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Linear Scaffolds for Multivalent Targeting of Melanocortin Receptors

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Supporting Information

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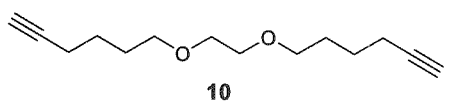

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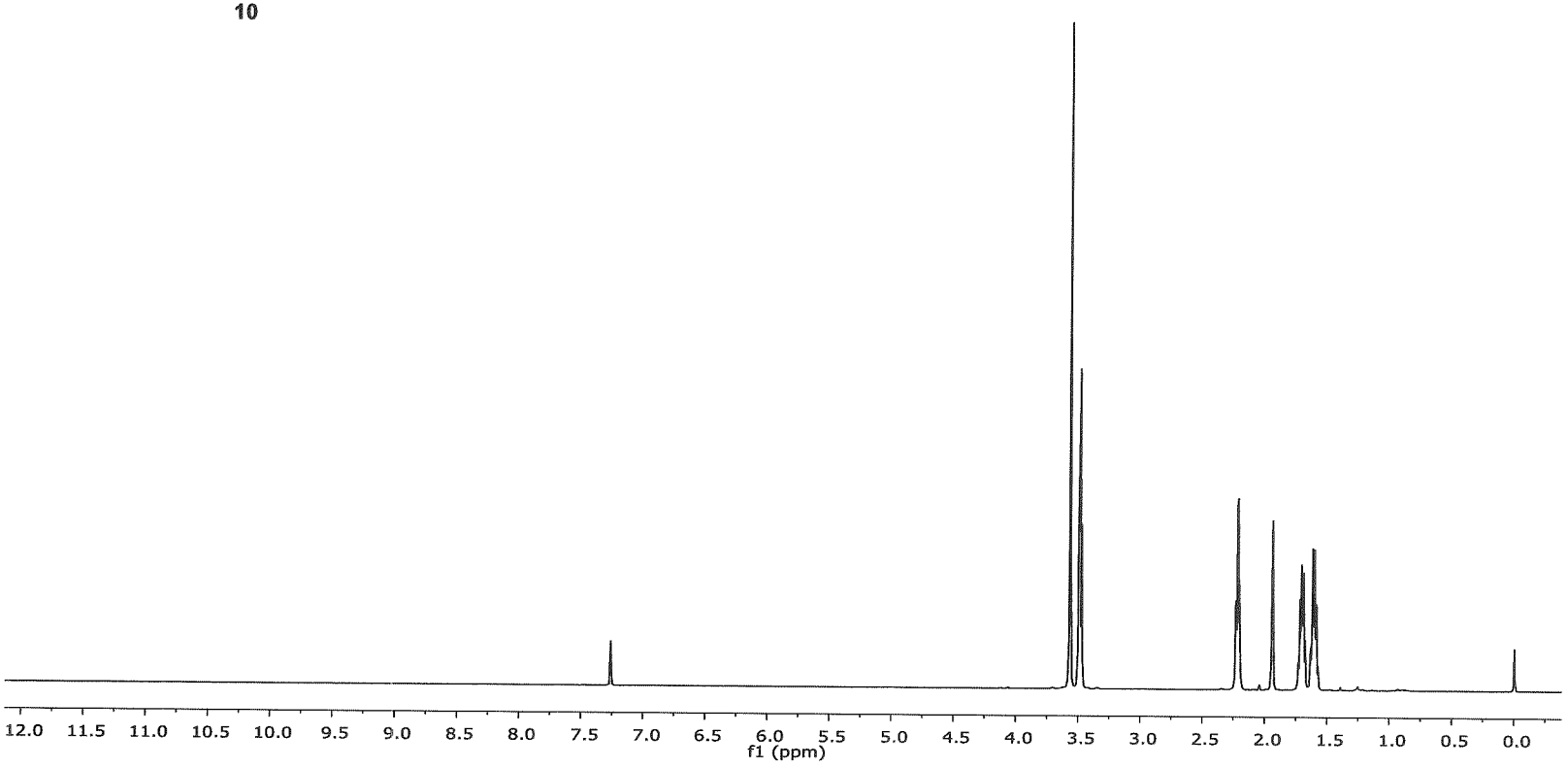
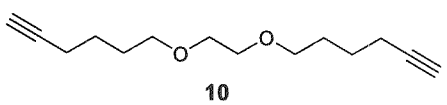


^{1}H NMR Spectrum of Compound **10**



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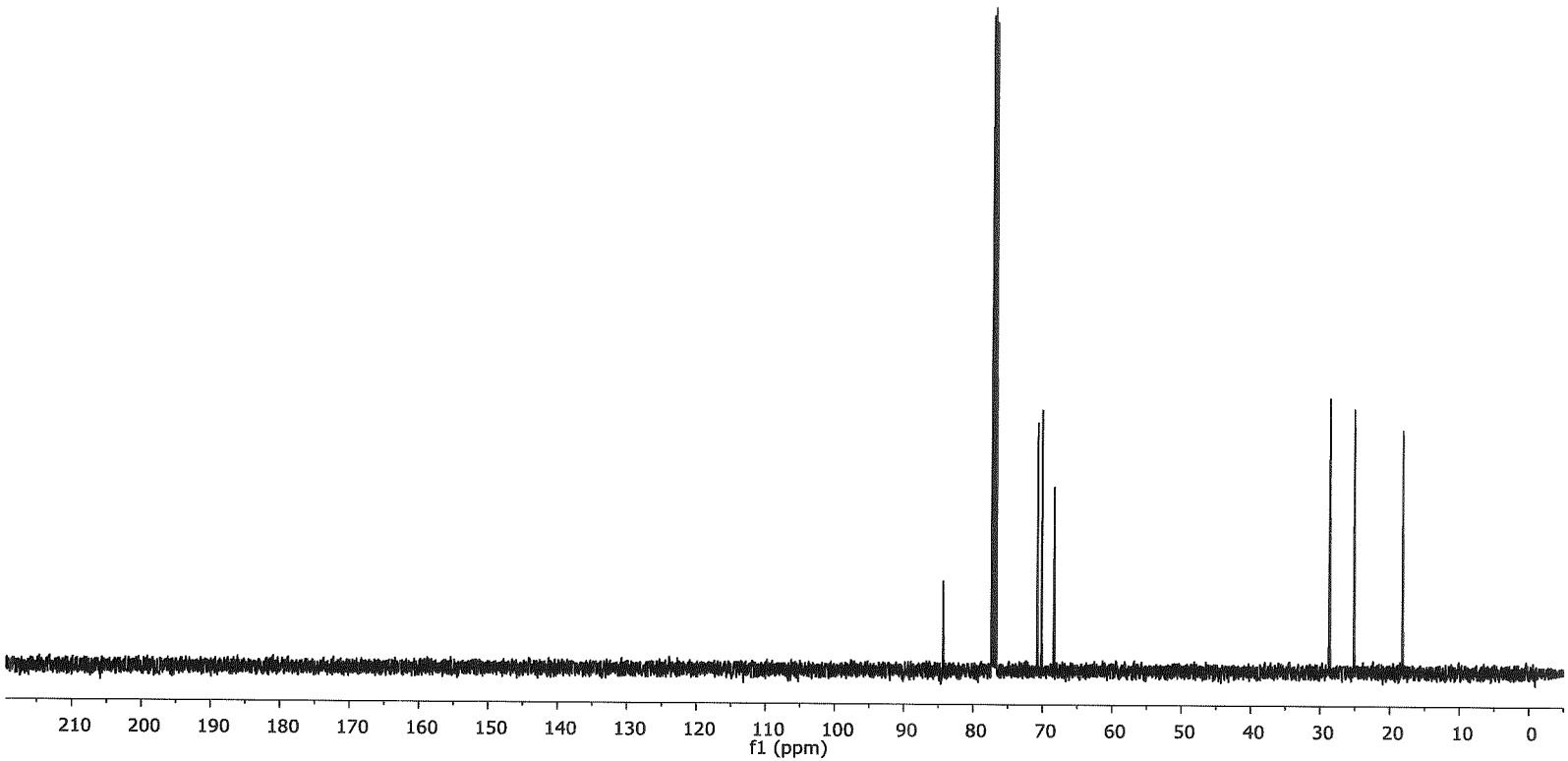

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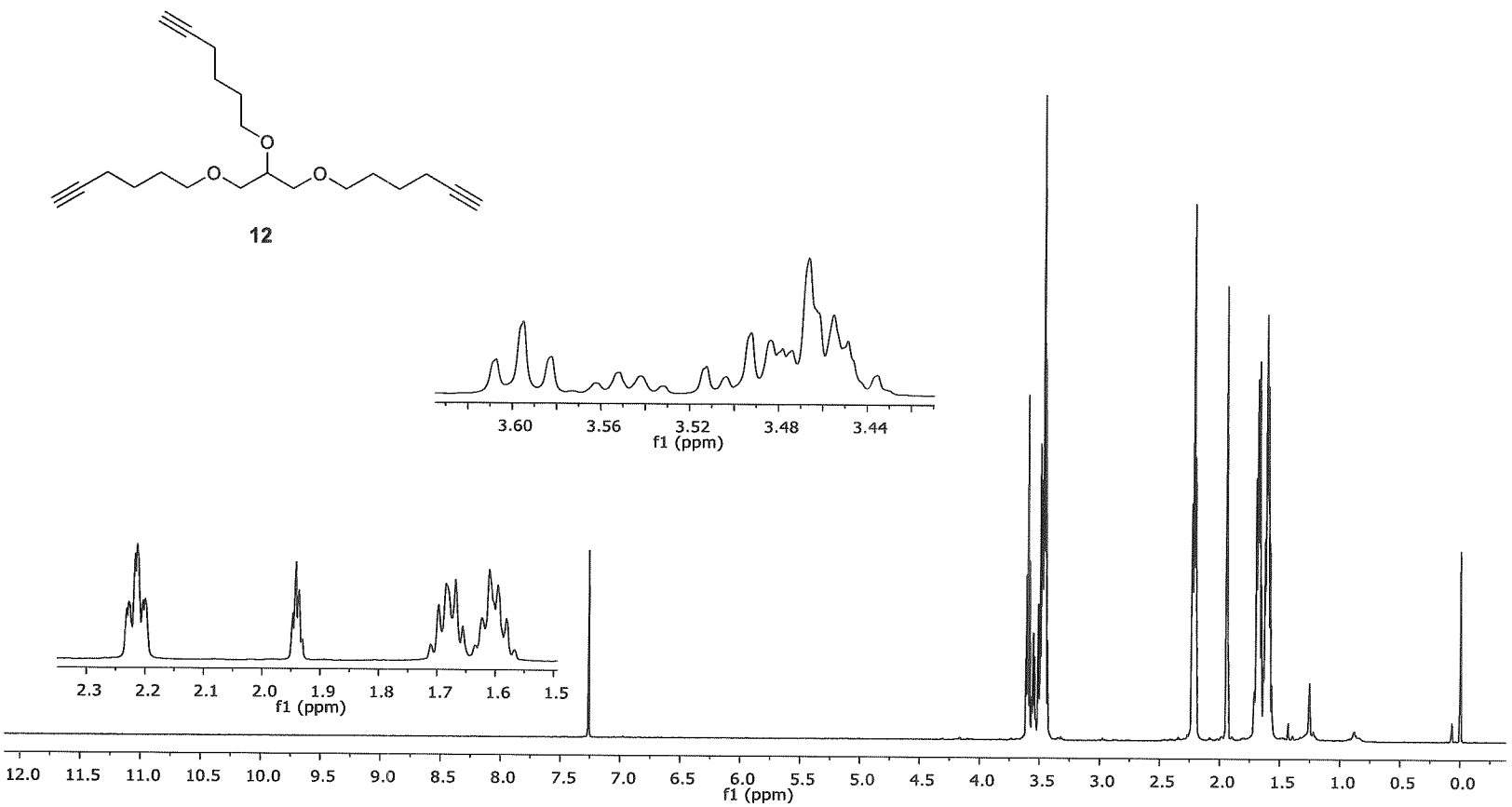
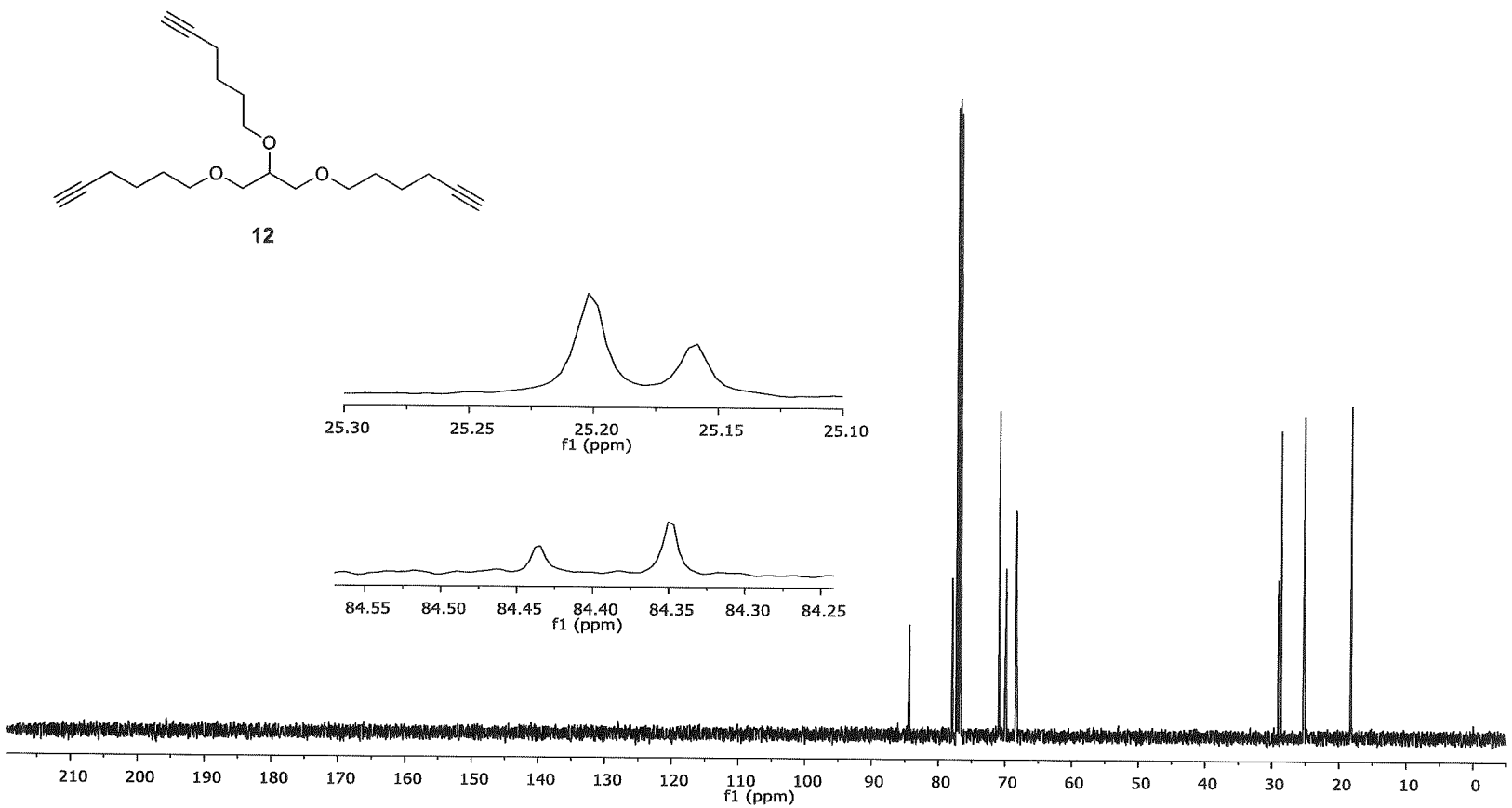
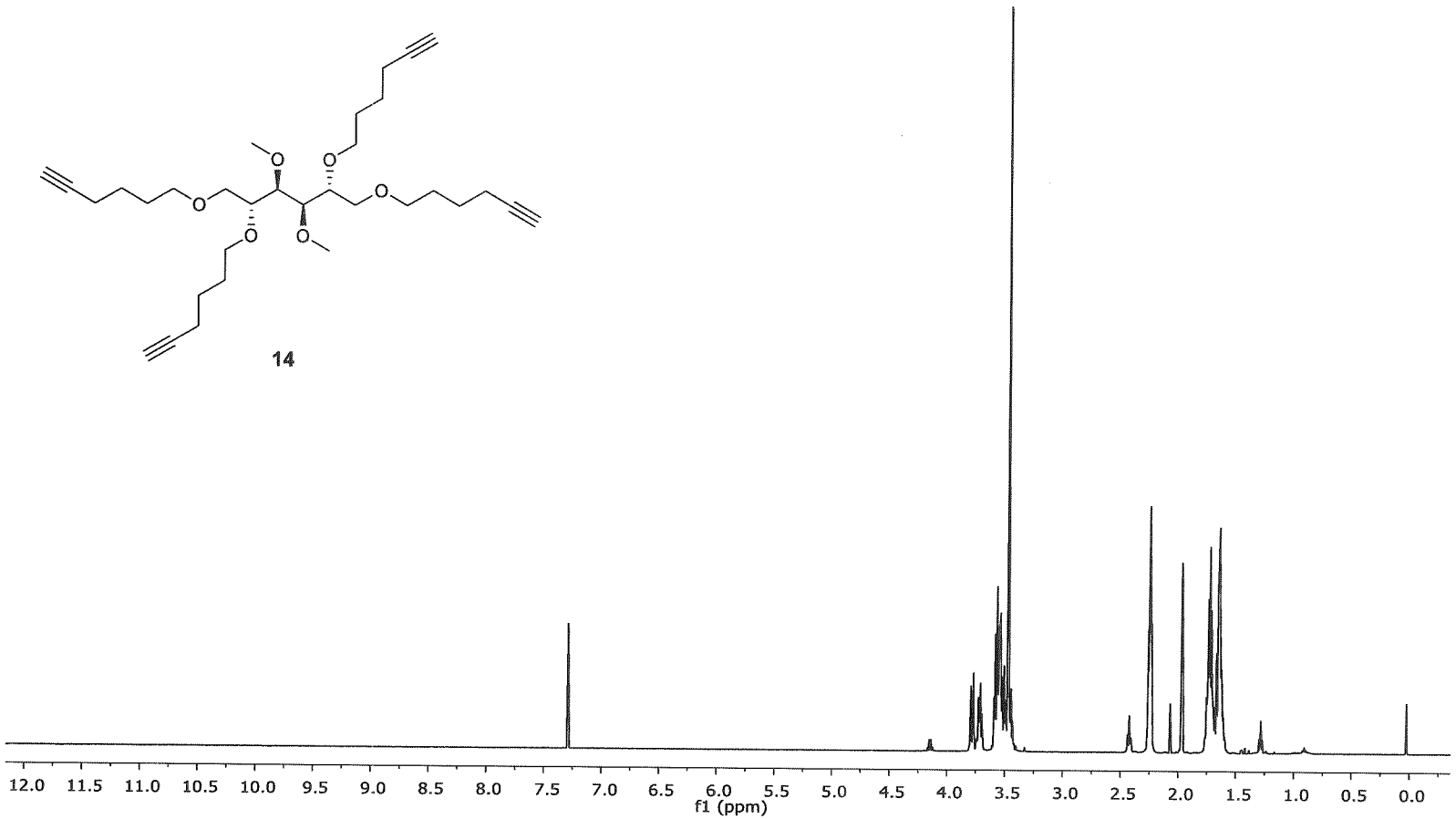


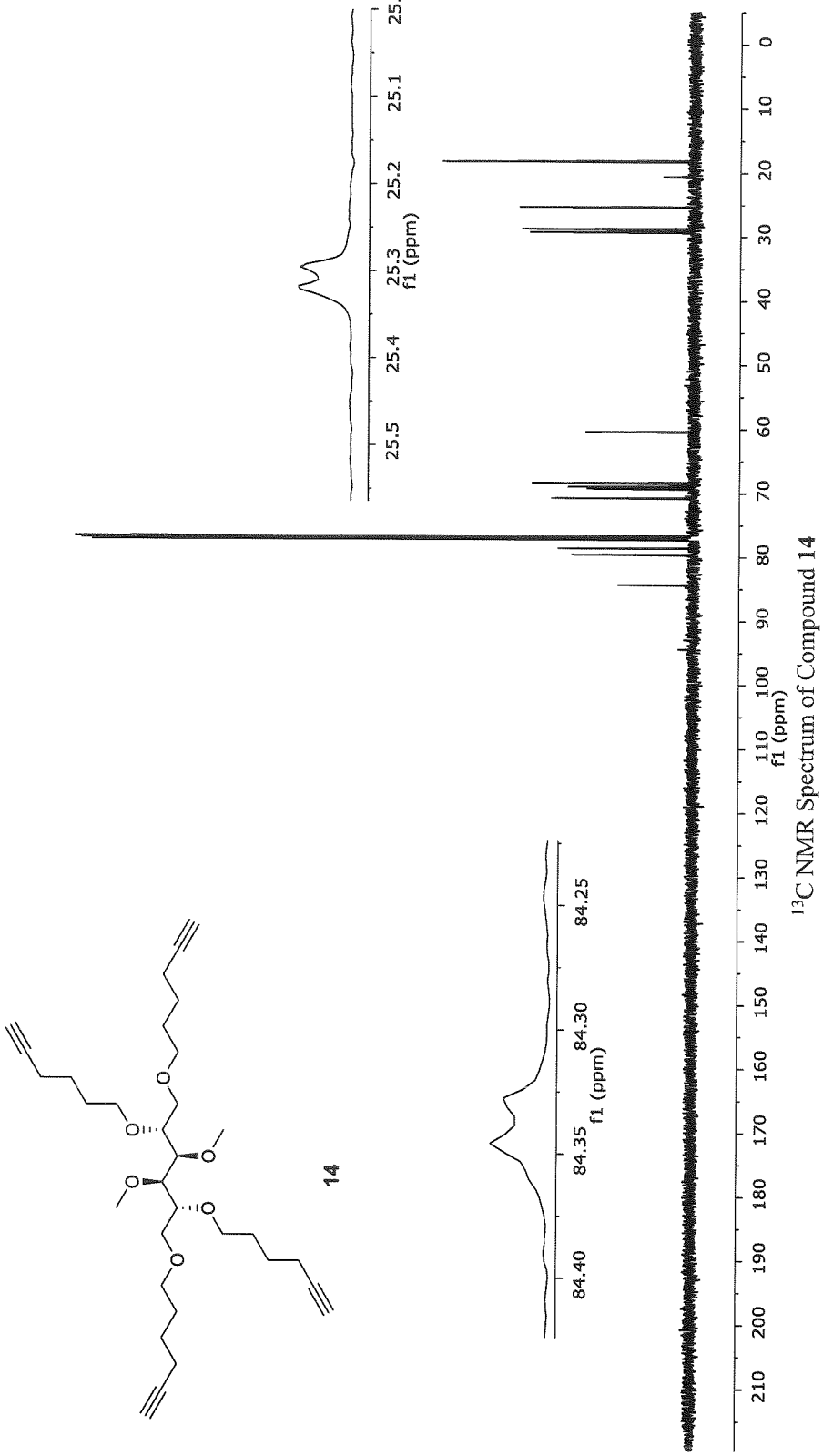
^{1}H NMR Spectrum of Compound **12**



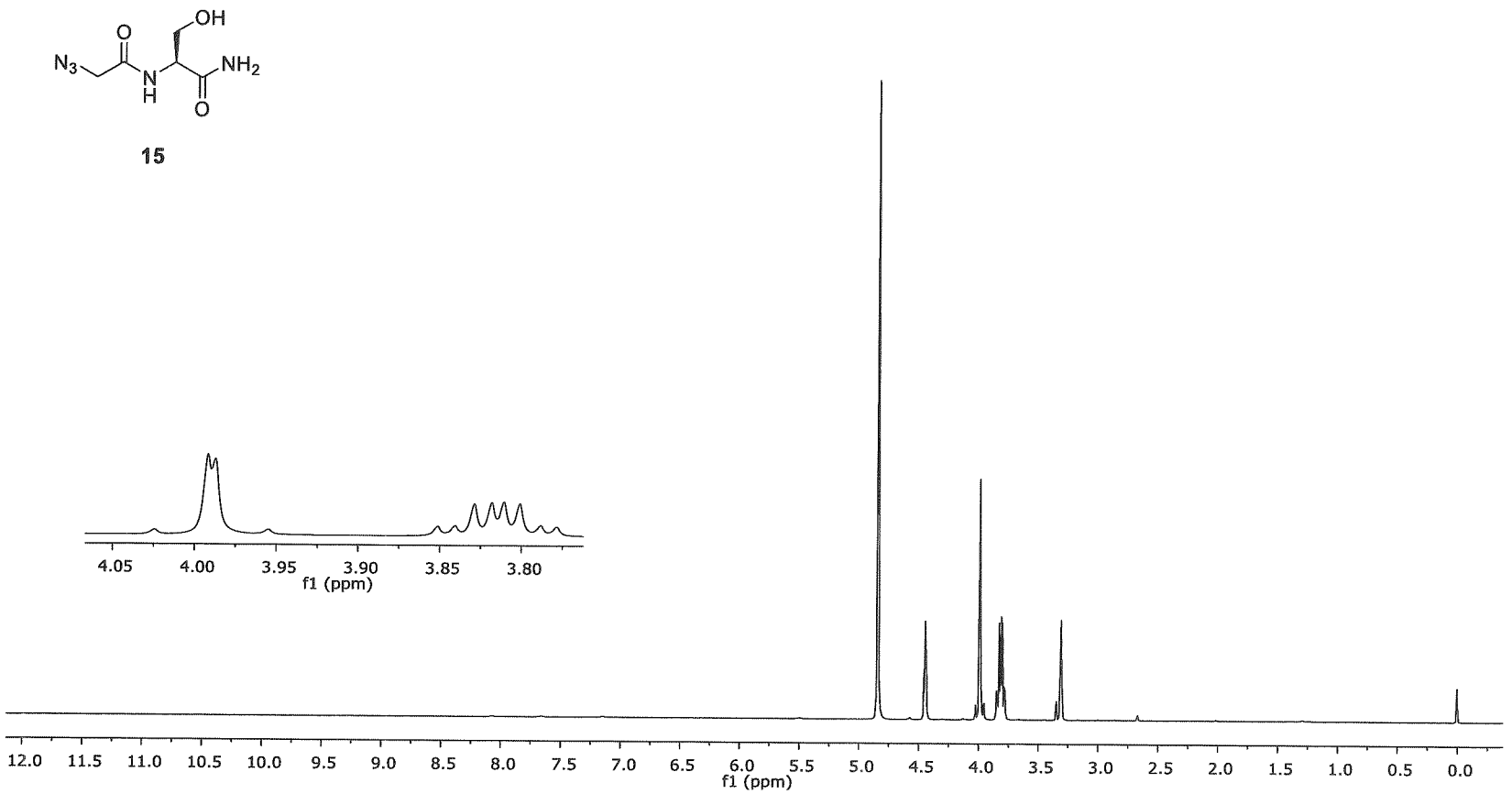
^{13}C NMR Spectrum of Compound **12**



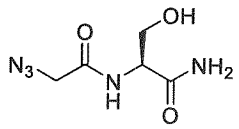
^{1}H NMR Spectrum of Compound **14**



^{13}C NMR Spectrum of Compound **14**



^{1}H NMR Spectrum of Compound **15**



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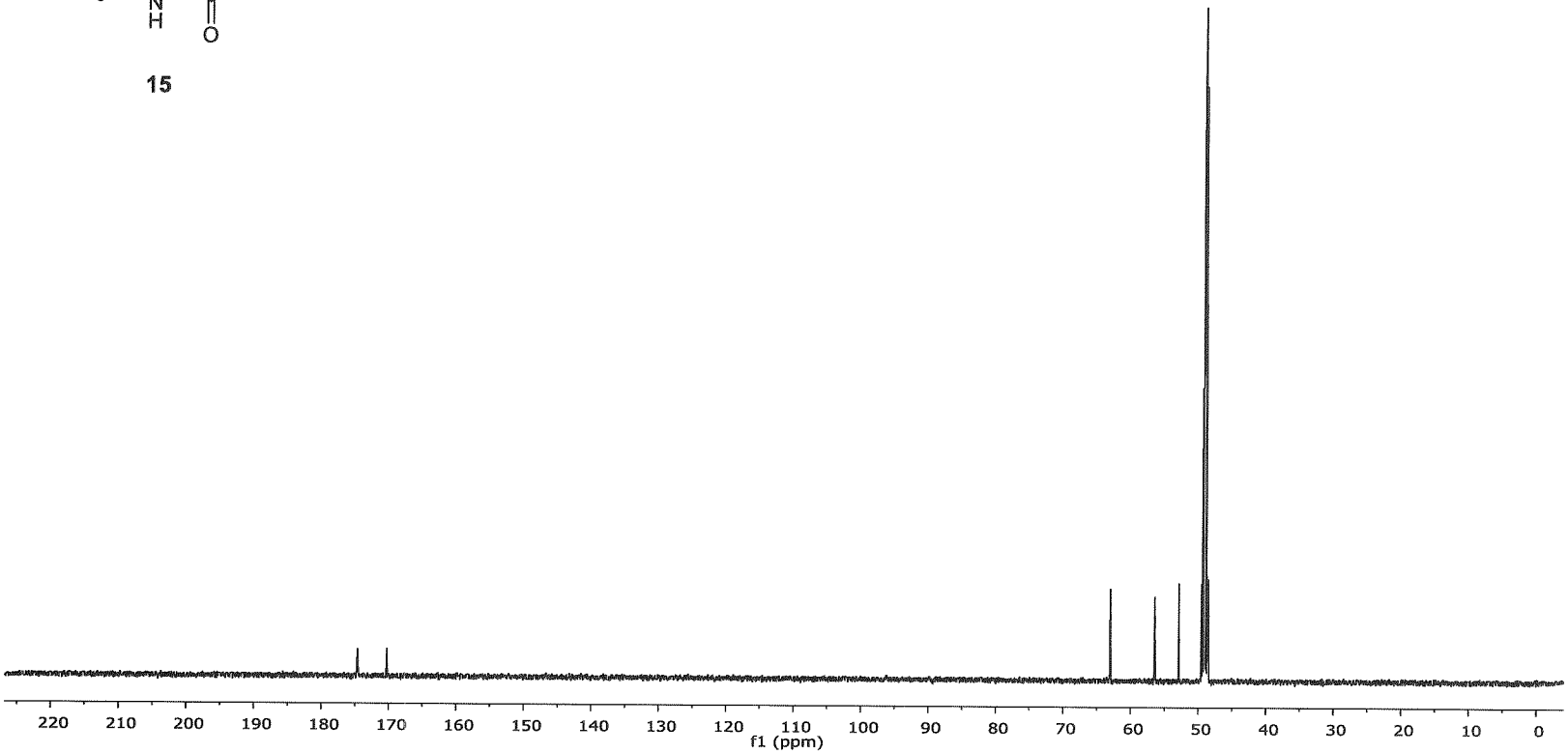
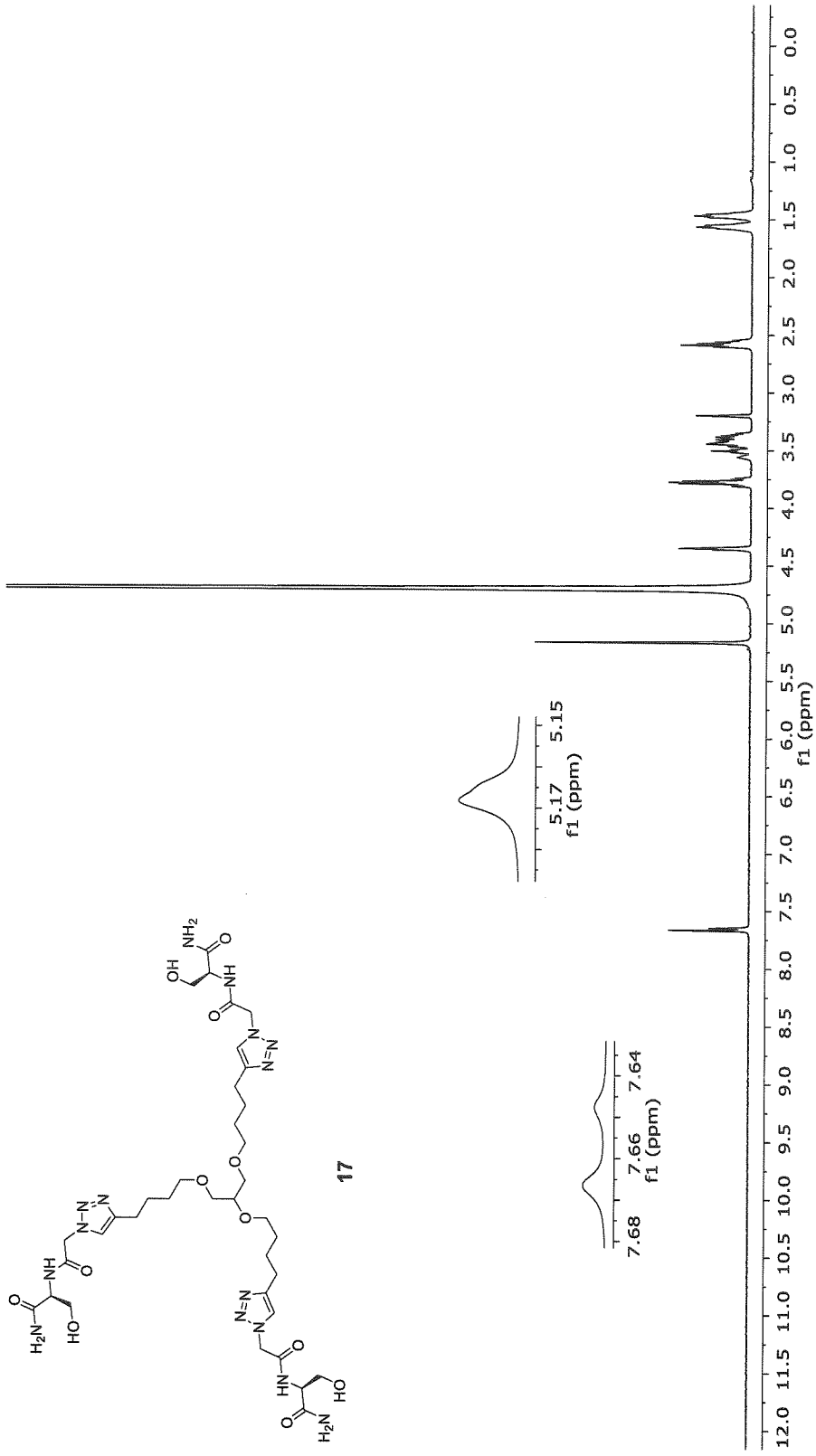
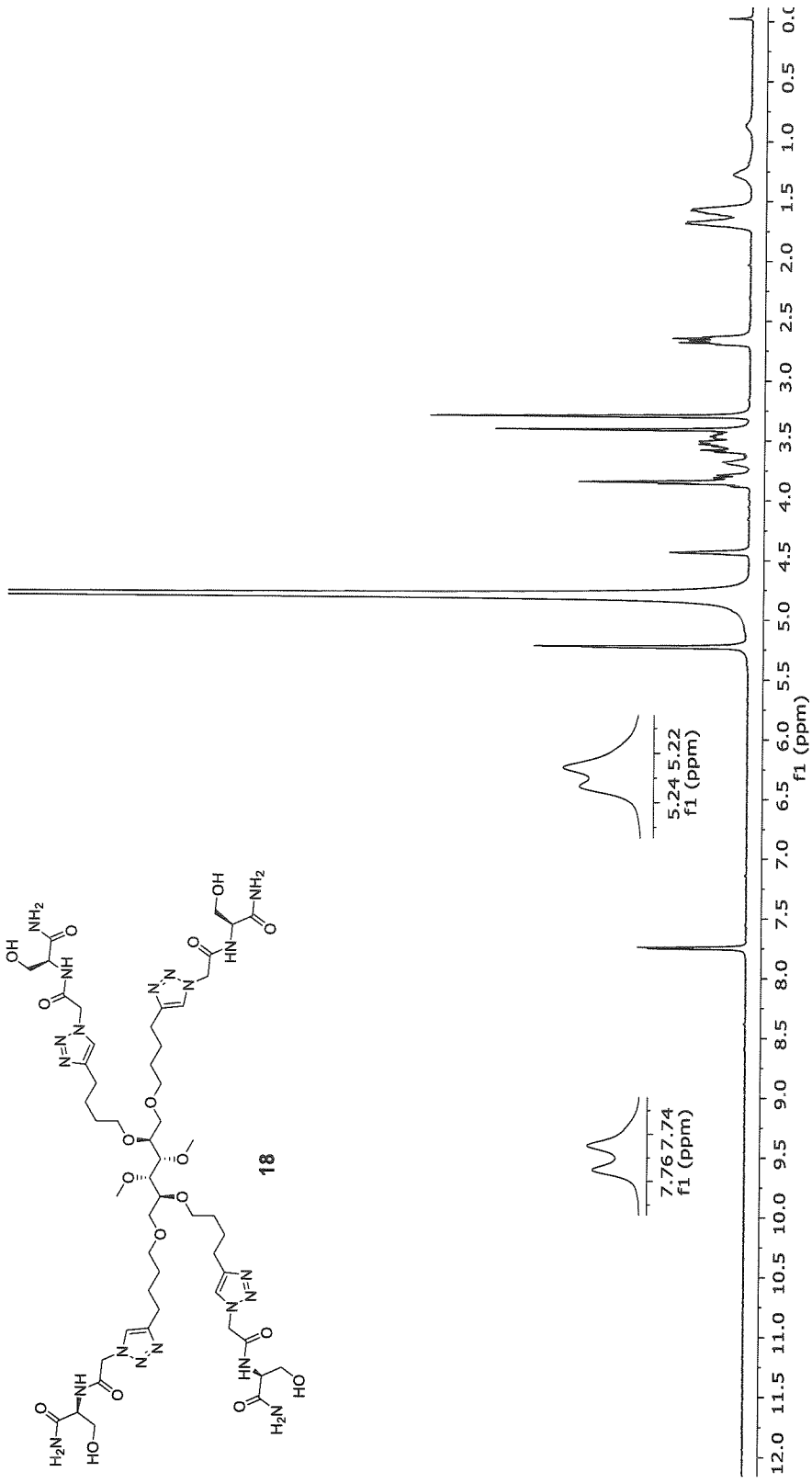


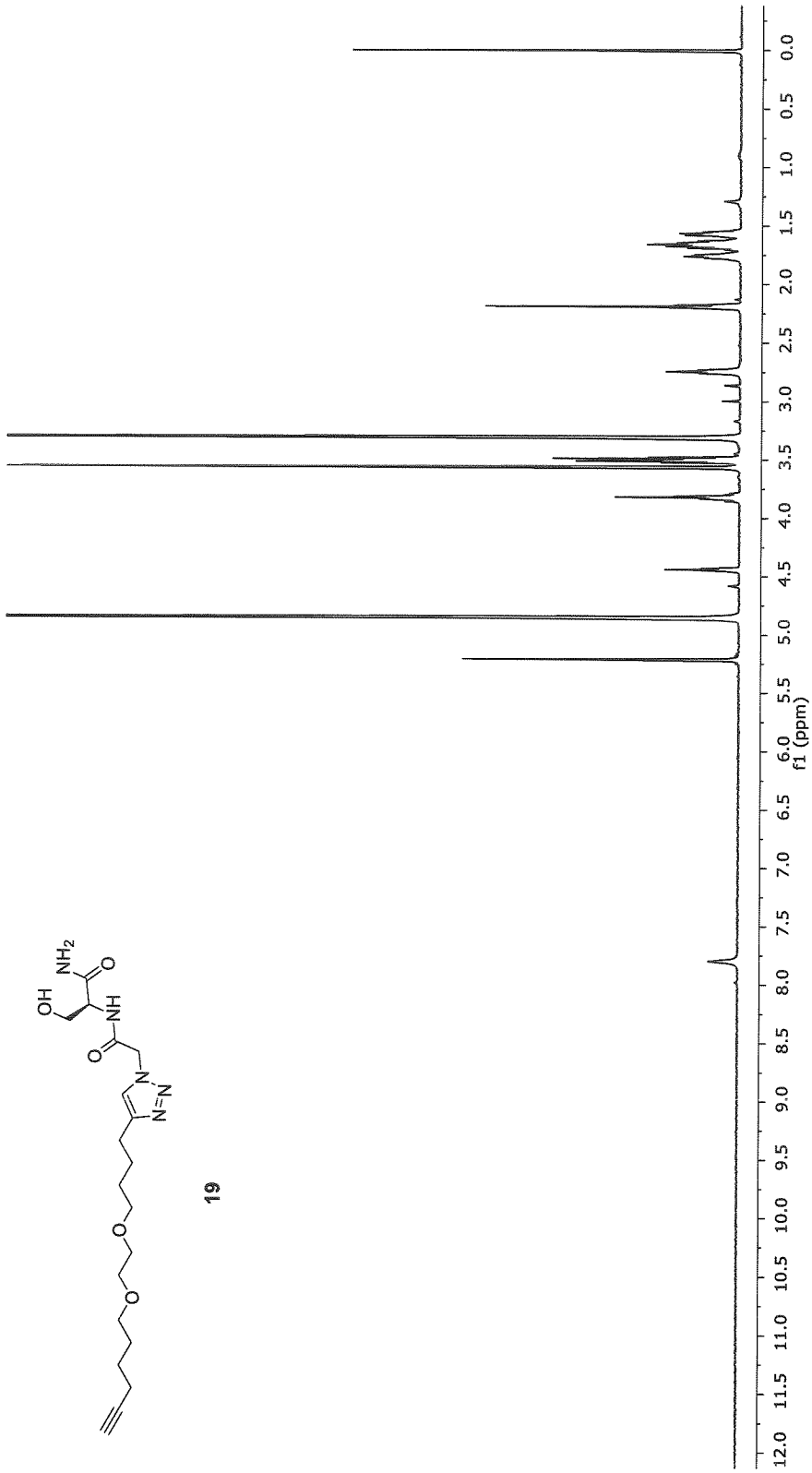
^{13}C NMR Spectrum of Compound **15**



^{1}H NMR Spectrum of Compound **17**

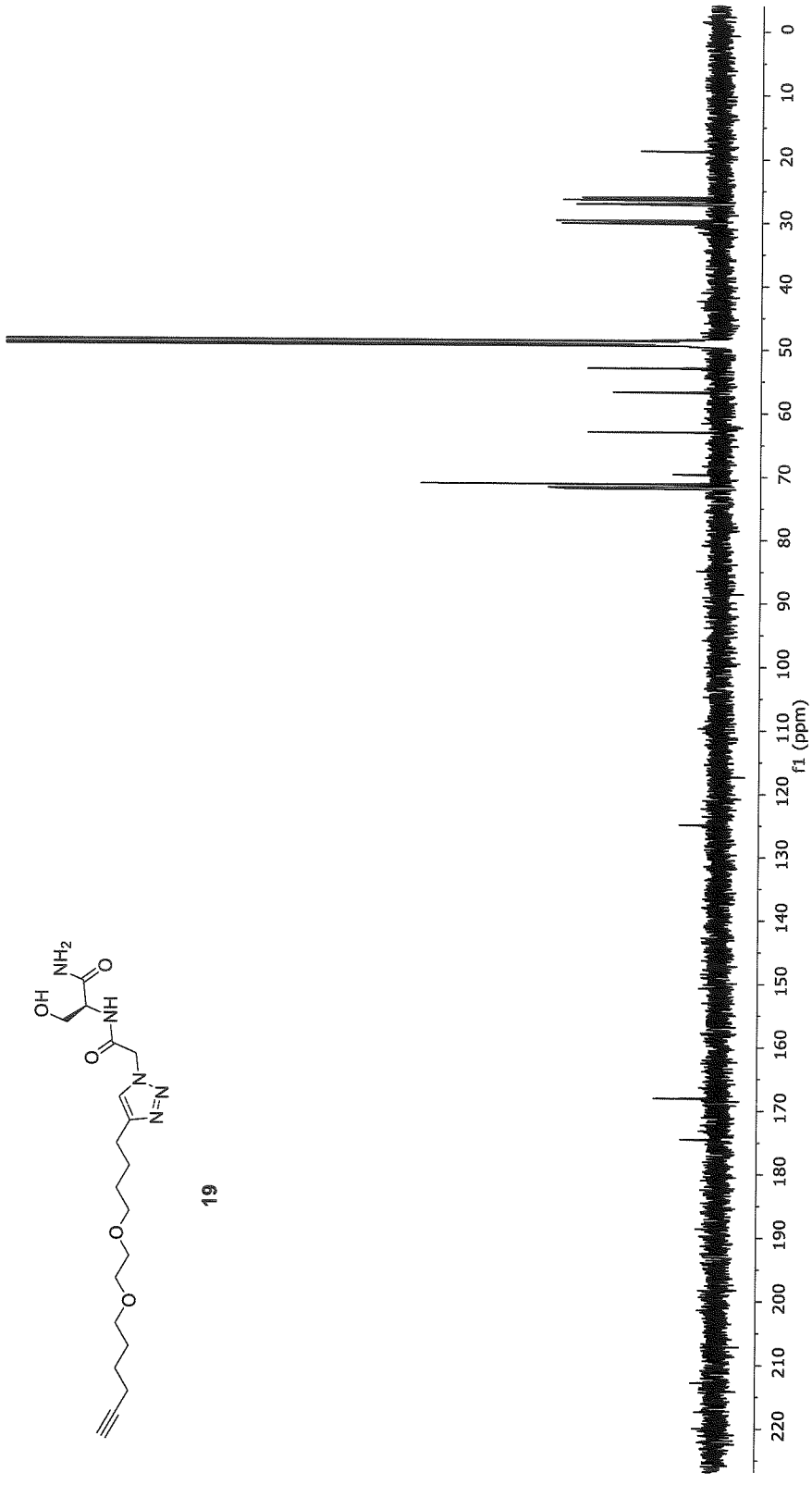


^{1}H NMR Spectrum of Compound **18**



19

^{1}H NMR Spectrum of Compound **19**



19

^{13}C NMR Spectrum of Compound **19**

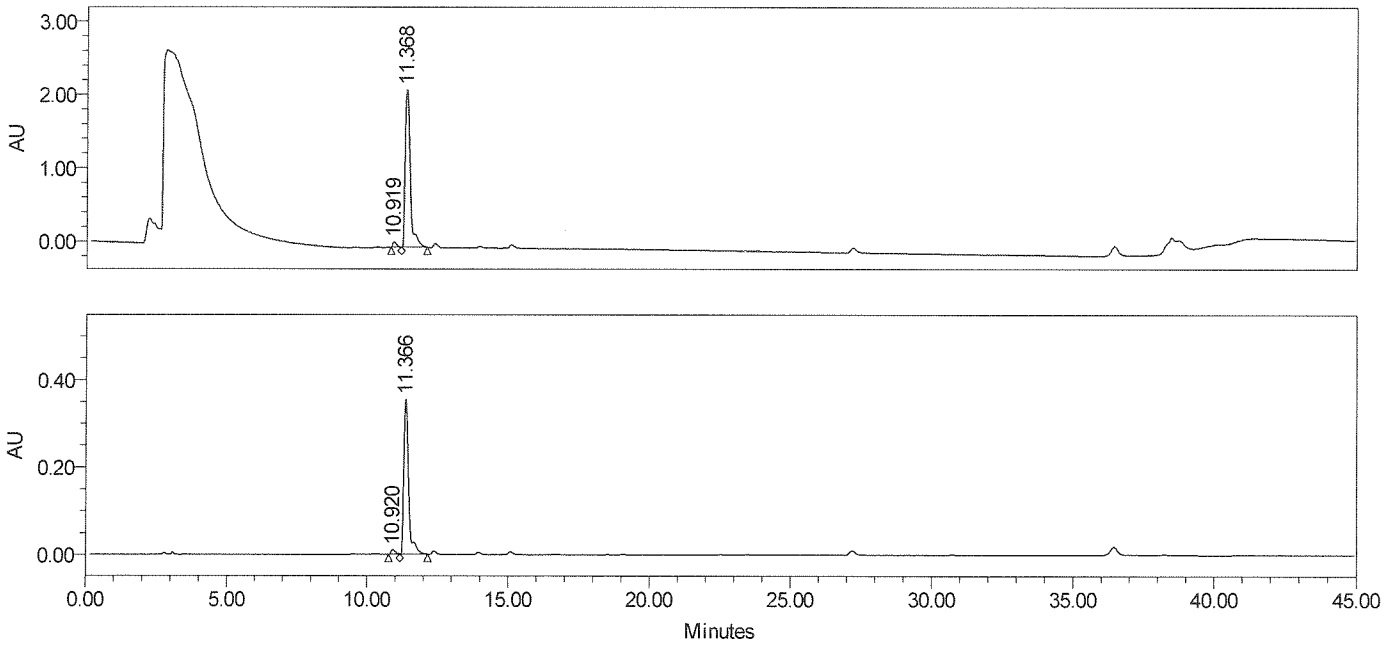
Dual Channel Summary

Reported by User: System

Project Name: BIO5_HPLC1

SAMPLE INFORMATION

Sample Name:	Elshan CH-1-226	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/5/2014 4:21:08 PM MDT
Vial:	80	Acq. Method Set:	10_90B_in
Injection #:	1	Date Processed:	6/11/2014 1:41:38 PM MDT,
Injection Volume:	10.00 ul	Processing Method	Peptide_general
Run Time:	45.0 Minutes	Channel Name:	2487Channel 1, 2487Channel 2
Sample Set Name	Prime_Run_07_19_07	Proc. Chnl. Descr.:	220nm, 280nm



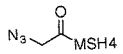
Channel 220 nm
Channel: 2487Channel 1

	RT	Area (µV*sec)	% Area	Channel
1	11.368	2.74e+007	97.69	2487Channel 1

Channel 280 nm
Channel: 2487Channel 2

	RT	Area (µV*sec)	% Area	Channel
1	11.366	4.22e+006	97.45	2487Channel 2

NOTES:



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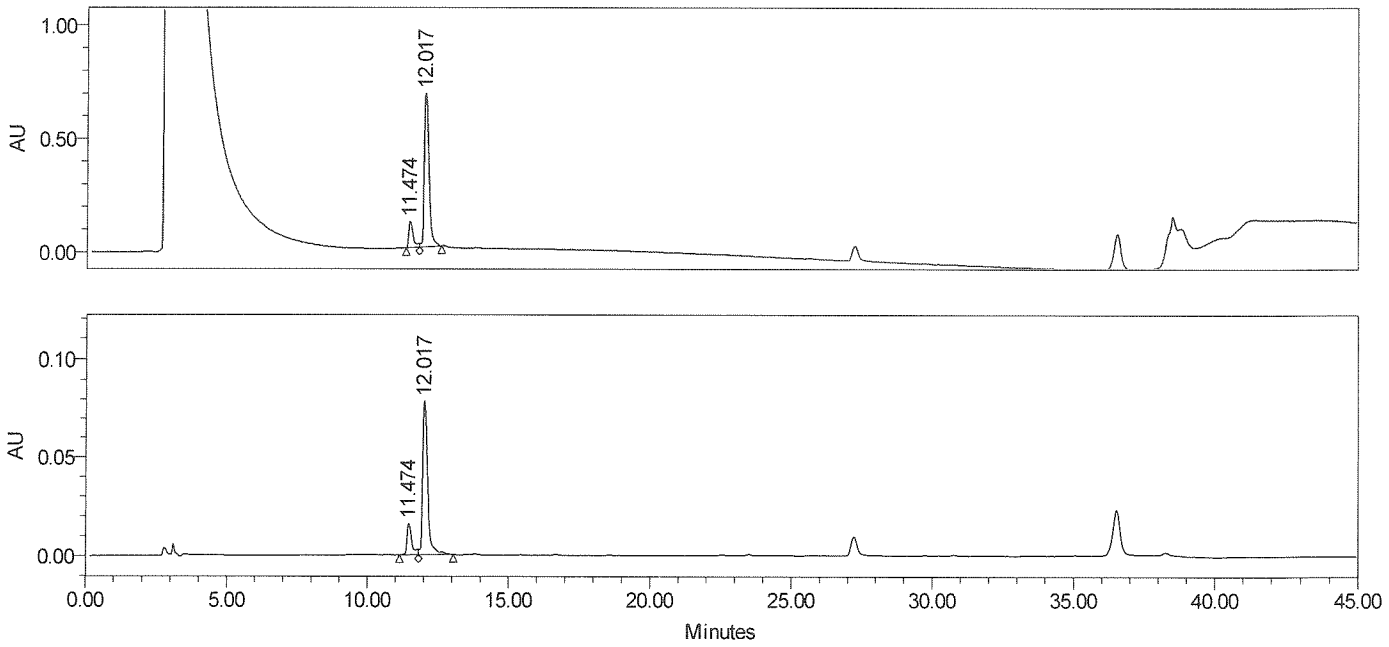
Dual Channel Summary

Reported by User: System

Project Name: BIO5_HPLC1

SAMPLE INFORMATION

Sample Name:	Elshan CH-1-54	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/5/2014 2:02:47 PM MDT
Vial:	77	Acq. Method Set:	10_90B_in
Injection #:	1	Date Processed:	6/11/2014 1:37:21 PM MDT,
Injection Volume:	10.00 ul	Processing Method	Peptide_general
Run Time:	45.0 Minutes	Channel Name:	2487Channel 1, 2487Channel 2
Sample Set Name	Prime_Run_07_19_07	Proc. Chnl. Descr.:	220nm, 280nm



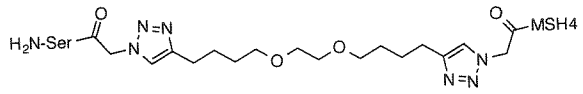
Channel 220 nm
Channel: 2487Channel 1

	RT	Area (µV*sec)	% Area	Channel
1	11.474	1.27e+006	13.77	2487Channel 1
2	12.017	7.94e+006	86.23	2487Channel 1

Channel 280 nm
Channel: 2487Channel 2

	RT	Area (µV*sec)	% Area	Channel
1	11.474	1.88e+005	16.22	2487Channel 2
2	12.017	9.73e+005	83.78	2487Channel 2

NOTES:



20

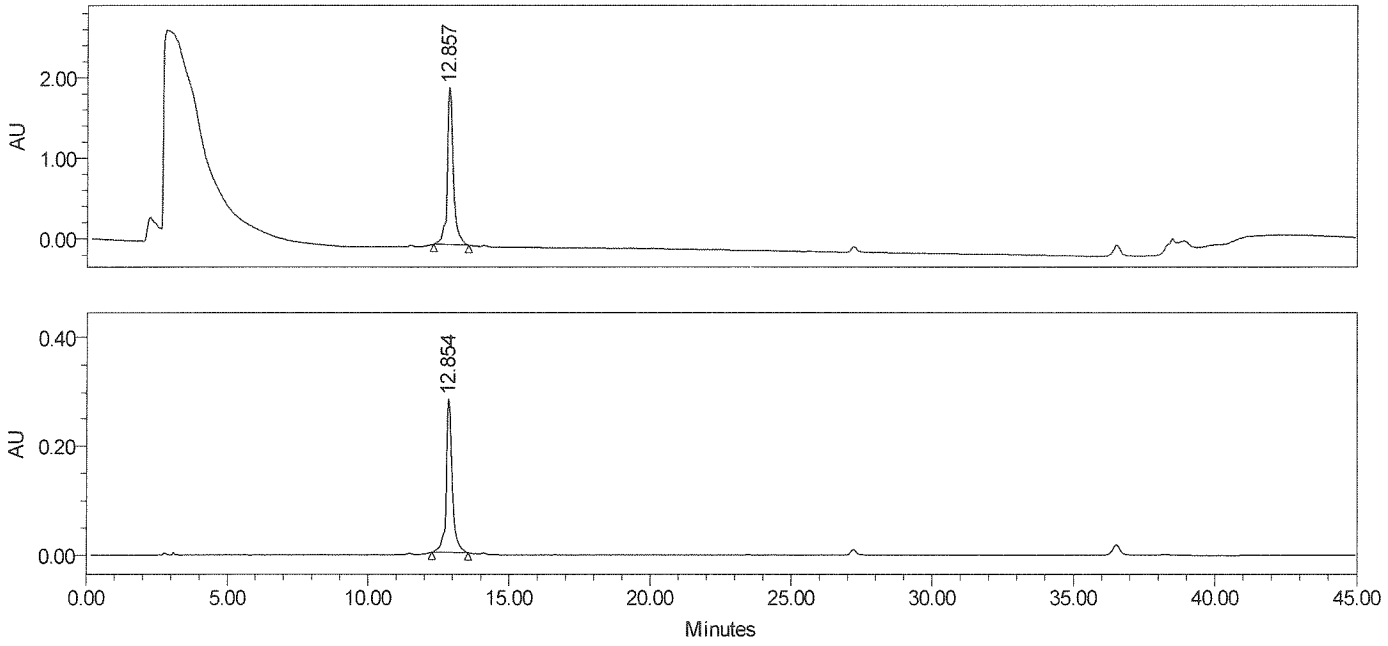
Dual Channel Summary

Reported by User: System

Project Name: BIO5_HPLC1

SAMPLE INFORMATION

Sample Name:	Elshan CH-1-267	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	6/5/2014 7:25:54 PM MDT
Vial:	84	Acq. Method Set:	10_90B_in
Injection #:	1	Date Processed:	6/11/2014 1:46:00 PM MDT,
Injection Volume:	10.00 ul	Processing Method	Peptide_general
Run Time:	45.0 Minutes	Channel Name:	2487Channel 1, 2487Channel 2
Sample Set Name	Prime_Run_07_19_07	Proc. Chnl. Descr.:	220nm, 280nm



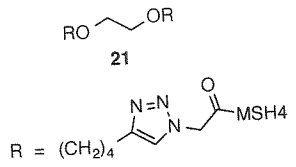
Channel 220 nm
Channel: 2487Channel 1

	RT	Area (µV*sec)	% Area	Channel
1	12.857	2.93e+007	100.00	2487Channel 1

Channel 280 nm
Channel: 2487Channel 2

	RT	Area (µV*sec)	% Area	Channel
1	12.854	4.17e+006	100.00	2487Channel 2

NOTES:



Dual Channel Summary

Reported by User: System

Project Name: BIO5_HPLC1

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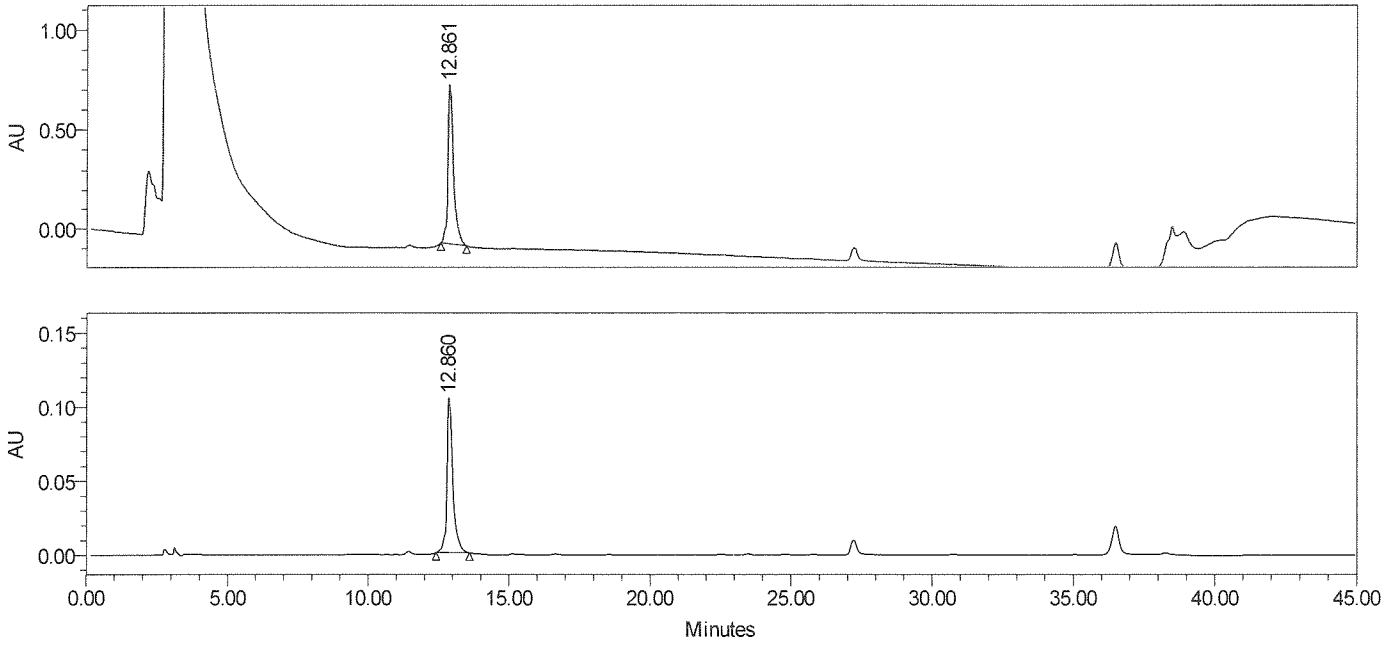
Sample Type:	Unknown	Date Acquired:	6/5/2014 5:53:42 PM MDT
Vial:	82	Acq. Method Set:	10_90B_in
Injection #:	1	Date Processed:	6/11/2014 1:43:55 PM MDT,
Injection Volume:	10.00 ul	Processing Method	Peptide_general
Run Time:	45.0 Minutes	Channel Name:	2487Channel 1, 2487Channel 2
Sample Set Name	Prime_Run_07_19_07	Proc. Chnl. Descr.:	220nm, 280nm



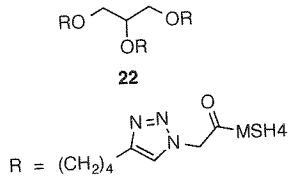
Channel 220 nm
Channel: 2487Channel 1

	RT	Area (µV*sec)	% Area	Channel
1	12.861	1.12e+007	100.00	2487Channel 1

Channel 280 nm
Channel: 2487Channel 2

	RT	Area (µV*sec)	% Area	Channel
1	12.860	1.53e+006	100.00	2487Channel 2

NOTES:



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Dual Channel Summary

Reported by User: System

Project Name: BIO5_HPLC1

SAMPLE INFORMATION

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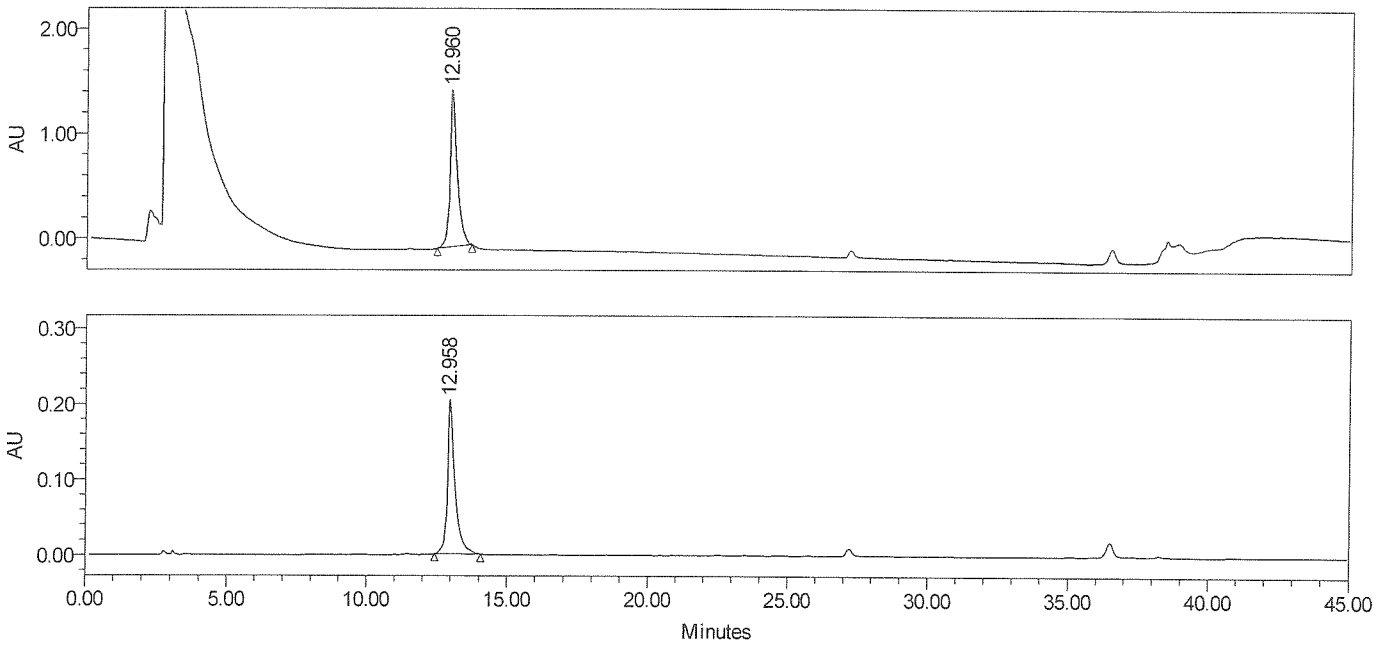
Sample Type:	Unknown	Date Acquired:	6/5/2014 6:39:47 PM MDT
Vial:	83	Acq. Method Set:	10_90B_in
Injection #:	1	Date Processed:	6/11/2014 1:44:56 PM MDT,
Injection Volume:	10.00 ul	Processing Method	Peptide_general
Run Time:	45.0 Minutes	Channel Name:	2487Channel 1, 2487Channel 2
Sample Set Name	Prime_Run_07_19_07	Proc. Chnl. Descr.:	220nm, 280nm



Channel 220 nm
Channel: 2487Channel 1

	RT	Area (µV*sec)	% Area	Channel
1	12.960	2.64e+007	100.00	2487Channel 1

Channel 280 nm
Channel: 2487Channel 2

	RT	Area (µV*sec)	% Area	Channel
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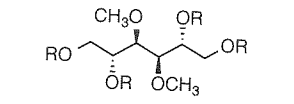
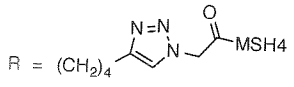
NOTES:



23



Data Analysis for the Binding Assays

Biological data analysis was performed using GraphPad Prism software (version 5.04) using the following analysis methods.

Saturation Binding Data

The Total Binding and Non-specific Binding curves in Figure 1 were generated from the binding assay data using nonlinear regression analysis and fitted to the "One site - Total and nonspecific binding" equations (Equations S1 and S2).

$$\text{Specific} = B_{max}*X / (X + K_d) \quad \text{(Equation S1)}$$

$$\text{Nonspecific} = NS*X + \text{Background} \quad \text{(Equation S2)}$$

For Total Binding: Y = specific + nonspecific

For Nonspecific binding: Y = nonspecific

Where

- B_{max} is the maximum specific binding in the same units as Y.
- K_d is the equilibrium binding constant, in the same units as X. It is the labeled ligand concentration needed to achieve a half-maximum binding at equilibrium.
- NS is the slope of nonspecific binding in Y units divided by X units.
- Background is the amount of nonspecific binding with no added labeled ligand. This represents counter background. If the counter automatically subtracts off the background signal, Background can be constrained to a constant value of zero.

The Specific Binding curve in Figure 1 was generated from data derived by taking the difference between the Total and Nonspecific binding data, using nonlinear regression analysis, and fitting to the "One site - Specific binding" equation (Equation S3).

$$\text{Specific} = B_{max}*X / (X + K_d) \quad \text{(Equation S3)}$$

Competitive Binding Data

Competitive binding data were analyzed using nonlinear regression analysis and fitted to the "One site - Fit K_i" equation.

$$\log EC_{50} = \log (10\text{^}\log K_i*(1 + [L]/[\text{Hot } K_d])) \quad \text{(Equation S4)}$$

$$Y = \text{Bottom} + (\text{Top-Bottom}) / (1+10\text{^}(X - \text{Log } EC_{50})) \quad \text{(Equation S5)}$$

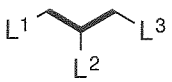
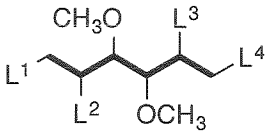
Where

- Top and Bottom are plateaus in the units of the Y axis.
- K_i is the molar equilibrium dissociation constant of the unlabeled ligand.
- [L] is the concentration of labeled ligand in nM. Here [L] = 20 nM
- [Hot K_d] is the equilibrium dissociation constant of the labeled ligand in nM. Here [Hot K_d] = 21 nM.

Molecular Dynamics Studies

Molecular dynamics (MD) calculation trials were performed in triplicate on compounds **21-23** using the AMBER99 force field and the MD program contained in MOE® to obtain representative conformations at 310K. The initial structure of each molecule was minimized, then solvated in a water sphere (compound **21**- 4487 H_2O, compound **22**- 5490 H_2O, compound **23**- 5308 H_2O), and again minimized. The results (depicted) were used as the starting structures for each calculation trial. One carbon of the core scaffold was fixed within the solvation sphere (**21**, ethylene glycol C1; **22**, glycerol C2; **23**, mannitol C3) to avoid migration of the molecule toward the edge of the water sphere during MD. Initial calculations performed using compound **22** showed convergence of the structure within 1 ns of a 5 ns production run, therefore subsequent production calculation durations were set at 1 ns. MD consisted of heating the molecule from 300 to 310 K over 250 ps, equilibration for 250 ps, and production for 1 ns. Trajectories were recorded every 0.5 ps.

The lowest energy structure of the production run for each trial was compared to the starting structure by graphical overlayment (see Figure 4 in the article). Inter-ligand distance measurements (from the N-terminal nitrogen atoms of the histidine residues) are shown for the starting structures. The graphs represent the inter-ligand distances for each individual trial with the exception of the graph for compound **21**, which contains all three trials on one graph.

scaffold origin	ligand labeling
ethylene glycol	
glycerol	
D-mannitol	

Average Inter-Ligand Distances[a]

Compound (inter-ligand distance measured)	Average Inter-Ligand Distance (Å)
21 (L_1-L_2)	21.06±0.62
22 (L_1-L_2)	22.99±0.28
23 (L_2-L_4)	27.84±0.64
[a]The values reported for compounds **22** and **23** are the averages for the two ligands giving the greatest inter-ligand distances for that compound.	

Compound **21**

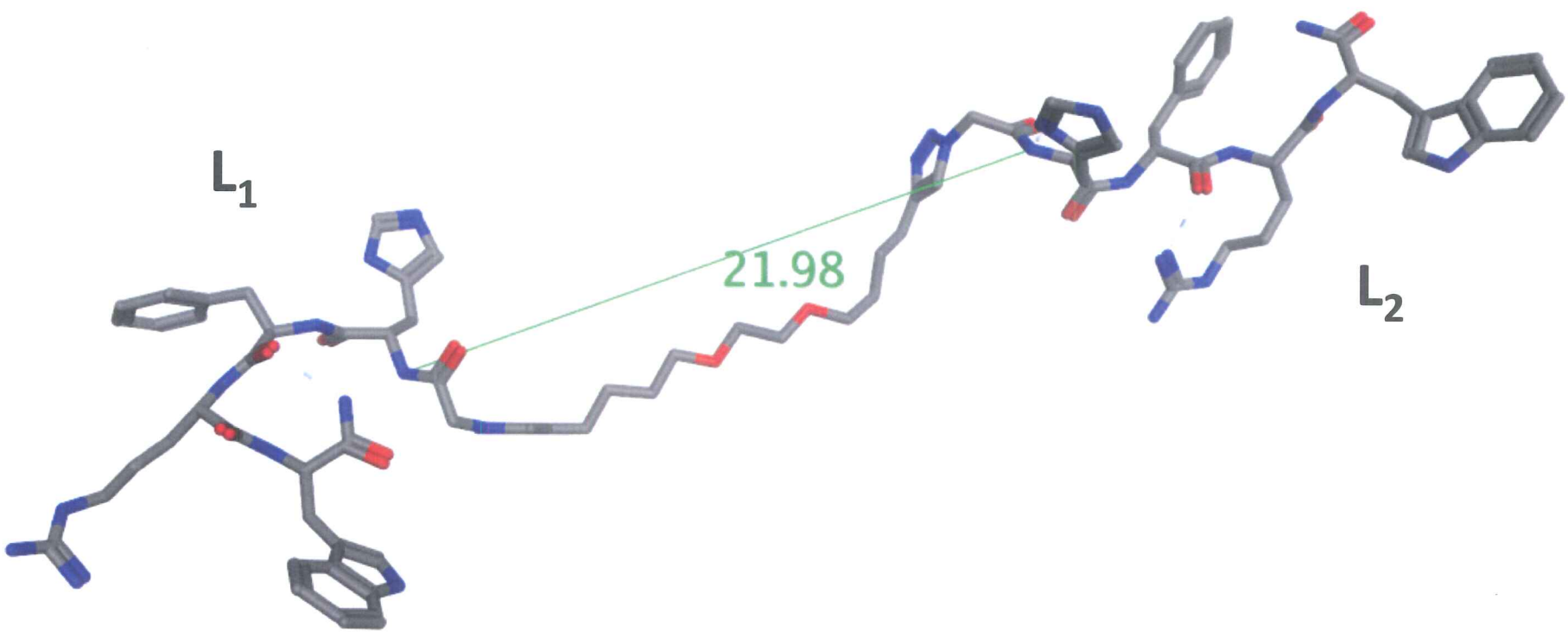
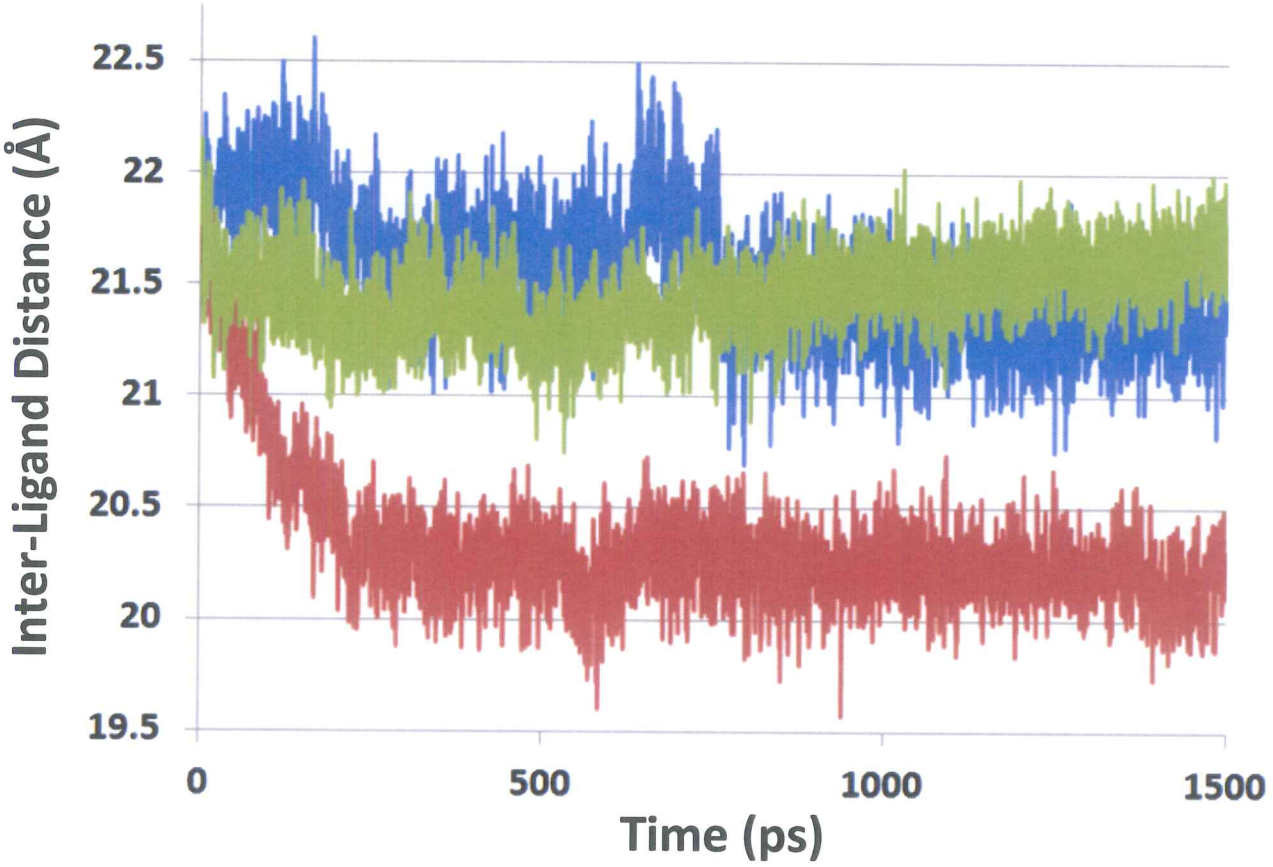


Figure S1: Starting minimized structure with inter-ligand distance measurement.

Compound **21**



Compound 21	**Trial 1**	**Trial 2**	**Trial 3**
L_1-L_2 Distance (Å)	21.45±0.26	20.23±0.16	21.49±0.18

Figure S2: Average Inter-Ligand Distance for Each Compound **21** Trial

Compound **21**

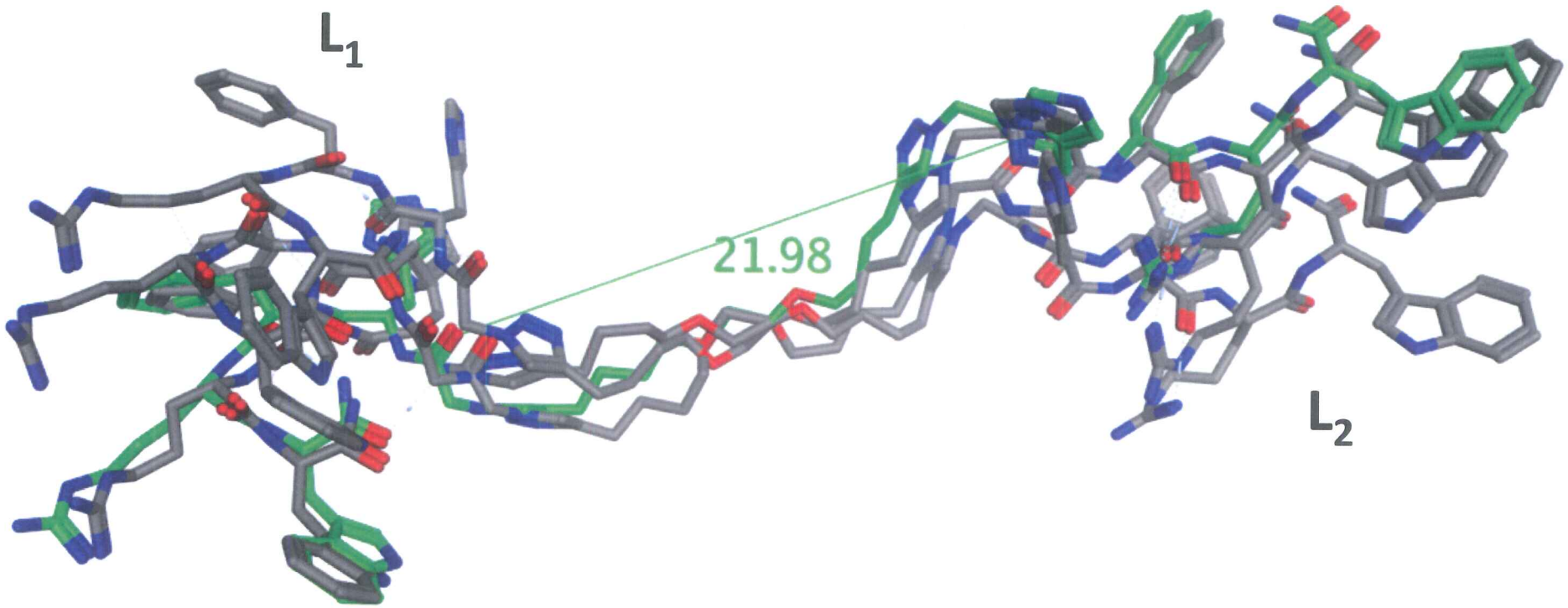


Figure S3: Lowest energy structure from each trial with starting minimized structure in green.

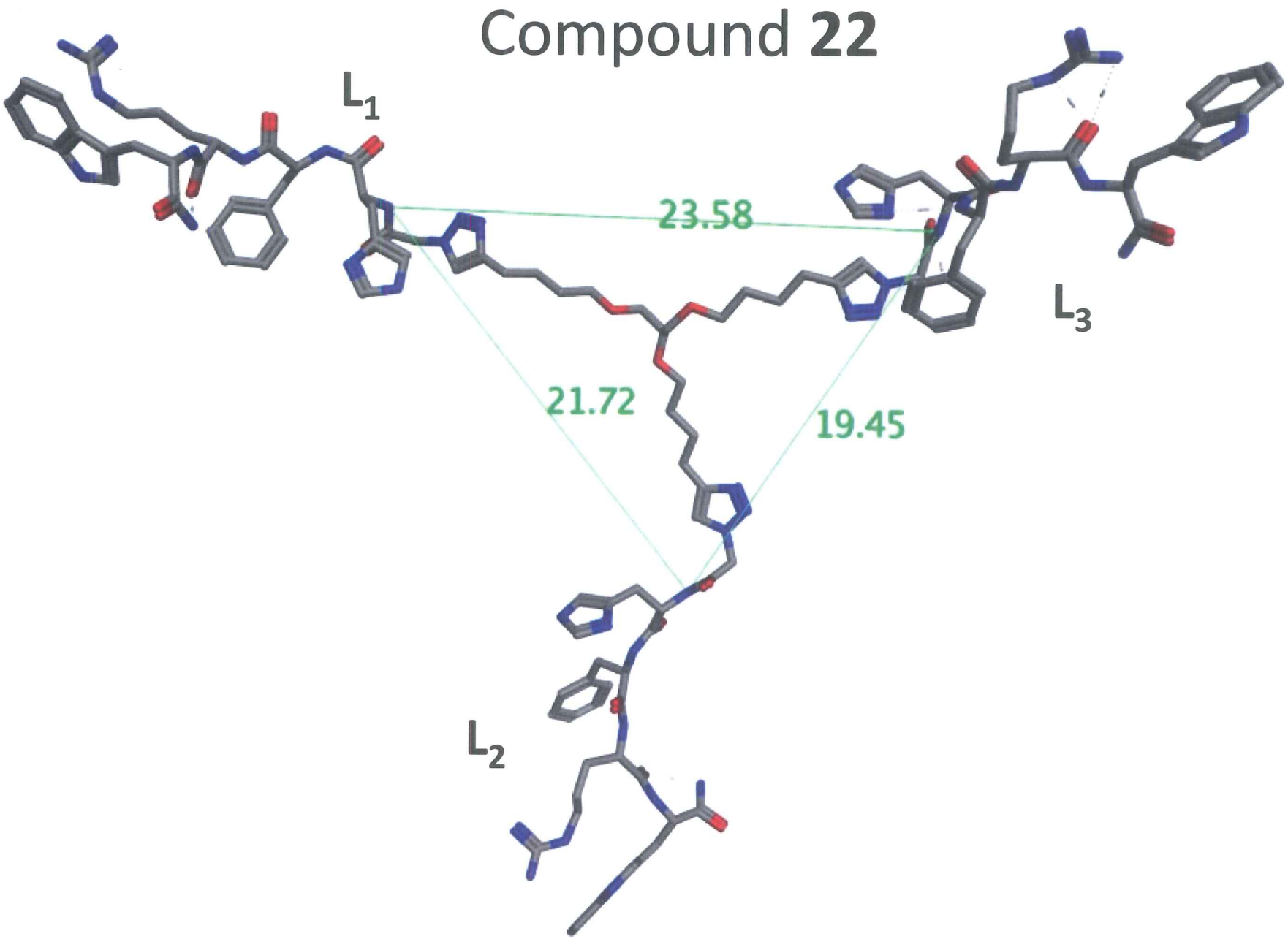
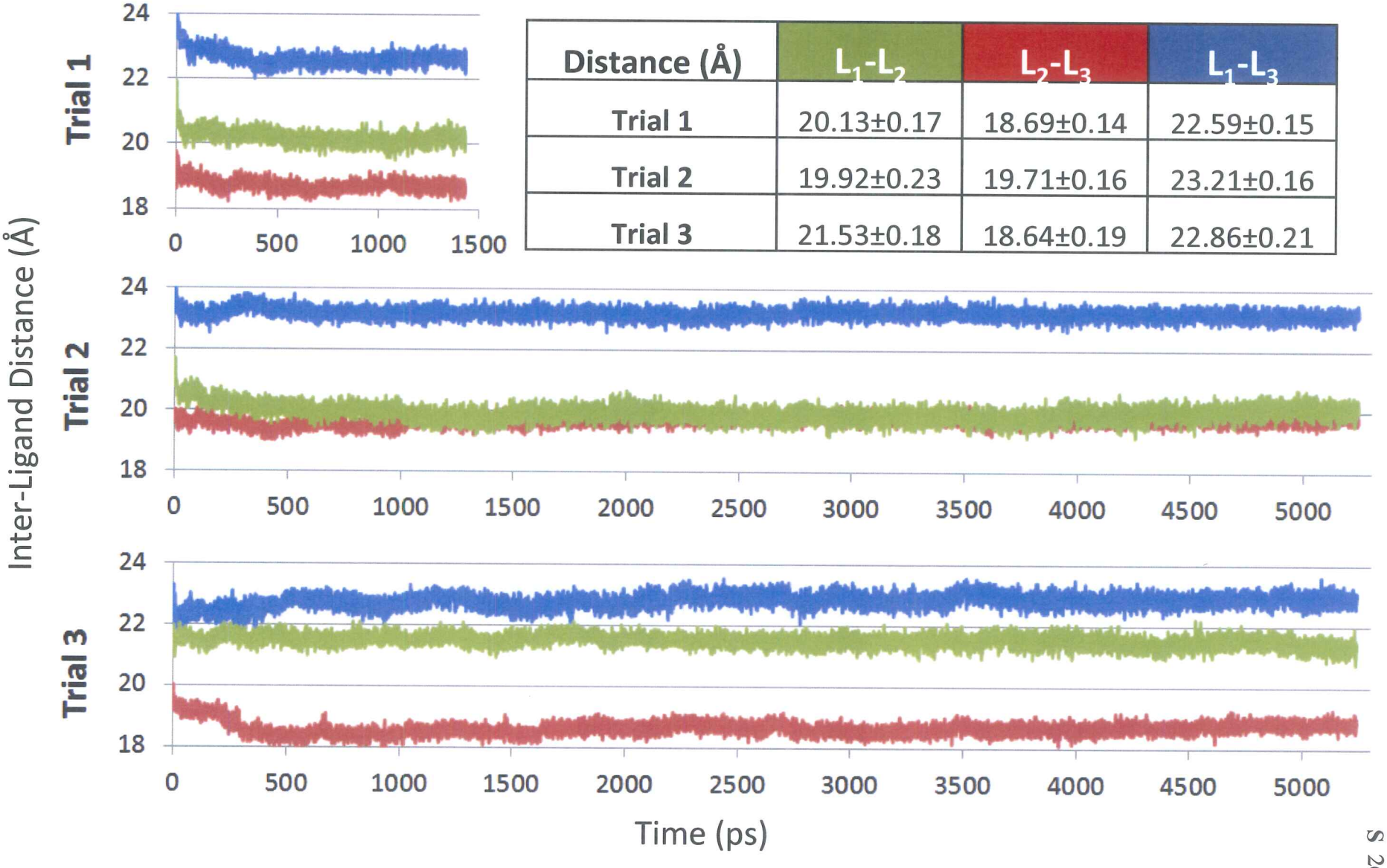


Figure S4: Starting minimized structure with inter-ligand distance measurements.

Compound **22**



Distance (Å)	L_1-L_2	L_2-L_3	L_1-L_3
Trial 1	20.13±0.17	18.69±0.14	22.59±0.15
Trial 2	19.92±0.23	19.71±0.16	23.21±0.16
Trial 3	21.53±0.18	18.64±0.19	22.86±0.21

Figure S5: Average Inter-Ligand Distances for Each Compound **22** Trial

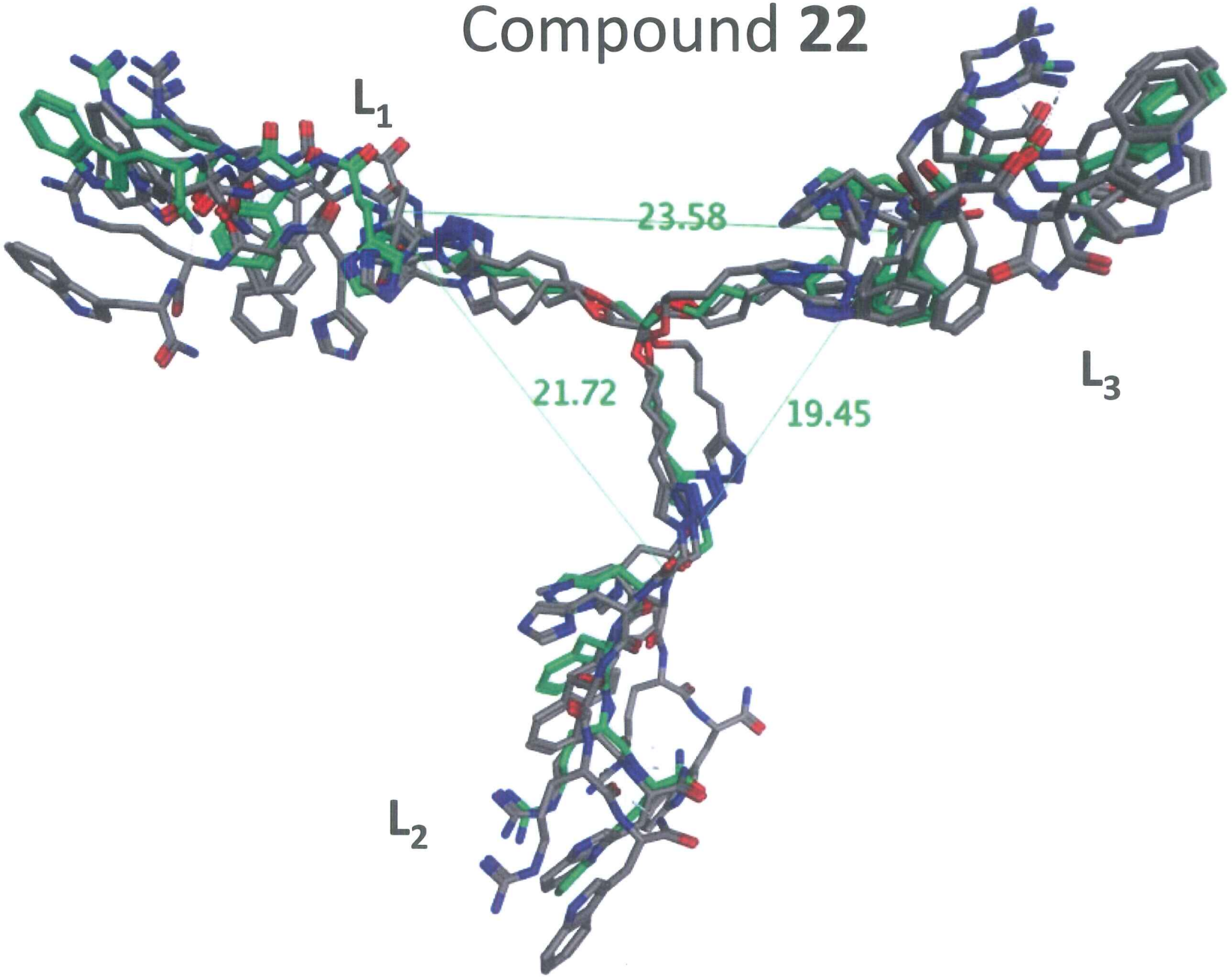


Figure S6: Lowest energy structure from each trial with starting minimized structure in green.

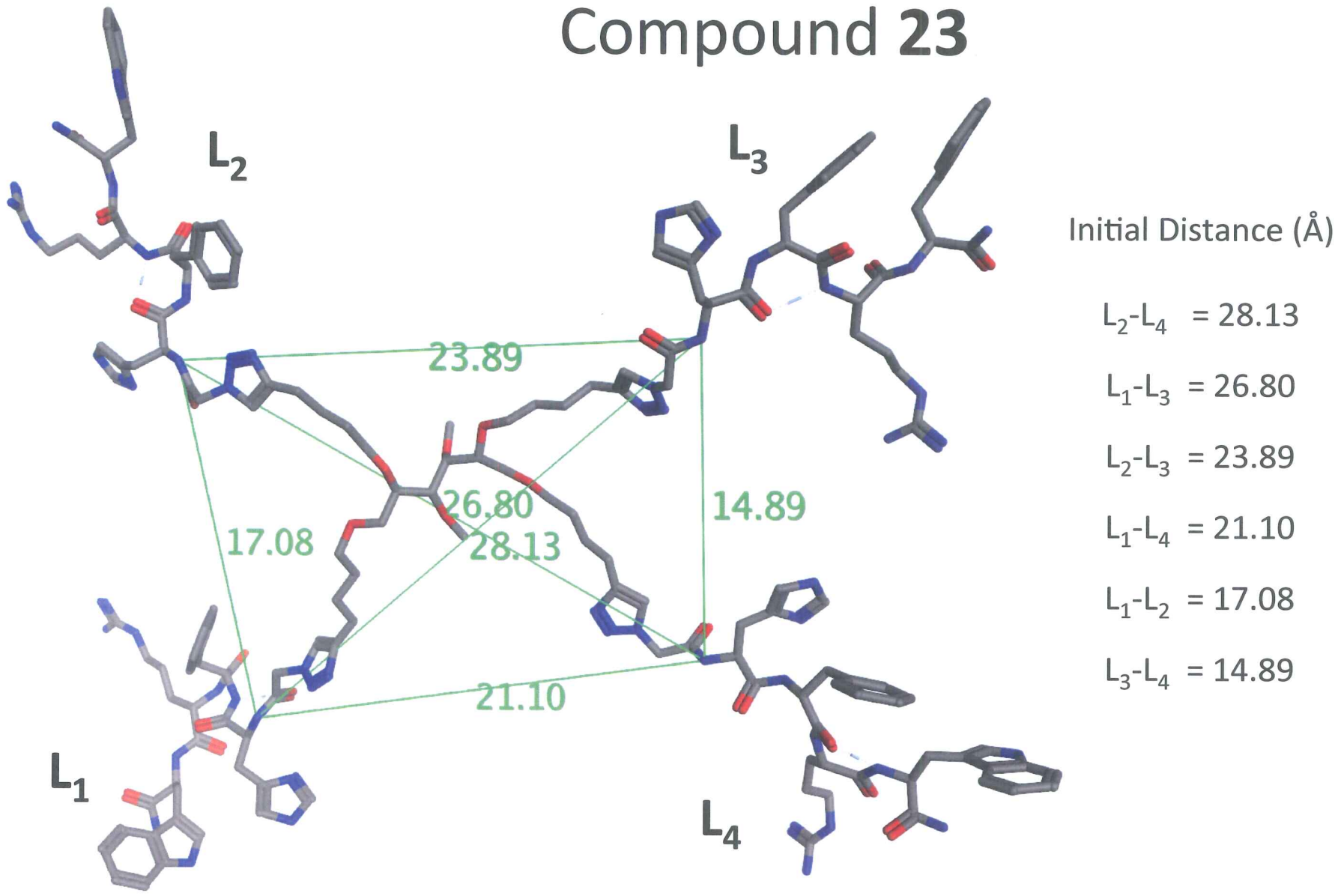
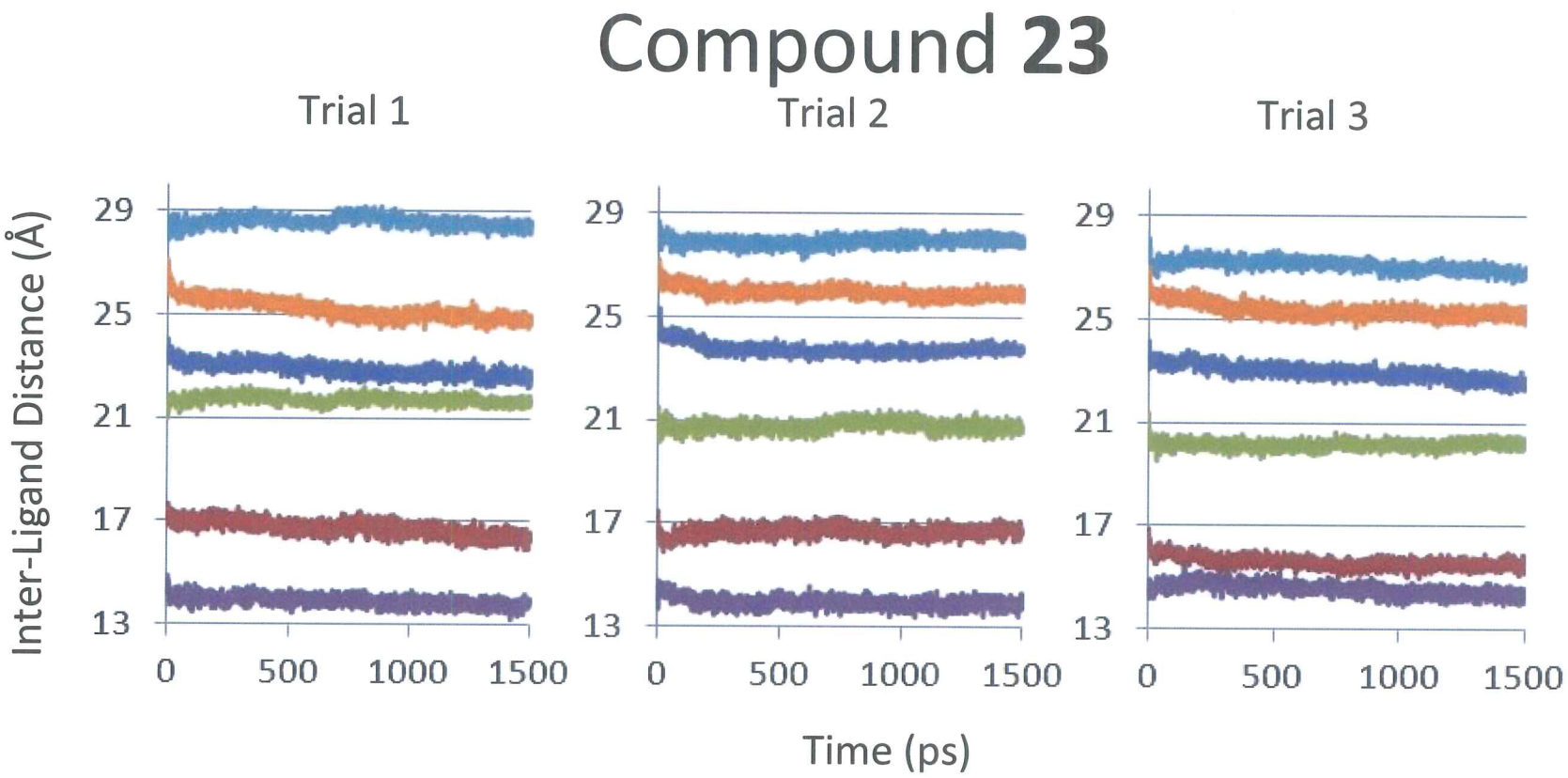


Figure S7: Starting minimized structure with inter-ligand distance measurements.



Distance (Å)	L_2-L_4	L_1-L_3	L_2-L_3	L_1-L_4	L_1-L_2	L_3-L_4
Trial 1	28.57±0.18	25.02±0.20	22.77±0.18	21.72±0.14	16.64±0.23	13.87±0.16
Trial 2	27.91±0.16	25.93±0.15	23.67±0.13	20.84±0.19	16.72±0.18	13.96±0.16
Trial 3	27.06±0.18	25.27±0.15	22.86±0.21	20.17±0.14	15.55±0.15	14.49±0.18

Figure S8: Average Inter-Ligand Distances for Each Compound **23** Trial

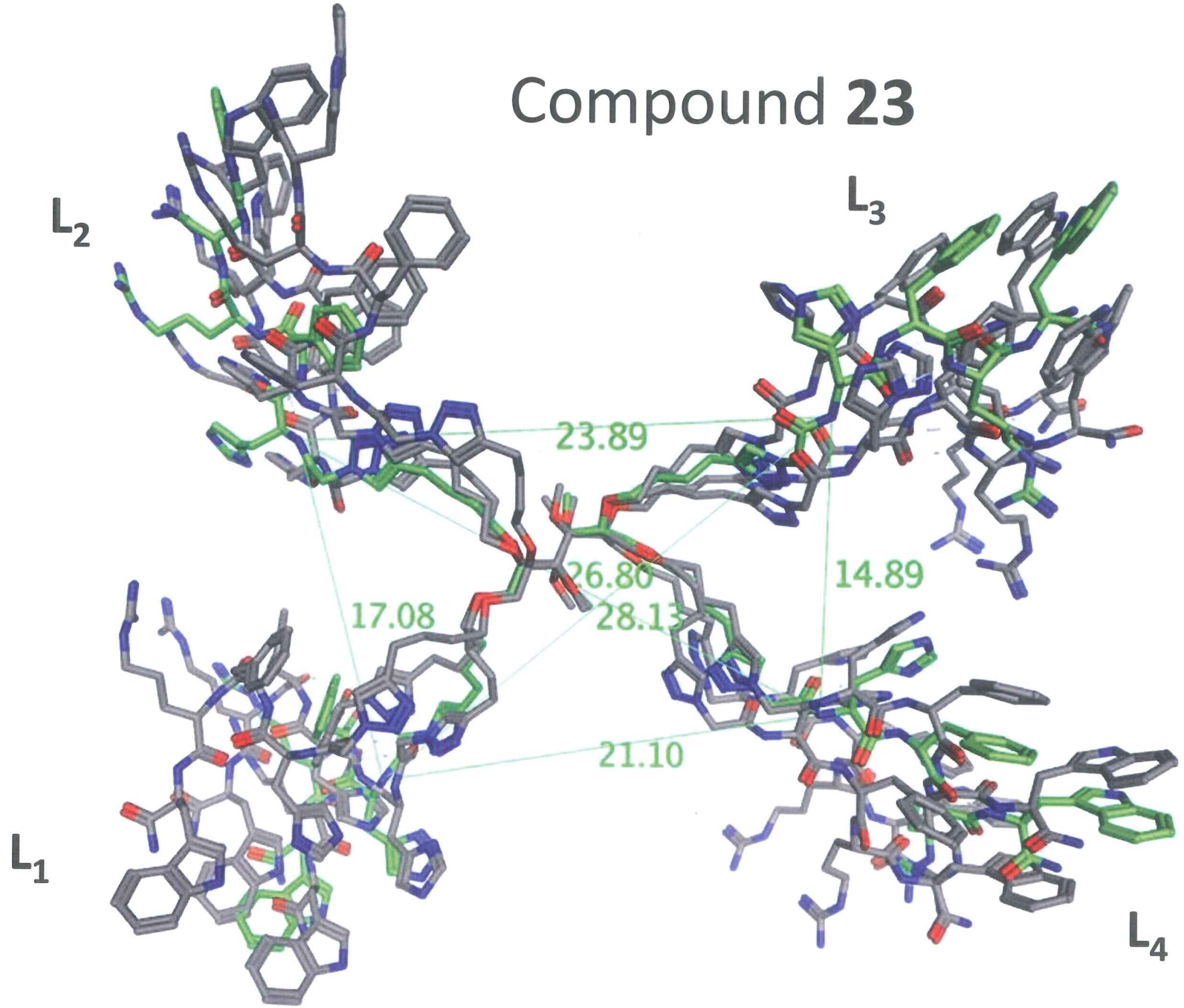


Figure S9: Lowest energy structure from each trial with starting minimized structure in green.