Electronic Supplementary Material (ESI) for RSC Advances.


# An unexpected re-arrangement of the antibiotic carbapenem core to new 1,4 diazepin-5-one scaffolds

Byron Peters[a], Rufaro Razuwika[a], Marivel Samipillai[a], Per I. Arvidsson[a],[b],[c], Hendrik G. Kruger[a] Thavendran Govender* and Tricia Naicker*[a]

Catalysis and Peptide Research Unit, University of KwaZulu Natal, Durban, South Africa, 4001 *govenderthav@ukzn.ac.za or naickert1@ukzn.ac.za

# Table of Contents

## Experimental

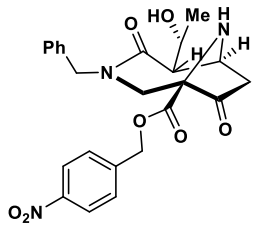
All the reactions were performed at room temperature and under atmospheric conditions unless specified. Reagents and solvents were purchased from Aldrich, Merck and Fluka and all crude solvents were distilled. The resulting products were recrystallized in ethanol unless stated otherwise. All the NMR spectra were recorded using a Bruker AVANCE III 400 MHz at room temperature and the sample concentrations were ~10mg in 0.5 ml $CDCl_3$ solution. Chemical shifts are expressed in ppm and coupling constants are reported in Hz. Optical rotations were measured out on a Bellingham + Stanley Polarimeter (Model 440+). High Resolution Mass Spectrometric masses were determined on the Bruker microTOF-Q II instrument. Column chromatography carried out with silica gel 60. Thin layer chromatography (TLC) was performed using Merck Kiesel gel 60 F254.

## Crystallization

Compound **3b** did not crystalize, therefore the PNB group was removed under hydrogenlysis conditions with Pd/C and $H_2$ (1 atm, 3 hours) in MeOH. The catalyst was filtered off using vacuum filtration. The solvent was removed under vacuo. 10mg of the crude was dissolved in 3mL of MeOH by sonication at 45°C for 2 min and the remaining undissolved material was filtered out. To the clear solution of the filtrate was added a few drops of toluene and mixed well. The system was then allowed to evaporate at ambient condition which yielded colorless block shaped crystals over the period of 7 days. Analysis of the crystal revealed it to be the methyl ester.

## Characterization data

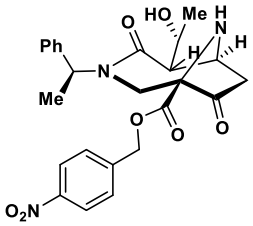
**4-nitrobenzyl (1R,5S,6S)-3-benzyl-5-((R)-1-hydroxyethyl)-4,8-dioxo-3,9-diazabicyclo[4.2.1]nonane-1-carboxylate (2a):**




Benzylamine (5.0 mmol, 545 µl), paraformaldehyde, (5.0 mmol, 150 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, *N*-methylene-1-phenylmethanamine (687.7 mg, 5 mmol). The resultant imine was reacted with *p*-nitrobenzyl-6-(1-hydroxyethyl)-1-azabicyclo(3.2.0)heptane-3,7-dione-2-carboxylate (carbapenem),

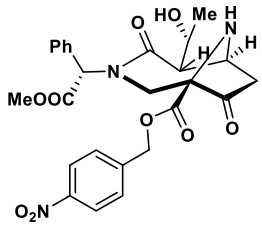
(955.6 mg, 2.5 mmol) in the presence of proline, (86 mg, 0.75 mmol) for 24 h to yield 620 mg of a white solid (53% yield). **$R_f$** (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{24}$ – 24 (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{24}H_{26}N_3O_7$ $[M+H]^+$: 468.1765; found 468.1767. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.19 (d, *J* = 6.6 Hz, 3H), 2.23 (d, *J* = 18.7 Hz, 1H), 2.47 (dd, *J* = 5.6 and 3.6 Hz, 1H) 2.69 (dd, *J* = 18.7 and 8.0 Hz, 1H), 3.54 (d, *J* = 15.7, 1H), 3.96 (d, *J* = 15.7 Hz, 1H), 4.37-4.19 (m, 2H), 4.36 (m, 1H), 4.82 (d, *J* = 14.6 Hz, 1H), 5.06 (d, *J* = 11.5 Hz, 2H), 7.18-7.25 (m, 5H), 7.35 (d, *J* = 8.7 Hz, 2H), 8.14 (d, *J* = 8.7 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 21.0, 43.6, 47.3, 49.8, 57.5, 65.1, 67.0, 123.2, 126.2, 128.1, 140.9, 129, 133.5, 135, 141.5, 148.3, 166.7, 174.7, 209 ppm.

**4-nitrobenzyl (1R,5S,6S)-5-((R)-1-hydroxyethyl)-4,8-dioxo-3-((S)-1-phenylethyl)-3,9-diazabicyclo[4.2.1]nonane-1-carboxylate (2b):**



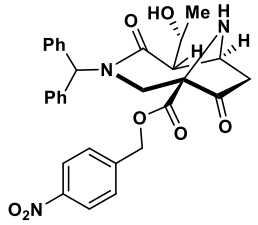

Methylbenzylamine (3.0 mmol, 370 µl), paraformaldehyde, (3.0 mmol, 90.09 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, *N*-methylene-1-phenylethanamine (687.7 mg, 5 mmol). The resultant imine was reacted with carbapenem, (955.6 mg, 2.5 mmol) in the presence of proline, (86 mg, 0.75 mmol) for 48 h to give a white solid of mass, 675 mg, (56% yield). **$R_f$** (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 40 (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{25}H_{28}N_3O_7$ $[M+H]^+$: 482.1922; found 482.1920. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.35 (d, *J* = 6.9 Hz, 1H), 1.49 (d, *J* = 6.9 Hz, 2H), 1.56 (d, *J* = 7.2 Hz, 3H), 2.77 (d, *J* = 6.9 Hz, 2H), 2.95 (d, *J* = 8.8 Hz, 1H), 3.02 (dd, *J* = 15.5 and 8.6 Hz, 1H), 4.01-4.18 (m, 2H), 4.18 (d, *J* = 13.4 Hz, 1H), 4.71 (dd, *J* = 9.9 and 6.0 Hz, 1H), 5.32 (d, *J* = 19.2 Hz, 2H), 6.06 (d, *J* = 14.3 Hz, 1H), 7.38-7.22 (m, 5H), 7.45 (d, *J* = 9.1 Hz, 2H), 8.32 (d, *J* = 6.8 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 20.5, 21.6, 36.5, 42.34, 36.5, 52.5, 66.5, 74.8, 124.2, 126.2, 127.8, 127.0, 128.4, 142.4, 141.8, 128.9, 168.3, 168.9, 170.3, 173.2 ppm.

**4-nitrobenzyl (1R,5S,6S)-5-((R)-1-hydroxyethyl)-3-((S)-2-methoxy-2-oxo-1-phenylethyl)-4,8-dioxo-3,9-diazabicyclo[4.2.1]nonane-1-carboxylate (2c):**




Methyl 2-amino-2-phenylacetate was in a form of a salt so 1.0 g was neutralized using sodium bicarbonate in water and then the resultant amine, (687 mg, 4.15 mmol) paraformaldehyde, (4.15 mmol, 124.8 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, methyl 2-(methyleneamino)-2-phenylacetate (752.4 mg, 4.15 mmol). The resultant imine was reacted with carbapenem, (800.4 mg, 2.07 mmol) in the presence of proline, (86 mg, 0.75 mmol) for 24 h to give 631 mg of a white solid, (48% yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 16. (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{26}H_{28}N_3O_9$ $[M+H]^+$: 526.1826; found 526.1851. **$^1$H NMR** (400 MHz, $CDCl_3$): δ = 1.19 (d, *J* = 6.7 Hz, 3H), 2.44 (d, *J* = 4.6 Hz, 1H), 3.59 (d, *J* = 16.6, 1H), 3.81 (s, 3H , H10), 4.22 (d, *J* = 16.6, 1H), 4.27 (d, *J* = 6.8 Hz, 1H), 4.45 (d, *J* =4.9 1H), 4.69 (d, 13.5 Hz, 1H), 4.96 (d, *J* = 18.2 Hz, 1H), 4.98 (d, *J* = 13.5 Hz, 1H), 5.01 (dd, *J* = 18.5 and 7.6 Hz, 1H), 6.49 (s, 1H), 7.24-7.39 (m, 7H), 7.39 (d, *J* = 8.5 Hz, 2H), 8.22 (d, *J* = 8.6 Hz, 2H) ppm. **$^{13}$C NMR** (400 MHz, $CDCl_3$): δ = 19.8 45.40, 46.8, 46.52, 52.7, 55.71, 60.22, 65.68, 66.5, 123.81, 127.99, 128.73, 129.01, 129.41, 133.92, 134.7, 141.7, 148.8, 165.8, 171.1, 175.6, 209.9 ppm.

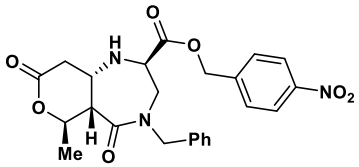
**4-nitrobenzyl (1R,5S,6S)-3-benzhydryl-5-((R)-1-hydroxyethyl)-4,8-dioxo-3,9-diazabicyclo[4.2.1]nonane-1-carboxylate (2d):**




Benzhydrylamine (3.0 mmol, 516 µl), paraformaldehyde, (3.0 mmol, 90.09 mg) and 1.0 g of magnesium sulphate were reacted for 24 h to obtain the imine, *N*-methylene-1-diphenylmethanamine (752.7 mg, 3 mmol). The resultant imine was reacted with carbapenem, (669.7 mg, 1.5 mmol) in the presence of proline, (86 mg, 0.75 mmol) for 48 h to get 979 mg of a white solid (72% yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 8. (*c* = 0.1,

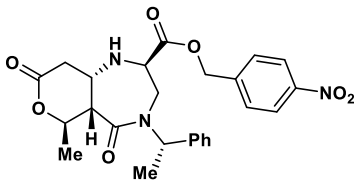
in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{30}H_{30}N_3O_9$ $[M+H]^+$: 544.2078; found 544.2040. **$^1$H NMR** (400 MHz, $CDCl_3$): δ = 1.18 (d, *J* = 6.7 Hz, 3H), 2.31 (d, *J* = 18.2 Hz, 1H), 2.73 (dd, *J* = 18.2 and 7.5 Hz, 1H), 3.59 (d, *J* = 16.1, 1H), 4.31 (d, *J* = 13.7 Hz, 1H), 4.29 (d , *J* = 13.7, 1H), 4.29 (s, 1H), 4.44 (m, 1H), 4.72 (d, *J* = 13.5 Hz, 1H), 5.02 (d, *J* = 13.5 Hz, 1H), 7.35-7.17 (m, 12H), 8.18 (d, *J* = 8.6 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 19.9 45.79, 46.82, 46.9, 55.8, 61.3, 65.7, 66.3, 123.7, 127.6, 127.8, 128, 128, 129, 138, 139, 141, 147, 165, 174, 209 ppm.

**4-nitrobenzyl (2R,5aS,6R,9aS)-4-benzyl-6-methyl-5,8-dioxodecahydropyrano[4,3-e][1,4]diazepine-2-carboxylate (3a):**



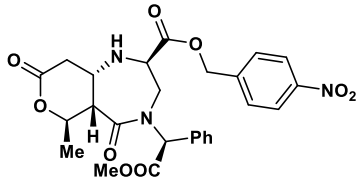

Benzylamine (5.0 mmol, 545 µl), paraformaldehyde, (5.0 mmol, 150 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, *N*-methylene-1-phenylmethanamine (687.7 mg, 5.0 mmol). The resultant imine was reacted with *p*-nitrobenzyl-6-(1-hydroxyethyl)-1-azabicyclo(3.2.0)heptane-3,7-dione-2-carboxylate (carbapenem), (955.6 mg, 2.5 mmol) in the presence of proline, (288 mg, 2.5 mmol) for 24 h to yield 702 mg of a white solid (60% yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 8. (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{24}H_{26}N_3O_7$ $[M+H]^+$: 468.1765; found 468.1802. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.30 (d, *J* = 6.2 Hz, 3H), 2.63 (dd, *J* = 5.7, 2.3 Hz, 1H), 2.73 (dd, *J* = 5.7, 2.3 Hz, 1H), 2.91 (d, *J* = 5.7, 2.3 Hz, 1H), 4.04-4.17 (m, 4H), 4.30-4.40 (qd, *J* = 5.7, 4.1 Hz, 2H), 5.19 (m, 2H), 6.49 (d, *J* = 5.40, 1H), 7.19-7.33 (m, 5H), 7.49 (d, *J* = 8.7 Hz, 2H), 8.19 (d, *J* = 8.7 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 21.5 39 42.3, 43.6, 53.8, 64.0, 53.8, 66.1, 76.8, 77.1, 77.4, 123.8, 127.7, 127.7, 128.5, 128.8, 137.7, 142.3, 147.8, 167.7, 168.4, 170.1 ppm.

**4-nitrobenzyl (2R,5aS,6R,9aS)-6-methyl-5,8-dioxo-4-((S)-1-phenylethyl)decahydropyrano[4,3-e][1,4]diazepine-2-carboxylate (3b):**



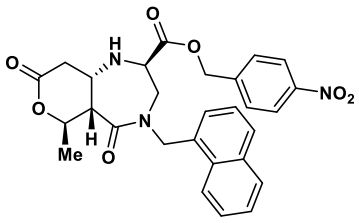
Methylbenzylamine (3.0 mmol, 370 µl), paraformaldehyde, (3.0 mmol, 90.09 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, N-methylene-1-phenylethanamine (687.7 mg, 5 mmol). The resultant imine was reacted with carbapenem, (955.6 mg, 2.5 mmol) in the presence of proline, (288 mg, 2.5 mmol) for 48 h to give a white solid of mass 877 mg (73 % yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 12, (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{25}H_{28}N_3O_7$ $[M+H]^+$: 482.1922; found 482.1889. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.35 (d, *J* = 6.7 Hz, 3H), 1.50 (d, *J* = 7.0 Hz, 3H), 2.63 (dd, *J* = 6.8, 9.6 Hz, 1H), 2.73 (dd, *J* = 6.8, 9.6 Hz, 1H), 2.91 (dd, *J* = 8.2, 2.34 Hz, 1H), 3.92-4.26 (m, 4H), 5.03-5.31 (m, 3H), 5.92 (d, *J* =7.1, 1H), 7.26-7.49 (m, 5H), 7.56 (d, *J* = 8.3 Hz, 2H), 8.21 (d, *J* = 8.9 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 21.4, 39.1, 42.3, 49.1, 53.8, 64.2, 65.6, 66.6, 123.9, 126.1, 128.5, 128.7, 142.5, 167.5, 168.3, 169.0 ppm.

**4-nitrobenzyl (2R,5aS,6R,9aS)-4-((S)-2-methoxy-2-oxo-1-phenylethyl)-6-methyl-5,8-dioxodecahydropyrano[4,3-e][1,4]diazepine-2-carboxylate (3c):**




Methyl 2-amino-2-phenylacetate was in a form of a salt so 1.0 g was neutralized using sodium bicarbonate in water and then the resultant amine, (687 mg, 4.15 mmol) paraformaldehyde, (4.15 mmol, 124.8 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, methyl 2-(methylene amino)-2-phenylacetate (752.4 mg, 4.15mmol). The resultant imine was reacted with carbapenem, (800.4 mg, and 2.07 mmol) in the presence of proline, (288 mg, 2.5 mmol) for 24 h to give 630 mg of a white solid (48% yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 4. (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{26}H_{28}N_3O_9$ $[M+H]^+$: 526.1726; found 526.1851. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.36 (d, 3H), 2.50 (dd, *J* = 10.63, 6.72 1H), 2.58 (dd, *J* = 10.63, 6.72 1H), 2.98-3.08 (m, 2H), 3.76 (s, 3H), 4.15 (d, 13.5 Hz, 2H), 4.54 (d, *J* = 10.6 Hz, 1H), 5.34 (q, *J* = 9.5 Hz, 2H), 5.58 (d, *J* = 7.3 Hz, 1H), 6.11 (d, *J* = 33.72, 2H), 6.91 (d, *J* = 6.72, 1H), 7.30-7.42 (m, 5H), 7.56 (d, *J* = 9.0 Hz, 2H), 8.27 (d, *J* = 8.4 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 21.3 38.6, 53.1, 53.6, 55.6, 56.5, 64.3, 65.9, 67.1, 115.7, 124.0, 127.3, 128.7, 129.0, 129.2, 130.5, 135.5, 142.2, 146.3, 161.9, 165.2, 169.6, 171.3 ppm.

**4-nitrobenzyl (2R,5aS,6R,9aS)-6-methyl-4-(naphthalen-1-ylmethyl)-5,8-dioxodecahydropyrano[4,3-e][1,4]diazepine-2-carboxylate (3d):**




Naphthylmethylamine (3.0 mmol, 370 µl), paraformaldehyde, (3.0 mmol, 90.09 mg) and 1.0 g magnesium sulphate were reacted for 15 h to obtain the imine, *N*-methylene-1-(naphthalen-1-yl)methanamine (687.7 mg, 5mmol). The resultant imine was reacted with carbapenem, (955.6 mg, 2.5 mmol) in the presence of proline, (288 mg, 2.5 mmol) for 48 h to give a white solid of mass, 657 mg (52% yield). $\mathbf{R_f}$ (EtOAc/hexane, 70:30) = 0.4. $[\alpha]_D^{21}$ – 8. (*c* = 0.1, in $CHCl_3$). **HRMS** (ESI+) *m/z* calculated for $C_{28}H_{28}N_3O_7$ $[M+H]^+$: 518.1922; found 518.1936. **$^1$H NMR** (400 MHz, $CDCl_3$): δ 1.34 (d, *J* = 6.2 Hz, 3H), 2.63 (dd, *J* = 5.7,2.3 Hz, 2H), 2.73 (dd, *J* = 5.7,2.3 Hz, 2H), 2.95 (d, *J* = 5.7, 2.3 Hz, 1H), 4.02-4.19 (m, 4H), 4.80-4.94 (qd, *J* = 5.6, 4.1 Hz, 2H), 5.18 (q, 2H), 6.04 (t, 1H), 7.42-7.60 (m, 6H), 7.83 (d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 2H) ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 21.5 39.1, 41.9, 42.4, 53.8, 64.2, 65.7, 66.5, 123.2, 123.9, 125.4, 126.2, 126.9, 128.6, 128.9, 131.3, 132.8, 133.9, 142.3, 167.6, 168.4, 169.6 ppm.

**Crystallography refinement for 3b**

Single crystal data was collected by mounting a suitable single crystal on a Bruker Smart Apex II CCD diffractometer using graphite-monochromated Mo-Kα radiation (l = 0.71073 Å) in a stream of cold nitrogen at 173 (1)K using Oxford Cryostream cooling system (Oxford Cryostat). Data reduction and cell refinement were performed using the program SAINT-Plus [27]. The data were scaled and absorption correction performed using SADABS [27]. The space groups were determined from systematic absences by XPREP [28]and further justified by the refinement results. The structure was solved by direct methods using SHELXS-97 [29] and refined by full-matrix least-squares methods based on F2 using SHELXL-97.[29] The program ORTEP-3 Windows version 1.08 [29] was used to prepare molecular graphic image and publication materials. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms, except H10A, were placed in idealized positions and refined in riding models with Uiso assigned the values to be 1.2 or 1.5 times those of their parent atoms and the constraint distances of C-H ranging from 0.95 Å to 1.00 Å. The hydrogen atom H10A

was located in the difference electron density maps and refined independently. CIF of crystal structure of 3b has been deposited to CCDC (CCDC 1455806).

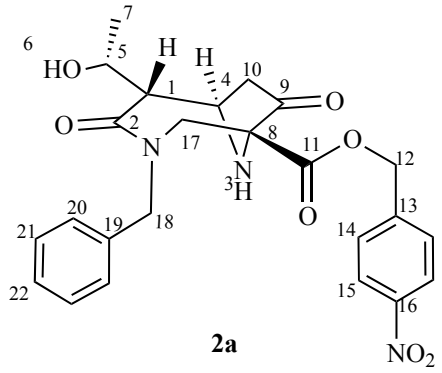
**References**


1. Sorrell, T.N., *Organic chemistry*. 2006, Sausalito, Calif.: University Science Books.
2. Radchenko, D.S., et al., *Conformationally Restricted Nonchiral Pipecolic Acid Analogues.* The Journal of Organic Chemistry, 2009. **74**(15): p. 5541-5544.
3. Denisenko, A.V., et al., *3-Benzyl-3-azabicyclo[3.1.1]heptan-6-one: A Promising Building Block for Medicinal Chemistry.* Organic Letters, 2010. **12**(19): p. 4372-4375.
4. Gante, J., *Peptidomimetics—Tailored Enzyme Inhibitors.* Angewandte Chemie International Edition in English, 1994. **33**(17): p. 1699-1720.
5. Heinrich, N., et al., *Early phase evaluation of SQ109 alone and in combination with rifampicin in pulmonary TB patients.* Journal of Antimicrobial Chemotherapy, 2015.
6. Li, K., et al., *Oxa, Thia, Heterocycle, and Carborane Analogues of SQ109: Bacterial and Protozoal Cell Growth Inhibitors.* ACS Infectious Diseases, 2015. **1**(5): p. 215-221.
7. Södergren, M.J. and P.G. Andersson, *Chiral, bicyclic proline derivatives and their application as ligands for copper in the catalytic asymmetric allylic oxidation of olefins.* Tetrahedron Letters, 1996. **37**(42): p. 7577-7580.
8. Phelan, R.M. and C.A. Townsend, *Mechanistic Insights into the Bifunctional Non-Heme Iron Oxygenase Carbapenem Synthase by Active Site Saturation Mutagenesis.* Journal of the American Chemical Society, 2013. **135**(20): p. 7496-7502.
9. Arya, S.C. and N. Agarwal, *Apropos: The Challenge of Antimicrobial Resistance in Brazil.* Clinical Infectious Diseases, 2011. **53**(4): p. 401-402.
10. Cele, Z.E.D., et al., *Organocatalytic Mannich Reactions on a Carbapenem Core – Synthesis of Mannich Bases and Bicyclic Diazanonanes.* European Journal of Organic Chemistry, 2014. **2014**(11): p. 2253-2260.
11. Khanyase, S., et al., *l-Proline organocatalyzed Michael synthesis of monobactam and carbapenem β-lactam cores.* Tetrahedron: Asymmetry, 2014. **25**(13–14): p. 969-973.
12. Pawar, S.A., et al., *Organocatalyzed stereospecific C-C bond formation of [small beta]-lactams.* Organic & Biomolecular Chemistry, 2013. **11**(48): p. 8294-8297.
13. Cele, Z.E.D., et al., *Applied Enantioselective Aminocatalysis: α-Heteroatom Functionalization Reactions on the Carbapenem (β-Lactam Antibiotic) Core.* European Journal of Organic Chemistry, 2015. **2015**(3): p. 638-646.

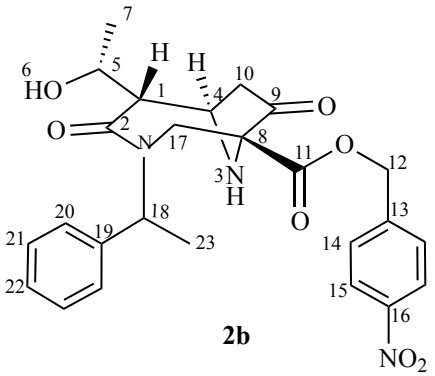
14. Bradley, J.S., et al., *Carbapenems in clinical practice: a guide to their use in serious infection.* International Journal of Antimicrobial Agents, 1999. **11**(2): p. 93-100.
15. Rodloff, A.C., E.J.C. Goldstein, and A. Torres, *Two decades of imipenem therapy.* Journal of Antimicrobial Chemotherapy, 2006. **58**(5): p. 916-929.
16. Shah, P.M. and R.D. Isaacs, *Ertapenem, the first of a new group of carbapenems.* Journal of Antimicrobial Chemotherapy, 2003. **52**(4): p. 538-542.
17. Viehman, J.A., M.H. Nguyen, and Y. Doi, *Treatment options for carbapenem-resistant and extensively drug-resistant Acinetobacter baumannii infections.* Drugs, 2014. **74**(12): p. 1315-1333.
18. Batirel, A., et al., *Comparison of colistin–carbapenem, colistin–sulbactam, and colistin plus other antibacterial agents for the treatment of extremely drug-resistant Acinetobacter baumannii bloodstream infections.* European journal of clinical microbiology & infectious diseases, 2014. **33**(8): p. 1311-1322.
19. Chen, A., et al., *An approach to the total synthesis of lankacidins: synthesis of advanced macrocyclic precursors.* Tetrahedron Letters, 2001. **42**(7): p. 1251-1254.
20. Brain, C.T., et al., *An approach to the total synthesis of lankacidins: synthesis of the requisite building blocks.* Tetrahedron Letters, 2001. **42**(7): p. 1247-1250.
21. Ye, T. and M.A. McKervey, *Organic Synthesis with .alpha.-Diazo Carbonyl Compounds.* Chemical Reviews, 1994. **94**(4): p. 1091-1160.
22. Reekie, T.A., et al., *Synthesis of biologically active seven-membered-ring heterocycles.* Synthesis (Germany), 2013. **45**(23): p. 3211-3227.
23. Pollini, G.P., et al., *Synthetic Approaches to Enantiomerically Pure 8-Azabicyclo[3.2.1]octane Derivatives.* Chemical Reviews, 2006. **106**(6): p. 2434-2454.
24. Nortcliffe, A. and C.J. Moody, *Seven-membered ring scaffolds for drug discovery: Access to functionalised azepanes and oxepanes through diazocarbonyl chemistry.* Bioorganic & Medicinal Chemistry, 2015. **23**(11): p. 2730-2735.
25. Reekie, T.A., et al., *Synthesis of Biologically Active Seven-Membered-Ring Heterocycles.* Synthesis, 2013. **45**(23): p. 3211-3227.
26. Sano, S., H. Shimizu, and Y. Nagao, *Facile generation method for conjugated allenyl esters based on retro-Dieckmann-type ring-opening reactions.* Tetrahedron Letters, 2005. **46**(16): p. 2883-2886.
27. *Bruker (2001). SAINT*, Madison, Wisconsin, USA.
28. *Bruker (1997)XPREP. Version 5.1/NT. ,* , Bruker AXS Inc.: Madison, Wisconsin, USA.

29. Farrugia, L., *ORTEP-3 for Windows - a version of ORTEP-III with a Graphical User Interface (GUI)*. Journal of Applied Crystallography, 1997. **30**(5 Part 1): p. 565.

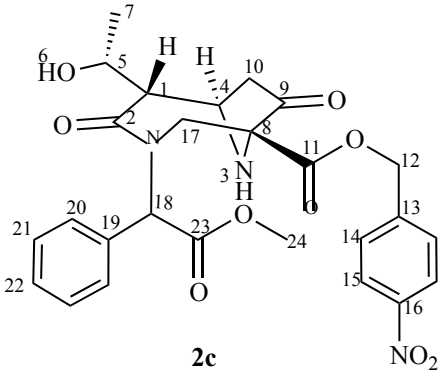
## 2D NMR elucidation of 2a-d and 3a-d



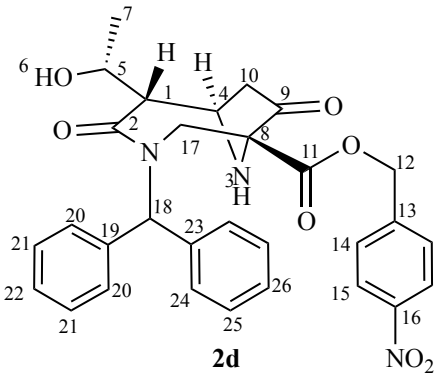
**2a**



**2b**



**2c**



**2d**

Structures of the synthesized compounds **2a-d**

The bicyclic diazanonane ring was first elucidated, followed by the side chains. For the elucidation, the methyl protons, H7, were used as a convenient starting point as the protons exhibits a COSY and HMBC correlations with the protons and the carbon nuclei in the bicyclic diazanonane ring. From the $^{13}$C-APT spectra, it was to differentiate between the methine (CH) protons and the methylene ($CH_2$) protons. The protons, which could not be assigned using the COSY spectrum, were assigned from the NOESY spectrum. The corresponding carbons atoms were elucidated from the HSQC spectrum. Some of the quaternary carbons, (C) were elucidated from the HMBC spectrum.

For the side chain on C8, a convenient handle was the distinct aromatic protons, especially the ones (H15) next to the nitro group. The COSY spectrum enabled us to assign the rest of the protons on this side chain.

Compound **2a** has only one $CH_3$ group and the corresponding protons (H7), were assigned from the $^{1}$H NMR and the $^{13}$C-APT spectra as the peak at 1.19 ppm. The corresponding carbon C7 was deduced from the HSQC spectrum.

There are three more methine (CH) signals (H1, H4 and H5) excluding the aromatic protons, with two of them on the bicyclic ring (H1 and H4). From the COSY spectrum, the H7 proton shows a correlation with the proton at 4.39 ppm (H5), this in turn correlates with a proton at 2.49 ppm (H1),

which in turn interacts with the proton at 4.23 ppm (H4). Of the three signals, the signal for H5 was the most deshielded due to the neighboring oxygen atom, and therefore its signal appears at a higher frequency. As H4 is close to nitrogen in the ring (NH), the signal appears at a relatively lower frequency as compared to that of H5 and also at a relatively higher frequency as compared to H1, which was on a carbon directly attached to only carbons.

The chirality at C5 was already known form the starting material as ordered. From the NOESY spectrum, H4 correlates with H7 indicating that they are on the same side of the ring. H1 and H5 also show a through space NOESY correlation confirming the previous assignment. The chirality on C8 was deduced from the X-ray crystal structure of **2a**.[97]

Within the bicyclic diazanonane scaffold there were only two $CH_2$ signals, H10 (2.24 and 2.68 ppm) and H17 (3.96 and 3.55 ppm), whilst the remaining $CH_2$ signals were from H12 (5.07 ppm) and H18 (4.23 and 4.82 ppm) belonged to side chains. These signals were identified from the $^{13}$C-APT spectrum.

From the proton spectrum, the peaks with the same coupling constants were first noted as $CH_2$ signals. Signals at 2.24 and 2.68 ppm (H10) have the coupling constant (J) of 18.65 Hz. these protons exhibit a NOESY correlation with H4 and a HMBC correlation with H1. Signals at 3.96 and 3.55 ppm (H17) also shared the same coupling constant (J = 15.70 Hz). These protons (H17) show a HMBC correlation with C1 and C2 and also a NOESY correlation with H4.

From the $^{13}$C-APT spectrum, 7 signals were identified as belonging to quaternary carbons and three of these are in the bicyclic diazanonane scaffold, C2 (174.7 ppm) and C8 (136.3 ppm) and C9 (209.1 ppm). C2 exhibits a HMBC correlation with H1, H17 and H18 and is also characteristic of a carbonyl peak in the region of 170.0 ppm. C8 shows a HMBC correlation to H17 and the signal at 141.7 ppm (C13) and C9 exhibits HMBC correlation with H10. This concluded the elucidation of the bicyclic ring.

Elucidation of the remainder of the side chain on C8 was achieved using the aromatic protons (H15, 8.14 ppm, 2 equivalent protons) as the starting point. These protons are more shielded due to the adjacent nitro group (on C16), so the signal appears at the highest frequency on the proton spectrum. H15 protons show a NOESY correlation to H14 (7.35 ppm). The only peak that integrated for two protons, 5.07 ppm, was assigned to H12, since the protons exhibit COSY correlation with the aromatic protons, H14. The signal at 166.7 ppm (C11) exhibits HMBC correlation to H12. C16 (148.3 ppm) shows HMBC correlation with H15. The quaternary signal at 141.7 ppm (C13) reveals HMBC correlation with H14.

For the last side chain, (on the nitrogen), the benzylic protons (H18) at 4.23 and 4.82 ppm are adjacent to nitrogen therefore the chemical shift for the signal is at higher frequency than the expected one of range 2.2-3.0 ppm as there is more deshielding on the proton nuclei. These protons show a COSY correlation with the protons in the aromatic protons, H20 and H21. The corresponding carbon signals were deduced from the HSQC spectrum. The multiplet from 7.14-7.24 ppm constituted the signals for the protons on the other aromatic side chain (H20, H21 and H22). The corresponding carbons were deduced from the HSQC spectrum. C19 (136.1 ppm) exhibits a HMBC correlation to the aromatic protons H20 and H21 the benzylic protons (H18).

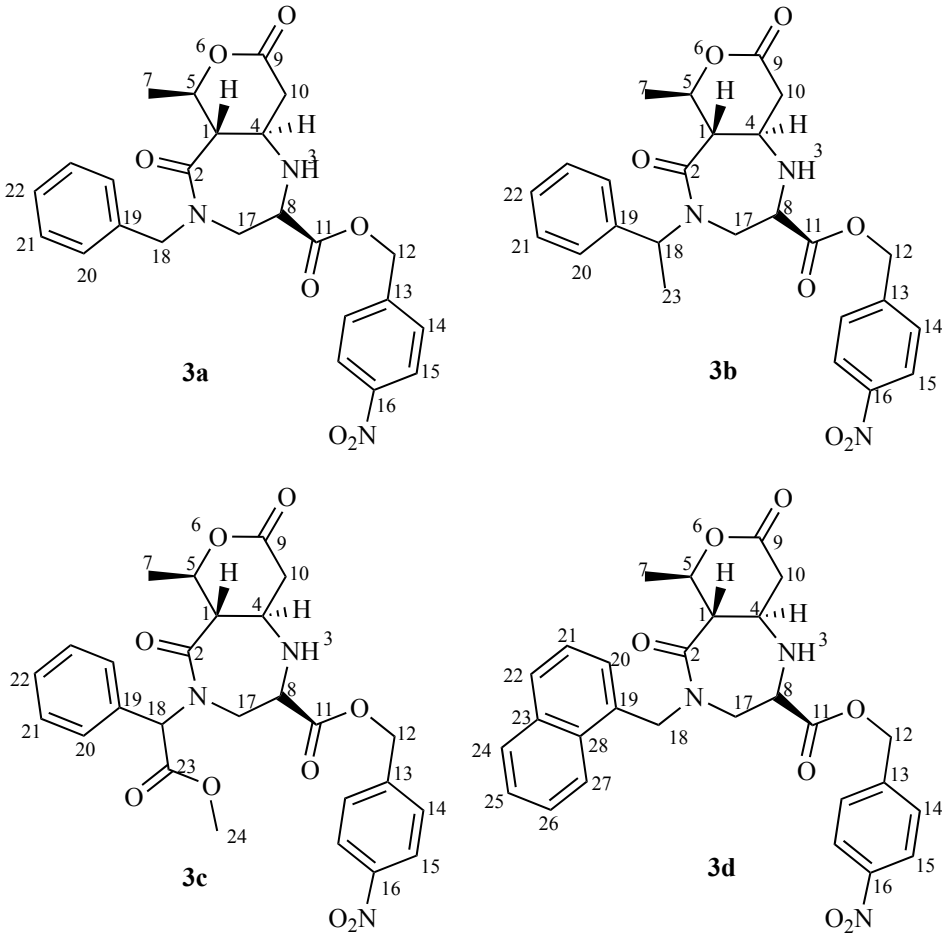
The elucidation of **2d, 2c** and **2b** followed the same approach used for **2a**. For **2d**, the difference in this compound was the substitution of one proton on the C18 atom with an aromatic ring. Due to this change, the signal of H18 shifted to a higher frequency, (multiplet at 7.18-7.38 ppm), which is in the aromatic region. This is due to the conjugation of electrons extending to the benzylic proton. The signals for the protons in the aromatic ring from H24-26 occurred in the same multiplet region as H18. H/C23, H/C24, H/C25, H/C26 had the same chemical shift as H/C19, H/C20, H/C21and H/C22 as they were equivalent carbons and protons.

For **2c**, the change of the compound was also at C18. In this compound, the proton was substituted with a carboxyl methyl ester group. The signal for H18 appears at a lower frequency (6.51 ppm) than the one in **2d (**7.18-7.38 ppm**)** because the conjugation of electrons was destroyed and the proton nucleus is less deshielded. There is only one additional signal in the proton NMR spectrum at 3.82 ppm, which was assigned to H24. From the HMBC spectrum, the carbonyl carbon at 133.9 ppm (C23) correlates with H18 at 6.51 ppm.

In **2b**, methyl group (C23) was attached to C18 and the methyl protons (H23) are registered as a doublet at 1.49 and at 1.35 ppm and the corresponding carbon at 51.4 ppm (C23) from HSQC spectrum.

**Table 1**. NMR data for compounds **2a-d**

| $^{1}$H NMR | | | | | $^{13}$C NMR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Compound δ (ppm) | | | | | Compound δ (ppm) | | | |
| | **2a** | **2d** | **2c** | **2b** | | **2a** | **2d** | **2c** | **2b** |
| 1 | 2.49 | 2.45 | 2.45 | 2.77 | 1 | 57.5 | 55.8 | 55.7 | 64.3 |
| 2 | - | - | - | - | 2 | 174.7 | 174.9 | 175.4 | 169.0 |
| 3 | - | - | - | - | 3 | NH | NH | NH | NH |
| 4 | 4.23 | 3.62 | 4.26 | 4.18 | 4 | 47.4 | 46.9 | 46.5 | 52.5 |
| 5 | 4.39 | 4.45 | 4.45 | 4.71 | 5 | 66.5 | 65.7 | 65.6 | 74.5 |
| 6 | - | - | - | - | 6 | OH | OH | OH | OH |
| 7 | 1.19 | 1.21 | 1.20 | 1.56 | 7 | 20.7 | 19.9 | 19.9 | 20.1 |
| 8 | - | - | - | - | 8 | 136.3 | 138.9 | 134.0 | 168.3 |
| 9 | - | - | - | - | 9 | 209.6 | 209.9 | 209.7 | |
| 10 | 2.24, 2.68 | 2.76, 2.33 | 2.31, 2.72 | 2.95, 3.02 | 10 | 43.9 | 45.7 | 46.5 | 36.4 |
| 11 | - | - | - | - | 11 | 166.6 | 165.9 | 166.2 | 170.2 |
| 12 | 5.07 | 4.72, 5.02 | 4.68, 4.99 | 5.32 | 12 | 66.6 | 66.3 | 66.6 | 66.2 |
| 13 | - | - | - | - | 13 | 141.7 | 141.8 | 142.2 | 128.8 |
| 14 | 7.35 | 7.18-7.38 | 7.40 | 7.45 | 14 | 128.2 | 127.7 | 128.0 | 128.5 |
| 15 | 8.14 | 8.21 | 8.25 | 8.32 | 15 | 123.8 | 123.7 | 123.8 | 124.0 |
| 16 | - | - | - | - | 16 | 148.3 | 147.8 | 147.0 | 141.8 |
| 17 | 3.96, 3.55 | 4.29, 4.31 | 4.68, 4.99 | 4.18, 4.01 | 17 | 49.8 | 46.8 | 46.8 | 42.34 |
| 18 | 4.82, 4.23 | 7.18-7.38 | 6.51 | 6.03 | 18 | 51.8 | 61.3 | 60.24 | 51.1 |
| 19 | - | - | - | - | 19 | 136.1 | 139.0 | 134.0 | 142.4 |
| 20 | 7.14-7.24 | 7.18-7.38 | 7.27-7.34 | 7.38-7.22 | 20 | 127.8 | 129.9 | 129.7 | 126.1 |
| 21 | 7.14-7.24 | 7.18-7.38 | 7.27-7.34 | 7.38-7.22 | 21 | 128.3 | 128.7 | 129.0 | 127.1 |
| 22 | 7.14-7.24 | 7.18-7.38 | 7.27-7.34 | 7.38-7.22 | 22 | 127.8 | 127.8 | 128.7 | 127.8 |
| 23 | - | - | - | 1.49, 1.35 | 23 | - | 139.0 | 52.7 | 21.4, 21.7 |
| 24 | - | 7.18-7.38 | 3.82 | - | 24 | - | 129.9 | 45.4 | - |
| 25 | - | 7.18-7.38 | - | - | 25 | - | 128.7 | - | - |
| 26 | - | 7.18-7.38 | - | - | 26 | - | 127.8 | - | - |




Structures of the synthesized compounds **3a-d**

First the methyl protons, (H7, 1.28 ppm) were assigned and used as the starting point for the elucidation of the structure. The protons in the bicyclic rings were assigned first. H7 exhibits a COSY correlation with H5, the multiplet from 4.03-4.18 ppm. H5 shows correlation with the methine proton H1 (2.91 ppm). H1 also shows a COSY correlation with H4 (4.03-4.18 ppm), which also exhibits a COSY correlation with the methylene protons H10 (the multiplet 2.60-2.76 ppm). C9 (168.4. ppm) exhibits a HMBC correlation with H10 protons.

C2 (167.8ppm) shows a HMBC correlation with the H1 proton. H17 (4.30-4.42 ppm) are the only methylene protons left, showing a COSY correlation to a methine proton, H8 (6.50 ppm). This is the main difference between **2a** and **3a.** Compared to the **2a,** which does not have a proton at C8, in **3a,** C8 has a proton bonded and is now a methine carbon.

For the branches, on the branch with the *para*-nitro protecting group, C11 (170.1ppm) shows a HMBC correlation with H8. H12 (5.20 ppm) is the only other methylene proton left and it shows a COSY correlation with C13 (147.8 ppm). C13 also shows a HMBC correlation with H14 (7.49 ppm). H14 and H15 (8.19 ppm), shows a COSY correlation and H15 also shows a HMBC correlation with C16 (142.2 ppm).

On the remaining branch, H18 (5.03-5.31 ppm) is the only remaining methylene group and it shows a HMBC correlation with C19 (128.7ppm). H20-22 signals are in the multiplet, 7.21-7.33 ppm. H19 also show a HMBC correlation with H20, which also exhibits a COSY correlation with H21. H21 shows a COSY correlation with H22.

Other important correlations to confirm the rearrangement of the bicyclic rings were the HMBC correlation between C9 and H5 (confirmed that a new ring was formed), the NOESY correlation between H10 and H8, H10 and H17 (confirms the configuration of the molecule as shown by the crystal structure obtained), and also that of H7 and H1 (confirmed the chirality on C5 and C1). H8, shows a NOESY correlation with H10 and from the confirmation of the molecule from the X-ray structure, the chirality at C8 was deduced.

The same approach was used in elucidating the structures of **3b, 3c** and **3d** as above. The change in the structure of **3b** is at C18, which is now a methine instead of methylene. H18 (5.92 ppm) shows a COSY correlation with the methyl protons H23 (1.50 ppm) and also a HMBC correlation with C19 (142.8 ppm). The protons are less shielded due to the methyl group, C23 that is electron donating, and so the signal appears at a higher frequency, 5.09 ppm, than in **3a**.

In **3c**, there is a methyl ester group on the methine group C18. The signal for H18 appears at a higher frequency (6.91 ppm) than the one in **2a (**5.03-5.30 ppm**)** due to the adjacent electron withdrawing ester group on C18. There is only one additional signal in the proton spectrum at 3.76 ppm (H24). From the HMBC spectrum, the carbonyl carbon at 161.9 ppm (C23) correlates with H18 (6.91 ppm).

In **3d**, instead of a benzyl ring on the nitrogen of the amide bond, there is a naphthyl group. Compared to the spectra of **3a,** there are additional protons in the aromatic region. H20 (multiplet from 7.42-7.60 ppm), which showed a HMBC correlation with C18. Signals for H26 and H25 are also included in this multiplet and H27, H24 and H22 signals appear at 8.00, 7.90 and 7.83 ppm respectively.

The results from the two-dimensional NMR elucidation of the structures are shown in **Table 2**.

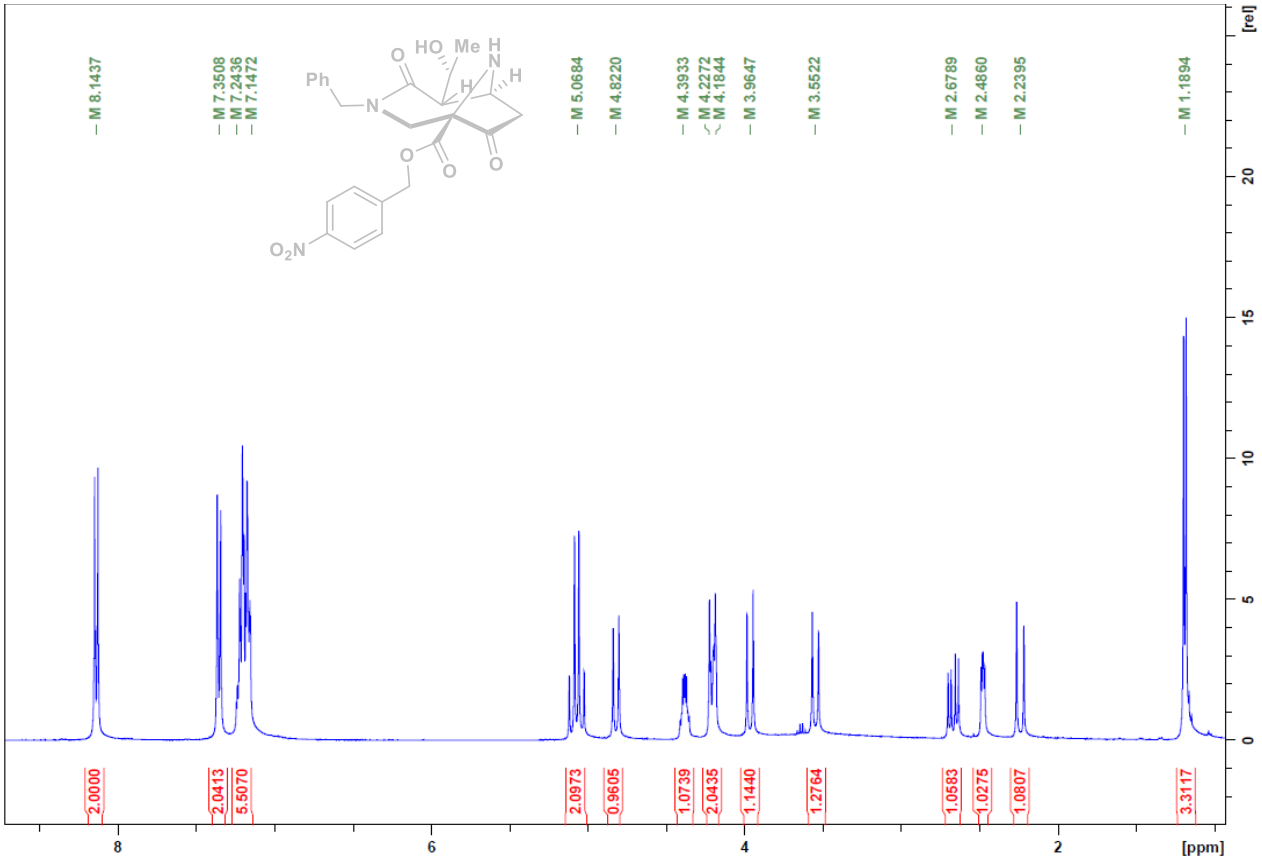
**Table 2:** NMR data for compounds **3a-d**

| $^{1}$H NMR | | | | | $^{13}$C NMR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Compound δ (ppm) | | | | | Compound δ (ppm) | | | |
| | **3a** | **3b** | **3c** | **3d** | | **3a** | **3b** | **3c** | **3d** |
| 1 | 2.91 | 2.95 | 2.98-3.08 | 2.95 | 1 | 63.9 | 64.2 | 64.3 | 64.2 |
| 2 | - | - | - | - | 2 | 167.8 | 167.5 | 165.2 | 167.6 |
| 3 | - | - | - | | 3 | NH | NH | NH | NH |
| 4 | 4.03-4.18 | 4.00-4.19 | 4.54 | 4.03-4.20 | 4 | 53.8 | 49.1 | 55.6 | 53.8 |
| 5 | 4.03-4.18 | 4.00-4.19 | 4.15 | 4.03-4.20 | 5 | 66.1 | 66.3 | 67.2 | 66.5 |
| 6 | - | - | - | - | 6 | O | O | O | O |
| 7 | 1.28 | 1.35 | 1.37 | 1.35 | 7 | 21.5 | 21.4 | 21.3 | 21.5 |
| 8 | 6.50 | 5.93 | 5.57 | 6.04 | 8 | 43.6 | 49.1 | 56.5 | 41.9 |
| 9 | | | | - | 9 | 168.4 | 168.3 | 169.6 | 168.4 |
| 10 | 2.76-2.60 | 2.59-2.75 | 2.58, 3.04 | 2.78-2.64 | 10 | 39.0 | 39.1 | 38.7 | 39.1 |
| 11 | | | | - | 11 | 170.1 | 169.0 | 171.3 | 169.6 |
| 12 | 5.20 | 5.18 | 5.34 | 5.19 | 12 | 65.6 | 65.6 | 65.9 | 65.7 |
| 13 | - | - | - | - | 13 | 147.8 | 147.8 | 146.3 | |
| 14 | 7.49 | 7.56 | 7.57 | 7.42-7.60 | 14 | 128.9 | 128.7 | 128.7 | 128.6 |
| 15 | 8.19 | 8.21 | 8.27 | 8.20 | 15 | 123.8 | 123.9 | 124.0 | 123.9 |
| 16 | - | - | - | - | 16 | 142.2 | 142.1 | 142.1 | 142.3 |
| 17 | 4.30-4.42 | 4.00-4.19 | 6.07, 6.15 | 4.03-4.20 | 17 | 42.3 | 42.3 | | 42.2 |
| 18 | 5.30-5.03 | 5.09 | 6.91 | 4.94 | 18 | 43.6 | 64.2 | 56.5 | 41.9 |
| 19 | - | - | - | - | 19 | 137.7 | 142.8 | 135.5 | 134.0 |
| 20 | 7.21-7.33 | 7.27-7.37 | 7.30-7.42 | 7.42-7.60 | 20 | 128.5 | 128.5 | 129.2 | 129.0 |
| 21 | 7.21-7.33 | 7.27-7.37 | 7.30-7.42 | 7.42-7.60 | 21 | 127.7 | 127.6 | 128.9 | 126.9 |
| 22 | 7.21-7.33 | 7.27-7.37 | 7.30-7.42 | 7.83 | 22 | 127.6 | 126.1 | 127.3 | 126.3 |
| 23 | - | 1.50 | - | - | 23 | - | 21.7 | 161.9 | 132.8 |
| 24 | - | - | 3.76 | 7.90 | | - | - | 53.1 | 129.0 |
| 25 | - | - | - | 7.42-7.60 | | - | - | - | 125.5 |
| 26 | - | - | - | 7.42-7.60 | | - | - | - | 125.5 |
| 27 | - | - | - | 8.00 | | - | - | - | 123.2 |
| 28 | - | - | - | | | - | - | - | 131.3 |

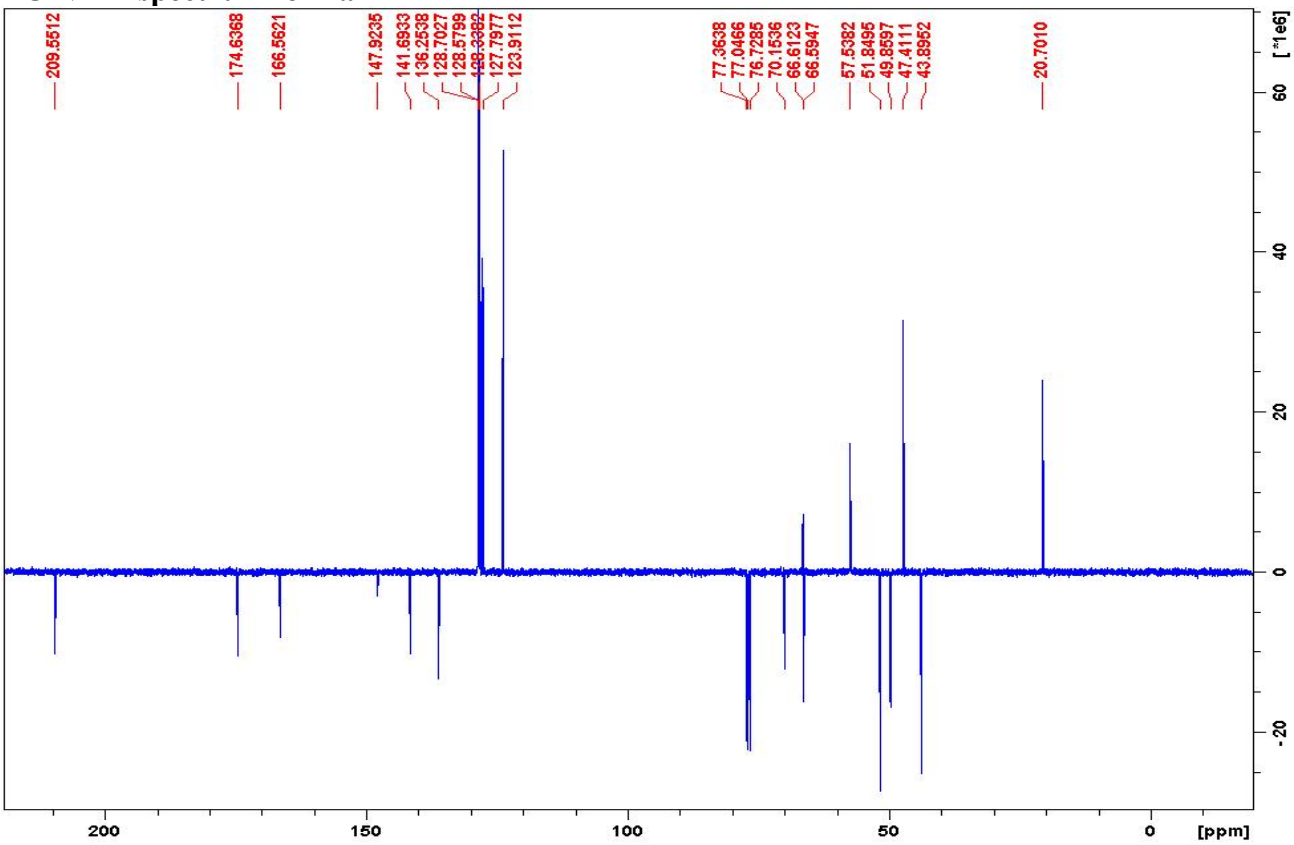
## Appendix

1. NMR spectra
2. HRMS spectra

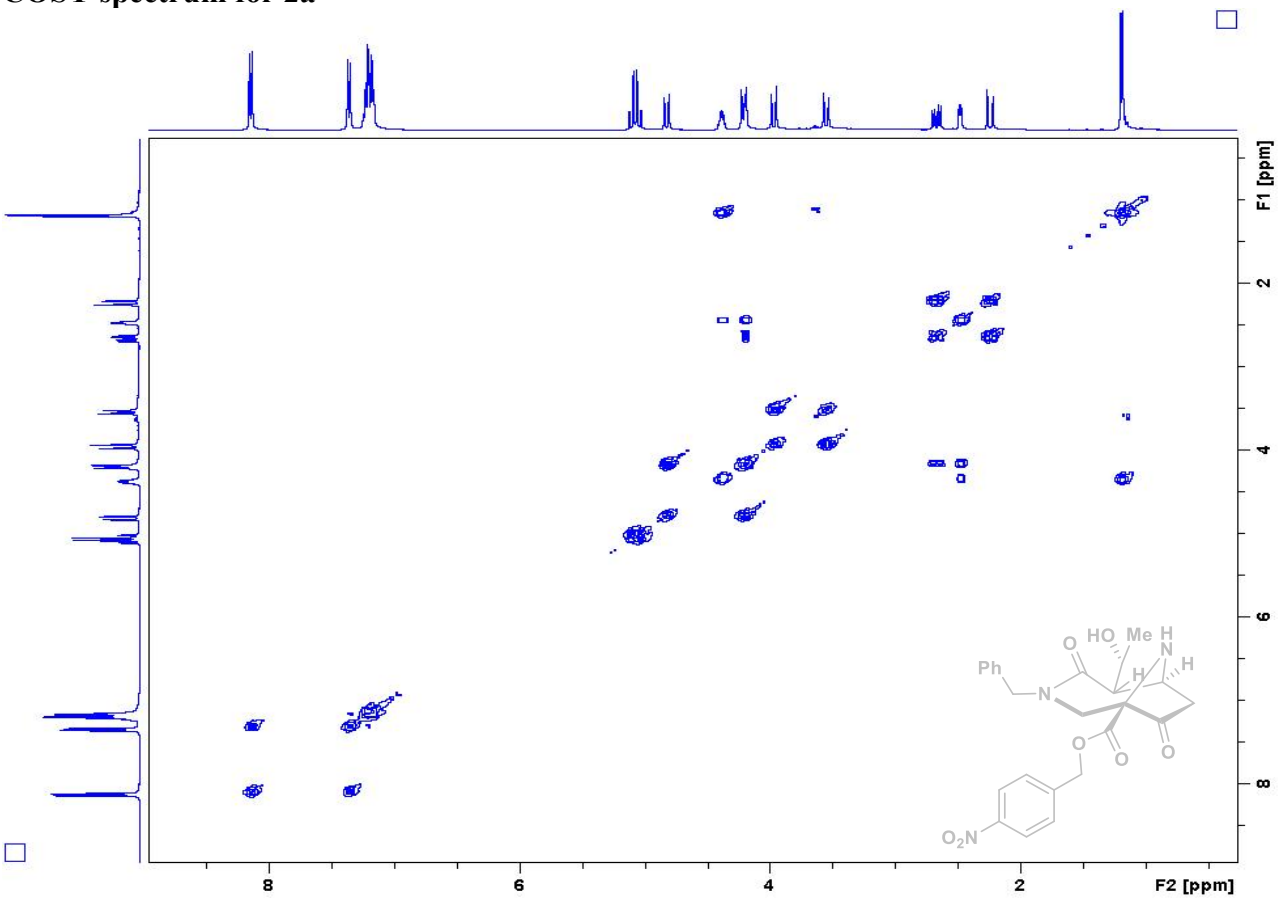
## $^{1}$H NMR spectrum for 2a



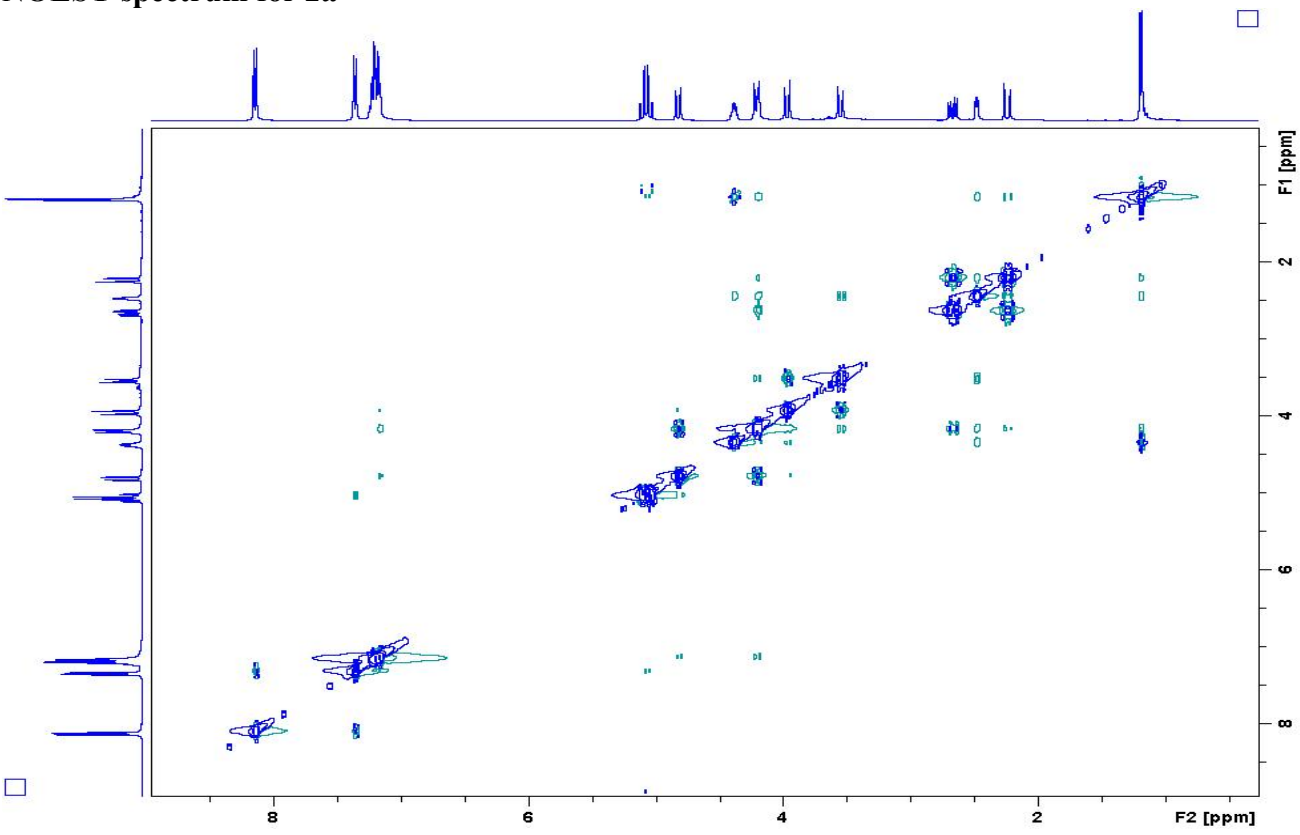

## $^{13}$C NMR spectrum for 2a



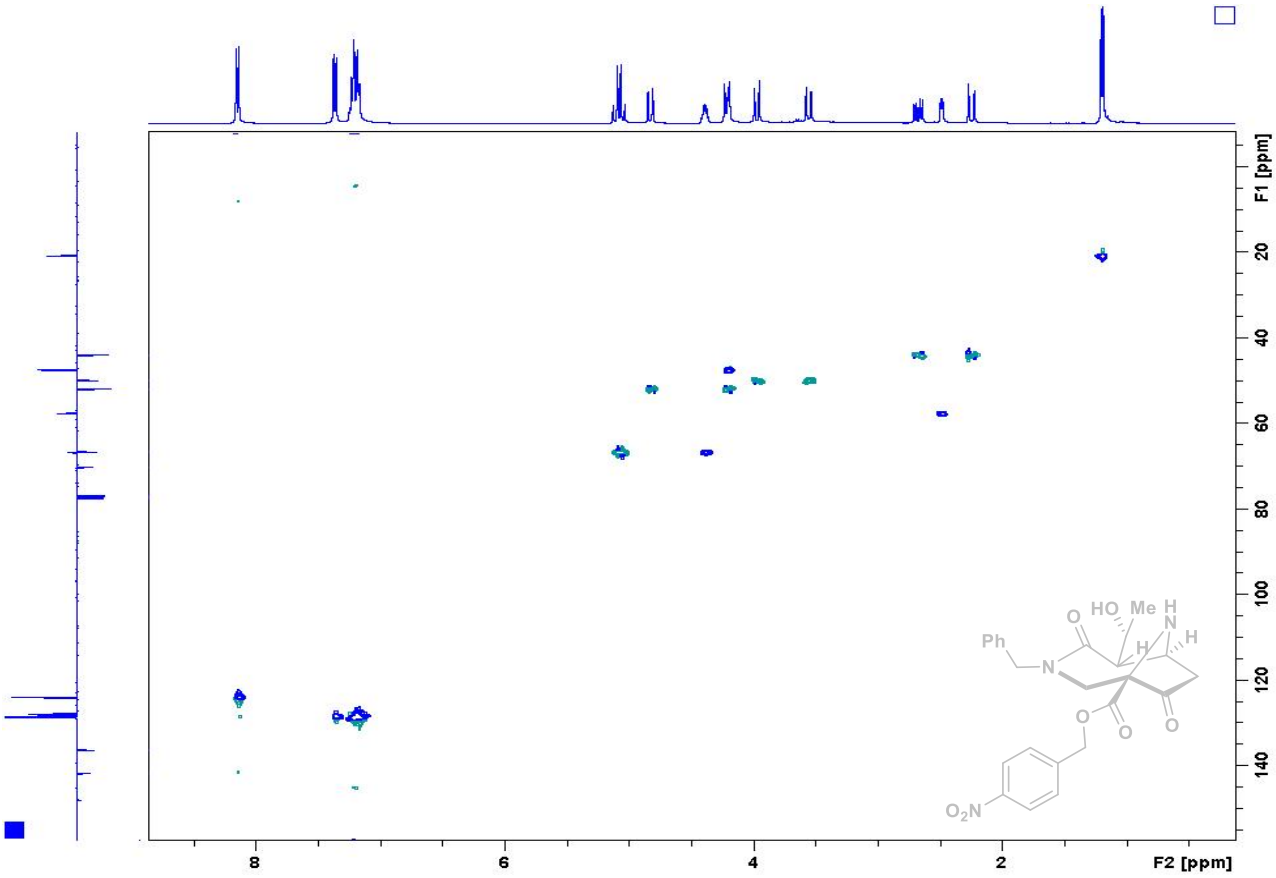
**COSY spectrum for 2a**



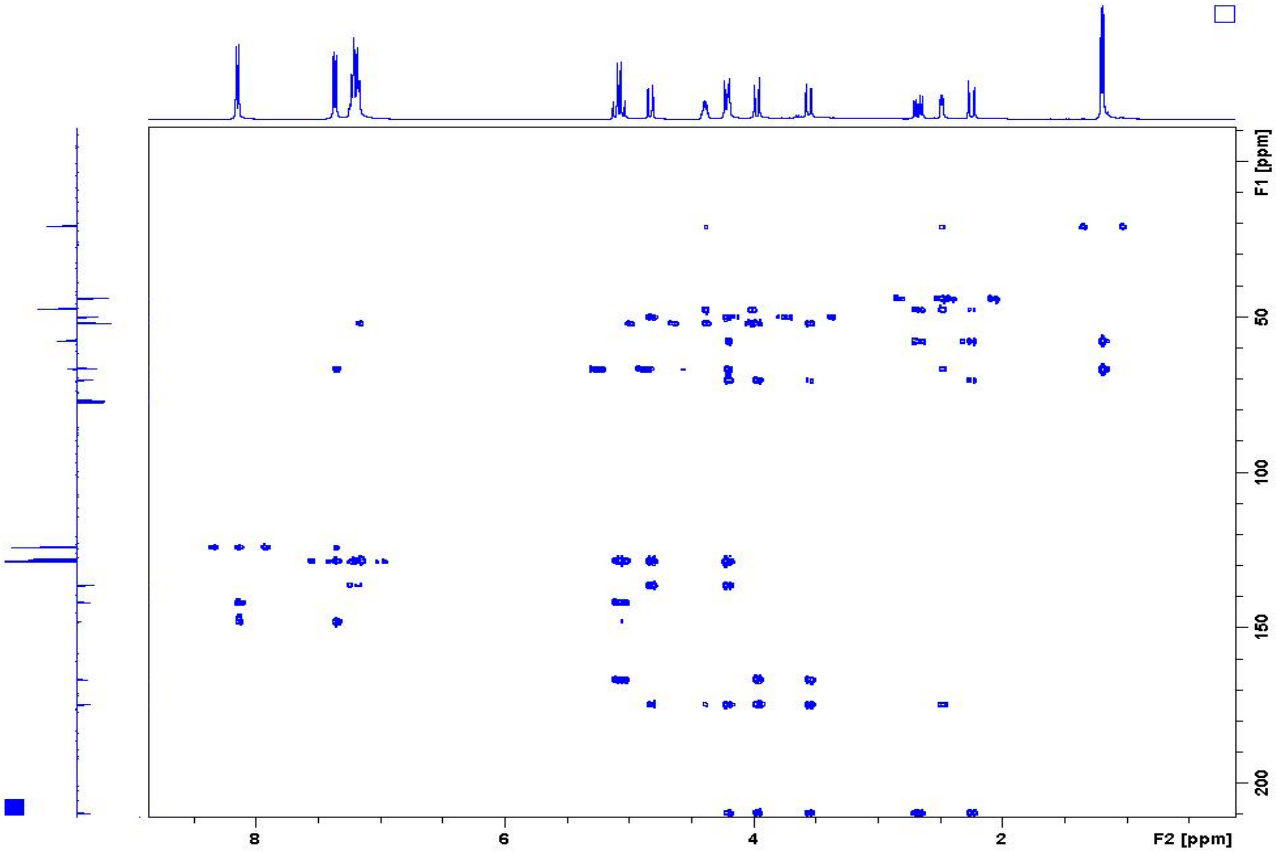

**NOESY spectrum for 2a**



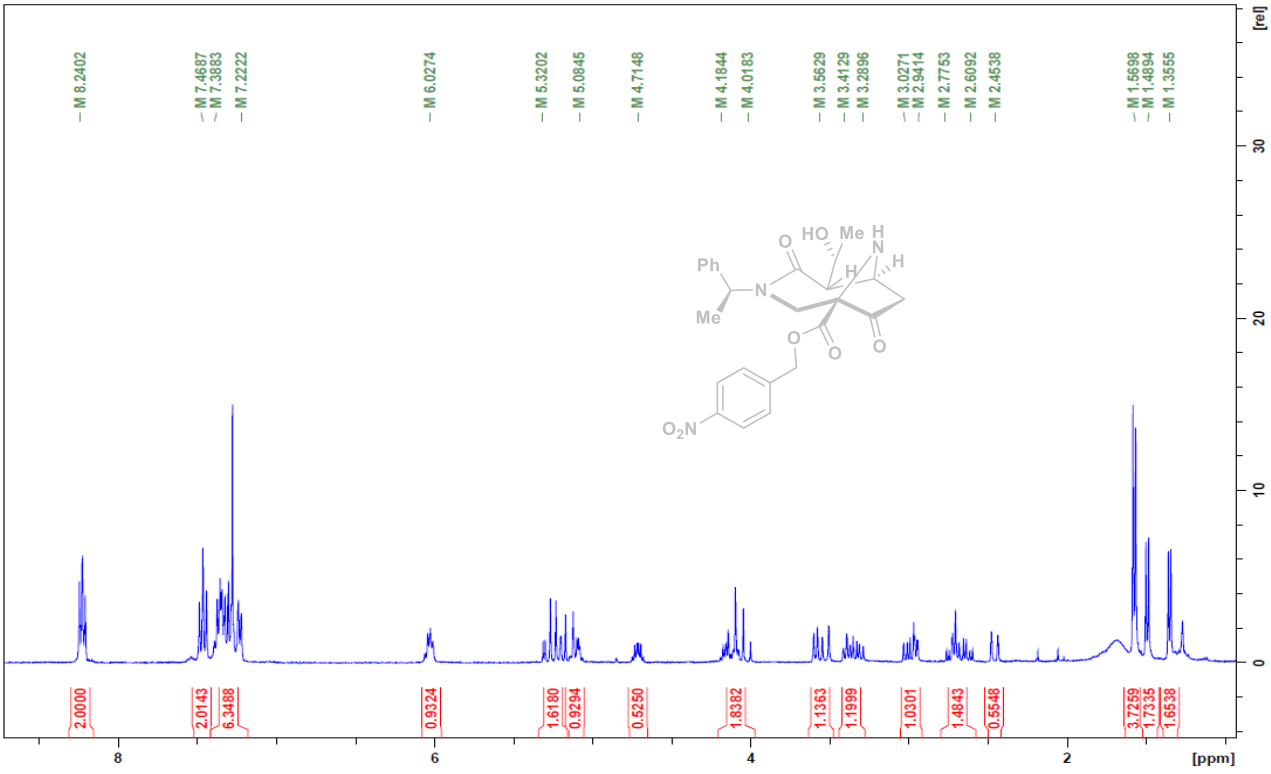
**HSQC spectrum for 2a**



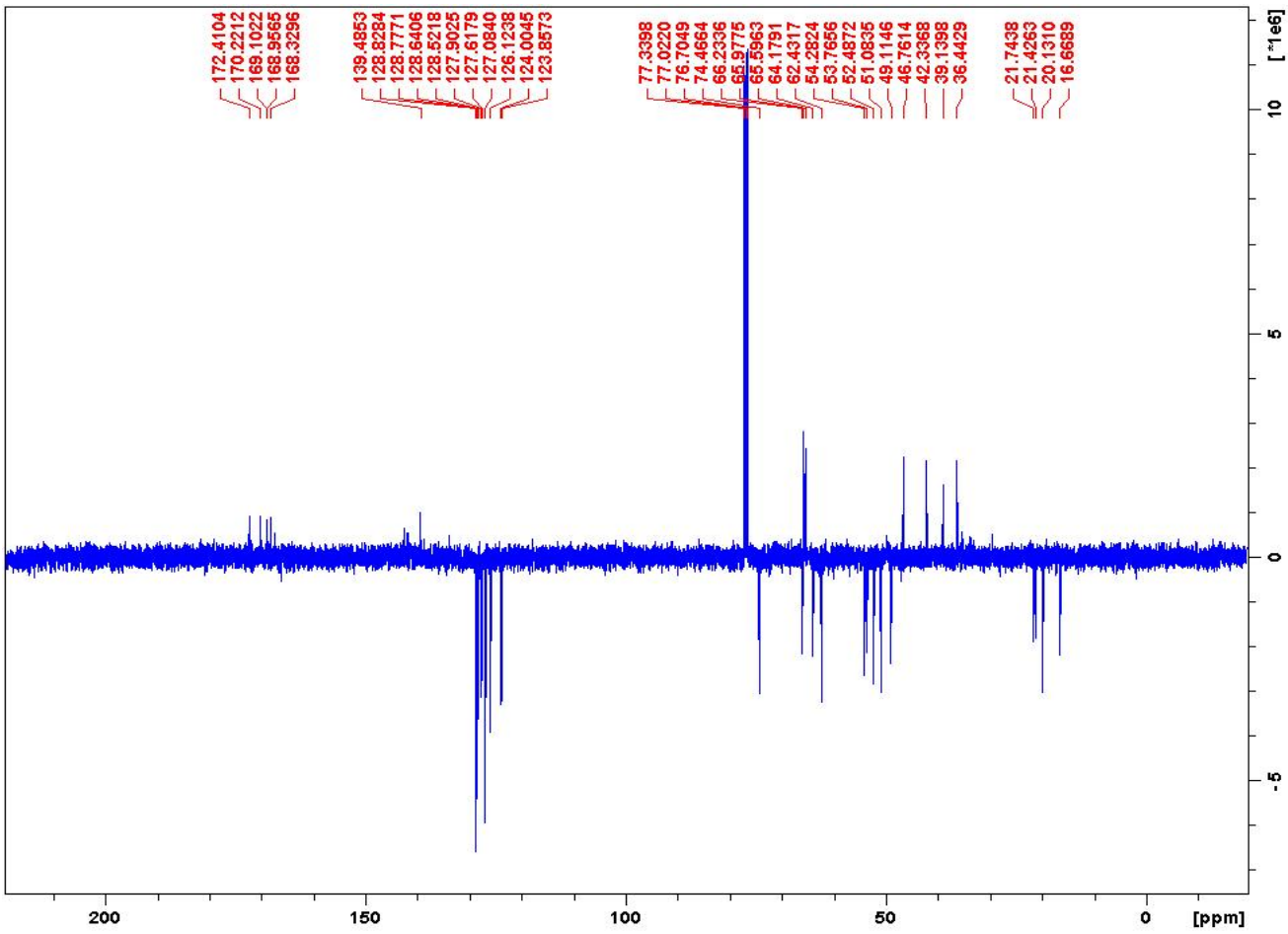

**HMBC spectrum for 2a**



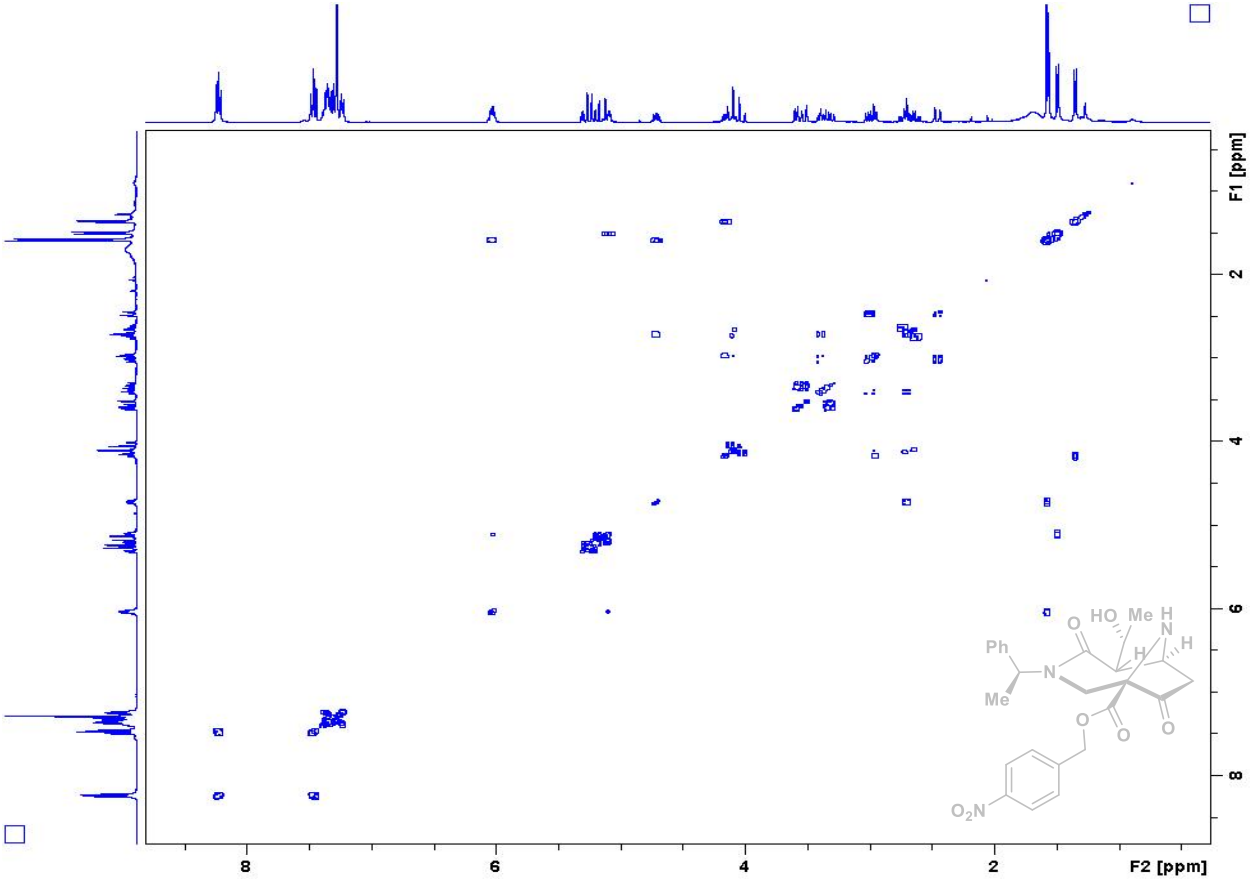
## $^{1}$H NMR spectrum for 2b



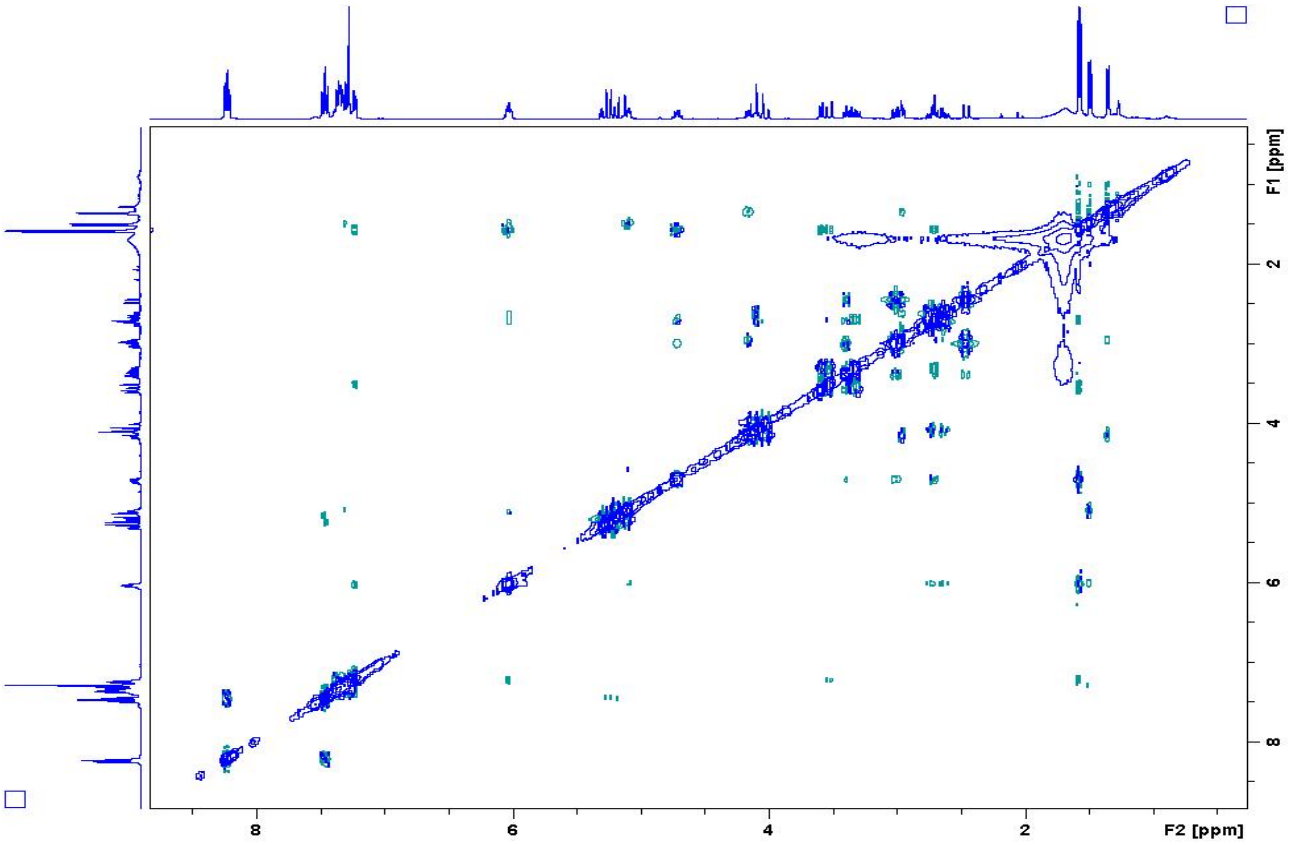

## $^{13}$C NMR spectrum for 2b



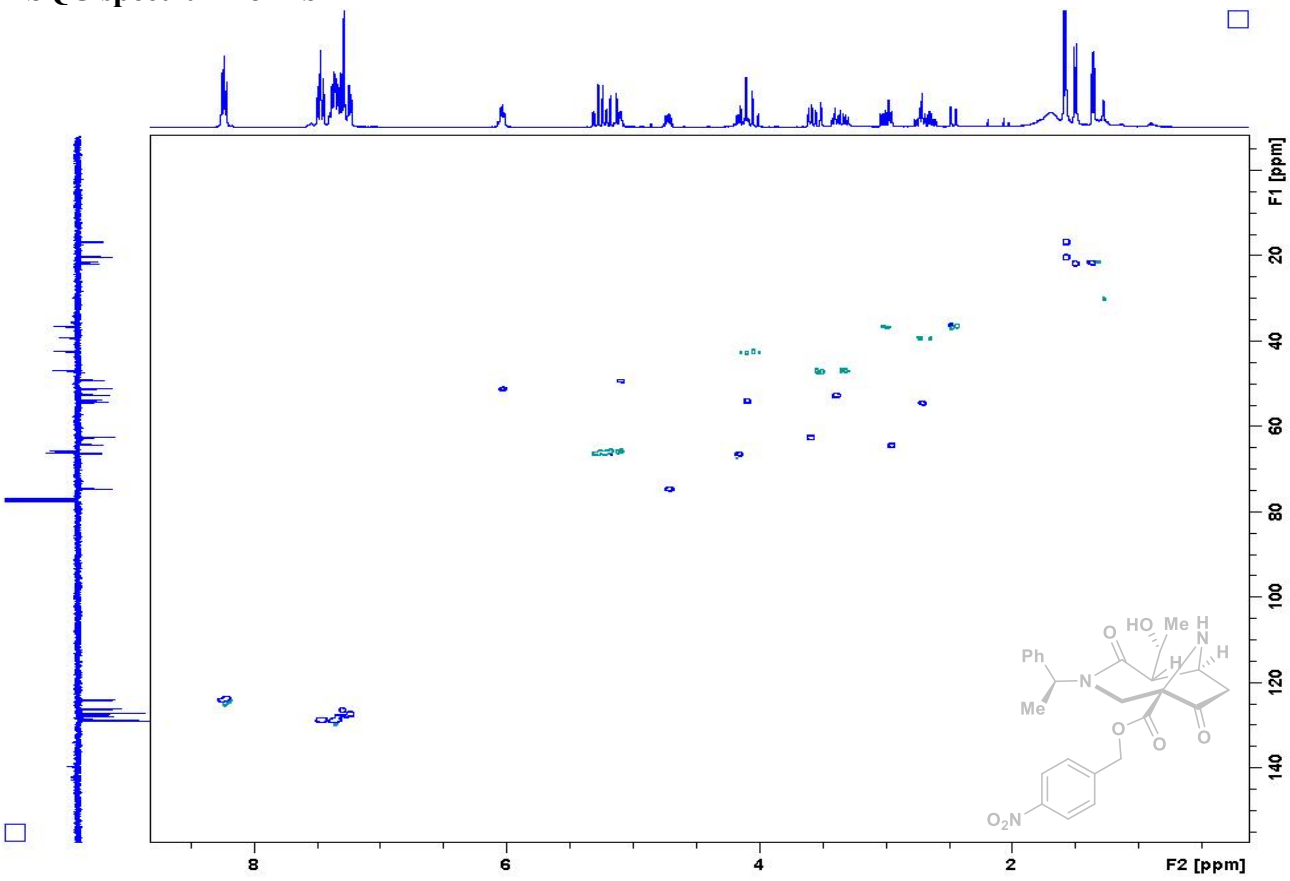
**COSY spectrum for 2b**



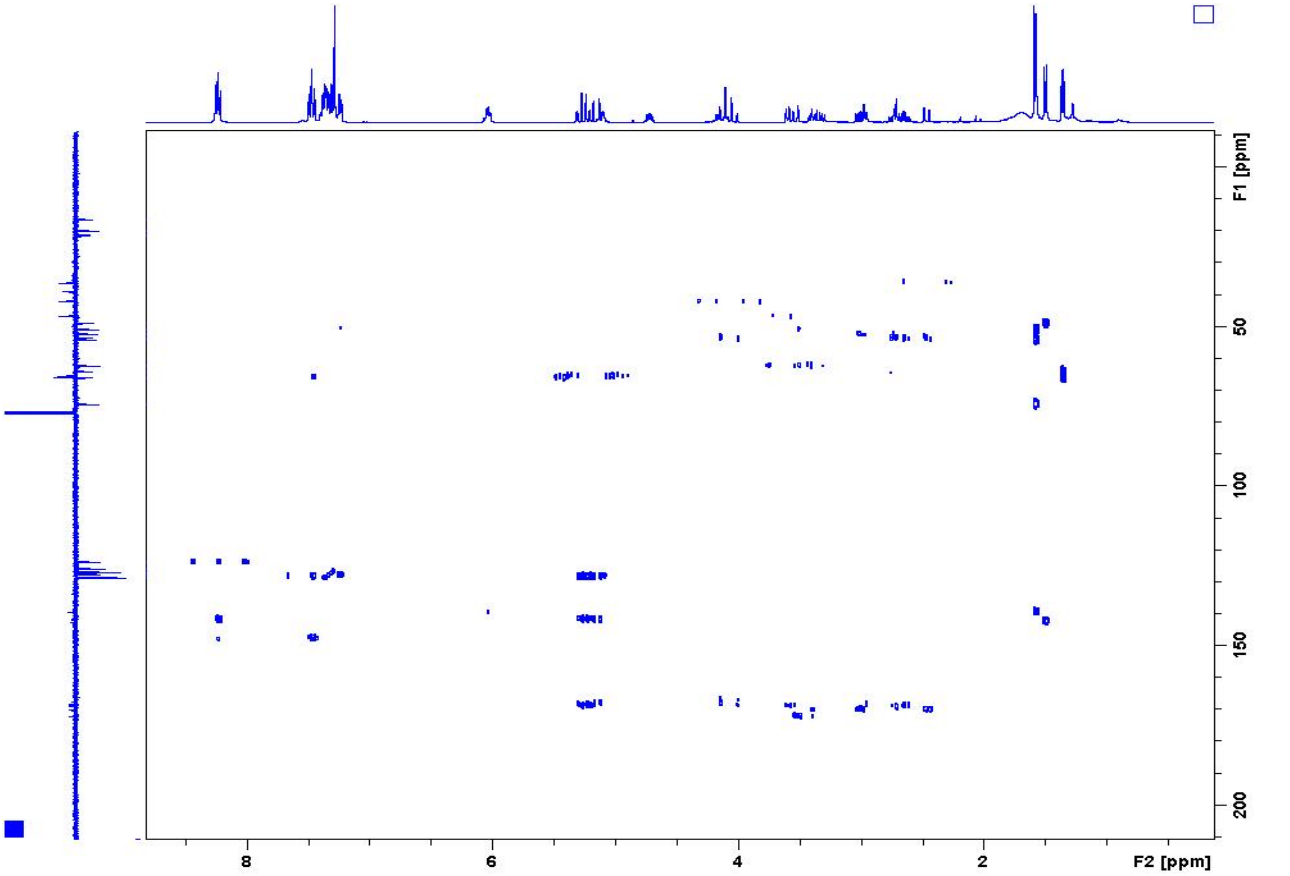

**NOESY spectrum for 2b**



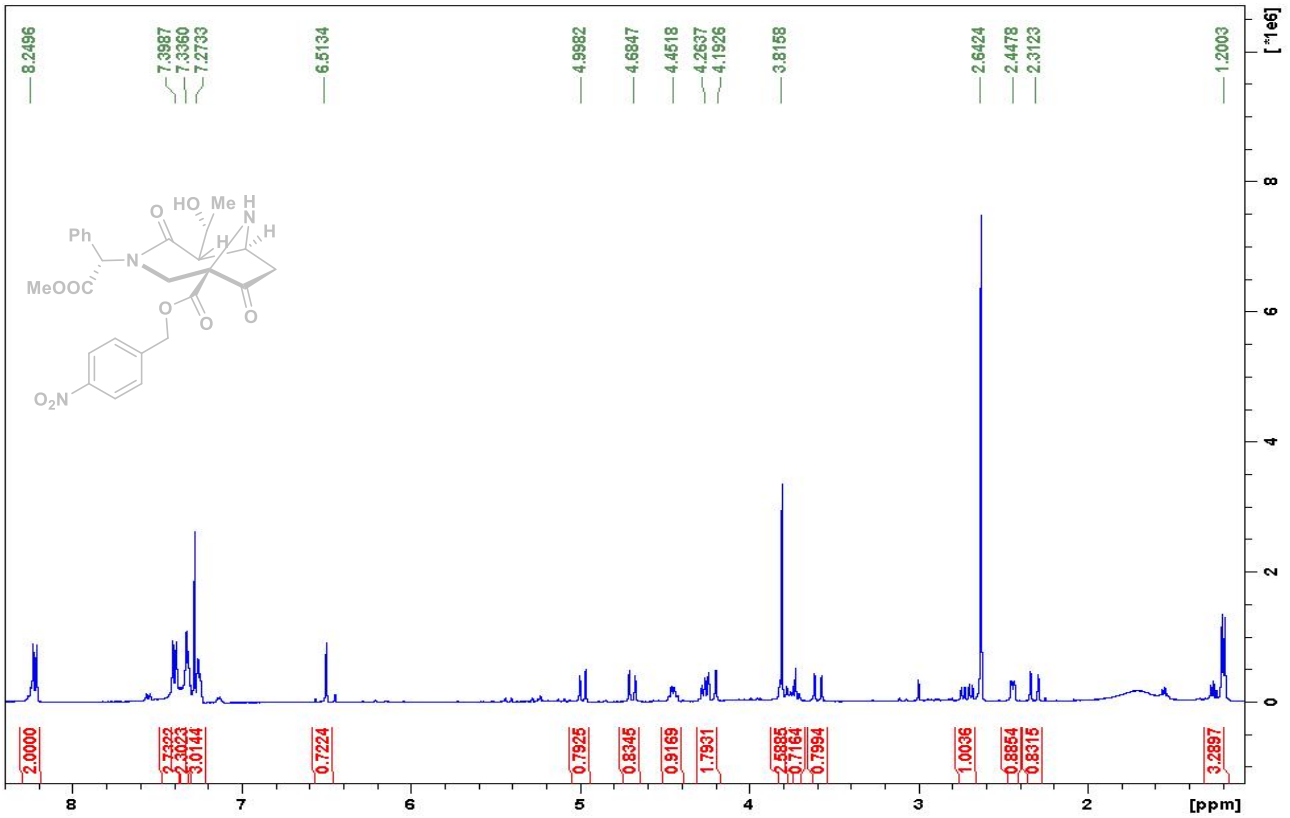
**HSQC spectrum for 2b**



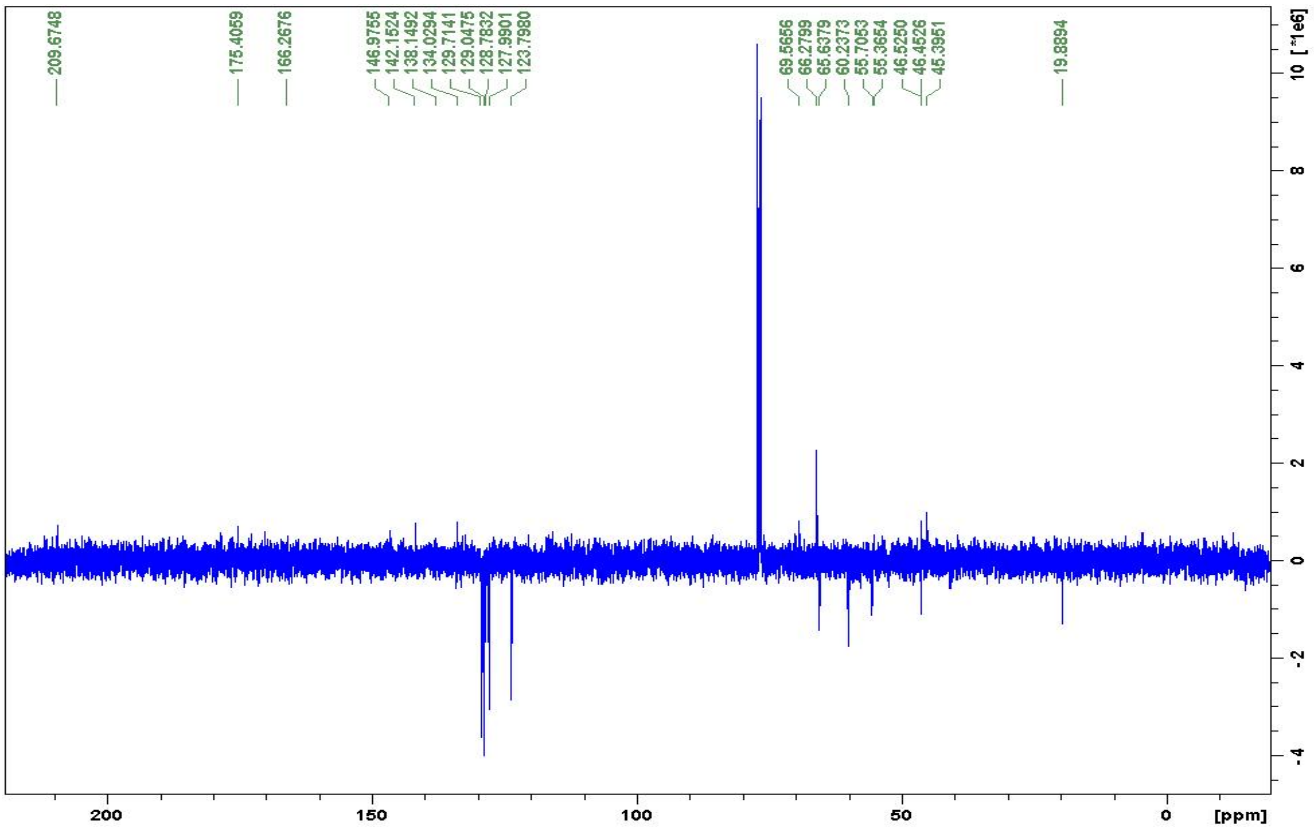

**HMBC spectrum for 2b**



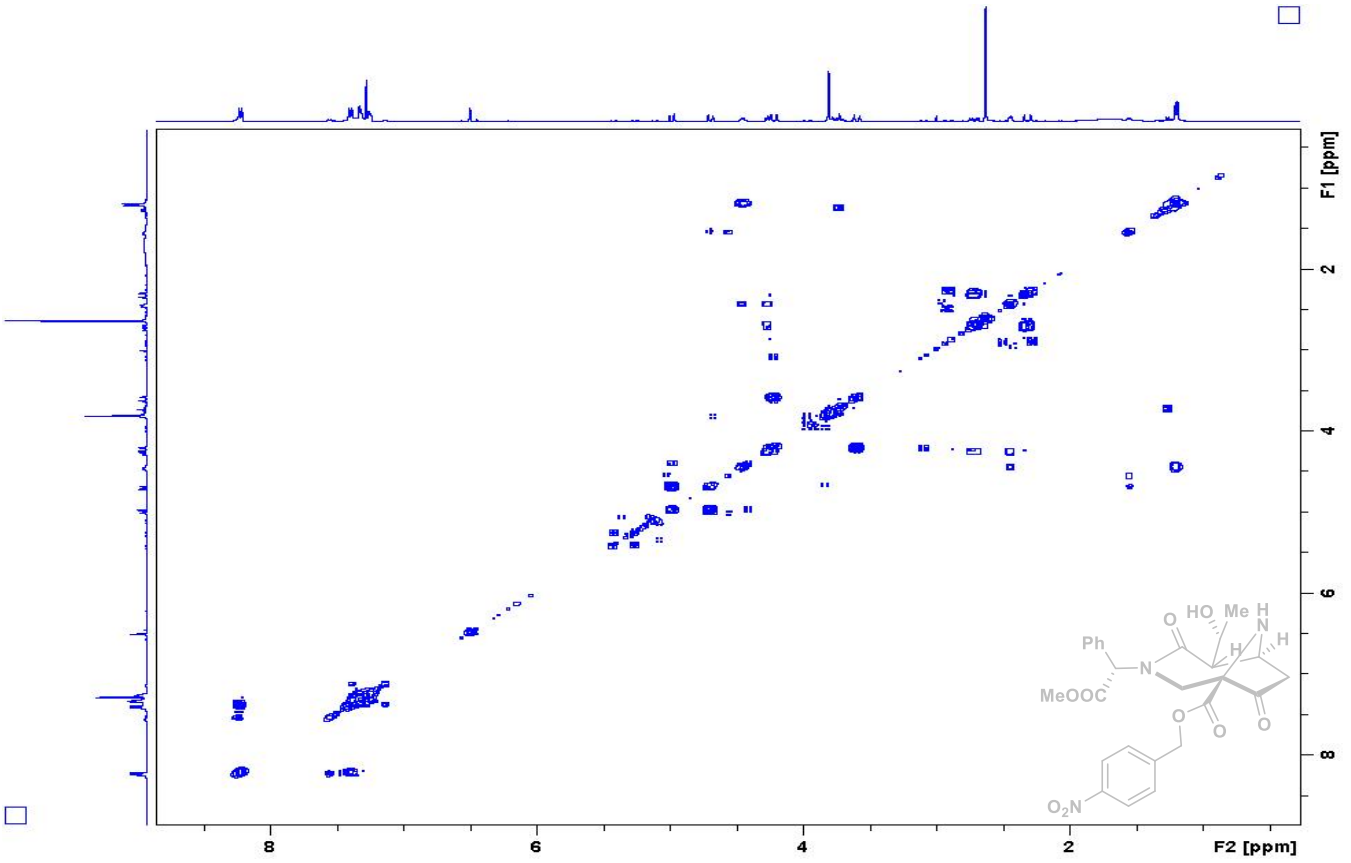
## $^{1}$H NMR spectrum for 2c



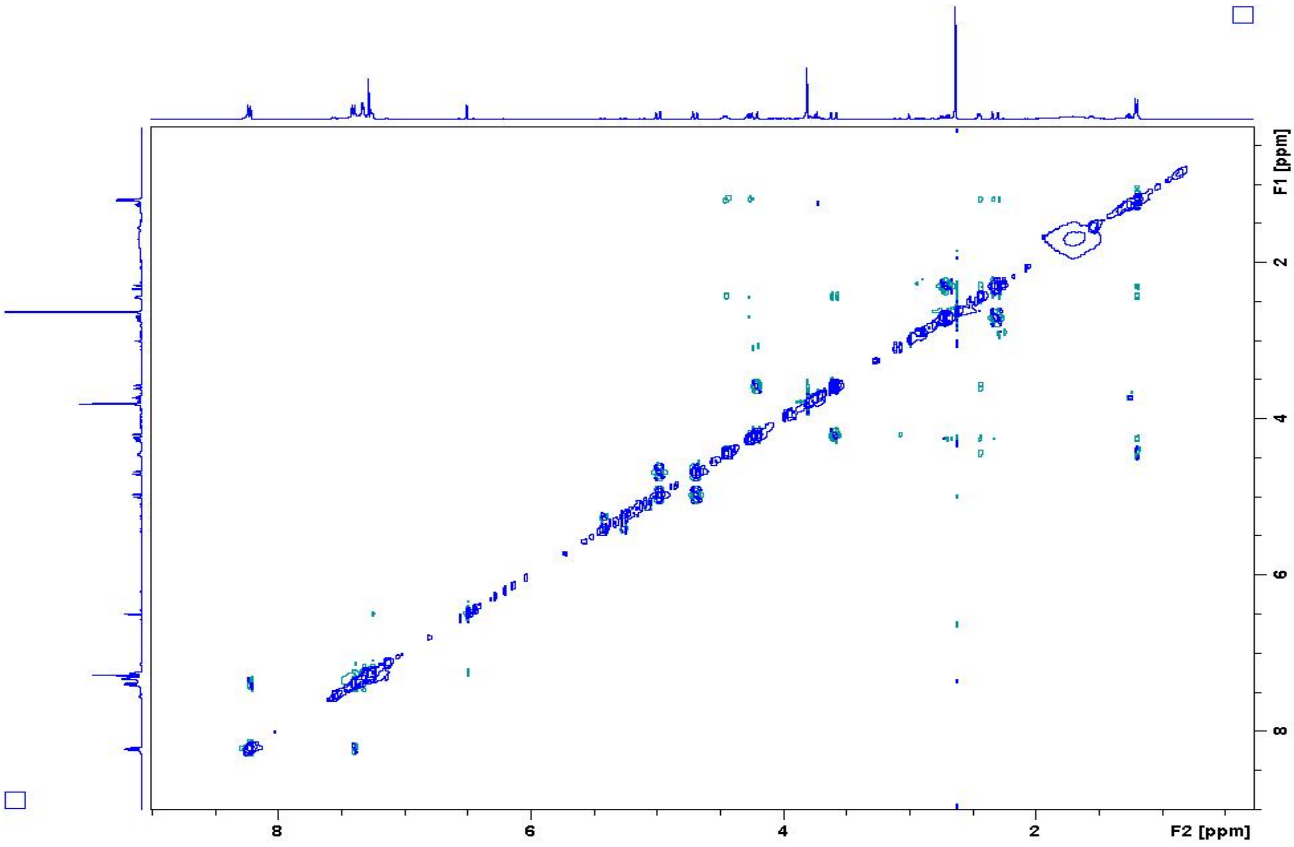

## 13C NMR spectrum for 2c



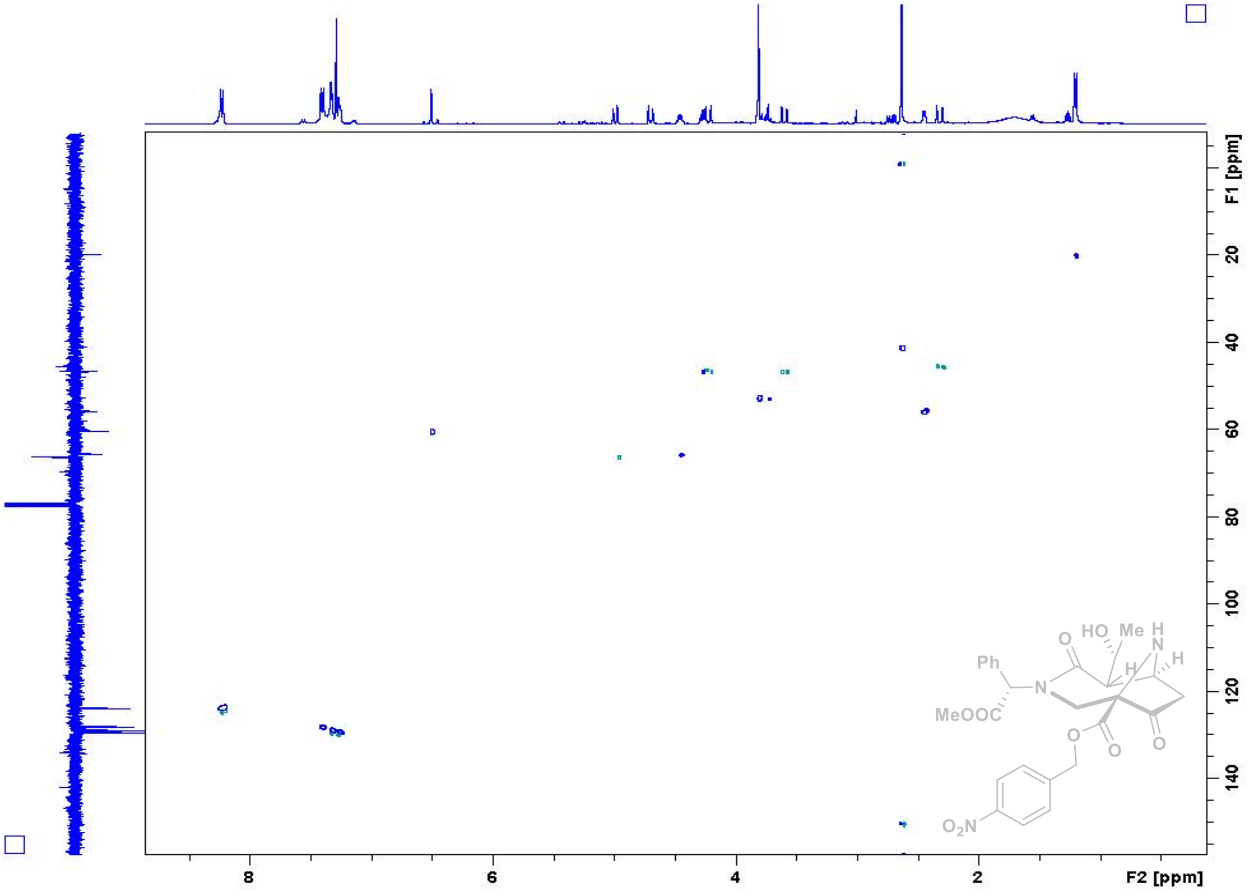
**COSY spectrum for 2c**



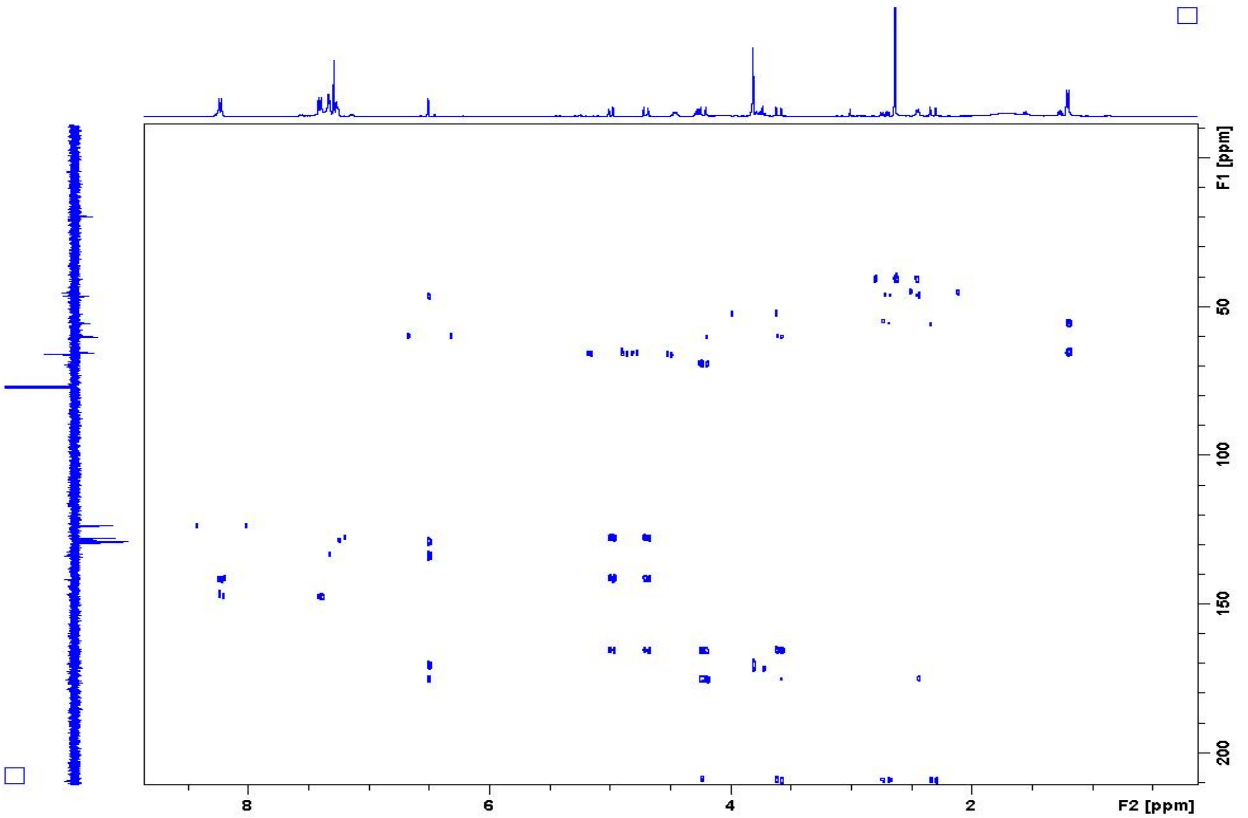

**NOESY spectrum for 2c**



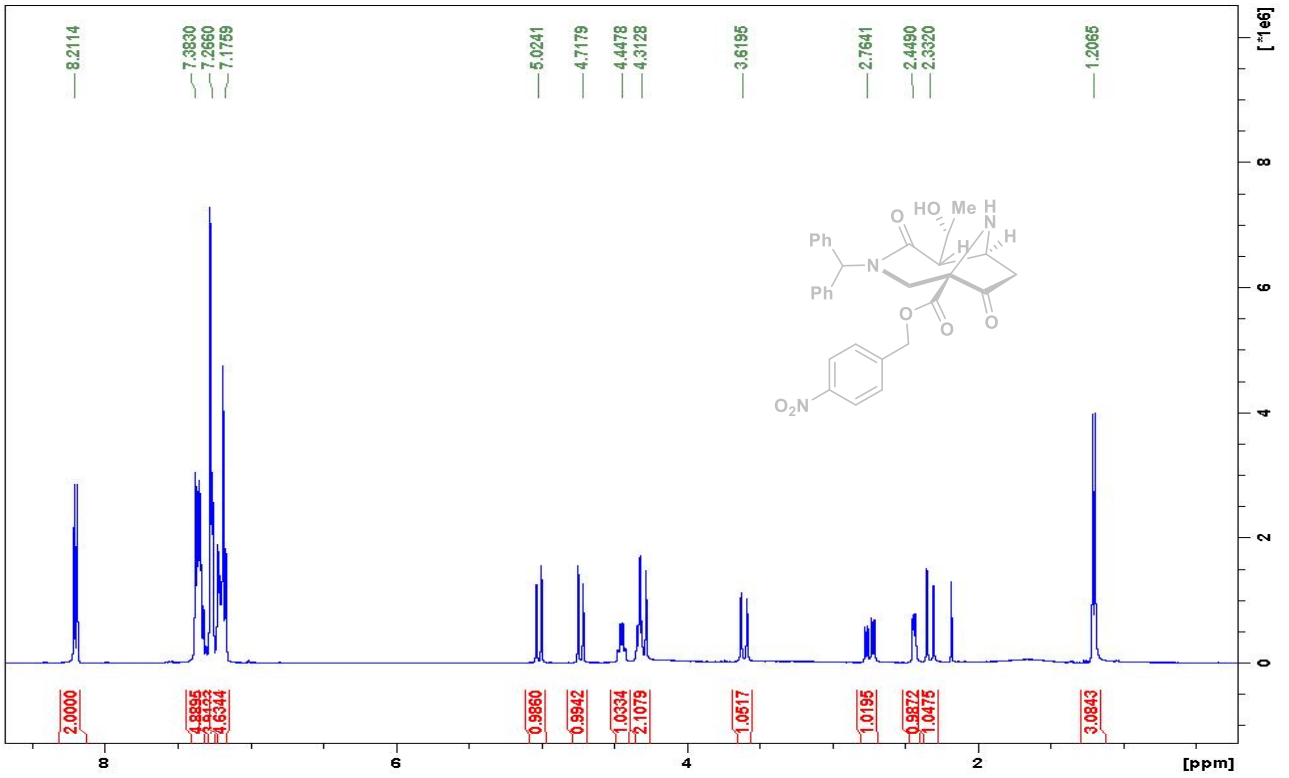
**HSQC spectrum for 2c**



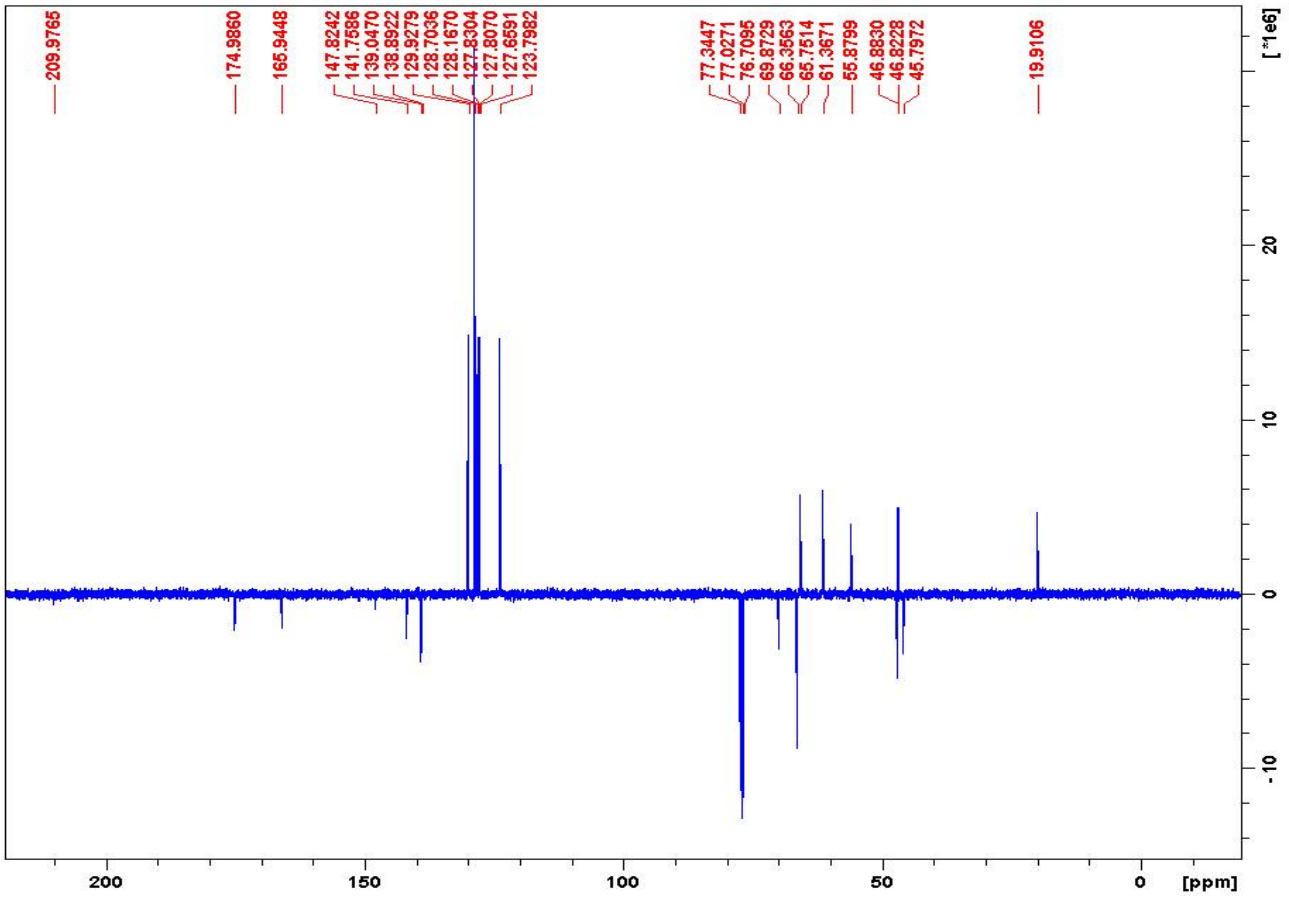

**HMBC spectrum for 2c**



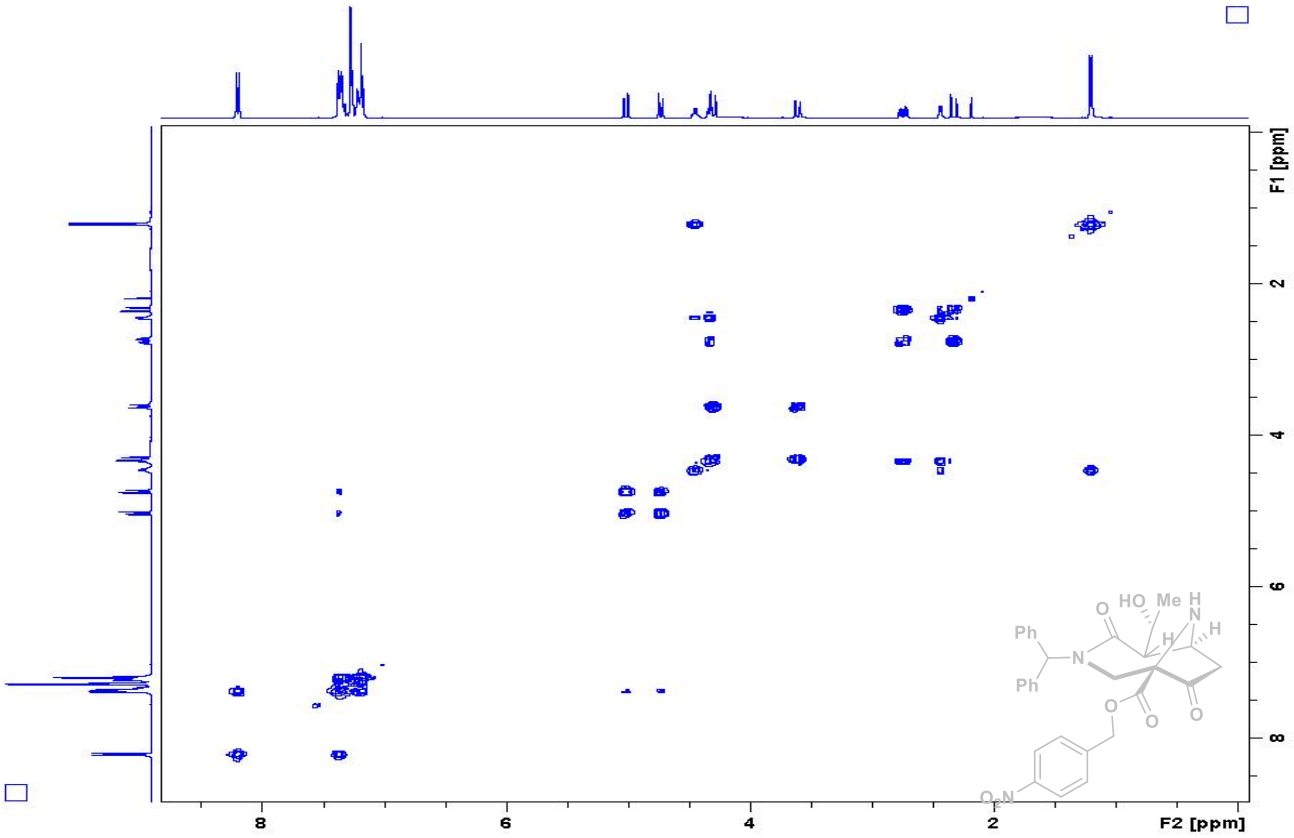
## $^{1}$H NMR spectrum for 2d



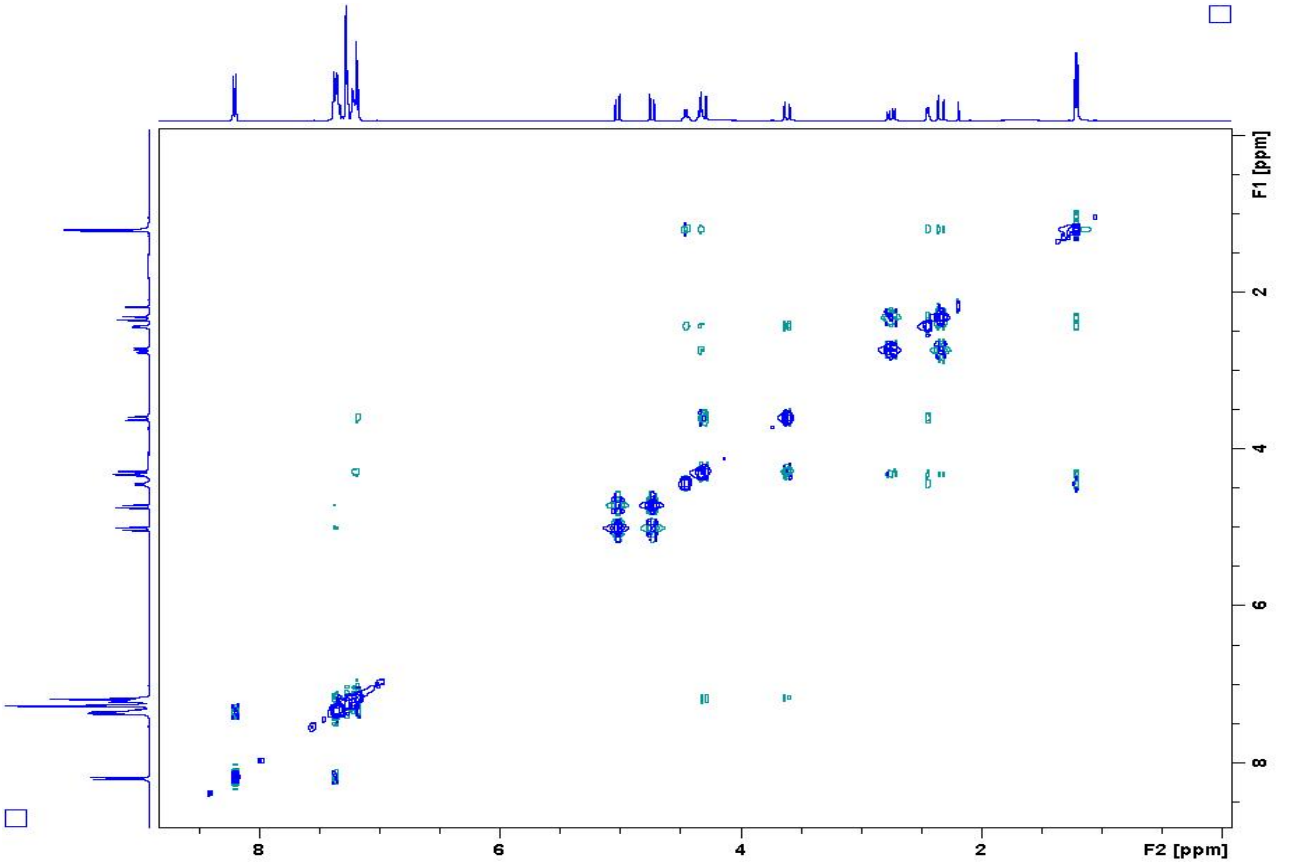

## $^{13}$C NMR spectrum for 2d



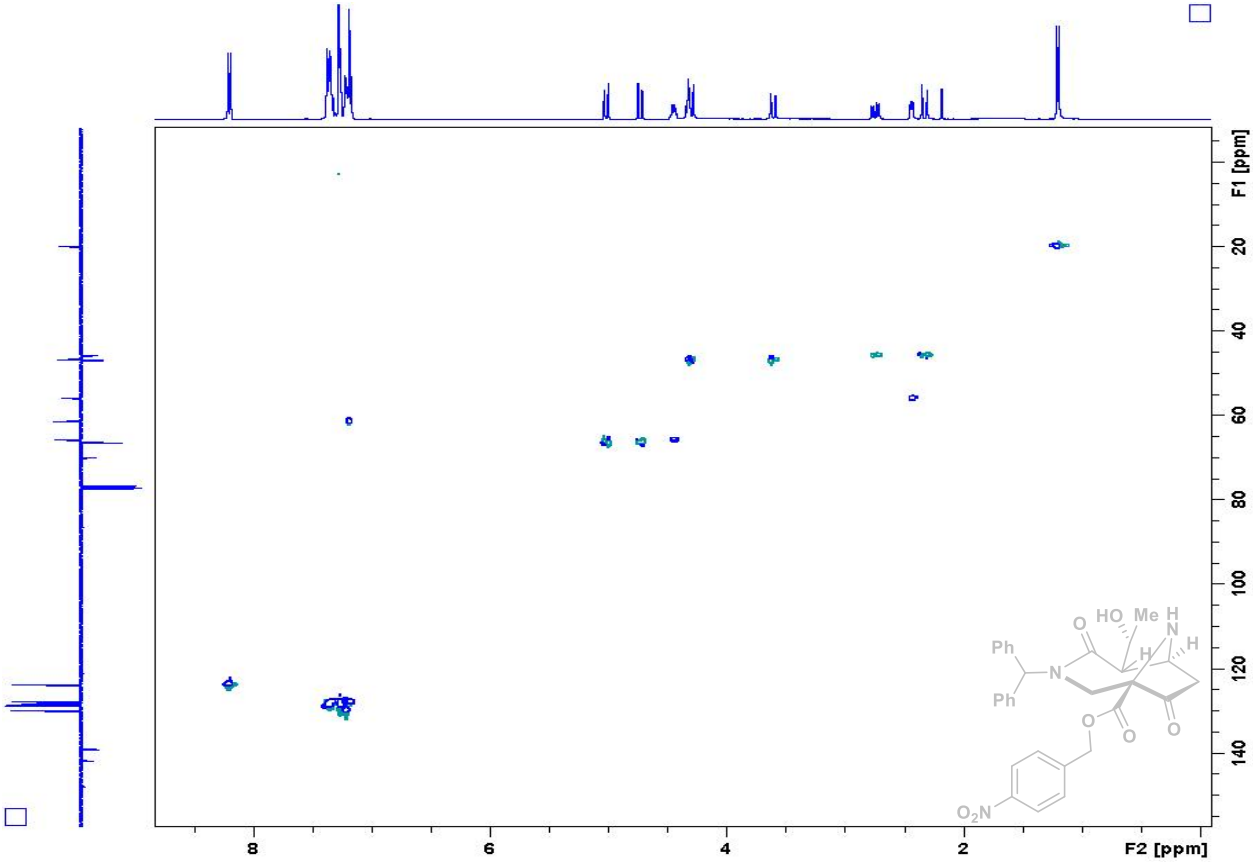
**COSY spectrum for 2d**



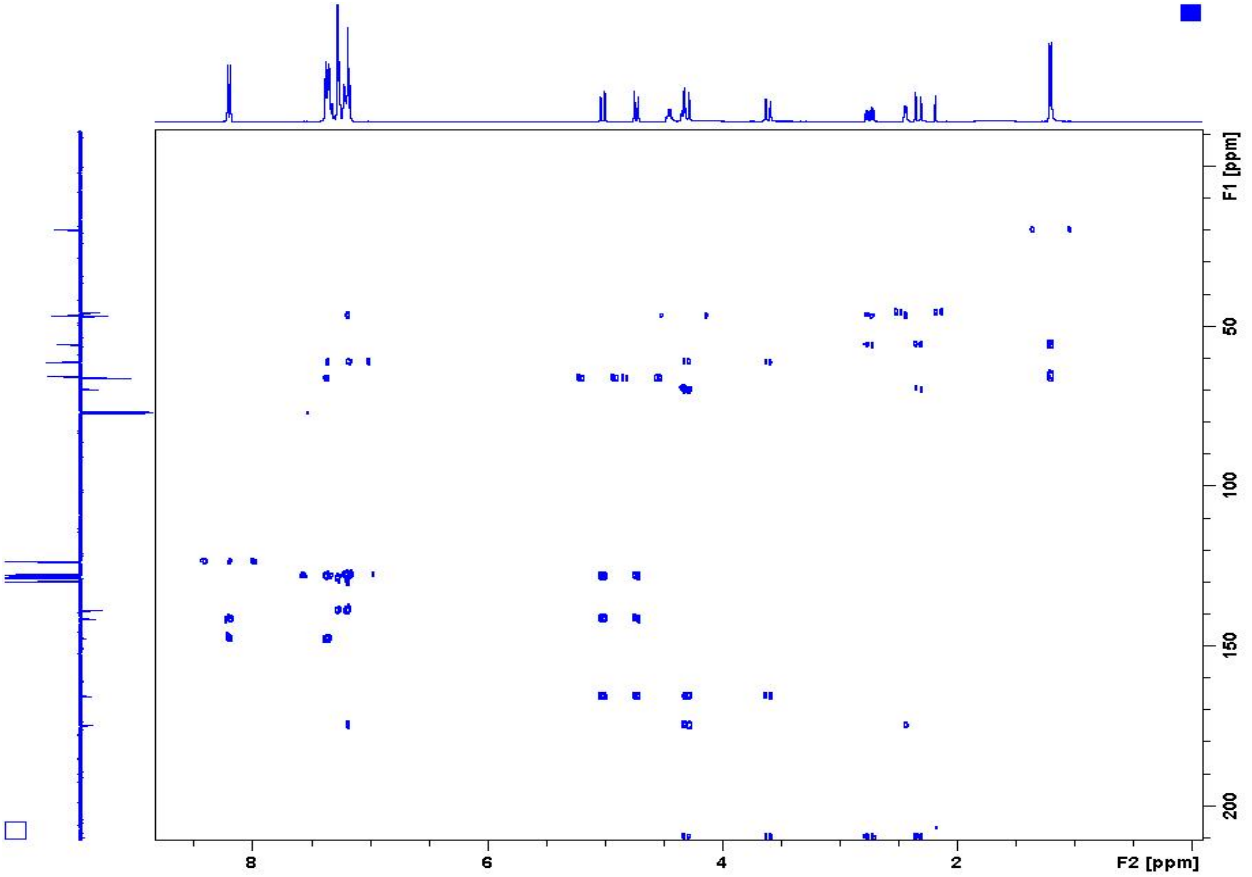

**NOESY spectrum for 2d**



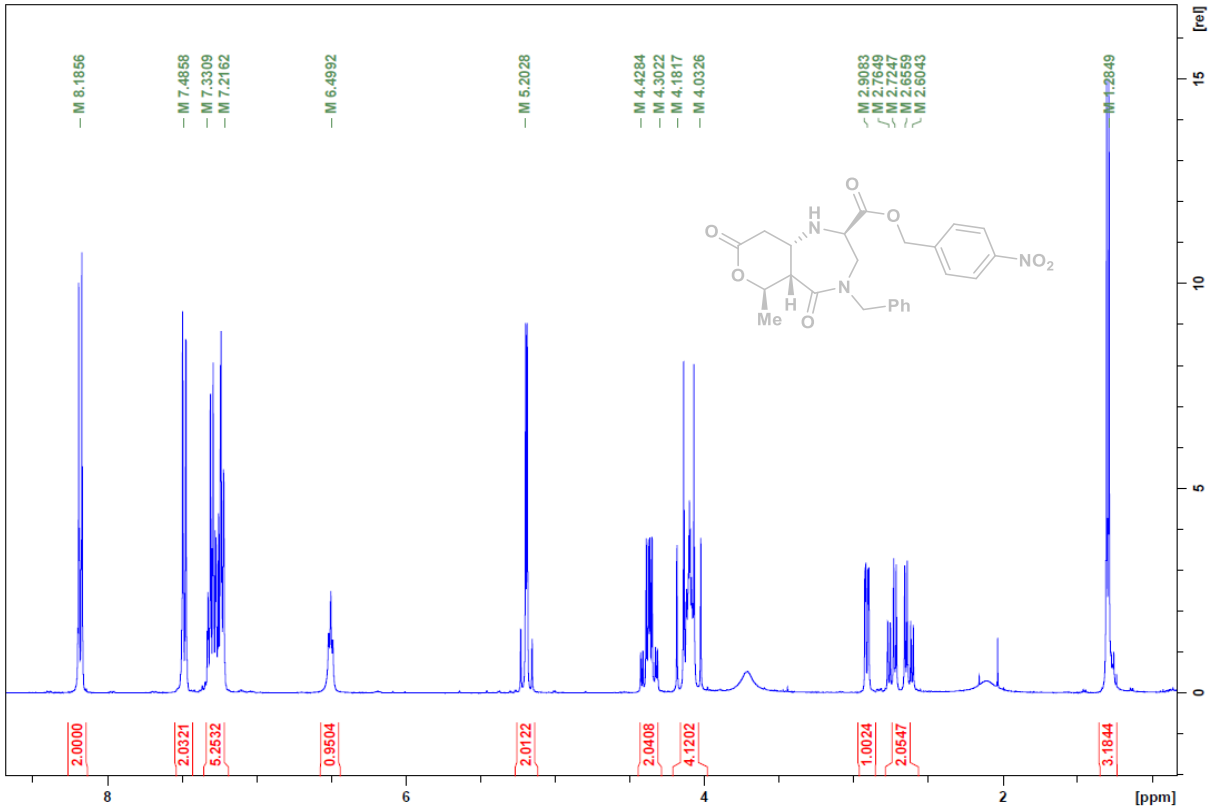
**HSQC spectrum for 2d**



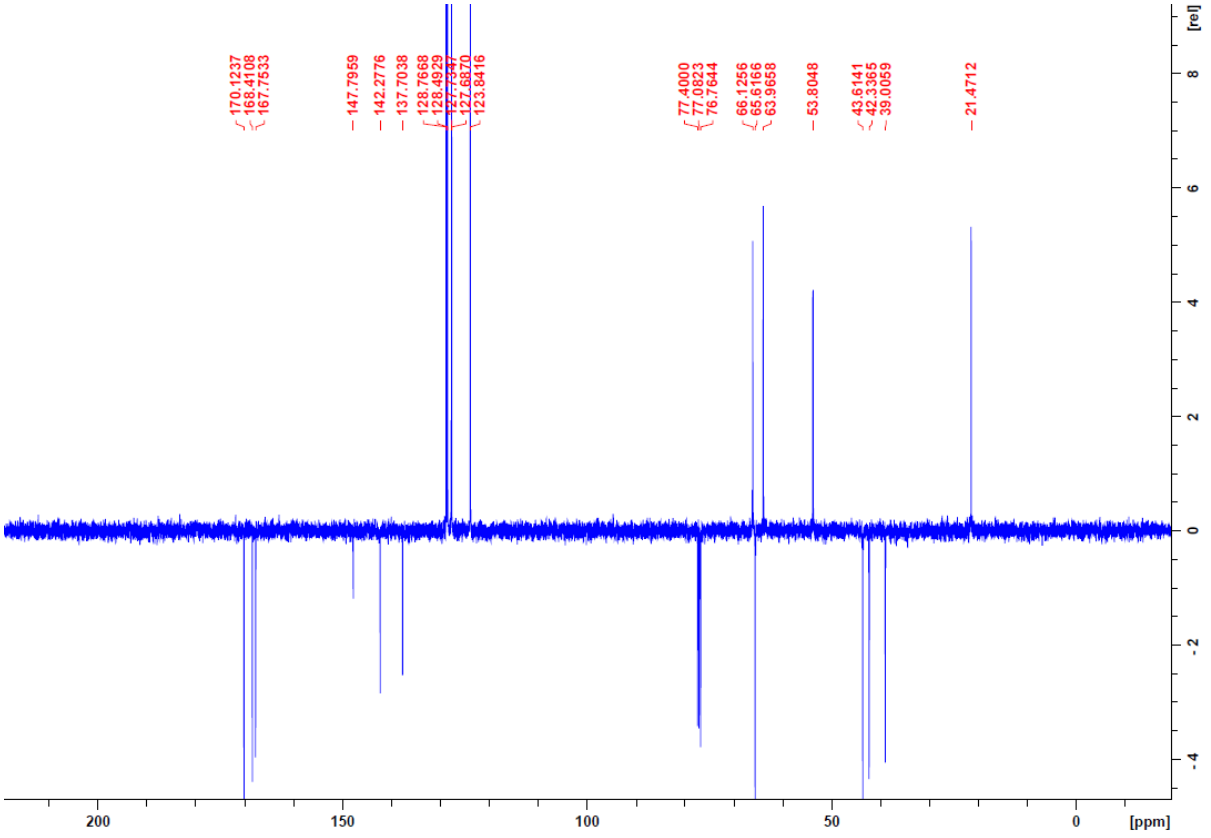

**HMBC spectrum for 2d**



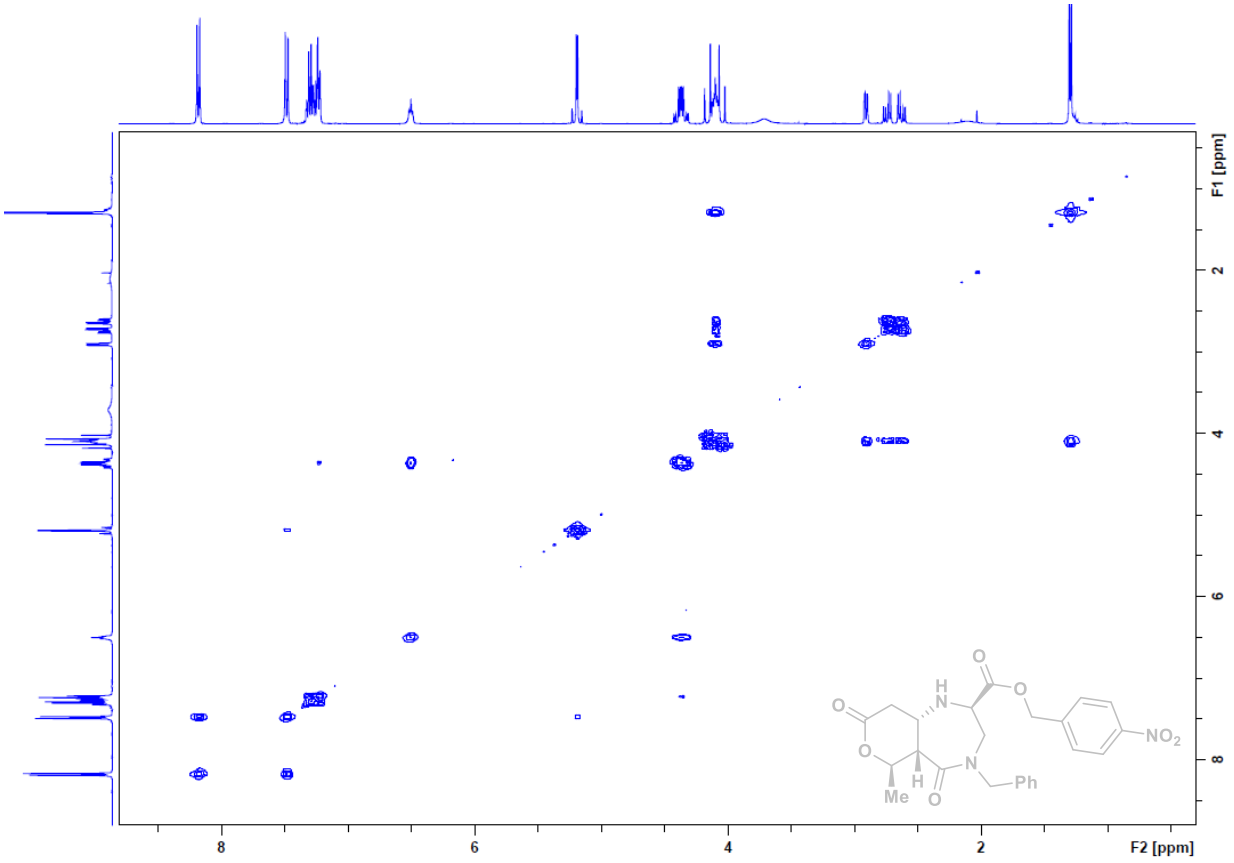
## $^{1}$H NMR spectrum for 3a



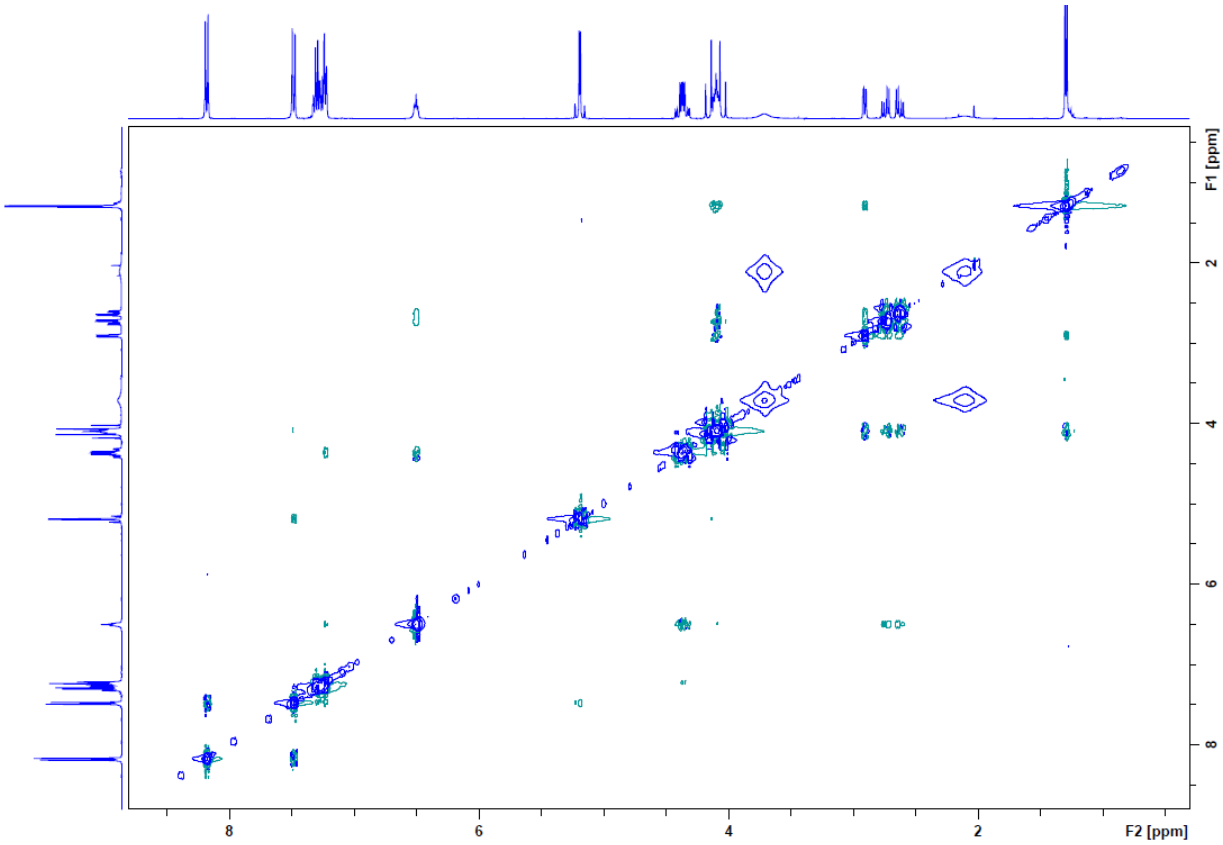

## $^{13}$C NMR spectrum for 3a



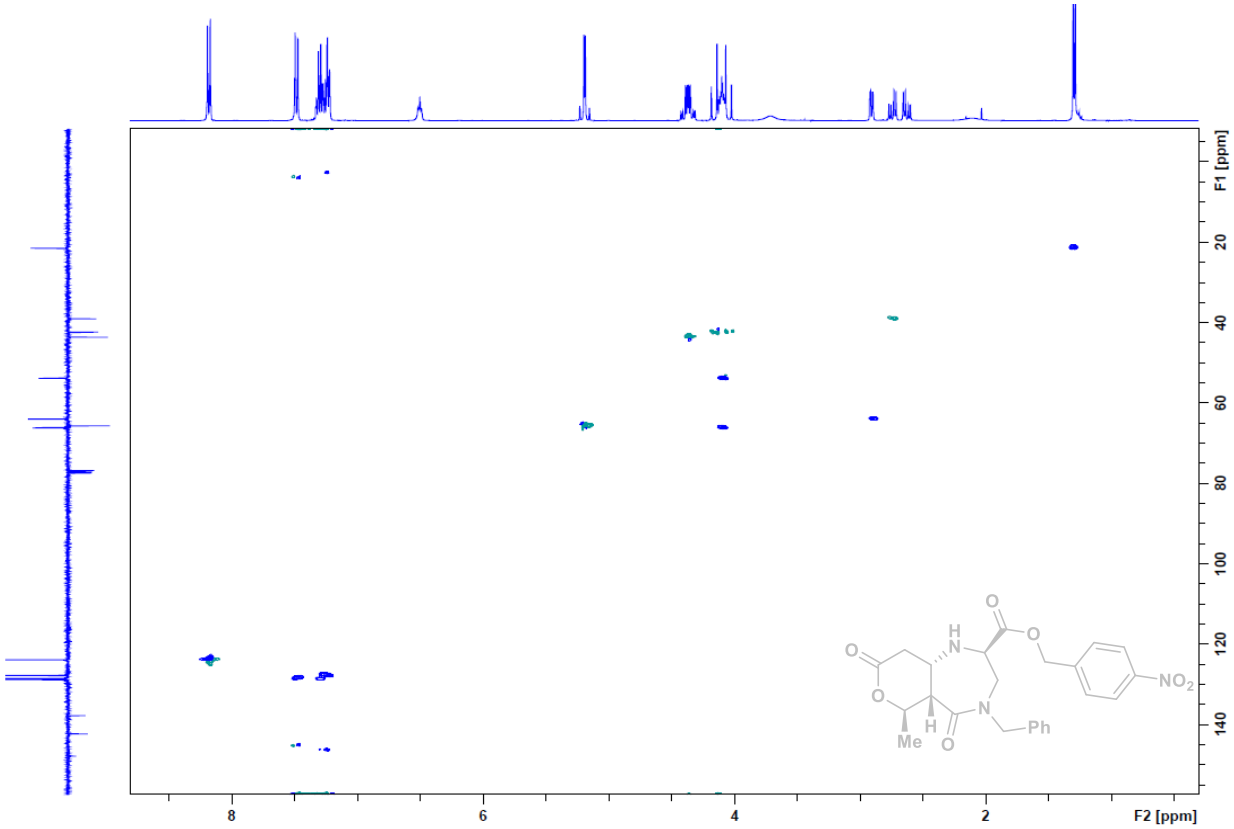
**COSY spectrum for 3a**



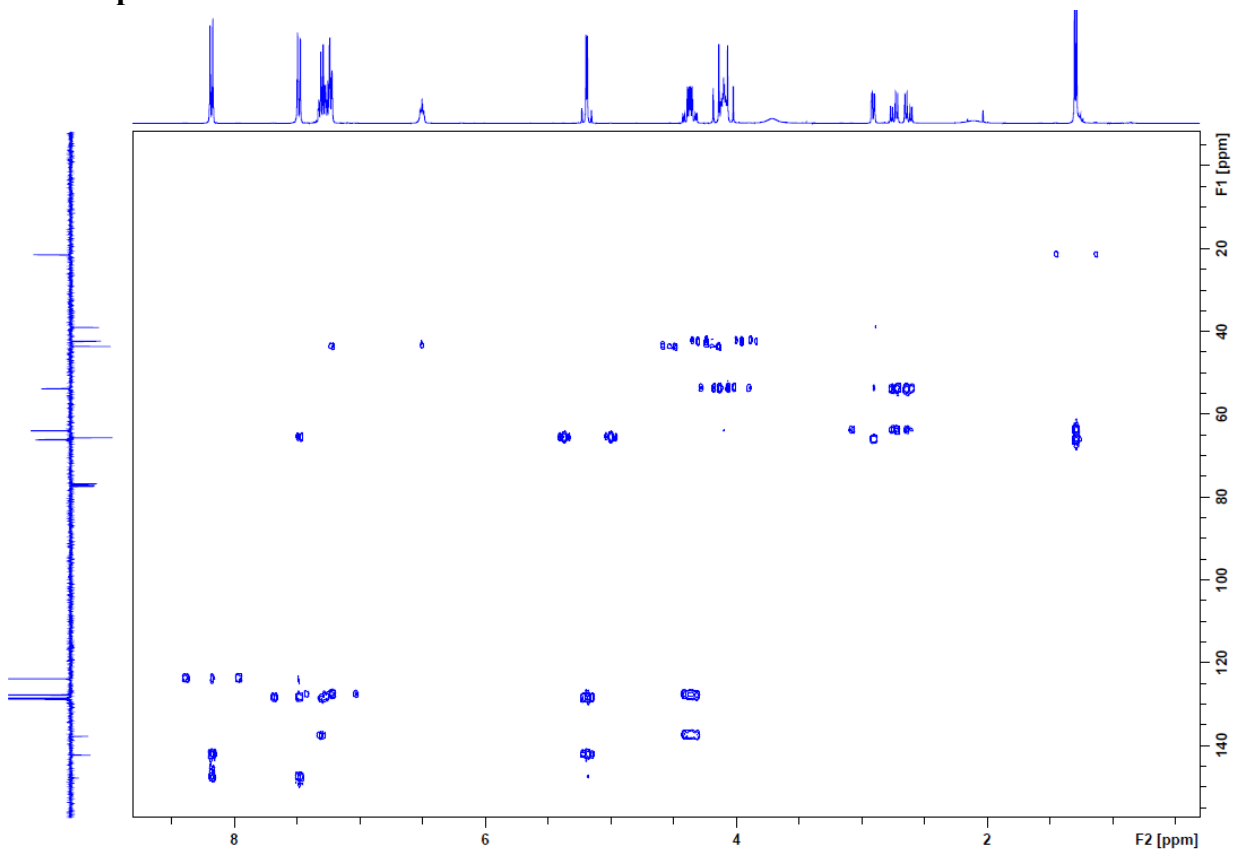

**NOESY spectrum for 3a**



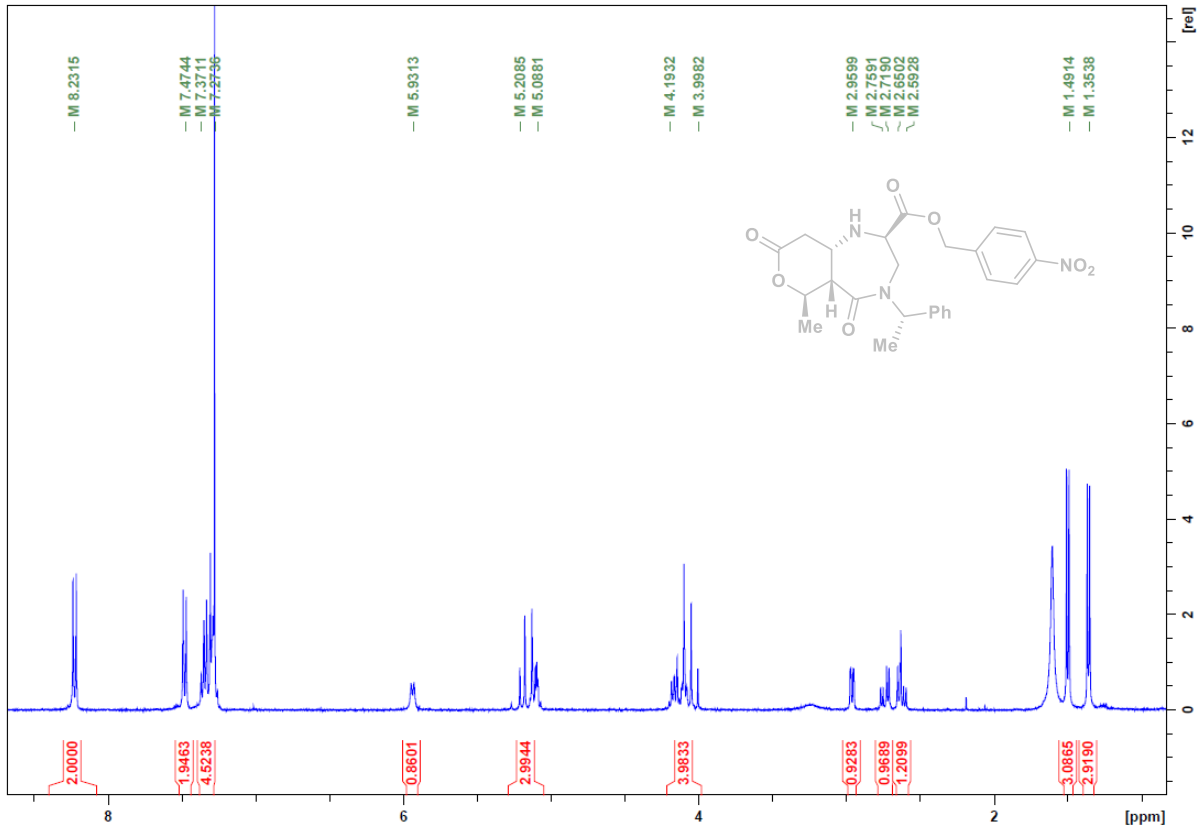
**HSQC spectrum for 3a**



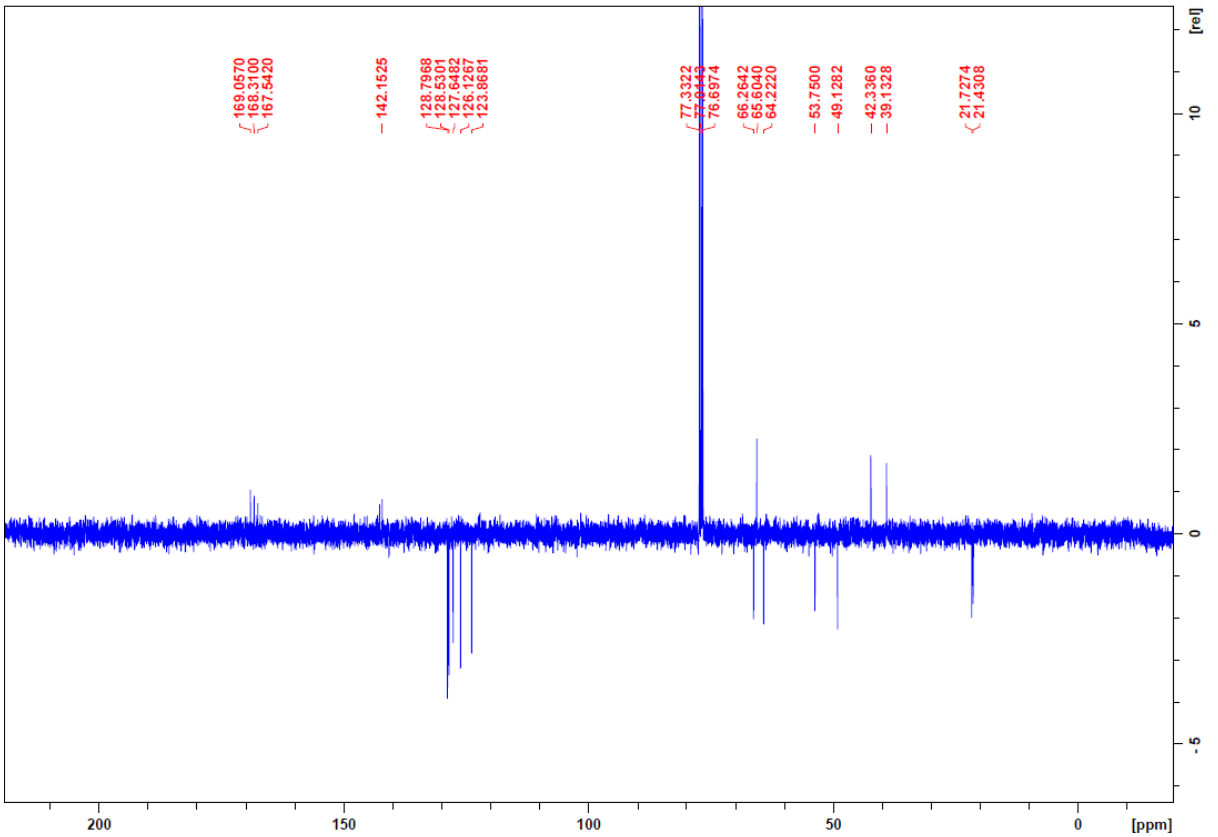

**HMBC spectrum for 3a**



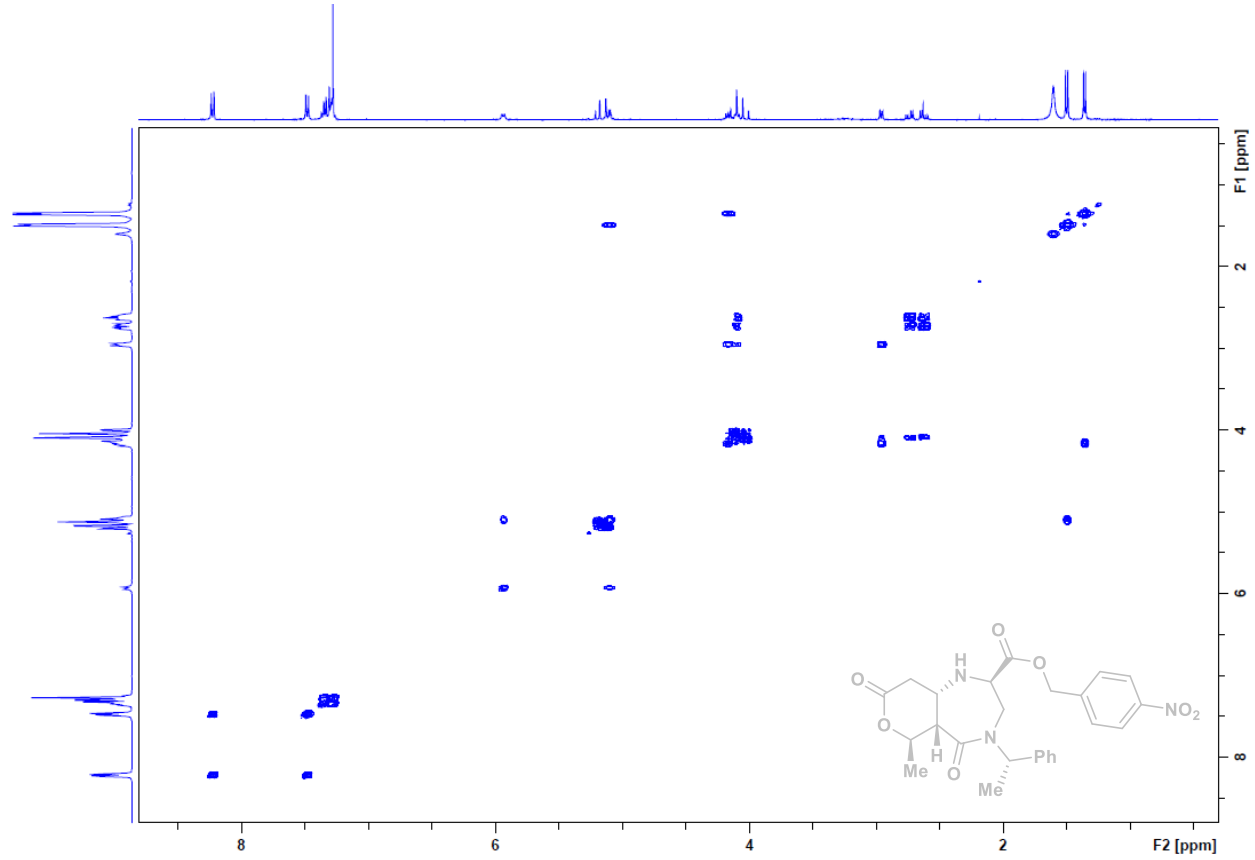
## $^{1}$H NMR spectrum for 3b



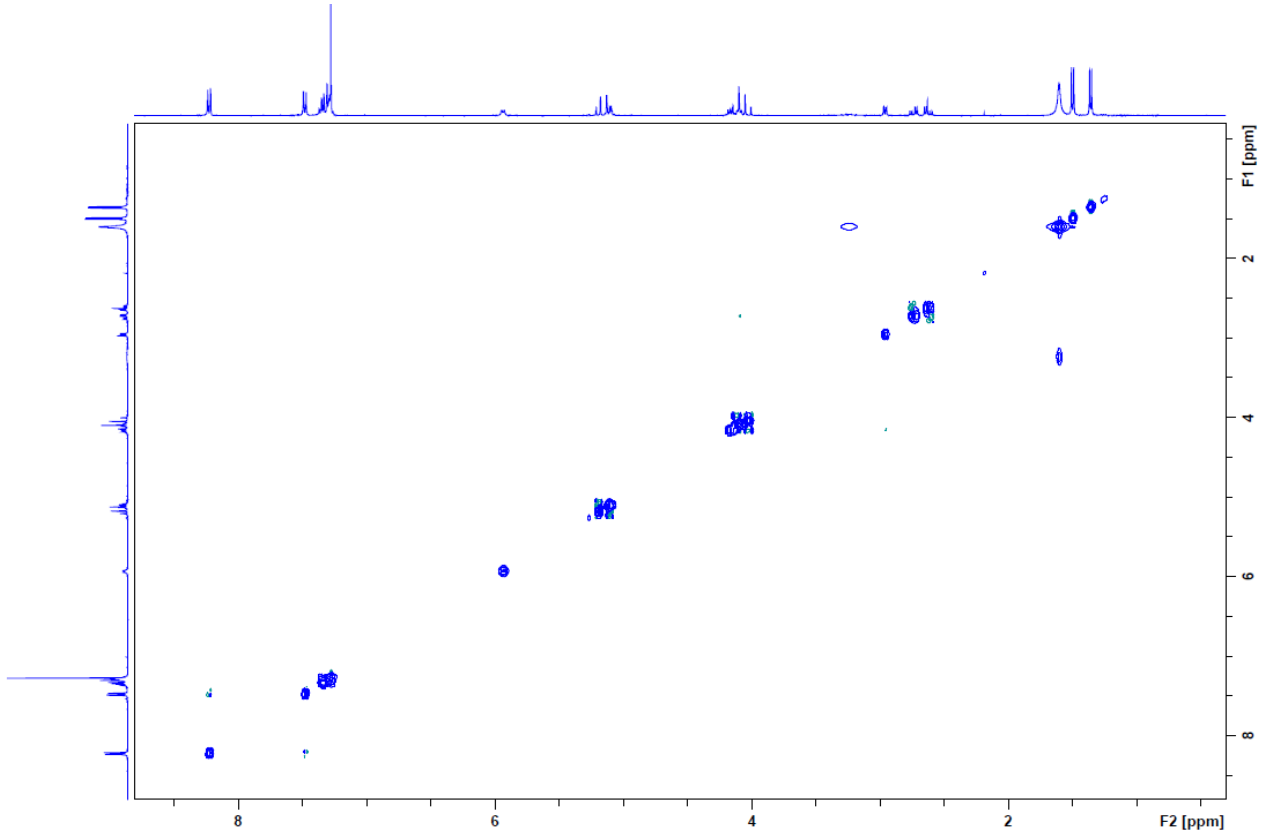

## $^{13}$C NMR spectrum for 3b



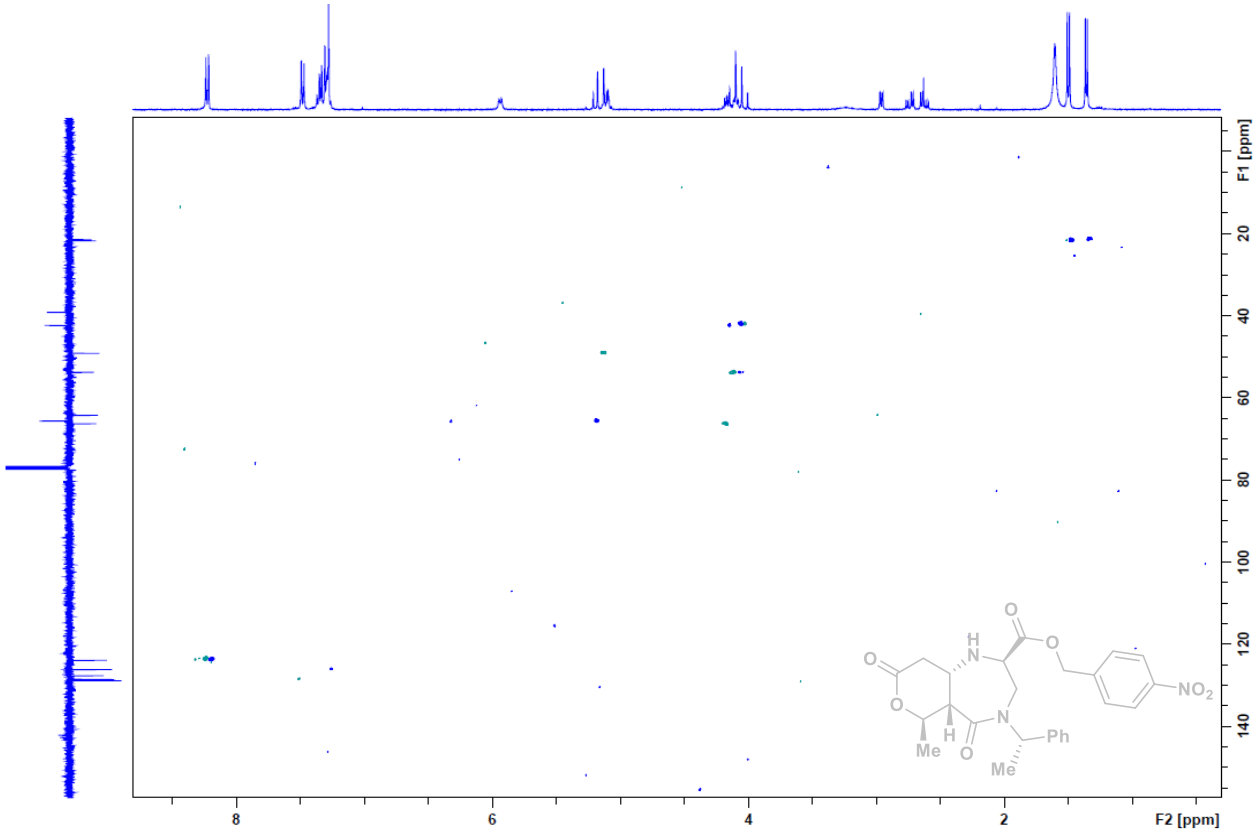
**COSY spectrum for 3b**



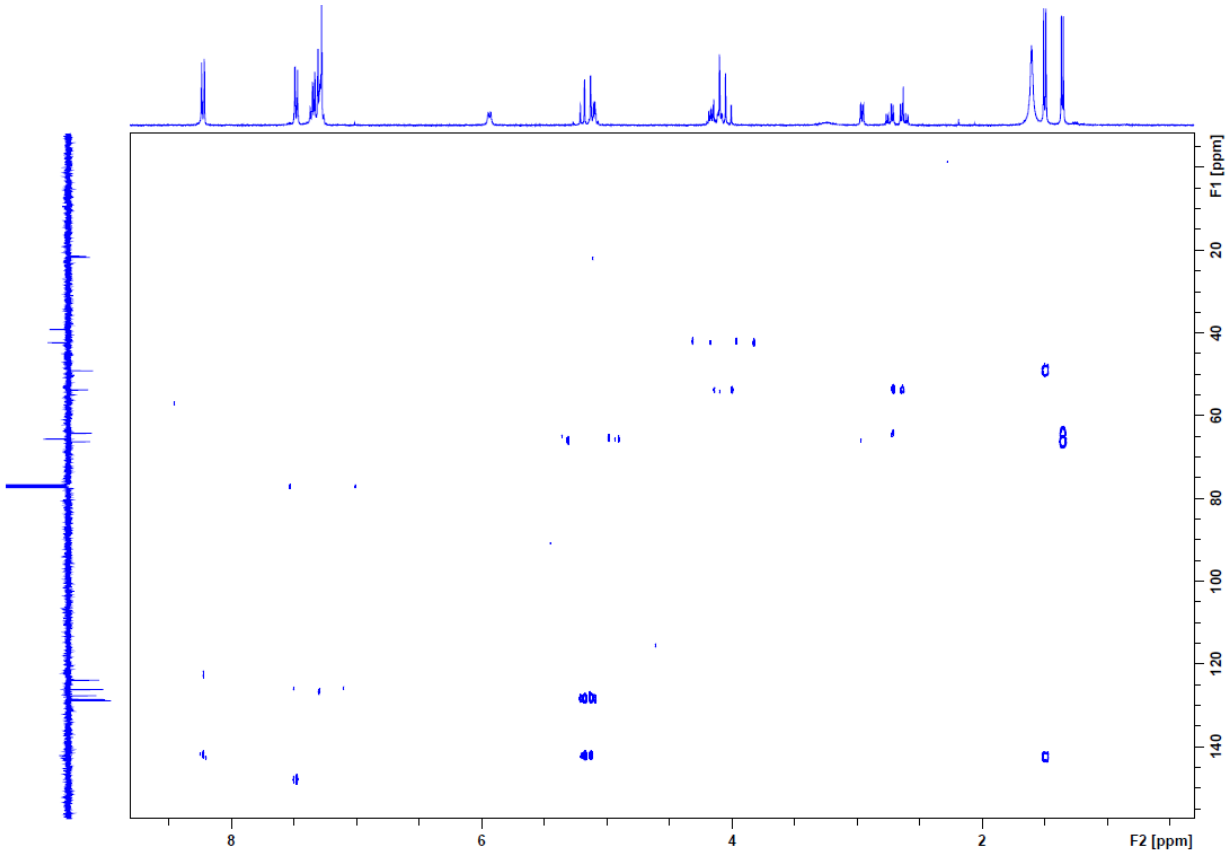

**NOESY spectrum for 3b**



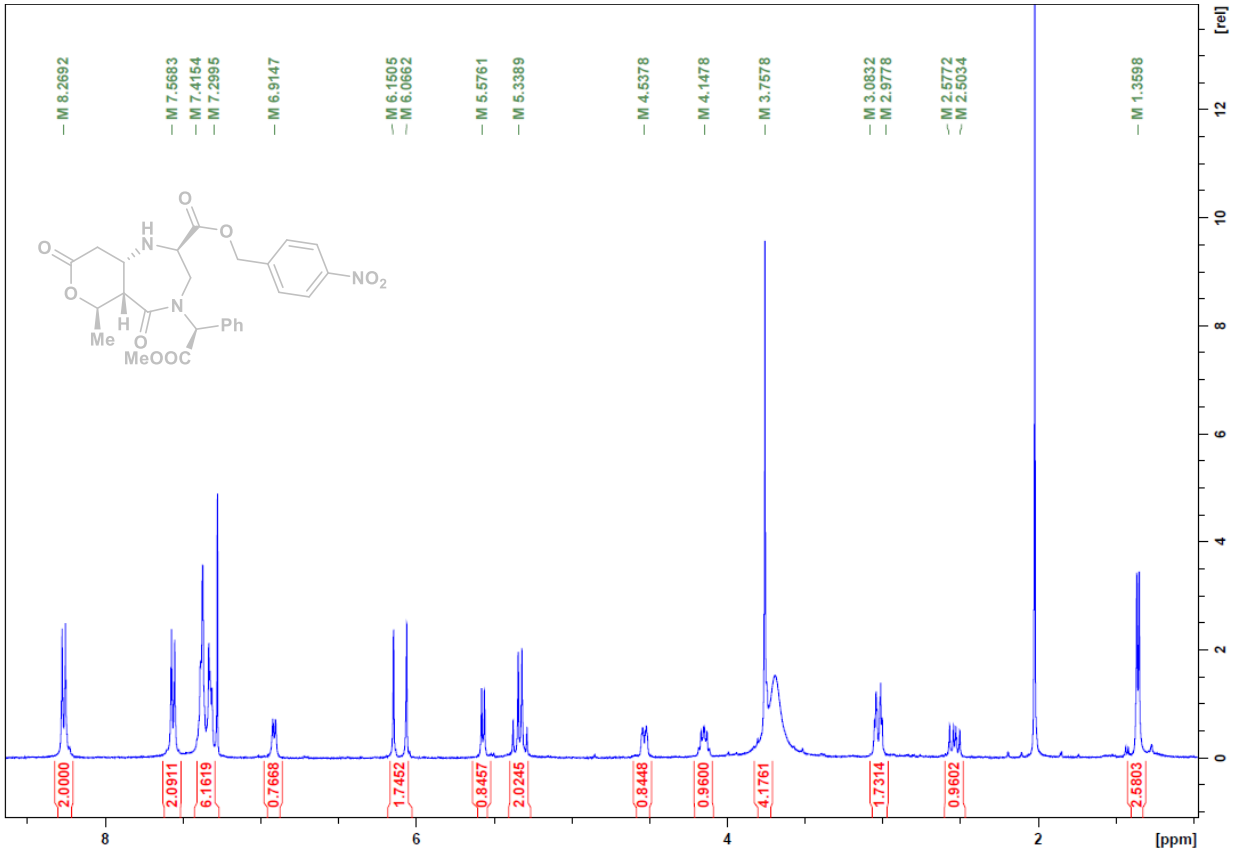
**HSQC spectrum for 3b**



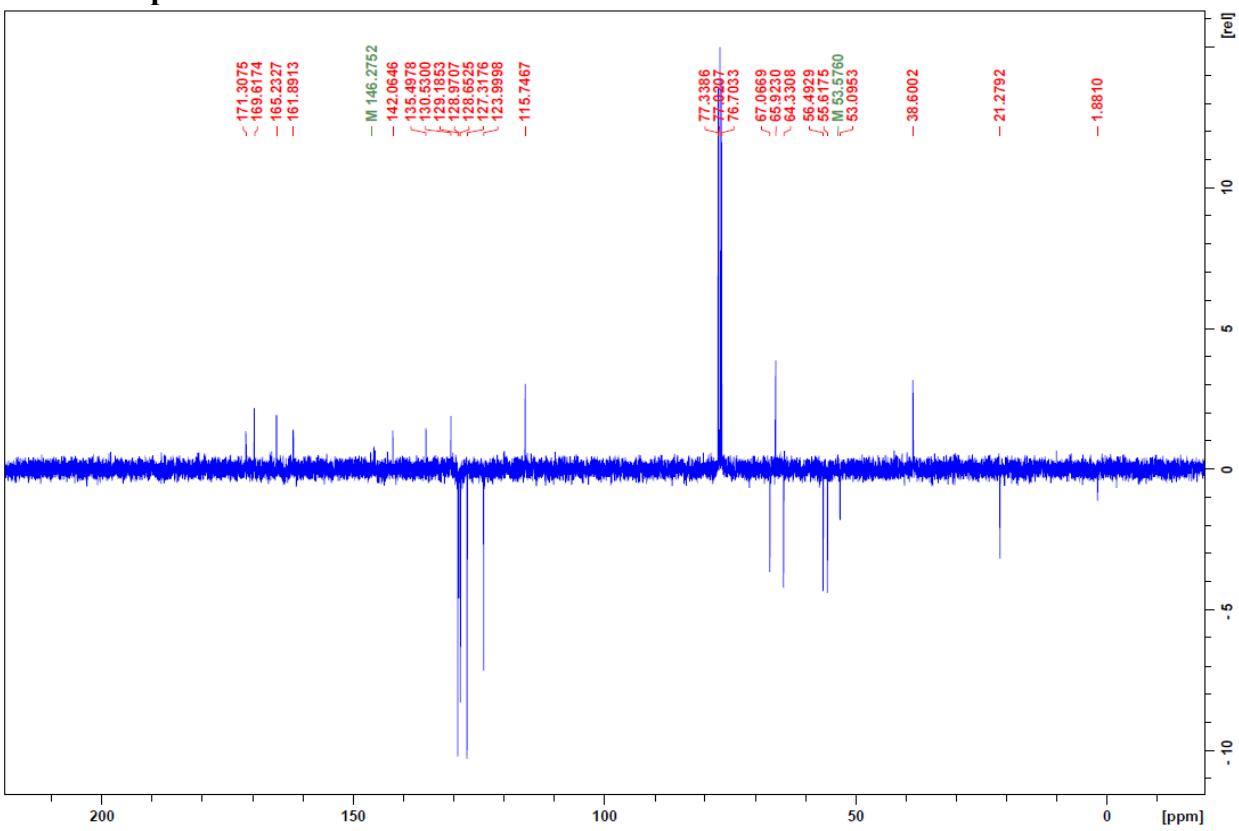

**HMBC spectrum for 3b**



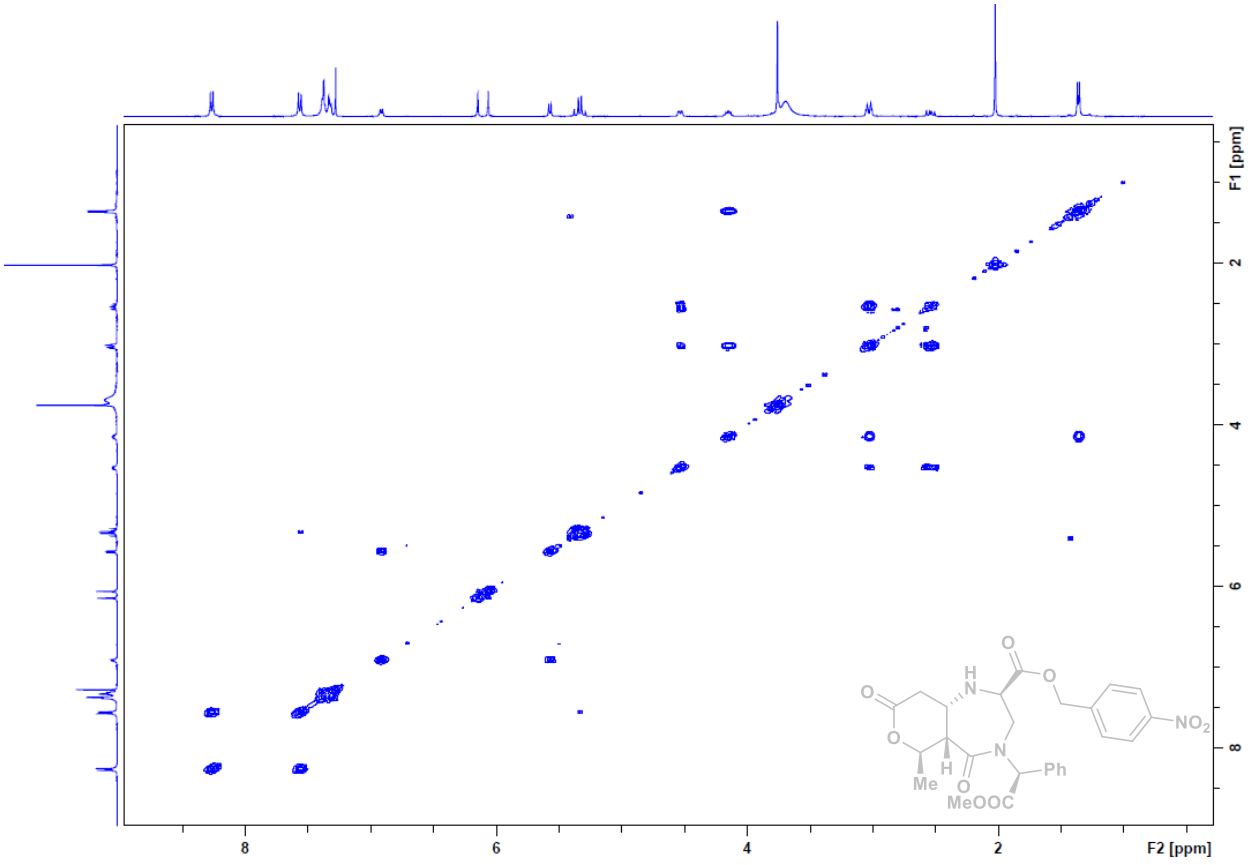
## $^{1}$H NMR spectrum for 3c



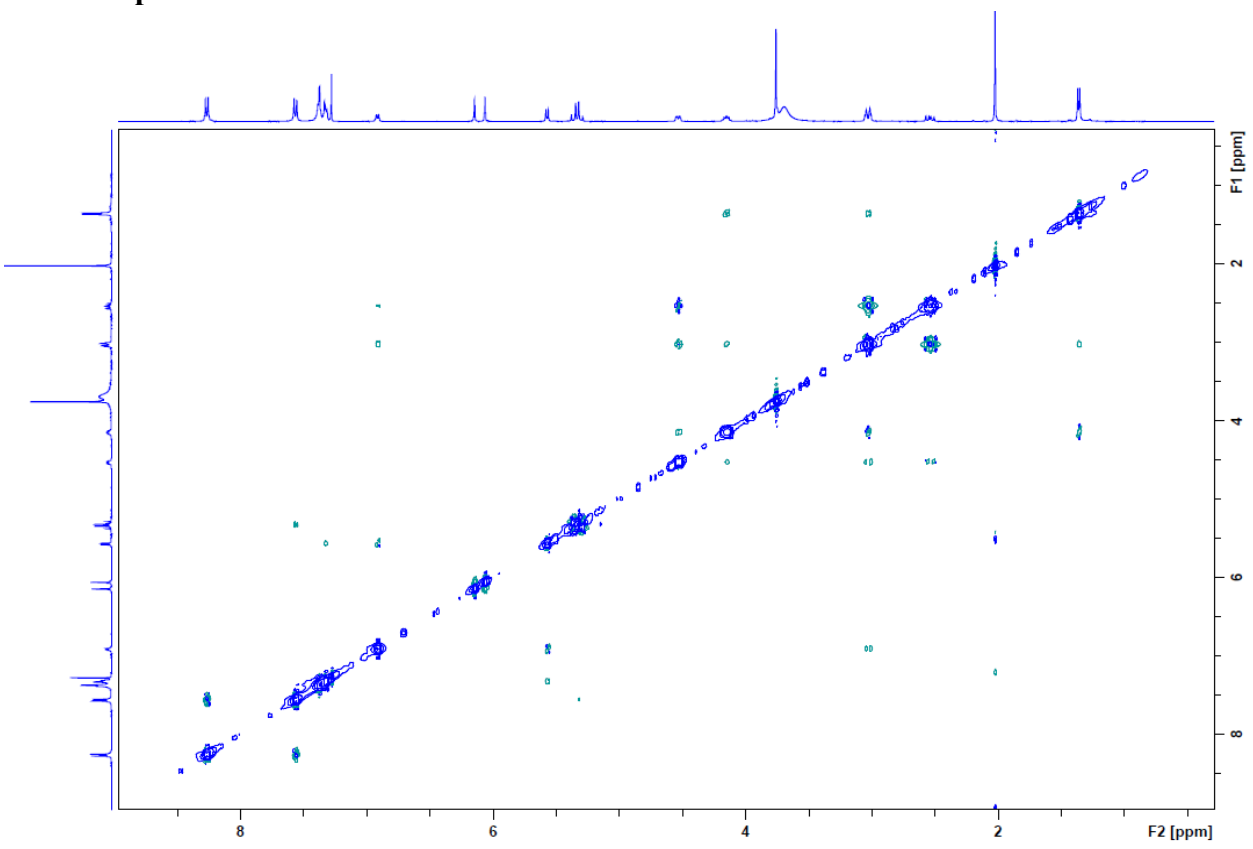

## $^{13}$C NMR spectrum for 3c



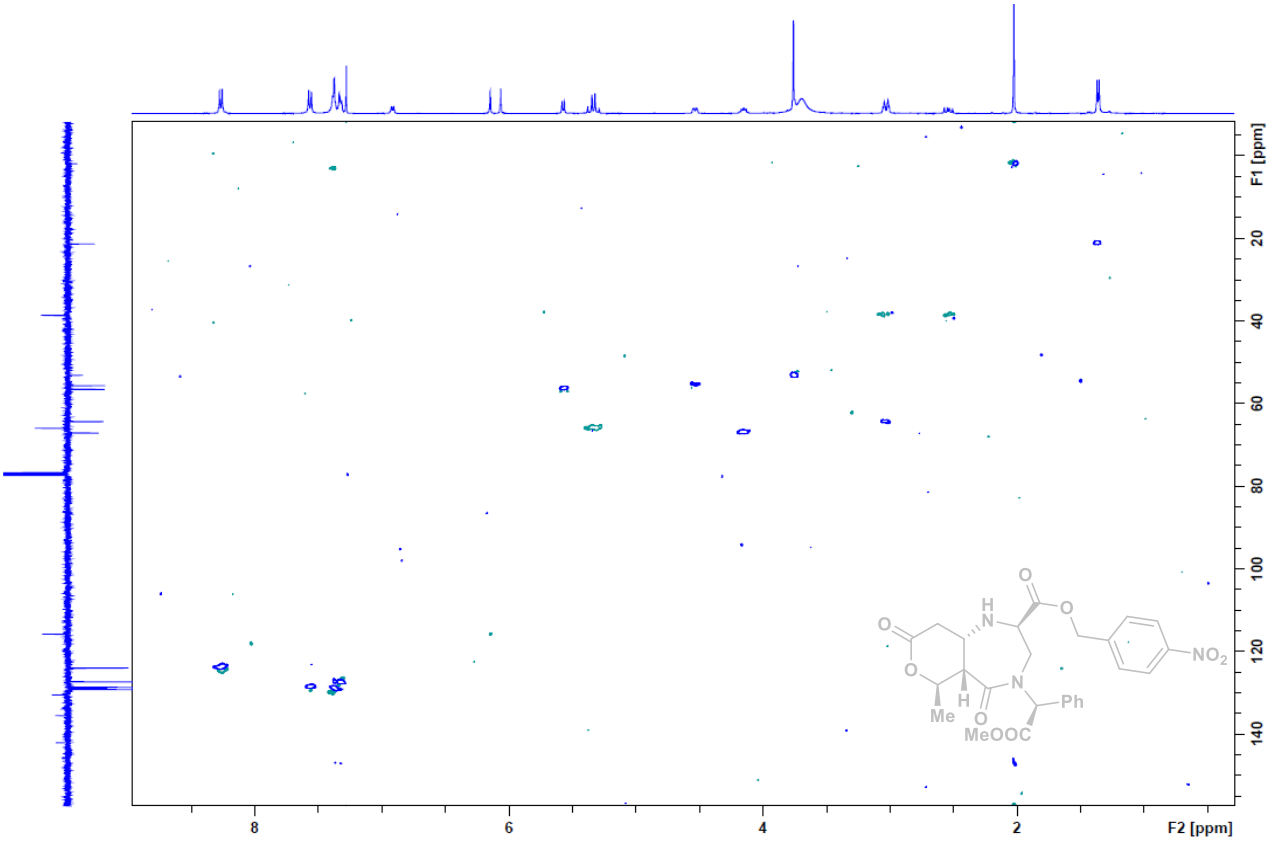
**COSY spectrum for 3c**



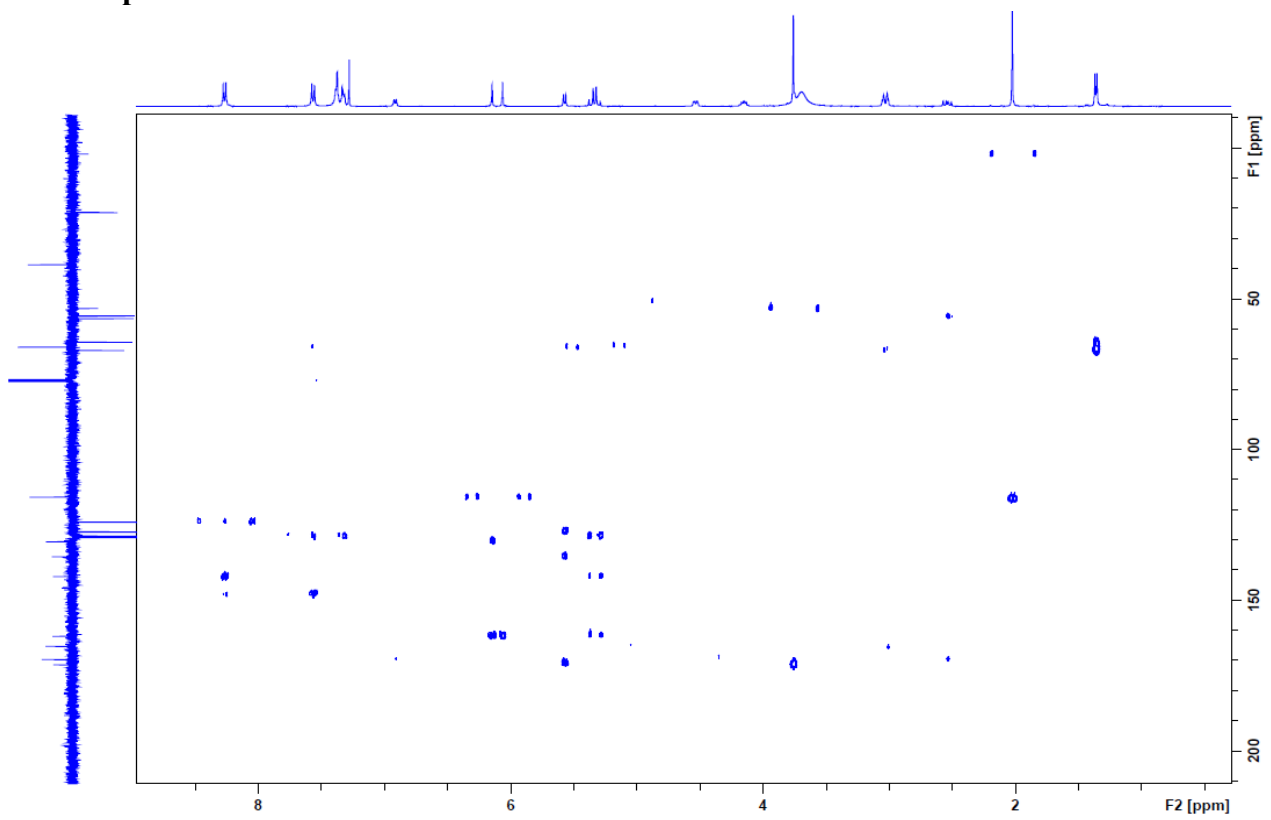

**NOESY spectrum for 3c**



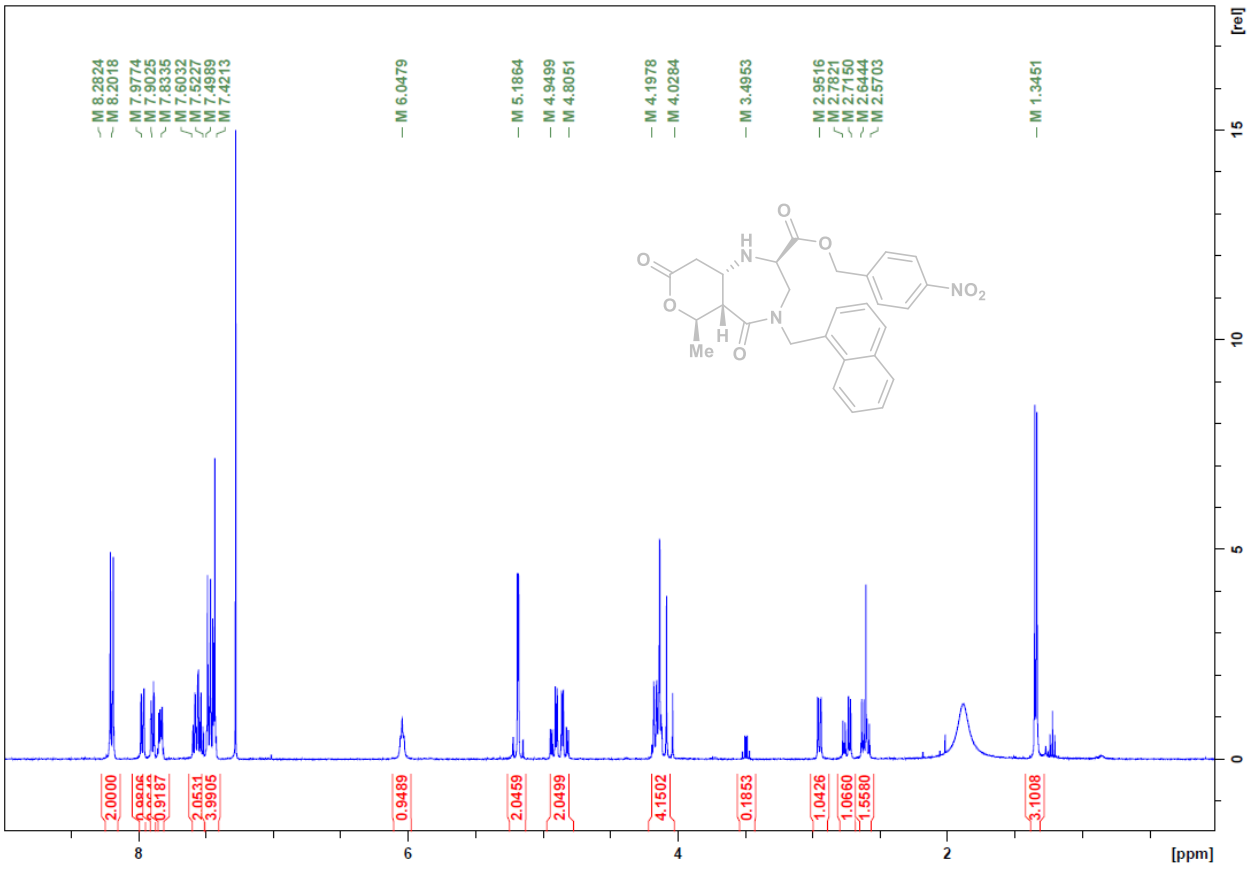
**HSQC specrum for 3c**



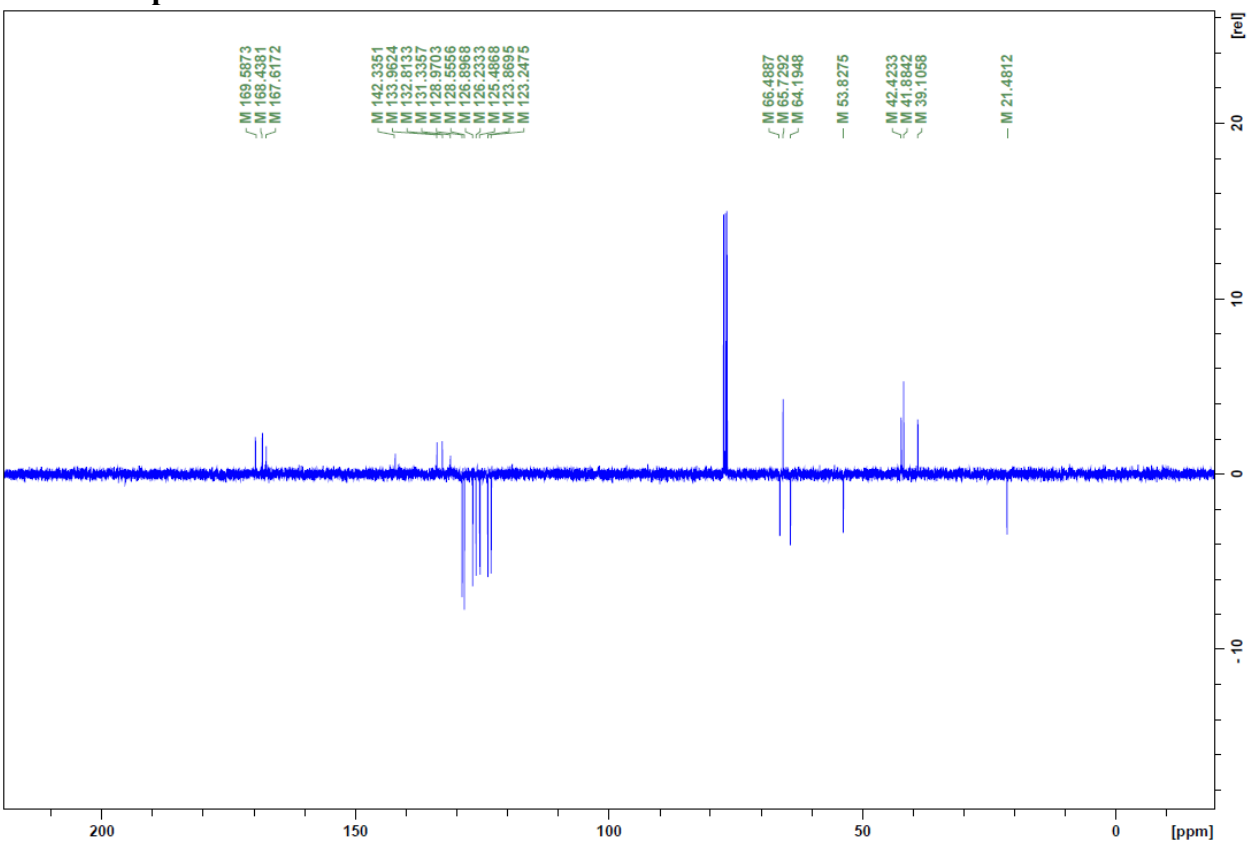

**HMBC spectrum for 3c**



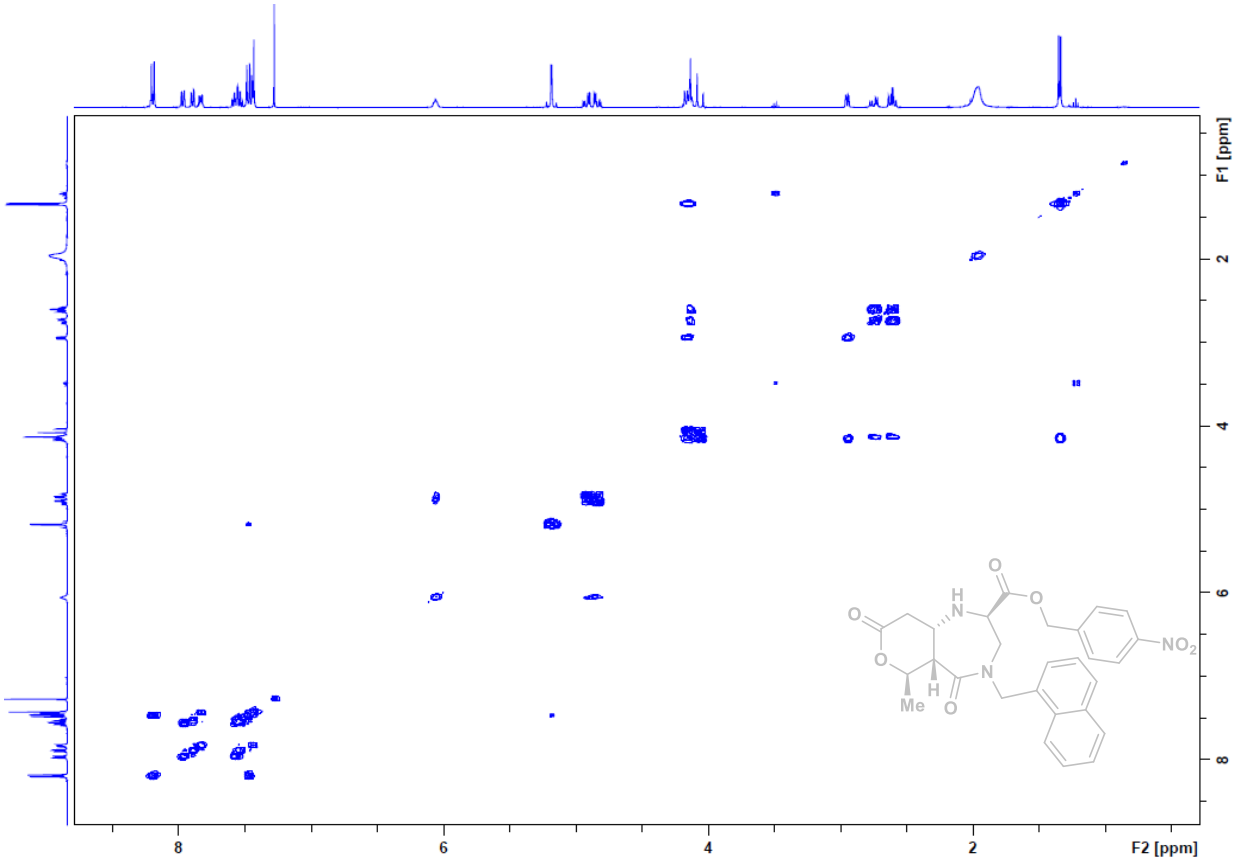
## $^{1}$H NMR spectrum for 3d



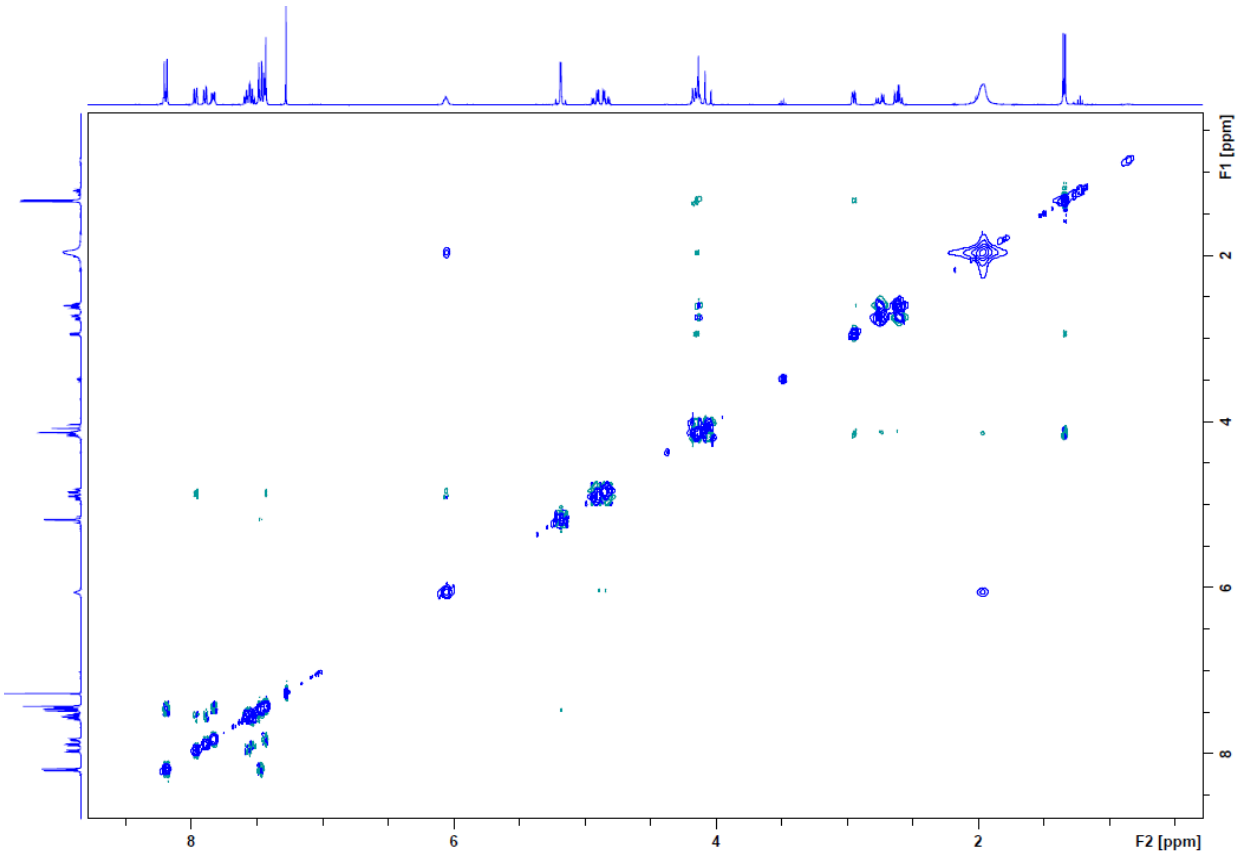

## $^{13}$C NMR spectrum for 3d



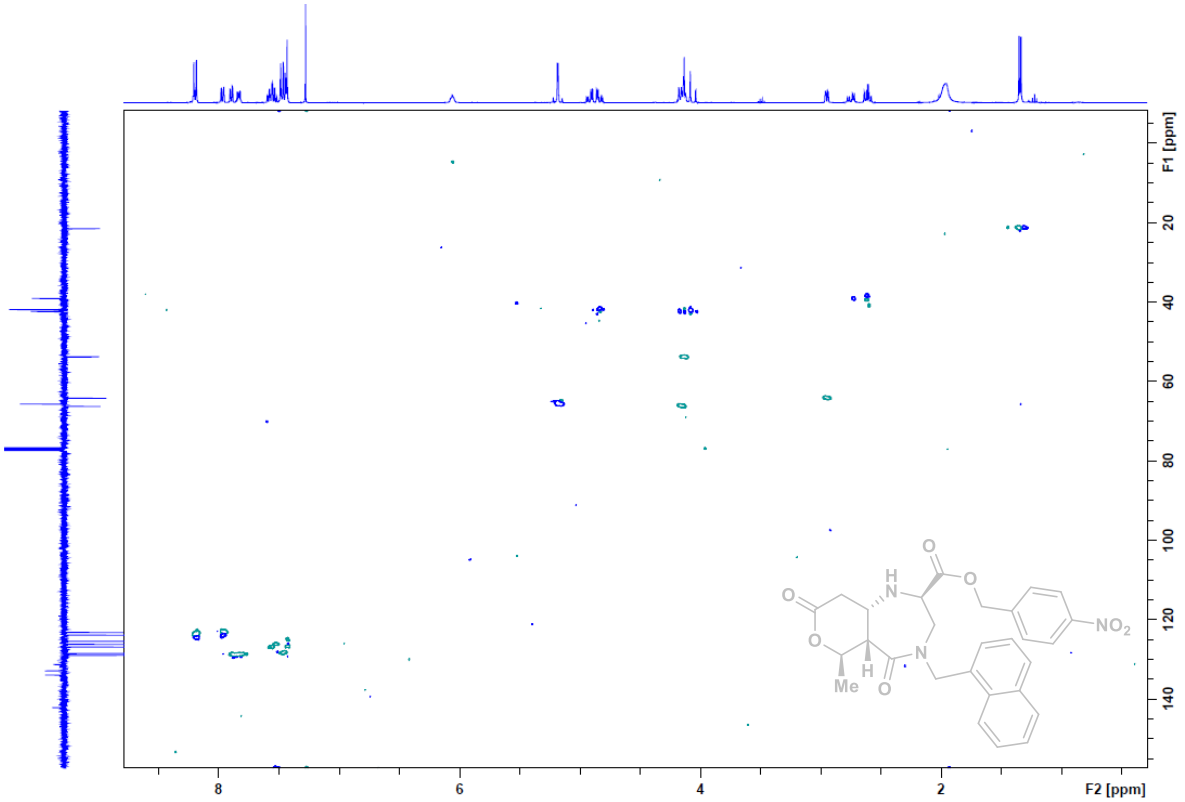
**COSY spectrum for 3d**




**NOESY spectrum for 3d**



**HSQC spectrum for 3d**




**HMBC spectrum for 3d**