Supplementary Information

Synthesis of regio- and stereoselectively deuterium-labelled derivatives of L-glutamate semialdehyde for studies on carbapenem biosynthesis

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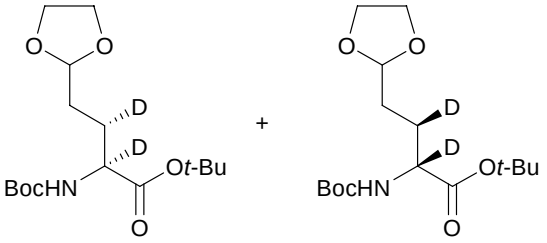
Additional Syntheses

***tert*-Butyl *N*-Boc-glycinate 12.**[1] To a solution of *N*-Boc-glycine **11**[2] (12.6 g, 71.9 mmol) and *t*-BuOH (90 mL, 0.94 mol) in toluene (160 mL), *N*,*N*-DMF-dineopentylacetal (60 mL, 0.21 mol) was added dropwise over 30 min at reflux temperature. The reaction mixture was heated under reflux for a further 5 h. After cooling to rt, the solution was washed with saturated Na_2CO_3 solution (2 x 150 mL) and water (2 x 150 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant yellow-brown oil (14.4 g) was purified by short column chromatography (hexane-EtOAc, 10 : 1) to give **12** (12.4 g, 75 %) as a colourless oil; δ_H (400 MHz; DMSO-d_6) 1.38 (9 H, s, *t*-Bu), 1.40 (9 H, s, *t*-Bu), 3.53 (2 H, d, *J* 6.0, NHCH_2) and 7.10 (1 H, t, J 6.0, NHCH_2); δ_C (101 MHz; DMSO-d_6) 27.7, 28.2, 42.6, 78.0, 80.4, 155.8 and 169.5; *m/z* (HR-ESI$^+$) 254.1363 (M+Na$^+$. $C_{11}H_{21}NNaO_4$ requires 254.1363).

[1] While this compound is known (see *e. g.* C. A. M. Afonso, *Tetrahedron Lett.*, 1995, **36**, 8857), it should be noted that the method of preparation described here is very convenient and turned out to be superior to some literature procedures.
[2] R. Houssin, J.-L. Bernier and J.-P. Hénichart, *Synthesis*, 1988, 259.

2-(1,3-Dioxolan-2-yl)-ethanal 15.[3] To a solution of methyl (1,3-dioxolan-2-yl)-acetate **17**[4] (437 mg, 3.24 mmol) in anhydrous CH_2Cl_2 (30 mL), a 1.0 M solution of DIBAL-H in THF (3.56 mL, 3.56 mmol) was added at -78 °C. The reaction mixture was stirred at -78 °C, and after 2 h and 4 h, respectively, the same volume of DIBAL-H solution was added again. After an overall reaction time of 6 h, the reaction was quenched by sequential addition of MeOH (3 mL) and water (1.5 mL) at -78 °C. The reaction mixture was warmed to rt and filtered through a thin pad of silica (Et_2O). The filtrate was evaporated to give crude **15** (346 mg, estimated purity > 85 % according to ^{1}H-NMR) as a yellowish oil. Due to significant sensitivity of the aldehyde, it was used directly in the subsequent Horner-Wadsworth-Emmons reaction after NMR characterisation; δ_H (400 MHz; $CDCl_3$) 2.76 (2 H, dd, *J* 2.0 and 4.5, CHC*H*$_2$), 3.82-4.02 (4 H, m, OC*H*$_2$C*H*$_2$O), 5.24 (1 H, t, *J* 4.5, C*H*CH$_2$) and 9.77 (1 H, t, *J* 4.5, CH$_2$C*H*O); δ_C (101 MHz; $CDCl_3$) 47.3, 65.1, 100.1 and 199.3.

Racemic *tert*-Butyl (2*S*,3*S*)- and (2*R*,3*R*)-2-(*tert*-butyloxycarbonyl-amino)-2,3-[$^2H_2$]-4-(1,3-dioxolan-2-yl)-butanoate *rac*-21. A solution of didehydro-amino acid **(*Z*)-19** (52 mg, 0.16 mmol) and Wilkinson's catalyst (7.3 mg, 7.9 μmol) in $C^2H_3O^2H$ (10 mL) was stirred under an atmosphere of $^2H_2$ (1 bar) at rt for 8 days with addition of more Wilkinson's catalyst (6.1 mg, 6.6 μmol) after 4 days. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (hexane-EtOAc, 4 : 1) to give ***rac*-21** (40 mg, 75 %) as a colourless oil; spectroscopic data were identical with those for **21**.



***rac*-21**

Assignment of the absolute configurations of 21 and 22

It is established that rhodium-catalysed homogenic hydrogenation reactions occur in a *syn* manner.[5] The (*Z*) stereochemistry of the protected didehydro amino acid precursors **(*Z*)-19**

[3] While this compound is known (see *e. g.* A. D. Baxter, F. Binns, T. Javed, S. M. Roberts, P. Sadler, F. Scheinmann, B. J. Wakefield, M. Lynch and R. F. Newton, *J. Chem. Soc. Perkin Trans. I*, 1986, 889), it should be noted that the method of preparation described here is very convenient and turned out to be superior to some literature procedures.
[4] T. Hosokawa, T. Ohta, S. Kanayama and S.-I. Murahashi, *J. Org. Chem.*, 1987, **52**, 1758.
[5] see *e. g.* W. S. Knowles, *Acc. Chem. Res.*, 1983, **16**, 106.

and **(*Z*)-20** was determined unambigously by NMR analyses (see main text). Hence, the relative stereochemistry of the stereocentres at the C-2- and C-3-positions of **21** and **22** could be assigned. The only stereochemical aspect unassigned therefore was the L-configuration (2*S*) of both **21** and **22**. One source of evidence comes from the knowledge that the chiral hydrogenation catalyst (+)-1,2-bis-((2*S*,5*S*)-2,5-dimethyl-phospholano)-benzene (cycloocta-diene)-rhodium(I) tetrafluoroborate ((*S*,*S*)-Me-DUPHOS-Rh) is known to yield L-amino acids.[6,7]

Attempts were made to determine the enantiomeric purities of asymmetric deuteration and hydrogenation products **21** and **22** by chiral HPLC. First, the separation of racemic reference ***rac*-21** was evaluated. Although a range of standard chiral columns and elution conditions were investigated (Chiralpak™ IA and IC and Chiralcel™ OD columns, Chiral Technologies Europe, *n*-hexane/*iso*-propanol eluents), satisfactory separations were not obtained. Hence, an alternative method to (indirectly) assess the enantiomeric purities of **21** and **22** using the stereochemical preference of CarB (*via* assays with the deprotected forms **6** and **7**) was employed.

Conversion of L-GSA **1** with CarB was found to occur quantitatively under standard assay conditions (*i. e.* no unconverted GSA was detected by LC-MS assays). However, incubation of D-GSA did not result in formation of a *t*-CMP derivative.[8] Thus, because only L-GSA **1** is a substrate of CarB, complete conversion of a sample of unkown enantiomeric purity would imply that no detectable amount of the D-GSA enantiomer was present. Hence, any detectable unconverted GSA found in CarB assays with **6** and **7** should represent the D-isomer.

When ***rac*-6** (obtained from ***rac*-21** *via* standard deprotection, see Experimental Section in the main text) was analysed by LC-MS, a single peak with *m/z* = 132 ([M-H]$^-$ for [$^2H_2$]-GSA, ESI$^-$) was observed (*vide infra*, Fig. S1, bottom). After ***rac*-6** had been incubated with CarB, a peak corresponding to a *t*-CMP product (*m/z* = 174 [M-H]$^-$ for [$^2H_2$]-*t*-CMP, ESI$^-$) and a peak corresponding to unreacted starting material (*m/z* = 132 [M-H]$^-$ for [$^2H_2$]-GSA, ESI$^-$) were observed (Figure S1, top).

After either **6** or **7** were incubated with CarB and malonyl-CoA for 10 min under standard conditions (Figure S2), no peak corresponding to the mass of the respective GSA derivative was observed by LC-MS. The detection limit for unconverted GSA on the LC-MS system was investigated *via* injection of suitably diluted solutions of **6**. It was found that even 5 % of

[6] M. J. Burk, *J. Am. Chem. Soc.*, 1991, **113**, 8518.
[7] T. Masquelin, E. Broger, K. Müller, R. Schmid and D. Obrecht, *Helv. Chim. Acta*, 1994, **77**, 1395.
[8] J. L. Sorensen, M. C. Sleeman and C. J. Schofield, *Chem. Commun.*, 2005, 1155.

the original concentration of the deprotection solution used for the assays still gave a detectable peak with *m/z* = 132 ([M-H]$^-$). Therefore, because no peak corresponding to either **6** or **7** was observed after CarB incubation, it can be concluded that the enantiomeric ratios of both precursors **21** and **22** are > 95 : 5. This result is in agreement with the excellent stereoselectivities reported for the hydrogenation of *N*-Cbz-protected didehydro-amino acid *tert*-butyl esters in the presence of (*S*,*S*)-Me-DUPHOS-Rh.[9]

Representative CarB/ThnE assays

The CarB assays described above are representative examples of the assay methodology. LC-MS data for the assays with ***rac*-6** and **6** are given below (Figures S1 and S2). Chromatograms for the respective *m/z* values were calculated from the total ion count (TIC). Small changes in retention times were found to occur when using the Primesep 100 column (Sielc).

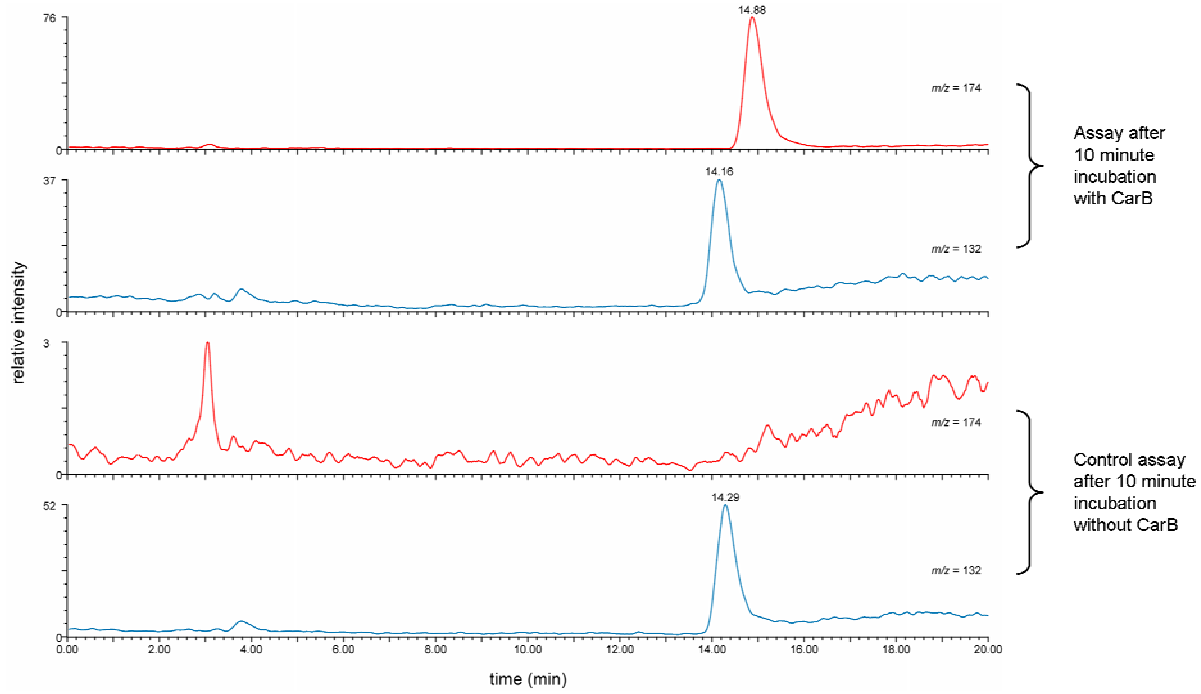


Fig. S1 LC-MS chromatograms for a typical CarB assay employing ***rac*-6**. The blue traces represent unreacted substrates (control assay: GSA derivative ***rac*-6** in racemic form (*m/z* = 132 [M-H]$^-$, ESI$^-$). In a CarB assay, only the D-enantiomer of ***rac*-6** (*m/z* = 132 [M-H]$^-$) remains unreacted. The red traces represent L-configured *t*-CMP derivative **34** (*m/z* = 174 [M-H]$^-$).

[9] T. Masquelin, E. Broger, K. Müller, R. Schmid and D. Obrecht, *Helv. Chim. Acta*, 1994, **77**, 1395.

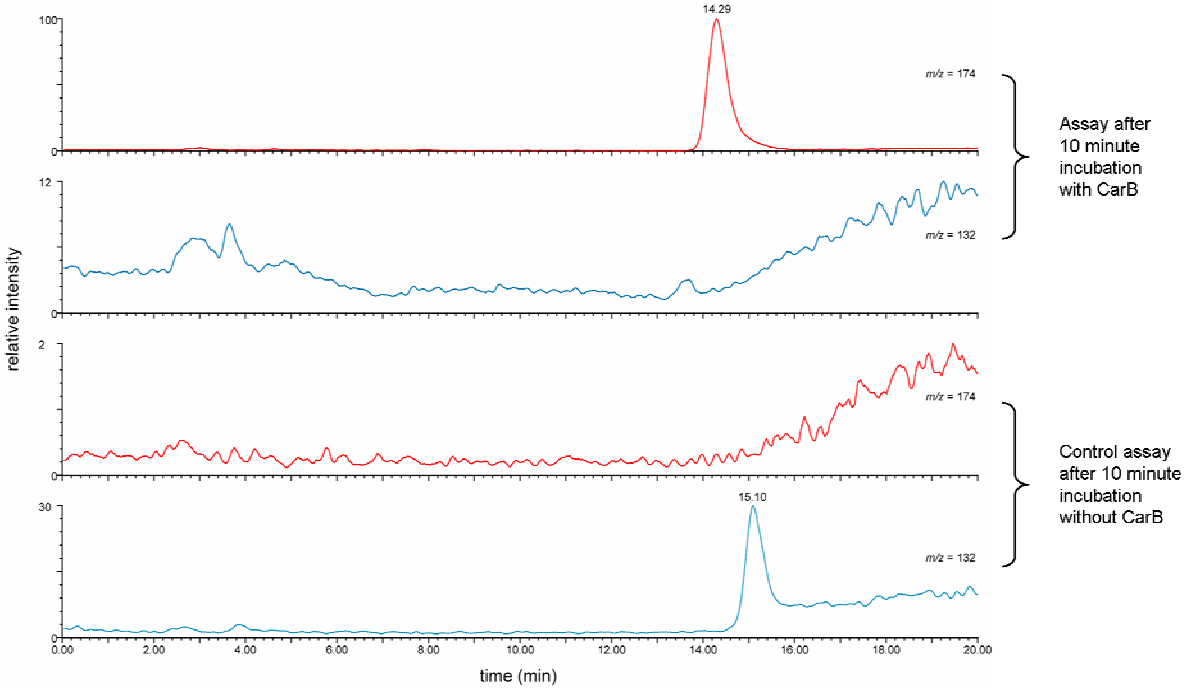


Fig. S2 LC-MS chromatograms for a typical CarB assay employing **6**. The blue traces represent unreacted substrate (labelled L-GSA derivative **6** (*m/z* = 132 $[M-H]^-$, ESI^-), red: *t*-CMP derivative **34** (*m/z* = 174 $[M-H]^-$).

MS data for *t*-CMP products 37, 38 and 4

MS data for the *t*-CMP products **37**, **38** and **4** isolated from ThnE assays with L-GSA derivatives **10**, **8** and **1**, respectively, are given in Figure S3. The MS data correspond to the ^{1}H-NMR data for the same compounds displayed in Figure 3 (main text).

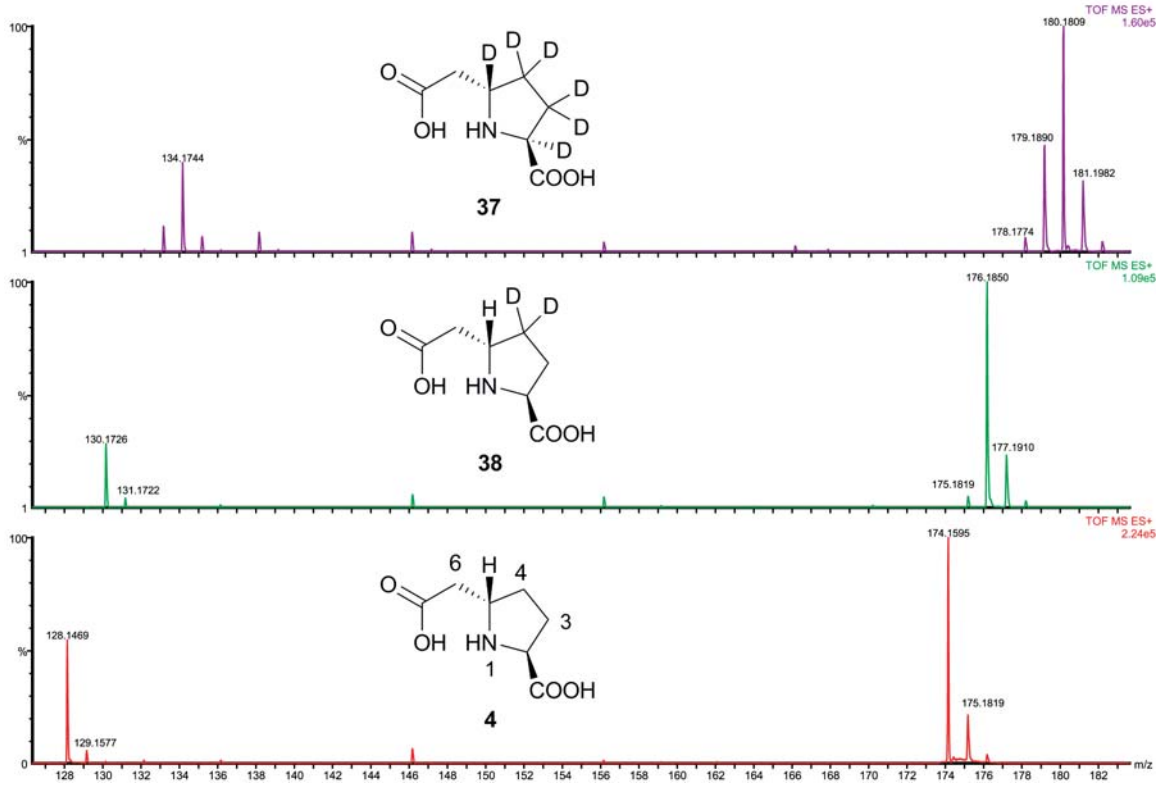


Fig. S3 ESI-MS data of the deuterium-labelled *t*-CMP products **37** (purple trace, top) and **38** (green trace, middle) as well as of non-labelled *t*-CMP **4** (red trace, bottom) resulting from incubations of the appropriate GSA derivatives with ThnE and malonyl-CoA (ESI^{+} conditions).

Additional spectroscopic data for compound 38

The structural assignment of *t*-CMP product **38** was further supported by 2D-NMR analysis (Figure S4).

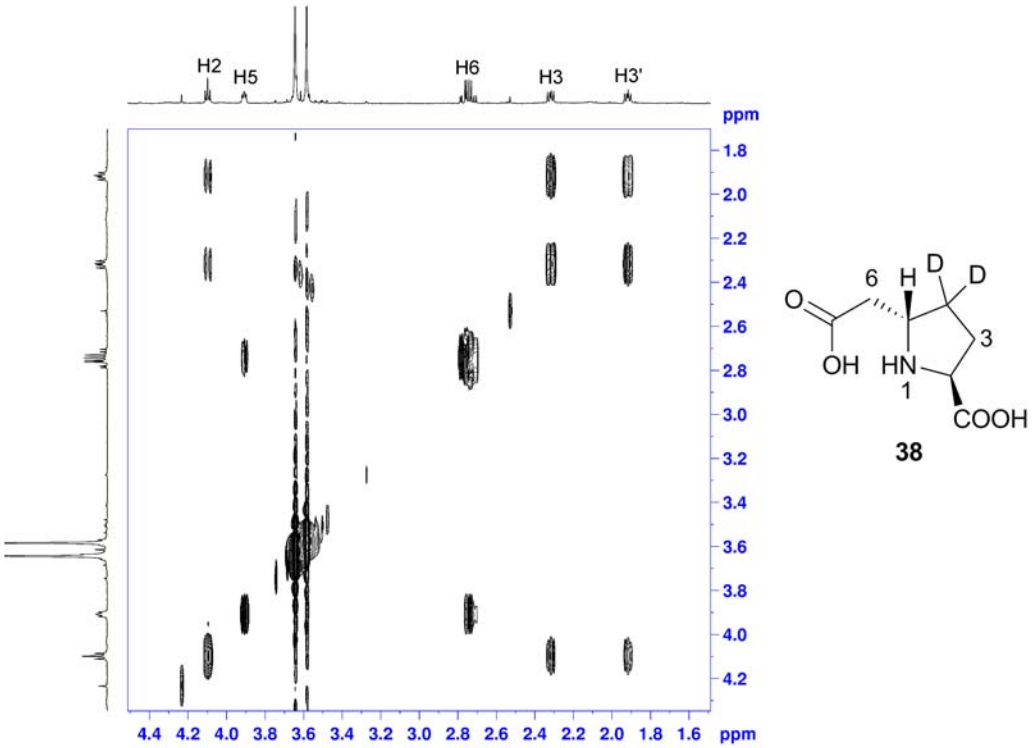
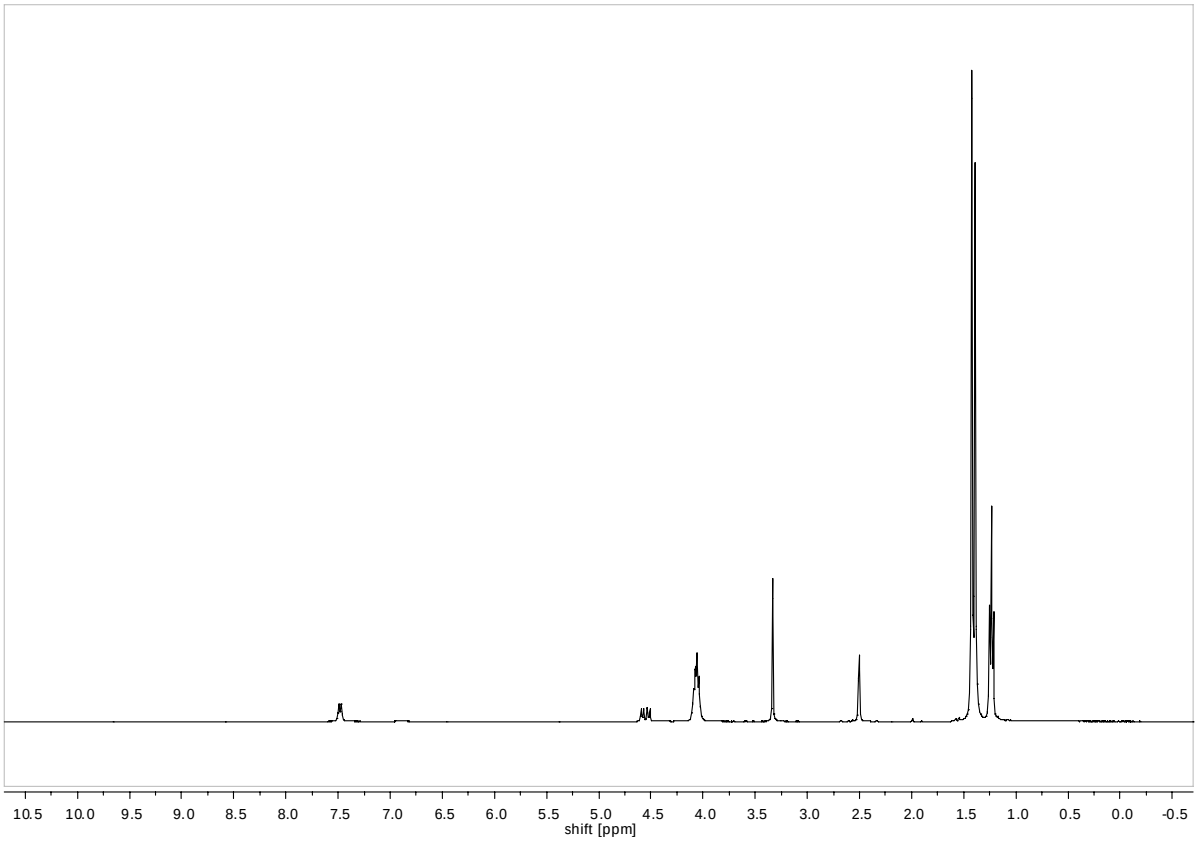
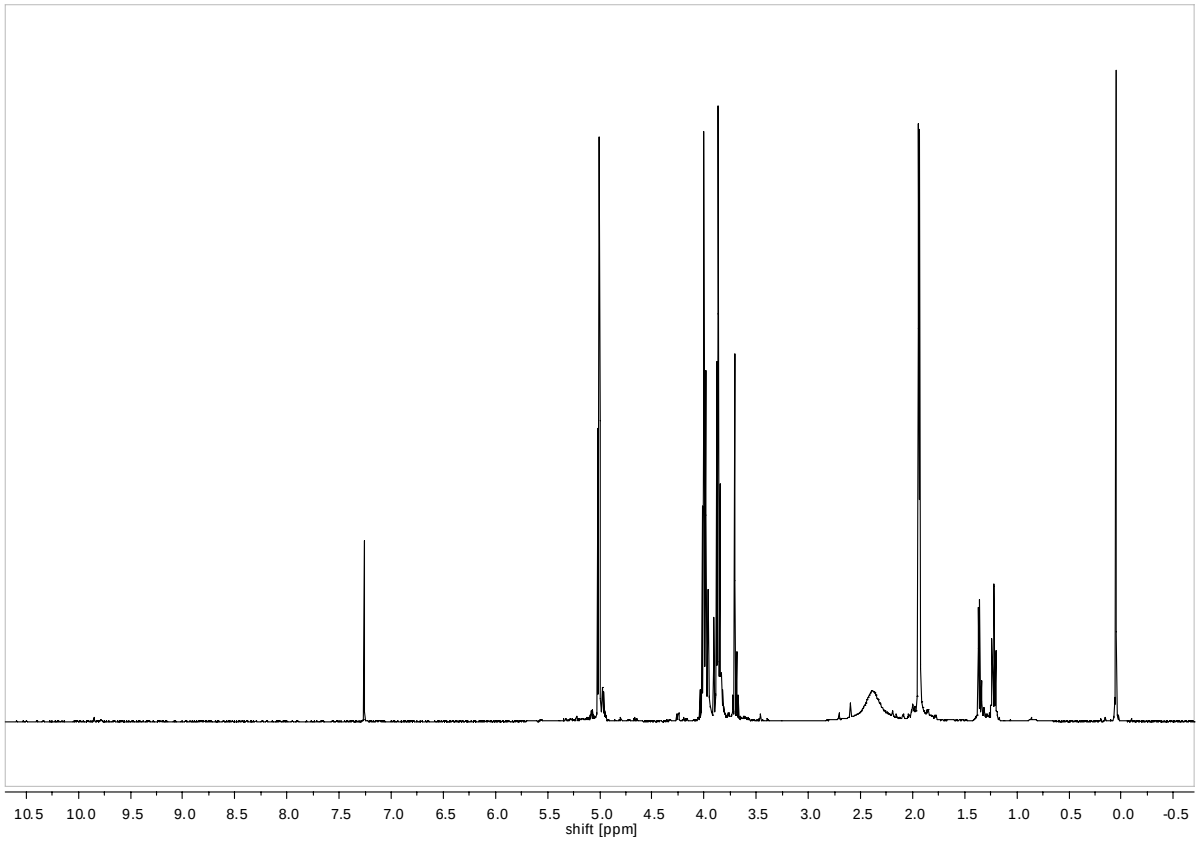


Fig. S4 ^{1}H,^{1}H-COSY NMR spectrum of *t*-CMP product **38**.

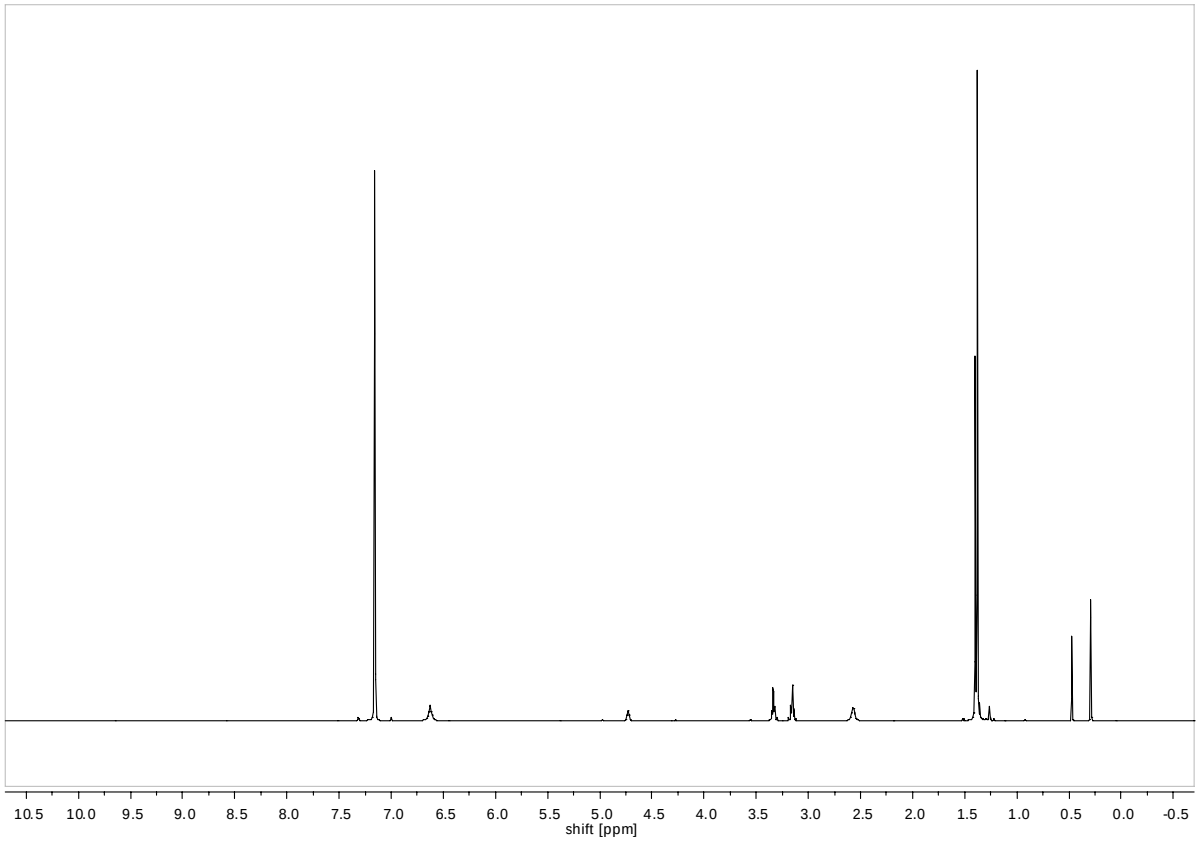
^{1}H-NMR spectrum of compound **14**



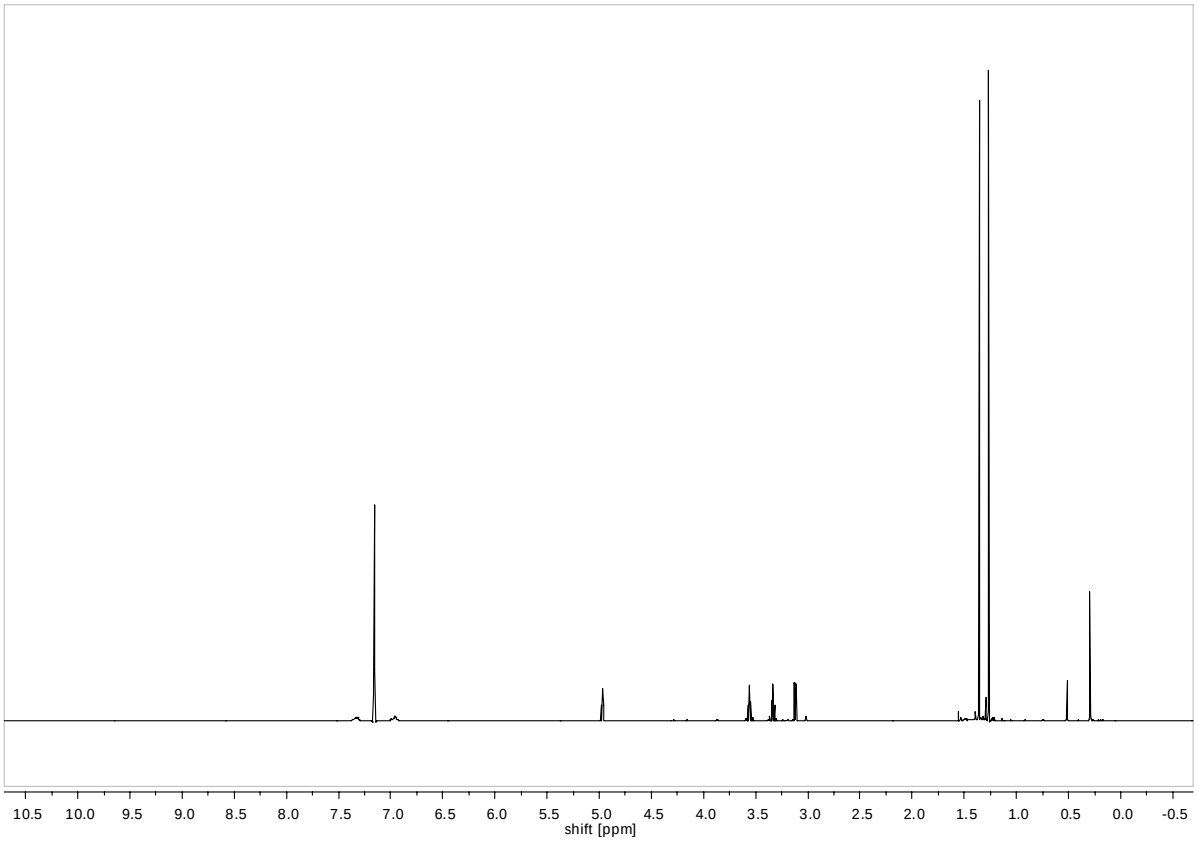
^{1}H-NMR spectrum of compound **18**



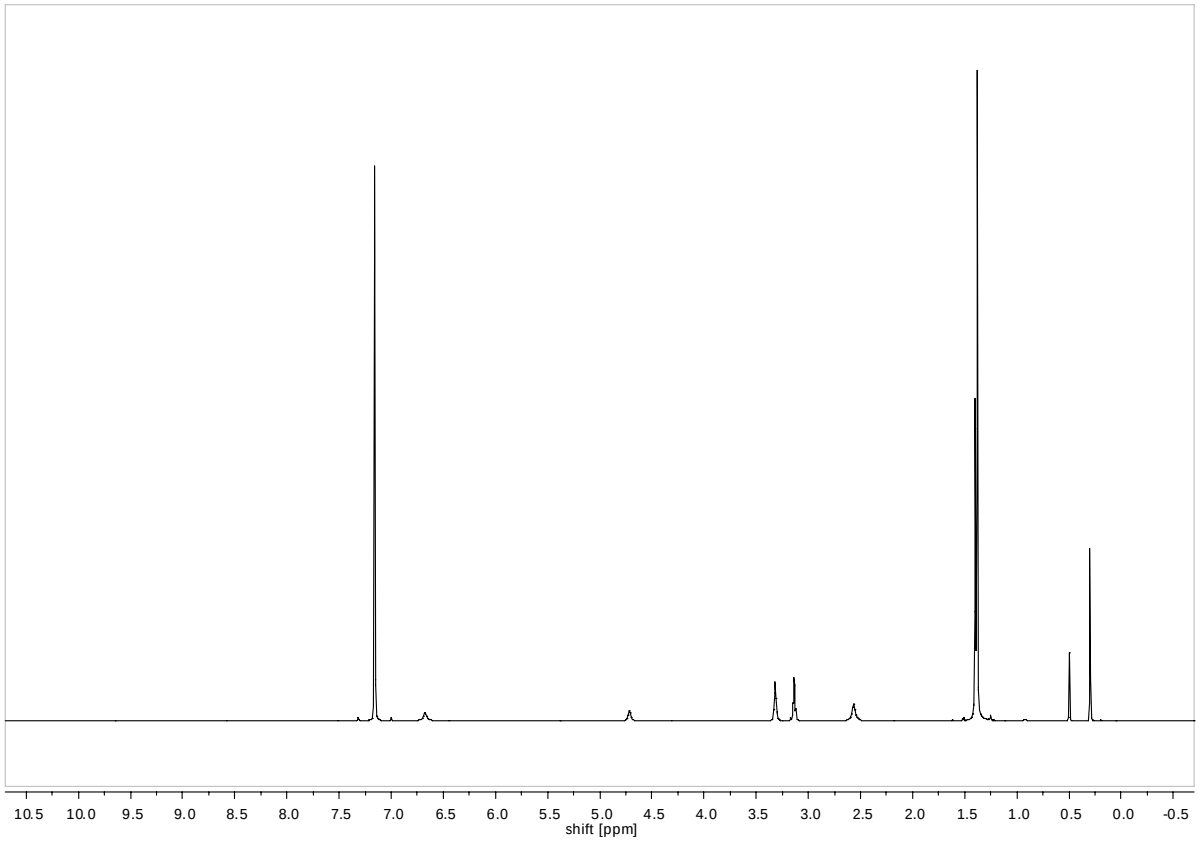
^{1}H-NMR spectrum of compound **(*Z*)-19**



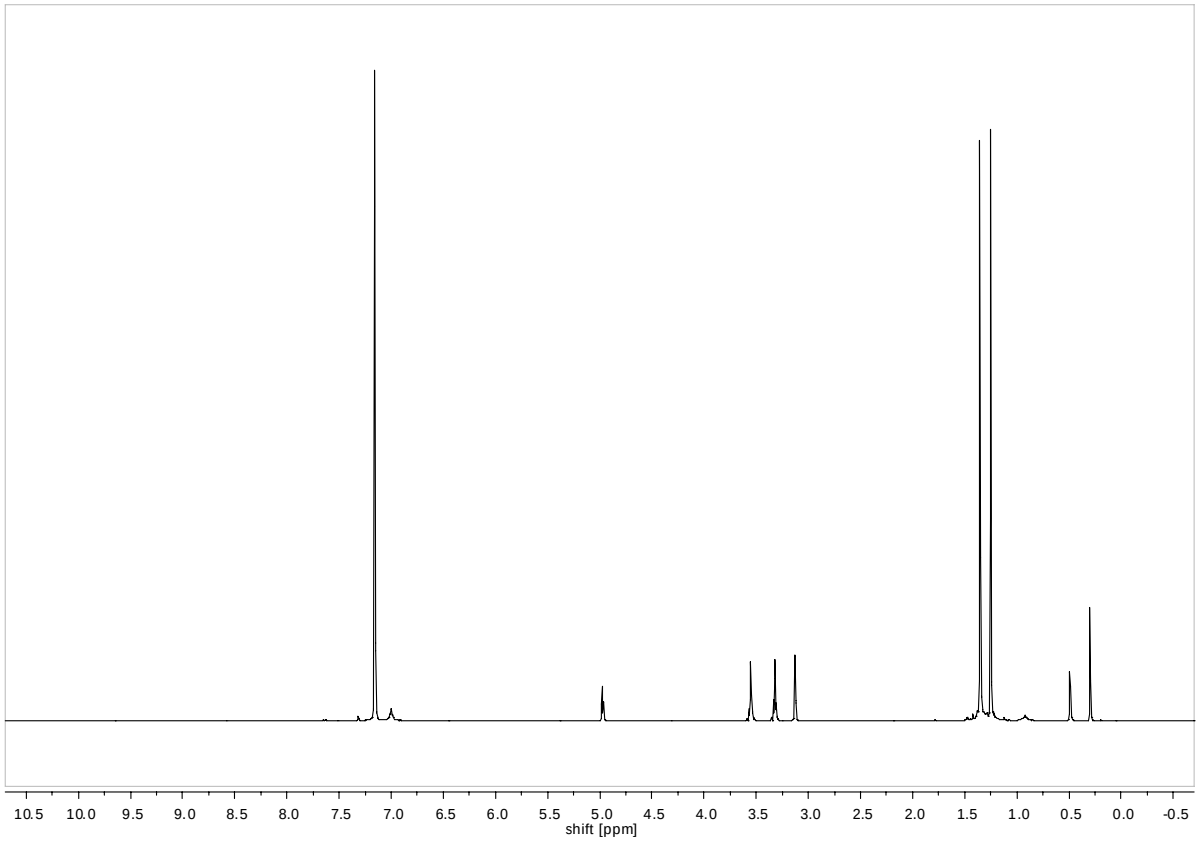
^{1}H-NMR spectrum of compound **(*E*)-19**



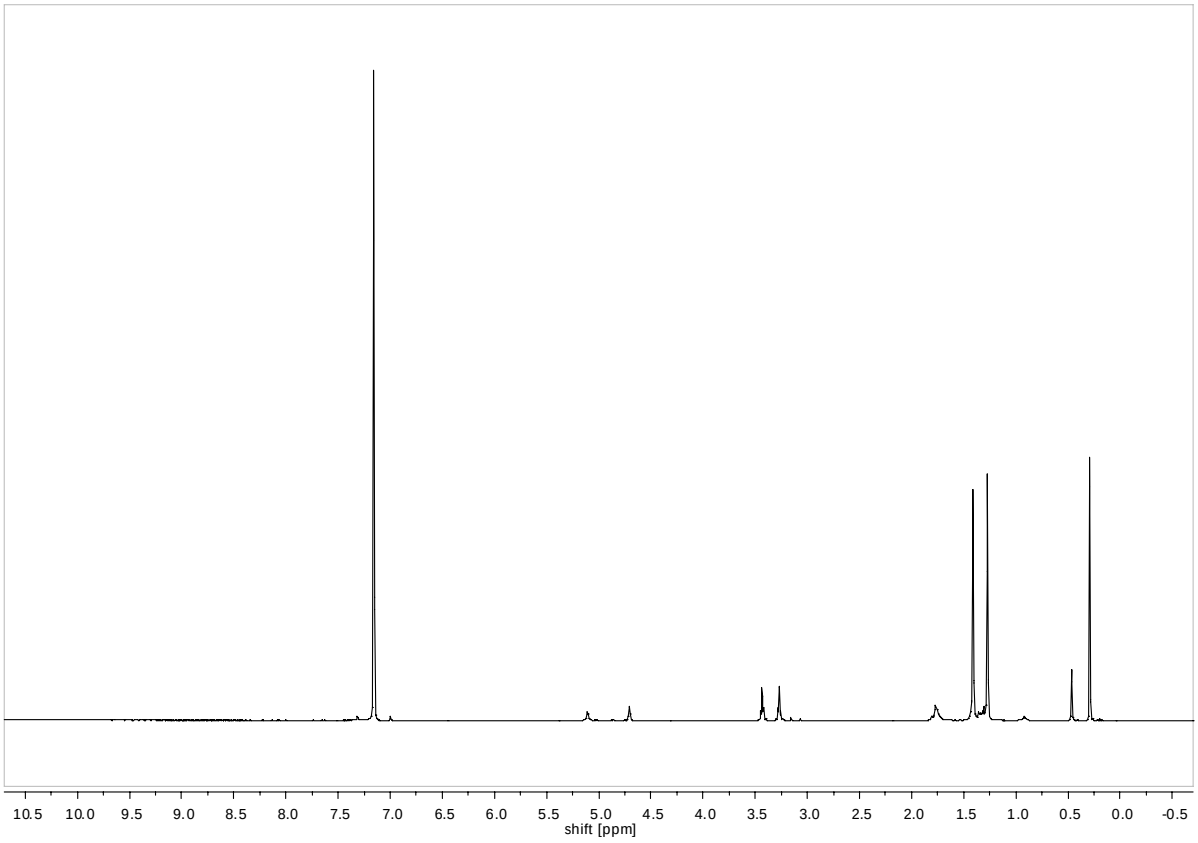
^{1}H-NMR spectrum of compound **(*Z*)-20**



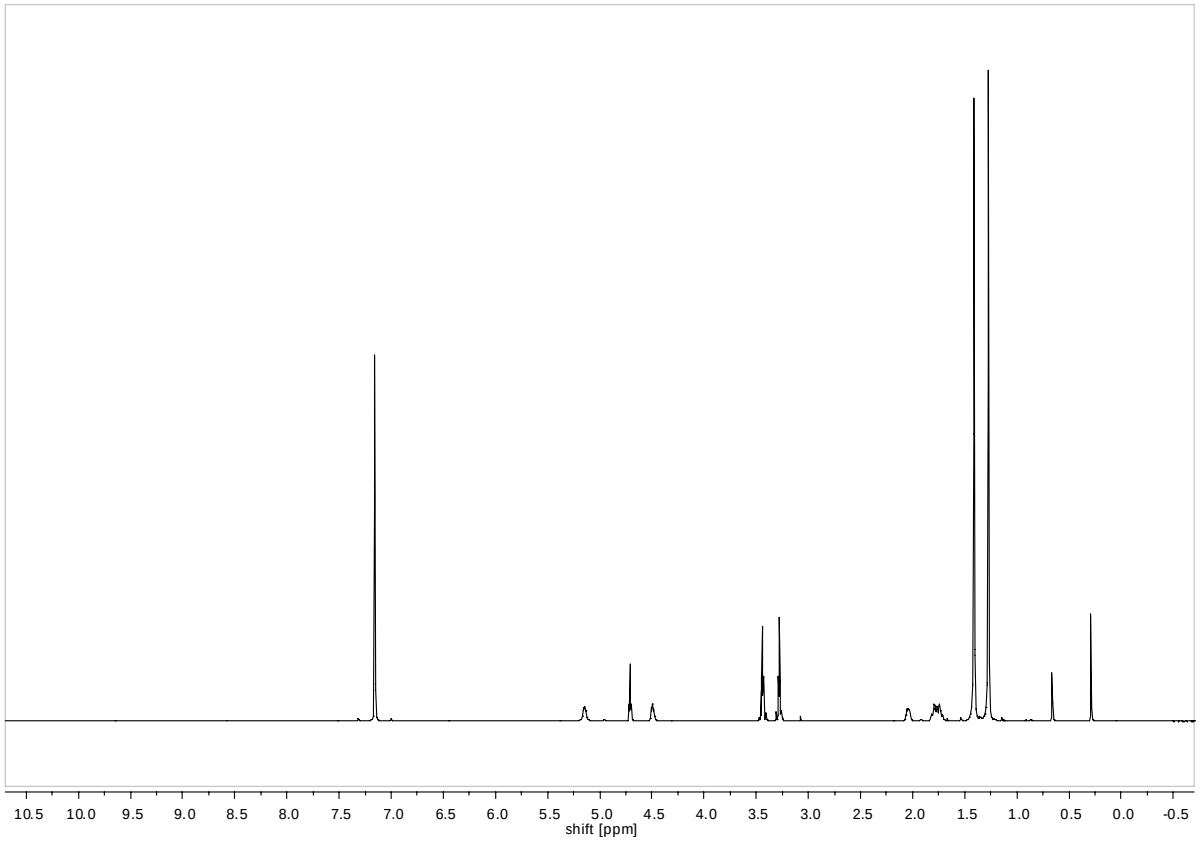
^{1}H-NMR spectrum of compound **(*E*)-20**



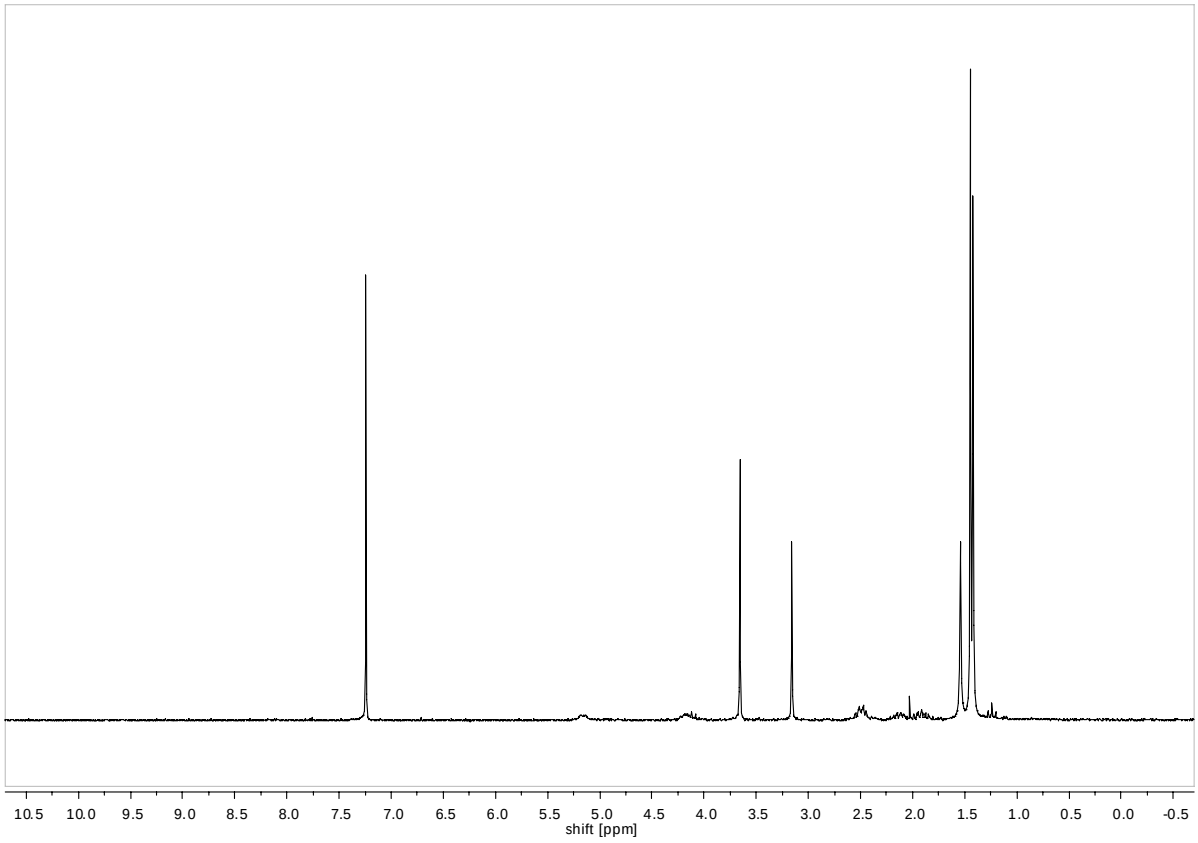
^{1}H-NMR spectrum of compound **21**



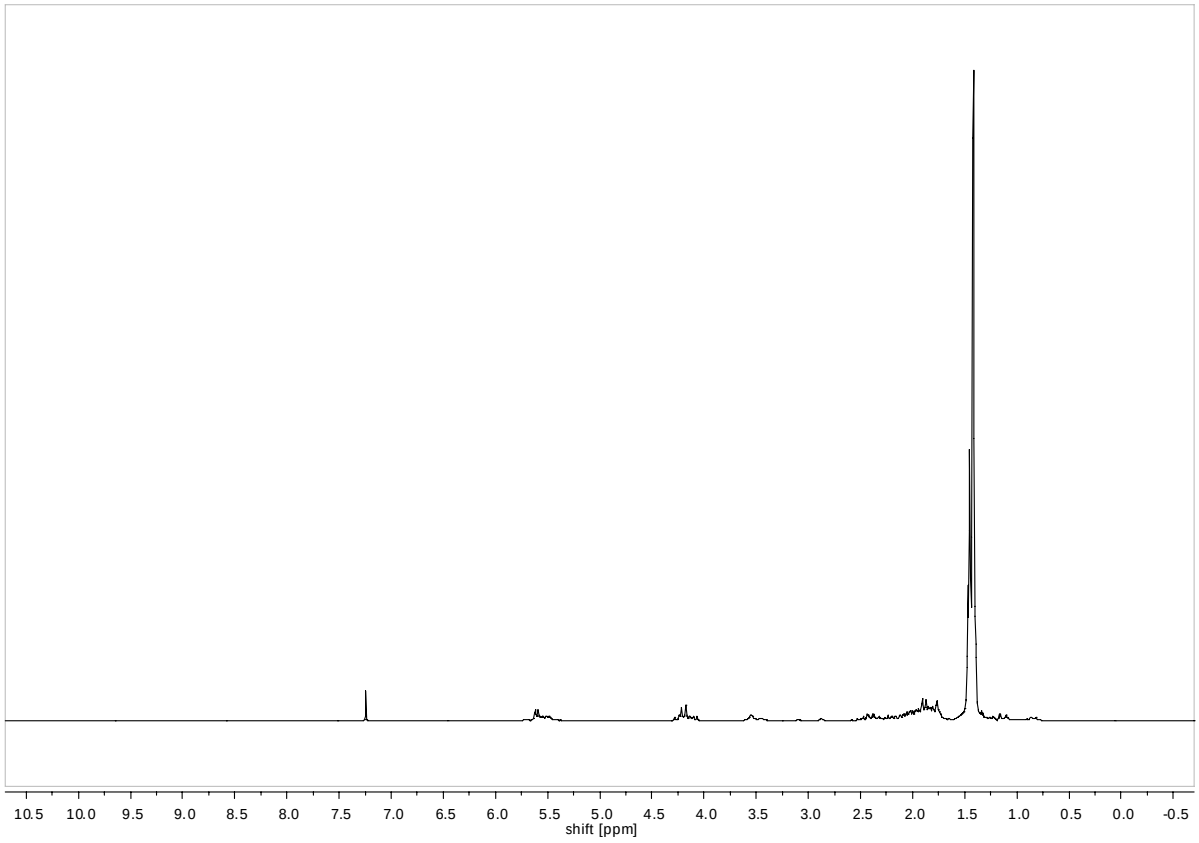
^{1}H-NMR spectrum of compound **22**



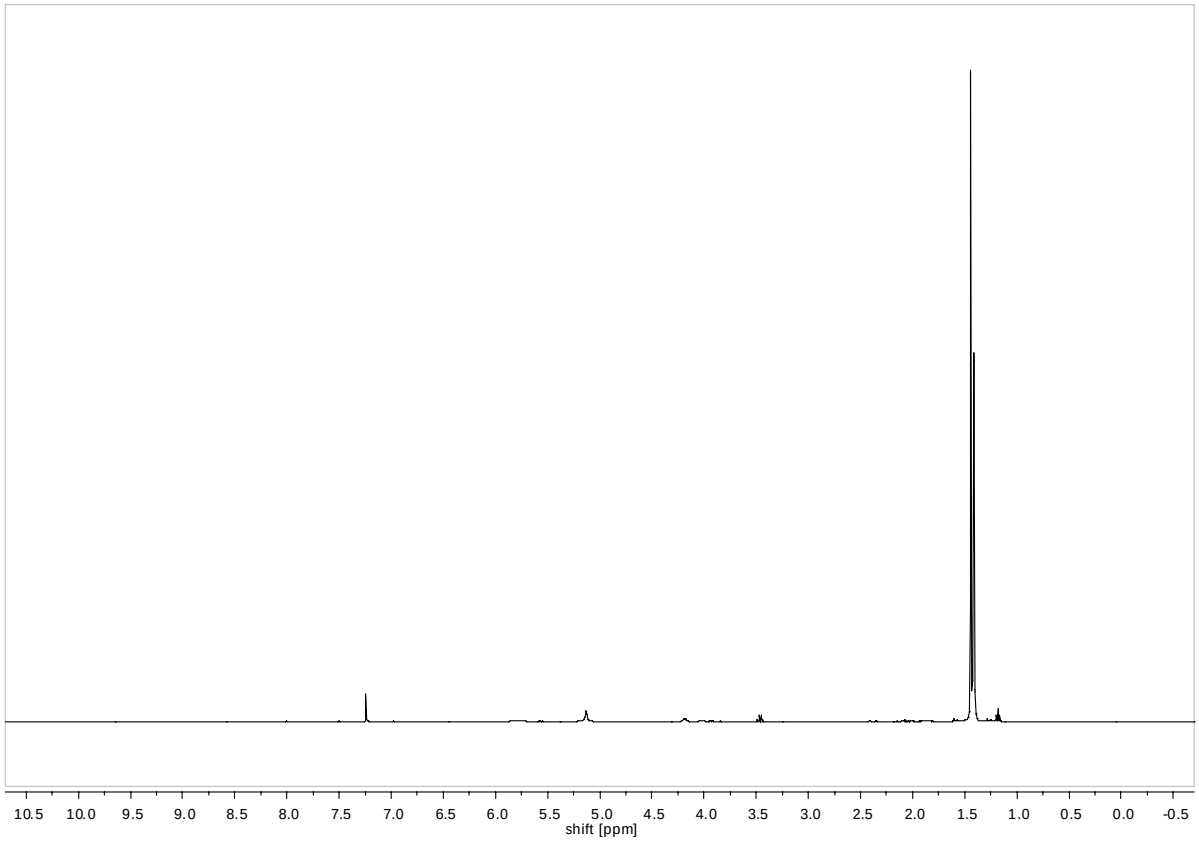
^{1}H-NMR spectrum of compound **25**



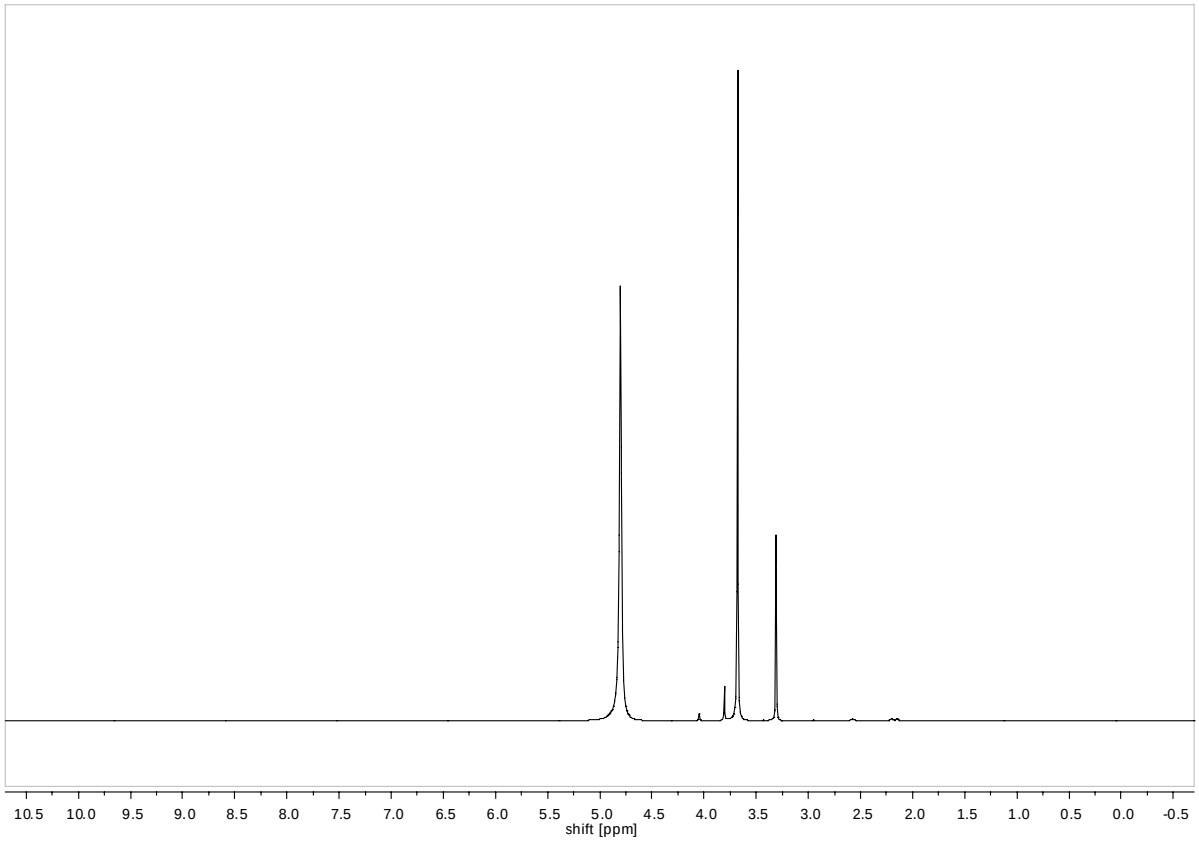
^{1}H-NMR spectrum of compound **26**



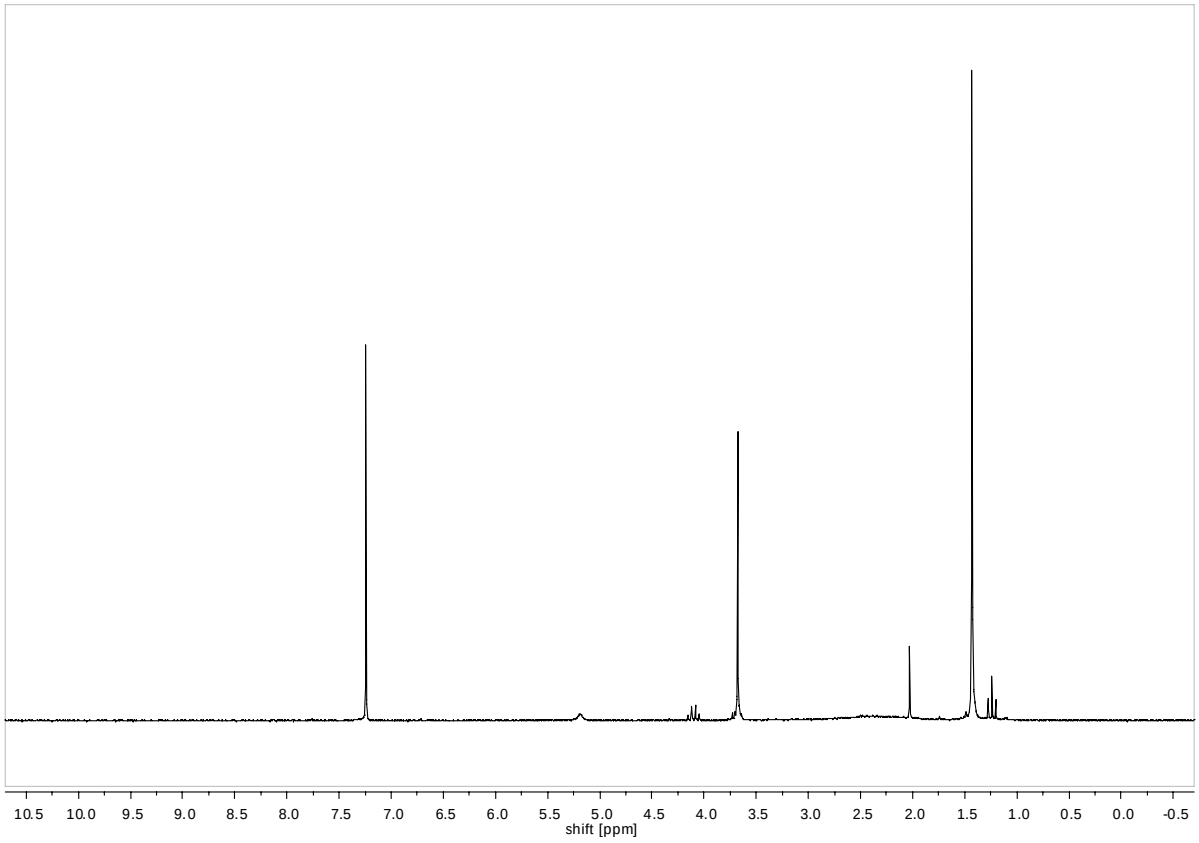
^{1}H-NMR spectrum of compound **27**



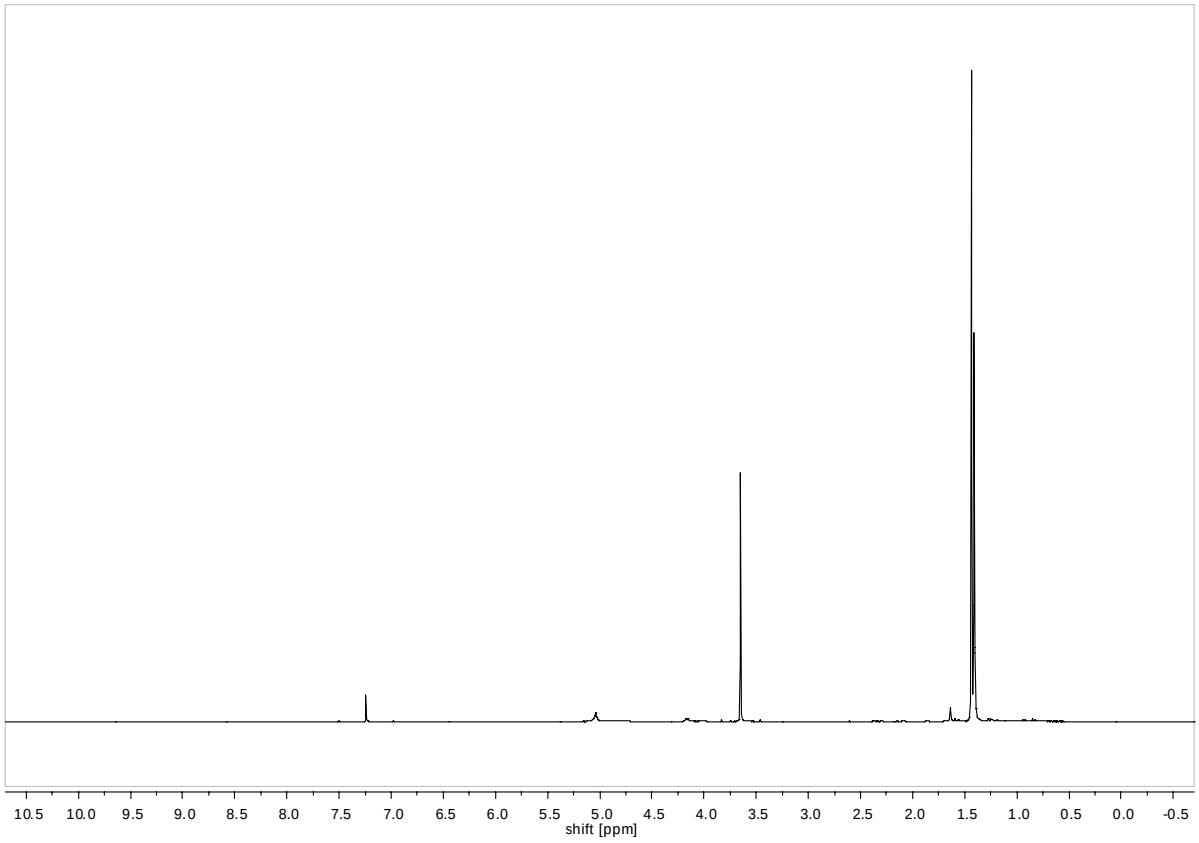
^{1}H-NMR spectrum of compound **29**



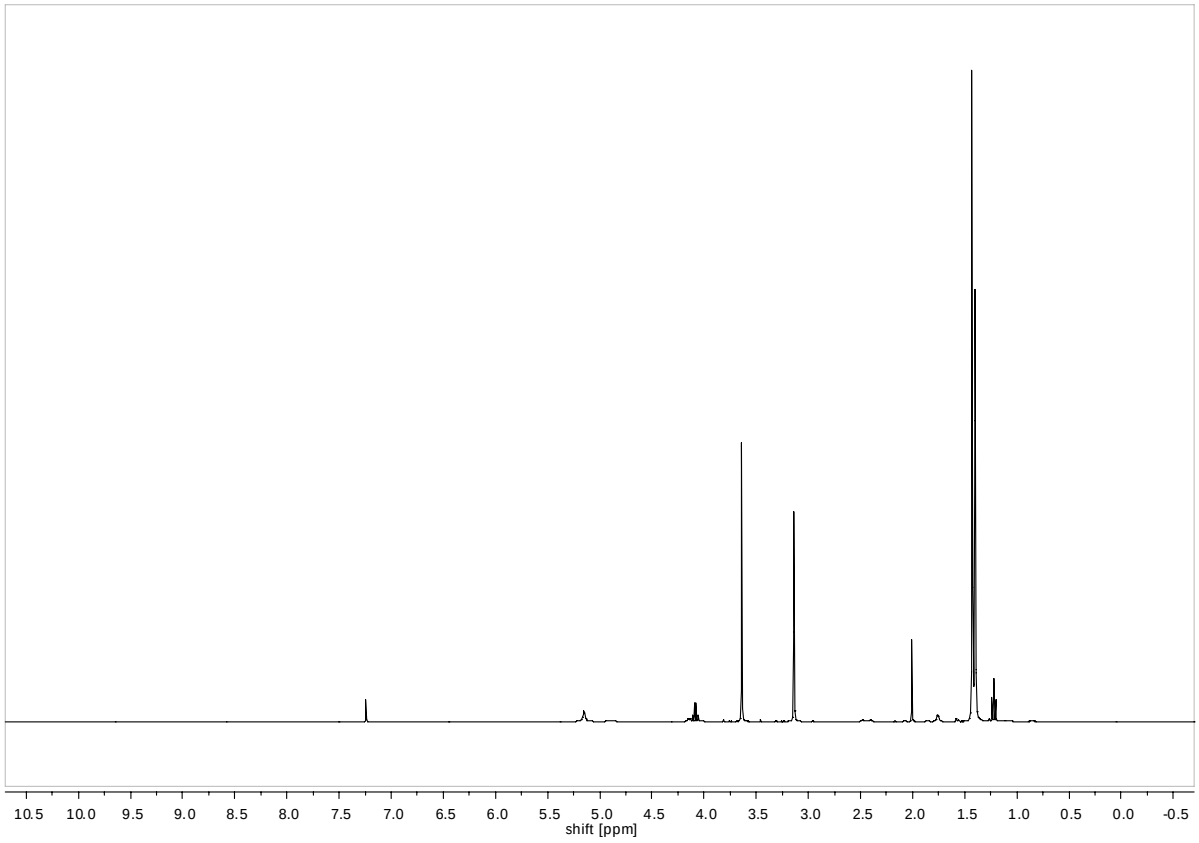
^{1}H-NMR spectrum of compound **30**



^{1}H-NMR spectrum of compound **31**



^{1}H-NMR spectrum of compound **32**



^{1}H-NMR spectrum of compound **33**