
Supplementary Information

Synthesis and Bioactivities of Marine Pyran-Isoindolone Derivatives as Potential Antithrombotic Agents

Yinan Wang ^{1,†}, Hui Chen ^{2,†}, Ruilong Sheng ³, Zhe Fu ¹, **Junting Fan** ⁵, Wenhui Wu ¹, Qidong Tu ^{4,*} and Ruihua Guo ^{1,6,7,*}

¹ College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China; wynshou@163.com (Y.W.); fzshou2019@163.com (Z.F.); whwu@shou.edu.cn (W.W.)

² Shanghai Engineering Research Center of Hadal Science and Technology, College of Marine Sciences, Shanghai Ocean University, Shanghai 201306, China; h-chen@shou.edu.cn

³ CQM-Centro de Química da Madeira, Campus da Penteada, Universidade da Madeira, 9000-390 Funchal, Portugal; ruilong.sheng@staff.uma.pt

⁴ School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, China

⁵ School of Pharmacy, Nanjing Medical University, Nanjing 211166, China; juntinfan@njmu.edu.cn

⁶ Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China

⁷ Laboratory of Quality and Safety Risk Assessment for Aquatic Products on Storage and Preservation (Shanghai), Ministry of Agriculture, Shanghai 201306, China

* Correspondence: 1020100994@jxstnu.edu.cn (Q.T.); rhguo@shou.edu.cn (R.G.)

† These two authors contributed equally to this work.

1. General procedure for preparation of derivatives F1–F7[1,2]

Halogenated hydrocarbon or substituted benzyl (5.0 mmol, RI or RBr) was added drop wise to a solution of compound FGFC1 (50 mg, 0.057 mmol) and K₂CO₃ (79.5mg, 0.57 mmol) in acetone (2.0 mL). The reaction mixture was reacted at room temperature and the reaction was determined by TLC. The reaction mixture was diluted with 50 mL of CH₂Cl₂ and washed three times with 50 mL of brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was chromatographed on the silica gel to yield derivatives F1–F7.

1.1

Methyl(R)-5-((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-5-methoxy-2-methyl

-7-oxo-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)-yl)-2-((2R,3R)-2-((E)-4,8dimethylnona-3,7-dien-1-yl)-3-hydroxy-5-methoxy-7-oxo-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)-yl)pentanoate(F1).

White amorphous powder, yield 62% (after chromatography with petroleum ether/ethyl acetate, 1:3); ¹H NMR (CDCl₃, 500 MHz) δ: 6.88 (1H, s), 6.83 (1H, s), 5.09–5.05 (4H, m), 4.41 (1H, d, *J* = 17.0 Hz), 4.24 (1H, d, *J* = 17.0 Hz), 4.205–4.208 (2H, m), 3.93 (2H, dd, *J* = 10.5, 5.0 Hz), 3.83 (3H, s), 3.82 (3H, s), 3.70 (3H, s), 3.59–3.53 (1H, m), 2.96–2.91 (2H, m), 2.76–2.68 (2H, m), 2.21–2.12 (6H, m), 2.06–2.02 (5H, m),

1.96–1.90 (5H, m), 1.66 (6H, s), 1.62–1.61 (6H, m), 1.57 (6H, s), 1.36 (3H, s), 1.33 (3H, s). ¹³C NMR (125 MHz, CDCl₃) δ: 169.7, 168.5, 167.9, 156.6, 156.5, 147.5, 147.3, 135.0, 134.9, 134.7, 134.6, 133.3, 131.3, 128.7, 127.8, 123.1, 122.5, 121.3, 120.8, 111.6, 111.1, 96.7, 96.6, 78.3, 78.2, 68.5, 68.4, 66.5, 66.4, 65.2, 52.7, 46.5, 43.5, 40.9, 38.6 (2C), 36.1, 30.9, 28.7, 28.3, 26.2, 25.9, 25.6, 24.7, 24.4, 21.7, 20.6 (2C), 18.4, 18.1, 16.7 (2C), 15.1, 15.0. ESI-MS: *m/z* 911 (C₅₄H₇₄N₂O₁₀, [M + H]⁺, Calc. 910.53).

1.2

Ethyl(R)-5-((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-5-ethoxy-3-hydroxy-2-methyl-7-oxo-3,4,7,9-tetrahydropyrano[2,3-*e*]isoindol-8(2H)-yl)-2-((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-5-ethoxy-3-hydroxy-7-oxo-3,4,7,9-tetrahydropyrano[2,3-*e*]isoindol-8(2H)yl)pentanoate (F2).

White amorphous powder, yield 75% (after chromatography with petroleum ether/ethyl acetate, 1:2); ¹H NMR (CDCl₃, 500 MHz) δ: 6.88 (1H, s), 6.84 (1H, s), 5.10–5.05 (4H, m), 4.44 (1H, d, *J* = 16.5 Hz), 4.22 (1H, d, *J* = 16.5 Hz), 4.209–4.207 (2H, m), 4.06 (2H, t, *J* = 6.5 Hz), 3.97–3.92 (7H, m), 3.72–3.67 (1H, m), 3.59–3.54 (1H, m), 3.00–2.96 (2H, m), 2.80–2.72 (2H, m), 2.18–2.11 (5H, m), 2.06–2.01 (5H, m), 1.96–1.89 (5H, m), 1.83–1.78 (5H, m), 1.66 (6H, s), 1.57 (12H, s), 1.36 (3H, s), 1.32 (3H, s), 1.05–1.02 (6H, m), 0.87 (3H, t, *J* = 7.5 Hz). ¹³C NMR (125 MHz, CDCl₃) δ: 171.0, 169.6, 169.0, 158.2, 158.1, 148.3, 148.1, 135.6, 135.5, 132.2, 132.1, 131.3, 130.3, 124.1 (2C), 123.7, 123.6, 122.0, 121.3, 112.3, 112.0, 97.3, 97.2, 79.1, 79.0, 69.8 (2C), 67.5, 67.4, 66.8, 53.7, 47.5, 44.4, 41.9, 39.6 (2C), 37.1, 37.0, 27.3, 26.8, 26.6 (2C), 25.5 (2C), 25.4, 22.5, 21.8, 21.5, 19.2, 18.9, 17.6 (2C), 15.9 (2C), 10.5 (2C), 10.2. ESI-MS: *m/z* 953 (C₅₇H₈₀N₂O₁₀, [M + H]⁺, Calc. 952.58).

1.3

Propyl(R)-5-((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-2-methyl-7-oxo-5-propoxy-3,4,7,9-tetrahydropyrano[2,3-*e*]isoindol-8(2H)-yl)-2-((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-7-oxo-5-propoxy-3,4,7,9-tetrahydropyrano[2,3-*e*]isoindol-8(2H)yl)pentanoate (F3)

White amorphous powder, yield 70% (after chromatography with petroleum ether/ethyl acetate, 1:2); ¹H NMR (CDCl₃, 500 MHz) δ: 6.87 (1H, s), 6.84 (1H, s), 5.11–5.05 (4H, m), 4.46 (1H, d, *J* = 16.5 Hz), 4.23 (1H, d, *J* = 16.5 Hz), 4.22–4.20 (2H, m), 4.18–4.14 (2H, m), 4.09–4.01 (4H, m), 3.94–3.92 (3H, m), 3.72–3.67 (1H, m), 3.59–3.54 (1H, m), 3.04–2.93 (2H, m), 2.80–2.72 (2H, m), 2.15–2.11 (8H, m), 2.06–2.02 (5H, m), 1.96–1.93 (5H, m), 1.66 (12H, s), 1.63–1.61 (2H, m), 1.57 (12H, s), 1.42–1.39 (6H, m), 1.36 (3H, s), 1.32 (3H, s), 1.23 (3H, t, *J* = 7.5 Hz). ¹³C NMR (125 MHz, CDCl₃) δ: 170.9, 169.6, 169.0, 158.1, 158.0, 148.3, 148.1, 135.7, 135.6, 132.1, 131.4, 131.33, 131.32, 124.2, 123.6, 122.1, 121.3, 119.7, 119.5, 111.8, 111.8, 97.3, 97.2, 79.1, 79.0, 67.6,

67.5, 64.0, 63.9, 61.3, 53.6, 47.5, 44.4, 41.9, 39.6 (2C), 37.1, 37.0, 27.3, 26.8 (2C), 26.6 (2C), 25.6 (2C), 25.5, 22.5 (2C), 21.6 (2C), 20.1, 19.3, 19.0, 17.6 (2C), 15.9, 15.8, 14.7 (2C), 14.1. ESI-MS: m/z 1017 ($C_{60}H_{86}N_2O_{10}$, $[M + Na]^+$, Calc. 994.63).

1.4

Cyanobenzyl (R)-2,5-Bis((2R,3R)-5-((4-cyanobenzyl)oxy)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-2-methyl-7-oxo-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)yl)pentanoate(F4)

White amorphous powder, yield 56 % (after chromatography with petroleum ether/ethyl acetate,); 1H NMR ($CDCl_3$, 500 MHz) δ : 7.62 (2H, d, $J = 7.5$ Hz), 7.59 (2H, d, $J = 7.5$ Hz), 7.55 (3H, d, $J = 7.5$ Hz), 7.51 (3H, d, $J = 7.5$ Hz), 7.46 (2H, d, $J = 7.5$ Hz), 6.94 (1H, s), 6.90 (1H, s), 5.17–5.05 (6H, m), 4.74 (4H, s), 4.40 (1H, d, $J = 16.5$ Hz), 4.27–4.18 (3H, m), 3.98–3.96 (2H, m), 3.78–3.45 (3H, m), 3.07–3.03 (2H, m), 2.87–2.81 (2H, m), 2.21–2.14 (8H, m), 2.04–2.01 (6H, m), 1.96–1.95 (6H, m), 1.65 (6H, s), 1.57 (12H, s), 1.26 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ : 170.6, 169.5, 168.8, 157.4, 157.3, 148.6, 148.4, 138.3, 138.2, 136.8, 135.9, 135.8, 132.1, 131.9, 131.6, 131.5, 131.3, 131.0, 130.9, 130.4, 130.3, 130.1, 129.9, 129.7, 129.4, 129.2 (2C), 124.1, 123.4, 123.3, 122.9, 122.3, 118.8 (2C), 118.6 (2C), 118.3 (2C), 112.7, 112.4 (2C), 112.1, 97.5, 97.4, 79.4, 79.3, 68.9, 68.8, 67.4, 65.6, 63.9, 53.7, 47.6, 44.7, 41.8, 39.6, 37.0, 35.9, 31.9, 29.6, 29.1, 27.2, 27.1, 26.9, 26.6, 25.7, 25.4, 22.7, 21.6, 19.4, 19.2, 17.6 (2C), 16.0, 14.1. ESI-MS: m/z 1214 ($C_{75}H_{83}N_5O_{10}$, $[M + H]^+$, Calc. 1213.61).

1.5

4-(Trifluoromethoxy)benzyl(R)-2,5-bis((2R,3R)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-2-methyl-7-oxo-5-((4-(trifluoromethoxy)benzyl)oxy)-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)-yl) pentanoate(F5)

White amorphous powder, yield 72.6 % (after chromatography with petroleum ether/ethyl acetate); 1H NMR ($CDCl_3$, 500 MHz) δ : 7.47–7.45 (4H, m), 7.32 (2H, d, $J = 8.5$ Hz), 7.24–7.22 (4H, m), 7.16–7.12 (2H, m), 6.96 (1H, s), 6.94 (1H, s), 5.14 (2H, s), 5.09–5.05 (8H, m), 4.42 (1H, d, $J = 16.5$ Hz), 4.27–4.20 (3H, m), 3.96–3.93 (2H, m), 3.73–3.69 (1H, m), 3.55–3.52 (2H, m), 3.05–3.01 (2H, m), 2.85–2.77 (2H, m), 2.23–2.11 (6H, m), 2.06–1.99 (6H, m), 1.97–1.93 (6H, m), 1.65 (6H, s), 1.64–1.62 (2H, m), 1.57 (12H, s), 1.42 (3H, s), 1.37 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ : 170.8, 169.4, 168.8, 157.7, 157.6, 149.1, 148.9, 147.1, 139.2, 138.6, 136.0, 135.9, 135.4, 134.1, 132.5, 131.5, 129.6 (2C), 128.7 (2C), 128.1, 127.9, 127.8, 127.3, 127.2, 124.5, 124.4, 124.2, 124.1, 124.0 (2C), 123.5 (2C), 122.9, 122.8, 122.3, 121.1 (2C), 119.1 (2C), 112.5, 112.0, 97.7, 97.6, 79.3, 79.2, 69.5, 69.4, 67.6, 67.4, 66.0, 53.7, 47.5, 44.5, 41.9, 39.7, 37.1, 34.8, 34.5, 29.7 (2C), 29.4, 27.0, 26.6 (2C), 22.7 (2C), 21.6, 21.1, 19.4, 19.1, 17.6 (2C), 16.0, 14.1. ESI-MS: m/z 1343 ($C_{75}H_{83}F_9N_2O_{10}$, $[M + H]^+$, Calc. 1342.59).

1.6

4-Bromobenzyl(R)-2,5-bis((2R,3R)-5-((4-bromobenzyl)oxy)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-2-methyl-7-oxo-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)yl)pentanoate(F6)

White amorphous powder, yield 82.3%(after chromatography with petroleum ether/ethyl acetate);
¹H NMR (CDCl₃, 500 MHz) δ:7.50-7.48 (4H, m), 7.40 (2H, d, *J*=8.5Hz), 7.30-7.28 (4H, m), 7.14 (2H, d, *J*=8.5Hz), 6.93 (1H, s), 6.90 (1H, s), 5.13–5.01 (10H, m), 4.40 (1H, d, *J*=17.0Hz), 4.25–4.15 (3H, m), 3.95–3.91 (2H, m), 3.71–3.65 (1H, m), 3.55–3.51 (2H, m), 3.04–2.99 (2H, m), 2.85–2.77 (2H, m), 2.20–2.10 (7H, m), 2.05–2.00 (5H, m), 1.97–1.91 (6H, m), 1.65 (6H, s), 1.63–1.61 (2H, m), 1.56 (12H, s), 1.37 (3H, s), 1.34 (3H, s). ¹³C NMR (125 MHz, CDCl₃) δ: 170.1, 169.5, 168.8, 157.6, 157.5, 148.4, 148.3, 135.9, 135.8, 135.6, 135.5, 134.3, 132.2, 131.7 (2C), 131.59, 131.6, 131.4, 131.3, 131.1, 130.4, 130.3, 129.7, 129.1, 128.8, 128.5, 124.1, 124.0, 123.5, 123.4, 122.7, 122.3, 122.0, 121.9, 121.8, 119.0, 118.9, 112.5, 112.1, 97.6, 97.5, 79.3, 79.2, 69.4, 69.3, 67.4, 67.3, 66.1, 53.6, 47.5, 44.5, 41.8, 39.6, 37.0, 33.4, 31.9, 29.7, 29.3, 27.1, 26.9, 26.6, 25.7, 25.4, 23.2, 22.7, 21.6, 19.4, 19.1, 17.6 (2C), 16.0, 14.1. ESI-MS: *m/z* 1373 (C₇₂H₈₃Br₃N₂O₁₀, [M + H]⁺, Calc. 1372.36).

1.7

2-bromobenzyl(R)-2,5-bis ((2R,3R)-5-((2-Bromobenzyl)oxy)-2-((E)-4,8-dimethylnona-3,7-dien-1-yl)-3-hydroxy-2-methyl-7-oxo-3,4,7,9-tetrahydropyrano[2,3-e]isoindol-8(2H)-yl)pentanoate(F-7).

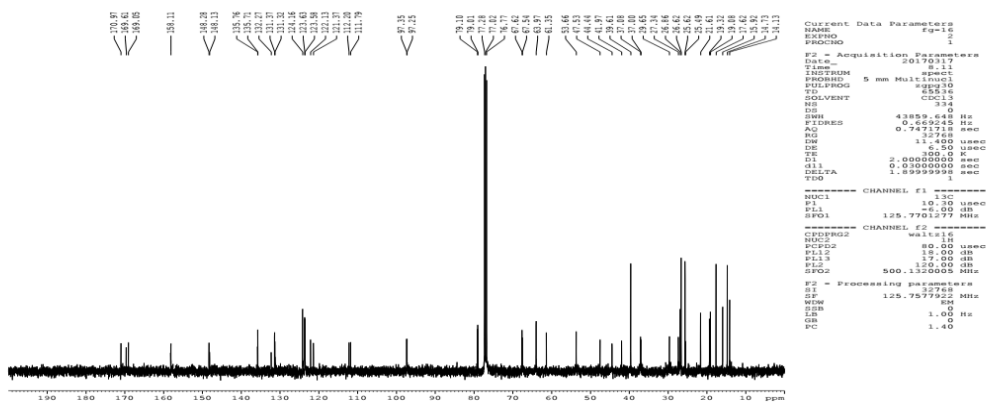
White amorphous powder, yield 73.2% (after chromatography with petroleum ether/ethyl acetate);
¹H NMR (CDCl₃, 500 MHz) δ:7.58–7.55 (4H, m), 7.51 (1H, d, *J*=8.0Hz), 7.34–7.32 (3H, m), 7.25 (1H, t, *J*=8.0 Hz), 7.17–7.13 (3H, m), 7.01 (1H, s), 6.98 (1H, s), 5.22–5.05 (10H, m), 4.48 (1H, d, *J* = 16.5Hz), 4.29–4.18 (3H, m), 3.96–3.95 (2H, m), 3.75–3.47 (3H, m), 3.10–3.06 (2H, m), 2.91–2.83 (2H, m), 2.22–2.13 (7H, m), 2.05–2.01 (5H, m), 1.97–1.93 (6H, m), 1.65 (6H, s), 1.62–1.60 (2H, m), 1.57 (12H, s), 1.38 (3H, s), 1.34 (3H, s). ¹³C NMR (125 MHz, CDCl₃) δ: 170.6, 169.5, 168.8, 157.49, 157.50, 148.4, 148.3, 136.0, 135.91, 135.93, 135.8, 134.6, 132.8 (2C), 132.6, 132.4, 131.4, 131.3, 130.1, 129.9, 129.8 (2C), 129.3, 129.2, 128.6, 127.5, 127.4, 124.2, 124.1, 123.5, 123.4, 123.3, 122.9, 122.3, 122.1, 119.1, 119.0, 112.5, 112.1, 97.8, 97.7, 79.3, 79.2, 69.8, 69.7, 67.5, 67.4, 66.6, 53.6, 47.5, 44.5, 41.9, 39.6 (2C), 37.1, 37.0, 29.7, 29.3, 27.3, 27.2, 26.9, 26.6, 25.7, 25.5, 22.7, 21.6, 19.4, 19.1, 17.7 (2C), 16.0, 14.1. ESI-MS: *m/z* 1373 (C₇₂H₈₃Br₃N₂O₁₀, [M + H]⁺, Calc. 1372.36).

References:

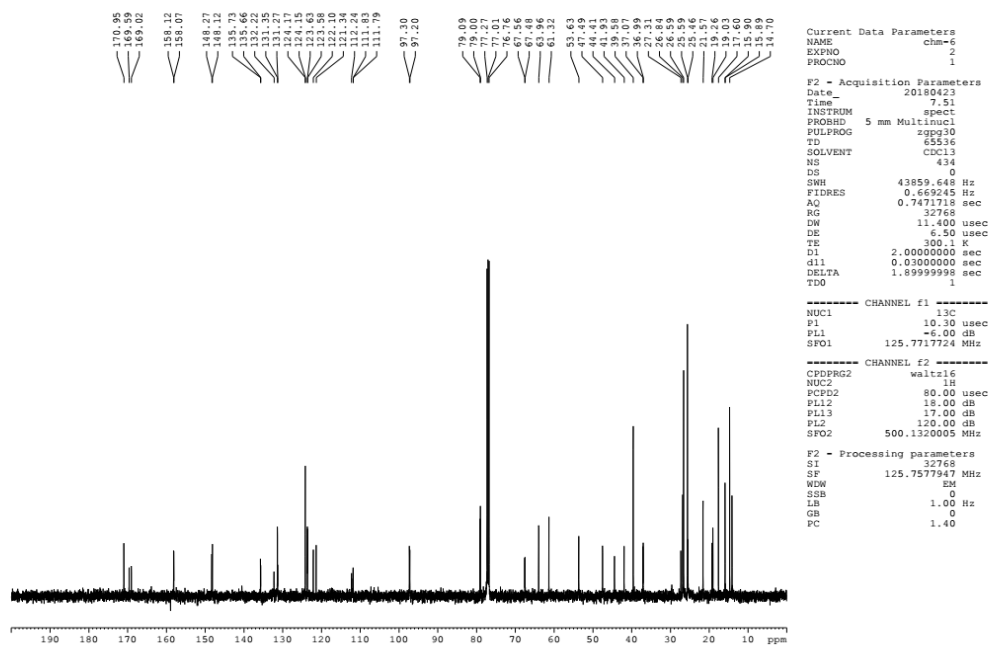
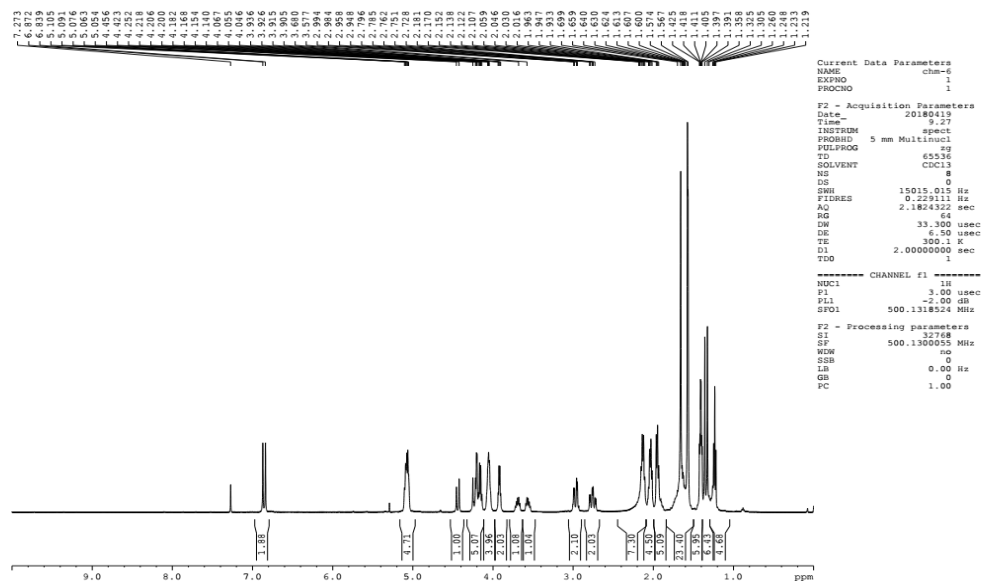
[1] Amanda, S.R.; Gabriel, N.V.; Sergio, H.F.; María, Y.R.; Maximiliano, I.B.; Samuel, E.S. Semisynthesis, ex vivo evaluation, and SAR studies of coumarin derivatives as potential

[2] Jane, A.P. Cell sensitivity assays: The MTT assay. *Methods. In. Mol. Med.* 1999, 80.

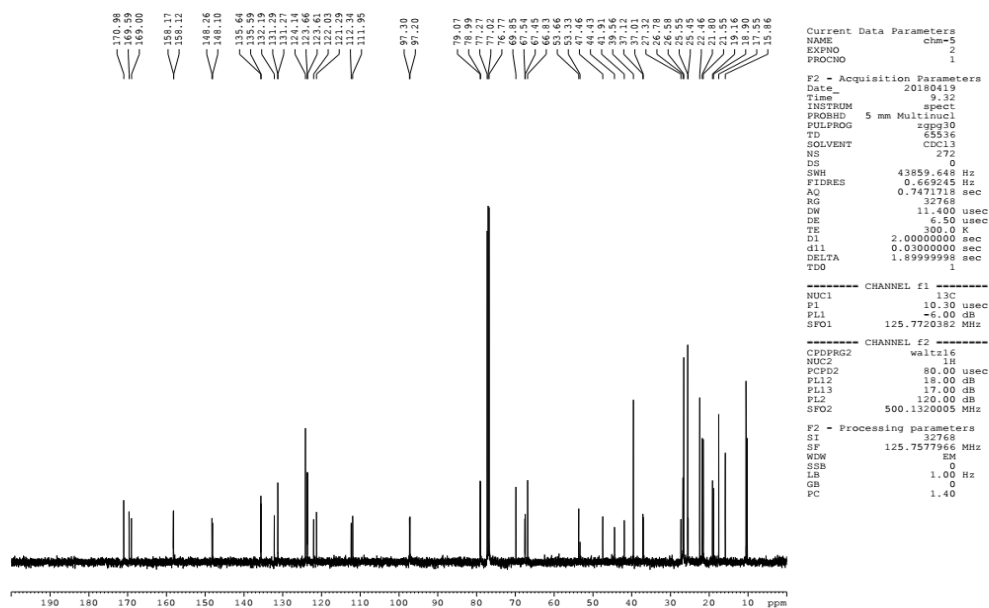
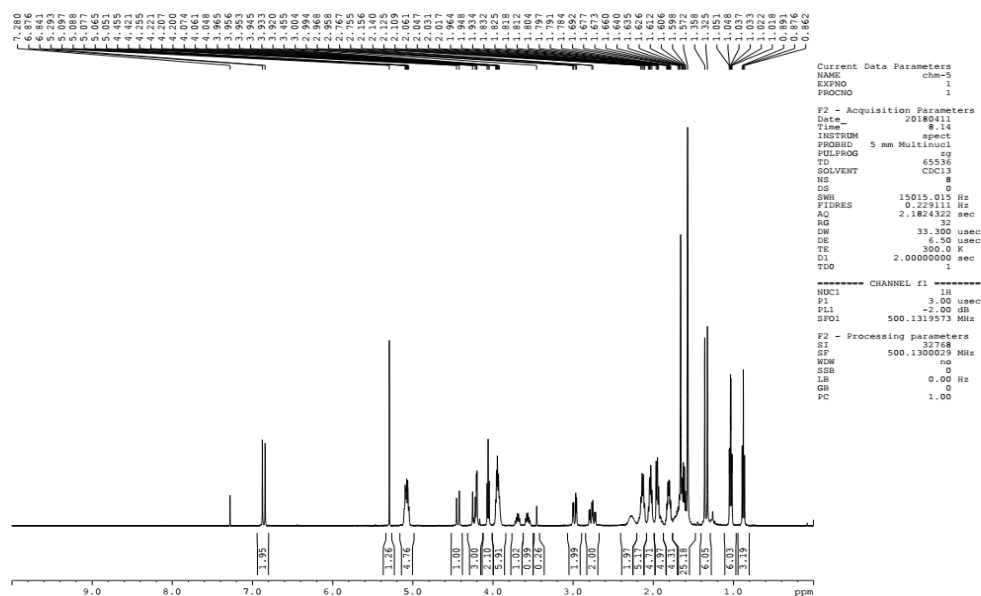
2.1 F1 NMR Spectra



2.2 F2 NMR Spectra



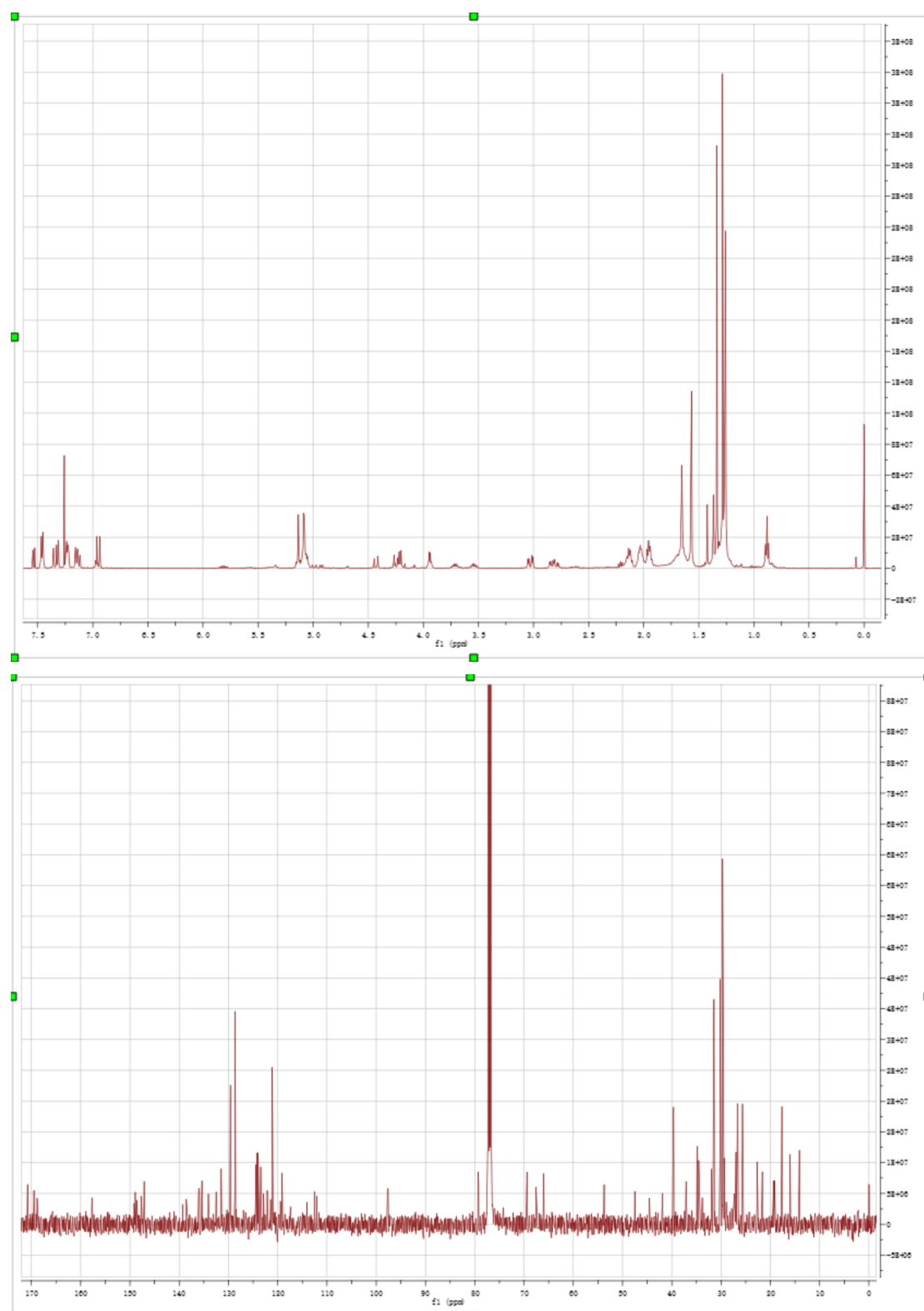
2.3 F3 NMR Spectra



2.4 F4 NMR Spectra



2.5 F5 NMR Spectra



2.6 F6 NMR Spectra



2.7 F7 NMR Spectra

