Electronic Supplementary Material (ESI) for MedChemComm.

Biochemical and microbiological evaluation of *N*-aryl urea derivatives against Mycobacteria and Mycobacterial hydrolases

Abhishek Vartak,[a†] Christopher Goins,[b†] Vinicius Calado Nogueira de Moura,[c] Celine M. Schreidah,a Alexander D. Landgraf,[a] Boren Lin,[d] Jianyang Du,[d] Mary Jackson,[c] Donald R. Ronning[a*] and Steven J. Sucheck [a*]

[a.] *Department of Chemistry and Biochemistry, University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606, USA.*
[b.] *Center for Therapeutic Discovery, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, 44195, USA*
[c.] *Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA*
[d.] *Department of Biological Sciences, University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606, USA.*

Supplementary Information

Table of contents

Biological assays:

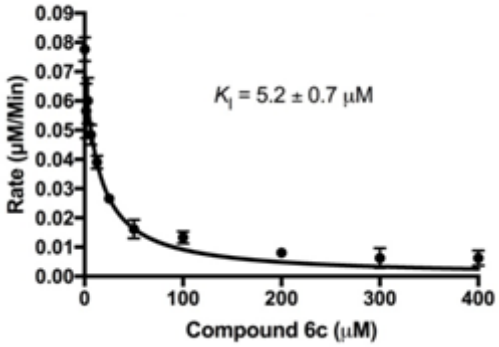


Figure **S1**: K_i determination for **6c** against Rv3802c

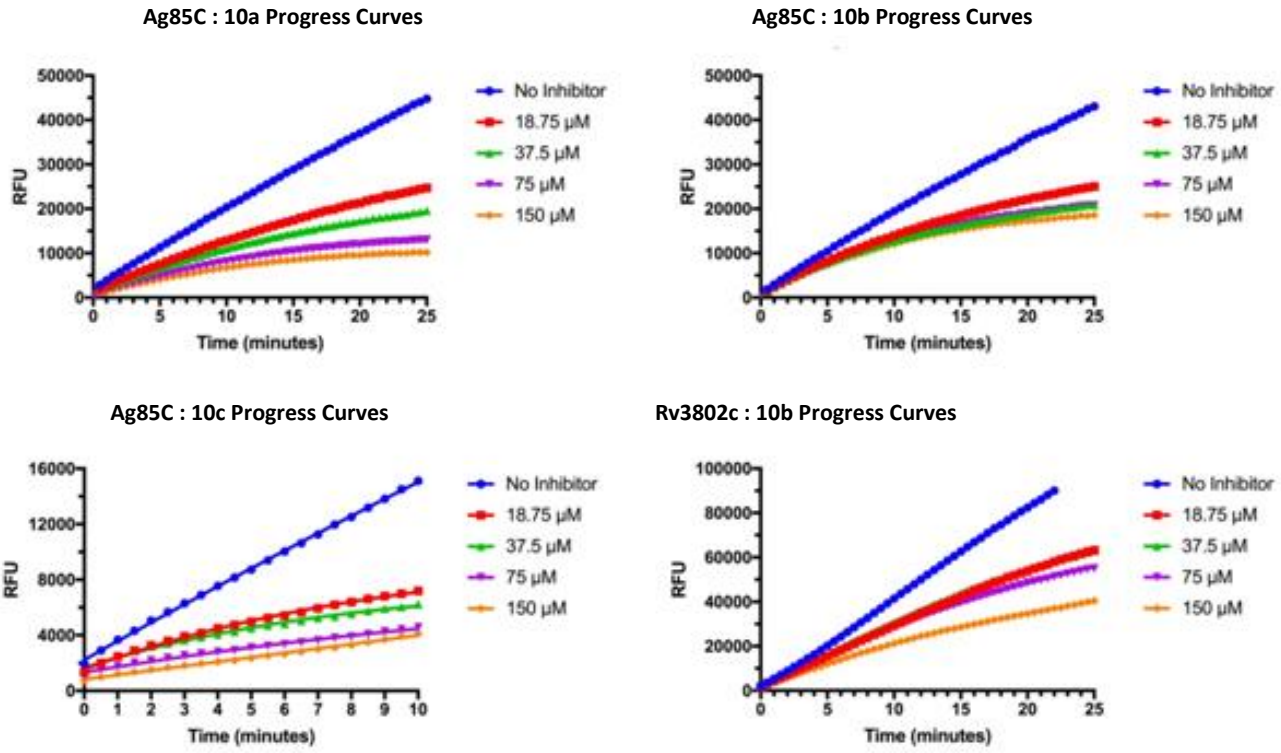


Figure **S2**: Dose dependent progress curves of covalent inhibitor **10a**, **10b**, **10c** against Ag85C and Rv3802c

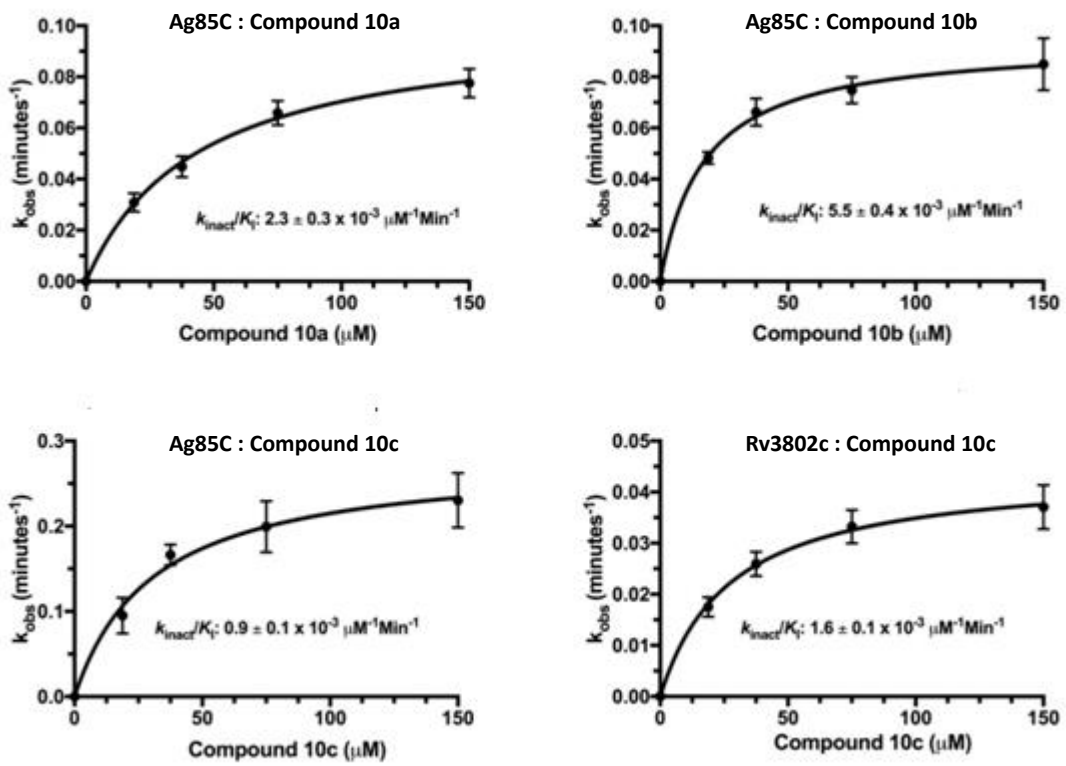


Figure **S3**: k_{inact}/K_I determination for covalent inhibitors

Minimum inhibitory concentration (MIC) determination

The MIC values of compounds against *M. abscessus* ATCC19977 and *M. tuberculosis* H37Rv mc^{2}6206 were determined in Mueller-Hinton II broth and 7H9-OADC–0.05% tyloxapol medium supplemented with 0.2% casaminoacids, 48 µg/ml pantothenate and 50 µg/ml L-leucine, respectively, in 96-well microtiter plates using the colorimetric resazurin microtiter assay and visually scanning for growth.[27] (Table **S1**)

The MIC values of compounds against *M. smegmatis* Mc^{2}155 were determined in LB broth containing tyloxapol (1-2 drops) in 96-well microtiter plates using resazurin assay as well. All compounds were screened in duplicates. (Table **S2**)

MICs were determined for *M. tuberculosis* H37Ra (ATCC 25177) in Dilfco™ Middlebrook Broth 7H9-OADC-0.05% Tween™ 80, performed in a 96 well microtiter plate via colorimetric resazurin microtiter assay with visual scanning for growth.[27] (Table **S3**)

Table **SI1**. MIC against *M. tuberculosis mc*^{2}6206

Compound	MIC (μM)		Compound	MIC (μM)		Compound	MIC (μM)
2a	nd		2g	nd		8b	nd
2b	25-50		6a	50-100		8c	nd
4a	3.12		4c	1.5		8d	nd
4b	50-100		6b	nd		10a	100
2c	nd		4d	nd		10b	50-100
2d	nd		6c	25-50		10c	100
2e	100		4e	3.12		12a	nd
2f	100		8a	nd		12b	nd

Table **SI2**. MIC against *M. smegmatis* Mc^{2}155

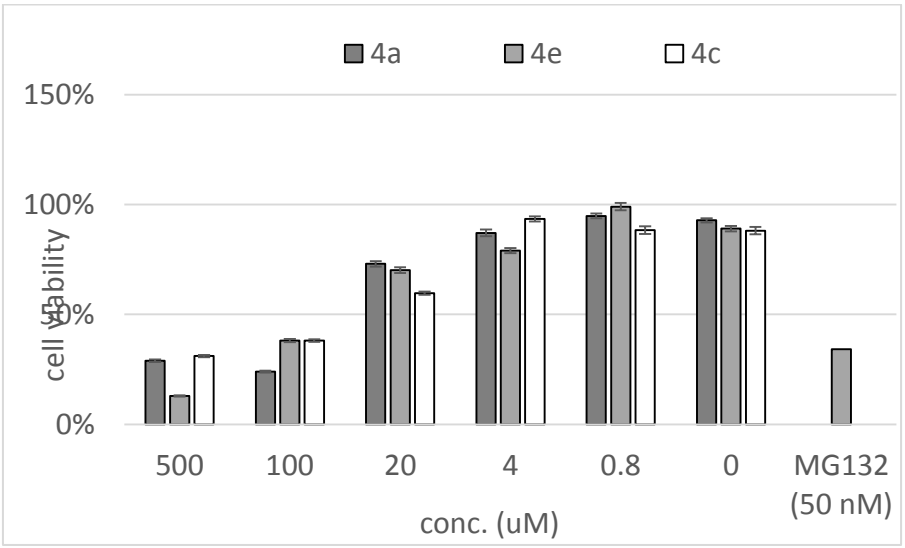
Compound	MIC (μM)		Compound	MIC (μM)		Compound	MIC (μM)
2a	>250		4a	15.6		6a	>250
2b	>250		4b	>250		6b	>250
2c	>250		4c	>250		6c	250
2d	>250		4d	>250		8a	>250
2e	>250		4e	62.5		8b	>250
2f	>250		10a	>250		8c	250
2g	>250		10b	>250		8d	>250
12a	>250		10c	>250			
12b	>250						

Table **SI3**. MIC against *M. tuberculosis* H37Ra (ATCC 25177)

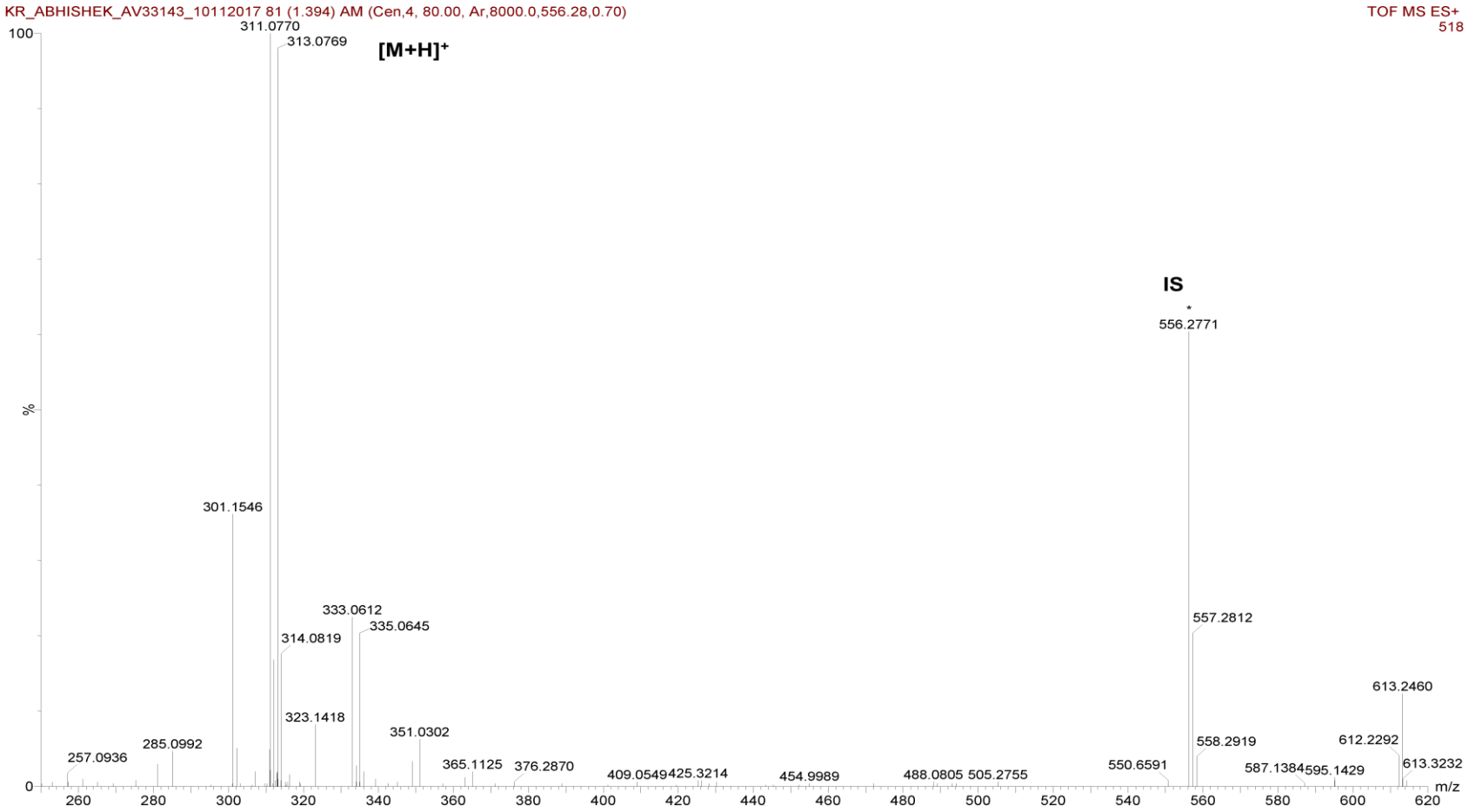
Compound	MIC (μM)		Compound	MIC (μM)
2a	>100		8b	>100
2b	>100		8a	>100
4a	25		8d	>100
4b	>100		8c	>100
2c	>100		6a	>100
2d	>100		4c	12.5
2e	>100		6b	>100
2f	>100		4d	>100
2g	>100		6c	>100
			4e	25

Cell viability assay

L929 murine fibroblast cell line was a kind gift from Dr. Guillermo Vazquez (the University of Toledo, OH, USA) and maintained in minimum essential medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 5% CO_2 and 37 °C. 10^3 cells per well were seeded in 96-well plates 24 hours prior to the experiment. To treat the cells, serial dilutions of each compound in DMSO were added to the cell culture, and after a 24 hour incubation, the cells were washed with PBS and treated with thiazolyl blue tetrazolium bromide (Sigma-Aldrich, St. Louis, MO, USA) for 4 hours, insoluble formazan dissolved in DMSO, and the optical density measured at 570 nm wavelength. For each compound at each concentration, the viability of treated cells was expressed as a percentage relative to the untreated cells, and a dose–response curve was obtained and the concentration causing 50% cell viability inhibition (IC_{50}) value was calculated.

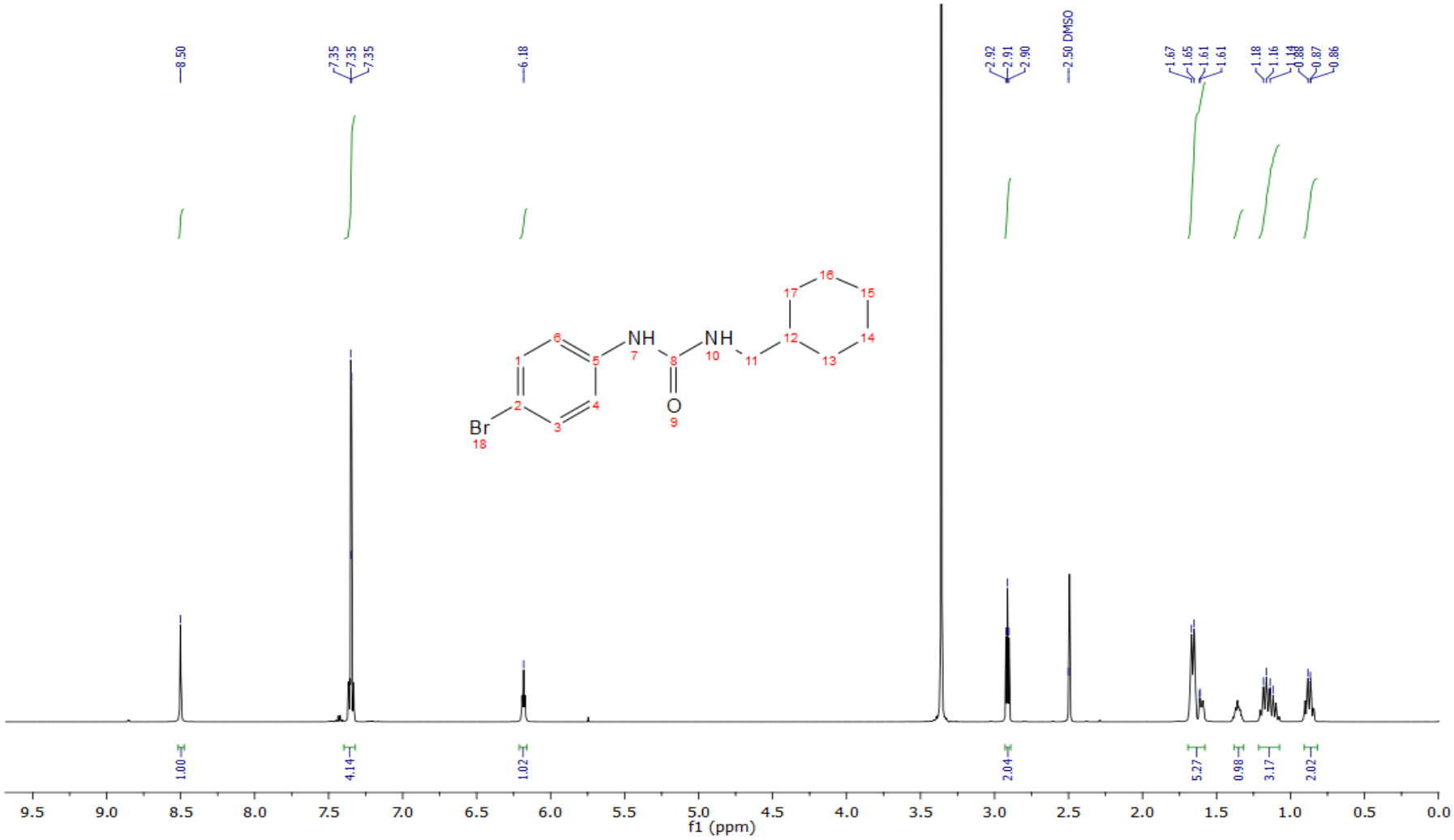


HRMS data for 1-(4-bromophenyl)-3-(cyclohexylmethyl)urea (**4b**)

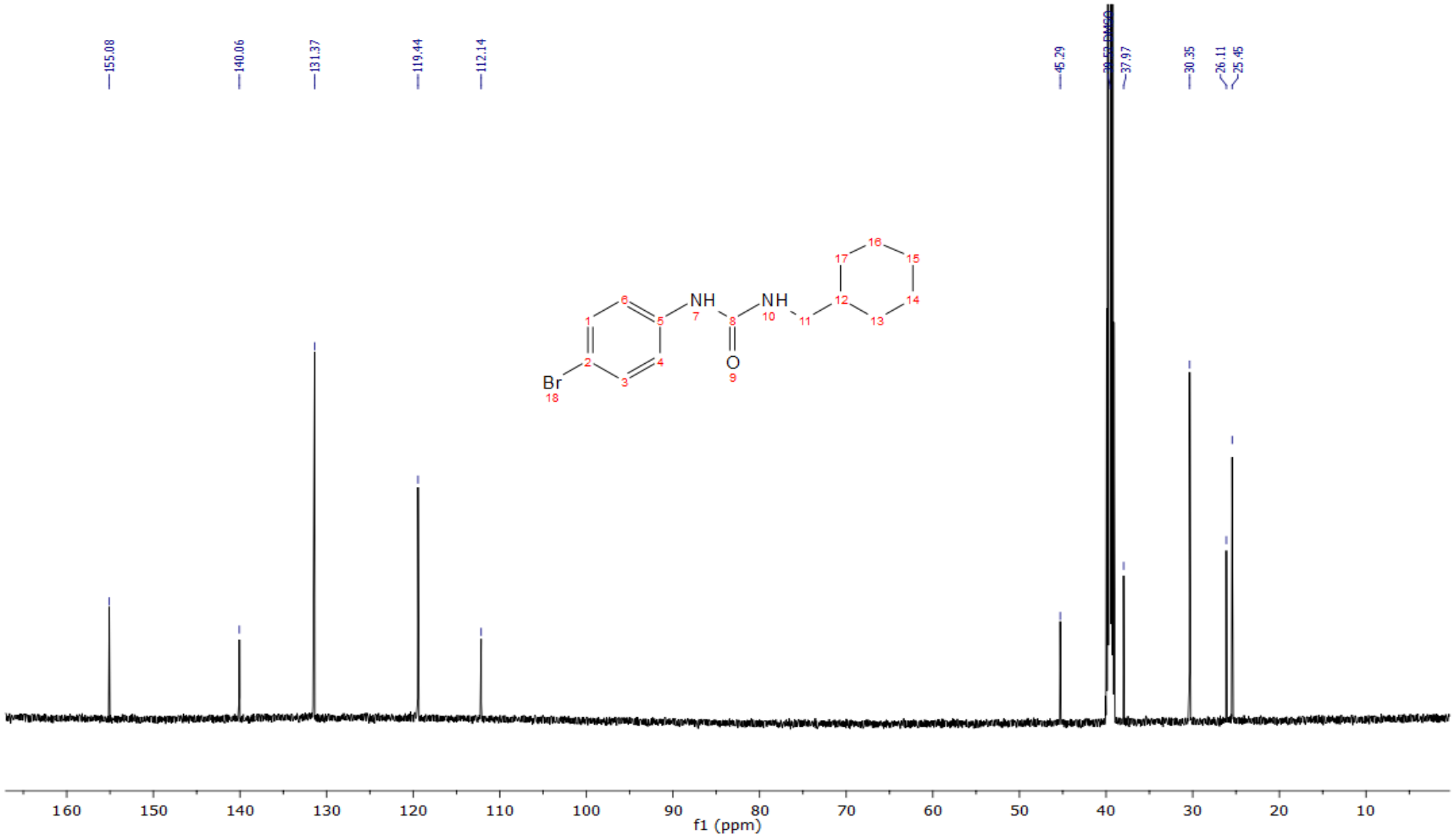


Mass accuracy = ((311.0759-311.0770)/311.0759)* 10^6 = 3.5 ppm

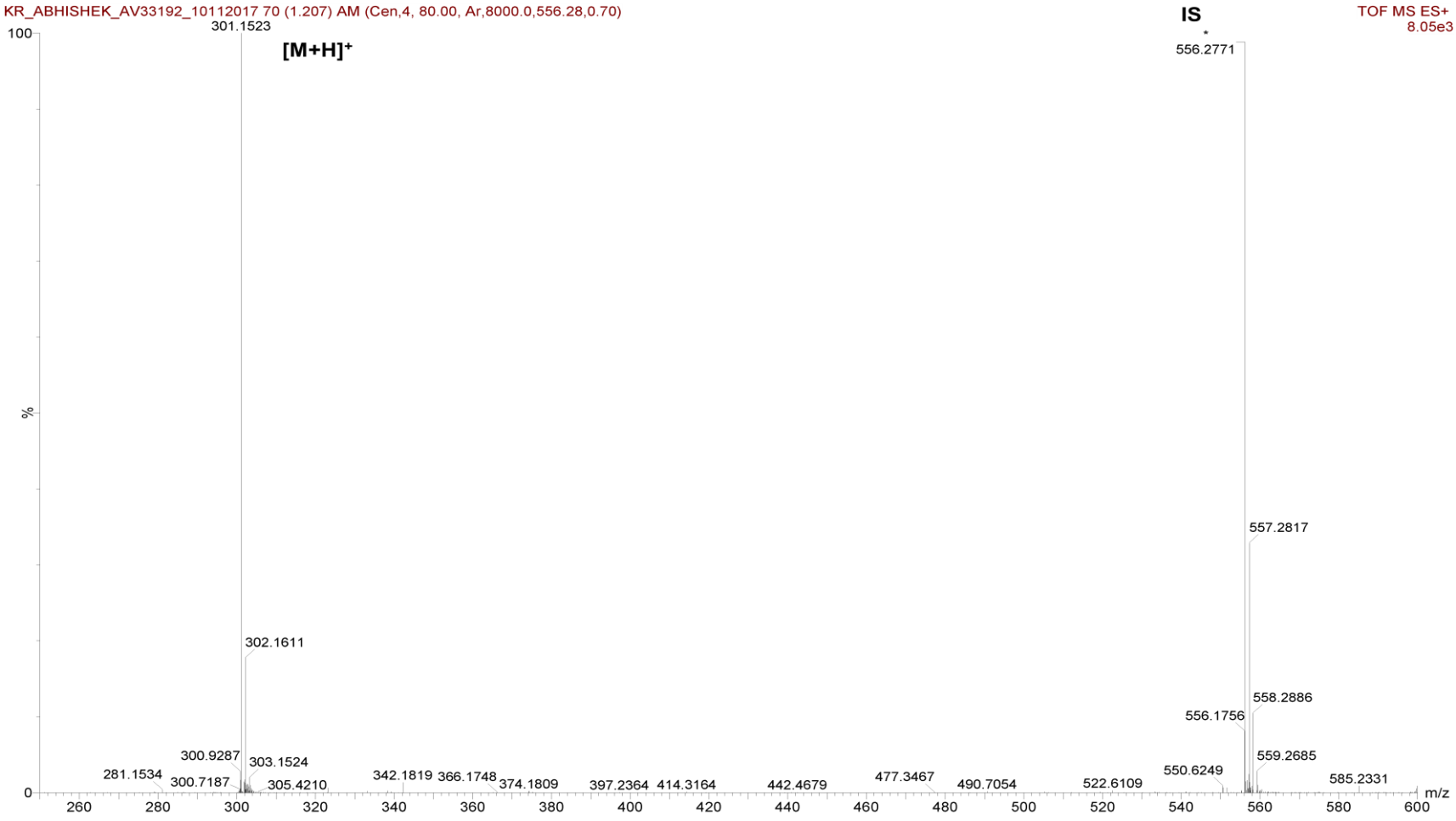
^{1}H NMR of 1-(4-bromophenyl)-3-(cyclohexylmethyl)urea (**4b**)



^{13}C NMR of 1-(4-bromophenyl)-3-(cyclohexylmethyl)urea (**4b**)

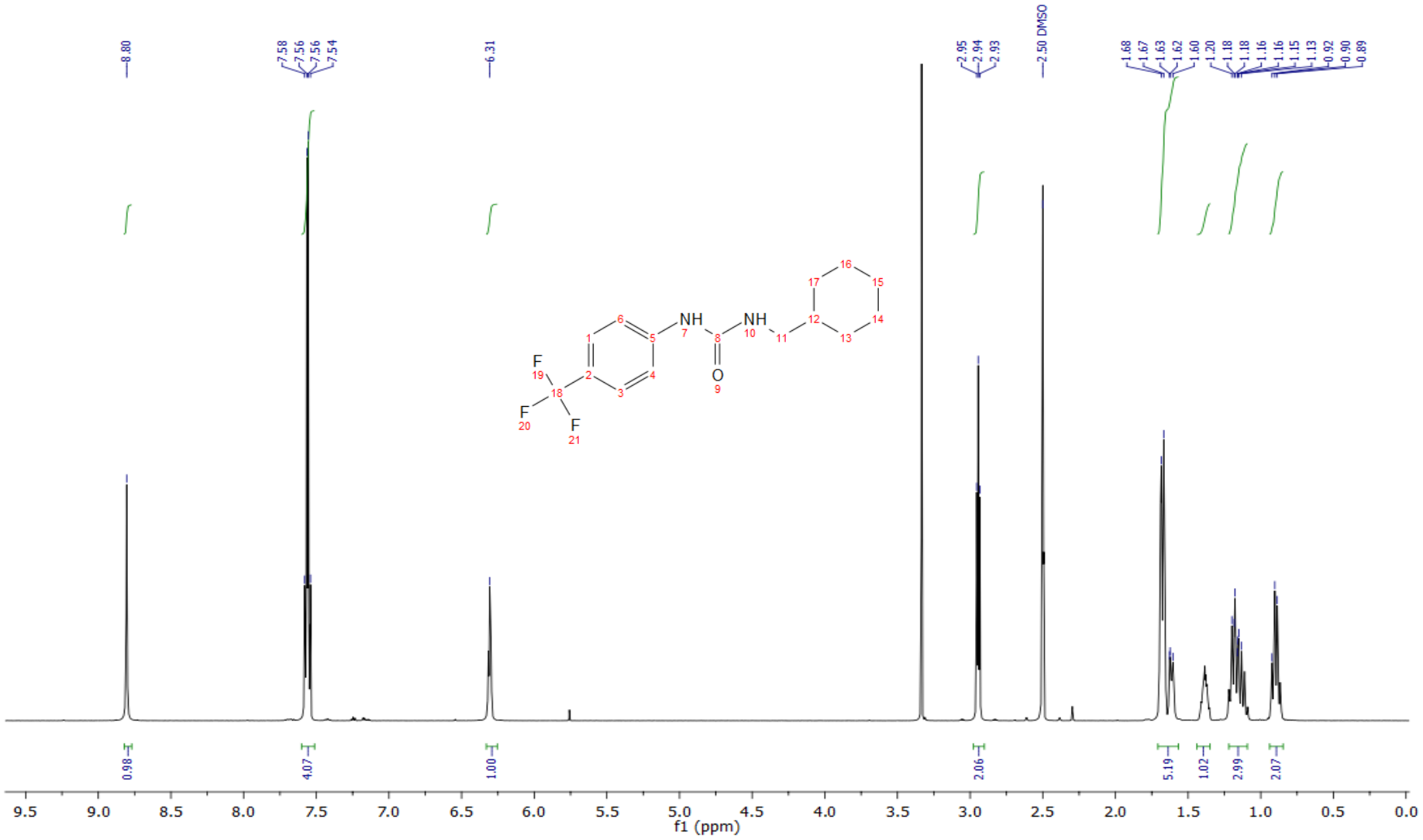


HRMS data for 1-(cyclohexylmethyl)-3-(4-(trifluoromethyl)phenyl)urea (**4c**)

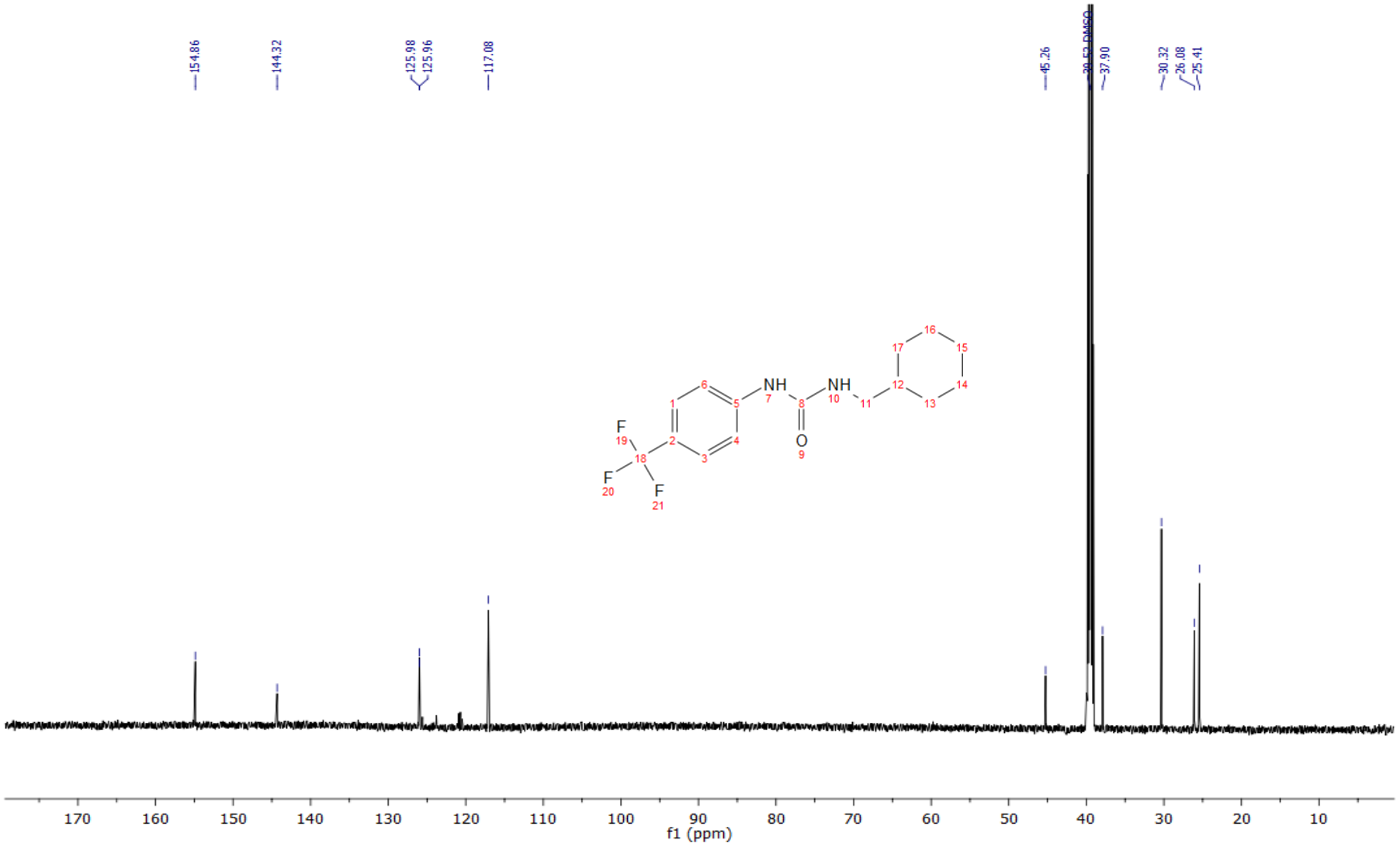


Mass accuracy = ((301.1528-301.1523)/301.1528)* 10^6 = 1.7 ppm

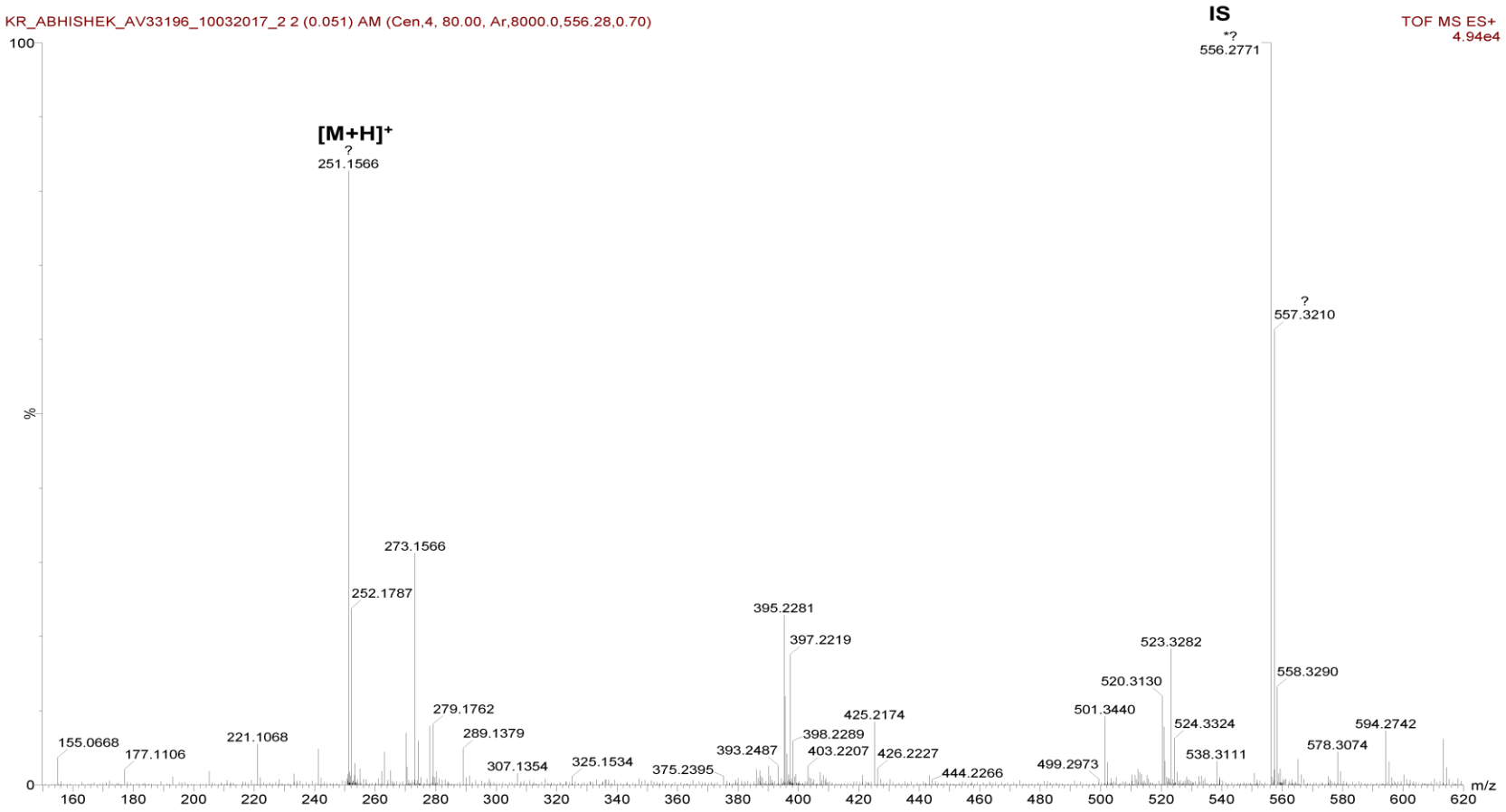
^{1}H NMR of 1-(cyclohexylmethyl)-3-(4-(trifluoromethyl)phenyl)urea (**4c**)



^{13}C NMR of 1-(cyclohexylmethyl)-3-(4-(trifluoromethyl)phenyl)urea (**4c**)

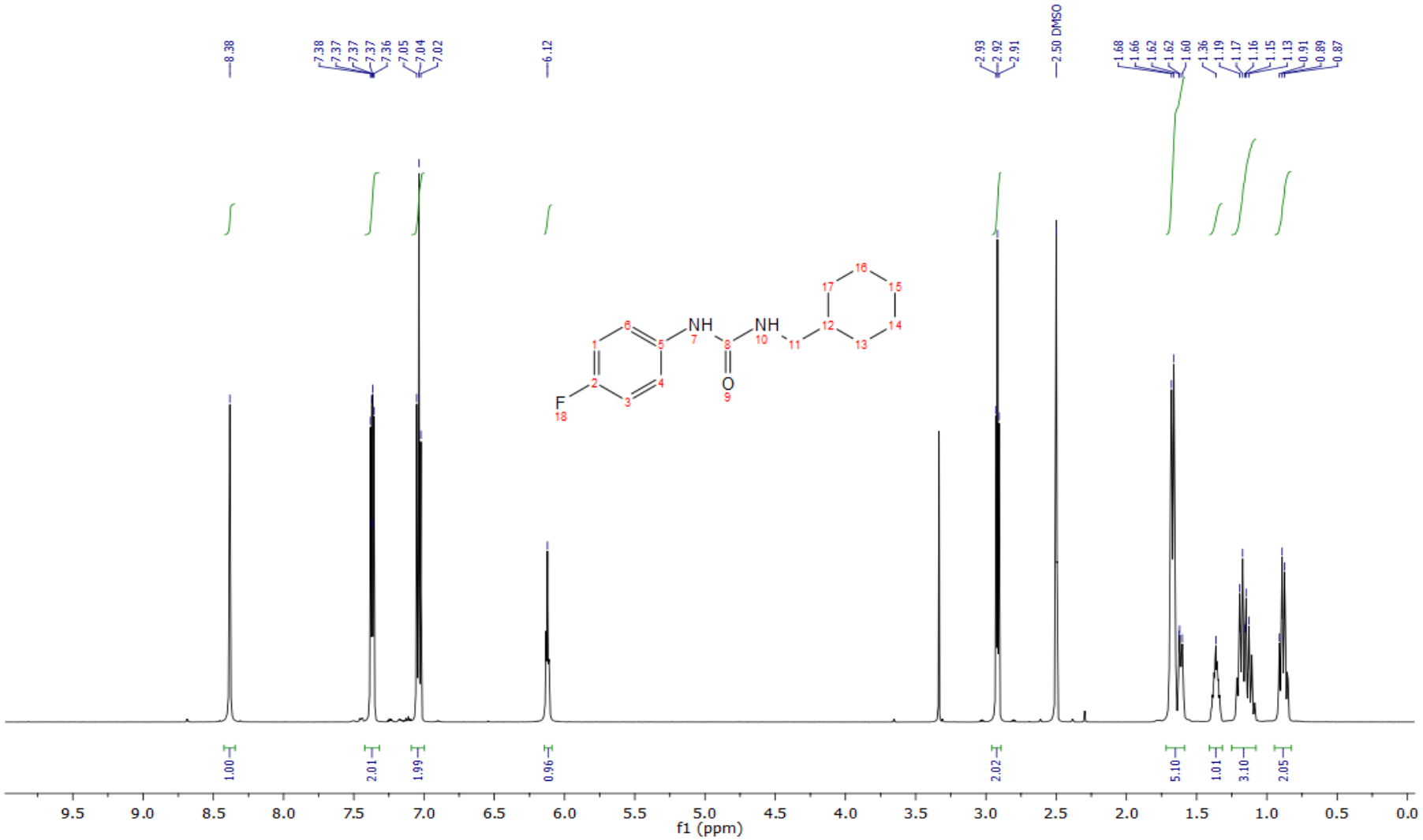


HRMS data for 1-(cyclohexylmethyl)-3-(4-fluorophenyl)urea (**4d**)

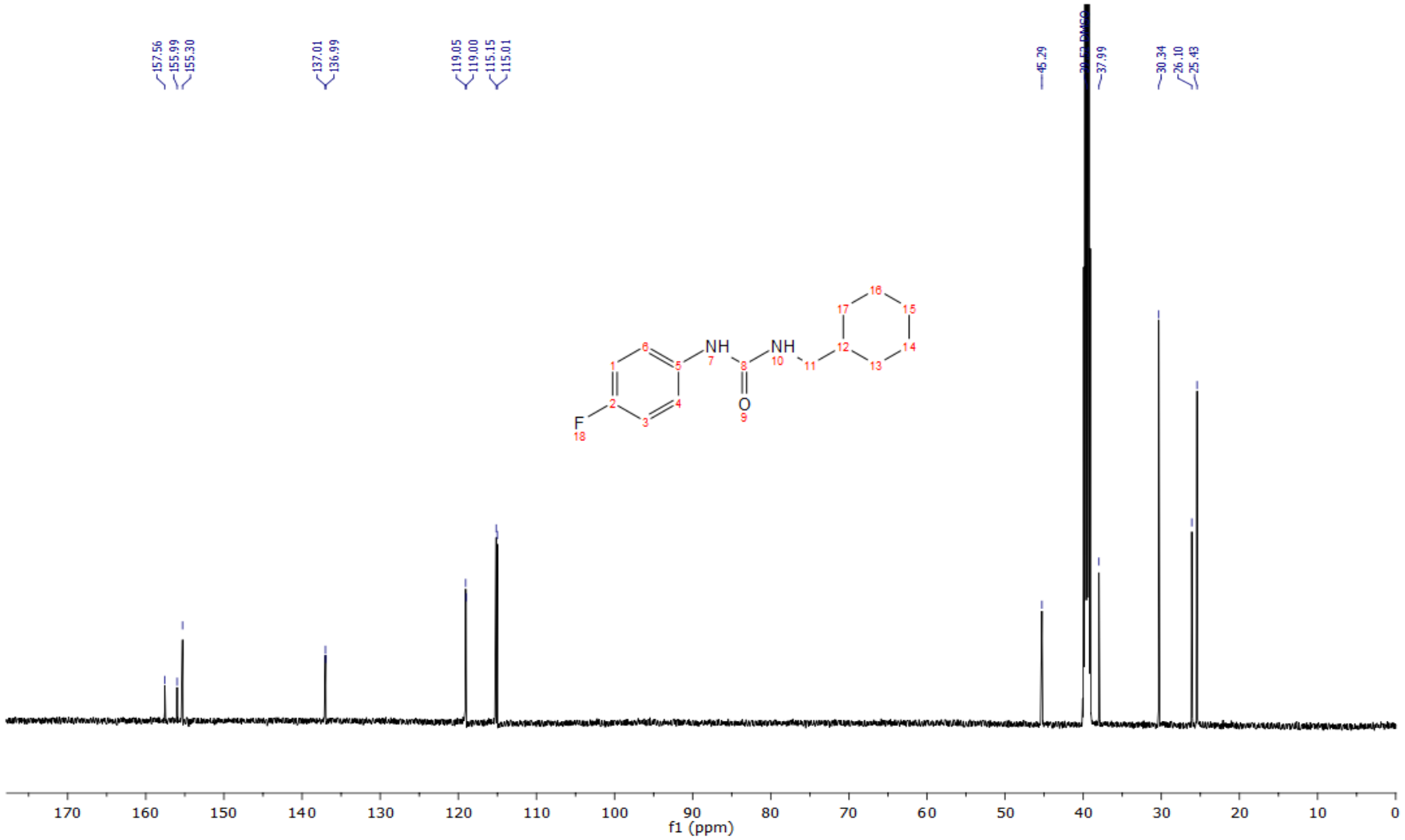


Mass accuracy = ((251.1560-251.1566)/251.1560)* 10^6 = 2.4 ppm

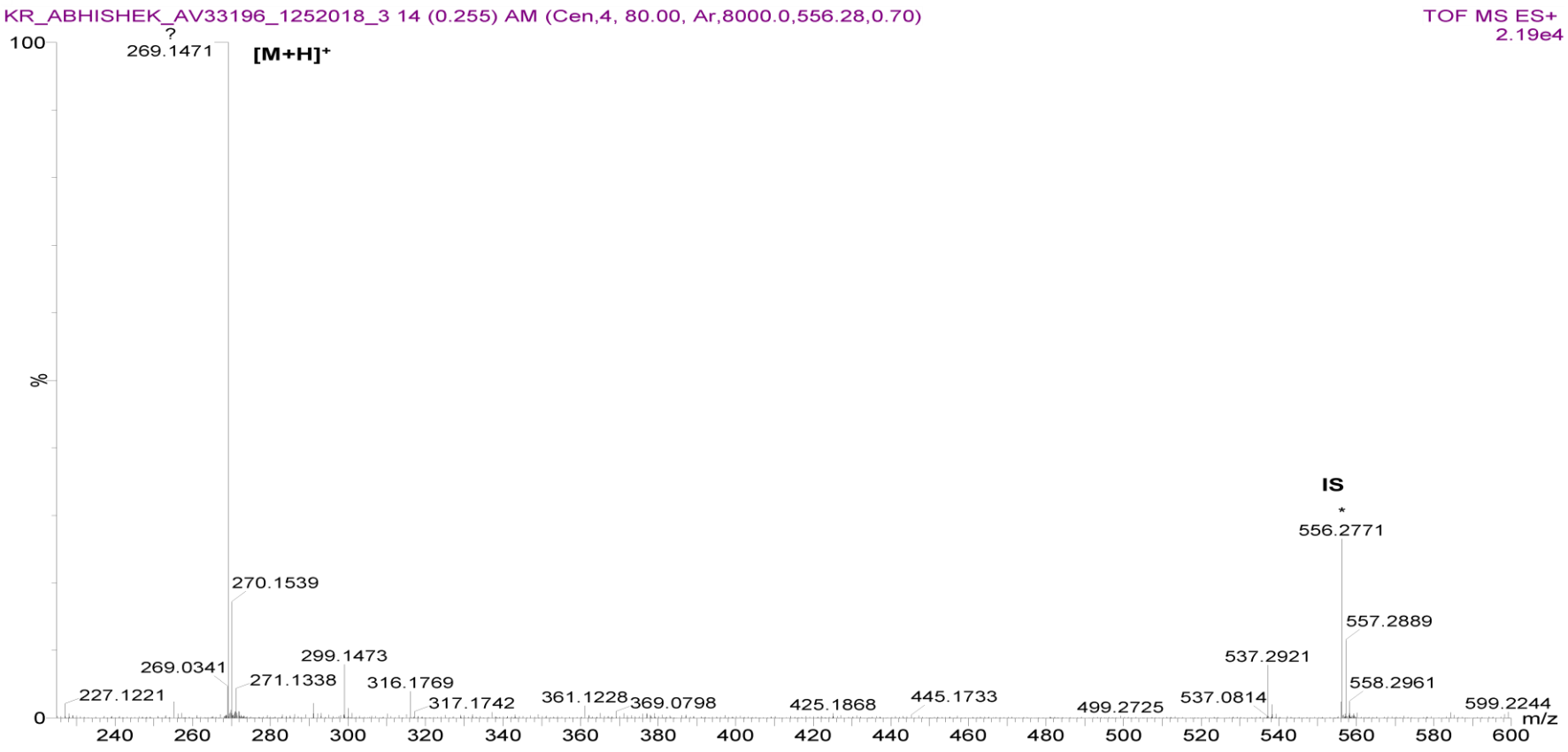
^{1}H NMR of 1-(cyclohexylmethyl)-3-(4-fluorophenyl)urea (**4d**)



^{13}C NMR of 1-(cyclohexylmethyl)-3-(4-fluorophenyl)urea (**4d**)

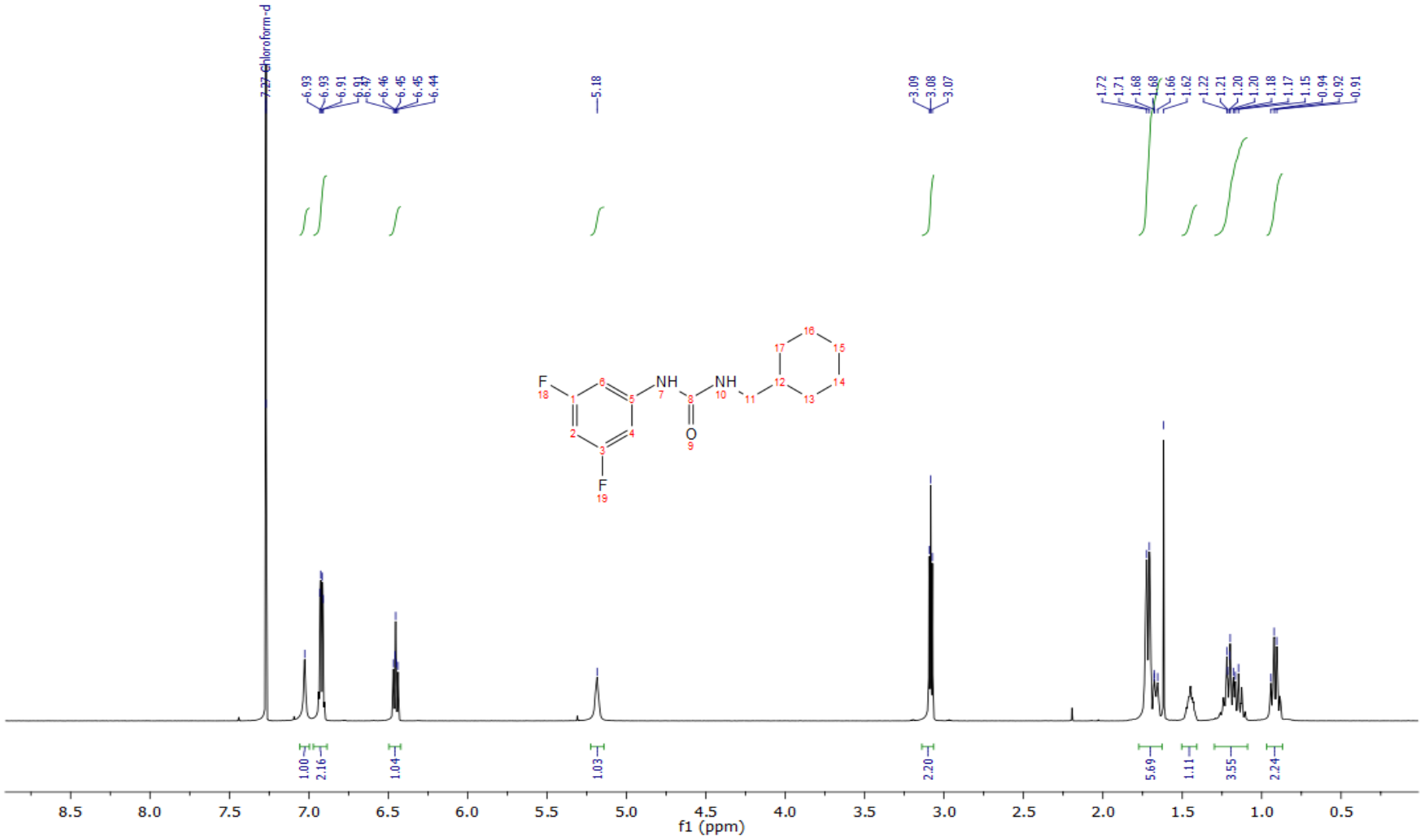


HRMS data for 1-(cyclohexylmethyl)-3-(3,5-difluorophenyl)urea (**4e**)

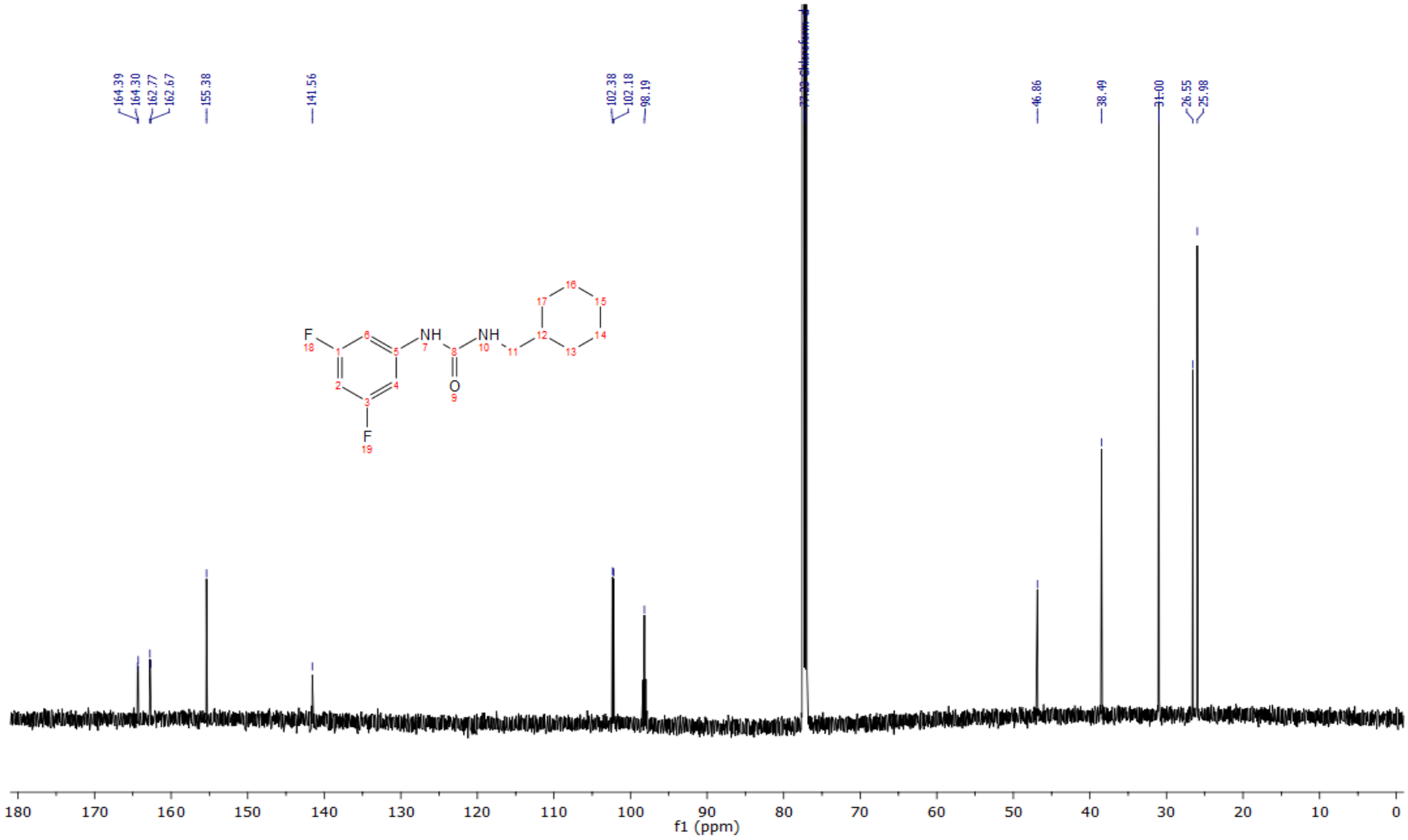


Mass accuracy = ((269.1465-269.1471)/269.1465)* 10^6 = 2.2 ppm

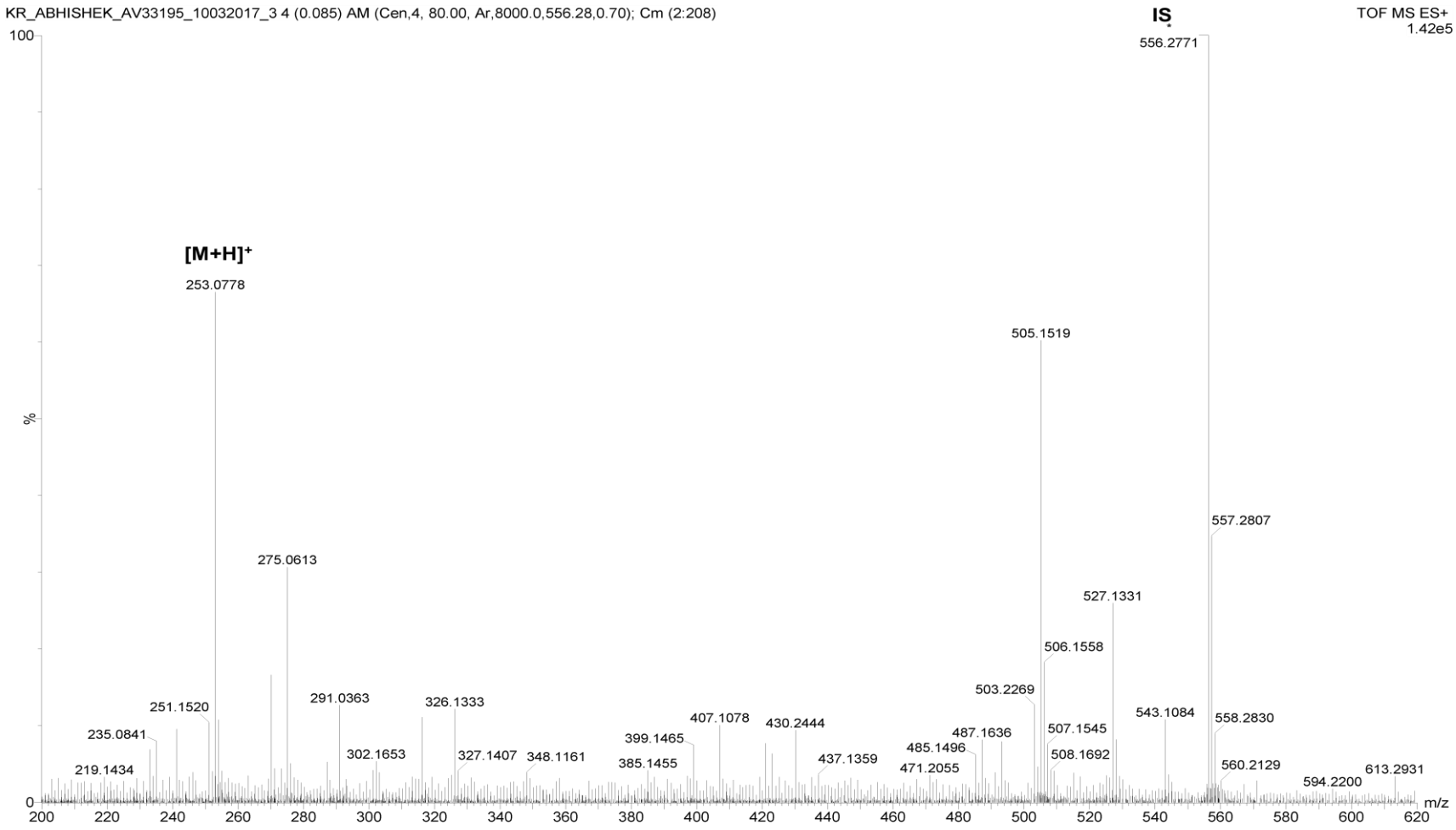
^{1}H NMR of 1-(cyclohexylmethyl)-3-(3,5-difluorophenyl)urea (**4e**)



^{13}C NMR of 1-(cyclohexylmethyl)-3-(3,5-difluorophenyl)urea (**4e**)

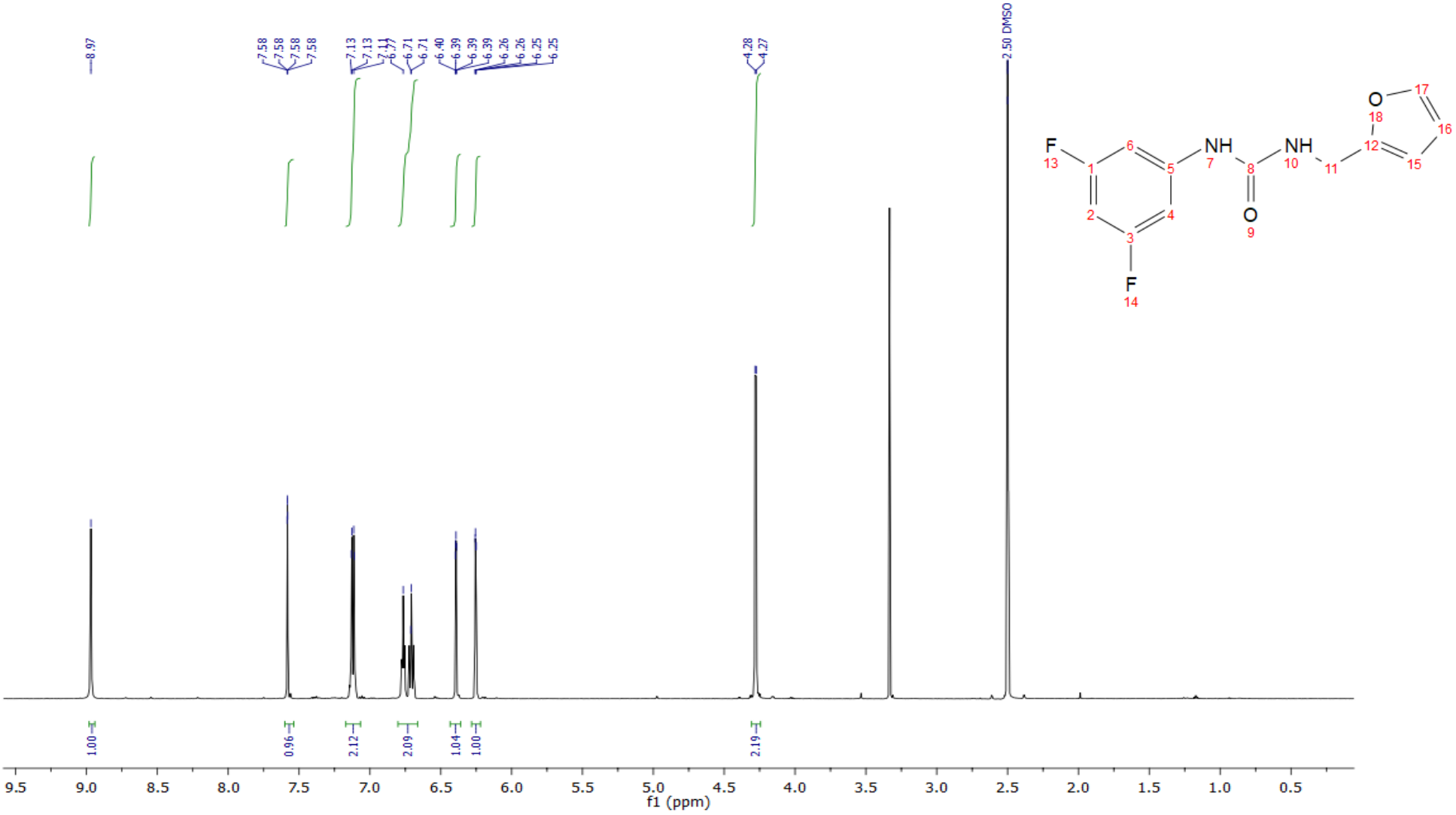


HRMS data of 1-(3,5-difluorophenyl)-3-(furan-2-ylmethyl)urea (**6c**)

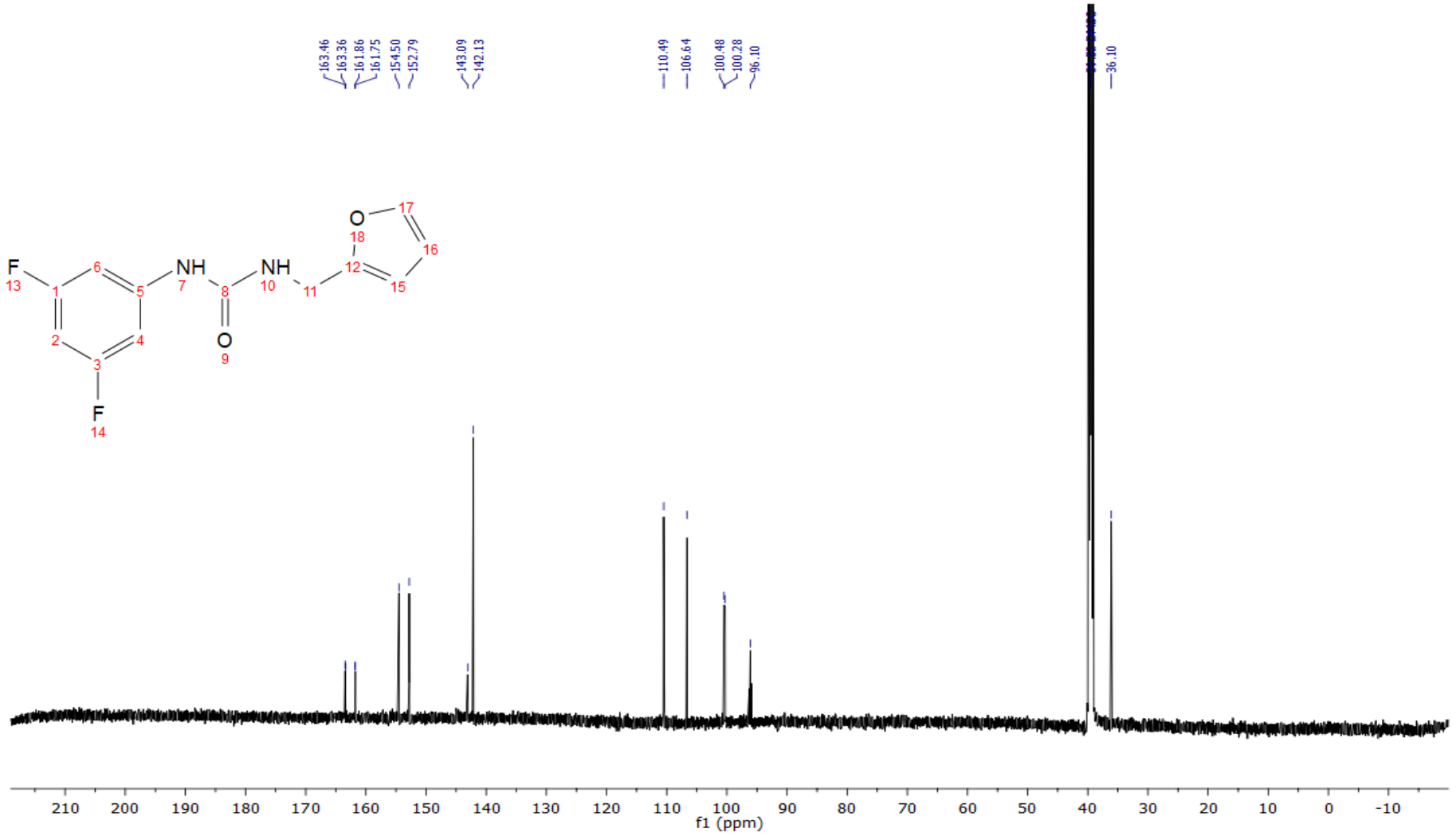


Mass accuracy = ((253.0789-253.0778)/253.0789)* 10^6 = 4.4 ppm

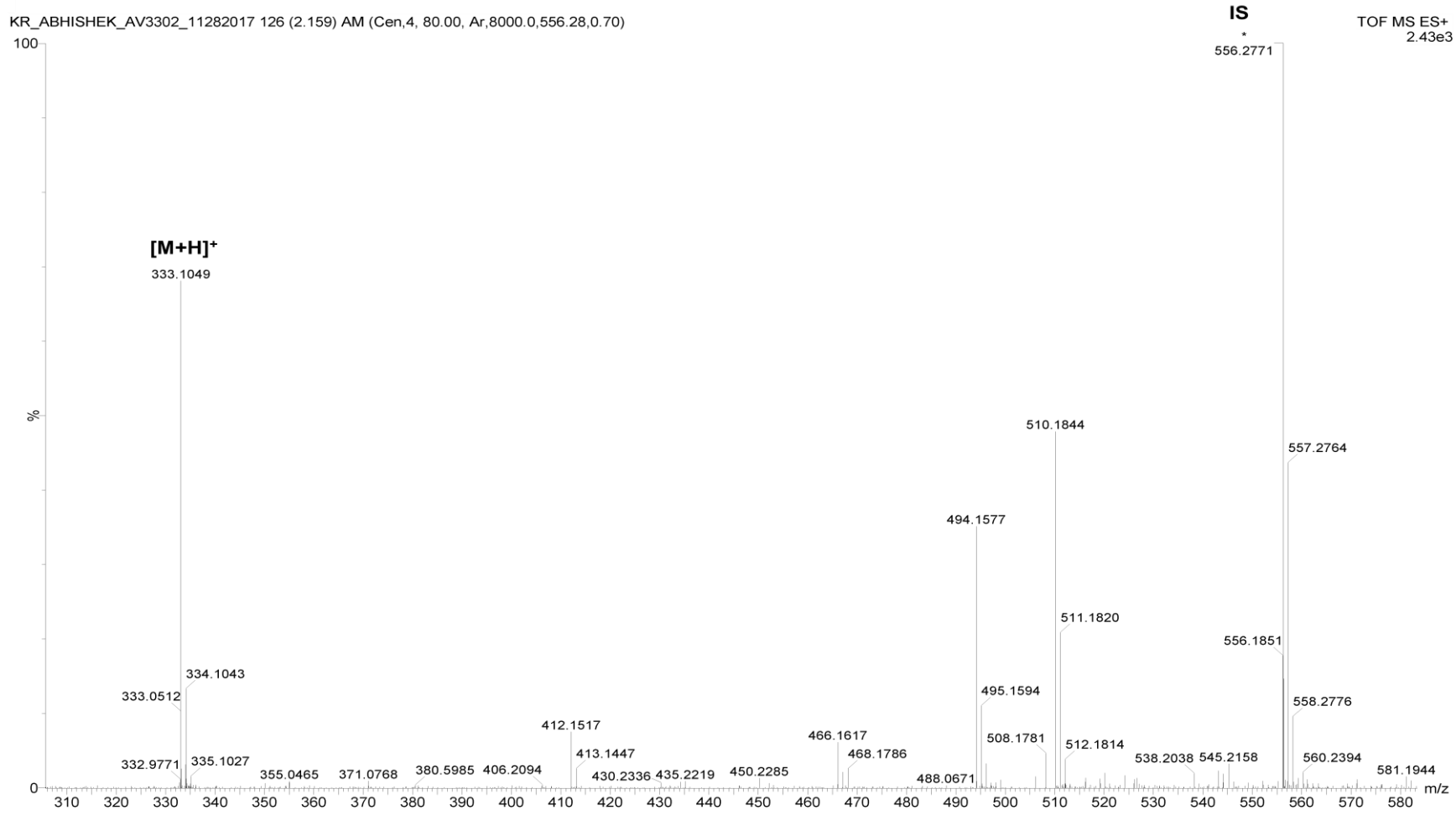
^{1}H NMR of 1-(3,5-difluorophenyl)-3-(furan-2-ylmethyl)urea (**6c**)



^{13}C NMR of 1-(3,5-difluorophenyl)-3-(furan-2-ylmethyl)urea (**6c**)

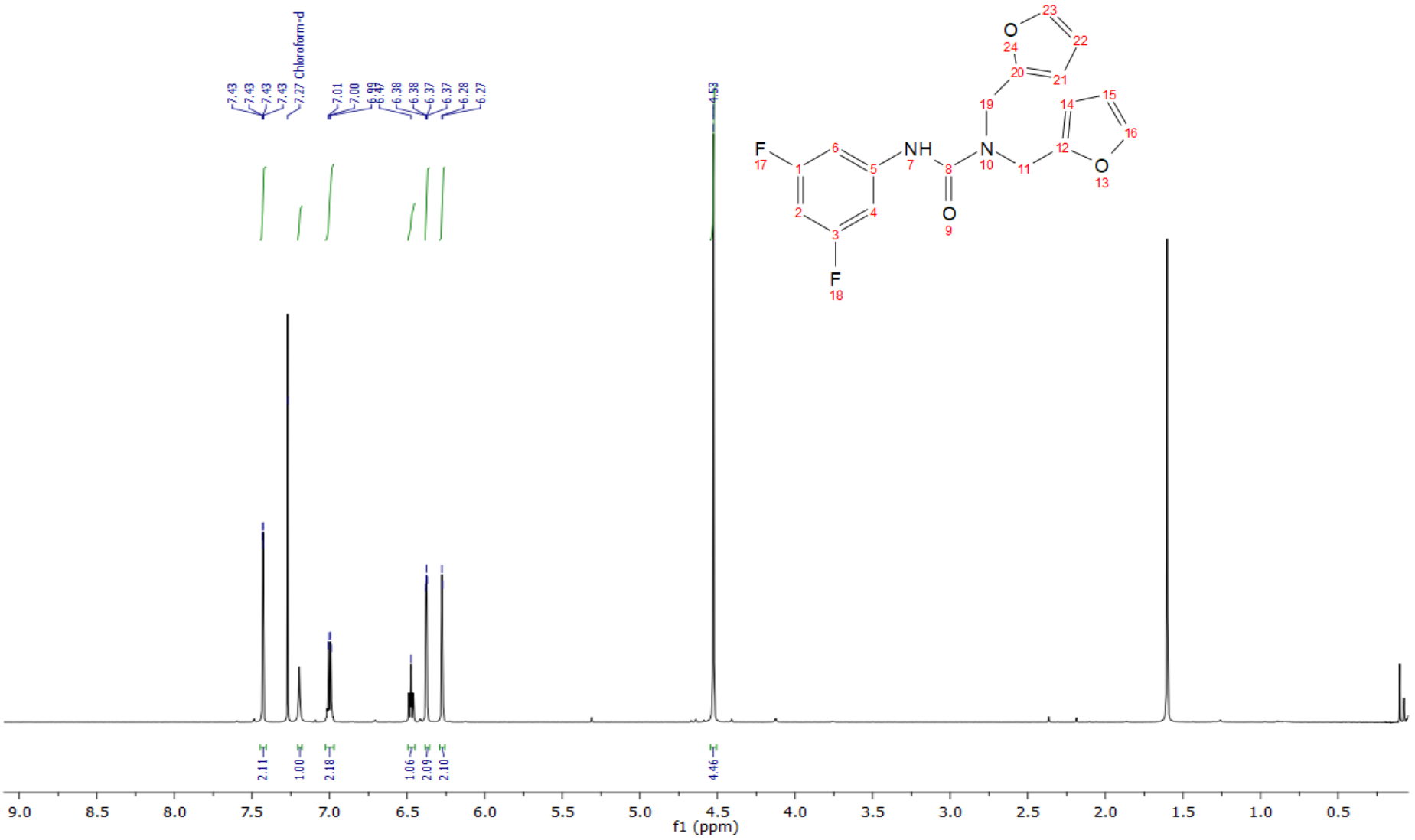


HRMS data of 3-(3,5-difluorophenyl)-1,1-bis(furan-2-ylmethyl)urea (**8a**)

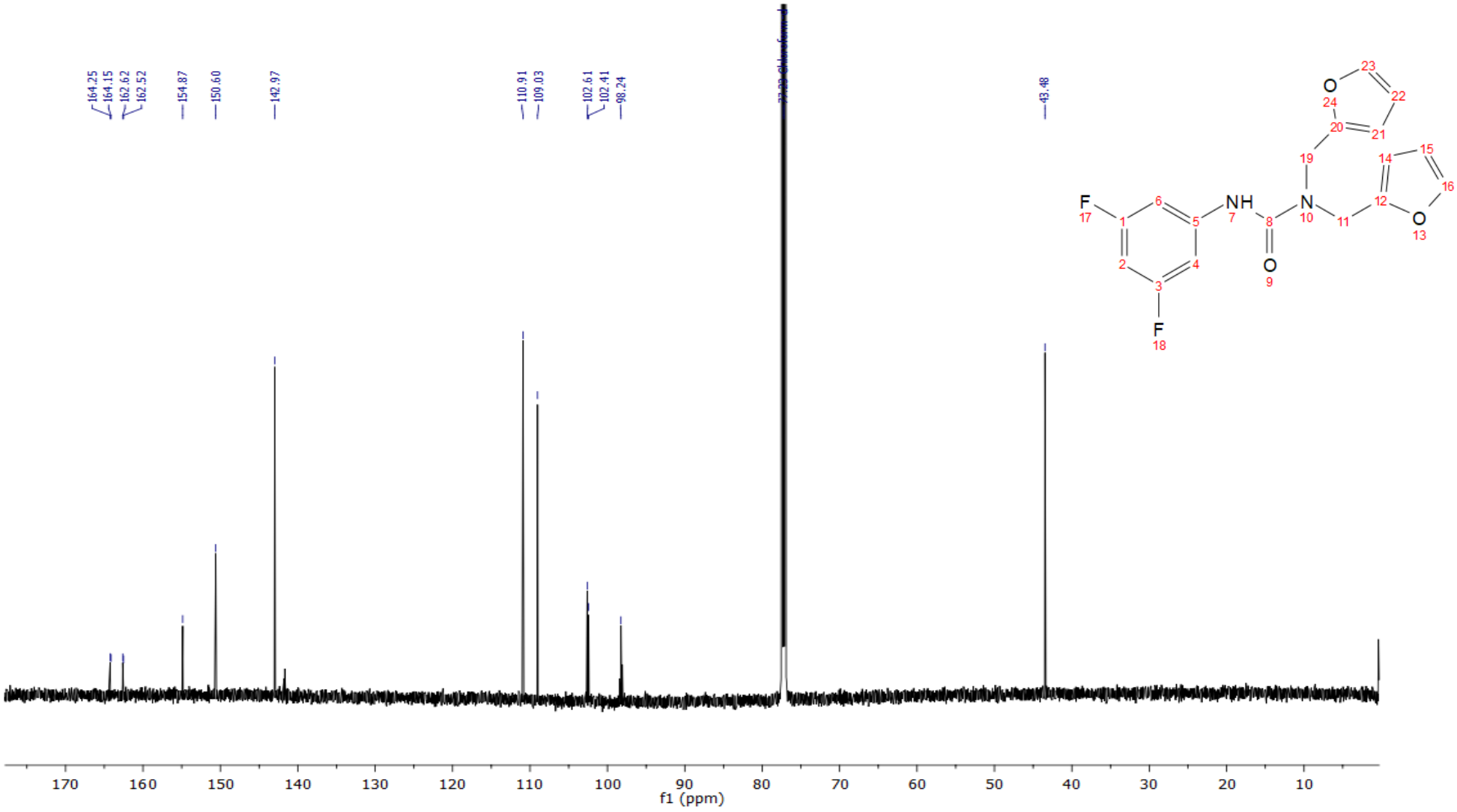


Mass accuracy = ((333.1051-333.1049)/333.1051)* 10^6 = 0.6 ppm

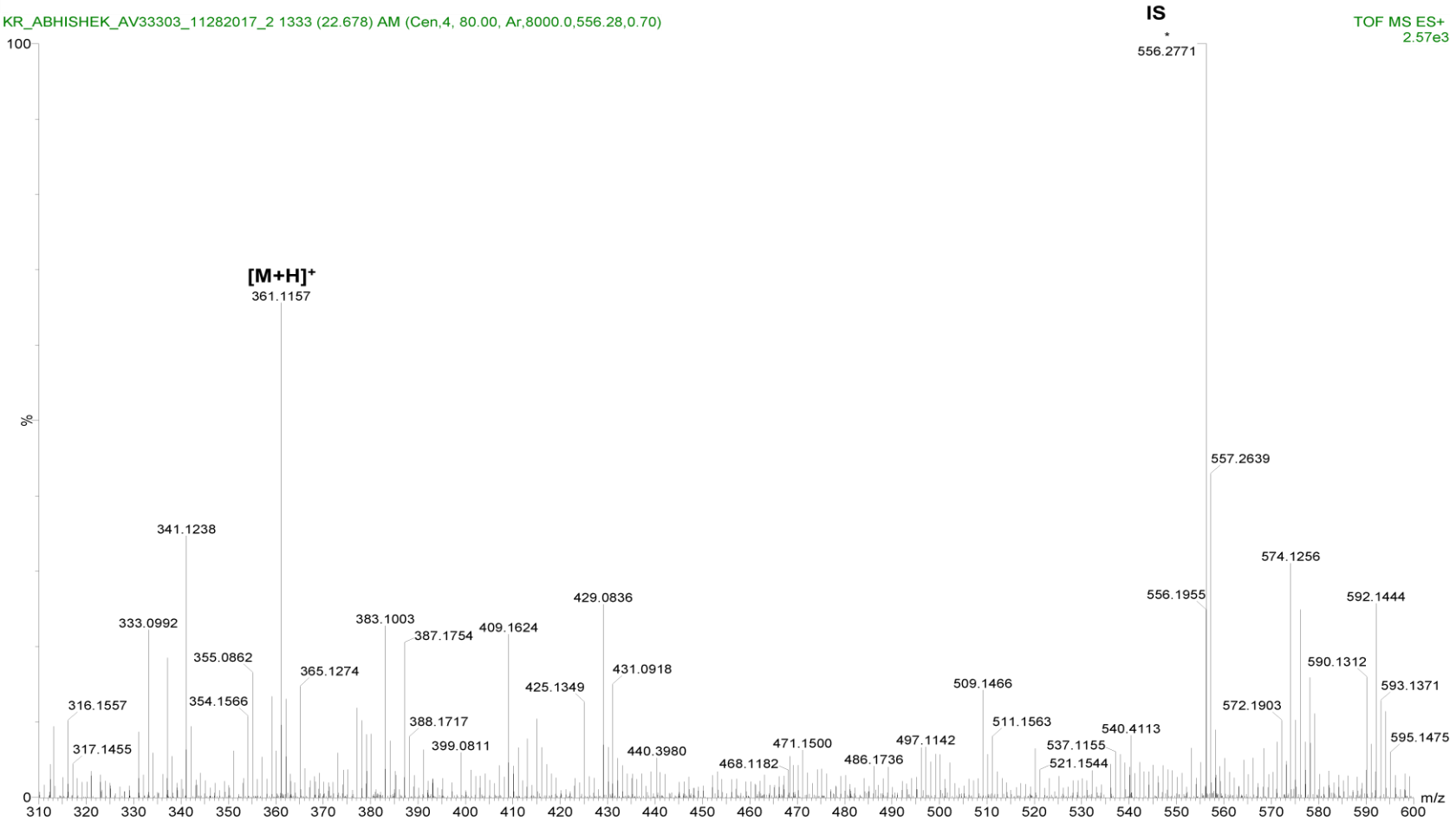
^{1}H NMR of 3-(3,5-difluorophenyl)-1,1-bis(furan-2-ylmethyl)urea (**8a**)



^{13}C NMR of 3-(3,5-difluorophenyl)-1,1-bis(furan-2-ylmethyl)urea (**8a**)

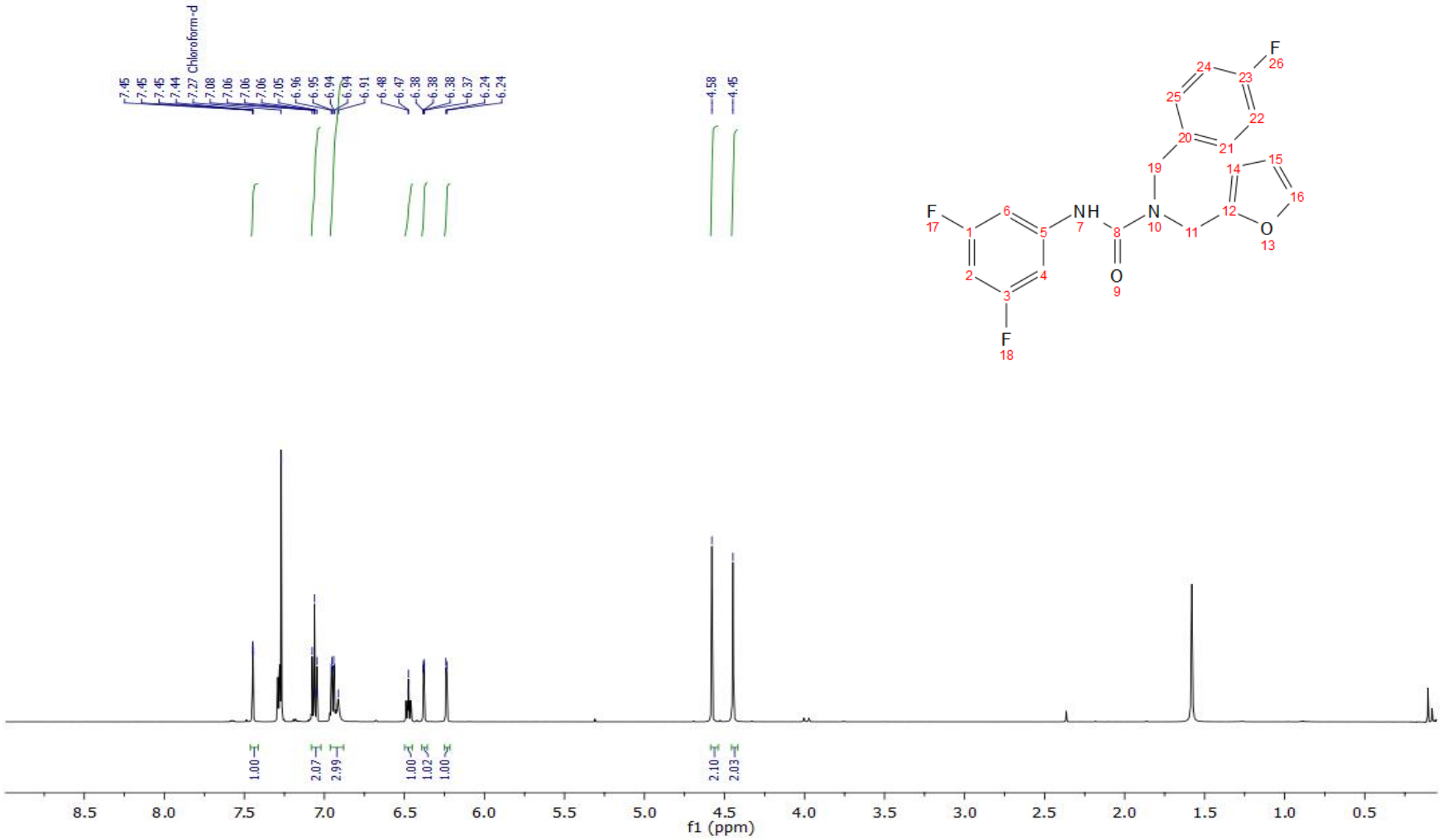


HRMS data of 3-(3,5-difluorophenyl)-1-(4-fluorobenzyl)-1-(furan-2-ylmethyl)urea (**8b**)

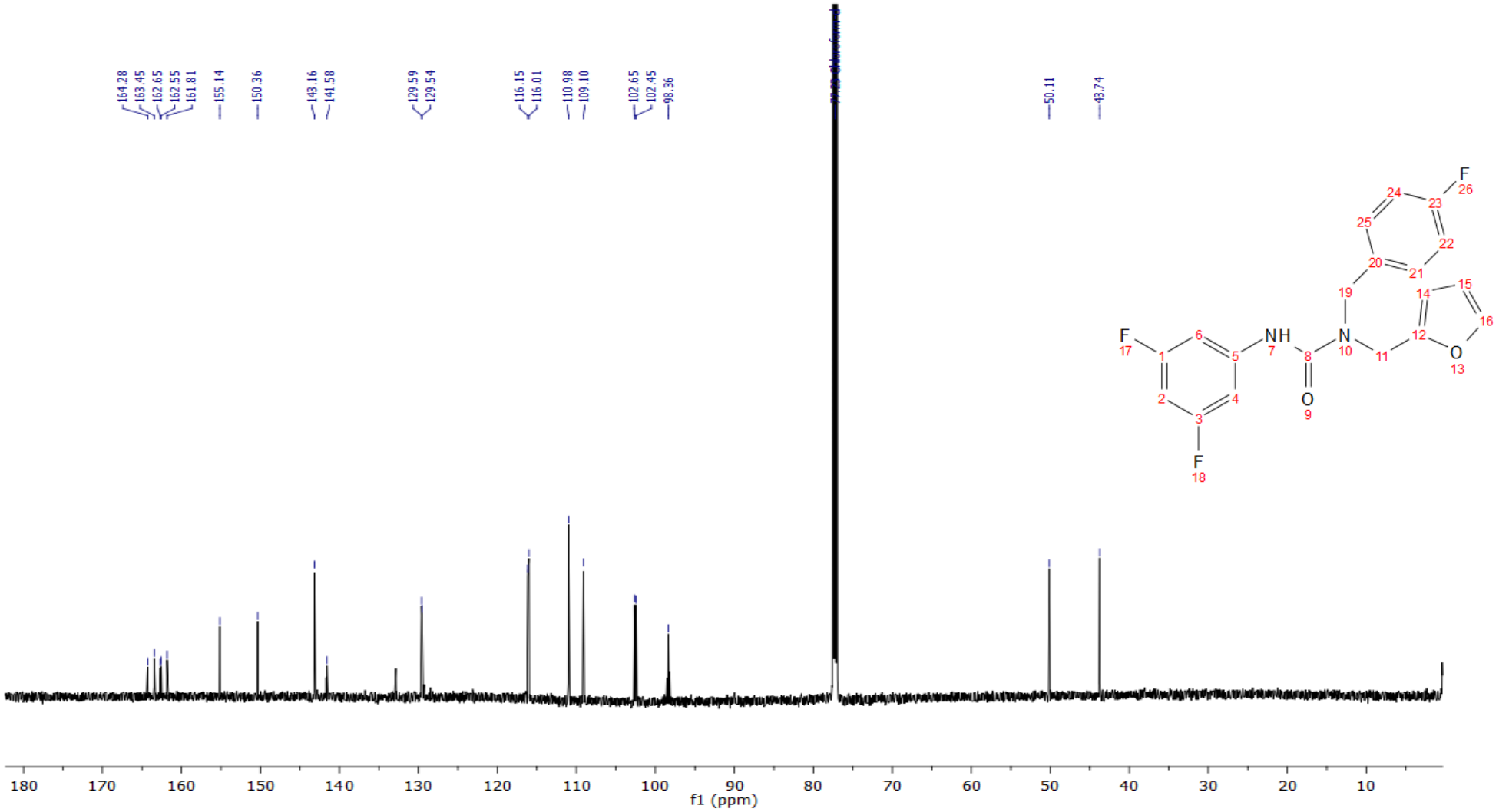


Mass accuracy = ((361.1164-361.1157)/361.1164)* 10^6 = 1.9 ppm

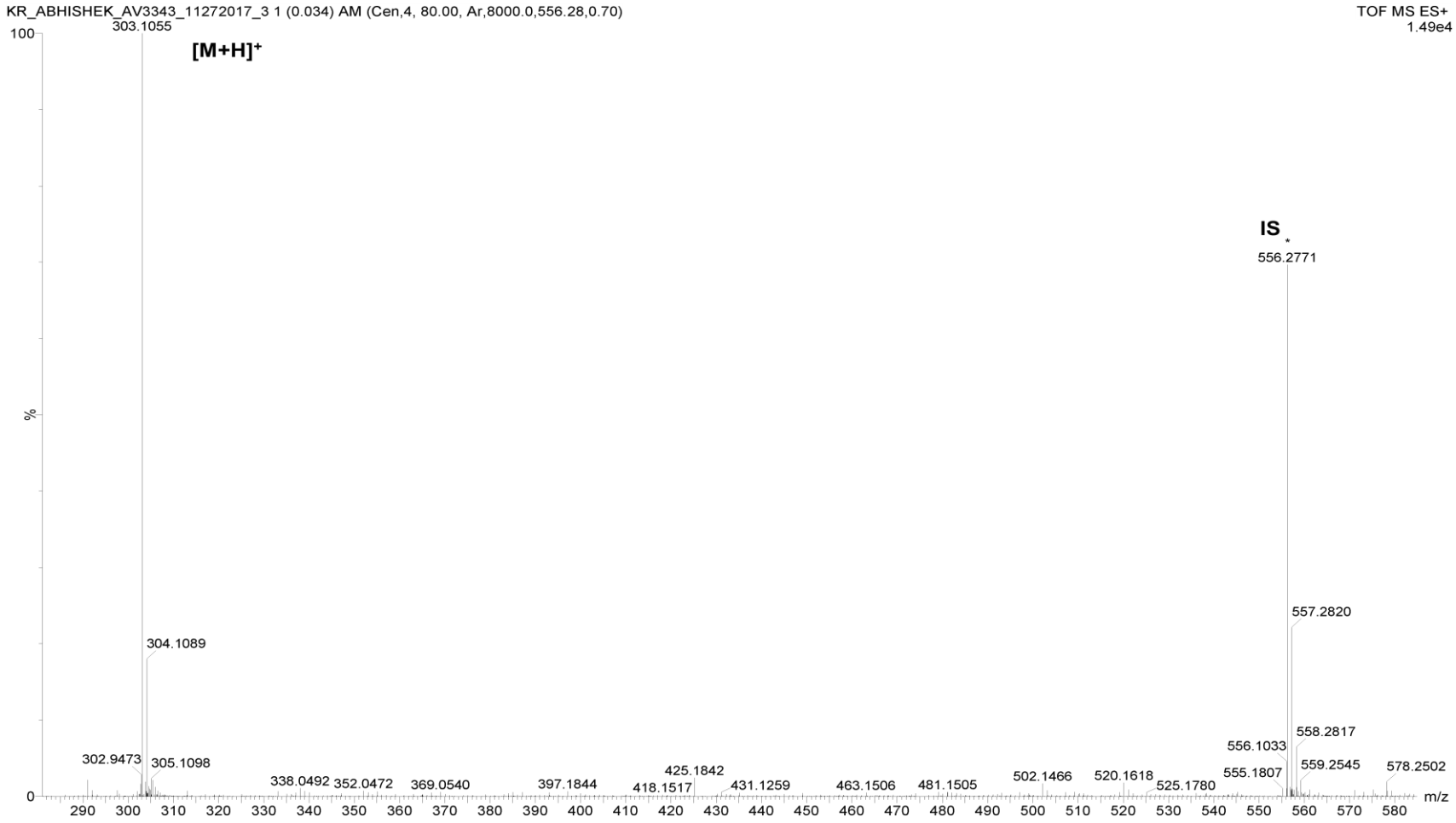
^{1}H NMR of 3-(3,5-difluorophenyl)-1-(4-fluorobenzyl)-1-(furan-2-ylmethyl)urea (**8b**)



^{13}C NMR of 3-(3,5-difluorophenyl)-1-(4-fluorobenzyl)-1-(furan-2-ylmethyl)urea (**8b**)

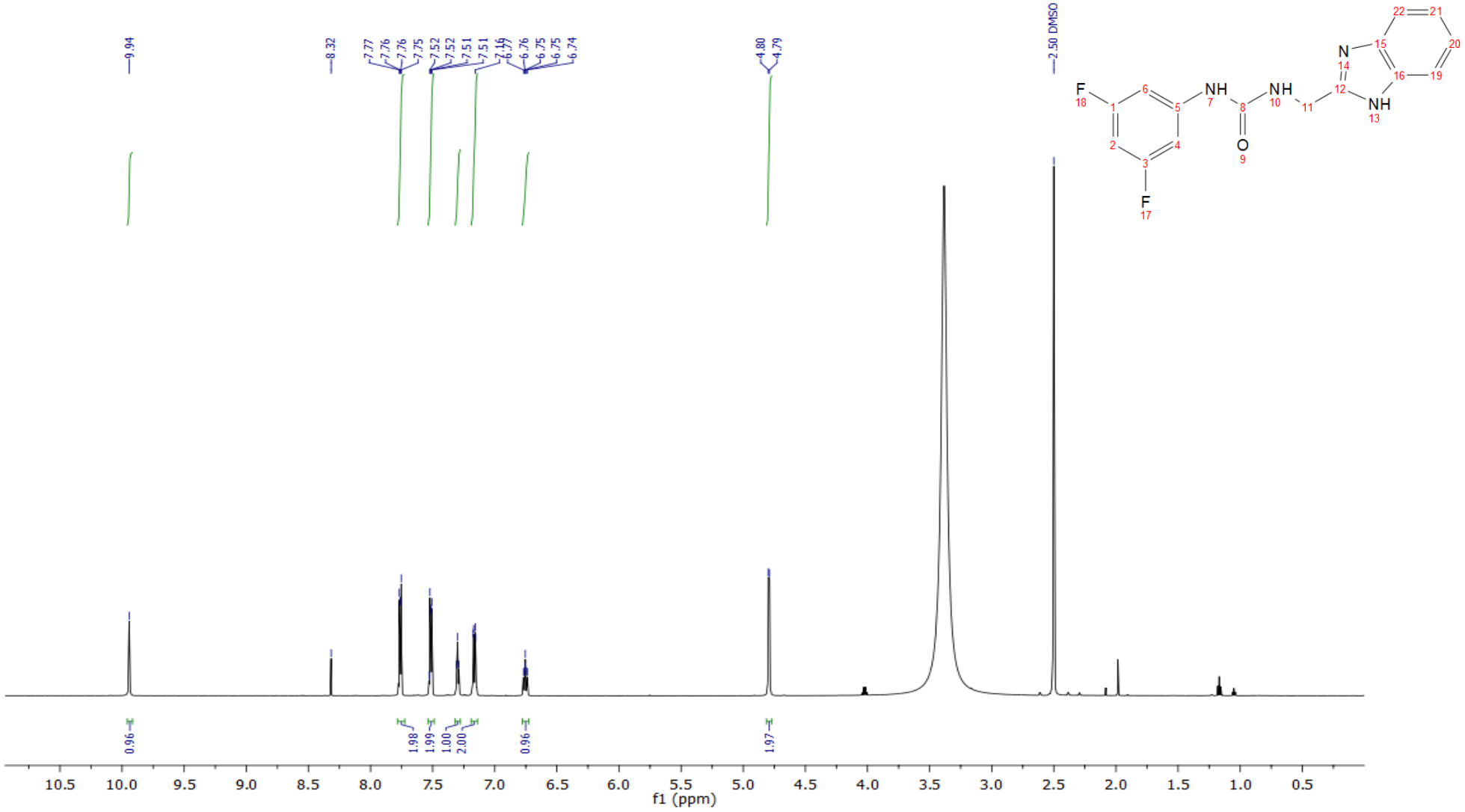


HRMS data of 1-((1H-benzimidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8c**)

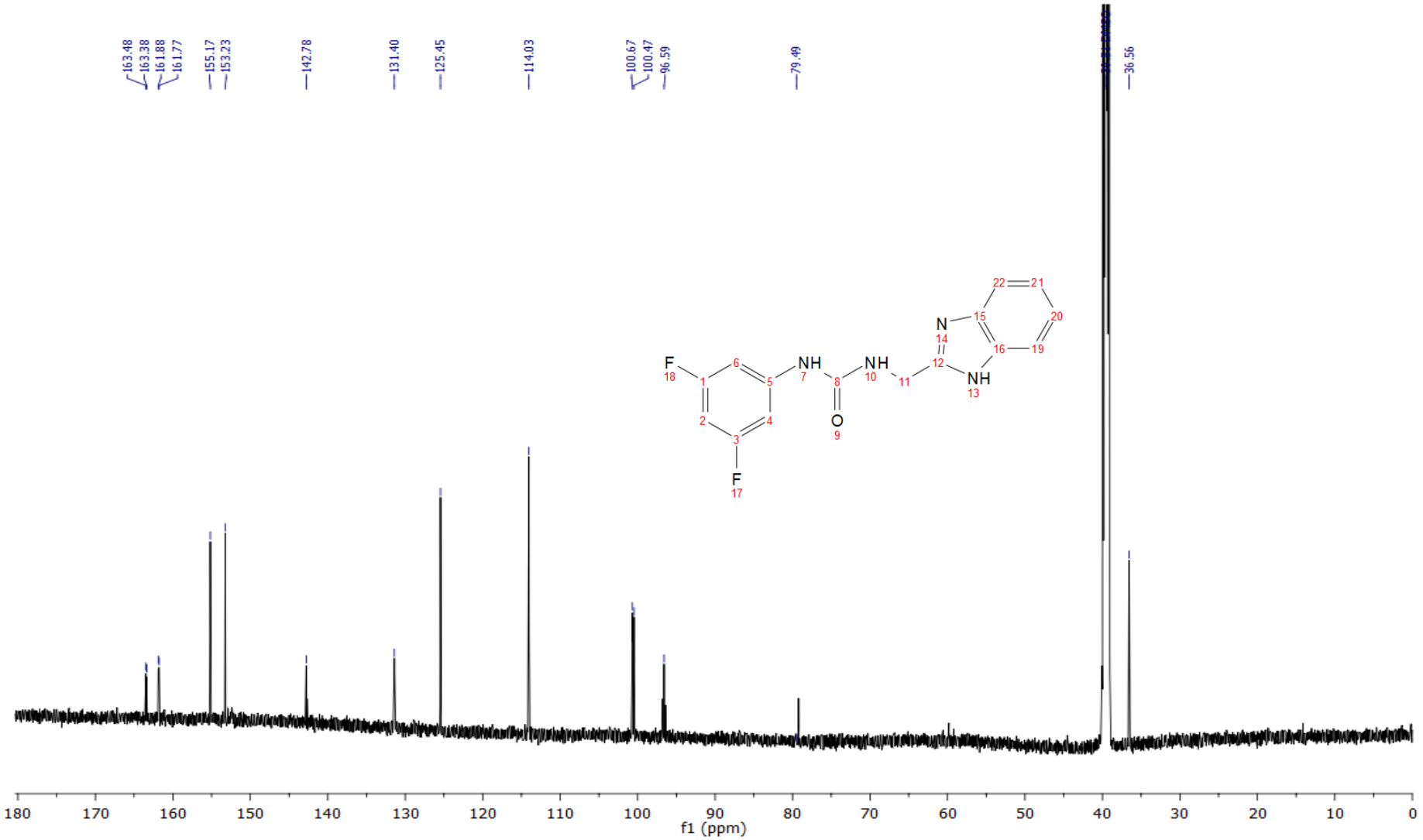


Mass accuracy = ((303.1057-303.1055)/303.1057)* 10^6 = 0.7 ppm

^{1}H NMR of 1-((1H-benzimidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8c**)



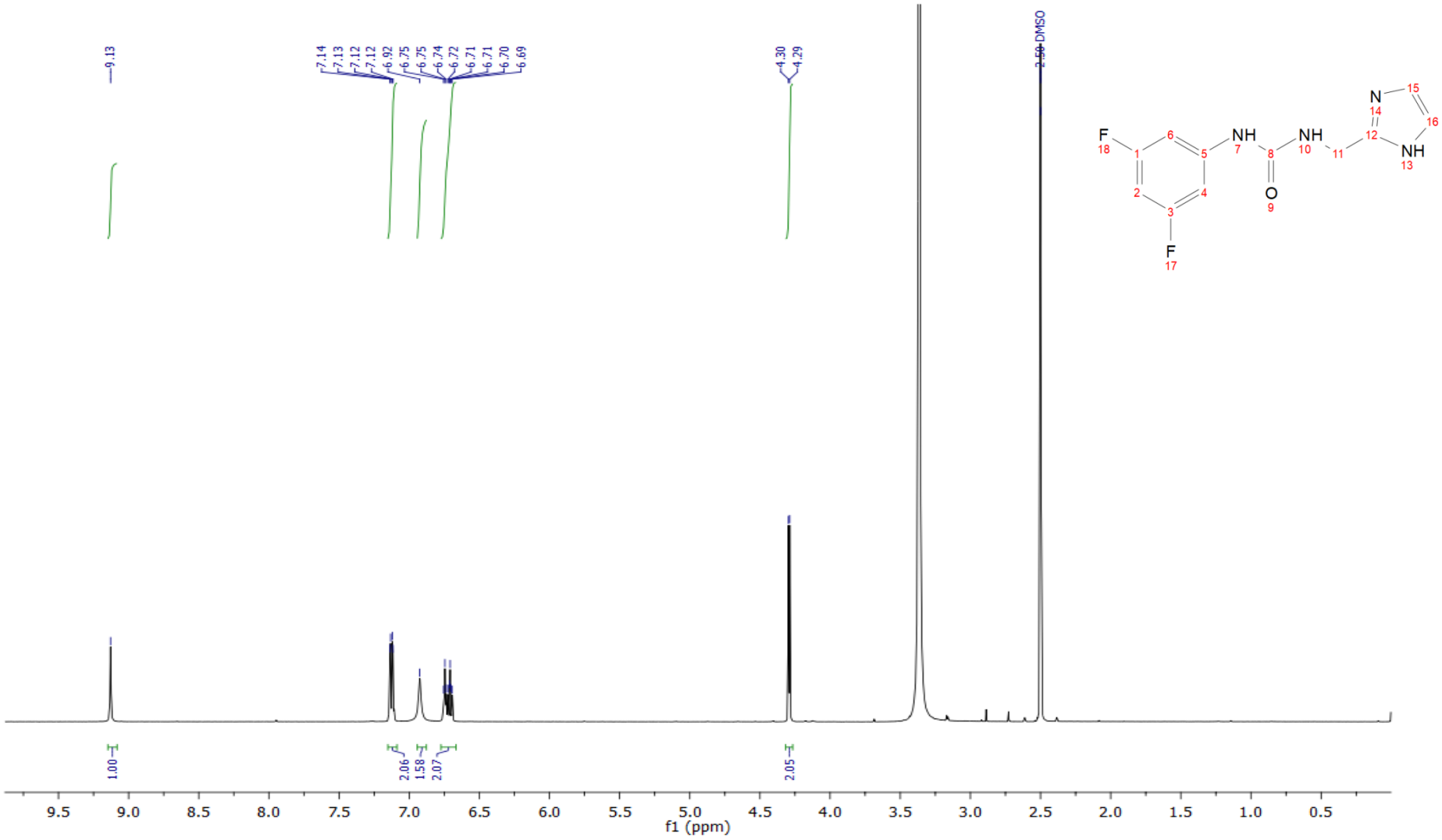
^{13}C NMR of 1-((1H-benzimidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8c**)



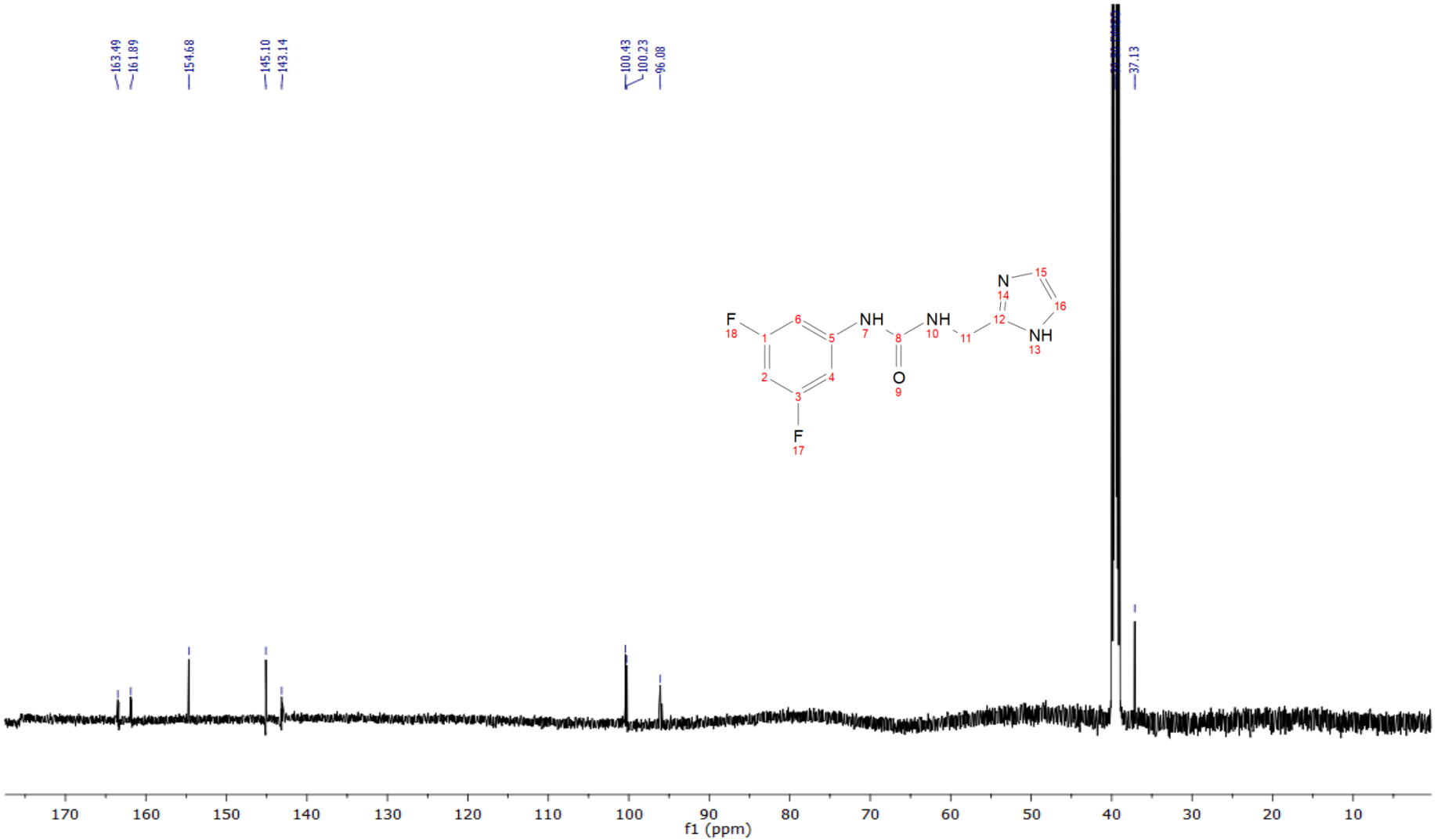
HRMS data of 1-((1H-imidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8d**)

Mass accuracy = ((253.0901-253.0898)/253.0901)* 10^6 = 1.2 ppm

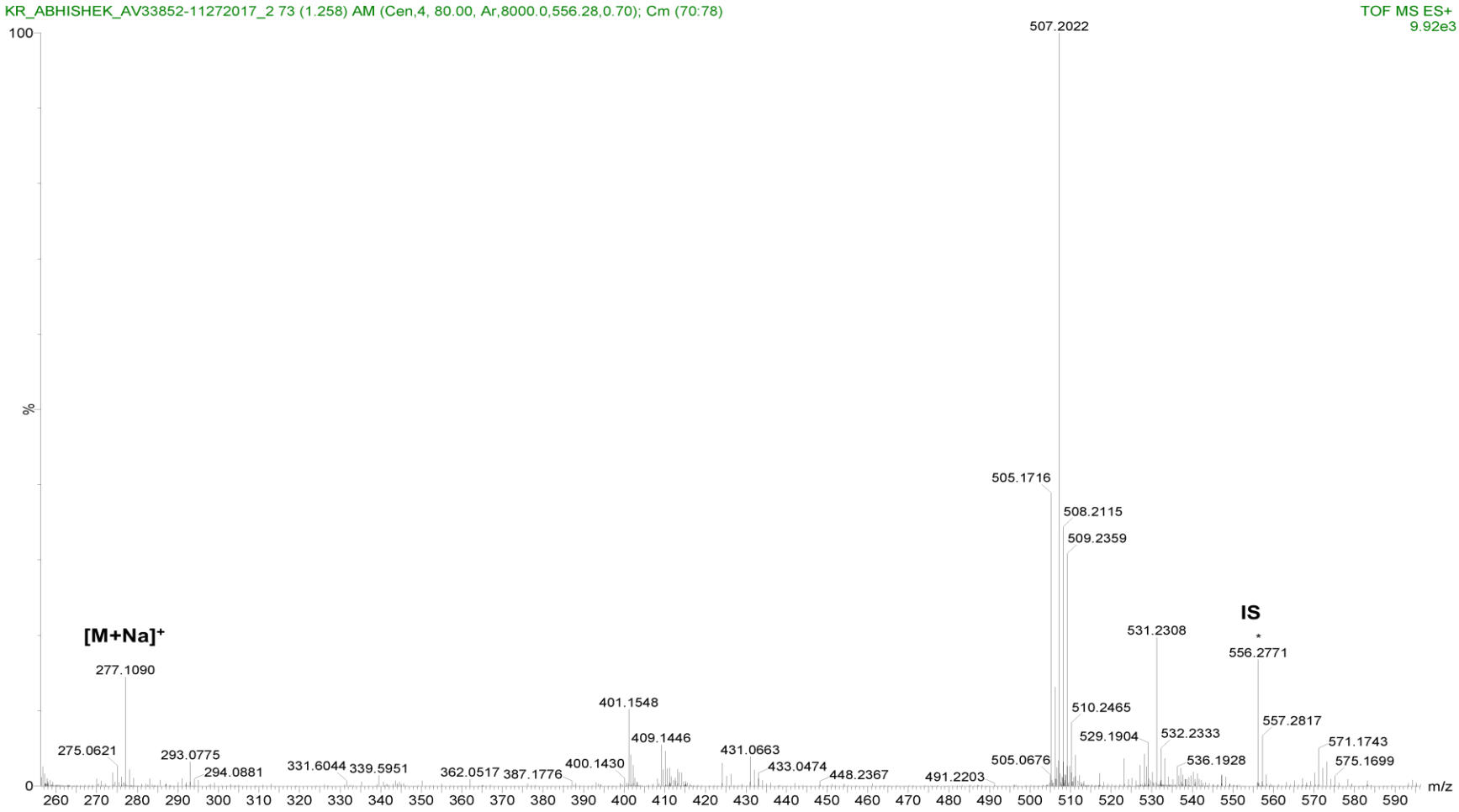
^{1}H NMR of 1-((1H-imidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8d**)



^{13}C NMR of 1-((1H-imidazol-2-yl)methyl)-3-(3,5-difluorophenyl)urea (**8d**)

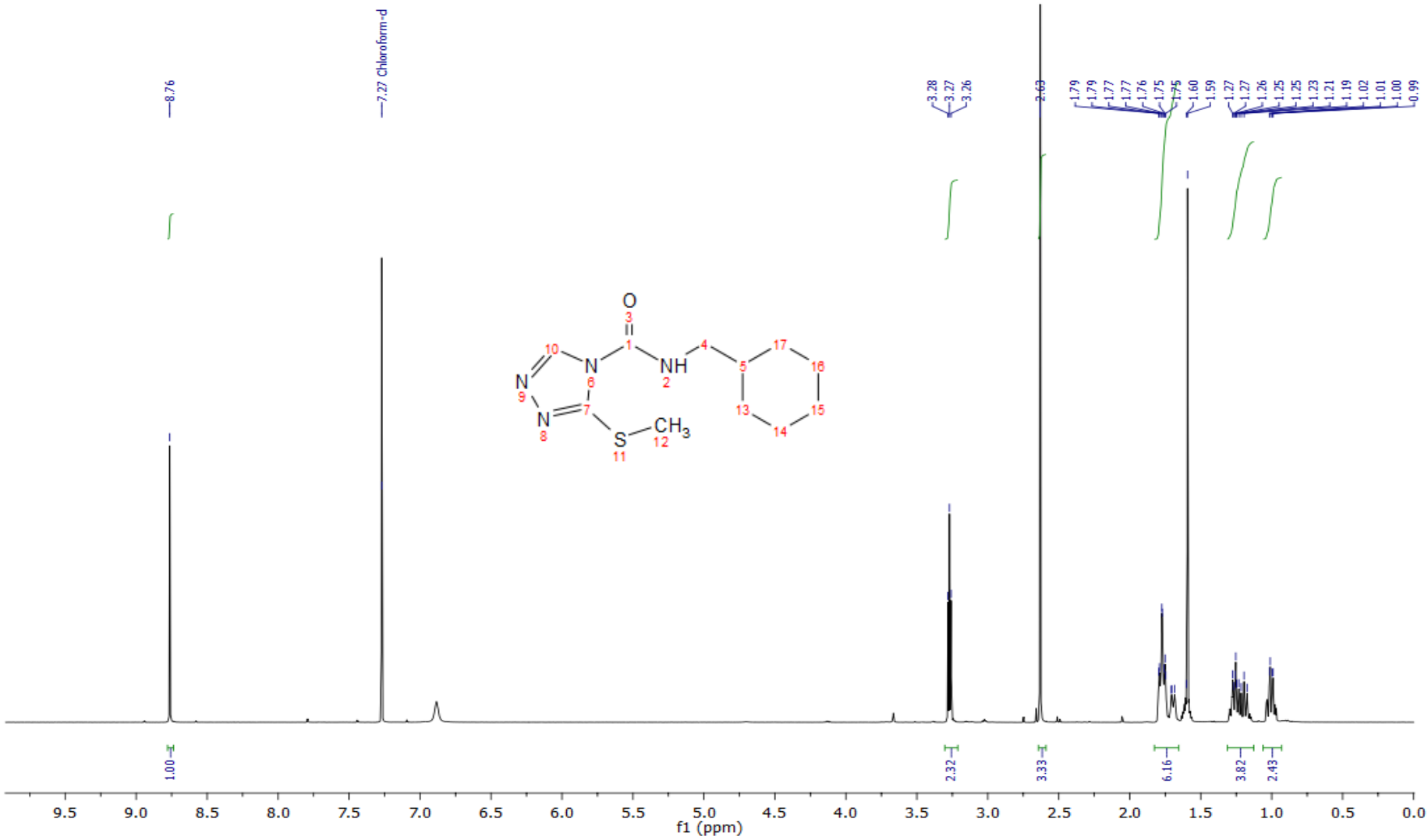


HRMS data of N-(cyclohexylmethyl)-3-(methylthio)-4H-1,2,4-triazole-4-carboxamide (**10b**)

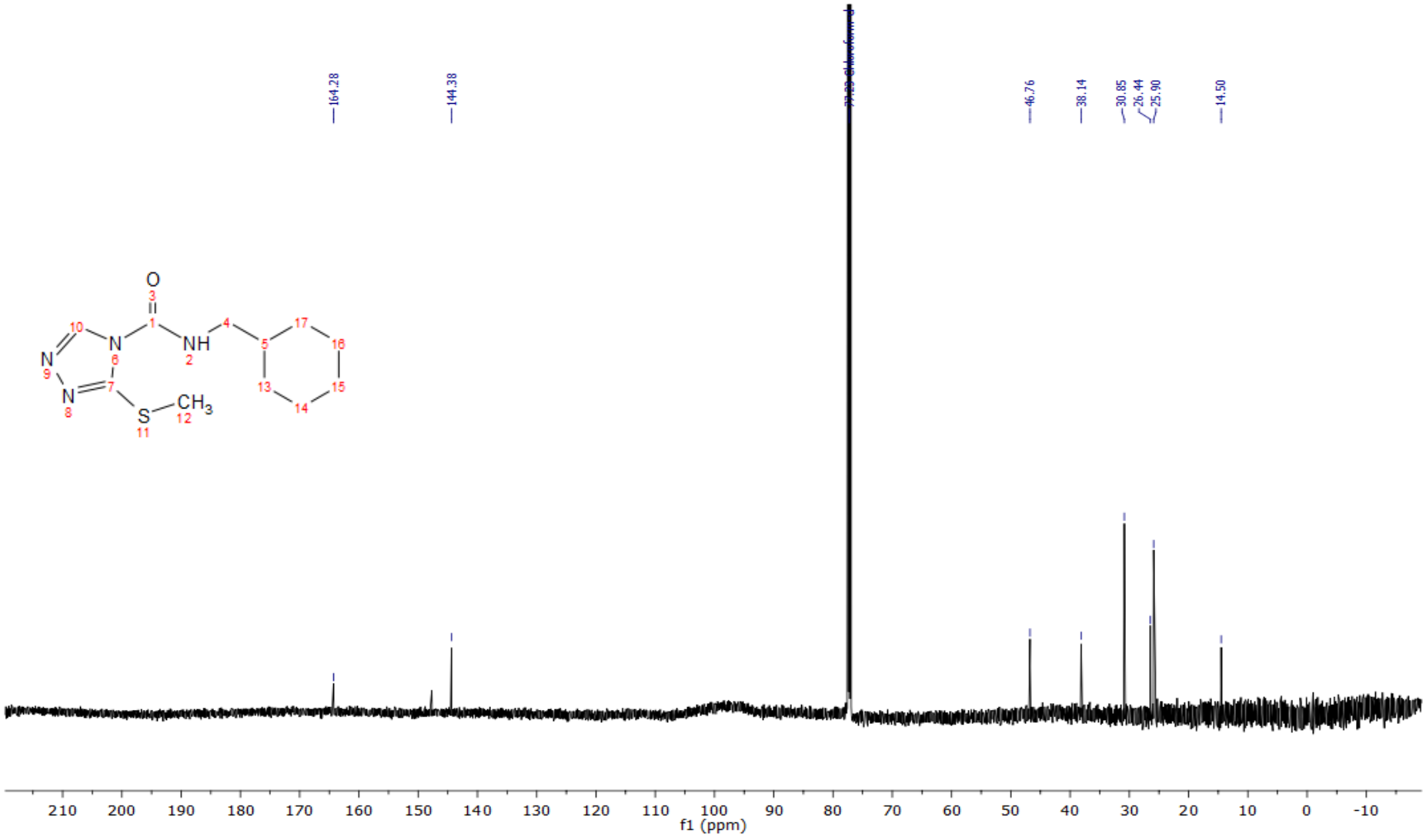


Mass accuracy = ((277.1099-277.1090)/277.1099)* 10^6 = 3.2 ppm

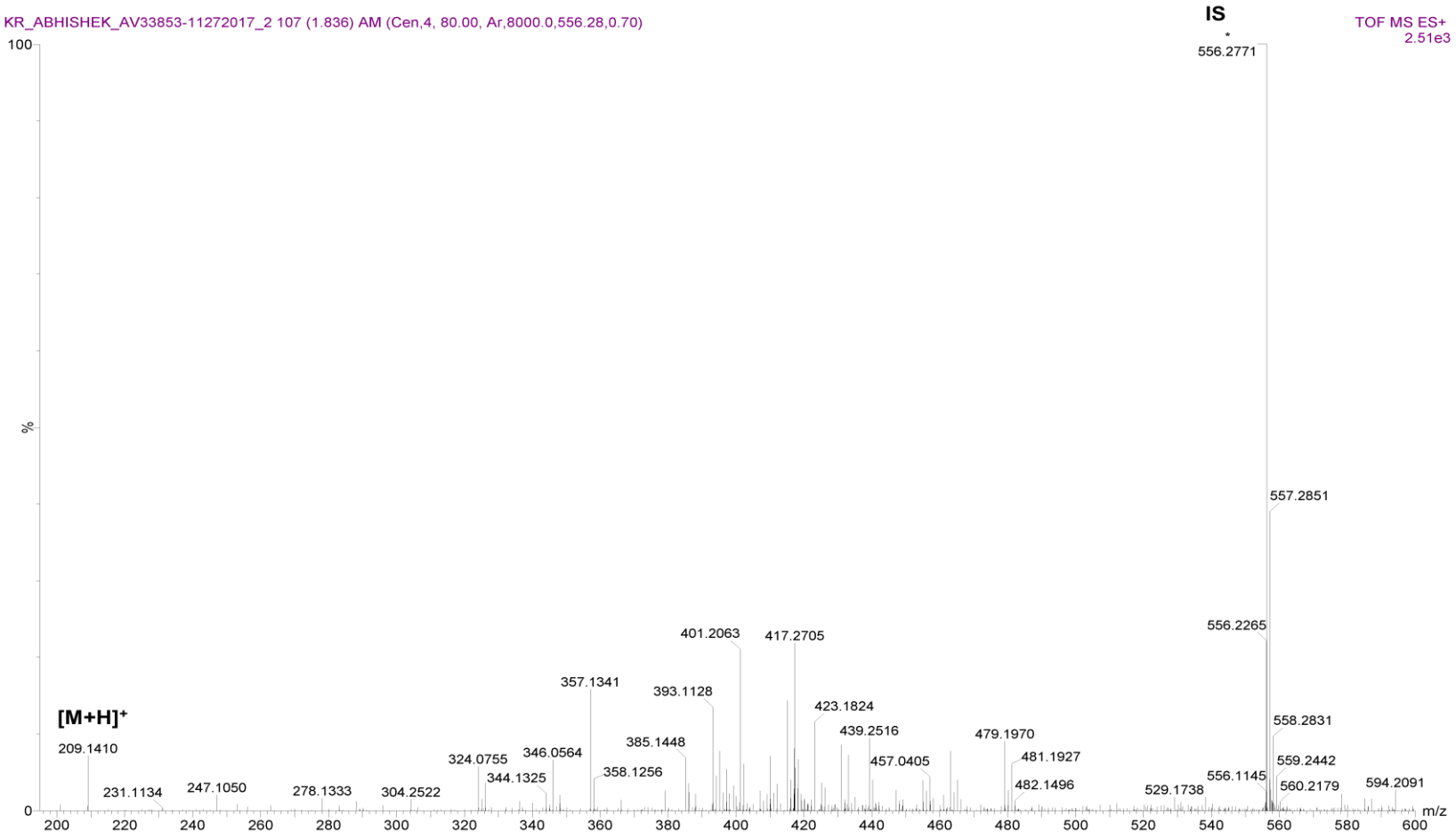
^{1}H NMR of N-(cyclohexylmethyl)-3-(methylthio)-4H-1,2,4-triazole-4-carboxamide (**10b**)



^{13}C NMR of N-(cyclohexylmethyl)-3-(methylthio)-4H-1,2,4-triazole-4-carboxamide (**10b**)

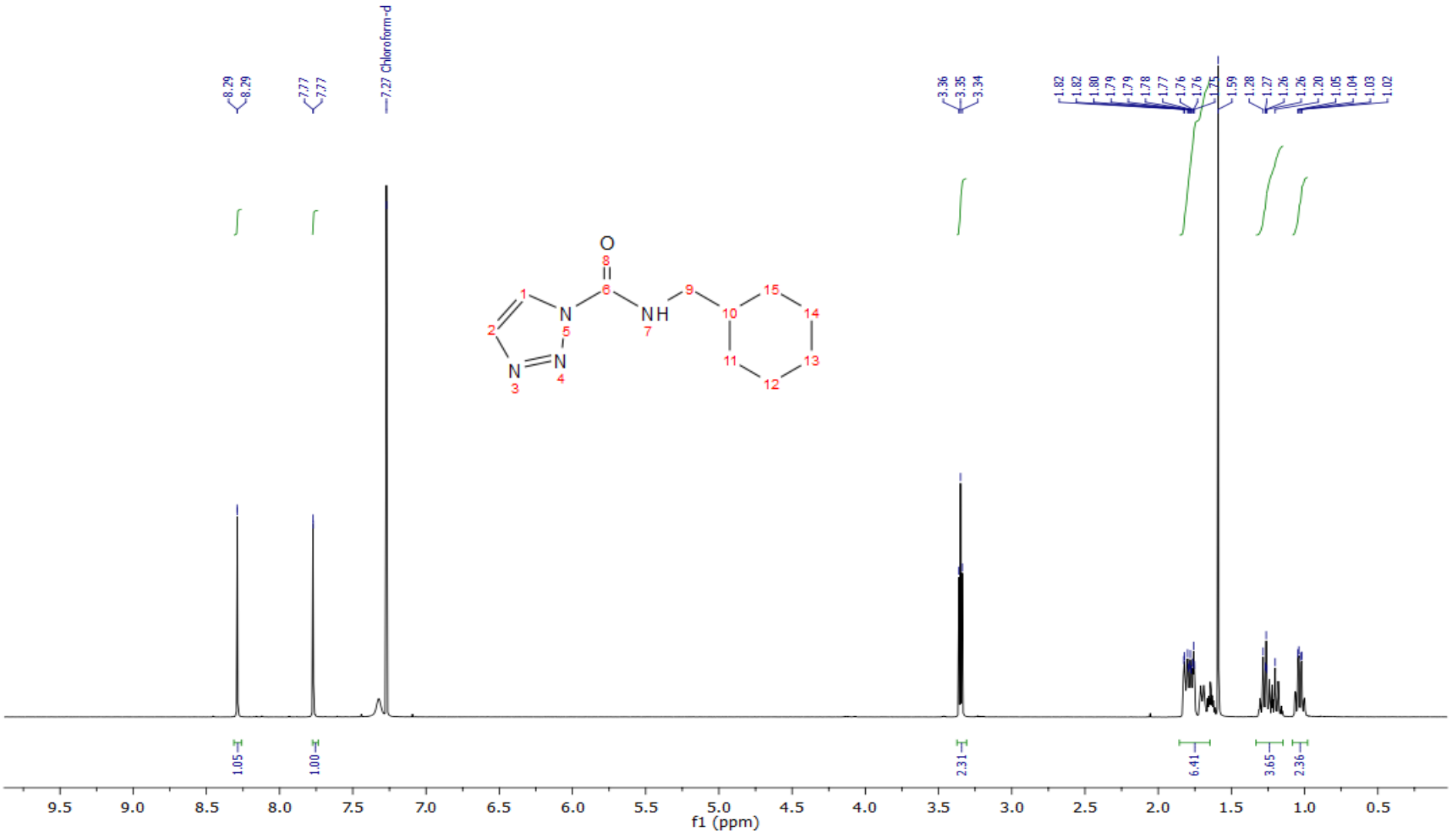


HRMS data of N-(cyclohexylmethyl)-1H-1,2,3-triazole-1-carboxamide (**10c**)



Mass accuracy = ((209.1402-209.1410)/209.1402)* 10^6 = 3.8 ppm

^{1}H NMR of N-(cyclohexylmethyl)-1H-1,2,3-triazole-1-carboxamide (**10c**)



^{13}C NMR of N-(cyclohexylmethyl)-1H-1,2,3-triazole-1-carboxamide (**10c**)