Org.Commun. **10:1 (2017) 40-45**

A pre-biotinylated linker assembly for single-step preparation of novel biosensors

Brent A. Banasik

Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182-1030, USA

Table of Contents	**Page**
S1: General Information	2
S2: Spectral data of reported compounds	3-4
S3: Spectra of reported compounds	5-13

S1: General Information

IR spectra were recorded on Perkin Elmer FT-IR spectrum RXI. ^{1}H NMR spectra were recorded on a Varian 400, 500, or 600-MHz instrument using $CDCl_3$ or DMSO-d6 with TMS as internal standard (δ = 0 ppm). $CDCl_3$ (δ = 77.00 ppm) or DMSO-d6 (δ = 39.52 ppm) was used as internal references for ^{13}C (100, 126 and 151 MHz) NMR. Preparative HPLC was carried out using Shimadzu SCL-10A/SPD-10A instrument with preparative Varian pursuit 10 C8 50-G 50mm column. Mass spectra were recorded using APCI Expressions instrument. Elemental analysis was conducted by Intertek Pharmaceutical Services (USA) on a Perkin-Elmer 2400 Elemental Analyzer with NIST traceable organic standard. Analytical thin-layer chromatography was performed on Silicycle glass backed 60Å ultra pure silica gel. Flash chromatography was conducted using a Biotage Isolera one instrument with pre-packed silica gel columns (AnaLogix, Sepra Si 50) or self-packed Luknova and Biotage snap columns filled with silica gel (Sorbent Technologies, 60Å, 230-400 mesh). All reactions were conducted under an argon atmosphere and in septum-capped oven-dried glassware unless otherwise specified. All solvents and reagents were purchased from Aldrich, Fisher Scientific, Combi-blocks, TCI America, Chem Impex or Oakwood Scientific.

S2: Spectral data of reported compounds

*3.1. Benzyl 4-((tert-butoxycarbonyl) amino) butanoate (**1**):* R_f = 0.5, 25% EtOAc in hexanes; ^{1}H NMR (400 MHz, Chloroform-d) δ 7.37 – 7.23 (m, Ar-H x 5, 5H), 5.09 (s, CH_2Ph, 2H), 4.79 (s, NH, 1H), 3.13 (q, J = 6.6 Hz, NCH_2, 2H), 2.37 (t, J = 7.4 Hz, RCH_2R, 2H), 1.80 (p, J = 7.1 Hz, RCH_2R, 2H), 1.41 (s, OtBu, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.93, 155.92, 135.83, 128.42, 128.09, 128.05, 78.88, 66.13, 39.73, 31.39, 28.30, 25.17; FTIR (neat, cm^{-1}) 3320, 2933, 1731, 1708, 1683, 1540, 1365, 1163, 995, 851, 754, 699, 675. This spectral data is identical to the previously reported literature compound.[1]

*3.2. Benzyl 4- (4- ((tert- butoxycarbonyl) amino) butanamido) butanoate (**2**):* R_f = 0.4, 100% EtOAc; ^{1}H NMR (400 MHz, Chloroform-d) δ 7.43 – 7.28 (m, Ar-H x 5, 5H), 6.37 (s, NH, 1H), 5.12 (s, CH_2Ph, 2H), 4.79 (s, NH, 1H), 3.29 (td, J = 6.9, 5.7 Hz, RCH_2R, 2H), 3.14 (q, J = 6.1 Hz, RCH_2R, 2H), 2.43 (t, J = 7.3 Hz, RCH_2R, 2H), 2.18 (t, J = 7.0 Hz, RCH_2R, 2H), 1.86 (p, J = 7.1 Hz, RCH_2R, 2H), 1.81 – 1.71 (m, RCH_2R, 2H), 1.43 (s, OtBu, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.24, 172.82, 156.61, 135.84, 128.59, 128.29, 128.22, 79.47, 66.41, 39.66, 38.97, 33.49, 31.76, 28.39, 26.41, 24.60; FTIR (neat, cm^{-1}) 3422, 2984, 2942, 1709, 1653, 1540, 1366, 1253, 1229, 1167, 847; CHN calc. (%): C (63.47), H (7.99), N (7.40); found: (%): C (65.17), H (7.69), N (7.32).

*3.3. Benzyl 2, 2- dimethyl- 4, 9, 14-trioxo-3-oxa- 5, 10, 15-triazanonadecan-19-oate (**3**):* R_f = 0.6, 100% EtOAc; ^{1}H NMR (500 MHz, Methanol-d4) δ 7.98 (dt, J = 10.3, 5.7 Hz, NH, 1H), 7.33 (dd, J = 21.2, 4.4 Hz, Ar-H x 5, 5H), 5.11 (s, NH x 2, 2H), 4.83 (s, CH_2Ph, 2H), 3.25 – 3.15 (m, RCH_2R x 2, 4H), 3.07 (t, J = 6.9 Hz, RCH_2R, 2H), 2.41 (t, J = 7.4 Hz, RCH_2R, 2H), 2.21 (t, J = 7.5 Hz, RCH_2R x 2, 4H), 1.86 – 1.69 (m, RCH_2R x 3, 6H), 1.43 (s, OtBu, 9H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.37, 175.35, 175.27, 174.36, 158.32, 137.50, 129.45, 129.10, 129.09, 79.79, 67.15, 40.72, 39.74, 39.55, 34.28, 34.26, 32.26, 28.77, 27.21, 26.66, 25.67; FTIR (neat, cm^{-1}) 3327, 2944, 2486, 1740, 1708, 1690, 1652, 1633, 1542, 1526, 1452, 1418, 1365, 1279, 1254, 1224, 1162, 1027, 1003, 852, 736, 693; CHN calc. (%): C (62.18), H (8.05), N (9.06); found: (%): C (61.94), H (7.89), N (9.17).

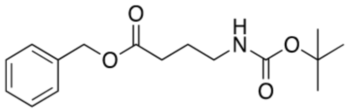
*3.4. Benzyl 4-(4-(4-(5- ((3aS, 4S, 6aR)-2- oxohexahydro- 1H- thieno [3,4-d]imidazol-4-yl) pentanamido) butanamido) butanamido) butanoate (**4**):* ^{1}H NMR (500 MHz, DMSO-d6) δ 7.81 (t, J = 5.7 Hz, NH, 1H), 7.77 (t, J = 5.4 Hz, NH x 2, 2H), 7.41 – 7.29 (m, Ar-H x 5, 5H), 6.41 (s, NH, 1H), 6.35 (s, NH, 1H), 5.09 (s, CH_2Ph, 2H), 3.09 (m, RCH_2R, 6H), 2.37 (t, J = 7.5 Hz, RCH_2R, 2H), 2.08 – 2.01 (m, RCH_2R, 6H), 1.66 (p, J = 7.1 Hz, RCH_2R, 2H), 1.59 (p, J = 7.2 Hz, RCH_2R, 4H), 1.55 – 1.40 (m, RCH_2R, 2H), 1.31 (dq, J = 15.8, 7.1 Hz, RCH_2R, 2H); ^{13}C NMR (101 MHz, DMSO) δ 172.50, 171.92, 171.72, 171.61, 162.68, 136.22, 128.40, 127.95, 127.88, 65.37, 61.02, 59.18, 55.38, 38.11, 37.73, 35.21, 32.91, 32.87, 30.93, 28.21, 28.01, 25.49, 25.28, 24.56; CHN calc. (%): C (59.06), H (7.35), N (11.88); found: (%): C (58.98), H (7.46), N (11.63).

*3.5. 4- (4- (4- (5- ((3aS, 4S, 6aR)-2-oxohexahydro-1H-thieno [3,4-d]imidazol-4-yl) pentanamido) butanamido) butanamido) butanoic acid (**5**):* ^{1}H NMR (500 MHz, Methanol-d4) δ 4.49 (ddd, J = 7.9, 5.0, 0.9 Hz, 1H), 4.30 (dd, J = 7.9, 4.5 Hz, 1H), 3.19 (td, J = 7.0, 2.1 Hz, 8H), 2.92 (dd, J = 12.7, 4.9 Hz, 1H), 2.70 (d, J = 12.8 Hz, 1H), 2.20 (dtd, J = 11.2, 9.1, 8.2, 2.3 Hz, 9H), 1.78 (dddd, J = 15.2, 8.8, 5.3, 1.7 Hz, 6H), 1.69 – 1.54 (m, 4H), 1.48 – 1.40 (m, 2H); APCI-MS *m/z* calcd for $C_{22}H_{36}N_5O_6S$ [M-H] 498.6, found 498.6; CHN calc. (%): C (52.89), H (7.46), N (14.02); found: (%): C (53.04), H (7.64), N (14.20).

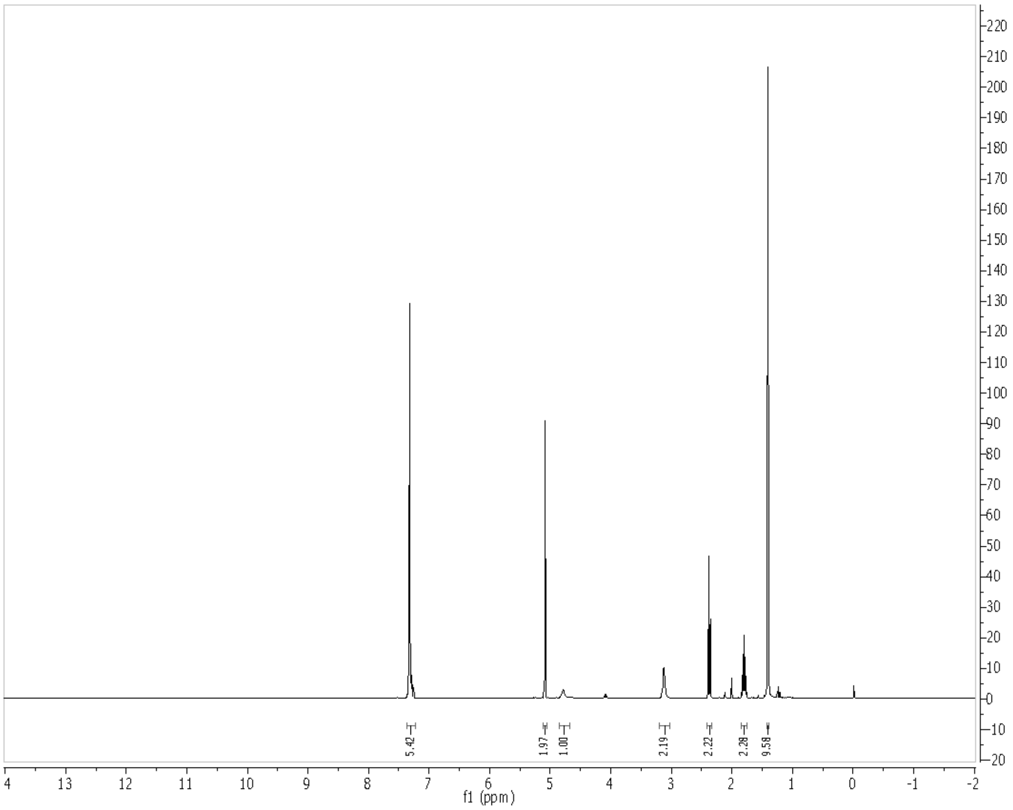
R:eferences

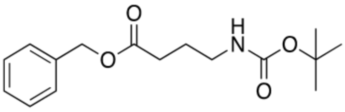
1. Barbayianni, E.; Fotakopoulou, I.; Schmidt, M.; Constantinou-Kokotou, V.; Bornscheuer, U.; Kokotos, G. Enzymatic Removal of Carboxyl Protecting Groups. Cleavage of the Benzyl and Methyl Moieties. *J. Org. Chem.* **2005**, *70*, 22, 8730-8733.

S4: Spectra of reported compounds

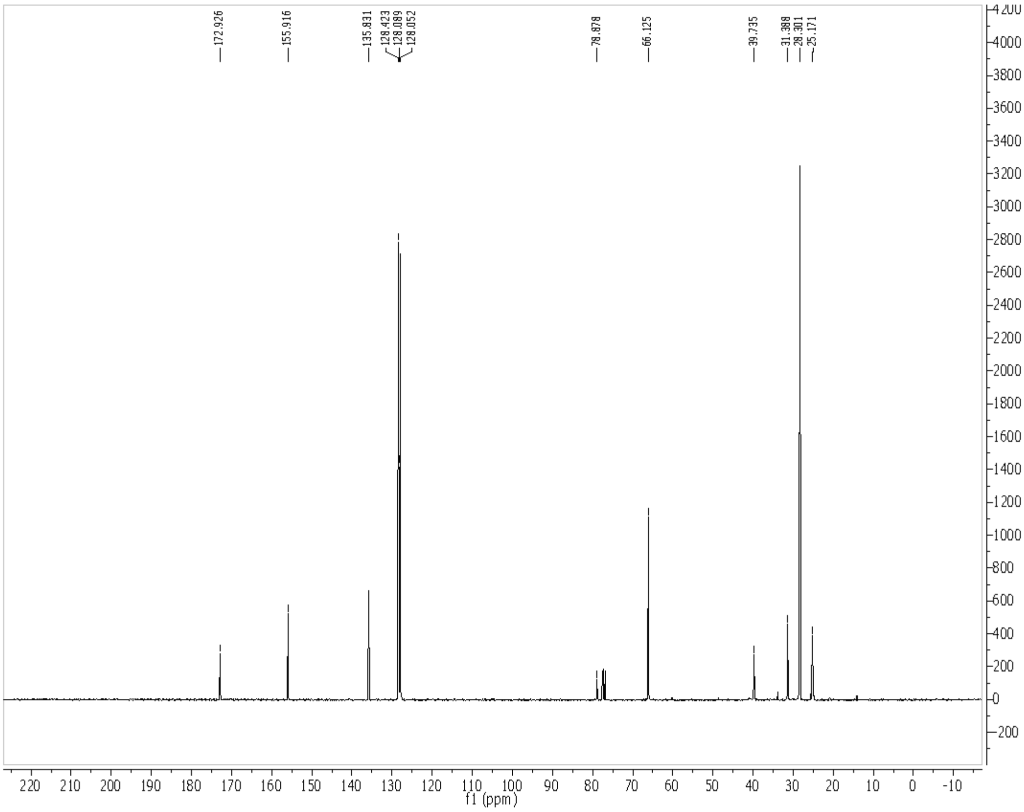


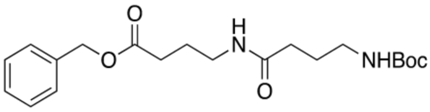
1



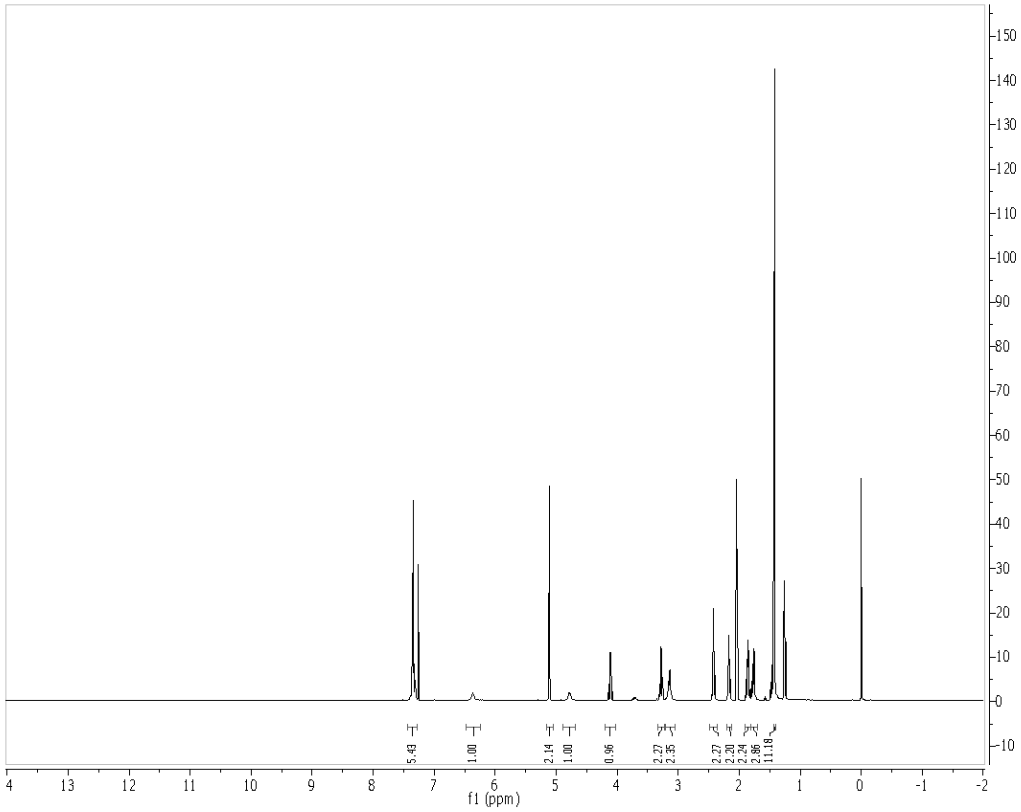

O
H
N
O
O
O

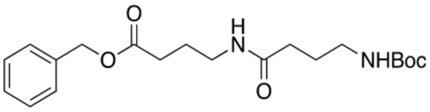
1


172.926
155.916
135.831
128.423
128.089
128.052
78.878
66.125
39.735
31.388
28.301
25.171
4200
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

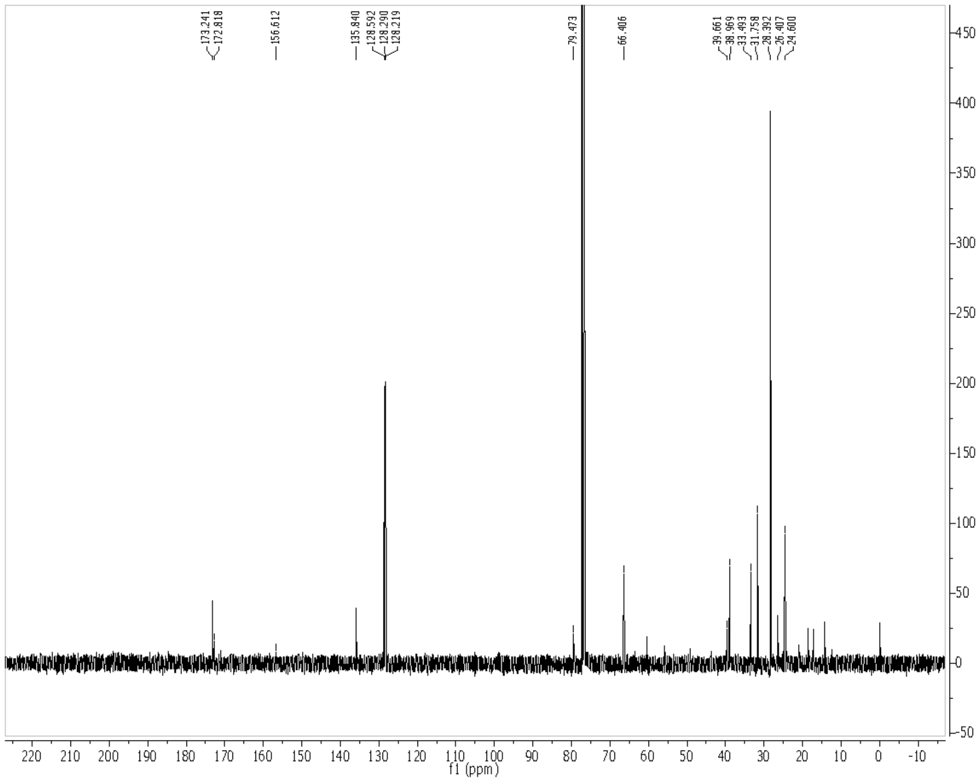

O
H
N
O
O
NHBoc

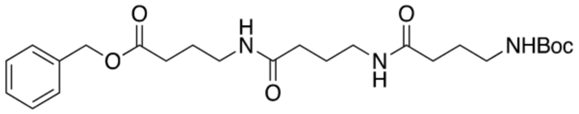
2


150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
5.43
1.00
2.14
1.00
0.96
2.27
2.35
2.27
2.20
2.24
2.86
11.18
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)

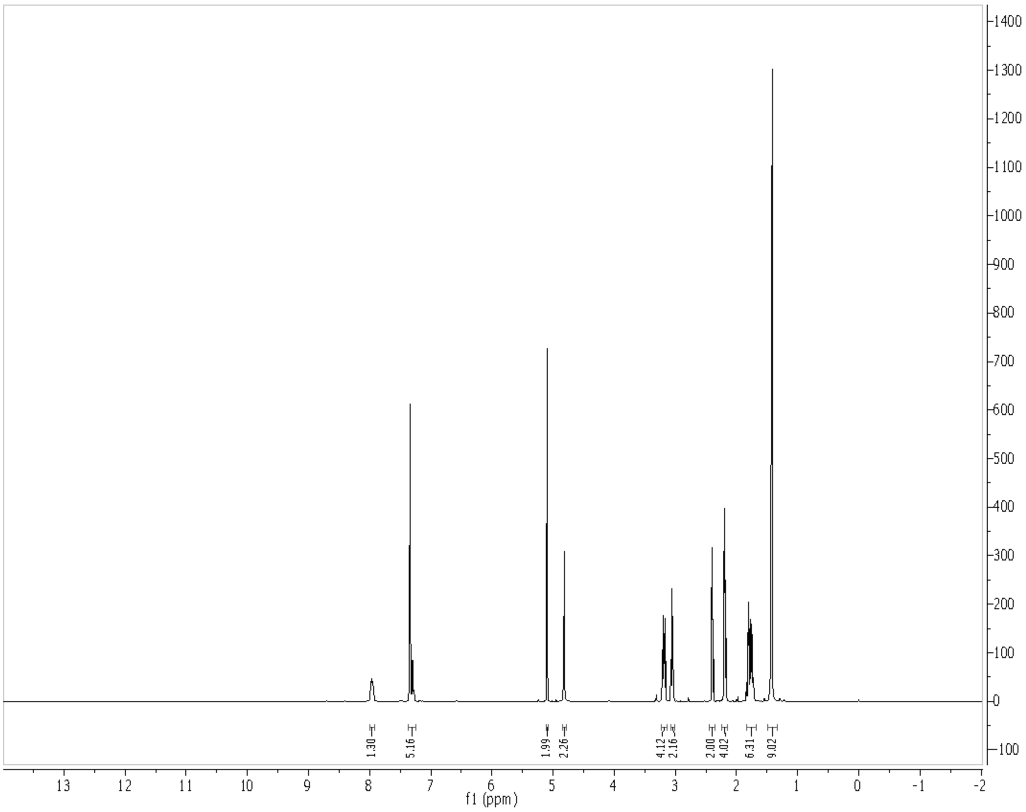

O
H
N
O
NHBoc
O

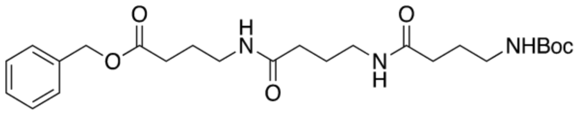
2


173.241
172.818
156.612
135.840
128.592
128.290
128.219
79.473
66.406
39.661
38.969
33.493
31.758
28.392
26.407
24.600
450
400
350
300
250
200
150
100
50
0
-50
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

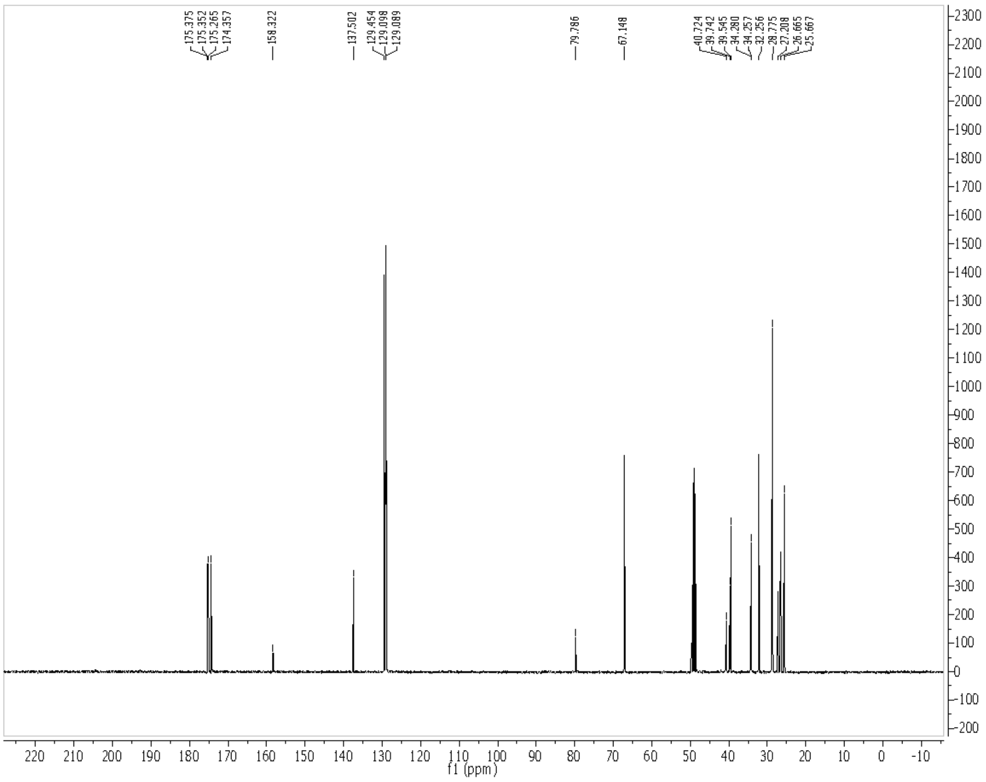

O
H
N
O
NHBoc
O
O
N
H

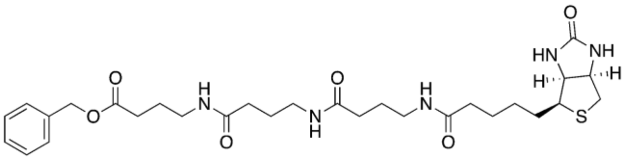
3


1.30
5.16
1.99
2.26
4.12
2.16
2.00
4.02
6.31
9.02
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100

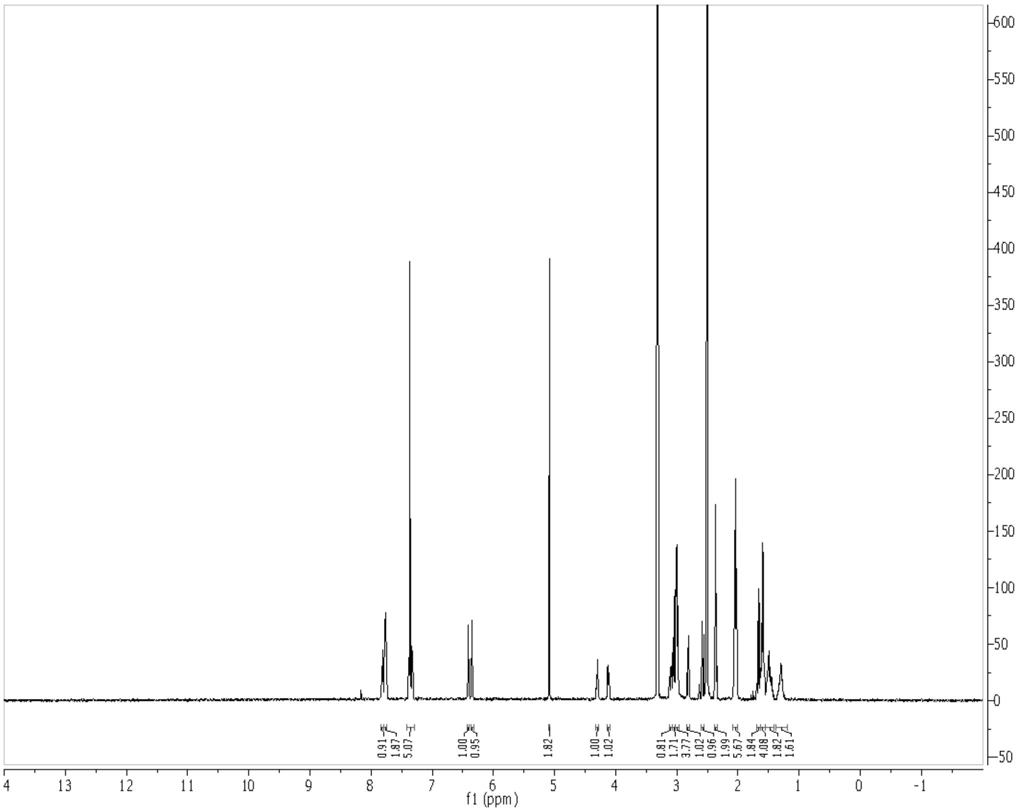

O
O
H
N
O
N
H
O
NHBoc

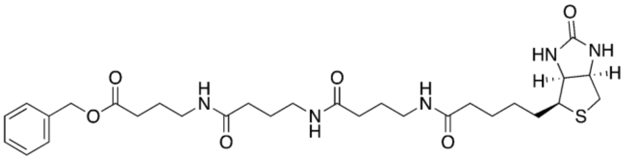
3


175.375
175.352
175.265
174.357
158.322
137.502
129.454
129.098
129.089
79.786
67.148
40.724
39.742
39.545
34.280
34.257
32.256
28.775
27.208
26.665
25.667
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

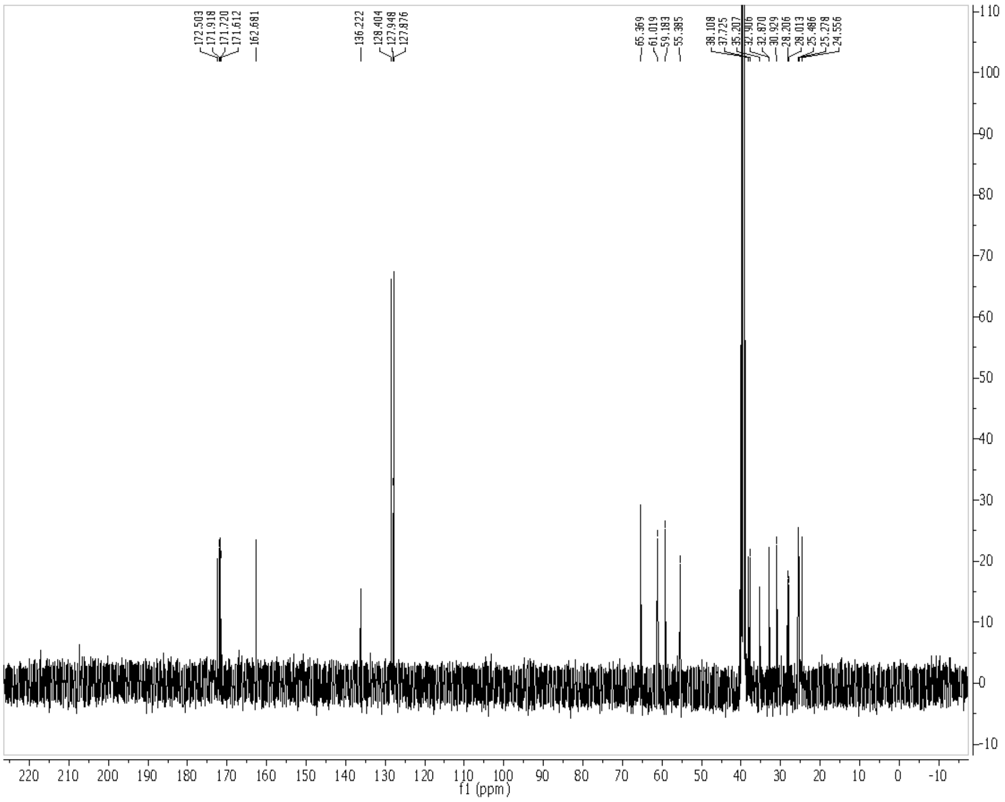

O
H
N
O
O
N
H
O
H
N
O
HN
NH
O
H
H
S

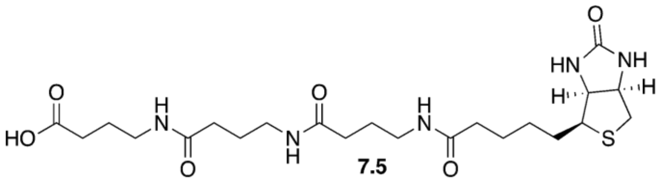
4


600
550
500
450
400
350
300
250
200
150
100
50
0
-50
0.91
1.87
5.07
1.00
0.95
1.82
1.00
1.02
0.81
1.71
3.77
1.02
0.96
1.99
5.67
1.84
4.08
1.82
1.61
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

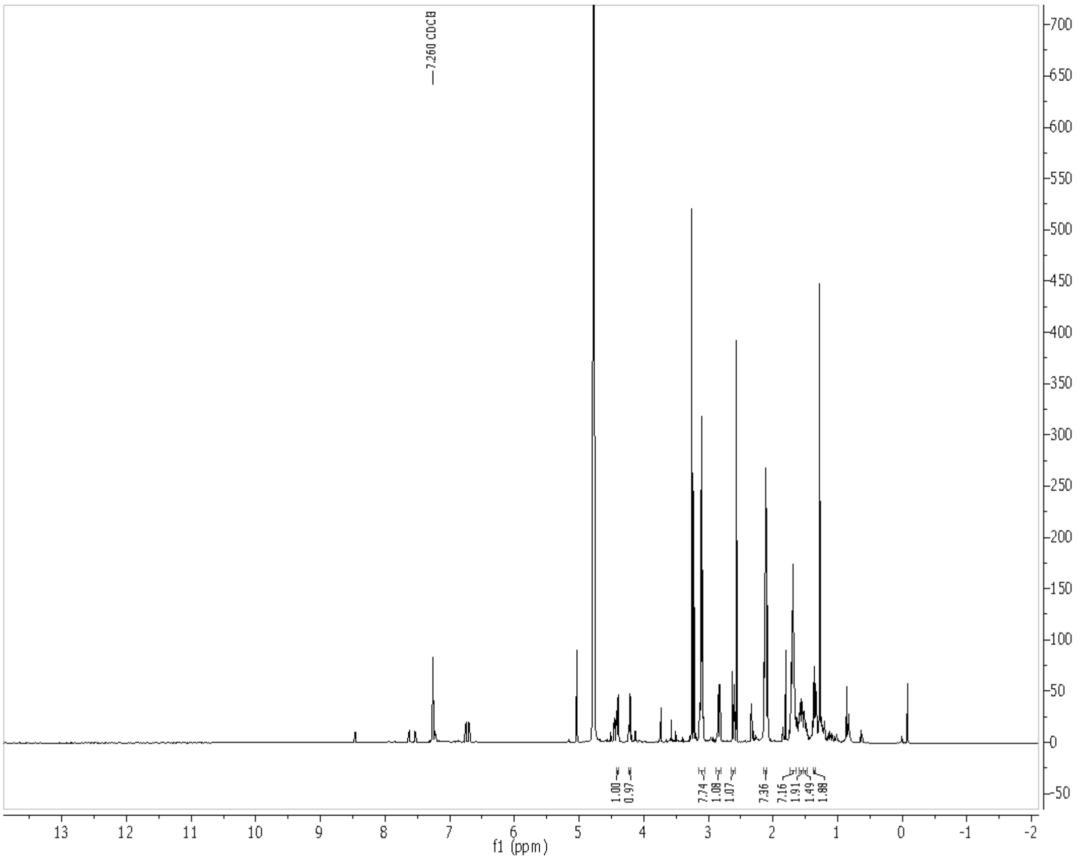

O
HN
NH
H
H
O
H
N
O
O
N
H
O
H
N
S
O

4


172.503
171.918
171.720
171.612
162.681
136.222
128.404
127.948
127.876
65.369
61.019
59.183
55.385
38.108
37.725
35.207
32.906
32.870
30.929
28.206
28.013
25.486
25.278
24.556
110
100
90
80
70
60
50
40
30
20
10
0
-10
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


HO
O
H
N
O
N
H
O
7.5
H
N
O
O
HN
NH
H
H
S

5


7.260 CDCl3
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
1.00
0.97
7.74
1.08
1.07
7.36
7.16
1.91
1.49
1.88
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)