SUPPORTING INFORMATION

Environmental Photochemistry of Dienogest: Phototransformation to Estrogenic Products and Increased Environmental Persistence via Reversible Photohydration

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TRANSFORMATION PRODUCT ISOLATION

pH 2 Scale-up and Isolation: 10.6 mg of crude reaction residue was purified by HPLC which resulted in 4 fractions, identified as: (i). Photohydrate B (1.6 mg), (ii). 11β-hydroxydienogest (2.4 mg), (iii). Aromatic B (1.8 mg) and (iv). ~ 1:2.5:5 mixture (2.1 mg) of an unknown, 11β-hydroxydienogest and Diene A respectively. Fraction (iv) was further purified to give 3 sub-fractions: (4.1). unknown (0.7 mg), (4.2). 11β-hydroxydienogest (0.4 mg), and (4.3). Diene A (1.0 mg).

pH 5 Scale-up and Isolation: 13.0 mg of crude reaction residue was purified by HPLC which resulted in 5 fractions, identified as: (i). Photohydrate Aβ (2.1 mg), (ii). Photohydrate B (2.1 mg), (iii). Photohydrate Aα (5.2 mg), (iv). DNG (2.0 mg) and (v). ~ 1:0.4:0.85 mixture (1.7 mg) of an unknown, 11β-hydroxydienogest and Diene A respectively.

pH 7 Scale-up and Isolation: 13.0 mg of crude reaction residue was purified by HPLC which resulted in 5 fractions, identified as: (i). Photohydrate Aβ (1.2 mg), (ii). Photohydrate Aα (9.7 mg), (iii). Aromatic A (0.7 mg), (iv). DNG (0.6 mg) and (v). unknown (0.6 mg).

TABLES

Table S1: ^{1}H NMR data of DNG,[40] Photohydrates Aβ/Aα/B, and 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

Proton	Dienogest δ_H mult.	Photohydrate Aβ δ_H mult. (*J* in Hz)	Photohydrate Aα δ_H mult. (*J* in Hz)	Photohydrate B δ_H mult. (*J* in Hz)	11β-hydroxydienogest δ_H mult. (*J* in Hz)
1	2.52 m	2.35 m	2.46 m	2.02 ddd (13.8, 9.4, 4.7)	2.81 m
	2.89 m	2.72 m	2.69 m	2.11 m	2.92 dt (15.4, 5.7)
2	2.42 m	2.49 m	2.47 m	2.27 m	2.48 m
	2.47 m	2.55 m	2.50 m	2.49 m	2.49 m
4	5.69 s	2.77 d (20.8)	2.72 d (20.8)	5.98 t (2.1)	5.82 s
	-	2.83 d (20.8)	2.8 d (20.8)	-	-
6	2.17 ddd	1.85 m	1.97 m	2.29 m	2.47 m
	2.38 m	1.95 m	2.44 m	2.52 m	2.48 m
7	1.25 m	1.56 m	1.45 m	1.44 m	1.35 m
	1.93 m	2.01 m	1.55 m	1.66 m	1.99 m
8	2.29 m	1.89 m	1.58 m	1.64 m	1.94 m
10	-	-	-	2.33 m	-
11	2.14 ddd	1.72 td (14.1, 3.9)	1.49 td (12.3, 4.2)	1.57 m	5.26 bd (4.6)
	2.86 m	2.72 m	2.00 m	1.89 m	-
12	1.30 m	0.91 td (14.3, 3.6)	1.47 td (11.6, 4.3)	1.51 m	1.44 dd (14.6, 5.0)
	1.78 m	1.52 m	1.63 m	1.52 m	2.40 dd (14.6, 1.5)
14	1.27 m	1.51 m	1.88 m	1.71 m	1.32 m
15	1.44 dq	1.46 m	1.40 m	1.42 m	1.53 m
	1.73 m	1.64 m	1.65 m	1.59 m	1.74 m
16	1.95 m	1.91 m	1.90 m	1.92 m	1.92 m
	2.13 ddd	2.15 m	2.15 ddd (14.8, 9.5, 6.6)	2.14 m	2.14 ddd (15.1, 9.8, 6.6)
18	1.08 s	1.07 s	0.96 s	1.00 s	1.16 s
20	2.48 d	2.40 d (16.4)	2.59 d (16.3)	2.56 d (16.3)	2.49 m
	2.62 dd	2.52 d (16.4)	2.67 dd (16.3, 1.3)	2.63 dd (16.3, 1.3)	2.60 dd (16.4, 0.8)
11-OH	-	-	-	-	8.02 s

Table S2: ^{13}C NMR data of DNG,[40] Photohydrates Aβ/Aα/B, and 11β-hydroxydienogest in $CDCl_3$ (150 MHz).

carbon	Dienogest δ_C	Photohydrate Aβ δ_C	Photohydrate Aα δ_C	Photohydrate B δ_C	11β-hydroxydienogest δ_C
1	25.9	24.6	24.4	20.5	26.1
2	37.1	39.3	38.9	36.0	37.0
3	199.2	210.3	210.5	200.0	199.3
4	122.7	44.6	44.4	127.4	124.7
5	156.3	132.3	134.1	164.0	157.0
6	30.7	26.3	30.3	34.5	30.5
7	27.4	19.1	20.3	25.0	27.1
8	40.1	41.9	42.3	44.0	37.0
9	144.2	72.3	70.0	74.0	139.0
10	126.4	130.6	130.7	47.5	134.0
11	25.5	31.1	30.3	32.0	79.0
12	31.9	29.3	27.0	27.5	34.2
13	46.3	46.1	46.5	46.0	45.2
14	51.3	43.2	42.4	43.0	50.3
15	23.5	23.6	22.6	23.0	23.3
16	37.4	37.4	37.0	37.0	37.0
17	81.6	81.4	81.9	82.0	81.8
18	13.5	13.8	13.5	13.0	15.2
20	28.2	28.4	28.4	28.5	28.2
21	118.1	118.4	118.7	118.2	118.0

Table S3: HMBC correlations for Photohydrates Aβ/Aα/B, and 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

Proton	Photohydrate Aβ H#→C#	Photohydrate Aα H#→C#	Photohydrate B H#→C#	11β-hydroxydienogest H#→C#
1	3	3,5,10	2,3,5,9,10	2,3,5,9,10
2	1,3,10	1,3,4	1,3,4,10	1,3,4
4	3,5,6,10	2,3,5,6,10	2,6,10	2,6,10
6	4,5,7,8,10	4,5,7,8,10	4,5,7,8,10	4,5,7,8
7	5,9,14	5,6,8,9,14	5,6,8,9,14	5,6,8,9,14
8	6,9,10,15	6,7,9,10,11,13,14	6,7,9,13,14,15	6,7,9,14,15
10	-	-	1,4,5,9	-
11	8,9,10,12,13	8,9,10,12,13	8,9,10,12,13	8,9,10,12,13
12	9,11,13,14,18	9,11,13,14,17,18	9,11,13,14,17,18	9,11,13,14,17,18
14	8,9,12,13,15,18	7,8,9,12,13,15,17,18	8,9,12,13,15,17,18	7,8,9,12,13,15,16,17,18
15	8,13,14,16	8,13,14,16,17	8,13,14,16,17	8,13,14,16,17
16	13,15,17,20	13,14,15,17,20	13,14,15,17,20	13,15,17,20
18	12,13,14,17	12,13,14,17	12,13,14,17	12,13,14,17
20	13,16,17,21	13,16,17,21	13,16,17,21	13,16,17,21

Table S4: HRMS results from DNG photolysis.

Compound	Retention Time (min)	Observed ($M^{+\bullet}$) [Calc'd]	Observed $(M+H)^+$ [Calc'd]	Observed $(M+Na)^+$ [Calc'd]	Observed $(M+K)^+$ [Calc'd]
Photohydrate Aβ	3.9		330.2068 [330.2069]		
Photohydrate B	4.8		330.2070 [330.2069]		
Photohydrate Aα	4.9			352.1869 [352.1888]	368.1617 [368.1628]
11β-hydroxydienogest	5.1	327.1838 [327.1834]			
Aromatic A	6.2	311.1885 [311.1885]			
Dienogest	7.3		312.1955 [312.1963]		
Aromatic B	7.9	309.1729 [309.1729]			
Diene A	8.6		312.1953 [312.1963]		

FIGURES

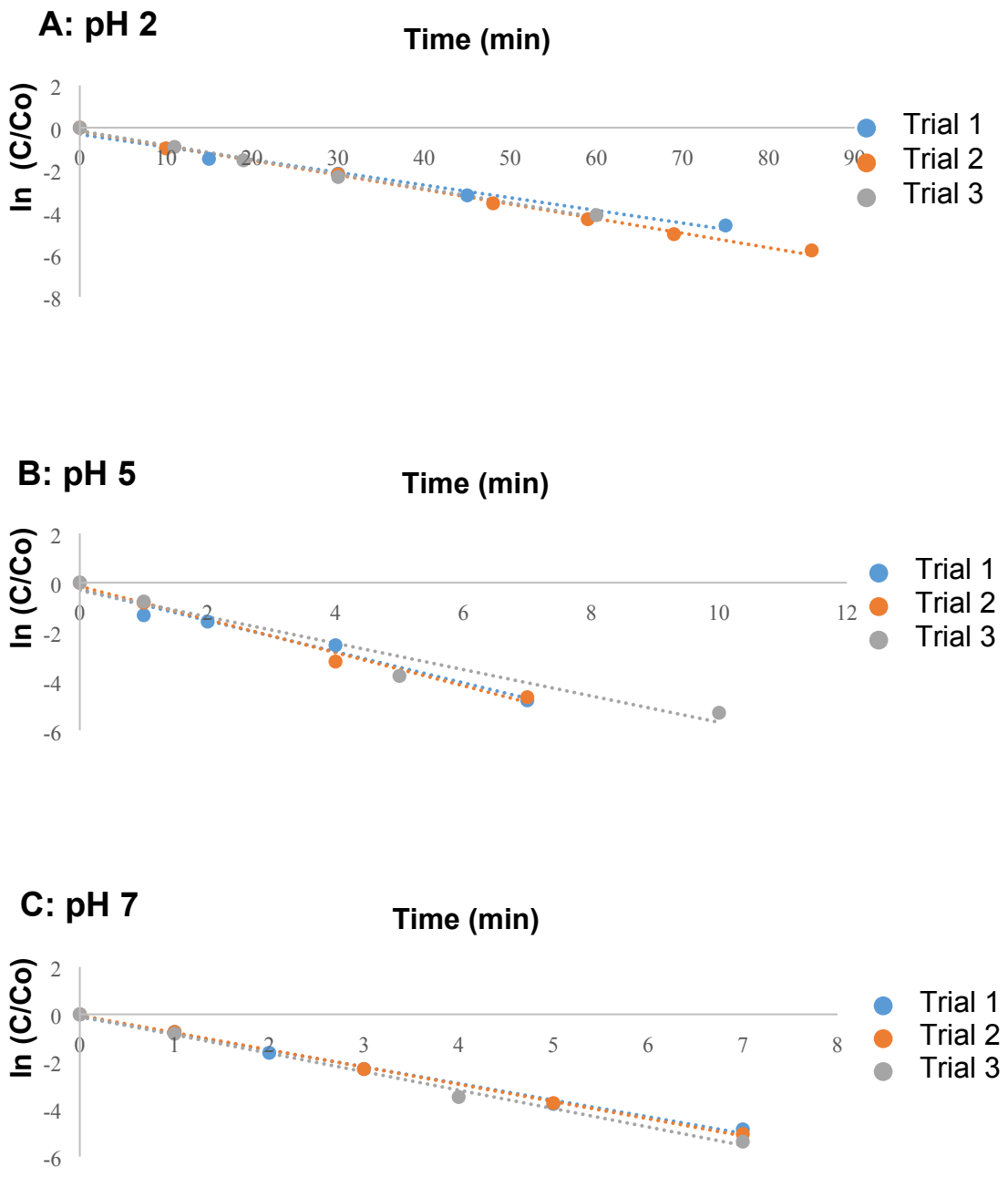

Figure S1: Semi-log plot of normalized DNG concentration versus time for (A) pH 2, (B) pH 5 and (C) pH 7. Linear trendline fits are shown as dashed lines for replicate (n = 3) trials. Measured pseudo-first-order rate coefficients, or k_{obs} values [pH 7 = 1.2 (± 0.07) × 10^{-2} s^{-1}; pH 5 = 1.0 (± 0.1) × 10^{-2} s^{-1}; and pH 2 = 1.1 (± 0.08) × 10^{-3} s^{-1}], were estimated from linear regression analyses.

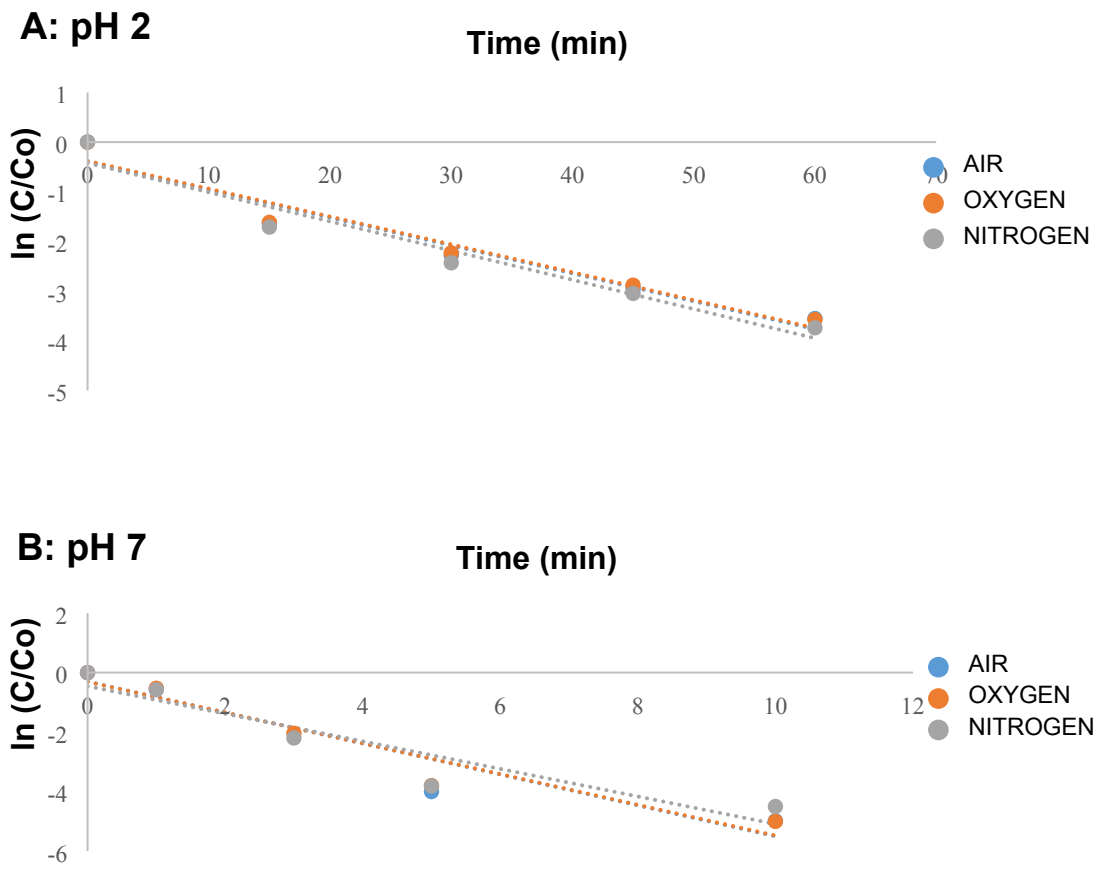

Figure S2: Semi-log plot of normalized DNG concentration versus time as a function of oxygen concentration (i.e., open to atmosphere or purged with oxygen or nitrogen) for (A) pH 2 and (B) pH 7.

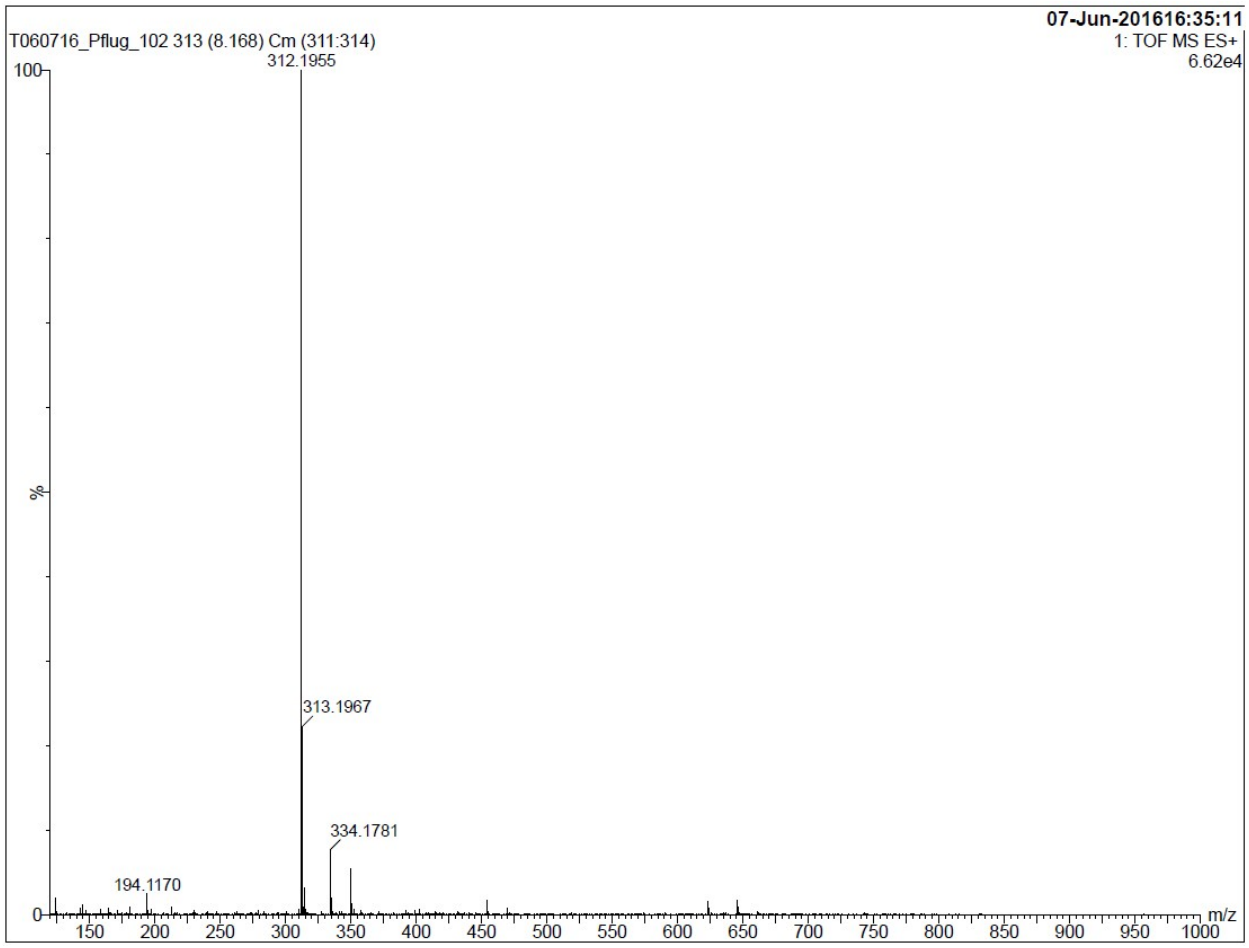

Figure S3: HR-ESI-TOFMS of DNG standard.

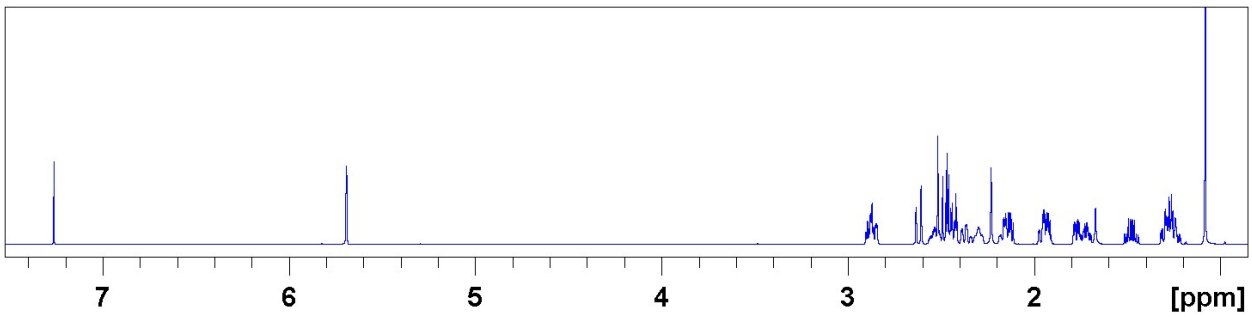

Figure S4: ^{1}H NMR spectrum of DNG standard in $CDCl_3$ (600 MHz).

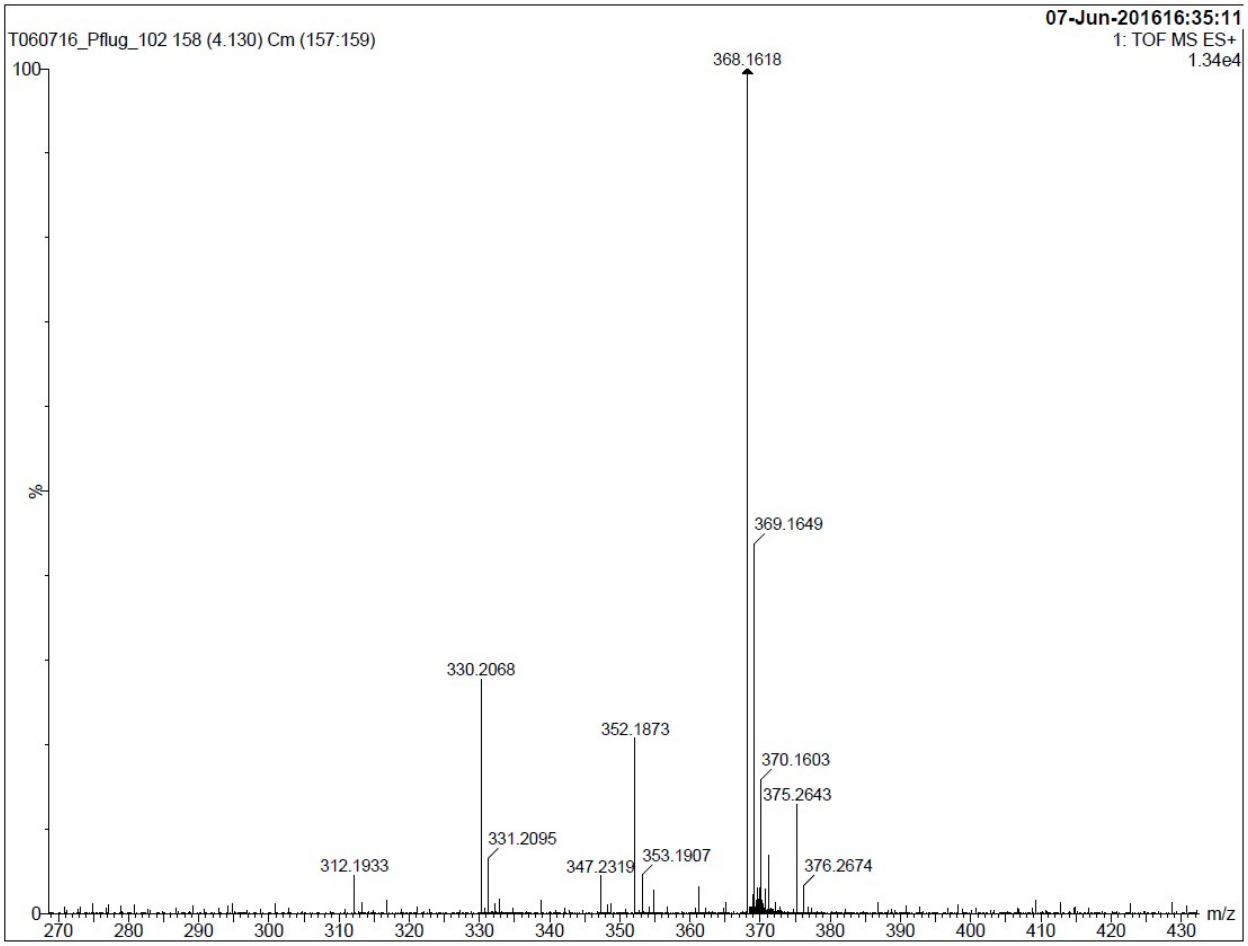

Figure S5: HR-ESI-TOFMS of Photohydrate Aβ.

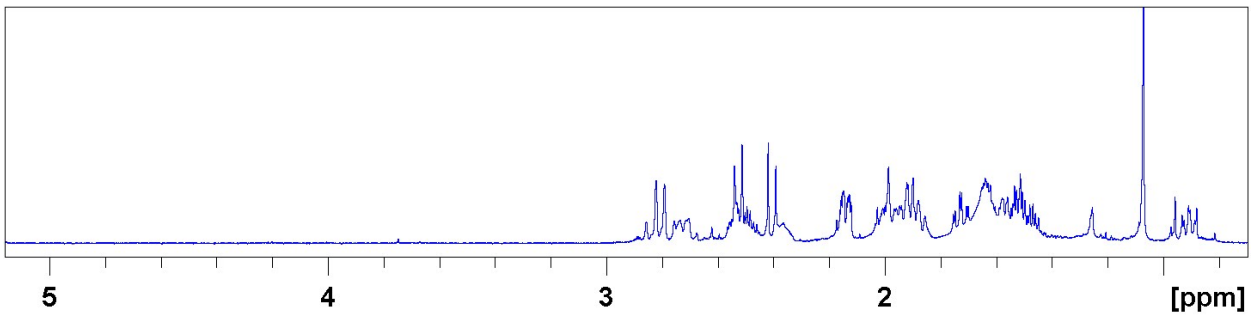

Figure S6: ^{1}H NMR spectrum of Photohydrate Aβ in $CDCl_3$ (600 MHz).

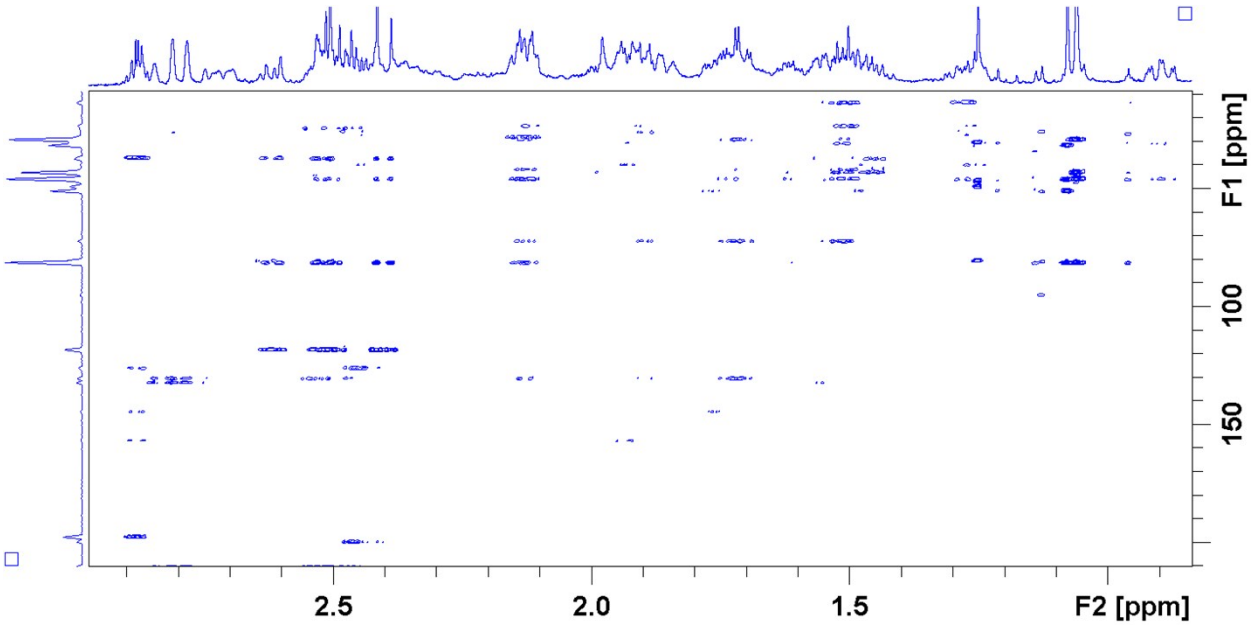

Figure S7: HMBC spectrum of Photohydrate Aβ in $CDCl_3$ (600 MHz).

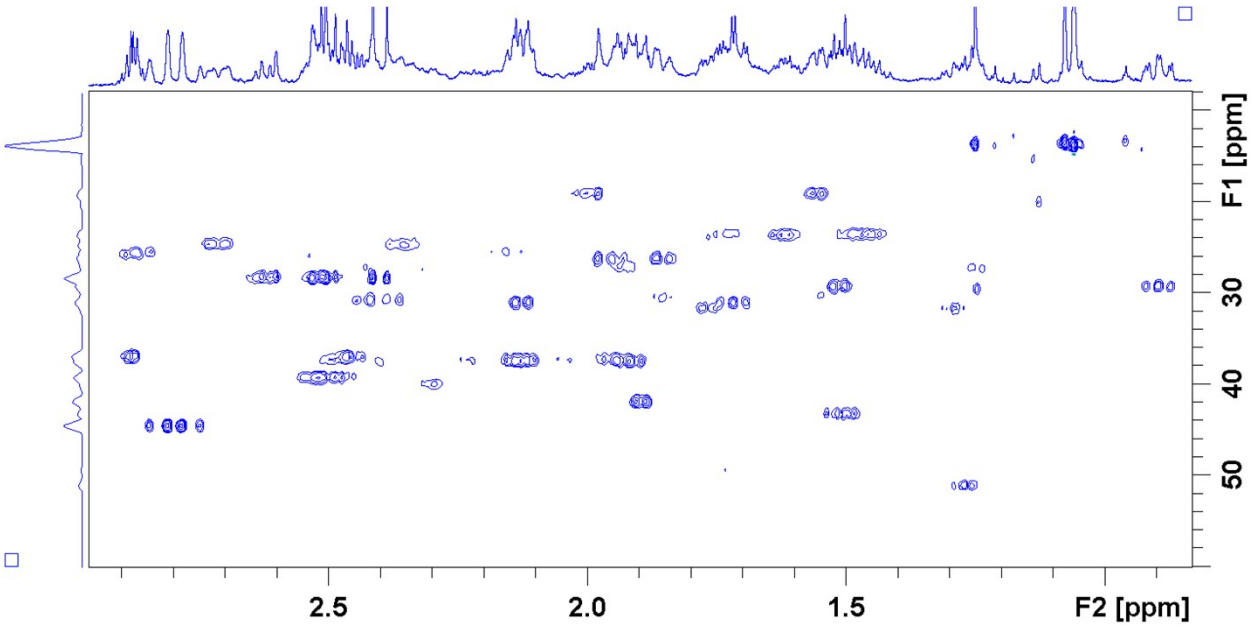

Figure S8: HSQC spectrum of Photohydrate Aβ in $CDCl_3$ (600 MHz).

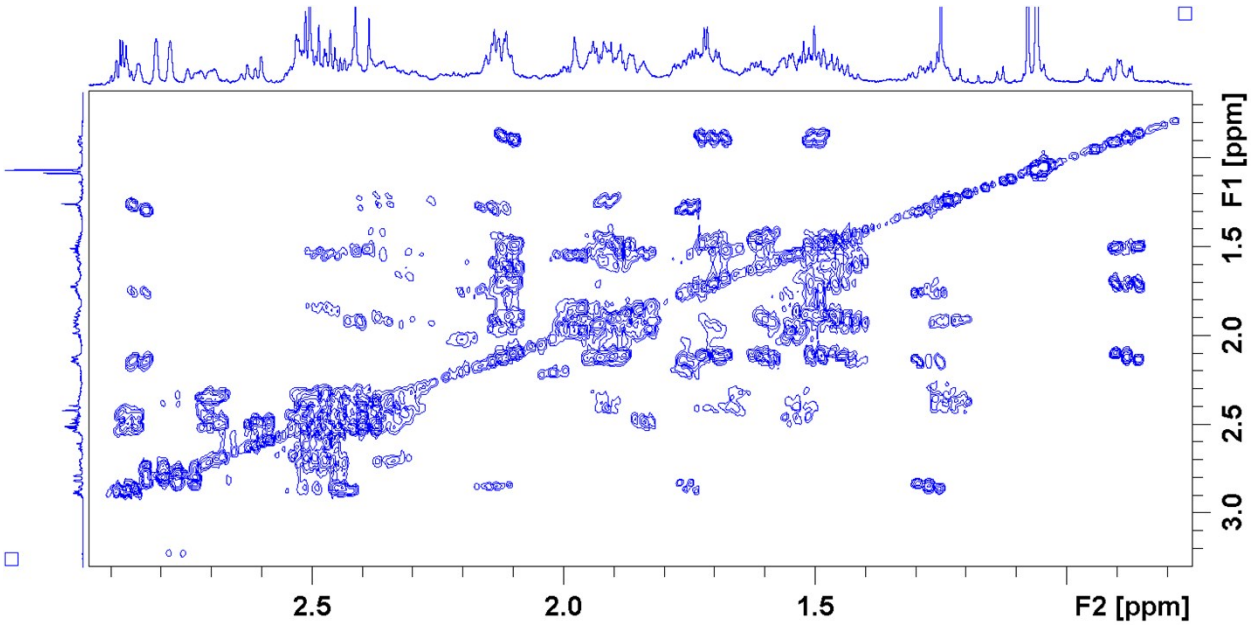

Figure S9: COSY spectrum of Photohydrate Aβ in $CDCl_3$ (600 MHz).

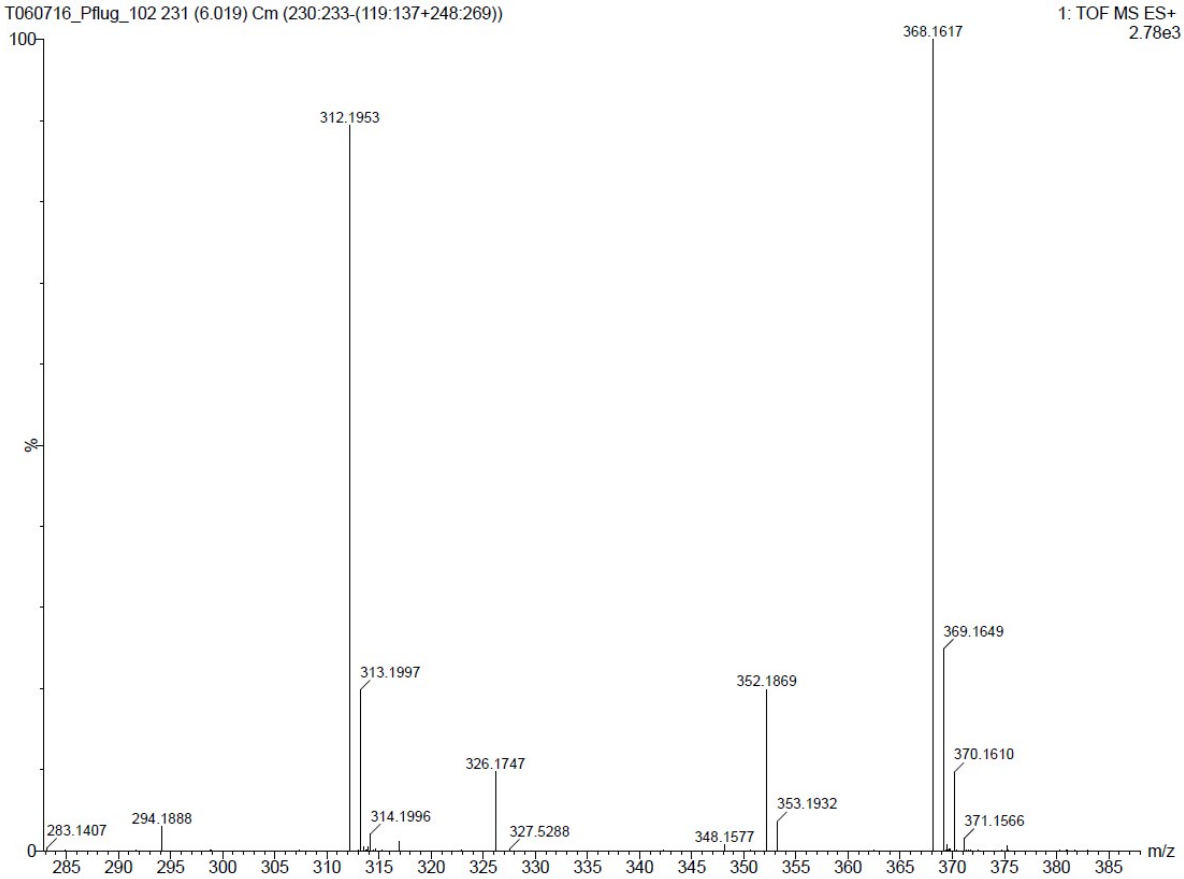

Figure S10: HR-ESI-TOFMS of Photohydrate Aα.

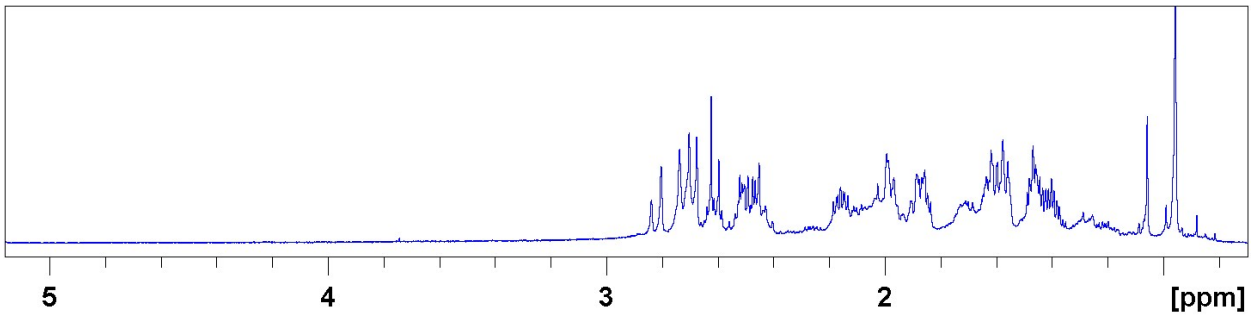

Figure S11: ^{1}H NMR spectrum of Photohydrate Aα in $CDCl_3$ (600 MHz).

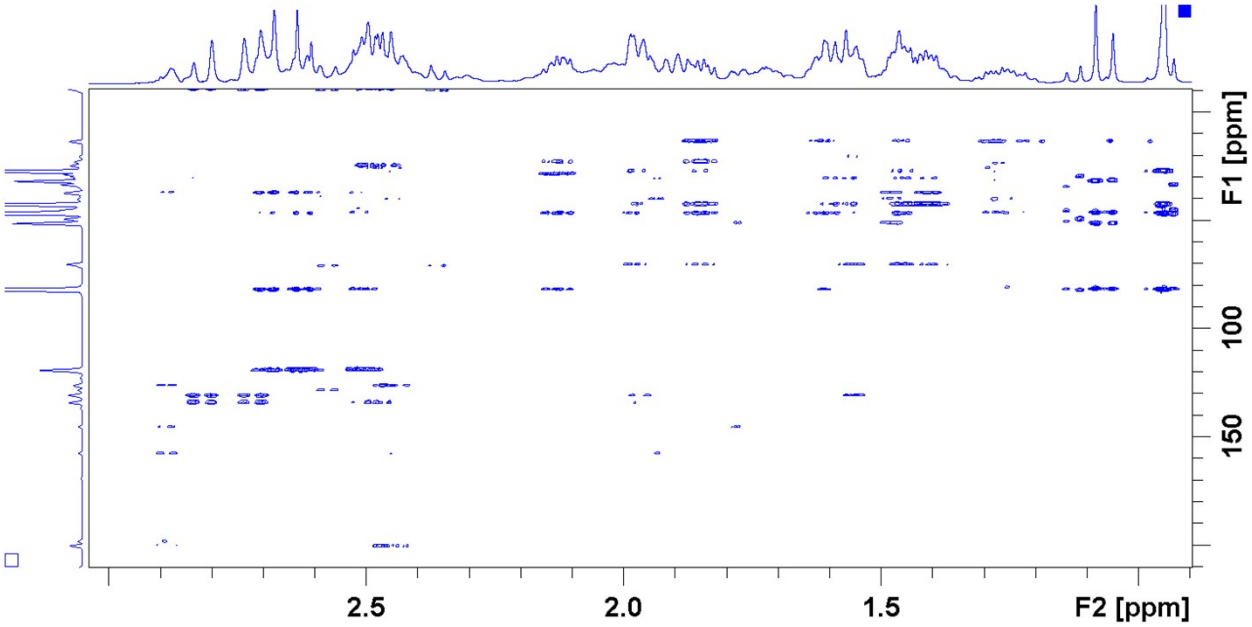

Figure S12: HMBC spectrum of Photohydrate Aα in $CDCl_3$ (600 MHz).

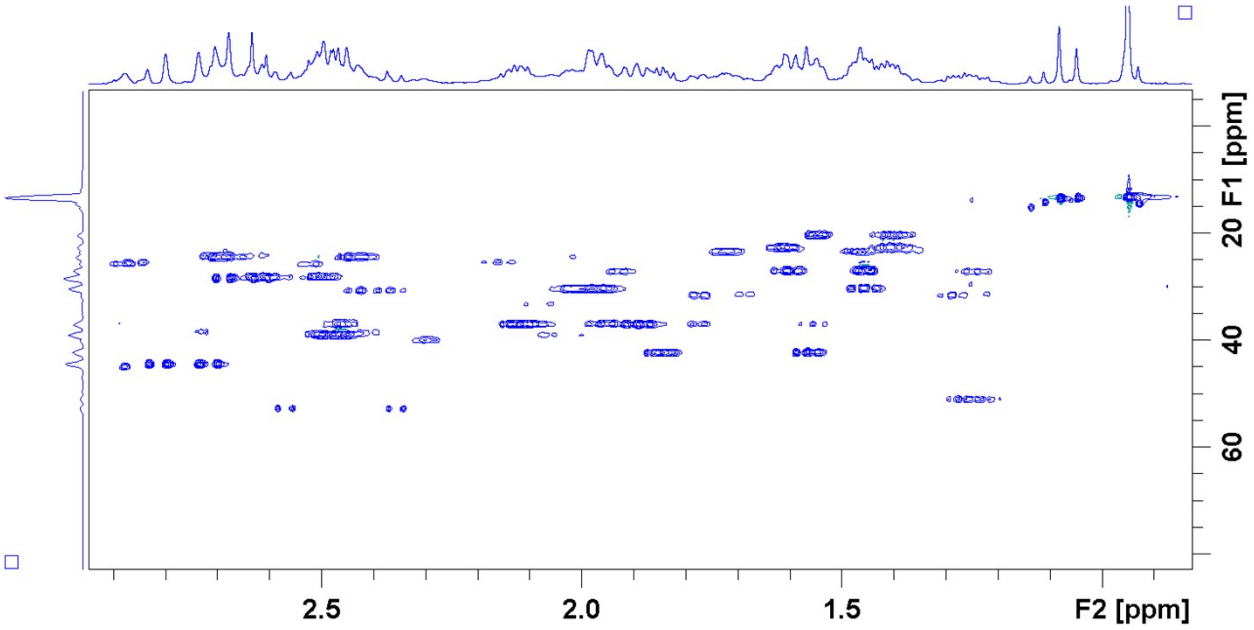

Figure S13: HSQC spectrum of Photohydrate Aα in $CDCl_3$ (600 MHz).

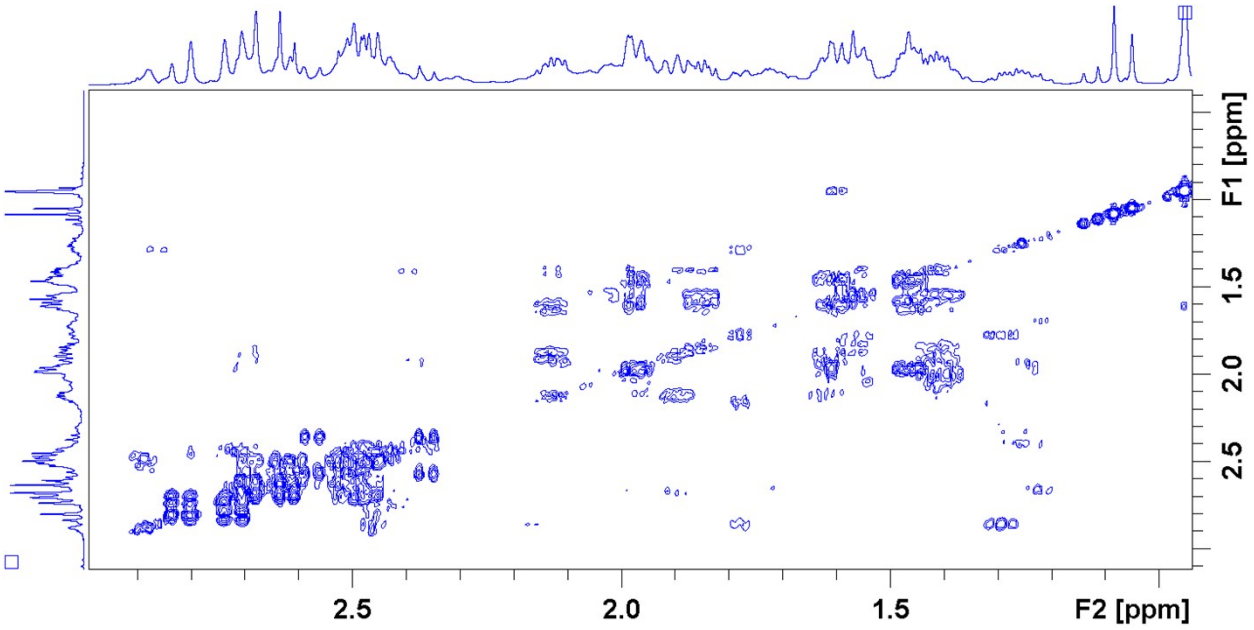

Figure S14: COSY spectrum of Photohydrate Aα in $CDCl_3$ (600 MHz).

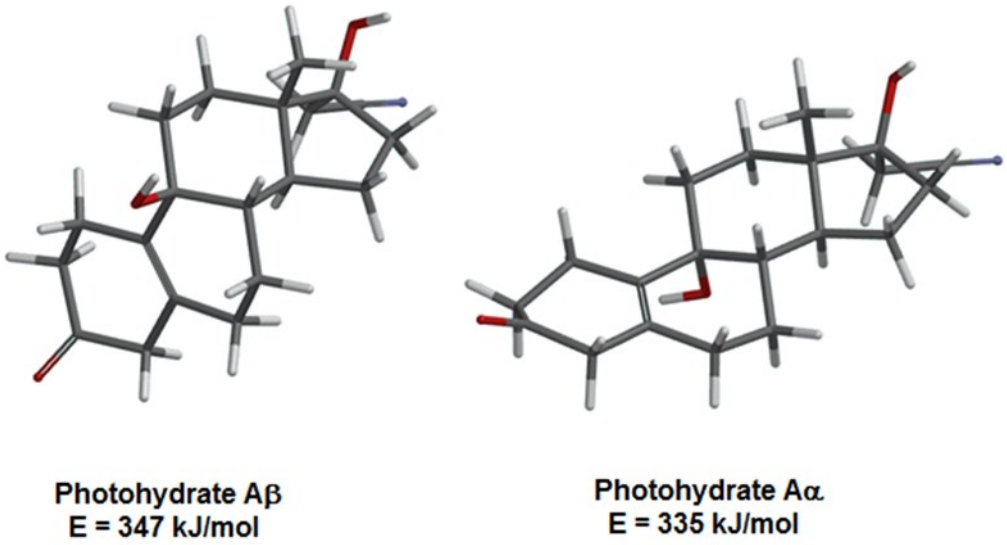

Figure S15: Energy minimized models of Photohydrates Aβ and Aα, using MMFF molecular mechanics with Monte-Carlo conformational searching.

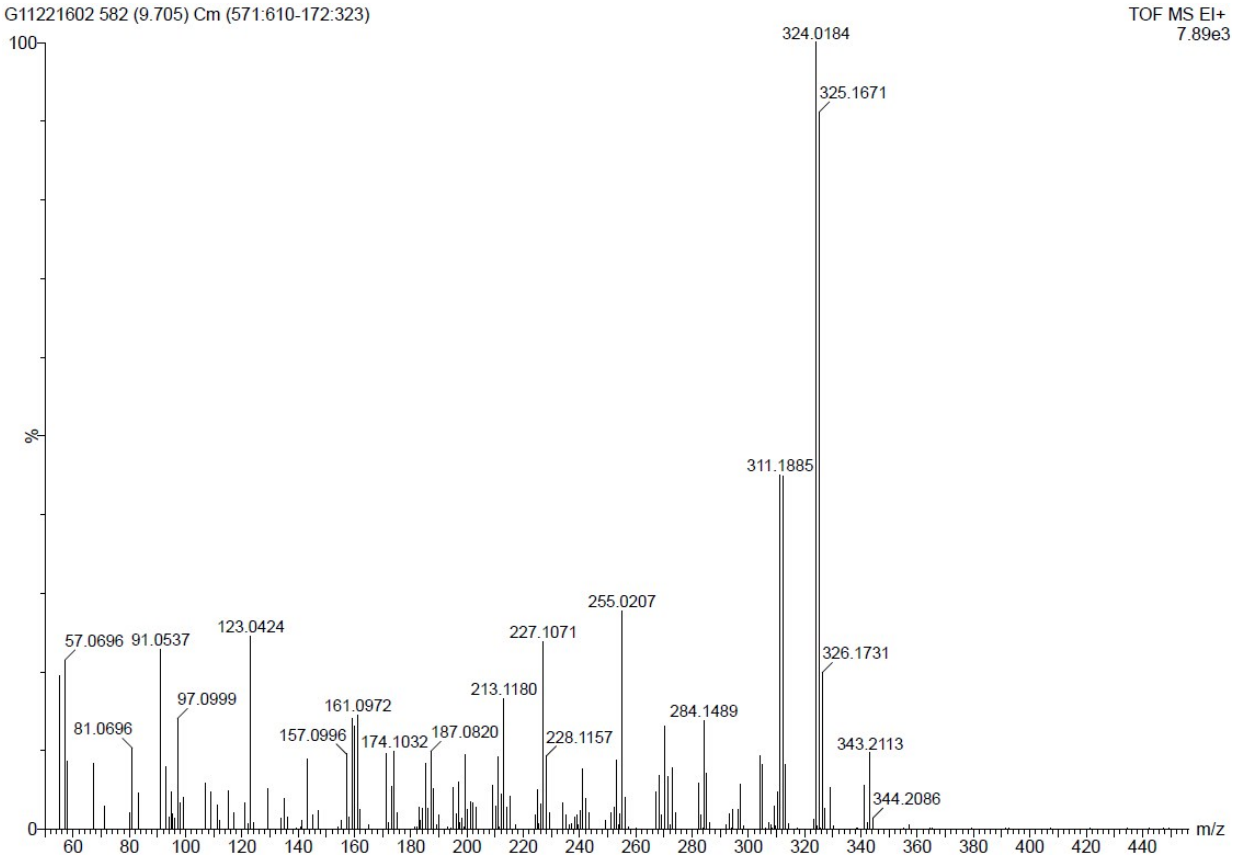

Figure S16: HR-EI-TOFMS of Aromatic A. Ion at 324.0184 Da is from impurity in sample.

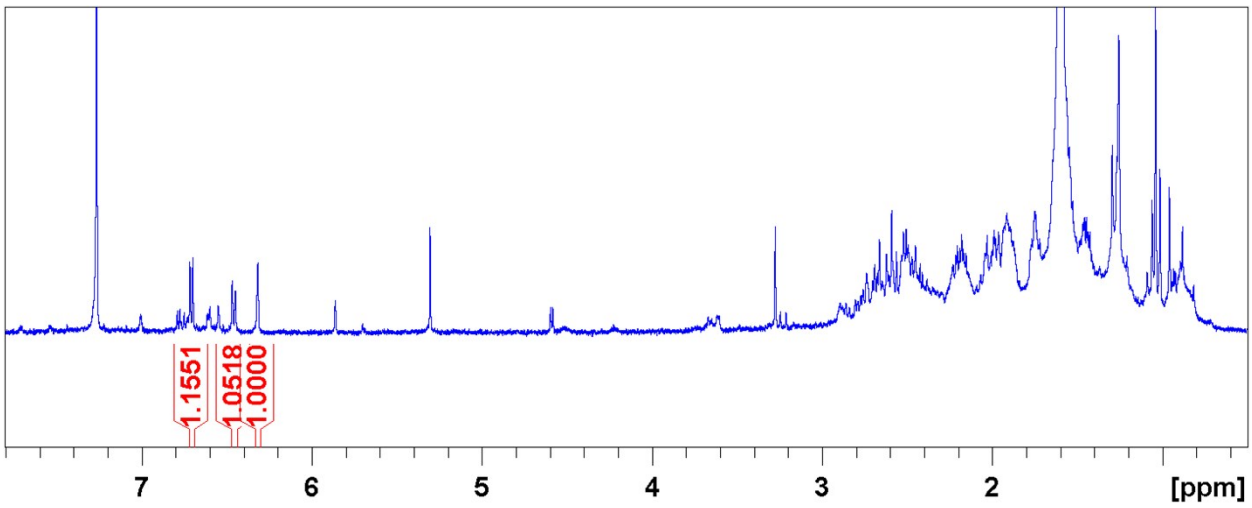

Figure S17: ^{1}H NMR spectrum of Aromatic A in $CDCl_3$ (600 MHz).

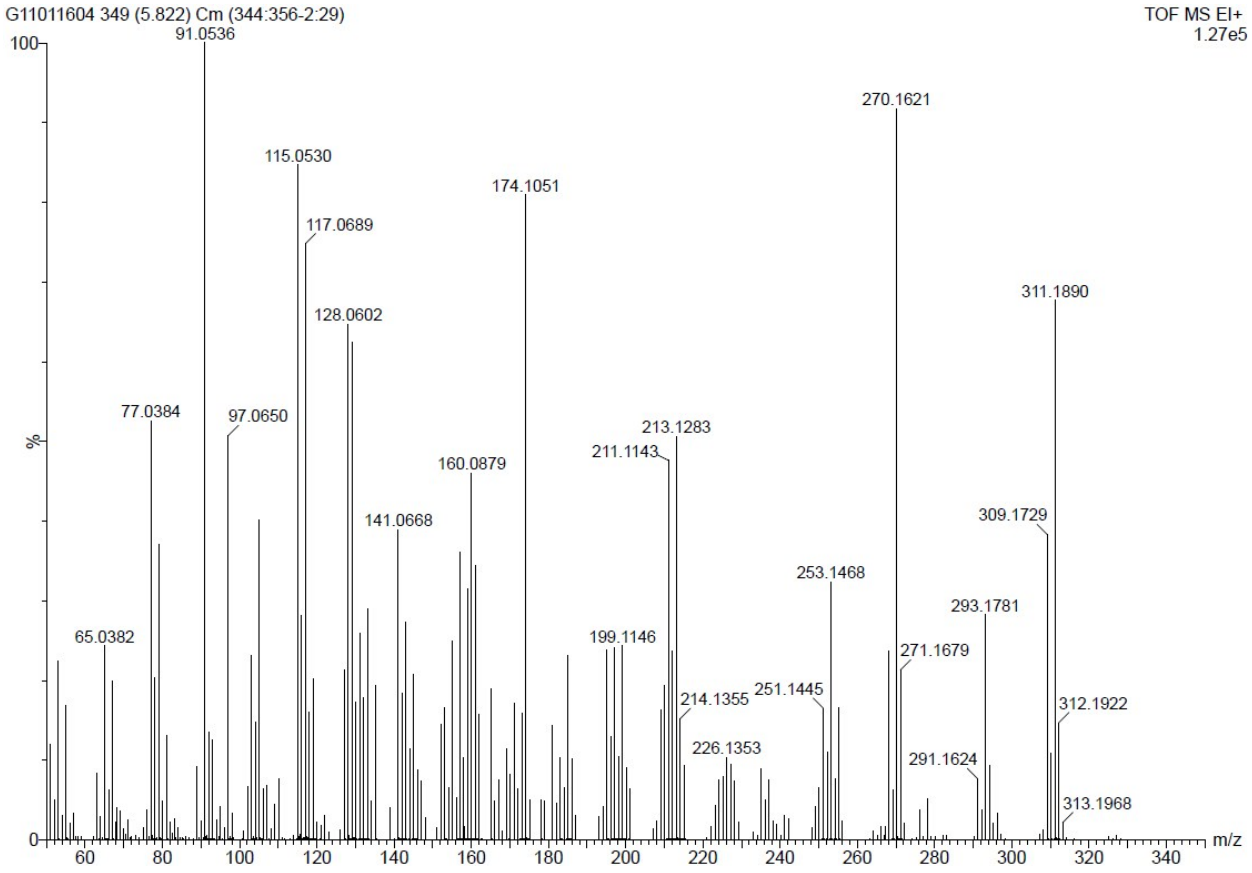

Figure S18: HR-EI-TOFMS of Aromatic B. Ion at 311.1890 Da is from parent DNG in sample.

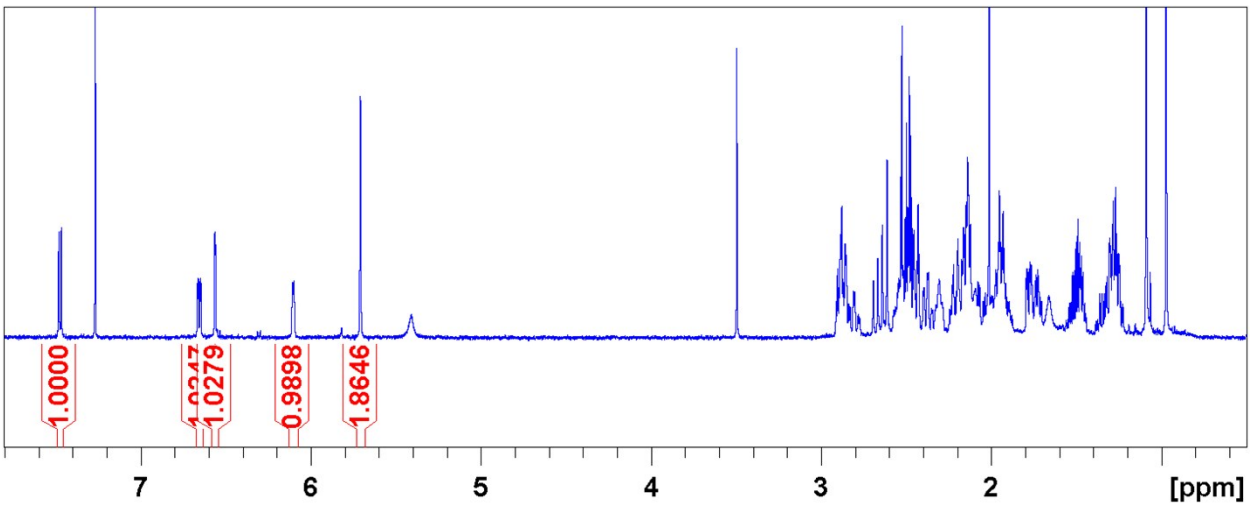

Figure S19: ^{1}H NMR spectrum of Aromatic B in $CDCl_3$ (600 MHz). Signal at 5.69 ppm corresponds to H4 of DNG, while signal at 3.49 ppm corresponds to residual methanol.

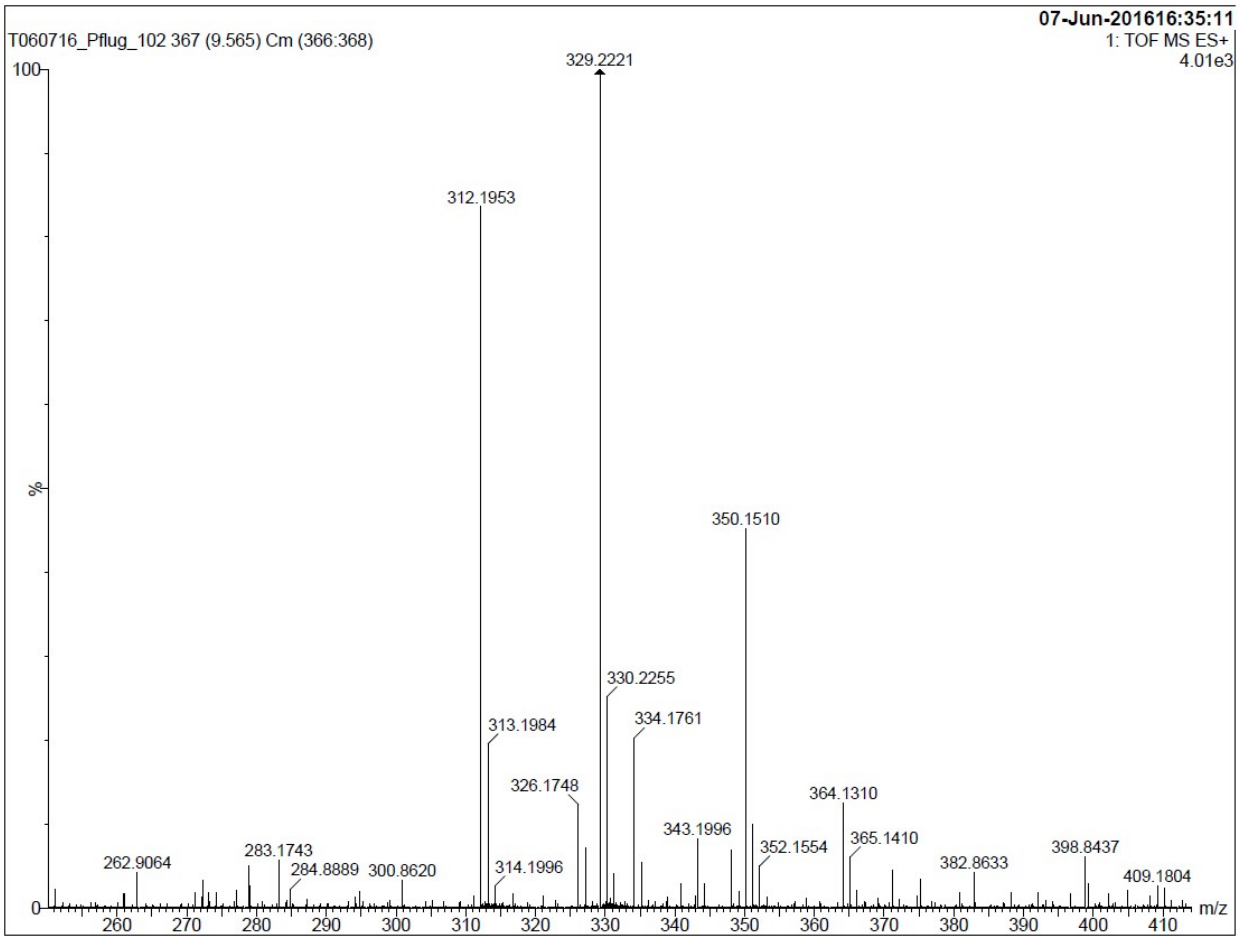

Figure S20: HR-ESI-TOFMS of Diene A. Ion at 329.2221 Da is from unidentified compound and Ion at 326.1748 Da is from Aromatic C in sample.

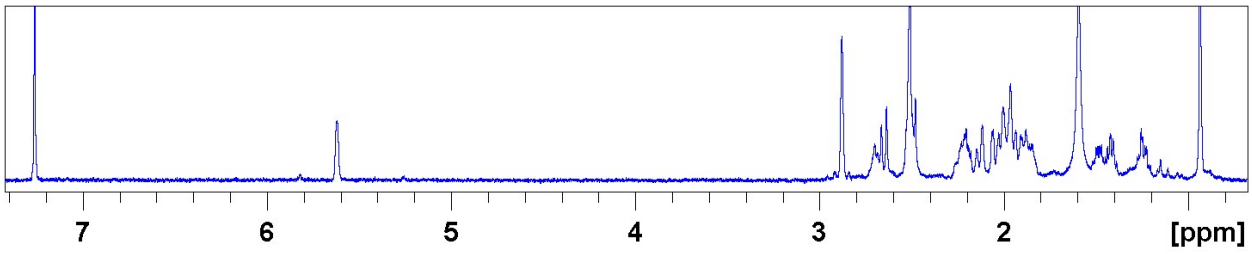

Figure S21: ^{1}H NMR spectrum of Diene A in $CDCl_3$ (600 MHz).

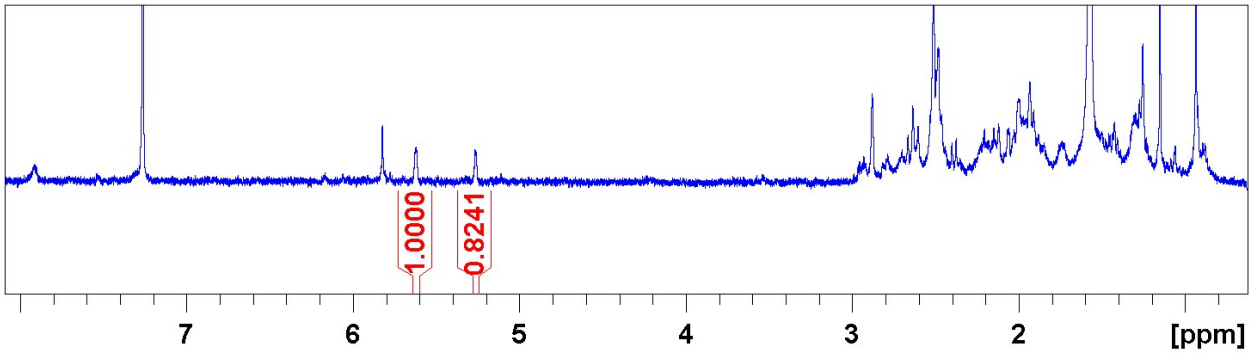

Figure S22: ^{1}H NMR spectrum of Diene A after 3 days in $CDCl_3$ (600 MHz). Signal at 5.26 ppm corresponds to H11 of 11β-hydroxydienogest.

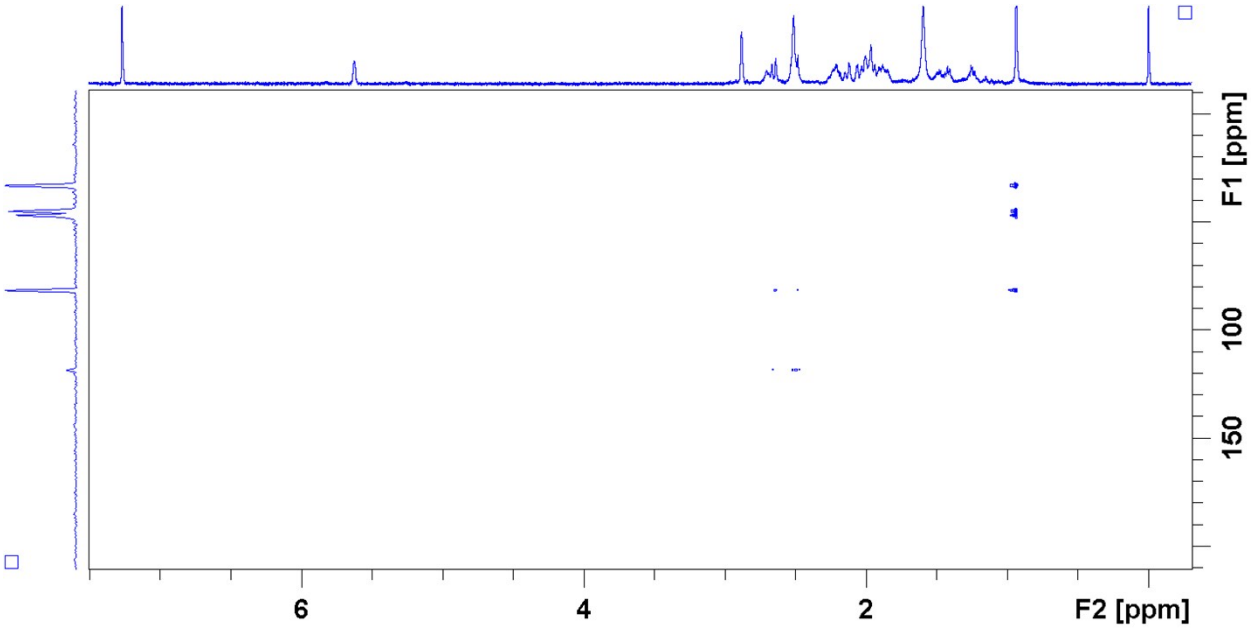

Figure S23: HMBC spectrum of Diene A in $CDCl_3$ (600 MHz).

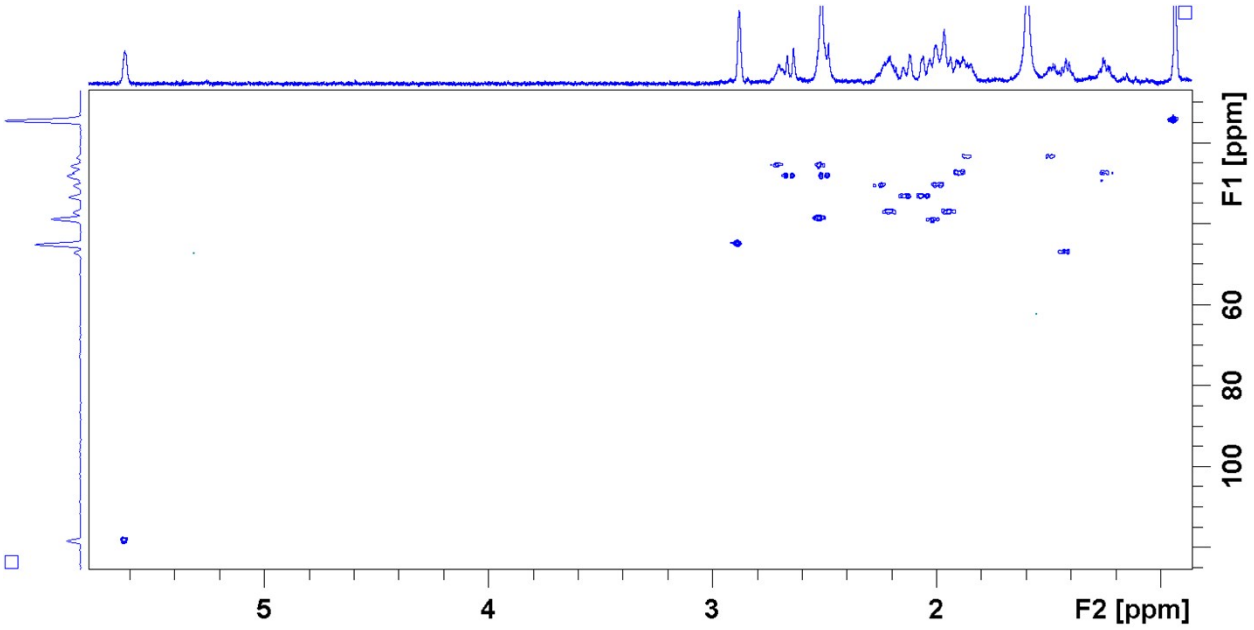

Figure S24: HSQC spectrum of Diene A in $CDCl_3$ (600 MHz).

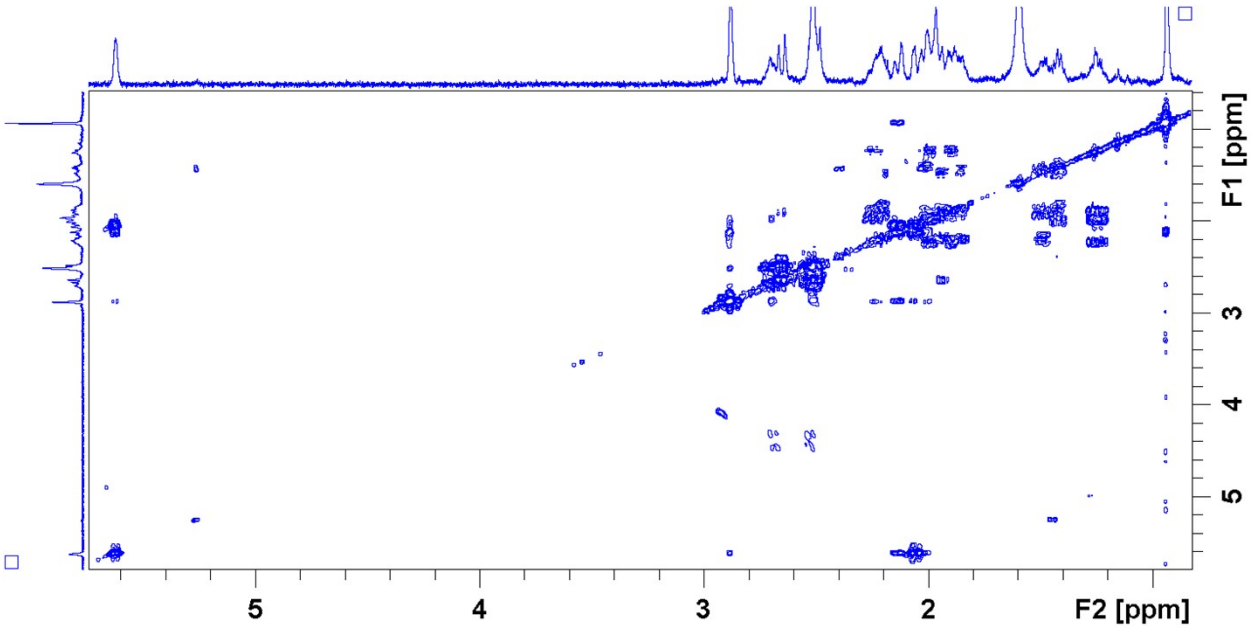

Figure S25: COSY spectrum of Diene A in $CDCl_3$ (600 MHz).

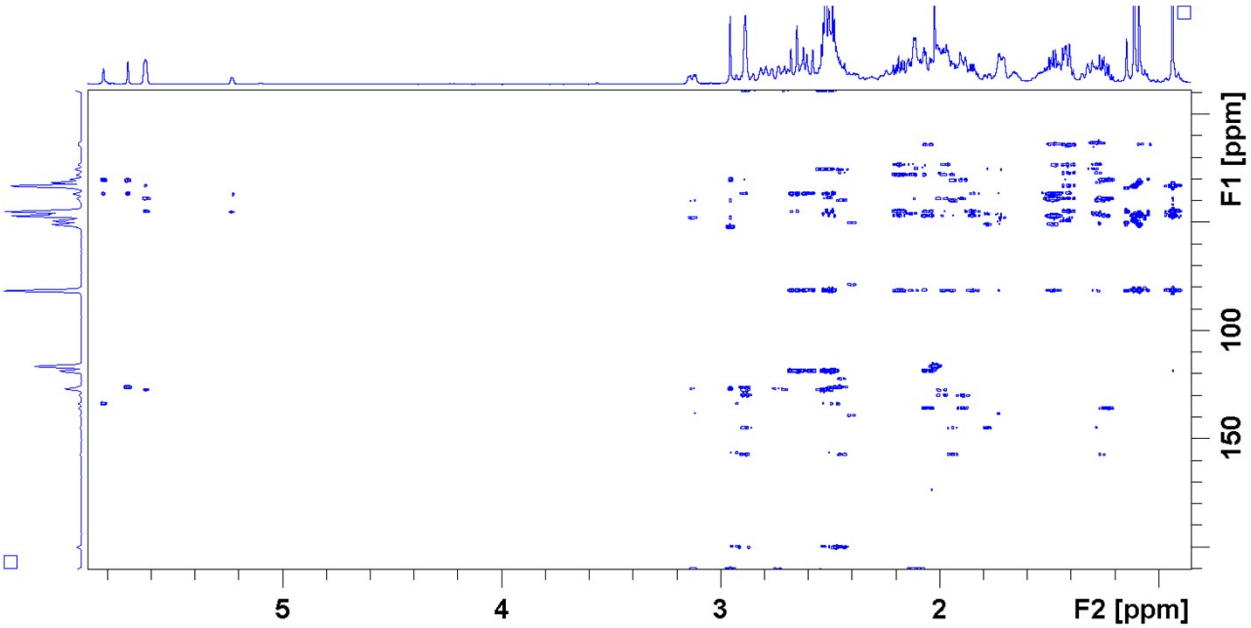

Figure S26: HMBC spectrum of impure sample of Diene A in $CDCl_3$ (600 MHz).

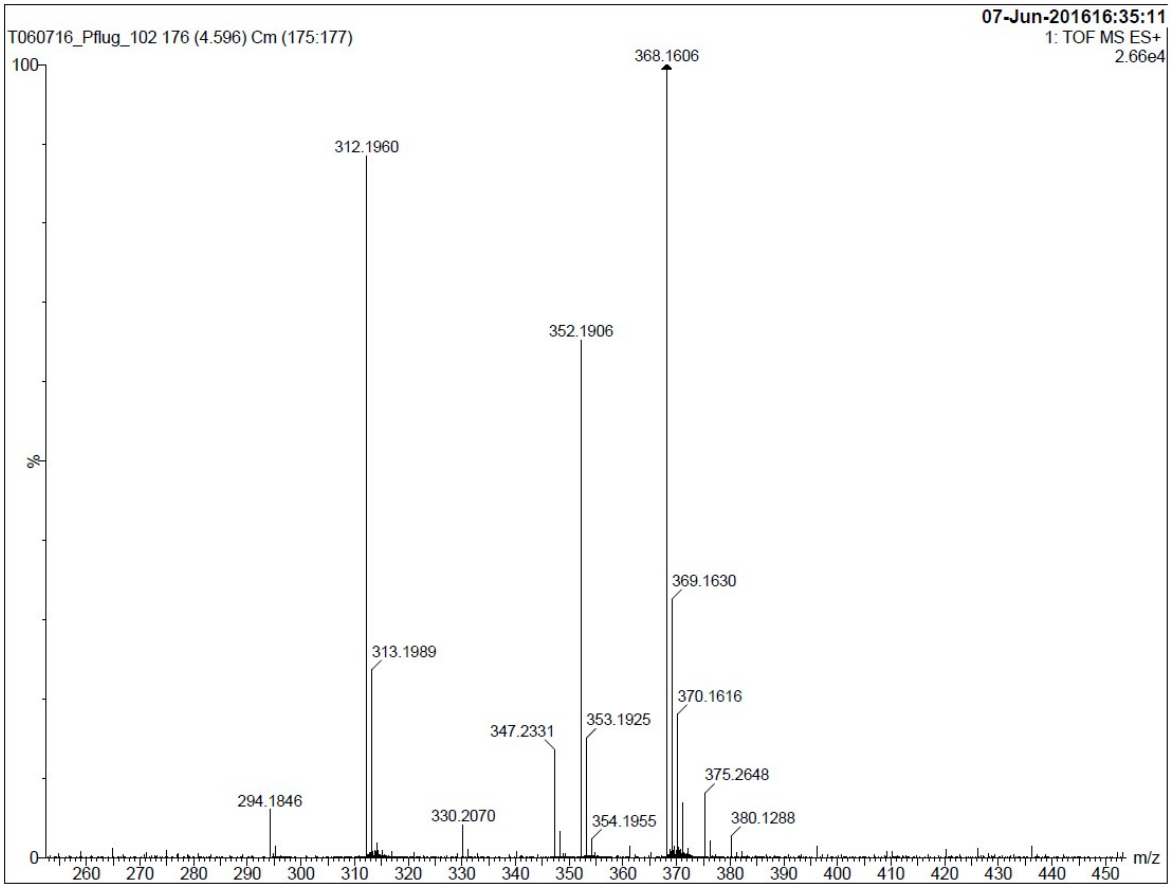

Figure S27: HR-ESI-TOFMS of Photohydrate B.

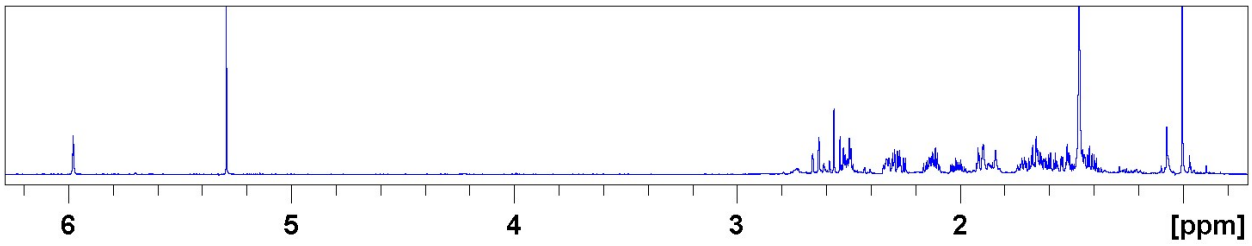

Figure S28: ^{1}H NMR spectrum of Photohydrate B in $CDCl_3$ (600 MHz). Signal at 5.30 ppm is of residual dichloromethane in sample.

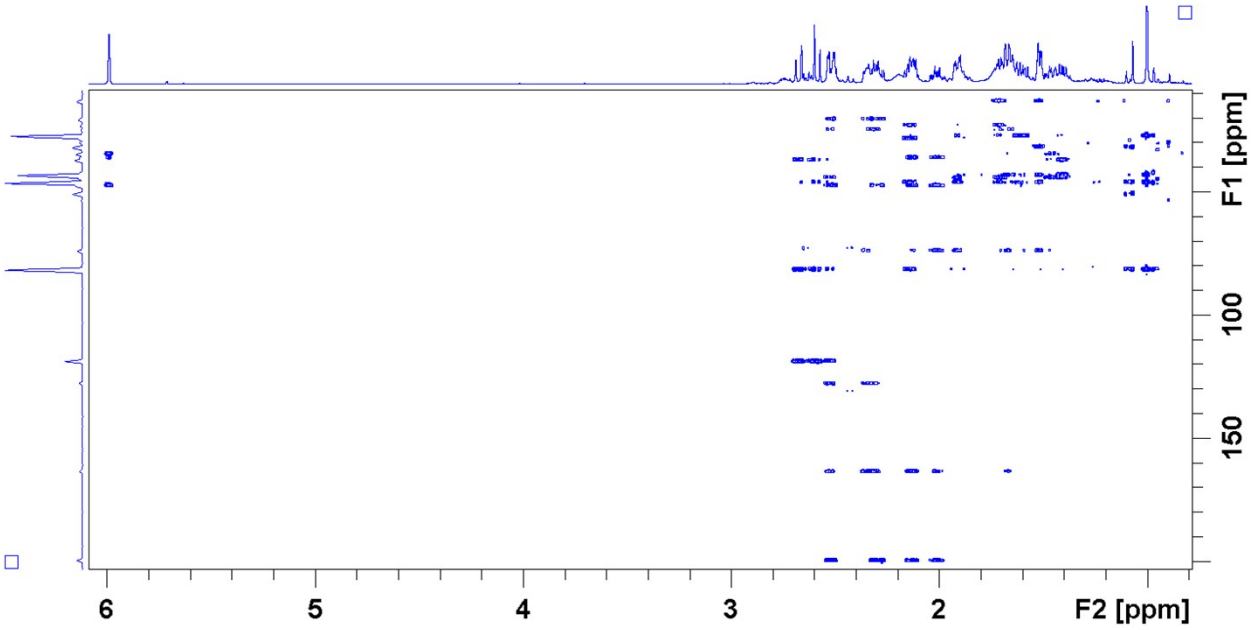

Figure S29: HMBC spectrum of Photohydrate B in $CDCl_3$ (600 MHz).

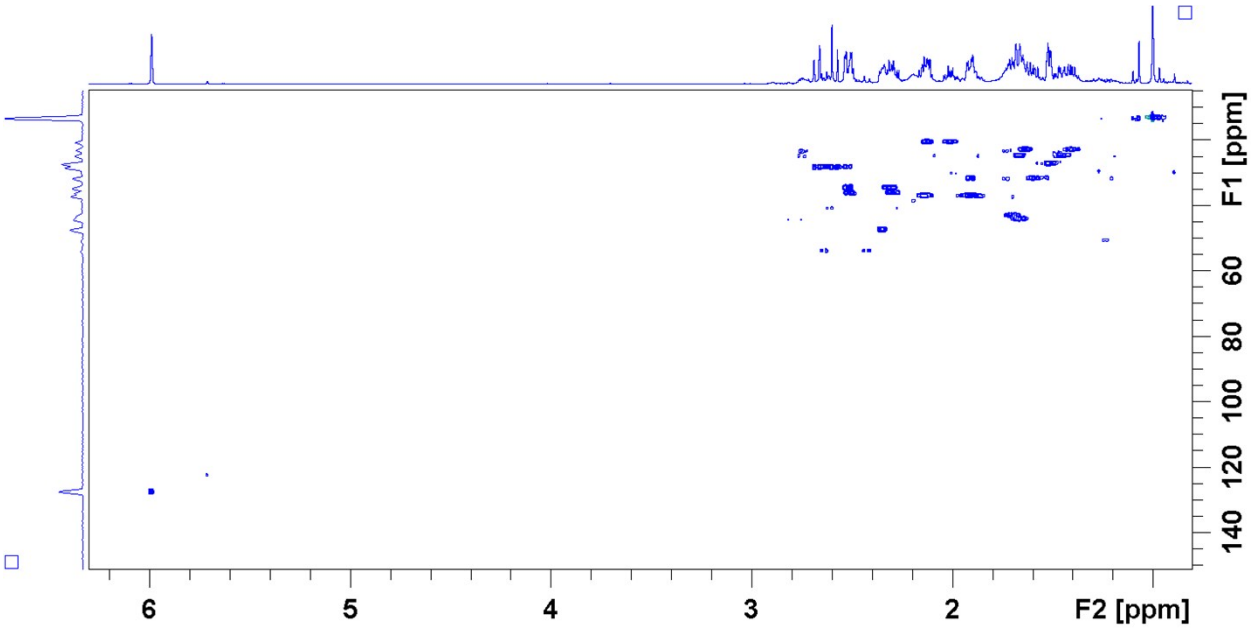

Figure S30: HSQC spectrum of Photohydrate B in $CDCl_3$ (600 MHz).

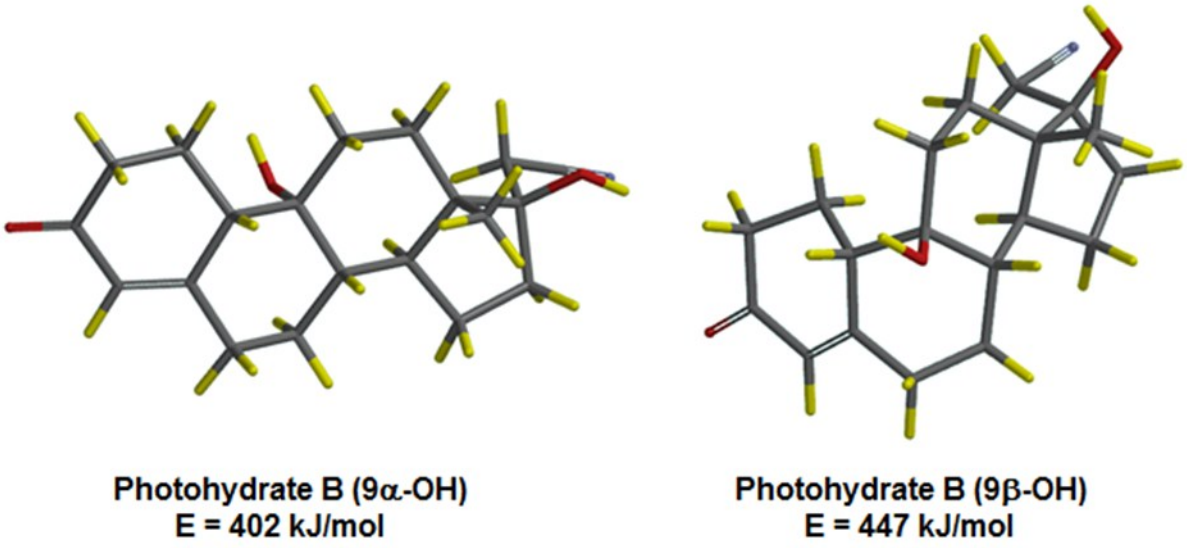

Figure S31: Energy minimized models of 9α-hydroxy versus 9β-hydroxy Photohydrate B, using MMFF molecular mechanics with Monte-Carlo conformational searching.

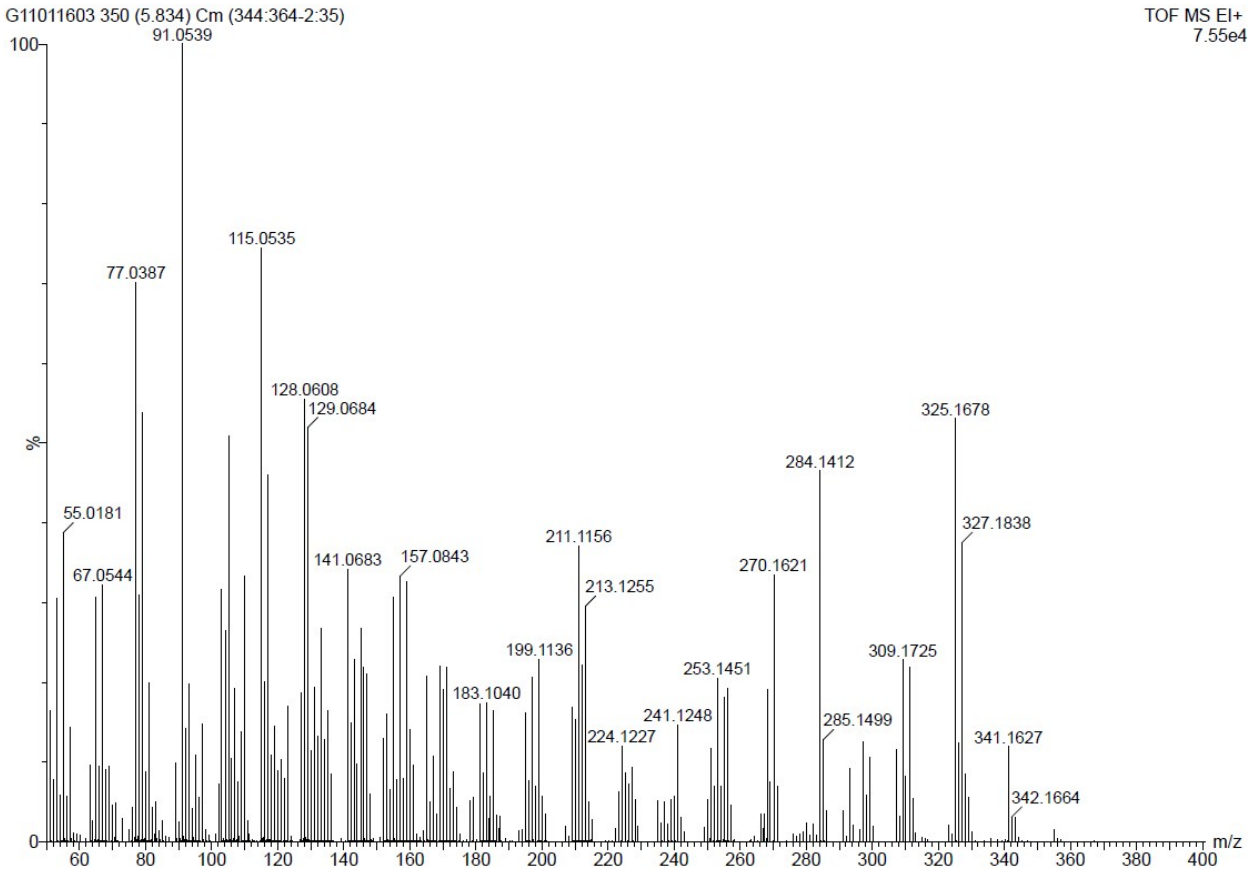

Figure S32: HR-EI-TOFMS of 11β-hydroxydienogest.

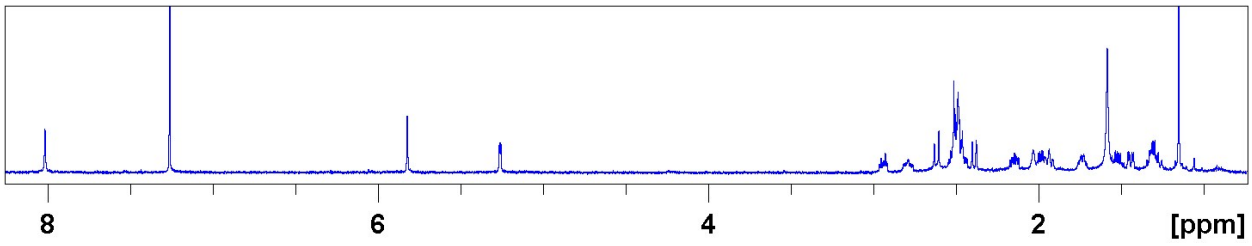

Figure S33: ^{1}H NMR spectrum of 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

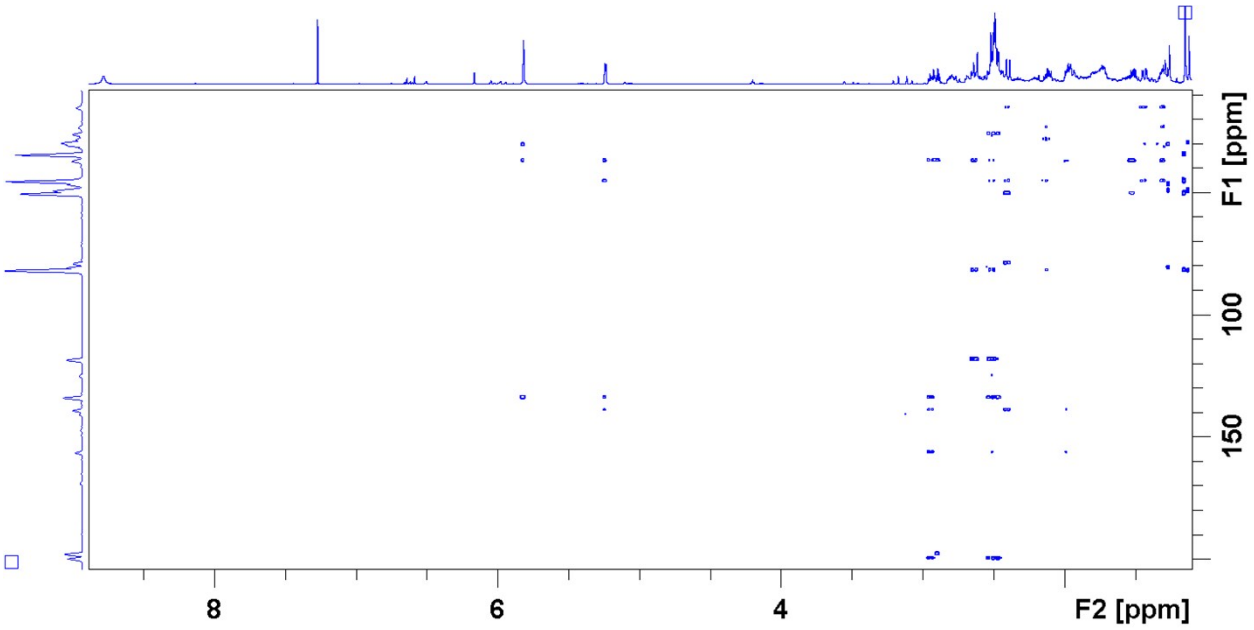

Figure S34: HMBC spectrum of 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

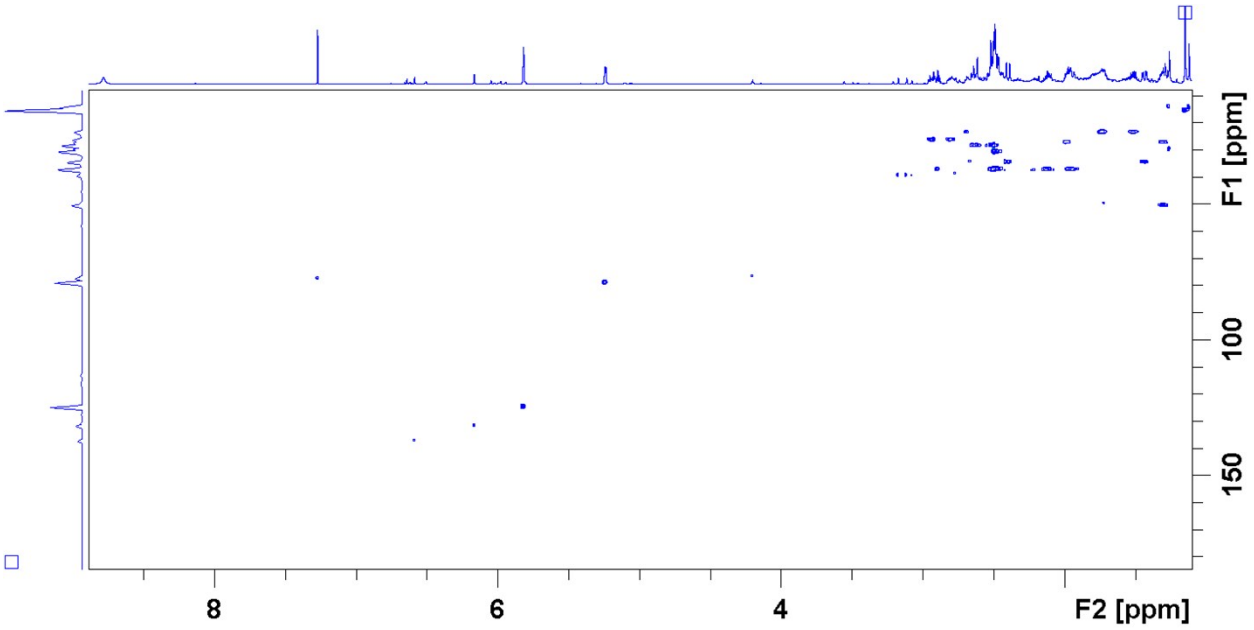

Figure S35: HSQC spectrum of 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

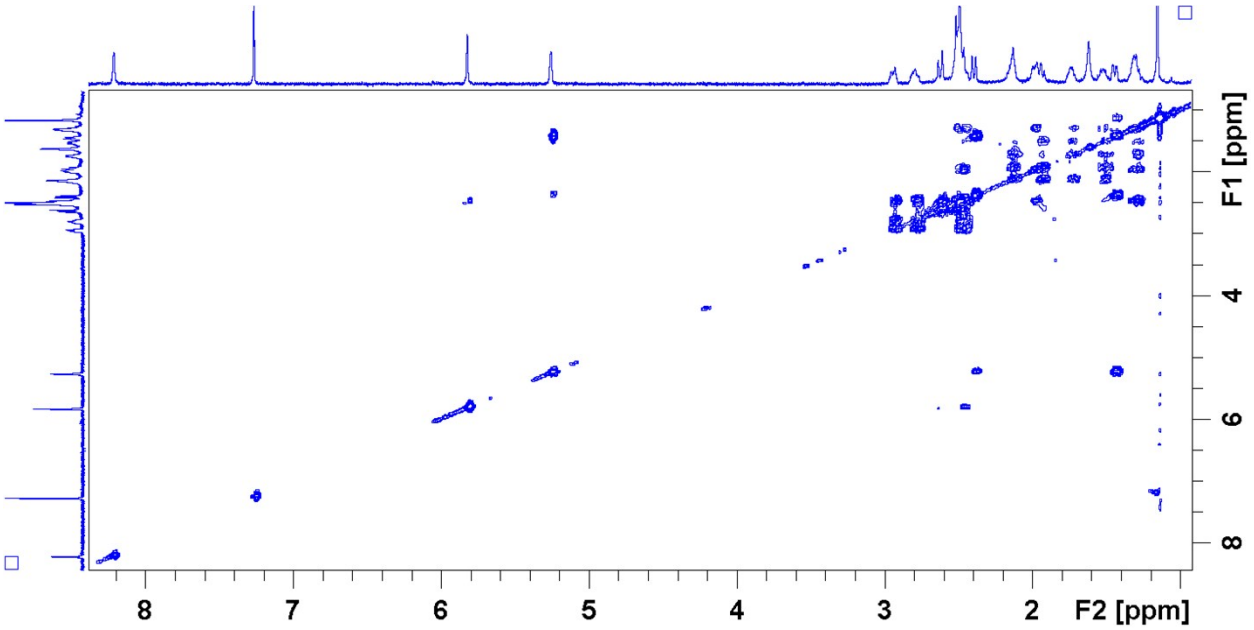

Figure S36: COSY spectrum of 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

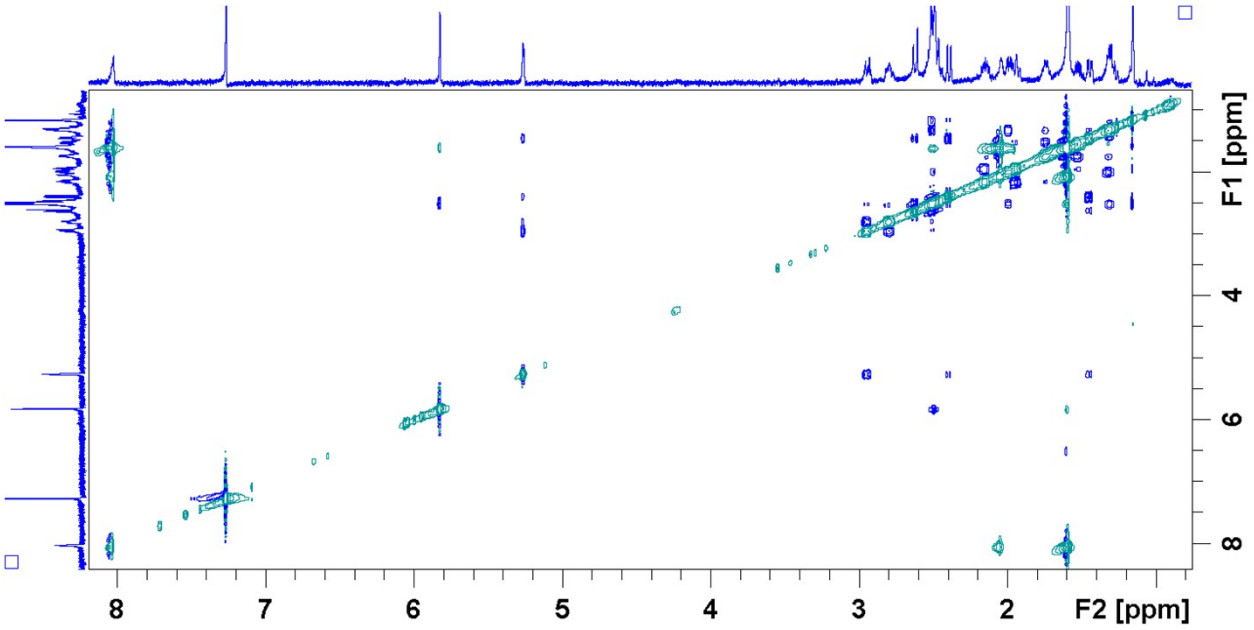

Figure S37: NOESY spectrum of 11β-hydroxydienogest in $CDCl_3$ (600 MHz).

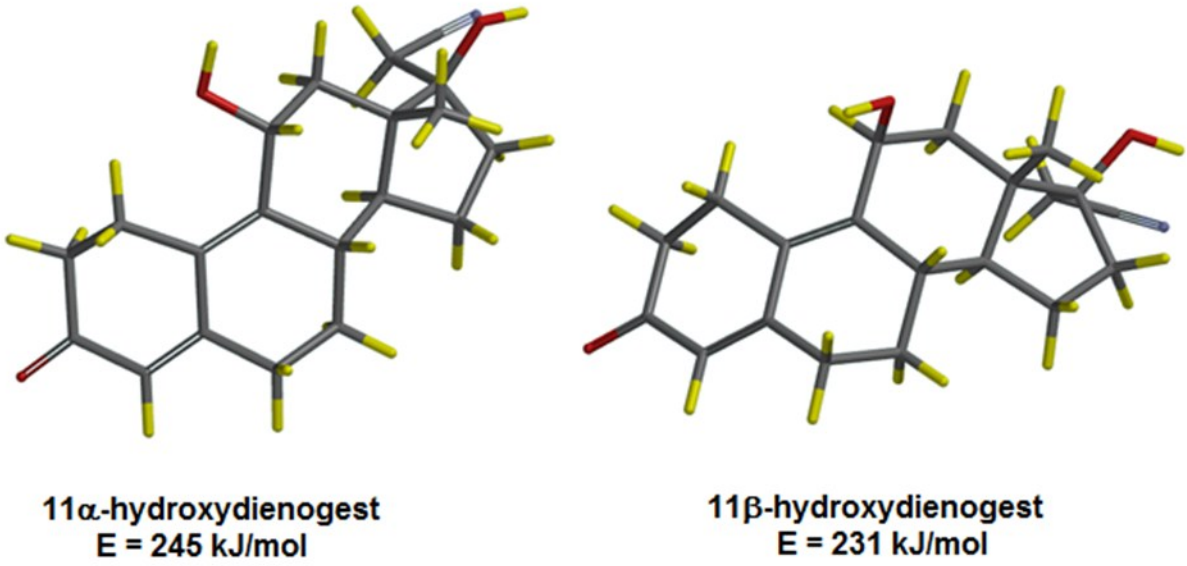

Figure S38: Energy minimized models of 11α-hydroxy versus 11β-hydroxydienogest, using MMFF molecular mechanics with Monte-Carlo conformational searching.

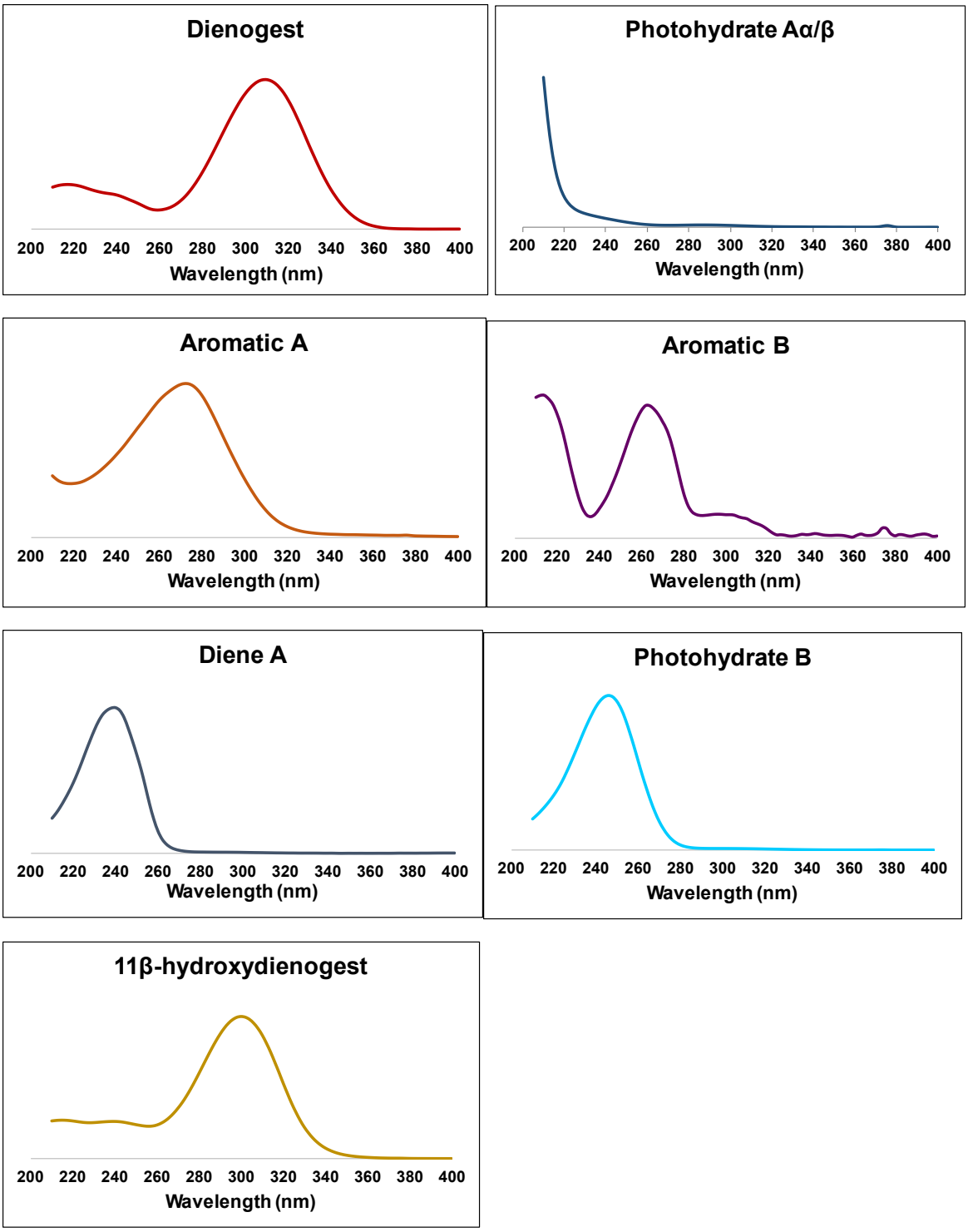

Figure S39: UV-VIS spectra of DNG and all identified primary and secondary products

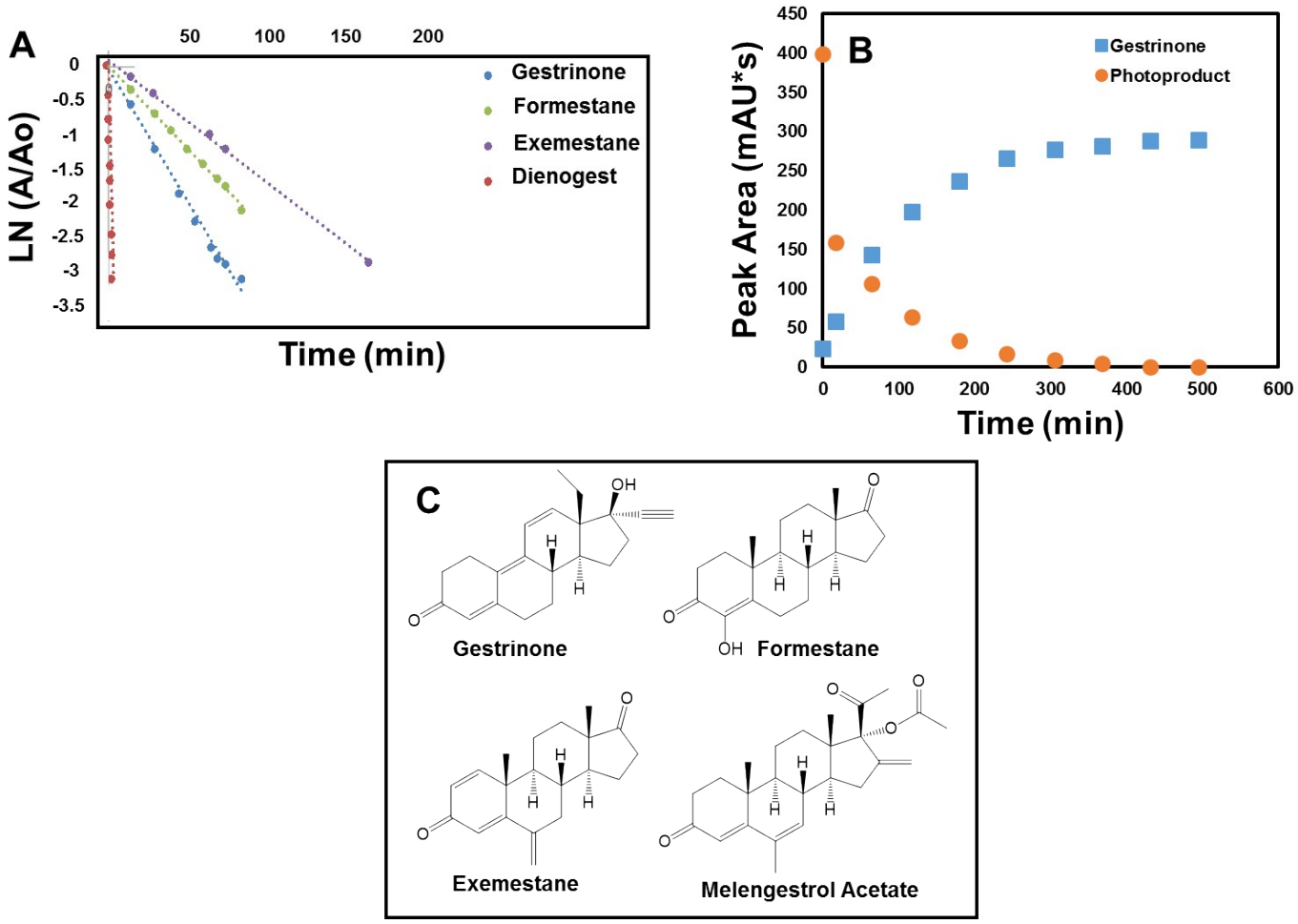

Figure S40: (A) Direct photolysis of various enones at pH 7, (B) Dark (thermal) stability of gestrinone pH 7 photoproduct mixture, and (C) structures of some conjugated enones examined for parent regeneration after photolysis.