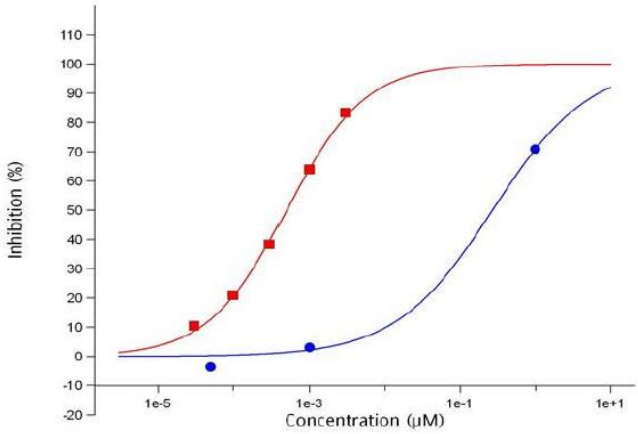
Supplementary Materials

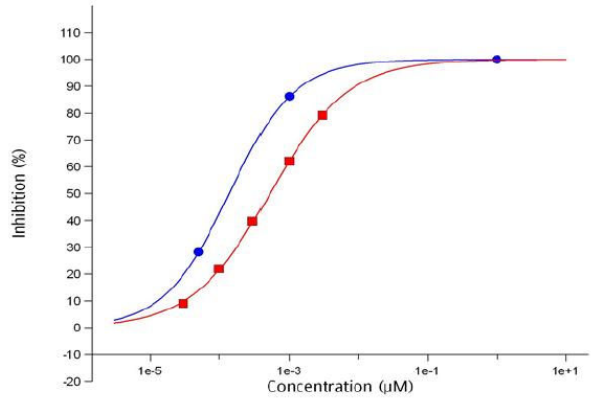
Synthesis of New 2,3-Dihydroindole Derivatives and Evaluation of Its Melatonin Receptor Binding Affinity

S1. Binding curves of compounds 7a and 7d to MT2 (A,B) and MT1 (C)



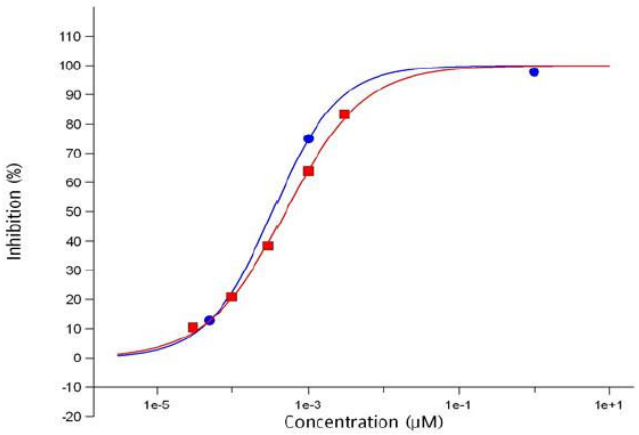
Compound	IC_{50}	K_i	n_H
7a	0.268 μM	0.139 μM	0.676
4-P-PDOT	0.497 nM	0.258 nM	0.840

Figure A



Compound	IC_{50}	K_i	n_H
7d	0.138 nM	0.0716 nM	0.925
Melatonin	0.527 nM	0.274 nM	0.774

Figure B



Compound	IC_{50}	K_i	n_H
7d	0.338 nM	0.176 nM	1.01
4-P-PDOT	0.497 nM	0.258 nM	0.840

Figure C

METHODS - RADIOLIGAND BINDING ASSAYS

■ 251600 Melatonin MT_1

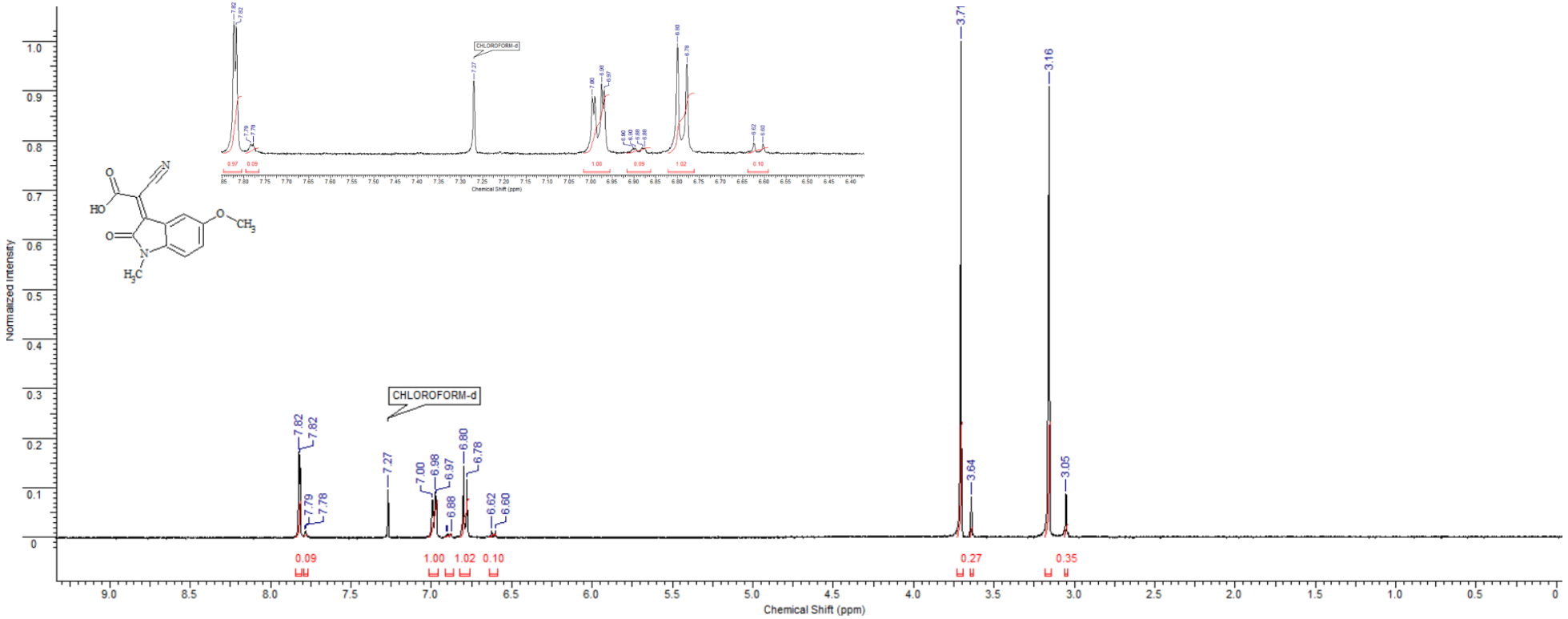
Source:	Human recombinant CHO-K1 cells
Ligand:	0.05 nM [^{125}I] 2-Iodomelatonin
Vehicle:	1% DMSO
Incubation Time/Temp:	3 hours @ 25°C
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA
Non-Specific Ligand:	1 µM 6-Chloromelatonin
K_D:	0.054 nM *
B_{MAX}:	3.5 pmole/mg Protein *
Specific Binding:	97% *
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

■ 251700 Melatonin MT_2

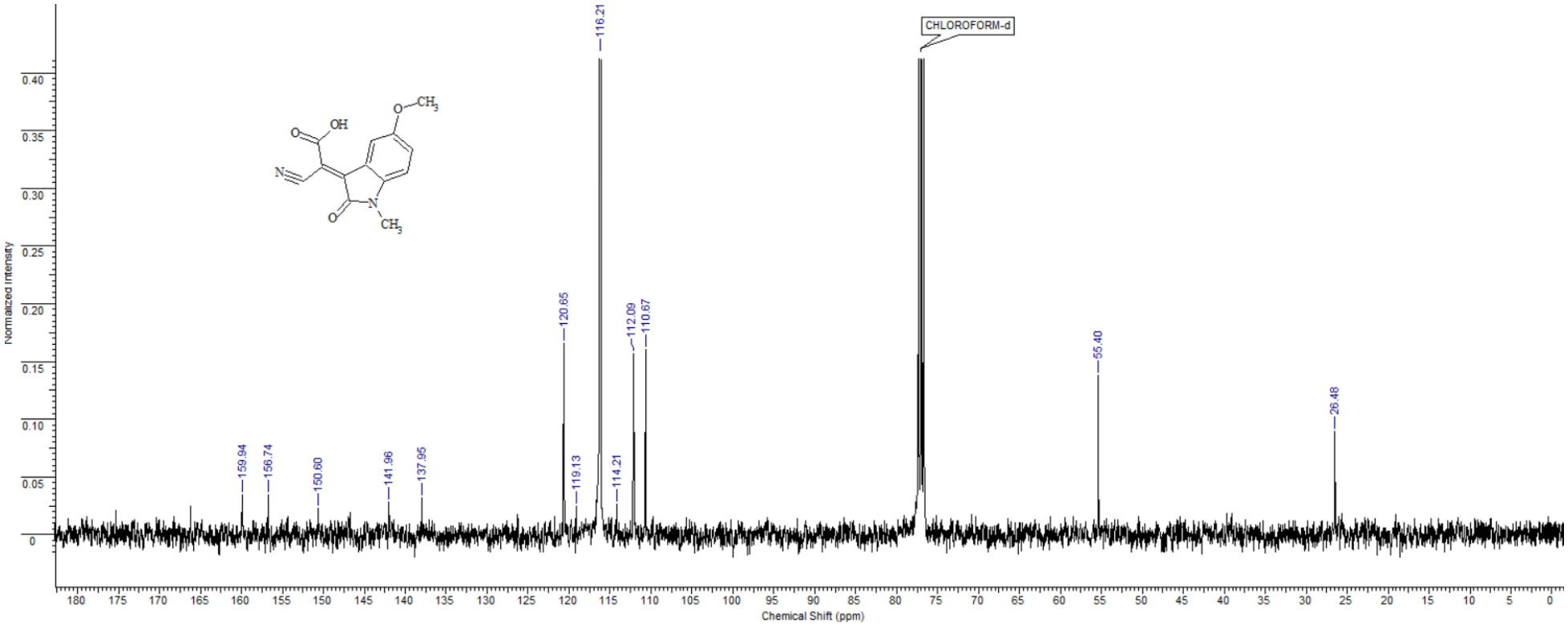
Source:	Human recombinant CHO-K1 cells
Ligand:	0.05 nM [^{125}I] 2-Iodomelatonin
Vehicle:	1% DMSO
Incubation Time/Temp:	4 hours @ 25°C
Incubation Buffer:	25 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA
Non-Specific Ligand:	1 µM 6-Chloromelatonin
K_D:	0.054 nM *
B_{MAX}:	0.93 pmole/mg Protein *
Specific Binding:	98% *
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Selected NMR spectra of synthesized compounds

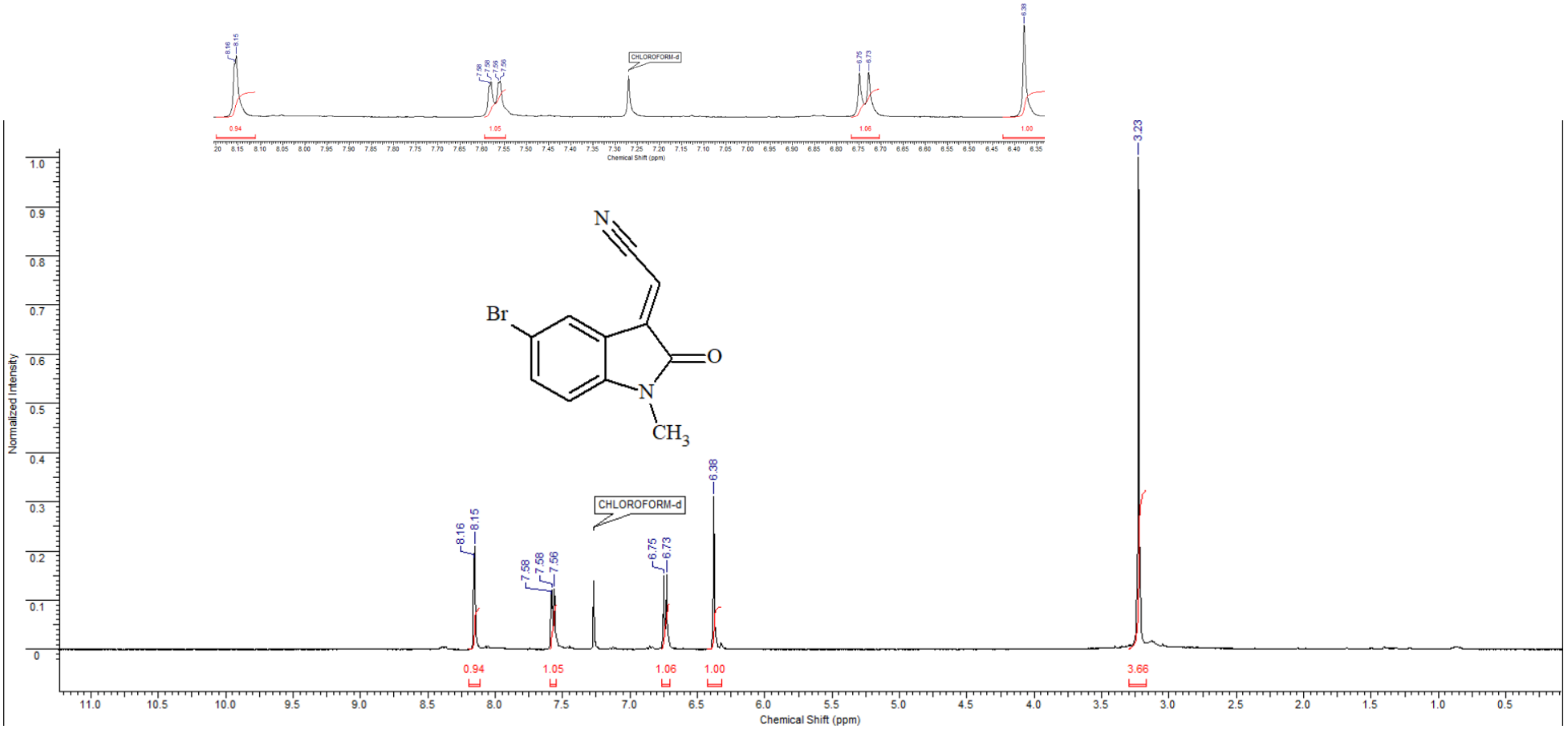
S2. 1H NMR spectrum of cyano(*N*-methyl-2-oxo-5-methoxy indolin-3-ylidene)acetic acid (**2g**)



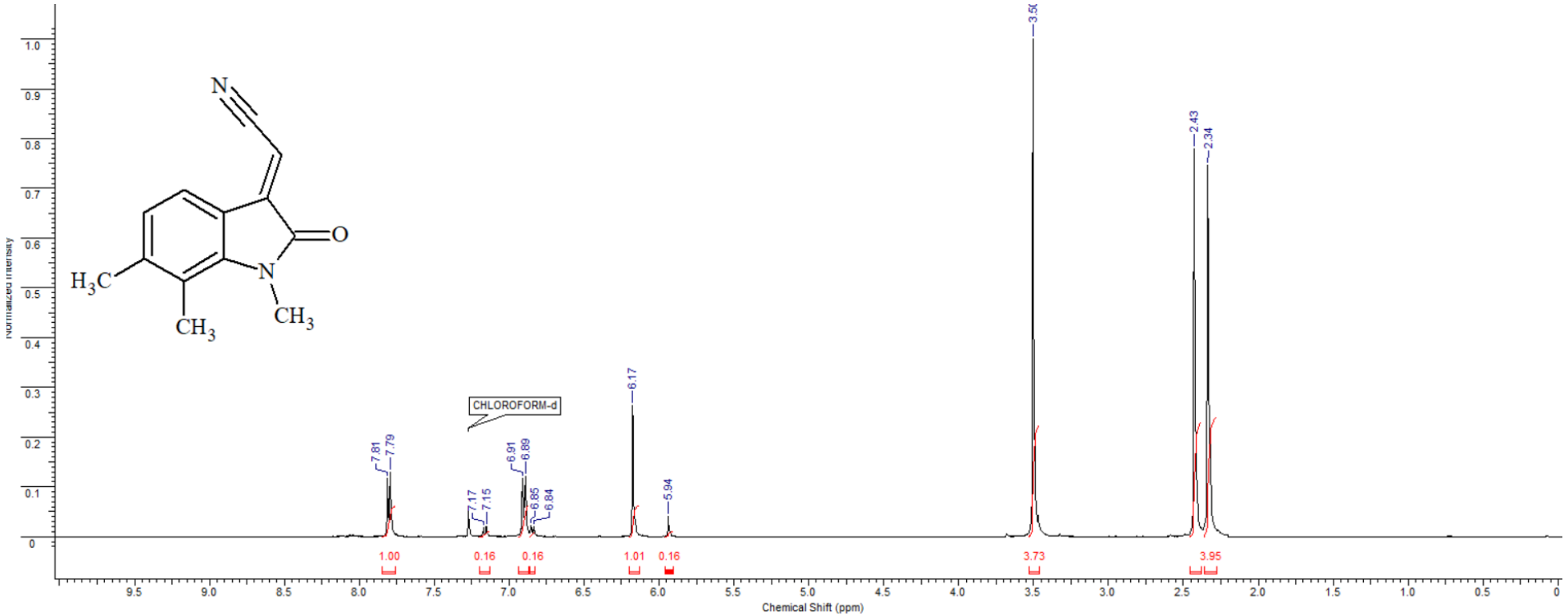
S3. 13C NMR spectrum of cyano(*N*-methyl-2-oxo-5-methoxy indolin-3-ylidene)acetic acid (**2g**)



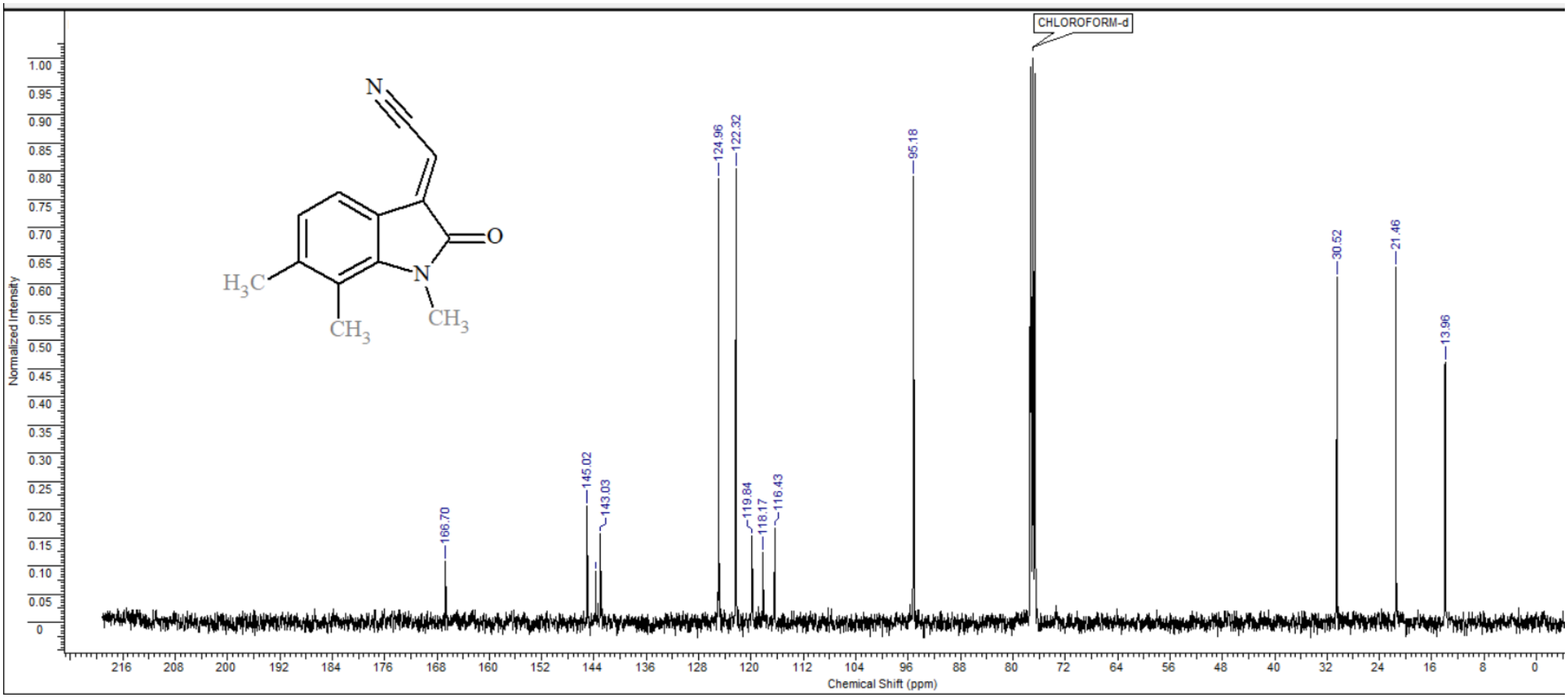
S4. 1H NMR spectrum of 2-(5-Bromo-1-methyl-2-oxo-2,3- dihydro-1H-indol-3-ylidene)acetonitrile (**3i**)



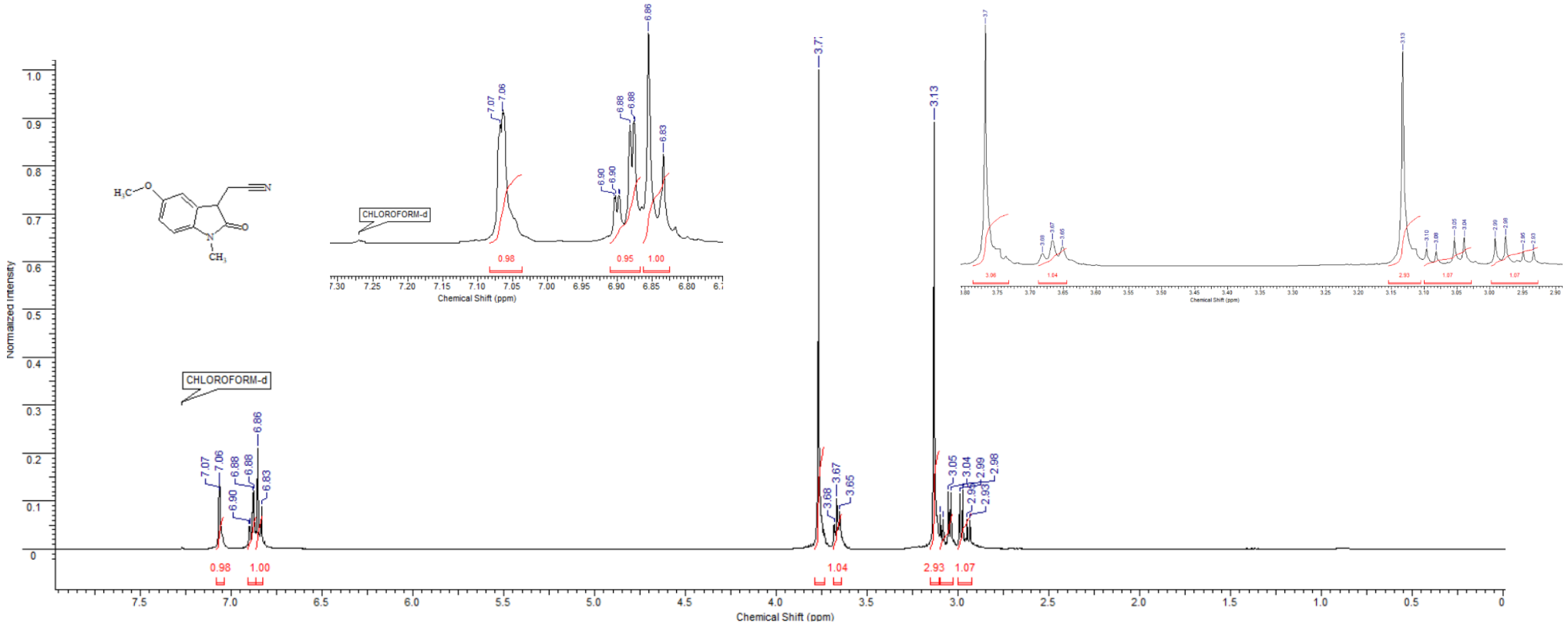
S5 1H NMR spectrum of 2-(1,6,7-Trimethyl-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene)acetonitrile (**3j**)



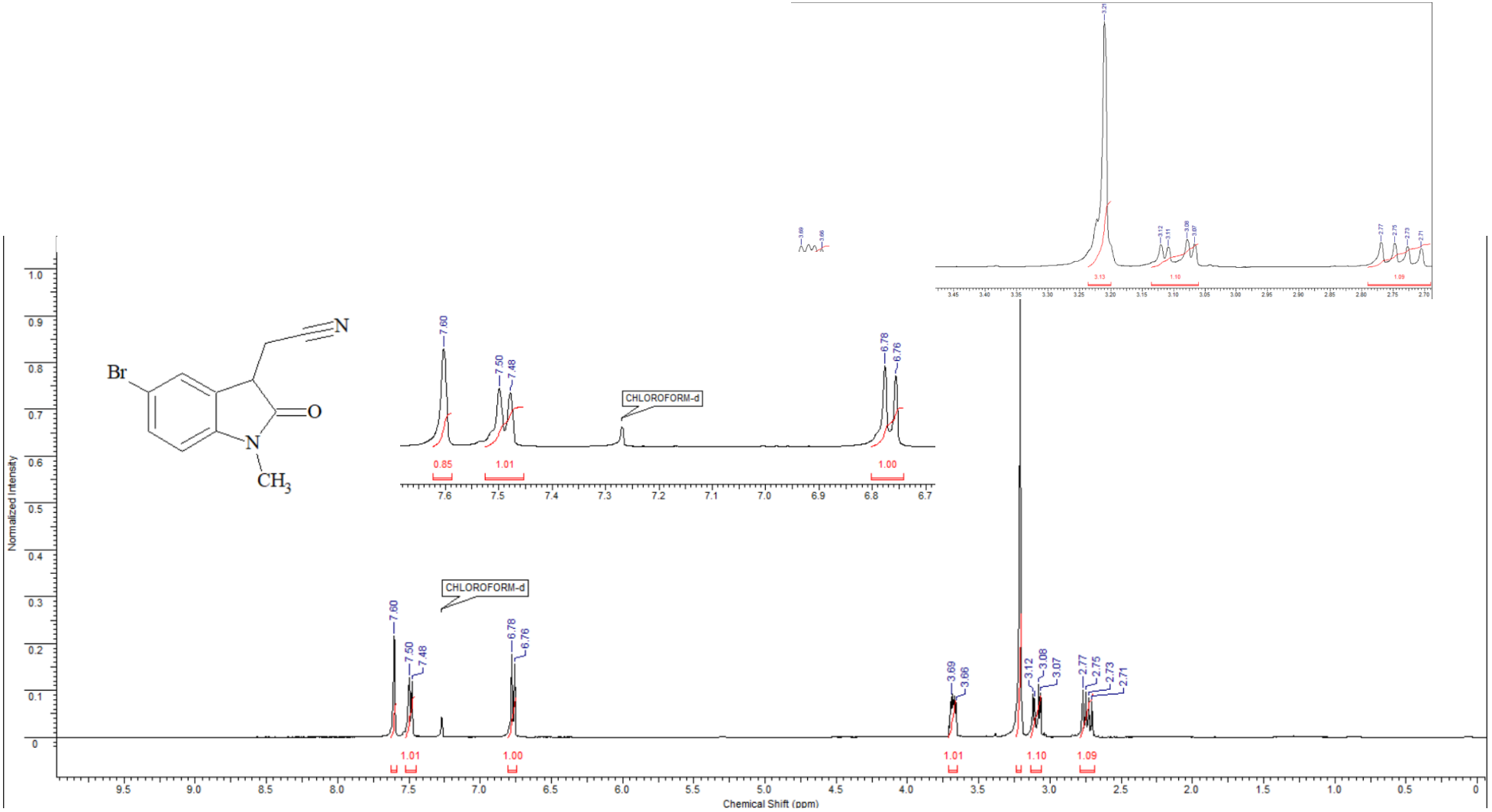
S6 13C NMR spectrum of 2-(1,6,7-Trimethyl-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene)acetonitrile (**3j**)



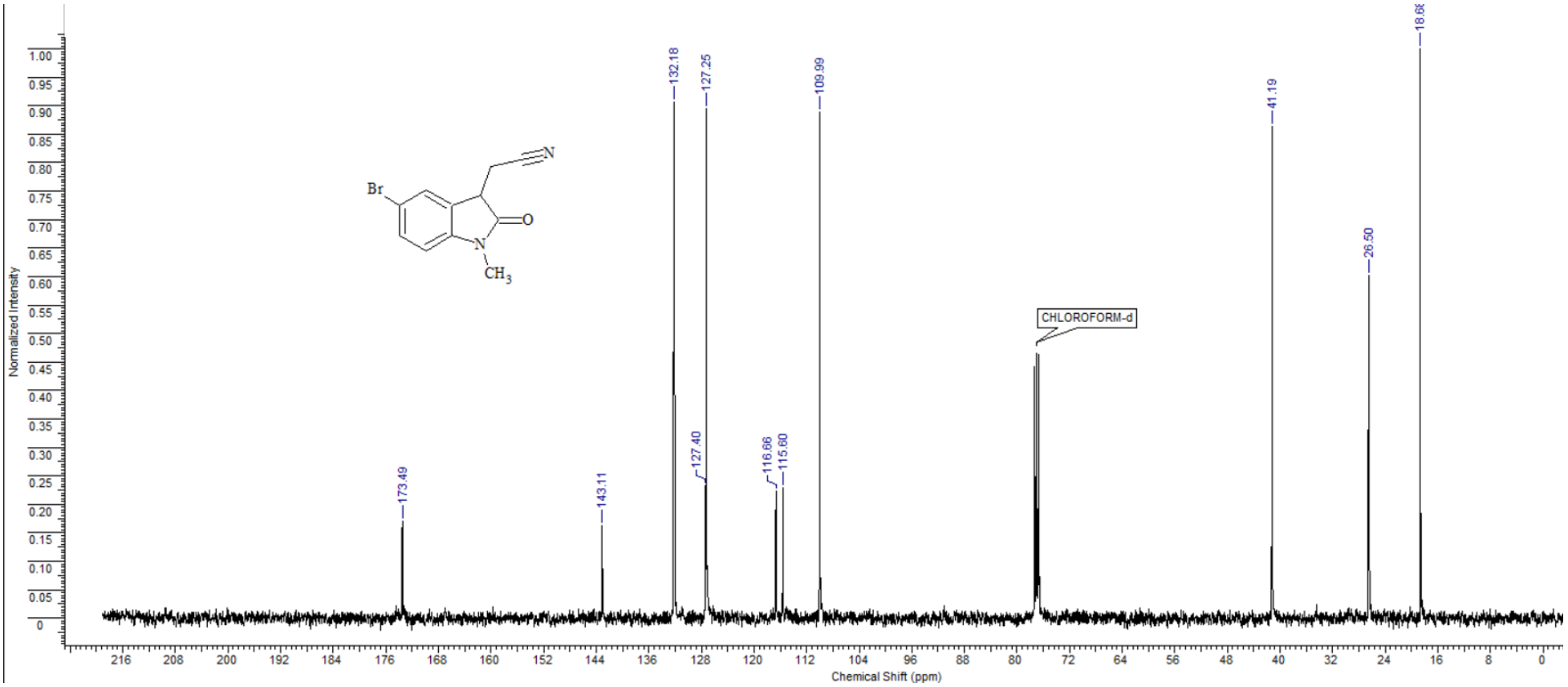
S7 1H NMR spectrum of 2-(1-methyl-5-methoxy-2-oxo-2,3-dihydro-1*H*-indol-3-yl)acetonitrile (**4g**)


Normalized Intensity
Chemical Shift (ppm)
CHLOROFORM-d
7.07
7.06
6.90
6.88
6.88
6.86
6.83
3.68
3.67
3.65
3.13
3.05
3.04
2.99
2.98
0.98
1.00
1.04
2.93
1.07

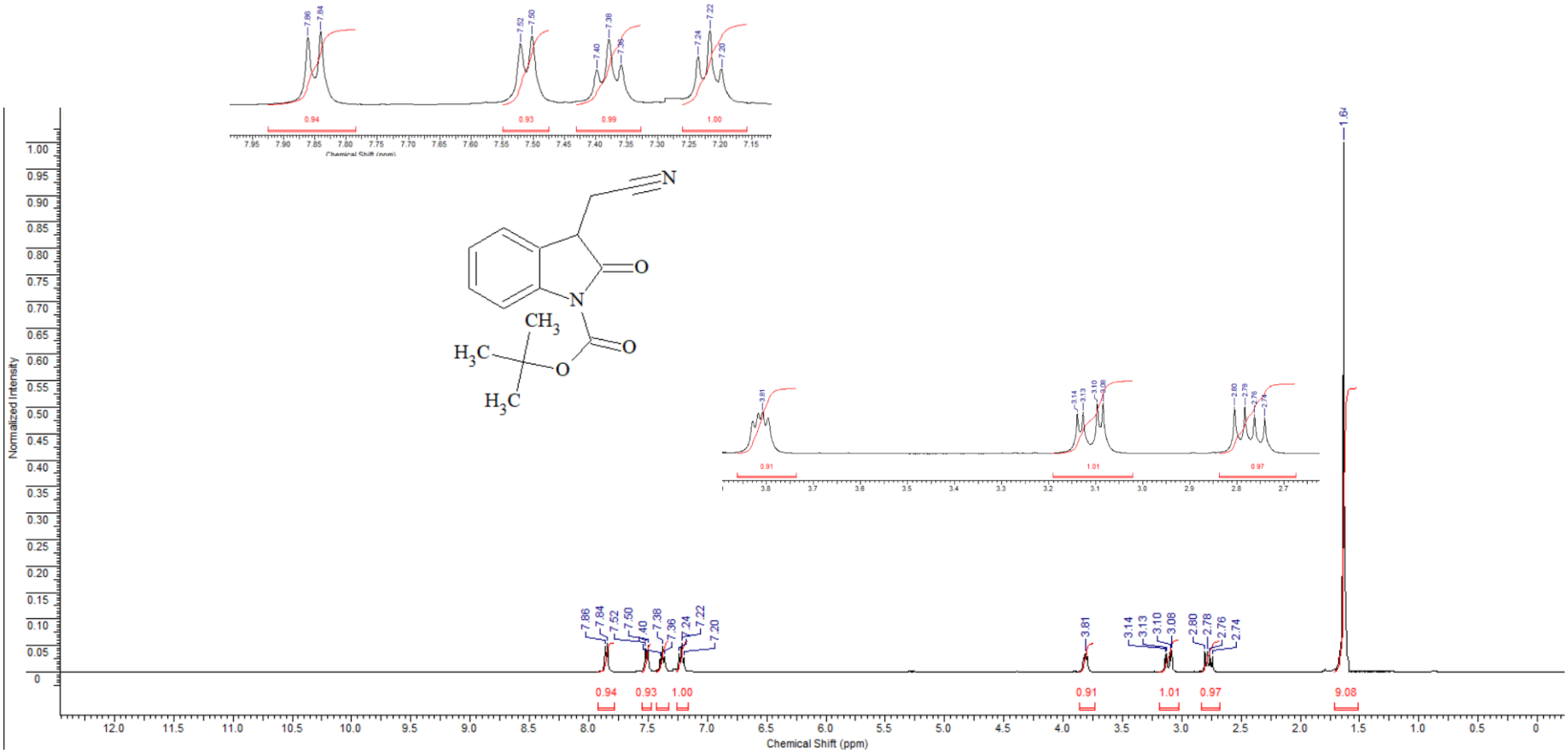
S8 1H NMR spectrum of 2-(5-bromo-1-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl)acetonitrile (**4i**)



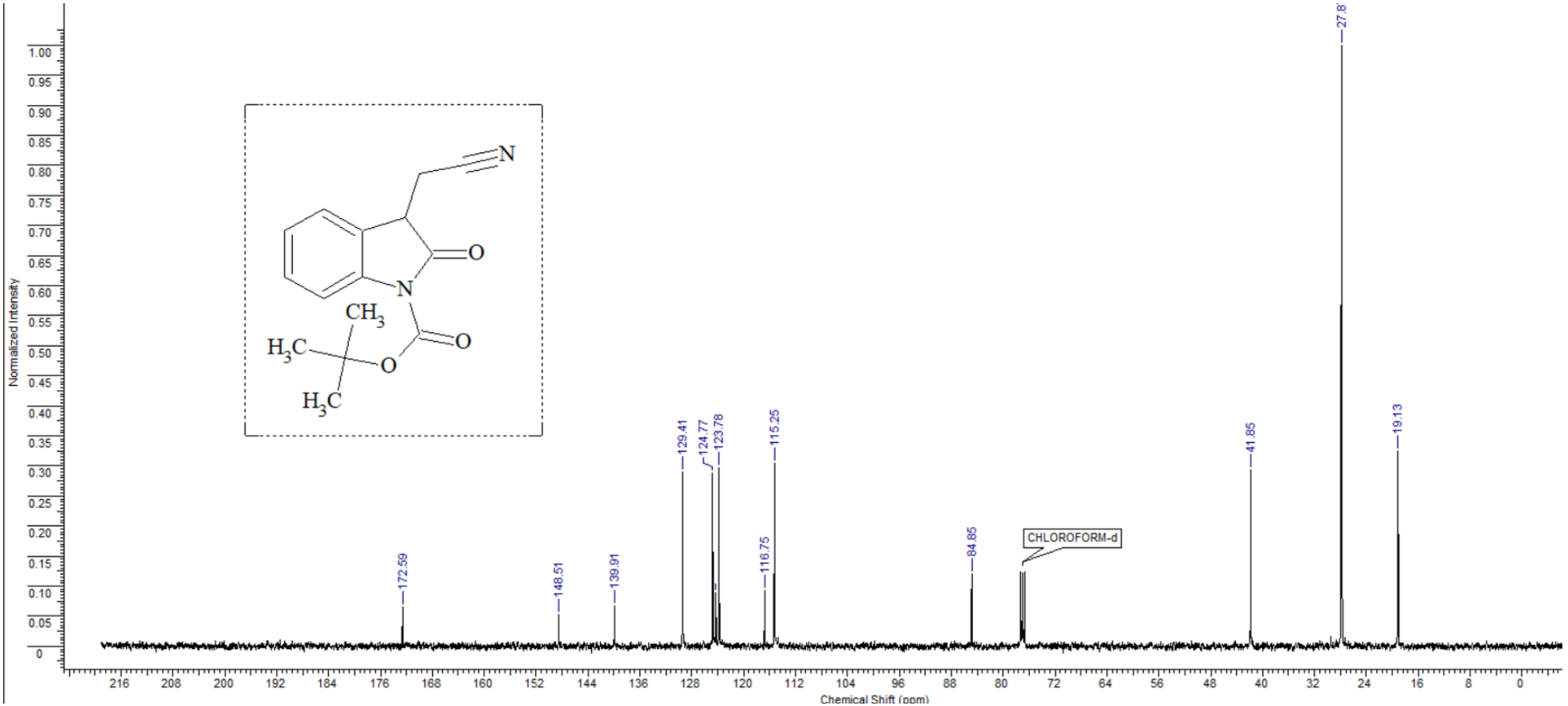
S9 13C NMR spectrum of 2-(5-Bromo-1-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**4i**)



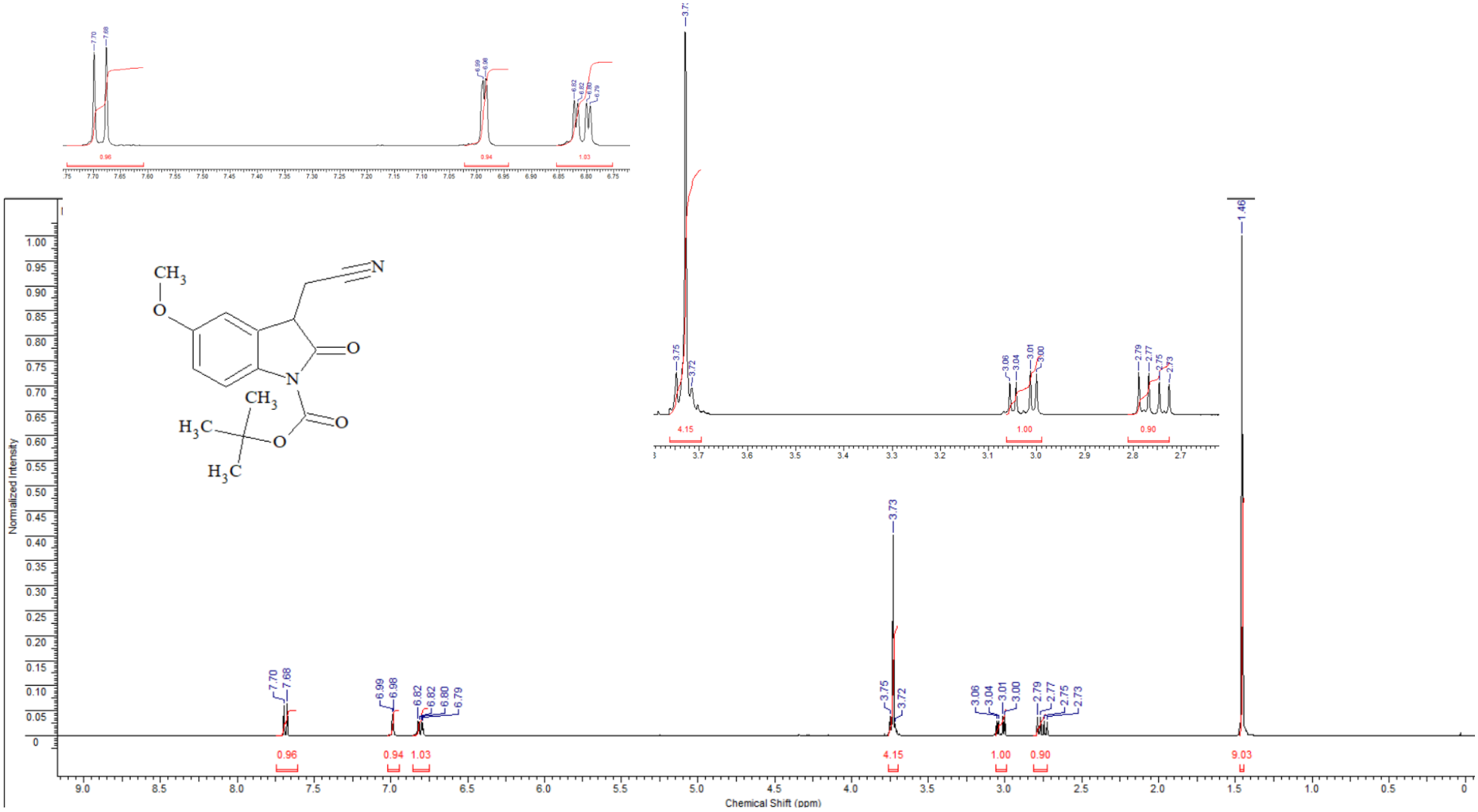
S10 1H NMR spectrum of Tert-butyl 3-(cyanomethyl)-2-oxo-2,3-dihydro-*1H*-indol-1-carboxylate (**4k**)



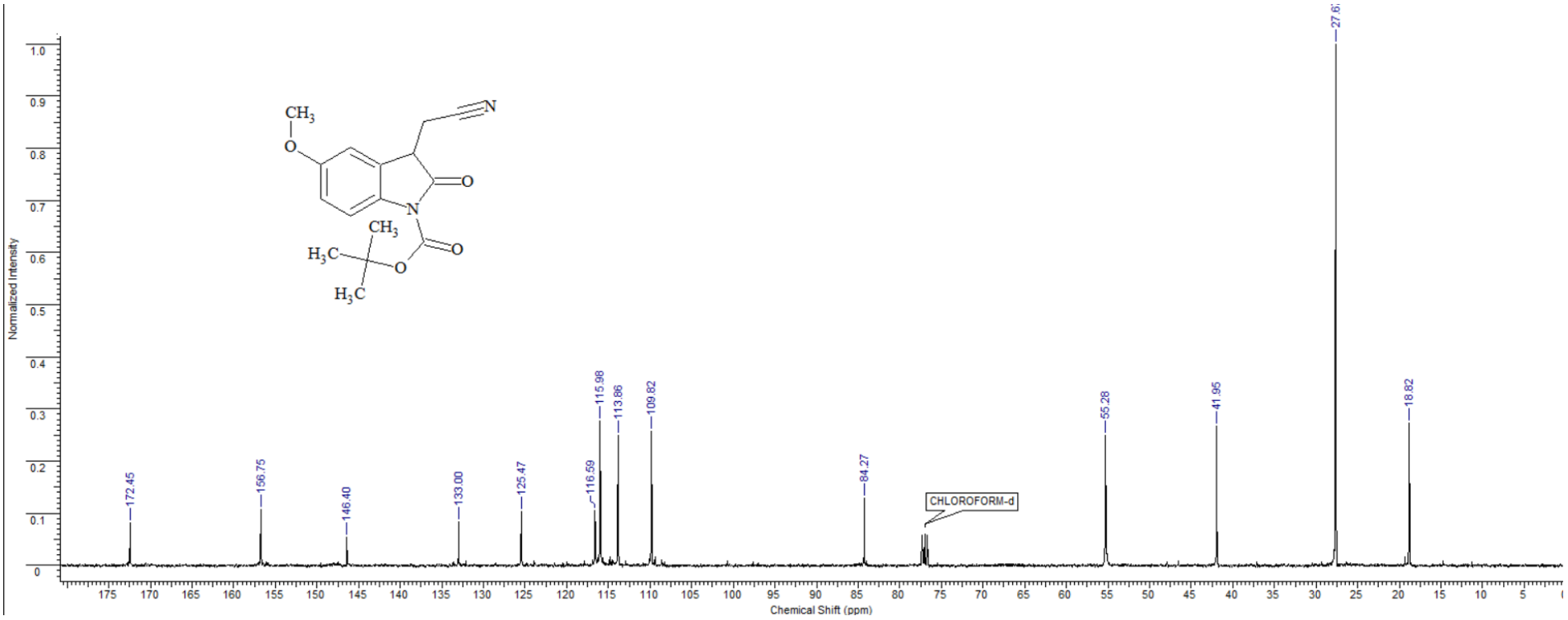
S11 13C NMR spectrum of Tert-butyl 3-(cyanomethyl)-2-oxo-2,3-dihydro-*1H*-indol-1-carboxylate (**4k**)



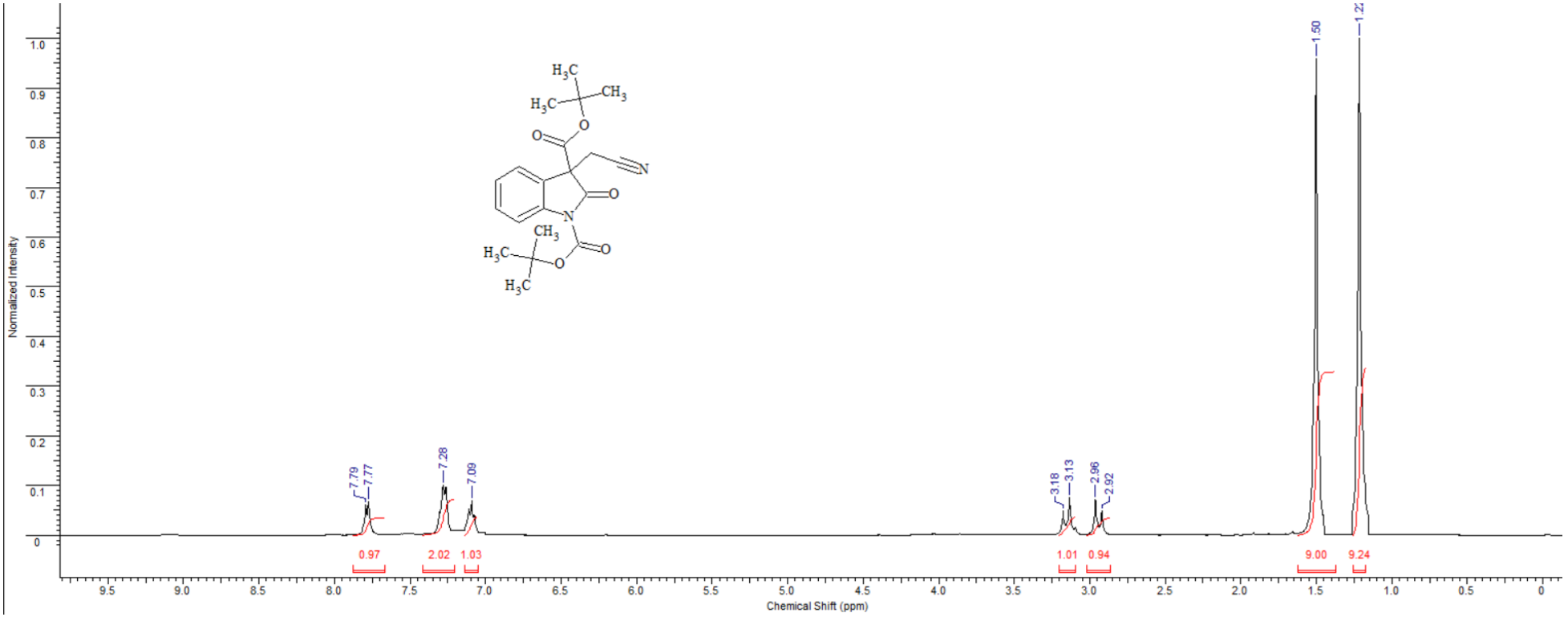
S12 1H NMR spectrum of Tert-butyl 5-methoxy-3-(cyanomethyl)-2-oxo-2,3-dihydro-1H-indol-1-carboxylate (**41**)



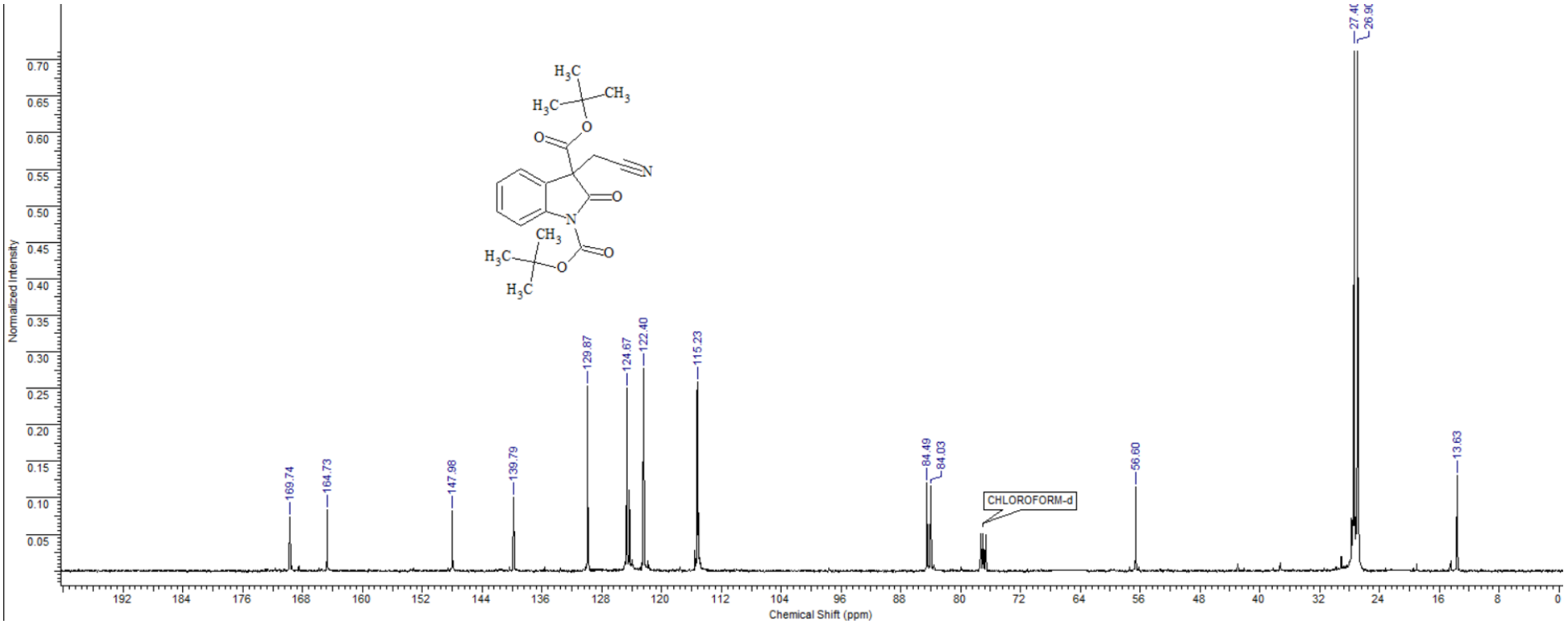
S13 13C NMR spectrum of Tert-butyl 5-methoxy-3-(cyanomethyl)-2-oxo-2,3-dihydro-1H-indol-1-carboxylate (**4l**)



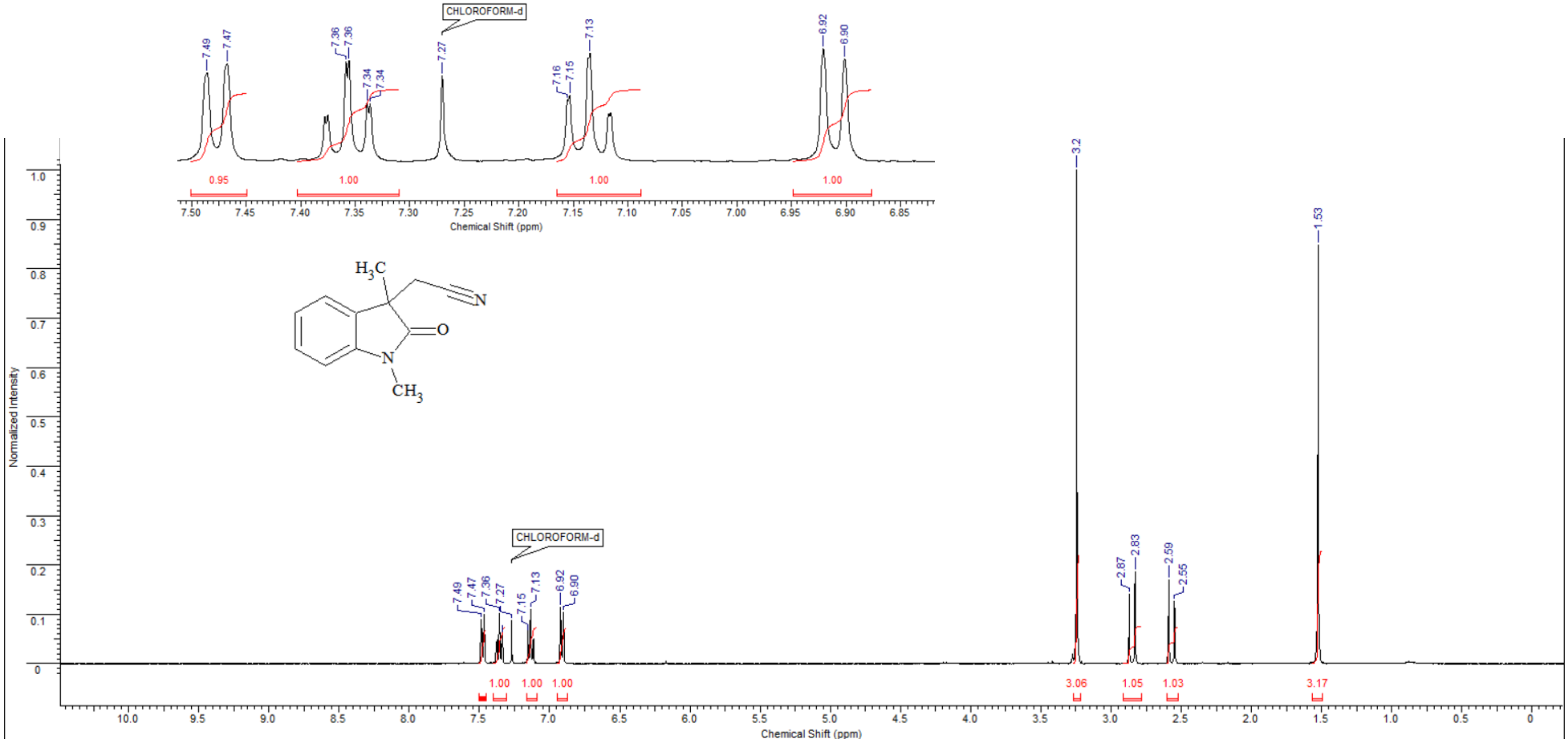
S14 1H NMR spectrum of 1,3-Di-tert-butyl 3-cyanomethyl-2-oxo-2,3-dihydro-1H-indol-1,3-dicarboxylate (**4m**)



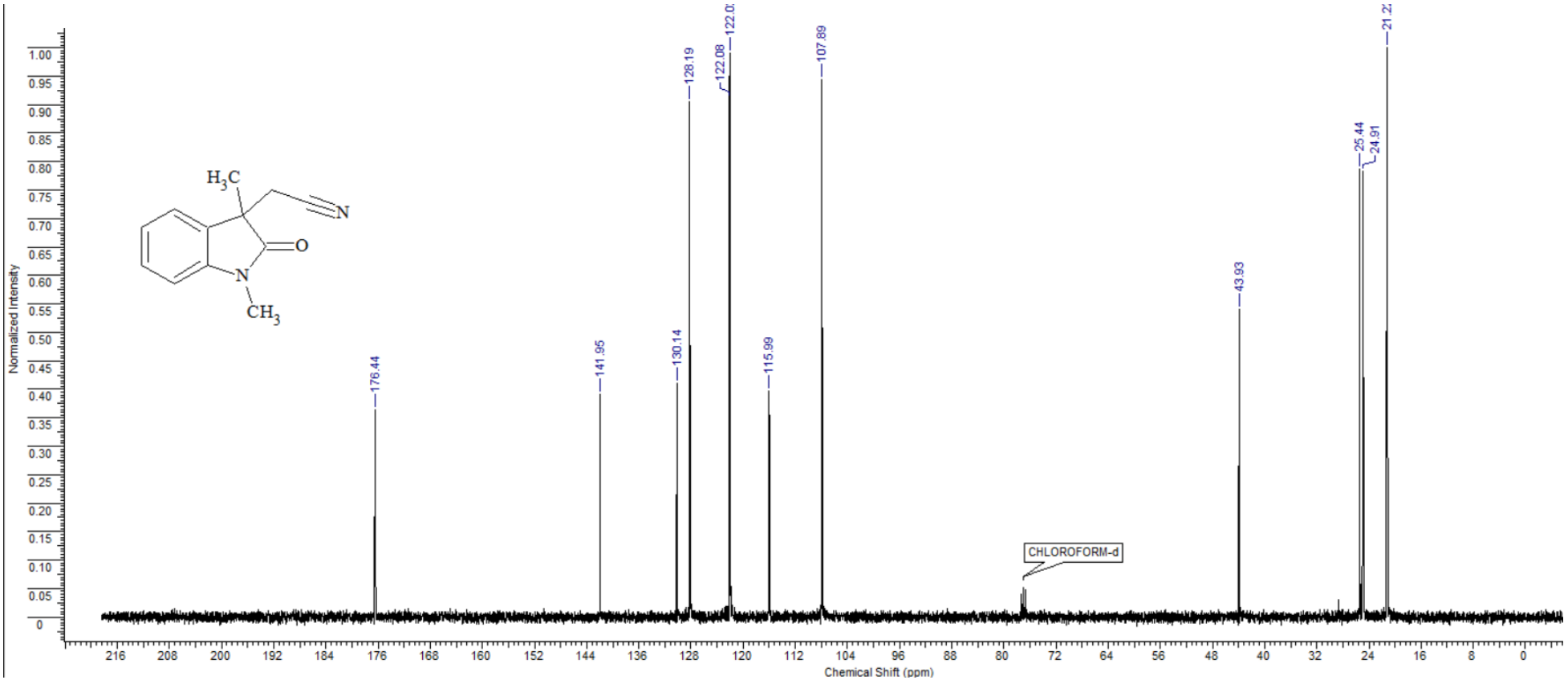
S15. 13C NMR spectrum of 1,3-Di-tert-butyl 3-cyanomethyl-2-oxo-2,3-dihydro-1H-indol-1,3-dicarboxylate (**4m**)



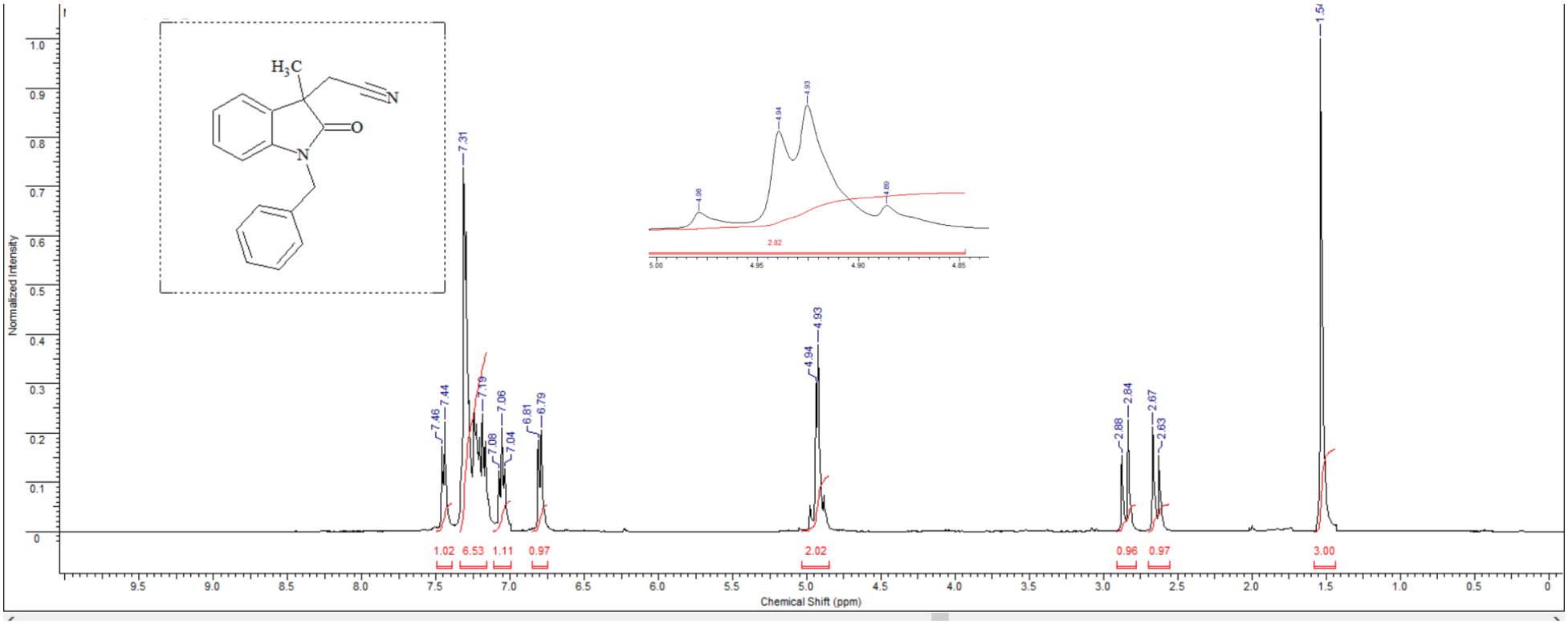
S16 1H NMR spectrum of 2-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5a**)



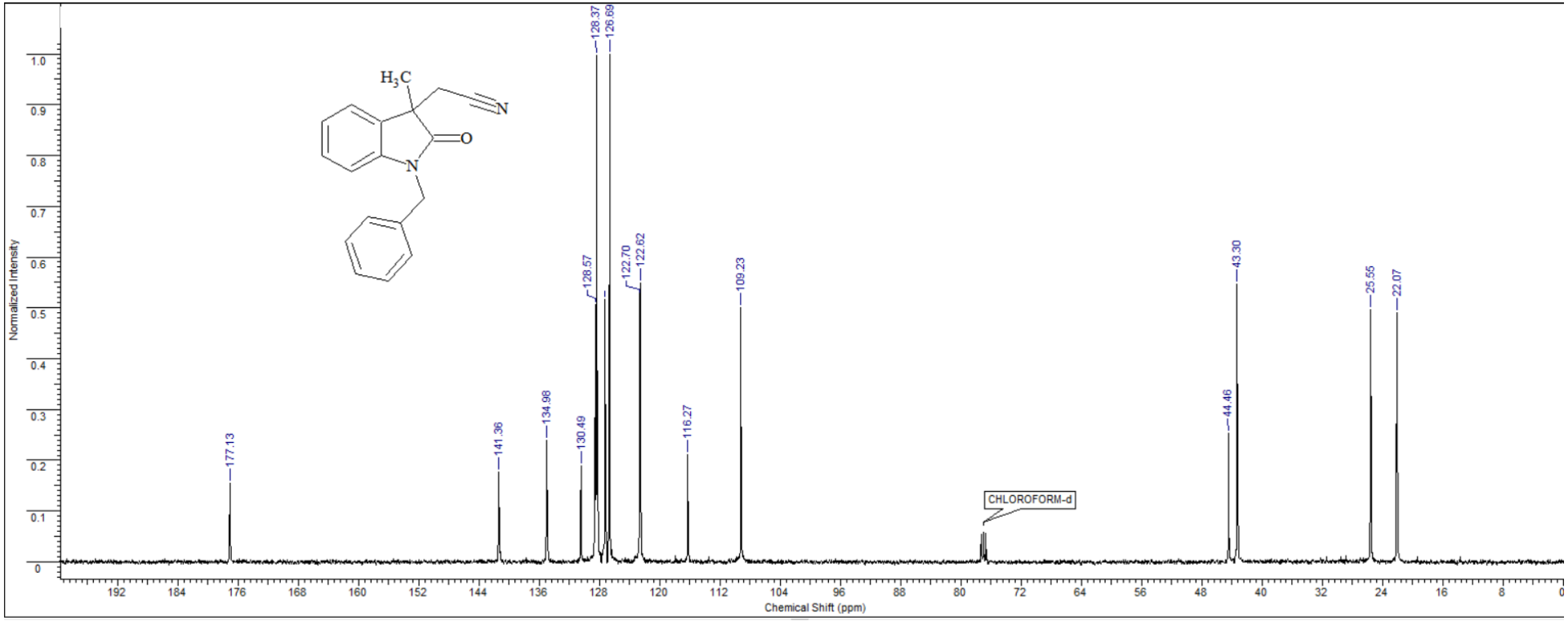
S17 13C NMR spectrum of 2-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5a**)



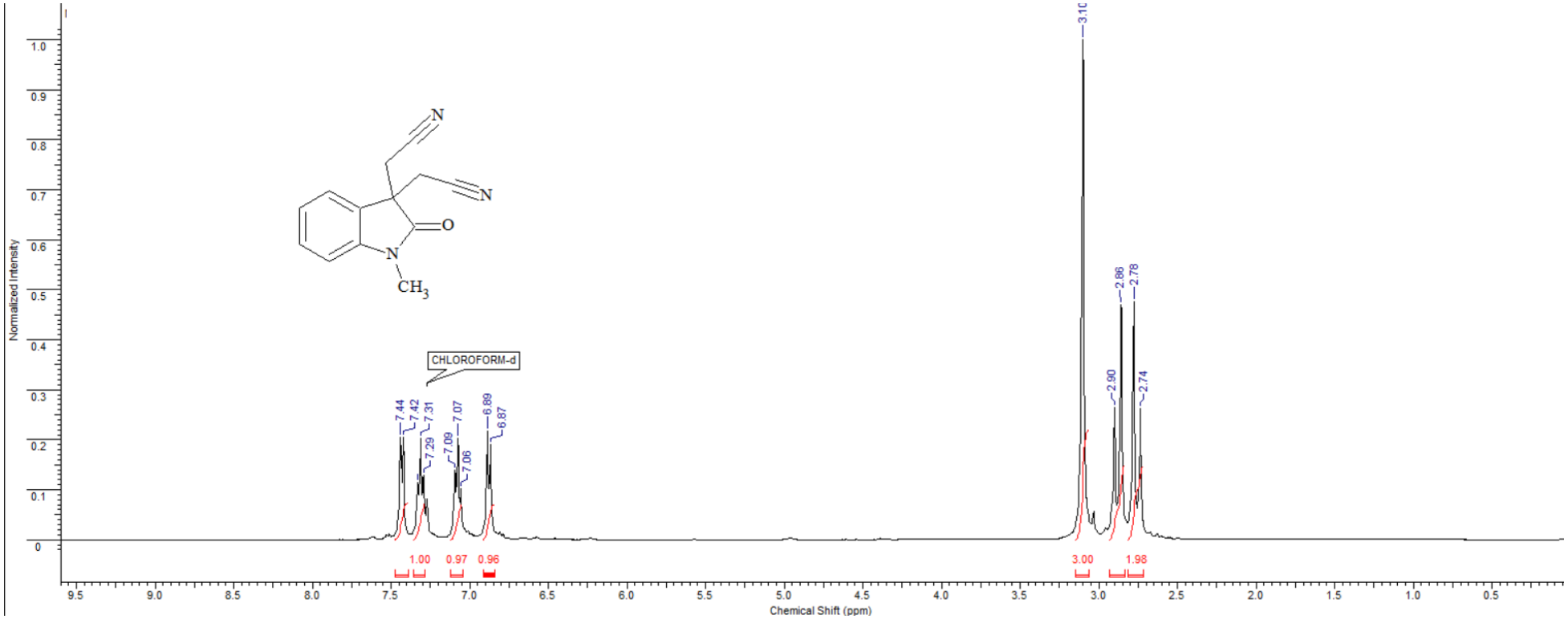
S18 1H NMR spectrum of 2-(1-Benzyl-3-methyl-2-oxo-2,3-dihydro-*1H*-indol-3-yl)acetonitrile (**5b**)



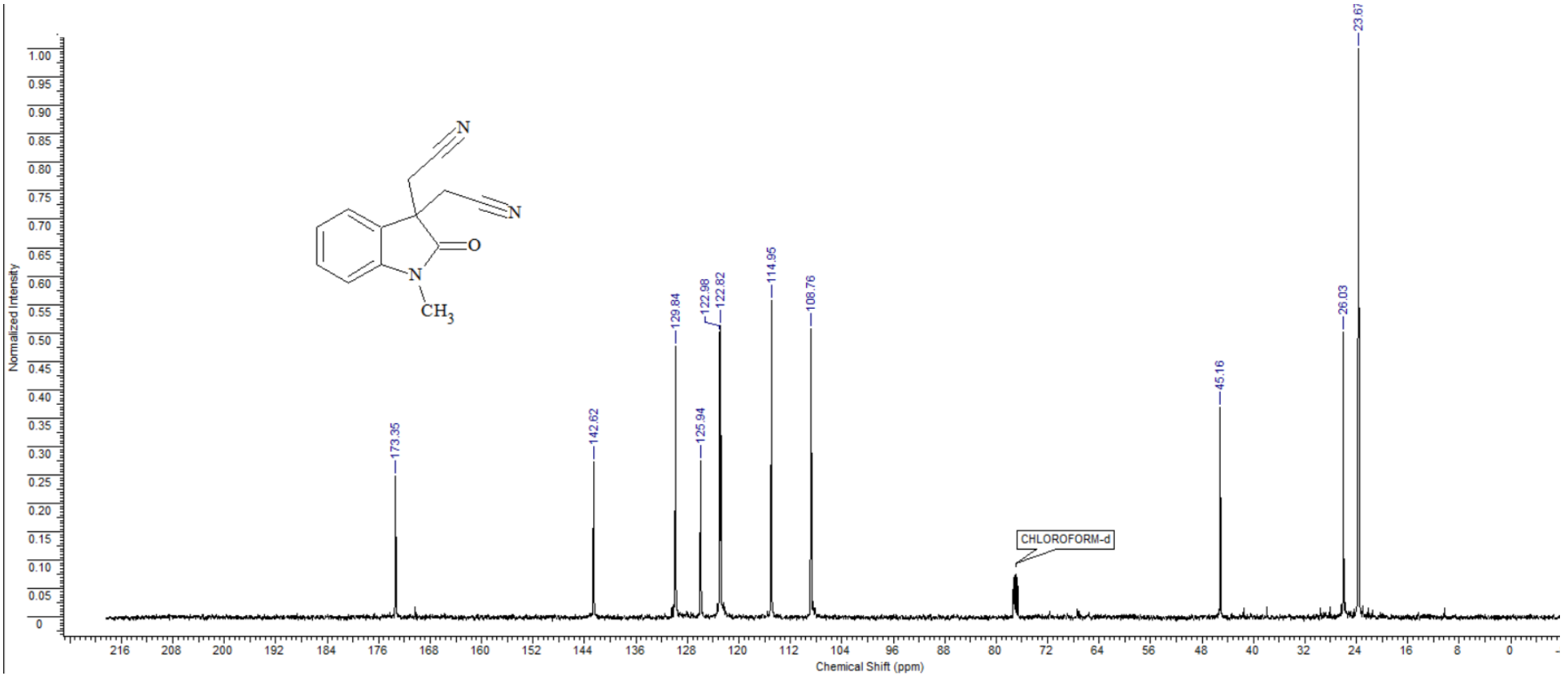
S19 13C NMR spectrum of 2-(1-Benzyl-3-methyl-2-oxo-2,3-dihydro-*1H*-indol-3-yl)acetonitrile (**5b**)



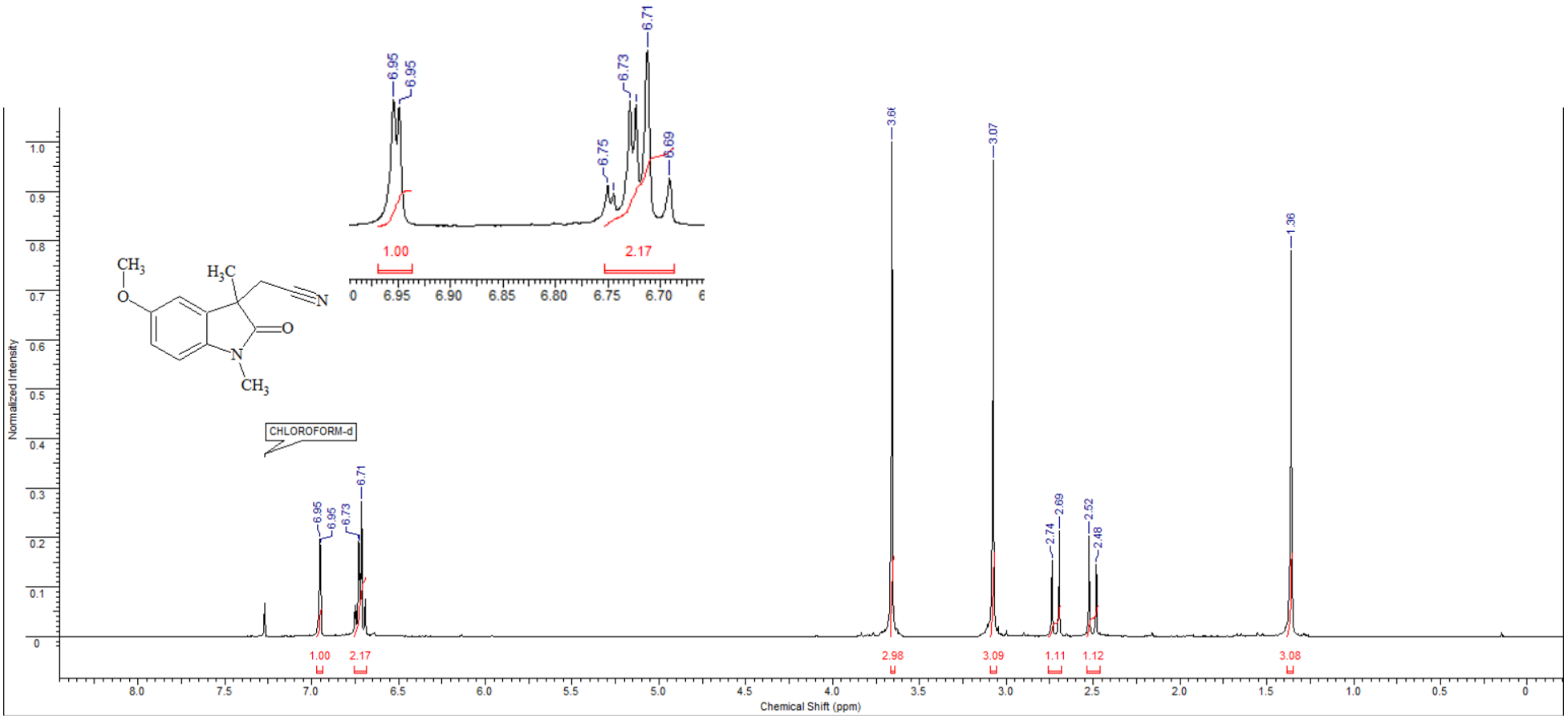
S20 1H NMR spectrum of 2-(1-Methyl-3-cyanomethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5d**)



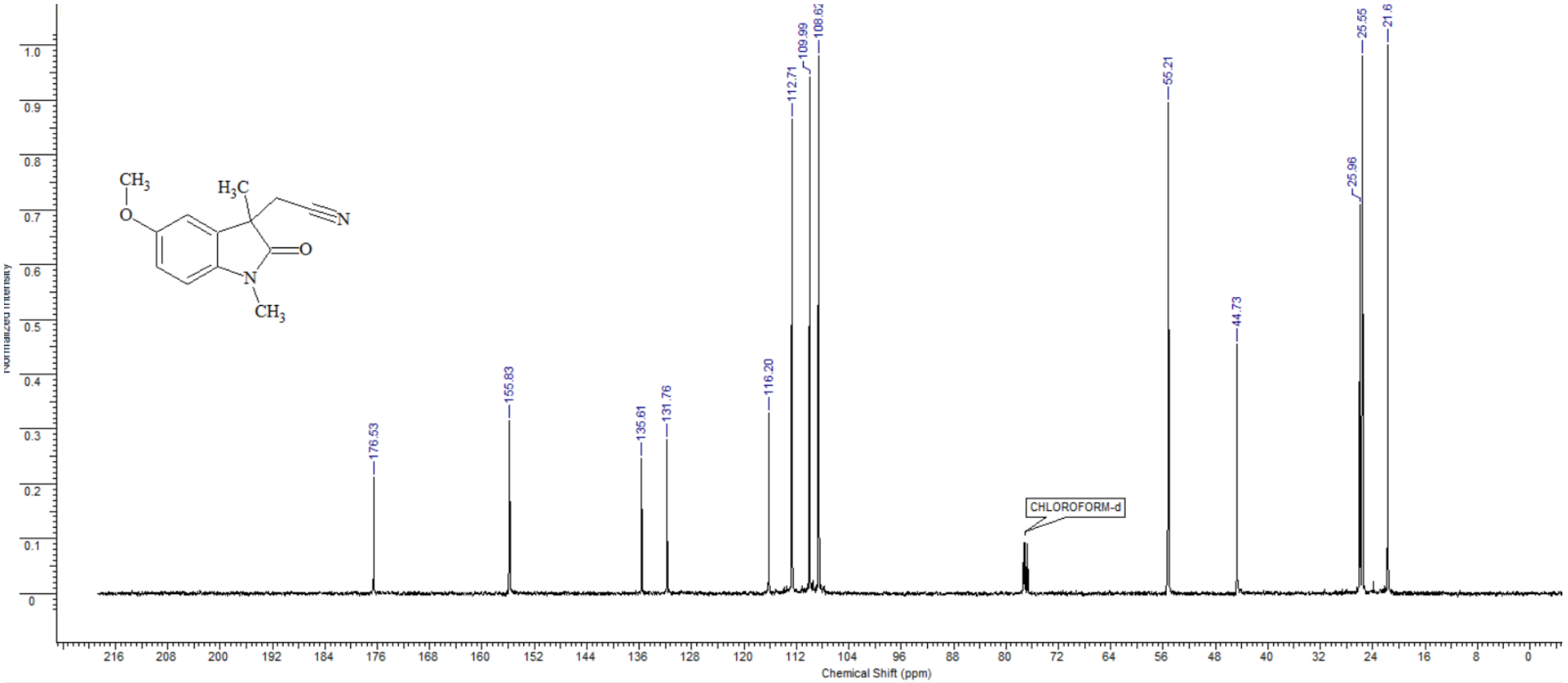
S21 13C NMR spectrum of 2-(1-Methyl-3-cyanomethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5d**)



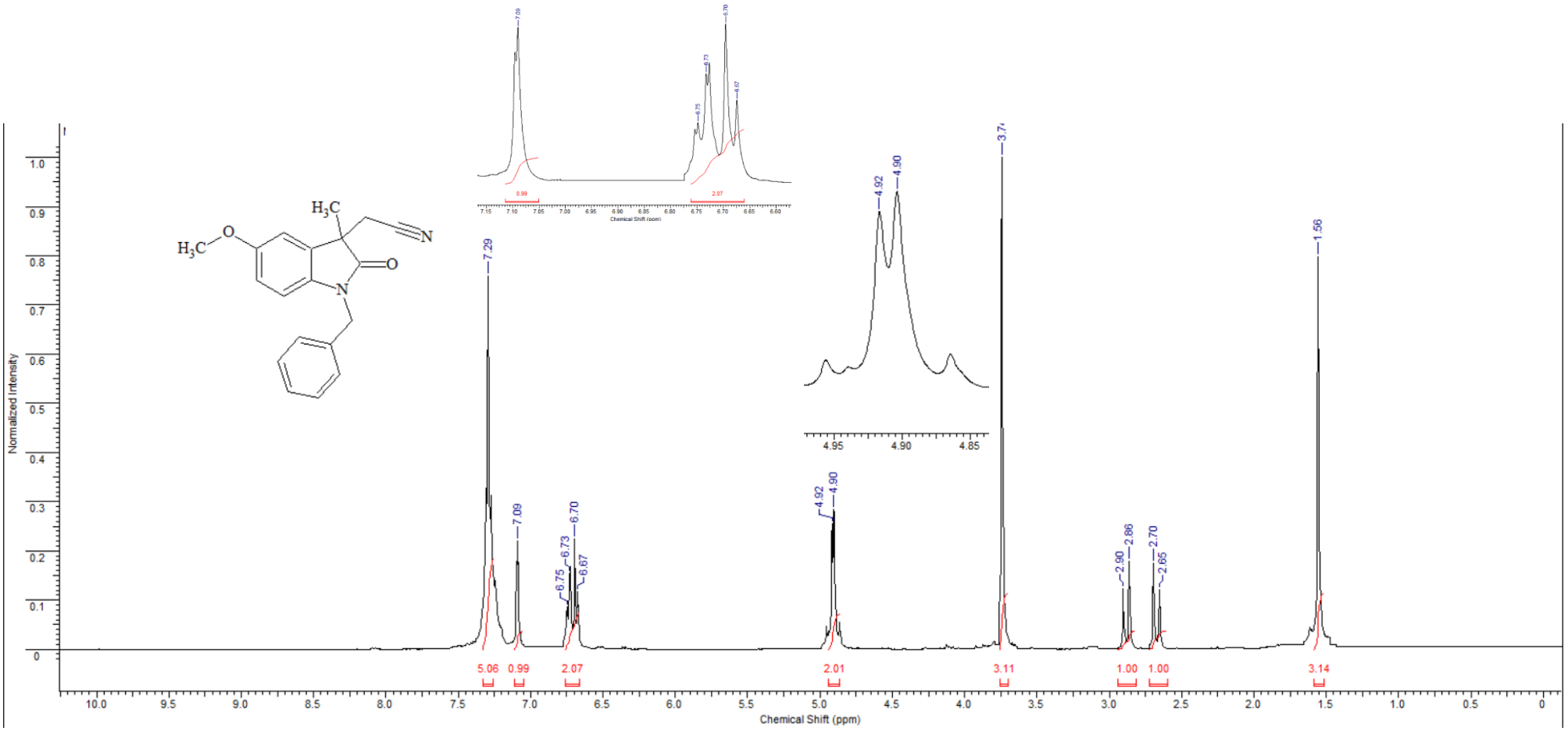
S22 1H NMR spectrum of 2-(1,3-Dimethyl-5-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5e**)



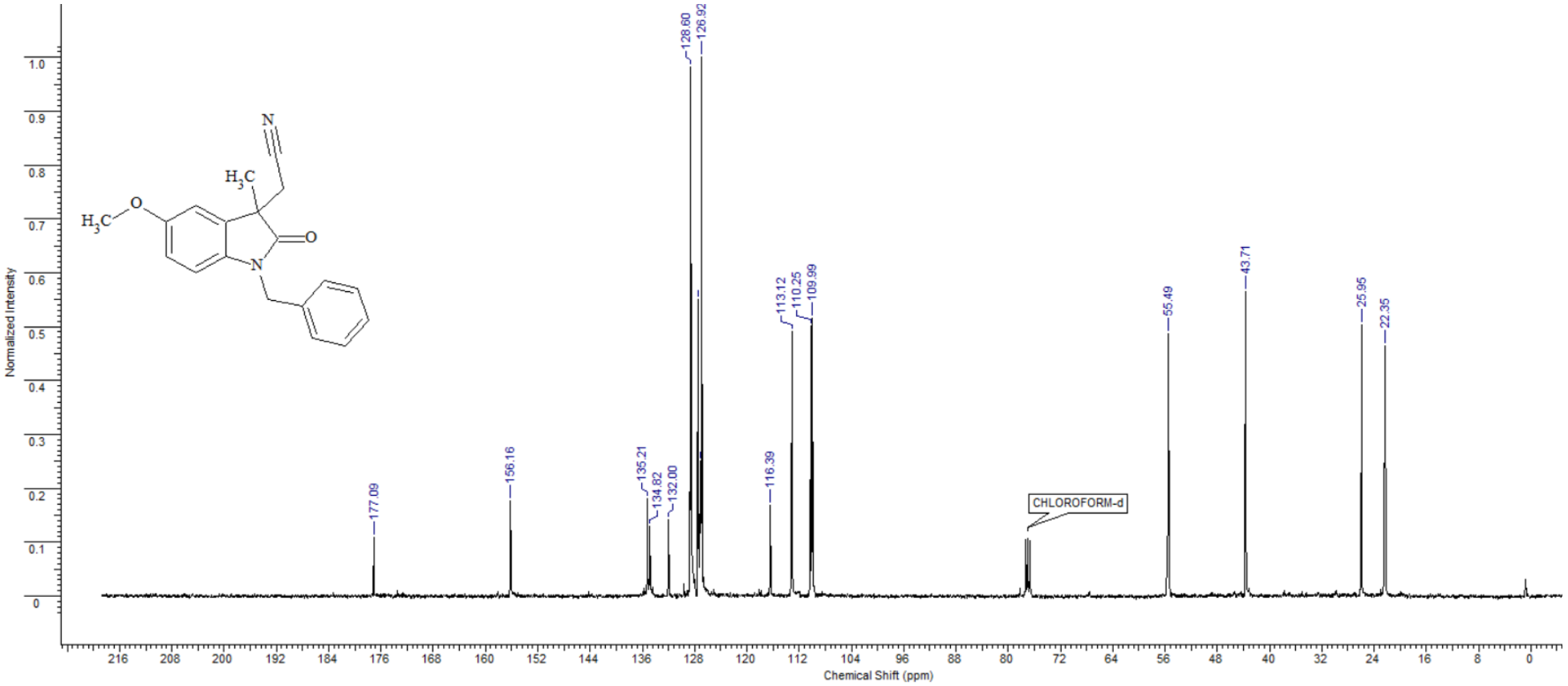
S23 13C NMR spectrum of 2-(1,3-Dimethyl-5-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5e**)



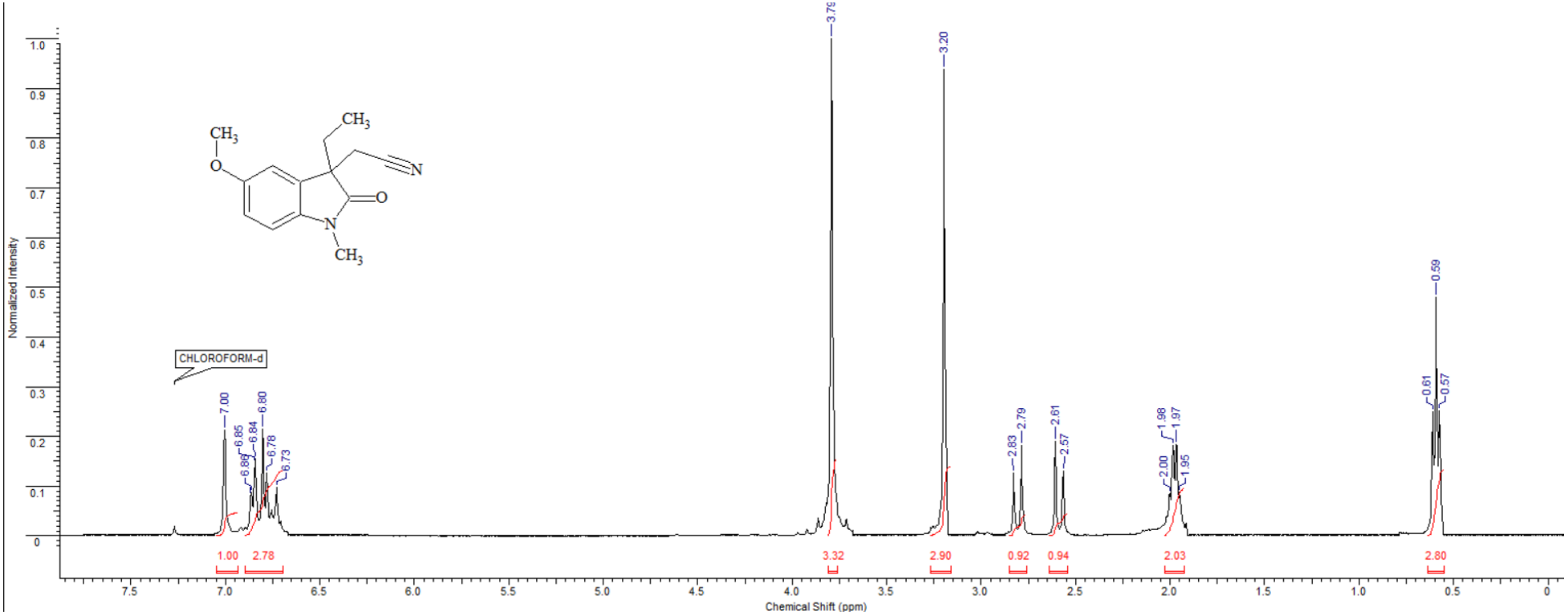
S24 1H NMR spectrum of 2-(1-Benzyl-3-methyl-5-methoxy-2-oxo-2,3-dihydro-*1H*-indol-3-yl)acetonitrile (**5f**)



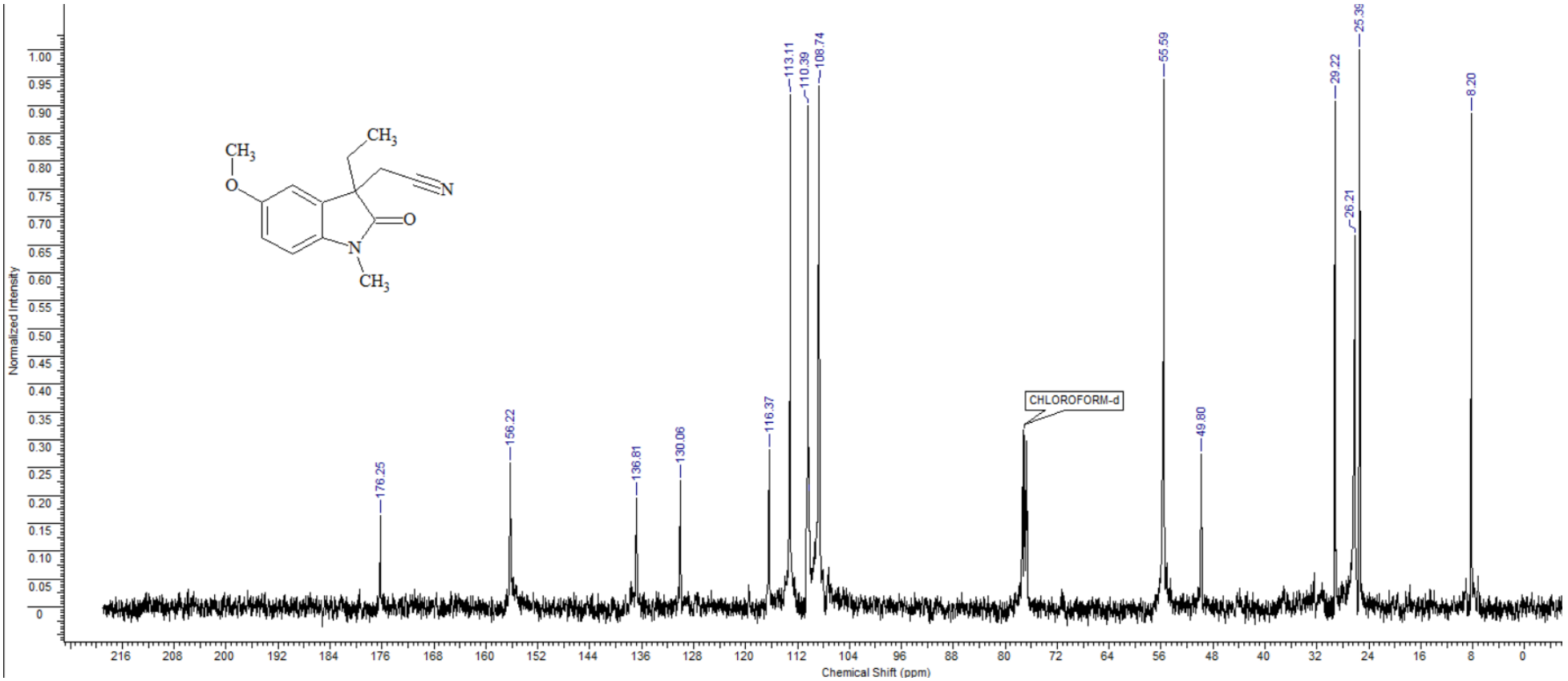
S25 13C NMR spectrum of 2-(1-Benzyl-3-methyl-5-methoxy-2-oxo-2,3-dihydro-*1H*-indol-3-yl)acetonitrile (**5f**)



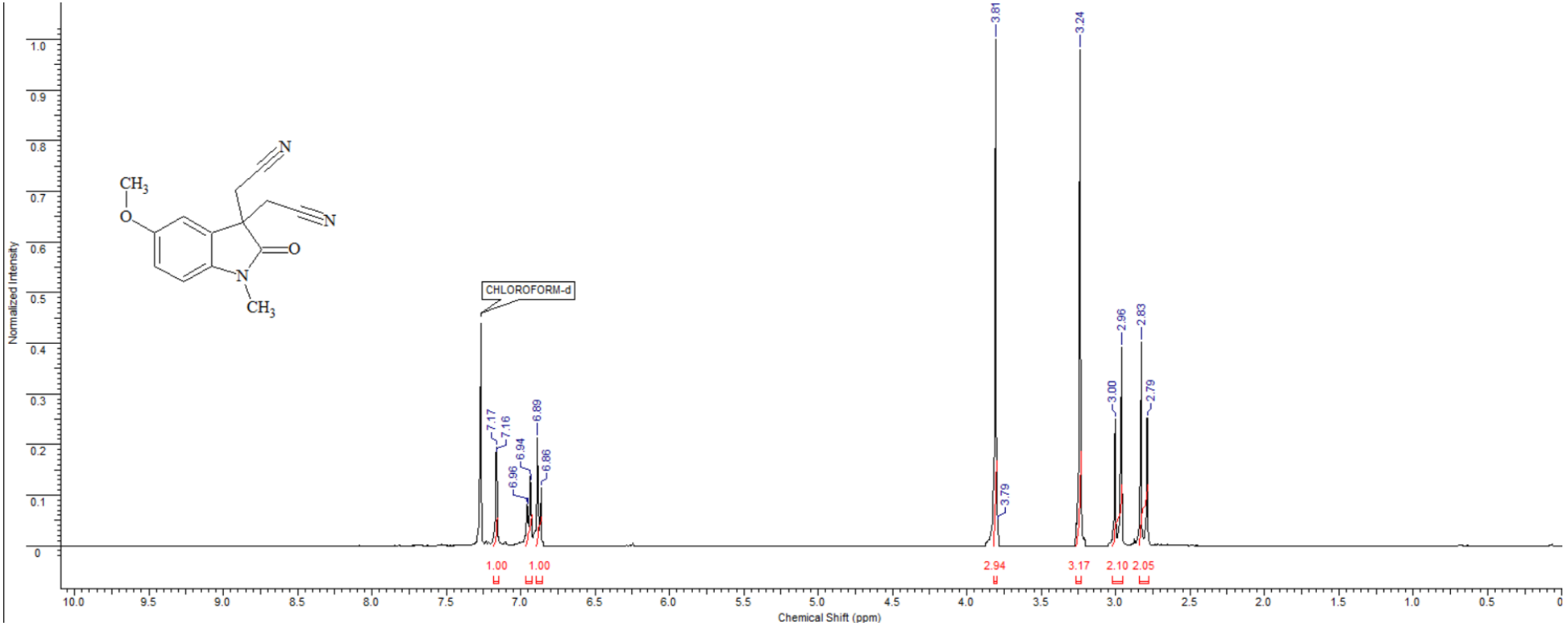
S26 1H NMR spectrum of 2-(3-Ethyl-1-methyl-5-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5g**)



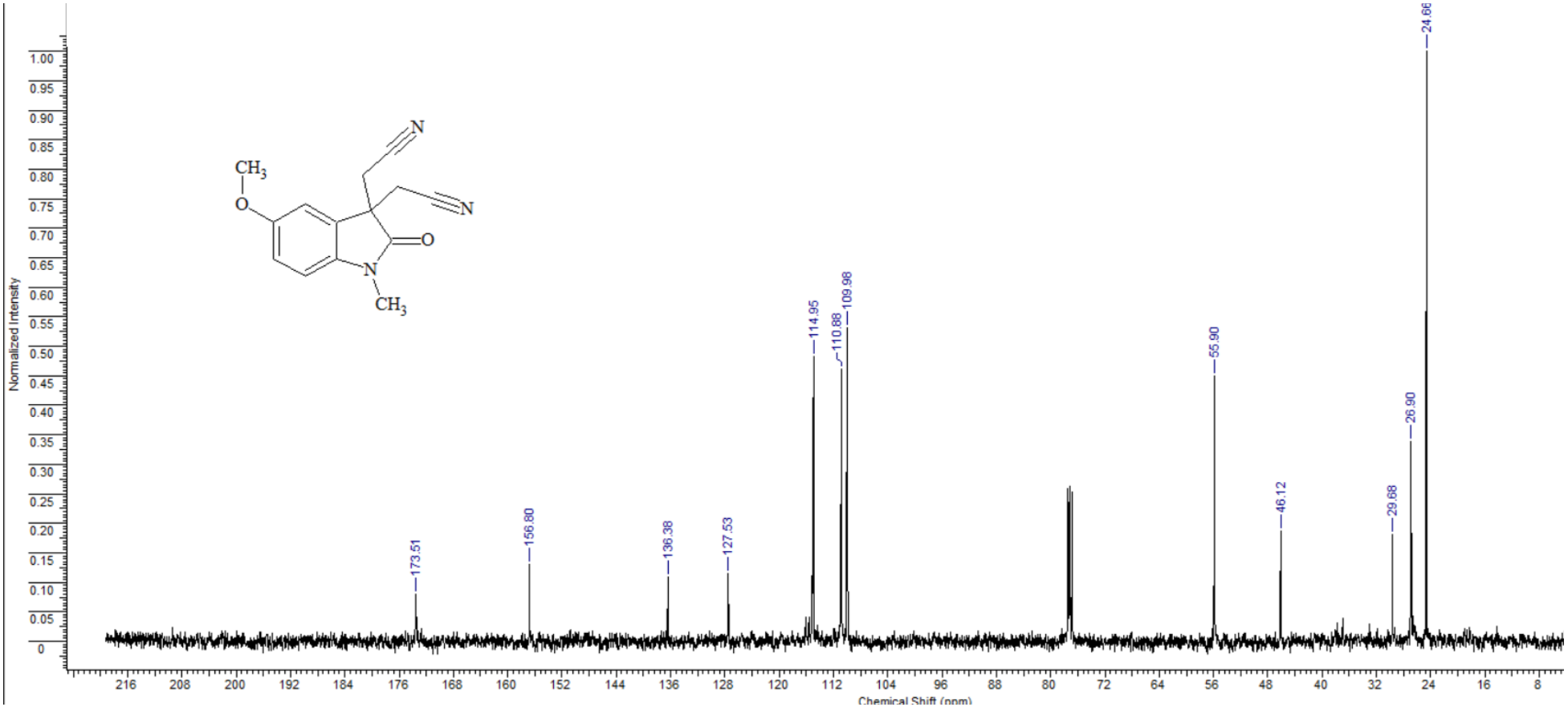
S27 13C NMR spectrum of 2-(3-Ethyl-1-methyl-5-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5g**)



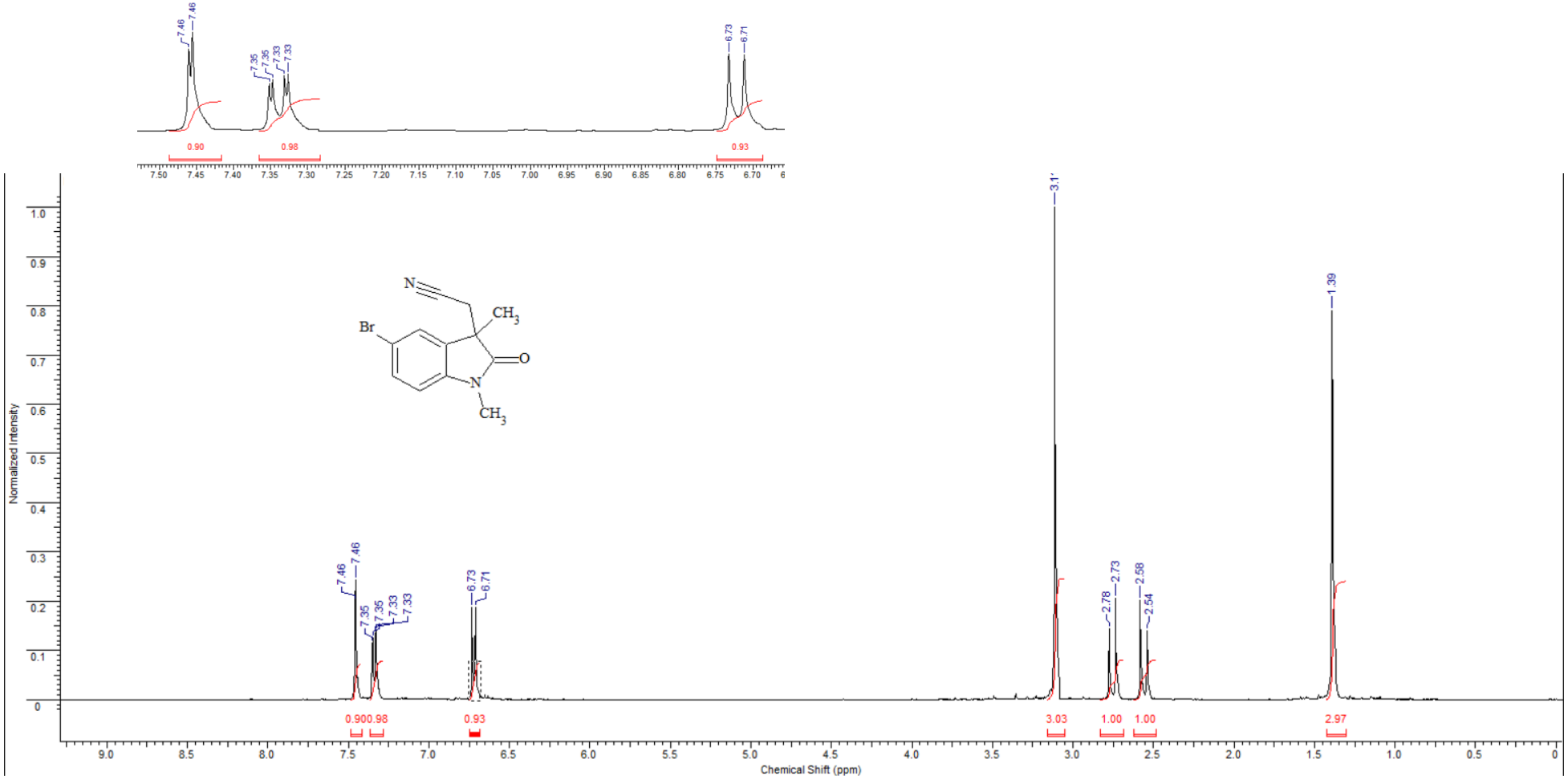
S28 1H NMR spectrum of 2-(1-Methyl-5-methoxy-3-(cyanomethyl)-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5h**)



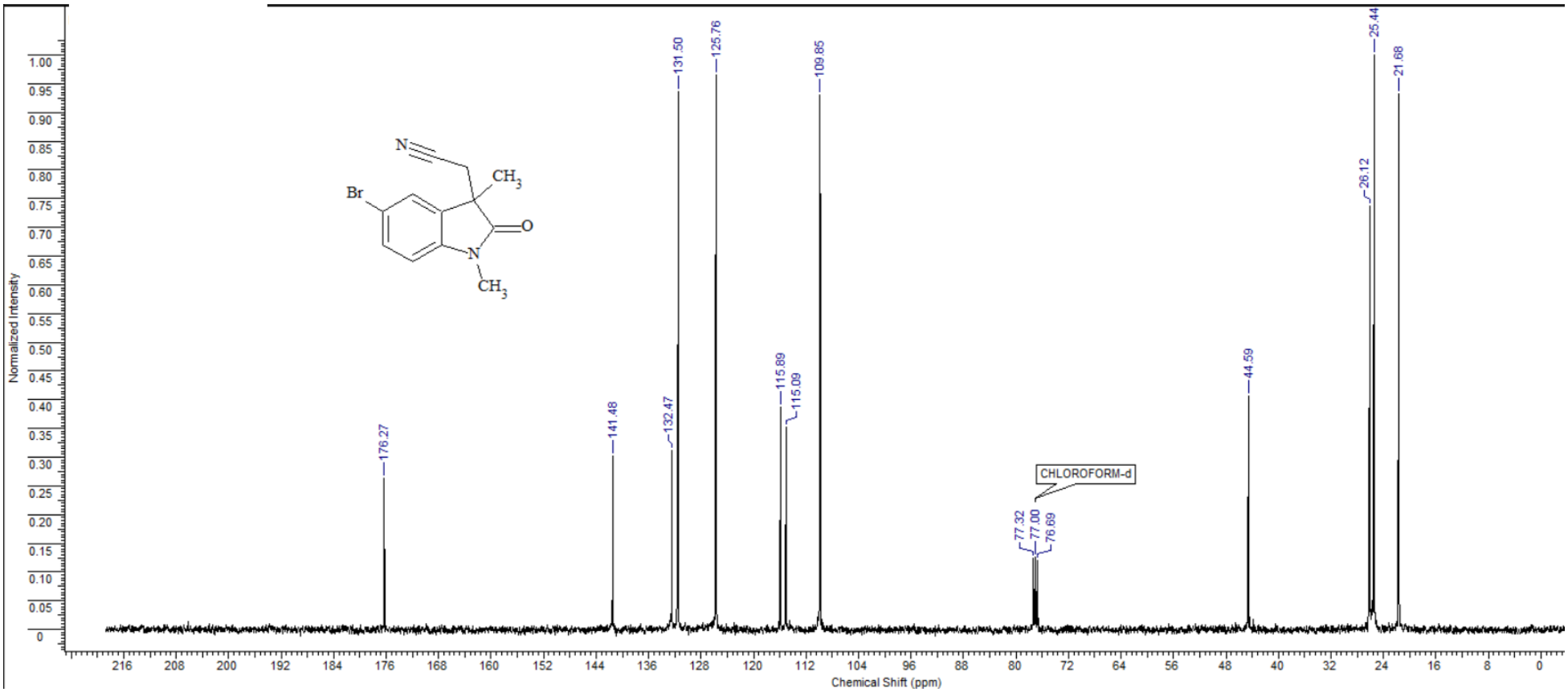
S29 13C NMR spectrum of 2-(1-Methyl-5-methoxy-3-(cyanomethyl)-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5h**)



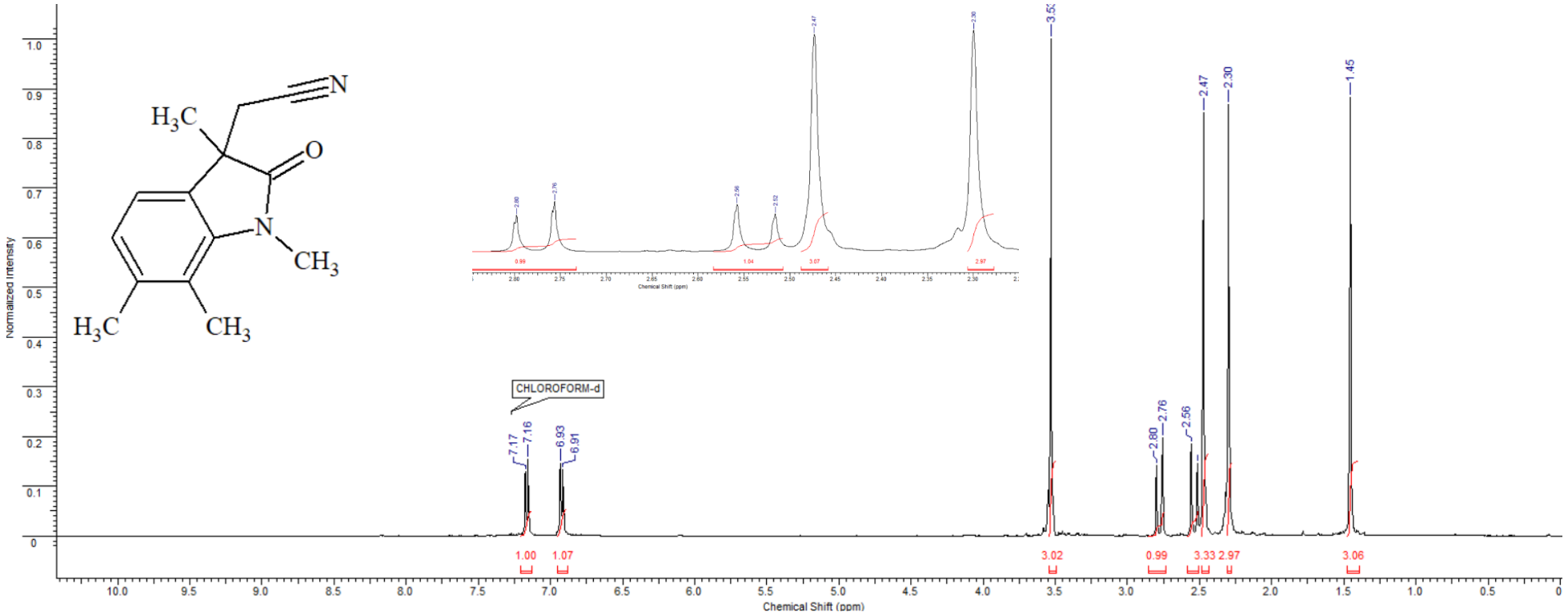
S30 1H NMR spectrum of 2-(5-Bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5j**)



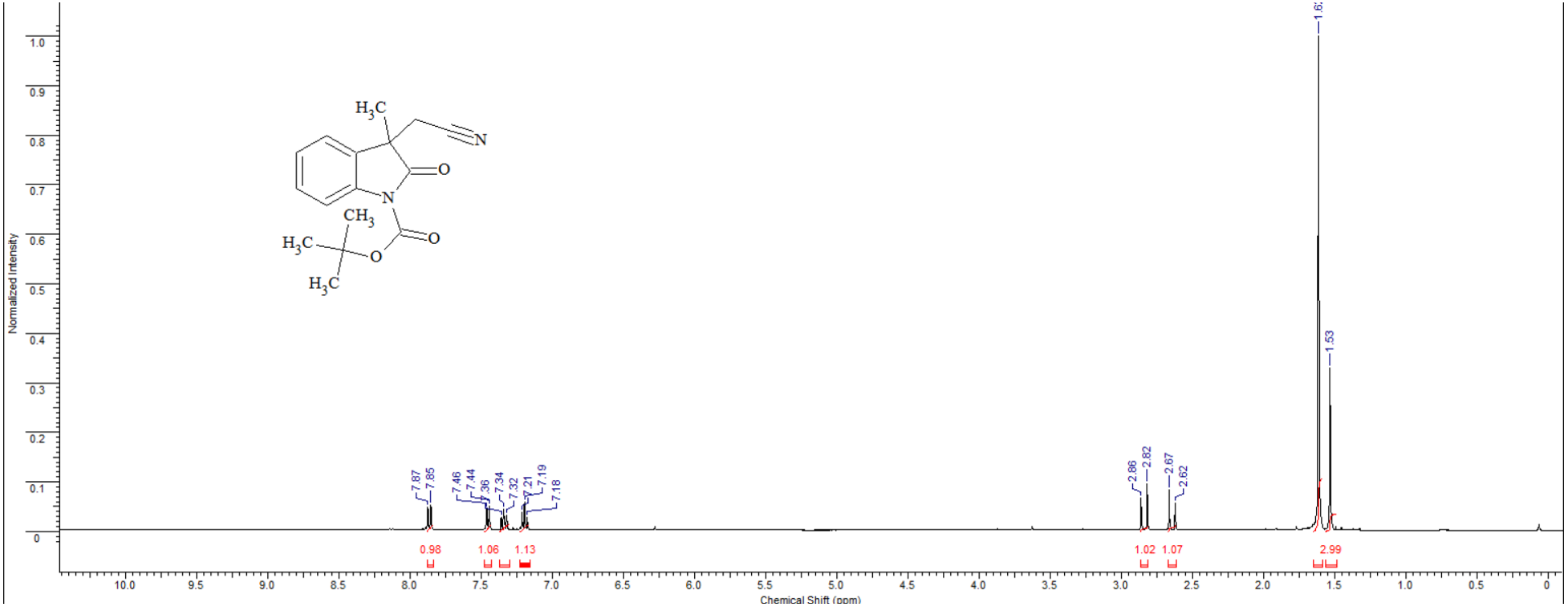
S31 13C NMR spectrum of 2-(5-Bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetonitrile (**5j**)



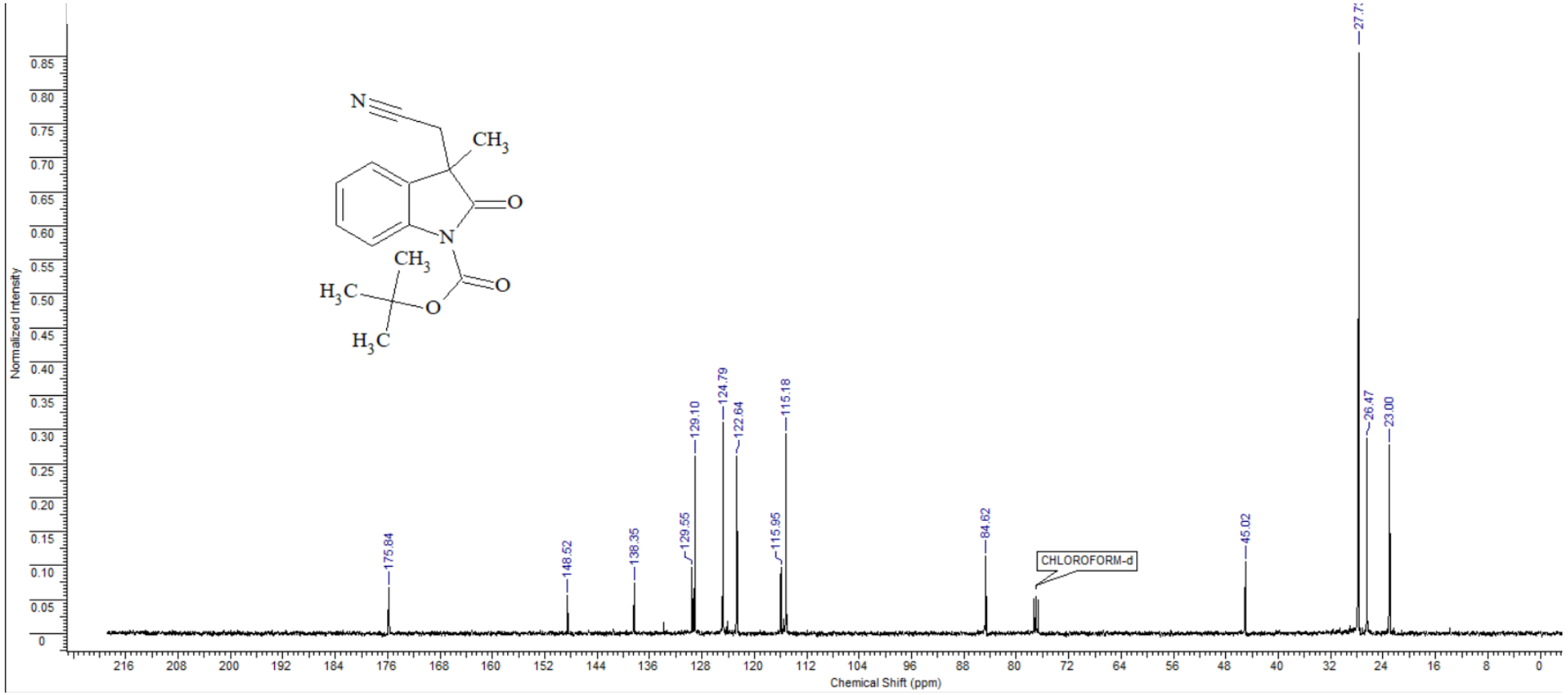
S32 1H NMR spectrum of 2-(1,3,6,7-Tetramethyl-2-oxo-2,3-dihydro-*1H*-indol-3-yl)acetonitrile (**5k**)



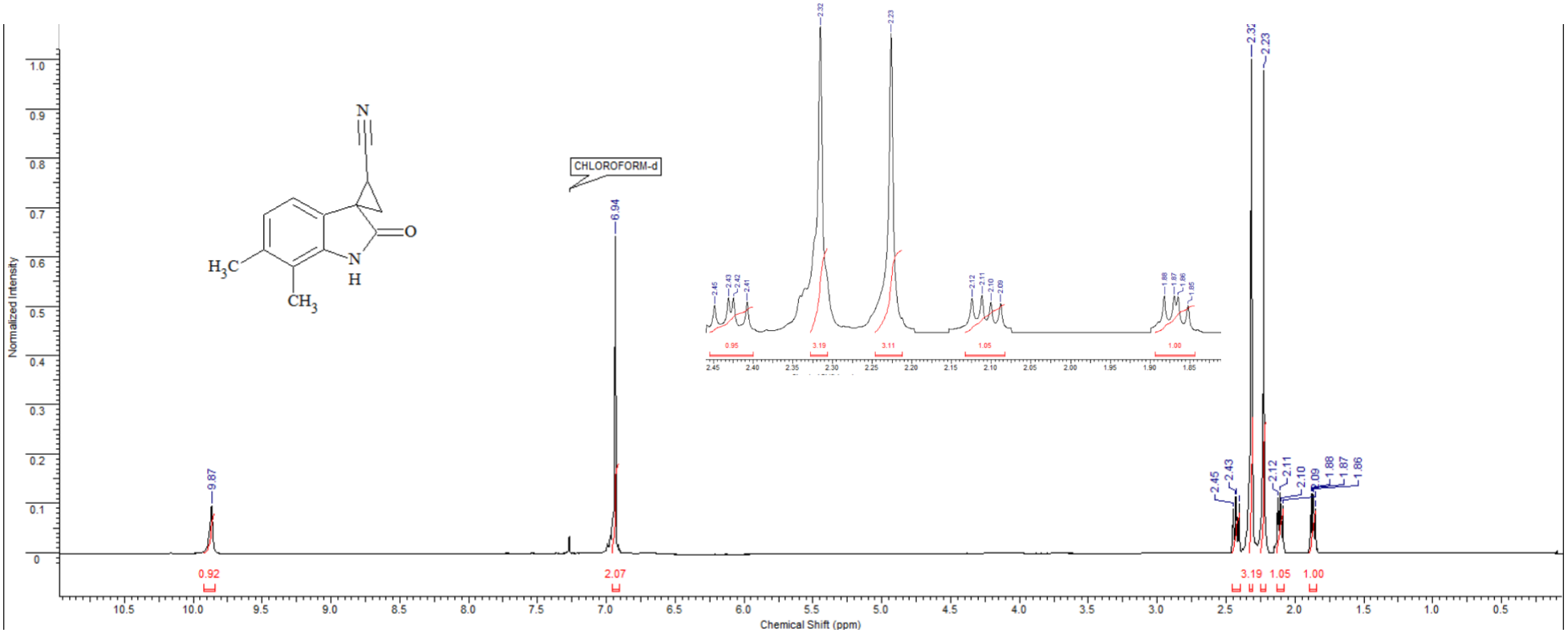
S33 1H NMR spectrum of Tert-butyl 3-(cyanomethyl)-3-methyl-2-oxo-2,3-dihydro-*1H*-indole-1-carboxylate (**5l**)



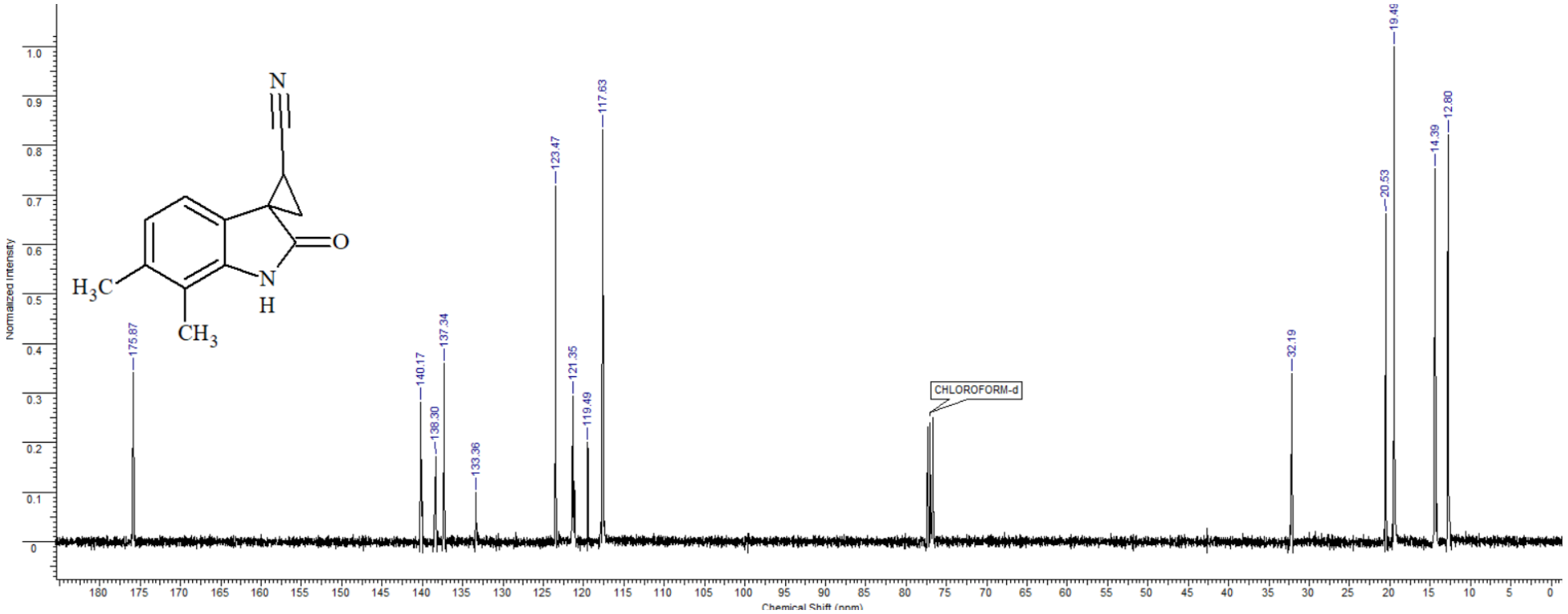
S34 13C NMR spectrum of Tert-butyl 3-(cyanomethyl)-3-methyl-2-oxo-2,3-dihydro-*1H*-indole-1-carboxylate (**5l**)



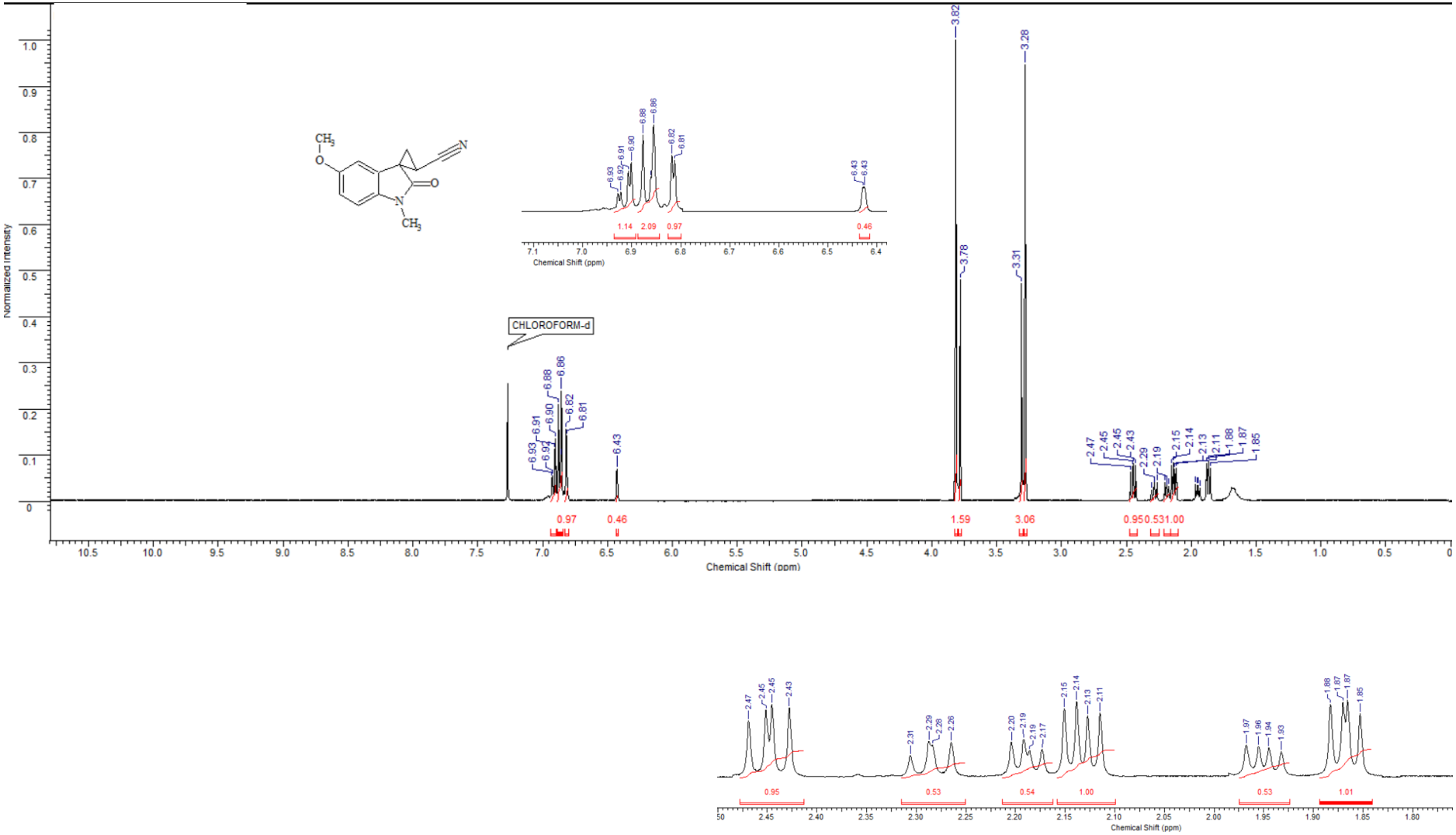
S35 1H NMR spectrum of (1R*,2R*)-6′,7′-Dimethyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (**6d**)



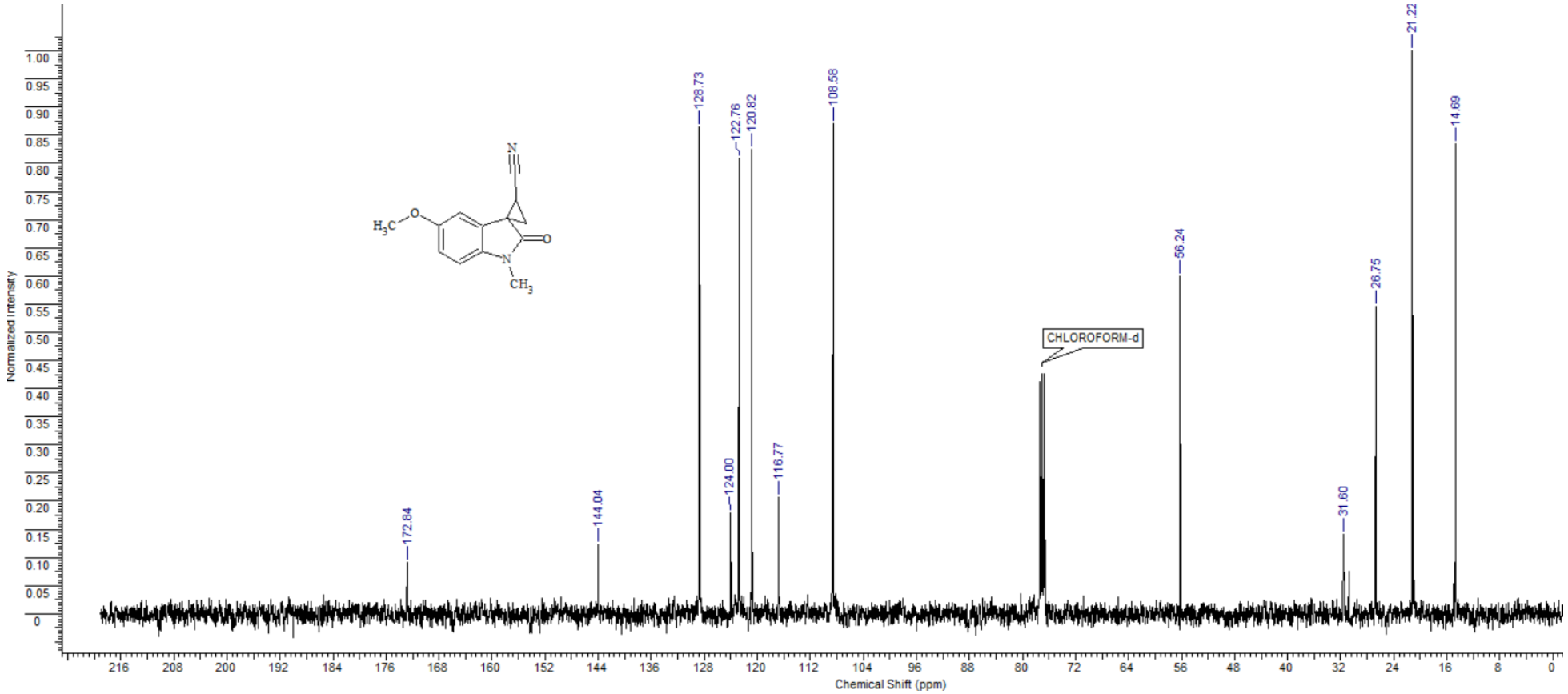
S36 13C NMR spectrum of (1R*,2R*)-6’,7’-Dimethyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (**6d**)



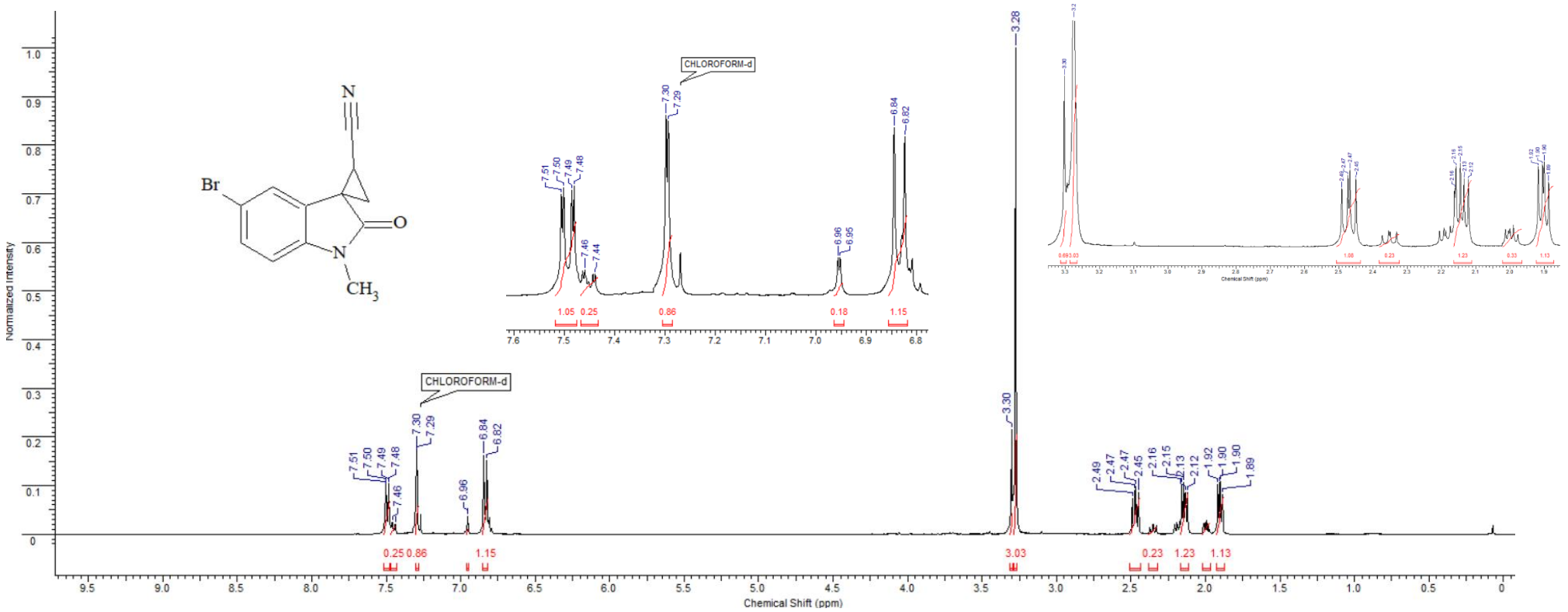
S37 1H NMR spectrum of 1’-Methyl-5’-methoxy-2’-oxo-1’,2’-dihydrospiro[cyclopropane-1,3’-indole]-3-carbonitrile (**6g**) (mixture of isomers)



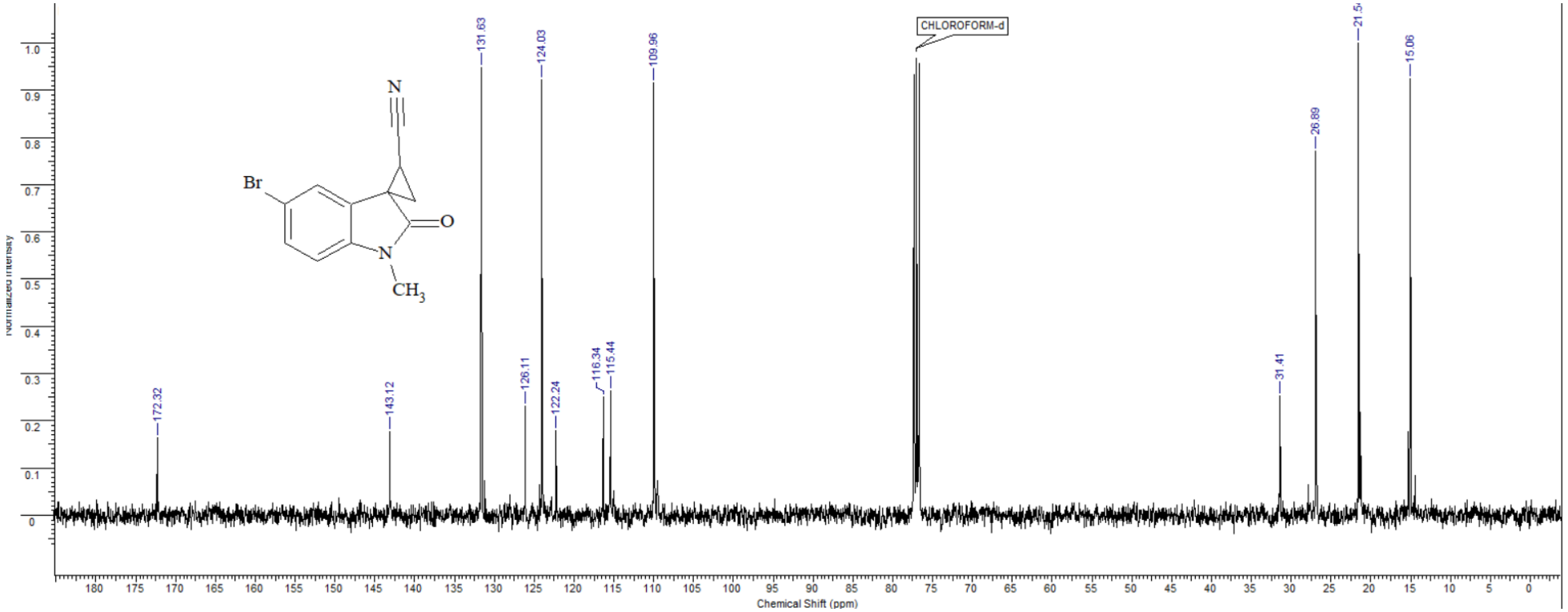
S38 13C NMR spectrum of 1’-Methyl-5’-methoxy-2’-oxo-1’,2’-dihydrospiro[cyclopropane-1,3’-indole]-3-carbonitrile (**6g**) (single isomers)



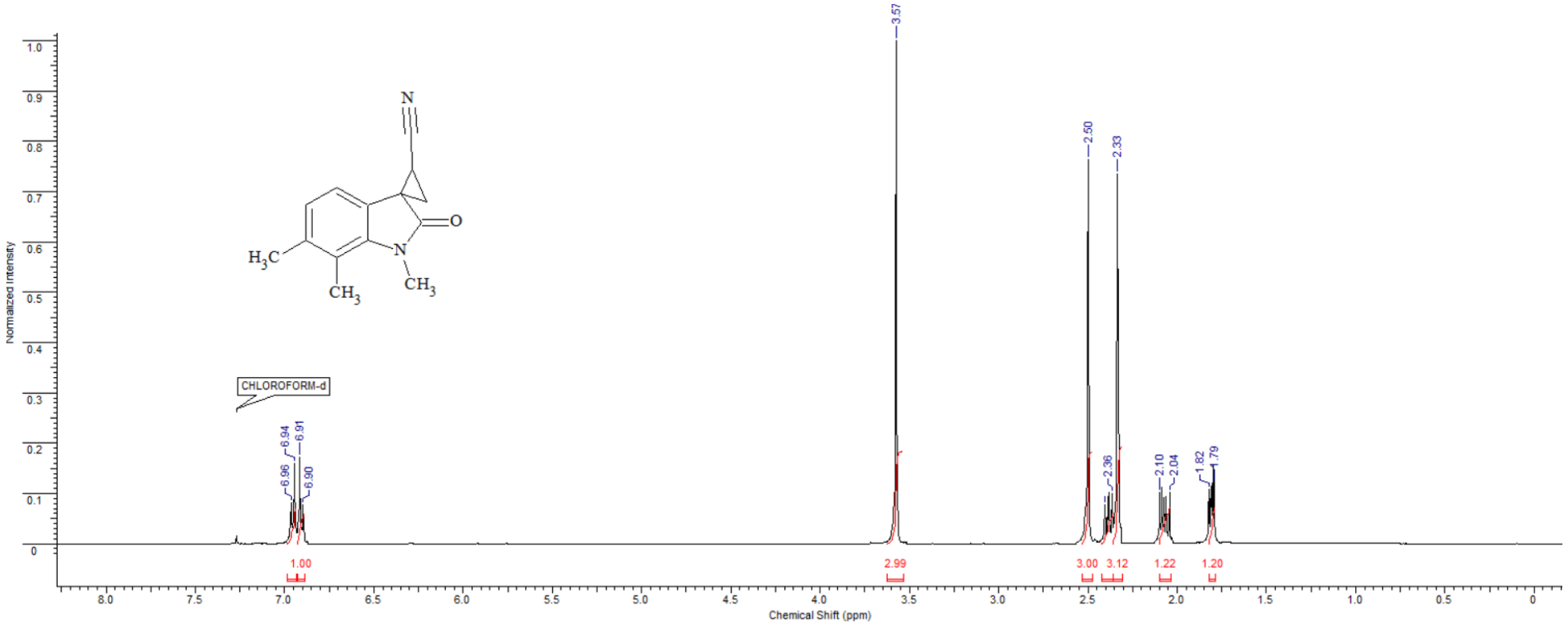
S39 1H NMR spectrum of 5'-Bromo-1-methyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (**6i**)



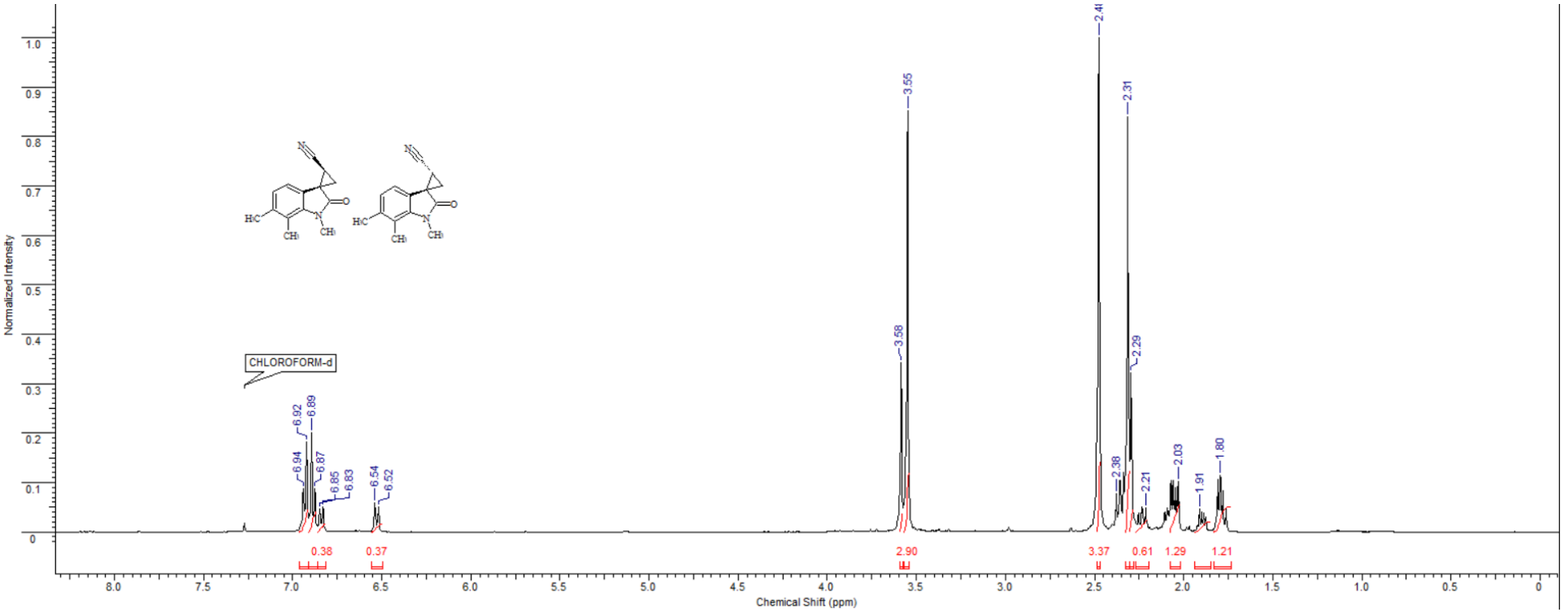
S40 13C NMR spectrum of 5'-Bromo-1-methyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (**6i**)



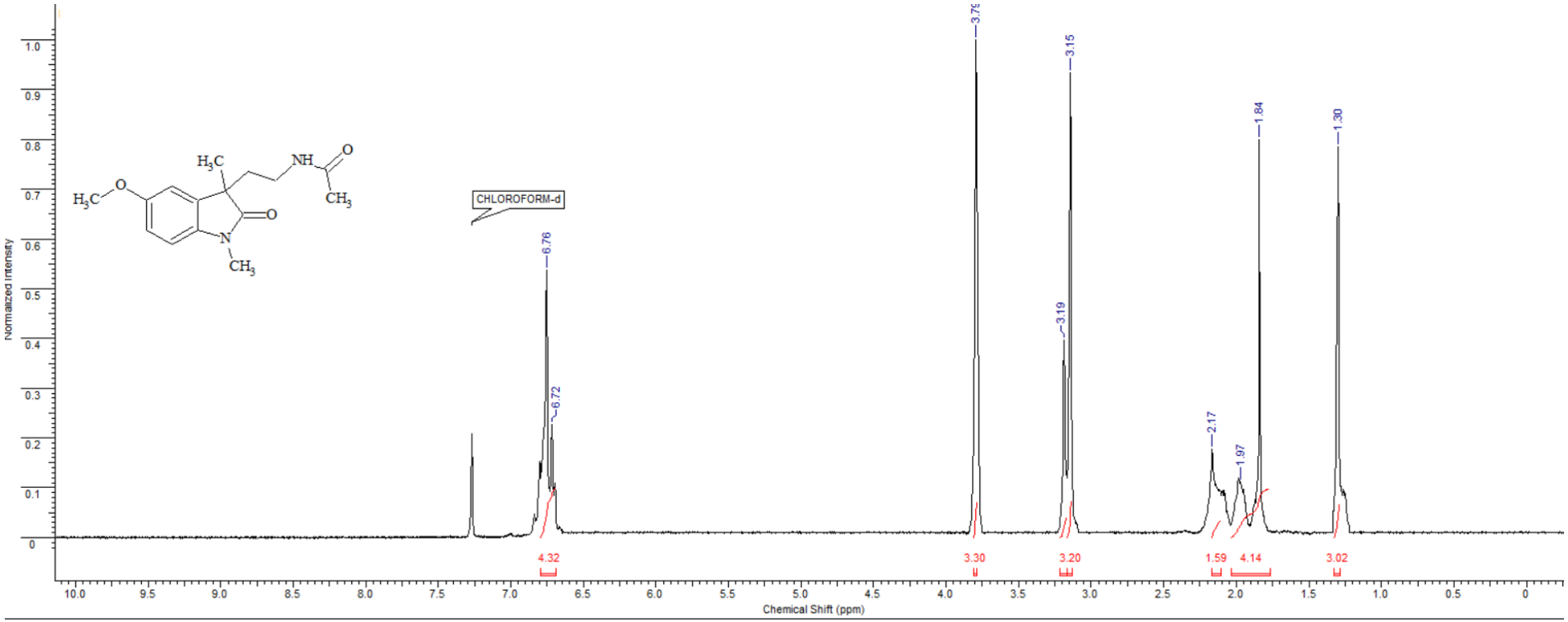
S41 1H NMR spectrum of 1’,6’,7’-Trimethyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (single isomer from 6d) (**6j**)



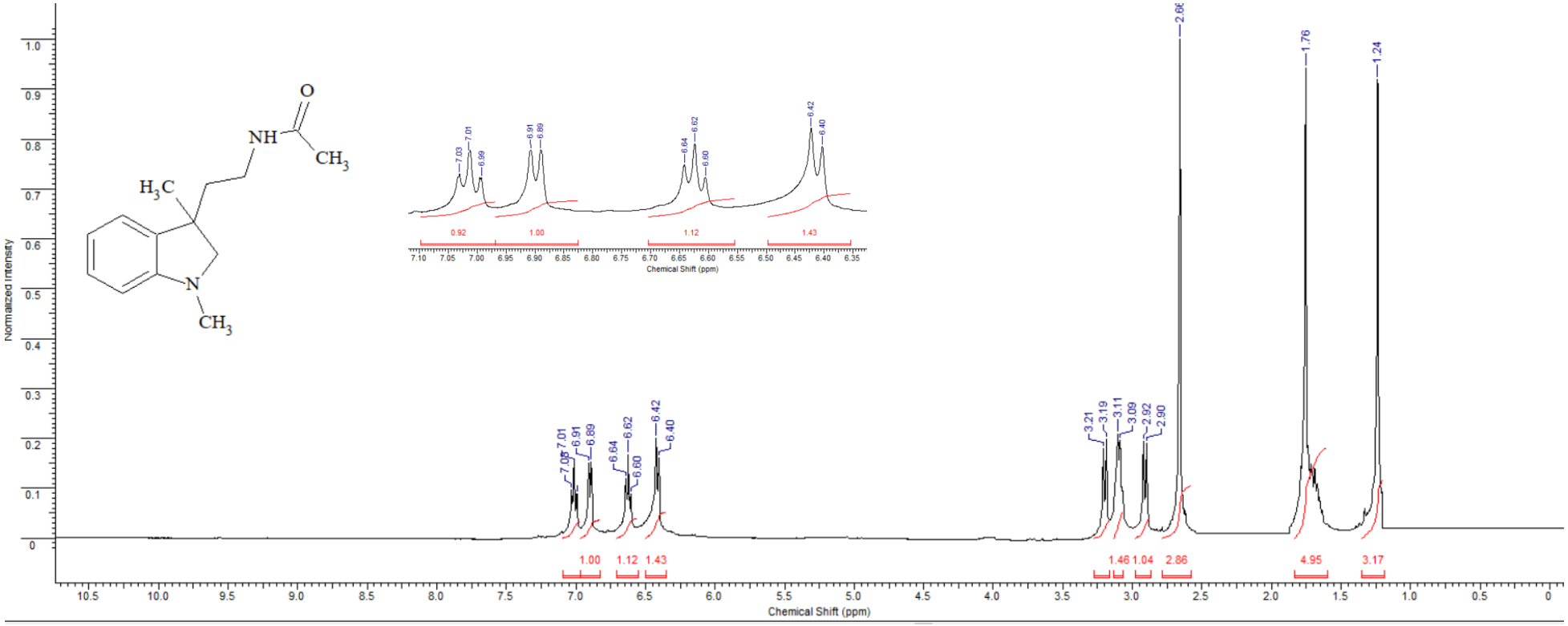
S42 1H NMR spectrum of 1’,6’,7’-Trimethyl-2'-oxo-1',2'-dihydrospiro[cyclopropane-1,3'-indole]-3-carbonitrile (mixture of isomers from **3j**) (**6j**)

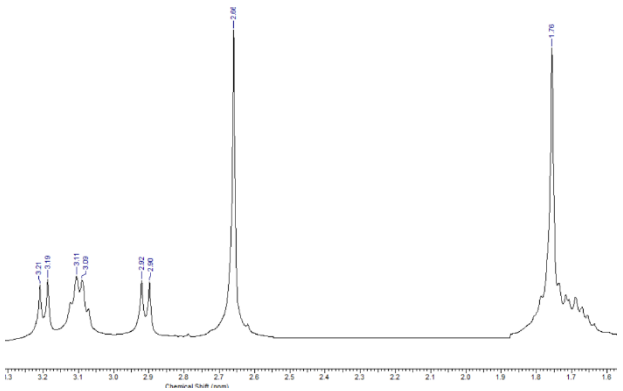


S43 1H NMR spectrum of N-[(1,3-dimethyl-5-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**7b**)

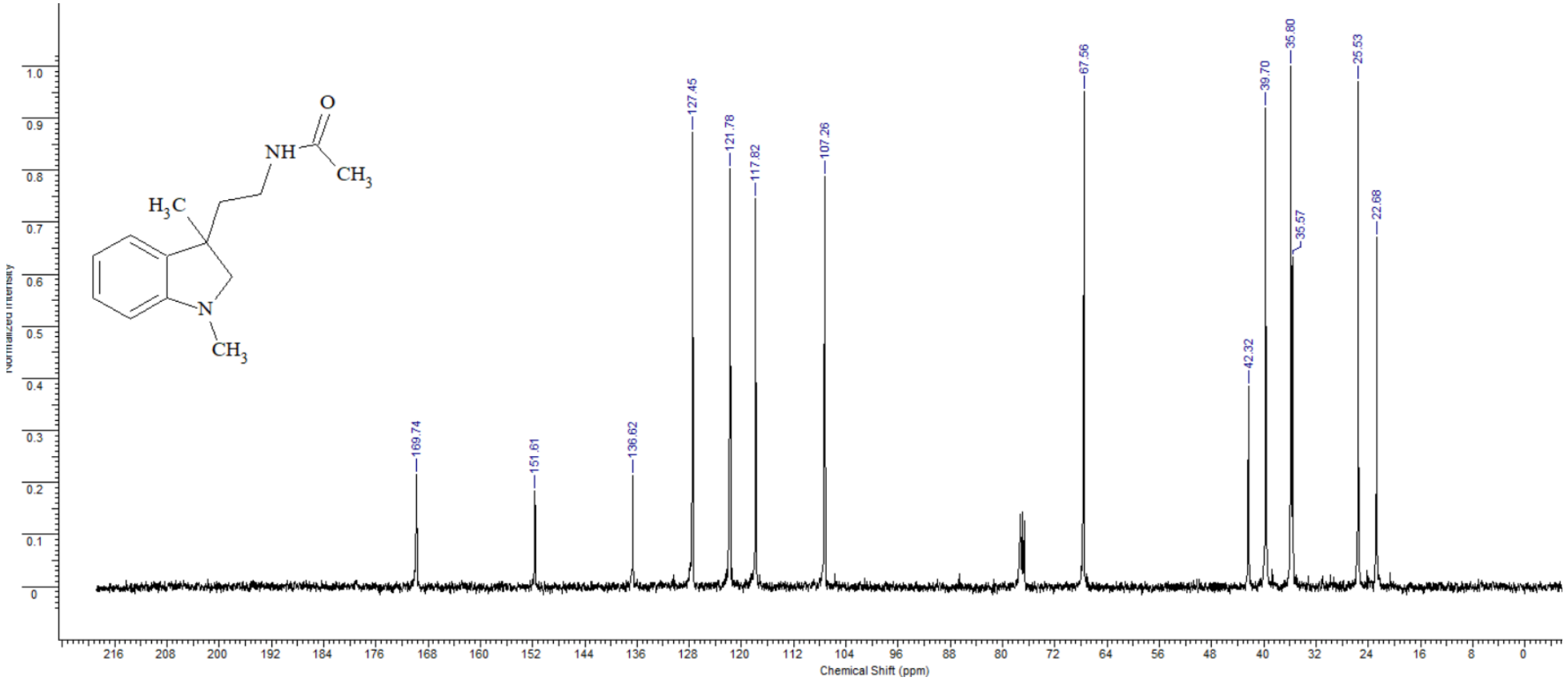


S44 1H NMR spectrum of N-[2-(1,3-Dimethyl-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8a**)

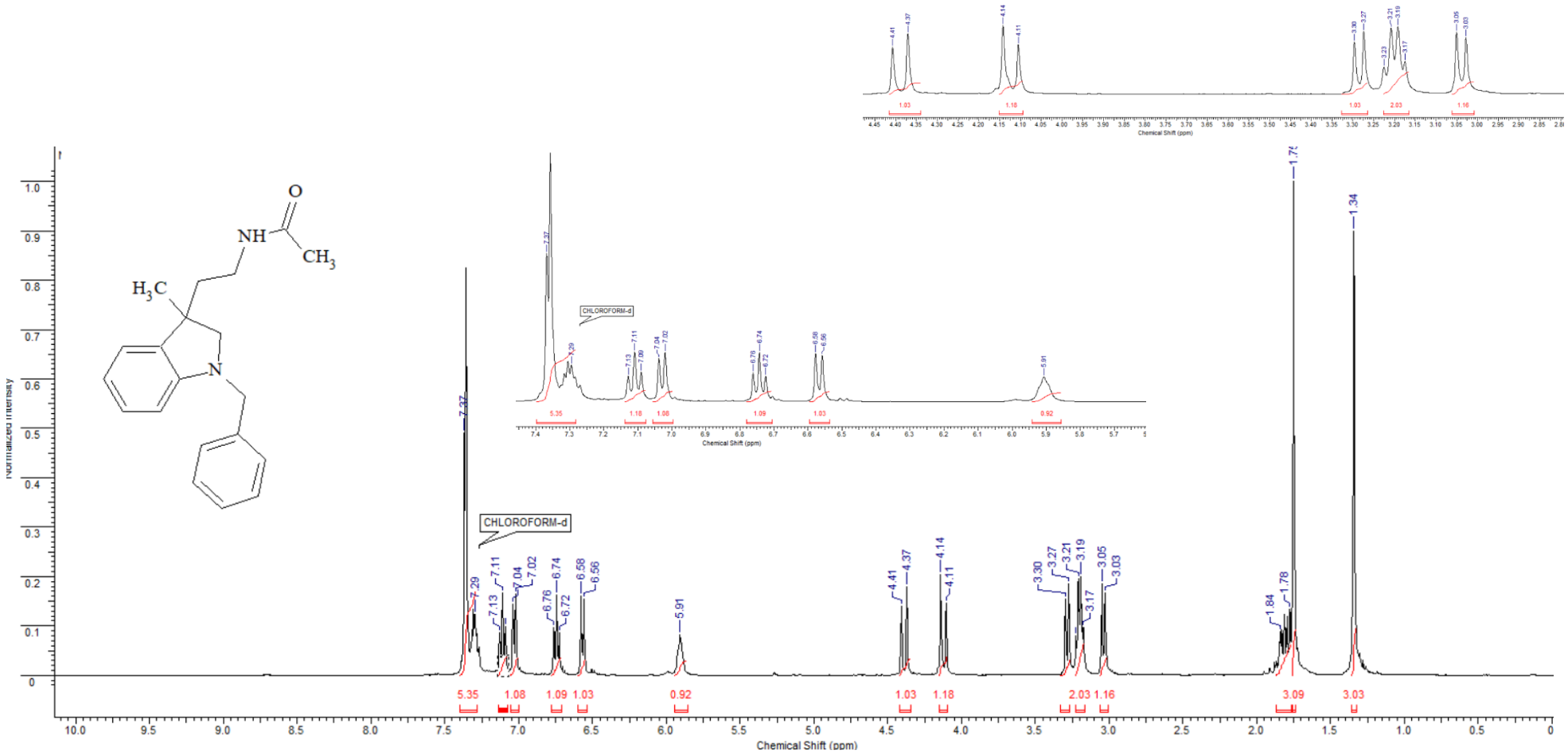




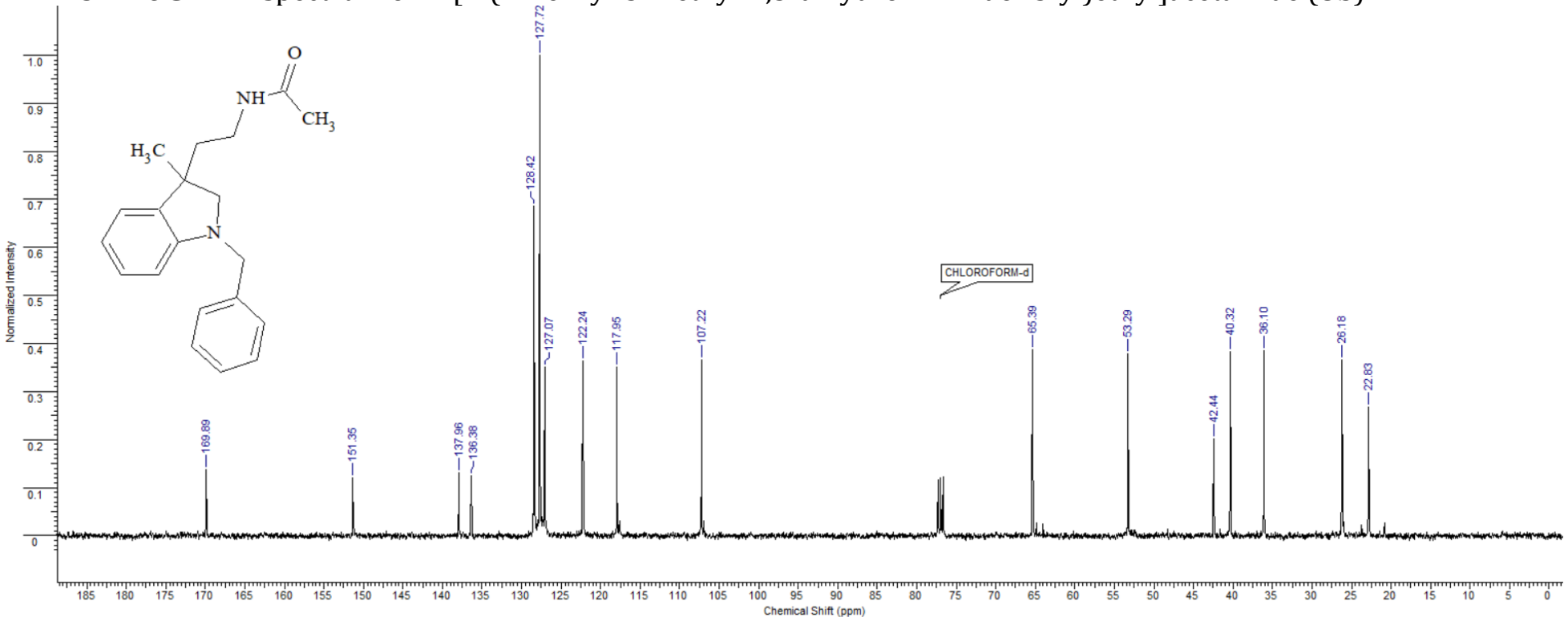
S45 13C NMR spectrum of N-[2-(1,3-Dimethyl-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8a**)



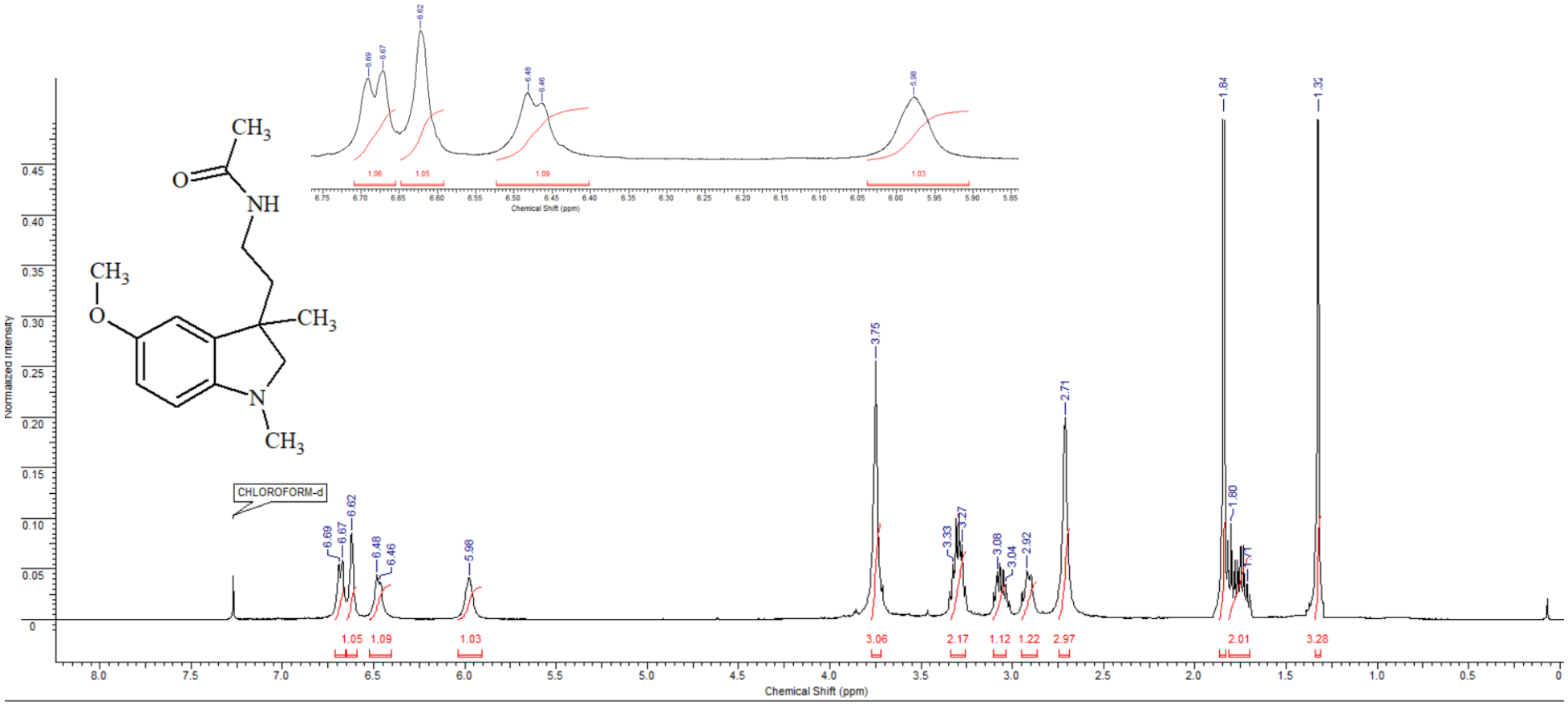
S46 1H NMR spectrum of N-[2-(1-Benzyl-3-methyl-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8b**)



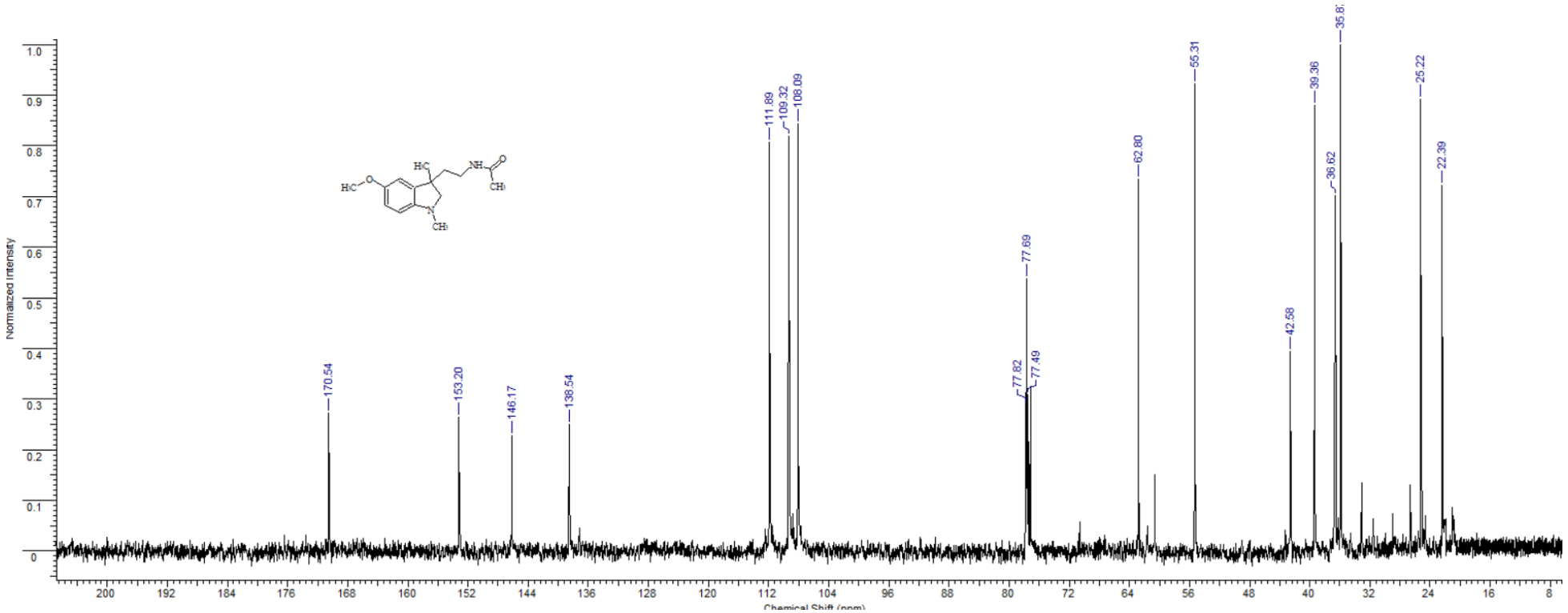
S47 13C NMR spectrum of N-[2-(1-Benzyl-3-methyl-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8b**)



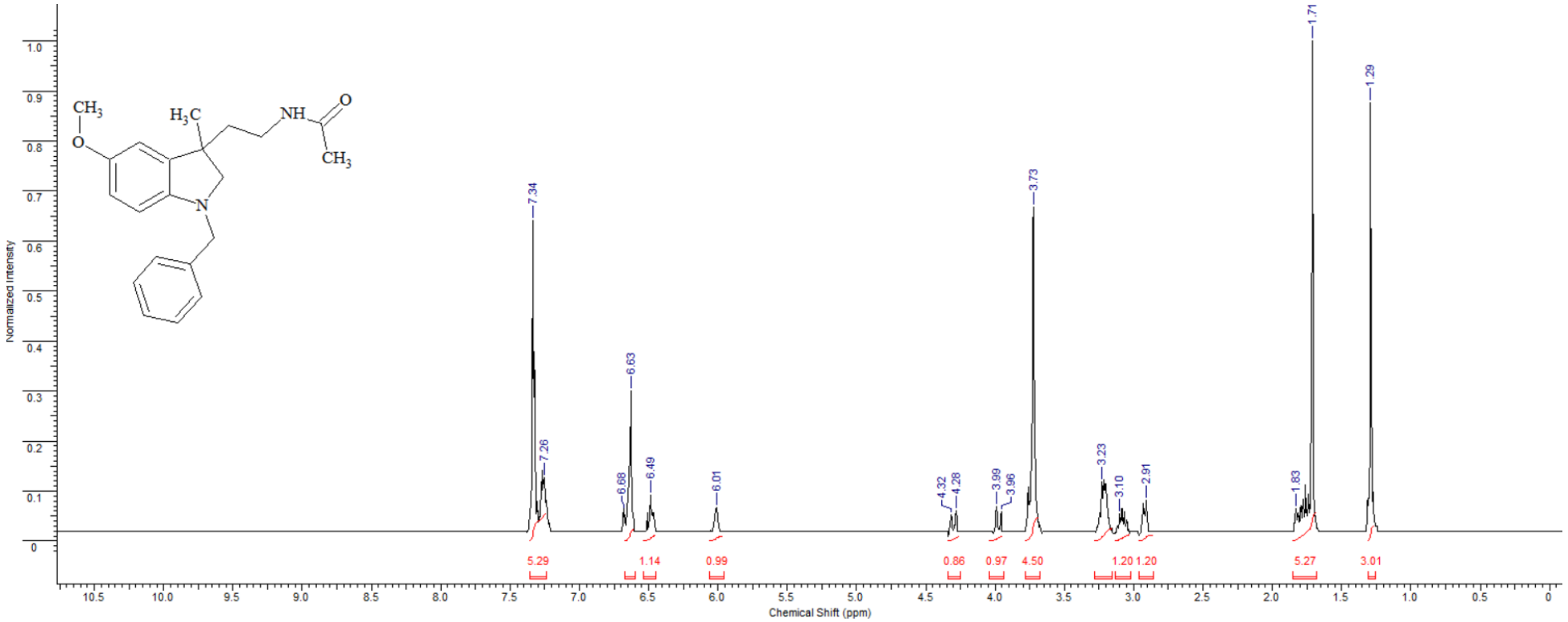
S48 1H NMR spectrum of N-[2-(1,3-Dimethyl-5-methoxy-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8e**)



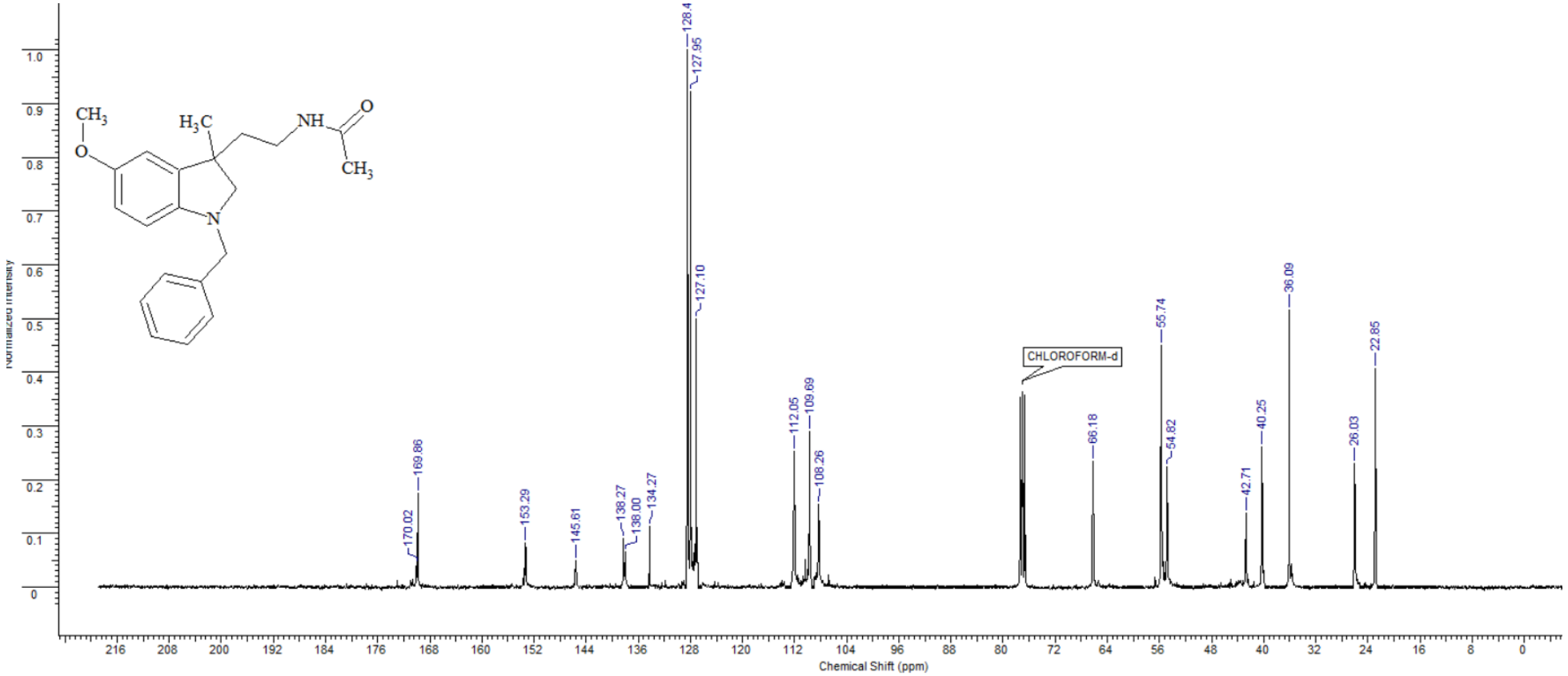
S49 13C NMR spectrum of N-[2-(1,3-Dimethyl-5-methoxy-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8e**)



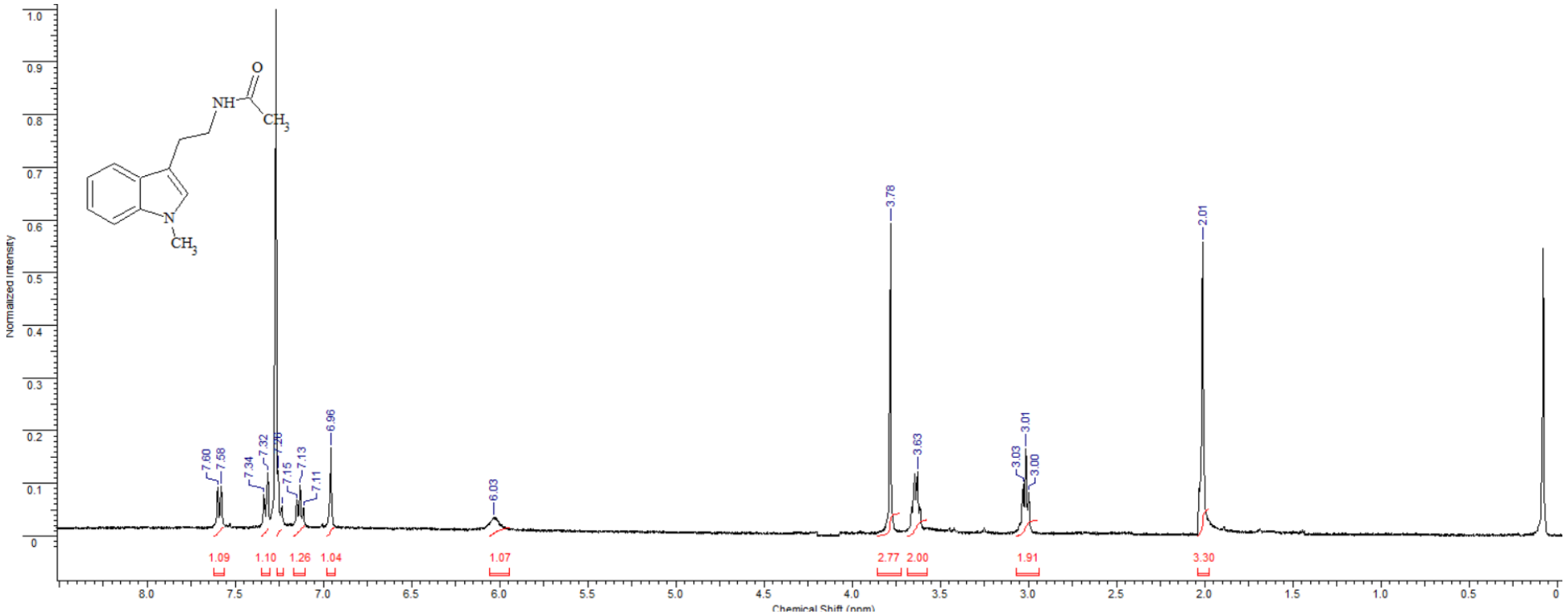
S50 1H NMR spectrum of *N*-[2-(1-Benzyl-3-methyl-5-methoxy-2,3-dihydro-*1H*-indol-3-yl)ethyl]acetamide (**8f**)



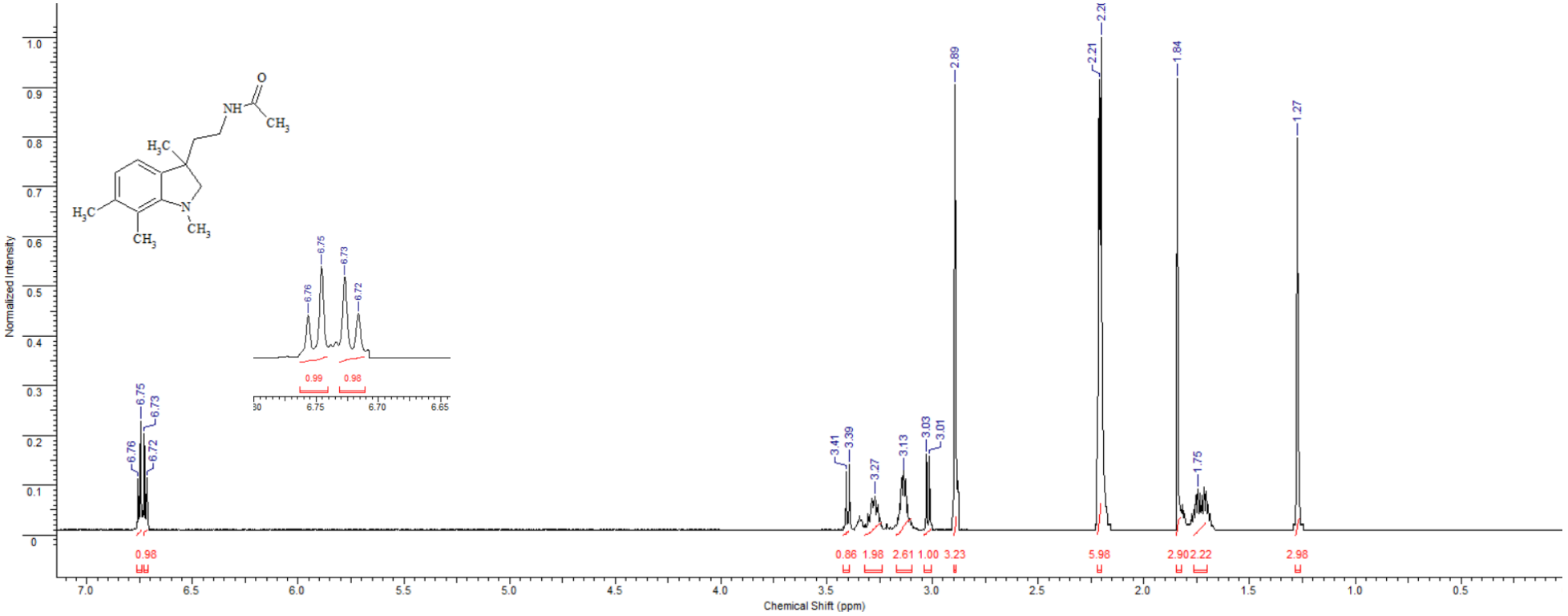
S51 13C NMR spectrum of *N*-[2-(1-Benzyl-3-methyl-5-methoxy-2,3-dihydro-*1H*-indol-3-yl)ethyl]acetamide (**8f**)



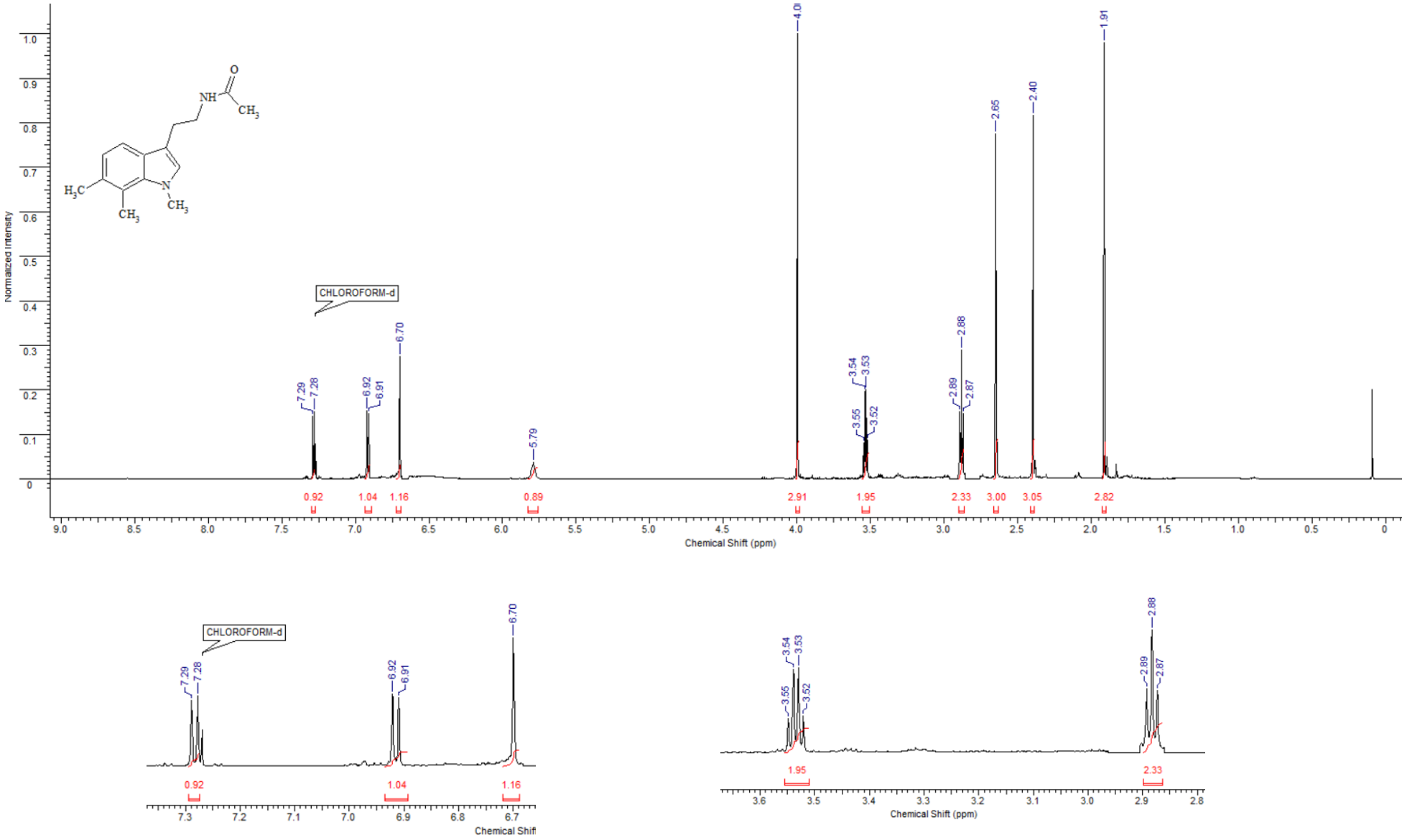
S52 1H NMR spectrum of *N*-[2-(1-Methyl-*1H*-indol-3-yl)ethyl]acetamide (**9i**)



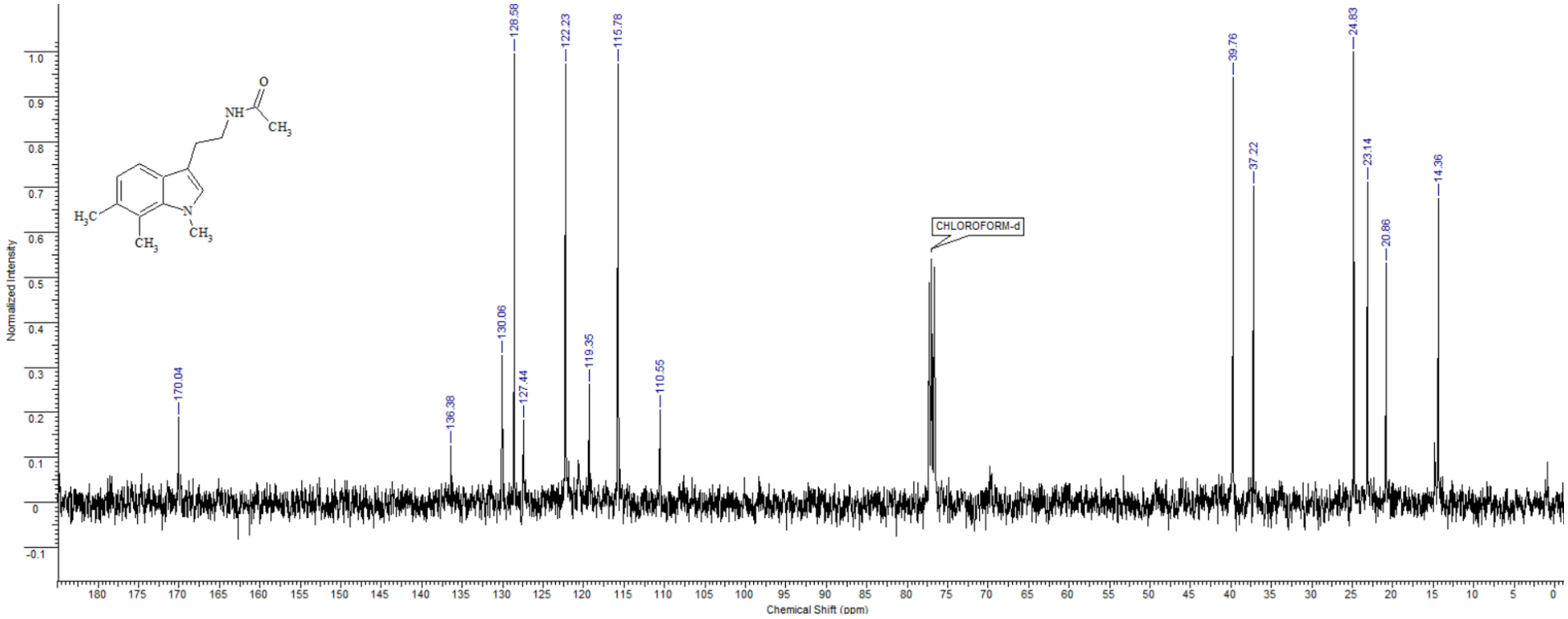
S53 1H NMR spectrum of N-[2-(1,3,6,7-Tetramethyl-2,3-dihydro-1H-indol-3-yl)ethyl]acetamide (**8k**)



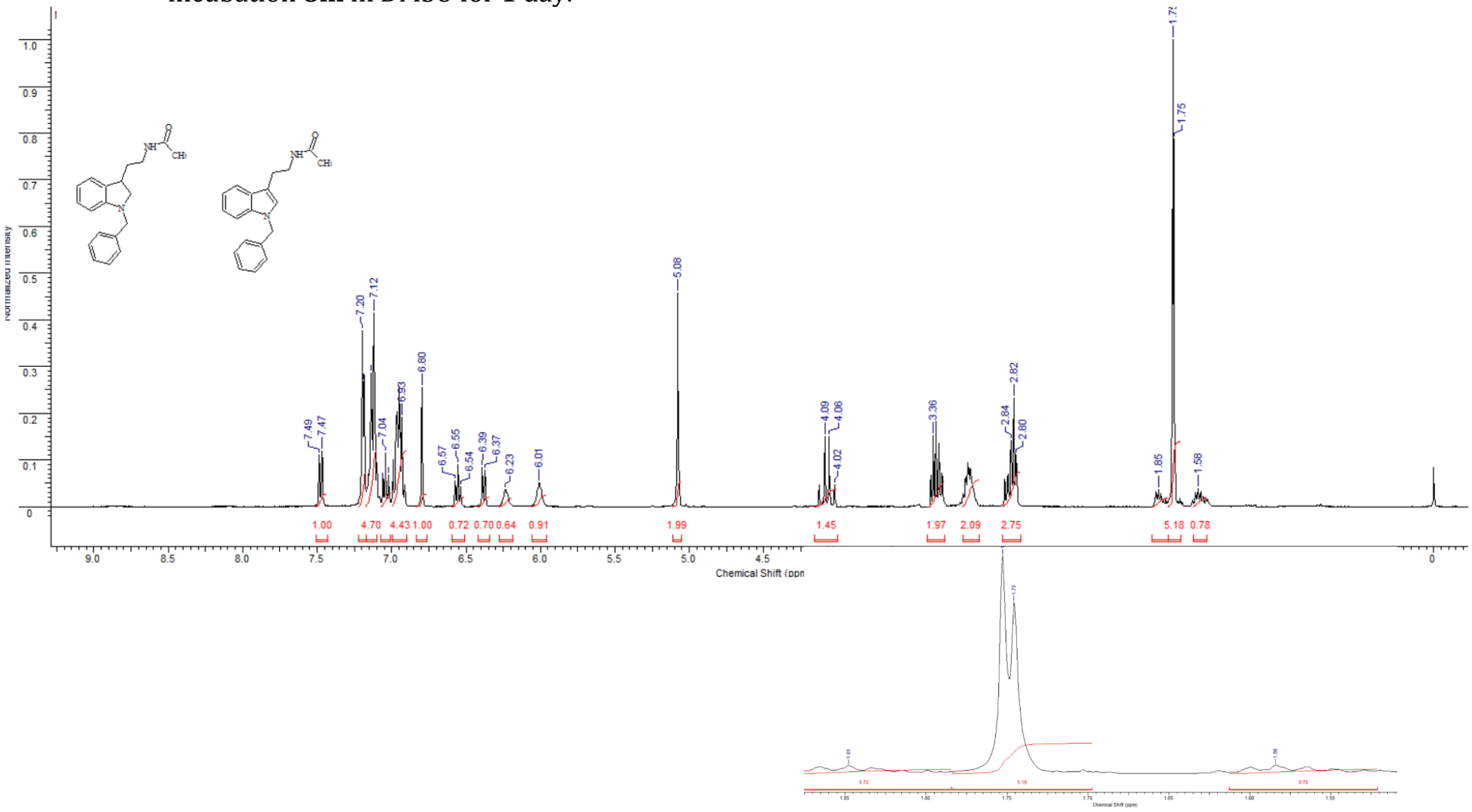
S54 1H NMR spectrum of *N*-[2-(1,6,7-Trimethyl-*1H*-indol-3-yl)ethyl]acetamide (**9l**)



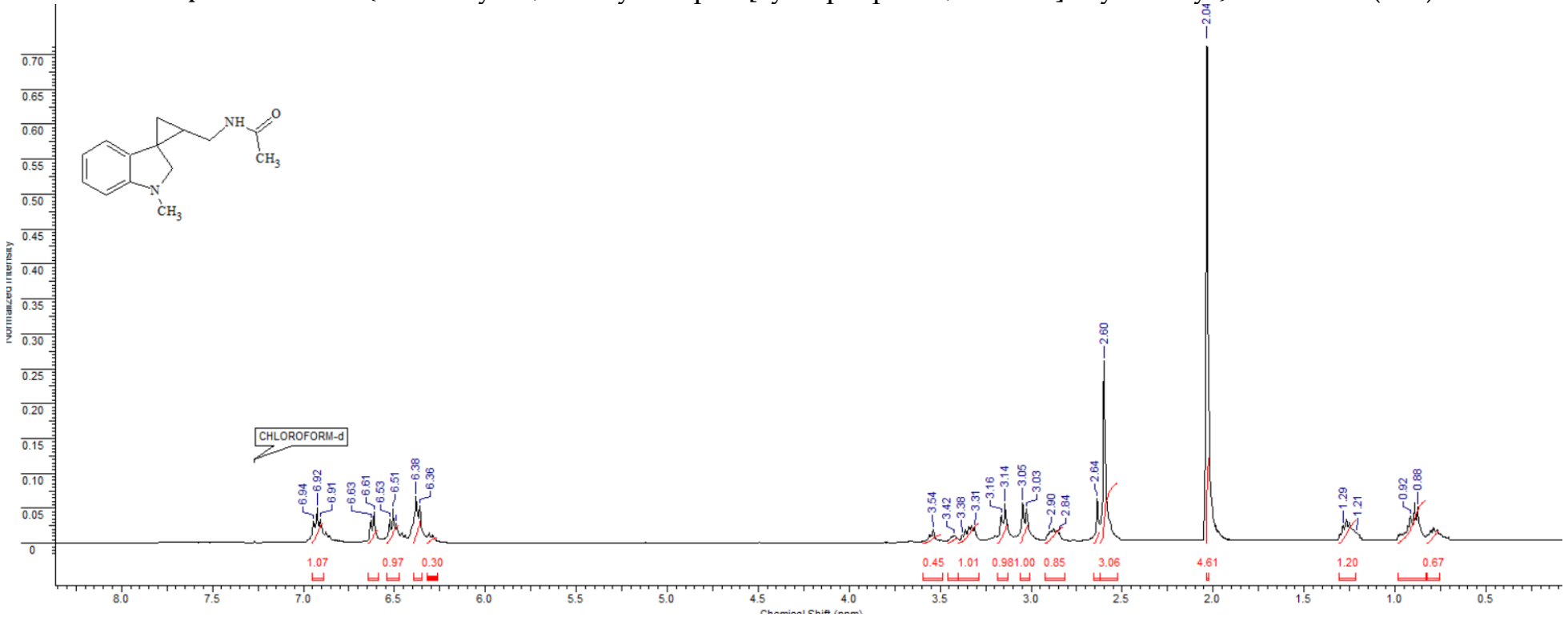
S55 13C NMR spectrum of *N*-[2-(1,6,7-Trimethyl-*1H*-indol-3-yl)ethyl]acetamide (**9l**)



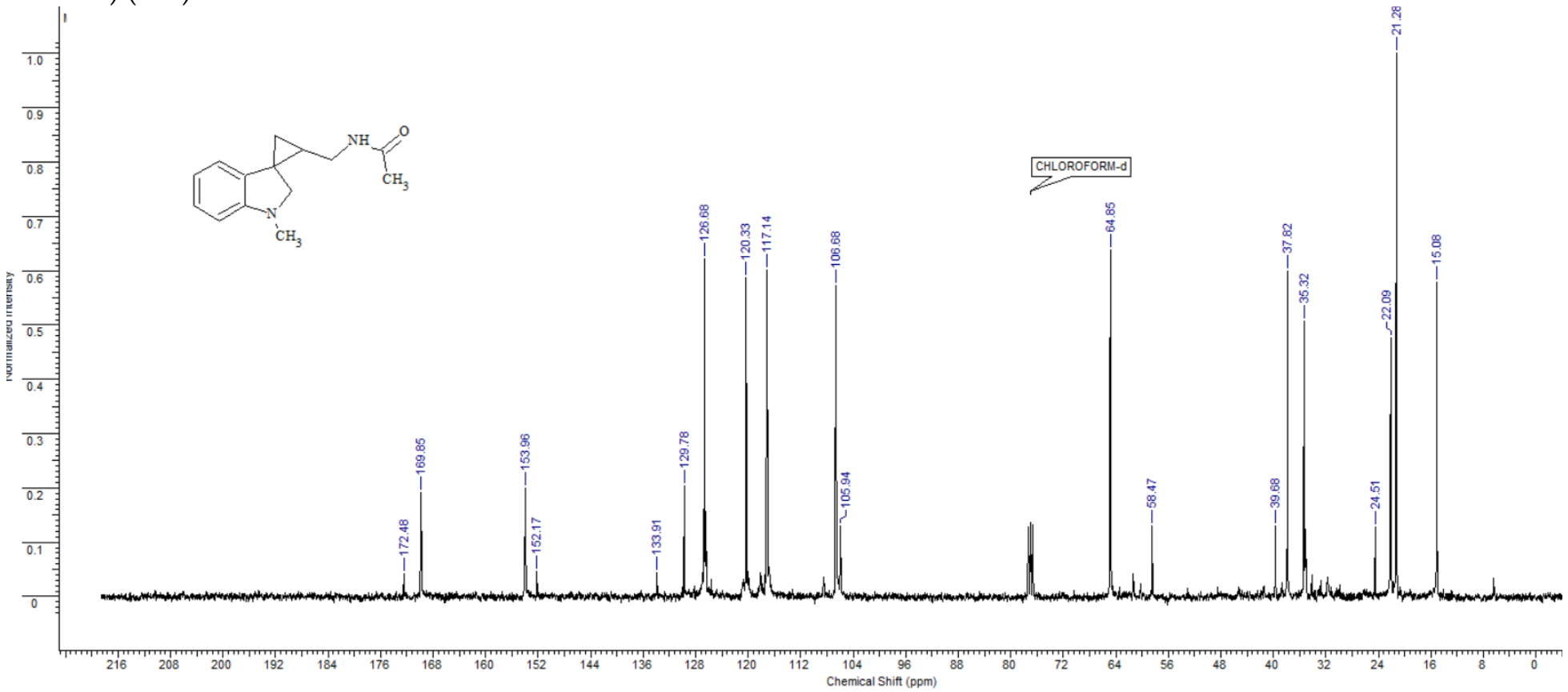
S56 1H NMR spectrum of *N*-[2-(1-benzyl-2,3-dihydro-*1H*-indol-3-yl)ethyl]acetamide (**8m**) and indol (**9m**) after incubation **8m** in DMSO for 1 day.



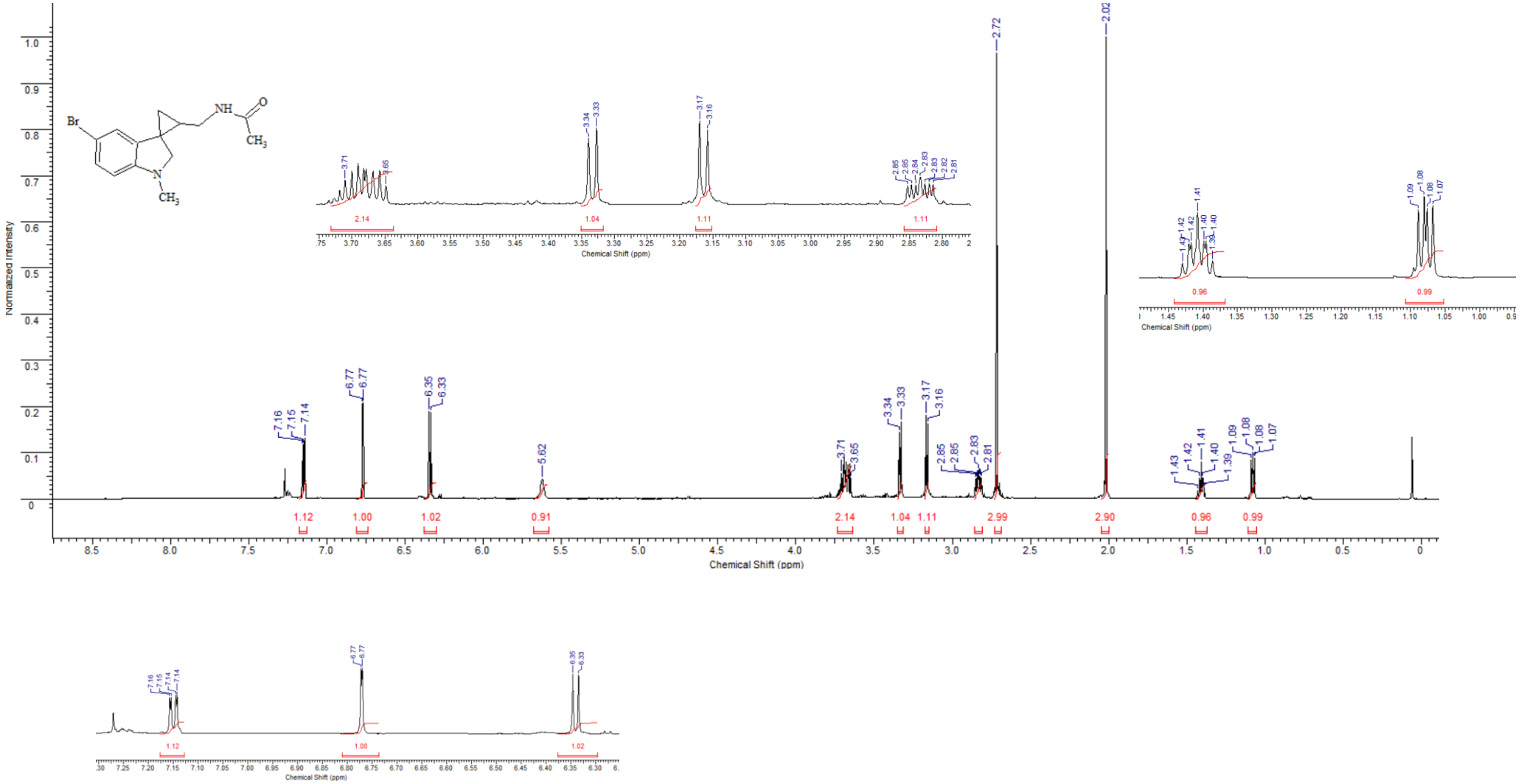
S57 1H NMR spectrum of *N*-{1'-Methyl-1',2'-dihydrospiro[cyclopropan-1,3'-indol]-3-ylmethyl}acetamide (**10a**)



S58 13C NMR spectrum of *N*-{1'-Methyl-1',2'-dihydrospiro[cyclopropan-1,3'-indol]-3-ylmethyl}acetamide (two isomers) (**10a**)



S59 1H NMR spectrum of *N*-{5'-Bromo-1'-methyl-1',2'-dihydrospiro[cyclopropan-1,3'-indol]-3-ylmethyl}acetamide (**10b**)



S60 13C NMR spectrum of *N*-{5'-Bromo-1'-methyl-1',2'-dihydrospiro[cyclopropan-1,3'-indol]-3-ylmethyl}acetamide (**10b**)