SYNTHESIS OF AN AMMONIUM ION-SELECTIVE FLUOROIONOPHORE

By

Mustafa Selman YAVUZ

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Chemistry

By

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Approved:

Dr. W. Grant McGimpsey, Major Advisor

Dr. James P. Dittami, Department Head

ABSTRACT:

The drawbacks of nonactin, the current commercial standard receptor for ammonium ion necessitate the development of new ammonium ionophores. We have designed and attempted to synthesize fluoroionophores, I- III. Molecular modeling of I suggests superior selectivity over that of nonactin. III was synthesized as a selective ionophore for optical detection of ammonium ion. The synthetic strategies for III are two-fold: solid phase and solution phase. Solution phase synthesis was performed with two different protecting groups (t-butyl ester and benzyl ester). A methyl-amino substituted anthracene molecule will be covalently coupled to the secondary amine group to provide an optical signaling moiety that operates on the basis of an "off-on" fluorescence emission mechanism. Compound IV was also synthesized in order to provide a sample reaction for the covalent coupling of the chromophore and to provide a fast route to an ammonium fluoroionophore.

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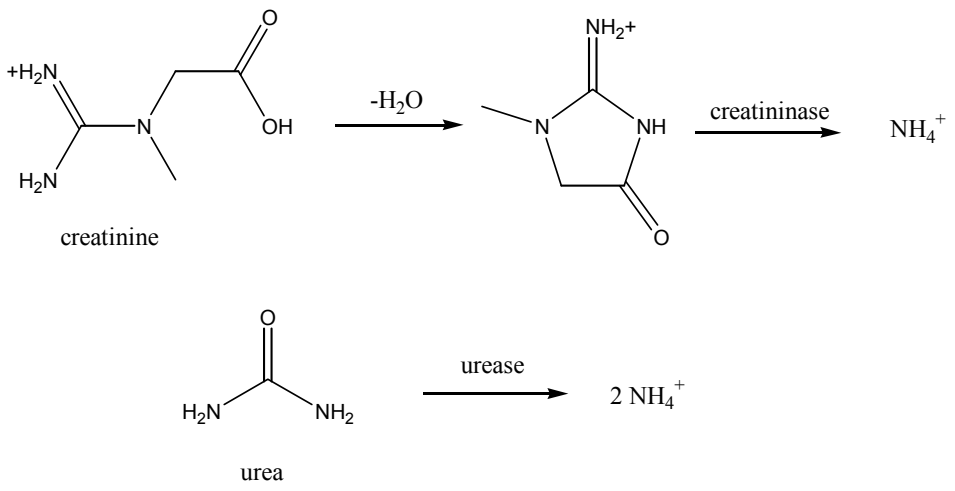
INTRODUCTION:

Over the past several decades, there has been continued interest in developing sensors, either electrochemical or optical, for ammonium cations (NH_4^+). The ammonium ion can be produced by the enzymatic conversion of urea and other nitrogen containing molecules (creatinine, L-phenylalanine, adenosine) found in the blood (Blood Urea Nitrogen or BUN). Its detection, then, is an important diagnostic for disease, particularly kidney disease. [1, 4, 8, 11, 13, 15] The ammonium ion is also an indicator for environmental contamination. For these reasons - clinical diagnostic and environmental monitoring - much effort has been expended in the development of ammonium ion sensors.

In clinical diagnostic applications, ammonium sensors consist of a molecule with an ammonium complexation site that is incorporated into a membrane plus a second membrane that contains an enzyme or enzymes that are capable of converting molecules containing amine and amides to ammonium ion. See for example, scheme 1.



Scheme-1: Enzymatic Degradation of urea and creatinine

Several compounds have been synthesized and identified previously as ammonium ion-selective ionophores. In particular, Lehn et al [2, 3, 7] investigated the ammonium binding properties of SC-24 and SC-25, macrotricyclic cryptands shown in Figure 1.

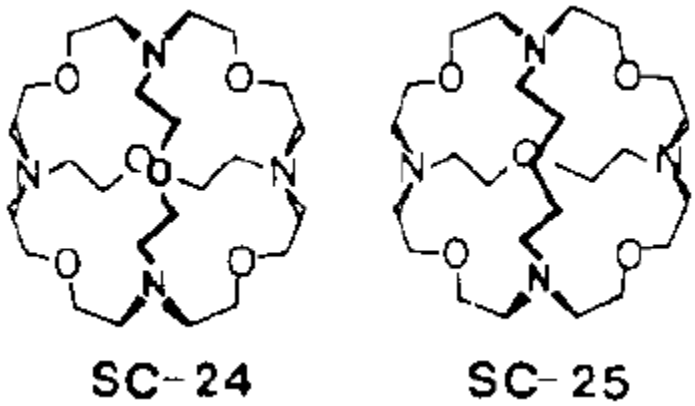


Figure 1: Structures of SC-24 and SC-25

In these molecules, the ammonium cation is bound by a tetrahedral array of N^+-H----N hydrogen bonding sites and by 10 electrostatic interactions to the five oxygens in SC-25 and 12 electrostatic interactions to the six oxygens in SC-24 [2, 7]. The most stable position of the NH_4^+ is centered inside the cavity with N^+-H----N = 3.13 A^o).[7] Despite high NH_4^+/ K^+ selectivity of SC-24(NH_4^+/K^+ = 500)[7] and SC-25 (NH_4^+/K^+ = 63) [7], a characteristic that is required for efficient sensing, the complexation equilibrium constant is extremely high, making these molecules ammonium ion sinks and useless as sensors.

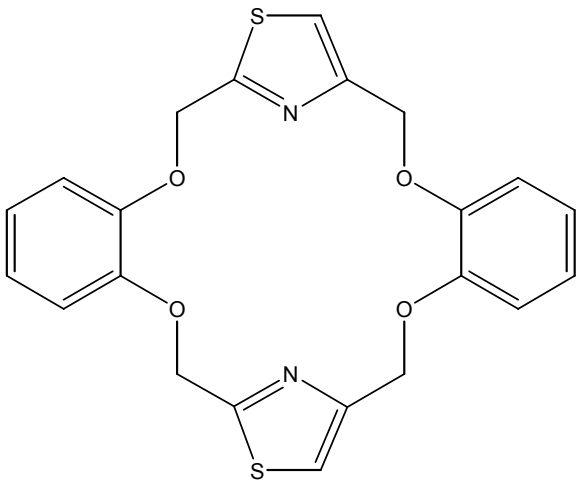


Figure-2: Structure of Thiazole containing dibenzo-18-crown-16 (TDB18C6)

Kim et al [15] synthesized the thiazole-containing dibenzo-18-crown-6 shown in Figure 2. This molecule provides both an appropriate sized cavity and binding interactions that are conformationally favorable for binding ammonium ion. Enhanced selectivity over alkali metal ions, particularly potassium which has a similar atomic radius to ammonium ion, was reported.

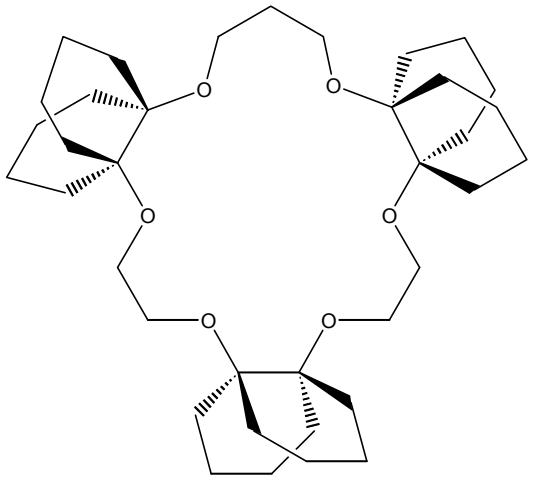


Figure-3: Structure of 19-crown-6-ether with decalino subunits (TD19C6)

Another attempt to increase ionophore selectivity was made by Suzuki et al [9] who took a rational approach to ionophore design by considering molecular conformational factors.

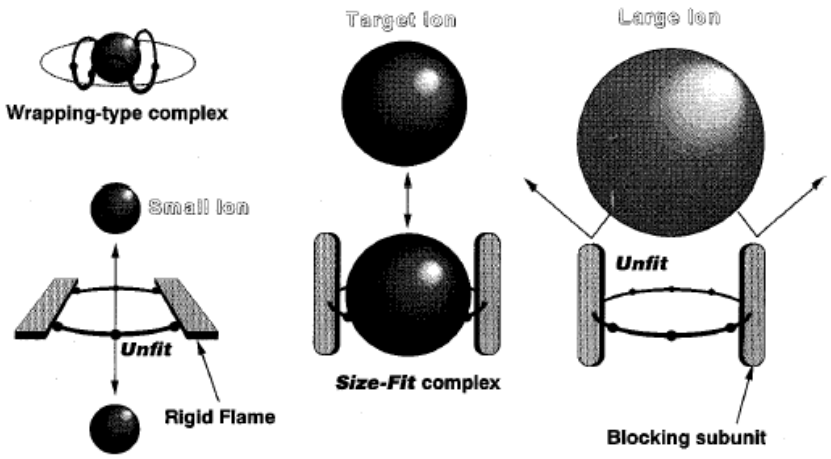


Figure-4: Molecular Structure Design [9]

Figures 3 and 4 show the results of these attempts. The molecule shown in Figure 3 was designed with a rigid frame and bulky block wall in order to prevent the formation of both a wrapping complex with smaller ions and a sandwich complex with larger ions.

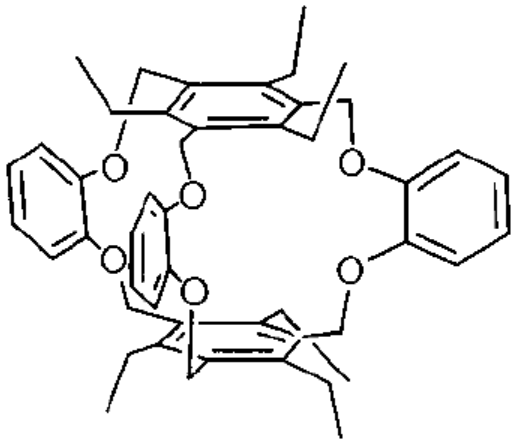


Figure-5: Structure of Tris (pyrazol-1-ylmethyl) benzene

Kim et al [4] reported high selectivity of tris (pyrazol-1-ylmethyl) benzene (Figure 5) for ammonium ion over alkali metal ions. Design factors included a consideration of the strong cation-π interaction provided by the phenyl rings and a rigid frame with a correctly sized cavity for binding. Tris (pyrazol-1-ylmethyl) benzene was highly selective for binding NH_4^+ over alkali metal ions via a cation-π interaction between the substrate and the central benzene ring of the receptor. In contrast to the nitrogen containing ionophores described above, the phenolic oxygens here (H-bonding acceptors) have larger pK_a values, potentially making the binding ability of this molecule less pH-dependent, an important factor in sensor performance for physiological applications.

The ammonium ionophore currently used in commercial sensor applications is nonactin, [4, 6, 8, 9, 11, 15] a natural antibiotic (Figure 7). However, nonactin has serious drawbacks. Due to its flexibility, the binding site in nonactin can accommodate other ions particularly potassium which is a major interfering analyte (only ~10 times more selective for ammonium over potassium). In addition, nonactin can form wrapping type complexes with smaller ions. [6, 8, 11]

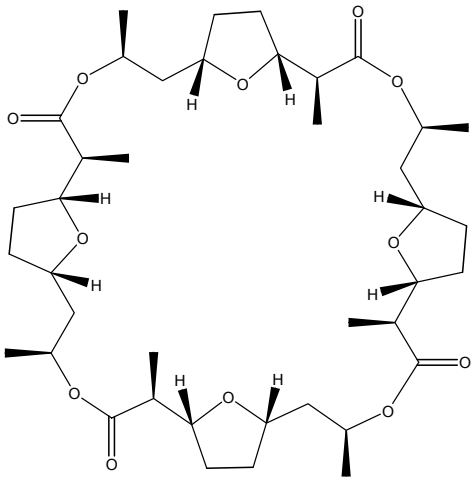
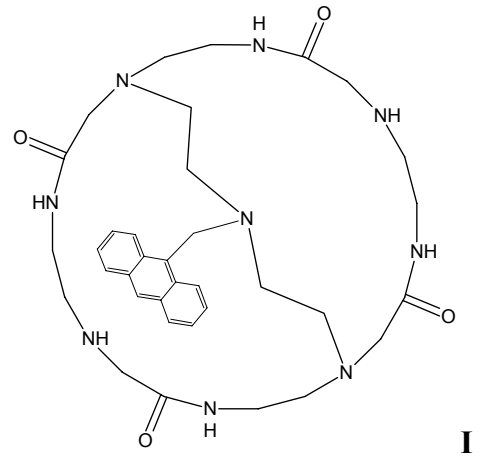
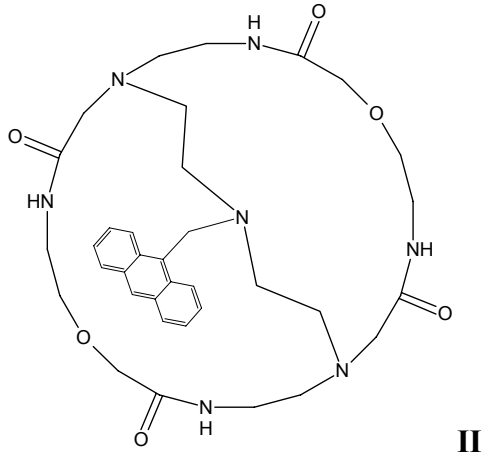


Figure-6: Structure of Nonactin

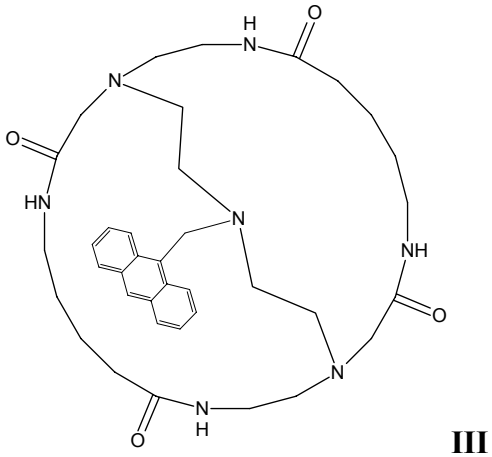
We have undertaken the design and synthesis of new ammonium ionophores and fluoroionophores to address the drawbacks of nonactin, particularly the lack of selectivity. In particular, we have designed and modeled I, II, III.



I



II



III

Figure-7: Structures of Target molecules; I, II, III

Modeling studies that we report in this work predict 10-100 times better $NH_4^+\backslash K^+$ selectivity for these compounds as compared to Nonactin.

The results of these studies are shown in Figure-8. Docking energies were determined on K^+ and NH_4^+ complexes with various ammonium ionophores including I, II and III. Published selectivity data [7] and predicted selectivity of I were plotted as log $NH_4^+\backslash K^+$ selectivity vs. Docking energy as shown below.

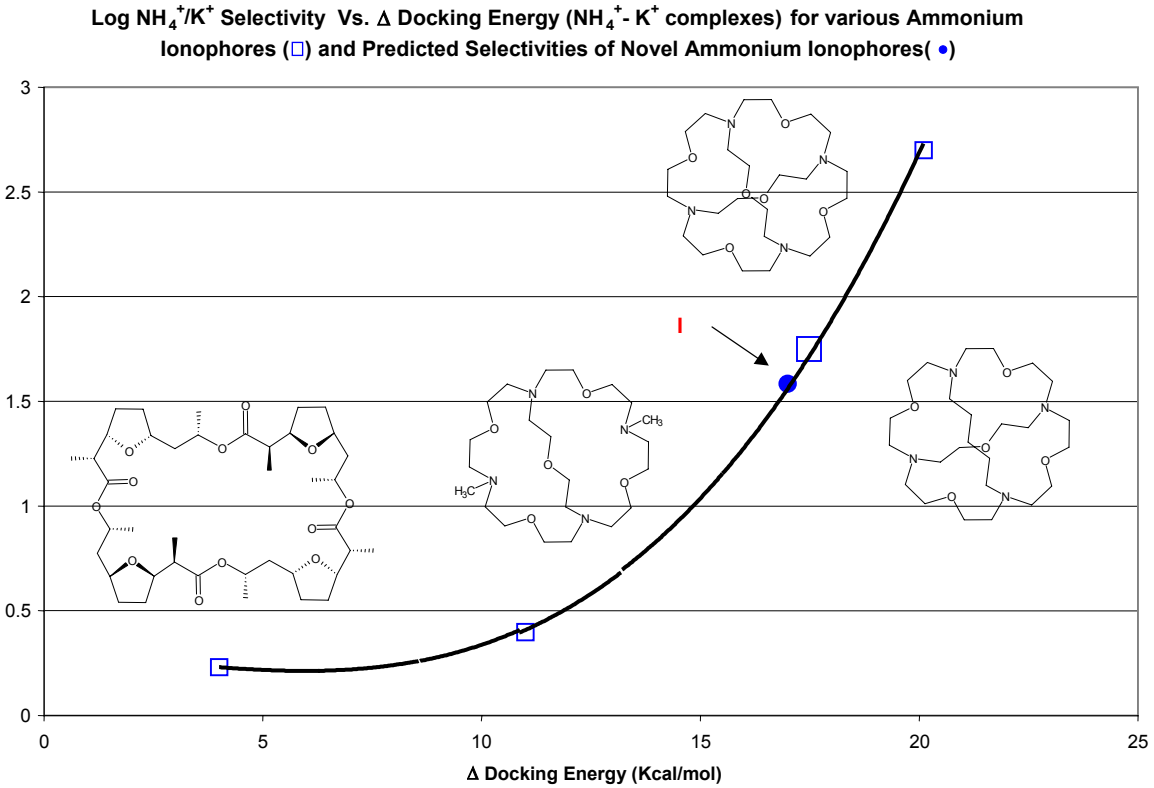


Figure-8: Graph of Log NH_4^+/K^+ selectivity vs. Docking Energy

In the figure-8 above [16], the tricyclic compounds are the most selective structures known today. (synthesized by Lehn and co-workers [7]). They show high selectivity but as mentioned above they can not be used for ammonium sensor applications due to irreversible complexation. In addition, nonactin and Bicyclic compound (BC-25) (synthesized by Lehn et al [7]) have poor selectivity for ammonium cations due to formation of weak complexes.

We expect I, II and III will have high ammonium cation selectivity (theoretically 10-100 times more selective than nonactin) because they incorporate H-bond donors (amide groups) in tetrahedrally symmetric complexation sites. We also expect lower complexation constants leading to reversibility.

In Figure-9, **I** is shown complexed with ammonium and potassium ions. When bound, the ammonium cation sits in the cavity of **I** with tetrahedral complexation and a minimum of four H-bonds. On the other hand, the potassium cation does not occupy a position in the cavity that is conducive to favorable complexation.

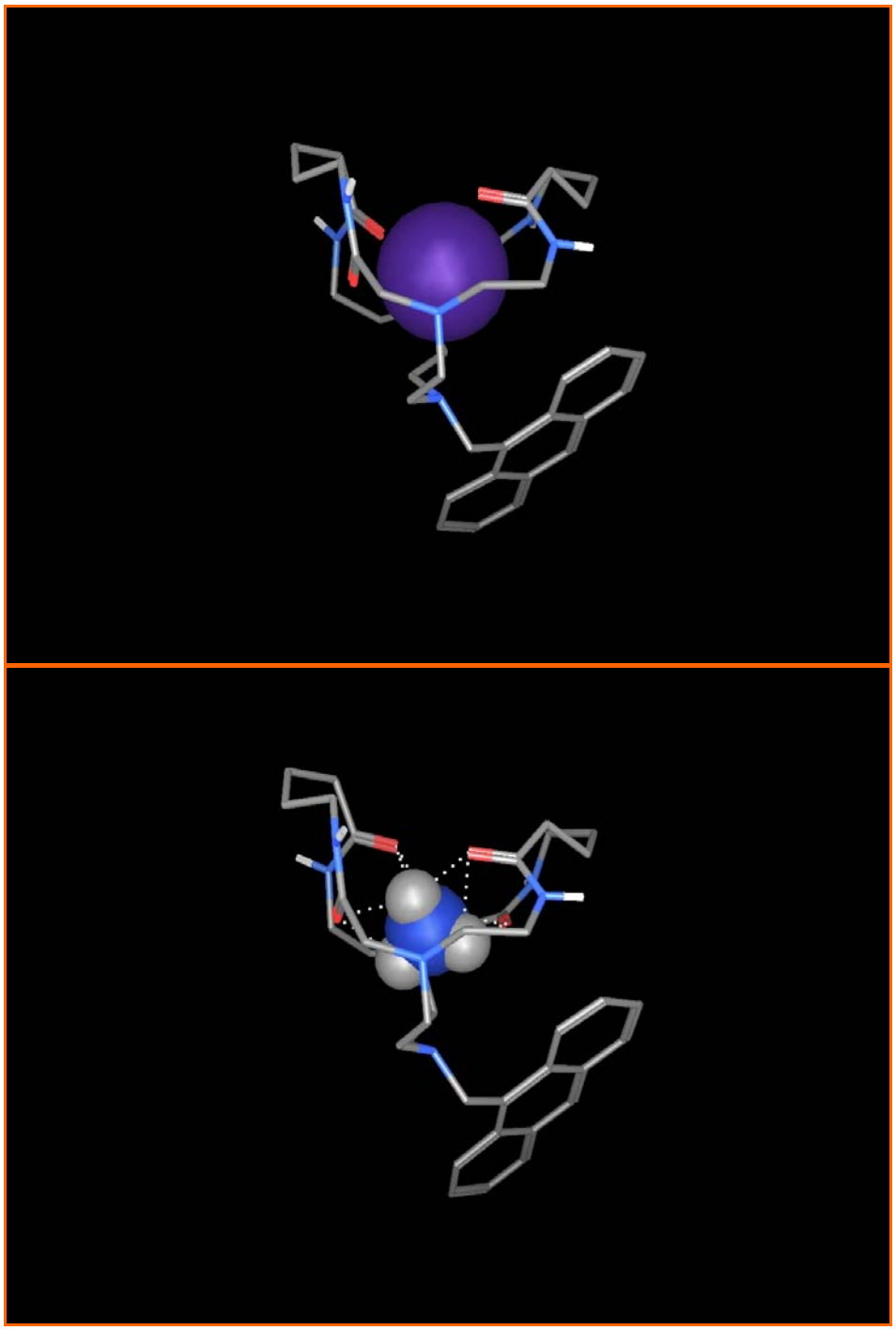
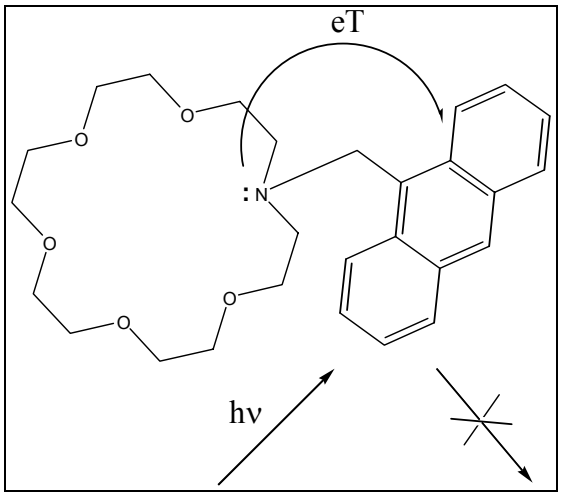
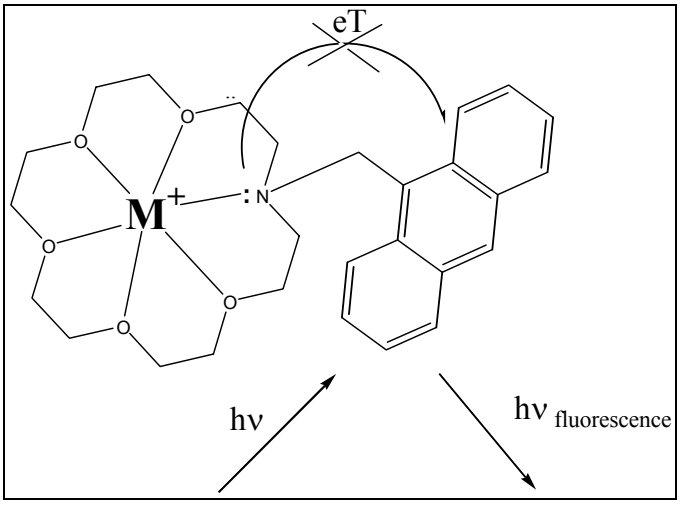


Figure-9: Complexation of **I** with NH_4^+ (down) and K^+ (up)

While most ammonium sensors are currently electrochemical and in fact a major effort of our group is to fabricate ammonium ion selective electrodes, we have undertaken here the creation of an optical ammonium ionophore, or fluoroionophore. In order to use optical detection, our fluoroionophore will incorporate an anthracene chromophore covalently attached to the binding site at one of the secondary amines. This molecule will signal binding by an 'off-on' photoelectron transfer switching mechanism (PET).



“ OFF ” state



“ ON ” state

Scheme-2: Model of Photo Electron Transfer Mechanism

In ion-free conditions, the lone pair of electrons on the nitrogen atom connected to the anthracene will quench the anthracene excited state by electron transfer, leading to low fluorescence intensity. When ammonium

ion is bound, however, its electrostatic field will disrupt the transfer and cause fluorescence to increase dramatically in intensity.

We report here the results of our efforts to design and synthesize I, II and III.

EXPERIMENTAL SECTION

I) Materials

All reagents were used as received without further purification. Dimethylformamide (DMF), 1,4 dioxane, diisopropylethylamine (DIPEA), piperidine, diethanolamine (99%), triethylamine (99.5%), and benzene (99%) were all purchased from Aldrich Chemicals. Coupling reagents (PyBOP, HOBt), Fmoc-OSu, using t-butyl-2,2,2 trichloroacetimidate [TBTA] and wang resin were purchased from Nova Biochem. δ-Aminovaleric acid-benzyl ester p-tosylate (**3**),N-β-aminoethyl-Gly-OH (**1**) was purchased from BaChem**.** Fmoc-5-Ava-OH (**5**) was purchased from Advanced Chemtech. Diethyliminodiacetate(**13**), borontrifluoride ($BF_3.Et_2O$), thionyl chloride(99.5%), 5-aminovaleric acid(**7**)(97%), t-butanol(99.5%), 9-(chloro methyl)anthracene (98%), diaza-18-crown-6 (98%) was purchased from Acros. Anhydrous magnesium sulfate, chloroform was purchased from Mallinckrodt. Methanol, ethanol, dichloromethane (DCM) was purchased from Pharmco. Trifluoro-acetic acid (TFA) was purchased from J.T.Baker. Hexane was purchased from Burdick&Jackson. N-Fmoc-iminodiacetic acid was purchased from Fluka. Iminodiacetic acid was purchased from Avocado.

II) General Methods

i) NMR

^{1}Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker AVANCE 400 (400 MHz) NMR spectrometer. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS) at 0.00 ppm. ^{13}Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 100 MHz on the same spectrometer

ii) Mass Spectrometry

Mass spectra (electrospray) were performed by BAYER Corporation, MA. The system is a Waters QuattroMicro MS with ESI and uses a triple quadrapole.

iii) Thin Layer Chromatography

Analytical thin layer chromatography(TLC) was performed using pre-coated silica gel plates (Whatman 200 μm KCF18 silica gel 60A reverse phase plates or Whatman 250 μm thickness KF6F silica gel 60A normal phase plates) with visualization by UV-lamp or in a glass chamber containing iodine.

iv) Preparative Thin Layer Chromatography

Preparative thin layer chromatography was performed using 1000 μm precoated Whatman PK6F 60 Å silica gel plates.

v) Flash Chromatography

Flash chromatography was performed on a Biotage Flash 12i using KP-Sil 32-63 μm 60A silica gel under N_2 pressure.

vi) Melting Point

All melting points were obtained using a Fisher-Johns Melting point apparatus and are uncorrected.

vii) UV-Visible Spectroscopy

Ground state absorption spectra were measured in a quartz cuvette (1cm x 1cm) with a Shimadzu UV 2100 Spectrophotometer. Samples were measured in single beam mode and compared with a blank obtained with pure solvent.

viii) Molecular Modeling Calculations

Compounds I-II-III were designed by molecular modeling using the Molecular Operating Environment (MOE) program. Molecular modeling was performed on an SGI 320 running Windows NT. Calculations were carried out using the MOE program version 2000.02 computing package (Chemical Computing Group Inc., Montreal, Quebec, Canada). Structures were minimized first using the AMBER94 potential control under a solvent dielectric of 5. PEF95SAC was used to calculate partial charges. Molecular structures were then subjected to a 30 ps molecular dynamics simulation employing the NVT statistical ensemble. The bicyclic structures were heated to 400 K, equilibrated at 310 K (37 ^{o}C, Body Temperature) and cooled down to 290 K in the dynamics thermal cycle at a rate of 10 K/ps.

The lowest energies of the bicyclic structures obtained from these dynamics calculations were then minimized again. The same procedure applied to minimized bicyclic structures with NH_4^+ and K^+ inside the cavity, respectively. The docking energies of ammonium and potassium cations were calculated by employing the default parameters supplied with the program.

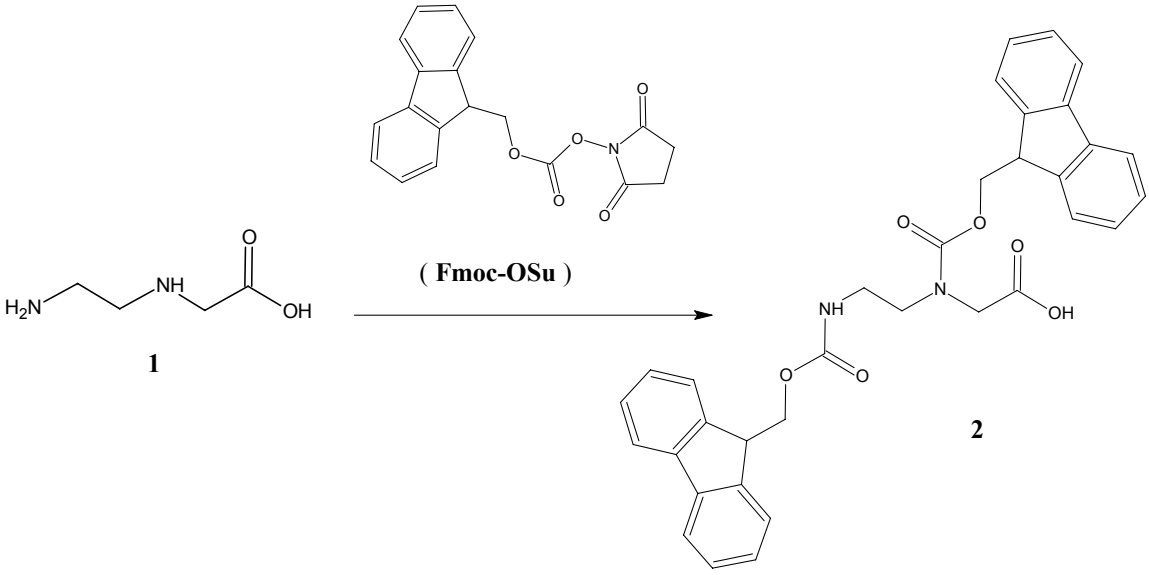
ix) Kaiser Test:

The Kaiser test is most widely used qualitative resin test for the presence or absence of free amino groups, especially primary amines.

In order to perform the Kaiser test, a few dry resin beads were placed in a test tube and two drops of each of the solutions of 2 ml of a 0.001M aqueous solution of KCN dissolved in 98 ml pyridine, 80 g of liquefied phenol dissolved in 100 ml ethanol and 5 g of ninhydrin dissolved in 100 ml ethanol were added to the test tube.

We took the resulting pale yellow mixture, and heated it in an oven at 120 ^{o}C for 5 minutes. If the resin beads appear clear or yellow at the end of this process, then there is no primary amine. However, if there is free unprotected amine, a dark blue solution will result [22].

III.Synthesis:

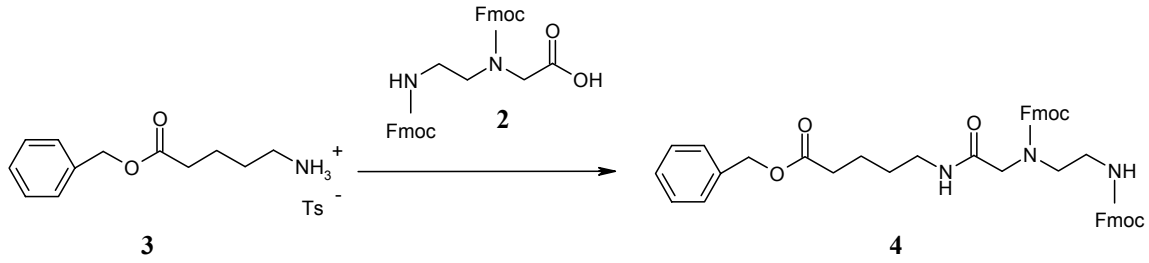


Scheme-3: Formation of 2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9-fluorenyl methoxy)carbonyl] aminoethyl)amino]acetic acid (**2**)

i) 2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9-fluorenyl methoxy) carbonyl]aminoethyl)amino]aceticacid(**2**)(Scheme-3)**:**

2-[(2-aminoethyl) amino] acetic acid (2g, 16.9 mmol) was dissolved in 40 ml of 9% Na_2CO_3 solution. Fmoc-OSu (14.25 g, 42.25 mmol) in 120 ml 1,4 Dioxane was added at once forming a suspension. The suspension was stirred over night at room temperature. The white precipitate was filtered and the filtrate was acidified with HCl up to pH: 2. After evaporating the solvents *in vacuo*, the solution was dissolved in 60 ml DI water. The solution was extracted thrice with 60 ml dichloromethane. The combined

organic extracts were dried with Na_2SO_4. After evaporating the solvent *in vacuo*, the crude was reprecipitated with dichloromethane: hexane (1:3) to give white solid (5.9 g, 62%). mp: 152.7 ^oC; R_f : 0.35 (1:15, Acetic acid: Dichloromethane); ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 10.2(broad, 1H, COOH), 7.7(t, 2H, CH), 7.65(t, 2H, CH),7.49(d, 2H, CH), 7.4(d, 2H, CH), 7.34(d, 2H, CH), 7.3(d, 2H, CH), 7.27(t, 2H, CH), 7.23(t, 2H, CH), 4.4(t, H, CH), 4.29(t, H, CH),4.1(d, 2H, CH_2), 3.85(d, 2H, CH_2), 3.73(s, 2H, CH_2), 3.3&3.1(2H, CH_2), 2.86(t, 2H, CH_2); ^{13}C-NMR:(100 MHz, MeOD) δ 40.51(CH_2), 68.26(CH_2), 69.43(CH_2), 121.2(CH), 121.34(CH), 121.5 (CH), 126.35(CH), 126.6(CH), 128.5(CH), 129.08(CH), 129.26(CH), 142.98(C), 145.54(C), 145.65(C), 145.69(C),158.55(C=O), 159.3(C=O), 173.8(COOH) EIS MS: m/z ($M + H^+$) calcd. 562.62 found 562.96; ($M + Na^+$) calcd.585.6 Found 585.008

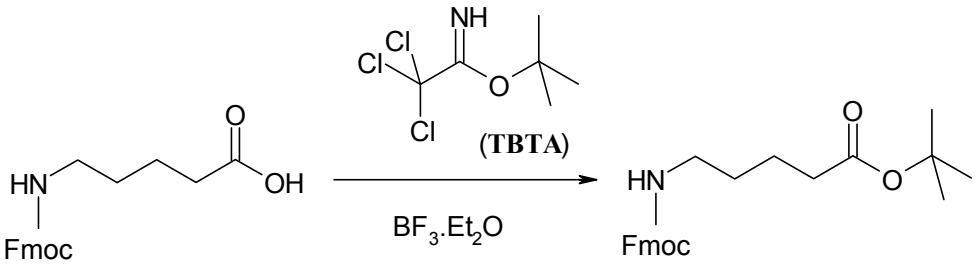


Scheme-4: Formation of Benzyl 5-(2-[[(9*H*-9-fluorenylmethoxy) carbonyl](2-[(9*H*-9-fluorenylmethoxy) carbonyl] aminoethyl)amino] acetylamino) pentanoate (**4**)

ii) Benzyl 5-(2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9-fluorenyl methoxy)carbonyl]aminoethyl)amino]acetylamino)pentanoate(4):

2-[[(9*H*-9-fluorenyl methoxy) carbonyl](2-[(9*H*-9-fluorenyl methoxy) carbonyl]aminoethyl)amino] acetic acid(**2**) (1.125 g, 2 mmol), (Benzo triazol-1-yloxy)tris (pyrrolidino) phosphonium hexafluorophosphate [PyBOP] (1.04 g, 2 mmol), 1-Hydroxybenzotriazole [HOBt] (0.27 g, 2 mmol) and N,N-Diisopropyl ethylamine [DIPEA] (1.1 ml, 6 mmol) was dissolved in 100 ml dichloromethane. Amino valeric acid-benzyl ester- δ-p-tosylate (0.76 g, 2 mmol) in 50 ml dichloromethane was added to the solution, and the mixture was stirred in room temperature for 16 hrs. The solution was washed thrice with 5% $NaHCO_3$ solution and thrice with 5% Citric acid solution. After dried with Na_2SO_4, the solvent was evaporated in vacuo. The crude was purified by reprecipitation with Dichloromethane: Hexane (1:3) to give a white solid (1.1 g, 73%). R_f: 0.45 (2:8 Hexane: Ethylacetate) mp.154.7 ^oC; ^1H-NMR (400 MHz, $CDCl_3$): δ 7.75-7.25 (m, 21H, Aromatic CH), 5.07(s,2H,CH_2), 4.48(d,2H,CH_2), 4.34 (d,2H, CH_2), 4.17 (t,2H,CH), 3.75&3.58(d,2H,CH_2), 3.23-3.08 (m,4H, CH_2), 2.34 (m, 2H, CH_2), 1.67-1.25(m,4H,CH_2) ; ^{13}C-NMR(100 MHz, $CDCl_3$): δ 22.34 (CH_2), 29.18(CH_2), 33.99(CH_2), 39.52(CH_2), 40(CH_2), 47.59(CH), 47.72 (CH), 49.87(CH_2), 52.56(CH_2), 53.12(CH_2), 66.8(CH_2), 67.8(CH_2), 120.37 (CH), 125.19(CH),125.32(CH), 125.52(CH), 127.46(CH), 127.62(CH), 128.08(CH), 128.21(CH), 128.63(CH), 128.69(CH),129(CH), 136(C), 141.7(C), 144(C), 156.83(C=O), 169.85(C=O), 173.71(C=O) ;

EIS MS: m/z (M + H^+) calcd. 751.8 found 752.236; (M + Na^+) calcd. 774.8 Found 775.226 (**Scheme-4**)

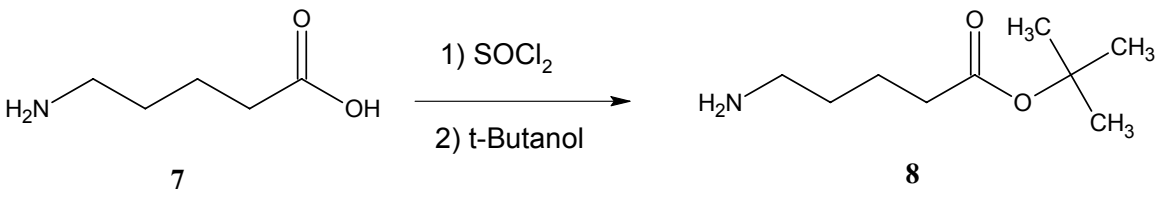


5 **6**

Scheme-5: Formation of *tert*-butyl 5-[(9*H*-9-fluorenyl methoxy) carbonyl] amino- pentanoate (**6**)

iii)*tert*-butyl5-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-pentanoate(6) (Scheme5)**:** 5-[(9*H*-9-fluorenylmethoxy)carbonyl]aminopentanoic acid (**5**) (1 g, 3 mmol) and t-butyl-2,2,2 trichloroacetimidate [TBTA] (1.965 g, 9 mmol) were dissolved in 100 ml Dichloromethane. After addition of 60 µl Boron trifluoride etherate [$BF_3.Et_2O$] (20 µl/mmol), the solution was stirred in room temperature for 24 hrs. After reaction was complete, a small amount of $NaHCO_3$ crystals was added to solution. After filtration the crystals, the solvent was evaporated *in vacuo*. The crude was purified by column chromatography (7.5:2.5, Hexane: Ethylacetate) to give a yellowish white solid (2.49 mg, 21%). R_f: 0.49 (7.5:2.5 Hexane:

Ethylacetate); mp.141.2 ^{o}C ; ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.77(d, 2H, CH), 7.58(d, 2H,CH), 7.38(t, 2H,CH), 7.31(t, 2H,CH), 4.4(d,2H,CH_2), 4.21(t,1H,CH), 3.19(t,2H,CH_2), 2.22(t,2H,CH_2), 1.6(t,2H,CH_2), 1.52 (t, 2H,CH_2), 1.44(t,9H,CH_3) ; ^{13}C-NMR(100 MHz, $CDCl_3$): δ 22.48(CH_2), 28.5(CH_3), 29.7(CH_2), 35.39(CH_2), 41.04(CH_2), 47.6(CH_2), 66.9(CH_2), 80.7(C), 120.37(CH), 125.47(CH), 127.43(CH), 128.06(CH), 141.7(C), 144.3(C), 156.8(C=O), 173.3(C=O) EIS MS: m/z ($M+Na^+$) calcd. 418.481 Found 418.22



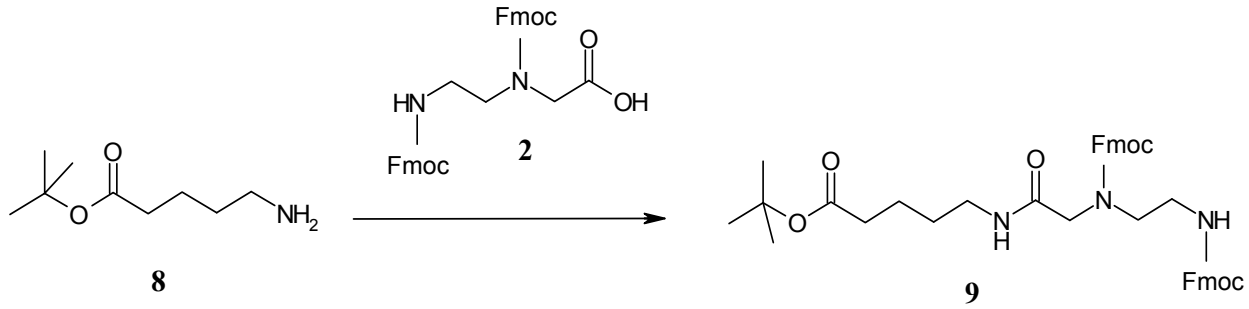
7 **8**

Scheme-6: Formation of *tert*-butyl 5-aminopentanoate(**8**)

iv) *tert*-butyl 5-aminopentanoate(8)(Scheme-6)**:**

Thionyl Chloride (10 ml, 137.6 mmol) was added to 5-aminopentanoic acid(**7**) (5 g, 43 mmol) in dropwise. The solution was stirred in room temperature for 2 hrs. After evaporation of Thionyl Chloride *in vacuo*, the residue was dissolved in 100 ml 2-methyl-2-propanol(t-Butanol). The solution was stirred over night in room temperature. The solution was made basic (pH: 13-14) with saturated NaOH solution. The solution was

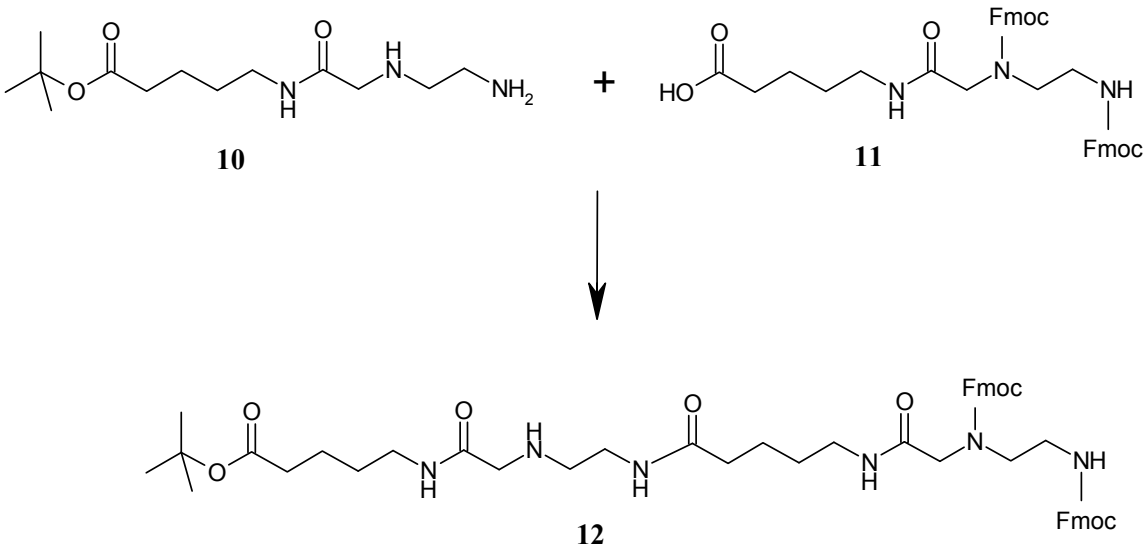
extracted thrice with 200 ml Dichloromethane, and then dried with Na_2SO_4. After evaporation of solvent *in vacuo*, the crude was purified by column chromatography (3:1:0.2, Dichloromethane: Methanol: Triethyl-amine) to give a liquid (1.5 g, 20%). R_f: 0.3 (3:1:0.2 Dichloromethane: Methanol: Triethylamine) ; ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 2.7(t,2H,CH_2), 2.25 (t,2H,CH_2), 1.83(s, 2H,NH_2), 1.64 (m, 2H, CH_2),1.48(m,2H,CH_2), 1.44(s,9H,CH_3) ; ^{13}C-NMR: (100 MHz, $CDCl_3$) δ 22.3 (CH_2), 28.3(CH_3), 32.17(CH_2), 35.73(CH_2), 41.85(CH_2), 80.22(C), 173.17(C=O)



Scheme-7: Formation of *tert*-butyl 5-(2-[[(9*H*-9-fluorenylmethoxy) carbonyl](2-[(9*H*-9-fluorenylmethoxy) carbonyl] amino ethyl) amino] acetyl amino) pentanoate(**9**)

v) *tert*-butyl 5-(2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9-fluorenylmethoxy) carbonyl] amino ethyl) amino] acetyl amino) pentanoate (**9**) (Scheme-7)**:** Mixture of 2-[[(9*H*-9-fluorenylmethoxy) carbonyl](2-[(9*H*-9-fluorenyl methoxy) carbonyl] amino ethyl) amino]

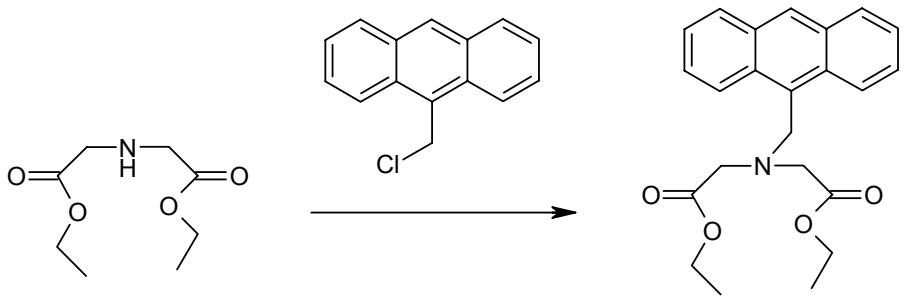
acetic acid (562 mg, 1 mmol), (Benzotriazol-1-yloxy) tris (pyrrolidino) phosphonium hexafluoro phosphate [PyBOP] (520 mg, 1 mmol), 1-Hydroxybenzotriazole [HOBt] (135 mg, 1 mmol), and N, N-Diisopropyl ethylamine [DIPEA] (0.35 ml, 2 mmol) were dissolved in 40 ml chloroform. A solution of *tert*-butyl 5-aminopentanoate (175 mg, 1 mmol) in 5 ml chloroform was added to the mixture. The mixture was stirred at room temperature for 15 hrs. After removing the chloroform and base (DIPEA) *in vacuo*, the crude was washed thrice with 75 ml of 5 % of $NaHCO_3$ solution, and thrice with 75 ml of 5 % citric acid solution. The combined organic extracts were dried with sodium sulfate and the solvent was removed *in vacuo*. The crude was purified by recrystalization in dichloromethane to give a white solid (490 mg, 68 %). R_f: 0.48 (2:8, Hexane-Ethyl Acetate) ; mp: 102.8 ^oC ; ^1H-NMR(400 MHz, $CDCl_3$): δ 7.74(d, 4H, CH), 7.58(d, 2H, CH), 7.5(d, 2H, CH), 7.38(t, 4H, CH), 7.26(t, 4H, CH), 6.08-5.75 (2H,CH), 4.5 (m,2H, CH_2), 4.3(m, 2H, CH_2), 4.19 (t, 2H, CH_2), 3.76 & 3.6(s,2H,CH_2), 3.4(d,2H,CH_2), 3.25-3.09(m,4H,CH_2), 2.2 (m, 2H, CH_2), 1.57(t, 2H, CH_2), 1.53(m, 2H, CH_2), 1.47(m, 2H, CH_2), 1.42 (s,9H,CH_3) ^{13}C-NMR(100 MHz, $CDCl_3$): δ 22.35(CH_2), 26.87(CH_2), 28.48(CH_3), 29.12(CH_2), 35.15 (CH_2), 39.58(CH_2), 46.66(CH_2), 47.58 (CH_2), 53.08(CH_2), 67.83(CH_2), 67.84(CH_2), 80.7(C), 120.34(CH), 125.15(CH), 125.28(CH), 125.5(CH), 127.43(CH), 127.6(CH), 128.04 (CH), 128.18(CH), 141.68(C), 144.3(C), 156.56(C=O), 169.6(C=O), 173.23 (2C=O) ; EIS MS: m/z calcd.($M + H^+$) 717.86 found 718.164



Scheme-8: Formation of *tert*-butyl 5-(2-[(2-[5-(2-[[(9*H*-9-fluorenyl methoxy)carbonyl](2-[(9*H*-9 fluorenylmethoxy)carbonyl]amino ethyl) amino]acetyl amino) pentanoyl] amino ethyl)amino]acetyl amino) pentanoate (**12**)

vi) *tert*-butyl 5-(2-[(2-[5-(2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9 fluorenyl methoxy)carbonyl]amino ethyl)amino]acetyl amino) pentanoyl] amino ethyl)amino]acetyl amino)pentanoate(12)(Scheme-8) **:** Mixture of 5-(2-[[(9*H*-9-fluorenylmethoxy)carbonyl](2-[(9*H*-9-fluorenyl methoxy) carbonyl] amino ethyl) amino]acetyl amino) pentanoic acid(**11**) (926.5 mg, 1.4 mmol), (Benzotriazol-1-yloxy)tris (pyrrolidino) phosphonium hexafluorophosphate [PyBOP] (728 mg, 1.4 mmol), 1-Hydroxybenzotriazole [HOBt] (189 mg, 1.4 mmol), and N,N-Diisopropyl ethylamine[DIPEA] (0.5 ml, 2.8 mmol) were dissolved in 25 ml chloroform. A solution of *tert*-butyl 5-(2-[(2-aminoethyl)amino]acetyl

amino) pentanoate(**10**) (382.7 mg, 1.4 mmol) in 35 ml chloroform was added to the mixture. The mixture was stirred at room temperature for 15 hrs. After removing the chloroform and base (DIPEA) *in vacuo*, the crude was washed thrice with 75 ml of 5 % of $NaHCO_3$ solution, and thrice with 75 ml of 5 % citric acid solution. The combined organic extracts were dried with sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (on silica gel) (8:1, Dichloromethane: Methanol, normal phase) to give white solid (350 mg, 38%). R_f: 0.5 (8:1, Dichloromethane: Methanol); mp. 117.7 ^oC (Decomposition) ; ^{1}H-NMR(400 MHz, $CDCl_3$): δ 7.74(t,4H,CH), 7.57 (d, 2H, CH), 7.5(d,2H,CH), 7.39(t,4H,CH), 7.27(d,4H,CH), 4.43(d,2H,CH_2), 4.33 (d,2H,CH_2), 4.18(d,2H,CH), 3.82(d,2H,CH_2), 3.48(t,2H,CH_2), 3.3-3.15(m,12H,CH_2), 2.71(t,2H,CH_2), 2.22-2.14(t,4H,CH_2), 1.67-1.47(m, 6H, CH_2), 1.42(s, 9H, CH_3) ; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 22.54(CH_2), 23.1 (CH_2), 28.49(CH_3), 28.94(CH_2), 29.35(CH_2), 30.1(CH_2), 35.28(CH_2), 35.95 (CH_2), 38.9(CH_2), 39.16(CH_2), 39.83(CH_2), 40.1(CH_2), 47.58(CH), 49.67(CH_2), 50.13(CH_2), 52.72(CH_2), 66.88(CH_2), 67.98(CH_2), 80.85(C), 120.34(CH), 125.17(CH), 125.39(CH), 125.53(CH), 127.44(CH), 127.62 (CH), 128.07(CH), 128.23(CH), 141.66(C), 144(C), 156.85(C=O), 172 (C=O), 173.6(C=O) ; EIS MS: m/z ($M + H^+$) calcd. 917.109 found 917.59; ($M+Na^+$) calcd.940.1 Found 940.56

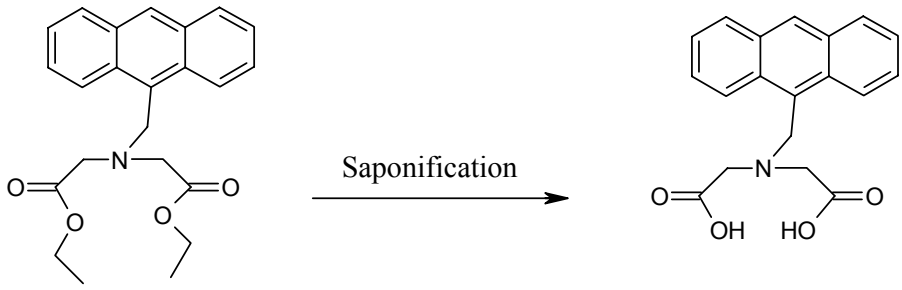


13 **14**

Scheme-9: Formation of Ethyl 2-[(9-anthrylmethyl)(2-ethoxy-2-oxoethyl) amino] acetate(**14**)

vii) Ethyl 2-[(9-anthrylmethyl)(2-ethoxy-2-oxoethyl) amino] acetate (**14**) (Scheme-9) **:** Mixture of diethyliminodiacetate(**13**) (1 g, 5.7 mmol) and triethylamine (2 ml, 11.4 mmol) were dissolved in 50 ml 1,4 dioxane. A solution of 9-(chloro methyl) anthracene (1.42 g, 6.27 mmol) in 50 ml 1,4 dioxane was added to the mixture. The mixture was heated to reflux 24 hrs. After removing 1,4 dioxane and base (triethylamine) *in vacuo*, the crude was made basic with 1 N NaOH solution (100 ml), and the solution was extracted with thrice with 75 ml dichloromethane. The combined organic extracts were dried with sodium sulfate and the solvent was removed *in vacuo*. The remained diethyliminodiacetate (starting compound) was removed by Kugelrohr distillation in 4 hrs. After recrystalization in ethyl ether, the precipitate was filtered. The solvent in filtrate was removed *in vacuo* to give brownish gummy solid (1.75 g, 81%) ; mp: 204 ^{o}C (Decomp.) ; ^{1}H-NMR: (400 MHz, DMSO) δ 8.65

(d,2H,CH), 8.43(s,1H,CH), 7.98(d,2H,CH), 7.52(t,2H,CH), 7.47 (t, 2H, CH), 4.95(s,2H,CH_2), 4.13(s,4H,CH_2), 3.58(s,4H,CH_2), 1.22(t,3H,CH3) ; ^{13}C-NMR: (100 MHz, DMSO) δ 14.88(CH_3), 50(CH_2), 54.81(CH_2), 61.14(CH_2), 125.66(CH), 125.78(CH), 126.63(CH), 128.73(CH), 129.45 (CH), 129.57 (C), 132.07(C), 132.38(C), 173.43(C=O)

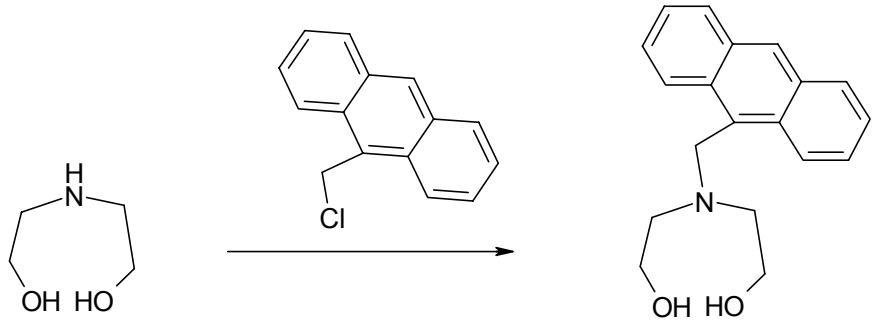


14 **15**

Scheme-10: Formation of 2-[(9-anthrylmethyl)(carboxymethyl) amino] acetic acid (**15**)

viii) 2-[(9-anthrylmethyl)(carboxymethyl) amino] acetic acid(15) (Scheme-10) **:** A solution of ethyl 2-[(9-anthrylmethyl)(2-ethoxy-2-oxoethyl) amino] acetate(14) (450 mg, 1.2 mmol) in 20 ml ethanol was combined with 3 ml of 2N NaOH solution. The suspension was heated to reflux 6 hrs. After filtration of suspension, the precipitate was dissolved DI water and made basic with 2N NaOH solution. The solution was

washed with thrice with 50 ml dichloromethane. After making the solution acidic with 2N HCl solution, the suspension was filtered to give yellow solid (240 mg, 62%). mp. 179 ^{o}C ; ^{1}H-NMR: (400 MHz, DMSO) δ 12.37(s,1H,OH), 8.7(d,2H,CH), 8.6(s,1H,CH), 8.08(d,2H,CH), 7.5 (t, 4H, CH), 4.84(s,2H,CH_2), 3.45(s,4H,CH_2) ; ^{13}C-NMR: (100 MHz, DMSO) δ 49.46 (CH_2), 54(CH_2), 125.95(CH), 125.99(CH), 126.77(CH), 128.48 (CH), 129.6(CH), 130.1 (CH), 131.78(C), 131.95(C), 173.54(COOH)



16 **17**

Scheme-11: Formation of 2-[(9-anthrylmethyl)(2-hydroxyethyl)amino]-1-ethanol (**17**)

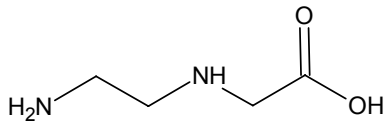
ix)2-[(9-anthrylmethyl)(2-hydroxyethyl)amino]-1-ethanol(17)

(Scheme-11) **:** Anthracene Chloride (5.47 g, 24.1 mmol) and Diethanol-amine (**16**) (2.79 g, 26.5 mmol) were dissolved in 100 ml of 1,4 Dioxane.

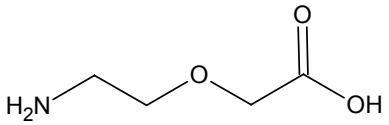
After addition of triethylamine (10 ml, 52.5 mmol), the suspension was heated to reflux for 24 hours. The precipitate was filtered. After evaporation of 1,4 Dioxane from filtrate , the crude was redissolved and made basic with 2 N NaOH solution (pH: 12-14). The solution was extracted thrice with 50 ml Dichloromethane. After drying the combined organic extracts with Na_2SO_4, the solvent was evaporated *in vacuo*. The crude was purified by recrystalizing with Dichloromethane to give brownish solid (3.84 g, 54 %). mp: 116.8 ^oC ; ^{1}H-NMR: (400 MHz,$CDCl_3$) δ 8.49 (s,1H,CH), 8.42(d,2H,CH), 7.98(d,2H,CH), 7.5 (t, 2H, CH), 7.44(t,2H,CH), 4.67 (s,2H,CH_2), 3.49(t,4H,CH_2), 2.77 (t, 4H, CH_2), 2.37 (s,1H,OH) ; ^{13}C-NMR: (100 MHz, $CDCl_3$) δ 51.9(CH_2), 56.2 (CH_2), 60.05(CH_2),124.6(CH), 125.35(CH), 126.65(CH), 128.5(CH), 129.7 (CH), 131.6(C), 131.8(C), 134.5(C)

RESULTS AND DISCUSSION

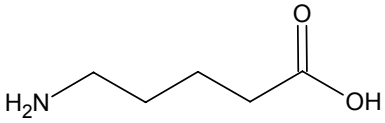
Three different amino acids were used to synthesize the monocyclic precursor compounds of the bicyclic final target molecules. Each of the monocyclics contain four amino acids (either **1**, **18**, **7** depending on the final target).



2-[(2-aminoethyl)amino]aceticacid(1)



(2-Amino-ethoxy)-aceticacid(18)

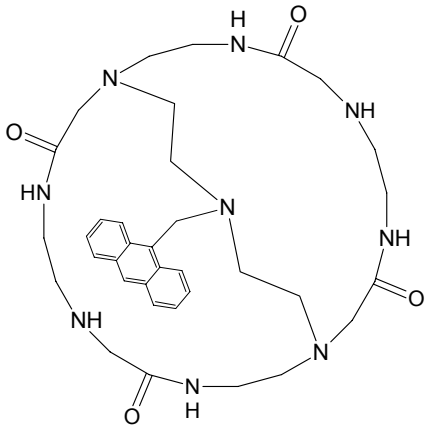


5-aminopentanoic acid(7)

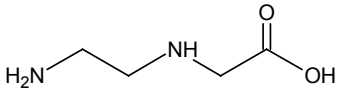
Figure-10: Structures of of 2-[(2-aminoethyl)amino]acetic acid(**1**), (2-aminoethoxy)-acetic acid(**18**), 5-aminopentanoic acid(**7**)

The monocyclic compounds contain two reactive sites (secondary amines) that facilitate addition of a bridging group to form the eventual bicyclic target compound. Therefore, all monocyclics contain at least two units of **1**. I is composed of four units of **1**; II contains one each of **1** and **18**; and III contains one each of **1** and **7**. Figures 11-14 summarize I, II, III and their precursors.

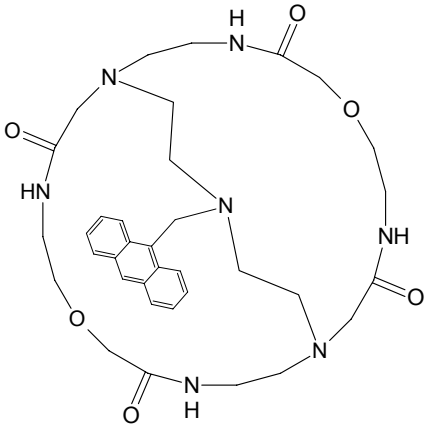
I



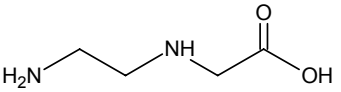
4 X



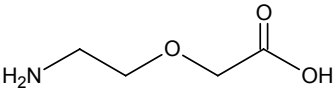
II



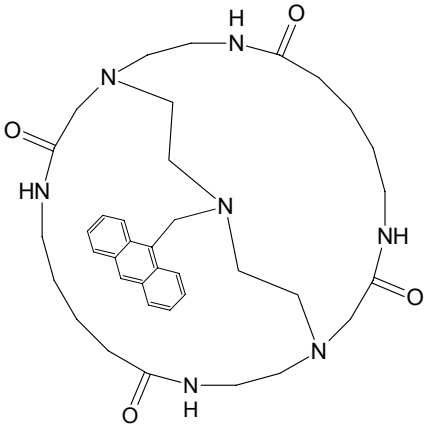
2 X



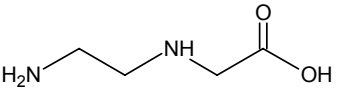
2 X



III



2 X



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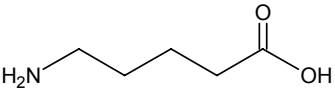


Figure 11: I,II,III and Aminoacids

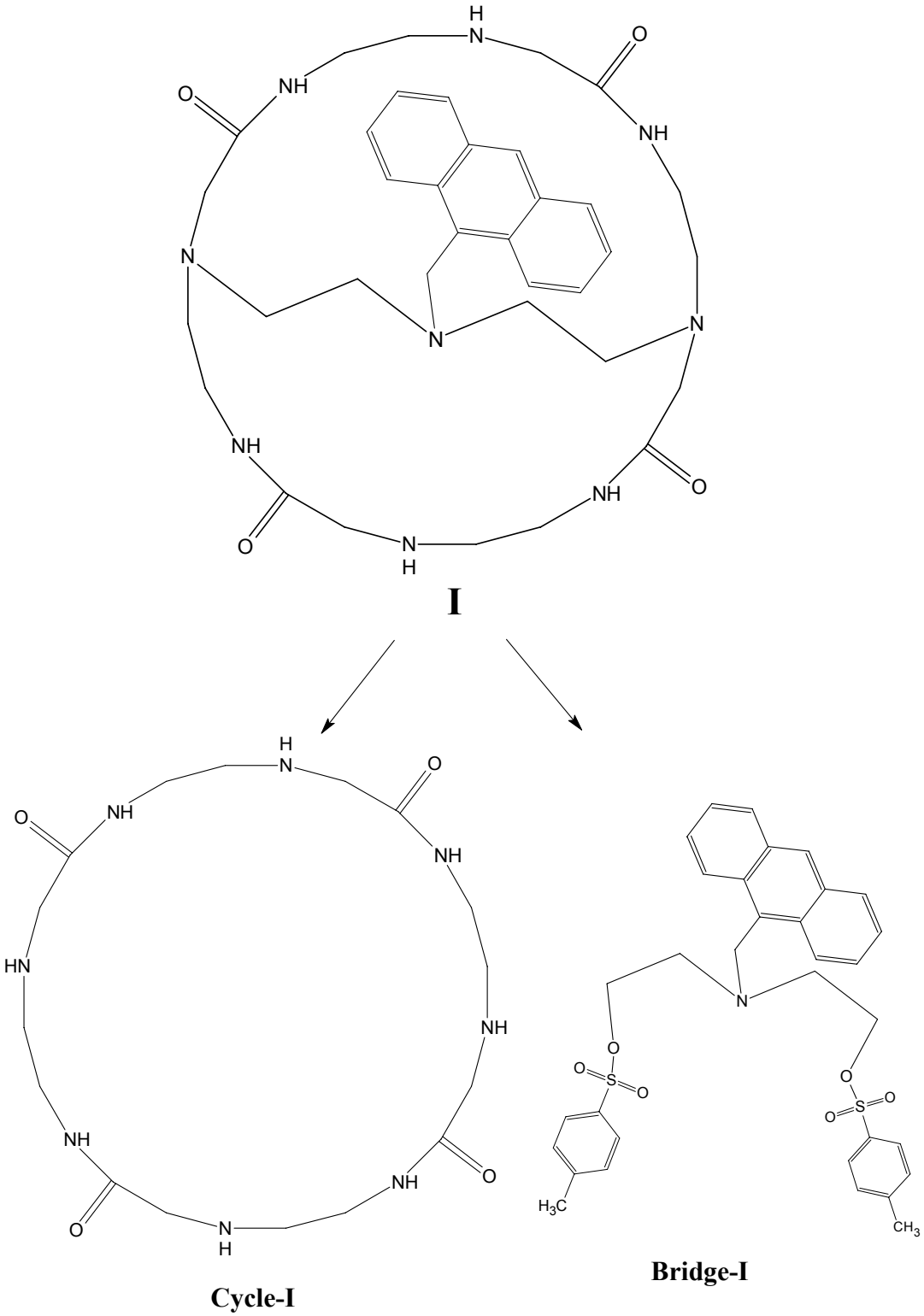


Figure-11: I and its precursors

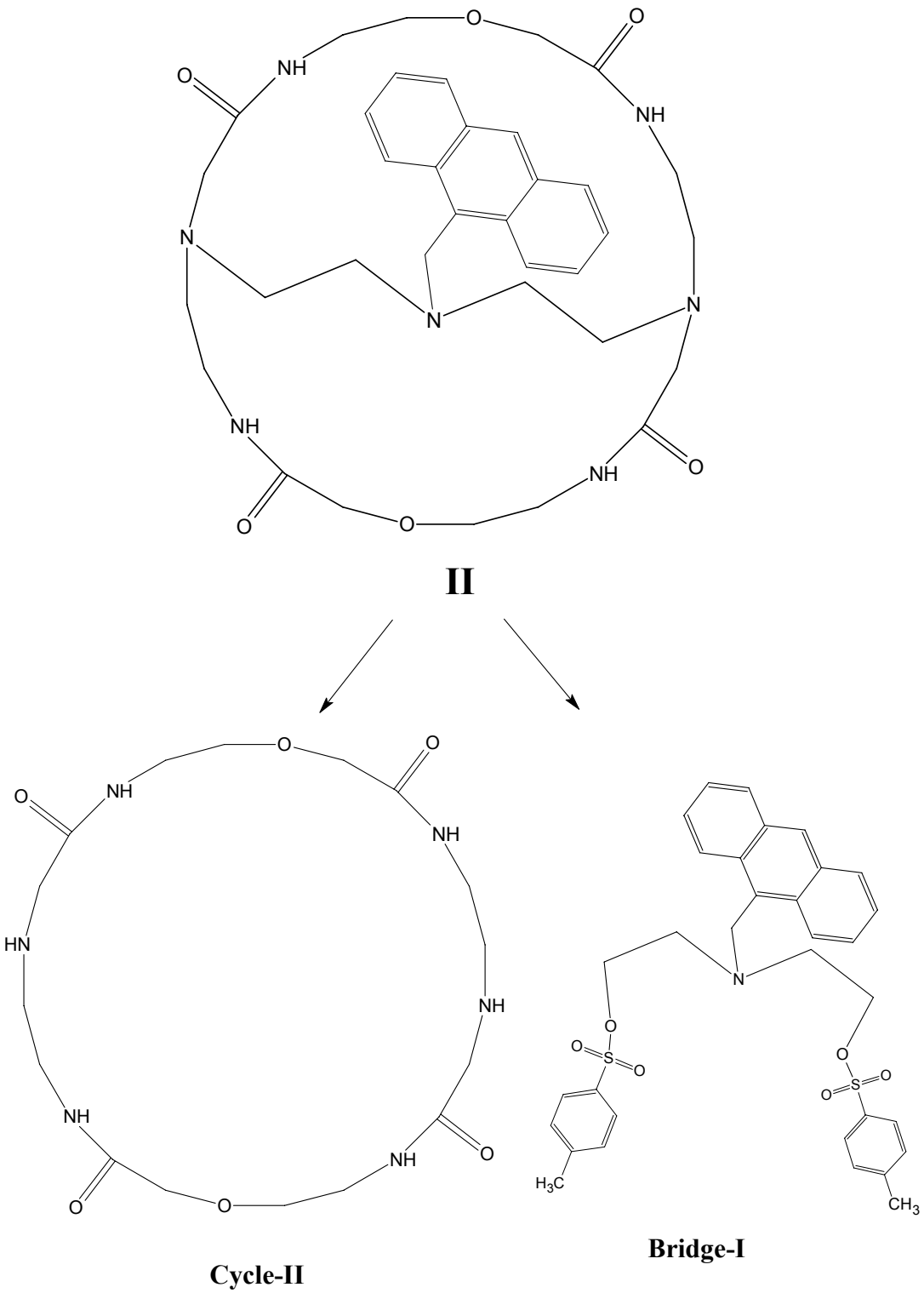


Figure-12: II and its precursors

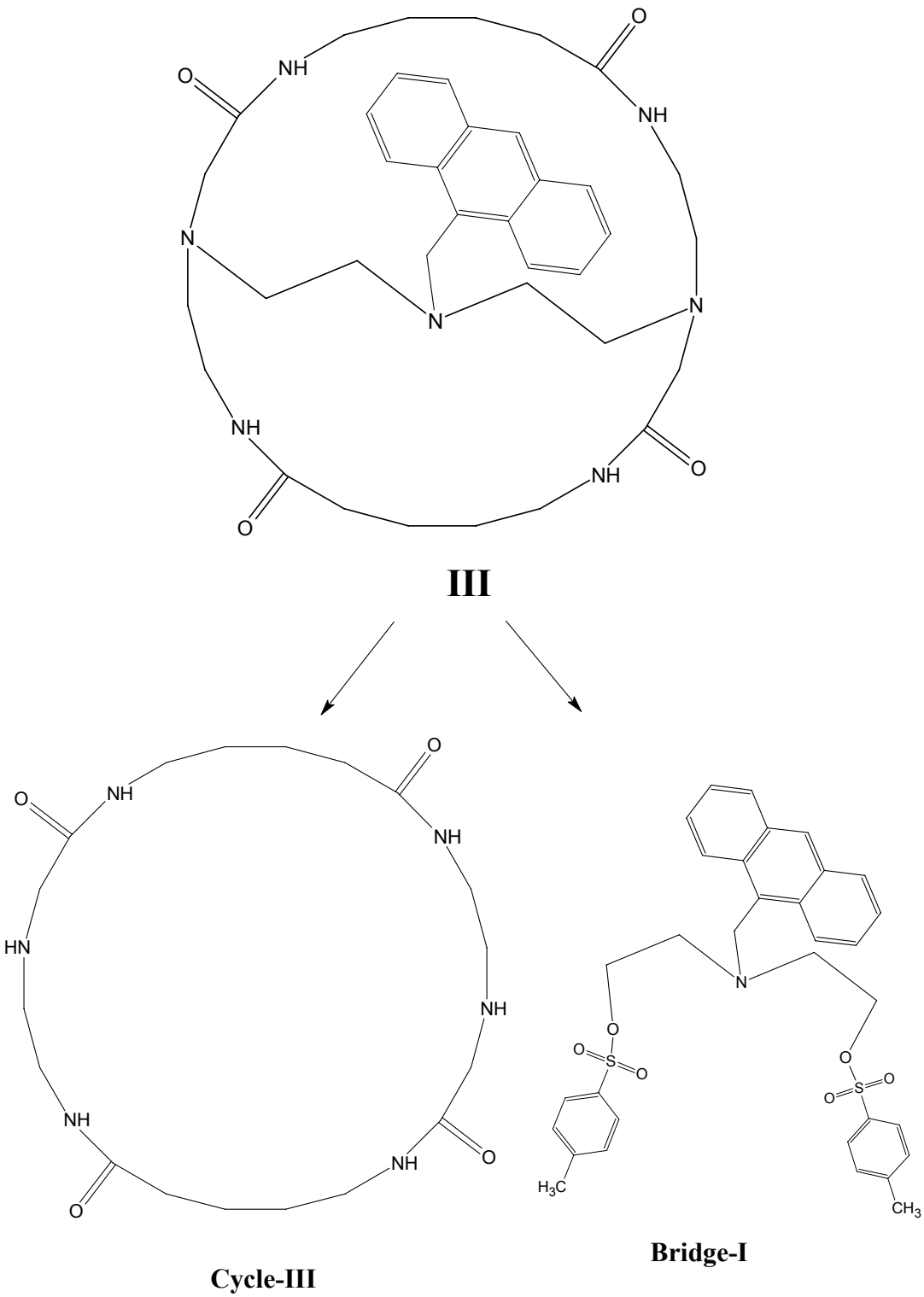
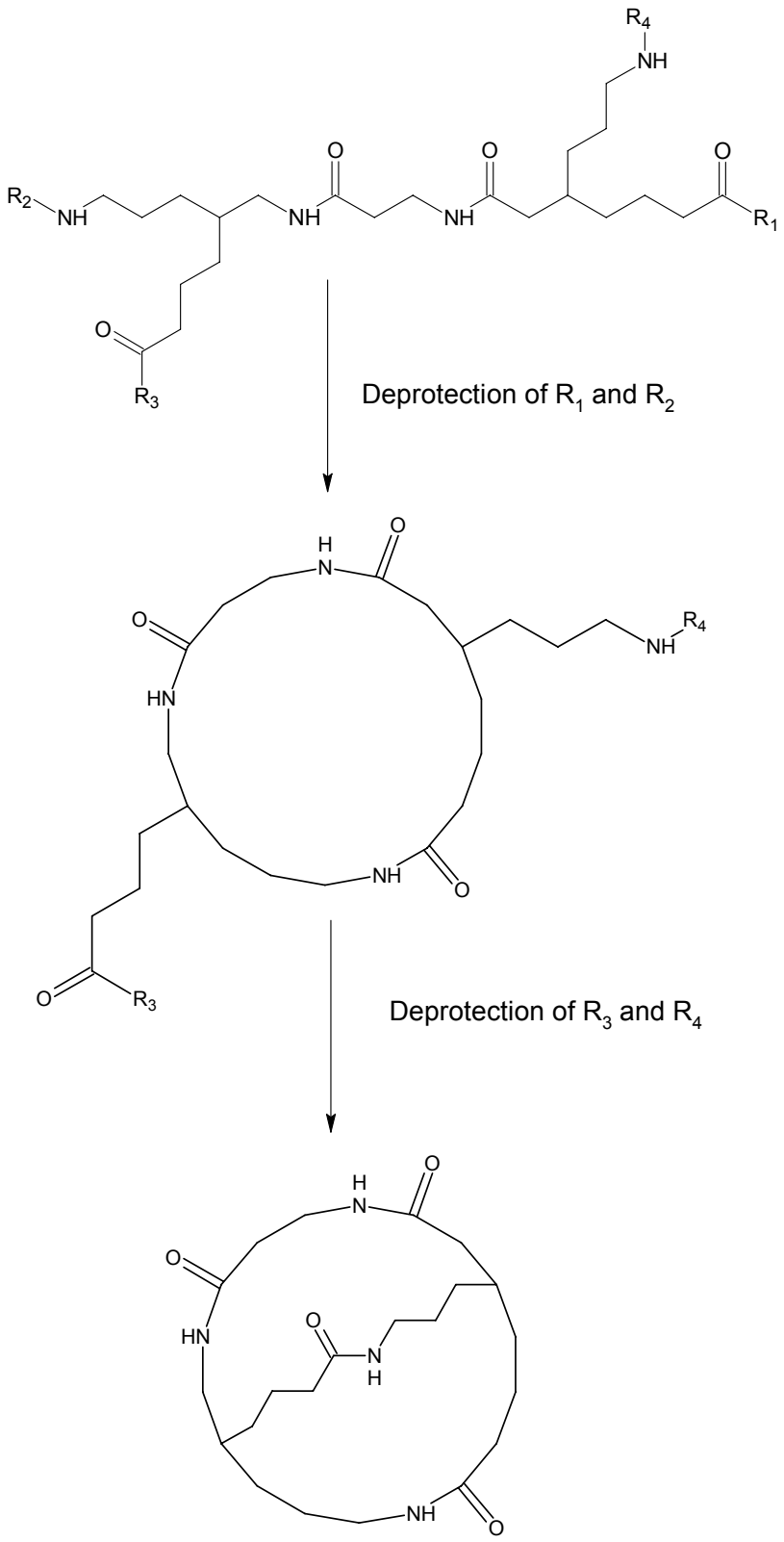


Figure-13: III and its precursors

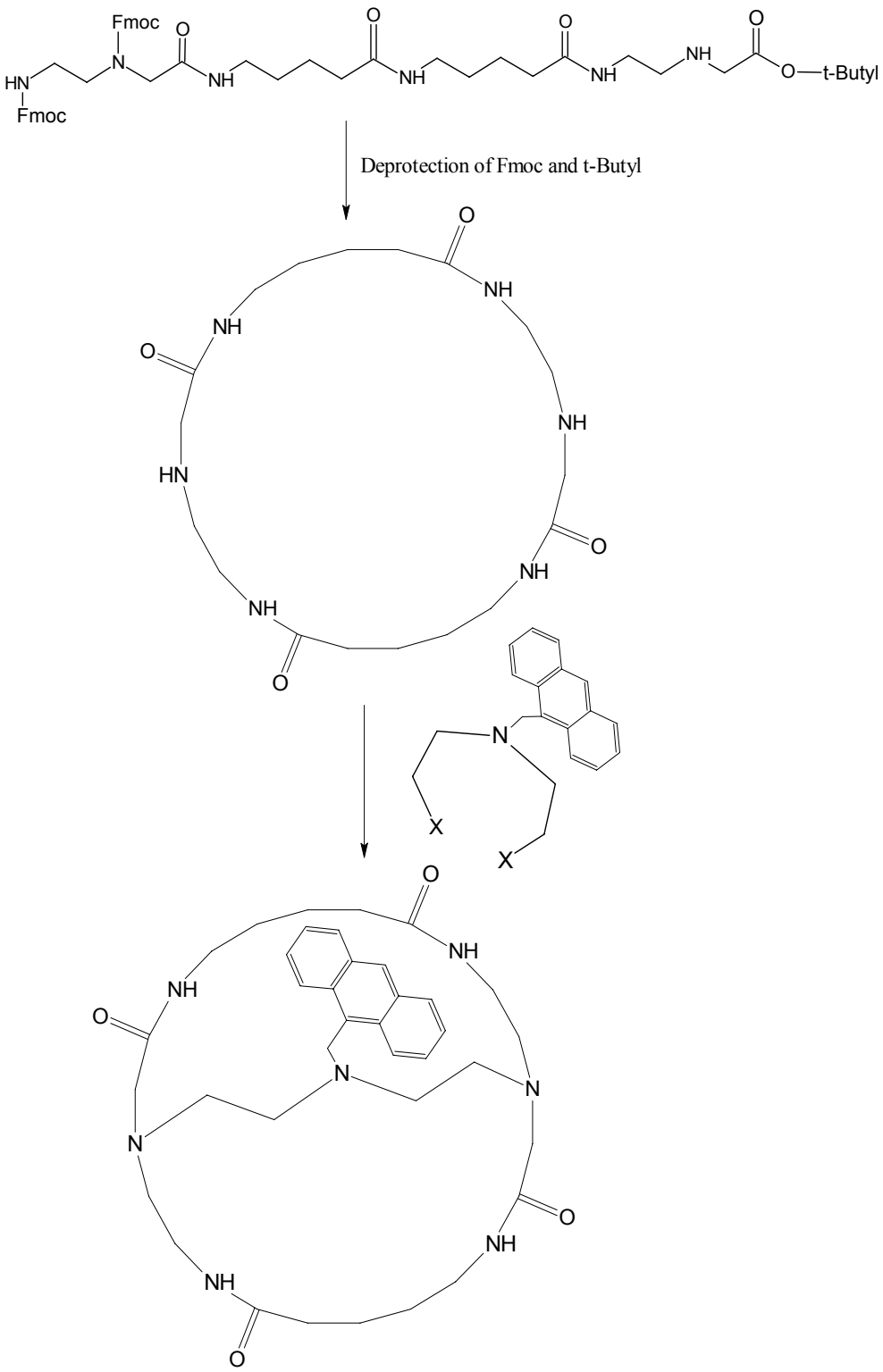
Synthetic Strategies:

Previous studies show that the bicyclic peptides can be synthesized by two strategies; Strategy-I involves building a linear peptide with two protected side chains. Cyclization of the linear chain then yields the cyclic peptide. Deprotection of the two side chains and coupling with the bridge yields the bicyclic target which can then be further functionalized by addition of the chromophore if desired. The scheme-12 for Strategy-I is shown below;



Scheme-12 : Strategy-I for Synthesizing Bicyclic Structures

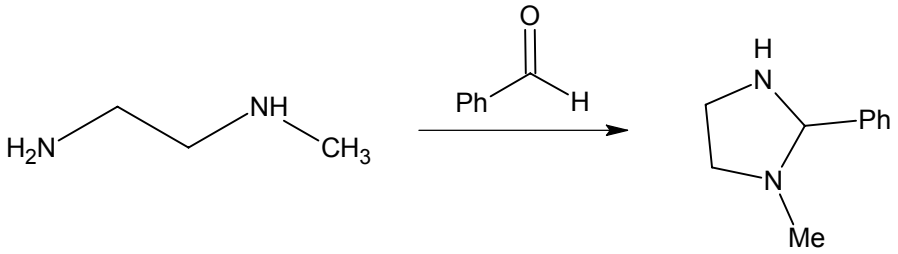
Strategy-II involves synthesis of the linear peptide, cyclization and then addition of the bridge and chromophore in one step. The scheme-13 for strategy-II is below;



Scheme-13: Strategy-II for Synthesizing Bicyclic Structures

Synthesis of bicyclic peptides I-II-III was attempted using this second strategy. The advantage here is that the starting materials are either easily prepared or available commercially. In this method, the bridging unit has a point of attachment for the chromophore that will be used in the optical signaling of ion binding

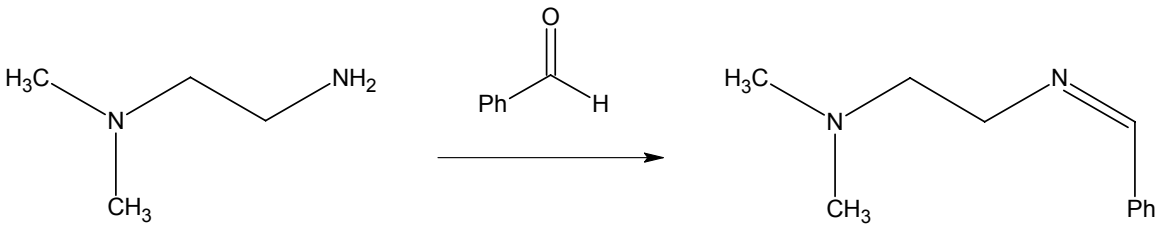
The major problem in the synthesis of this monocyclic compound is that **1** contains two reactive sites which are primary and secondary amines. There is no completely effective strategy that can protect only secondary amines or only primary amines in a mixture of secondary and primary amines. Therefore it can be anticipated that preparation of the target molecule will result in a significant number of side products. One method that could provide at least some differentiation between primary and secondary amines is benzaldehyde protection, shown in Scheme-14 below [17];



% 53

Scheme-14: Benzaldehyde protection reaction

The reaction of benzaldehyde with a molecule containing primary and secondary amines forms a five membered ring which results in only the primary amine remaining reactive for further coupling. However, deprotection of the five membered ring in subsequent steps can lead to side reactions or cleavage of the linear molecule. For example, reaction with benzaldehyde[19] can form side products that contain an imine function instead of a ring function (Scheme 15).



Scheme-15: Formation of Imine compound

Considering that the primary amine is more reactive than the secondary amine in coupling reactions a one-to-one ratio of the two peptides would likely yield the monocyclic peptide as the major product. The easiest system to synthesize should be III because **7** is commercially available and does not contain a secondary amine.

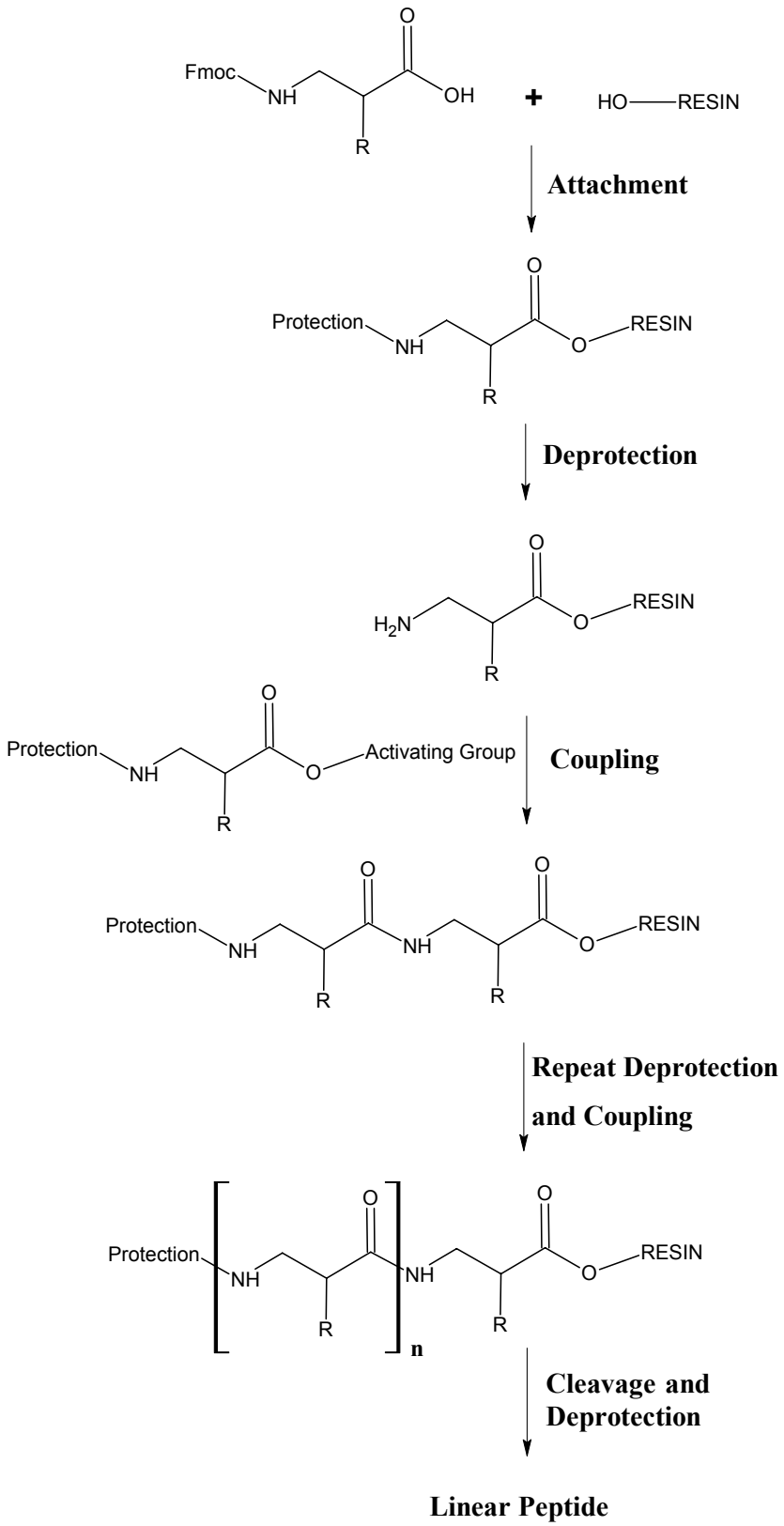
There are two general methods to synthesize the monocyclic compounds; solid phase peptide synthesis (SPPS) and solution phase peptide synthesis.

III:

Trial-I:

The decision to use Solid Phase Peptide Synthesis (SPPS) for the synthesis of bicyclic III is based on a well known method. It usually provides high yields and relatively pure products, because all excess reagents are easily removed by washing the resin with different solvents.[20,21] Solid Phase Peptide Synthesis (SPPS) is based on sequential addition of amino acids and side chain protected amino acid residues to a variety of possible insoluble resins (solid or polymeric supports). The only requirements for the resin are their stability in the solvent systems used and toward the coupling reagents as well as the ability to easily cleave the peptide from the resin at the end of the synthesis. With this method, only a few reactions and procedures are repeatedly used: swelling the resin; removal of protecting groups; coupling with protected amino acids; and cleaving the peptide from the support.[21,22]

The general scheme of SPPS is below;



Scheme-16: General SPPS route

i)

Synthetic Route

The decision to use Fmoc as a protecting group is due to its high coupling efficiency leading to easy purification [23] and the commercial availability of Fmoc-protected amino acids. For the solid support, a Wang Resin was chosen since cleavage from this resin does not involve basic conditions that would remove Fmoc groups.

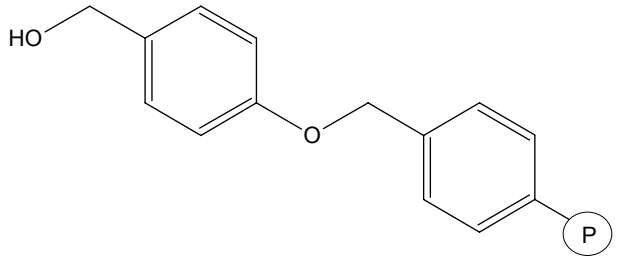
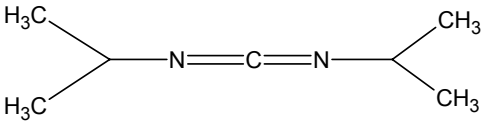


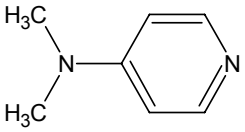
Figure-15: Wang Resin (100-200 mesh)

[**P** : polymer matrix(copoly(styrene-1 % DVB), 100-200 mesh]

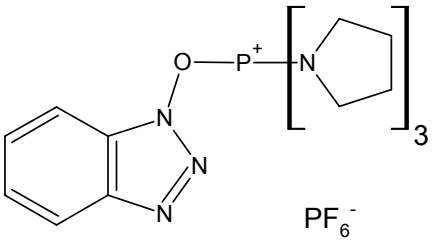
The structures of the coupling reagents that were used during the SPPS, are below;



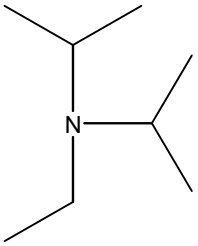
Diisopropylcarbodiimide



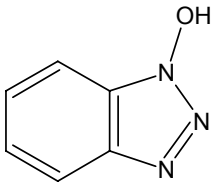
DiMetylAminoPyridine (DMAP)



Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)



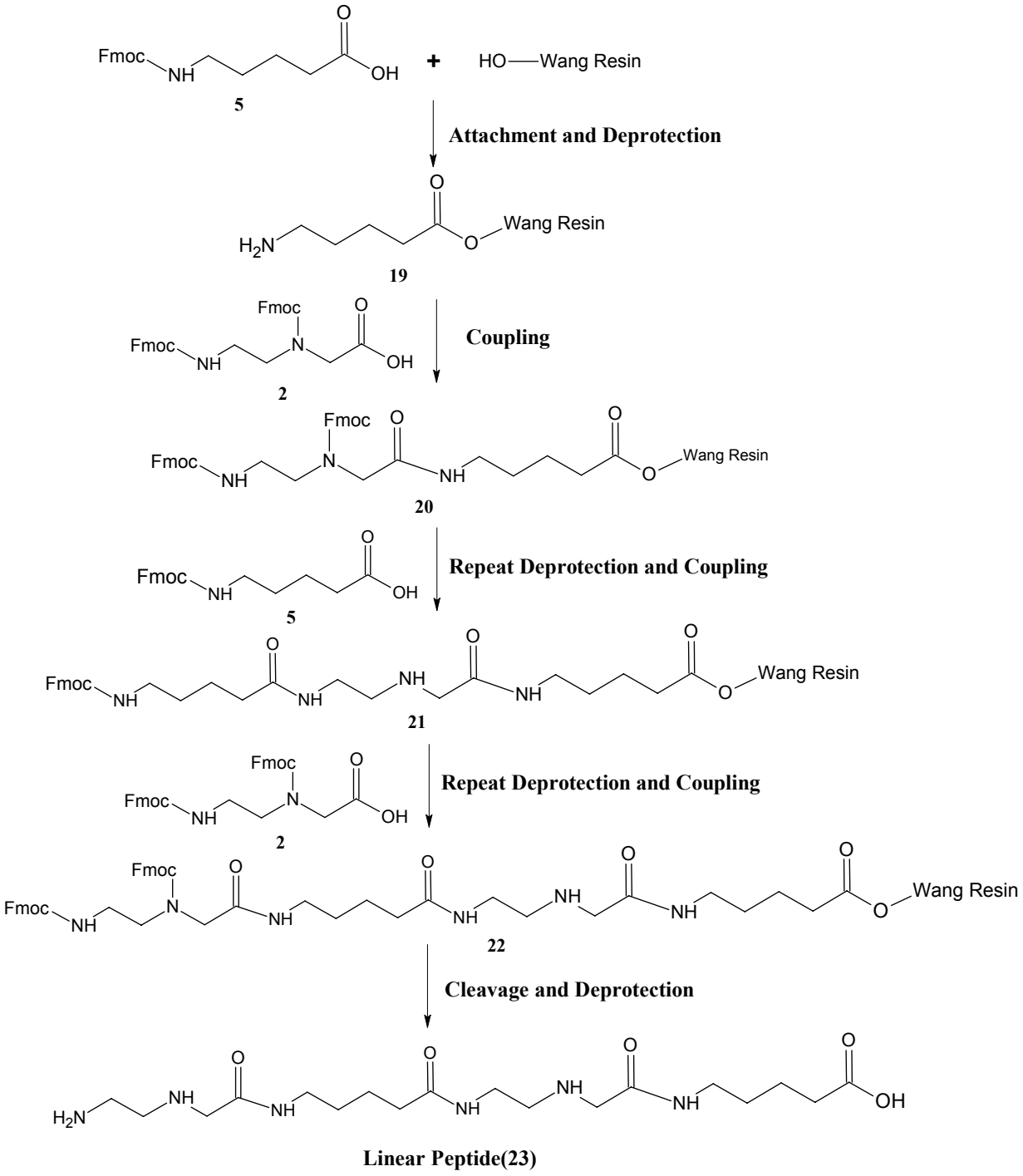
N,N-DiIsoPropyl-N-Ethyl Amine (DIPEA)



N-HydroxyBenzoTriazole (HOBt)

Figure-16: Coupling Reagents

The synthetic route is below;



Scheme-17: SPPS route

ii) Synthesis, Results and Discussion for the SPPS route :

For SPPS of bicyclic peptides, it was necessary to protect **1** with Fmoc groups. The reaction was performed according to the procedure described in the literature and given in detail in the experimental section.[24]

Solid phase synthesis was carried out on 1 g of Wang resin [100-200 mesh] (loading 0.5-1.30 mmol/g resin). The resin was stirred under N_2 in DMF for 30 minutes in order to swell the resin. DMF was removed by filtration. During this time, **5** (1.7 g, 5 mmol) was mixed with Diisopropylcarbodiimide (0.783g, 5 mmol) in DCM in a round bottom flask. The solution was stirred for 30 minutes and the DCM was removed under *vacuo*. The dried residue was dissolved in DMF and the solution was subsequently added to the resin. 4-Dimethylaminopyridine (DMAP) (12.27 mg, 0.1 mmol) was added as a catalyst and the mixture was stirred under N_2 for 2 hours. After this, the acid function of the amino acid was attached to the resin while the amine function of the amino acid was still protected with the Fmoc-protecting group.

The reaction solution was filtered off and the resin was washed three times each with DMF and methanol, once with ethanol and dried under *vacuo*. The loading was tested by cleaving the Fmoc-protection group from a known mass of resin (20 mg) with 20% piperidine in DMF and monitoring the UV absorption at 290 nm. The cleaved Fmoc group forms fluorene

which absorbs UV light at 290 nm. The loading of the resin was calculated according to the following formula;

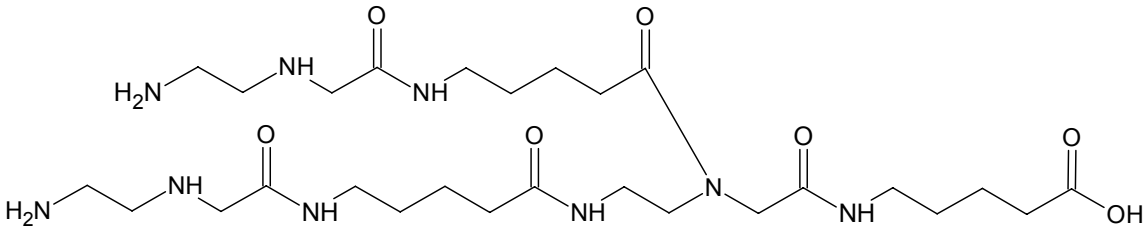
$$A = \text{const} * b * [C]$$

Here, the constant is the molar extinction coefficient, b is the path length in centimeters and [C] is the concentration of the fluorene. The absorbance yields the concentration of the fluorene allowing calculation of the amount of amino acid attached to the resin. Our results indicated a loading of ~100 %.

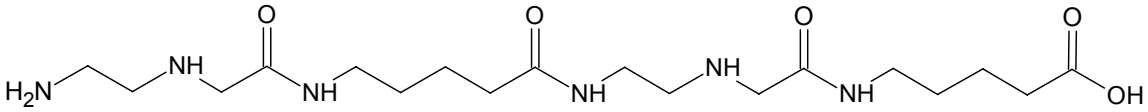
The remaining resin was reswelled again in DMF for 10 min. After washing the resin with DMF again, the Fmoc-protecting group was cleaved with 20 % piperidine in DMF for 10 min. The solution was filtered off and the resin washed three times with DMF. **2** (1.47 g, 2.5 mmol) and coupling reagents; PyBOP (1.3 g, 2.5 mmol), HOBt (0.338g, 2.5 mmol), and Diisopropylethylamine (DIPEA) (0.87 ml, 5 mmol) were dissolved in DMF. The mixture was then added to the resin and stirred overnight with N_2. The solution was filtered off and the resin was washed three times with DMF, three times with methanol, once with ethanol and dried under *vacuo*. Complete coupling was confirmed by the Kaiser test for free amine.

The general procedure was repeated again for addition of **5** (0.68 g, 2 mmol), and **2** (1.17 g, 2 mmol) respectively. After each Fmoc-deprotection, the resin was washed three times each with DMF MeOH, DMF,and EtOH and dried under *vacuo*. After the last coupling and deprotection step, the resin was transferred into a round bottom flask and stirred in 95% TFA solution for 3 hours. The resin was removed by filtration. The TFA in the filtrate was evaporated to a final volume of 5 ml. The crude peptide was precipitated with ethyl ether to give 100 mg of white crystals. MS of the white crystals [Appendix-A], a mixture of two major compounds(**24** and **23(**Linear Peptide)) was obtained.

Further analysis of crude the mixture with HPLC showed two major and one minor product in the crude mixture. One of the two major products was the desired linear peptide **(23)** (MW:416.516).But obviously, there was also a product that resulted from a double coupling during the third coupling step. The excess of **5** not only coupled with primary amine but also with unprotected secondary amine. The result was a branched peptide (**24**) according to the figure-17 below with a MW: 615.766.



24 (Formula Weight = 615.766)



23 (Linear Peptide) (Formula Weight = 416.516)

Figure-17: Structures of **23** (Linear Peptide) and **24** (Branched peptide)

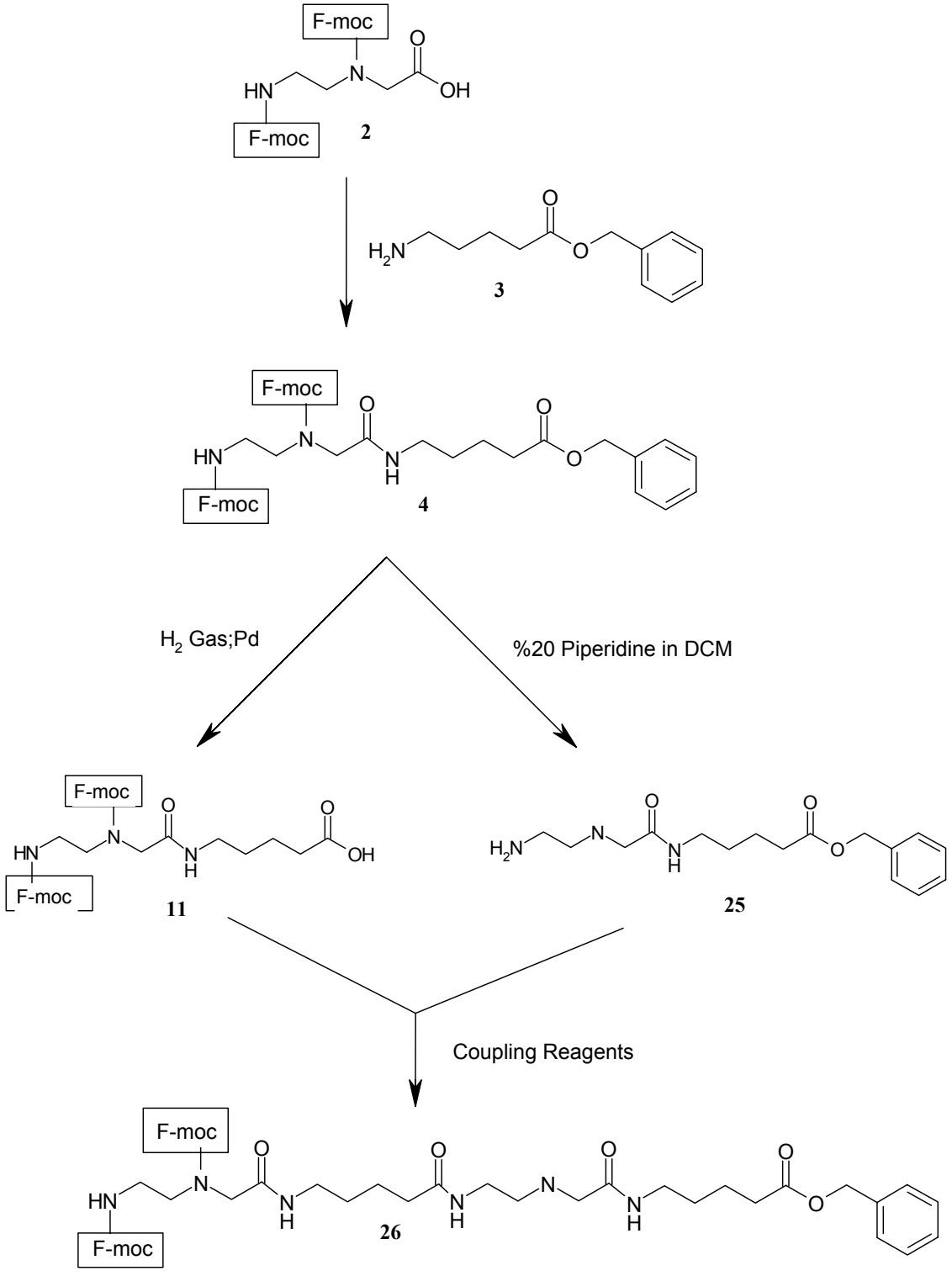
Low yield and difficulty in purification of the crude mixture of SPPS forced the use of solution phase peptide synthesis.

Trial II:

i) Synthetic Route

In order to use a solution phase approach for the synthesis of peptides, a second protecting group for the protection of the acid function is necessary. We chose the benzyl-ester protecting group because it is stable to the cleavage conditions for the Fmoc group and can be easily removed by H_2/Pd on activated carbon.

The coupling reagents were the same as used in SPPS. The synthetic route is shown below.



Scheme-18: Synthetic Route for solution phase synthesis with benzylester protection

ii) Synthesis, Results and Discussion

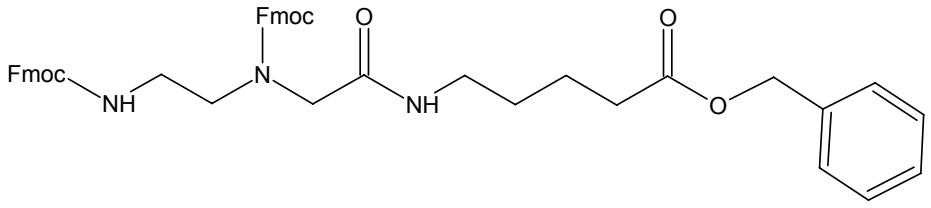
3 [δ-aminovaleric acid-benzyl ester p-tosylate] is commercial while **2** was synthesized according to the procedure described in the experimental experimental section.

In a round bottom flask, **2** was coupled with **3** according to the procedure for **4** described in the experimental section. The crude mixture was purified by reprecipitation with dichloromethane: hexane (1:3) to give a white solid (**4**).

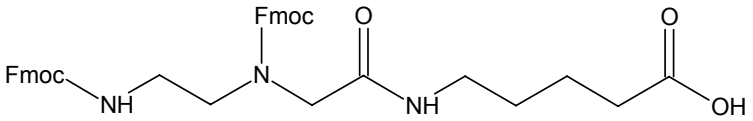
The resulting compound (**4**) was divided in half with one half undergoing deprotection of the Fmoc group and the other half undergoing deprotection of the benzylester group. Fmoc deprotection of **4** was performed in the same way as in the solid phase approach described above. The product, **25,** was used without further purification.

For deprotection of the benzyl ester group **4** was dissolved in chloroform and the emulsion was stirred under H_2 gas at room temperature for 3 hours after addition of palladium on activated carbon. After filtration of the Pd and evaporating the solvents, a crude mixture was obtained. Analysis of the crude mixture with prep-TLC and H-NMR showed that one product was fluorene indicating that the Fmoc is at least partially cleaved during the benzylester deprotection step. MS of the crude mixture[Appendix-A]

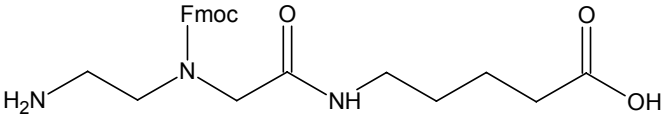
showed the presence of three compounds - starting material**(4)** (MW: 751.865), expected product**(11)** (MW: 661.743) and the product with the benzylester group and one of the Fmoc protecting groups removed (MW: 439.504);



4 (Formula Weight = 751.865)

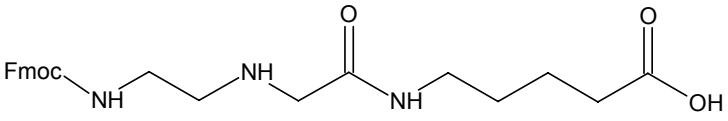


11 (Formula Weight = 661.743)



27

OR



28

Formula Weight = 439.504

Figure-18: Structures of Products from Benzylester Deprotection

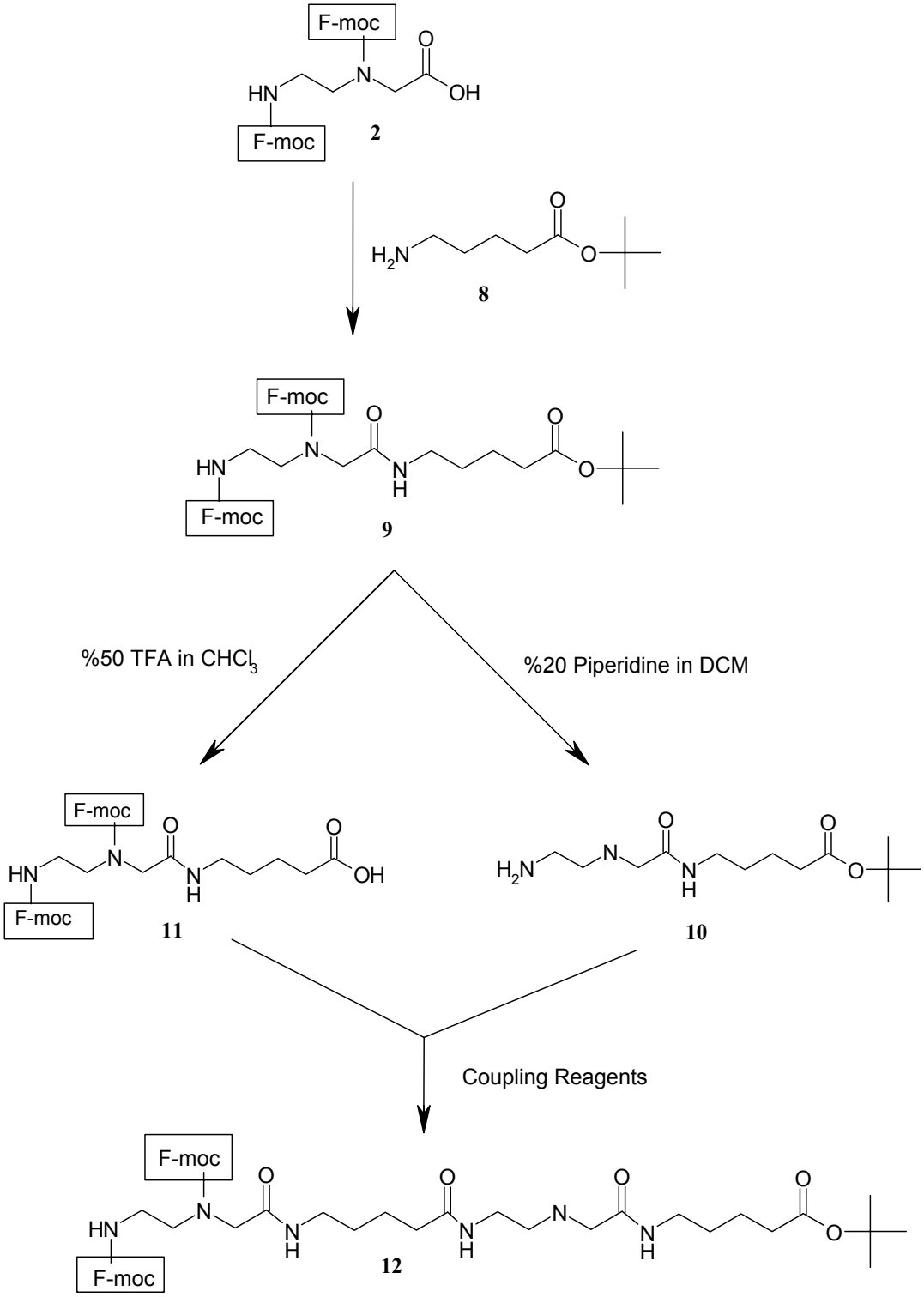
This method did not work properly and in further deprotection of Benzyl ester, we could face deprotection problem again.

Trial-III:

Low yield and deprotection of Fmoc during the deprotection of benzyl ester forced us to switch to solution phase peptide synthesis with a different acid protection group.

i) Synthetic Route

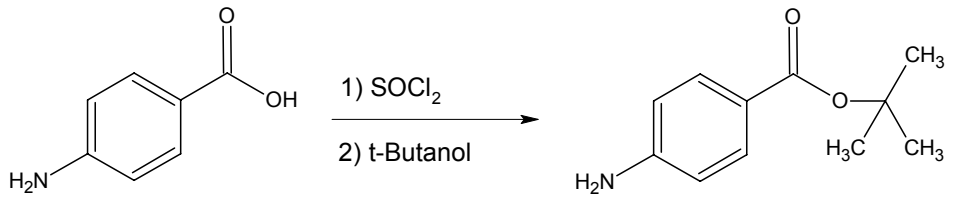
We chose to use *tert*-Butyl protection of the acid function of **7** because it can be easily removed by 50% TFA solution and it is stable to the conditions for Fmoc deprotection. The coupling reagents were the same used in SPPS. The synthetic route is shown below.



Scheme-19: Synthetic Route for Solution P.P.S. with t-Butylester Protection

ii) Synthesis, Results and Discussion

It was necessary to protect **5** with the *tert*-Butyl protecting group using t-butyl-2,2,2 trichloroacetimidate [TBTA]. The reaction was performed according to the procedure described by Armstrong, A.[26] However, this two step reaction was found to have a low yield. Taylor and coworkers [27] synthesized *tert*-butyl p-aminobenzoate from 4-amino benzoic acid by using thionyl chloride and t-butanol.



Scheme-20: Sample t-Butylester protection reaction

This reference reaction was reasonable to synthesize the *tert*-butyl protected amino acid-III (**8**) from this procedure. **8** was synthesized according to the procedure described in the experimental part. **8** was coupled with **2** according to the procedure described in the experimental part in order to get half linear peptide.

The resulting compound(**9**) was splitted into two parts. One part designated to undergo Fmoc deprotection to give **10** according to the

procedure described in SPPS. The other part designated to undergo tert-Butyl deprotection to give **11**. **9** was dissolved in 50% trifloroacetic acid (TFA) solution (v/v, chloroform) and stirred at room temperature for 45 min. After removing the solvent and acid, **11** was obtained and it was used further reactions without purification.

After deprotection reactions, we synthesized **12** by coupling **11** (*tert*-Butyl deprotected) with **10** (Fmoc deprotected). The coupling procedure and reagents were the same used in SPPS. After coupling reaction, the crude was purified by flash chromatography to give white solid (**12**). The coupling yield was low(38%) if we compared previous coupling reactions because we had primary amine with secondary amine in **10** which could react with the acid function of **11**.

Before further cyclization of the linear, and then synthesis of the bicyclic peptide, we decided to perform a sample reaction that was putting a bridge onto a commercial cycle that could have two secondary amine. We found 1,4,10,13-Tetraoxa-7,16-diaza-cyclooctadecane [Cycle-IV] that was;

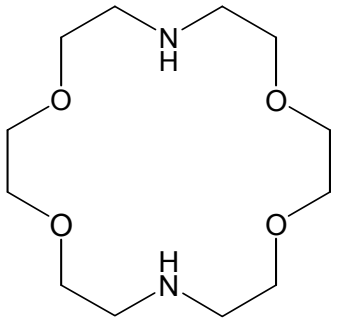
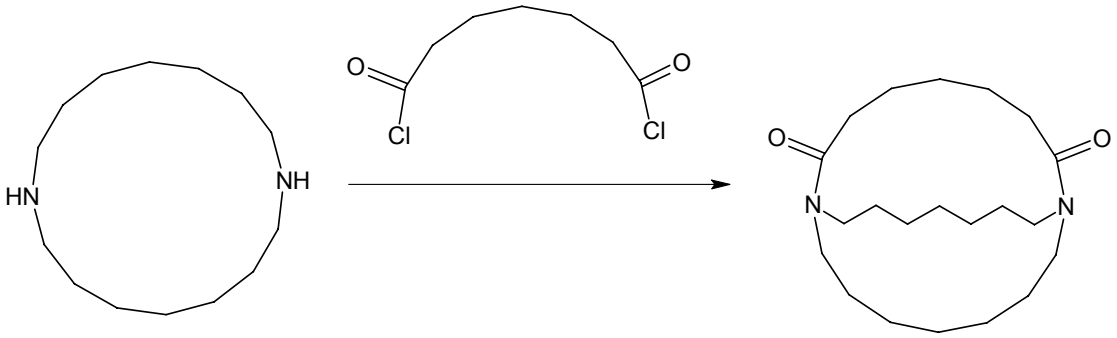


Figure-19: Structure of 1,4,10,13-Tetraoxa-7,16-diaza-cyclooctadecane (**Cycle-IV)**

This azacrown could be a sample for us because it has two secondary amines and it was composed of 18 atoms.

SAMPLE BICYCLIC :

In literature, E. Simmons and C. H. Park [28] synthesized bicyclic structures by putting a bridge that has two acid chloride arms, on to the cycle. The reaction scheme-21 was below;



Scheme-21: Sample Reaction for Formation of Bicyclic Structures

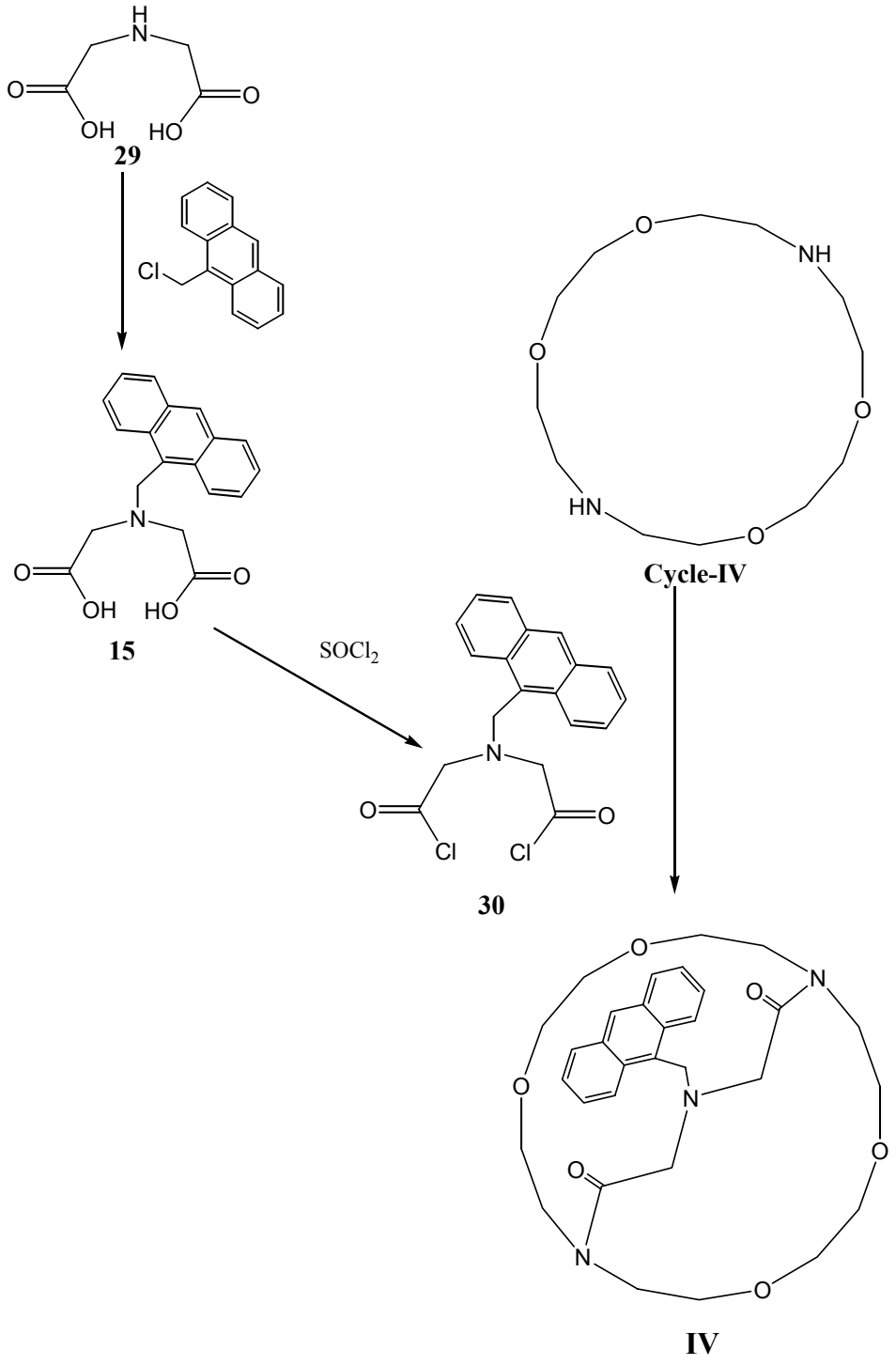
At this point, we would find a commercial bridge compound that would be similar to bridge-I.

Trial- I:

i) Synthetic Route

We tried to synthesize **15** by using imino-diaceticacid according to the procedure described in Organikum.[29]

The synthetic route was below;



Scheme-22: Synthetic Route-I for **IV**

ii) Synthesis, Results and Discussion

Anthracene Chloride(1.25 eq) and Iminodiacetic acid(**29**) were dissolved in acetonitrile and the solution was reflux for 4 hours after addition of triethylamine. After evaporation of solvent and base, the crude was obtained.

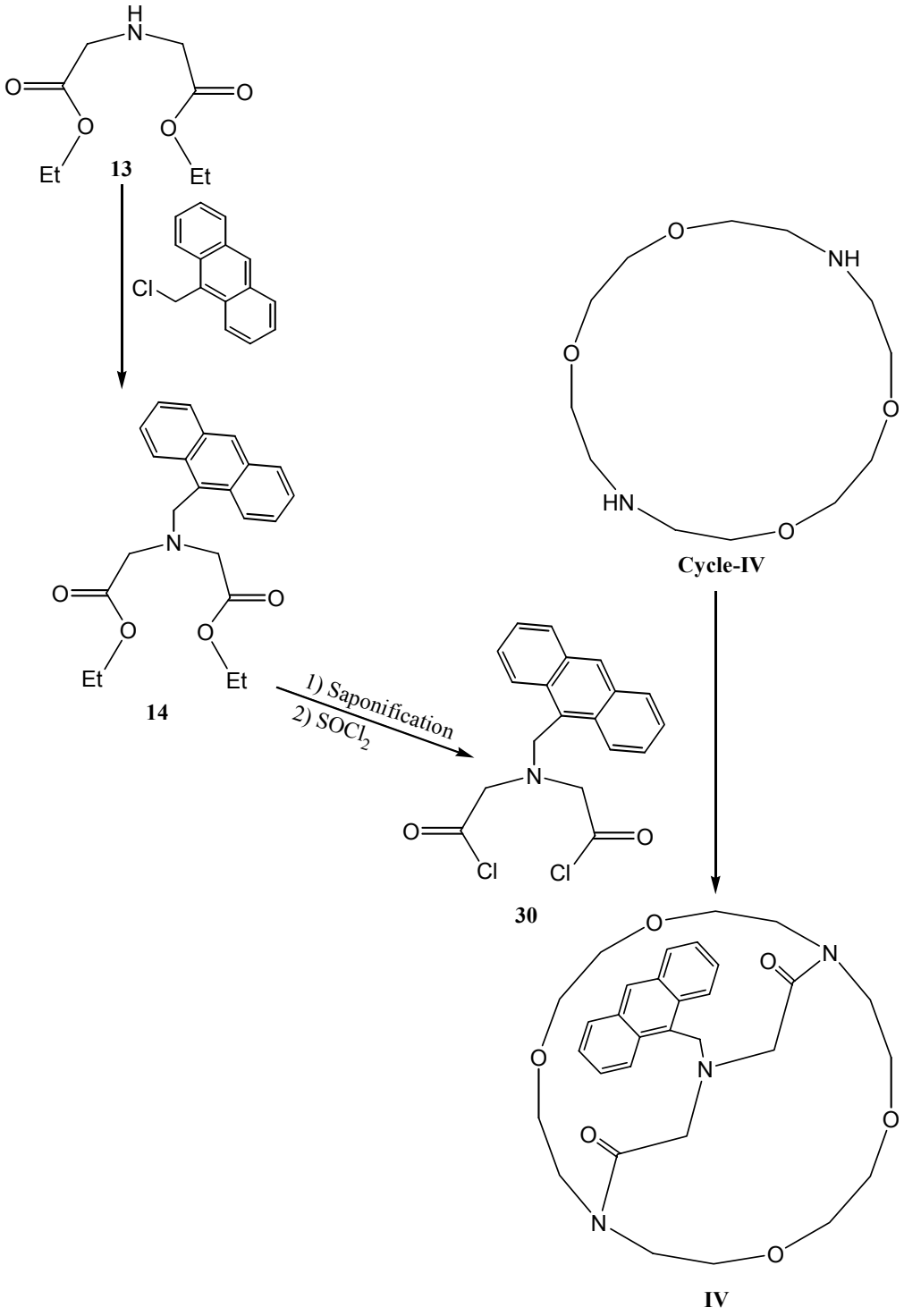
The NMR spectras of prep-TLC layers of the crude showed that the reaction did not work. We predicted that during the reaction, carboxylic acid parts could neutralize the secondary amine and prevent the anthracene chloride reaction with secondary amine.

Trial II:

i) Synthetic Route

In order to synthesize **15**, a protecting group for the protection of the acid function is necessary. We chose the ethyl-ester protecting group because it is stable to anthracene chloride reaction conditions and can be easily removed by saponification reaction.

The synthetic route was below;



Scheme-23: Synthetic Route-I for **IV**

ii) Synthesis, Results and Discussion

14 was synthesized by using **13** and anthracene chloride, and **30** was synthesized by saponification process of **14**. The procedures of these two reactions were described in the experimental part.

(Anthracen-9-ylmethyl-carboxymethyl-amino)diaceticacid (**15**) was dissolved in excess thionyl chloride and the mixture was heated to reflux for 30 min. After evaporating thionyl chloride, the solid was mixed with cycle-IV (1 eq) in excess benzene(more than 100 ml). After adding triethylamine, the mixture was stirred under N_2 at room temperature for 24 hrs. After evaporating base and benzene, the crude was dissolved in 2 N NaOH solution and extracted with DCM. After drying the combined organic phase with $MgSO_4$ and evaporating the solvent, the crude mixture was obtained.

The purification by using normal phase prep-TLC or doing normal phase TLC was really hard because anthracene was high fluorescent character and the anthracene compounds showed a lot of dots in normal phase TLC. The mass-spectra of the crude showed that the reaction did not work and we had only starting materials left.

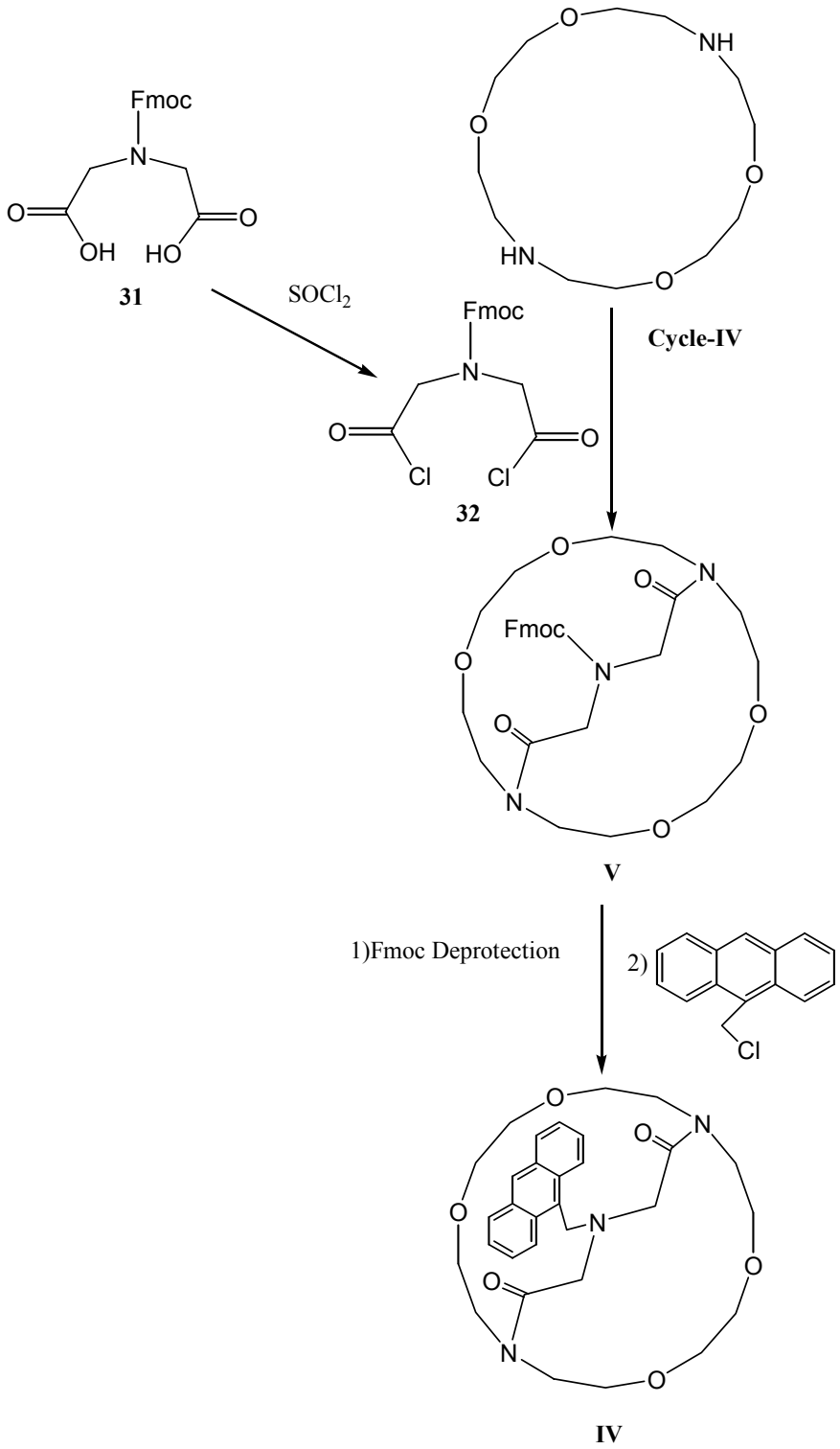
Trial III:

We could struggle to synthesize the bicyclic structure but we would face the purification problem of the bicyclic compound due to high fluorescent character of anthracene group. But, if we synthesize the bicylic structure which bridge amine group was protected by Fmoc protecting group, we could add anthracene after cleaving Fmoc group and we could easily purify the crude by removing the anthracene chloride (starting material) and HCl (only side product).

i) Synthetic Route

We found commercially Fmoc protected aminodiacetic acid (**31**). We chose the Fmoc protecting group because it was stable to the reaction conditions (acidic, HCl) and could be easily removed by %20 piperidine solution.

The synthetic route was below;



Scheme-24: Synthetic Route for **V** and **IV**

ii) Synthesis, Results and Discussion

N-Fmoc-iminodiacetic acid (**31**) was dissolved in excess thionyl chloride and the mixture was heated to reflux for 30 min. After evaporating thionyl chloride, the solid was mixed with cycle-IV (1 eq) in excess benzene. We made a very dilute solution in order to get a good yield. After addition of DIPEA(3 eq), the mixture was stirred under N_2 at room temperature for 7 hours. We used DIPEA as a base instead of triethylamine that was described in literature because DIPEA has bulky groups that could not cleave Fmoc protection. After evaporating the solvent and base, the crude was redissolved in dichloromethane and washed with 2 N HCl solution. The organic phase was dried with $MgSO_4$. We used $MgSO_4$ for drying instead of Na_2SO_4 because Mg has a large size than Na or NH_4^+ that can not fit to the cavity of the bicyclic compound. The purification is in progress.

BRIDGE COMPOUND:

During the cycle reactions, we tried to synthesize the bridge-I. In the bridge compound, there should be fluorescent groups and two reaction parts for putting the bridge compound onto the cycles. We tried to synthesize the bridge-I;

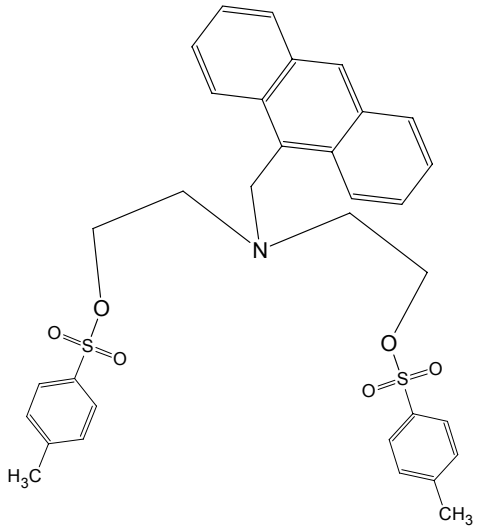
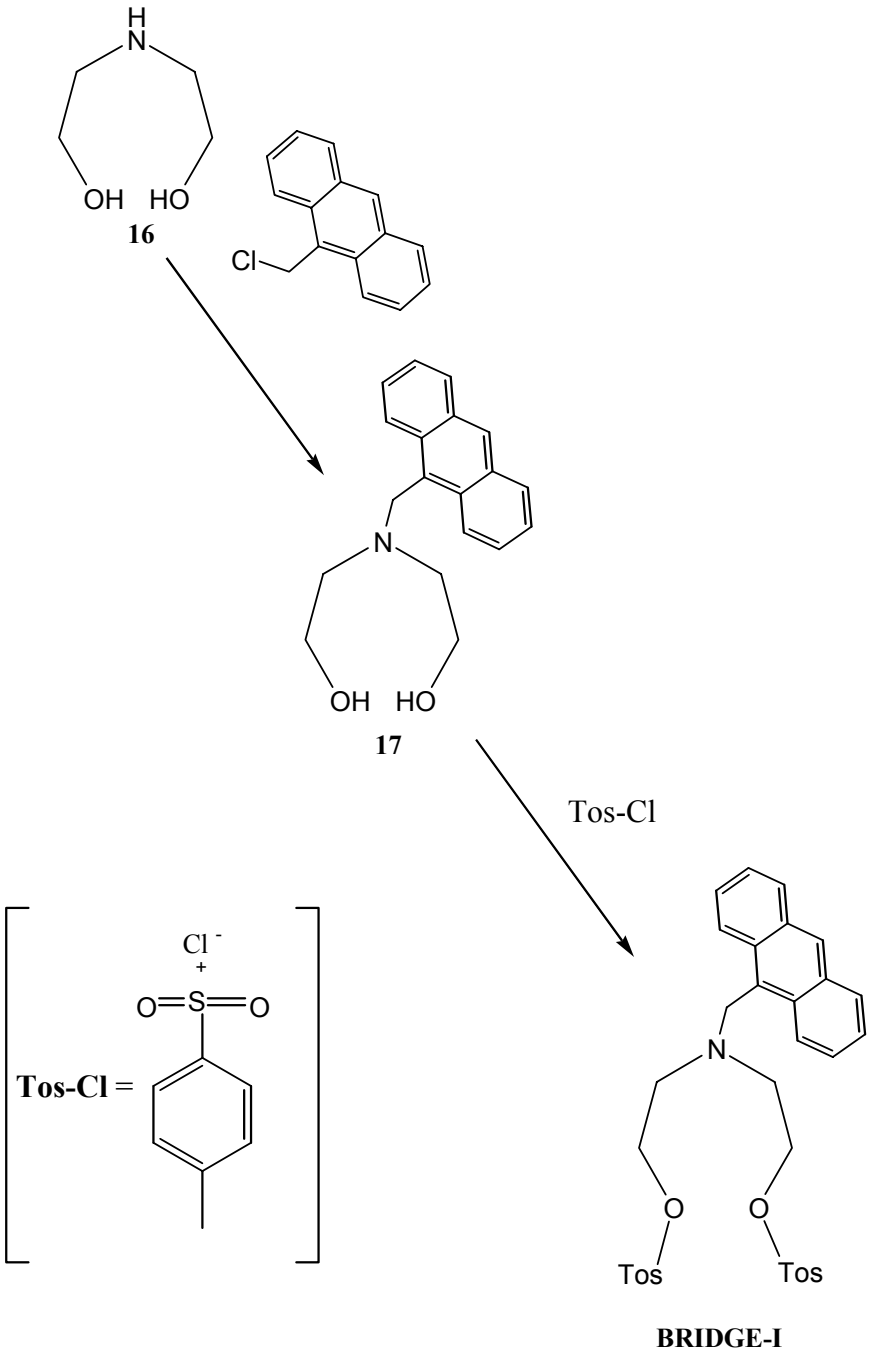


Figure-20: Structure of Bridge-I

i) Synthetic Route

We chose anthracene group due to having high fluorescent character, and the tosylate due to being very good leaving group. The reaction was done according to the procedure in Organikum.[29]

The synthetic route was below;



Scheme-25: Synthetic Route for Bridge-I

ii) Synthesis, Results and Discussion

17 was synthesized by using anthracene chloride and diethanolamine(**16**) according to the procedure described in the experimental part.

ii-A) Tosylation Reaction:

Trial I:

In a round bottom flask, p-Toluenesulfonylchloride (2.25 eq) were mixed with 2-[(9-anthrylmethyl) (2-hyroxyethyl) amino]-1-ethanol (**17)** in chloroform and the mixture was stirred a few minutes and cooled below 5 ^{o}C. This cooling was due to heat production. After addition of triethylamine in dropwise to the solution, the mixture was stirred at room temperature for 3 hours. A mixture of 6 g ice and 2.4 ml conc. HCl was added carefully to the solution, and then the mixture was stirred at room temperature for 30 min. The chloroform part was washed with DI water. After drying with Na_2SO_4,and evaporating the solvent, the yellowish brown solid was obtained. The H-NMR and C-NMR spectras showed that this brownish solid was the same as starting compound.

Trial II:

We tried the same procedure but we heated the mixture to reflux for 3 hrs instead of stirring in room temperature for 3 hours. The NMR spectras

showed that the reaction did not work again. We got only starting material left.

In literature, one tosylate attachment to alcohols gave good yields. But, in our case, the two tosylate attachment did not work. We predicted that one tosylate attached to the bridge arms could prevent the other attachment by its bulky character.

Further reactions for bridge compound, we could use other good leaving groups that do not have bulky character, for instance; mesolate, halogens

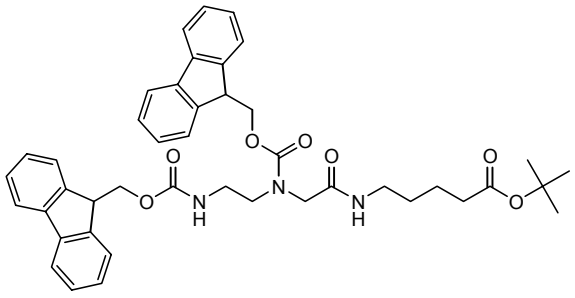
CONCLUSION:

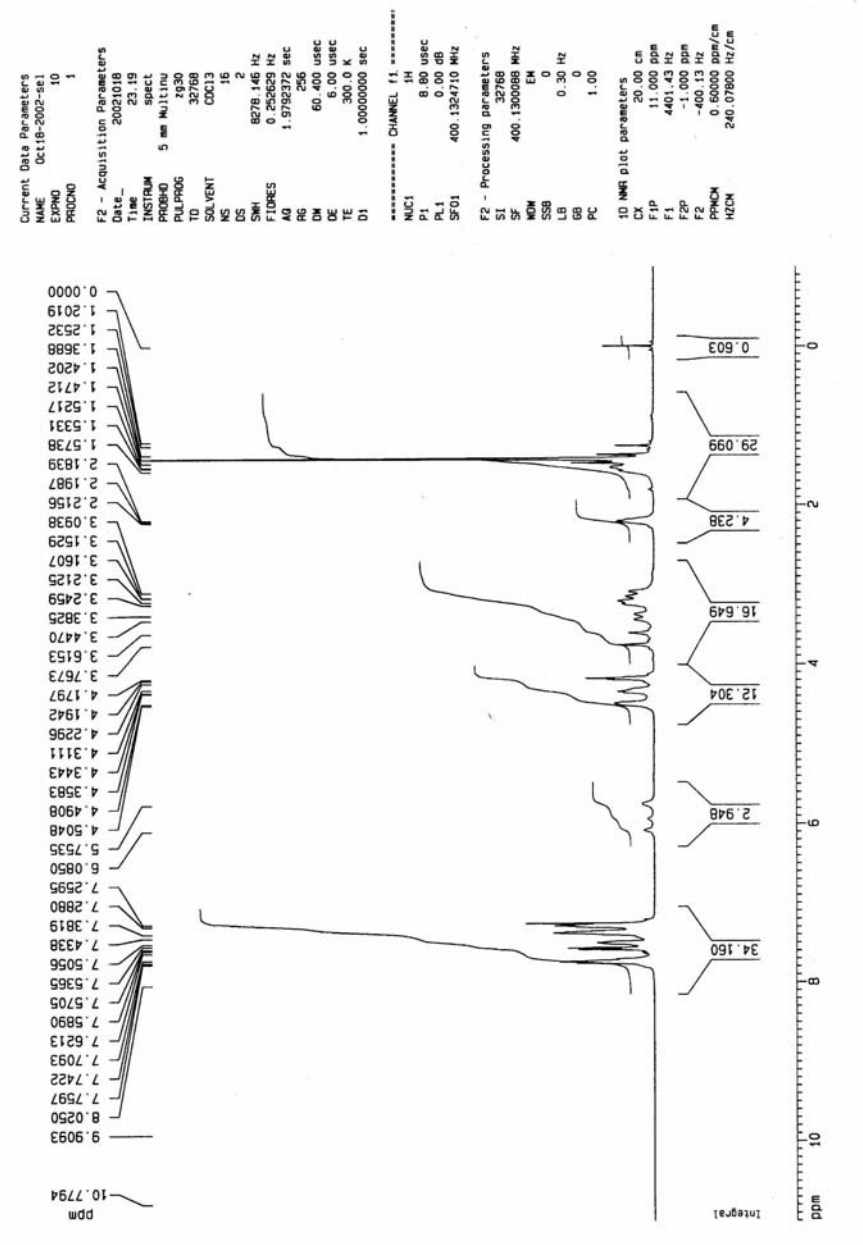
III was tried to synthesize in three different ways, and the linear that will form the cycle-III after cyclization, was synthesized recently. Up to now, Benzyl 5-(2-[[(9H-9-fluorenylmethoxy)carbonyl](2-[(9H-9-fluorenyl methoxy) carbonyl] aminoethyl)amino]acetylamino)pentanoate (**4**), tert-butyl 5-[(9H-9-fluorenyl methoxy)carbonyl]amino- pentanoate (**6**), t-butyl 5-(2-[[(9H-9-fluorenyl methoxy)carbonyl](2-[(9H-9-fluorenylmethoxy) carbonyl] amino ethyl) amino]acetyl amino) pentanoate (**9**), tert-butyl 5-(2-[(2-[5-(2-[[(9H-9-fluorenylmethoxy) carbonyl] (2-[(9H-9 fluorenylmethoxy)carbonyl]amino ethyl)amino]acetyl amino) pentanoyl] amino ethyl)amino]acetyl amino)pentanoate (**12**), ethyl 2-[(9-anthrylmethyl)(2-ethoxy-2-oxoethyl) amino] acetate (**14**)**,** 2-[(9-anthrylmethyl)(carboxymethyl) amino] acetic acid (**15**) which were not in literature, were synthesized. Future work will focus on;

- Synthesizing Bicyclic-V
- Synthesizing Bicyclic-IV from Bicyclic-V
- Cyclization the linear peptide(**12**)
- Synthesizing the Bridge-I
- Synthesizing **III**

After synthesizing the bicyclic molecules, their photophysical properties in the presence of ammonium cation will be characterized.

APPENDIX-A

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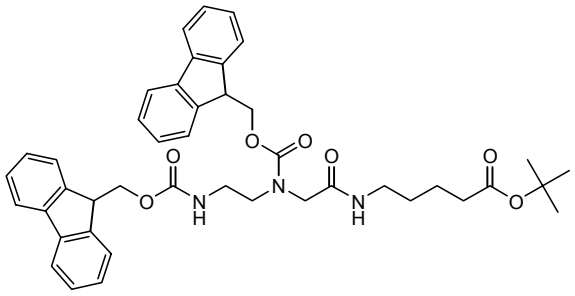

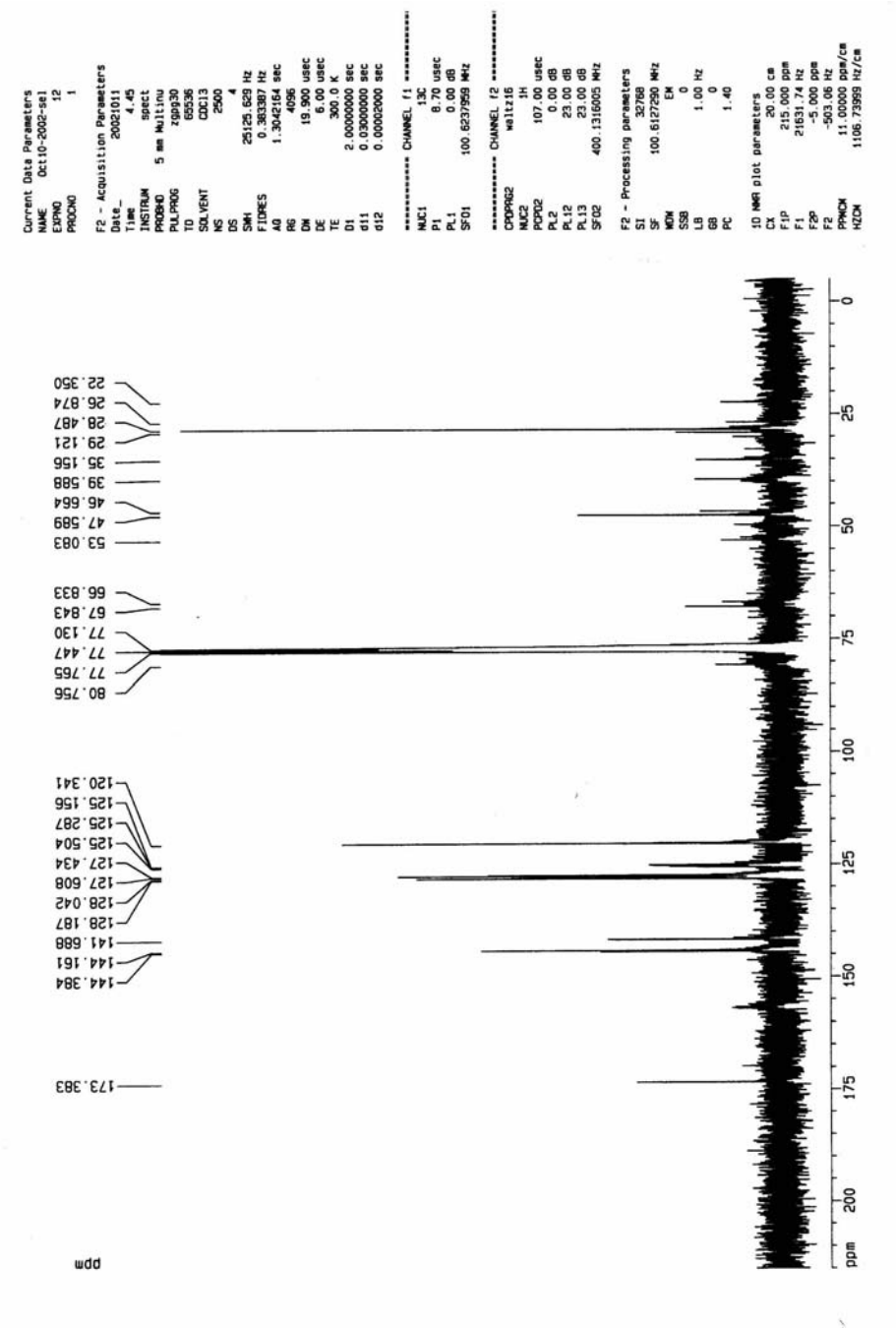
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NUC2 1H
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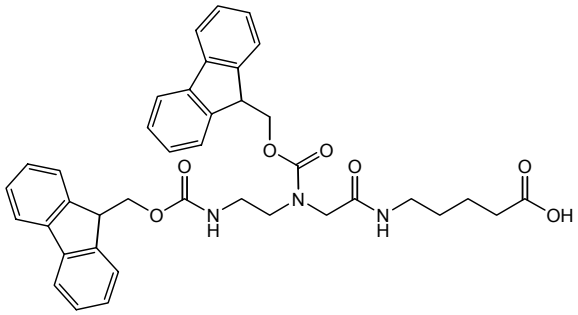

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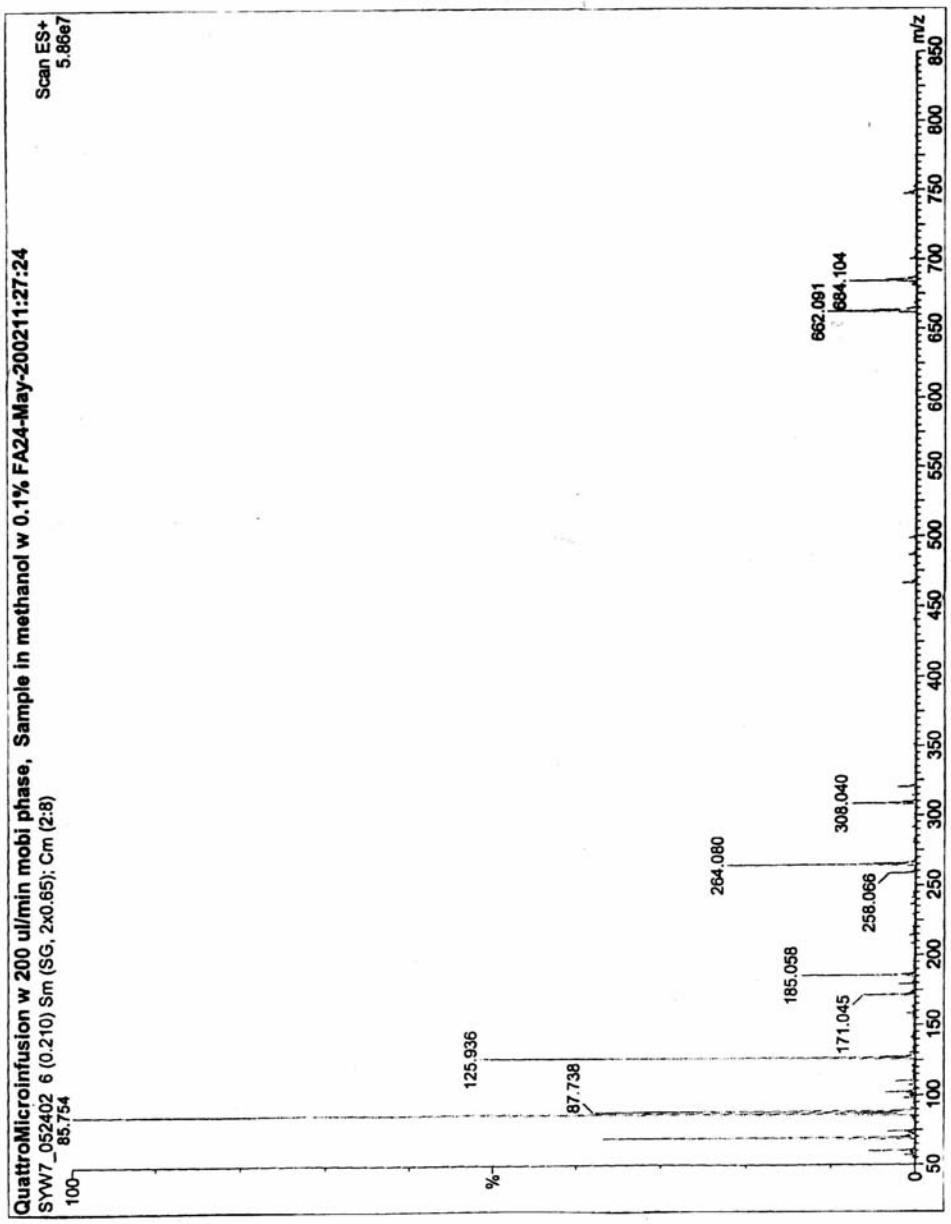
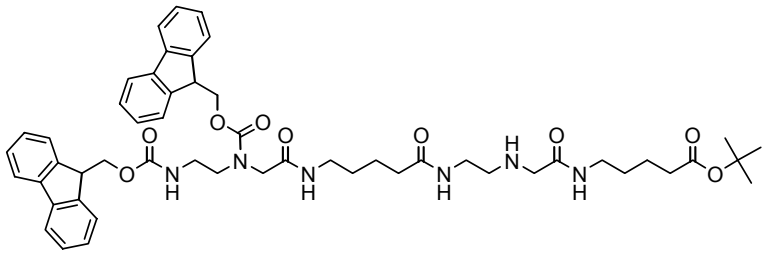
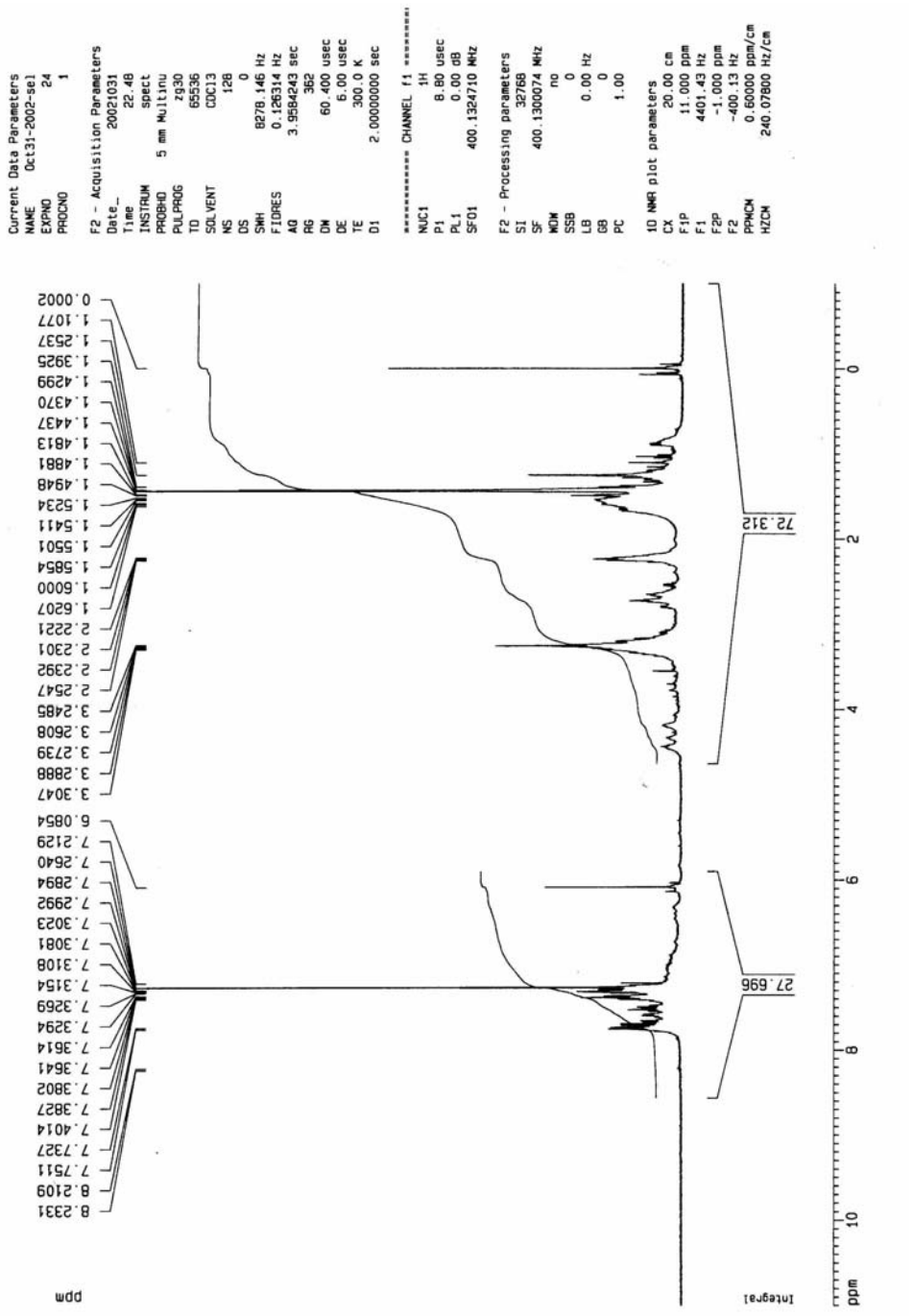
M + Na : Mol. Wt.: 684.73



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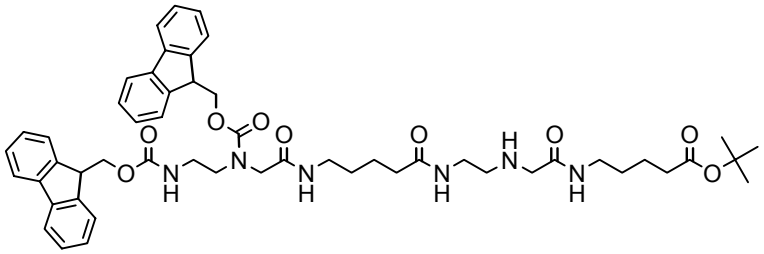

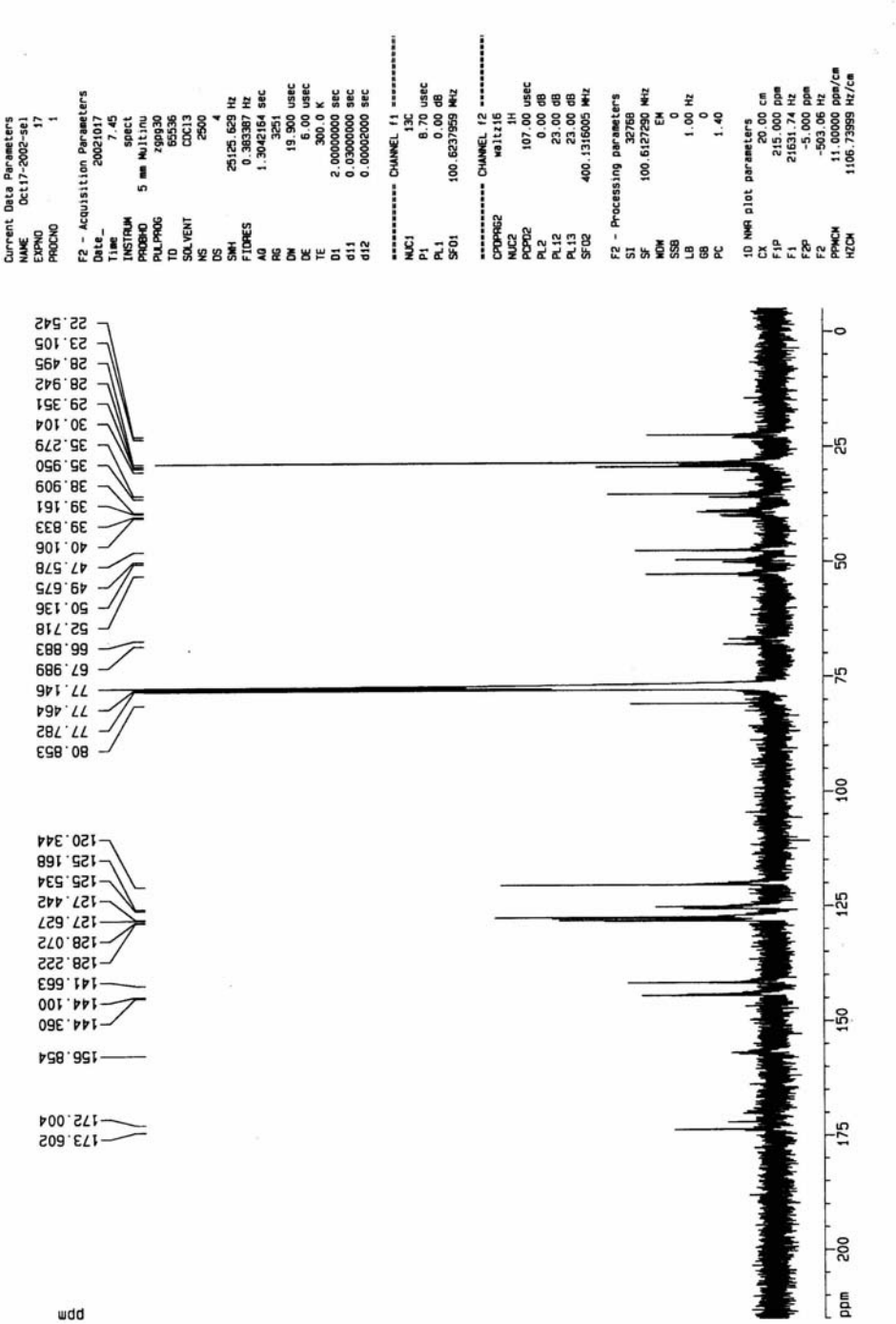
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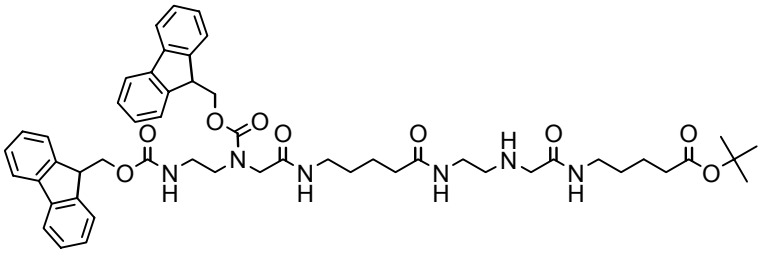

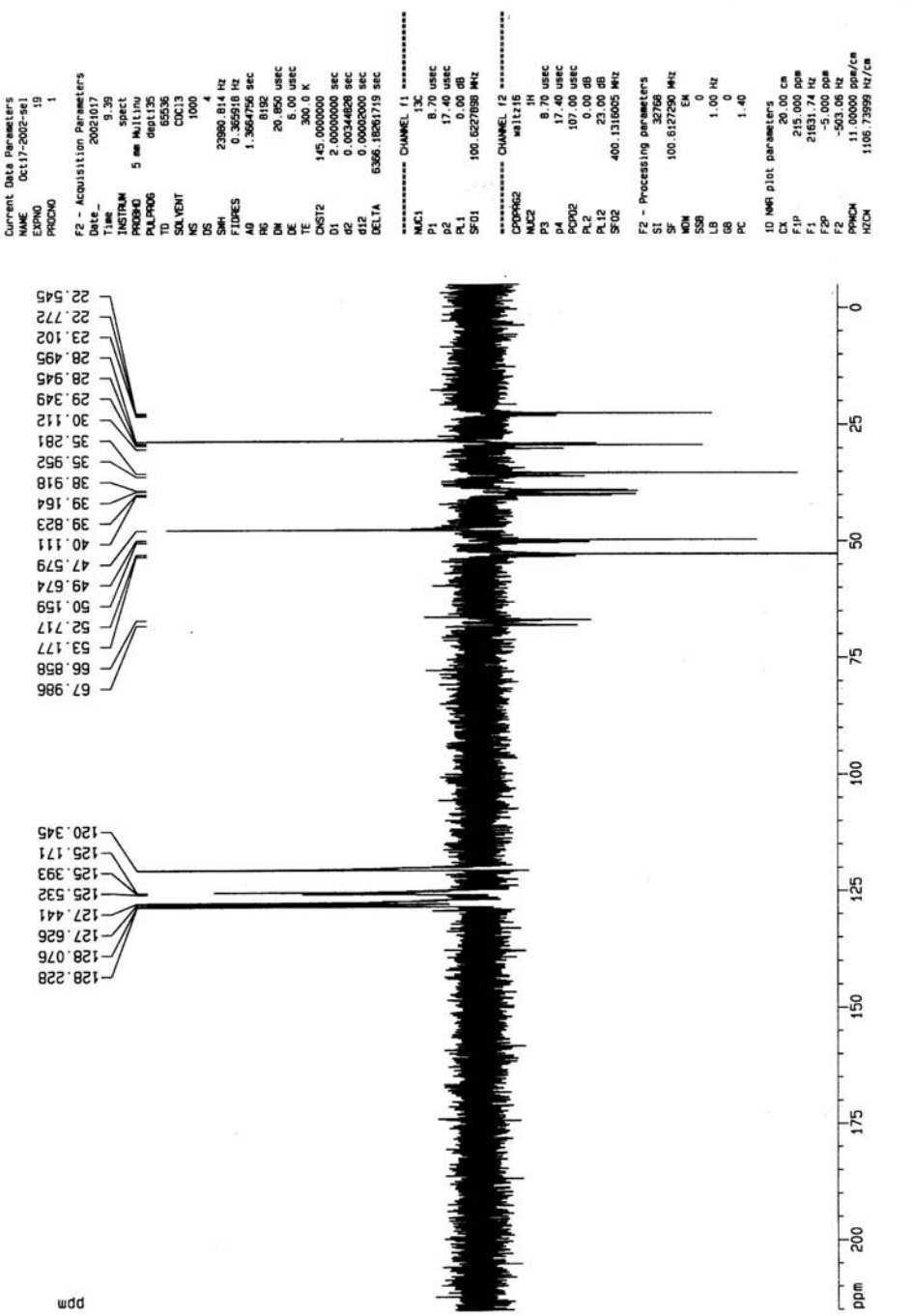
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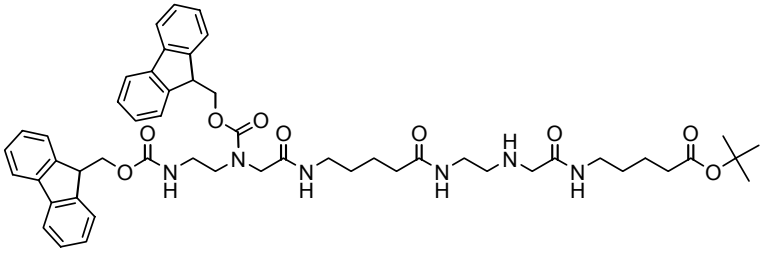

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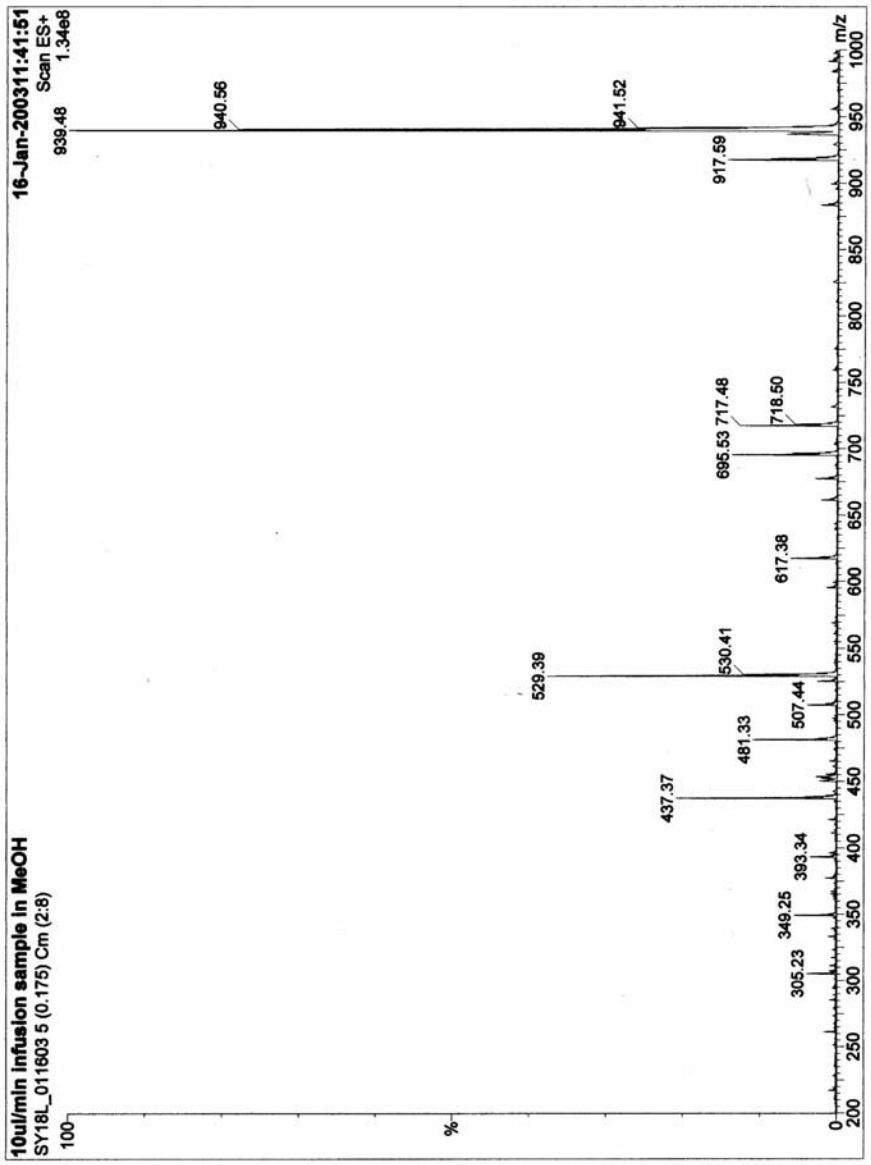
M + Na : Mol. Wt.: 940.09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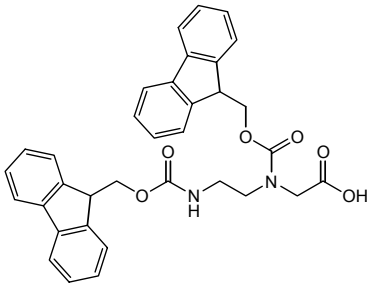
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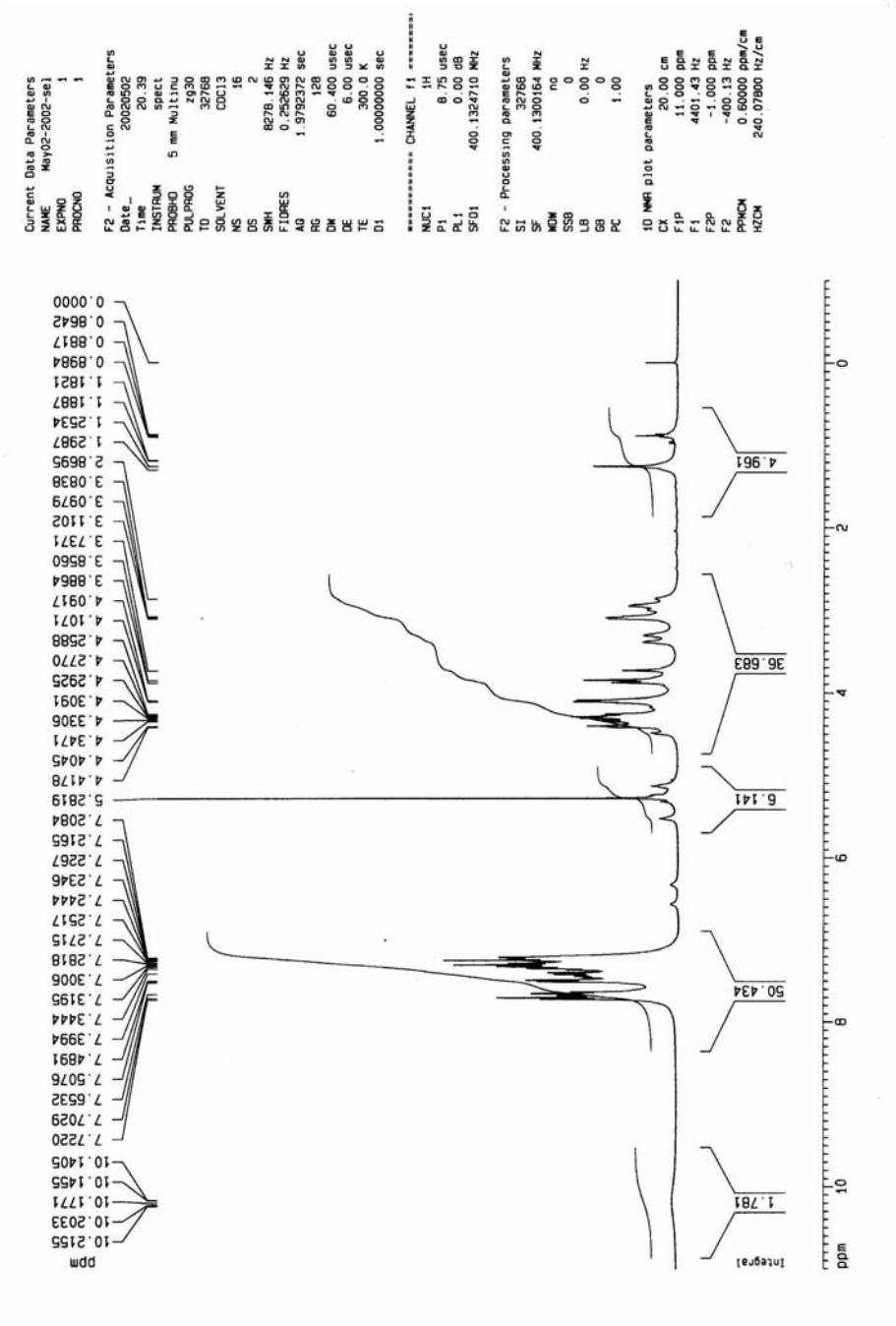
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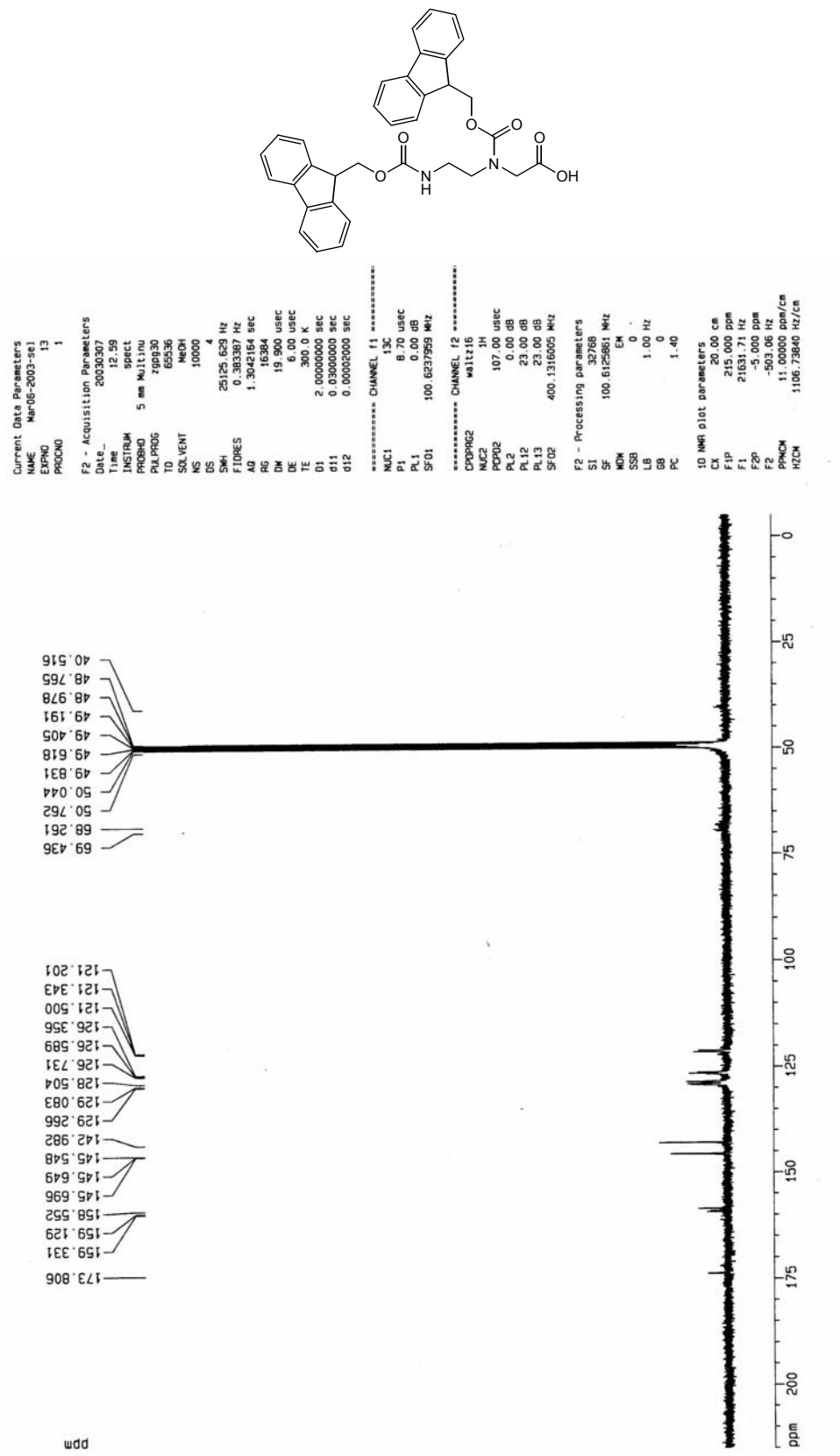
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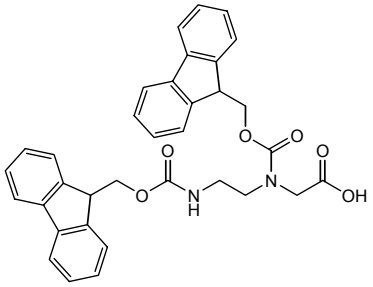
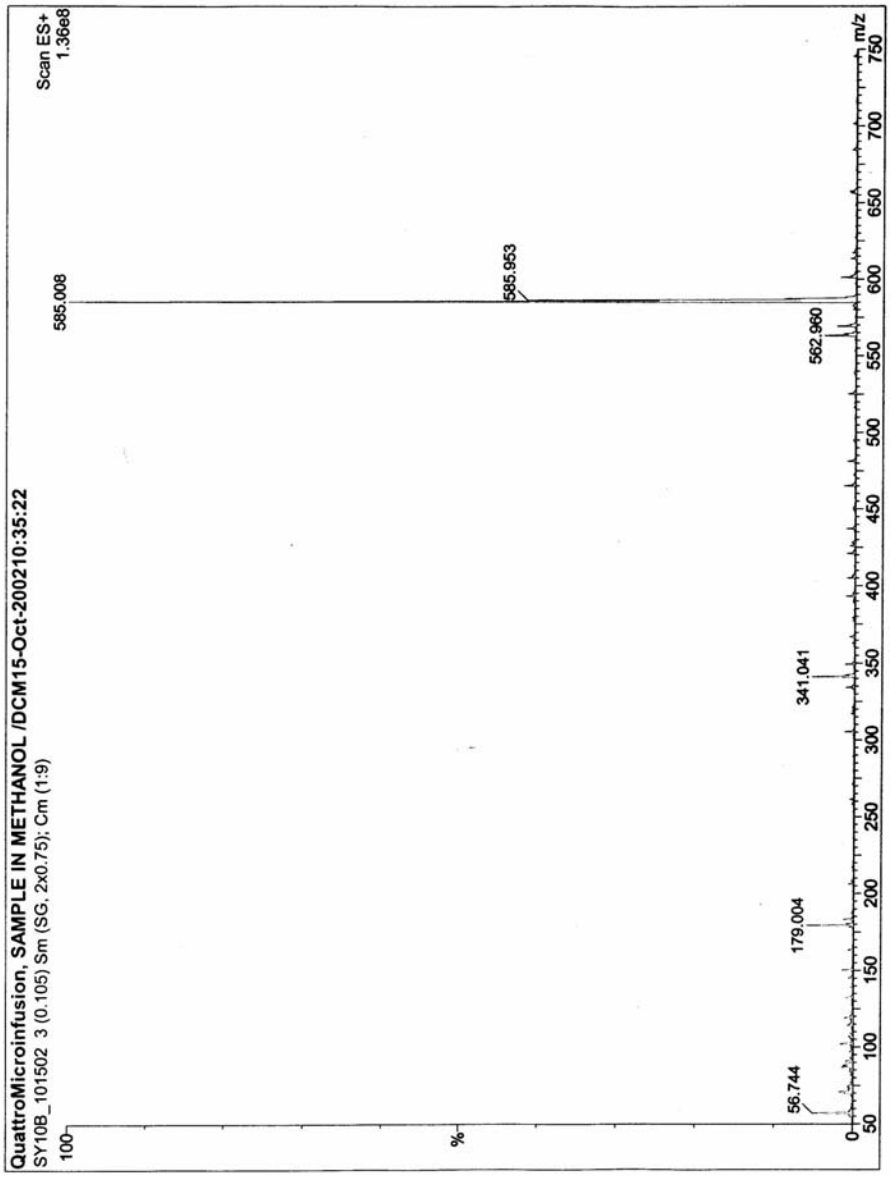
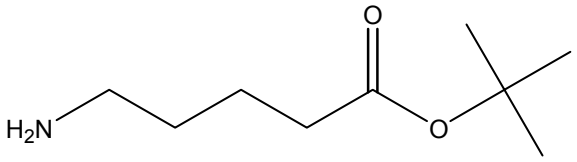
50.044
50.762
68.261
69.436
121.201
121.343
121.500
126.356
126.589
126.731
128.504
129.083
129.266
142.982
145.548
145.649
145.696
158.552
159.129
159.331
173.806
ppm
0
25
50
75
100
125
150
175
200
ppm

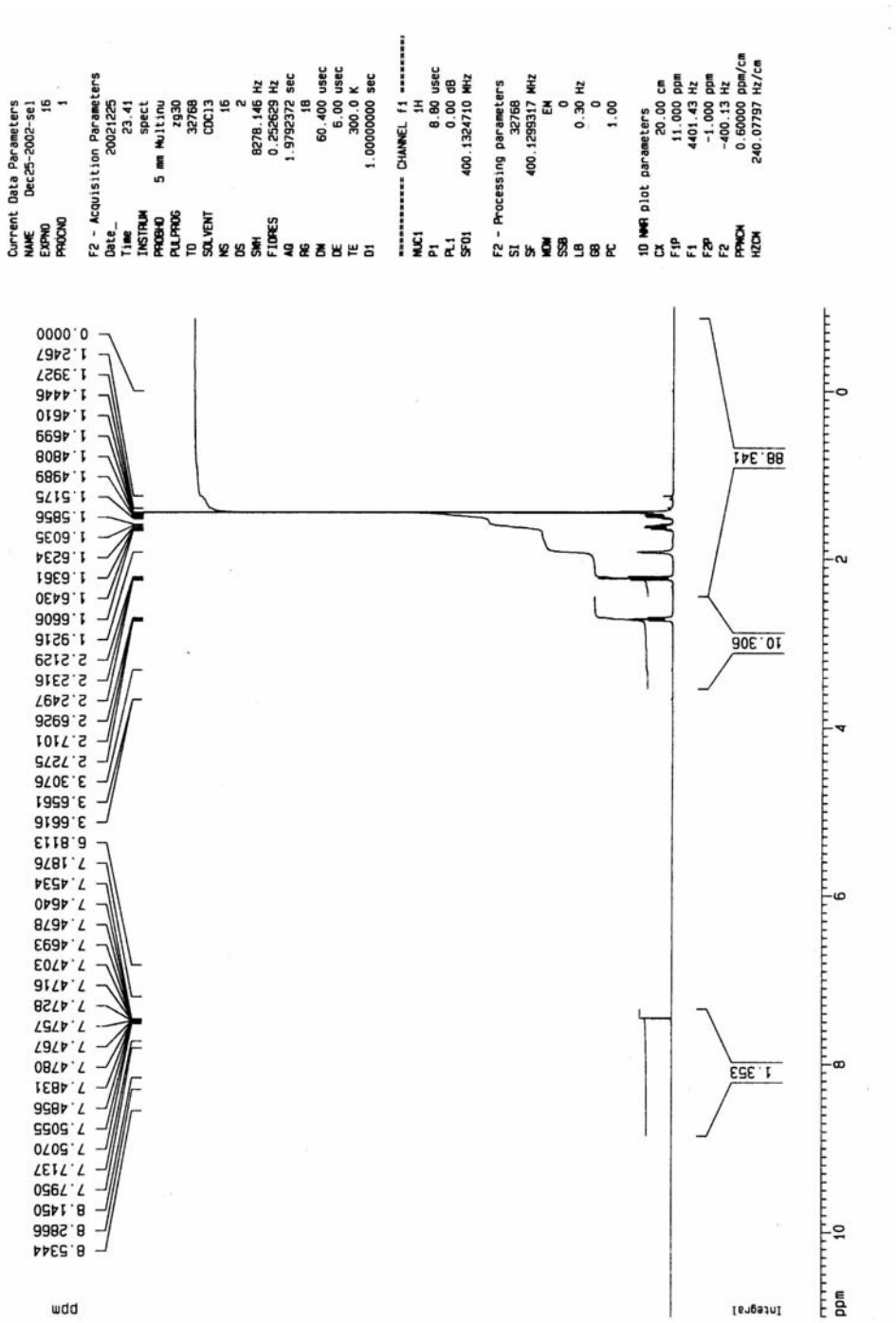


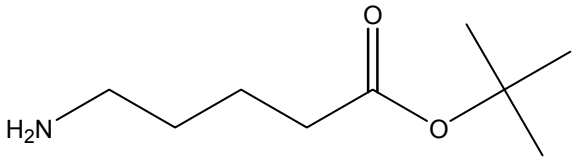
M : Mol. Wt.: 562.61

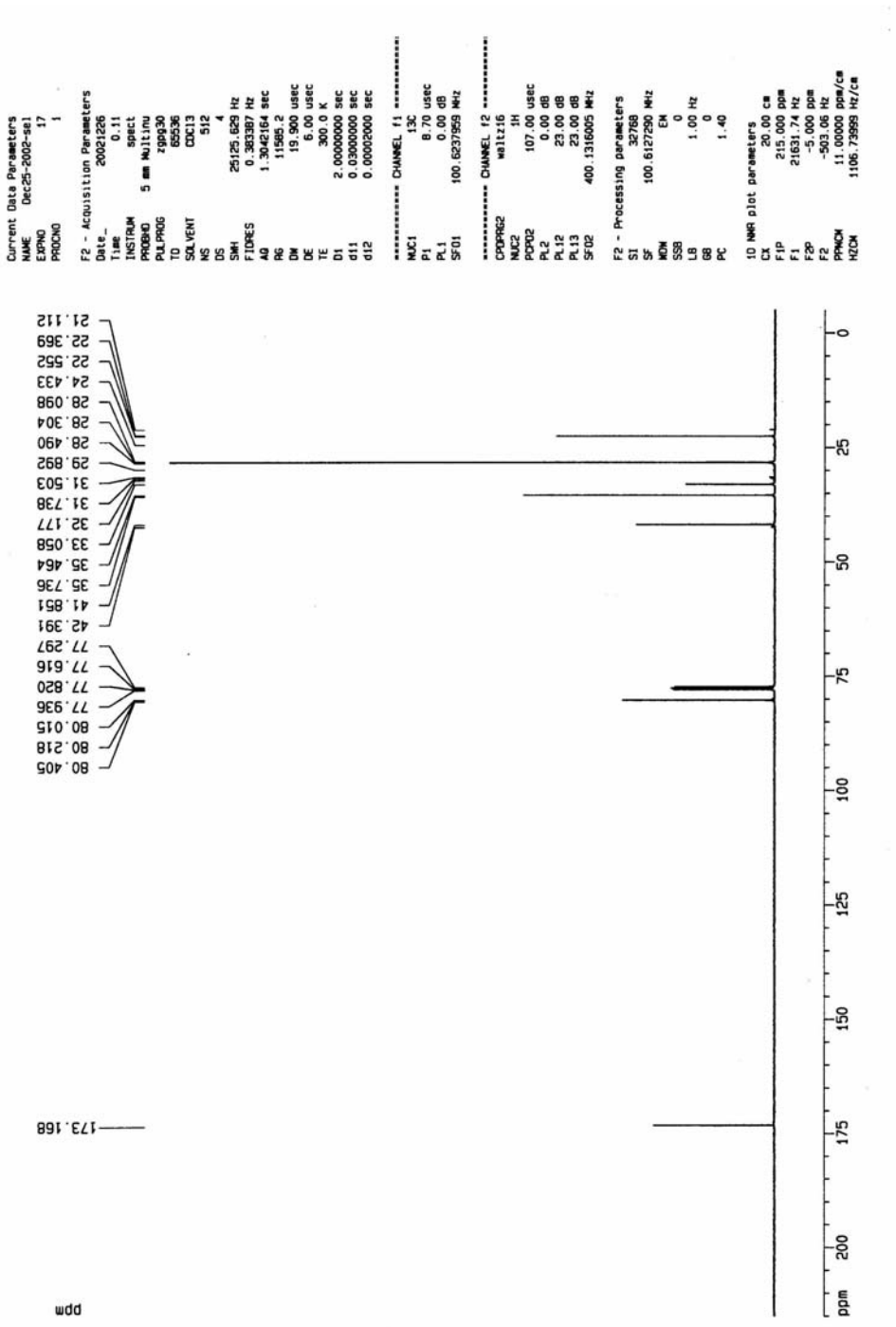
M + Na : Mol. Wt.: 585.60

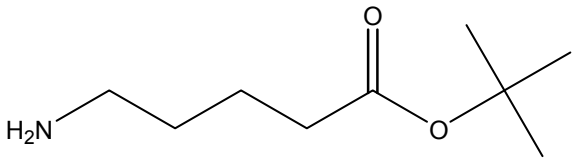

QuattroMicroinfusion, SAMPLE IN METHANOL /DCM15-Oct-200210:35:22
SY10B_101502 3 (0.105) Sm (SG, 2x0.75); Cm (1:9)
Scan ES+
1.36e8
100
%
0
585.008
585.953
562.960
341.041
179.004
56.744
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
m/z

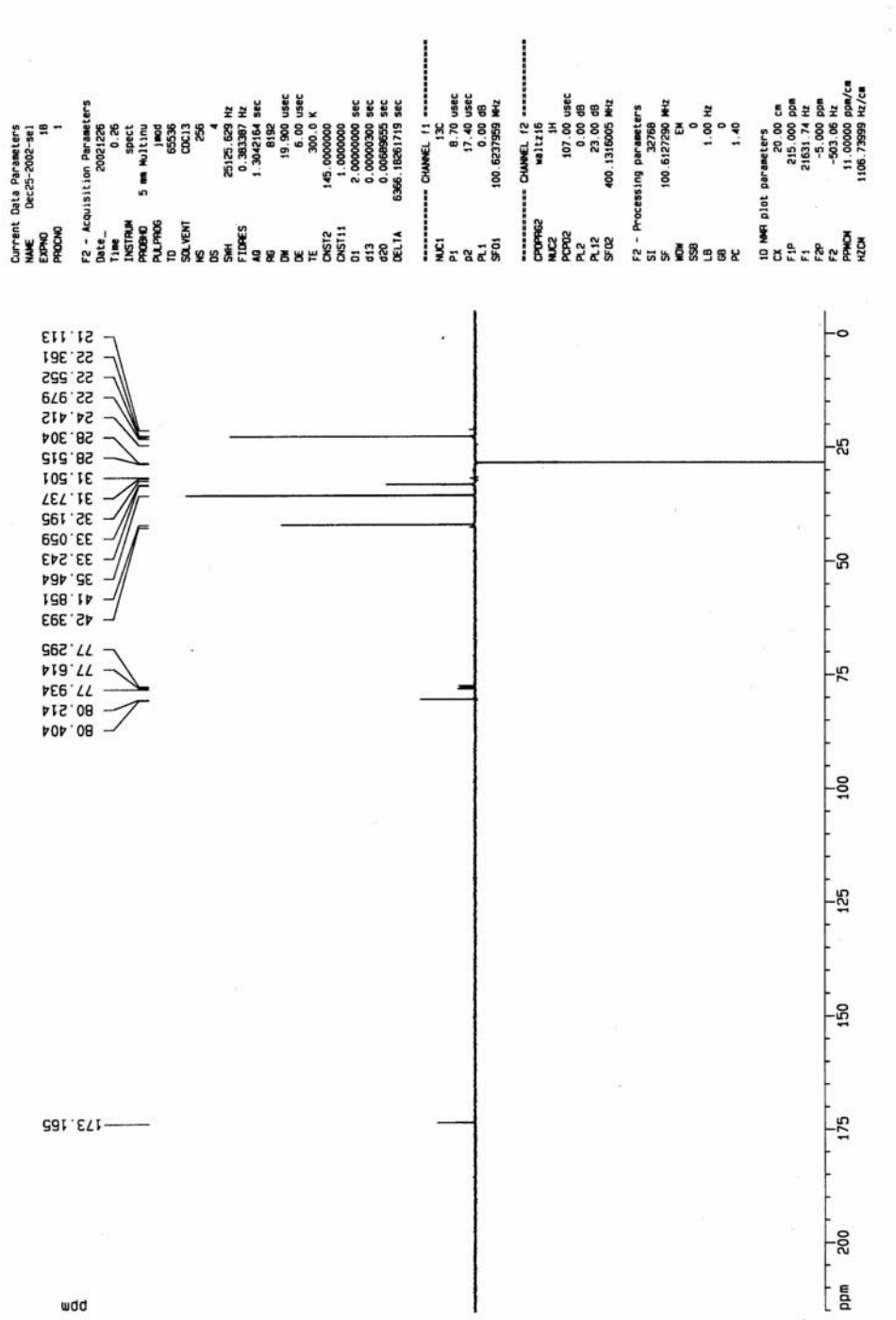

O
H2N
O

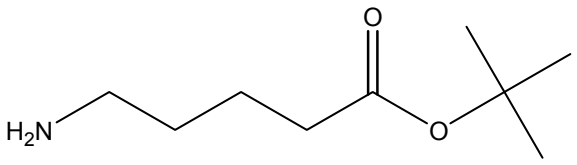

Current Data Parameters
NAME Dec25-2002-sel
EXPNO 16
PROCNO 1
F2 - Acquisition Parameters
Date_ 20021225
Time 23.41
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.252629 Hz
AQ 1.9792372 sec
RG 18
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 8.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1299317 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 4401.43 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 240.07797 Hz/cm
8.5344
8.2866
8.1450
7.7950
7.7137
7.5070
7.4856
7.4831
7.4780
7.4767
7.4757
7.4728
7.4716
7.4703
7.4693
7.4678
7.4640
7.4534
7.1876
6.8113
3.6616
3.6561
3.3076
2.7275
2.7101
2.6926
2.4997
2.3316
2.2129
1.9216
1.6606
1.6430
1.6361
1.6234
1.6035
1.5856
1.5175
1.4989
1.4808
1.4699
1.4610
1.4446
1.3927
1.2467
0.0000
88.341
10.306
1.353
ppm
Integral

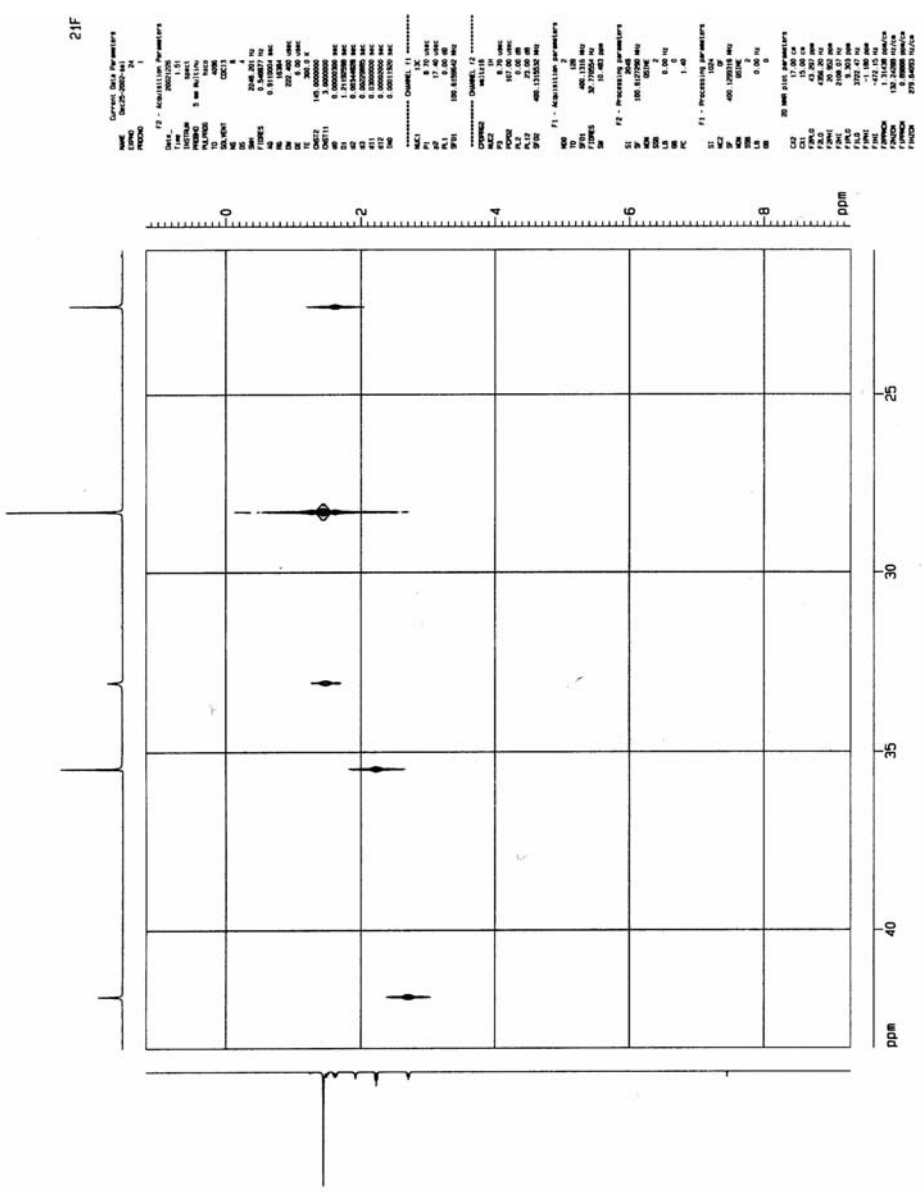

O
H2N
O

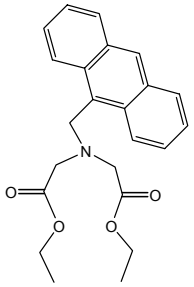

Current Data Parameters
NAME Dec25-2002-sel
EXPNO 17
PROCNO 1
F2 - Acquisition Parameters
Date_ 20021226
Time 0.11
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 11585.2
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
CHANNEL f1
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SF01 100.6237959 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
21.112
22.369
22.552
24.433
28.098
28.304
28.490
29.892
31.503
31.738
32.177
33.058
35.464
35.736
41.851
42.391
77.297
77.616
77.820
77.936
80.015
80.218
80.405
173.168
ppm
0
25
50
75
100
125
150
175
200

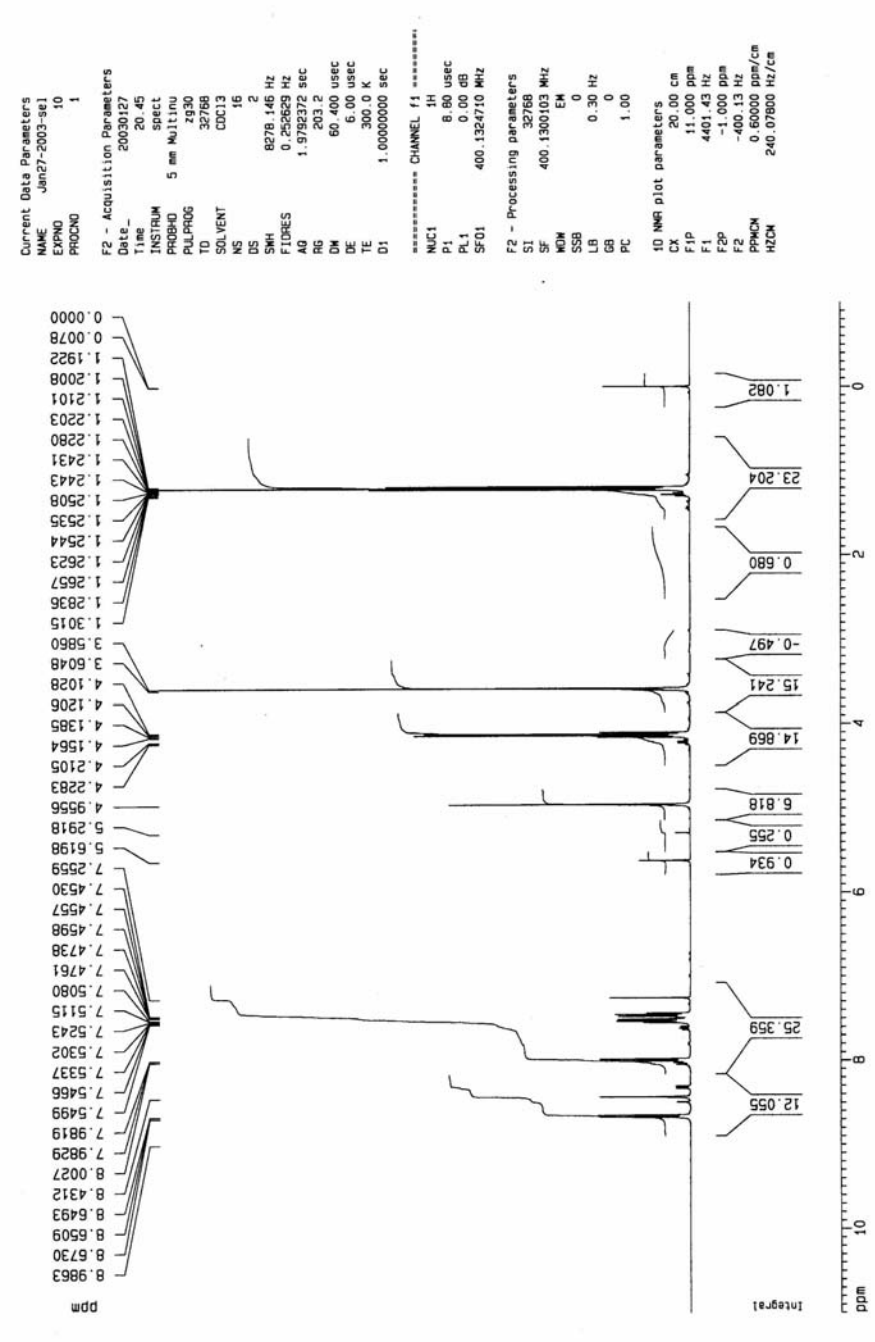

O
H2N
O

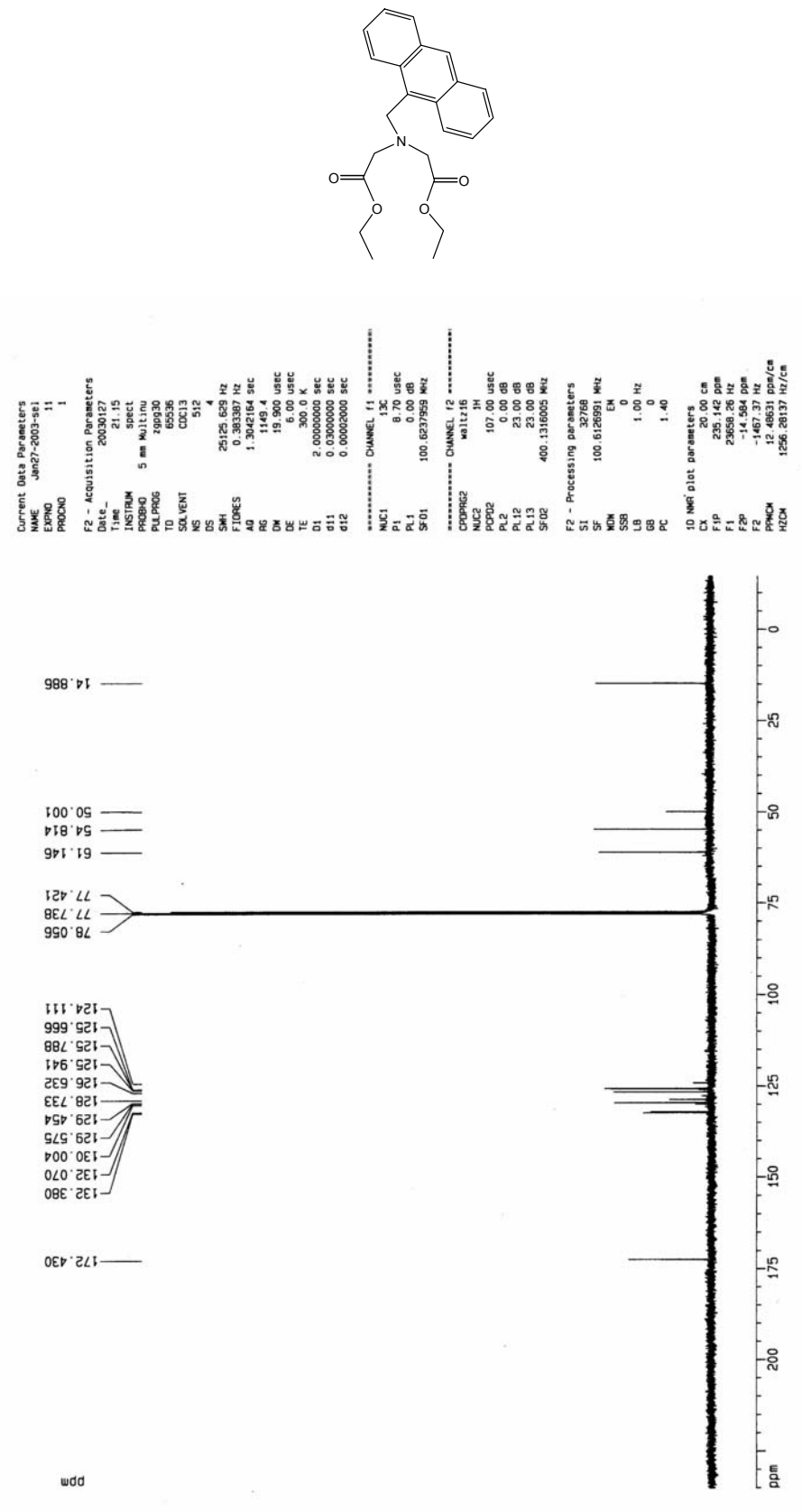

Current Data Parameters
NAME Dec25-2002-sel
EXPNO 18
PROCNO 1
F2 - Acquisition Parameters
Date_ 20021226
Time 0.26
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG jmod
TD 65536
SOLVENT CDCl3
NS 256
DS 4
SWH 25125.628 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
d13 0.00000300 sec
d20 0.00689655 sec
DELTA 6366.18261719 sec
CHANNEL f1
NUC1 13C
P1 8.70 usec
P2 17.40 usec
PL1 0.00 dB
SFO1 100.6237959 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
21.113
22.361
22.552
22.979
24.412
28.304
28.515
31.501
31.737
32.195
33.059
33.243
35.464
41.851
42.393
77.295
77.614
77.934
80.214
80.404
173.165
ppm
0
25
50
75
100
125
150
175
200
ppm

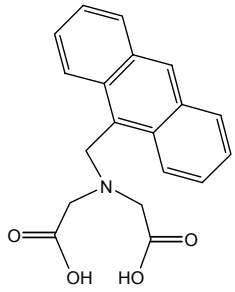

O
H2N
O

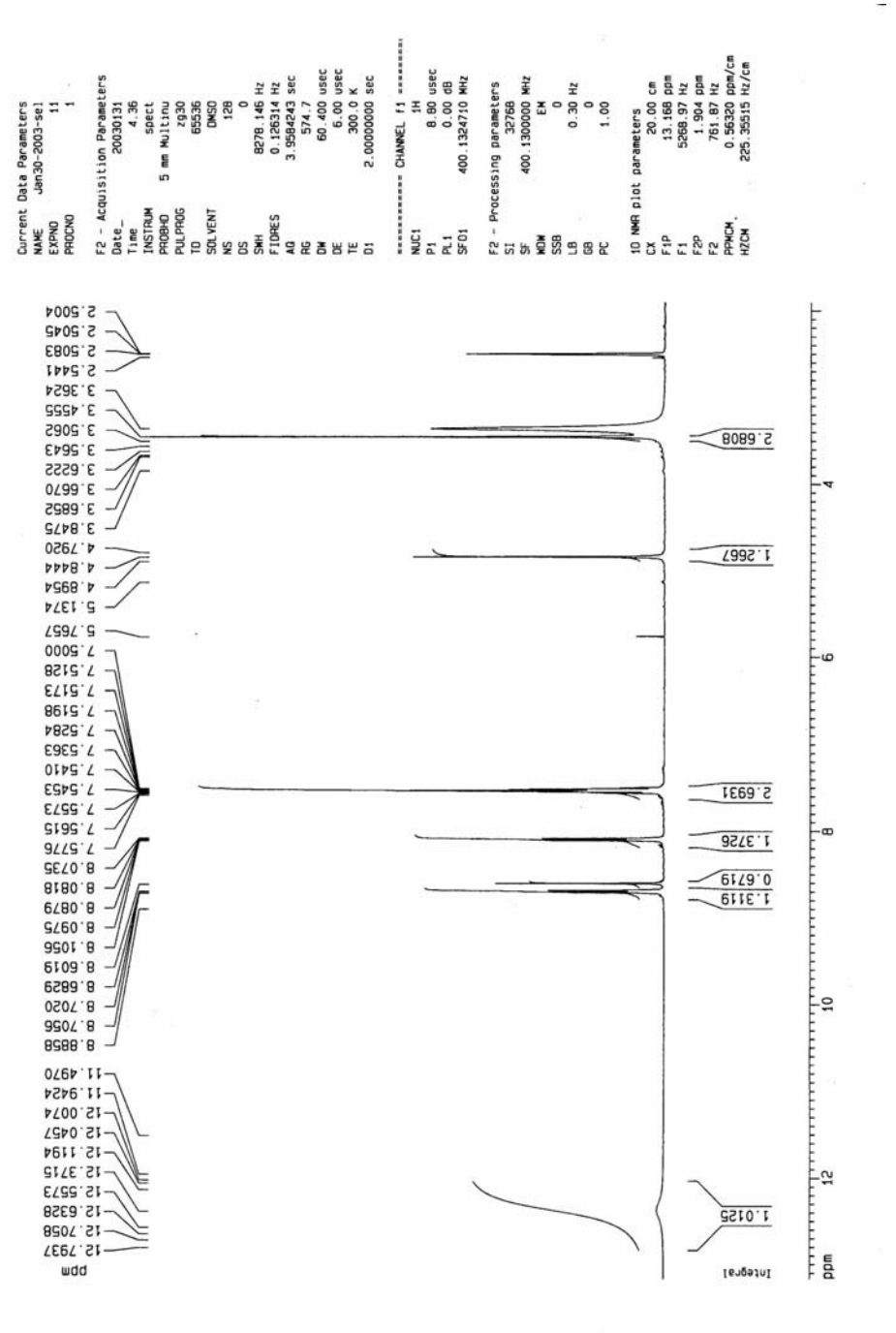

21F
ppm
ppm

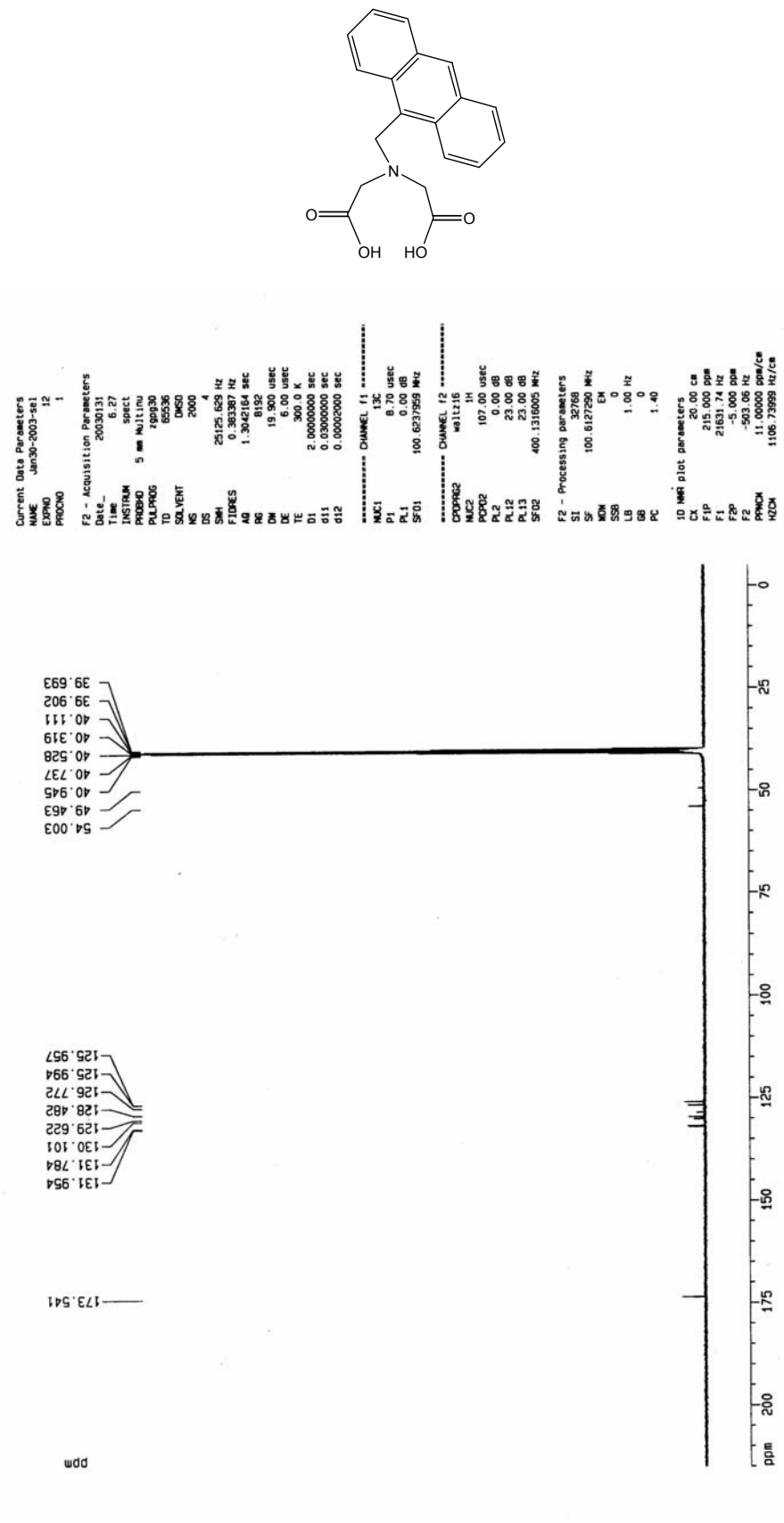


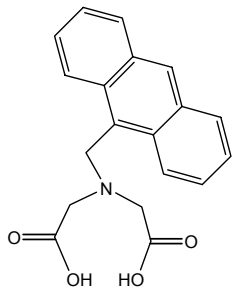

Current Data Parameters
NAME Jan27-2003-sel
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030127
Time 20.45
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.252629 Hz
AQ 1.9792372 sec
RG 203.2
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
========== CHANNEL f1 ========
NUC1 1H
P1 8.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300103 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 4401.43 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 240.07800 Hz/cm
ppm
Integral
1.082
23.204
0.680
-0.497
15.241
14.869
6.818
0.255
0.934
25.359
12.055

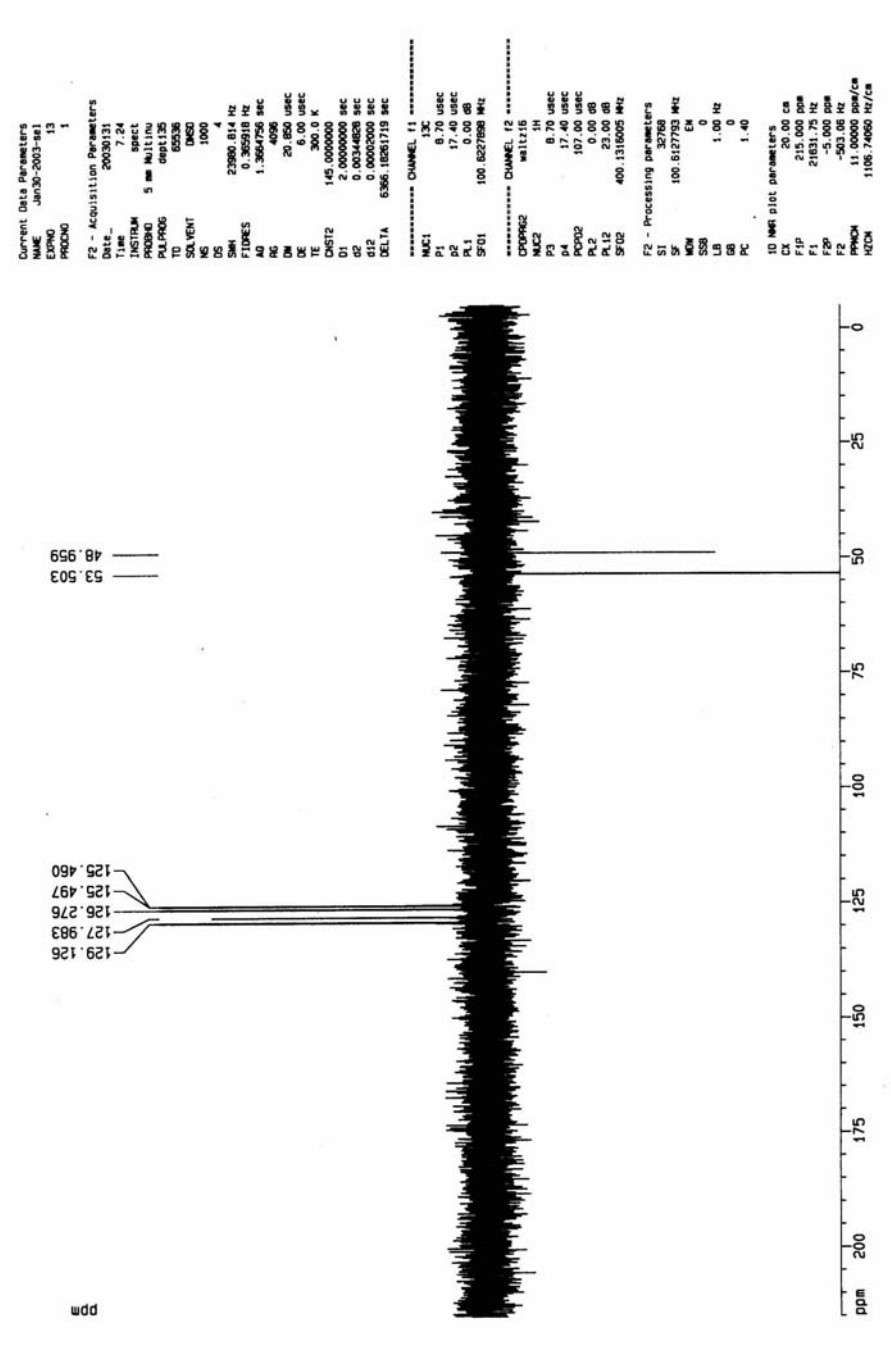

Current Data Parameters
NAME Jan27-2003-sei
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030127
Time 21.15
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 1149.4
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
CHANNEL f1
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SF01 100.6237959 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6126991 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 235.142 ppm
F1 23658.26 Hz
F2P -14.584 ppm
F2 -1467.37 Hz
PPMCM 12.48631 ppm/cm
HZCM 1256.28137 Hz/cm
14.886
50.001
54.814
61.146
77.421
77.738
78.056
124.111
125.666
125.788
125.941
126.632
128.733
129.454
129.575
130.004
132.070
132.380
172.430
ppm

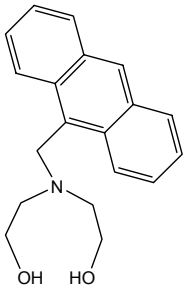

N
O
OH
HO
O

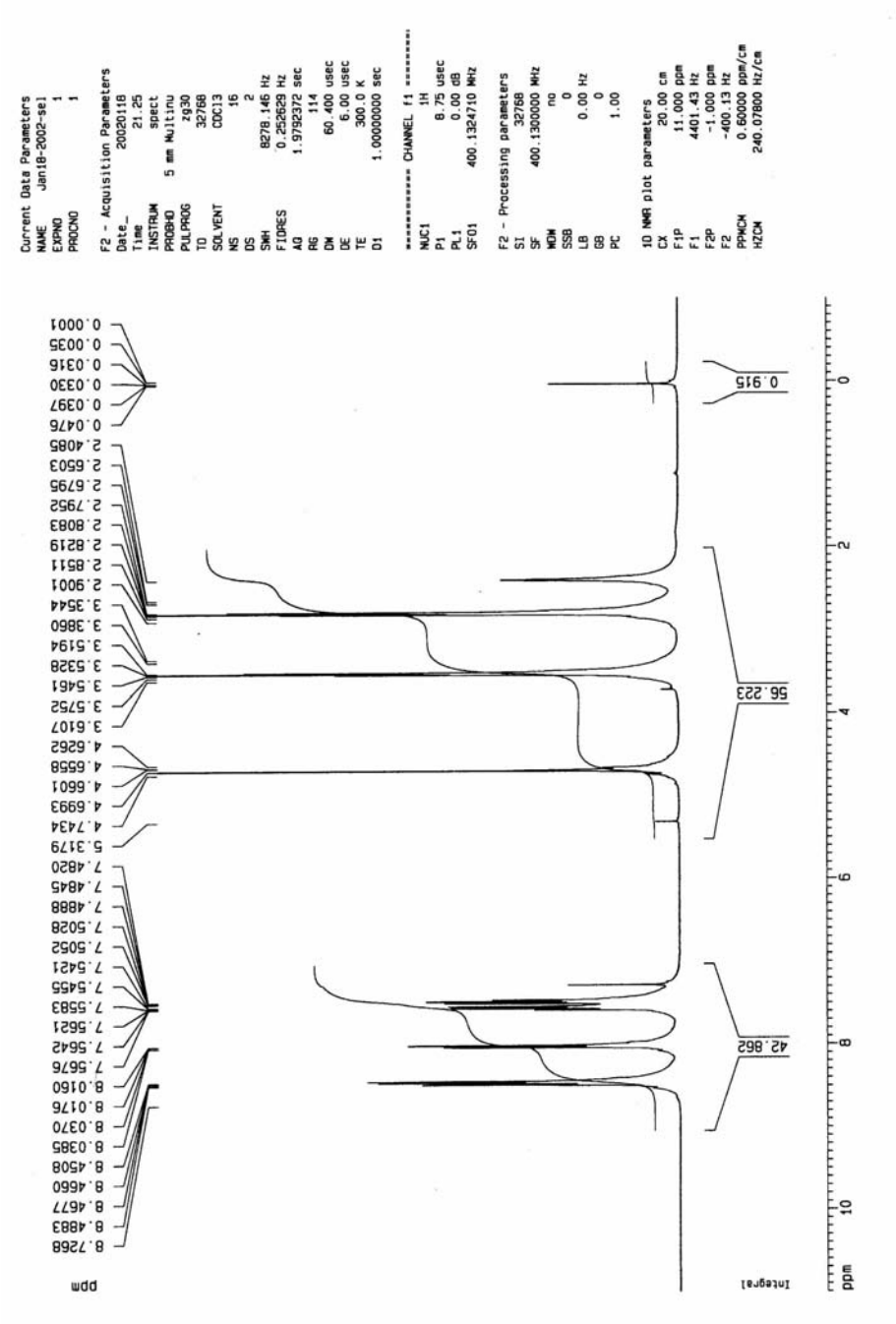

Current Data Parameters
NAME Jan30-2003-sel
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030131
Time 4.36
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 128
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 574.7
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
========== CHANNEL f1 ========
NUC1 1H
P1 8.80 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 13.168 ppm
F1 5268.97 Hz
F2P 1.904 ppm
F2 761.87 Hz
PPMCM 0.56320 ppm/cm
HZCM 225.35515 Hz/cm
2.5004
2.5045
2.5083
2.5441
3.3624
3.4555
3.5062
3.5643
3.6222
3.6670
3.6852
3.8475
4.7920
4.8444
4.8954
5.1374
5.7657
7.5000
7.5128
7.5173
7.5198
7.5284
7.5363
7.5410
7.5453
7.5573
7.5615
7.5776
8.0735
8.0818
8.0879
8.0975
8.1056
8.6019
8.6829
8.7020
8.7056
8.8858
11.4970
11.9424
12.0074
12.0457
12.1194
12.3715
12.5573
12.6328
12.7058
12.7937
ppm
2.6808
1.2667
2.6931
1.3726
0.6719
1.3119
1.0125
Integral
ppm

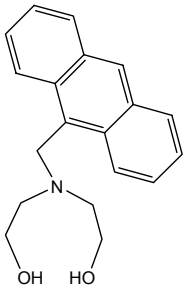

N
O
OH
HO
O
Current Data Parameters
NAME Jan30-2003-sel
EXPNO 12
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030131
Time 6.27
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2000
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
---------- CHANNEL f1 ----------
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SF01 100.6237959 MHz
---------- CHANNEL f2 ----------
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
39.693
39.902
40.111
40.319
40.528
40.737
40.945
49.463
54.003
125.957
125.994
126.772
128.482
129.622
130.101
131.784
131.954
173.541
ppm
0
25
50
75
100
125
150
175
200
ppm

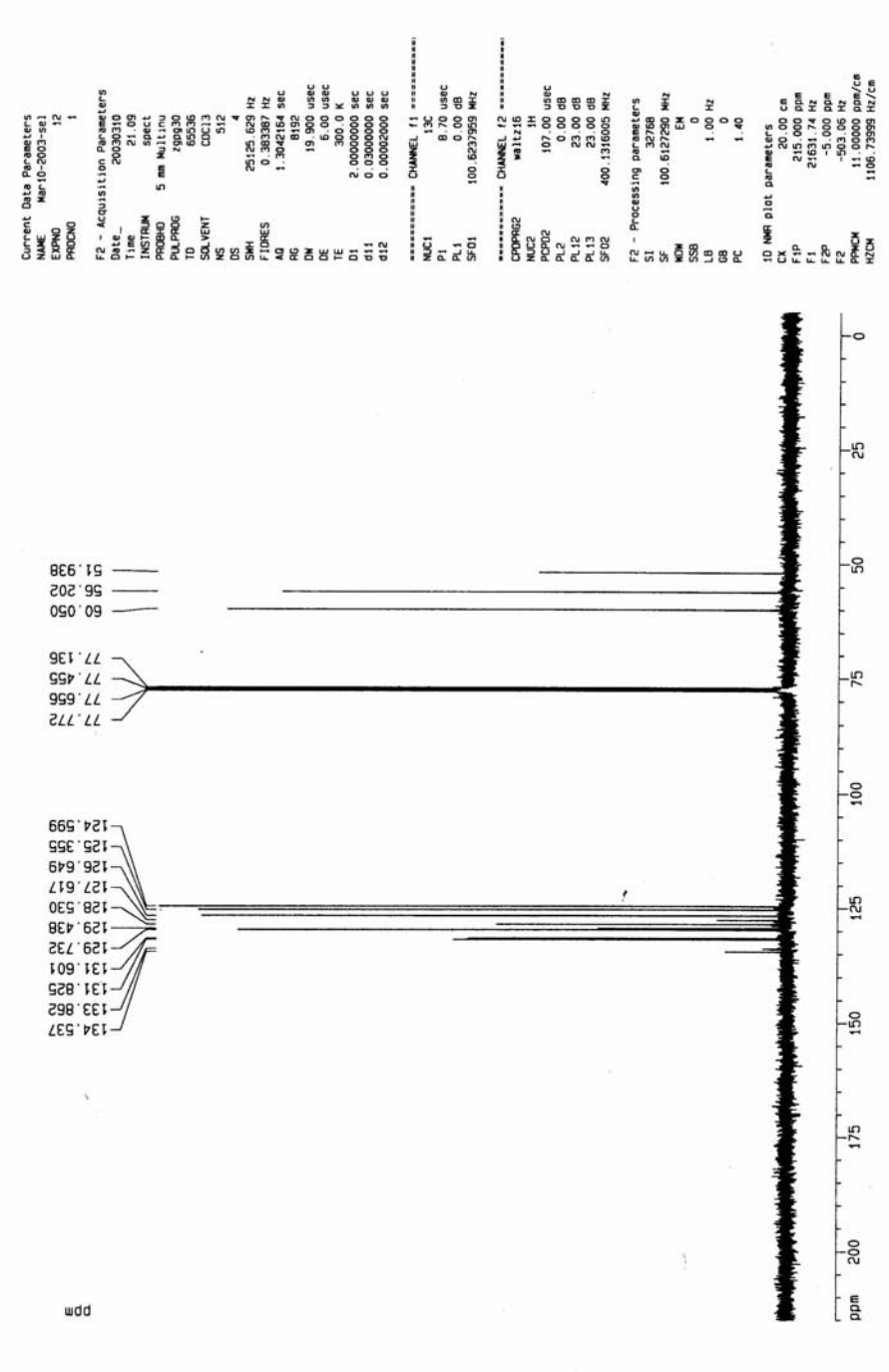

N
O
OH
HO
O


Current Data Parameters
NAME Jan30-2003-sel
EXPNO 13
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030131
Time 7.24
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG dept135
TD 65536
SOLVENT DMSO
NS 1000
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664736 sec
RG 4096
DW 20.850 usec
DE 6.00 usec
TE 300.9 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 6386.18261719 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 8.70 usec
P2 17.40 usec
PL1 0.00 dB
SFO1 100.6227898 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
P3 8.70 usec
P4 17.40 usec
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127703 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.75 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.74060 Hz/cm
48.959
53.503
125.460
125.497
126.276
127.983
129.126
ppm
0
25
50
75
100
125
150
175
200


N
OH
HO


Current Data Parameters
NAME Jan18-2002-sel
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020118
Time 21.25
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.252829 Hz
AQ 1.9792372 sec
RG 114
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
========== CHANNEL f1 ==========
NUC1 1H
P1 8.75 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300000 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 4401.43 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 240.07800 Hz/cm
ppm
Integral
0.915
56.223
42.862


N
OH
HO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NAME Mar10-2003-sel
EXPNO 12
PROCNO 1
F2 - Acquisition Parameters
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Time 21.09
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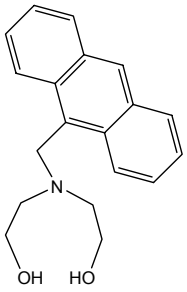
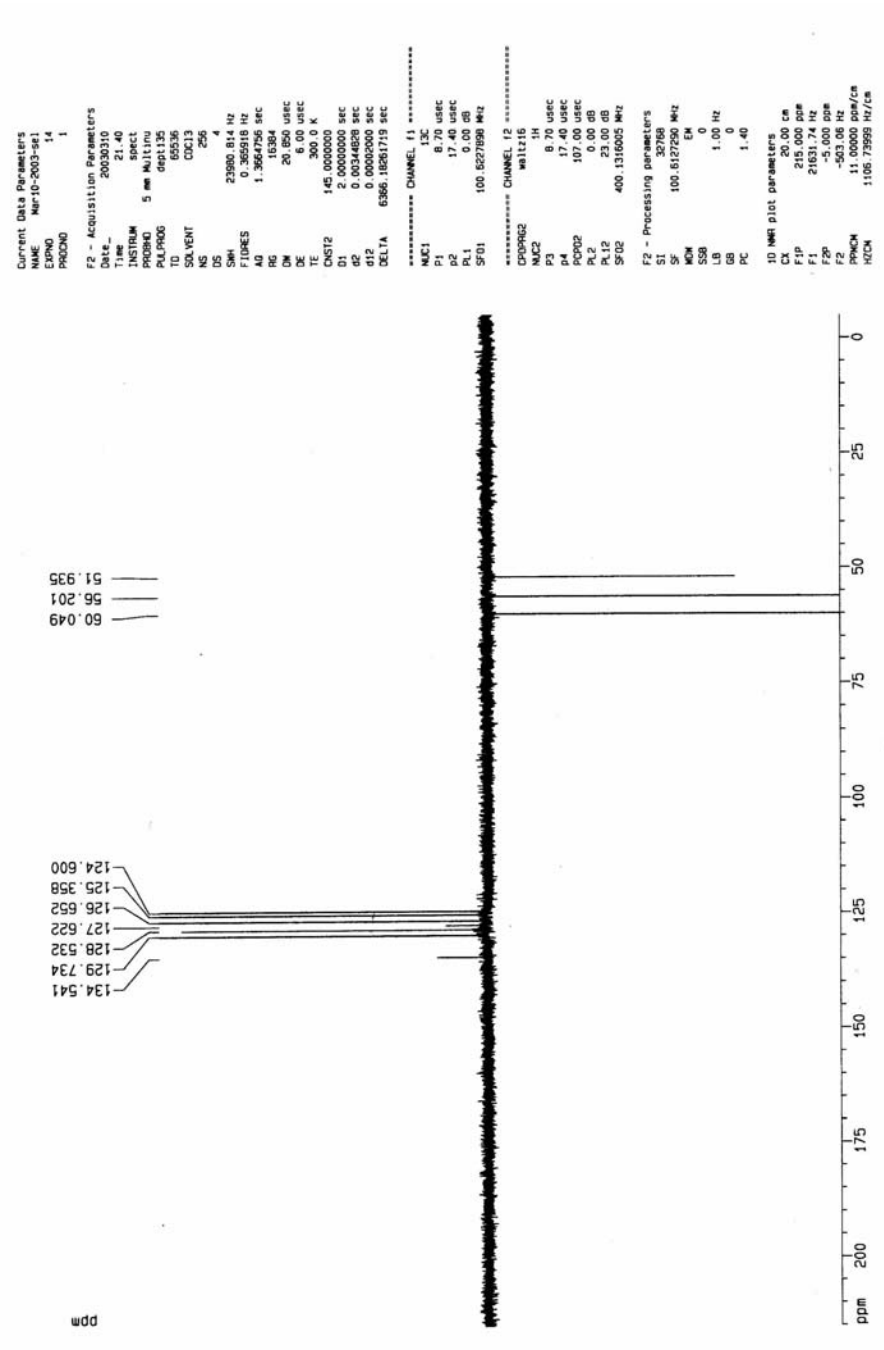
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
CHANNEL f1
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SFO1 100.6237959 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
51.938
56.202
60.050
77.136
77.455
77.656
77.772
124.599
125.355
126.649
127.617
128.530
129.438
129.732
131.601
131.825
133.862
134.537
ppm
0
25
50
75
100
125
150
175
200


N
OH
HO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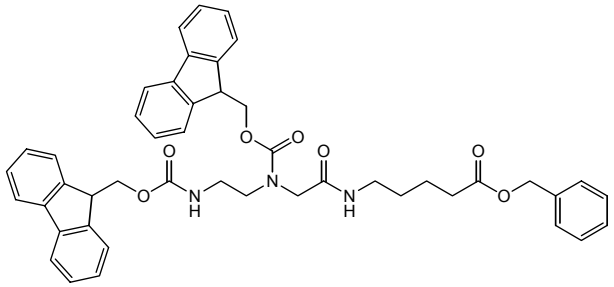
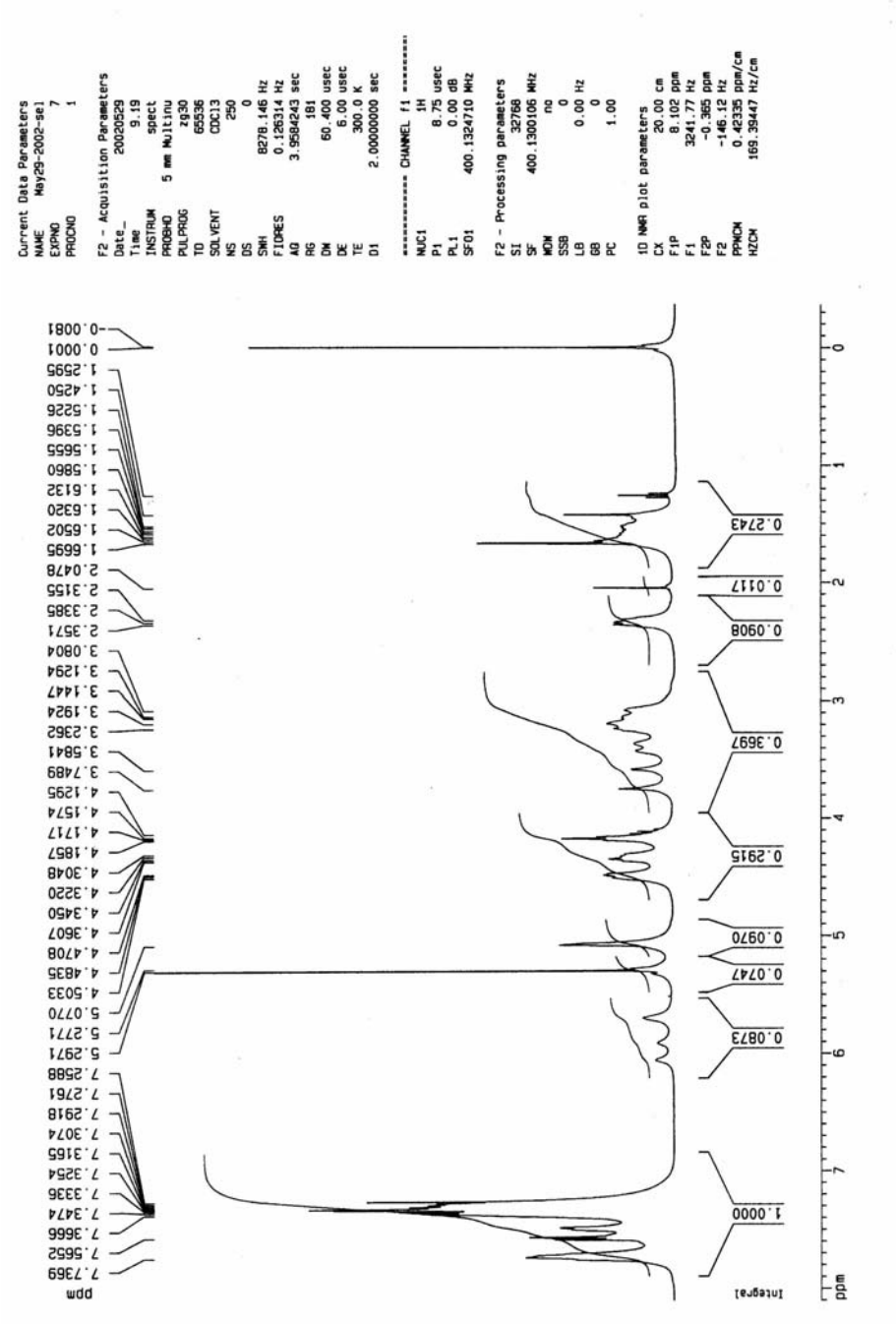
NAME Mar10-2003-sel
EXPNO 14
PROCNO 1
F2 - Acquisition Parameters
Date_ 20030310
Time 21.40
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG deptl35
TD 65536
SOLVENT CDCl3
NS 256
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 16384
DW 20.850 usec
DE 6.00 usec
TE 300.0 K
CNST12 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 6366.18261719 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 8.70 usec
p2 17.40 usec
PL1 0.00 dB
SFO1 100.5227890 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
P3 8.70 usec
P4 17.40 usec
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -563.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
51.935
56.201
60.049
124.600
125.358
126.652
127.622
128.532
129.734
134.541
ppm
0
25
50
75
100
125
150
175
200




Current Data Parameters
NAME May29-2002-sel
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020529
Time 9.19
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 250
DS 0
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9584243 sec
RG 181
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
CHANNEL f1
NUC1 1H
P1 8.75 usec
PL1 0.00 dB
SF01 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300106 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 8.102 ppm
F1 3241.77 Hz
F2P -0.365 ppm
F2 -146.12 Hz
PPMCM 0.42335 ppm/cm
HZCM 169.39447 Hz/cm
ppm
Integral
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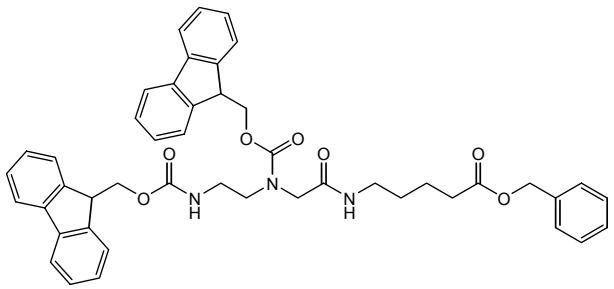
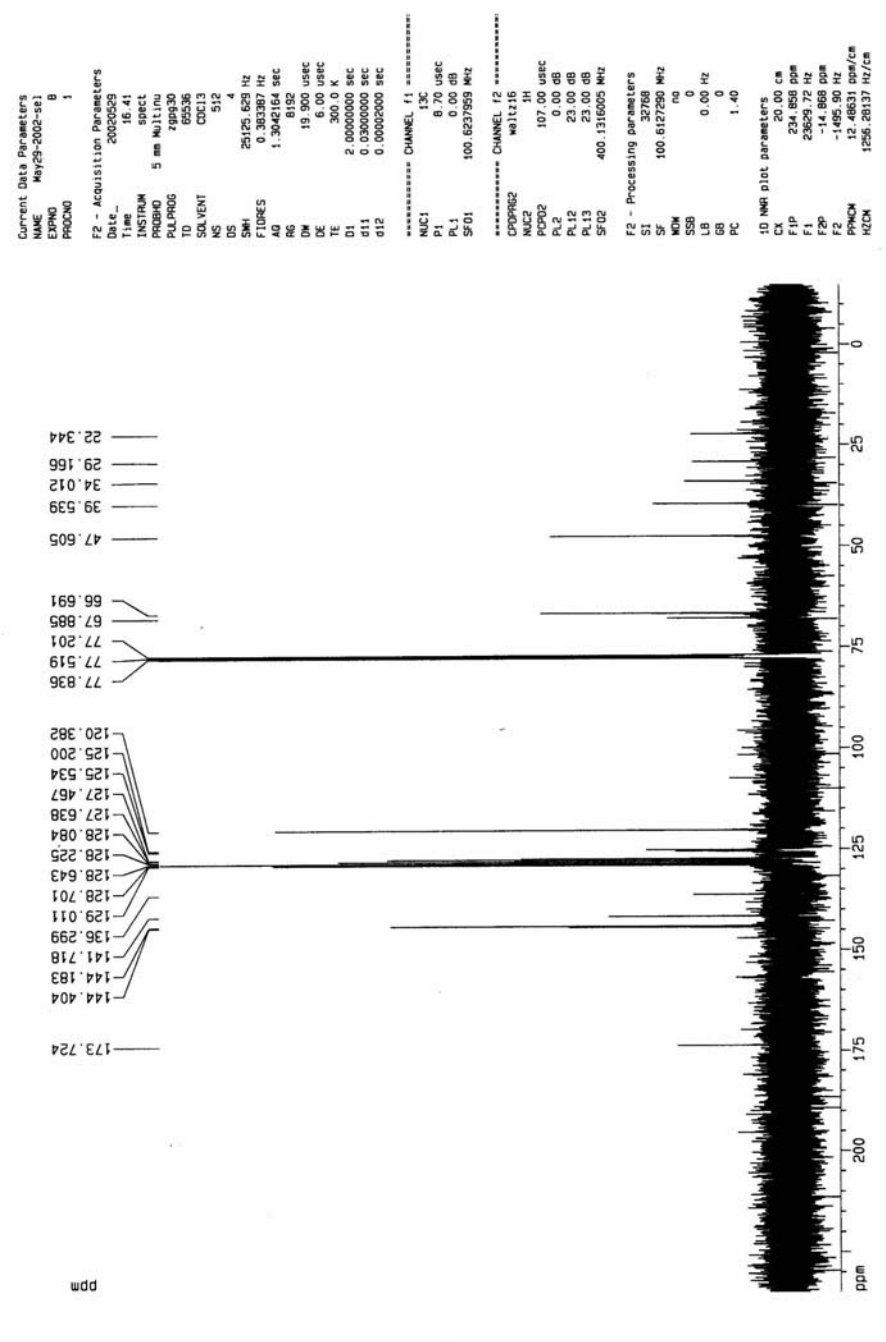
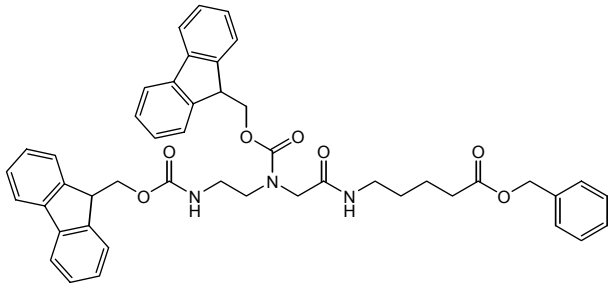
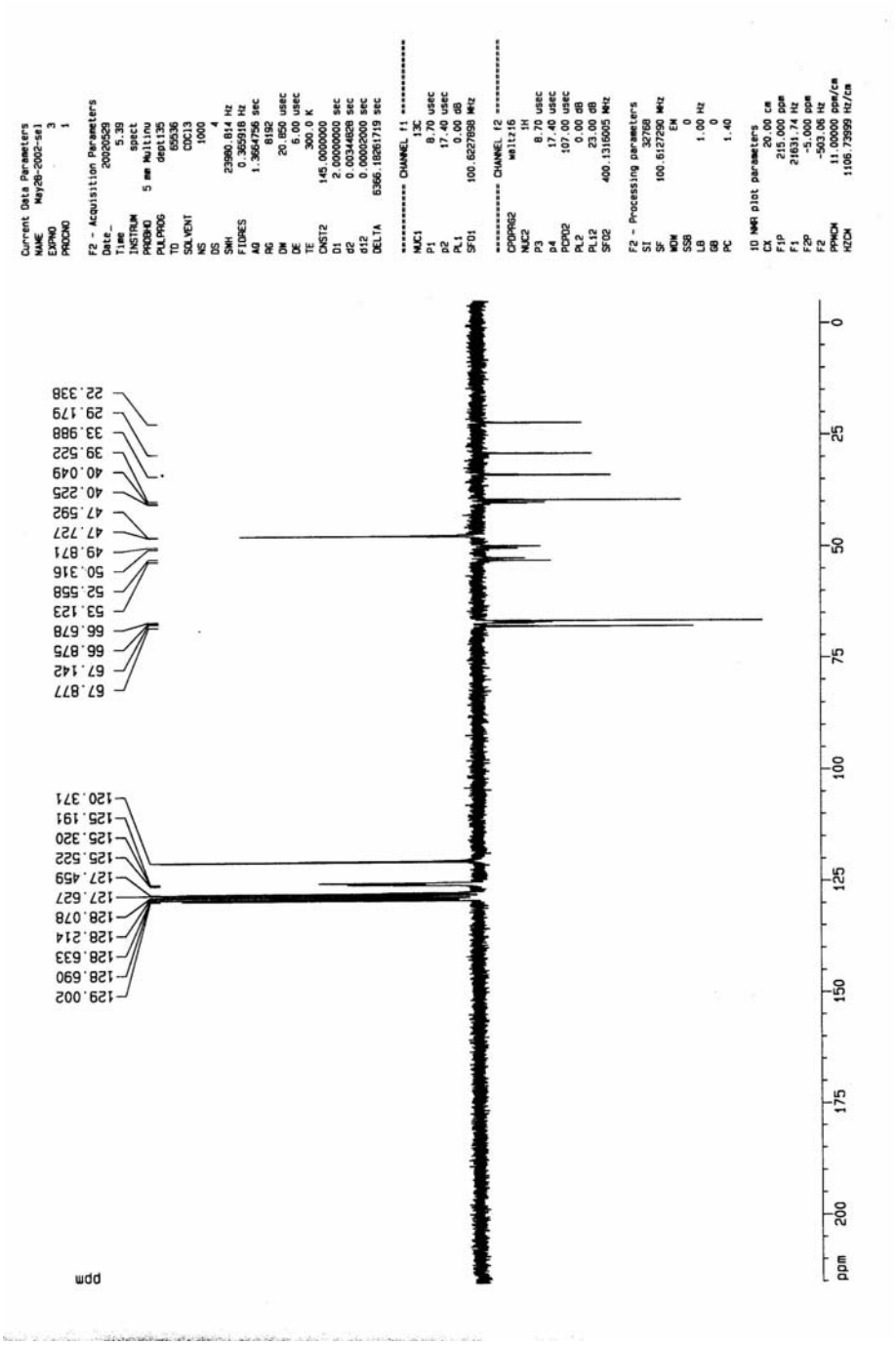
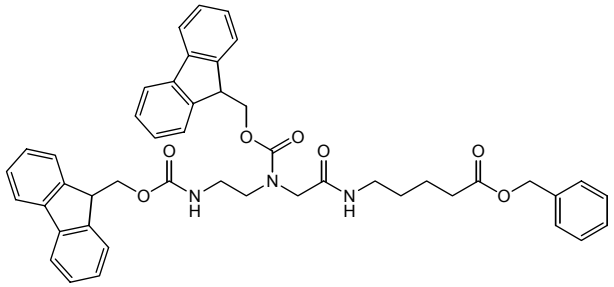
0.0873
0.0747
0.0970
0.2915
0.3697
0.0908
0.0117
0.2743




Current Data Parameters
NAME May29-2002-sel
EXPNO 8
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020529
Time 16.41
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 512
DS 4
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 8192
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SFO1 100.6237959 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 234.858 ppm
F1 23629.72 Hz
F2P -14.868 ppm
F2 -1495.90 Hz
PPMCM 12.48631 ppm/cm
HZCM 1256.28137 Hz/cm
22.344
29.166
34.012
39.539
47.605
66.691
67.885
77.201
77.519
77.836
120.382
125.200
125.534
127.467
127.638
128.084
128.225
128.643
128.701
129.011
136.299
141.718
144.183
144.404
173.724
wdd
ppm

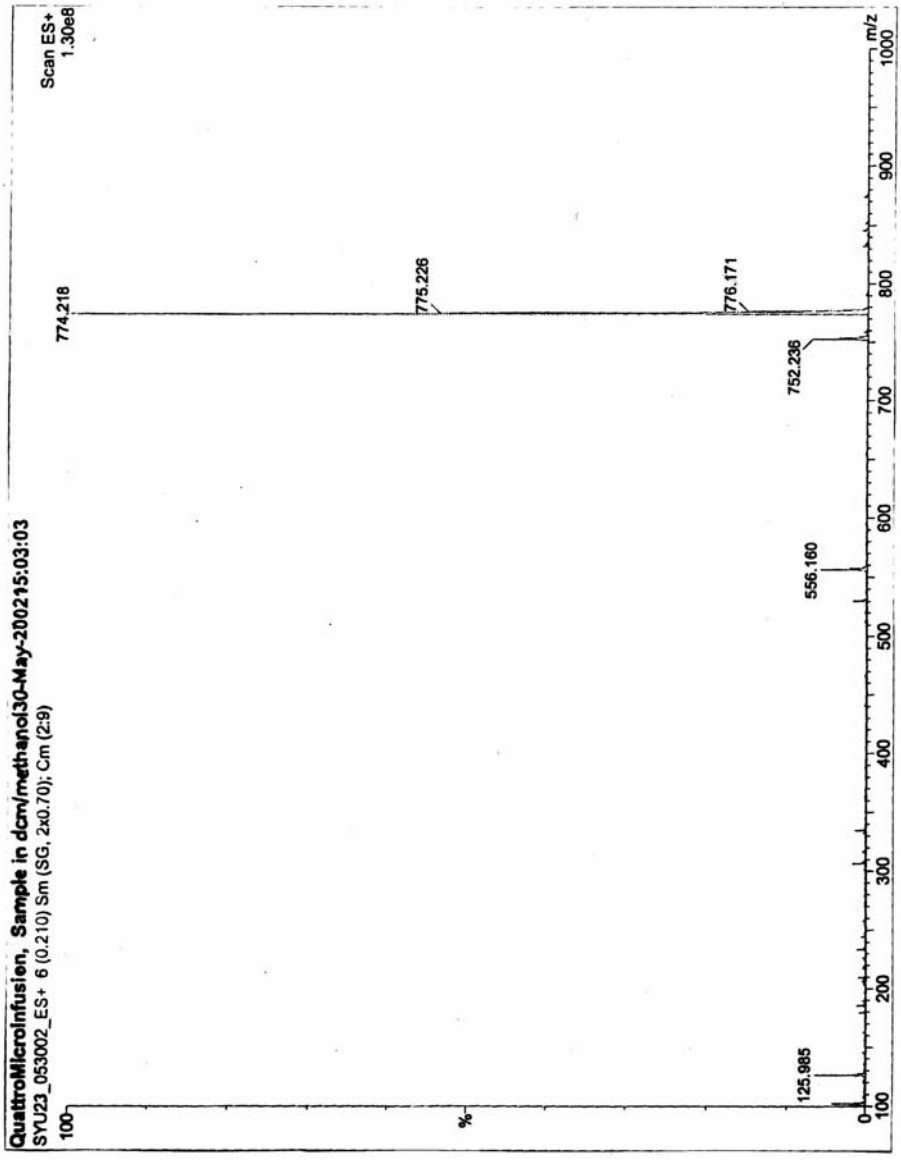


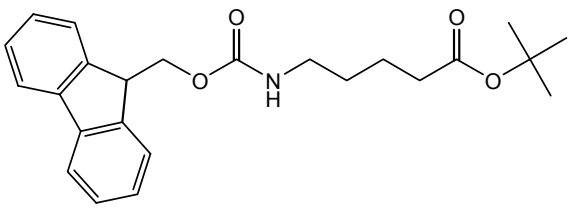

Current Data Parameters
NAME May26-2002-sal
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020529
Time 5.39
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 1000
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 8192
DW 20.850 usec
DE 6.00 usec
TE 300.0 K
CNST2 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 5360.10281719 sec
========== CHANNEL f1 ==========
NUC1 13C
P1 8.70 usec
p2 17.40 usec
PL1 0.00 dB
SF01 100.6227898 MHz
========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
P3 8.70 usec
P4 17.40 usec
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
SF02 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
22.338
29.179
33.988
39.522
40.049
40.225
47.592
47.727
49.871
50.316
52.558
53.123
66.678
66.875
67.142
67.877
120.371
125.191
125.320
125.522
127.459
127.627
128.078
128.214
128.633
128.690
129.002
ppm
0
25
50
75
100
125
150
175
200

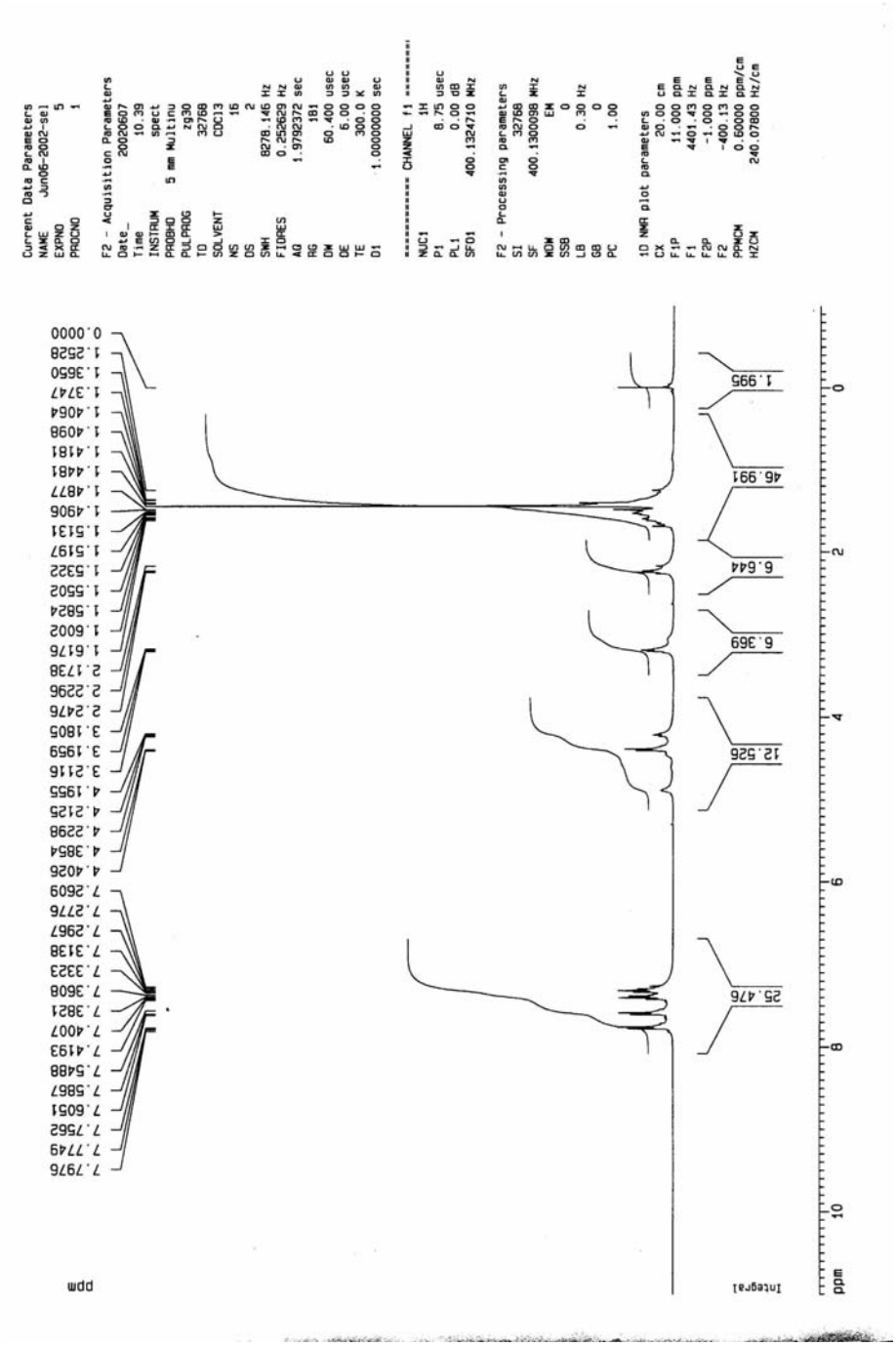


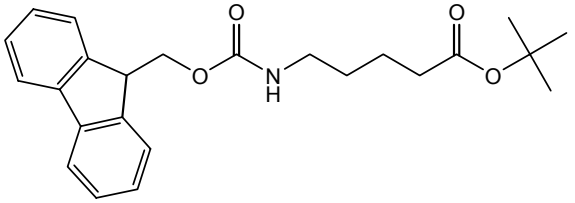
M : Mol. Wt.: 751.87

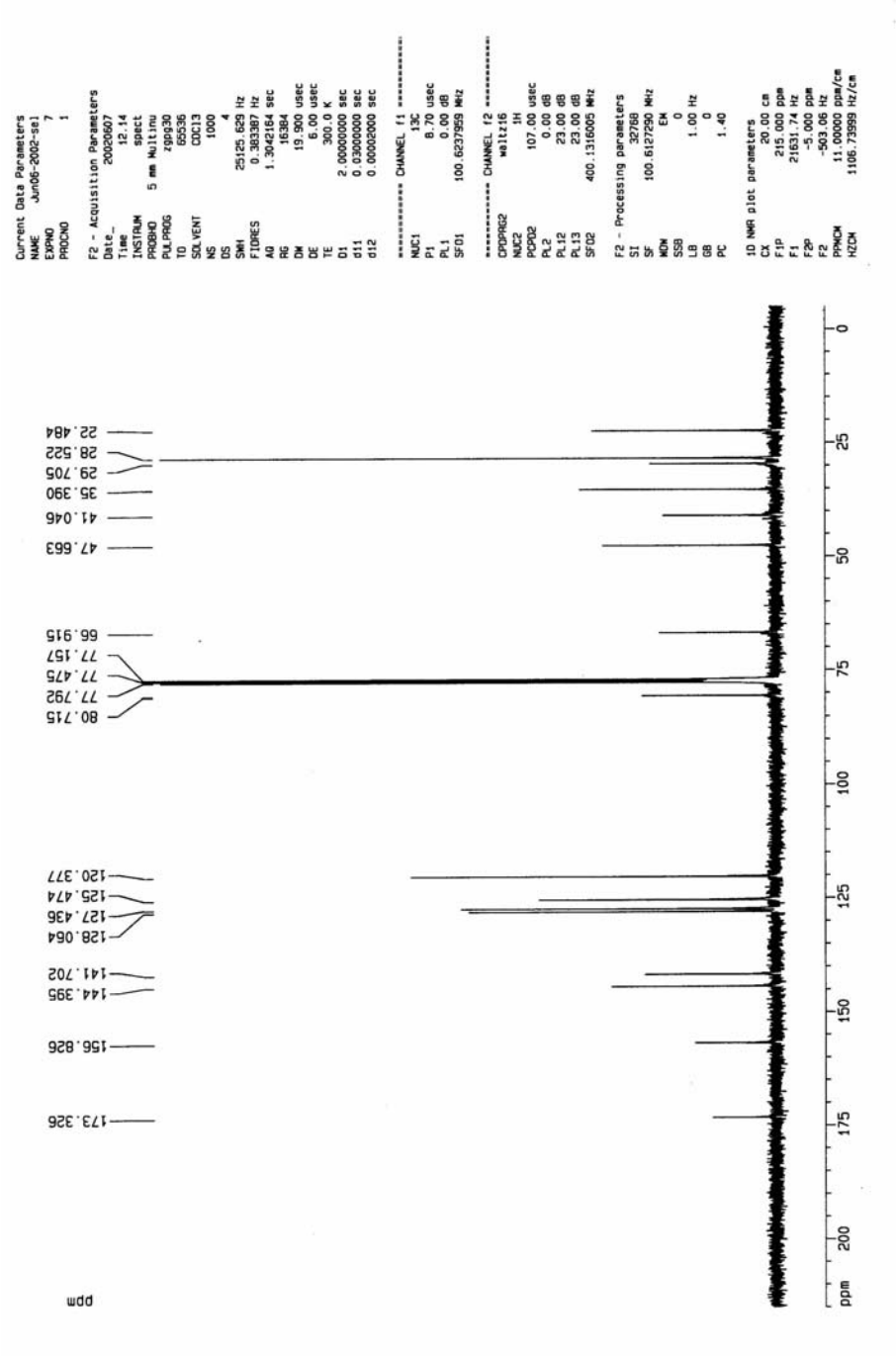
M + Na : Mol. Wt.: 774.86




O
O
O
N
H


Current Data Parameters
NAME Jun06-2002-sel
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020607
Time 10.39
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.252629 Hz
AQ 1.9792372 sec
RG 181
DW 60.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
========== CHANNEL f1 ==========
NUC1 1H
P1 8.75 usec
PL1 0.00 dB
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 32768
SF 400.1300098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 4401.43 Hz
F2P -1.000 ppm
F2 -400.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 240.07800 Hz/cm
0.0000
1.2528
1.3650
1.3747
1.4064
1.4098
1.4181
1.4481
1.4877
1.4906
1.5131
1.5197
1.5322
1.5502
1.5824
1.6002
1.6176
2.1738
2.2296
2.2476
3.1805
3.1959
3.2116
4.1955
4.2125
4.2298
4.3854
4.4026
7.2609
7.2776
7.2967
7.3138
7.3323
7.3608
7.3821
7.4007
7.4193
7.5488
7.5867
7.6051
7.7562
7.7749
7.7976
ppm
Integral
1.995
46.991
6.644
6.369
12.526
25.476
ppm


O
O
O
N
H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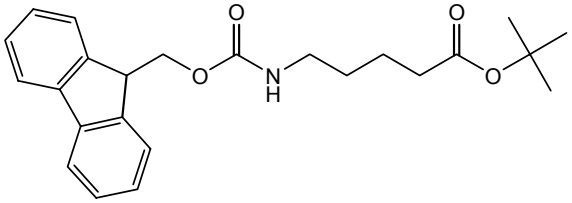
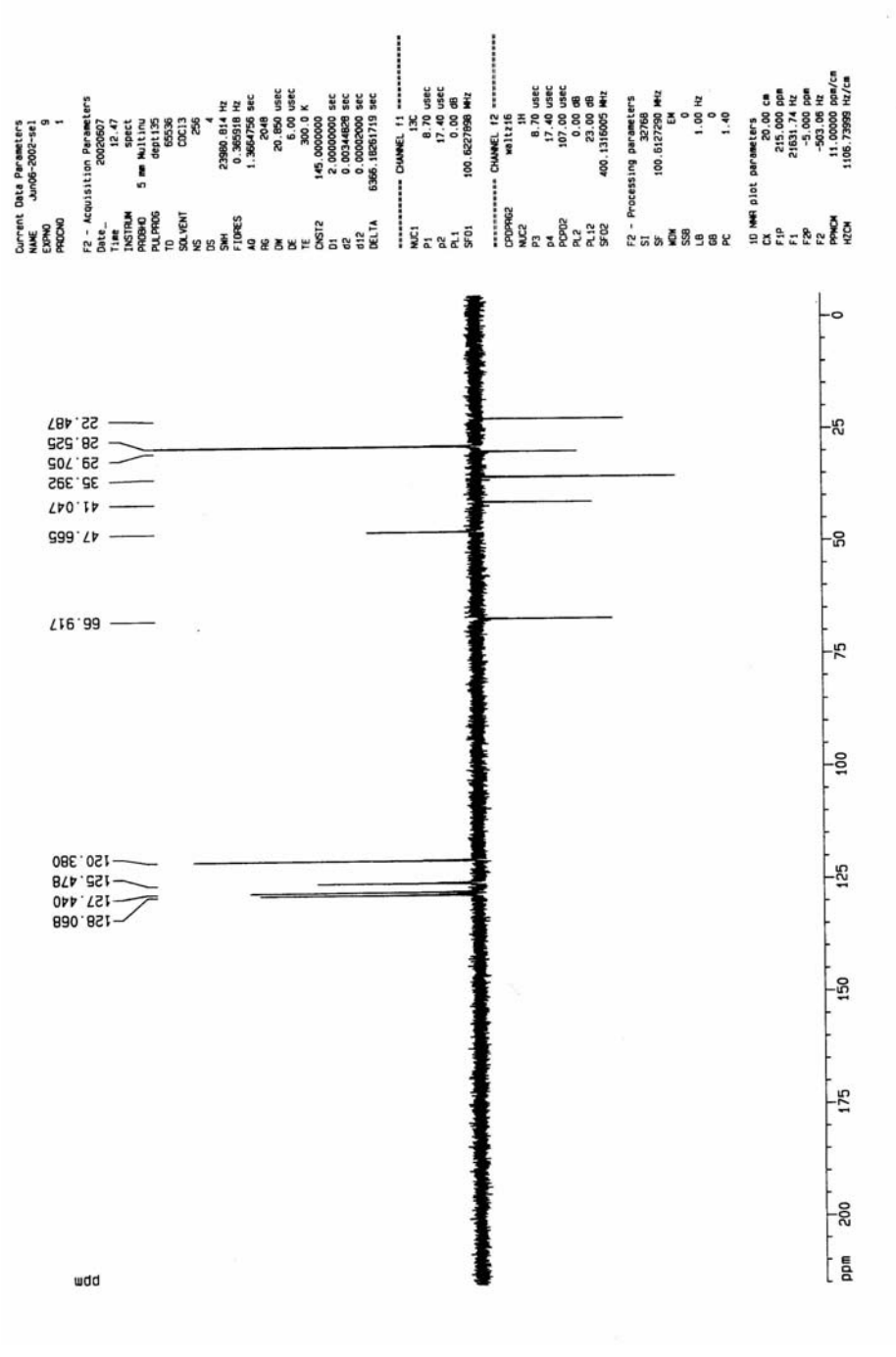
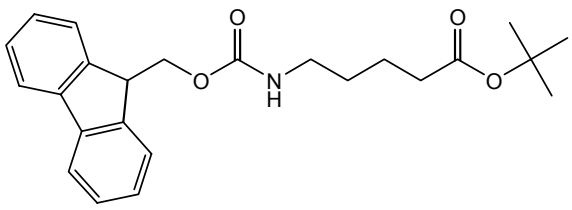
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020607
Time 12.14
INSTRUM spect
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1000
DS 4
SWH 25125.628 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 16384
DW 19.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
CHANNEL f1
NUC1 13C
P1 8.70 usec
PL1 0.00 dB
SFO1 100.6237959 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
PL13 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
22.484
28.522
29.705
35.390
41.046
47.663
66.915
77.157
77.475
77.792
80.715
120.377
125.474
127.436
128.064
141.702
144.395
156.826
173.326
ppm
0
25
50
75
100
125
150
175
200

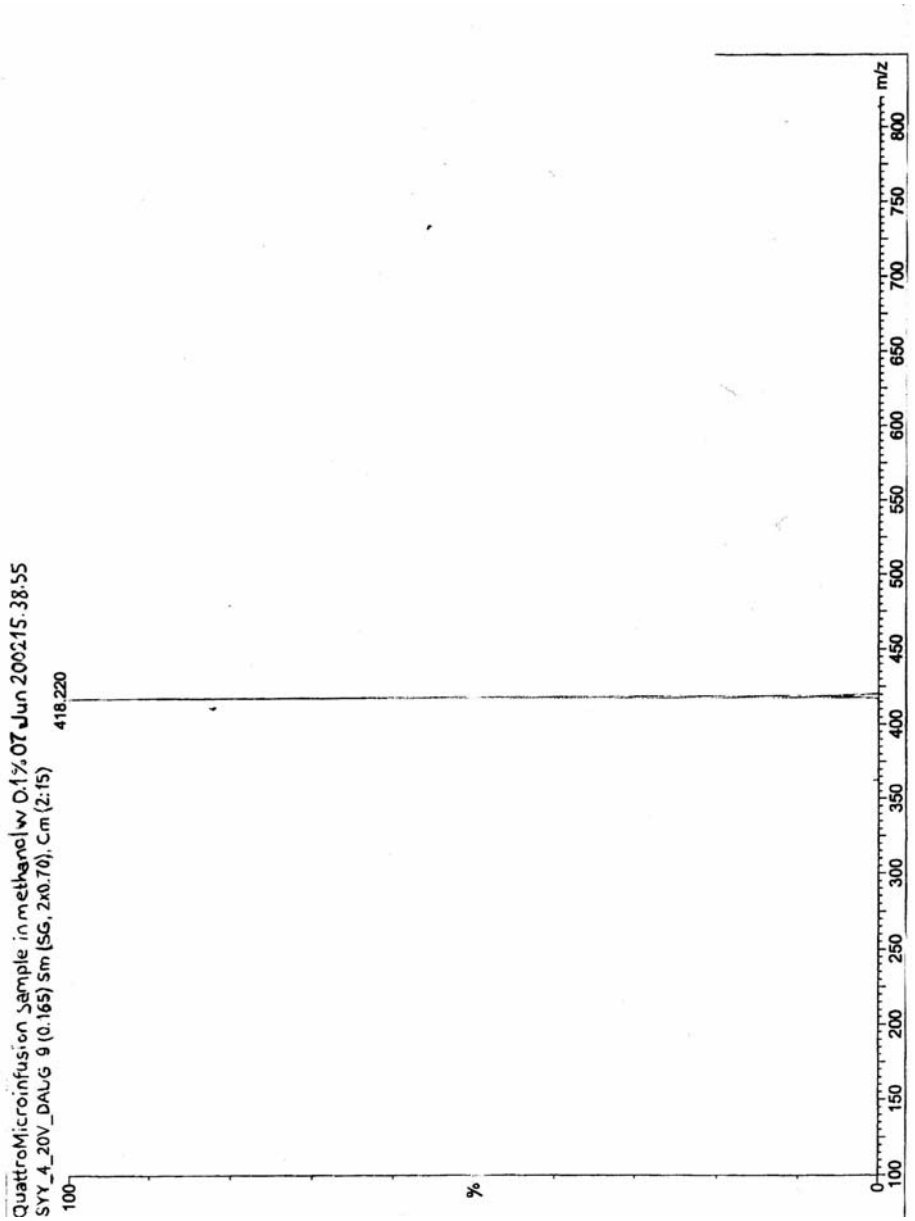

O
O
O
N
H

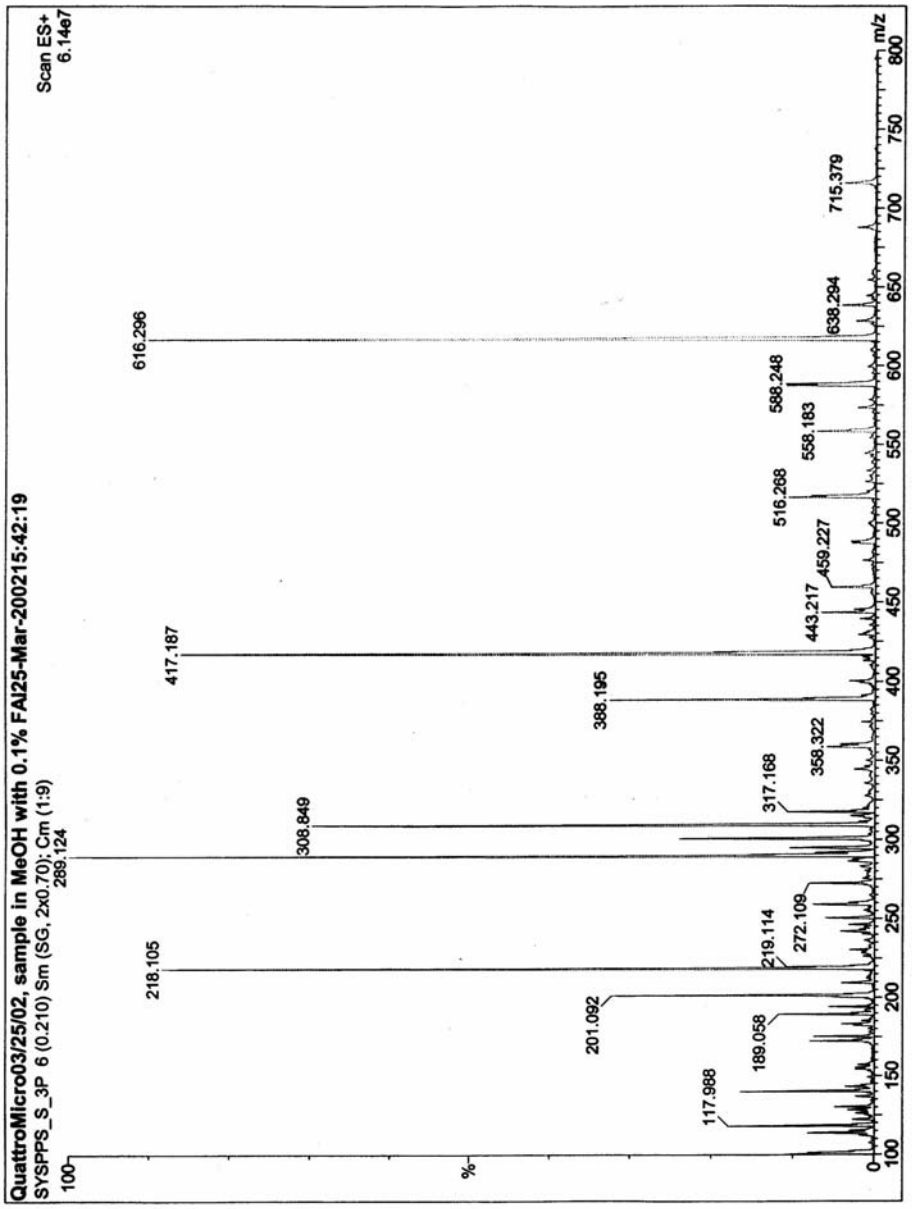

Current Data Parameters
NAME Jun06-2002-sel
EXPNO 9
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020607
Time 12.47
INSTRUM spect
PROBHD 5 mm Nulliu
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 256
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 2048
DW 20.850 usec
DE 6.00 usec
TE 300.0 K
CNST12 145.0000000
D1 2.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 6366.18261719 sec
---------- CHANNEL f1 ----------
NUC1 13C
P1 8.70 usec
p2 17.40 usec
PL1 0.00 dB
SFO1 100.6227898 MHz
---------- CHANNEL f2 ----------
CPDPRG2 waltz16
NUC2 1H
P3 8.70 usec
p4 17.40 usec
PCPD2 107.00 usec
PL2 0.00 dB
PL12 23.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127290 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 215.000 ppm
F1 21631.74 Hz
F2P -5.000 ppm
F2 -503.06 Hz
PPMCM 11.00000 ppm/cm
HZCM 1106.73999 Hz/cm
22.487
28.525
29.705
35.392
41.047
47.665
66.917
120.380
125.478
127.440
128.068
ppm
0
25
50
75
100
125
150
175
200
ppm

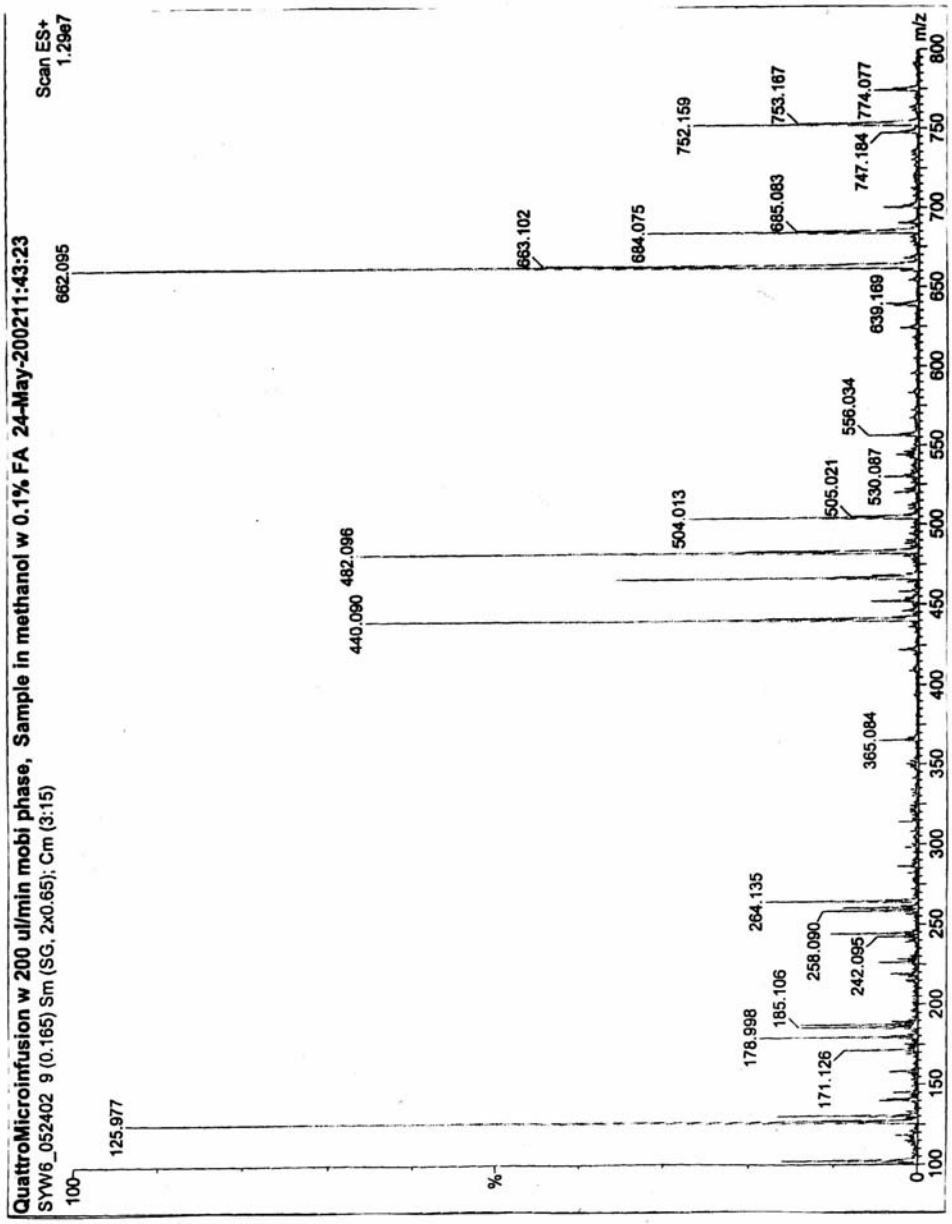


M : Mol. Wt.: 395.49

M + Na : Mol. Wt.: 418.48




QuattroMicro03/25/02, sample in MeOH with 0.1% FAI25-Mar-200215:42:19
SYSPPS_S_3P 6 (0.210) Sm (SG, 2x0.70); Cm (1:9)
Scan ES+
6.14e7
100
%
0
289.124
218.105
308.849
417.187
616.296
201.092
189.058
117.988
219.114
272.109
317.168
358.322
388.195
443.217
459.227
516.268
558.183
588.248
638.294
715.379
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z


QuattroMicroinfusion w 200 ul/min mobi phase, Sample in methanol w 0.1% FA 24-May-200211:43:23
SYW6_052402 9 (0.165) Sm (SG, 2x0.65); Cm (3:15)
Scan ES+
1.29e7
100
%
0
125.977
171.126
178.998
185.106
242.095
258.090
264.135
365.084
440.090
482.096
504.013
505.021
530.087
556.034
639.169
662.095
663.102
684.075
685.083
747.184
752.159
753.167
774.077
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z

REFERENCES:

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[2] Graf, E.; Lehn, J. M. *J. Am. Chem. Soc.* **1975**, *97(17)*, 5022-4

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