Supporting Information

[^{18}F]Fluorobenzoyl-Lysine-Pentanedioic Acid Carbamates: Novel PET Imaging Agents for the Prostate-Specific Membrane Antigen

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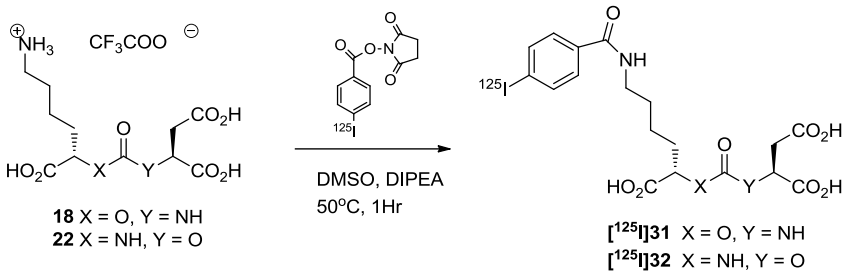
Radiosynthesis of (S)-2-(((((S)-1-carboxy-5-(4-[^{125}I]iodobenzamido)pentyl)oxy)carbonyl)amino) pentanedioic acid, [^{125}I]33 and (S)-2-(((((S)-1-carboxy-5-(4-[^{125}I]iodobenzamido)pentyl) carbamoyl)oxy)pentanedioic acid, [^{125}I]34.



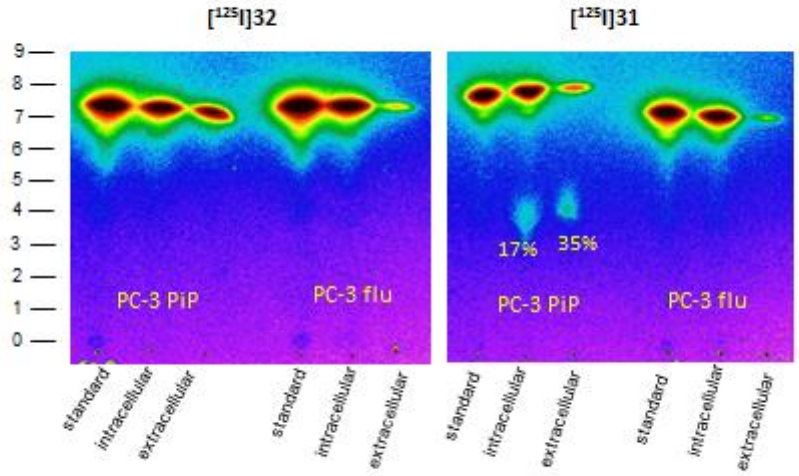
N-succinimidyl-4-[^{125}I]iodobenzoate was prepared by a modification of the method of Dekker et al (1). In particular, to a solution of 0.1mg N-succinimidyl-4-tributylstannylbenzoate (1) in 100µL methanol was added 2µL glacial acetic acid, 7.3mCi of Na[^{125}I] (Perkin Elmer, Billerica, MA), and 5µL of solution of N-chlorosuccinimide in methanol (10mg N-chlorosuccinide in 1.5mL methanol). This was allowed to stand at room temperature for 20 min, then diluted with 200µL methanol and injected onto a semi-preparative-HPLC (10 X 250mm, 10 micron, Phenomenex Luna C18 column, 55/45/0.1 water/acetonitrile/trifluoroacetic acid, flow = 4mL/m). N-succinimidyl-4-[^{125}I]iodobenzoate eluted at 14 min. This was diluted with 20mL water, loaded onto an activated Waters C18 Sep-Pak Plus cartridge, washed with 10mL water, dried with a stream of nitrogen for 2min, then eluted with 2mL methylene chloride through a Na_2SO_4 drying cartridge. The methylene chloride solution of N-succinimidyl-4-[^{125}I]iodobenzoate (5.9mCi) was stored at 0-2^{o}C.

The methylene chloride solution was then evaporated to dryness under a stream of nitrogen and to this was added a solution of **18** or **22** (2mg/200µL DMSO). To this solution is added 5µL diisopropylethylamine. Reaction is shaken and allowed to stand at room temperature for one hour. The reaction is then acidified by the addition of 20µL TFA and diluted with 1mL water. This is injected onto a semi-preparative-HPLC (10 X 250mm, 10 micron, Phenomenex Luna C18 column, 72/28/0.1 water/acetonitrile/trifluoroacetic acid, flow = 4mL/m). Retention times of **[^{125}I]31** and **[^{125}I]32** were 12 min and 11 min respectively. Product fraction was diluted with 40mL water, loaded onto an activated Waters C18 Sep-Pak Plus cartridge, washed with 10mL water, dried with a stream of nitrogen for 2min, then eluted with 2mL ethanol. Ethanol solution was concentrated under a stream of nitrogen until dryness and reconstituted in buffer for in-vitro assay. Starting with 2.0mCi and 2.2mCi of N-succinimidyl-4-[^{125}I]iodobenzoate, 1.8 and 2.0mCi of **[^{125}I]32** and **[^{125}I]31** was prepared.

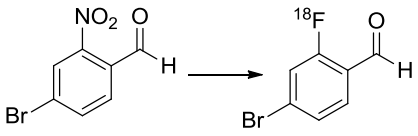
In-vitro metabolism of [^{125}I]31 and [^{125}I]32 in PC-3 PIP (PSMA+) and PC-3 flu (PSMA-) cells

PC-3 PIP (PSMA+) and PC-3 flu (PSMA-) cells were cultured as previously described (2). 300,000 PIP or flu cells were seeded into three wells each of a 6 well plate using RPMI 1640 + 10% fetal bovine serum + 1 % Penicillin-Streptomycin (Corning Cellgro, Manassas, VA) and were grown to 80% confluency. At the time of assay, the culture medium was refreshed and 50 µCi (1.35 kBq) of **[^{125}I]31** or **[^{125}I]32** was added to both a PIP- and flu-containing well. After radiotracer addition, the plate was returned to the incubator (humidified 37˚ C, 5% CO_2) for 30 minutes. The medium was then carefully removed and saved for counting in a LKB Wallac 1282 Compugamma gamma counter (Mount Waverly, Vic, Australia). The cells were washed twice with ambient temperature PBS, pH 7.4 followed by the addition of ddH_2O to lyse the cells. Lysis took place over 30 minutes inside the incubator. The lysates were then collected and counted using the gamma counter. Equal amounts of radioactivity from the supernatant and lysates were spotted onto silica gel 60 RP-18 F254S glass TLC plates (EMD Millipore Corp., Billerica, MA) and the plates were developed using a mobile phase consisting of 55% acetonitrile, 45% water and 0.1% trifluoroacetic acid. The TLC plate was dried and exposed to Kodak Biomax x-ray film (Fisher Scientific) prior to digitizing using the MCID Core package (Interfocus Imaging, Cambridge, UK). Standards solutions of **[^{125}I]31** and **[^{125}I]32** had Rf values of 0.8. Intracellular and extracellular metabolites of **[^{125}I]31** in PC-3 PIP cells had an Rf value of approximately 0.44.

Figure S2. TLC analysis of Metabolism of **[^{125}I]31** and **[^{125}I]32** in PC 3 PIP (PSMA+) and PC3 flu (PSMA-) cells.



Radiofluorination conditions for the preparation of 2-[^{18}F]fluoro-4-bromobenzaldehyde



28b **[^{18}F]29b**

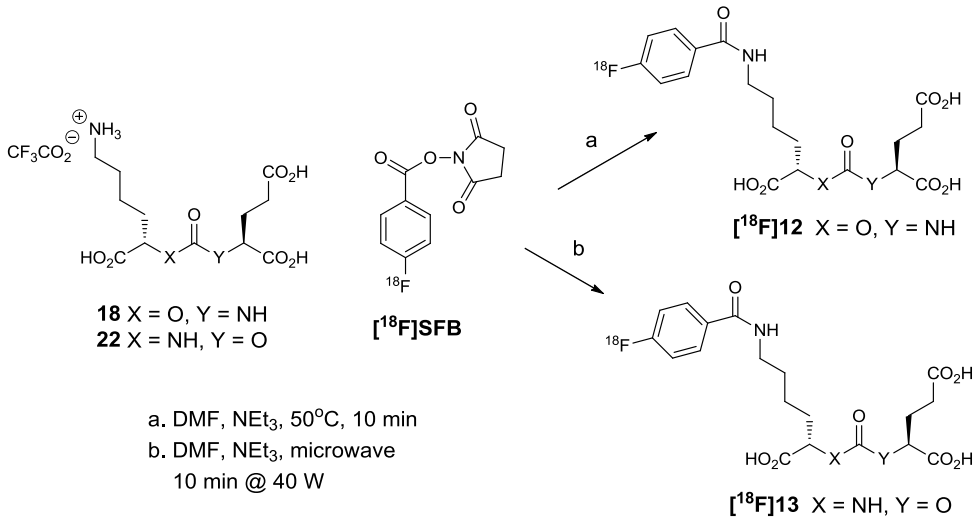
^{18}F-Fluoride was produced by a General Electric PETtrace biomedical cyclotron (GE HealthCare) using 18MeV proton bombardment on an ^{18}O-H_2O target and trapped on a Chromafix 30-PS-HCO3 QMA cartridge. The cartridge was eluted with 0.5mL of a solution of potassium carbonate or potassium bicarbonate(4.5mg/0.5mL) into a 3mL Wheaton reaction vial. To this was added 15-18 mg 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane ($K_{2.2.2}$) in 1mL of acetonitrile and heated to 100 ^{o}C under a stream of Argon gas to dryness. Further drying was accomplished by azeotropic distillation using 3 × 0.5 mL additions of acetonitrile. The vial is cooled to room temperature and a solution of 8-16 mg 4-bromo-2-nitrobenzaldehyde (**28b**) in 250 µL DMSO or acetonitrile is added and heated at various temperatures for 5-20 min then cooled to room temperature and diluted to a volume of 2 mL with 25% acetonitrile/water for purification by radio-HPLC (10 × 250 mm Phenomenex Luna C18 column, 54/46/0.1 water/acetonitrile/TFA, 4 mL/m). 4-bromo-2-[^{18}F]fluorobenzaldehyde (**[^{18}F]29b**) eluted at 18.5 min. Results are given is Table S1 below.

Table S1. Radiofluorination Conditions for 4-bromo-2-[^{18}F]fluorobenzaldehyde (**29b**)

Entry	Solvent	Temp ^{o}C	Base	Reaction time	% yield[a]
1	DMSO	rt	K_2CO_3	5 min	6
2	DMSO	65	K_2CO_3	5 min	6
3	DMSO	65	K_2CO_3	20 min	7
4	DMSO	100	K_2CO_3	5 min	15
5	DMSO	100	K_2CO_3	15 min	13
6	MeCN	100	K_2CO_3	5 min	4
7	DMSO	140	K_2CO_3	20 min	12
8	DMSO	140[b]	K_2CO_3	5min	0
9	DMSO	95	$KHCO_3$	5 min	22
10	DMSO	95	$KHCO_3$	20 min	30
11	DMSO	120	$KHCO_3$	20 min	50
12	DMSO	140	$KHCO_3$	20 min	32
13	DMSO	140	$KHCO_3$	20 min	33
14	DMSO	140[b]	$KHCO_3$	10 min	30

[a] non-decay corrected [b]microwave

Radiosynthesis of (*S*)-2-(((((*S*)-1-carboxy-5-(4-[^{18}F]fluorobenzamido)pentyl)oxy) carbonyl)amino)pentanedioic acid **[^{18}F]12** and (*S*)-2-((((*S*)-1-carboxy-5-(4-[^{18}F]fluorobenzamido)pentyl)carbamoyl)oxy) pentanedioic acid **[^{18}F]13**.



Radiosynthesis of **[^{18}F]12**.

[^{18}F]SFB was prepared according to a literature procedure (3). A methylene chloride solution of [^{18}F]SFB was evaporated under a stream of argon gas. To this residue was added 3 mg of (S)-2-((((S)-5-amino-1-carboxypentyl)oxy)carbonyl)amino)-pentanedioic acid **18** in 200 µL dry DMF and 5 µL triethylamine. This was heated for 10 min at 50 °C, cooled to room temperature, acidified with trifluoroacetic acid, diluted with water, and injected onto a radio-HPLC (10 × 250 mm Phenomenix Luna C18 column, mobile phase 75/25/0.1% water/acetonitrile/TFA, flow 4 mL/min). **[^{18}F]12** eluted at 12 min. The product HPLC fraction was collected, neutralized with sodium bicarbonate, concentrated under vacuum, and dissolved in sterile saline for injection. Radiochemical yield from **[^{18}F]SFB** ranged from 27-28%. Specific activity ranged from 2900-3900Ci/mmol (107,300-144,300GBq/mmol).

Radiosynthesis of **[^{18}F]13**.

A methylene chloride solution of [^{18}F]SFB was evaporated under a stream of argon gas. To this residue was added 3 mg of (S)-2-((((S)-5-amino-1-carboxypentyl)carbamoyl)oxy)pentanedioic acid **22** in 200 µL dry DMF and 5 µL triethylamine. This was heated for 10 min at 50 °C in a 40 W microwave (Resonance Instruments), cooled to room temperature, acidified with trifluoroacetic acid, diluted with water, and injected onto a radio-HPLC (10-mm × 250mm Phenomenix Luna C18 column, mobile phase 75/25/0.1% water/acetonitrile/TFA, flow 4 mL/min). **[^{18}F]13** eluted at 11.5 min. The product HPLC fraction was

collected, neutralized with sodium bicarbonate, concentrated under vacuum, and dissolved in sterile saline for injection. The non-decayed corrected radiochemical yield from **[^{18}F]SFB** was 5%.

N-Succinimidyl 4-bromo/iodo-2-fluorobenzoate:

4-bromo-2-fluorobenzoic acid (or 4-iodo-2-fluorobenzoic acid) 1 mmol and N-hydroxysuccinimide 125 mg (1.08 mmol) were dissolved in 2 mL dry DMF. To the solution, N,N-dicyclohexylcarbodiimide 170 µL (1.10 mmol) was added and the reaction was kept at room temperature overnight. After a flash column chromatography with ethyl acetate/hexane, 1:1, the N-succinimidyl 4-bromo/4-iodo-2-fluorobenzoates were obtained as white solids. TLC: silica gel, 1:1 ethylacetate:hexane. N-succinimidyl 4-bromo-2-fluorobenzoate (205mg) was obtained in a yield of 65%; Rf = 0.6. N-succinimidyl 4-iodo-2-fluorobenzoate (330mg) was obtained in a yield of 52%; Rf = 0.6.

N-Succinimidyl 4-bromo-2-fluorobenzoate:

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.97-7.94 (m, 1H), 7.48-7.44 (m, 2H), 2.92 (s, 4H).

N-Succinimidyl 4-iodo-2-fluorobenzoate

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.78-7.75 (m, 1H), 7.68-7.64 (m, 2H), 2.92 (s, 4H).

Figure S2. Preparative Radio-HPLC chromatogram of 2-[^{18}F]fluoro-4-bromo-benzaldehyde (**[^{18}F]29b**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 54/46/0.1 water/acetonitrile/TFA, 4mL/m. Double radioactive peak @ 18-19.5 min is due to saturation of the radioactivity detector.

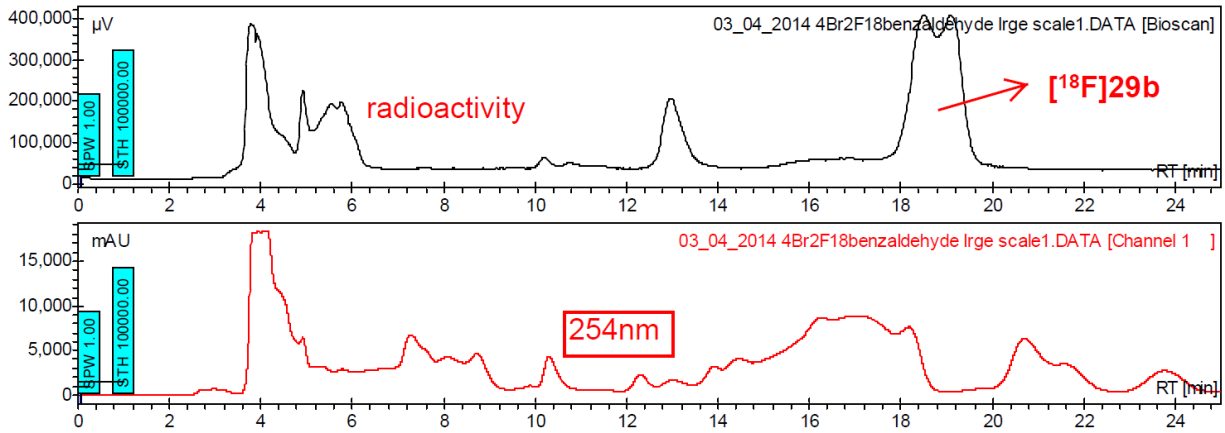


Figure S3. Preparative Radio-HPLC chromatogram of N-succinimidyl 2-[^{18}F]fluoro-4-bromobenzoate (**[^{18}F]30b**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 50/50/0.1 water/acetonitrile/TFA, 4mL/m.

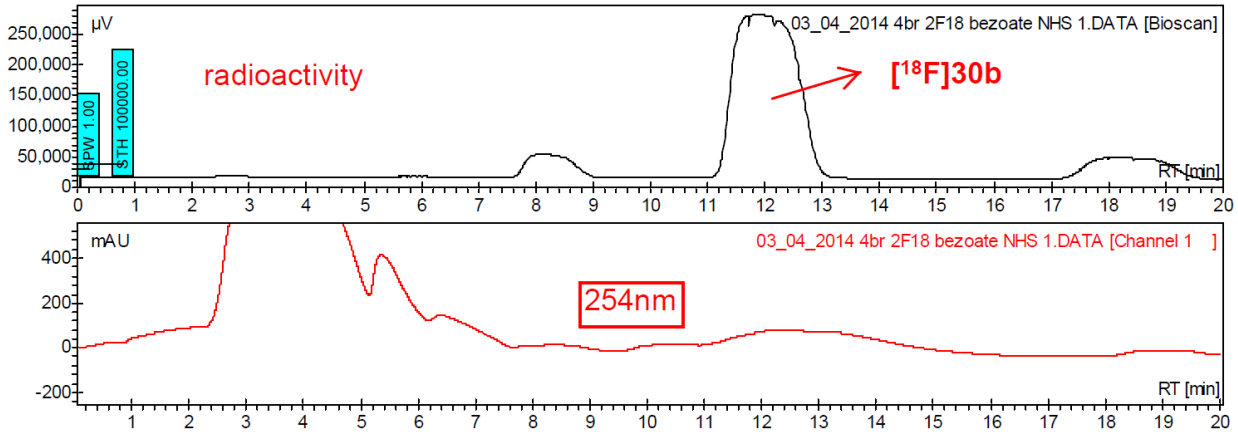


Figure S4. Preparative Radio-HPLC chromatogram of (S)-2-((((S)-5-(4-bromo-2-[^{18}F]fluorobenzamido)-1-carboxypentyl)carbamoyl)oxy)pentanedioic acid (**[^{18}F]23**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 70/30/0.1 water/acetonitrile/TFA, 4mL/m.

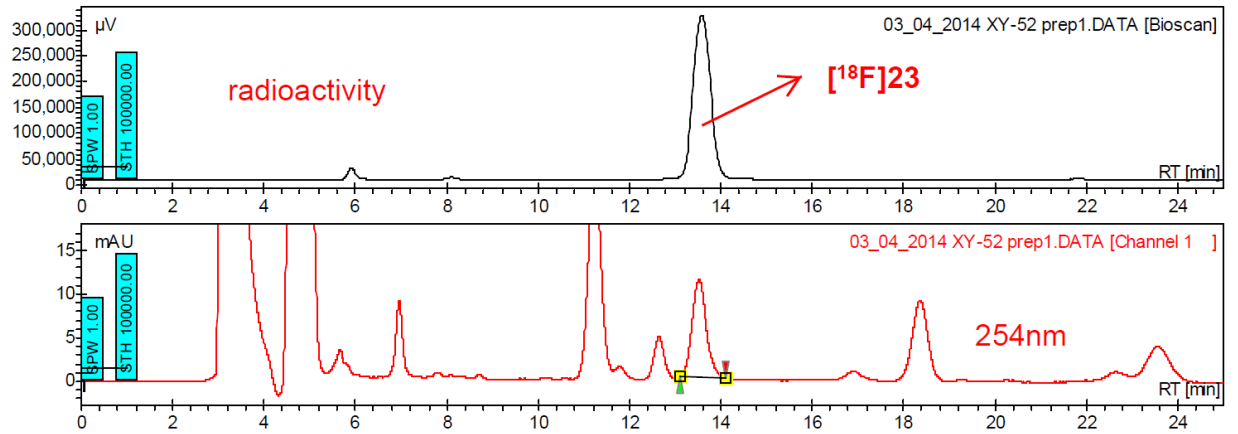


Figure S5. Quality control analytical radio-HPLC chromatogram of purified (S)-2-((((S)-5-(4-bromo-2-[^{18}F]fluorobenzamido)-1-carboxypentyl)carbamoyl)oxy)pentanedioic acid (**[^{18}F]23.** 4.6 X 150mm, 10 micron Phenomenex Luna C18 column, 70/30/0.1 water/acetonitrile/TFA, 1mL/m.

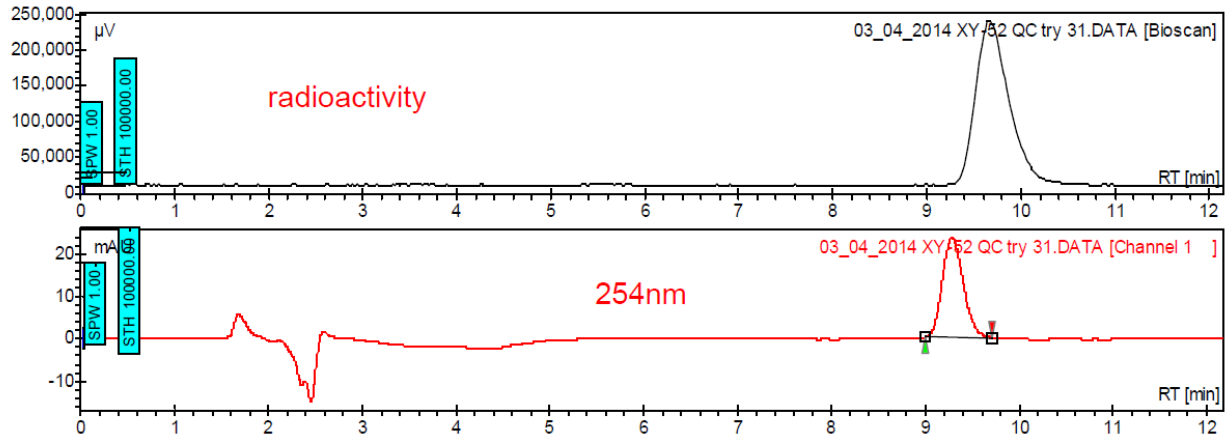


Figure S6. Preparative Radio-HPLC chromatogram of 2-[^{18}F]fluoro-4-iodo-benzaldehyde (**[^{18}F]29c**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 54/46/0.1 water/acetonitrile/TFA, 4mL/m. Double radioactive peak @ 21-23 minutes is due to saturation of the radioactivity detector.

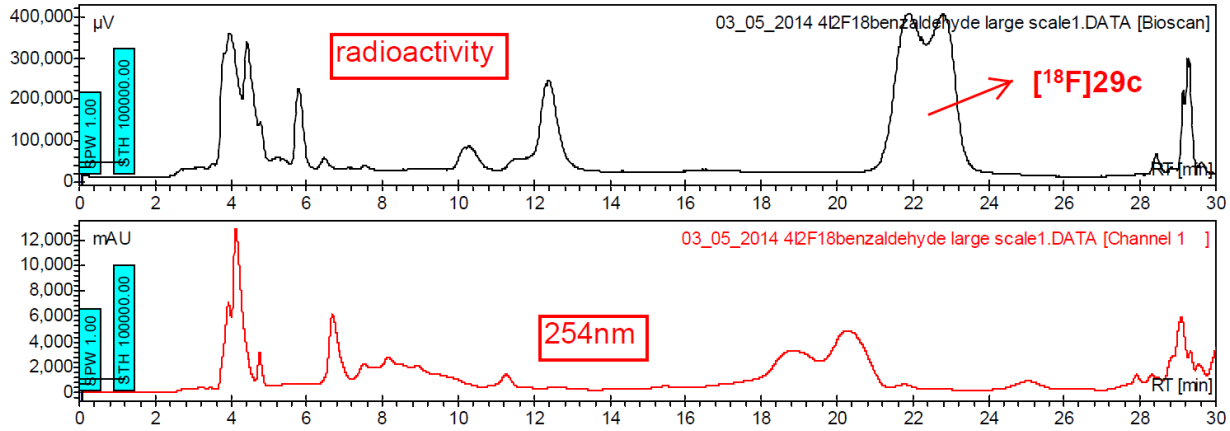


Figure S7. Preparative Radio-HPLC chromatogram of N-succinimidyl 2-[^{18}F]fluoro-4-iodobenzoate (**[^{18}F]30c**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 50/50/0.1 water/acetonitrile/TFA, 4mL/m.

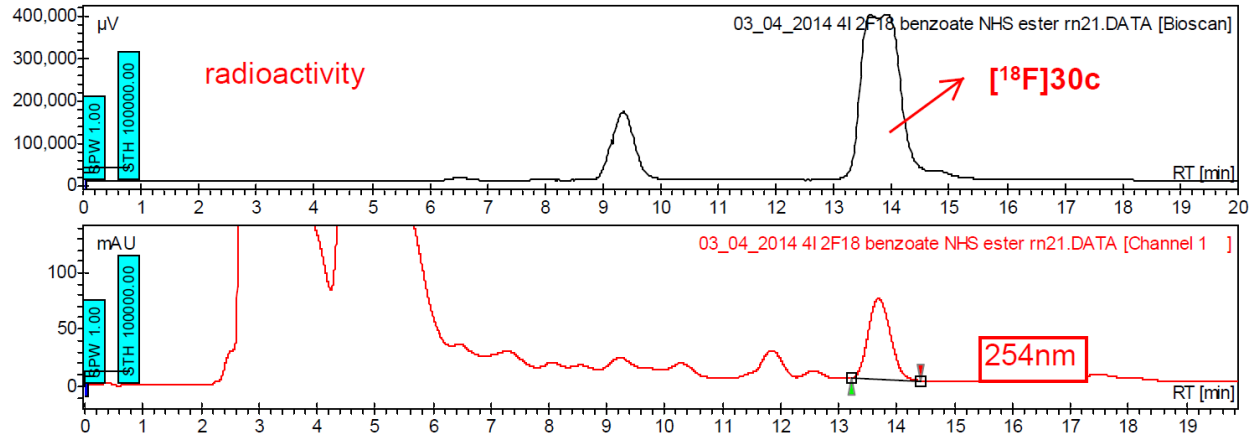


Figure S8. Preparative Radio-HPLC chromatogram of (S)-2-((((S)-5-(4-iodo-2-[^{18}F]fluorobenzamido)-1-carboxypentyl)carbamoyl)oxy)pentanedioic acid (**[^{18}F]24**. 10 X 250mm, 10 micron Phenomenex Luna C18 column, 70/30/0.1 water/acetonitrile/TFA, 4mL/m.

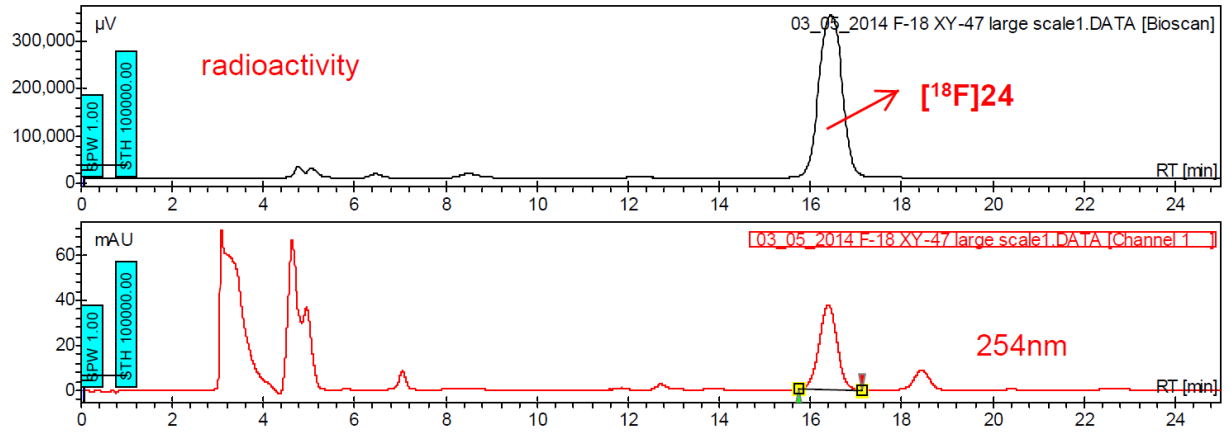
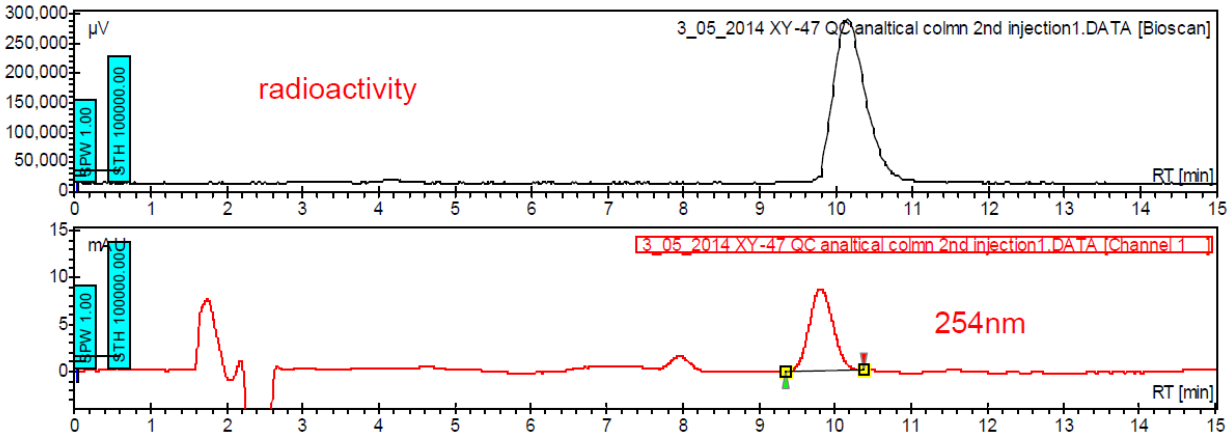
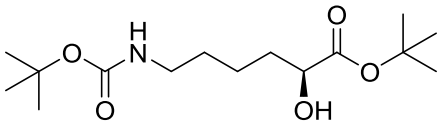


Figure S9. Quality control analytical radio-HPLC chromatogram of purified (S)-2-((((S)-5-(4-iodo-2-[^{18}F]fluorobenzamido)-1-carboxypentyl)carbamoyl)oxy)pentanedioic acid (**[^{18}F]24.** 4.6 X 150mm, 10 micron Phenomenex Luna C18 column, 70/30/0.1 water/acetonitrile/TFA, 1mL/m.



NMR spectra of synthesized compounds:



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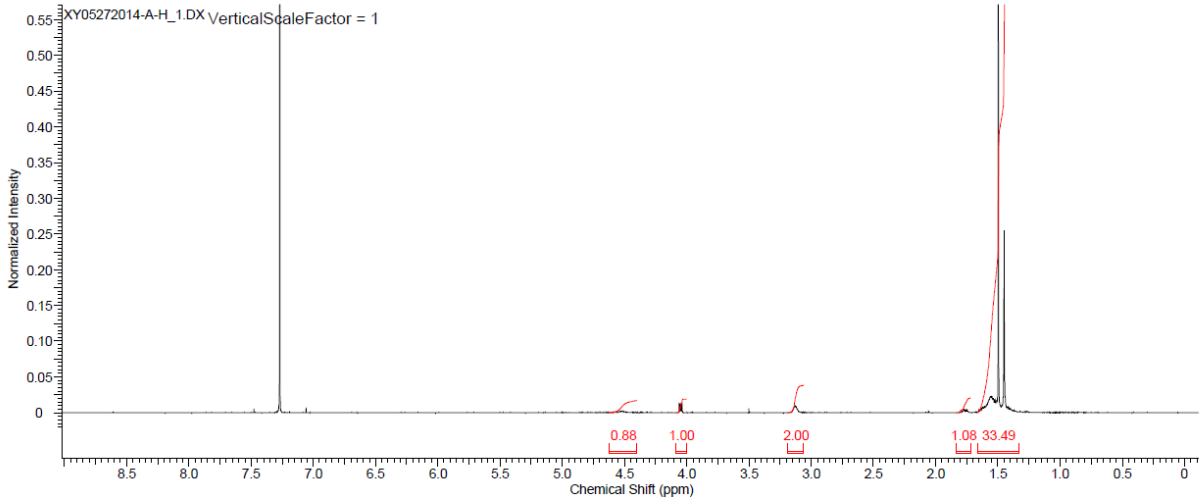

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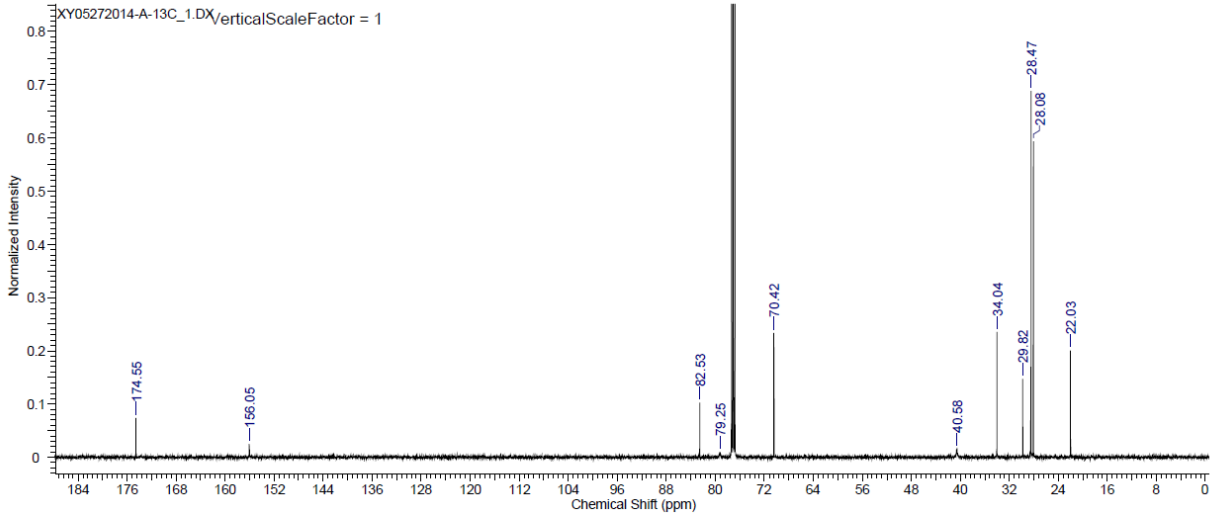

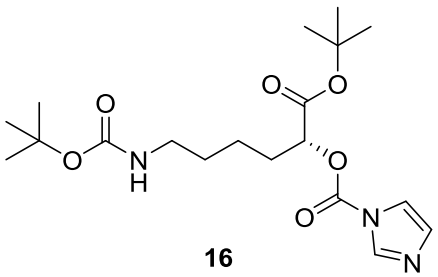

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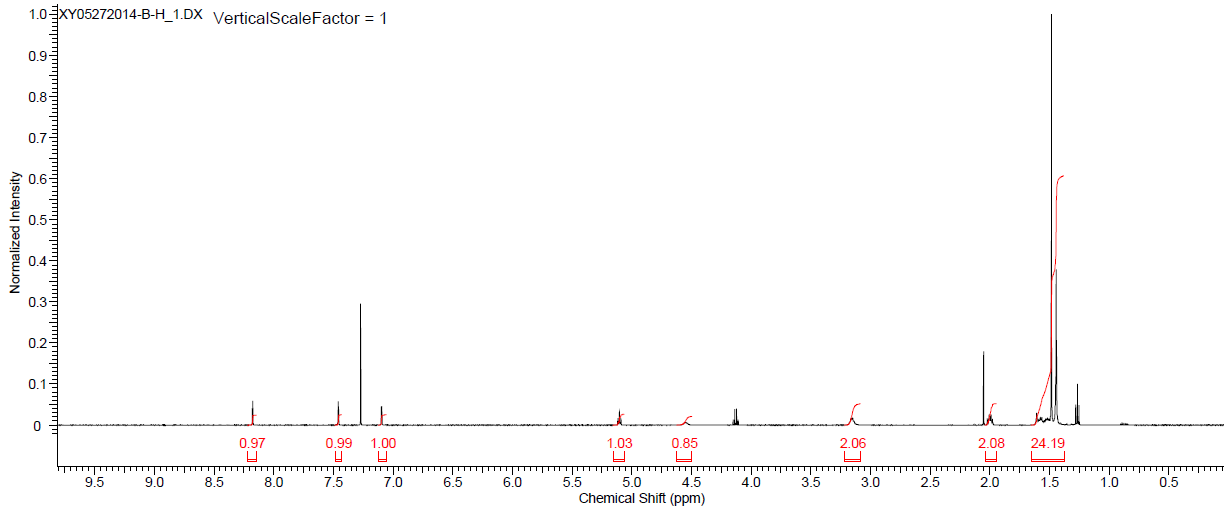

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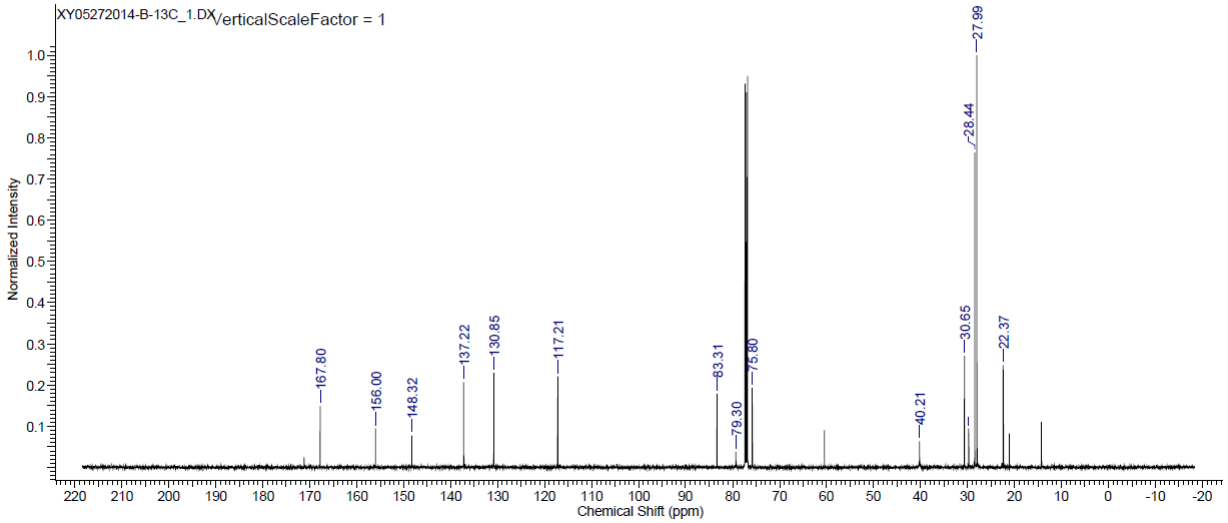

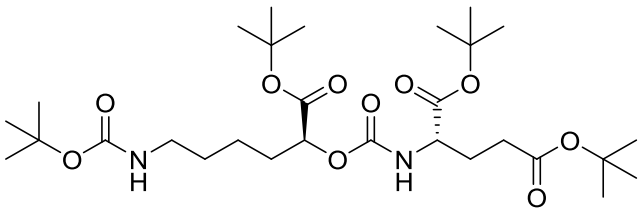

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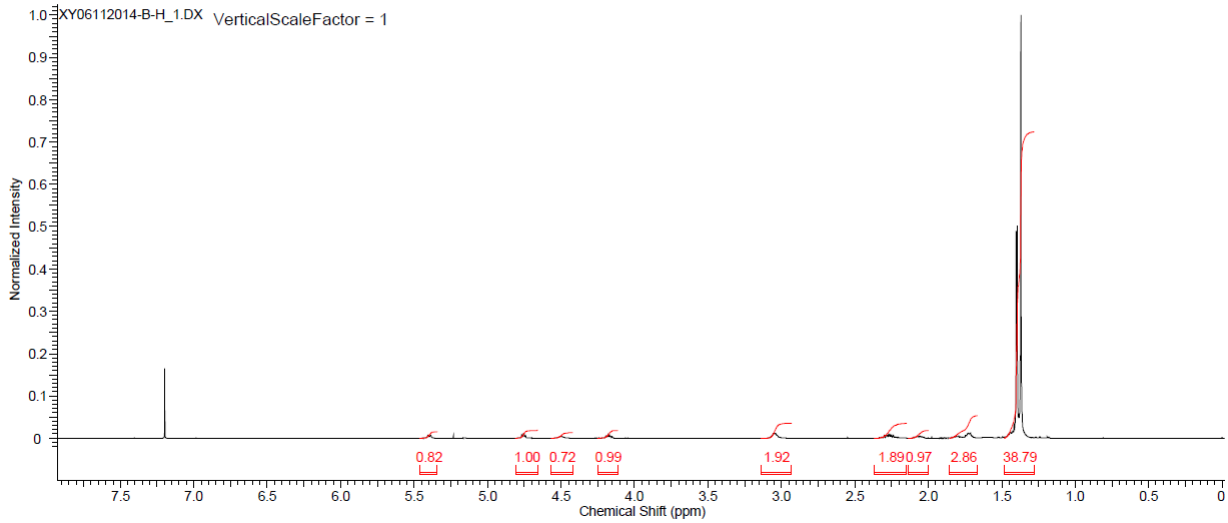

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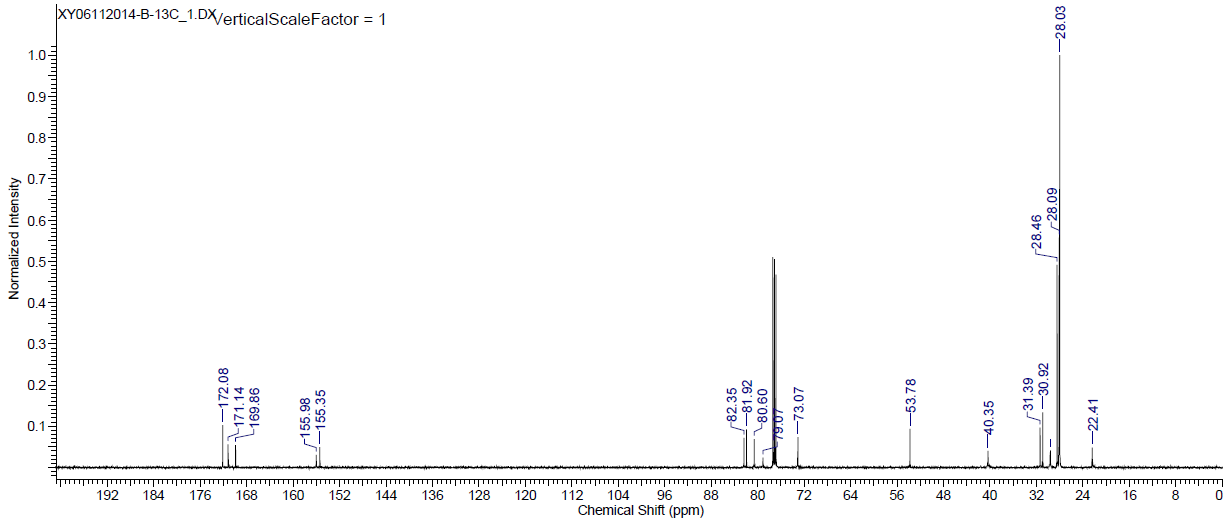
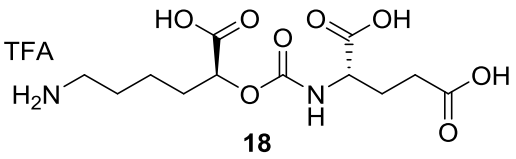
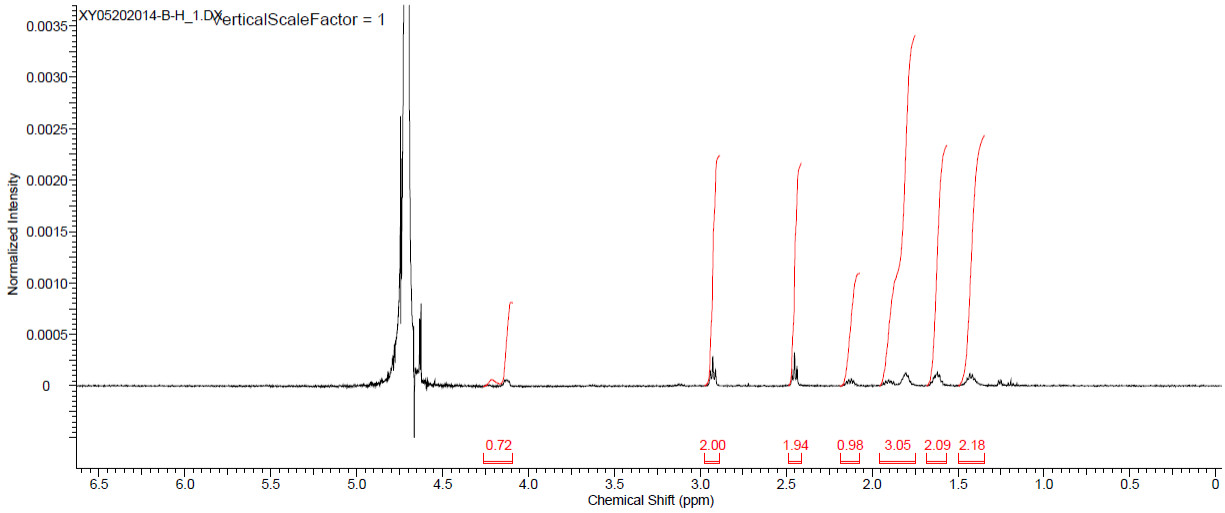


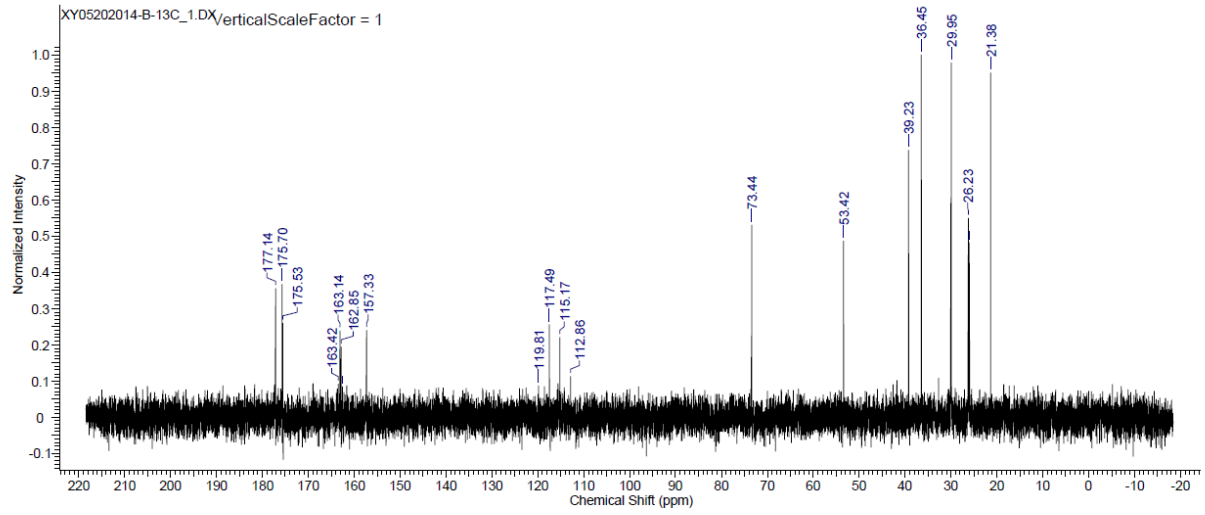


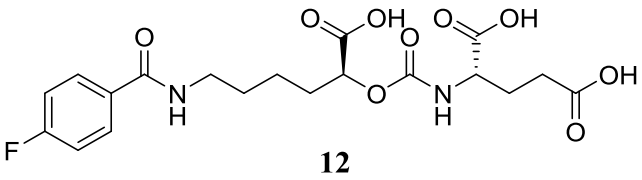
18

Acquisition Time (sec)	6.3615	***Comment***	1H 1D D2O {C:\data\Martin Pomper} MP xing 12			***Date***	20 May 2014 17:13:58
Date Stamp	20 May 2014 17:13:58						
File Name	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY05202014-B-H 1.DX					***Frequency (MHz)***	499.80
Nucleus	1H	***Number of Transients***	32	***Origin***	MP xing	***Original Points Count***	65536
Owner	MP xing	***Points Count***	65536	***Solvent***	DEUTERIUM OXIDE	***Spectrum Offset (Hz)***	3086.5986
Sweep Width (Hz)	10302.04	***Temperature (degree C)***	23.299				



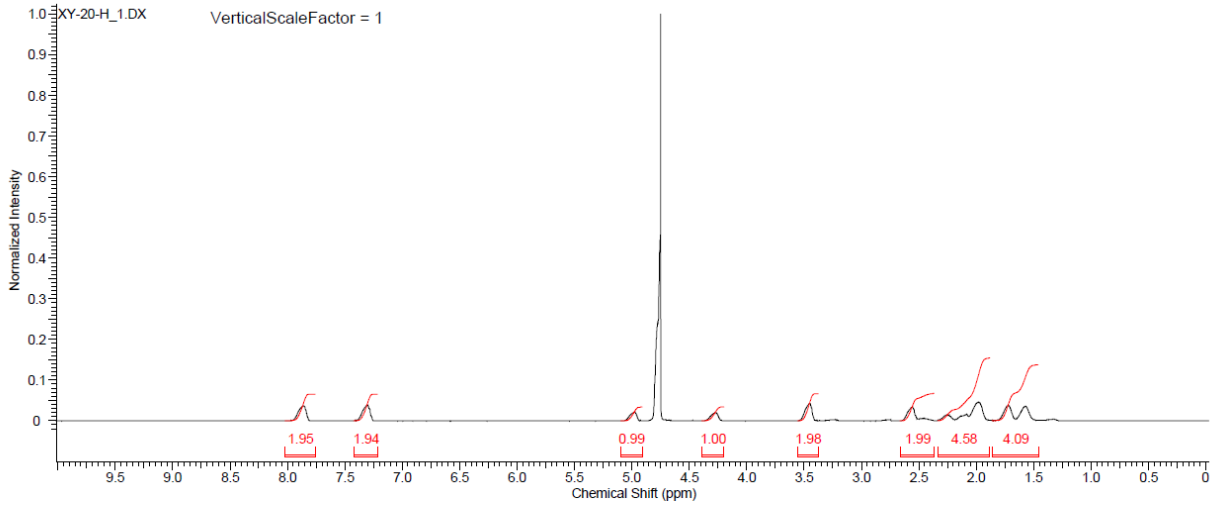
Acquisition Time (sec)	1.1010	***Comment***	13C 1D D2O {C:\data\Martin Pomper} MP xing 13			***Date***	20 May 2014 21:53:51
Date Stamp	20 May 2014 21:53:51	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY05202014-B-13C 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	DEUTERIUM OXIDE
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



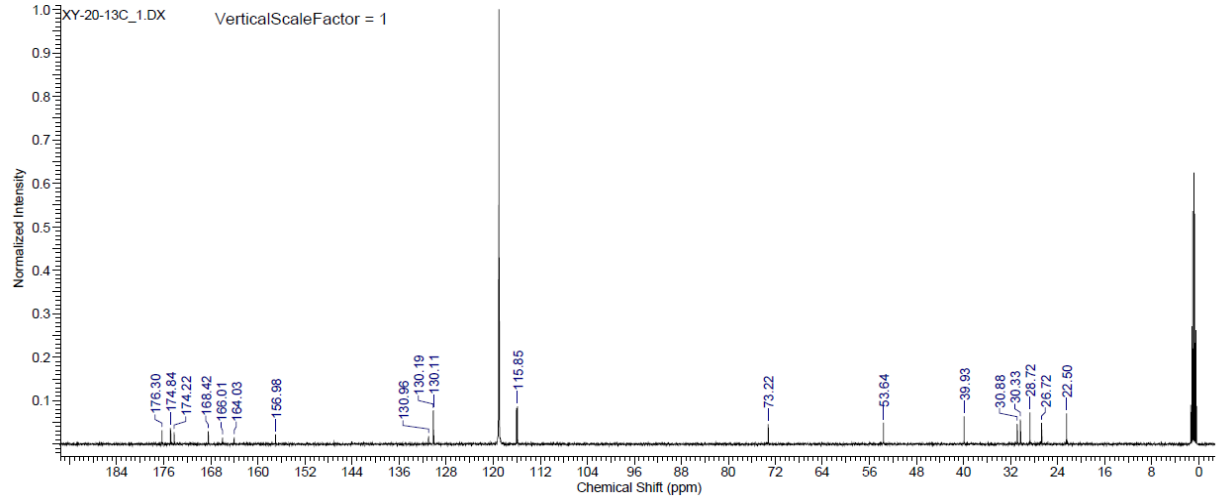


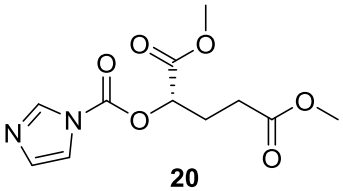
12

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 14			***Date***	27 May 2014 17:36:27
Date Stamp	27 May 2014 17:36:27						
File Name	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY-20-H 1.DX					***Frequency (MHz)***	499.80
Nucleus	1H	***Number of Transients***	32	***Origin***	MP xing	***Original Points Count***	65536
Owner	MP xing	***Points Count***	65536	***Solvent***	CH3CN+D2O	***Spectrum Offset (Hz)***	3115.1797
Sweep Width (Hz)	10302.04	***Temperature (degree C)***	23.299				



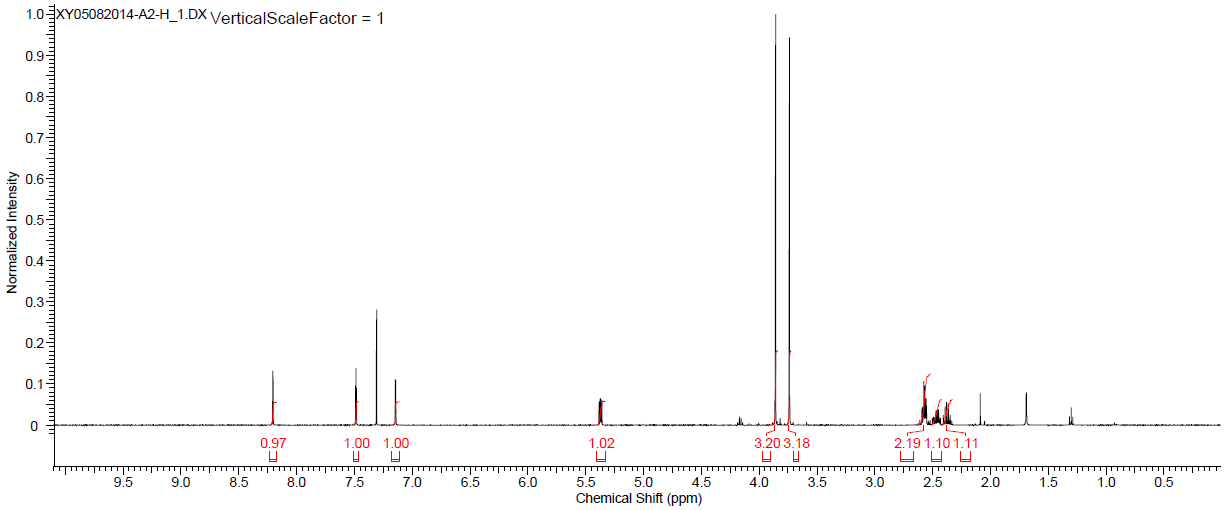
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 15			***Date***	28 May 2014 00:40:19
Date Stamp	28 May 2014 00:40:19						
File Name	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY-20-13C 1.DX					***Frequency (MHz)***	125.69
Nucleus	13C	***Number of Transients***	2048	***Origin***	MP xing	***Original Points Count***	32768
Owner	MP xing	***Points Count***	32768	***Solvent***	CH3CN+D2O	***Spectrum Offset (Hz)***	12570.0527
Sweep Width (Hz)	29761.00	***Temperature (degree C)***	23.299				



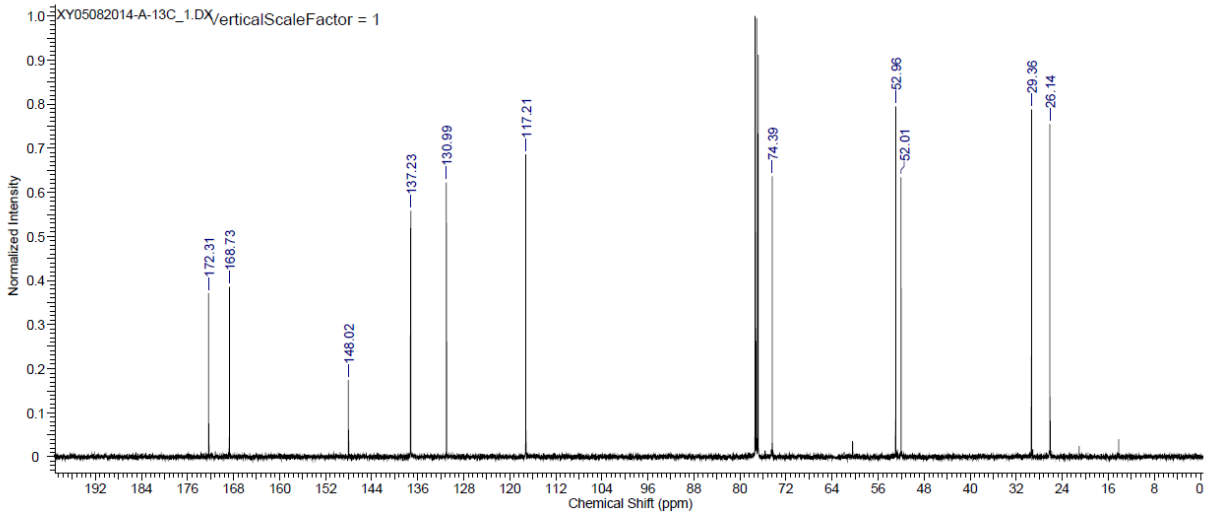


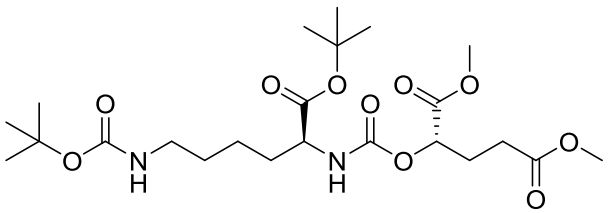
20

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CDCl3 {C:\data\Martin Pomper} MP xing 13			***Date***	08 May 2014 19:02:43
Date Stamp	08 May 2014 19:02:43						
File Name	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY05082014-A2-H 1.DX					***Frequency (MHz)***	499.80
Nucleus	1H	***Number of Transients***	16	***Origin***	MP xing	***Original Points Count***	65536
Owner	MP xing	***Points Count***	65536	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	3086.5986
Sweep Width (Hz)	10302.04	***Temperature (degree C)***	23.299				



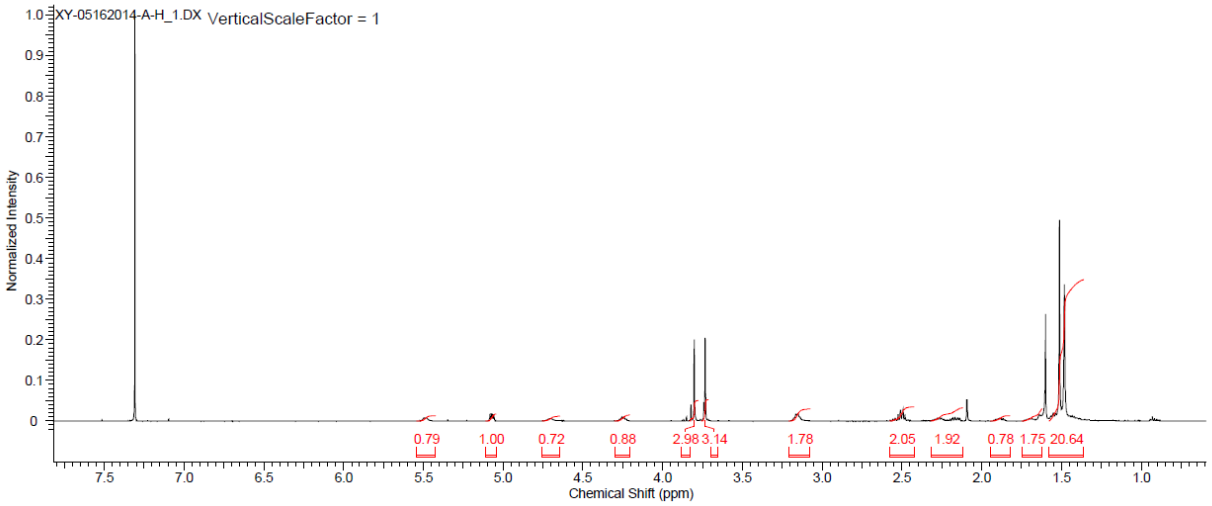
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CDCl3 {C:\data\Martin Pomper} MP xing 12			***Date***	08 May 2014 19:33:25
Date Stamp	08 May 2014 19:33:25	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY05082014-A-13C 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	512	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	CHLOROFORM-d
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



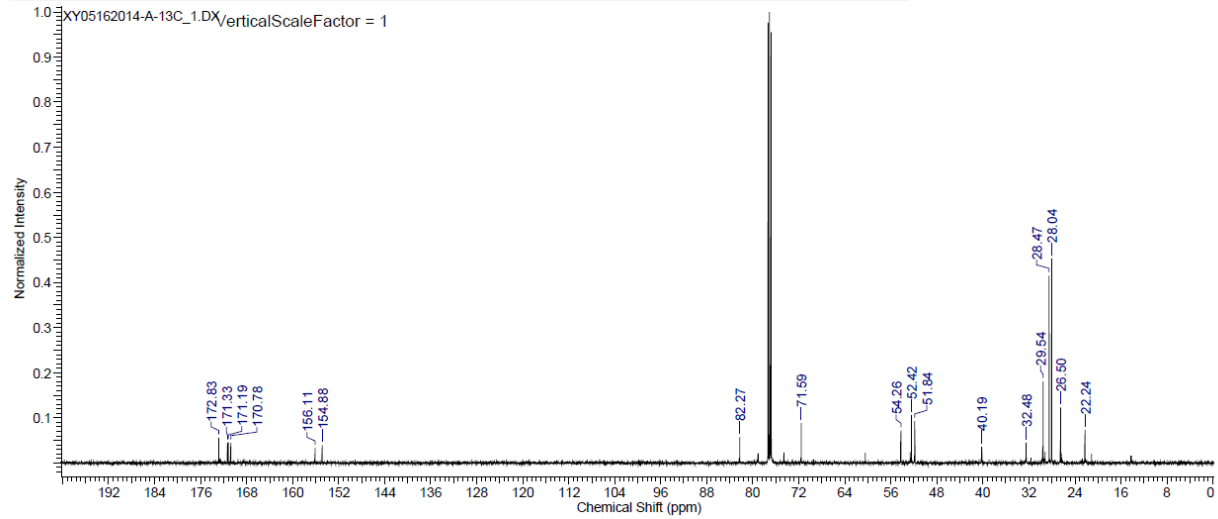


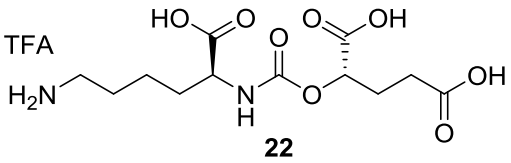
21

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CDCl3 {C:\data\Martin Pomper} MP xing 1			***Date***	16 May 2014 20:35:17
Date Stamp	16 May 2014 20:35:17						
File Name	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY-05162014-A-H 1.DX					***Frequency (MHz)***	499.80
Nucleus	1H	***Number of Transients***	32	***Origin***	MP xing	***Original Points Count***	65536
Owner	MP xing	***Points Count***	65536	***Solvent***	CHLOROFORM-d	***Spectrum Offset (Hz)***	3086.5986
Sweep Width (Hz)	10302.04	***Temperature (degree C)***	23.299				



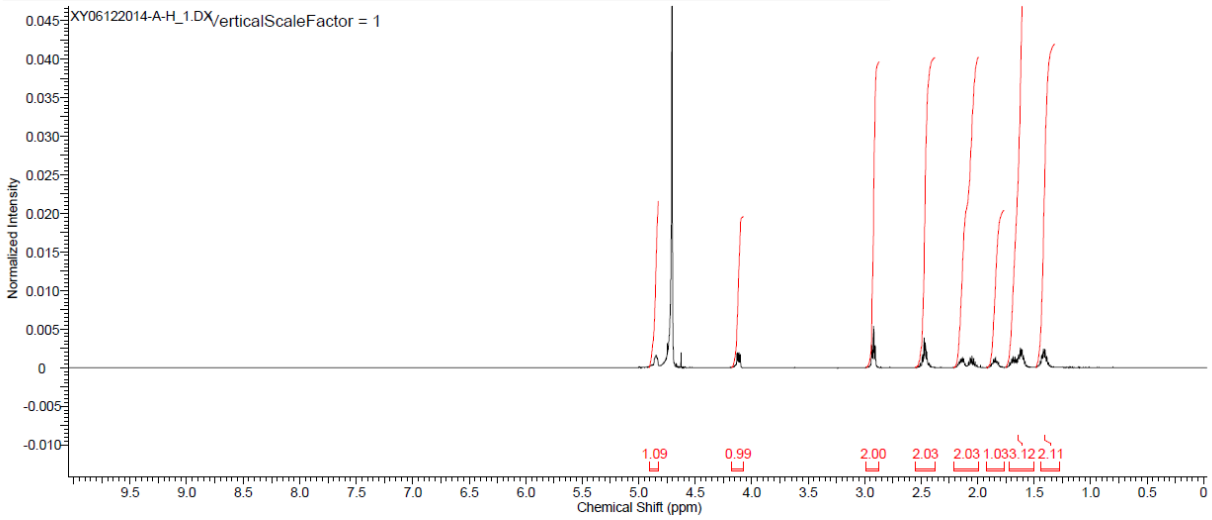
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CDCl3 {C:\data\Martin Pomper} MP xing 2			***Date***	16 May 2014 22:26:54
Date Stamp	16 May 2014 22:26:54	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY05162014-A-13C 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	CHLOROFORM-d
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



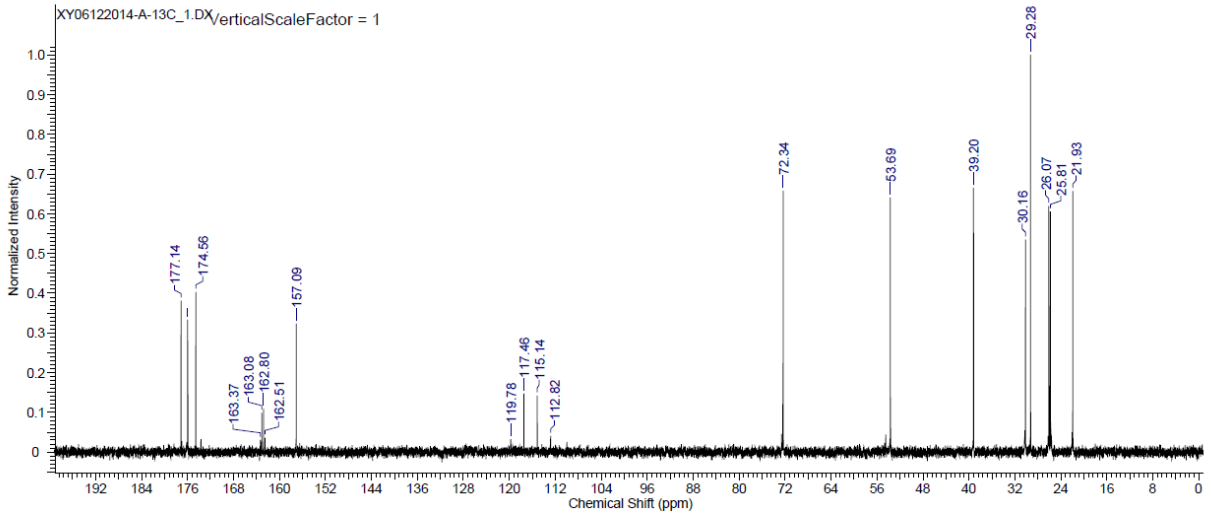


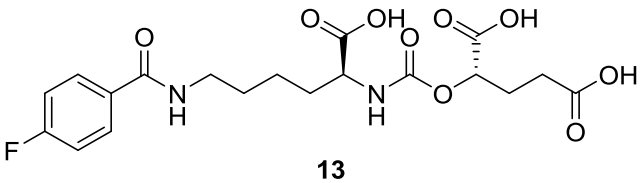
22

Acquisition Time (sec)	6.3615	***Comment***	1H_1D D2O {C:\data\Martin_Pomper} MP_xing 13			***Date***	12 Jun 2014 18:13:18
Date Stamp	12 Jun 2014 18:13:18	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY06122014-A-H_1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP_xing
Original Points Count	65536	***Owner***	MP_xing	***Points Count***	65536	***Solvent***	DEUTERIUM OXIDE
Spectrum Offset (Hz)	3086.5986	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		



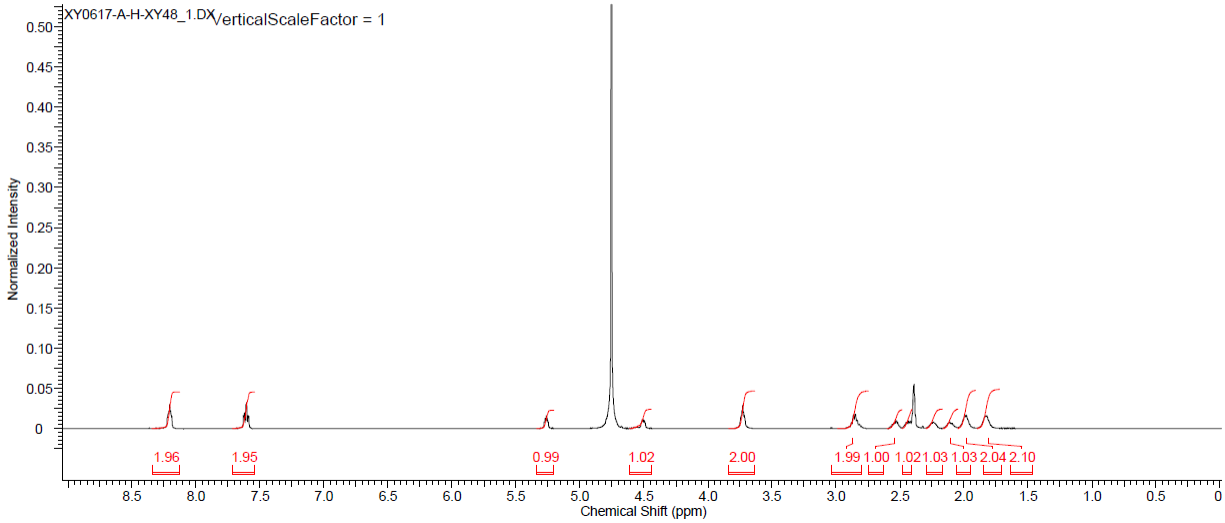
Acquisition Time (sec)	1.1010	***Comment***	13C_1D D2O {C:\data\Martin_Pomper} MP_xing 14			***Date***	12 Jun 2014 20:50:51
Date Stamp	12 Jun 2014 20:50:51	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY06122014-A-13C_1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP_xing
Original Points Count	32768	***Owner***	MP_xing	***Points Count***	32768	***Solvent***	DEUTERIUM OXIDE
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



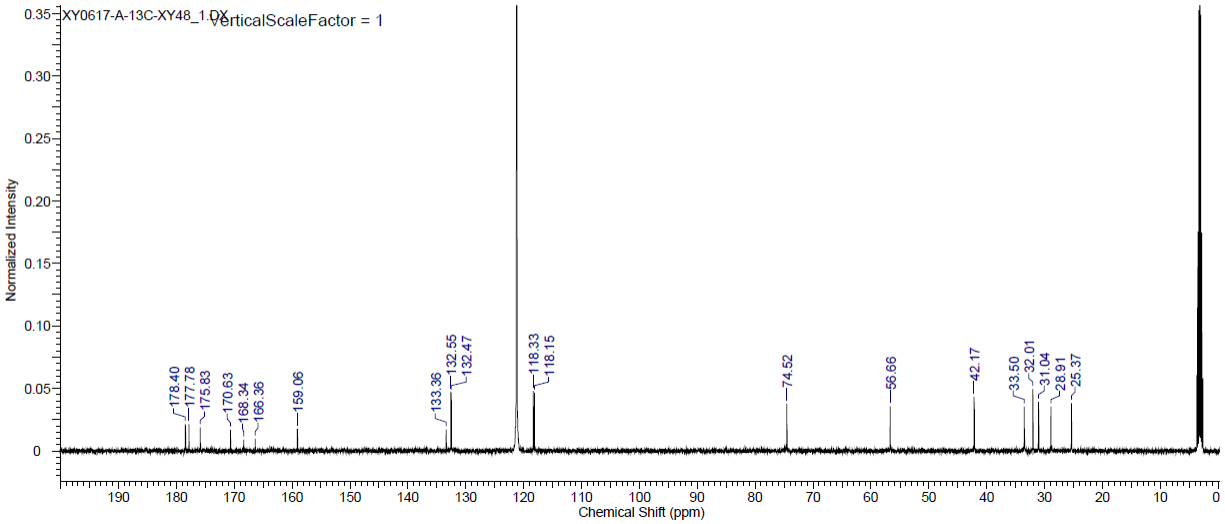


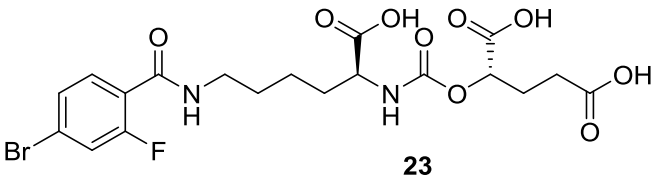
13

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 2			***Date***	17 Jun 2014 17:21:38
Date Stamp	17 Jun 2014 17:21:38	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0617-A-H-XY48 1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP xing
Original Points Count	65536	***Owner***	MP xing	***Points Count***	65536	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	3113.7649	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		



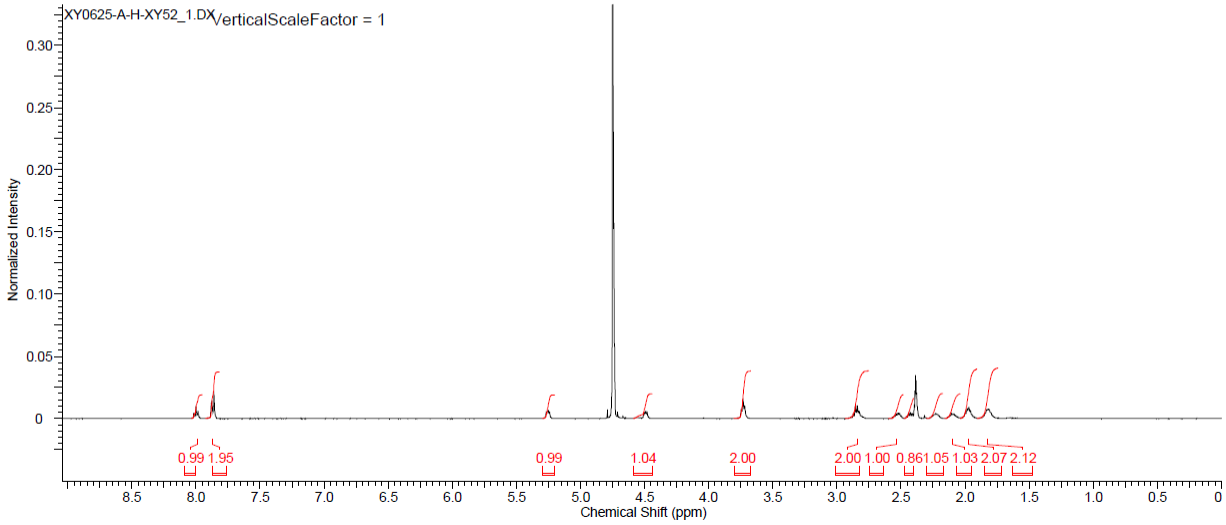
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 3			***Date***	17 Jun 2014 20:51:56
Date Stamp	17 Jun 2014 20:51:56	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0617-A-13C-XY48 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



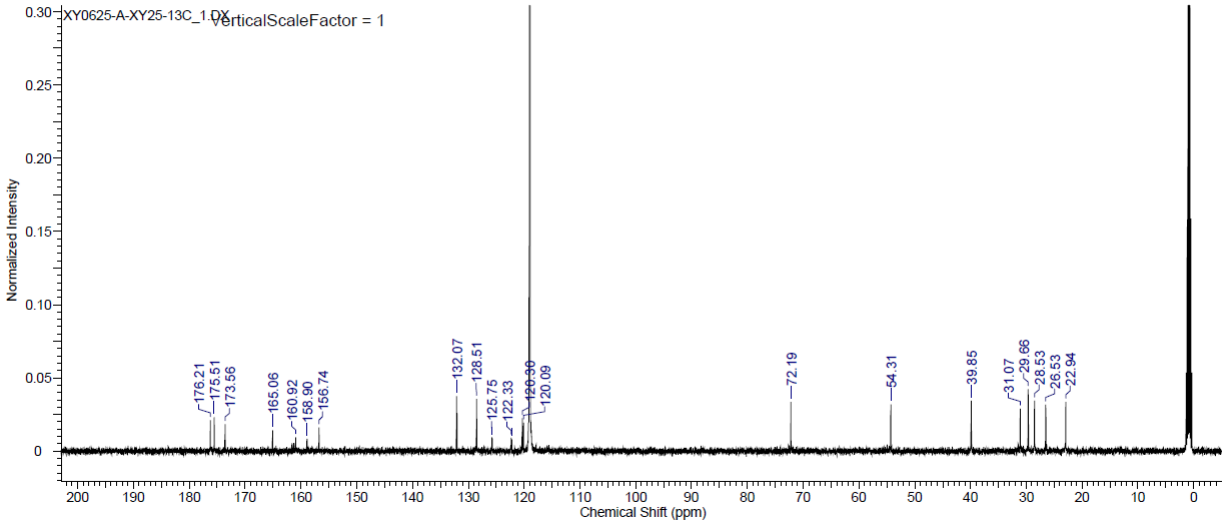


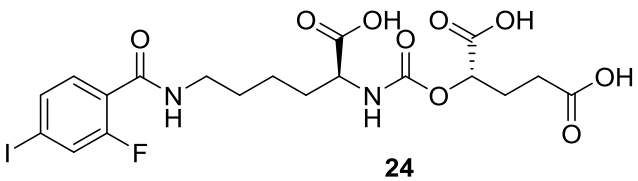
23

Acquisition Time (sec)	6.3615	***Comment***	1H_1D CH3CN+D2O {C:\data\Martin_Pomper} MP_xing 6			***Date***	25 Jun 2014 16:38:48
Date Stamp	25 Jun 2014 16:38:48	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0625-A-H-XY52_1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP_xing
Original Points Count	65536	***Owner***	MP_xing	***Points Count***	65536	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	3110.4639	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		



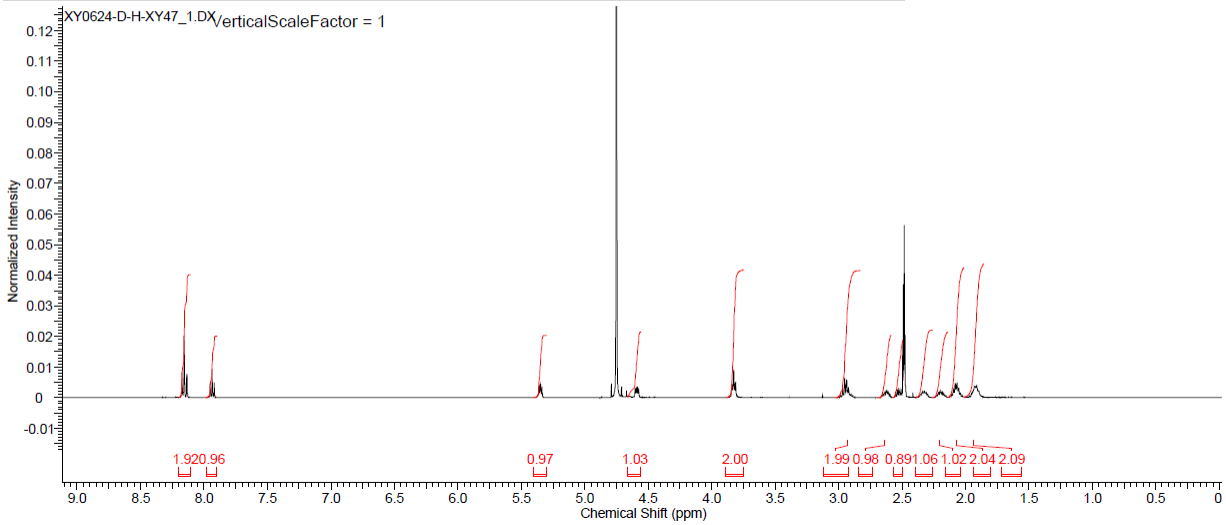
Acquisition Time (sec)	1.1010	***Comment***	13C_1D CH3CN+D2O {C:\data\Martin_Pomper} MP_xing 7			***Date***	25 Jun 2014 21:47:34
Date Stamp	25 Jun 2014 21:47:34	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0625-A-XY25-13C_1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP_xing
Original Points Count	32768	***Owner***	MP_xing	***Points Count***	32768	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



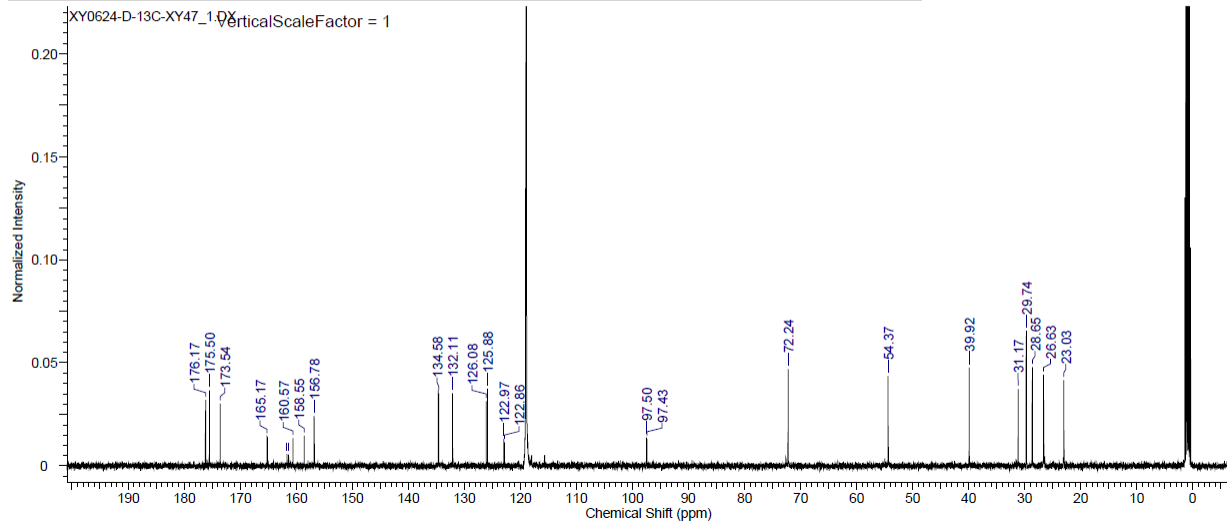


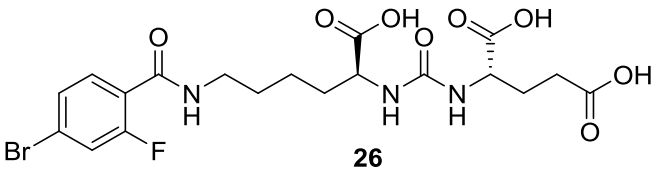
24

Acquisition Time (sec)	6.3615	***Comment***	1H_1D CH3CN+D2O {C:\data\Martin_Pomper} MP_xing 11			***Date***	24 Jun 2014 17:02:10
Date Stamp	24 Jun 2014 17:02:10	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0624-D-H-XY47_1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP_xing
Original Points Count	65536	***Owner***	MP_xing	***Points Count***	65536	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	3112.8218	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		



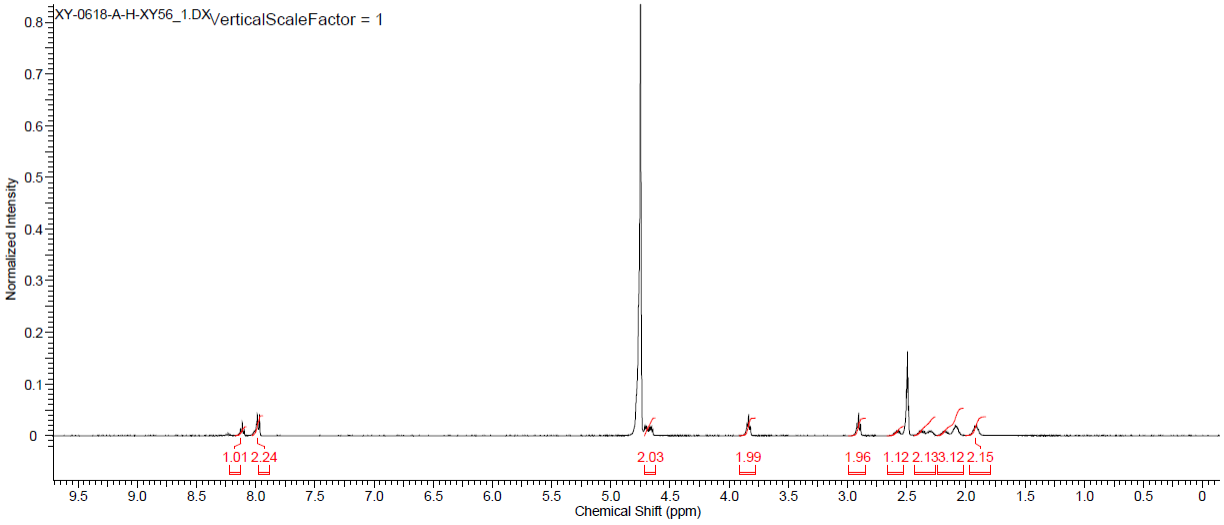
Acquisition Time (sec)	1.1010	***Comment***	13C_1D CH3CN+D2O {C:\data\Martin_Pomper} MP_xing 12			***Date***	24 Jun 2014 20:51:51
Date Stamp	24 Jun 2014 20:51:51	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY0624-D-13C-XY47_1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP_xing
Original Points Count	32768	***Owner***	MP_xing	***Points Count***	32768	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



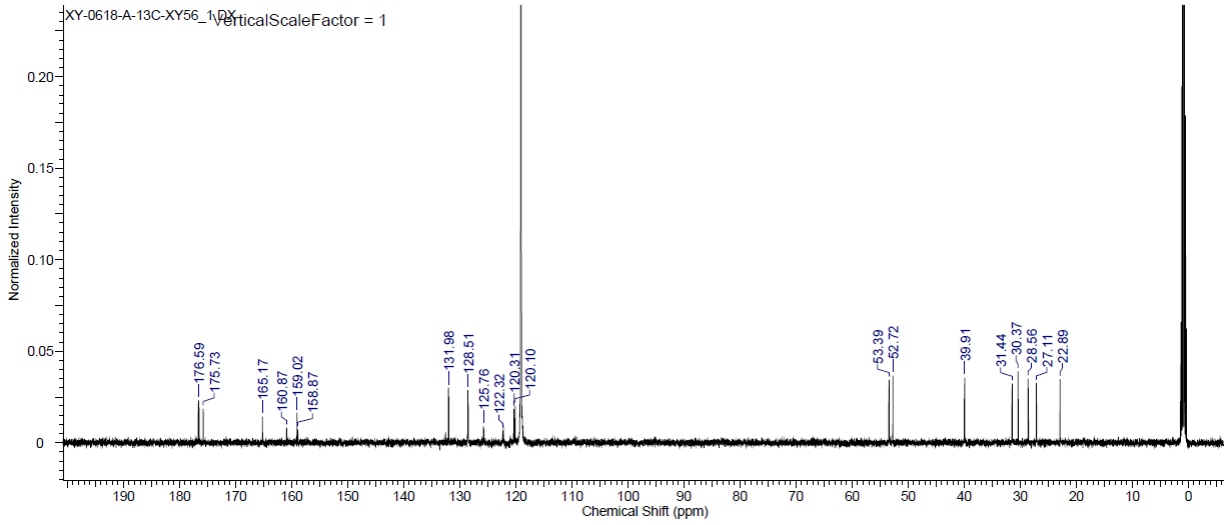


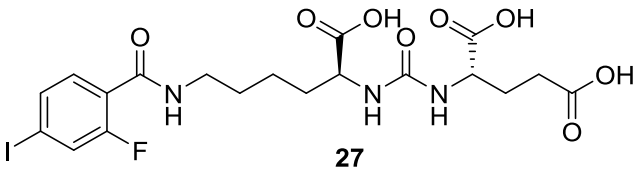
26

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 2			***Date***	18 Jun 2014 17:04:33
Date Stamp	18 Jun 2014 17:04:33	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY-0618-A-H-XY56 1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP xing
Original Points Count	65536	***Owner***	MP xing	***Points Count***	65536	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	3113.9221	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		



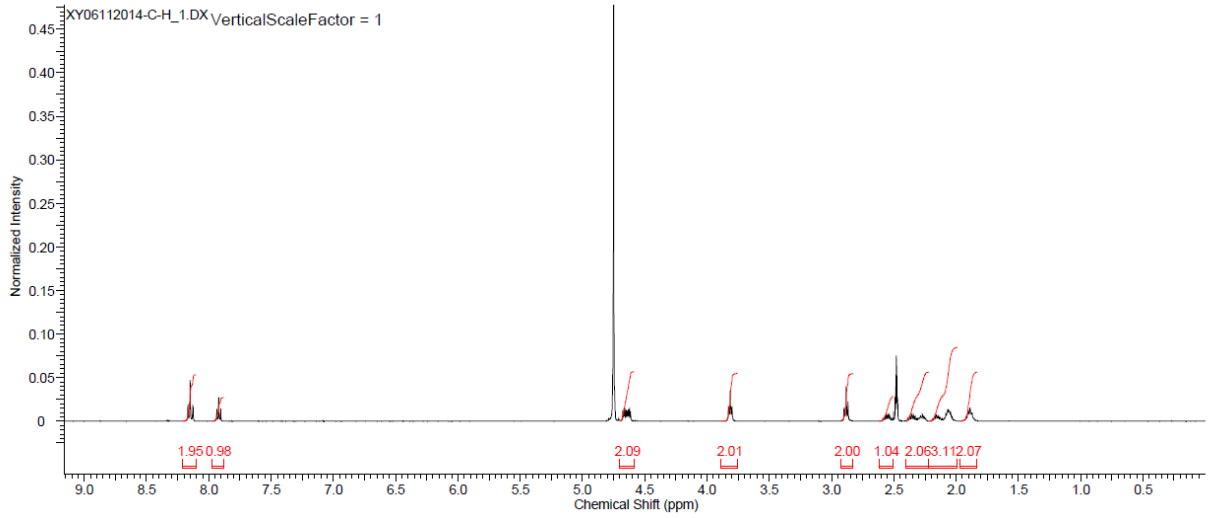
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 3			***Date***	18 Jun 2014 20:51:24
Date Stamp	18 Jun 2014 20:51:24	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY-0618-A-13C-XY56 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	2048	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



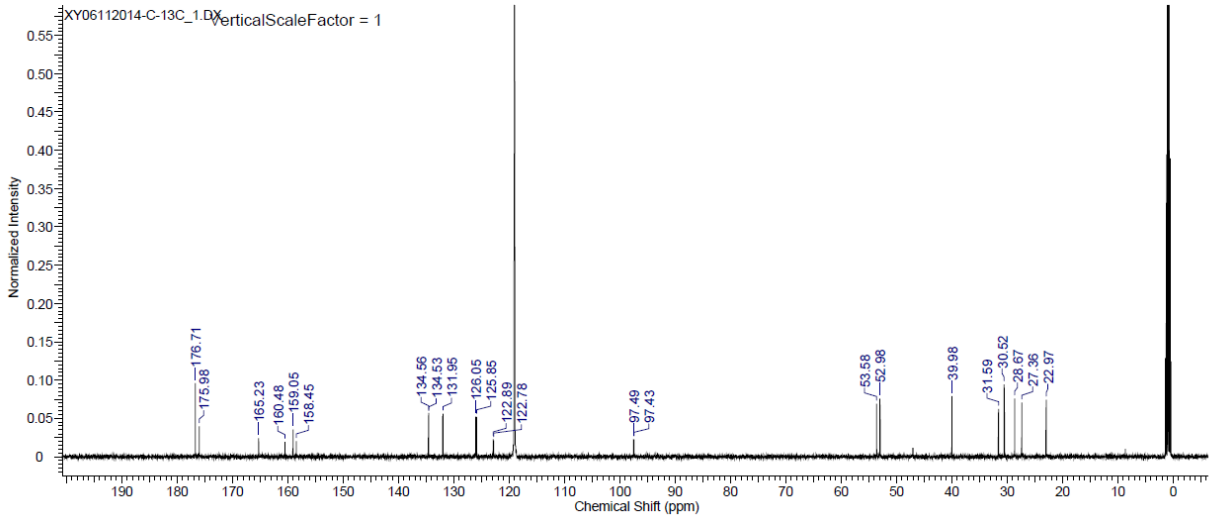


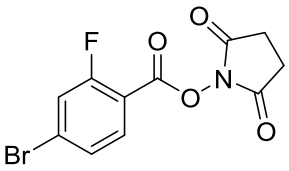
27

Acquisition Time (sec)	6.3615	***Comment***	1H 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 15			***Date***	11 Jun 2014 16:15:45
Date Stamp	11 Jun 2014 16:15:45	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY06112014-C-H 1.DX				
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	16	***Origin***	MP xing
Original Points Count	65536	***Owner***	MP xing	***Points Count***	65536	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	3113.4504	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	23.299		

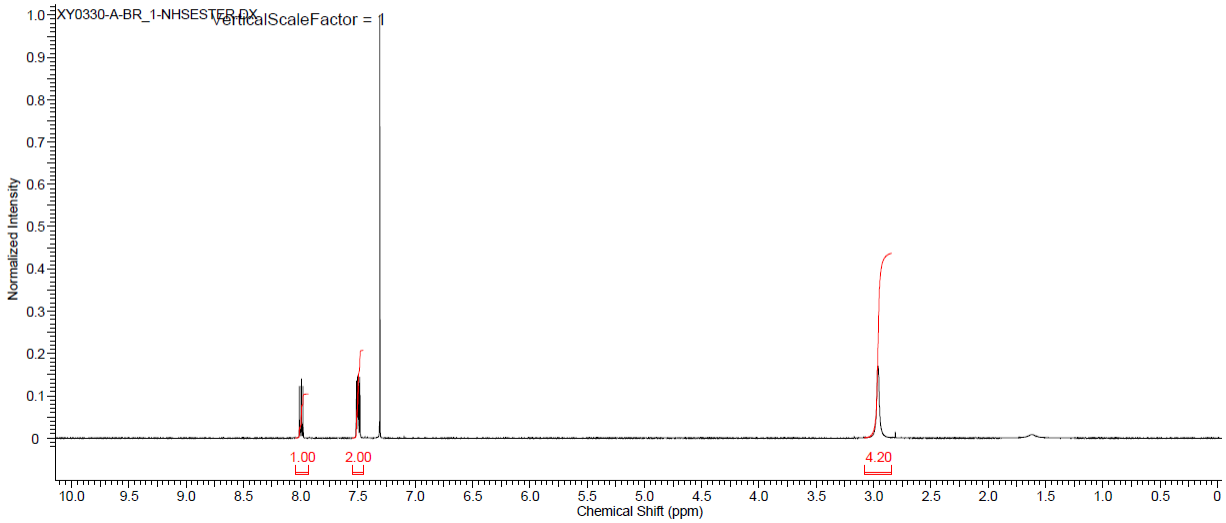


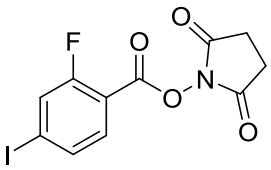
Acquisition Time (sec)	1.1010	***Comment***	13C 1D CH3CN+D2O {C:\data\Martin Pomper} MP xing 16			***Date***	11 Jun 2014 21:52:03
Date Stamp	11 Jun 2014 21:52:03	***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\CARBAMATE CHARACTERIZATION\XY06112014-C-13C 1.DX				
Frequency (MHz)	125.69	***Nucleus***	13C	***Number of Transients***	1024	***Origin***	MP xing
Original Points Count	32768	***Owner***	MP xing	***Points Count***	32768	***Solvent***	CH3CN+D2O
Spectrum Offset (Hz)	12570.0527	***Sweep Width (Hz)***	29761.00	***Temperature (degree C)***	23.299		



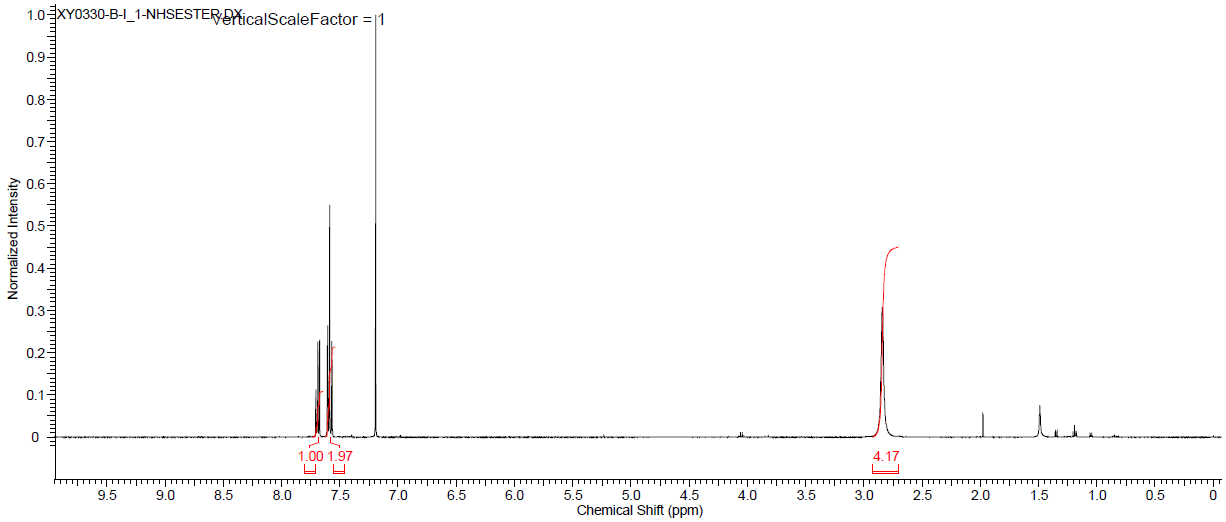


Acquisition Time (sec)	6.3615	***Comment***	1H 1D CDCl3 {C:\data\Martin Pomper} MP xing 15			***Date***	30 Mar 2015 12:50:48
Date Stamp	30 Mar 2015 12:50:48			***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\XY0330-A-BR 1-NHSESTER.DX		
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP xing
Original Points Count	65536	***Owner***	MP xing	***Points Count***	65536	***Solvent***	CHLOROFORM-d
Spectrum Offset (Hz)	3086.5986	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	21.496		





Acquisition Time (sec)	6.3615	***Comment***	1H 1D CDCl3 {C:\data\Martin Pomper} MP xing 16			***Date***	30 Mar 2015 12:55:51
Date Stamp	30 Mar 2015 12:55:51			***File Name***	C:\USERS\XYANG45\JHU FILES\NMR\XY0330-B-I 1-NHSESTER.DX		
Frequency (MHz)	499.80	***Nucleus***	1H	***Number of Transients***	32	***Origin***	MP xing
Original Points Count	65536	***Owner***	MP xing	***Points Count***	65536	***Solvent***	CHLOROFORM-d
Spectrum Offset (Hz)	3029.0415	***Sweep Width (Hz)***	10302.04	***Temperature (degree C)***	21.496		



1. Dekker, B., Keen, H., Shaw, D., Disley, L. et al. Functional comparison of annexin V analogues labeled indirectly and directly with iodine-124. *Nucl. Med. Biol*. **2005**, 32, 403-413.
2. Banerjee, S.R, Pullambhatla, M., Foss, C.A., Nimmagadda, S. et al. ^{64}Cu-labeled inhibitors of prostate-specific membrane antigen for PET imaging of prostate cancer. *Journal of medicinal chemistry*, 2014, 57(6), 2657-69.
3. Tang, G., Zeng, W., Yu, M., Kabalka, G. Facile synthesis of N-succinimidyl-4-[`F]fluorobenzoate ([^{18}F]SFB for protein labeling. J Label Comp Radiopharm, 2008, 51, 68-71.