CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

# Design, Synthesis and Evaluation of Oxazaborine Inhibitors of the NLRP3 Inflammasome

Alex G. Baldwin,[a] Victor S. Tapia,[b] Tessa Swanton,[b] Claire S. White,[b] James A. Beswick,[a] David Brough,*[b] and Sally Freeman*[a]

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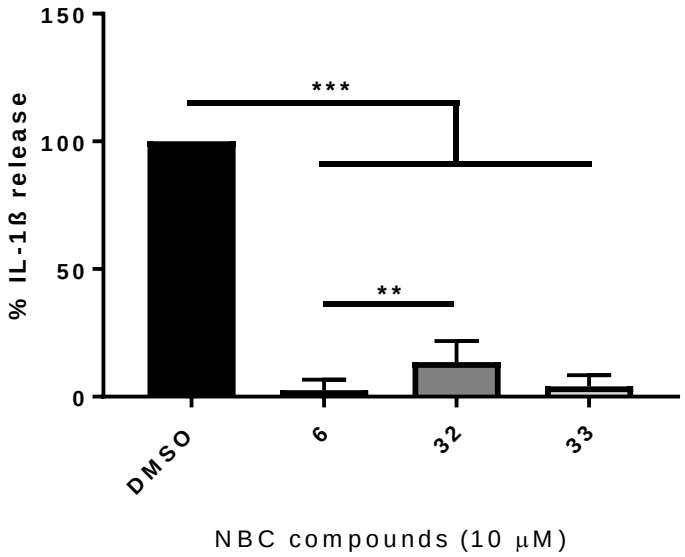



**Figure S1:** Effects of NBC32 and NBC33 on IL-1β release. THP-1 cells were primed with LPS (1 µg/ml, 4 h) and then treated with vehicle or NBC molecule (NBC6, NBC32 or NBC33) at 10 µM for 15 min before stimulation with nigericin (10 µM, 1h). IL-1β was measured by ELISA. The effects of the NBC molecules on IL-1β release was normalised to nigericin-induced IL-1β in the absence of any inhibitor. Data are presented as the mean ± s.e.m. with a minimum of 3 experiments per group. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test.

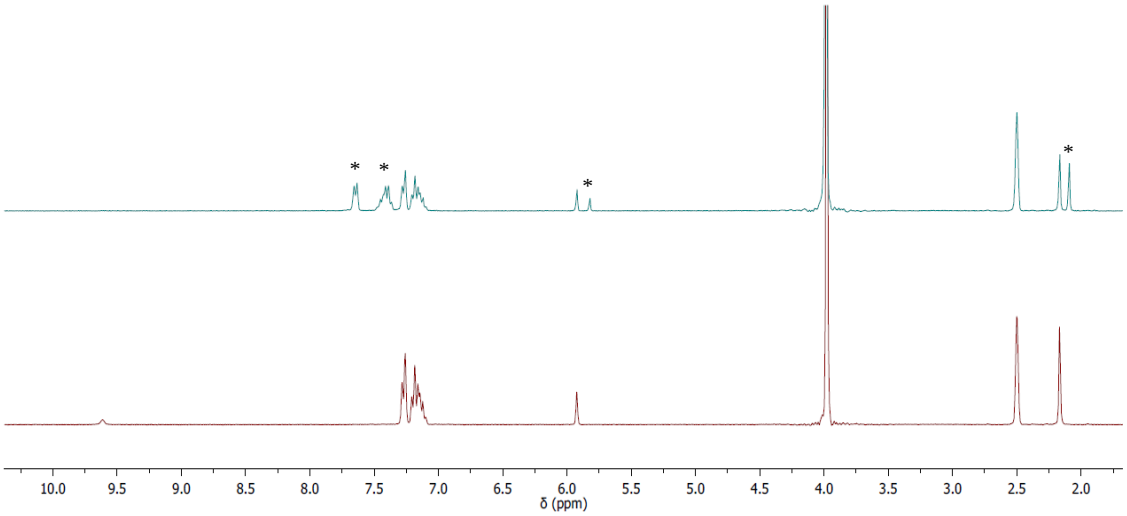
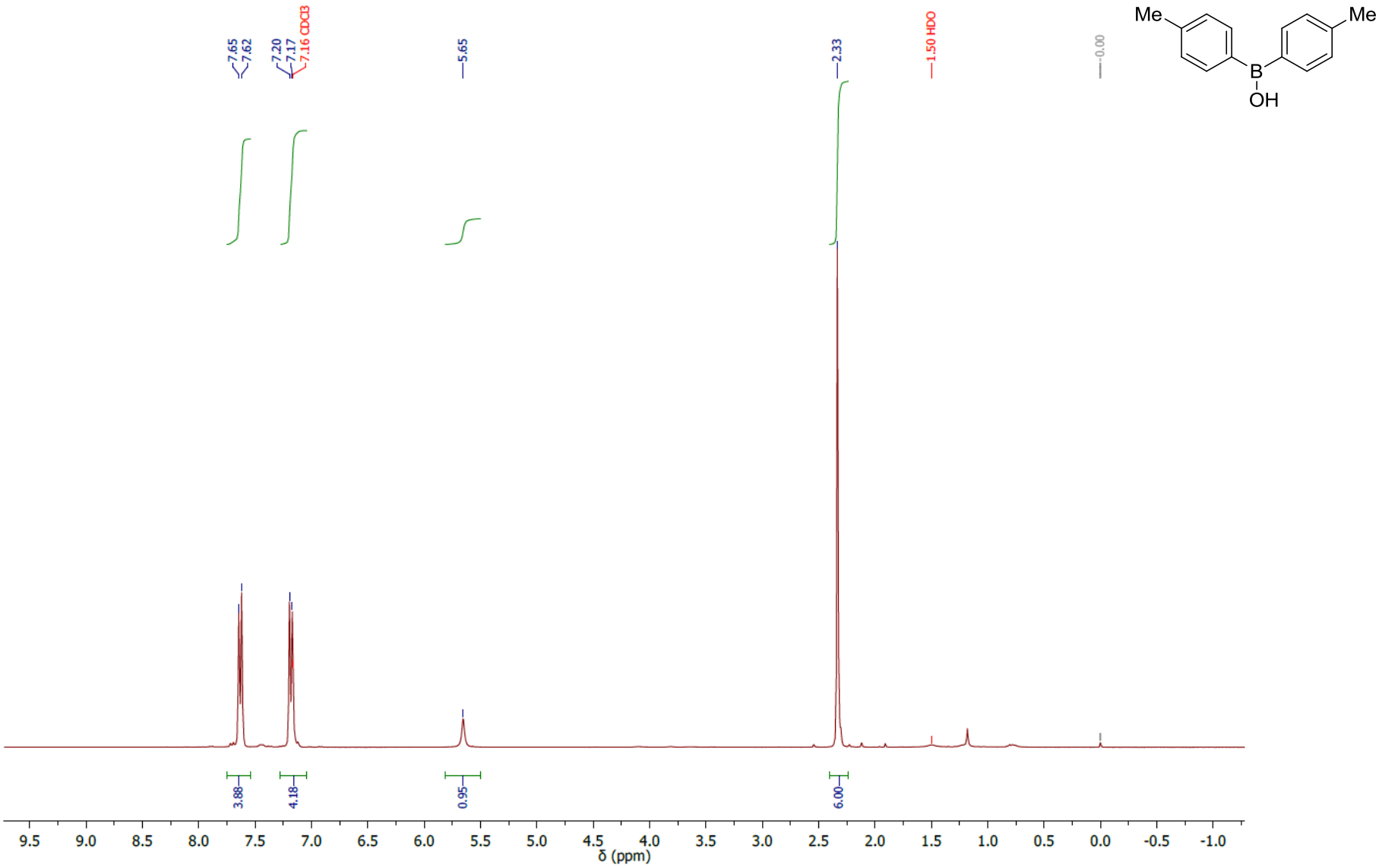
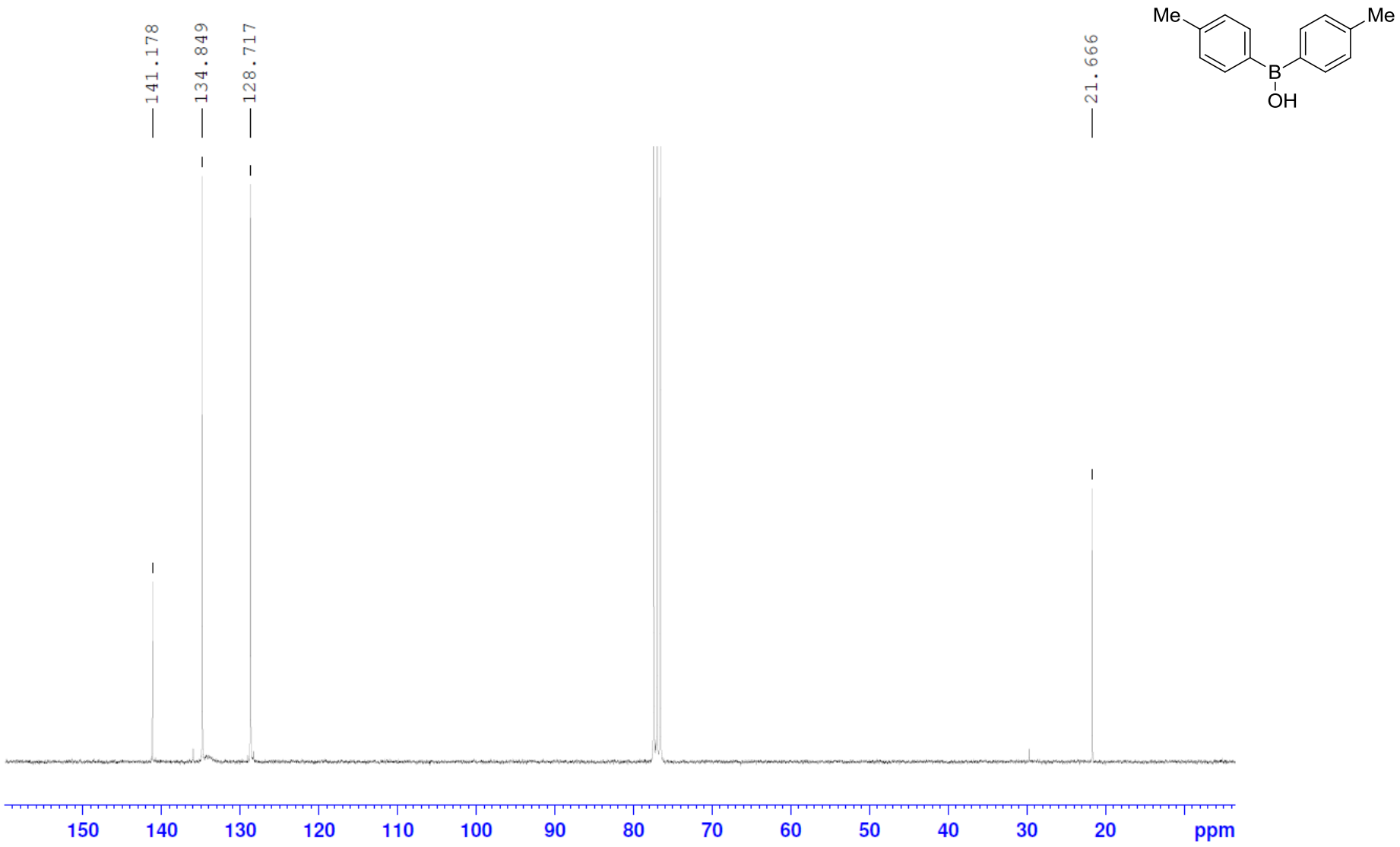


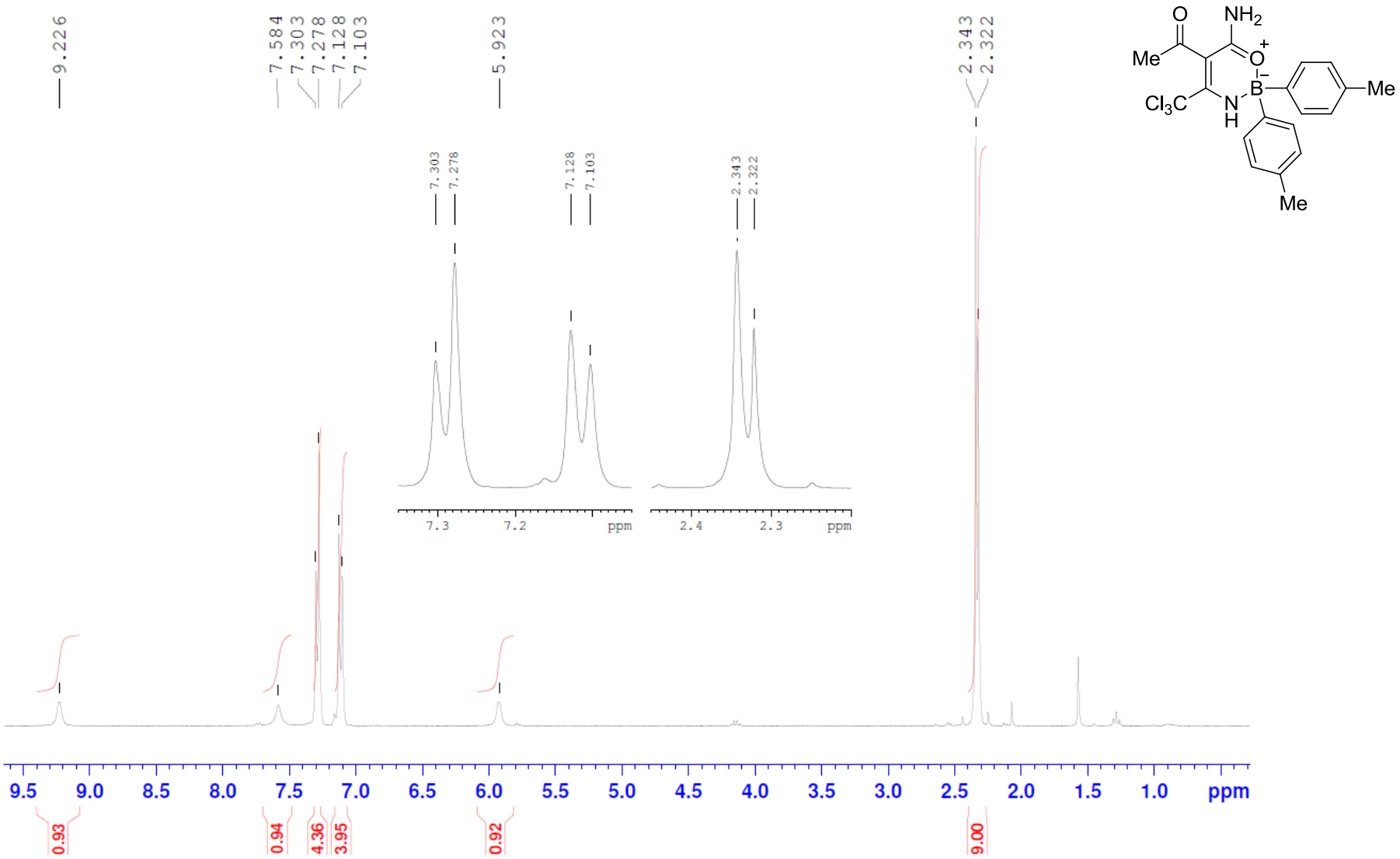

**Figure S2:** Stacked $^{1}H$ NMR spectra of BC23 (0.02 mmol) in 9:1 DMSO-$d_6$/$D_2O$ (700 µL) at 37 $^{o}C$; red: 0 h, blue: 24 h. Chemical shifts are defined in parts per million and referenced against residual undeuterated DMSO-$d_6$ (δ = 2.50 ppm). * Indicates new peaks associated with hydrolysis products of BC23.



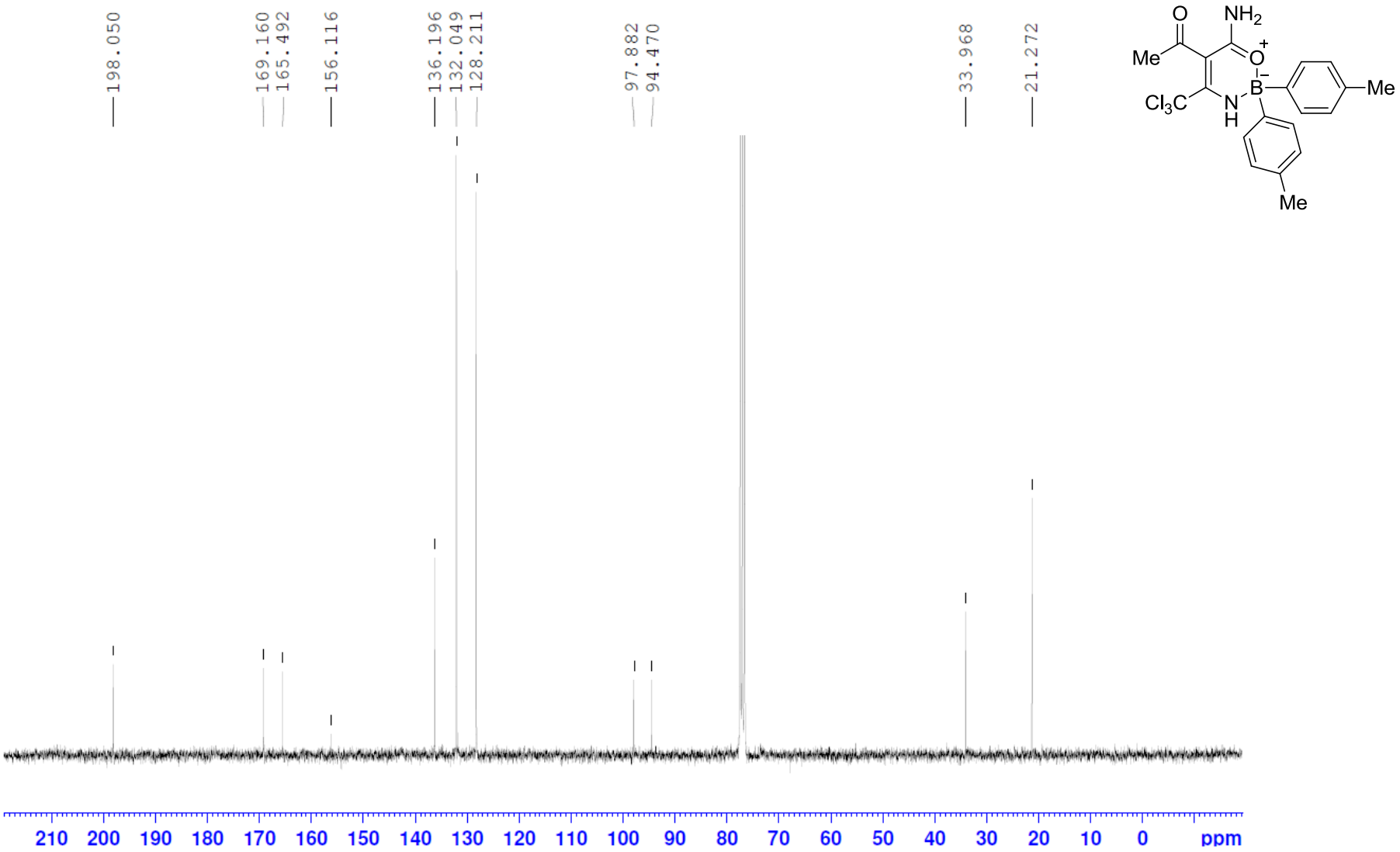

$^{1}$H NMR spectrum ($CDCl_3$) of di-*p*-tolylborinic acid (**5a**)



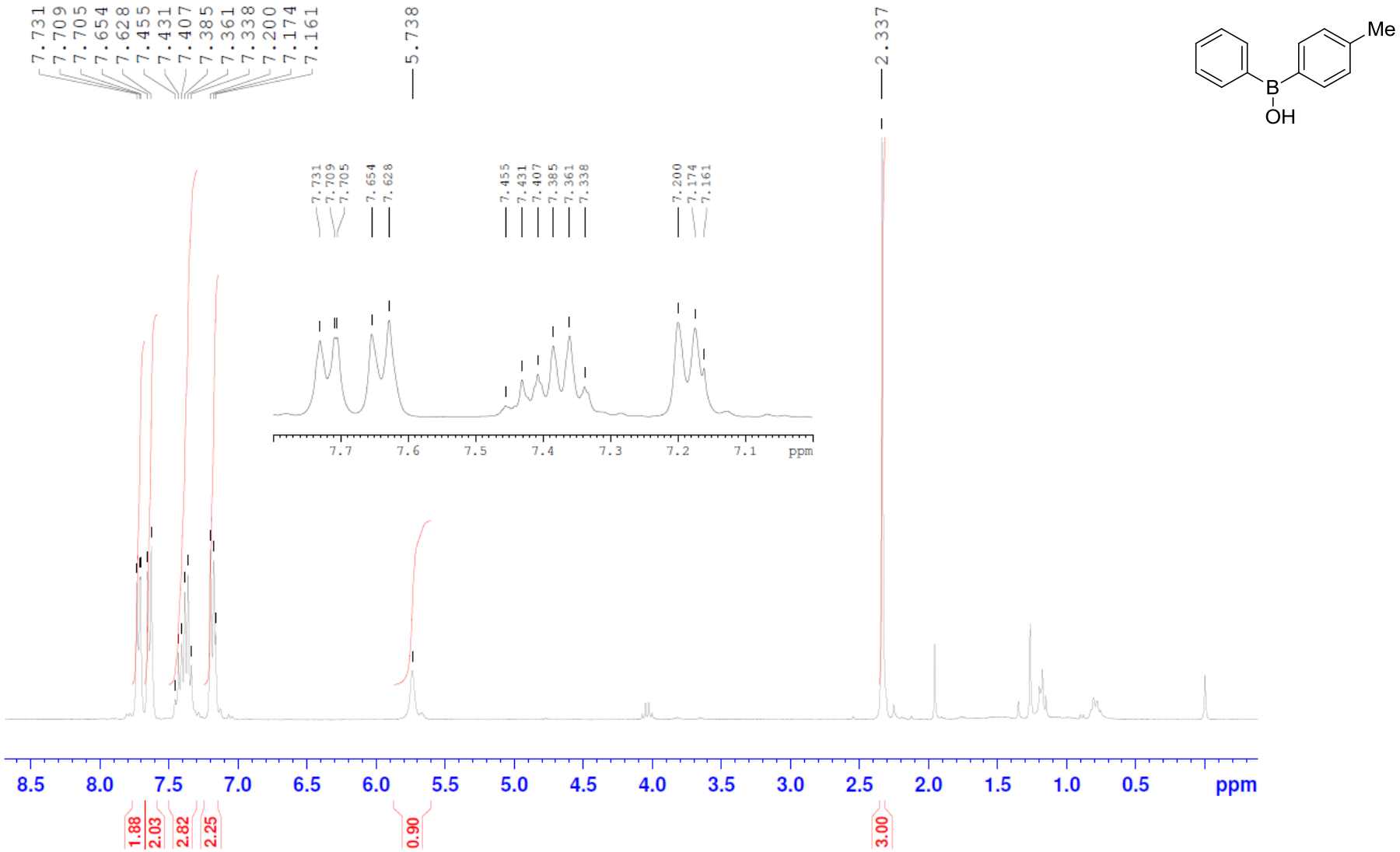

$^{13}$C NMR spectrum ($CDCl_3$) of di-*p*-tolylborinic acid (**5a**)



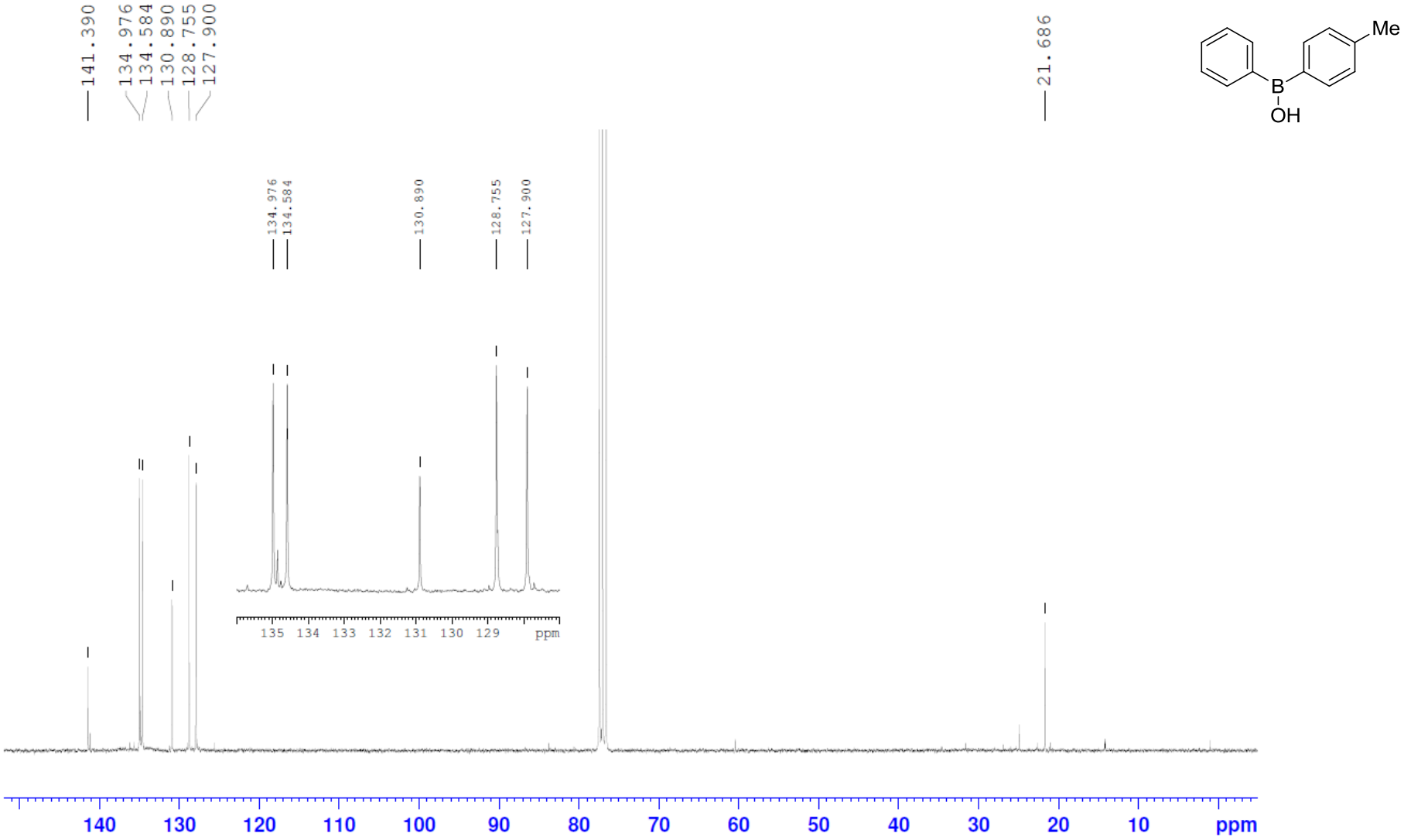

$^{1}$H NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2,2-bis(*p*-tolyl)-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC32)



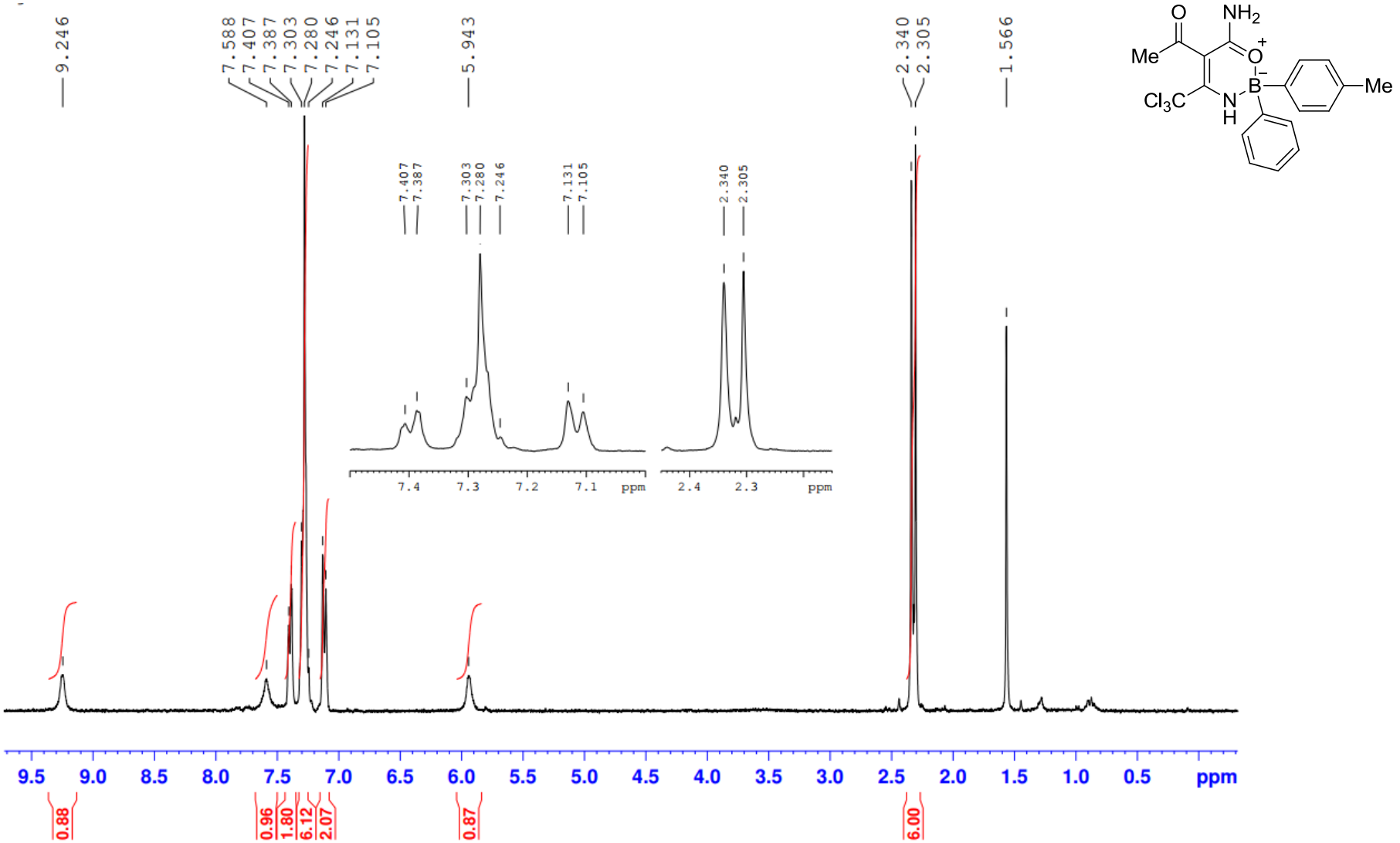

$^{13}$C NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2,2-bis(*p*-tolyl)-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC32)



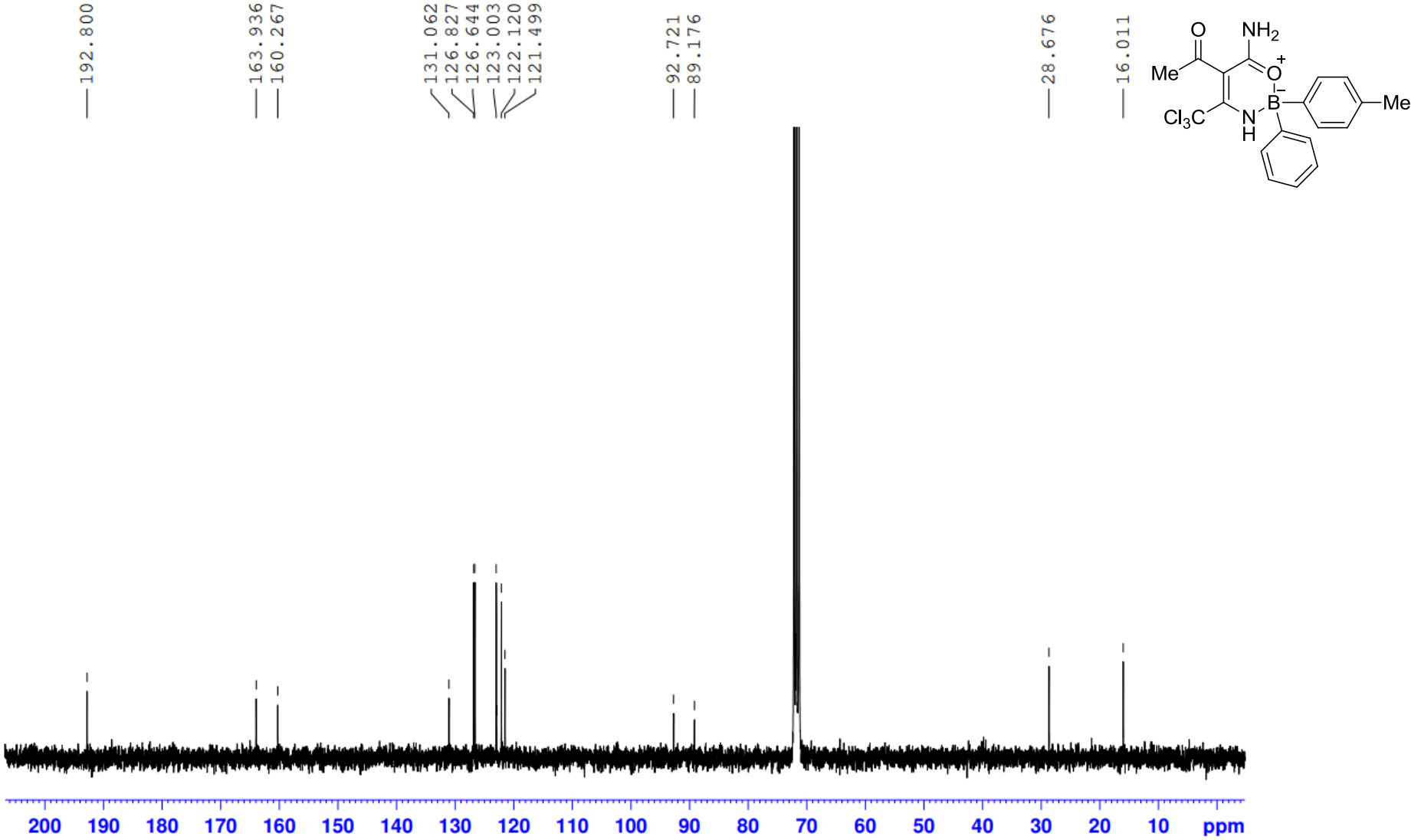

$^{1}$H NMR spectrum ($CDCl_3$) of (phenyl)(*p*-tolyl)borinic acid (**6a**)



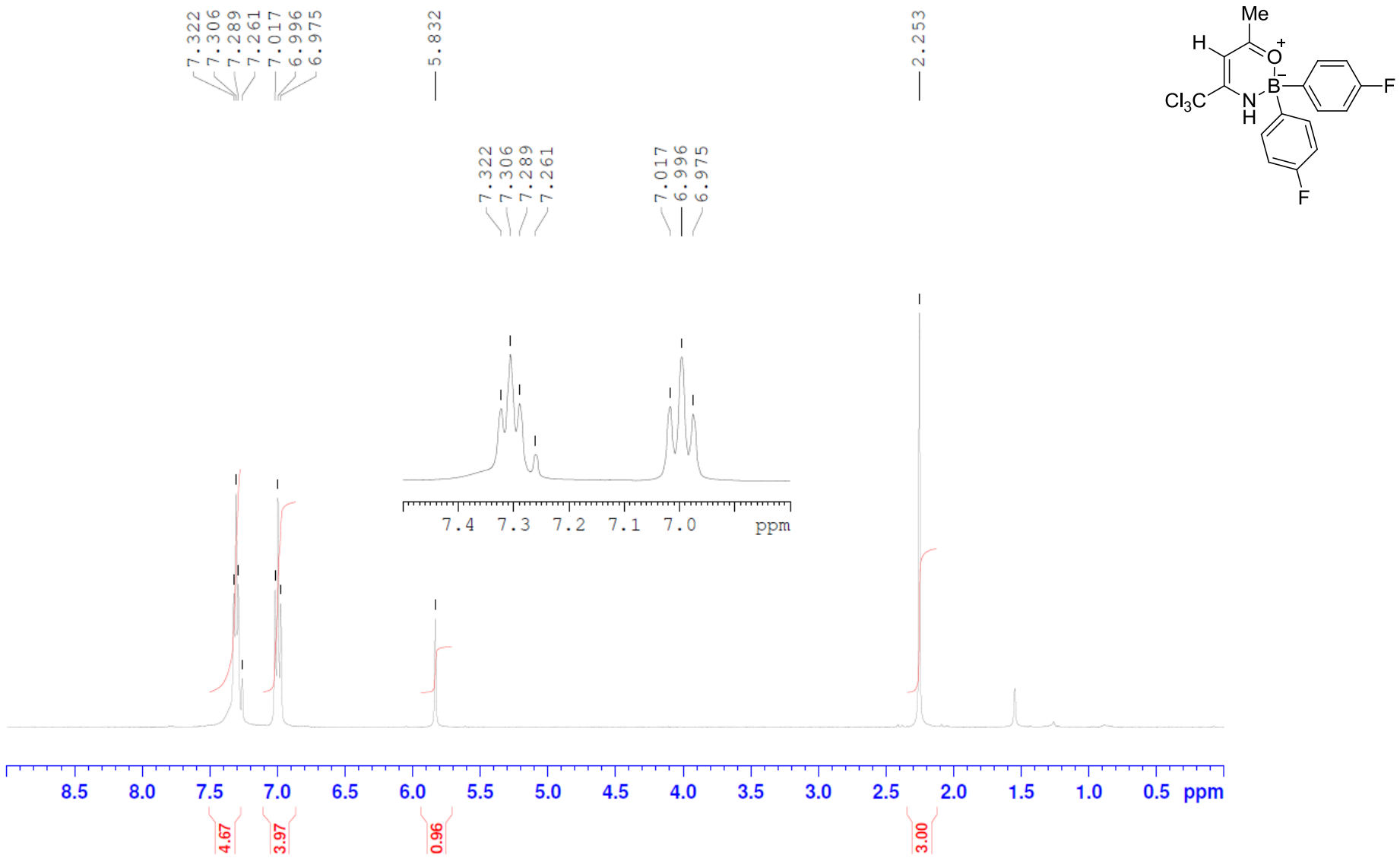

$^{13}$C NMR spectrum ($CDCl_3$) of (phenyl)(*p*-tolyl)borinic acid (**6a**)



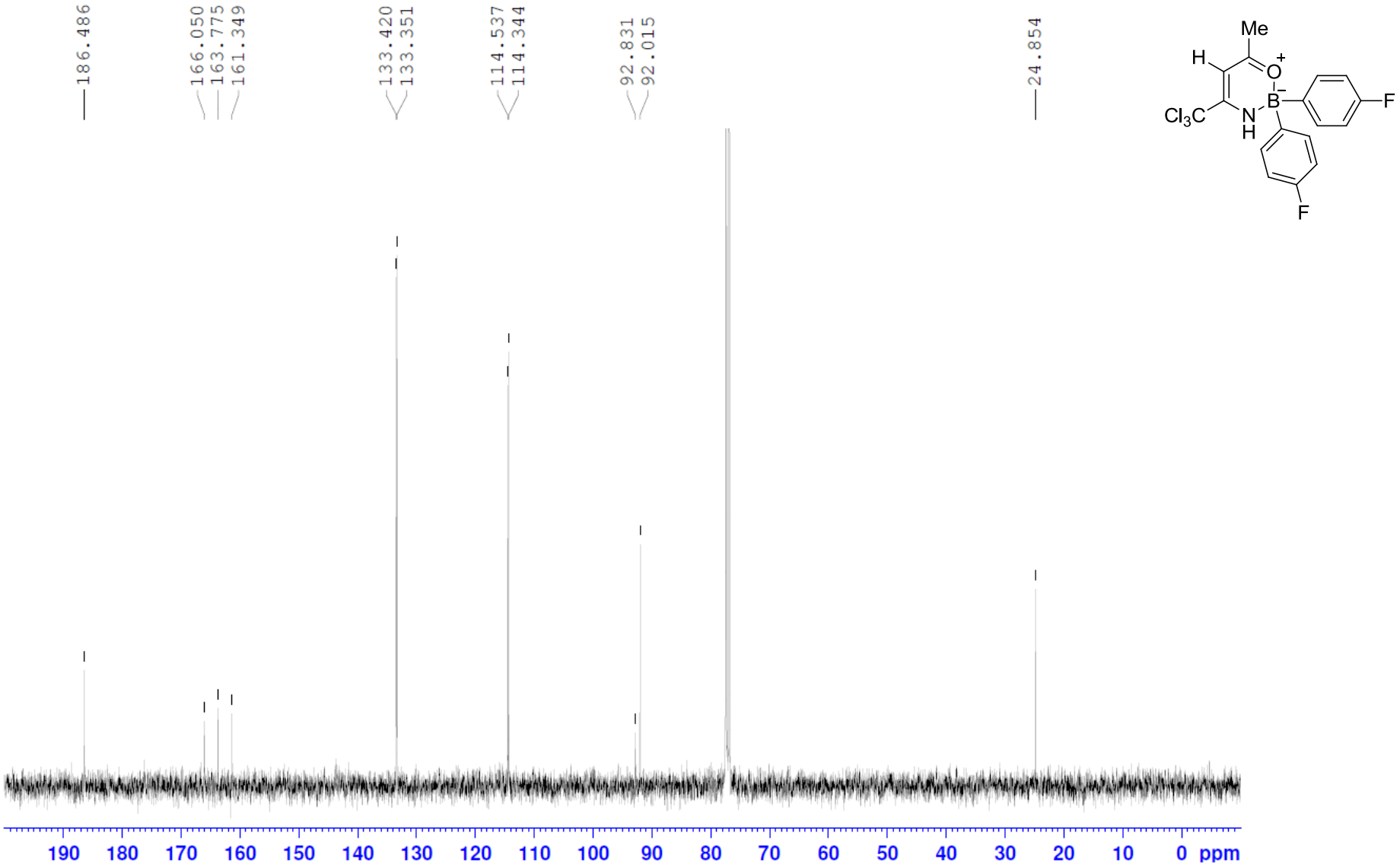

$^{1}$H NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2-(phenyl)-2-(*p*-tolyl)-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC33)



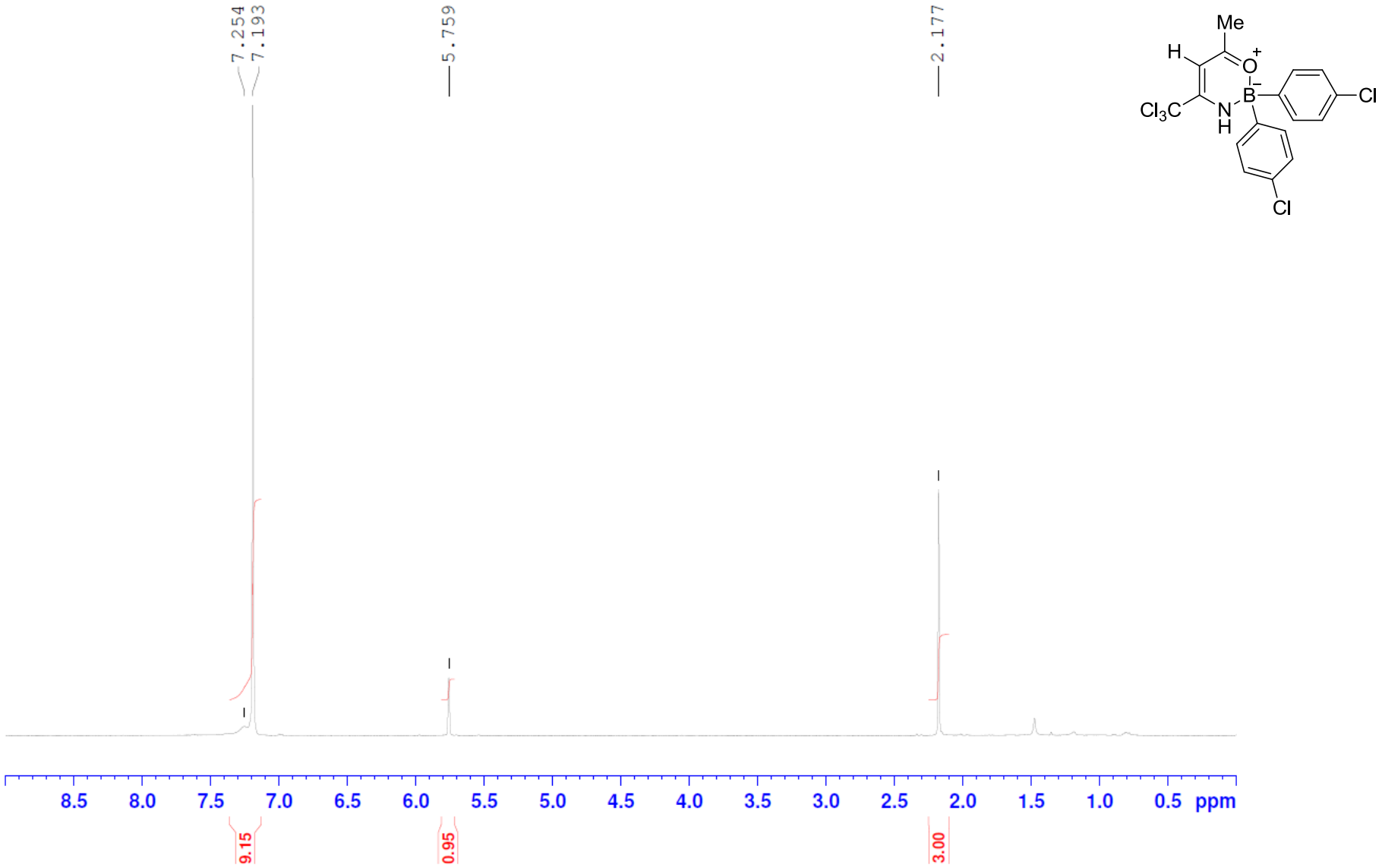

$^{13}$C NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2-(phenyl)-2-(*p*-tolyl)-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC33)



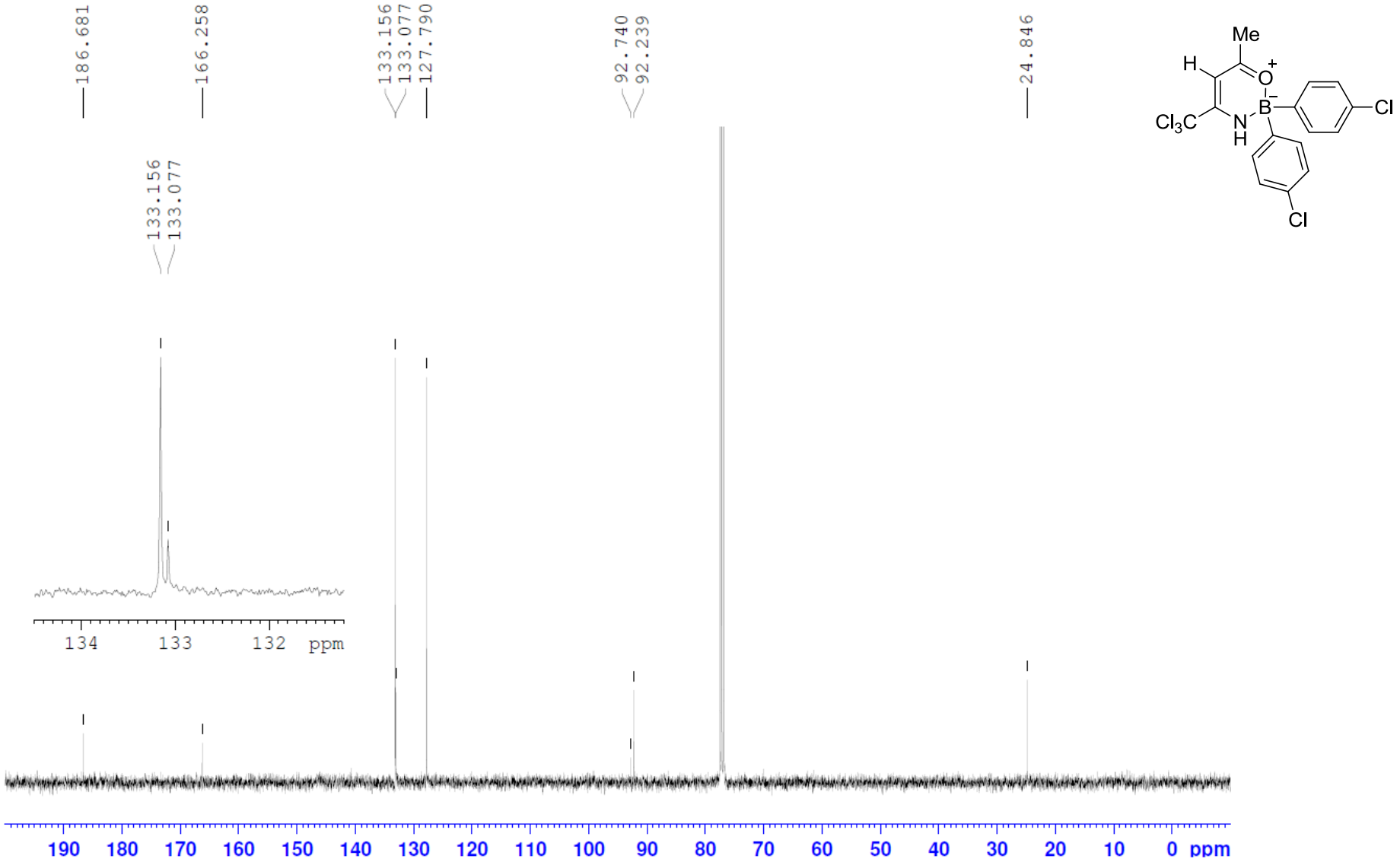

$^{1}$H NMR spectrum ($CDCl_3$) of 2,2-bis(4-fluorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC35)



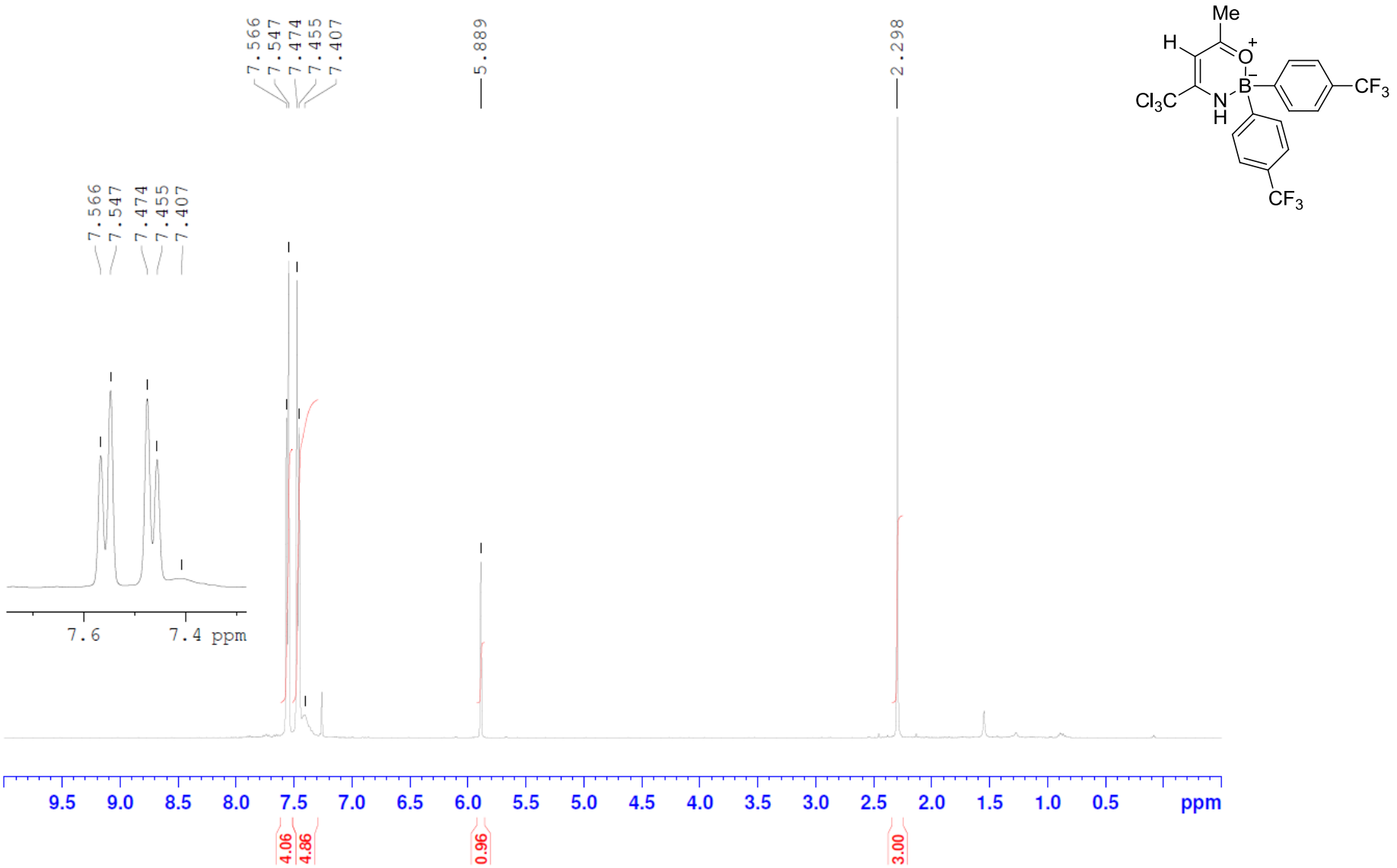

$^{13}C$ NMR spectrum ($CDCl_3$) of 2,2-bis(4-fluorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC35)



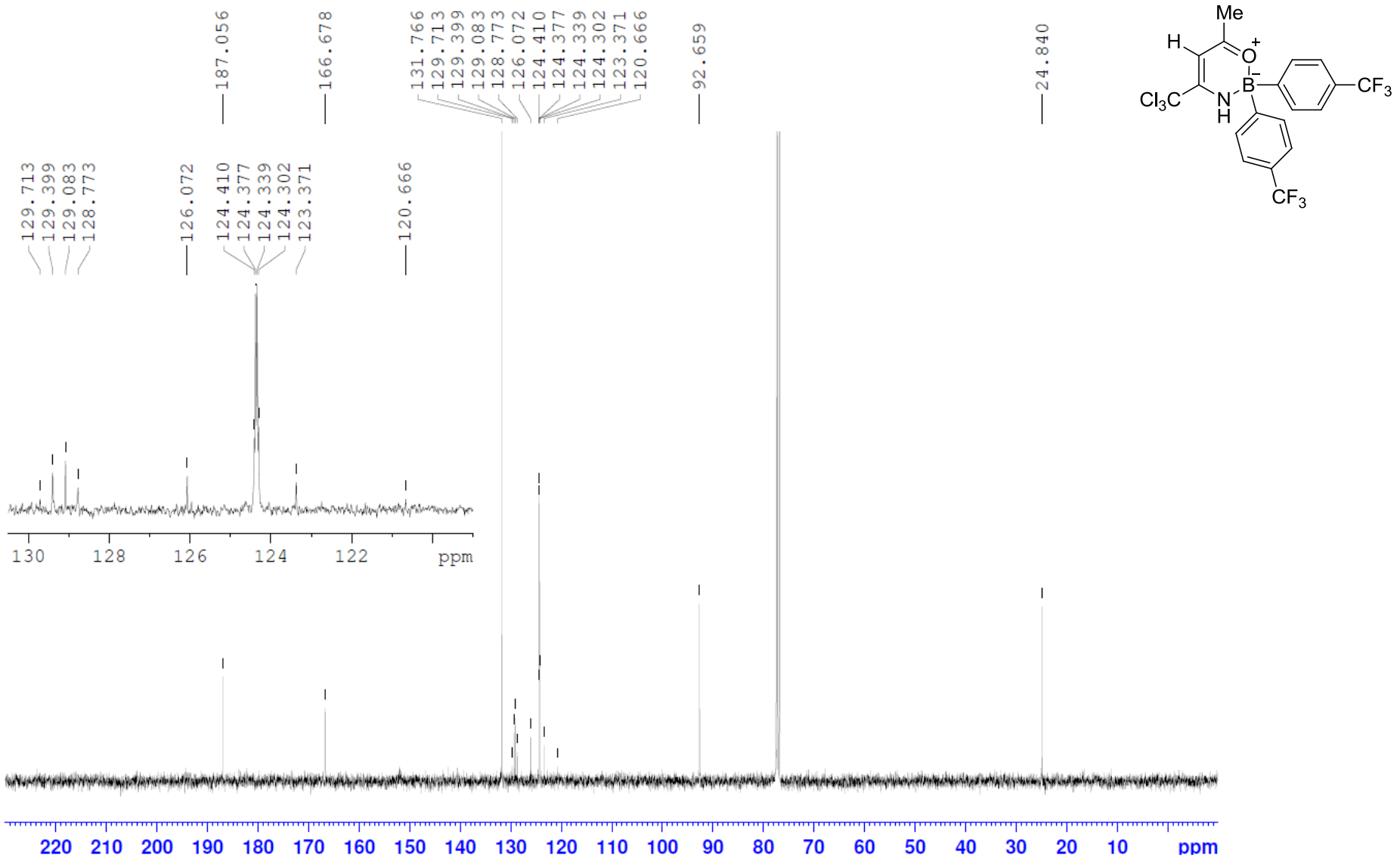

$^{1}$H NMR spectrum ($CDCl_3$) of 2,2-bis(4-chlorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC36)



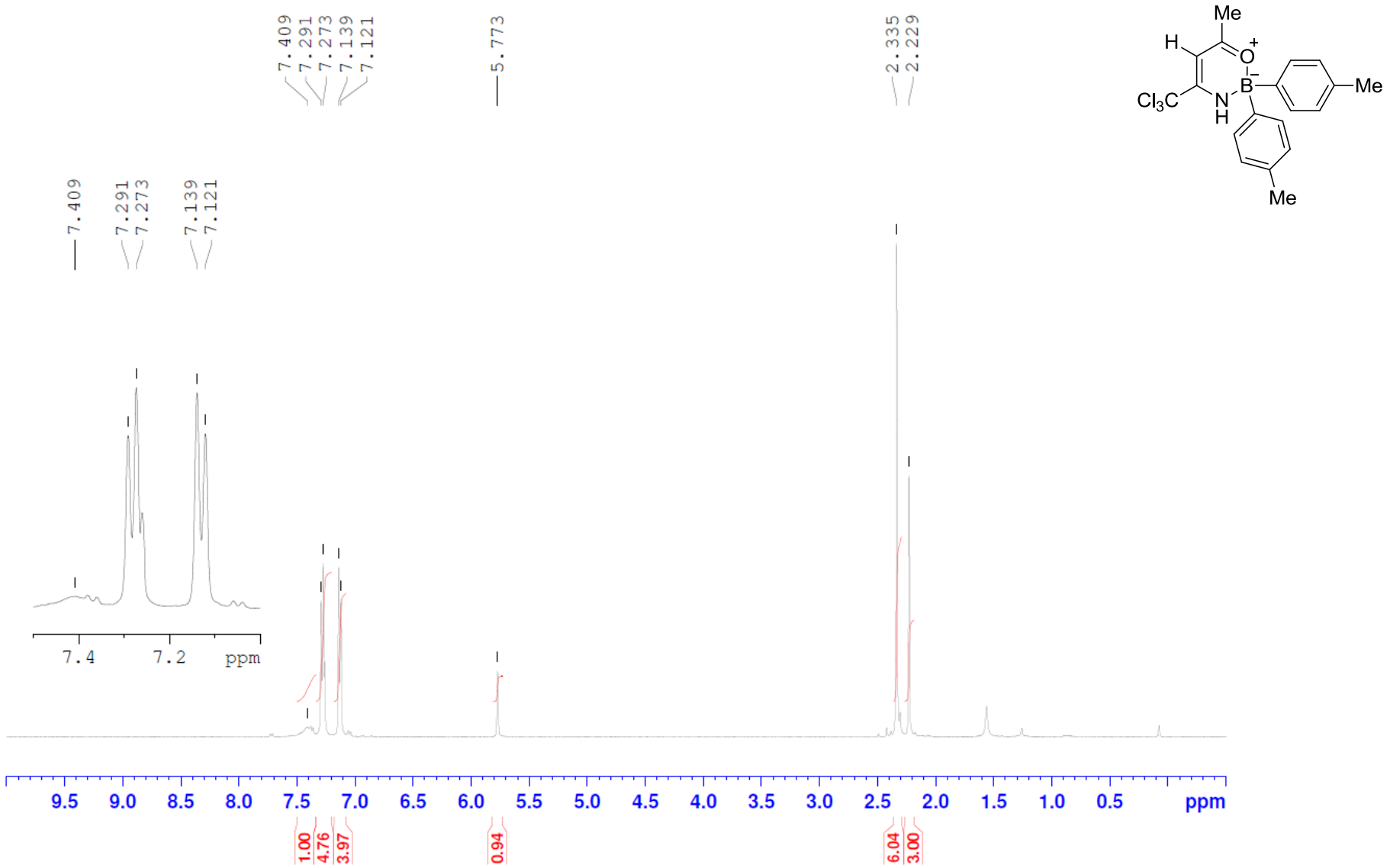

$^{13}$C NMR spectrum ($CDCl_3$) of 2,2-bis(4-chlorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC36)



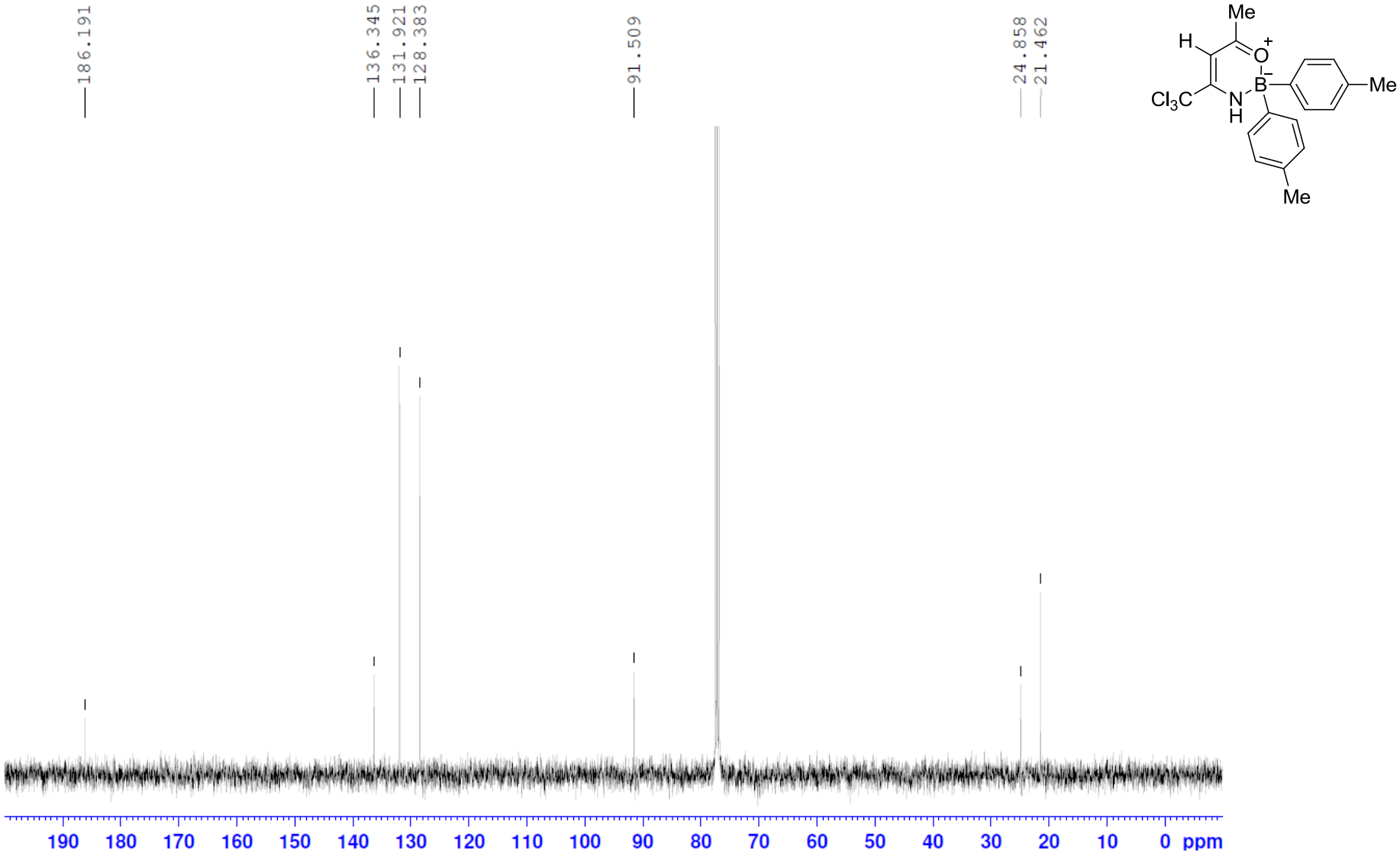

$^{1}$H NMR spectrum ($CDCl_3$) of 6-methyl-4-(trichloromethyl)-2,2-bis(4-(trifluoromethyl)phenyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC37)



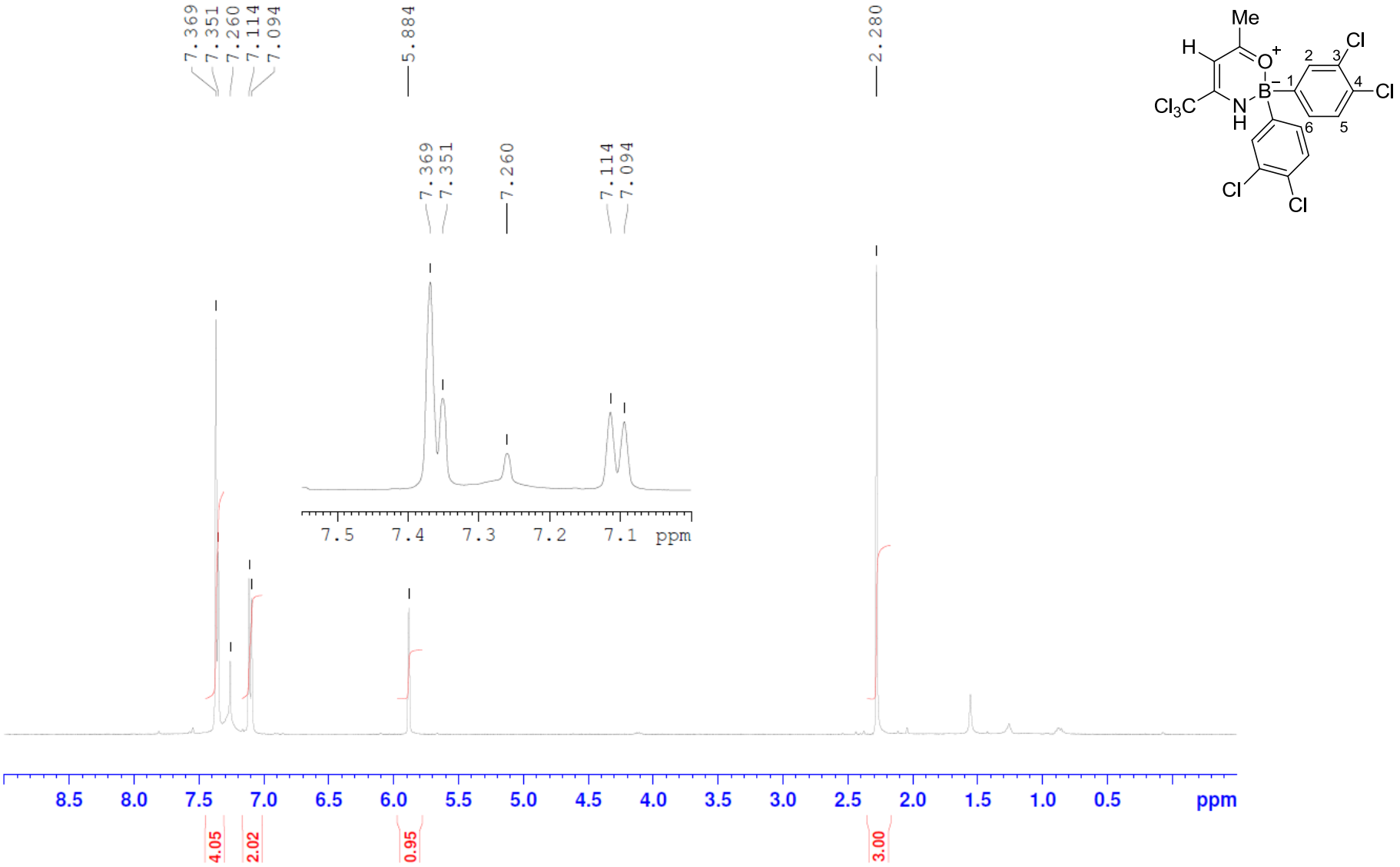

$^{13}C$ NMR spectrum ($CDCl_3$) of 6-methyl-4-(trichloromethyl)-2,2-bis(4-(trifluoromethyl)phenyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC37)



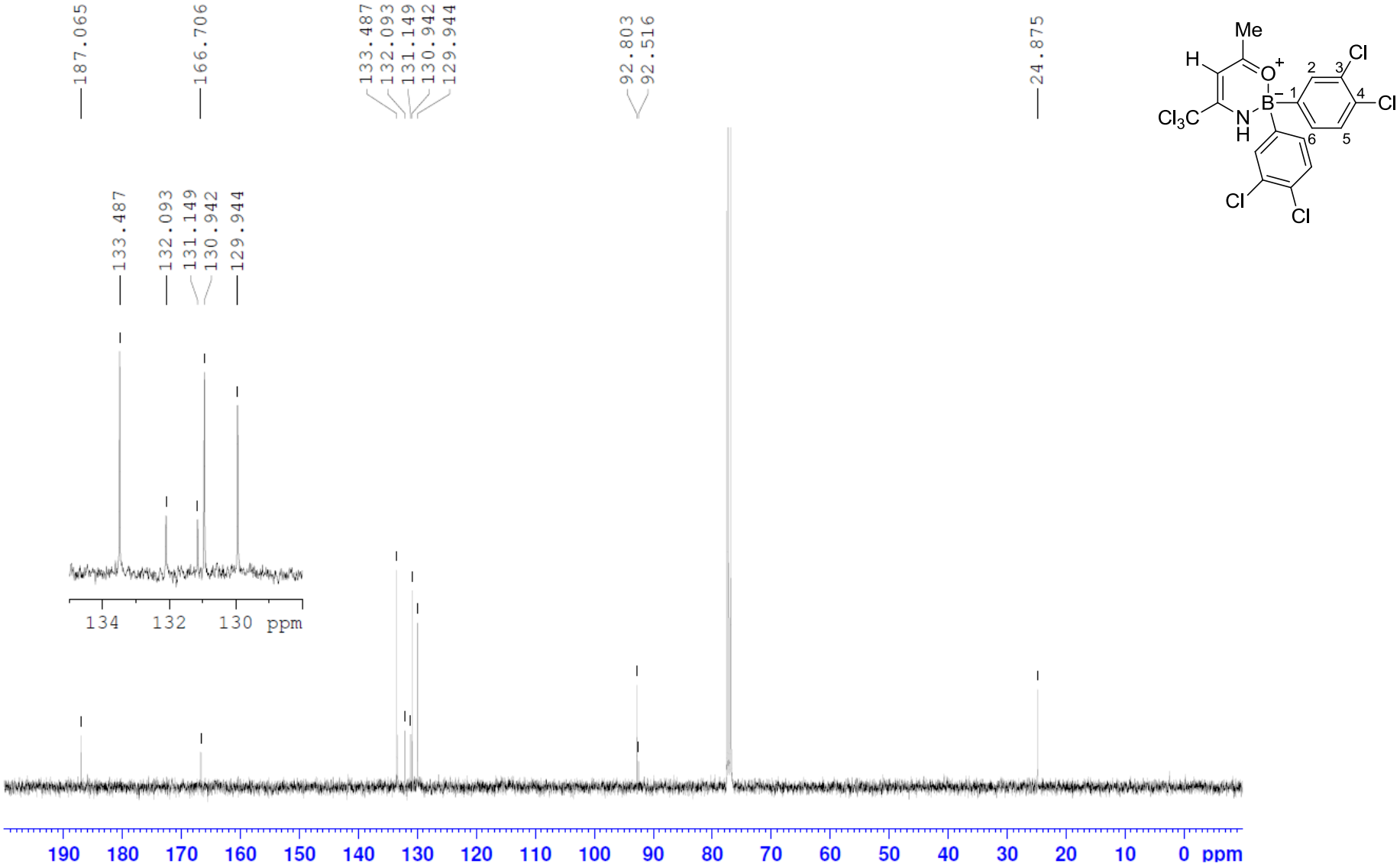

$^{1}$H NMR spectrum ($CDCl_3$) of 2,2-bis(4-methylphenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC38)



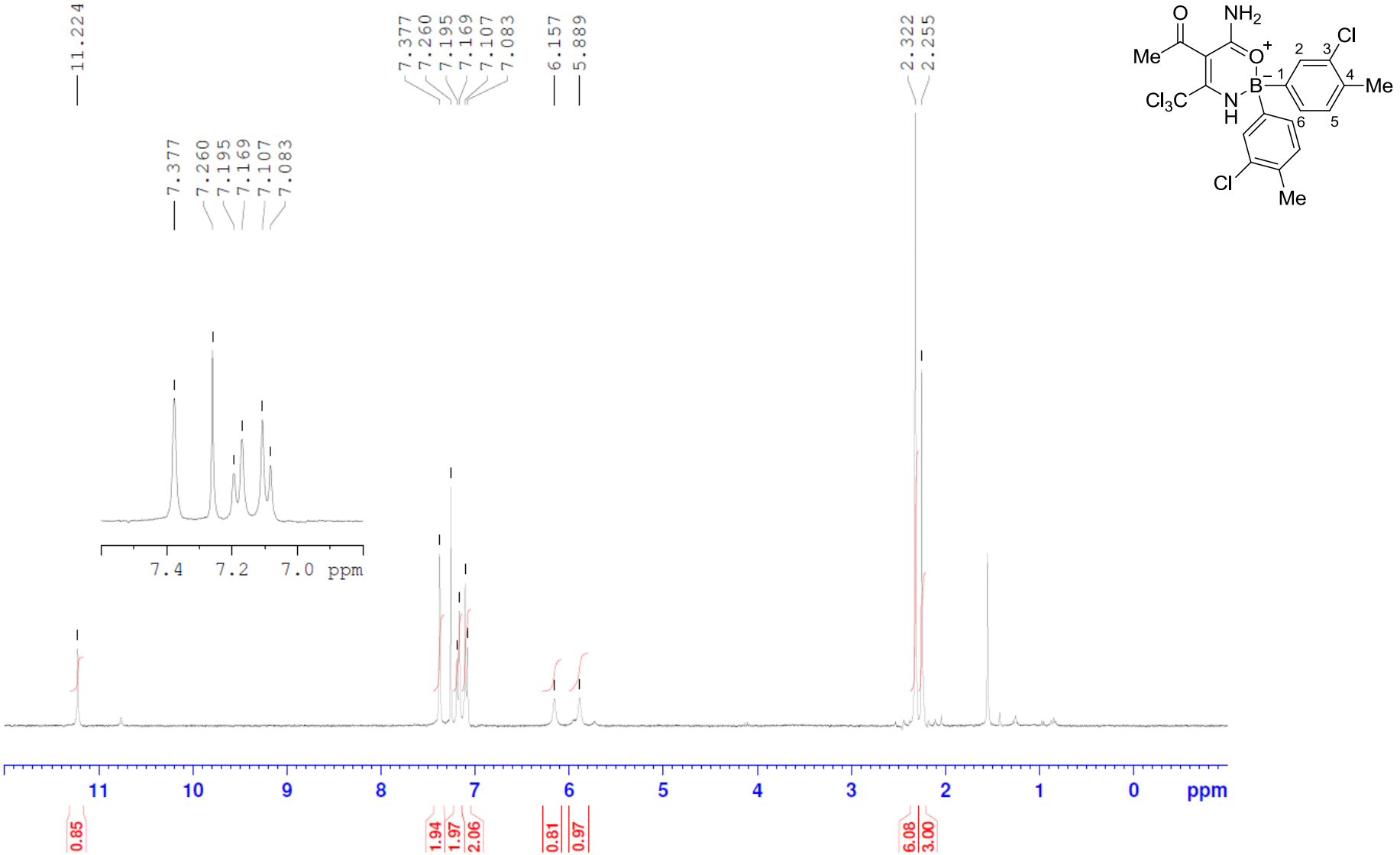

$^{13}C$ NMR spectrum ($CDCl_3$) of 2,2-bis(4-methylphenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC38)




$^{1}$H NMR spectrum ($CDCl_3$) of 2,2-bis(3,4-dichlorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC39)




$^{13}C$ NMR spectrum ($CDCl_3$) of 2,2-bis(3,4-dichlorophenyl)-6-methyl-4-(trichloromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC39)

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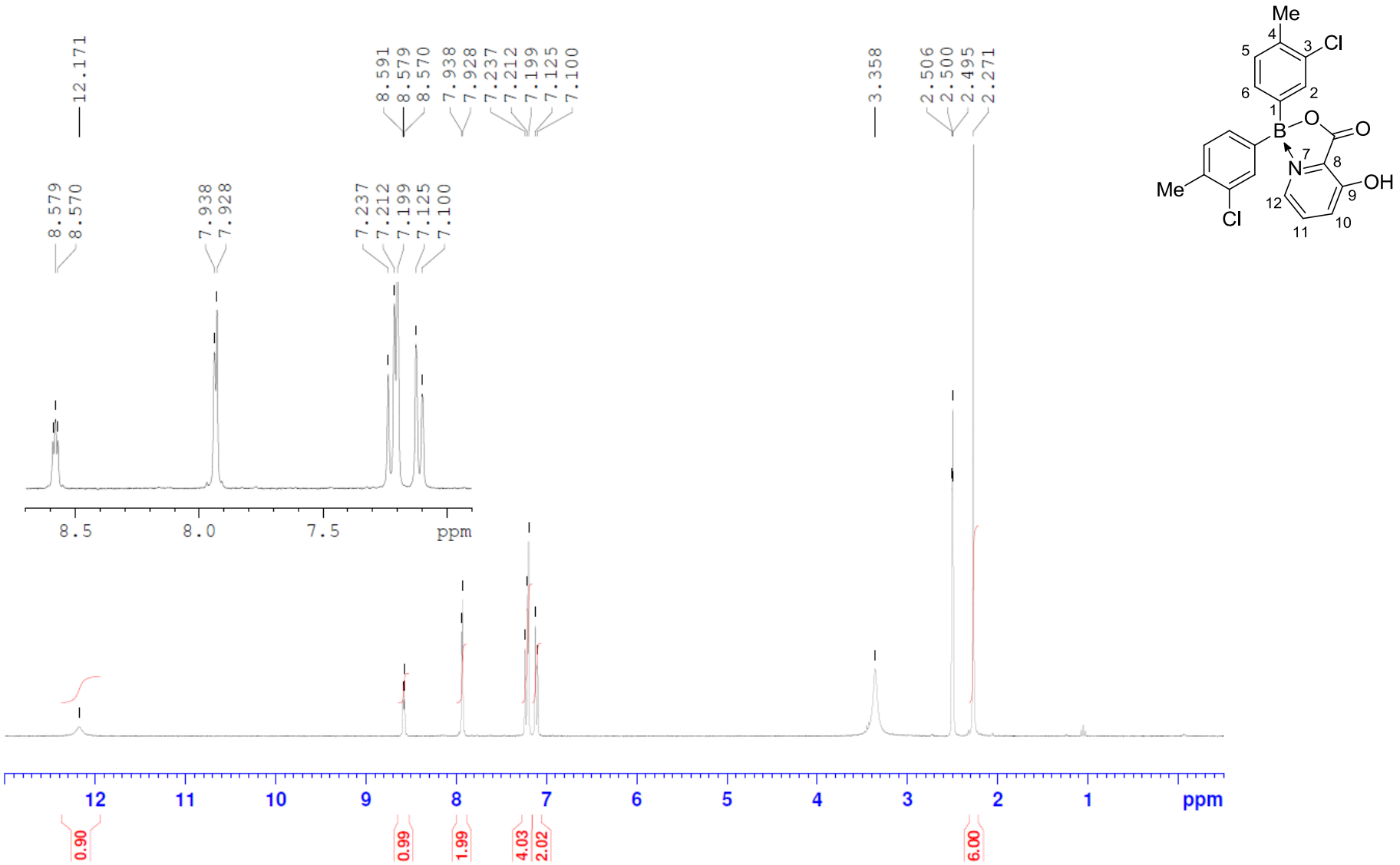
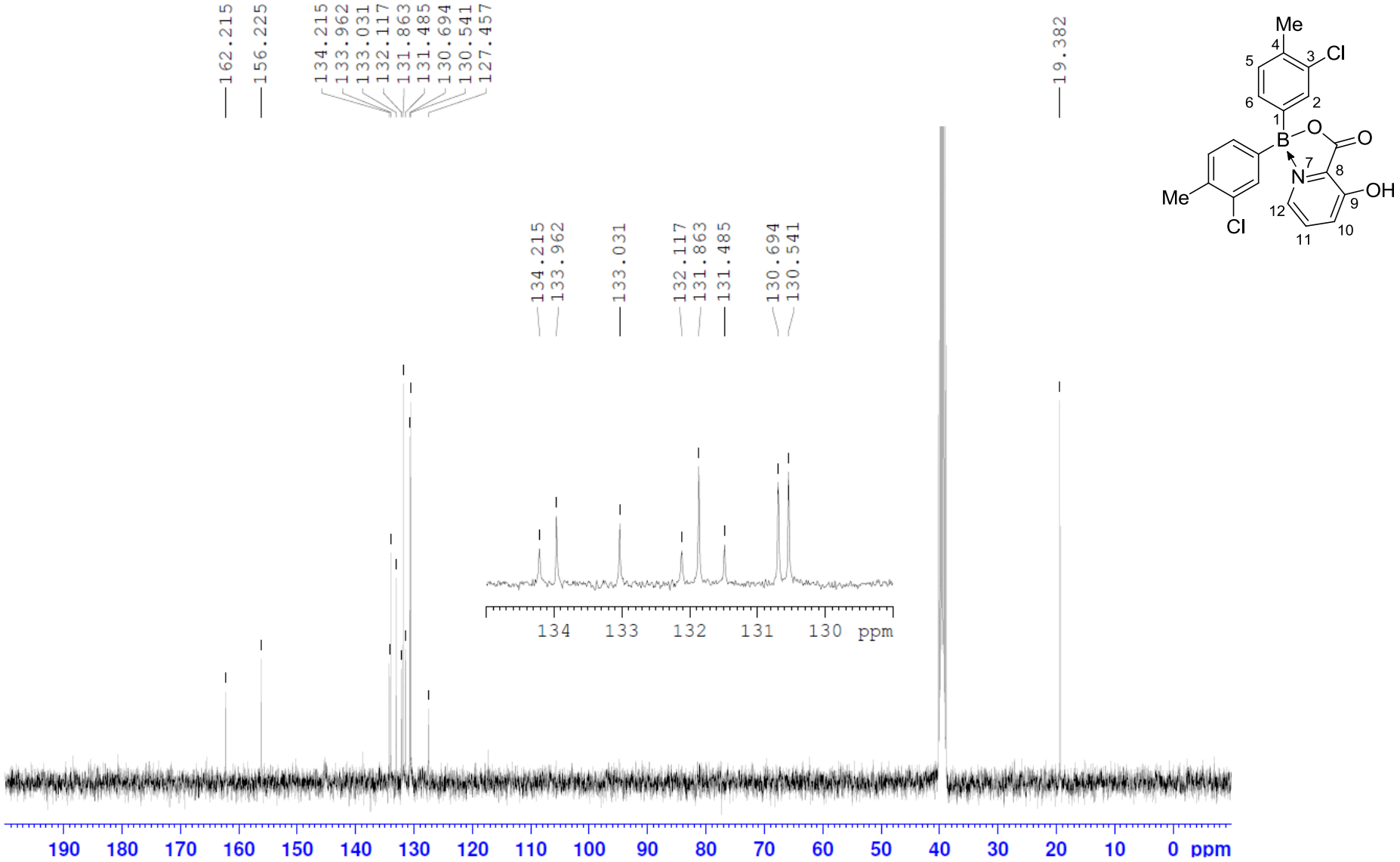
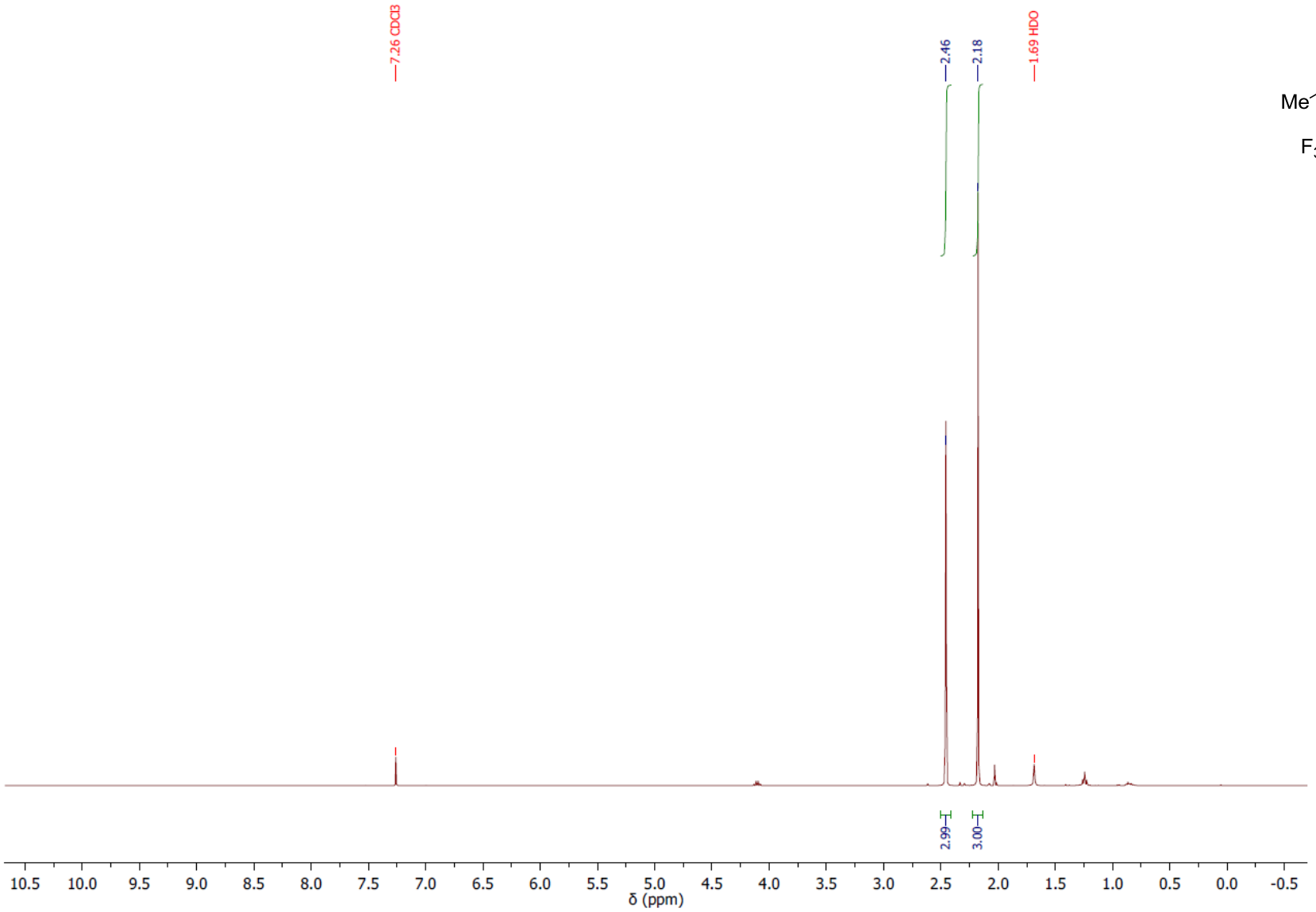
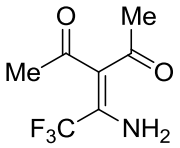



$^{1}$H NMR spectrum (DMSO-$d_6$) of 3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl) borane (AN0128)

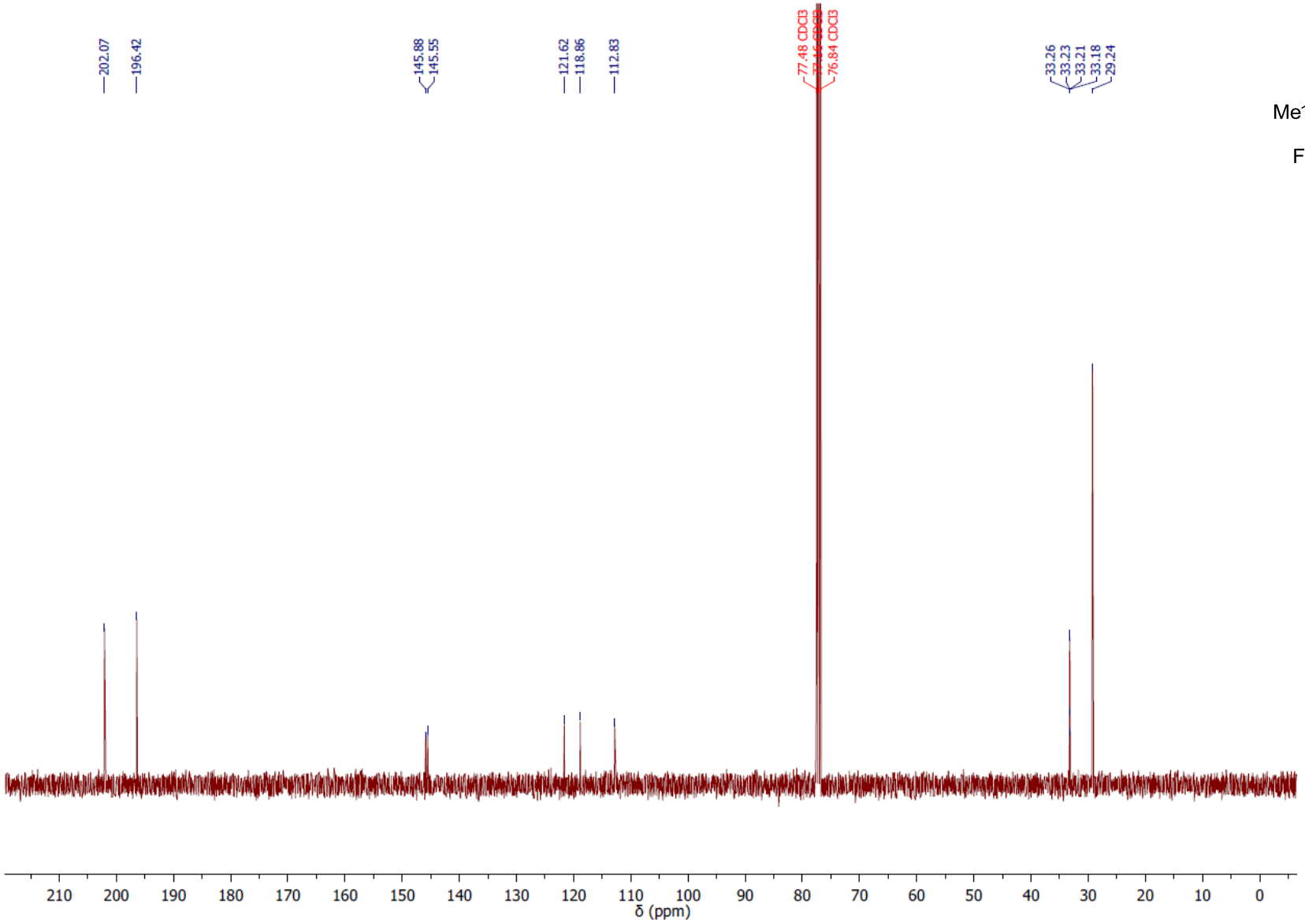



$^{13}$C NMR spectrum (DMSO-$d_6$) of 3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl) borane (AN0128)

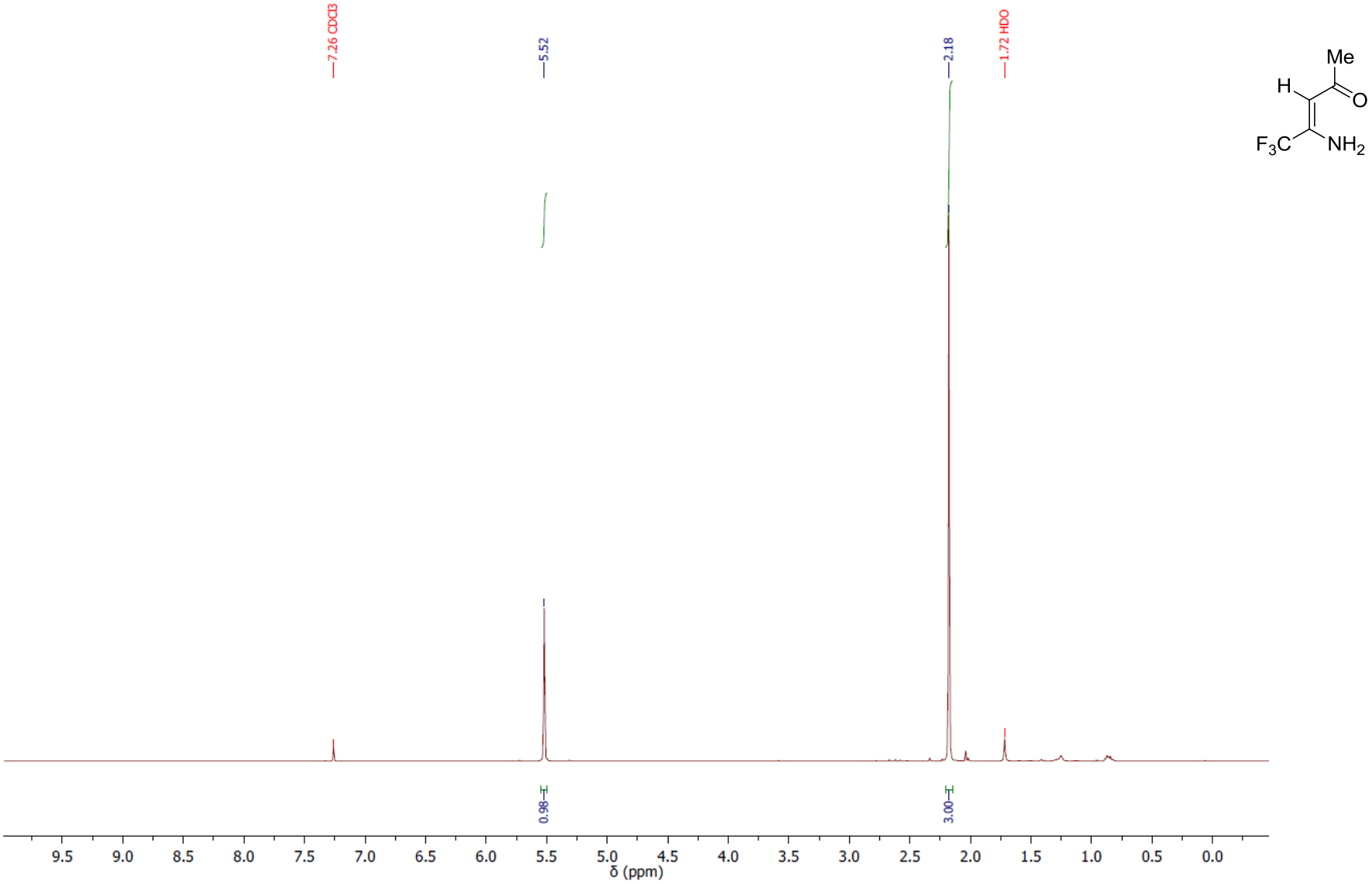


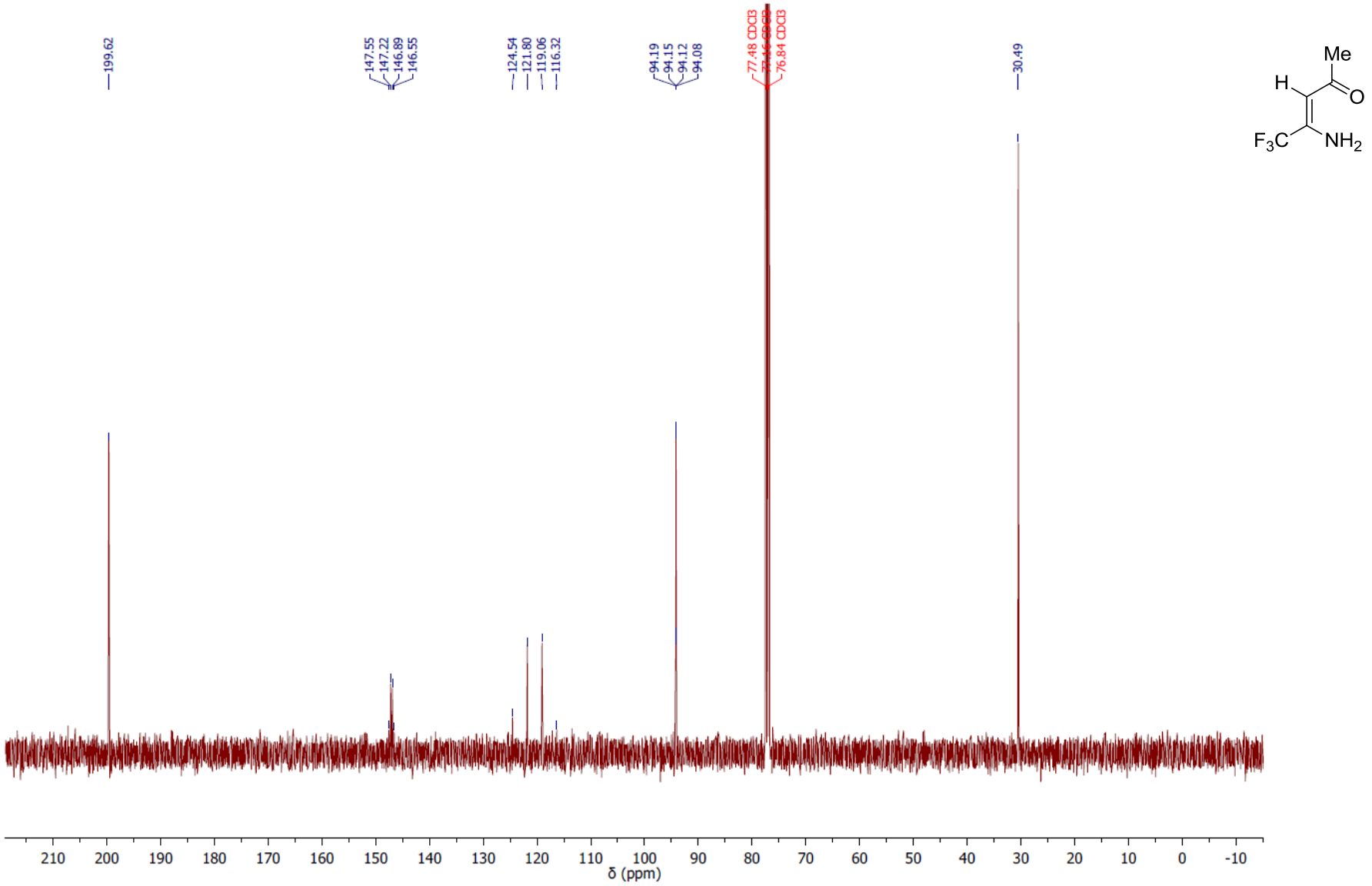

$^{1}$H NMR spectrum ($CDCl_3$) of 3-(1-amino-2,2,2-trifluoroethylidene)pentane-2,4-dione (**8**)



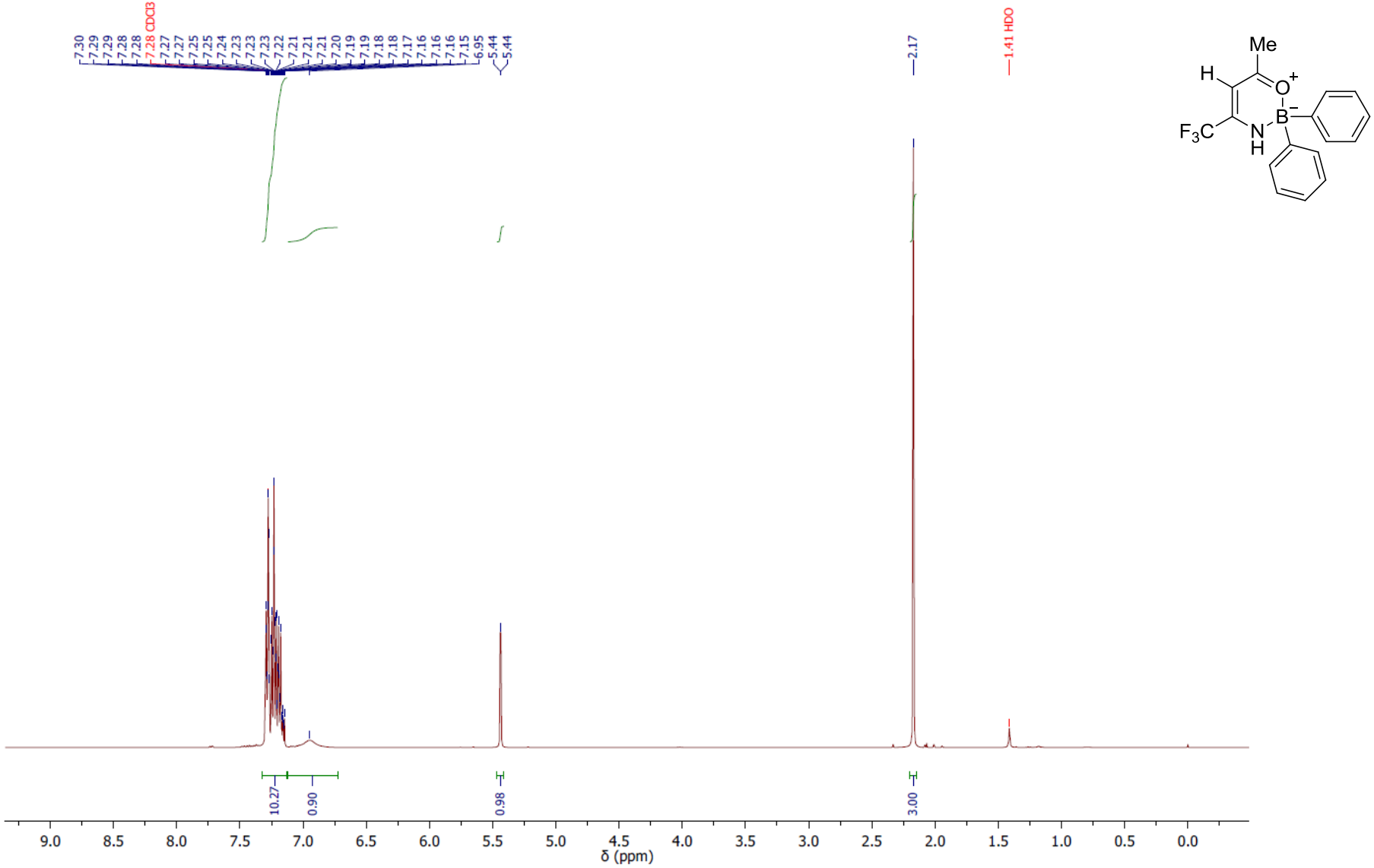

$^{13}C$ NMR spectrum ($CDCl_3$) of 3-(1-amino-2,2,2-trifluoroethylidene)pentane-2,4-dione (**8**)



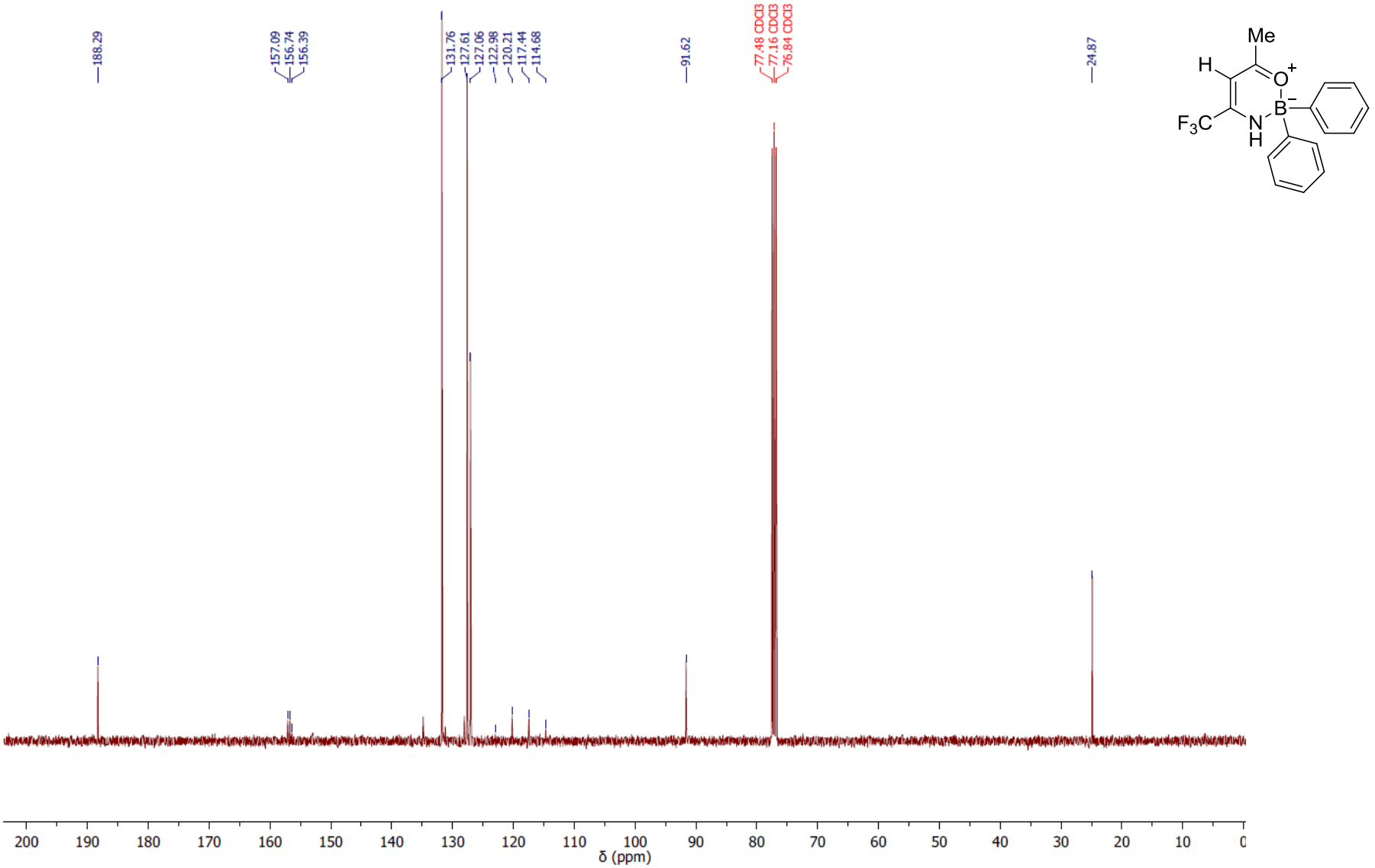

$^{1}$H NMR spectrum ($CDCl_3$) of (*Z*)-4-amino-5,5,5-trifluoropent-3-en-2-one (**10**)



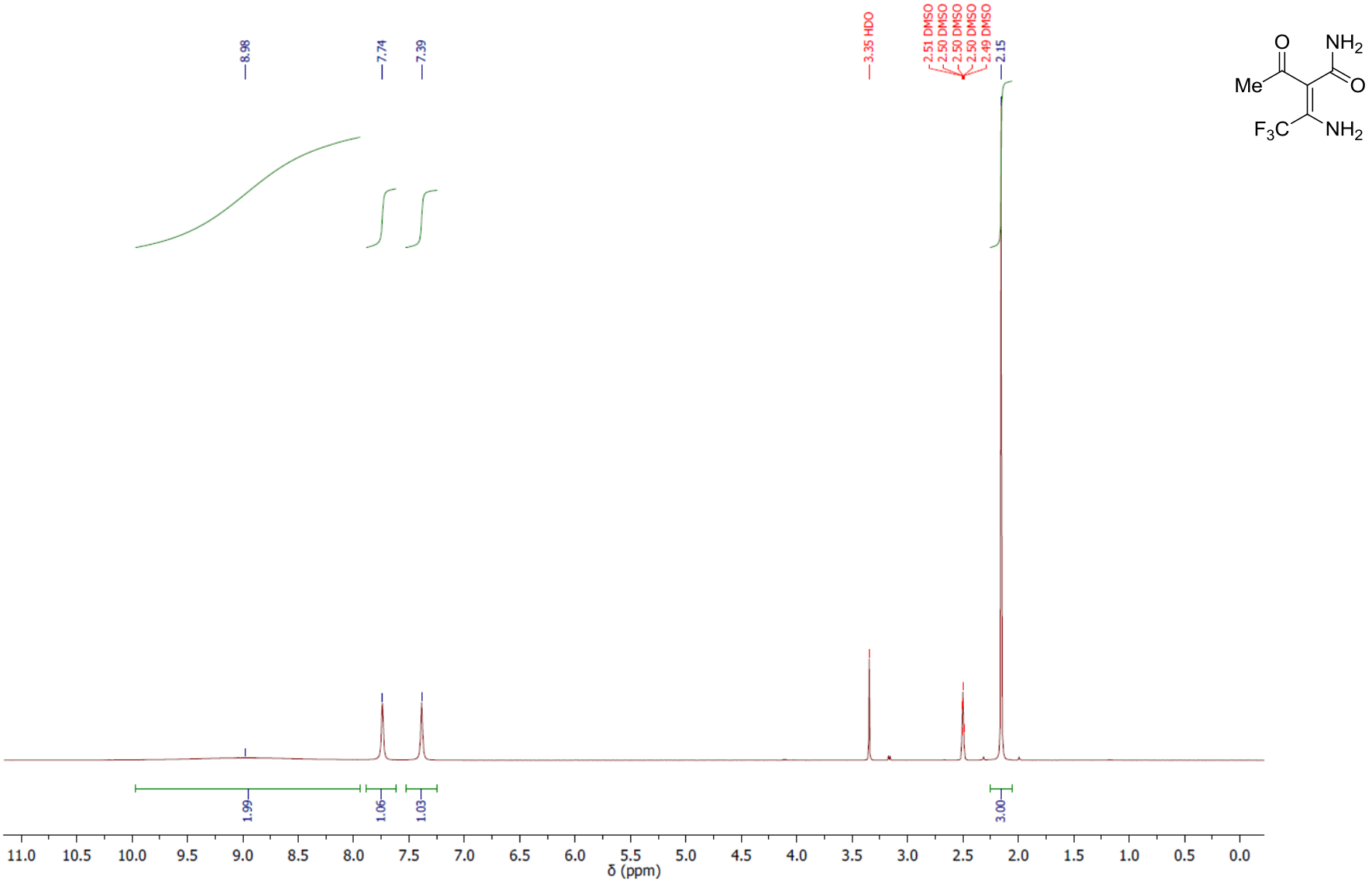

$^{13}C$ NMR spectrum ($CDCl_3$) of (*Z*)-4-amino-5,5,5-trifluoropent-3-en-2-one (**10**)



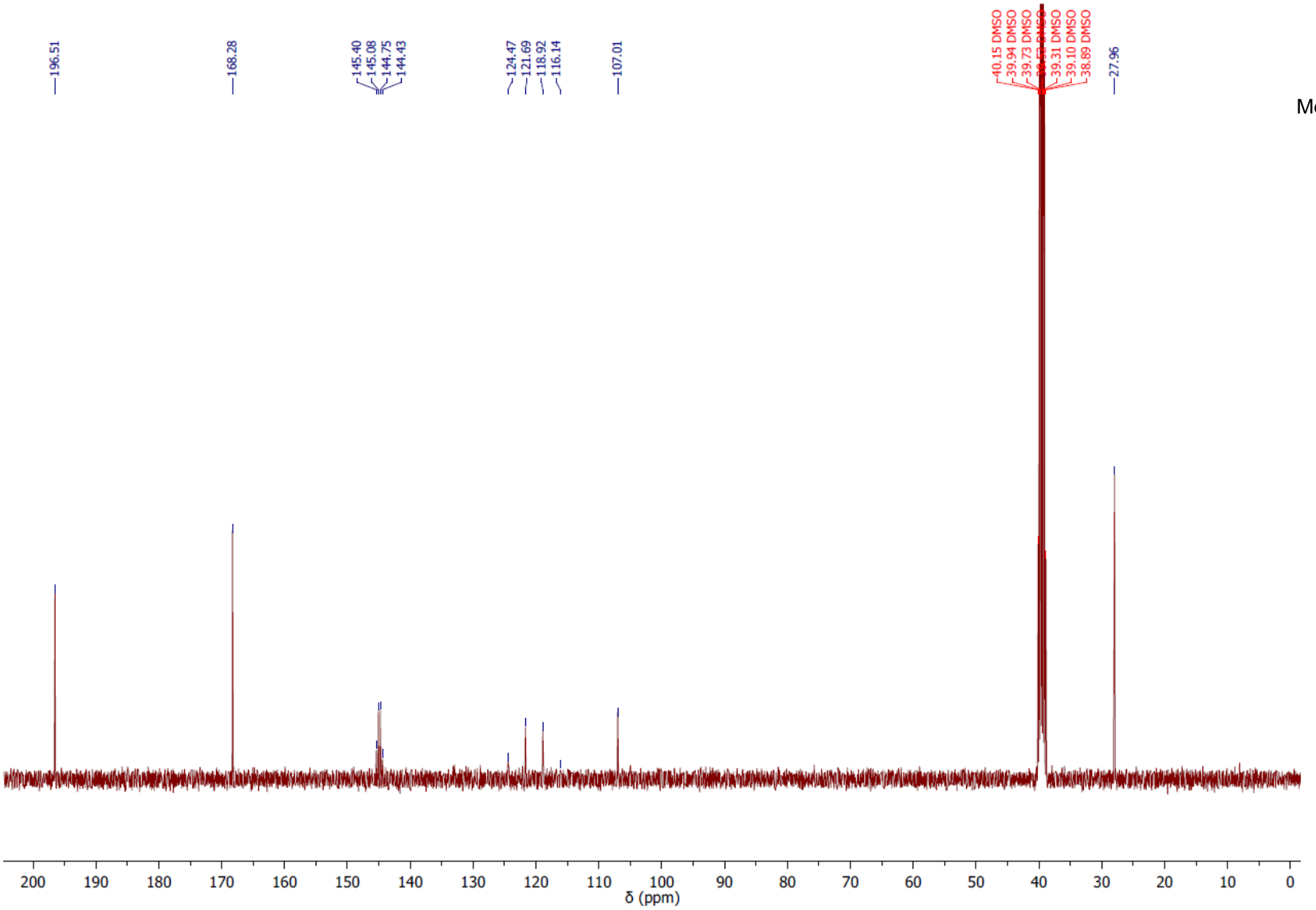

$^{1}$H NMR spectrum ($CDCl_3$) of 6-methyl-2,2-diphenyl-4-(trifluoromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC41)




$^{13}C$ NMR spectrum ($CDCl_3$) of 6-methyl-2,2-diphenyl-4-(trifluoromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC41)




$^{1}$H NMR spectrum (DMSO-$d_6$) of (*Z*)-2-acetyl-3-amino-4,4,4-trifluorobut-2-enamide (**9**)




$^{13}$C NMR spectrum (DMSO-$d_6$) of (*Z*)-2-acetyl-3-amino-4,4,4-trifluorobut-2-enamide (**9**)

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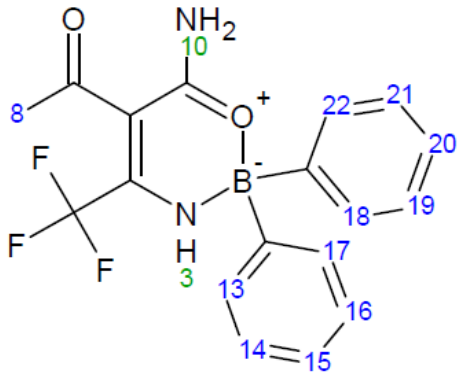


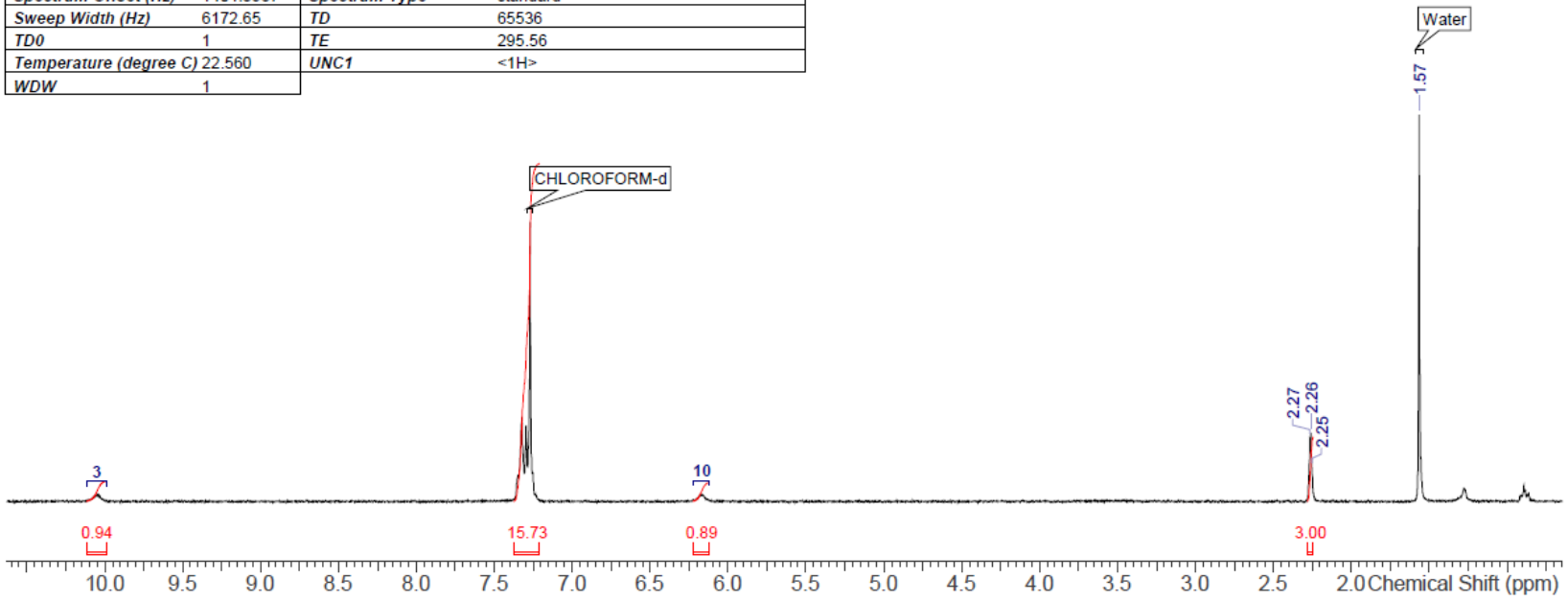


$^{1}$H NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2,2-diphenyl-4-(trifluoromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC42)

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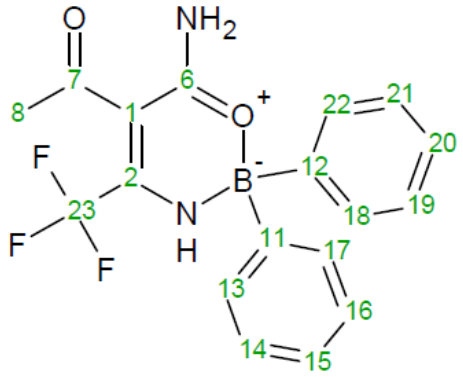


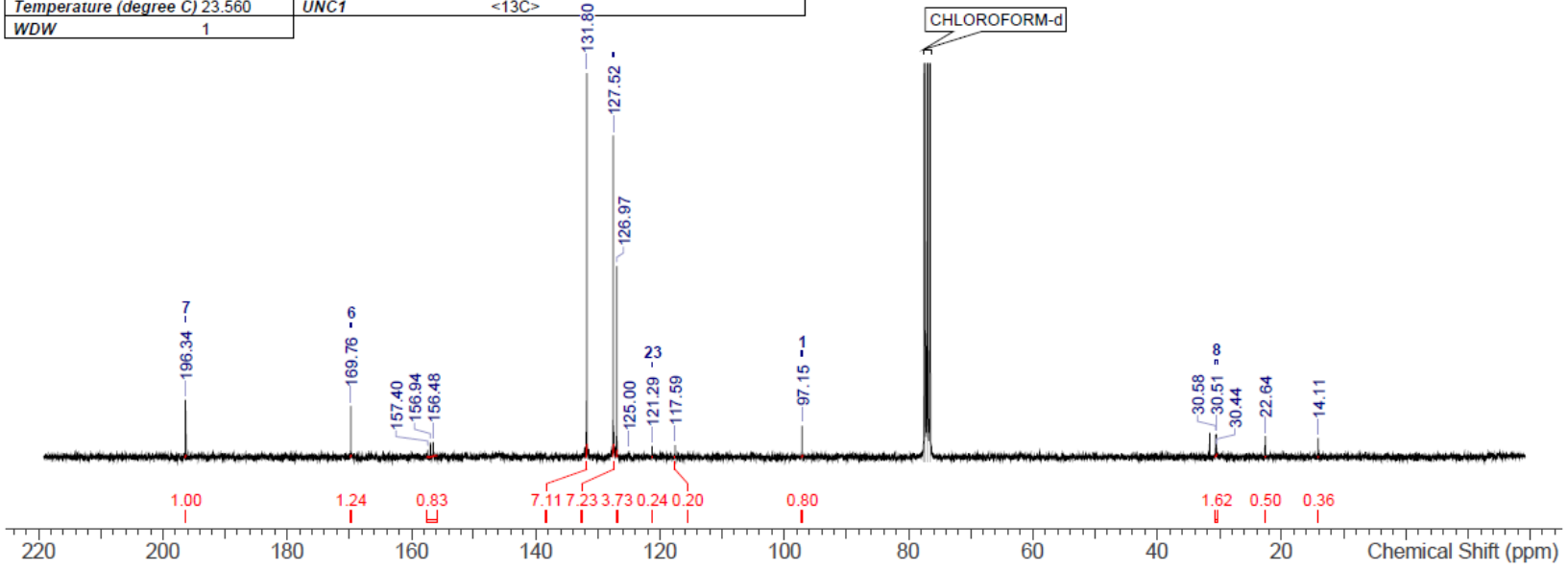


$^{13}$C NMR spectrum ($CDCl_3$) of 5-acetyl-6-amino-2,2-diphenyl-4-(trifluoromethyl)-2,3-dihydro-1,3,2-oxazaborinin-1-ium-2-uide (NBC42)