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Electronic Supplementary Information

Synthesis of the Bioactive Tetramic Acid JBIR-22 using a Late Stage Diels-Alder Reaction

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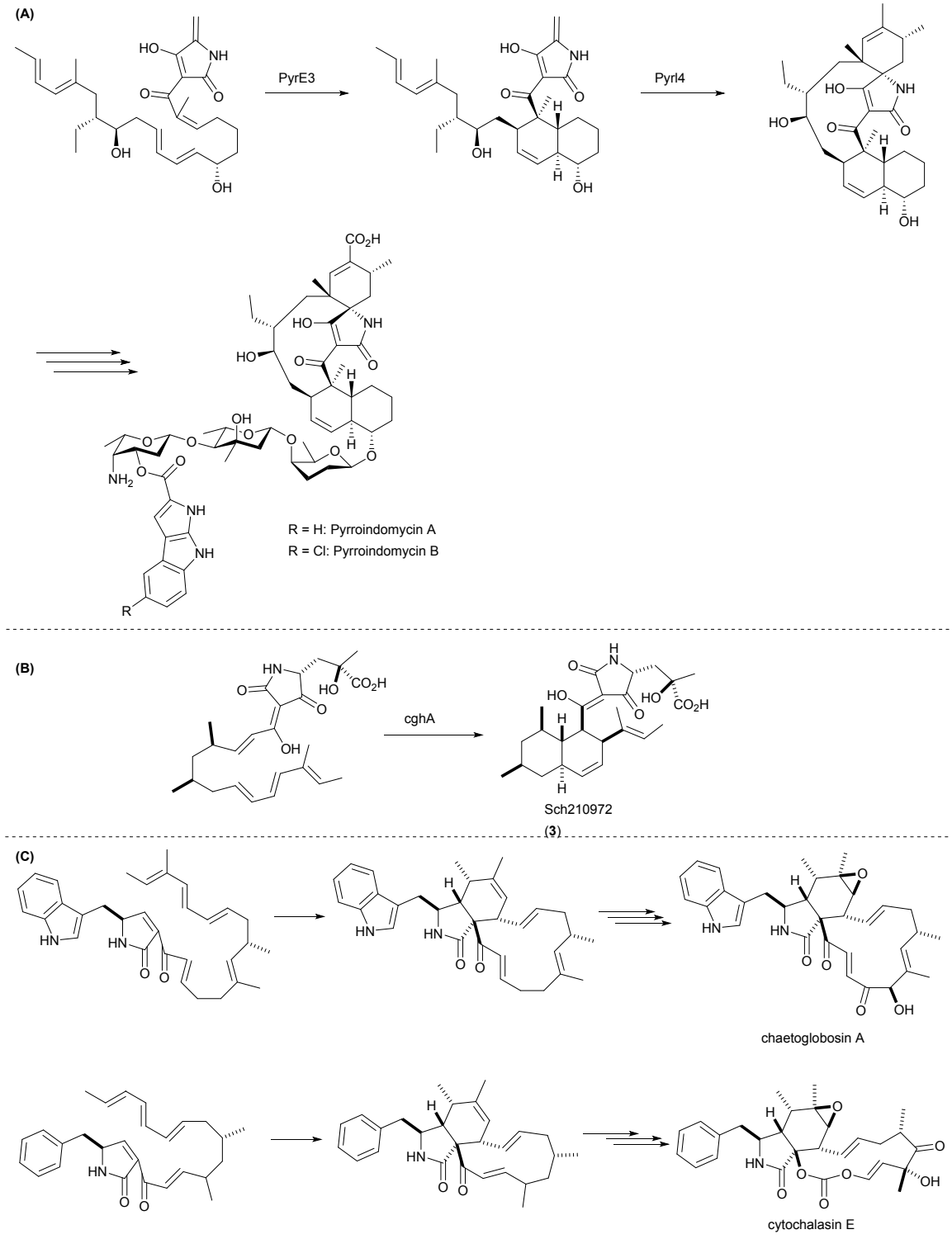
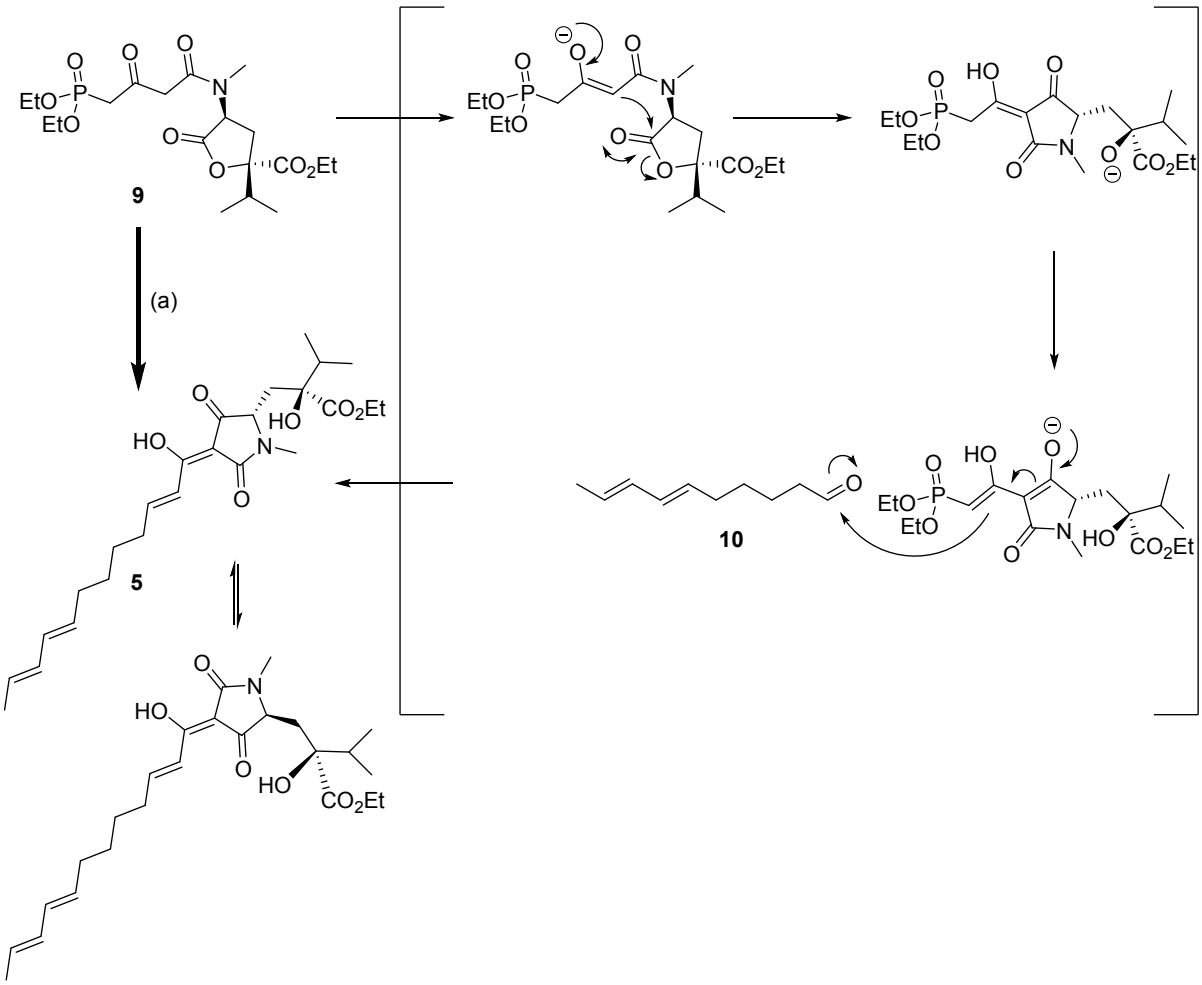
1. Late-stage DA cyclisations in the biosynthesis of tetramic acid derived natural products.



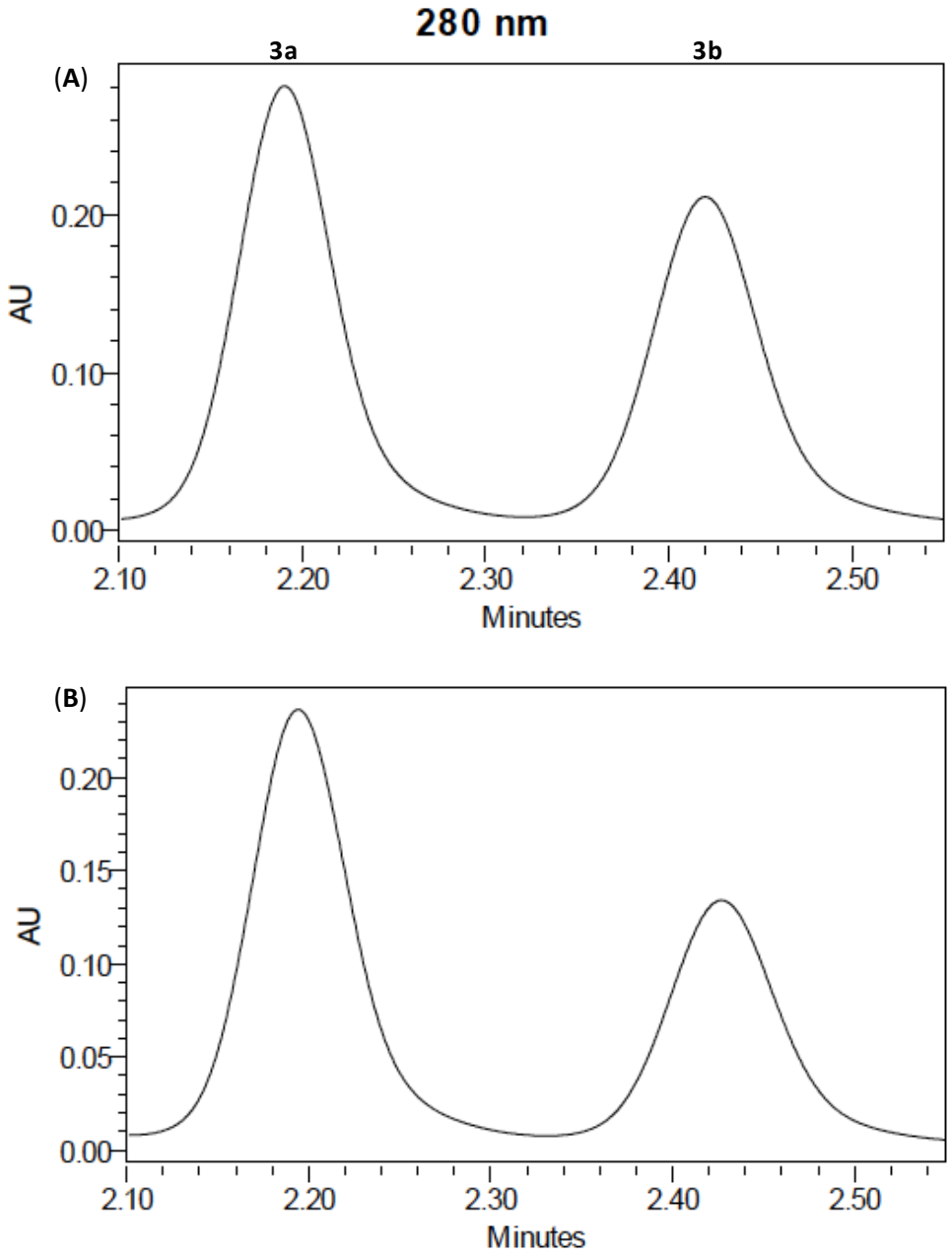
Scheme S1 (A) Dedicated cyclases "Diels-Alderases" PyrE3 and Pyrl4 have very recently been show to act in tandem to catalyse the formation of two cyclohexene rings in the biosynthesis of Pyrroindomycins A and B.[1] These enzyme-catalysed [4+2] cycloadditions occur after the formation of the tetramic acid ring core. (B) Proposed Sch210972 (**3**) biosynthetic pathway involving a cghA catalysed late-stage Diels-Alder cycloaddition.[2] (C) An enzymatic IMDA cycloaddition is proposed in the biosynthesis of chaetoglobosin A and cytochalasin E involving a partially reduced tetramic acid ring system.[2–4a]



Scheme S2. One-pot domino cyclisation-HWE olefination. *Reagents and conditions*: (**a**) (i) ^{t}BuOK, THF, 0 °C, 1 hour. (ii) **10**, THF, 0 °C → r.t., 16 h, 85%. For a very similar mechanism for a related transformation see reference 4b

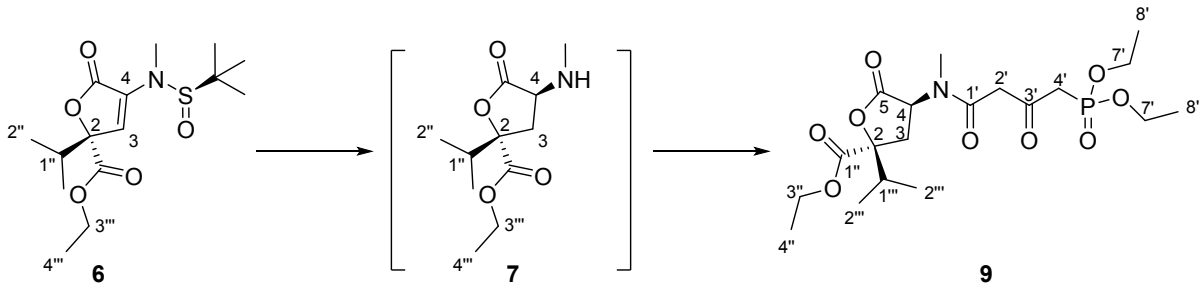
2. UPLC Analysis

Figure S1. The UPLC traces were obtained on an ACQUITY UPLC BEH C_{18} column (0.6 ml/min; solvent: 55 % MeCN (0.1% TFA)). (**A**) UPLC trace of the $BF_3.OEt_2$ catalysed IMDA cycloaddition (Table 1, entry 3). (**B**) UPLC trace of the Mg(II)-bis(oxazoline) complex **12** catalysed IMD cycloaddition (Table 1, entry 4).



3. Experimental procedures

***Iso*propyl (2*S*,4*S*)-4-(4-(diethoxyphosphoryl)-*N*-methyl-3-oxobutanamido)-2-*iso*propyl-5-oxotetrahydrofuran-2-carboxylate (9)**

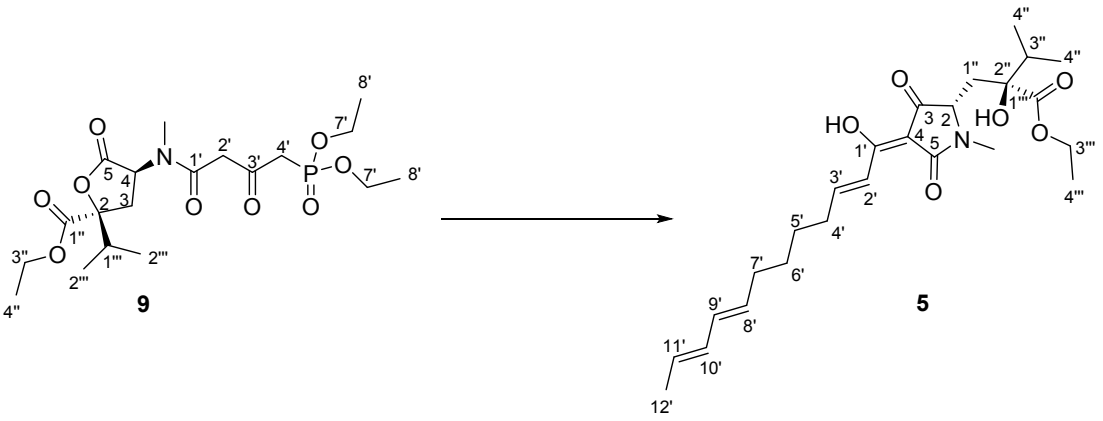


6 **7** **9**

To a solution of **6** (205 mg, 0.62 mmol, 1.0 eq.) in THF (1.5 mL) at 0 °C was added HCl (0.62 mL, 4.0 eq., 4 N in dioxane). The reaction was stirred for 10 minutes at this temperature before the addition of a solution of $NaBH_3CN$ (117 mg, 1.86 mmol, 3.0 eq.) in MeOH (3 mL). The reaction was stirred for a further 1 hour at 0 °C before being concentrated *in vacuo*. The residue was partitioned between a saturated aqueous solution of $NaHCO_3$ (5 mL) and EtOAc (5 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL) and the combined organic extracts were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the crude free amine **7**. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.25 (qd, *J* = 7.1, 2.2 Hz, 2H, C3'''-H_2), 3.54 (dd, *J* = 11.5, 8.4 Hz, 1H, C4-H), 2.74 (dd, *J* = 12.8, 8.4 Hz, 1H, C3-H_2), 2.46 (s, 3H, NHCH_3), 2.25 (hept, *J* = 6.9 Hz, 1H, C1''-H), 2.05 (dd, *J* = 12.8, 11.5 Hz, 1H, C3-H_2), 1.78 (br s, 1H, NHCH$_3$), 1.30 (t, *J* = 7.1 Hz, 3H, C4'''-H_3), 1.02 (d, *J* = 6.9 Hz, 3H, C2'-H_3), 0.99 (d, *J* = 6.9 Hz, 1H, C2'-H_3); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 175.9 (C5), 171.2 (C1'''), 87.3 (C2), 62.2 (C3'''), 58.1 (C4), 35.9 (C3), 34.4 (NCH_3), 34.3 (C1''), 17.0 (C2'), 16.4 (C2'), 14.3 (C4'''). To a solution of **7** in anhydrous MeCN (6 mL) was added a solution of **8** in anhydrous MeCN (3 mL), and the reaction was heated at reflux for 2.5 hours. The reaction was concentrated *in vacuo* and purified *via* the Biotage SP4 (silica-packed SNAP column 12 g; 0-8% MeOH/DCM) to give the title product **9** as an orange oil (220 mg, 79%). In $CDCl_3$ at room temperature the title compound **9** exists as a (7 : 3) *enol* : *keto* mixture. The NMR signals are reported for the major *keto* tautomer. ^{1}H and ^{13}C spectra are complicated by ^{31}P splitting. **IR** (thin film) ν_{max}: 2978, 2936, 1784 (C=O), 1734 (C=O), 1636 (C=O), 1236, 1184, 1022; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 4.83 – 4.71 (m, 1H, C4-H), 4.33 – 4.21 (m, 2H, C3''-H_2), 4.21 – 4.07 (m, 4H, (C7'-H_2)$_2$), 3.81 (s, 2H, C2'-H_2), 3.25 (dd, *J* = 22.6, 4.4 Hz, 2H, C4'-H_2), 3.01 (s, 3H, NCH_3), 2.68 (dd, *J* = 12.8, 9.1 Hz, 1H, C3-H_2), 2.47 (dd, *J* = 12.8, 11.4 Hz, 1H, C3-H_2), 2.38 – 2.29 (m, 1H, C1'''-H), 1.37 – 1.28 (m, 9H, C4''-H_3, (C8'-H_3)$_2$), 1.05 (d, *J* = 6.9 Hz, 3H, C2'''-H_3), 1.02 (d, *J* = 6.9 Hz, 3H, C2'''-H_3); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 195.7 (C3'), 172.1 (C5), 171.0 (C1''), 167.3 (C1'), 87.2 (C2), 63.0 (d, *J* = 6.5 Hz, C7'), 62.4 (C3''), 56.7 (C4), 49.7 (C2'), 42.5 (d, *J* = 125.9 Hz, C4'), 35.3 (NCH_3), 34.2 (C1'''), 31.5 (C3), 17.0 (C2'''), 16.5 (C2'''), 16.4 (C8'), 14.3 (C4''). **^{31}P NMR** (202 MHz, Chloroform-*d*)

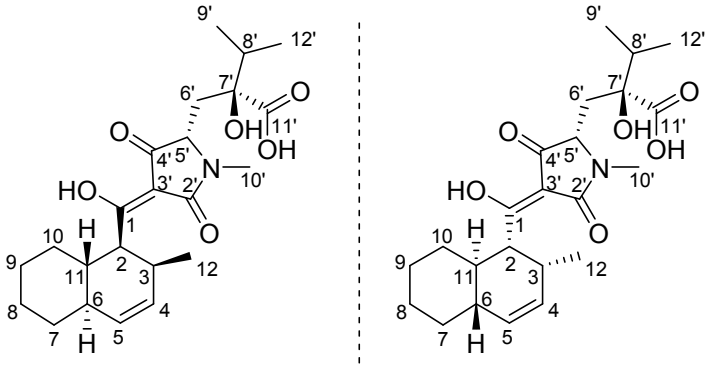
δ 19.2. ***m/z*** (ES$^+$) 472.17 ([M+Na]$^+$, 100 %); **HRMS** (ES$^+$) Calcd for $C_{19}H_{32}O_9NPNa$ [M+Na]$^+$: 472.1707, found 472.1696; $[\alpha]_D^{20}$ = -9.2 (*c* 1.0, MeOH).

Ethyl (*S*)-2-hydroxy-2-(((*S*,*E*)-4-((2*E*,8*E*,10*E*)-1-hydroxydodeca-2,8,10-trien-1-ylidene)-1-methyl-3,5-dioxopyrrolidin-2-yl)methyl)-3-methylbutanoate (5)



To a solution of **9** (100 mg, 0.22 mmol, 1.0 eq.) in THF (2.5 mL) at 0 °C was added ^{t}BuOK (0.24 mL, 1.1 eq., 1M in THF) and the reaction was stirred for 40 minutes. To the mixture was added a solution of **10**[5] (102 mg, 0.67 mmol, 3.0 eq.; 85% *E*,*E*-diene geometry) in THF (1 mL) and the reaction was stirred for a further 15 minutes before being warmed to room temperature and stirred for 16 hours. The reaction was quenched by the addition of an aqueous solution of HCl (3 mL, 1N) and extracted with DCM (3 × 10 mL). The organic extracts were combined, washed with brine (10 mL), dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified *via* the Biotage SP4 (Reverse-phase silica-packed SNAP column 4 g; 20-100% H_2O/(MeOH:MeCN)) to give the title product **5** as an yellow oil (83 mg, 85%). In $CDCl_3$ at room temperature the ^{1}H and ^{13}C NMR spectra of **5** are complicated by the presence of *keto*/*enol* tautomers and a minor *E*,*Z*,*E* isomer. The NMR signals are reported for the major tautomer. **IR** (thin film) ν $_{max}$: 2964, 2931, 1717 (C=O), 1690 (C=O), 1645 (C=O), 1586, 1449, 1242, 989; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.24 – 7.13 (m, 1H, C3'-H), 7.08 – 7.03 (m, 1H, C2'-H), 6.04 – 5.90 (m, 2H, C9'-H, C10'-H), 5.62 – 5.45 (m, 2H, C8'-H, C11'-H), 4.24 (q, *J* = 7.1 Hz, 2H, C3'''-H_2), 3.67 (dd, *J* = 9.6, 2.2 Hz, 1H, C2-H), 2.97 (s, 3H, NCH_3), 2.37 – 2.29 (m, 2H, C4'-H_2), 2.30 – 2.28 (m, 1H, C1''-H_2), 2.10 – 2.00 (m, 3H, C3''-H, C7'-H_2), 1.90 (dd, *J* = 14.5, 9.6 Hz, 1H, C1''-H_2), 1.71 (d, *J* = 6.4 Hz, 3H, C12'-H_3), 1.57 – 1.45 (m, 2H, C5'-H_2), 1.46 – 1.37 (m, 2H, C6'-H_2), 1.31 (t, *J* = 7.1 Hz, 3H, C4'''-H_3), 0.94 (d, *J* = 7.3 Hz, 3H, C4''-H_3), 0.93 (d, 7.3 Hz, 3H, C4''-H_3); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.3 (C3), 175.5 (C1'''), 175.1 (C1'), 173.4 (C5), 151.8 (C3'), 131.6 (C10'), 131.4 (C8'), 130.8 (C9'), 127.2 (C11'), 121.6 (C2'), 98.9 (C4), 78.8 (C2''), 64.5 (C2), 61.6 (C3'''), 36.8 (C3''), 34.9 (C1''), 33.3 (C4'), 32.4 (C7'), 29.1 (C6'), 27.7 (C5'), 26.8 (NCH_3), 18.1 (C12'), 17.5 (C4''), 16.4 (C4''), 14.5 (C4'''); ***m/z*** (ES$^-$) 446.25 ([M-H]$^-$, 100 %); **HRMS** (ES$^-$) Calcd for $C_{25}H_{36}O_6N$ [M-H]$^-$: 446.2548, found 446.2549.

JBIR-22 (**3a**/**b**)

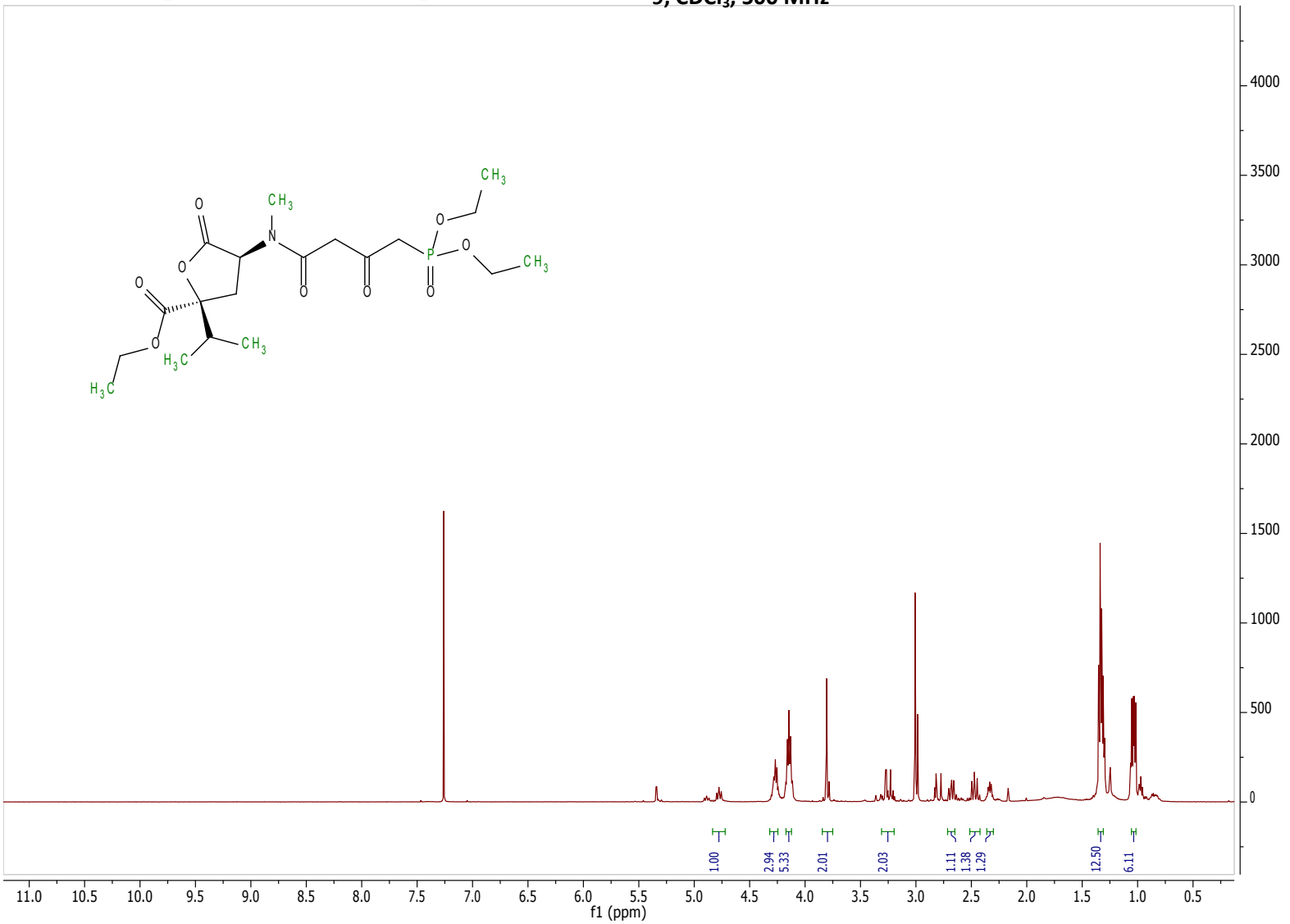


3a **3b**

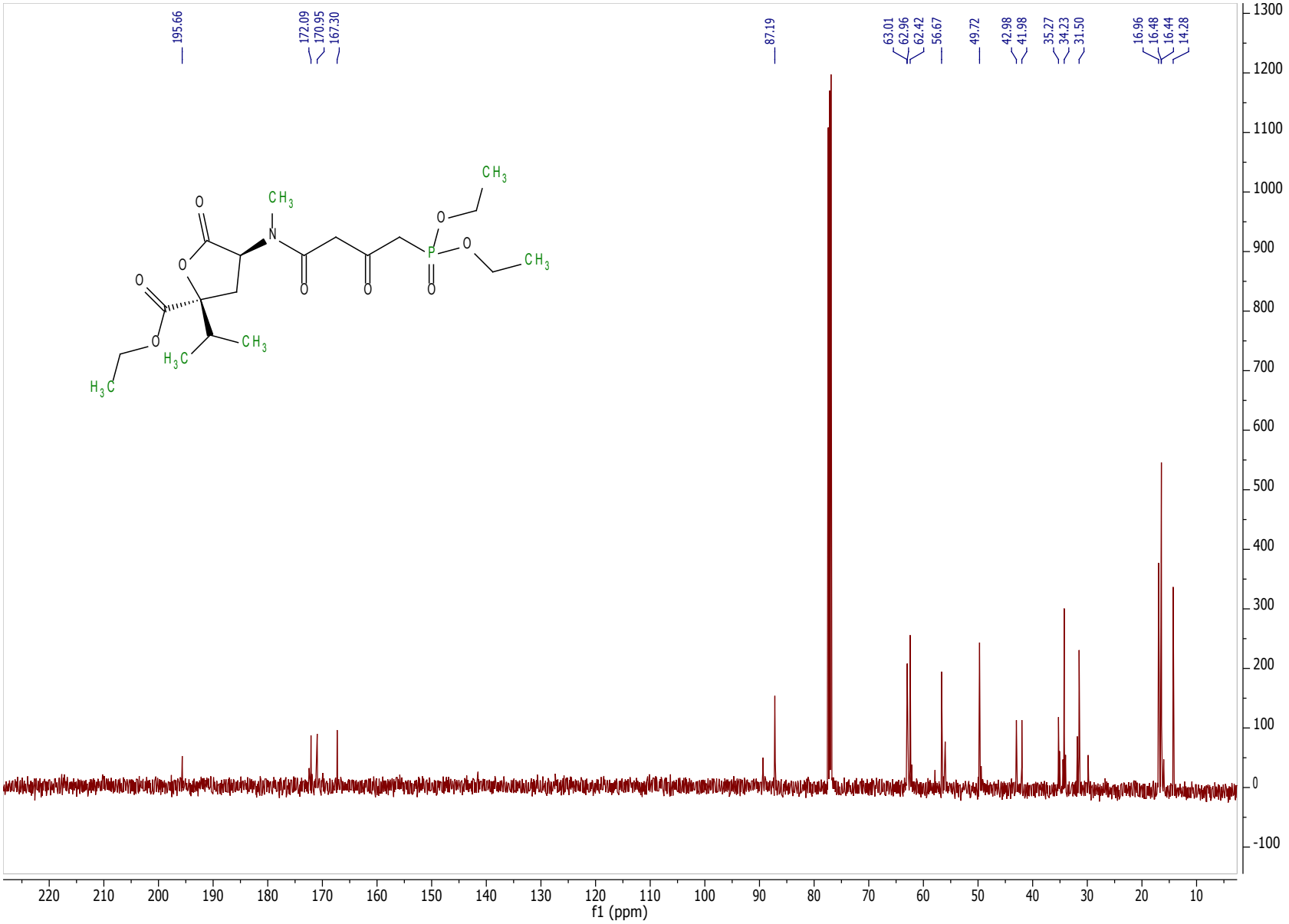
The 1:1 mixture of JBIR-22 (**3a**/**b**) was synthesised as reported in Ref[8]. **3a**/**b** was obtained as an amorphous white solid (73 mg). Spectroscopic data was obtained for JBIR-22 **3a**/**b**-Et_2NH salt. $[\alpha]_D^{23}$ = +22.0 (*c* 0.7, MeOH); **^{1}H NMR** (500 MHz, Acetone-d_6) δ 5.57 – 5.48 (m, 2H, 2 × C4-H), 5.33 – 5.27 (m, 2H, 2 × C5-H), 3.93 (dd, *J* = 11.1, 5.5 Hz, 1H, C2-H (**3b**)), 3.90 (dd, *J* = 11.1, 5.5 Hz, 1H, C2-H (**3a**)), 3.37 (dd, *J* = 10.1, 1.3 Hz, 2H, 2 × C5'-H), 2.72 (s, 6H, 2 × C10'-H_3), 2.67 – 2.56 (m, 2H, 2 × C3-H), 2.31 (dt, *J* = 13.9, 1.5 Hz, 2H, 2 × C6'-H_2), 2.04 – 1.94 (m, 4H, 2 × C8'-H, 2 x 1 of C10-H_2), 1.76 – 1.66 (m, 8H, 2 × 1 of C7-H_2, 2 × C6-H, 2 × 1 of C8-H_2, 2 × 1 of C9-H_2), 1.63 (dd, *J* = 13.8, 10.1 Hz, 2H, 2 × 1 of C6'-H_2), 1.51 – 1.41 (m, 2H, 2 × C11-H), 1.30 – 1.28 (m, 4H, 2 × 1 of C8-H_2, 2 × 1 of C9-H_2), 1.09 – 0.98 (m, 2H, 2 × 1 of C7-H_2), 0.91 (d, *J* = 6.7 Hz, 6H, 2 × C9'-H_3), 0.77 (d, *J* = 7.2 Hz, 3H, C12-H_3 (**3a**)), 0.75 (d, *J* = 7.2 Hz, 3H, C12-H_3 (**3b**)), 0.73 – 0.67 (m, 2H, 2 × 1 of C10-H_2); **^{13}C NMR** (126 MHz, Acetone) δ 196.40 (C1), 196.36 (C1), 196.0 (C4'), 195.8 (C4'), 180.5 (C11'), 180.4 (C11'), 174.8 (C2'), 174.7 (C2'), 133.6 (C4), 133.5 (C4), 131.2 (C5), 131.1 (C5), 101.5 (2 × C3'), 80.0 (C7'), 79.9 (C7'), 63.3 (C5'), 63.2 (C5'), 51.07 (C2), 50.98 (C2), 43.4 (C6), 43.3 (C6), 37.4 (C11), 37.3 (C11), 37.2 (C6'), 37.1 (C6'), 36.62 (C8'), 36.60 (C8'), 34.4 (C7), 34.3 (C7), 32.3 (C3), 32.2 (C3), 31.1 (C10), 31.0 (C10), 27.7 (C9/C8), 27.6 (C9/C8), 18.7 (C9'), 18.7 (C9'), 18.34 (C12), 18.33 (C12), 16.8 (2 × C12'); ***m/z*** (ES^-) 418.23 ($[M-H]^-$, 100 %); **HRMS** (ES^-) Calcd for $C_{23}H_{32}O_6N$ $[M-H]^-$: 418.2235, found 418.2225. Spectroscopic data are in agreement with that reported previously by us for the single diastereomers JBIR-22 **3a** and **3b**.[5] See Section 6 for the NMR spectra of the 1:1 mixture of **3a**/**b**.

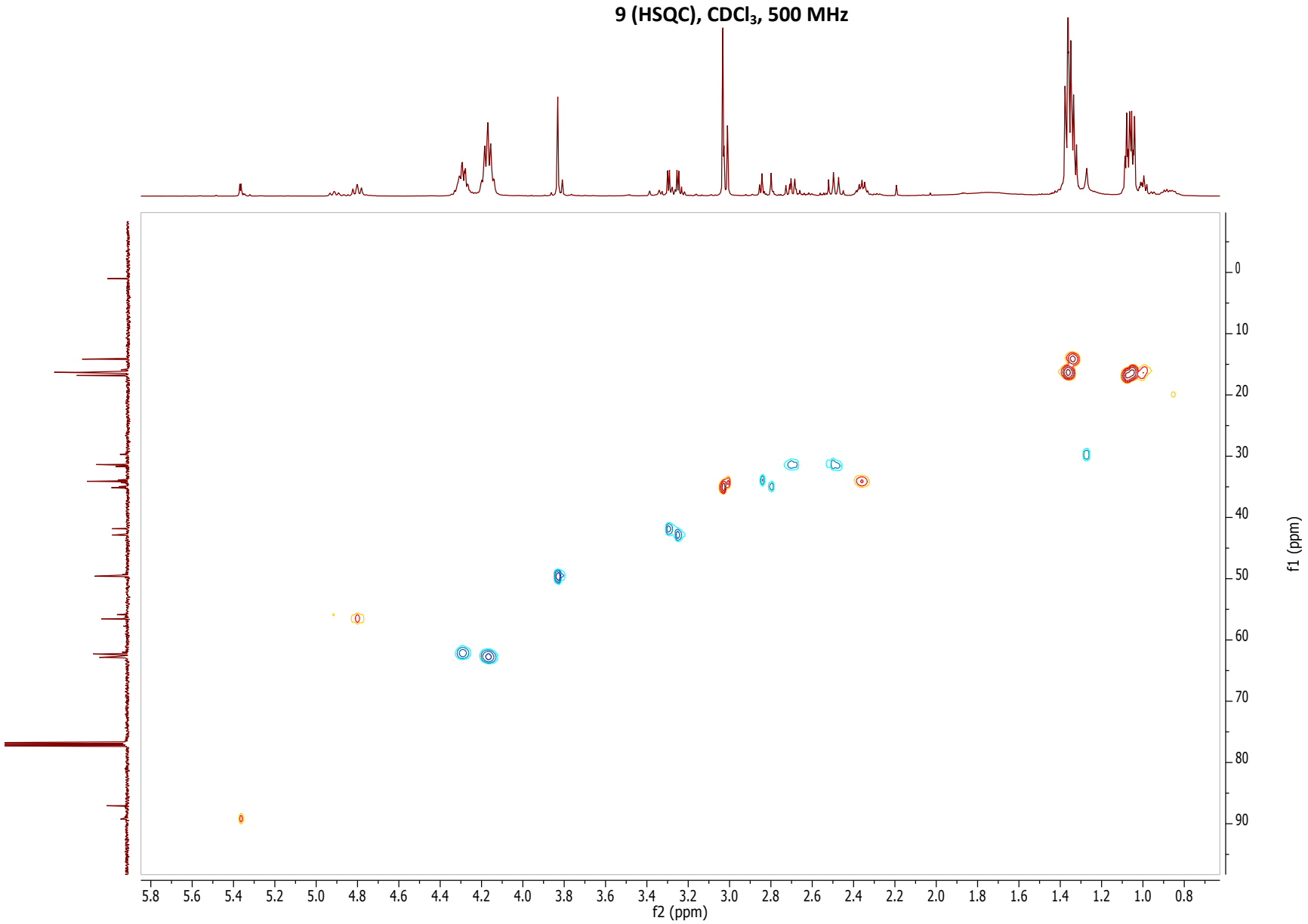
4. NMR spectra of novel compounds

9, $CDCl_3$, 500 MHz

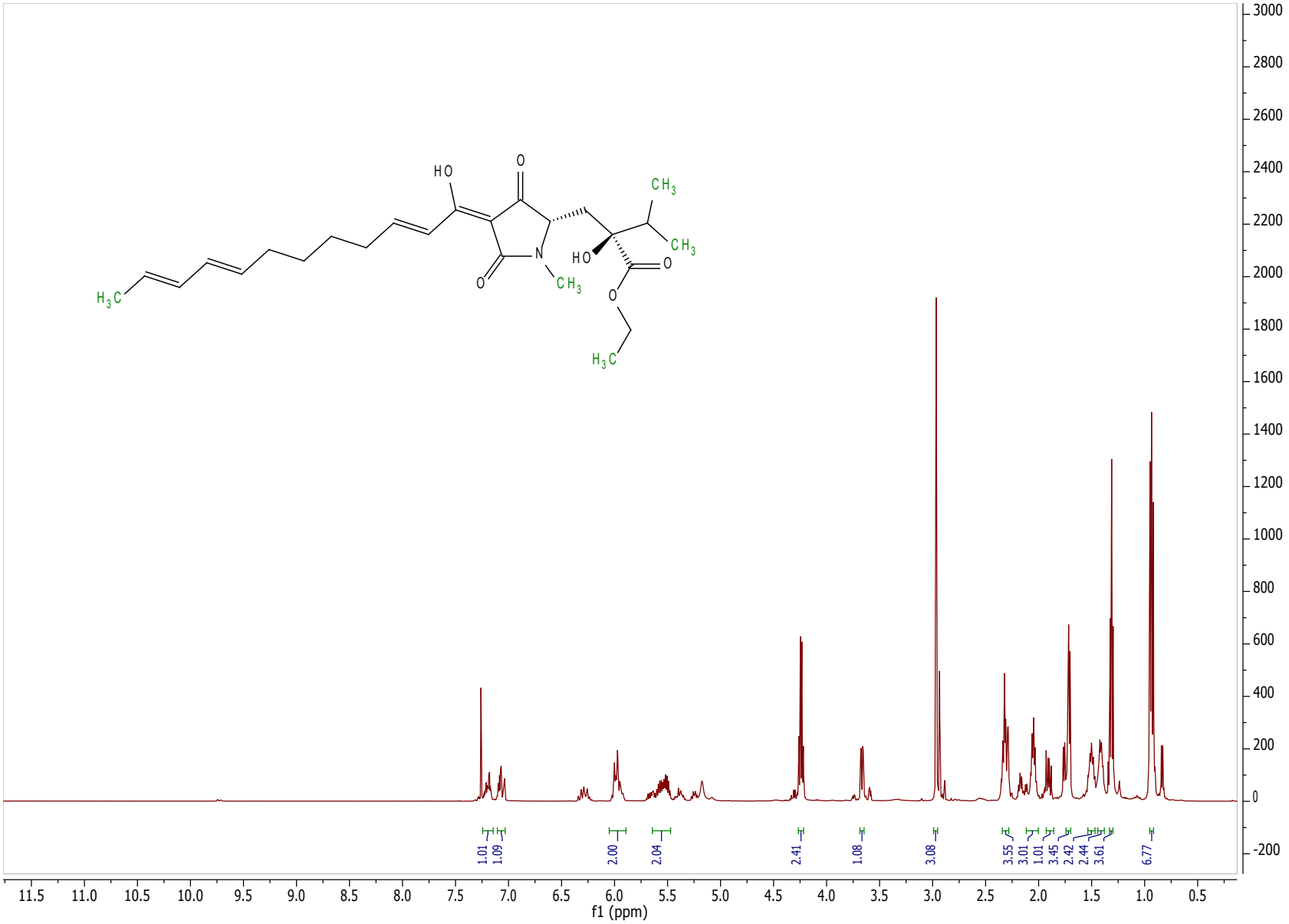


9, $CDCl_3$, 500 MHz

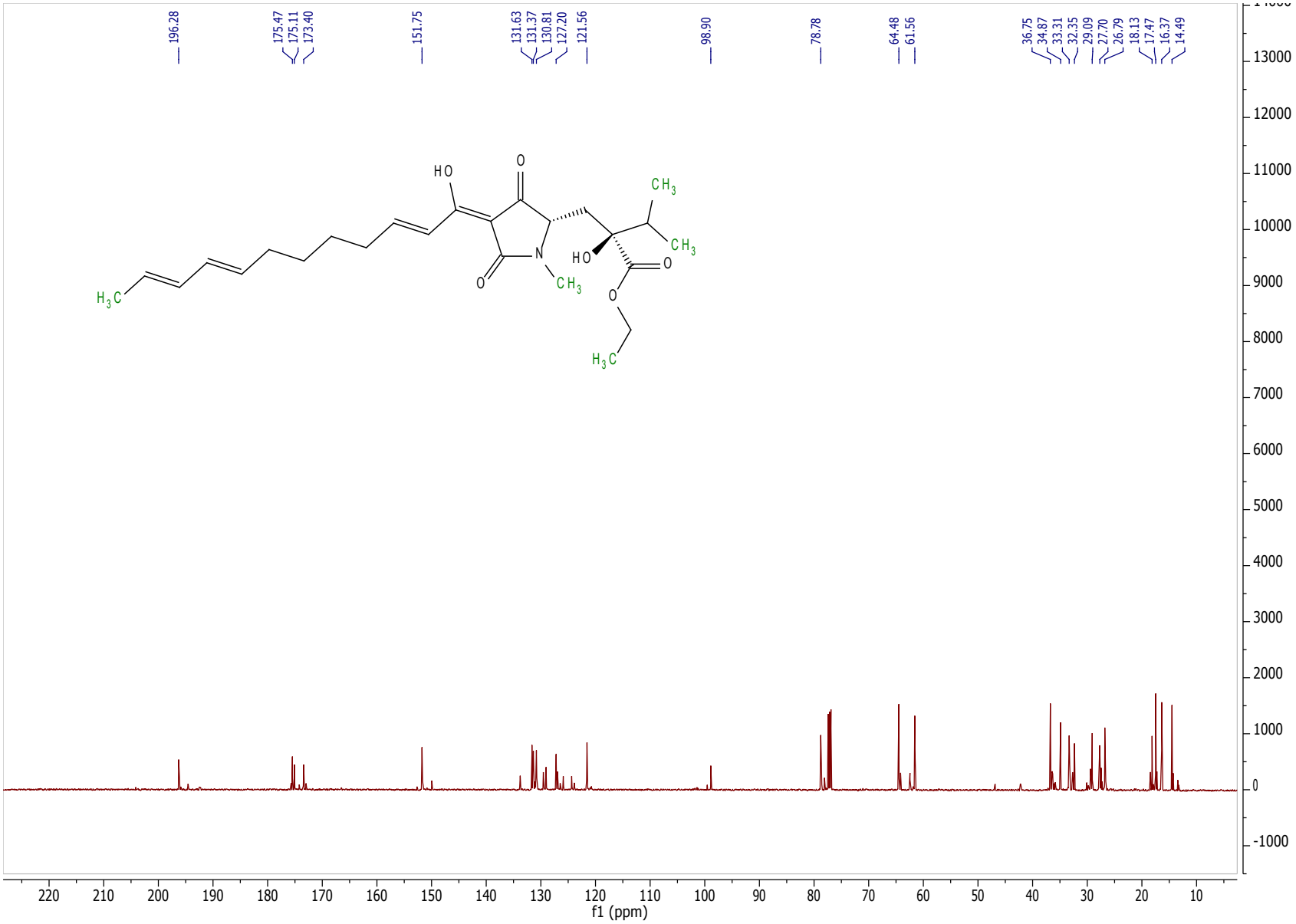



9 (HSQC), CDCl3, 500 MHz
f2 (ppm)
f1 (ppm)
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
10
20
30
40
50
60
70
80
90

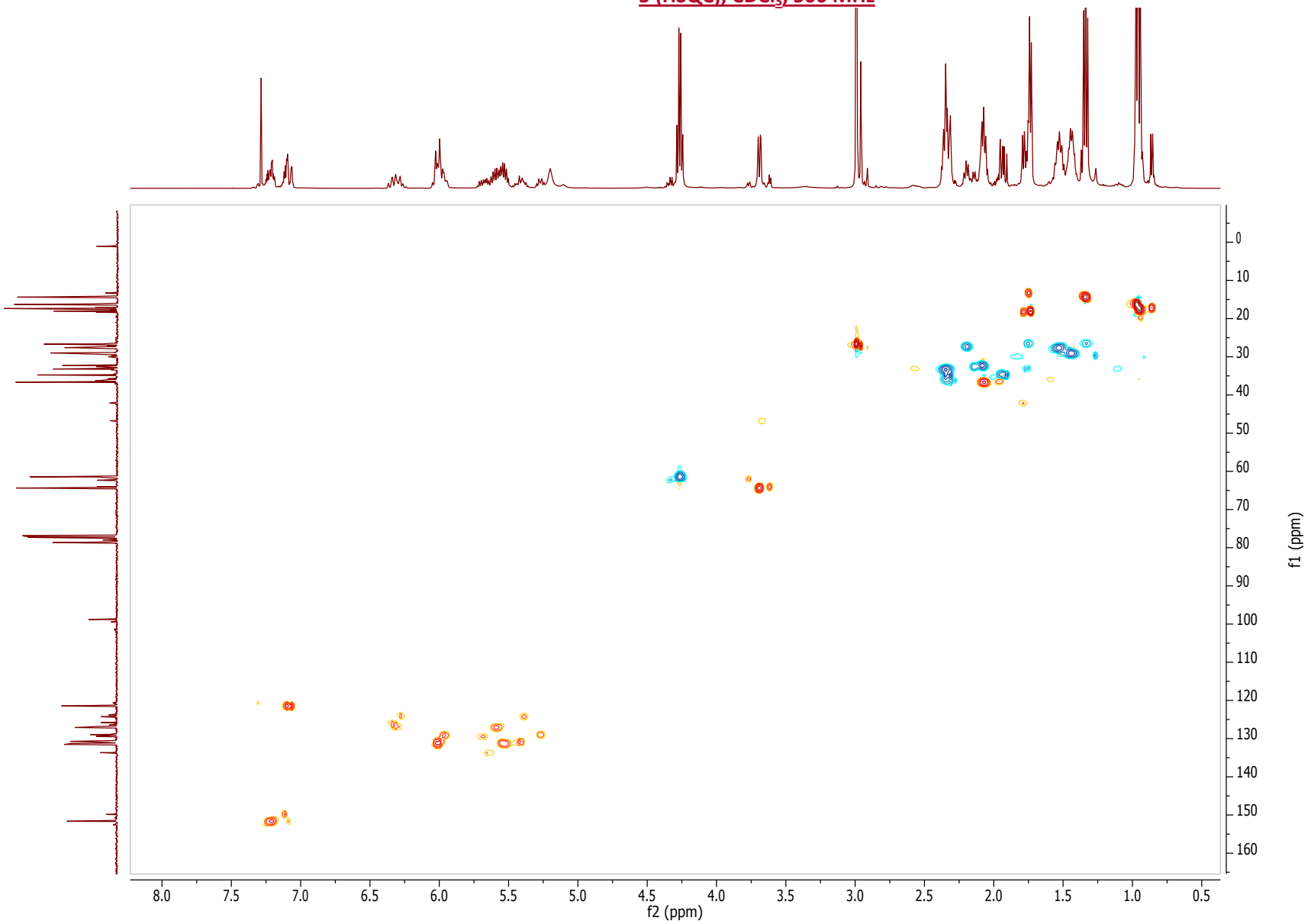
5, $CDCl_3$, 500 MHz



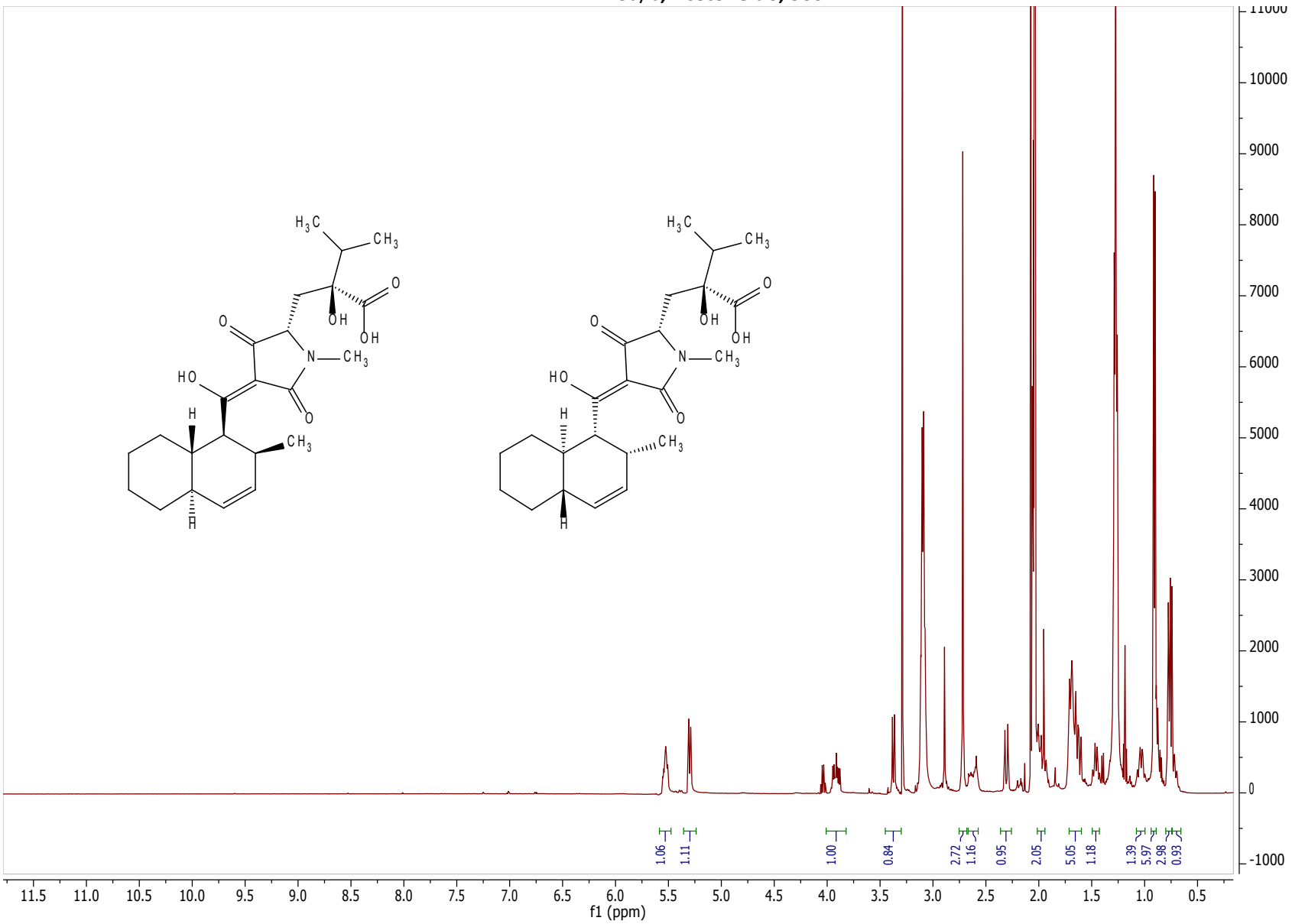
5, $CDCl_3$, 500 MHz



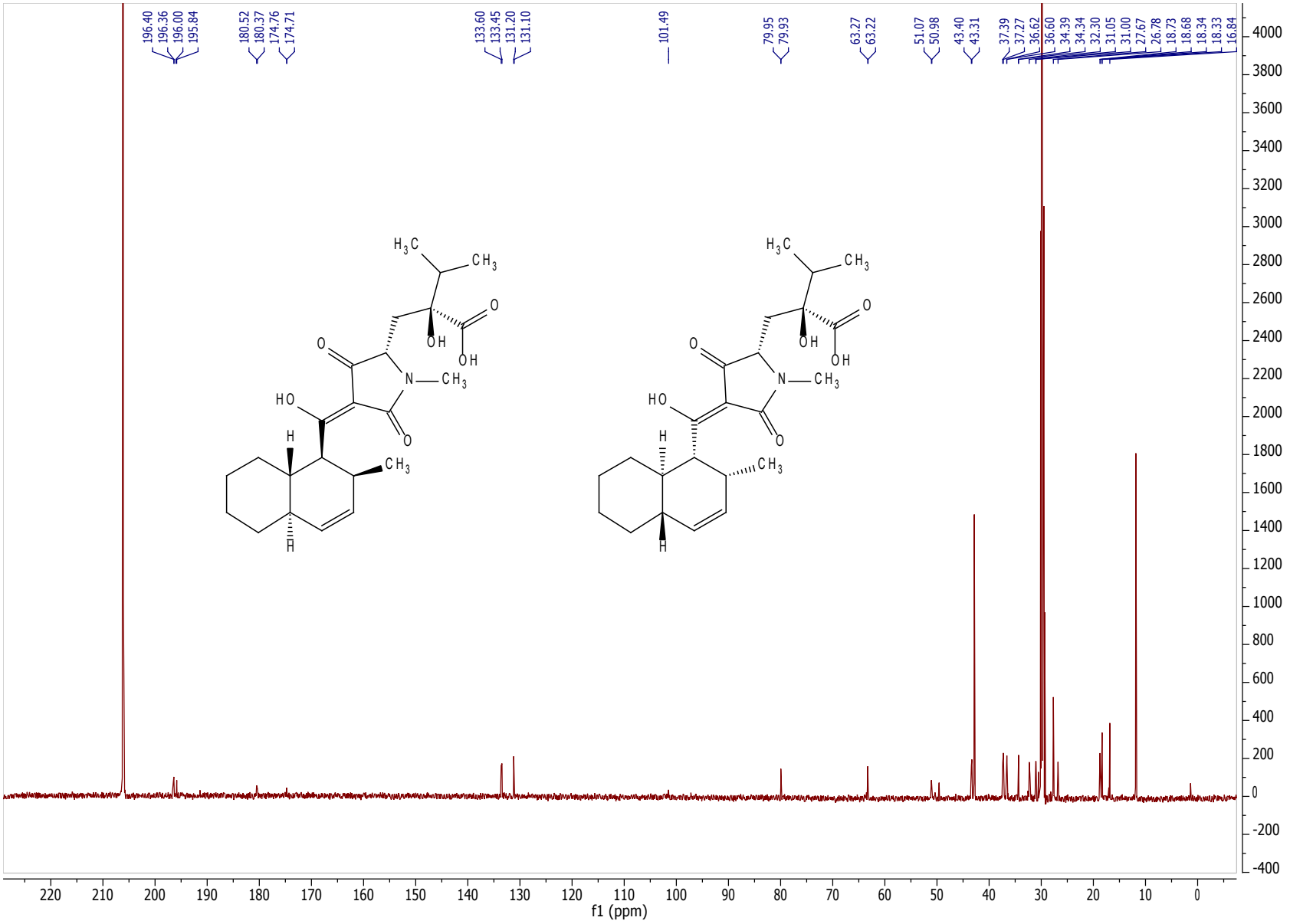
5 (HSQC), $CDCl_3$, 500 MHz



3a/b, Acetone-*d*6, 500 MHz



3a/b, Acetone-*d*6, 500 MHz



5. Bibliography

1 Z. Tian, P. Sun, Y. Yan, Z. Wu, Q. Zheng, S. Zhou, H. Zhang, F. Yu, X. Jia, D. Chen, A. Mándi, T. Kurtán and W. Liu, *Nat. Chem. Biol.*, 2015, 1–10.

2 K. Watanabe, *Chem. Pharm. Bull.*, 2014, **62**, 1153–1165.

3 W. L. Kelly, *Org. Biomol. Chem.*, 2008, **6**, 4483–93.

4 G. Li, S. Kusari and M. Spiteller, *Nat. Prod. Rep.*, 2014, **31**, 1175–1201.

5 A. R. Healy, M. Izumikawa, A. M. Z. Slawin, K. Shin-ya and N. J. Westwood, *Angew. Chemie Int. Ed.*, 2015, **54**, 4046–4050.