

Supplementary Material

Microwave-assisted synthesis and evaluation of antibacterial activity of novel 6-fluoroaryl substituted [1,2,4]triazolo[1,5-*a*]pyrimidines

Egor V. Verbitskiy,^{a,b,*} Svetlana A. Baskakova,^a Nikolay A. Rasputin,^a
Natal'ya A. Gerasimova,^c Polina G. Amineva,^c Natal'ya P. Evstigneeva,^c Natal'ya V.
Zil'berberg,^c Nikolay V. Kungurov,^c Marionella A. Kravchenko,^d Sergey N. Skorniyakov,^d
Gennady L. Rusinov,^{a,b} Oleg N. Chupakhin,^{a,b} and Valery N. Charushin^{a,b}

^a Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences
S. Kovalevskoy Str., 22, Ekaterinburg, 620990, Russia

^b Ural Federal University, Mira St. 19, Ekaterinburg, 620002, Russia

^c Ural Research Institute for Dermatology, Venereology and Immunopathology, Scherbakova St., 8,
Ekaterinburg, 620076, Russia

^d Ural Research Institute for Phthisiopulmonology, 22 Parts'ezda St., 50, Ekaterinburg, 620039,
Russia

E-mail: Verbitsky@ios.uran.ru

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Antimycobacterial assay

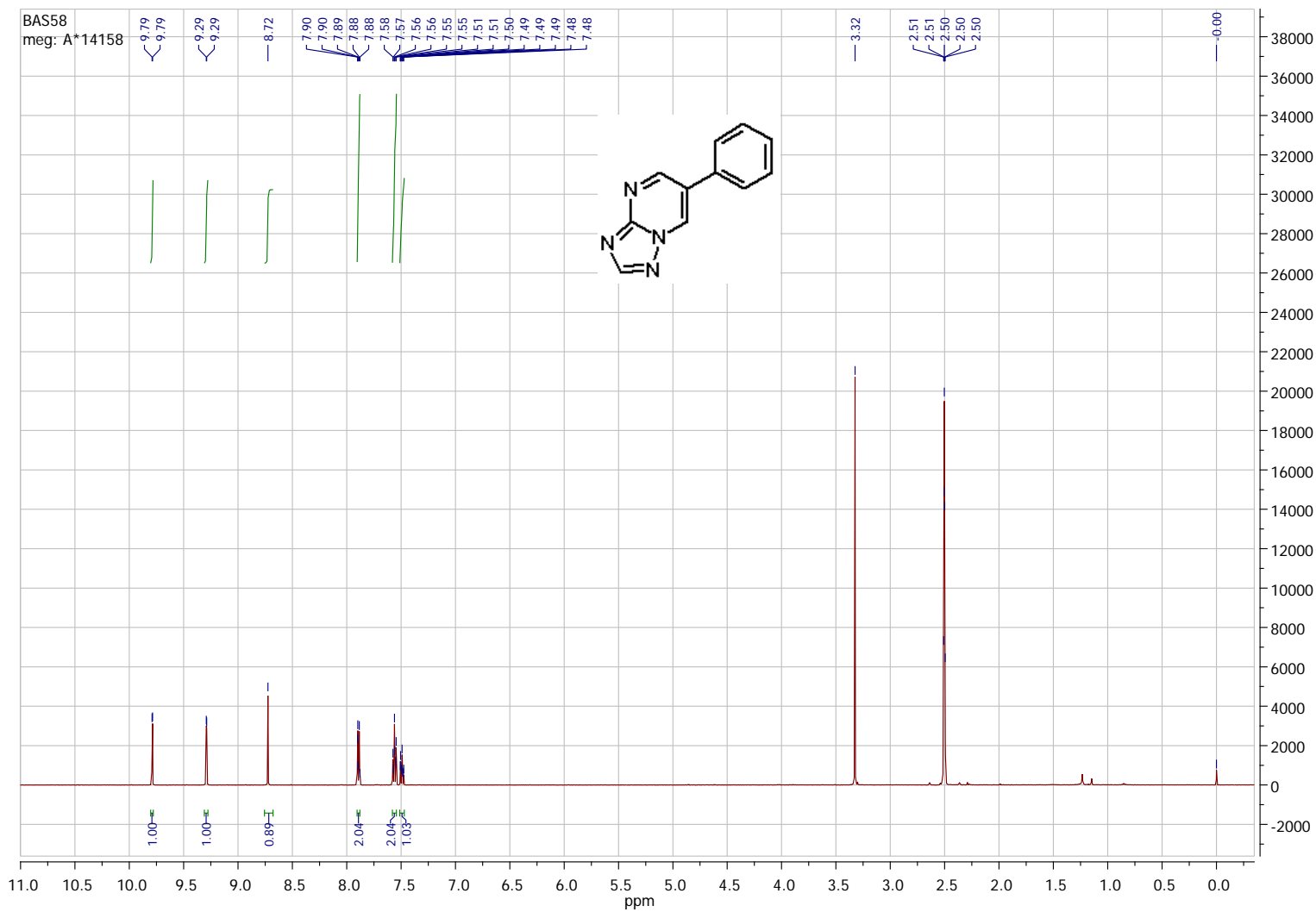
To evaluate the inhibitory efficiency of compounds on *Mycobacterium tuberculosis* (MTB), *M. tuberculosis H₃₇Rv*, which is susceptible to all classical antituberculosis drugs, was used. The minimal inhibitory concentration (MIC) for *M. tuberculosis H₃₇Rv* for each compound was determined by the micro broth dilution method. All molecules tested were dissolved in dimethylsulfoxide and their 1/2 dilutions were prepared in 5 mL tubes, using the Löwenstein-Jensen medium. A few colonies from freshly grown *M. tuberculosis H₃₇Rv* were suspended in Löwenstein-Jensen medium to obtain 1.0 McFarland turbidity, and then diluted ten times using the same

medium. After that the tubes were incubated at 37°C, using medium with different concentrations of the tested compound, and a positive control tube containing only clear growth medium. After 24 hours tubes were put in a vertical position, and the free edge of the buried 0.3 mL of the substance in the following concentrations of the test compound: 12.5, 6.2, 3.1, 1.5, 0.7, 0.37, 0.15 µg/mL. The tubes were then placed in a thermostat at 37 °C and incubated for 10 days. Growth estimate for the MTB were determined by the standard methods, where the appearance of zones of growth retardation MTB (over 10 mm) indicated the presence of tuberculostatic properties in concentrations of the compound under study. Penetration size stunting MTB (in mm) is proportional to the degree of tuberculostatic activity. Growth delay of 100 mm or more is considered as a complete growth inhibition MTB. The multi-drug-resistant (MDR) tuberculosis strains have been isolated from tuberculosis patients in Ural Research Institute for Phthisiopulmonology (Russia). The minimal inhibitory concentrations against *Mycobacterium avium*, *Mycobacterium terrae*, and MDR tuberculosis strains were evaluated similarly.

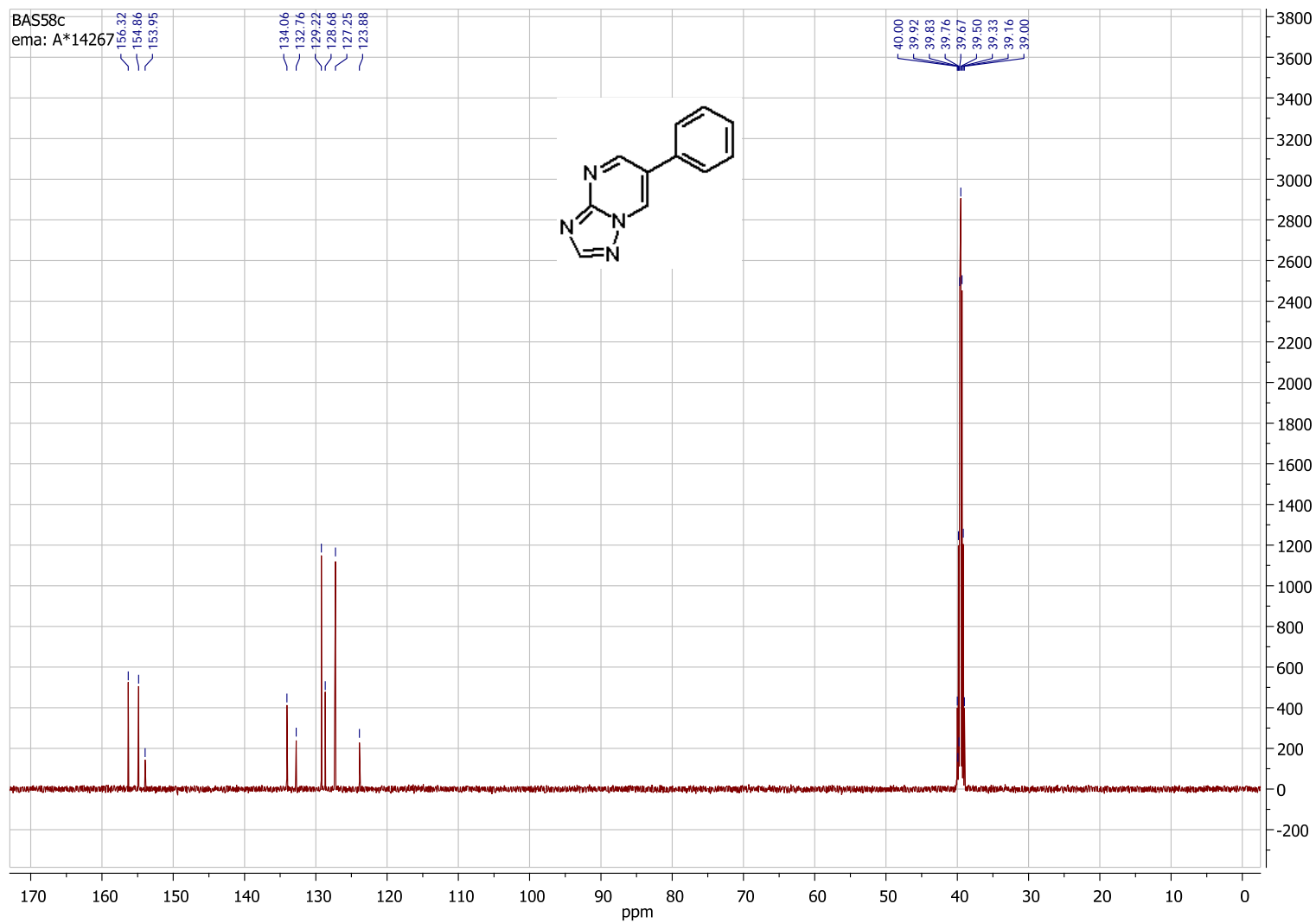
Anti-gonorrhea activity assay

The two fold serial dilution technique recommended by Clinical and Laboratory Standards Institute (CLSI)¹ was used to evaluate the inhibitory efficiency of molecules on *Neisseria gonorrhoeae*. The medium for testing *Neisseria gonorrhoeae* consists of GC agar to which a 1% defined growth supplement. Adjust the density of the suspension to contain 10⁸ CFU/mL by comparison with a 0.5 McFarland turbidity standard. For suspension using colonies from an overnight (20- to 24-hour) chocolate agar plate incubated in 5% CO₂ 36±1°. Dilute this suspension 1:10 in Muller-Hinton to give 10⁷ CFU/mL. The test compounds concentrations: 1000, 500, 250, 125, 62.5, 31.2, 15.0, 7.5, 3.8, 1.9, 0.99 µg/mL (solvent – DMSO, diluent – H₂O and GC agar base).

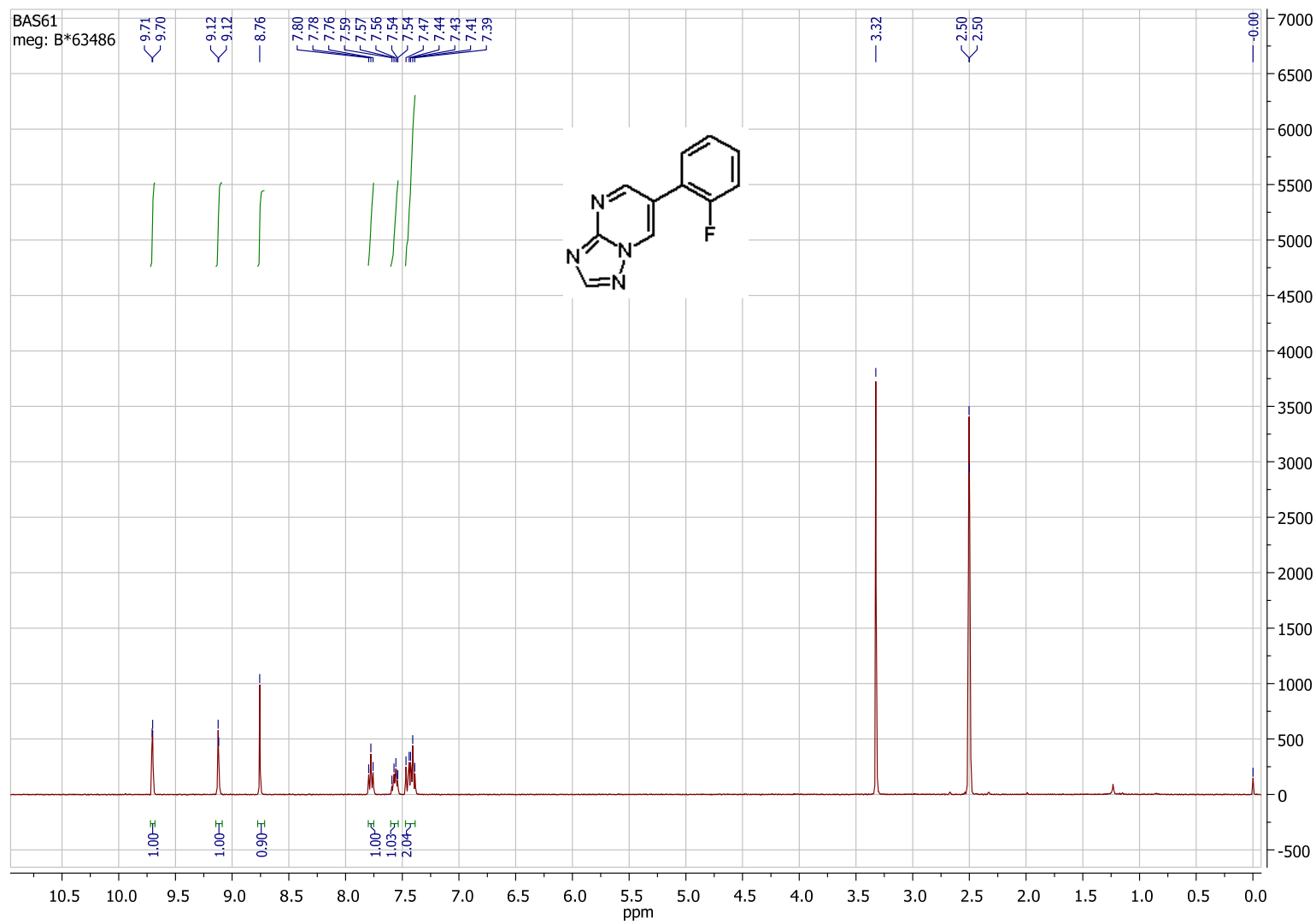
¹ Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement*; Clinical and Laboratory Standards Institute: Wayne, PA, **2014**. (<http://www.cdc.gov/std/gonorrhea/arg/b88-feb-2005.pdf>).



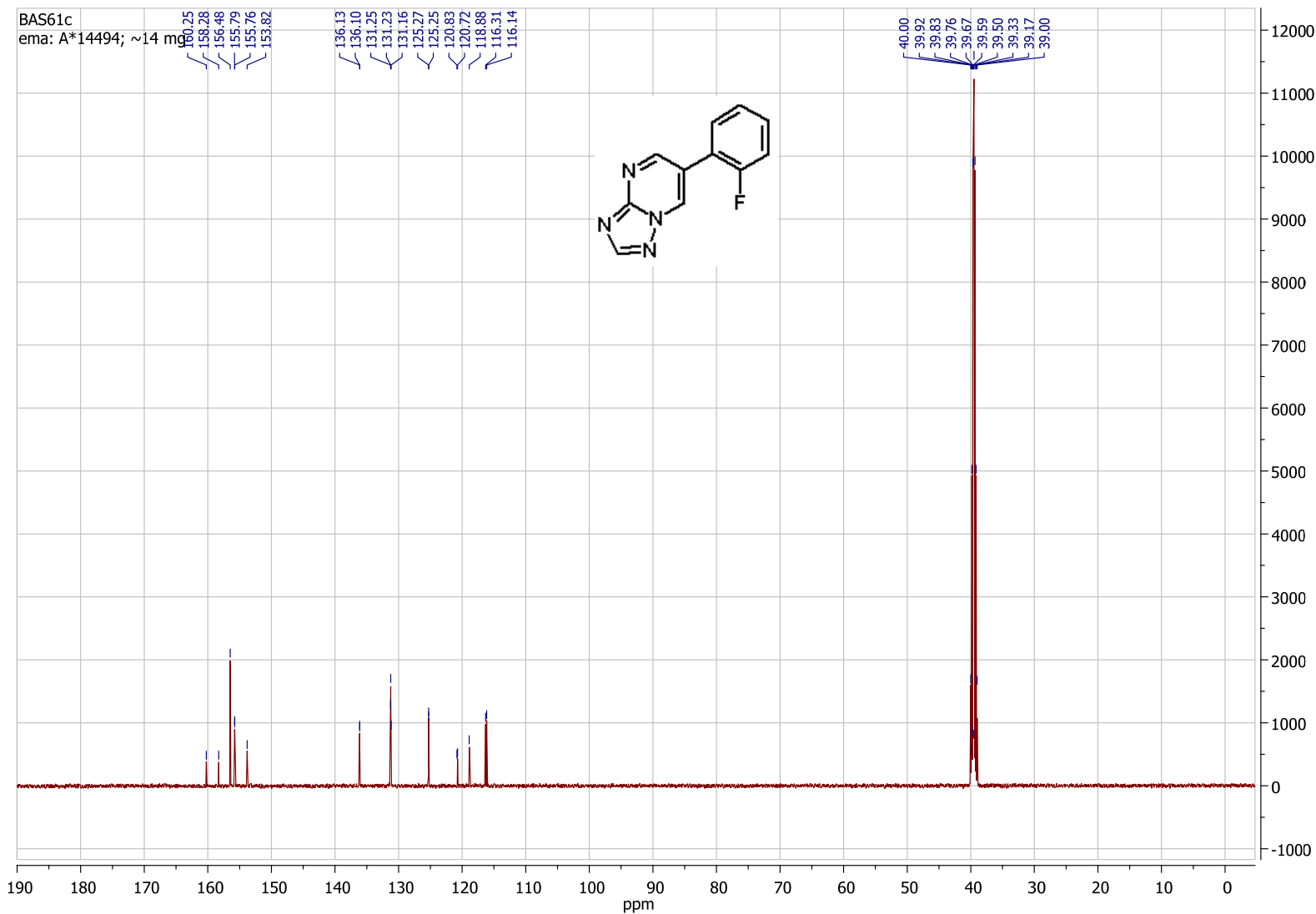
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5a**.



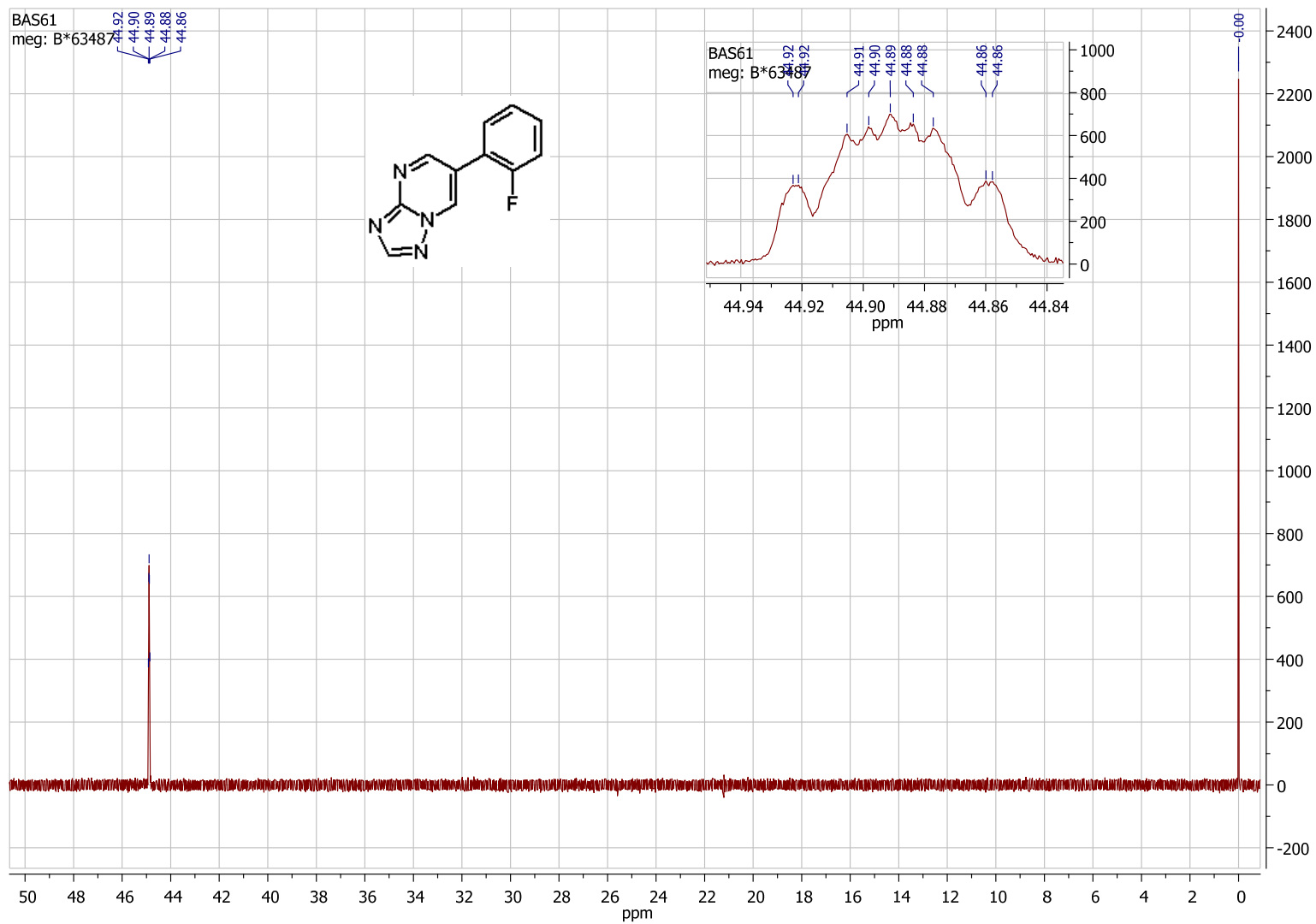
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) spectrum of **5a**.



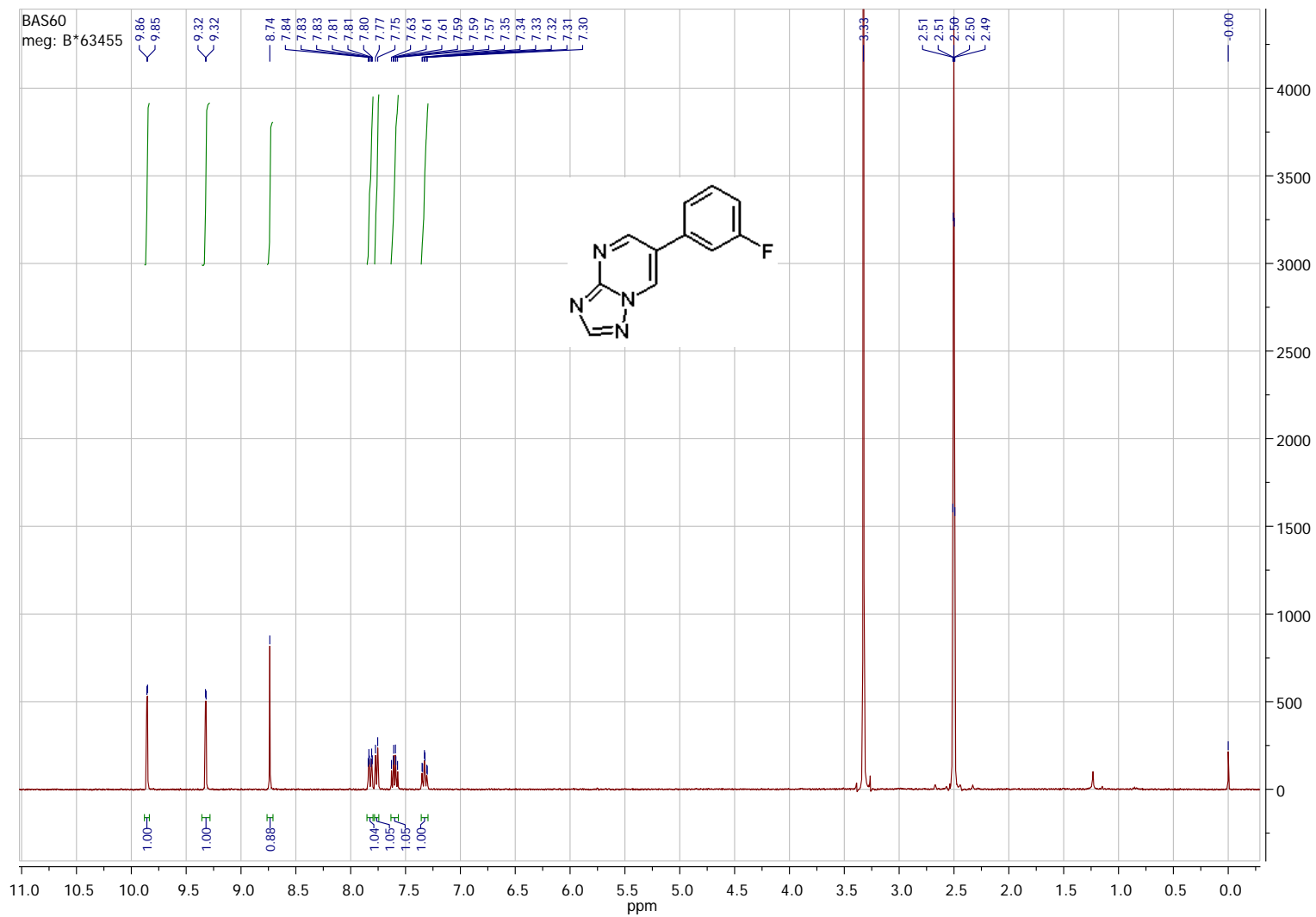
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5b**.



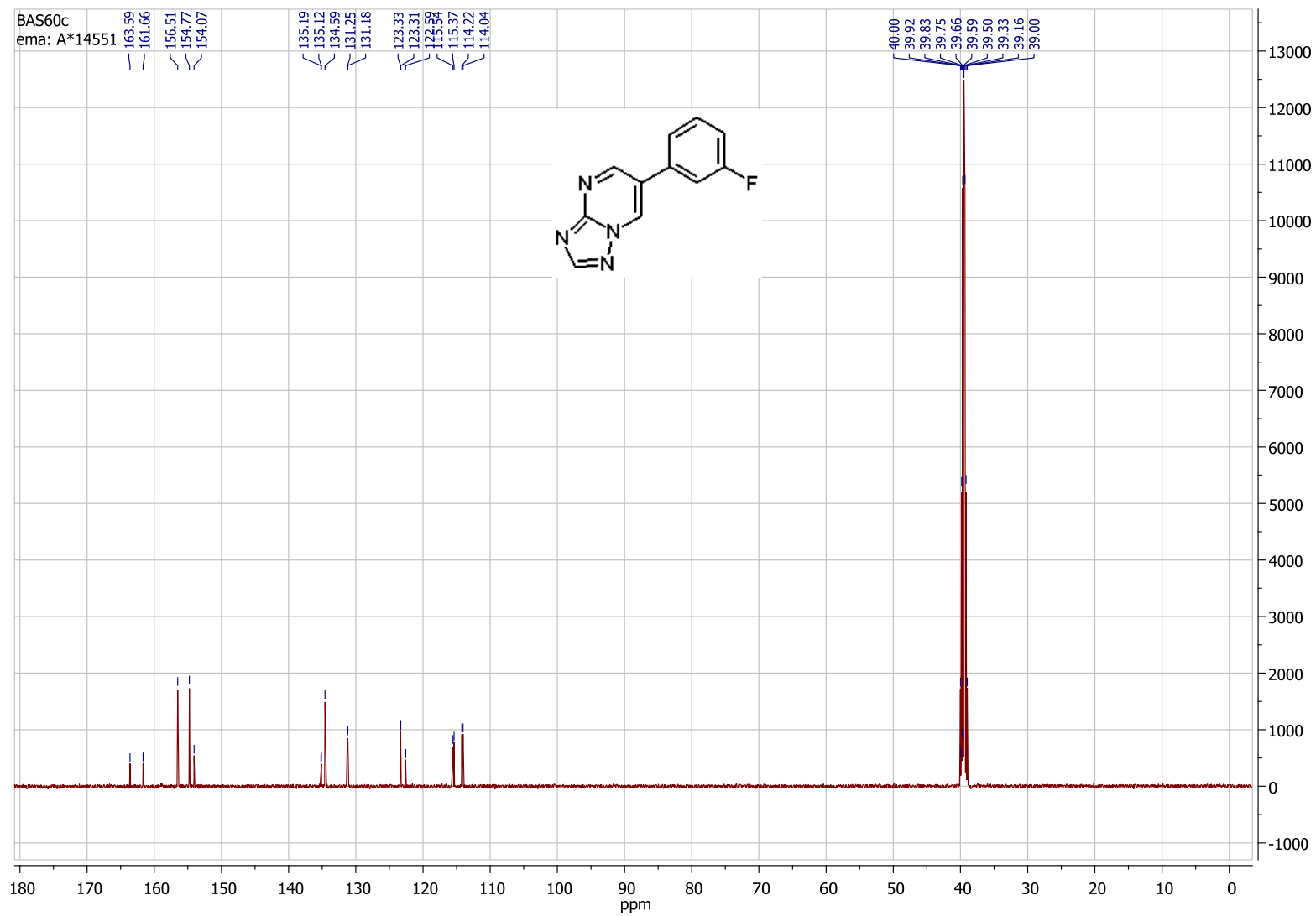
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) spectrum of **5b**.



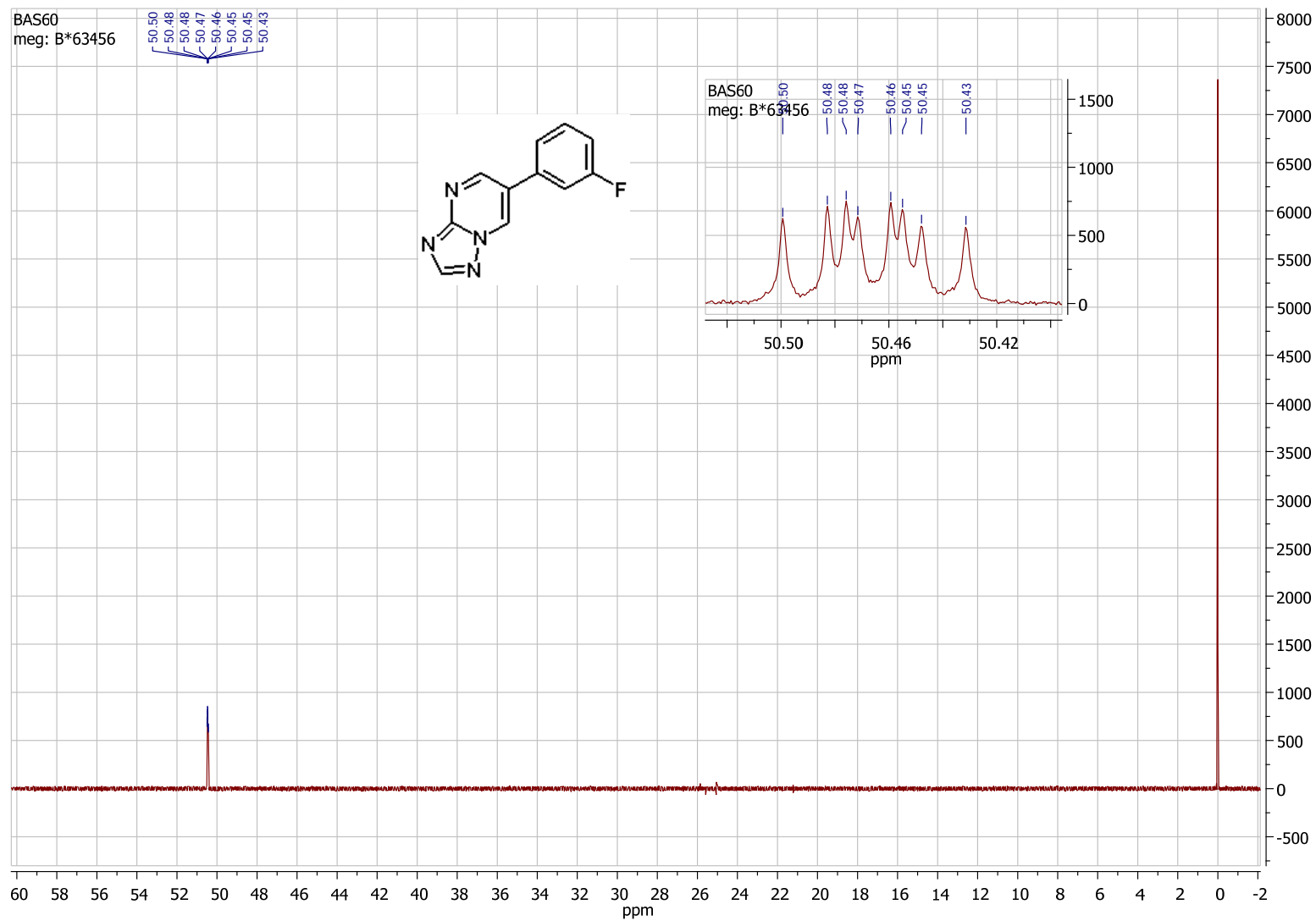
^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5b**.



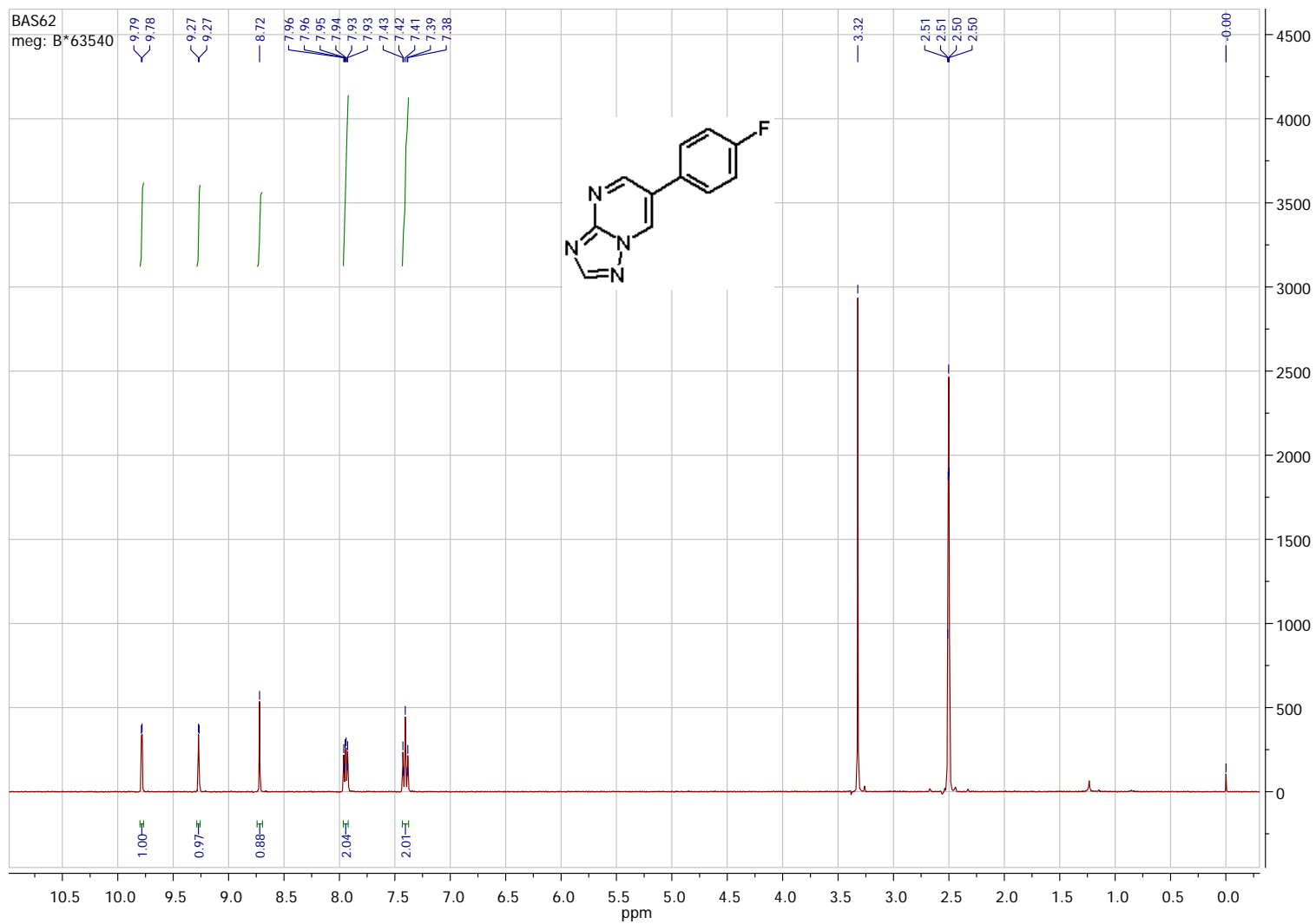
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5c**.



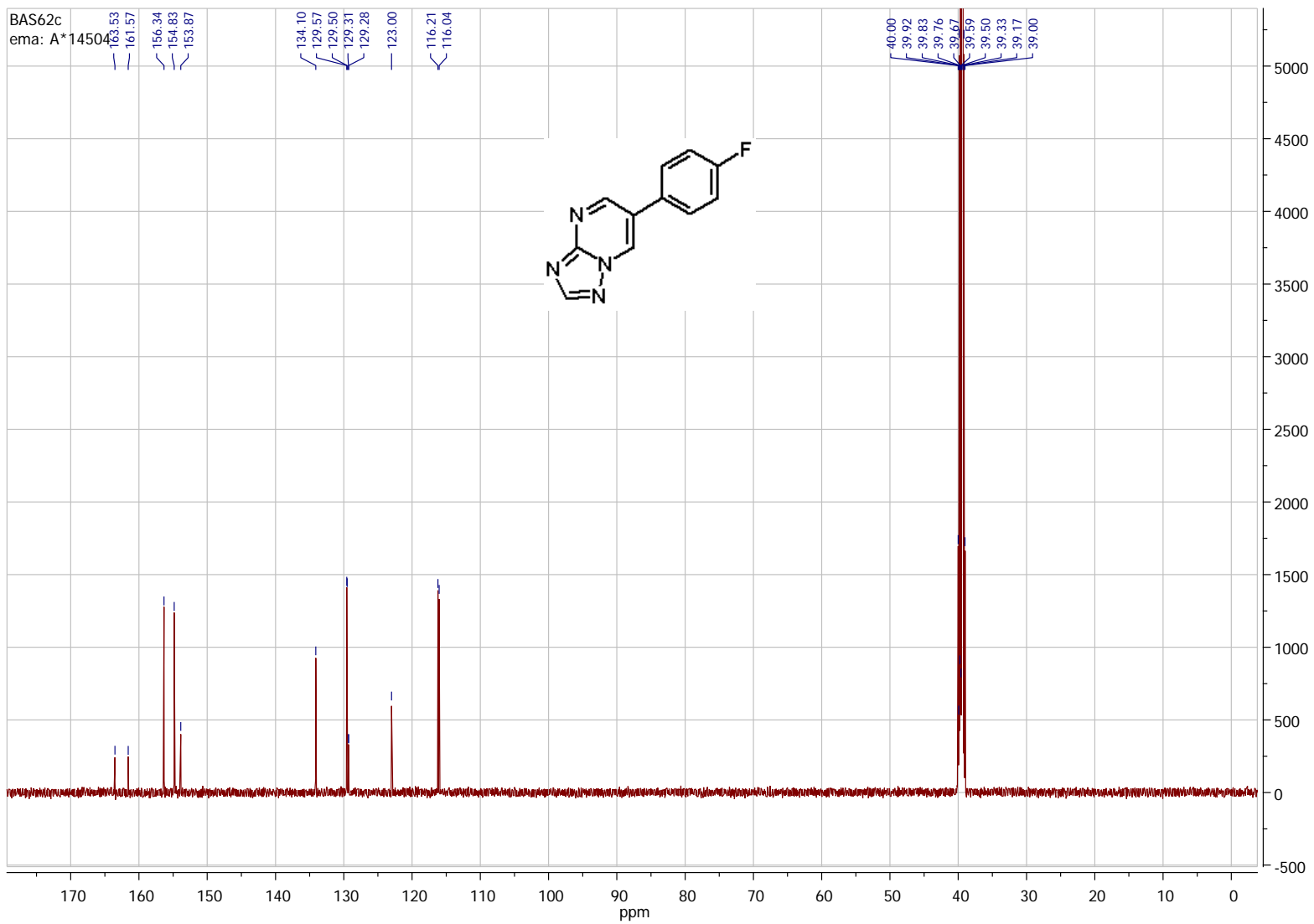
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5c**.



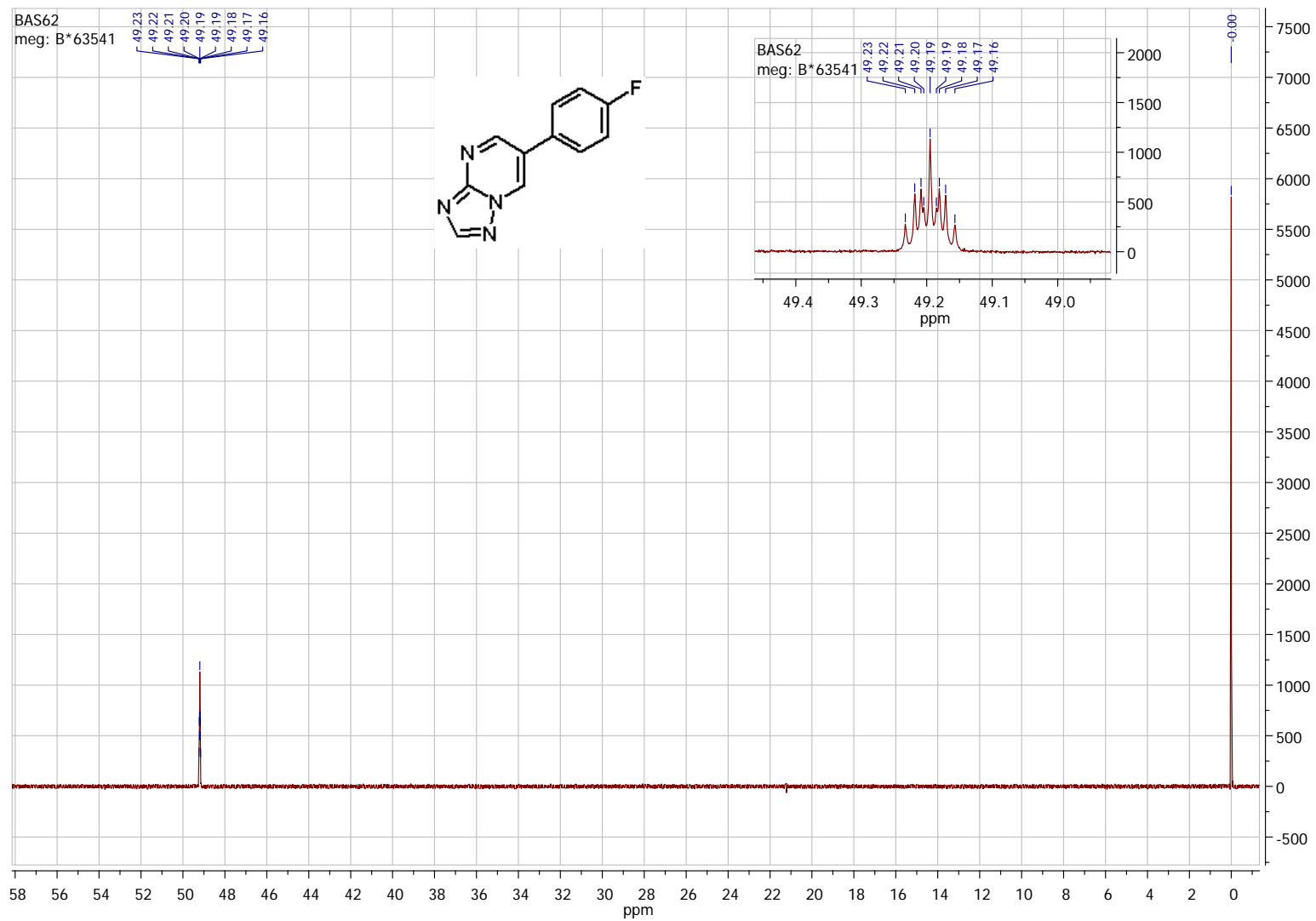
^{19}F NMR (470.5 MHz, $\text{DMSO}-d_6$) spectrum of **5c**.



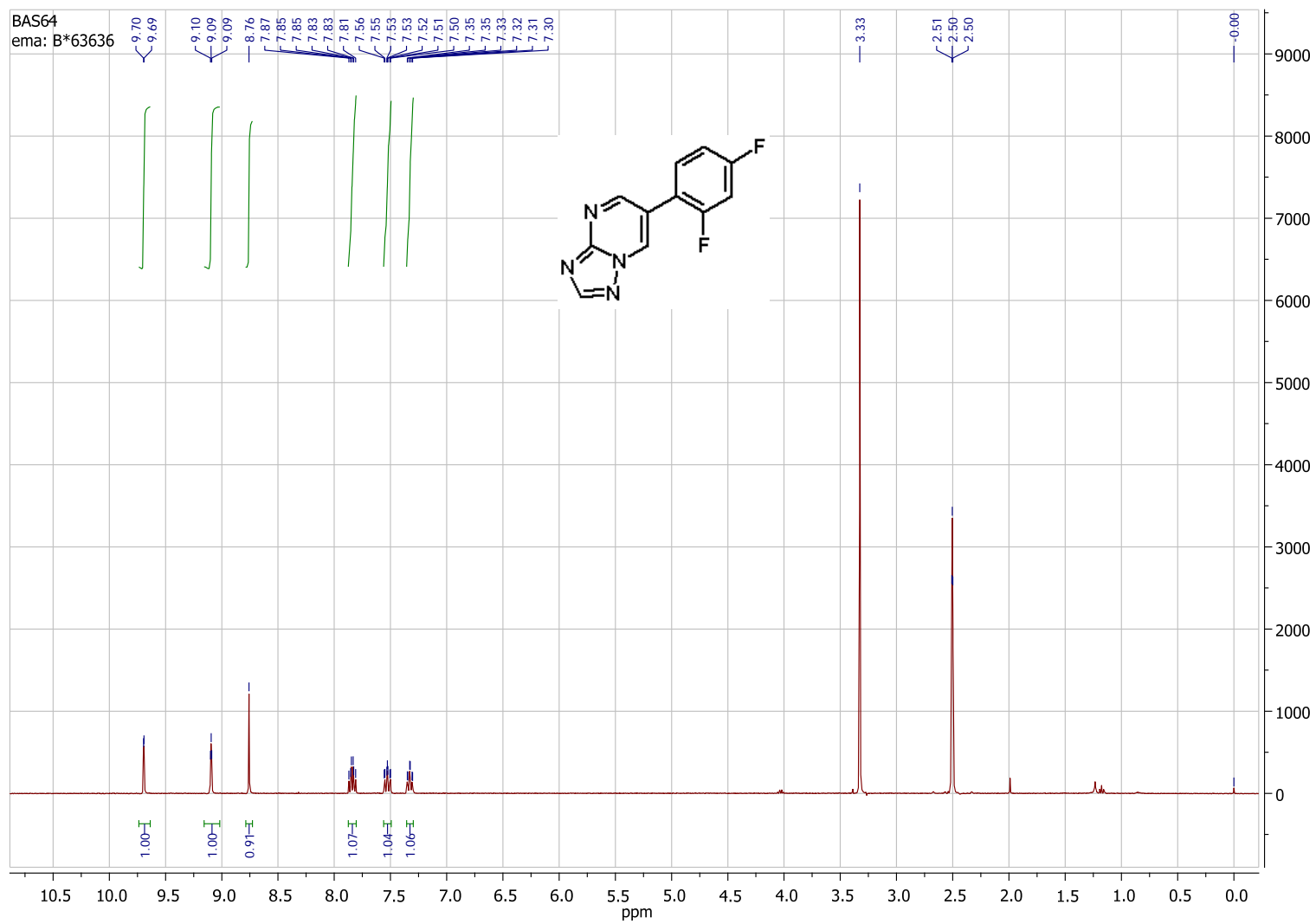
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5d**.



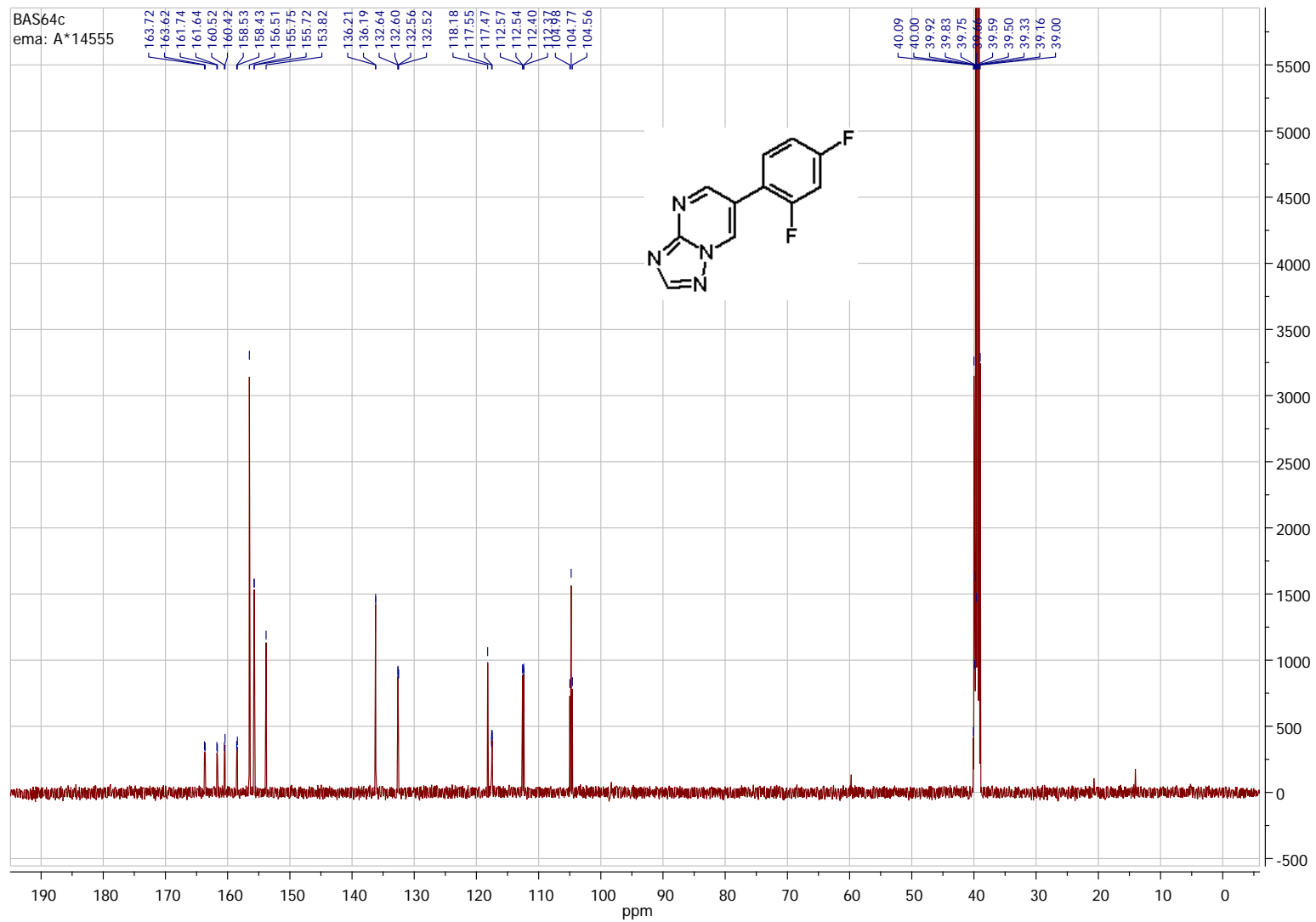
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5d**.

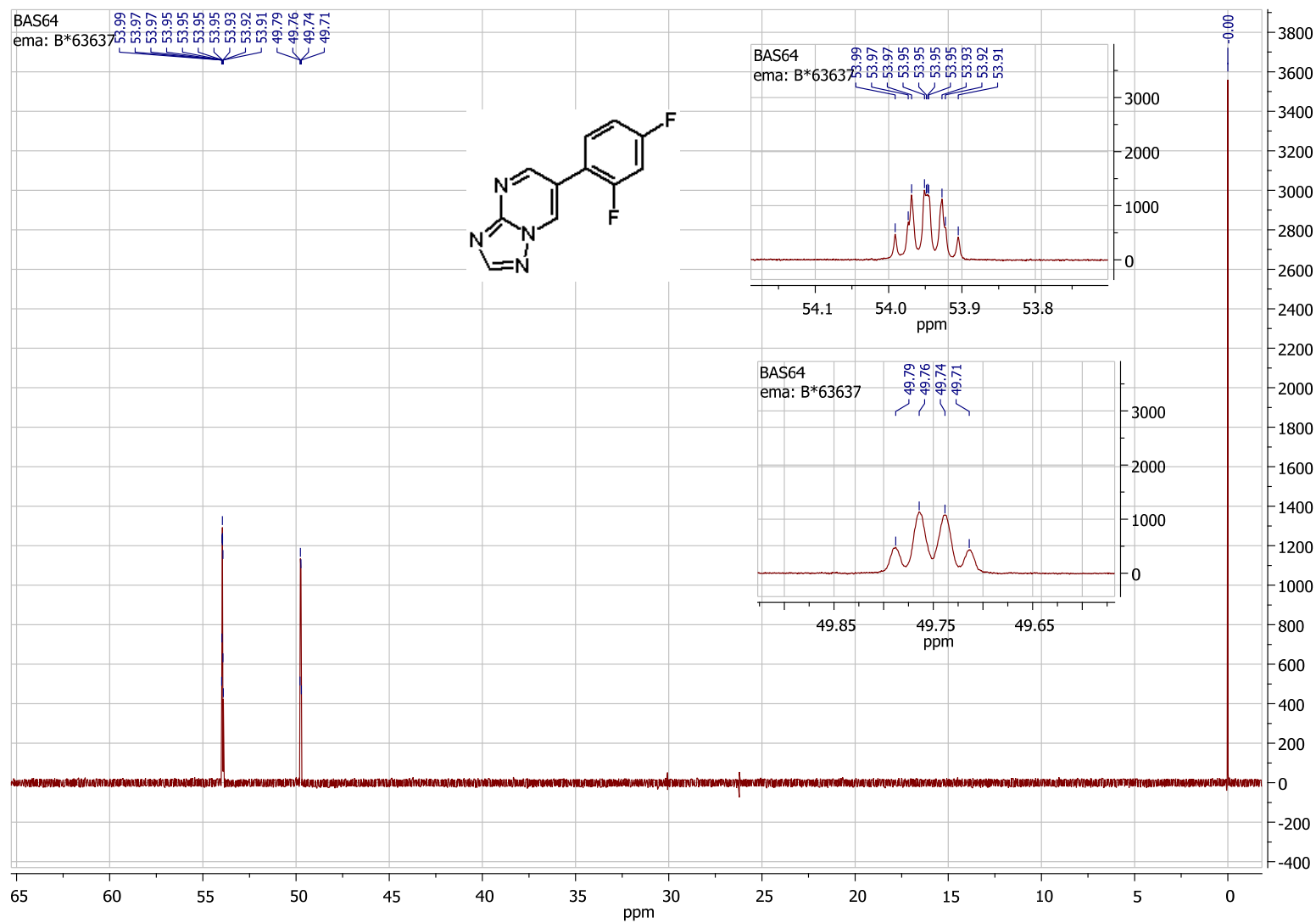


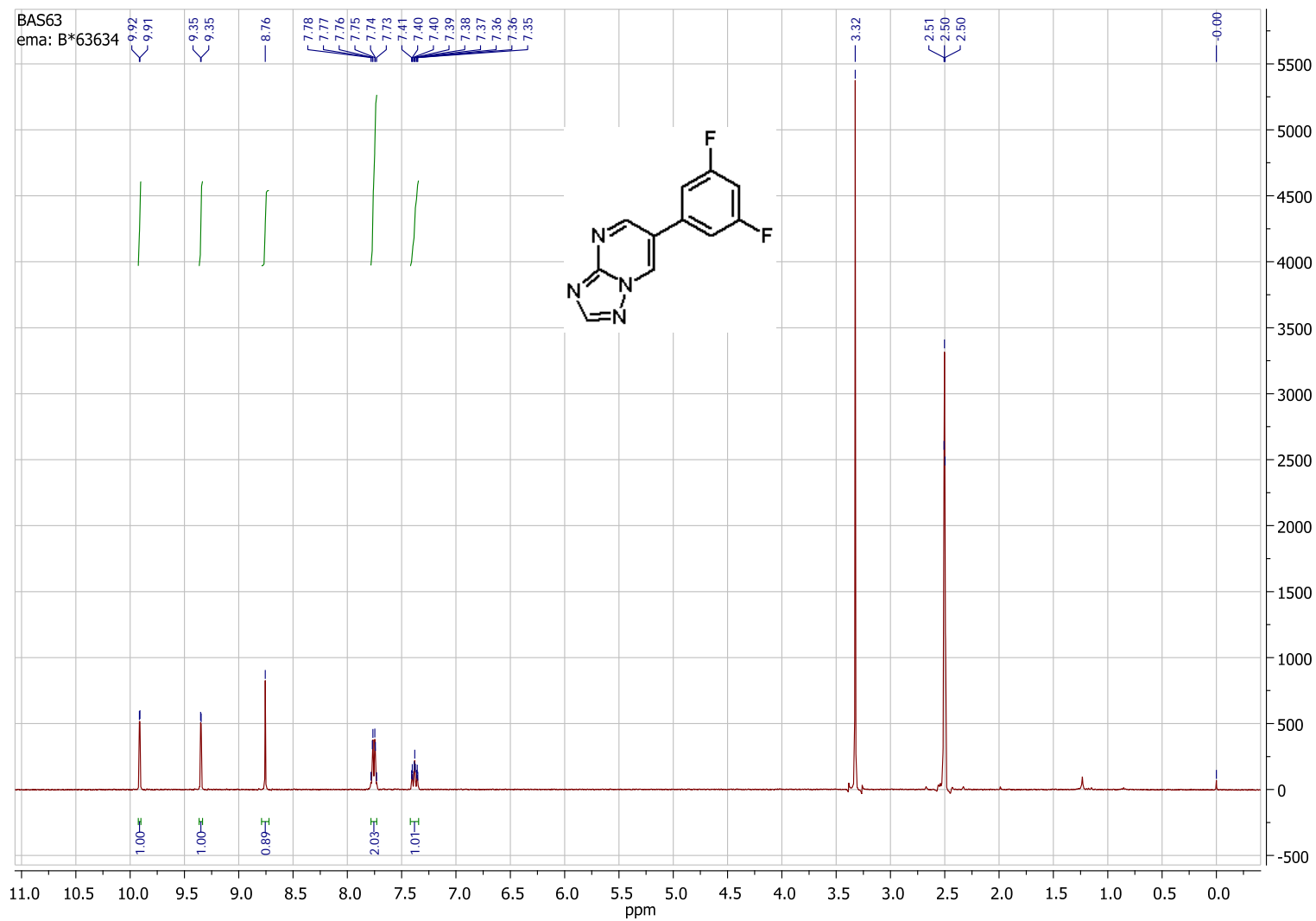
^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5d**.



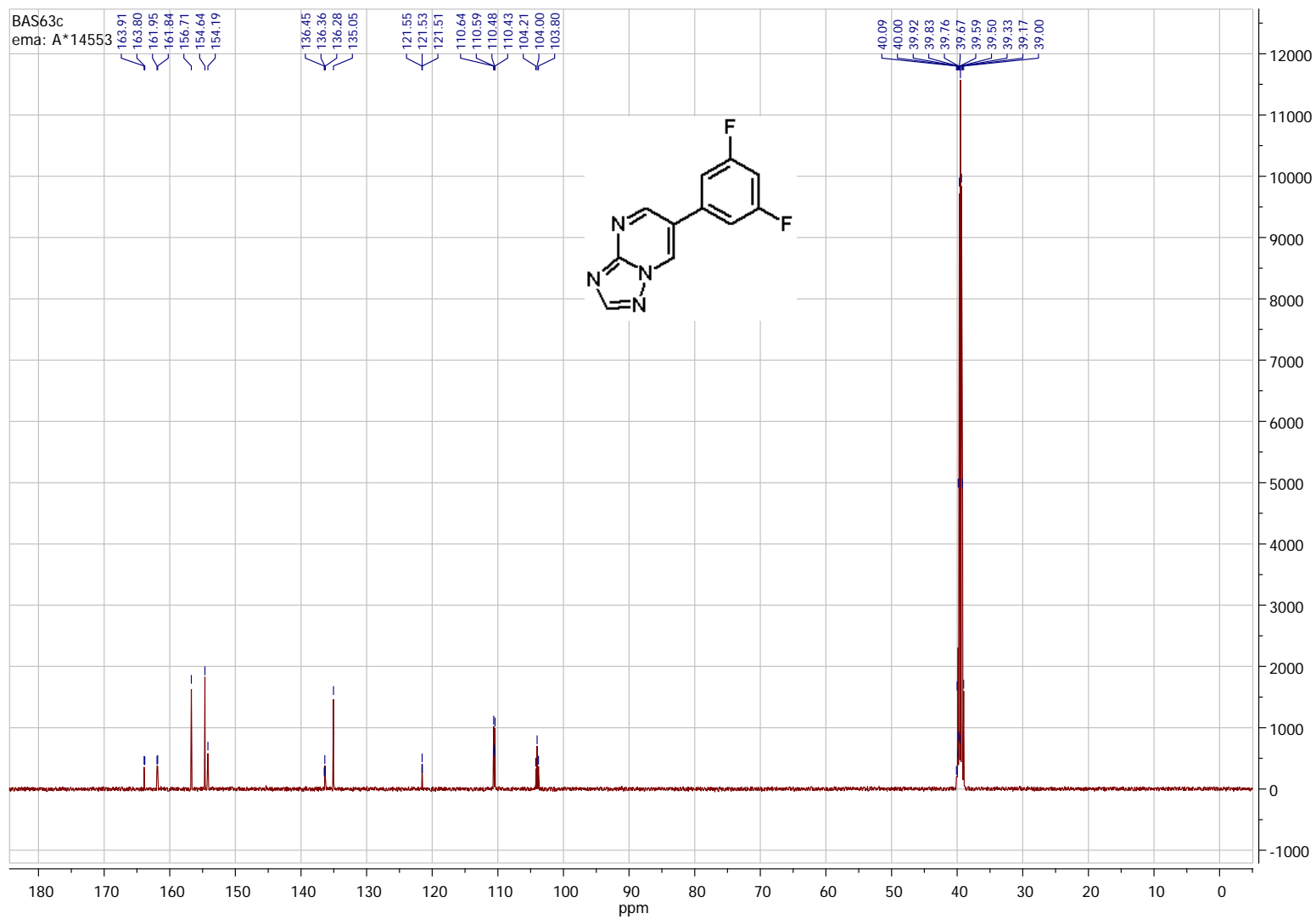
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5e**.



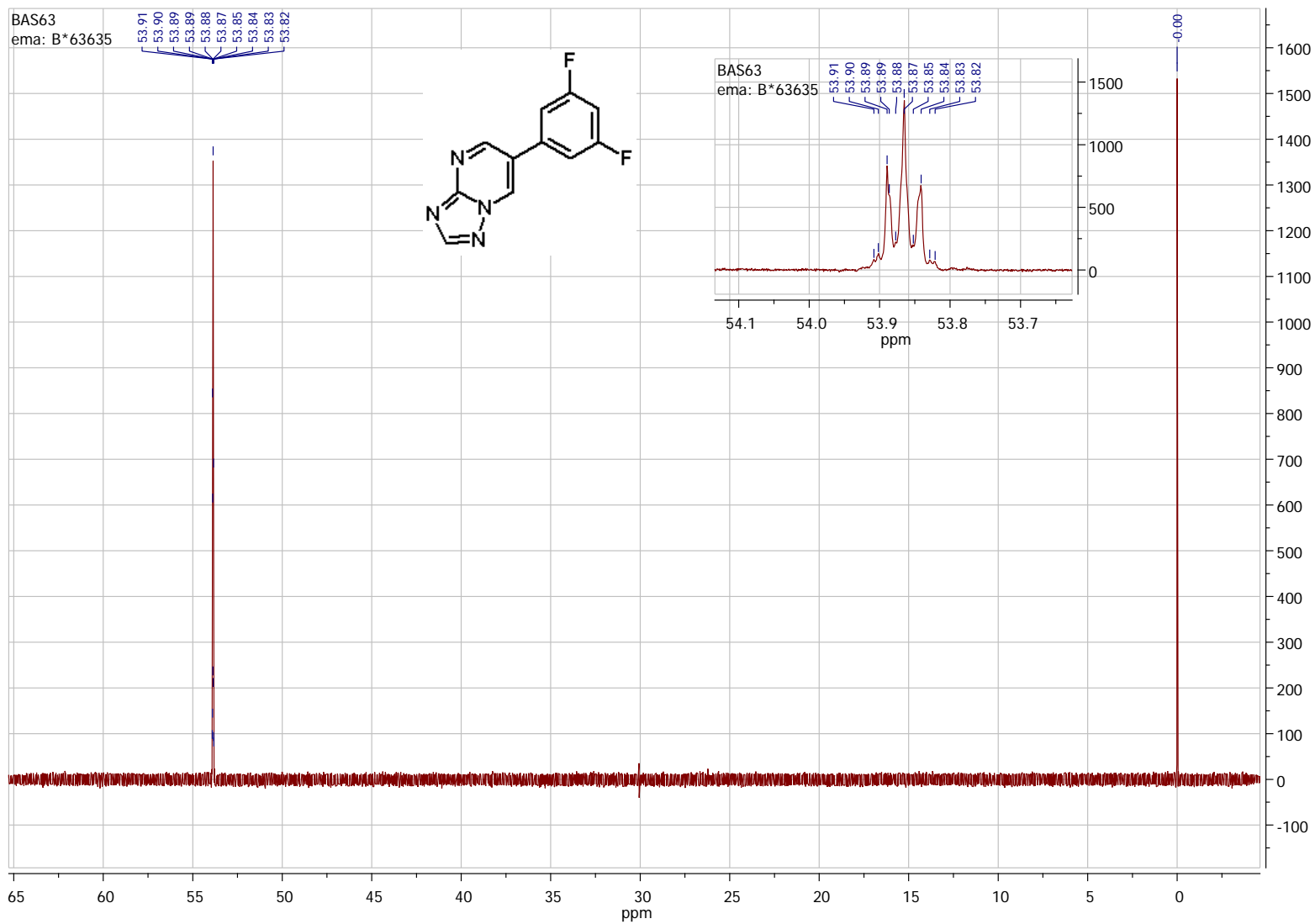
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5e**. ^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5e**.



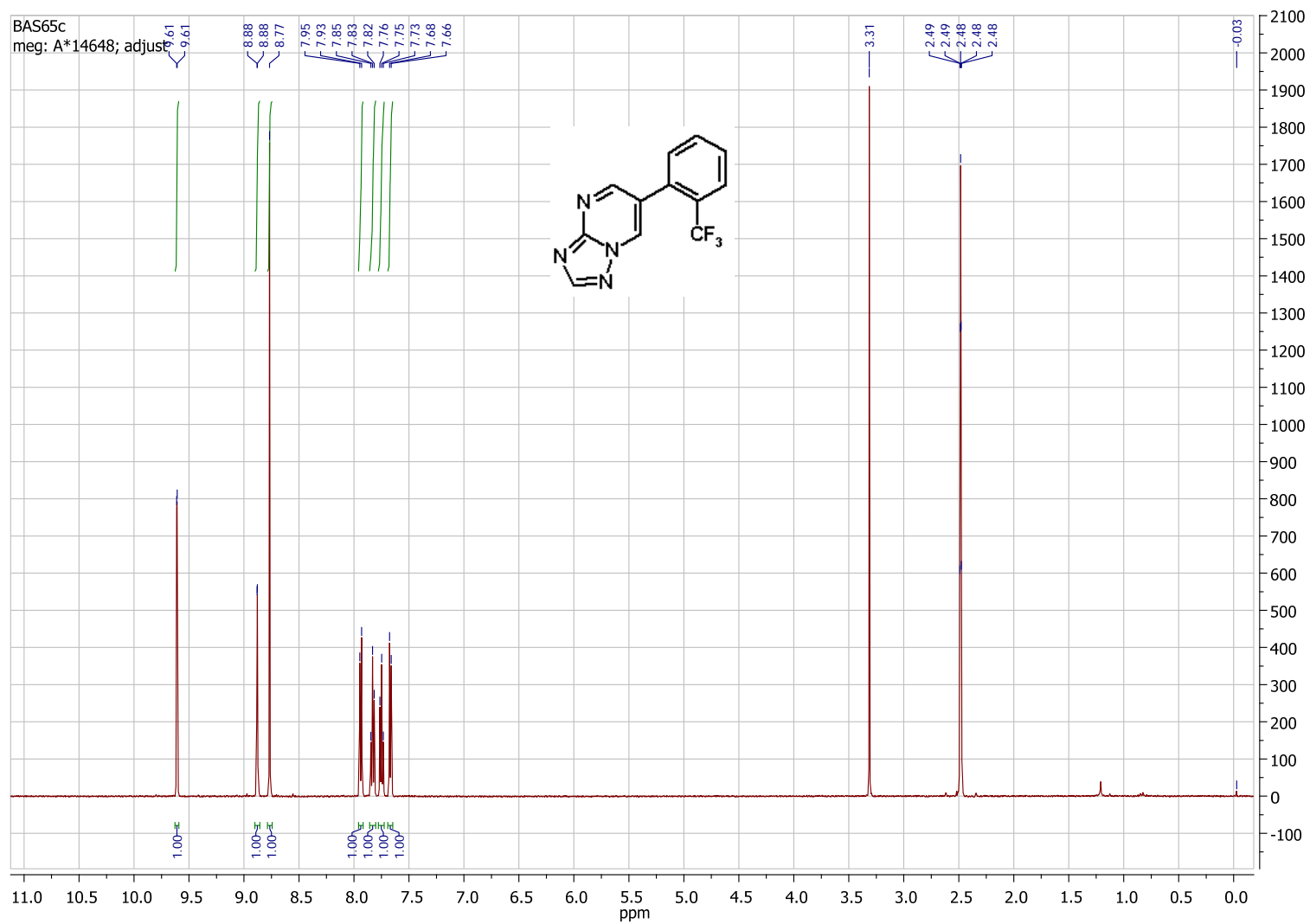
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5f**.



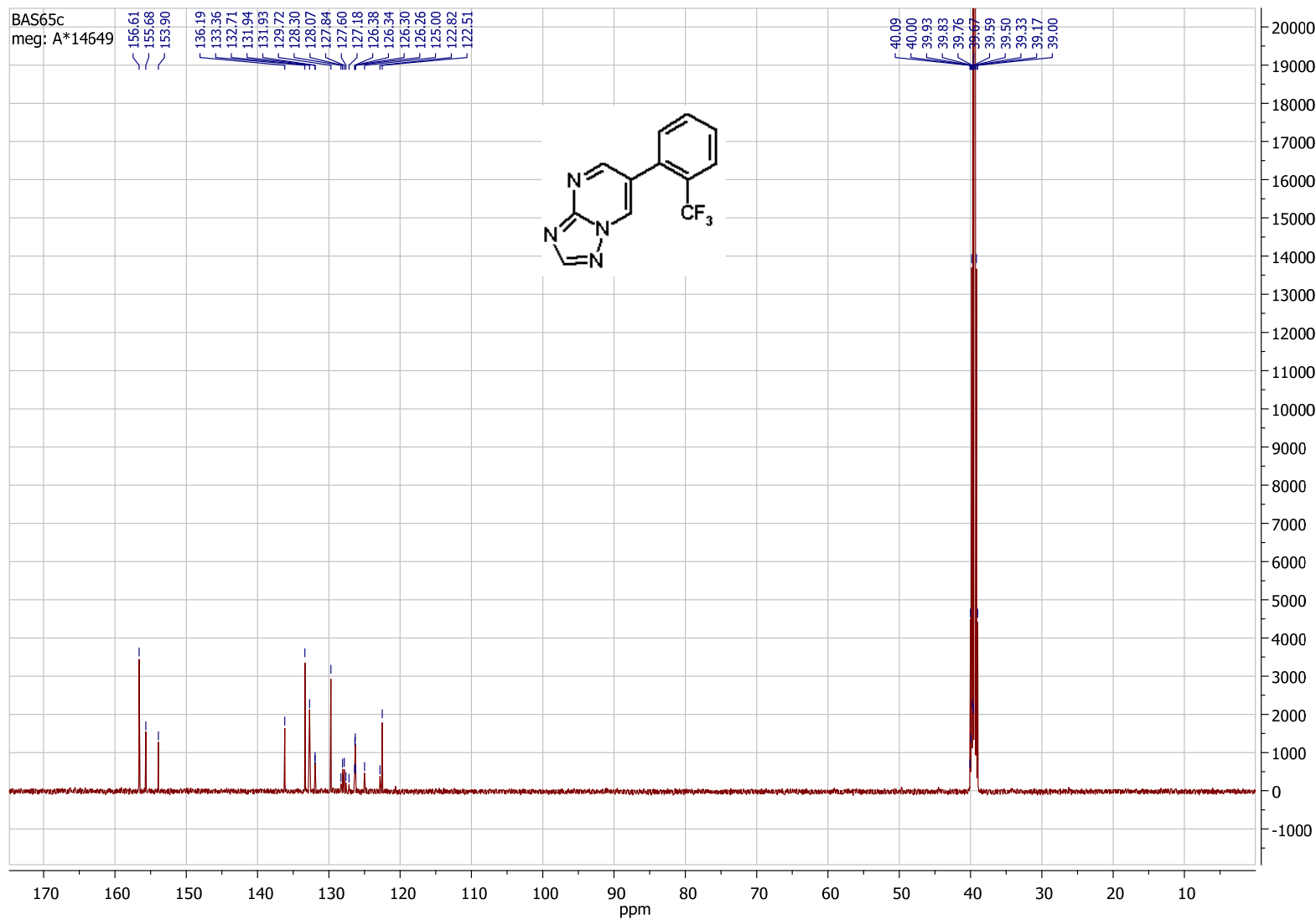
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5f**.



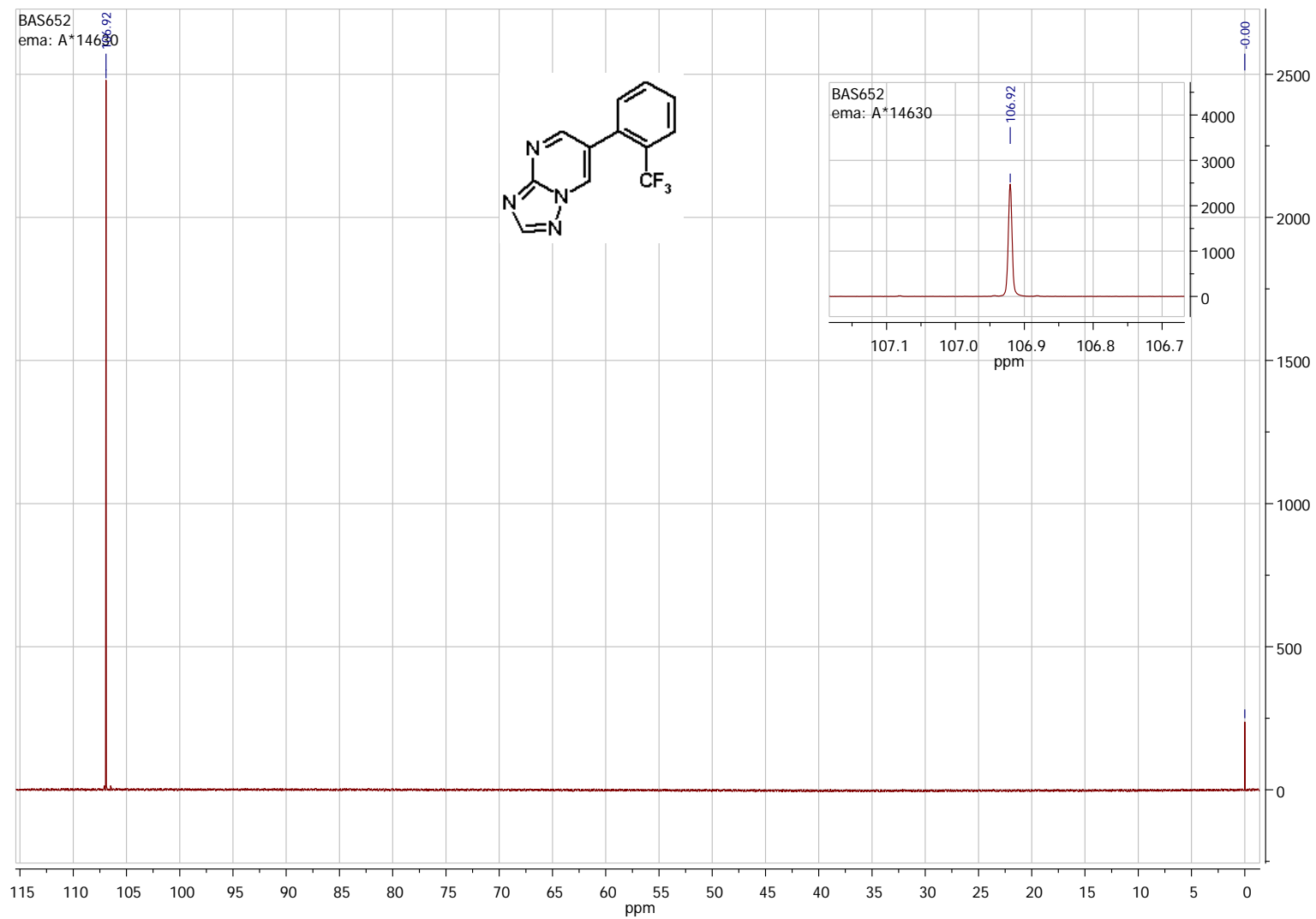
^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5f**.



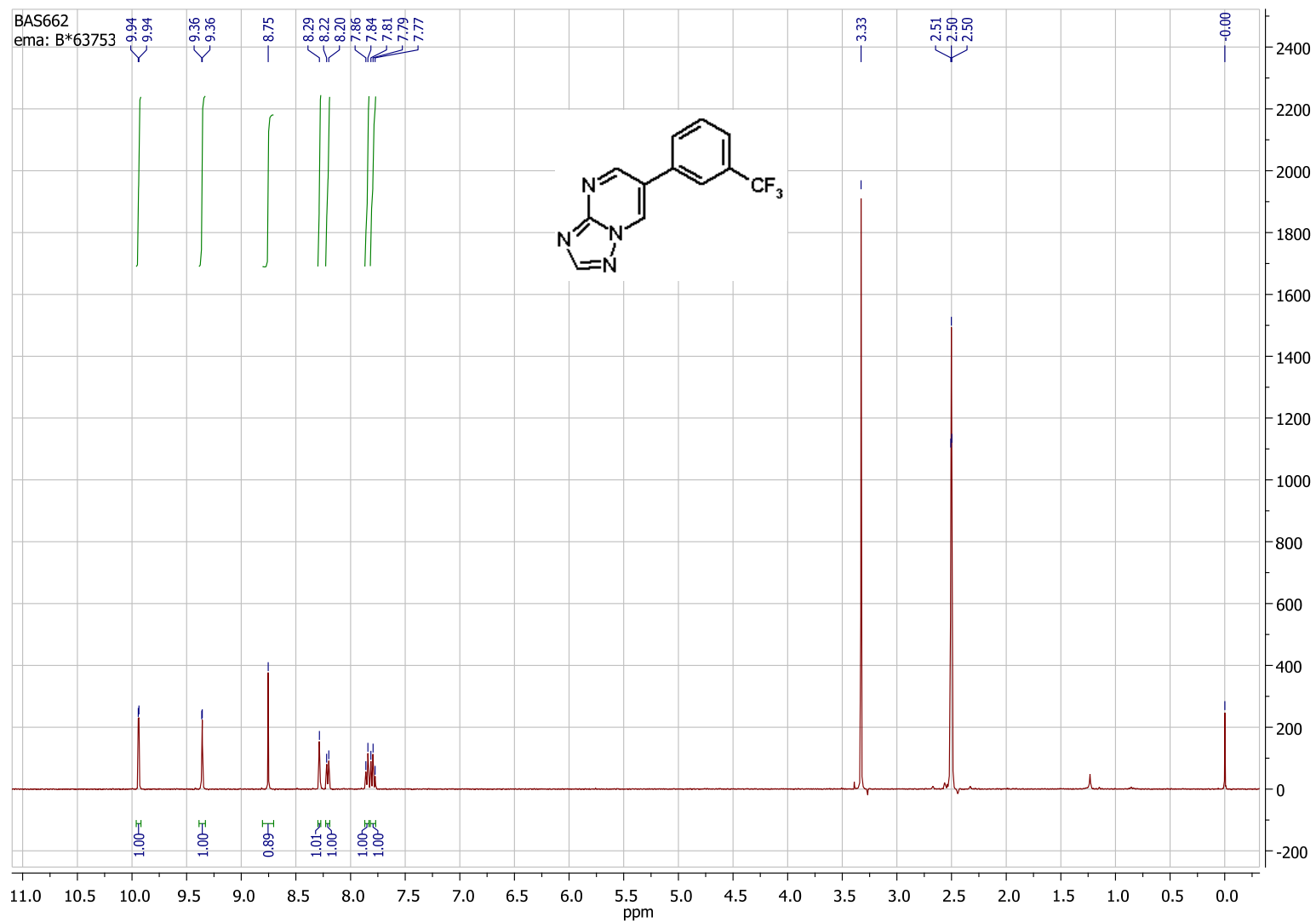
^1H NMR (500 MHz, $\text{DMSO}-d_6$) spectrum of **5g**.



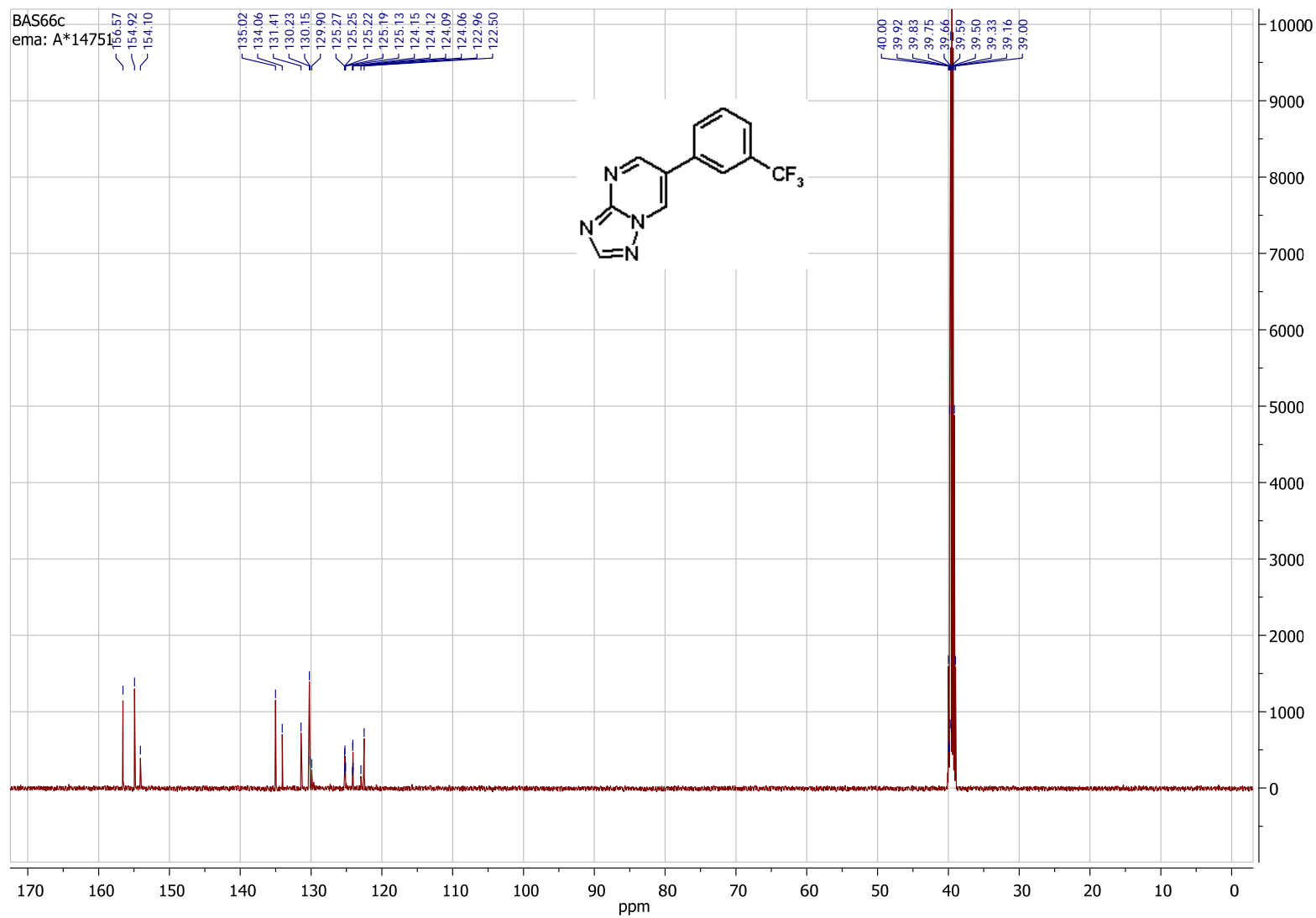
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5g**.



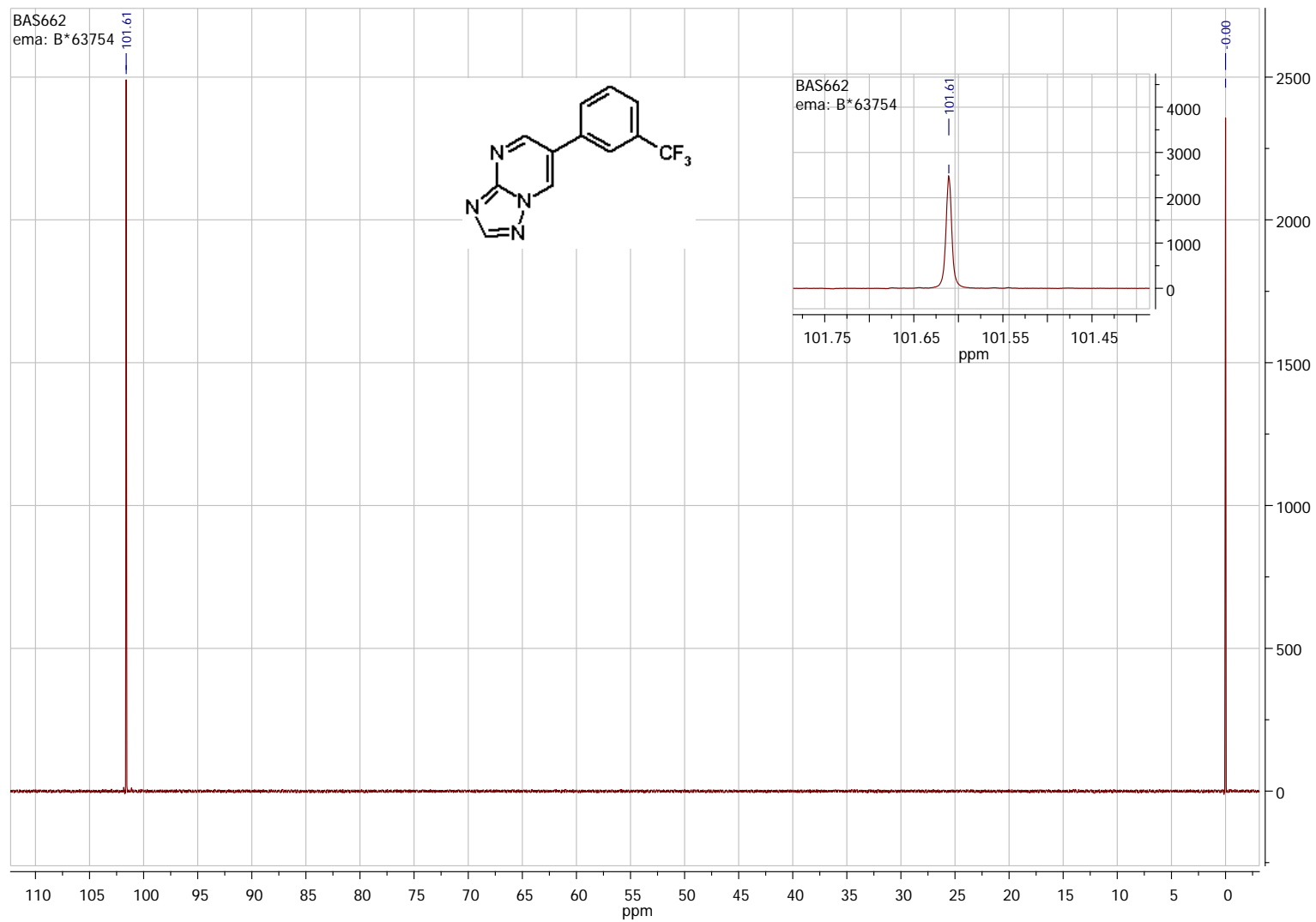
^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5g**.



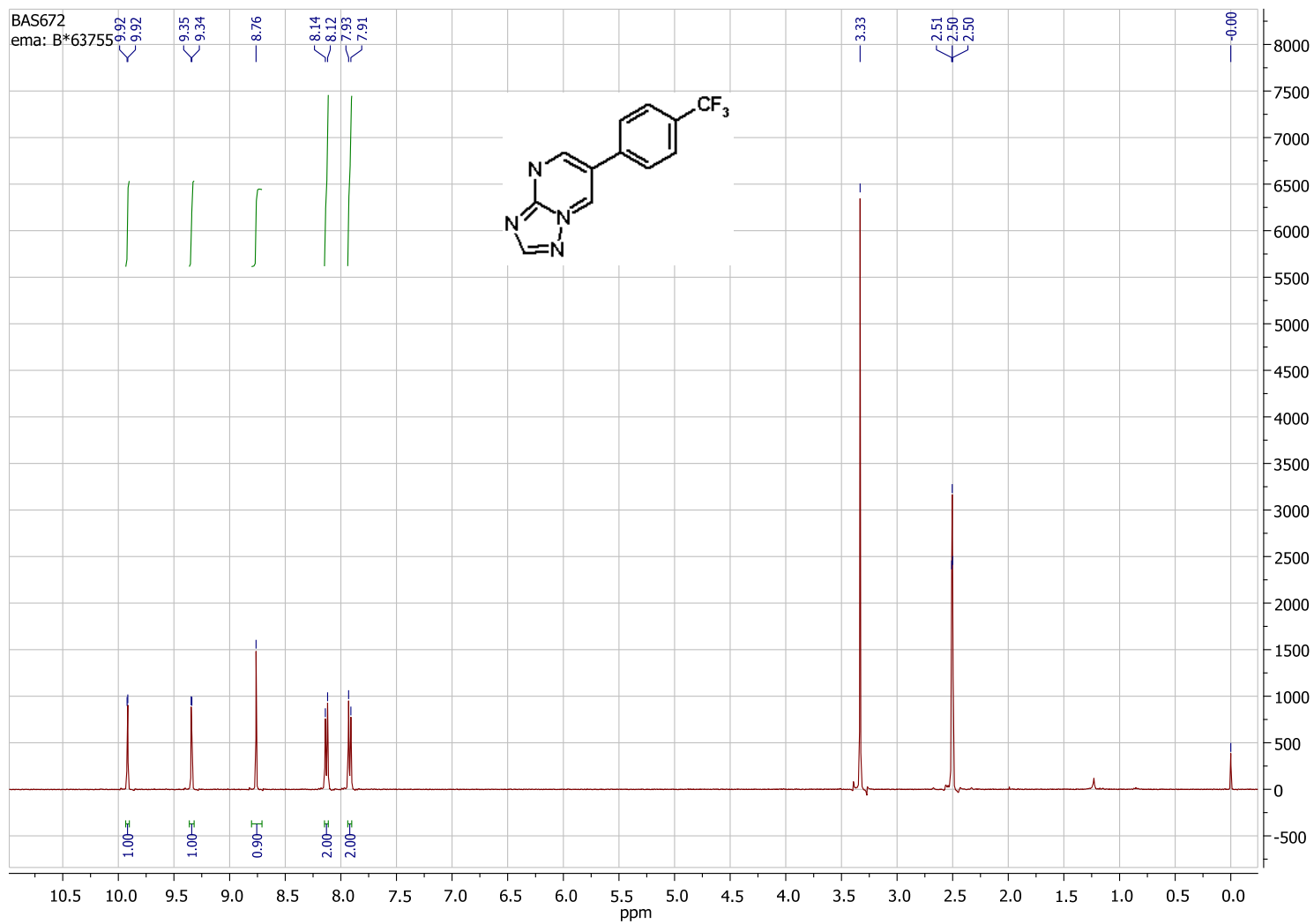
^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of **5h**.



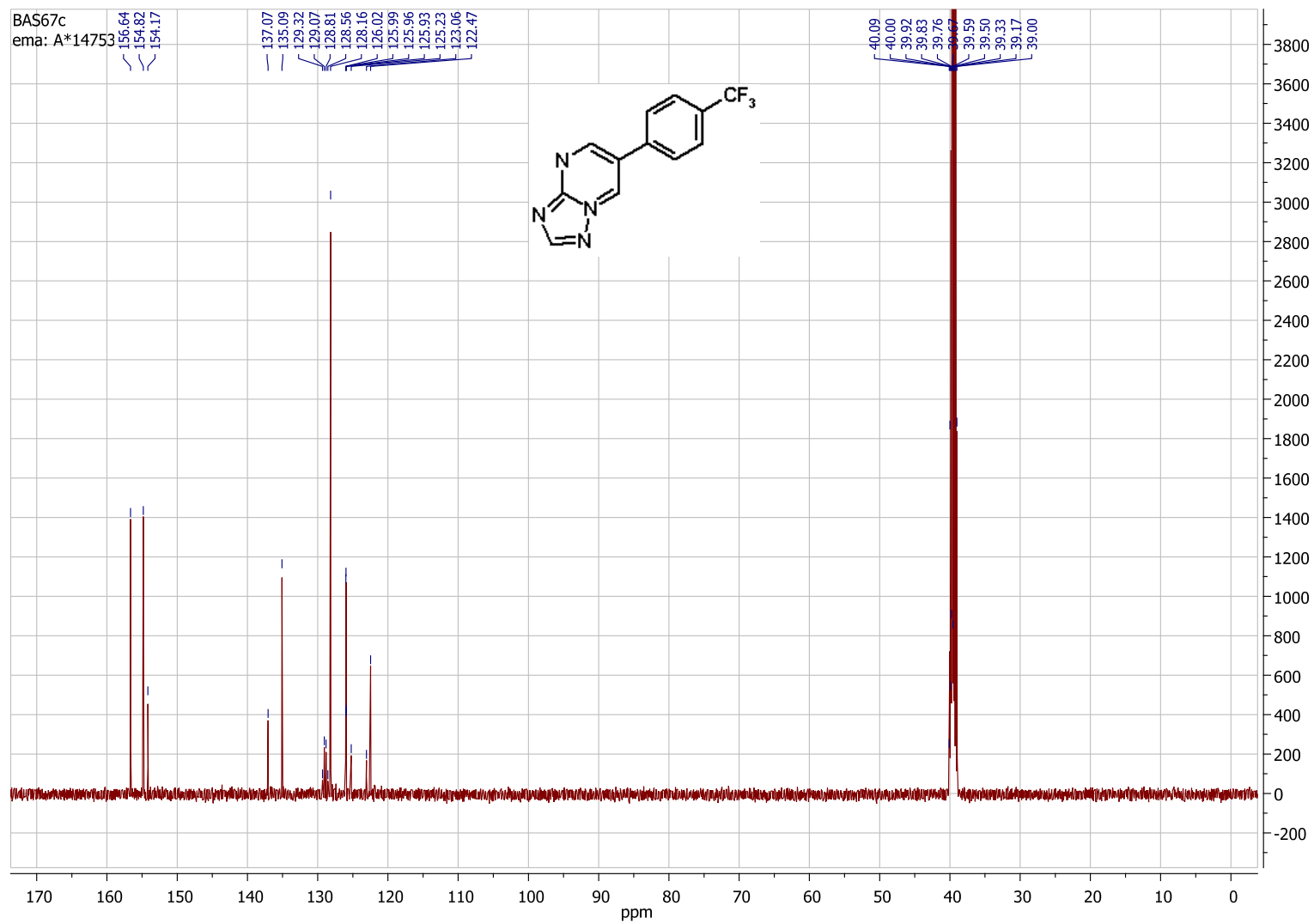
^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5h**.



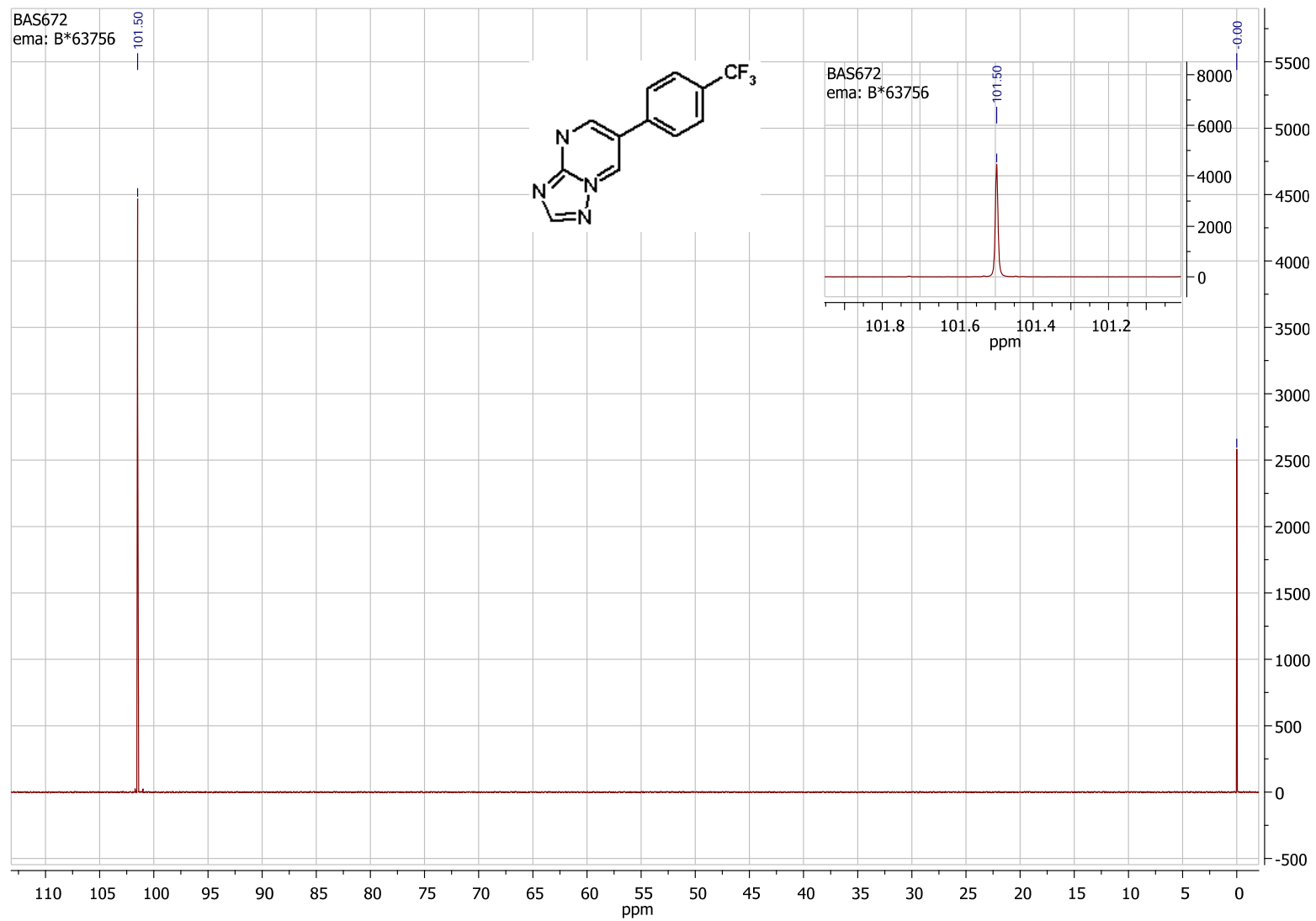
¹⁹F NMR (470.5 MHz, DMSO-*d*₆) spectrum of **5h**.



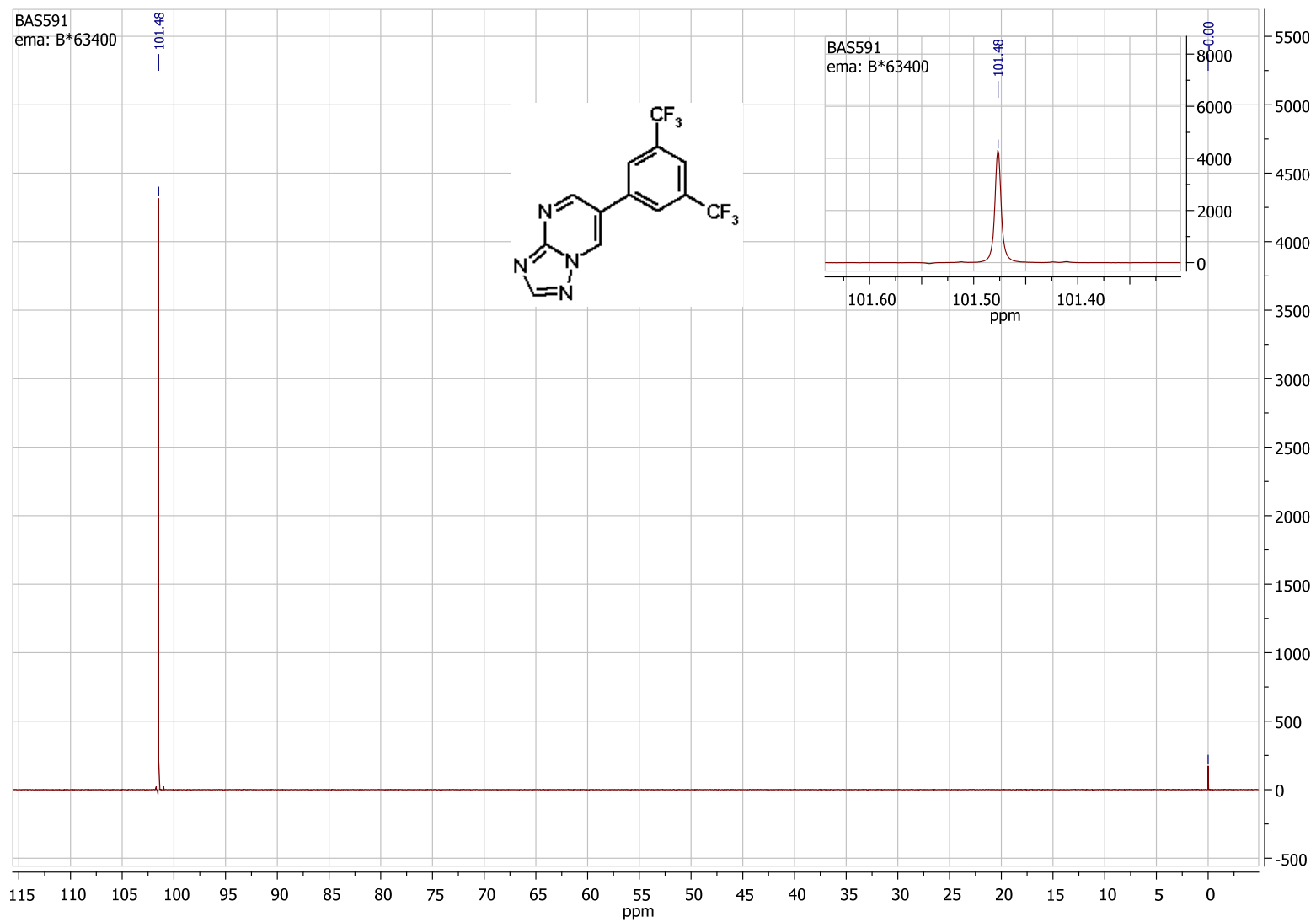
^1H NMR (500 MHz, $\text{DMSO}-d_6$) spectrum of **5i**.



^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) spectrum of **5i**.



¹⁹F NMR (470.5 MHz, DMSO-*d*₆) spectrum of **5i**.



^{19}F NMR (470.5 MHz, $\text{DMSO-}d_6$) spectrum of **5j**.