
Supplementary information

A condensate-hardening drug blocks RSV replication in vivo

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Supplementary information Guide

for

A condensate-hardening drug blocks RSV replication *in vivo*.

by

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1. Chemical synthesis of A3M, A3E, A3P

1.1. List of compounds

Compound 1 (Cyclopamine)

Compound 2 (intermediate 1)

Compound 3 (intermediate 2)

Compound 4 (A3M)

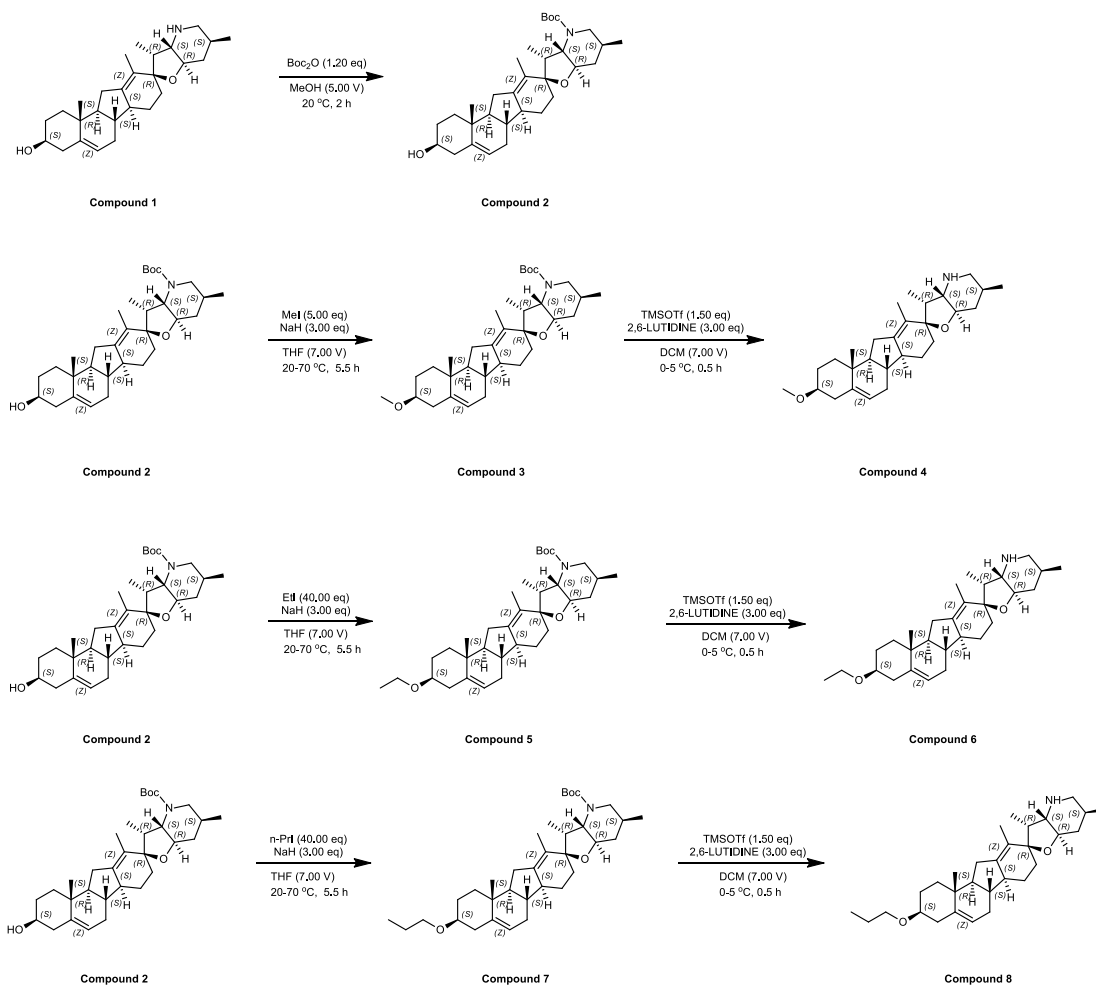
Compound 5 (intermediate 3)

Compound 6 (A3E)

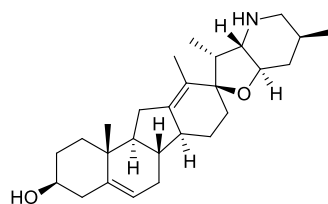
Compound 7 (intermediate 4)

Compound 8 (A3P)

1.2. Synthesis scheme



71 **Compound 1 (Cyclopamine)**

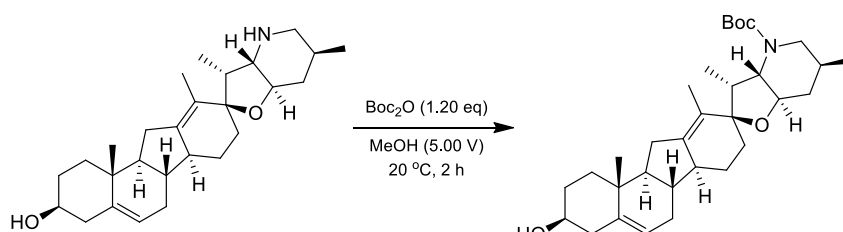


72 cyclopamine

73 m.p.: 217.5–220.2 °C; TLC (CH₂Cl₂:MeOH, 5:1 v/v): R_F = 0.33; [α]₂₀^D (deg cm³ g⁻¹
 74 dm⁻¹) = –56.07 (c = 0.01016 g cm⁻³ in MeOH); ¹H-NMR (CDCl₃ 400 MHz): δ 5.37-
 75 5.36 (m, 1 H), 3.53-3.52 (m, 1 H), 3.22-3.19 (m, 1 H), 3.07-3.05 (m, 1 H), 2.65-2.62
 76 (m, 1 H), 2.34-2.18 (m, 8 H), 1.80-1.72 (m, 11 H), 1.70-1.20 (m, 8 H), 0.95-0.92 (m, 9
 77 H); ¹³C NMR (101 MHz, CHLOROFORM-d) δ 142.96, 141.69, 126.62, 121.85,
 78 85.44, 75.45, 71.71, 66.18, 54.61, 52.07, 49.26, 41.81, 41.54, 39.62, 39.09, 38.17,
 79 36.56, 31.97, 31.64, 31.37, 31.13, 28.95, 24.54, 18.93, 18.70, 13.15, 10.73; IR (KBr):
 80 3282.27 cm⁻¹ ν(O-H) ; 1633.16 cm⁻¹ ν(C=C); UV/vis: λ_{max} 196.0 nm; HRMS(m/z):
 81 [M+H]⁺ calculated for C₂₇H₄₂NO₂: 412.3216; found: 412.3190.

82

83 **Synthesis of compound 2**



84 cyclopamine

2

85

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.1	441.62	5.00 g	12.15 mmol	1.00	
(Boc) ₂ O	218.25	3.18 g	14.58 mmol	1.20	
MeOH	-	25.00 mL	-	-	
Cpd 2	511.73	2.16 g	10.65 mmol	-	Yld 87.68%

86

87 Cyclopamine (5 g, 12.15 mmol, 1.00 eq) was dissolved in MeOH (25.00 mL) followed
 88 by addition of Boc₂O (3.18 g, 14.58 mmol, 3.35 mL, 1.20 eq). The reaction mixture
 89 was stirred at 20 °C for 2.00 hrs until the suspension solution turned clear. LCMS

(ET46937-2-P1a2) shows ~97.5% of desired compound 2 (product: RT = 3.231 min; M-55=456.4).

The reaction mixture was quenched by addition of H₂O (10.00 mL) at 25 °C, and then diluted with EtOAc (10.00 mL) and extracted with EtOAc (10.00 mL * 3). The combined organic layers were washed with brine (20 mL * 2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=50/1 to 5/1).

Compound 2 (5.45 g, 10.65 mmol, 87.68% yield) was obtained as a white solid.

m.p.: 118.6–130.6 °C; TLC (PE:EtOAc, 3:1 v/v): RF = 0.45; $[\alpha]_{20}^D$ (deg cm³ g⁻¹ dm⁻¹)

= -5.40 (c = 0.009458 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, MeOD): δ 5.38 (t, *J* =

2.6 Hz, 1H), 4.09-4.12 (s, 1H), 3.471-3.676 (s, 2H), 3.36 (m, 1H), 3.185-3.211 (m, 1H),

2.818-2.836 (m, 1H), 2.200-2.314 (m, 6H), 1.480-1.912 (m, 22H), 1.224-1.260 (m, 4H),

0.931-1.016 (m, 4H); ¹³C NMR (101 MHz, METHANOL-d₄) δ 159.01, 144.66, 143.30,

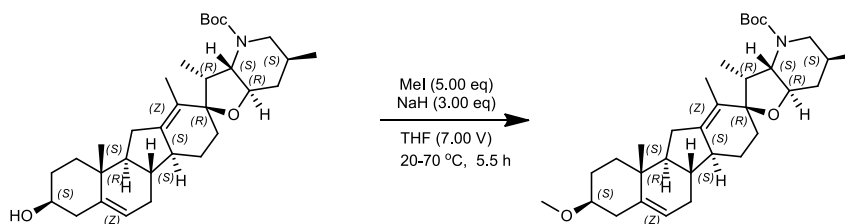
127.72, 122.81, 86.80, 81.63, 73.67, 72.61, 64.46, 61.67, 53.57, 51.05, 50.28, 43.59,

43.20, 42.71, 39.61, 37.88, 35.70, 33.56, 32.19, 30.02, 29.56, 28.82, 25.79, 21.33, 21.02,

19.14, 14.63, 13.85, 10.88; IR (KBr): 1740.87, 1705.26 cm⁻¹ ν(C=O); 1153.14 cm⁻¹

ν(C-O); UV/vis: λ_{max} 196.5 nm; HRMS (m/z): [M-Boc+H]⁺ calculated for C₂₇H₄₂NO₂:

412.3210; found: 412.3186.

110 **Synthesis of compound 3**

111

Compound 2

Compound 3

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.2	511.73	1.50 g	2.93 mmol	1.00	-
MeI	141.94	2.08 g	14.66 mmol	5.00	-
NaH	24.00	351.71 mg	8.79 mmol	3.00	60% w/w
THF	-	10.5 mL	-	-	
Cpd 3	525.76	1.00 g	1.90 mmol	-	Yld 64.89%

112

113 Compound 2 (1.50 g, 2.93 mmol, 1.00 eq), dry THF (10.5 mL) and NaH (351.71 mg,
 114 8.79 mmol, 60% w/w, 3.00 eq) was added to the flask at 20 °C and stirred at 70 °C for
 115 2.5 hrs. MeI (2.08 g, 14.66 mmol, 912.39 uL, 5.00 eq) was added to the mixture and
 116 stirred at 70 °C for 3.0 hrs. LCMS (ET46937-7-P1A8) show ~100% of desired
 117 compound 3 (product: RT = 3.694 min; M-55=470.4).

118 The reaction was quenched carefully by H₂O (10 mL), extracted with ethyl acetate (20
 119 mL), washed with brine (10 mL), dried over Na₂SO₄, and concentrated to give the crude
 120 product. The crude product was purified by trituration from n-hexane (3.00 mL) at 20
 121 °C.

122 Compound 3 (1.00 g, 1.90 mmol, 64.89% yield) was obtained as a white solid.

123 m.p.: 164.9–170.6 °C; TLC (PE:EtOAc, 3:1 v/v): RF = 0.70; $[\alpha]_{20}^D$ (deg cm³ g⁻¹ dm⁻¹)

124 = -4.63 (c = 0.009074 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, CDCl₃): δ 5.37 (t, J =

125 2.2 Hz, 1H), 3.52-3.56 (m, 2H), 3.35 (s, 3H), 3.11-3.14 (m, 2H), 2.81-2.83 (m, 2H),

126 2.44-2.47 (m, 1H), 2.17-2.24 (m, 5H), 1.69-1.90 (m, 7H), 0.91-1.47 (m, 26H); ¹³C NMR

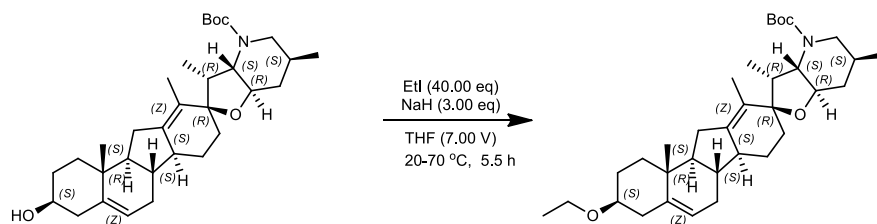
127 (101 MHz, CHLOROFORM-d) δ 157.42, 143.12, 141.77, 126.73, 121.79, 84.85, 80.41,

128 80.01, 72.58, 63.24, 55.68, 52.12, 50.70, 49.31, 41.87, 41.53, 38.25, 38.10, 36.96, 35.20,

129 32.58, 31.16, 29.03, 28.41, 27.71, 24.62, 20.59, 18.69, 13.52, 10.48; IR (KBr): 1703.23

130 cm⁻¹ ν(C=O); 1168.28 cm⁻¹ ν(C-O); UV/vis: λ_{max} 194.5 nm; HRMS(m/z): [M-

131 Boc+H]⁺ calculated for C₂₈H₄₄NO₂: 426.3367; found: 426.3369.

132 **Synthesis of compound 5**

133

Compound 2

Compound 5

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.2	511.73	0.50 g	0.977 mmol	1.00	-
EtI	155.97	6.10g	39.08 mmol	40.0	-
NaH	24.00	117.25 mg	2.93 mmol	3.00	60% w/w
THF	-	3.50 mL	-	-	
Cpd 5	539.79	0.30 g	0.556 mmol	-	Yld 56.88%

134

135 Compound 2 (500 mg, 977.07 μmol , 1 *eq*), THF (3.50 mL) and NaH (117.25 mg, 2.93
 136 mmol, 60% w/w, 3.00 *eq*) were added to the flask at 20 $^\circ\text{C}$ and the mixture was stirred
 137 at 70 $^\circ\text{C}$ for 2.5 hrs. EtI (6.10 g, 39.08 mmol, 3.13 mL, 40.00 *eq*) was added to the
 138 mixture and stirred at 70 $^\circ\text{C}$ for another 3.0 hrs.

139 LCMS (ET46937-6-P1a9) data show ~96.8% of desired compound 5. (product: RT =
 140 1.524 min; M-55=484.3)

141 Another the same 2 reactions (500 mg each) were combined here. The reaction was
 142 quenched by carefully adding H_2O (5.00 mL), extracted with ethyl acetate (10.00 mL),
 143 wash with brine (5.00 mL), dried over Na_2SO_4 , concentrate to give the crude product.
 144 The crude product was purified by trituration with n-hexane (1.00 mL) at 20 $^\circ\text{C}$.
 145 Compound 5 (900 mg, 1.67 mmol, 56.88% yield) was obtained as a white solid.

146 m.p.: 155.0–161.1 $^\circ\text{C}$; TLC (PE:EtOAc, 3:1 v/v): RF = 0.65; $[\alpha]_{20}^D$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$)

147 1) = -0.07 ($c = 0.09866 \text{ g cm}^{-3}$ in MeOH); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 5.36 (d, J

148 = 2.0 Hz, 1H), 3.50-3.56 (m, 4H), 3.14-3.16 (m, 2H), 2.76-2.90 (m, 2H), 2.39-2.49

149 (m, 1H), 2.19-2.24 (m, 5H), 1.69-1.90 (m, 7H), 0.92-1.47 (m, 31H); $^{13}\text{C NMR}$ (101

150 MHz, CHLOROFORM-d) δ 157.41, 143.15, 142.01, 126.71, 121.62, 84.84, 80.00,

151 78.81, 72.57, 63.24, 52.14, 50.69, 49.32, 41.87, 41.53, 38.78, 38.22, 36.97, 35.20,

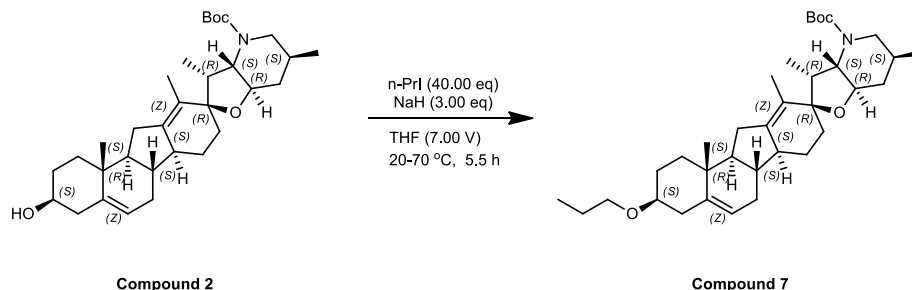
152 32.58, 31.16, 29.03, 28.40, 28.23, 24.62, 20.59, 18.68, 15.75, 13.51, 10.47; IR (KBr):

153 1735.23, 1698.35 cm^{-1} $\nu(\text{C=O})$; 1157.52, 1104.58 cm^{-1} $\nu(\text{C-O})$; UV/vis: λ_{max} 194.

154 nm; HRMS(m/z): [M-Boc+H]⁺ calculated for C₂₉H₄₆NO₂: 440.3523; found:
 155 440.3519.

156

157 Synthesis of compound 7



158

159

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.2	511.73	2.00 g	3.91 mmol	1.00	-
n-PrI	169.99	26.58 g	156.33 mmol	40.0	-
NaH	24.00	468.99 mg	11.72 mmol	3.00	60% w/w
THF	-	14.00 mL	-	-	
Cpd 7	553.81	0.80 g	1.44 mmol	-	Yld 36.96%

160

161 Compound 2 (2.00 g, 3.91 mmol, 1.00 eq), THF (14.00 mL) and NaH (468.99 mg,
 162 11.72 mmol, 60% w/w, 3.00 eq) were added to the flask at 20 °C and the mixture was
 163 stirred at 70 °C for 2.5 hrs. n-PrI (26.58 g, 156.33 mmol, 15.27 mL, 40.00 eq) was added
 164 to the mixture and stirred at 70 °C for another 3.0 hrs.

165 LCMS (**ET46937-12-p1a2**) showed ~21.70% of desired compound 7 was detected
 166 (product: RT = 1.083 min; M-55=498.4).

167 The reaction was quenched by carefully adding H₂O (20.00 mL), extracted with ethyl
 168 acetate (20.00 mL), wash with brine (10.00 mL), dry over Na₂SO₄, and concentrate to
 169 give the crude product. The crude product was purified by trituration with n-hexane
 170 (3.00 mL) at 20 °C. Compound 7 (800 mg, 1.44 mmol, 36.96% yield) was obtained as
 171 a white solid.

172 m.p.: 171.2–176.5 °C; TLC (PE:EtOAc, 3:1 v/v): RF = 0.50; [α]₂₀^D (deg cm³ g⁻¹ dm⁻

173 ¹) = 0.82 (c = 0.00973 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, CDCl₃): δ 5.36 (s, 1H),

174 3.53-3.56 (t, *J* = 6.6 Hz, 2H), 3.40-3.43 (t, *J* = 6.6 Hz, 2H), 3.14-3.16 (t, *J* = 5.0 Hz,

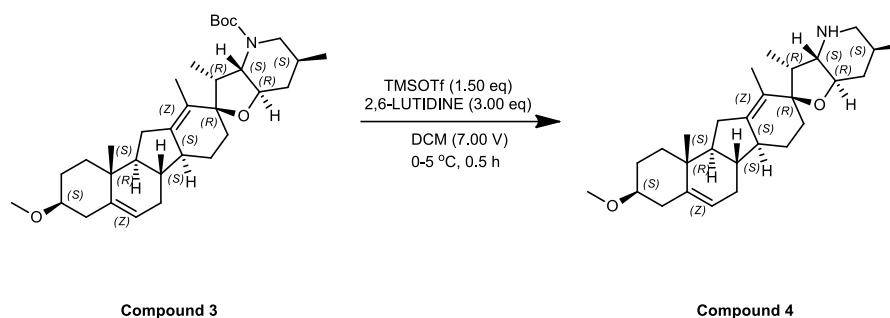
175 2H), 2.76-2.90 (m, 2H), 2.39-2.49 (m, 1H), 2.18-2.22 (m, 5H), 1.55-1.90 (m, 7H),

176 0.90-1.47 (m, 33H); ¹³C NMR (101 MHz, CHLOROFORM-d) δ 157.42, 143.17,

177 142.07, 126.70, 121.57, 84.85, 80.01, 78.98, 72.58, 69.86, 63.24, 52.15, 50.70, 49.33,
 178 41.87, 41.53, 38.77, 38.23, 36.98, 35.20, 32.59, 31.17, 29.04, 28.40, 28.22, 24.62,
 179 23.40, 20.59, 18.70, 13.52, 10.68, 10.47; IR (KBr): 1705.90 cm^{-1} $\nu(\text{C=O})$;
 180 1151.72, 1103.17 cm^{-1} $\nu(\text{C-O})$; UV/vis: λ_{max} 195.5 nm; HRMS (m/z): $[\text{M-Boc}+\text{H}]^+$
 181 calculated for $\text{C}_{30}\text{H}_{48}\text{NO}_2$: 454.3680; found: 454.3659.

182

183 Synthesis of compound 4 (A3M)



184

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.3	525.76	0.60 g	1.14 mmol	1.00	
TMSOTf	222.26	380.4 mg	1.71 mmol	1.50	
2,6-LUTIDNE	107.15	366.84 mg	3.42 mmol	3.00	
DCM	-	4.2 mL	-	-	
Compound 4 A3M	425.64	200 mg	0.469 mmol	-	Yld 41.17%

185

186 Compound 3 (600 mg, 1.14 mmol, 1.00 *eq*), 2, 6-dimethylpyridine (366.84 mg, 3.42
 187 mmol, 398.74 μL , 3.00 *eq*) and DCM (4.20 mL) were added to the flask. The reaction
 188 was cooled to 0 $^{\circ}\text{C}$ in an ice-bath under N_2 . TMSOTf (380.47 mg, 1.71 mmol, 309.32
 189 μL , 1.50 *eq*) was added and the mixture stirred at 0-5 $^{\circ}\text{C}$ for 0.5 hr.

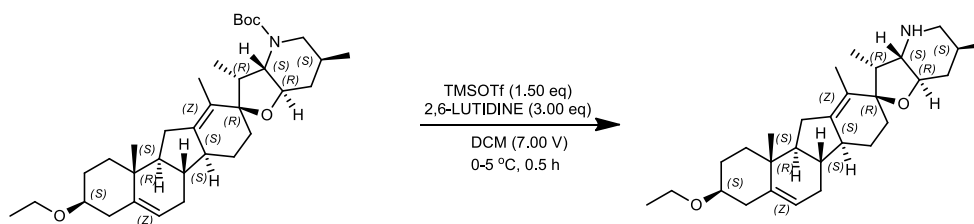
190 LCMS (**ET46937-10-Pa6**) show ~95.10% of desired compound (product: RT = 1.228
 191 min; $\text{M}+1=426.3$).

192 The reaction mixture was poured into sat. NaHCO_3 (5.00 mL), extracted with EtOAc
 193 (5.00 mL $\times$ 2), washed with brine (5.00 mL), dried over Na_2SO_4 , and concentrate to give
 194 the crude product. The crude product was purified by trituration with MTBE (3.00 mL)
 195 at 20 $^{\circ}\text{C}$.

196 Compound 4 **A3M** (200 mg, 469.88 μmol , 41.17% yield) was obtained as a white solid.

m.p.: 198.5–199.2 °C; TLC (DCM:MeOH, 90.9:9.0 v/v): RF = 0.45; $[\alpha]_{20}^D$ (deg cm³ g⁻¹ dm⁻¹) = -46.72 (c = 0.00974 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, CDCl₃): δ 5.38-5.37 (m, 1 H), 3.54-3.51 (m, 1 H), 3.36 (s, 3 H), 3.36-3.32 (m, 1 H), 3.07-3.07 (m, 1 H), 2.88-2.85 (m, 1 H), 2.52-2.52 (m, 1 H), 2.49-2.46 (m, 2 H), 2.27-2.21 (m, 5 H), 2.21-1.74 (m, 7 H), 1.71 (m, 3 H), 1.7-1.24 (m, 7 H), 1.10-1.08 (m, 3 H), 1.02-1.01 (m, 3 H), 0.93 (m, 3 H); ¹³C NMR (101 MHz, CHLOROFORM-d) δ 144.11, 141.73, 125.55, 121.68, 86.11, 80.36, 77.24, 73.23, 64.93, 55.64, 52.96, 52.07, 49.18, 41.56, 38.48, 38.22, 38.09, 36.91, 31.62, 31.10, 29.12, 28.97, 27.68, 24.42, 18.64, 18.45, 13.06, 10.98; IR (KBr): 3317.53 cm⁻¹ ν(N-H); 1107.35 cm⁻¹ ν(C-O); UV/vis: λ_{max} 196.0 nm; HRMS (m/z): [M+H]⁺ calculated for C₂₈H₄₄NO₂: 426.3367; found: 426.3339.

Synthesis of compound 6 (A3E)



Compound 5

Compound 6

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.5	539.79	0.60 g	1.11 mmol	1.00	
TMSOTf	222.26	370.58 mg	1.67 mmol	1.50	
2,6-LUTIDNE	107.15	357.31 mg	3.33 mmol	3.00	
DCM	-	4.20 mL	-	-	
Compound 6 A3E	439.67	160 mg	0.364 mmol	-	Yld 32.94%

Compound 5 (600 mg, 1.11 mmol, 1.00 eq), 2, 6-dimethylpyridine (357.31 mg, 3.33 mmol, 388.38 uL, 3.00 eq) and DCM (4.20 mL) were added to the flask. The reaction mixture was cooled to 0 °C in an ice-bath under N₂. TMSOTf (370.58 mg, 1.67 mmol, 301.28 uL, 1.50 eq) was added and the mixture stirred at 0~5 °C for 0.5 hr.

LCMS (ET46937-11-P1z2) shows ~97.53% of desired compound 6 (product: RT = 3.888 min; M+1=440.4).

The reaction mixture was poured into sat.NaHCO₃ (5.00 mL), extracted with EtOAc (5.00 mL×2), washed with brine (5.00 mL), dried over Na₂SO₄, and concentrated to

219 give the crude product. The crude product was purified by trituration with MTBE (3.00
220 mL) at 20 °C.

221 Compound 6 **A3E** (160 mg, 363.91 μmol , 32.74% yield) was obtained as a white solid.
222 m.p.: 217.4–220.5 °C; TLC (DCM:MeOH, 90.9:9.0 v/v): RF = 0.30; $[\alpha]_{20}^D$ (deg cm³ g
223 ⁻¹ dm⁻¹) = -48.42 (c = 0.009522 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, CDCl₃):
224 δ 5.36 (s, 1H), 3.53-3.56 (t, *J* = 6.6 Hz, 2H), 3.40-3.43 (t, *J* = 6.6 Hz, 2H), 3.14-3.16 (t,
225 *J* = 5.0 Hz, 2H), 2.76-2.90 (m, 2H), 2.39-2.49 (m, 1H), 2.18-2.22 (m, 5H), 1.55-1.90
226 (m, 7H), 0.90-1.47 (m, 33H); ¹³C NMR (101 MHz, CHLOROFORM-*d*) δ 142.93,
227 142.00, 126.69, 121.62, 85.40, 78.81, 75.57, 66.32, 63.22, 54.80, 52.18, 49.31, 41.52,
228 39.73, 39.17, 38.77, 38.22, 36.97, 32.02, 31.75, 31.18, 28.95, 28.23, 24.55, 18.96,
229 18.68, 15.75, 13.14, 10.73; IR (KBr): 1148.67 cm⁻¹ ν (C-O); UV/vis: λ_{max} 196.0 nm;
230 HRMS (m/z): [M+H]⁺ calculated for C₂₉H₄₆NO₂: 440.3523; found: 440.3531.
231

232 Synthesis of compound 8 (**A3P**)



233

Reagent	MW.	amount	mmol	ratio	Other Info.
Cpd.7	553.41	0.50 g	0.902 mmol	1.00	
TMSOTf	222.26	300.99 mg	1.35 mmol	1.50	
2,6-LUTIDNE	107.15	290.22 mg	2.71 mmol	3.00	
DCM	-	3.50 mL	-	-	
A3P	453.70	150.0 mg	0.330 mmol	-	Yld 36.62%

234

235 Compound 7 (500 mg, 902.83 μmol , 1.00 *eq*), 2, 6-dimethylpyridine (290.22 mg, 2.71
236 mmol, 315.45 μL , 3.00 *eq*) and DCM (3.50 mL) were added to the flask and the
237 reaction was cooled to 0 °C in an ice-bath under N₂. TMSOTf (300.99 mg, 1.35 mmol,
238 244.71 μL , 1.50 *eq*) was added and the mixture was stirred at 0-5 °C for 0.5 hr.

239 LCMS (**ET46937-14-P1a5**) showed ~91.77% of desired compound 8 (product: RT =

240 3.346 min; M+1=454.4).

241 The reaction mixture was poured into sat. NaHCO₃ (5.00 mL), extracted with EtOAc
242 (5.00 mL×2), washed with brine (5.00 mL), dried over Na₂SO₄, and concentrated to
243 give the crude product. The crude product was purified by trituration with MTBE (3.00
244 mL) at 20°C.

245 Compound 8 **A3P** (150 mg, 330.62 umol, 36.62% yield) was obtained as a white solid.
246

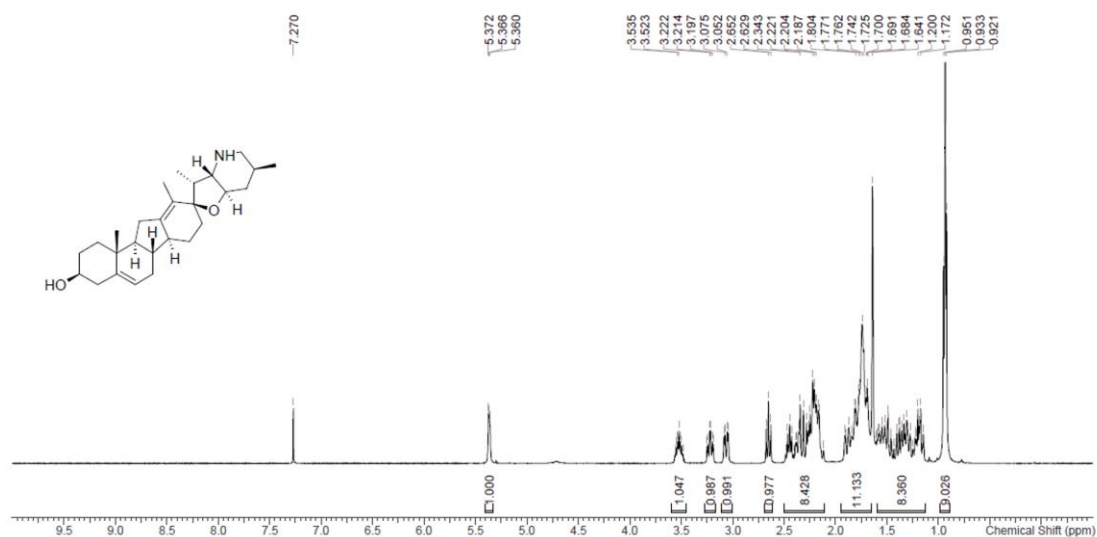
247 m.p.: 198.7–205.2 °C; TLC (DCM:MeOH, 90.9:9.0 v/v): RF = 0.30; $[\alpha]_{20}^D$ (deg cm³ g
248 ⁻¹ dm⁻¹) = -42.15 (c = 0.009752 g cm⁻³ in MeOH); ¹H-NMR (400 MHz, CDCl₃):
249 δ 9.576 (s, 1H), 8.518 (s, 1H), 5.36-5.37 (s, 1H), 3.70-3.71 (m, 1H), 3.40-3.47 (m,
250 3H), 3.22 (m, 1H), 2.76 (m, 1H), 2.45 (m, 1H), 2.31 (m, 1H), 2.28 (m, 1H), 2.22-
251 2.31 (m, 6H), 1.063-1.930 (m, 26H), 0.907-0.944 (m, 6H); ¹³C NMR (101 MHz,
252 CHLOROFORM-d) δ 144.93, 142.01, 124.80, 121.43, 86.60, 78.92, 71.71, 69.85,
253 64.05, 52.07, 51.75, 49.13, 41.59, 38.73, 38.21, 37.69, 37.43, 36.91, 31.35, 31.08,
254 29.01, 28.18, 27.39, 24.35, 23.37, 18.65, 18.19, 13.05, 11.34, 10.64; IR (KBr):
255 1606.82 cm⁻¹ ν(C=C); 1168.94 cm⁻¹ ν(C-O); UV/vis: λ_{max} 196.5 nm; HRMS (m/z):
256 [M+H]⁺ calculated for C₃₀H₄₈NO₂: 454.3680; found: 454.3673.
257

258
259

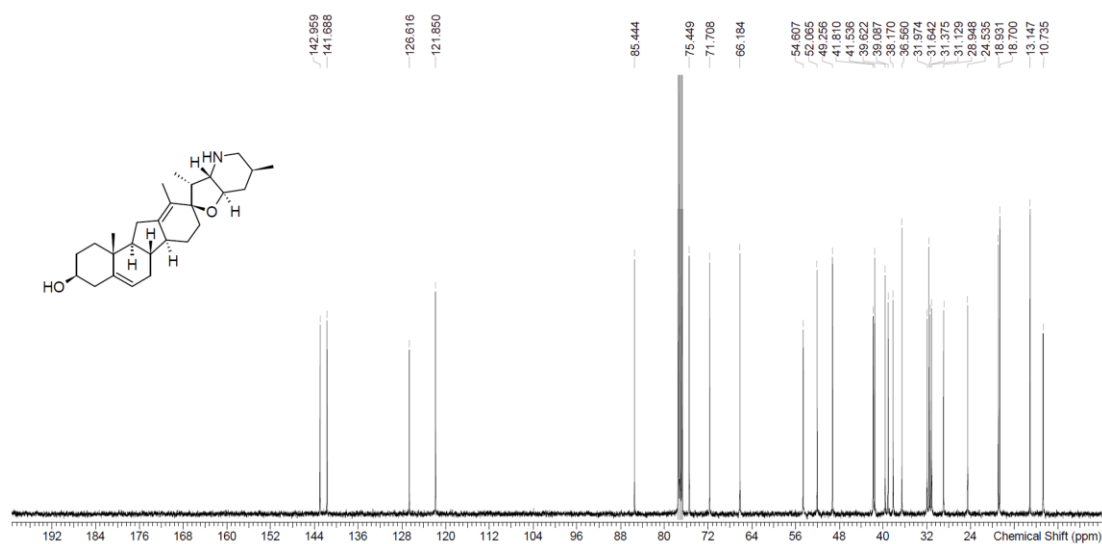
1.3. Images of analytical data

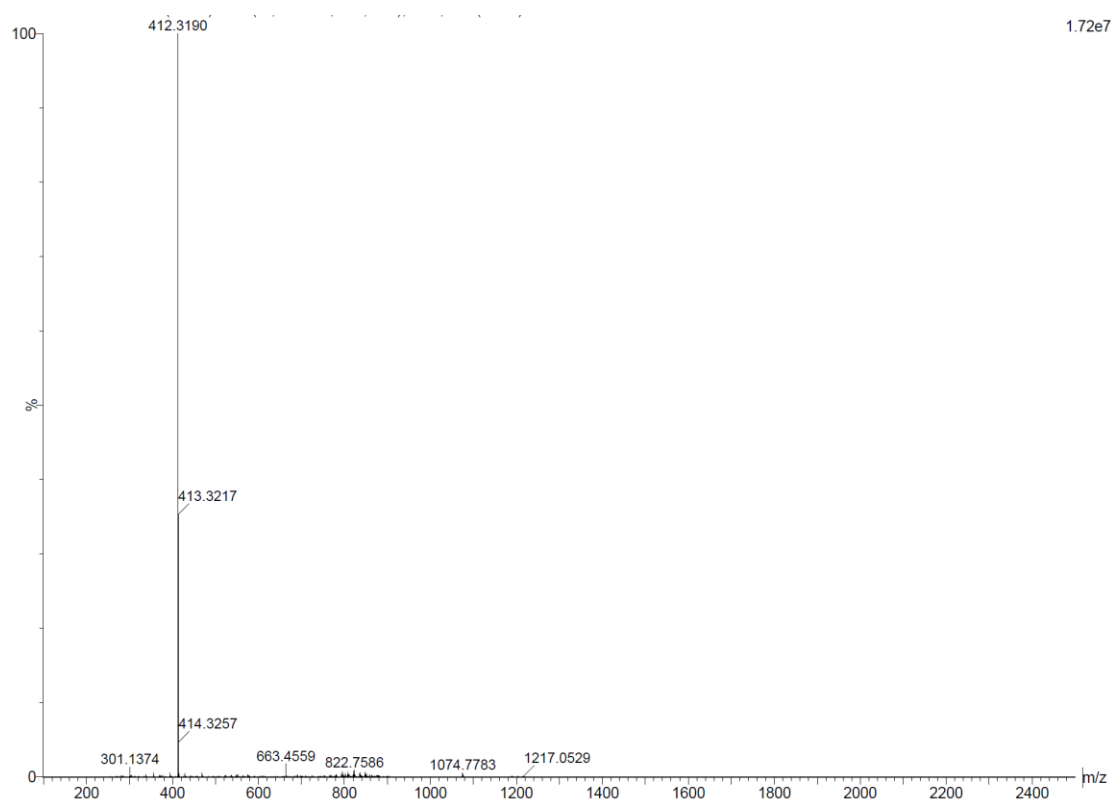
1.3.1. Compound 1 (cyclopamine)

Compound 1 ^1H -NMR



Compound 1 ^{13}C -NMR



270 **Compound 1 HRMS**

271

272

273

274

275 **Compound 1 Melting point**

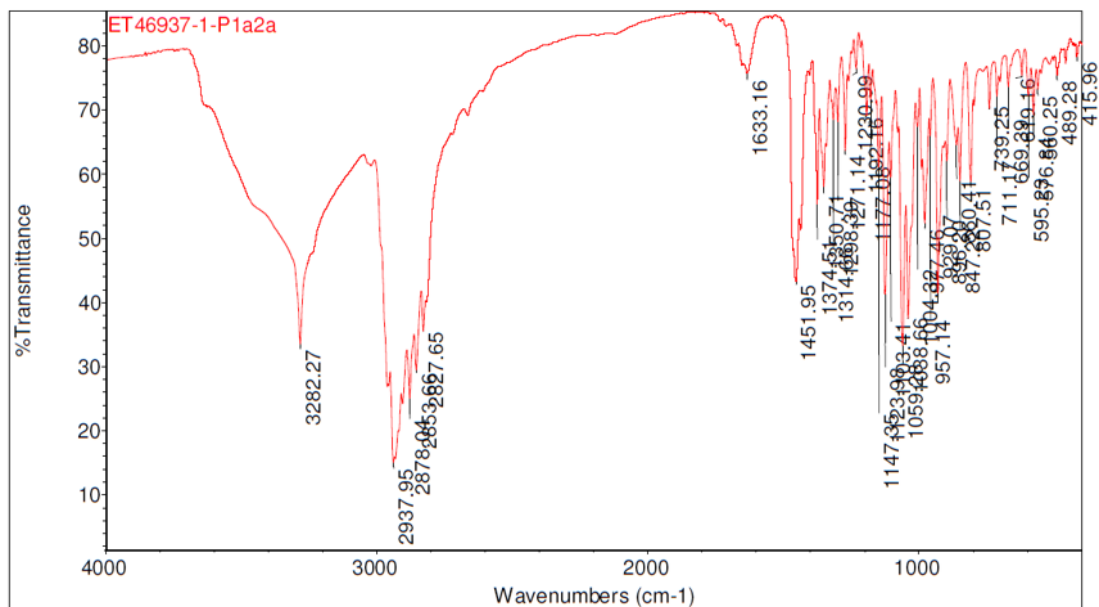
Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	217.6	217.5	217.5	217.5
End of Melting	220.2	220.2	220.2	220.2

276

277

278

Compound 1 IR



Compound 1 Optical rotation

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ /(°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	1.0166	-0.285	-56.07	-56.07
2	1.0166	-0.285	-56.07	

- Samples diluted with methanol
- L = 0.5 dm

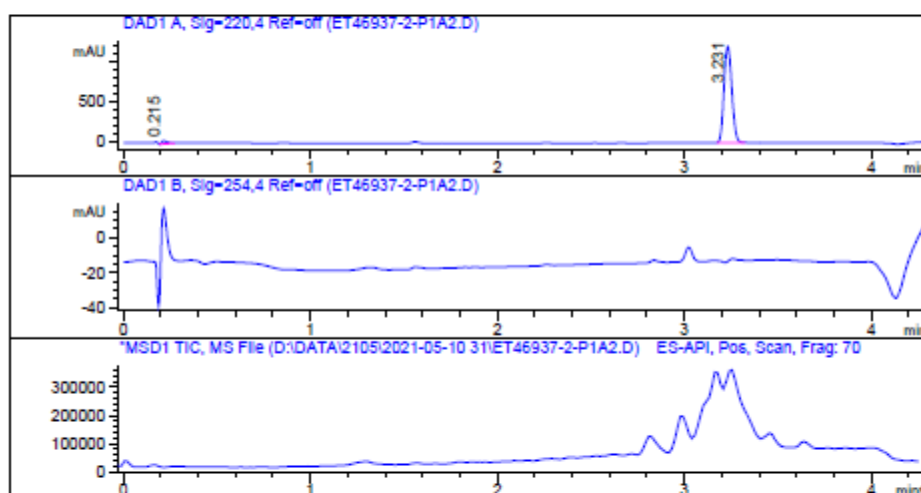
287 1.3.2. Compound 2

288

289 Compound 2 (ET46937-2-P1a2) IPC-LCMS

Method Info : Instrument: Agilent1200+6110MS
 Column: XBridge C18 Sum 2.1*50mm
 Column Temp: 40
 Mobile Phase: A: 10mM NH₄HCO₃ in H₂O
 Mobile Phase: B: ACN
 Flow Rate: 1ml/min

Time	B%	Flow (ml/min)
0.00	5	1.0
3.00	95	1.0
3.50	95	1.0
3.51	5	1.0
4.00	5	1.0

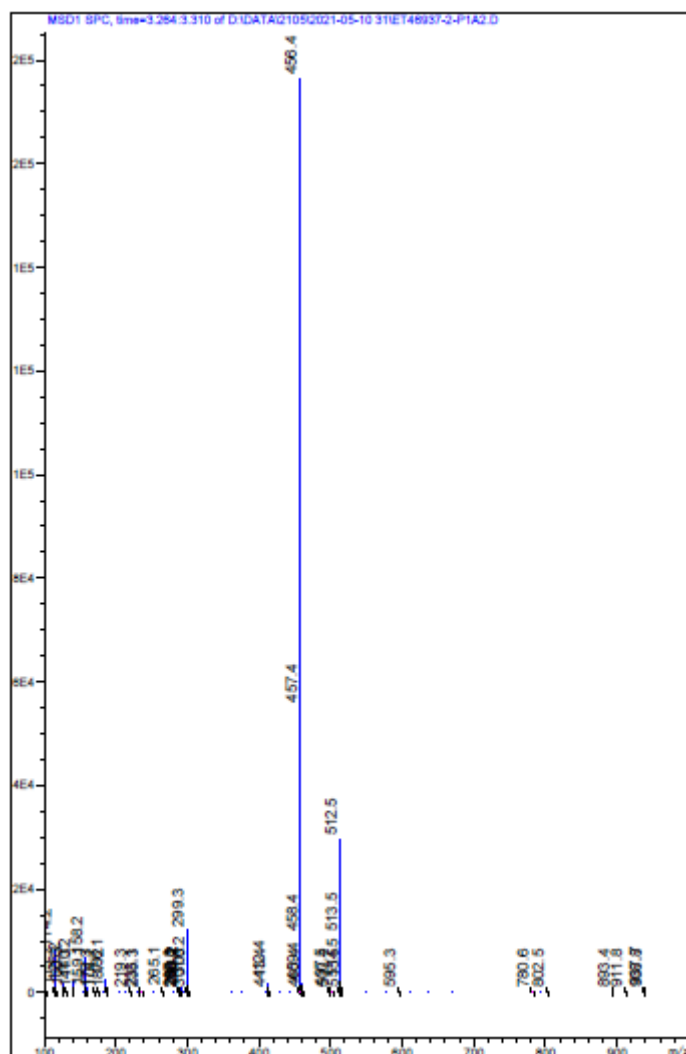


Integration Result

Signal 1 : DAD1 A, Sig=220,4 Ref=off

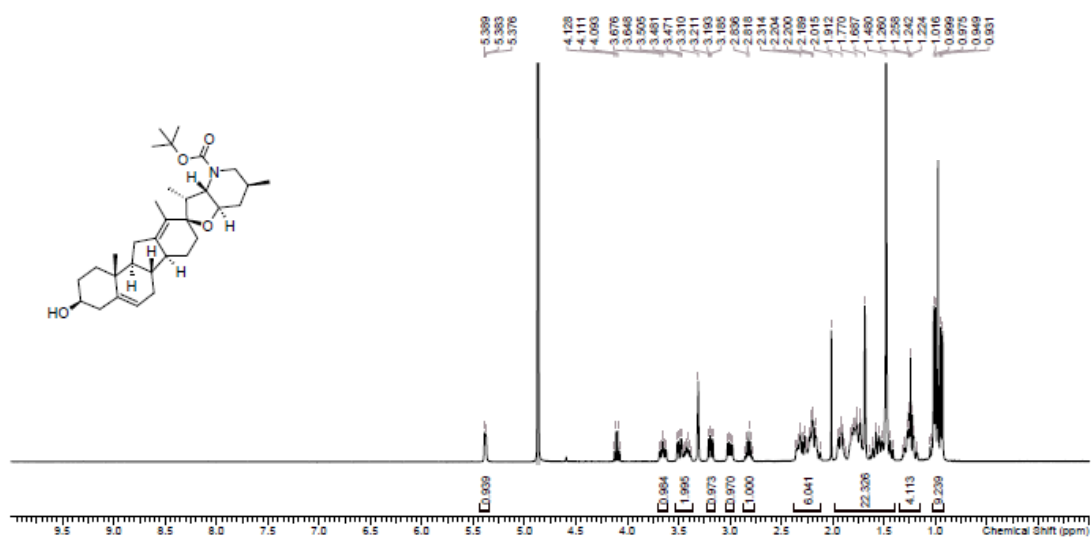
Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.215	86.710	37.636	3.061	0.035	2.425
2	3.231	3489.063	1191.973	96.939	0.047	97.575

290

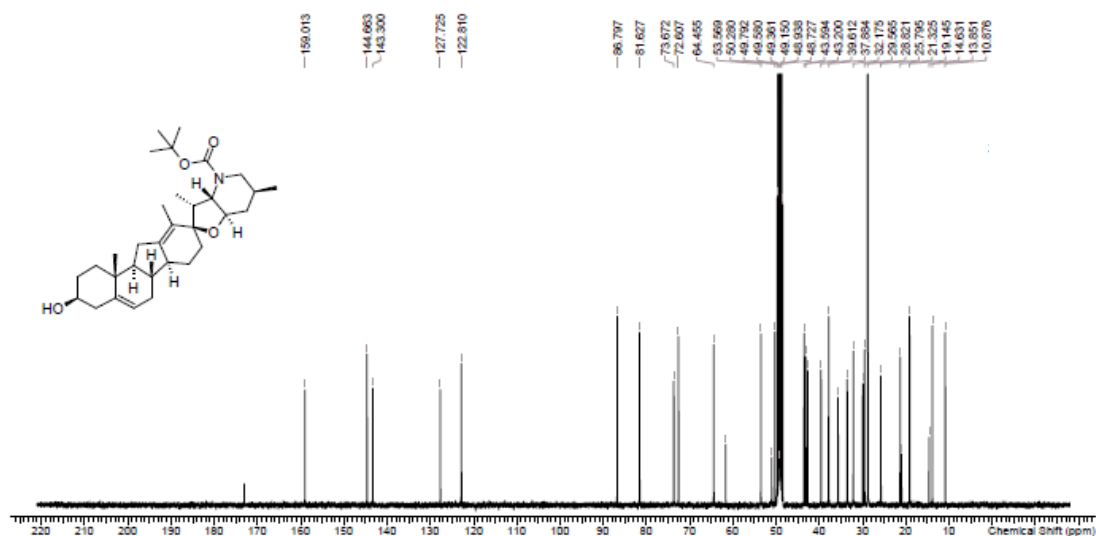


291

292

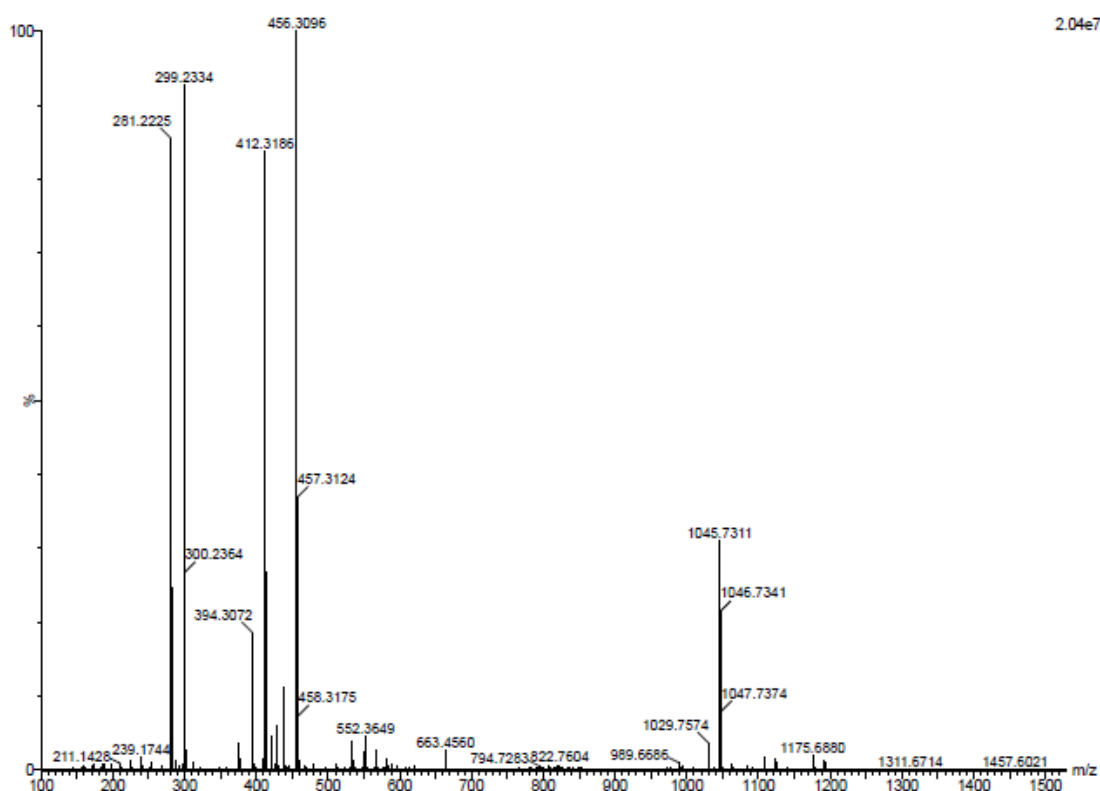
293 **Compound 2** ^1H -NMR

294

295 **Compound 2** ^{13}C -NMR

296

297

298 **Compound 2** HRMS

299

300

301

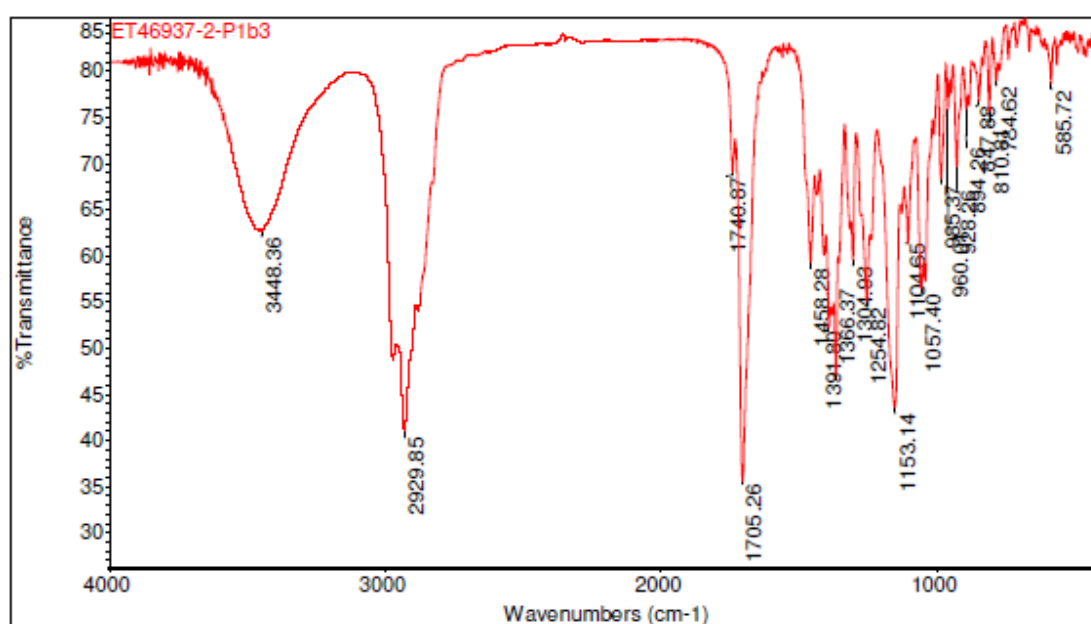
302 **Compound 2 Melting point**

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	118.6	118.6	118.6	118.6
End of Melting	130.6	130.6	130.6	130.6

303

304

305 **Compound 2 IR**



306

307

308 **Compound 2 Optical rotation**

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ /(°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	0.9458	-0.025	-5.29	-5.40
2	0.9458	-0.026	-5.50	

1. Samples diluted with MeOH

2. $L = 0.5$ dm

309

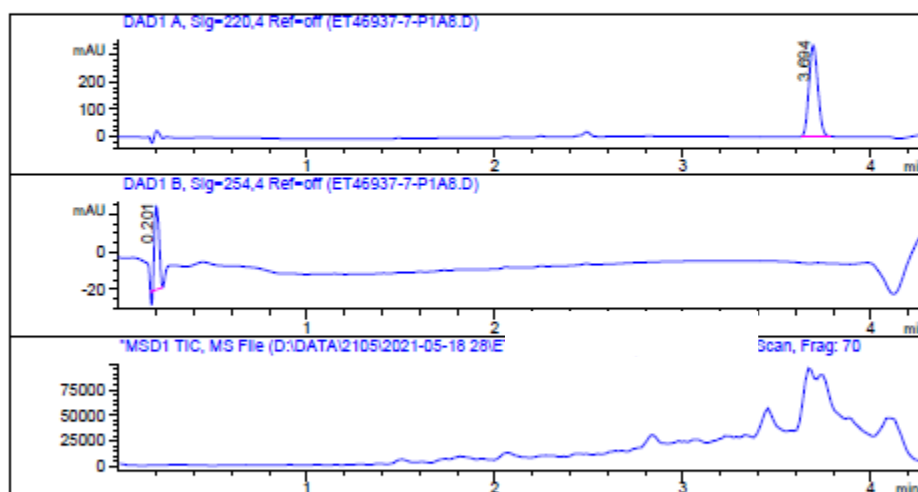
310

1.3.3. Compound 3

Compound 3 (ET46937-7-P1A8) IPC-LCMS

Method Info : Instrument: Agilent1200+6110MS
 Column: XBridge C18 Sum 2.1*50mm
 Column Temp: 40
 Mobile Phase: A: 10mM NH₄HCO₃ in H₂O
 Mobile Phase: B: ACN
 Flow Rate: 1ml/min

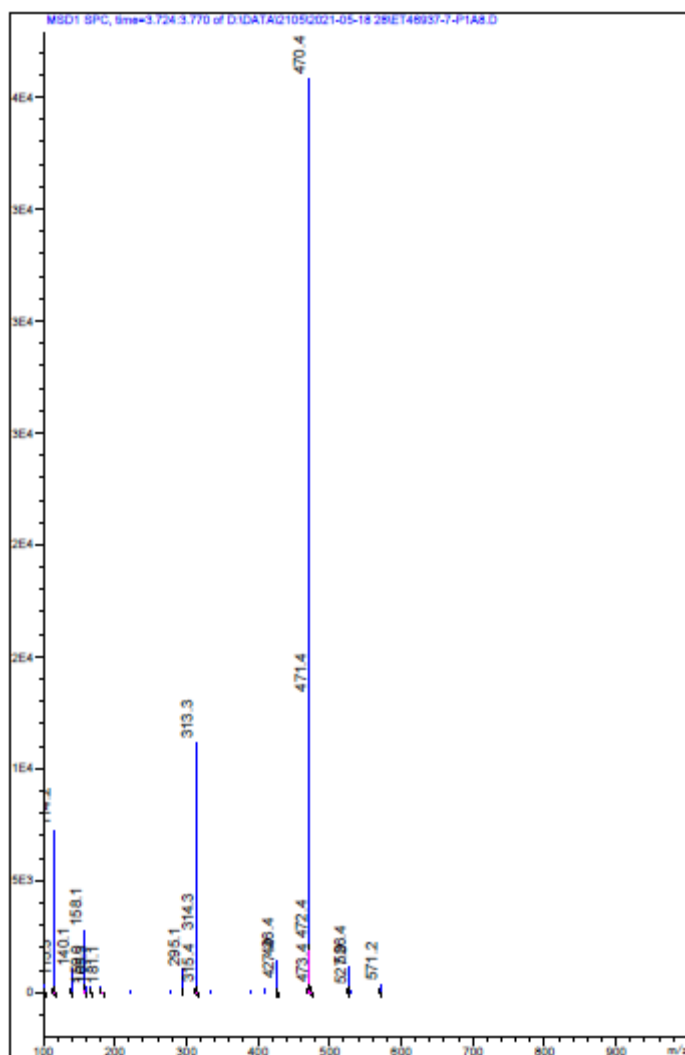
Time	B%	Flow (ml/min)
0.00	5	1.0
3.00	95	1.0
3.50	95	1.0
3.51	5	1.0
4.00	5	1.0



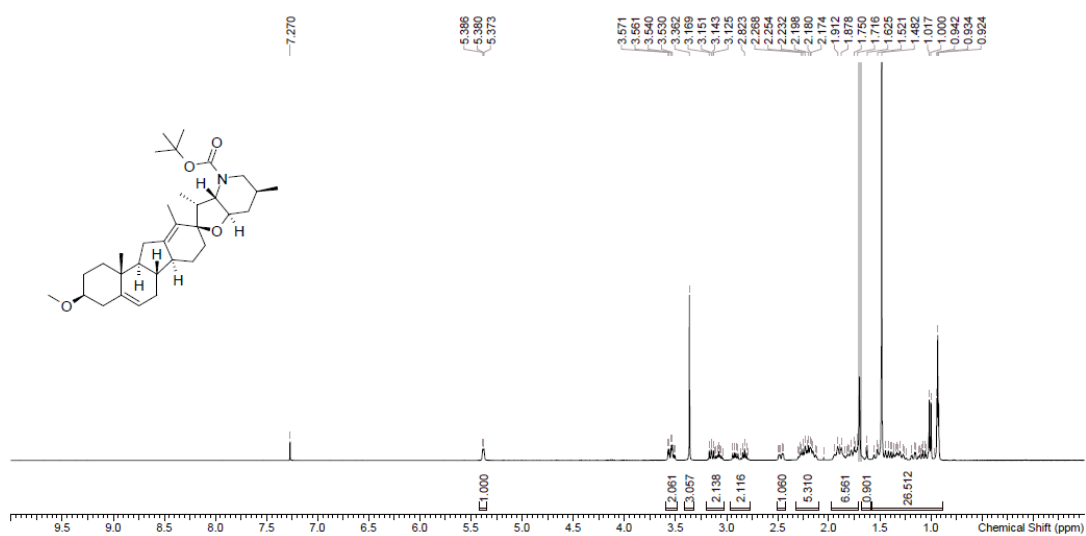
Integration Result

Signal 1 : DAD1 A, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	3.694	1108.599	334.027	100.000	0.052	100.000

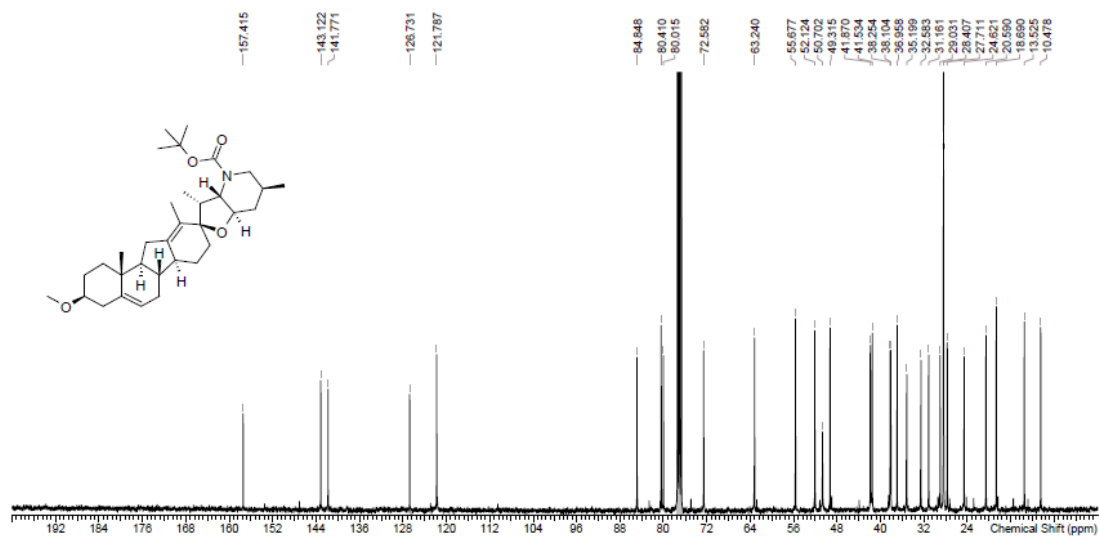


315

316 **Compound 3 ^1H -NMR**

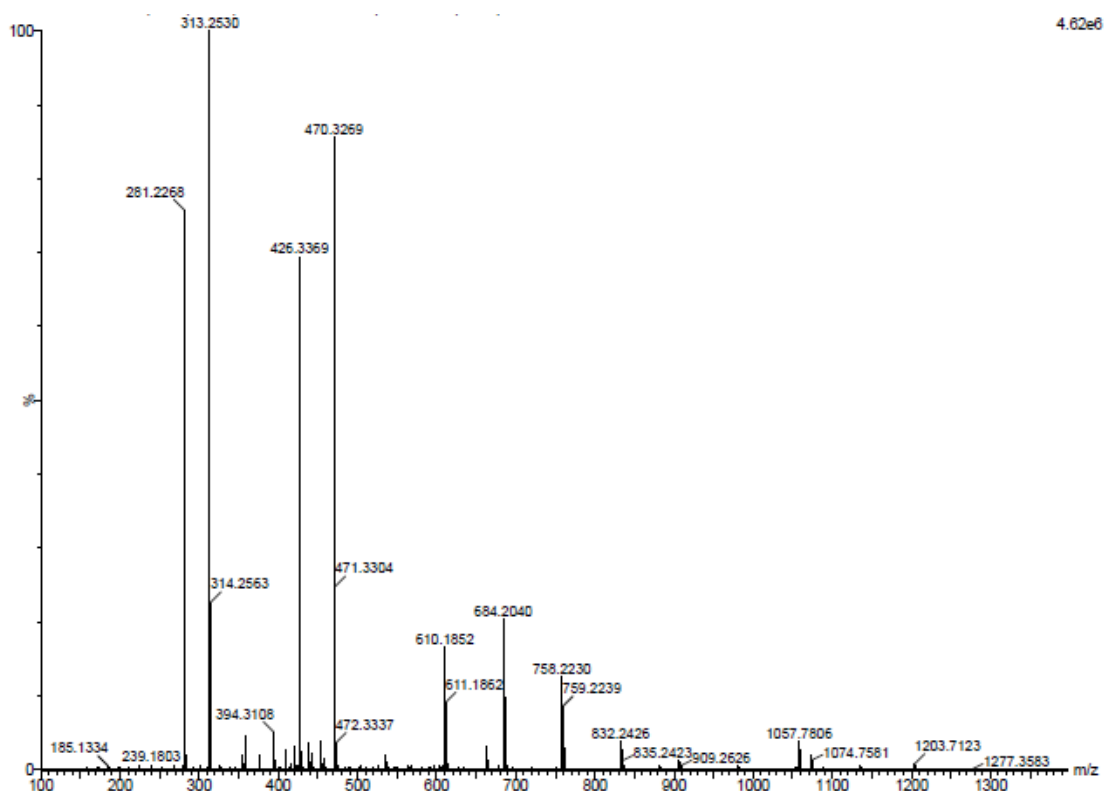
317

318

319 **Compound 3** ^{13}C -NMR

320

321

322 **Compound 3** HRMS

323

324

325

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	165.3	165.1	164.2	164.9
End of Melting	170.6	170.7	170.6	170.6

	$c / (\text{g}/100\text{mL})$	$\alpha / (^\circ)$	$[\alpha]_D^{20} (^\circ)$	$[\bar{\alpha}]_D^{20} / (^\circ)$
1	0.9074	-0.021	-4.63	-4.63
2	0.9074	-0.021	-4.63	

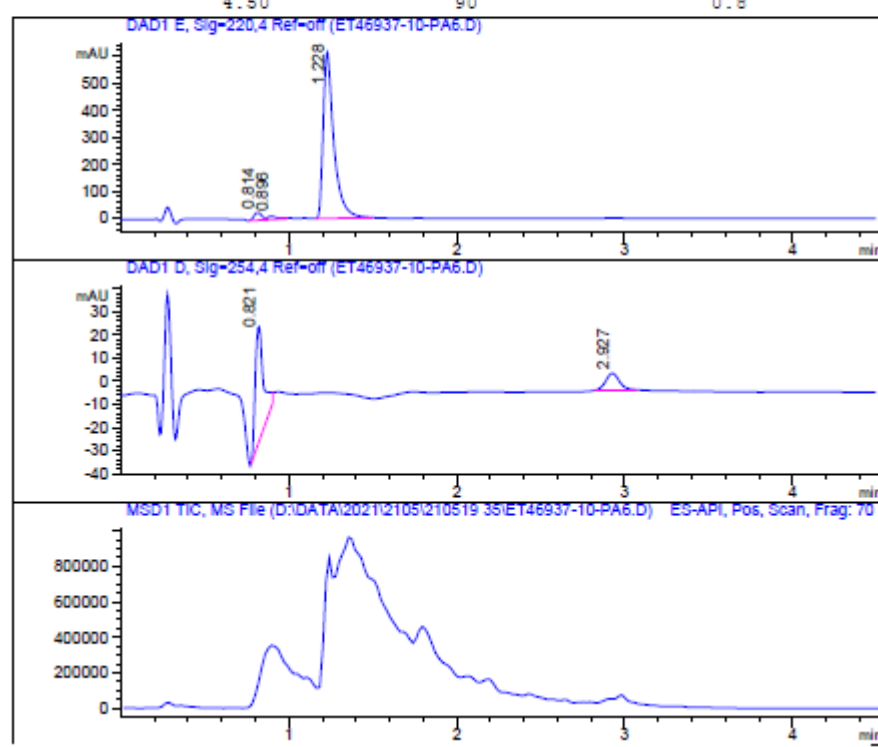
1. Samples diluted with methanol
2. $L = 0.5 \text{ dm}$

1.3.4. Compound 4 (A3M)

Compound 4 (ET46937-10-Pa6) IPC-LCMS

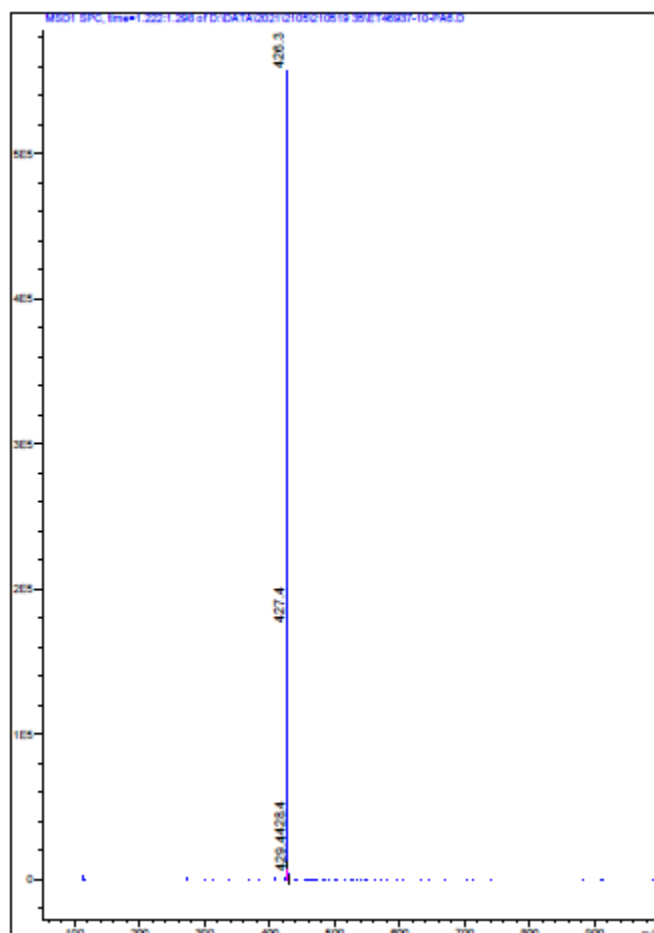
Method Info ->Instrument:Agilent 1200 HPLC MSD:6120 single quadrupole
MSD
Column:XBridge C18,2.1*50mm, 5µm
Column Temp: 40 °C
Mobile Phase:A:H2O+10mM NH4HCO3
Mobile Phase:B: ACN
Flow Rate: 0.8ml/min

Time	B%	Flow(ml/min)
0.00	90	0.8
0.40	90	0.8
3.40	100	0.8
3.85	100	0.8
3.86	90	0.8
4.50	90	0.8

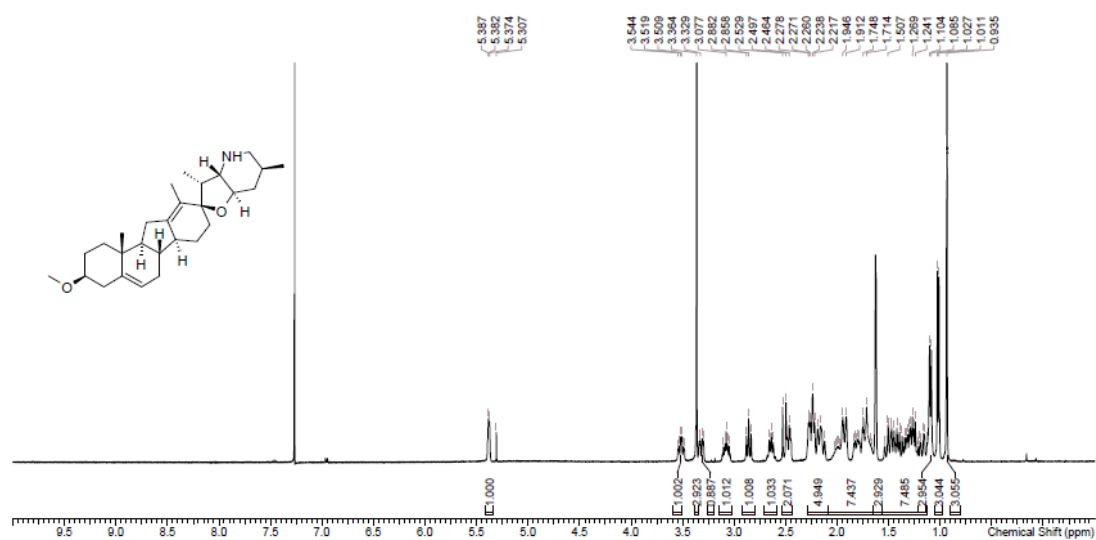


Signal 1 :DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Height	Height %	Width [min]	Area	Area %
1	0.814	27.944	4.264	0.050	90.119	3.157
2	0.896	11.720	1.788	0.059	49.004	1.717
3	1.228	615.738	93.948	0.065	2715.188	95.126



341

342 **Compound 4 ^1H -NMR**

343

344

345



348

349 **Compound 4 HRMS**



351

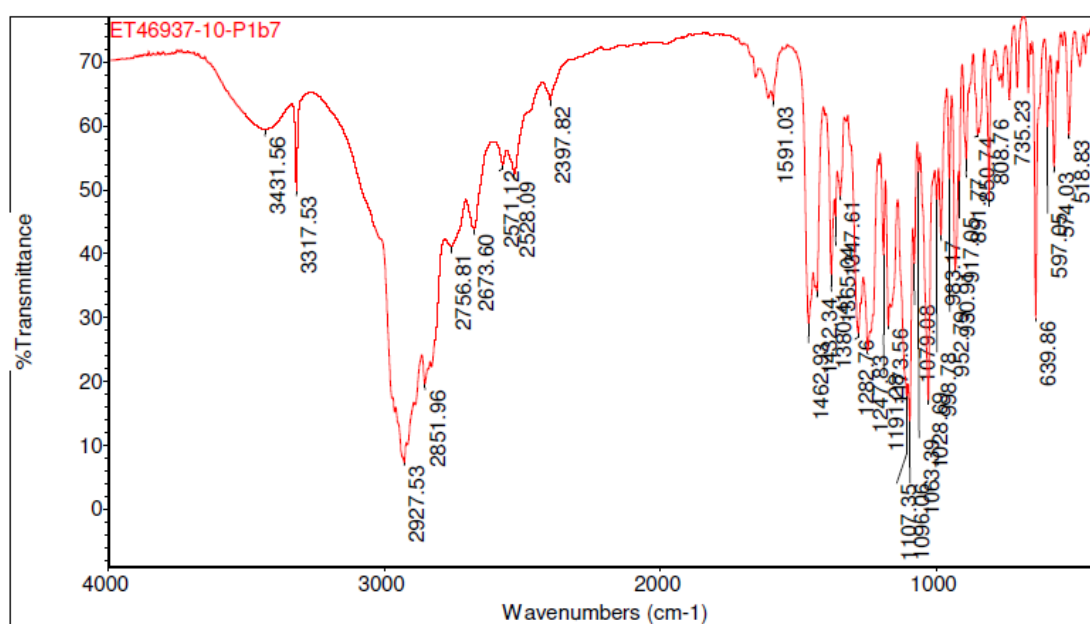
352 **Compound 4 Melting point**

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	198.5	198.5	198.5	198.5
End of Melting	199.2	199.2	199.2	199.2

353

354

355 **Compound 4 IR**



356

357

358 **Compound 4 Optical rotation**

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ /(°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	0.9974	-0.233	-46.72	-46.72
2	0.9974	-0.233	-46.72	

1. Samples diluted with methanol

2. $L = 0.5$ dm

359

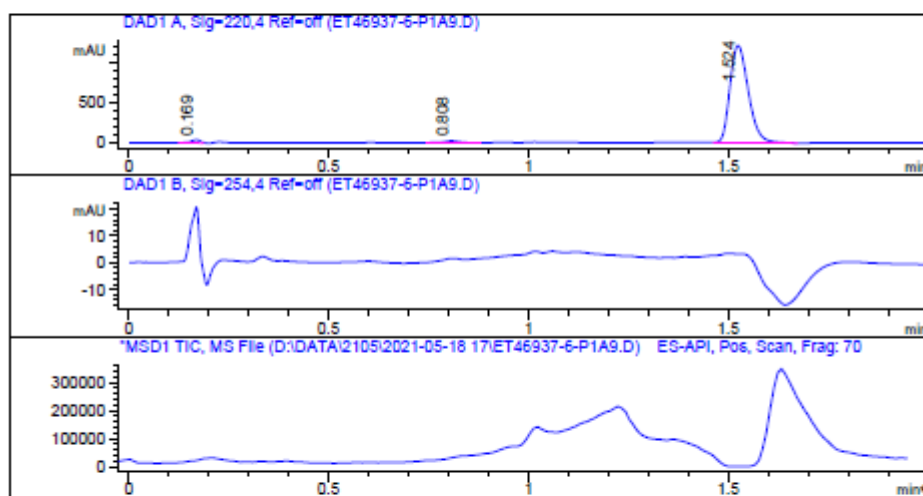
360

1.3.5. Compound 5

Compound 5 (ET46937-6-P1a9) IPC-LCMS

Method Info : Instrument: Agilent1200+6110MS
 Column: XBridge C18 Sum 2.1*50mm
 Column Temp: 40
 Mobile Phase: A: 10mM NH₄HCO₃ in H₂O
 Mobile Phase: B: ACN
 Flow Rate: 1ml/min

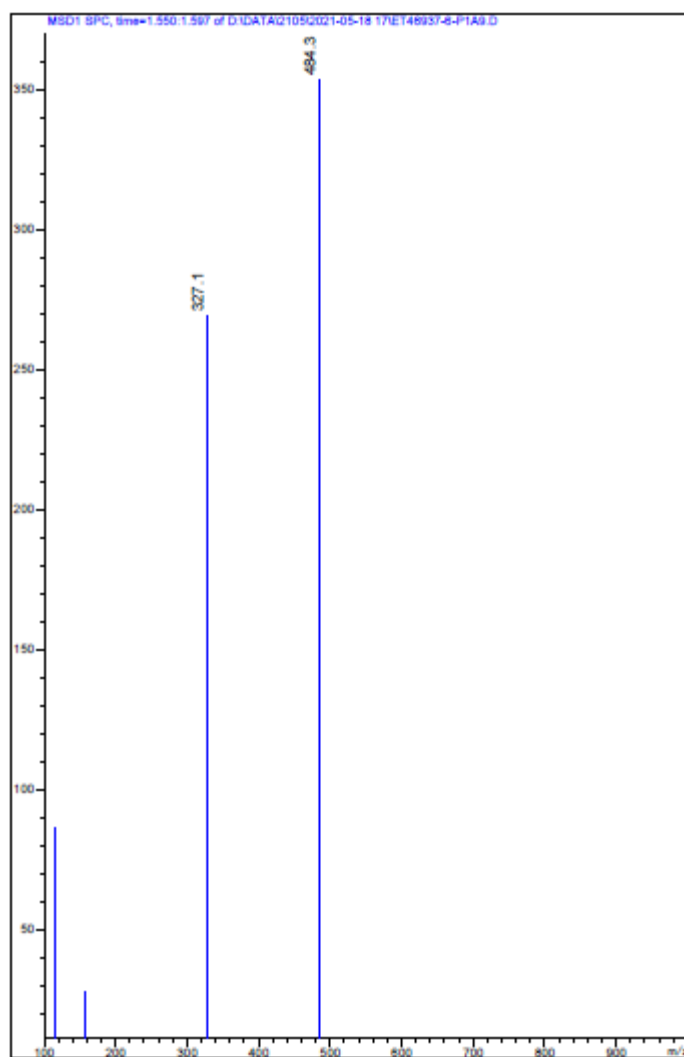
Time	B%	Flow (ml/min)
0.00	50	1.0
0.70	100	1.0
1.10	100	1.0
1.11	50	1.0
2.00	50	1.0



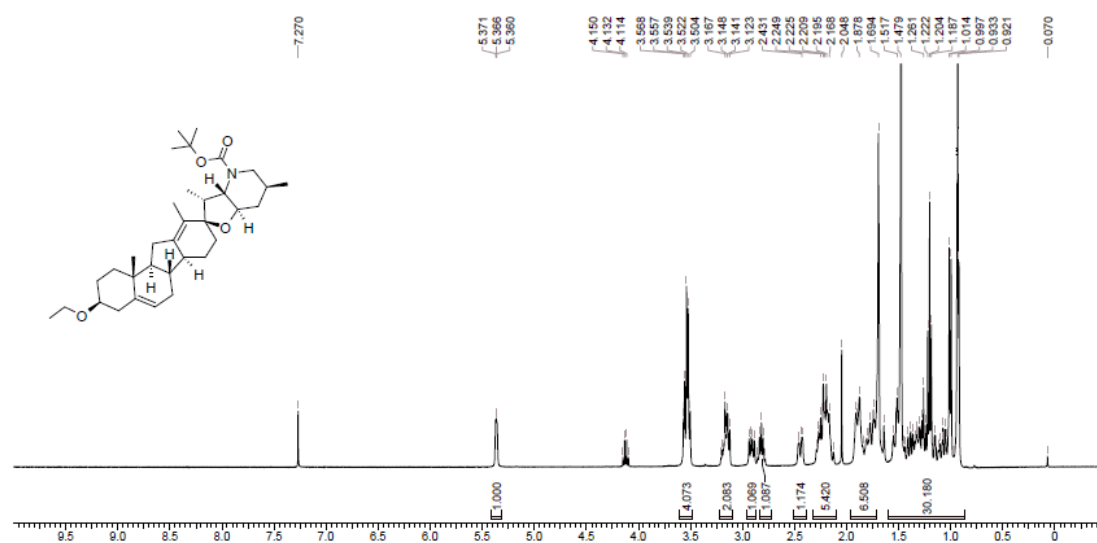
Integration Result

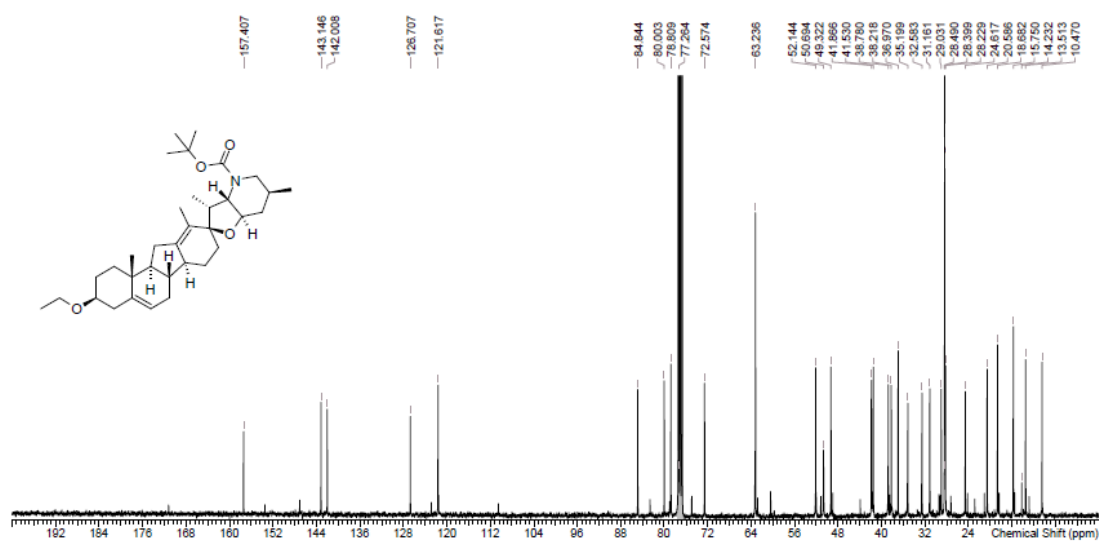
Signal 1 : DAD1 A, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.169	65.747	39.081	3.097	0.026	1.653
2	0.808	57.773	21.285	1.687	0.041	1.452
3	1.524	3854.300	1201.532	95.216	0.050	96.895



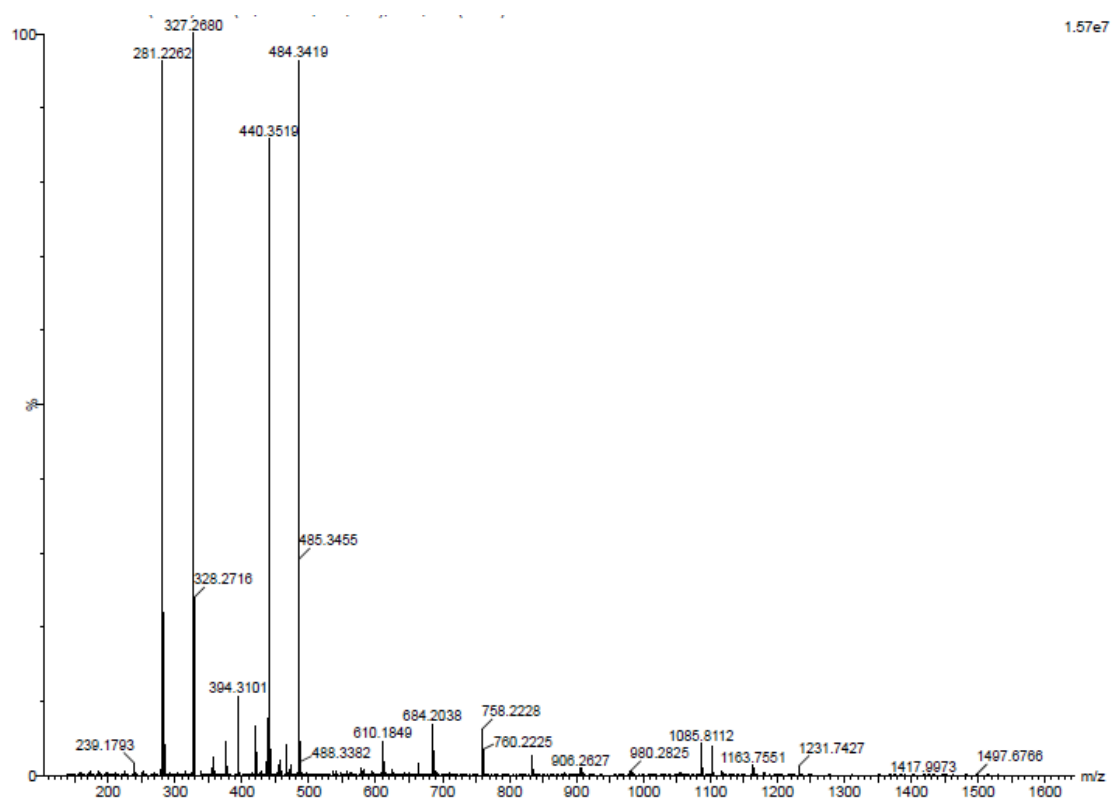
Compound 5 ^1H -NMR



369 **Compound 5** ^{13}C -NMR

370

371

372 **Compound 5** HRMS

373

374

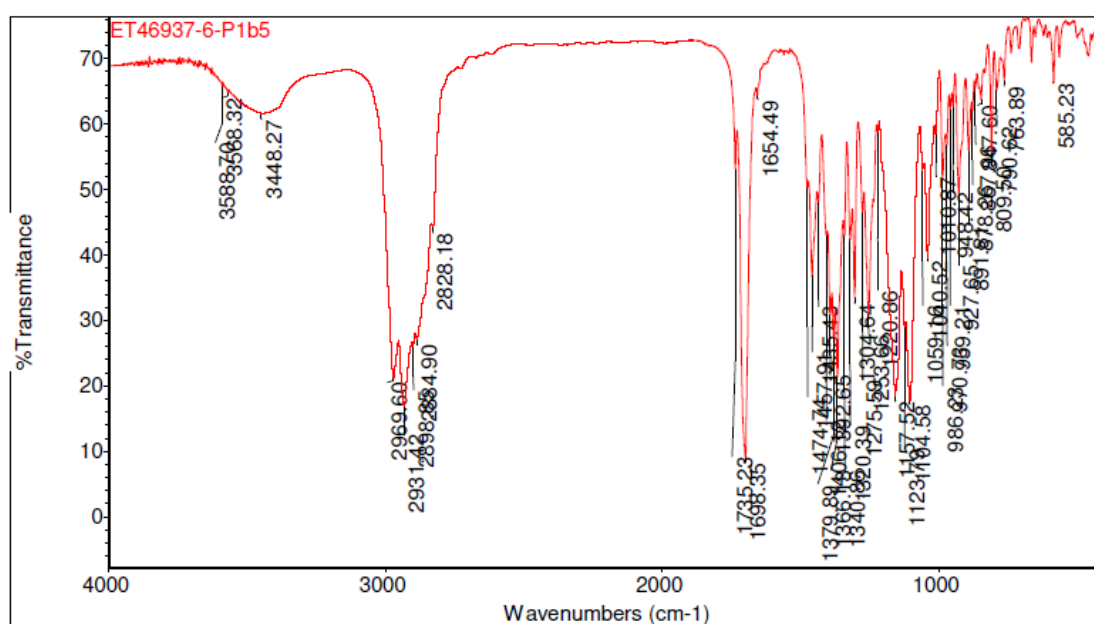
375

376

Compound 5 Melting point

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	154.9	155.0	155.1	155.0
End of Melting	160.7	161.3	161.3	161.1

Compound 5 IR



Compound 5 Optical rotation

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ (°)	$[\bar{\alpha}]_D^{20}$ (°)
1	9.8660	-0.004	-0.08	-0.07
2	9.8660	-0.003	-0.06	

1. Samples diluted with methanol

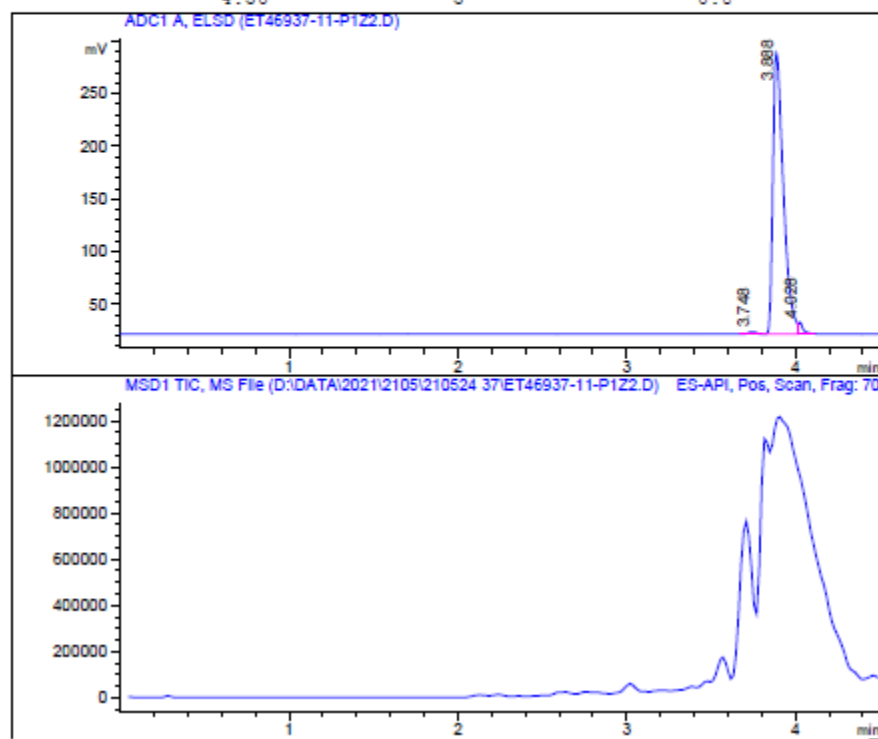
2. $L =$ 0.5 dm

1.3.6. Compound 6 (A3E)

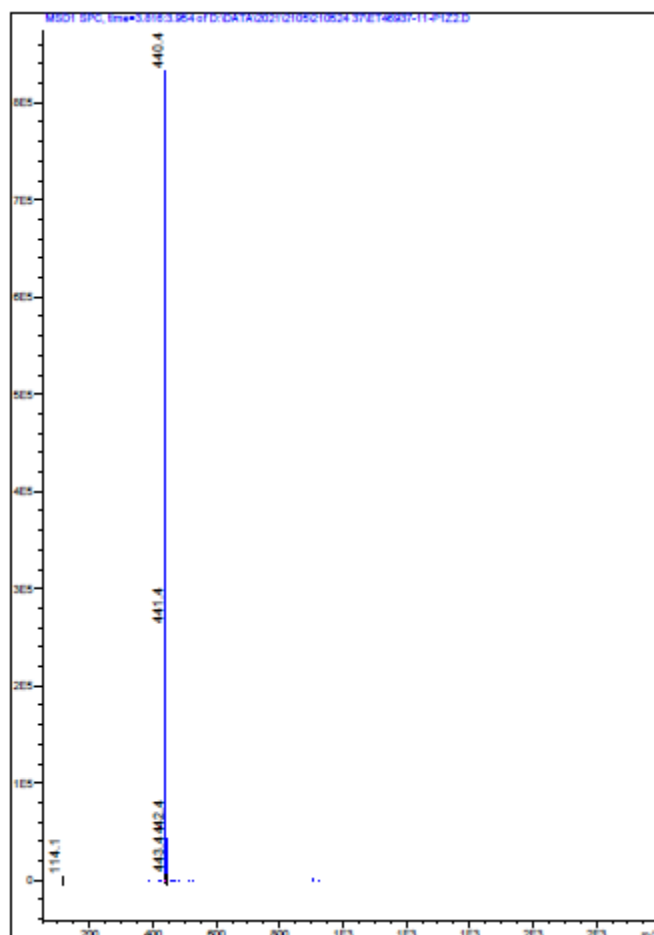
Compound 6 (ET46937-11-P1z2) IPC-LCMS

Method Info ->Instrument:Agilent 1200 HPLC
MSD:6120 single quadrupole MSD
Column:XBridge C18,2.1*50mm, 5µm
Column Temp: 40 °C
Mobile Phase:A:H2O+10mM NH4HCO3
Mobile Phase:B:ACN
Flow Rate: 0.8ml/min

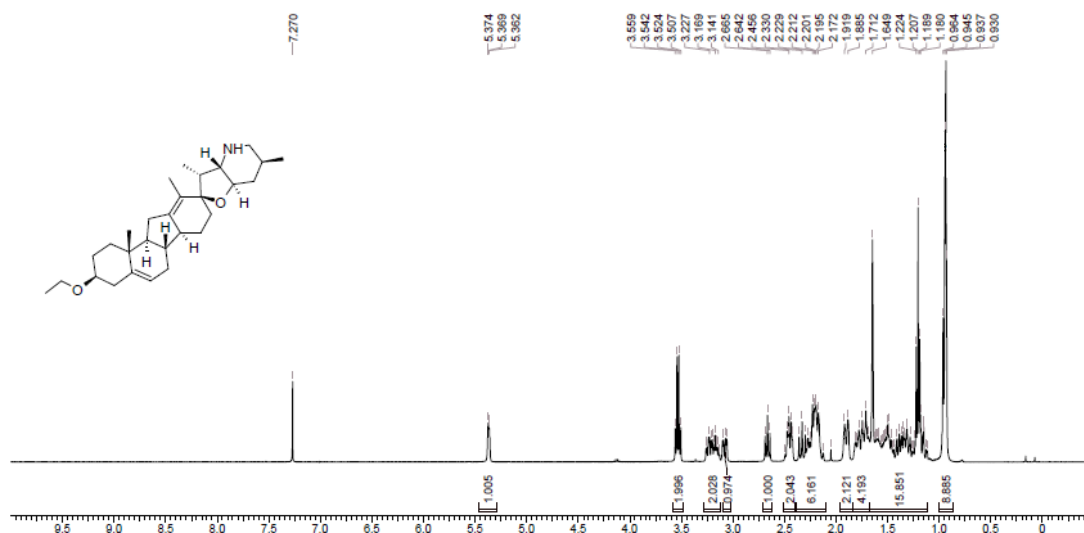
Time	B%	Flow(ml/min)
0.00	5	0.8
3.40	95	0.8
3.85	95	0.8
3.86	5	0.8
4.50	5	0.8

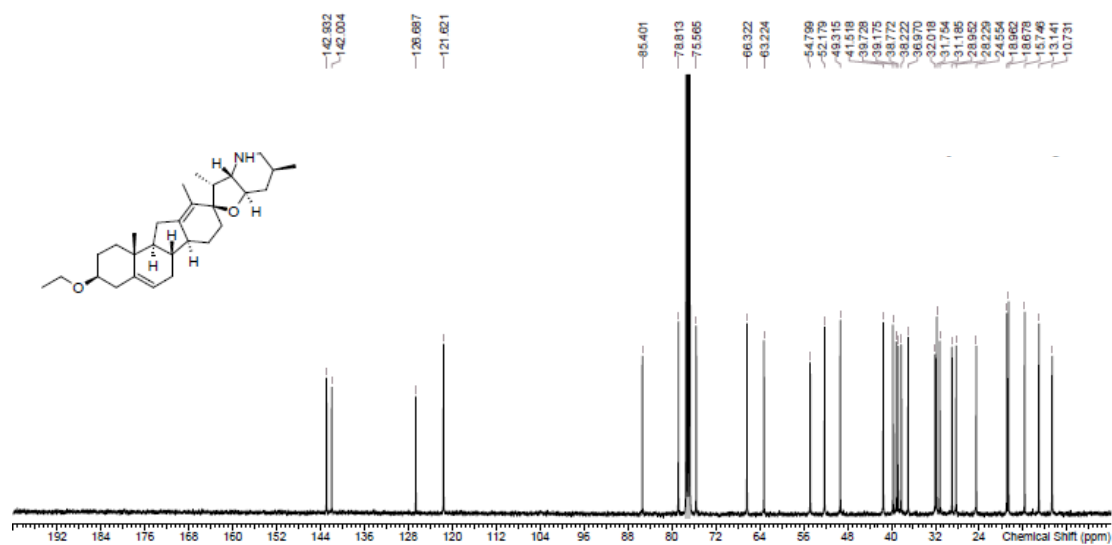


Peak #	RT [min]	Height	Height %	Width [min]	Area	Area %
1	3.748	2.207	0.790	0.056	7.828	0.665
2	3.888	266.312	95.306	0.064	1148.578	97.533
3	4.028	10.910	3.904	0.028	21.224	1.802



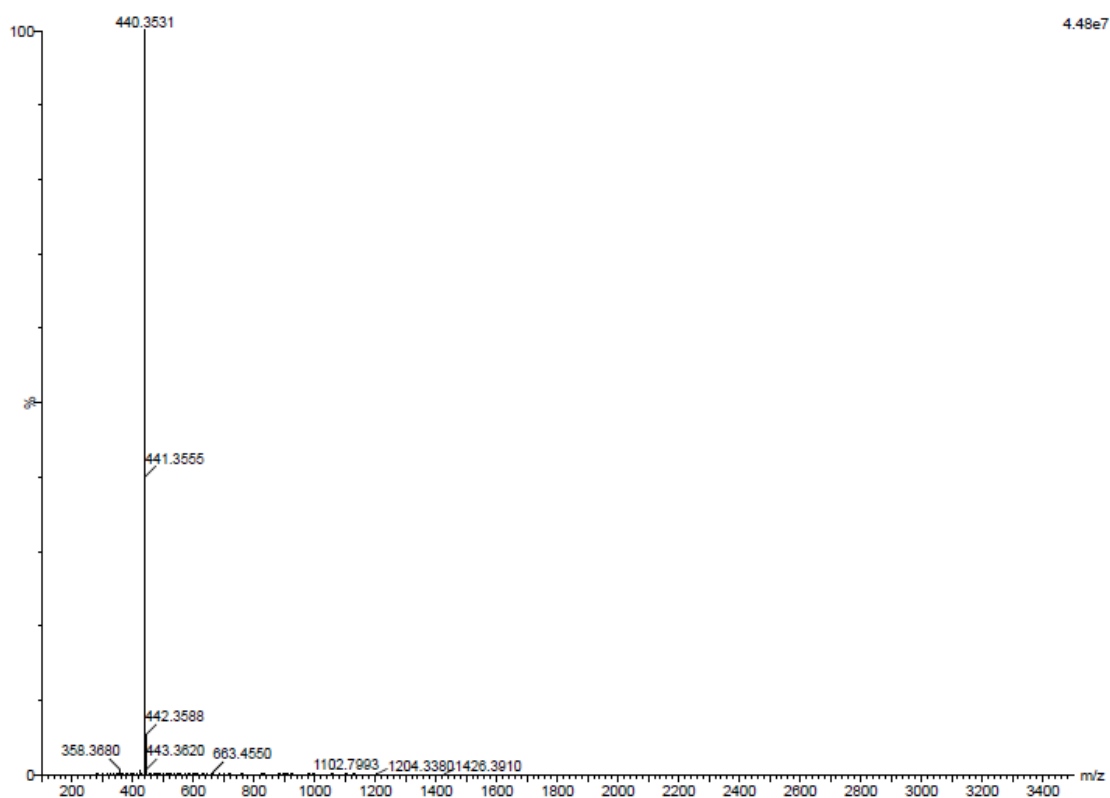
Compound 6 ^1H -NMR



396 **Compound 6** ^{13}C -NMR

397

398

399 **Compound 6** HRMS

400

401

402

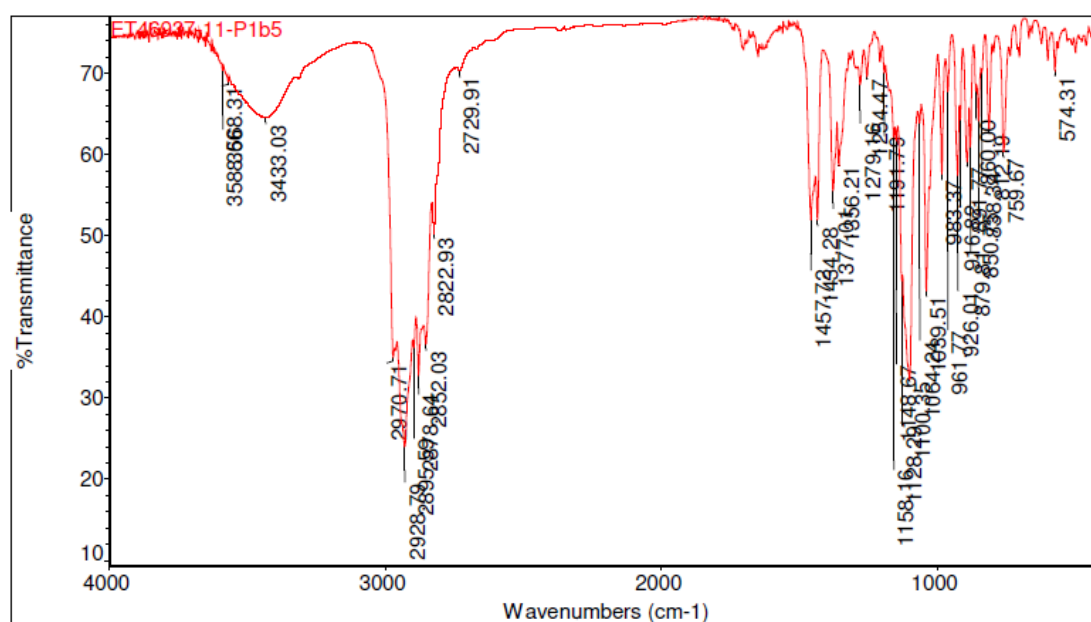
403 **Compound 6 Melting point**

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	217.8	217.2	217.1	217.4
End of Melting	220.4	220.4	220.6	220.5

404

405

406 **Compound 6 IR**



407

408 **Compound 6 Optical rotation**

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ (°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	0.9522	-0.230	-48.31	-48.42
2	0.9522	-0.231	-48.52	

1. Samples diluted with methanol

2. $L = 0.5$ dm

409

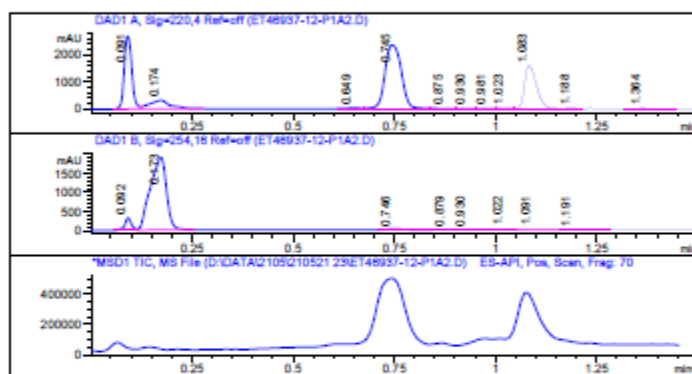
410

411

1.3.7. Compound 7

Compound 7 (ET46937-12-p1a2) IPC-LCMS

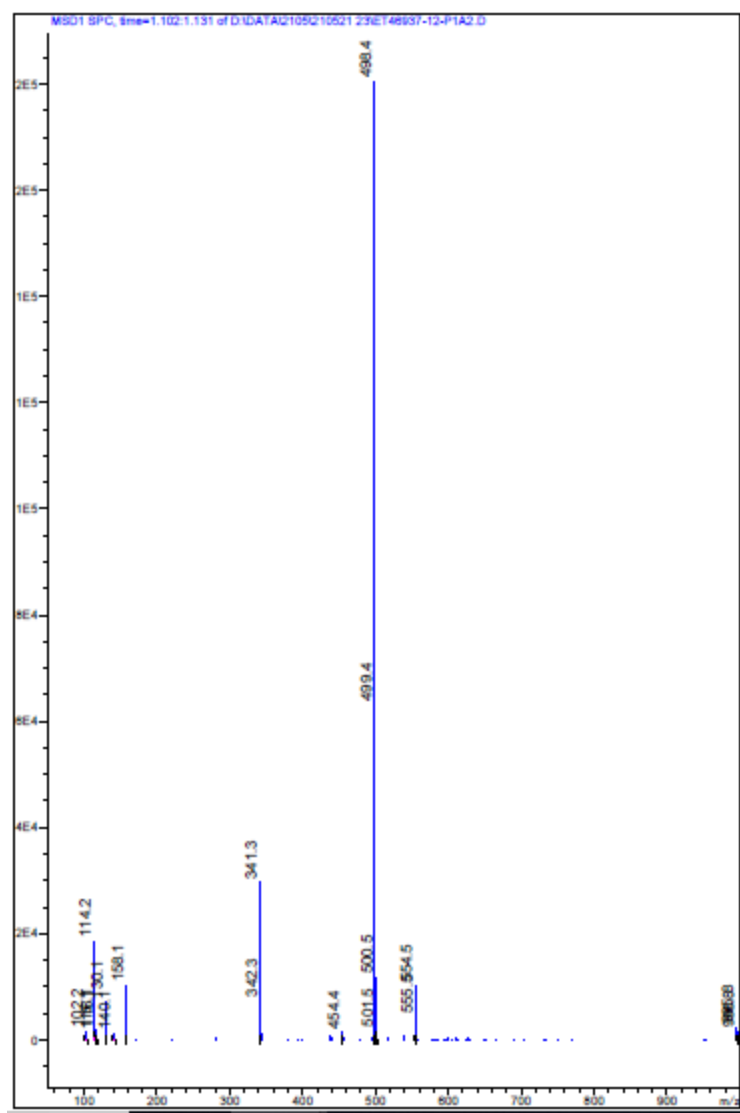
Method Info : Instrument:Agilent1200+6120MS
 Column:Kinetex EVO C18 5um 30*2.1mm
 Column:40
 Mobile Phase:A:0.04%TFA in H2O
 Mobile Phase:B:0.02%TFA in ACN
 Flow Rate: 1.5ml/min
 Time B% Flow(ml/min)
 0.00 50 1.5
 0.80 100 1.5
 1.20 100 1.5
 1.21 50 1.5
 1.50 50 1.5



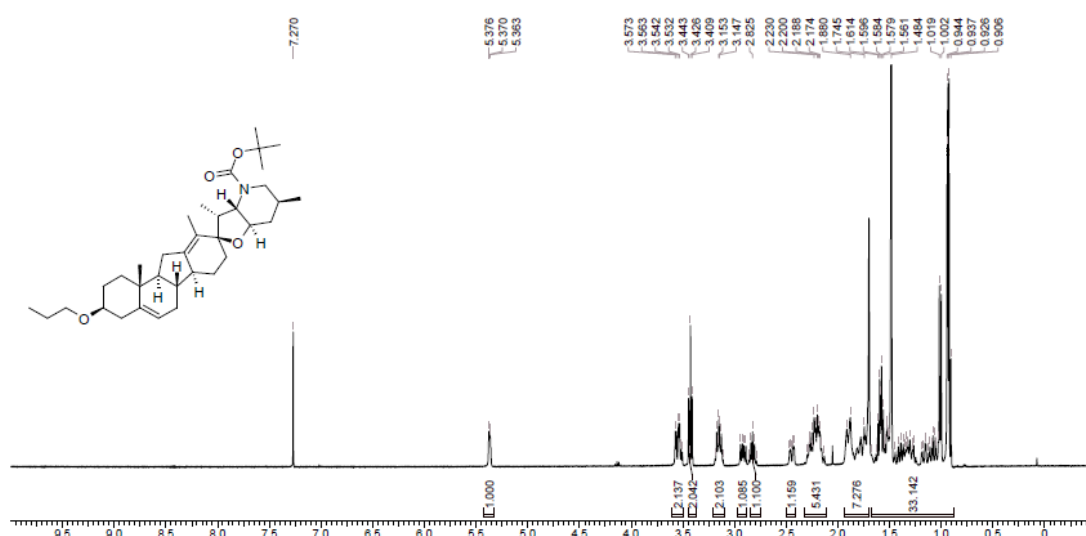
Integration Result

Signal 1 : DAD1 A, Sig=220.4 Ref-off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.091	3528.972	2704.217	37.488	0.021	23.342
2	0.174	1132.732	317.904	4.407	0.048	7.492
3	0.649	55.039	25.126	0.348	0.032	0.364
4	0.745	6601.385	2350.949	32.591	0.045	43.663
5	0.875	71.615	28.661	0.397	0.037	0.474
6	0.930	105.723	47.506	0.659	0.032	0.699
7	0.981	144.043	57.186	0.793	0.035	0.953
8	1.023	120.705	53.058	0.736	0.033	0.798
9	1.083	3279.306	1598.359	22.158	0.032	21.690
10	1.188	25.053	12.427	0.172	0.031	0.166
11	1.364	54.209	18.122	0.251	0.047	0.359

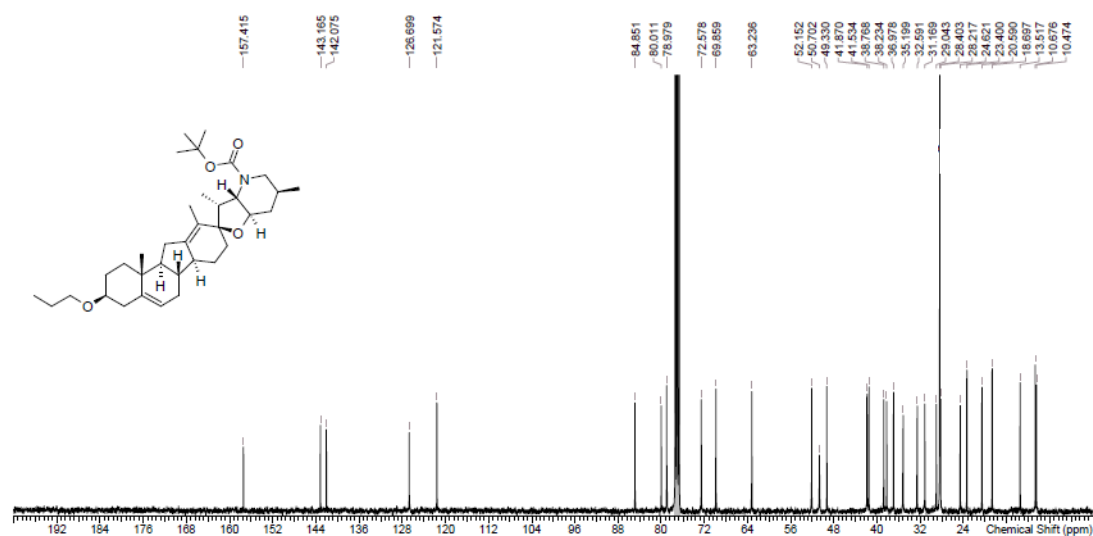


416

417 **Compound 7** ^1H -NMR

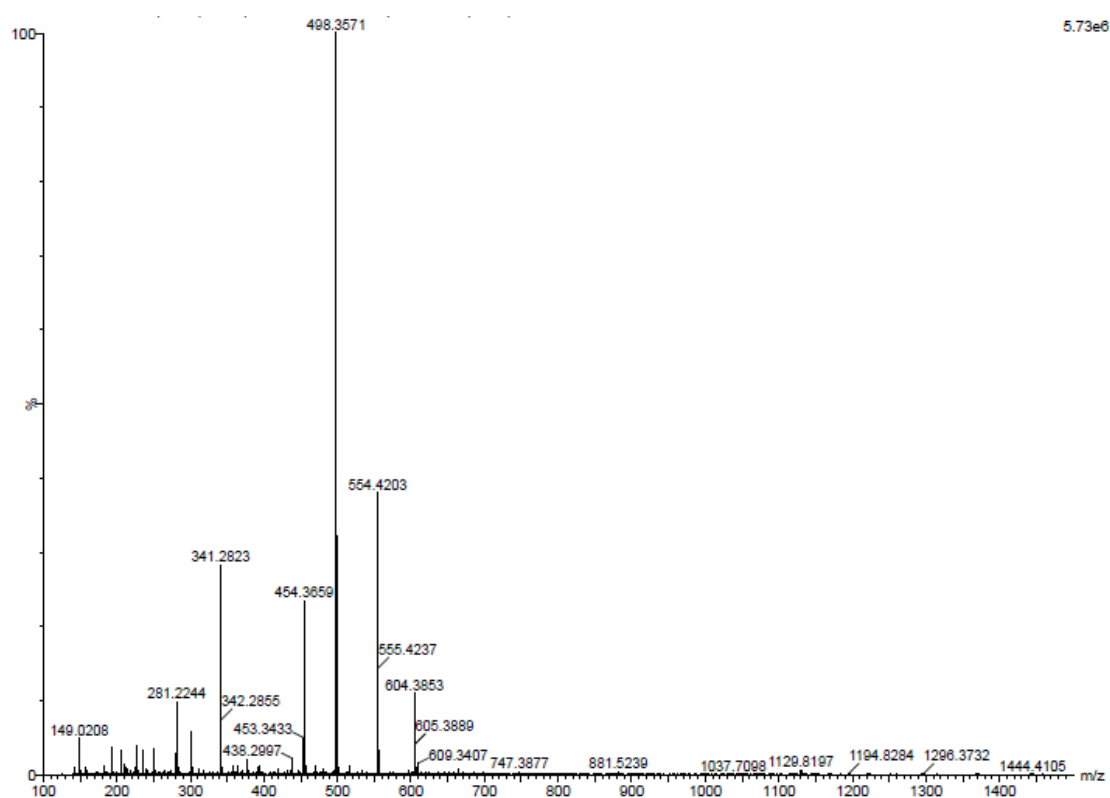
418

419

420 **Compound 7** ^{13}C -NMR

421

422

423 **Compound 7** HRMS

424

425

426

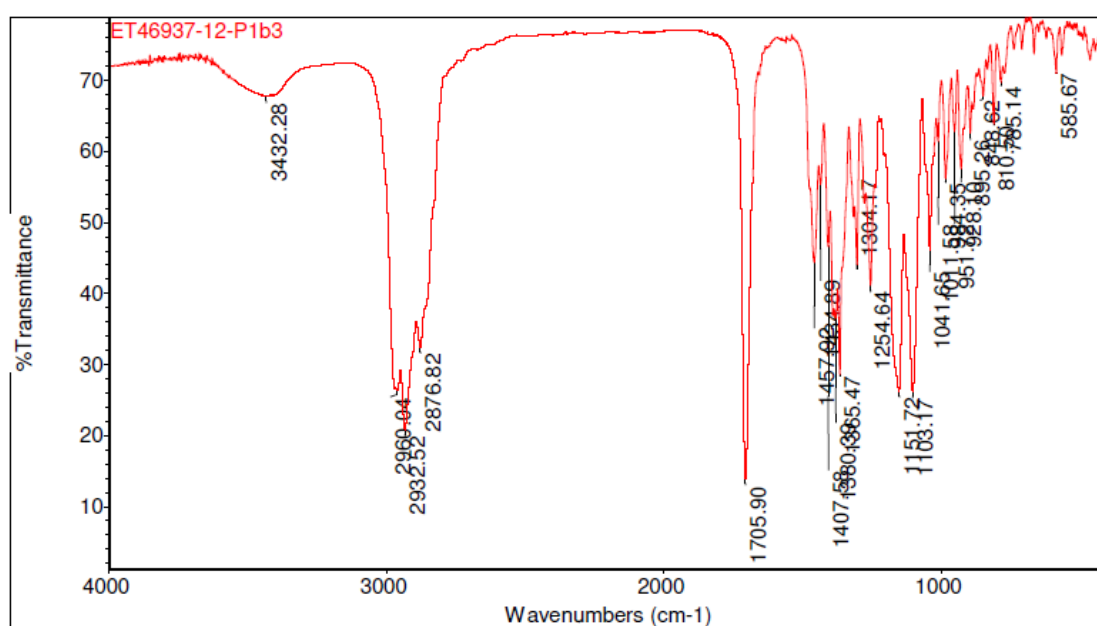
427 **Compound 7 Melting point**

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	171.0	171.6	170.9	171.2
End of Melting	176.8	176.3	176.3	176.5

428

429

430 **Compound 7 IR**



431

432

433 **Compound 7 Optical rotation**

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ /(°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	0.9730	0.004	0.82	0.82
2	0.9730	0.004	0.82	

1. Samples diluted with methanol

2. $L = 0.5$ dm

434

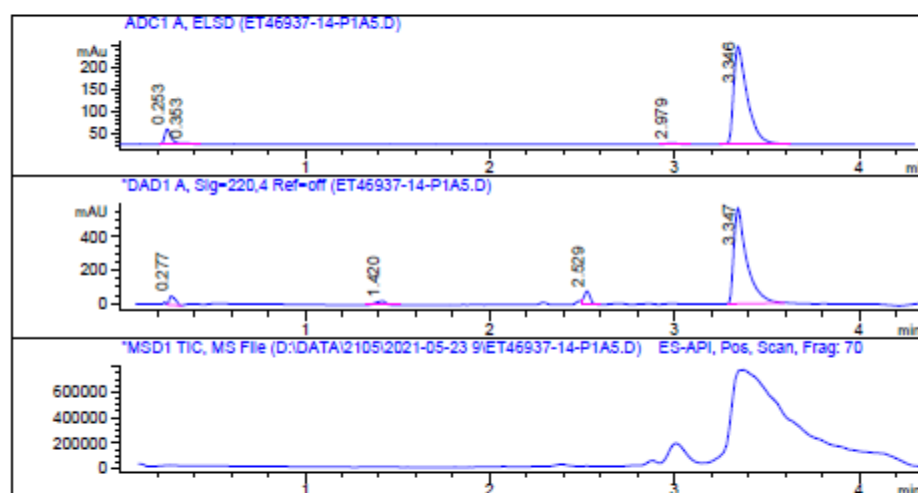
435

1.3.8. Compound 8 (A3P)

Compound 8 (ET46937-14-P1a5) IPC-LCMS

Method Info : Instrument: Agilent1200+6110MS
 Column: XBridge C18 Sum 2.1*50mm
 Column Temp: 40
 Mobile Phase: A: 10mM NH₄HCO₃ in H₂O
 Mobile Phase: B: ACN
 Flow Rate: 1ml/min

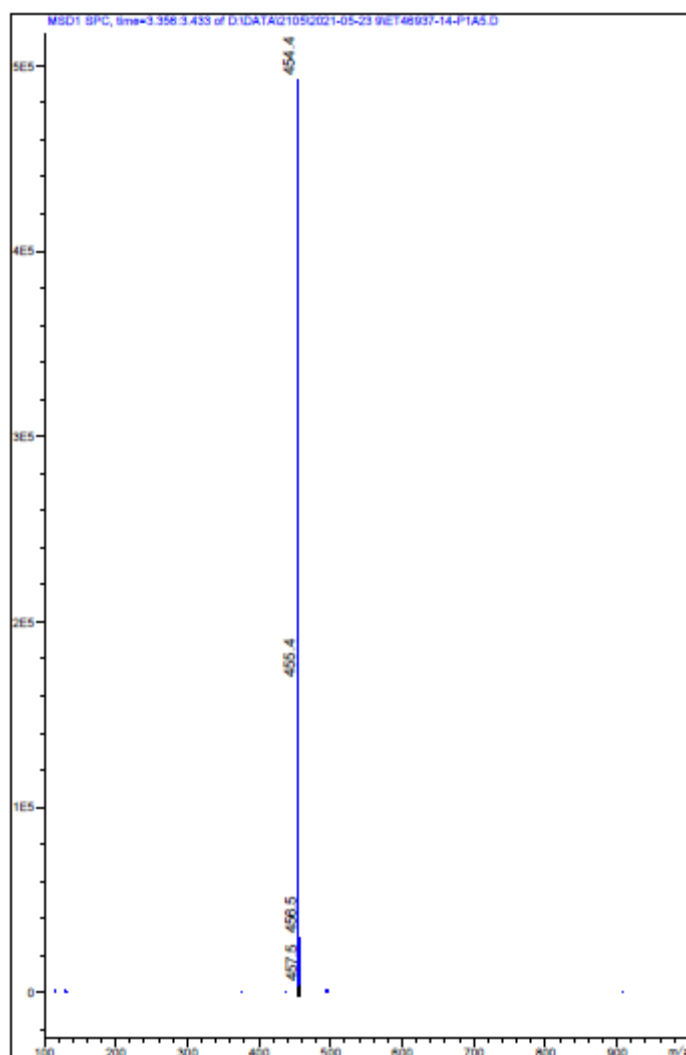
Time	B%	Flow (ml/min)
0.00	5	1.0
3.00	95	1.0
3.50	95	1.0
3.51	5	1.0
4.30	5	1.0



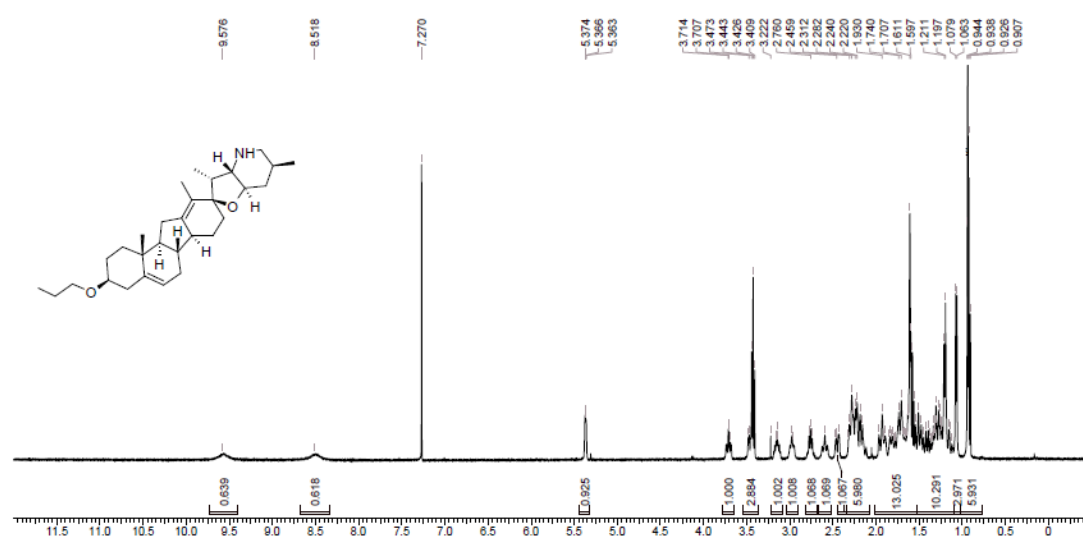
Integration Result

Signal 1 : ADCL A, ELSD

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.253	86.349	34.596	13.177	0.037	6.916
2	0.353	6.937	2.321	0.884	0.044	0.556
3	2.979	9.346	2.326	0.886	0.056	0.749
4	3.346	1145.831	223.313	85.053	0.075	91.779

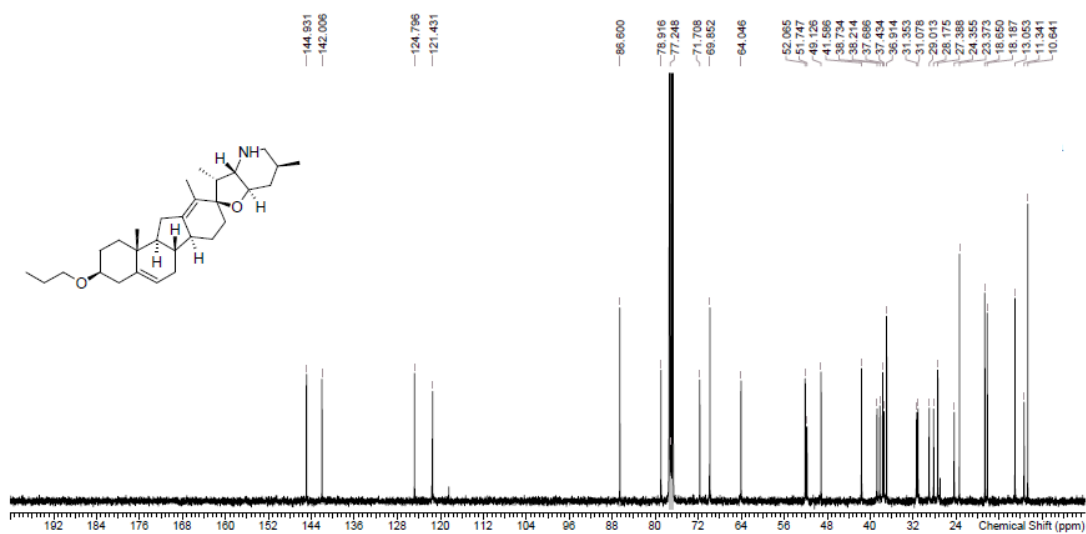


440

441 **Compound 8** ^1H -NMR

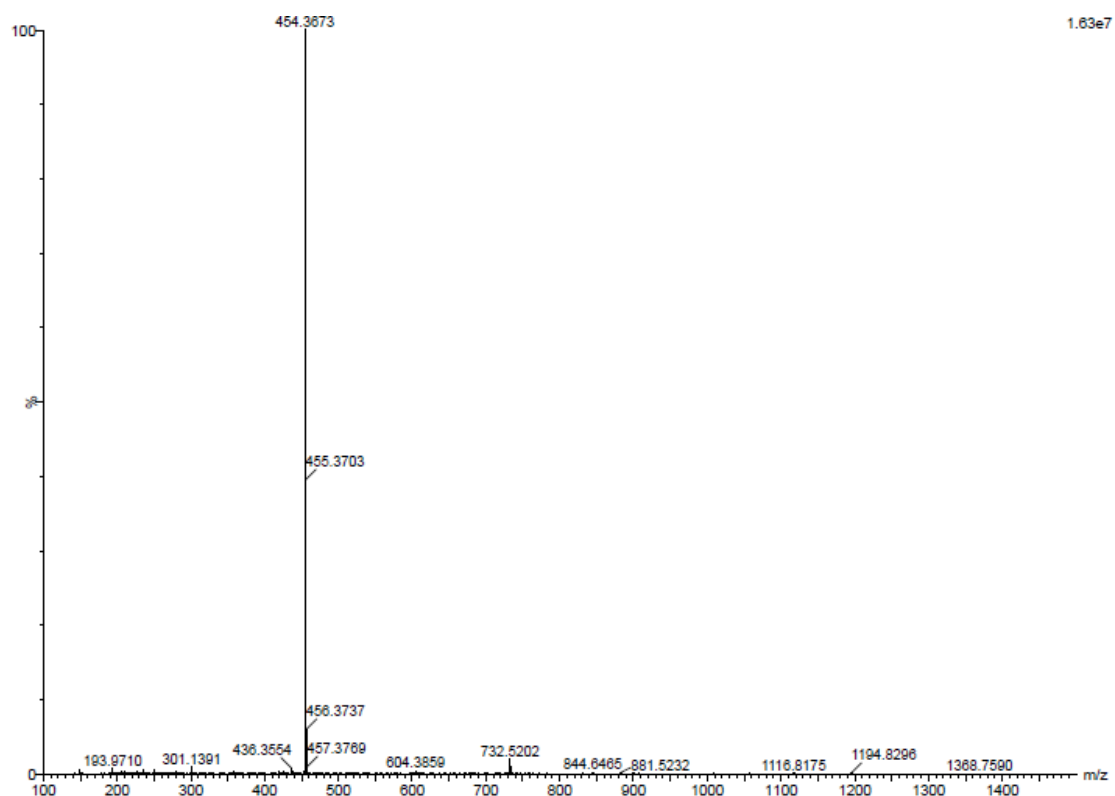
442

443

444 **Compound 8** ^{13}C -NMR

445

446

447 **Compound 8** HRMS

448

449

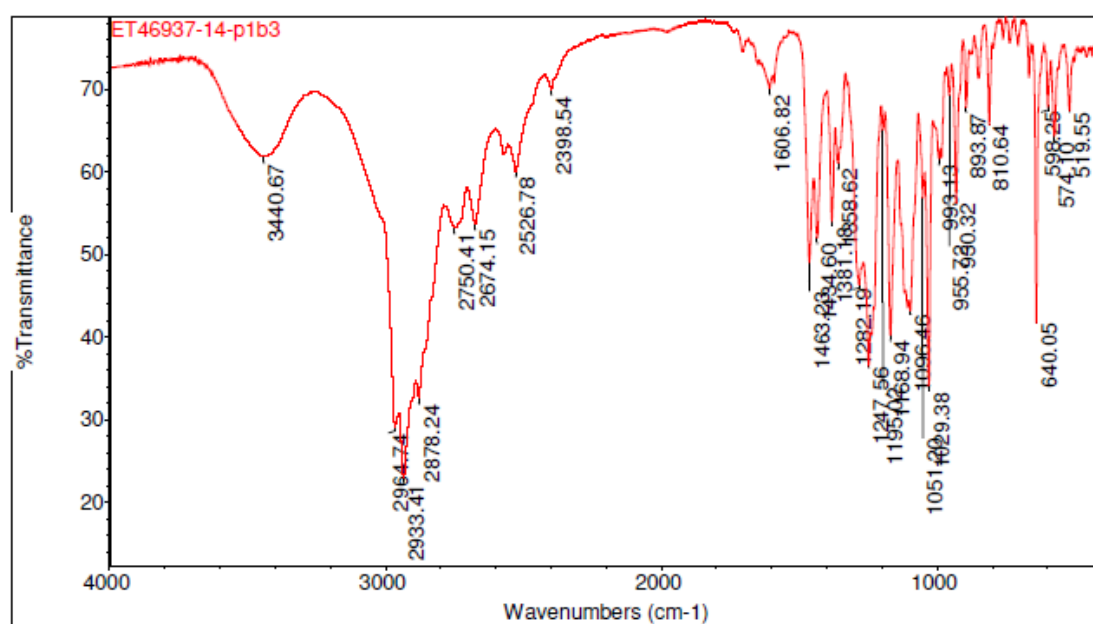
450

451 **Compound 8 Melting point**

Items	1 (°C)	2 (°C)	3 (°C)	$\bar{x}$ (°C)
Start of Melting	198.7	198.7	198.7	198.7
End of Melting	205.2	205.2	205.2	205.2

452

453

454 **Compound 8 IR**

455

456

457 **Compound 8 Optical rotation**

	c /(g/100mL)	α /(°)	$[\alpha]_D^{20}$ /(°)	$[\bar{\alpha}]_D^{20}$ /(°)
1	0.9752	-0.205	-42.04	-42.15
2	0.9752	-0.206	-42.25	

1. Samples diluted with methanol

2. L = 0.5 dm

458

2. Supplementary video legends

2.1. Supplementary videos 1, 2, 3. IBAG dynamics in compound-treated cells

Time-lapse microscopy of IBAGs in HEp-2 cells infected with RSV-M2-1-mGFP. At 24 hpi, images were taken every 6 s in a CO₂-controlled chamber heated at 37 °C, with an Olympus FV3000 inverted confocal microscope. After five minutes, cells were treated with DMSO (mock, video 1), 5 µM CPM (video 2) or 25 µM A3E (video 3) and subsequently images were taken for another 20 min. The resulting videos were visualized using Image J software (4 fps). A representative video from three independent experiments in which 3 videos were acquired is shown. Scale bar 10 µm.

2.2. Supplementary video 4. IB dynamics

Time-lapse microscopy of IBs in HEp-2 cells infected with RSV-P-BFP. At 24 hpi, cells were imaged every 30 s in a CO₂-controlled chamber heated at 37°C, with an Olympus FV3000 inverted confocal microscope. The resulting videos were visualized under the Image J software (4 pfs). A representative video from 10 videos from 2 independent experiments is shown. Scale bar 10 µm.

2.3. Supplementary videos 5, 6 and 7. IB dynamics and mobility

Time-lapse microscopy of IBs in HEp-2 cells infected with RSV-P-BFP. At 24 hpi, cells were treated for 1 h with DMSO (mock, video 5), 5 µM CPM (video 6) or 25 µM A3E (video 7) and subsequently imaged every 30s for 15 min in a CO₂-controlled chamber heated at 37°C, with an Olympus FV3000 inverted confocal microscope. The resulting videos were visualized under Image J and ICY software (10 pfs). A representative video from 10 videos from 2 independent experiments is shown. Scale bar 10 µm.