Supporting Information

Hydrazone and Hydrazide-Containing *N*-Substitued Glycines as Peptoid Surrogates for Expedited Library Synthesis: Application to the Preparation of Tsg101-Directed HIV-1 Budding Antagonists

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Human Tsg101 UEV Domain *E.coli* Expression and Purification

The human Tsg101 UEV domain with a C-terminal His-Tag (hTsg101-UEV-His) was expressed in *E.coli* using the pET expression system and host strain BL21(DE3)pLysS (Novagen). Briefly, hTsg101-UEV was PCR amplified using the following primers; 5'-GGCCATGGCGGTGTCGGAGAGCCAGC-3' and 5'-CCTCGAGAGGACGAGAGAA GACTGG-3' and cloned into pET21b (Novagen), placing hTsg101-UEV in-frame with a C-terminal His-Tag. Protein expression was induced at $O.D_{600}$ 0.4-0.6 with 1 mM IPTG (Novagen) for 3 hours at 37° C. The resultant bacterial pellet was sonicated in 1 x phosphate buffer containing 20mM Imidazole and Benzoase nuclease (Novagen). The hTsg101-UEV-His protein was purified to approximately 90% purity using the HisTrap HP kit (Amersham Biosciences) (Figure 1). The manufacture's protocol was optimized with a pre-elution wash using 1 x phosphate buffer containing 80 mM imidazole and elution performed with 1 x phosphate buffer containing 300 mM Imidazole. Buffer exchange into PBS and protein concentration was performed using vivaspin 20, 5000 MWCO columns (Vivascience). The purified protein was verified by SDS-PAGE stained with GelCode Blue (Pierce; Figure 1) and protein concentration estimated with the Quick start Bradford protein assay (Bio-Rad).

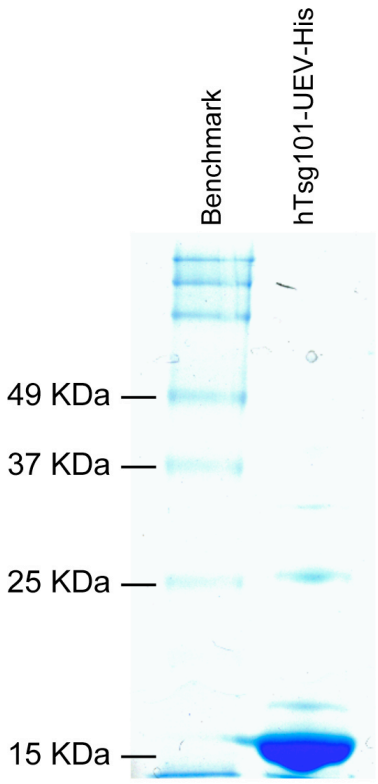


Figure 1. The human Tsg101-UEV domain with a C-terminal His-Tag was expressed in *E.coli* using the pET expression system and host strain BL21(DE3)pLysS (Novagen). Protein purification was performed using the His-tagged HP kit (Amersham Biosciences). The purified and concentrated protein was verified by SDS-PAGE stained with GelCode blue (Pierce).

Fluorescence Anisotropy Assays

Fluorescence anisotropy is based on the observation that when a fluorescent molecule is excited with plane-polarized light, it emits polarized fluorescent light into a fixed plane if the molecules are stationary between excitation and emission.[1] Because molecules are not stationary but tumble and rotate the fluorescence anisotropy of the molecule is proportional to the molecule's molecular volume (if viscosity and temperature are constant). Smaller molecules rotate fast but when they interact with large molecules and there rotation is slowed and the anisotropy of the smaller molecule is increased. This phenomenon can be used to study molecular interactions. The fluorescence anisotropy is a measure of the ratio of the emission intensities from the parallel and perpendicular planes.

Equilibrium binding isotherms for TSG101 binding to peptoids were monitored by fluorescence anisotropy using methods developed previously.[2] Serial dilutions of TSG101 protein were mixed in 96-well Costar polypropylene plates (Corning, NY) with 20 – 50 nM peptoids labeled at the N-terminus with fluorescein in 20 mM HEPES, 150 mM NaCl, pH 7.5, 5 mM β-mercaptoethanol, 100 μM TCEP. After 10min incubation, 2

aliquots of 50 µL were removed and transferred into 384-well Costar polypropylene plates (Corning, NY) and read using a Tecan Ultra plate reader (Durham, NC) with excitation and emission wavelengths at 485 nm and 535 nm, respectively. Curves were fit assuming a single binding site per oligonucleotide, using the relation:

$$An(C) = \frac{\left(\left(R \cdot An_{bound} - An_{free} \right) \cdot \left(\left\{ K_d + C + Lt \right) - \left[\left(K_d + C + Lt \right)^2 - 4 \cdot Lt \cdot C \right]^{1/2} \right\} / 2Lt \right) + An_{free} \right)}{\left(\left(R - 1 \right) \cdot \left(\left\{ K_d + C + Lt \right) - \left[\left(K_d + C + Lt \right)^2 - 4Lt \cdot C \right]^{1/2} \right\} / (2Lt) \right) + 1 \right)} \quad (1)$$

where $An(C)$ is the measured anisotropy as a function of concentration, Lt is the oligonucleotide concentration, An_{free} is the anisotropy of unbound peptoid, An_{bound} is the anisotropy of the bound peptoid solution, R is the ratio of the fluorescence intensity of bound peptoid relative to free peptoid, C is the protein concentration, and K_d is the measured dissociation constant resulting from the fit.[1] A plot of binding data is shown in Figures 2 and 3.

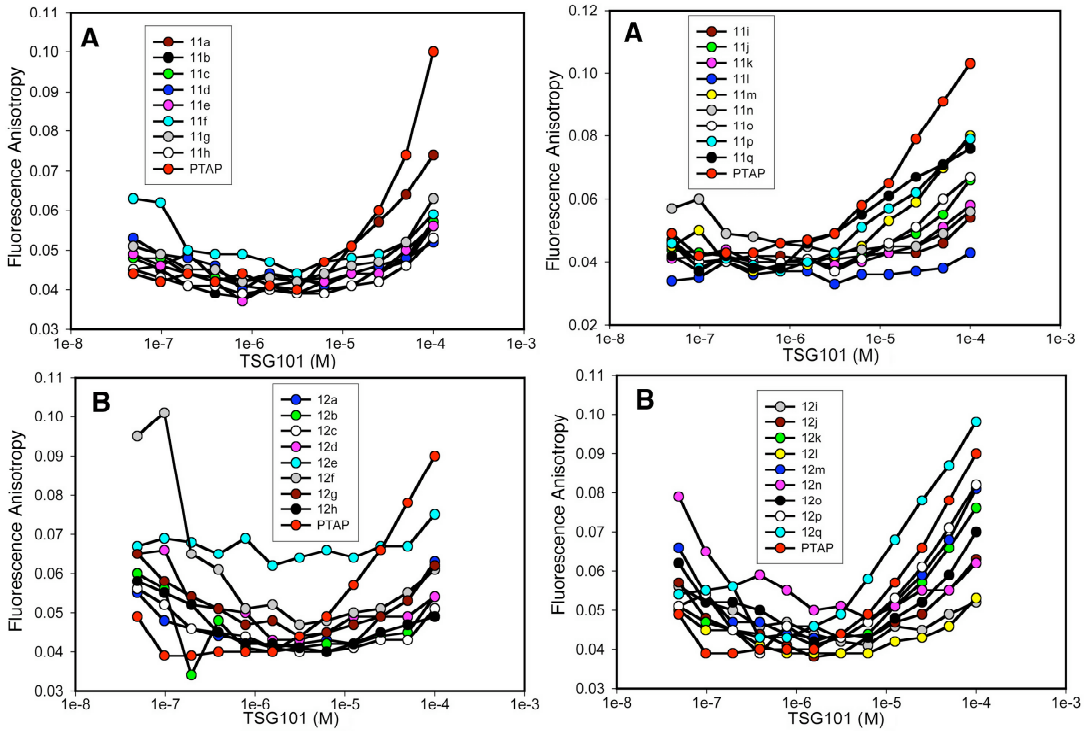


Figure 2. Results of FA assays measuring the binding of peptide analogues to Tsg101 protein: (A) Peptoid hydrazones; (B) Peptoid hydrazides.

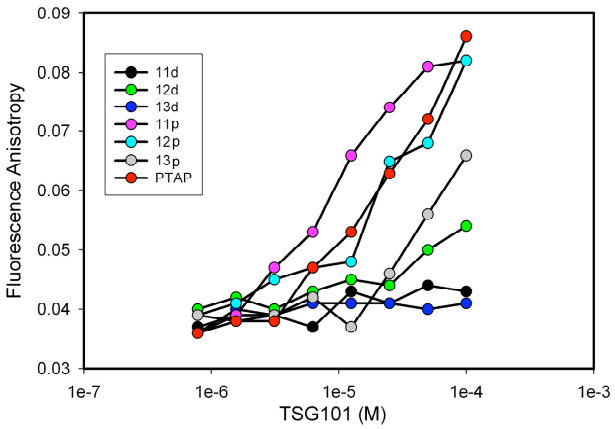
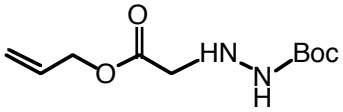


Figure 3. Results of FA assays measuring the binding of peptide analogues to Tsg101 protein.

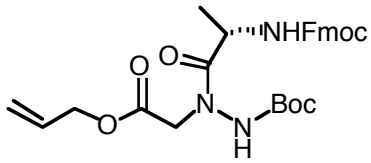
Synthetic Experimental

Bromoacetic acid 2-propenyl ester (6). To a soluton of allyl alcohol (20.5 mL, 0.30 mol), triethylamine (46 mL, 0.33 mol) and 4-dimethylaminopyridine (1.83 g, 1.50 mmol) in dichloromethane (600 mL) at 0° C, was added bromoacetyl bromide (26.0 mL, 0.30 mol) dropwise then the reaction mixture was warmed to room temperature, and stirred overnight. The solution was washed with H_2O (2 x 150 mL) and brine (150 mL), dried (Na_2SO_4) and solvent was removed under vacuum. Distillation (80° C at 3.5 Torr) afforded known **6**[3] as a colorless oil (27.2 g, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (m, 1 H), 5.35 (m, 2 H), 4.67 (m, 2 H), 3.87 (s, 2 H).



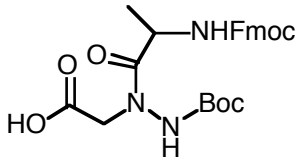
$C_{10}H_{18}N_2O_4$
Exact Mass: 230.13
Mol. Wt.: 230.26

2-[(2'-Propenyloxy)-2-oxoethyl]hydrazinecarboxylic acid 1,1-dimethylethyl ester (7). A mixture of ester **6** (25.0 g, 0.140 mol) and *tert*-butyl carbazate (17.5 g, 0.126 mol) in *N, N*-diisopropylethylamine (150 mL) was stirred at room temperature (48 h). Solvent was removed under vacuum, the residue was dissolved in ethyl acetate (200 mL), washed with H_2O (2 x100 mL) and brine (50 mL), dried (Na_2SO_4) and solvent was removed under vacuum. Purification by silical flash chromatography (hexane and ethyl acetate) afforded **7** as a viscous colorless oil (21.0 g, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (brs, 1 H), 5.92 (m, 1 H), 5.32 (m, 2 H), 4.65 (m, 2 H), 3.70 (s, 2 H), 1.45 (s, 9H). FAB-MS (+VE) *m/z*: 231.1 $(M + H)^+$. HR-MS (NH_3/Cl) $(M + H)^+$: 231.1355, Calc: 231.1350.



$C_{28}H_{33}N_3O_7$
Exact Mass: 523.23
Mol. Wt.: 523.58

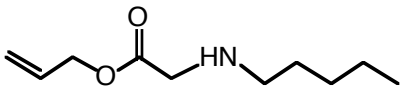
2-[(1,1-Dimethylethoxy)carbonyl]-1-[2-(2'-propenyloxy)-2-oxo-1-ethyl]hydrazide-*N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine (8). To a solution of *N*-Fmoc-L-Ala-OH (5.42 g, 17.4 mmol) in *N,N*-dimethylforamide (30 mL) at 0° C was added 4-methylmorpholine (2.40 mL, 21.7 mmol) followed by isobutyl chloroformate (2.50 mL, 19.1 mmol) and the reaction mixture was stirred at 0° C (10 minutes). A solution of **7** (2.00 g, 8.70 mmol) in *N,N*-dimethylforamide (10 mL) was added then the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was diluted by ethyl acetate (200 mL), washed with H_2O (3 x 50 mL) and brine, dried (Na_2SO_4) and solvent was removed under vacuum. Purification by silical flash chromatography (hexane and ethyl acetate) afforded **8** as a colorless oil (3.60 g, 79% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.80 (brs, 1 H), 7.84 (d, J = 7.2 Hz, 2 H), 7.70 (t, J = 7.4 Hz, 2 H), 7.45 (d, J = 8.4 Hz, 1 H), 7.64 (m, 2 H), 7.28 (t, J = 7.6 Hz, 2 H), 5.85 (m, 1 H), 5.25 (d, J = 15.6 Hz, 1 H), 5.15 (d, J = 10.4 Hz, 1 H), 4.70 – 4.40 (brs, 3 H, rotamers), 4.40 – 3.50 (brs, 5 H, rotamers), 1.50 – 1.30 (m, 9 H), 1.20 (m, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 175.2, 170.6, 169.5, 169.3, 168.1, 155.8, 144.4, 144.2, 141.1, 132.9, 132.6, 128.0, 127.5, 125.7, 120.5, 118.2, 81.0, 79.0, 66.1, 65.4, 64.9, 47.1, 46.9, 28.5, 28.3. FAB-MS (+VE) m/z: 524.2 (M + H)+. HR-FAB (M + Na)$^+$: 546.2255, Calc: 546.2236.



$C_{25}H_{29}N_3O_7$
Exact Mass: 483.20
Mol. Wt.: 483.51

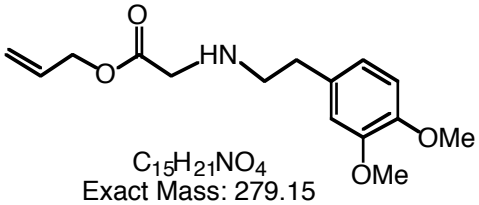
2-[(1,1-Dimethylethoxy)carbonyl]-1-[2-carboxy-1-ethyl]hydrazide-*N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine (9). A solution of dipeptide **8** (3.60 g, 6.88 mmol) in anhydrous tetrahydrofuran (120 mL) was degassed under argon for 5 minutes, then morpholine (3.10 mL, 34.3 mmol) and tetrakis(triphenylphosphine)palladium (0)

(824 mg, 0.71 mmol) was added and the mixture was stirred at room temperature (30 minutes). The reaction mixture was acidified with aqueous HCl (0.10 N, 50 mL) then tetrahydrofuran was removed under vacuum. The residue was extracted with ethyl acetate (2 x100 mL), washed with aqueous HCl (0.10 N, 2 x 50 mL), brine (50 mL) and purified by silical gel flash chromatography (dichloromethane and methanol) to afford a viscous yellow oil. This was dissolved in 50% aqueous acetonitrile (50 mL) and lyophilized to provided **9** as a pale yellow powder in (3.00 g, 90% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.70 (brs, 1 H), 7.83 (d, *J* = 7.6 Hz, 2 H), 7.69 (t, *J* = 8.0 Hz, 2 H), 7.60 – 7.35 (m, 3 H), 7.28 (dt, *J* = 7.6, 1.2 Hz, 2 H), 4.70 – 4.40 (brs, 2 H, rotamers), 4.25 – 4.15 (m, 3 H), 4.00 – 3.40 (m, 1 H, rotamers), 1.50 – 1.30 (m, 9 H), 1.18 (m, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 175.0, 170.3, 155.8, 155.0, 144.4, 144.2, 141.1, 133.5, 132.5, 132.0, 129.2, 129.1, 128.1, 127.5, 125.8, 120.5, 81.0, 66.2, 50.5, 47.1, 46.8, 28.3. FAB-MS (-VE) *m/z*: 482.1 $(M - H)^{-}$. HR-FAB $(M + Na)^{+}$: 506.1907, Calc: 506.1905.



$C_{10}H_{19}NO_2$
Exact Mass: 185.14
Mol. Wt.: 185.26

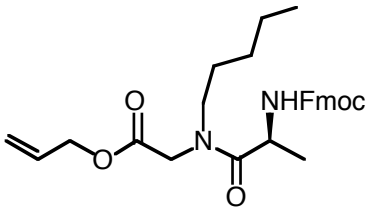
Pentylamine-acetic acid allyl ester (14a). To a solution of Bromoacetic acid 2-propenyl ester **6** (2.0 g, 11.2 mmol) in dichloromethane (50 mL), was added triethylamine (3.2 mL, 22.4 mmol) and pentylamine (1.94 mL, 16.8 mmol). The mixture was stirred overnight and diluted by ethyl acetate (150 mL), washed by H_2O (50 mL x 2) and brine (50 mL), dried over sodium sulfate and purified by silical gel column (hexane and ethyl acetate) to afford 1.40 g pale yellow oil in 63% yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (m, 1 H), 5.30 (m, 2 H), 4.63 (dt, *J* = 5.6, 1.2 Hz, 2 H), 3.44 (s, 2 H), 2.60 (t, *J* = 7.2 Hz, 2 H), 1.65 (brs, 1 H), 1.50 (m, 2 H), 1.34 – 1.29 (m, 4 H), 0.90 (m, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 172.3, 133.0, 118.0, 64.7, 50.7, 49.1, 29.6, 29.4, 22.5, 14.3. FAB-MS (+VE) *m/z*: 186.2 $(M + H)^{+}$. HR-MS (NH_3/Cl) $(M + H)^{+}$: 186.1482, Calc: 186.1488.



$C_{15}H_{21}NO_4$
Exact Mass: 279.15
Mol. Wt.: 279.33

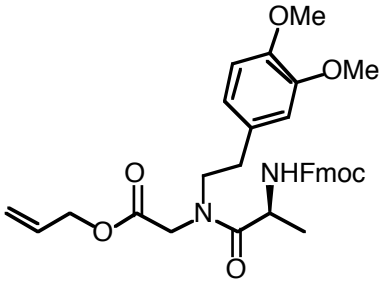
(3,4-Dimethoxy-phenethylamino)-acetic acid allyl ester (14b). Nuclearphilic replacement of **6** to **14b** as reported above for conversion **6** to **14a** yielded the XX in 86% yield as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 – 6.75 (m, 3 H), 5.90 (m, 1 H), 5.26 (m, 2 H), 4.62 (dt, *J* = 5.6, 1.2 Hz, 2 H), 3.88 (s, 3 H), 3.86 (s, 3 H), 3.46 (s, 2

H), 2.88 (m, 2 H), 2.77 (t, J = 7.0 Hz, 2 H), 1.80 (brs, 1 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 172.3, 149.1, 147.5, 133.1, 120.8, 118.1, 112.9, 112.3, 64.8, 55.9, 55.7, 50.7, 50.5, 35.8. FAB-MS (+VE) *m/z*: 280.3 $(M + H)^+$. HR-MS (NH_3/Cl) $(M + H)^+$: 280.1555, Calc: 280.1552.



$C_{28}H_{34}N_2O_5$
Exact Mass: 478.25
Mol. Wt.: 478.58

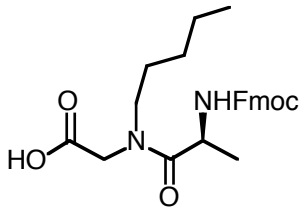
***N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine-N-pentyl-glycine allyl ester (15a).** Coupling **14a** with Fmoc-Ala-OH as reported above for conversion **7** to **8** yielded the product in 42% yield as a white wax.^{1}H NMR (400 MHz, DMSO-d6) δ 7.83 (d, J = 7.6 Hz, 2 H), 7.70 – 7.60 (m, 3 H), 7.36 (t, J = 7.6 Hz, 2 H), 7.27 (t, J = 7.6 Hz, 2 H), 5.84 (m, 1 H), 5.23 (dt, J = 17.2, 1.6 Hz, 1 H), 5.16 (m, 1 H), 4.55 – 4.45 (m, 3 H, rotamers), 4.30 – 4.10 (m, 4.2 H, rotamers), 3.85 (AB, J_{AB} = 17.2 Hz, 0.8 Hz), 3.35 – 3.20 (m, 2 H), 1.53 (m, 1.3 H, rotamers), 1.35 (m, 0.7 H, rotamers), 1.25 – 1.05 (m, 7 H), 1.05 – 0.80 (m, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 173.2, 173.0, 170.0, 169.3, 156.0, 144.2, 141.1, 132.8, 128.0, 127.4, 125.7, 120.5, 118.1, 66.2, 65.1, 48.7, 48.2, 47.1, 46.6, 28.8, 28.6, 22.3, 18.2, 14.3. FAB-MS (+VE) *m/z*: 479.5 $(M + H)^+$. HR-FAB $(M + Na)^+$: 501.2411, Calc: 501.2388.



$C_{33}H_{36}N_2O_7$
Exact Mass: 572.25
Mol. Wt.: 572.65

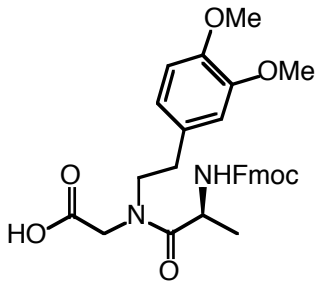
***N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine-N-(3,4-Dimethoxy-phenenthyl)-glycine allyl ester (15b).** Coupling **14b** with Fmoc-Ala-OH as reported above for conversion **7** to **8** yielded the product in quantitive yield as a white wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2 H), 7.59 (d, J = 7.6 Hz, 2 H), 7.38 (m, 2 H), 7.29 (m, 2 H), 6.80 – 6.69 (m, 3 H), 5.95 – 5.85 (m, 2 H), 5.26 (m, 2 H), 4.67 – 4.60 (m, 3 H), 4.40 – 4.30 (m, 3 H), 4.20 (m, 1 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.73 – 3.52 (m, 3 H), 2.88 (t, J = 4.8 Hz, 1.3 H, rotamers), 2.80 (t, J = 4.8 Hz, 0.7 H, rotamers), 1.46 (d, J

= 6.8 Hz, 0.5 H, rotamers, 1.33 (d, J = 6.8 Hz, 2.5 H). FAB-MS (+VE) *m/z*: 573.3 $(M + H)^+$. HR-FAB $(M + Na)^+$: 595.2474, Calc: 595.2447.



$C_{25}H_{30}N_2O_5$
Exact Mass: 438.22
Mol. Wt.: 438.52

***N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine-N-pentyl-glycine (16a).** Deprotection of **15a** as reported above for conversion **8** to **9** yielded the product in 82% yield as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.83 (d, J = 7.6 Hz, 2 H), 7.69 (m, 2 H), 7.59 (m, 1 H), 7.36 (t, J = 7.2 Hz, 2 H), 7.28 (t, J = 7.6 Hz, 2 H), 4.48 – 4.36 (m, 1 H, rotamers), 4.25 – 4.13 (m, 3.3 H, rotamers), 4.08 – 3.94 (m, 1 H, rotamers), 3.69 (AB, J_{AB} = 17.2 Hz, 0.7 H, rotamers), 3.35 – 3.10 (m, 2 H), 1.52 (m, 1.2 H, rotamers), 1.36 (m, 0.8 Hz, rotamers), 1.25 – 1.05 (m, 7 H), 0.79 (m, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 172.9, 171.7, 171.0, 156.0, 144.2, 141.1, 133.6, 132.5, 131.9, 129.1, 128.0, 127.5, 125.7, 120.4, 66.2, 48.5, 48.0, 47.2, 46.6, 28.8, 28.4, 22.3, 18.2, 14.3. FAB-MS (-VE) *m/z*: 437.1 $(M - H)^-$. HR-FAB $(M + Na)^+$: 461.2029, Calc: 461.2041.



$C_{30}H_{32}N_2O_7$
Exact Mass: 532.22
Mol. Wt.: 532.58

***N*-[(9H-fluorenyl-9-ylmethoxy)carbonyl]-L-alanine-N-(3,4-Dimethoxy-phenenthyl)-glycine (16b).** Deprotection of **15b** as reported above for conversion **8** to **9** yielded the product in 86% yield as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.84 (d, J = 7.2 Hz, 2 H), 7.70 – 7.62 (m, 3 H), 7.36 (t, J = 7.6 Hz, 2 H), 7.28 (m, 2 H), 6.87 – 6.65 (m, 3 H), 4.46 (m, 1 H), 4.32 – 4.13 (m, 4 H, rotamers), 4.04 – 3.85 (m, 1 H, rotamers), 3.67 – 3.63 (m, 6 H), 3.47 (m, 1.6 H, rotamers), 3.32 (m, 0.4 H, rotamers),

2.78 (m, 1.4 H, rotamers), 2.59 (m, 0.6 H, rotamers), 1.10 (d, J = 6.8 Hz, 3 H). ^{13}C NMR (400 MHz, DMSO-d6) δ 174.9, 173.0, 171.7, 171.1, 156.1, 149.4, 147.8, 144.3, 141.1, 133.6, 132.5, 132.0, 131.4, 129.2, 128.1, 127.5, 125.7, 121.2, 120.5, 113.2, 113.0, 112.2, 66.2, 55.8, 50.5, 50.0, 48.5, 47.0, 46.6, 35.0, 18.2. FAB-MS (-VE) *m/z*: 531.3 $(M - H)^-$. HR-FAB $(M + H)^+$: 533.2302, Calc:533.2295.

General procedure for solid-phase peptide synthesis. Peptides and peptoids were synthesize on NovaSyn®TGR resin (purchased from Novabiochem, cat. no. 01-64-0060) using standard Fmoc solid-phase protocols using active ester coupling (1-hydroxybenzotriazole (HOBT) and *N, N'*-diisopropylcarbodiimide (DIC) as coupling reagents, once, 2 hours) for primary amines and bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP) (double coupling, 2 hours each) for the secondary amines. All peptides and peptoids were tagged with FITC for fluorescence anisotropy assays by reacting with 5 equivalents of fluorescein isothiocyanate in the presence of 5 equivalents of *N, N*-diisopropylethylamine in *N, N*-dimethylforamide (overnight). The final resin was washed with *N, N*-dimethylforamide, methanol, dichloromethane and ether then dried under vacuum (overnight). Peptides and peptoids was cleaved from resin (200 mg) by treatment with 5 mL of trifluoroacetic acid : triisbutylsilane : H_2O (90 : 5 : 5) (4 h). The resin was filtered off and the filtrate was concentrated under vacuum, then precipitated with ether and the precipitate washed with ether. The resulting solid was dissolved in 50% aqueous acetonitrile 5 mL) and purified by reverse phase preparative HPLC using a Phenomenex C_{18} column (21 mm dia x 250 mm, cat. no: 00G-4436-P0) with a linear gradient from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 50% acetonitrile (0.1% trifluoroacetic acid) over 35 minutes at a flow rate of 10.0 mL/minute (detection at 254 nm). Lyophilization provided products as yellow powders.

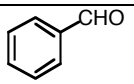
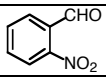
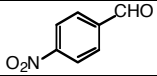
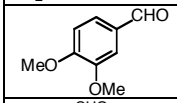
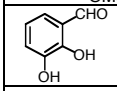
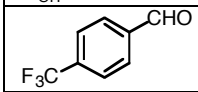
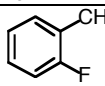
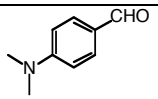
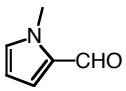
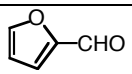
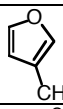
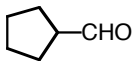
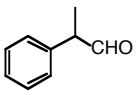
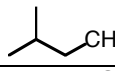
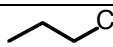
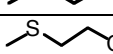
Synthesis of peptide hydrazones 11a – 11r. To a solution of unsubstitued peptide hydrazide **10** (1.0 mg, 0.00070 mmol) in 50% aqueous acetonitrile (0.5 mL) was added the appropriate aldehyde (10 equivalents) and the mixture was gently agitated at room temperature (overnight). The product mixture was subjected directly to purificaiton by reverse phase preparative HPLC using a VYDAC Protein and Peptide C_{18} column (10 mm dia x 250 mm; catalog no. 218TP510) with a linear gradient of from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 50% acetonitrile (0.1% trifluoroacetic acid) over 20 minutes at a flow rate of 8.0 mL/minute (detection at 254 nm). Lyophilization provided the peptoid hydrazones **11a** – **11q** as yellow powders (mass spectral data, Table S1).

Reduction of peptoid hydrazones to yield *N*-substituted peptoid hydrazides 12a – 12r. Samples of peptide hydrazones **11a – 11r** (~ 0.50 mg, 0.00035 mmol) were dissolved in aqueous acetonitrile (0.5 mL) containing 0.1% trifluoroacetic acid and 20 equivalents of sodium cyanoborohydride ($NaBH_3CN$, 1 M, in H_2O, 8 uL) was added and the mixtures were agitated at room temperature (overnight). Purification by preparative HPLC was as indicated above for peptoid hydrazones (mass spectral data, Table S1).

References

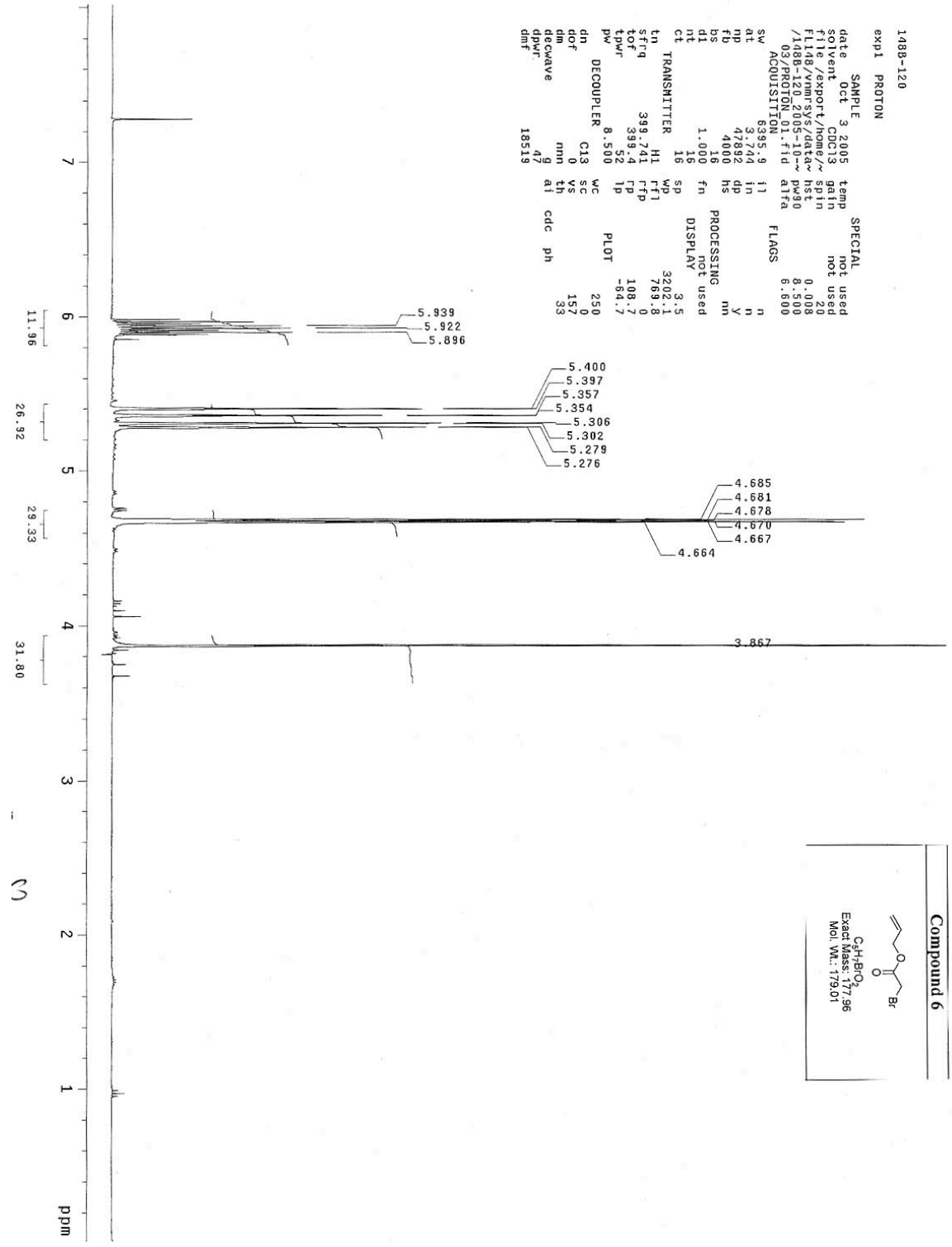
(1) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Second Edition ed.; Plenum Publishers, 1999.
(2) Fisher, R. J.; Fivash, M. J.; Stephen, A. G.; Hagan, N. A.; Shenoy, S. R.; Medaglia, M. V.; Smith, L. R.; Worthy, K. M.; Simpson, J. T.; Shoemaker, R.; McNitt, K. L.; Johnson, D. G.; Hixson, C. V.; Gorelick, R. J.; Fabris, D.; Henderson, L. E.; Rein, A. *Nucleic Acids Res.* **2006**, *34*, 472-484.
(3) Toke, L.; Jaszay, Z. M.; Petnehazy, I.; Clementis, G.; Vereczkey, G. D.; Kovesdi, I.; Rockenbauer, A.; Kovats, K. *Tetrahedron* **1995**, *51*, 9167-78

Table S1. **Mass spectral data for peptoid hydrazones and N-substitued peptoid hydrazides.**

R	number	M.W. (theoretical)	M.W. (observed)[a]	number	M.W. (theoretical)	M.W. (observed)
	**11a**	1529.58	1530.79	**12a**	1531.60	1533.51
	**11b**	1574.57	1575.30	**12b**	1576.58	1545.90[b]
	**11c**	1574.57	1575.54	**12c**	1576.58	1546.25[b]
	**11d**	1589.60	1590.80	**12d**	1591.62	1592.90
	**11e**	1561.57	1562.48	**12e**	1563.59	1564.96
	**11f**	1597.57	1598.88	**12f**	1599.58	1601.05
	**11g**	1547.57	1570.17[c]	**12g**	1549.59	1550.27
	**11h**	1572.62	1573.88	**12h**	1574.64	1599.12 [c]
	**11i**	1532.59	1533.66	**12i**	1534.61	1535.07
	**11j**	1519.56	1520.78	**12j**	1521.58	1523.39
	**11k**	1519.56	1520.37	**12k**	1521.58	1522.28
	**11l**	1523.59	1524.40	**12l**	1525.61	1548.22 [c]
	**11m**	1521.61	1522.24	**12m**	1523.63	1524.20
	**11n**	1557.61	1558.34	**12n**	1559.63	1583.70 [c]
	**11o**	1509.61	1510.52	**12o**	1511.63	1512.09
	**11p**	1495.60	1496.57	**12p**	1497.61	1598.29
	**11q**	1527.57	1528.74	**12q**	1529.58	1530.51

[a]Observed mass obtained by MALDI-TOF operating in linear mode using α-cyano-4-hydroxy-cinnamic acid as matrix; [b]The NO_2 group was reduced to NH_2; [c]$(M + Na)^+$ peak.

NMR Spectra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fb 4000
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ct 16
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tn H1
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tof 399.4
tpwr 52
pw 8.500
DECOUPLER
dn C13
dof 0
dm nnn
decwave g
dpwr 47
dmf 18519
temp
gain
spin
hst
pw90
atfa
FLAGS
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not used
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6.600
sp
wp
rfl
rfp
rp
lp
PLOT
wc
sc
vs
th
ai
cdc
ph
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0
157
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PROCESSING
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DISPLAY
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3202.1
769.8
0
108.7
-64.7
n
y
y
nn
not used
5.939
5.922
5.896
5.400
5.397
5.357
5.354
5.306
5.302
5.279
5.276
4.685
4.681
4.678
4.670
4.667
4.664
3.867
11.96
26.92
29.33
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ppm
Compound 6
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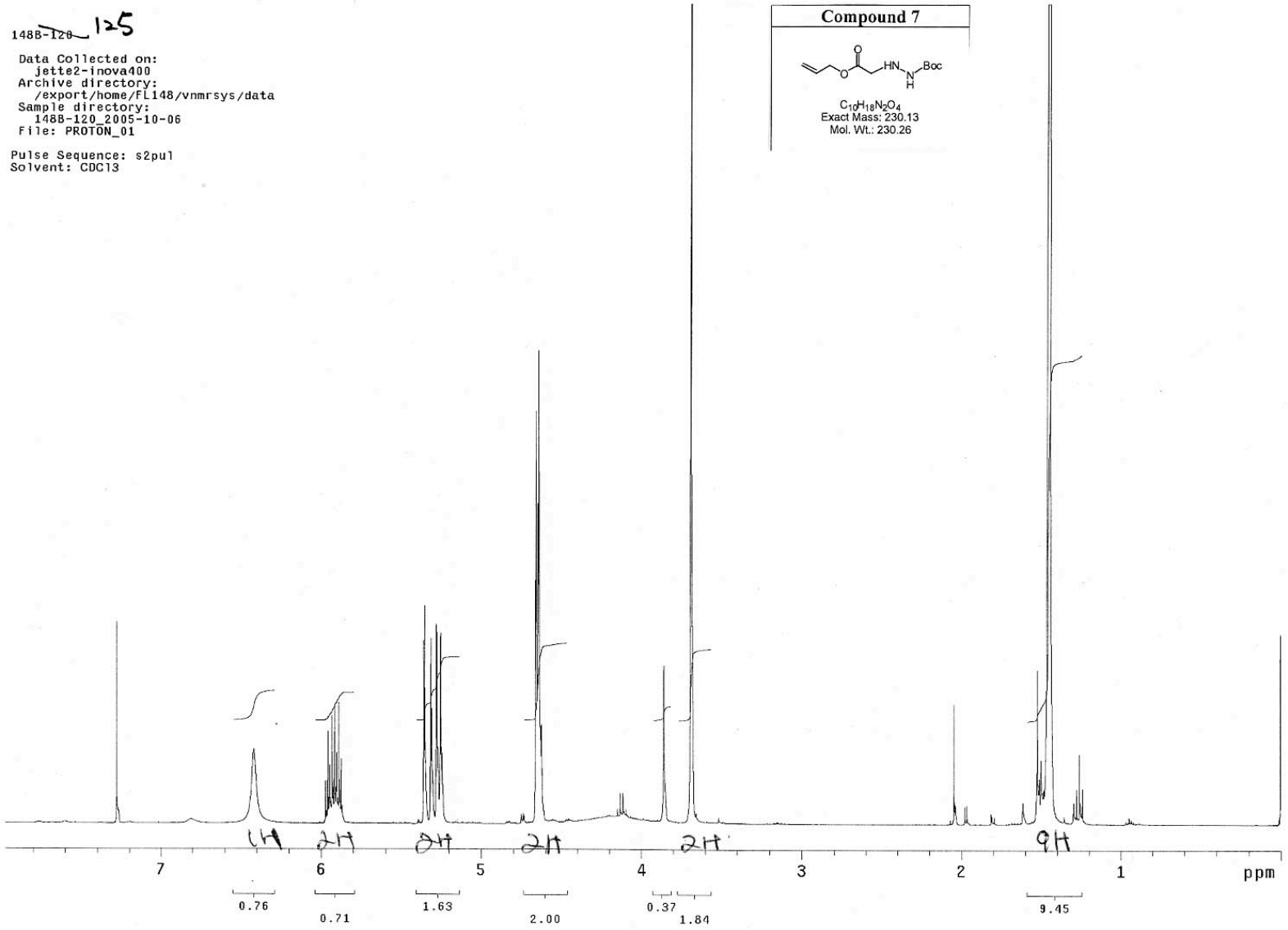
Exact Mass: 177.96
Mol. Wt.: 179.01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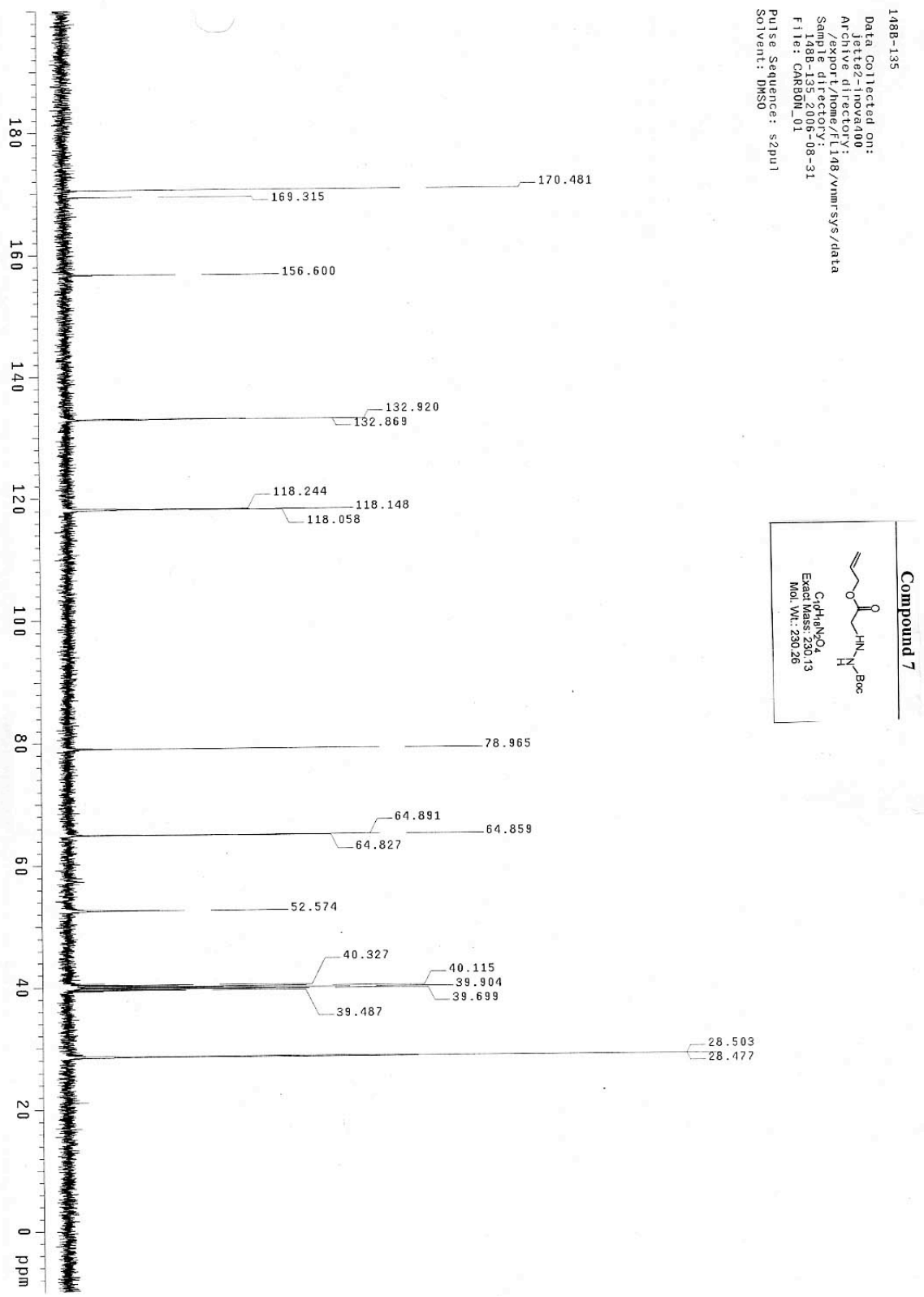
Solvent: CDCl3
Compound 7
C10H18N2O4
Exact Mass: 230.13
Mol. Wt.: 230.26
1H
2H
2H
2H
2H
9H
7
6
5
4
3
2
1
ppm
0.76
0.71
1.63
2.00
0.37
1.84
9.45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Pulse Sequence: s2pul
Solvent: DMSO
Compound 7
C10H18N2O4
Exact Mass: 230.13
Mol. Wt.: 230.26
170.481
169.315
156.600
132.920
132.869
118.244
118.148
118.058
78.965
64.891
64.859
64.827
52.574
40.327
40.115
39.904
39.699
39.487
28.503
28.477
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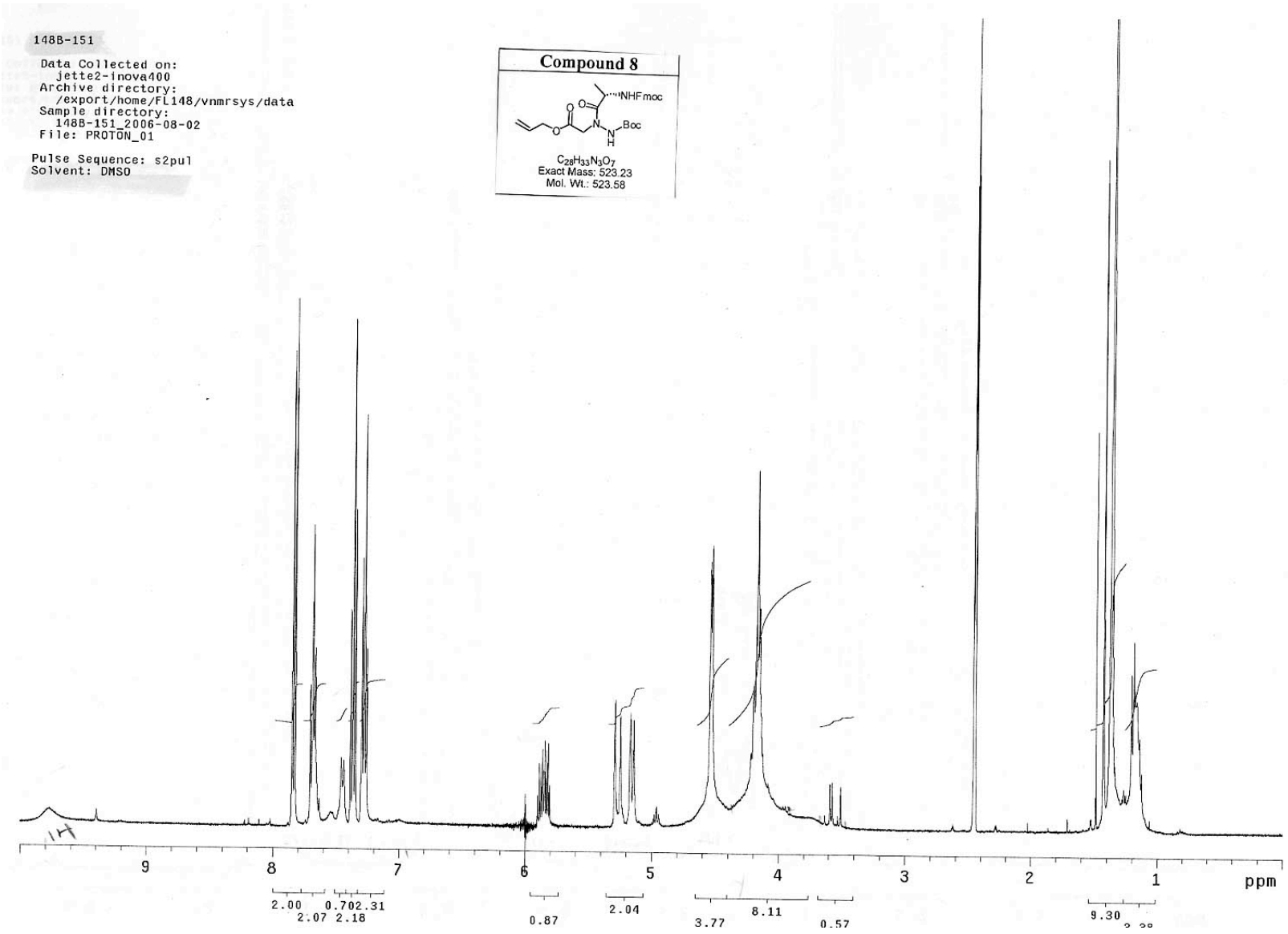
160
140
120
100
80
60
40
20
0
ppm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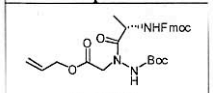
File: PROTON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 8
NHFmoc
Boc
C28H33N3O7
Exact Mass: 523.23
Mol. Wt.: 523.58
1H
9
8
7
6
5
4
3
2
1
ppm
2.00
2.07
0.70
2.18
2.31
0.87
2.04
3.77
8.11
0.57
9.30

Compound 8



$C_{28}H_{33}N_3O_7$
Exact Mass: 523.23
Mol. Wt.: 523.58

```
1488-151

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 File: CARBON_01

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Solvent: DMSO

 Temp. 25.0 C / 298.1 K
Operator: FL148

 Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 1.199 sec
 Width 21108.2 Hz
 512 repetitions
OBSERVE  C13, 100.5147441 MHz
DECOUPLE  H1, 399.7421628 MHz
 Power 36 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.0 Hz
FT size 65536
Total time 18 min
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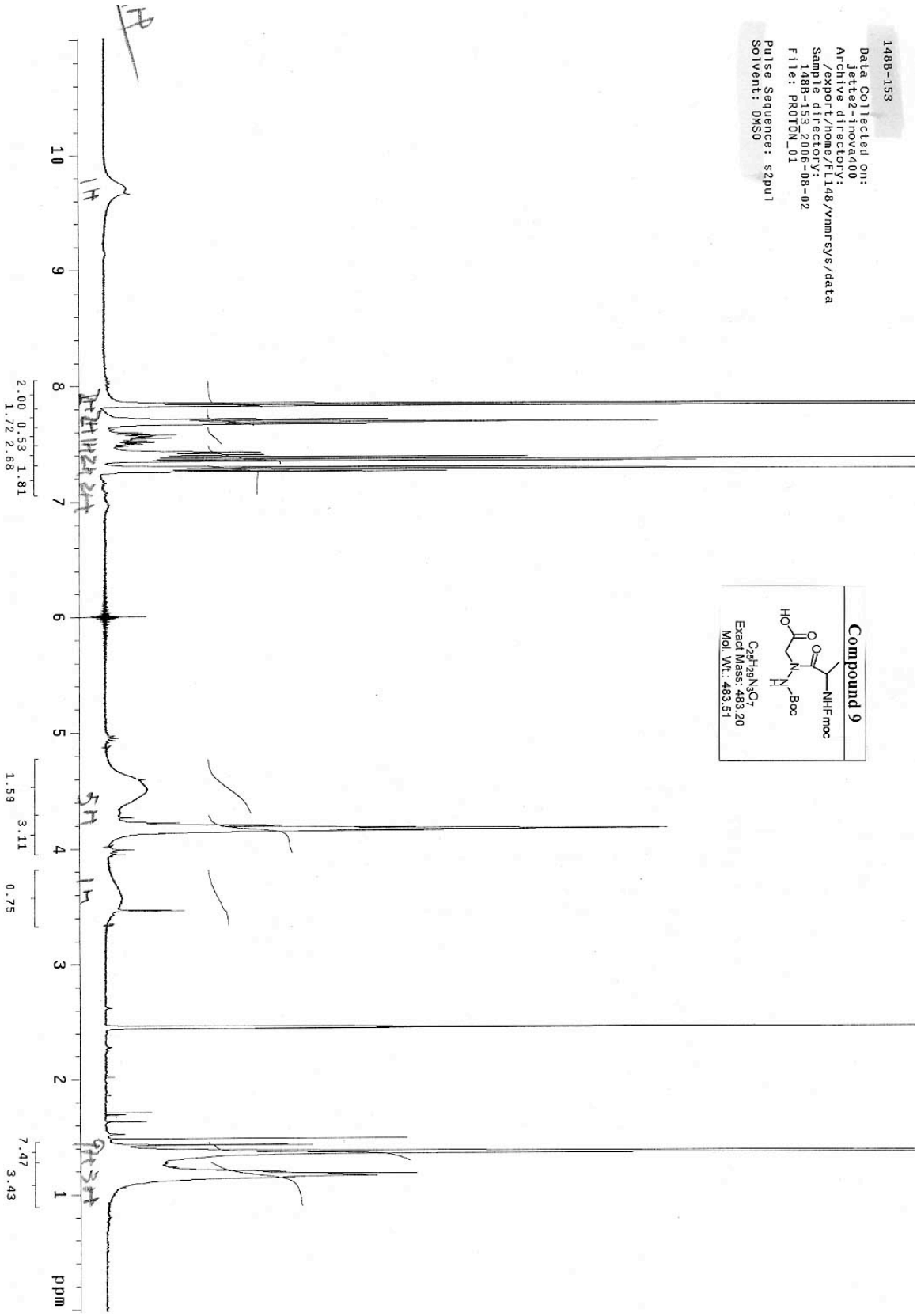
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File: PROTON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 9
NHFmoc
Boc
C25H29N3O7
Exact Mass: 483.20
Mol. Wt.: 483.51
2.00 0.53 1.81
1.72 2.68
1.59 3.11
0.75
7.47 3.43
ppm

148B-153

Data Collected on:
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Archive directory:
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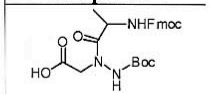
Sample directory:
148B-153_2006-08-31
File: CARBON_01

Pulse Sequence: s2pul
Solvent: DMSO

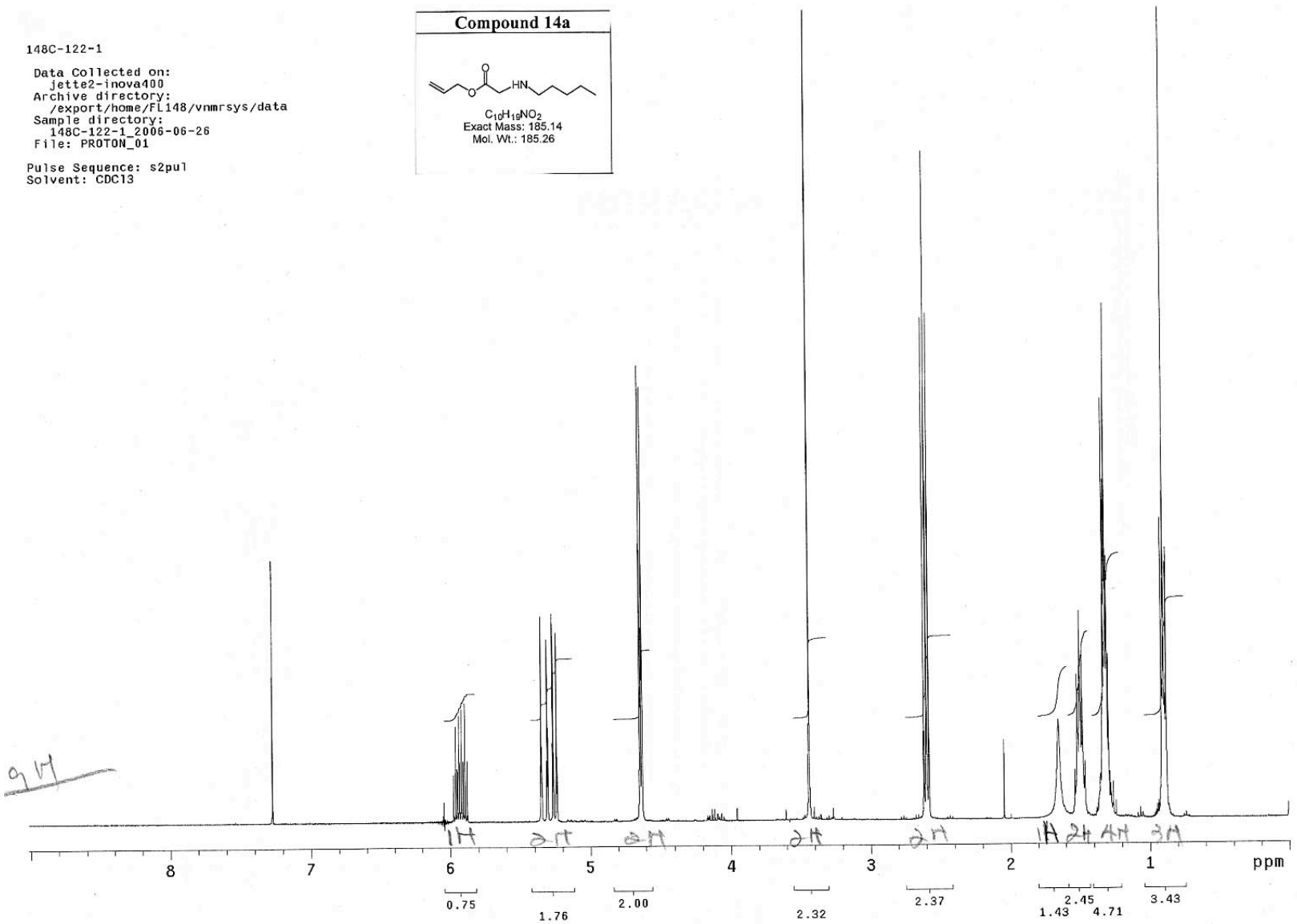
Temp. 25.0 C / 298.1 K
Operator: FL148

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 21108.2 Hz
512 repetitions
OBSERVE C13, 100.5147441 MHz
DECOUPLE H1, 399.7421628 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 27 min

Compound 9



$C_{25}H_{29}N_3O_7$
Exact Mass: 483.20
Mol. Wt.: 483.51


Compound 14a
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Data Collected on:
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Archive directory:
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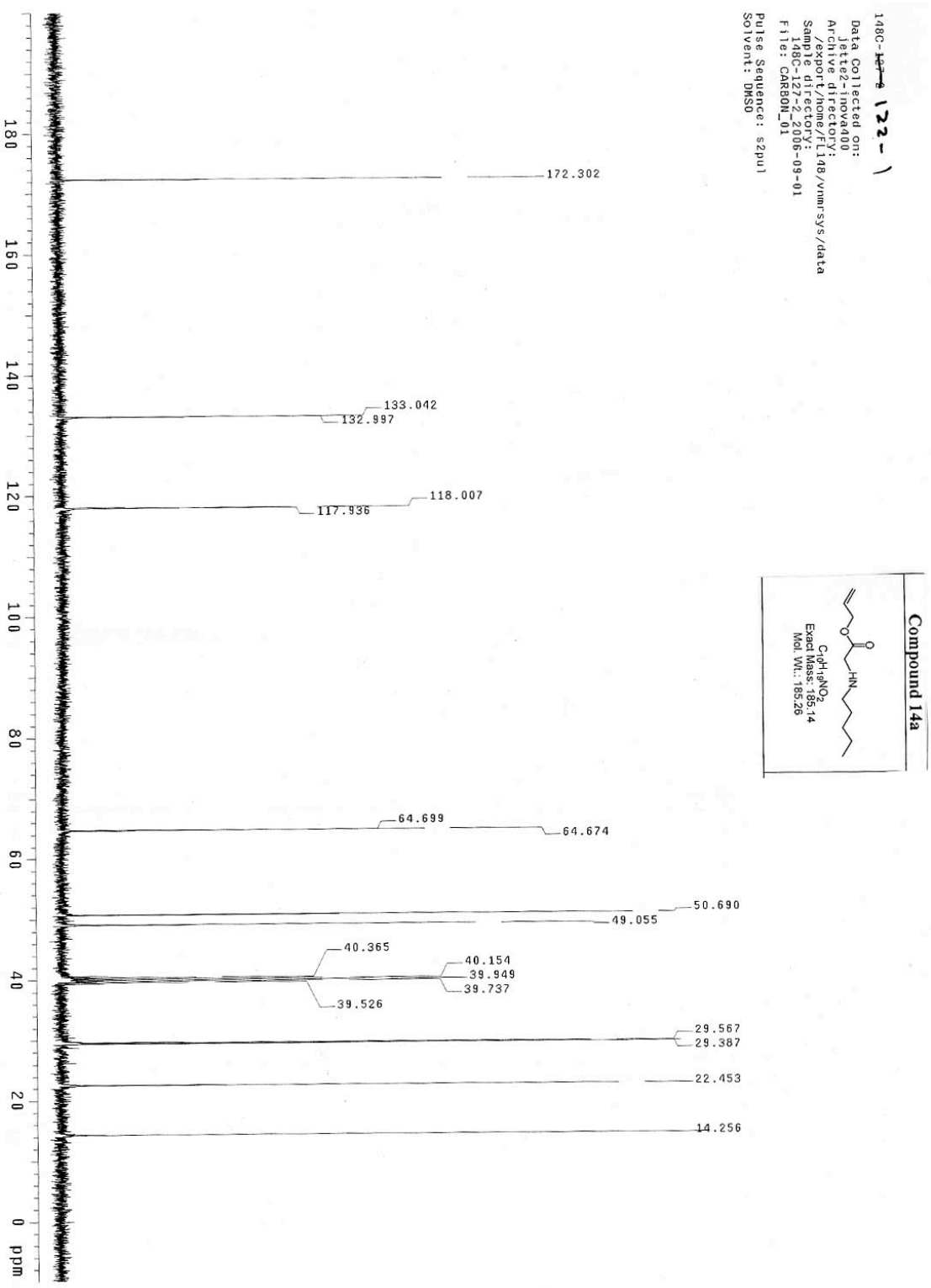
Sample directory:
148C-122-1_2006-06-26
File: PROTON_01
Pulse Sequence: s2pul
Solvent: CDCl3
C10H19NO2
Exact Mass: 185.14
Mol. Wt.: 185.26
HN
O
8
7
6
5
4
3
2
1
ppm
0.75
1.76
2.00
2.32
2.37
1.43
4.71
2.45
3.43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Jette2-inova400
Archive directory:
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File: CARBON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 14a
C9H15NO2
Exact Mass: 165.14
Mol. Wt.: 165.26
HN
172.302
133.042
132.997
118.007
117.936
64.699
64.674
50.690
49.055
40.365
40.154
39.949
39.737
39.526
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14.256
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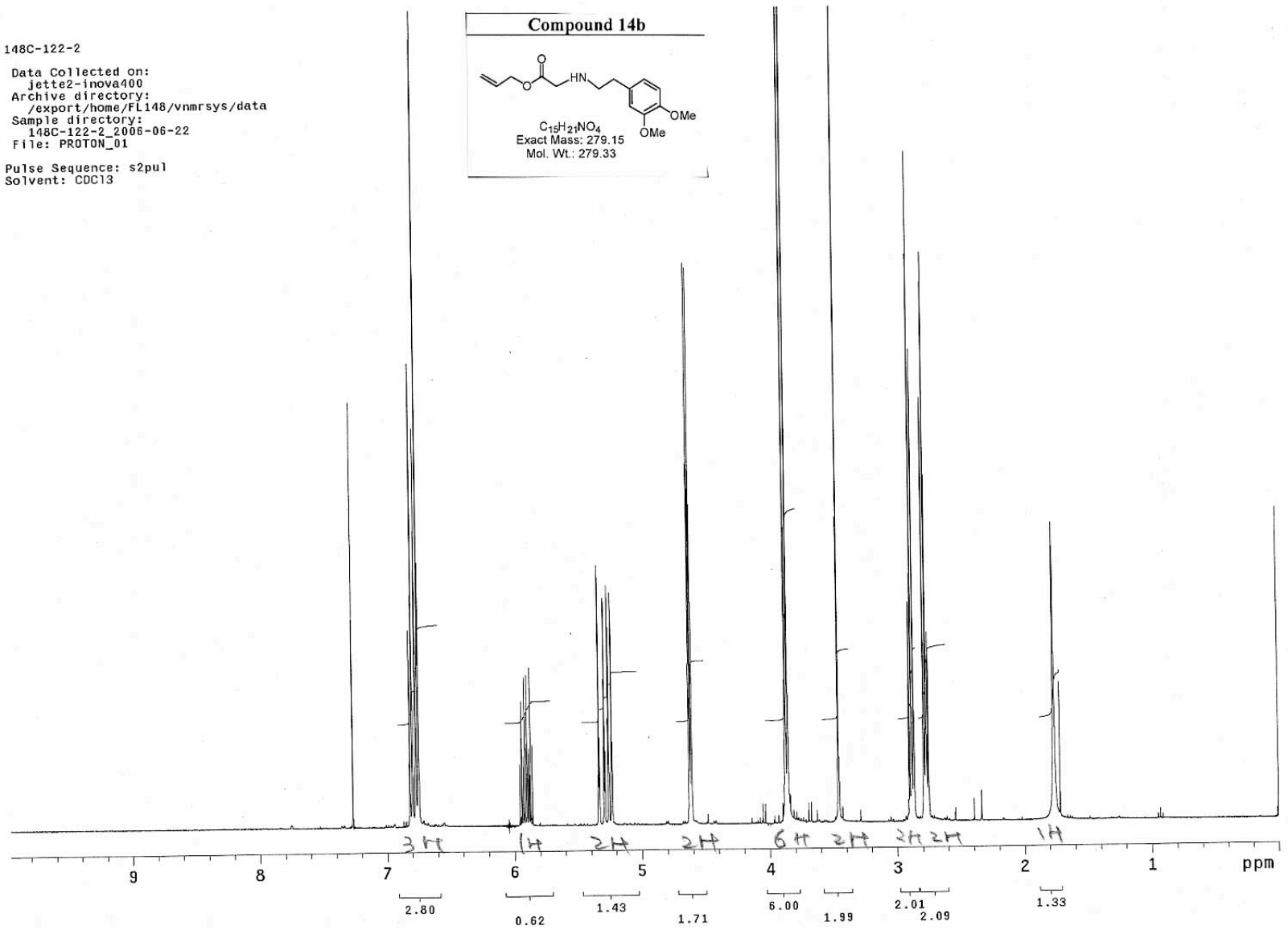
160
140
120
100
80
60
40
20
0
ppm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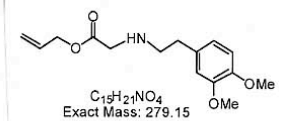
File: PROTON_01
Pulse Sequence: s2pul
Solvent: CDCl3
Compound 14b
HN
OMe
OMe
C15H21NO4
Exact Mass: 279.15
Mol. Wt.: 279.33
3H
1H
2H
2H
6H
2H
2H 2H
1H
9
8
7
6
5
4
3
2
1
ppm
2.80
0.62
1.43
1.71
6.00
1.99
2.01
2.09
1.33

148C-122-2

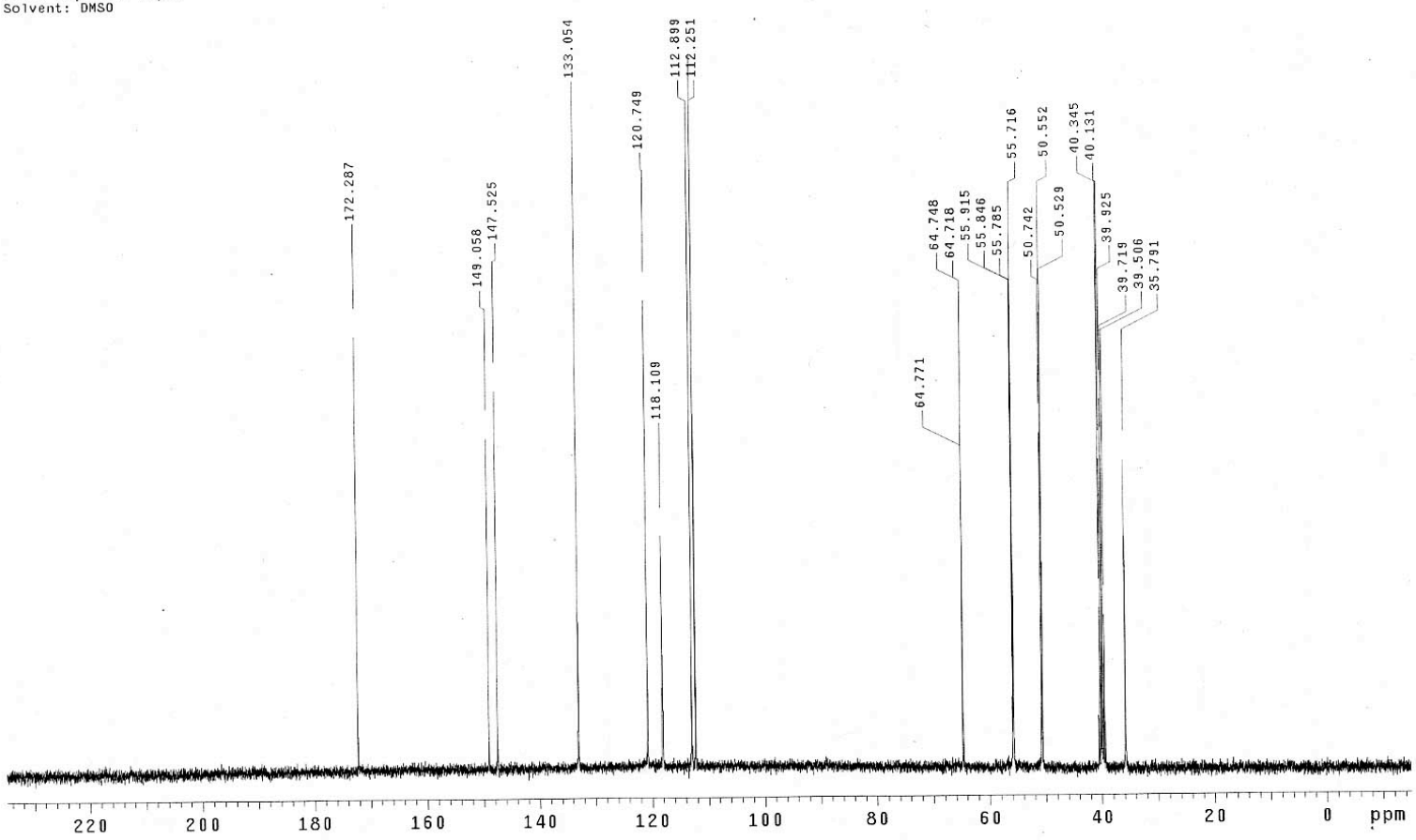
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-122-2_2006-09-01
File: CARBON_01

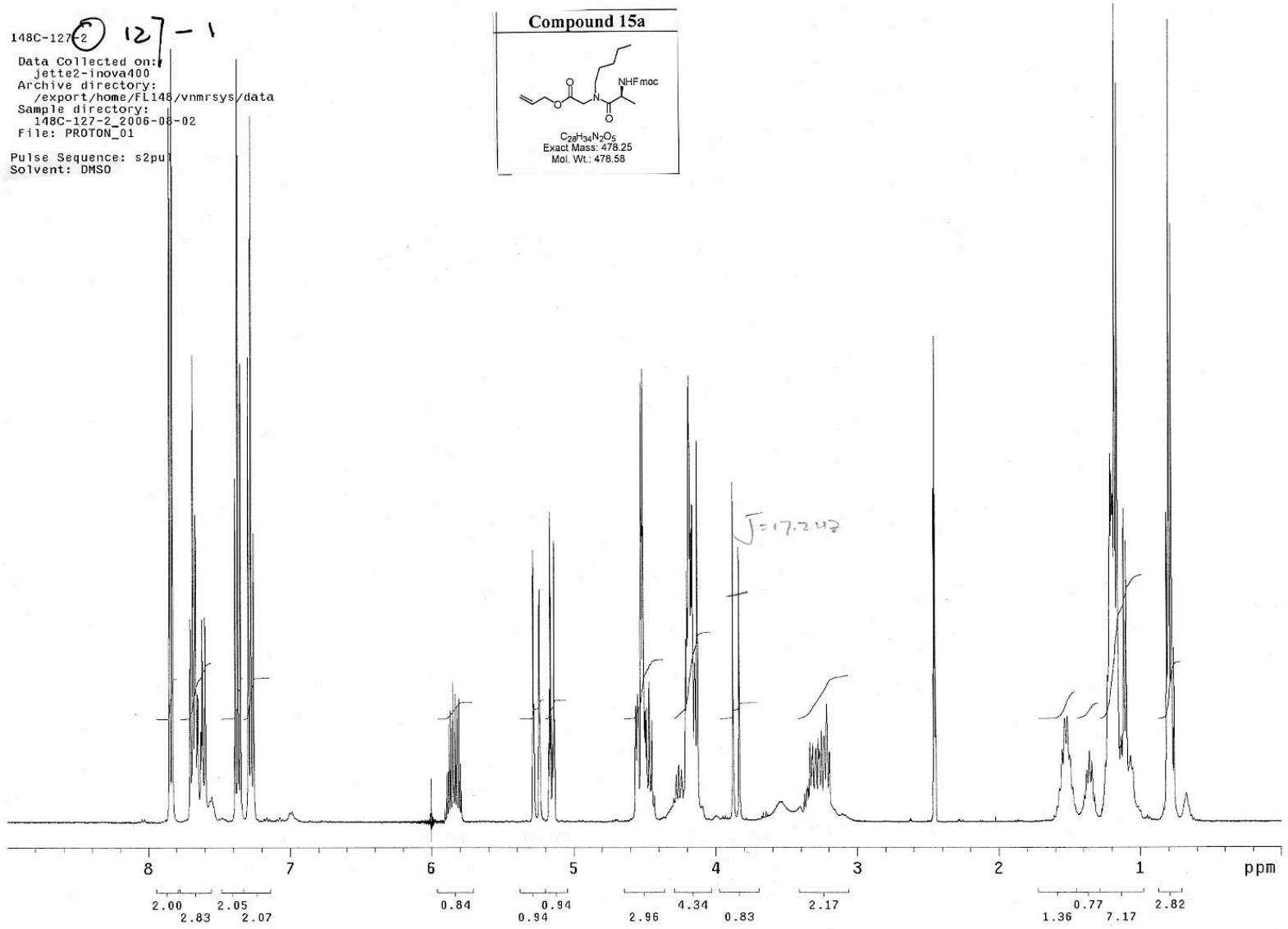
Pulse Sequence: s2pul
Solvent: DMSO

Compound 14b



$C_{15}H_{21}NO_4$
Exact Mass: 279.15
Mol. Wt.: 279.33




148C-127-2 (2) 127-1
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-127-2_2006-08-02
File: PROTON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 15a
NHFmoc
C28H34N2O5
Exact Mass: 478.25
Mol. Wt.: 478.58
J=17.2Hz
8
7
6
5
4
3
2
1
ppm
2.00
2.83
2.05
2.07
0.84
0.94
0.94
2.96
4.34
0.83
2.17
0.77
1.36
7.17
2.82

148C-127-1

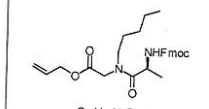
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-122-1_2006-09-01
File: CARBON_01

Pulse Sequence: s2pul
Solvent: DMSO

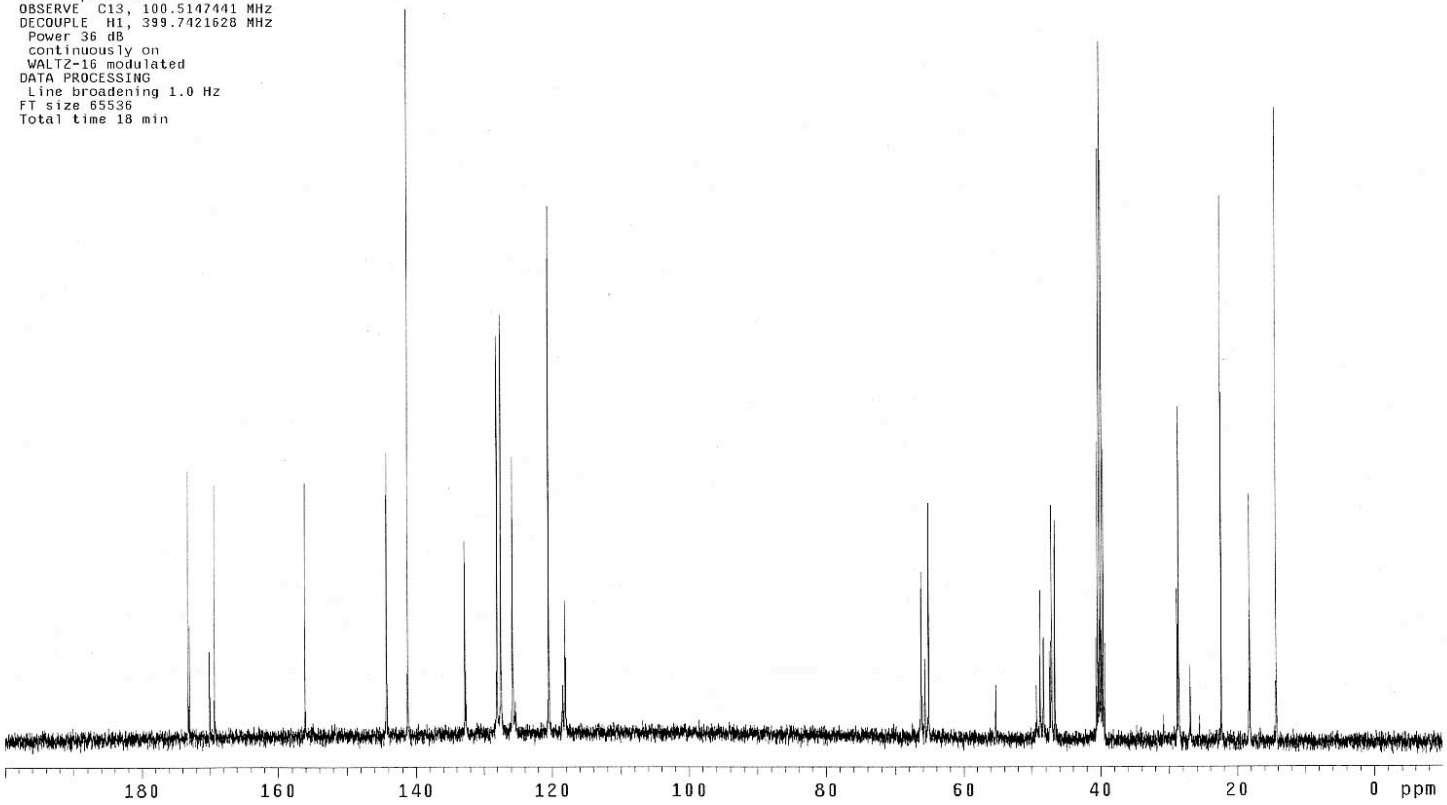
Temp. 25.0 C / 298.1 K
Operator: FL148

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 21108.2 Hz
384 repetitions
OBSERVE C13, 100.5147441 MHz
DECOUPLE H1, 399.7421628 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 18 min

Compound 15a



$C_{28}H_{34}N_2O_5$
Exact Mass: 478.25
Mol. Wt.: 478.58

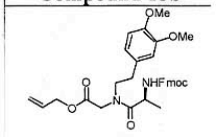


148C-127-2

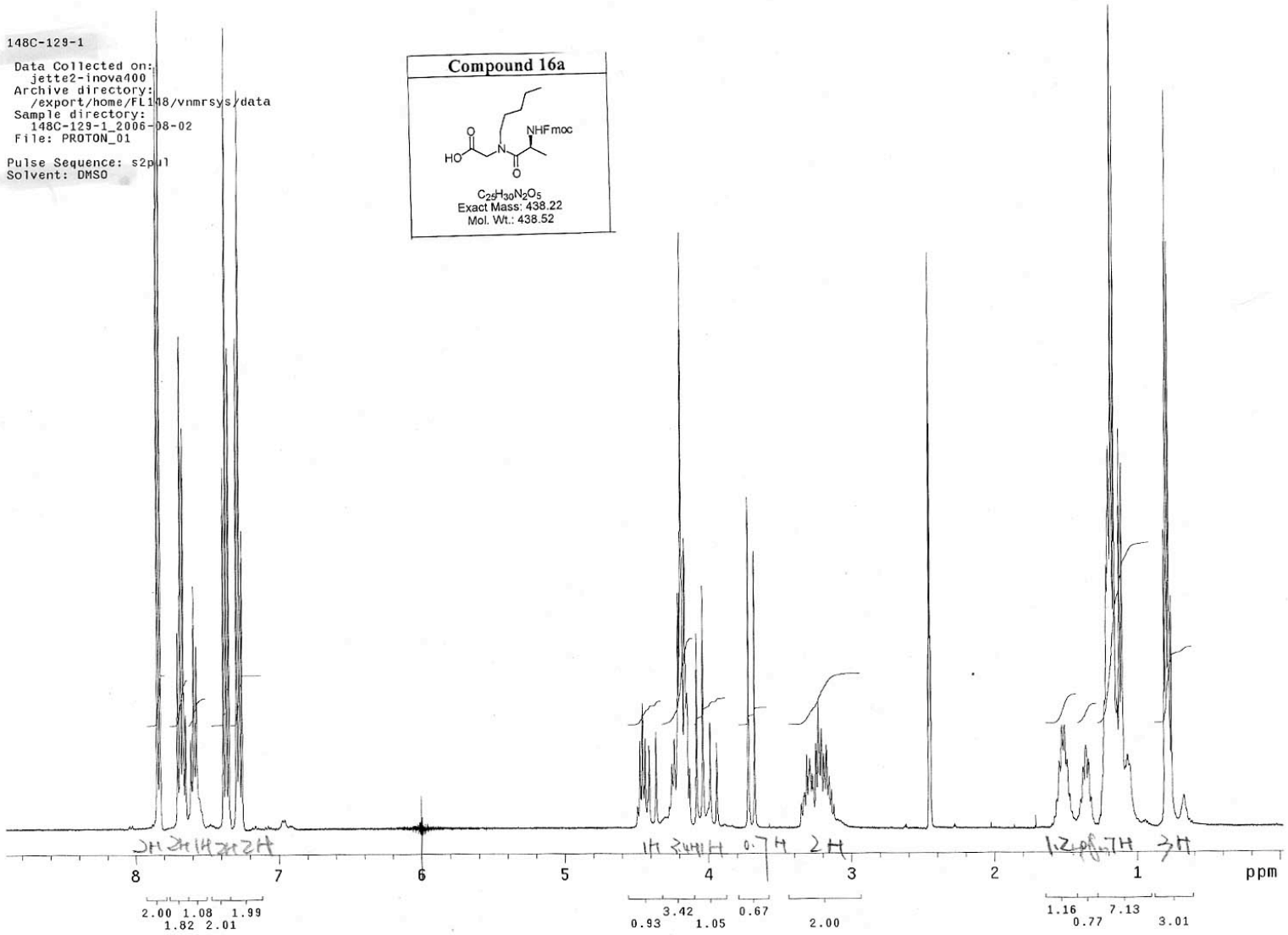
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-127-1_2006-06-26
File: PROTON_01

Pulse Sequence: s2pul
Solvent: CDCl3

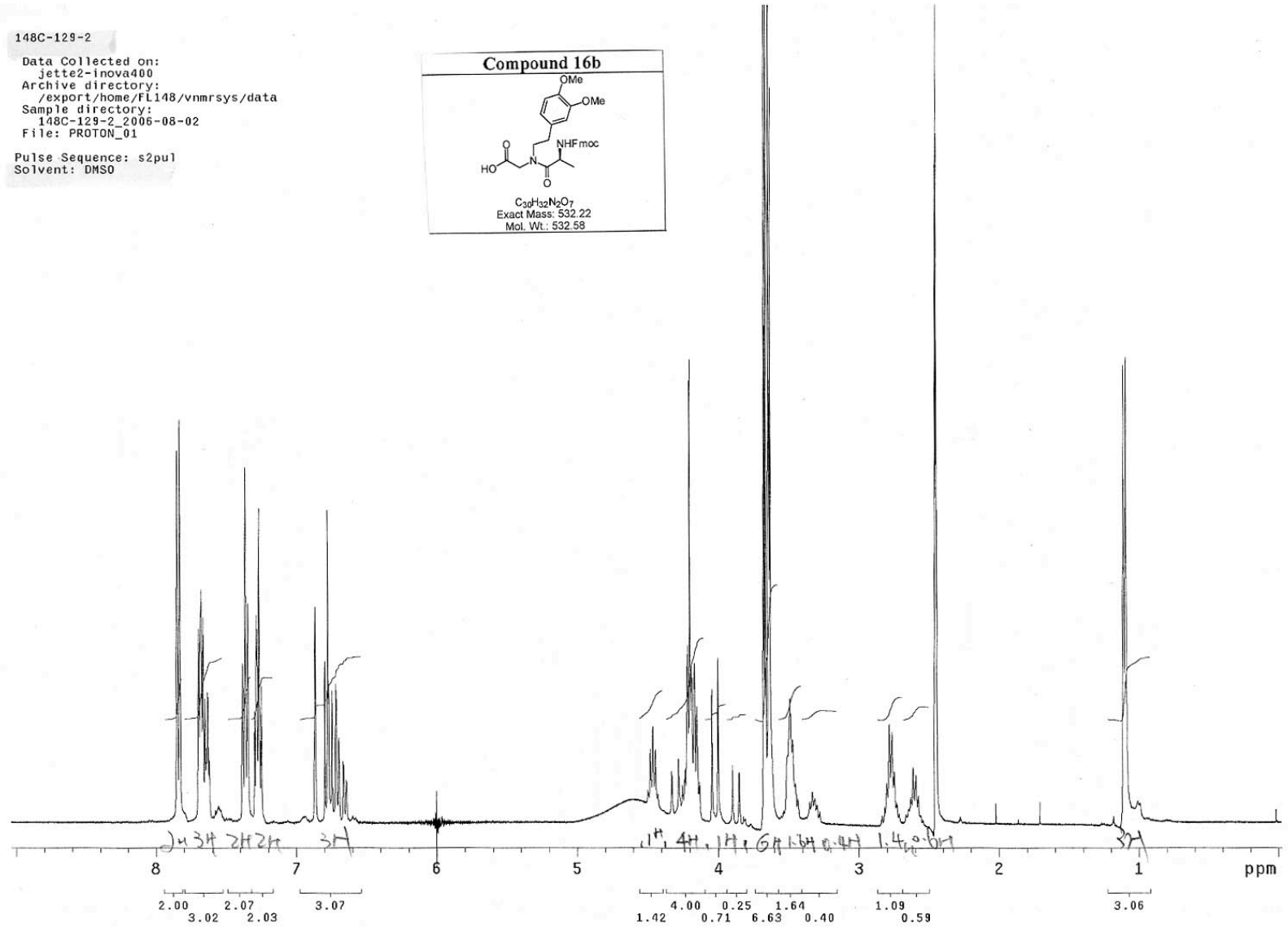
Compound 15b



$C_{33}H_{36}N_2O_7$
Exact Mass: 572.25
Mol. Wt.: 572.65


148C-129-1
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-129-1_2006-08-02
File: PROTON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 16a
NHFmoc
HO
O
N
O
C25H30N2O5
Exact Mass: 438.22
Mol. Wt.: 438.52
8
7
6
5
4
3
2
1
ppm
2.00 1.08 1.99
1.82 2.01
3.42
0.93 1.05
0.67
2.00
1.16 7.13
0.77 3.01

148C-129-1
Data Collected on:
Jette2-inova400
Archive directory:
/export/home/fl148/vnmrsys/data
Sample directory:
148C-129-1.000-08-31
File: CARBON_01
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
Operator: fl148
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 21008.2 Hz
512 repetitions
OBSERVE C13, 100.5147441 MHz
DECOUPLE H1, 399.7421628 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 18 min
Compound 16a
HO
O
N
O
NHFmoc
C29H30N2O5
Exact Mass: 438.22
Mol. Wt.: 438.52
180
160
140
120
100
80
60
40
20
0 ppm


148C-129-2
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-129-2_2006-08-02
File: PROTON_01
Pulse Sequence: s2pul
Solvent: DMSO
Compound 16b
OMe
OMe
NHFmoc
HO
O
N
O
C30H32N2O7
Exact Mass: 532.22
Mol. Wt.: 532.58
8
7
6
5
4
3
2
1
ppm
2.00
3.02
2.07
2.03
3.07
4.00
1.42
0.25
0.71
1.64
6.63
0.40
1.09
0.59
3.06

148C-129-2

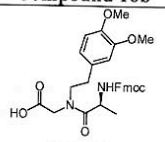
Data Collected on:
jette2-inova400
Archive directory:
/export/home/FL148/vnmrsys/data
Sample directory:
148C-129-2_2006-08-31
File: CARBON_01

Pulse Sequence: s2pul
Solvent: DMSO

Temp. 25.0 C / 298.1 K
Operator: FL148

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 25125.6 Hz
512 repetitions
OBSERVE C13, 100.5147441 MHz
DECOUPLE H1, 399.7421628 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 18 min

Compound 16b



$C_{30}H_{32}N_2O_7$
Exact Mass: 532.22
Mol. Wt.: 532.58