

Development of Mithramycin Analogues with Increased Selectivity towards ETS Transcription Factor Expressing Cancers

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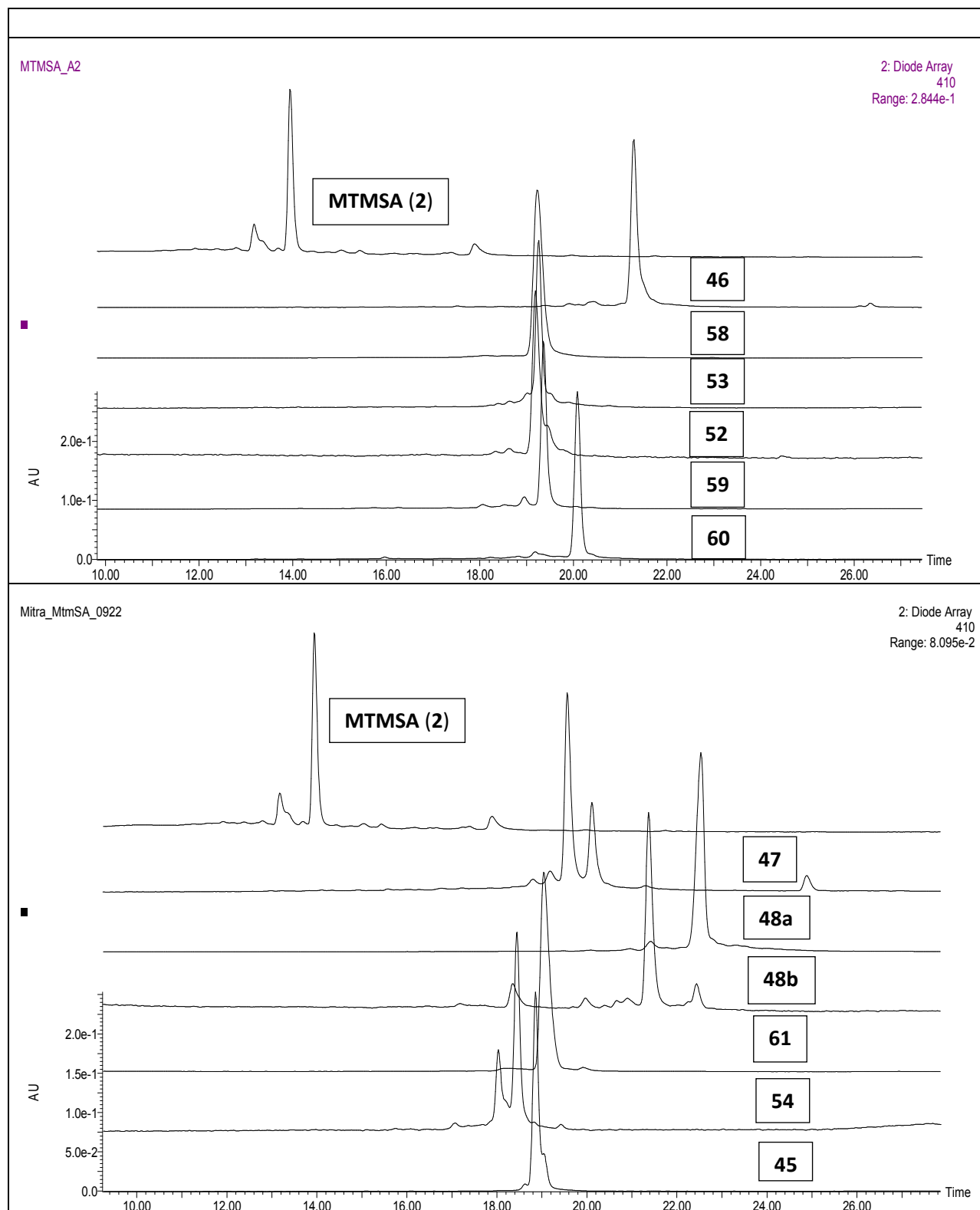
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Supporting Information (SI)

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1. HPLC profile of MTMSA analogues

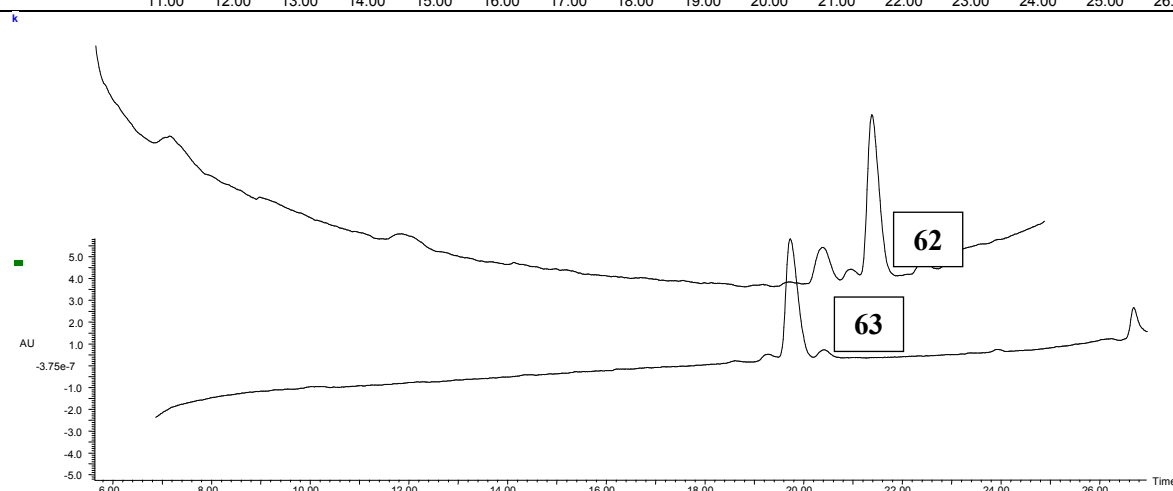
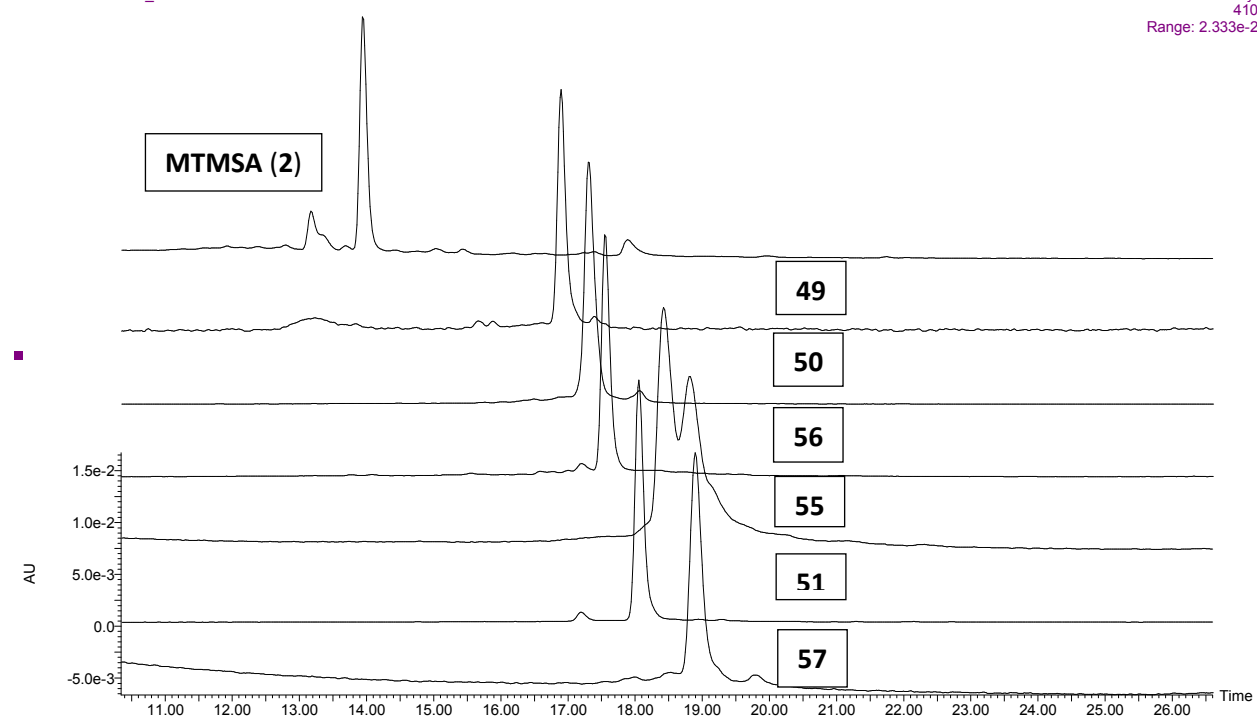


HPLC profile of MTMSA analogues

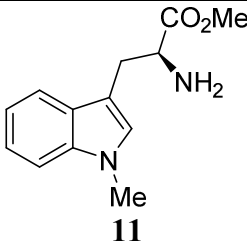
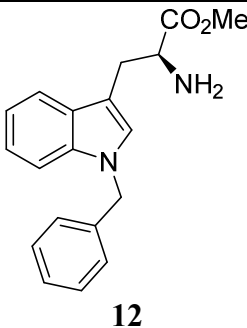
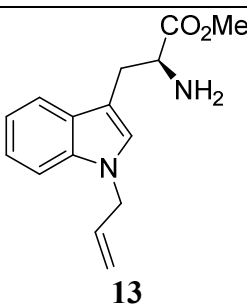
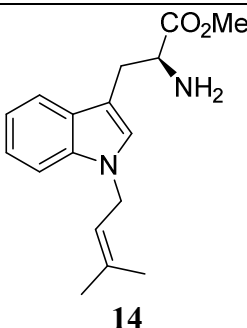
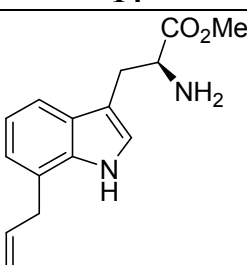
Prithiba

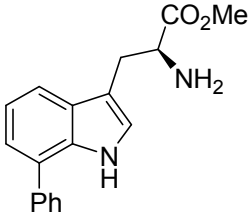
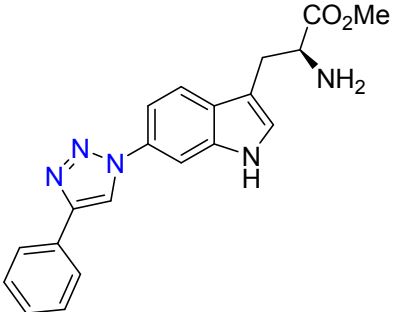
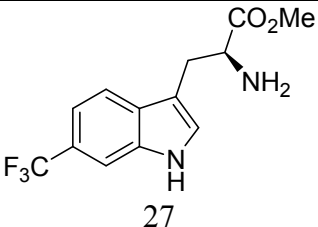
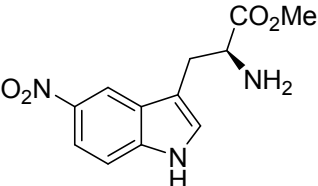
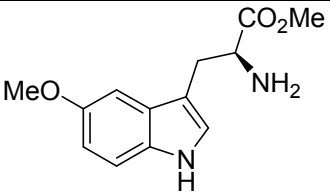
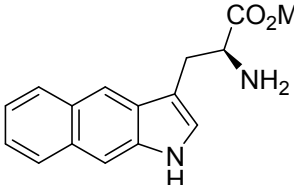
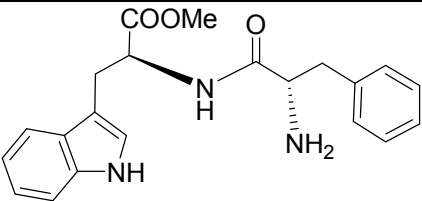
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410
Range: 2.333e-2



2. LCMS data of free amines

Entry	Amine	LCMS (ES+) m/z calcd for	LCMS Found
1	 11	$C_{13}H_{17}N_2O_2$ $[M+H]^+$ 233.1	233.1
2	 12	$C_{19}H_{21}N_2O_2$ $[M+H]^+$ 309.1	309.1
3	 13	$C_{15}H_{19}N_2O_2$ $[M+H]^+$ 259.1	259.1
4	 14	$C_{17}H_{23}N_2O_2$ $[M+H]^+$ 287.2	287.1
5		$C_{15}H_{19}N_2O_2$ $[M+H]^+$ 259.1	259.1

6		$C_{18}H_{19}N_2O_2$ $[M+H]^+$ 295.1	295.1
7	 23	$C_{20}H_{20}N_5O_2$ $[M]^+$ 362.1	362.1
8	 27	$C_{13}H_{14}F_3N_2O_2$ $[M+H]^+$ 287.1	287.0
9		$C_{12}H_{14}N_3O_4$ $[M+H]^+$ 264.1	264.0
10		$C_{13}H_{17}N_2O_3$ $[M+H]^+$ 249.1	249.1
11		$C_{16}H_{17}N_2O_2$ $[M+H]^+$ 269.1	269.0
12		$C_{21}H_{24}N_3O_3$ $[M+H]^+$ 366.1	366.1

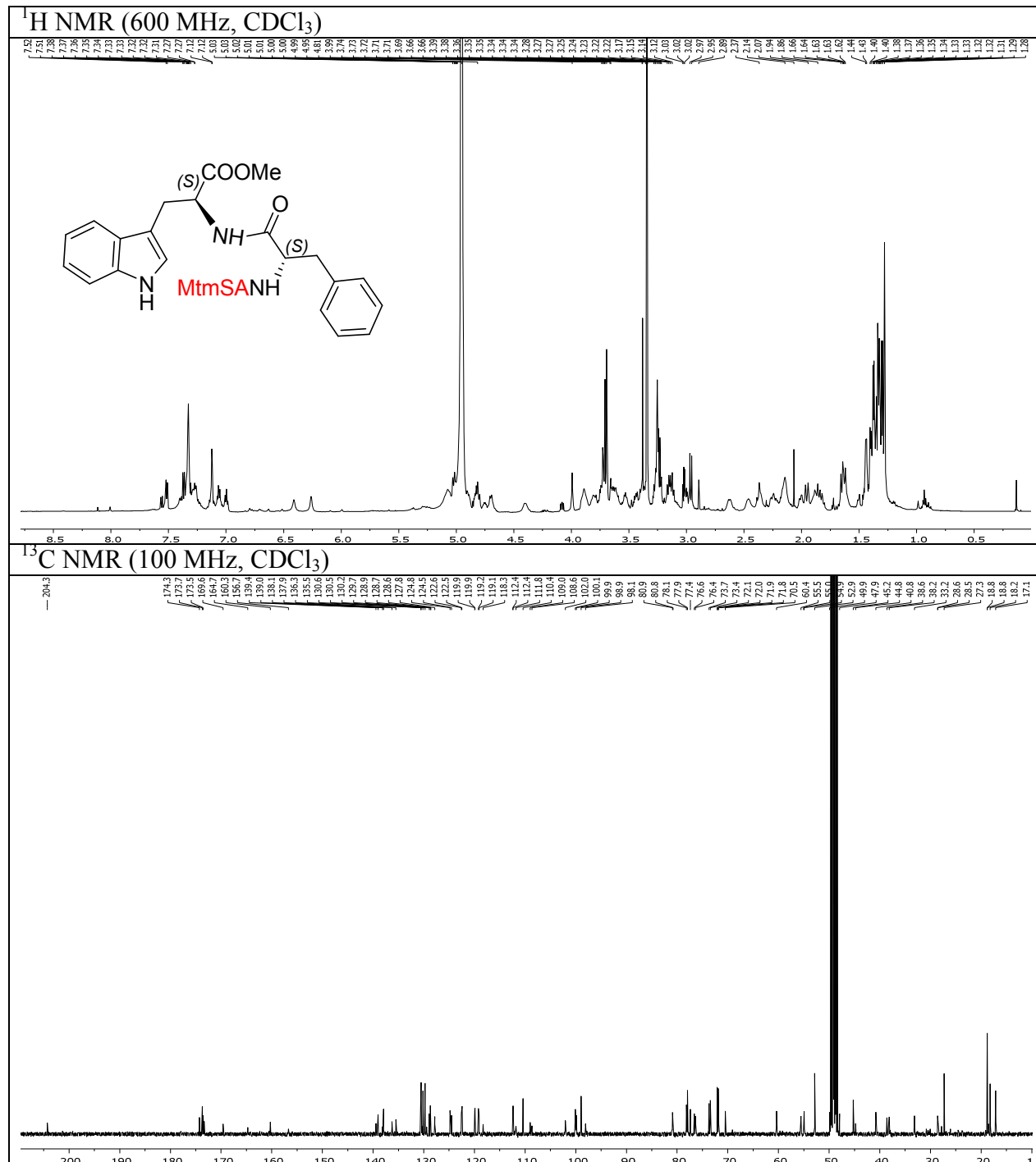
3. HRMS data of analogues

Entry	MTMSA Analogues	HRMS -TOF MS	Yield
1	50	[M-H] calcd for $C_{61}H_{80}N_3O_{26}$ 1270.5030; Found 1270.5024	0.9 mg, 14%
2	60	[M-H] calcd for $C_{70}H_{90}N_3O_{25}$ 1372.5863; Found 1372.5890	12 mg, 10%
3	59	[M-H] calcd for $C_{70}H_{90}N_3O_{25}$ 1372.5863; Found 1372.5905	0.6 mg, 12%
4	52	[M-H] calcd for $C_{67}H_{85}N_2O_{24}$ 1301.5492; Found 1301.5460	3.4 mg, 26%
5	53	[M-H] calcd for $C_{64}H_{85}N_2O_{24}$ 1265.5492; Found 1265.5509	0.5 mg, 11%
6	58	[M-H] calcd for $C_{63}H_{82}NO_{24}$ 1236.5227; Found 1236.5288	1.6 mg, 16%
7	46	[M-H] calcd for $C_{68}H_{87}N_2O_{24}$ 1315.5649; Found 1315.5654	0.6 mg, 13%
8	45	[M-H] calcd for $C_{62}H_{83}N_2O_{24}$ Exact Mass: 1239.5336; Found 1239.5309	0.4 mg, 10%
9	54	[M-H] calcd for $C_{69}H_{86}N_5O_{24}$ 1368.5663; Found 1368.5635	0.9 mg, 18%
10	61	[M-H] calcd for $C_{72}H_{91}N_4O_{25}$ 1411.5972; Found 1411.5985	17 mg, 14%

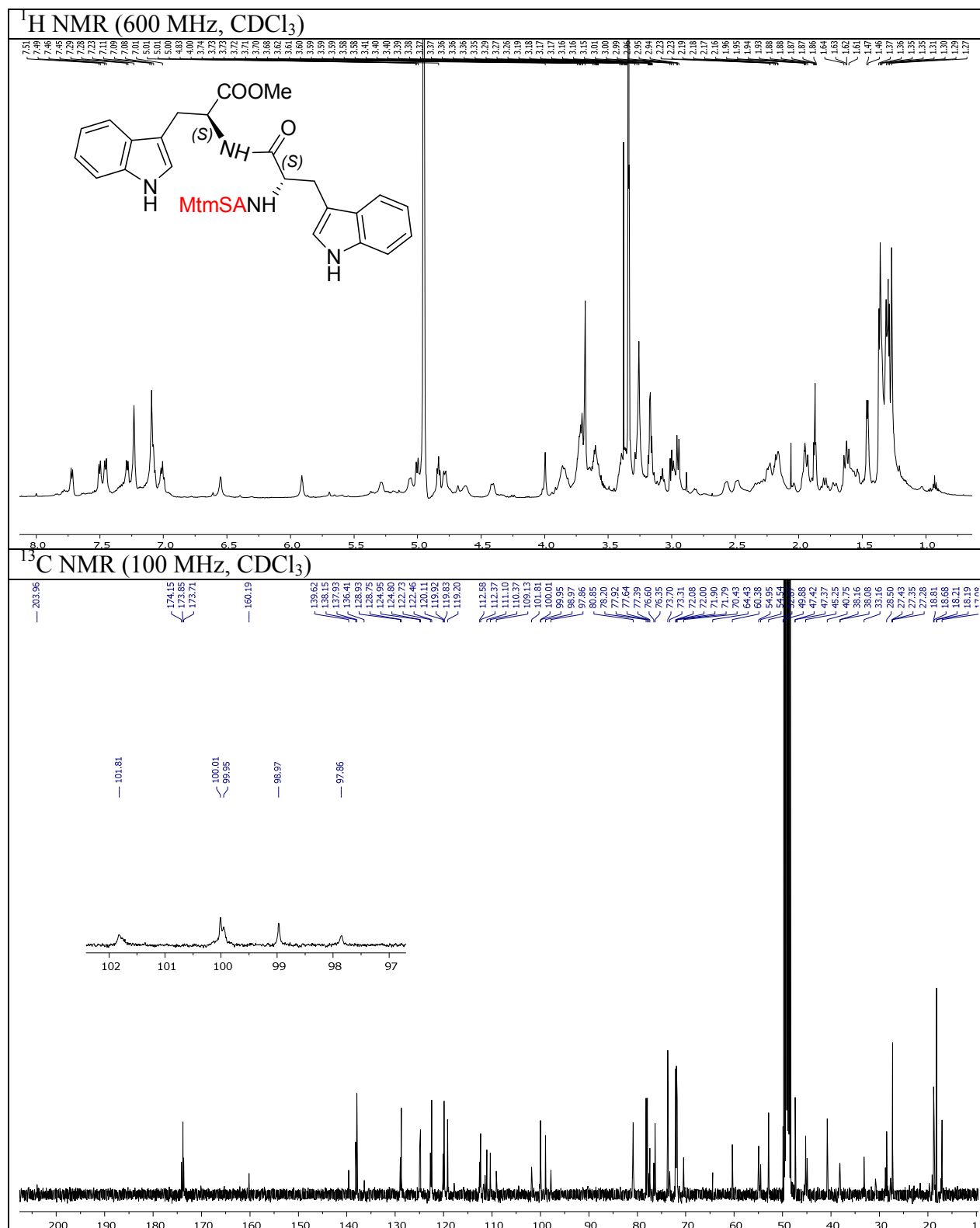
11	48a	[M-H] calcd for $C_{66}H_{89}N_2O_{24}$ 1293.5805; 1293.5876	0.6 mg, 12%
12	48b	[M-H] calcd for $C_{66}H_{89}N_2O_{24}$ 1293.5805; 1293.5824	2.2 mg, 22%
13	47	[M-H] calcd for $C_{64}H_{85}N_2O_{24}$ 1265.5492; Found 1266.5557	0.4 mg, 10%
14	57	[M-H] calcd for $C_{65}H_{83}N_2O_{24}$ 1275.5336; Found 1275.5374	1.8 mg, 20%
15	51	[M-H] calcd for $C_{64}H_{85}N_2O_{25}$ 1281.5441 Found 1281.5485	2.2 mg, 22%
16	55	[M-H] calcd for $C_{62}H_{80}F_3N_2O_{24}$ 1293.5053; Found 1293.5065	0.6 mg, 10%
17	56	[M-H] calcd for $C_{61}H_{80}FN_2O_{24}$ 1243.5085; Found 1243.5108	1.1 mg, 17%
18	49	[M-H] calcd for $C_{62}H_{83}N_2O_{25}$ 1255.5285; Found 1255.5267	1.5 mg, 14%
19	62	[M-H] calcd for $C_{74}H_{95}N_4O_{25}$ 1439.6285; Found 1439.6305	1.4 mg, 17%
20	63	[M-H] calcd for $C_{68}H_{89}N_2O_{25}$ 1333.5754; Found 1333.5756	0.4 mg, 10%

4. Select NMR spectra

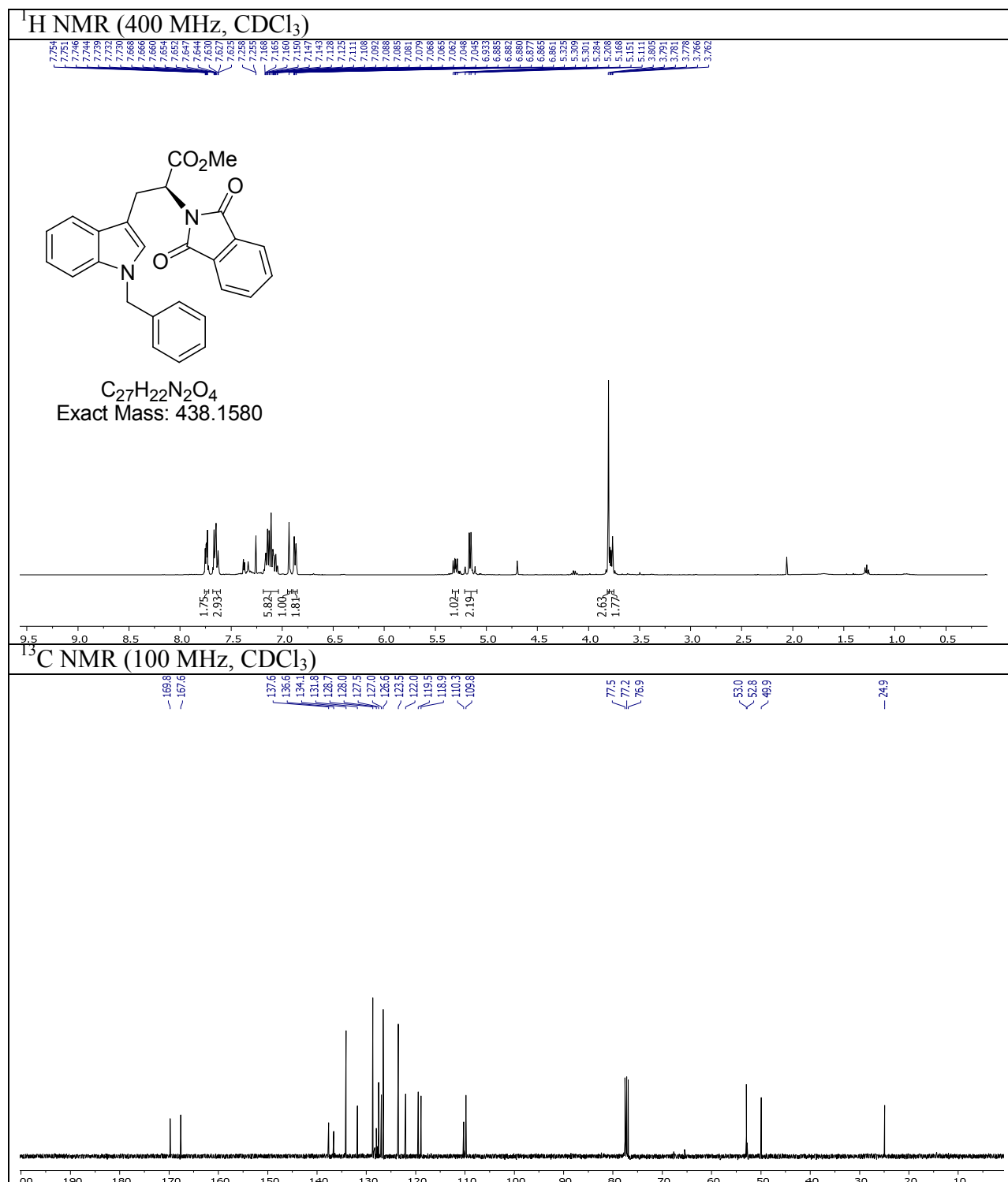
Analogue 60



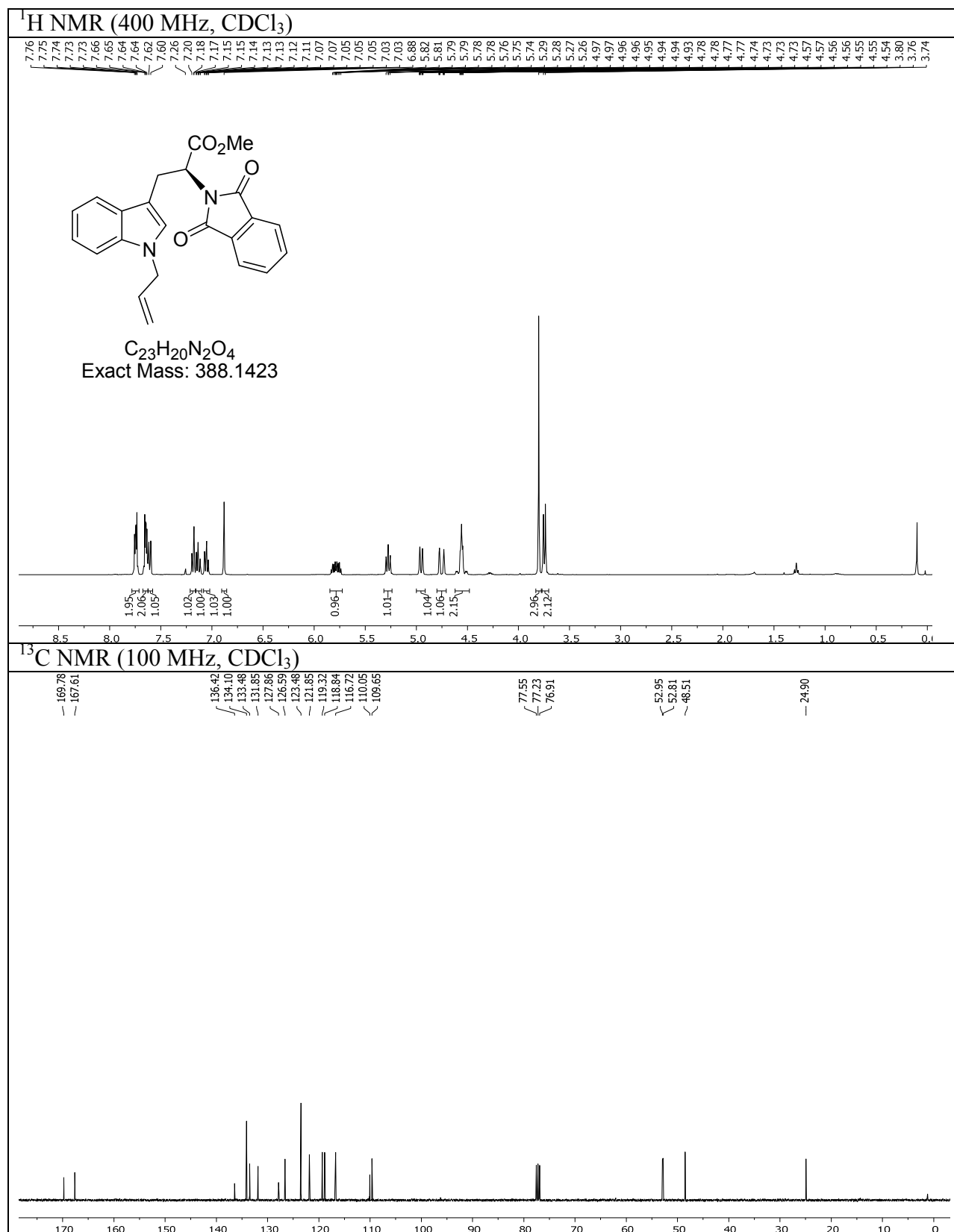
Analogue 61



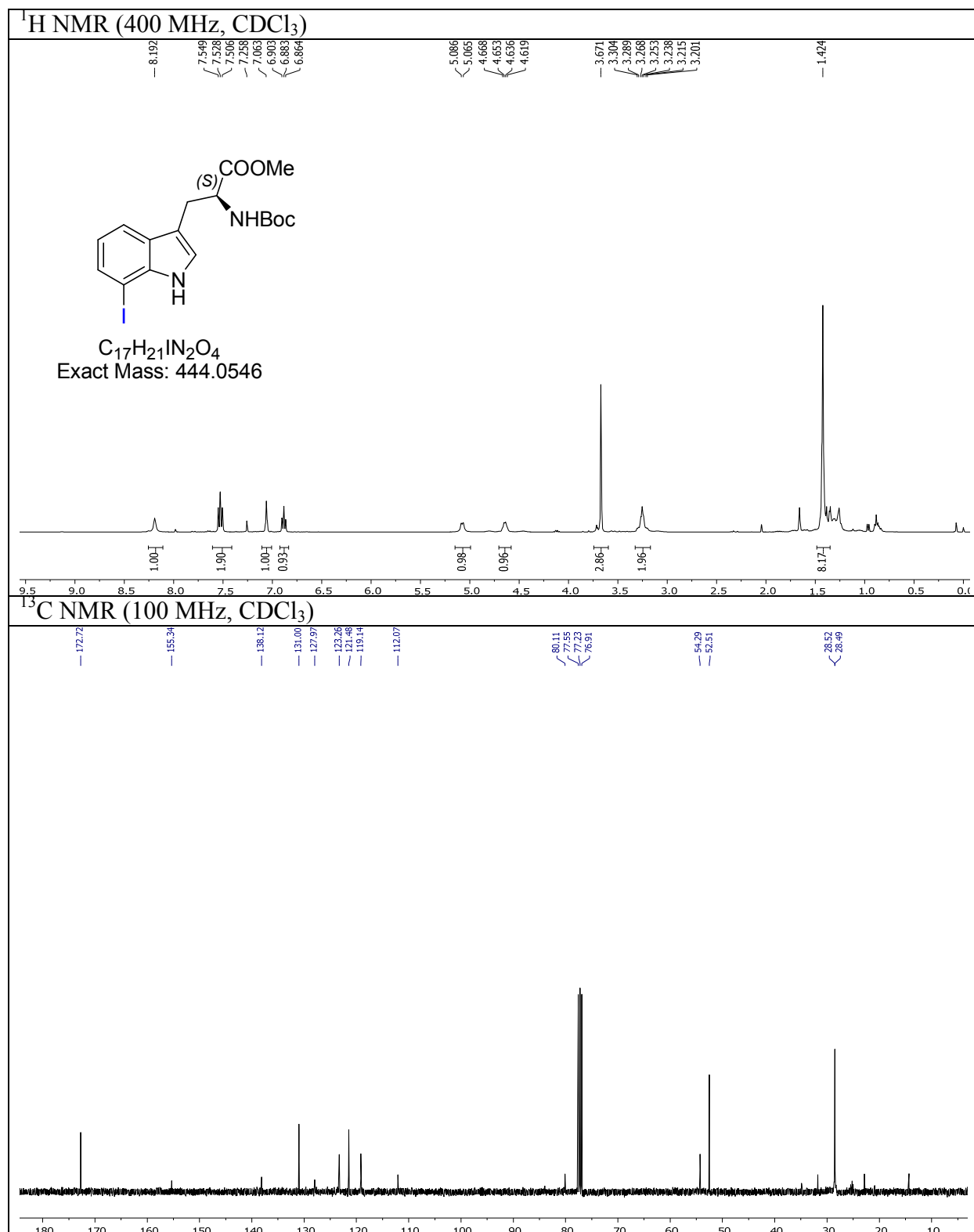
(S)-Methyl-3-(1-benzyl-1H-indol-3-yl)-2-(1,3-dioxoisindolin-2-yl)propanoate (8)



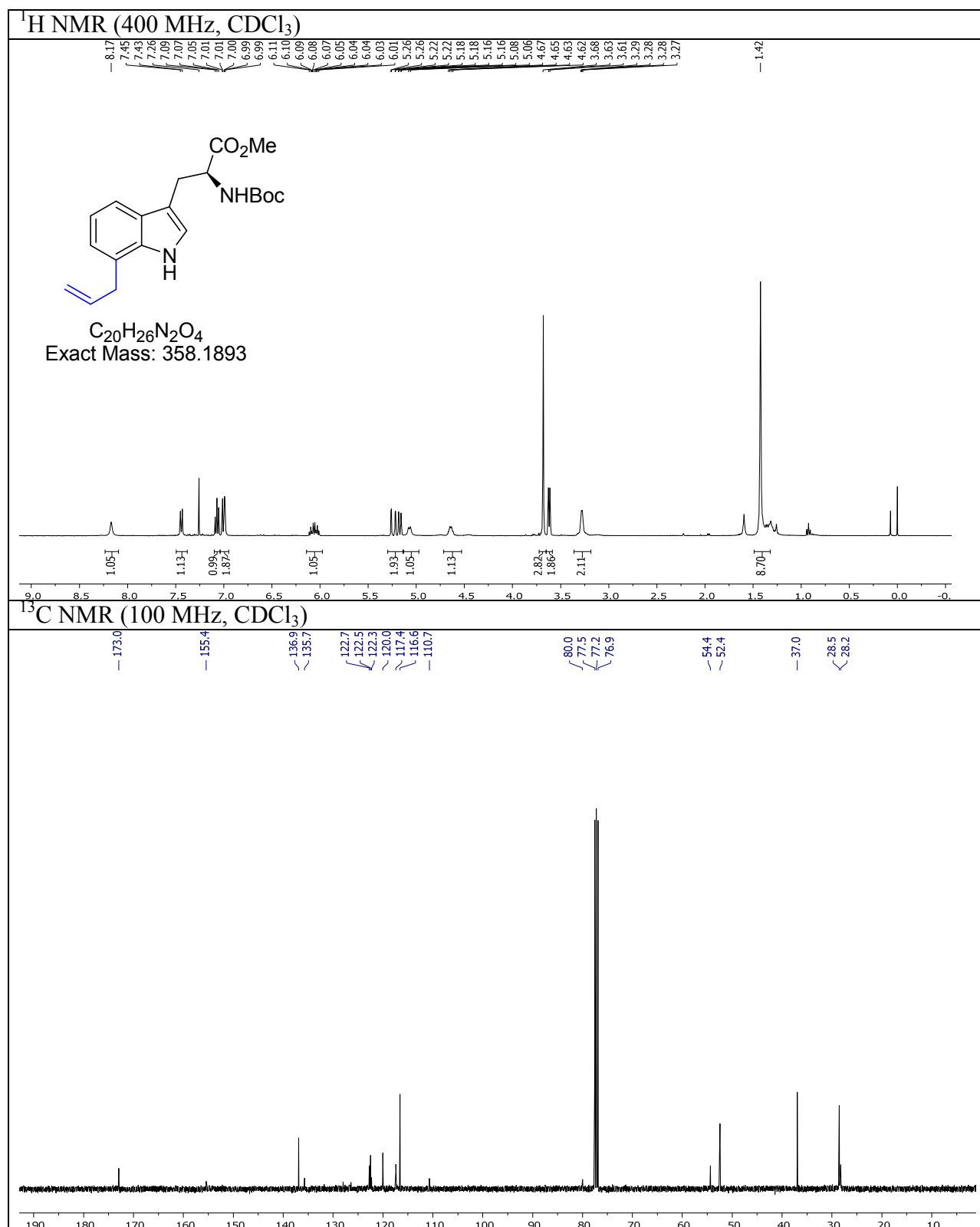
(S)-Methyl-3-(1-allyl-1*H*-indol-3-yl)-2-(1,3-dioxisoindolin-2-yl)propanoate (9)



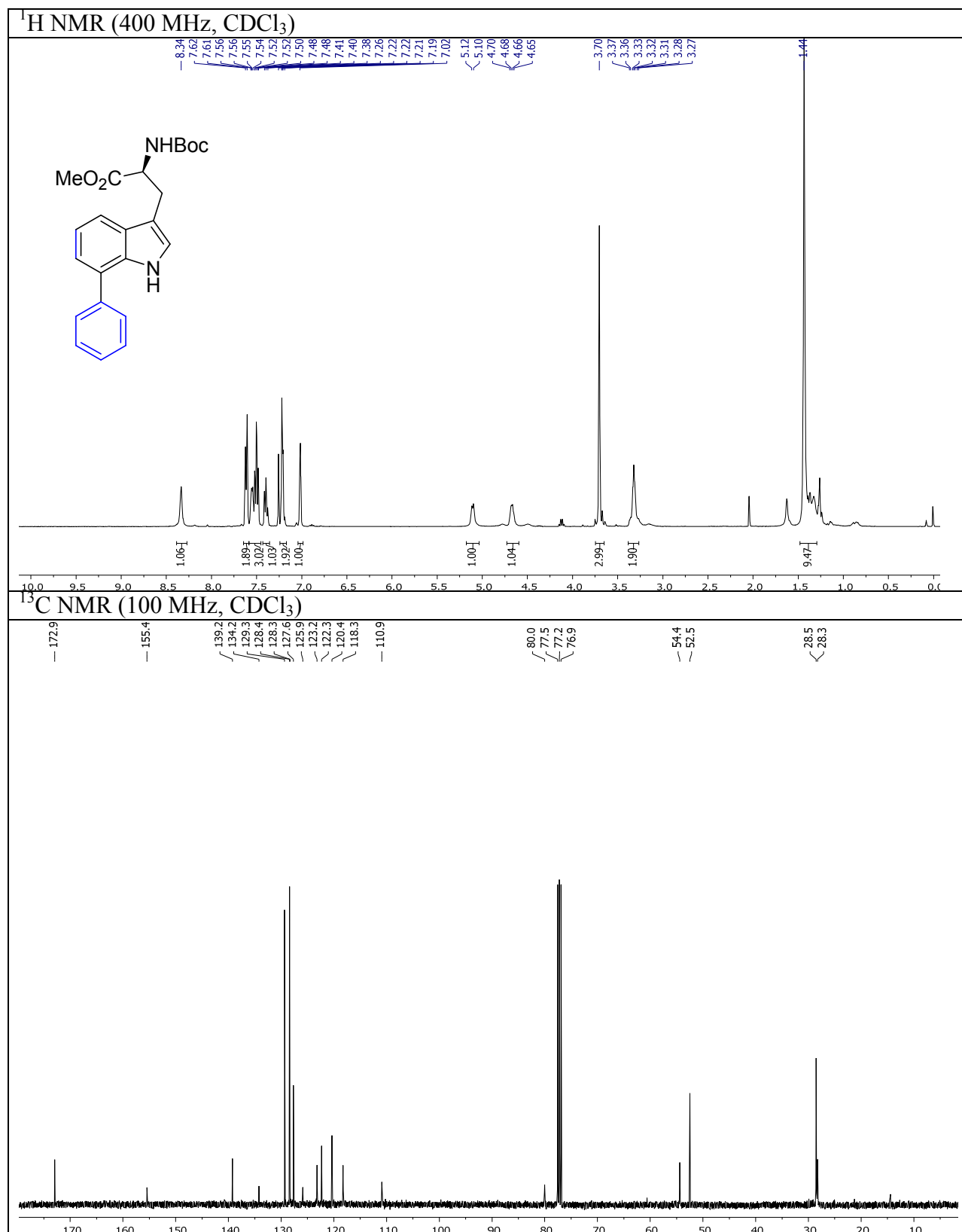
(R)-Methyl-2-(*tert*-butoxycarbonyl)-3-(7-iodo-1*H*-indol-3-yl)propanoate (18)



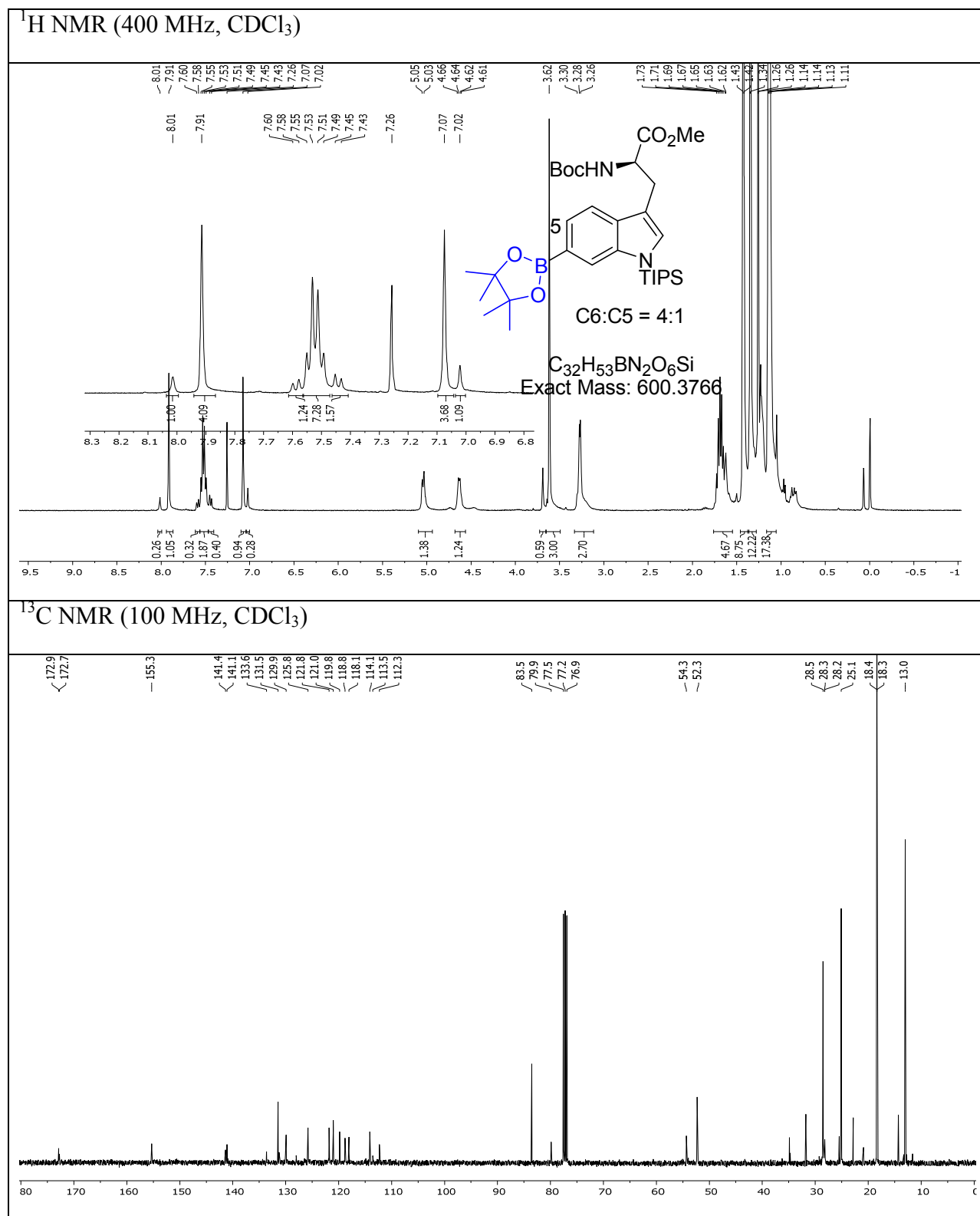
(*R*)-Methyl-3-(7-allyl-1*H*-indol-3-yl)-2-(*tert*-butoxycarbonyl)propanoate (17)



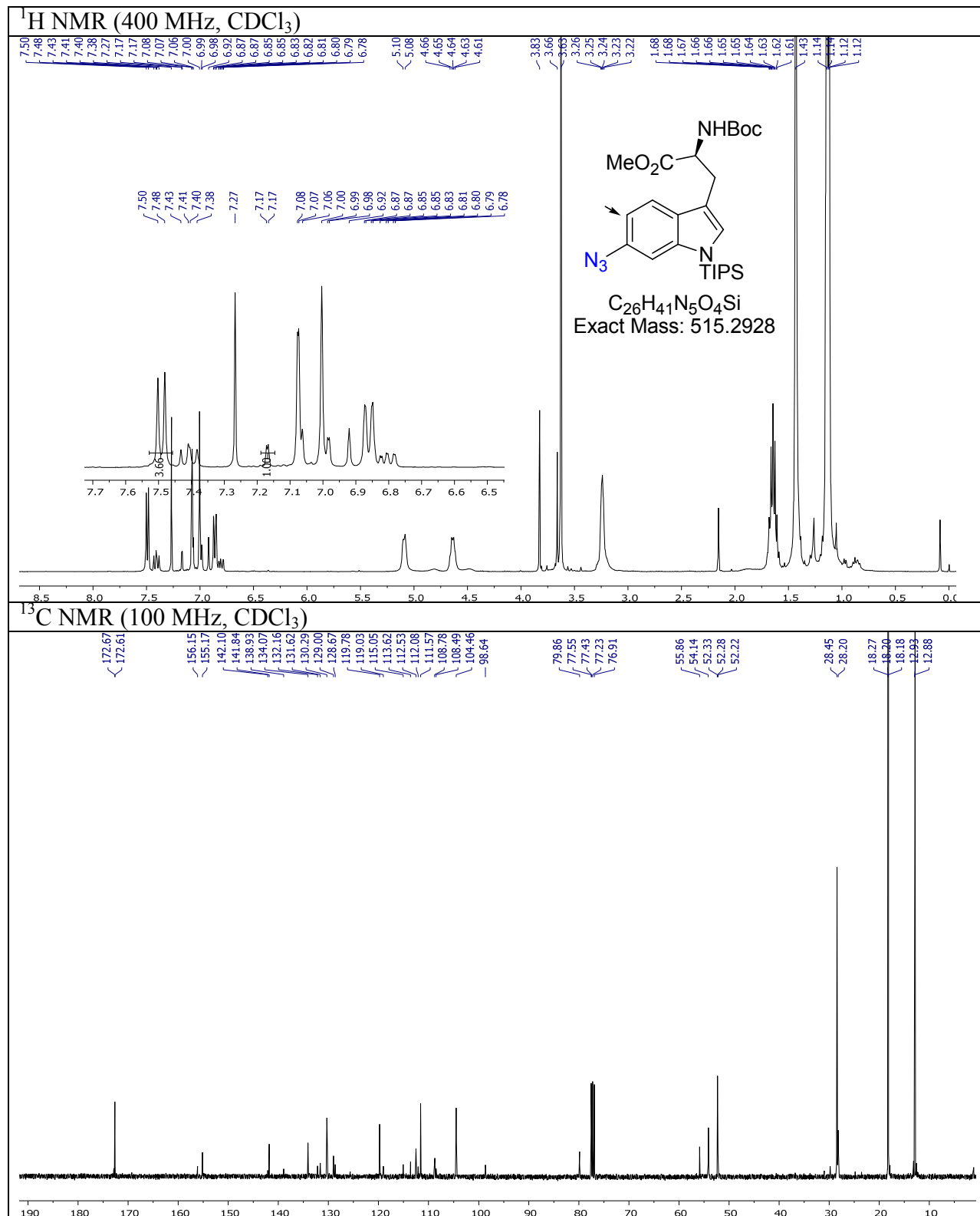
(*R*)-Methyl-2-(*tert*-butoxycarbonyl)-3-(7-phenyl-1*H*-indol-3-yl)propanoate (20)



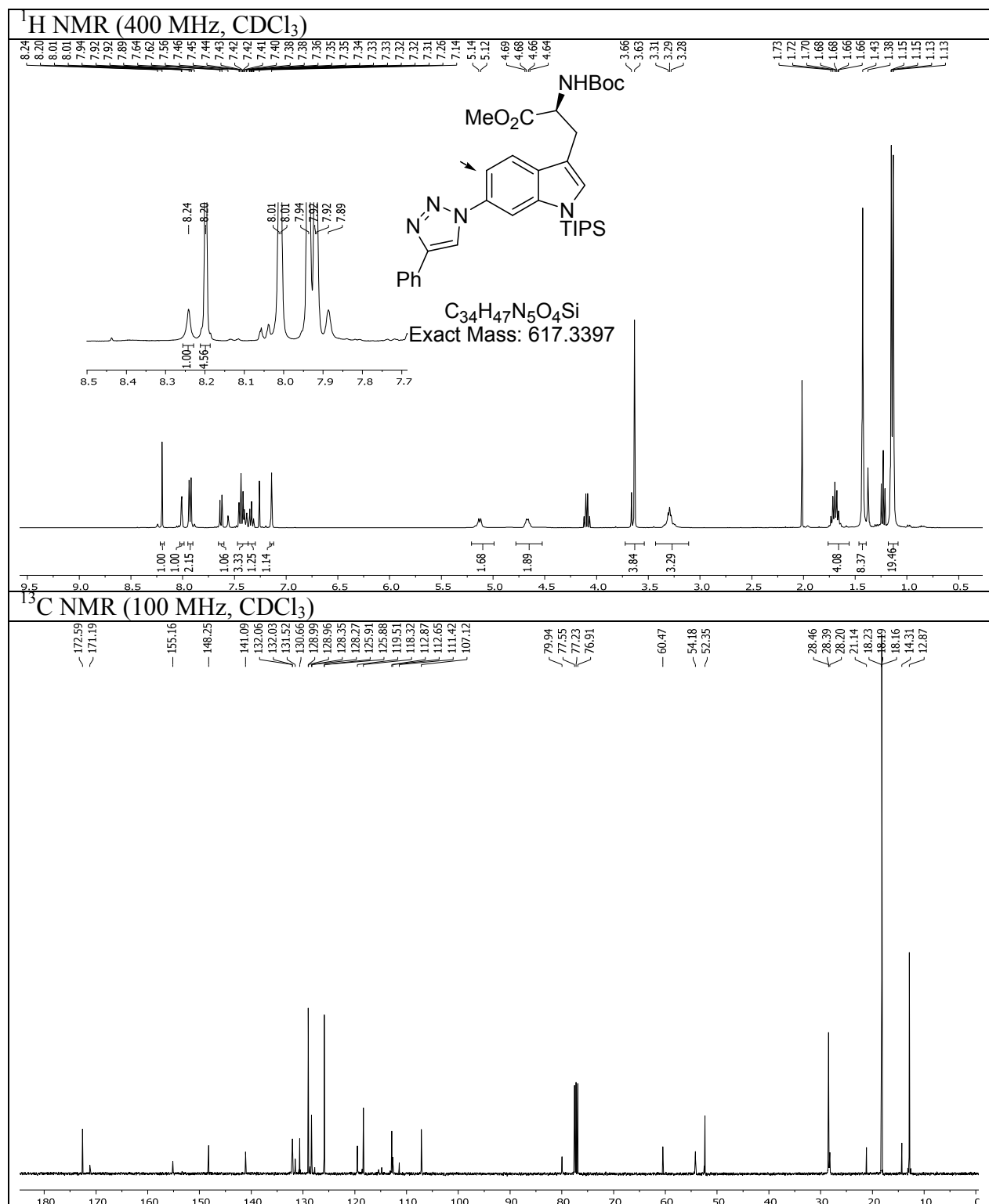
(S)-Methyl-2-((*tert*-butoxycarbonyl)amino)-3-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(triisopropylsilyl)-1*H*-indol-3-yl)propionate (22)



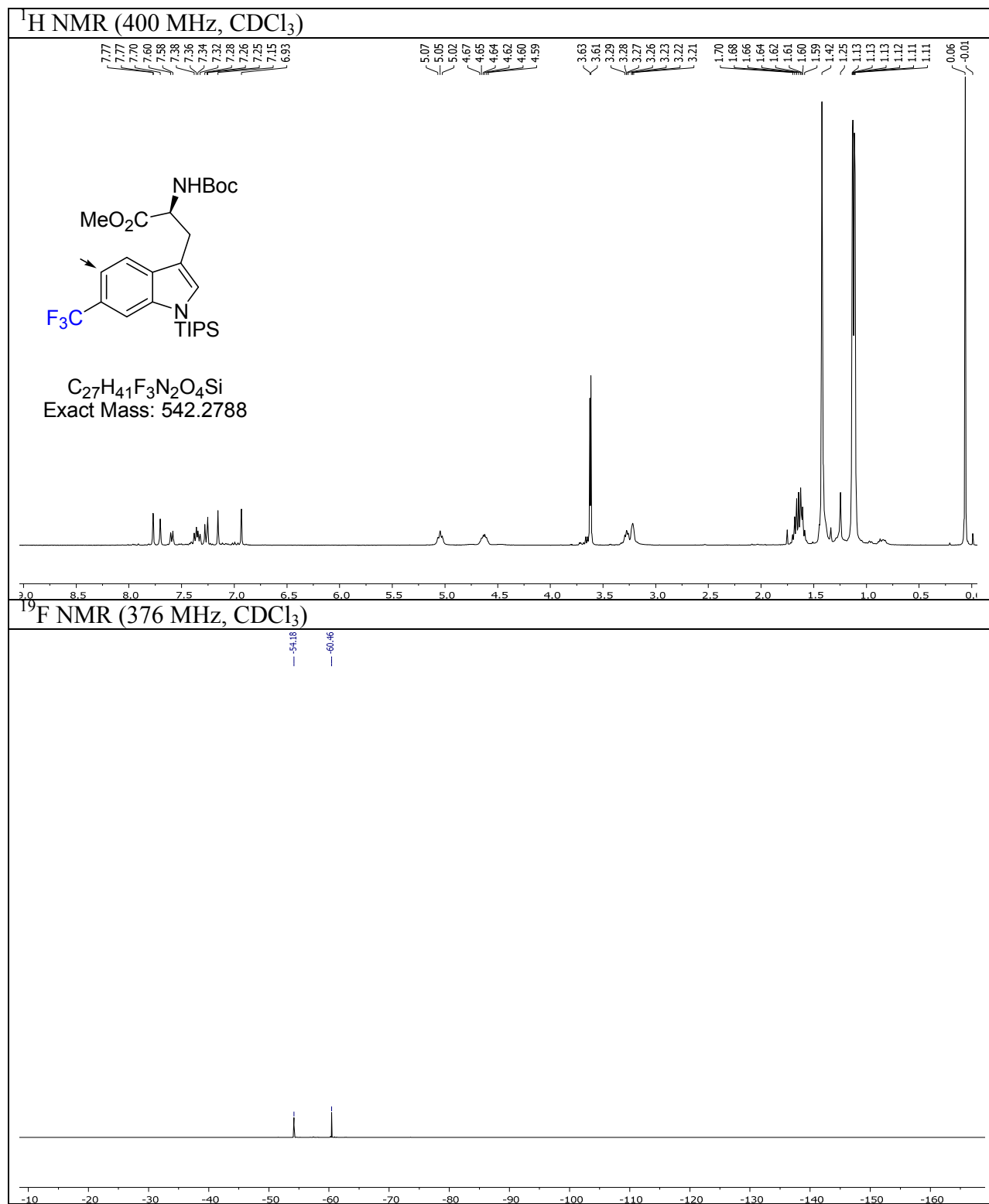
(*R*)-Methyl-3-(6-azido-1-(triisopropylsilyl)-1*H*-indol-3-yl)-2-(*ter*-butoxycarbonyl) propionate (24**, mixture of C6 and C5 isomers, major C6 isomer)**



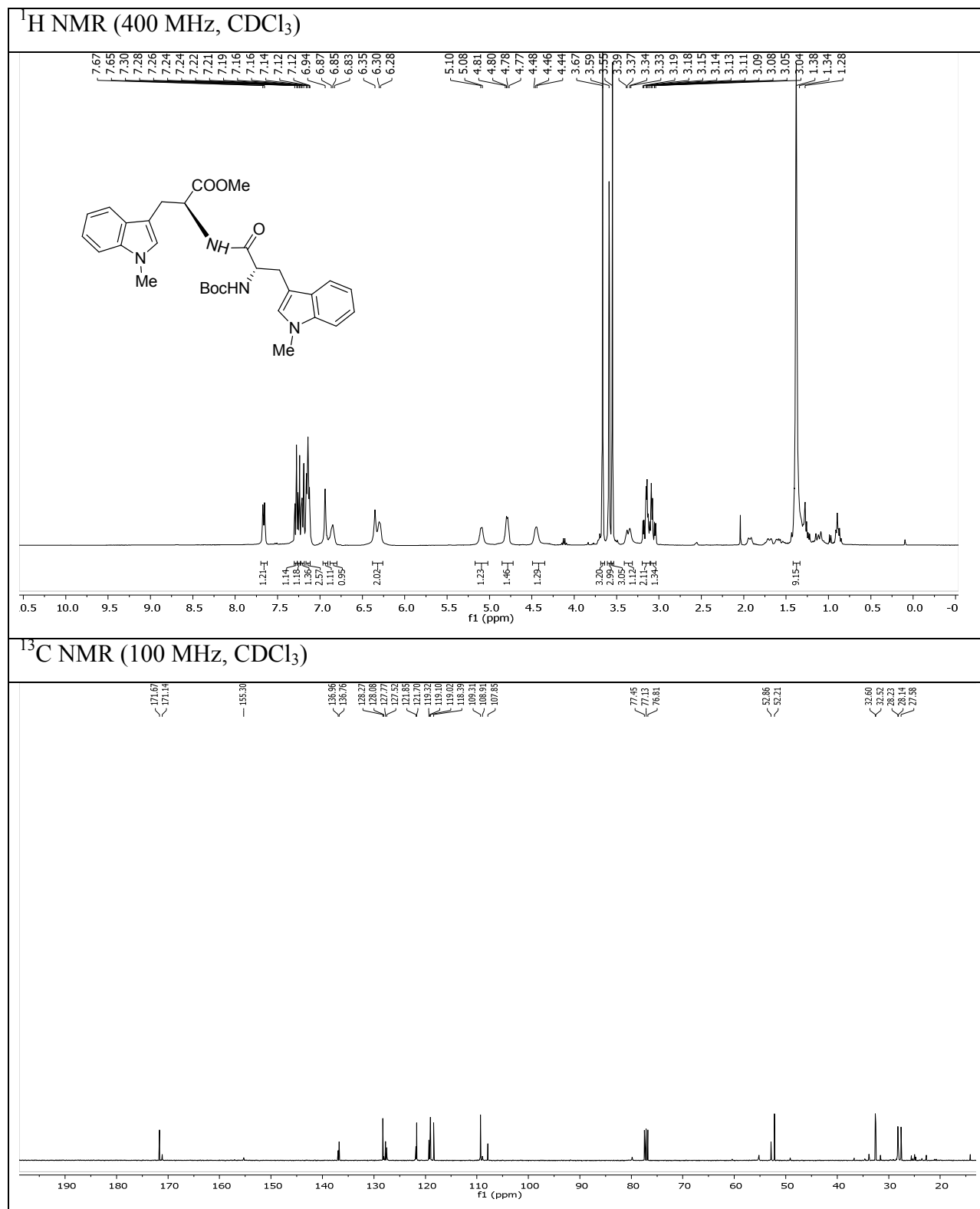
(2*R*)-Methyl-2-(*tert*-butoxycarbonyl)-3-(6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-(triisopropylsilyl)-1*H*-indol-3-yl)propionate (25, mixture of C6 and C5 isomers, major C6 isomer)



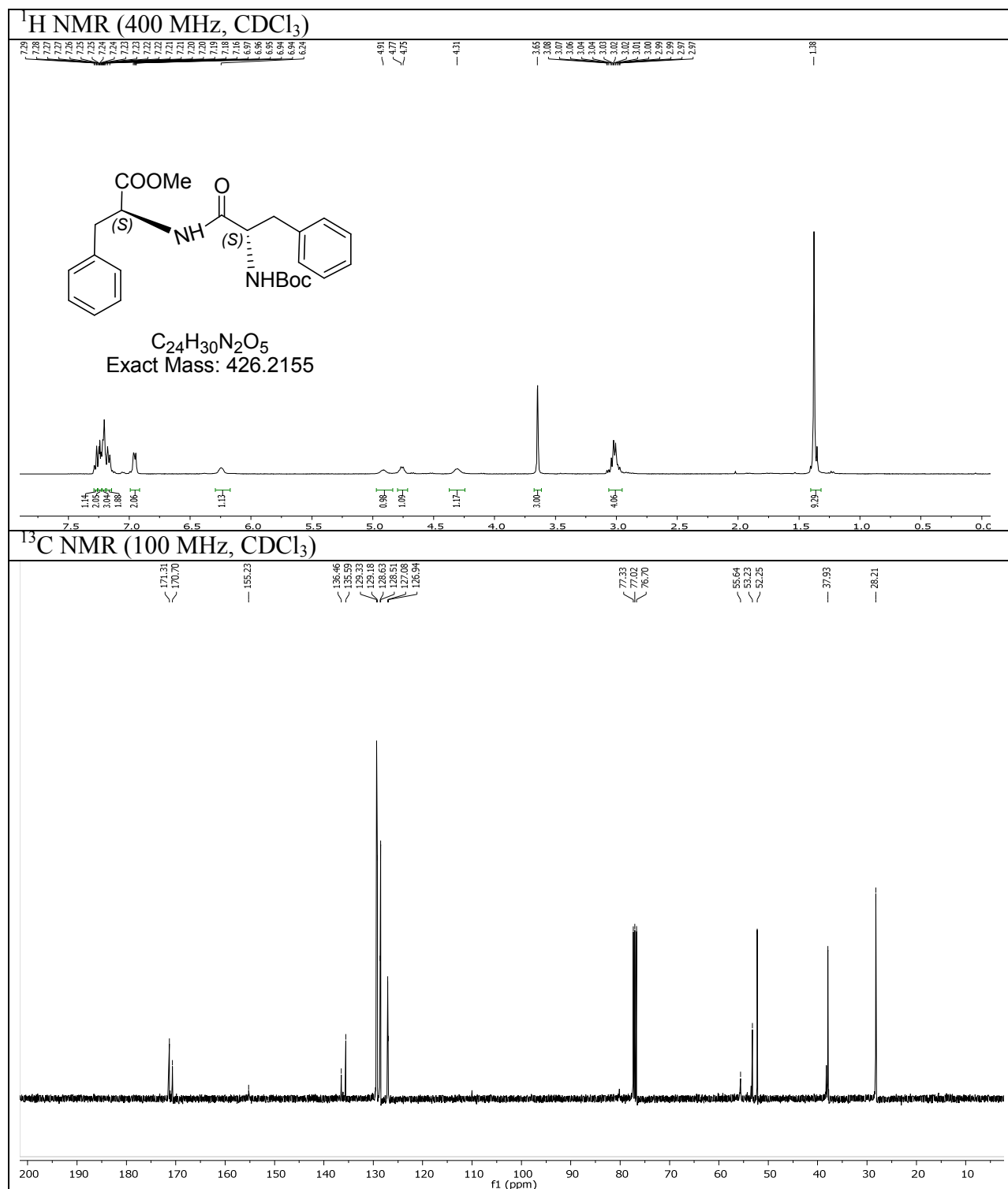
Methyl-2-*tert*-butoxycarbonylamino-3-(6-trifluoromethyl-1-triisopropylsilanyl-1*H*-indol-3-yl)propionate (26, mixture of C6 and C5 isomers, major C6 isomer)



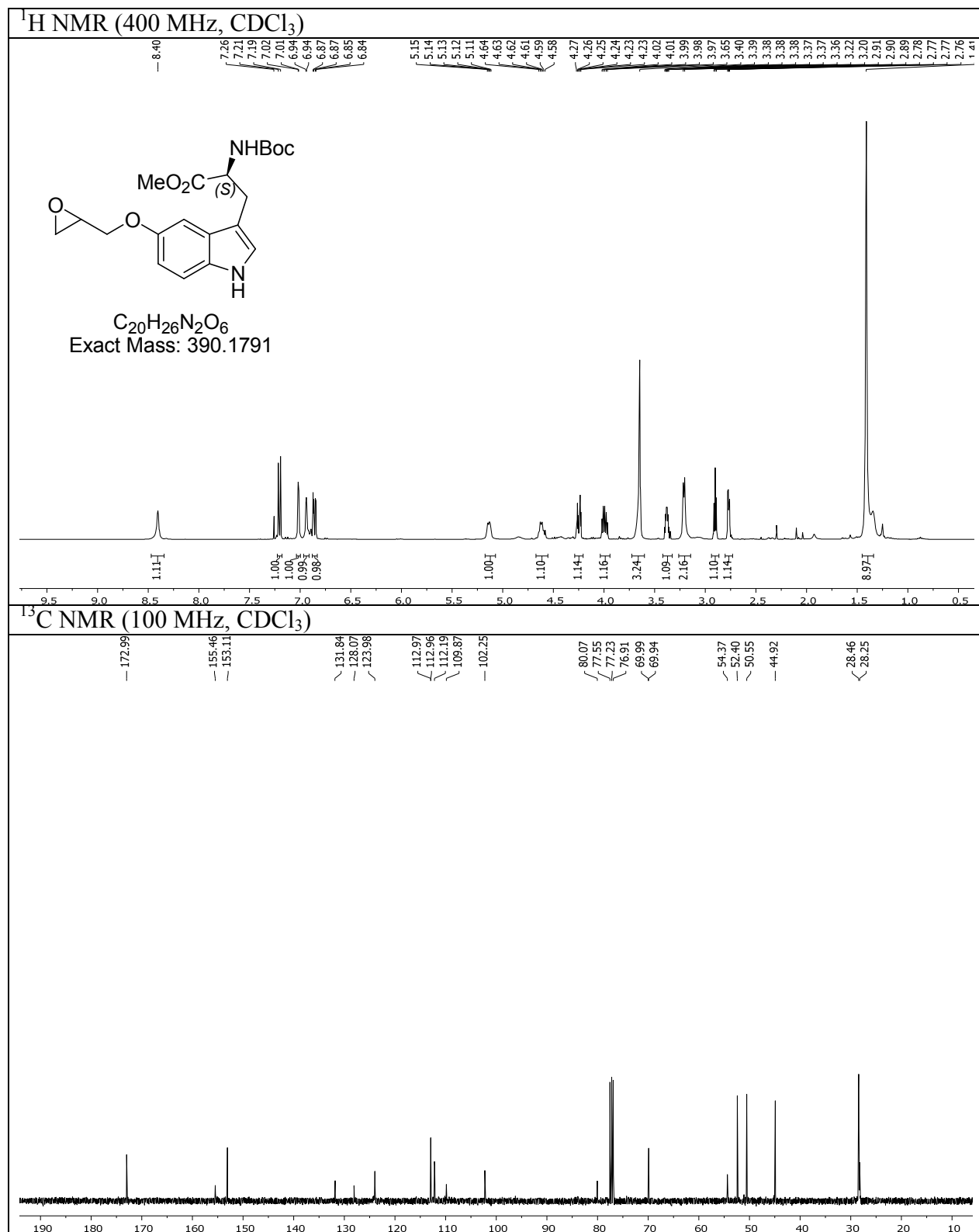
Methyl-2-(2-*tert*-butoxycarbonylamino-3-phenyl-propionylamino)-3-phenyl-propionate
(39)



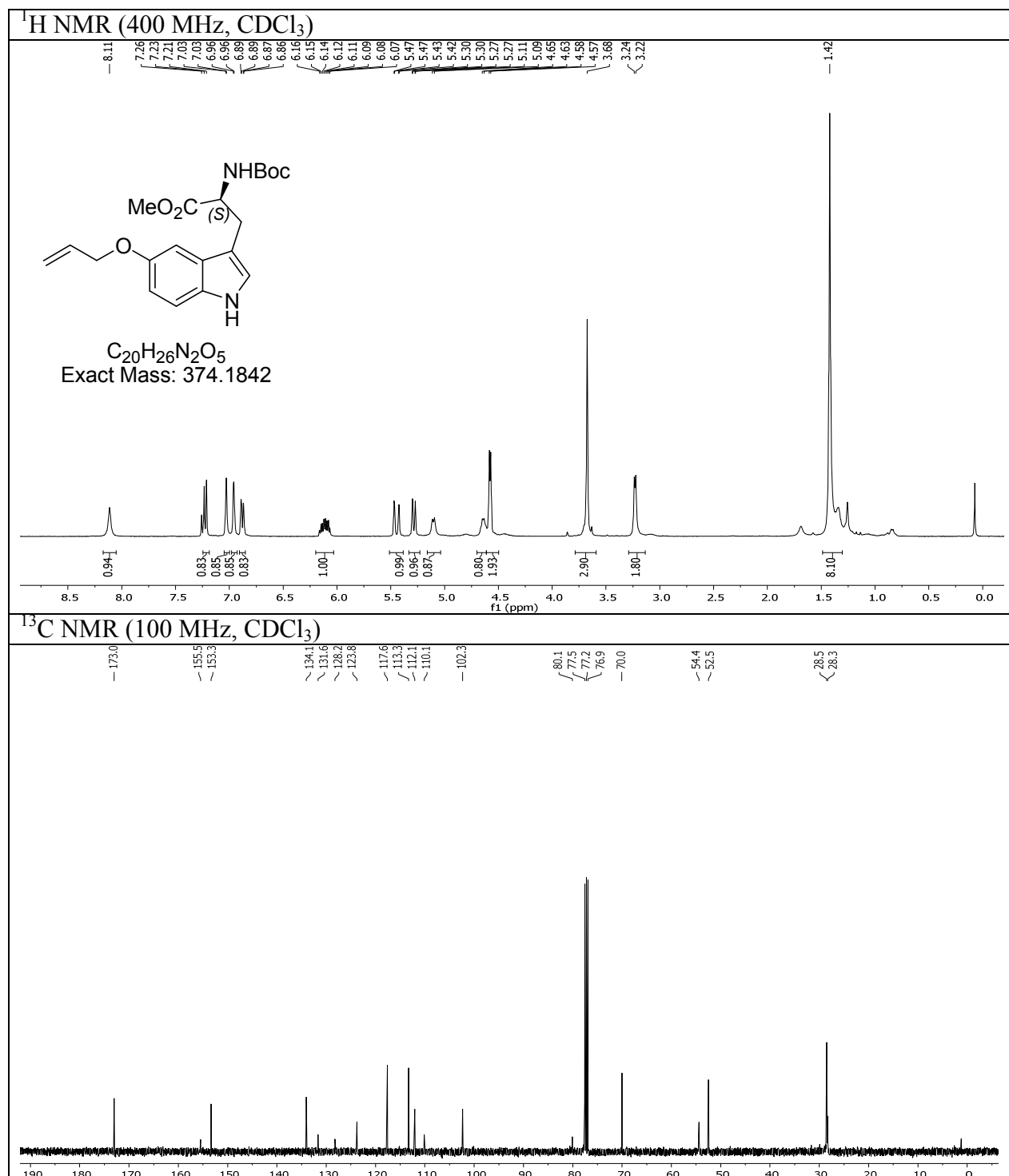
Methyl-2-(2-*tert*-butoxycarbonylamino-3-phenyl-propionylamino)-3-phenylpropionate (40)



Methyl-2-*tert*-Butoxycarbonylamino-3-(5-oxiranylmethoxy-1*H*-indol-3-yl)propionate (42)



Methyl-3-(5-Allyloxy-1*H*-indol-3-yl)-2-*tert*-butoxycarbonylaminopropionate (44)



5. Cytotoxicity data of MTMSA analogues

Table 1. Cytotoxicity (GI₅₀) screen in Ewing sarcoma cell lines of select MTMSA analogues

	RD-ES		TC-71		A-673		5838		ES-8		ES-2		ES-7	
	EWS-FLI1 Type 2		EWS-FLI1 Type 1		EWS-FLI1 Type 1		EWS-ERG		EWS-FLI1 Type 2		EWS-FLI1 Type 3		EWS-FLI1 Type 4	
MTMSA Analogues	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)
MTM (1)	40	30-53	55	44-68	41	30-56	43	30-61	54	43-68	63	45-89	46	28-76
MTMSAPhe (4)	91	68- 126	143	95- 216	146	92- 230	38	30-49	72	46- 115	261	117- 615	156	110- 222
MTMSATrp (3)	47	26-85	57	40-82	81	35- 186	6	4-10	99	60- 162	133	77- 231	32	12-92
60	50	32-76	44	25-82	168	124- 230	149	121- 186	53	46-61	78	66-91	46	22-104
61	57	44-75	35	18-68	364	186- 714	53	40-70	114	89- 147	123	74- 204	48	29-83
59	54	31-95	19	7-71	238	175- 321	39	25-60	74	46- 120	125	69- 228	Ne	Ne
45	539	416- 703	696	534- 987	1009	603- 1717	121	98- 151	1331	857- 2090	1501	1081- 2376	672	508- 855
51	843	567- 1264	636	436- 937	272	214- 357	24	20-29	90	54- 152	5374	> 1377	1664	1024- 2757
56	1004	700- 1455	640	429- 962	262	222- 299	466	229- 929	401	250- 652	3310	> 1231	1657	1093- 2547
63	3052	1026- 13442	211	175- 254	491	179- 1339	579	350- 966	542	426- 675	Ne	Ne	910	340- 2519

Table 2. Cytotoxicity (GI₅₀) screen in non-Ewing sarcoma cell lines of select MTMSA analogues

	DU 145		HCT 116		PANC-1		U-118 MG		HeLa		A549		DMS 114		LNCaP	
MTMSA Analogues	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)	GI ₅₀ (nM)	CI (95%)
MTM (1)	26	20-35	32	26-38	100	89-	80	52-	77	66-89	71	59-85	65	42-	48	31-75

						113		114						101		
MTMSAPhe (4)	315	222-482	256	189-311	261	213-317	1819	372-12206	545	307-982	1237	987-1952	483	254-935	732	382-1428
MTMSATrp (3)	46	39-56	30	24-36	161	102-254	167	87-325	323	233-448	89	62-130	151	56-425	109	73-164
60	400	199-816	449	289-699	574	453-742	Ne	> 422	557	200-1616	2849	1905-41225	1135	> 366	1607	1054-2454
61	246	139-439	198	125-314	640	519-786	Ne	Ne	951	498-1858	1399	758-2646	1115	543-2384	856	603-1218
59	67	39-115	82	73-84	152	74-311	830	569-987	397	282-625	1938	1668-2220	18	6-54	697	457-1064
45	761	629-894	826	> 620	1731	> 988	Ne	Ne	1758	1463-2082	2372	1538-5217	1054	851-1404	1863	1263-2808
51	298	237-389	380	254-596	532	330-873	291	191-449	1709	1242-2490	1540	253-9711	2840	1316-6612	2228	1445-3526
56	157	108-203	419	225-770	386	255-643	303	191-484	1551	695-3647	941	363-2466	2468	> 649	1965	866-4942
63	159	130-195	437	176-1052	1956	1793-2126	2204	1302-3846	1774	1195-2674	Ne	Ne	2310	1174-4815	Ne	Ne

6. Primers used for qRT-PCR analysis

Table 1: Primers used for qRT-PCR analysis

Gene	Forward Primer	Reverse Primer
EWS-FLI1	TCCTACAGCCAAGCTCCAAGTC	ACTCCCCGTTGGTCCCCTCC
NR0B1	TGATGCTGGAAATGCTCTGT	TTACACTCTTTTGCCACAGC
Sp1	GGAGAGCAAAACCAGCAGAC	AAGGTGATTGTTTGGGCTTG
BCL-2	GAGACAGCCAGGAGAAATCA	CCTGTGGATGACTGAGTACC
CCK	CTGCGTCCTAATCCAAAAGC	CATTCGTCCAGAAGGAGC
GAPDH	AAGGTGAAGGTCGGAGTCAA	GATCTCGCTCCTGGAAGATG