# Structure-activity relationship studies of pyrimidine-4-carboxamides as inhibitors of *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD)

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## Supporting Information

### Contents:

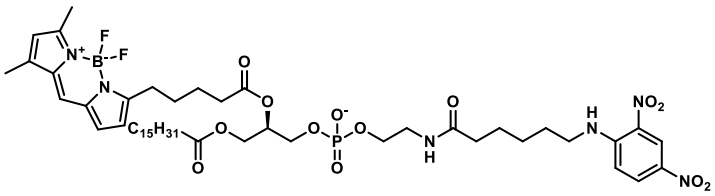


**Figure S1.** Structure of fluorescence-quenched substrate PED6.

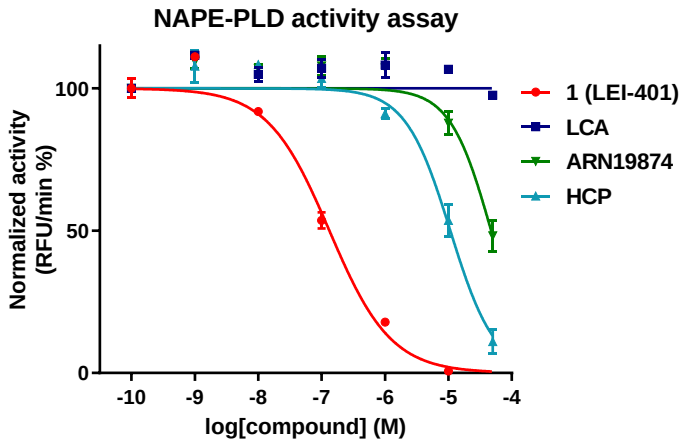



**Figure S2.** NAPE-PLD PED6 activity assay dose-response curves for **1** (**LEI-401**) and reference inhibitors lithocholic acid (LCA), ARN19874 and hexachlorophene (HCP). Data represent mean values ± SEM (N = 2, n = 2).

**Table S1.** NAPE-PLD inhibitory activities of **1** (**LEI-401**) and reference inhibitors.

| ID | PED6 assay | | | | | Reported $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|
| | $pIC_{50}$ ± SEM | $IC_{50}$ (μM) | $K_i$ (μM) | cLogP[a] | LipE[b] | |
| **1 (LEI-401)** | 7.14 ± 0.04 | 0.072 | 0.027 | 3.46 | 3.68 | - |
| **Lithocholic acid** | < 4.3 | - | - | 6.60 | - | 68[1] |
| **Hexachlorophene** | 4.94 ± 0.07 | 11 | 4.27 | 7.03 | -2.09 | 1.6[2] |
| **ARN19874** | 4.27 ± 0.07 | 54 | 20.1 | 2.13 | 2.08 | 34[3] |

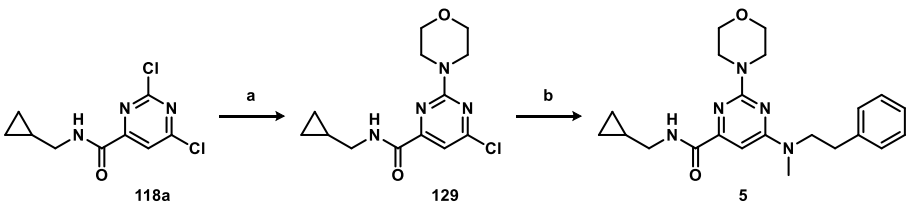
[a] cLogP was calculated using Chemdraw 15; [b] Lipophilic efficiency (LipE) = $pIC_{50}$ – cLogP.

## References

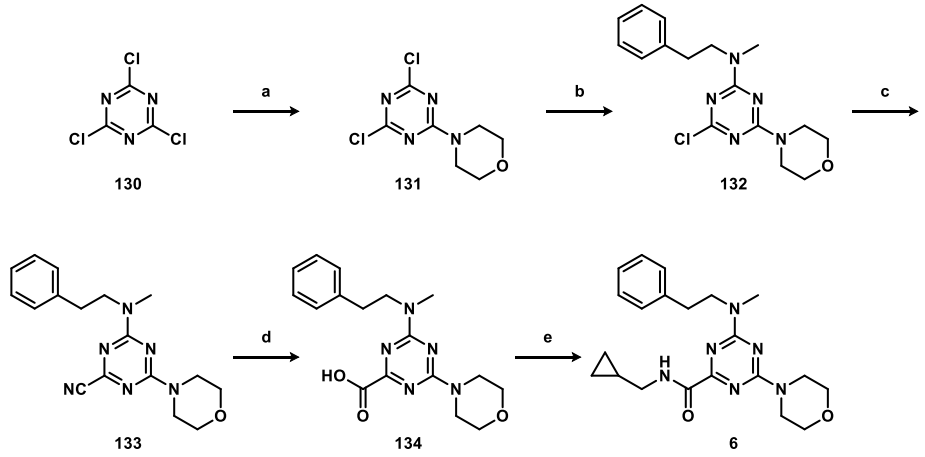

(1) Margheritis, E.; Castellani, B.; Magotti, P.; Peruzzi, S.; Romeo, E.; Natali, F.; Mostarda, S.; Gioiello, A.; Piomelli, D.; Garau, G. Bile acid recognition by NAPE-PLD. *ACS Chem. Biol.* **2016,** 11, 2908-2914.

(2) Aggarwal, G.; Zarrow, J. E.; Mashhadi, Z.; Flynn, C. R.; Vinson, P.; Weaver, C. D.; Davies, S. S. Symmetrically substituted dichlorophenes inhibit *N*-acyl-phosphatidylethanolamine phospholipase D. *J. Biol. Chem.* **2020,** 295, 7289-7300.

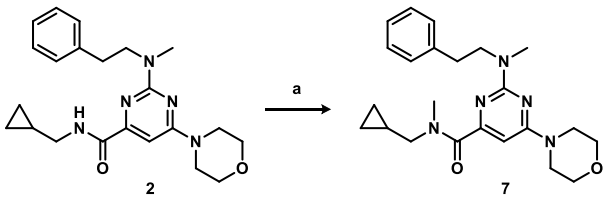
(3) Castellani, B.; Diamanti, E.; Pizzirani, D.; Tardia, P.; Maccesi, M.; Realini, N.; Magotti, P.; Garau, G.; Bakkum, T.; Rivara, S.; Mor, M.; Piomelli, D. Synthesis and characterization of the first inhibitor of *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD). *ChemComm* **2017,** 53, 12814-12817.



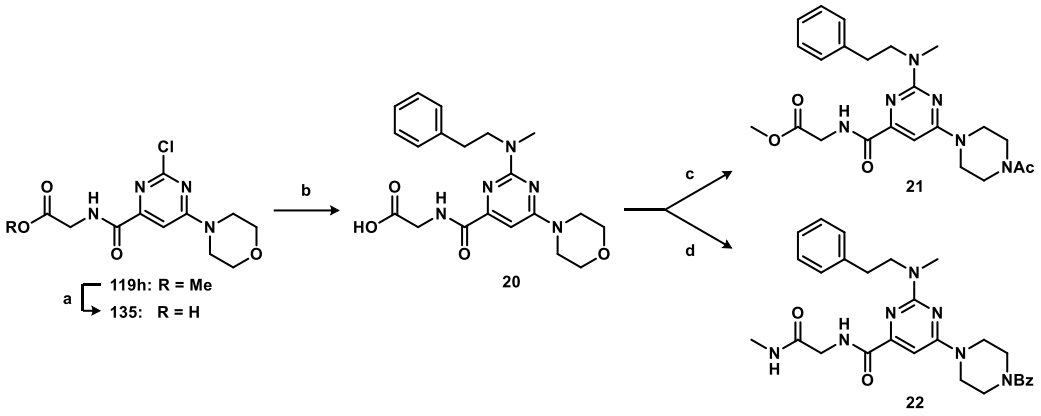

**Scheme S1.** Synthesis of pyrimidine regioisomer **5**. Reagents and conditions: a) morpholine, DiPEA, MeOH, 0 °C, 5% (+ 89% regioisomer **29**); b) *N*-methylphenethylamine, DiPEA, MeOH, 70 °C, 71%.



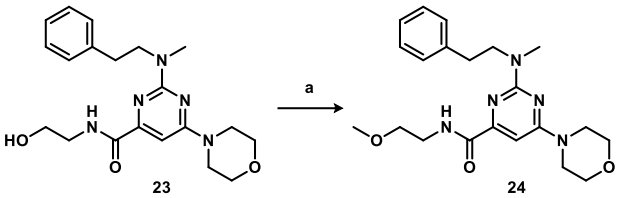

**Scheme S2:** Synthesis of triazine **6**. Reagents and conditions: a) morpholine, DiPEA, DCM, rt, 72%; b) *N*-methylphenethylamine, $K_2CO_3$, acetone, rt, 68%; c) KCN, DABCO, DMF, rt, 53%; d) NaOH, $H_2O$, THF, 60 °C, 85%; e) cyclopropylmethanamine, PyBOP, DiPEA, DMF, rt, 67%.



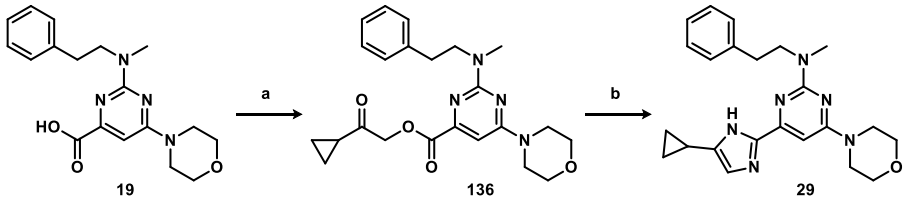

**Scheme S3.** Synthesis of $R_1$ cyclopropylmethylamine analogue **7**. Reagents and conditions: a) NaH, MeI, DMF, 0 °C to rt, 48%.



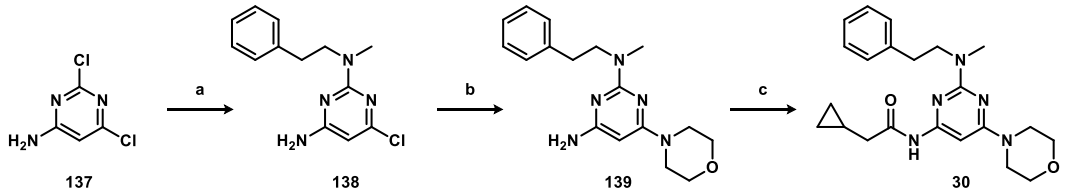

**Scheme S4.** Synthesis of $R_1$ cyclopropylmethylamine analogues **20-22**. Reagents and conditions: a) NaOH, THF, MeOH, $H_2O$, rt, 99%; b) *N*-methylphenethylamine, DiPEA, *n*-BuOH, 120 °C, 20%; c) EDC·HCl, HOBt, MeOH, DCM, rt, 62%; d) $MeNH_2$·HCl, PyBOP, DiPEA, DMF, 48%.



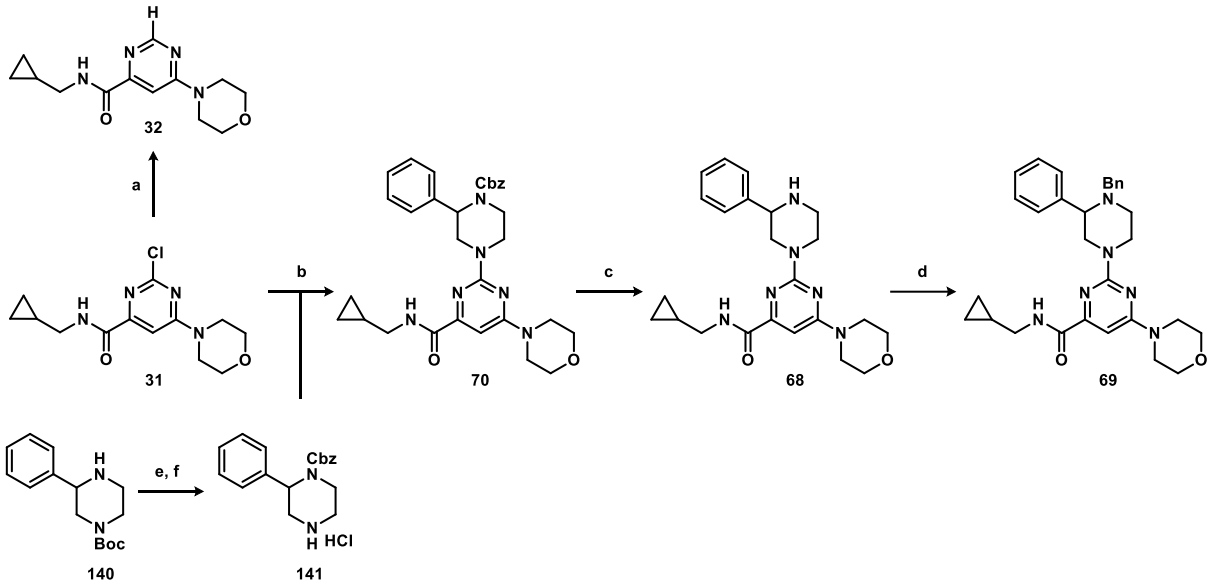

**Scheme S5.** Synthesis of $R_1$ cyclopropylmethylamine analogue **24**. Reagents and conditions: a) NaOtBu, MeI, DMF, 0 °C to rt, 28%.



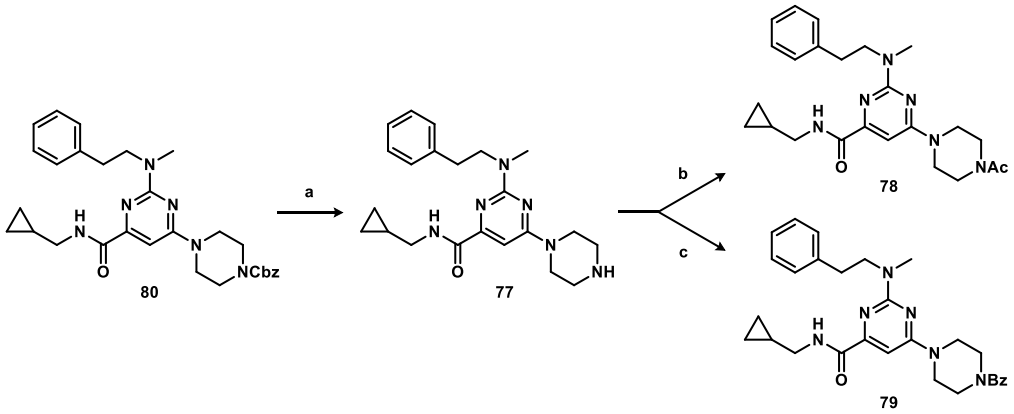

**Scheme S6.** Synthesis of $R_1$ cyclopropylmethylamine analogue **29**. Reagents and conditions: a) 2-bromocyclopropylethanone, $Cs_2CO_3$, DMF, rt, 53%; b) $NH_4OAc$, xylene, 140 °C, 6%.



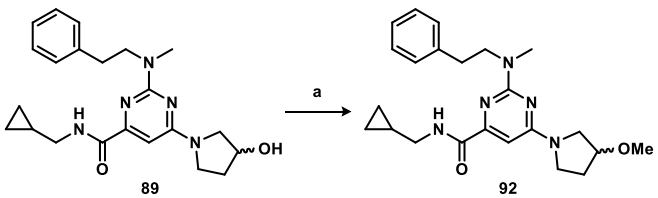

**Scheme S7.** Synthesis of **30**. Reagents and conditions: a) *N*-methylphenethylamine, DiPEA, 2-PrOH, reflux, 64%; b) morpholine, DiPEA, *n*-BuOH, µW, 180 °C, 89%; c) *i.* 2-cyclopropylacetic acid, oxalyl chloride, DCM-$d_2$, rt; *ii.* **139**, DCM, rt, 34%.




**Scheme S8.** Synthesis of $R_2$ analog **32** and 3-phenylpiperazine analogues **68-70**. Reagents and conditions: a) Pd/C, $H_2$, MeOH, rt, 95%; b) **141**, DiPEA, *n*-BuOH, 120 °C, 99%; c) Pd/C, $H_2$, MeOH, rt, 90%; d) BnBr, DiPEA, $CH_3CN$, rt, 74%; e) CbzCl, $NaHCO_3$, THF, $H_2O$, 0 °C to rt, 90%; f) 4 M HCl, 1,4-dioxane, rt, quant.




**Scheme S9.** Synthesis of $R_3$ morpholine analogues **77-79**. Reagents and conditions: a) Pd/C, $H_2$, MeOH, AcOH, rt, 63%; b) $Ac_2O$, DiPEA, DCM, rt, 78%; c) BzCl, $Et_3N$, DCM, rt, 74%.




**Scheme S10.** Synthesis of $R_3$ morpholine analogue **92**. Reagents and conditions: a) NaH, MeI, DMF, 0 °C to rt, 44%.

## NMR Spectra

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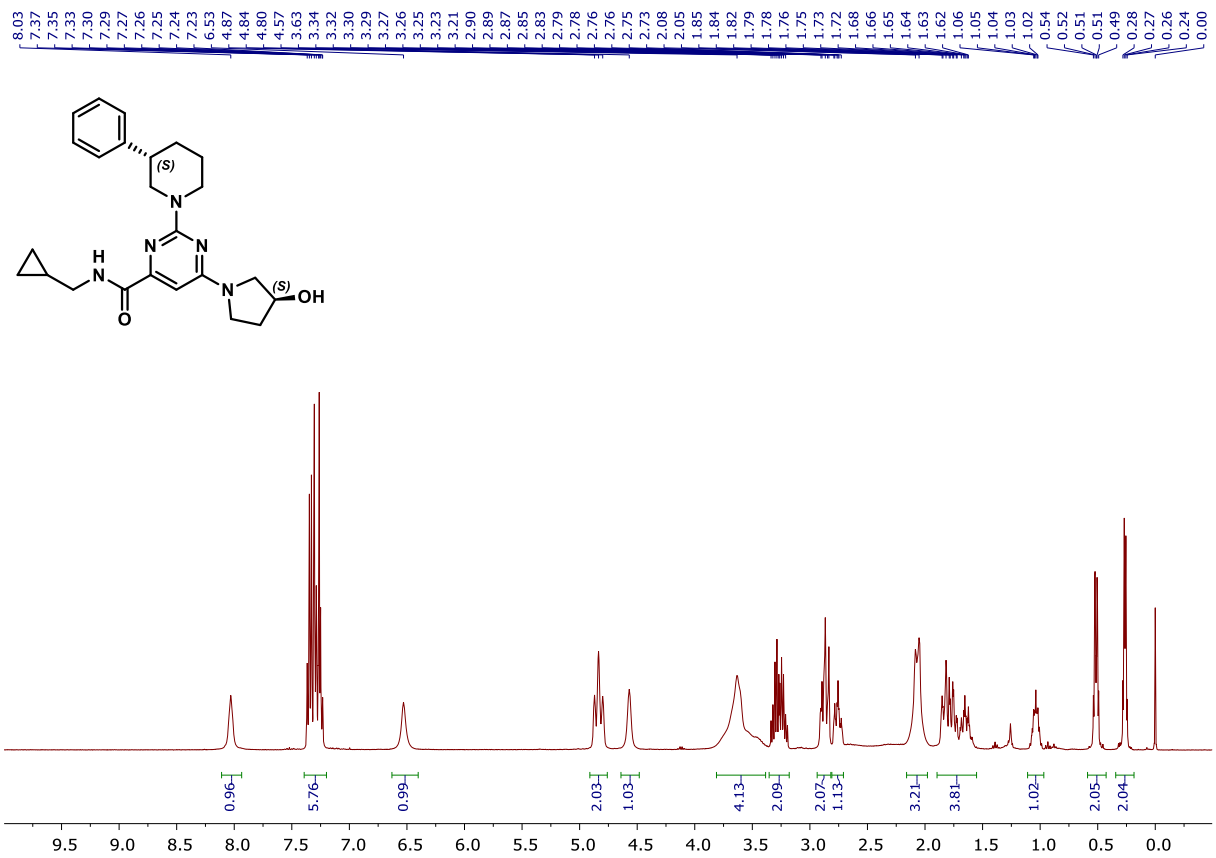


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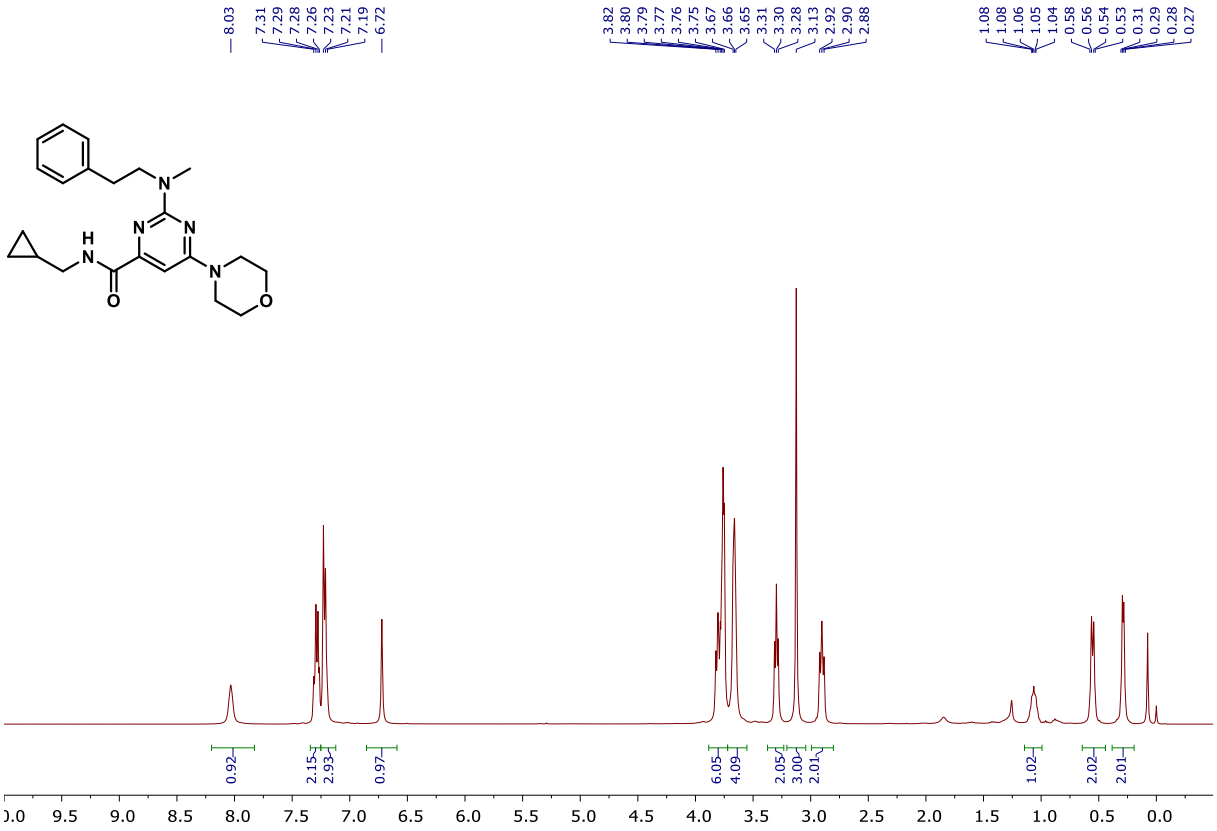


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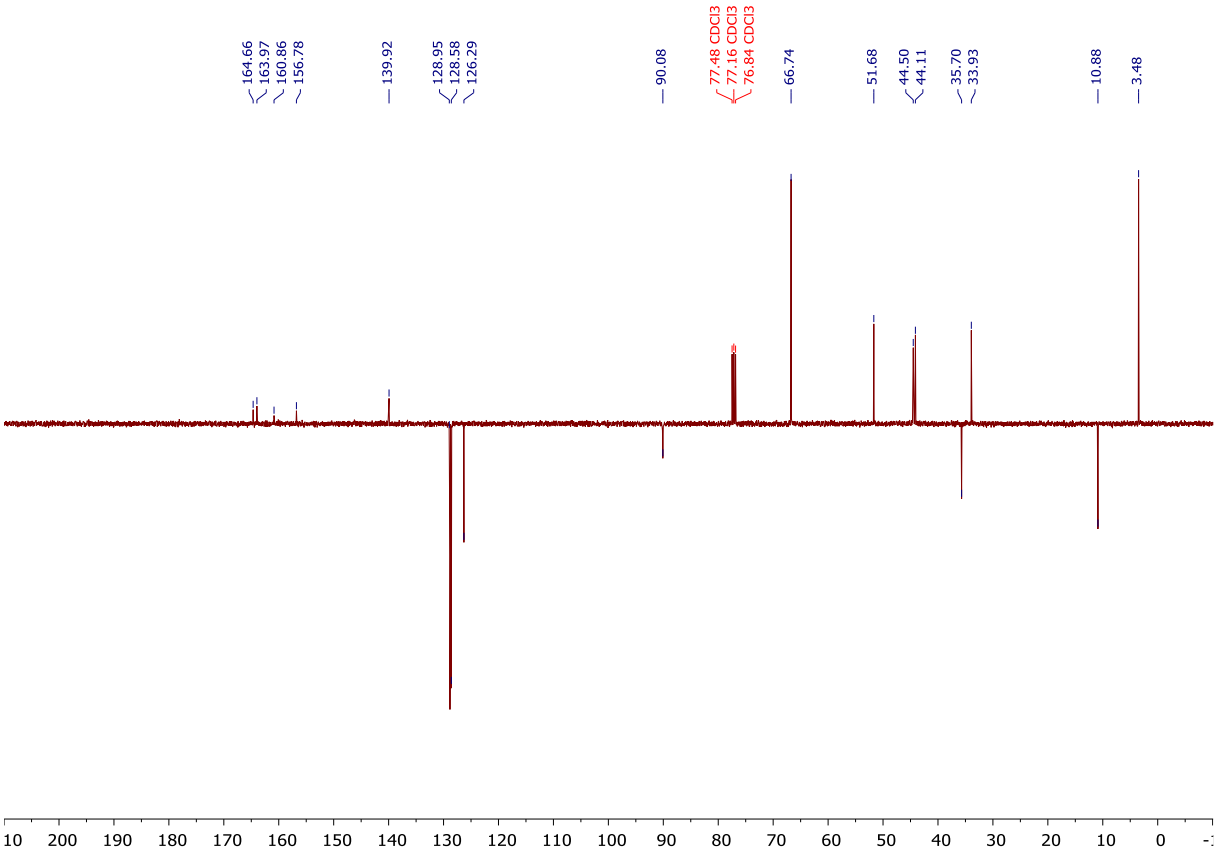

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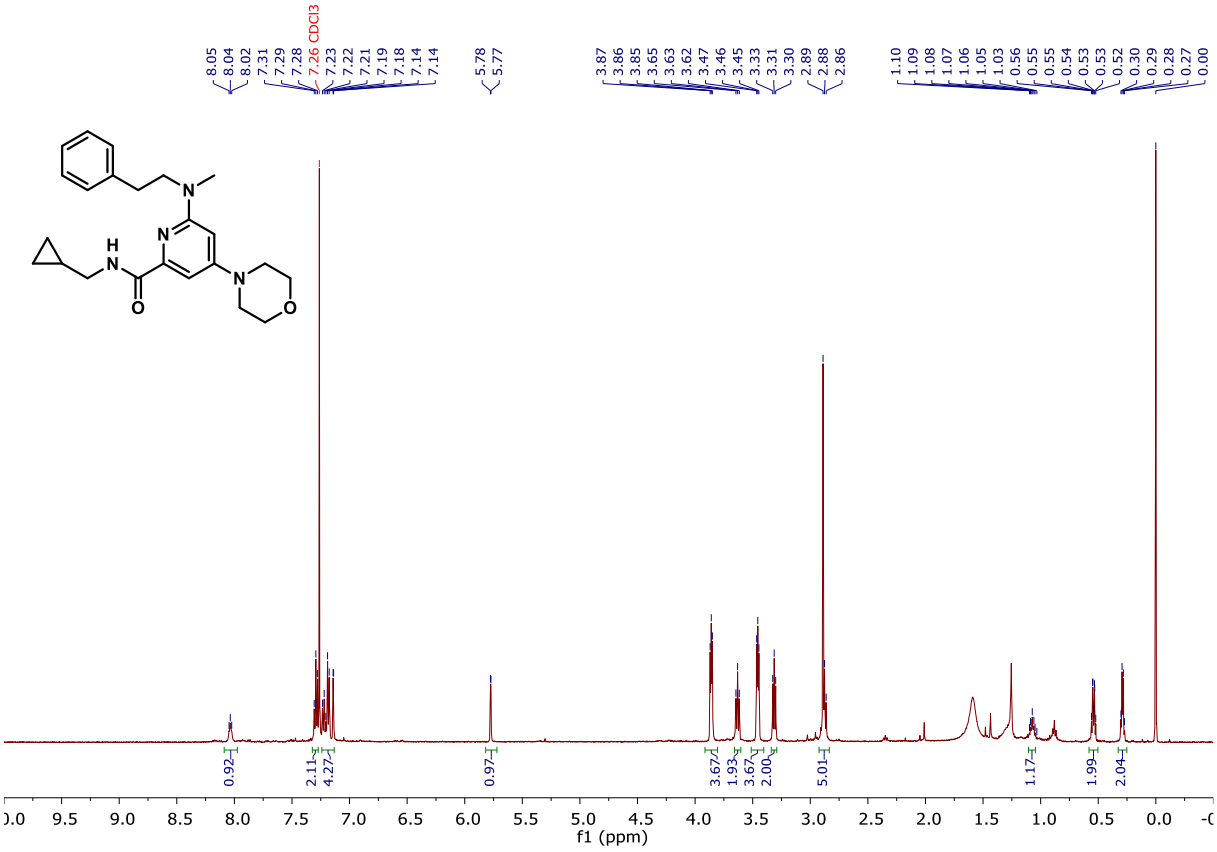


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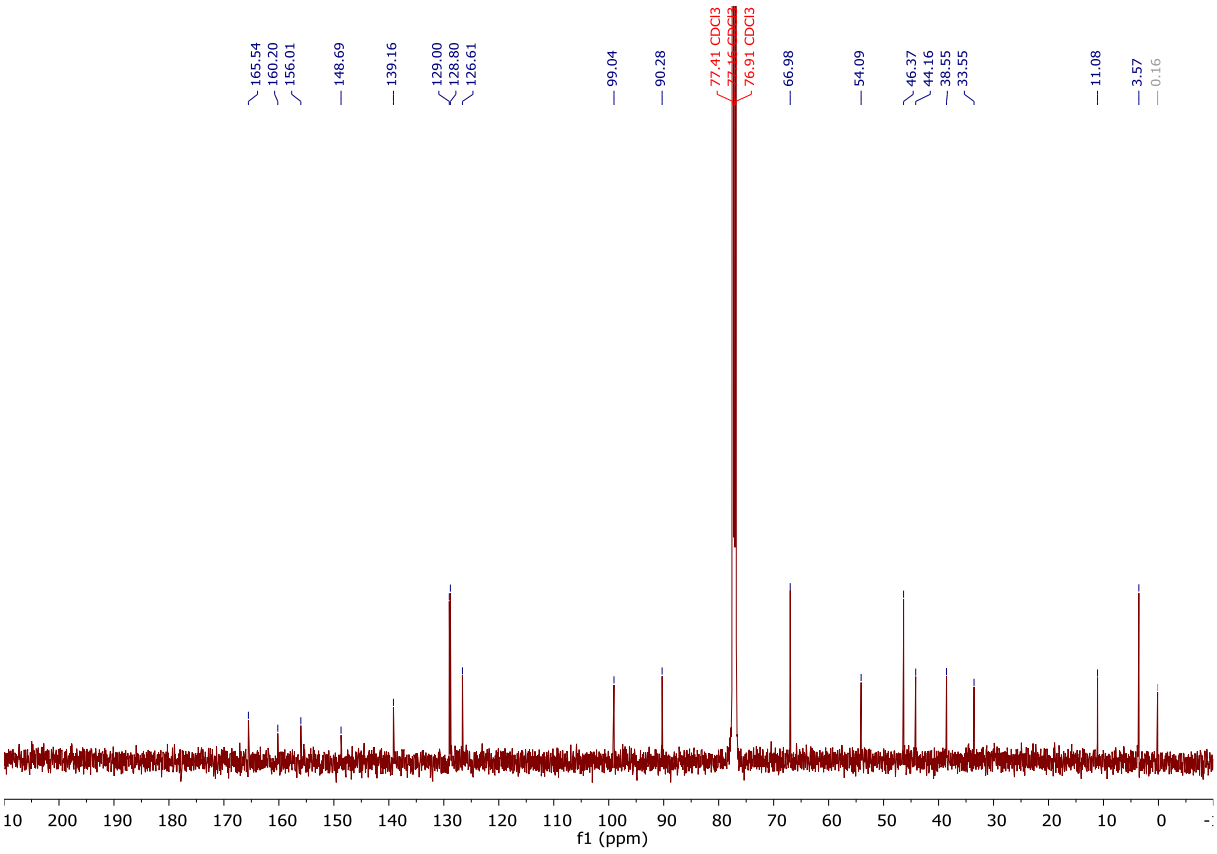

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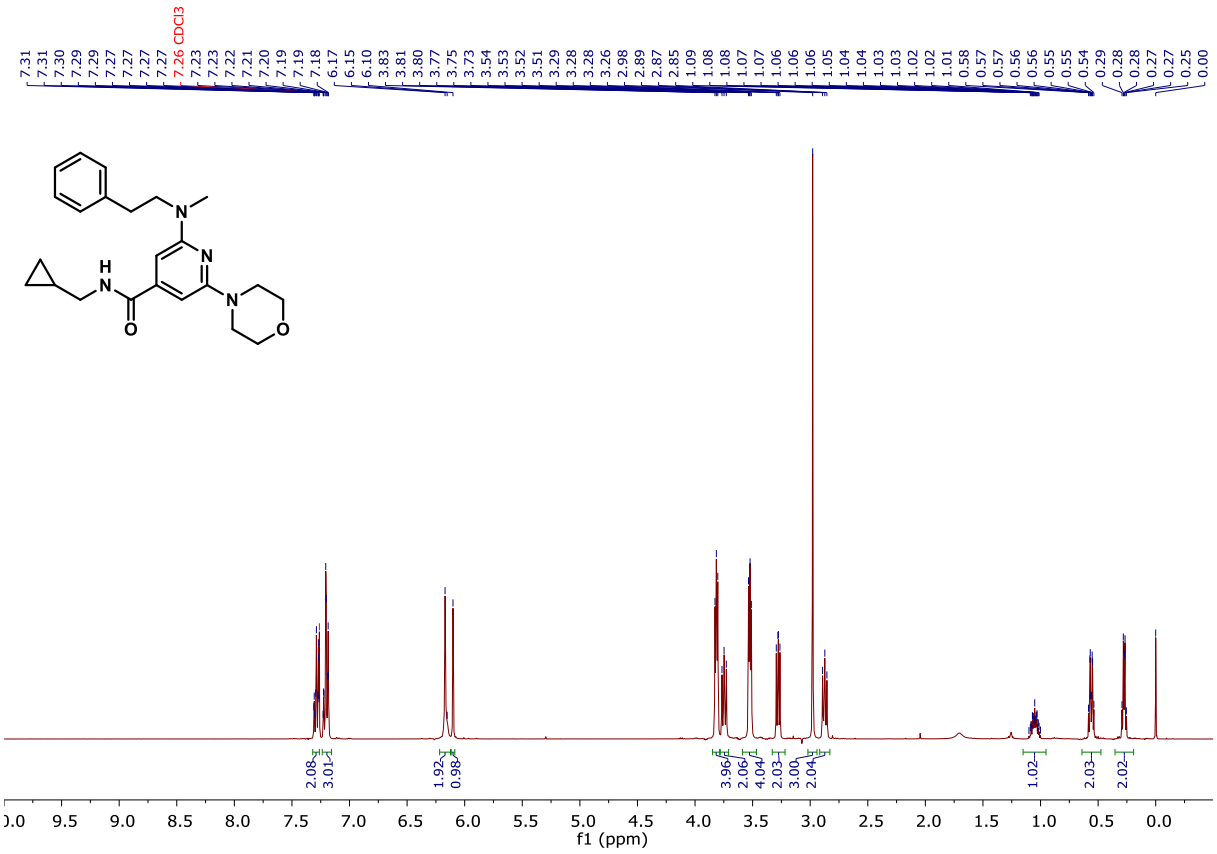


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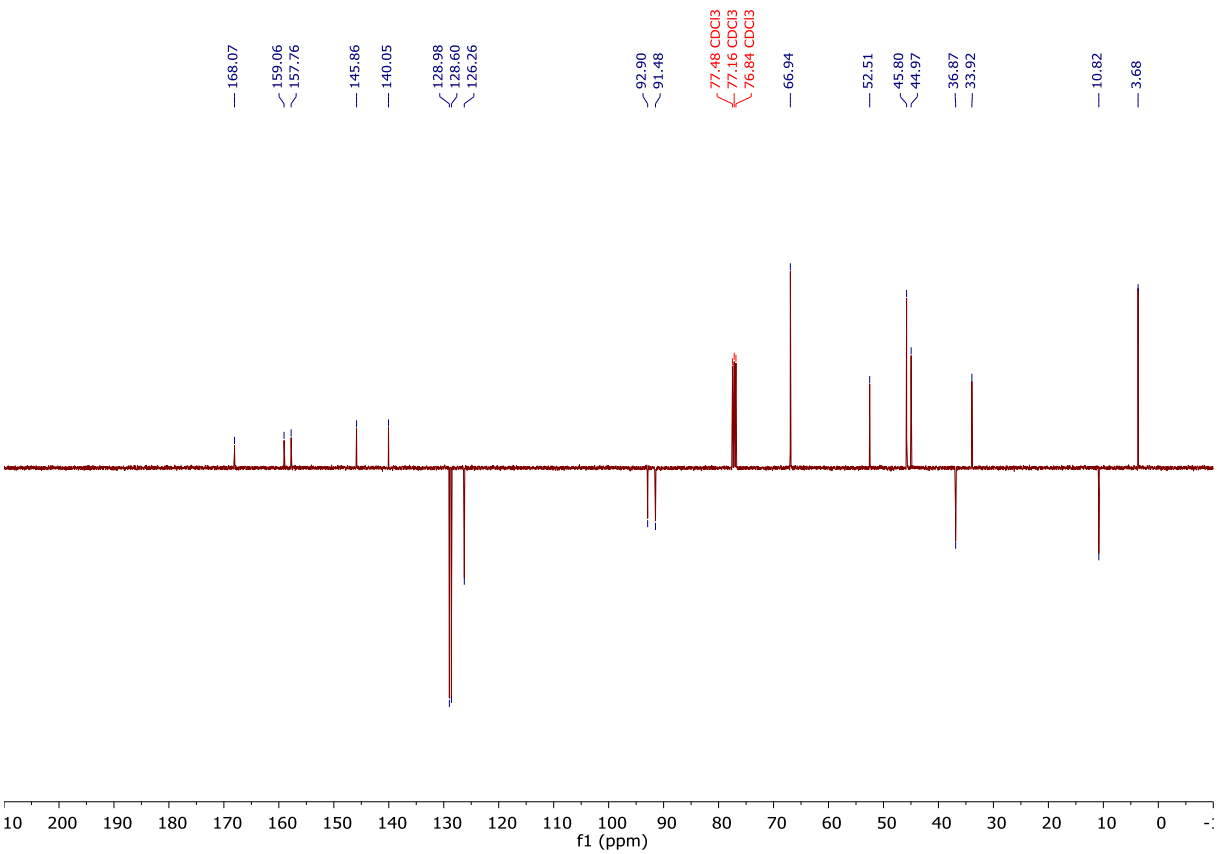

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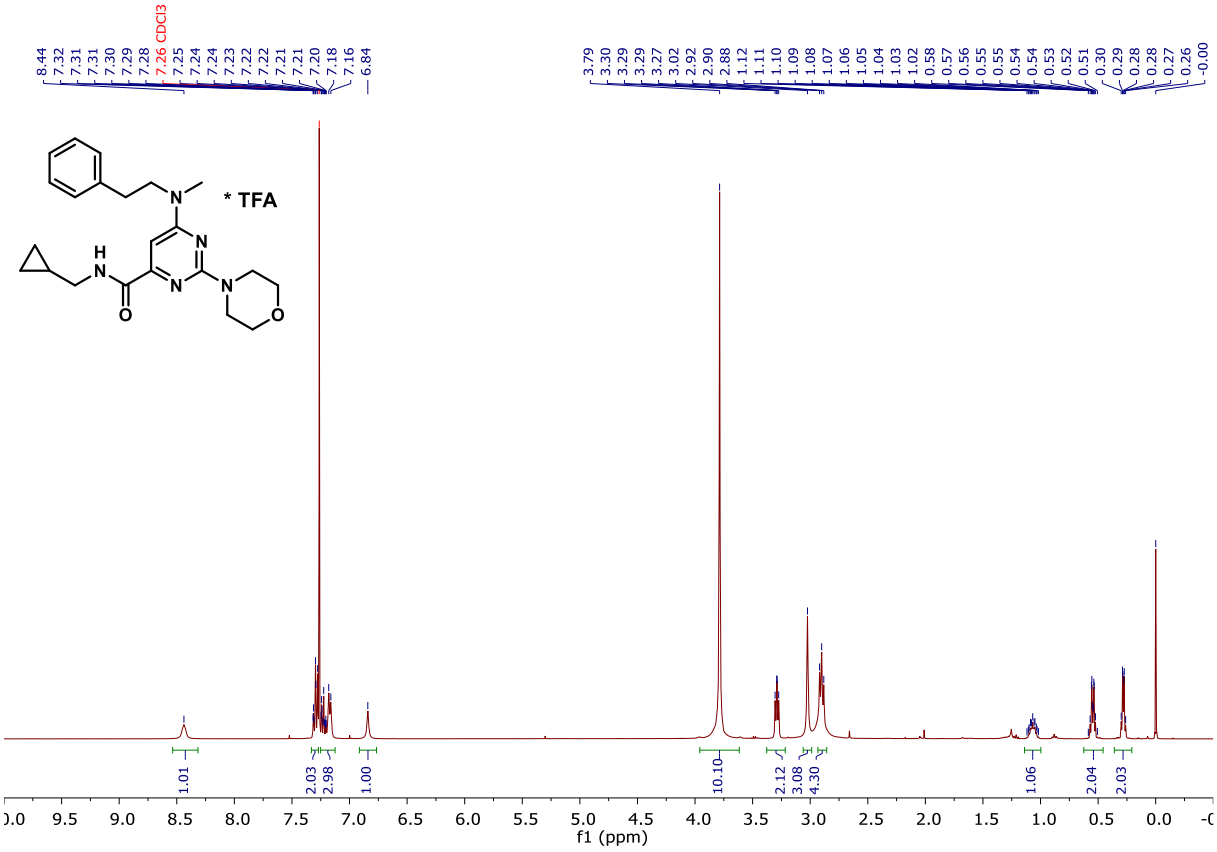


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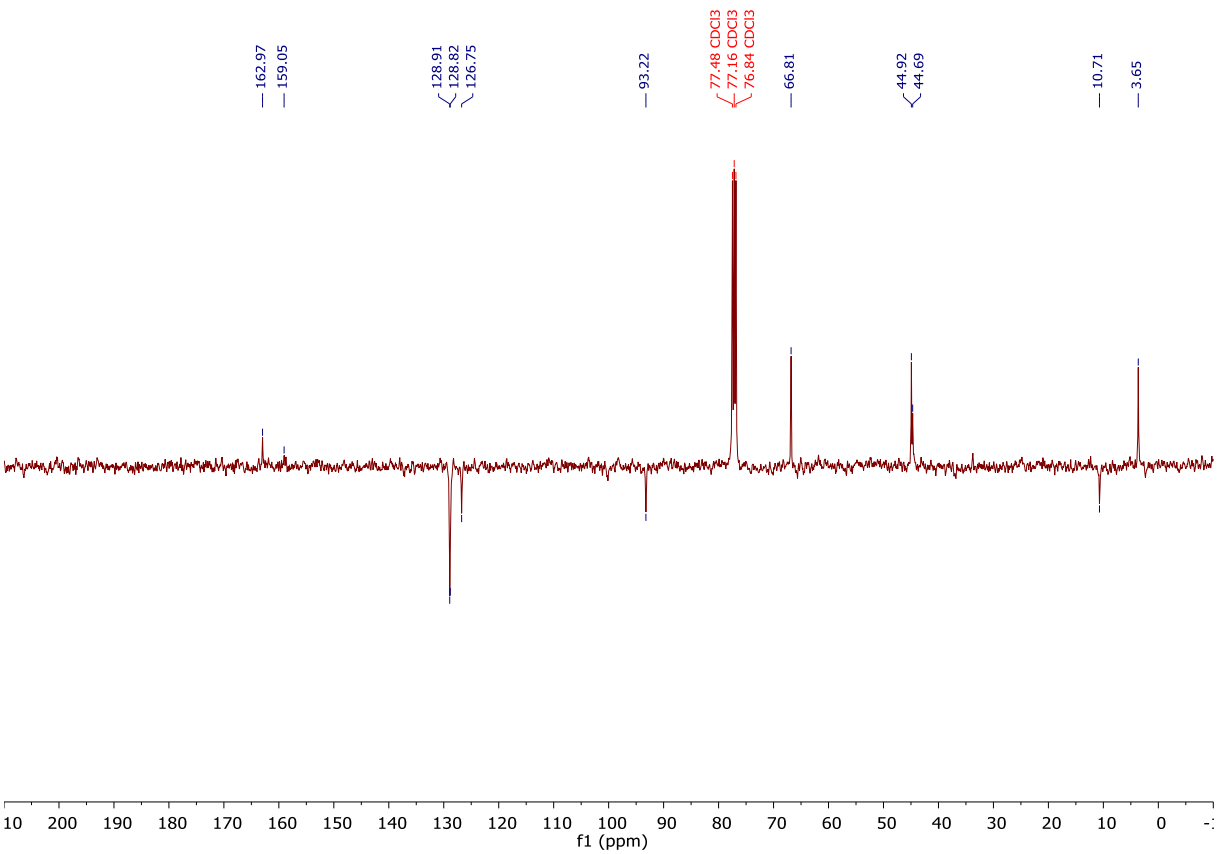

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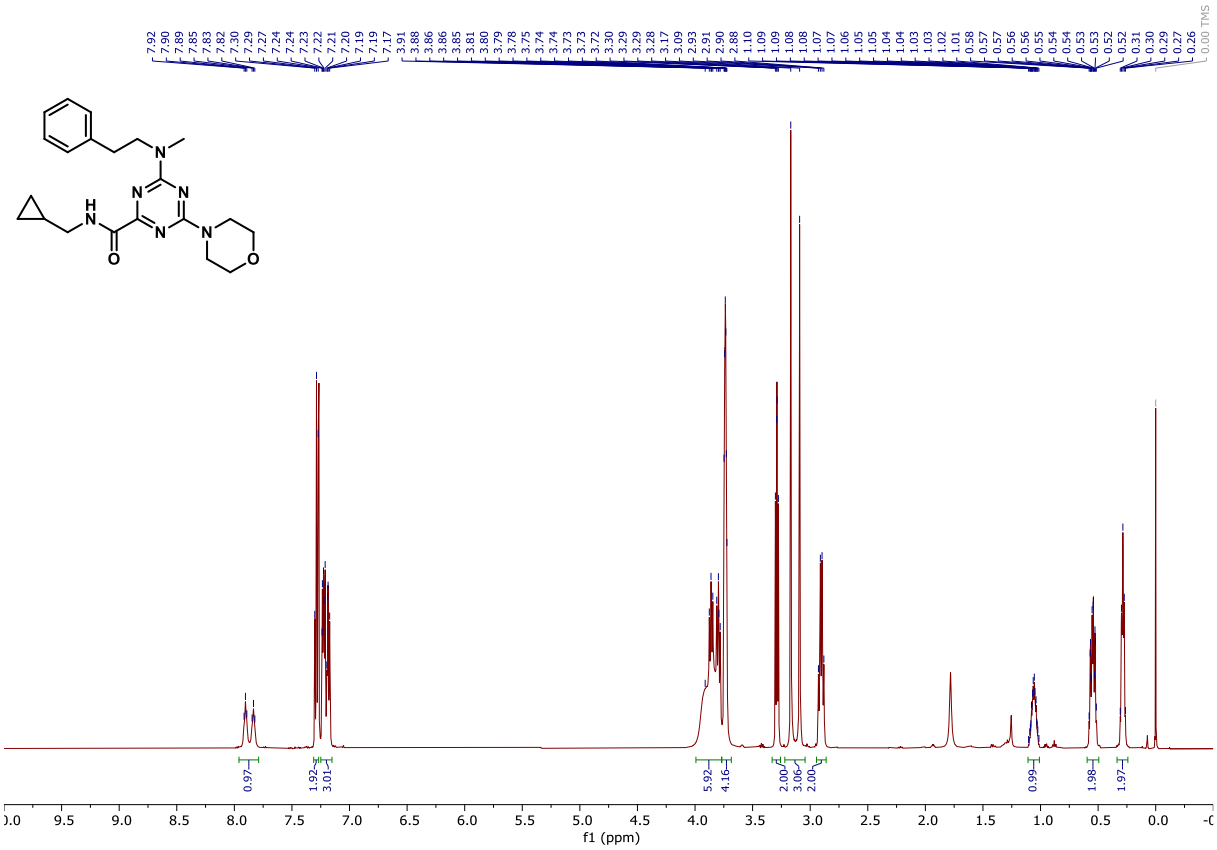


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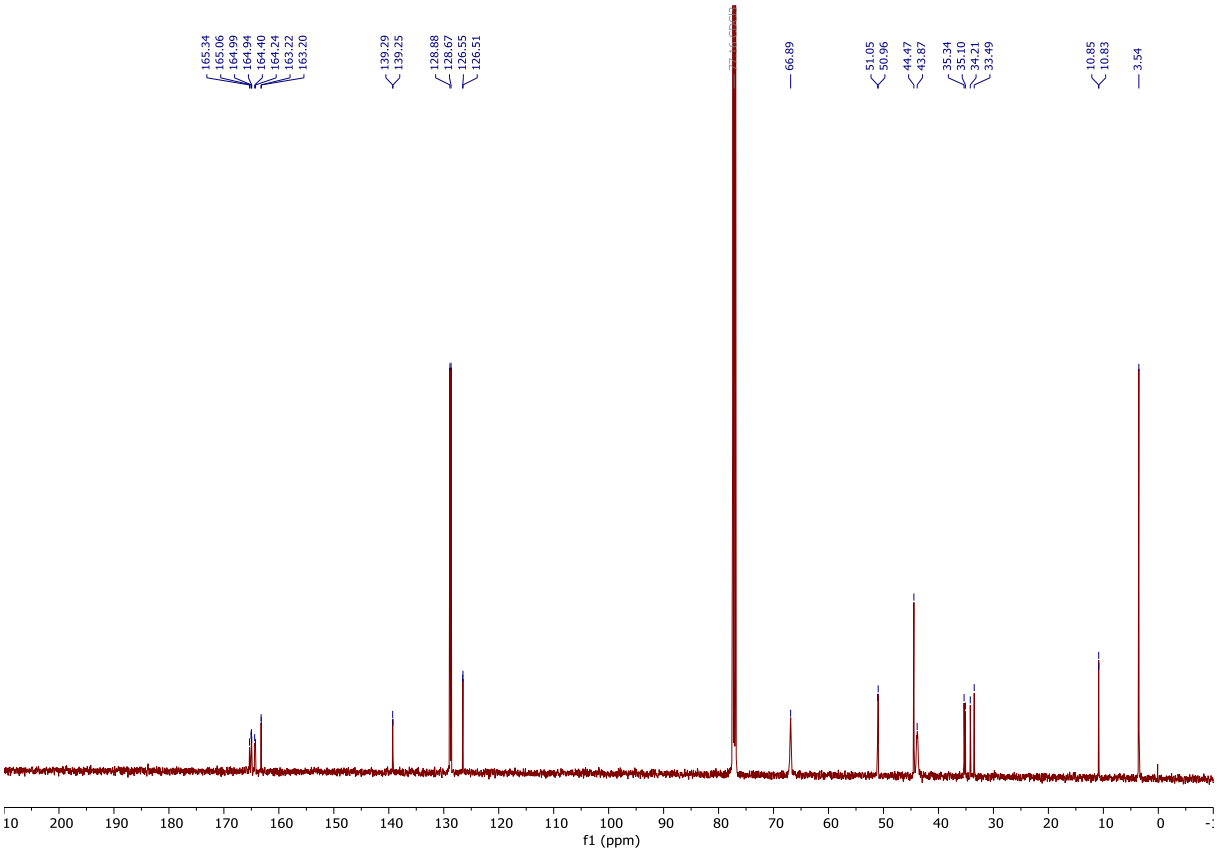

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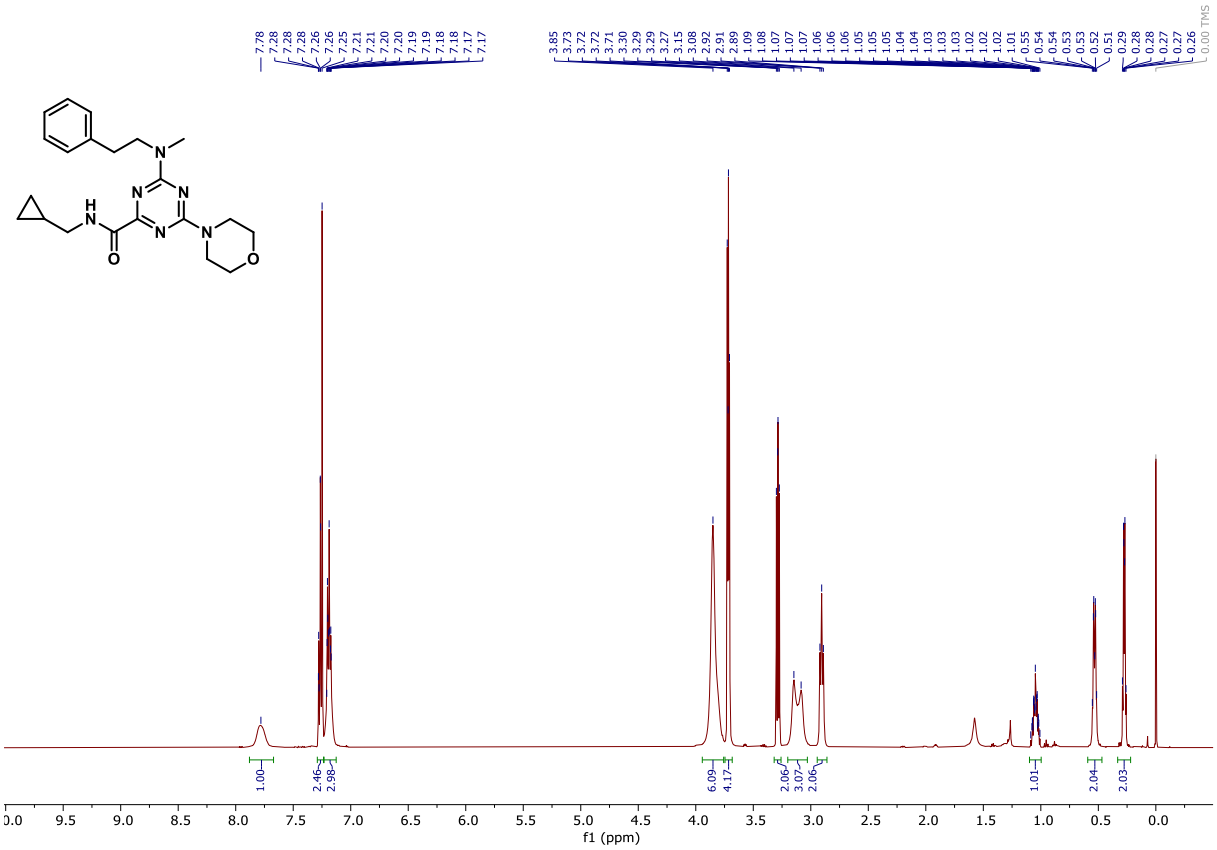


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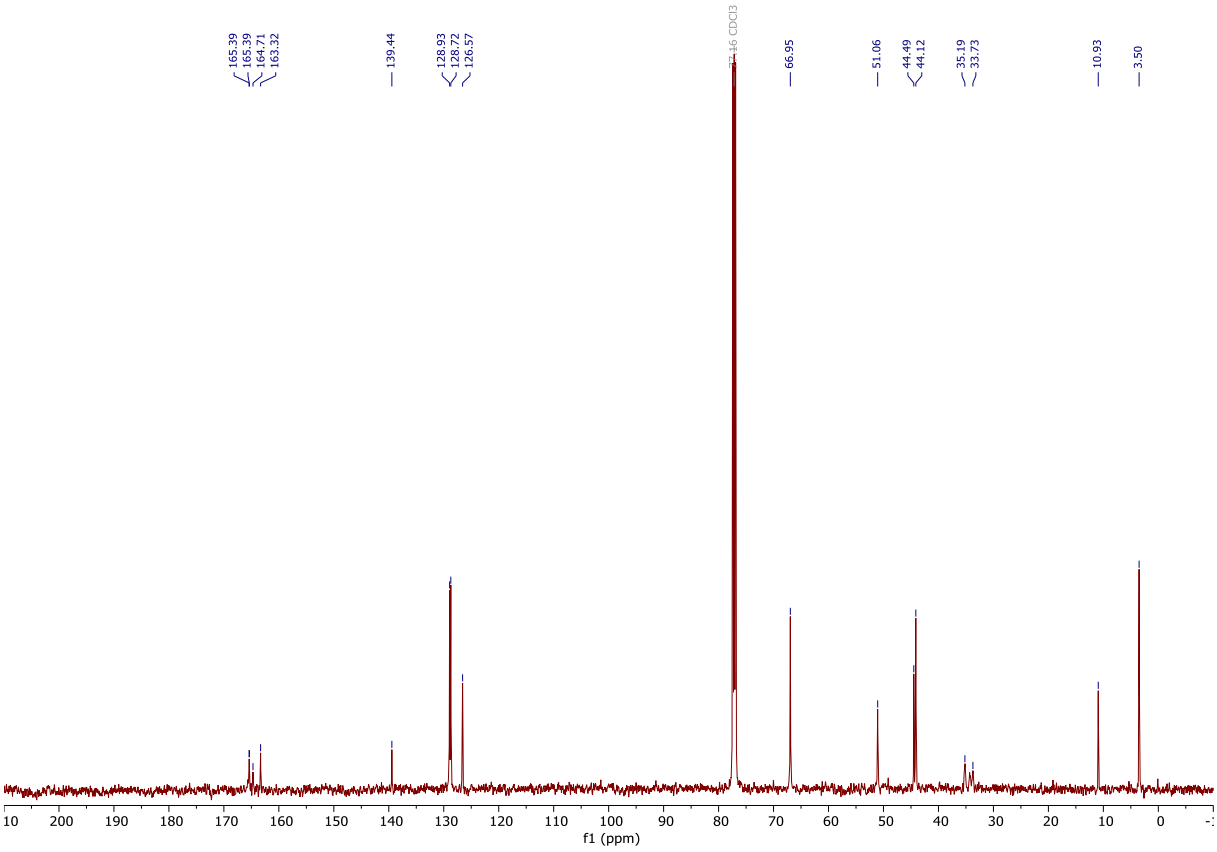
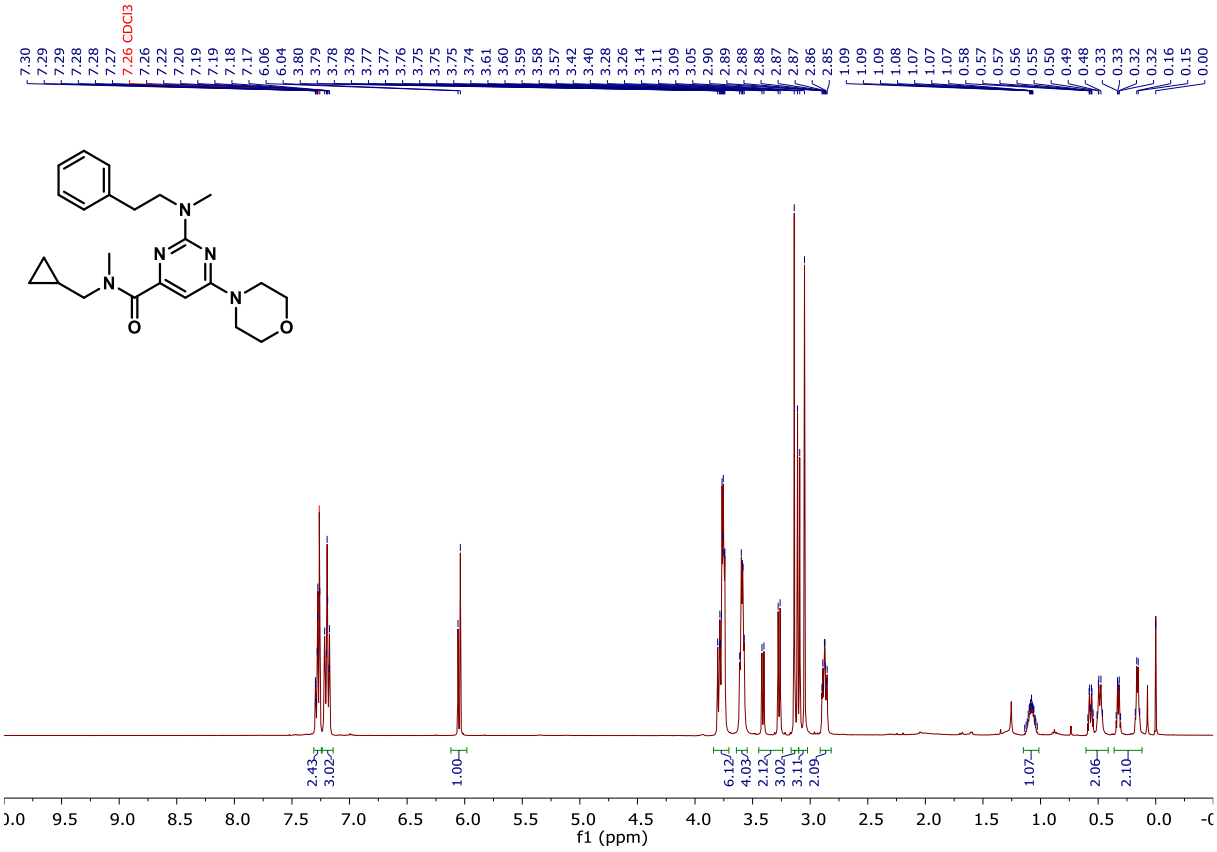


**$^{1}$H-NMR: 7** (two rotamers in a ratio of 6:4 in $CDCl_3$ at T = 298 K)

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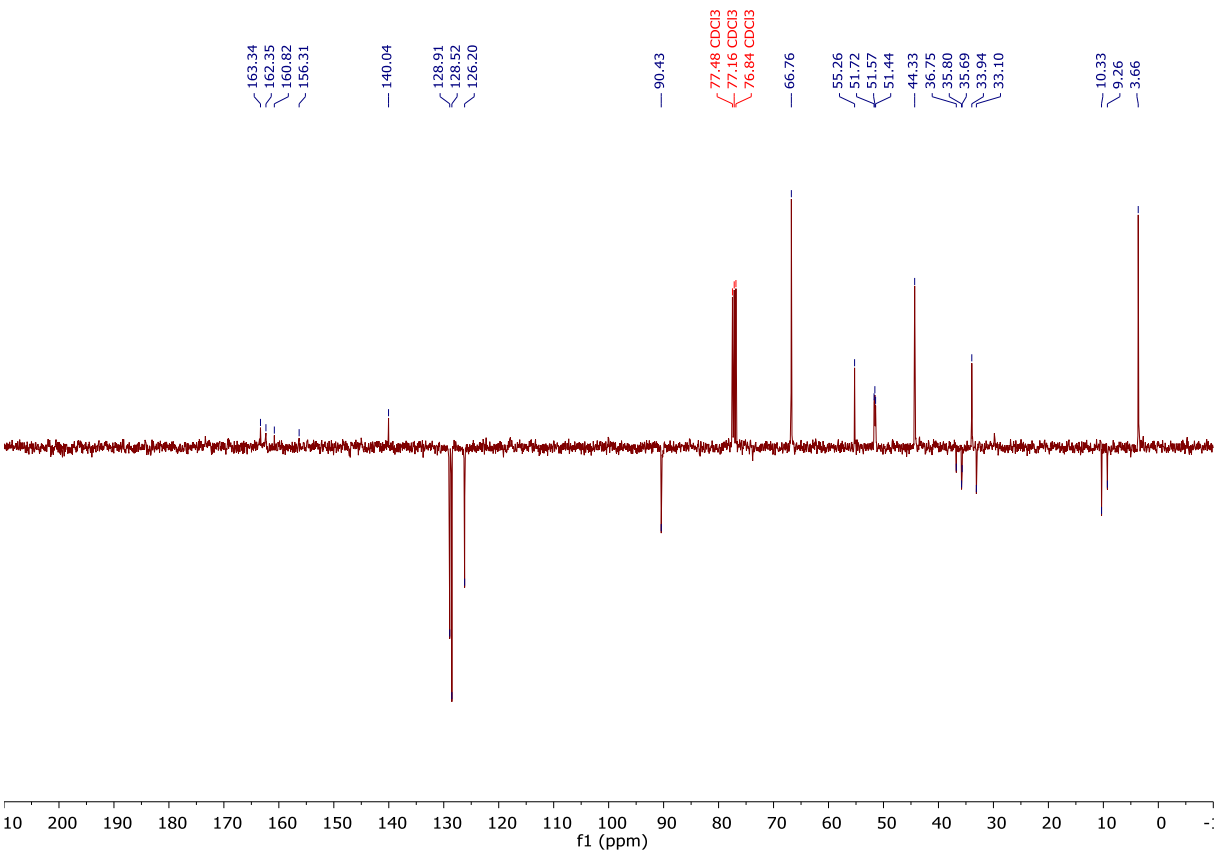

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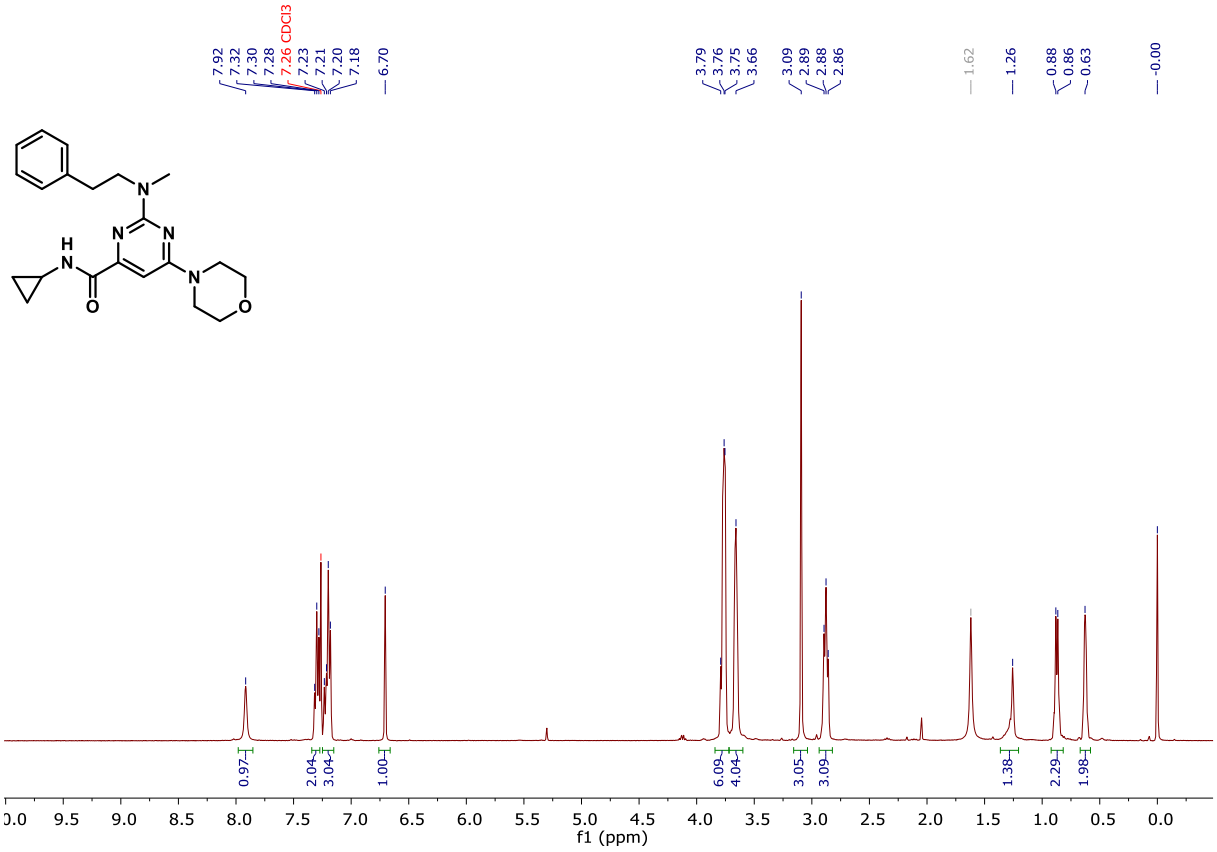


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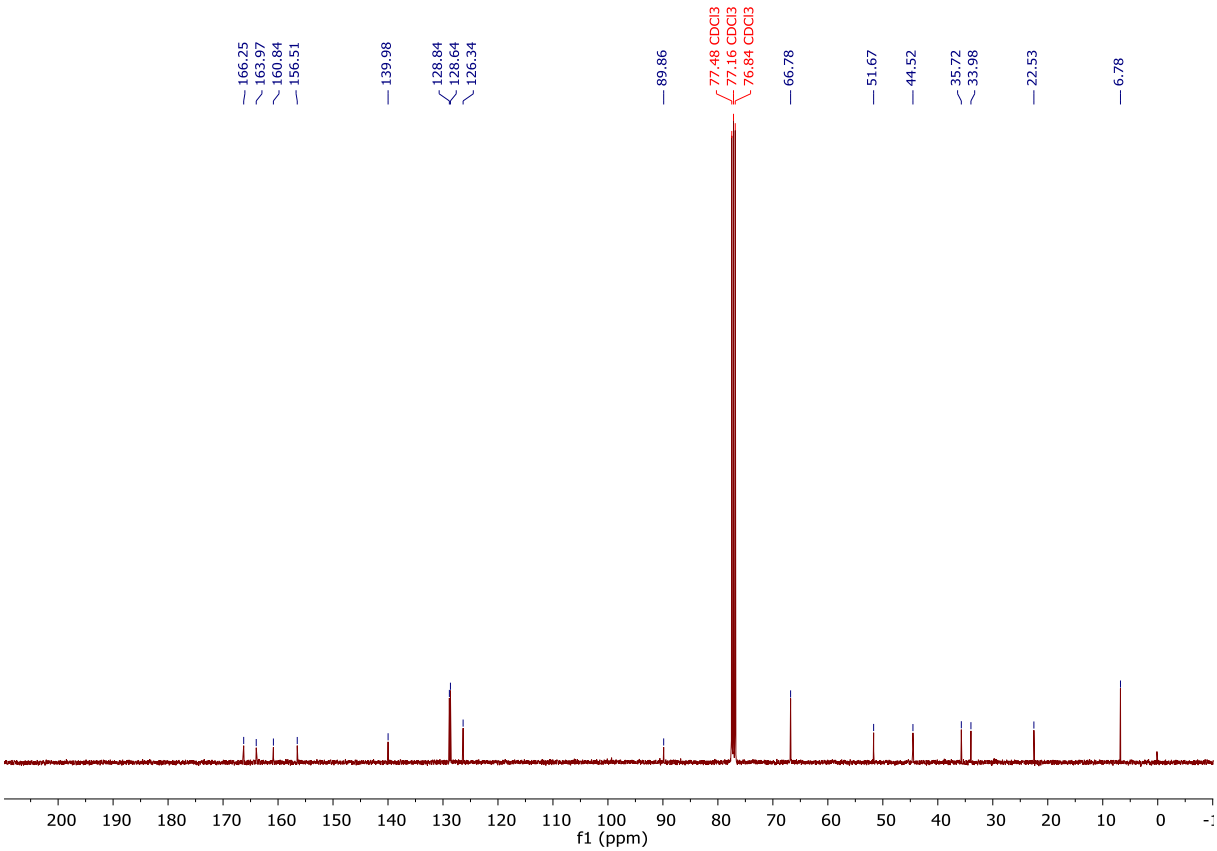

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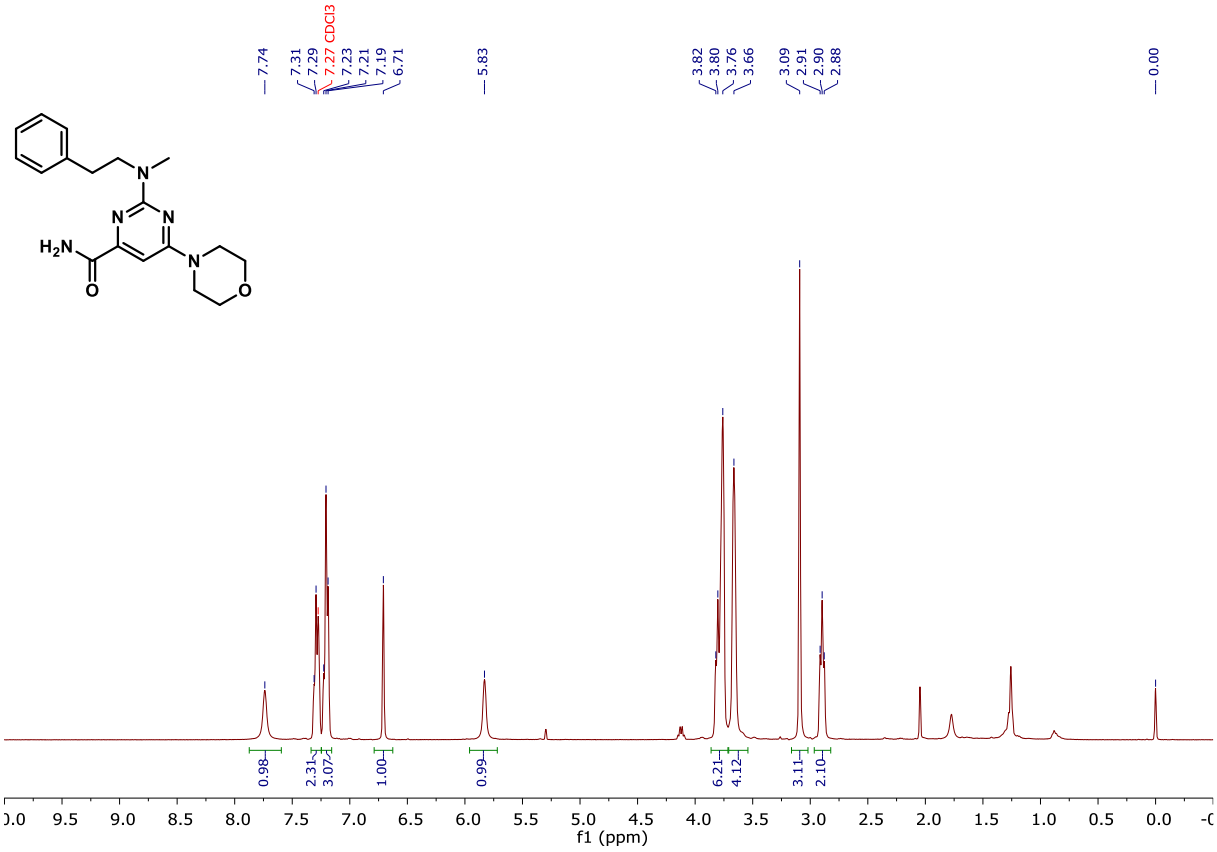


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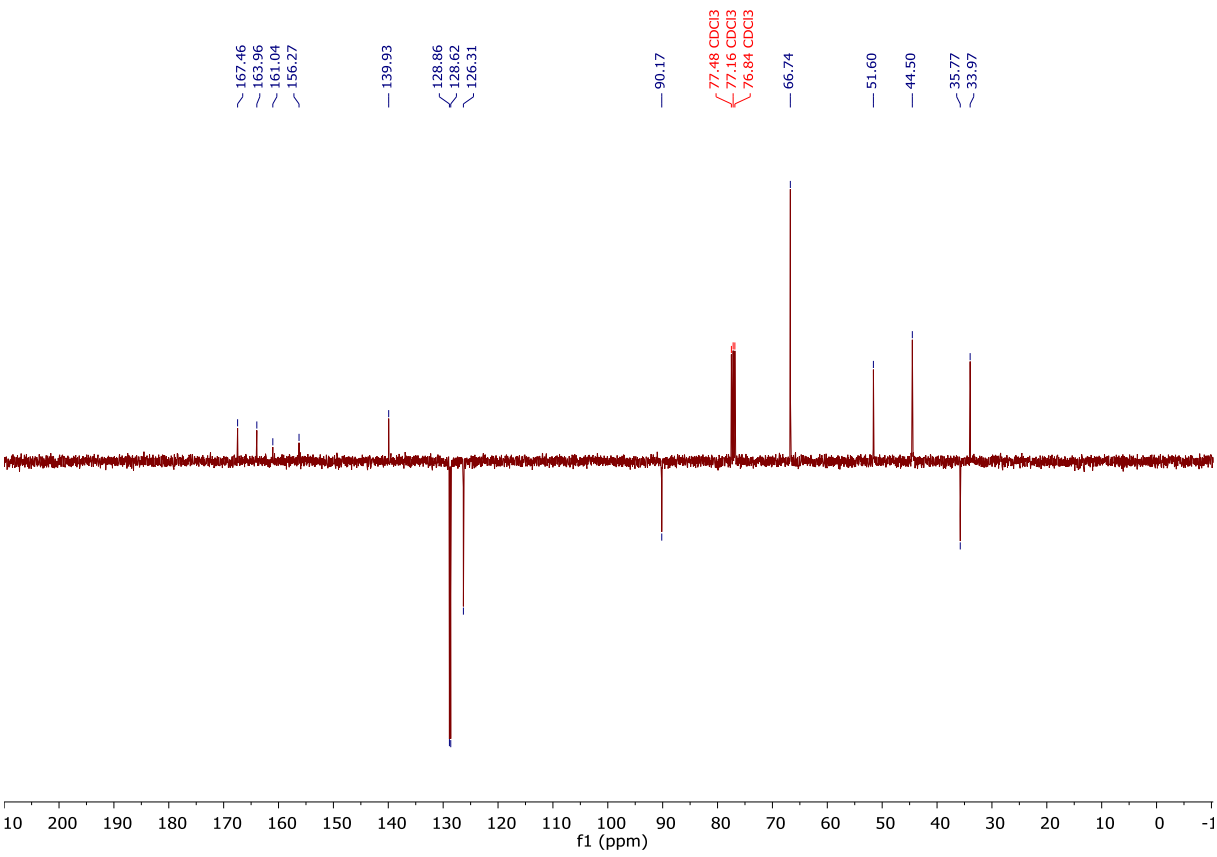

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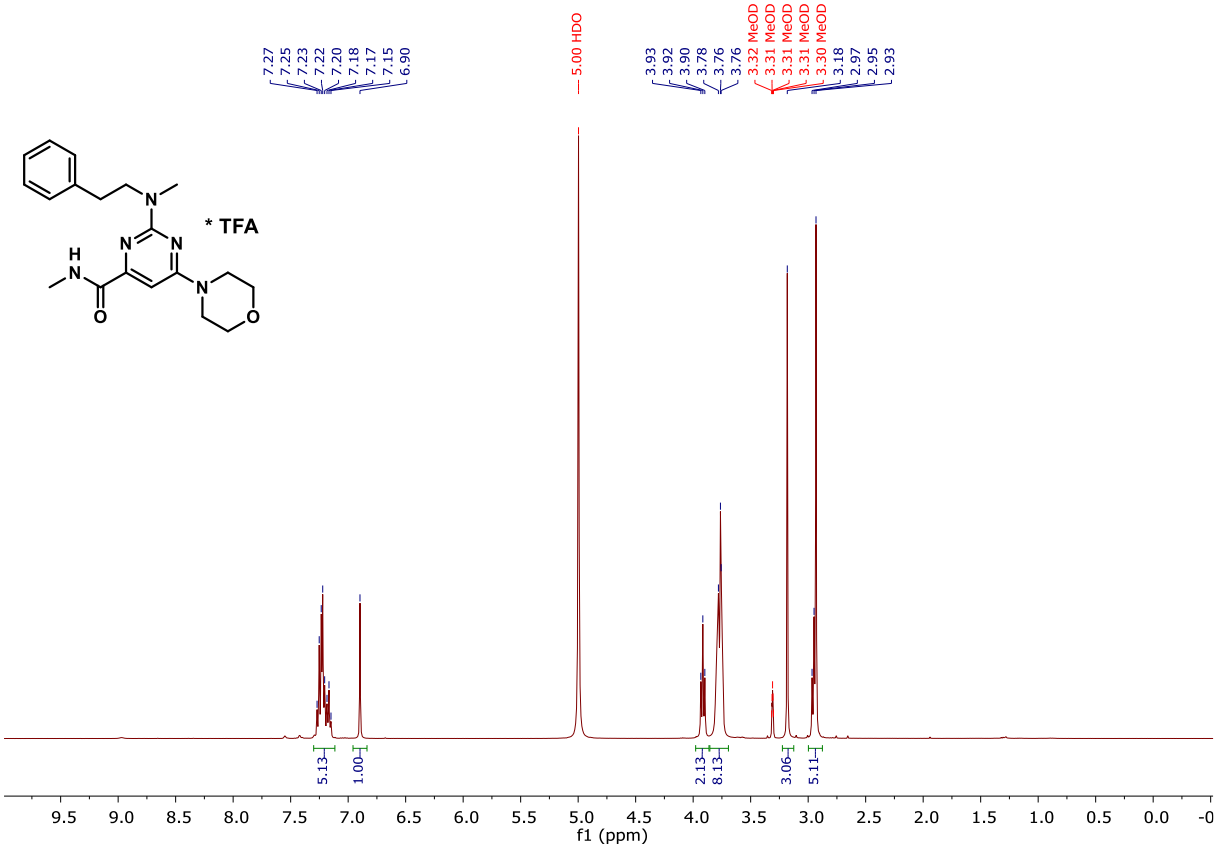


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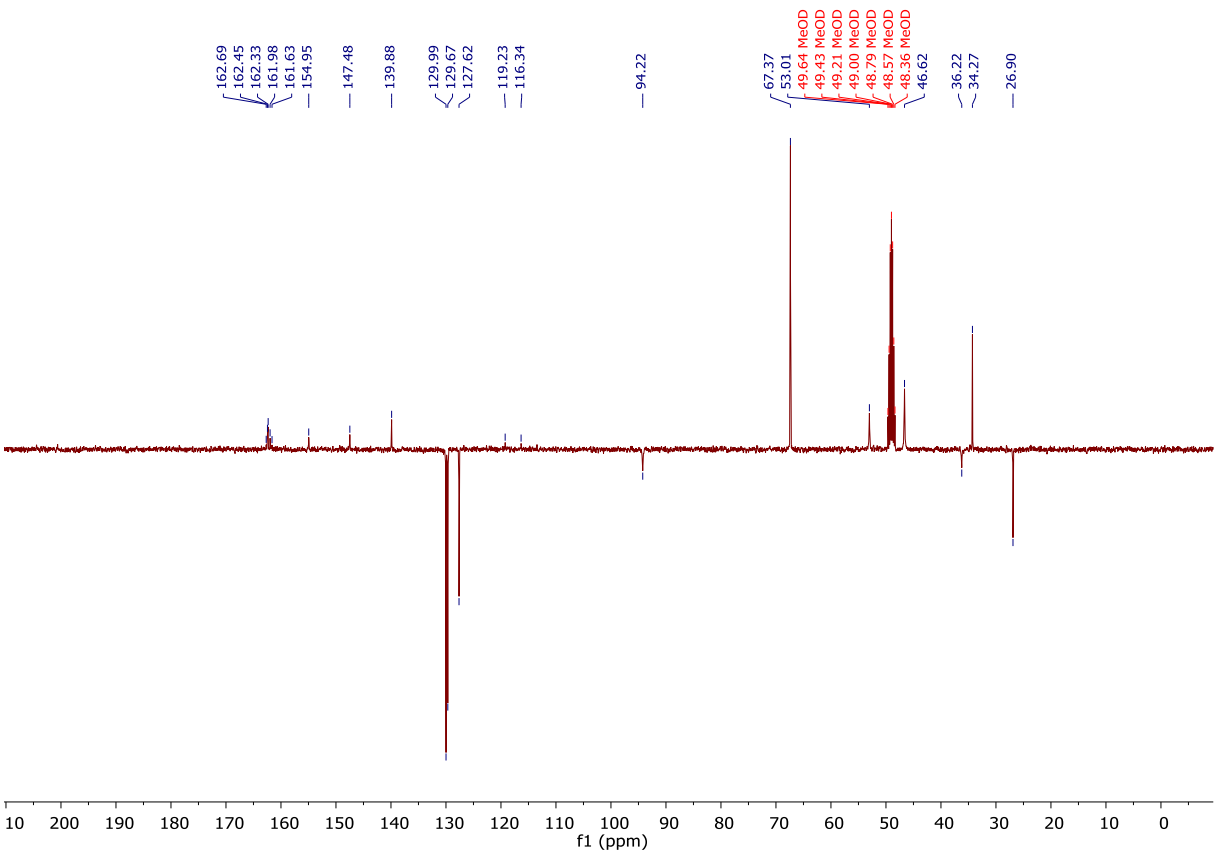

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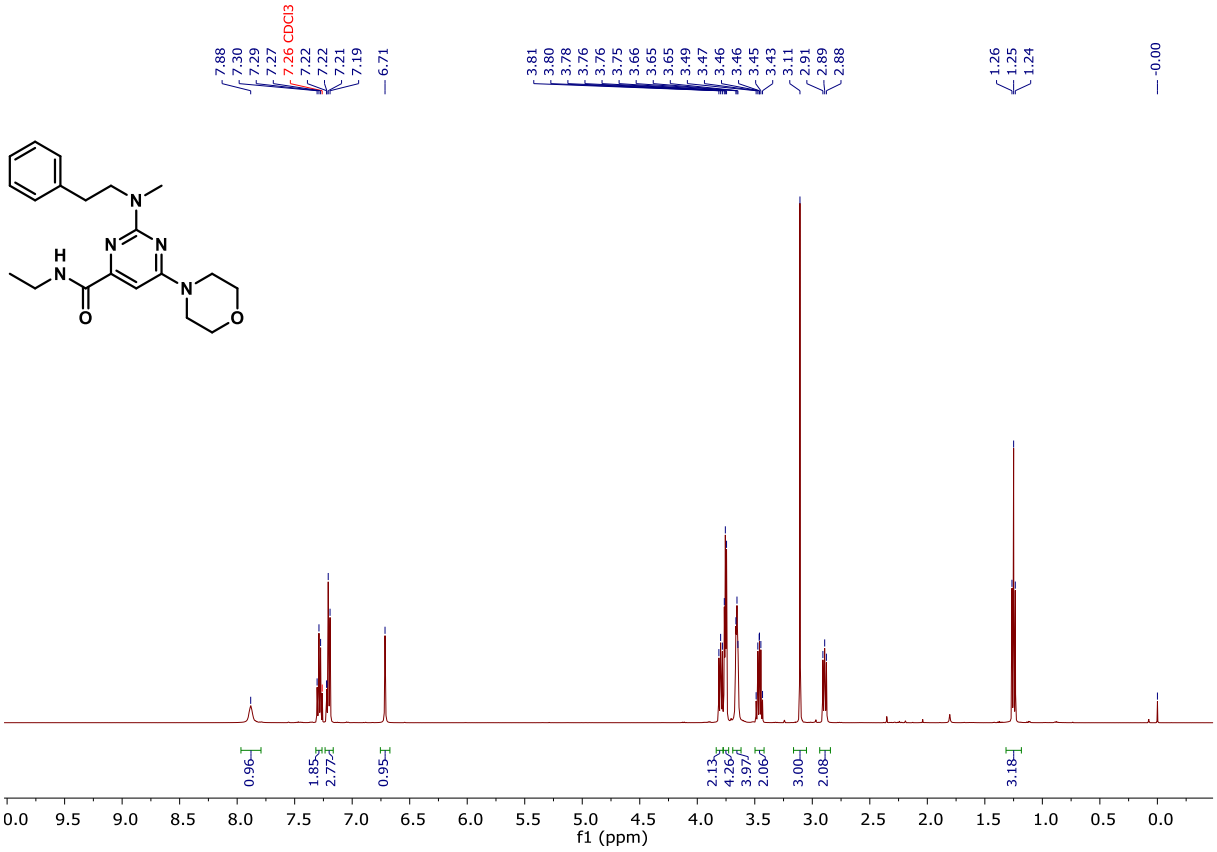


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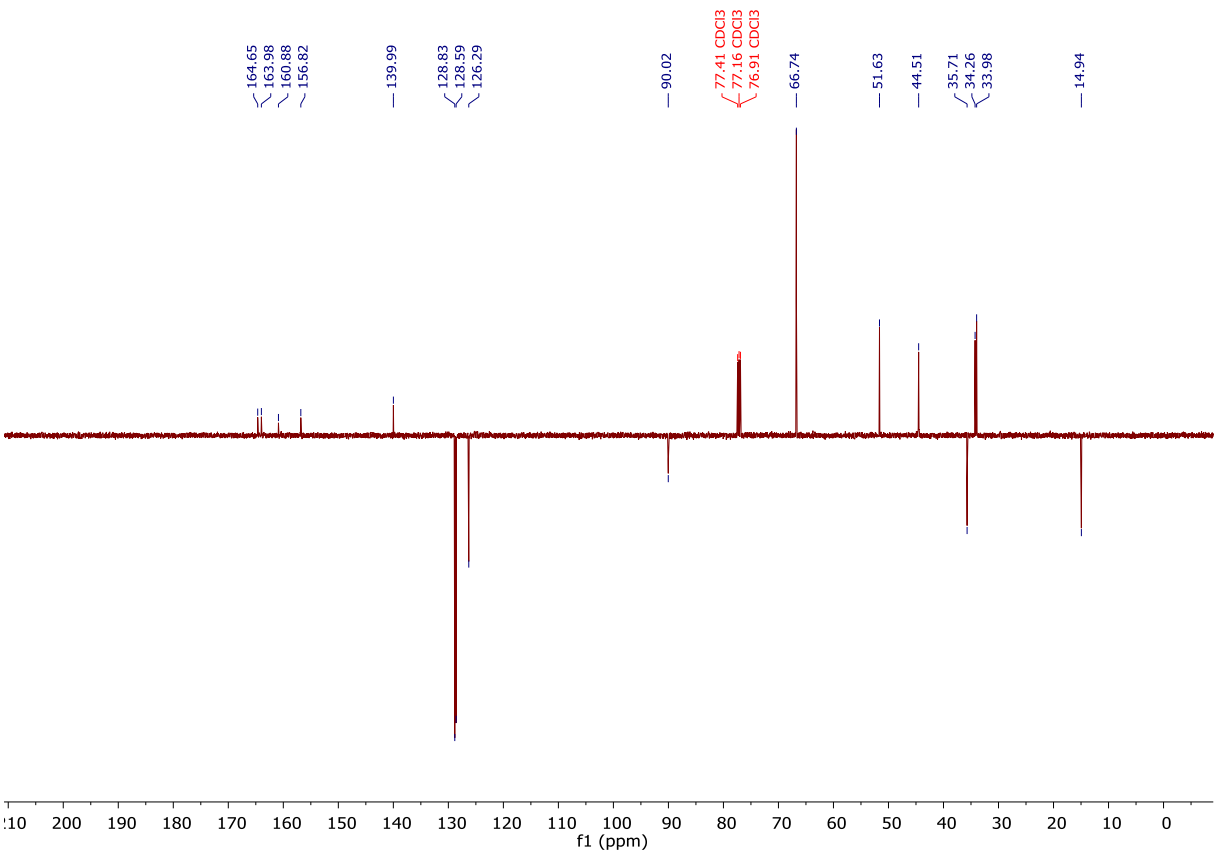

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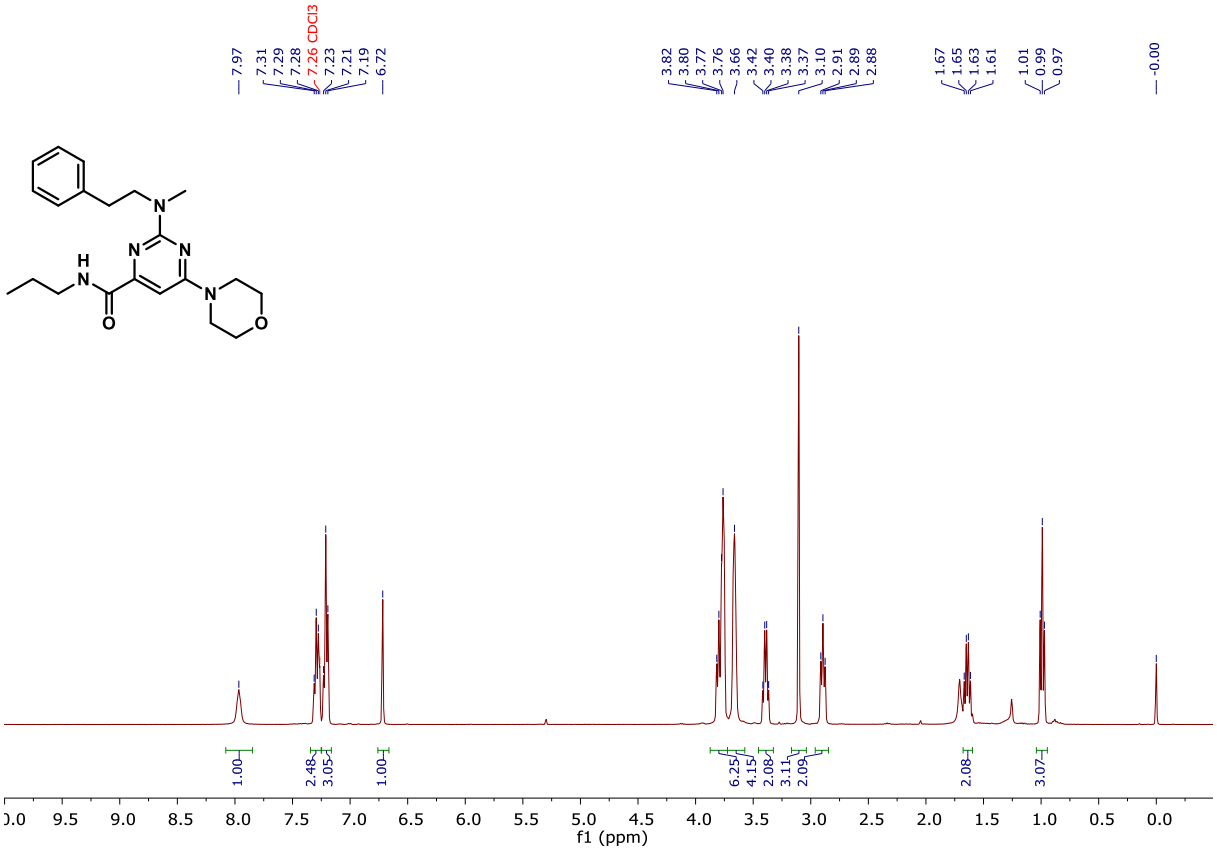


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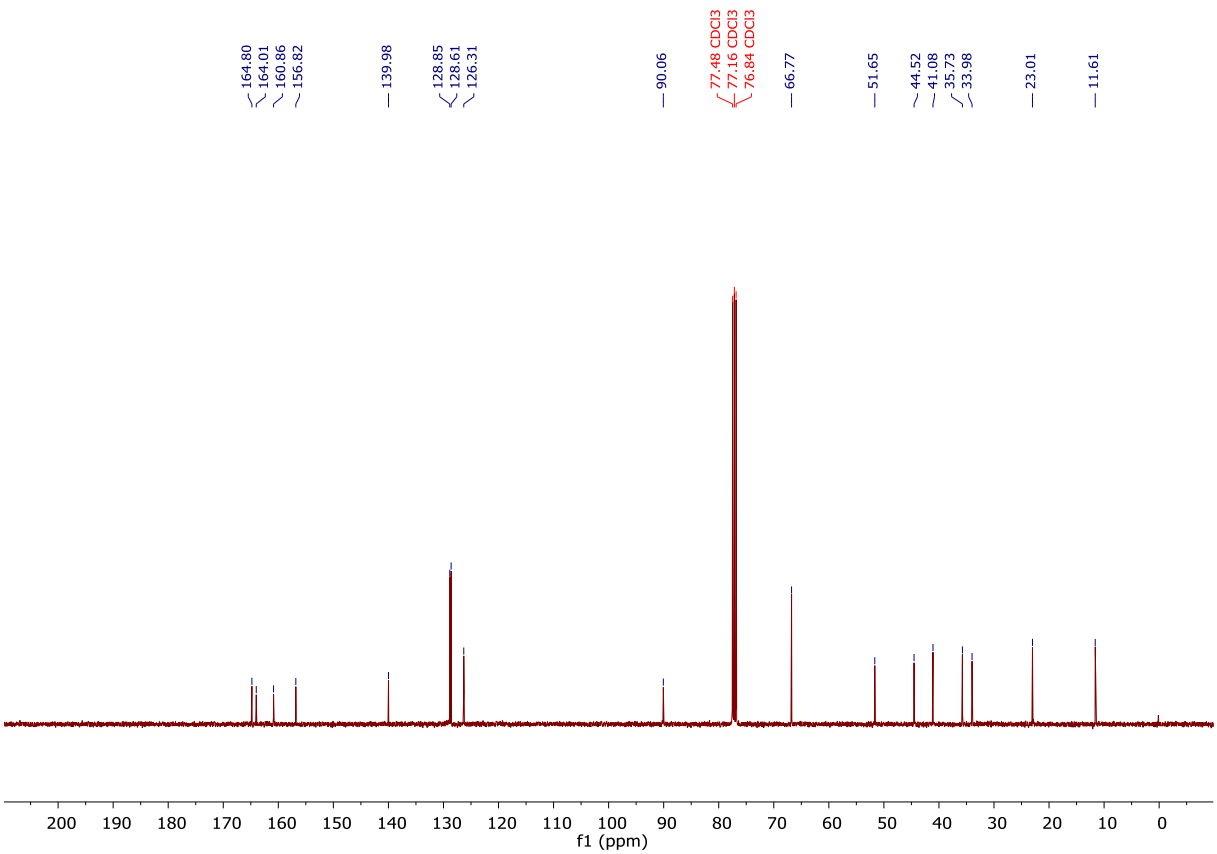

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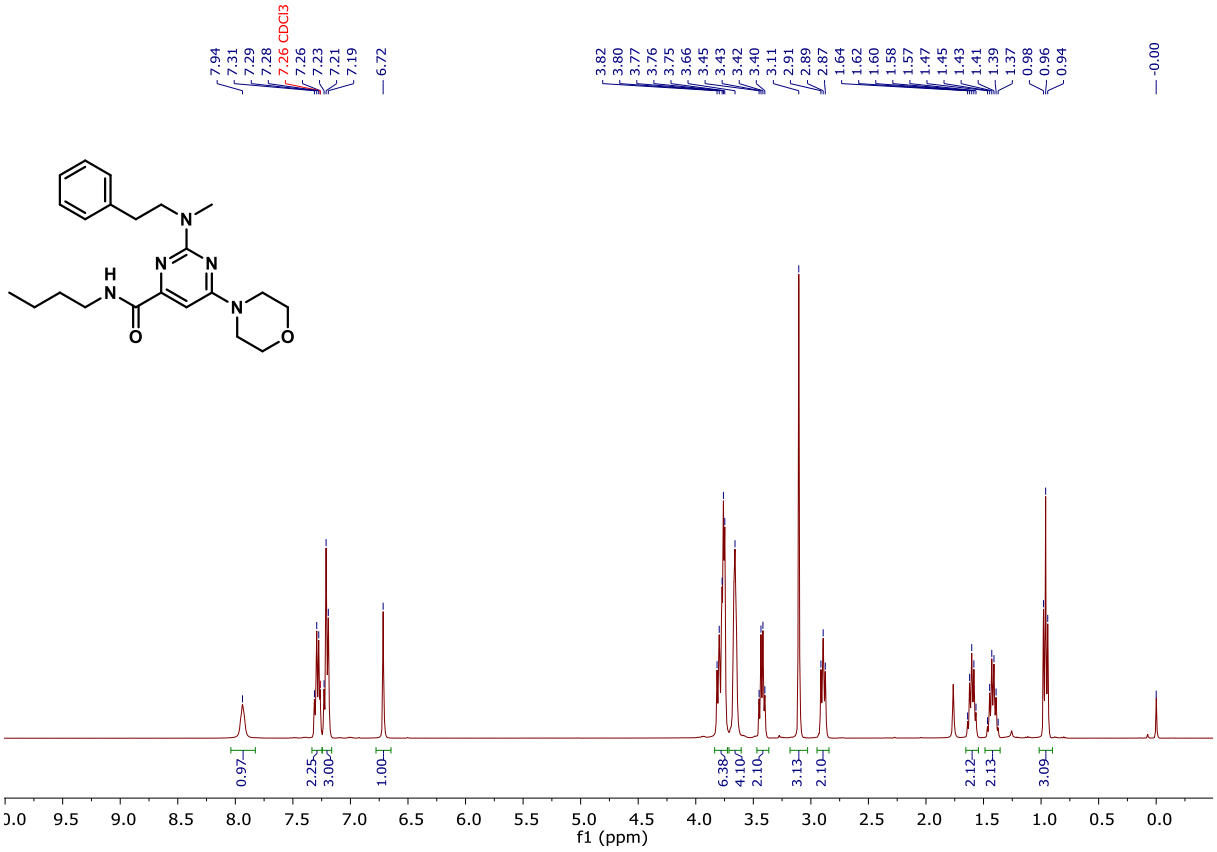


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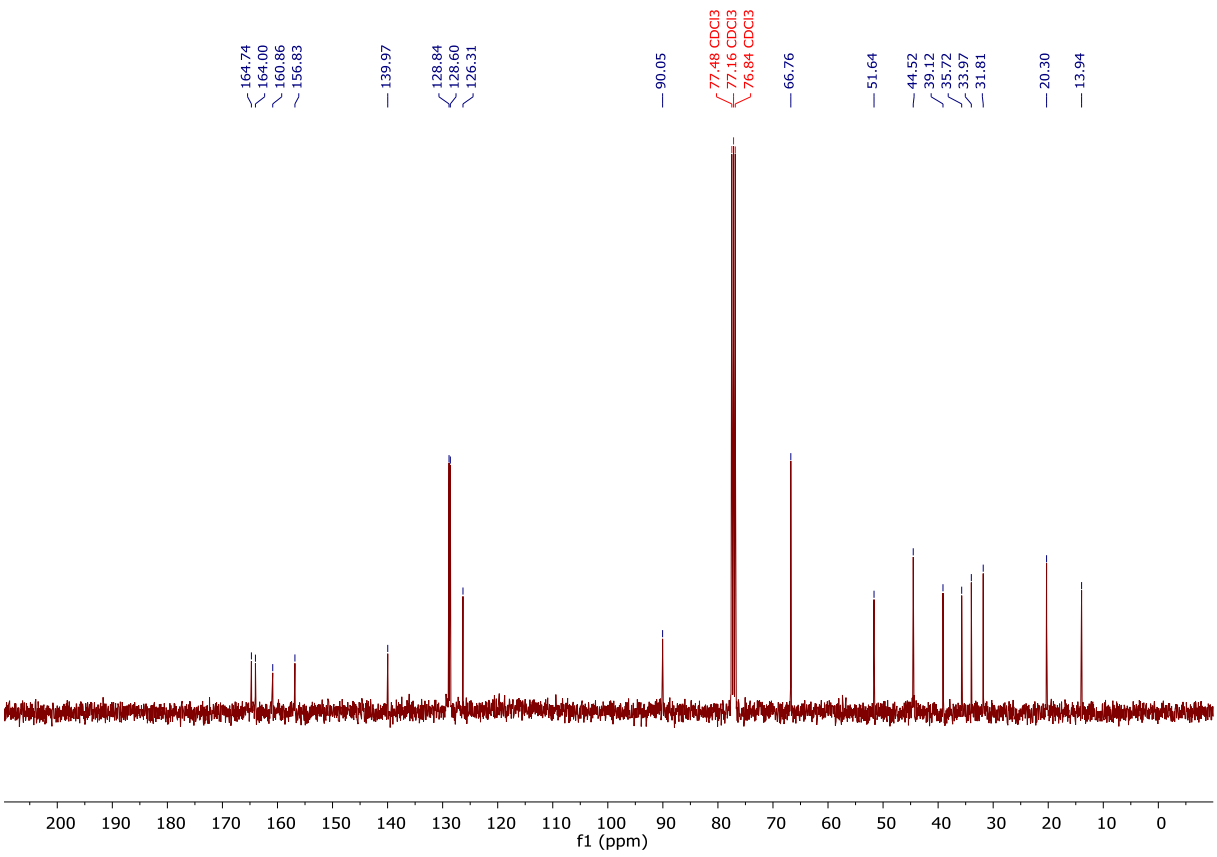

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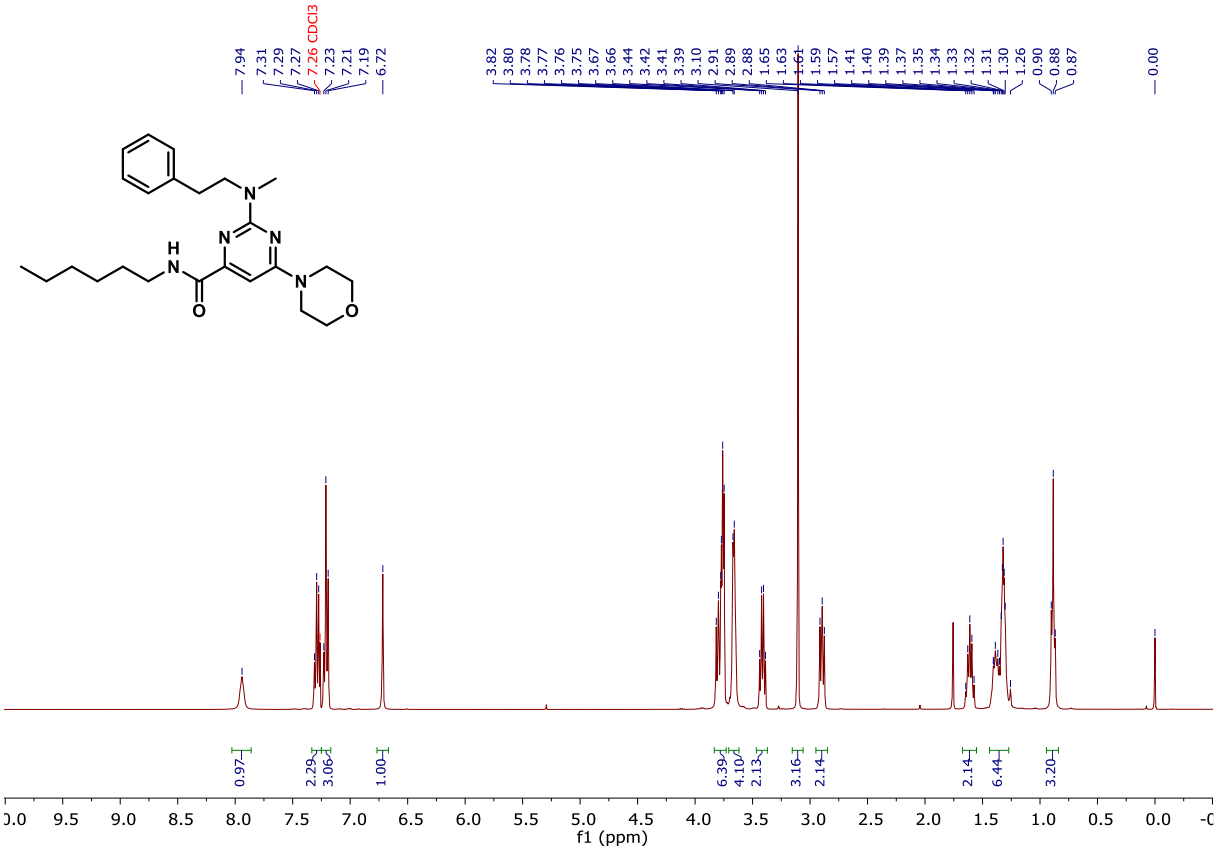


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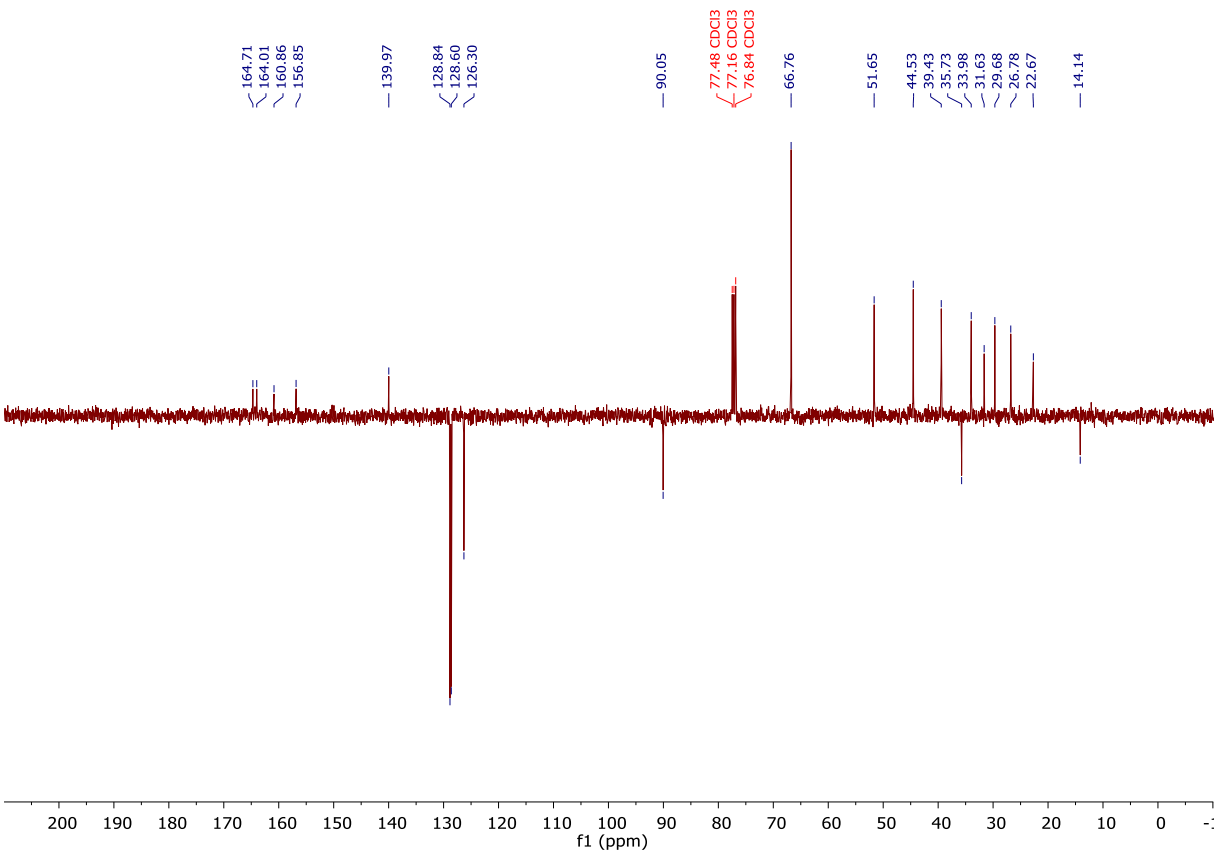

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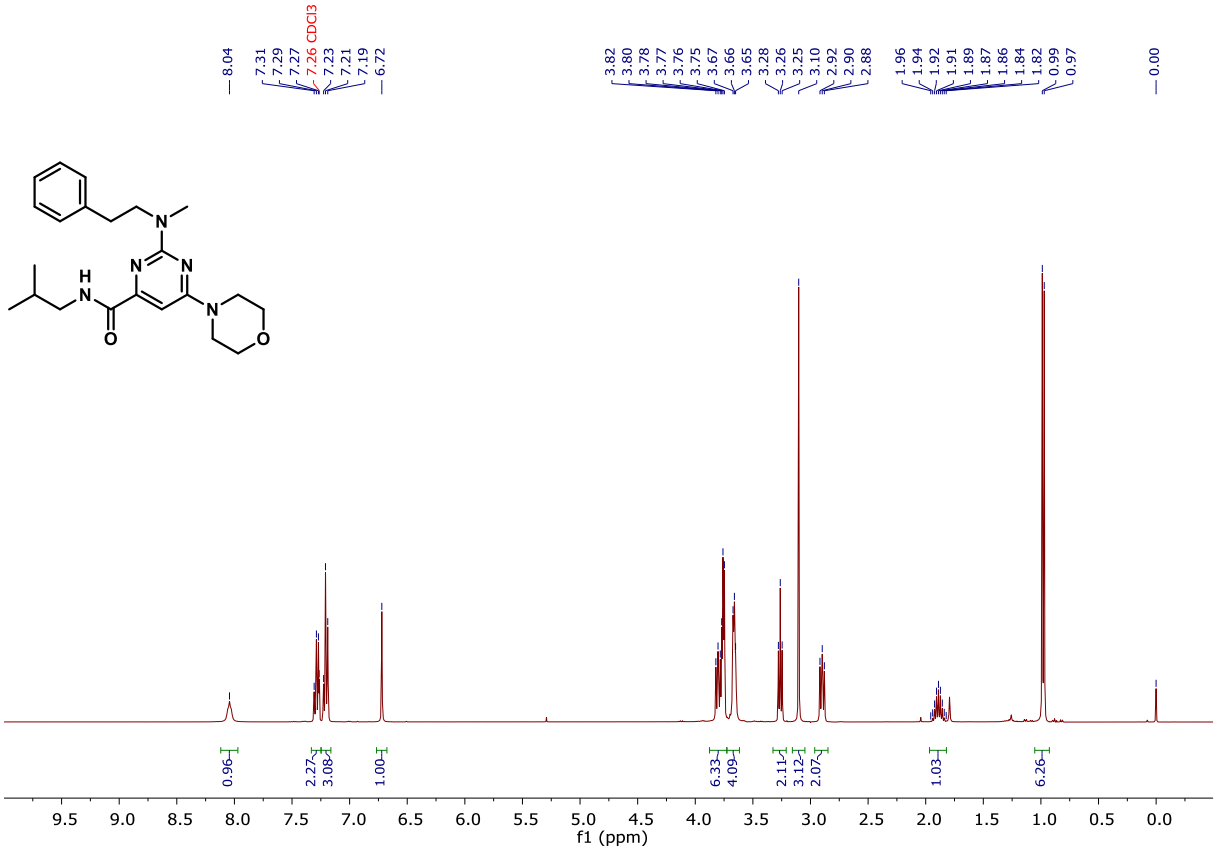


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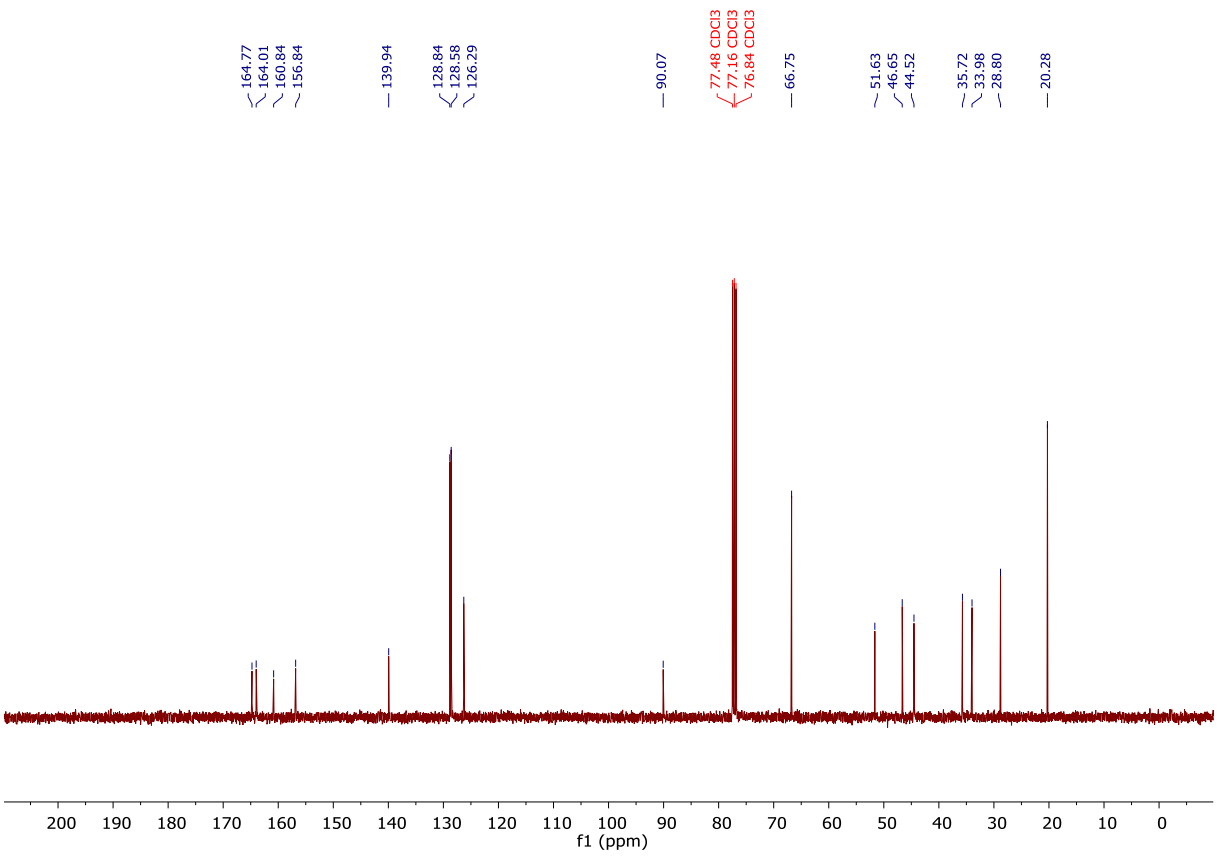

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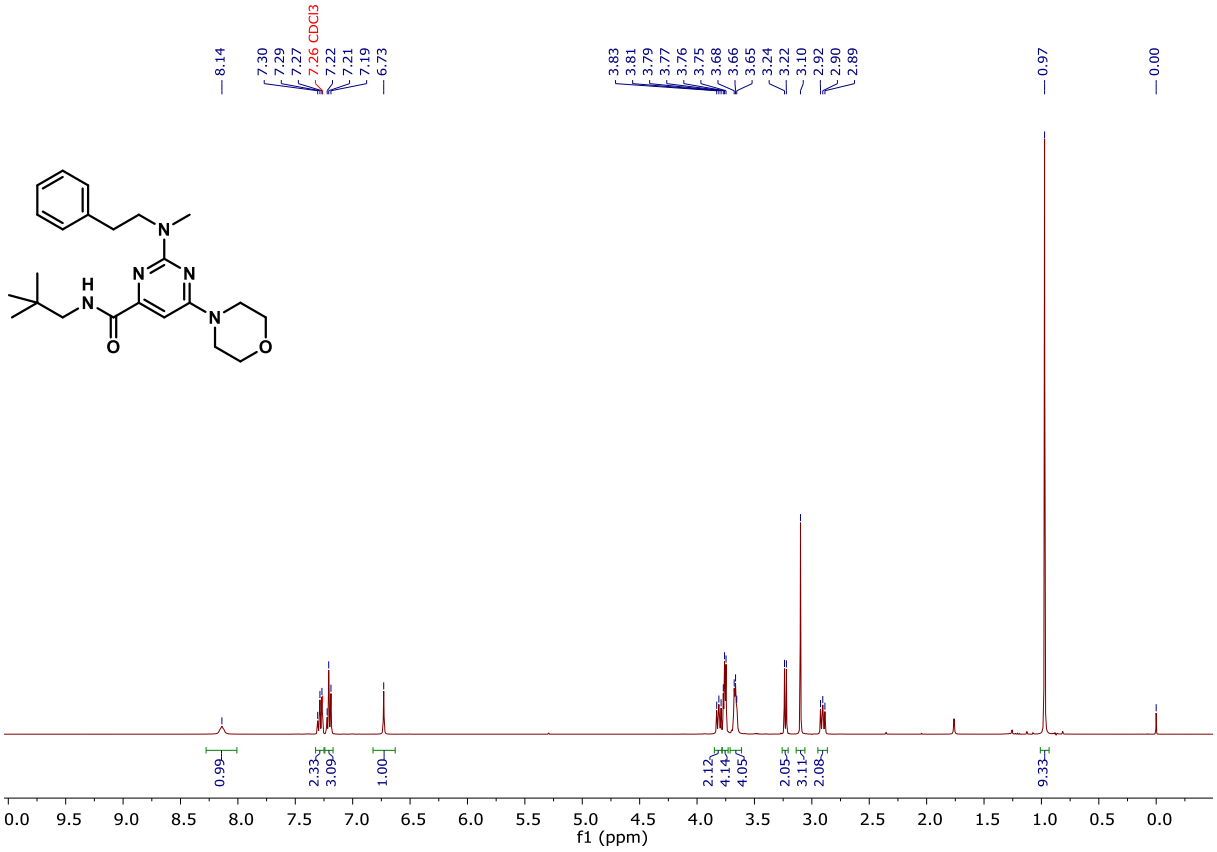


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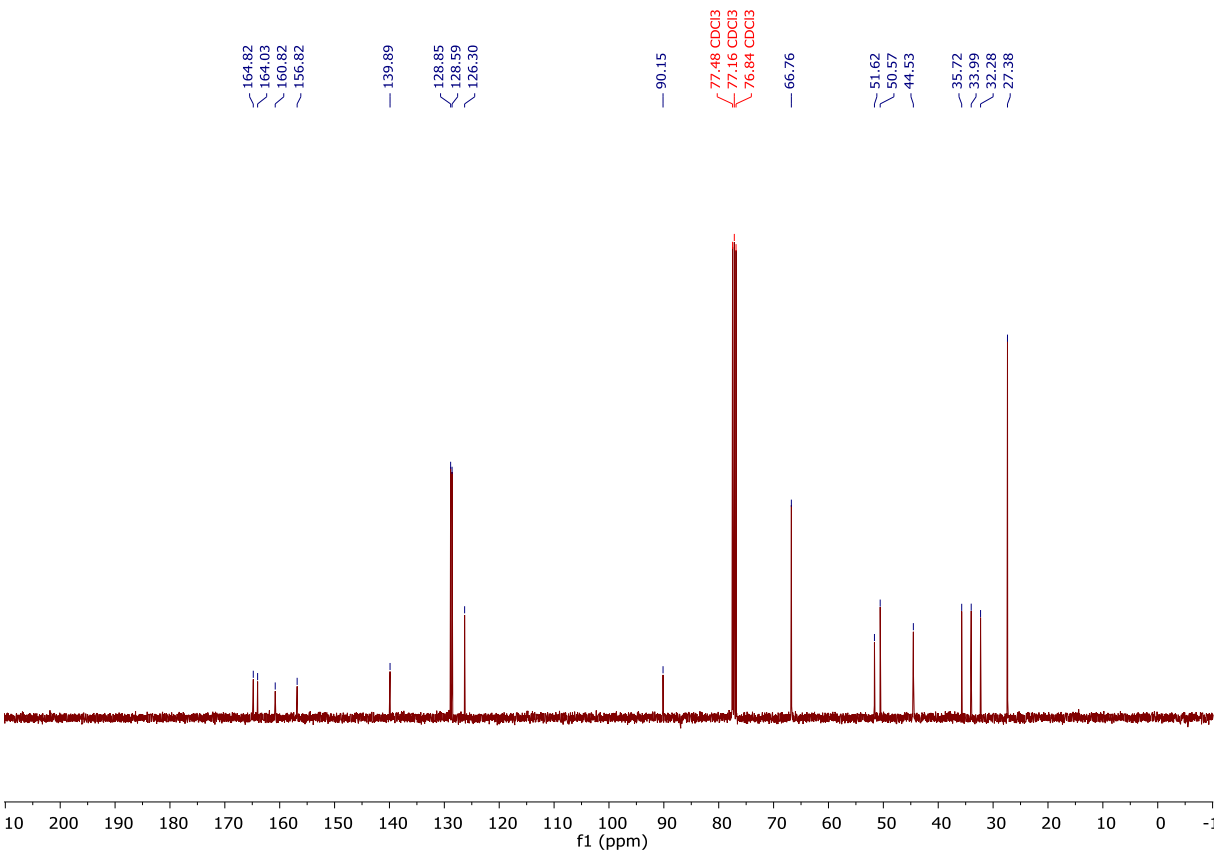

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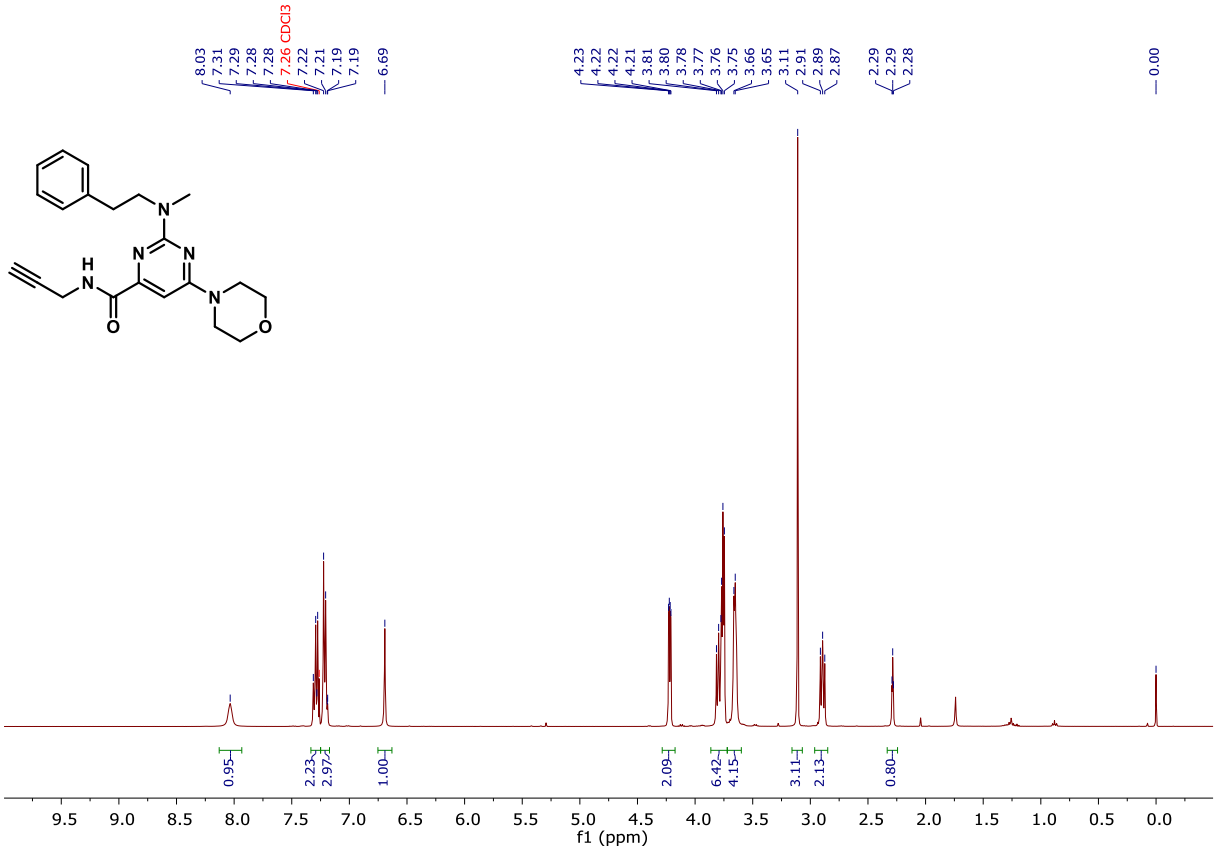
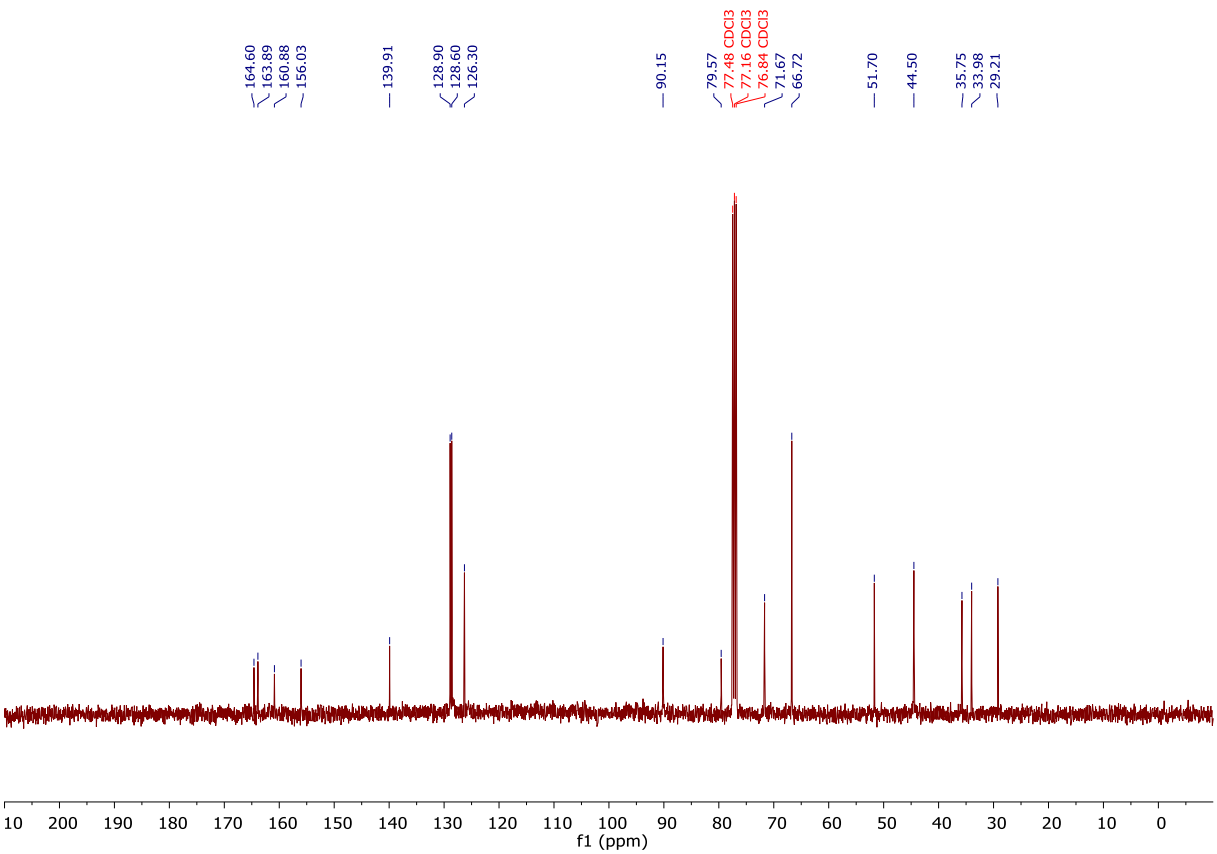
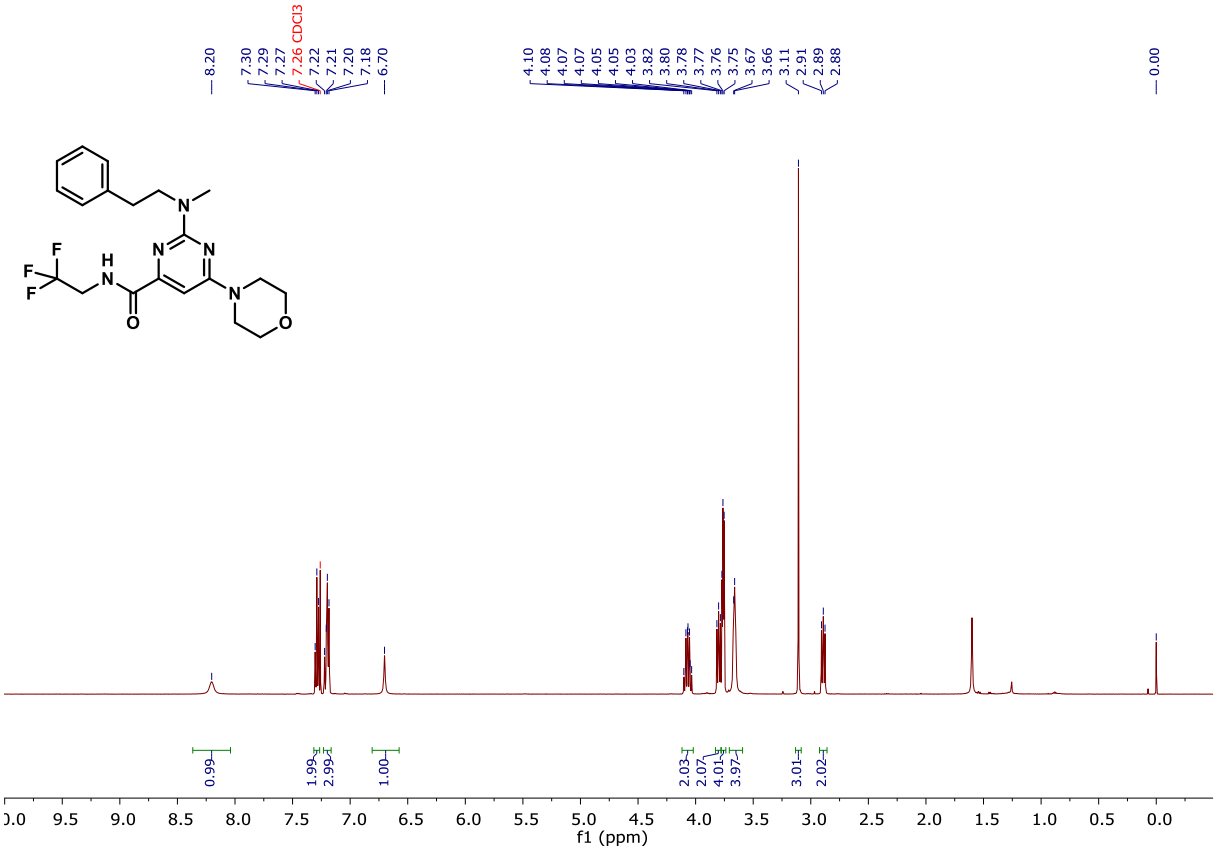


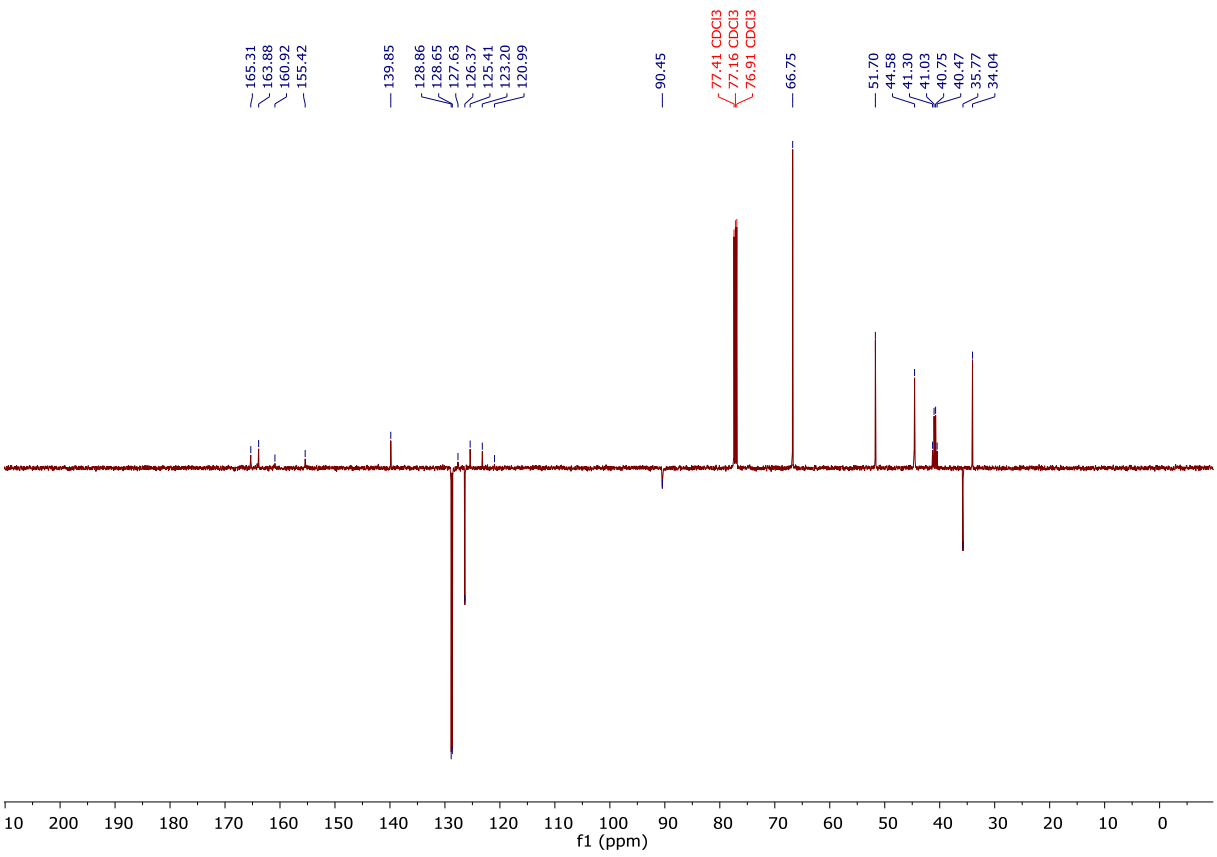

**$^{13}$C-NMR**



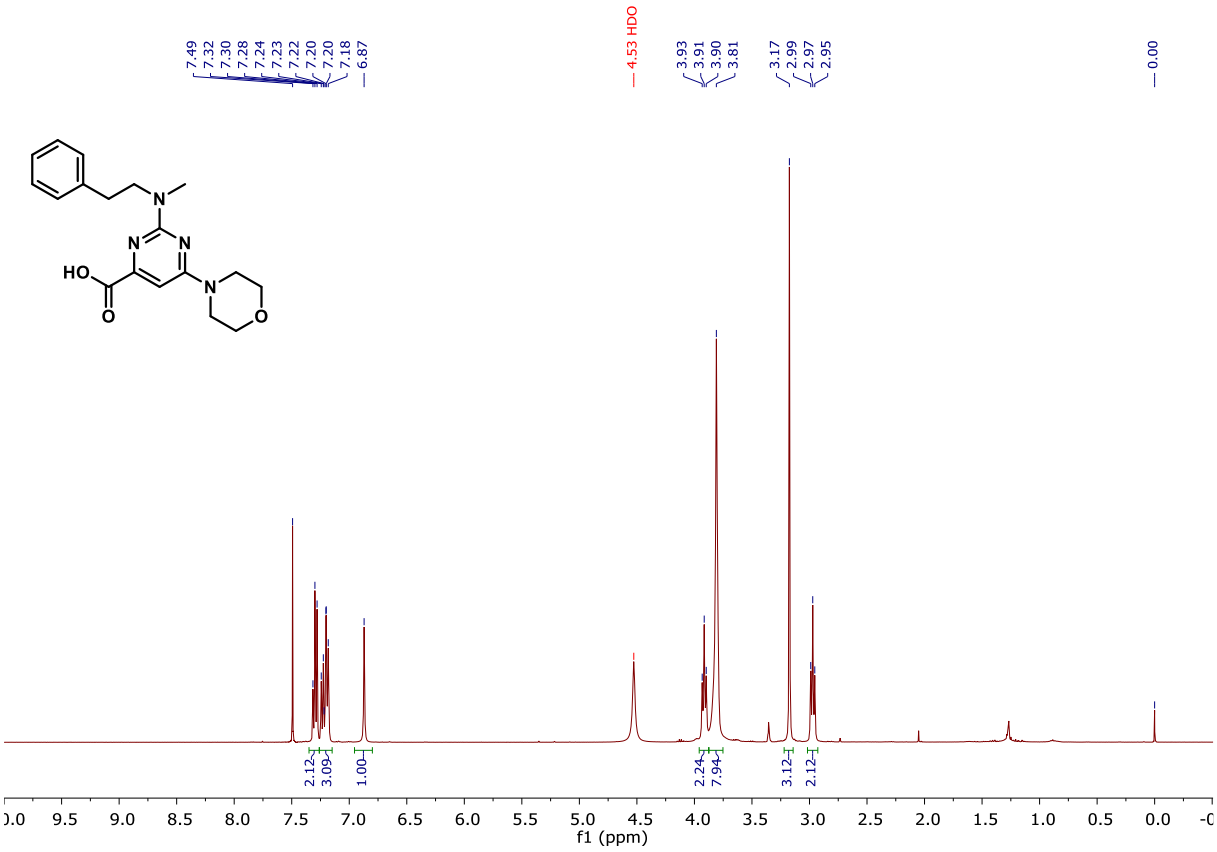
**$^{1}$H-NMR: 18**



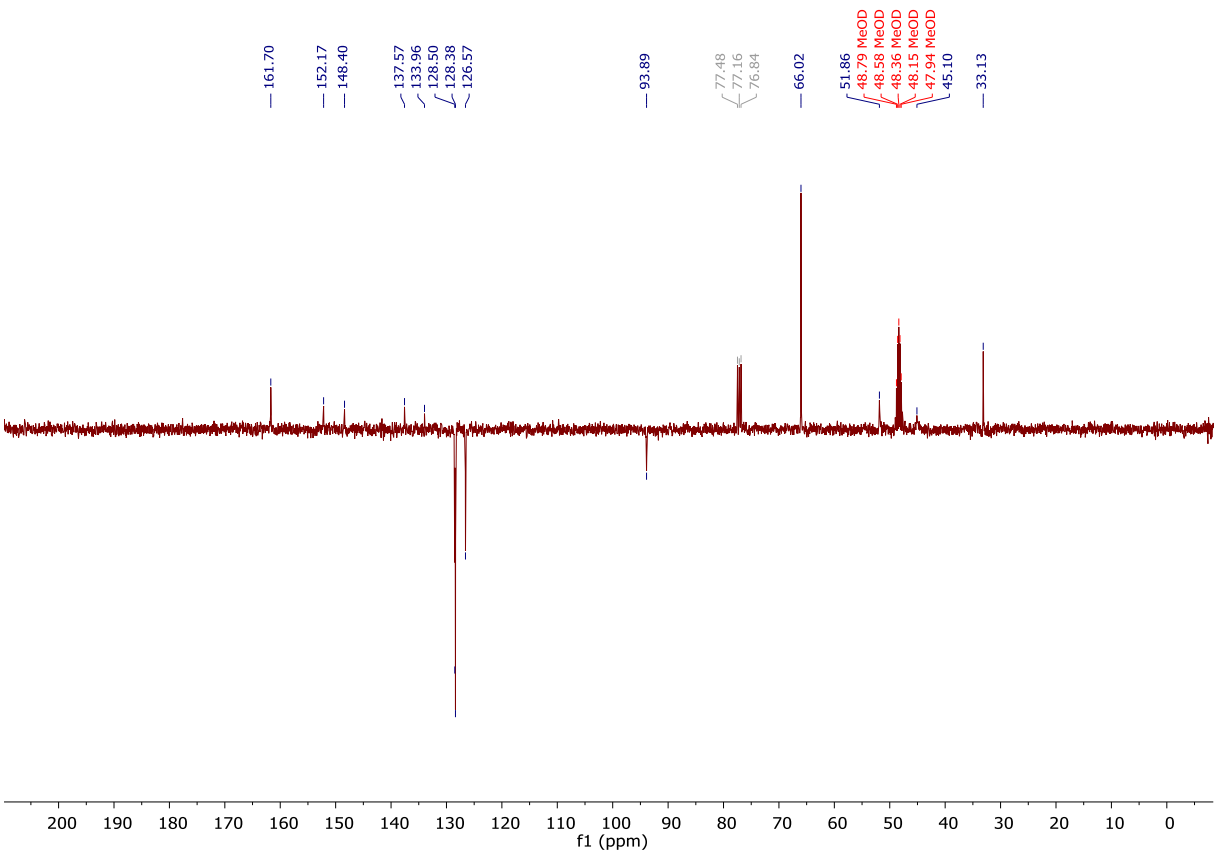

**$^{13}$C-NMR**



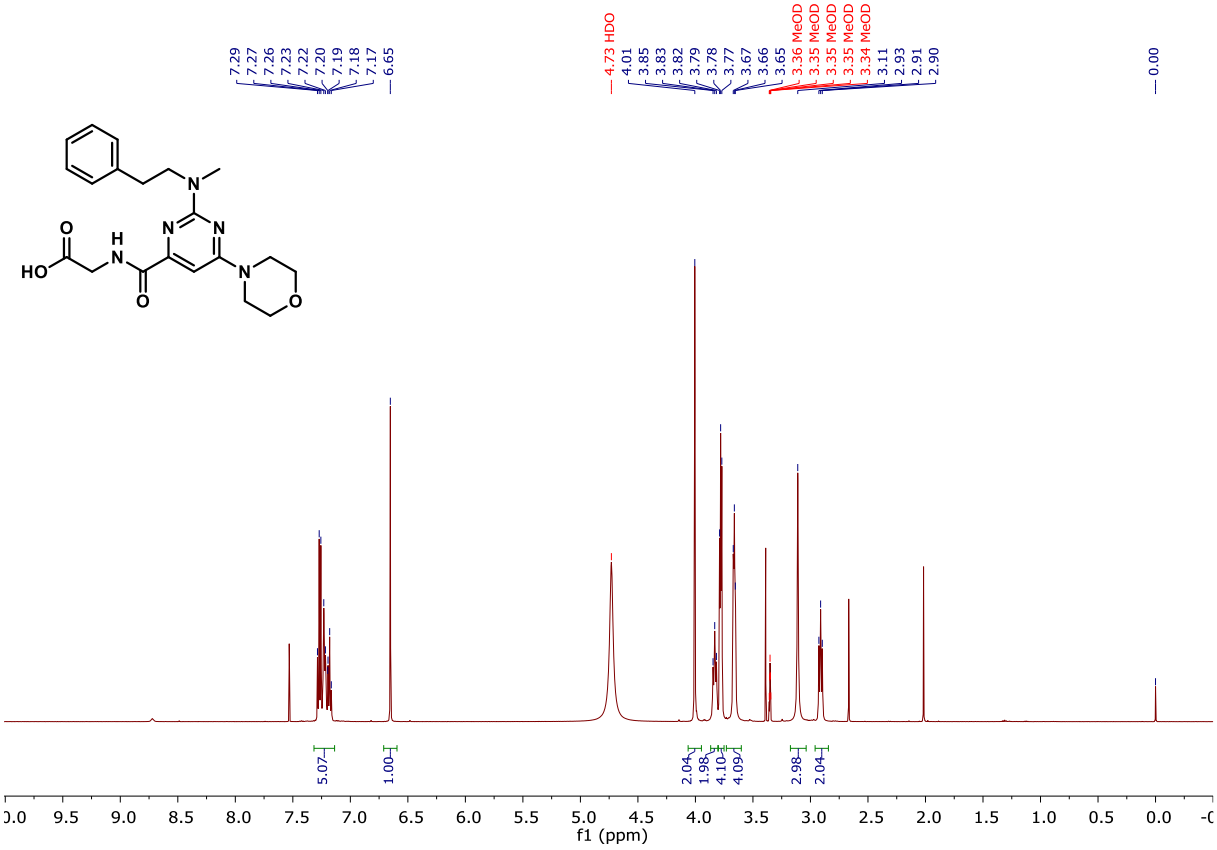
**$^{1}$H-NMR: 19 ($CDCl_3$ + MeOD)**



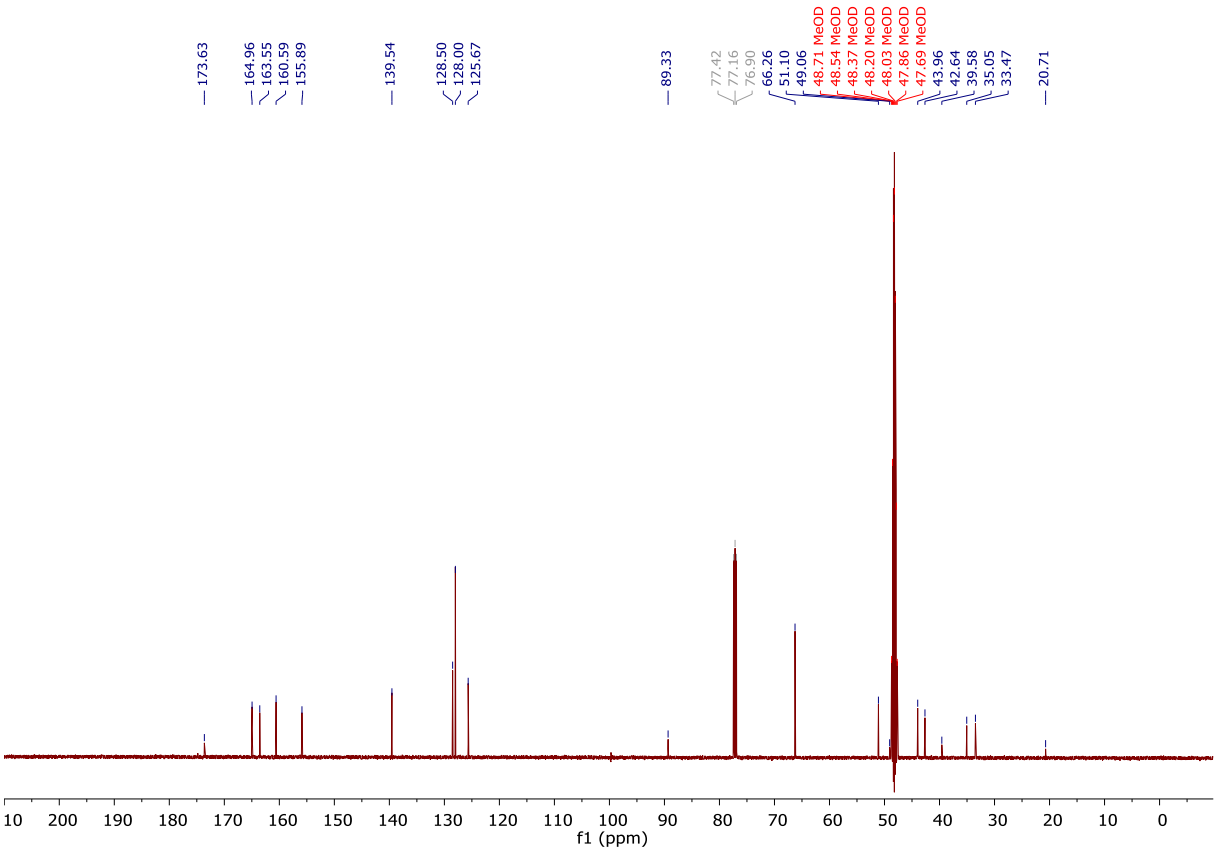

**$^{13}$C-NMR**



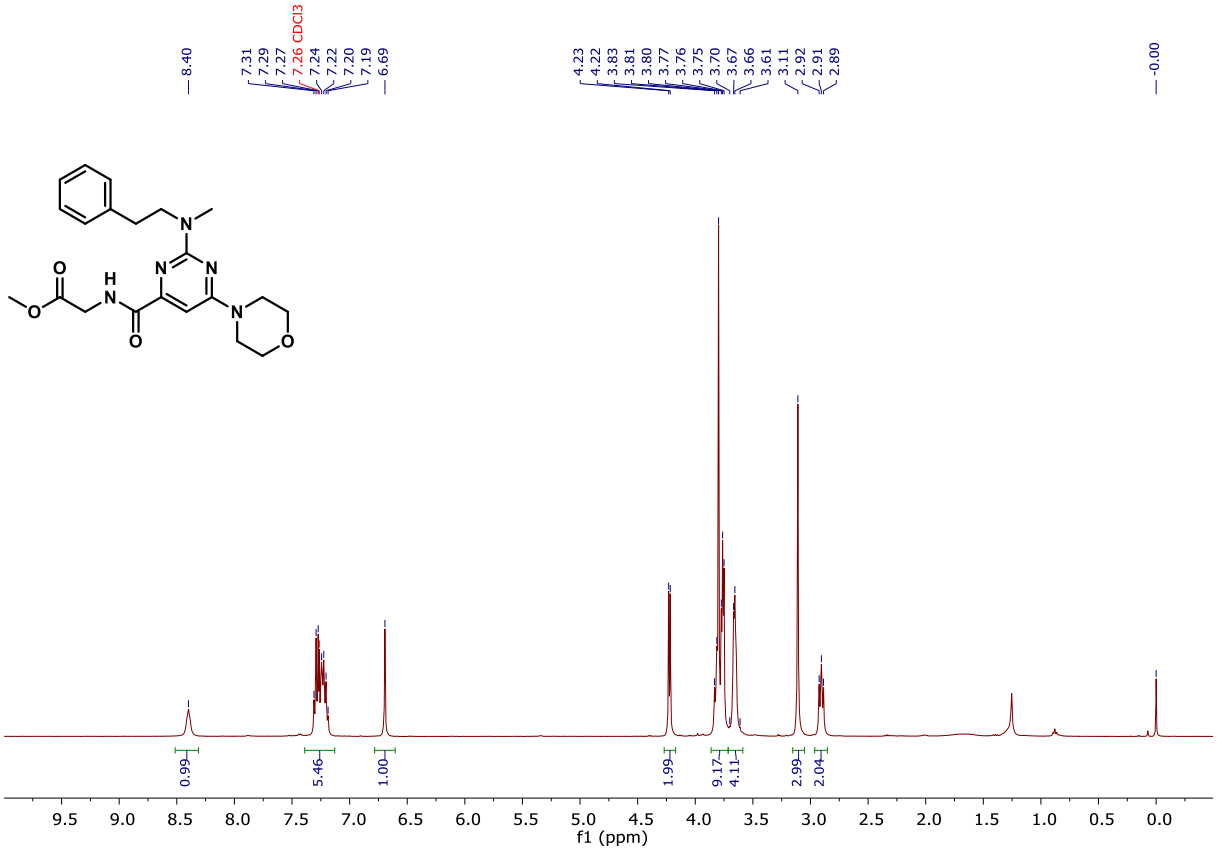
**$^{1}$H-NMR: 20 ($CDCl_3$ + MeOD)**



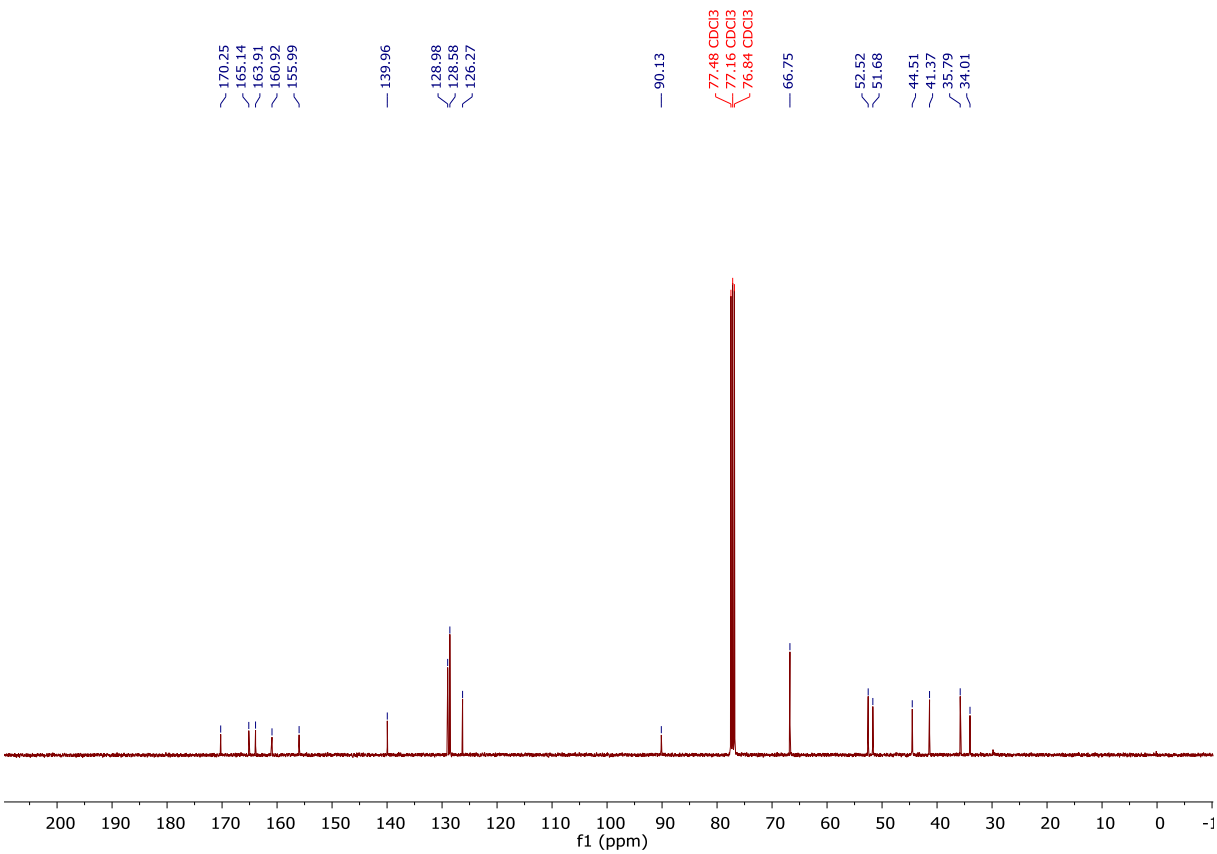

**$^{13}$C-NMR**



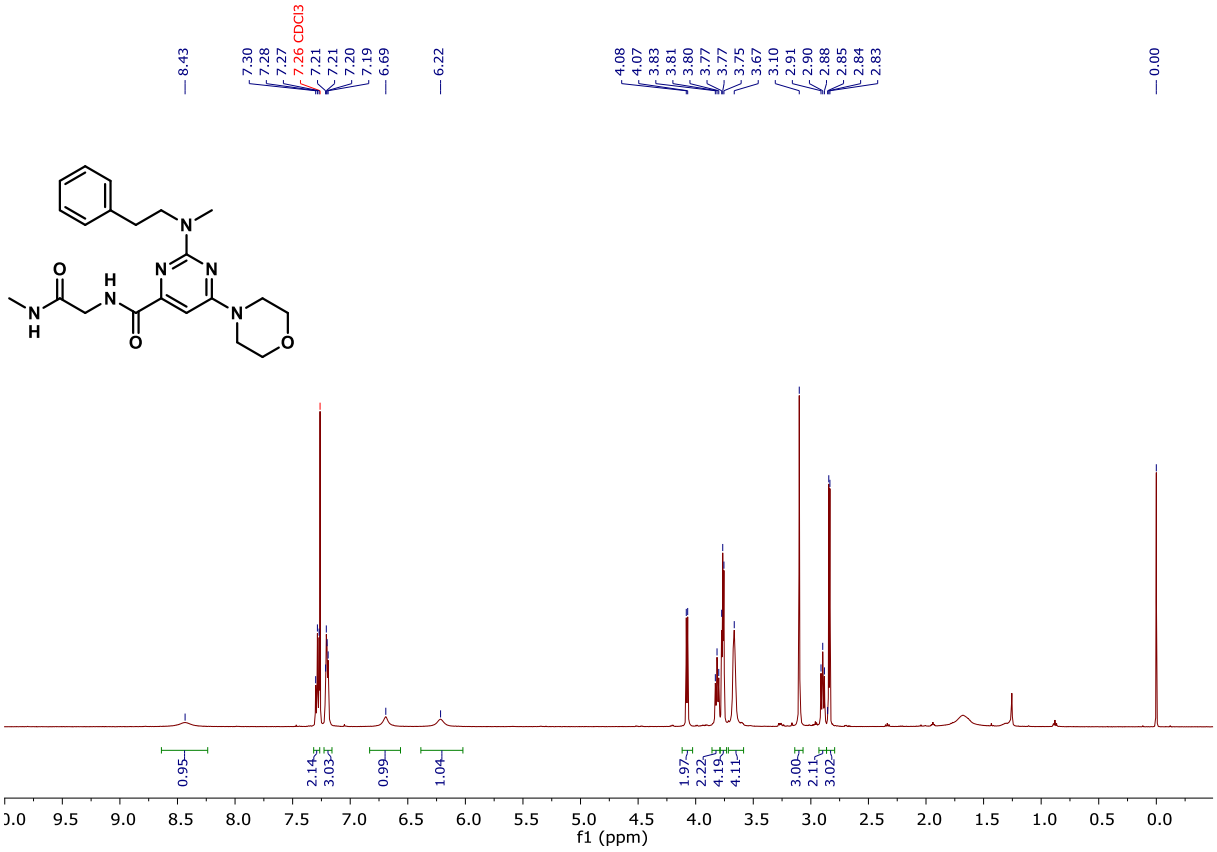
**$^{1}$H-NMR: 21**



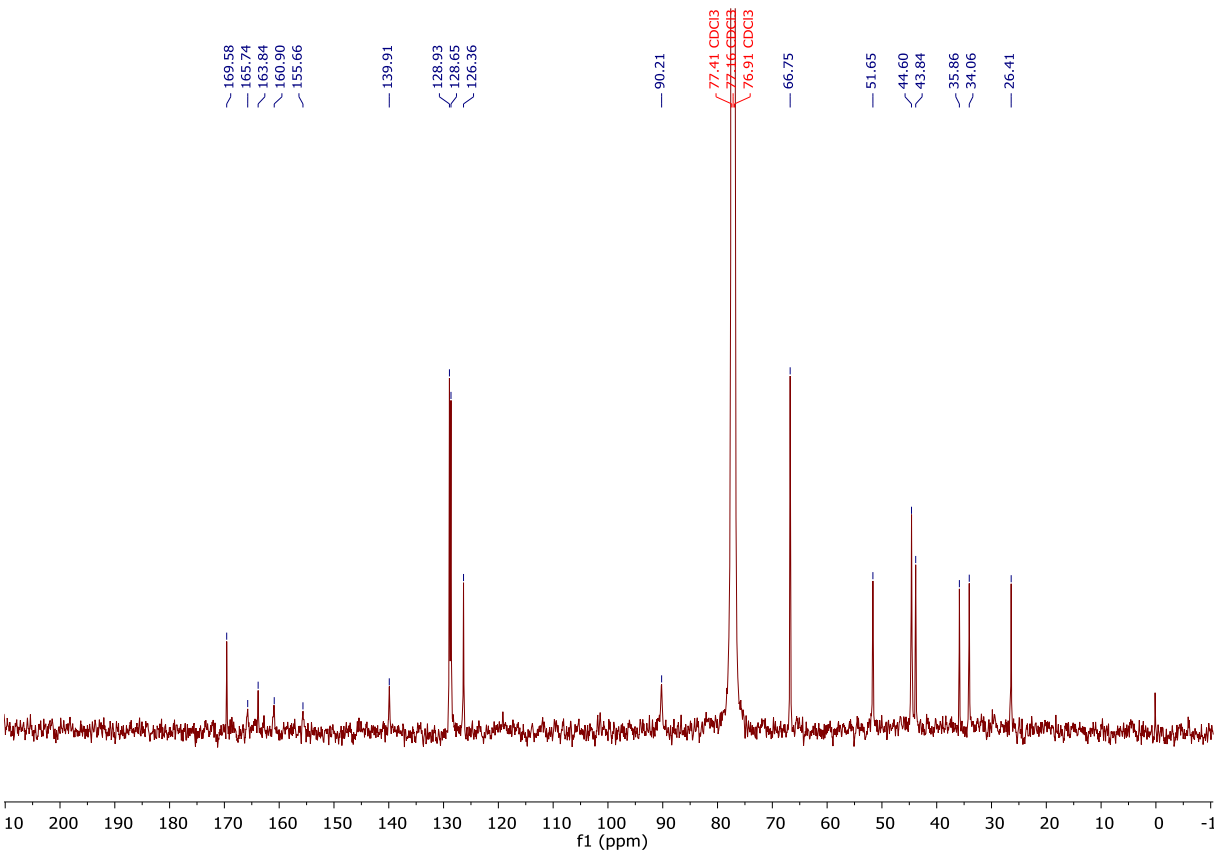

**$^{13}$C-NMR**



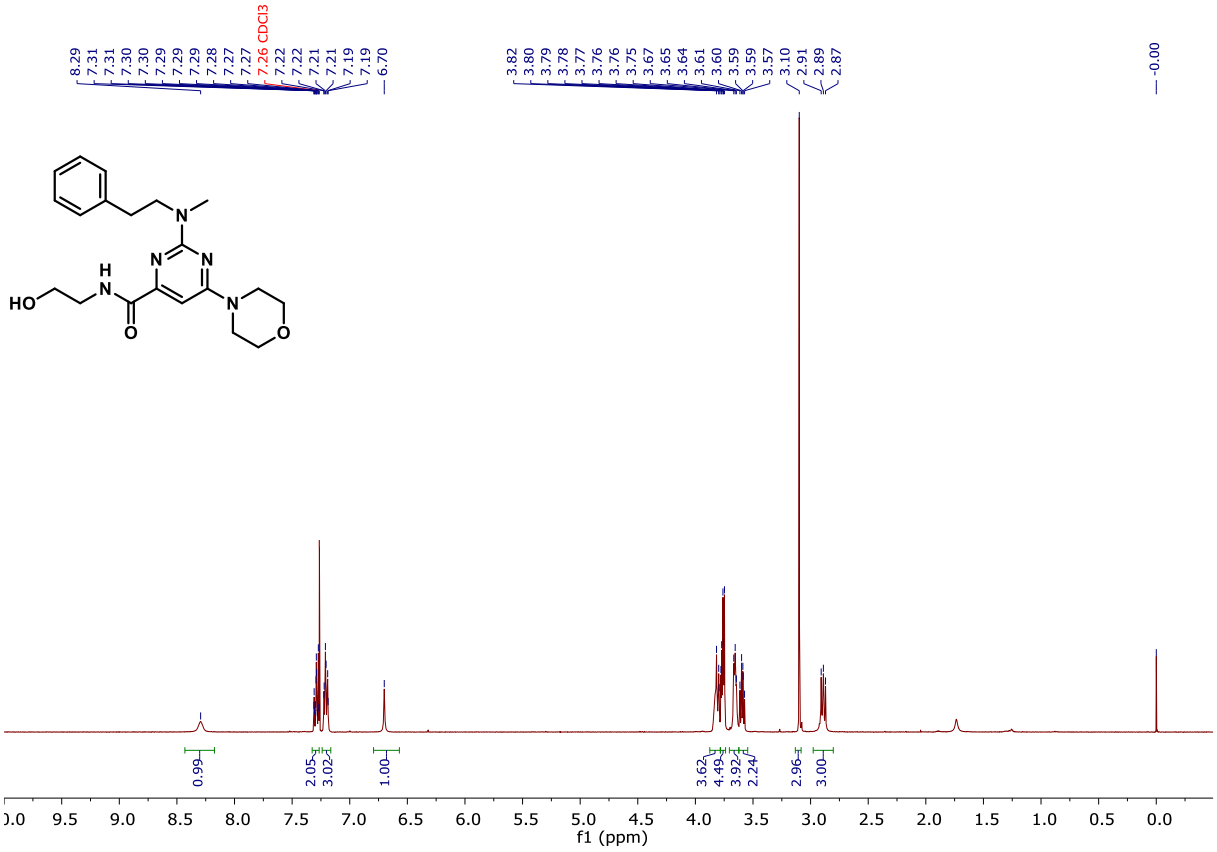
**$^{1}$H-NMR: 22**



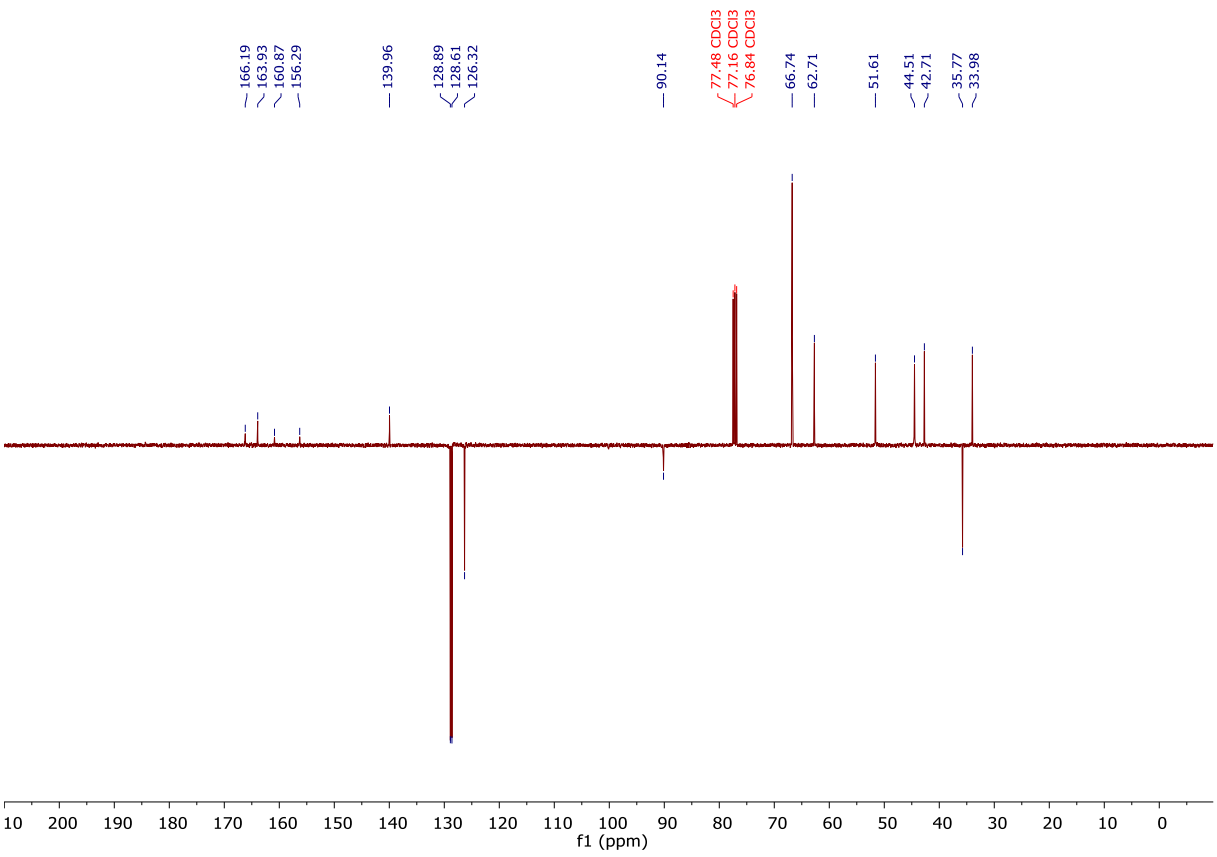

**$^{13}$C-NMR**



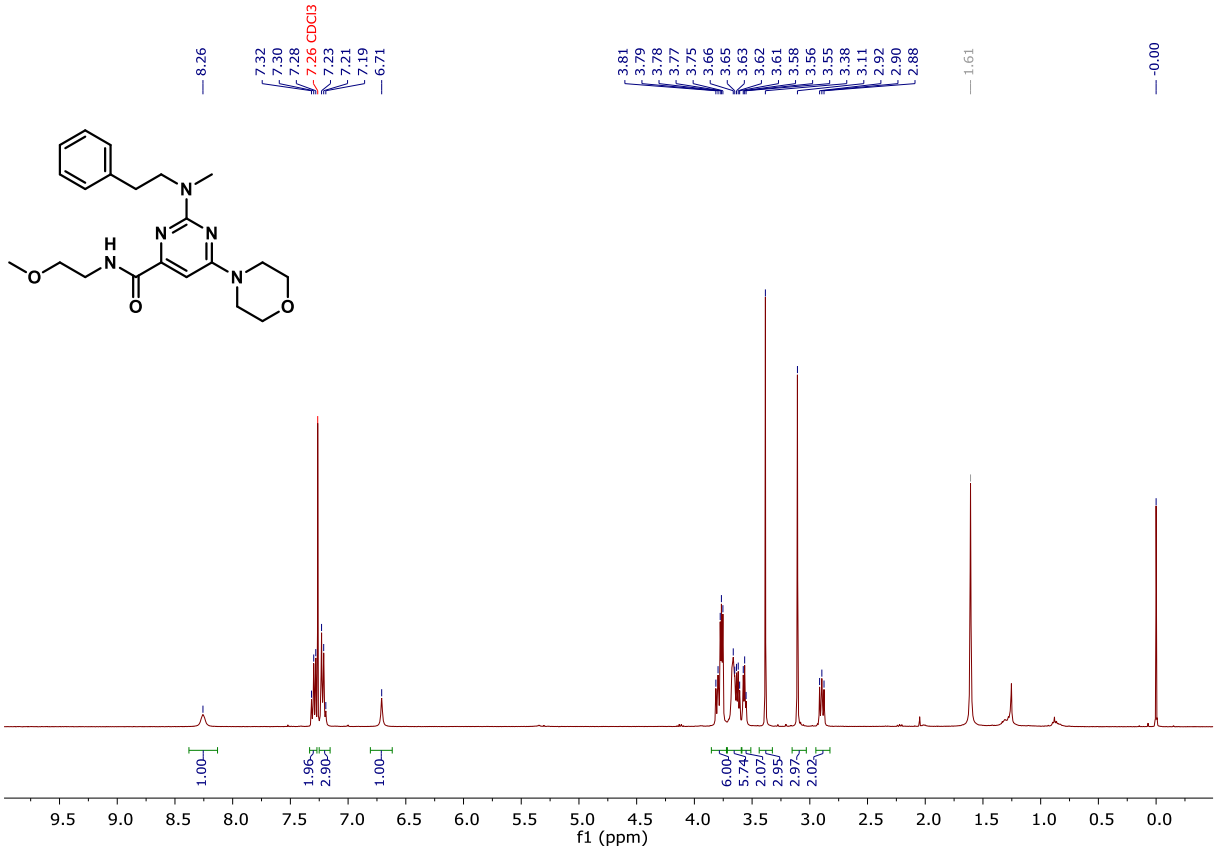
**$^{1}$H-NMR: 23**



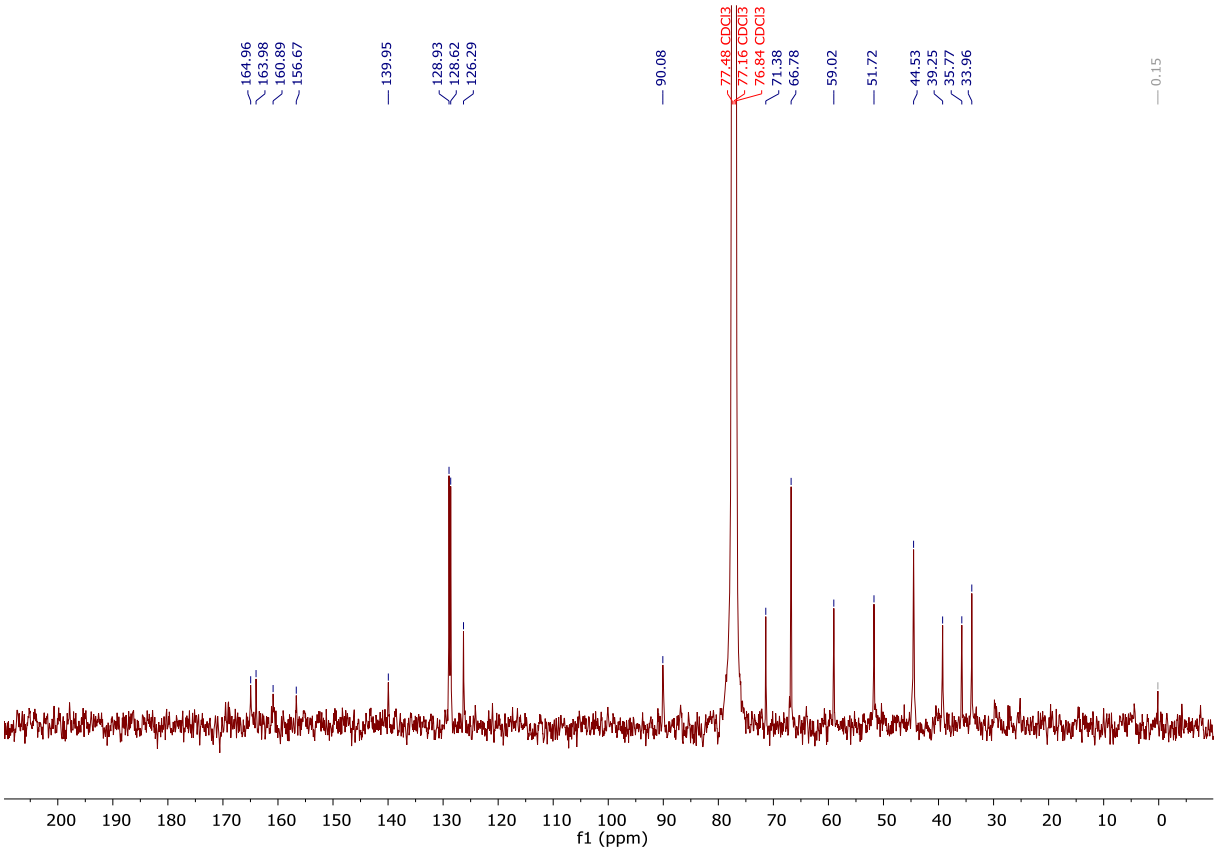

**$^{13}$C-NMR**



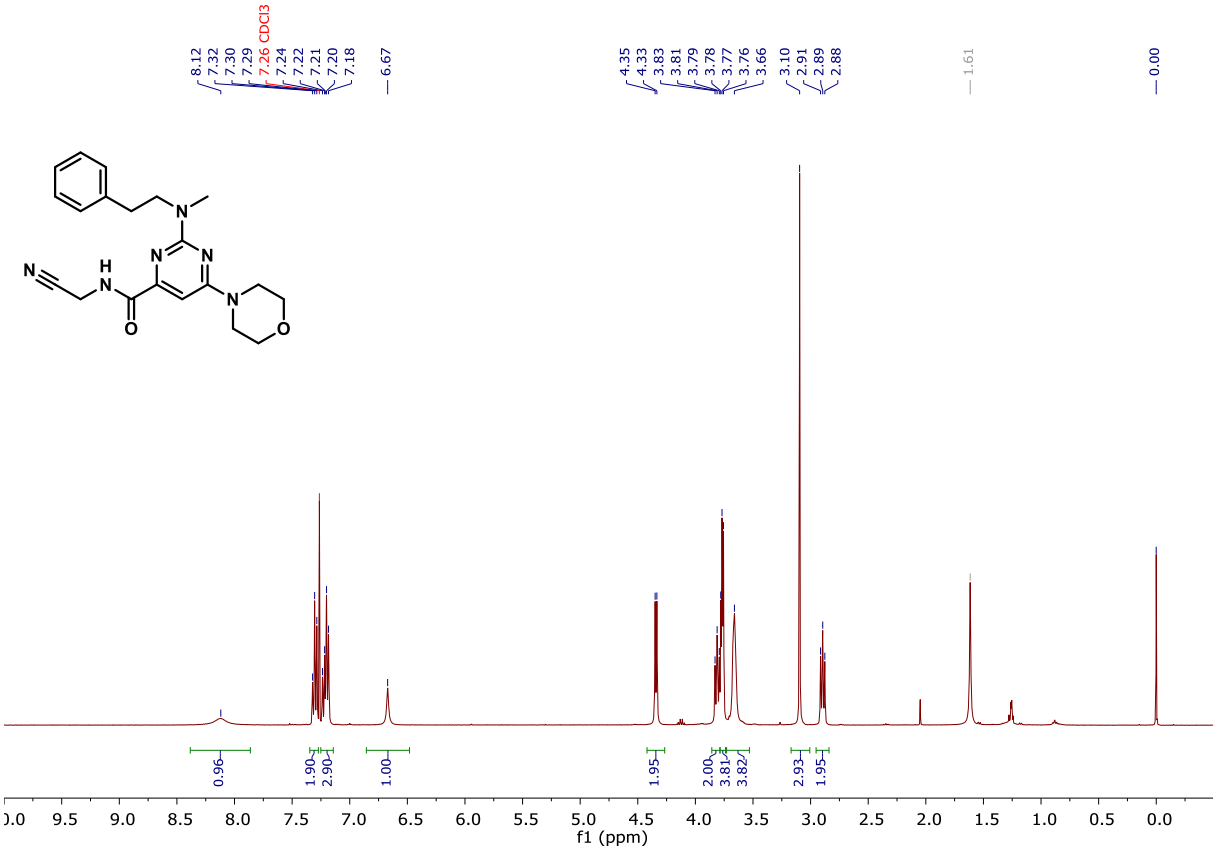
**$^{1}$H-NMR: 24**



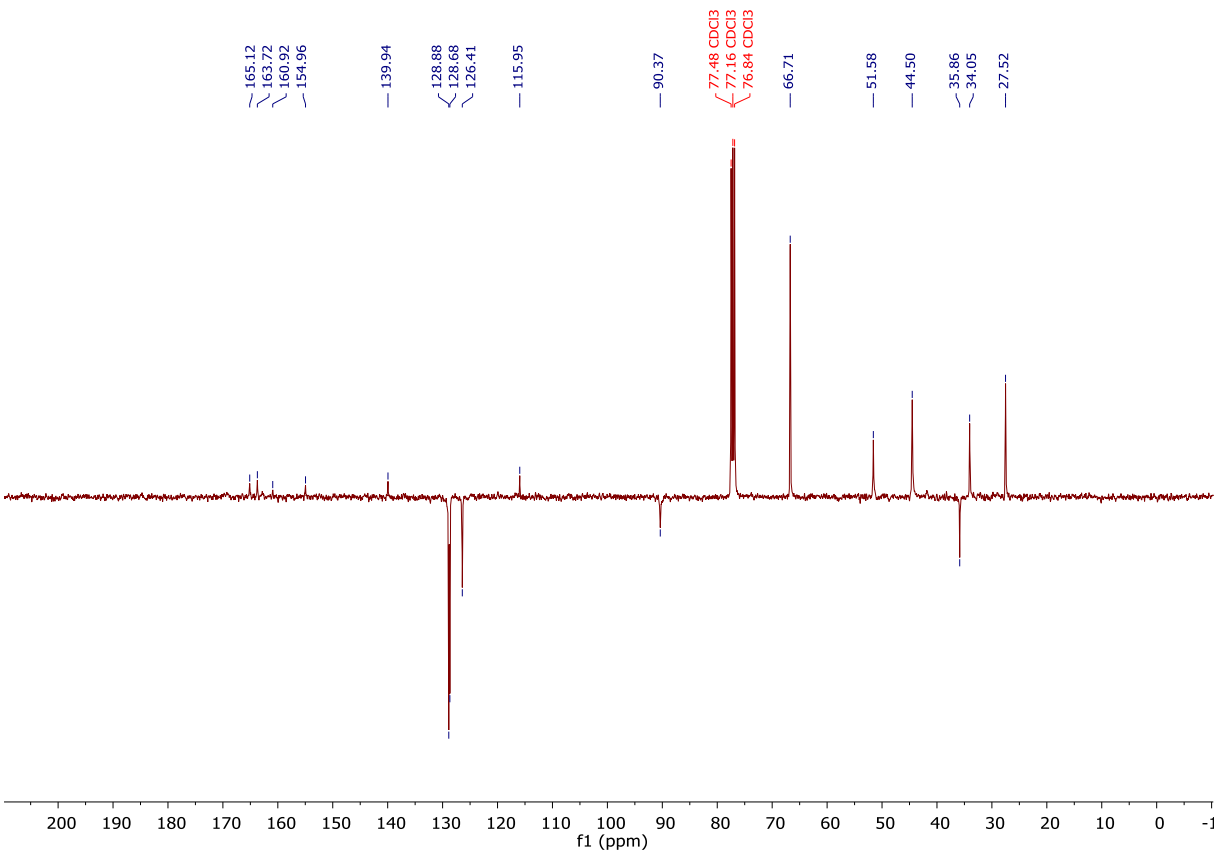

**$^{13}$C-NMR**



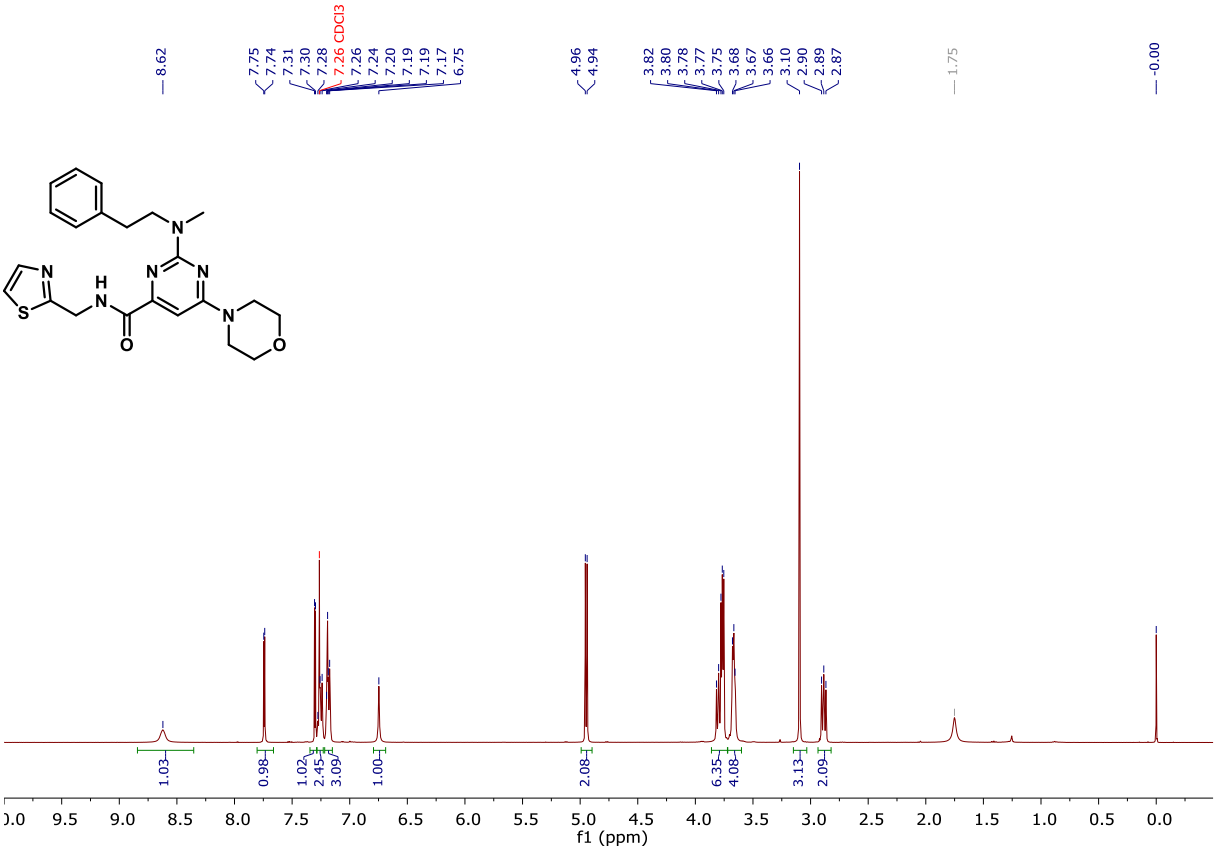
**$^{1}$H-NMR: 25**



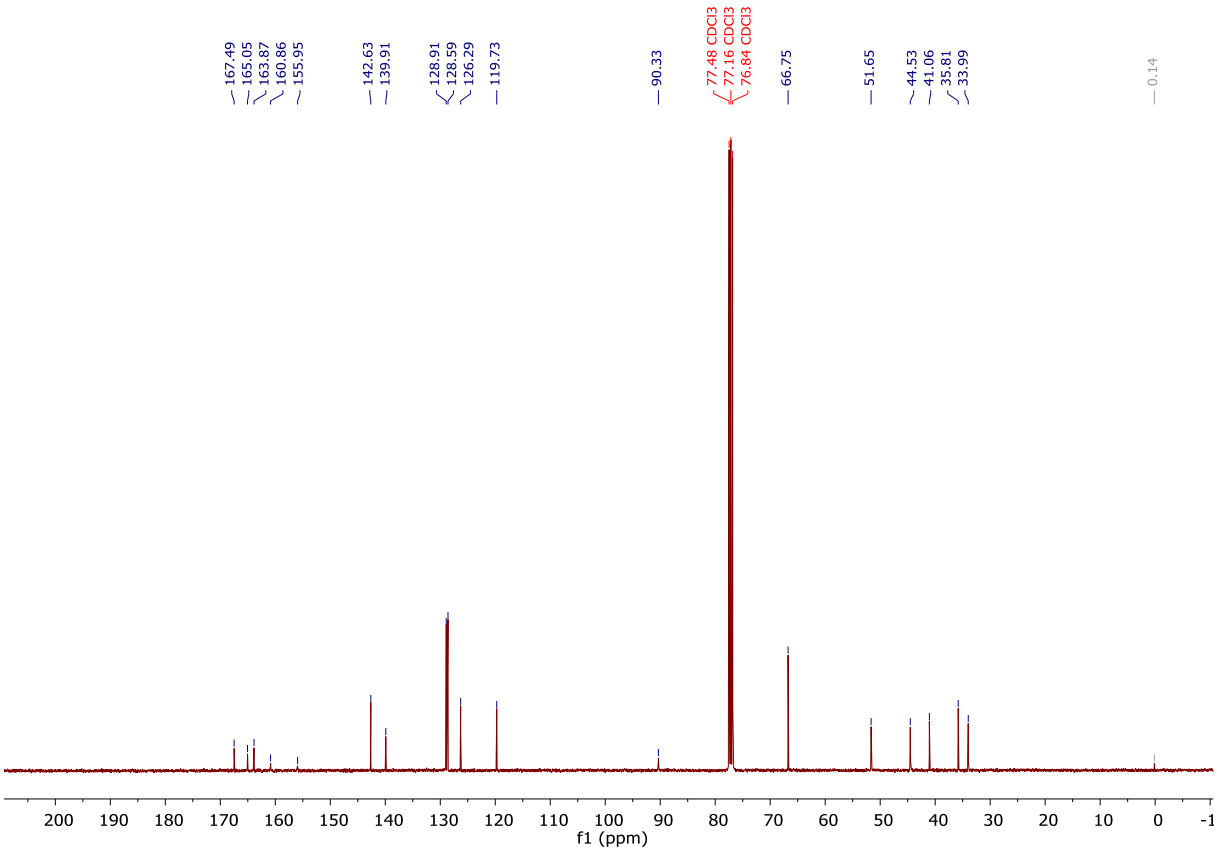

**$^{13}$C-NMR**



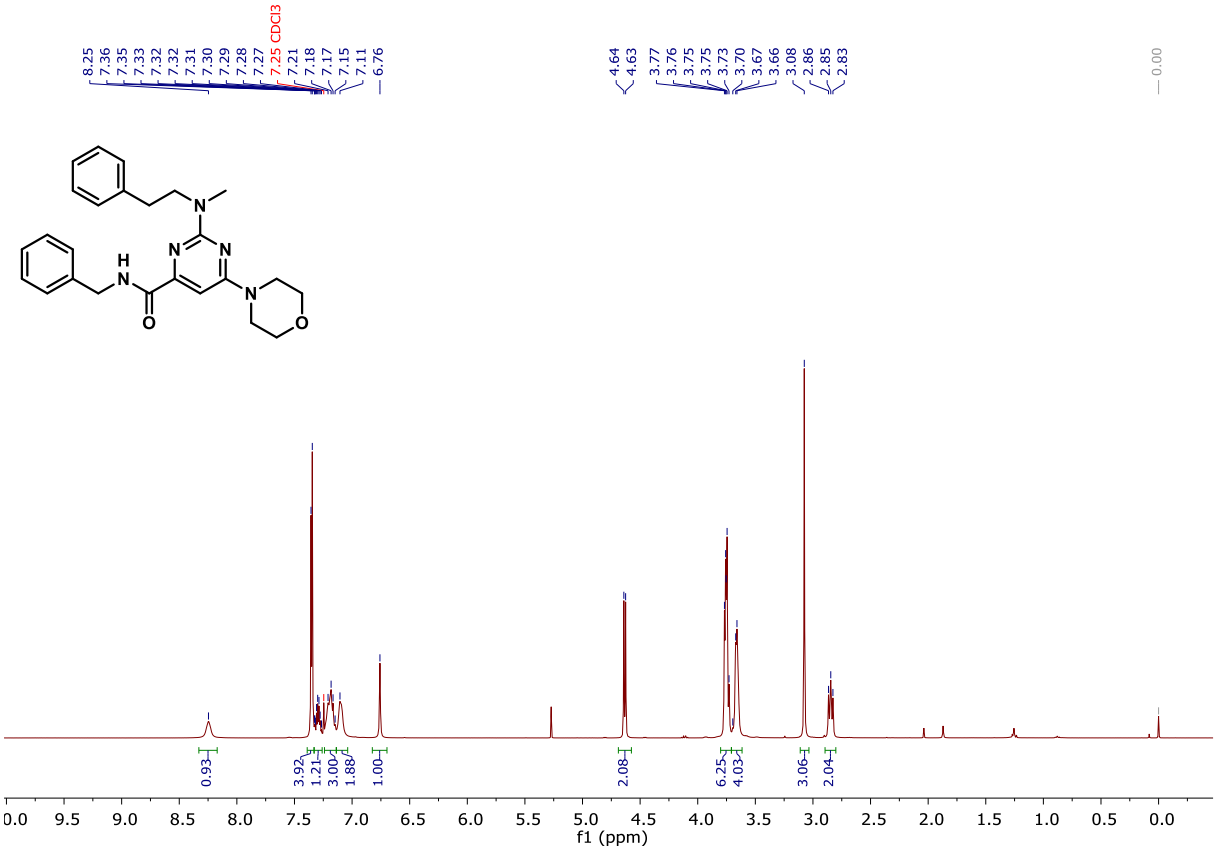
**$^{1}$H-NMR: 26**



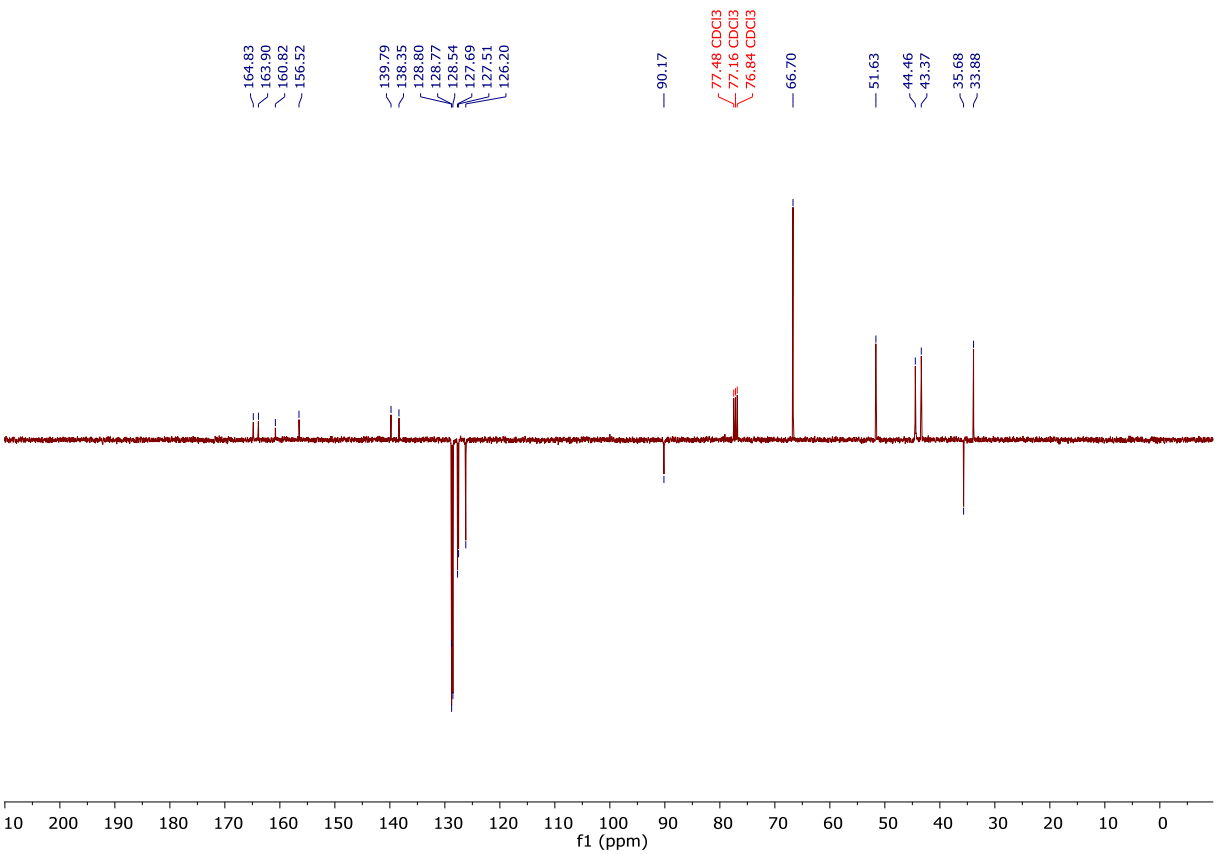

**$^{13}$C-NMR**



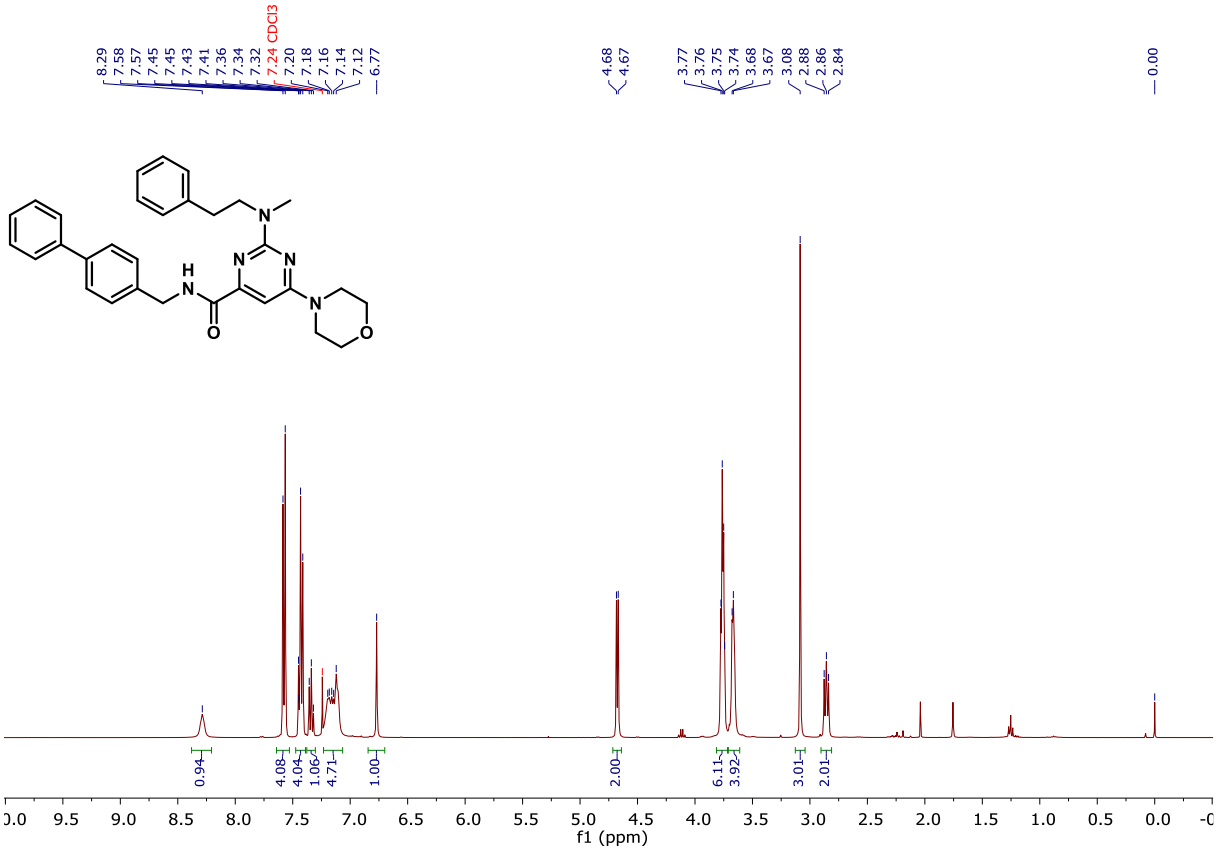
**$^{1}$H-NMR: 27**



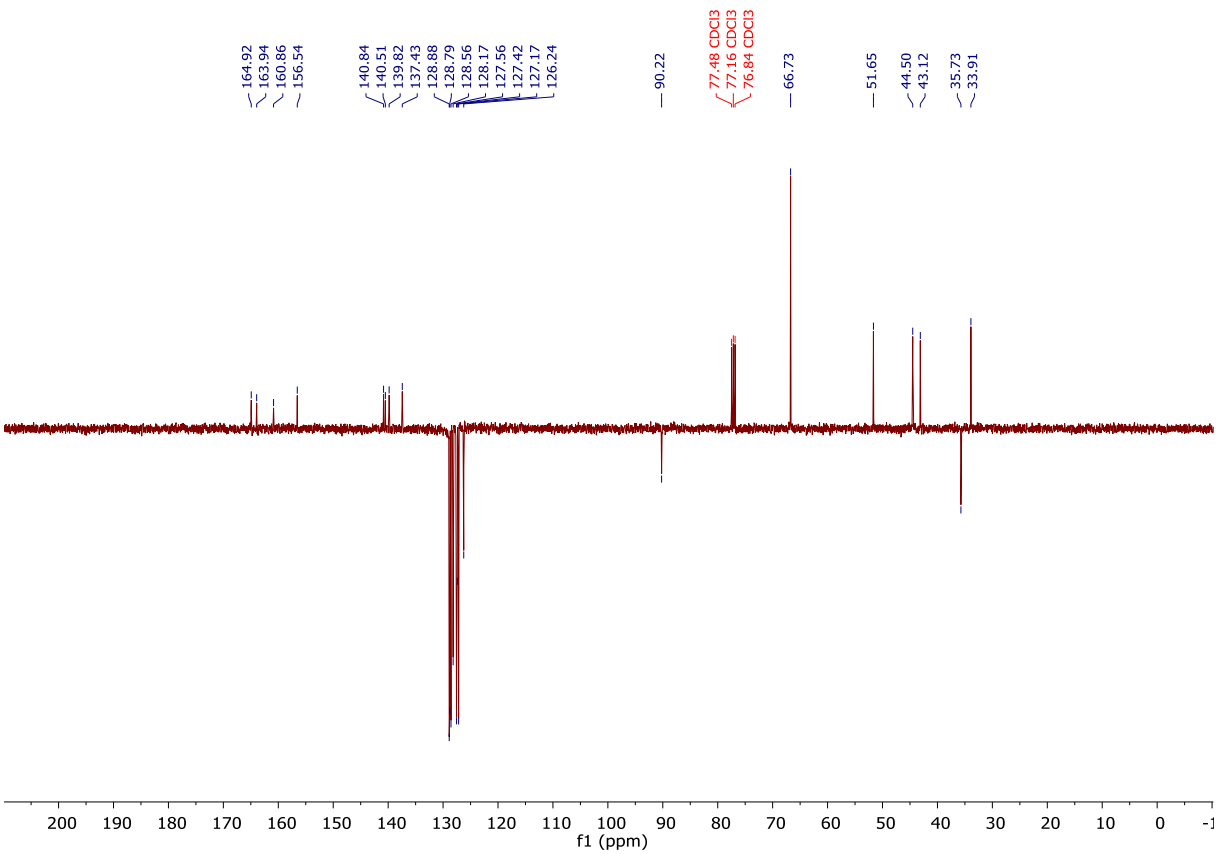

**$^{13}$C-NMR**



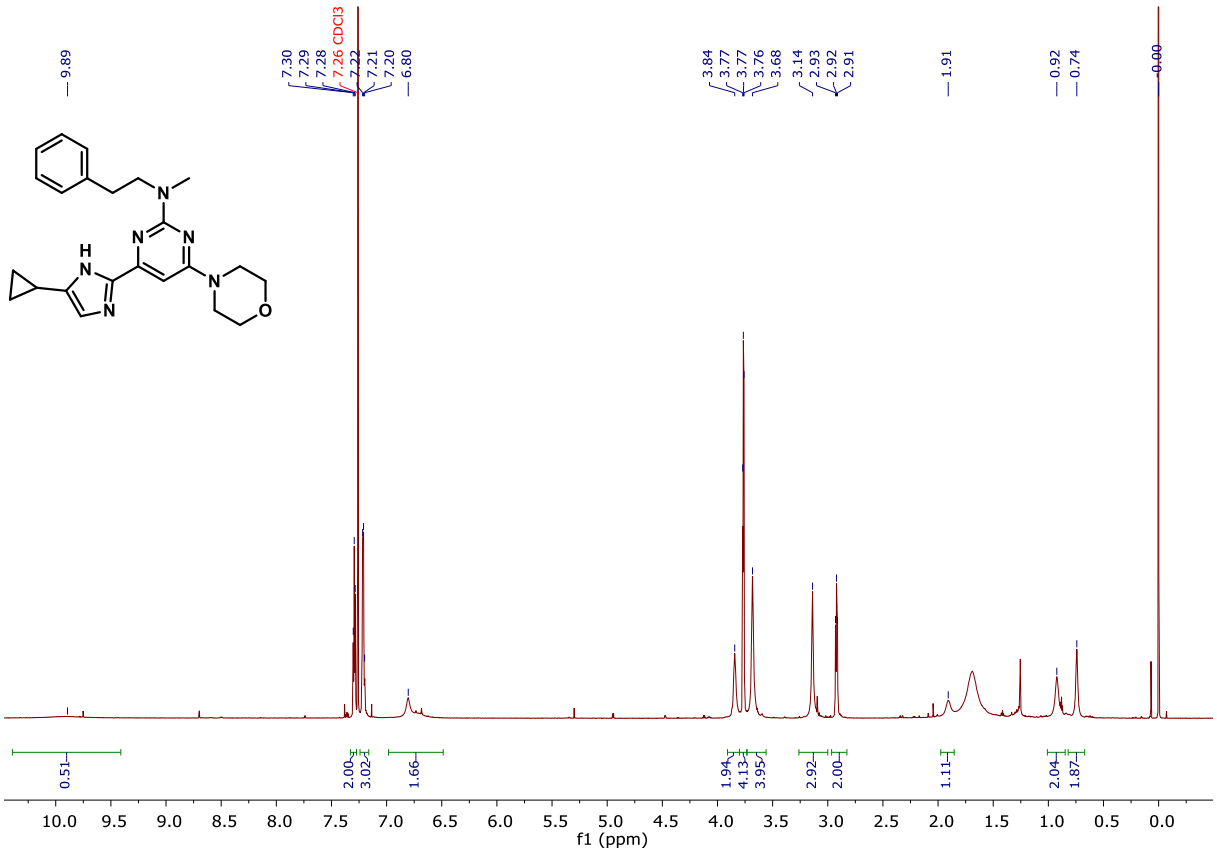
**$^{1}$H-NMR: 28**



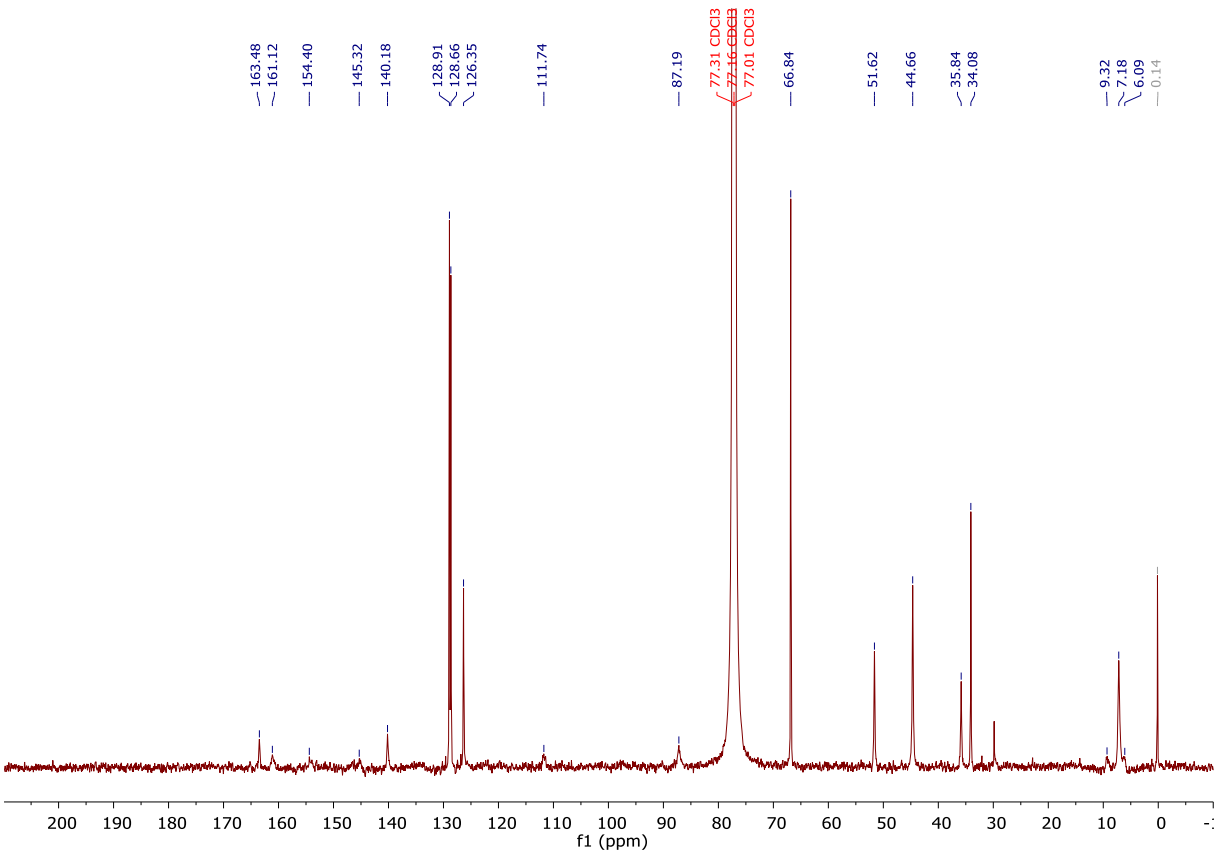

**$^{13}$C-NMR**



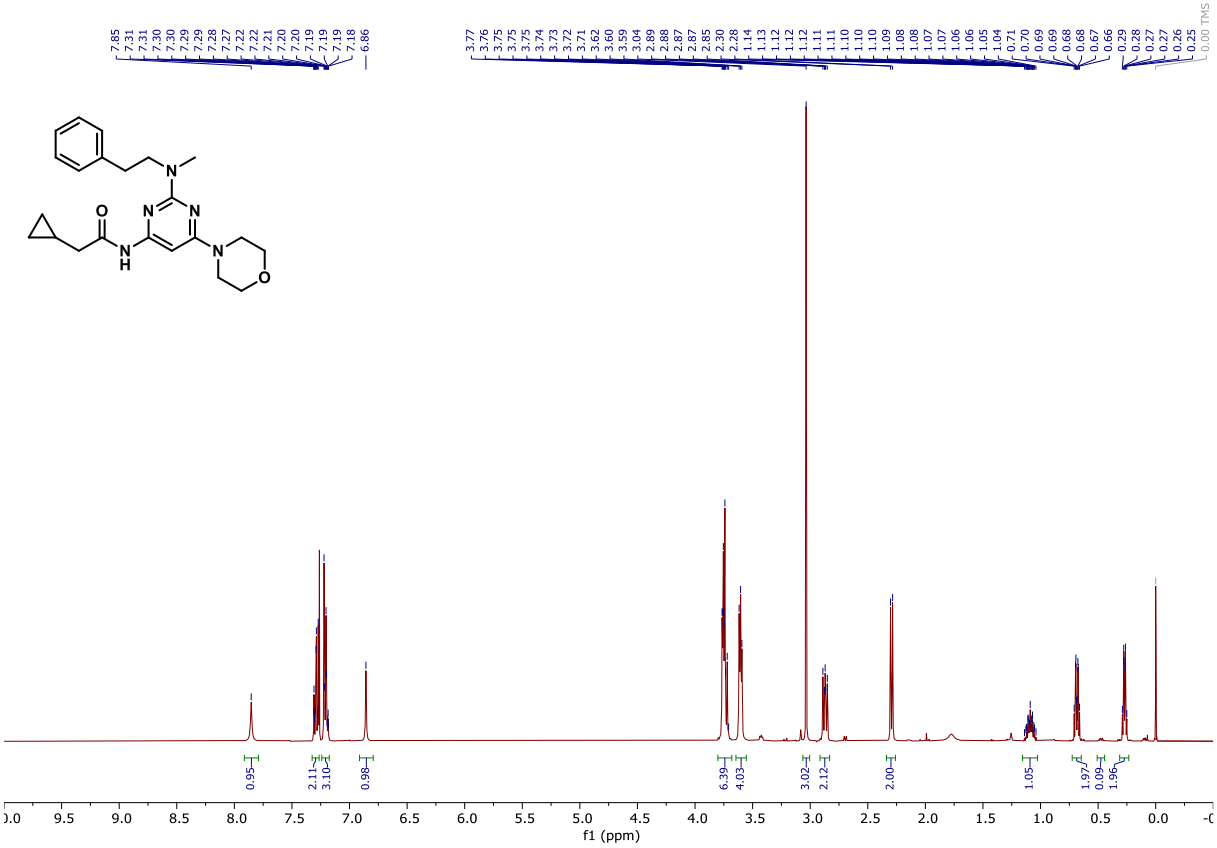
**$^{1}$H-NMR: 29**



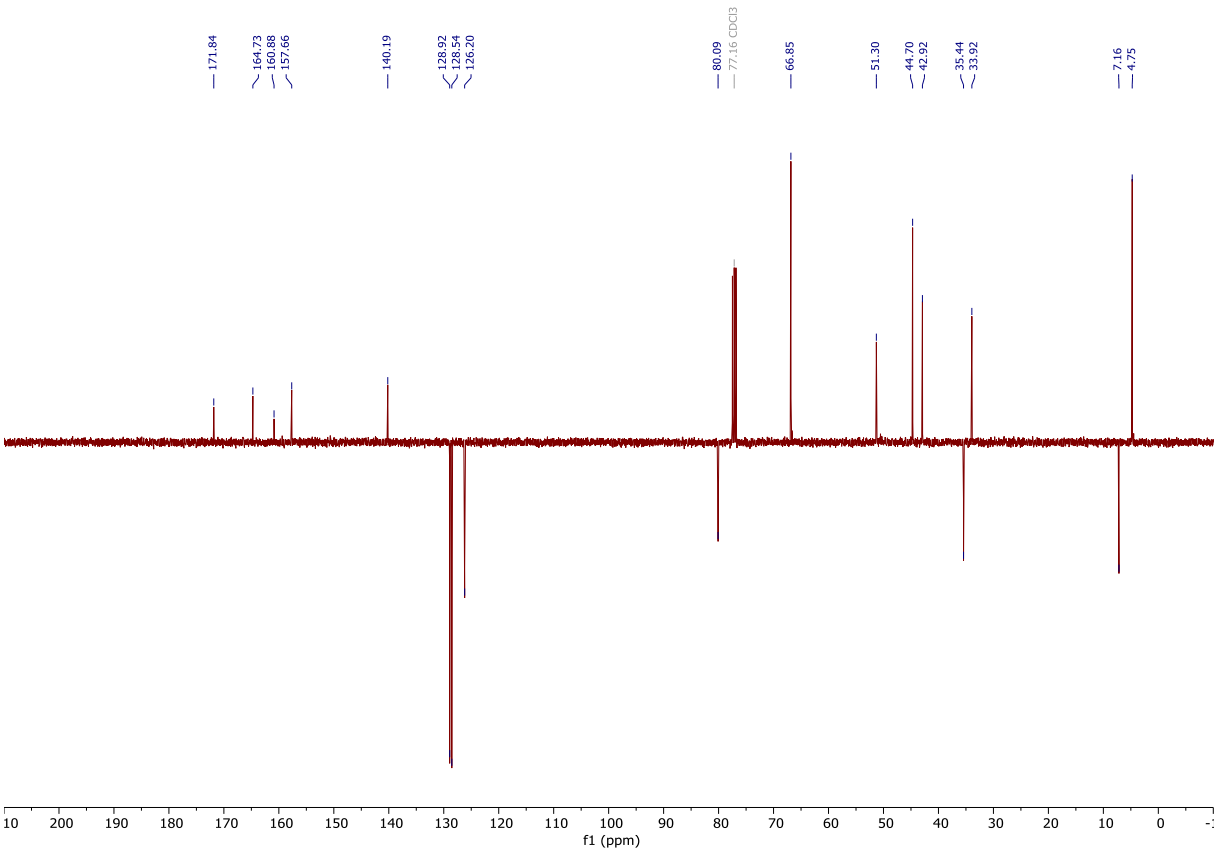

**$^{13}$C-NMR**



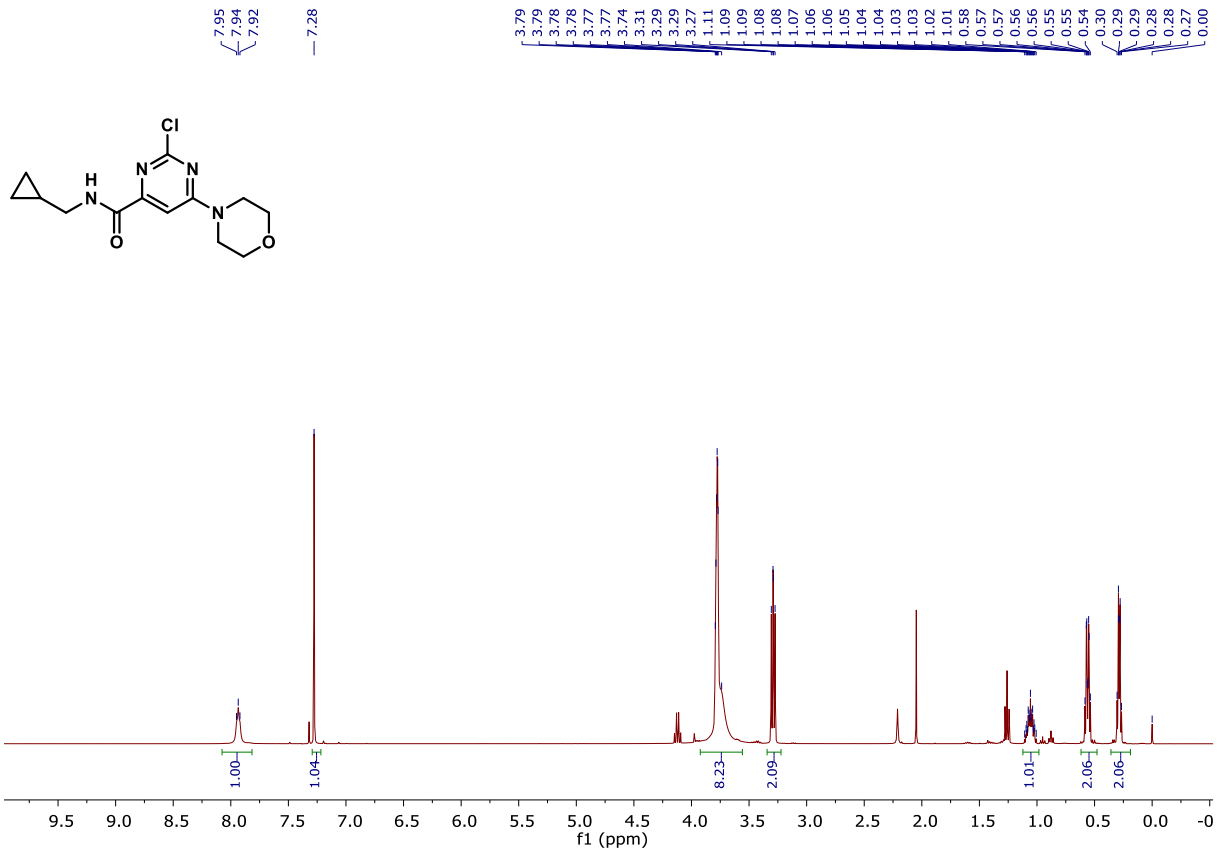
**$^{1}$H-NMR: 30**



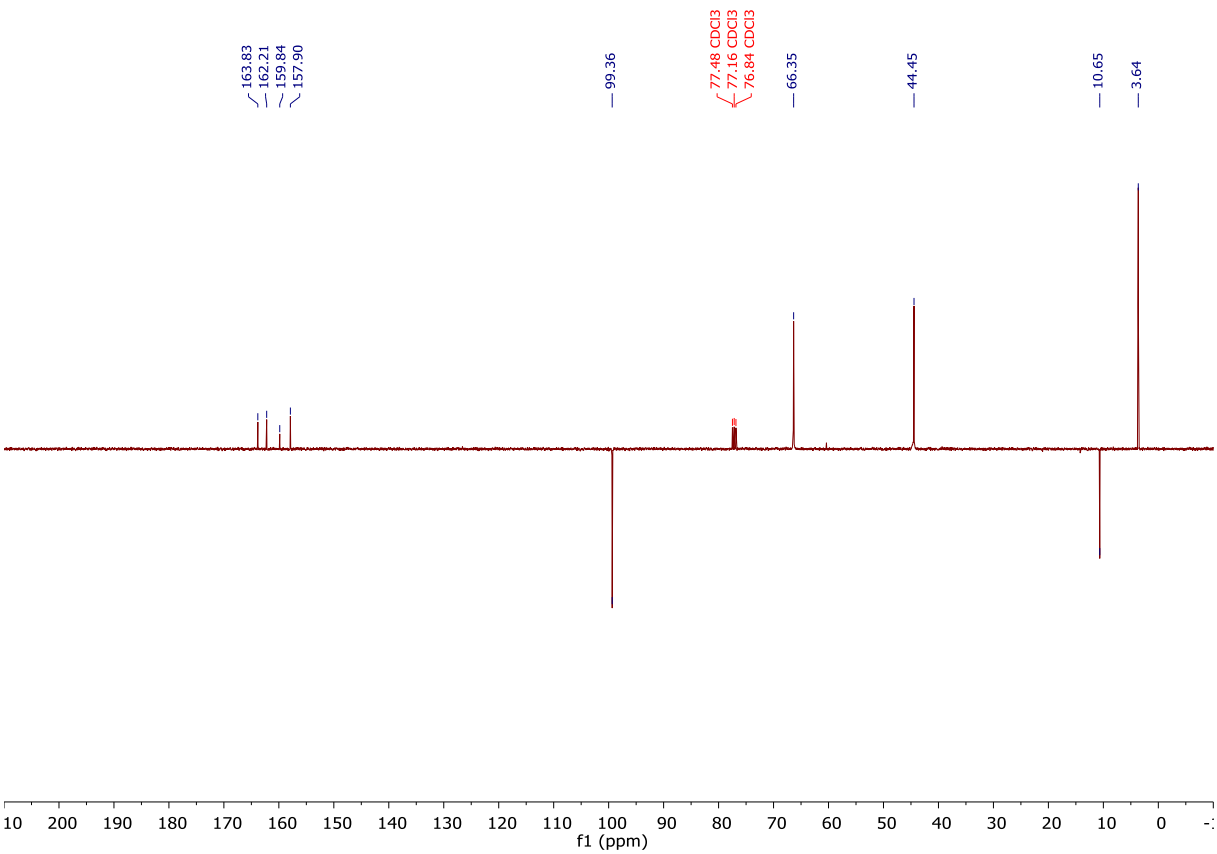

**$^{13}$C-NMR**



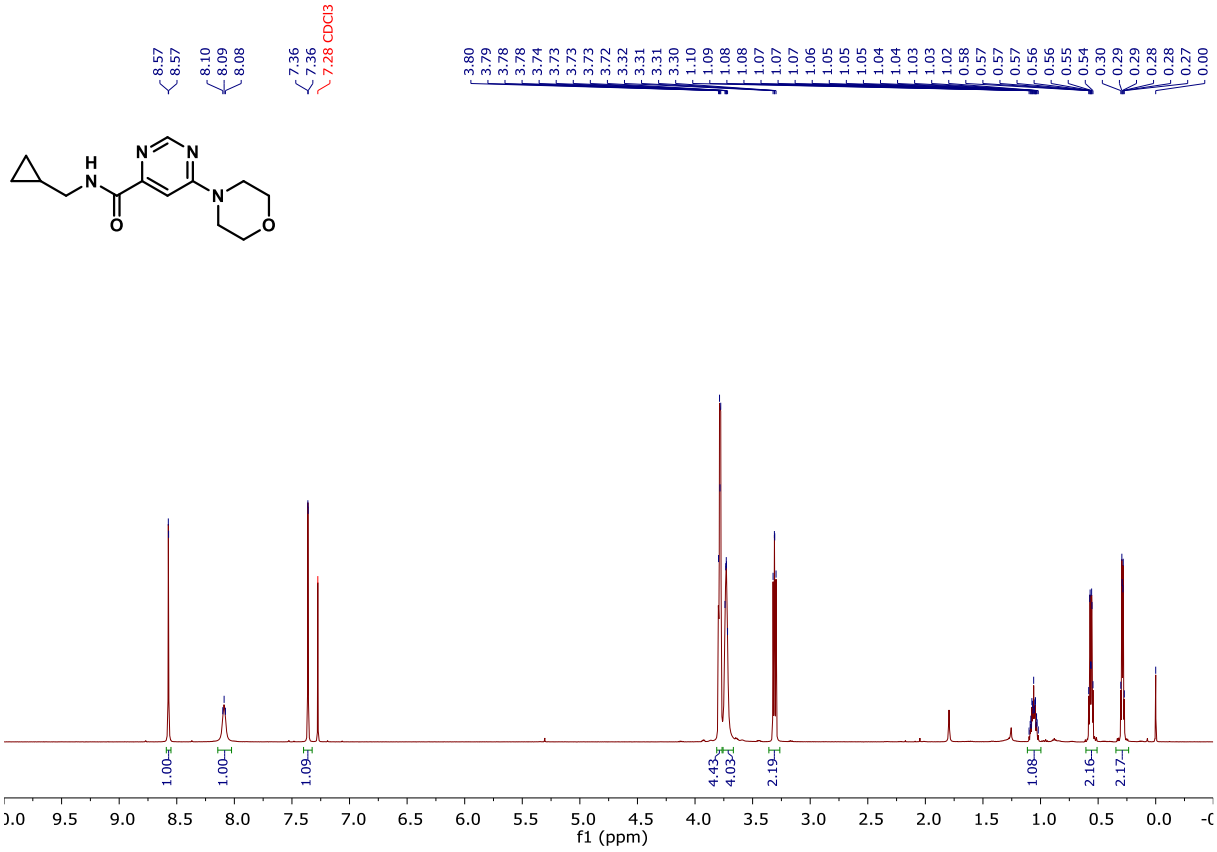
**$^{1}$H-NMR: 31**



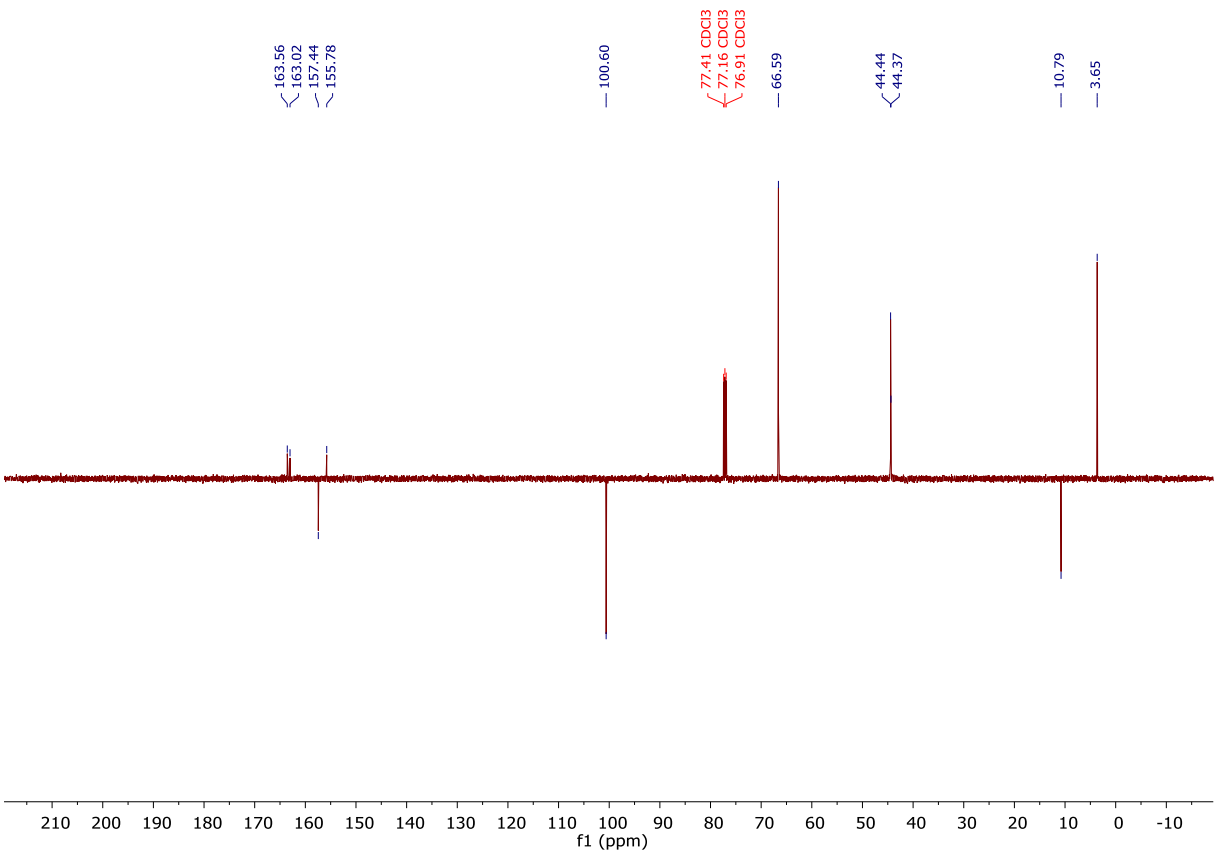

**$^{13}$C-NMR**



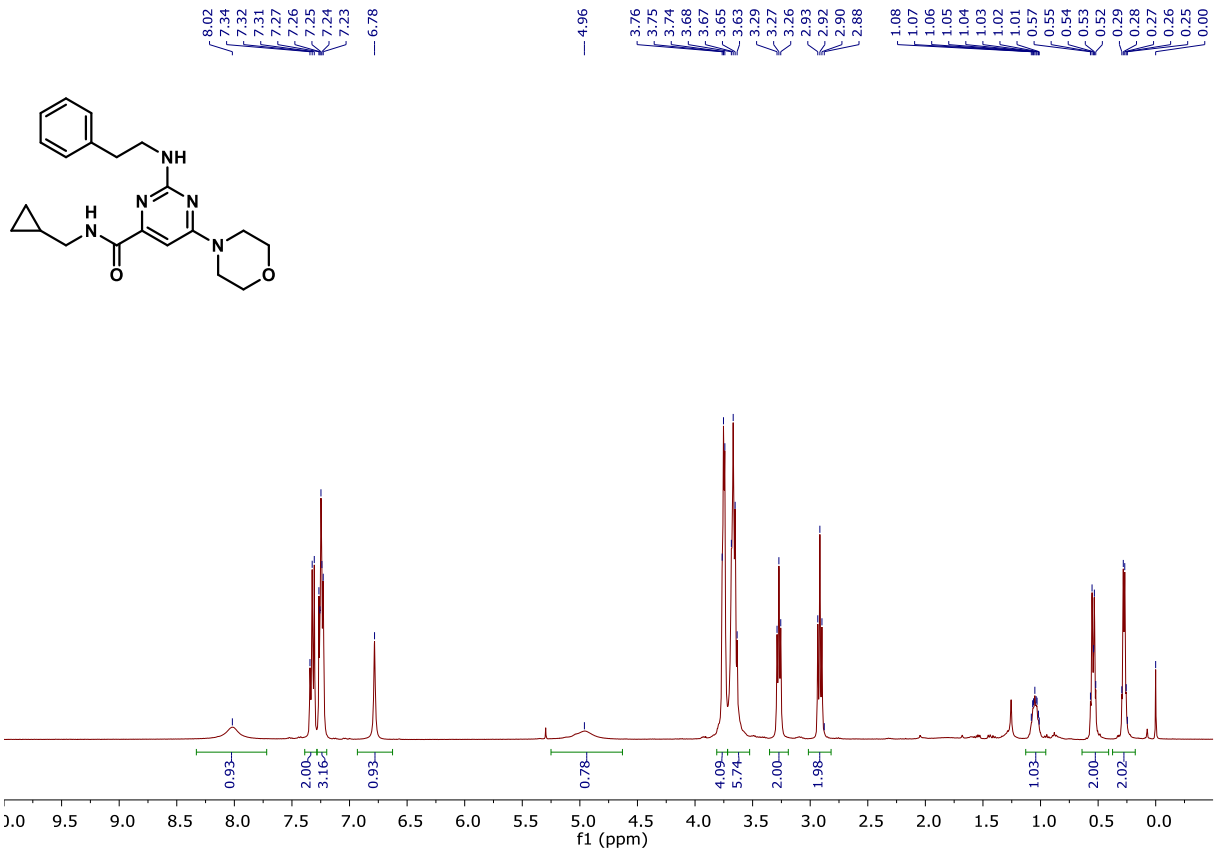
**$^{1}$H-NMR: 32**



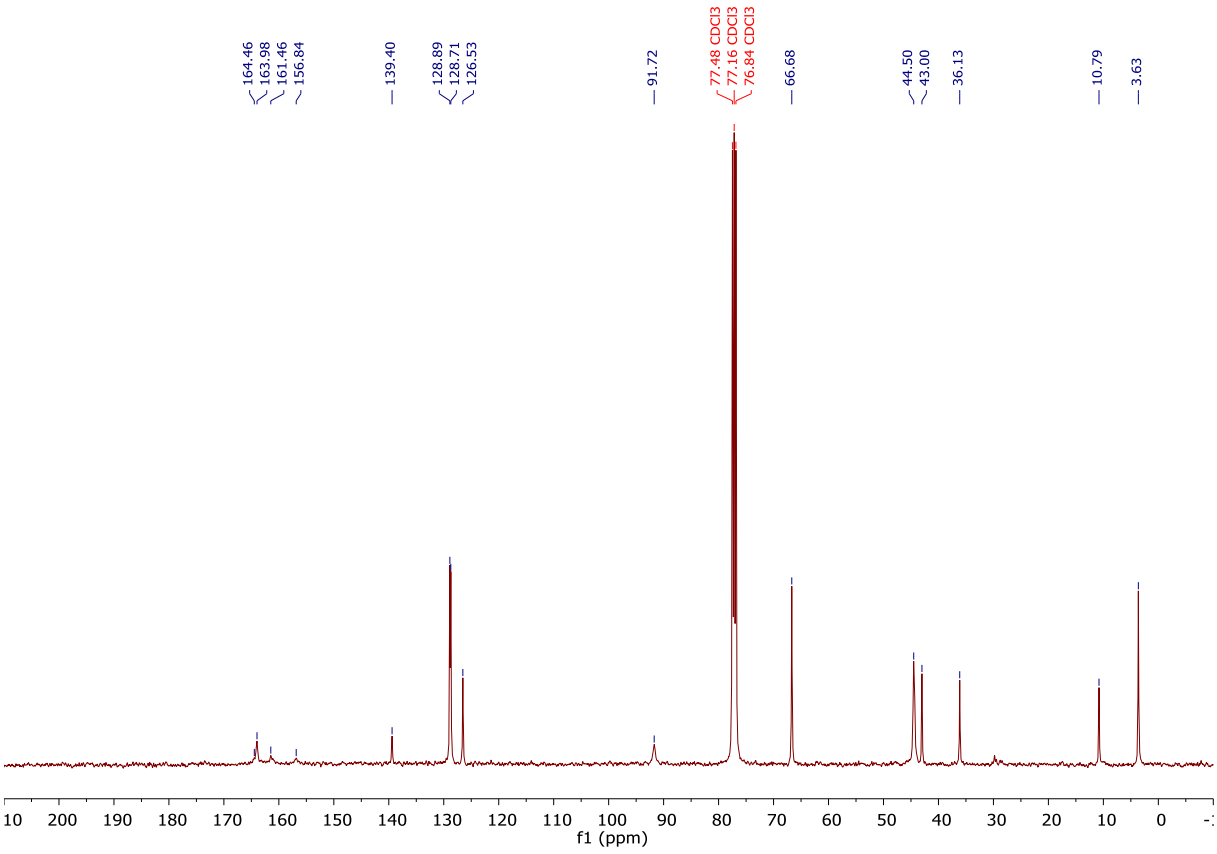

**$^{13}$C-NMR**



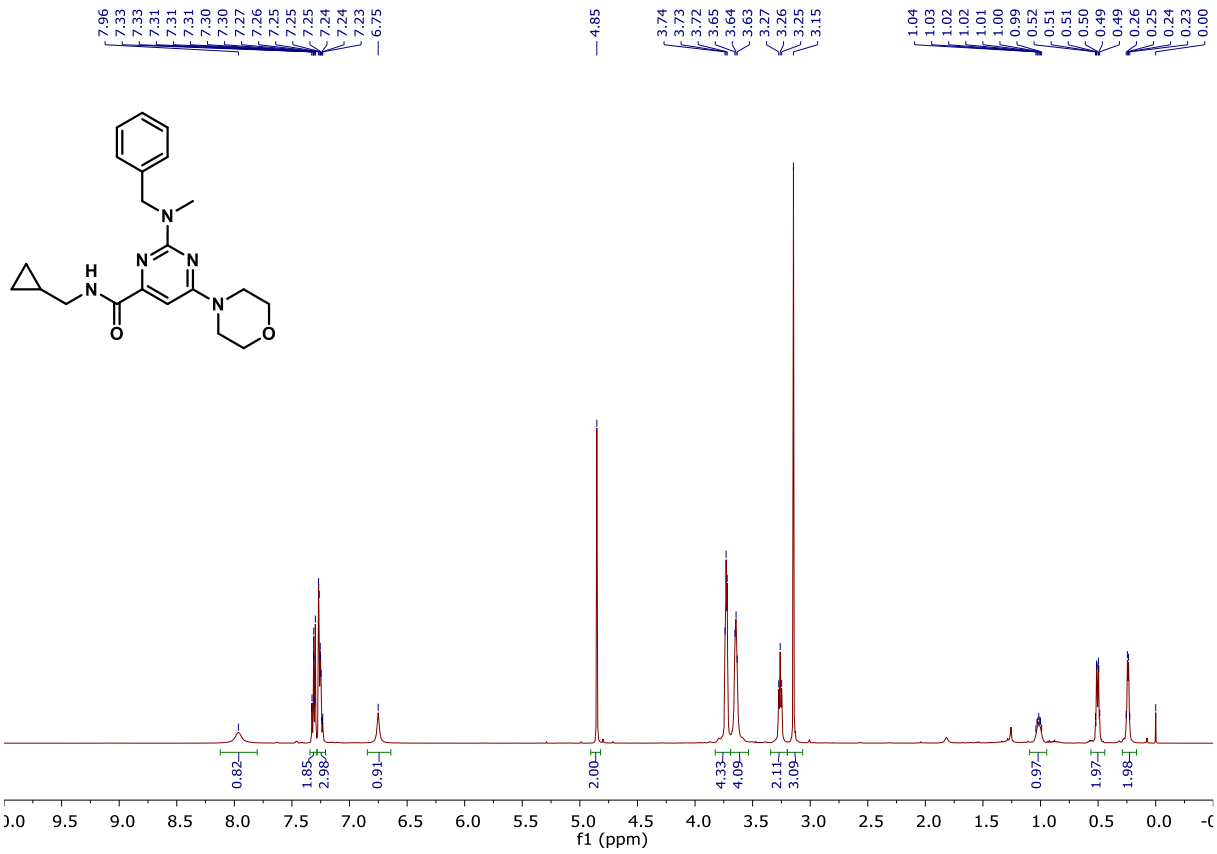
**$^{1}$H-NMR: 33**



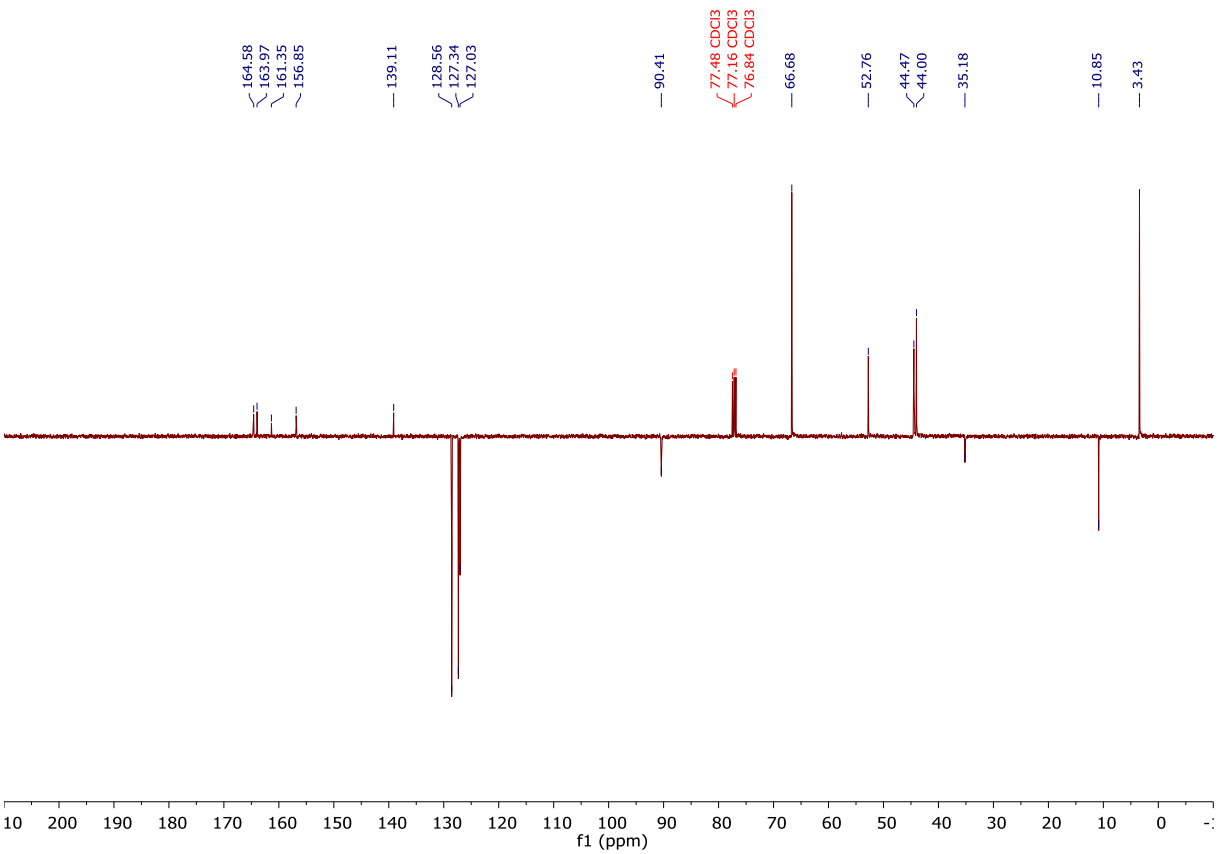

**$^{13}$C-NMR**



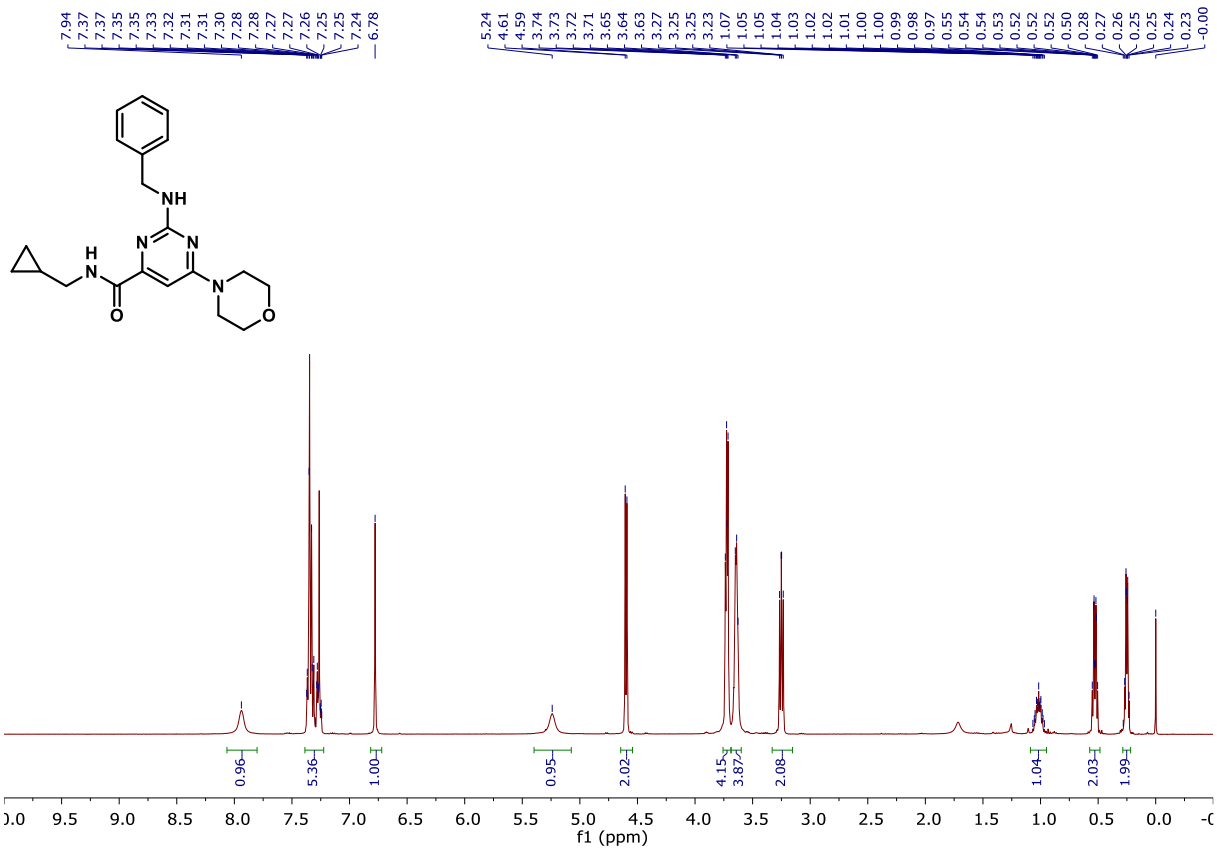
**$^{1}$H-NMR: 34**



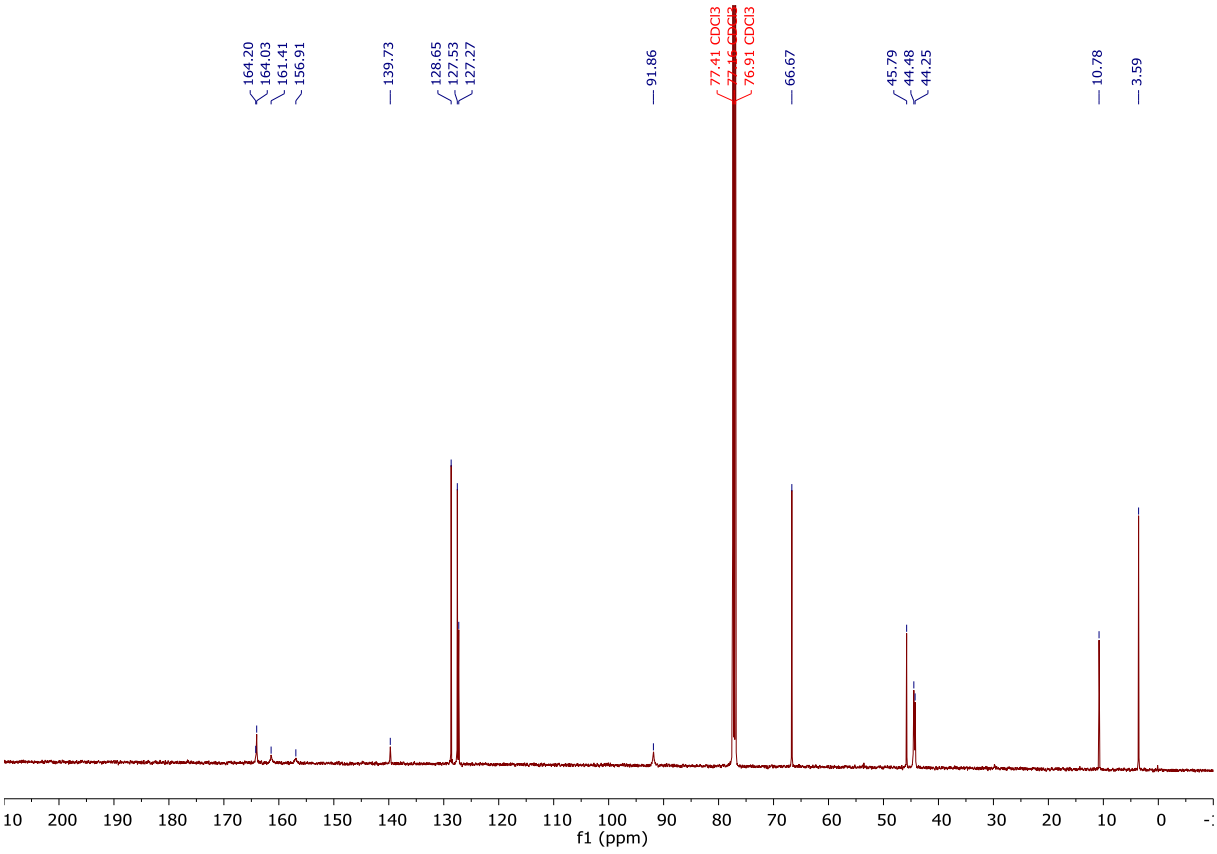

**$^{13}$C-NMR**



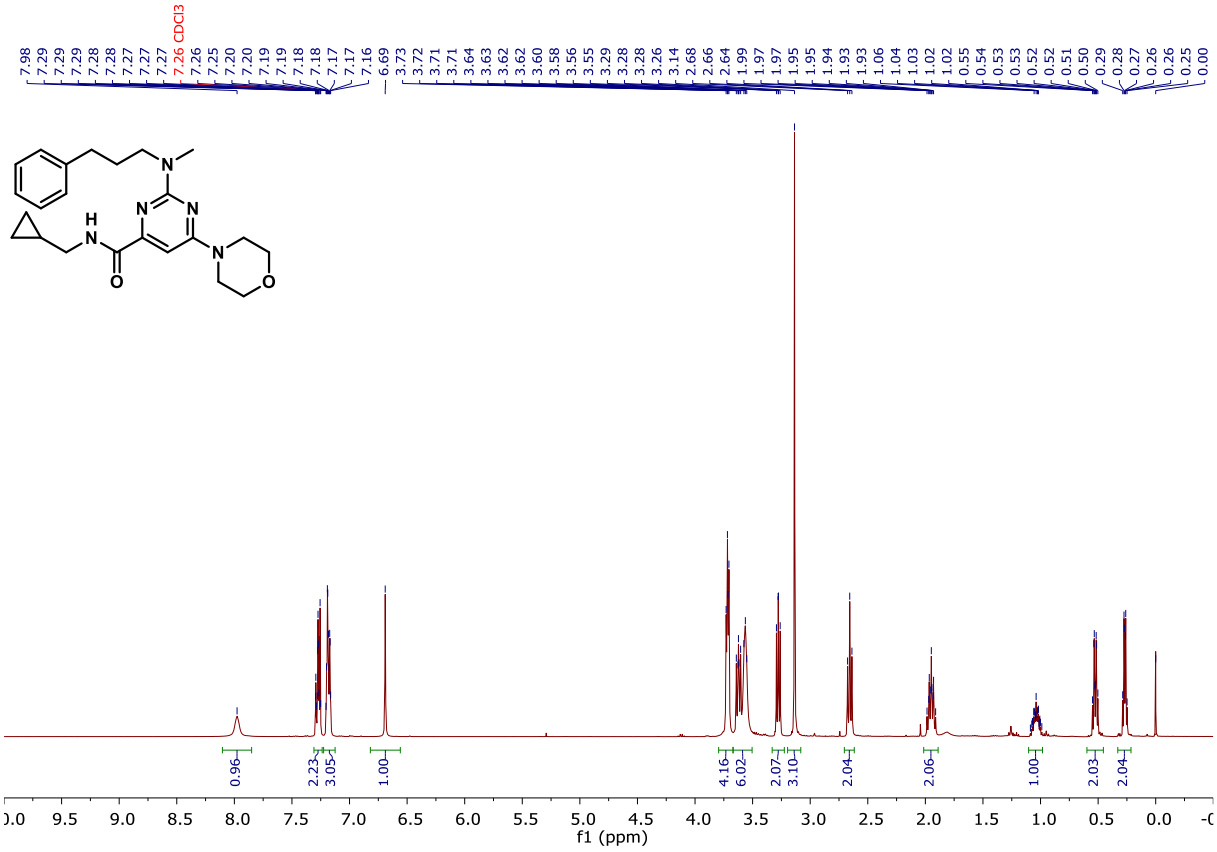
**$^{1}$H-NMR: 35**



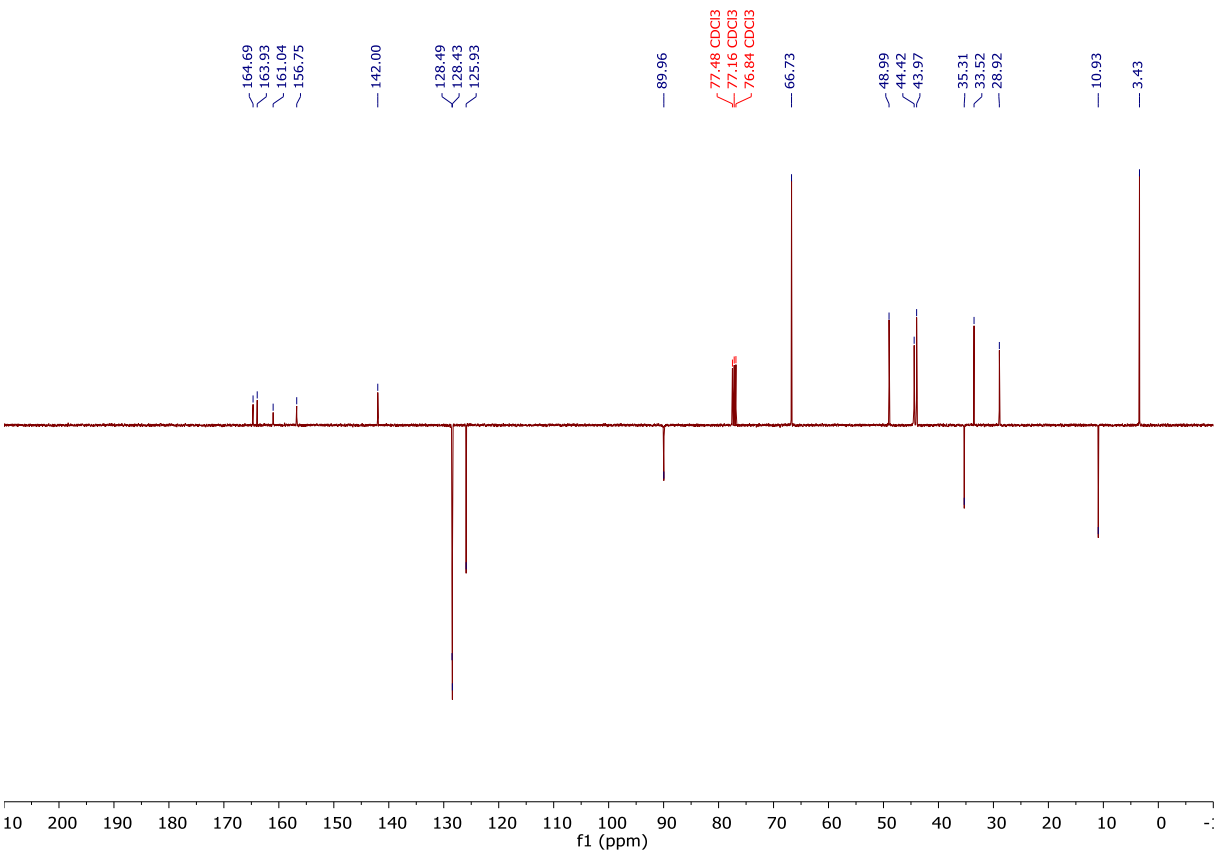

**$^{13}$C-NMR**



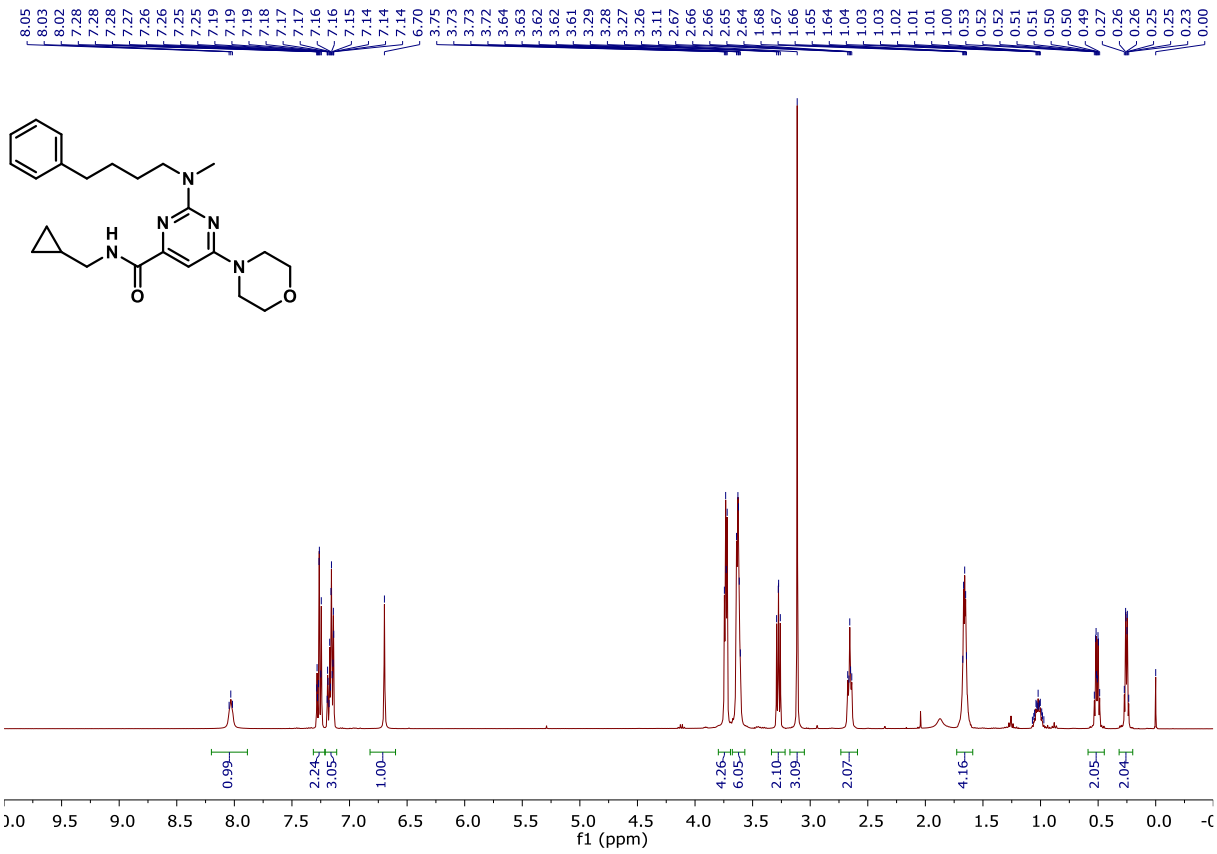
**$^{1}$H-NMR: 36**



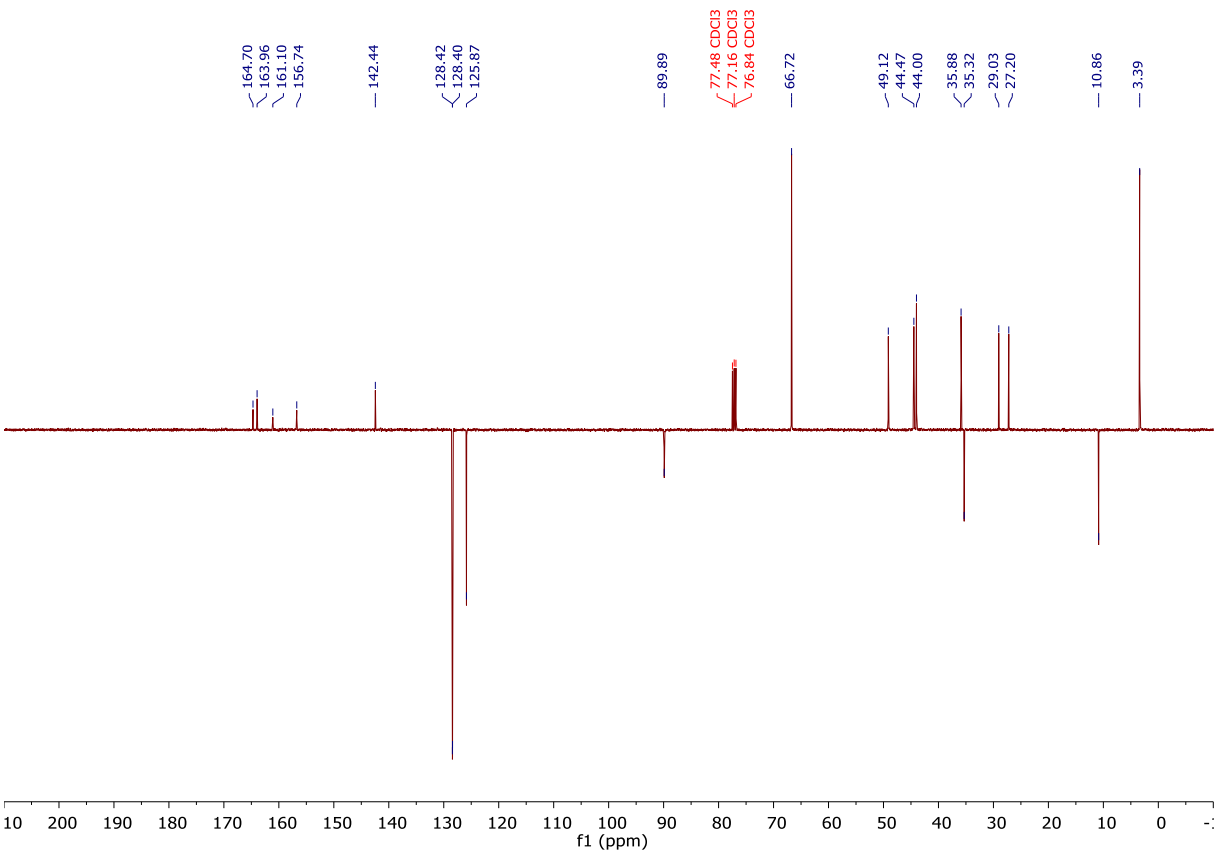

**$^{13}$C-NMR**



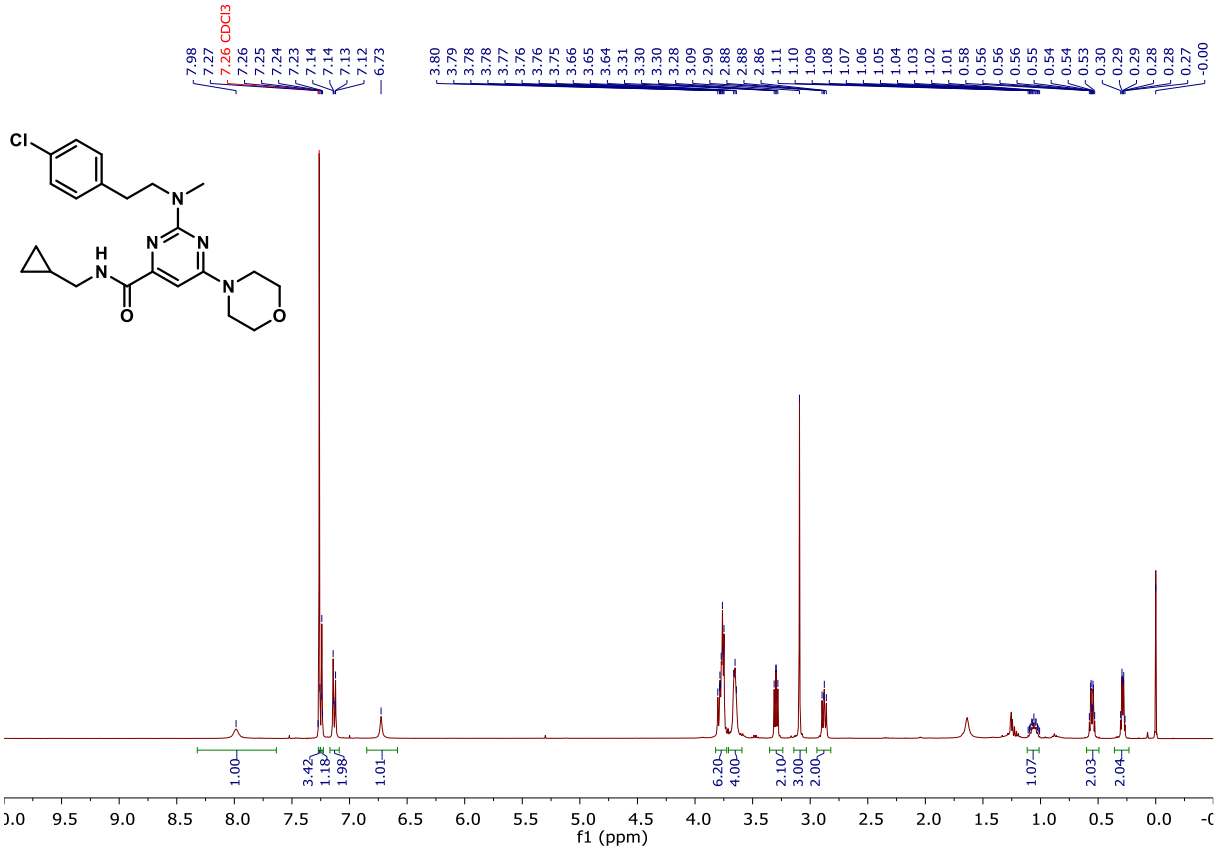
**$^{1}$H-NMR: 37**



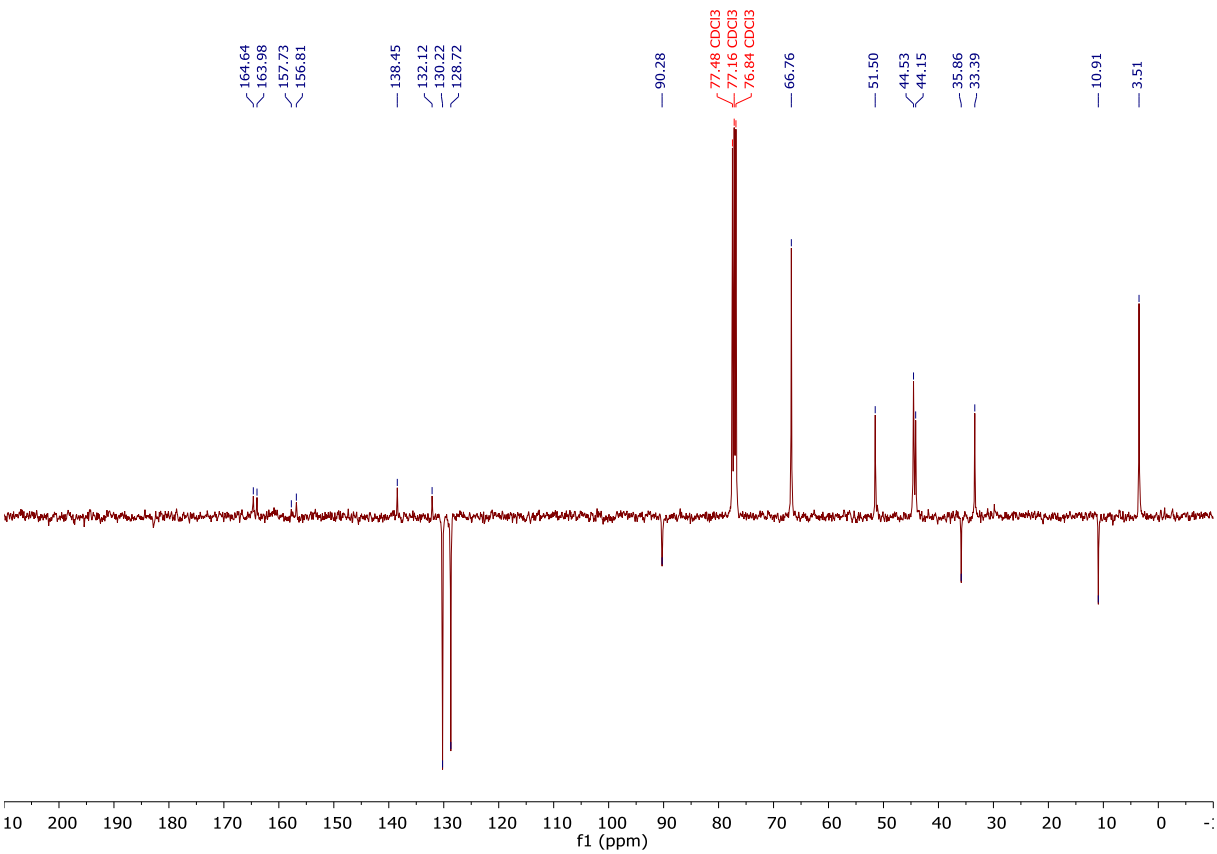

**$^{13}$C-NMR**



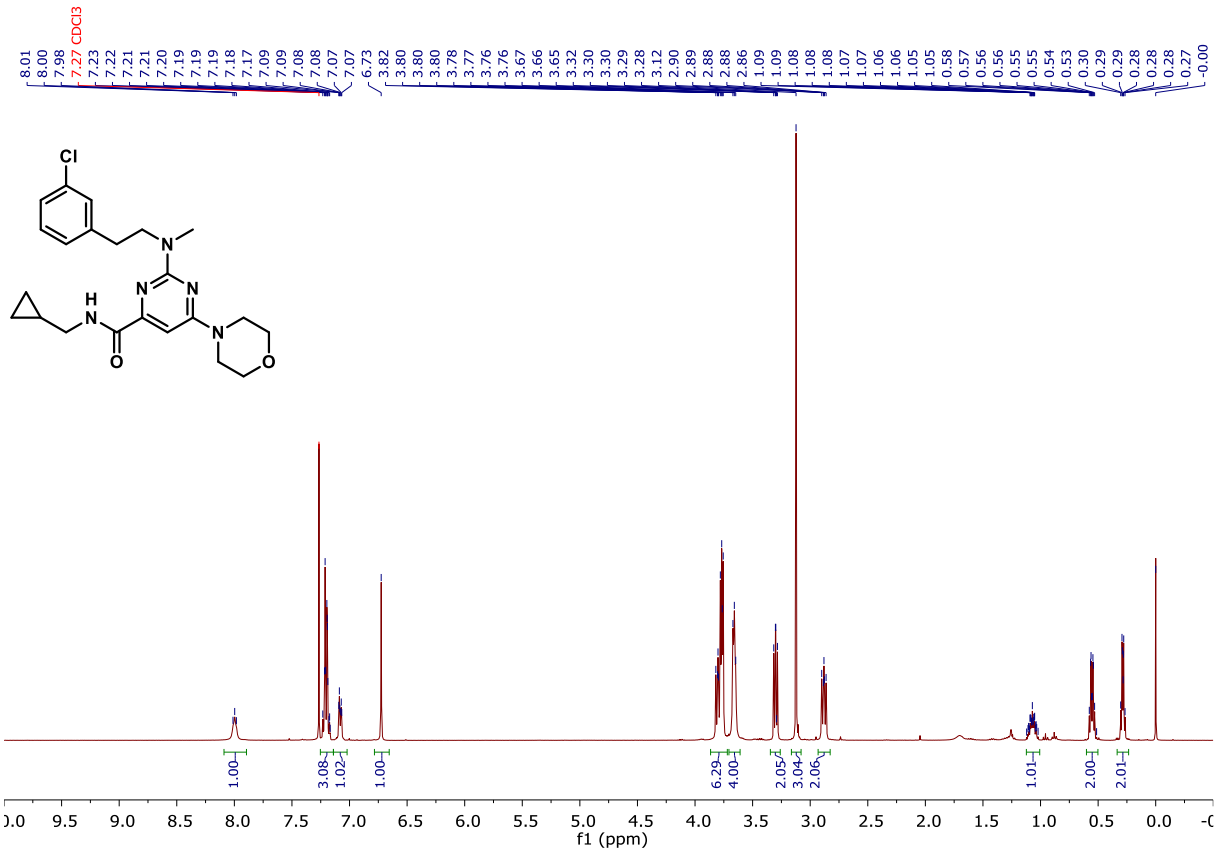
**$^{1}$H-NMR: 38**



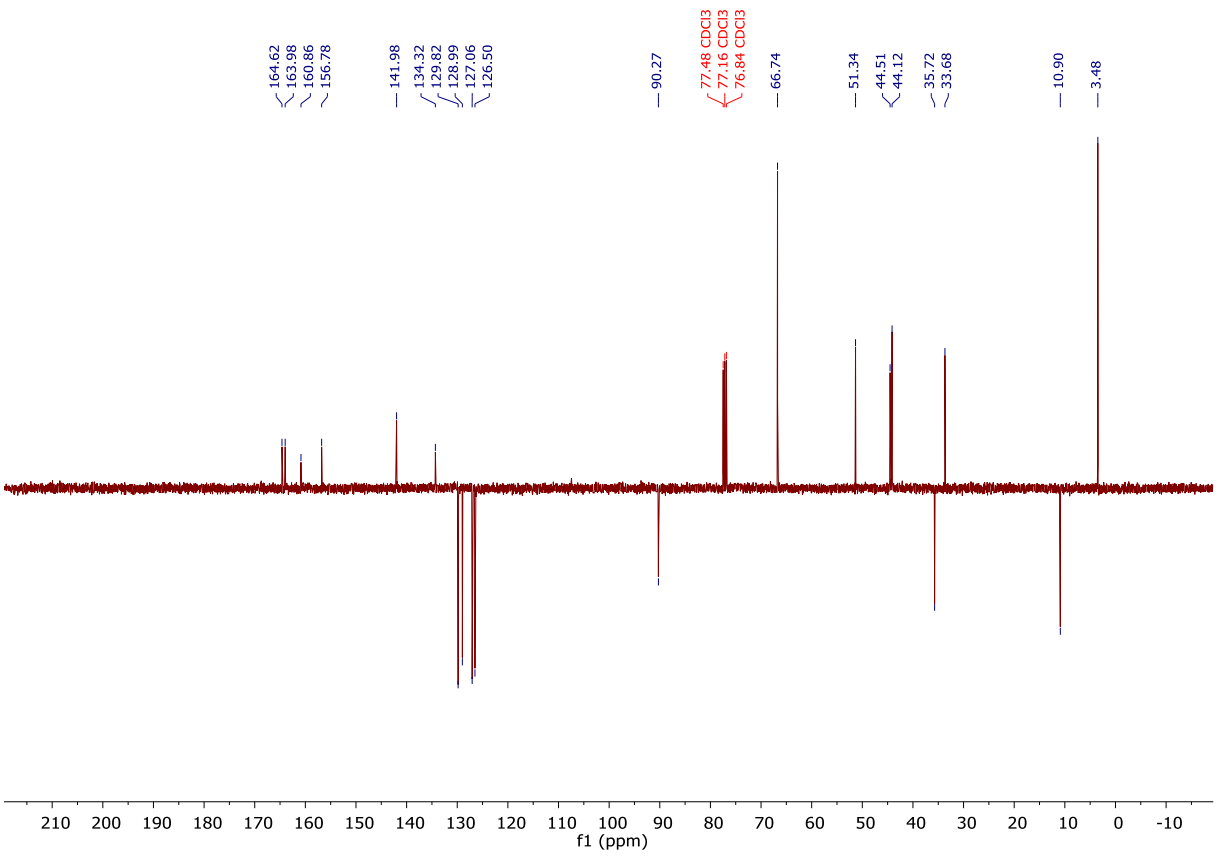

**$^{13}$C-NMR**



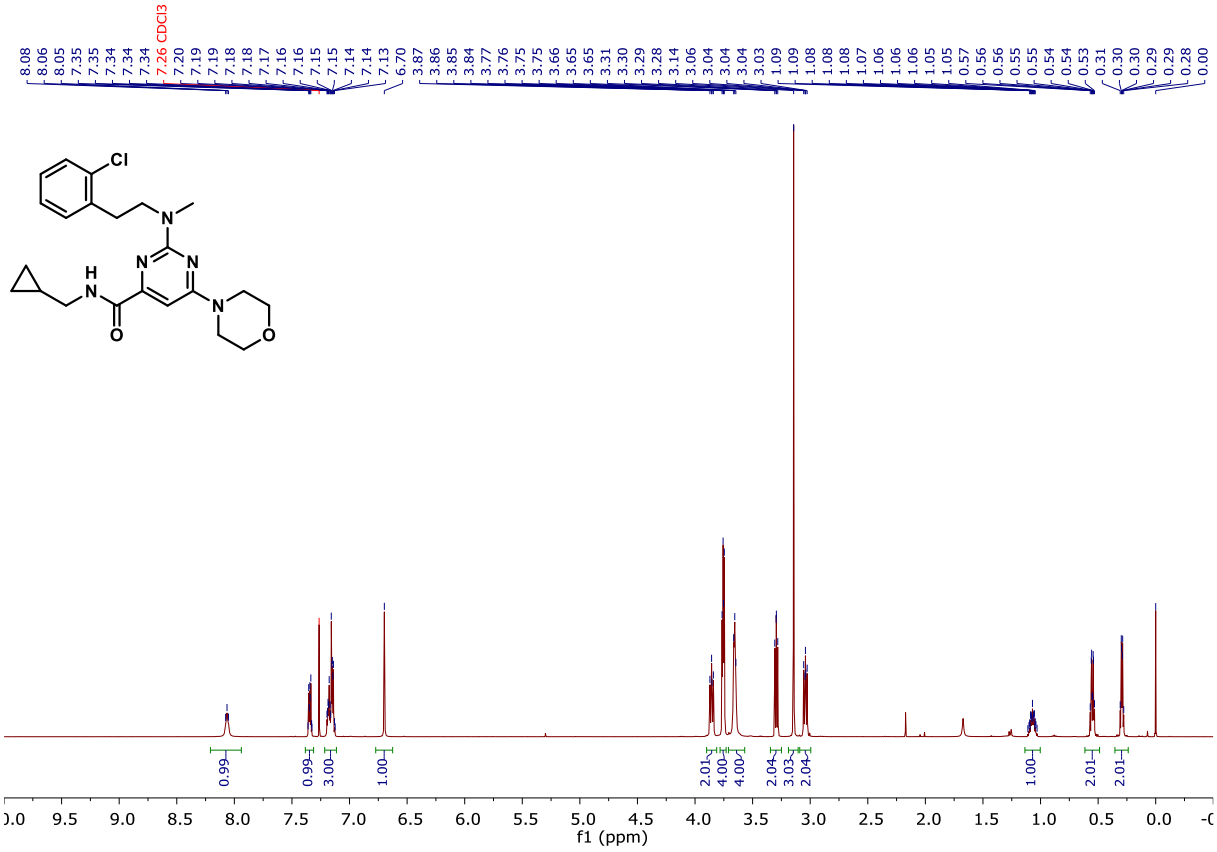
**$^{1}$H-NMR: 39**



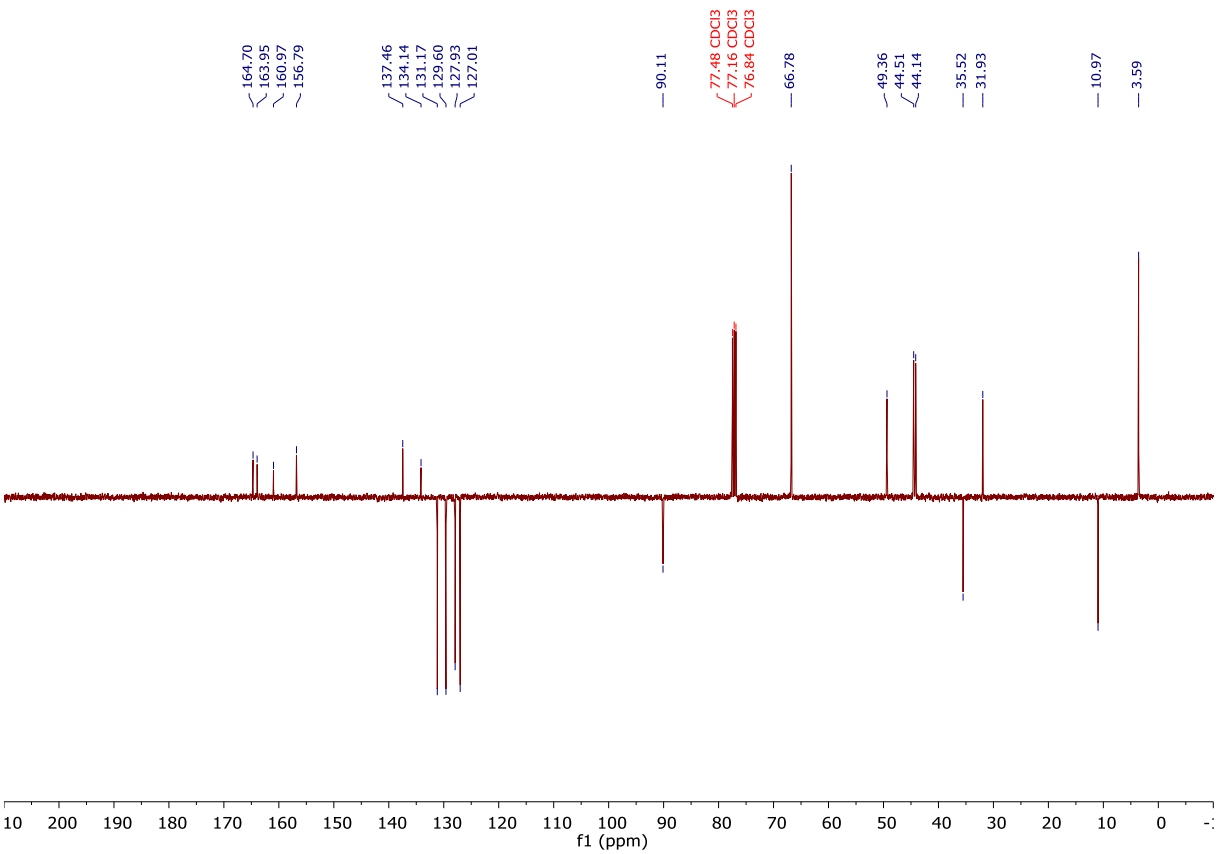

**$^{13}$C-NMR**



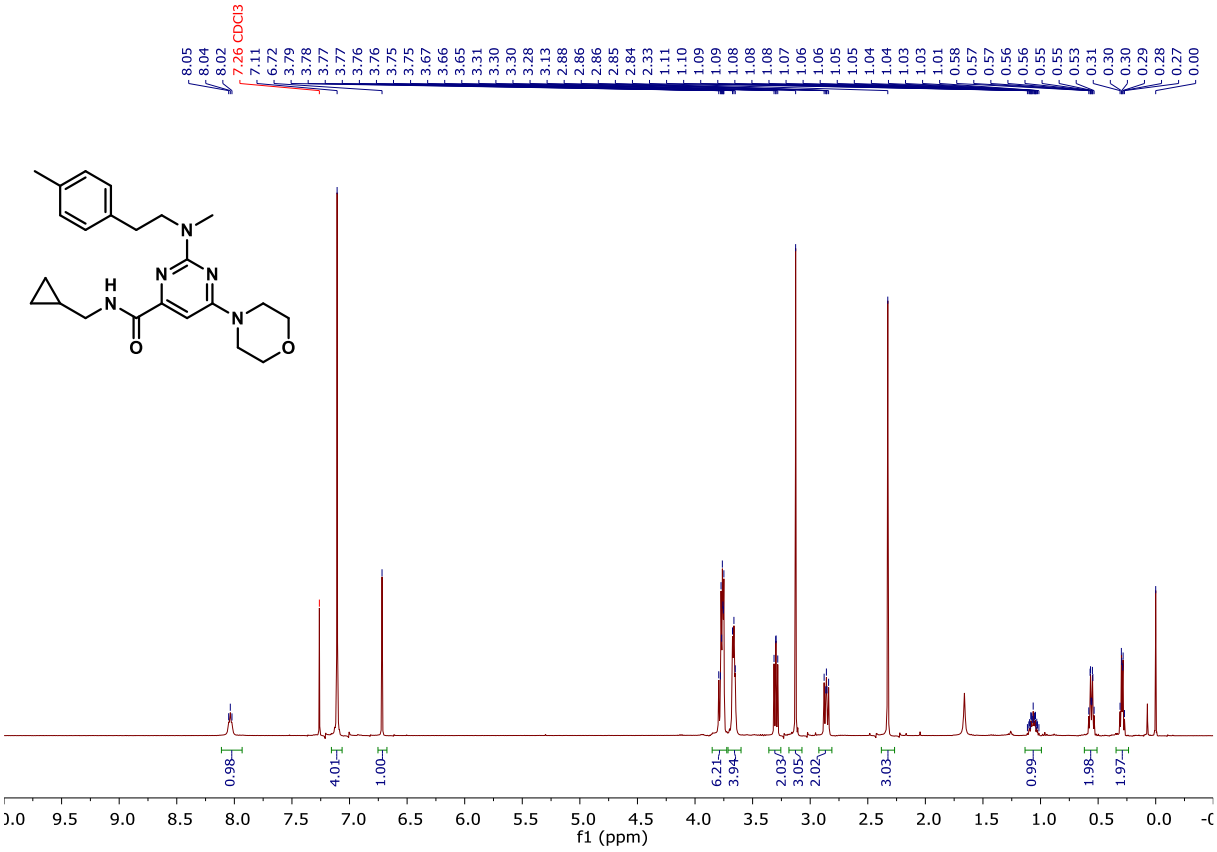
**$^{1}$H-NMR: 40**



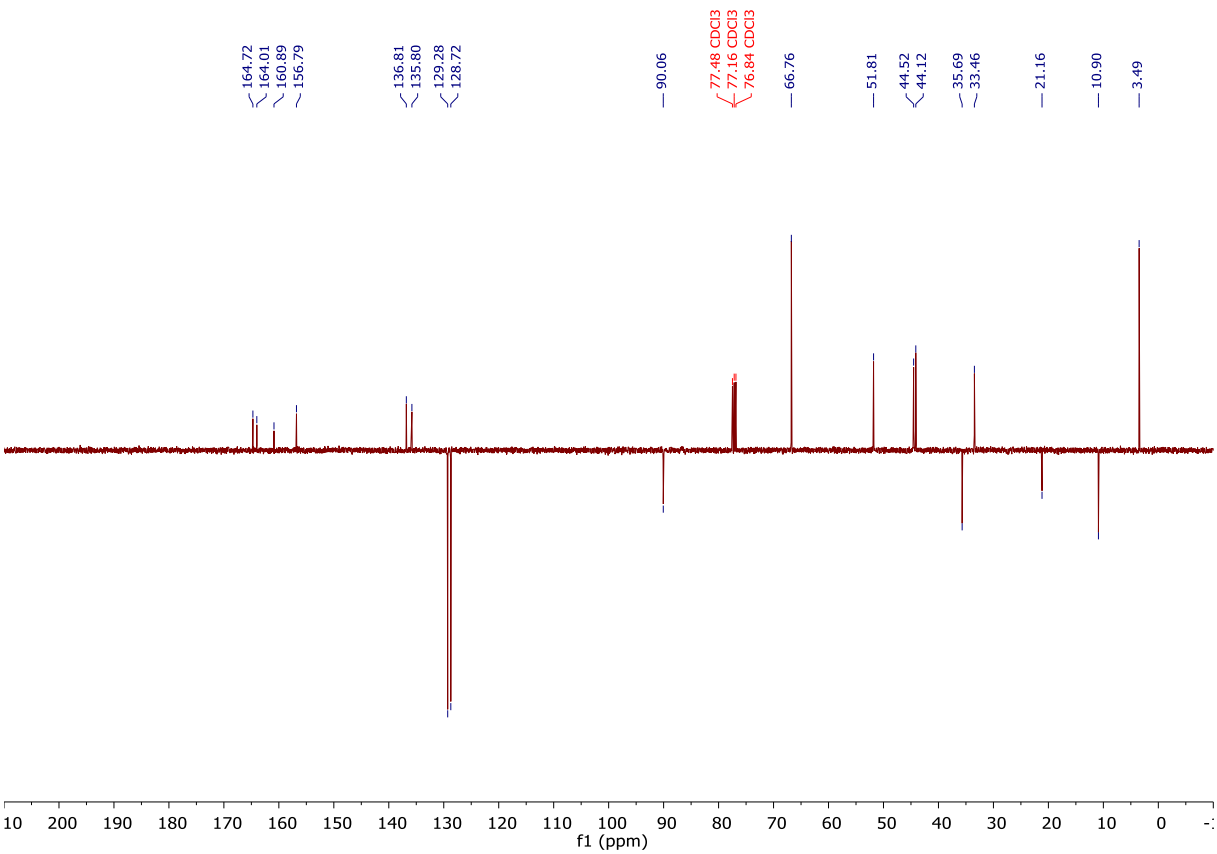

**$^{13}$C-NMR**



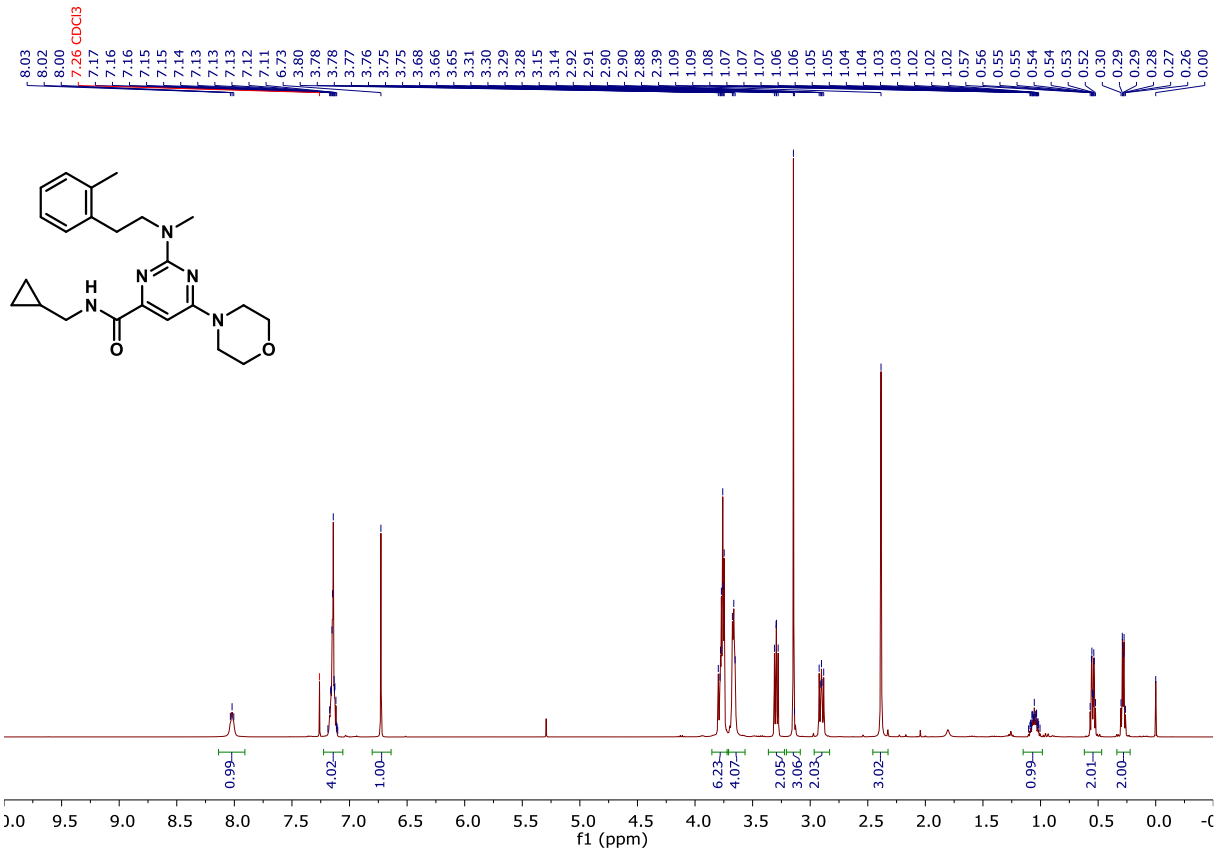
**$^{1}$H-NMR: 41**



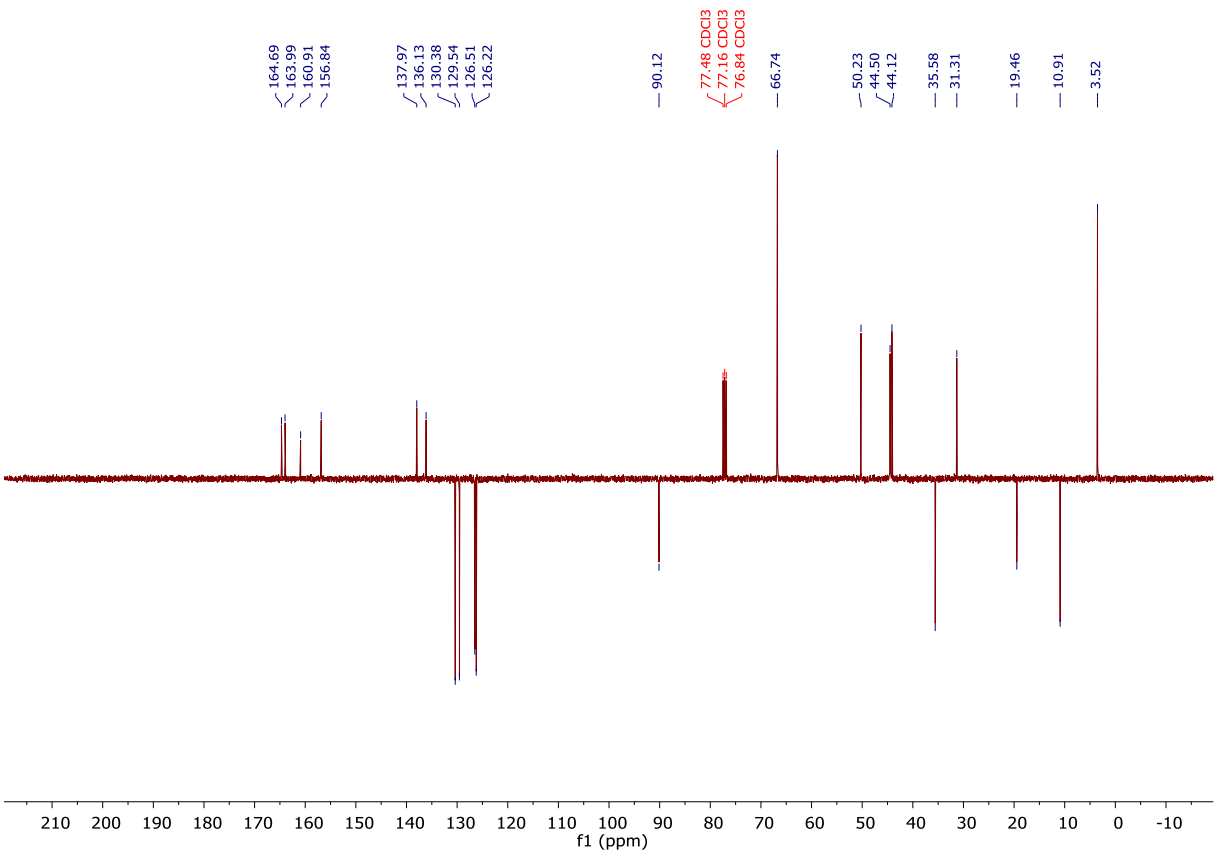

**$^{13}$C-NMR**



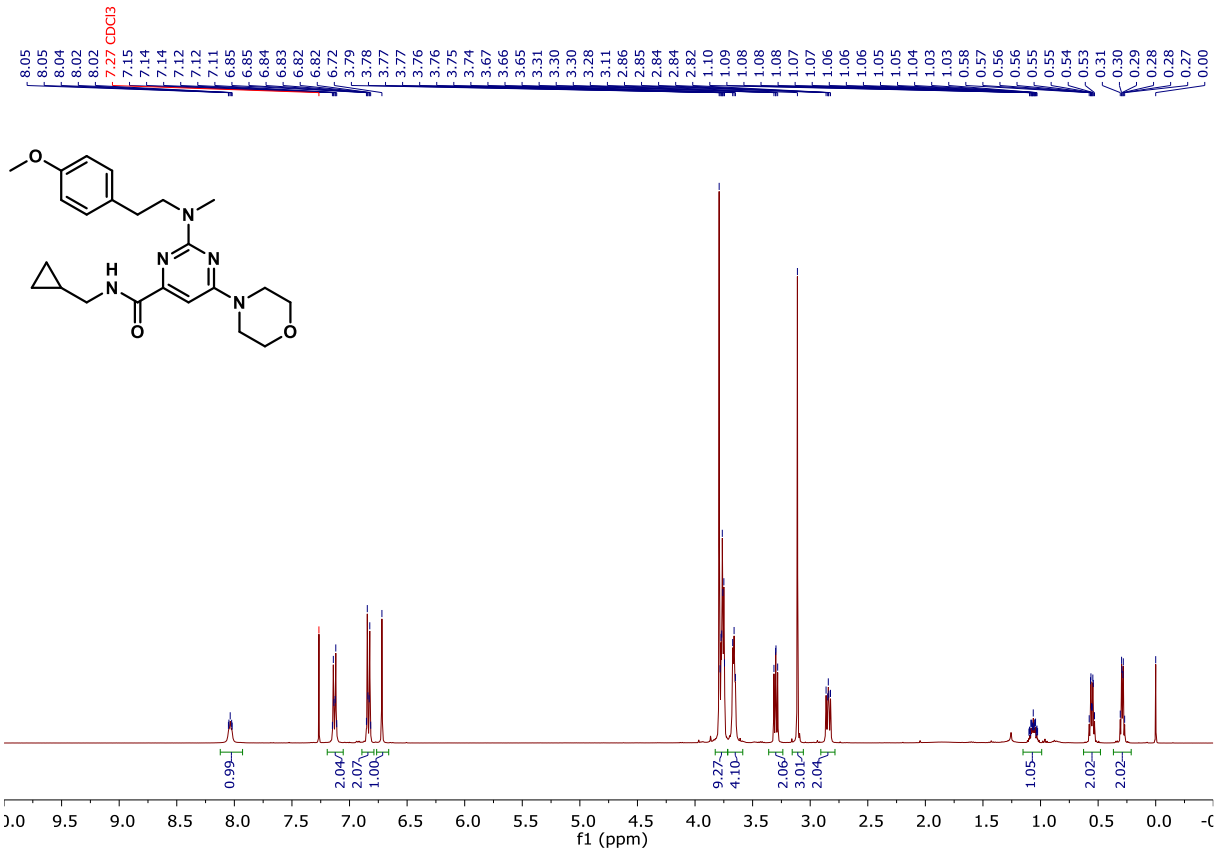
**$^{1}$H-NMR: 42**



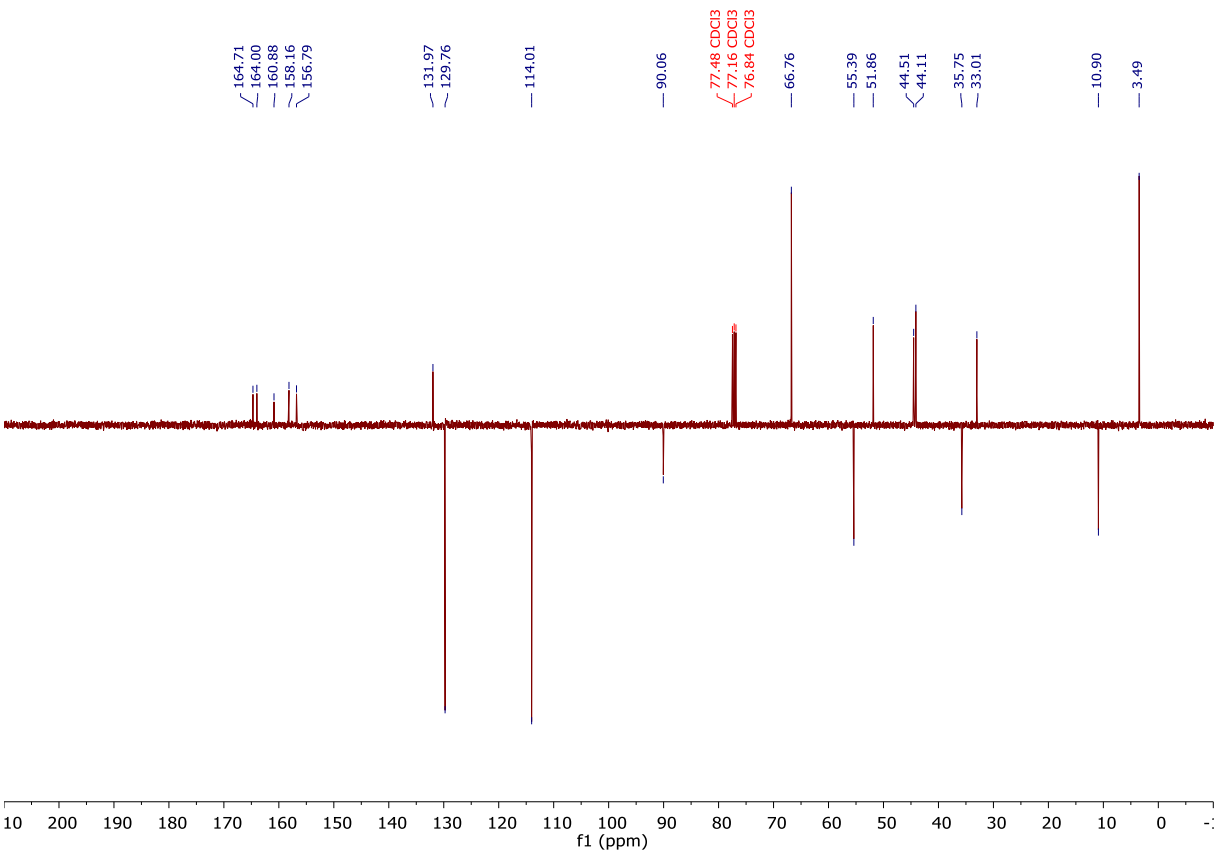

**$^{13}$C-NMR**



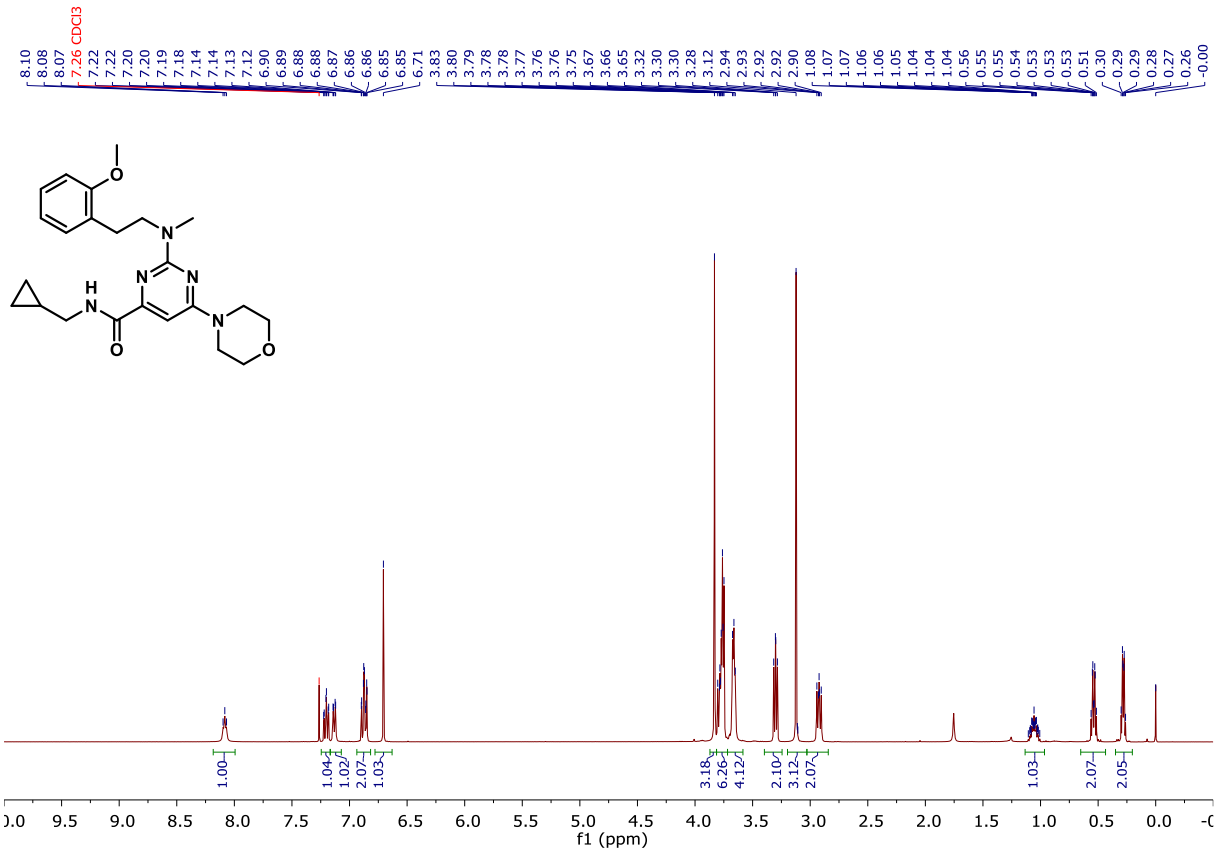
**$^{1}$H-NMR: 43**



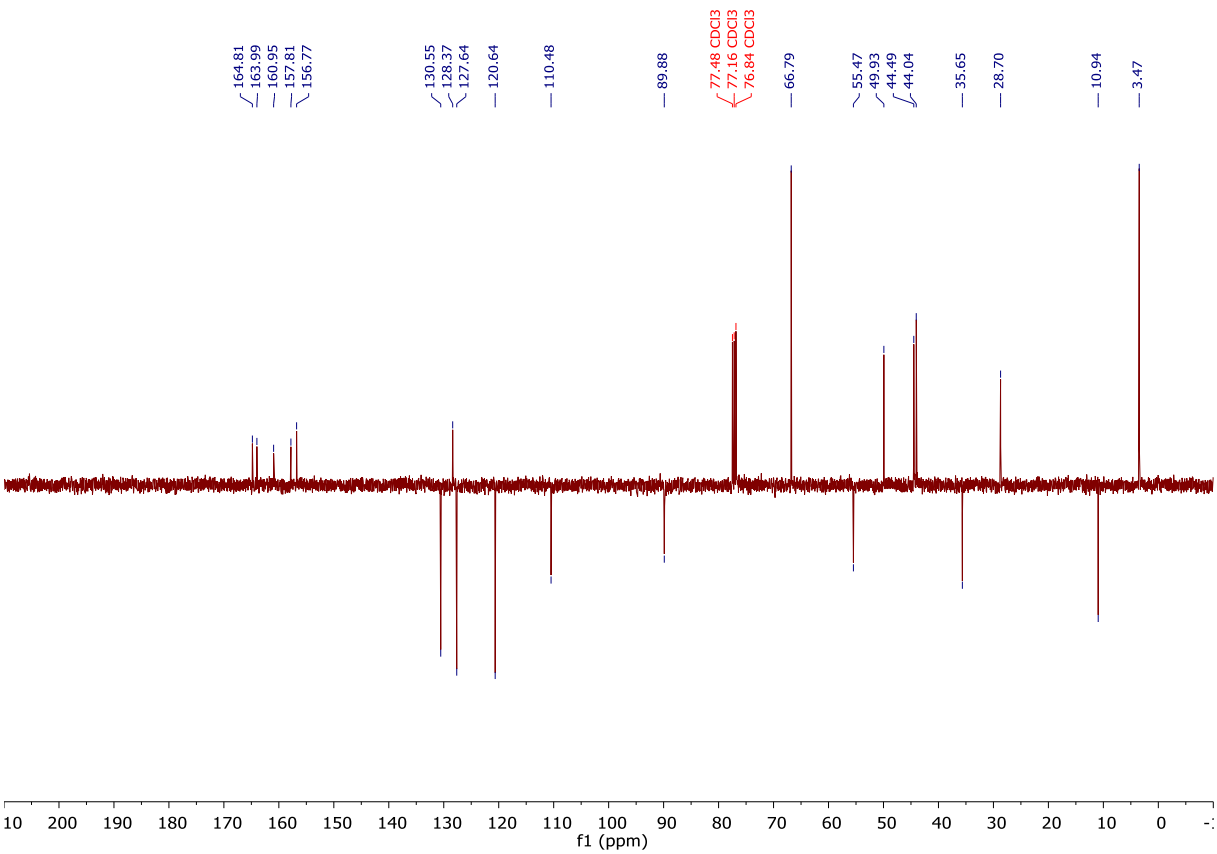

**$^{13}$C-NMR**



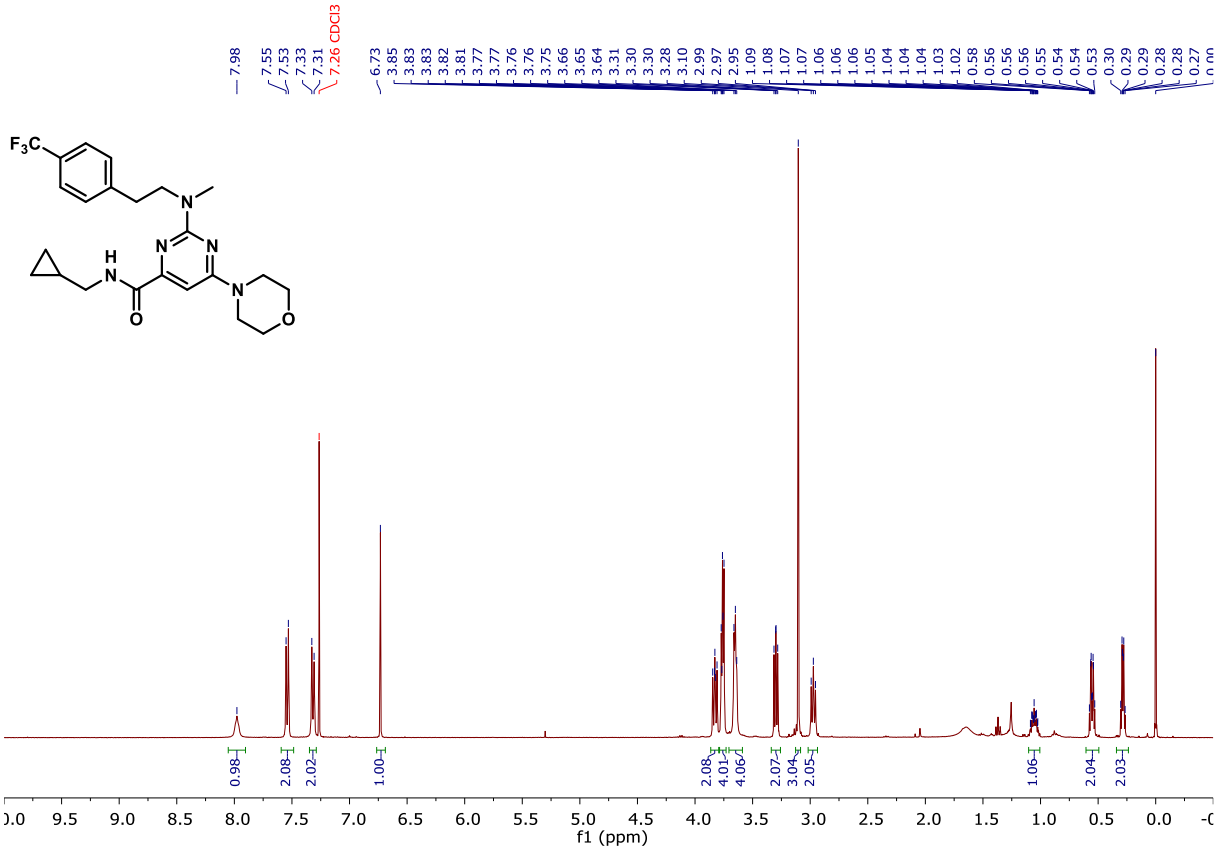
**$^{1}$H-NMR: 44**



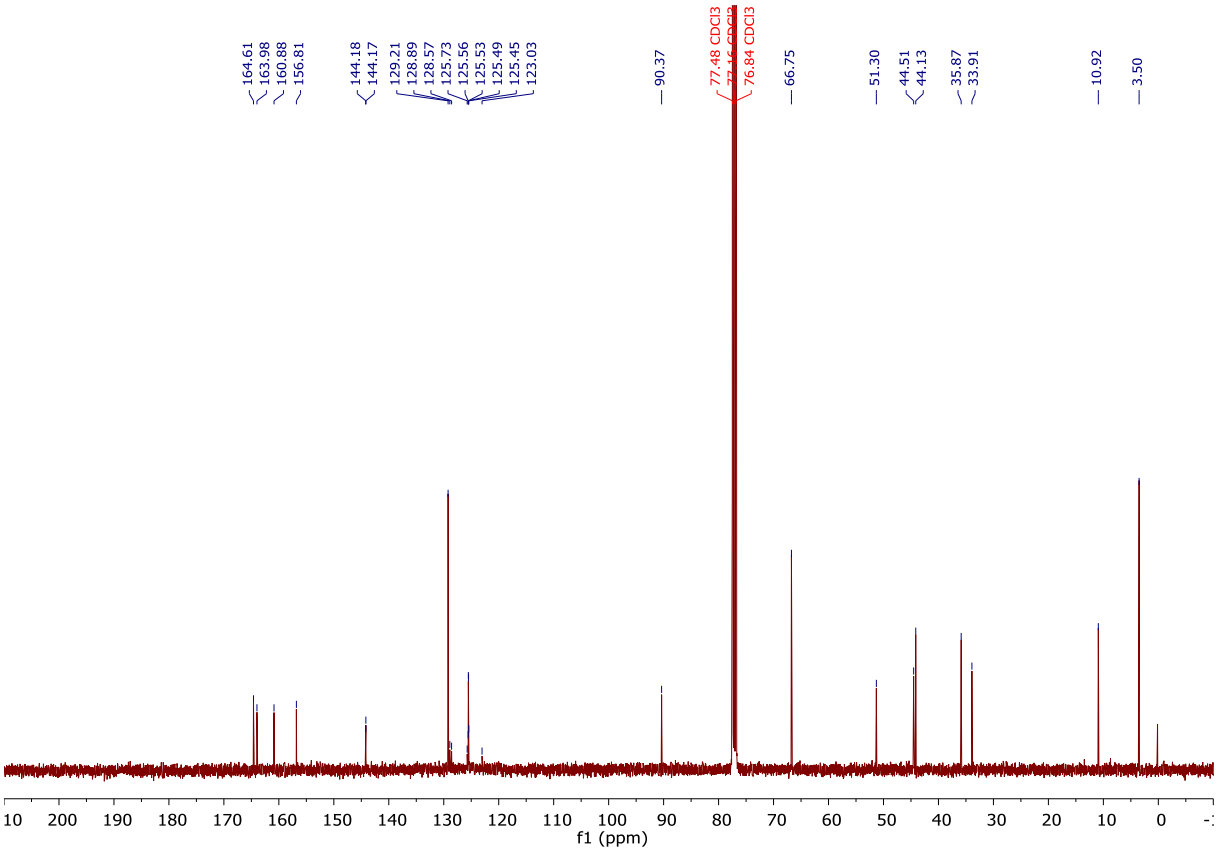

**$^{13}$C-NMR**



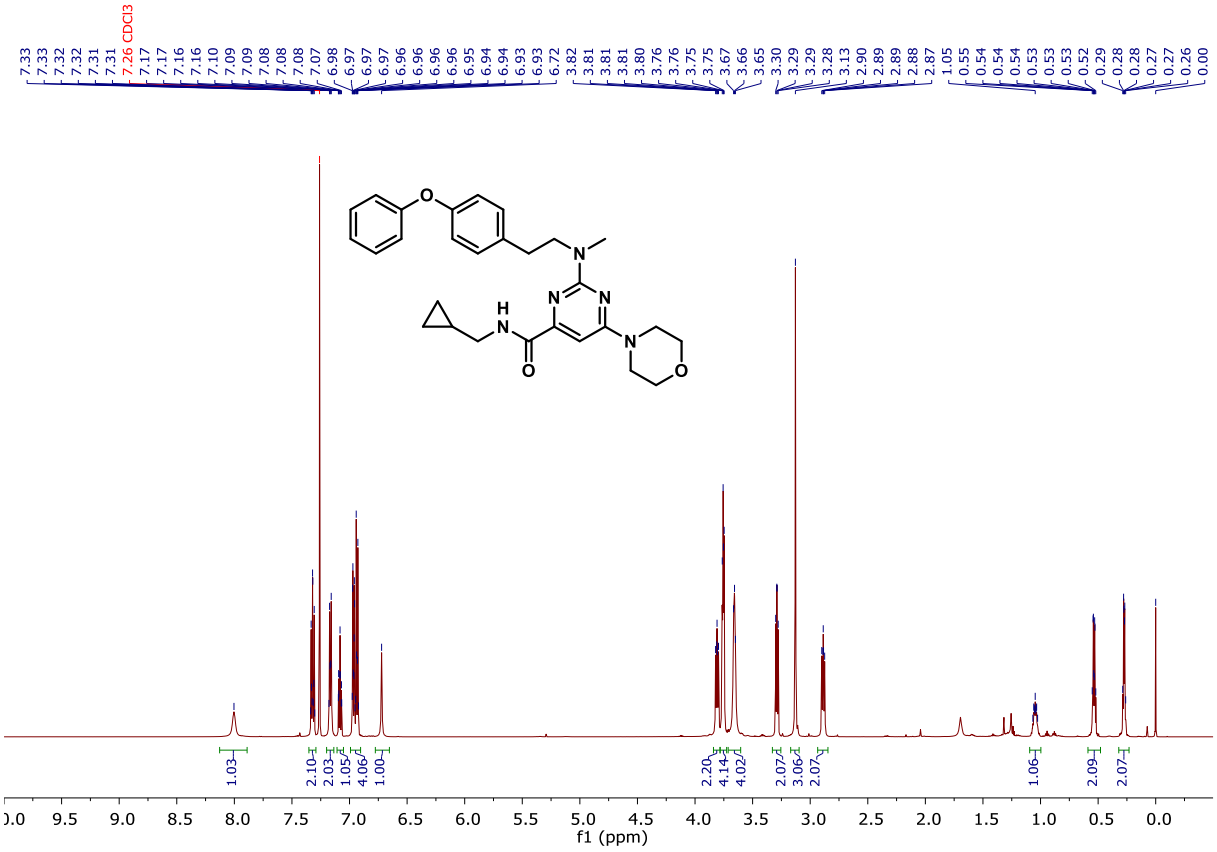
**$^{1}$H-NMR: 45**



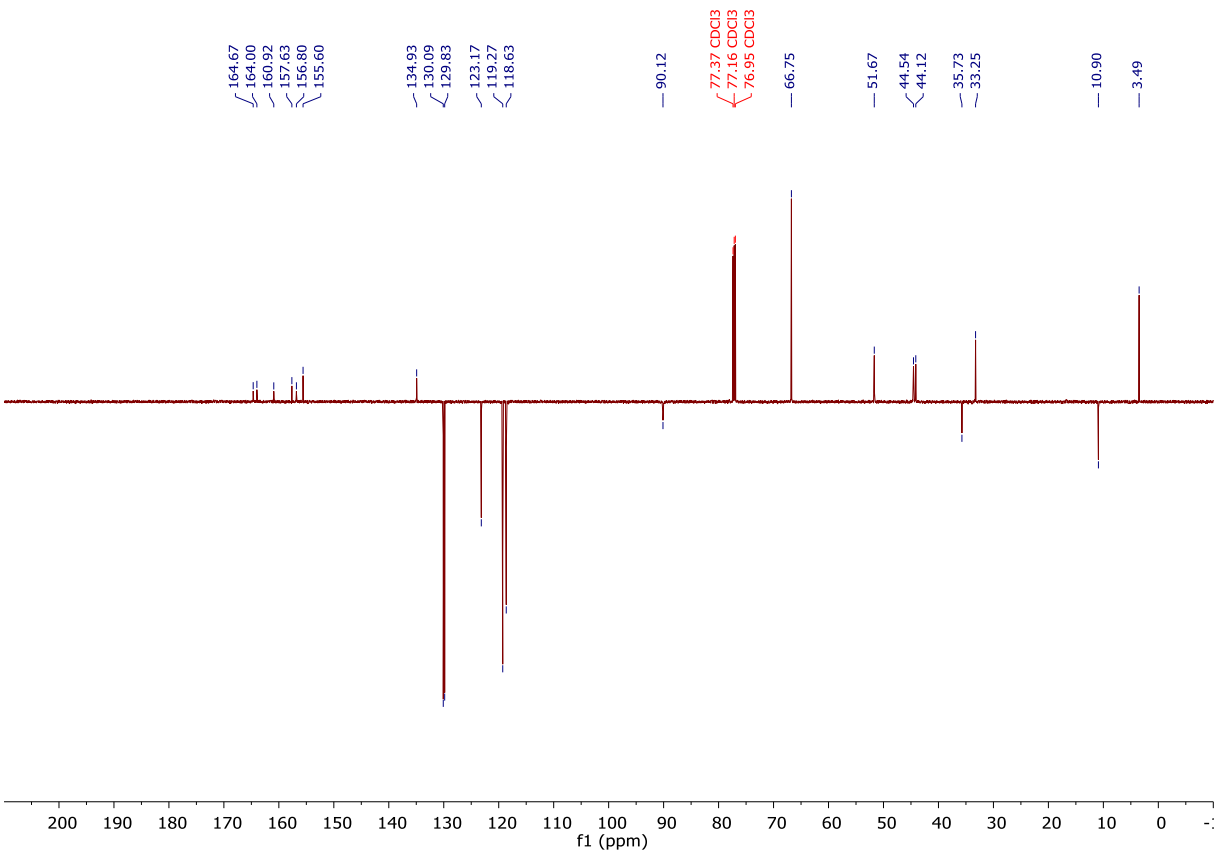

**$^{13}$C-NMR**



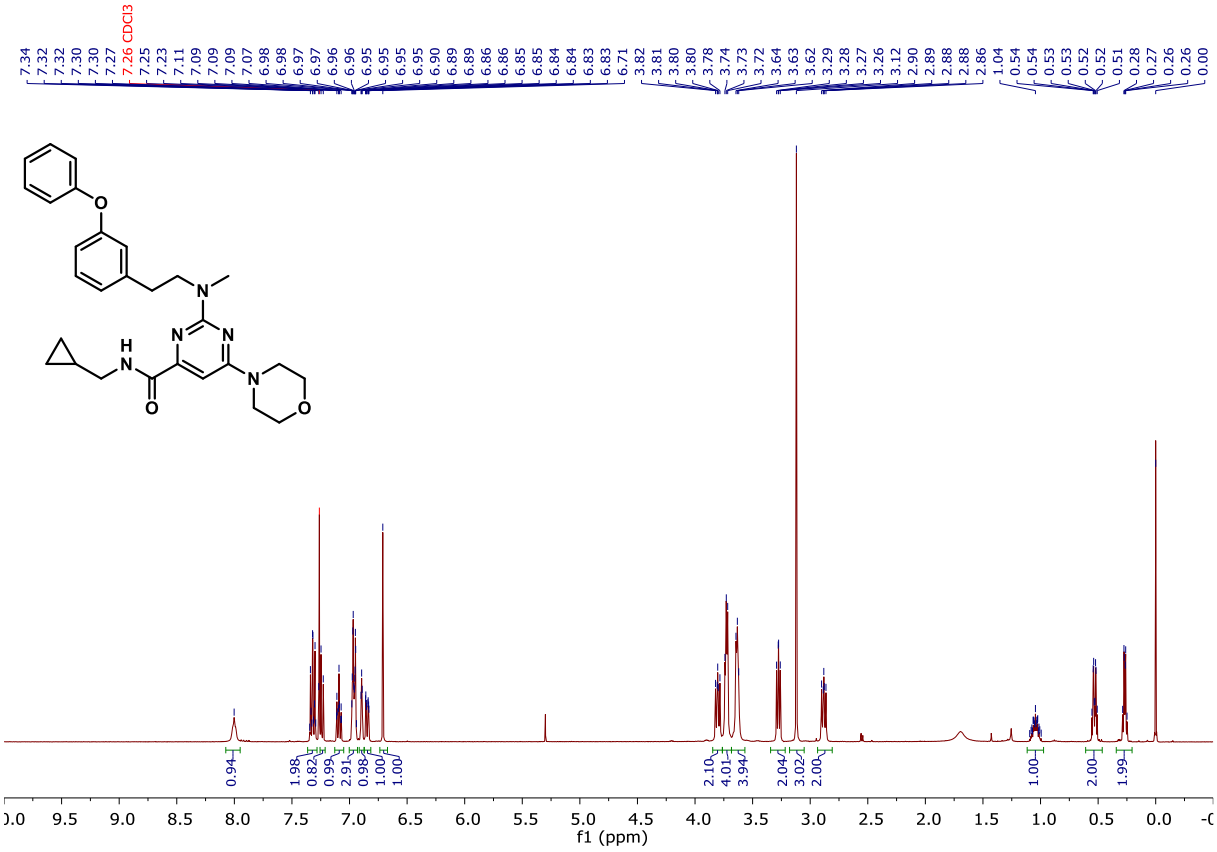
**$^{1}$H-NMR: 46**



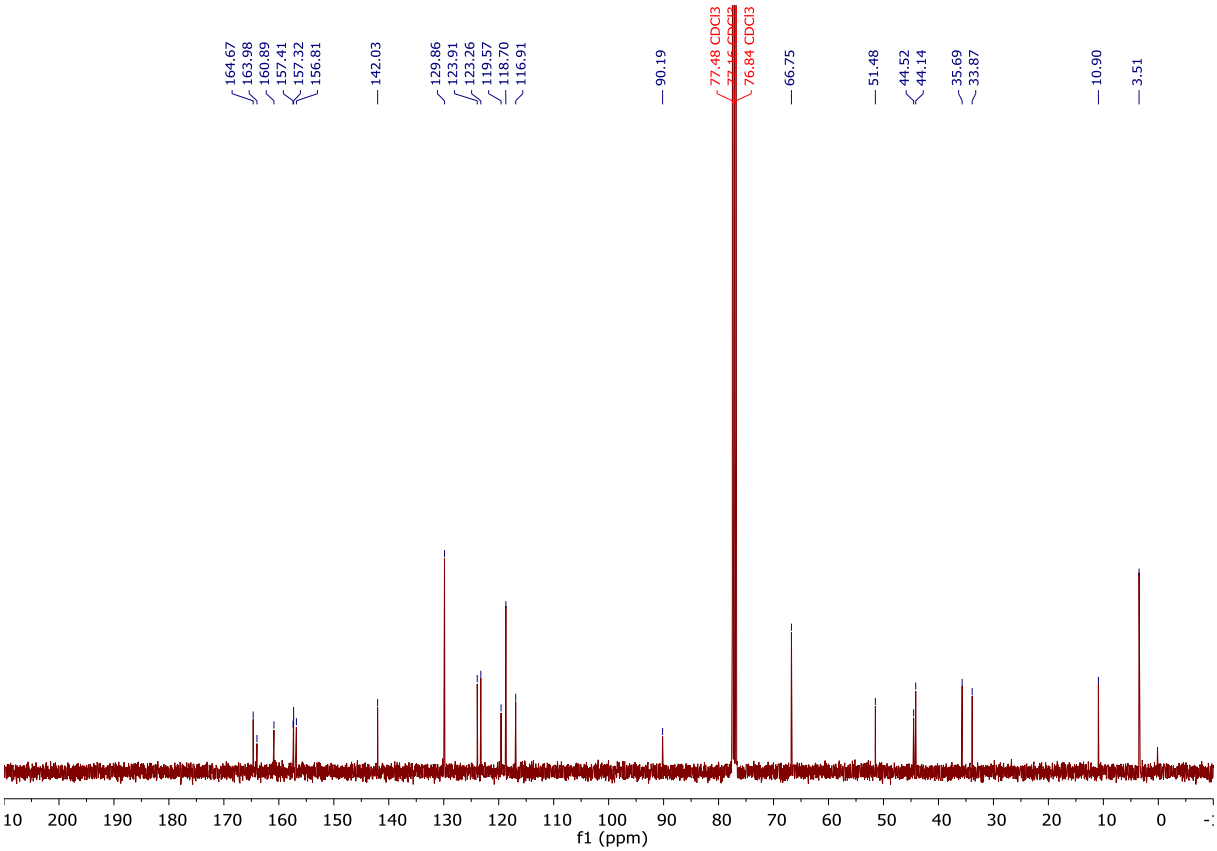

**$^{13}$C-NMR**



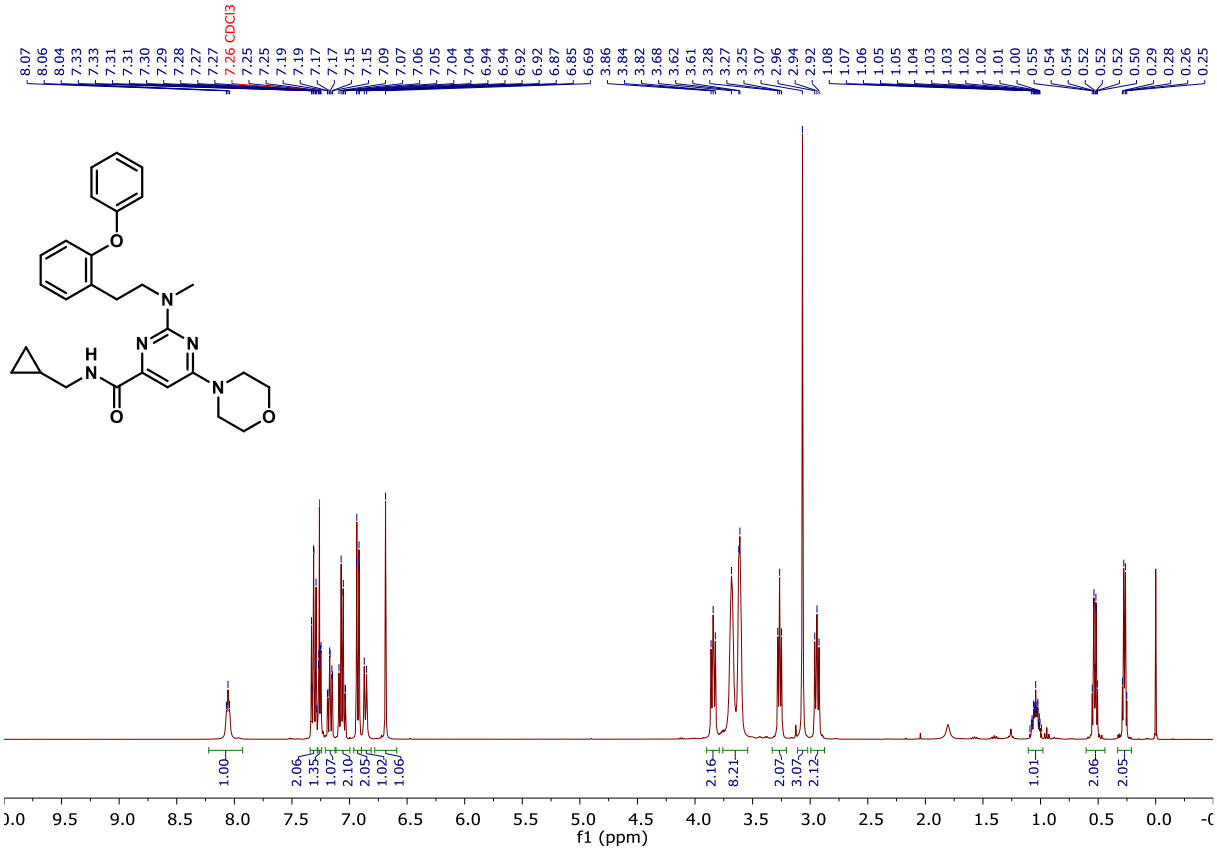
**$^{1}$H-NMR: 47**



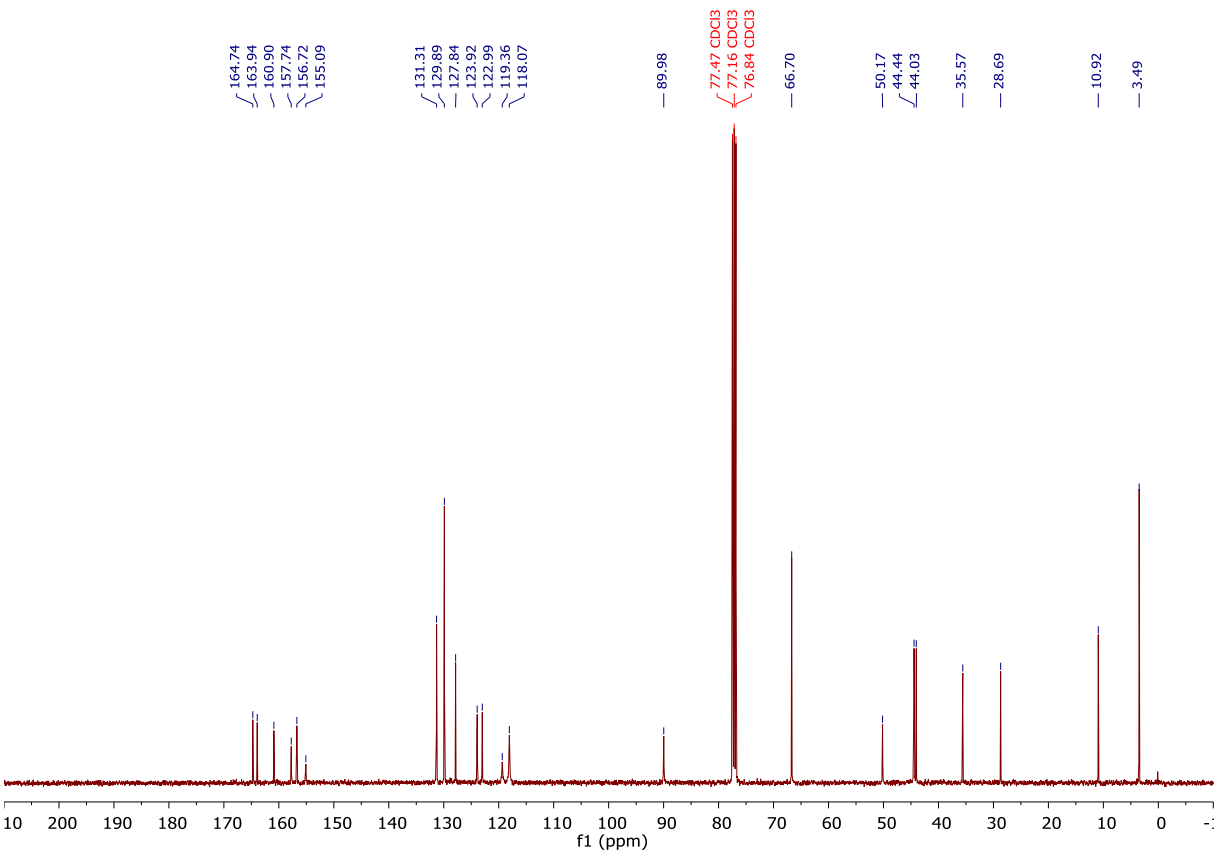

**$^{13}$C-NMR**



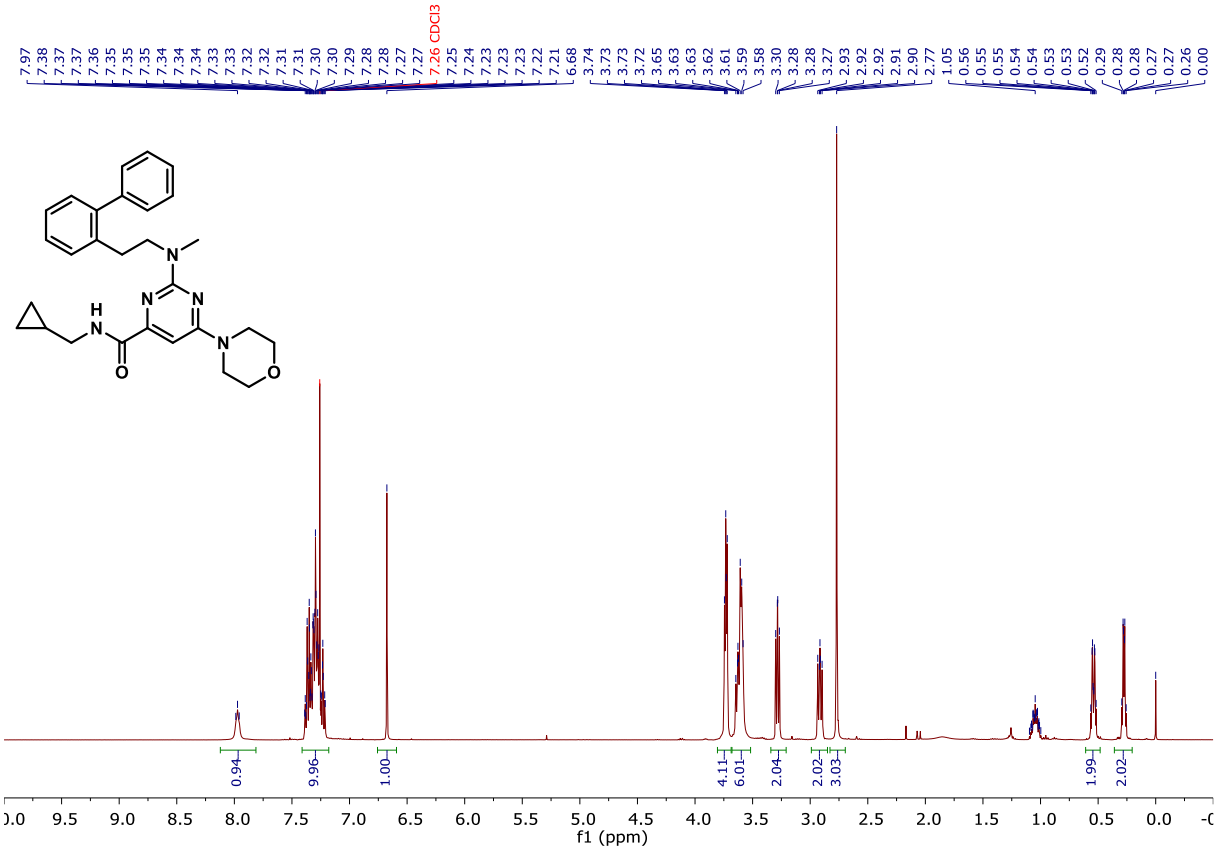
**$^{1}$H-NMR: 48**



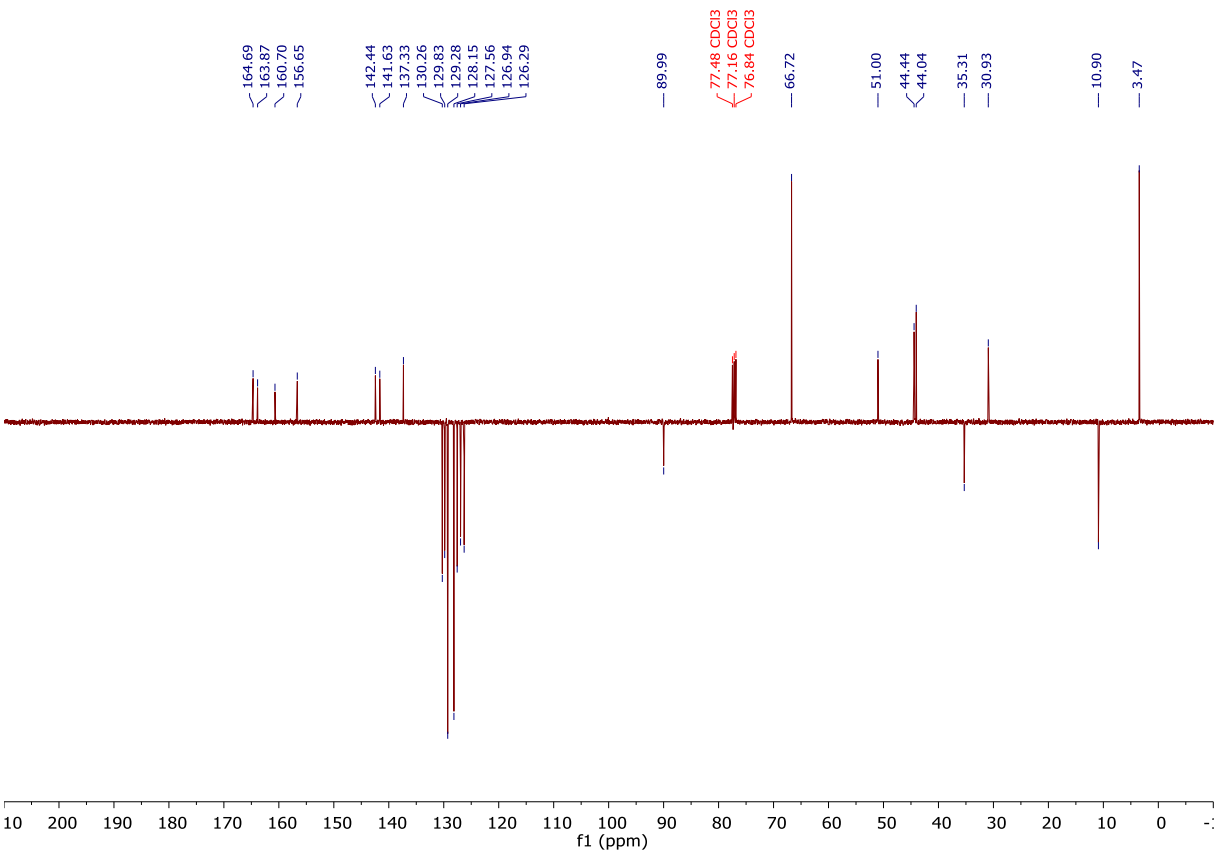

**$^{13}$C-NMR**



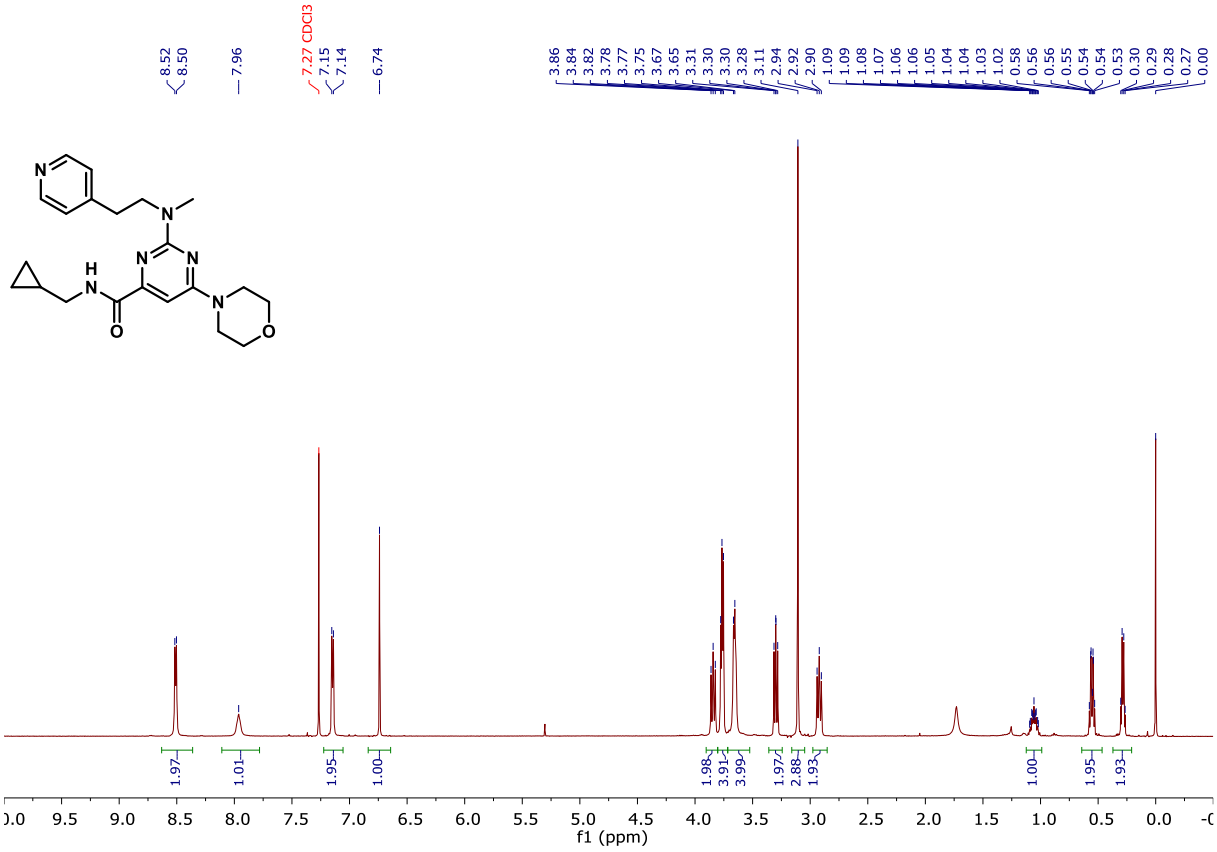
**$^{1}$H-NMR: 49**



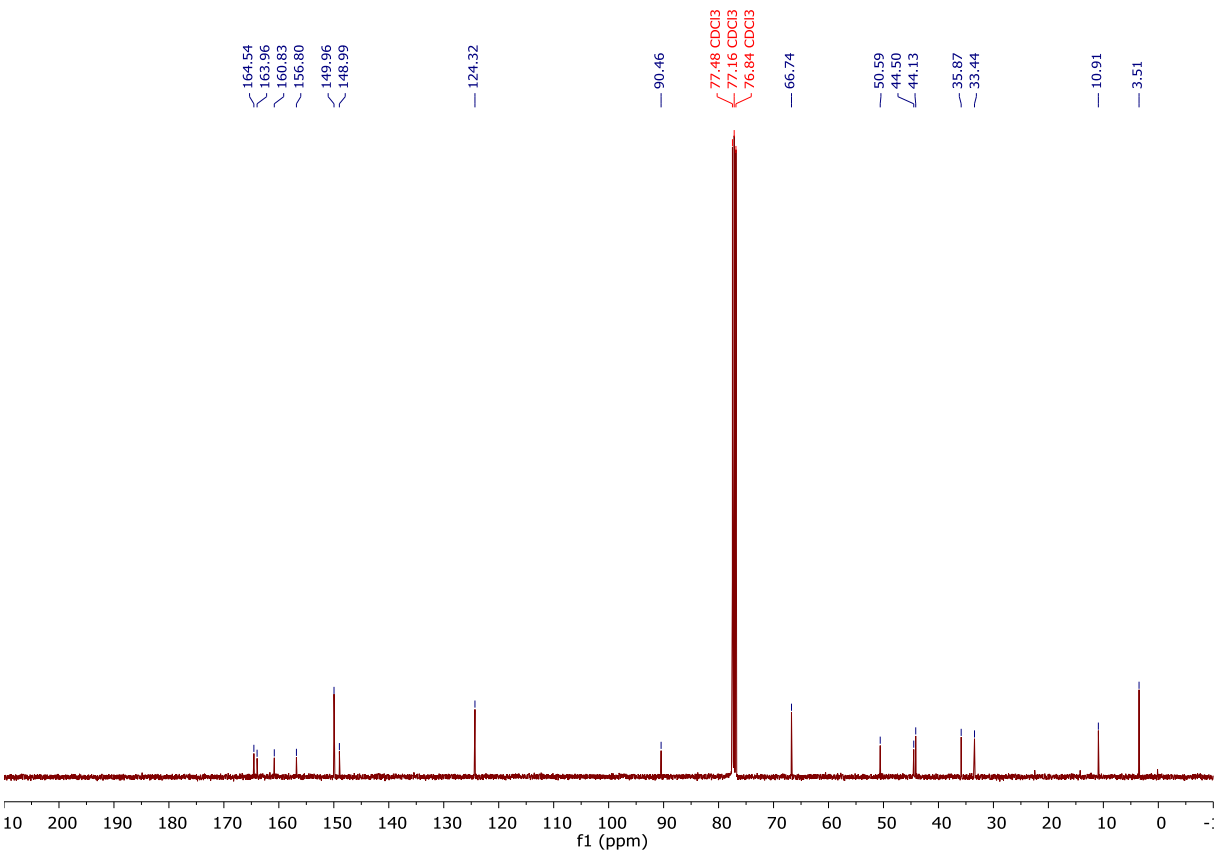

**$^{13}$C-NMR**



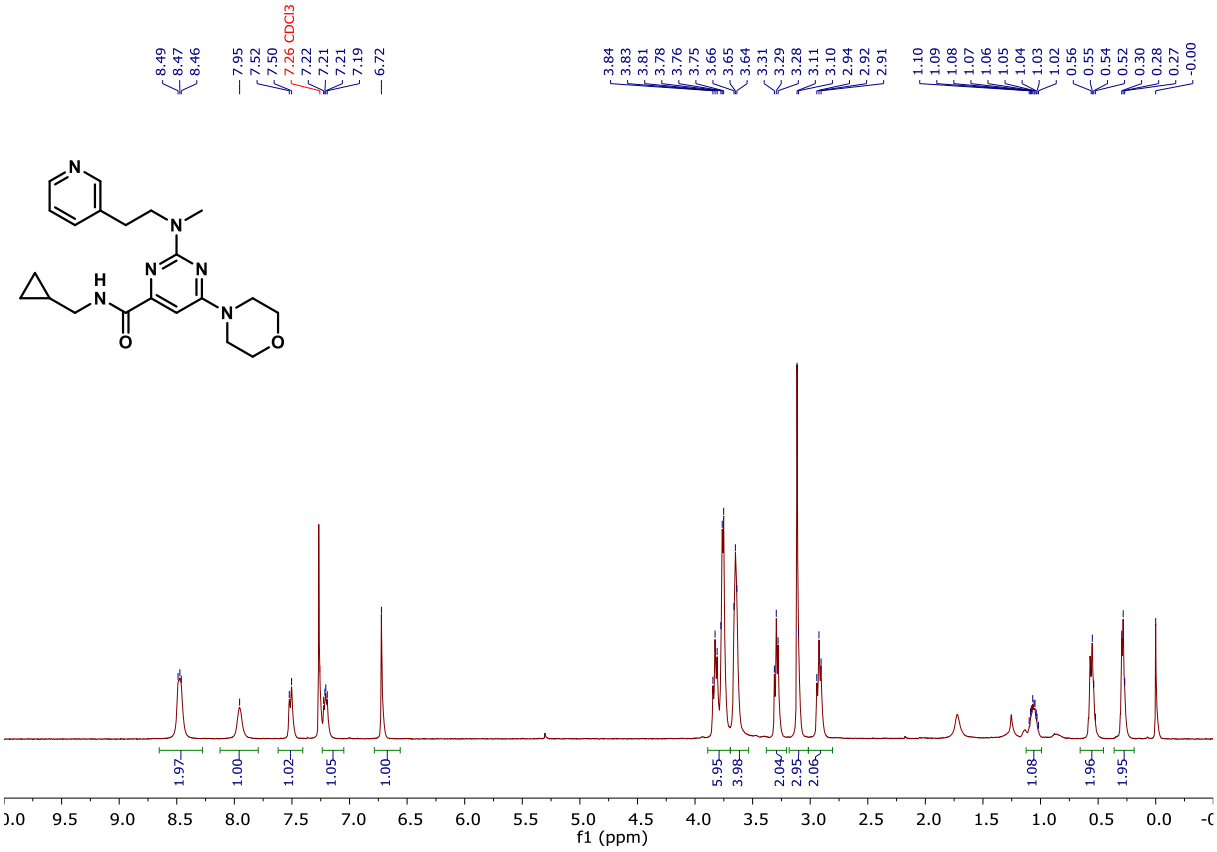
**$^{1}$H-NMR: 50**



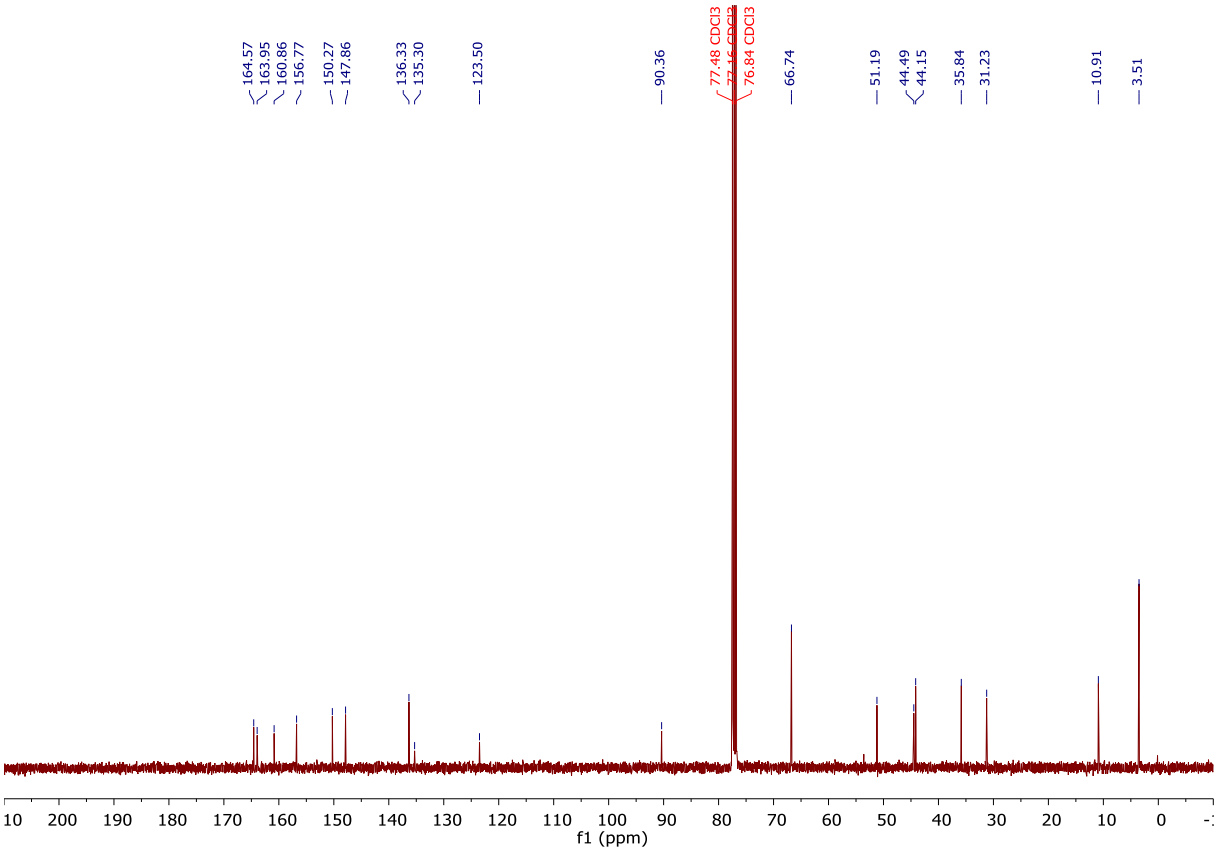

**$^{13}$C-NMR**



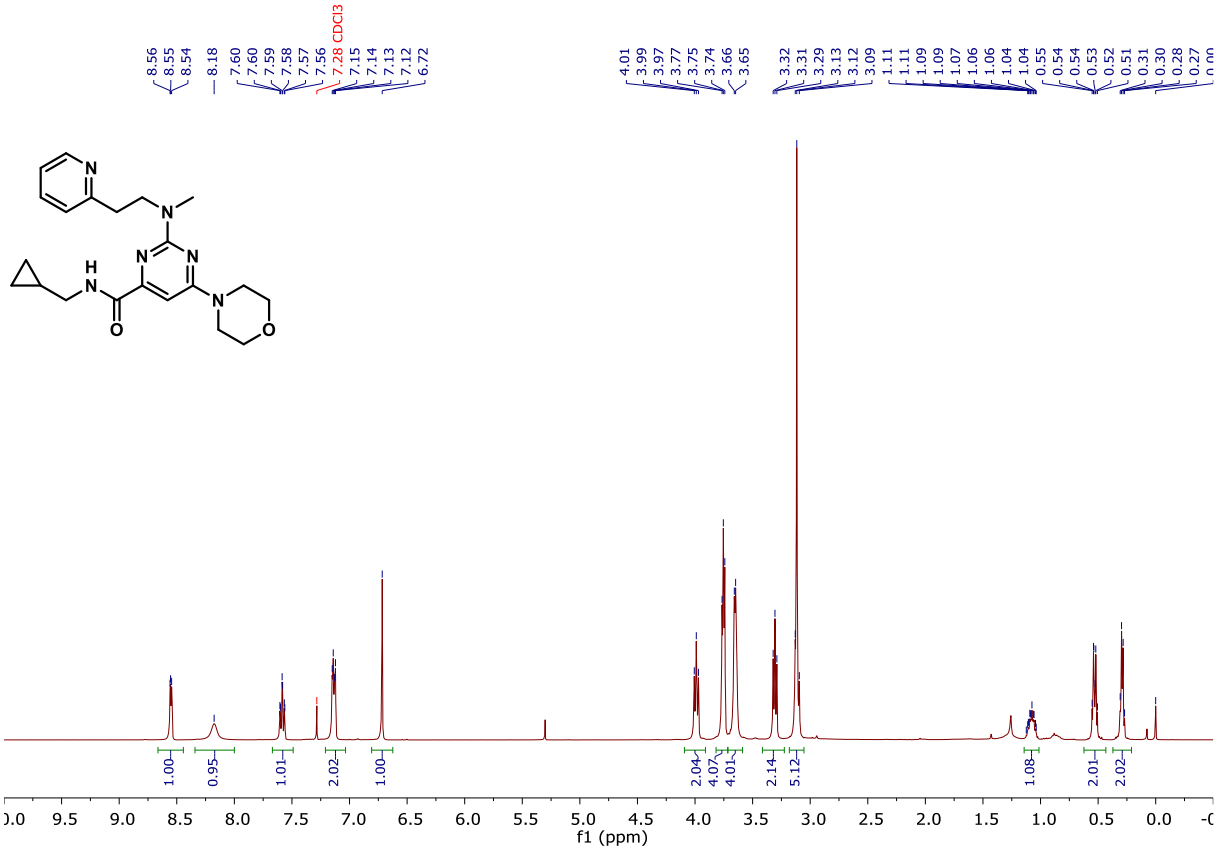
**$^{1}$H-NMR: 51**



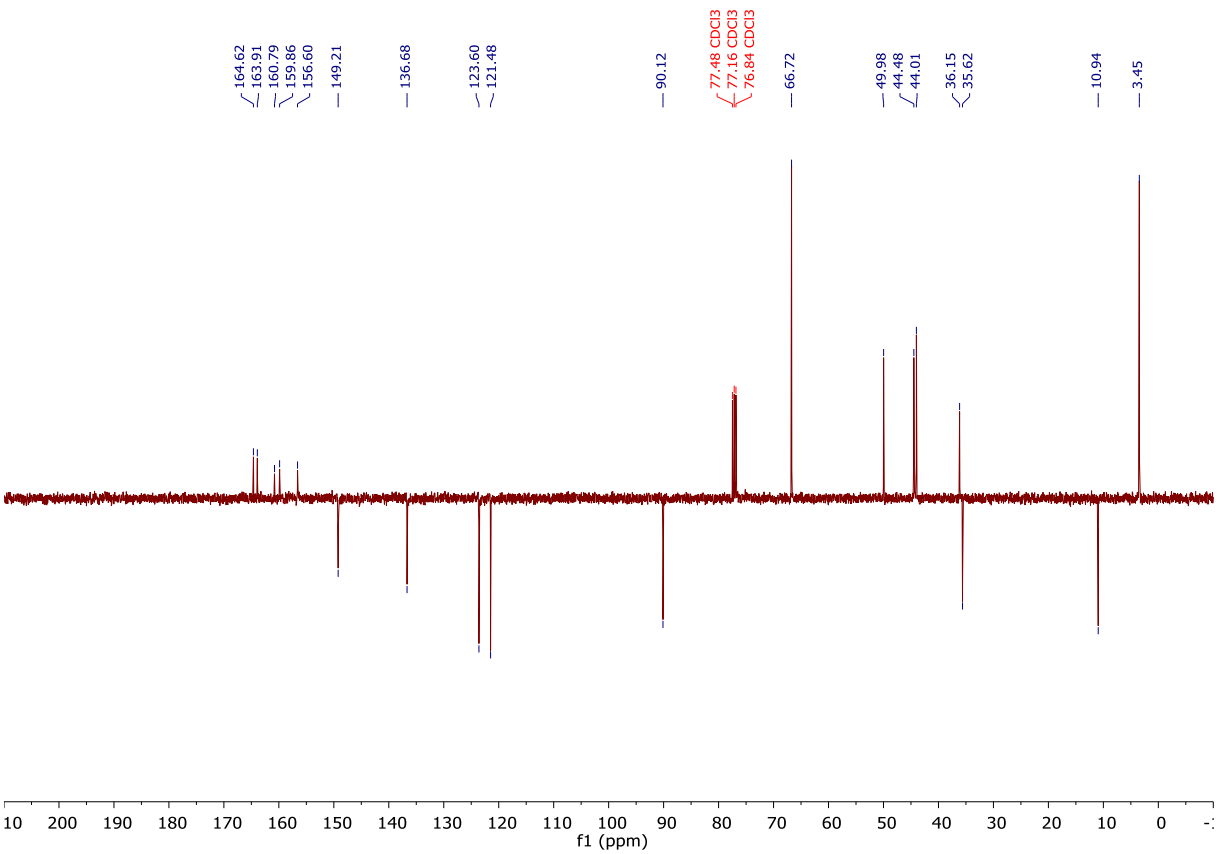

**$^{13}$C-NMR**



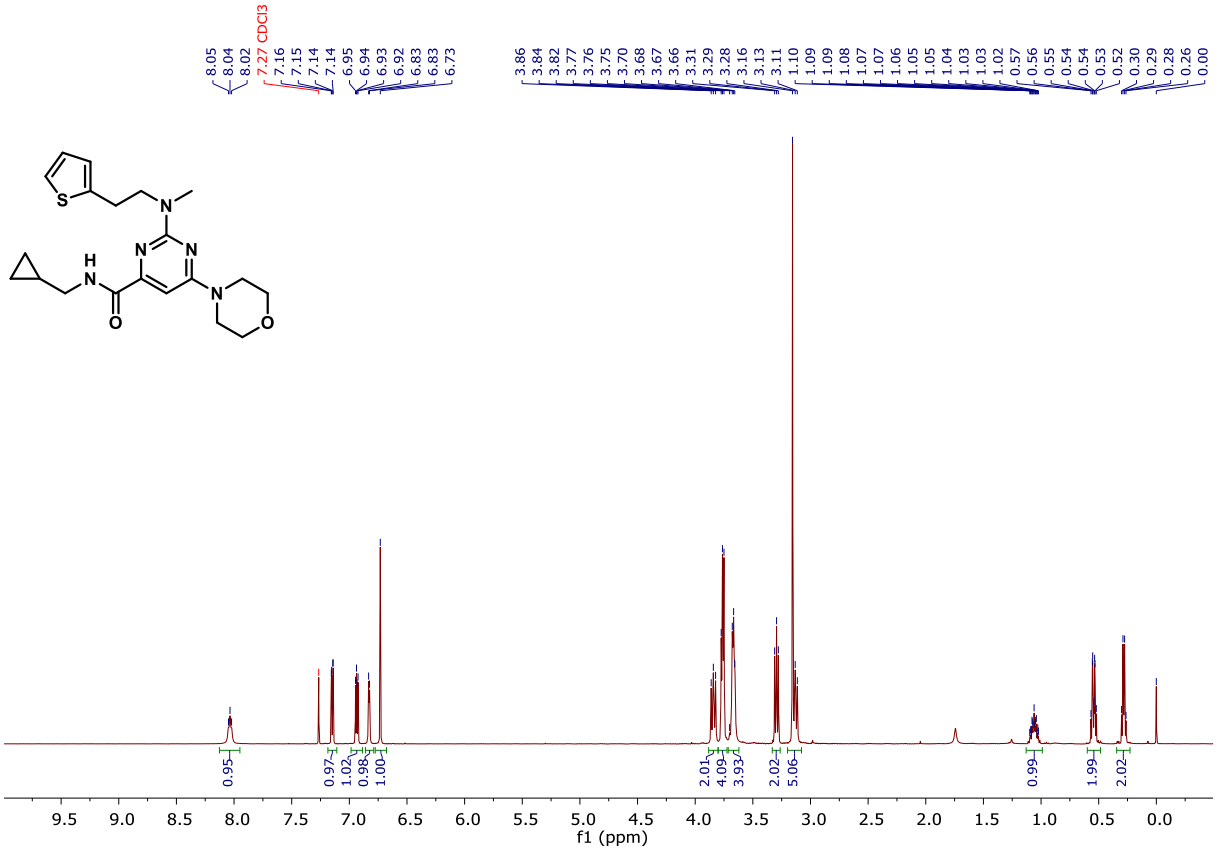
**$^{1}$H-NMR: 52**



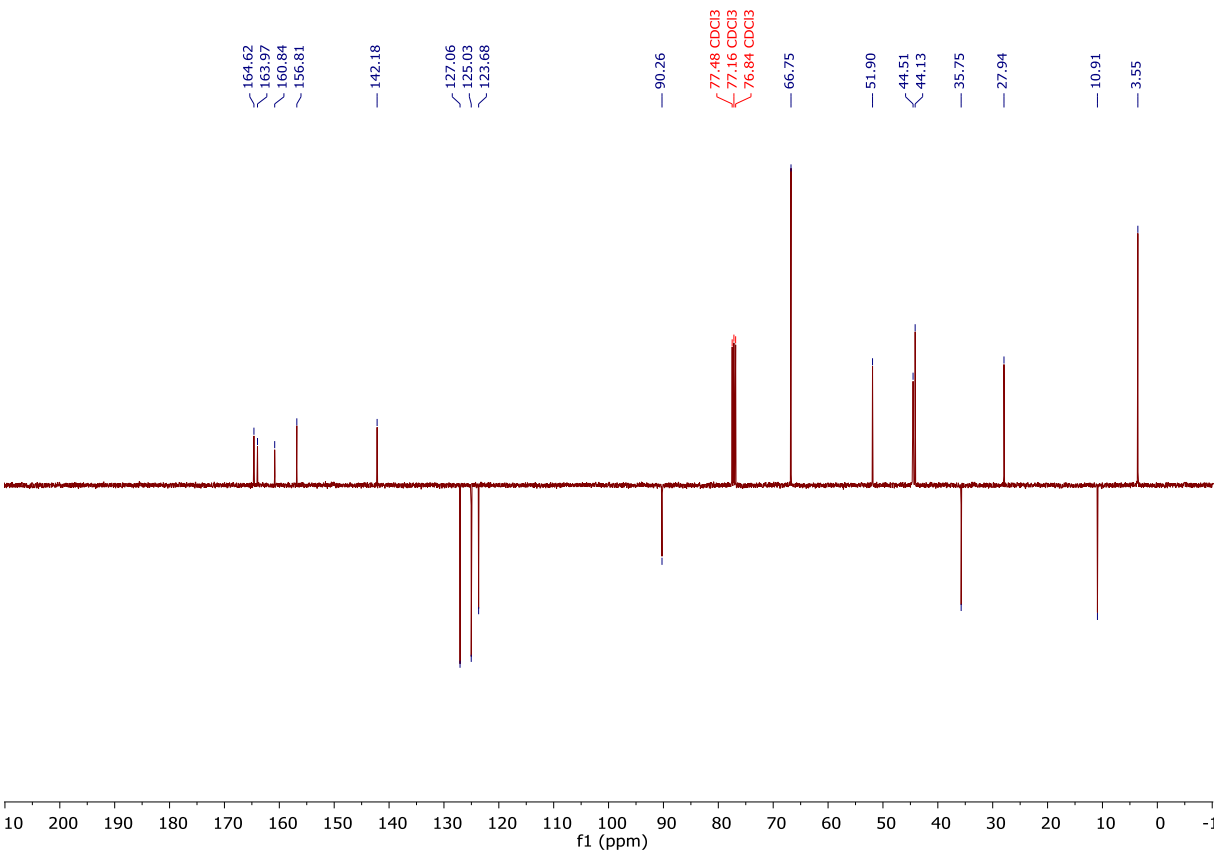

**$^{13}$C-NMR**



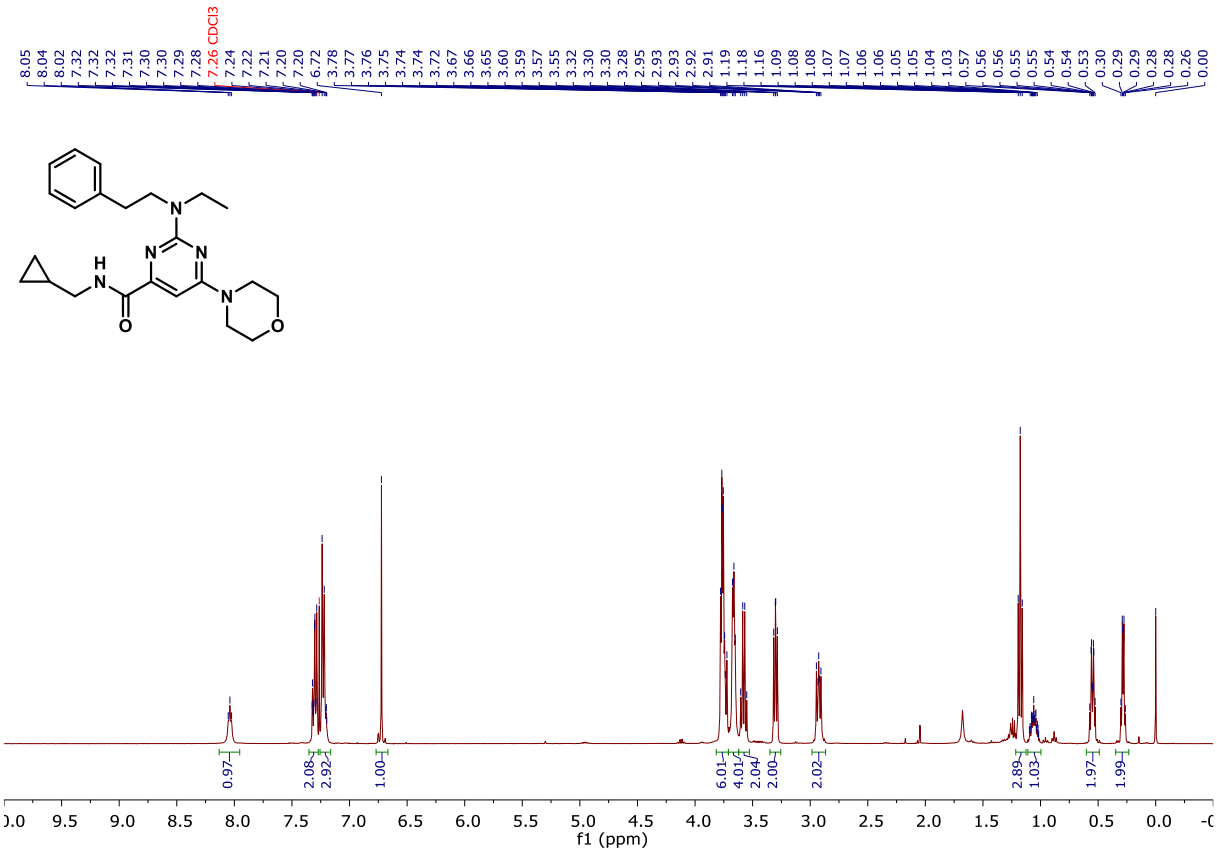
**$^{1}$H-NMR: 53**



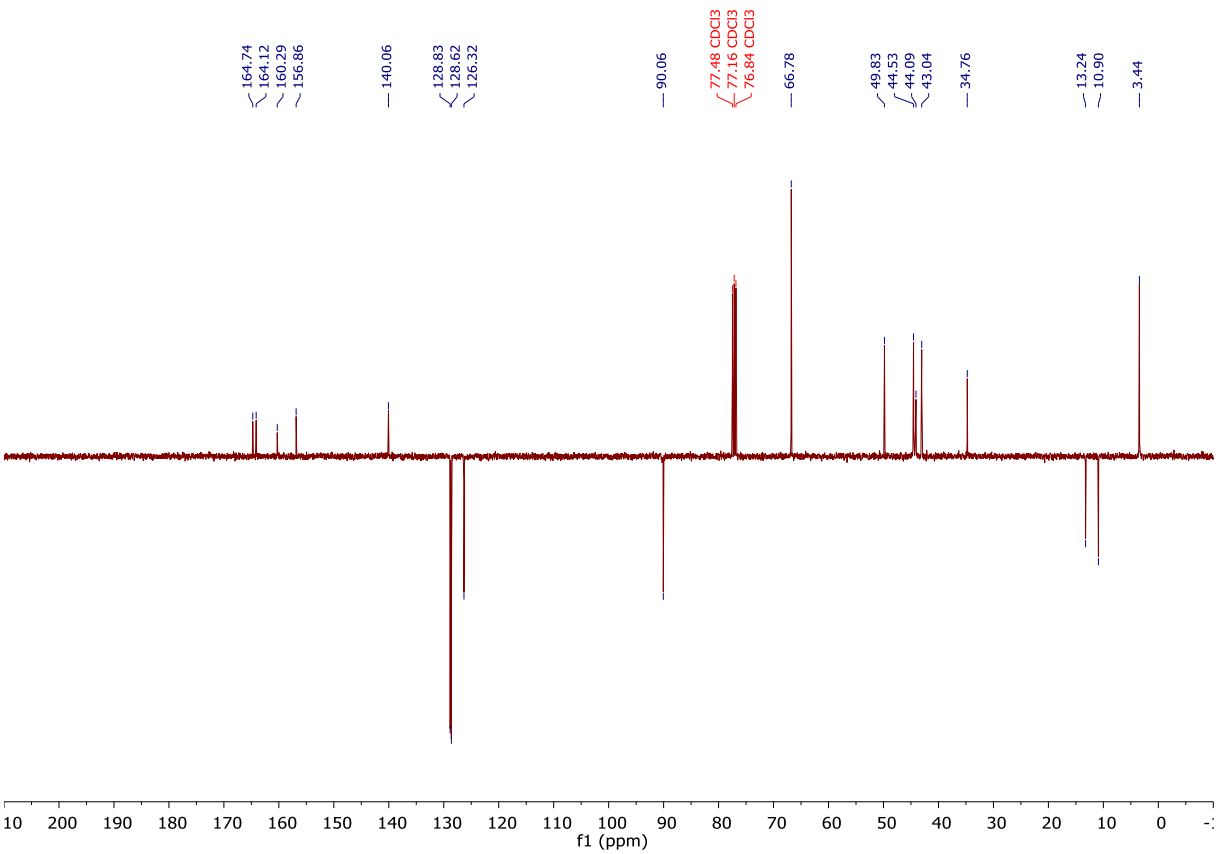

**$^{13}$C-NMR**



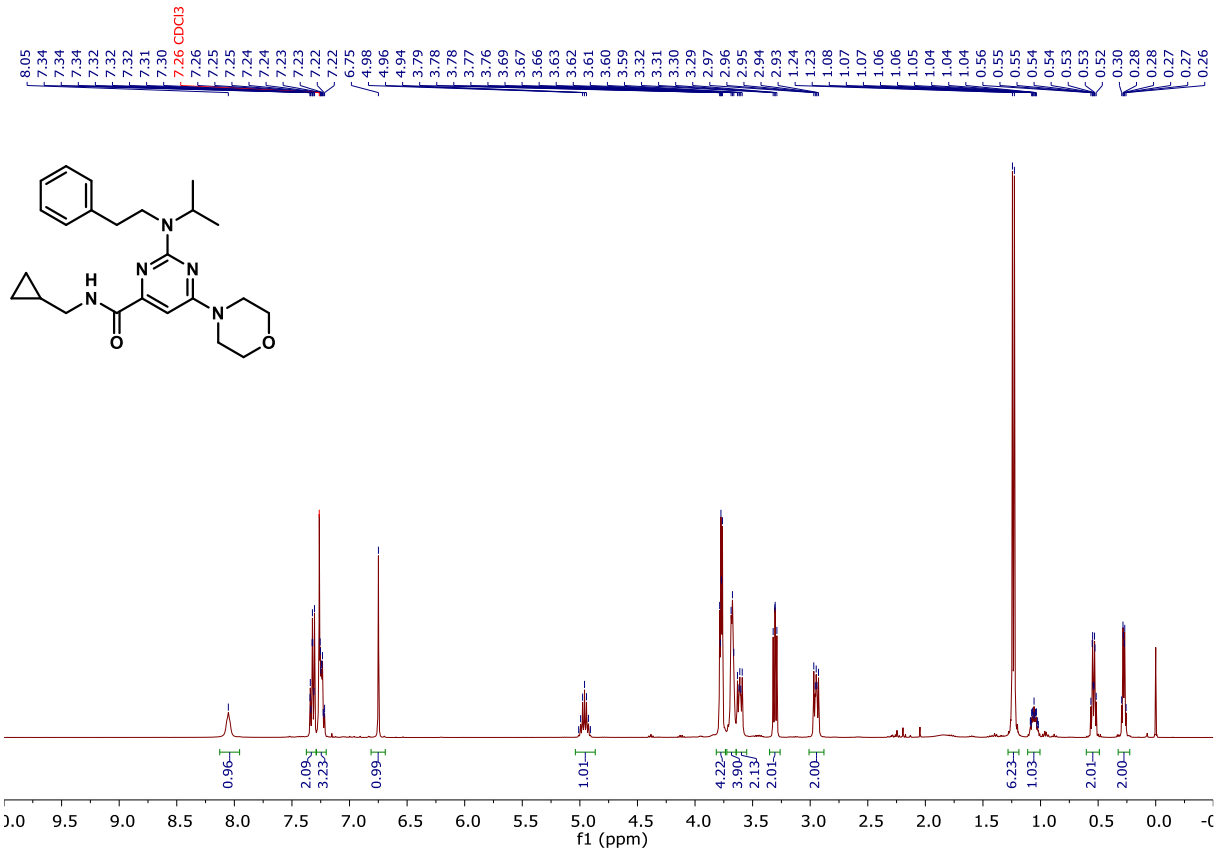
**$^{1}$H-NMR: 54**



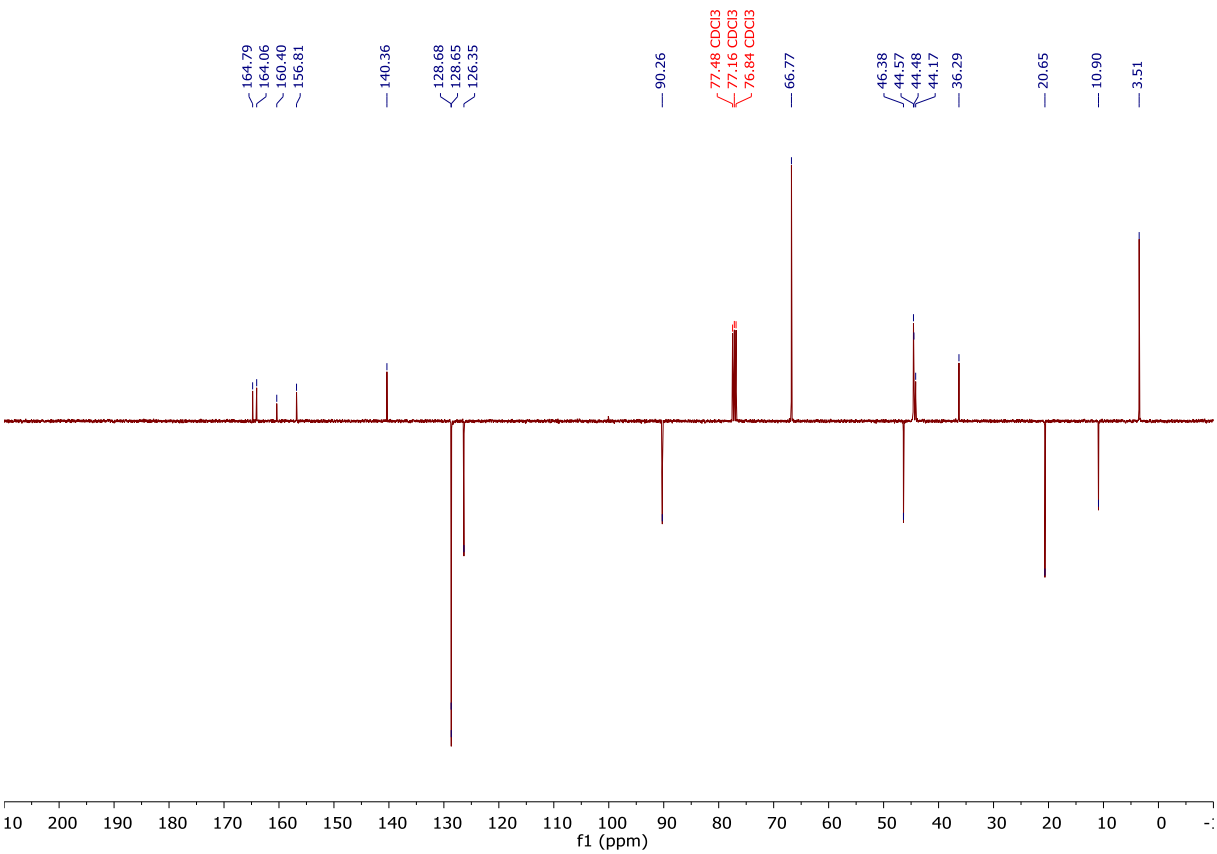

**$^{13}$C-NMR**



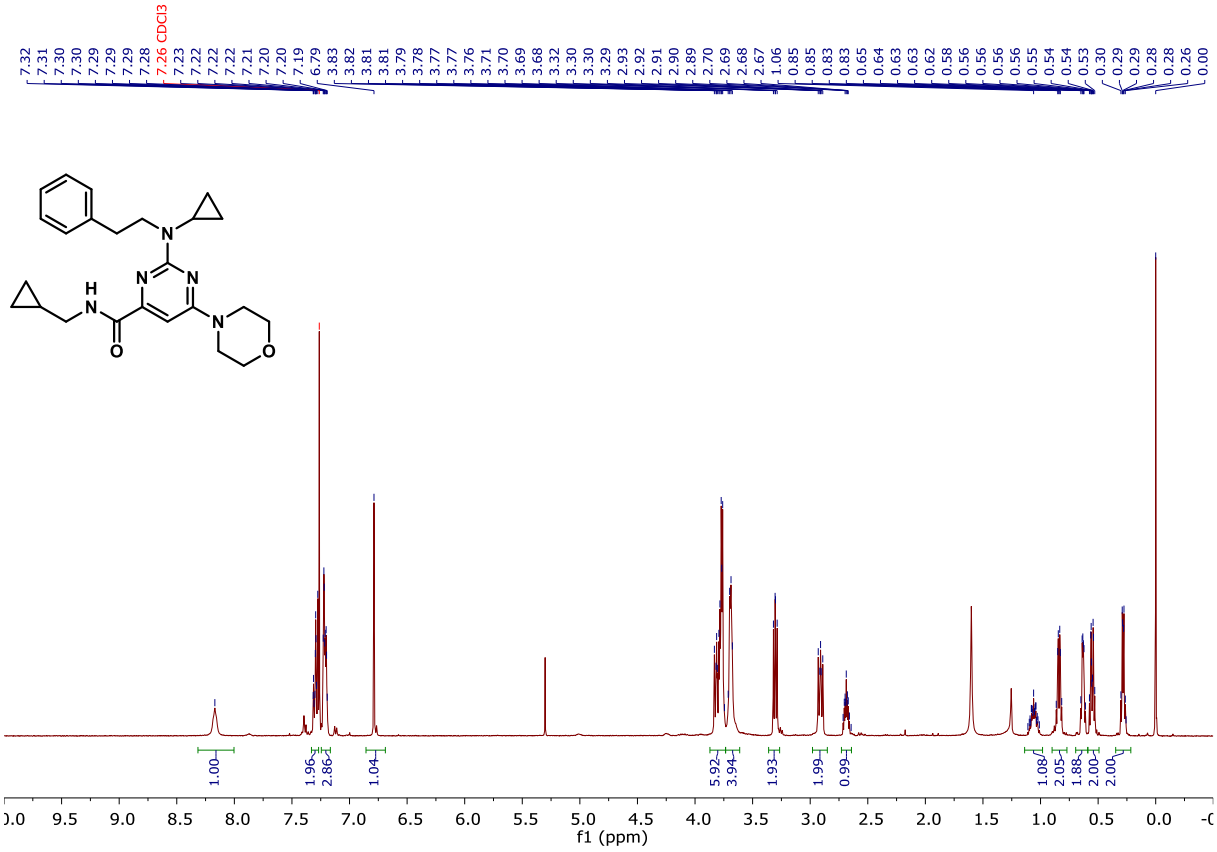
**$^{1}$H-NMR: 55**



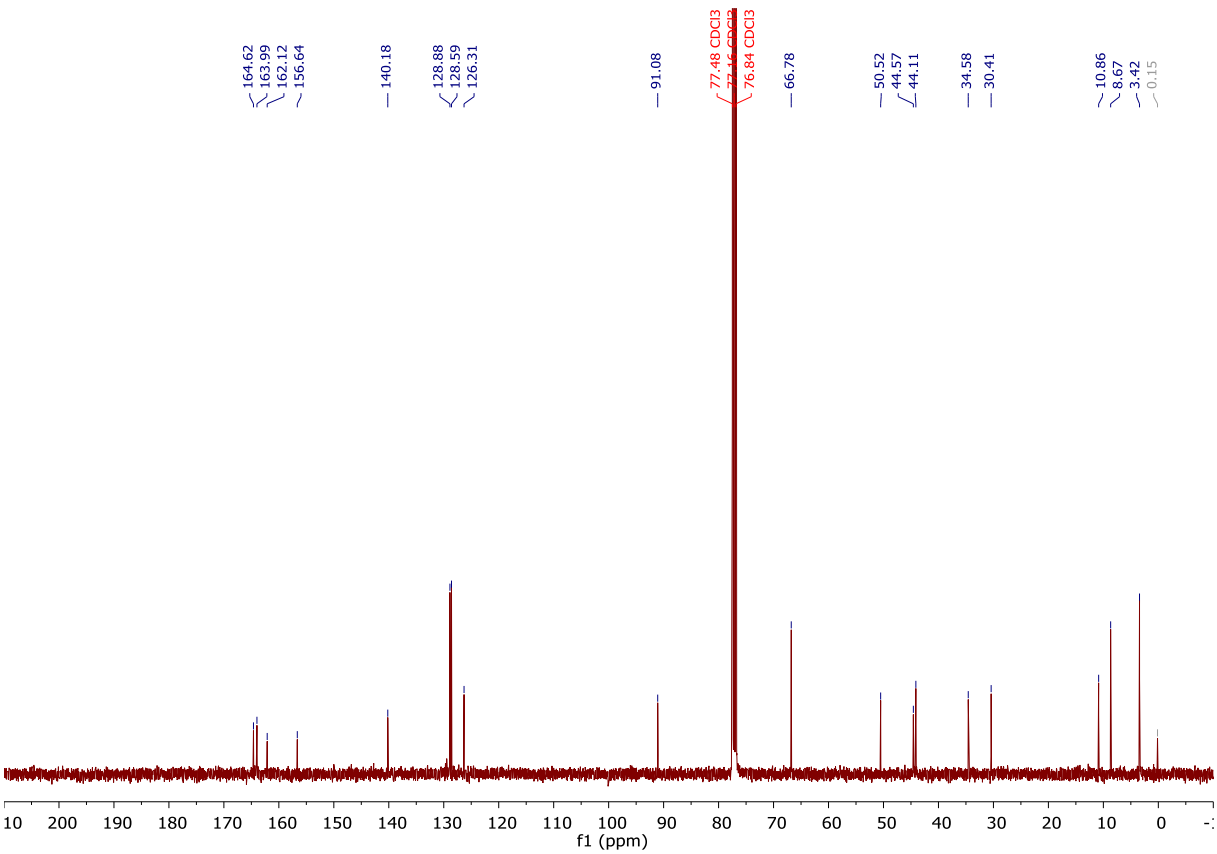

**$^{13}$C-NMR**



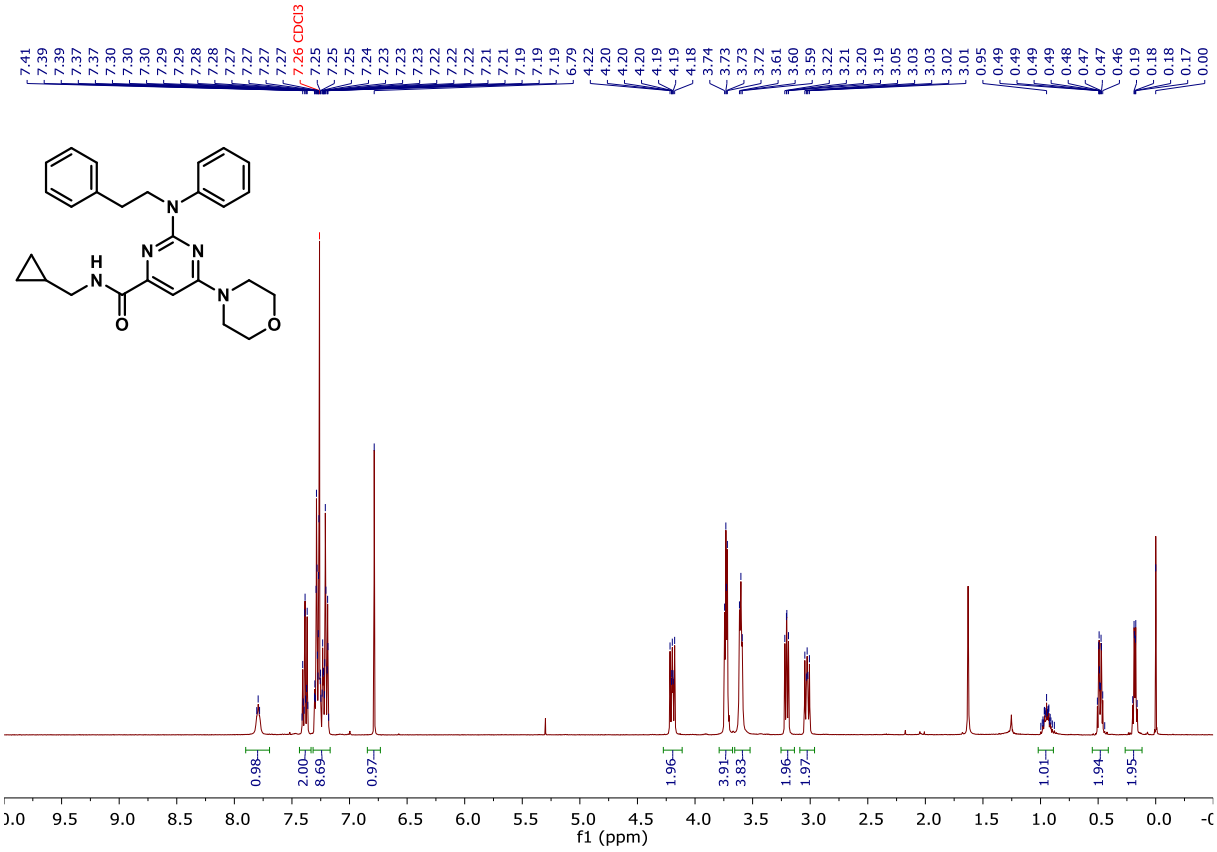
**$^{1}$H-NMR: 56**



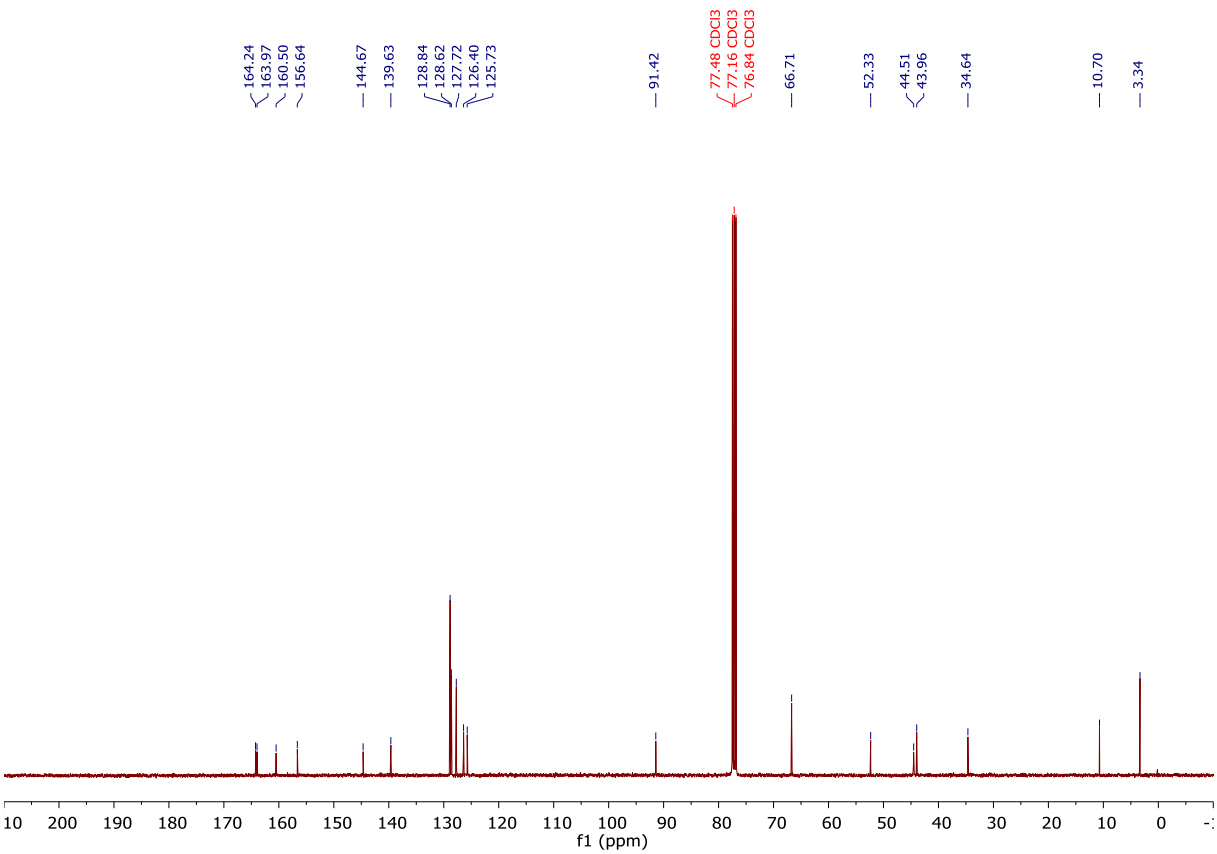

**$^{13}$C-NMR**



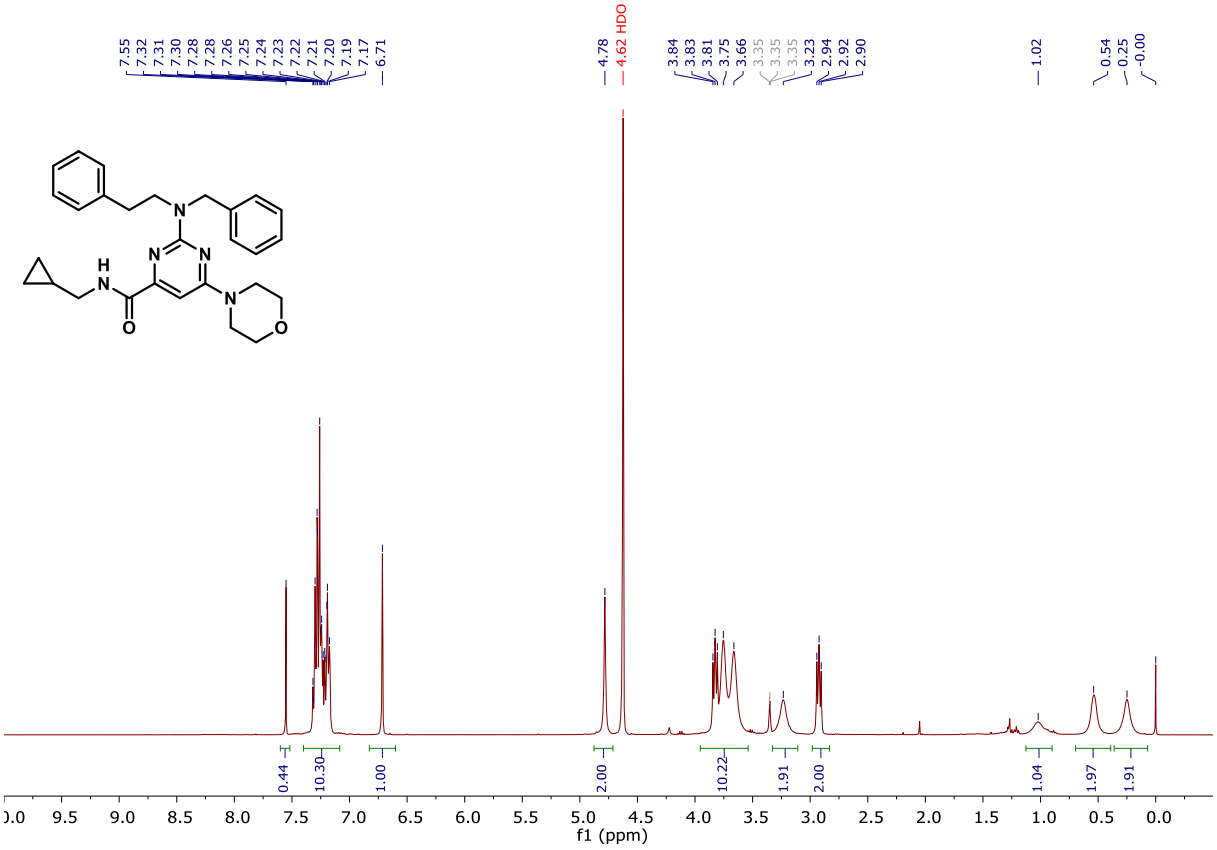
**$^{1}$H-NMR: 57**



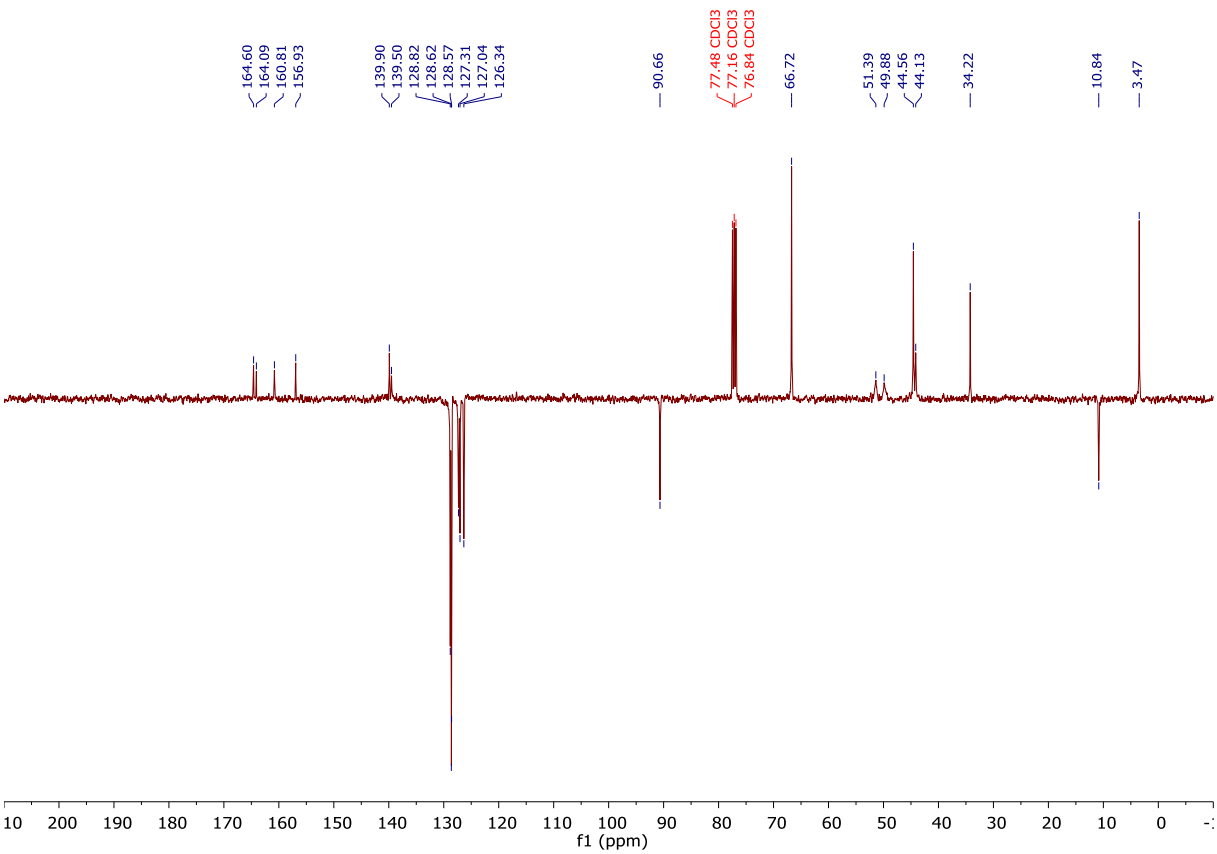

**$^{13}$C-NMR**



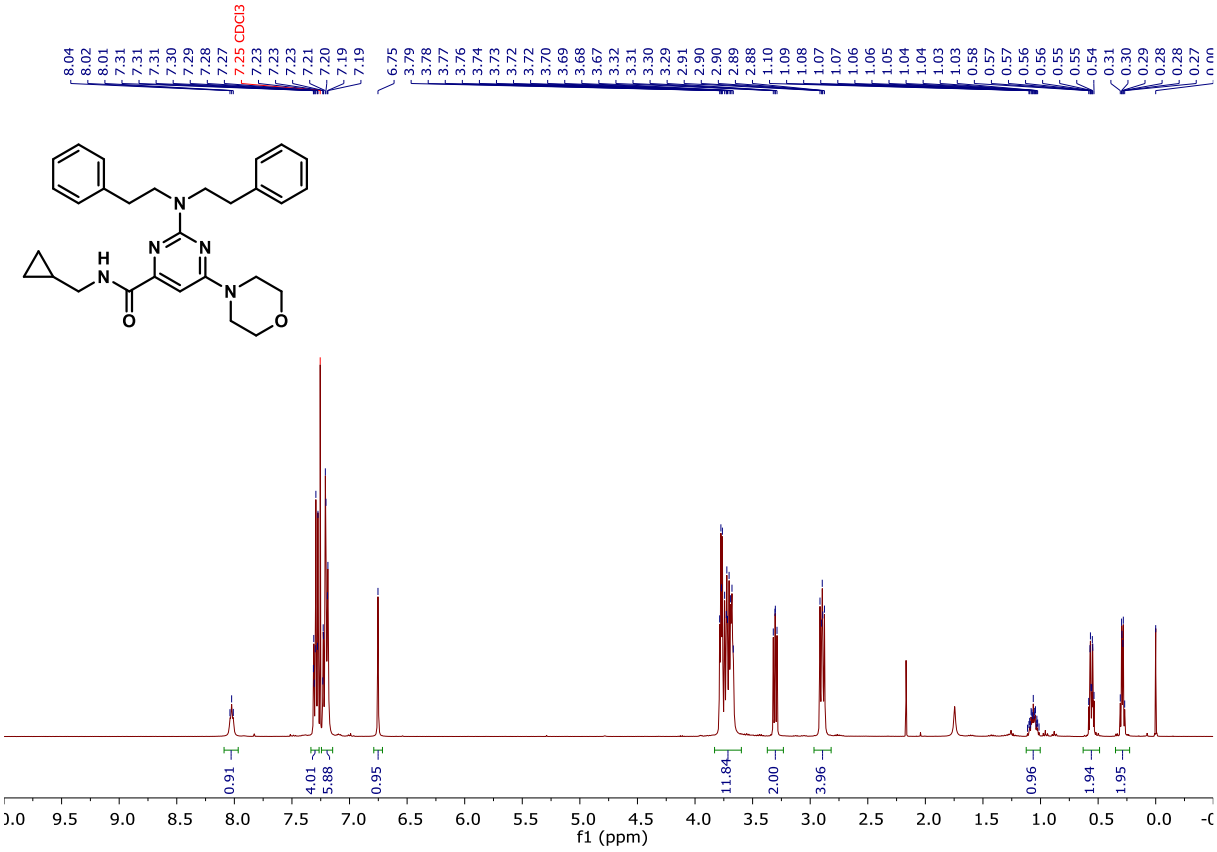
**$^{1}$H-NMR: 58** (MeOD + $CDCl_3$)



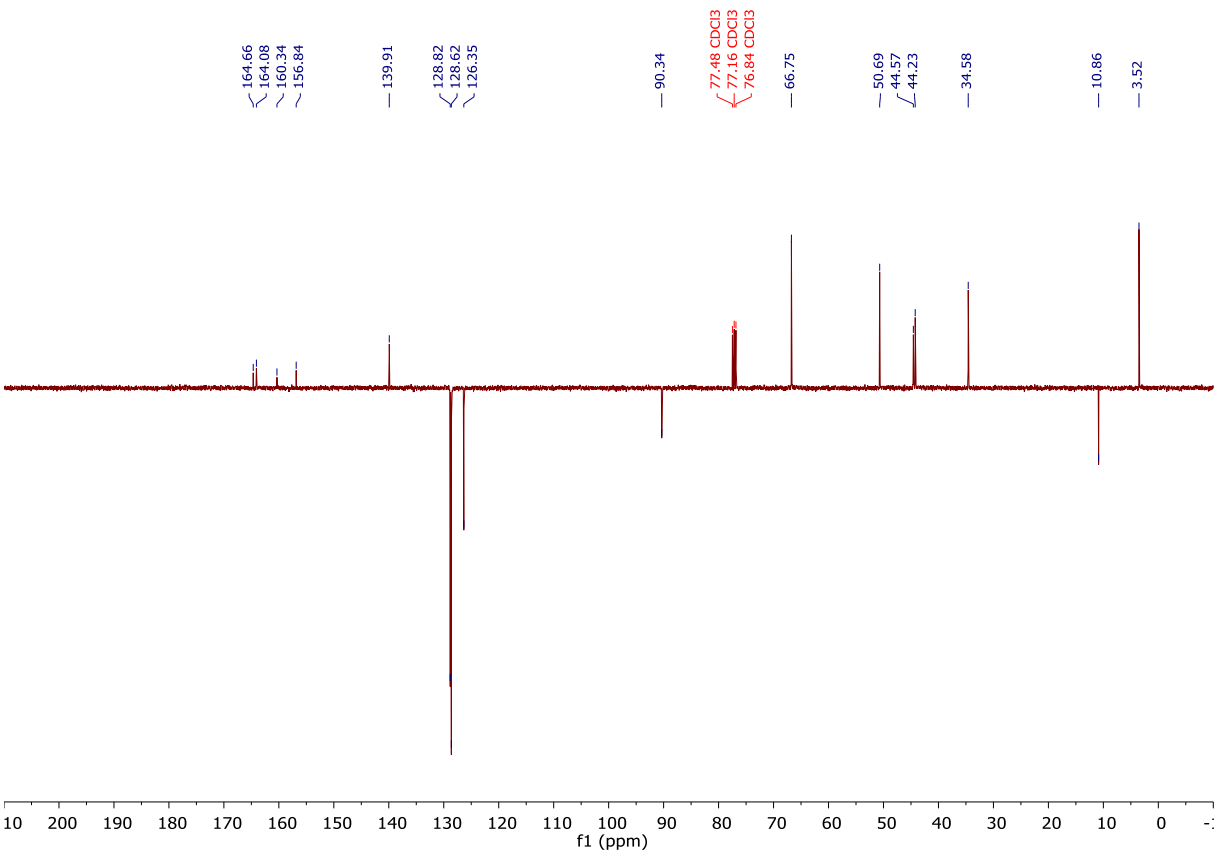

**$^{13}$C-NMR** ($CDCl_3$)



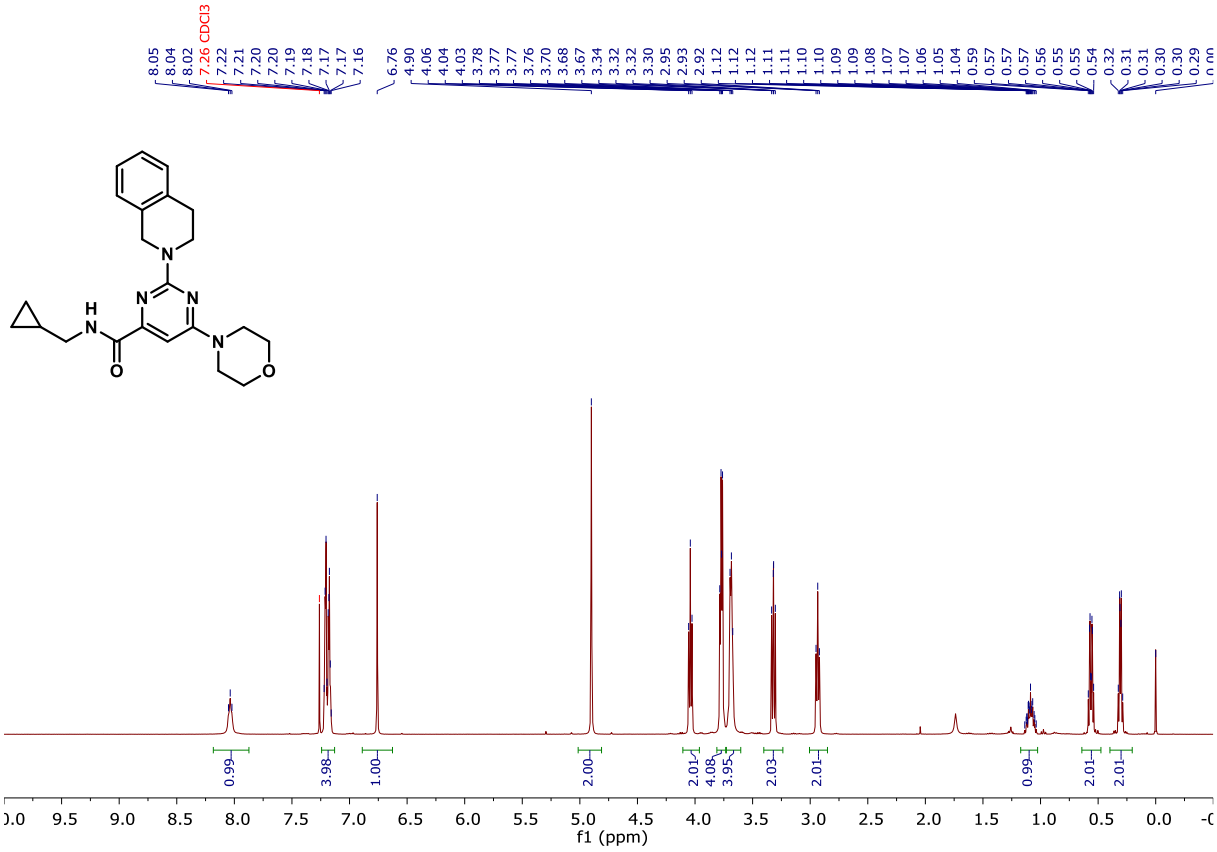
**$^{1}$H-NMR: 59**



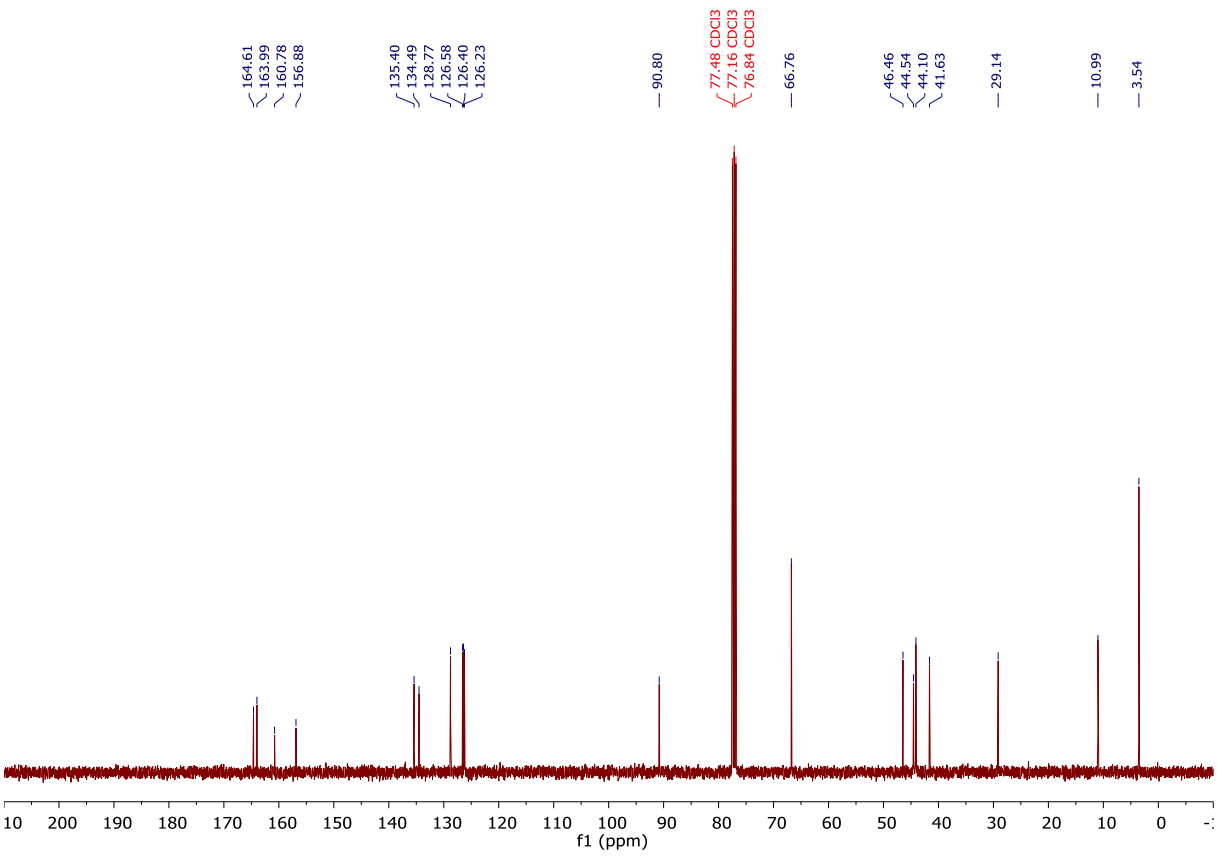

**$^{13}$C-NMR**



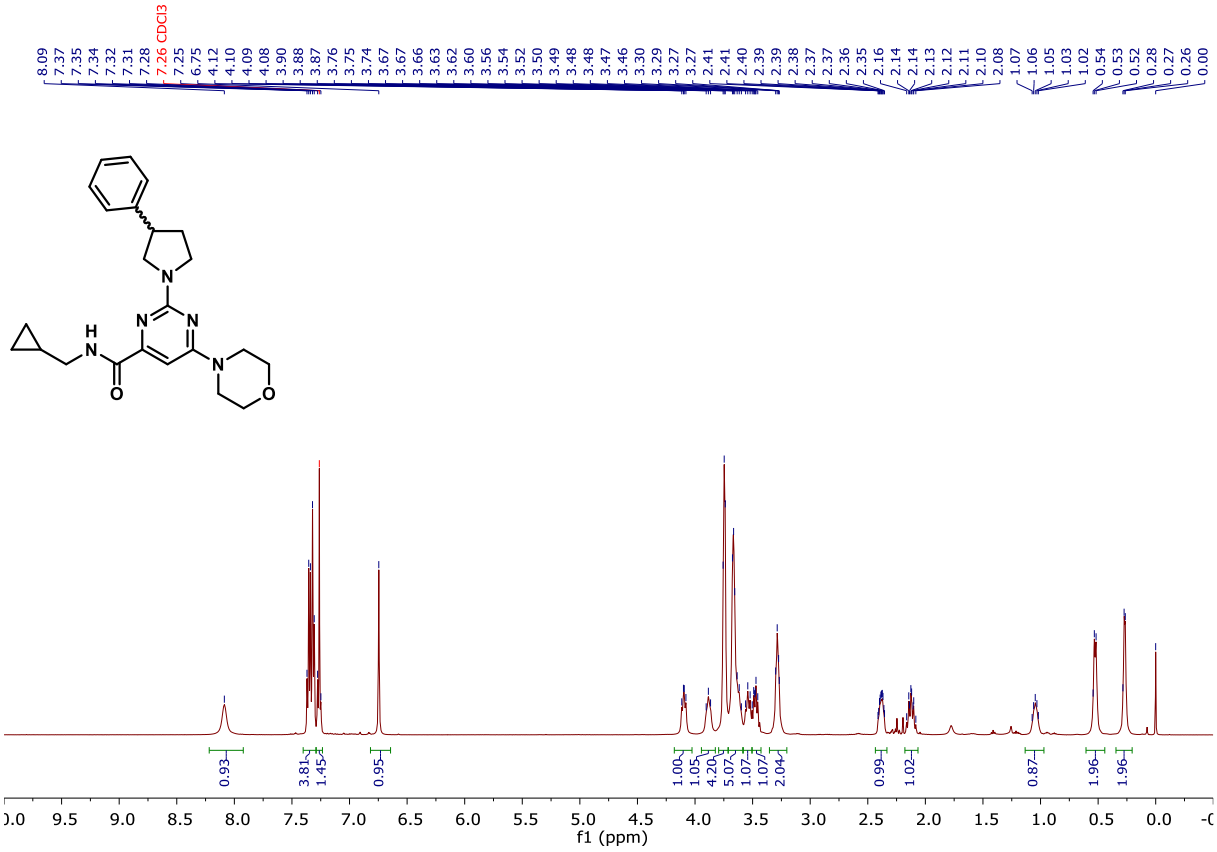
**$^{1}$H-NMR: 60**



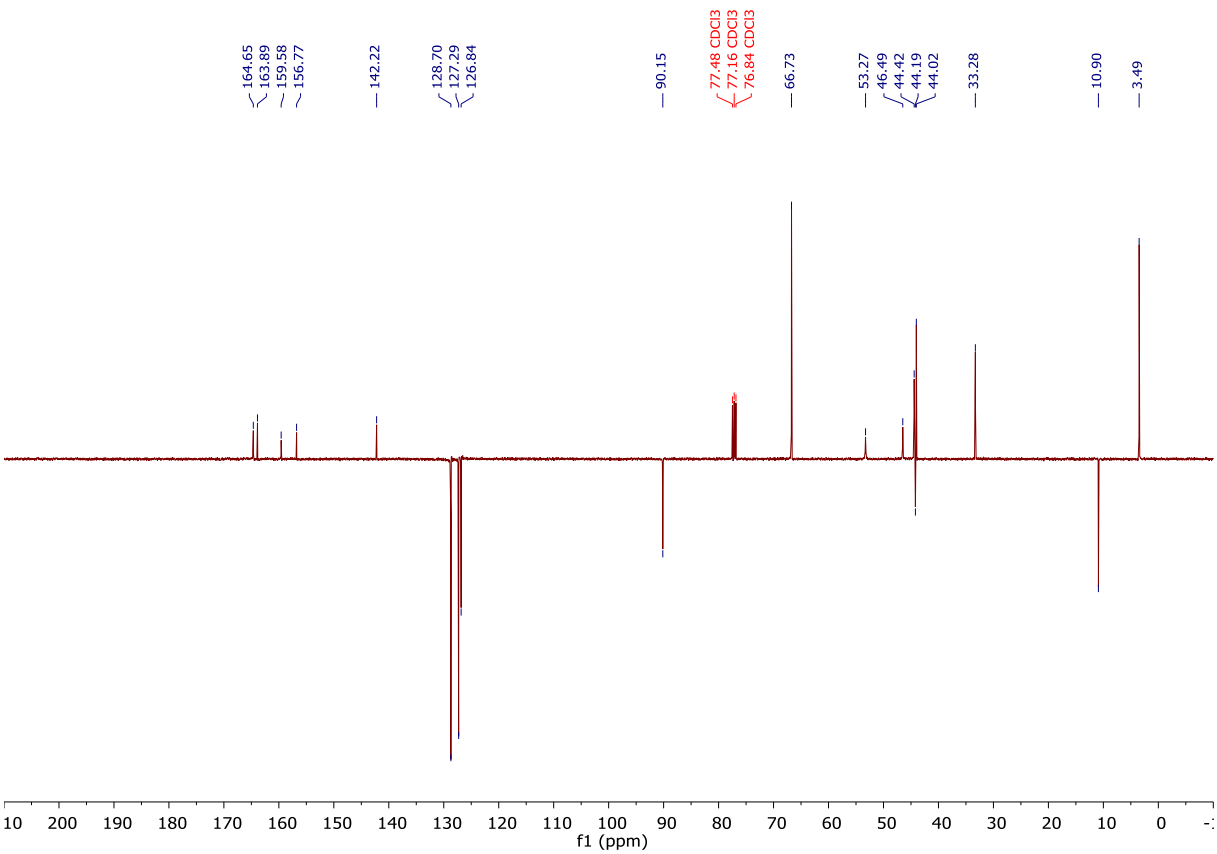

**$^{13}$C-NMR**



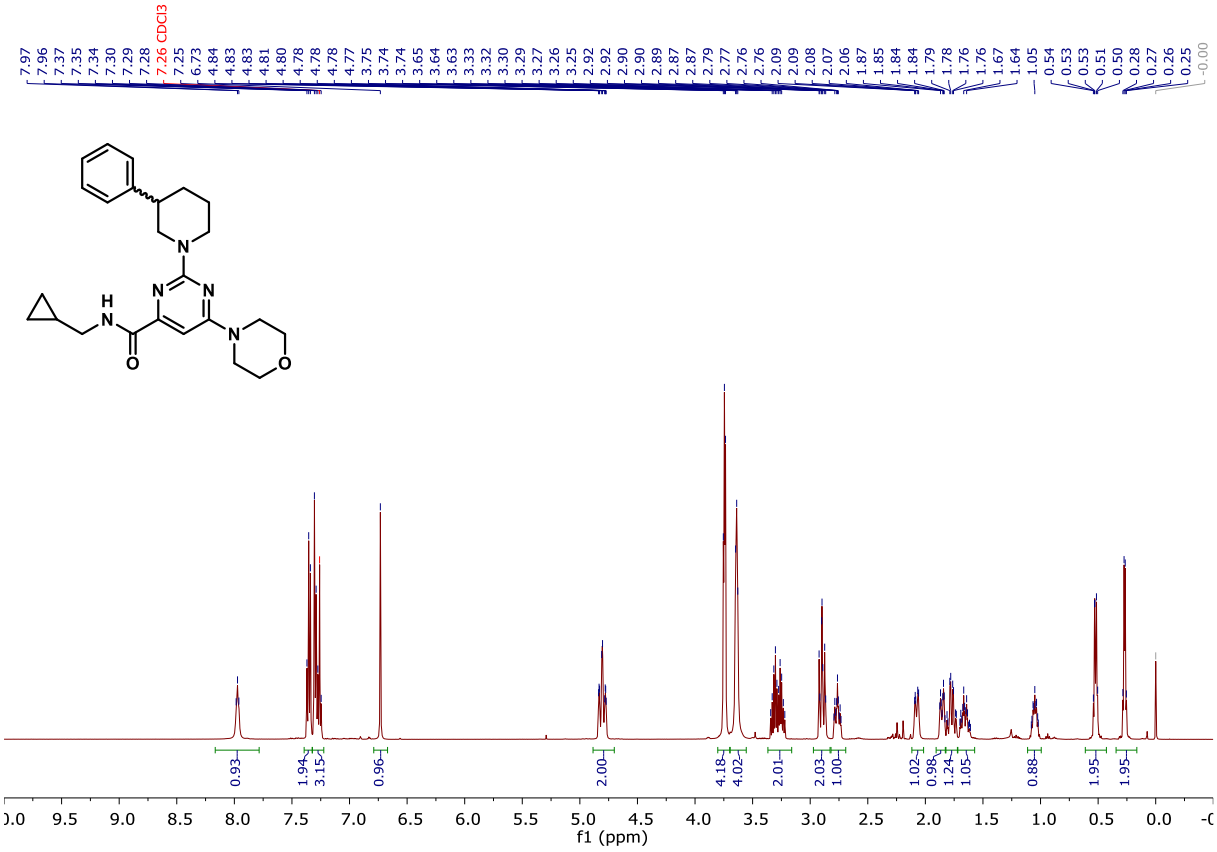
**$^{1}$H-NMR: 61**



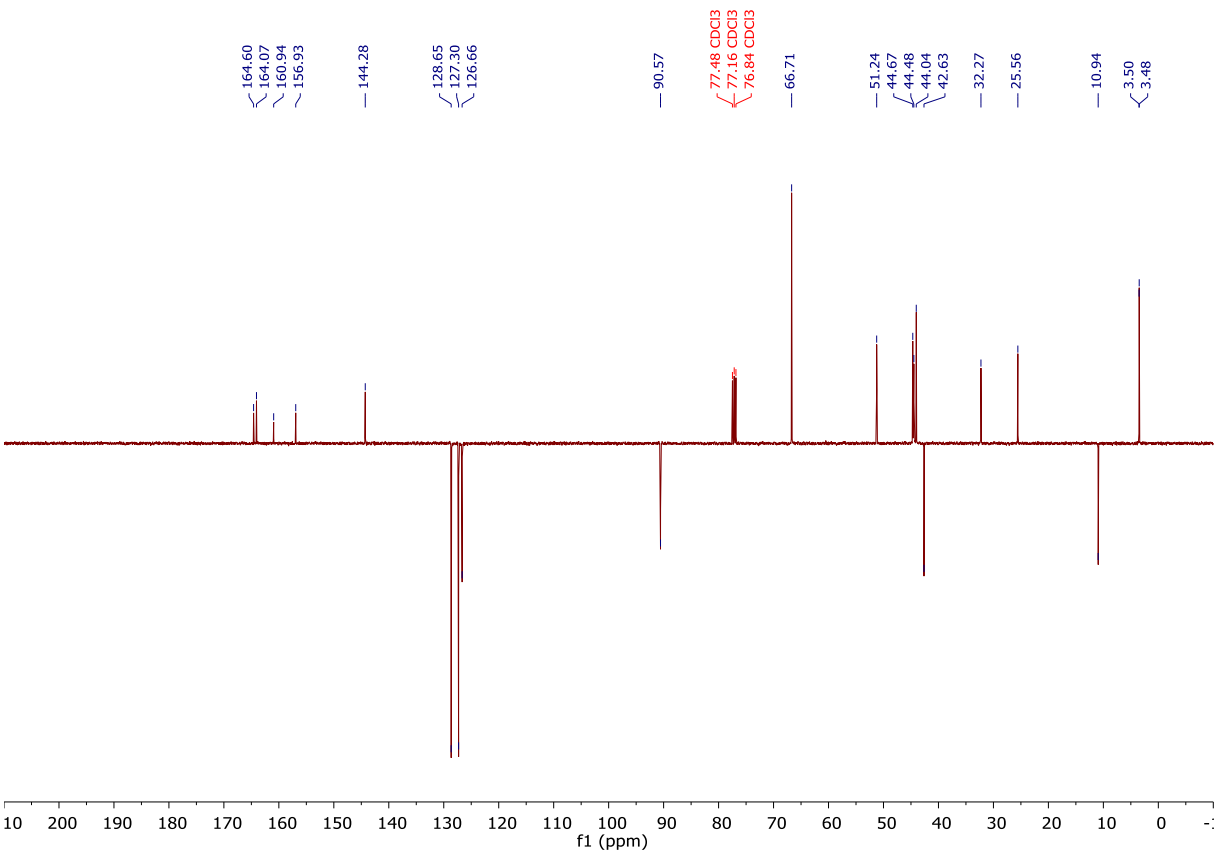

**$^{13}$C-NMR**



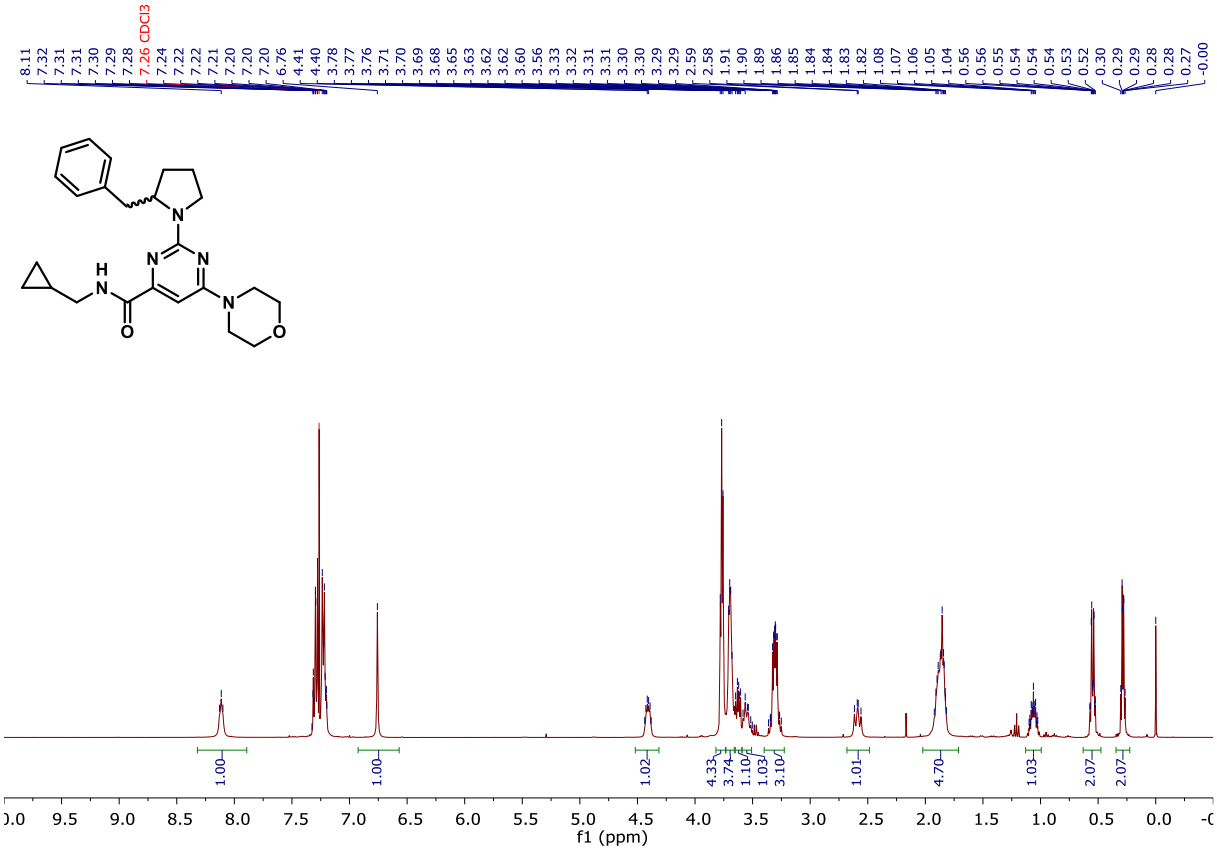
**$^{1}$H-NMR: 62**



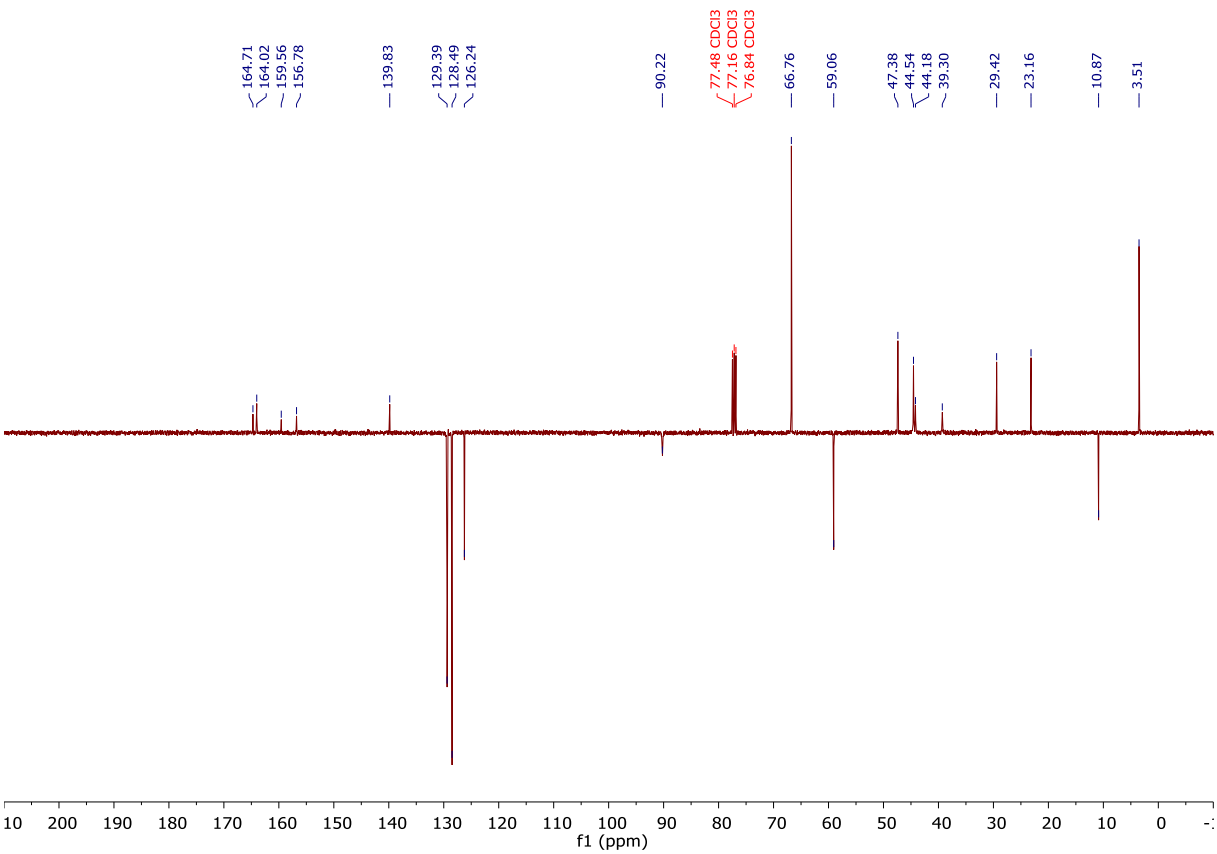

**$^{13}$C-NMR**



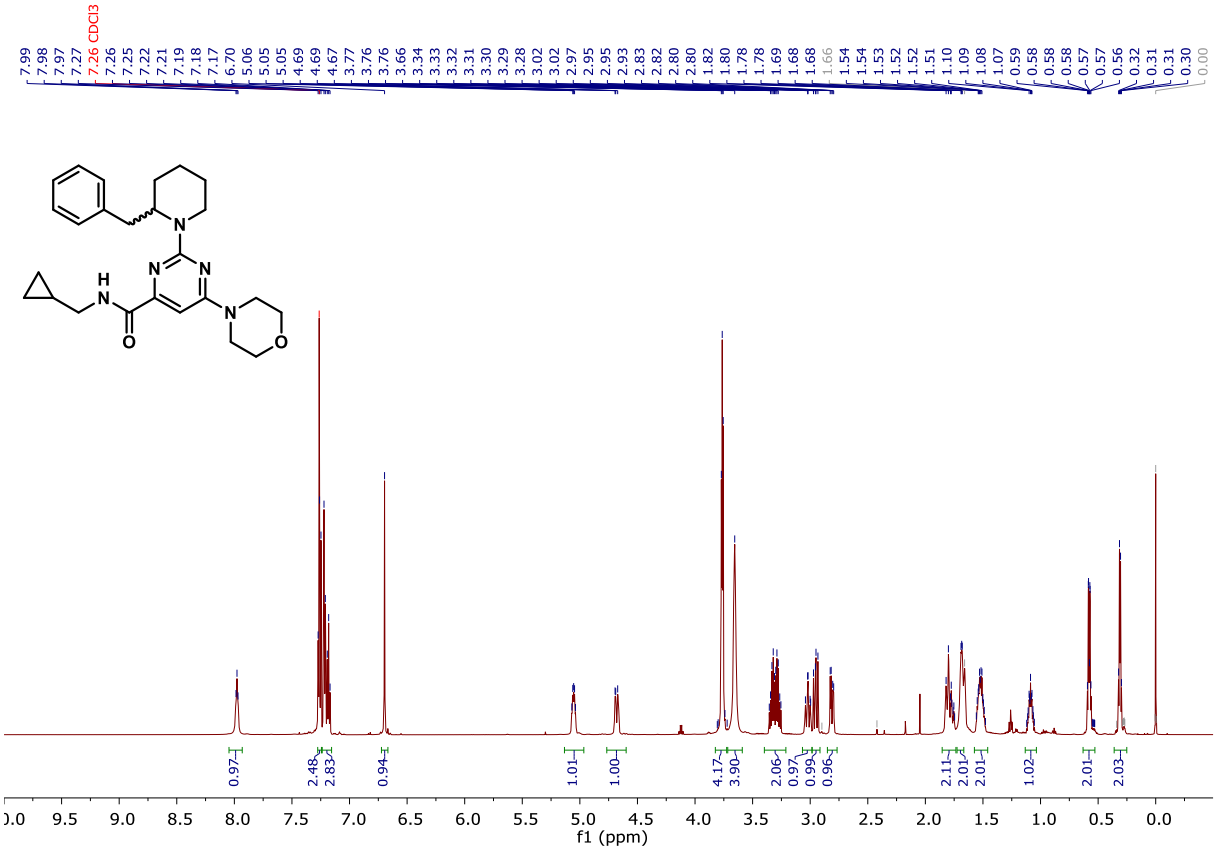
**$^{1}$H-NMR: 63**



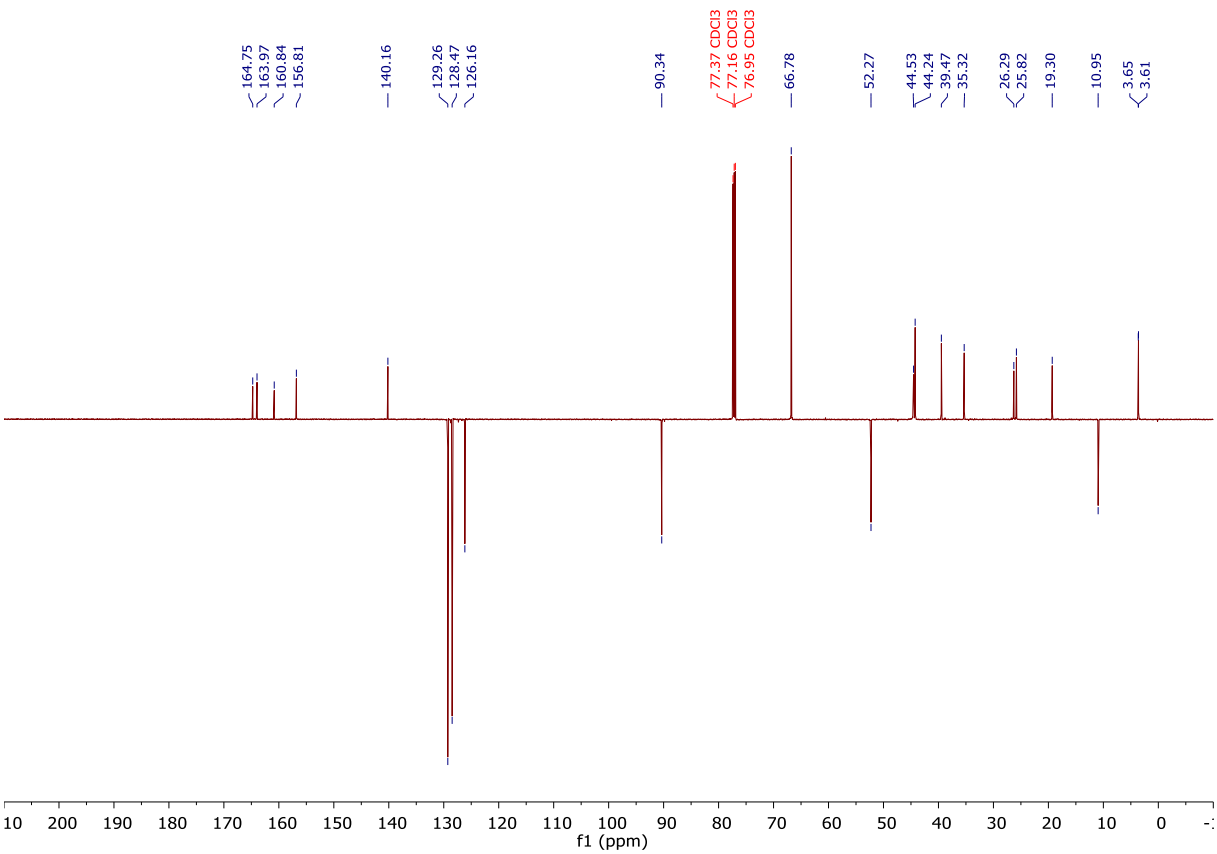

**$^{13}$C-NMR**



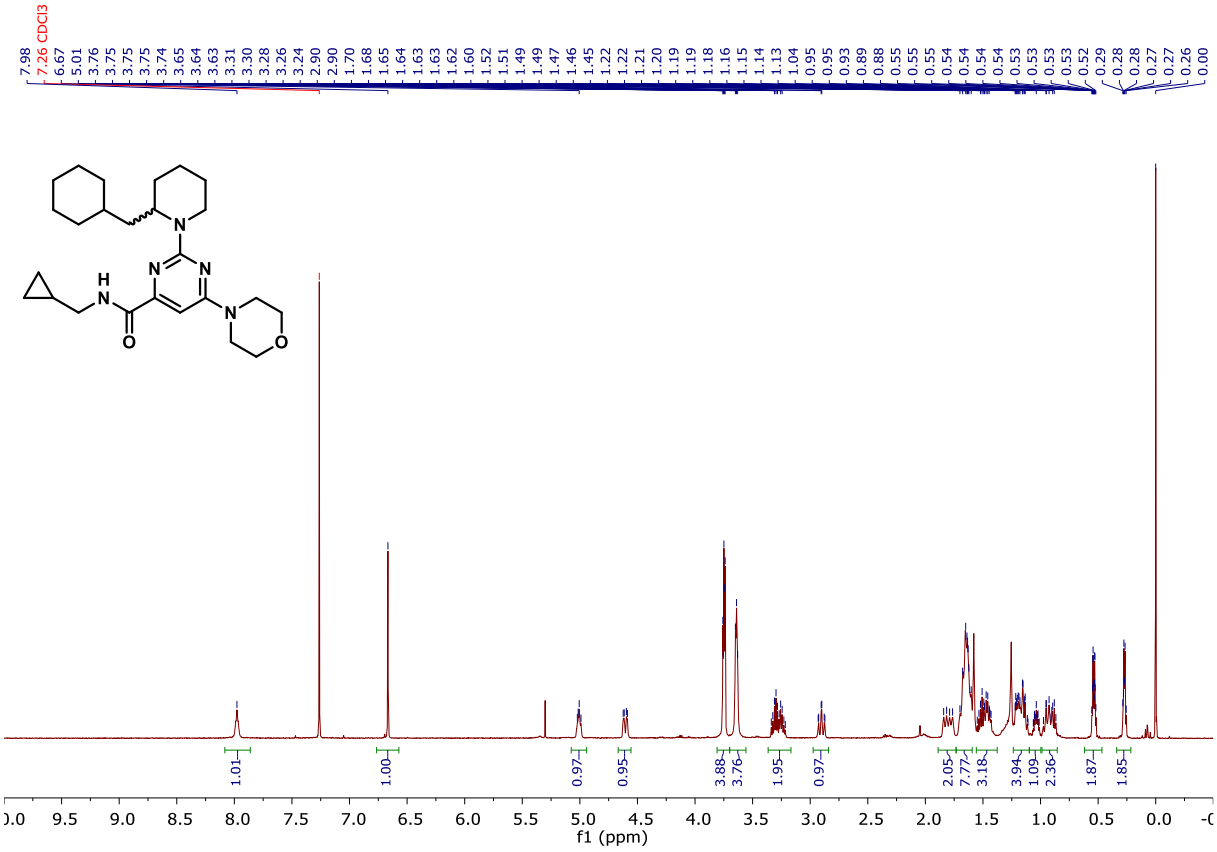
**$^{1}$H-NMR: 64**



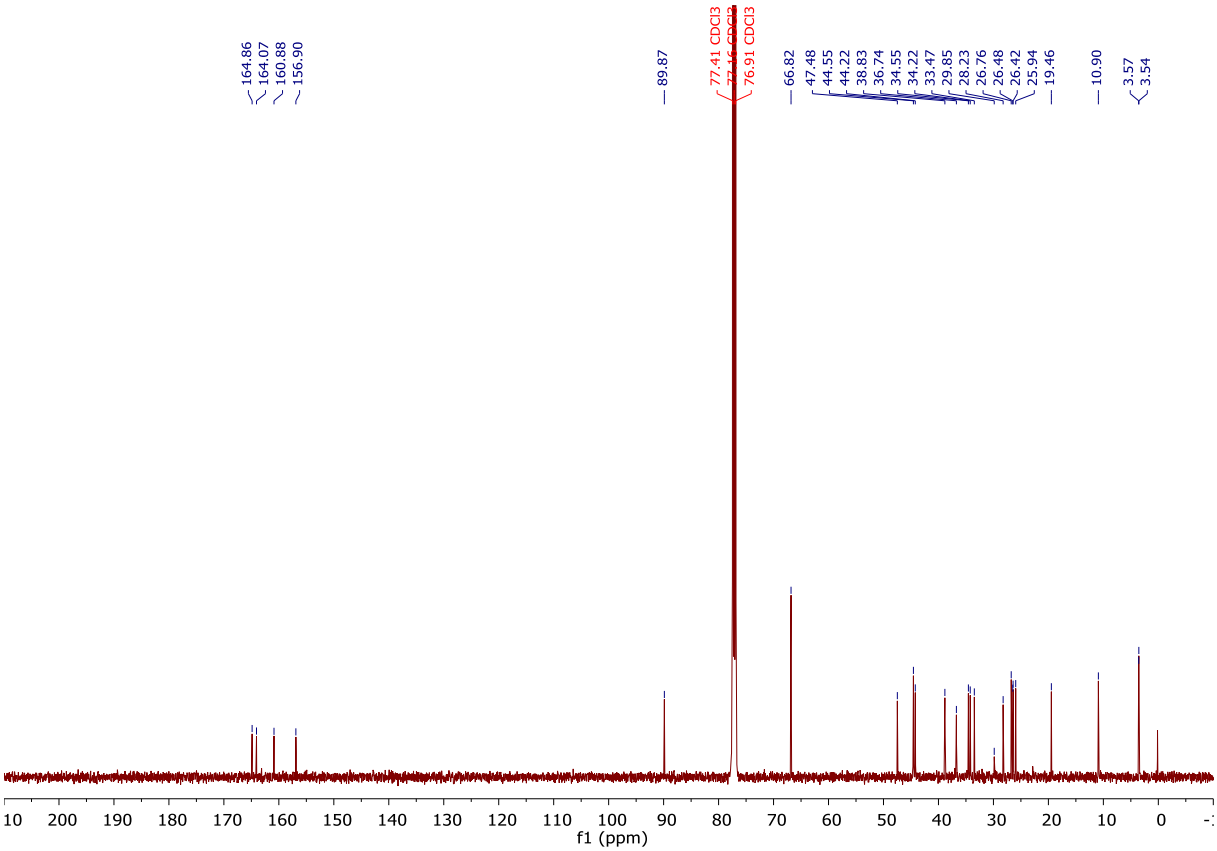

**$^{13}$C-NMR**



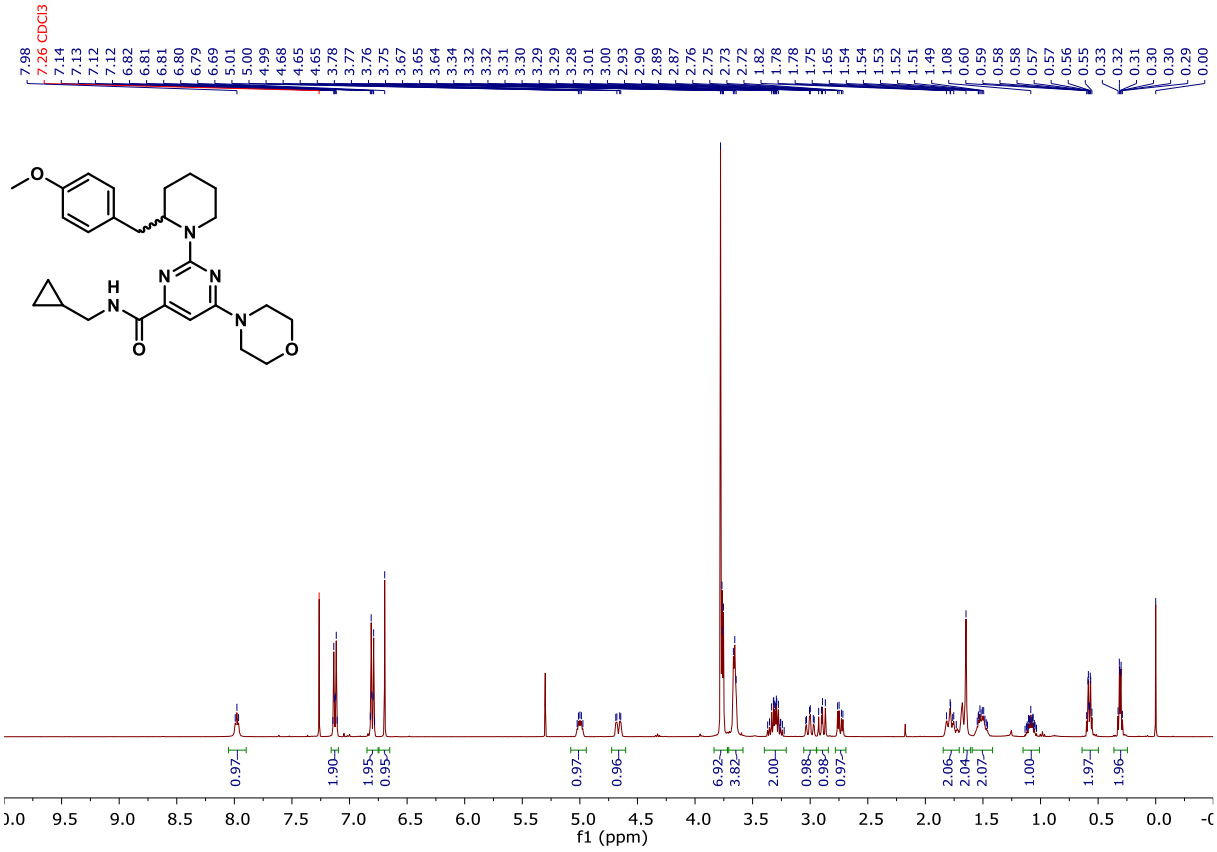
**$^{1}$H-NMR: 65**



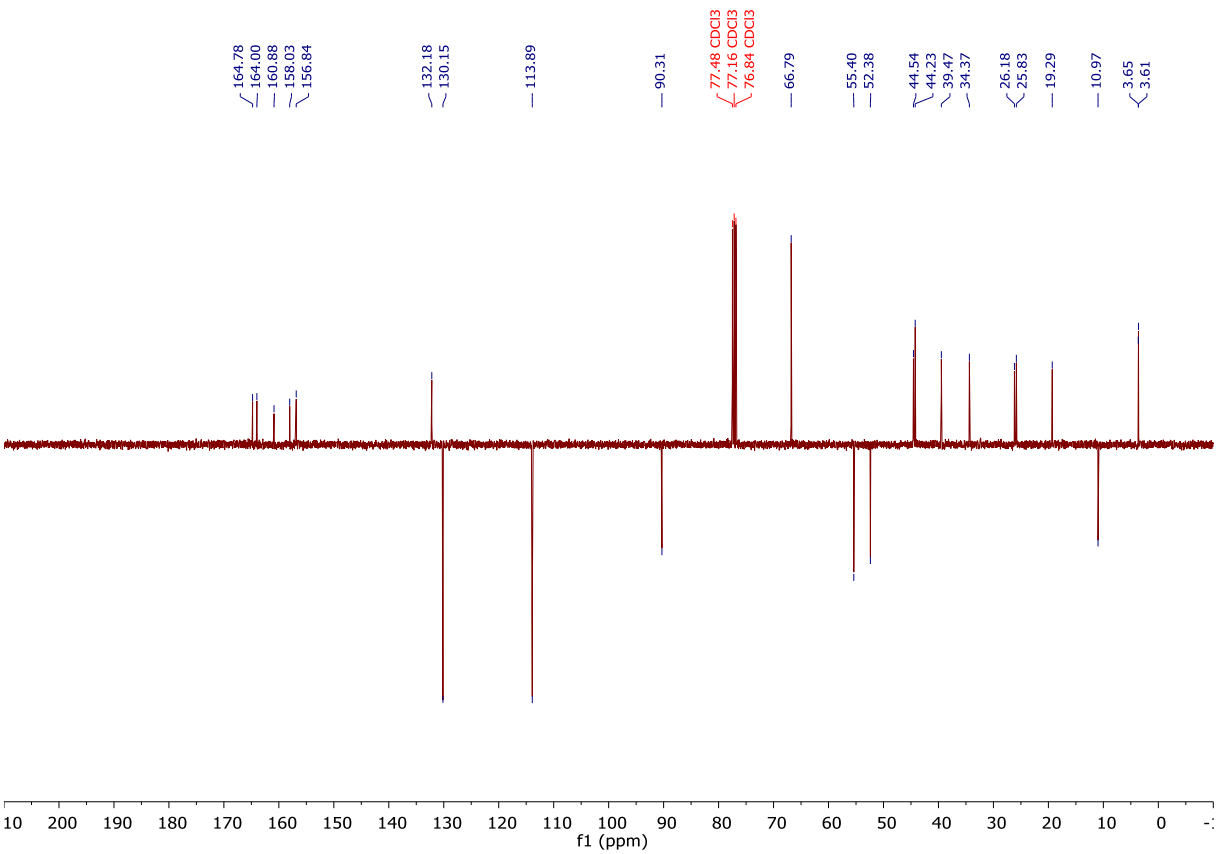

**$^{13}$C-NMR**



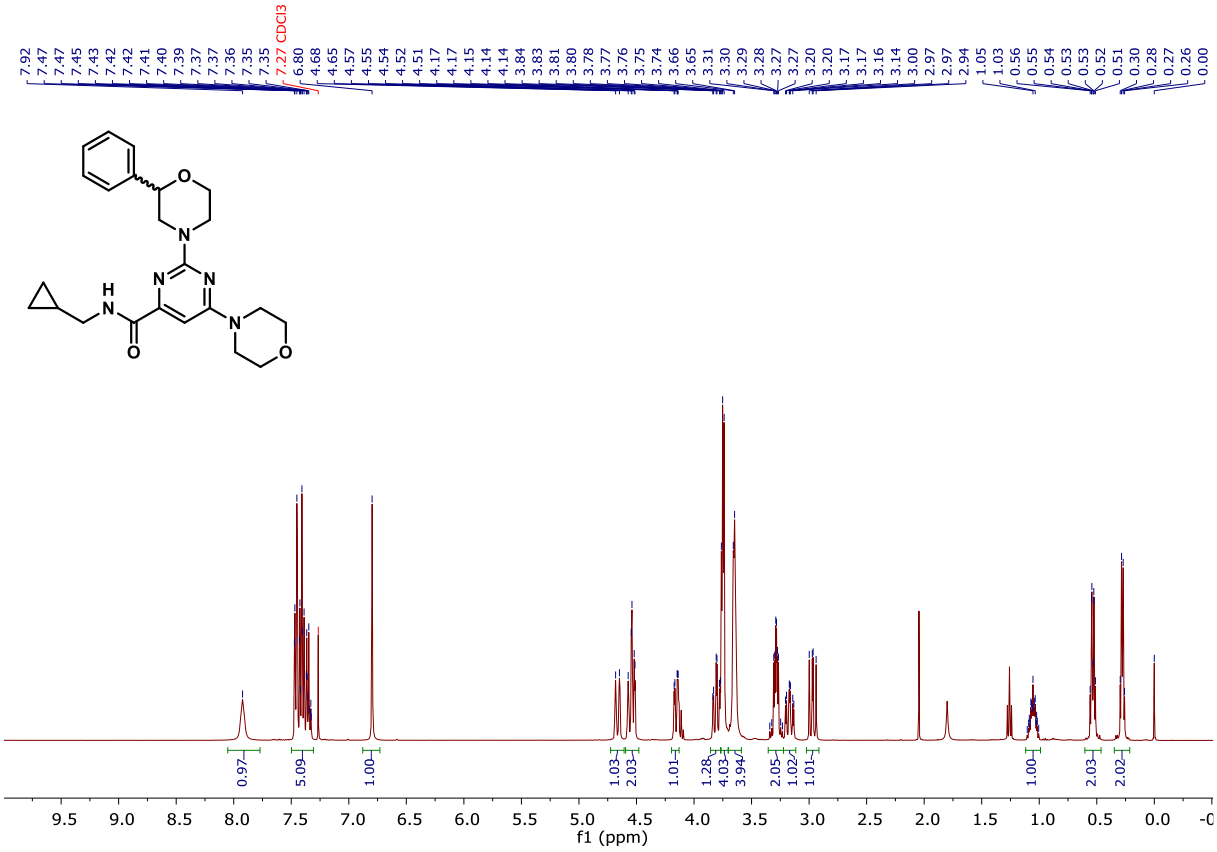
**$^{1}$H-NMR: 66**



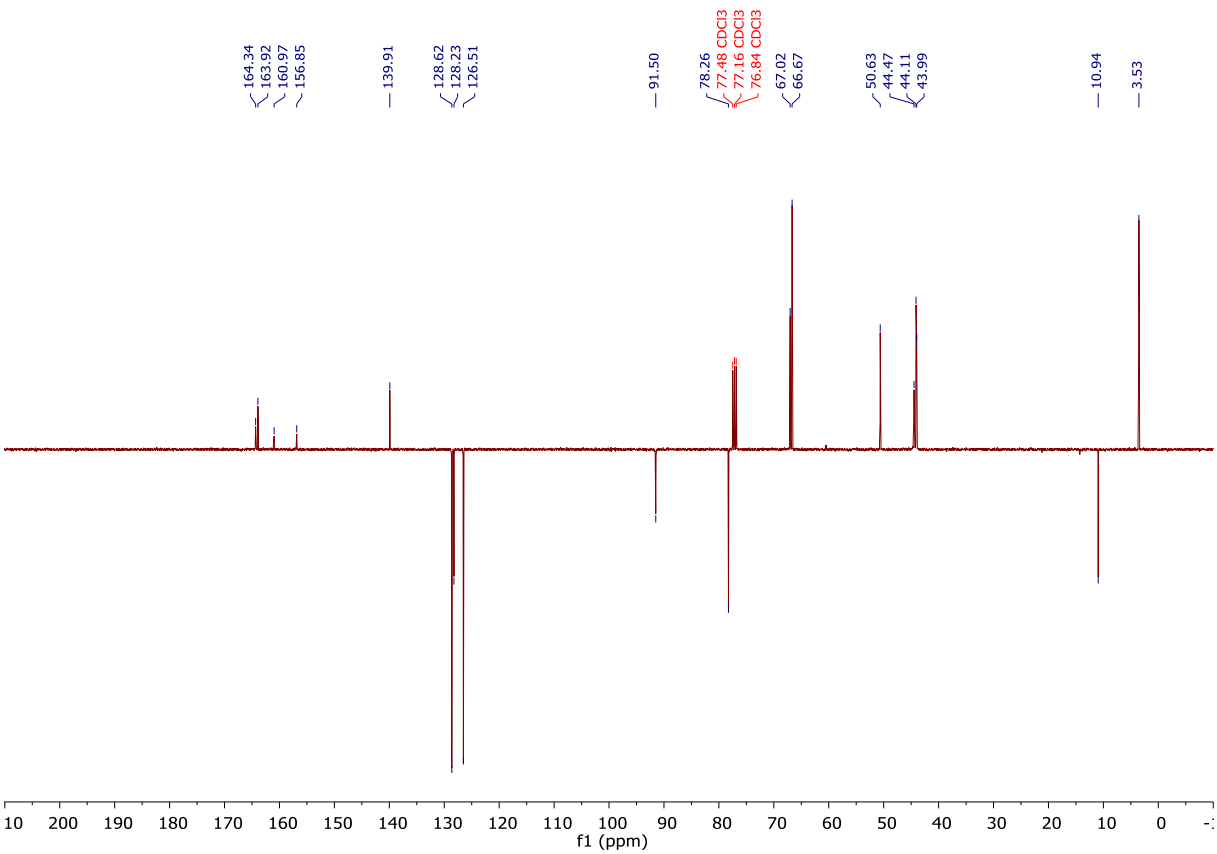

**$^{13}$C-NMR**



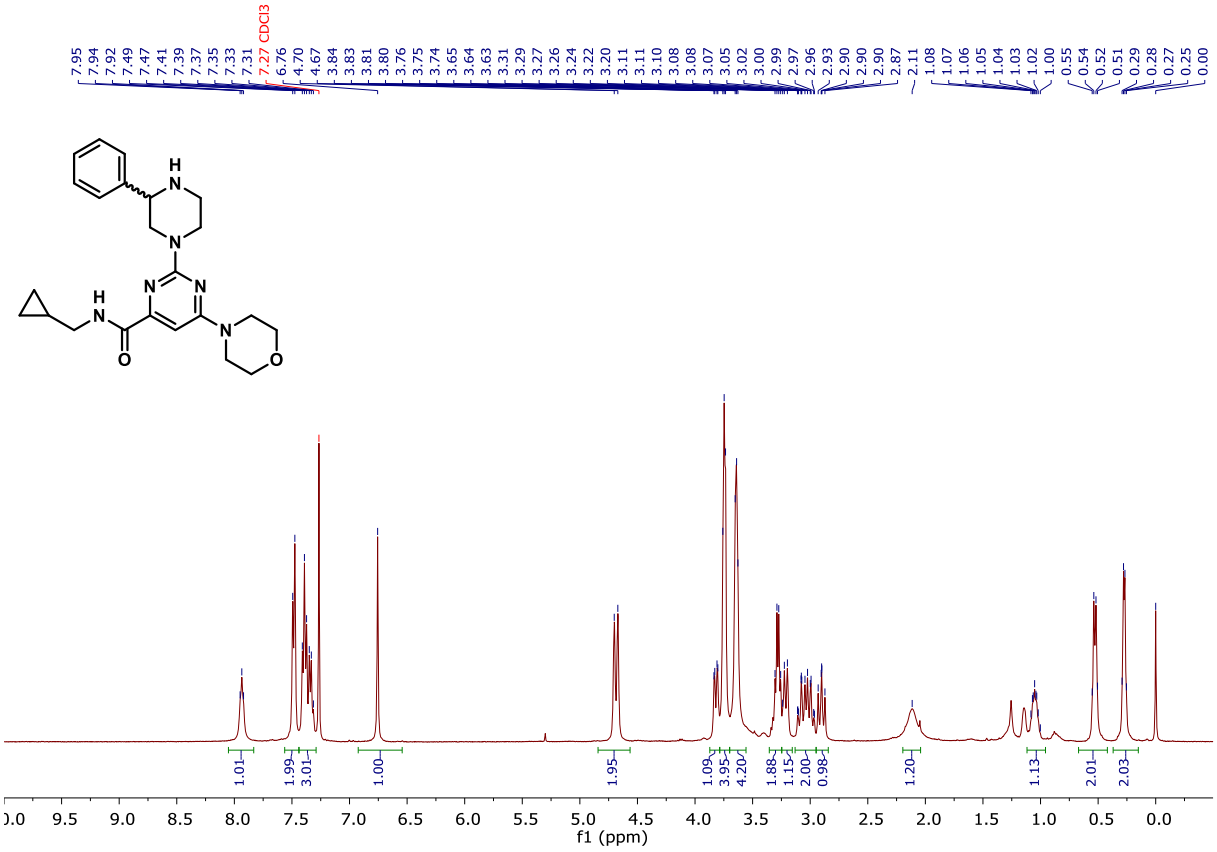
**$^{1}$H-NMR: 67**



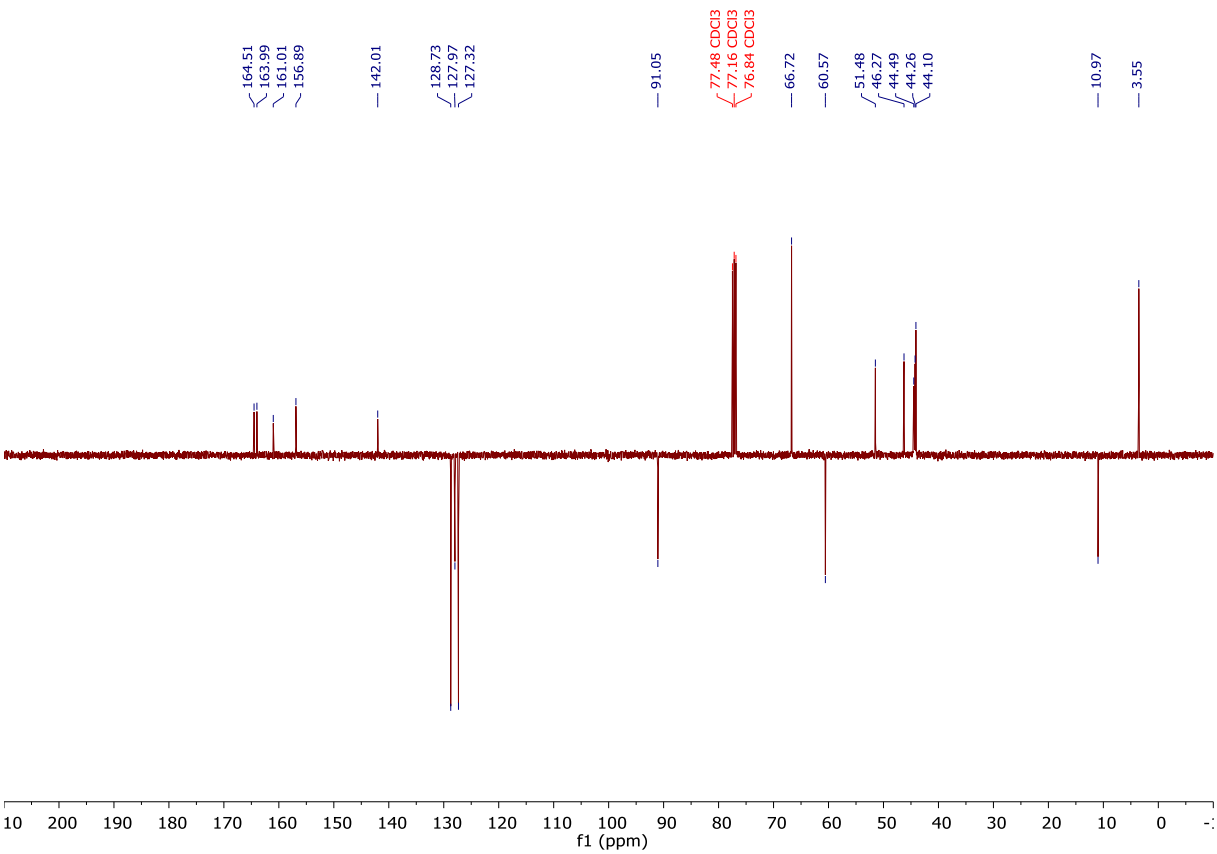

**$^{13}$C-NMR**



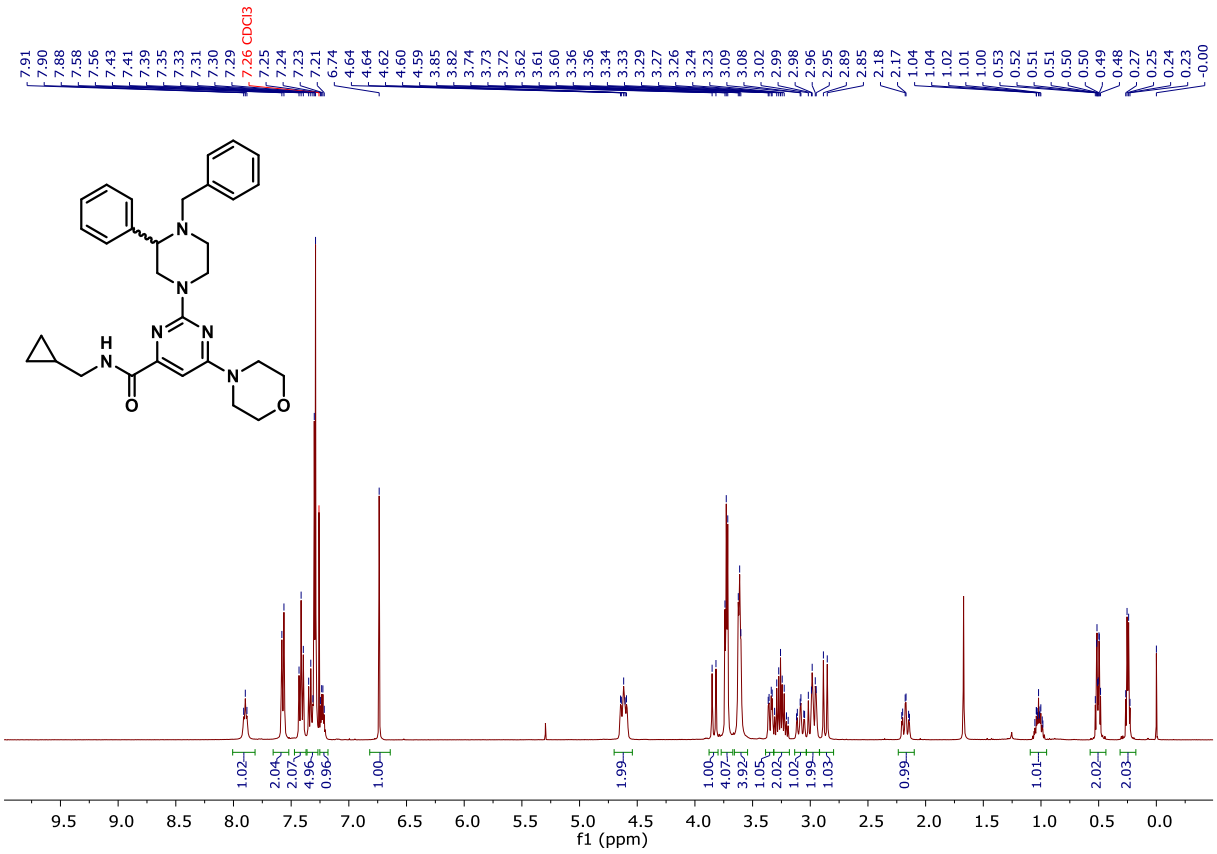
**$^{1}$H-NMR: 68**



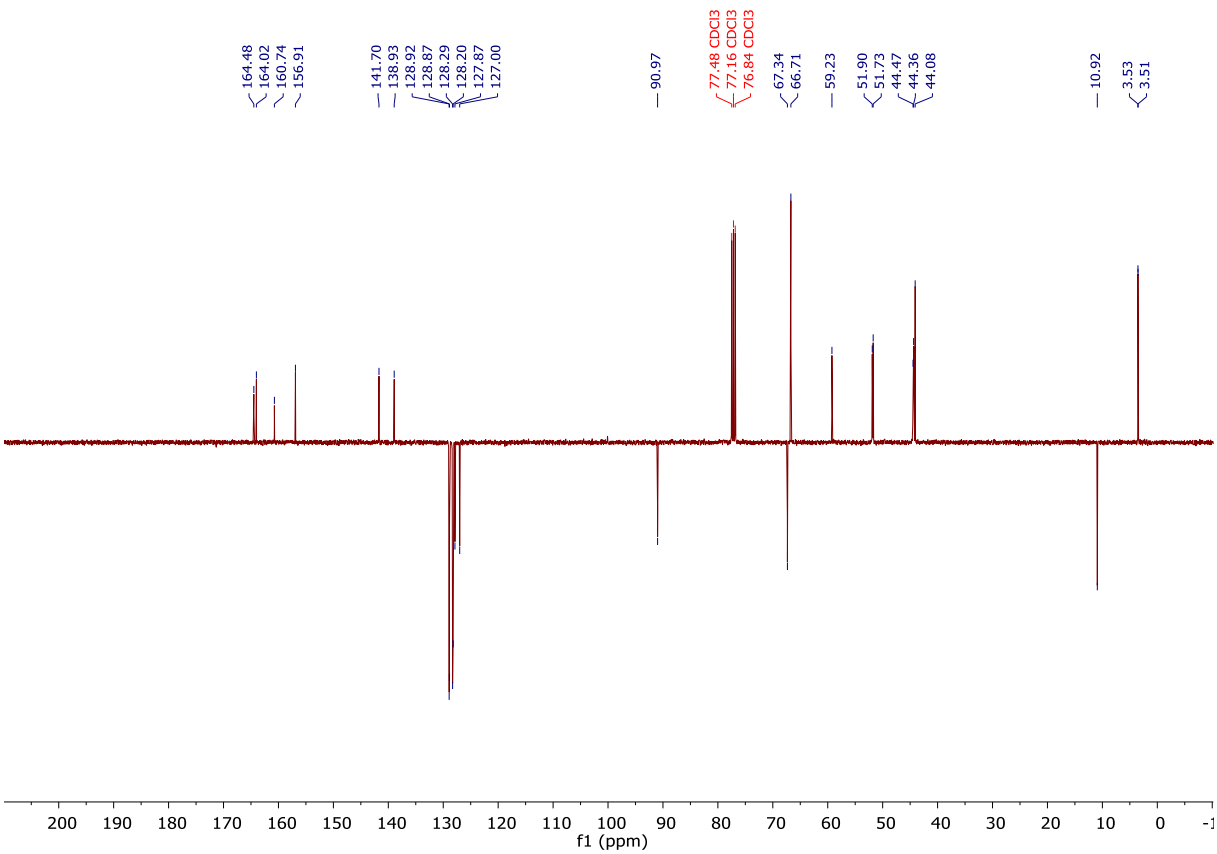

**$^{13}$C-NMR**



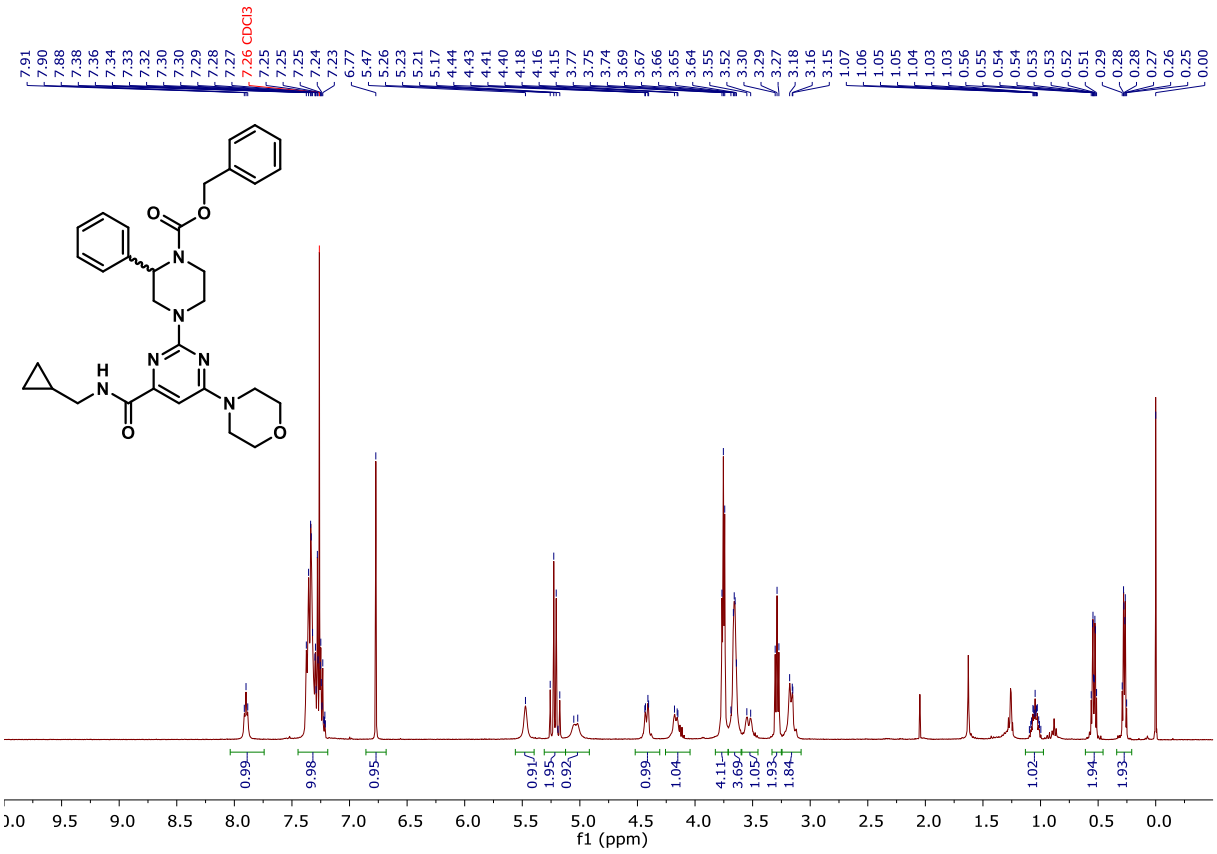
**$^{1}$H-NMR: 69**



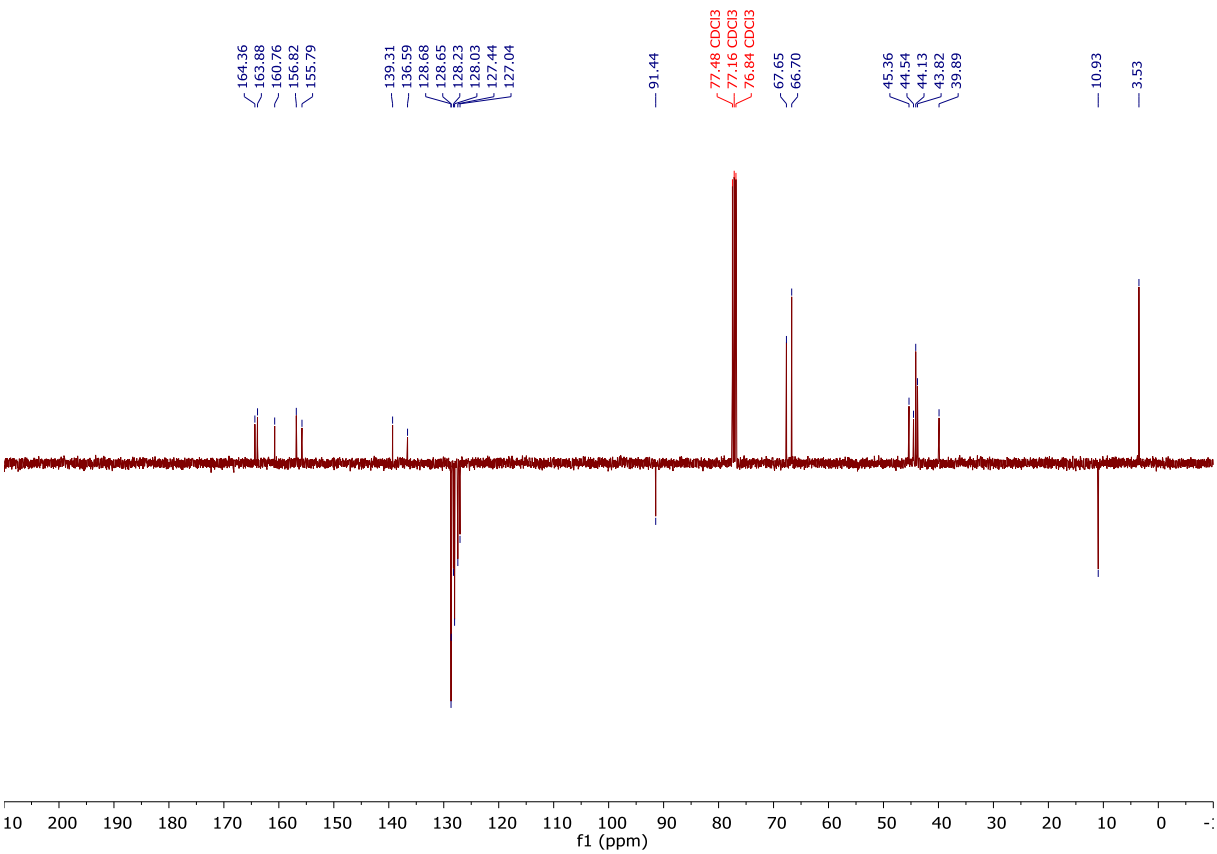

**$^{13}$C-NMR**



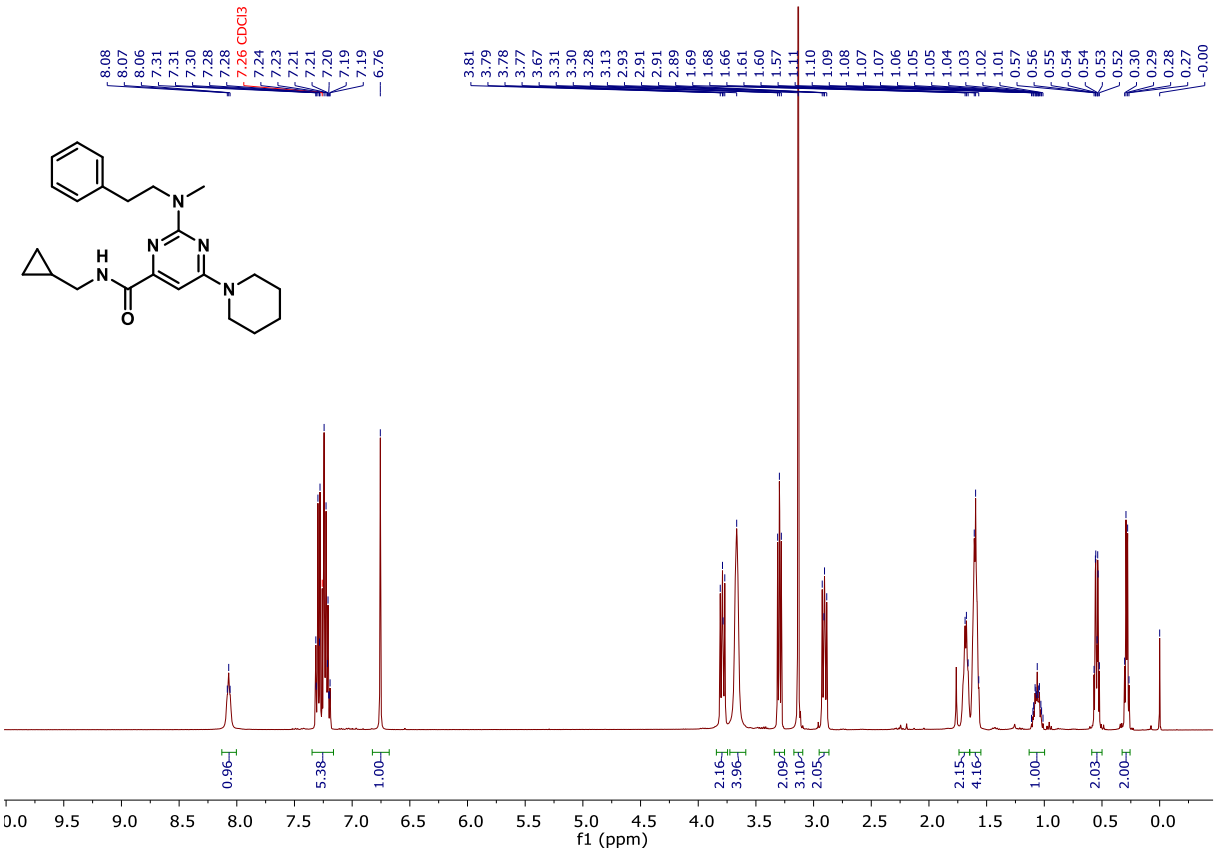
**$^{1}$H-NMR: 70**



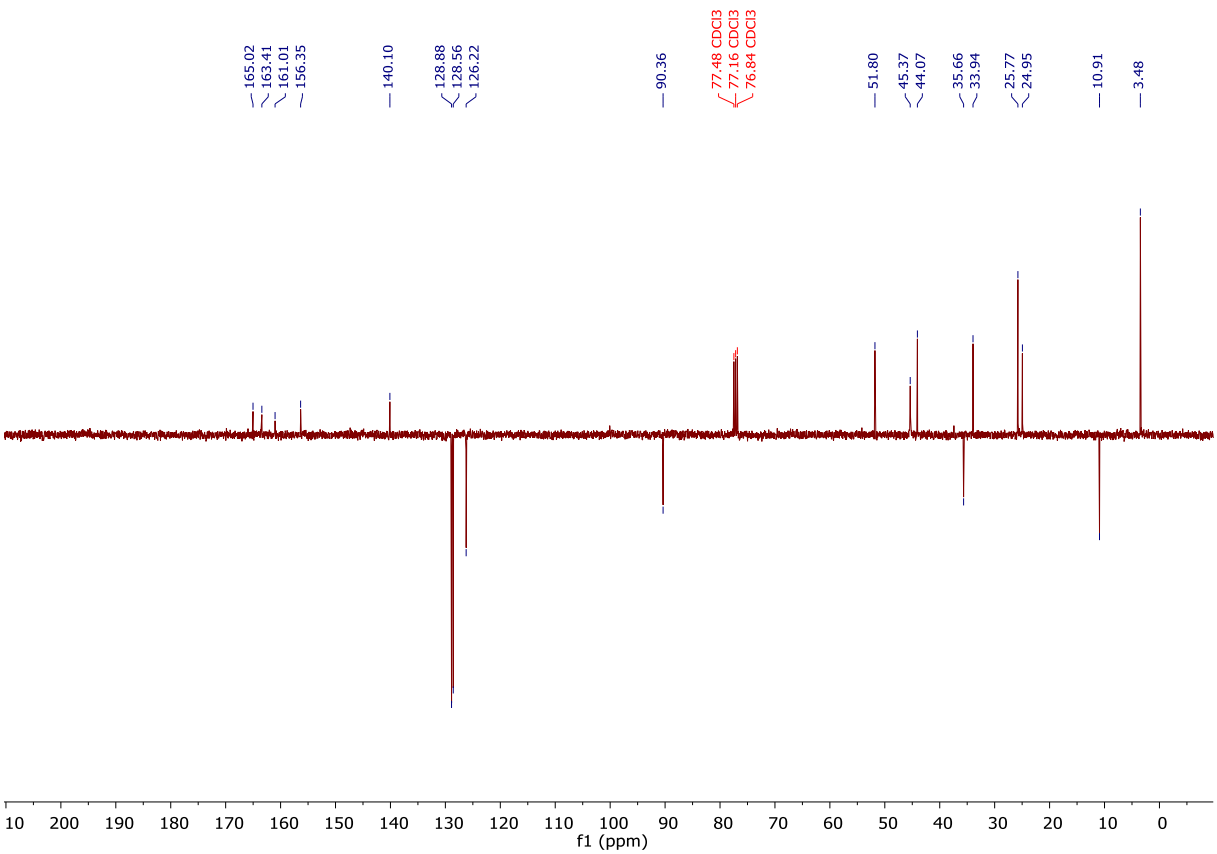

**$^{13}$C-NMR**



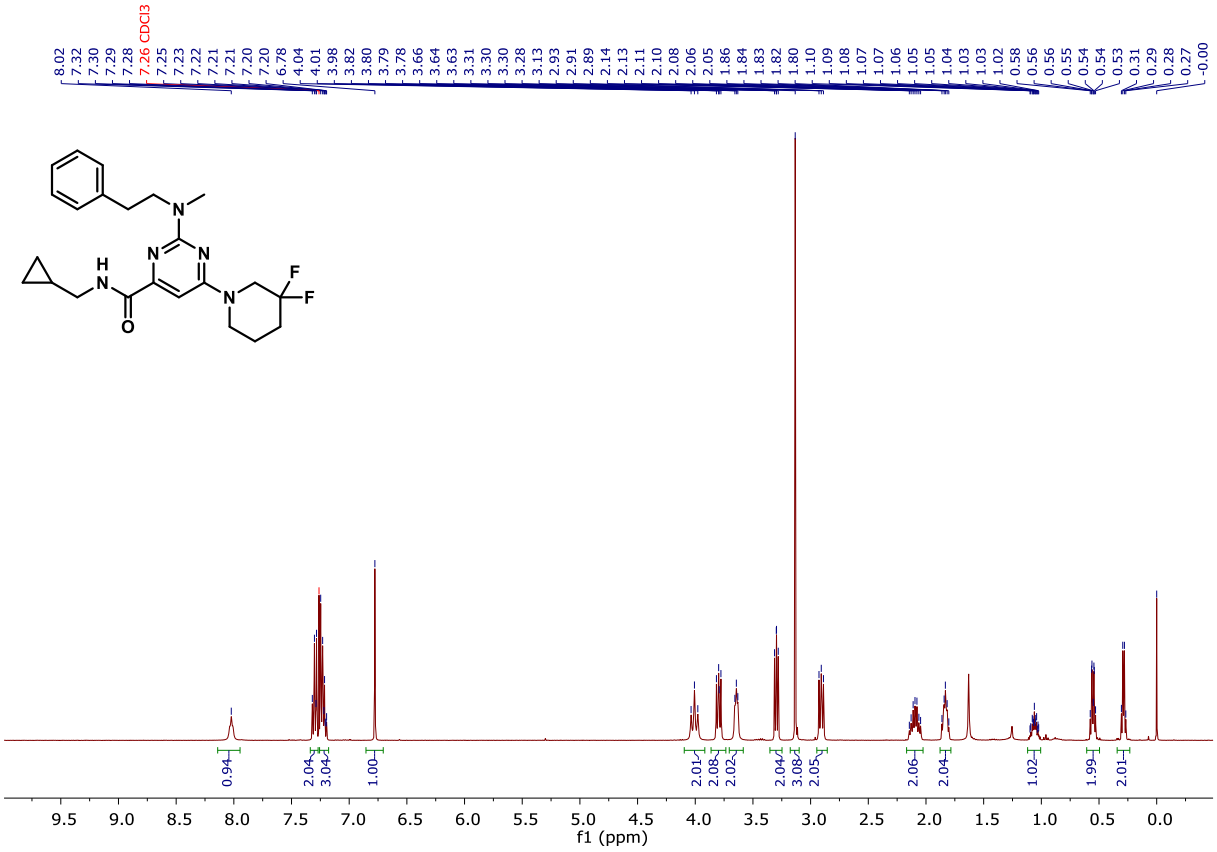
**$^{1}$H-NMR: 71**



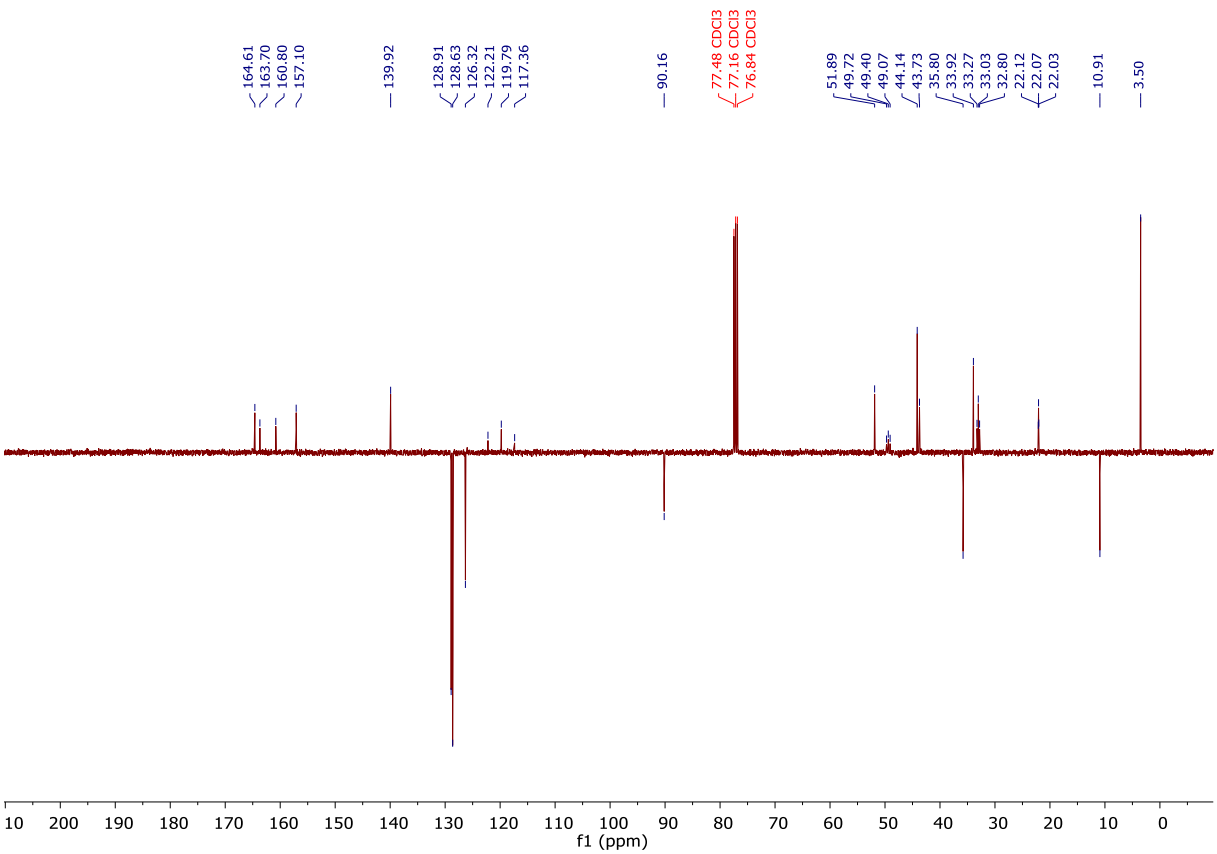

**$^{13}$C-NMR**



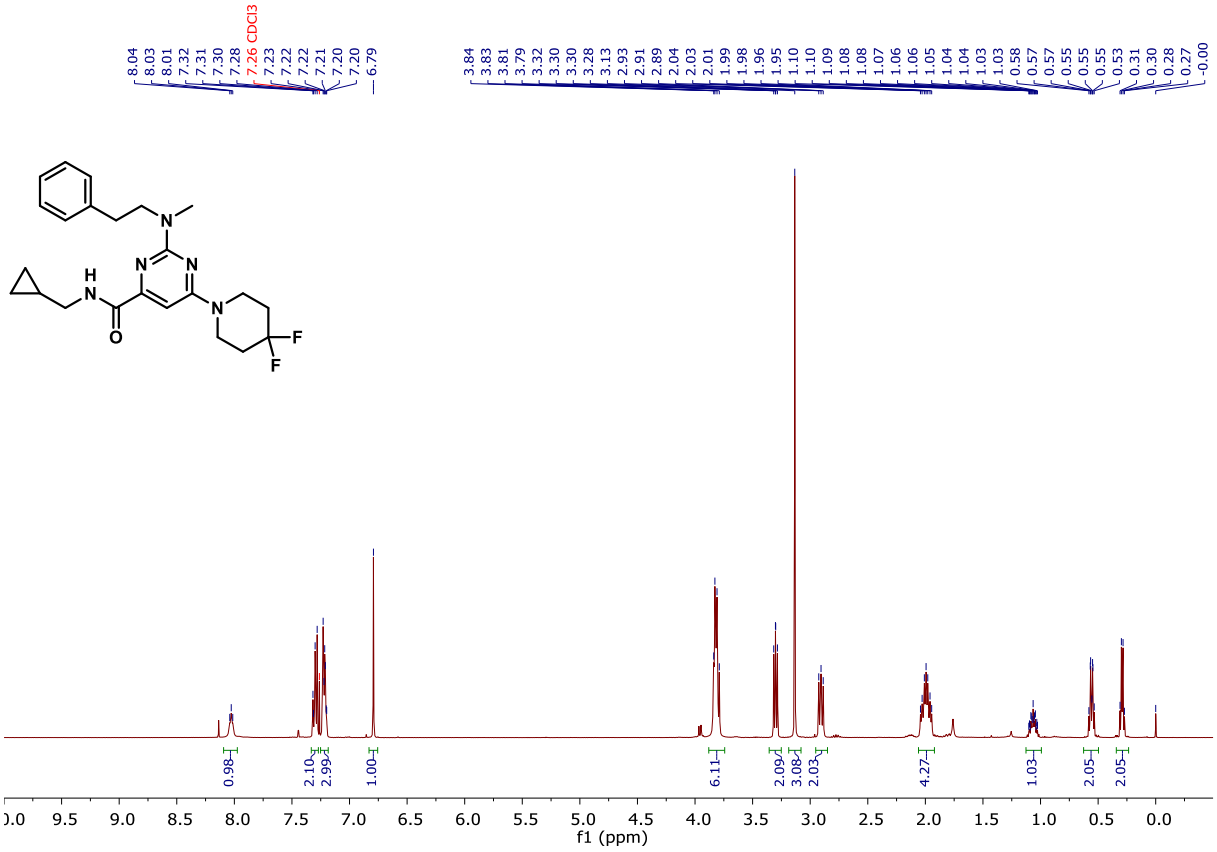
**$^{1}$H-NMR: 72**



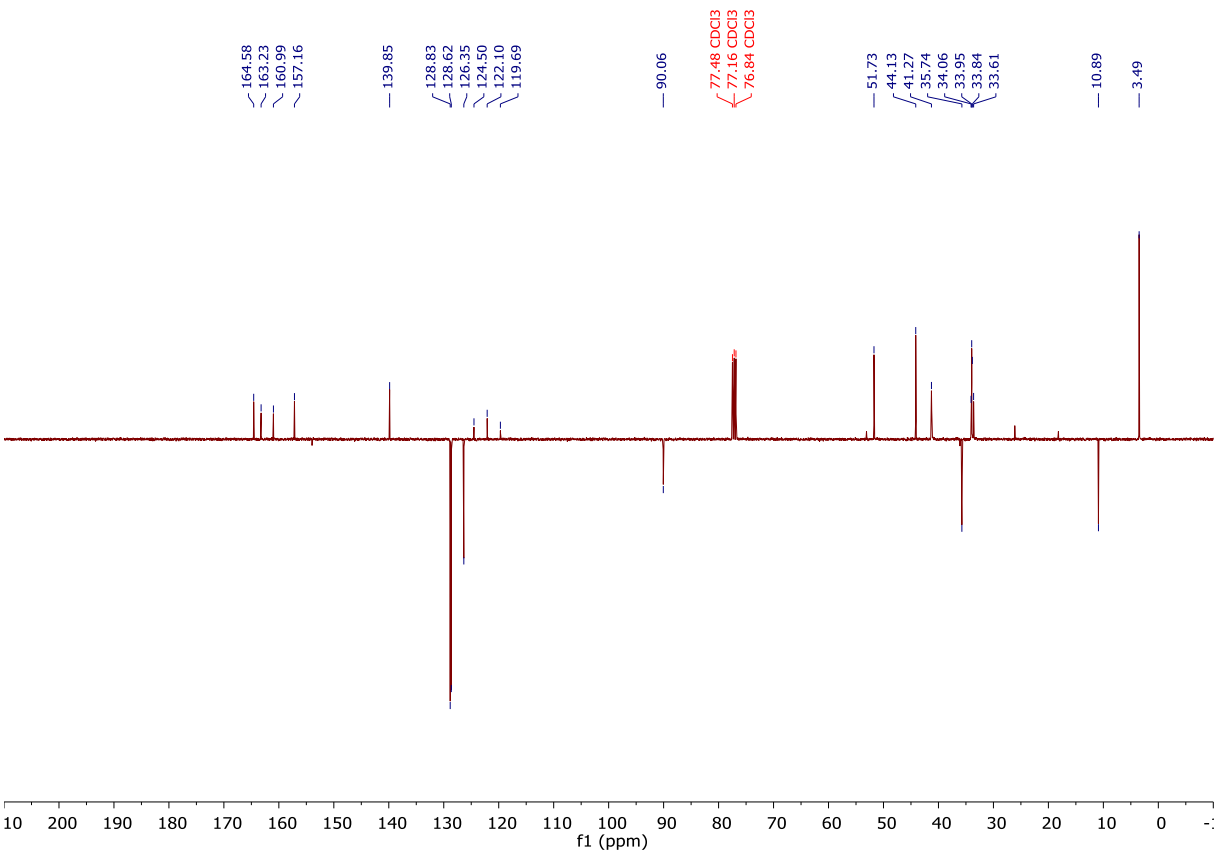

**$^{13}$C-NMR**



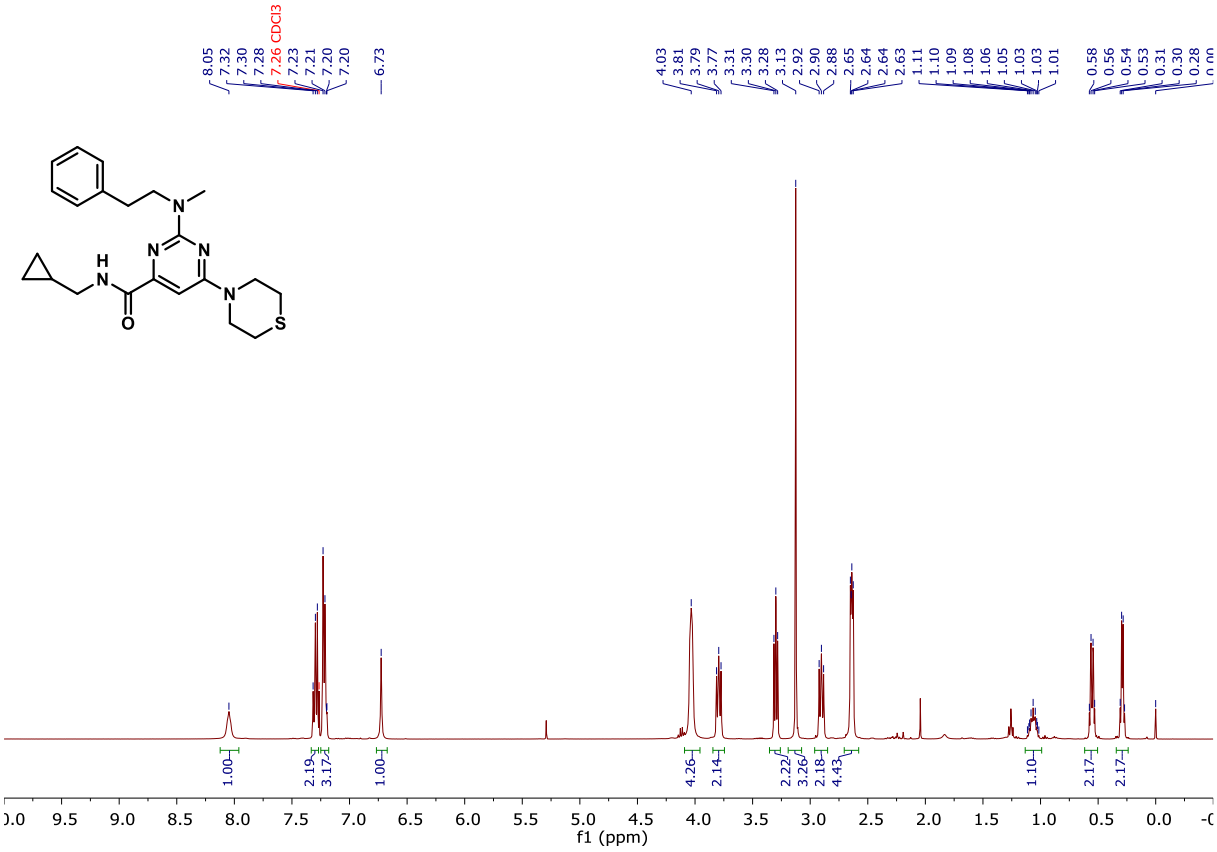
**$^{1}$H-NMR: 73**



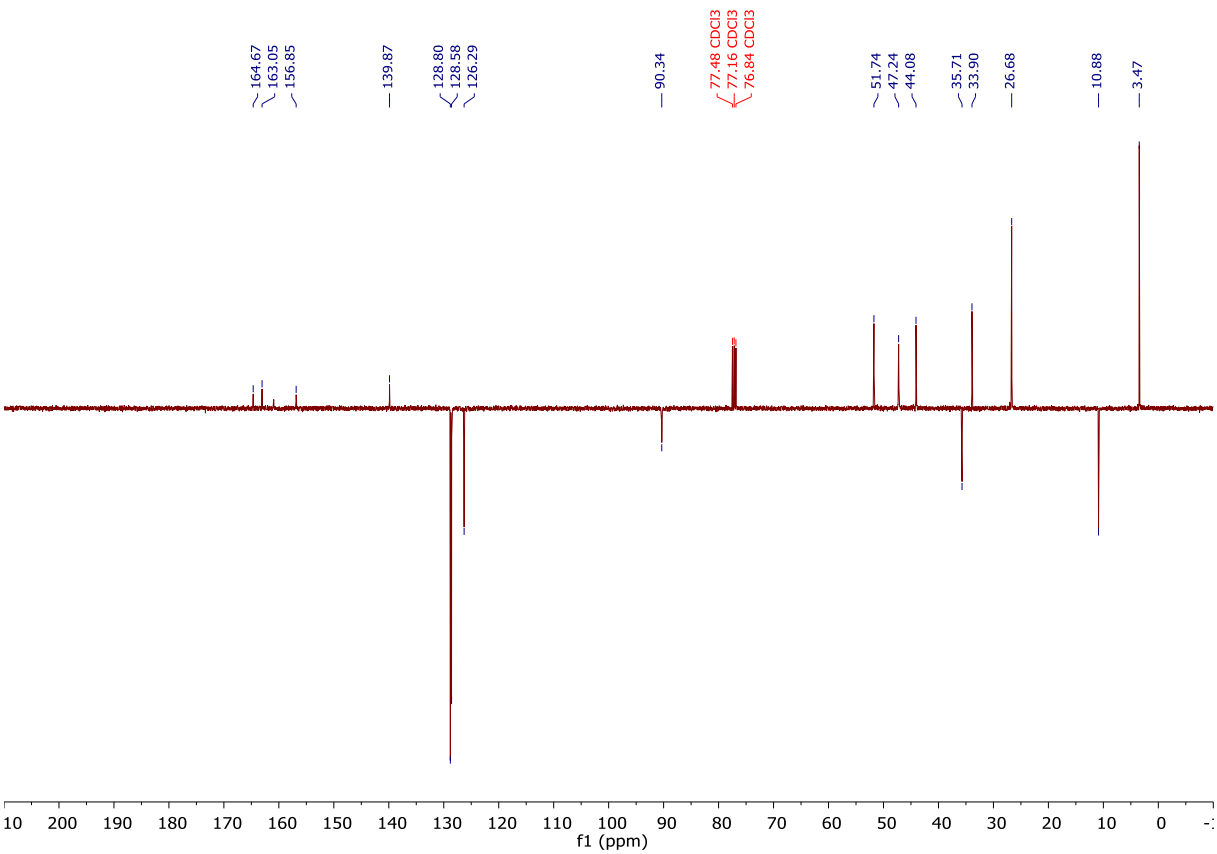

**$^{13}$C-NMR**



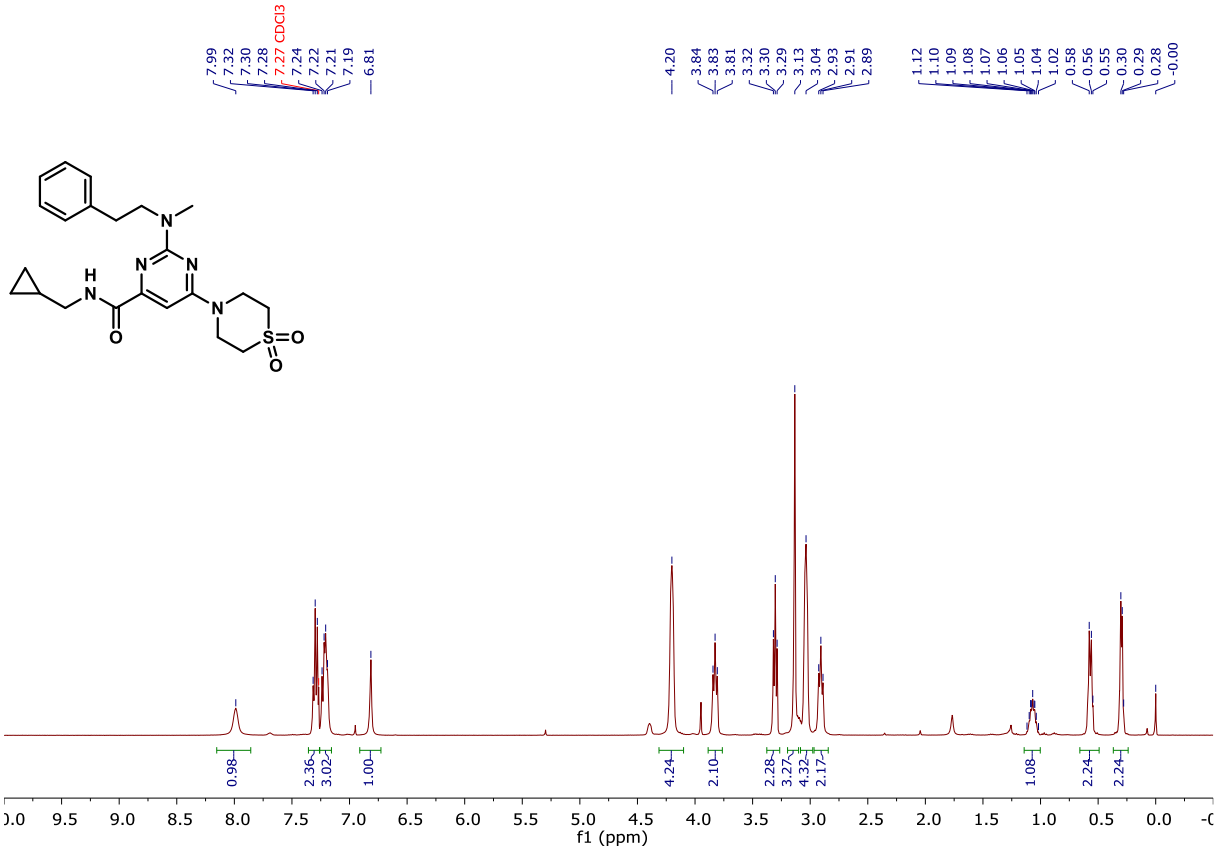
**$^{1}$H-NMR: 74**



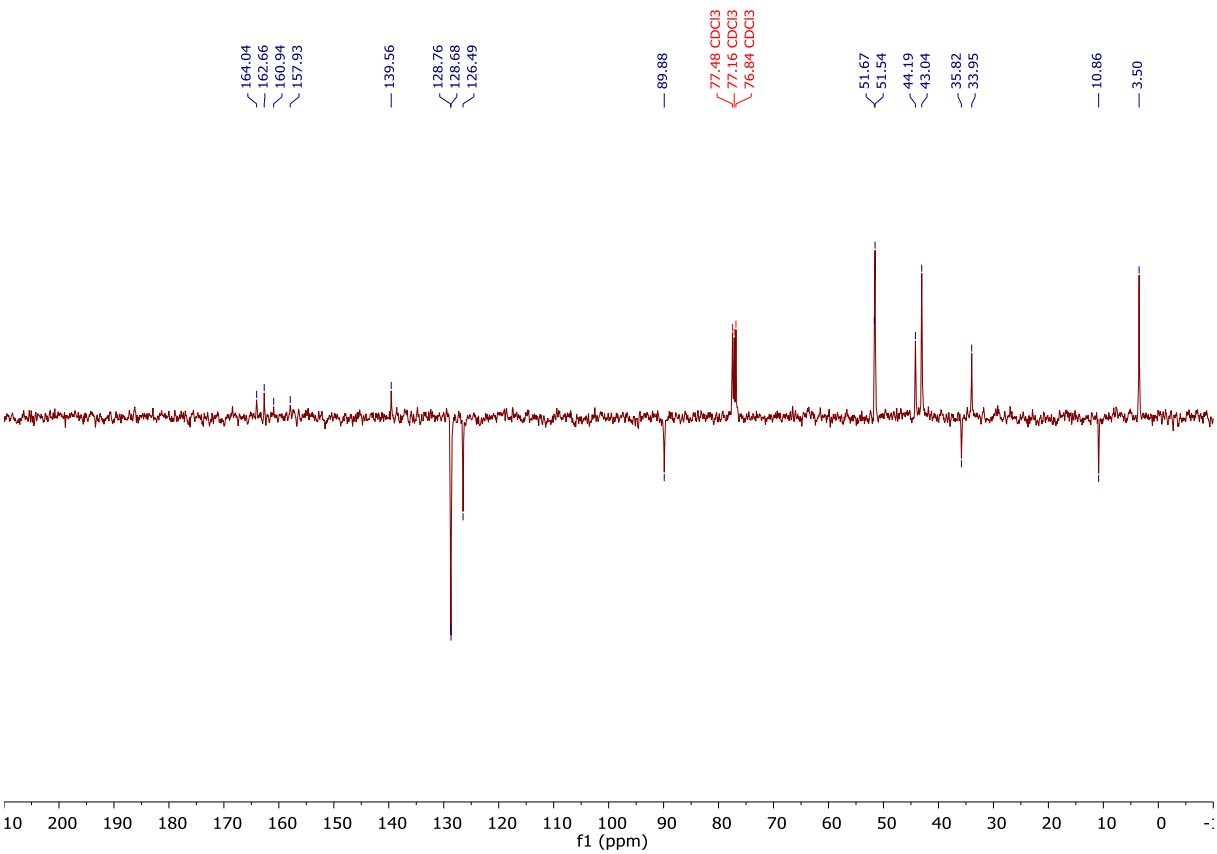

**$^{13}$C-NMR**



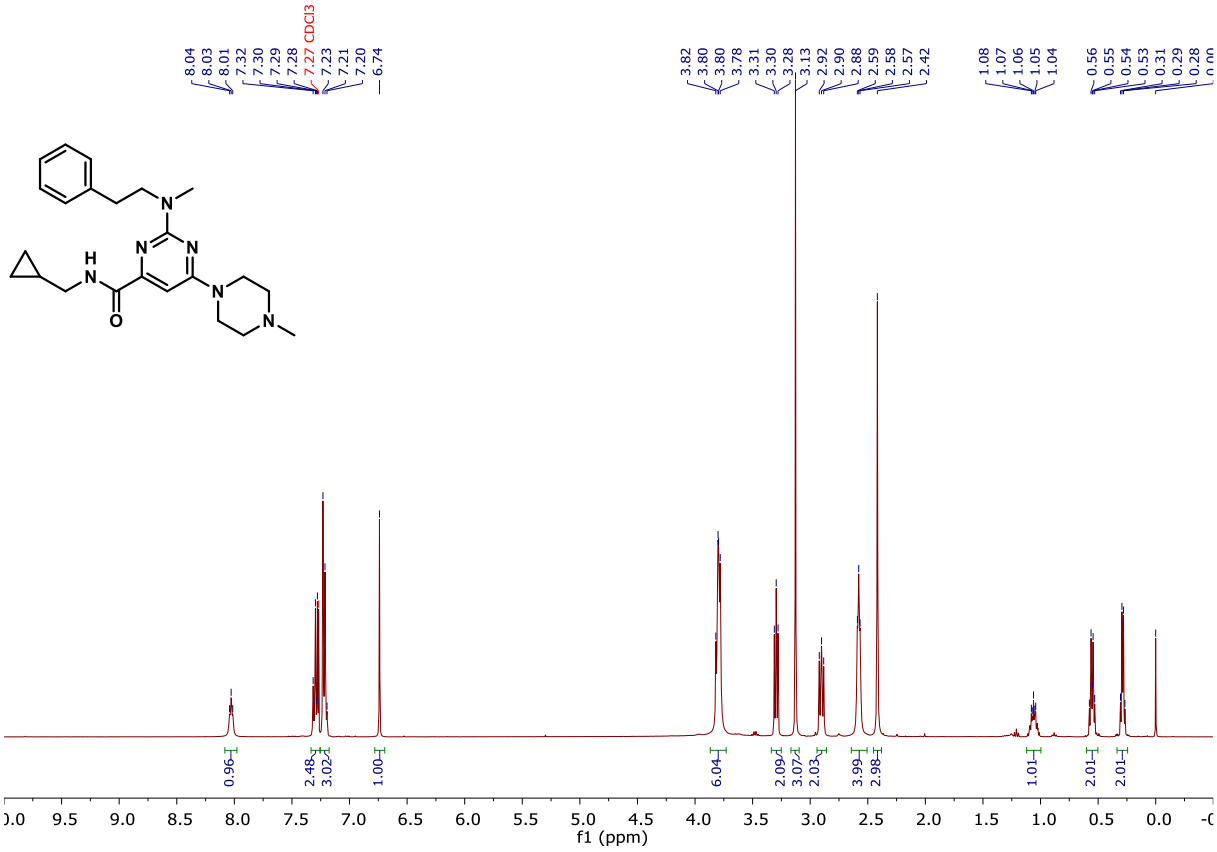
**$^{1}$H-NMR: 75**



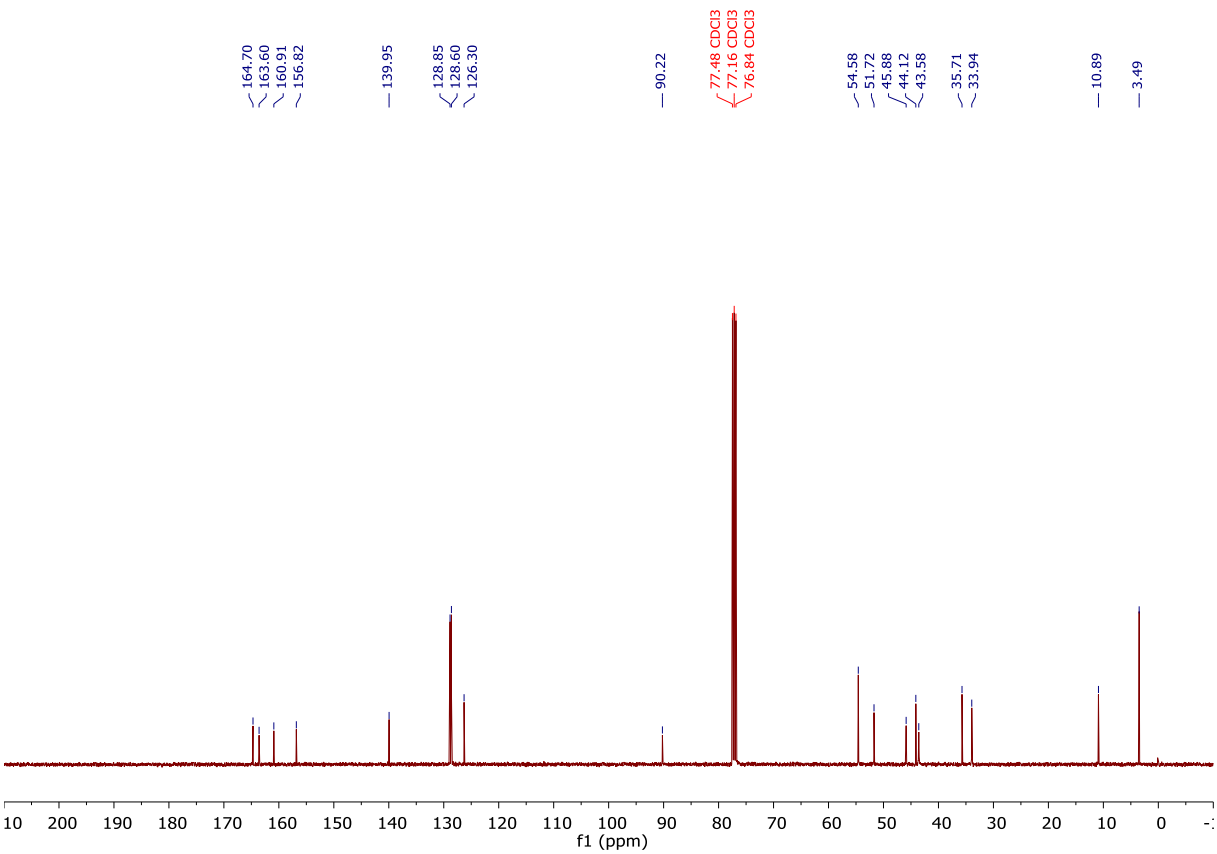

**$^{13}$C-NMR**



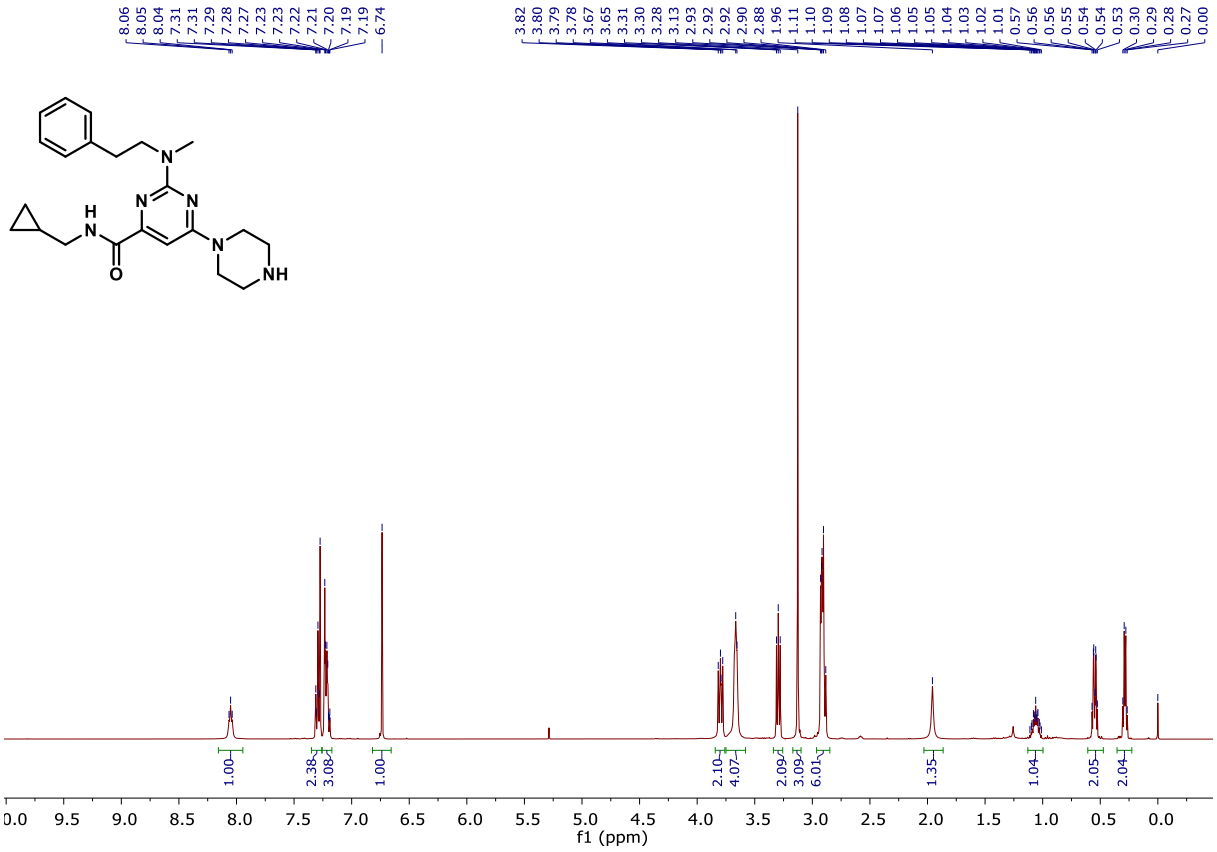
**$^{1}$H-NMR: 76**



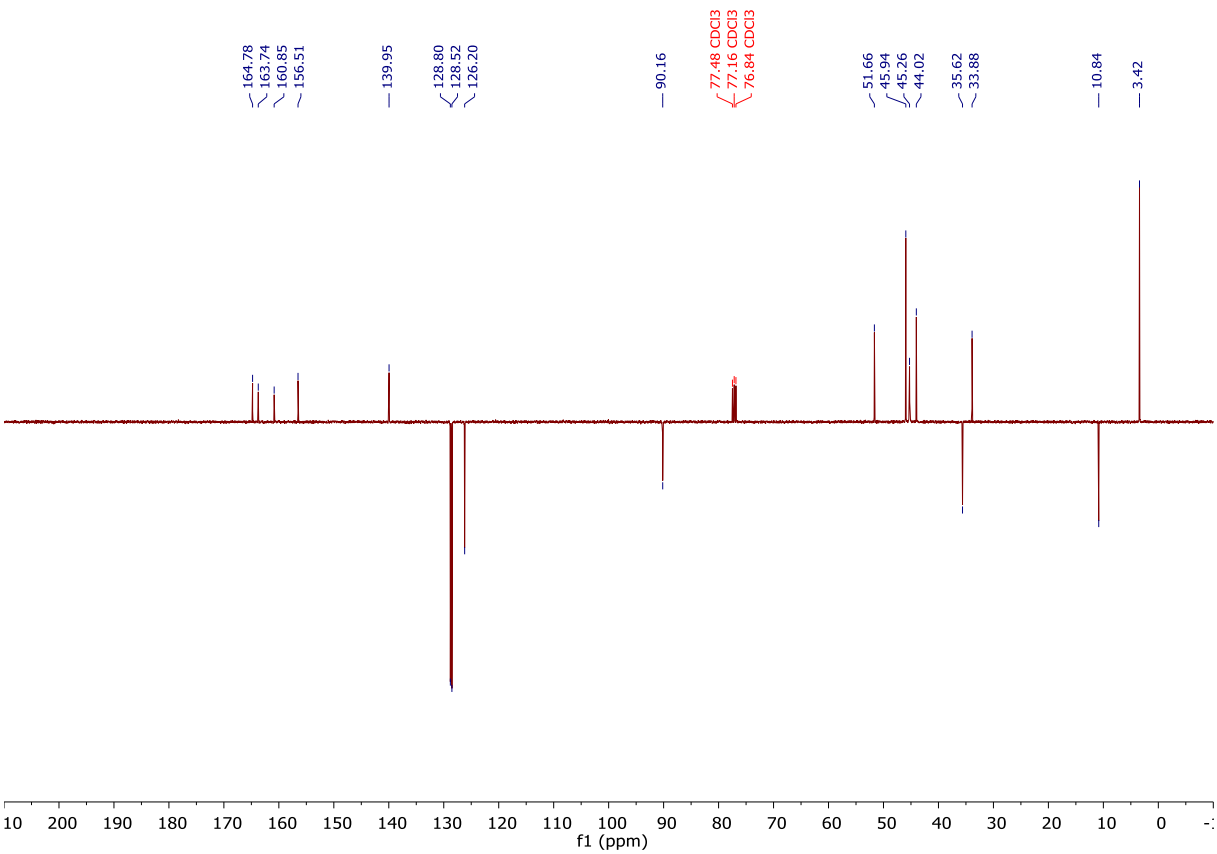

**$^{13}$C-NMR**



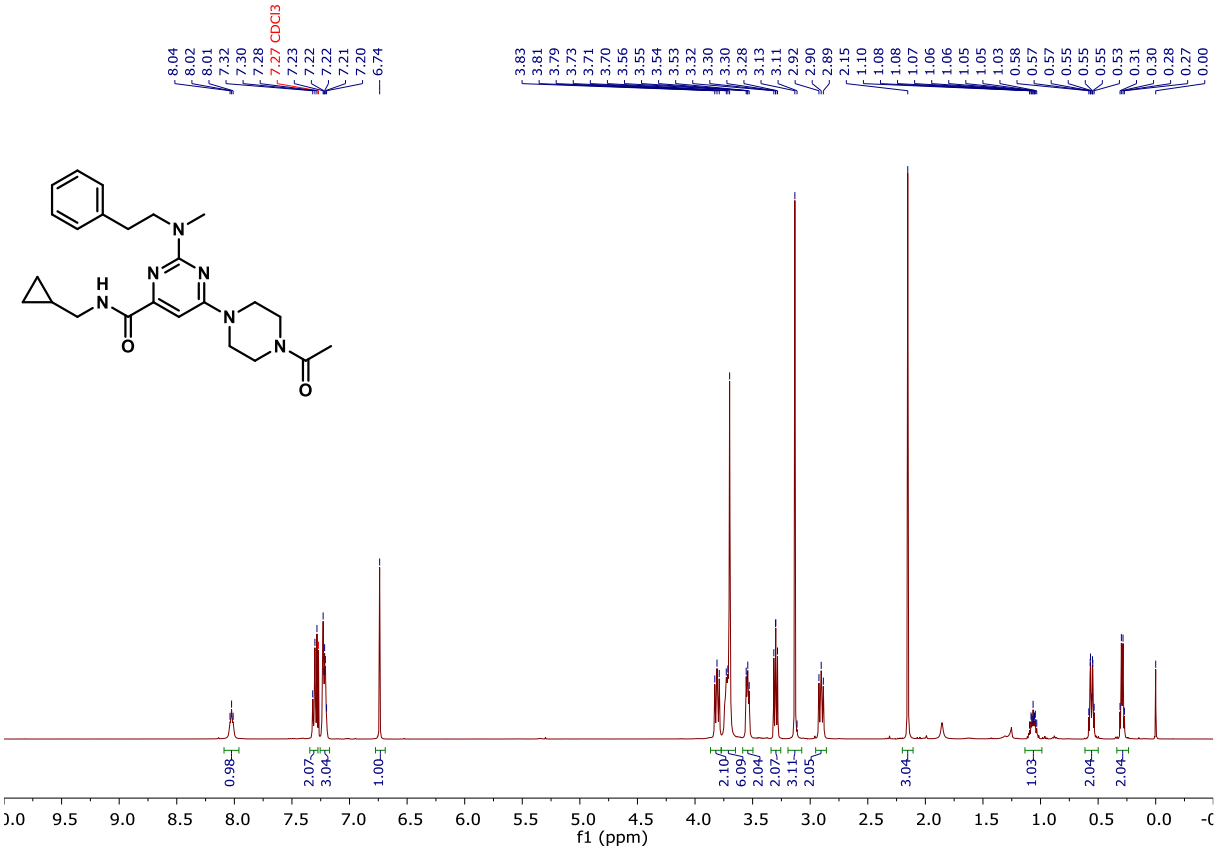
**$^{1}$H-NMR: 77**



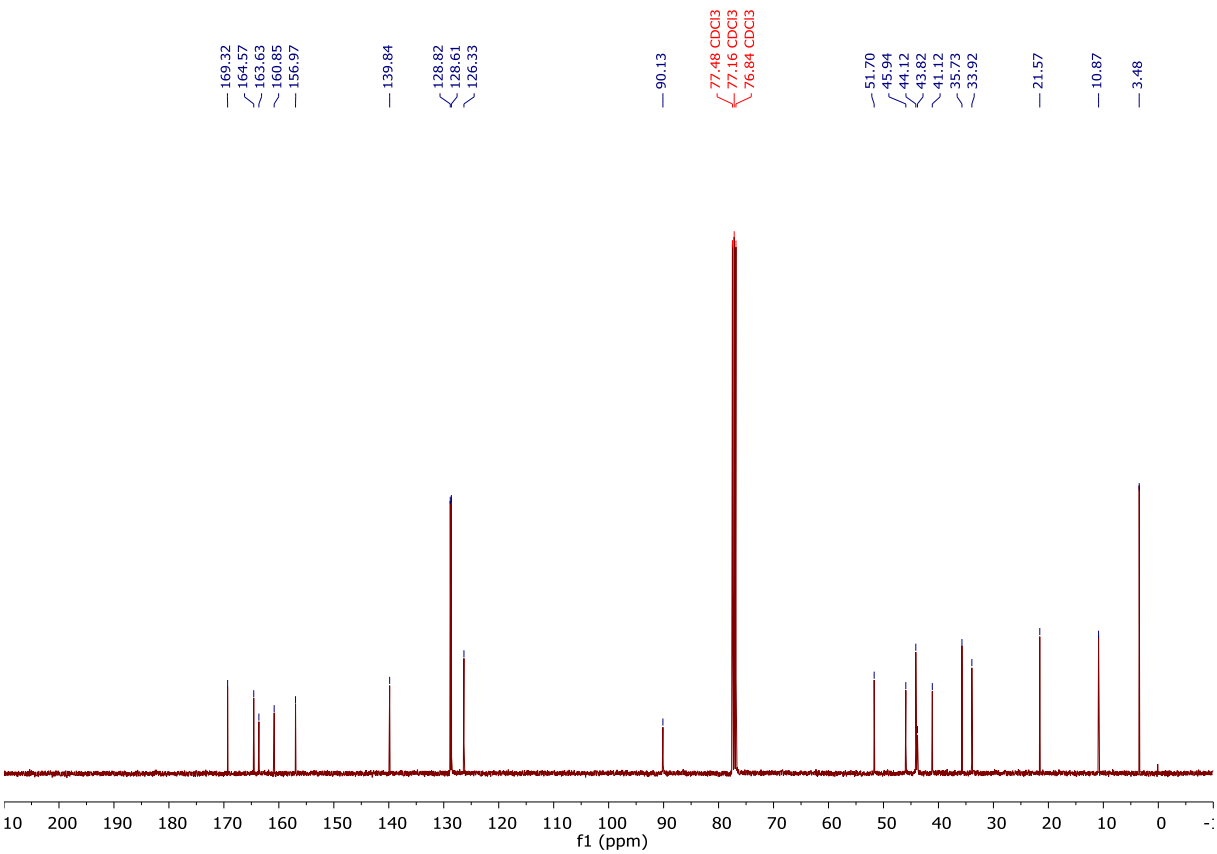

**$^{13}$C-NMR**



**$^{1}$H-NMR: 78**




**$^{13}$C-NMR**



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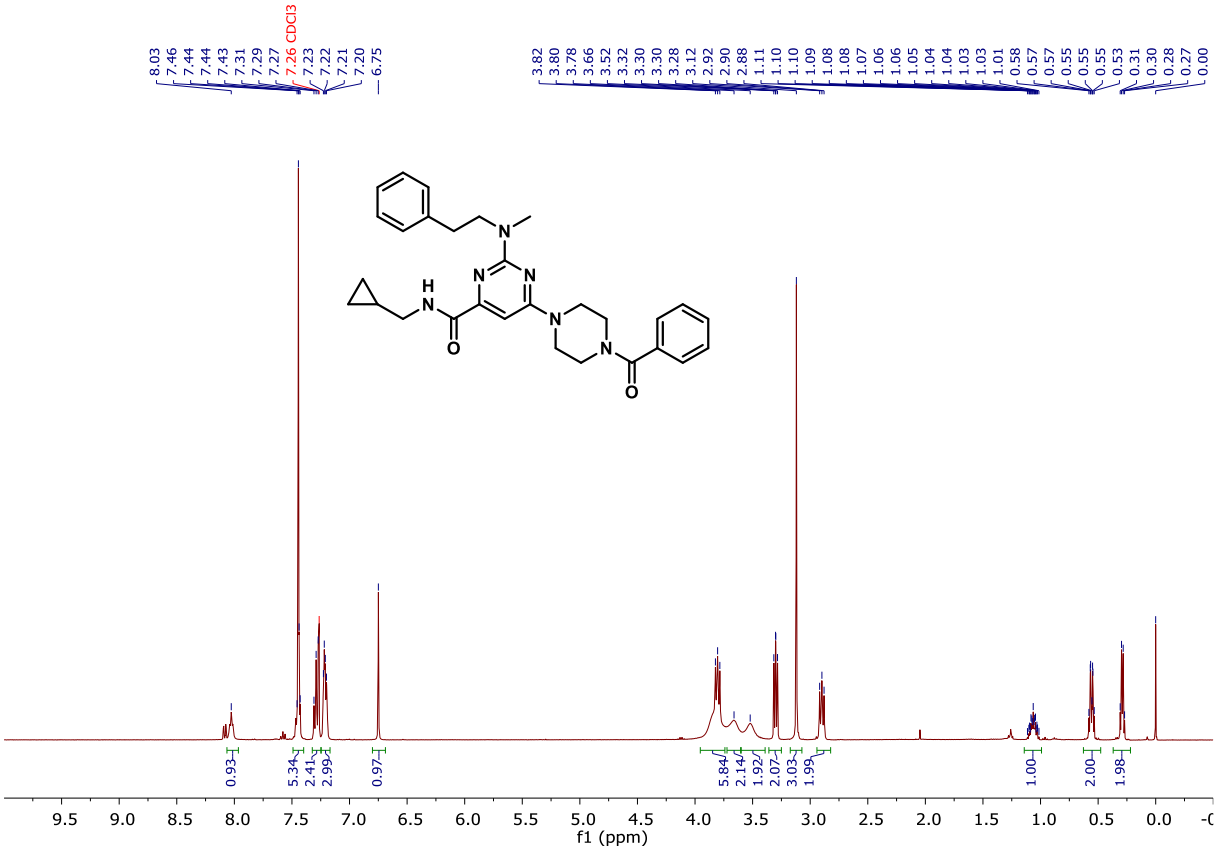
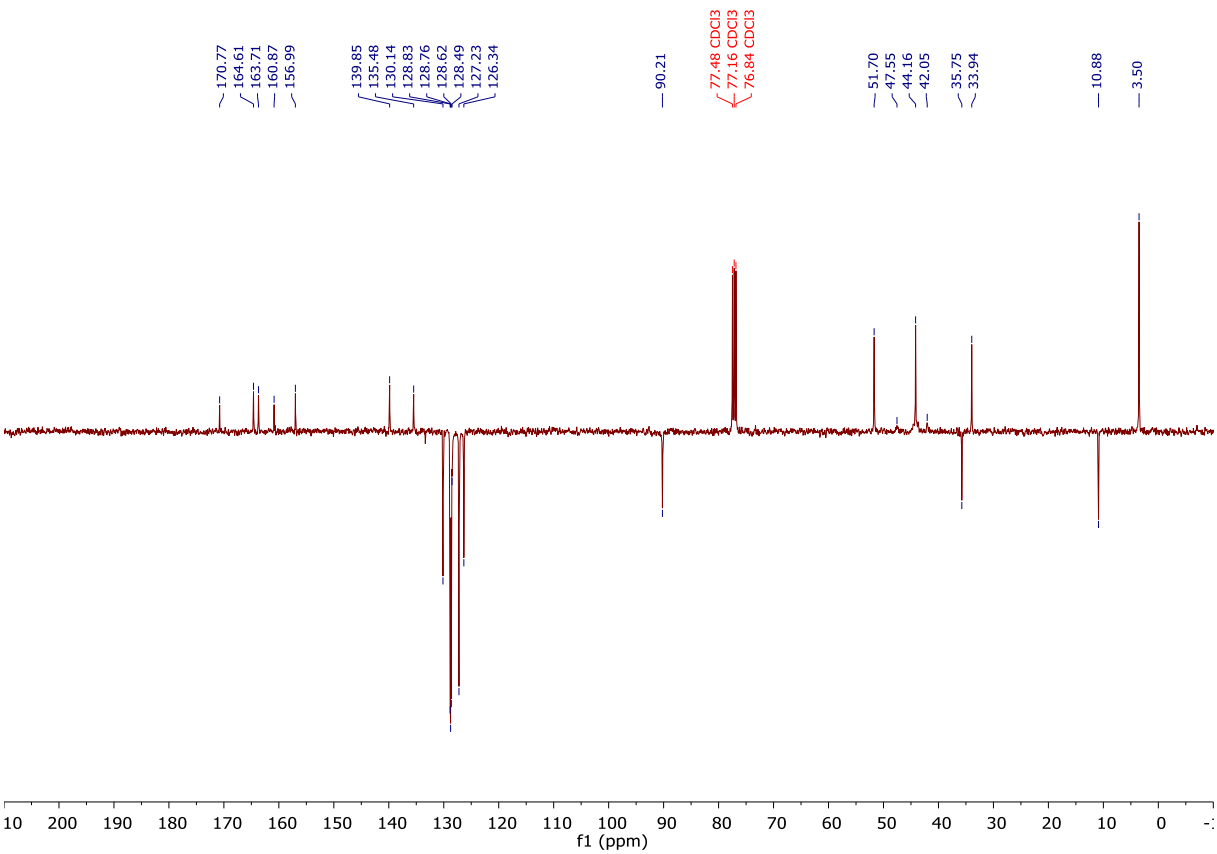
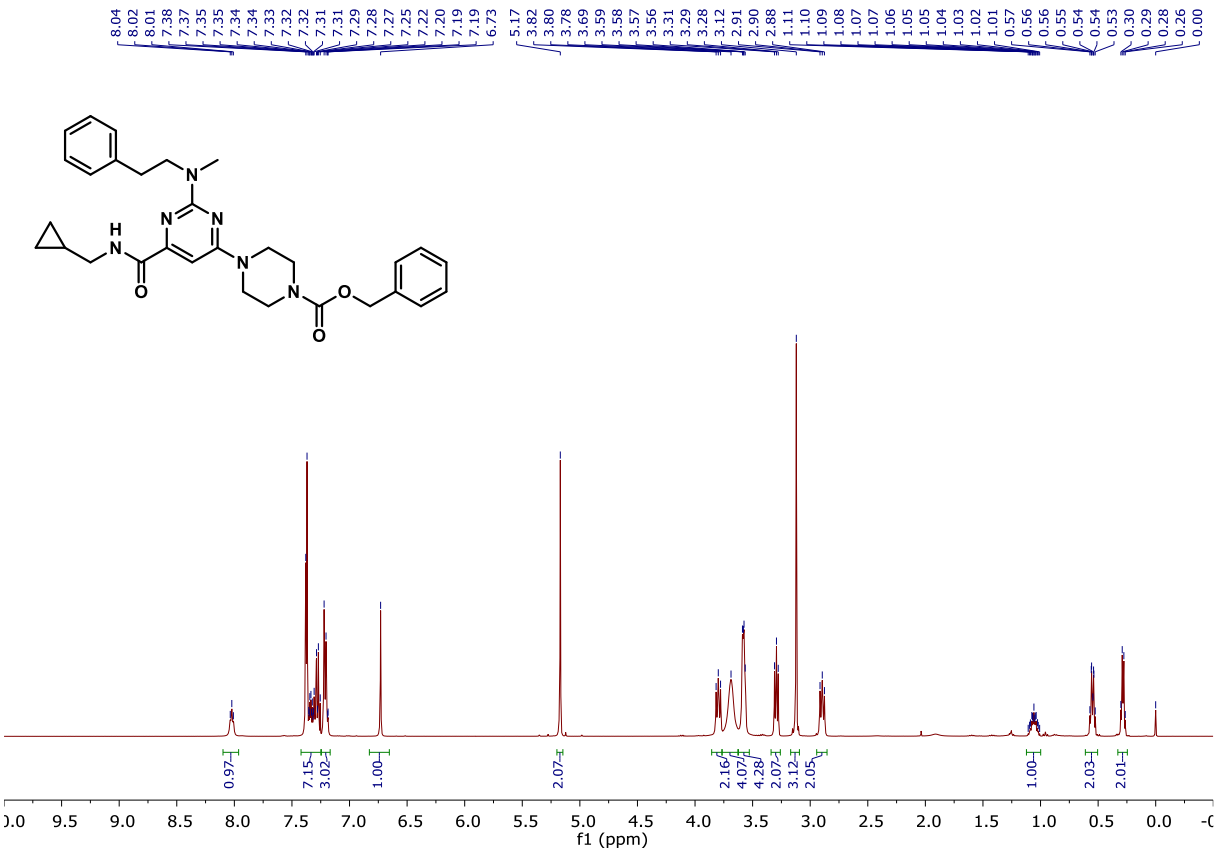


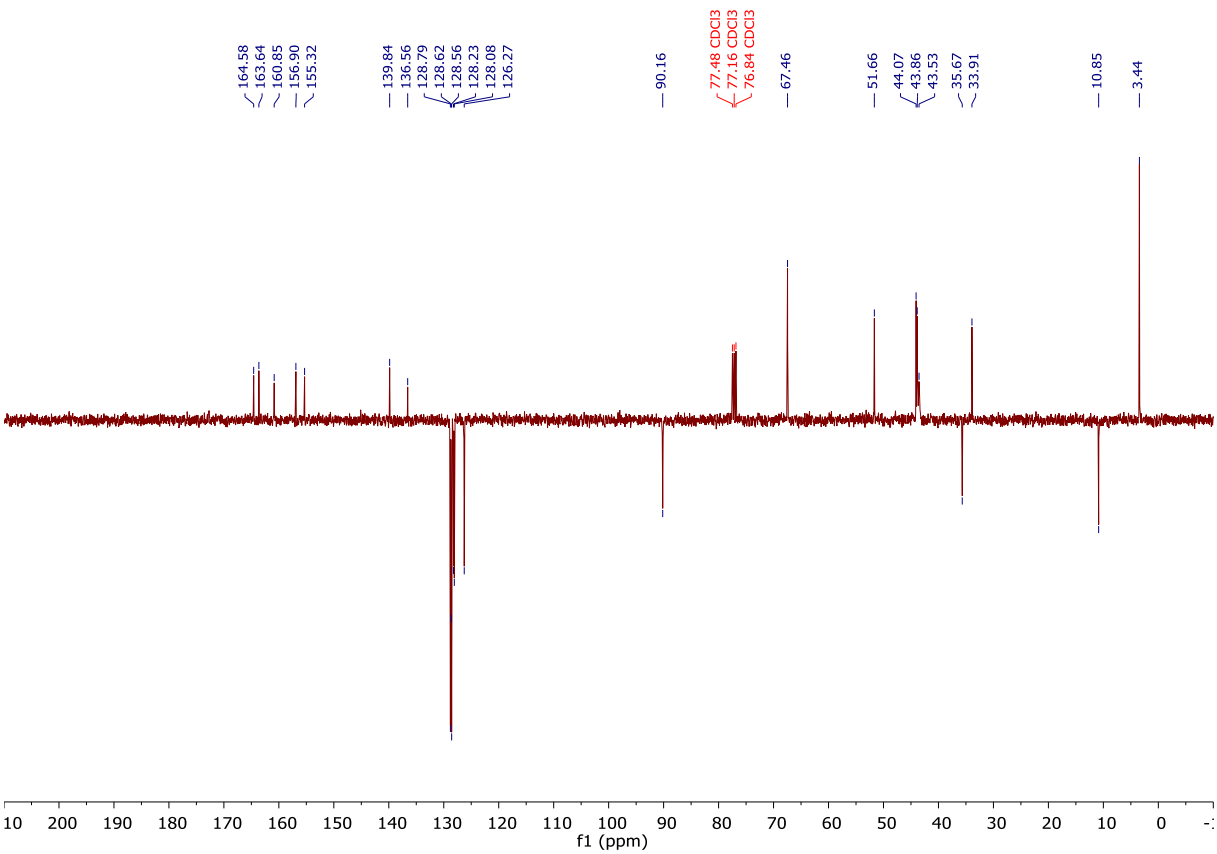

**$^{13}$C-NMR**



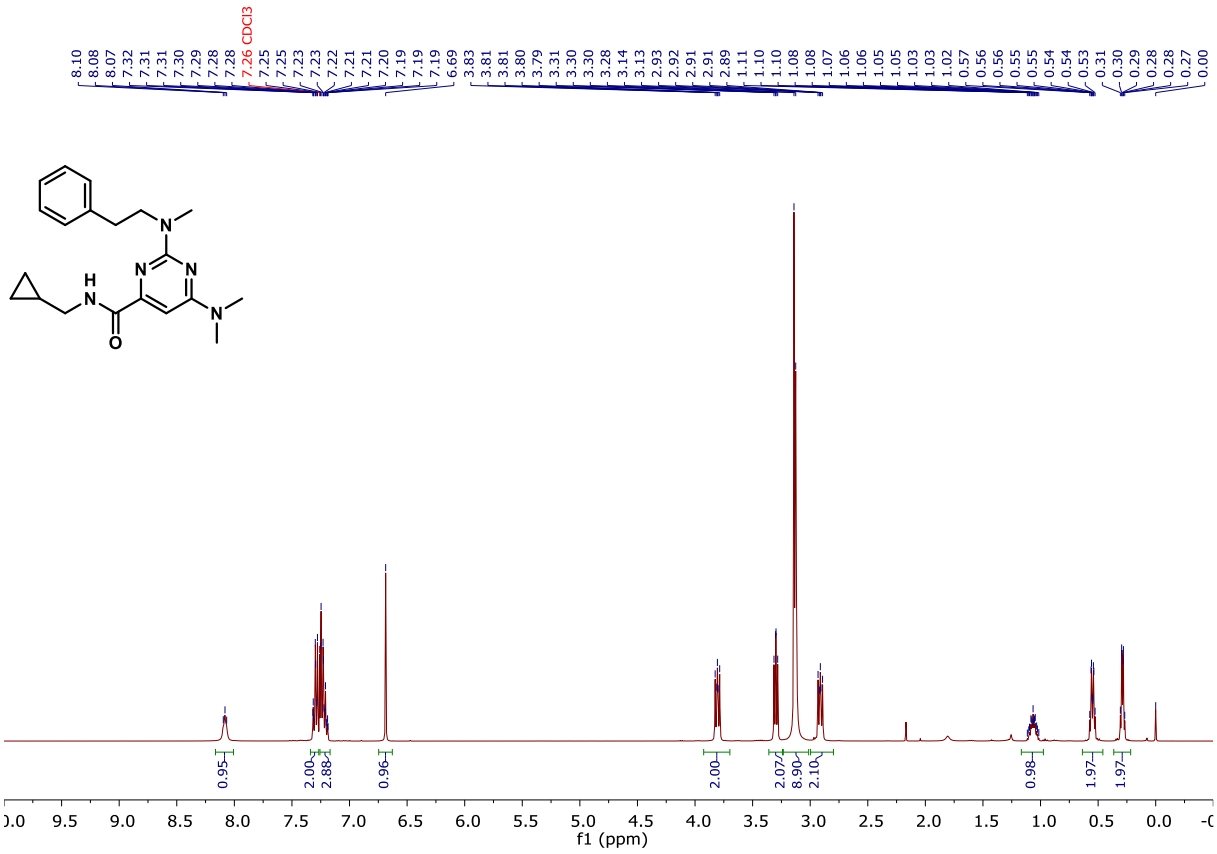
**$^{1}$H-NMR: 80**



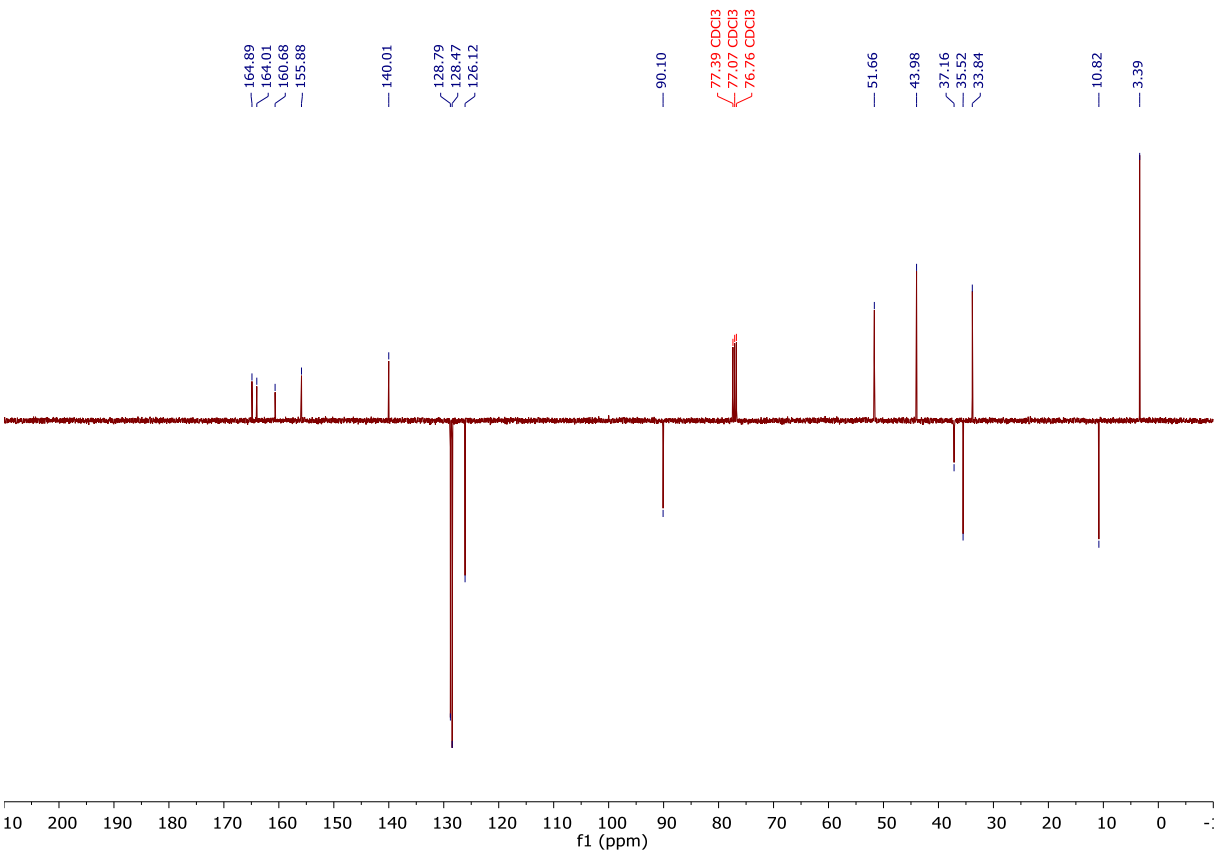

**$^{13}$C-NMR**



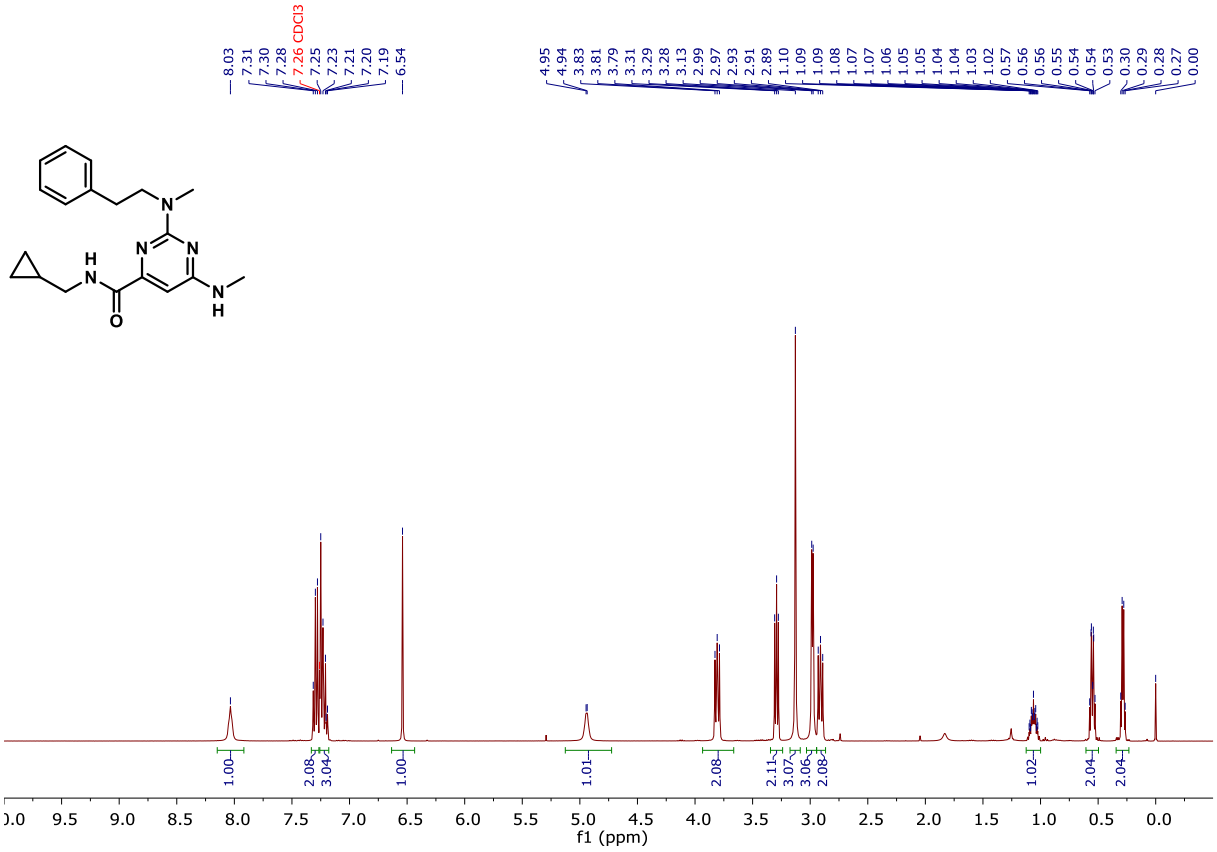
**$^{1}$H-NMR: 81**



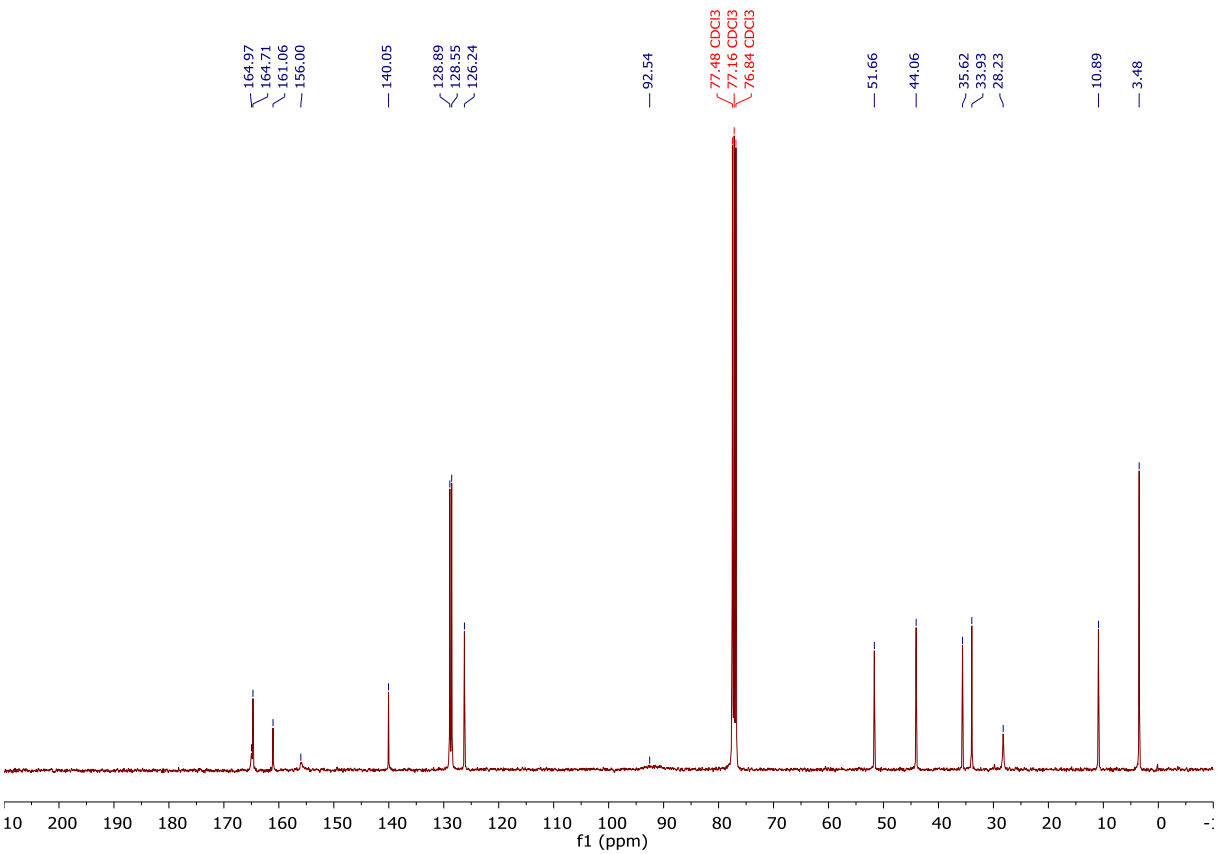

**$^{13}$C-NMR**



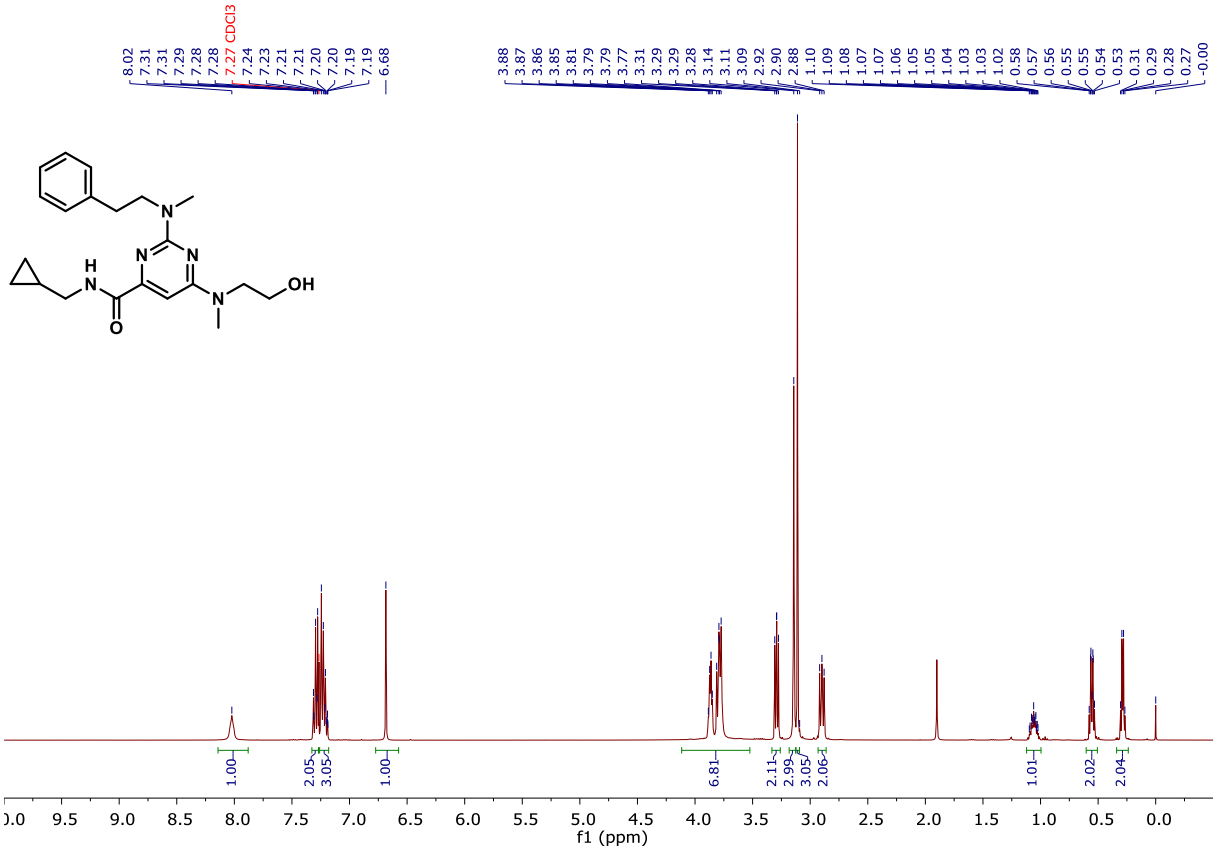
**$^{1}$H-NMR: 82**




**$^{13}$C-NMR**



**$^{1}$H-NMR: 83**

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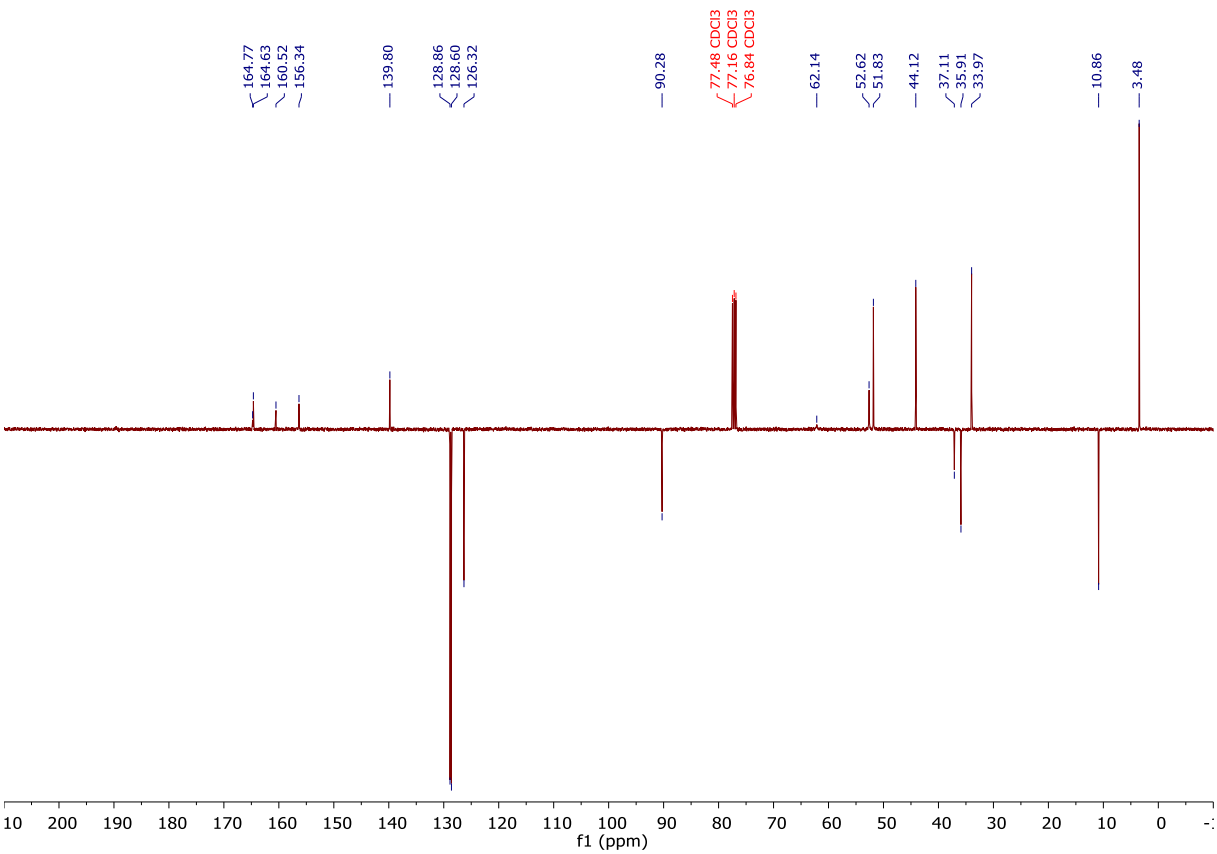
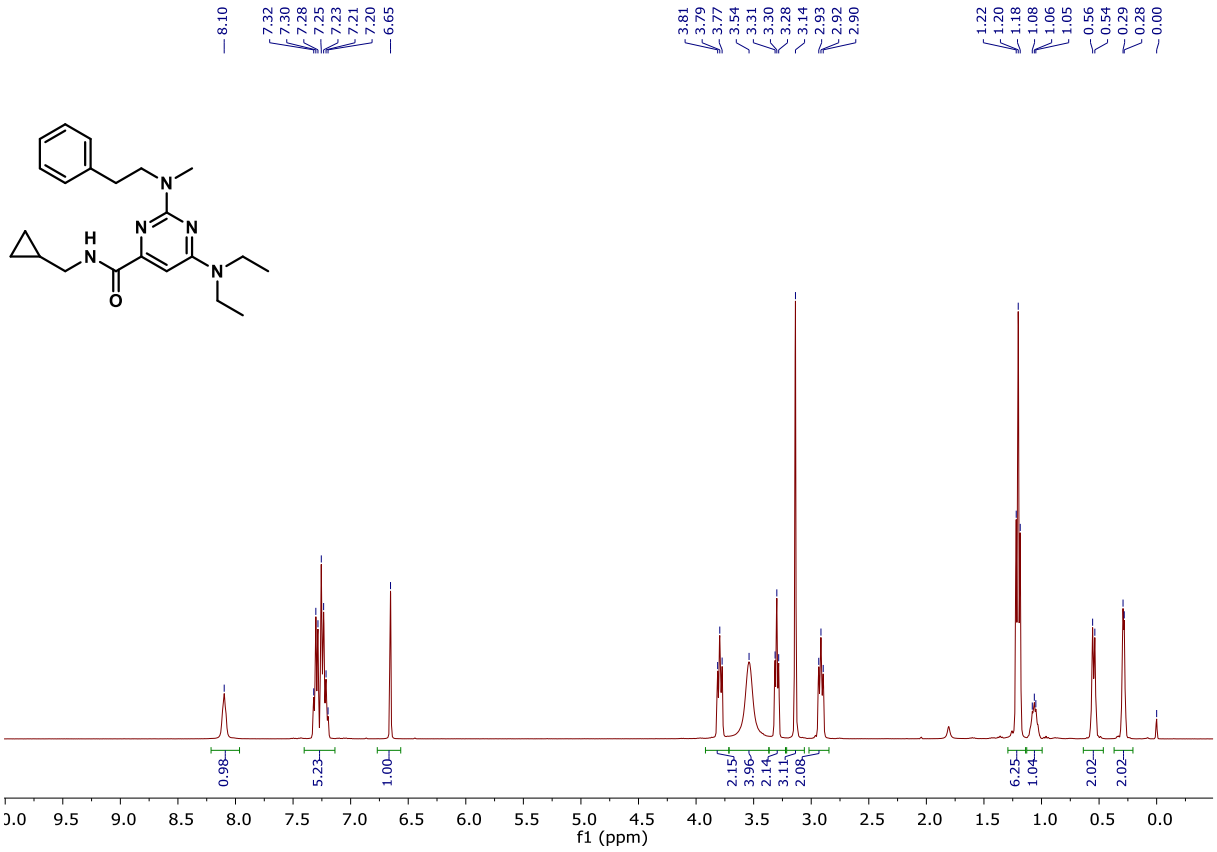
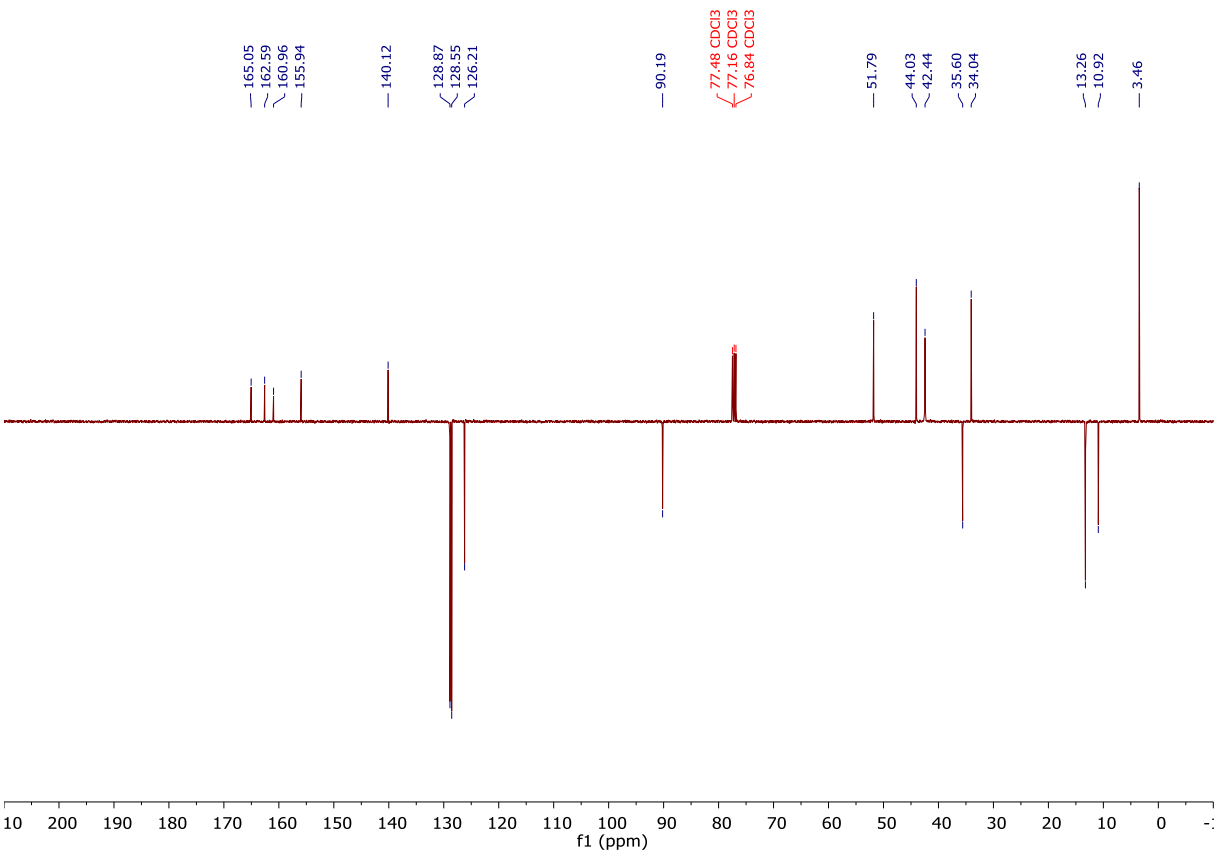


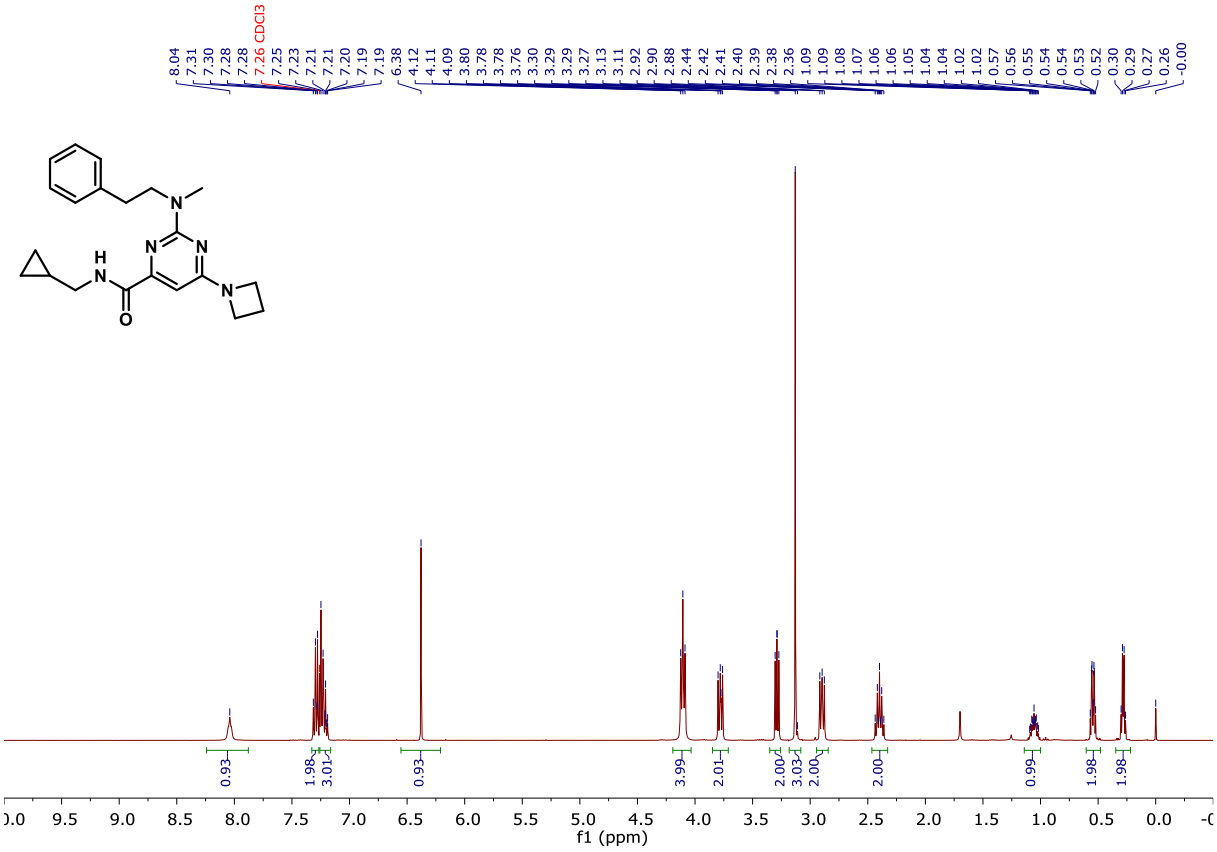
**$^{1}$H-NMR: 84**



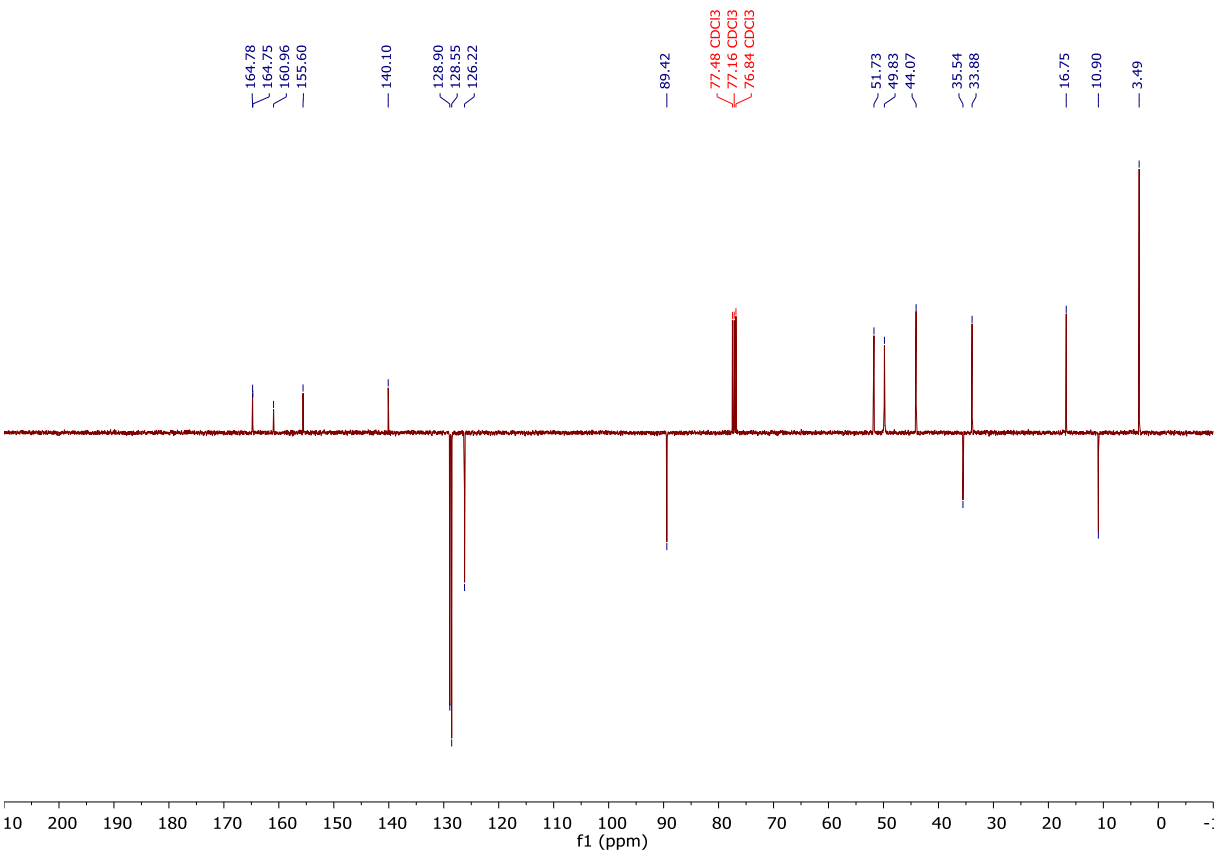

**$^{13}$C-NMR**



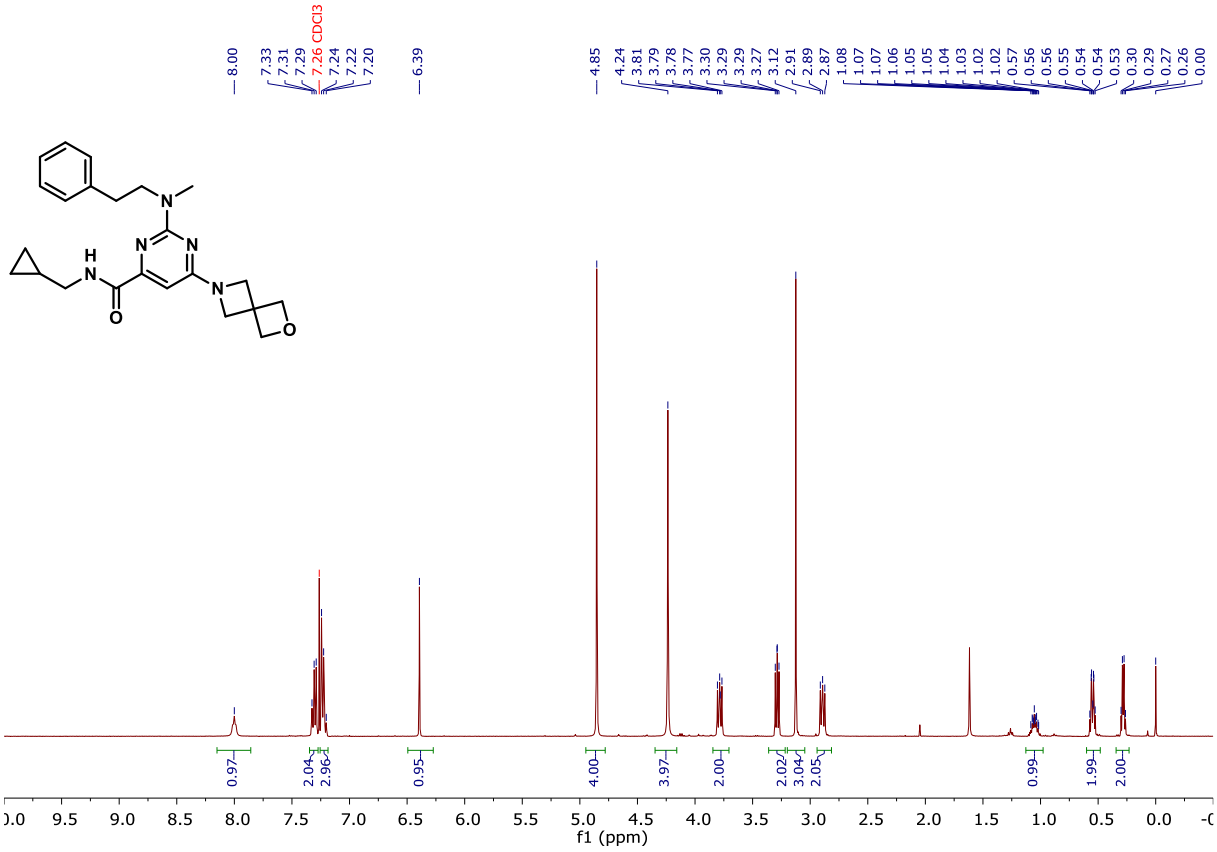
**$^{1}$H-NMR: 85**



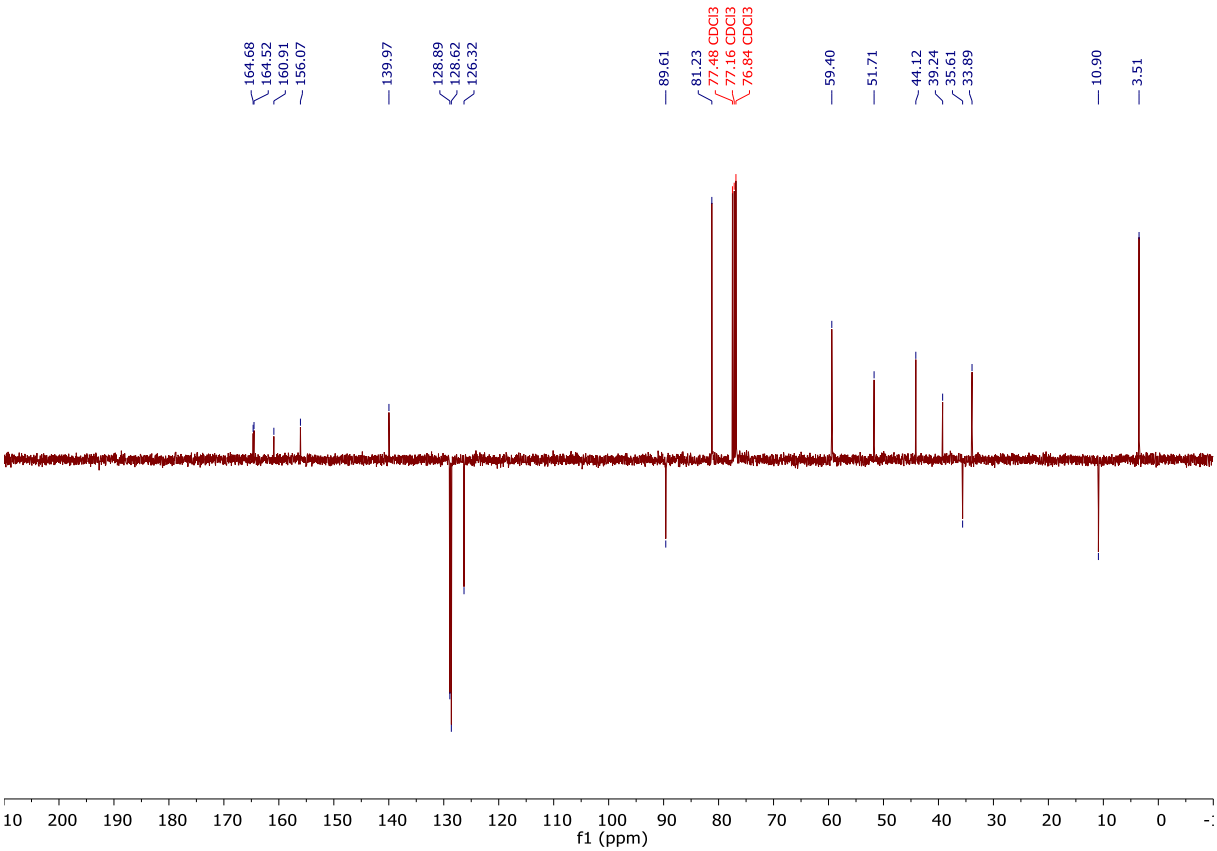

**$^{13}$C-NMR**



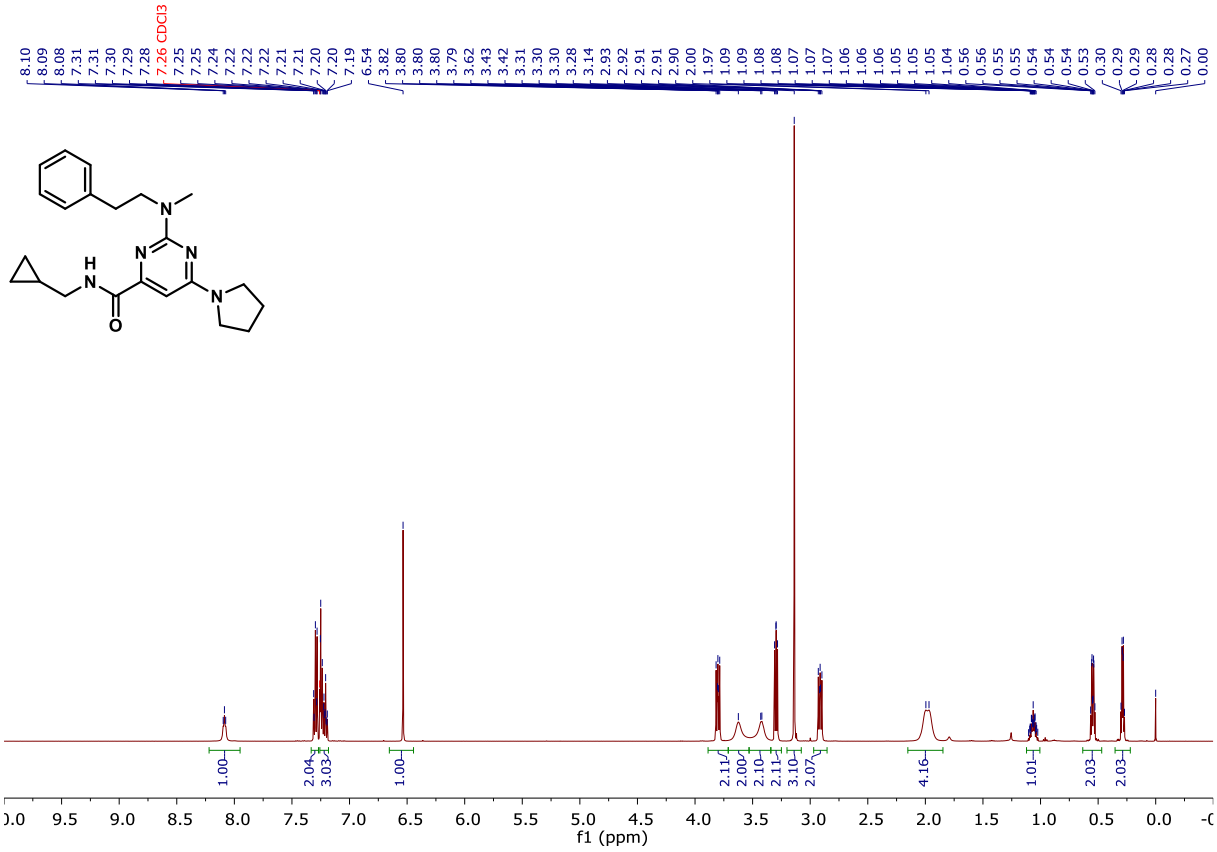
**$^{1}$H-NMR: 86**



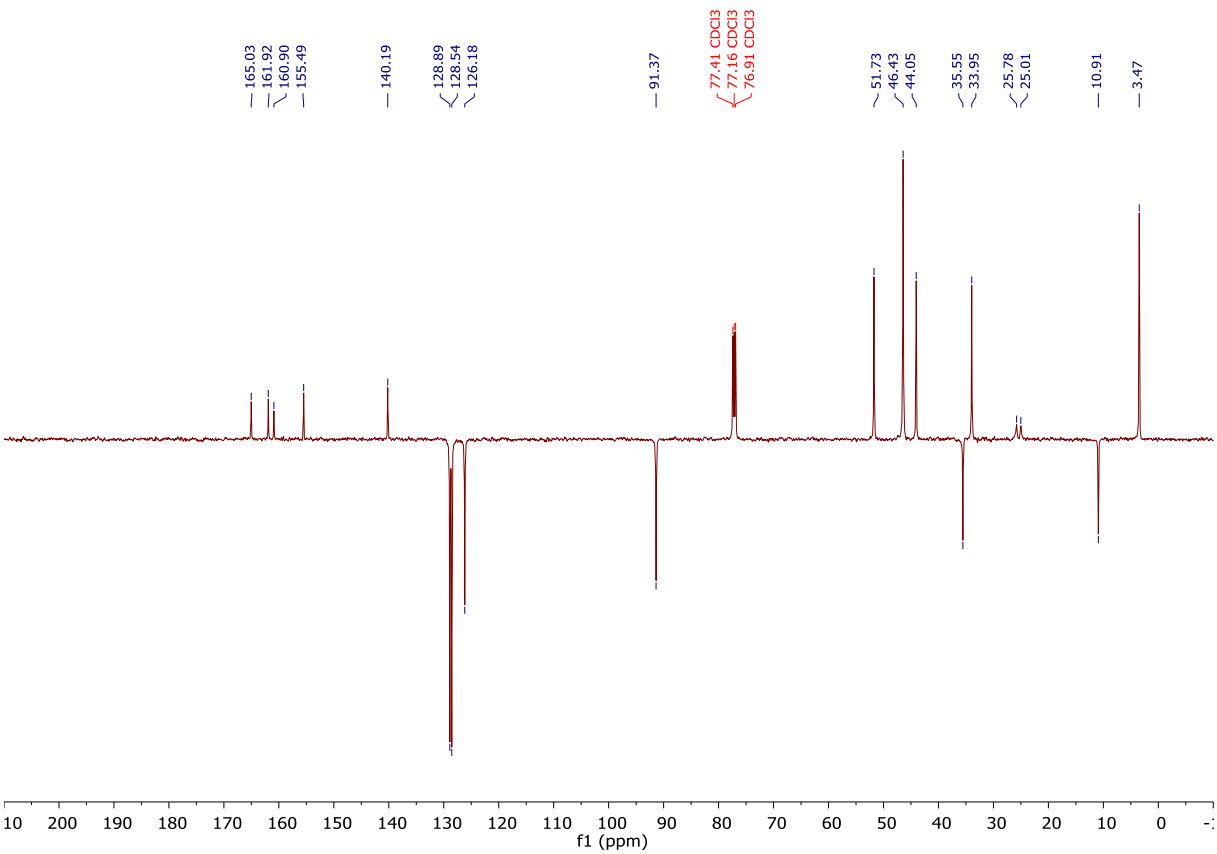

**$^{13}$C-NMR**



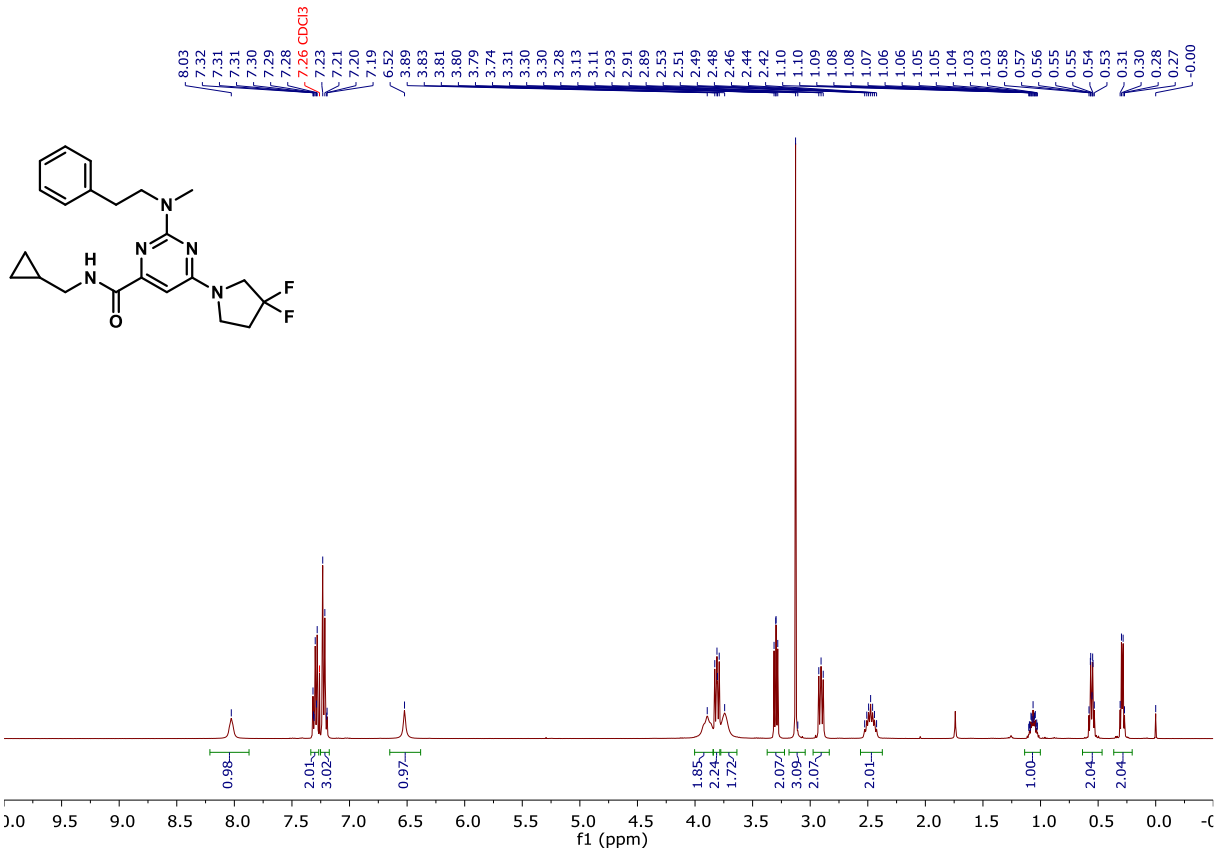
**$^{1}$H-NMR: 87**



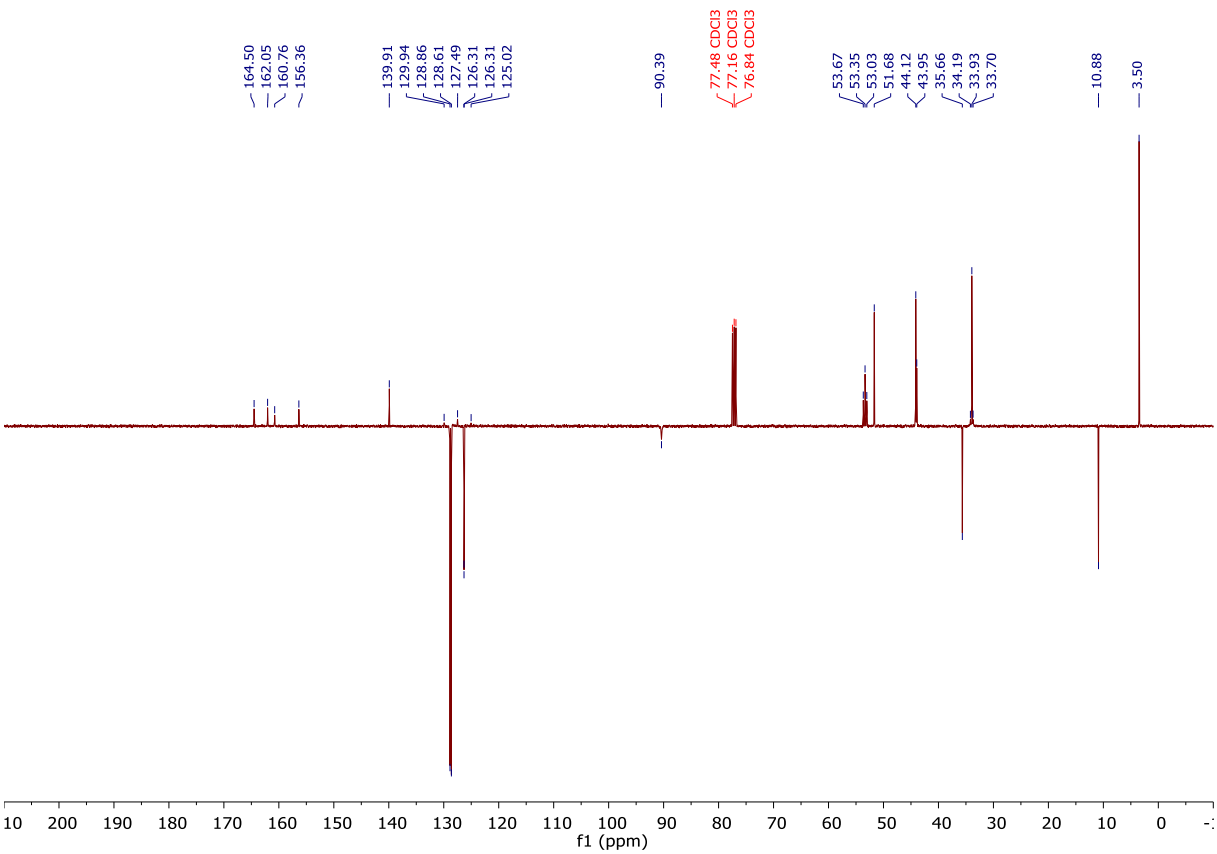

**$^{13}$C-NMR**



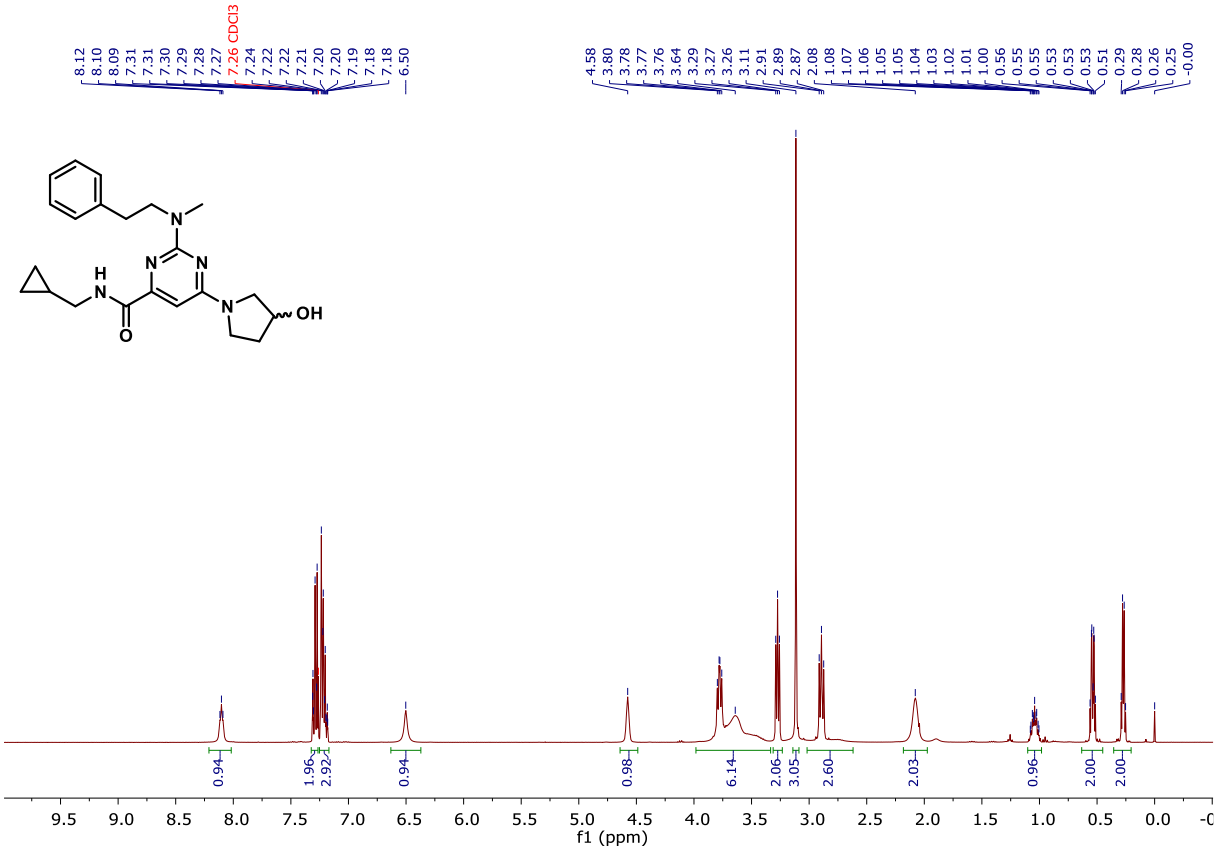
**$^{1}$H-NMR: 88**



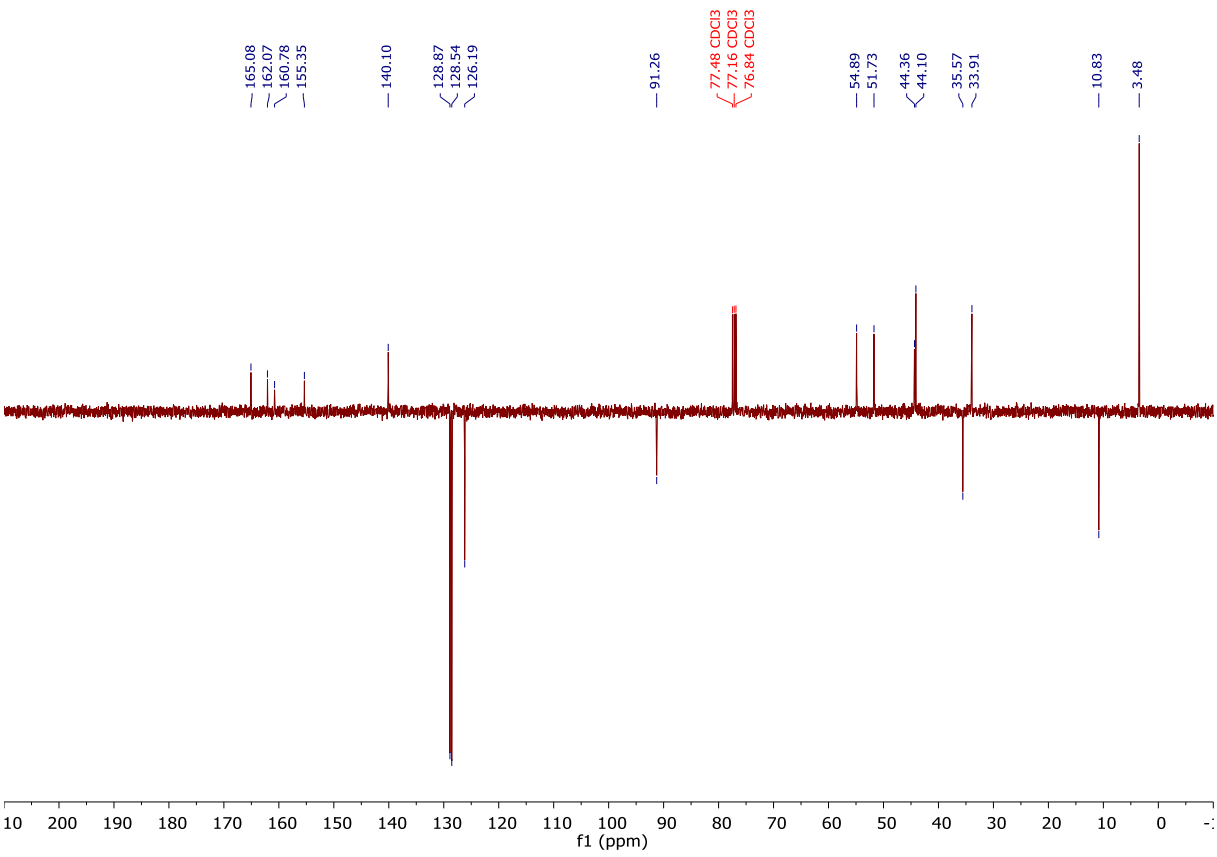

**$^{13}$C-NMR**



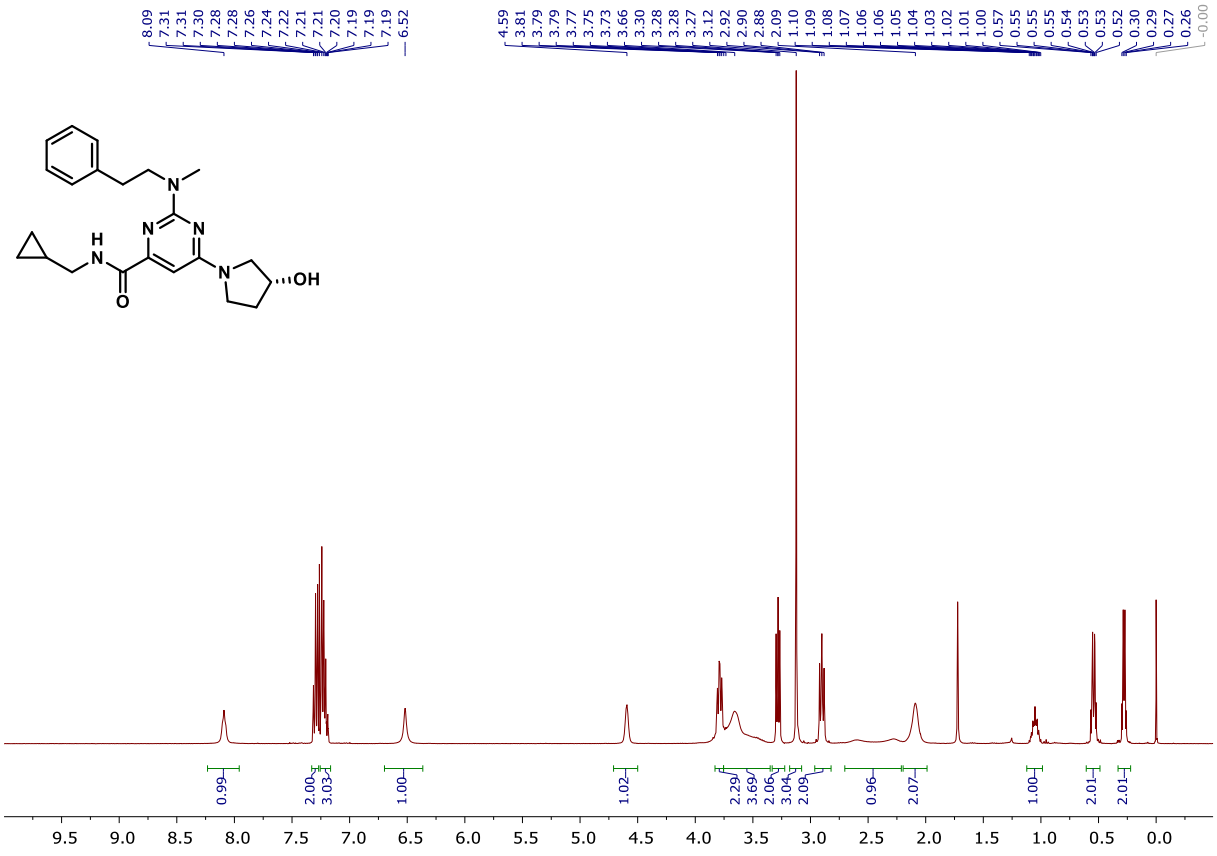
**$^{1}$H-NMR: 89**




**$^{13}$C-NMR**

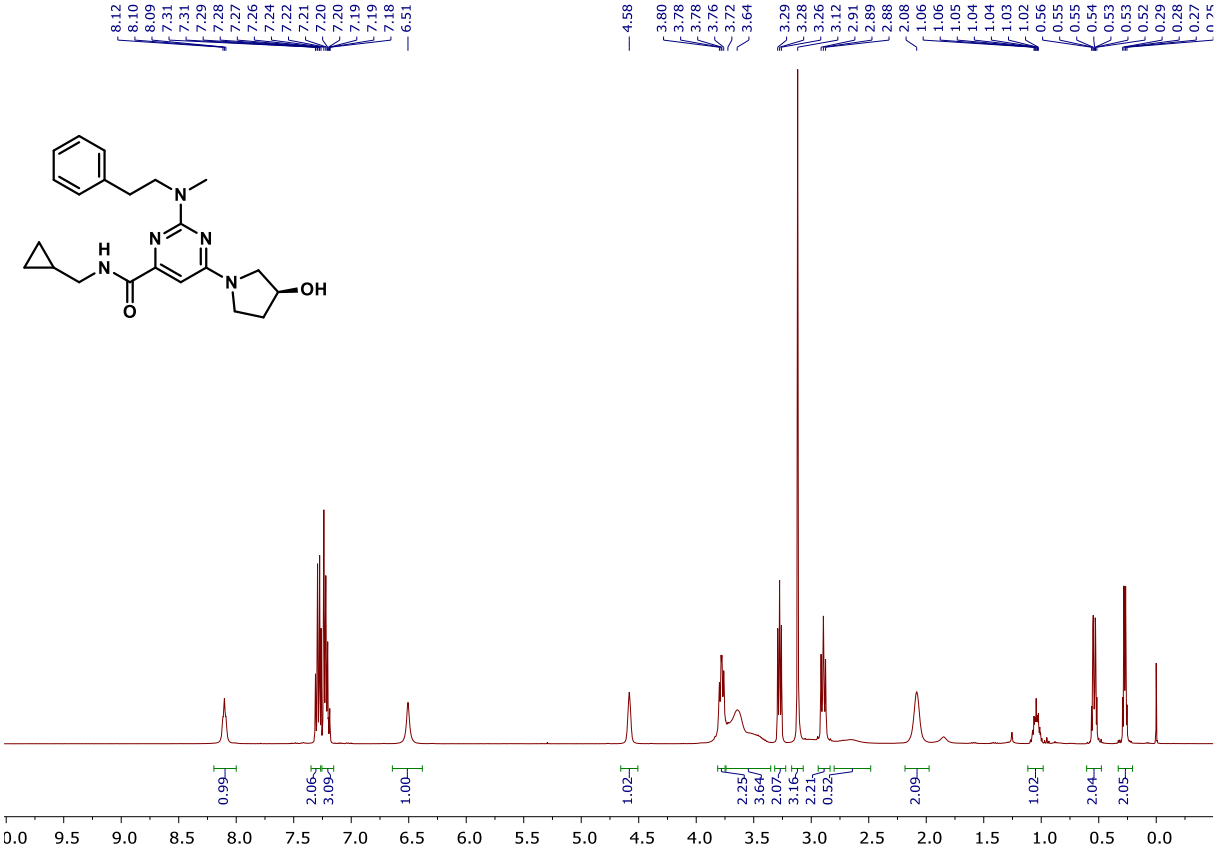


**$^{1}$H-NMR: (*R*)-90**



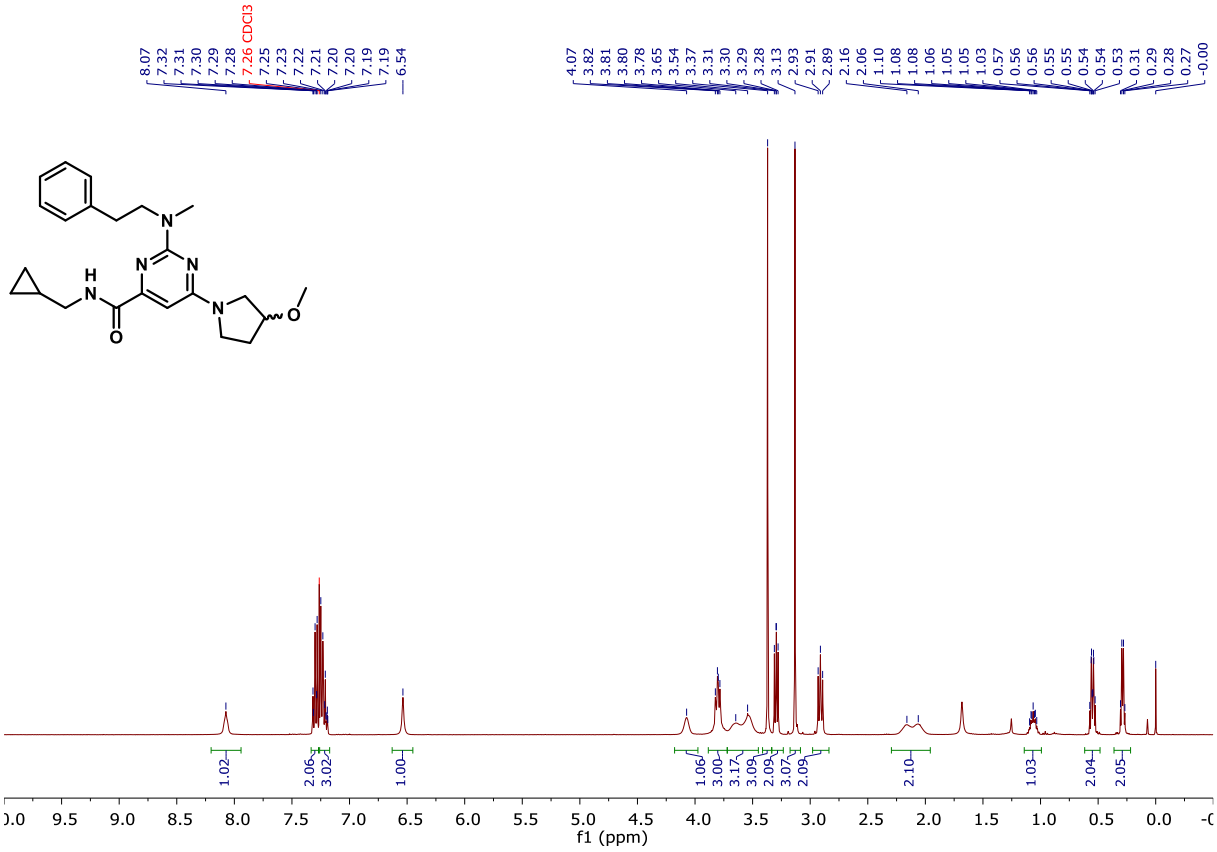

**$^{13}$C-NMR**

**$^{1}$H-NMR: (*S*)-91**

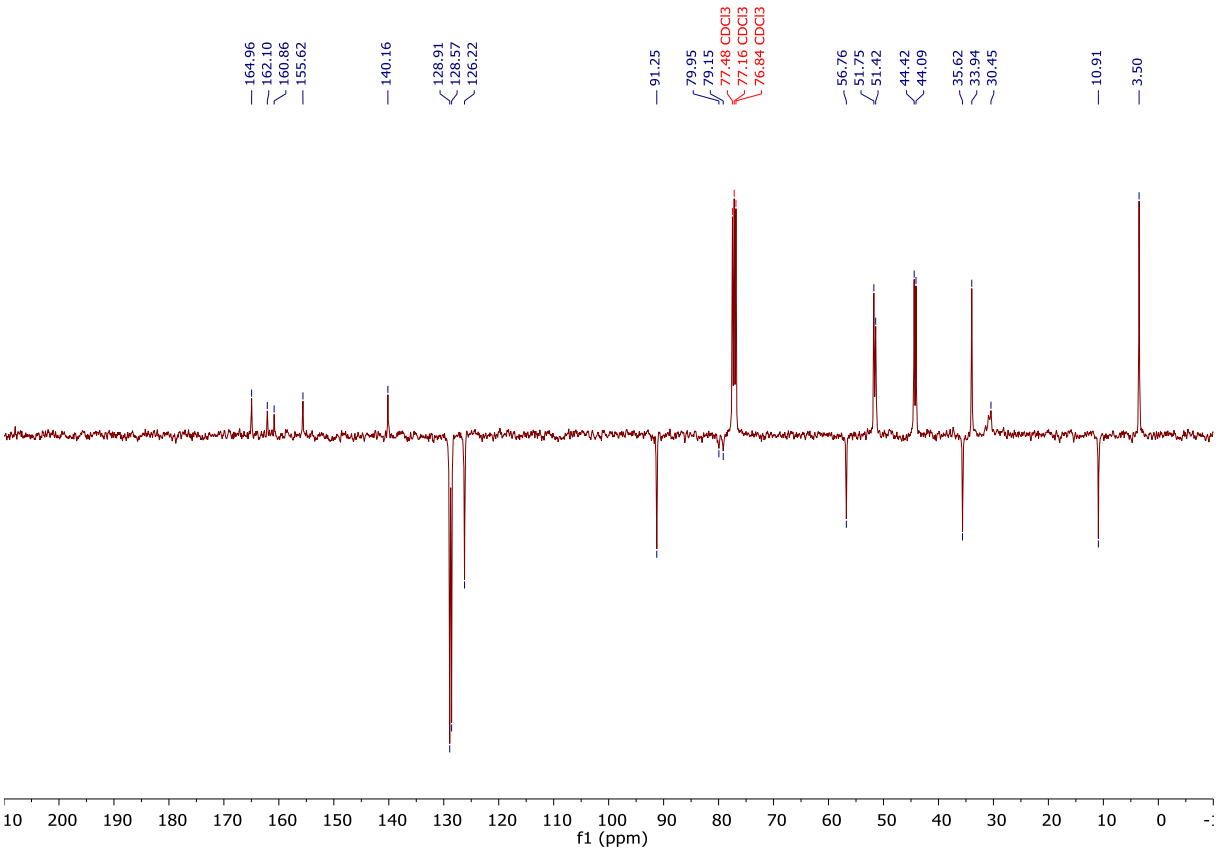



**$^{13}$C-NMR**

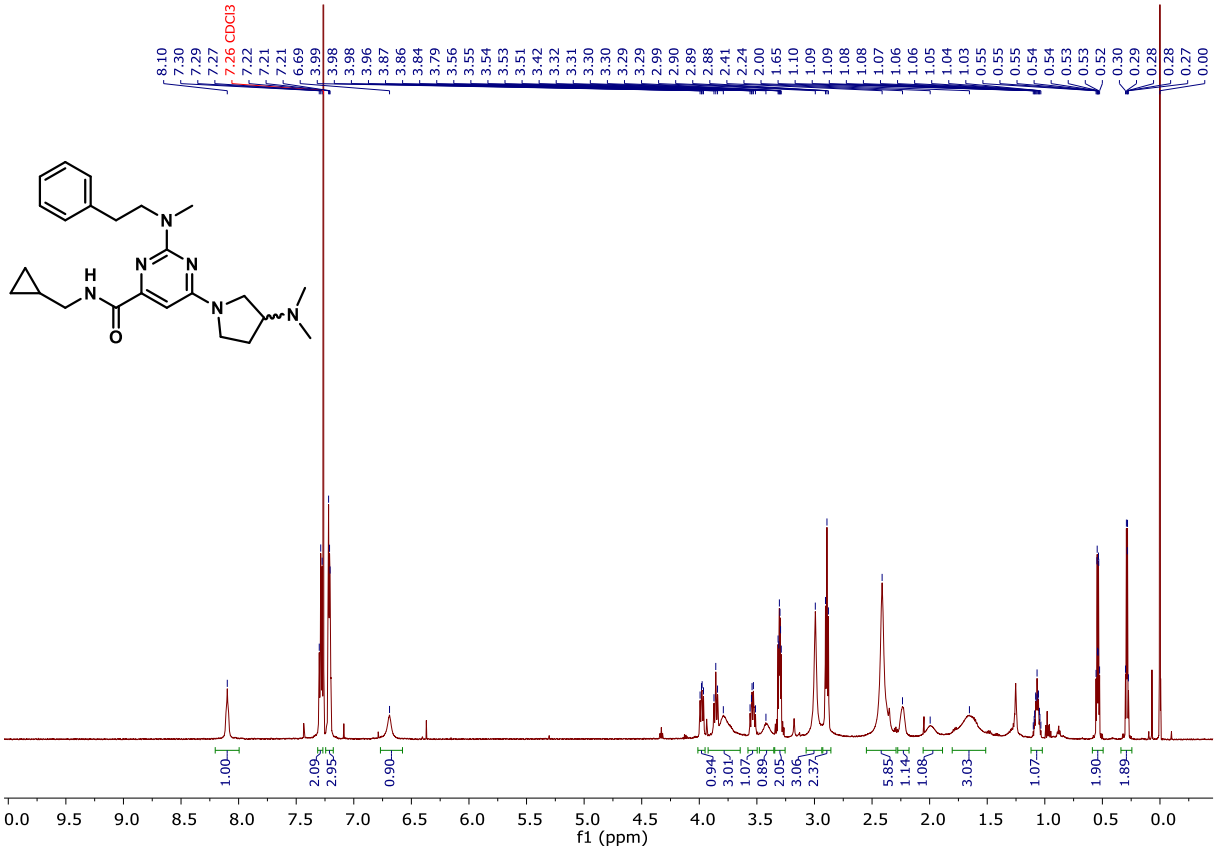
**$^{1}$H-NMR: 92**



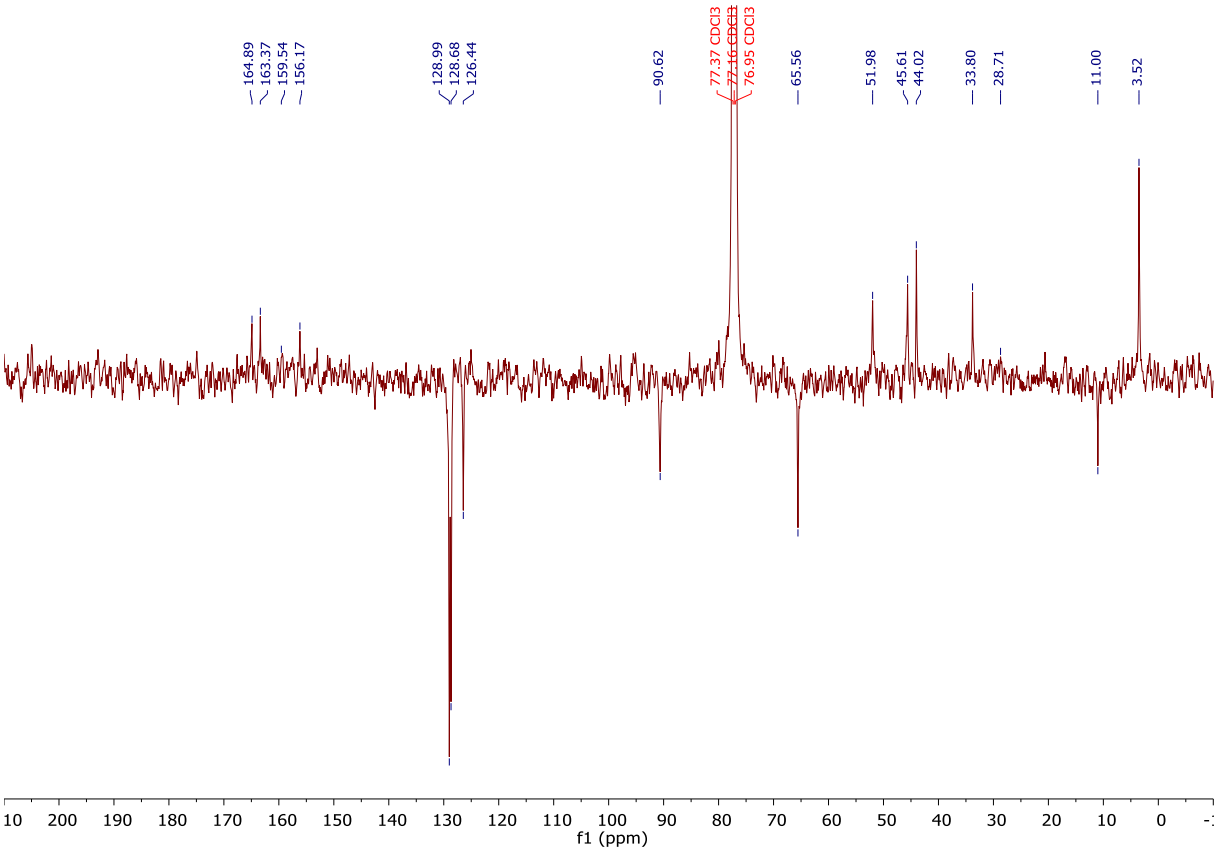

**$^{13}$C-NMR**



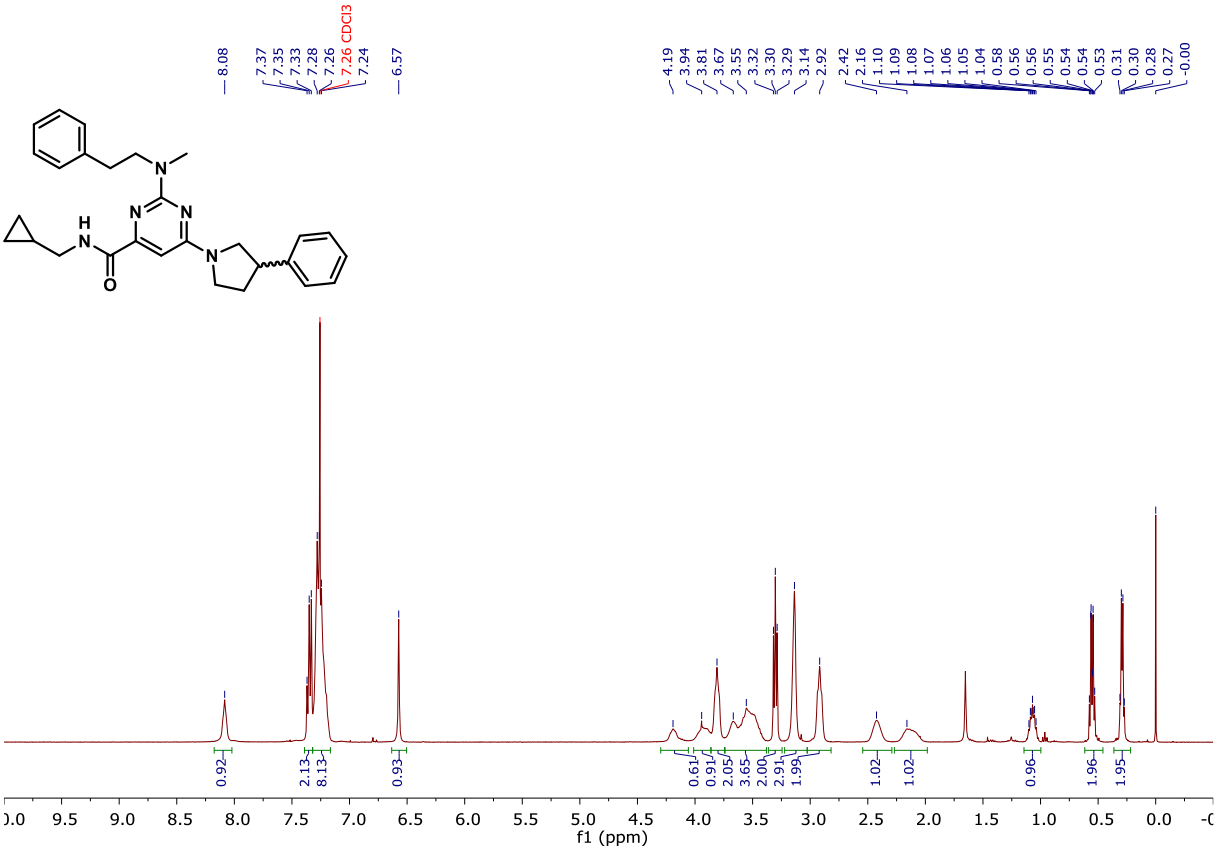
**$^{1}$H-NMR: 93**



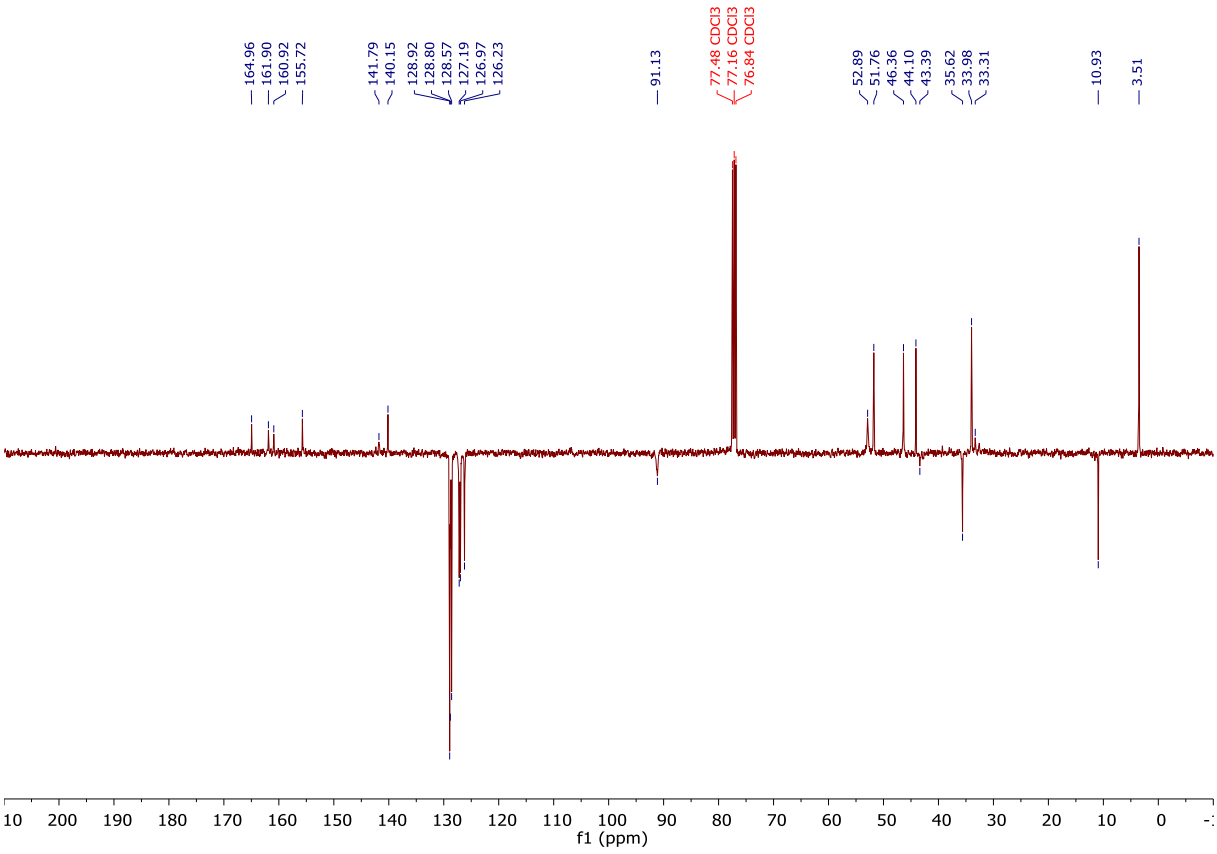

**$^{13}$C-NMR**



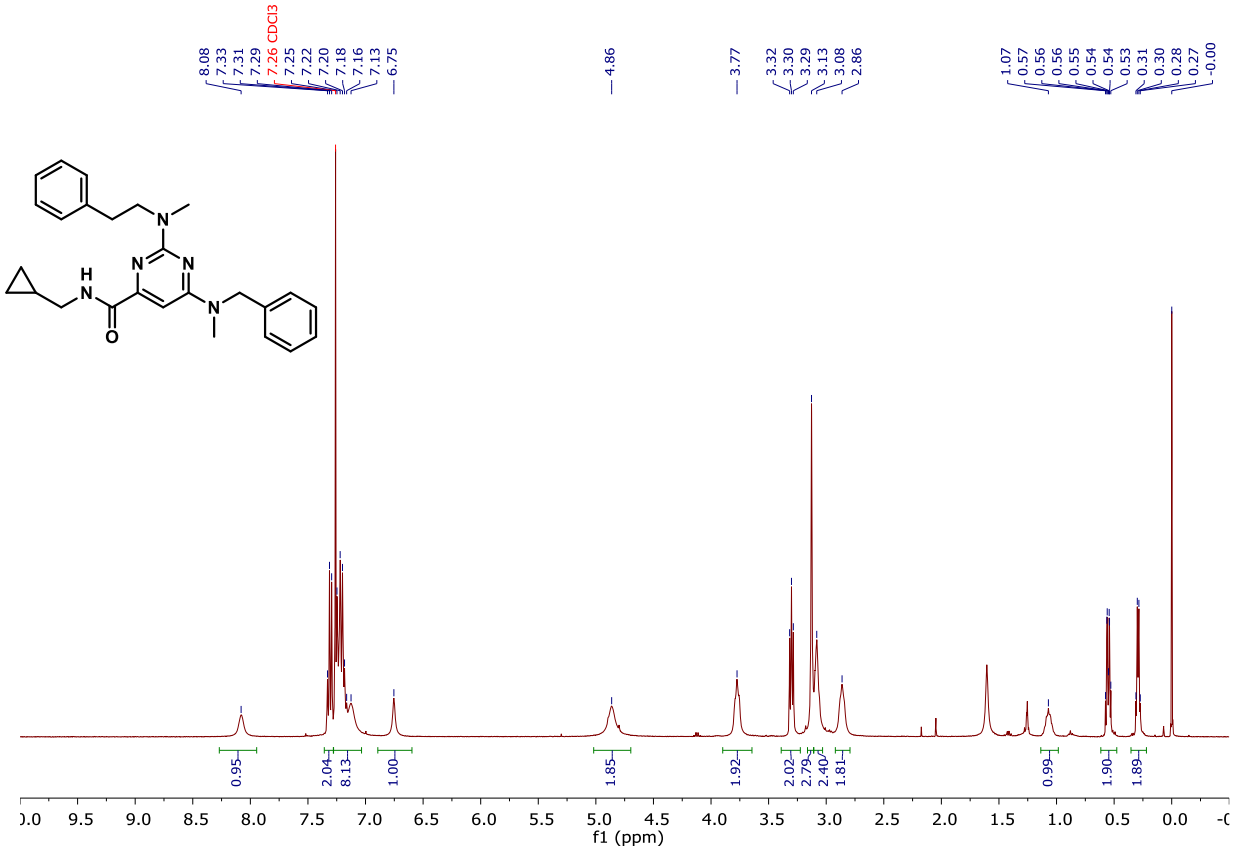
**$^{1}$H-NMR: 94**



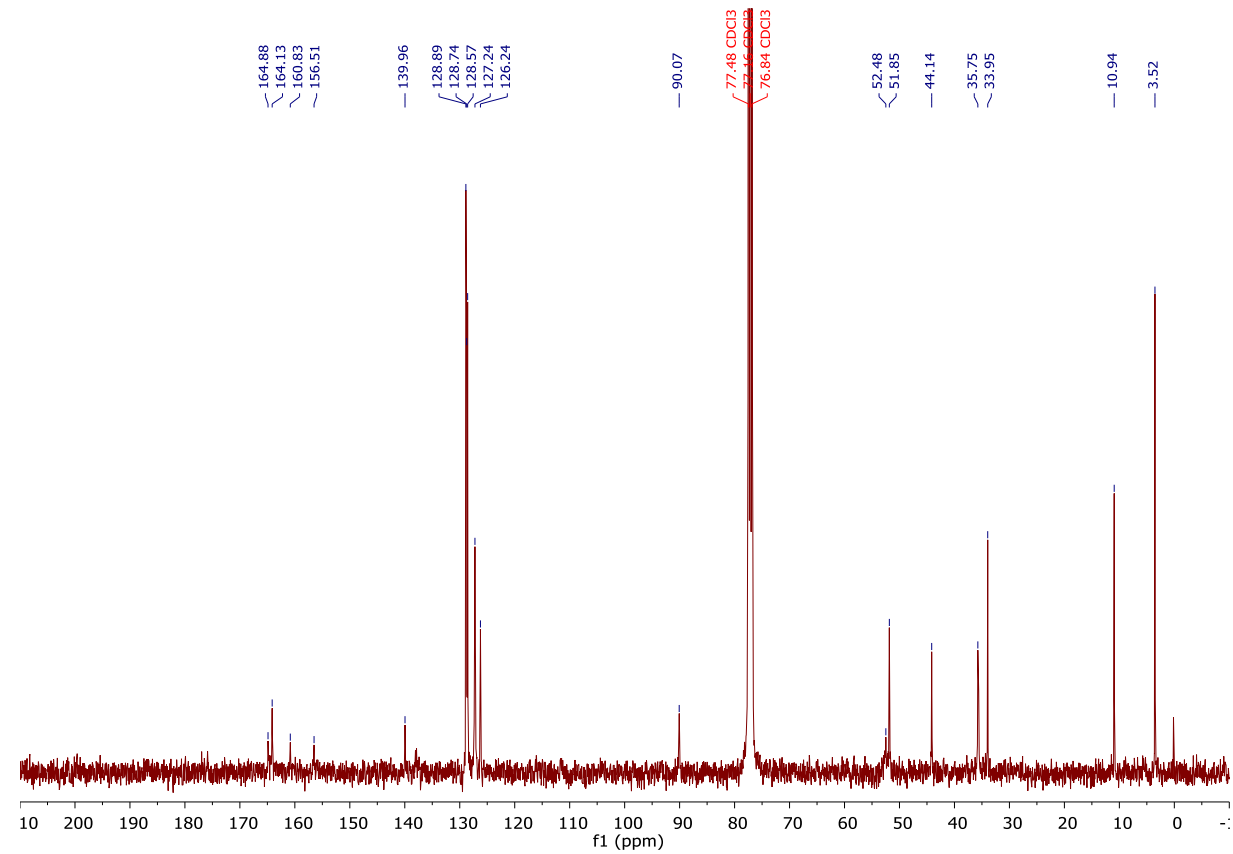

**$^{13}$C-NMR**



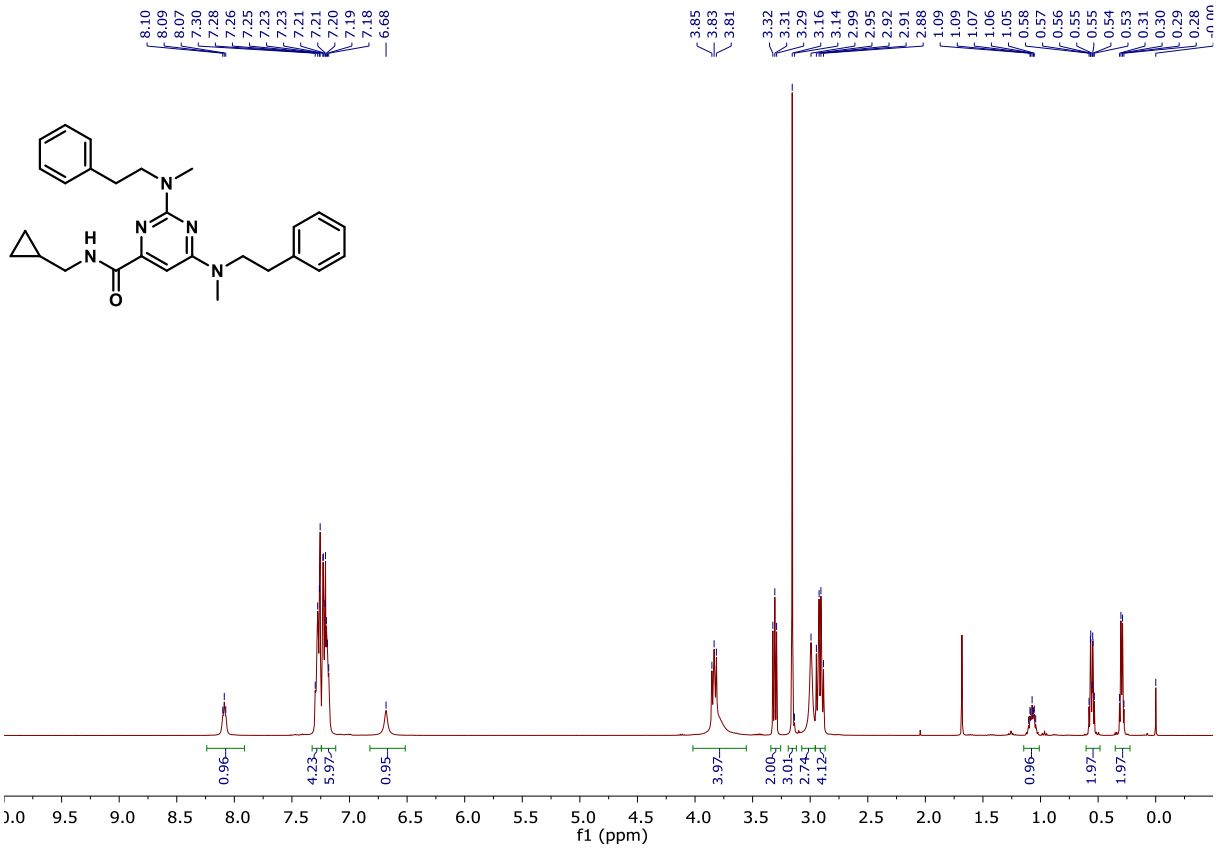
**$^{1}$H-NMR: 95**



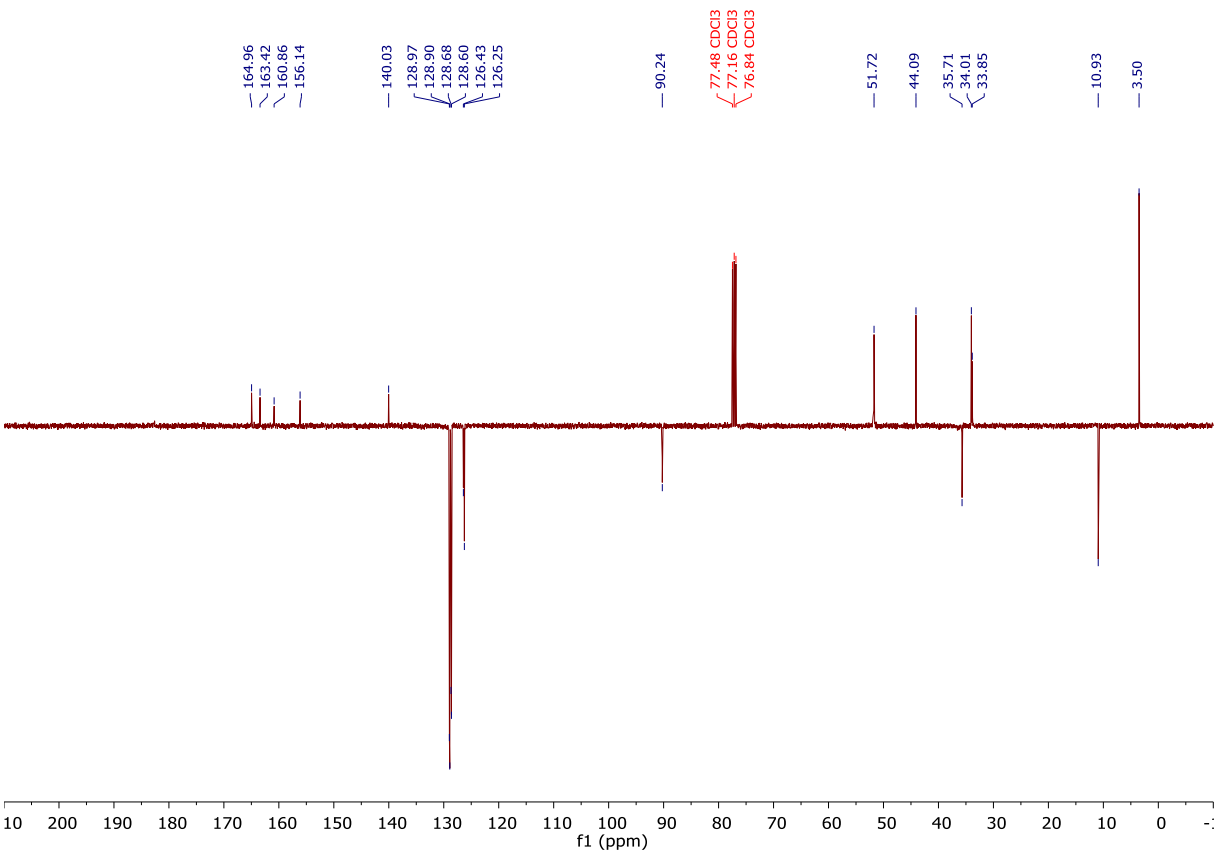

**$^{13}$C-NMR**



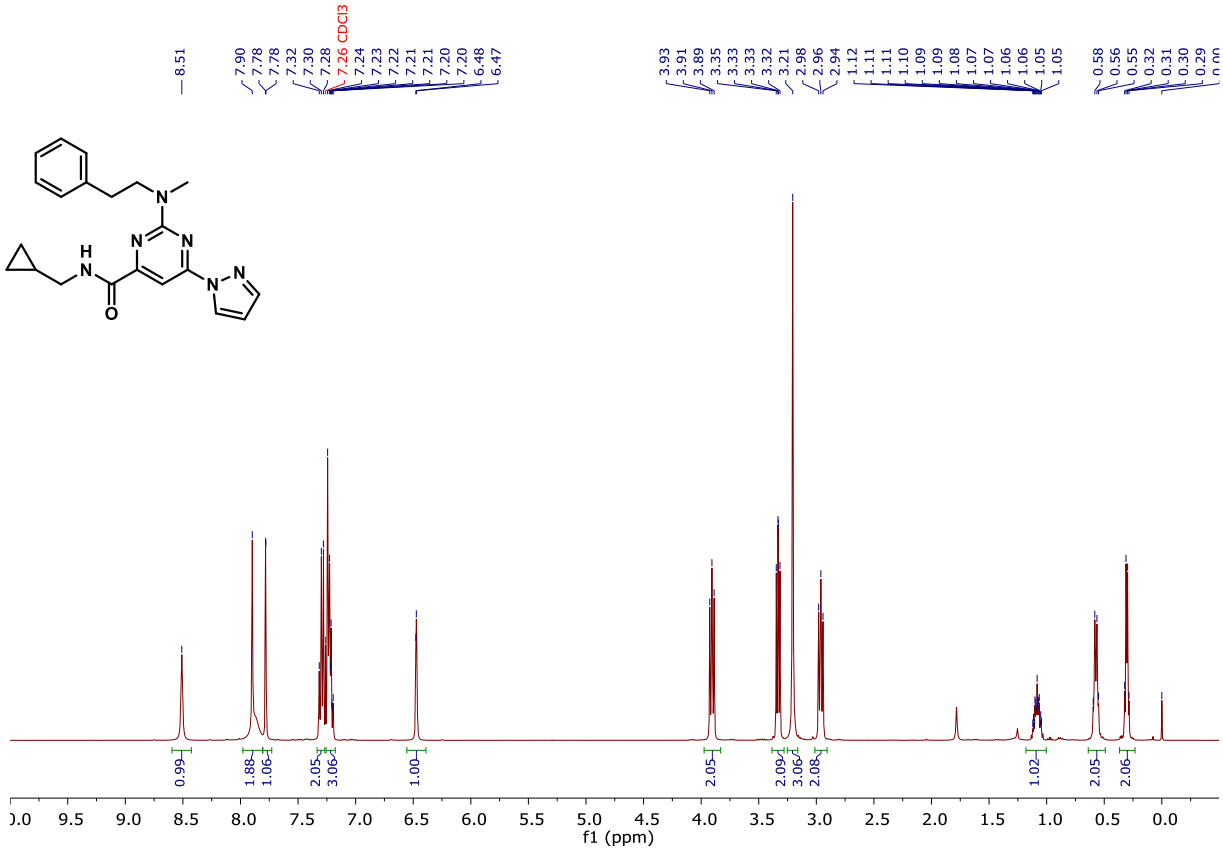
**$^{1}$H-NMR: 96**



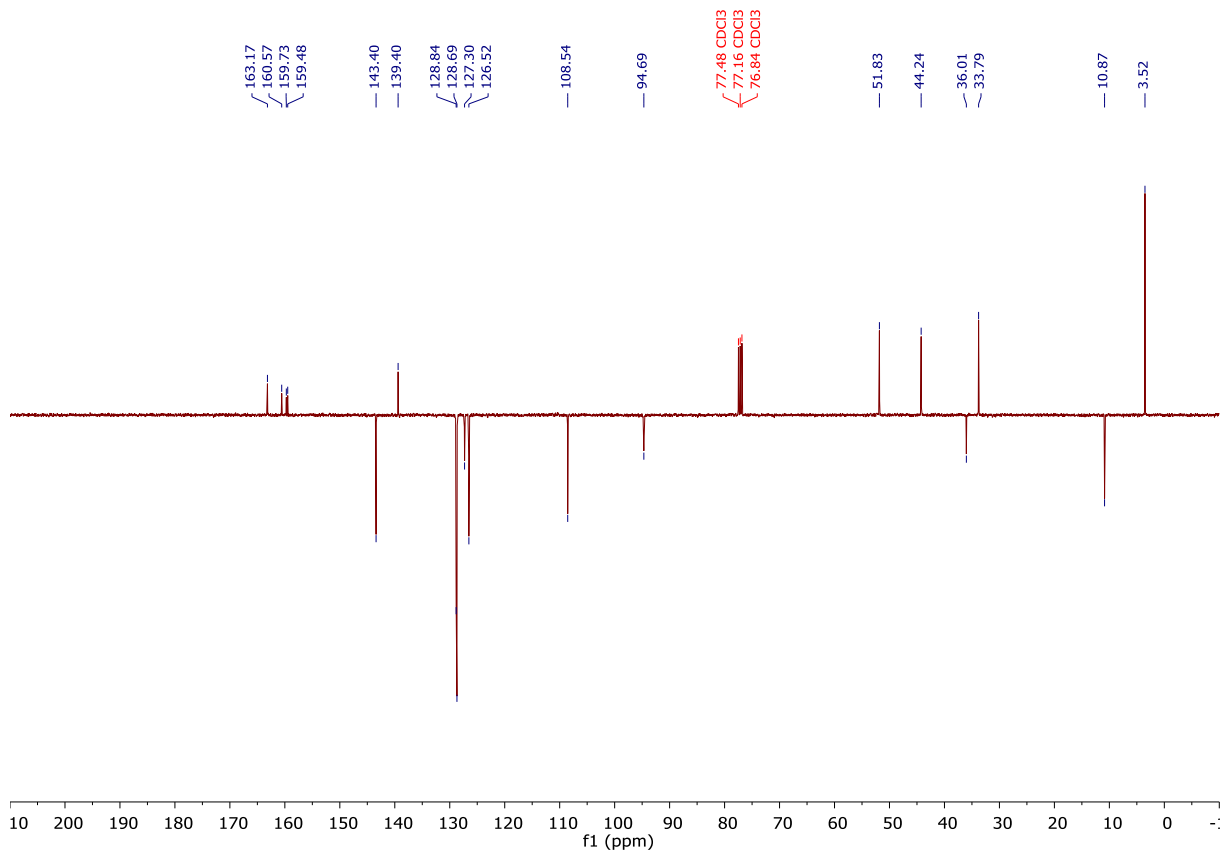

**$^{13}$C-NMR**



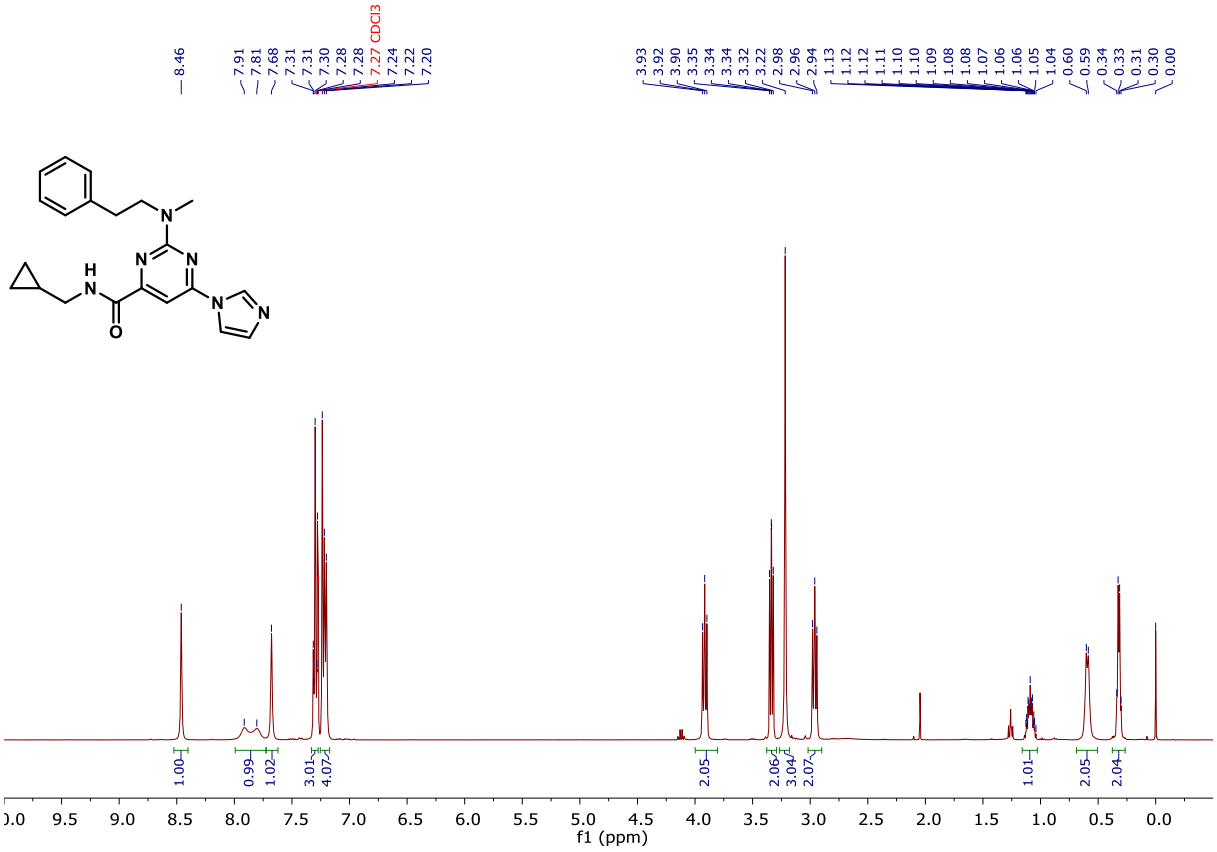
**$^{1}$H-NMR: 97**



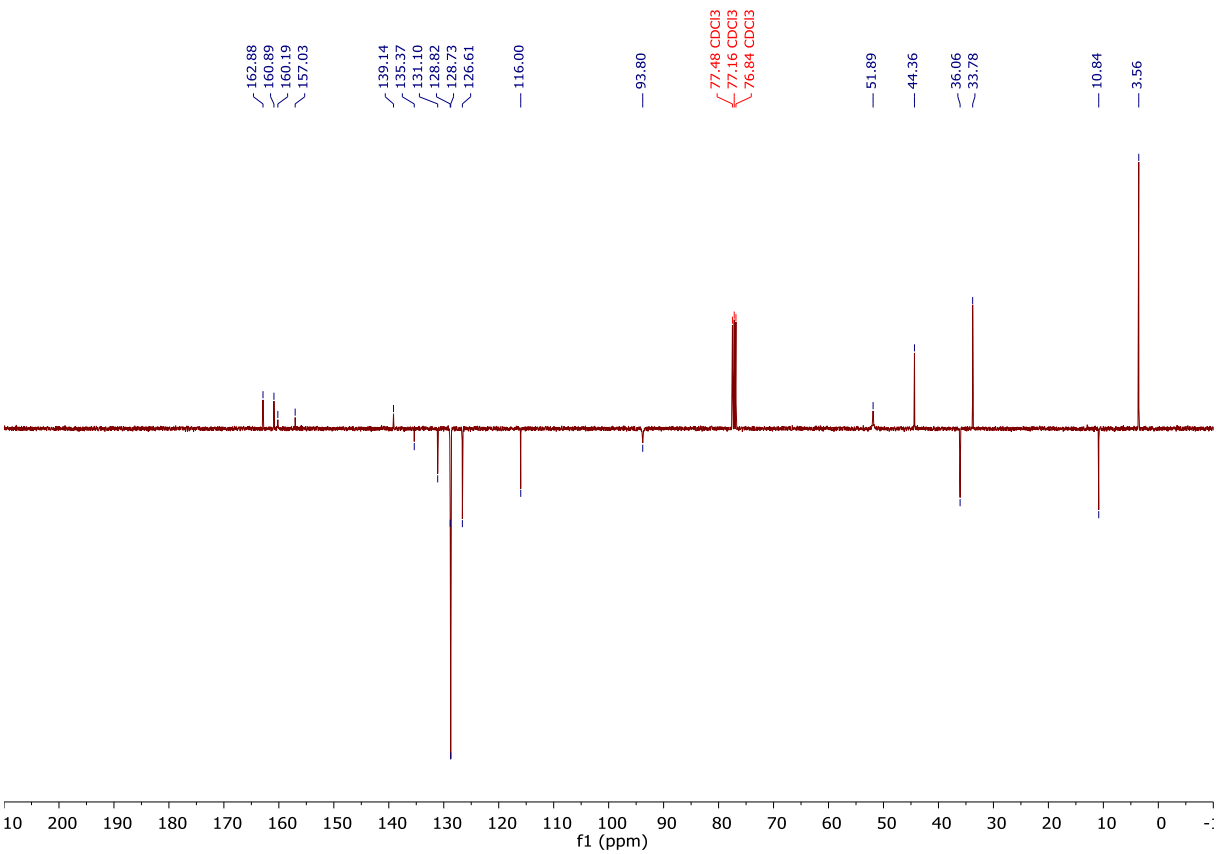

**$^{13}$C-NMR**



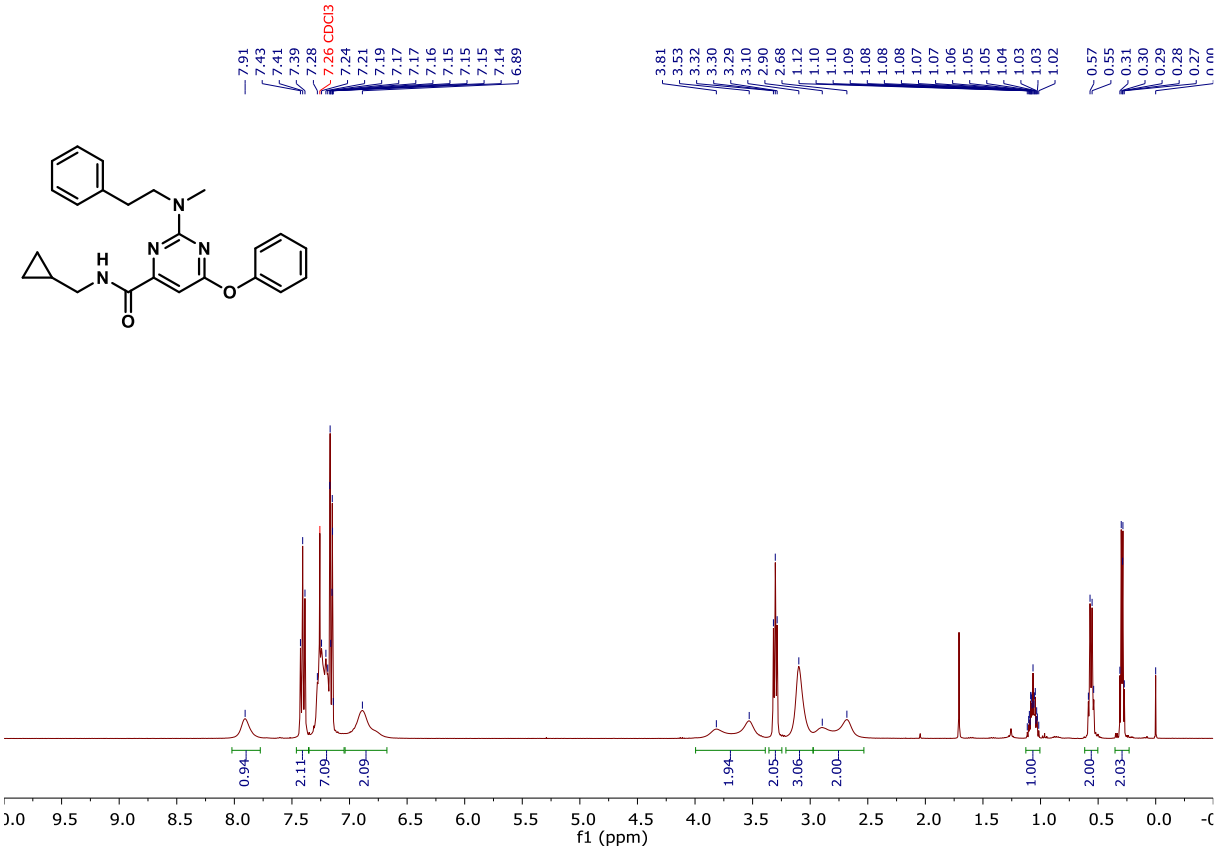
**$^{1}$H-NMR: 98**



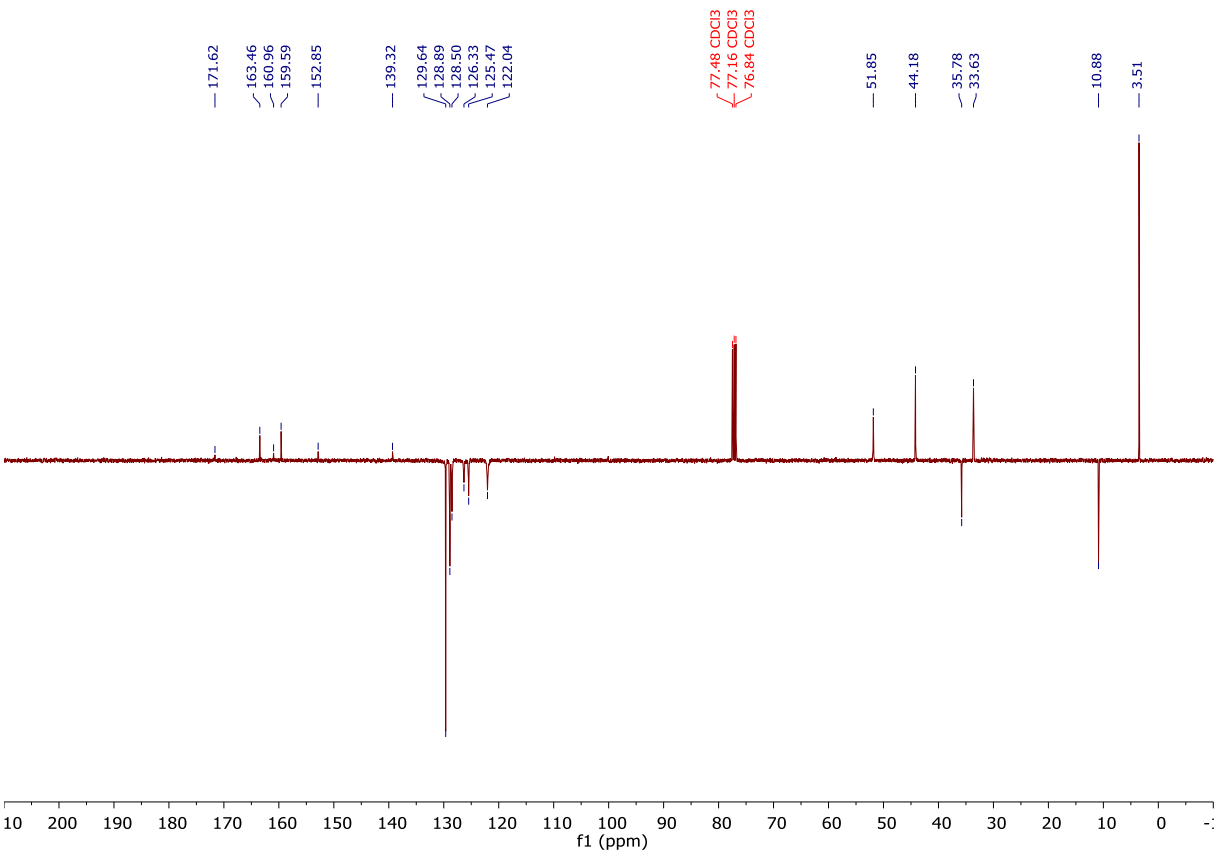

**$^{13}$C-NMR**



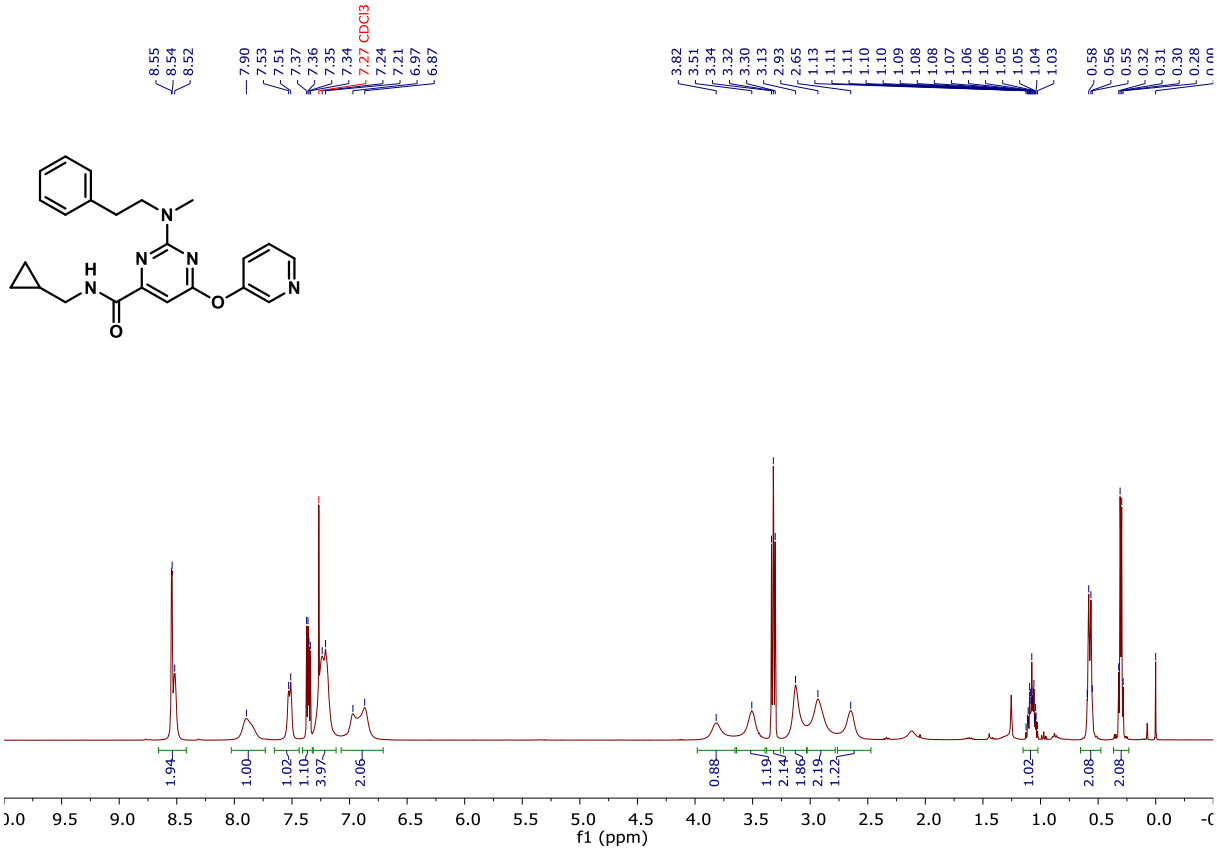
**$^{1}$H-NMR: 99**



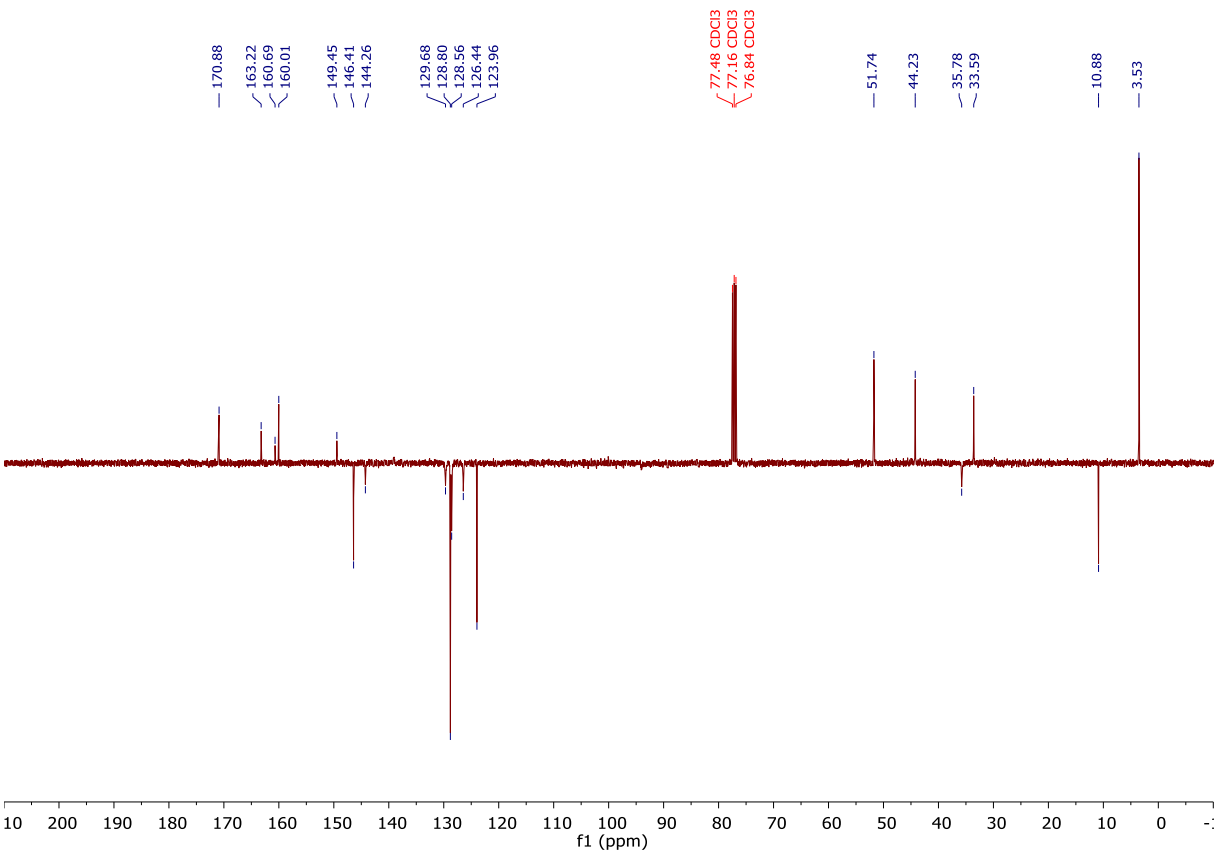

**$^{13}$C-NMR**



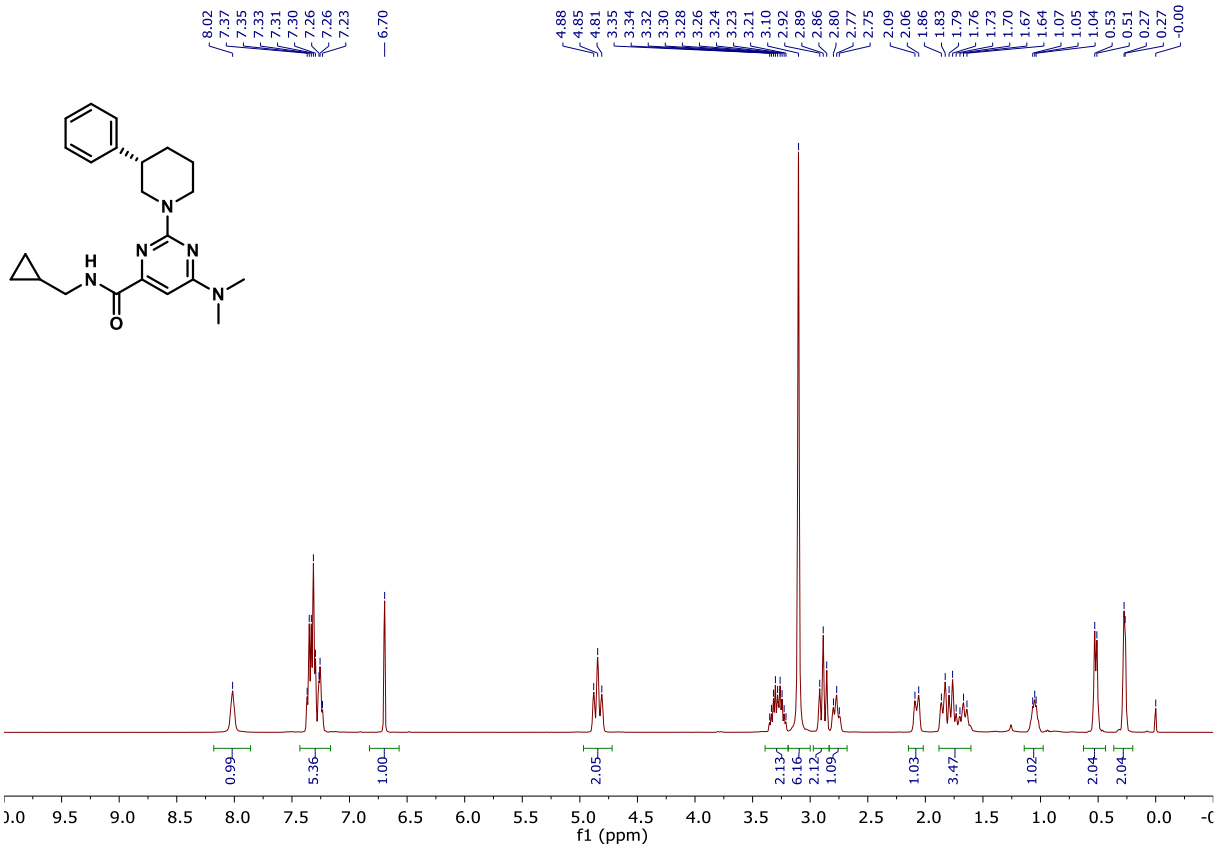
**$^{1}$H-NMR: 100**



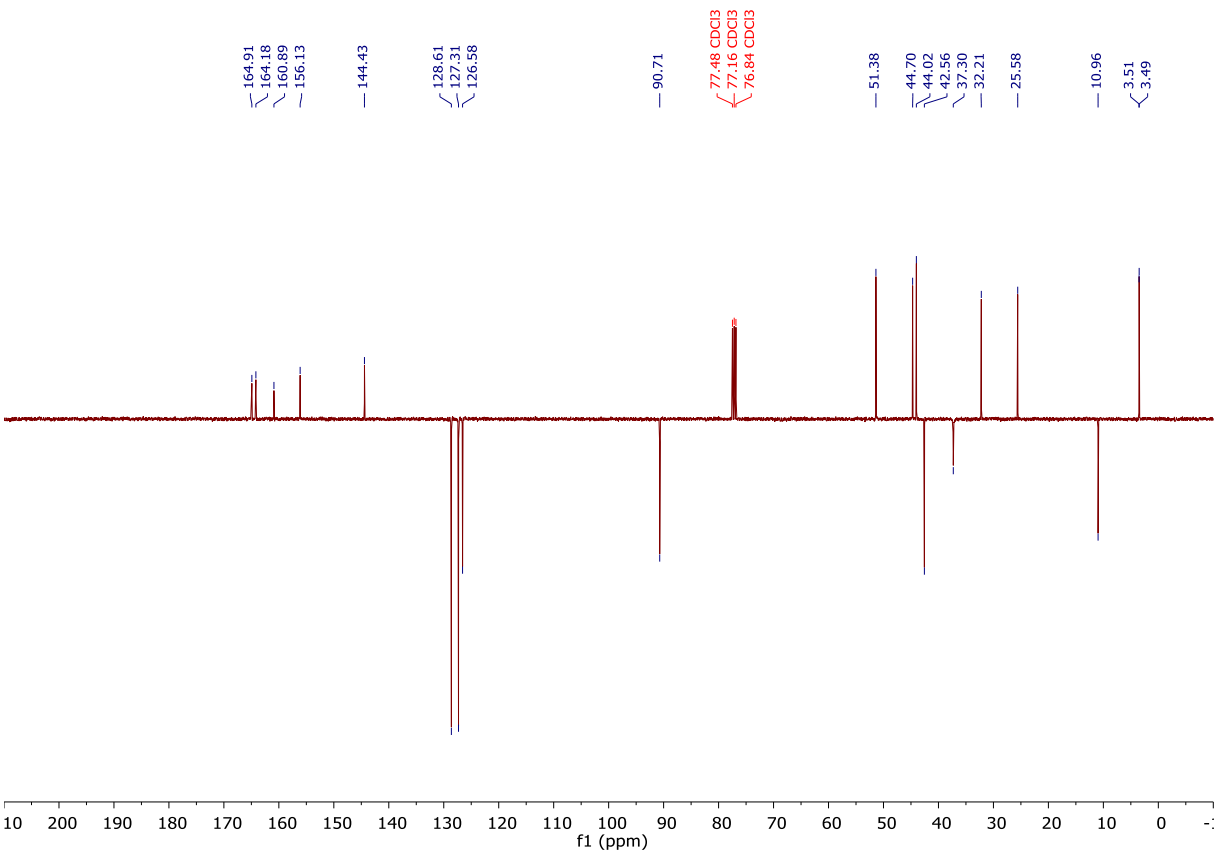

**$^{13}$C-NMR**



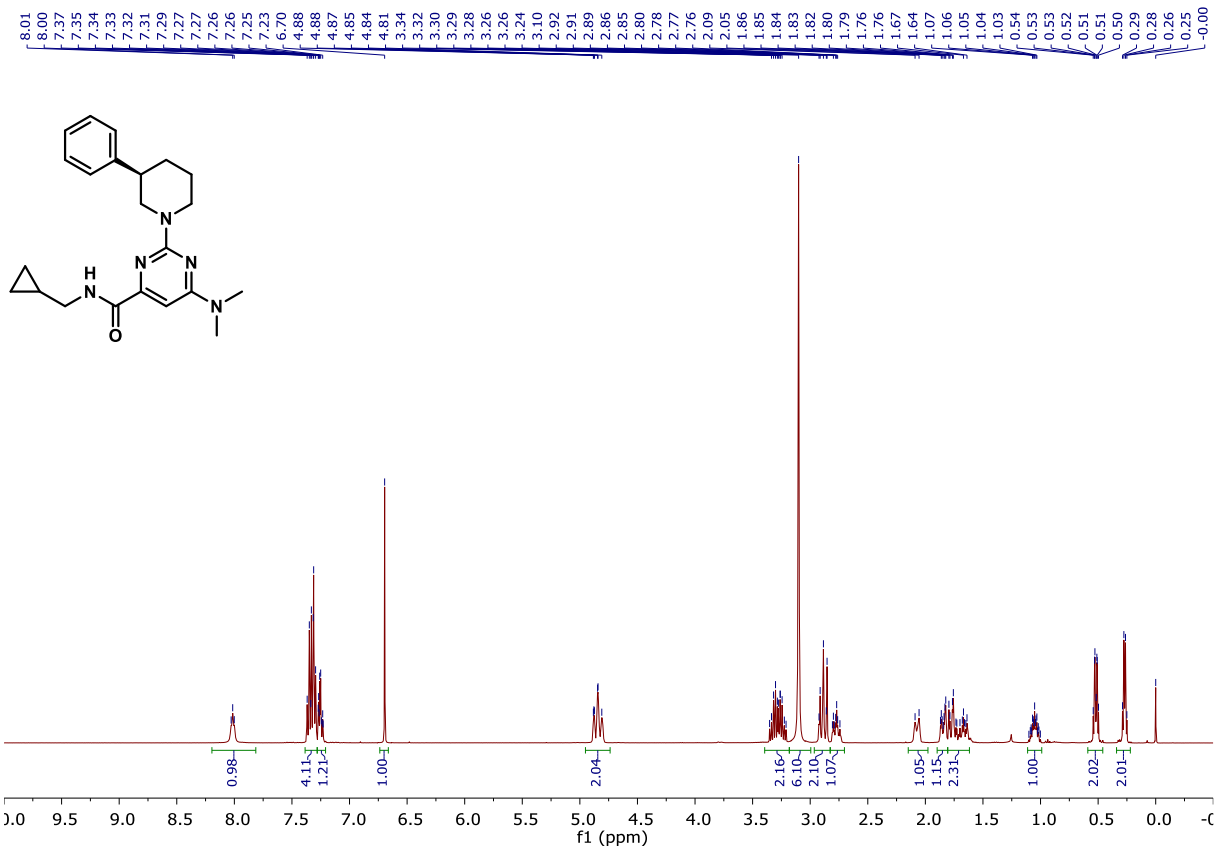
**$^{1}$H-NMR: (*S*)-101**



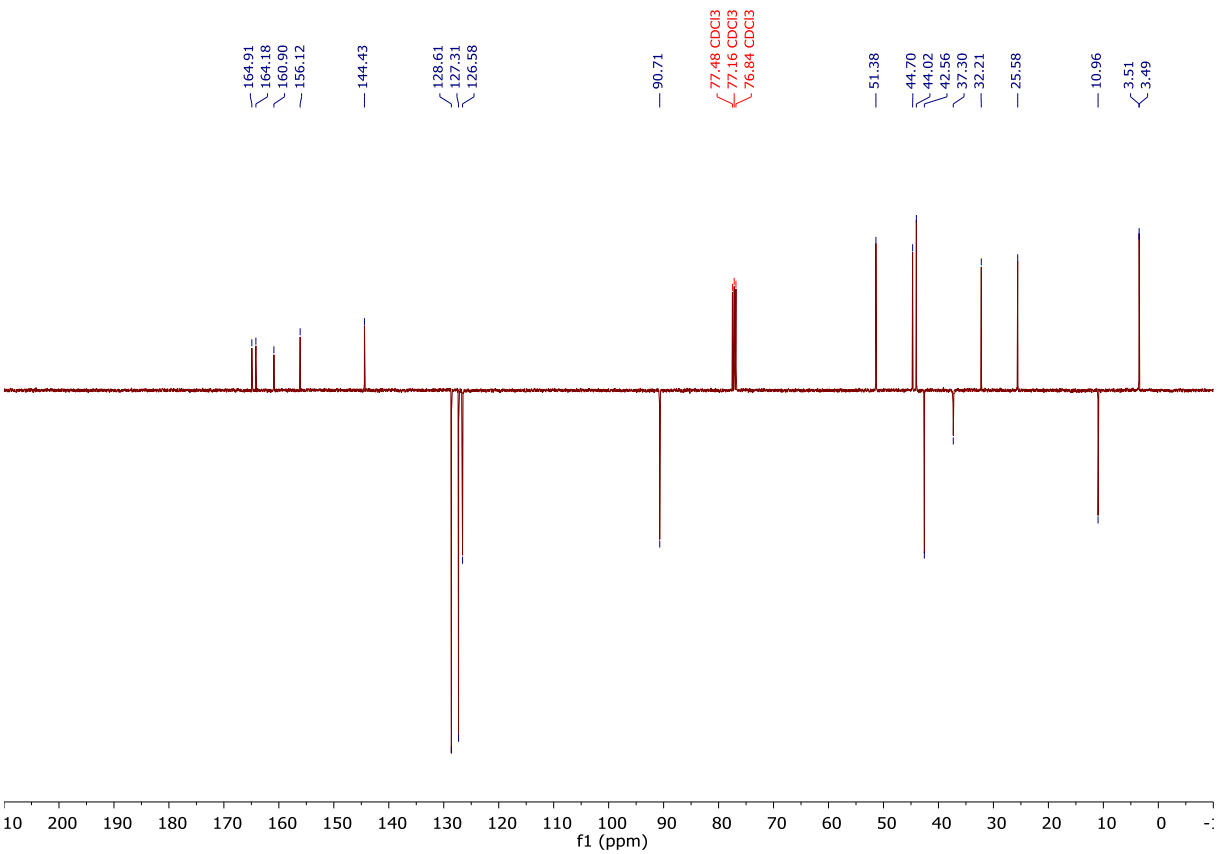

**$^{13}$C-NMR**



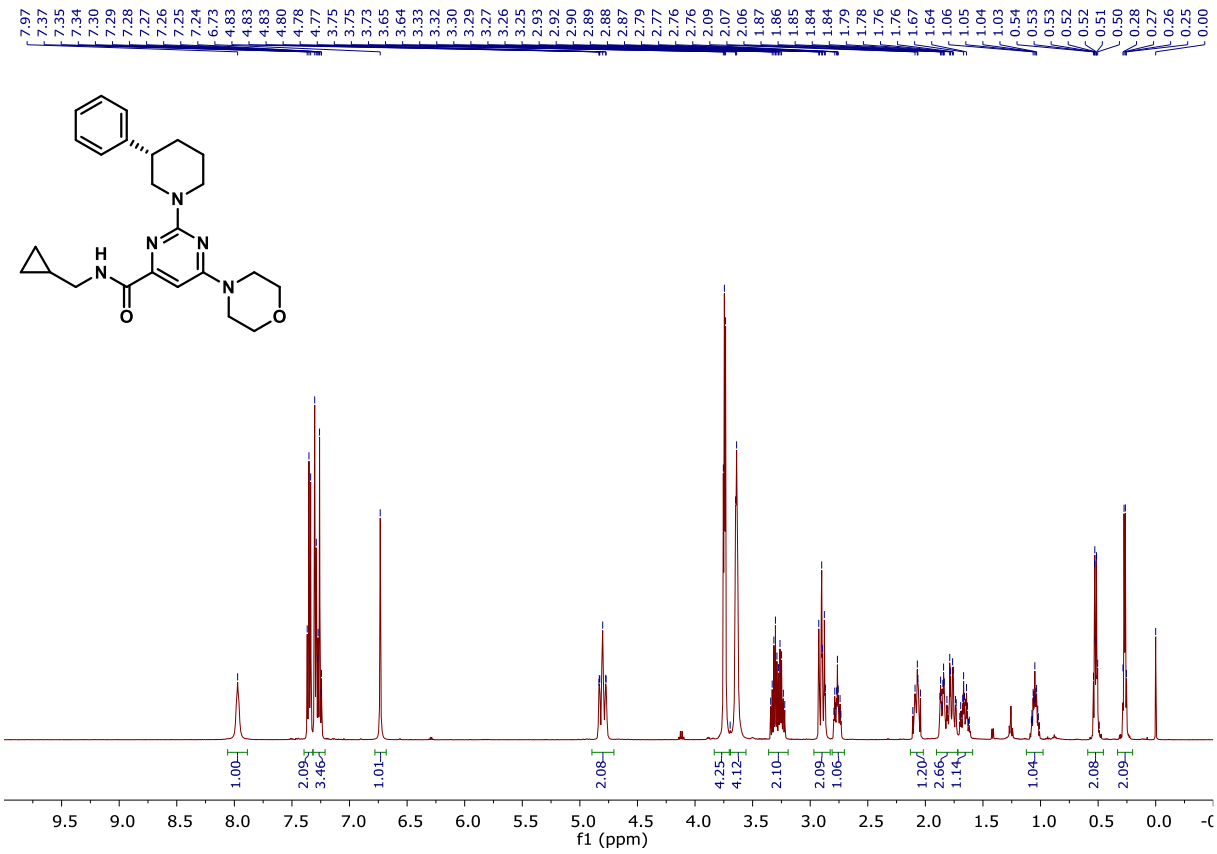
**$^{1}$H-NMR: (*R*)-102**



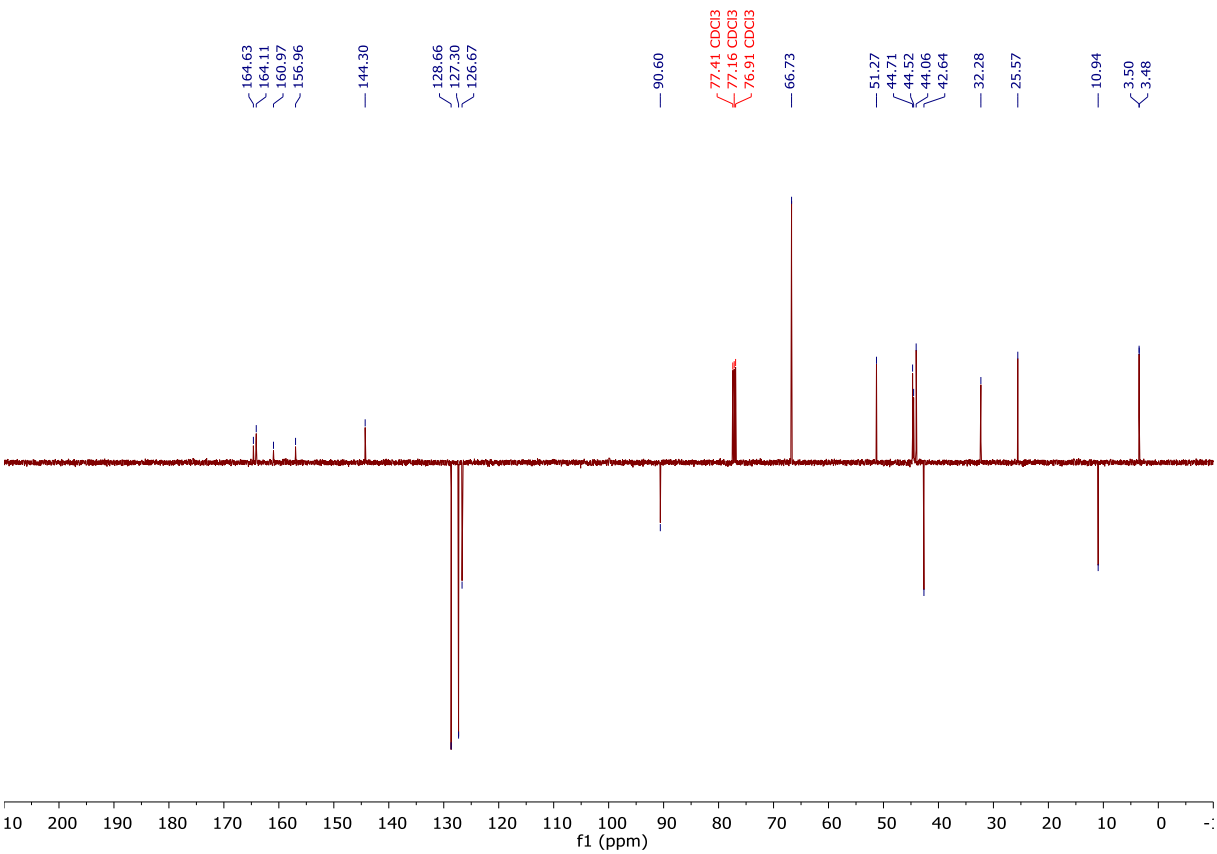

**$^{13}$C-NMR**



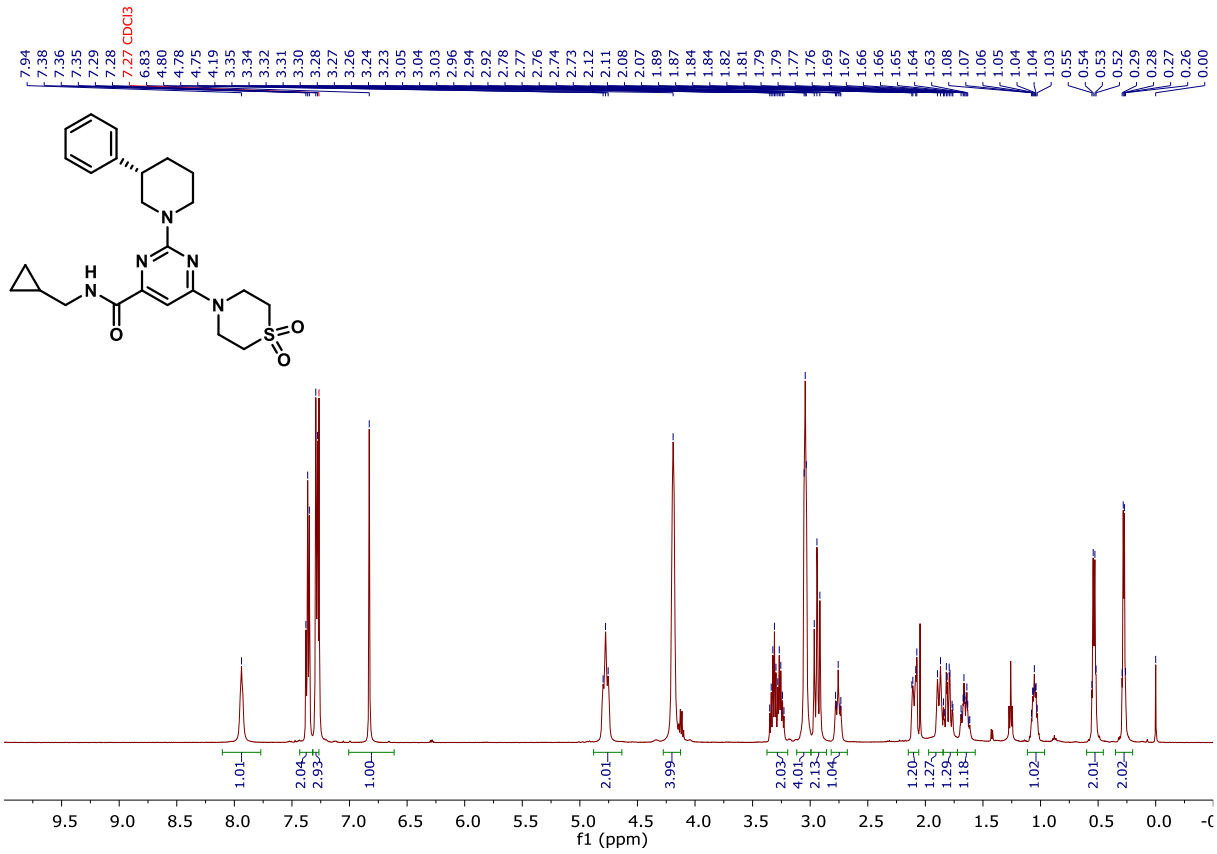
**$^{1}$H-NMR: (*S*)-103**



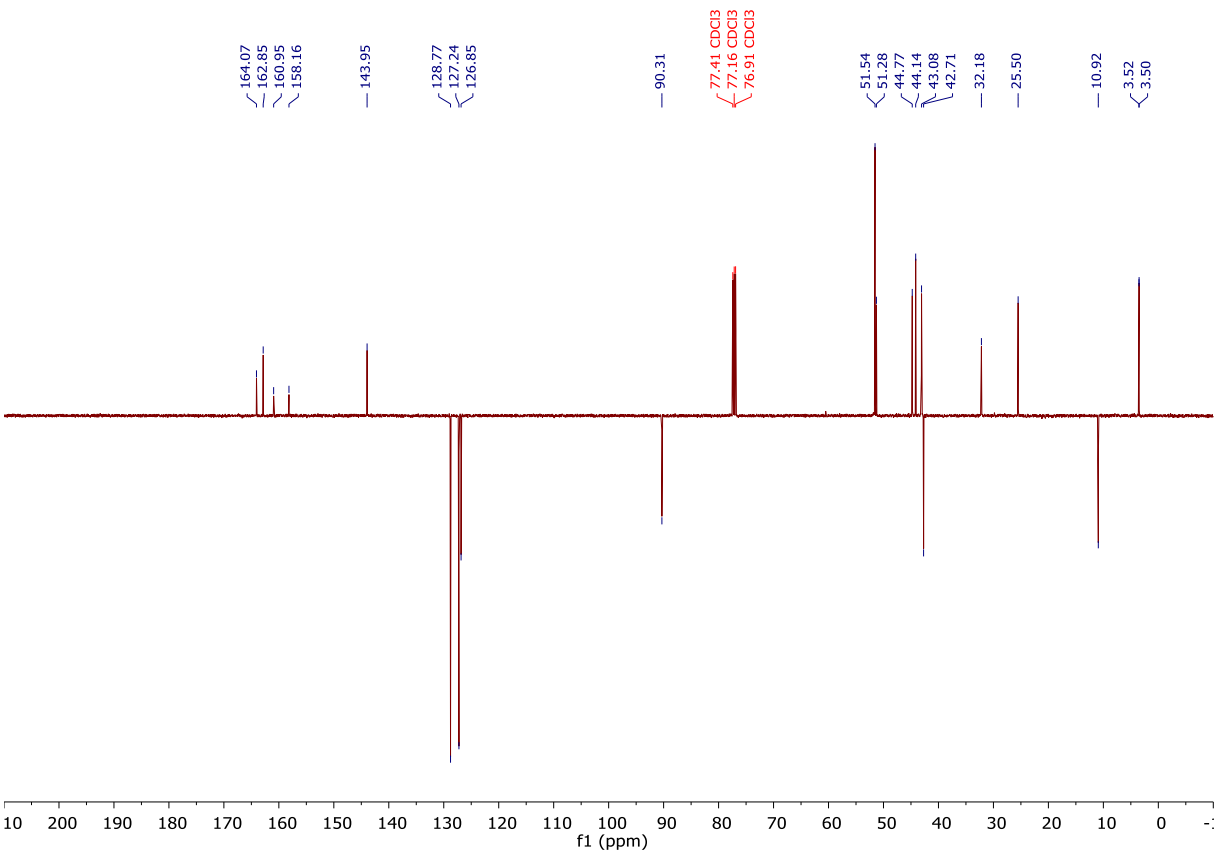

**$^{13}$C-NMR**



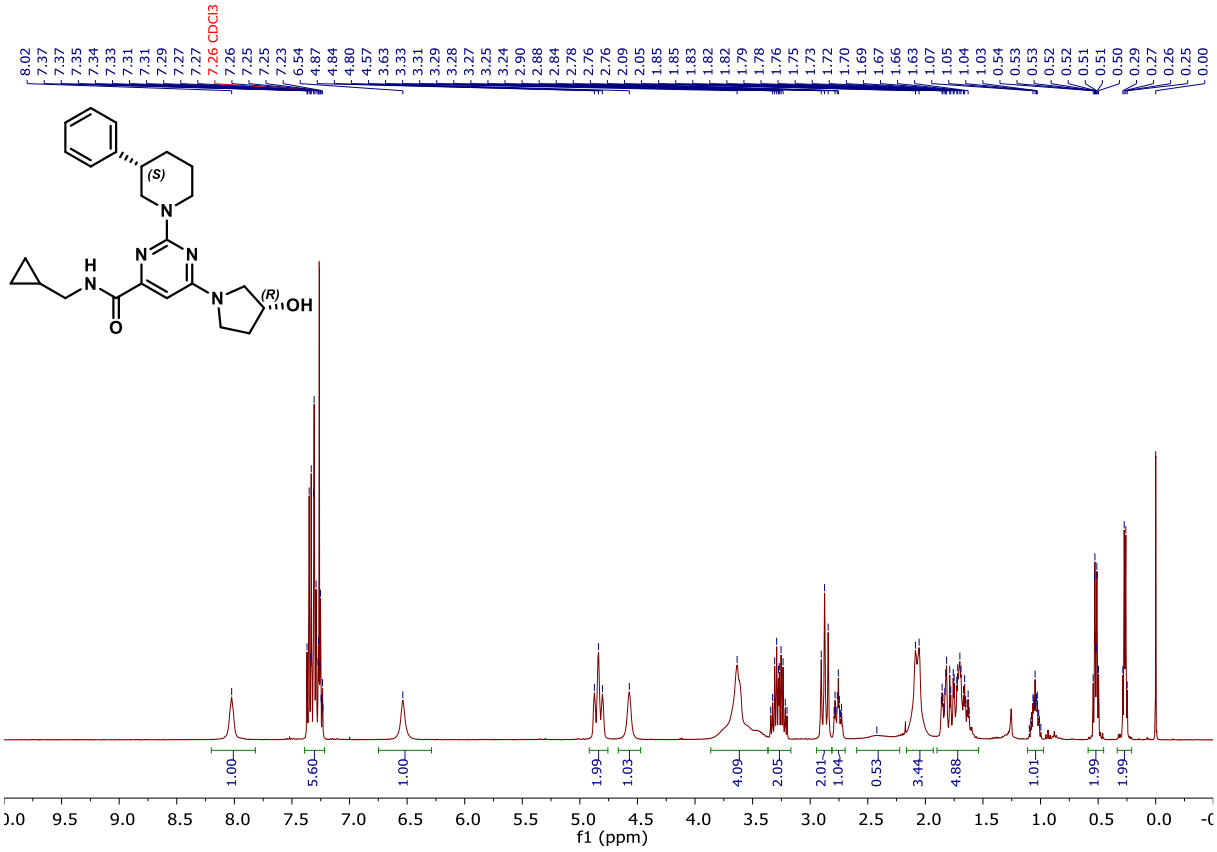
**$^{1}$H-NMR: (*S*)-104**



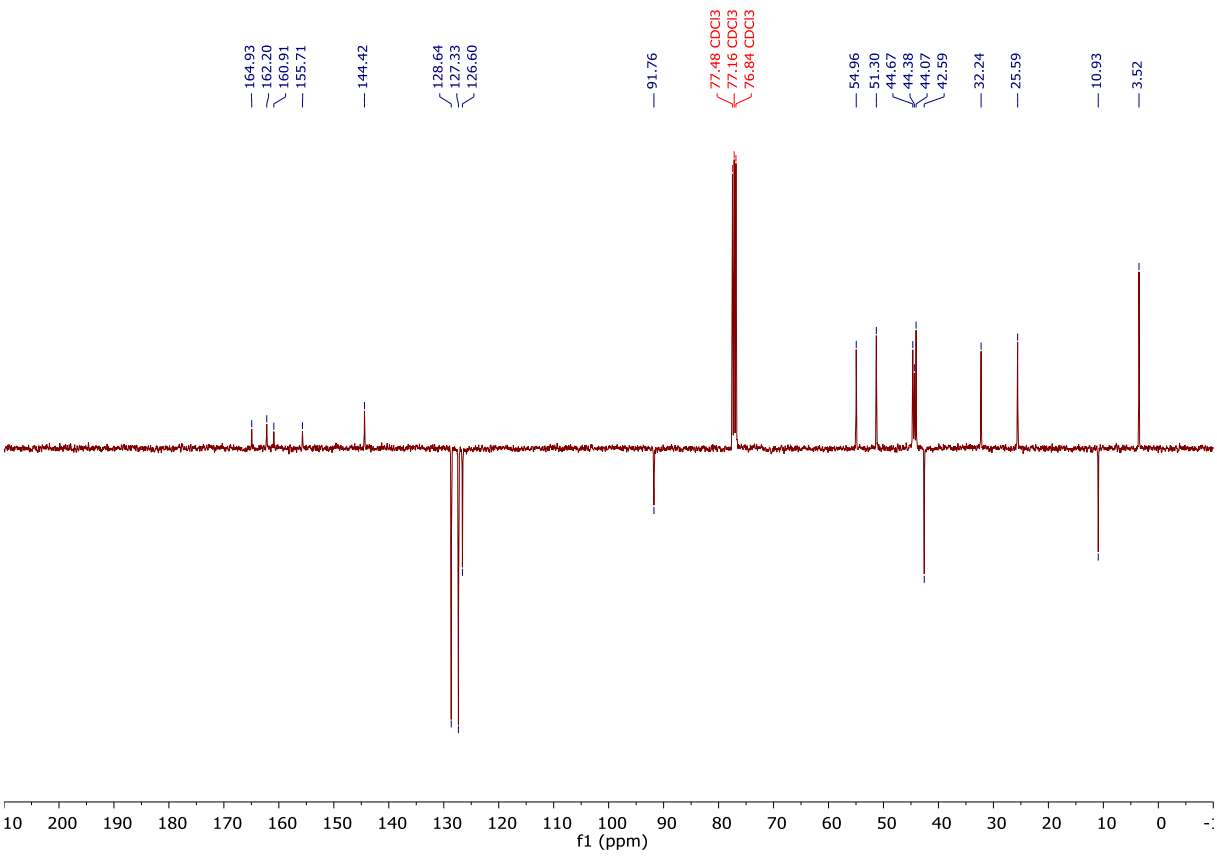

**$^{13}$C-NMR**



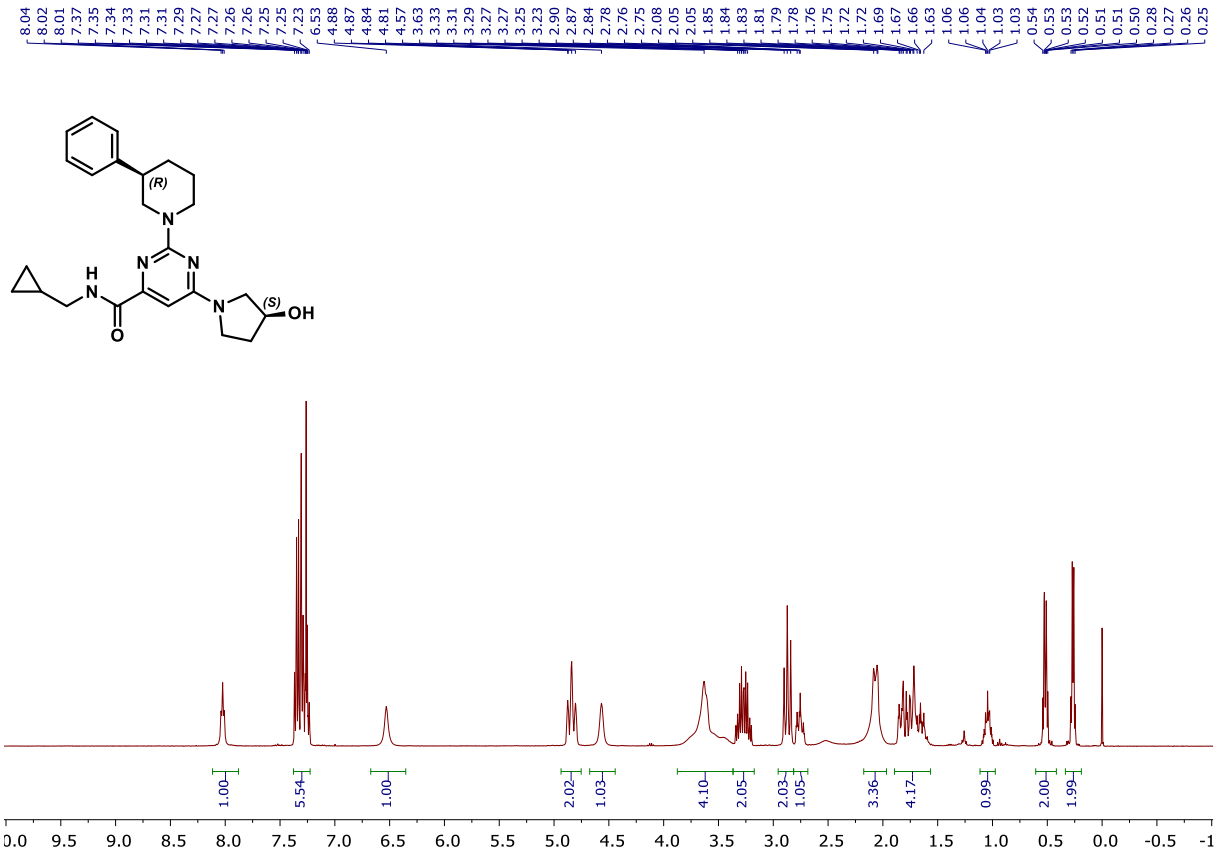
**$^{1}$H-NMR: 105**




**$^{13}$C-NMR**

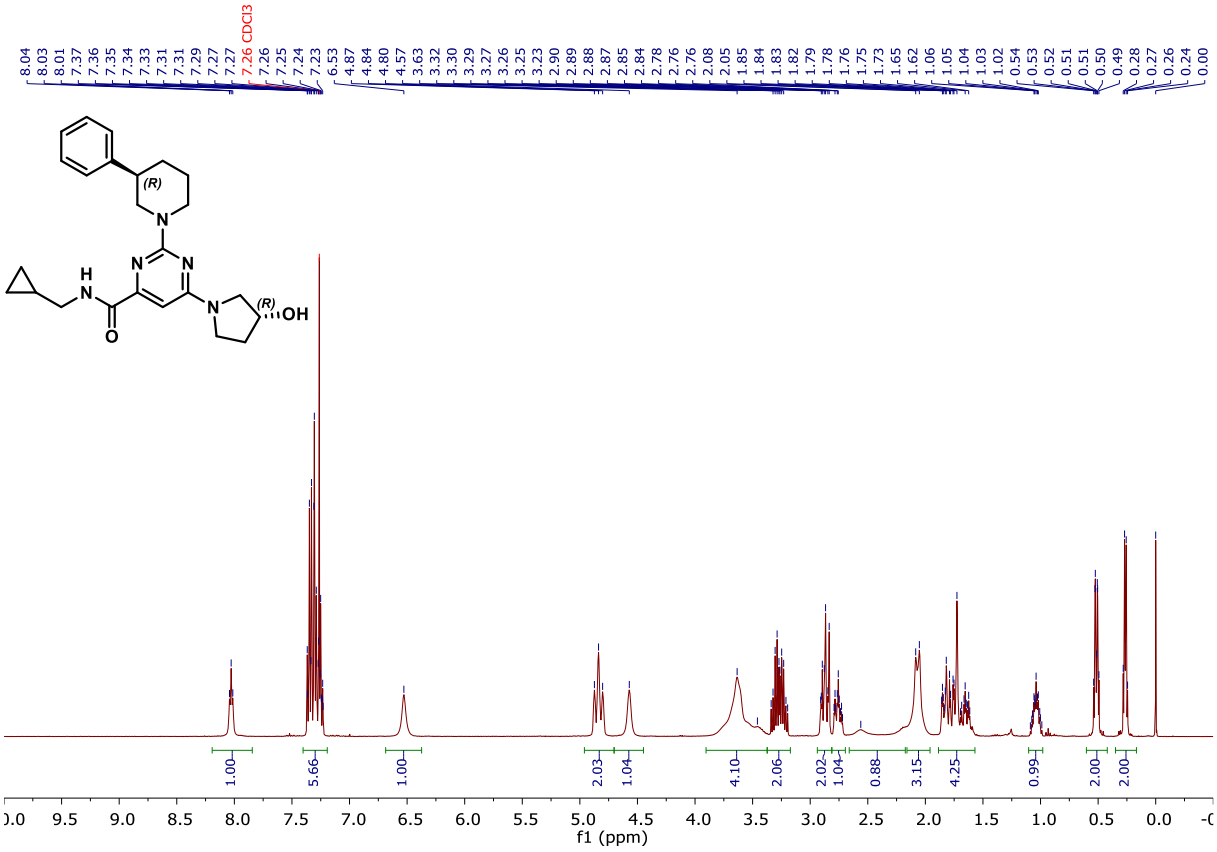


**$^{1}$H-NMR: 106**

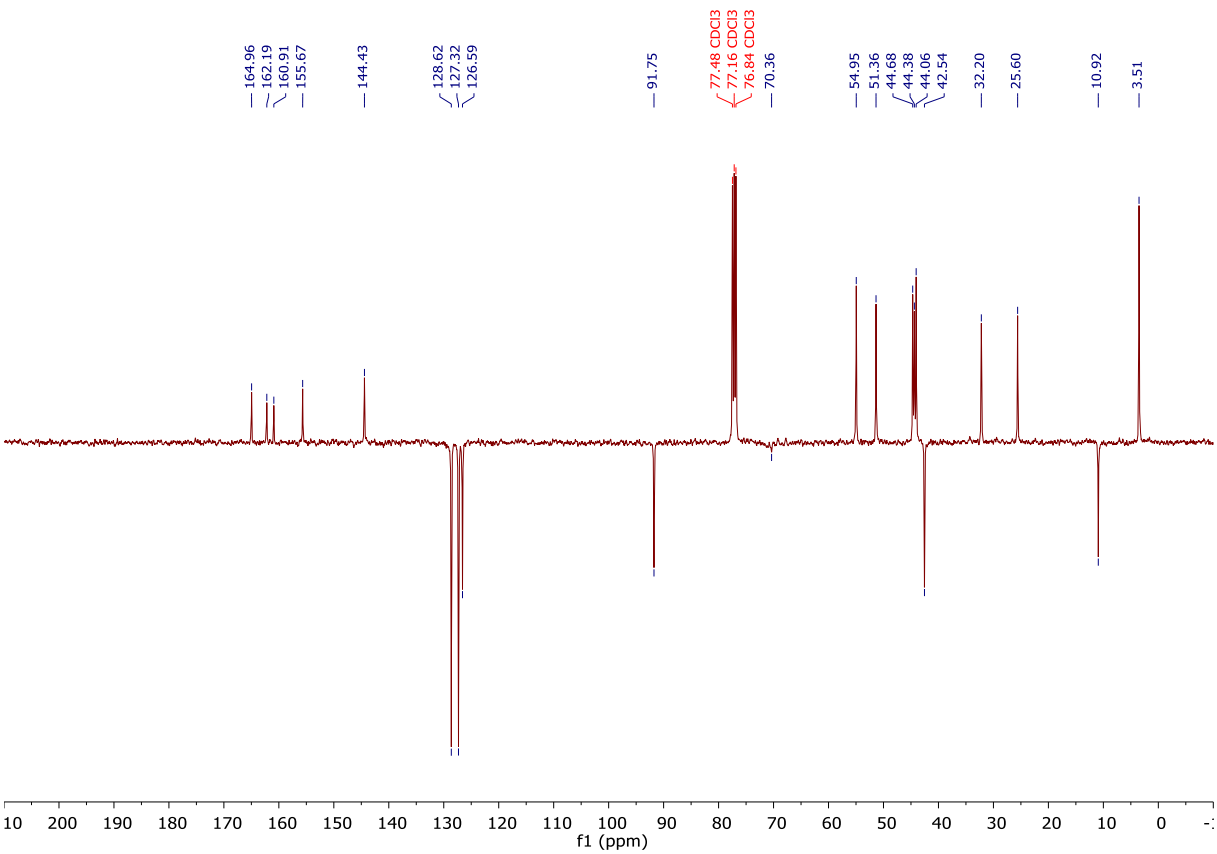



**$^{13}$C-NMR**

**$^{1}$H-NMR: 107**

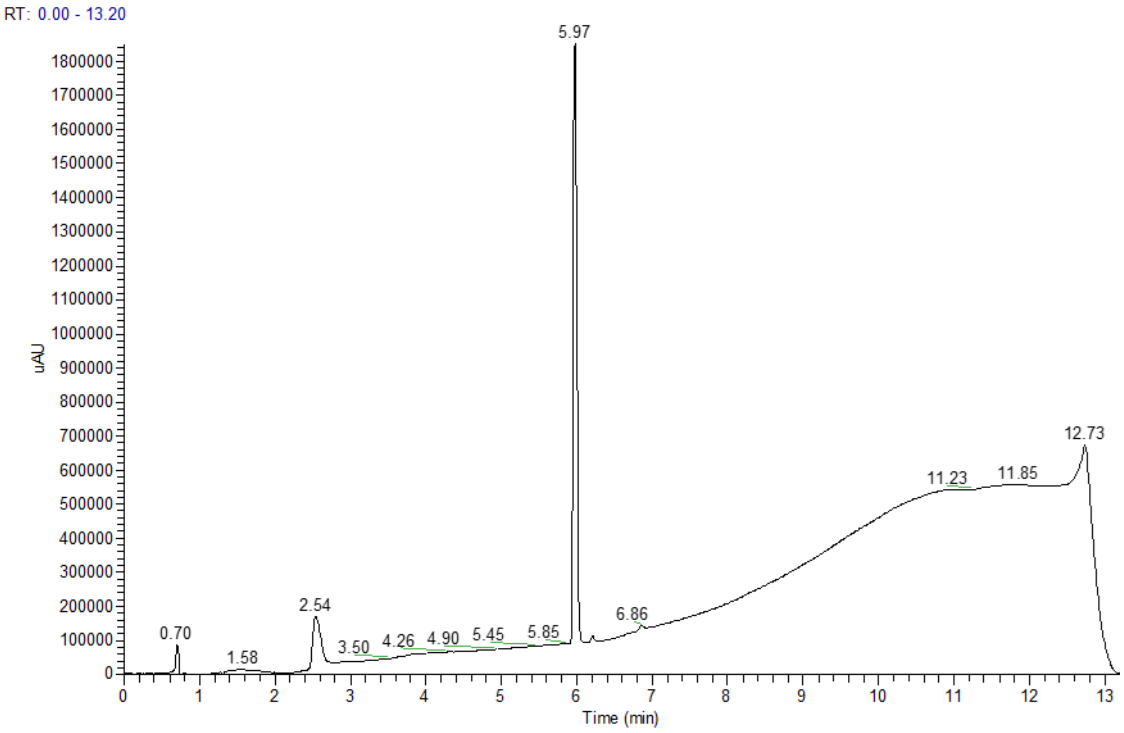



**$^{13}$C-NMR**



**HPLC-traces:**

**(*S*,*S*)-1, LEI-401:** 10-90% ($CH_3CN$ in $H_2O$ + 0.1% TFA), RT = 5.97 min, Purity: >95%.




**2:** 10-90% ($CH_3CN$ in $H_2O$ + 0.1% TFA), RT = 6.12 min, Purity: >95%.