Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Total synthesis of the proposed structures of the natural DNA methyl transferase (DNMT) inhibitors peyssonenynes, and structural revision of peyssonenyne B

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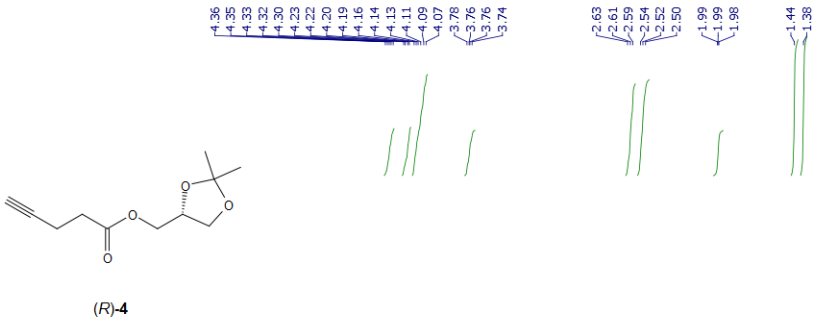
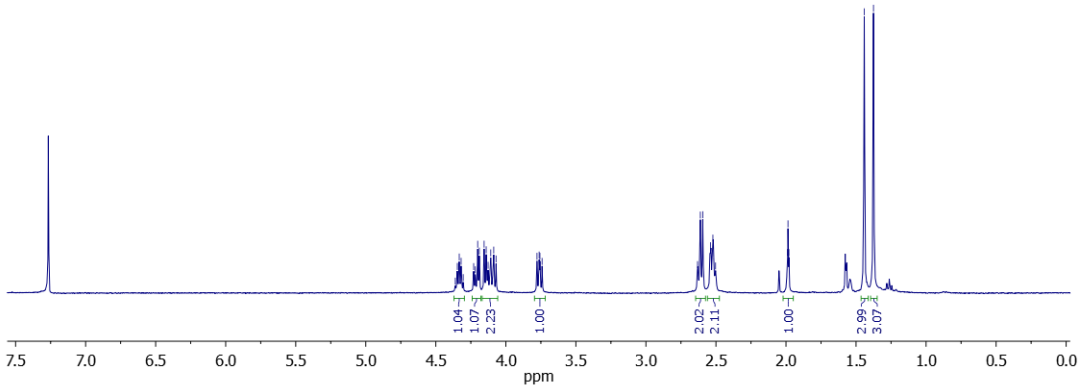
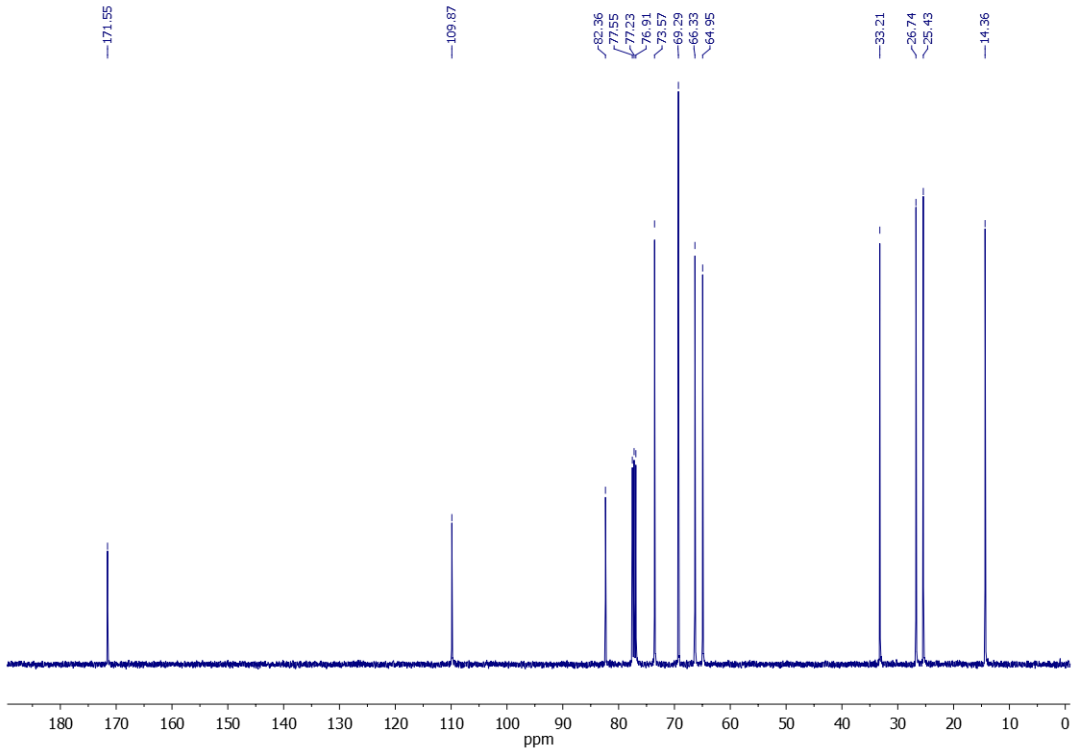
NMR Spectra

(2'*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl Pent-4-ynoate ((*R*)-4)

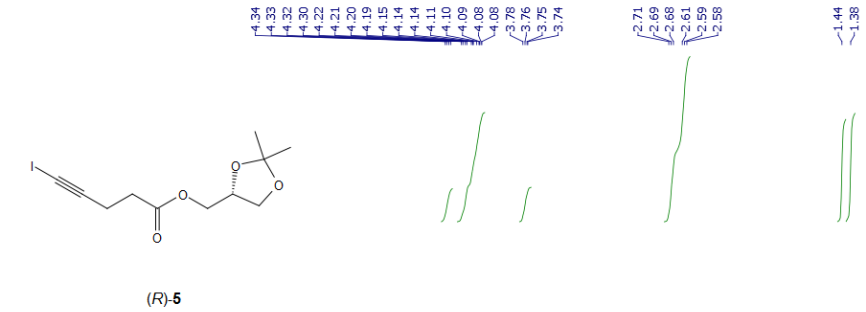


(*R*)-**4**





(2'*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 5-Iodopent-4-ynoate ((*R*)-5)



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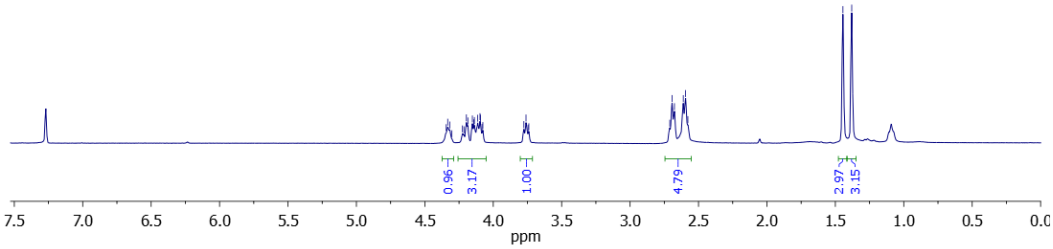
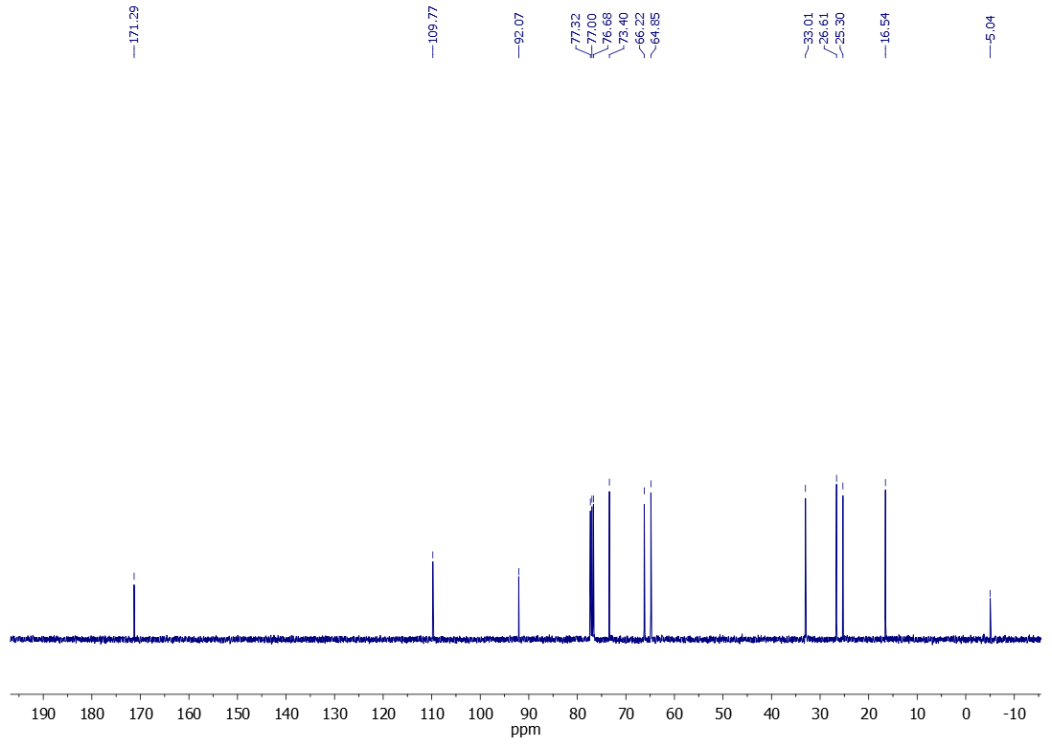
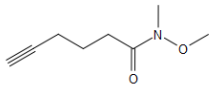




N-Methoxy-*N*-methylhex-5-ynamide (7)



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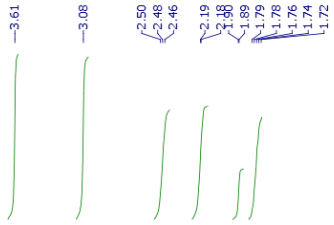
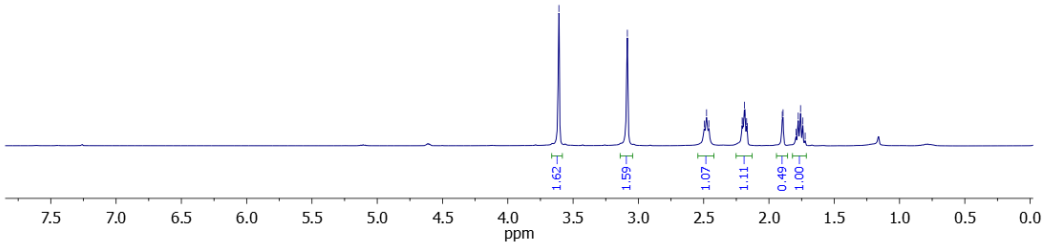
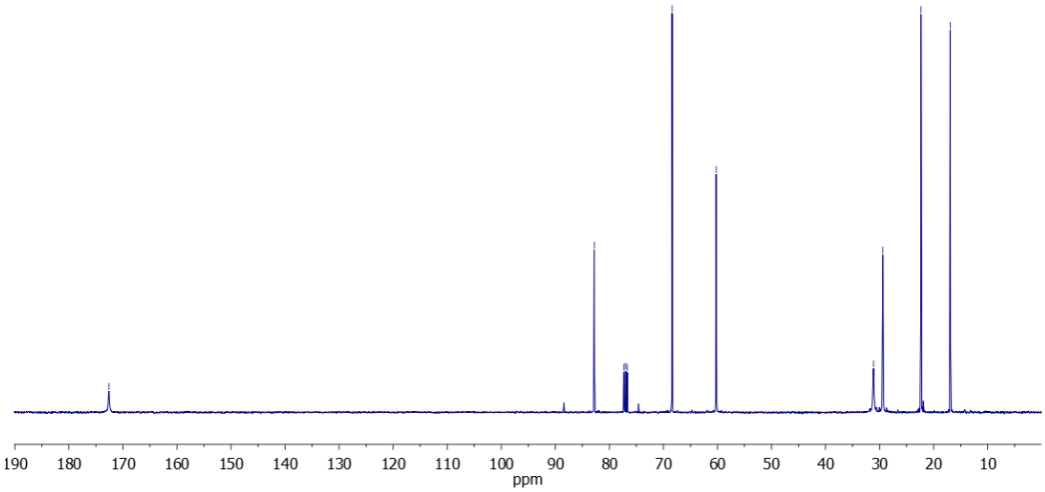
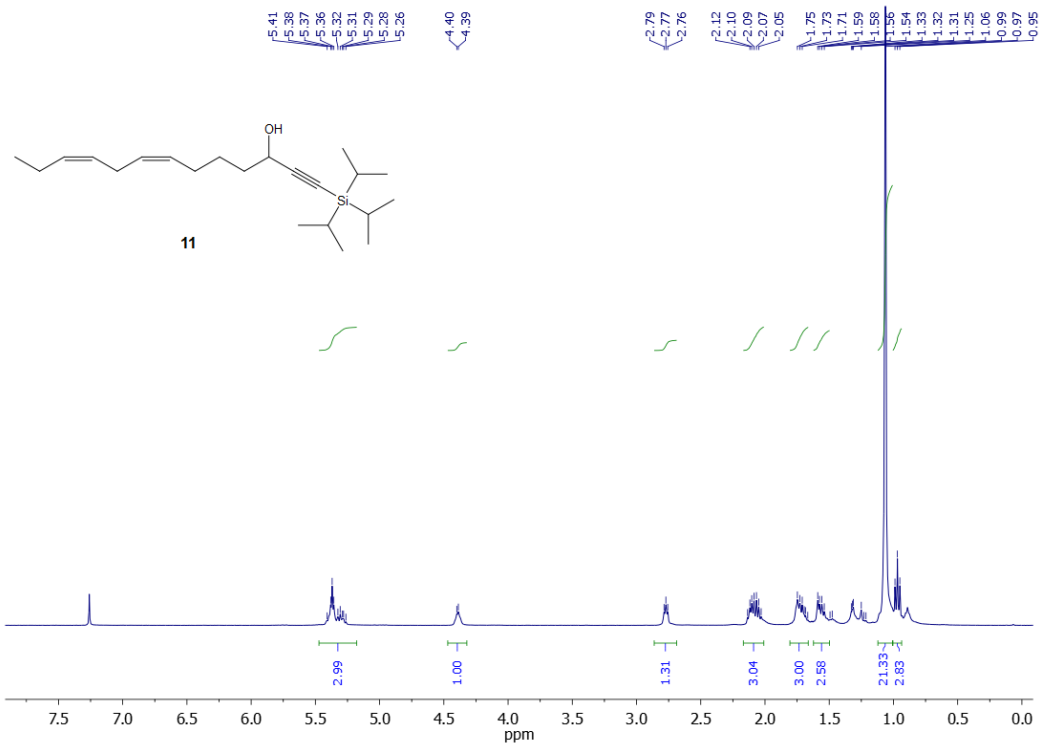
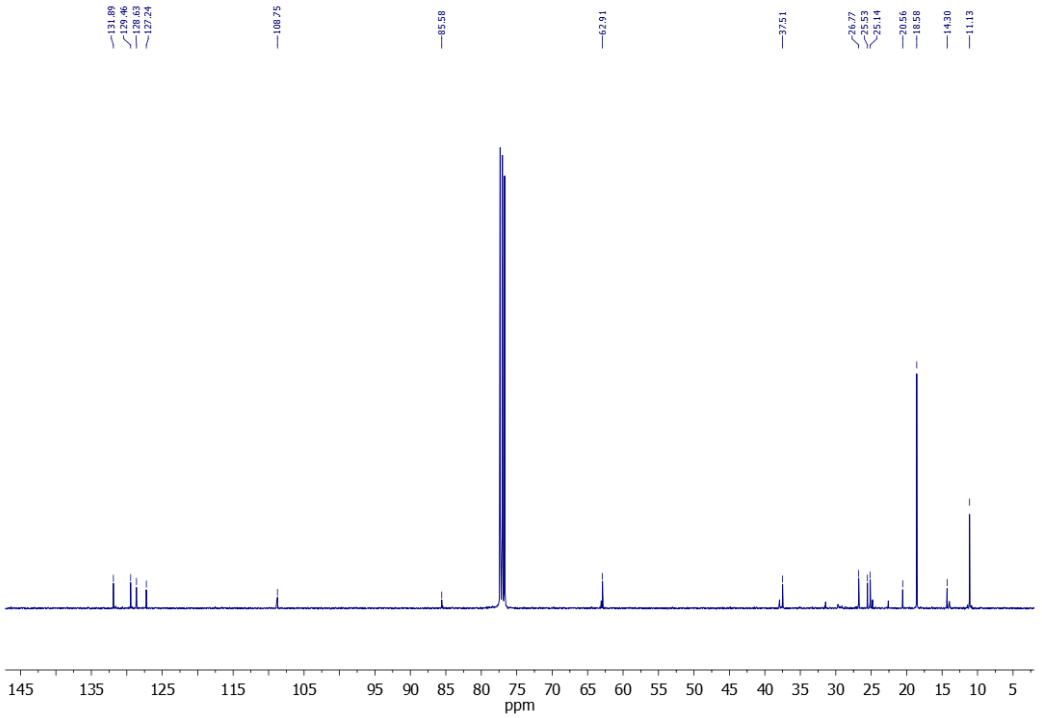




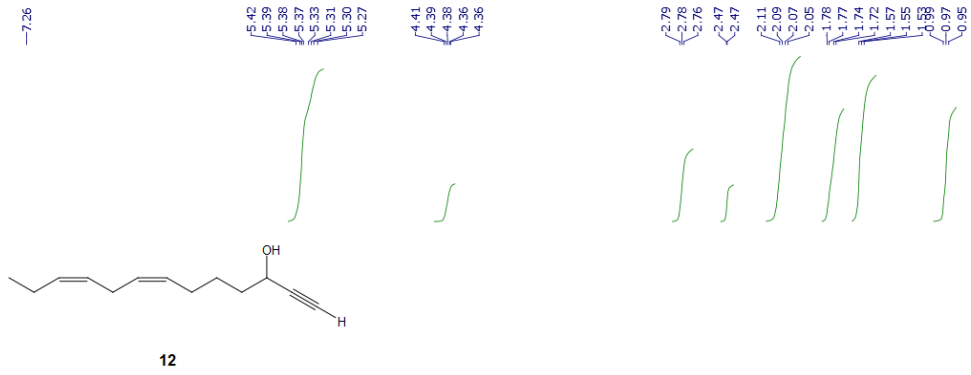


(7*Z*,10*Z*)-1-(Triisopropylsilyl)trideca-7,10-dien-1-yn-3-ol (11)





(7*Z*,10*Z*)-Trideca-7,10-dien-1-yn-3-ol (12)



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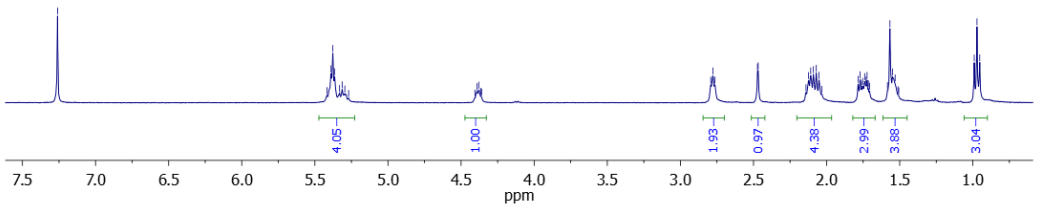
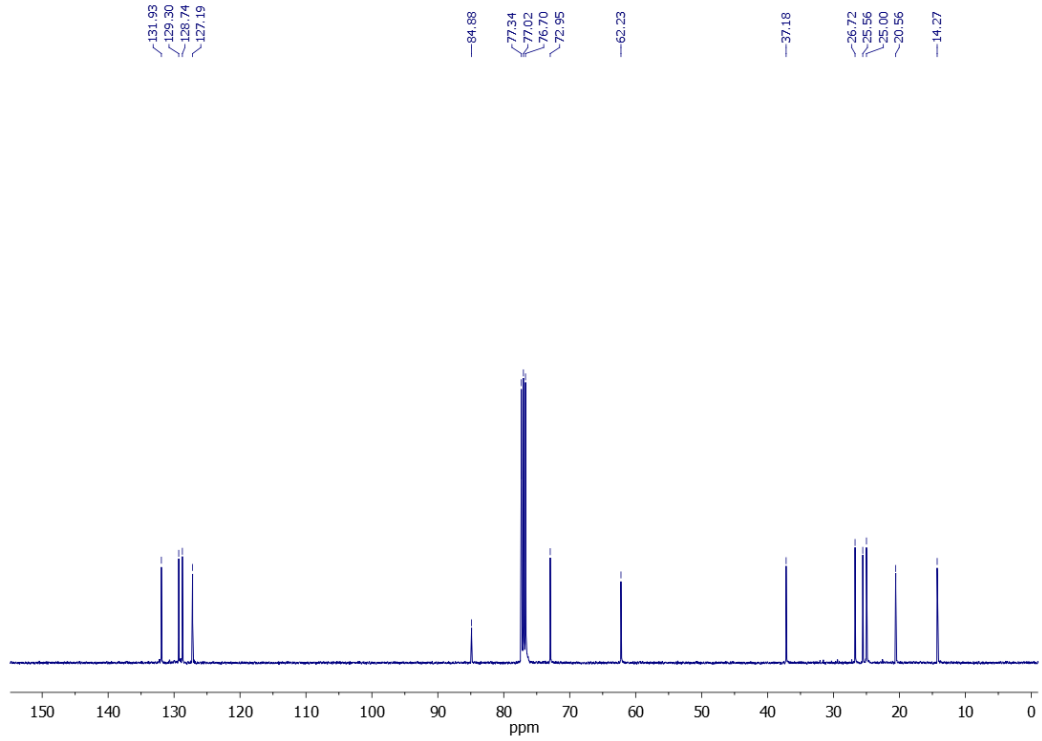
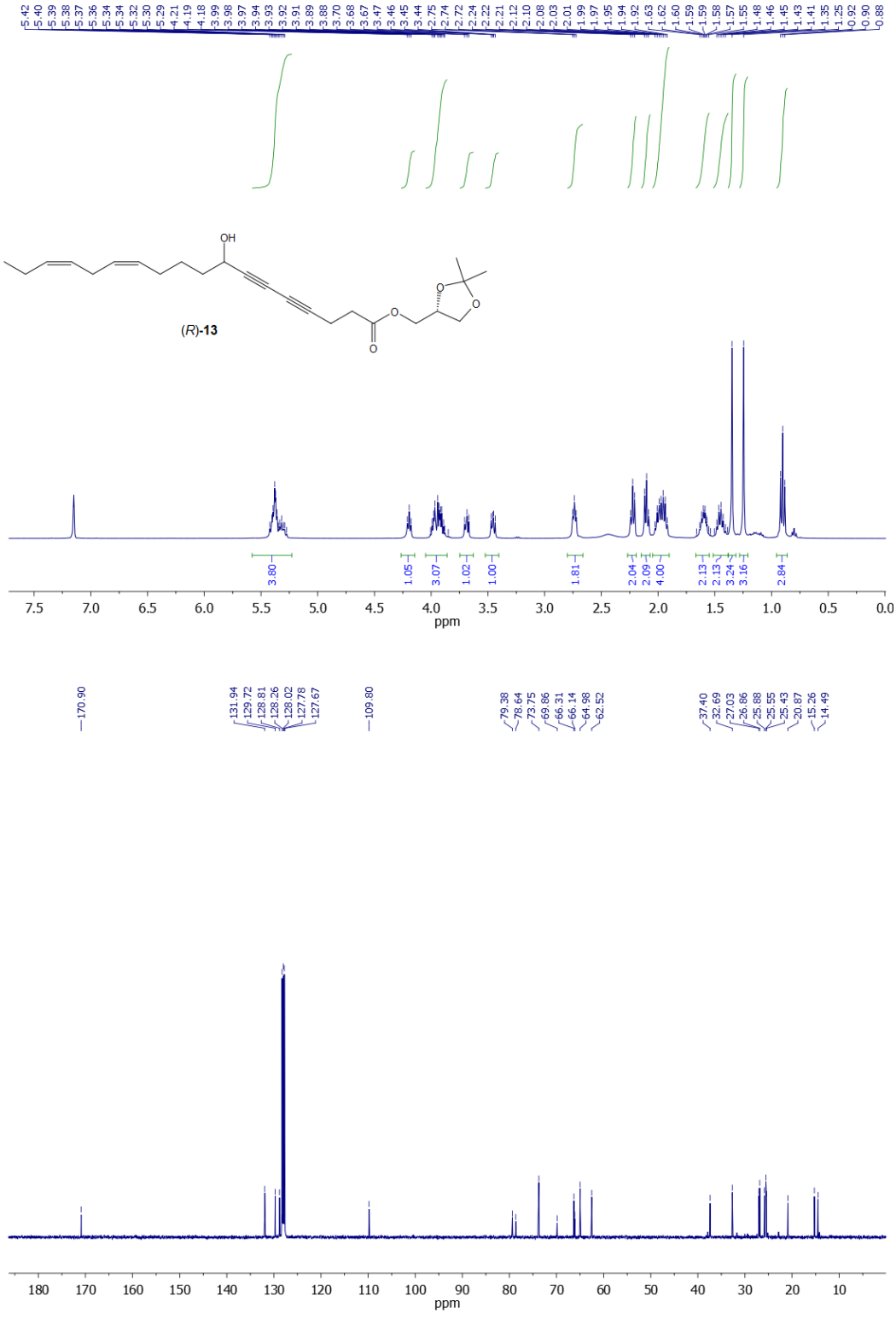
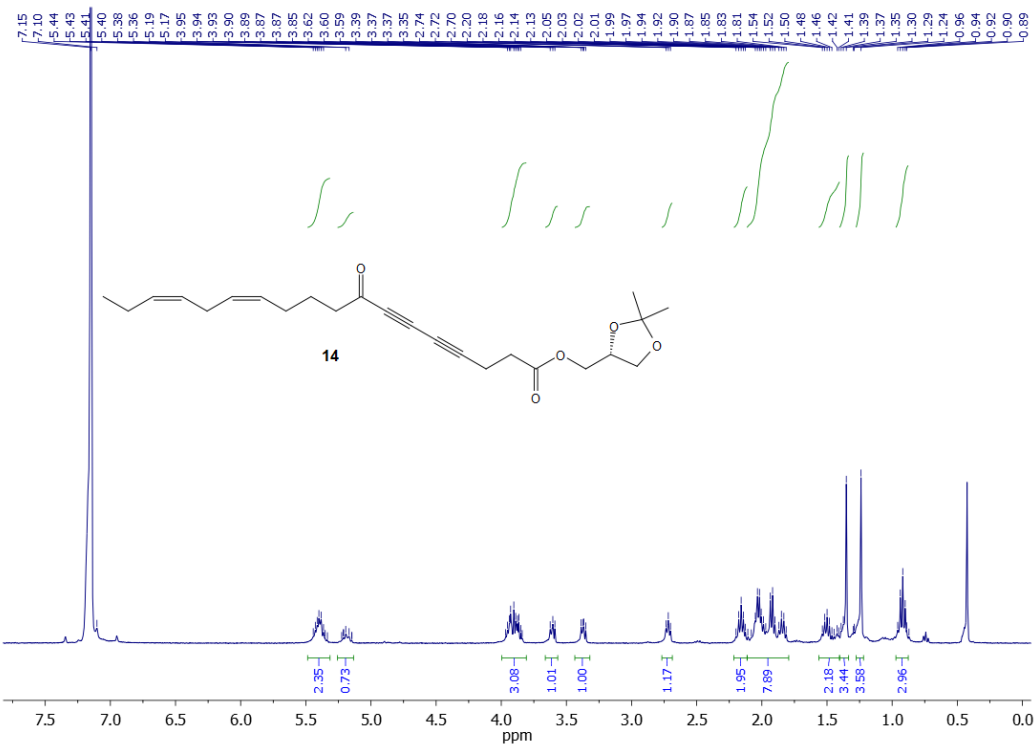


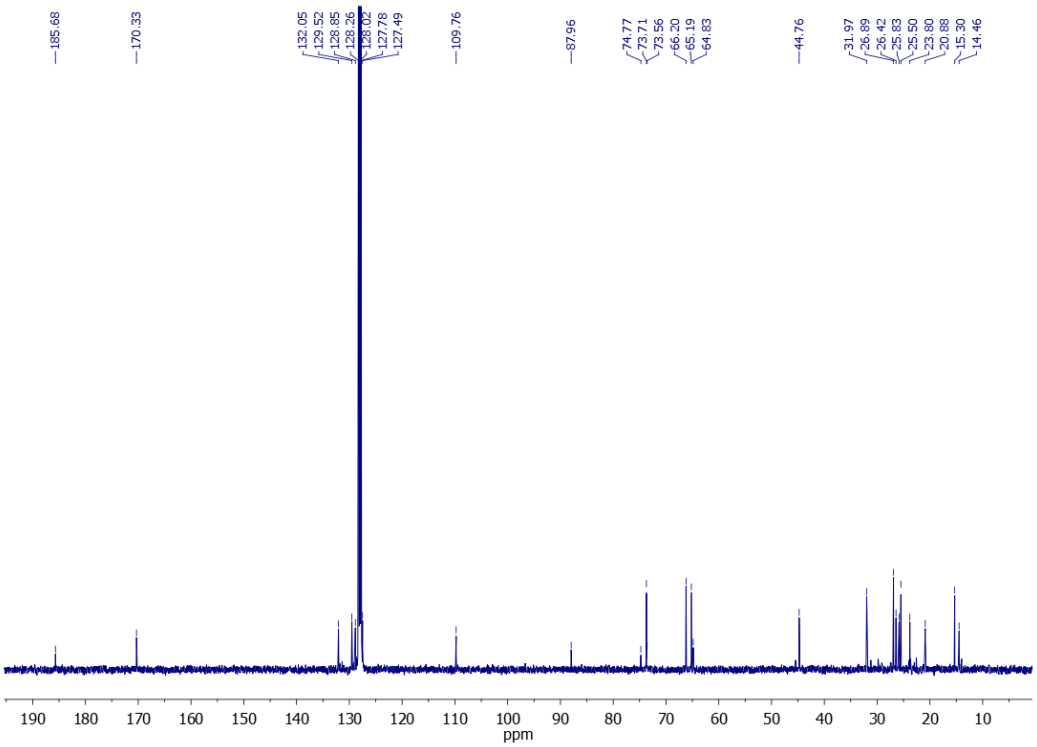


(12*Z*,15*Z*)-(2'*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-Hydroxyoctadeca-12,15-dien-4,6-diynoate ((*R*)-13)

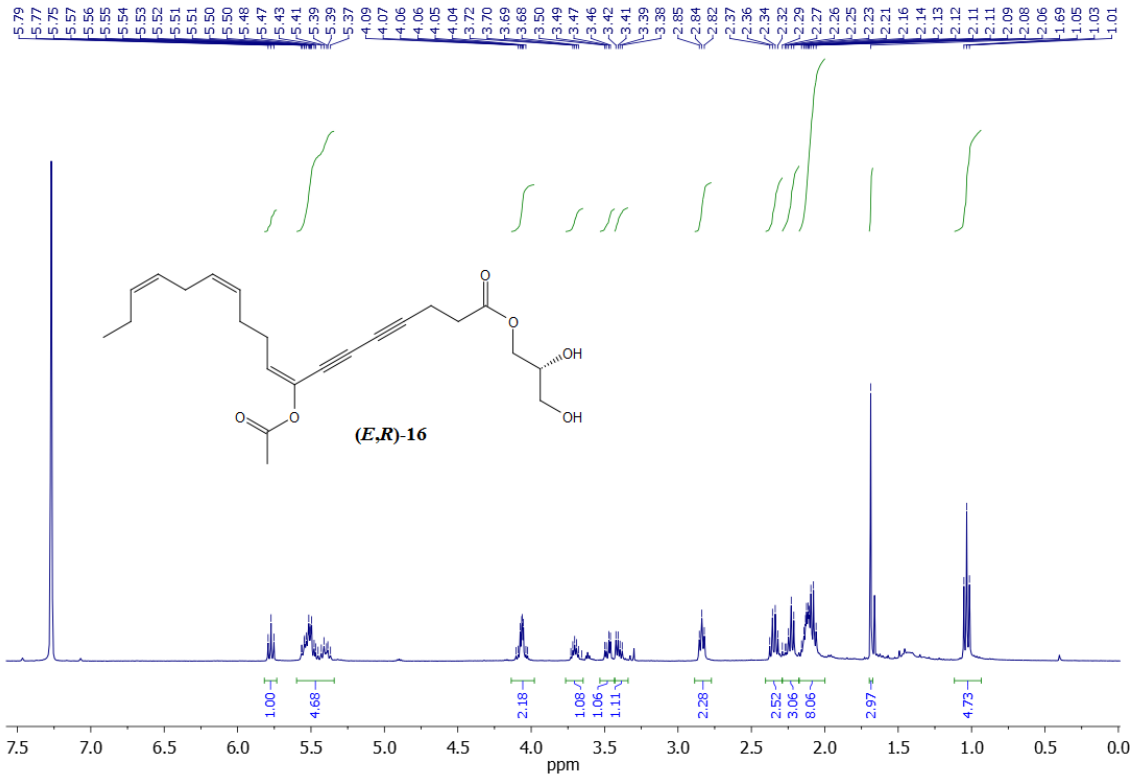


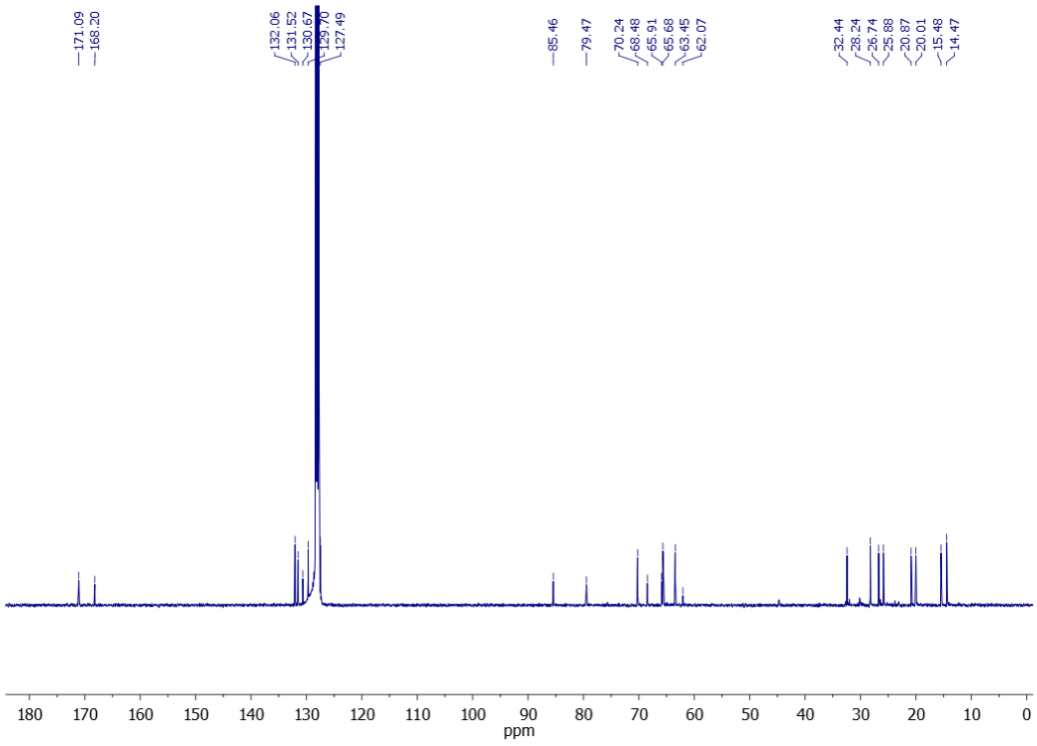
(12*Z*,15*Z*)-(2'*R*)-2,3-*O*-Isopropylidene-2,3-dihydroxy-1-prop-1-yl 8-Oxooctadeca-12,15-dien-4,6-diynoate ((*R*)-14)



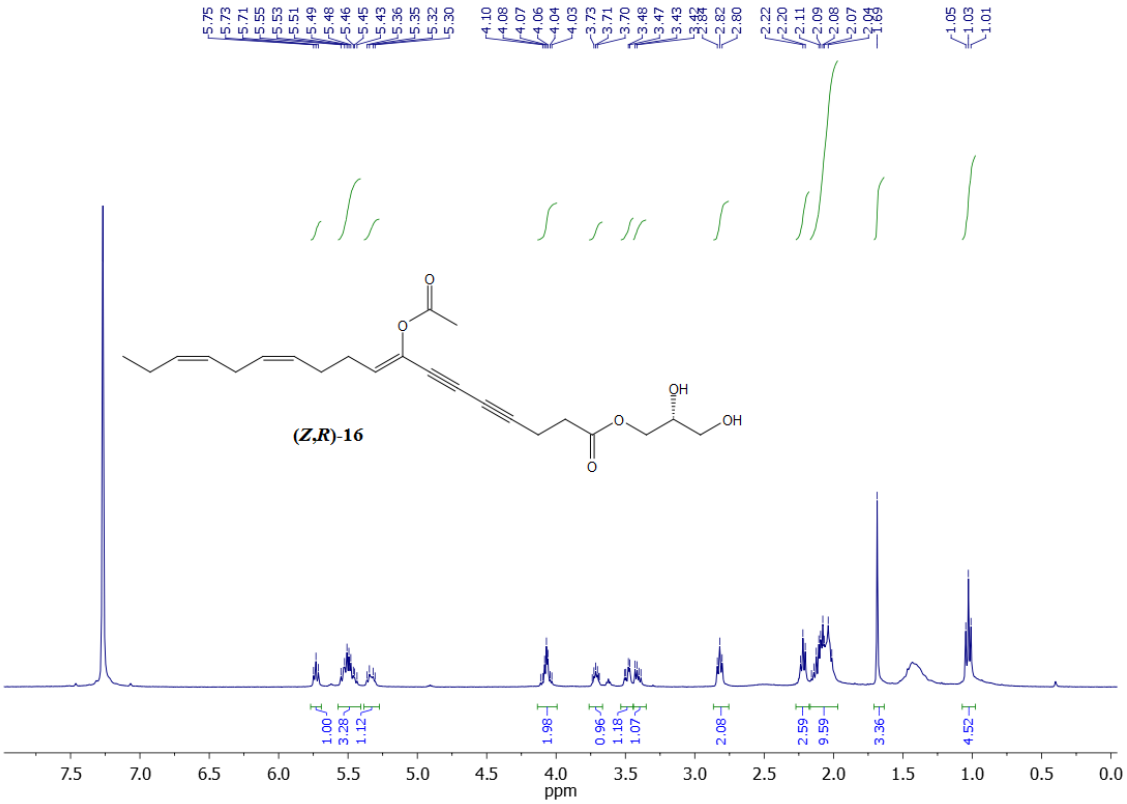


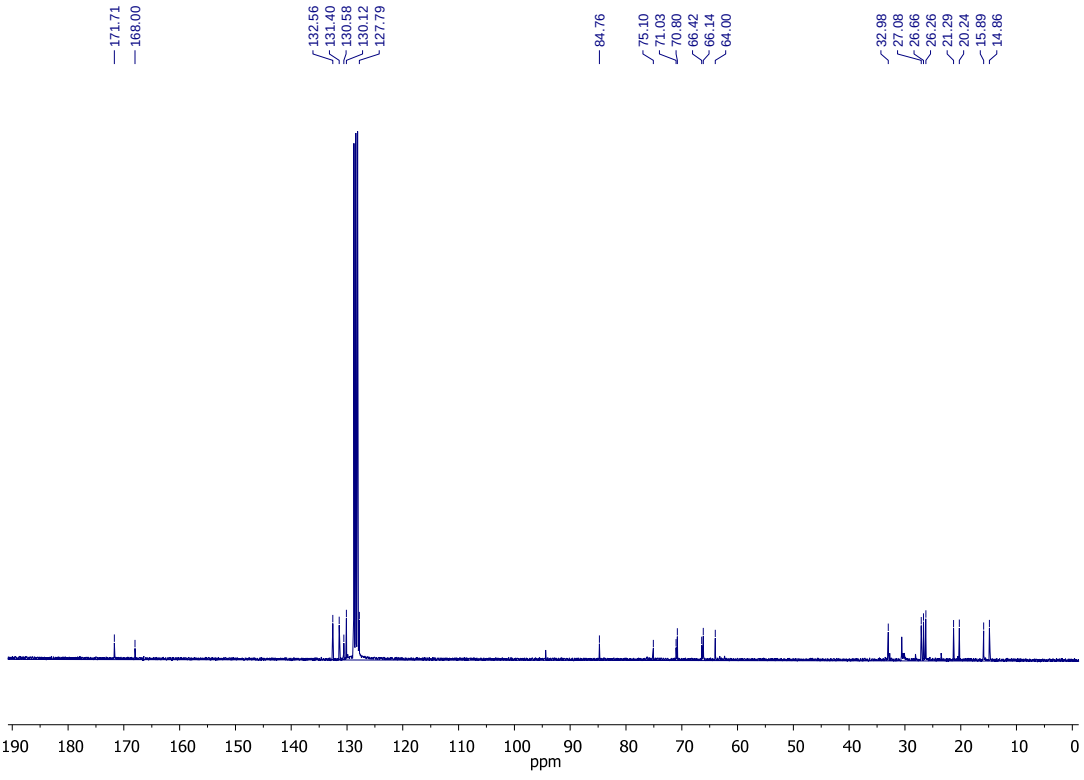
(8*E*,12*Z*,15*Z*)-(2'*R*)-2,3-Dihydroxyprop-1-yl 8-Acetoxyoctadeca-8,12,15-trien-4,6-diynoate ((*E*,*R*)-16).



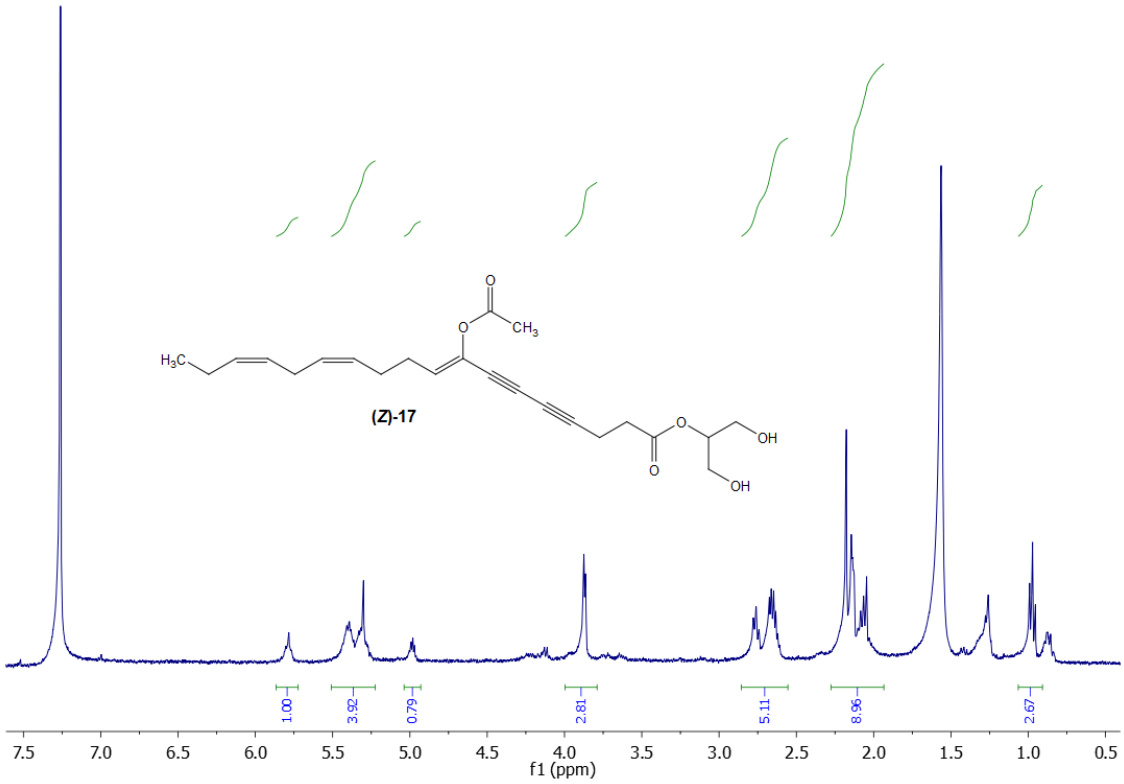


(8*Z*,12*Z*,15*Z*)-(2'*R*)-2,3-Dihydroxyprop-1-yl 8-Acetoxyoctadeca-8,12,15-trien-4,6-diynoate ((*Z*,*R*)-16).

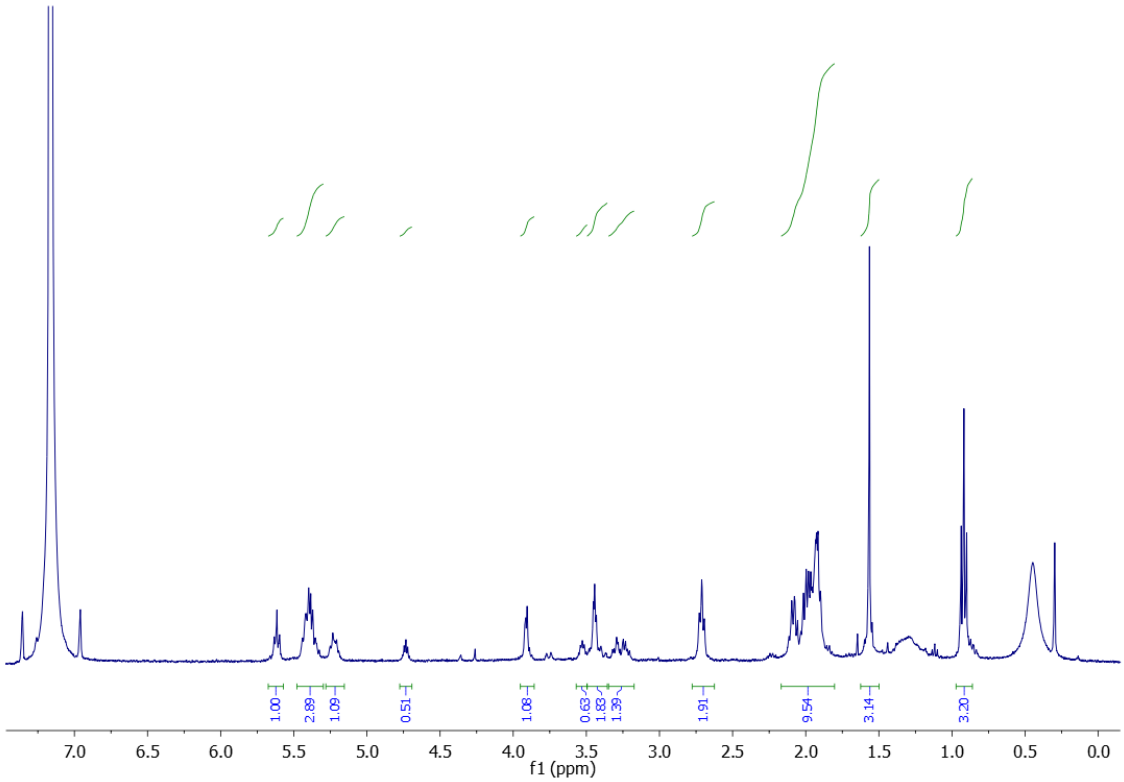


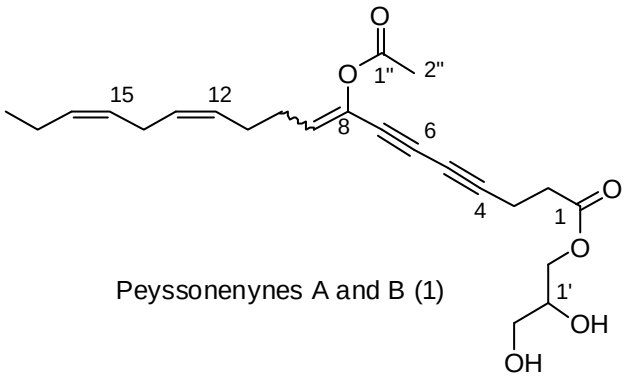


(*R*)-17 ^{1}H-RMN in $CDCl_3$



(*R*)-17 ^{1}H-RMN in C_6D_6





Peyssonenynes A and B (1)

Table 1. ^{13}C and ^{1}H NMR Data for Peyssonenyne A (**1**, $CDCl_3$), Peyssonenyne B (**2**, $CDCl_3$) at 400 MHz, respectively

	Natural source				**Synthetic compounds**			
	Peyssonenyne A (1)		**Peyssonenyne B (2)**		**Peyssonenyne *E***		**Peyssonenyne *Z***	
Atom no.	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)
1	171.8 C		171.8 C		171.8 C		171. 8 C	
2	33.0 CH_2	2.61 (ddd, 14 .4, 2.0, 1.0)	32.8 CH_2	2.62 (m)	32.8 CH_2	2.72 – 2.54 (m)	32.8 CH_2	2.70 – 2.56 (m)
		2.63 (ddd, 14 .4, 9.1, 1.4)				2.72 – 2.54 (m)		2.70 – 2.56 (m)
3	15.8 CH_2	2.65 (ddd, 14 .4, 2.0, 1.4)	15.6 CH_2	2.66 (m)	15.8 CH_2	2.72 – 2.54 (m)	15.7 CH_2	2.70 – 2.56 (m)
		2.66 (ddd, 14 .4, 9.1, 1.0)				2.72 – 2.54 (m)		2.70 – 2.56 (m)
4	83.5 C		83.3 C		84.6 C		83.4 C	
5	65.81 C		65.7 C		65.6 C		65.6 C	
6	74.1 C		73.6 C		78.9 C		74.0 C	
7	69.8 C		70.0 C		67.5 C		70.0 C	
8	129.5 C		128.9 C		129.9 C		129.4 C	
9	131.1 CH	5.78 (t, 9.4)	131.1 CH	5.78 (m)	131.6 CH	5.81 (t, 7.9)	131.1 CH	5.78 (t, 7.4)
10	26.2 CH_2	2.13 (d, 10.5)	26.2 CH_2	2.13 (m)	26.6 CH_2	2.38 – 2.30 (m)	26.1 CH_2	2.16 – 2.11 (m)
11	26.6 CH_2	2.13 (d, 10.5)	26.2 CH_2	2.13 (m)	28.1 CH_2	2.25 – 2.16 (m)	26.5 CH_2	2.16 – 2.11 (m)
12	129.9 CH	5.40 (m)	130.5 CH	5.40 (m)	129.7 CH	5.46 – 5.23 (m)	129.7 CH	5.45 – 5.23 (m)

13	128.2 CH	5.31 (m)	128.0 CH	5.31 (m)	128.2 CH	5.46 – 5.23 (m)	128.1 CH	5.45 – 5.23 (m)
14	25.8 CH_2	2.75 (br t, 7.1)	b	2.75 (t, 7.4)	25.7 CH_2	2.77 (t, 6.6)	25.6 CH_2	2.75 (t, 7.1)
15	127.1 CH	5.29 (m)	127.2 CH	5.29 (m)	127.1 CH	5.46 – 5.23 (m)	127.0 CH	5.45 – 5.23 (m)
16	132.3 CH	5.38 (m)	132.2 CH	5.38 (m)	132.2 CH	5.46 – 5.23 (m)	132.2 CH	5.45 – 5.23 (m)
17	20.8 CH_2	2.06 (br p, 7. 6)	20.5 CH_2	2.06 (m)	20.7 CH_2	2.12 – 2.01 (m)	20.7 CH_2	2.10 – 2.00 (m)
18	14.5 CH_3	0.97 (t, 7.6)	14.1 CH_3	0.97 (t, 7.4)	14.4 CH_3	0.97 (t, 7.5)	14.4 CH_3	0.97 (t, 7.5)
1'	65.83 CH_2	4.19 (dd, 14. 4, 7.5)	*b*	4.19 (m)	65.8 CH_2	4.19 (dd, 11.6, 6.0)	65.7 CH_2	4.24 (dd, 11.6, 4.8)
		4.24 (dd, 14. 4, 5.8)				4.25 (dd, 11.6, 4.7)		4.18 (dd, 11.6, 5.9)
2'	70.3 CH	3.96 (br s)	*b*	3.96 (m)	70.2 CH	4.00 – 3.92 (m, 1H)	70.2 CH	3.99 – 3.91 (m)
3'	63.5 CH_2	3.63 (dd, 14. 1, 7.6)	*b*	3.63 (m)	63.4 CH_2	3.62 (dd, 11.3, 6.1)	63.4 CH_2	3.61 (dd, 11.4, 6.1)
		3.72 (dd, 14. 1, 4.5)		3.72 (m)		3.72 (dd, 11.3, 3.9)		3.72 (dd, 10.6, 3.0)
1"	168.4 C		168.1 C		169.1 C		168.4 C	
2"	20.8 CH_3	2.17 (s)	*b*	2.17 (s)	20.9 CH_3	2.14 (s)	20.6 CH_3	2.17 (s)

Table 2. ^{13}C and ^{1}H NMR Data for Peyssonenyne B (**2**, C_6D_6), Peyssonenyne *E* and *Z* (C_6D_6) at 400 MHz, respectively

	Natural source		**Synthetic**			
	Peyssonenyne B (2)		**Peyssonenyne *E***		**Peyssonenyne *Z***	
Atom no.	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)
1	170.9 C		171.1 C		171.7 C	
2	32.4 CH_2	1.90 (m)	32.4 CH_2	2.0-1.9 (m, 6H, $2H_2$ + $2H_{10}$ + $2H_{11}$)	33.0 CH_2	2.0-1.9 (m, 8H, $2H_2$ + $2H_{10}$ + $2H_{11}$ + $2H_{17}$)
3	15.4 CH_2	2.07 (m)	15.5 CH2	2.3-2.2 (m, 2H, $2H_3$)	15.9 CH_2	2.11 (t, *J* = 7.0 Hz, 2H, $2H_3$)
4	83.8 C		85.5 C		84.8 C	
5	65.7 C		65.9 C		66.4 C	
6	74.4 C		79.5 C		75.1 C	
7	70.5 C		68.5 C		71.0 C	
8	129.9 CH		130.7 C		130.6 C	
9	130.6 CH	5.62 (t, 6.9)	132.1 CH	5.65 (t, *J* = 8.1 Hz, 1H, H_9)	132.6 CH	5.62 (t, *J* = 7.3 Hz, 1H, H_9)
10	26.2 CH_2	1.99 (m)	26.7 CH_2	2.0-1.9 (m, 6H, $2H_2$ + $2H_{10}$ + $2H_{11}$)	26.7 CH_2	2.0-1.9 (m, 8H, $2H_2$ + $2H_{10}$ + $2H_{11}$ + $2H_{17}$)
11	26.4 CH_2	1.92 (m)	28.2 CH_2	2.0-1.9 (m, 6H, $2H_2$ + $2H_{10}$ + $2H_{11}$)	27.1 CH_2	2.0-1.9 (m, 8H, $2H_2$ + $2H_{10}$ + $2H_{11}$ + $2H_{17}$)
12	128.1 CH	5.23 (m)	128.4 CH	5.5-5.2 (m, 4H, H_{12} + H_{13} + H_{15} + H_{16})	131.4 CH	5.3-5.2 (m, 1H)
13	129.9 CH	5.42 (m)	129.7 CH	5.5-5.2 (m, 4H, H_{12} + H_{13} + H_{15} + H_{16})	128.8 CH	5.4-5.3 (m, 3H)
14	25.4 CH_2	2.71 (t, 6.6)	25.9 CH_2	2.72 (t, *J* = 6.5 Hz, 2H, $2H_{14}$)	26.2 CH_2	2.71 (t, *J* = 6.8 Hz, 2H, $2H_{14}$)
15	126.8 CH	5.37 (m)	127.5 CH	5.5-5.2 (m, 4H, H_{12} + H_{13}	127.8 CH	5.4-5.3 (m, 3H)

				+ H_{15} + H_{16})		
16	130.4 CH	5.42 (m)	131.5 CH	5.5-5.2 (m, 4H, H_{12} + H_{13} + H_{15} + H_{16})	130.1 CH	5.4-5.3 (m, 3H)
17	21.3 CH_2	2.02 (m)	20.0 CH_2	2.10 (t, *J* = 7.0 Hz, 2H, $2H_{17}$)	21.3 CH_2	2.0-1.9 (m, 8H, $2H_2$ + $2H_{10}$ + $2H_{11}$ + $2H_{17}$)
18	15.2 CH_3	0.92 (t, 7.5)	14.5 CH_3	0.91 (t, *J* = 7.4 Hz, 3H, CH_3)	14.8 CH_3	0.91 (t, *J* = 7.5 Hz, 3H, CH_3)
1'	65.3 CH_2	3.91 (d, 5.8)	65.7 CH_3	4.0-3.9 (m, 2H, $2H_{1'}$)	66.1 CH_2	4.0-3.9 (m, 2H, $2H_{1'}$)
2'	69.9 CH	3.52 (br s)	70.2 CH	3.6-3.5 (m, 1H, $H_{2'}$)	70.8 CH	3.6-3.5 (m, 1H, $H_{2'}$)
3'	63.2 CH_2	3.29 (m)	63.4 CH_2	3.37 (dd, *J* = 11.0, 3.6 Hz, 1H, $H_{3'}$)	64.0 CH_2	3.37 (dd, *J* = 11.2, 3.9 Hz, 1H, $H_{3'}$)
		3.23 (m)		3.29 (dd, *J* = 11.3, 6.0 Hz, 1H, H3')		3.29 (dd, *J* = 11.2 and 6.0 Hz, 1H, $H_{3'}$)
1"	167.2 C		168.2 C		168.0 C	
2"	19.5 CH_3	1.55 (s)	20.9 CH_3	2.0-1.9 (m, 6H, $2H_2$ + $2H_{10}$ + $2H_{11}$)	20.2 CH_3	1.57 (s, 3H, OC(O)$\underline{C}H_3$)

Biology

Bisulfite treatment and PCR for RARβ methylation

20 µg of genomic DNA (or in vitro methylated DNA) were mixed with 5.5 µL of 3M NaOH, the volume was adjusted to 55.5 µL with filtered water. After 15 min incubation at 42 °C, 500 µL of 3M sodium bisulfite solution containing 0.05M hydroquinone were added. The mixture was incubated at 54 °C for 16 h. The modified DNA was purified with Nucleospin extract purification kit and eluted with 50 µL of filtered water. The eluate is desulfonated with 5.5 µL of 3M NaOH for 15 min at room temperature. 16.8 µL of 10M ammonium acetate, 1µL glycol-blue and 200 µL ethanol were added for the DNA precipitation. The DNA is recovered after centrifugation, washed, air dried and resuspended with 50 µL of filtered water. PCR was performed as follows: 5 min for denaturation at 94 °C, 35 cycles, 1 min denaturation at 94 °C, 1 min annealing at 57.5 °C for the methylated primer and 52 °C for the unmethylated primer, 1 min elongation at 72 °C, 5 min further elongation at 72 °C. The PCR product (10µL + 5µL loading dye) is analyzed on 1.5% agarose gel.

RARß2 methylated primer S: 5’ TCG AGA ACG CGA GCG ATT CG; AS: 5’ GAC CAA TCC AAC CGA AAC GA

RARß2 unmethylated primer S: 5’TTG AGA ATG TGA GTG ATT TG; AS: 5’ AAC CAA TCC AAC CAA AAC AA

Below are the results for compounds (*R*)-**16A** and (*R*)-**16B**

Msp RARß
HCT116 cell line

(*R*)-1A and (*R*)-1B 10µM
5AZAdC 5µM