Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Discovery of a low affinity thyrotropin-releasing hormone (TRH)-like peptide that exhibits potent inhibition of scopolamine-induced memory impairment in mice

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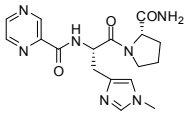
CONTENTS

Experimental

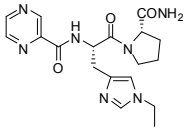
The reaction monitoring and compounds purity was checked on pre-coated silica gel G_{254} TLC plates (Merck) through the spotting and visualization under UV spectrophotometer or by exposing them to iodine vapors. Column chromatographic purification was carried out on Merck silica gel (230-400 mesh) or neutral alumina. IR spectra (λ_{max} in cm^{-1}) were recorded on Nicolet FT-IR Impact 410 instrument either as neat or with KBr pellets. ^1H NMR spectra were recorded on 400 MHz Bruker FT-NMR (Advance DP X 400) spectrometer-using tetramethylsilane as the internal standard and the chemical shifts are reported in δ (ppm) units. Coupling constants are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet. The sample concentration in each case was approximately 7 mg in 0.5 mL of the solvent. Mass spectra were recorded on either GCMS (Shimazdu QP 5000 spectrometer) auto sampler/direct injection (EI/CI) or LC (Finnigan Mat LCQ spectrometer) (APCI/ESI). The HRMS spectra were recorded on Bruker Maxis mass spectrometer. The melting points were recorded on capillary melting point apparatus or on the PerkinElmer DSC instrument and are uncorrected.

General method for the synthesis of R_1-L-His(1-alkyl)-L-ProNH$_2$ (6a-w)

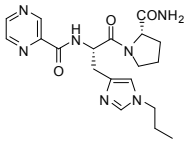
The respective dipeptides (**4a-e**) were dissolved in 40% CF_3CO_2H in CH_2Cl_2 (5 mL) and stirred for 30 min at 0 ^oC. The resulting dipeptide salt (**5a-e**) were neutralized by added a solution of 7N methanolic ammonia solution (5 mL) and stirring the mixture for 10 min at ambient temperature. The solvent was evaporated under reduced pressure to afford free dipeptide, which was used in the next coupling step without isolation. The free dipeptide (1 mmol) was dissolved in DMF (5 mL) and cooled to 4°C. The requisite hetero ring-containing carboxylic acid (1 mmol), DIC (1.1 mmol) and 1-hydroxybenzotriazole (1.1 mmol) was added and the resulting mixture stirred at 4 ^oC for 36 h. The solvent was removed under reduced pressure and residue was purified by column chromatography using a stationary phase of neutral alumina and a mobile phase of 0-7% CH_3OH in CH_2Cl_2 to afford **6a-w**.



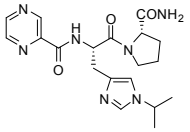
2-Pyz-L-His(1-methyl)-L-ProNH$_2$ (6a): Yield: 52%; white solid; mp.: 82-83°C; IR (KBr): 3433, 1634 cm^{-1}; ^1H NMR (CD_3OD): δ 9.18 (d, 1H, J = 4 Hz), 8.78 (d, 1H, J = 4 Hz), 8.67 (d,1H, J = 4 Hz), 7.49 (s, 1H), 6.97 (s, 1H), 5.06 (m, 1H), 4.45 (m, 1H), 3.57-3.87 (m, 2H), 3.67 (m, 3H) 3.02-3.20 (m, 2H), 2.22 (m, 2H), 2.00 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.67, 170.77, 163.24, 147.37, 144.37, 144.35, 143.44, 143.26, 137.64, 136.09, 119.08, 60.38, 51.67, 32.23, 29.66, 24.39; HRMS: *m/z* calcd for $C_{17}H_{21}N_7O_3$ $[M+H]^+$: 372.1784, found, 372.1780; R_f = 0.37 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90); HPLC: t_R = 3.78 min, purity: 99.54% [CH_3CN-H_2O-TFA (70:30:0.8%)].



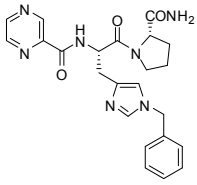
2-Pyz-L-His(1-ethyl)-L-ProNH$_2$ (6b): Yield: 45%; white solid mp.: 42-43°C; IR (KBr): 3429, 1645 cm^{-1}; ^1H NMR (CD_3OD): δ 9.18 (d 1H, J = 4 Hz) 8.78 (d, 1H, J = 4 Hz), 8.67 (d, 1H, J = 4 Hz), 7.56 (s, 1H), 7.05 (s, 1H), 5.07 (m, 1H), 4.60 (m, 1H), 3.99 (m, 2H), 3.67-3.86 (m, 2H), 3.08-3.17 (m, 2H), 2.00-1.96 (m, 4H), 1.39 (t, 3H, J = 4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 175.66, 170.78, 163.24, 147.37, 144.37, 144.35, 143.44, 143.26, 137.64, 136.094, 119.08, 60.38, 51.67, 32.23, 29.66, 24.39, 15.28; HRMS: *m/z* calcd for $C_{18}H_{23}N_7NaO_3$ $[M+Na]^+$: 408.1760, found, 408.1761; R_f = 0.42 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.143 min, purity: 96.302% [CH_3CN-H_2O-TFA (70:30:0.8%)].



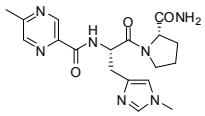
2-Pyz-L-His(1-propyl)-L-ProNH$_2$ (6c): Yield: 55%; white solid; mp.: 57-58°C; IR (KBr): 3433, 1634, cm-$^{-1}$; ^{1}H NMR ($CDCl_3$): δ 9.20 (d, 1H, *J* = 4 Hz), 8.80 (d, 1H, *J* = 4 Hz), 8.69 (d, 1H, *J* = 4 Hz), 7.65 (s, 1H), 7.05 (s, 1H), 5.30 (m, 1H), 5.10 (m, 1H), 4.91 (m, 2H), 3.67 (m, 2H), 3.12-3.60 (m, 2H), 2.30 (m, 2H), 2.15 (m, 2H), 2.10 (m, 2H), 0.92 (t, 3H, *J* = 8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.69, 170.76, 163.19, 147.36, 144.34, 143.47, 136.99, 136.00, 117.85, 60.41, 51.69, 31.40, 29.79, 24.41; HRMS: *m/z* calcd for $C_{19}H_{25}N_7NaO_3$ $[M+Na]^+$: 422.1917, found, 422.1918; R_f = 0.53 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90); HPLC: t_R = 4.17 min, purity: 99.76% [CH_3CN-H_2O-TFA (70:30:0.8.



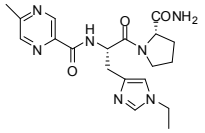
2-Pyz-L-His(1-isopropyl)-L-ProNH$_2$ (6d): Yield: 46%; white solid; mp.: 80-81°C; IR (KBr): 3434, 1634 cm^{-1}; ^{1}H NMR (CD_3OD): δ 9.20 (d, 1H, *J* = 6 Hz), 8.80 (m,1H), 8.70 (m, 1H, *J* = 4 Hz), 7.63 (s, 1H), 7.15 (s, 1H), 5.09 (m, 1H), 4.47 (m, 1H), 4.38 (m, 1H), 3.52 (m, 2H), 3.06-3.23 (m, 2H), 2.23 (m, 2H), 2.01 (m, 2H), 1.47 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.66, 170.76, 163.20, 147.37, 1.44.36, 143.47, 135.87, 135.073, 115.72, 60.42, 51.76, 49.37 29.90, 29.23, 29.36, 24.39; HRMS: *m/z* calcd for $C_{19}H_{25}N_7NaO_3$ $[M+Na]^+$: 422.1917, found, 422.1910; R_f = 0.51 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90); HPLC: t_R = 4.31 min, purity: 99.22% [CH_3CN-H_2O-TFA (70:30:0.8%)].



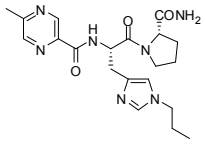
2-Pyz-L-His(1-benzyl)-L-ProNH$_2$ (6e): Yield: 52%; white solid; mp.: 70-71°C; IR (KBr): 3481, 1645 cm^{-1}; ^{1}H NMR (CD_3OD): δ 9.17 (d, 1H, *J* = 1.4 Hz), 8.80 (m, 1H), 8.63 (d, 1H, *J* = 4.04 Hz), 7.67 (s, 1H), 7.05-7.32 (m, 5H), 7.90 (s, 1H), 5.15 (s, 2H), 5.06 (m, 1H), 4.44 (m, 1H), 3.85 (m, 2H), 2.92-3.18 (m, 2H), 2.22 (m, 2H), 1.99 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.65, 170.73, 163.37, 143.23, 142.09, 141.58, 136.21, 128.03, 127.67,127.00, 118.17, 60.23, 51.54, 50.21, 29.91, 29.36, 29.36, 24.38, 20.20; HRMS: *m/z* calcd for $C_{23}H_{25}N_7NaO_3$ $[M+Na]^+$: 470.1917, found, 470.1918; R_f = 0.67 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.93 min, purity: 99.91% [CH_3CN-H_2O-TFA (70:30:0.8%)].



5-Mpyz-L-His(1-methyl)-L-ProNH$_2$ (6f): Yield: 50%; white solid; mp.: 51-52°C; IR (KBr): 3433, 1634 cm^{-1}; ^{1}H NMR (CD_3OD): δ 9.03 (s 1H), 8.78 (s 1H) 7.53 (s, 1H), 6.96 (s, 1H), 5.07 (m, 1H), 4.43 (m, 1H), 3.57-3.87 (m, 2H), 3.86 (m, 3H) 3.03-3.31 (m, 2H), 2.62 (s, 3H) 2.22 (m, 2H), 2.02 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.68, 170.88, 163.48, 155.57, 140.25, 140.25, 139.63, 136.11, 119.05, 60.38, 51.59, 32.23, 29.66, 24.39, 22.19; HRMS: *m/z* calcd for $C_{18}H_{23}N_7NaO_3$[M+Na]$^+$: 408.1760, found, 408.1763; R_f = 0.57 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.31 min, purity: 98.24% [CH_3CN-H_2O-TFA (70:30:0.8%)].

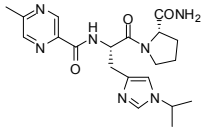


5-Mpyz-L-His(1-ethyl)-L-ProNH$_2$ (6g): Yield: 53%; white solid; mp.: 49-50°C; IR (KBr): 3418, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.23 (s, 1H), 8.40 (s, 1H), 7.35 (s, 1H), 6.80 (s, 1H), 5.07 (m, 1H), 4.66 (m, 1H), 3.96 (q, 2H, *J* =7.6 Hz), 3.63 (m, 2H), 3.10-3.23 (m, 2H), 2.65 (s, 3H), 2.27 (m, 2H), 2.13 (m, 2H) 1.46 (d, 3H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.67, 170.57, 163.37, 143.14, 142.63, 135.90, 116.92, 60.83, 51.29, 47.41, 41.97, 31.57, 29.25, 21.85, 16.23; HRMS: *m/z* calcd for $C_{19}H_{25}N_7NaO_3$ $[M+Na]^+$:422.1917, found, 422.1916; R_f = 0.67 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.42 min, purity: 97.94% [CH_3CN-H_2O-TFA (70:30:0.8%)].

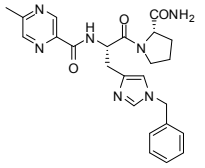


5-Mpyz-L-His(1-propyl)-L-ProNH$_2$ (6h): Yield: 43%; white solid; mp.: 47-48°C; IR (KBr): 3418, 1644 cm^{-1}; ^{1}H NMR (CD_3OD): δ 9.02 (s, 1H), 8.56 (s, 1H), 7.54 (s, 1H), 7.03 (s, 1H), 4.43 (m, 1H), 3.89 (m, 1H), 3.51 (s, 2H), 3.55 (m, 2H), 3.03-3.18 (m, 2H), 2.61 (s, 3H), 2.20 (m, 2H), 2.02 (m, 2H), 1.73 (m, 2H), 0.85 (m, 3H); ^{13}C NMR (100

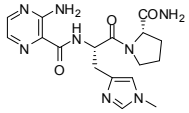
MHz, CD_3OD): δ 175.67, 170.82, 163.40, 143.24, 142.08, 141.63, 136.96, 136.04, 117.81, 60.41,51.61, 29.86, 24.39, 23.86, 20.20, 9.79; HRMS: *m/z* calcd for $C_{20}H_{27}N_7NaO_3$; $[M+Na]^+$: 436. 2073, found, 436.2073; R_f = 0.57 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.17 min, purity: 99.76% [CH_3CN-H_2O-TFA (70:30:0.8%)].



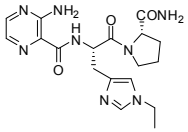
5-Mpyz-L-His(1-isopropyl)-L-ProNH$_2$ (6i): Yield: 48 %; white solid; mp: 52-53°C IR (KBr): 3434, 1634 cm^{-1}; ^1H NMR (CD_3OD): δ 9.20 (s, 1H), 8.80 (m, 1H), 7.63 (s, 1H), 7.15 (s, 1H), 5.09 (m, 1H), 4.47 (m, 1H), 4.38 (m, 1H), 3.52 (m, 2H), 3.06-3.23 (m, 2H), 2.65 (s, 3H), 2.23 (m, 2H), 2.01 (m, 2H), 1.47 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.66, 170.76, 163.20, 147.37, 1.44.36, 143.47, 135.87, 135.073, 115.72, 60.42, 51.76, 49.37 29.90, 29.23, 29.36, 24.39; HRMS: *m/z* calcd for $C_{20}H_{27}N_7NaO_3$ $[M+H]^+$: 414.2250, found, 414.2251; R_f = 0.43 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.31 min, purity: 97.42% [CH_3CN-H_2O-TFA (70:30:0.8%)].



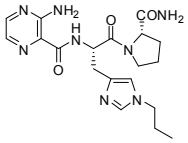
5-Mpyz-L-His(1-benzyl)-L-ProNH$_2$ (6j): Yield: 50%; mp.: 46-47°C; IR (KBr): 3481, 1645 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.00 (s, 1H), 8.51 (s, 1H), 7.64 (s, 1H), 7.20-7.27 (m, 5H), 7.02 (s, 1H), 5.13 (s, 2H), 5.04 (m, 1H), 4.43 (m, 1H) 3.79 (m, 2H), 3.01-3.19 (m, 2H), 2.63 (s, 3H), 2.19 (m, 2H), 1.99 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.64, 170.80, 163.37, 143.233, 142.091, 141.58, 136.21, 128.032, 127.67, 127.06, 118.17, 60.23, 51.54, 50.21, 29.91, 29.36, 29.36, 24.38, 20.20; HRMS: *m/z* calcd for $C_{24}H_{27}N_7NaO_3$ $[M+Na]^+$: 484.2073, found, 484.2076; R_f = 0.67 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.93 min, purity: 99.91% [CH_3CN-H_2O-TFA (70:30:0.8%)].



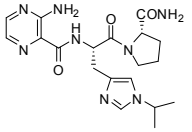
3-Apyz-L-His(1-methyl)-L-ProNH$_2$ (6k): Yield: 52%; colorless solid; mp.:75-76°C; IR (KBr): 3434, 1641 cm^{-1}; ^1H NMR (CD_3OD): δ 8.14 (d, 1H, J = 8 Hz) 7.72 (m,1H) 7.41 (s, 1H), 6.83 (s, 1H), 4.92 (m, 1H), 4.33 (m, 1H), 3.57 (s, 3H), 3.20 (m, 2H), 2.99-3.05 (m, 2H), 2.12 (m, 2H), 2.07 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.74, 171.23, 162.29, 146.49, 137.62, 136.19, 131.21, 119.03, 60.37, 52.09, 51.23, 32.26, 29.84, 29.34, 29.02, 24.37, 23.63; HRMS: *m/z* calcd for $C_{17}H_{22}N_8NaO_3$ $[M+Na]^+$: 409.1713, found, 409.1714; R_f = 0.37 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.82 min, purity: 97.83% [CH_3CN-H_2O-TFA (70:50:0.8%)].



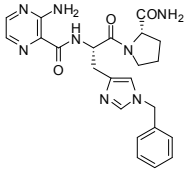
3-Apyz-L-His(1-ethyl)-L-ProNH$_2$ (6l): Yield: 47%; colorless solid; mp.: 70-72°C; IR (KBr): 3435, 1634 cm^{-1}, ^1H NMR ($CDCl_3$): δ 9.18 (bs, 2H), 8.70 (bs, 1H), 8.14 (d, 1H, J = 8 Hz), 7.82 (m,1H, J = 4 Hz,) 7.34 (s, 1H), 6.80 (s, 1H), 5.38 (bs,1H), 4.97 (m, 1H), 4.64 (m, 1H), 3.93 (t, 2H, J = 16 Hz), 3.63 (m, 2H), 3.05-3.31 (m, 2H), 2.28 (m, 2H), 2.09 (m, 2H), 1.44 (t, 3H, J = 2.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 175.74, 171.23, 162.29, 146.49, 137.62, 136.19, 131.24, 119.1, 60.37, 52.09, 51.23, 32.26, 29.84, 29.34, 29.02, 24.37, 23.63, 15.10; HRMS: *m/z* calcd for $C_{18}H_{24}N_8NaO_3$ $[M+Na]^+$: 433.1869, found, 433.1869; R_f = 0.42 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.12 min, purity: 99.95% [CH_3CN-H_2O-TFA (70:50:0.8%)].



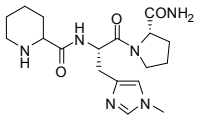
3-Apyz-L-His(1-propyl)-L-ProNH$_2$ (6m): Yield: 50%; white solid; mp.: 74-75°C IR (KBr): 3429 1638, cm^{-1}; ^1H NMR (CD_3OD): δ 8.14 (d, 1H, J = 4 Hz), 7.83 (d, 1H, J = 4 Hz), 7.56 (s, 1H), 7.04 (s, 1H), 5.04 (m, 1H), 4.48 (m, 1H), 3.94 (t, 2H, J = 7.04 Hz), 3.55 (m, 2H), 3.00-3.15 (m, 2H), 2.22 (m, 2H), 2.02 (m, 2H), 1.79 (m, 3H), 0.87 (t, 3H, J = 7.6 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 175.54, 171.20, 169.94, 165.81, 155.16, 146,49, 137.05, 136.14, 131.11, 60.40, 52.10, 51,24, 29.99, 29.35, 24.39, 9.80; HRMS: *m/z* calcd for $C_{19}H_{26}N_8NaO_3$ $[M+Na]^+$: 437.2026, found, 437.2020; R_f = 0.50 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.58 min, purity: 99.92% [CH_3CN-H_2O-TFA (70:50:0.8%)].



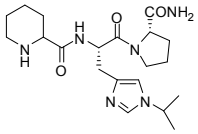
3-Apyz-L-His(1-isopropyl)-L-ProNH$_2$ (6n): Yield: 50%; white solid; mp.: 57-58 °C; IR (KBr): 3429 1638, cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.13 (d, 1H, J = 2.32 Hz), 7.83 (d, 1H, J = 2.36 Hz), 7.66 (s, 1H), 7.11 (s, 1H), 5.01 (m, 1H), 4.90 (m, 1H), 4.46 (m, 1H), 3.50 (m, 2H), 3.01-3.16 (m, 2H), 1.96-2.23 (m, 4H), 1.45 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.73, 171.20, 171.63, 166.19, 155.13, 146,50, 136.28, 136.14, 131, 125.83, 115.42, 60.42, 58.89, 55,22, 51.43, 49.43, 31.34, 30.06, 29.99, 28.30, 24.39, 21.99, 21.76; HRMS: *m/z* calcd for $C_{19}H_{26}N_8NaO_3$ $[M+Na]^+$: 437.2026, found, 437.2021; R_f = 0.50 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.68 min, purity: 99.84% [CH_3CN-H_2O-TFA (70:50:0.8%)].



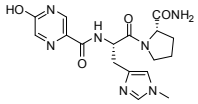
3-Apyz-L-His(1-benzyl)-L-ProNH$_2$ (6o): Yield: 50%; light brown solid; mp.: 59-61 °C; IR (KBr): 3430, 1641 cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.11 (m, 1H), 7.77 (m,1H), 7.64 (s, 1H), 7.25-7.29 (m, 5H), 7.02 (s, 1H), 5.14 (s, 2H), 4.99 (m, 1H), 4.41 (m, 1H), 3.80 (m, 2H), 3.31-3.02 (m, 2H), 2.20 (m, 2H), 1.98 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.58, 170.80, 166.01, 154.09, 146.49, 145.25, 135.44, 131.27, 128.40, 127.39, 126.0, 125.68, 92.17, 78.07, 60.63, 53.33, 50.87, 31.09, 29.39, 24.39; HRMS: *m/z* calcd for $C_{23}H_{26}N_8NaO_3$ $[M+Na]^+$: 485.2026, found 485.2023; R_f = 0.37 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.82 min, purity: 97.83% [CH_3CN-H_2O-TFA (70:50:0.8%)].



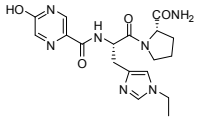
2-Pip-L-His(1-methyl)-L-ProNH$_2$ (6p): Yield: 42%; white solid; mp.: 127-128 °C; IR (KBr): 3481, 1645 cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.38 (s, 1H), 6.82 (s, 1H), 4.30 (m, 1H), 3.69 (m, 1H), 3.57 (s, 3H), 3.36 (m, 1H), 3.33 (m, 2H), 2.96-3.11 (m, 2H), 2.53 (m, 2H), 3.24 (m, 2H) 1.17-1.190 (m, 8H); ^{13}C NMR (100 MHz, CD_3OD): δ 177.08, 175.75, 174.10, 171.32, 137.57, 136.38, 88.89, 60.31, 59.23, 51.33, 45.00, 32.26, 29.81, 29.62, 25.27, 23.77, 23.65; HRMS: *m/z* calcd for $C_{18}H_{28}N_6NaO_3$ $[M+Na]^+$: 399.2121, found, 399.2120;R_f = 0.32 [CH_3OH:10% NH_4OH:CH_2Cl_2 (12:2:88)]; HPLC: t_R = 4.30 min, purity: 97.91% [CH_3CN-H_2O-TFA (70:30:0.8%)].



2-Pip-L-His(1-isopropyl)-L-ProNH$_2$ (6q): Yield: 23%; colorless solid; mp.: 102-103 °C; IR (KBr): 3481, 1645 cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.57 (s, 1H), 7.23 (s, 1H), 5.19 (m, 1H), 4.42 (m, 1H), 3.96 (m, 1H) 3.78 (m, 1H), 3.22 (m, 2H), 2.86-3.14 (m, 2H), 1.28-2.21 (m, 10H), 1.60 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.08, 169.94, 136.57, 135, 126.38, 67.31, 64.30, 59.23, 51.33, 45.10, 32.16, 29.81, 29.62, 25.27, 23.77, 23.0; HRMS: *m/z* calcd for $C_{18}H_{28}N_6NaO_3$ $[M+Na]^+$: 427.2540, found, 427.2545; R_f = 0.31 [CH_3OH:10% NH_4OH:CH_2Cl_2 (12:2:88)]; HPLC: t_R = 4.54 min, purity: 99.55% [CH_3CN-H_2O-TFA (70:30:0.8%)].

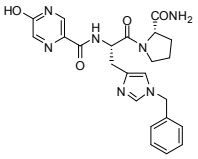


5-Hpyz-L-His(1-methyl)-L-ProNH$_2$ (6r): Yield: 42%; white solid; mp.: 157-158°C; IR (KBr): 3481, 1645 cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.50 (s, 1H), 8.13 (s, 1H), 7.99 (s, 1H), 7.30 (s, 1H), 5.14 (m, 1H), 4.47 (m, 1H), 3.85 (s, 3H), 3.80 (m, 2H), 3.16-3.22 (m, 2H), 2.30 (m, 2H), 1.96-2.01 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.85, 169.20, 163.08, 161.82,157.07, 146.89, 135.21,132.06, 129..06, 126.36, 121.37, 118.18 115.37, 60.14, 53.41, 50.41, 34.26, 29.69, 27.05, 24.60, 21.17; HRMS: *m/z* calcd for $C_{17}H_{21}N_7NaO_4$ $[M+Na]^+$: 410.1553, found, 410.1552; R_f = 0.20 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.86 min, purity: 99.83% [CH_3CN-H_2O-TFA (70:30:0.8%)].

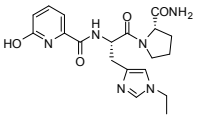


5-Hpyz-L-His(1-ethyl)-L-ProNH$_2$ (6s): Yield: 33%; white powder; mp.: 143-144°C; IR (KBr): 3481, 1645 cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.56 (s, 1H), 8.16 (s, 1H), 7.83 (s, 1H), 7.46 (s, 1H), 5.18 (m, 1H), 4.57 (m, 1H), 4.15 (m, 2H), 3.70 (m, 2H), 2.99-3.22 (m, 2H), 1.97-2.30 (m, 4H), 1.96-2.01 (m, 2H), 1.45 (t, 3H, J = 7.36 Hz); ^{13}C NMR

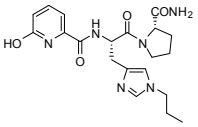
(100 MHz, CD_3OD): δ 175.83, 169.23, 161.83, 161.48, 157.08, 146.88, 134.74, 131.82, 130.71, 119.18, 118.28, 115.53, 60.17, 53.45, 43.75, 29.68, 27.24, 14.42; HRMS: *m/z* calcd for $C_{18}H_{23}N_7NaO_4$ $[M+Na]^+$: 424.1710, found 424.1711; R_f = 0.67 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 3.86 min, purity: 98.73% [CH_3CN-H_2O-TFA (70:30:0.8%)].



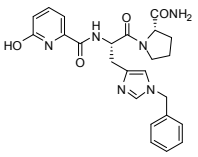
5-Hpyz-L-His(1-benzyl)-L-ProNH$_2$ (6t): Yield: 38%; white powder; mp.: 222-224°C; IR (KBr): 3481, 1645 cm^{-1}; ^1H NMR (CD_3OD): δ 8.44 (s, 1H), 7.66 (s, 1H), 7.60 (s, 1H), 7.69 (s, 1H), 7.10-7.23 (m, 5H), 7.10 (s, 1H), 5.18 (s, 2H), 4.98 (m, 1H), 4.35 (m, 1H), 3.80 (m, 2H), 3.01-3.12 (m, 2H), 2.14 (m, 2H), 1.96 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 175.82, 169.08, 162.92, 161.89, 162.24, 161.55, 147.69, 146.85, 135.21, 134.45, 130.03, 127.67, 126.11, 118.17, 60.23, 51.54, 50.21, 33.92, 29.65, 26.89, 24.38; HRMS: *m/z* calcd for $C_{23}H_{25}N_7NaO_4$ $[M+Na]^+$: 486.1866, found, 486.1860; R_f = 0.67 [CH_3OH:10% NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.56 min, purity: 97.83% [CH_3CN-H_2O-TFA (70:30:0.8%)].



6-Hpic-L-His(1-ethyl)-L-ProNH$_2$ (6u): Yield: 50%; white powder; mp.: 210-211°C; IR (KBr): 3472, 1646 cm^{-1}; ^1H NMR (CD_3OD): δ 8.75 (m, 1H) 7.44 (s, 1H), 7.37 (s, 1H), 6.81 (s, 1H), 6.79 (m, 1H), 5.16 (m, 1H), 4.87 (m, 1H), 3.82 (m, 2H), 3.49-3.62 (m, 2H), 3.19-3.26 (m, 2H), 2.65 (m, 2H), 1.80 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 177.36, 169.91, 165.47, 140.73, 139.47, 135.66, 127.87, 118.46, 118.17, 114.35, 61.23, 55.70, 49.52, 28.68, 25.52, 23.87; HRMS: *m/z* calcd for $C_{19}H_{24}N_6NaO_4$ $[M+Na]^+$:423.1757, found 423.1756; R_f = 0.37 [CH_3OH:10%, NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.32 min, purity: 96.62% [CH_3CN-H_2O-TFA (70:30:0.8%)].

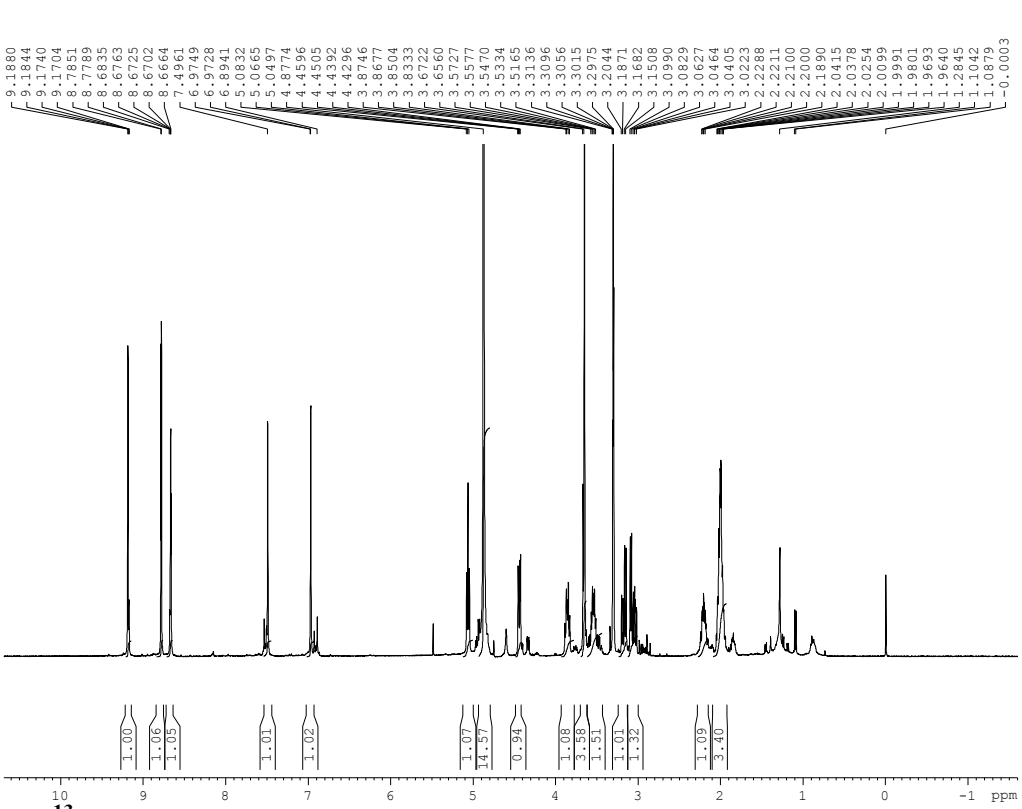


6-Hpic-L-His(1-propyl)-L-ProNH$_2$ (6v): Yield: 50%; white powder; mp.: 210-211°C; IR (KBr): 3472, 1646 cm^{-1}; ^1H NMR (CD_3OD): δ 8.58 (m, 1H) 7.52 (m, 1H), 7.10 (s, 1H), 6.60 (m, 1H), 6.67 (s, 1H), 5.40 (m, 1H), 4.98 (m, 1H), 3.93 (m, 2H), 3.64-3.47 (m, 2H), 3.06-3.16 (m, 2H), 2.11 (m, 2H), 1.10 (m, 2H), 0.68 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 168.96, 161.91, 161.57, 140.72, 134.76,129.87, 120.46, 118.17, 115.35, 60.23, 50.70, 50.52, 29.68, 26.52, 24.57, 23.02; HRMS: *m/z* calcd for $C_{20}H_{26}N_7NaO_4$ $[M+Na]^+$: 486.1860, found, 486.1862; R_f = 0.37 [CH_3OH:10%, NH_4OH:CH_2Cl_2 (8:2:90)]; HPLC: t_R = 4.00 min, purity: 99.60% [CH_3CN-H_2O-TFA (70:30:0.8%)].



6-Hpic-L-His(1-benzyl)-L-ProNH$_2$ (6w): Yield: 50%; white powder; mp.: 175-176°C; IR (KBr): 3481, 1645 cm^{-1}; ^1H NMR (CD_3OD): δ 8.44 (m, 1H), 7.58 (d, 1H, *J*= 7.28 Hz), 7.23 (s, 1H), 7.20-7.58 (s, 5H), 7.21 (s, 1H), 6.69 (m, 1H) 6.67 (s, 1H), 5.38 (s, 2H), 5.01 (m, 1H), 4.34 (m, 1H), 3.43-3.71 (m, 2H), 2.94-3.17 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 169.45, 162.92, 162.24, 161.89, 161.55, 147.69, 146.85, 135.21, 134.45, 130.10, 127.67, 126.00, 116.17, 60.23, 51.54, 50.21, 33.92, 29.65, 26.89, 24.38; HRMS: *m/z* calcd for $C_{19}H_{24}N_6NaO_4$ $[M+Na]^+$: 486.1960, found, 486.1960; R_f = 0.67 [CH_3OH:10%, NH_4OH:CH_2Cl_2 (8:2:90)] HPLC: t_R = 4.56 min, purity: 97.38% [CH_3CN-H_2O-TFA (70:30:0.8%)].

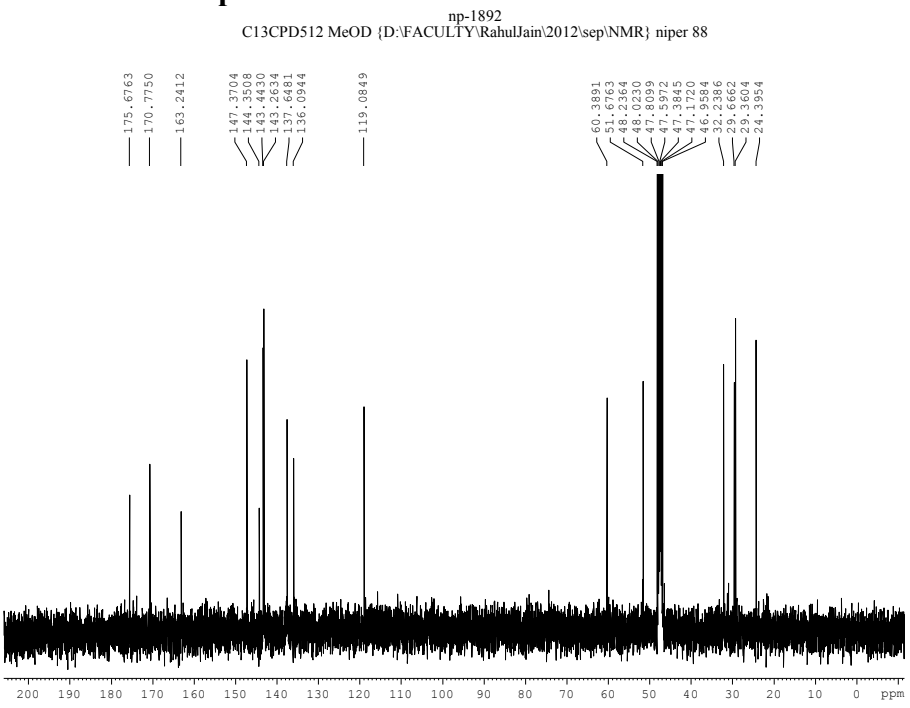
S1. ^{1}H-NMR Spectrum of 6a




```
NAME              np-1892
EXPNO                  10
PROCNO                  1
Date_            20121001
Time                 9.45
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               MeOD
NS                      16
DS                       2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ             3.9846387 sec
RG                     203
DW                60.800 usec
DE                 6.50 usec
TE                295.5 K
D1           1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.20 usec
PL1                -2.00 dB
PL1W         14.80958652 W
SFO1         400.1324710 MHz
SI                   32768
SF           400.1300097 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.40
```

S2. ^{13} C-NMR Spectrum of 6a



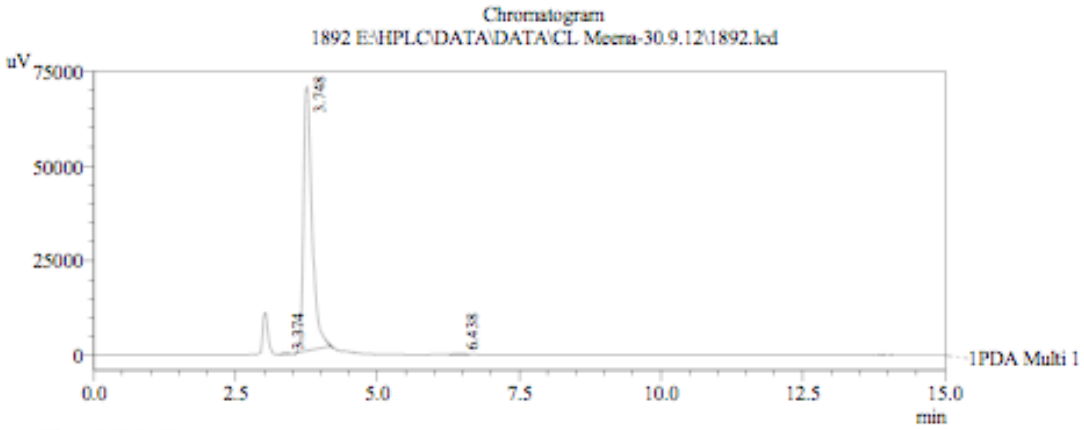

```
NAME              np-1892
EXPNO                  11
PROCNO                  1
Date_            20121001
Time                10.16
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG             zgpg30
TD                   65536
SOLVENT               MeOD
NS                     512
DS                       4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ             1.3631988 sec
RG                     203
DW                20.800 usec
DE                 6.50 usec
TE                296.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 9.50 usec
PL1               -1.00 dB
PL1W        44.90434265 W
SFO1       100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                -2.00 dB
PL12               14.33 dB
PL13               18.33 dB
PL2W        14.80958652 W
PL12W        0.34478071 W
PL13W        0.13725966 W
SFO2       400.1316005 MHz
SI                   32768
SF         100.6127690 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                   1.40
```

S3. HPLC chromatogram of 6a

Sample Information
Acquired by : CLM
Sample Name : 1892
Sample ID : 1892
Tray# : 1
Vail# : 12
Injection Volume : 10 uL
Data Filename : 1892.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 3:50:48 AM
Data Processed : 10/1/2012 12:12:32 PM
Column : RP-18
Flow Rate : 1.0 mL/min



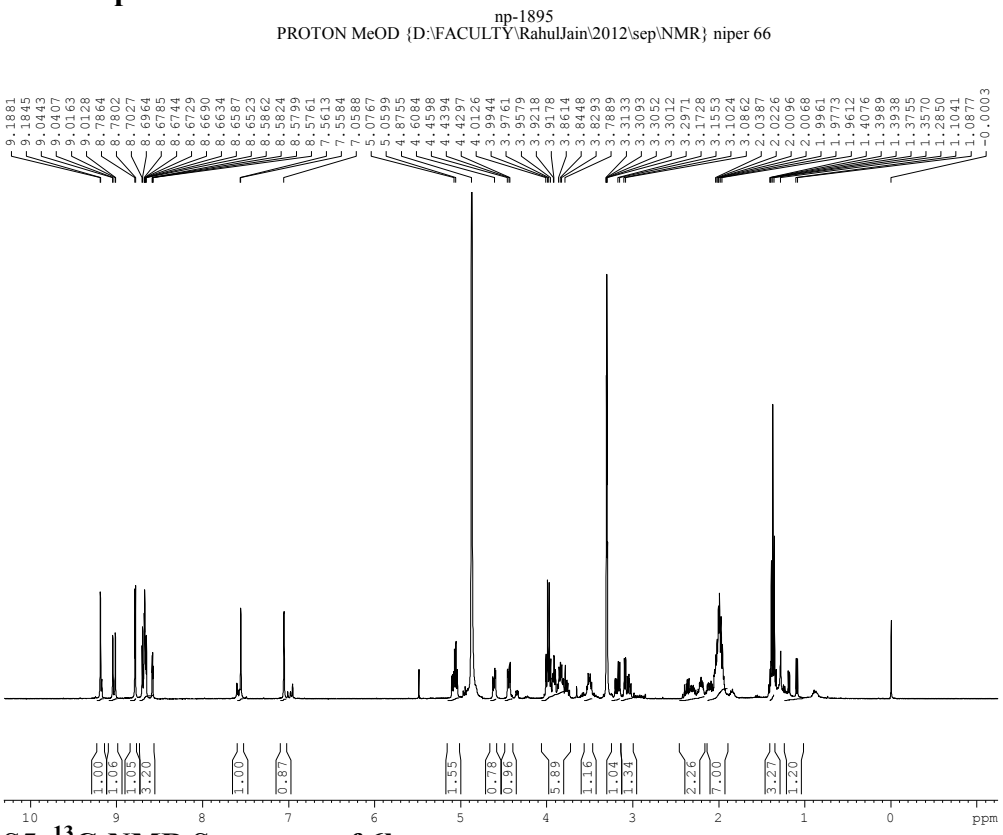
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.374	1641	251	0.224
2	3.748	728679	69936	99.547
3	6.438	1675	130	0.229
Total		731995	70317	100.000

S4. ^{1}H Spectrum of 6b

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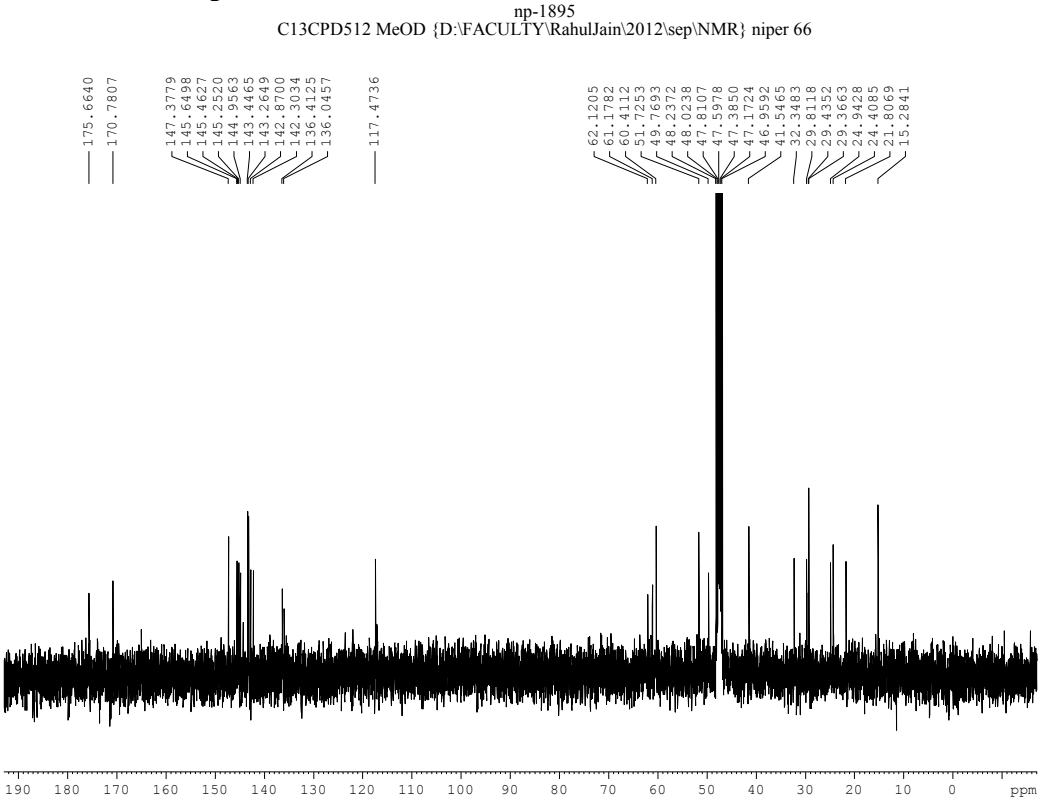
NAME            np-1895
EXPNO                10
PROCNO                1
Date_          20120928
Time              16.46
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                  203
DW                60.800 usec
DE                 6.50 usec
TE                295.5 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                12.20 usec
PL1               -2.00 dB
PL1W        14.80958652 W
SFO1       400.1324710 MHz
SI                32768
SF         400.1300098 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.40
```

S5. ^{13}C-NMR Spectrum of 6b



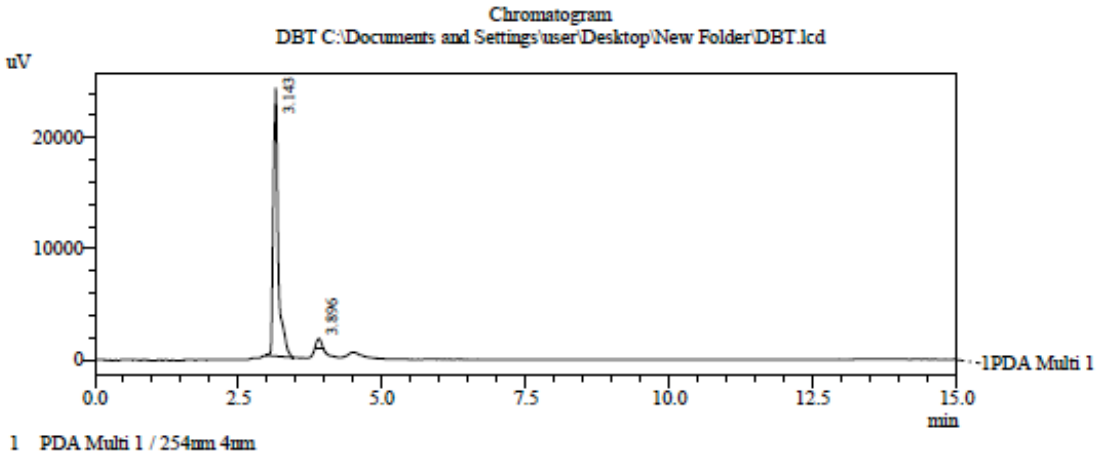

```
NAME            np-1895
EXPNO                11
PROCNO                1
Date_          20121001
Time              19.30
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                  512
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631988 sec
RG                  203
DW                20.800 usec
DE                 6.50 usec
TE                296.6 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                 9.50 usec
PL1               -1.00 dB
PL1W        44.90434265 W
SFO1       100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -2.00 dB
PL12              14.33 dB
PL13              18.33 dB
PL2W        14.80958652 W
PL12W        0.34478071 W
PL13W        0.13725966 W
SFO2       400.1316005 MHz
SI                32768
SF         100.6127690 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

S6. HPLC Spectrum of 6b

Sample Information
Acquired by : SChhuttan L. Meena
Sample Name : DBT
Sample ID : DBT
Tray# : 1
Vail# : 9
Injection Volume : 10 uL
Data Filename : DBT.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 3:04:14 AM
Data Processed : 10/1/2012 2:03:05 PM
Column : RP-18
Flow Rate : 1.0 mL/min



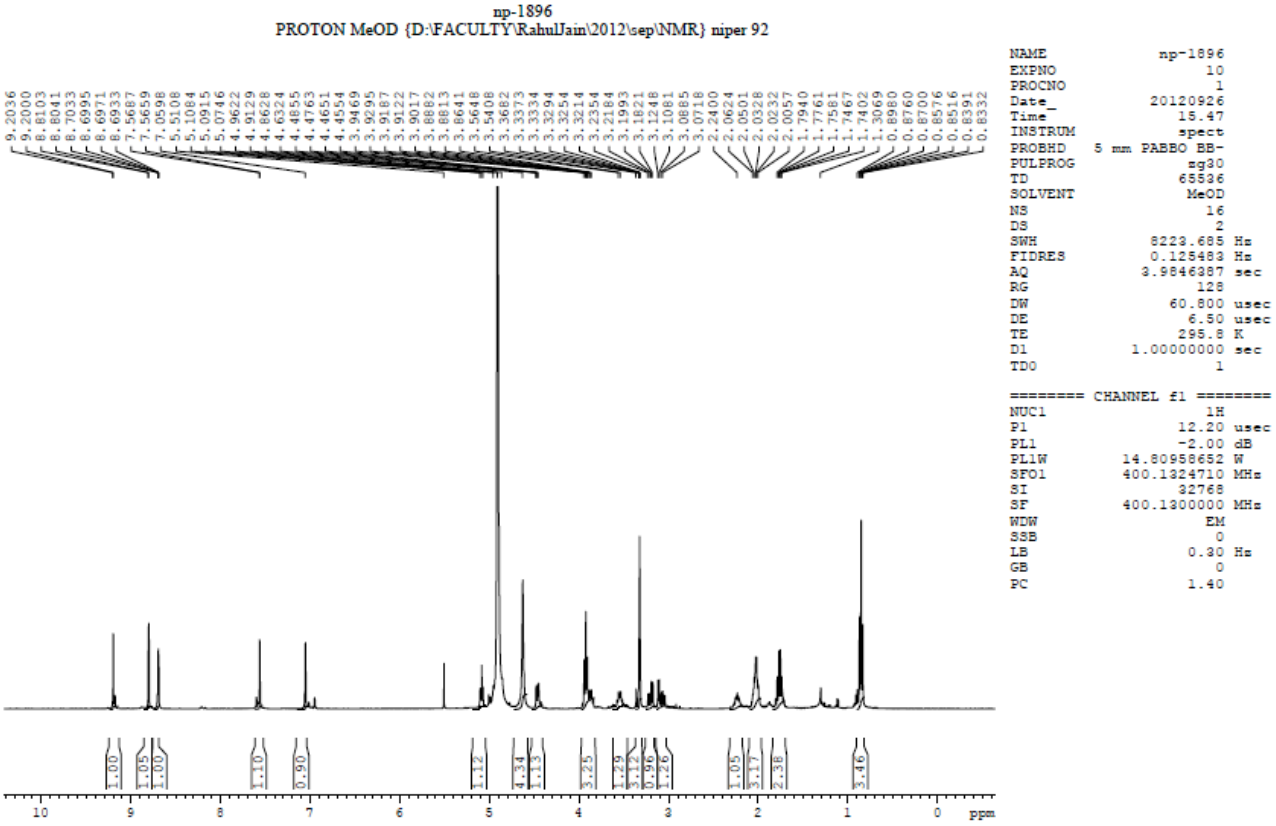
1 PDA Multi 1 / 254nm 4nm

PeakTable

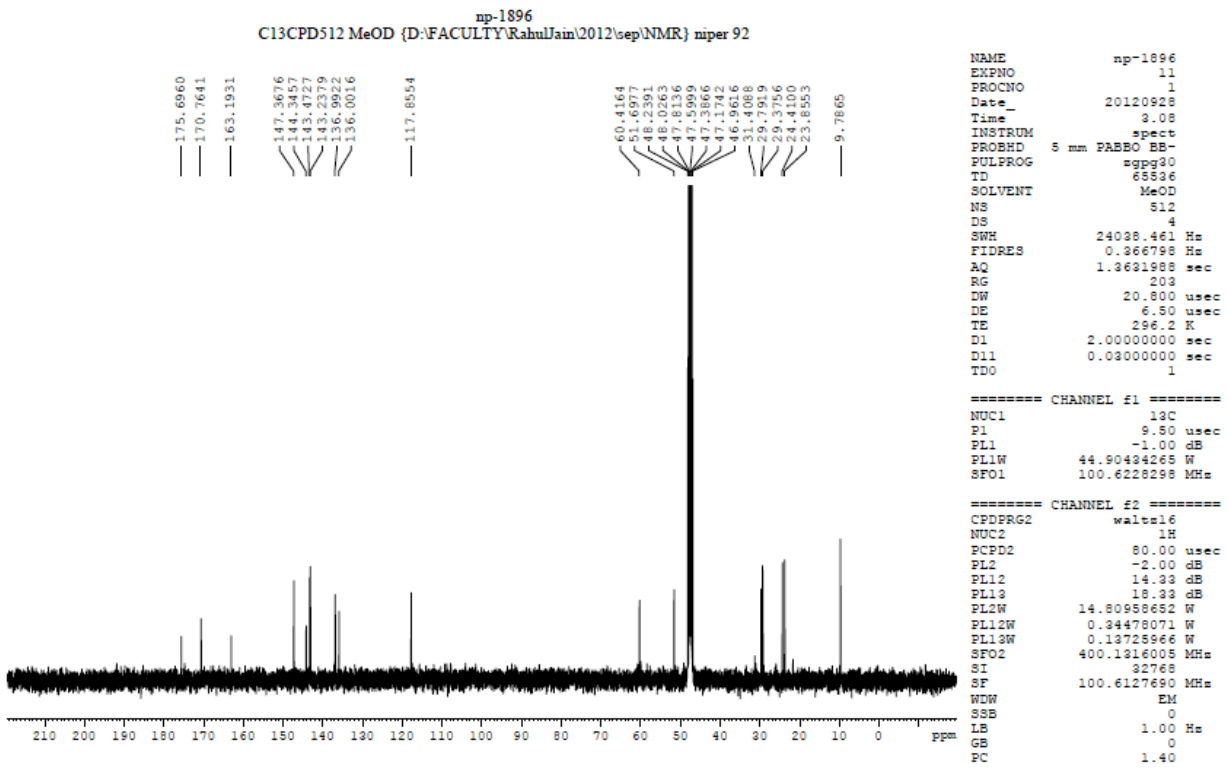
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.143	139763	24096	96.302
2	3.896	5367	937	3.698
Total		145130	25033	100.000

S7. ^{1}H-NMR Spectrum of 6c



S8. ^{13}C-NMR Spectrum of 6c

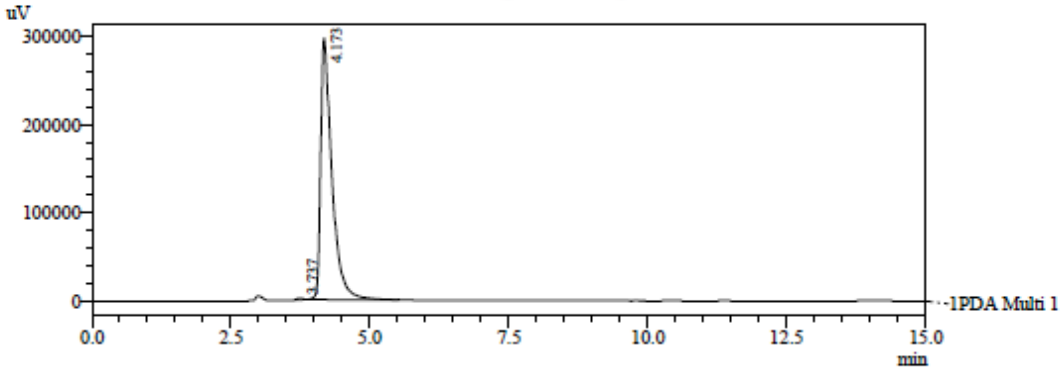


S9. HPLC chromatogram of 6c

NIPER

Sample Information
Acquired by : Chhuttan . Meena
Sample Name : NP-1896
Sample ID : NP-1896
Tray# : 1
Vail# : 7
Injection Volume : 10 uL
Data Filename : NP-1896.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B2.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 6:20:25 PM
Data Processed : 10/1/2012 2:06:00 PM
Column : RP-18
Flow Rate : 1.0 mL/min

Chromatogram
NP-1896 C:\Documents and Settings\user\Desktop\New Folder\NP-1896.lcd



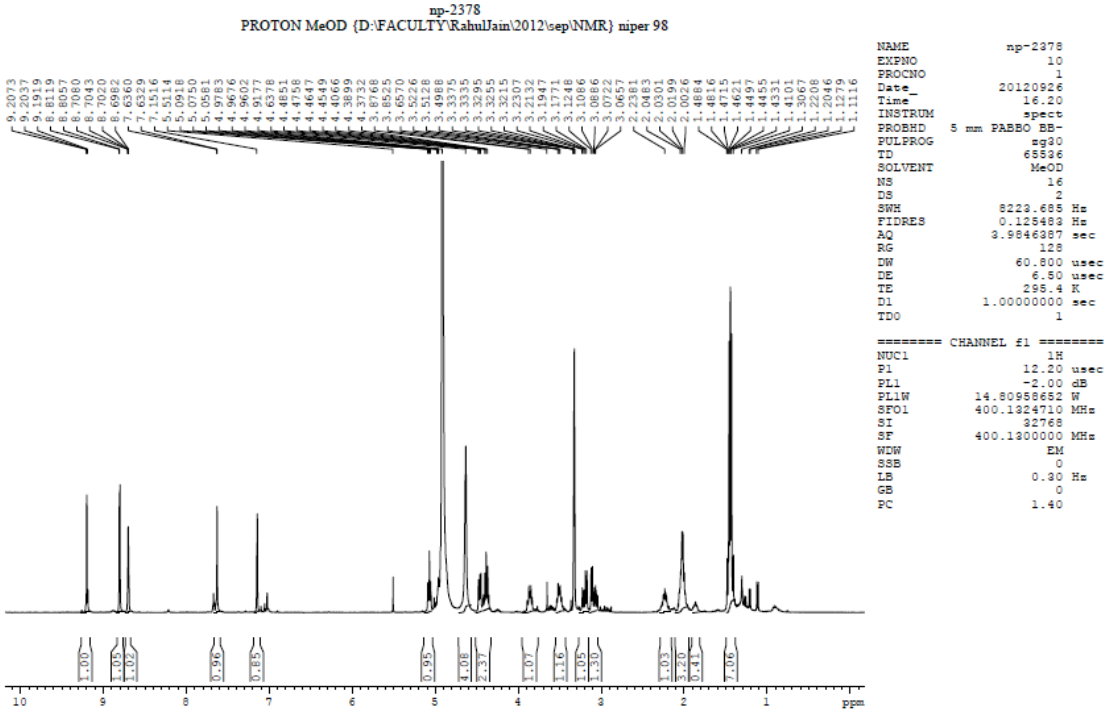
1 PDA Multi 1 / 254nm 4nm

PeakTable

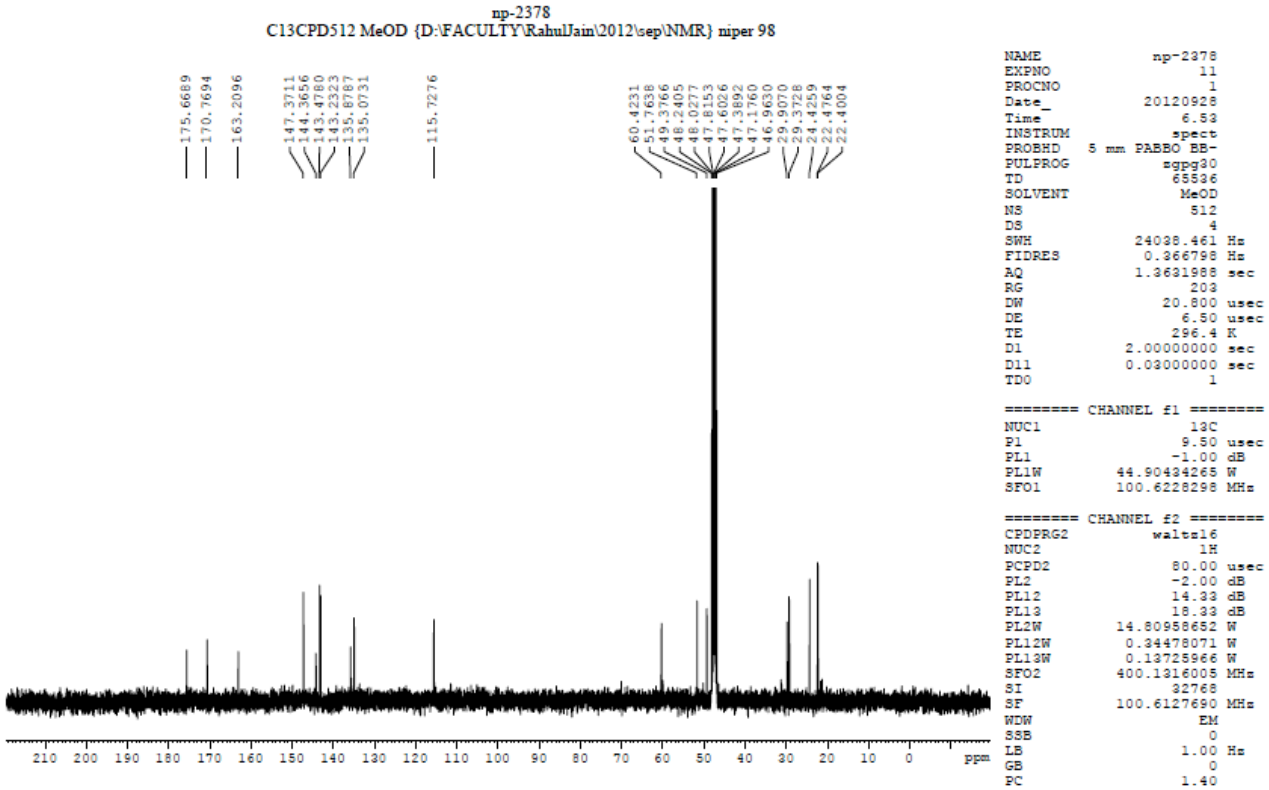
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.737	10005	1658	0.238
2	4.173	4190242	296170	99.762
Total		4200247	297827	100.000

S10. [1]H-NMR Spectrum of 6d



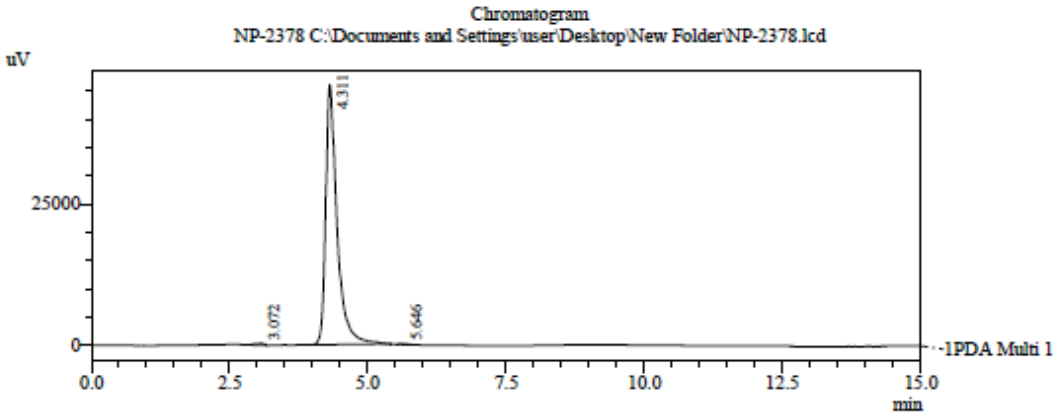
S11. [13]C-NMR Spectrum of 6d



S12. HPLC chromatogram of 6d

NIPER

Sample Information
Acquired by : cHHUTTAN l. mEENA
Sample Name : NP-2378
Sample ID : NP-2378
Tray# : 1
Vail# : 1
Injection Volume : 10 uL
Data Filename : NP-2378.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 1:00:07 AM
Data Processed : 10/1/2012 2:23:15 PM
Column : RP-18
Flow Rate : 1.0 mL/min



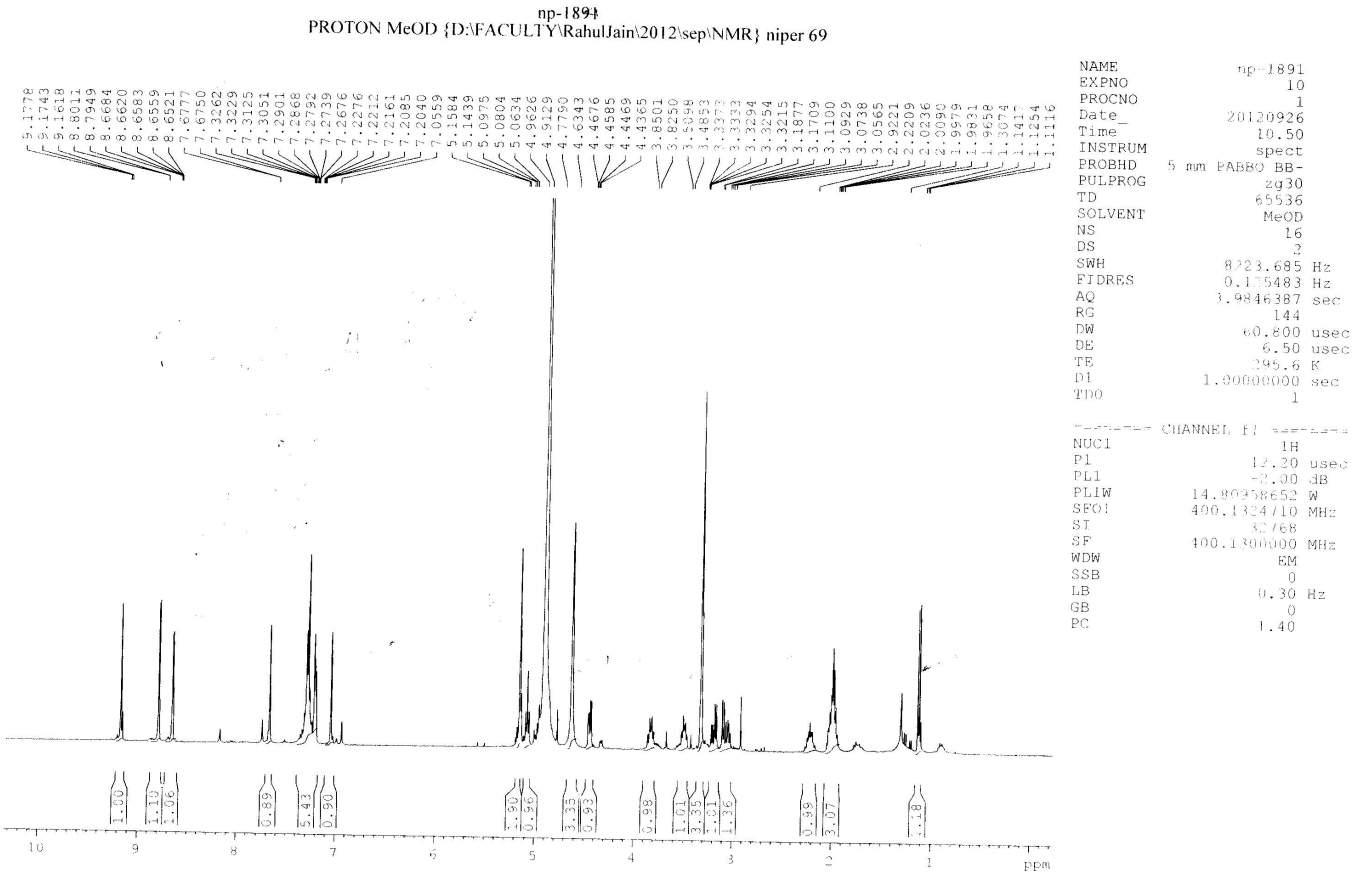
1 PDA Multi 1 / 254nm 4nm

PeakTable

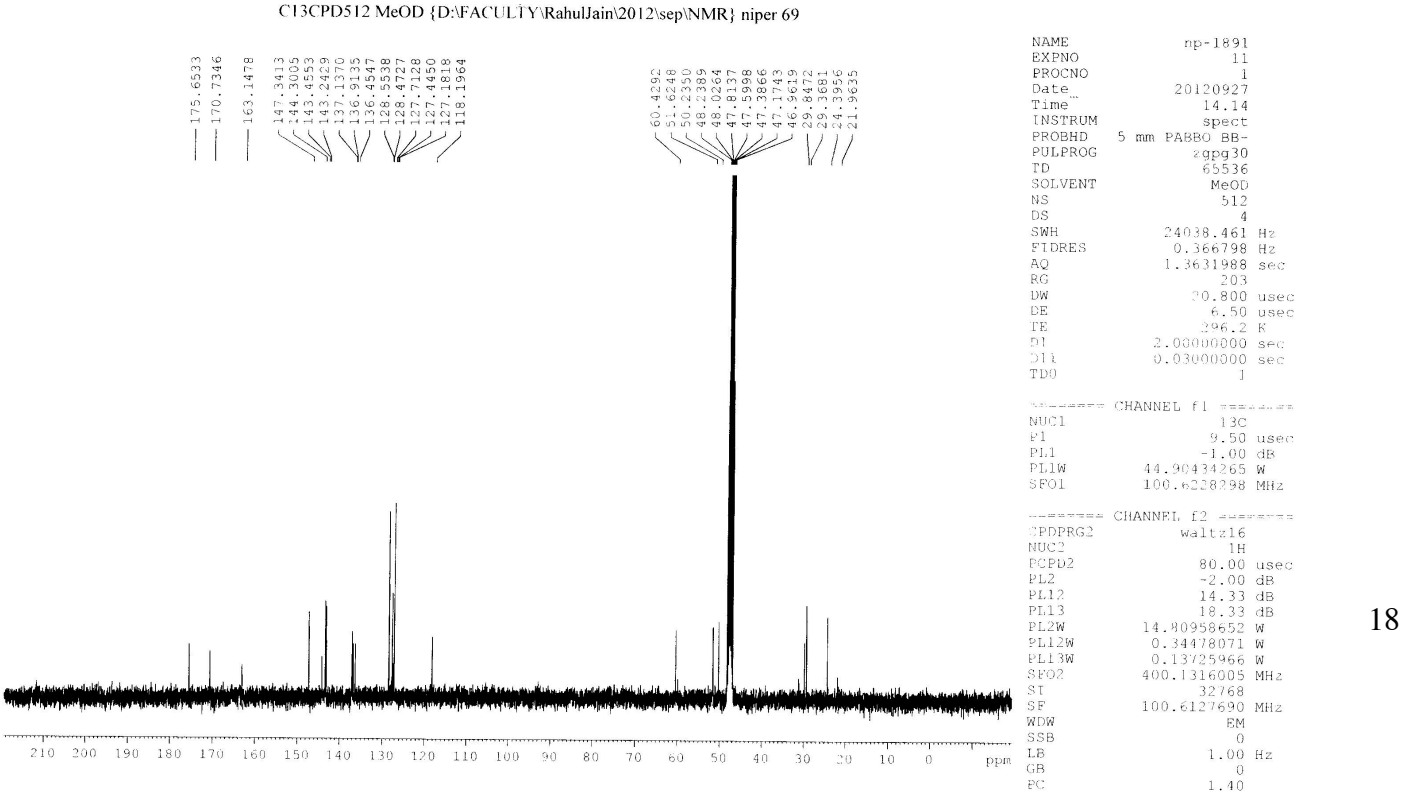
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.072	4225	374	0.632
2	4.311	662891	45992	99.226
3	5.646	942	109	0.141
Total		668058	46475	100.000

S13. ^{1}H-NMR Spectrum of 6e



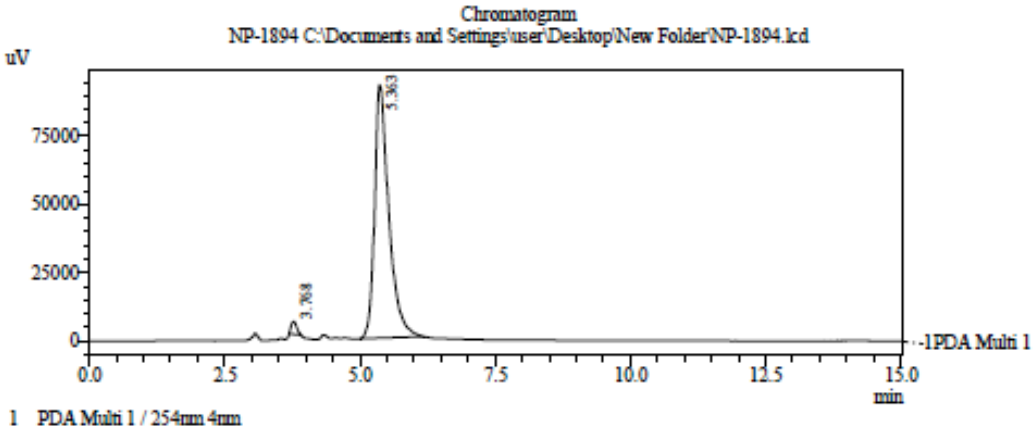
S14. ^{13}C-NMR Spectrum of 6e



S15. HPLC chromatogram of 6e

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : NP-1894
Sample ID : NP-1894
Tray# : 1
Vail# : 8
Injection Volume : 10 uL
Data Filename : NP-1894.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B2.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 6:35:58 PM
Data Processed : 10/1/2012 2:05:16 PM
Column : RP-18
Flow Rate : 1.0 mL/min



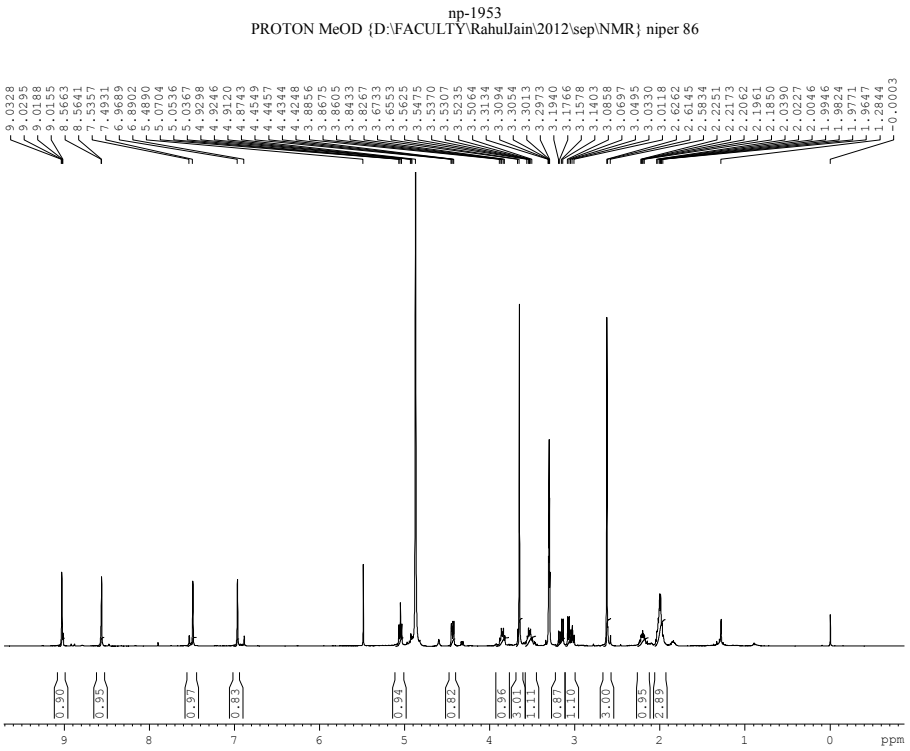
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.768	33318	4701	1.928
2	5.363	1694644	92717	98.072
Total		1727962	97418	100.000

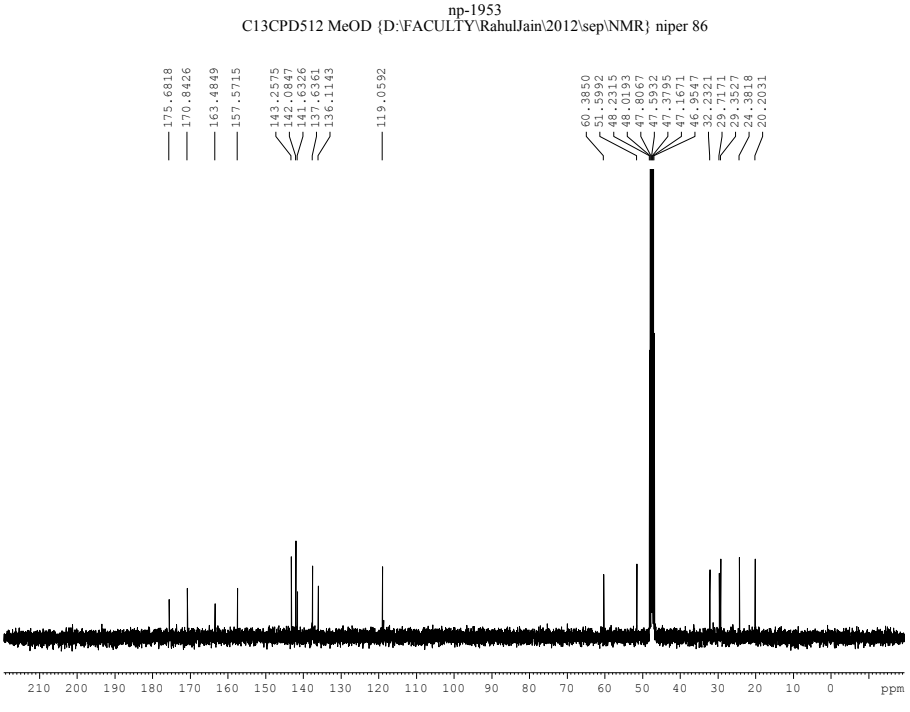
S16. ^{1}H-NMR Spectrum of 6f




```
NAME            np-1953
EXPNO                10
PROCNO                1
Date_          20120929
Time               1.31
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                  203
DW               60.800 usec
DE                6.50 usec
TE               295.7 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                12.20 usec
PL1               -2.00 dB
PL1W        14.80958652 W
SFO1        400.1324710 MHz
SI                32768
SF          400.1300098 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.40
```

S17. ^{13}C-NMR Spectrum of 6f




```
NAME            np-1953
EXPNO                11
PROCNO                1
Date_          20120929
Time               2.01
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                  512
DS                    4
SWH           24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631988 sec
RG                  203
DW               20.800 usec
DE                6.50 usec
TE               296.3 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

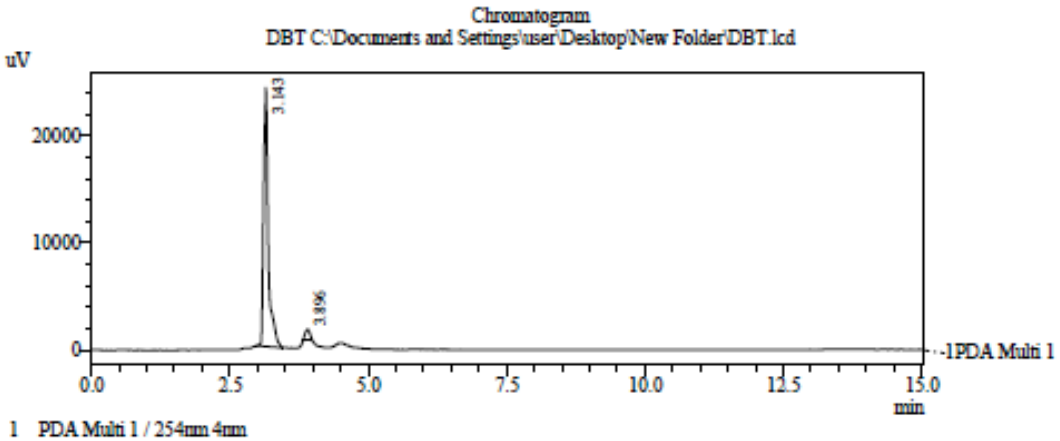
======== CHANNEL f1 ========
NUC1                13C
P1                 9.50 usec
PL1               -1.00 dB
PL1W        44.90434265 W
SFO1        100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -2.00 dB
PL12              14.33 dB
PL13              18.33 dB
PL2W        14.80958652 W
PL12W        0.34478071 W
PL13W        0.13725966 W
SFO2        400.1316005 MHz
SI                32768
SF          100.6127690 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

S18. HPLC chromatogram of 6f

NIPER

Sample Information
Acquired by : SChhuttan L. Meena
Sample Name : DBT
Sample ID : DBT
Tray# : 1
Vail# : 9
Injection Volume : 10 uL
Data Filename : DBT.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 3:04:14 AM
Data Processed : 10/1/2012 2:03:05 PM
Column : RP-18
Flow Rate : 1.0 mL/min



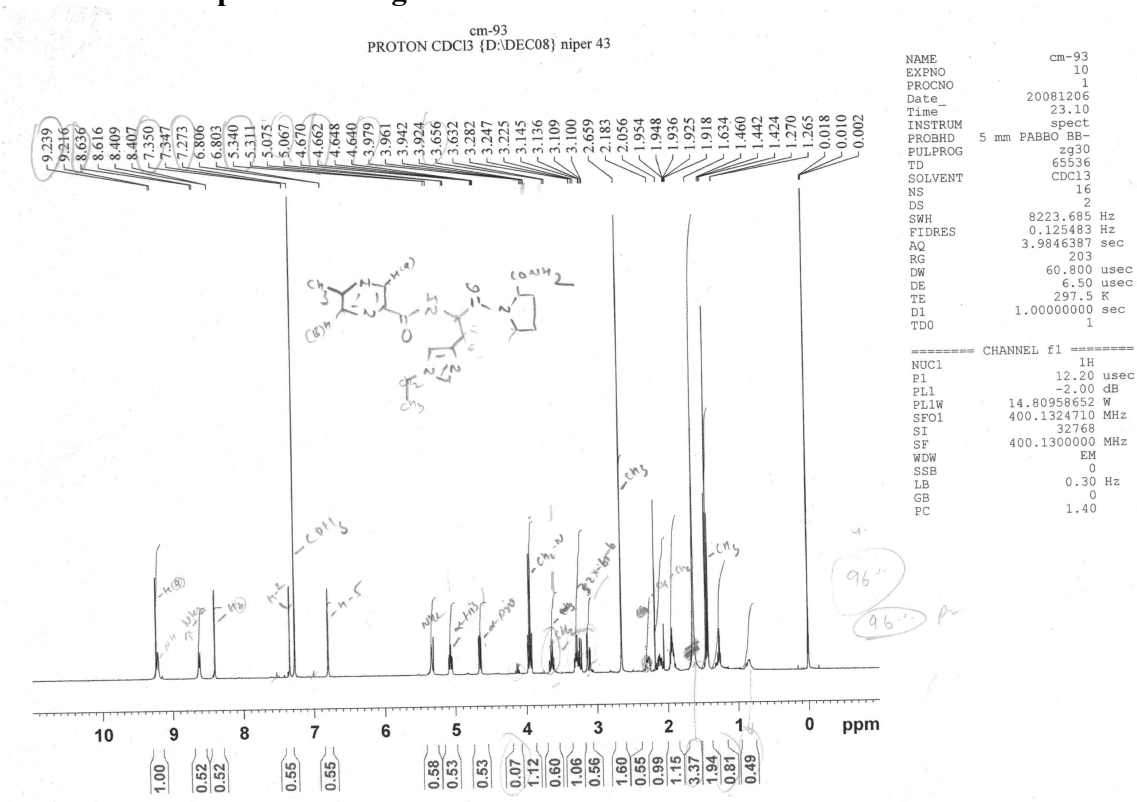
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.143	139763	24096	96.302
2	3.896	5367	937	3.698
Total		145130	25033	100.000

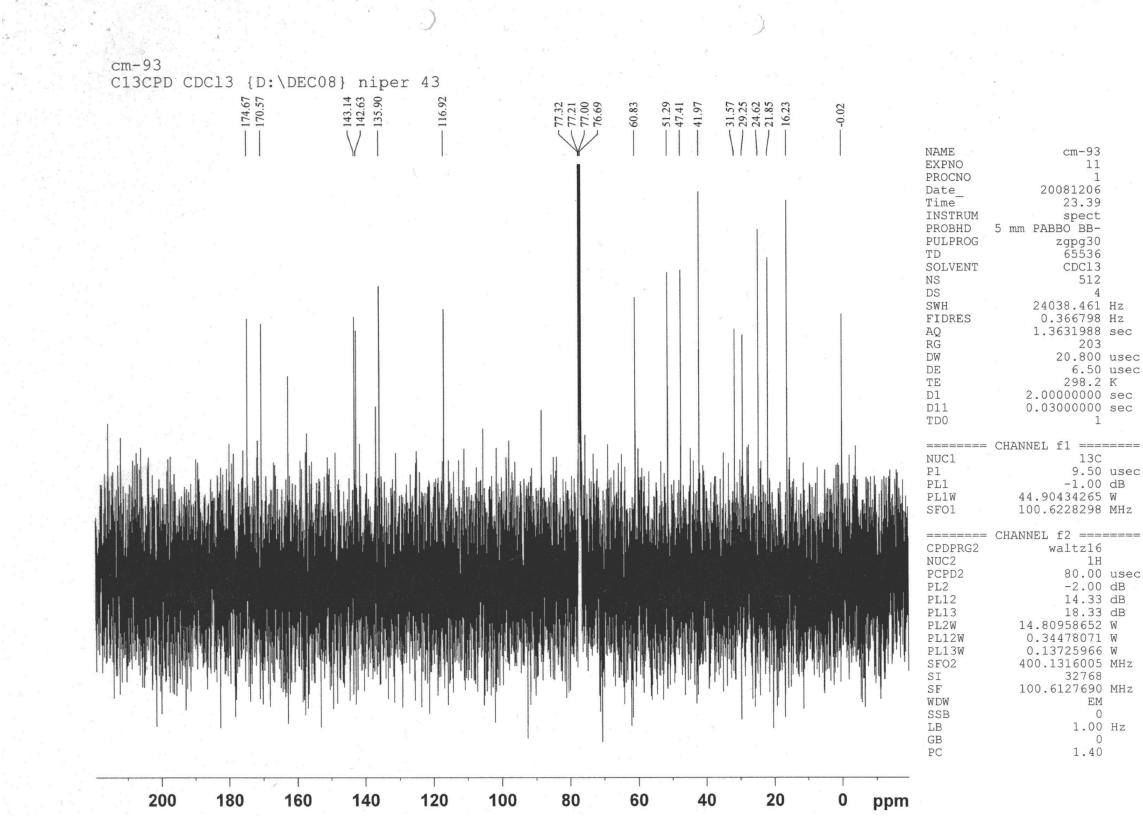
S19. ^{1}H-NMR Spectrum of 6g




```
NAME                cm-93
EXPNO                  10
PROCNO                  1
Date_            20081206
Time                23.10
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                    203
DW                60.800 usec
DE                 6.50 usec
TE                297.5 K
D1               1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.20 usec
PL1                -2.00 dB
PL1W          14.80958652 W
SFO1          400.1324710 MHz
SI                  32768
SF            400.1300000 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                 1.40
```

S20. ^{13}C-NMR Spectrum of 6g




```
NAME                cm-93
EXPNO                  11
PROCNO                  1
Date_            20081206
Time                23.39
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG           zgpg30
TD                 65536
SOLVENT             CDCl3
NS                    512
DS                      4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                    203
DW                20.800 usec
DE                 6.50 usec
TE                298.2 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                     1

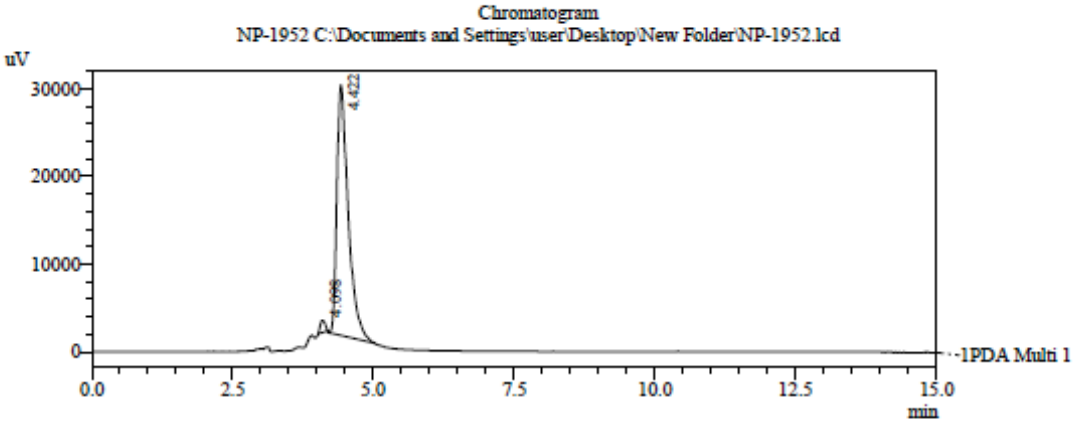
======== CHANNEL f1 ========
NUC1                  13C
P1                 9.50 usec
PL1                -1.00 dB
PL1W          44.90434265 W
SFO1          100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                -2.00 dB
PL12               14.33 dB
PL13               18.33 dB
PL2W          14.80958652 W
PL12W          0.34478071 W
PL13W          0.13725966 W
SFO2          400.1316005 MHz
SI                  32768
SF            100.6127690 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                 1.40
```

S21. HPLC chromatogram of 6g

NIPER

Sample Information
Acquired by : chhuttan
Sample Name : NP-1952
Sample ID : NP-1952
Tray# : 1
Vail# : 8
Injection Volume : 10 uL
Data Filename : NP-1952.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 2:48:46 AM
Data Processed : 10/1/2012 2:08:21 PM
Column : RP-18
Flow Rate : 1.0 mL/min



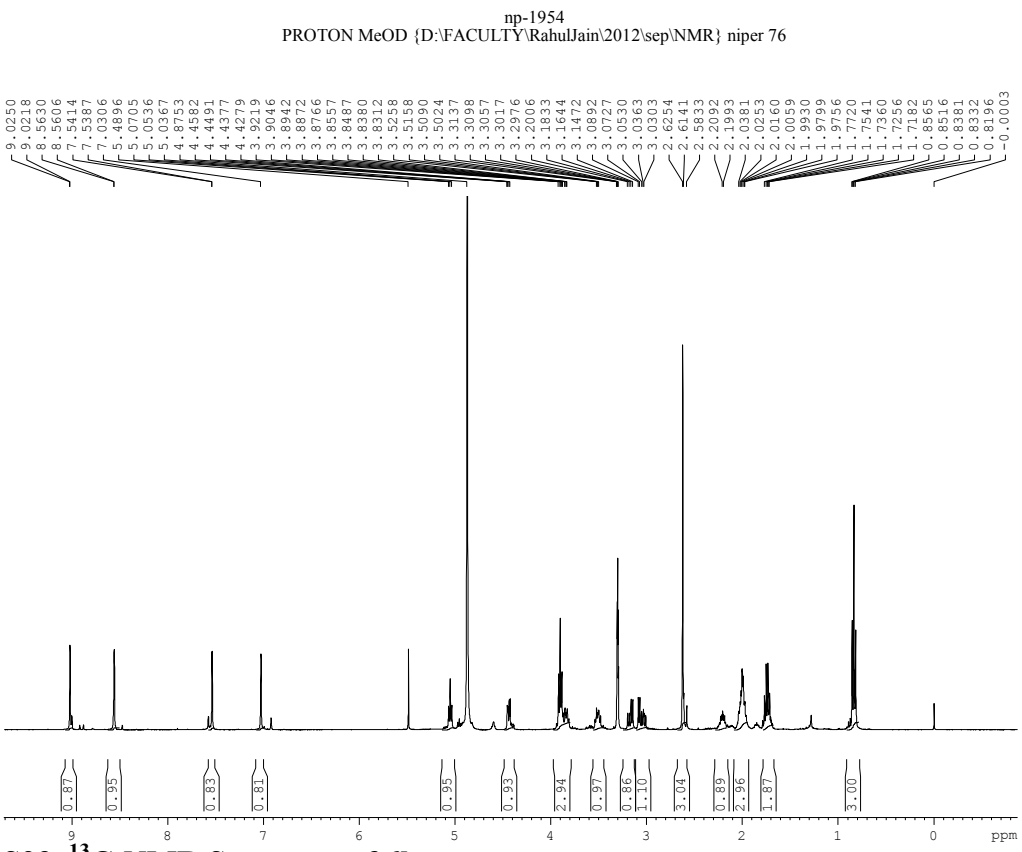
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	4.098	8404	1367	2.059
2	4.422	399858	28497	97.941
Total		408263	29865	100.000

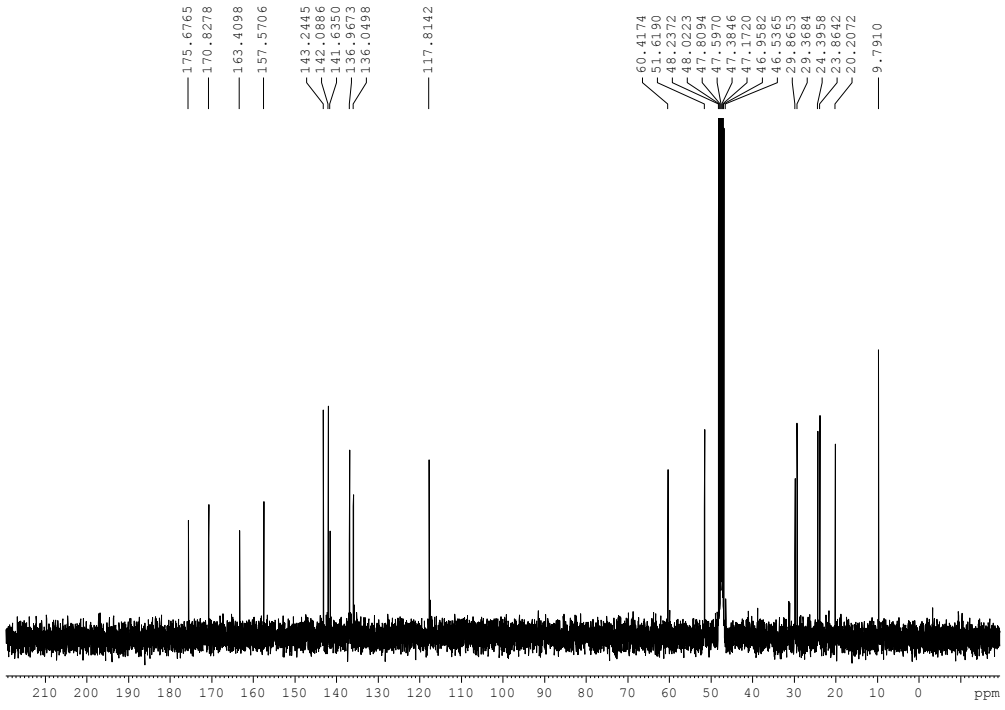
S22. ^{1}H-NMR Spectrum of 6h



NAME	np-1954
EXPNO	10
PROCNO	1
Date_	20120928
Time	19.09
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	MeOD
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	203
DW	60.800 usec
DE	6.50 usec
TE	295.7 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.20 usec
PL1	-2.00 dB
PL1W	14.80958652 W
SFO1	400.1324710 MHz
SI	32768
SF	400.1300095 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

S23. ^{13}C-NMR Spectrum of 6h

np-1954
C13CPD512 MeOD {D:\FACULTY\RahulJain\2012\sep\NMR} niper 76



NAME	np-1954
EXPNO	11
PROCNO	1
Date_	20120928
Time	19.39
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	MeOD
NS	512
DS	4
SWH	24038.461 Hz
FIDRES	0.366798 Hz
AQ	1.3631988 sec
RG	203
DW	20.800 usec
DE	6.50 usec
TE	296.3 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	9.50 usec
PL1	-1.00 dB
PL1W	44.90434265 W
SFO1	100.6228298 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-2.00 dB
PL12	14.33 dB
PL13	18.33 dB
PL2W	14.80958652 W
PL12W	0.34478071 W
PL13W	0.13725966 W
SFO2	400.1316005 MHz
SI	32768
SF	100.6127690 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

S24. HRMS Spectrum of 6h

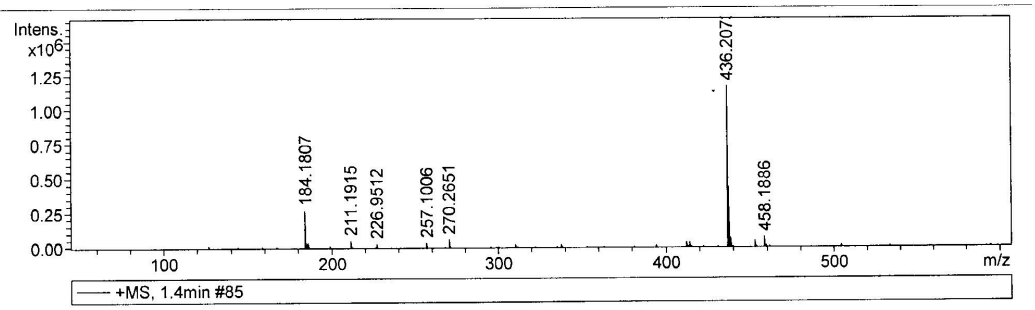
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\Rahul jain\12-09-05-NP-1954.d	Acquisition Date	9/5/2012 2:25:06 PM
Method	sodium formate tune_low.m	Operator	VIKAS GROVER
Sample Name	NP-1954	Instrument / Ser#	maXis 40
Comment			

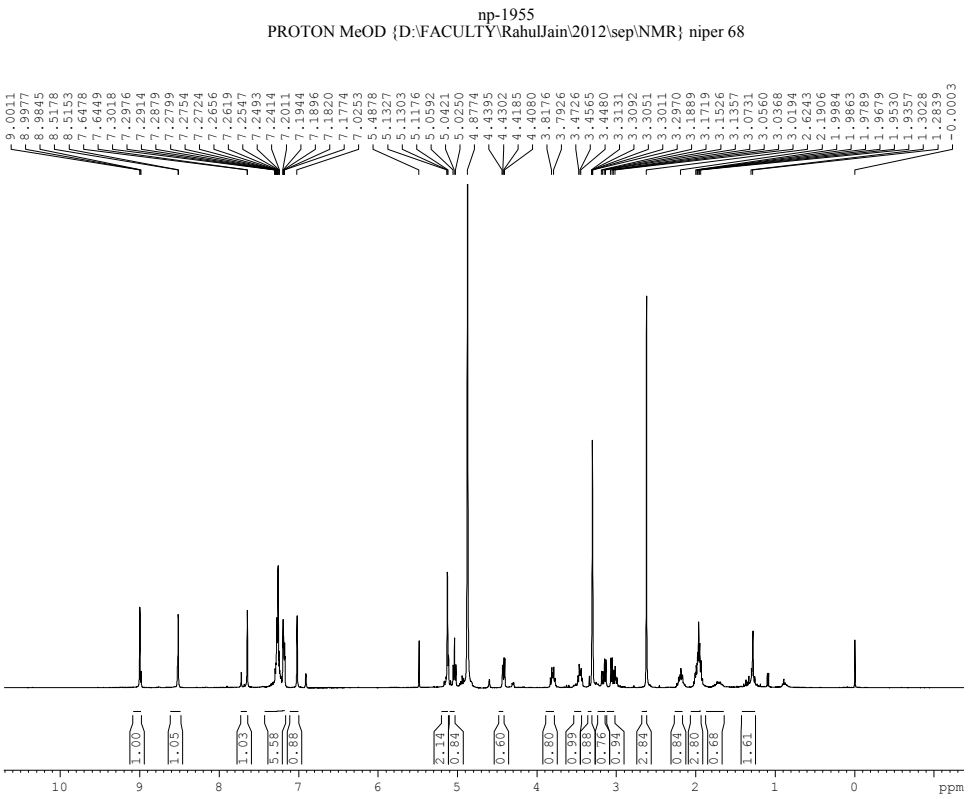
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	5.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	600 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source



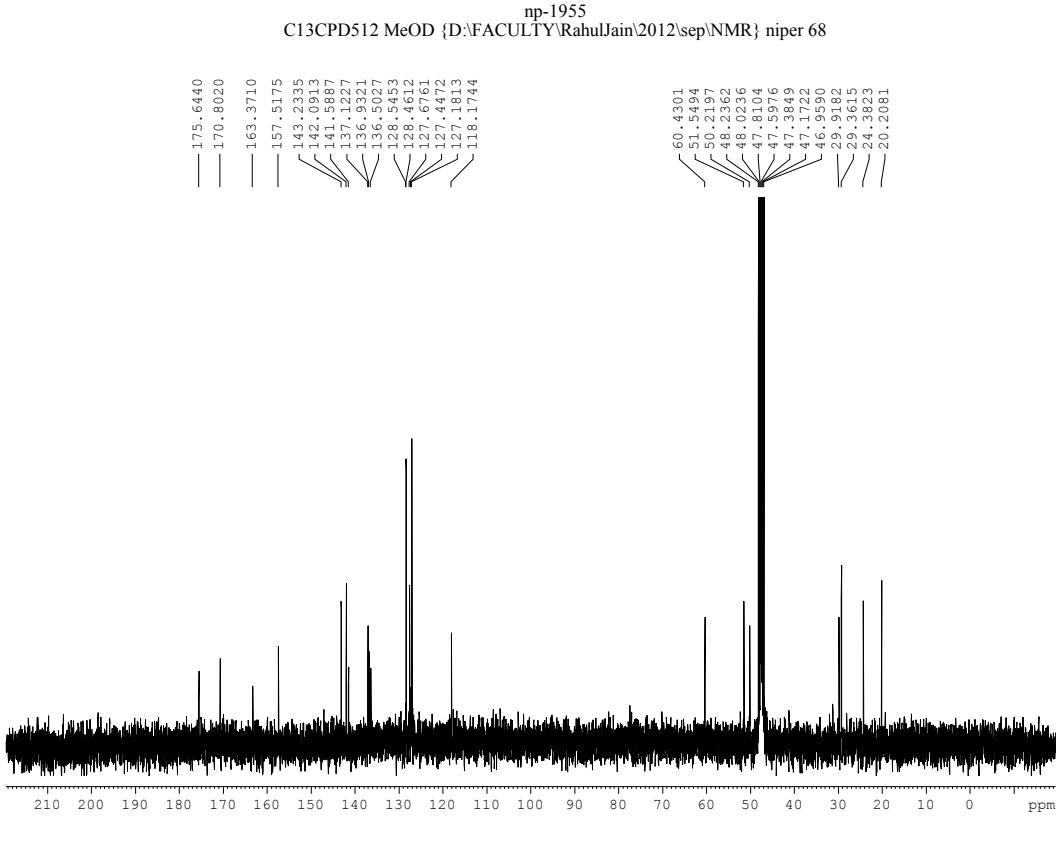
#	m/z	Res.	S/N	I	FWHM
1	184.1807	18490	3037.5	275348	0.0100
2	211.1915	19027	602.3	57808	0.0111
3	226.9512	19466	339.7	31904	0.0117
4	257.1006	20457	427.6	39693	0.0126
5	270.2651	20327	647.8	63873	0.0133
6	436.2073	17178	5938.2	1168376	0.0254
7	437.2094	20672	2226.2	441534	0.0211
8	438.2118	21621	346.4	67969	0.0203
9	452.1807	22482	267.9	44575	0.0201
10	458.1886	21800	495.3	76065	0.0210

S25. ^{1}H-NMR Spectrum of 6i



NAME	np-1955
EXPNO	10
PROCNO	1
Date_	20120928
Time	16.56
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	MeOD
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	203
DW	60.800 usec
DE	6.50 usec
TE	295.5 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.20 usec
PL1	-2.00 dB
PL1W	14.80958652 W
SFO1	400.1324710 MHz
SI	32768
SF	400.1300098 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

S26. ^{13}C-NMR Spectrum of 6i




```
NAME            np-1955
EXPNO                11
PROCNO                1
Date_          20121001
Time              20.41
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                  512
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631988 sec
RG                  203
DW               20.800 usec
DE                6.50 usec
TE                296.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                 9.50 usec
PL1               -1.00 dB
PL1W        44.90434265 W
SFO1       100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                -2.00 dB
PL12               14.33 dB
PL13               18.33 dB
PL2W        14.80958652 W
PL12W        0.34478071 W
PL13W        0.13725966 W
SFO2       400.1316005 MHz
SI               32768
SF         100.6127690 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

S27. HRMS Spectrum of 6i

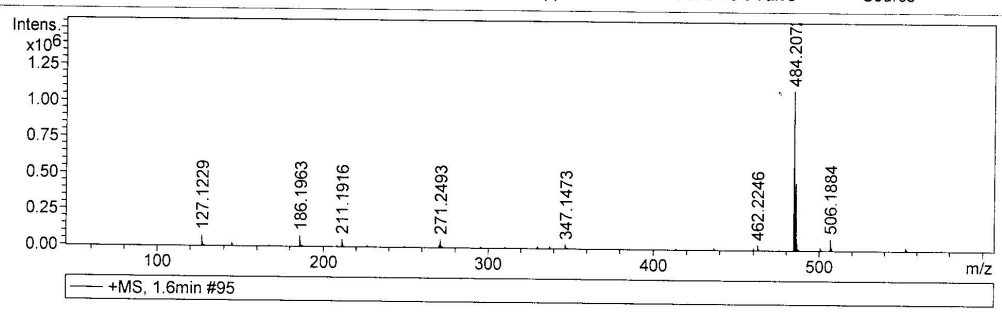
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\Rahul jain\12-09-05-NP-1955.d	Acquisition Date	9/5/2012 1:45:11 PM
Method	sodium formate tune_low.m	Operator	VIKAS GROVER
Sample Name	NP-1955	Instrument / Ser#	maXis 40
Comment			

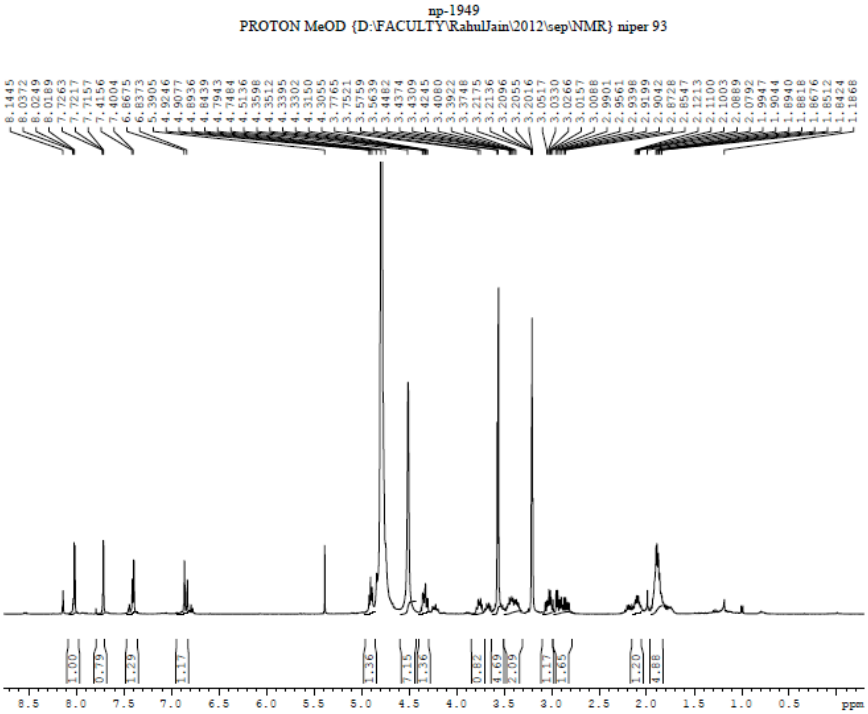
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	5.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	600 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

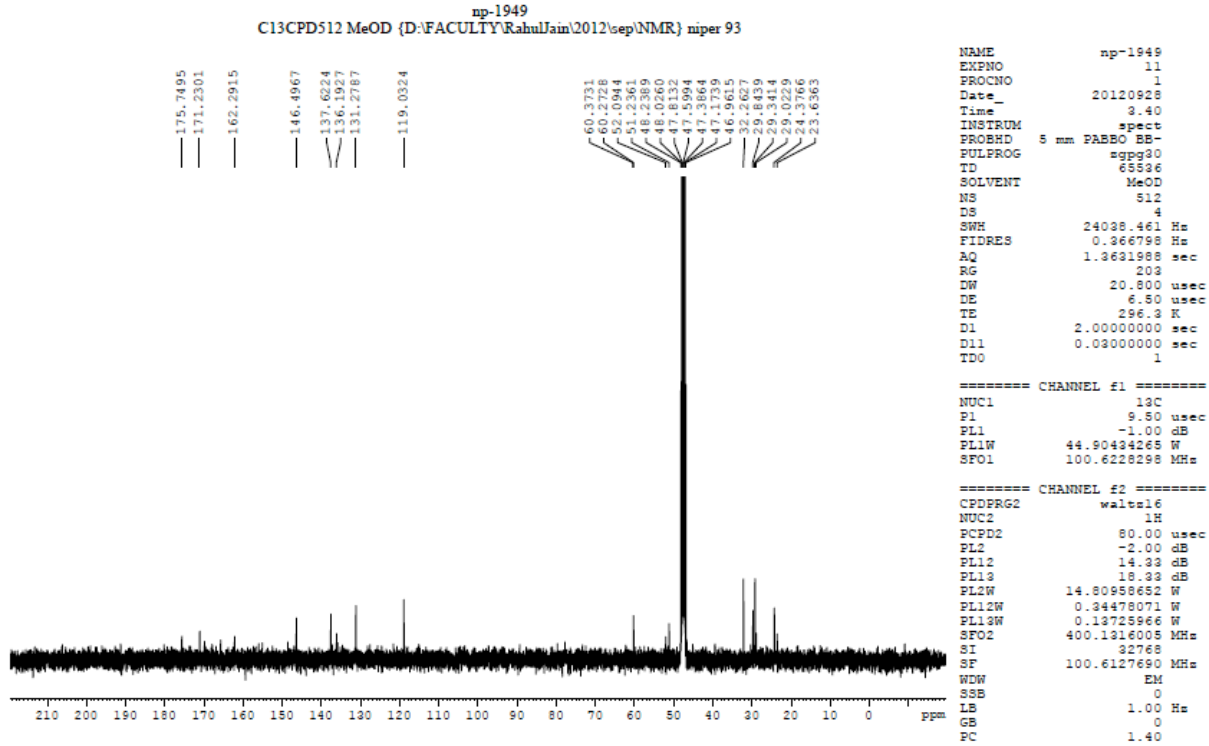


#	m/z	Res.	S/N	I	FWHM
1	127.1229	17232	917.7	75235	0.0074
2	186.1963	17699	897.1	73929	0.0105
3	211.1916	18556	620.1	52791	0.0114
4	257.1003	20027	35.0	3232	0.0128
5	271.2493	20704	592.0	55640	0.0131
6	330.1420	21411	141.1	12543	0.0154
7	337.3074	21057	134.8	12181	0.0160
8	347.1473	22362	288.6	26628	0.0155
9	462.2246	22398	222.5	33613	0.0206
10	484.2072	17916	6885.6	1096984	0.0270
11	485.2094	21283	2927.9	461309	0.0228
12	486.2120	22361	503.2	78397	0.0217
13	506.1884	22942	665.1	80277	0.0221

S28. ^{1}H-NMR Spectrum of 6j



S29. ^{13}C-NMR Spectrum of 6j

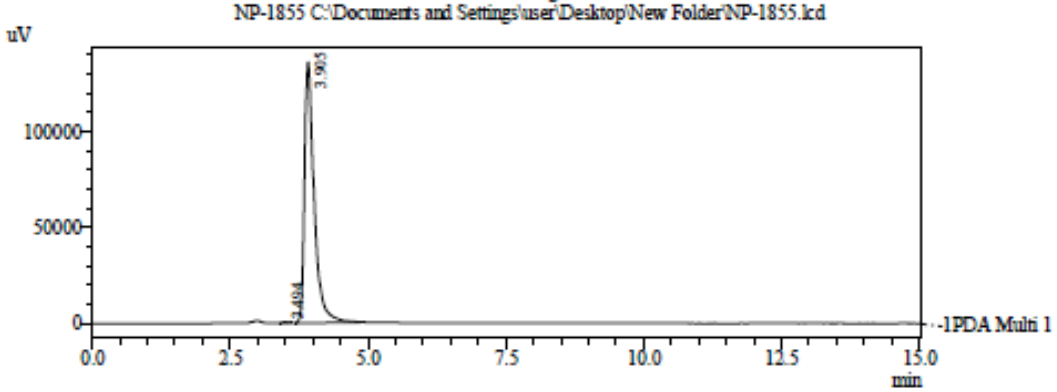


S30. HPLC chromatogram of 6j

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : NP-1855
Sample ID : NP-1855
Tray# : 1
Vail# : 2
Injection Volume : 10 uL
Data Filename : NP-1855.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B2.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 5:02:53 PM
Data Processed : 10/1/2012 2:03:42 PM
Column : RP-18
Flow Rate : 1.0 mL/min

Chromatogram
NP-1855 C:\Documents and Settings\user\Desktop\New Folder\NP-1855.lcd



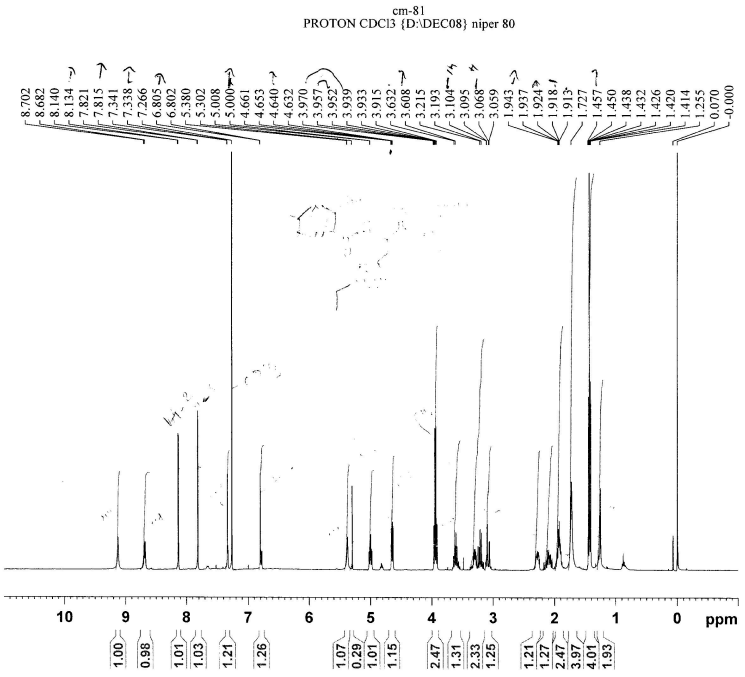
1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.494	1976	324	0.119
2	3.905	1660177	135567	99.881
Total		1662152	135892	100.000

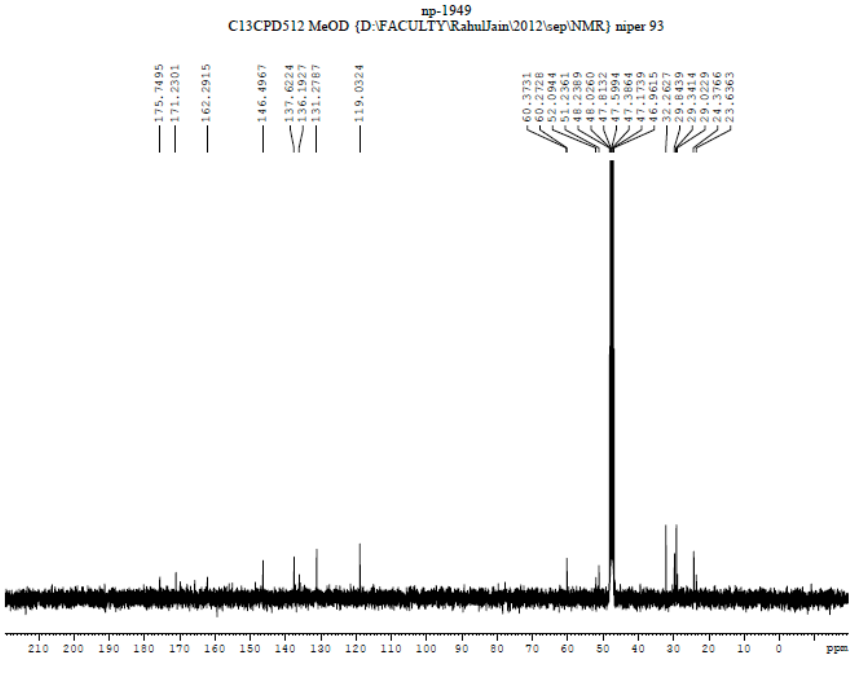
S31. ^{1}H-NMR Spectrum of 6k




```
NAME                cm-81
EXPNO                  10
PROCNO                  1
Date_            20081210
Time                7.00
INSTRUM            spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                    203
DW                 60.800 usec
DE                  6.50 usec
TE                 297.2 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.20 usec
PL1                -2.00 dB
PL1W         14.80958652 W
SFO1         400.1324710 MHz
SI                  32768
SF           400.1300031 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.40
```

S32. ^{13}C NMR spectrum of 6k




```
NAME                np-1949
EXPNO                  11
PROCNO                  1
Date_            20120928
Time                3.40
INSTRUM            spect
PROBHD    5 mm PABBO BB-
PULPROG            zgpg30
TD                  65536
SOLVENT              MeOD
NS                    512
DS                      4
SWH              24038.461 Hz
FIDRES           0.366798 Hz
AQ               1.3631988 sec
RG                    203
DW                 20.800 usec
DE                  6.50 usec
TE                 296.3 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1

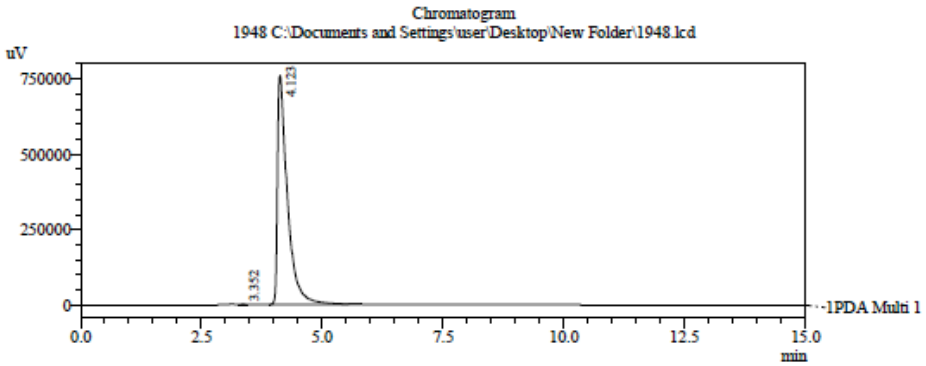
======== CHANNEL f1 ========
NUC1                  13C
P1                  9.50 usec
PL1                -1.00 dB
PL1W         44.90434265 W
SFO1         100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                -2.00 dB
PL12               14.33 dB
PL13               18.33 dB
PL2W         14.80958652 W
PL12W         0.34478071 W
PL13W         0.13725966 W
SFO2         400.1316005 MHz
SI                  32768
SF           100.6127690 MHz
WDW                   EM
SSB                    0
LB                 1.00 Hz
GB                     0
PC                 1.40
```

S33. HPLC chromatogram of 6k

NIPER

Sample Information
Acquired by : CLM
Sample Name : 1948
Sample ID : 1948
Tray# : 1
Vail# : 24
Injection Volume : 10 uL
Data Filename : 1948.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 6:57:03 AM
Data Processed : 10/1/2012 12:18:14 PM
Column : RP-18
Flow Rate : 1.0 mL/min



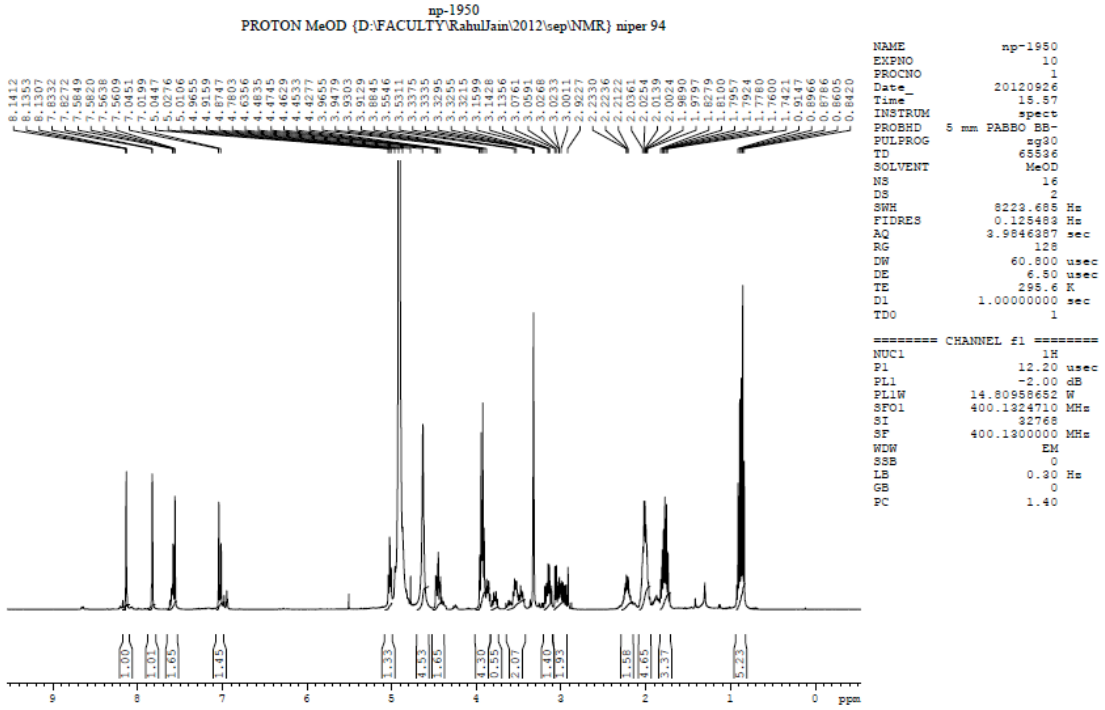
1 PDA Multi 1 / 254nm 4nm

PeakTable

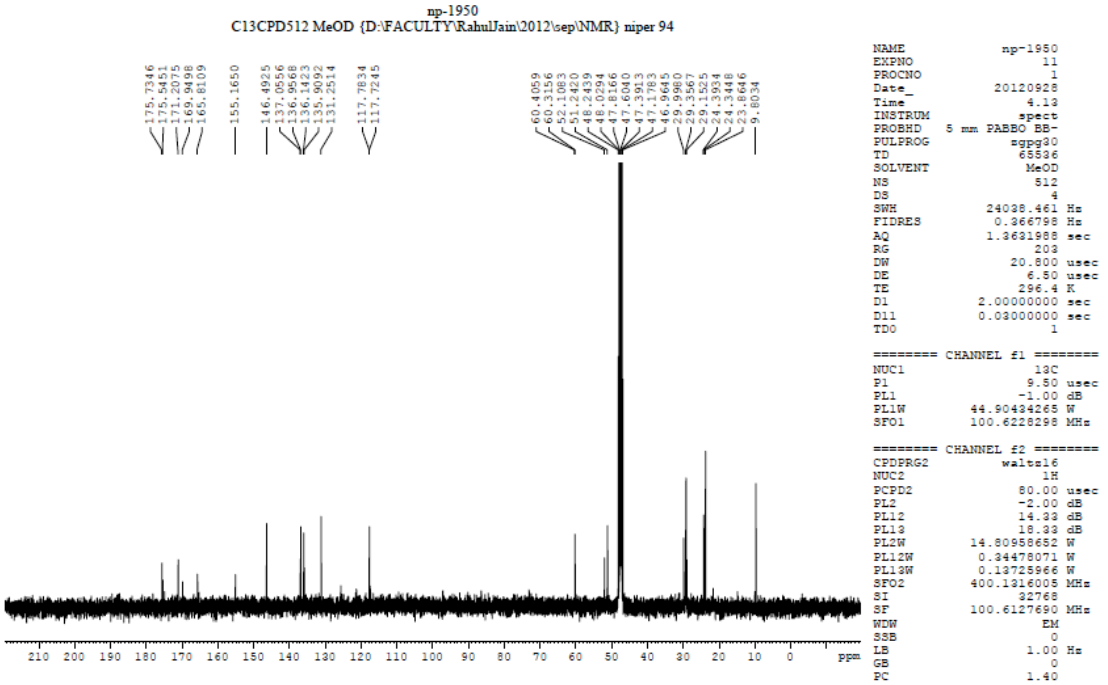
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.352	4836	802	0.044
2	4.123	10948132	759238	99.956
Total		10952968	760041	100.000

S34. ^{1}H-NMR Spectrum of 6l



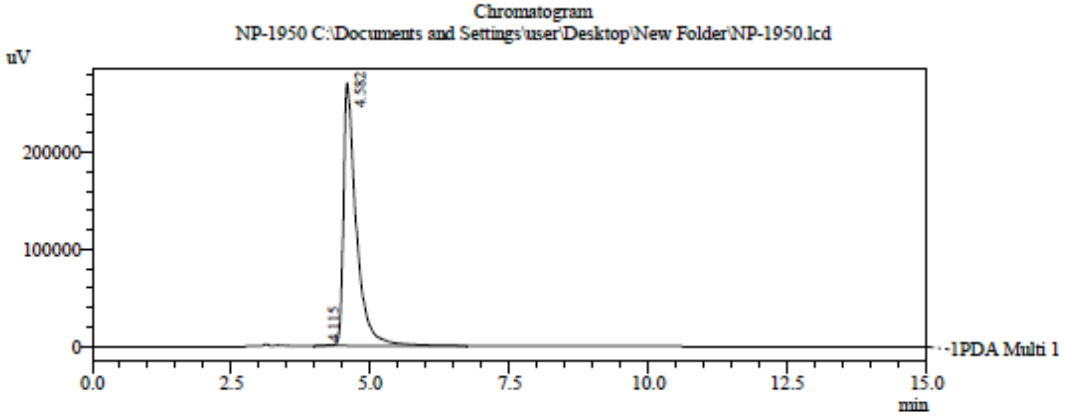
S35. ^{13}C-NMR Spectrum of 6l



S36. HPLC chromatogram of 6l

NIPER

Sample Information
Acquired by : Chhuttan L.Meena
Sample Name : NP-1950
Sample ID : NP-1950
Tray# : 1
Vail# : 9
Injection Volume : 10 uL
Data Filename : NP-1950.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B2.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 6:51:27 PM
Data Processed : 10/1/2012 2:07:25 PM
Column : RP-18
Flow Rate : 1.0 mL/min



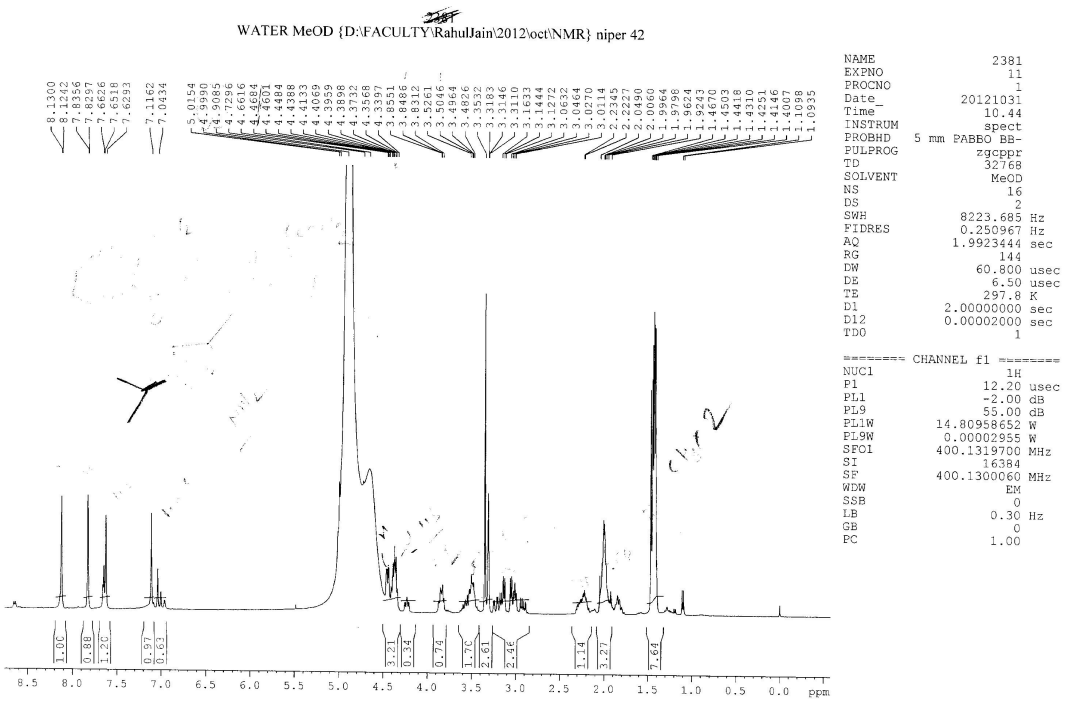
1 PDA Multi 1 / 254nm 4nm

PeakTable

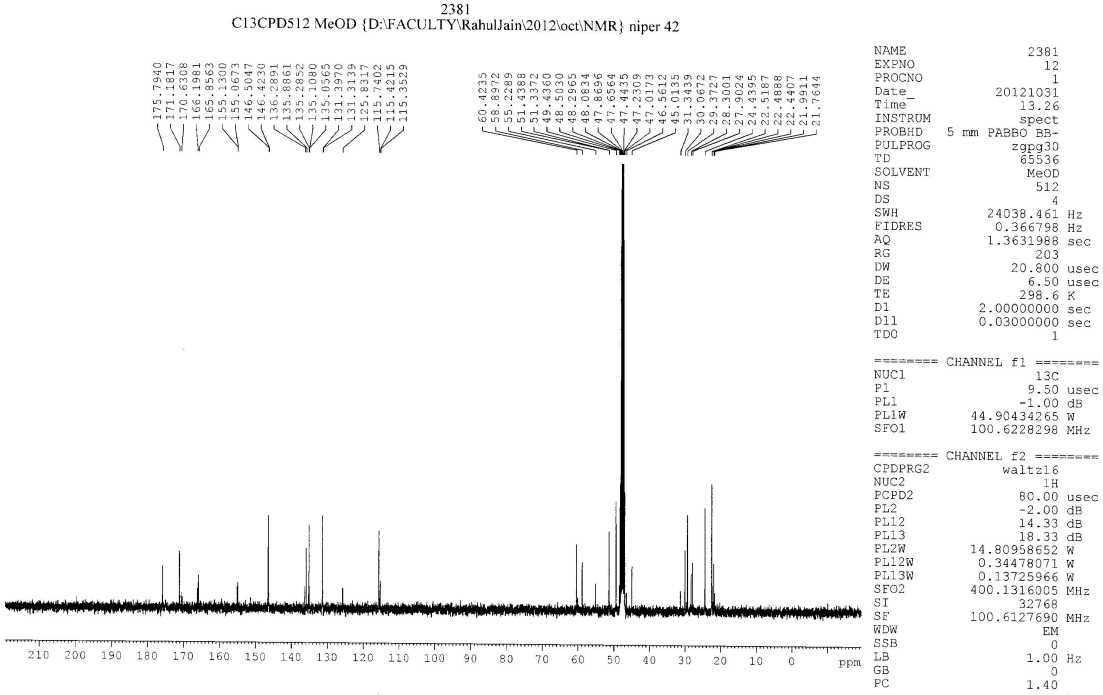
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	4.115	3194	416	0.074
2	4.582	4334056	270930	99.926
Total		4337250	271346	100.000

S37. ^{1}H-NMR Spectrum of 6m



S38. ^{13}C-NMR Spectrum of 6m

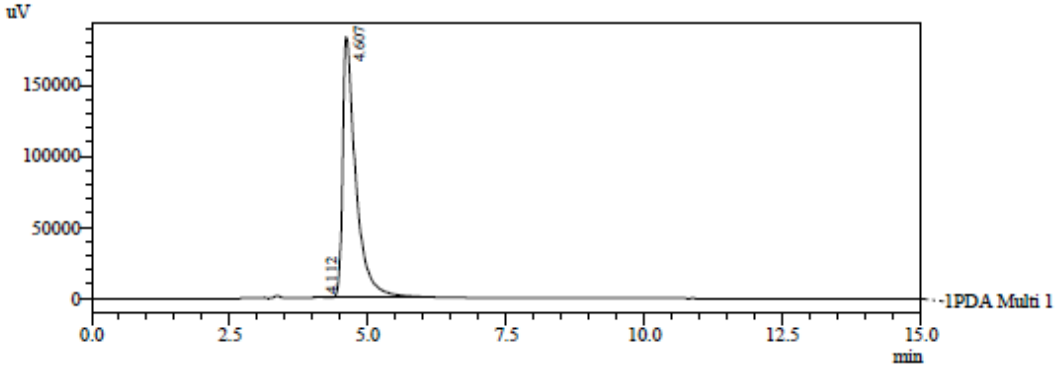


S39. HPLC chromatogram of 6m

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : 2381
Sample ID : 2381
Tray# : 1
Vail# : 19
Injection Volume : 10 uL
Data Filename : 2381.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 5:39:26 AM
Data Processed : 10/1/2012 2:00:37 PM
Column : RP-18
Flow Rate : 1.0 mL/min

Chromatogram
2381 C:\Documents and Settings\user\Desktop\New Folder\2381.lcd



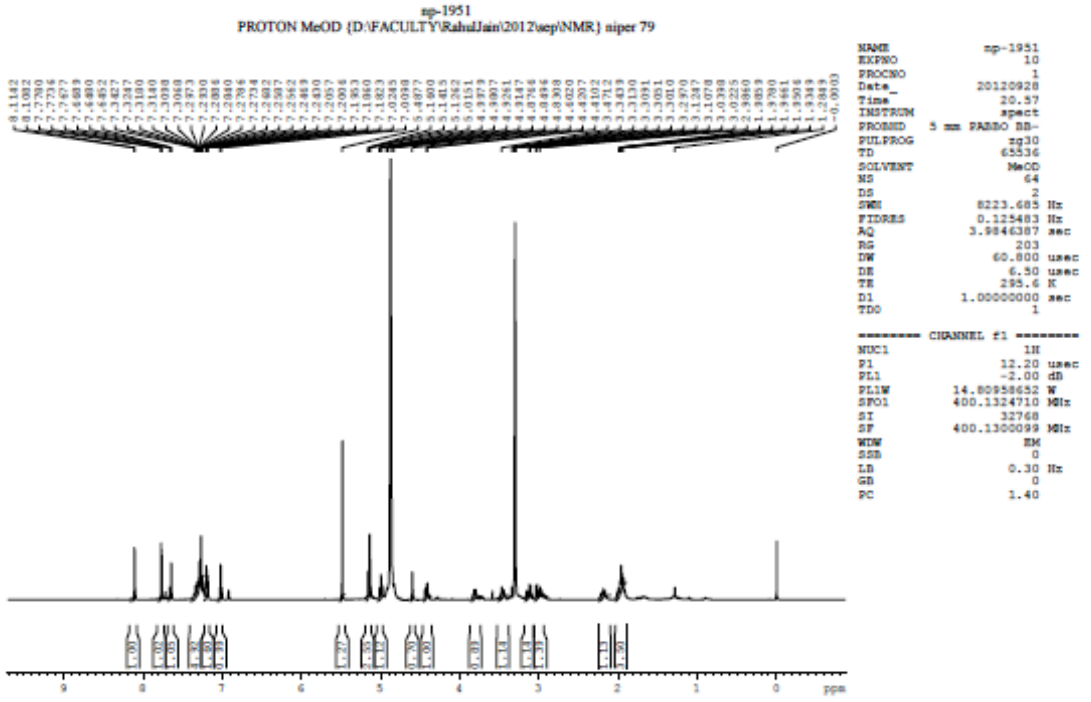
1 PDA Multi 1 / 254nm 4nm

PeakTable

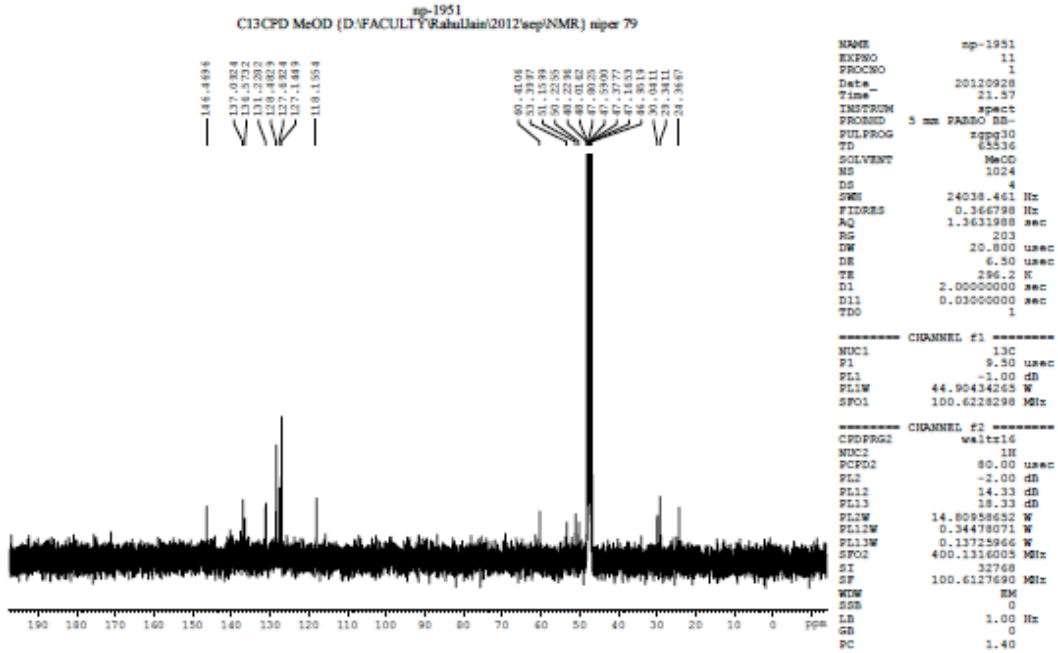
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	4.112	4778	557	0.156
2	4.607	3057485	183420	99.844
Total		3062262	183977	100.000

S40. ^{1}H-NMR Spectrum of 6n



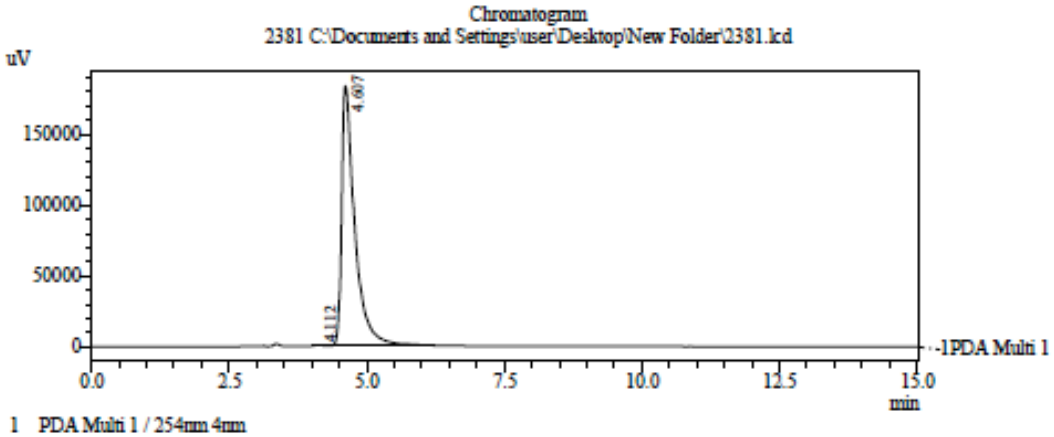
S41. ^{13}C-NMR Spectrum of 6n



S42. HRMS Spectrum of 6n

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : 2381
Sample ID : 2381
Tray# : 1
Vail# : 19
Injection Volume : 10 uL
Data Filename : 2381.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 5:39:26 AM
Data Processed : 10/1/2012 2:00:37 PM
Column : RP-18
Flow Rate : 1.0 mL/min



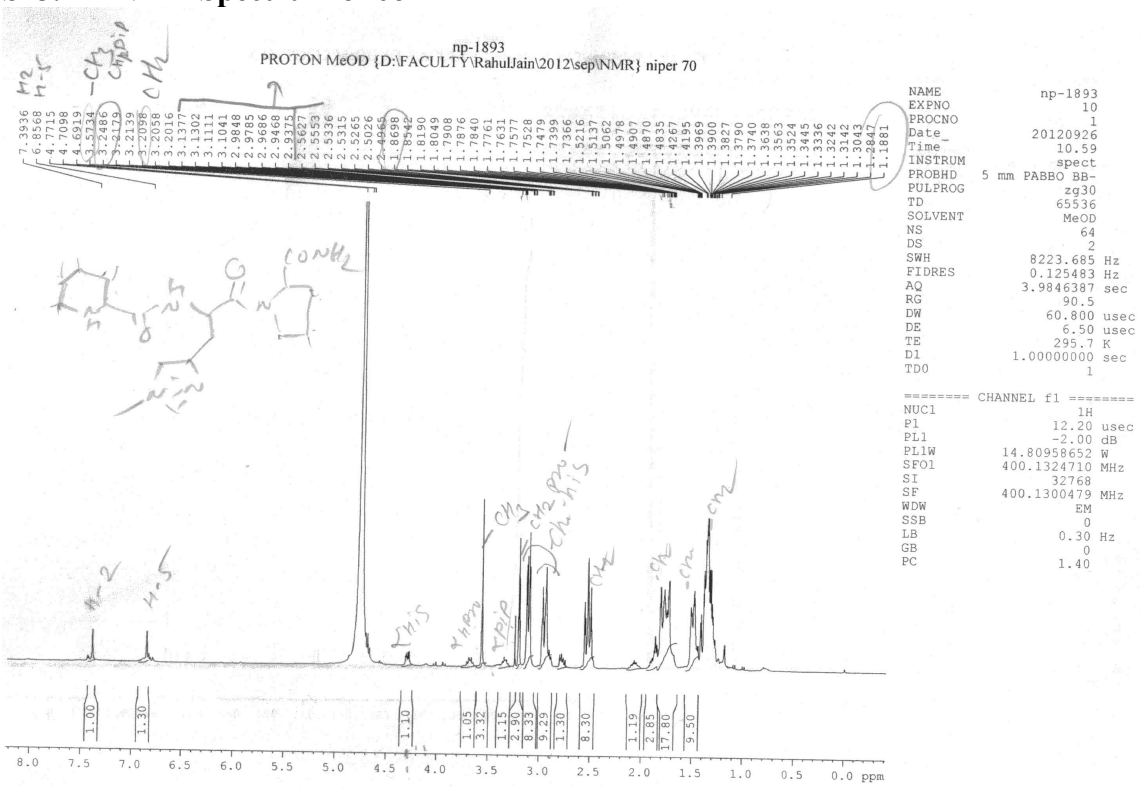
1 PDA Multi 1 / 254nm 4nm

PeakTable

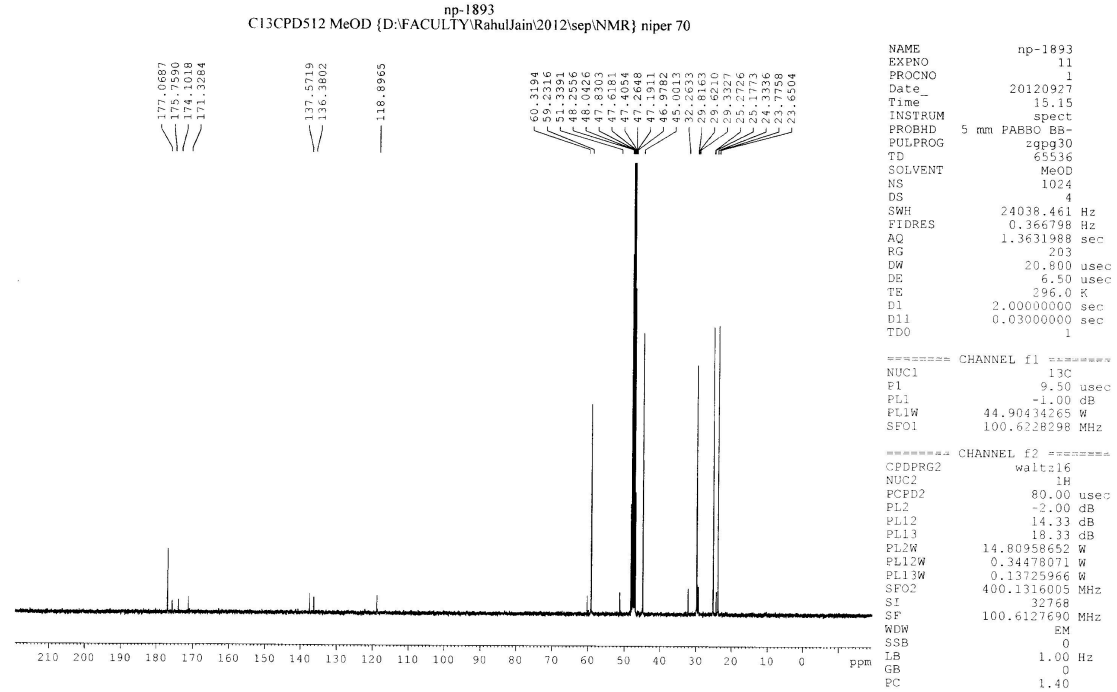
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	4.112	4778	557	0.156
2	4.607	3057485	183420	99.844
Total		3062262	183977	100.000

S43. ^{1}H-NMR Spectrum of 6o



S44. ^{13}C-NMR Spectrum of 6o

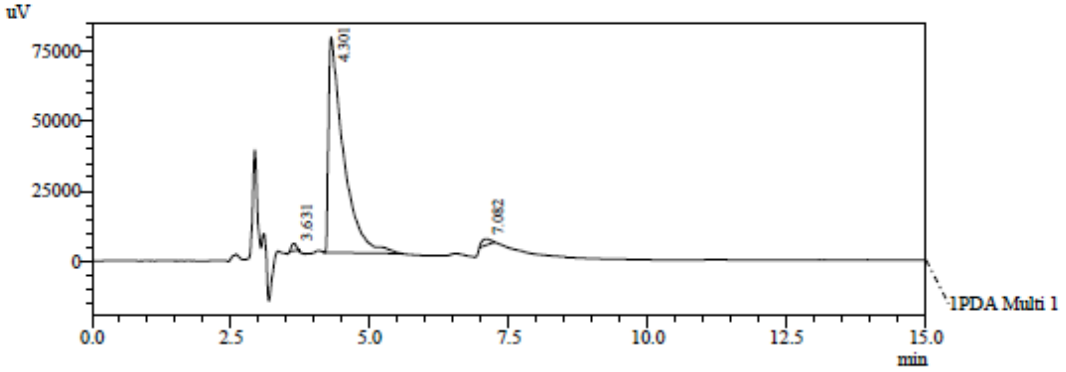


S45. HPLC chromatogram of 6o

NIPER

Sample Information
Acquired by : CLM
Sample Name : 1893
Sample ID : 1893
Tray# : 1
Vail# : 29
Injection Volume : 10 uL
Data Filename : 1893.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 8:14:41 AM
Data Processed : 10/1/2012 12:16:08 PM
Column : RP-18
Flow Rate : 1.0 mL/min

Chromatogram
1893 C:\Documents and Settings\user\Desktop\New Folder\1893.lcd



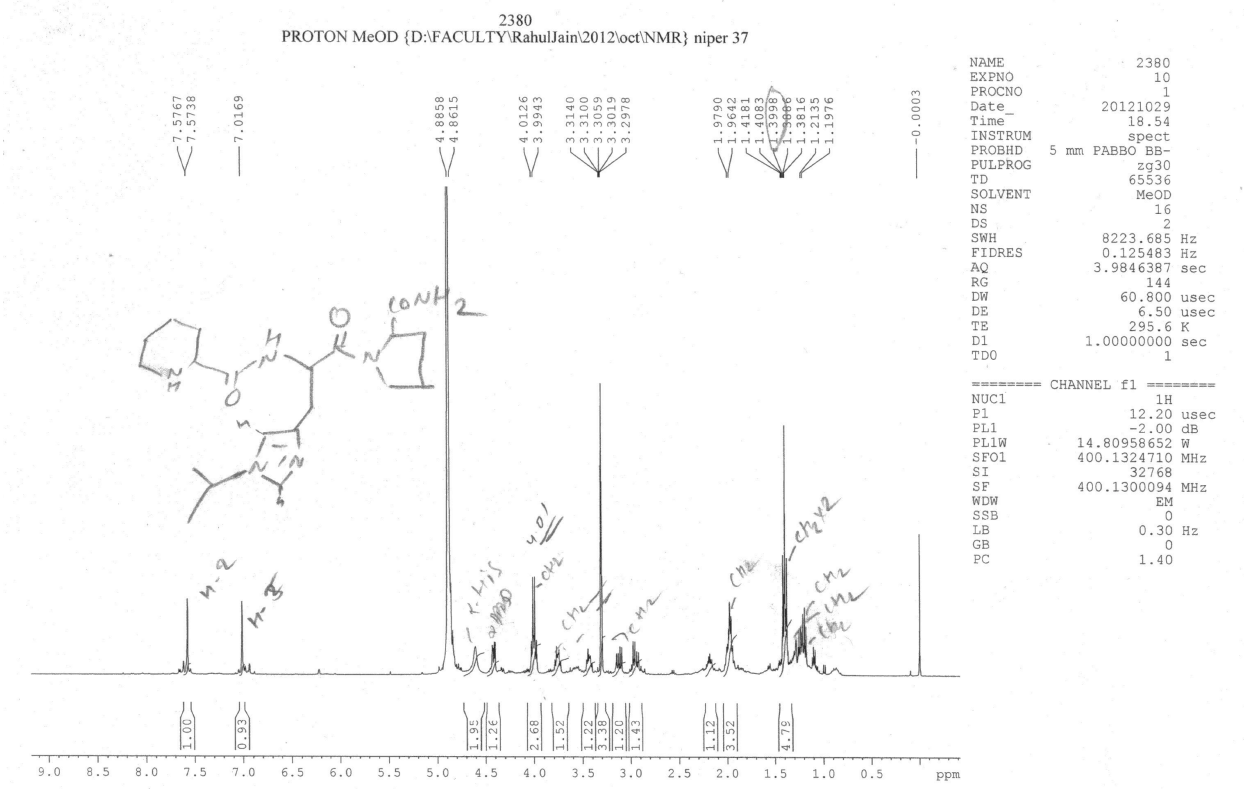
1 PDA Multi 1 / 215nm 4nm

PeakTable

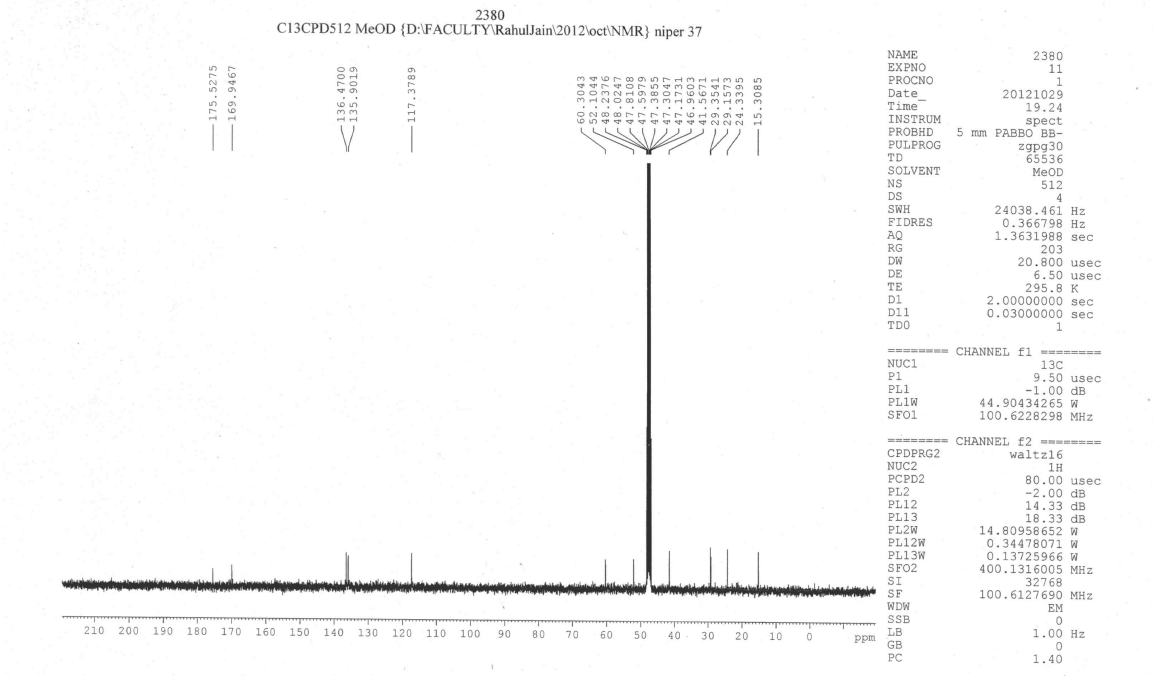
PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.631	14680	2599	0.990
2	4.301	1444351	77022	97.380
3	7.082	24185	2189	1.631
Total		1483216	81809	100.000

S46. ^{1}H-NMR Spectrum of 6p



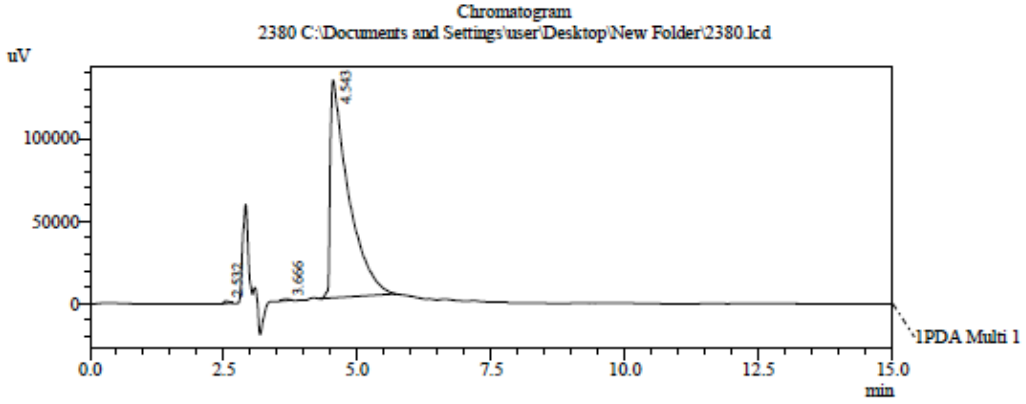
S47. ^{13}C-NMR Spectrum of 6p



S48. HPLC chromatogram of 6p

NIPER

Sample Information
Acquired by : chhuttan
Sample Name : 2380
Sample ID : 2380
Tray# : 1
Vail# : 21
Injection Volume : 10 uL
Data Filename : 2380.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 6:10:31 AM
Data Processed : 10/1/2012 1:59:55 PM
Column : RP-18
Flow Rate : 1.0 mL/min



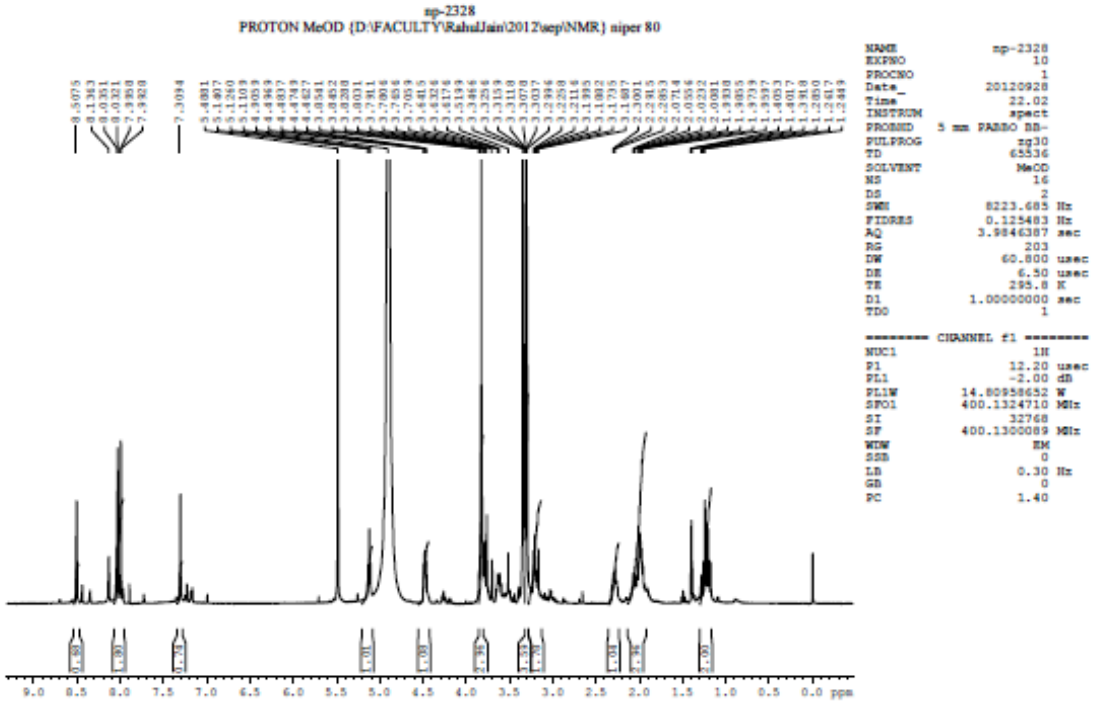
1 PDA Multi 1 / 215nm 4nm

PeakTable

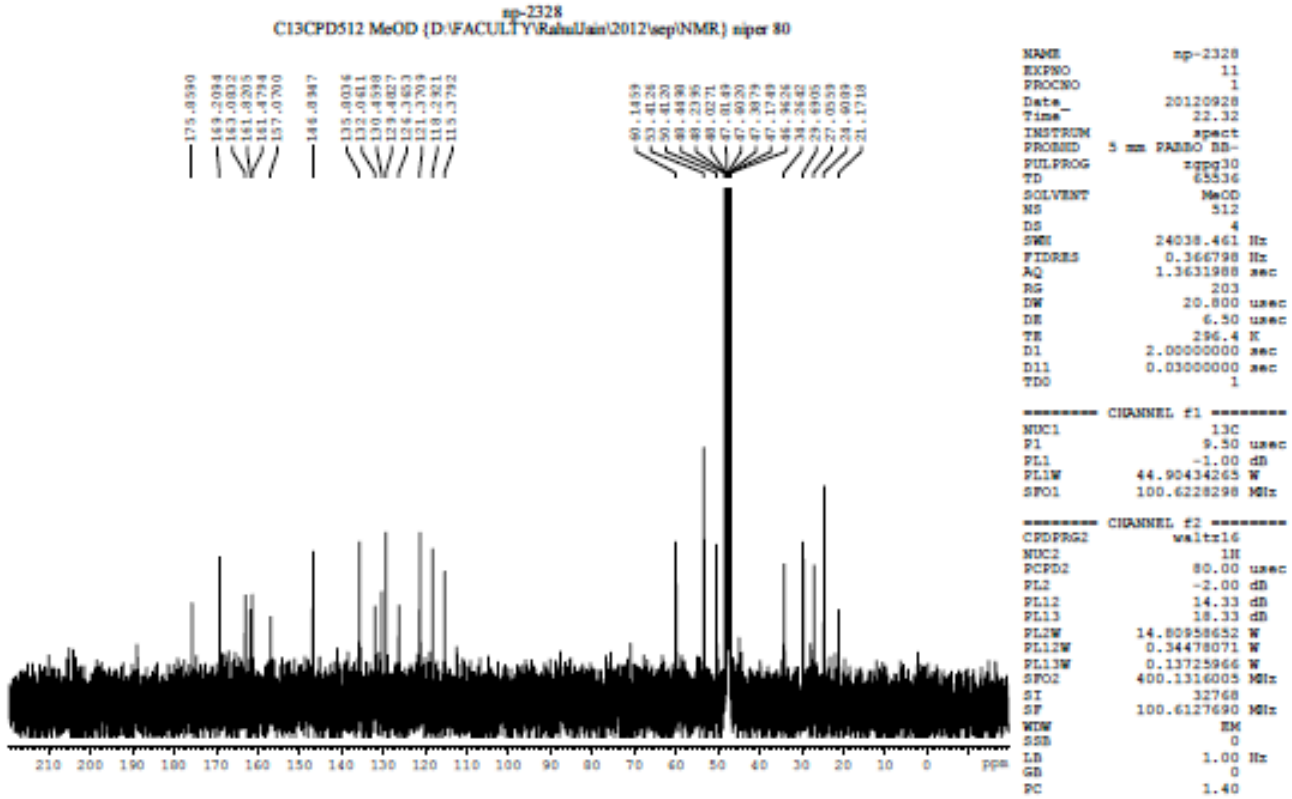
PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	2.532	7695	1460	0.245
2	3.666	6355	926	0.203
3	4.543	3121568	131990	99.552
Total		3135618	134376	100.000

S49.[1]H-NMR Spectrum of 6q



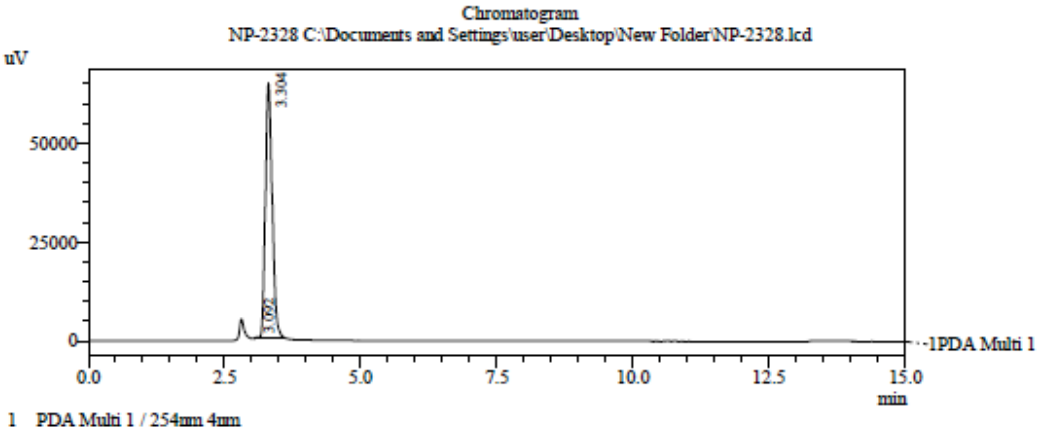
S50. [13] C-NMR Spectrum of 6q



S51.HPLC chromatogram of 6q

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : NP-2328
Sample ID : NP-2328
Tray# : 1
Vail# : 5
Injection Volume : 10 uL
Data Filename : NP-2328.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 2:02:11 AM
Data Processed : 10/1/2012 2:16:32 PM
Column : RP-18
Flow Rate : 1.0 mL/min



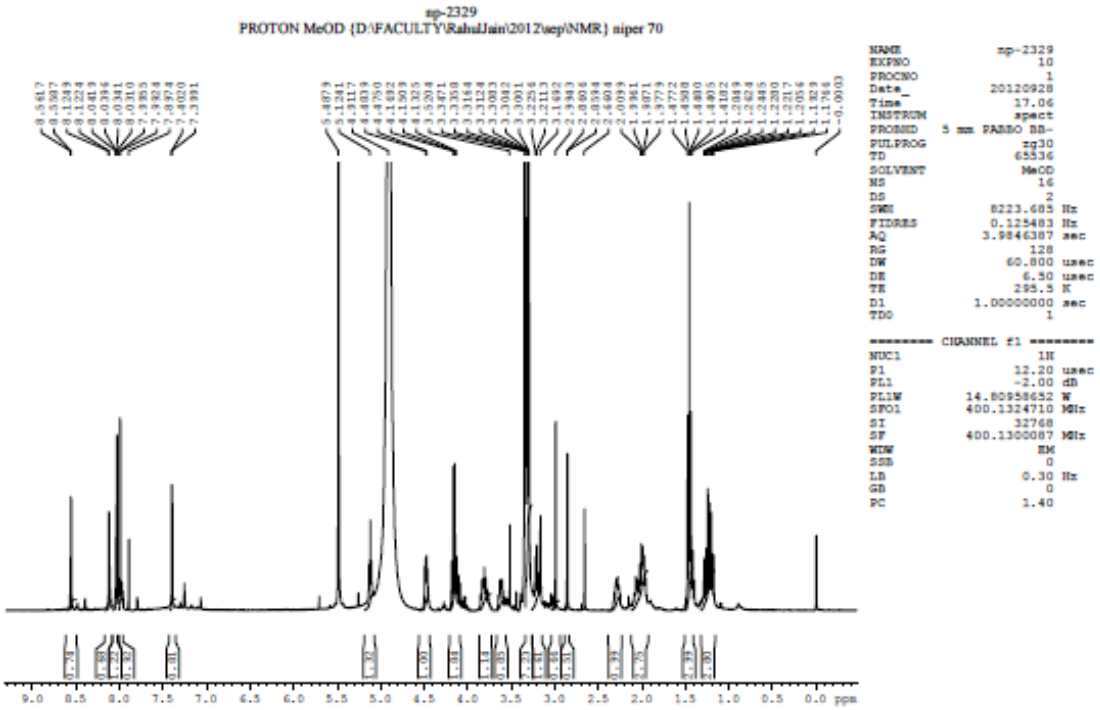
1 PDA Multi 1 / 254nm 4nm

PeakTable

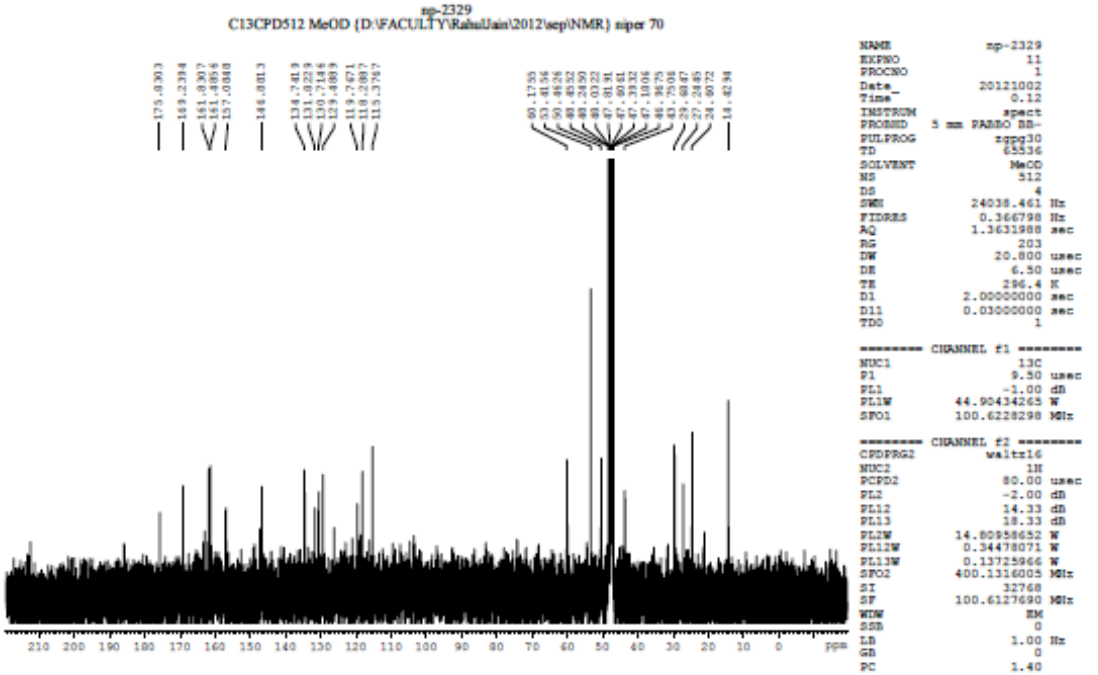
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.092	424	141	0.076
2	3.304	559879	64130	99.924
Total		560303	64270	100.000

S52.[1]H NMRSpectrum of 6r



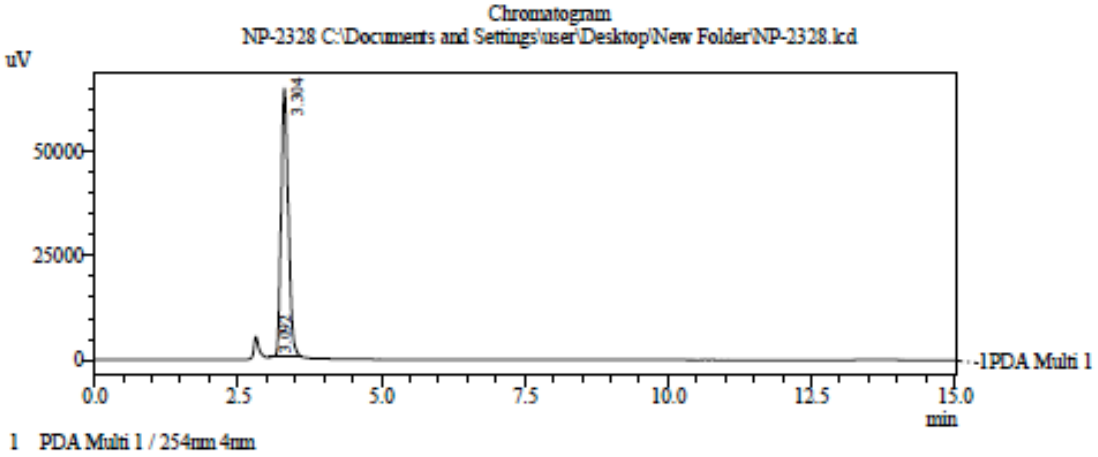
S53.[13]C-NMRSpectrum of 6r



S54. HPLC chromatogram of 6r

NIPER

Sample Information
Acquired by : Chhuttan L. Meena
Sample Name : NP-2328
Sample ID : NP-2328
Tray# : 1
Vail# : 5
Injection Volume : 10 uL
Data Filename : NP-2328.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 2:02:11 AM
Data Processed : 10/1/2012 2:16:32 PM
Column : RP-18
Flow Rate : 1.0 mL/min



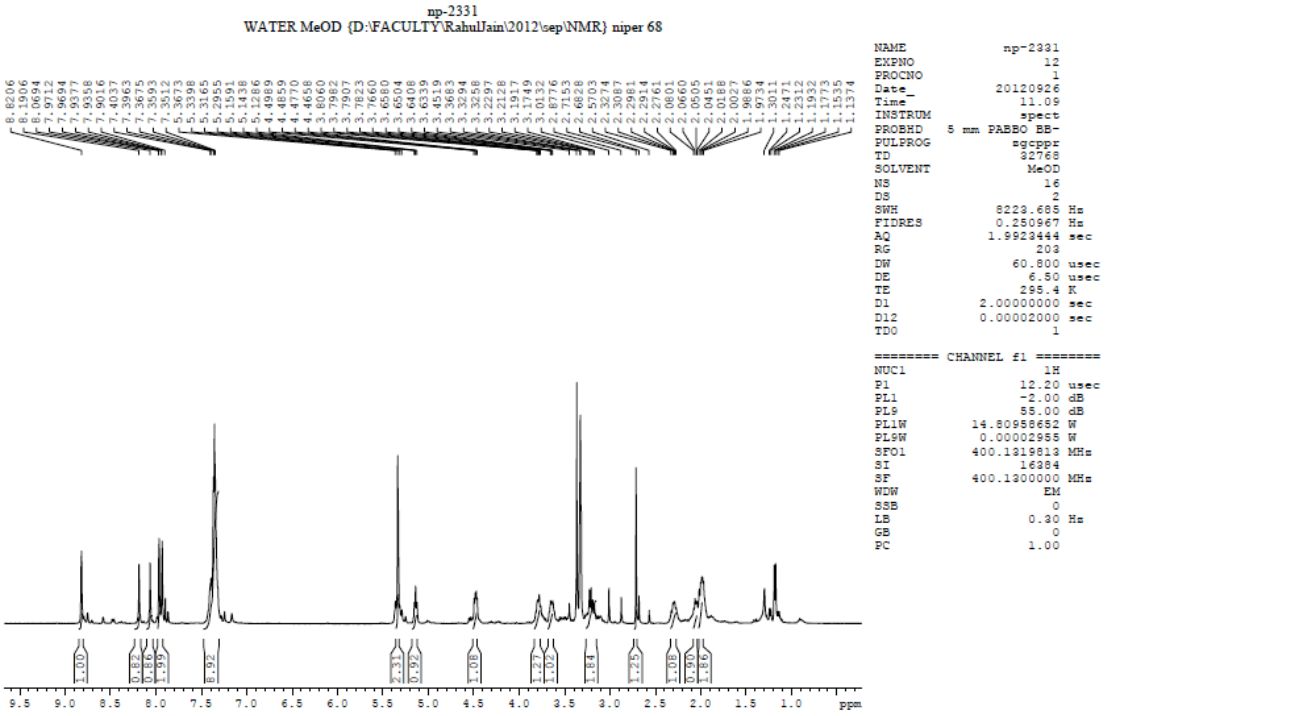
1 PDA Multi 1 / 254nm 4nm

PeakTable

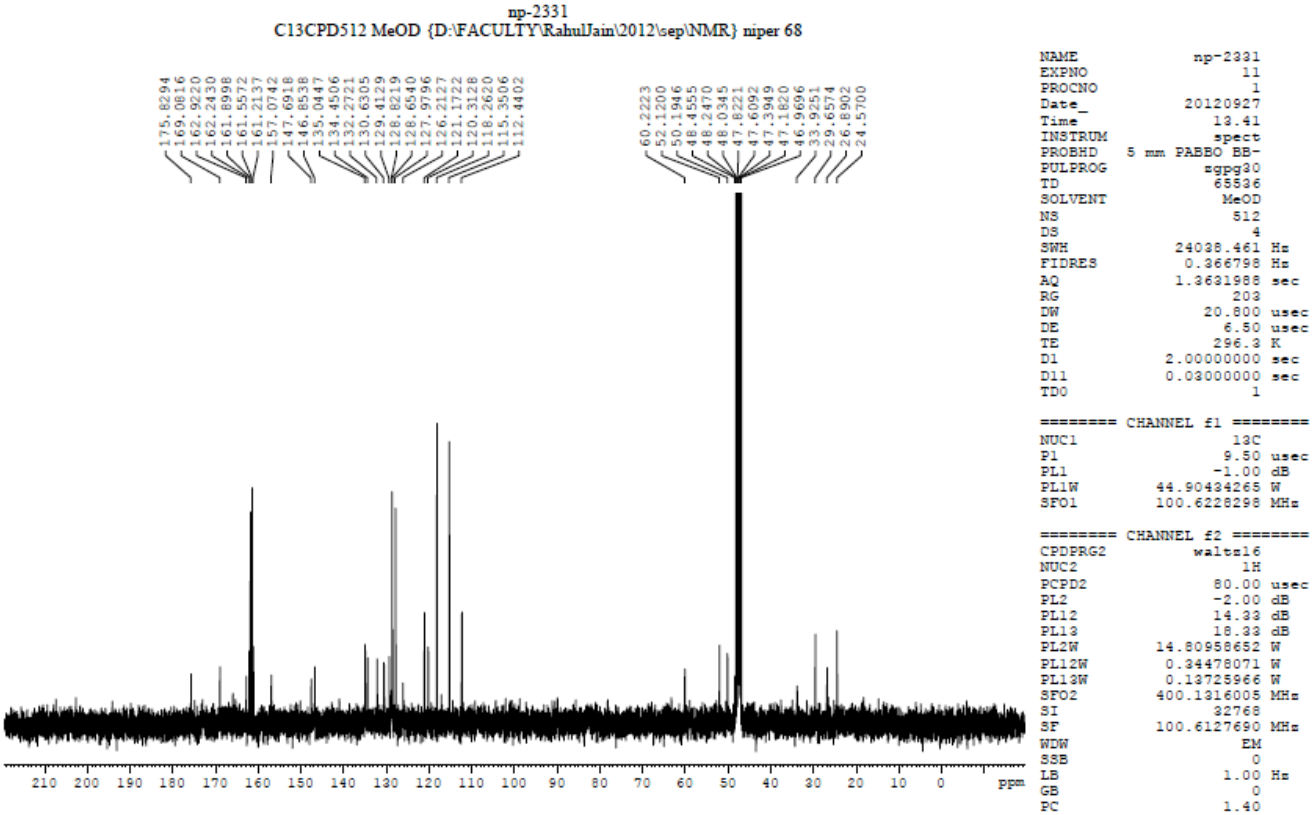
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.092	424	141	0.076
2	3.304	559879	64130	99.924
Total		560303	64270	100.000

S55.[1]H-NMR Spectrum of 6s



S56.[13]C-NMR Spectrum of 6s

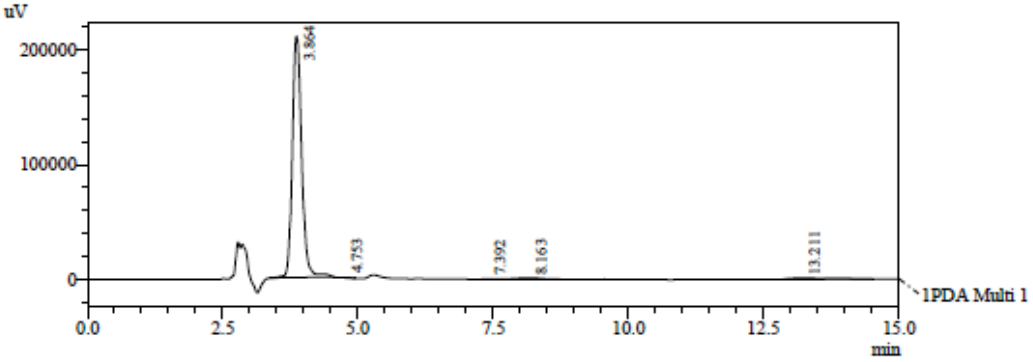


S57.HPLCchromatogram of 6s

NIPER

Sample Information
Acquired by : Chhuttan L. meena
Sample Name : NP-2331
Sample ID : NP-2331
Tray# : 1
Vail# : 3
Injection Volume : 10 uL
Data Filename : NP-2331.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B2.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 5:18:23 PM
Data Processed : 10/1/2012 2:17:27 PM
Column : RP-18
Flow Rate : 1.0 mL/min

Chromatogram
NP-2331 C:\Documents and Settings\user\Desktop\New Folder\NP-2331.lcd



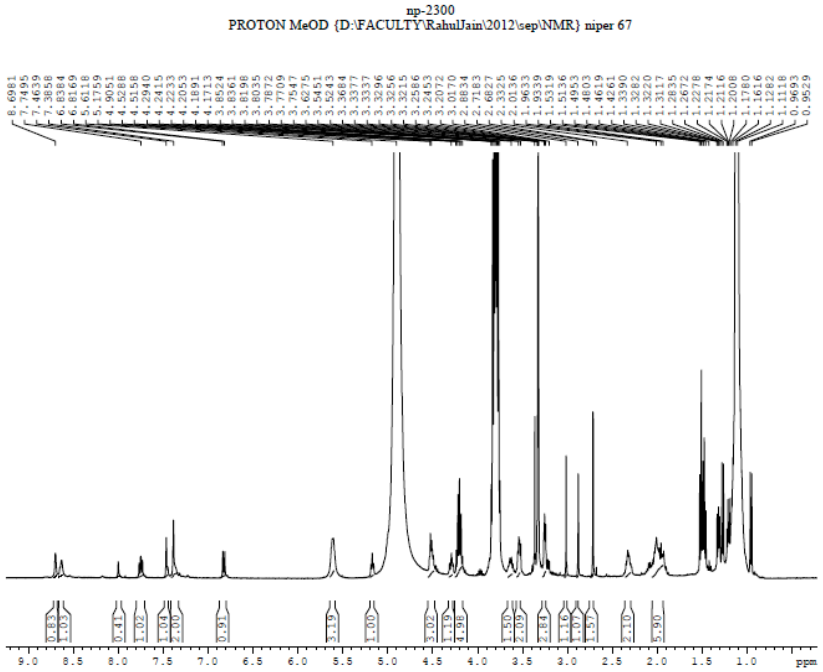
1 PDA Multi 1 / 224nm 4nm

PeakTable

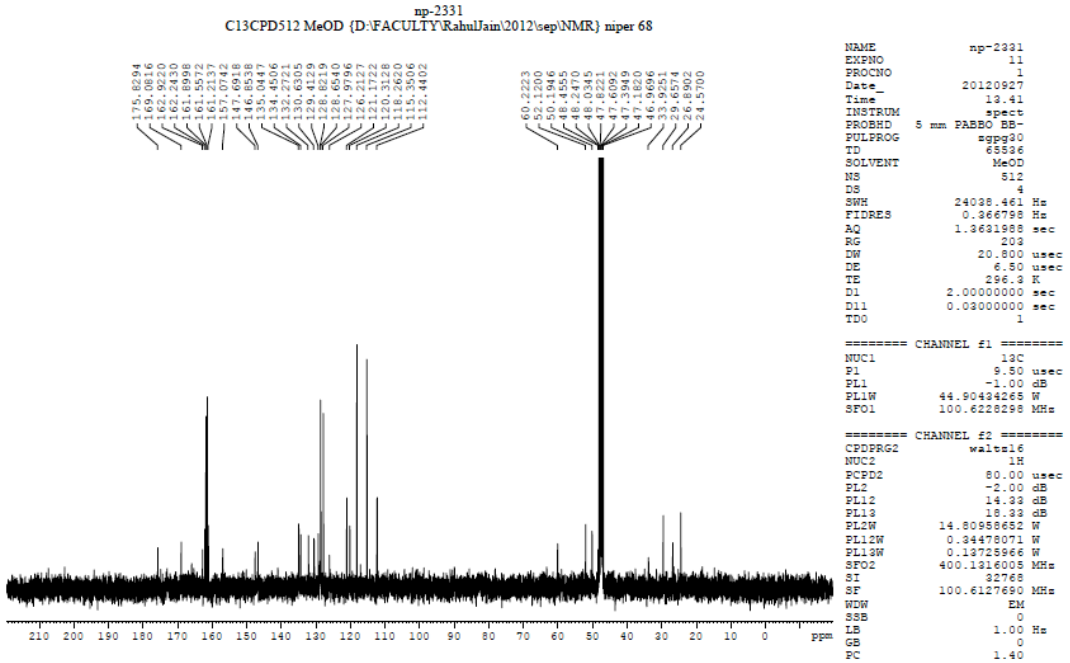
PDA Ch1 224nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.864	2534671	209669	95.546
2	4.753	5395	541	0.203
3	7.392	2766	132	0.104
4	8.163	46045	1415	1.736
5	13.211	63938	1156	2.410
Total		2652816	212913	100.000

S58.[1]H-NMR Spectrum of 6t



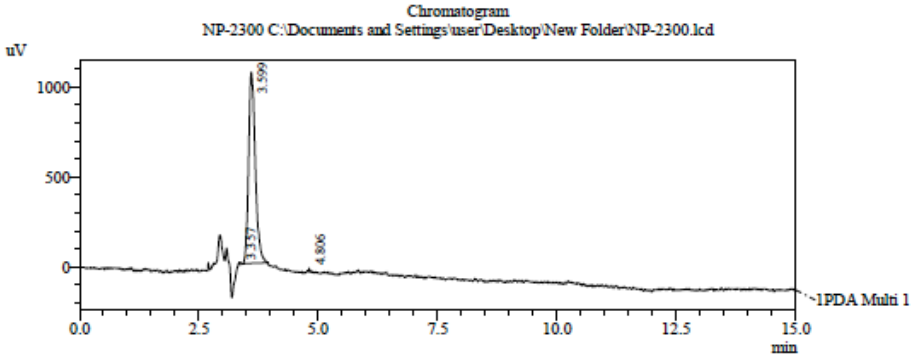
S59. 13C NMR spectrum of 6t



S60.HPLCchromatogram of 6t

NIPER

Sample Information
Acquired by : Chhuttan L. meena
Sample Name : NP-2300
Sample ID : NP-2300
Tray# : 1
Vail# : 3
Injection Volume : 10 uL
Data Filename : NP-2300.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 9/30/2012 2:58:58 PM
Data Processed : 10/1/2012 2:09:21 PM
Column : RP-18
Flow Rate : 1.0 mL/min



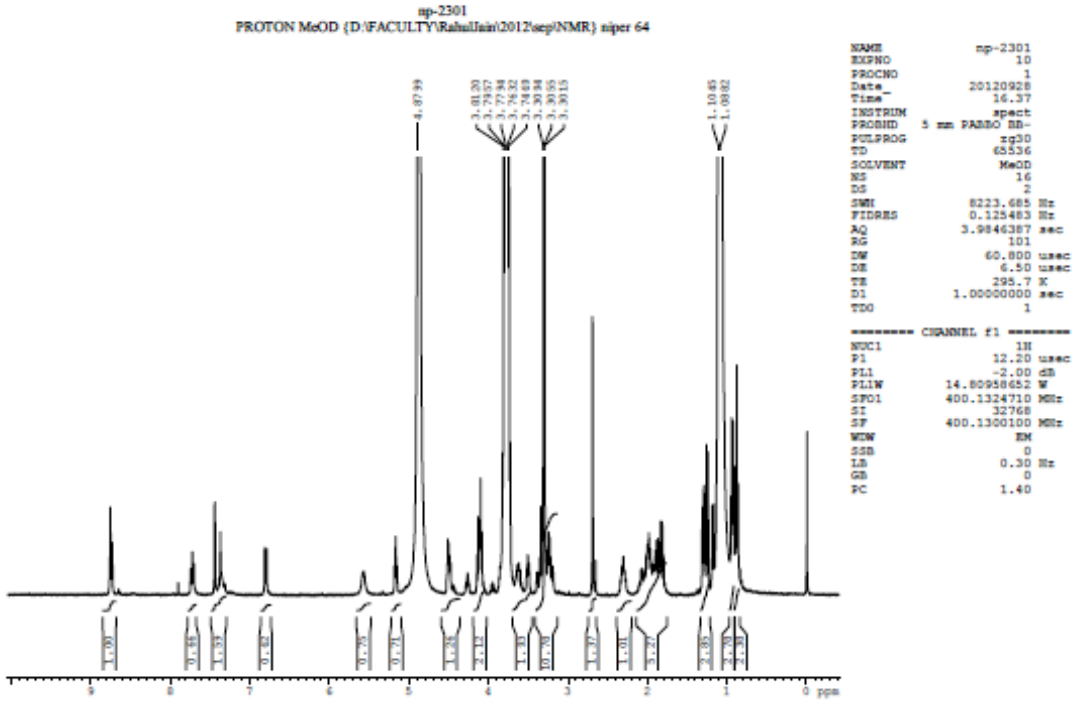
1 PDA Multi 1 / 254nm 4nm

PeakTable

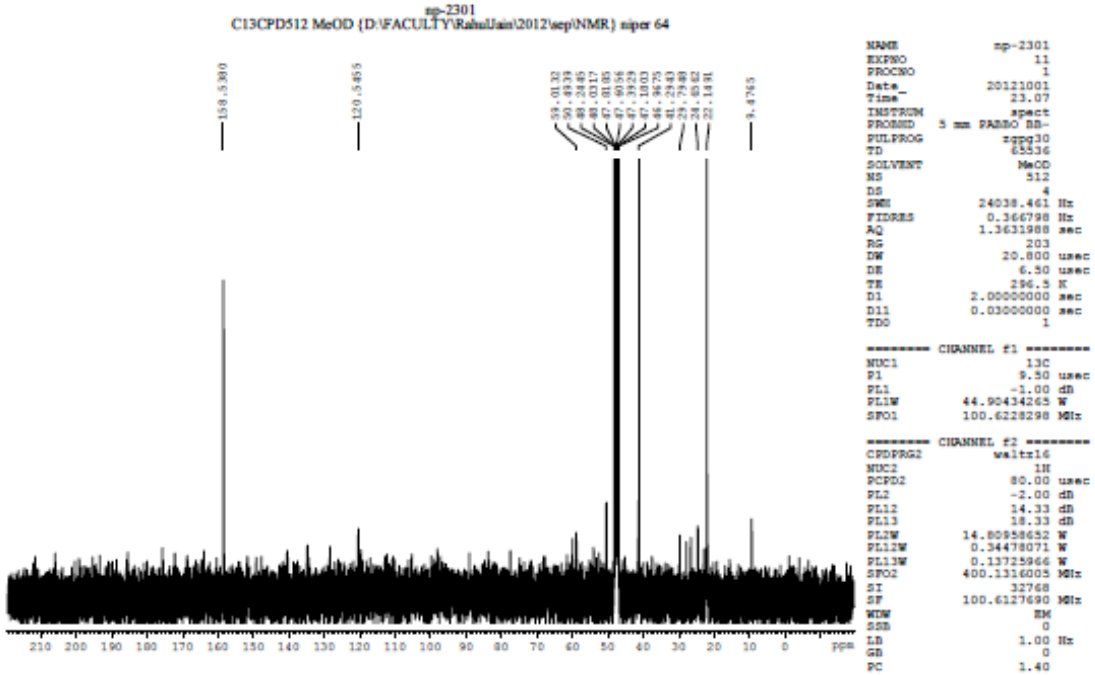
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.357	37	13	0.354
2	3.599	10385	1059	99.604
3	4.806	4	15	0.041
Total		10426	1087	100.000

S61.[1]H NMR Spectrum of 6u



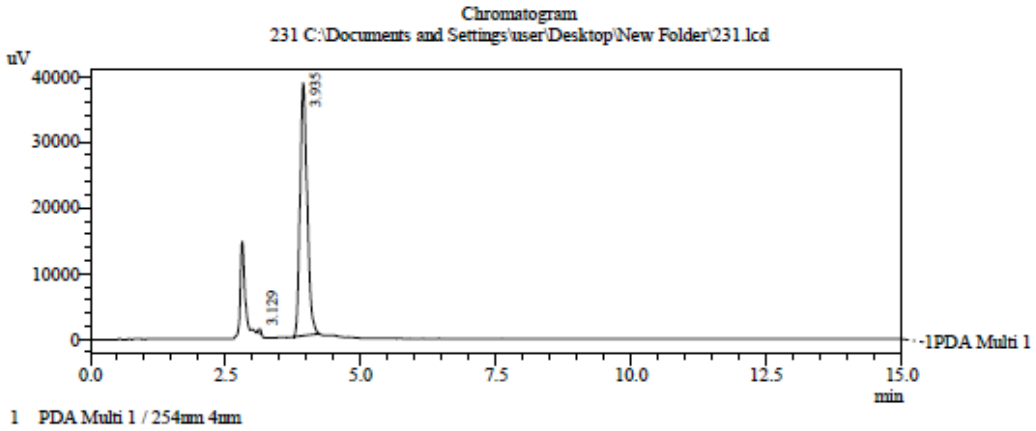
S62.[13]C-NMR Spectrum of 6u



S63.HPLC chromatogram of 6u

NIPER

Sample Information
Acquired by : Sunil
Sample Name : 231
Sample ID : 231
Tray# : 1
Vail# : 10
Injection Volume : 10 uL
Data Filename : 231.lcd
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 3:19:45 AM
Data Processed : 10/1/2012 12:26:47 PM
Column : RP-18
Flow Rate : 1.0 mL/min



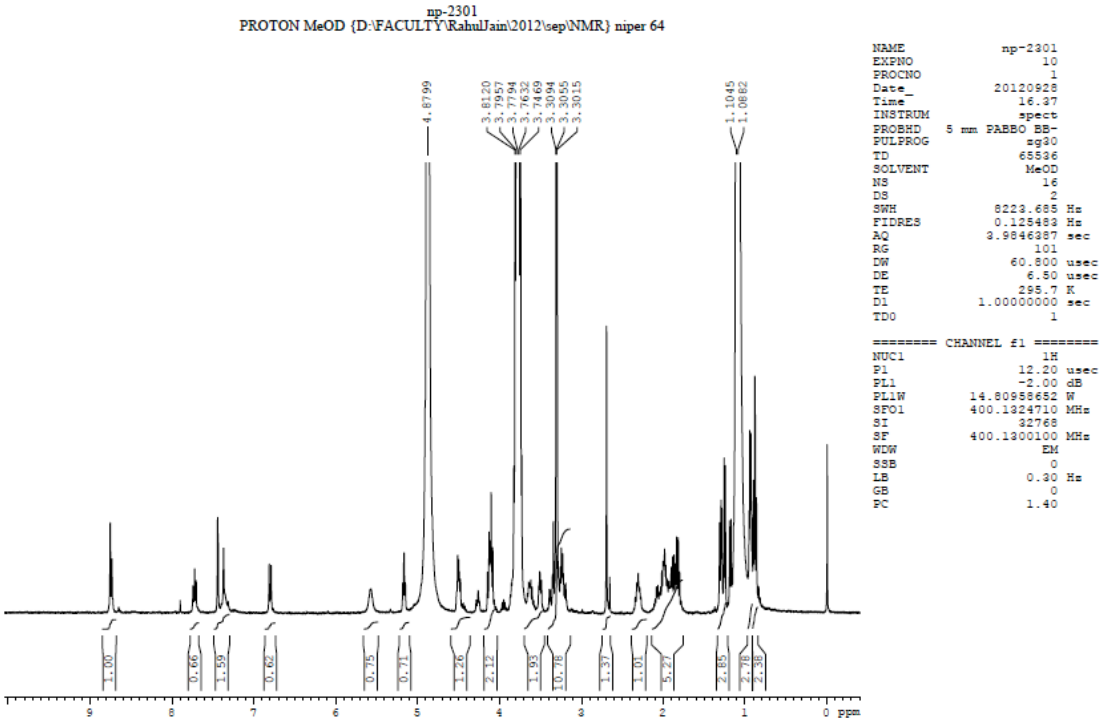
1 PDA Multi 1 / 254nm 4nm

PeakTable

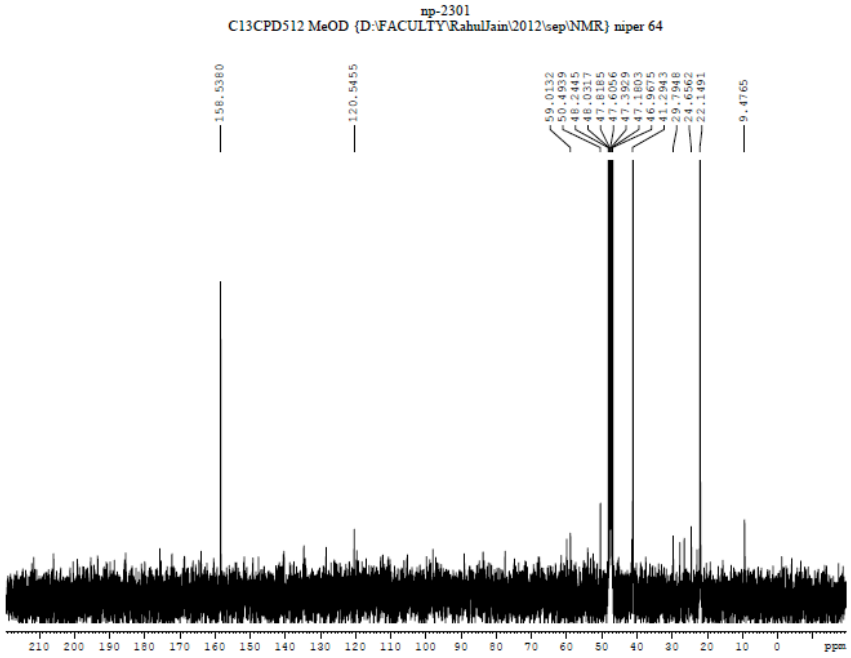
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.129	1877	661	0.528
2	3.935	353696	38399	99.472
Total		355573	39060	100.000

S64.[1]H-NMR Spectrum of 6v



S65.[13]C-NMR Spectrum of 6v



S66.HRMS of 6v

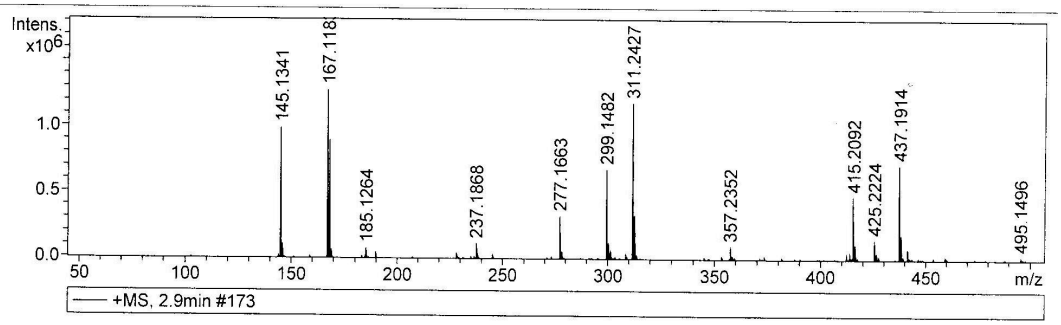
Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\Rahul jain\12-09-01-NP-2301.d	Acquisition Date	9/3/2012 4:27:42 PM
Method	sodium formate tune_low.m	Operator	VIKAS GROVER
Sample Name	NP-2301	Instrument / Ser#	maXis 40
Comment			

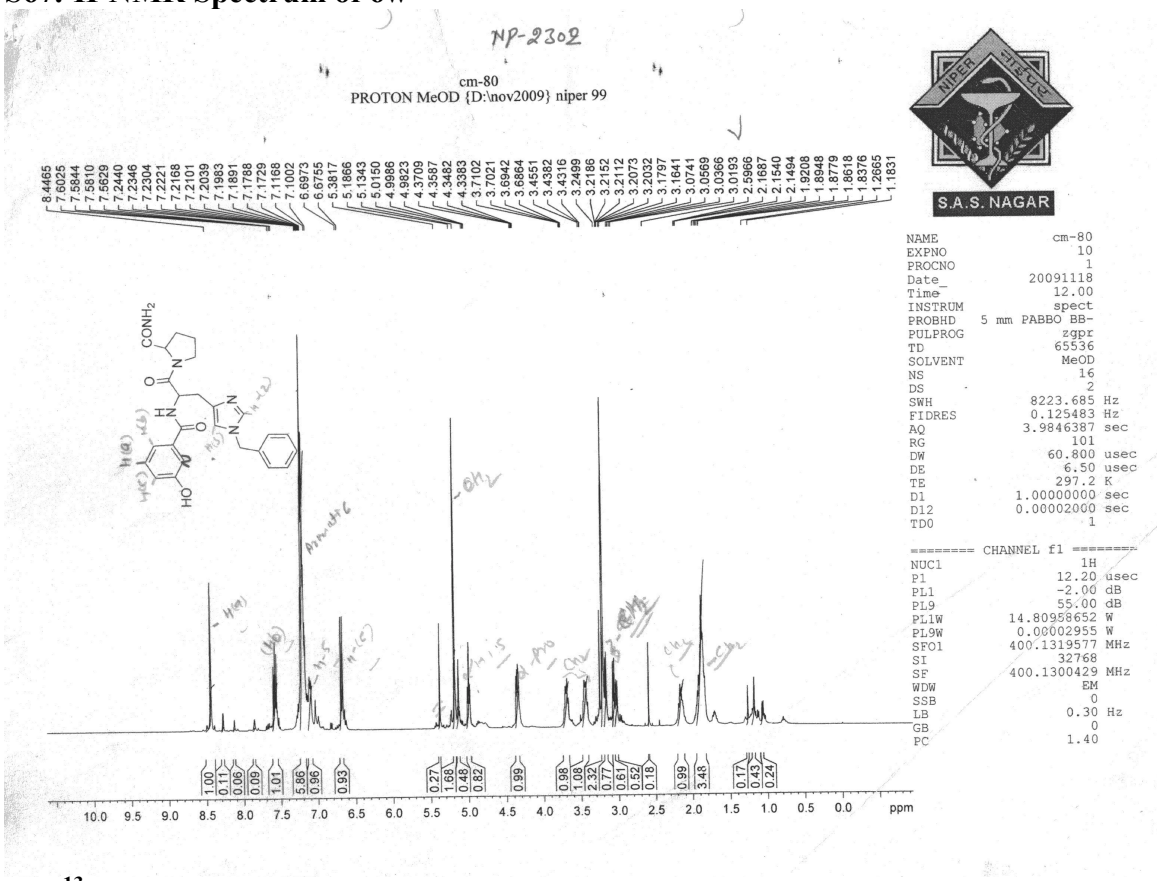
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	5.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

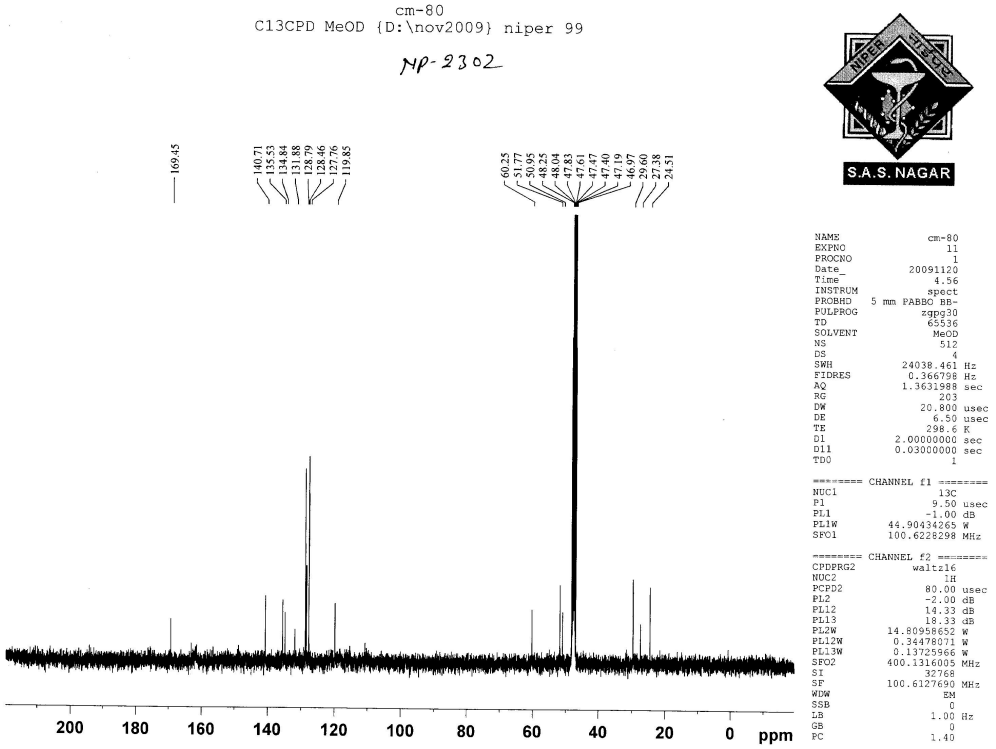


#	m/z	Res.	S/N	I	FWHM
1	145.1341	14803	4995.9	984187	0.0098
2	146.1370	16786	538.3	108085	0.0087
3	167.1183	7229	6887.6	1274659	0.0231
4	185.1264	19527	569.2	79399	0.0095
5	237.1868	20251	906.1	115816	0.0117
6	277.1663	20332	2177.8	325200	0.0136
7	299.1482	19283	2229.4	678424	0.0155
8	311.2427	16261	3843.8	1179791	0.0191
9	357.2352	21936	695.8	96993	0.0163
10	415.2092	21543	1923.7	474503	0.0193
11	425.2224	21894	480.1	152147	0.0194
12	437.1914	21330	2570.5	719268	0.0205
13	495.1496	22730	232.0	30364	0.0218

S67.[1]H-NMR Spectrum of 6w



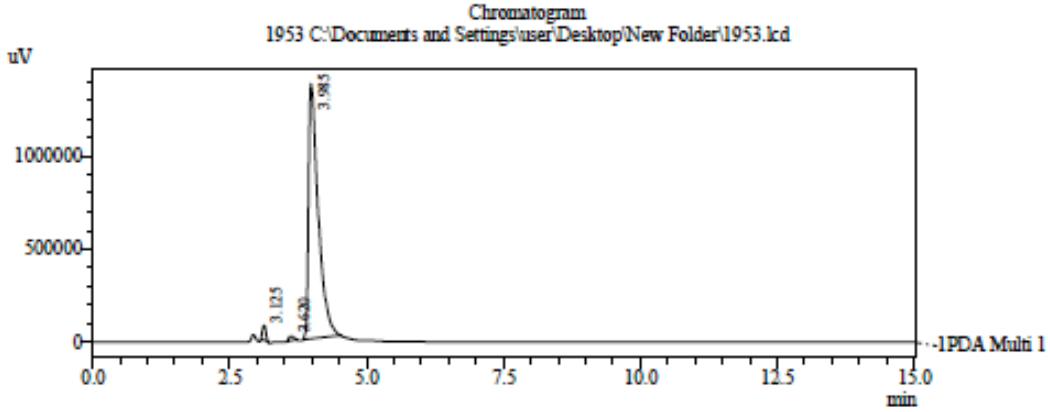
S68.[13]C-NMR Spectrum of 6w



S69.HPLC chromatogram of 6w

NIPER

Sample Information
Acquired by : Sunil
Sample Name : 2302
Sample ID : 2302
Tray# : 1
Vail# : 27
Injection Volume : 10 uL
Data Filename : 2302
Method Filename : 70%-B-PLOT-15 MIN.lcm
Batch Filename : 30.9.12B9.lcb
Report Filename : sunil C-18.lcr
Date Acquired : 10/1/2012 7:43:40 AM
Data Processed : 10/1/2012 12:24:30 PM
Column : RP-18
Flow Rate : 1.0 mL/min



1 PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	3.125	312829	77103	1.817
2	3.620	137311	22999	0.797
3	3.985	16767615	1365261	97.386
Total		17217755	1465364	100.000

S70. Receptor binding assay

Material and methods

All of the synthesized peptides were examined for both their affinity for mTRH-R1 and mTRH-R2 and their ability to serve as agonists and their selectivity for the receptors. Twenty-four hour before the experiment (human embryonic kidney cells 293) HEK293 cells stably expressing mTRH-R1 or mTRH-R2, were plated in appropriate cell culture plate 24 well plates. 300,000 Cells per well were seeded and incubated overnight at 37°C. The following day, the cell monolayer was washed 3 times and the plate and buffer were placed on ice for at least 45 min prior the experiment. The plates were kept at 0-4^{o}C and washed once with 1 mL/well of ice cold Hank's Balanced Salt Solution (HBSS) and with 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) without disturbing the cell monolayer. The compounds were added and mixed with [^{3}H]-1-MeTRH (100 µL), TRH analogues (250 µL) at 4 ºC before adding in plate (cold), all three-fold dilution in a single well totaling 350 μL (in duplicate).The plates were kept in the cold room in an ice tray up to 4 h and then the supernatant was aspirated and cells were carefully washed three times by adding 1 mL ice cold buffer per well (without disturbing monolayer of cells). 0.4-*N* NaOH solution (1 mL) was added to it and shaken for 30 min on the shaking platform for cell lysis. In small scintillation vial, 4 mL of scintillation fluids was taken and dispensed with 0.7 mL of lysate. It was shaken using a vortex mixer for 5 sec and placed in the scintillation counter. Affinities, reported as IC_{50} (μM), were determined as described earlier; briefly by measuring the concentration of the peptides required to displace 50% of 4nM [^{3}H]-1-MeTRH from the mTRH receptor.

S71. Material and method of FLIPRassay

HEK293 cells stably expressing mTRH-R1 or mTRH-R2 were seeded in black-walled, clear-bottomed 96-well plates (Corning, NY) at a density of 6×10^{4} cells/well in DMEM (10% FBS, 1% penicillin-streptomycin, 0.1% hygromycin) media and incubated for 24 h at 37^{o}C and 6% CO_2. On the following day, the culture media was replaced with 100 μL of HBSS supplemented with 20 mM HEPES, pH 7.5 and the cells were loaded with 100μL of calcium 3 fluorescent dye (Molecular Devices, Sunnyvale, CA) for 1 h at room temperature before addition of compounds. Transient changes in intracellular (Ca^{2+}) induced by ligands were measured. Changes in fluorescence were detected at the emission wavelength of 515–575 nm using FLIPR calcium assay kit according to the manufacturer's recommendation. This kit includes a calcium sensitive dye that is taken into the cytoplasm of the cell during incubation. The kit masking technology remains outside the cell and blocks background fluorescence. Upon ligand binding to the receptor, calcium released into the cytoplasm of the cell, the dye binds to the intracellular calcium and becomes fluorescent; fluorescence is measured by the FLIPRTETRA® high throughput cellular screening system (Molecular Devices, Sunnyvale, CA). Data are reported as EC_{50} (μM) values. All the results from receptor binding, FLIPR assays are discussed in Table 1.

S72. Material and methods of *in vivo* reversal of pentobarbital-induced sleeping time assay

Antagonism of pentobarbital-induced sleeping time one of the best trends CNS effects of TRH is its analeptic action manifested by the decrease in barbiturate narcosis. TRH is an effective analeptic agent, which reduces pentobarbital-induced sleeping time by 50% or more following peripheral administration of high doses or central injection of lower doses in rats, rabbits, and monkeys. The all synthesized TRH-like peptides were evaluated *in vivo* by using the antagonism of a pentobarbital induced sleeping time model as described. TRH analogues were injected intravenously through the tail vein at a dose of 10

mmolkg^{-1} (equivalent to 3.6 mgkg^{-1} TRH). Ten minutes after administration of the synthesized peptides, each animal received 50 mgkg^{-1} sodium pentobarbital intraperitoneally. The sleeping time was recorded as the time elapsed from the onset of loss of righting reflex until it returned (Table 2).

S73. Functional Observational Battery

Functional Observational Battery is a systematic study performed to determine the effect of any disease or drug treatment on different functions of central nervous system/functions. This consists of measurement of various behavioral parameters in the home cage, hand held and open field cages. The scoring patterns are a modified form of Irwin's scoring (Hubler et al., 2005; Irwin, 1968). Various functional parameters, which were studied, are summarized in Table 4 and 5.

S74. Statistical analysis

All the values are expressed as mean ± S.E.M. Statistical analysis was performed using Sigma Stat 2.0 statistical software. Statistical significance for multi group was assessed by using one-way ANOVA followed by Dunnet test. Median was determined in FOB and statistical significance was assessed by Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Dunnet test $P<0.05$ is considered as statistically significant.

Table 4 Effect of **6c** (TRH 10 µmol/kg i.v. and 10 µmol/kg i.v.) on FOB parameters at different time interval

Treatment	Saline						TRH 10 µmol/kg						6c- 5 µmol/kg i.v.					
Parameters	0 min	15 min	30 min	60 min	120 min	180 min	0 min	15 min	30 min	60 min	120 min	180 min	0 min	15 min	30 min	60 min	120 min	180 min
Home cage observation																		
Spontaneous activity level	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)
Home cage removal & Handling																		
Excitability/ handling reactivity	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)
Open field Observations (Neuromuscular activity)																		
Landing hind limb foot splay (mm)	39.3.± 0.80	37.3 ±0.67	37.16 ±0.81	37.89 ±0.98	35.27 ±0.71	34.88 ±0.83	42.61 ±1.09	42.55±103*	39.61±1.20	39.88 ±0.78	38.072±1.04	38.72 ±1.04*	38.22 ±0.80	35.61 ±1.02	37.16 ±0.81	35.77 ±0.83	37.83 ±1.14	35.0 ±0.79
Open field Observation (Sensory response)																		
Spontaeous activity level (mm)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	3.5 (3,4)	4.0 (4,4)	4.0 (4,4)	4.5 (4,5)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	3.5 (3,4)	3.5 (3,4)	4.5 (4,5)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)
Auditory response	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3,4)	3.0 (3,4)	4.0 (4,4)	3.5 (3,4)	3.5 (3,4)	3.0 (3,4)	3.5 (3,5)	3.5 (3,5)	4.0 (4,4)	3.0 (3,4)	3.5 (3,4)	3.5 (3,4)	3.5 (3,4)	3.0 (3,4)
Somatosensory response	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	4.0 (4,4)	3.5 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)*	3.5 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)*	4.0 (4,4)*	4.0 (4,4)*	3.0 (3,3)*	3.0 (3,3)
Visual approach	3.0 (3,3)	3.0 (3,3)	3.0 (2,3)	3.0 (3,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	4.0 (4,4)	3.5 (3,4)	3.0 (3,4)	3.5 (3,4)	3.0 (3,3)	3.5 (3,4)	4.0 (4,4)	3.5 (3,4)	3.5 (3,4)	3.0 (3,3)	3.0 (3,3)
Olfactory response	3.0 (3,3)	3.0 (3,3)	3.0 (2,3)	3.0 (3,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	3.0 (3,4)	3.5 (3,4)	3.0 (3,4)	3.0 (3,4)	3.5(3,4)*	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3.3)
Arousal	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)

Mice (6) were treated with saline or TRH/ Analogues and observed for 3 hrs. Values are expressed as average of medians of scores (25th, 75th percentile) except Landing hind limb foot splay (mean ± SEM), * $p < 0.05$ vs saline using Kruskal-Wallis One Way ANOVA followed by Dunnet test.

Table 5 Effect of **6c** (TRH 10 µmol/kg i.v. and 10 µmol/kg i.v.) on FOB parameters at different time interval

Treatment	Saline						TRH 10 µmol/kg						6c- 20µmol/kg i.v.					
Parameters	0 min	15 min	30 min	60 min	120 min	180 min	0 min	15 min	30 min	60 min	120 min	180 min	0 min	15 min	30 min	60 min	120 min	180 min
Home cage observation																		
Spontaneous activity level	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (2,3)	2.5 (3,3)	3.0 (3,3)	2.5 (3,3)
Home cage removal & Handling																		
Excitability/ handling reactivity	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)	2.0 (2,2)
Open field Observations (Neuromuscular activity)																		
Landing hind limb foot splay (mm)	39.3.± 0.80	37.3 ±0.67	37.16 ±0.81	37.89 ±0.98	35.27 ±0.71	34.88 ±0.83	38.2 ±0.80	34.88 ±0.83	37.05± 1.23	**33.88 ±0.6***	34.44± 0.97	38.72 ±1.04	40.94 ±1.07	38.05 ±1.17	36.72 ±1.28	37.5 ±0.83	35.55 ±0.65	36.11 ±0.80
Stimulus response (Open field)																		
Landing hind limb foot splay (mm)	4.0 (4,4)	4.0 (4,4)	4.0 (4,5)	3.5 (4,4)	4.0 (4,5)	4.0 (4,4)	4.5 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	3.5 (4,4)	3.5 (4,4)	4.5 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)
Spontaneous activity level	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3,4)	3.0 (3,4)	4.0 (4,4)	3.5 (3,4)	3.5 (3,4)	3.0 (3,4)	3.5 (3,5)	3.5 (3,5)	4.0 (4,4)	3.0 (3,4)	3.5 (3,4)	3.5 (3,4)	3.5 (3,4)	3.5 (3,4)
Somatosensory response	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.5 (4,4)	4.0 (3,4)	3.5 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4) *	3.5 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)*	4.0 (4,4)*	4.0 (4,4)*	3.0 (3,3)*	3.0 (3,3)
Visual approach	3.0 (3,3)	4.0 (3,3)	3.5 (2,3)	3.0 (3,3)	3.5 (3,3)	3.0 (2,3)	3.5 (3,3)	4.0 (4,4)	3.5 (3,4)	3.5 (3,4)	3.0 (3,4)	3.0 (3,3)	3.0 (3,4)	3.0 (4,4)	3.0 (3,4)	3.0 (3,4)	3.0 (3,3)	2.5 (3,3)
Olfactory response	3.0 (3,3)	3.0 (3,3)	3.0 (2,3)	3.0 (2,3)	3.0 (3,3)	2.5 (2,3)	3.0 (3,3)	3.0 (3,4)	3.5 (3,4)	3.0 (3,4)	3.0 (3,4)	3.5(3,4) *	3.0 (3,4)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3,3)	3.0 (3.3)
Arousal	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (3,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)	4.0 (4,4)

Mice (6) were treated with saline or TRH/ Analogues and observed for 3 hrs. Values are expressed as average of medians of scores (25th, 75th percentile) except Landing hind limb foot splay (mean ± SEM), * $p < 0.05$ vs saline using Kruskal-Wallis One Way ANOVA followed by Dunnet test.