

Supporting Information

Synthetic antibody protein mimics of infliximab by molecular scaffolding on novel CycloTriVeratrilene (CTV) derivatives

Ondřej Longin, Mohammed Hezwani, Helmus van de Langemheen, Rob M.J. Liskamp*

School of Chemistry, Joseph Black Building, University of Glasgow, University Avenue, Glasgow G12 8QQ (UK)

E-Mail: Robert.liskamp@glasgow.ac.uk

Table of Contents

1	Product characterisation.....	S3
1.1	Semi-orthogonally protected scaffold 2.....	S3
1.2	Semi-orthogonally protected scaffold 3.....	S4
1.3	Semi-orthogonally protected scaffold 4.....	S5
1.4	Propargyl-MEG-OH 7a.....	S6
1.5	<i>O</i> -THP-Propargyl-MEG 8a.....	S6
1.6	<i>O</i> -THP-(TES)Propargyl-MEG 9a.....	S7
1.7	<i>O</i> -THP-(TIPS)Propargyl-MEG 10a.....	S8
1.8	(TES)Propargyl-MEG bromide 11a.....	S9
1.9	(TIPS)Propargyl-MEG bromide 12a.....	S10
1.10	Propargyl-MEG iodide 13a.....	S11
1.11	Di(<i>O</i> -THP)- <i>O</i> -MEG-propargyl CTV 15a.....	S11
1.12	<i>O</i> -MEG-Propargyl CTV-diOH 16a.....	S12
1.13	<i>O</i> -MEG-Propargyl- <i>O</i> -MEG-(TIPS)propargyl CTV-OH 17a.....	S13
1.14	Side product 18a.....	S14
1.15	Side product 5.....	S15
1.16	Propargyl-DEG-OH 7b.....	S16
1.17	<i>O</i> -THP-Propargyl-DEG 8b.....	S17
1.18	<i>O</i> -THP-(TES)Propargyl-DEG 9b.....	S18
1.19	<i>O</i> -THP-(TIPS)Propargyl-DEG 10b.....	S19
1.20	(TES)Propargyl-DEG bromide 11b.....	S20
1.21	(TIPS)Propargyl-DEG bromide 12b.....	S21
1.22	Propargyl-DEG bromide 13b.....	S22
1.23	Di(<i>O</i> -THP)- <i>O</i> -DEG-propargyl CTV 15b.....	S23
1.24	<i>O</i> -DEG-Propargyl CTV-diOH 16b.....	S24
1.25	<i>O</i> -DEG-Propargyl- <i>O</i> -DEG-(TIPS)propargyl CTV-OH 17b.....	S25
1.26	Side product 18b.....	S26

1.27	Tri- <i>O</i> -Propargyl CTV 20.....	S27
1.28	Tri- <i>O</i> -MEG-Propargyl CTV 21.....	S27
1.29	Tri- <i>O</i> -DEG-Propargyl CTV 22.....	S28
1.30	Synthetic antibody 35	S29
1.31	Synthetic antibody 36	S30
1.32	Synthetic antibody 37	S31
1.33	Synthetic antibody 38	S32
1.34	Synthetic antibody 39	S33
1.35	Synthetic antibody 40	S34
1.36	Synthetic antibody 44	S35
1.37	Synthetic antibody 45	S36
1.38	Synthetic antibody 46	S37
1.39	Synthetic antibody 47	S38
1.40	Synthetic antibody 48	S39
1.41	Synthetic antibody 49	S40
2	Fitted SPR sensograms	S42
2.1	Synthetic antibody 35	S42
2.2	Synthetic antibody 36	S42
2.3	Synthetic antibody 37	S43
2.4	Synthetic antibody 44	S43
2.5	Synthetic antibody 47	S43
2.6	Synthetic antibody 48	S44
2.7	Synthetic antibody 49	S44
2.8	CDR mimic 31	S44

1 Product characterisation

1.1 Semi-orthogonally protected scaffold 2

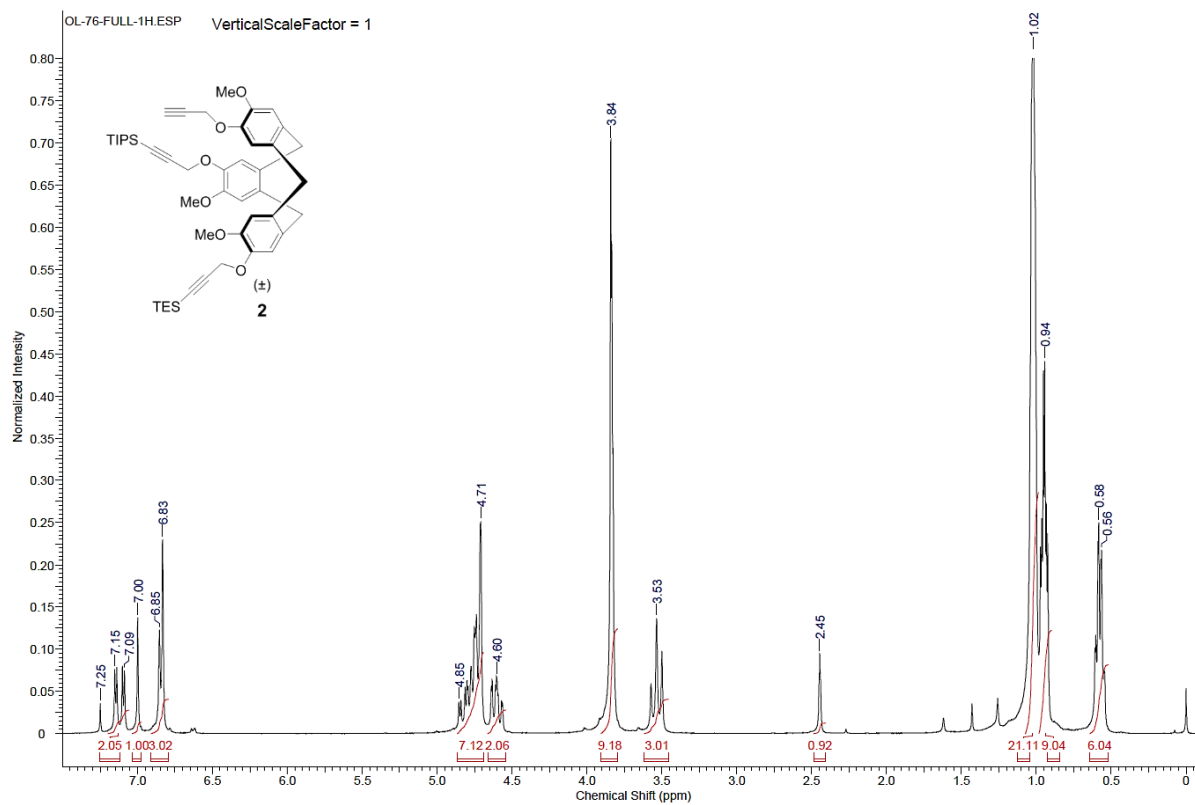


Figure S1: ¹H-NMR spectrum of semi-orthogonally protected scaffold 2.

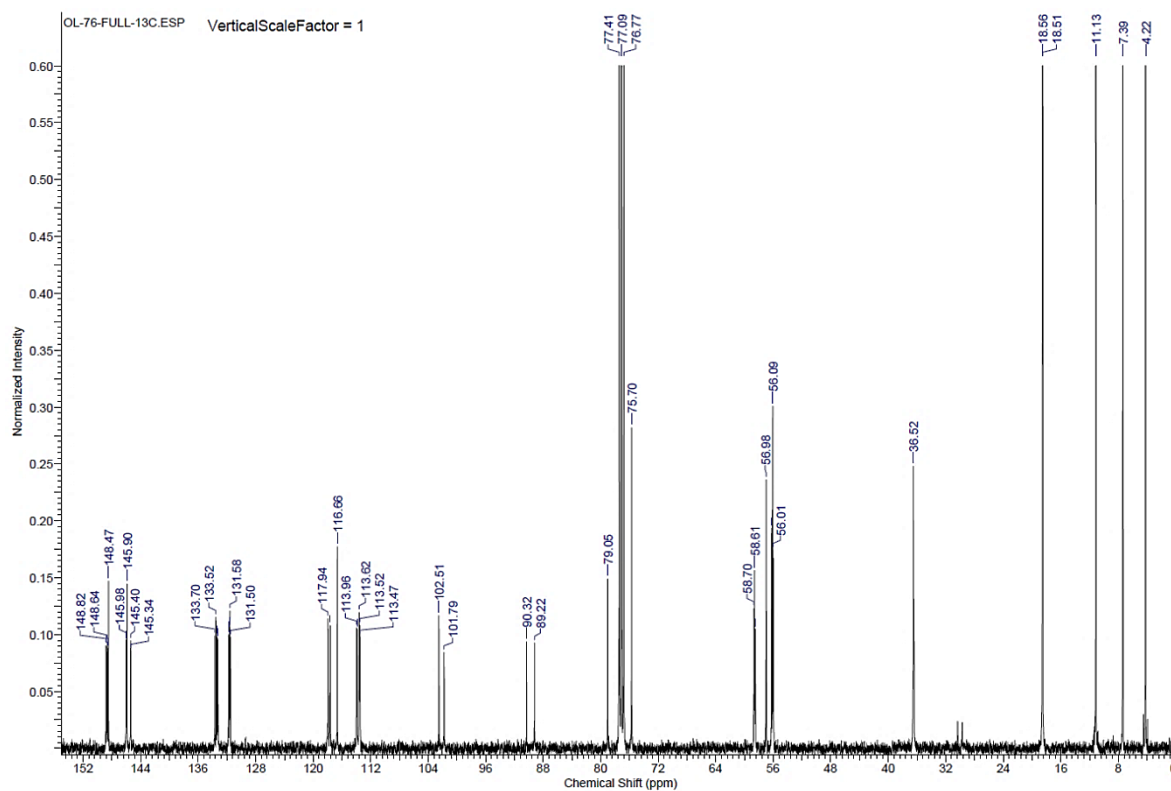


Figure S2: ¹³C-NMR spectrum of semi-orthogonally protected scaffold 2.

1.2 Semi-orthogonally protected scaffold 3

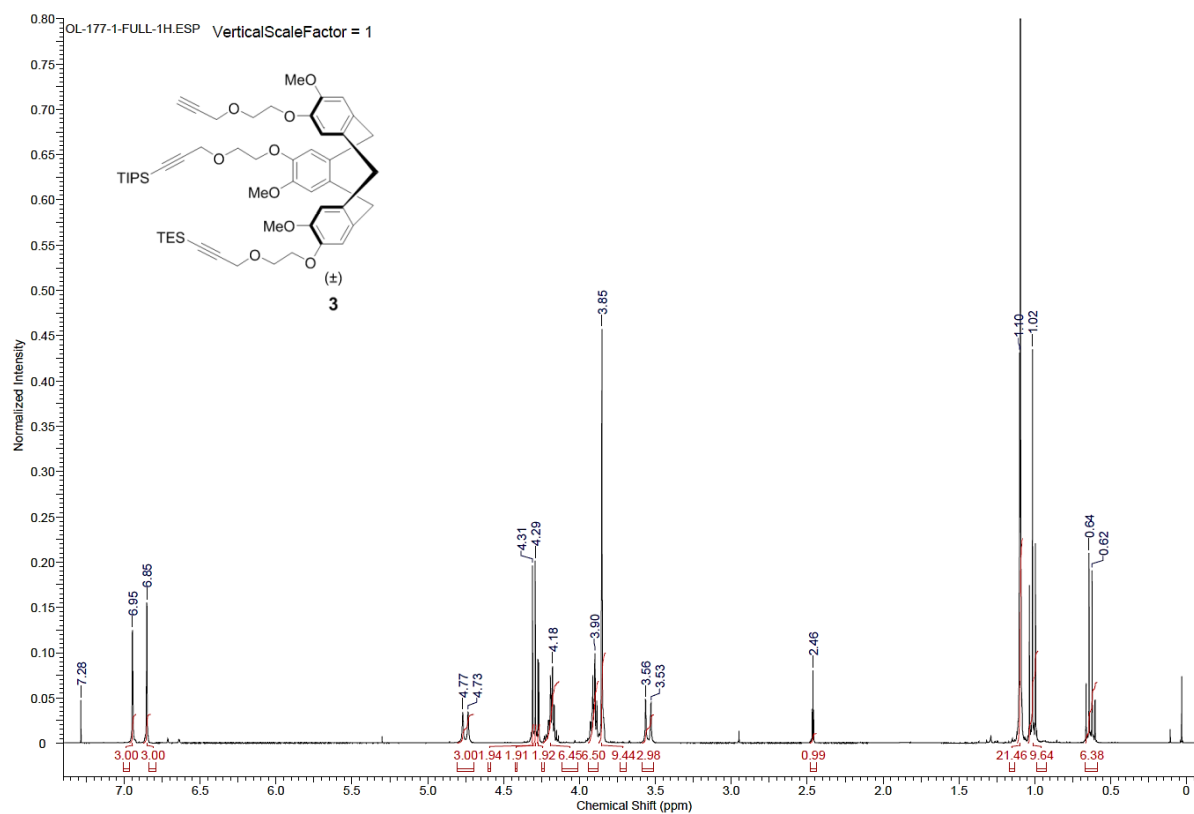


Figure S3: ¹H-NMR spectrum of semi-orthogonally protected scaffold 3.

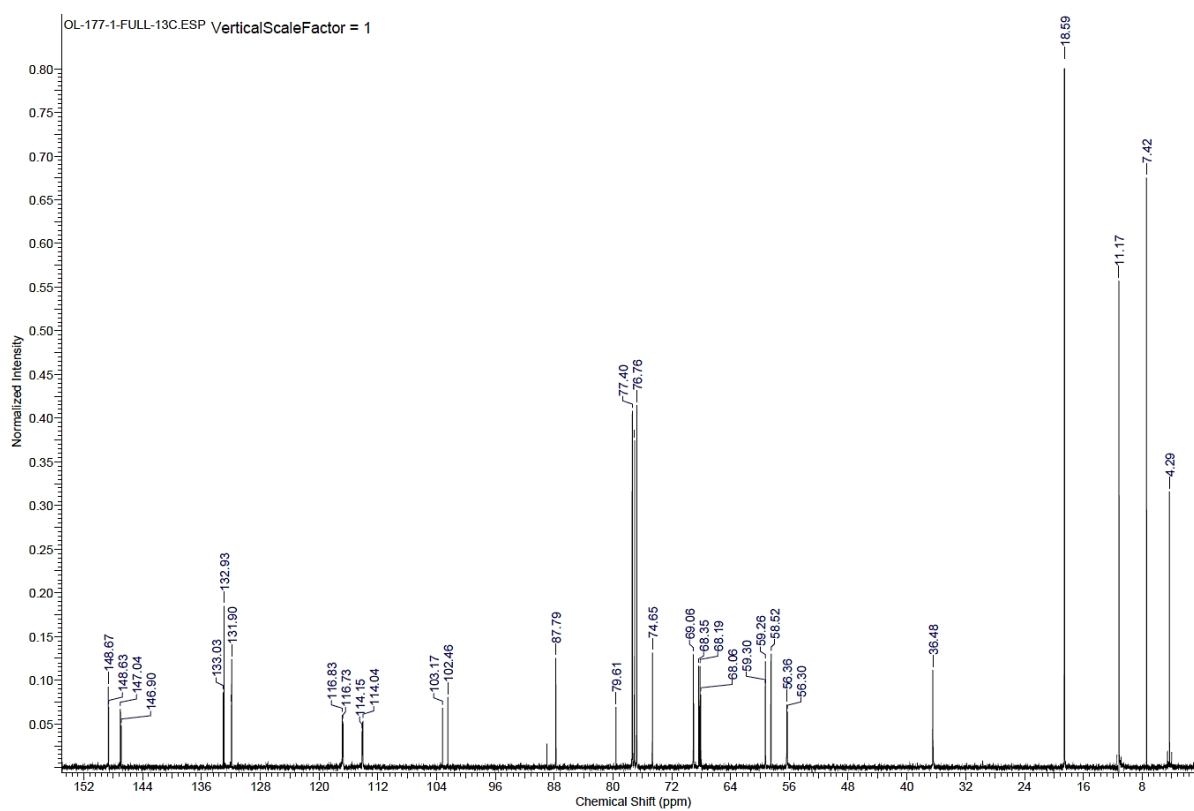


Figure S4: ¹³C-NMR spectrum of semi-orthogonally protected scaffold 3.

1.3 Semi-orthogonally protected scaffold 4

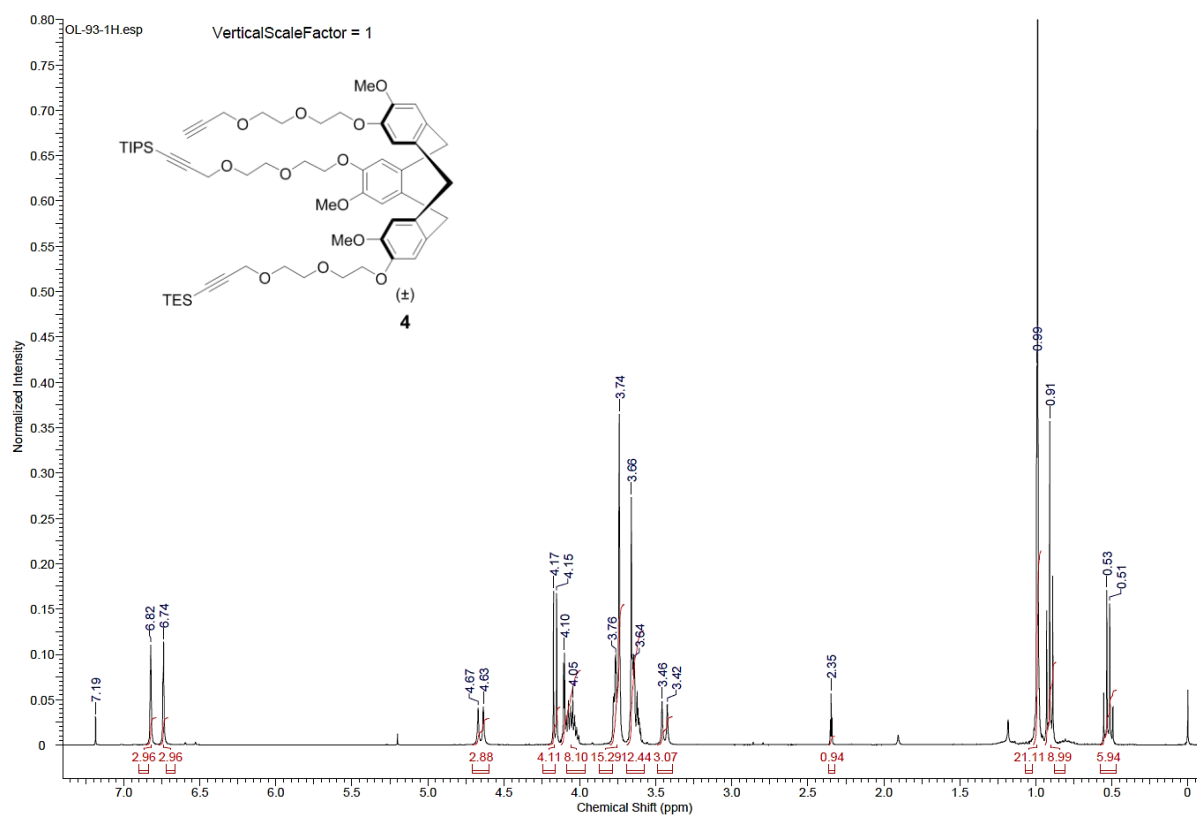


Figure S5: ¹H-NMR spectrum of semi-orthogonally protected scaffold 4.

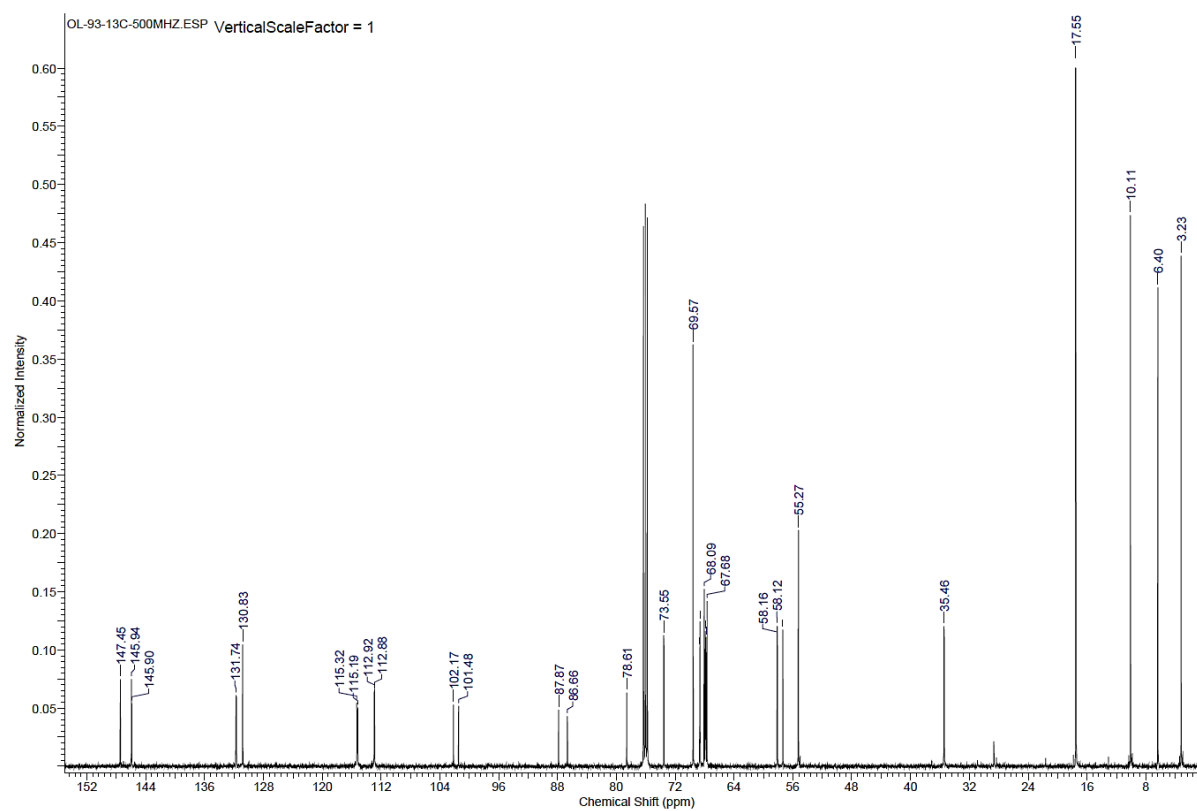


Figure S6: ¹³C-NMR spectrum of semi-orthogonally protected scaffold 4.

1.4 Propargyl-MEG-OH 7a

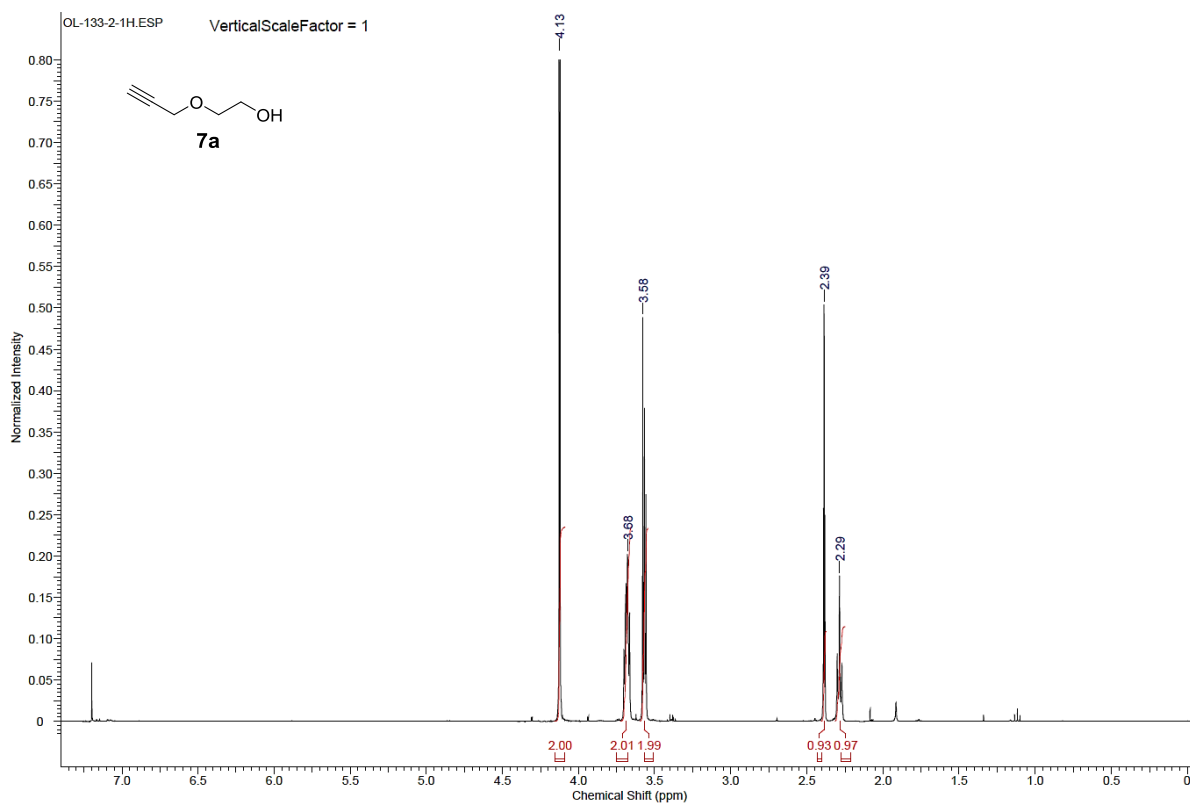


Figure S7: ¹H-NMR spectrum of compound 7a.

1.5 O-THP-Propargyl-MEG 8a

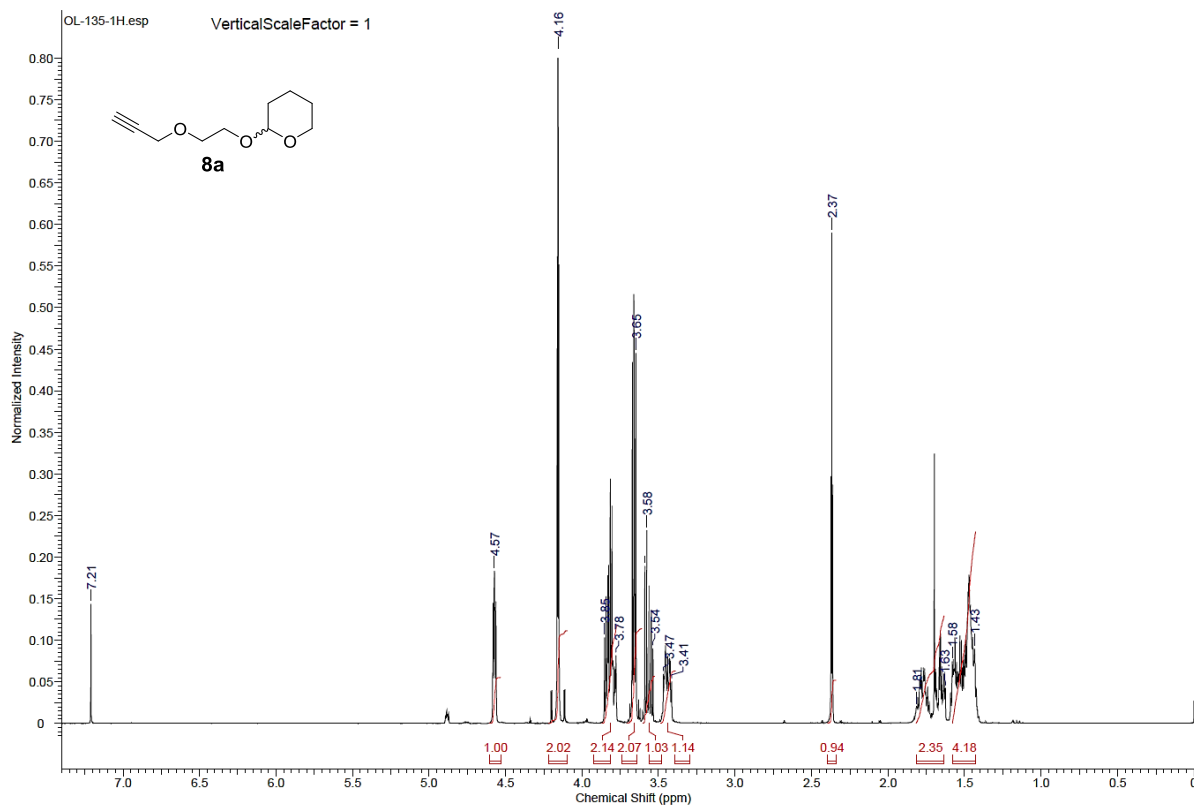


Figure S8: ¹H-NMR spectrum of compound 8a.

1.6 O-THP-(TES)Propargyl-MEG 9a

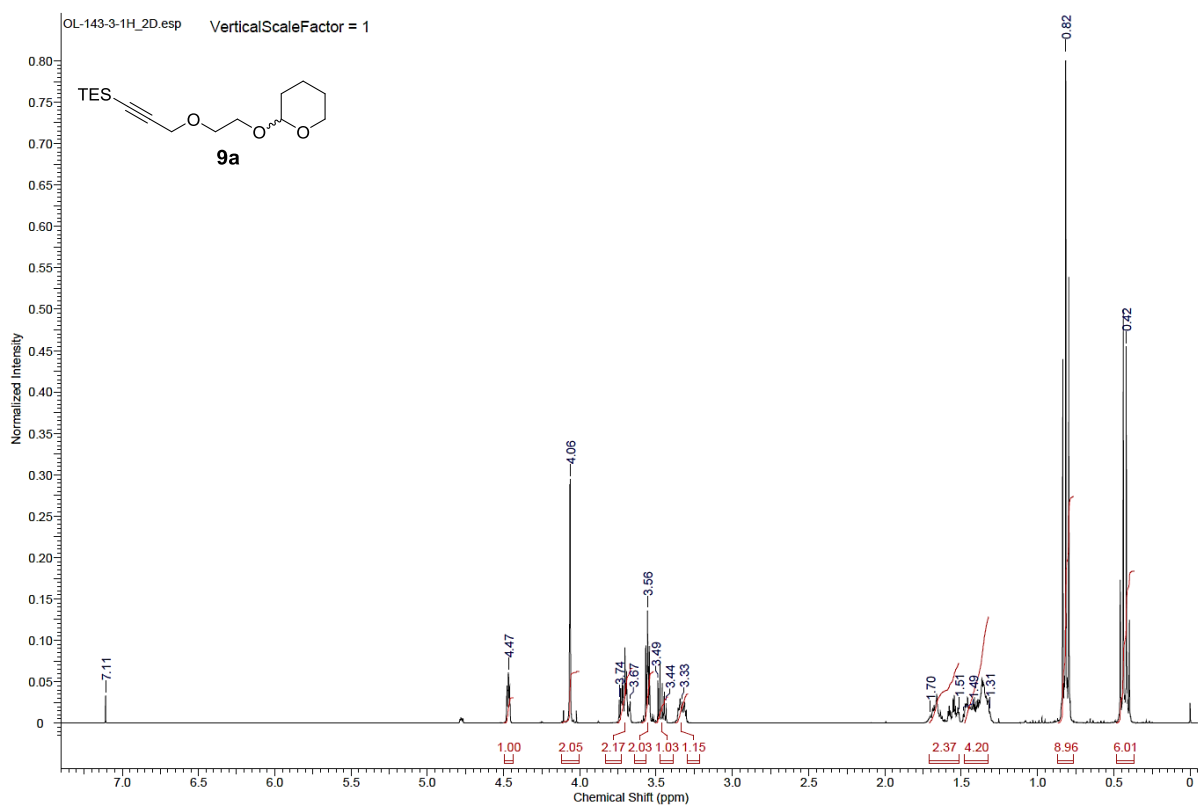


Figure S9: ¹H-NMR spectrum of compound 9a.

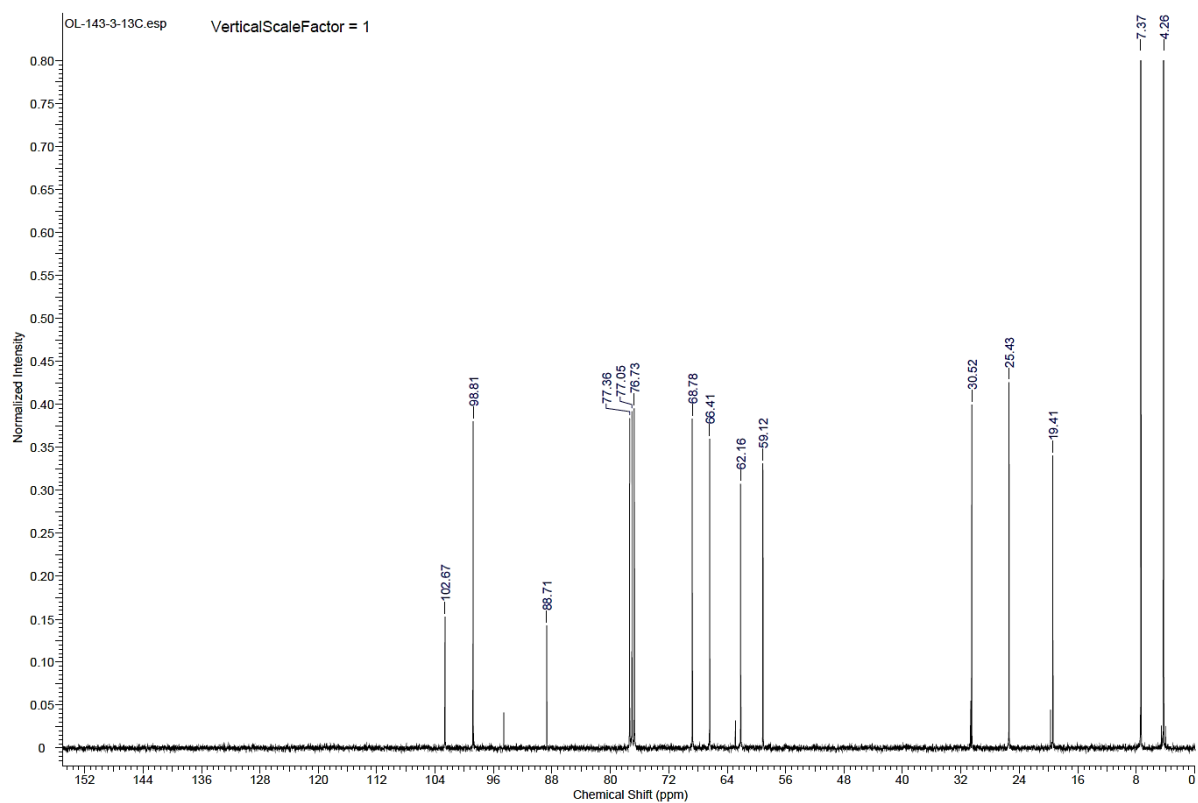


Figure S10: ¹³C-NMR spectrum of compound 9a.

1.7 O-THP-(TIPS)Propargyl-MEG 10a

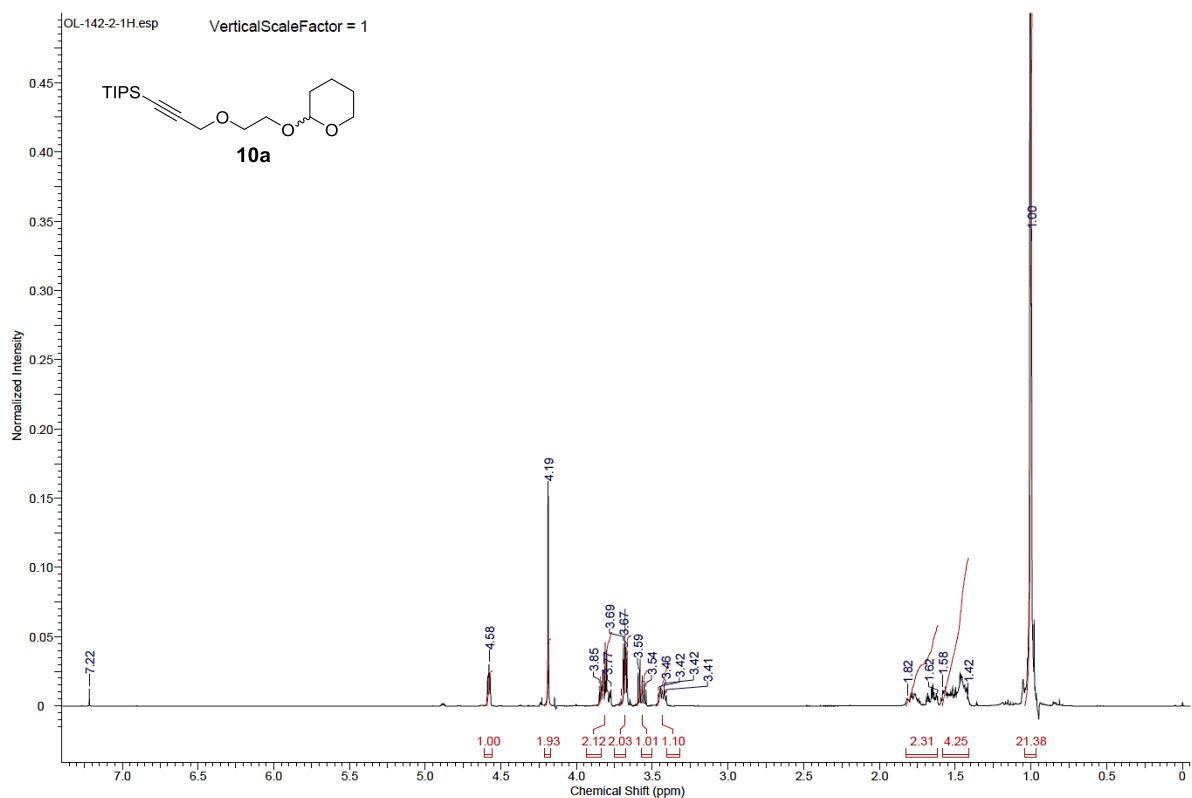


Figure S11: ¹H-NMR spectrum of compound 10a.

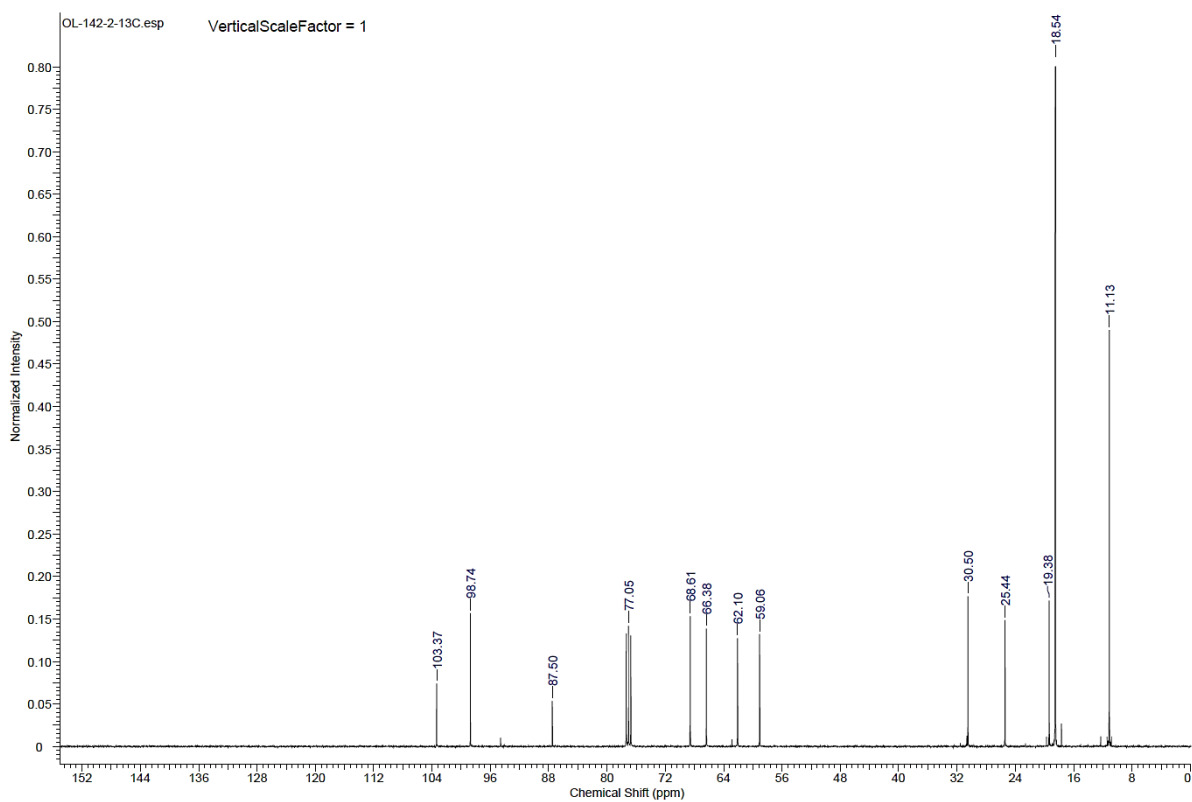


Figure S12: ¹³C-NMR spectrum of compound 10a.

1.8 (TES)Propargyl-MEG bromide 11a

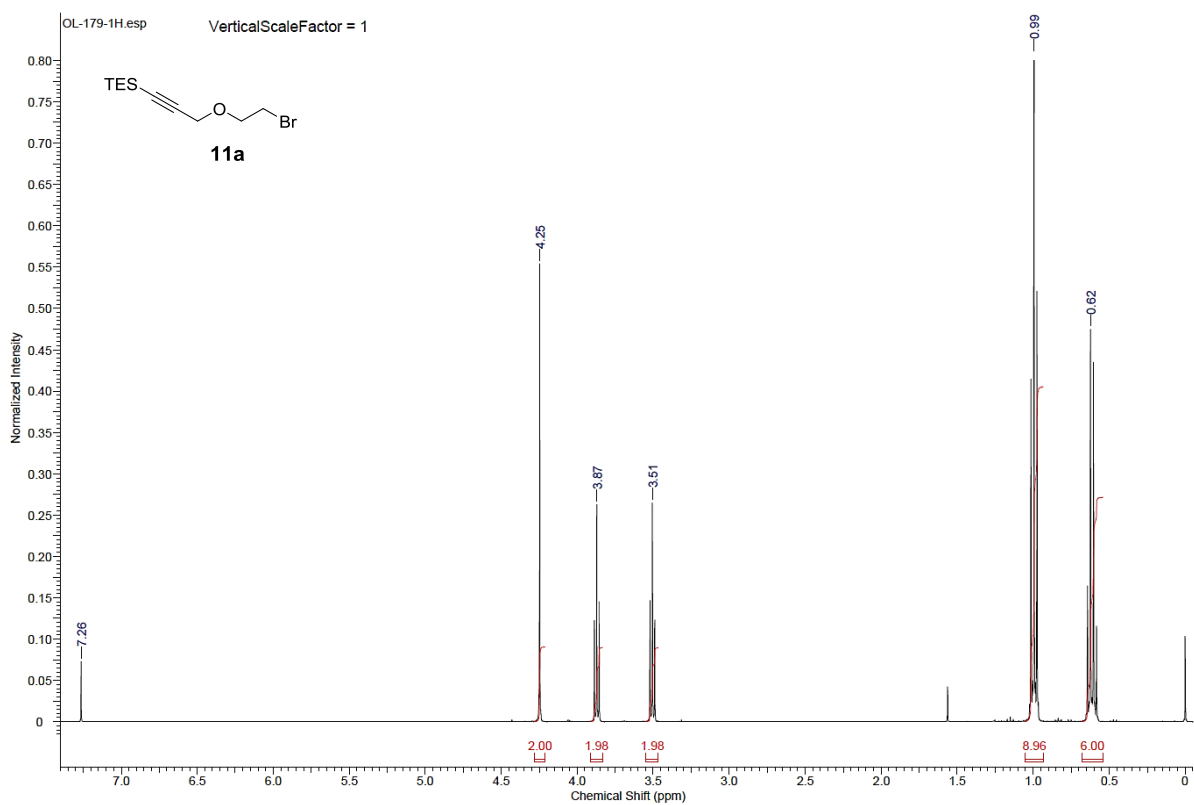


Figure S13: ¹H-NMR spectrum of compound 11a.

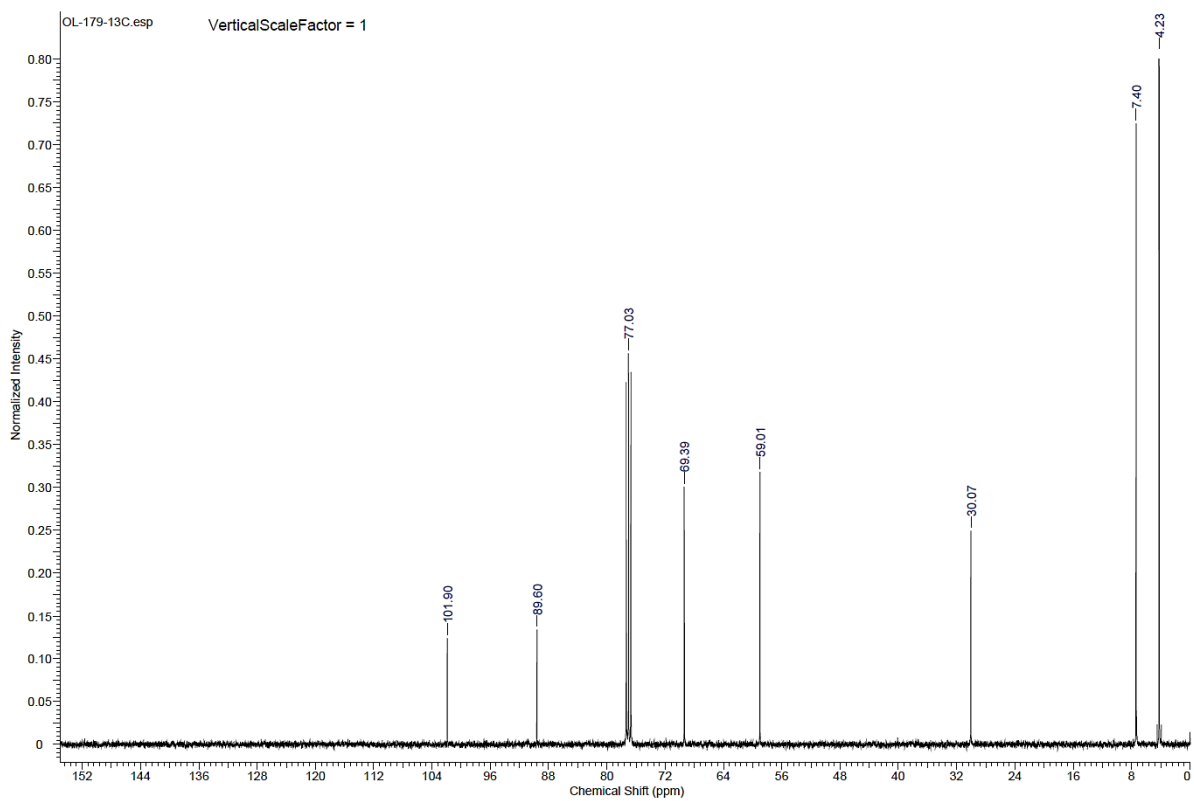


Figure S14: ¹³C-NMR spectrum of compound 11a.

1.9 (TIPS)Propargyl-MEG bromide 12a

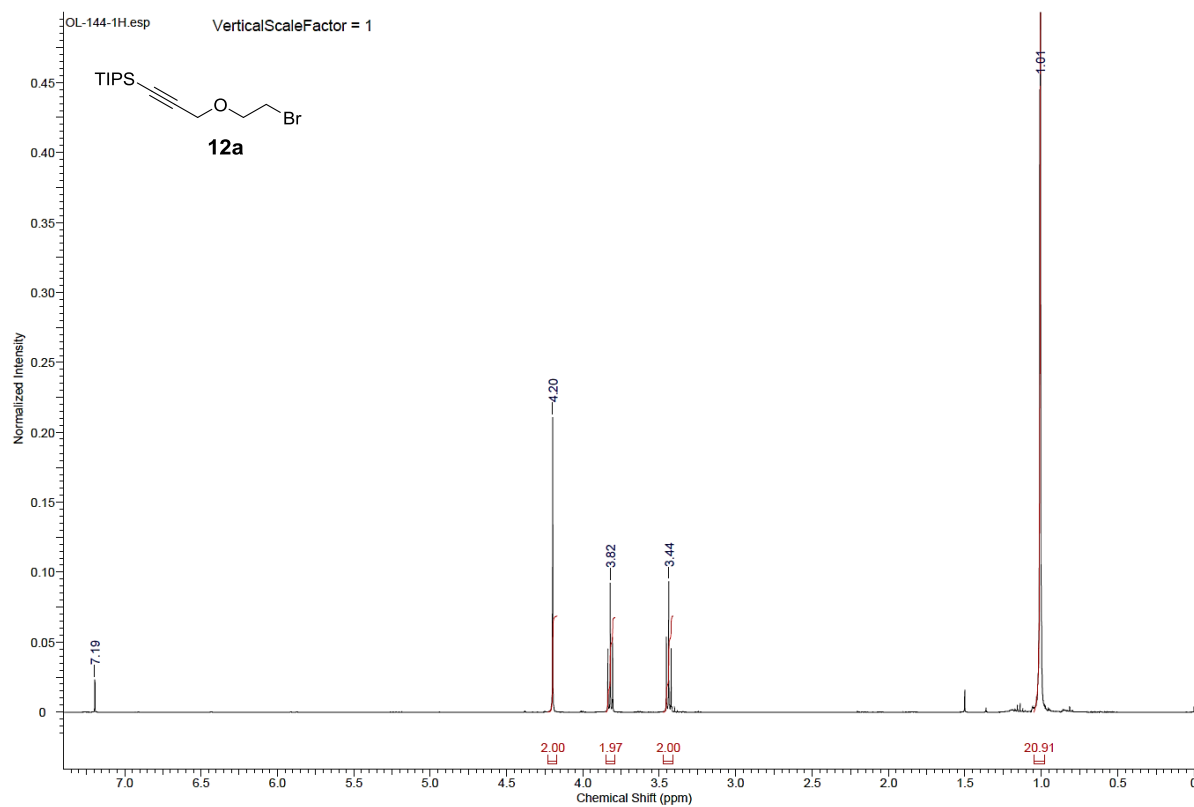


Figure S15: ¹H-NMR spectrum of compound 12a.

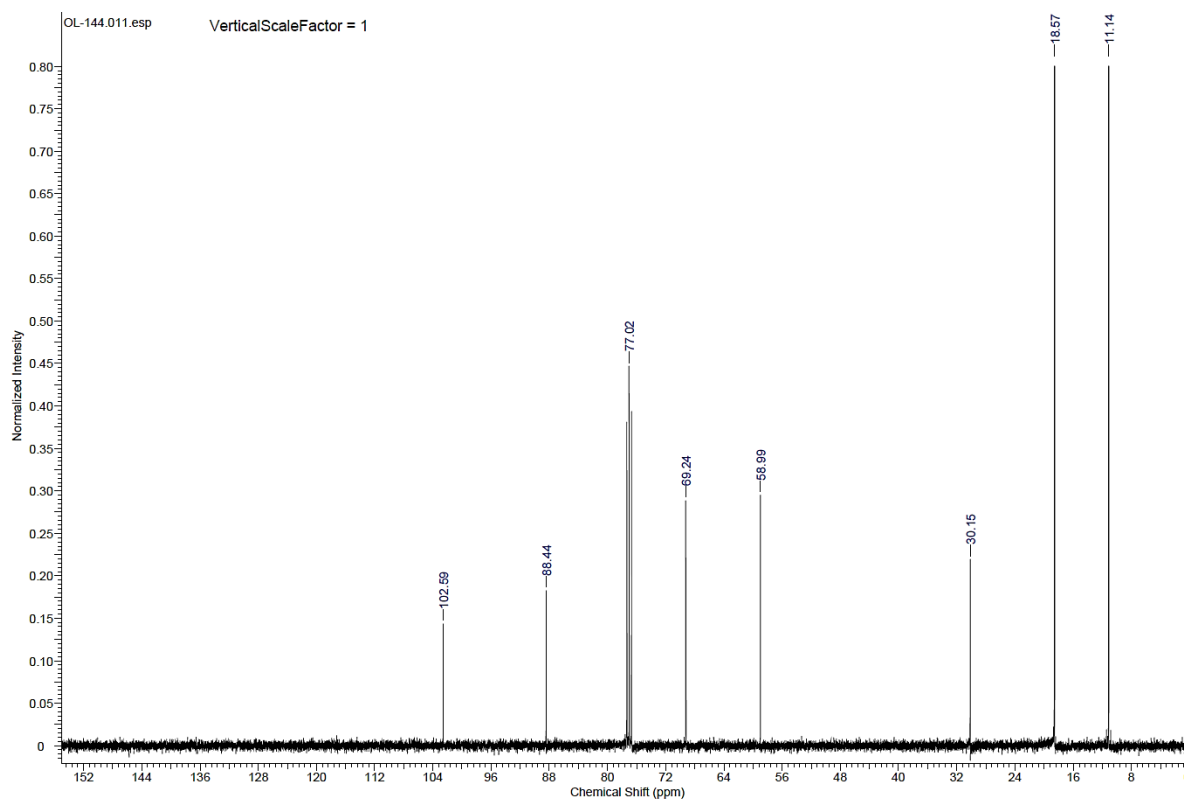


Figure S16: ¹³C-NMR spectrum of compound 12a.

1.10 Propargyl-MEG iodide 13a

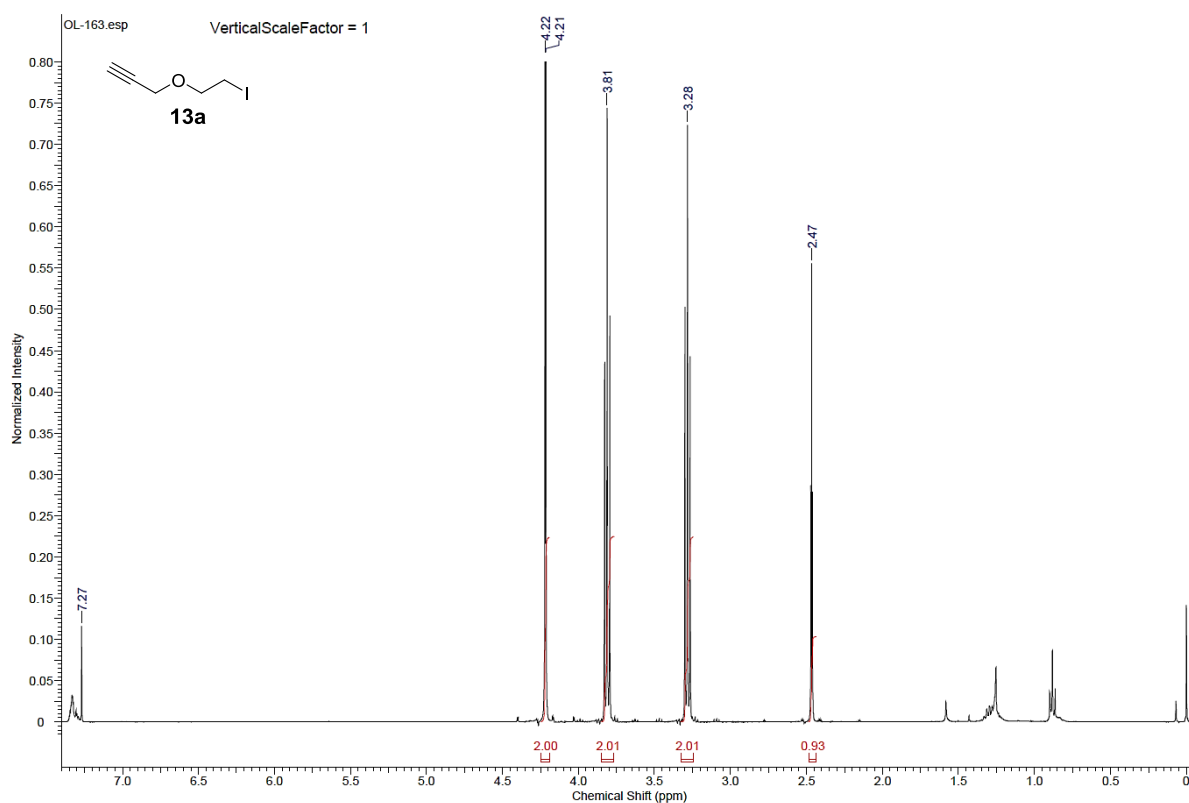


Figure S17: ¹H-NMR spectrum of compound 13a.

1.11 Di(O-THP)-O-MEG-propargyl CTV 15a

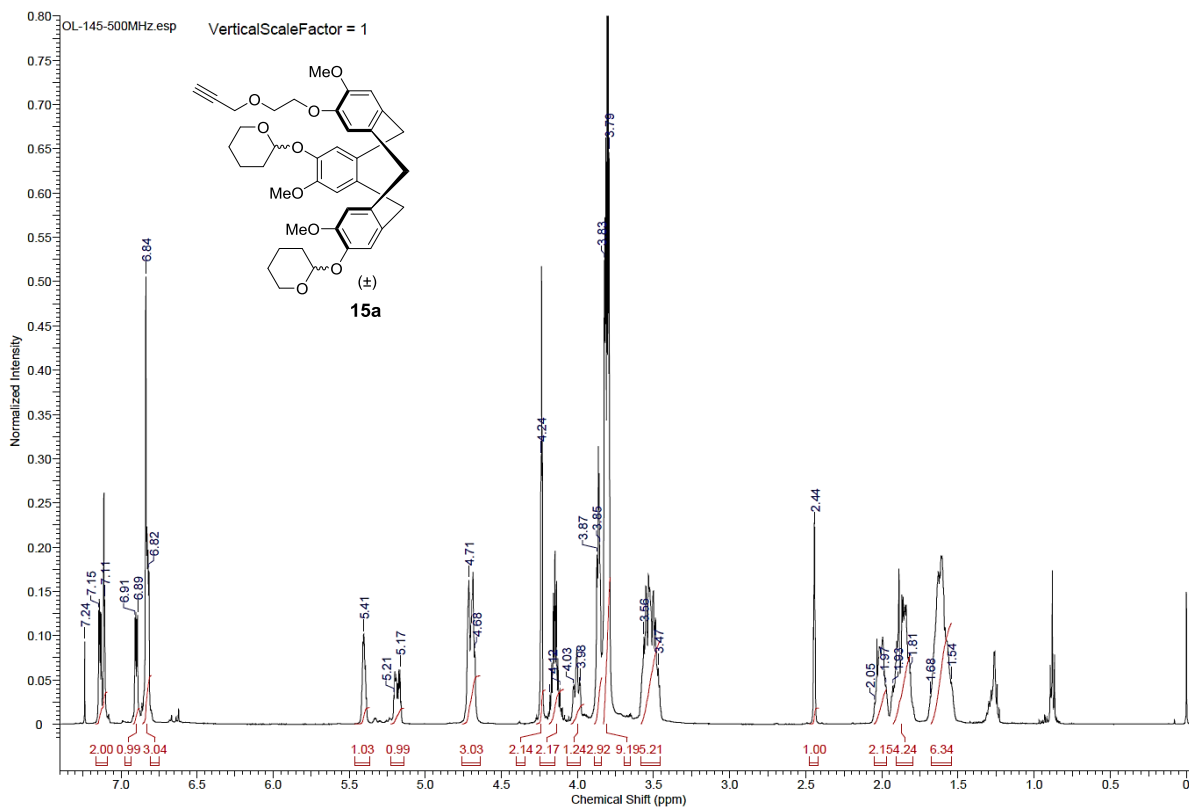


Figure S18: ¹H-NMR spectrum of compound 15a.

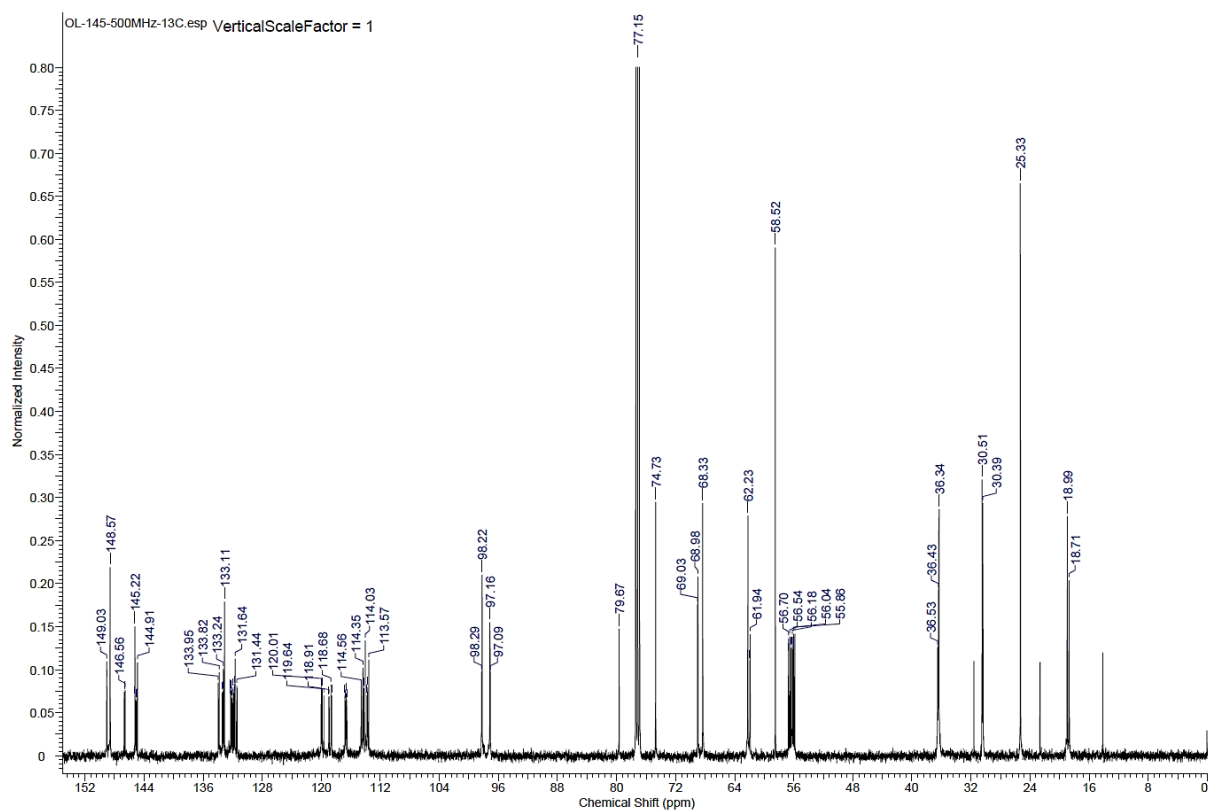


Figure S19: ^{13}C -NMR spectrum of compound 15a.

1.12 O-MEG-Propargyl CTV-diOH 16a

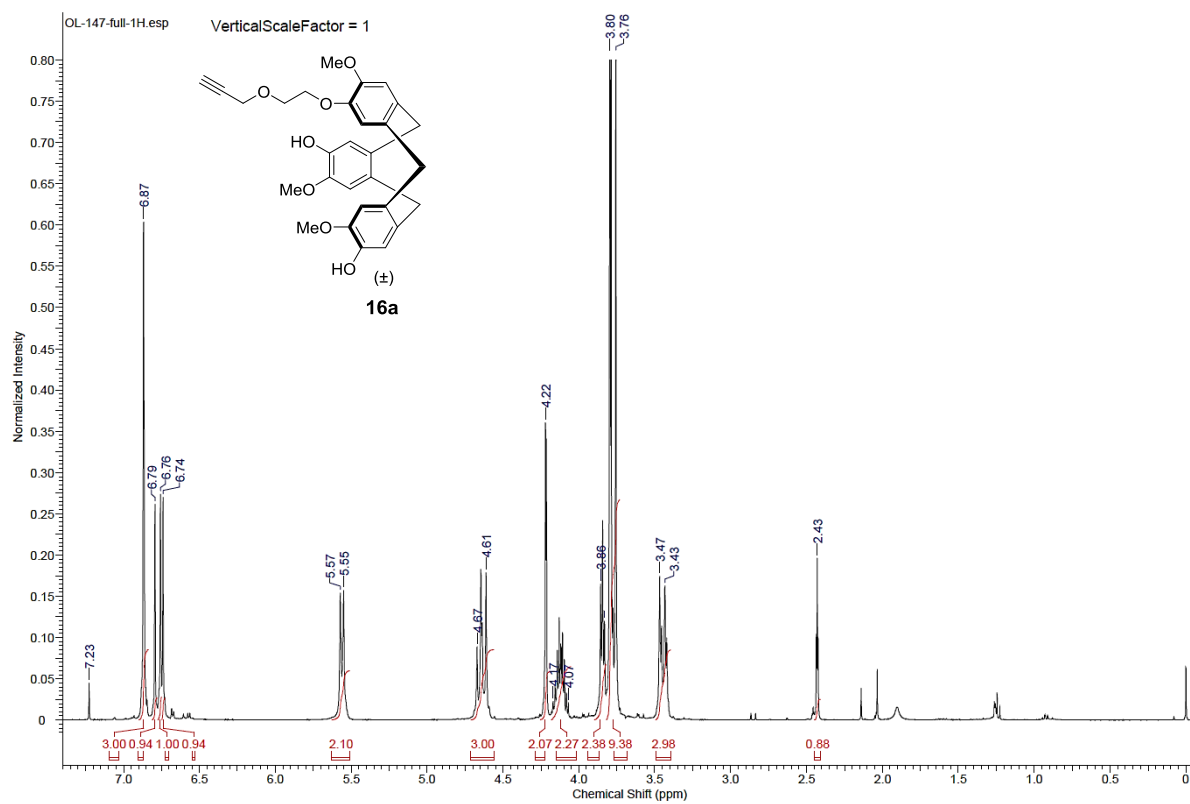


Figure S20: ^1H -NMR spectrum of compound 16a.

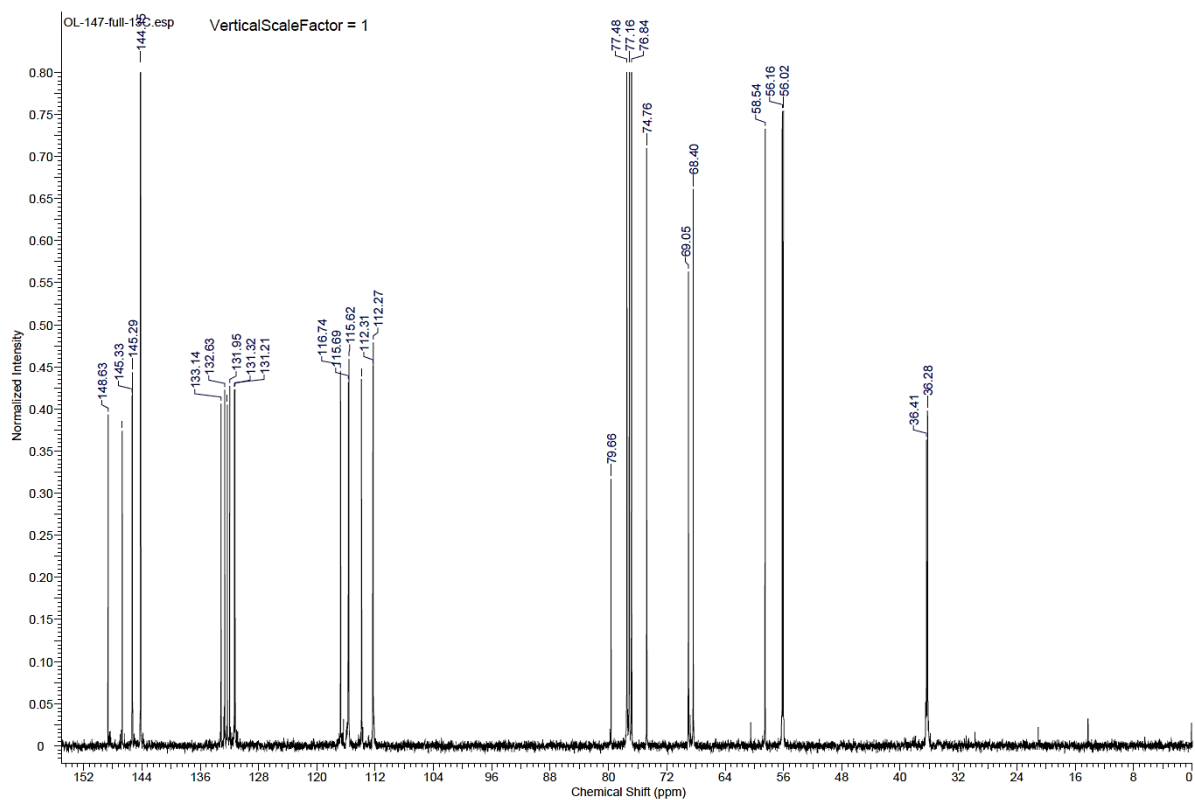


Figure S21: ^{13}C -NMR spectrum of compound 16a.

1.13 O-MEG-Propargyl-O-MEG-(TIPS)propargyl CTV-OH 17a

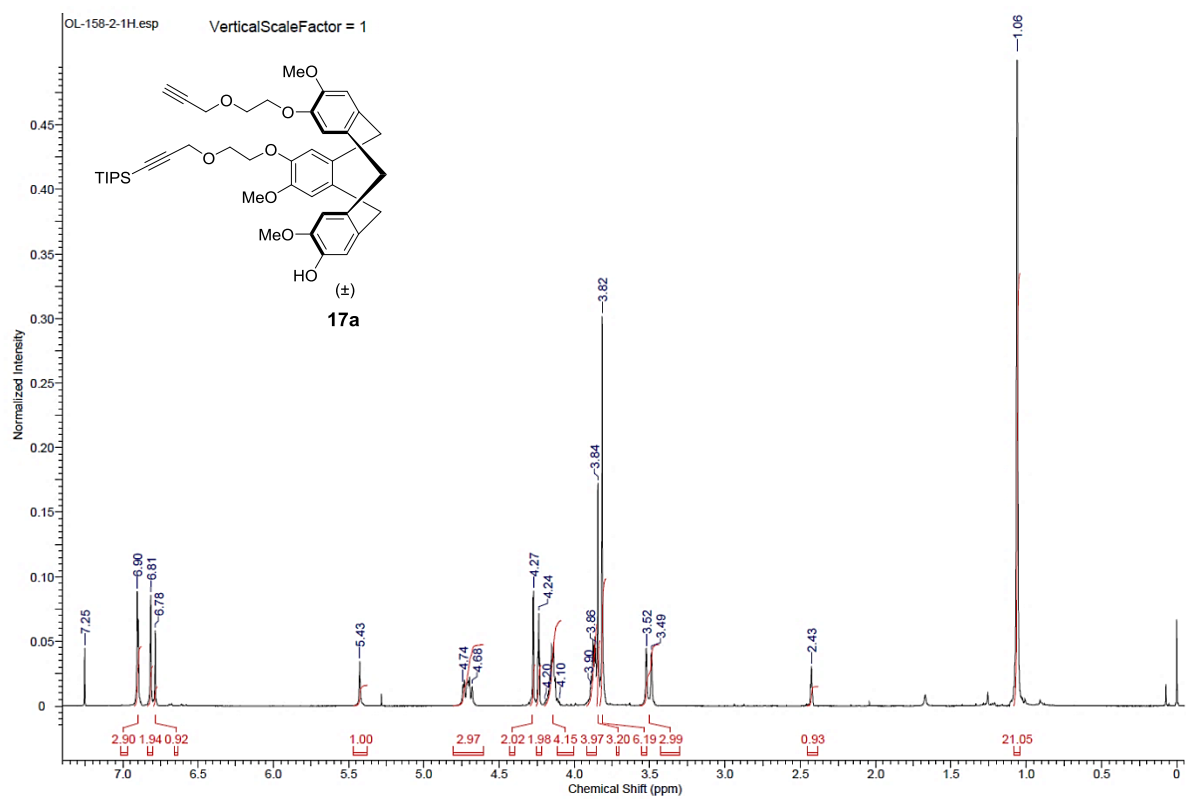


Figure S22: ^1H -NMR spectrum of compound 17a.

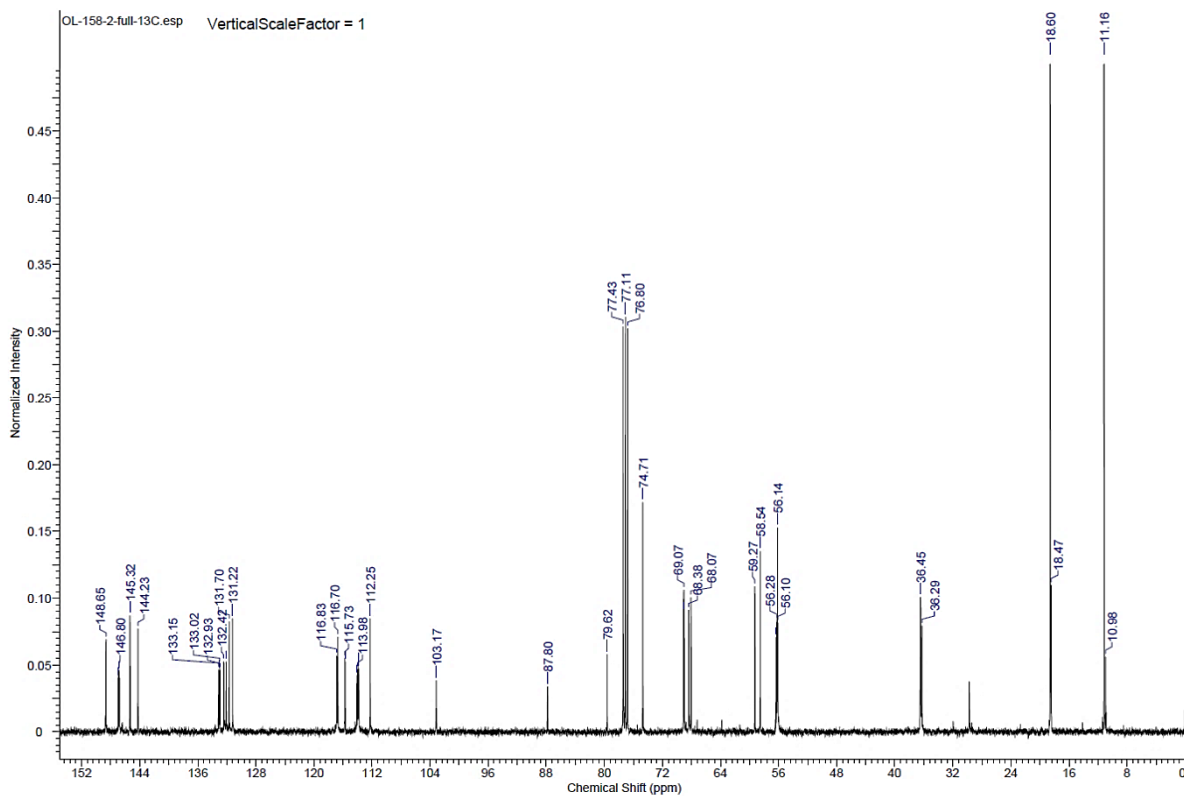


Figure S23: ^{13}C -NMR spectrum of compound 17a.

1.14 Side product 18a

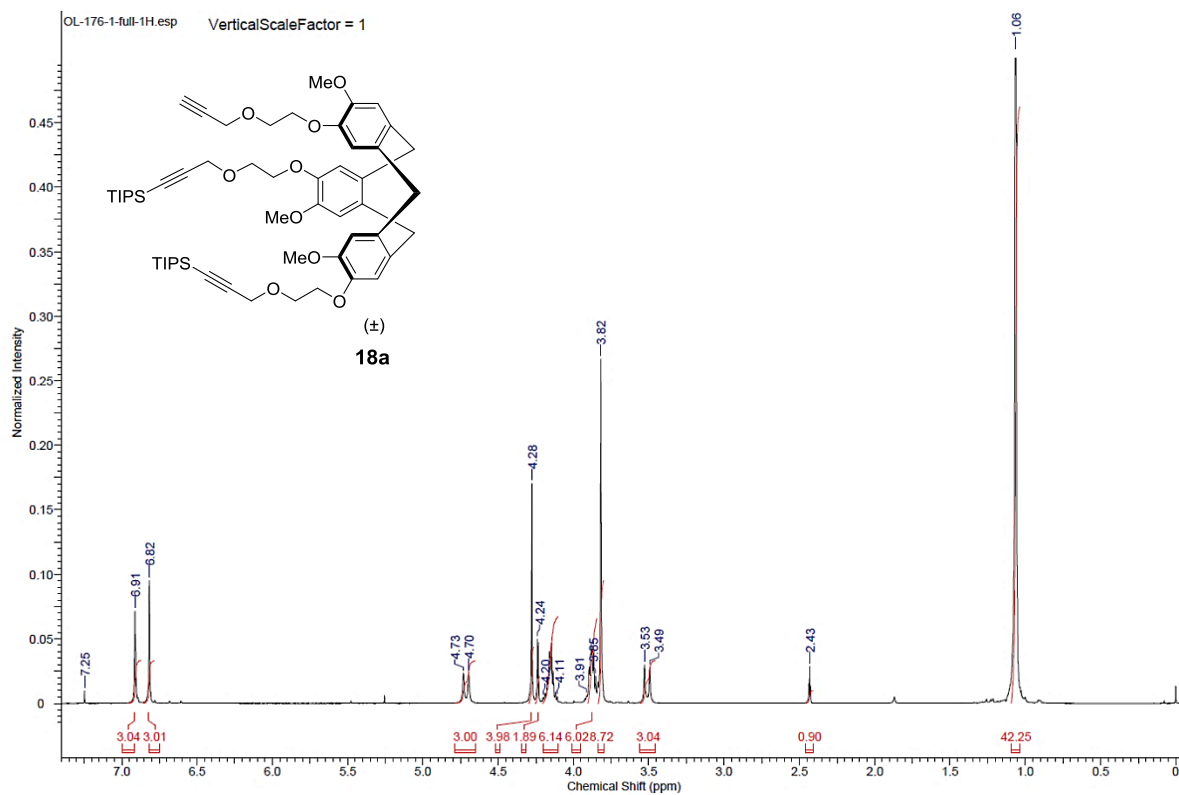


Figure S24: ^1H -NMR spectrum of compound 18a.

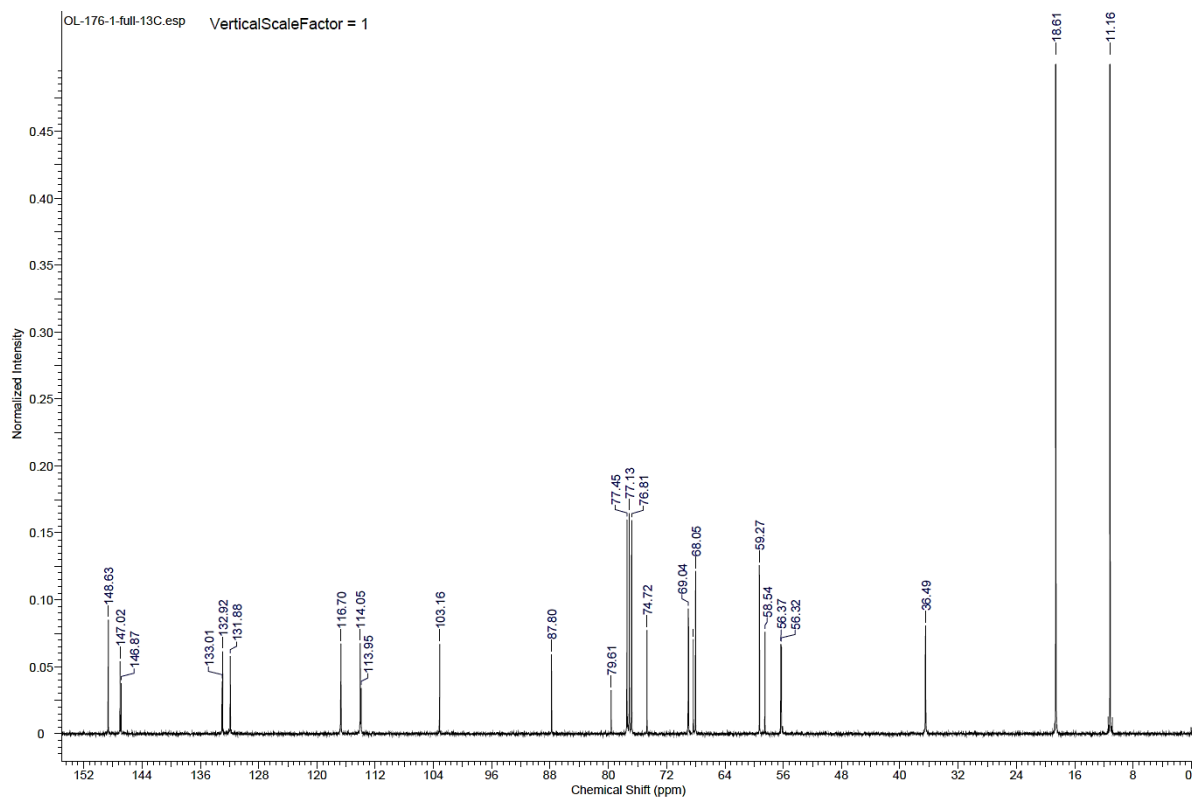


Figure S25: ^{13}C -NMR spectrum of compound 18a.

1.15 Side product 5

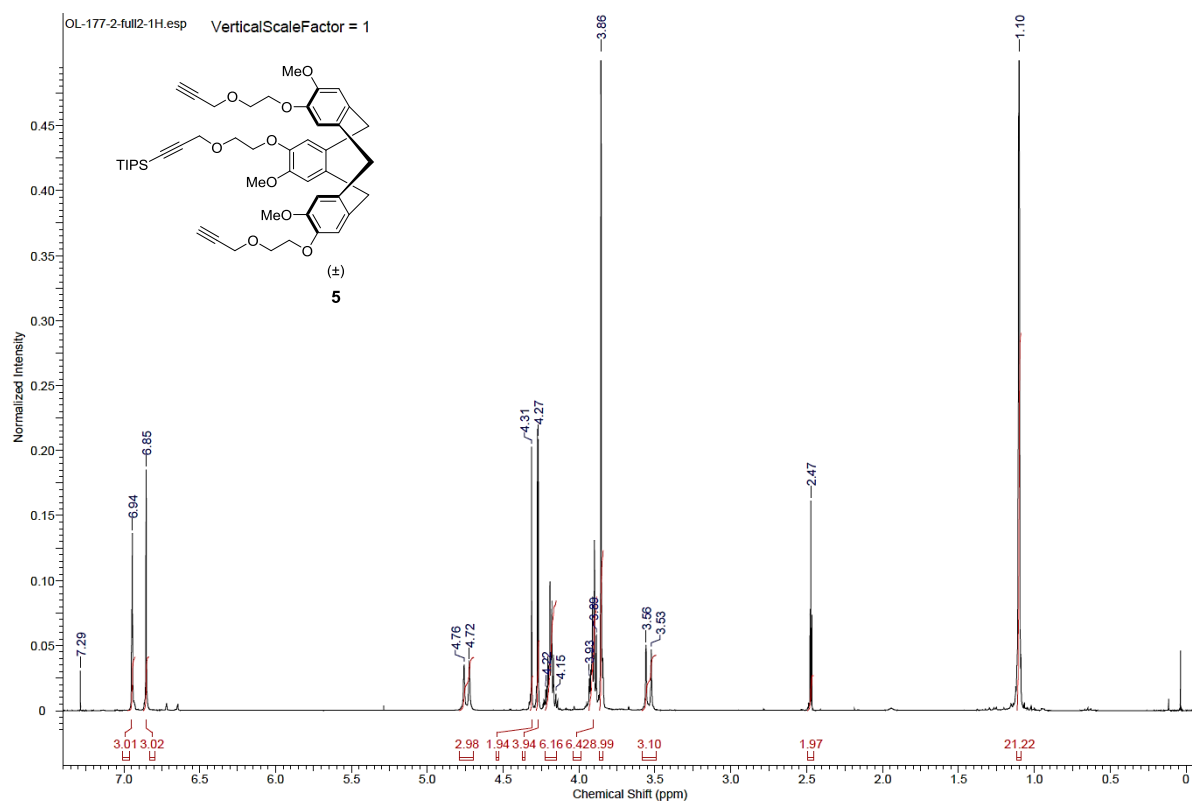


Figure S26: ^1H -NMR spectrum of compound 5.

1.17 O-THP-Propargyl-DEG 8b

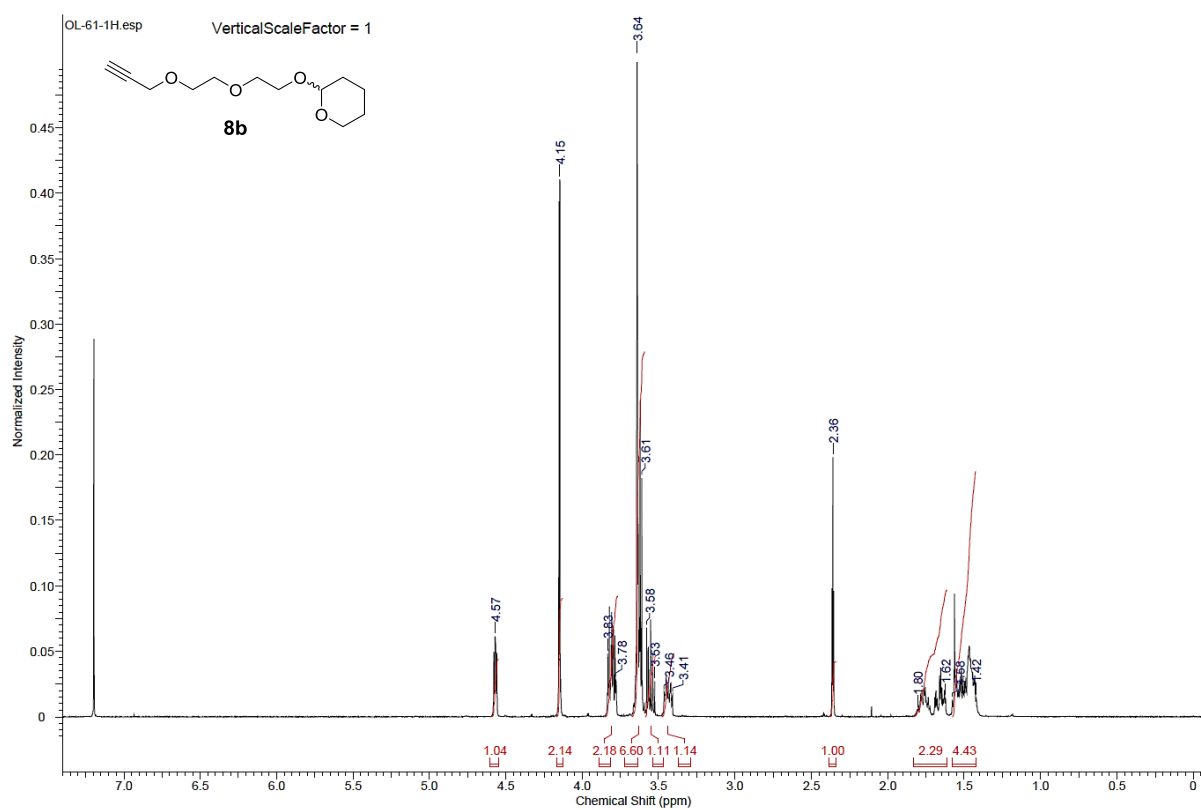


Figure S29: ¹H-NMR spectrum of compound 8b.

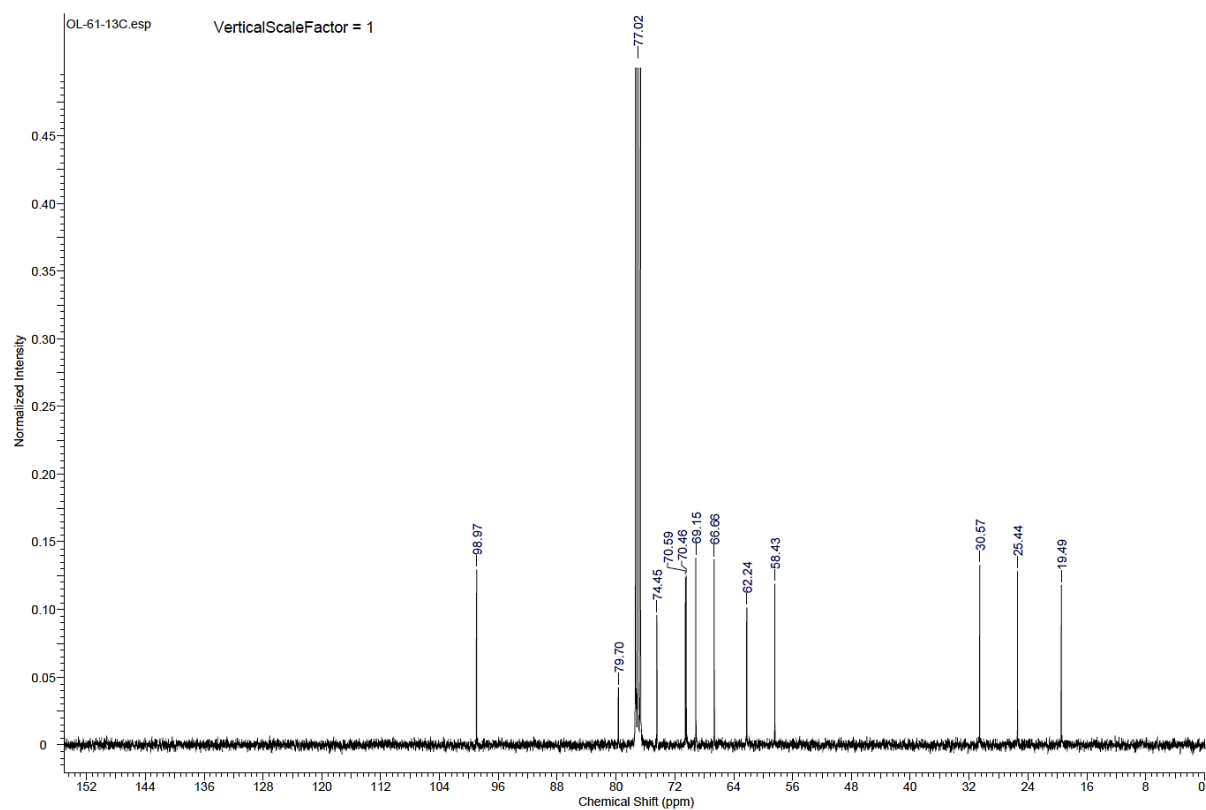


Figure S30: ¹³C-NMR spectrum of compound 8b.

1.18 O-THP-(TES)Propargyl-DEG 9b

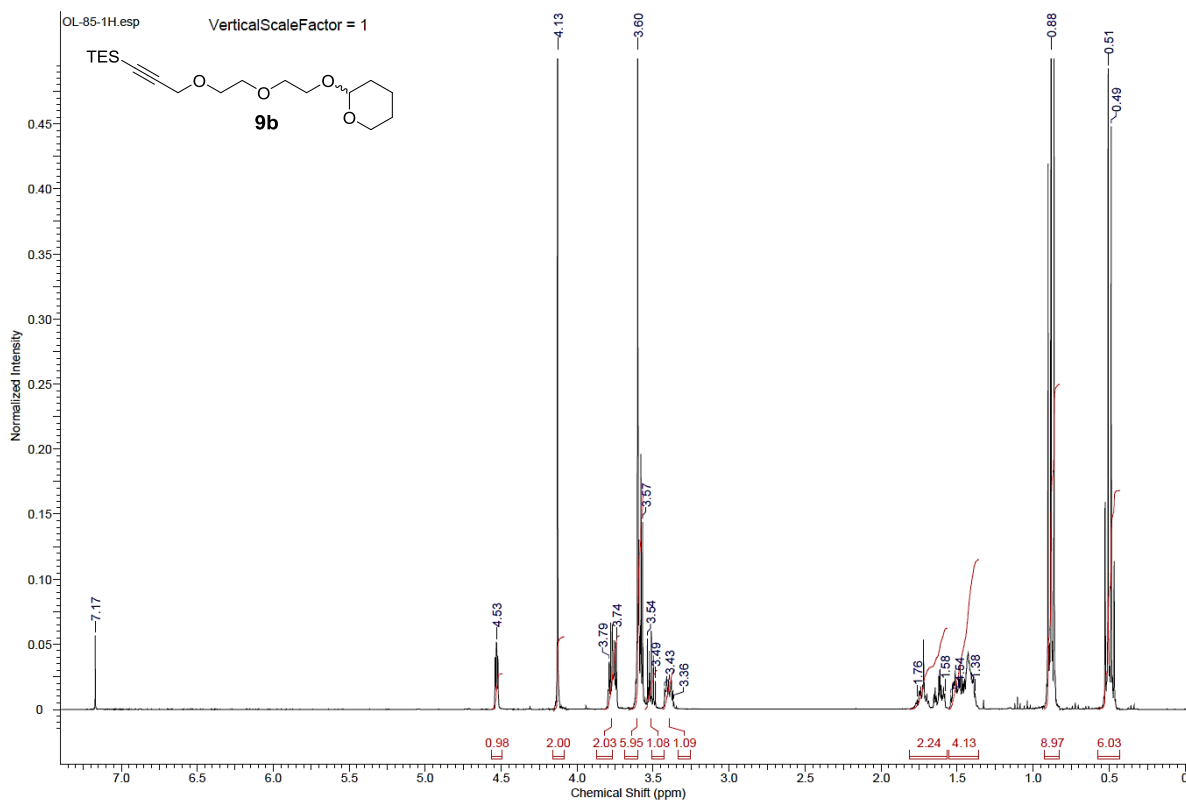


Figure S31: ¹H-NMR spectrum of compound 9b.

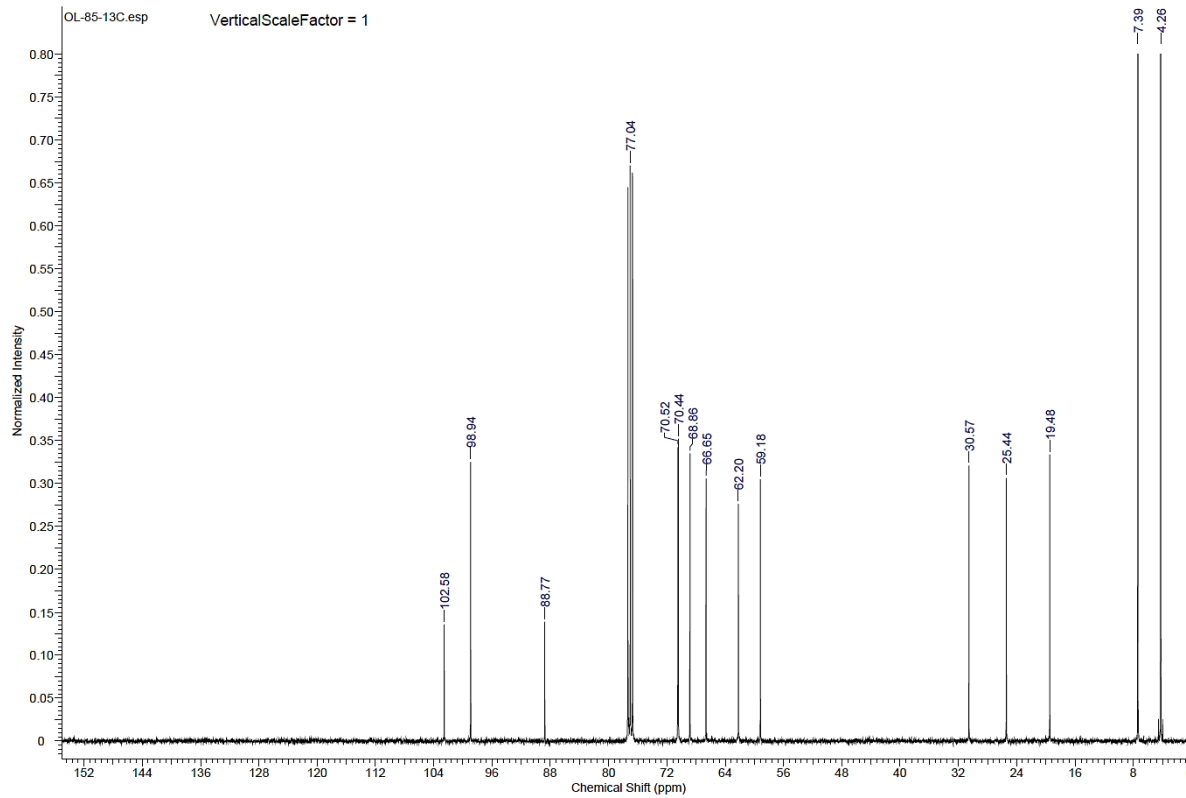


Figure S32: ¹³C-NMR spectrum of compound 9b.

1.19 O-THP-(TIPS)Propargyl-DEG 10b

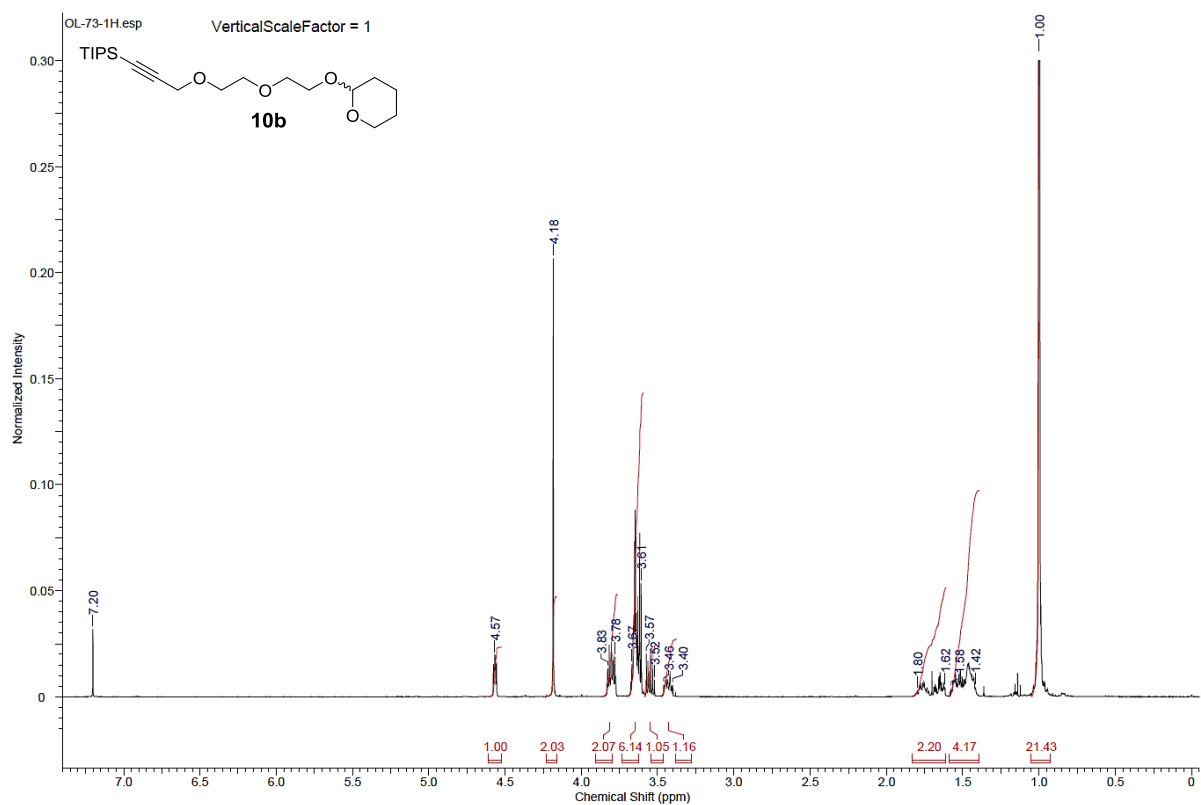


Figure S33: ¹H-NMR spectrum of compound 10b.

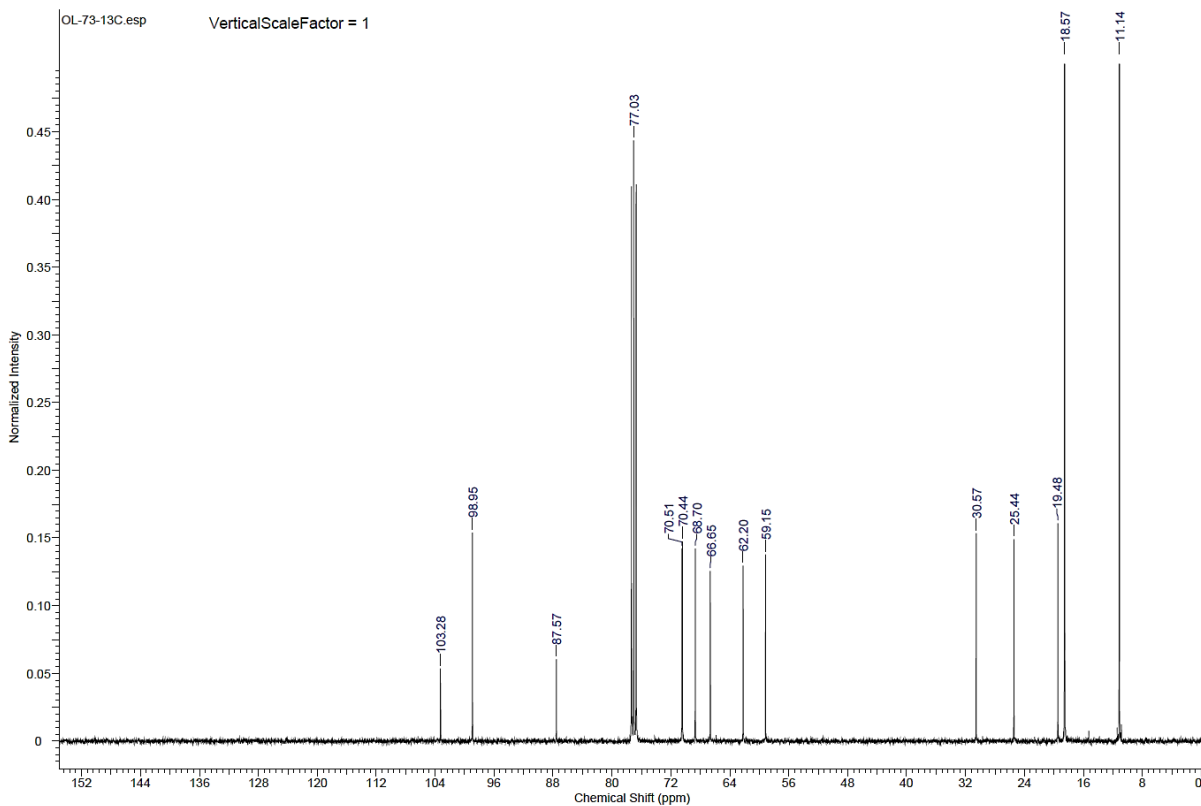


Figure S34: ¹³C-NMR spectrum of compound 10b.

1.20 (TES)Propargyl-DEG bromide 11b

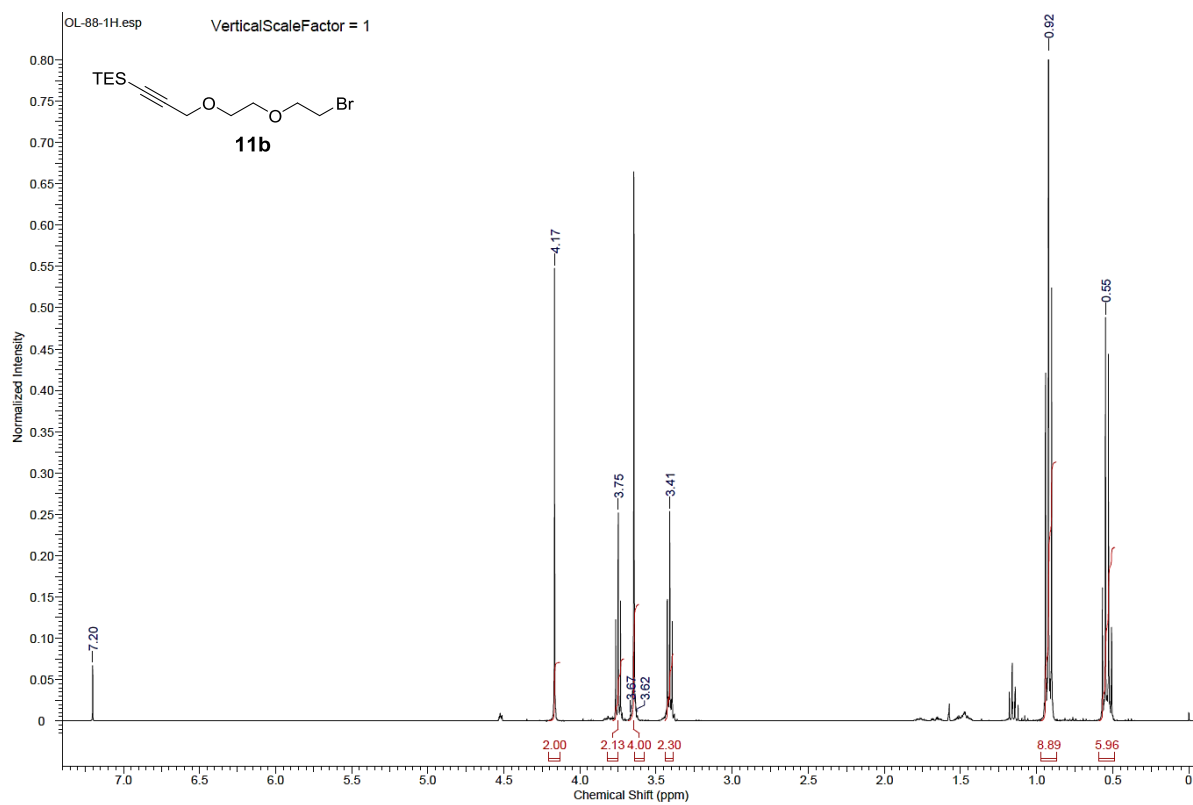


Figure S35: ¹H-NMR spectrum of compound 11b.

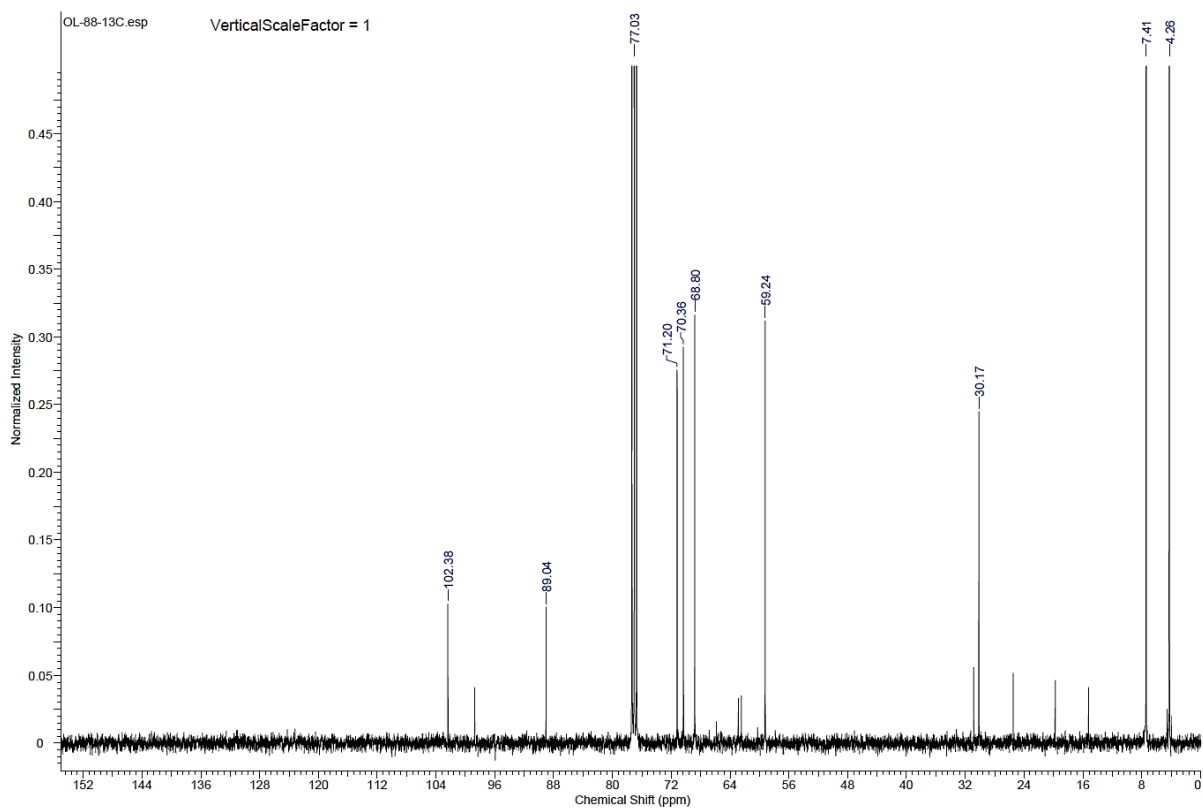


Figure S36: ¹³C-NMR spectrum of compound 11b.

1.21 (TIPS)Propargyl-DEG bromide 12b

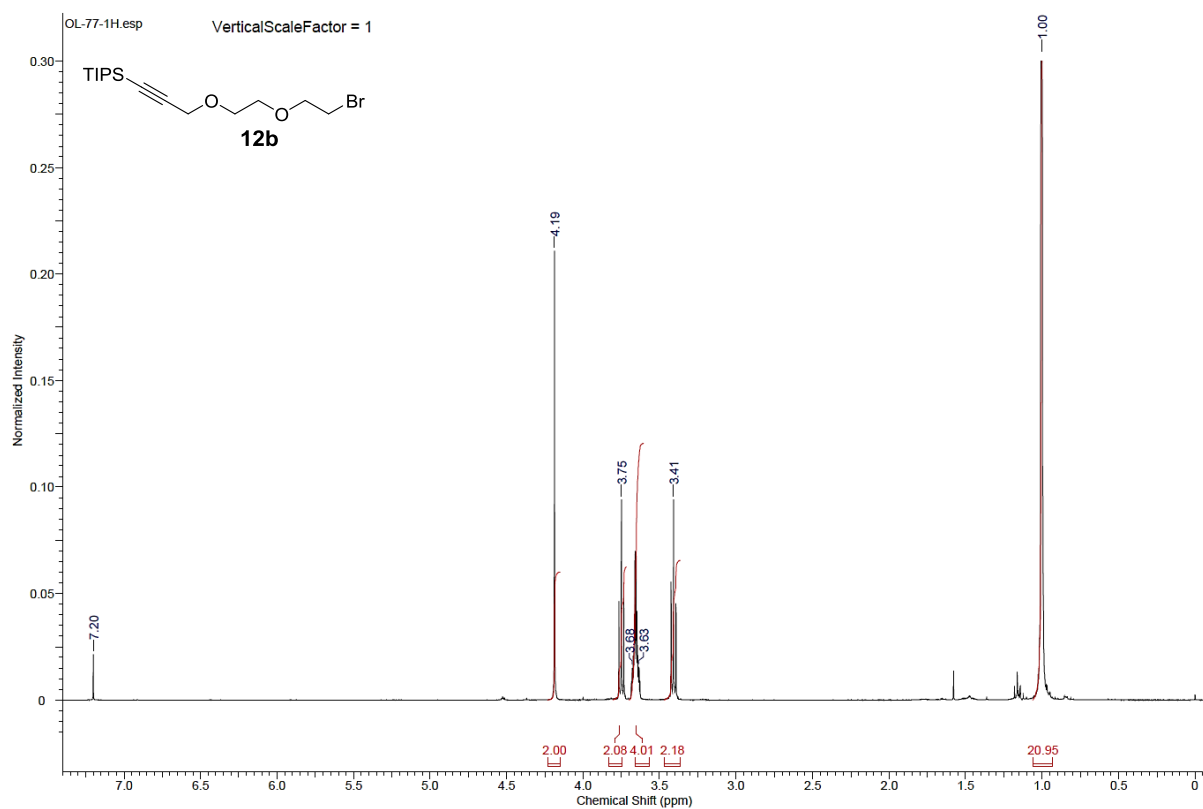


Figure S37: ¹H-NMR spectrum of compound 12b.

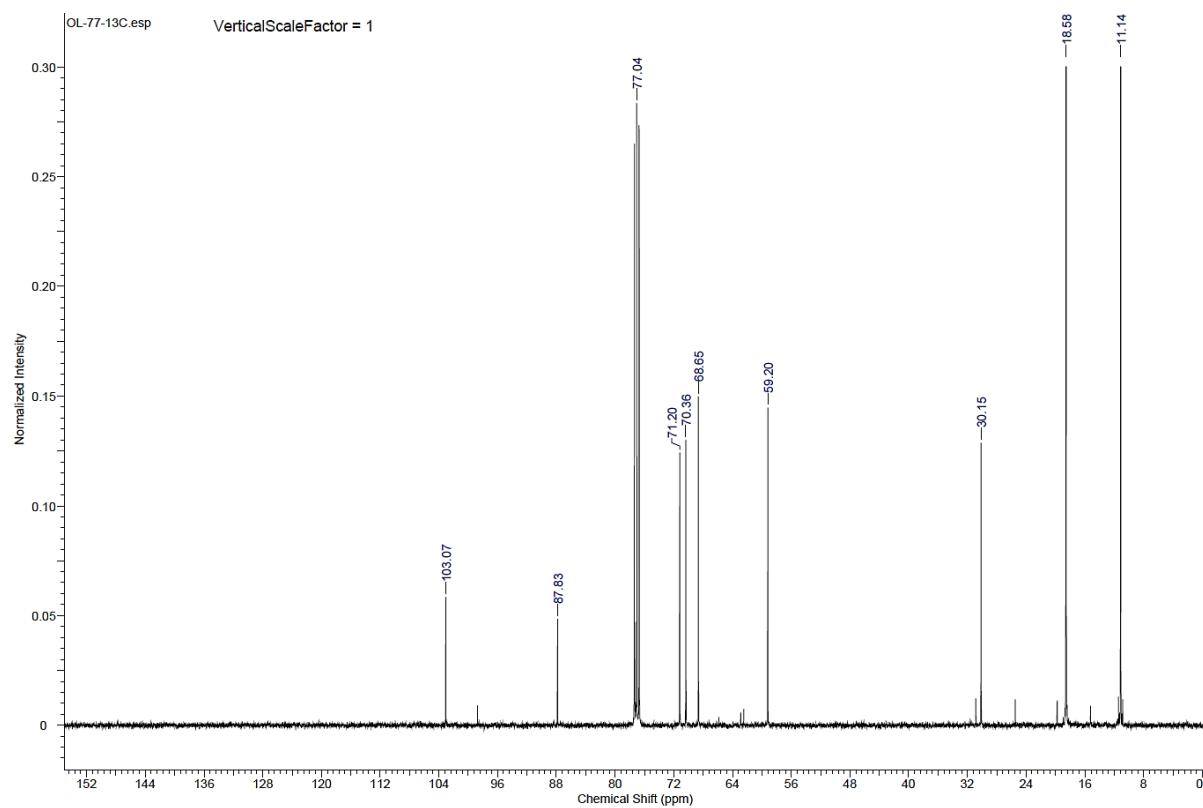


Figure S38: ¹³C-NMR spectrum of compound 12b.

1.22 Propargyl-DEG bromide 13b

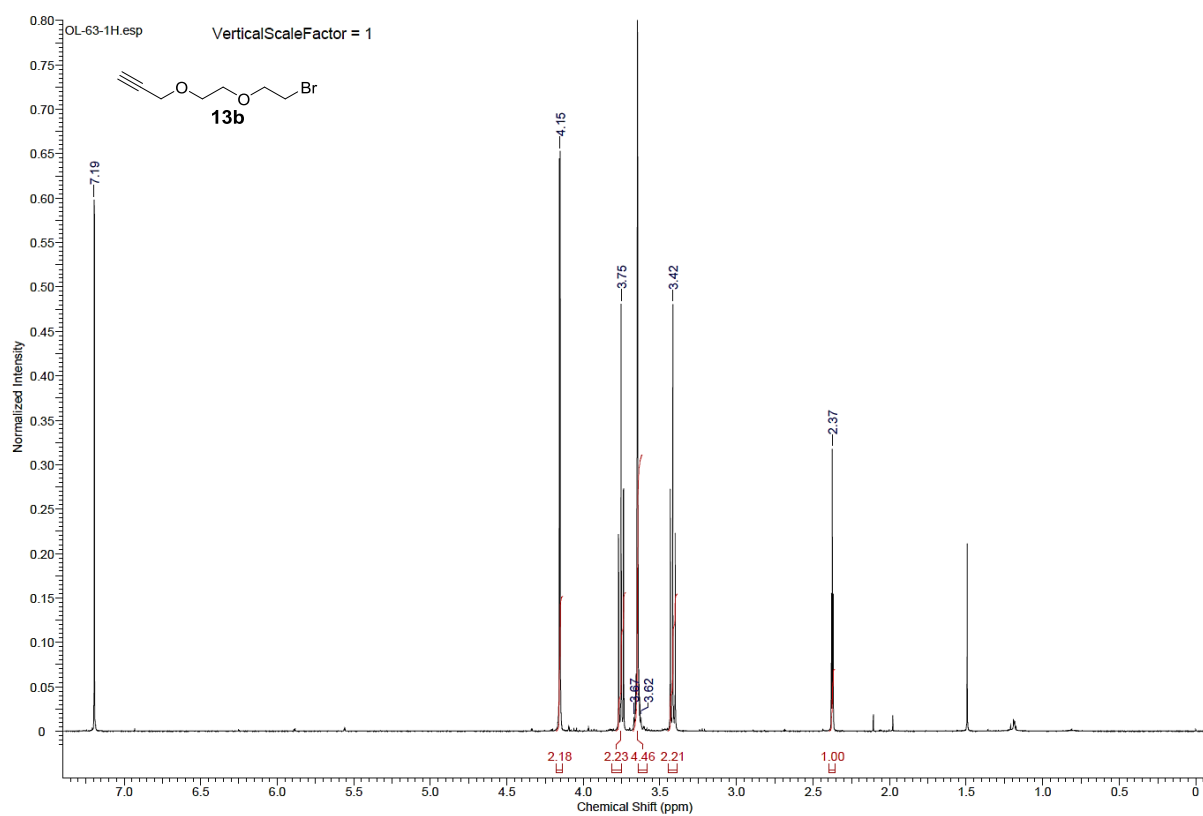


Figure S39: ¹H-NMR spectrum of compound 13b.

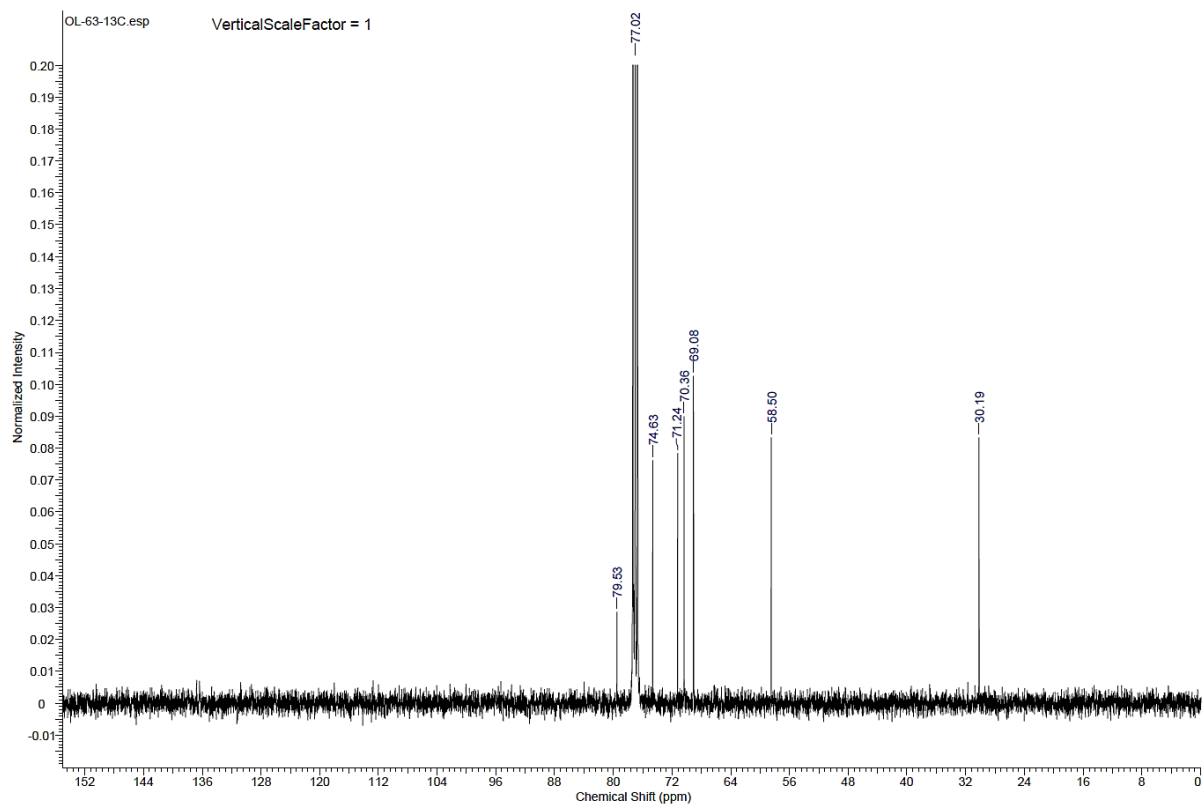


Figure S40: ¹³C-NMR spectrum of compound 13b.

1.23 Di(*O*-THP)-*O*-DEG-propargyl CTV 15b

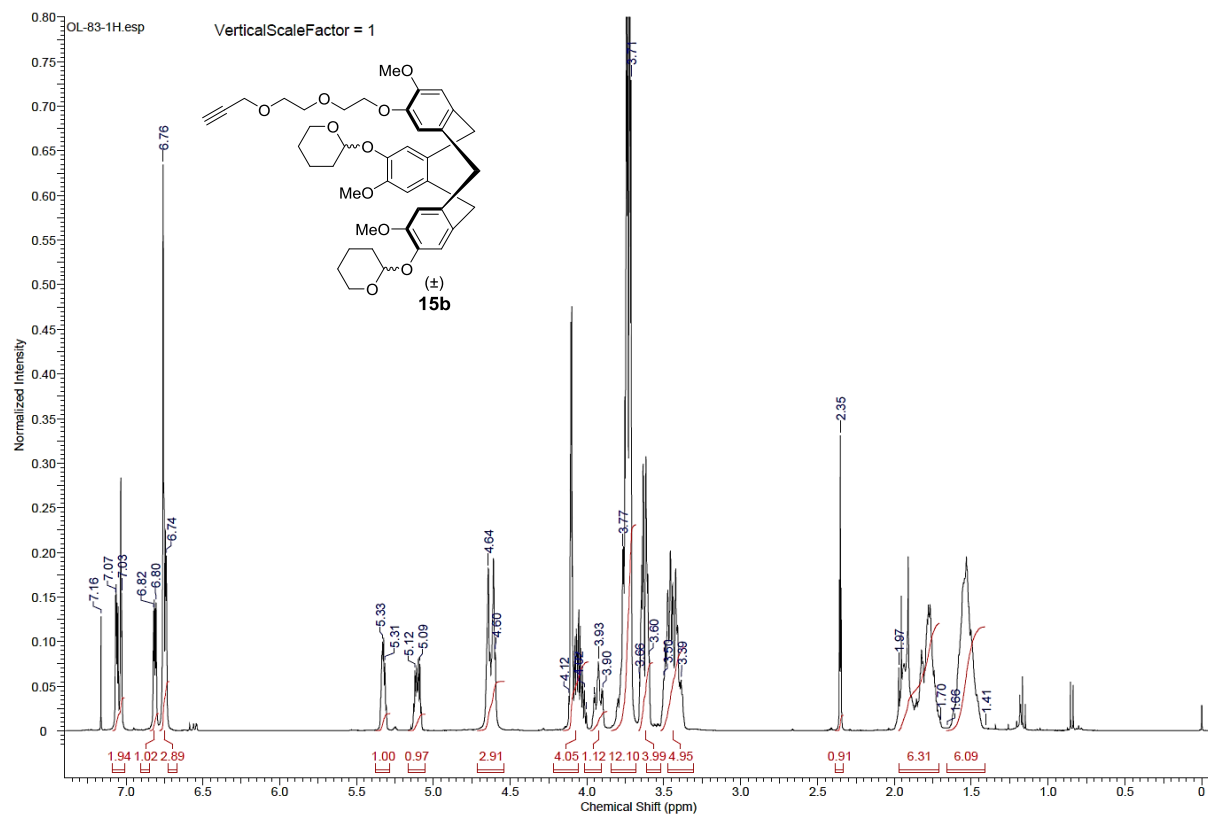


Figure S41: ¹H-NMR spectrum of compound 15b.

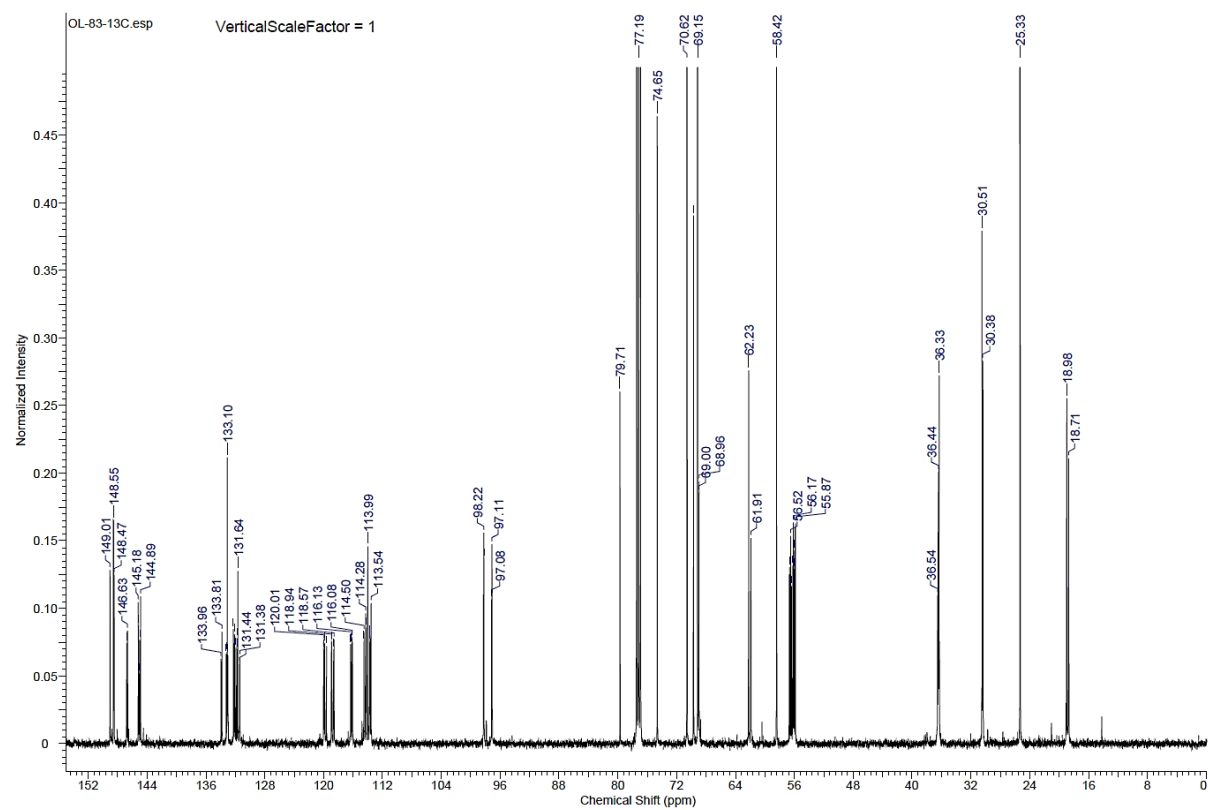


Figure S42: ¹³C-NMR spectrum of compound 15b.

1.24 O-DEG-Propargyl CTV-diOH 16b

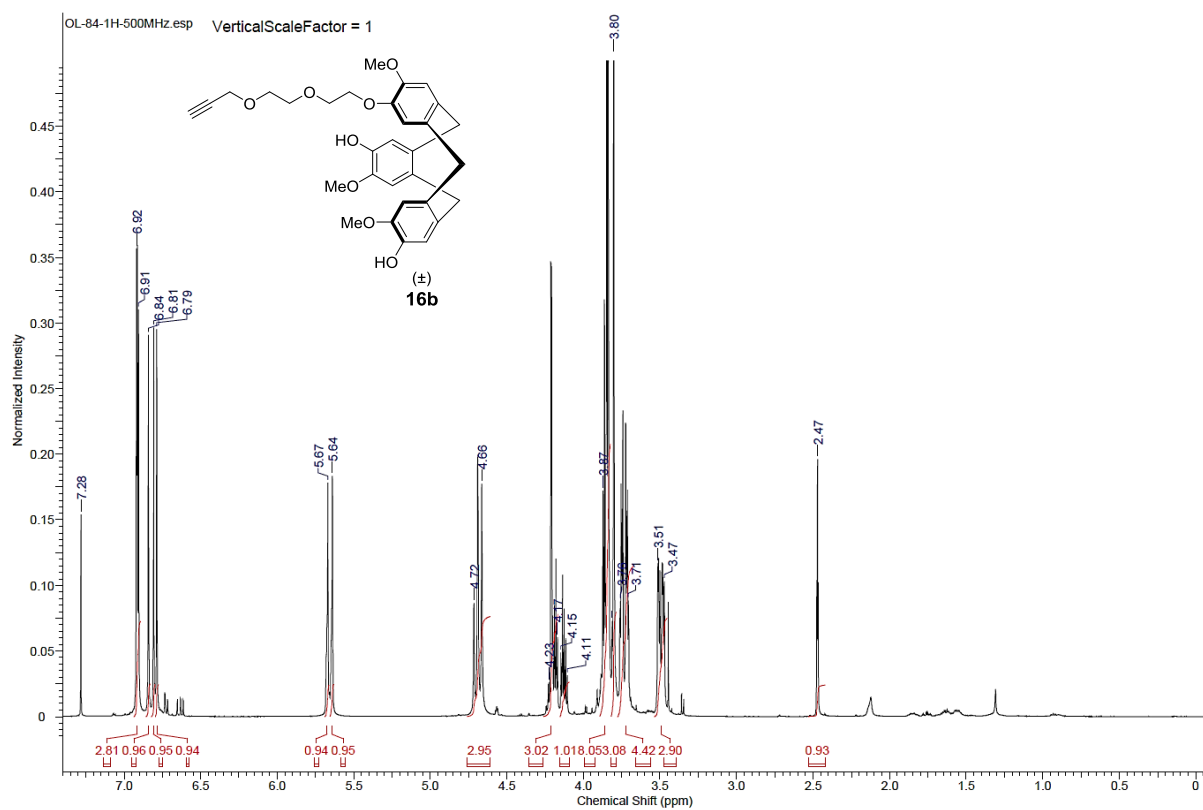


Figure S43: ¹H-NMR spectrum of compound 16b.

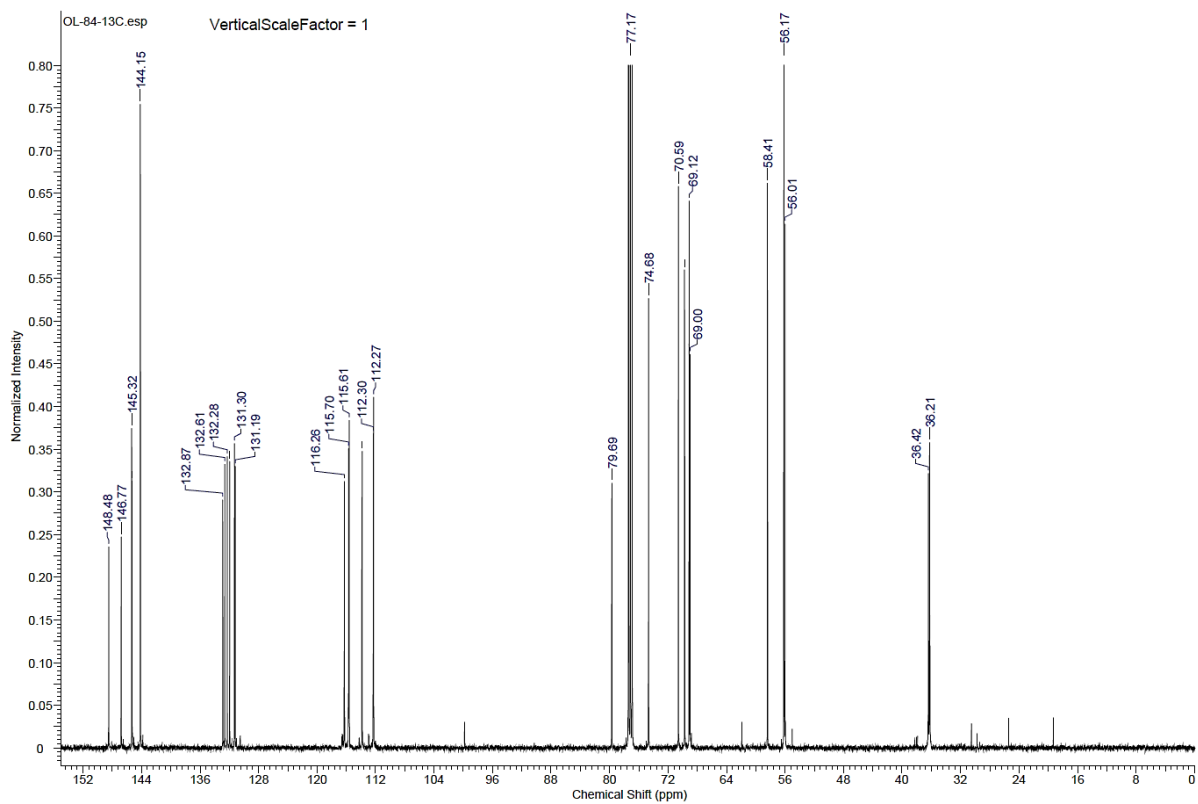


Figure S44: ¹³C-NMR spectrum of compound 16b.

1.25 O-DEG-Propargyl-O-DEG-(TIPS)propargyl CTV-OH 17b

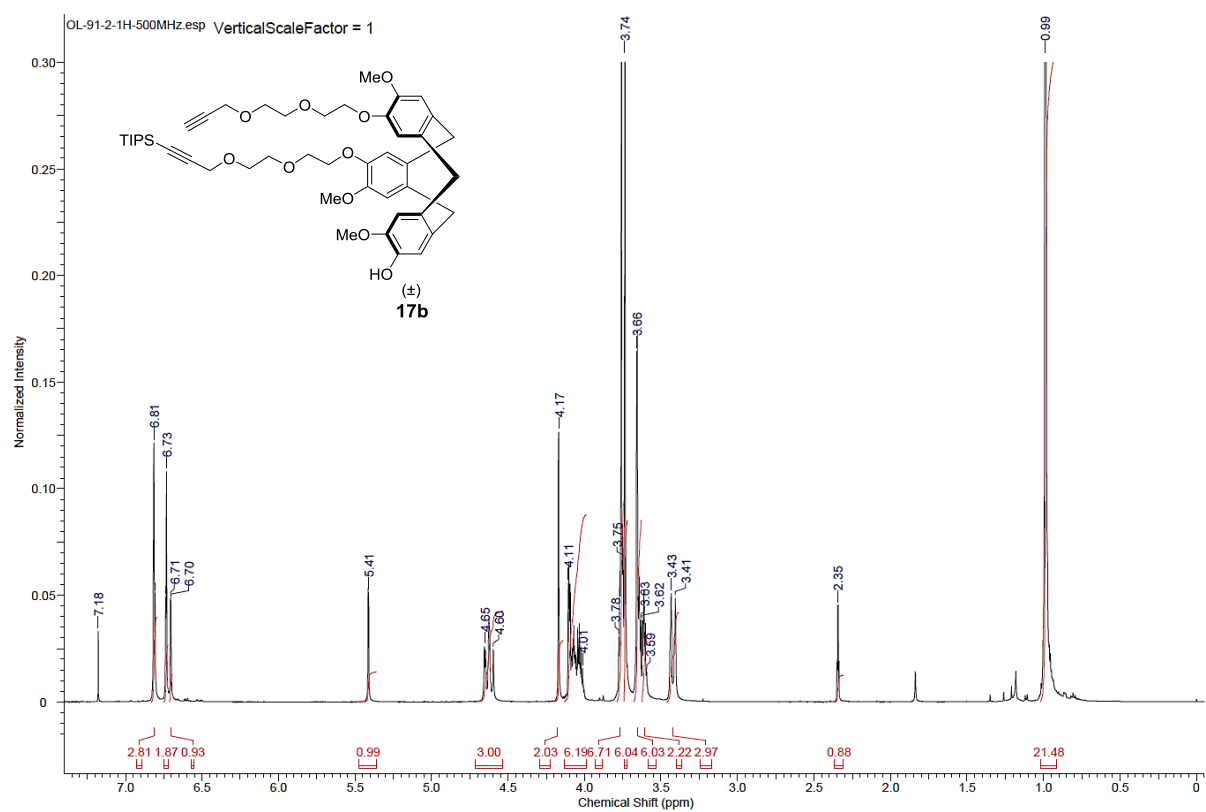


Figure S45: ¹H-NMR spectrum of compound 17b.

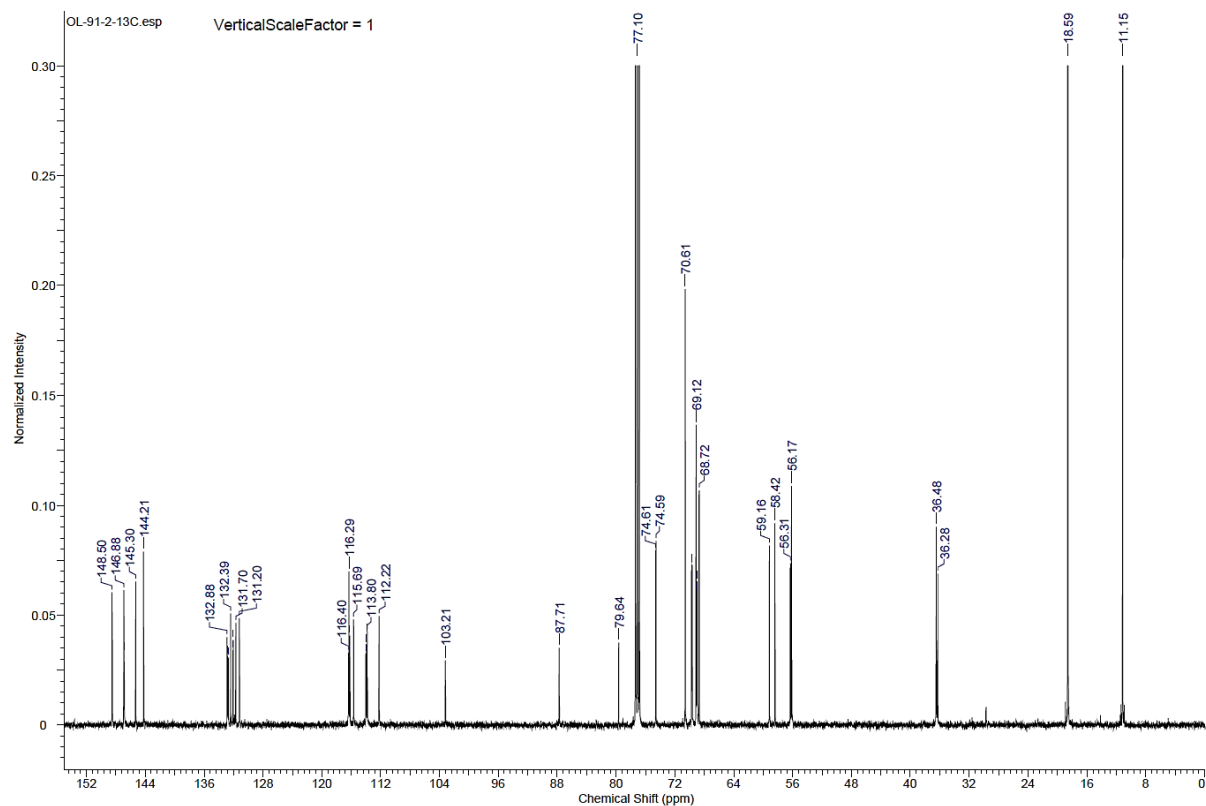


Figure S46: ¹³C-NMR spectrum of compound 17b.

1.26 Side product 18b

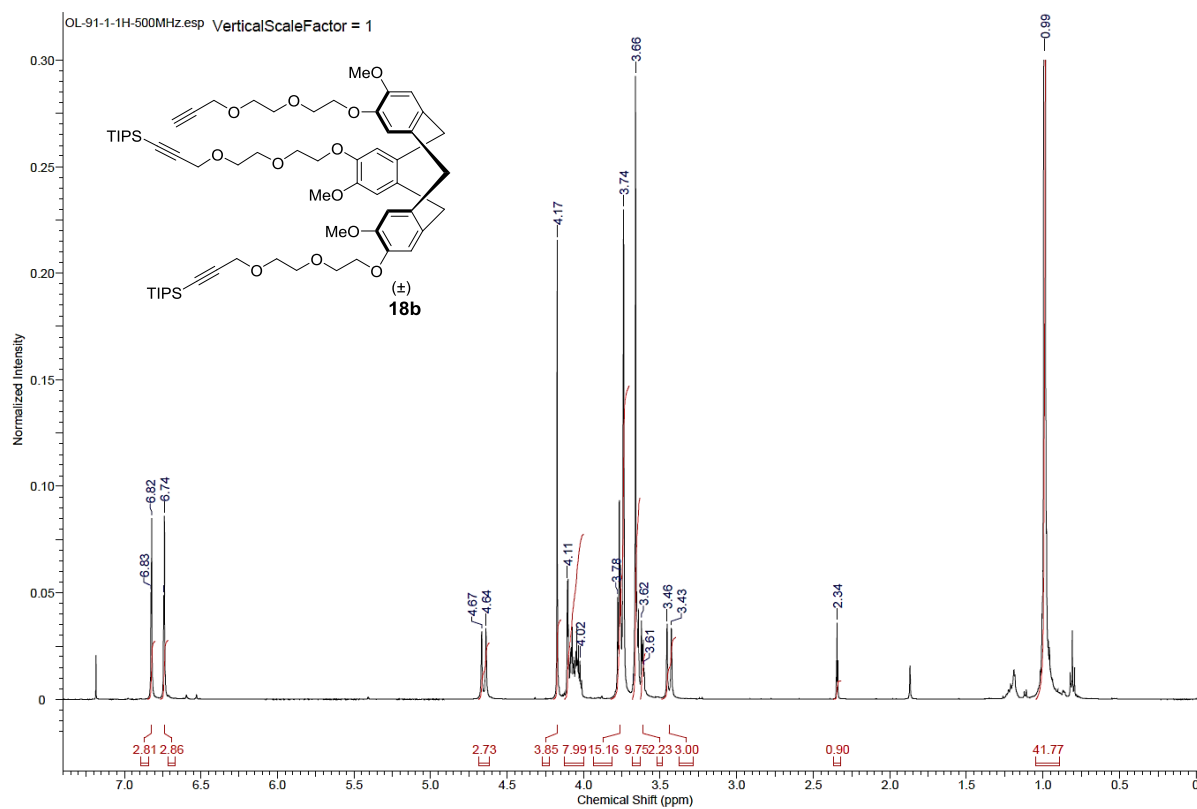


Figure S47: $^1\text{H-NMR}$ spectrum of compound **18b**.

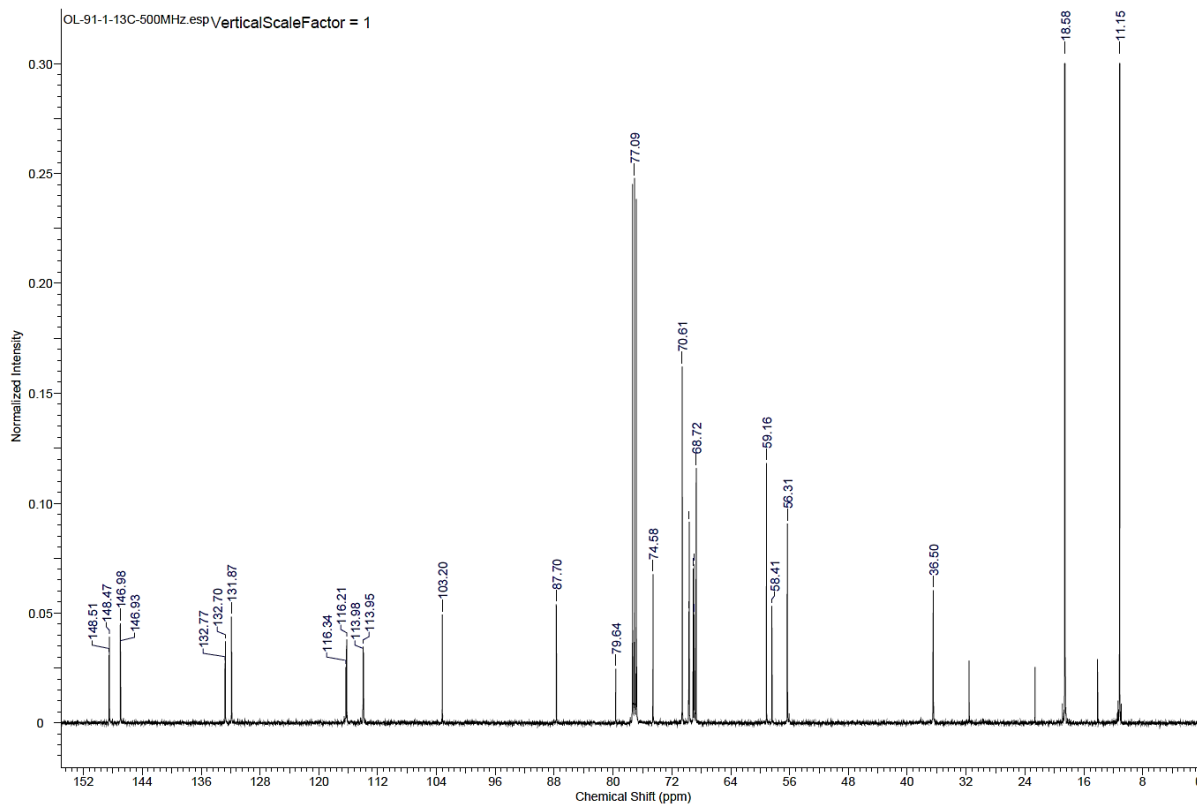


Figure S48: $^{13}\text{C-NMR}$ spectrum of compound **18b**.

1.27 Tri-O-Propargyl CTV 20

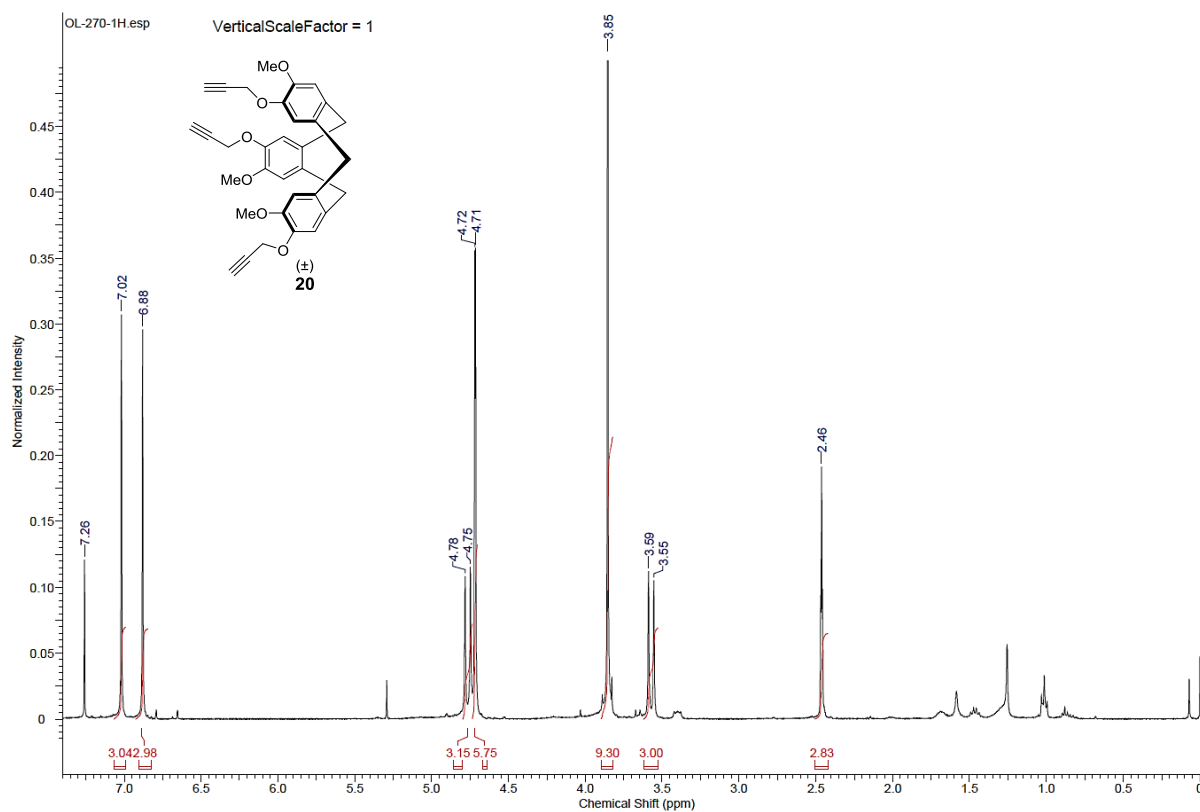


Figure S49: ¹H-NMR spectrum of compound 20.

1.28 Tri-O-MEG-Propargyl CTV 21

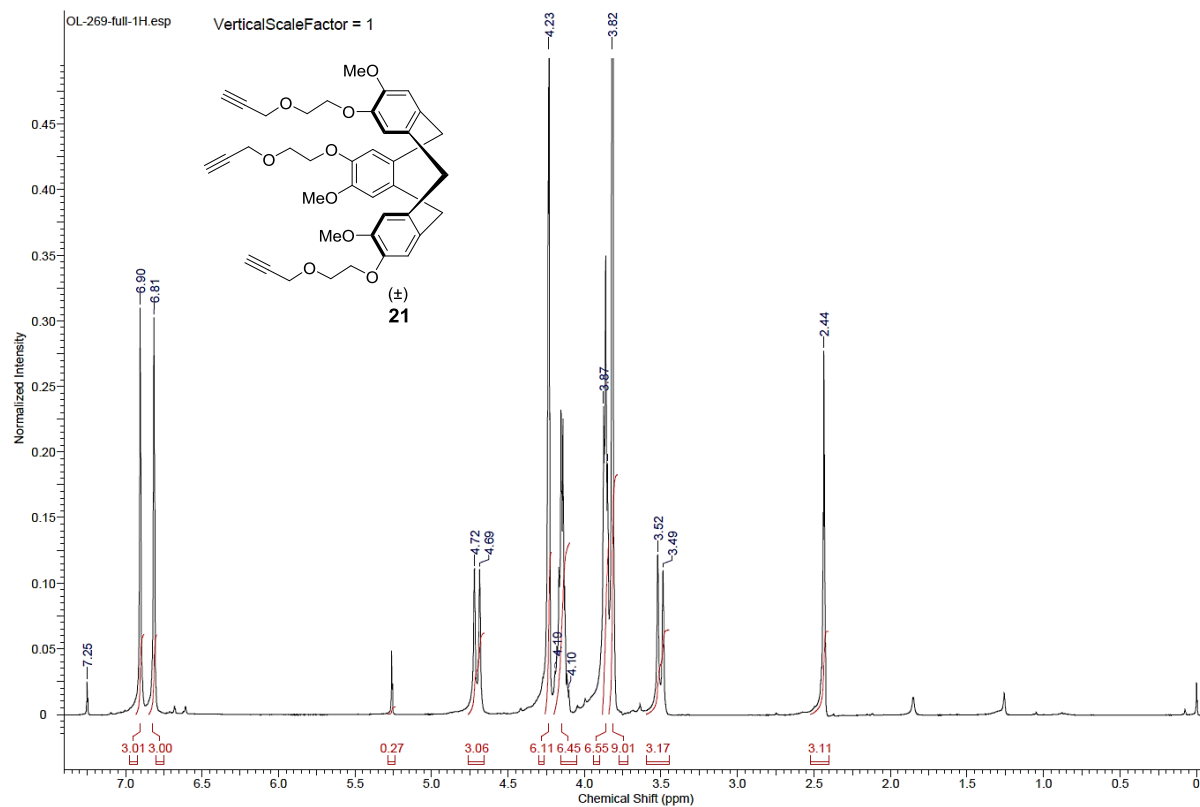


Figure S50: ¹H-NMR spectrum of compound 21.

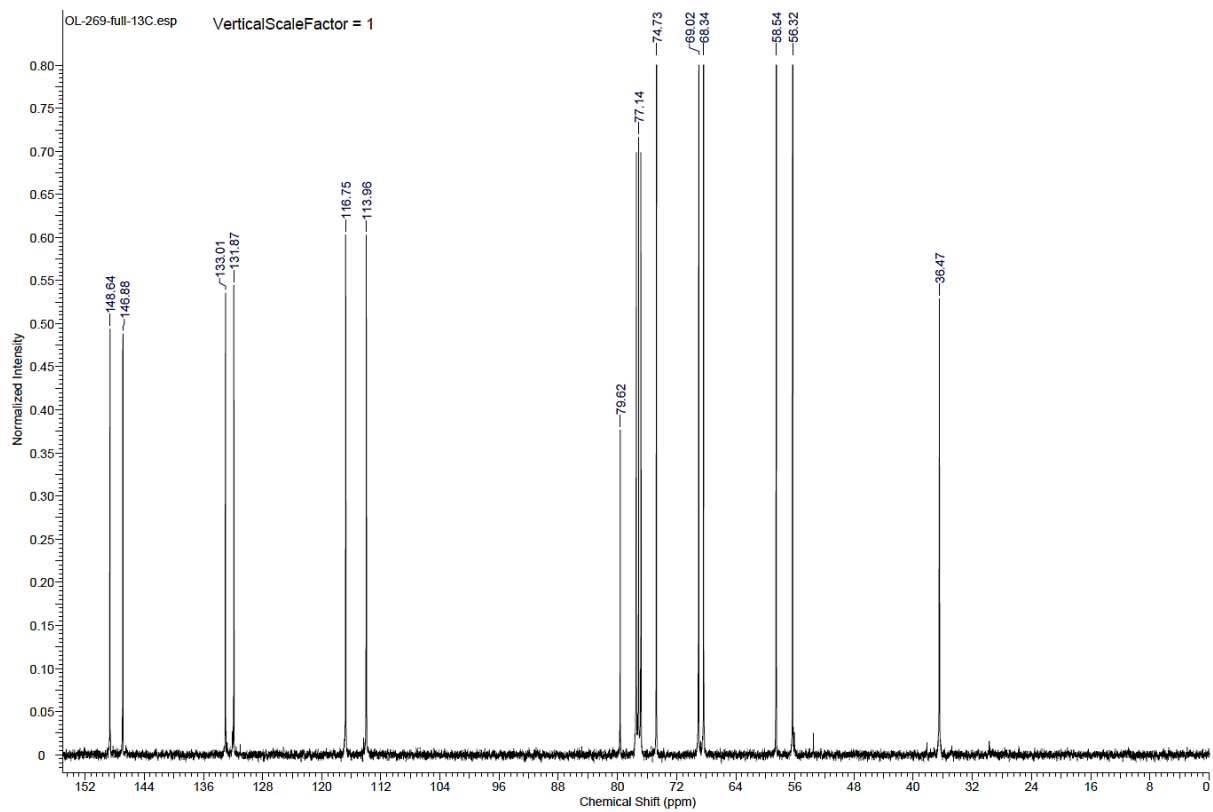


Figure S51: ¹³C-NMR spectrum of compound 21.

1.29 Tri-O-DEG-Propargyl CTV 22

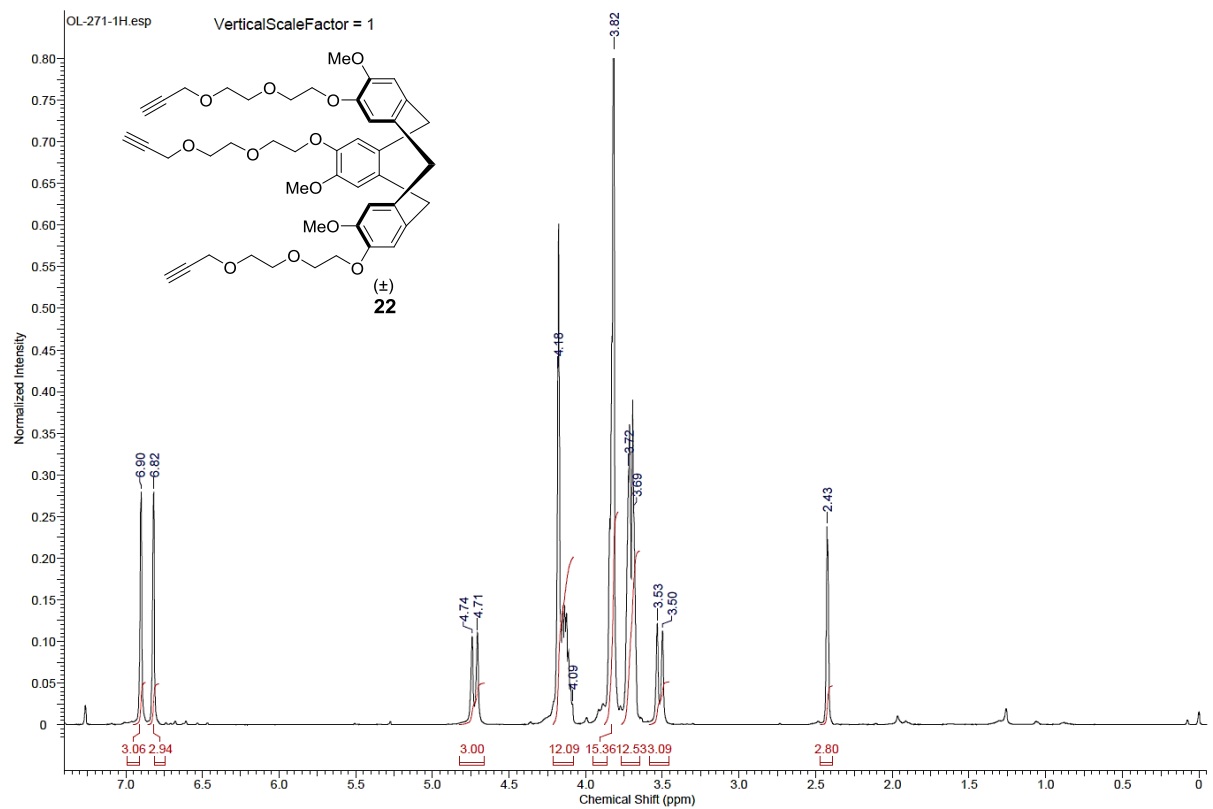


Figure S52: ¹H-NMR spectrum of compound 22.

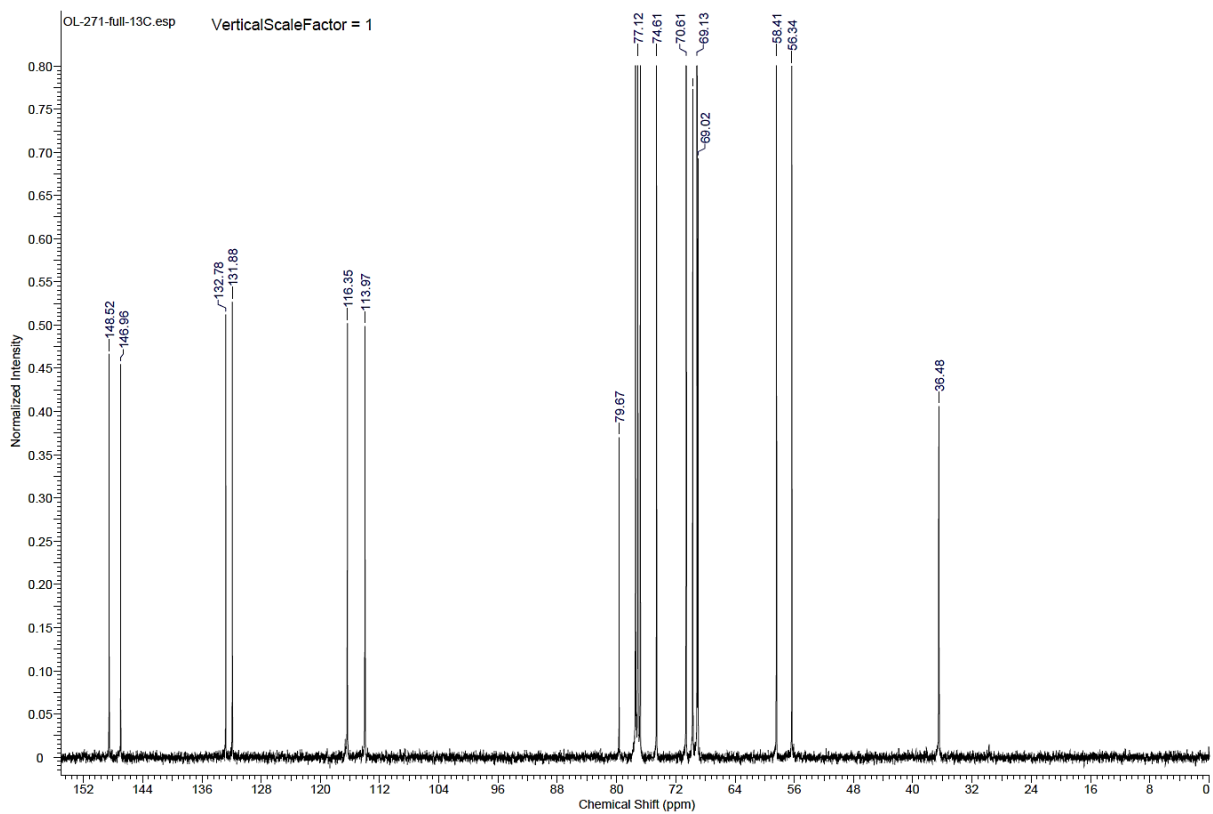


Figure S53: ^{13}C -NMR spectrum of compound 22.

1.30 Synthetic antibody 35

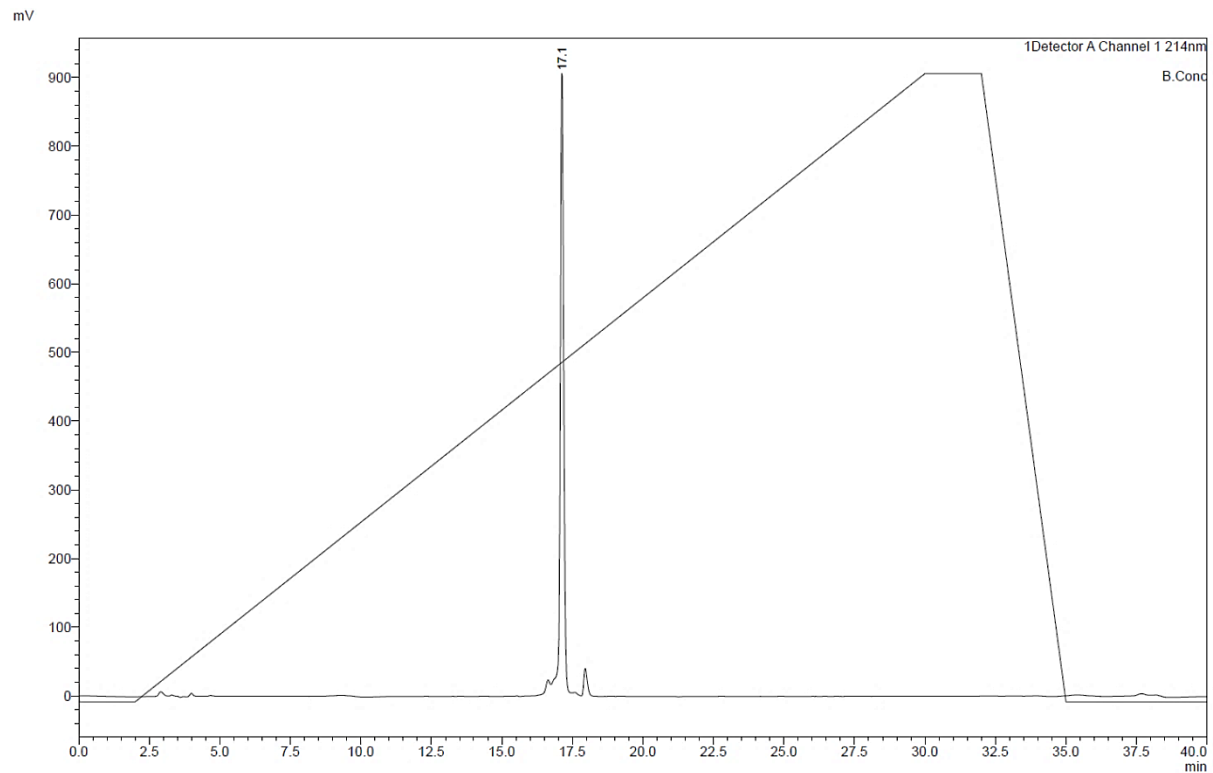


Figure S54: Analytical HPLC chromatogram of synthetic antibody 35.

Data: pk_OLP119_cyano_ref_pos0001.3L3[c] 16 Mar 2018 11:42 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 116, P.Ext. @ 4400 (bin 207)

%Int. 77 mV[sum= 13954 mV] Profiles 1-181 Unsmoothed -Baseline 50

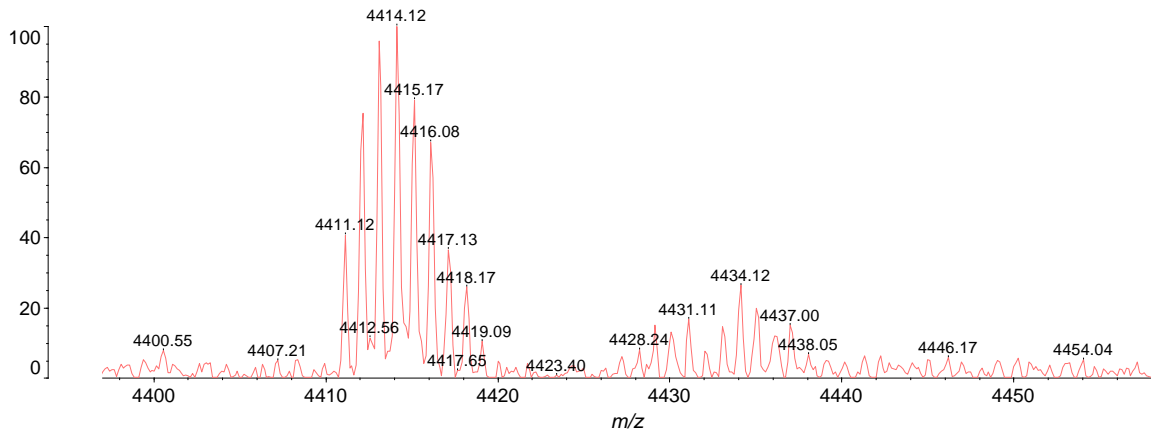


Figure S55: MALDI spectrum of synthetic antibody 35.

1.31 Synthetic antibody 36

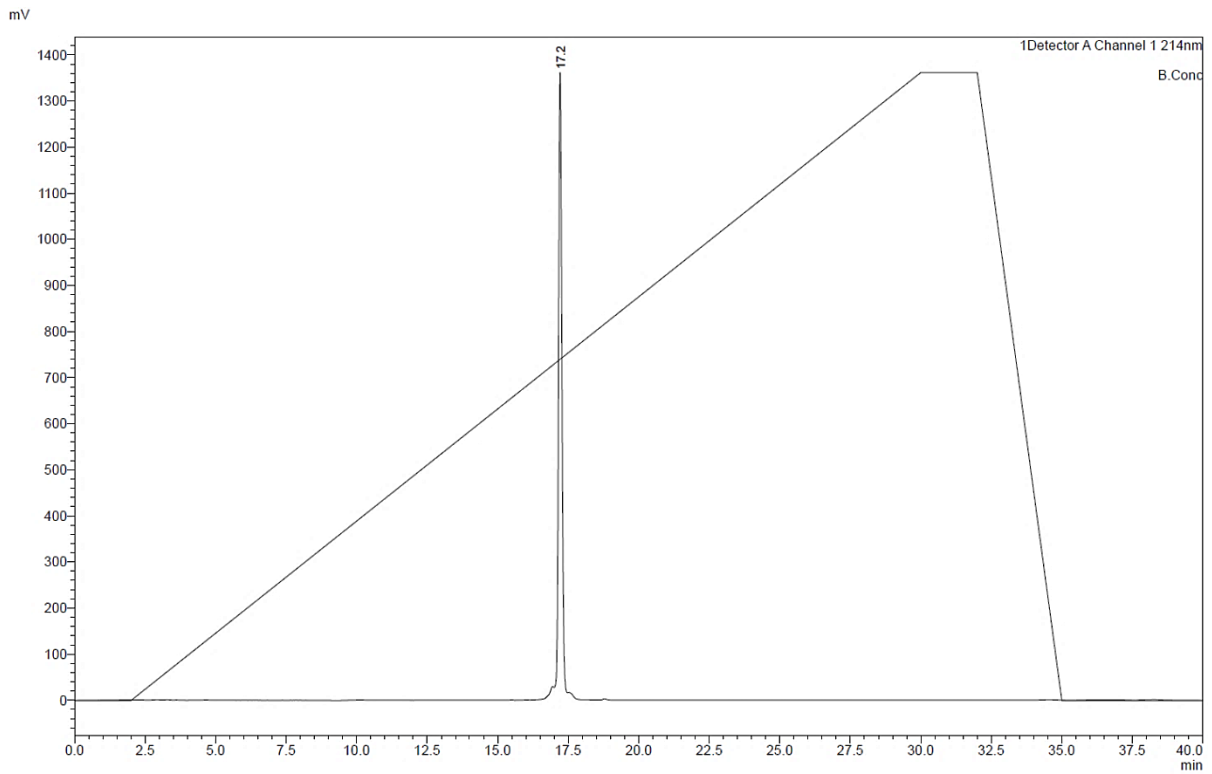


Figure S56: Analytical HPLC chromatogram of synthetic antibody 36.

Data: pk_OLP115_cyano_ref_pos0001.3L1[c] 16 Mar 2018 10:57 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 111, P.Ext. @ 4500 (bin 210)

%Int. 175 mV[sum= 31951 mV] Profiles 1-183 Unsmoothed -Baseline 50

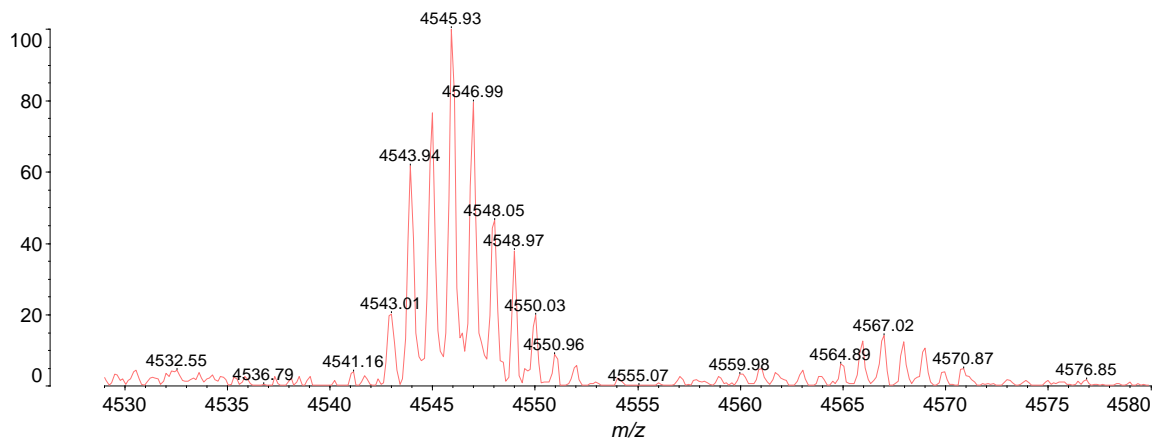


Figure S57: MALDI spectrum of synthetic antibody 36.

1.32 Synthetic antibody 37

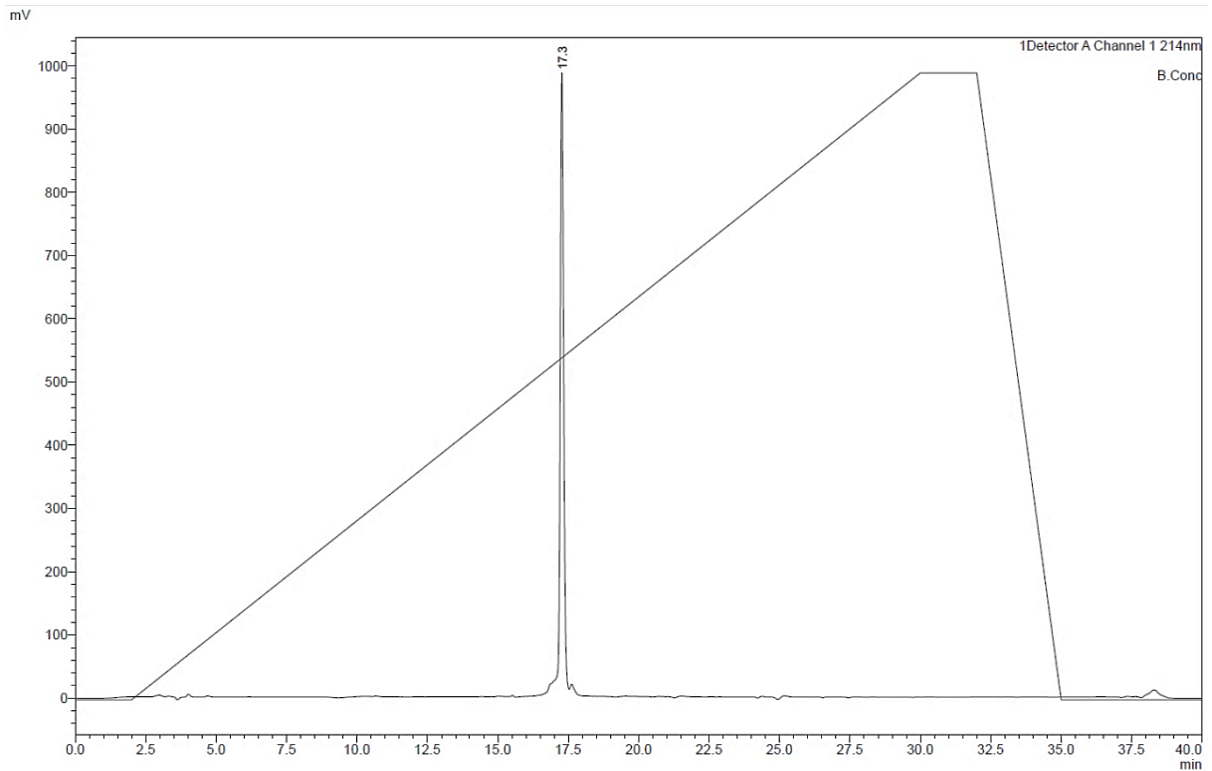


Figure S58: Analytical HPLC chromatogram of synthetic antibody 37.

Data: pk_OLP125_cyano_ref_pos0001.3J4[c] 16 Mar 2018 13:54 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4600 (bin 212)

%Int. 210 mV[sum= 21389 mV] Profiles 1-102 Unsmoothed -Baseline 50

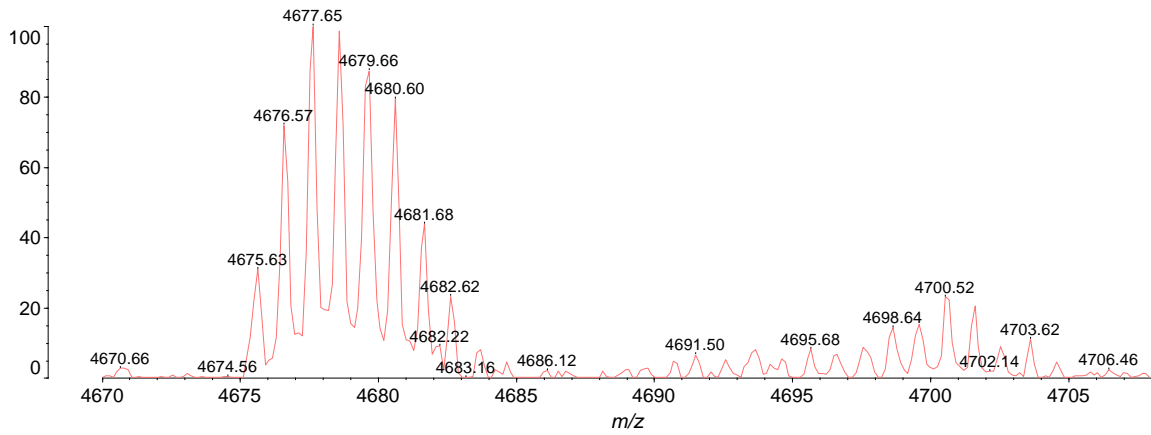


Figure S59: MALDI spectrum of synthetic antibody 37.

1.33 Synthetic antibody 38

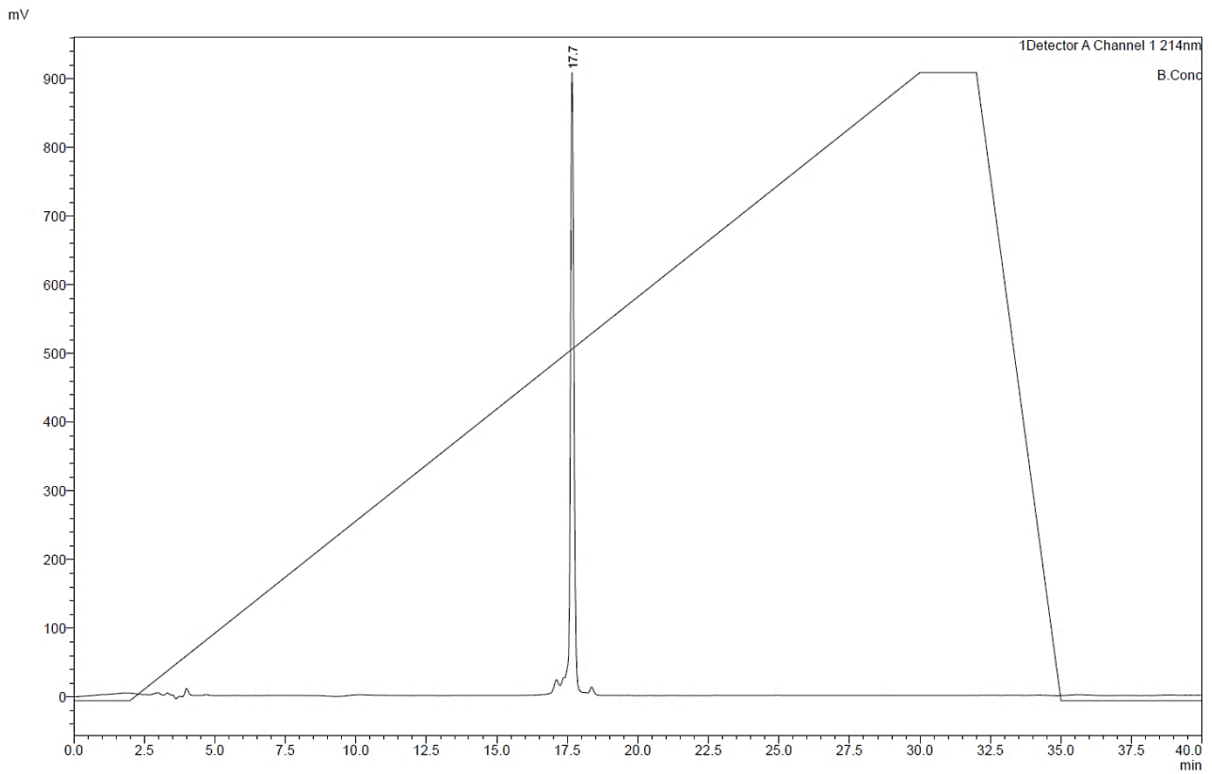


Figure S60: Analytical HPLC chromatogram of synthetic antibody 38.

Data: pk_OLP120_cyano_ref_pos0001.3L4[c] 16 Mar 2018 11:48 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 116, P.Ext. @ 4350 (bin 206)

%Int. 73 mV[sum= 8896 mV] Profiles 10-131 Unsmoothed -Baseline 50

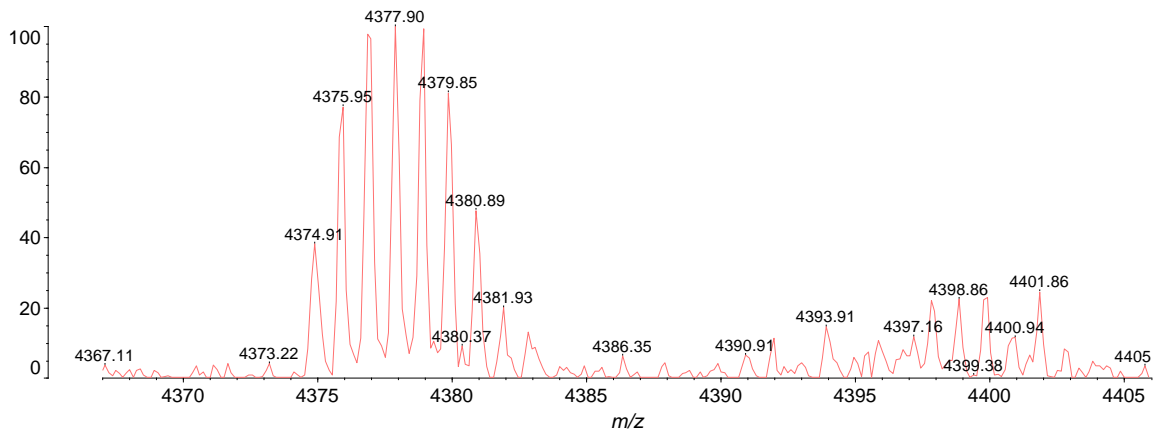


Figure S61: MALDI spectrum of synthetic antibody 38.

1.34 Synthetic antibody 39

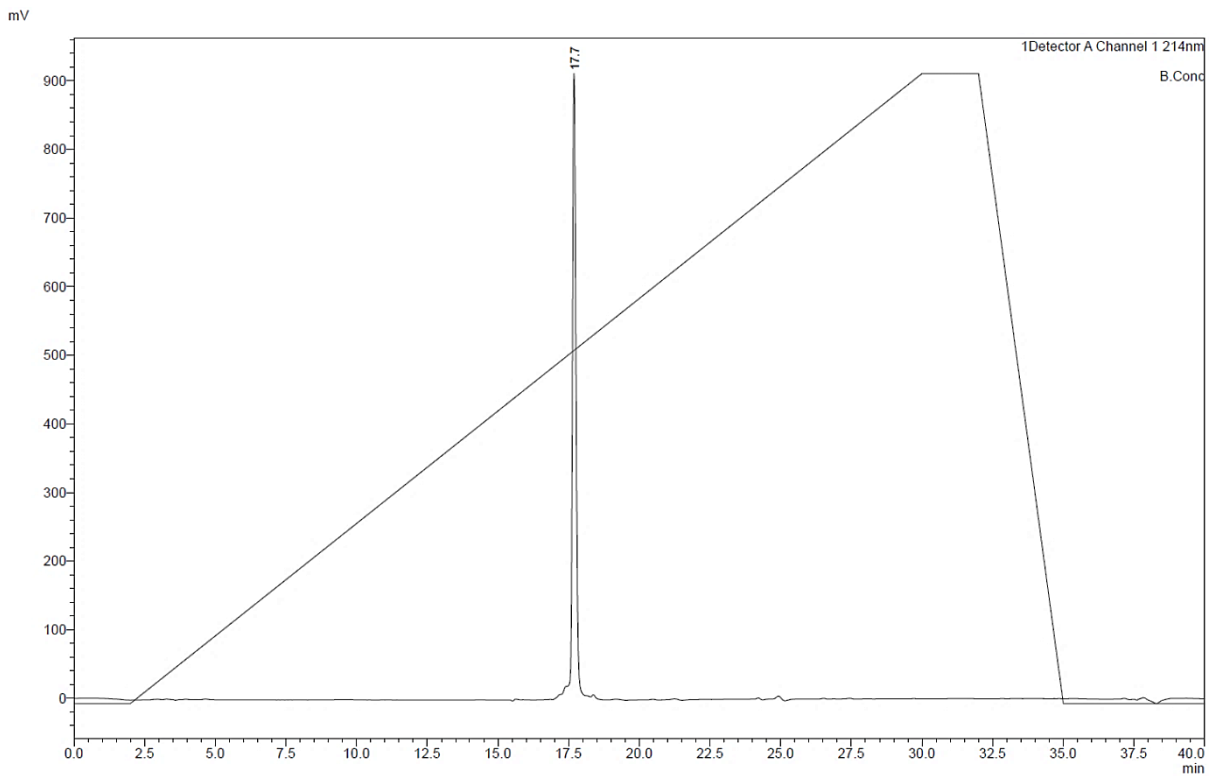


Figure S62: Analytical HPLC chromatogram of synthetic antibody 39.

Data: pk_OLP116_cyano_ref_pos0001.3L2[c] 16 Mar 2018 11:05 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 111, P.Ext. @ 4500 (bin 210)

%Int. 101 mV[sum= 9419 mV] Profiles 89-181 Unsmoothed -Baseline 50

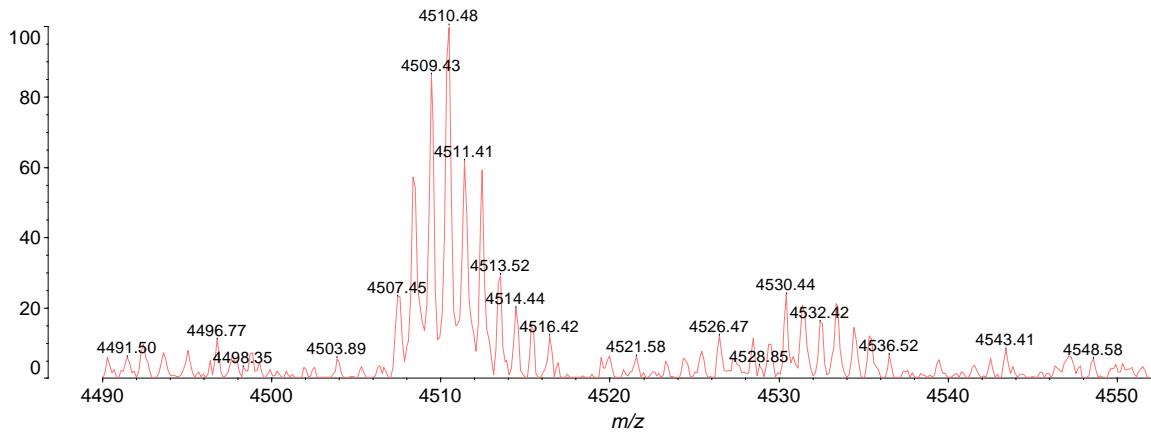


Figure S63: MALDI spectrum of synthetic antibody 39.

1.35 Synthetic antibody 40

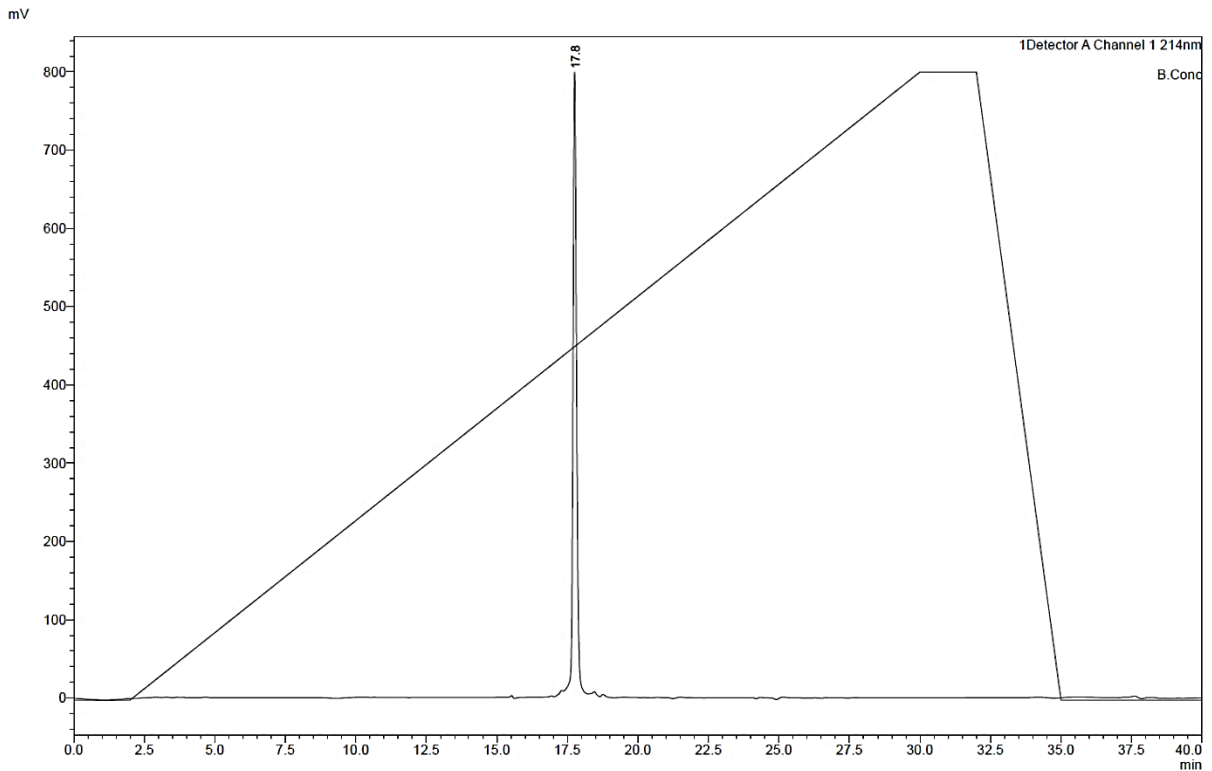


Figure S64: Analytical HPLC chromatogram of synthetic antibody 40.

Data: pk_OLP126_cyano_ref_pos0001.3H1[c] 16 Mar 2018 13:57 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4600 (bin 212)

%Int. 49 mV[sum= 6948 mV] Profiles 1-141 Unsmoothed -Baseline 50

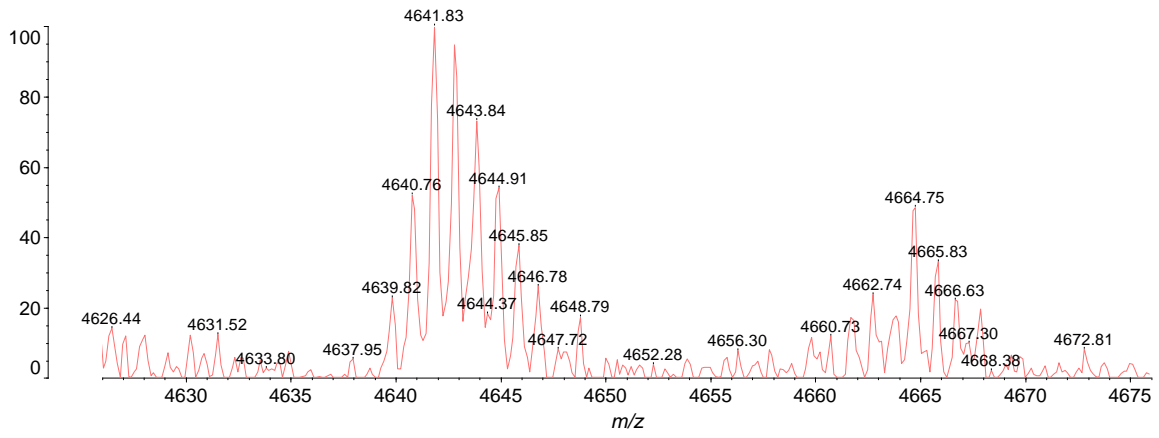


Figure S65: MALDI spectrum of synthetic antibody 40.

1.36 Synthetic antibody 44

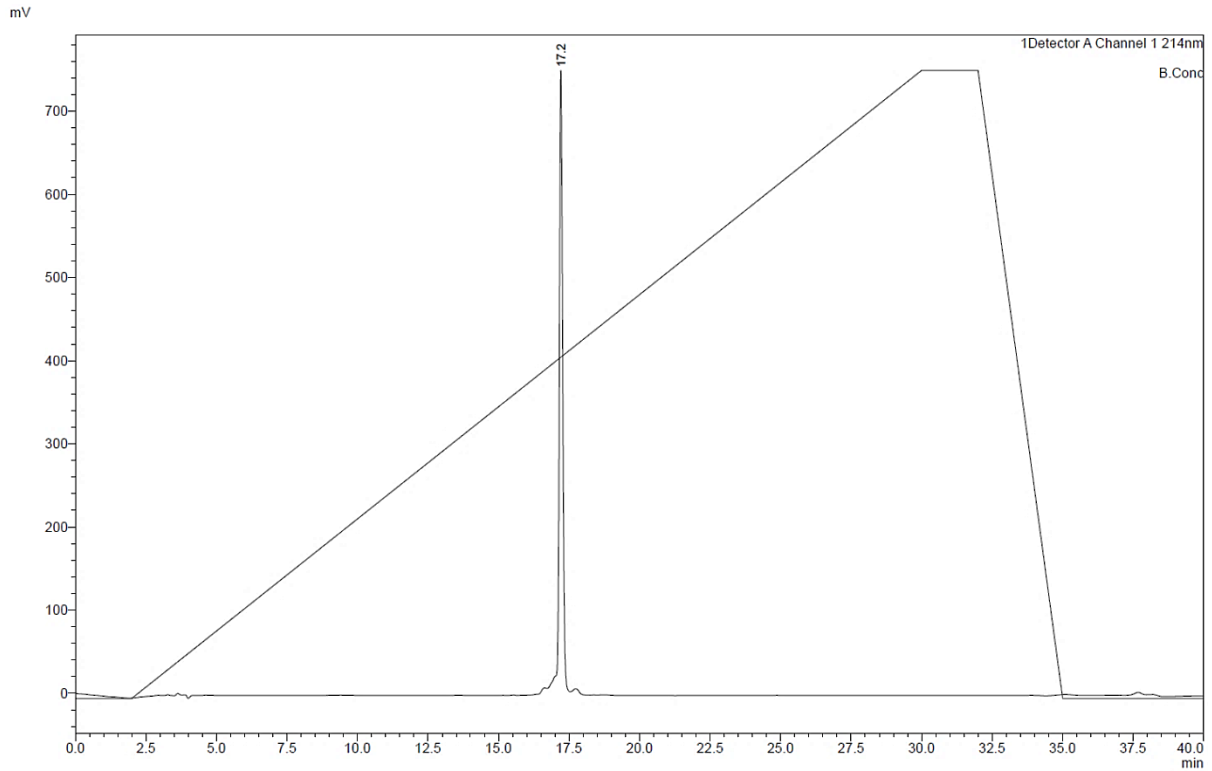


Figure S66: Analytical HPLC chromatogram of synthetic antibody 44.

Data: pk_OCP122R_cyano_ref_pos0002.3H2[c] 18 Jan 2018 15:15 Cal: 3 PEP MIX 18 Jan 2018 14:38
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 120, P.Ext. @ 4200 (bin 202)

%Int. 485 mV[sum= 51412 mV] Profiles 1-106 Unsmoothed

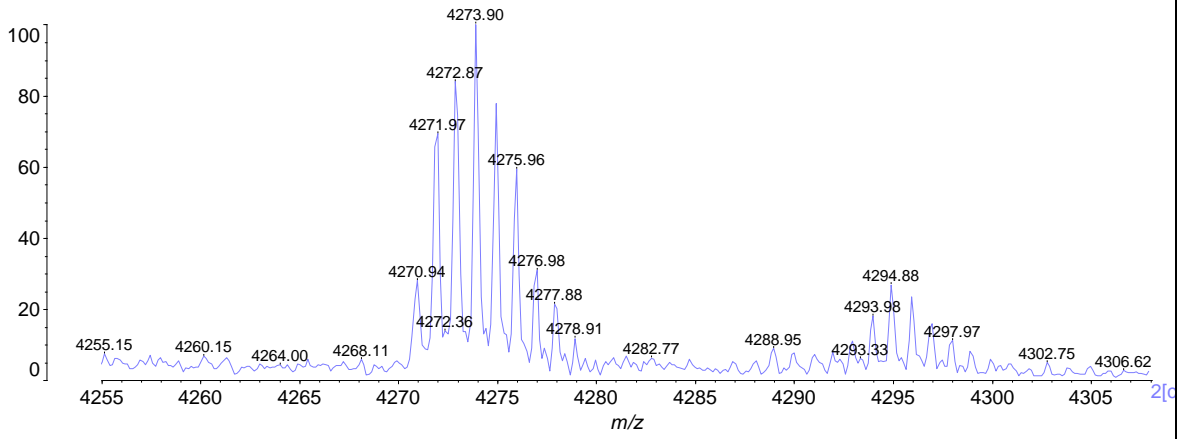


Figure S67: MALDI spectrum of synthetic antibody 44.

1.37 Synthetic antibody 45

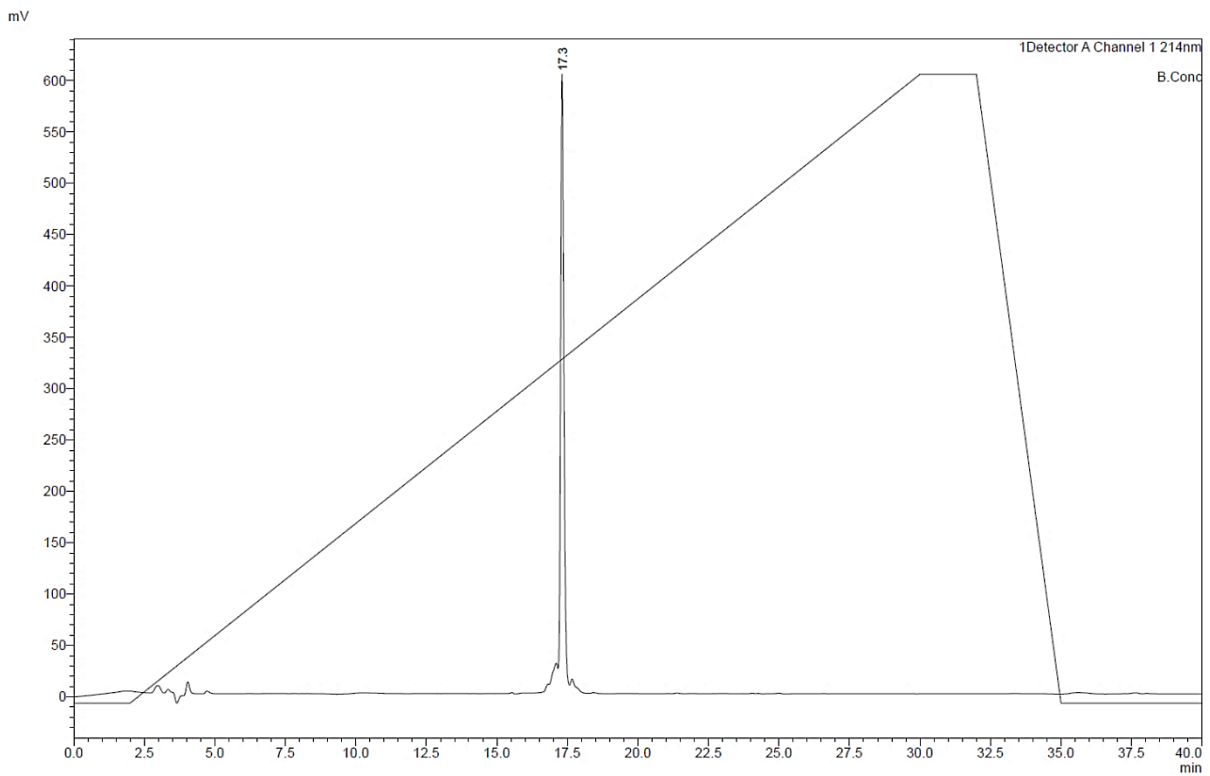


Figure S68: Analytical HPLC chromatogram of synthetic antibody 45.

Data: pk_OLP128_cyano_ref_pos0001.3H3[c] 16 Mar 2018 14:05 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4300 (bin 205)

%Int. 32 mV[sum= 9638 mV] Profiles 1-300 Unsmoothed -Baseline 50

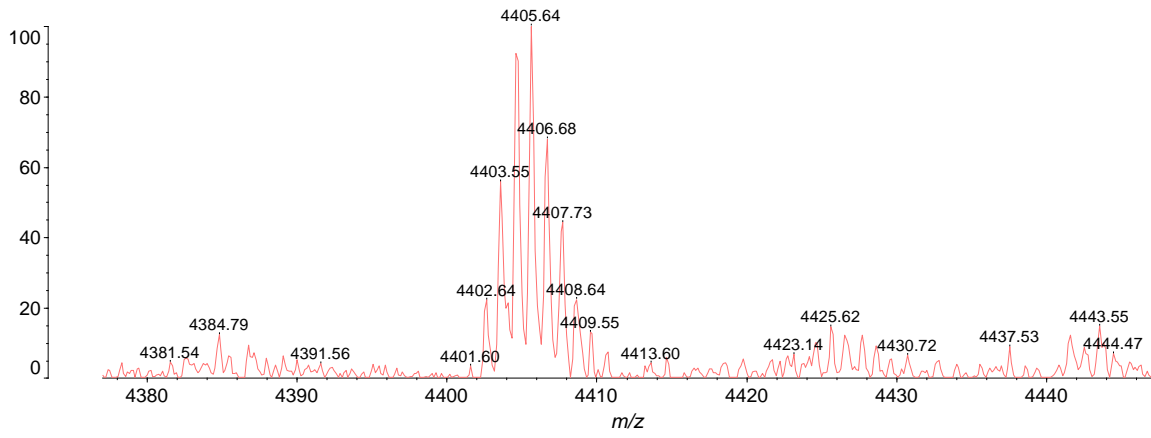


Figure S69: MALDI spectrum of synthetic antibody 45.

1.38 Synthetic antibody 46

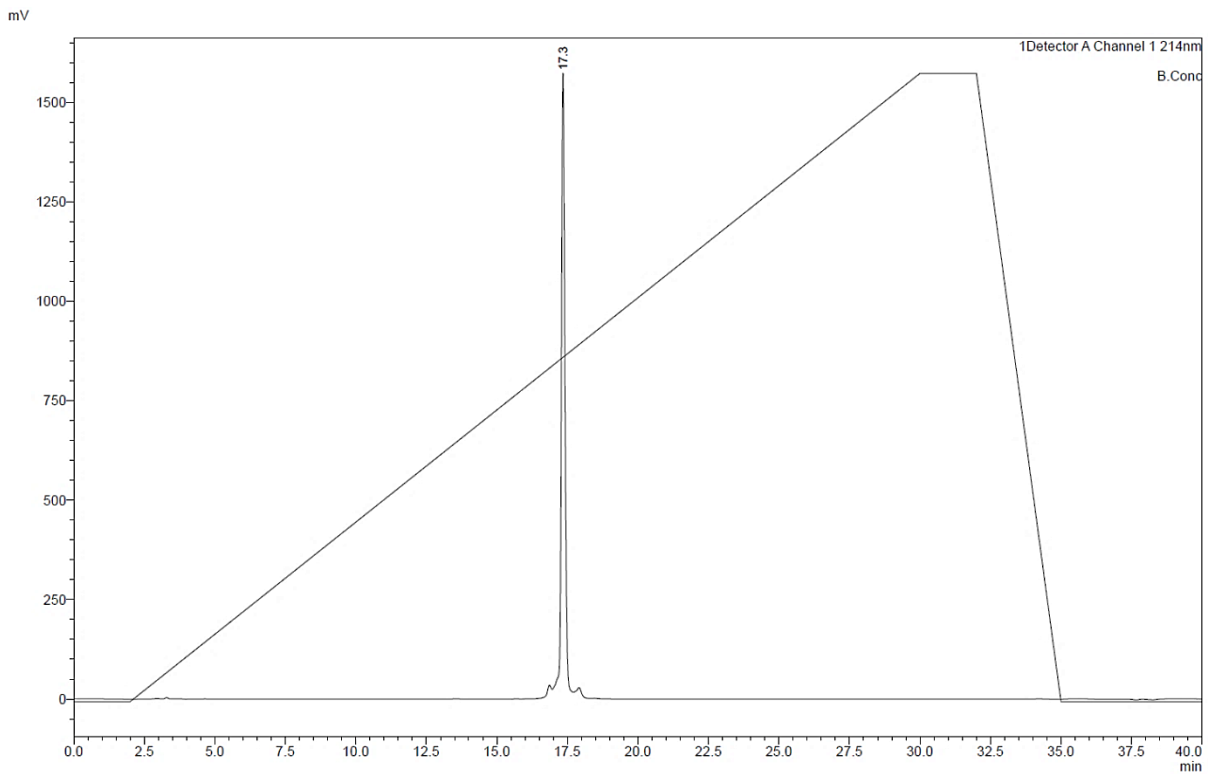


Figure S70: Analytical HPLC chromatogram of synthetic antibody 46.

Data: pk_OLP124_cyano_ref_pos0001.3J3[c] 16 Mar 2018 13:49 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4500 (bin 210)

%Int. 75 mV[sum= 12183 mV] Profiles 1-162 Unsmoothed -Baseline 50

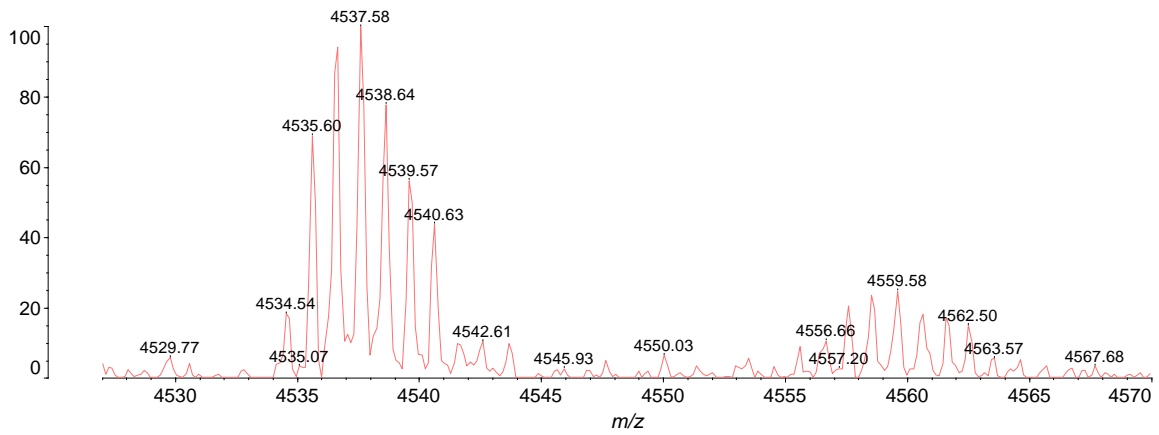


Figure S71: MALDI spectrum of synthetic antibody 46.

1.39 Synthetic antibody 47

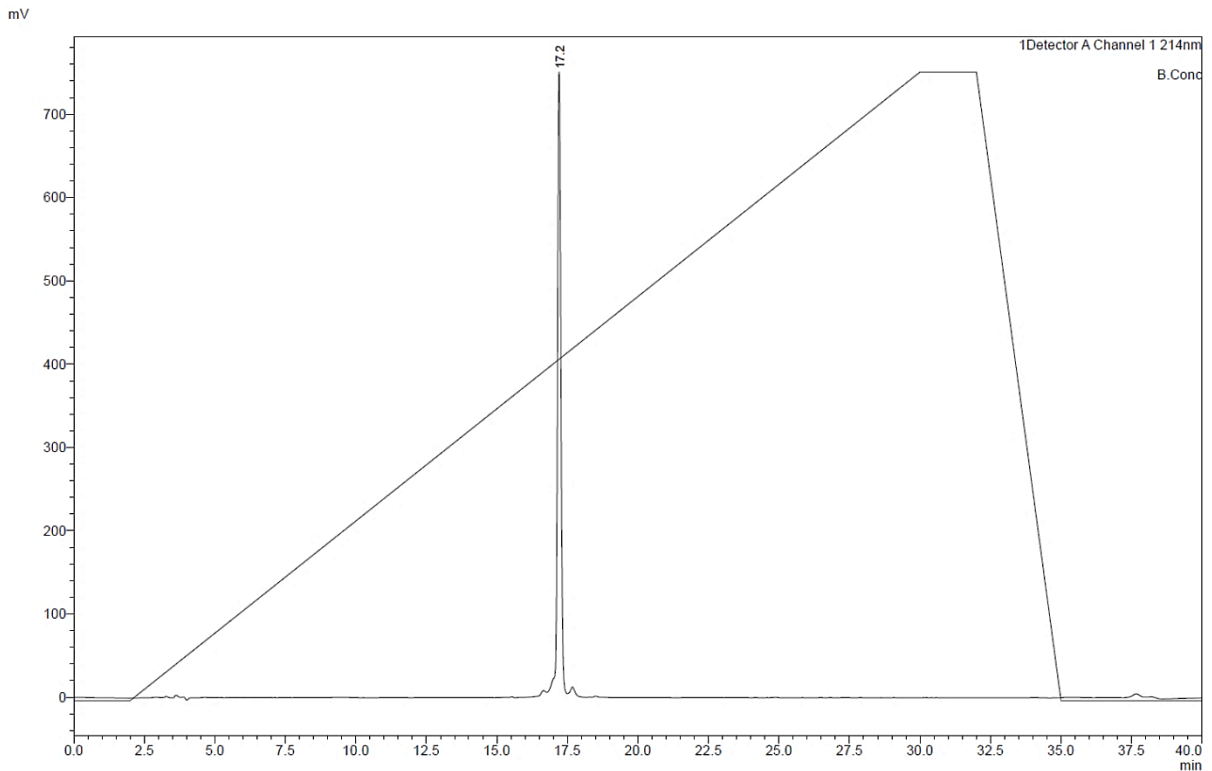


Figure S72: Analytical HPLC chromatogram of synthetic antibody 47.

Data: pk_OLP121_cyano_ref_pos0002.311[c] 16 Mar 2018 11:54 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 108, P.Ext. @ 4300 (bin 205)

%Int. 47 mV[sum= 8805 mV] Profiles 60-248 Unsmoothed -Baseline 50

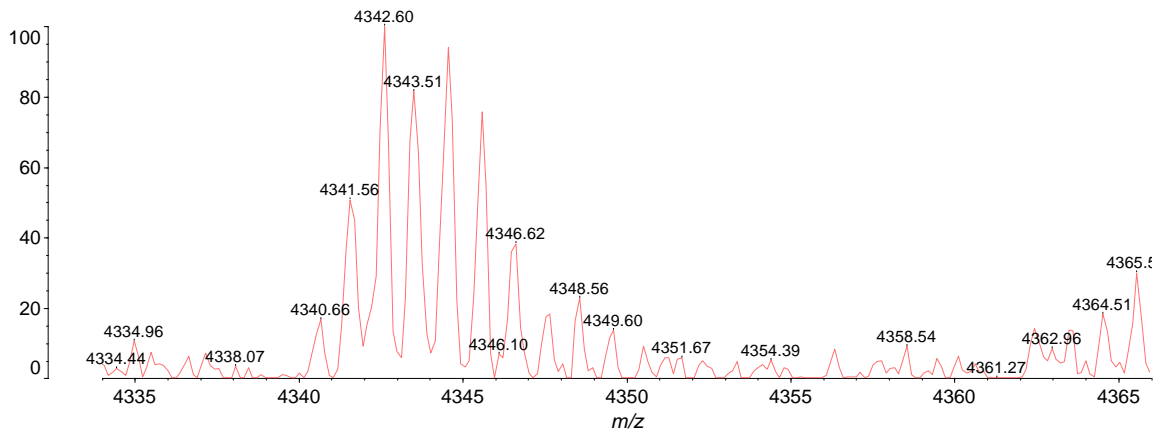


Figure S73: MALDI spectrum of synthetic antibody 47.

1.40 Synthetic antibody 48

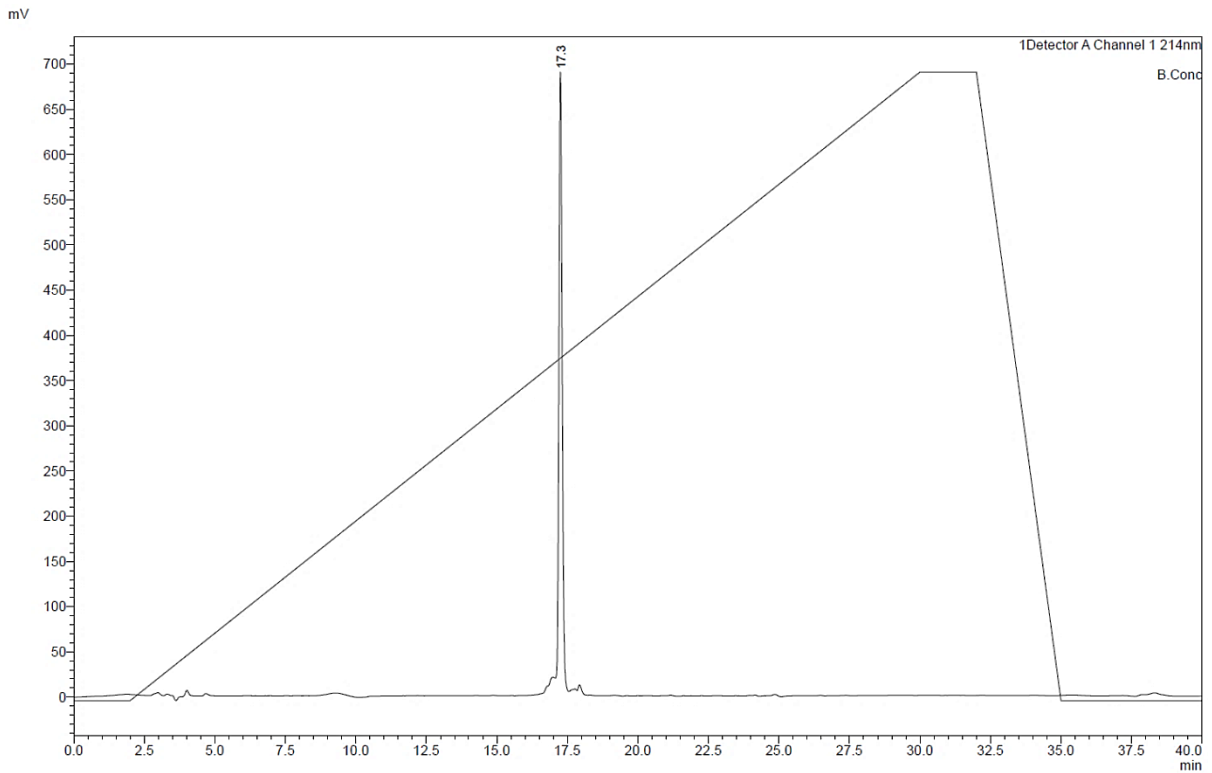


Figure S74: Analytical HPLC chromatogram of synthetic antibody 48.

Data: pk_OLP127_cyano_ref_pos0001.3H2[c] 16 Mar 2018 14:01 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4400 (bin 207)

%Int. 142 mV[sum= 19860 mV] Profiles 1-140 Unsmoothed -Baseline 50

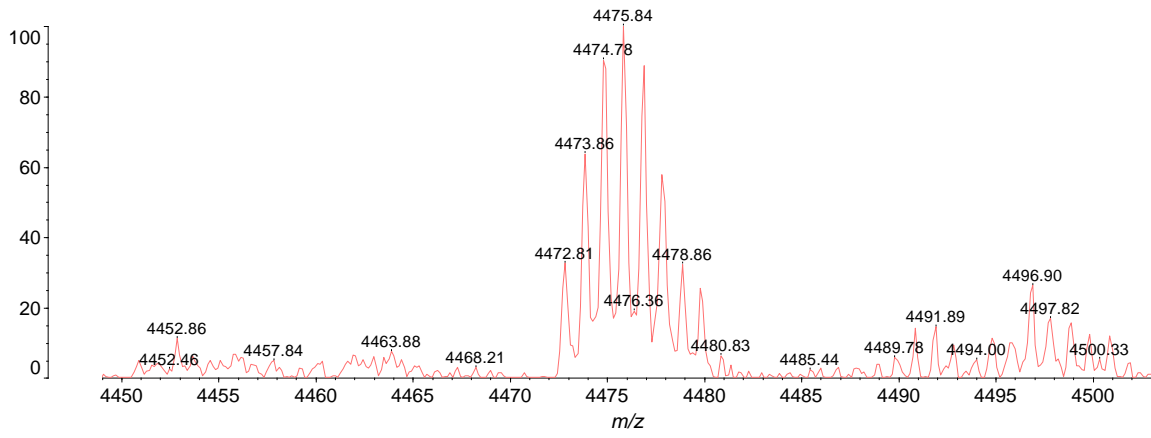


Figure S75: MALDI spectrum of synthetic antibody 48.

1.41 Synthetic antibody 49

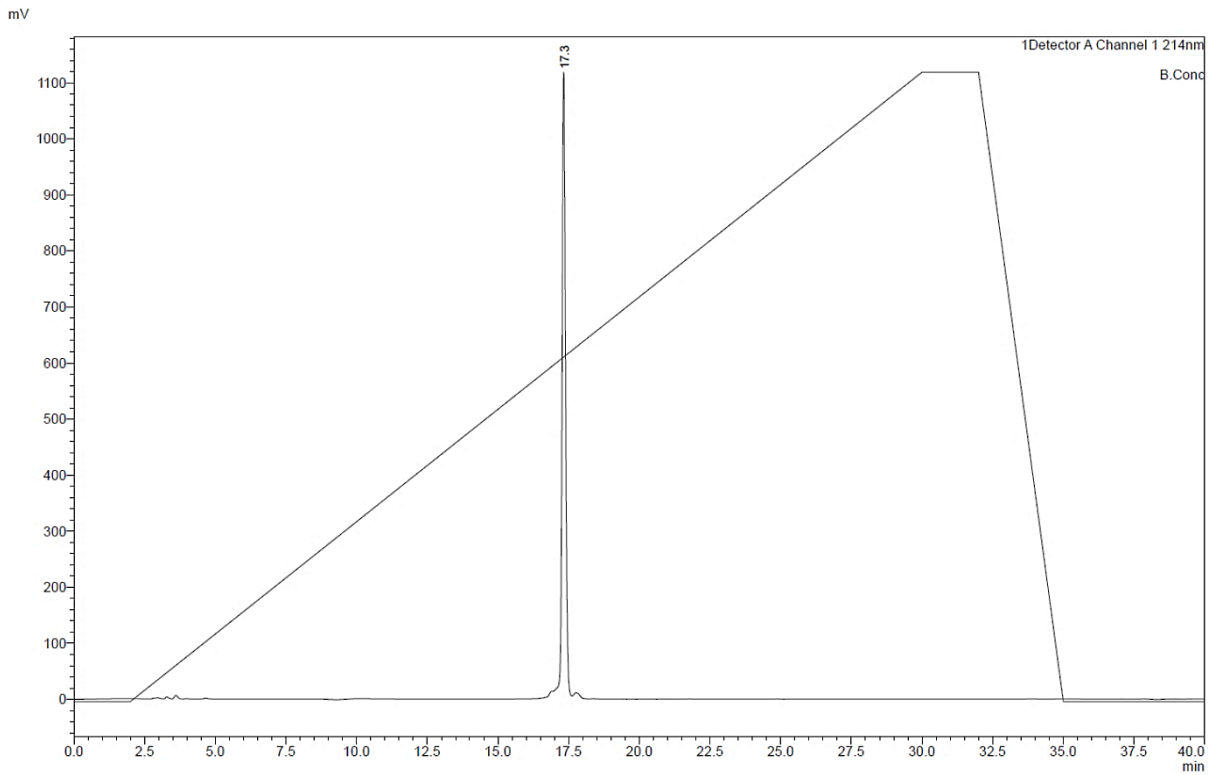


Figure S76: Analytical HPLC chromatogram of synthetic antibody 49.

Data: pk_OLP123_cyano_ref_pos0001.3J2[c] 16 Mar 2018 11:58 Cal: 3 PEP MIX 16 Mar 2018 10:53
Shimadzu Biotech Axima CFR 2.8.3.20080616: Mode reflectron, Power: 109, P.Ext. @ 4600 (bin 212)

%Int. 106 mV[sum= 21902 mV] Profiles 1-207 Unsmoothed -Baseline 50

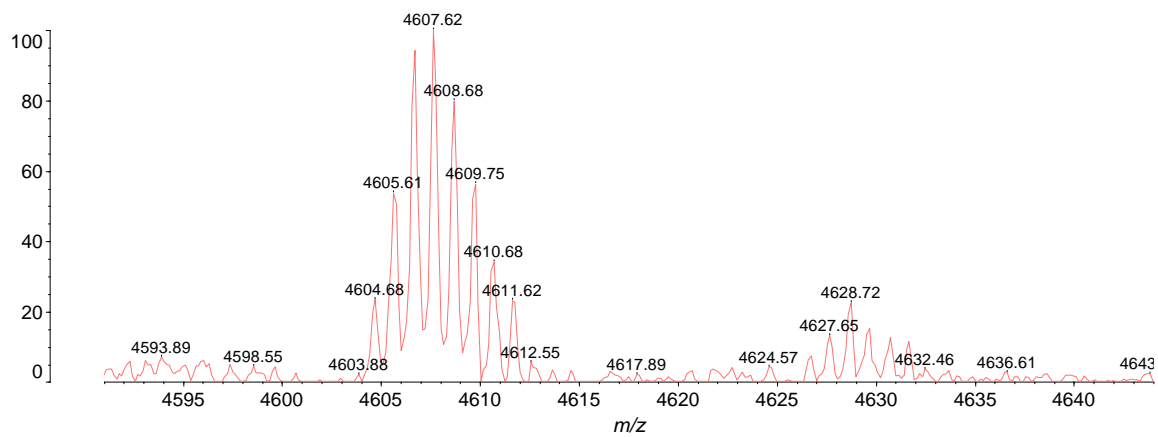


Figure S77: MALDI spectrum of synthetic antibody 49.

2 Fitted SPR sensograms

Data shown only for compounds which bound TNF α . One representative sensogram selected for each such compound. Fitting was performed using Biacore X100 Evaluation software version 2.0.1 on blank subtracted and injection peaks cleaned data with heterogeneous ligand fitting model.

2.1 Synthetic antibody 35

Kinetics: 'OLP119-1', fit: '1. Heterogeneous Ligand'

Sample: OLP119-1 Temp: 21°C Curve: Fc=2-1

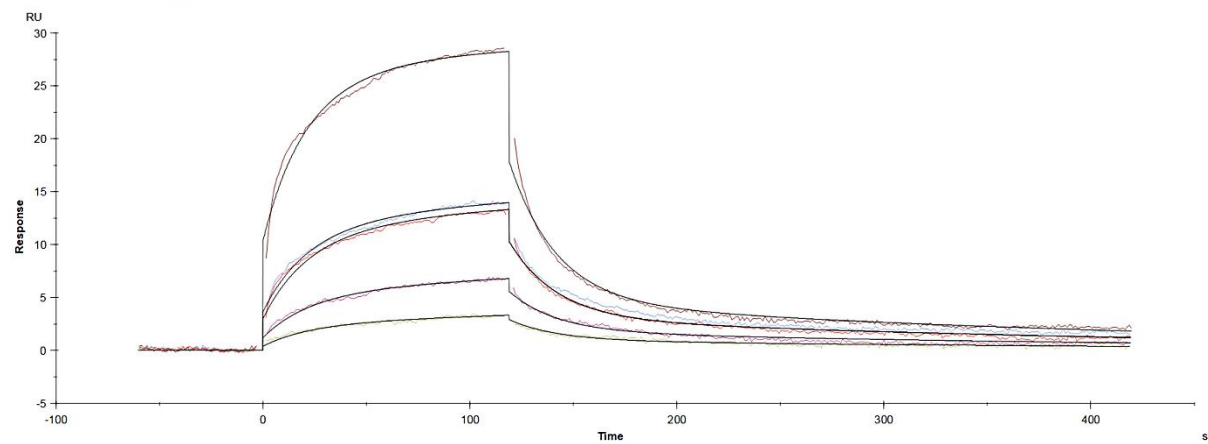


Figure S78: Representative sensogram for synthetic antibody 35 fitted with heterogeneous ligand model.

2.2 Synthetic antibody 36

Kinetics: 'OLP115-1', fit: '1. Heterogeneous Ligand'

Sample: OLP115-1 Temp: 21°C Curve: Fc=2-1

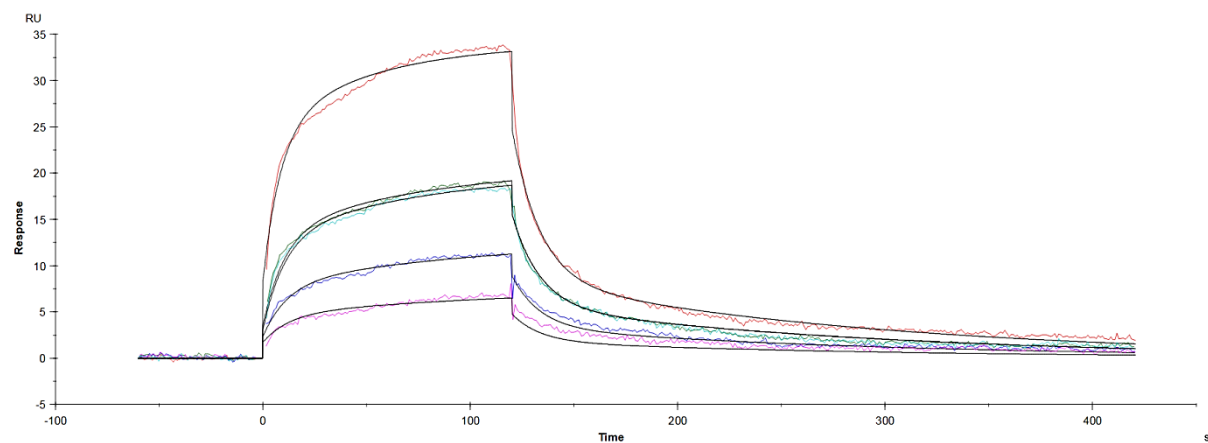


Figure S79: Representative sensogram for synthetic antibody 36 fitted with heterogeneous ligand model.

2.3 Synthetic antibody 37

Kinetics: 'OLP125-1', fit: '1. Heterogeneous Ligand'

Sample: OLP125-1 Temp: 21°C Curve: Fc=2-1

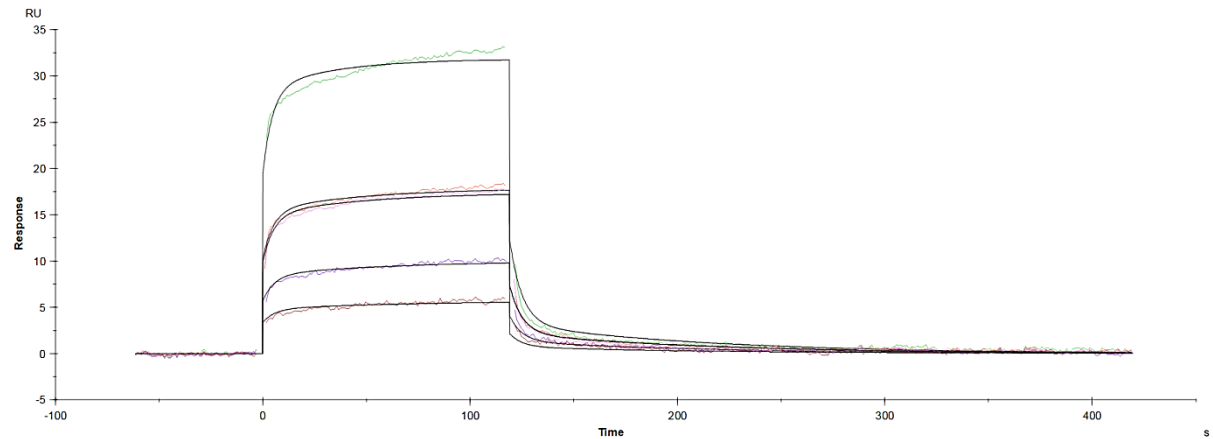


Figure S80: Representative sensogram for synthetic antibody 37 fitted with heterogeneous ligand model.

2.4 Synthetic antibody 44

Kinetics: 'OLP122-1', fit: '1. Heterogeneous Ligand'

Sample: OLP122-1 Temp: 21°C Curve: Fc=2-1

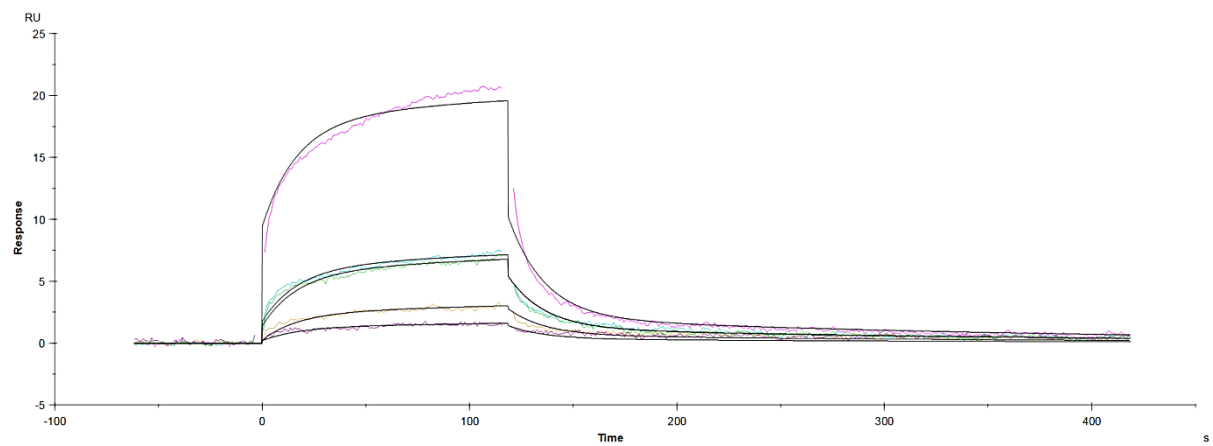


Figure S81: Representative sensogram for synthetic antibody 44 fitted with heterogeneous ligand model.

2.5 Synthetic antibody 47

Kinetics: 'OLP121-1', fit: '1. Heterogeneous Ligand - RI kept as below 20%'

Sample: OLP121-1 Temp: 21°C Curve: Fc=2-1

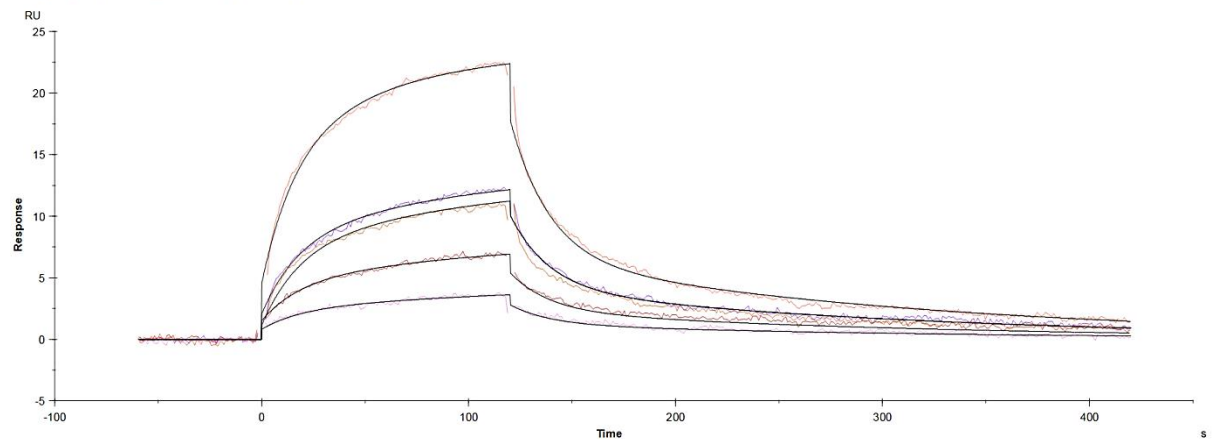


Figure S82: Representative sensogram for synthetic antibody 47 fitted with heterogeneous ligand model.

2.6 Synthetic antibody 48

Kinetics: 'OLP127-1 2', fit: '1. Heterogeneous Ligand'

Sample: OLP127-1 Temp: 21°C Curve: Fc=2-1

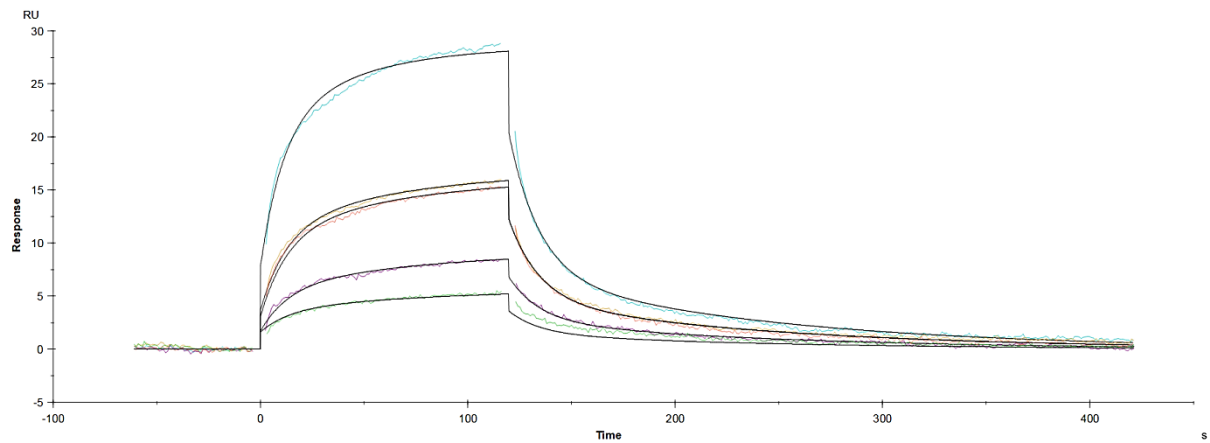


Figure S83: Representative sensogram for synthetic antibody 48 fitted with heterogeneous ligand model.

2.7 Synthetic antibody 49

Kinetics: 'OLP123-1', fit: '1. Heterogeneous Ligand'

Sample: OLP123-1 Temp: 21°C Curve: Fc=2-1

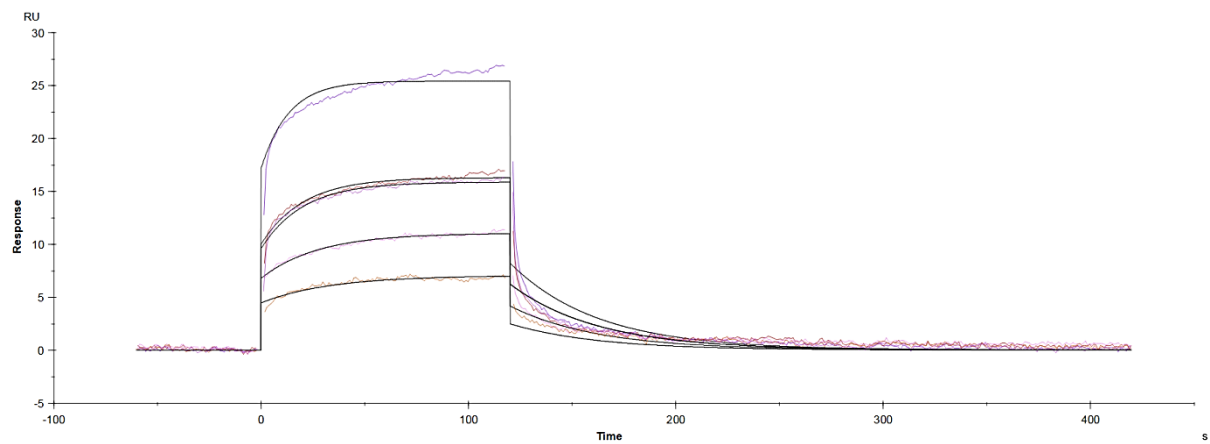


Figure S84: Representative sensogram for synthetic antibody 49 fitted with heterogeneous ligand model.

2.8 CDR mimic 31

Kinetics: 'OLP103_GHloop', fit: '3. Heterogeneous Ligand'

Sample: OLP103_GHloop Temp: 21°C Curve: Fc=2-1

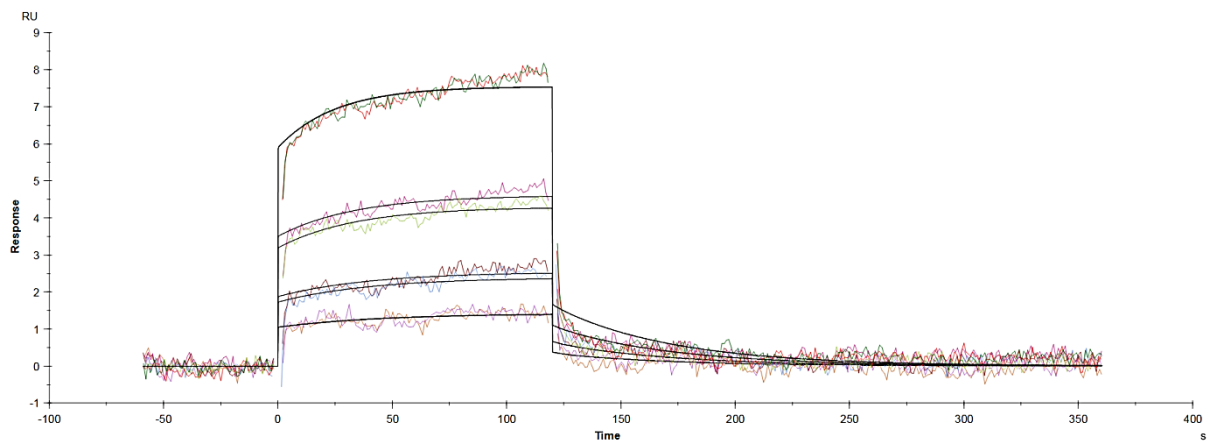


Figure S85: Representative sensogram for CDR loop mimic 31 fitted with heterogeneous ligand model.