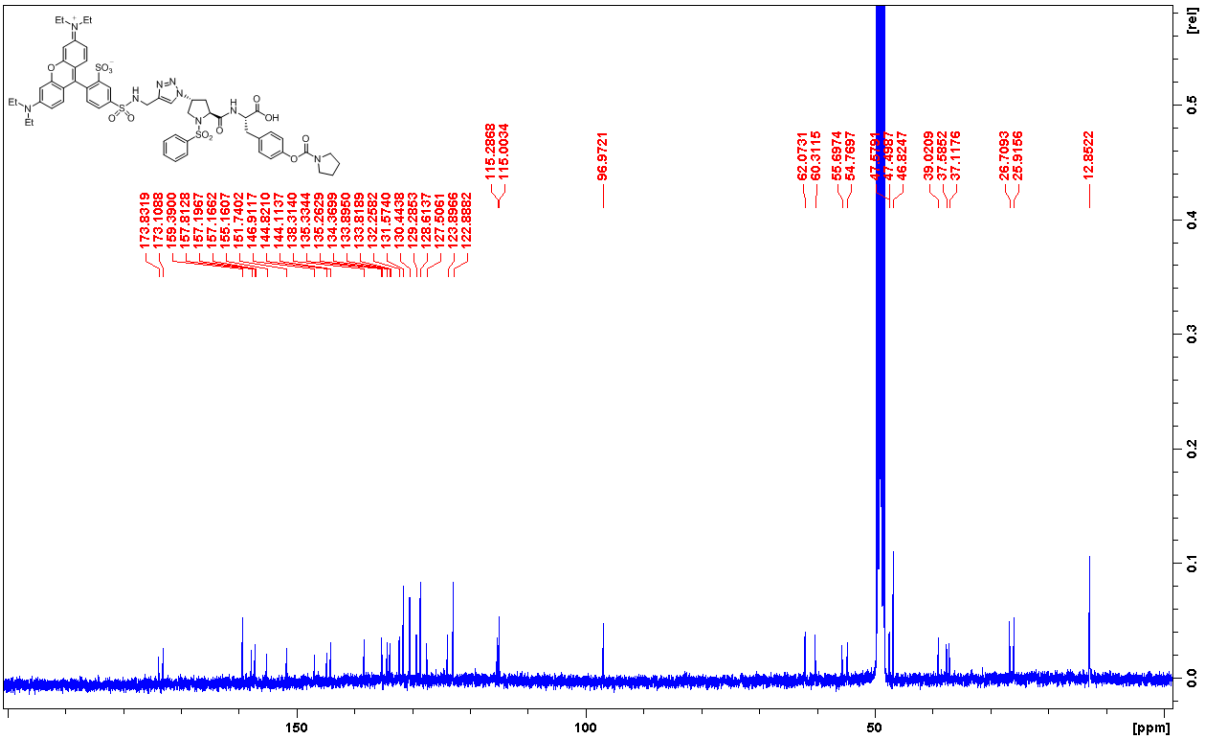


R-BC154 (25)

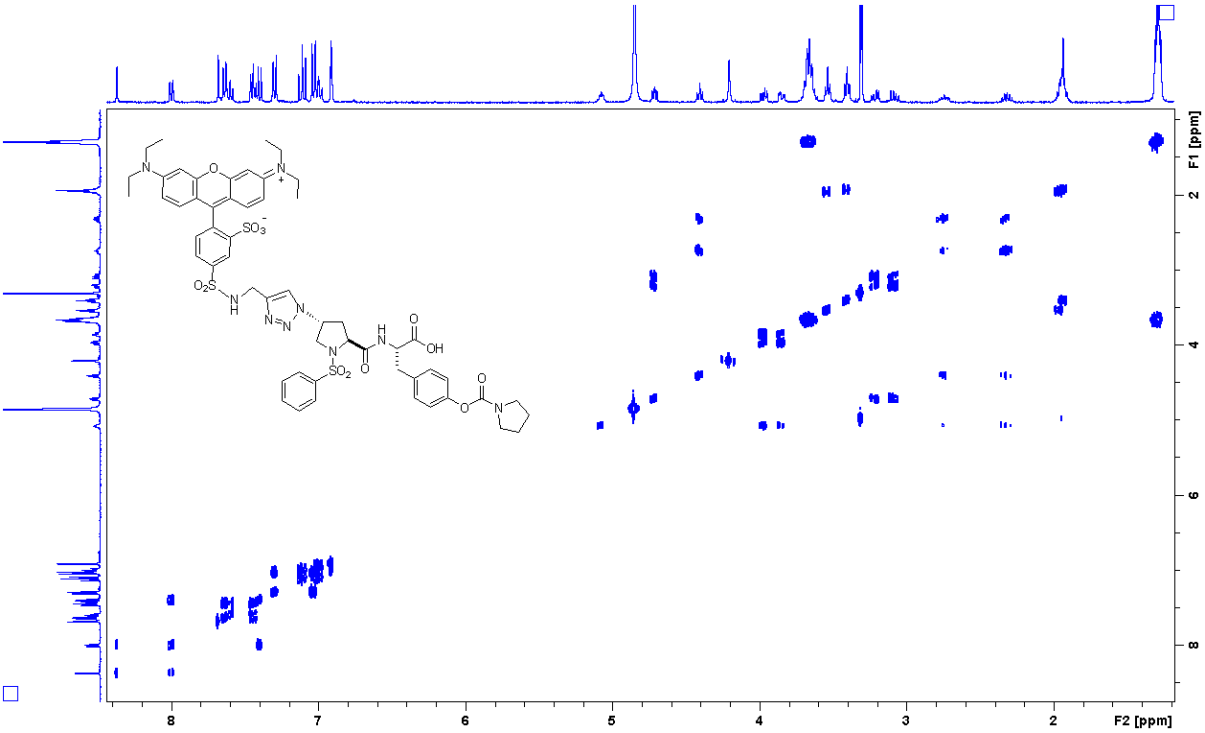
^{1}H NMR of 25



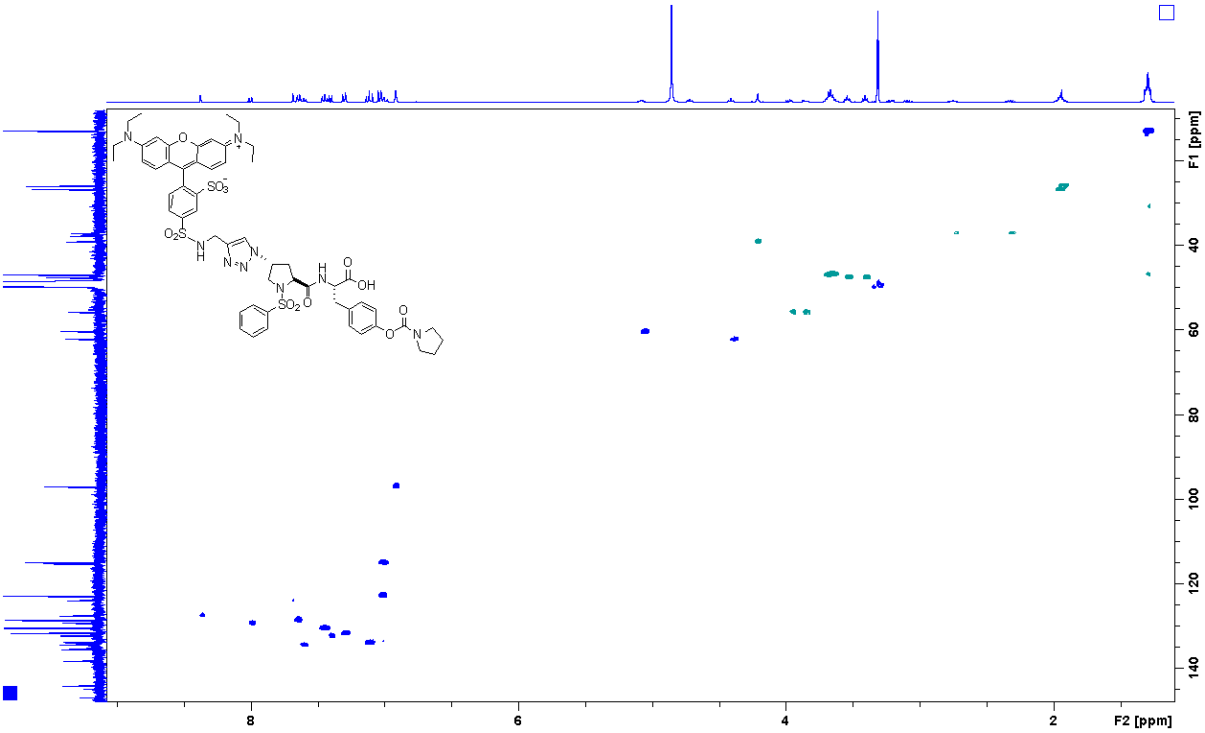
^{13}C NMR of R-BC154 (25)



^{1}H-^{1}H COSY of R-BC154 (25)



HSQC NMR of R-BC154 (25)



HPLC trace of R-BC154 (25)

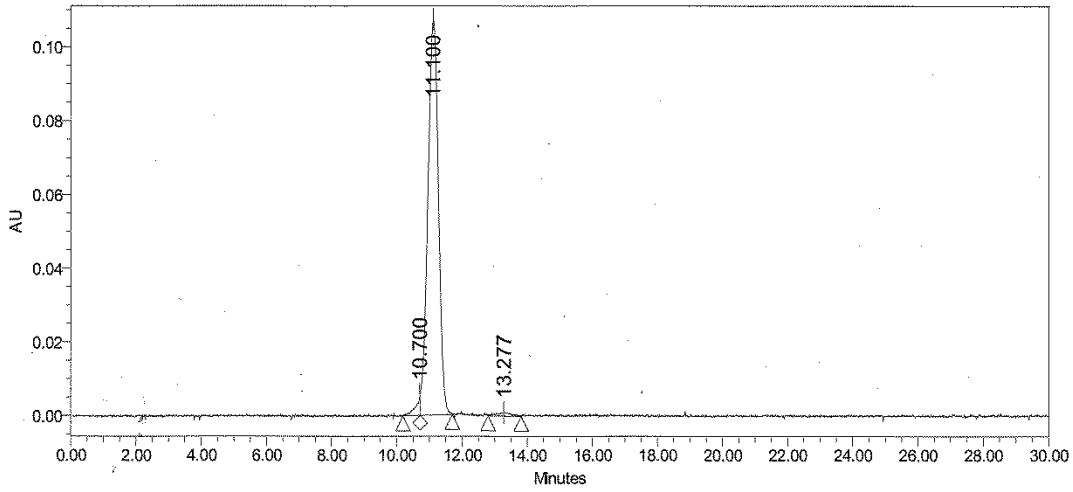
CSIRO Materials Science & Engineering

Run Information

Sample Name: BC1-49 fr 83 65%MeOH/TFA
Detector: PDA 565.0 nm
HplcColumn 150x4.6mm Alltima HP C18
FlowRate: 1.0 mL / min
MobilePhase: 65% MeOH / 0.1% TFA

Date Acquired: 7/09/2011 1:39:02 PM EST
Acq Method Set : AllianceE 100% A isocratic
Date Processed: 14/03/2012 10:30:27 AM EST
Processing Method: nkh

HPLC Chromatogram



The Results

	Name	R.Time	Area	% Area	Height	% Height
1		10.700	49368	1.98	5605	4.93
2		11.100	2410774	96.75	107152	94.24
3		13.277	31667	1.27	949	0.83

References

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3. H. Yang, S. Vasudevan, C. O. Oriakhi, J. Shields and R. G. Carter, *Synthesis*, 2008, 957-961.