Bioinspired Synthesis and biological evaluation of *ent*-Protulactones A and B

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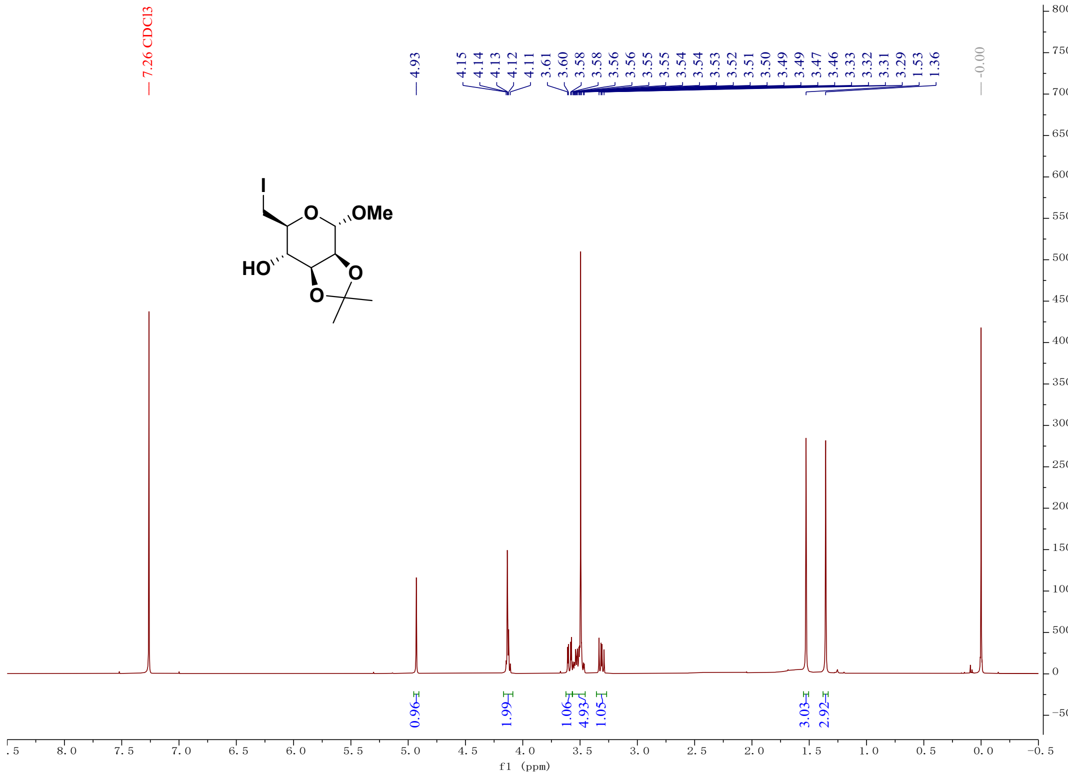


Figure 1. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **8**

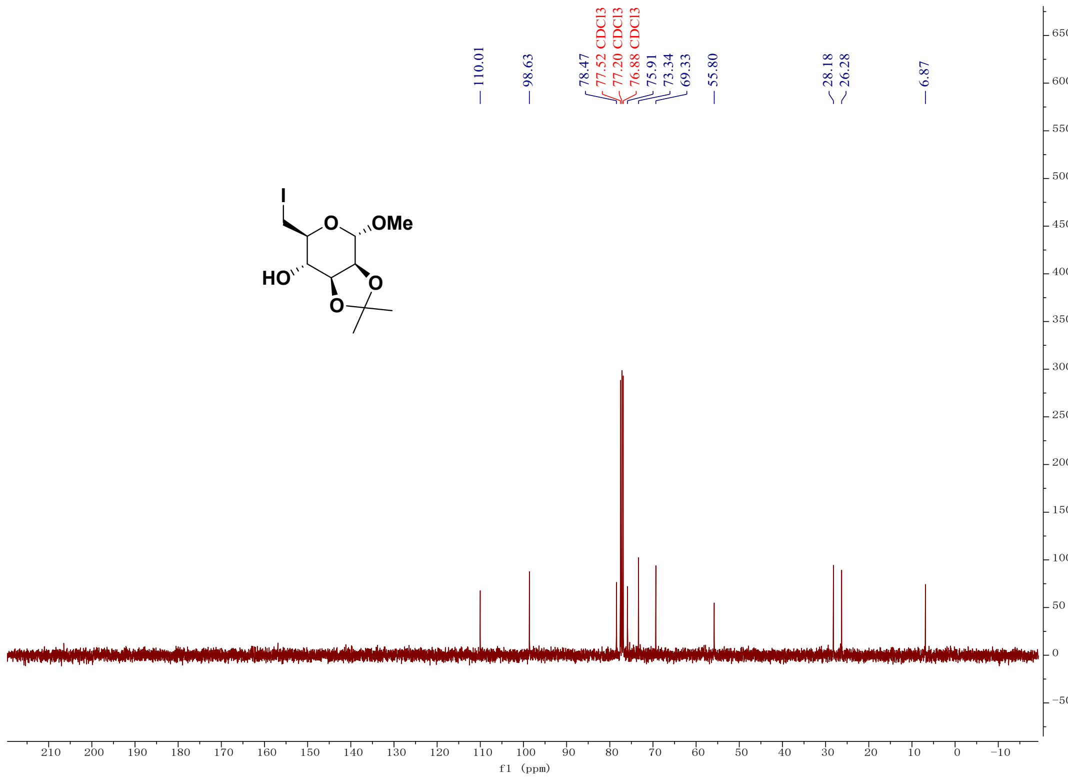
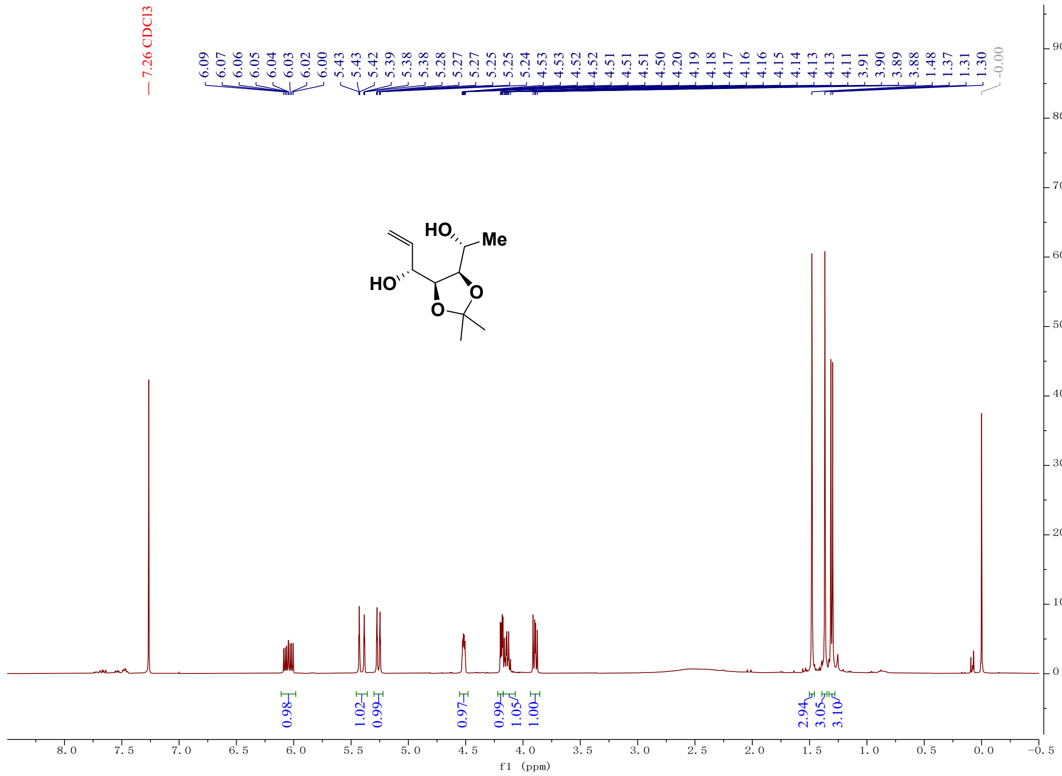


Figure 2. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **8**



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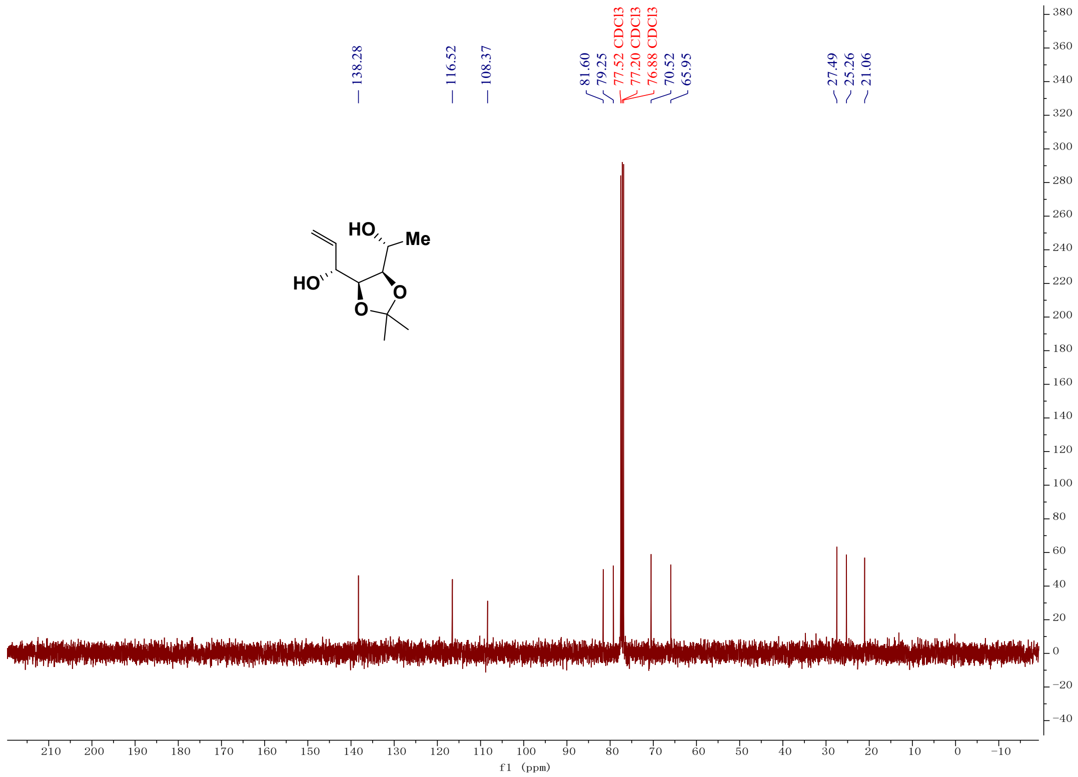

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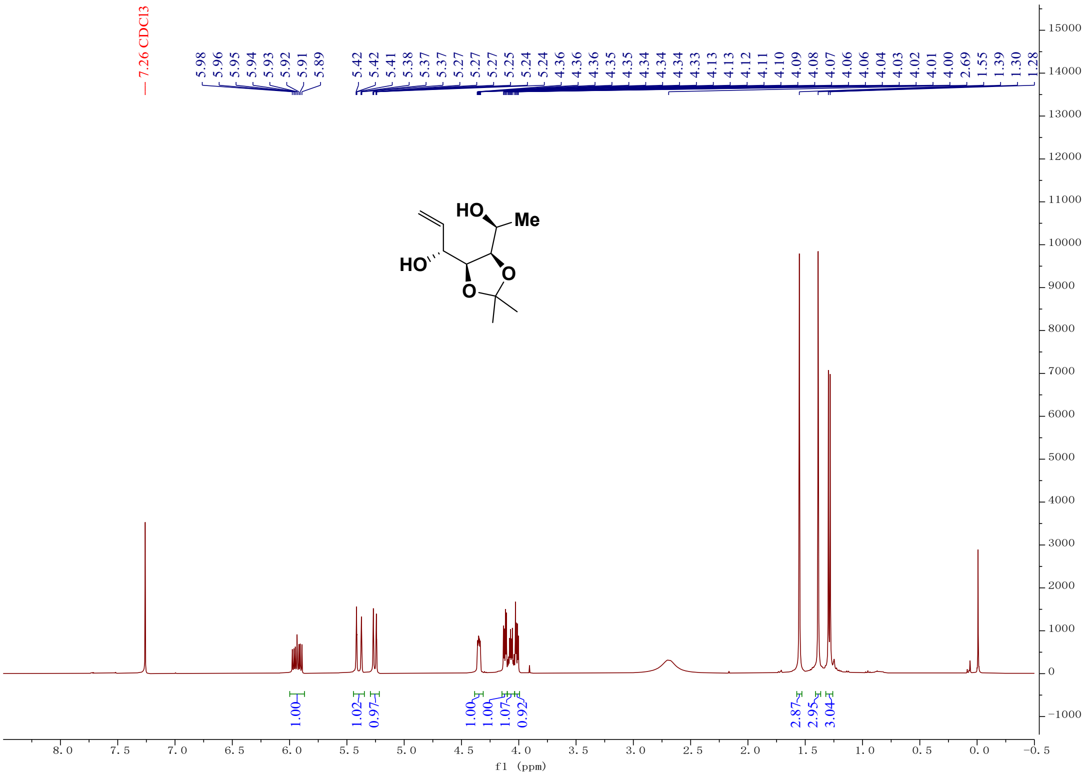

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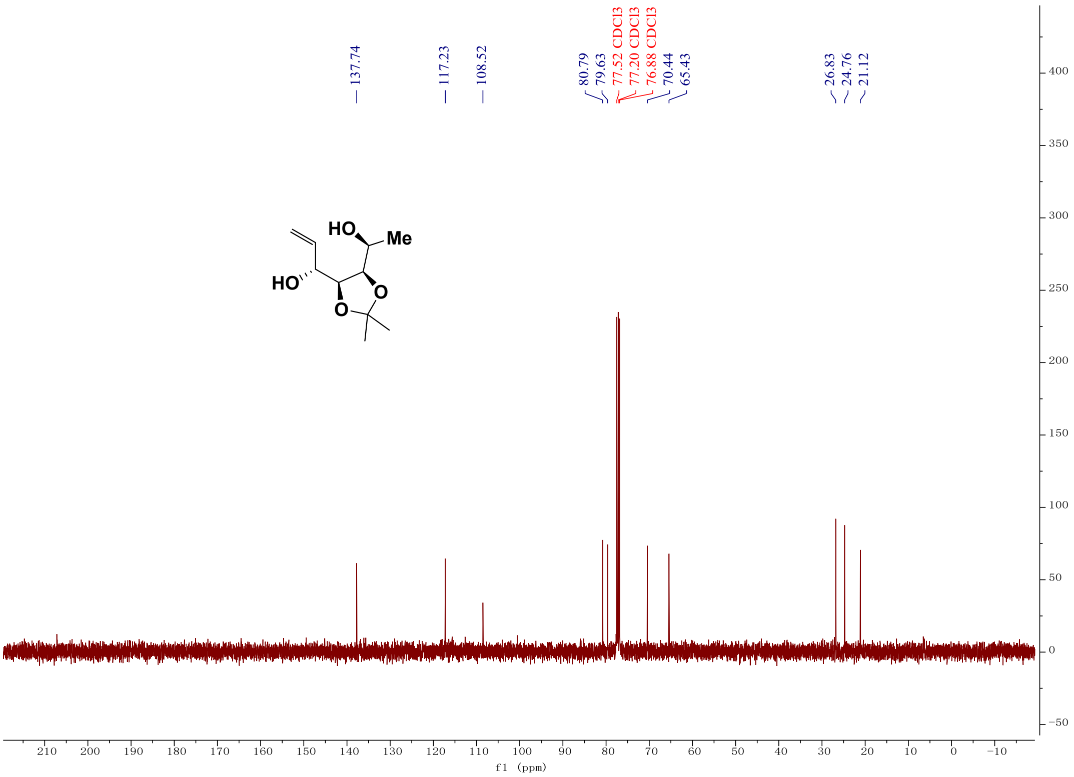

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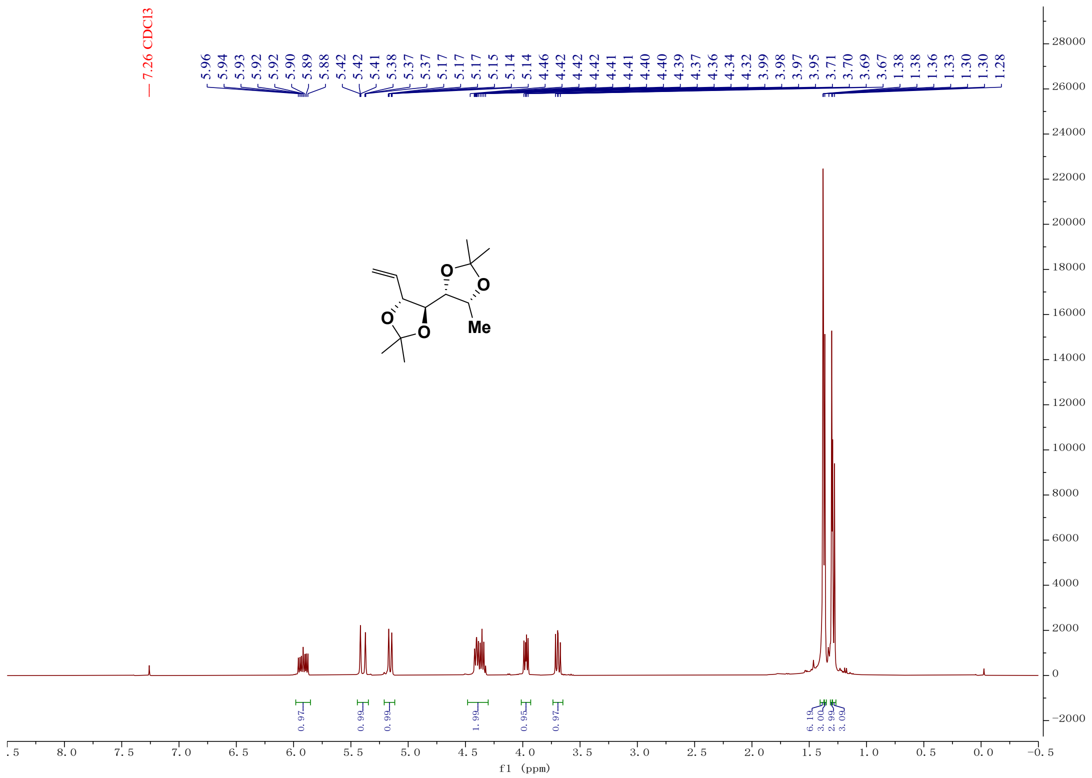

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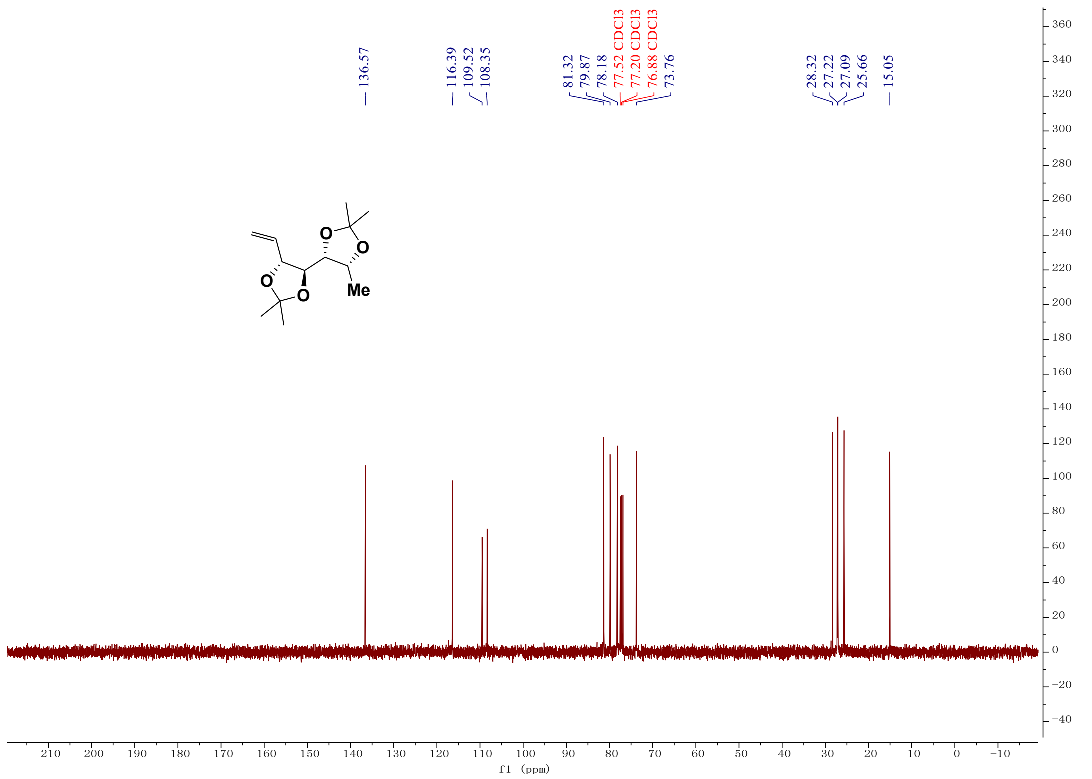

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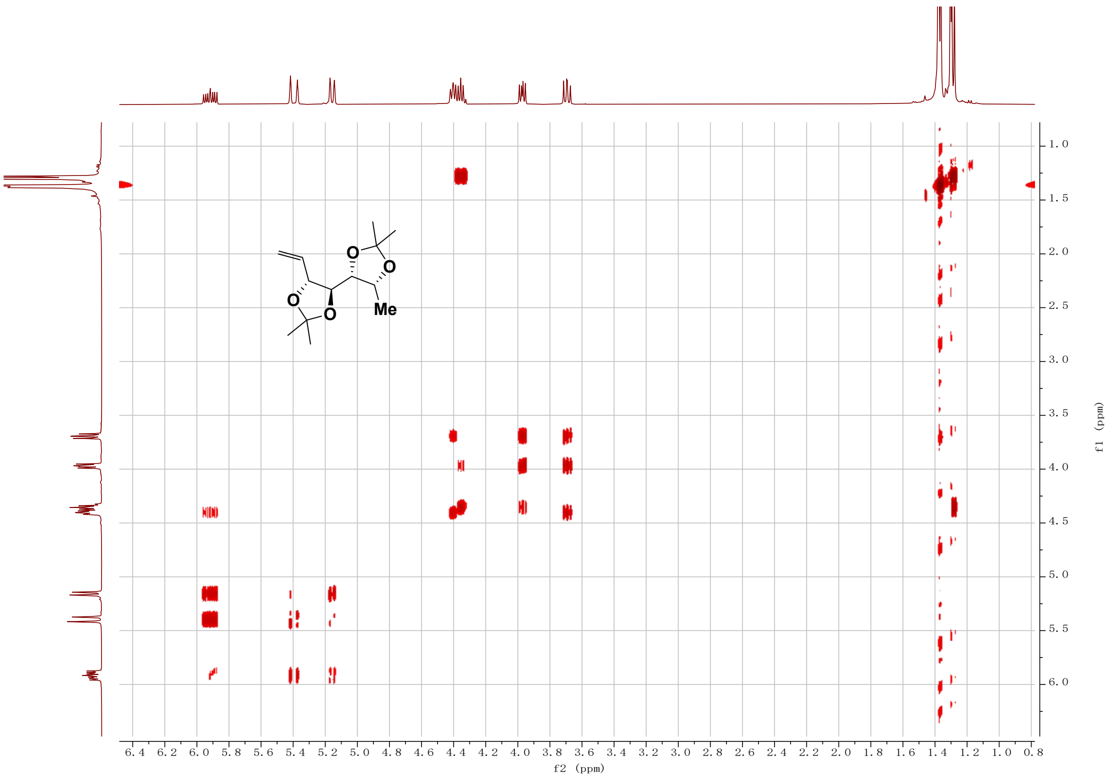

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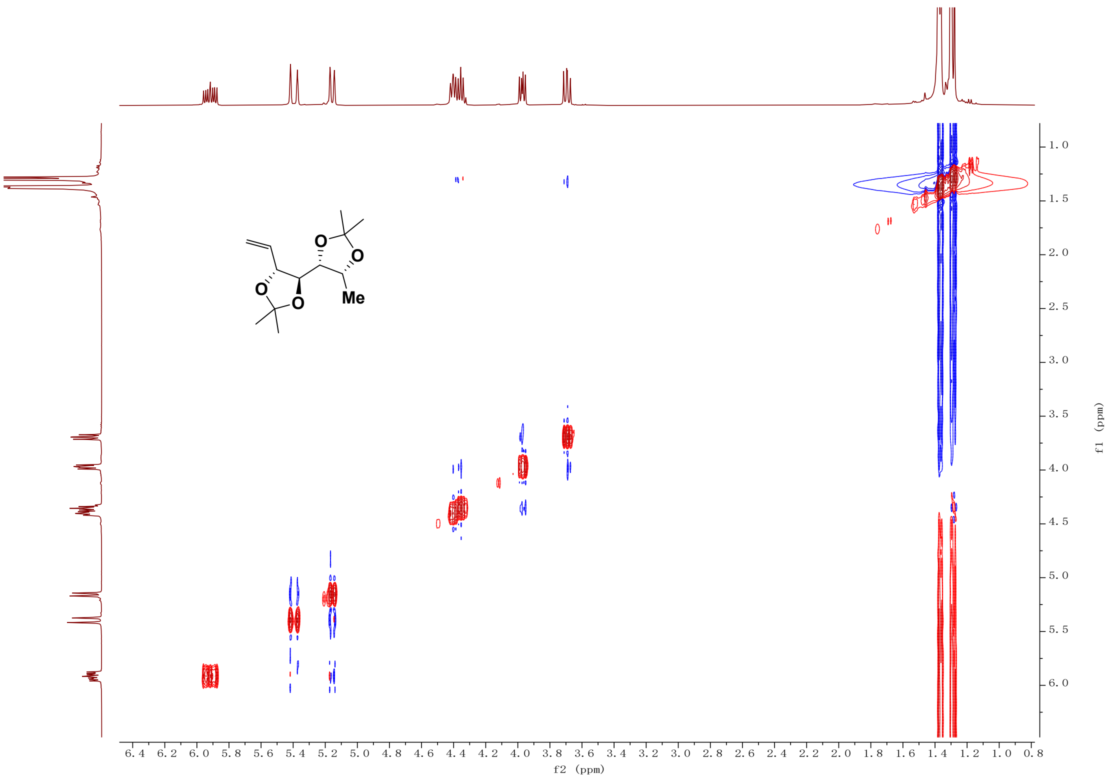

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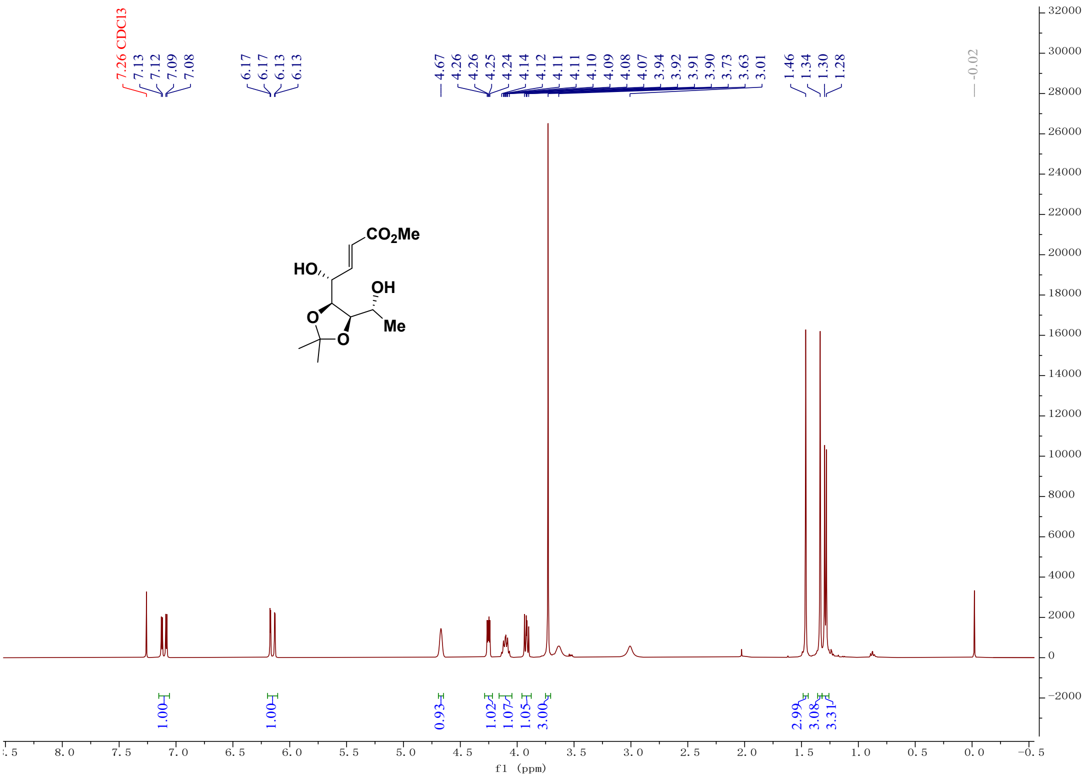

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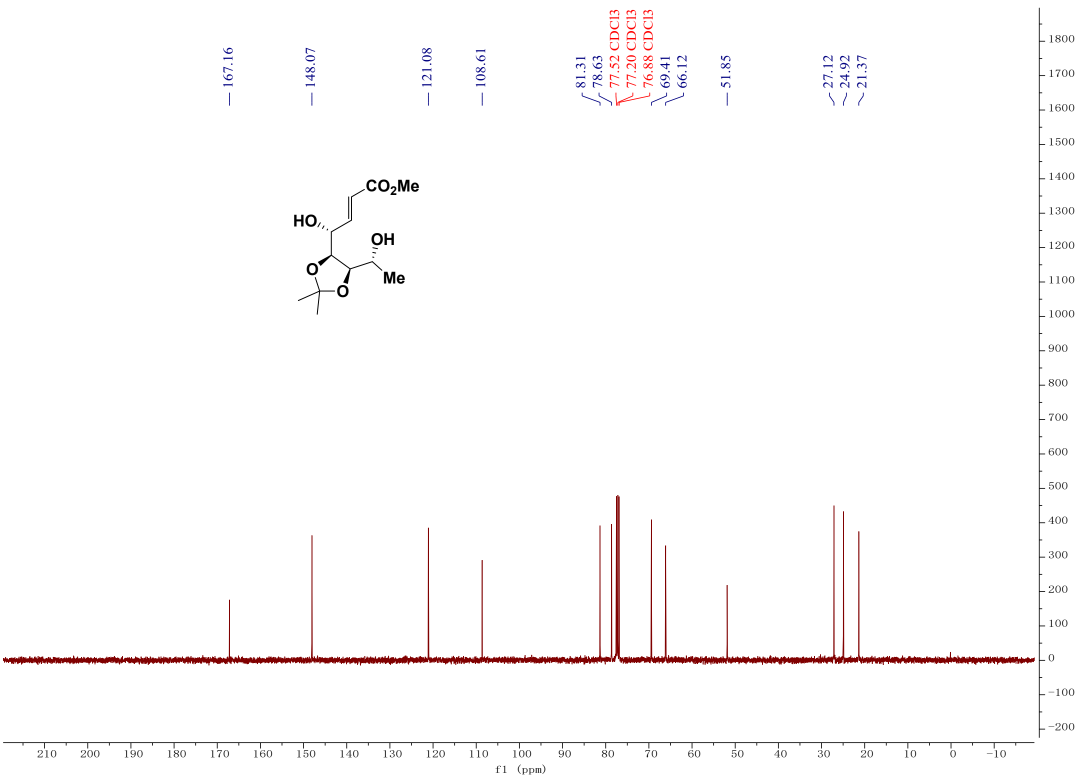

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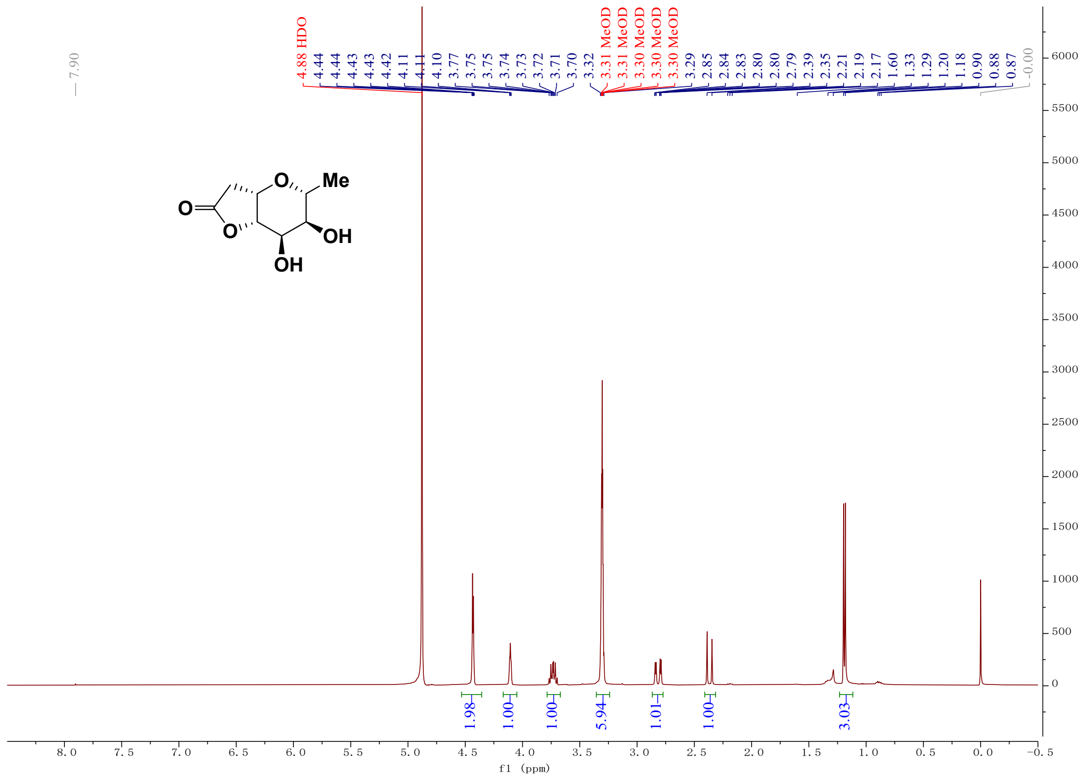

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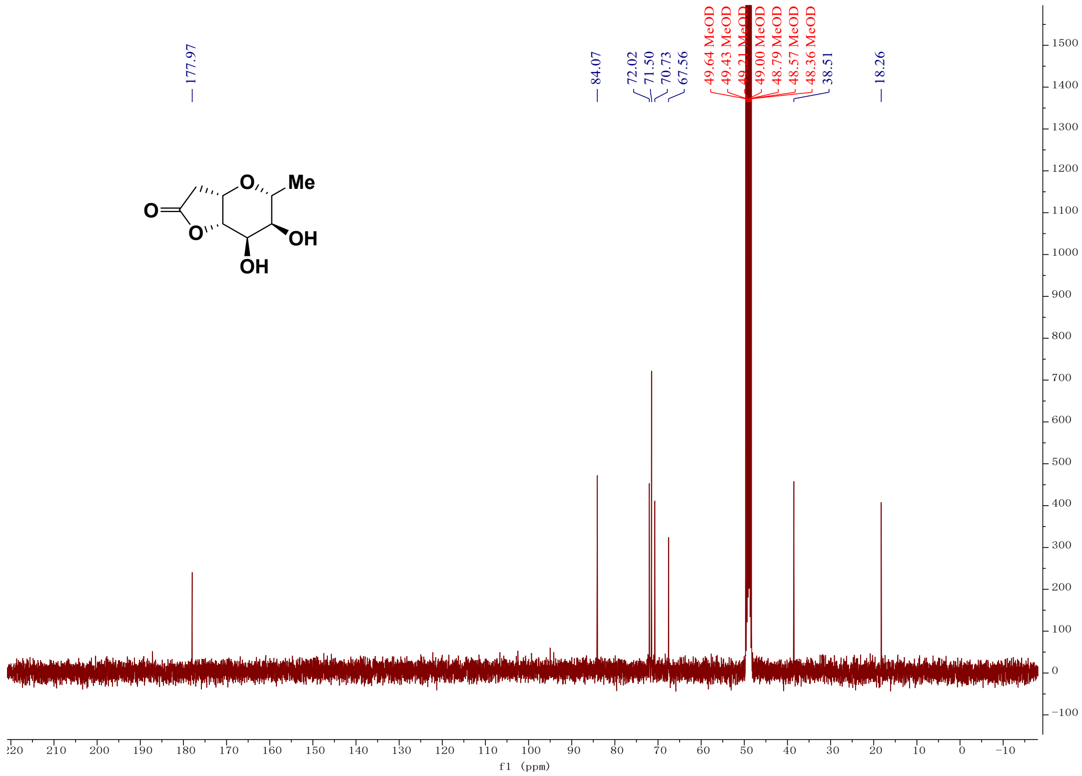

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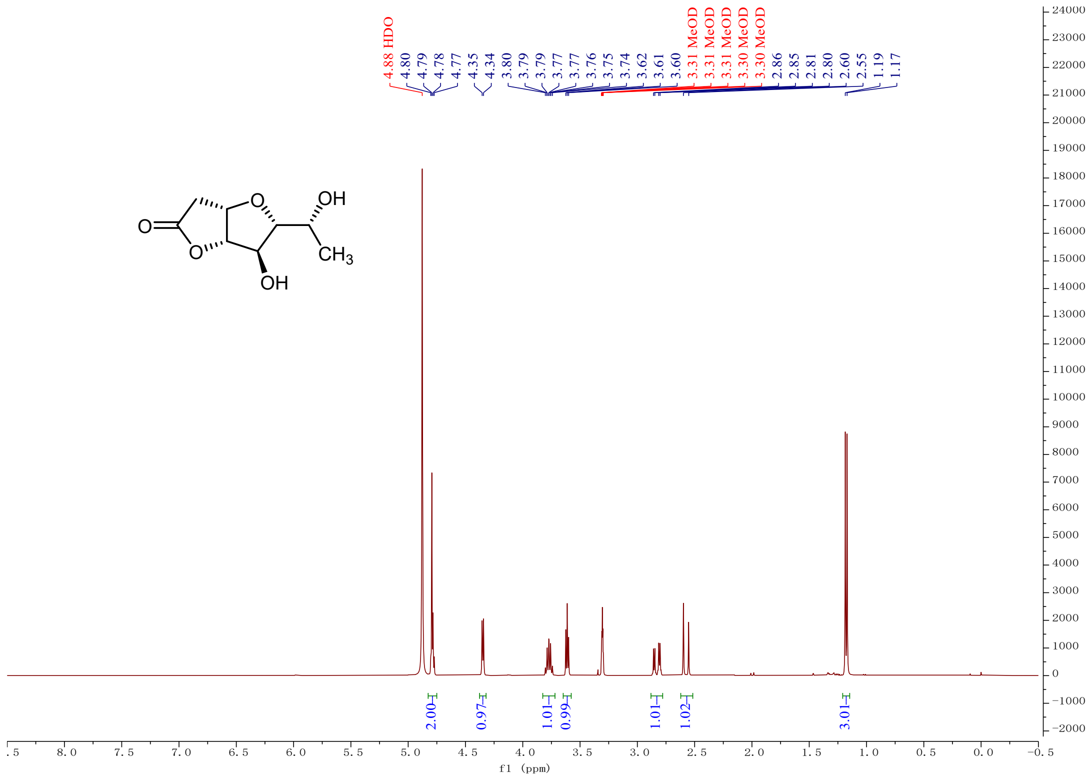

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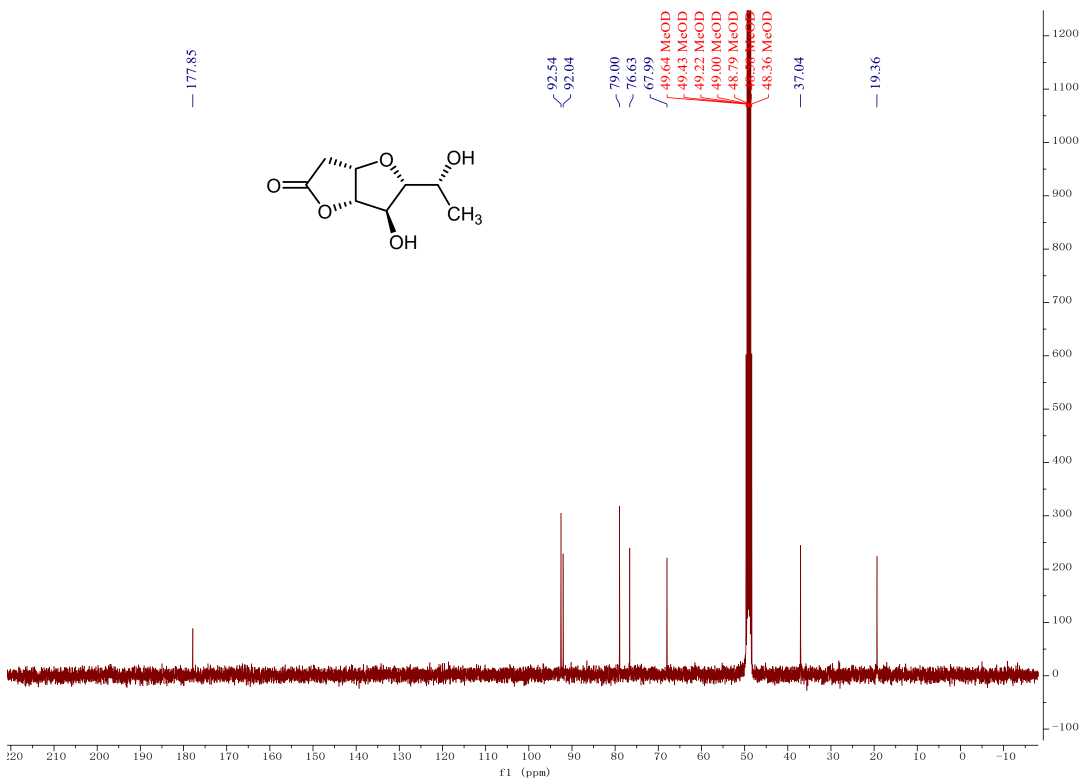

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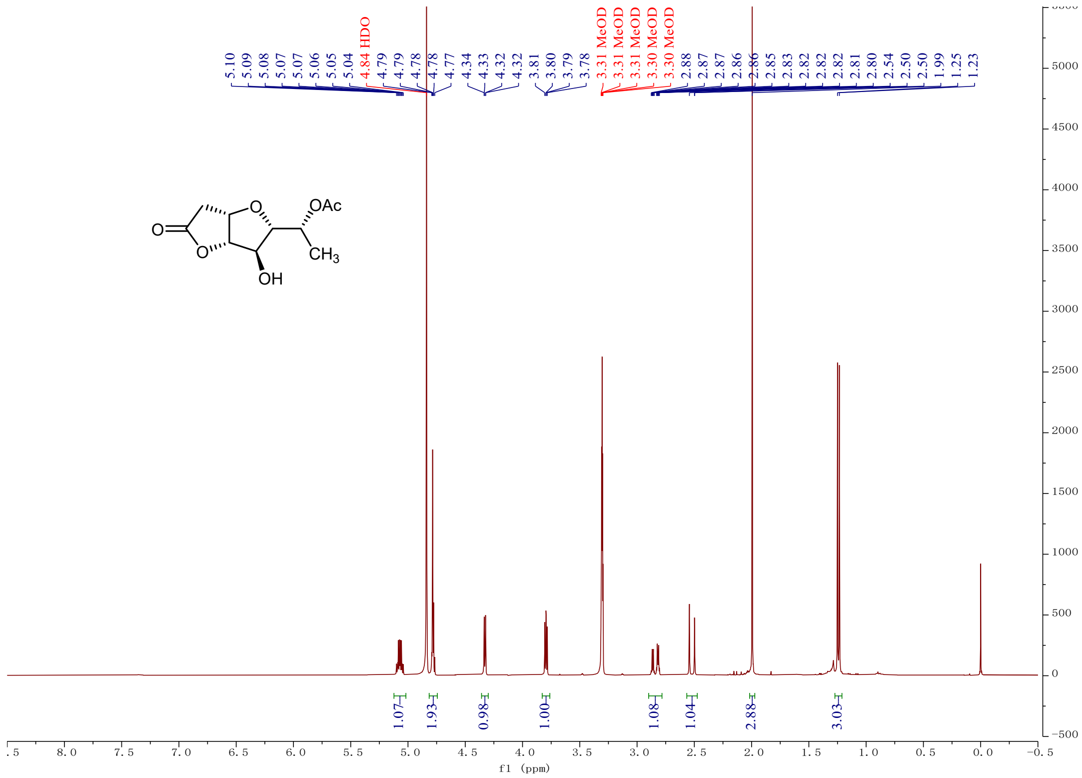

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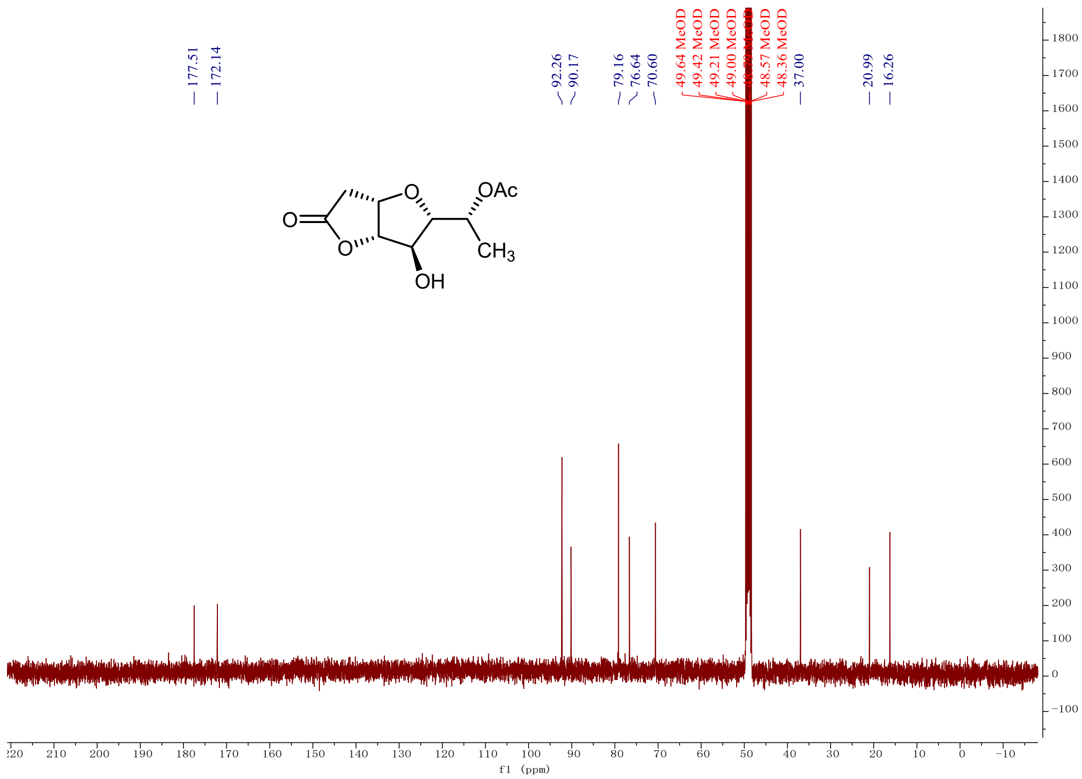

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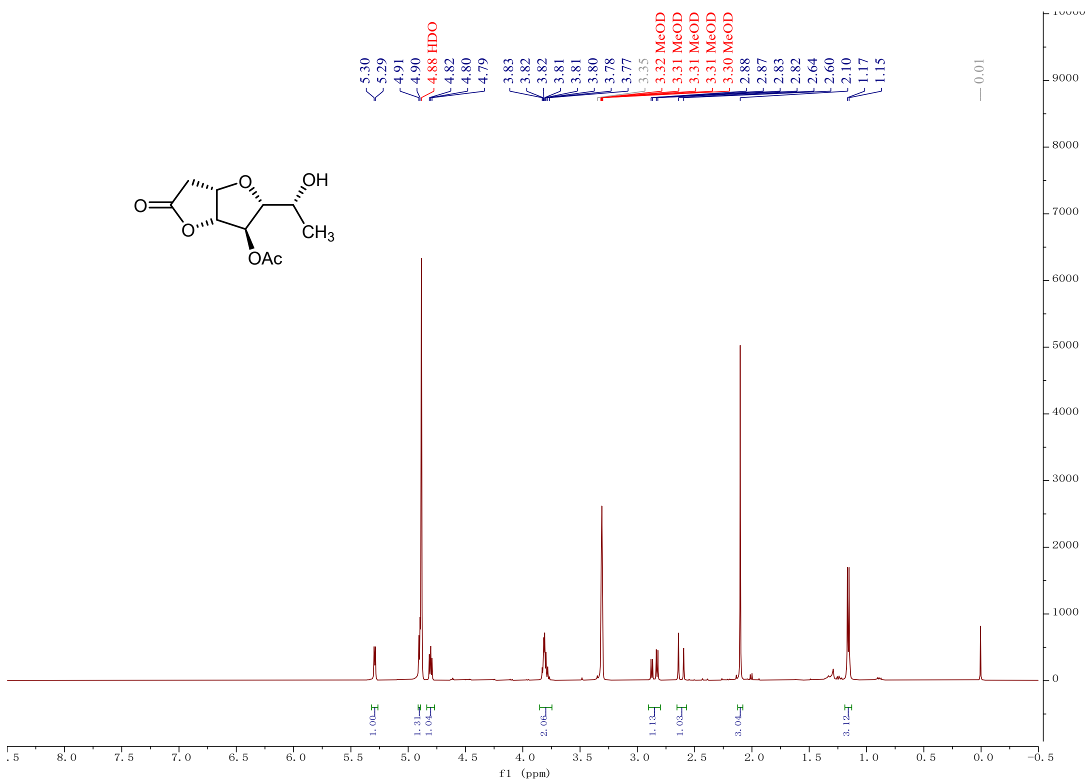

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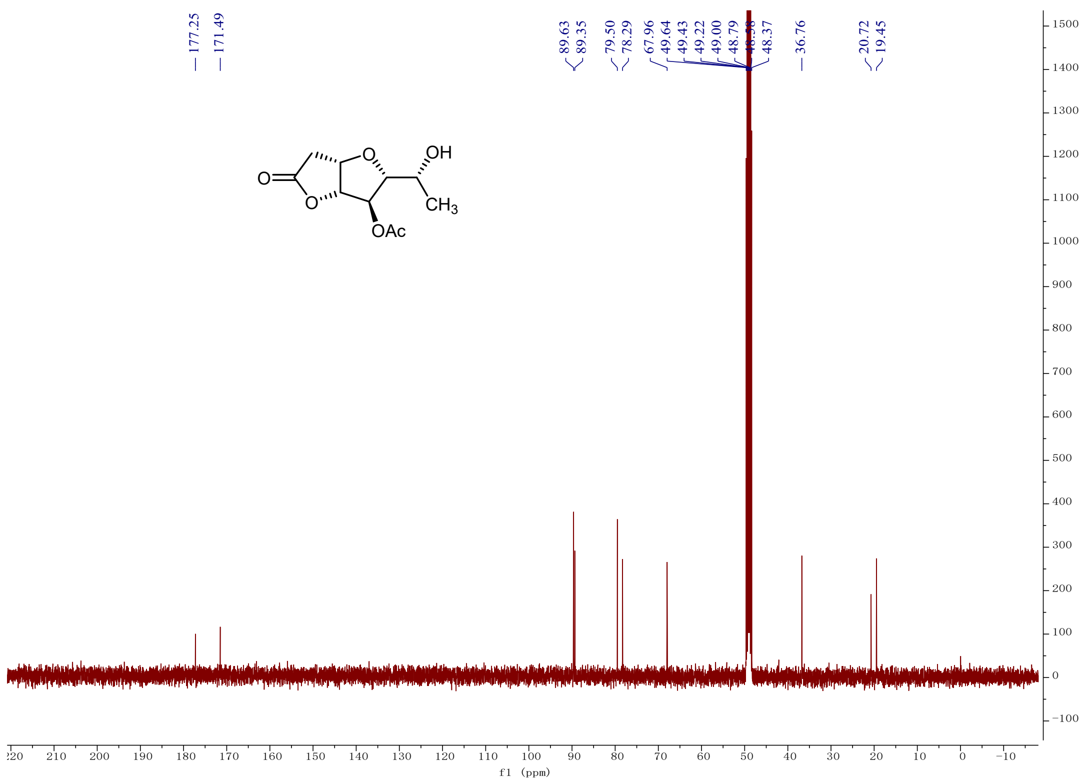

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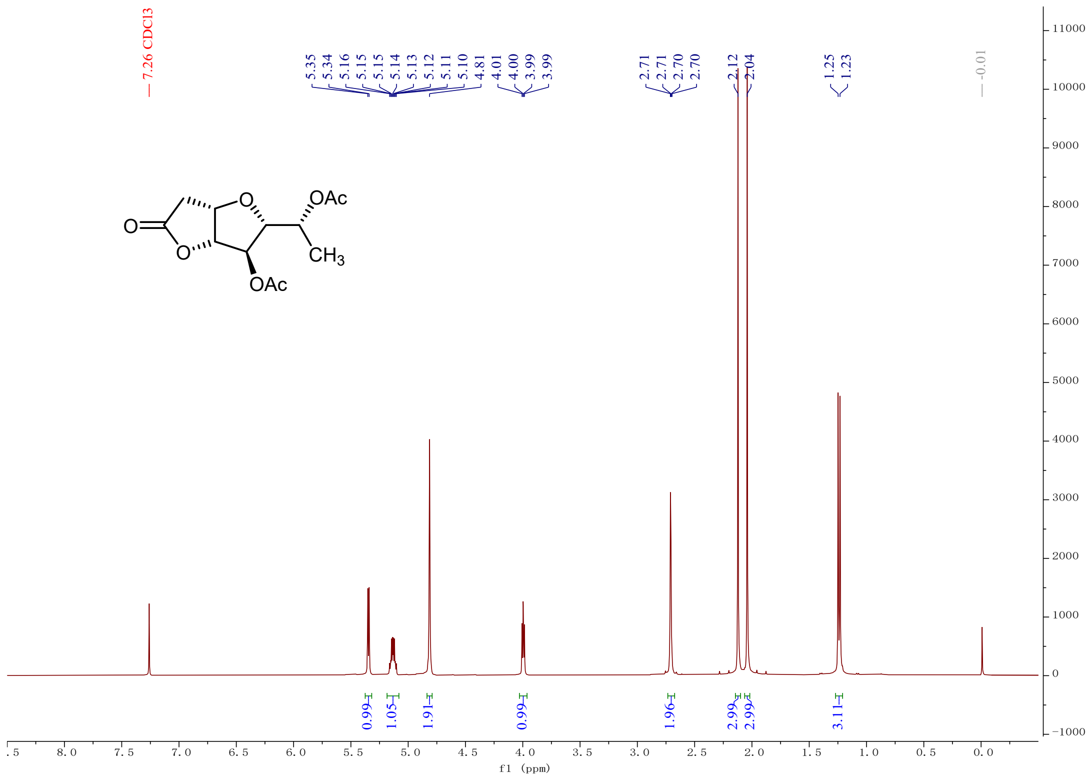

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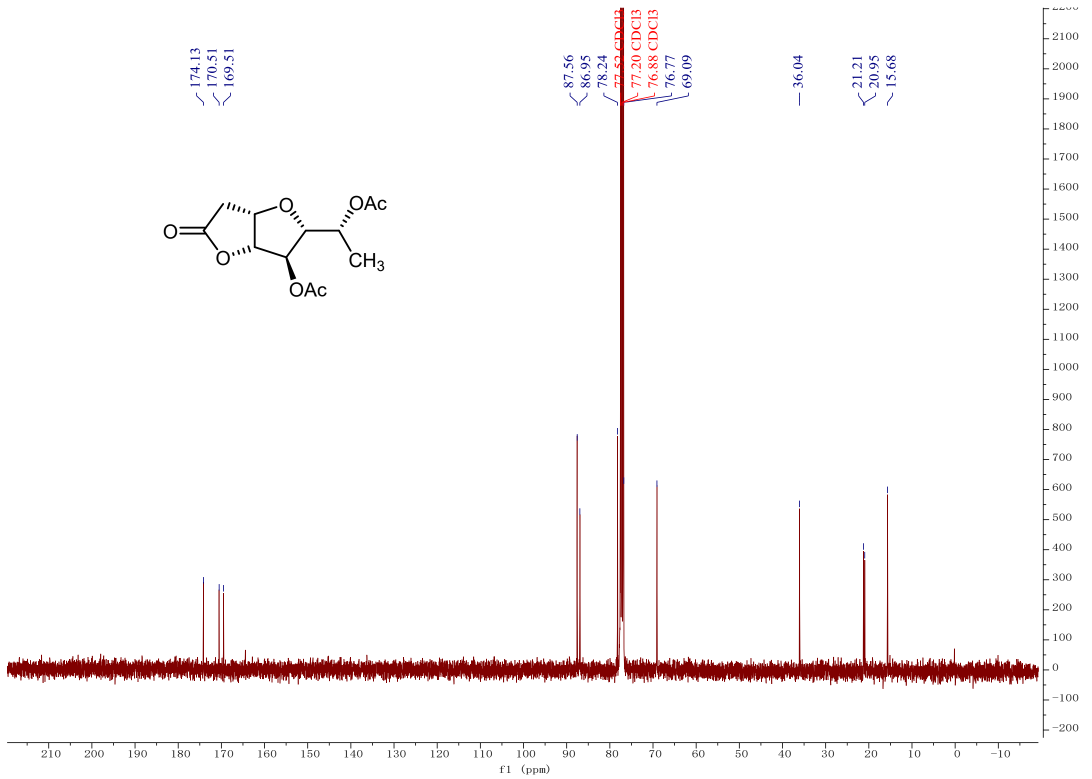

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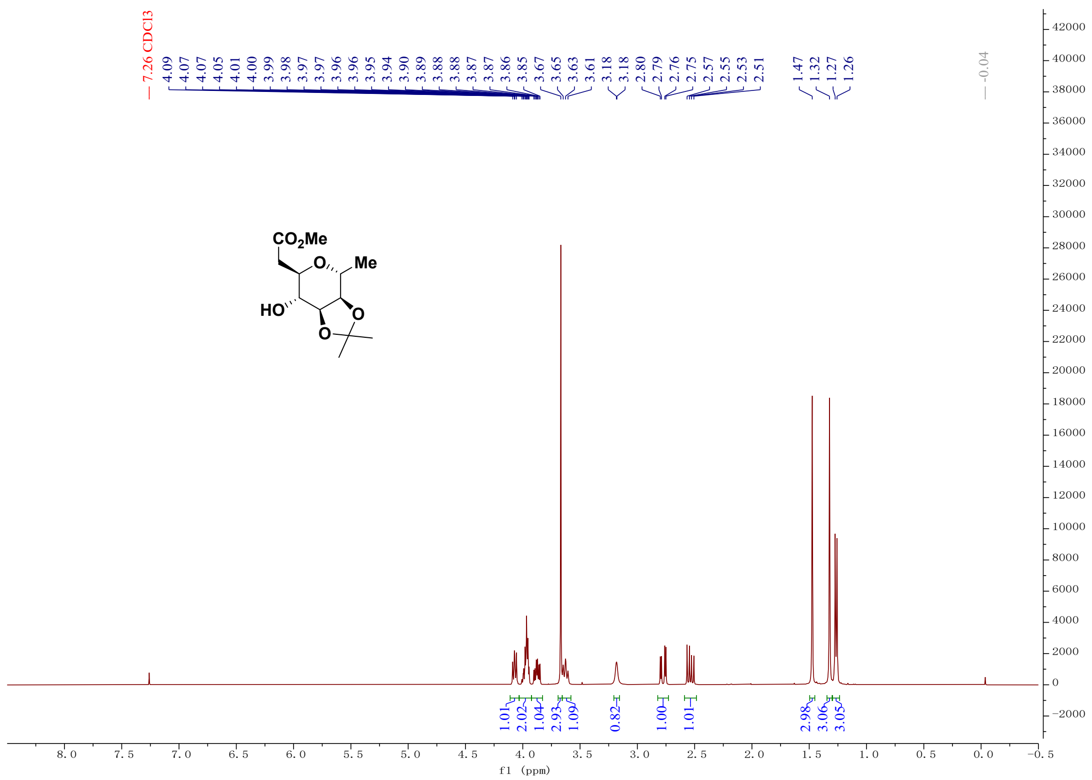

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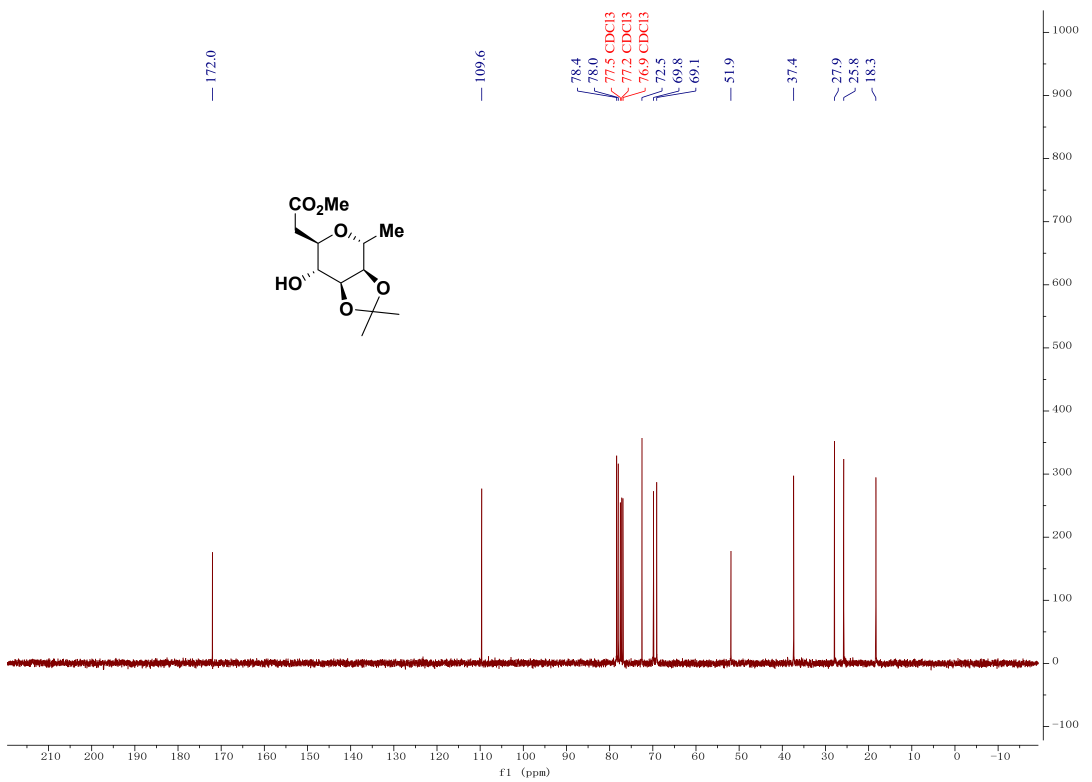

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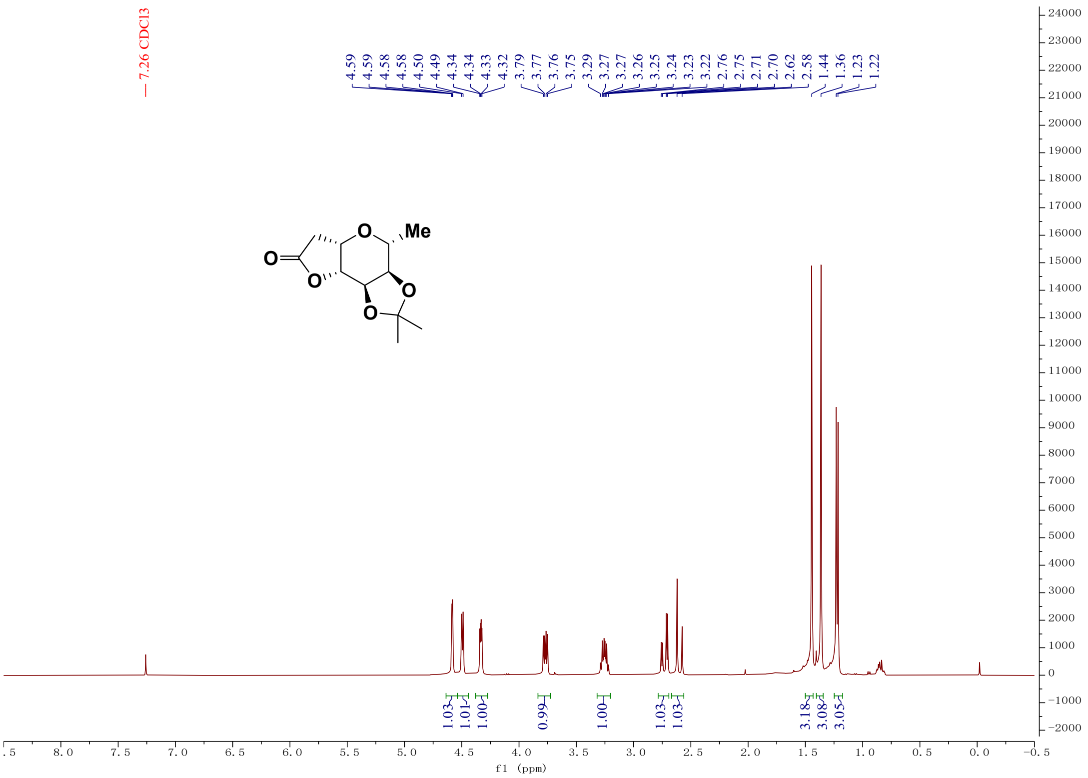

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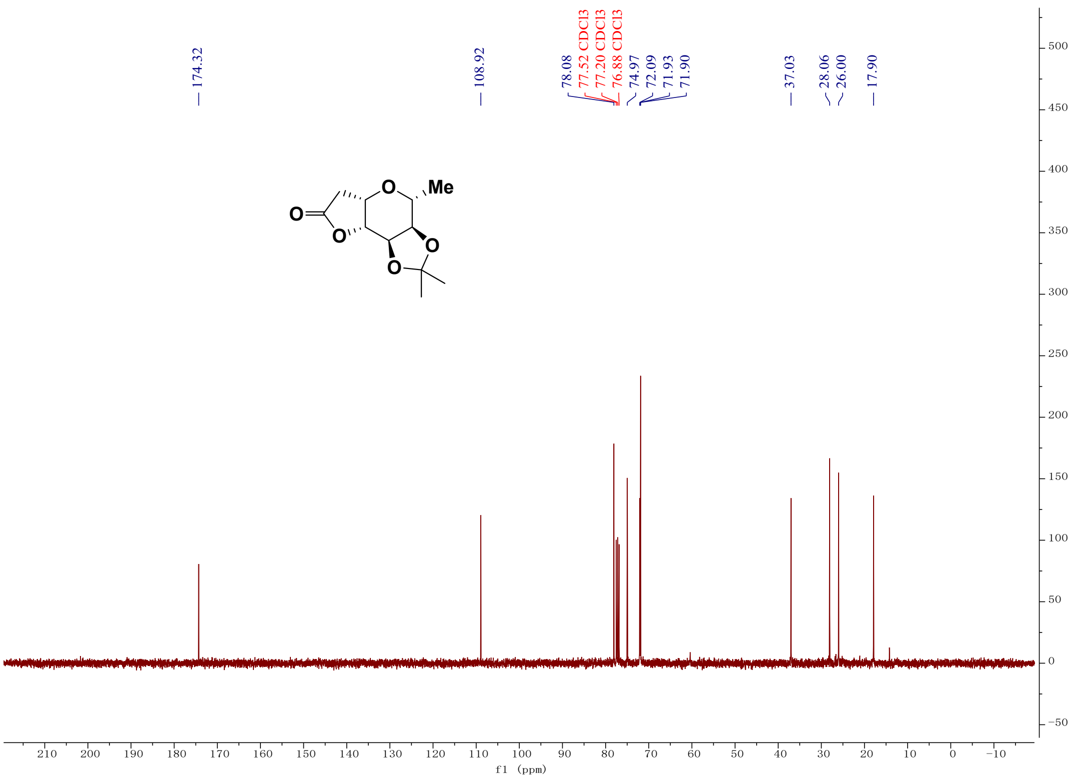

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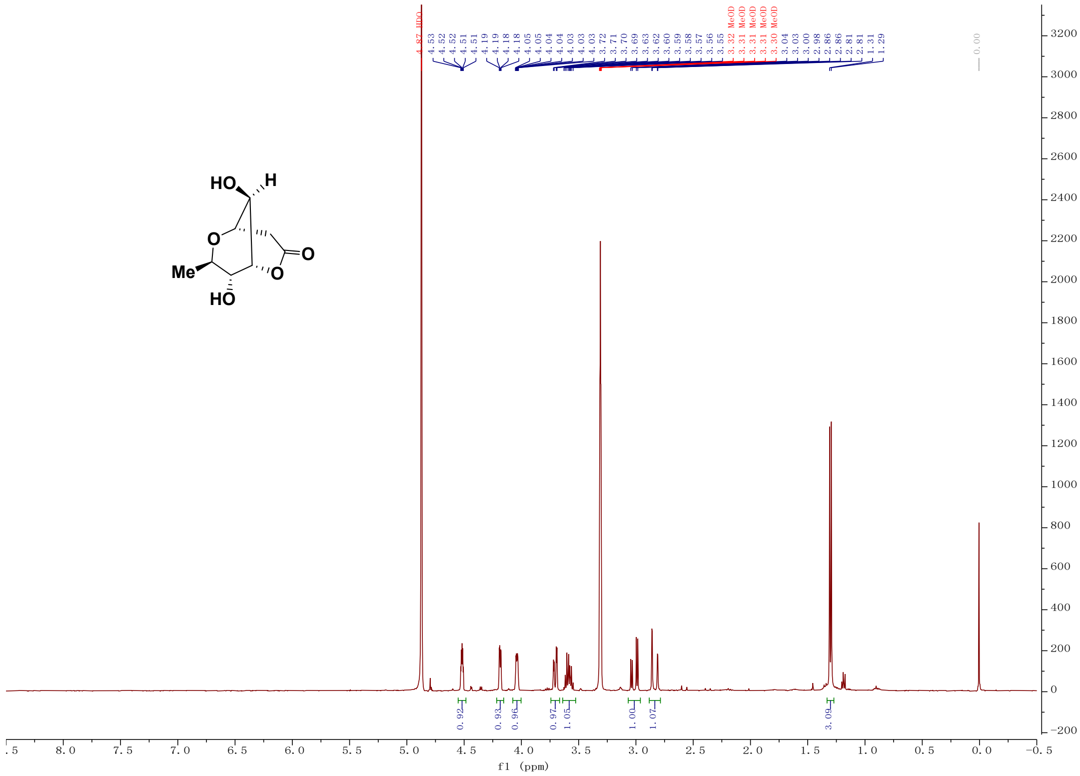

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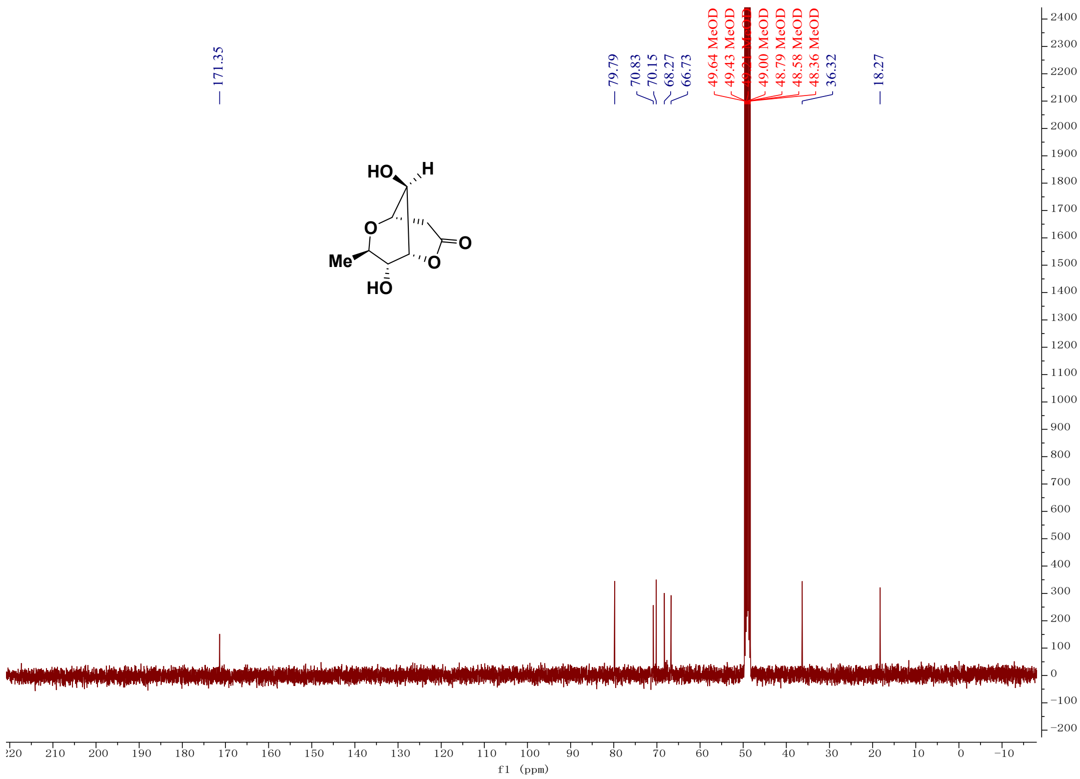

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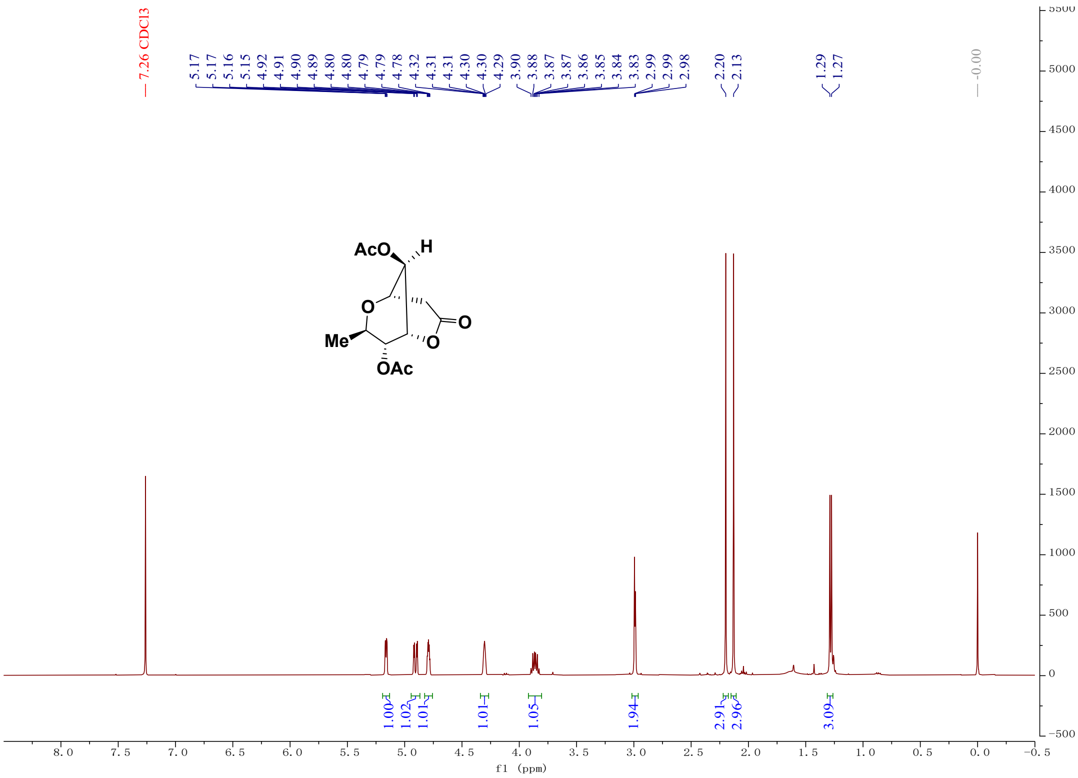

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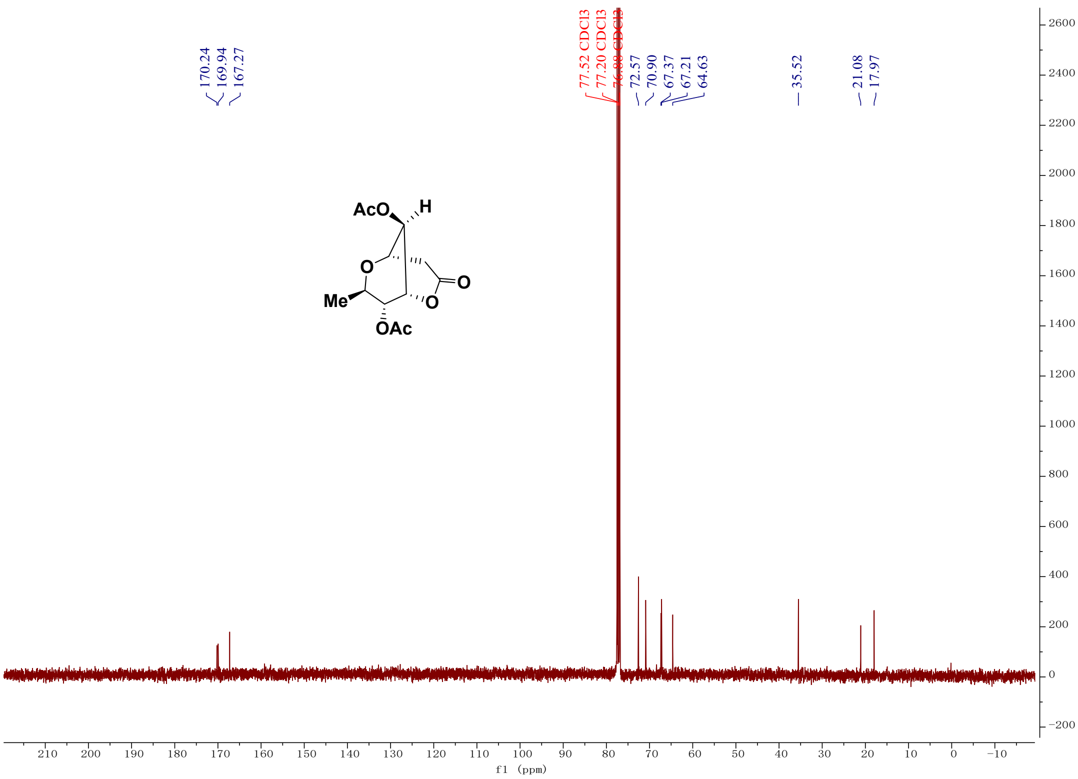

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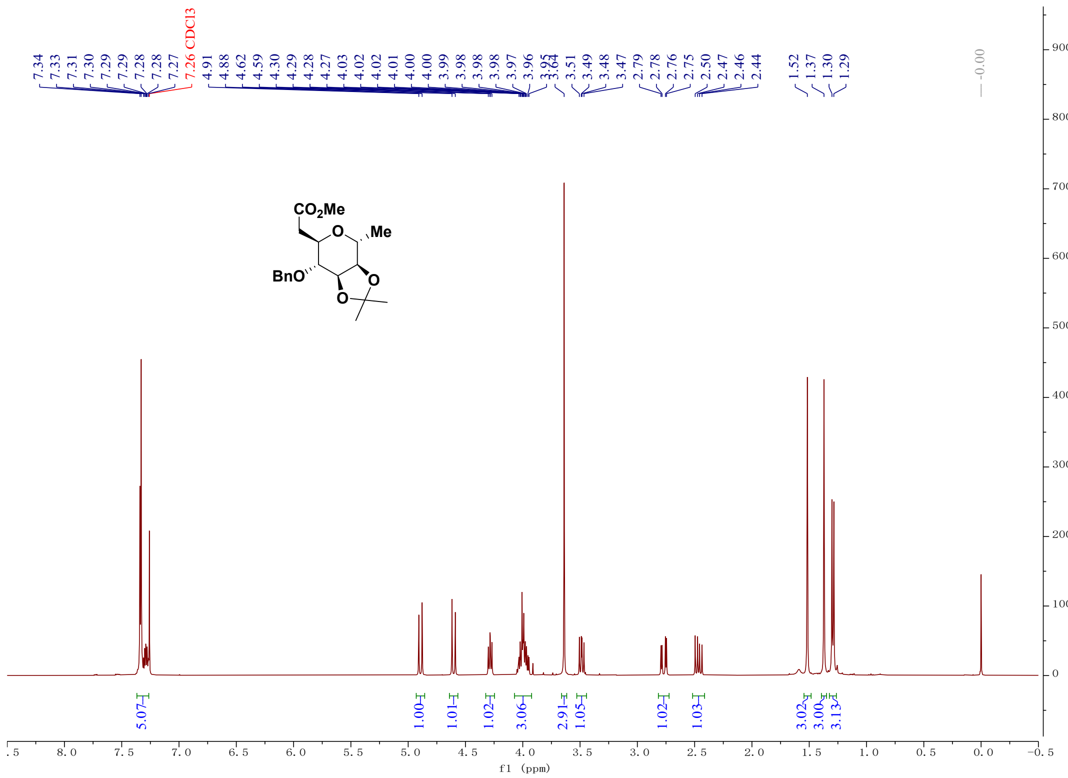

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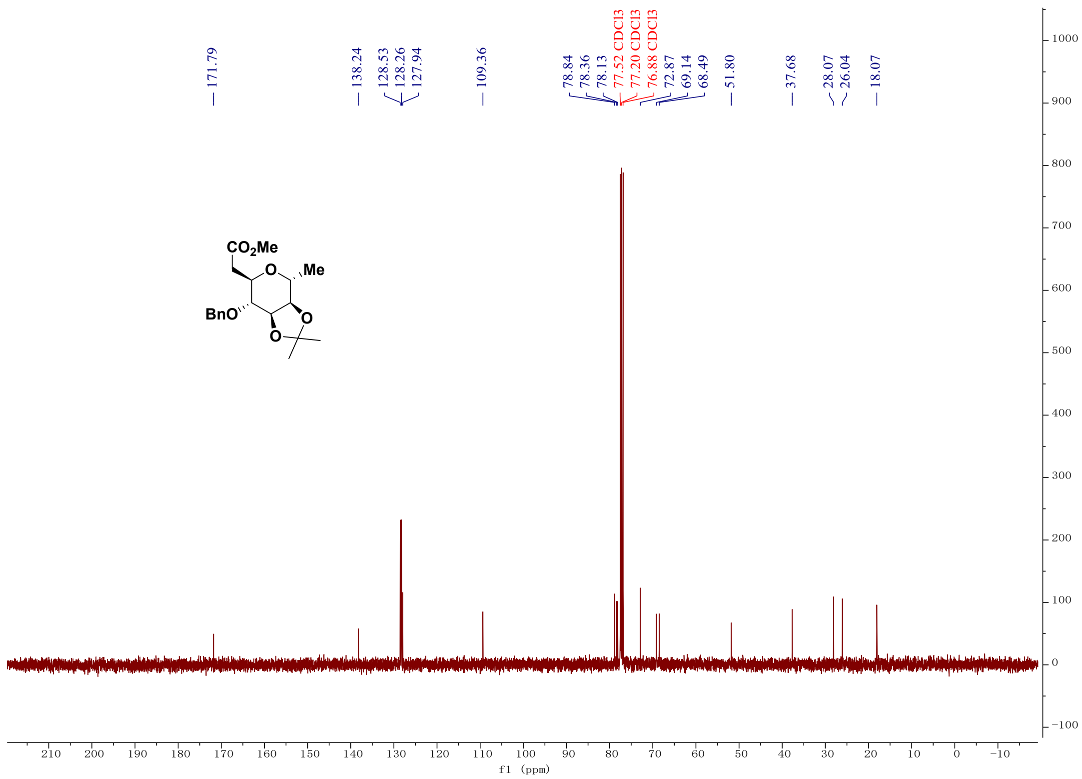

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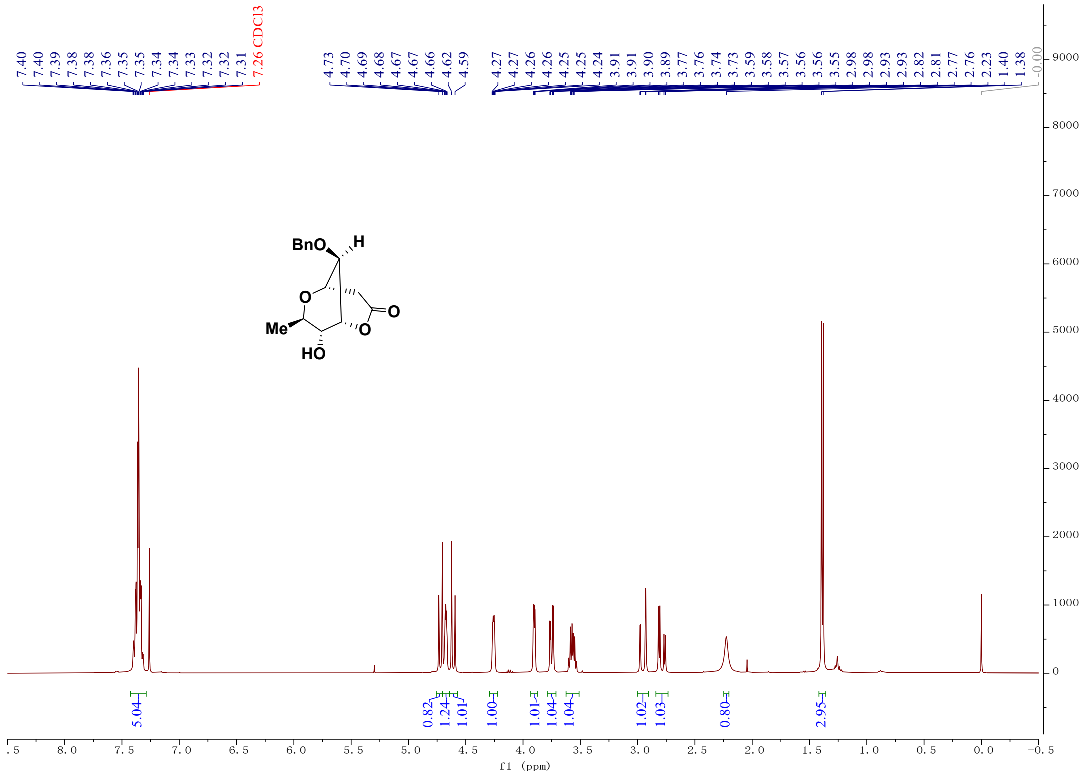

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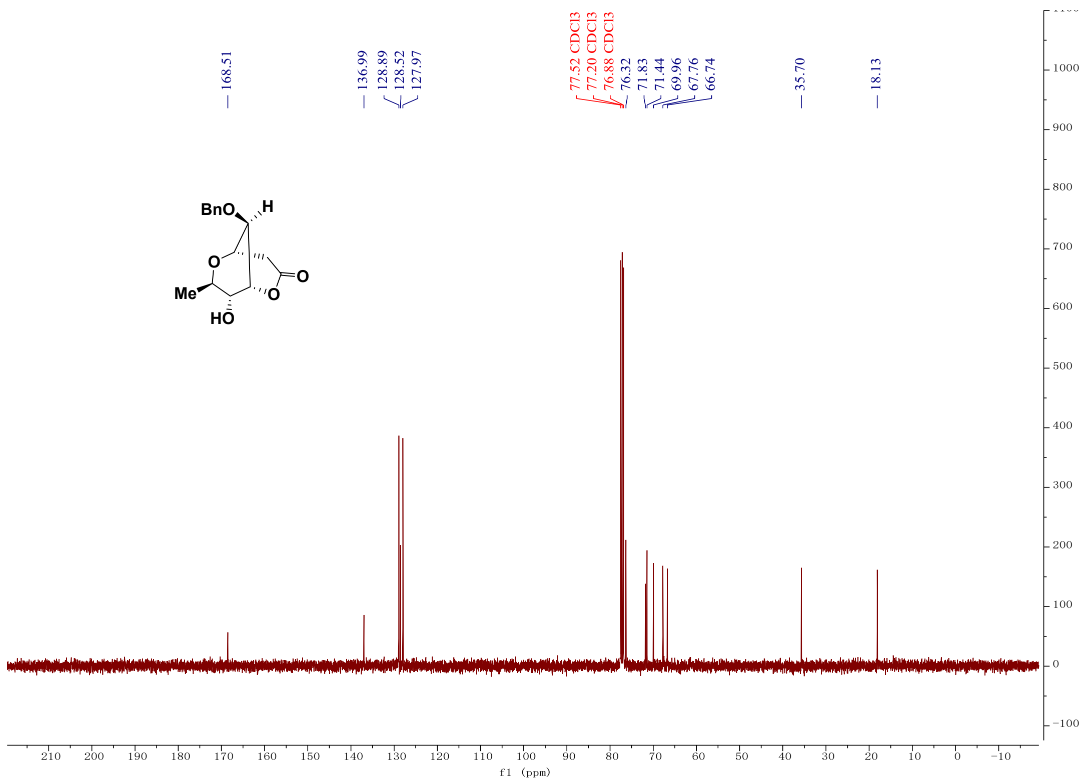


Figure 38. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **21**

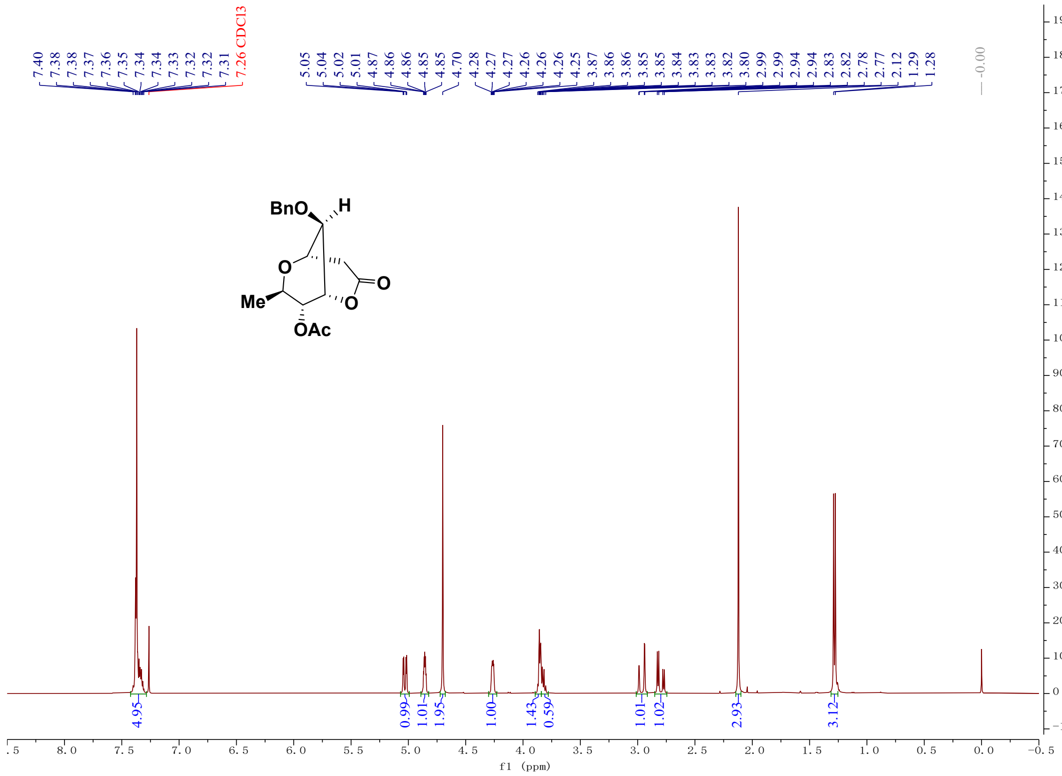


Figure 39. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **22**

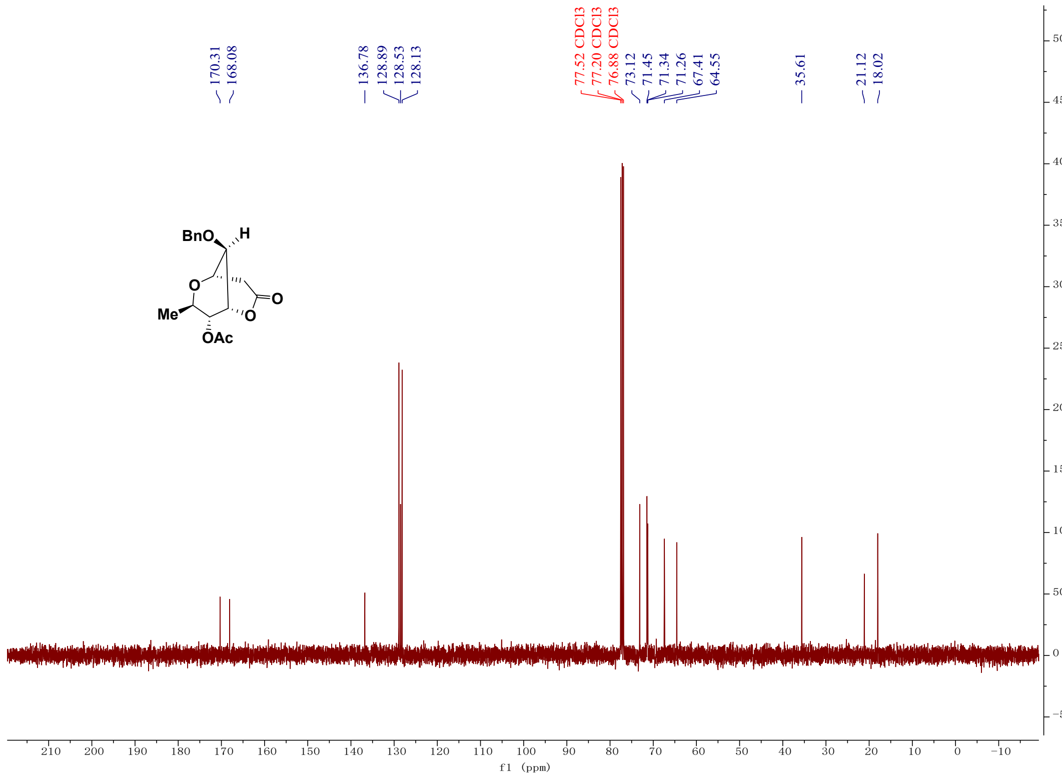


Figure 40. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **22**

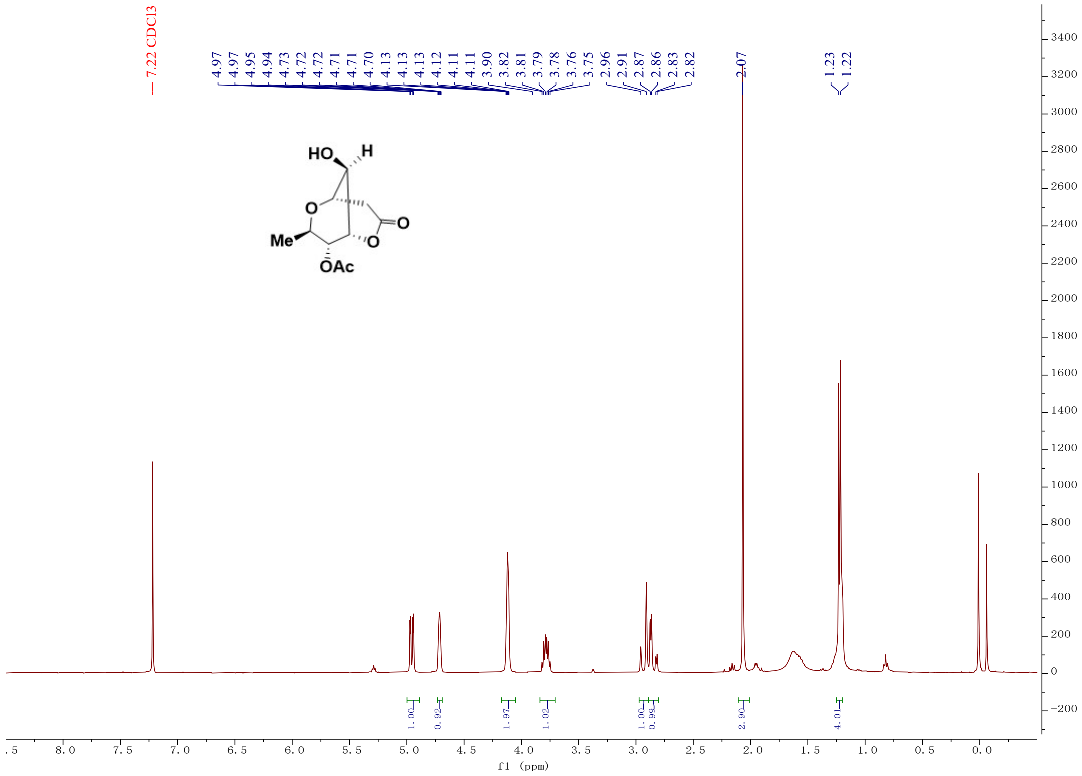


Figure 41. ^{1}H NMR (400 MHz, $CDCl_3$ with 15μL CD_3OD) spectrum of (+)-protulactone B (**2′**)

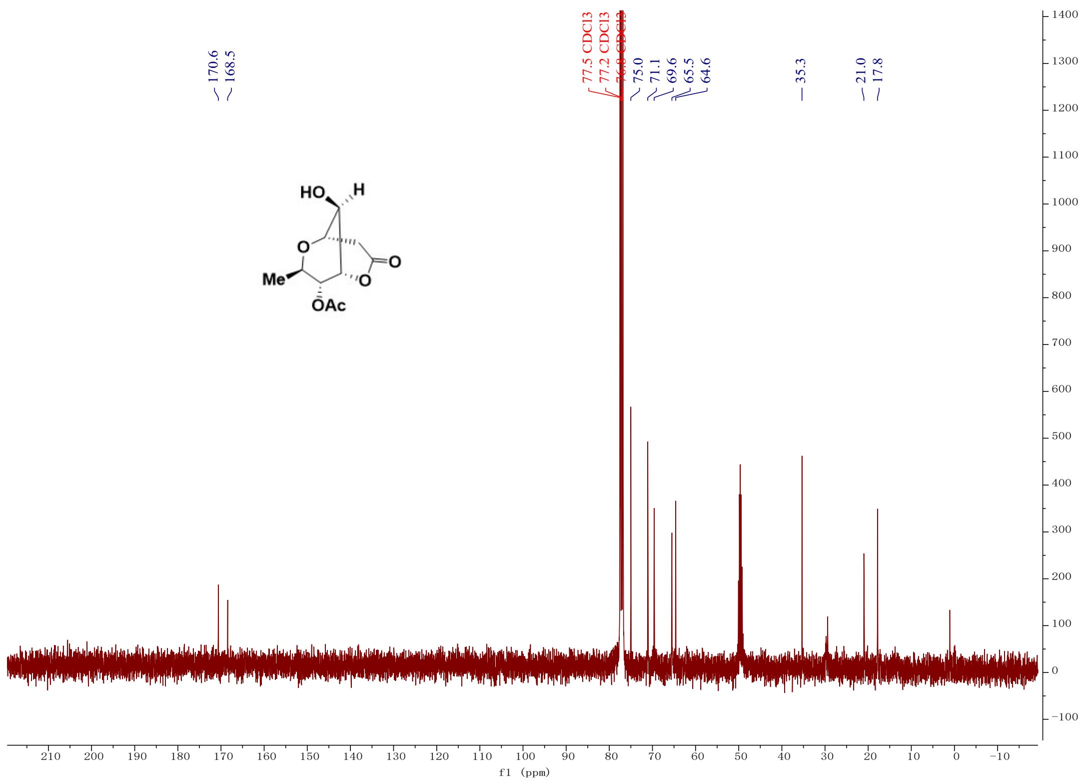


Figure 42. ^{13}C NMR (100 MHz, $CDCl_3$ with 15μL CD_3OD) spectrum of (+)-protulactone B (**2′**)

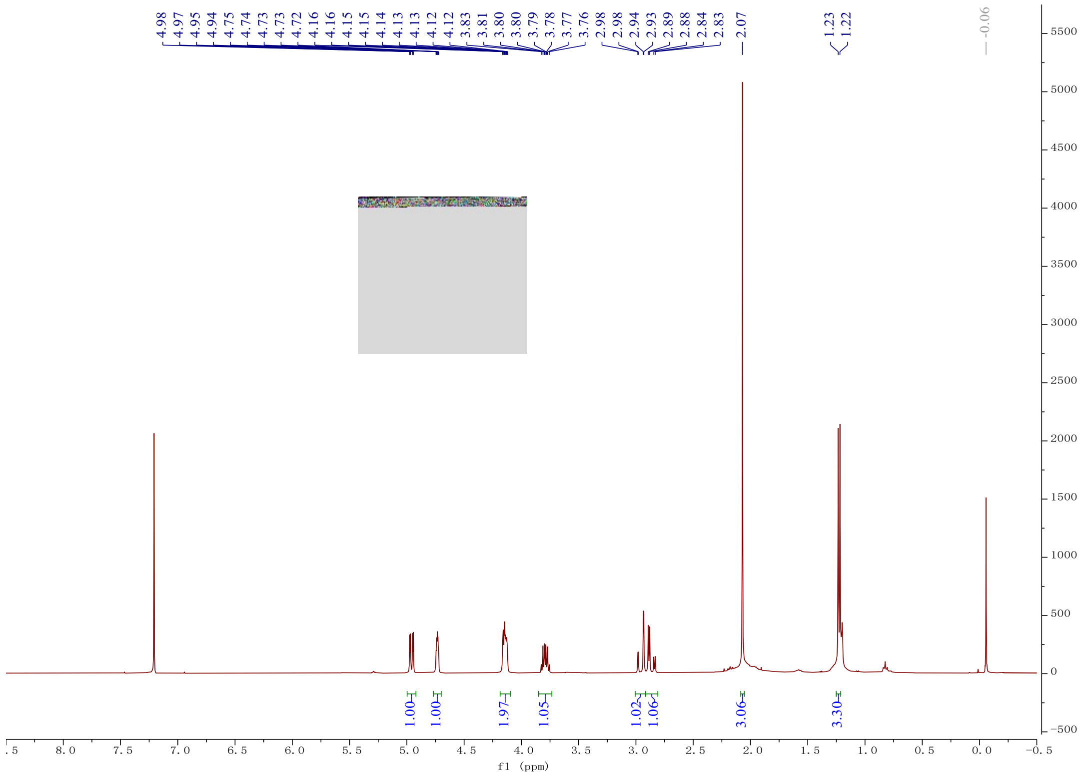
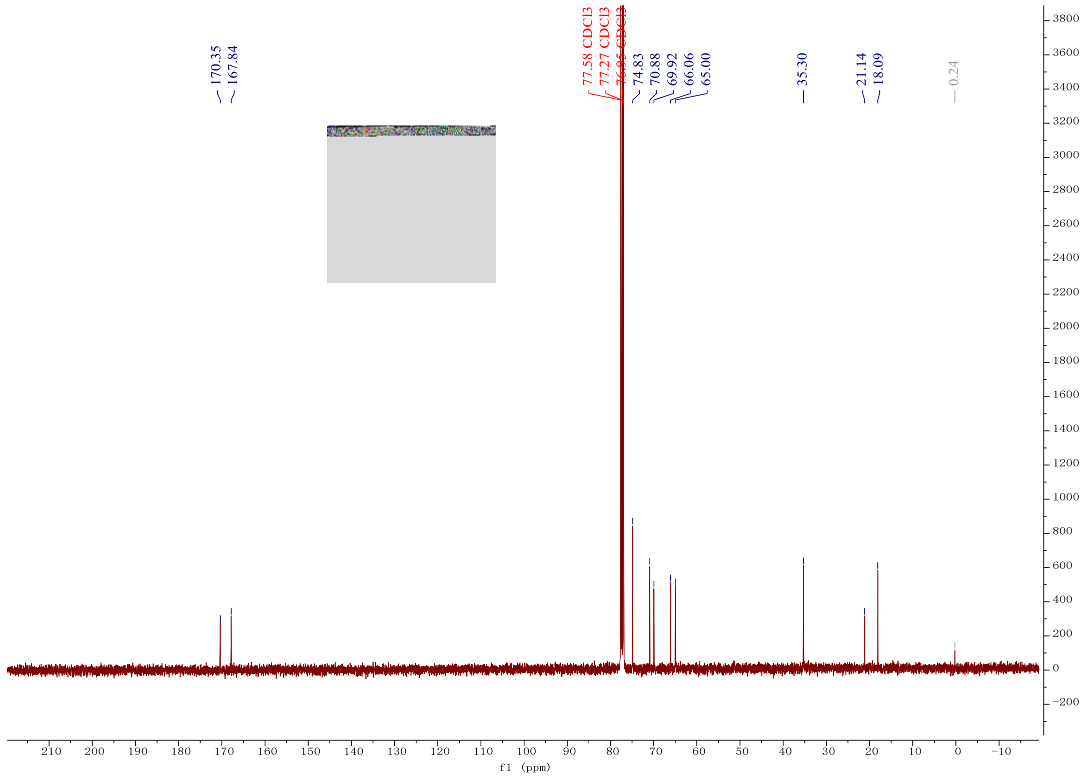


Figure 43. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of (+)-protulactone B (**2′**)



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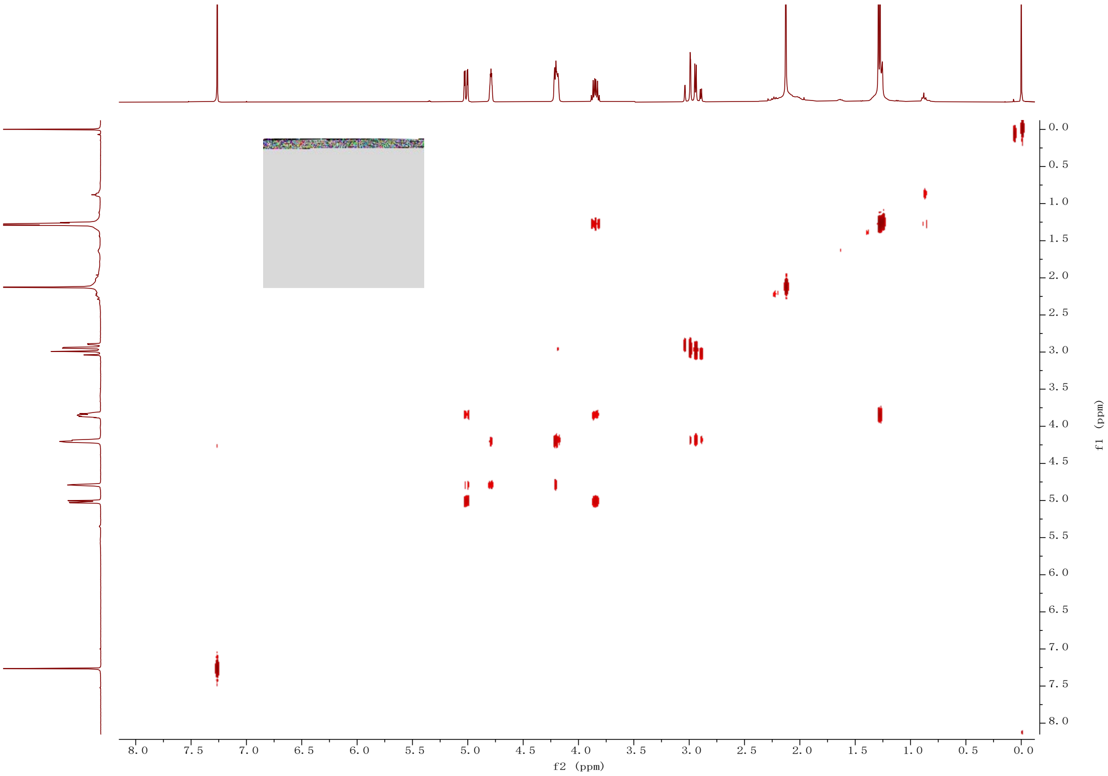


Figure 45. ^1H-^1H COSY (400 MHz, $CDCl_3$) spectrum of (+)-protulactone B (**2′**)

2. Comparison of ^{1}H and ^{13}C NMR data of Protulactone A and B

Table 1. Comparison of ^{1}H NMR data of natural and synthetic (−)-protulactone A (**1′**)

No	^{1}H-natural ^{a}	^{1}H-synthetic (Gracza) ^{b};	^{1}H-synthetic (our work) ^{c}	$\Delta_{\delta}=\delta_{a}-\delta_{c}$
1	-	-	-	
2	2.84 (1H, dd, 18.3, 4.8)	2.89−2.80 (m, 1H)	2.84 (1H, dd, 18.2, 4.8)	0
	2.52 (1H, br d, 18.3)	2.52 (dd, J = 18.1, 0.8 Hz, 1H)	2.57-2.47 (1H, m)	-
3	4.76 (1H, m)	4.82−4.77 (m, 2H)	4.82-4.74 (2H, m)	-
4	4.77 (1H, m)			
5	4.32 (1H, br d, 4.8)	4.33 (d, J = 4.7 Hz, 1H)	4.33 (1H, d, 4.8)	-0.01
6	3.79 (1H, dd, 4.8, 4.0)	3.80 (dd, J = 4.7, 4.0 Hz, 1H)	3.79 (1H, dd, 4.8, 4.0)	0
7	5.07 (1H, dq, 4.0, 6.6)	5.07 (dq, J = 4.0, 6.6 Hz, 1H)	5.07 (1H, dq, 4.0, 6.6)	0
8	1.24 (3H, d, 6.6)	1.25 (d, J = 6.6 Hz, 3H)	1.24 (3H, d, 6.6)	0
1’	-	-	-	-
2’	1.99 (3H, s)	2.00 (s, 3H)	1.99 (3H, s)	0

^{a} Spectra were recorded at 400 MHz (^{1}H NMR) in CD_3OD.[2]

^{b} Spectra were recorded at 300 MHz (^{1}H NMR) in CD_3OD.[4]

^{c} Spectra were recorded at 400 MHz (^{1}H NMR) in CD_3OD.

Table 2. Comparison of ^{13}C NMR data of natural and synthetic (−)-protulactone A (**1′**)

No	^{13}C-natural [a]	^{13}C-synthetic (Gracza) [b]	^{13}C-synthetic (our work) [c]	$\Delta_{\delta} = \delta_b - \delta_c$
1	176.6 (s)	177.5	177.5	0
2	35.7 (t)	37.0	37.0	0
3	77.8 (d)	79.1	79.2	-0.1
4	90.9 (d)	92.2	92.3	-0.1
5	75.3 (d)	76.6	76.6	0
6	88.8 (d)	90.1	90.2	-0.1
7	69.3 (d)	70.6	70.6	0
8	14.9 (q)	16.3	16.3	0
1’	171.0 (s)	172.1	172.1	0
2’	19.7 (q)	21.0	21.0	0

[a] Spectra were recorded at 100 MHz (^{13}C NMR) in CD_3OD.[2]

[b] Spectra were recorded at 75 MHz (^{13}C NMR) in CD_3OD.[4]

[c] Spectra were recorded at 100 MHz (^{13}C NMR) in CD_3OD.

Table 3. Comparison of ^{1}H NMR data of natural and synthetic (+)-protulactone B (**2′**)

No	^{1}H-natural [a]	^{1}H-synthetic (our work) [b]	^{1}H-synthetic (our work) [c]	$\Delta_{\delta} = \delta_{a} - \delta_{c}$
1	-	-	-	-
2	2.92 (1H, dd, 18.6, 1.1)	2.96 (1H, dd, 19.4, 1.6)	2.93 (1H, dd, 19.2, 0.8)	-0.01
	2.85 (1H, dd, 18.6, 4.7)	2.86 (1H, dd, 19.4, 5.0)	2.85 (1H, dd, 19.2, 4.6)	0
3	4.12 (1H, m)	4.14 (2H, m)	4.17-4.05 (2H, m)	-
4	4.09 (1H, m)			
5	4.70 (1H, ddd, 4.6, 2.3, 2.3)	4.73(1H, ddd, 4.8, 2.4, 2.4)	4.71 (1H, ddd, 4.6, 2.2, 2.2)	-0.01
6	4.97 (1H, dd, 10.1, 2.3)	4.96 (1H, dd, 10.0, 2.6)	4.96 (1H, dd, 10.0, 2.6)	+0.01
7	3.78 (1H, dq, 6.0, 10.1)	3.79 (1H, dq, 6.0, 10.0)	3.78 (1H, dq, 5.8, 10.6)	0
8	1.22 (3H, d, 6.0)	1.22 (3H, d, 6.0)	1.22 (3H, d, 6.0)	0
1’	-	-	-	-
2’	2.07 (3H, s)	2.07 (3H, s)	2.07 (3H, s)	0

[a] Spectra were recorded at 400 MHz (^{1}H NMR) in $CDCl_3$ with drops of CD_3OD.[2]

[b] Spectra were recorded at 400 MHz (^{1}H NMR) in $CDCl_3$.

[c] Spectra were recorded at 400 MHz (^{1}H NMR) in $CDCl_3$ with 15μL CD_3OD.

Table 4. Comparison of ^{13}C NMR data of natural and synthetic (+)-protulactone B (**2′**)

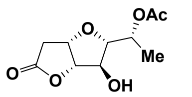
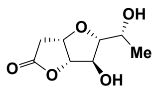
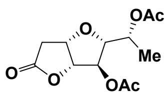
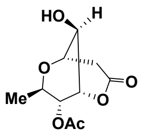
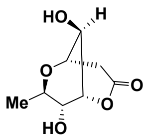
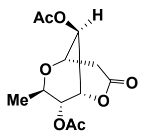
No	^{13}C-Natural [a]	^{13}C-Our work [b]	^{13}C-Our work [c]	$\Delta_{\delta} = \delta_a - \delta_c$
1	168.6	167.8	168.5	+0.1
2	35.3	35.3	35.3	0
3	69.5	69.9	69.6	-0.1
4	65.4	66.1	65.5	-0.1
5	75.0	74.8	75.0	0
6	71.1	70.9	71.1	0
7	64.5	65.0	64.6	-0.1
8	17.8	18.1	17.8	0
1’	170.7	170.4	170.6	+0.1
2’	21.0	21.1	21.0	0

[a] Spectra were recorded at 100 MHz (^{13}C NMR) in $CDCl_3$ with drops of CD_3OD.[2]

[b] Spectra were recorded at 100 MHz (^{13}C NMR) in $CDCl_3$.

[c] Spectra were recorded at 100 MHz (^{13}C NMR) in $CDCl_3$ with 15μL CD_3OD.

Table **5.** In vitro Cytotoxicity of protulactones A (**1′**) and B (**2′**) and their derivatives

Compounds		MCF-7 (x±s) [a]	Capan-2(x±s) [b]
 (-)-Protulactone A, (1')	30 μM	5.22%±5.27%	-3.44%±0.43%
	10 μM	0.38%±3.08%	-8.60%±1.22%
	3 μM	1.57%±3.03%	-5.74%±0.95%
 3	30 μM	13.87%±5.93%	-0.32%±2.85%
	10 μM	13.76%±5.22%	-6.79%±5.41%
	3 μM	2.71%±2.32%	-4.14%±2.43%
 13	30 μM	17.42%±1.48%	-5.70%±3.15%
	10 μM	9.64%±2.37%	-7.11%±1.58%
	3 μM	2.16%±6.06%	-5.99%±0.86%
 (+)-Protulactone B, (2')	30 μM	-4.28%±8.70%	-3.86%±3.98%
	10 μM	7.13%±4.62%	-7.39%±2.32%
	3 μM	-4.98%±3.63%	-4.56%±1.68%
 4	30 μM	4.61%±6.56%	-7.90%±2.22%
	10 μM	2.54%±1.21%	-9.62%±0.92%
	3 μM	-1.56%±3.88%	-13.00%±2.43%
 17	30 μM	11.32%±3.62%	-5.77%±4.20%
	10 μM	5.22%±4.36%	-8.00%±1.13%
	3 μM	-3.41%±7.70%	-9.43%±3.48%

[a] Mcf-7 was provided by Cell Culture Center at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

[b] Capan-2 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).