Supplementary Materials

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Synthetic design and biological evaluation of new p53-MDM2 interaction inhibitors based on imidazoline core.

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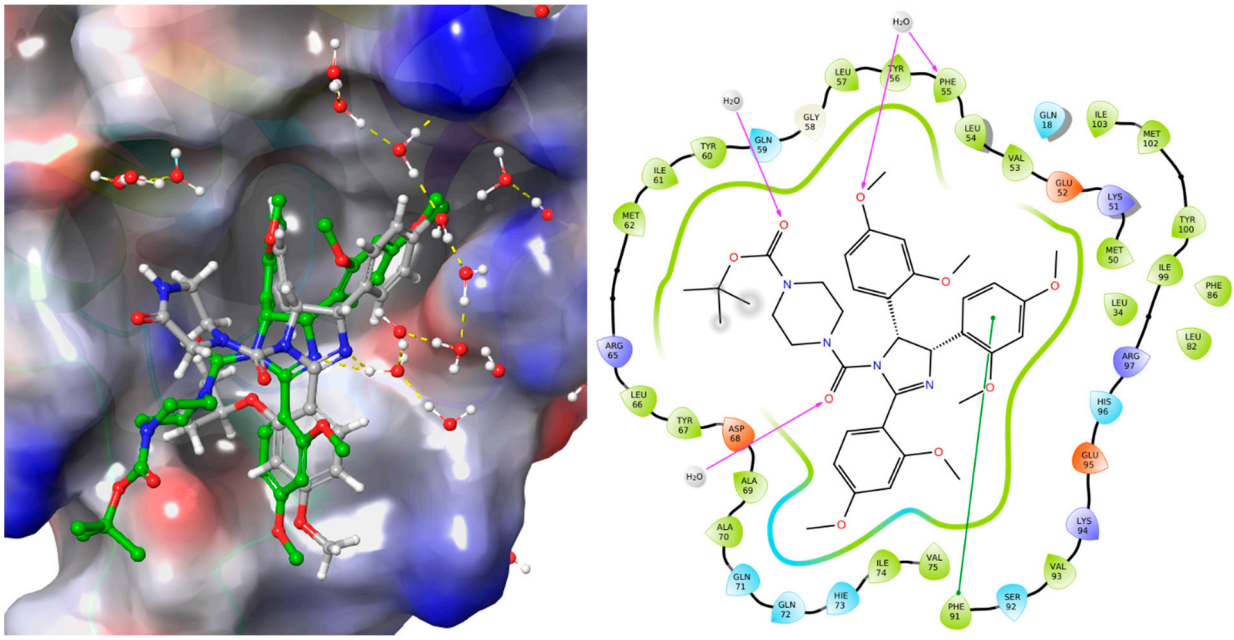
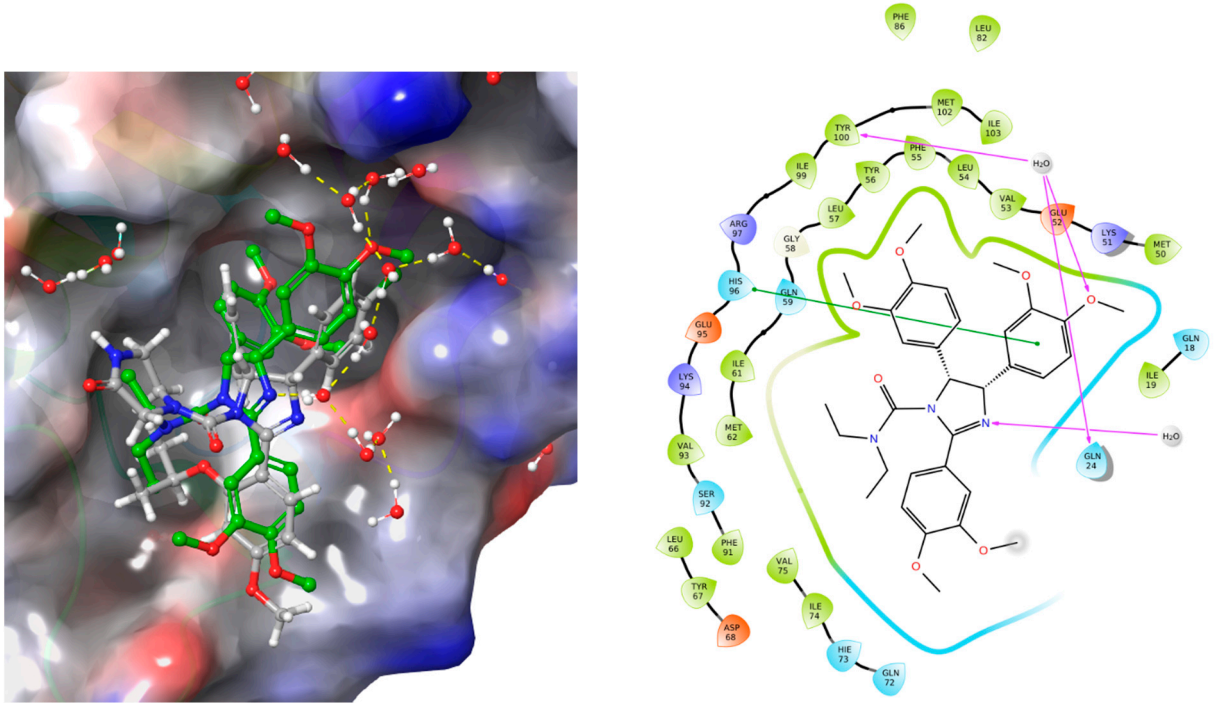
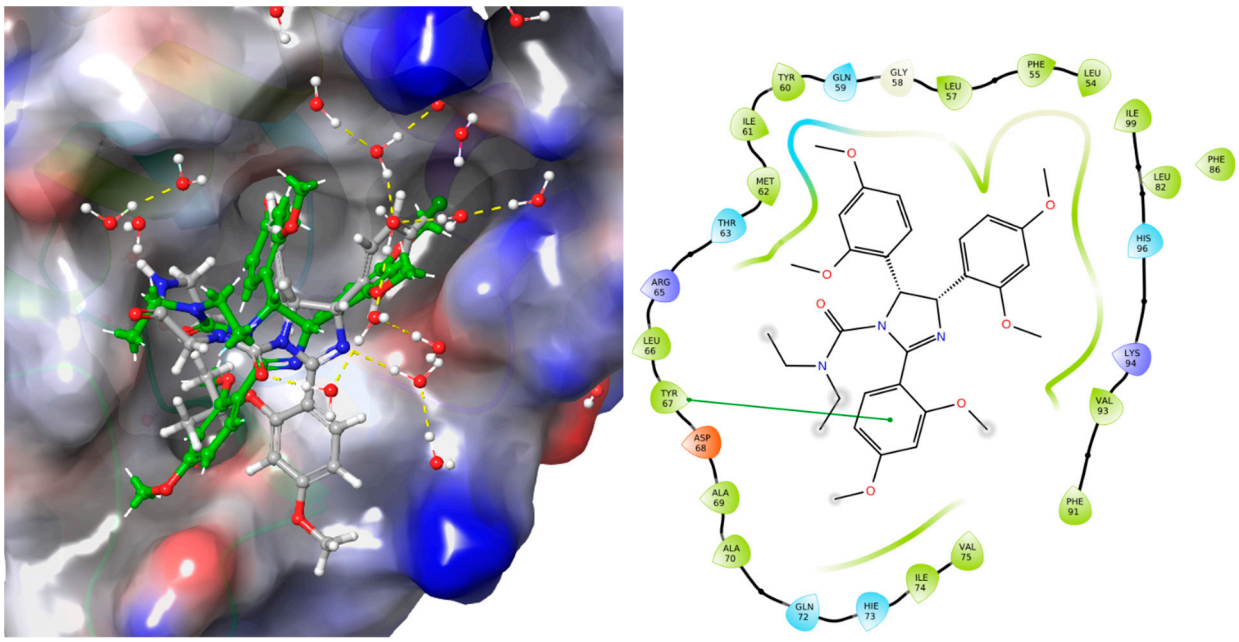
Molecular docking



Supplementary Figure S1 The docked pose (on the left) for **2m** (green) in p53 binding site of MDM2 protein in comparison to nutlin-3a (grey), as well as its ligand-protein interaction map (on the right)



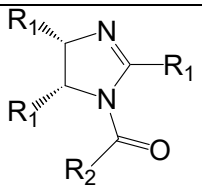
Supplementary Figure S2 The docked pose (on the left) for **2n** (green) in p53 binding site of MDM2 protein in comparison to nutlin-3a (grey), as well as its ligand-protein interaction map (on the right).



Supplementary Figure S3 The docked pose (on the left) for **2i** (green) in p53 binding site of MDM2 protein in comparison to nutlin-3a (grey), as well as its ligand-protein interaction map (on the right).

Biology

Supplementary Table S1. Cytotoxicity of synthesized 2,4,5-triaryl cis-imidazoline derivatives to HCT-116 cell line.



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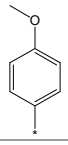
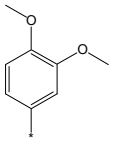
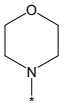
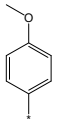
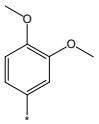
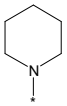
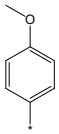
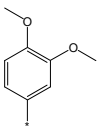
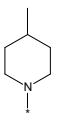
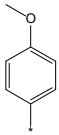
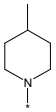
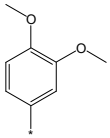
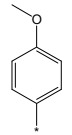
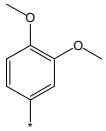
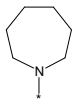
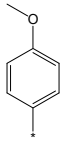
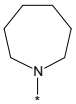
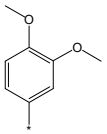
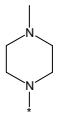
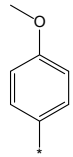
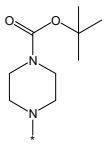
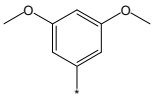
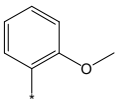
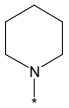
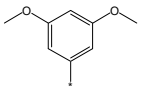
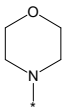
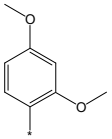
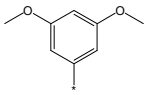
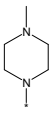
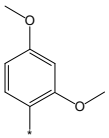
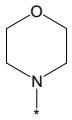
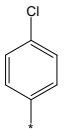
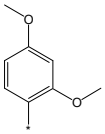
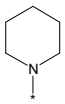
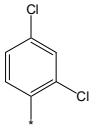
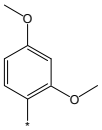
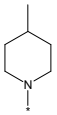
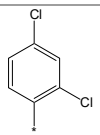
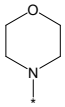
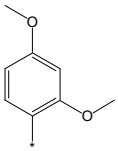
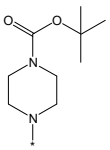
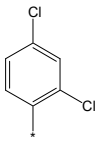
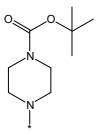
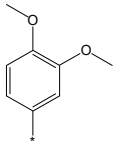
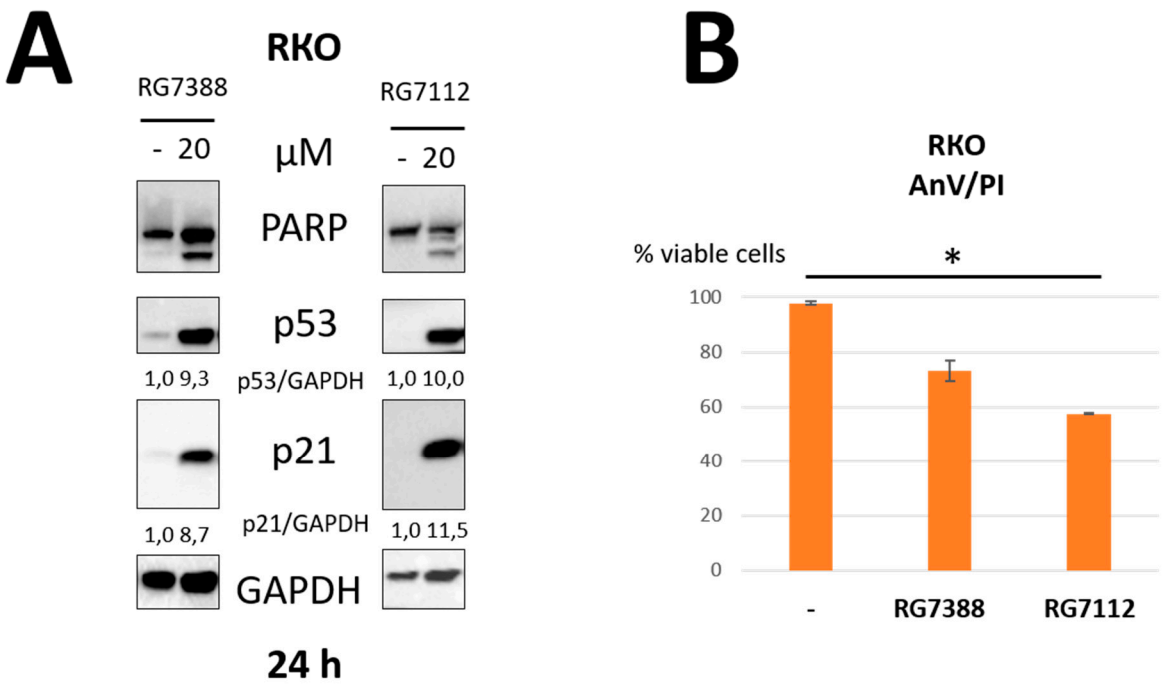
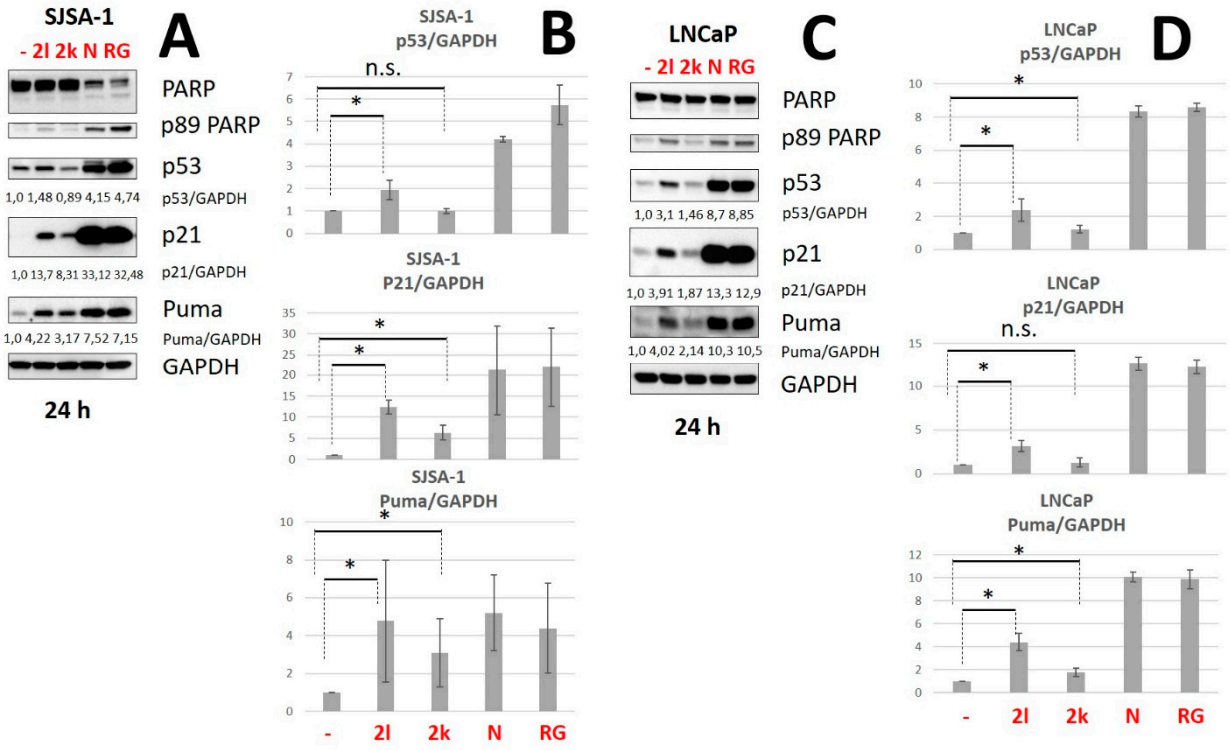
2a			54	**2o**			131
2b			>200	**2p**			102
2c			54	**2q**			112
2d			38	**2r**			>200

2e			115	**2s**			77
2f			27	**2t**			>200
2g			41	**2u**			9
2h			59	**2v**			98
2i			30	**2w**			65
2j			126	**2x**			11
2k			23	**2y**			24
2l			16	**2z**			17
2m			13	**2aa**			21
2n			51				



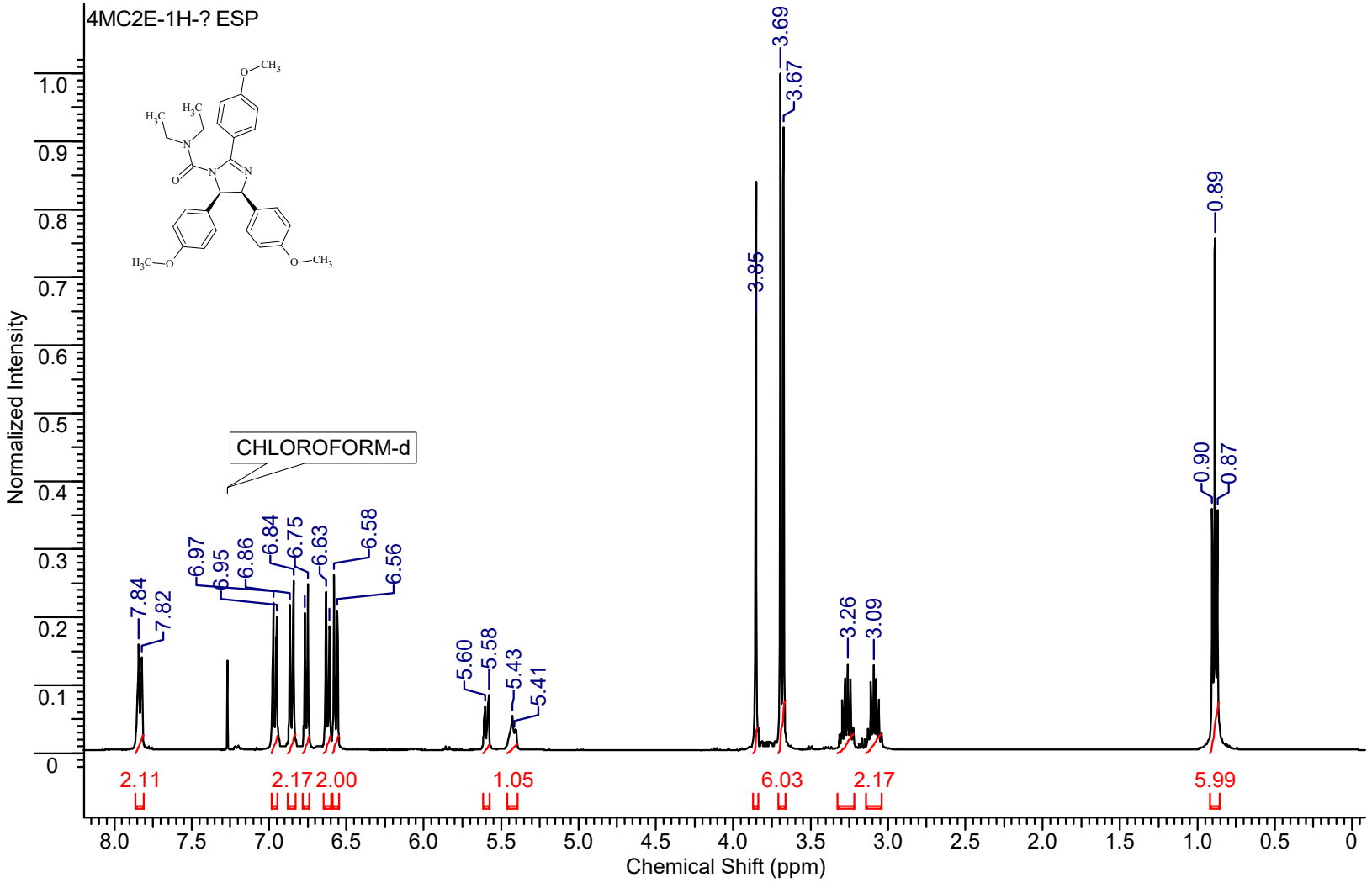
Supplementary Figure S4 A. Western Blot analysis of total cellular lysates from RKO cells upon treatment with RG7388 and RG7112. B. The histograms of flow cytometry (FC) analysis data for RKO cells using Annexin V-FITC / PI staining, % viable cells – cells negative for both Annexin V-FITC and propidium iodide (PI). Data from n = 3 biological replicates were shown as mean ± s.d., * $p < 0.05$, n.s. – not significant. PARP – poly (ADP-ribose)-polymerase; GAPDH – glyceraldehyde 3-phosphate dehydrogenase; p53/GAPDH and p21/GAPDH – densitometric analysis of p53 or p21 bands normalized to GAPDH, h – hours.



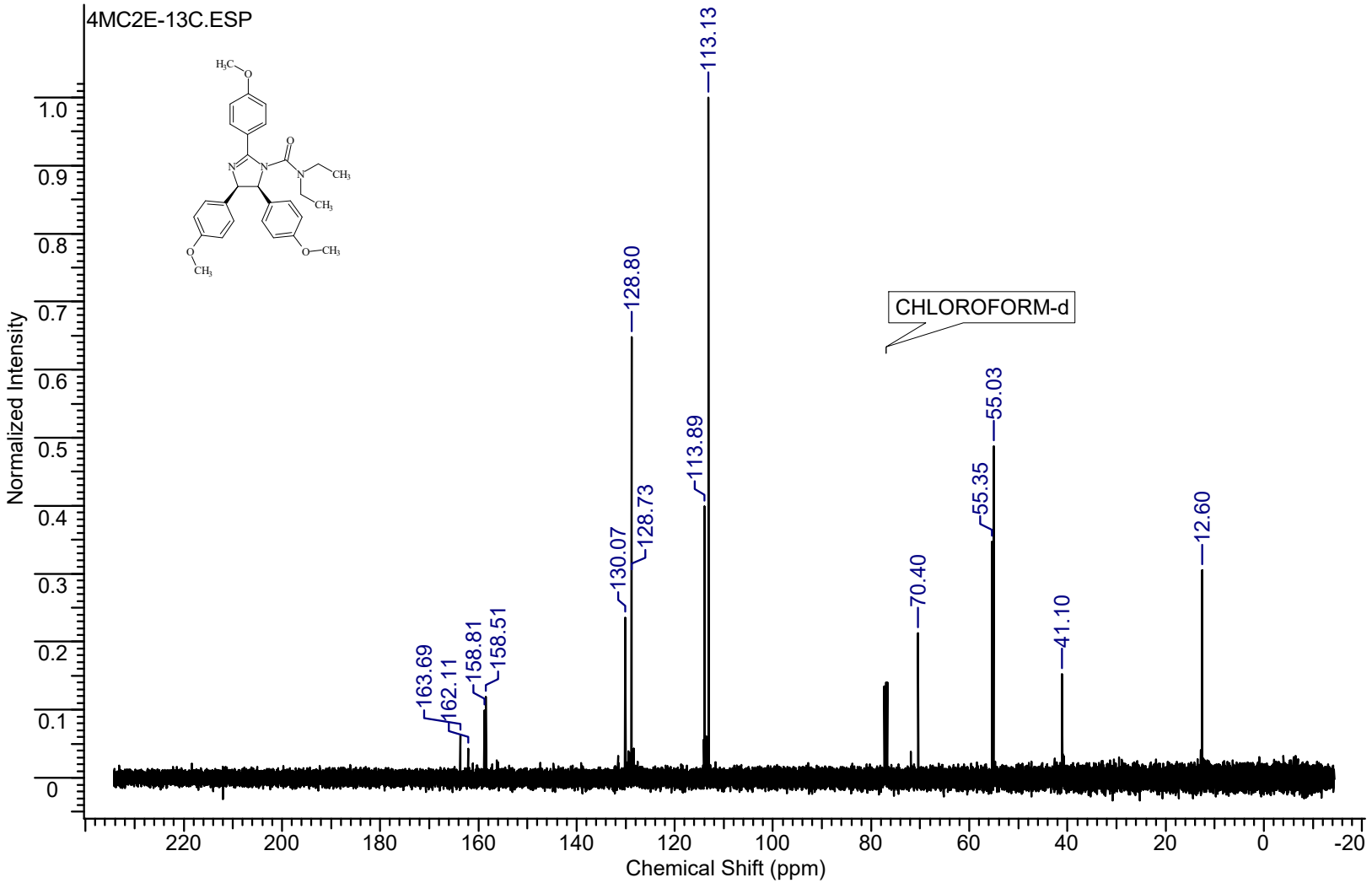
Supplementary Figure S5 A, C. Western Blot analysis of total cellular lysates from SJSA-1 (A) and LNCaP (C) cells upon treatment with compounds **2l, 2k**, Nutlin-3a and RG7388. B, D - Densitometric analysis of p53, p21 and Puma bands normalized to GAPDH in SJSA-1 (B) and LNCaP (D) cells. Data from n = 3 biological replicates were shown as mean ± s.d., * $p < 0.05$, n.s. – not significant. PARP – poly (ADP-ribose)-polymerase; GAPDH – glyceraldehyde 3-phosphate dehydrogenase; p53/GAPDH, p21/GAPDH and Puma/GAPDH – densitometric analysis of p53, p21 and Puma bands normalized to GAPDH, h – hours, N - Nutlin-3a (10 μM), R – RG7388 (5 μM).

Chemistry (1H and 13C NMR data for compounds 2a-2aa)

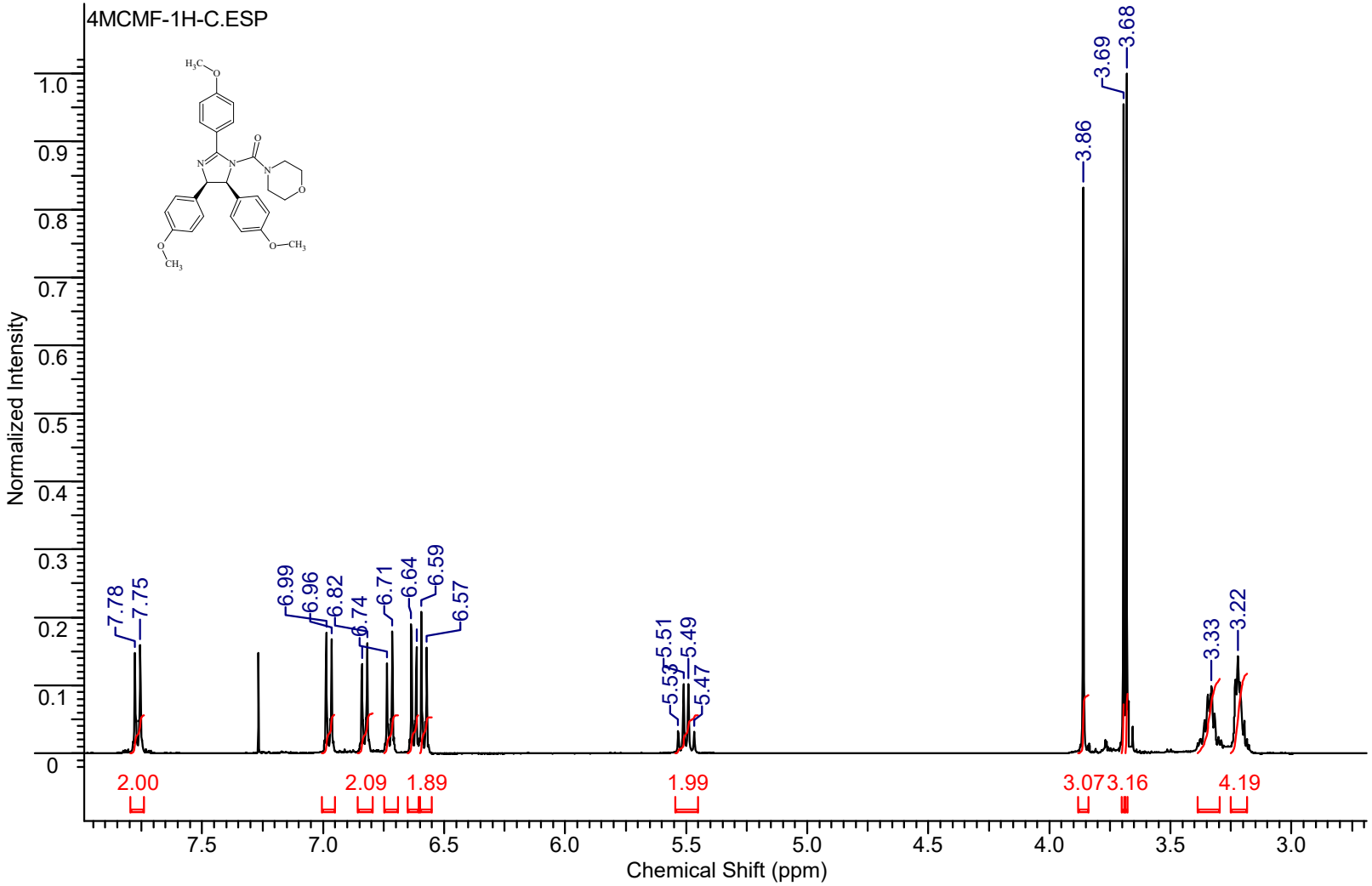
***1H NMR Cis-N,N*-diethyl-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carboxamide (2a)**



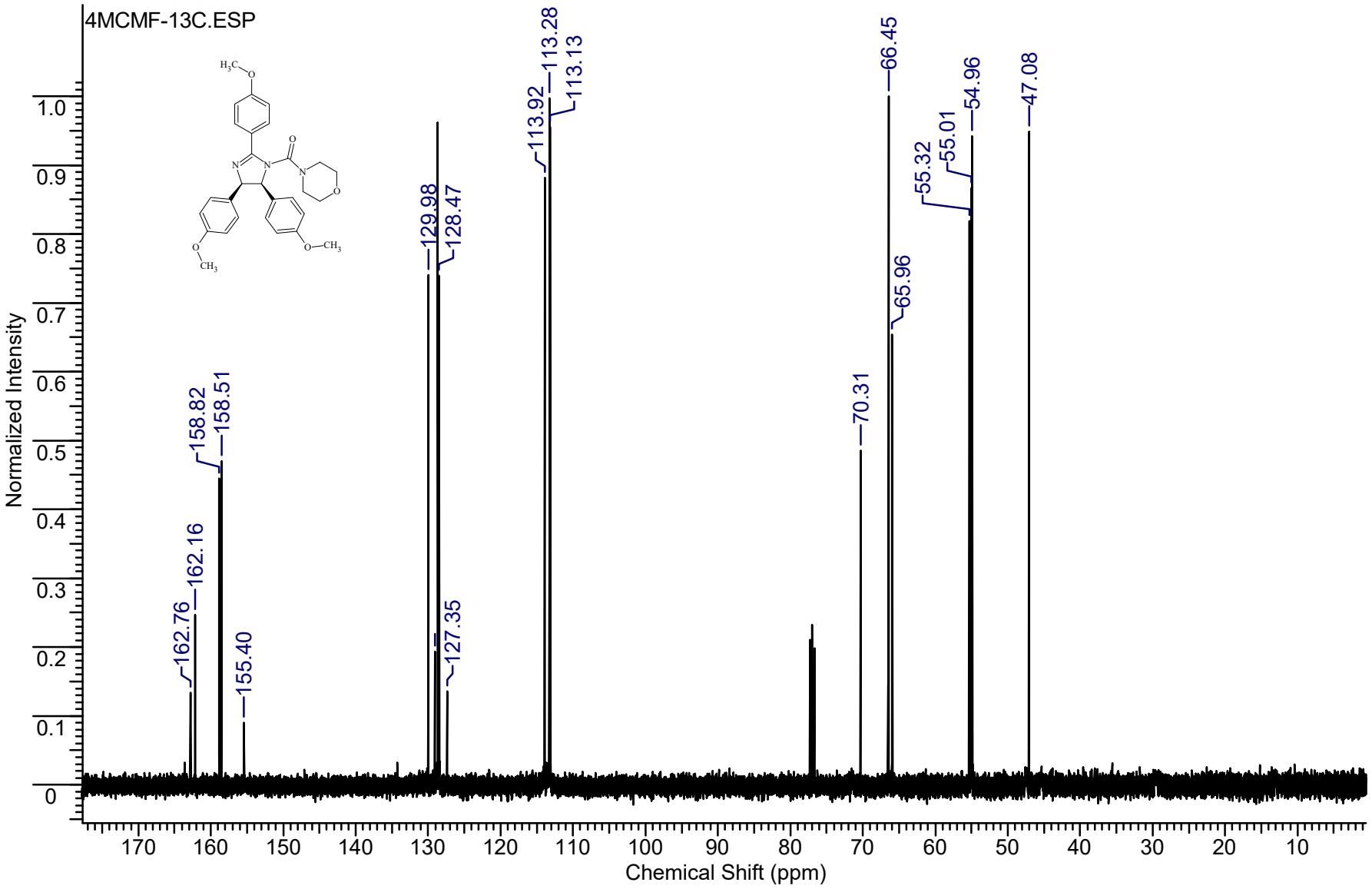
***13C NMR Cis-N,N*-diethyl-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carboxamide (2a)**



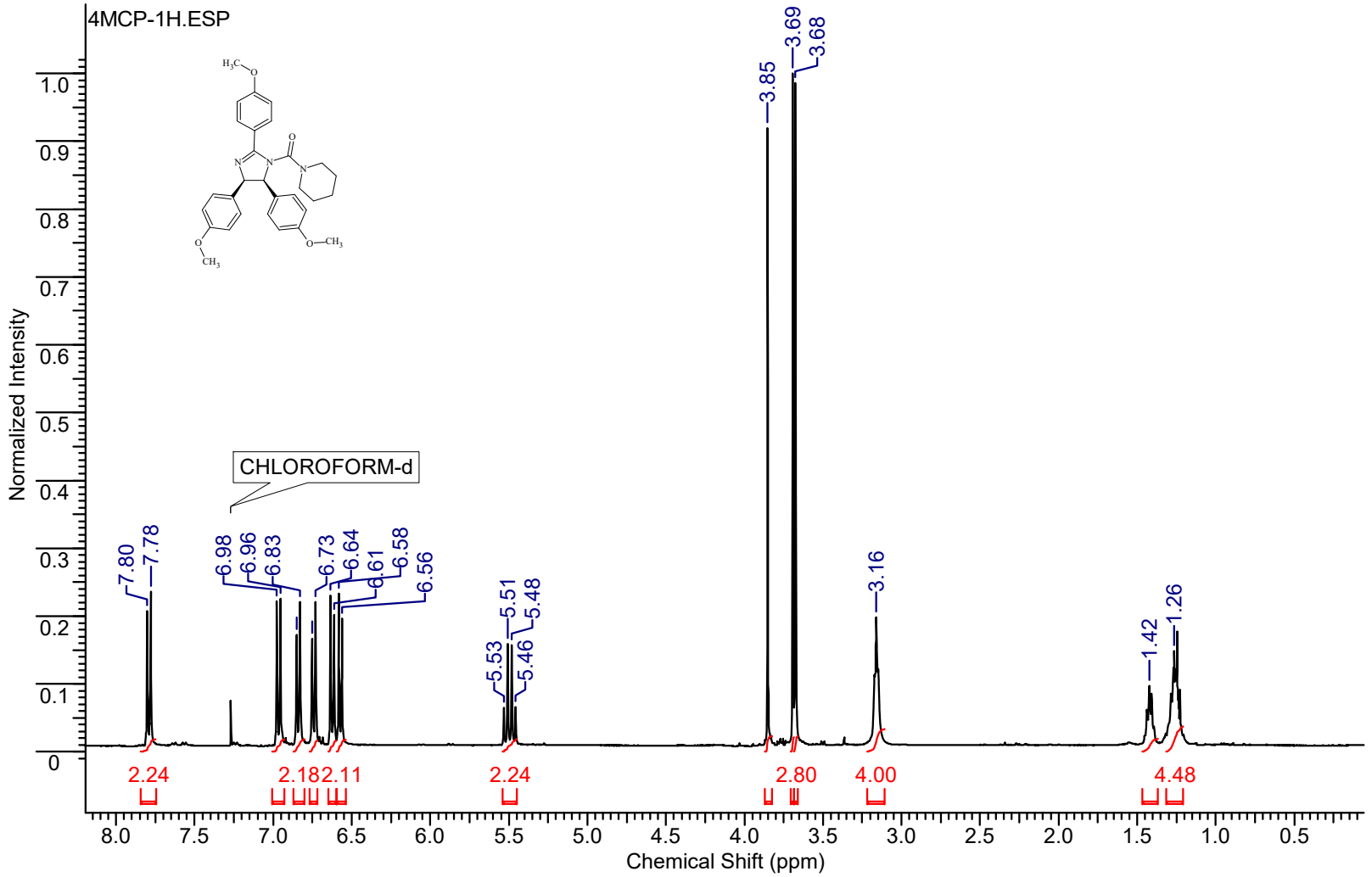
1H NMR 4-{[*Cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-1H-imidazol-1-yl]carbonyl}morpholine (2b).



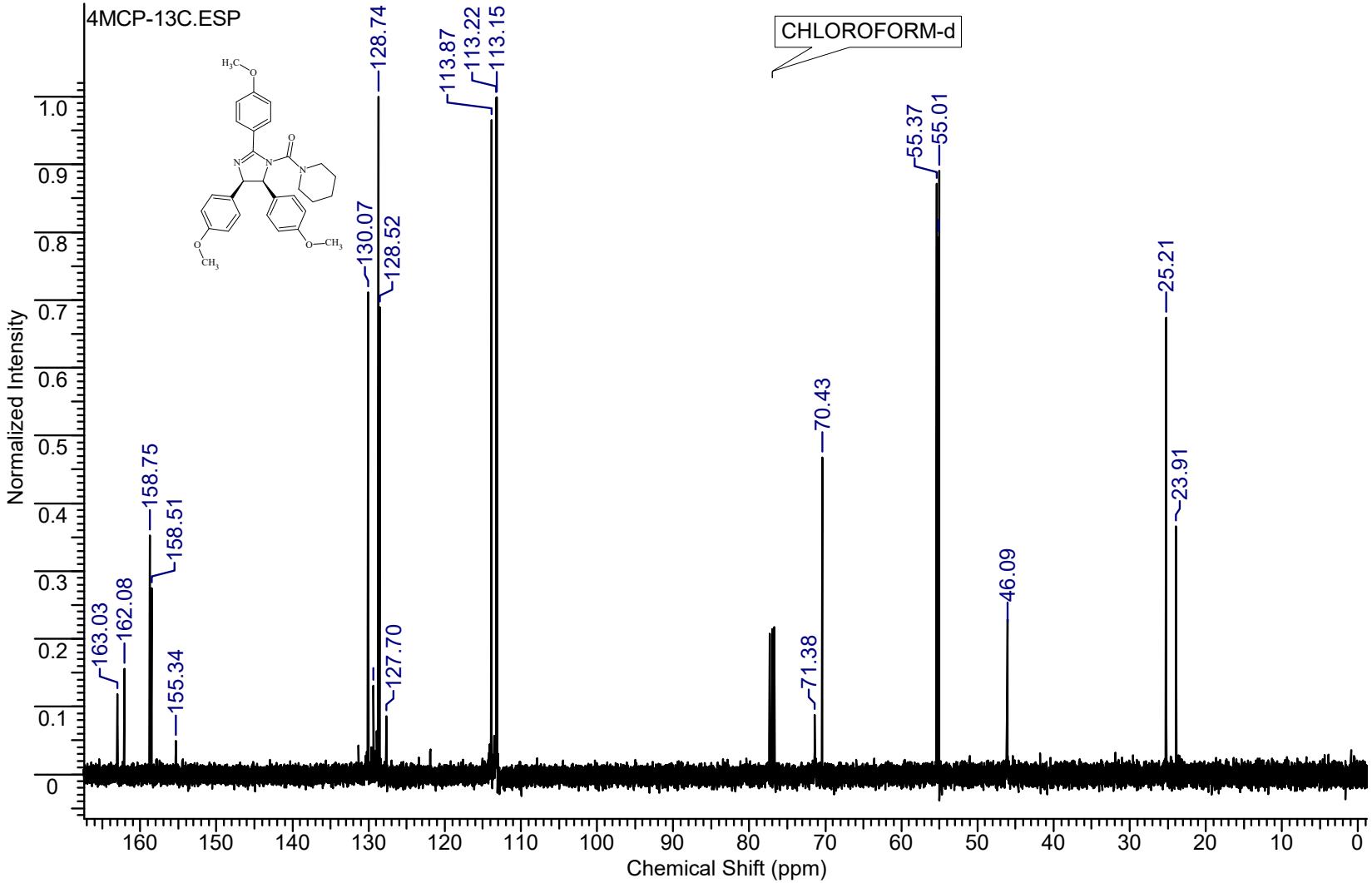
13C NMR 4-{[*Cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-1H-imidazol-1-yl]carbonyl}morpholine (2b).



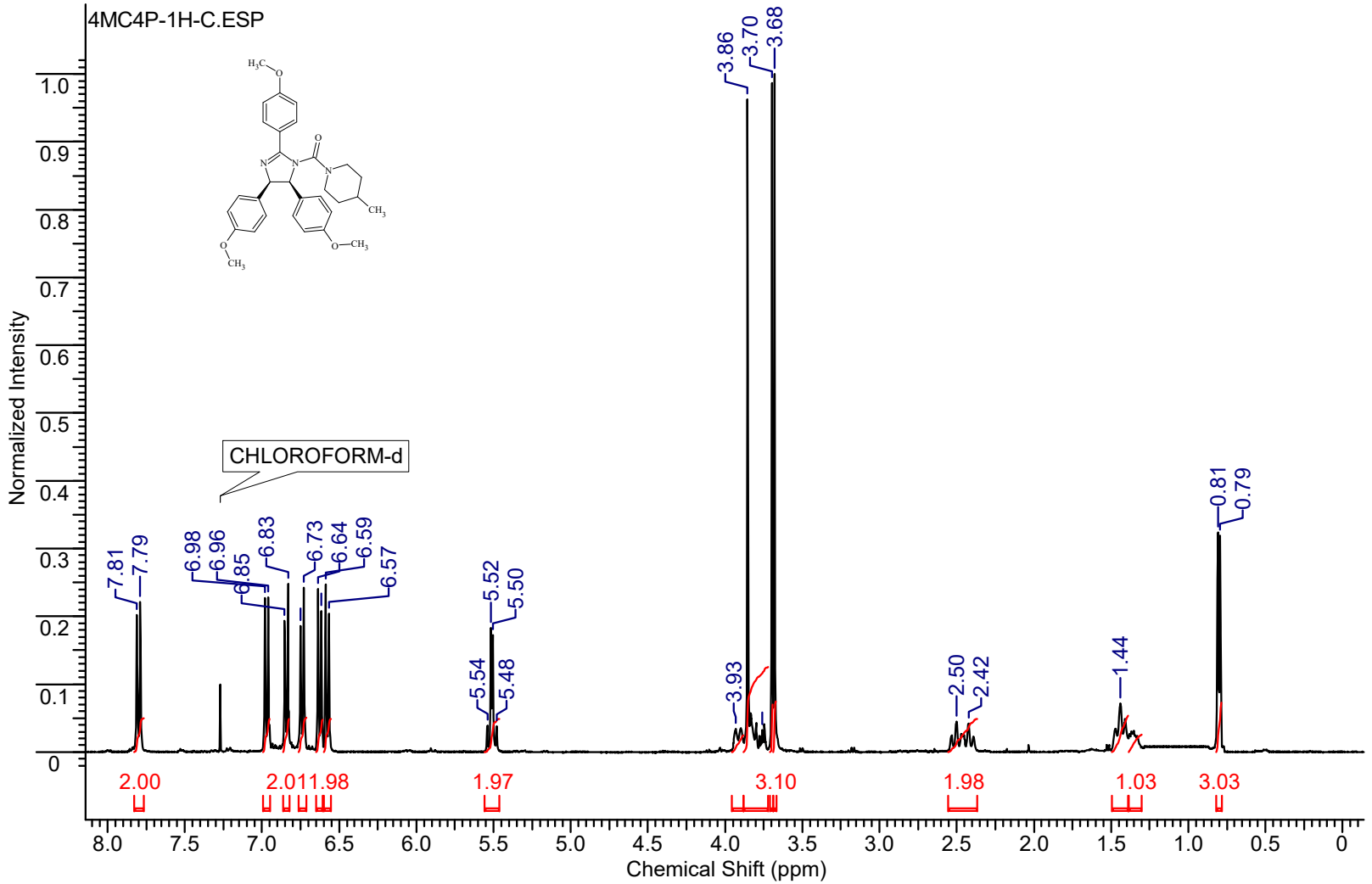
1H NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2c).



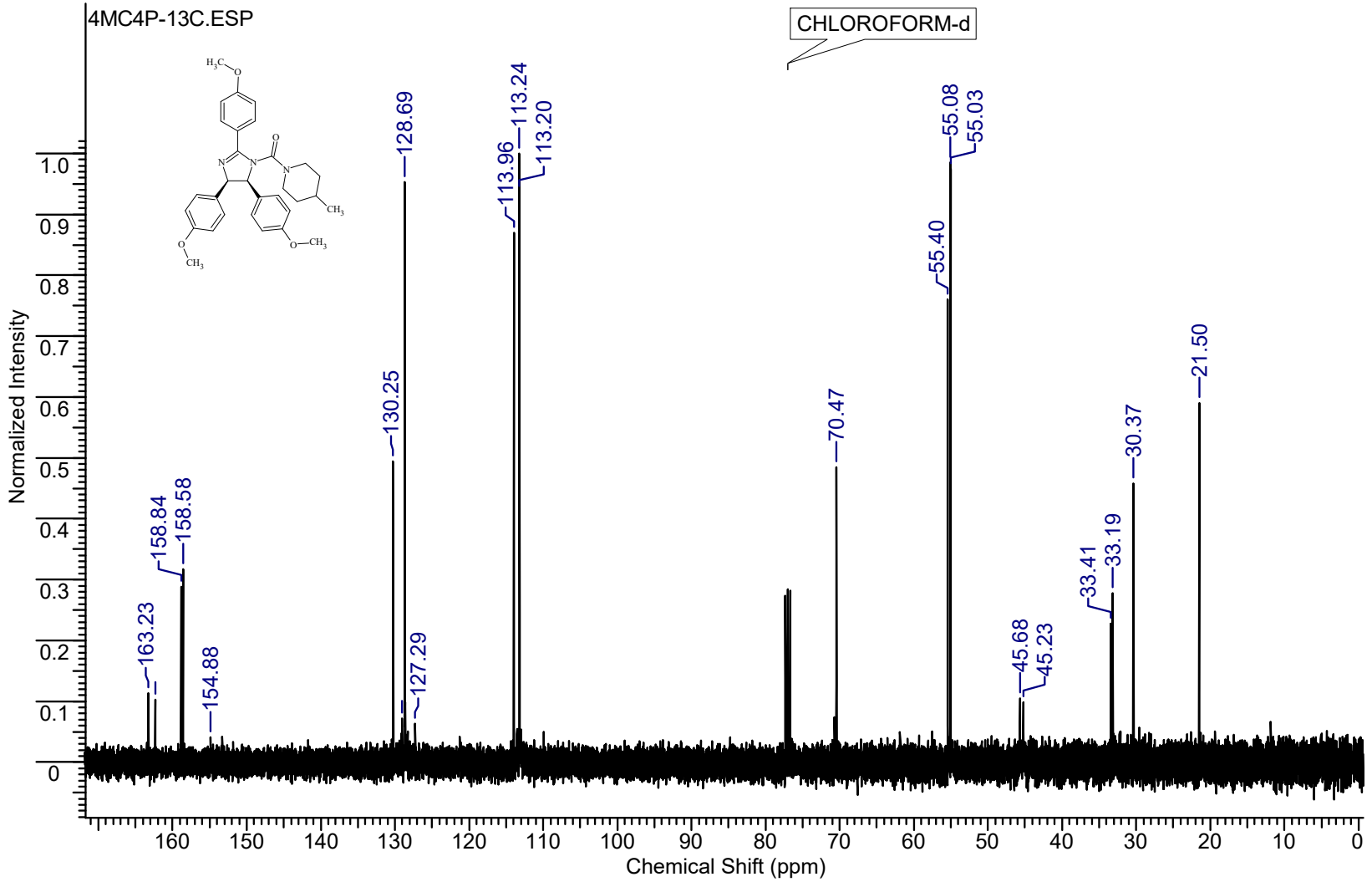
13C NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2c).



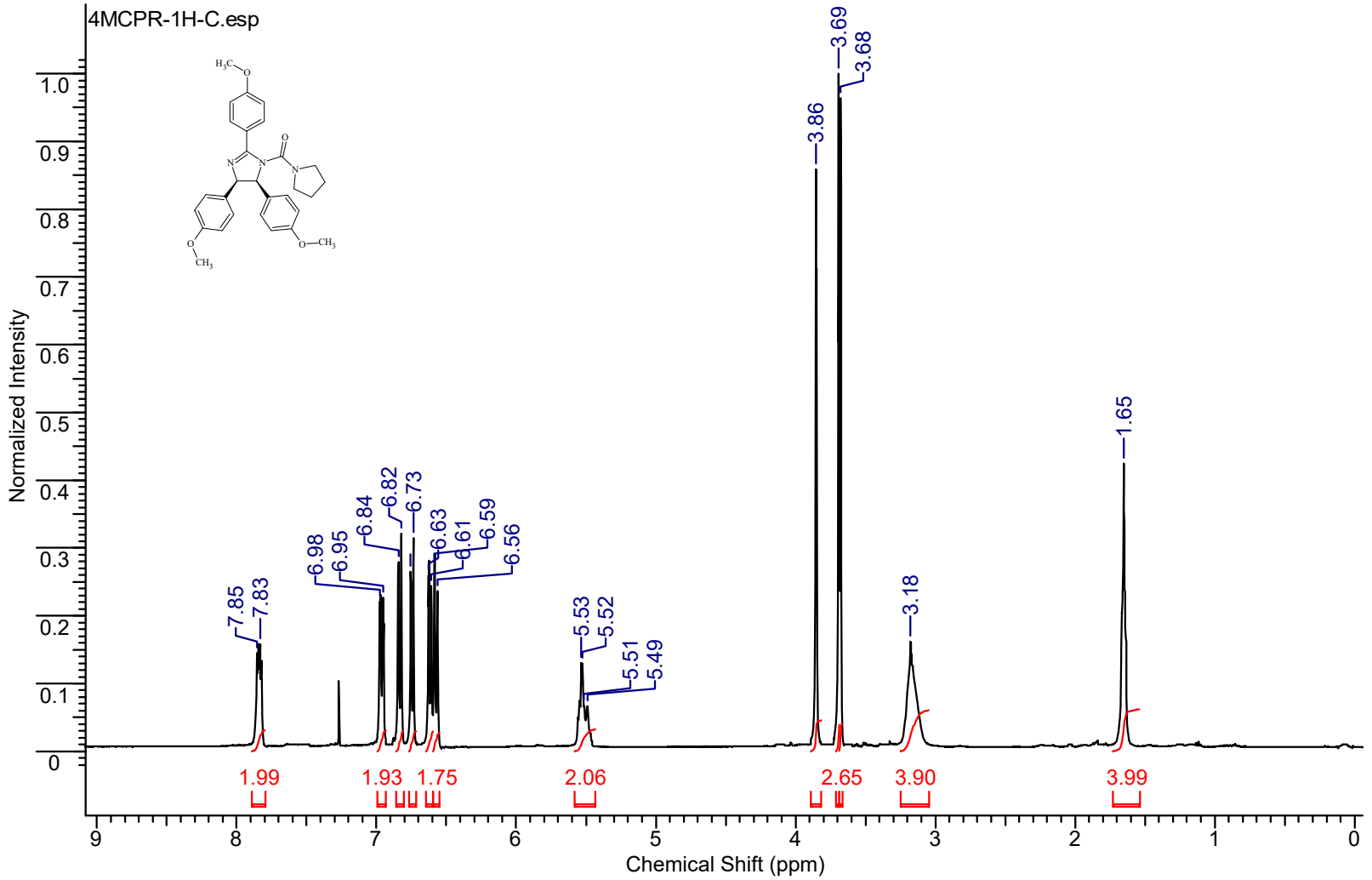
1H NMR (4-Methylpiperidin-1-yl) (*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2d).



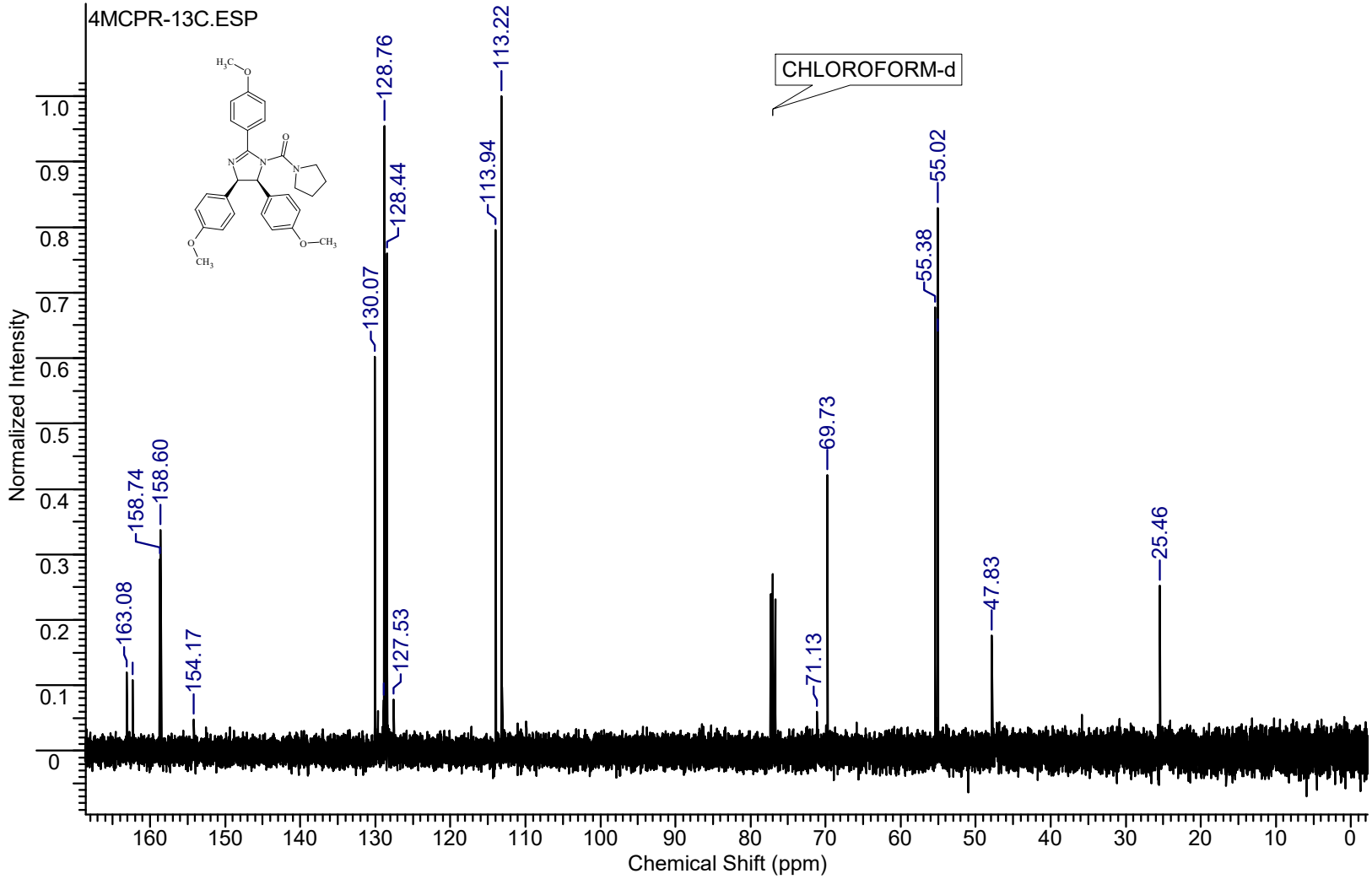
13C NMR (4-Methylpiperidin-1-yl) (*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2d).



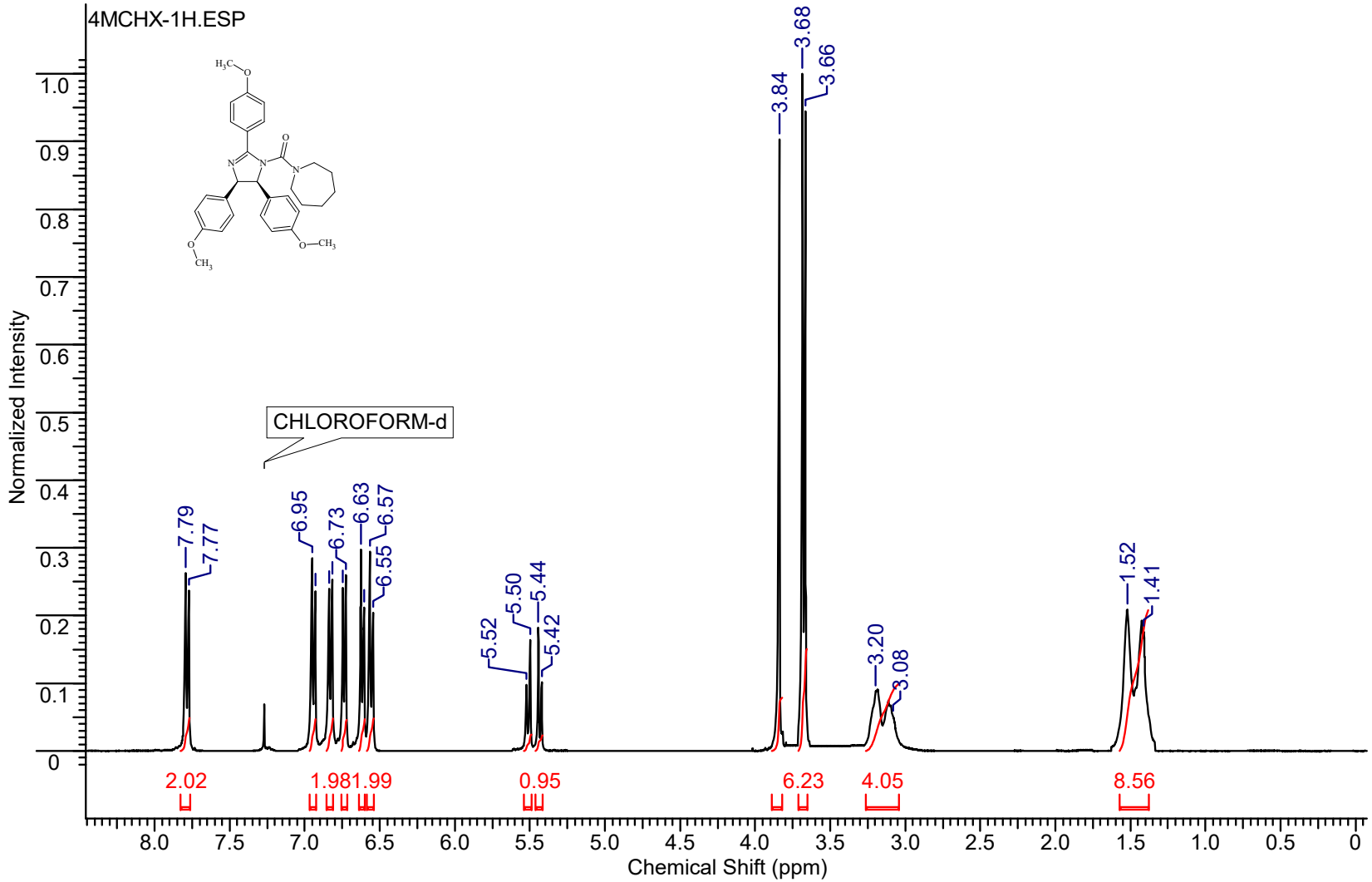
1H NMR Pyrrolidin-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2e).



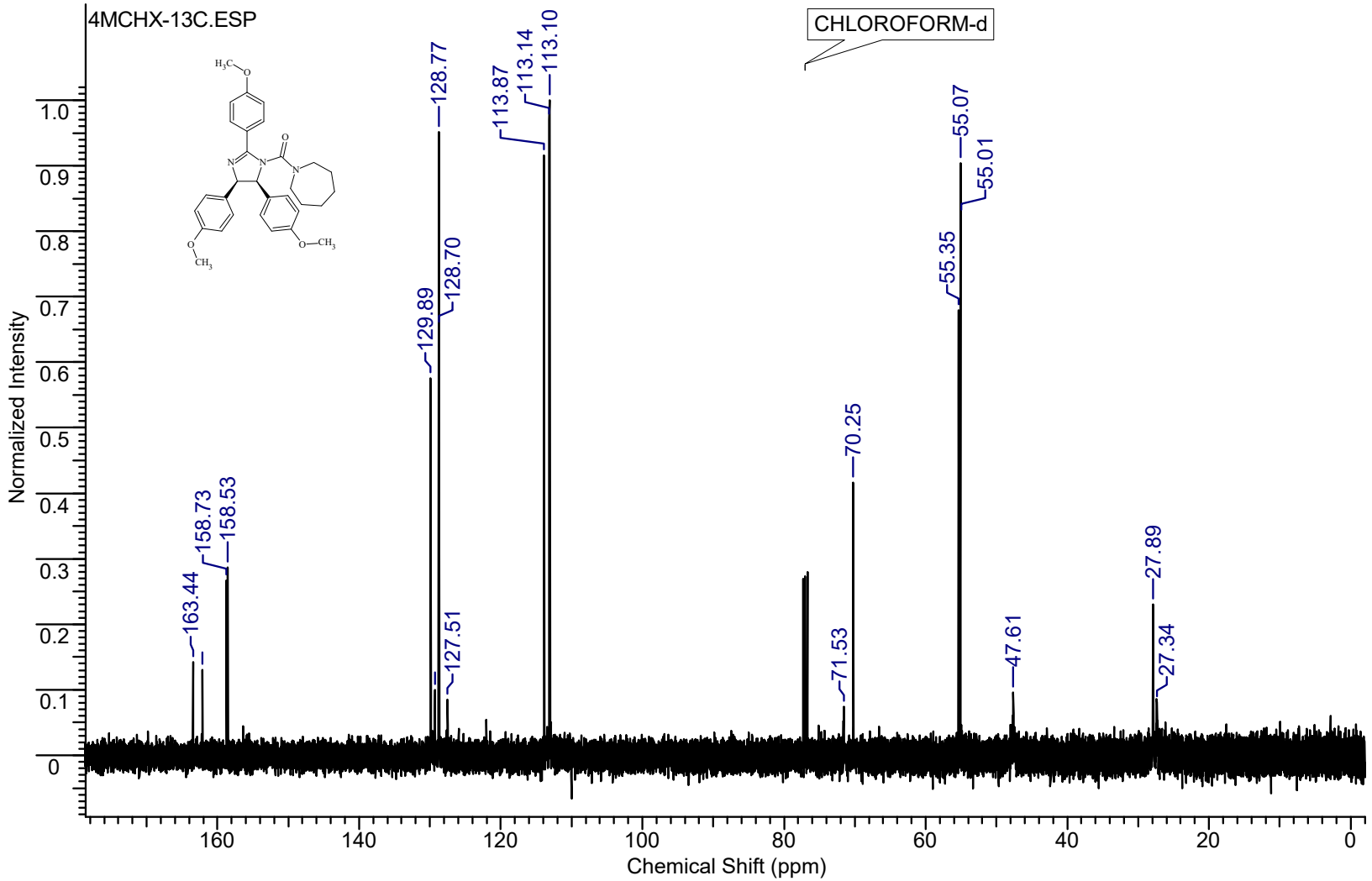
13C NMR Pyrrolidin-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2e).



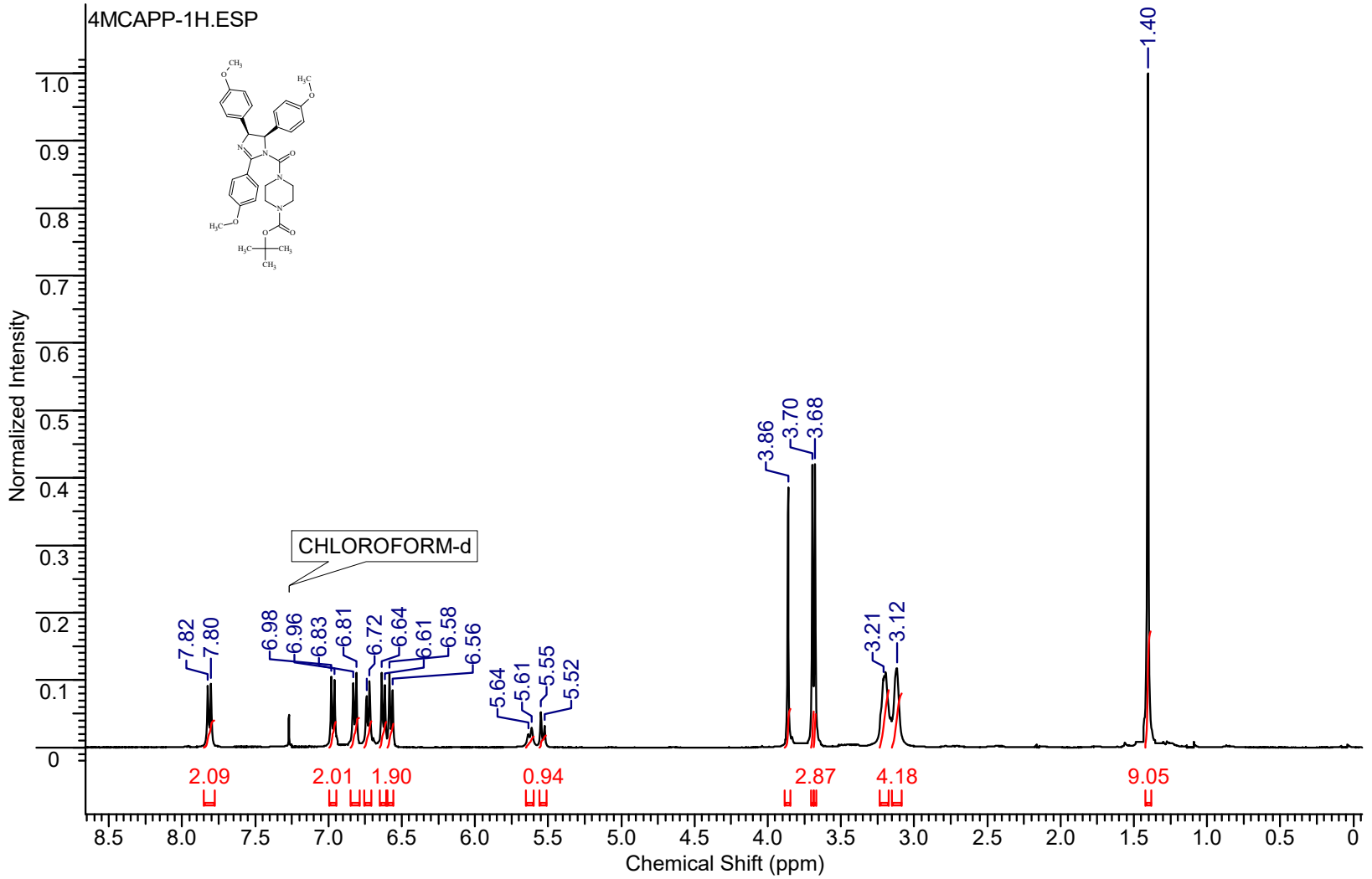
1H NMR Azepan-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2f).



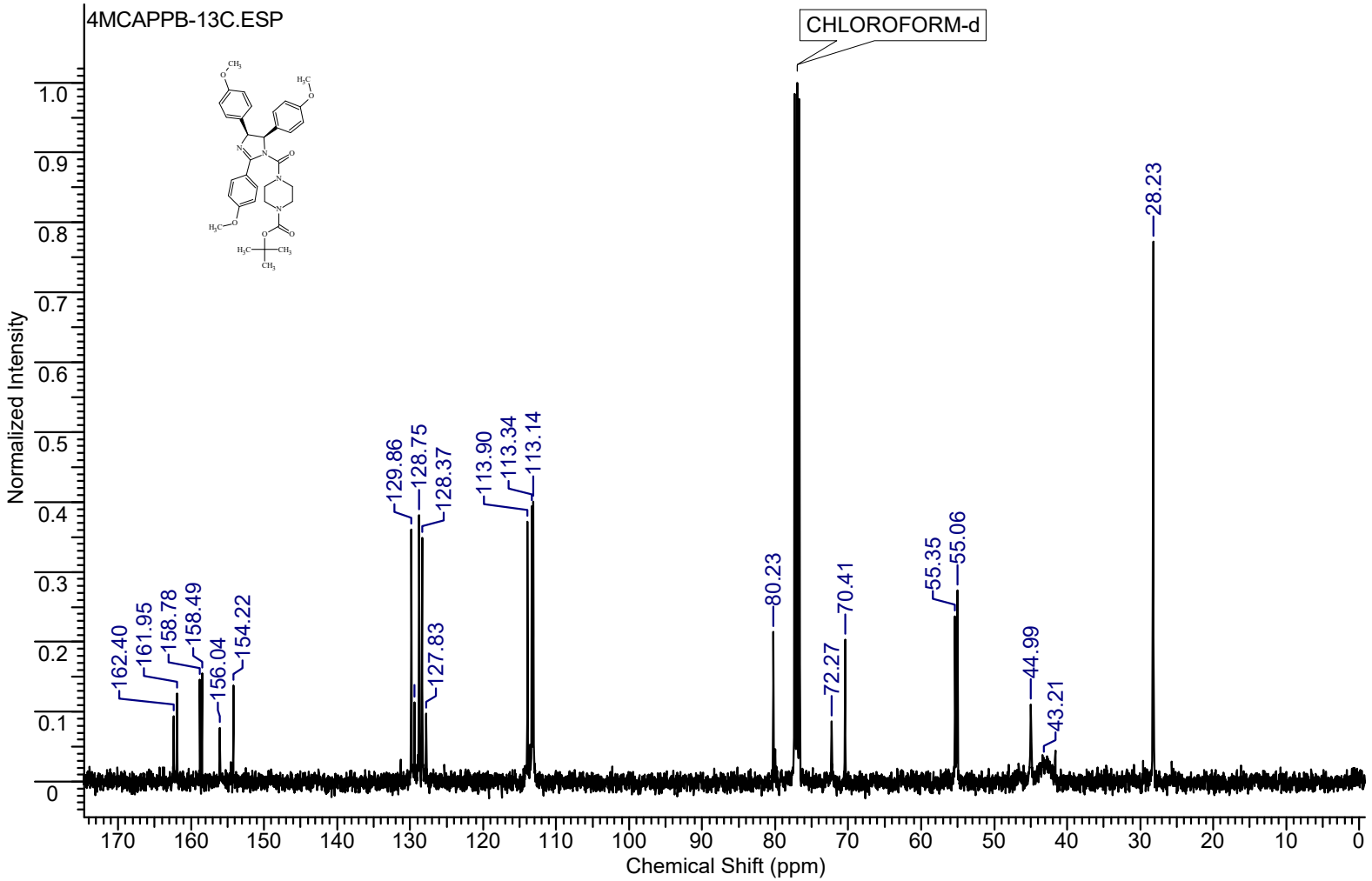
13C NMR Azepan-1-yl(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2f).



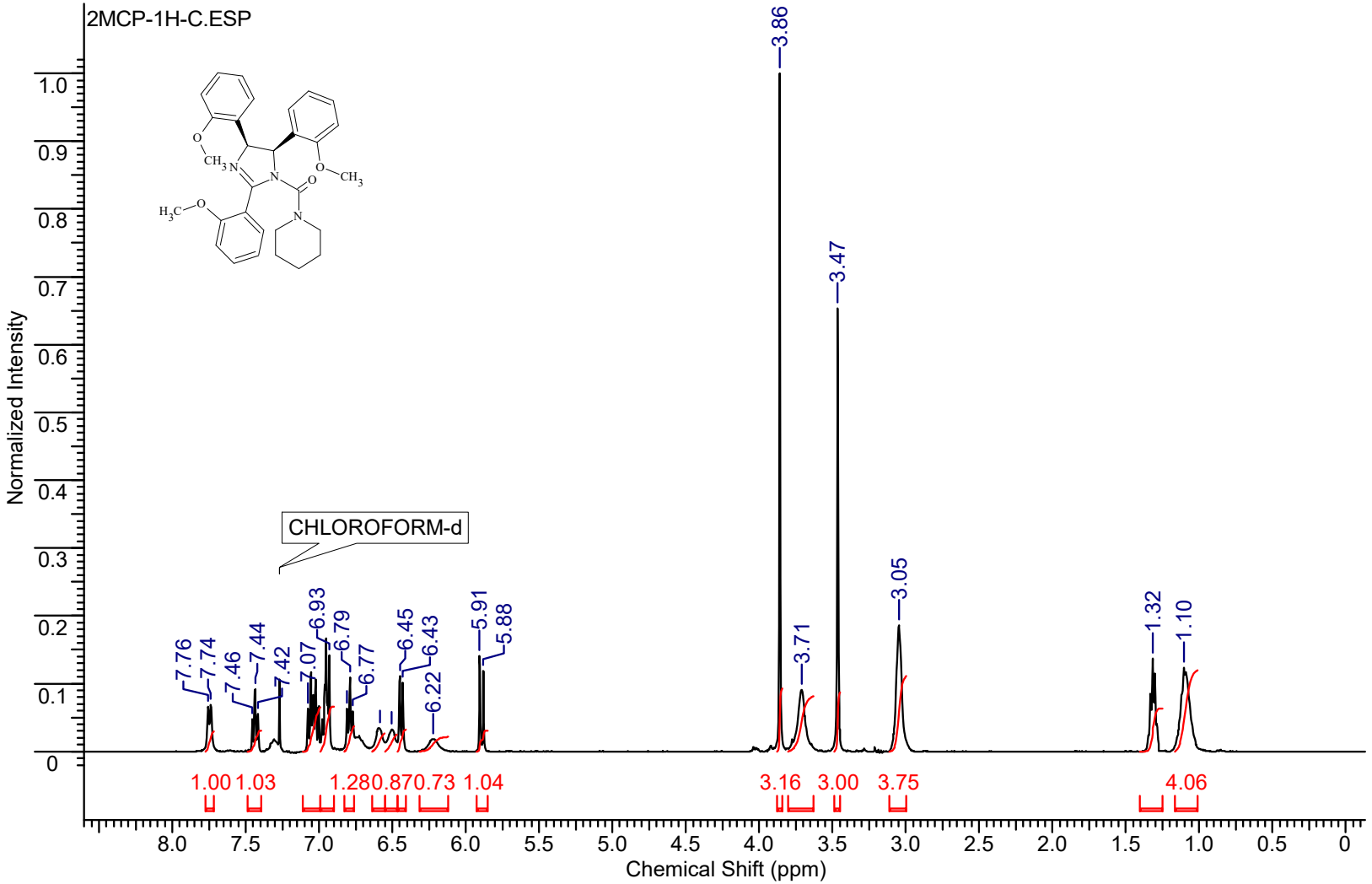
1H NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2g).



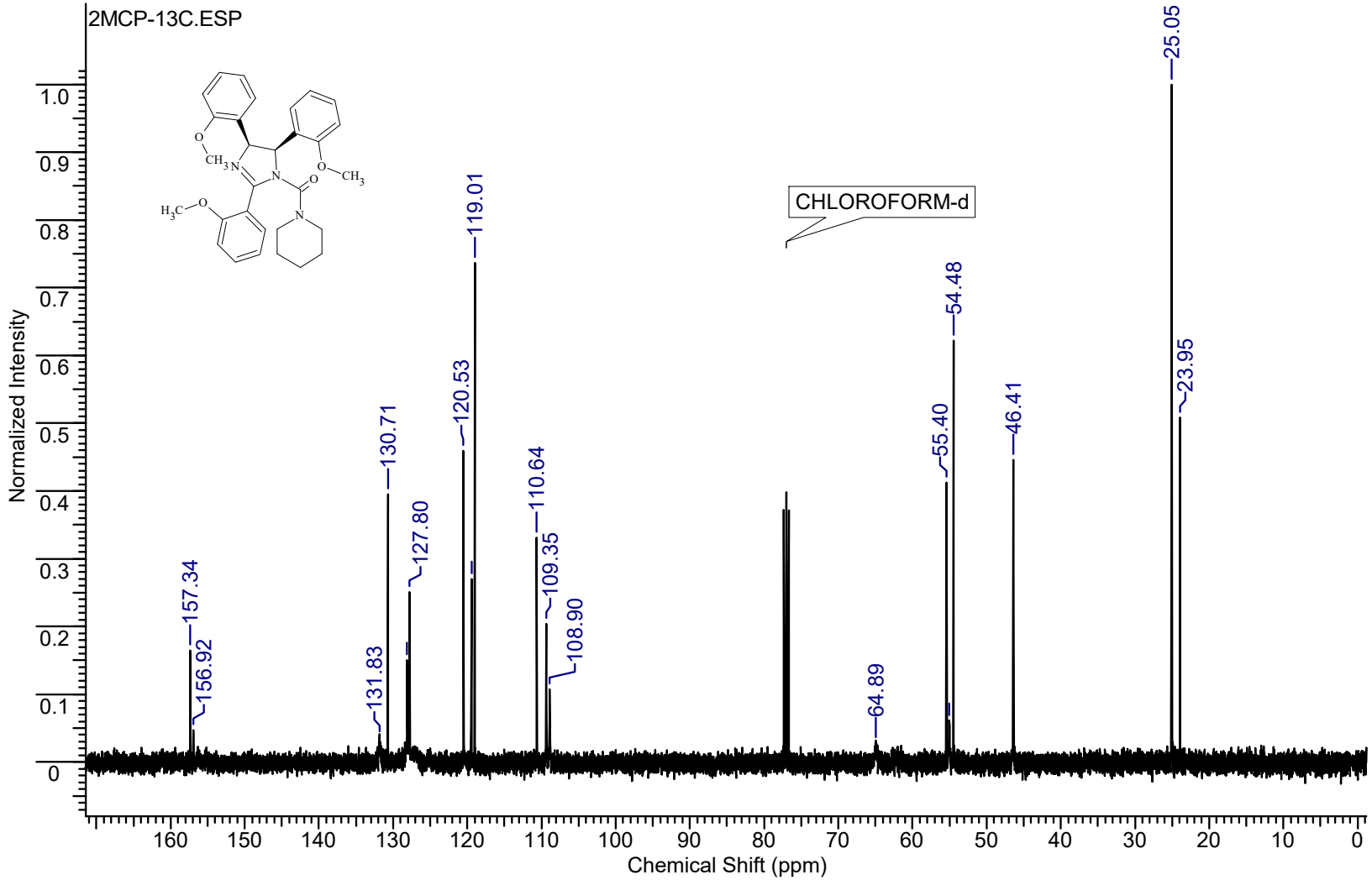
13C NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(4-methoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2g).



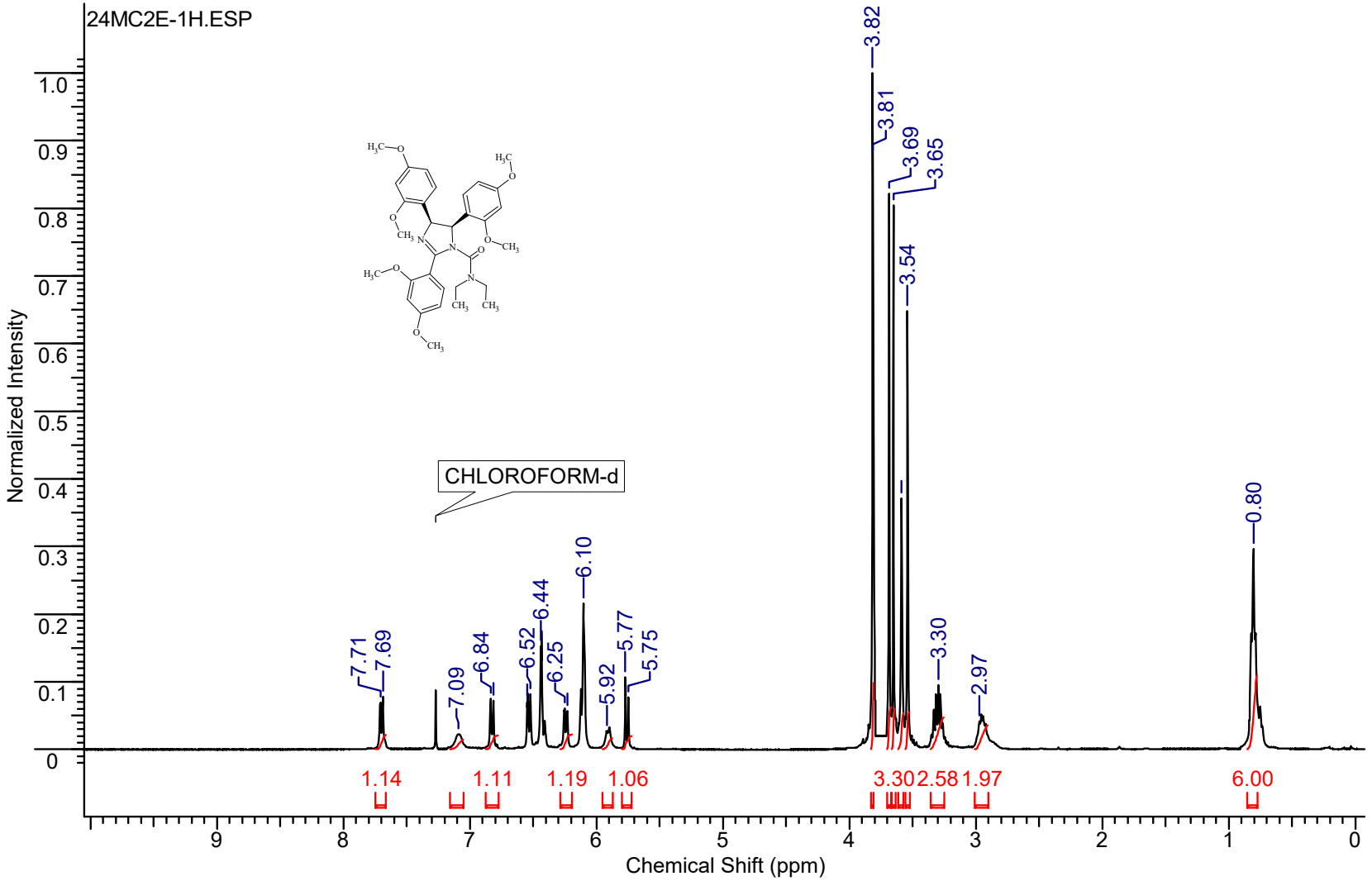
1H NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(2-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2h).



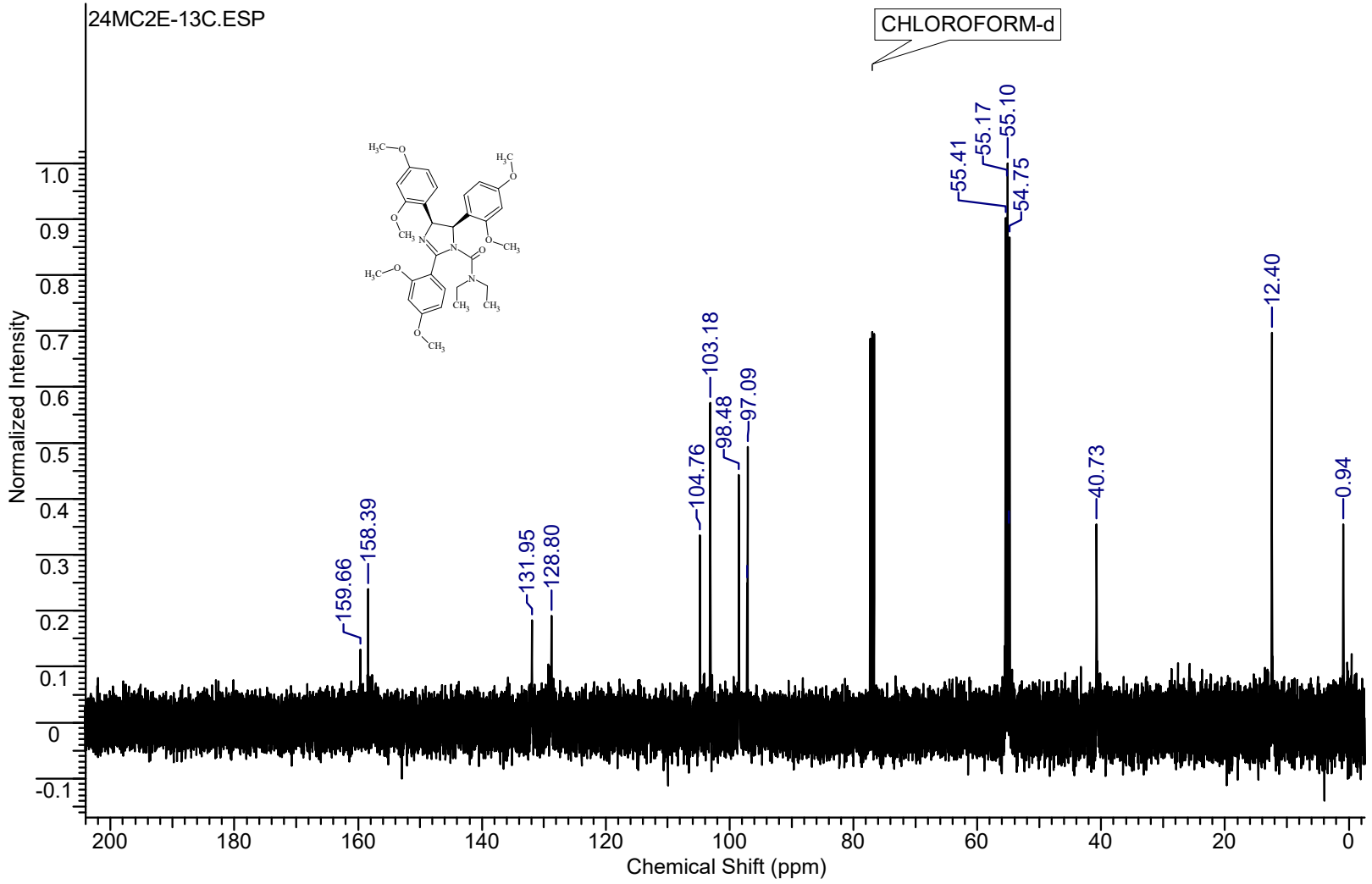
13C NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(2-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2h).



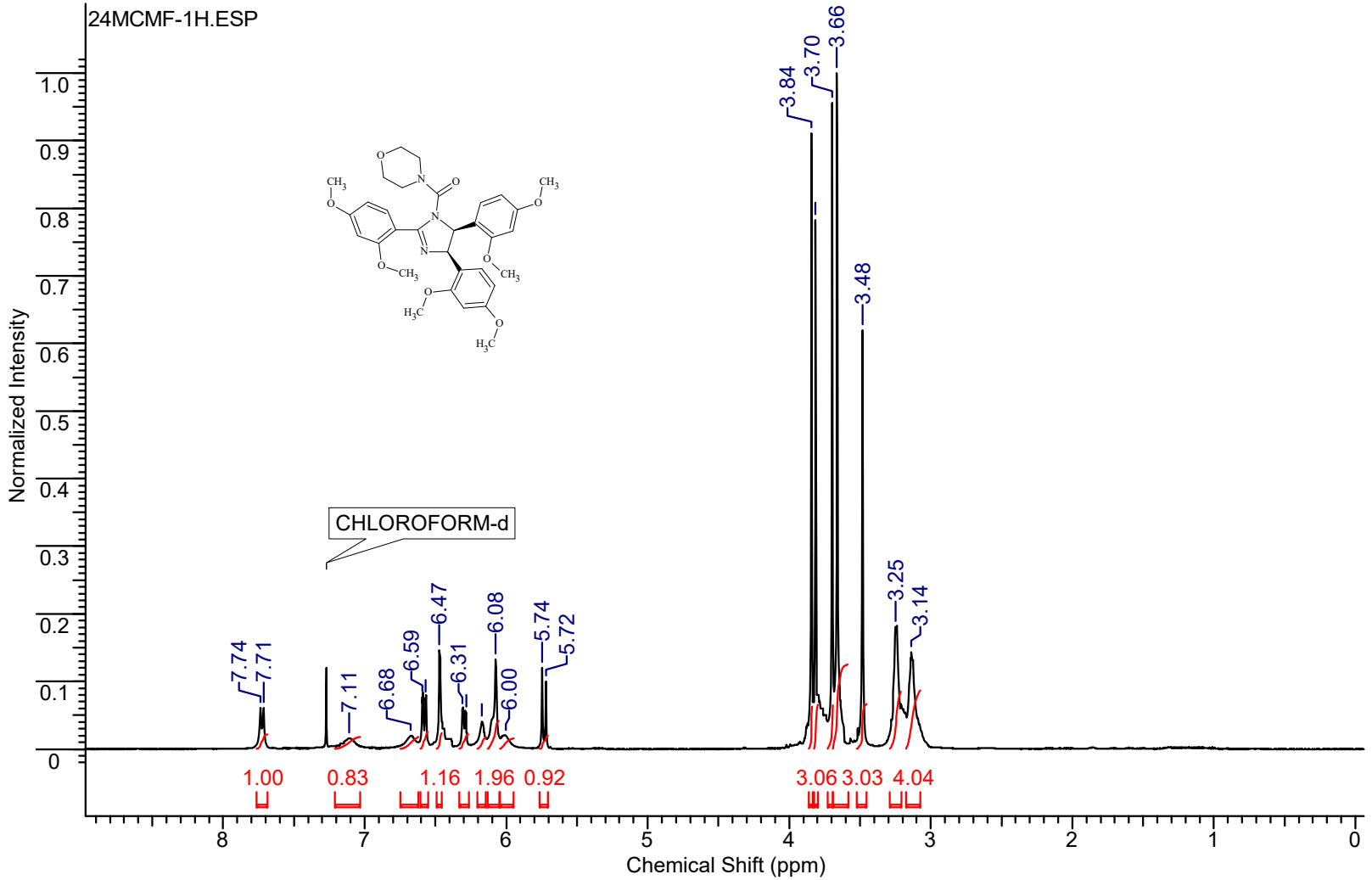
1H NMR *Cis-N,N*-diethyl-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2i).



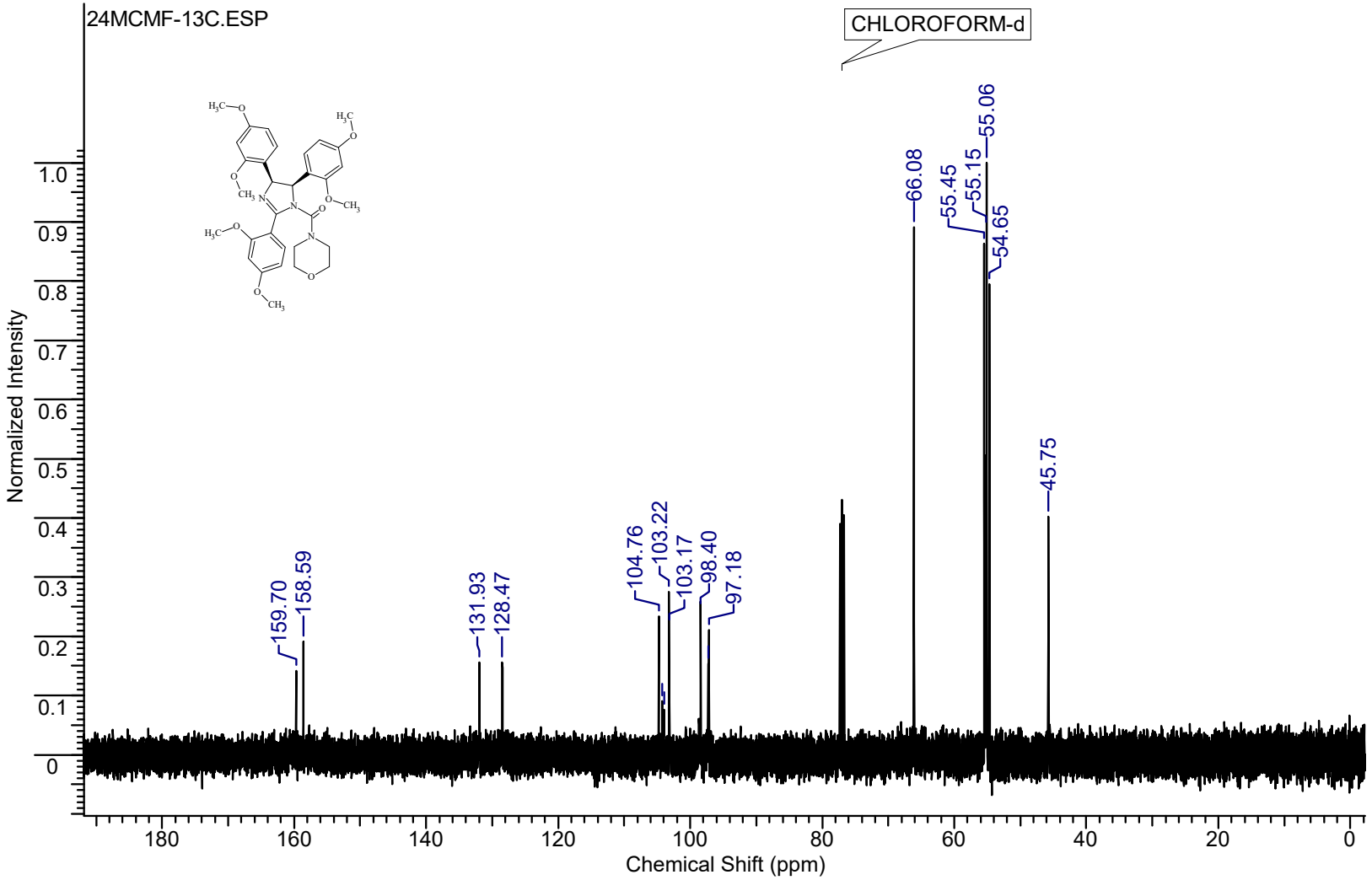
13C NMR *Cis-N,N*-diethyl-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2i).



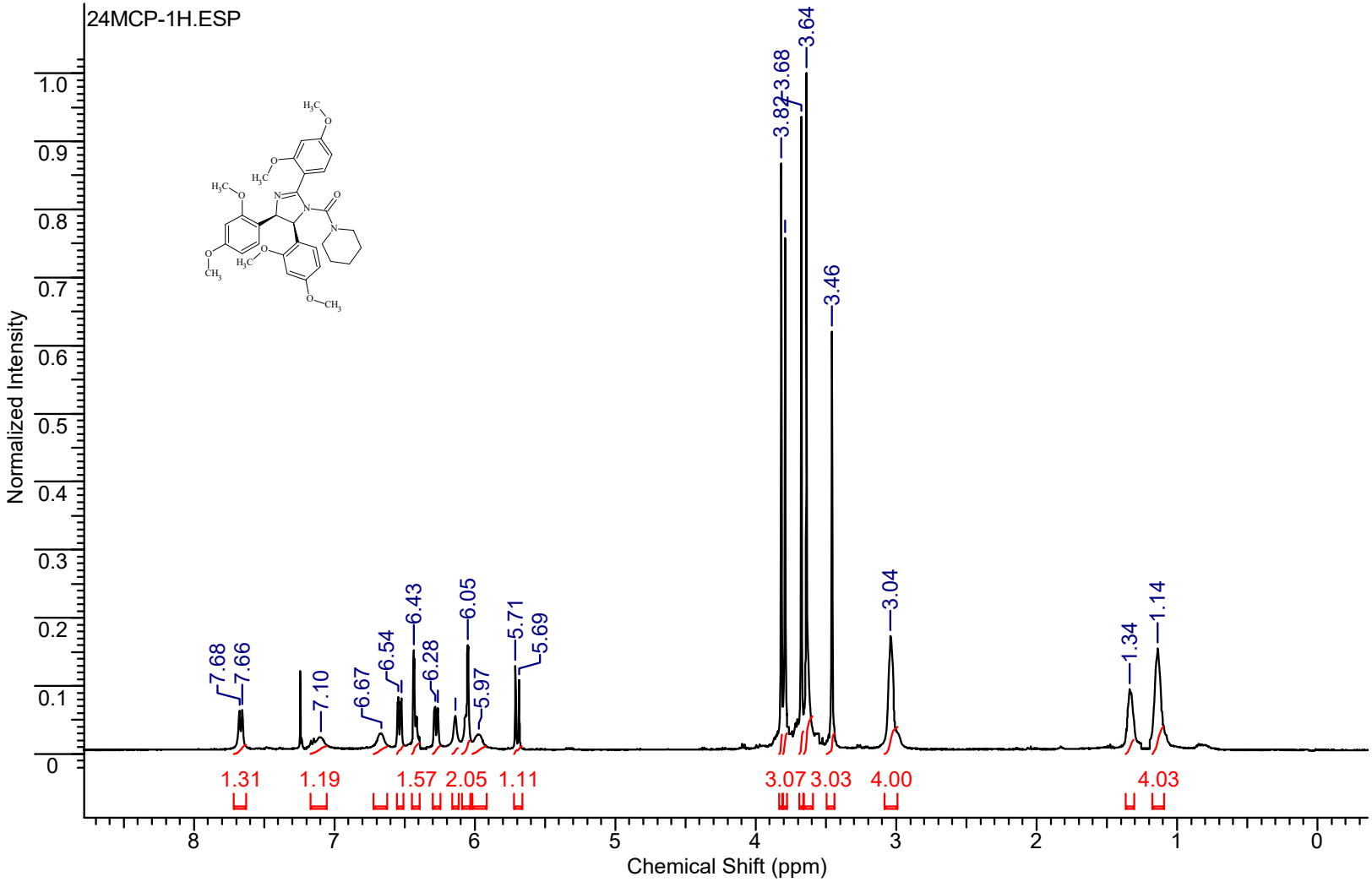
1H NMR 4-{[*Cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2j).



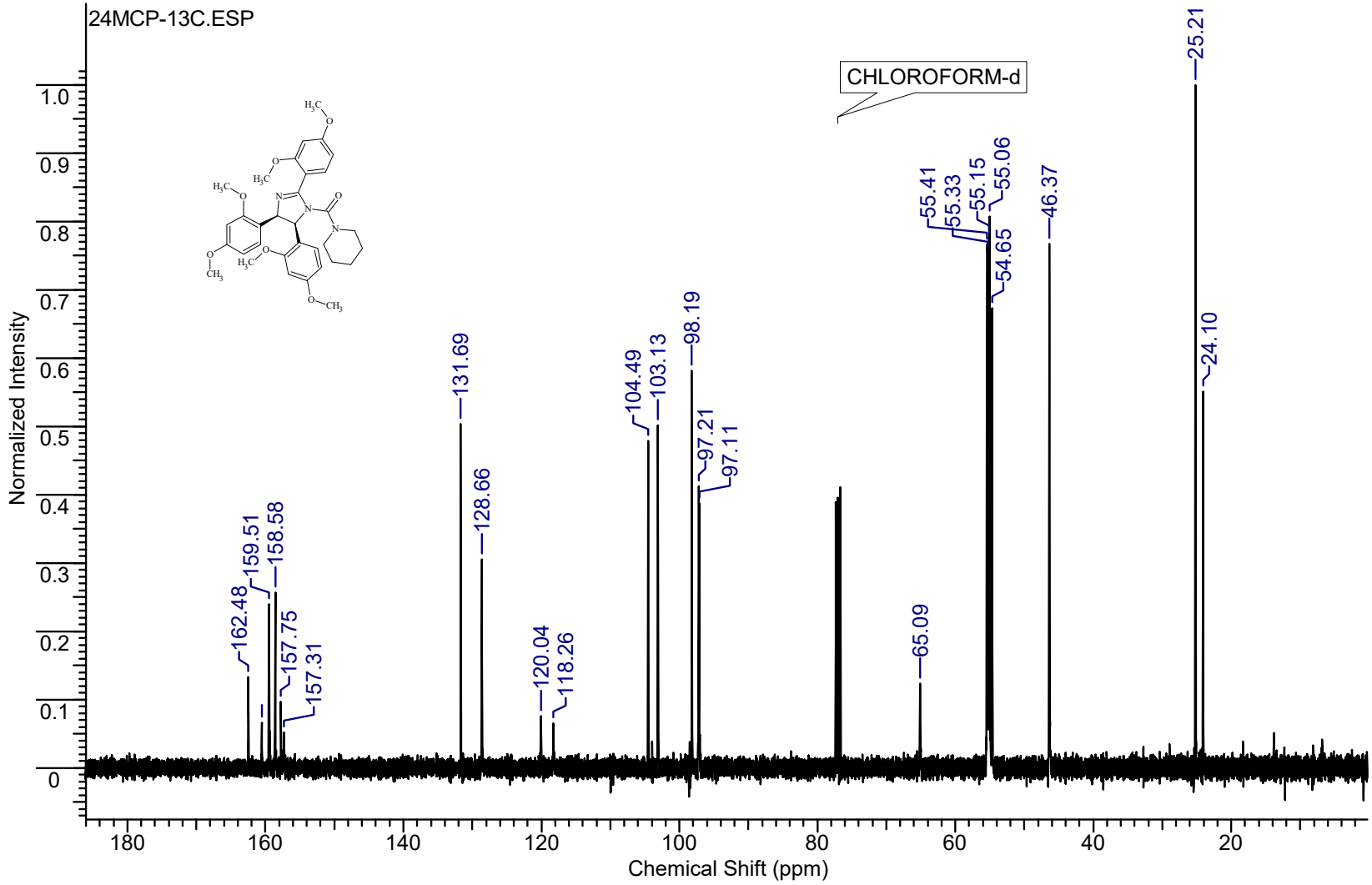
13C NMR 4-{[*Cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2j).



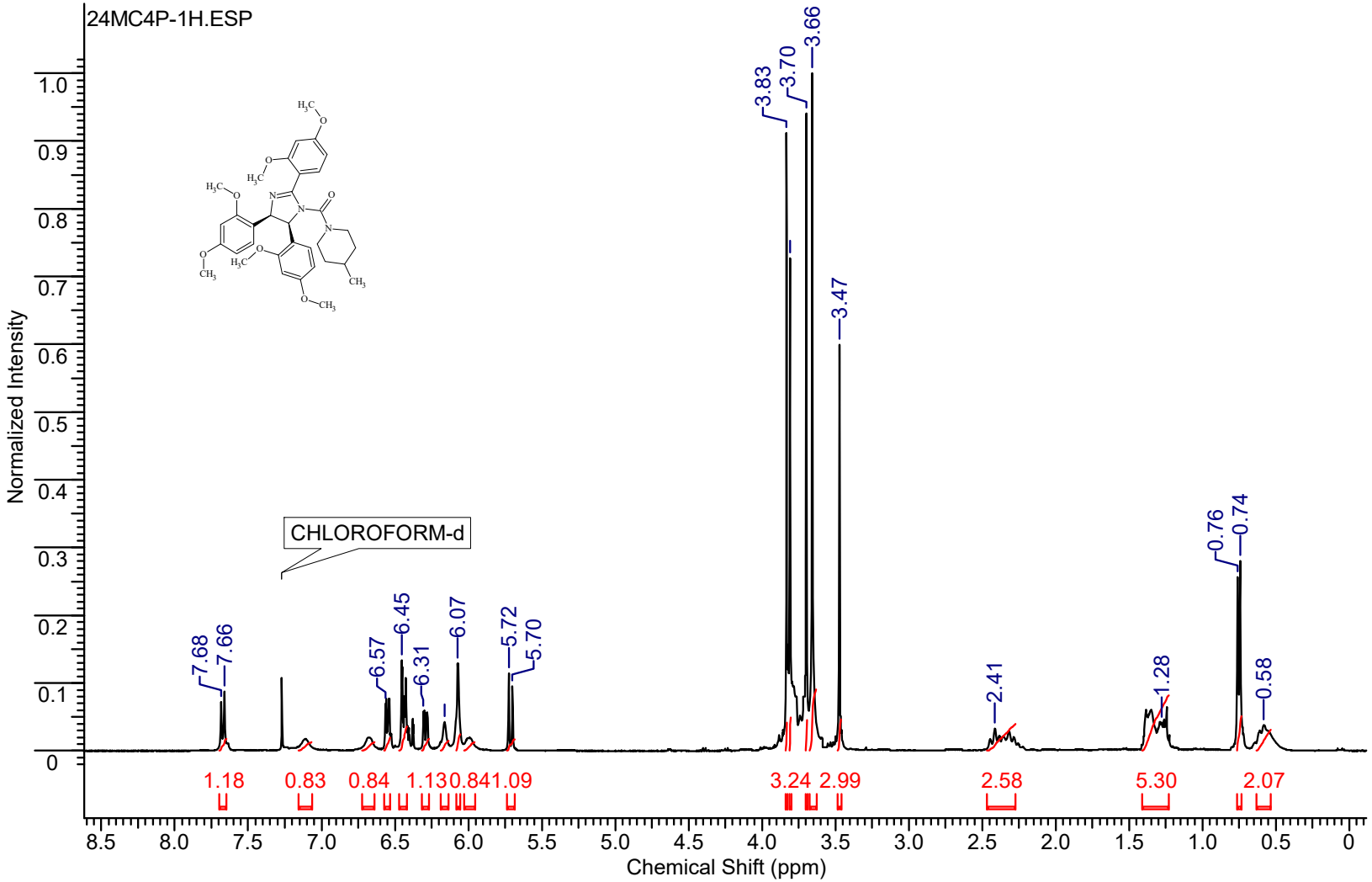
1H NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2k).



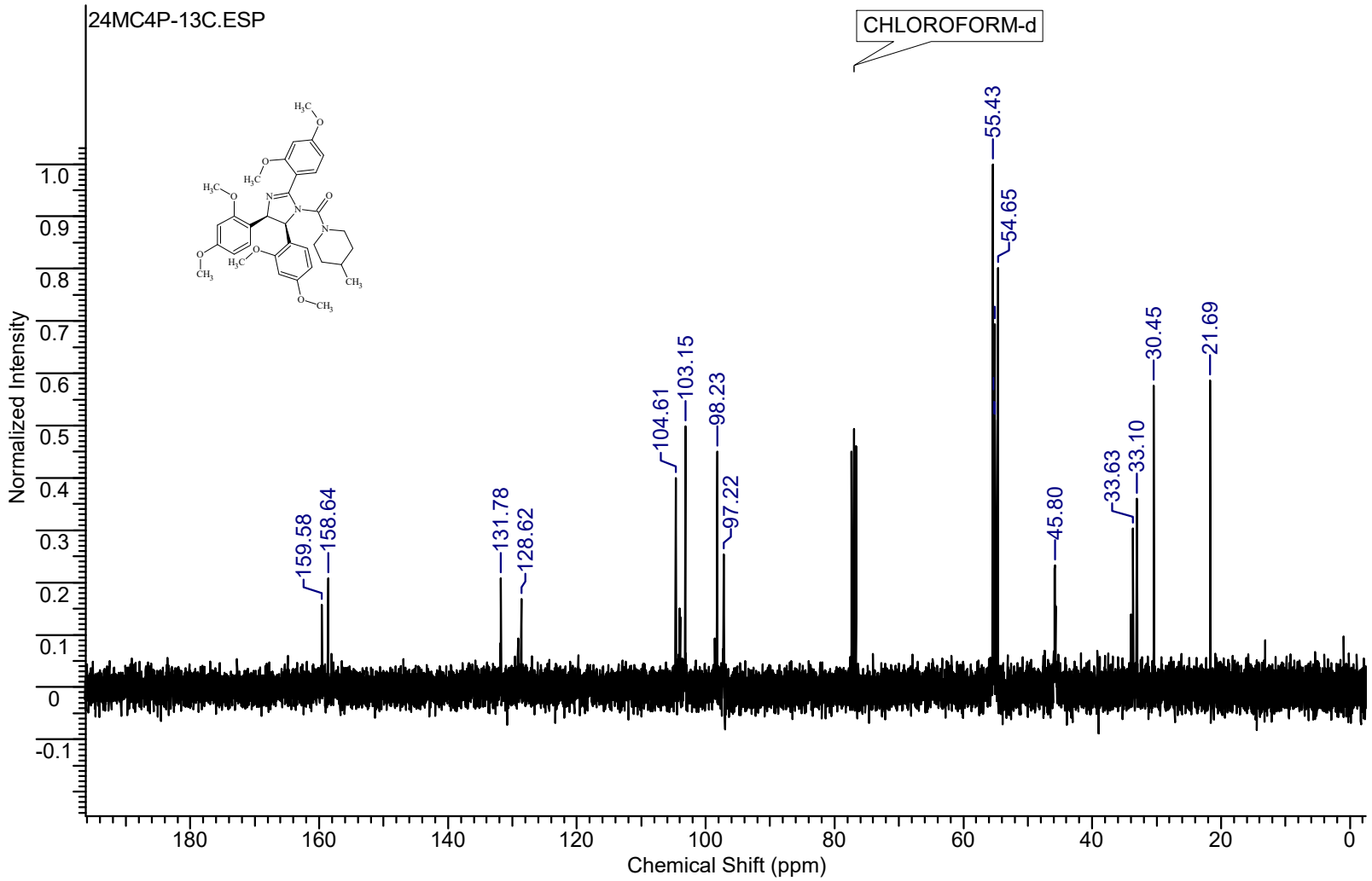
13C NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2k).



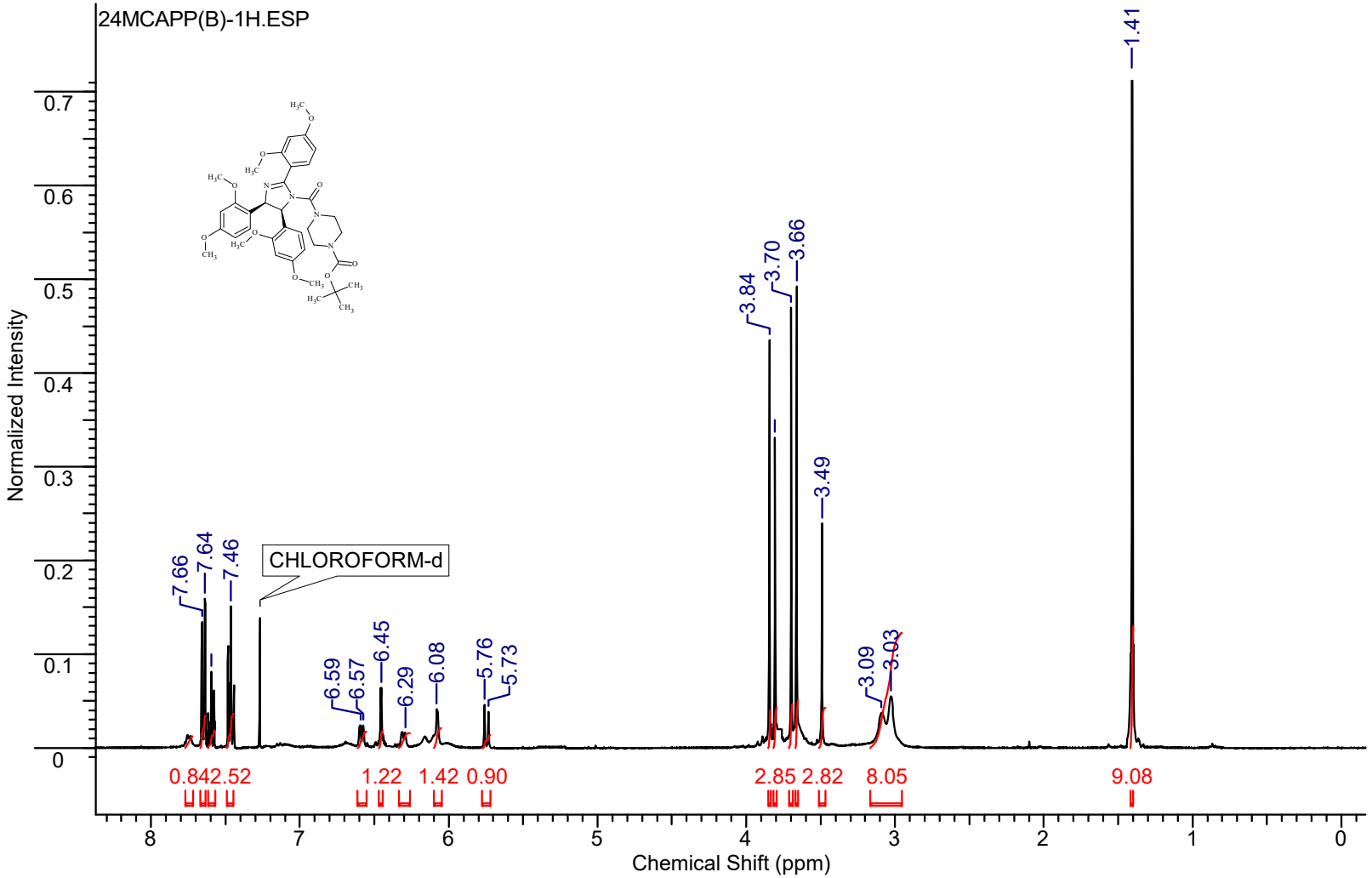
1H NMR (4-Methylpiperidin-1-yl)(*cis*-2,4,5-*tris*(2,4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2l).



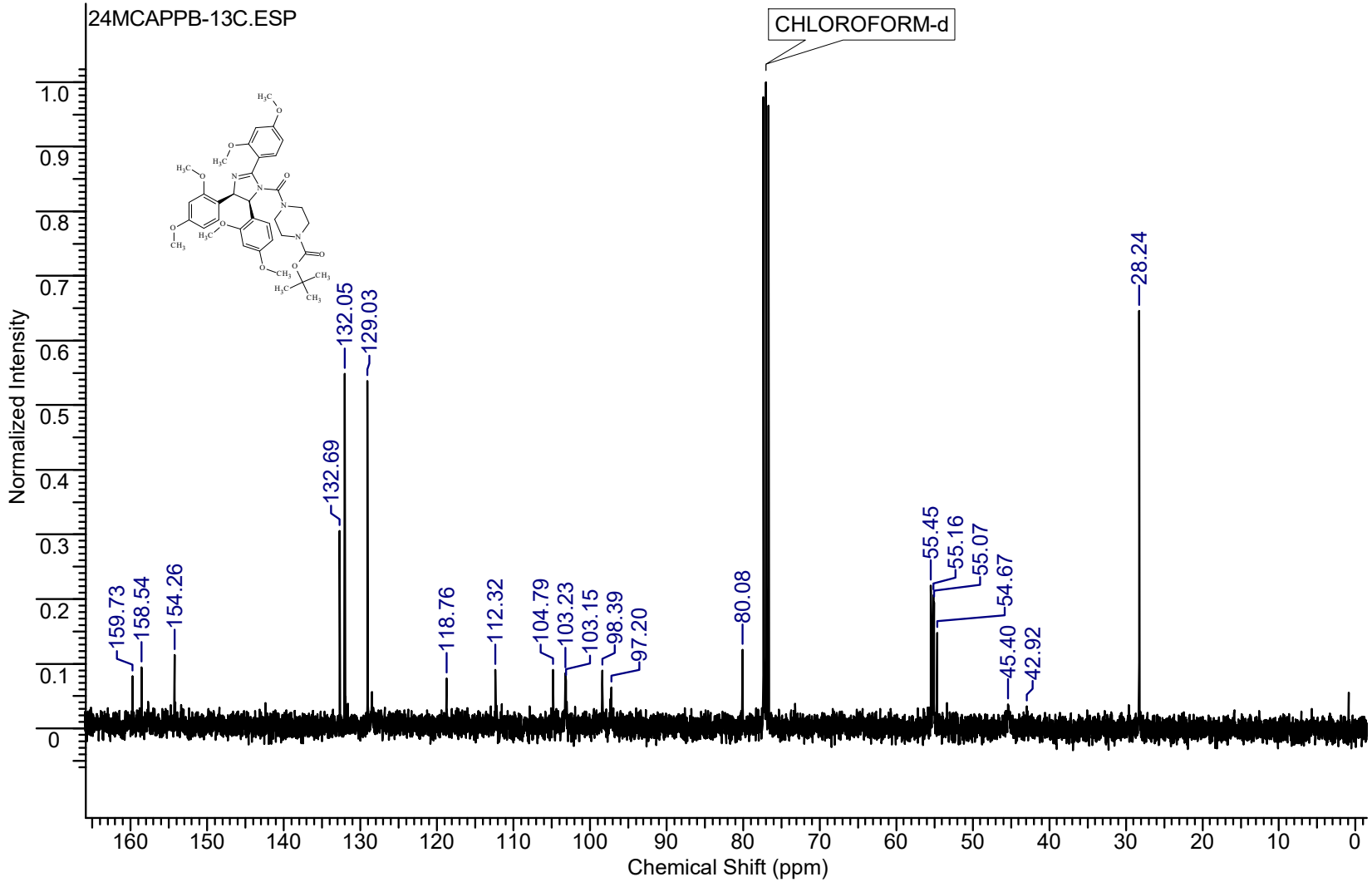
13C NMR (4-Methylpiperidin-1-yl)(*cis*-2,4,5-*tris*(2,4-methoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2l).



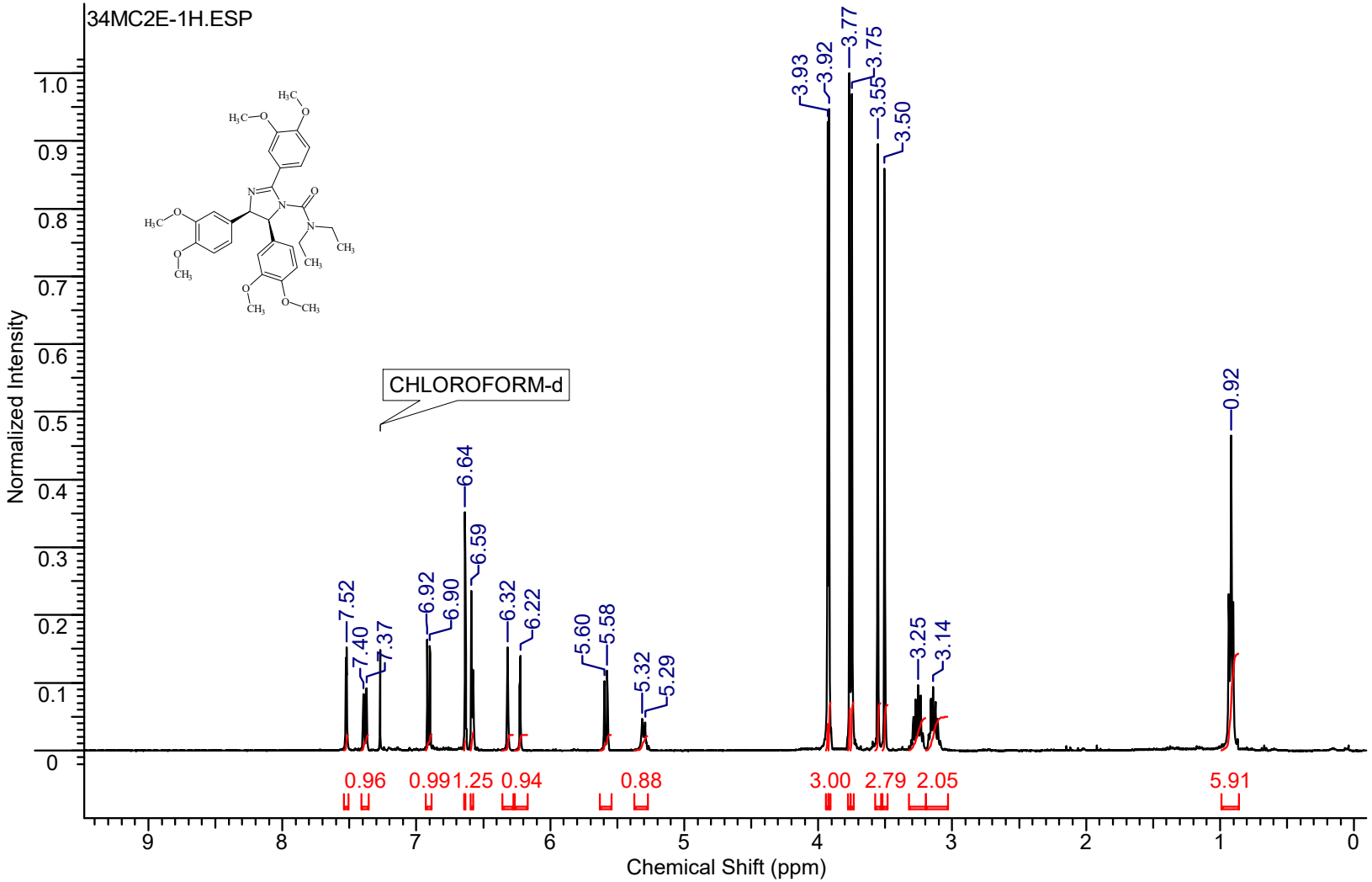
1H NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2m).



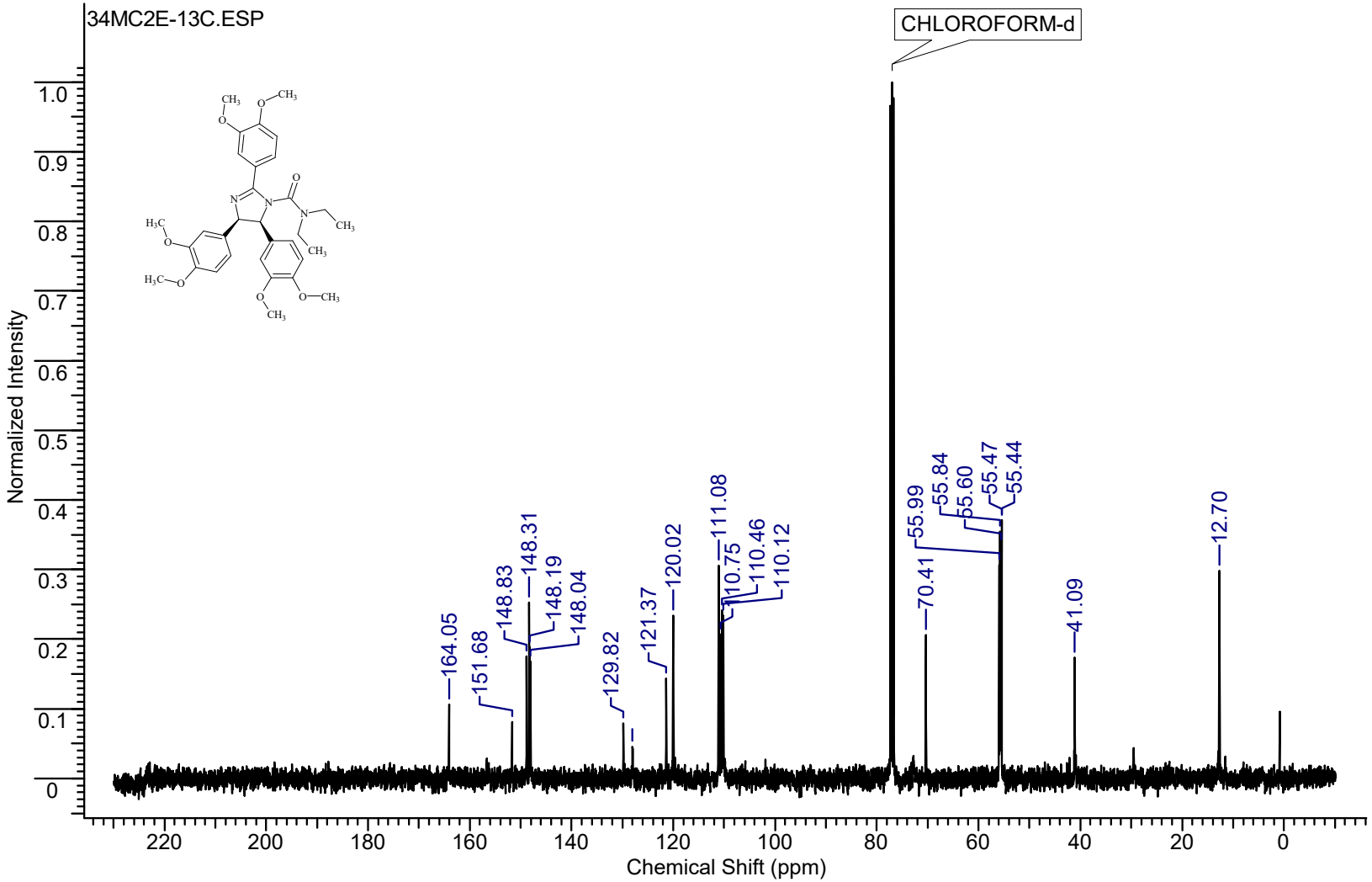
13C NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(2,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2m).



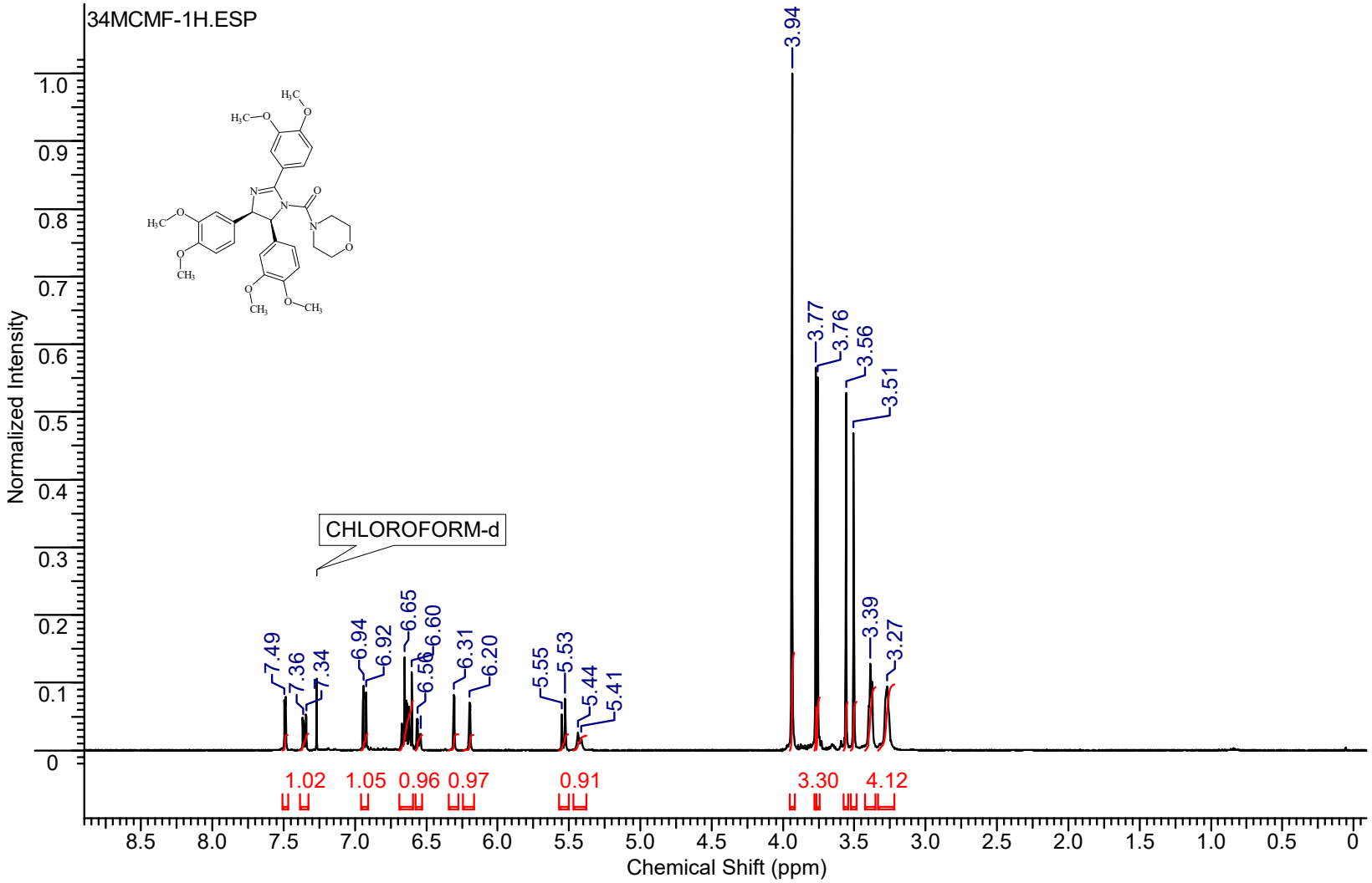
1H NMR *Cis*-*N,N*-diethyl-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2n).



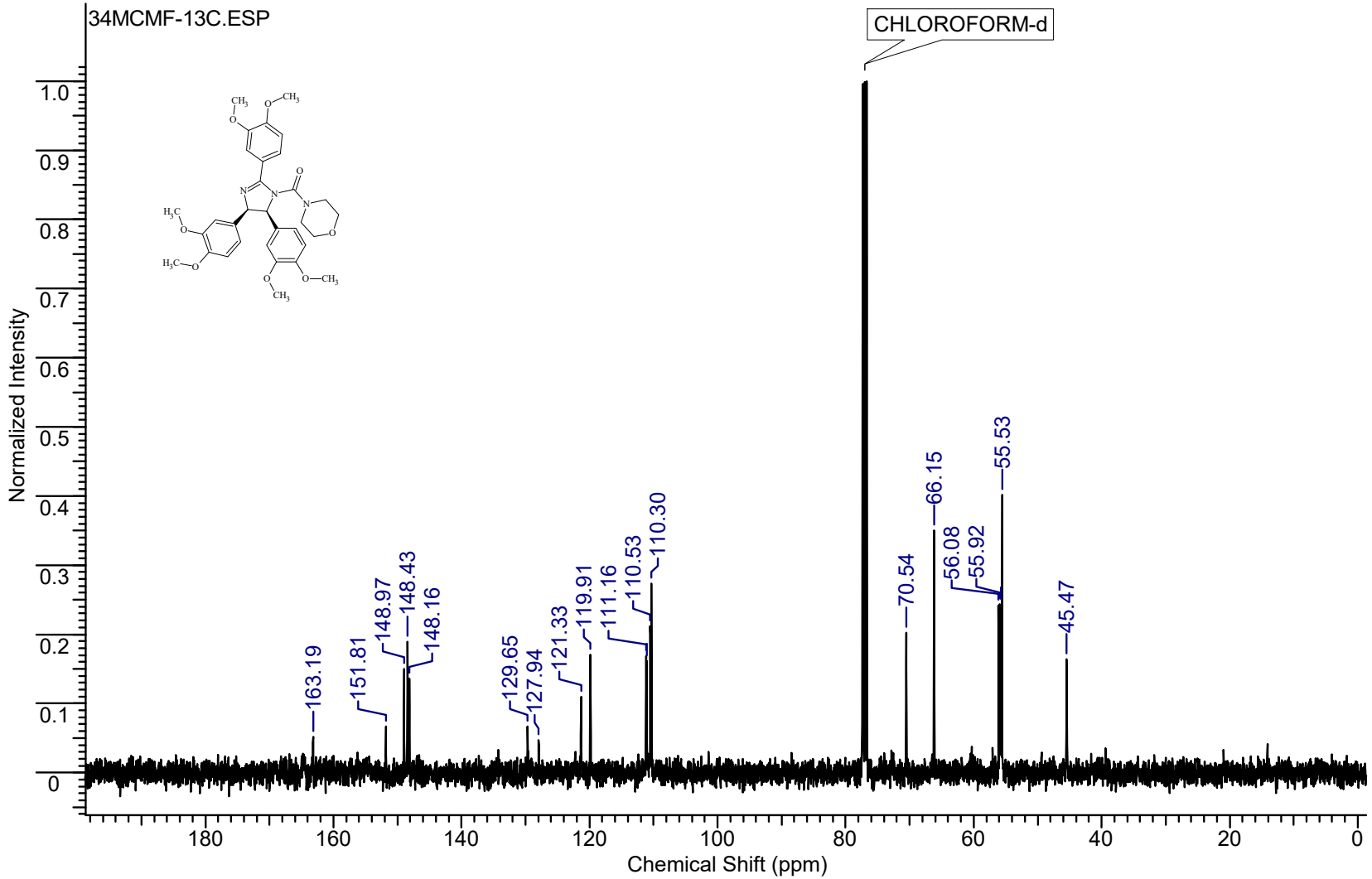
13C NMR *Cis-N,N*-diethyl-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2n).



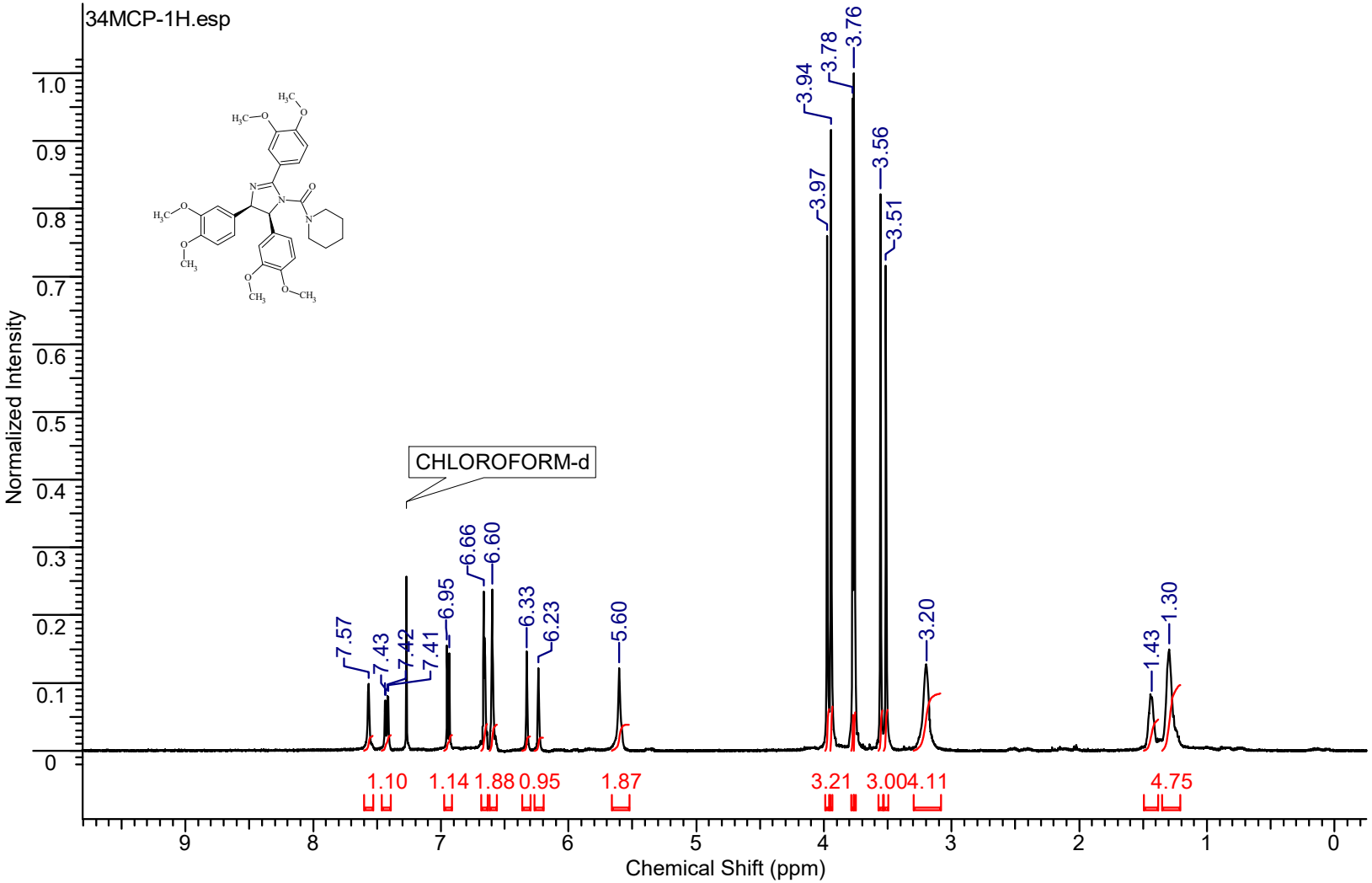
1H NMR 4-{[*Cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2o).



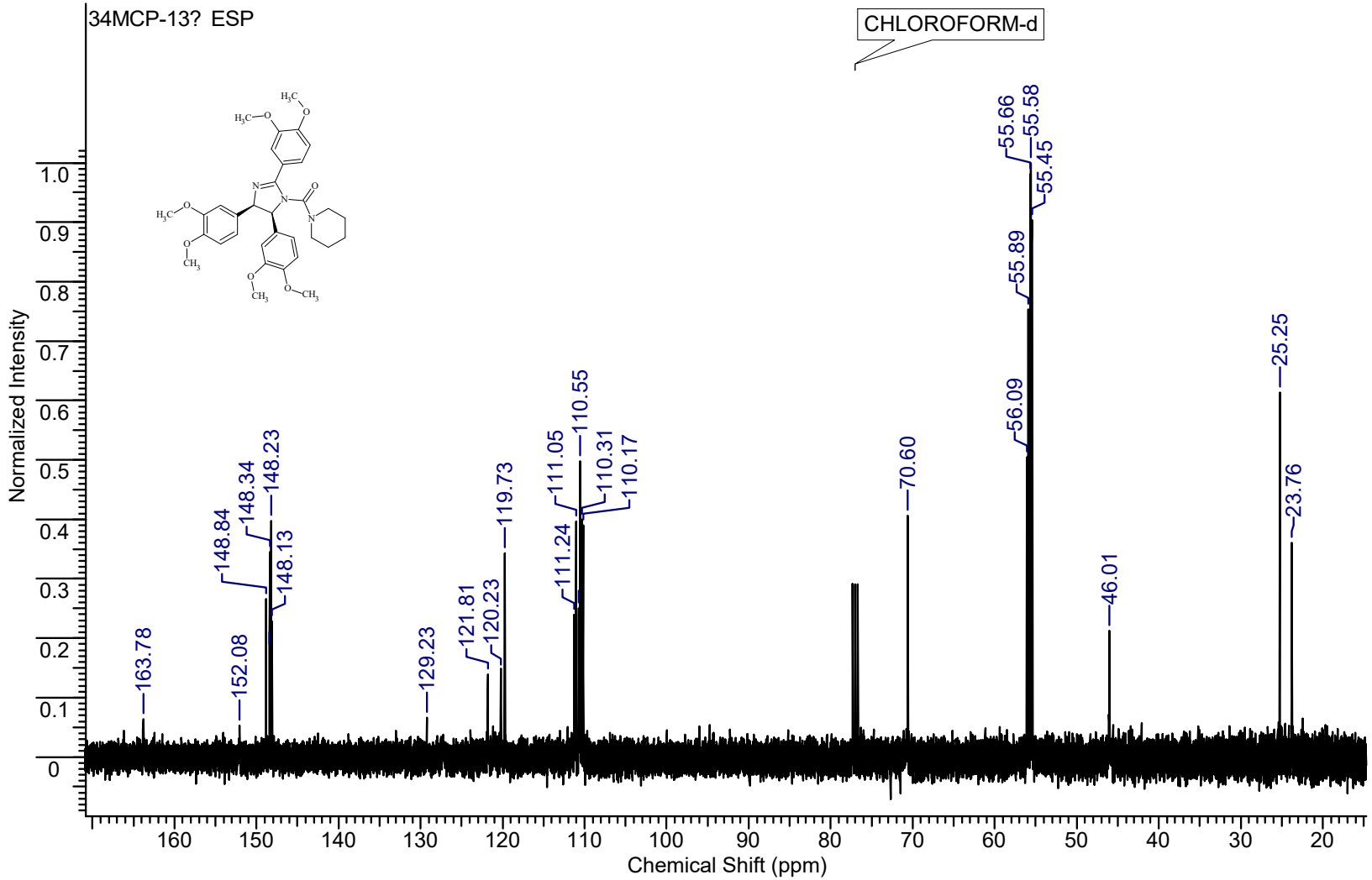
13C NMR 4-{[*Cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2o).



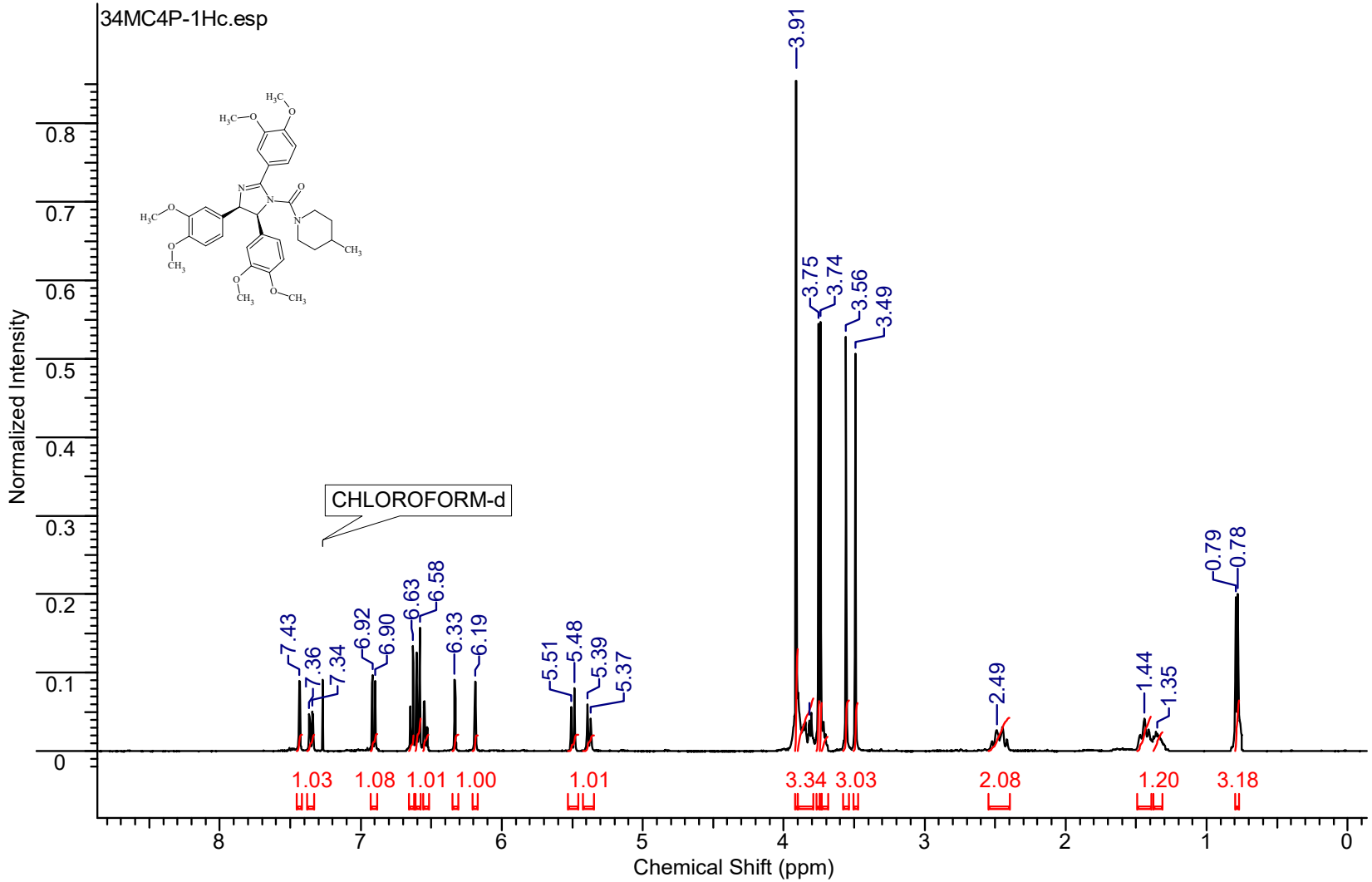
1H NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2p).



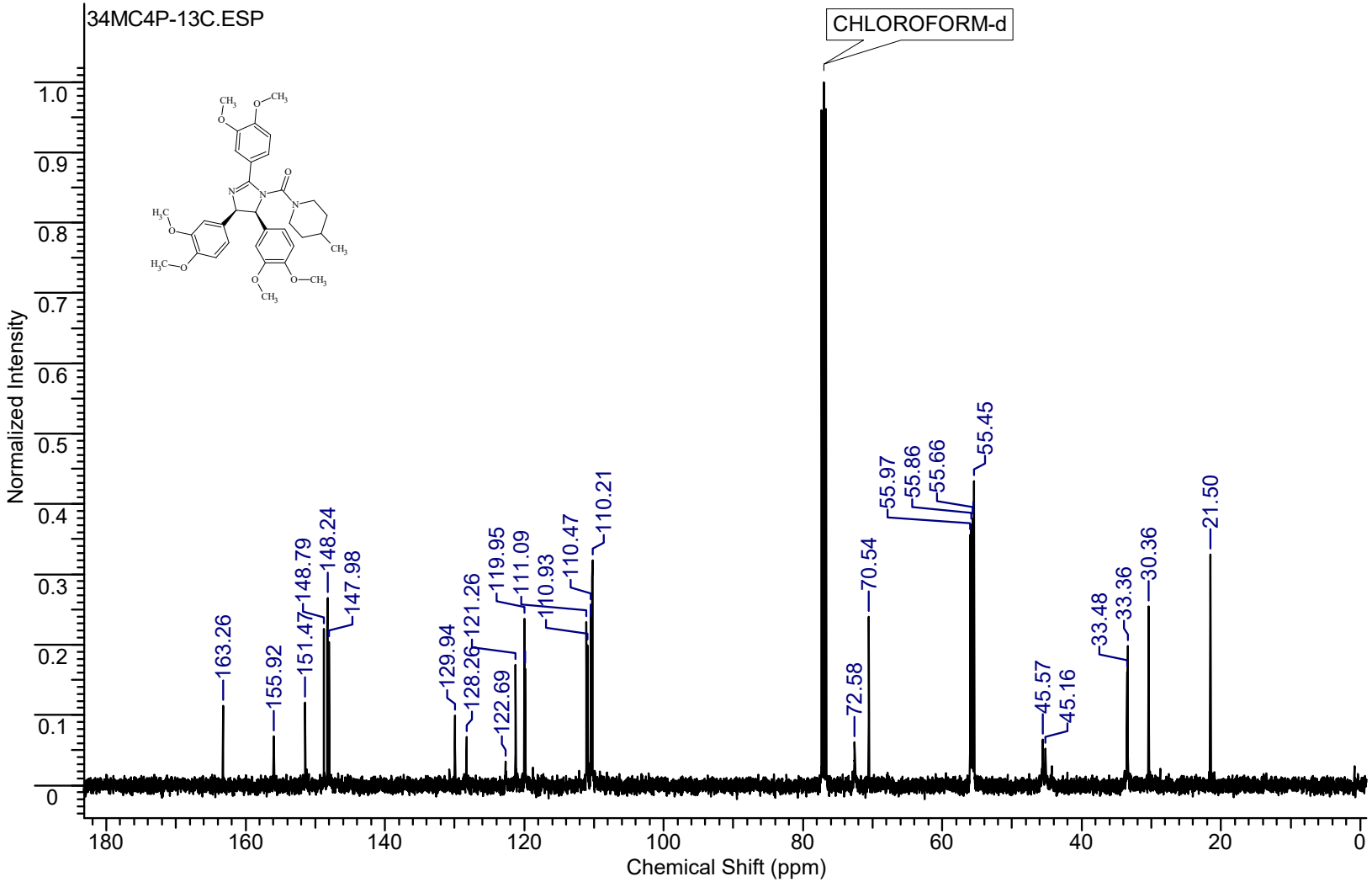
13C NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2p).



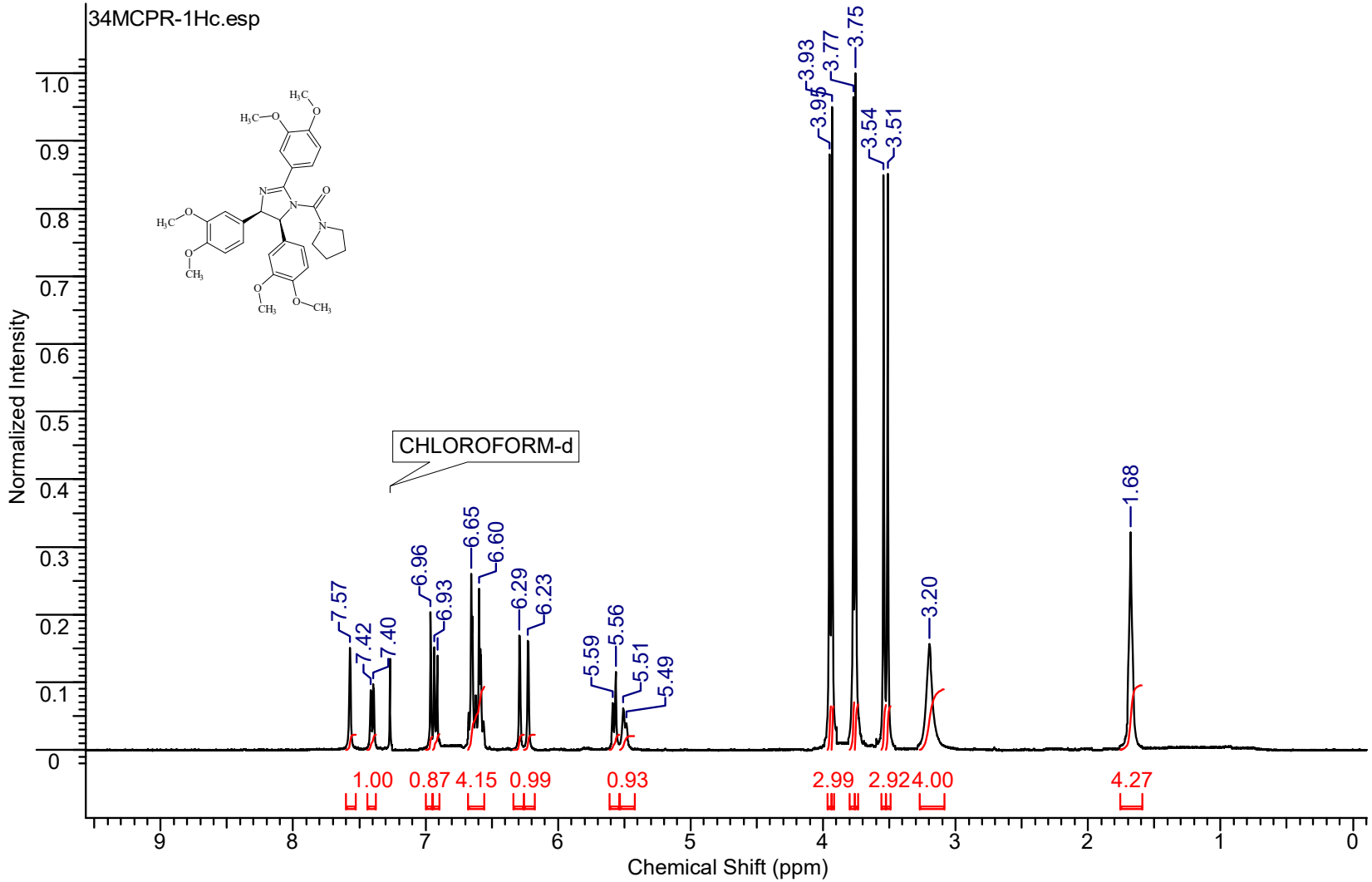
1H NMR (4-Methylpiperidin-1-yl)(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2q).



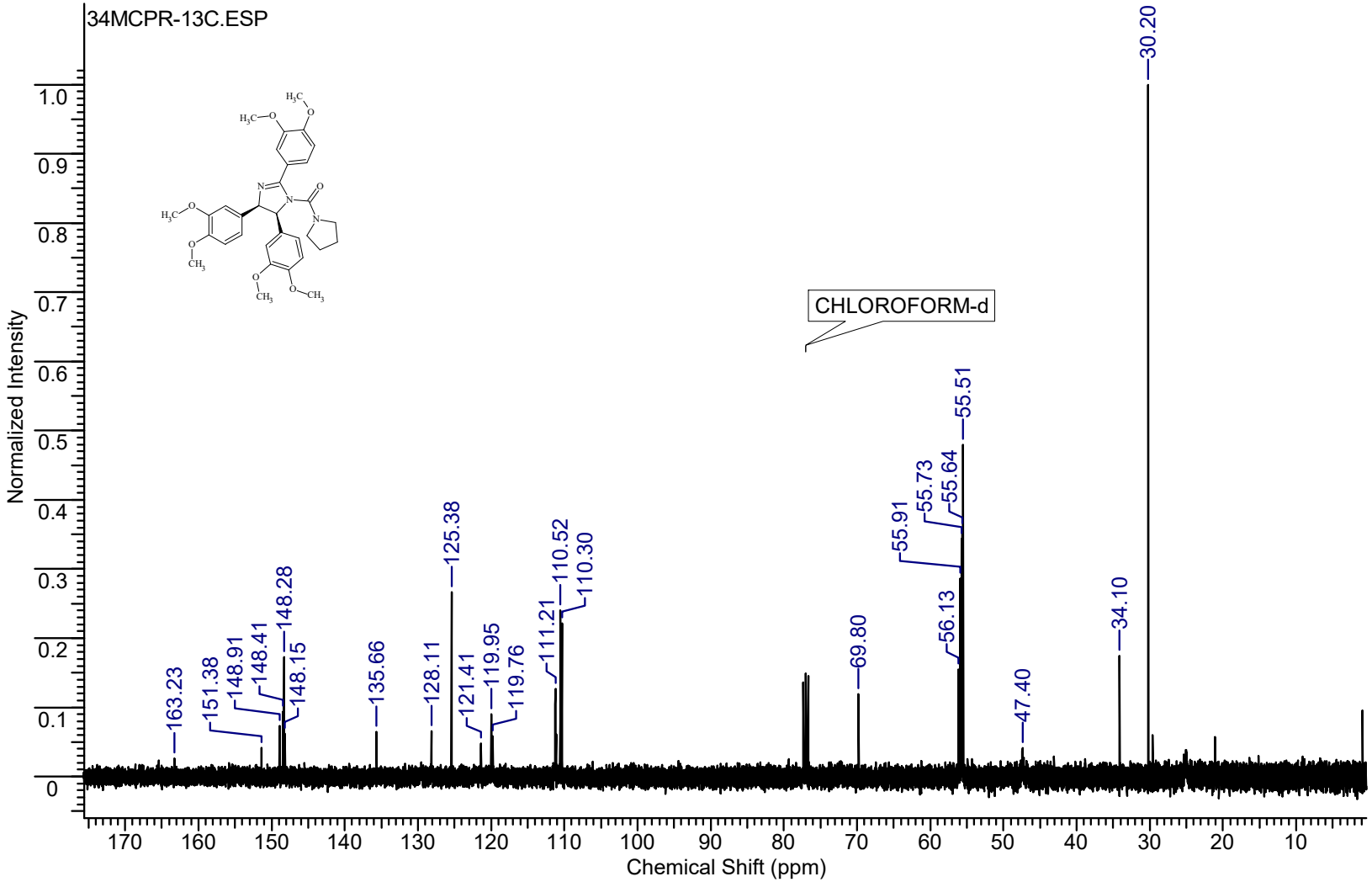
13C NMR (4-Methylpiperidin-1-yl)(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2q).



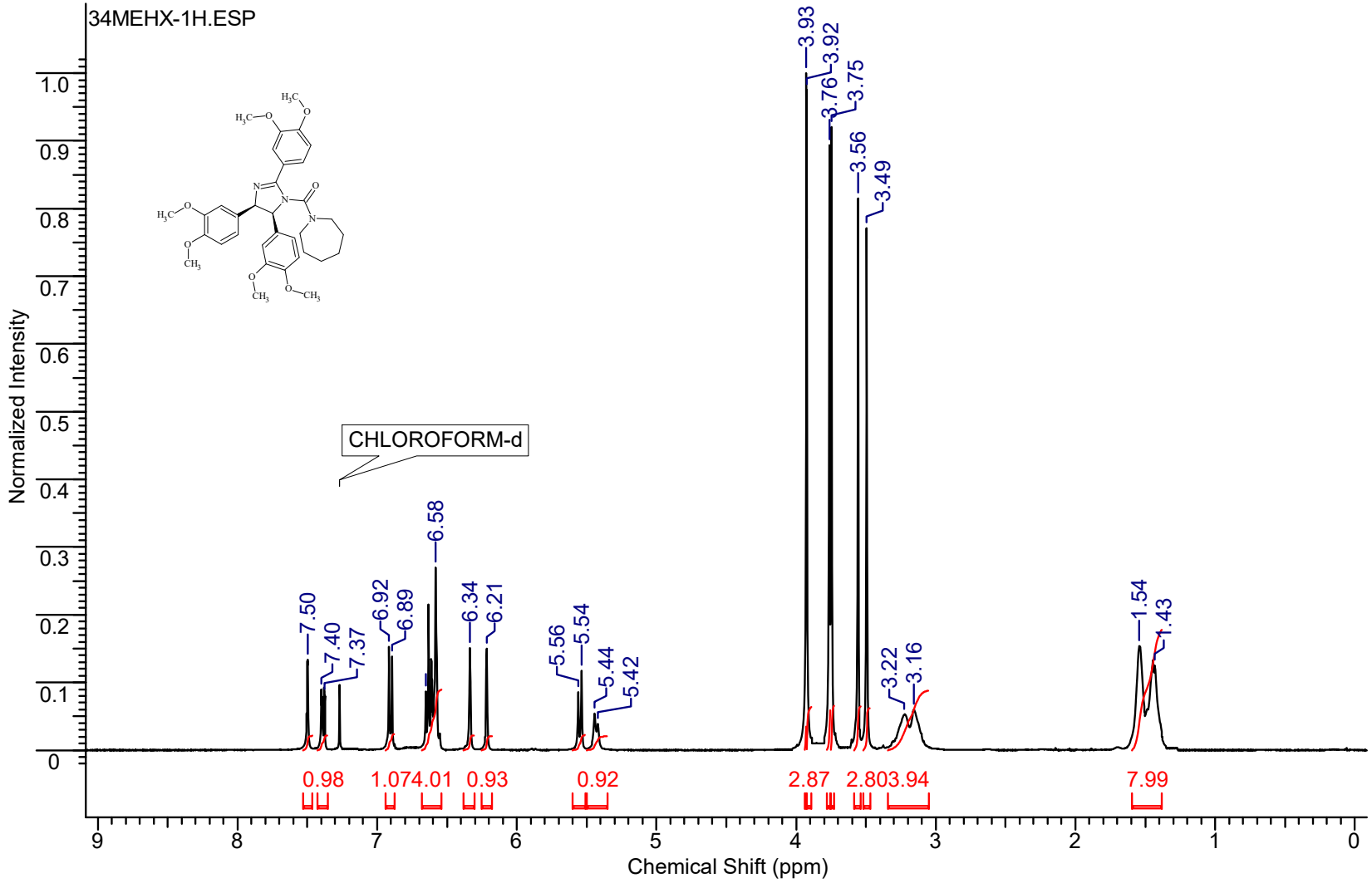
1H NMR Pyrrolidin-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2r).



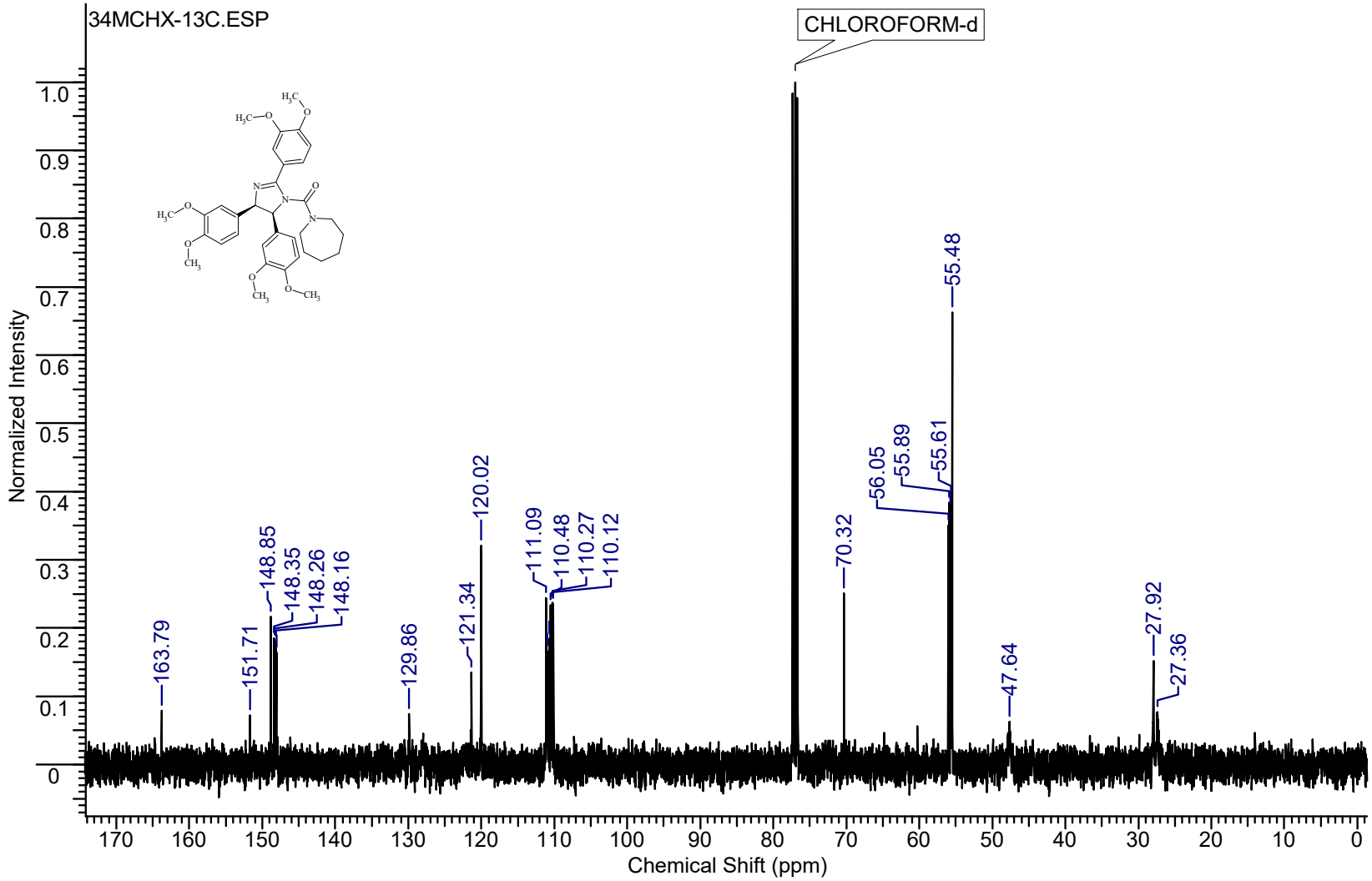
13C NMR Pyrrolidin-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2r).



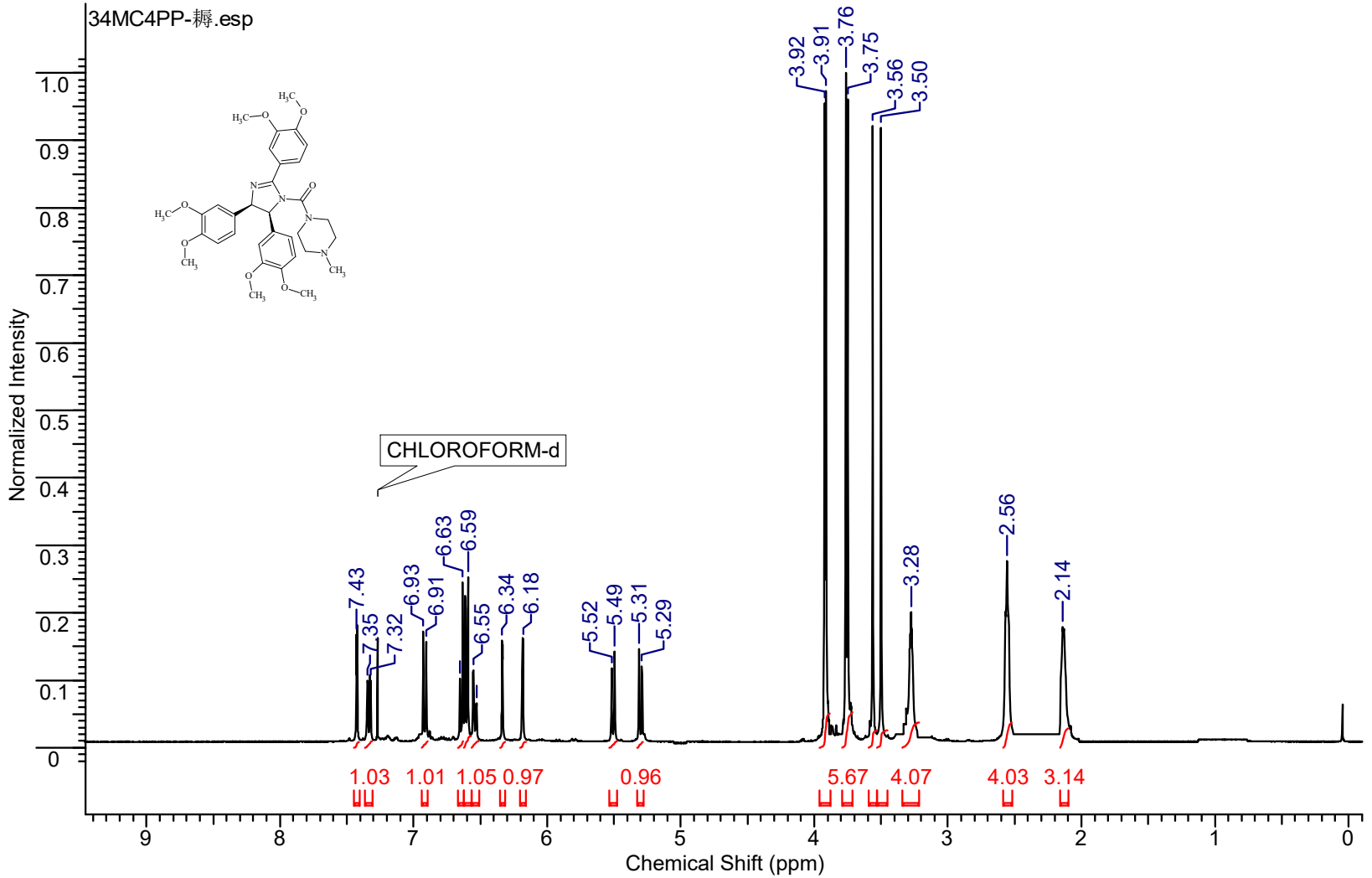
1H NMR Azepan-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2s).



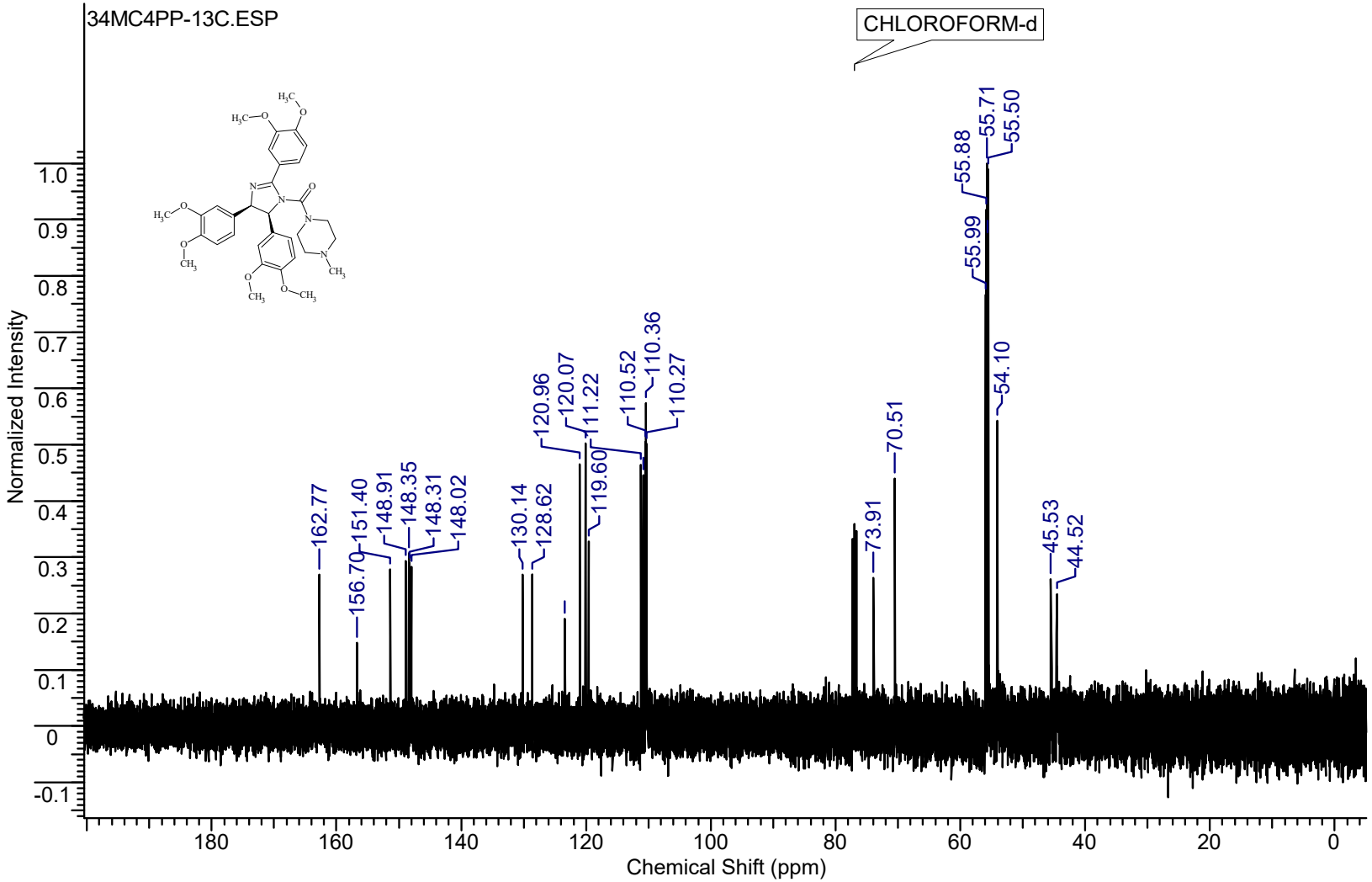
13C NMR Azepan-1-yl(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2*s*).



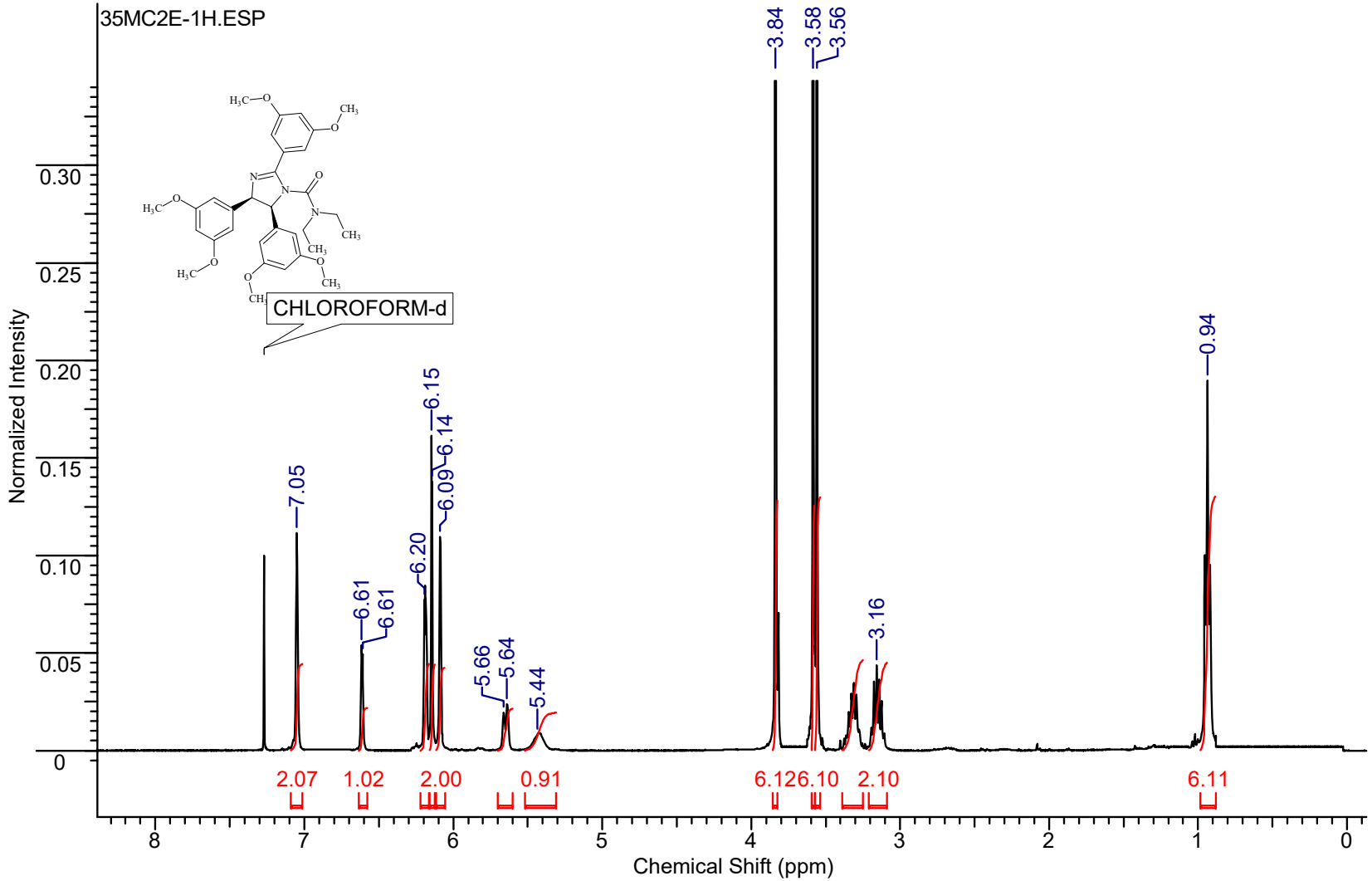
1H NMR (4-Methylpiperazin-1-yl)(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2t).



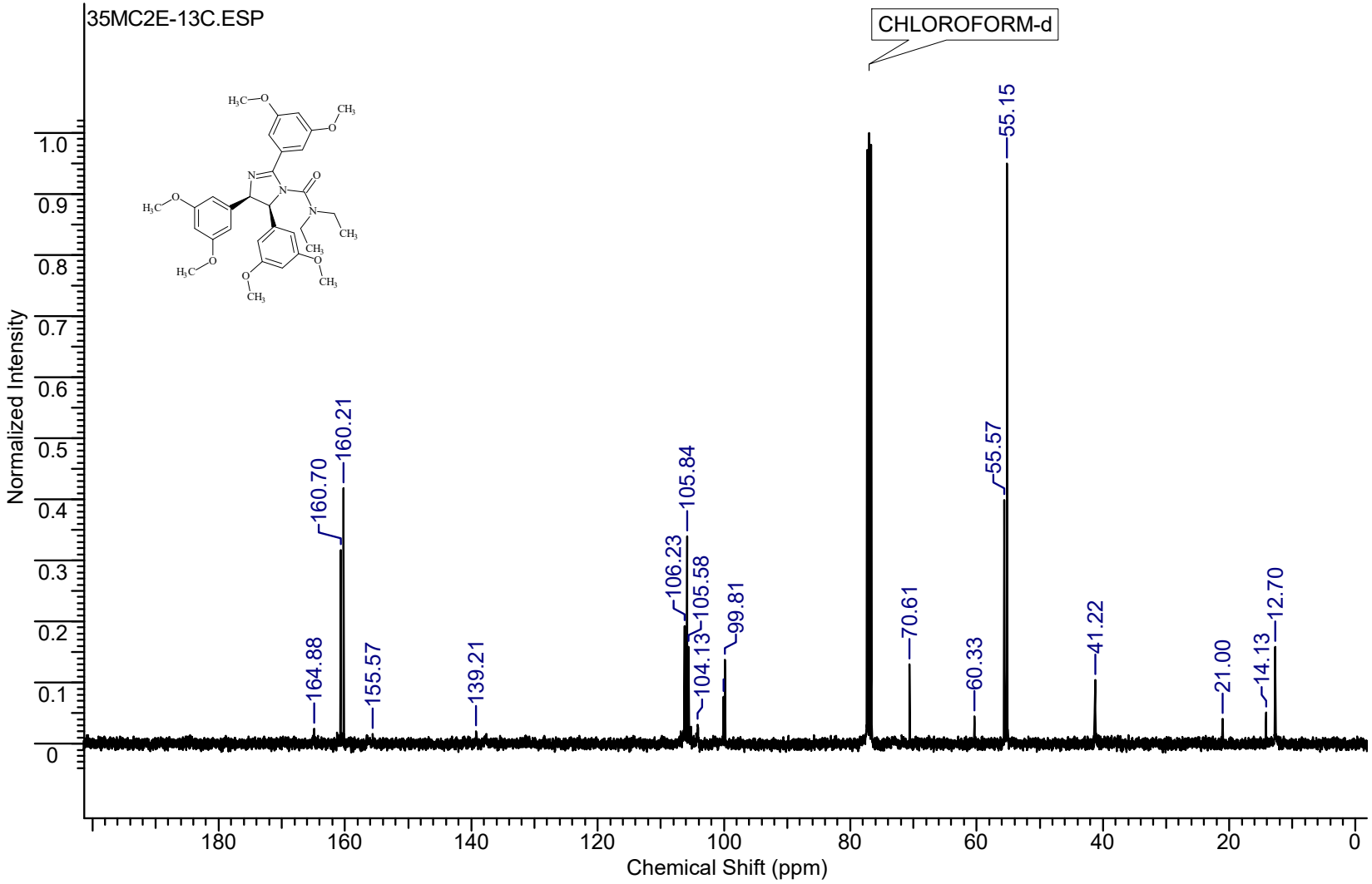
13C NMR (4-Methylpiperazin-1-yl)(*cis*-2,4,5-*tris*(3,4-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2t).



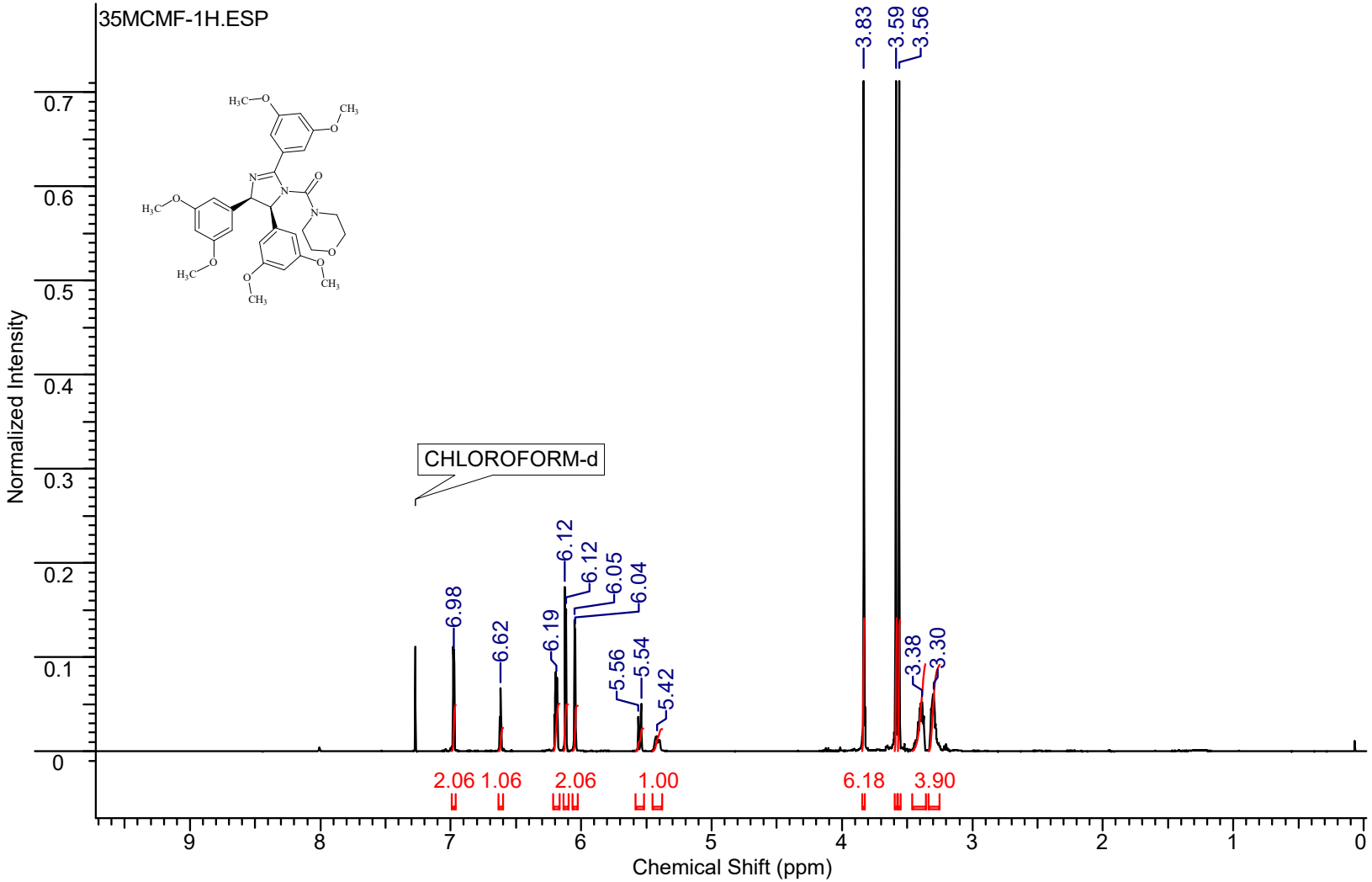
***1H NMR Cis-N,N*-diethyl-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2u).**



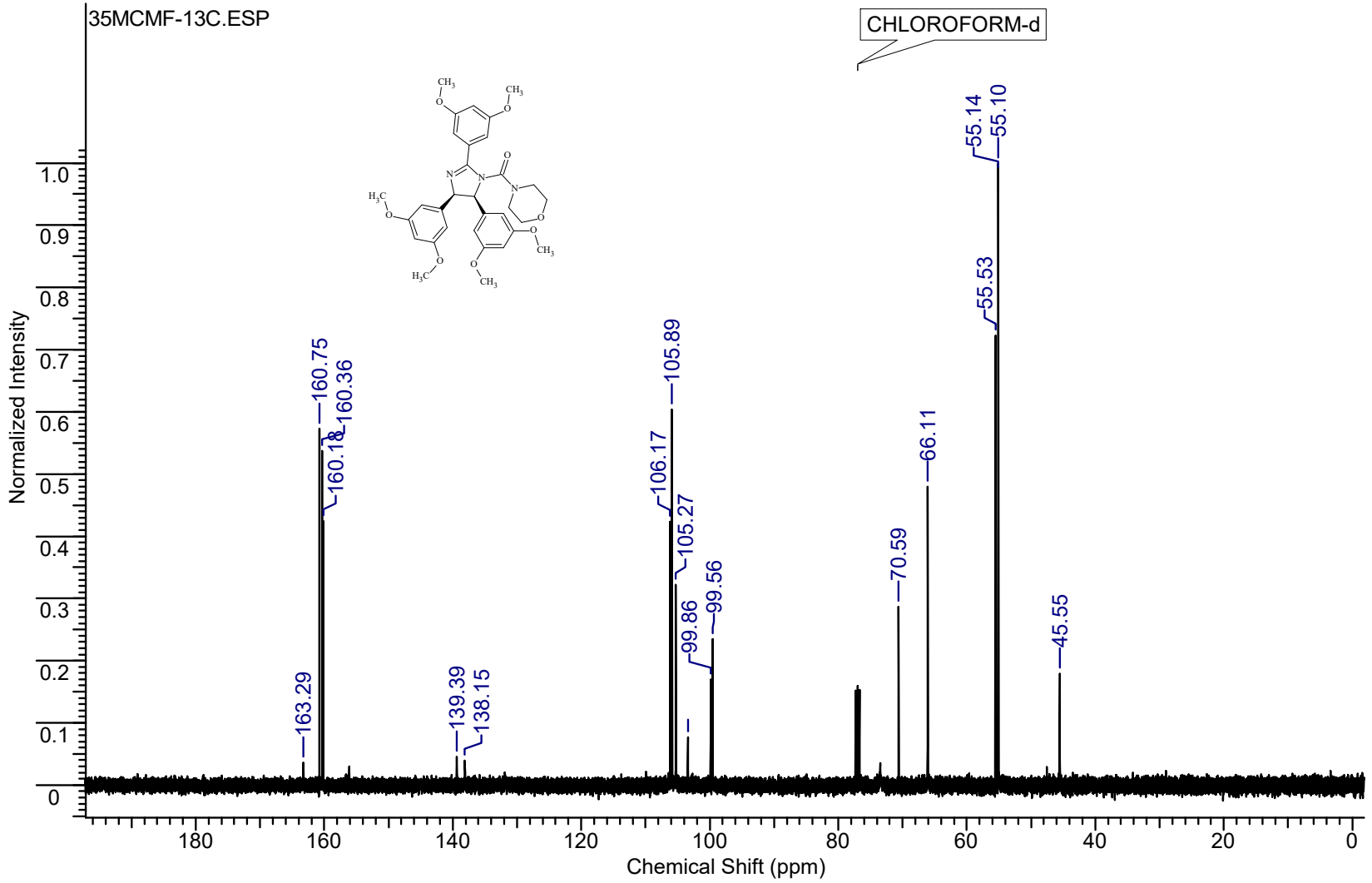
***13C NMR Cis-N,N*-diethyl-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2u).**



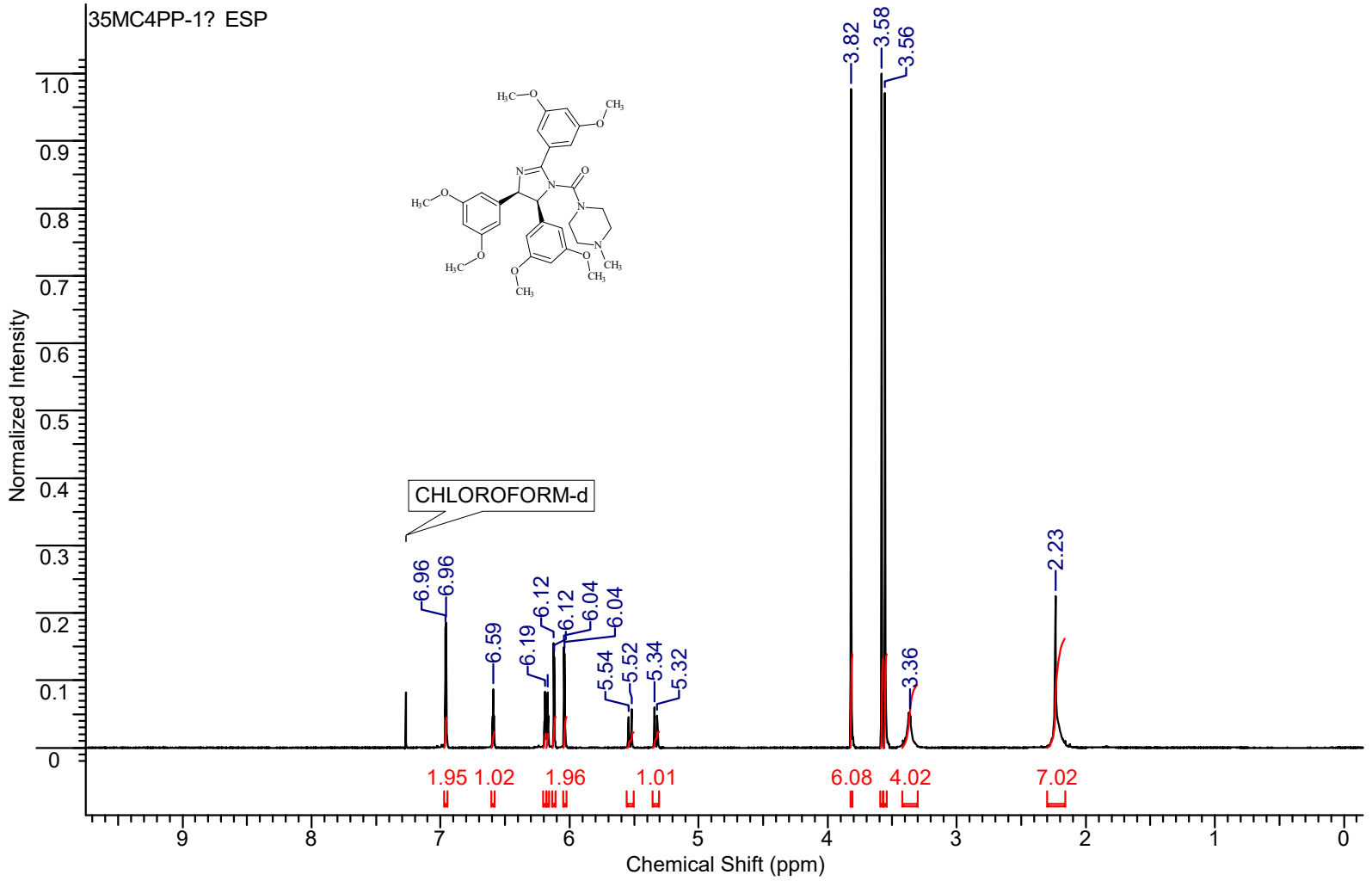
1H NMR 4-{[*Cis*-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2v).



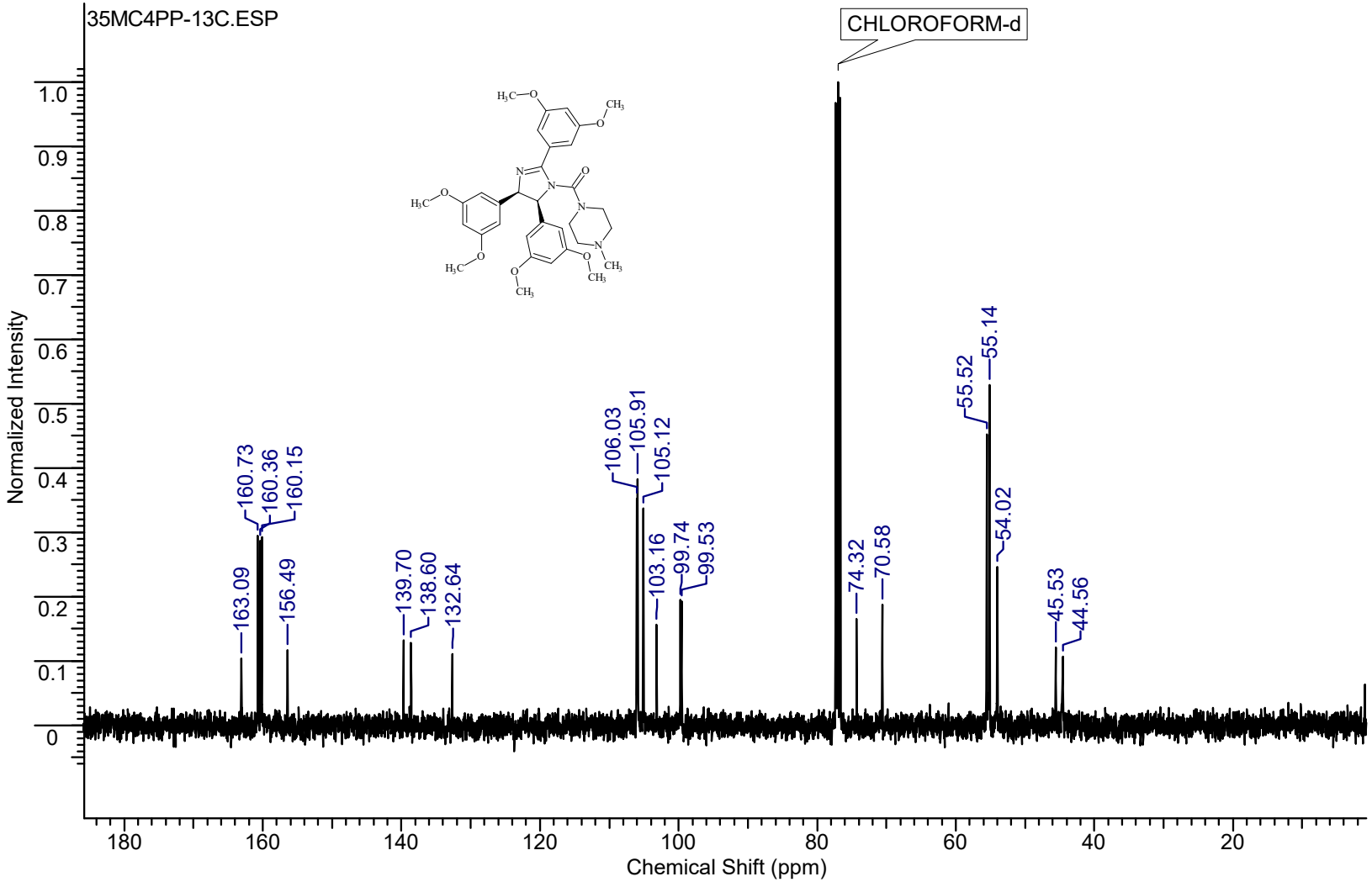
13C NMR 4-{[*Cis*-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2v).



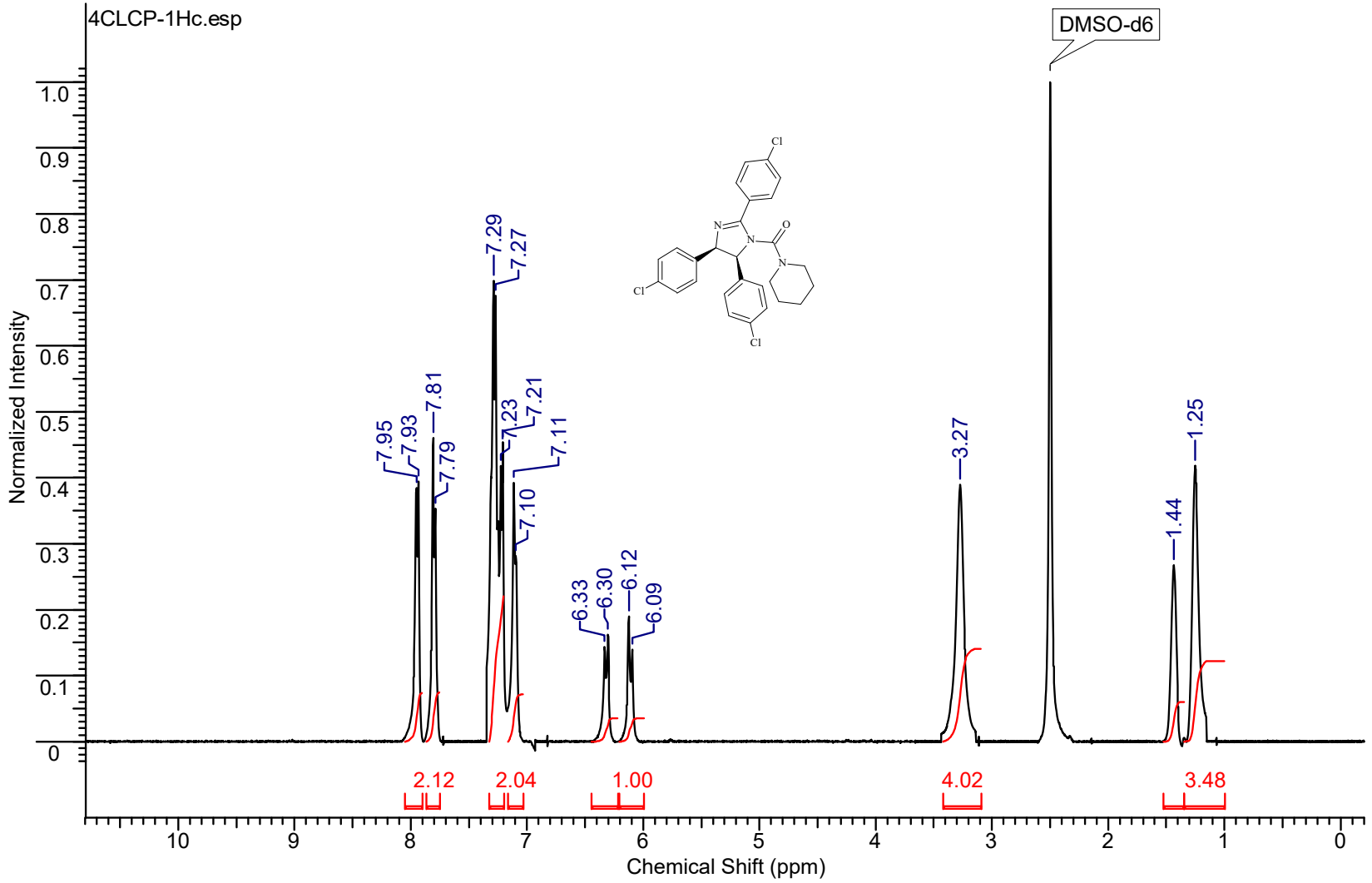
1H NMR (4-Methylpiperazin-1-yl)(*cis*-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2w).



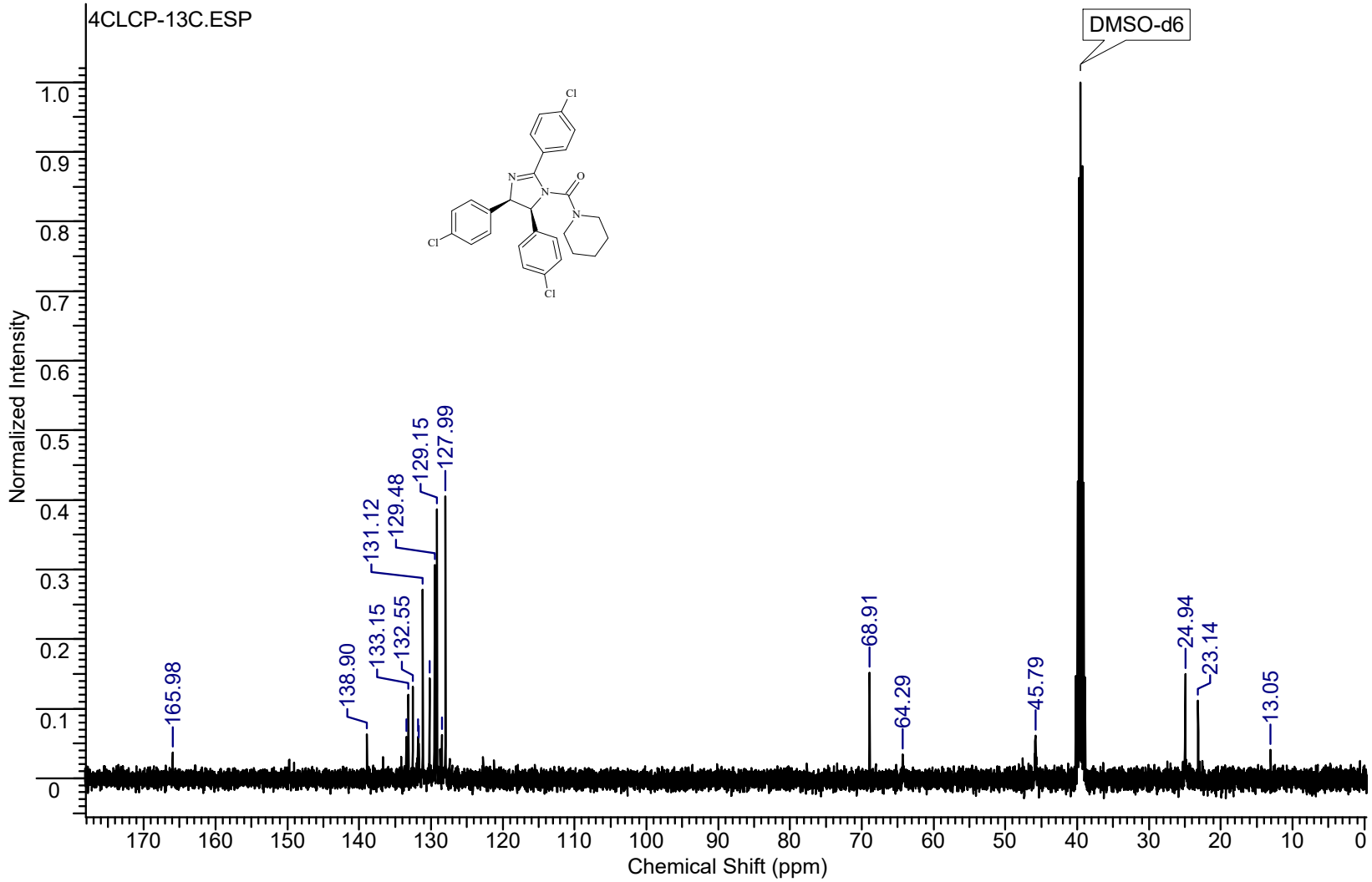
13C NMR (4-Methylpiperazin-1-yl)(*cis*-2,4,5-*tris*(3,5-dimethoxyphenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2w).



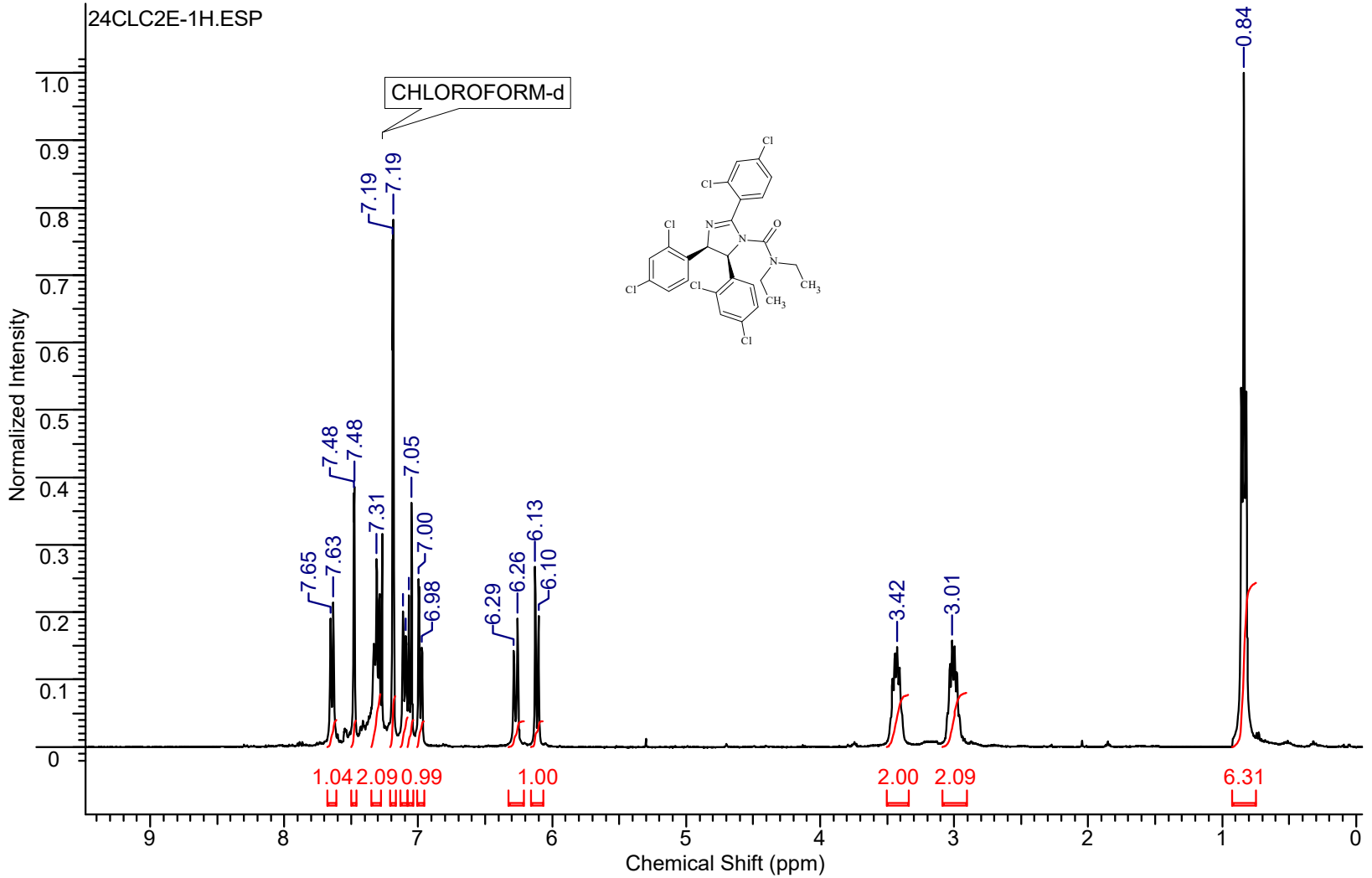
1H NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(4-chlorophenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2x).



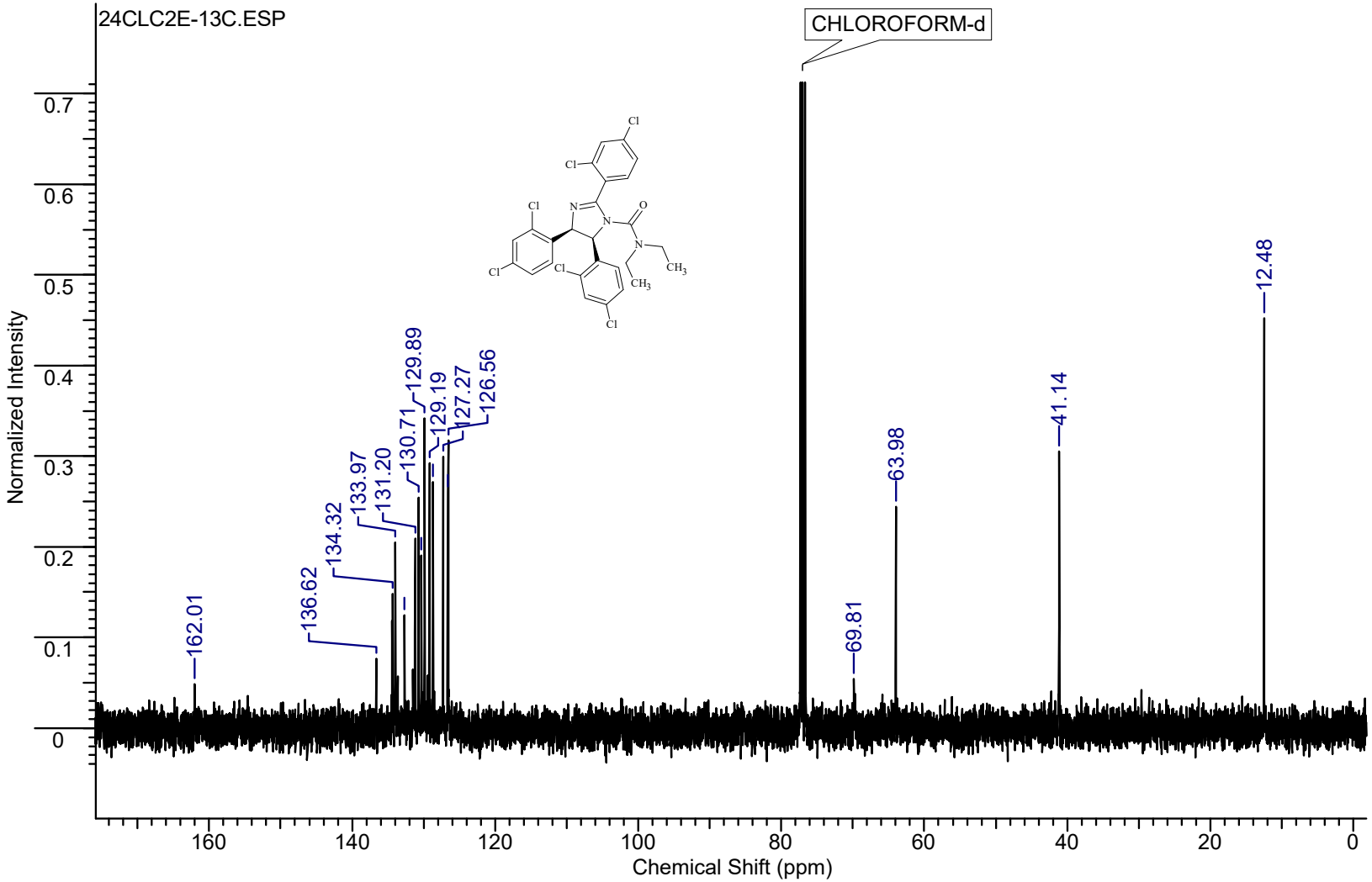
13C NMR Piperidin-1-yl(*cis*-2,4,5-*tris*(4-chlorophenyl)-4,5-dihydro-*1H*-imidazol-1-yl)methanone (2x).



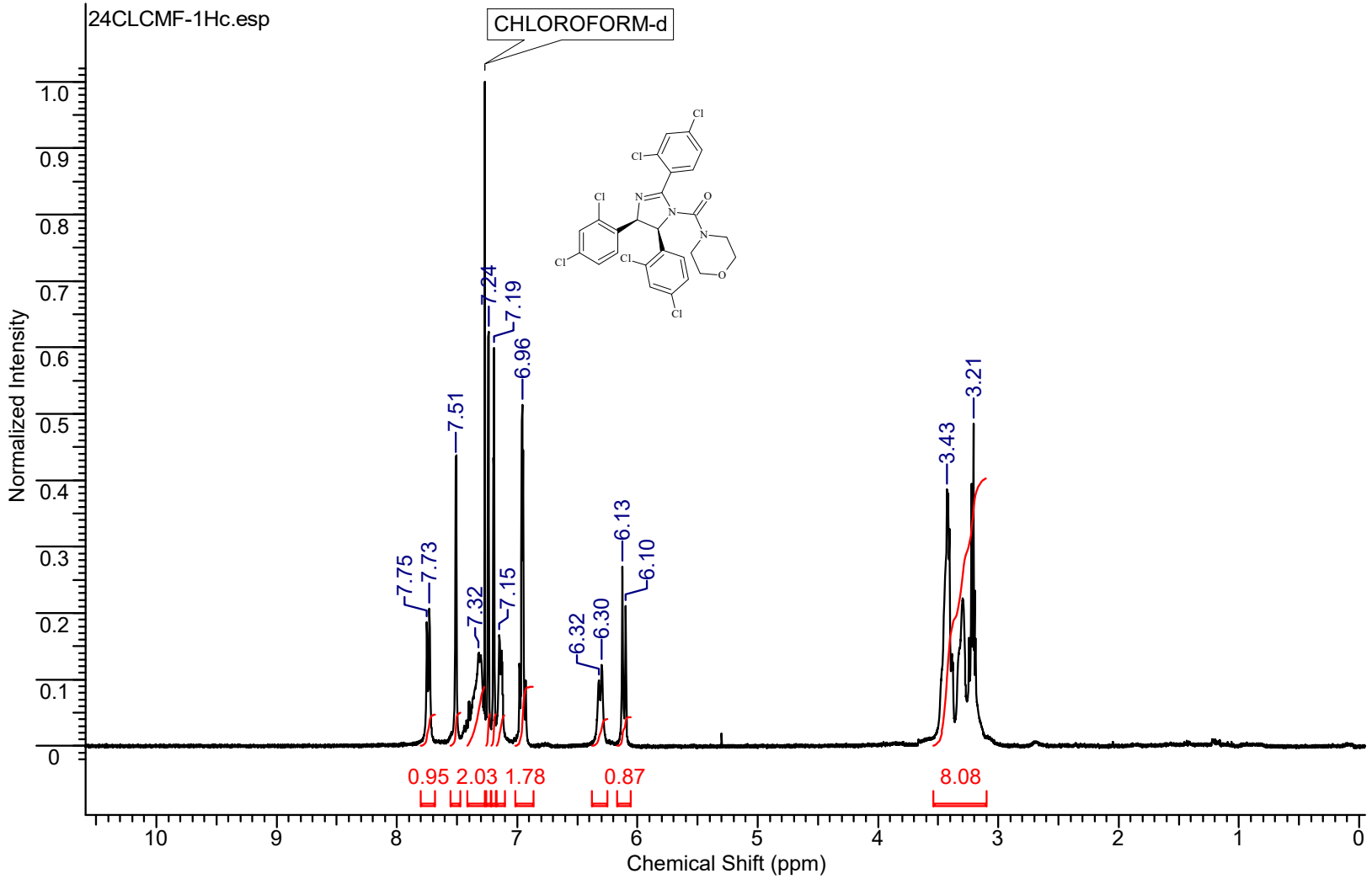
1H NMR *Cis*-*N,N*-diethyl-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2y).



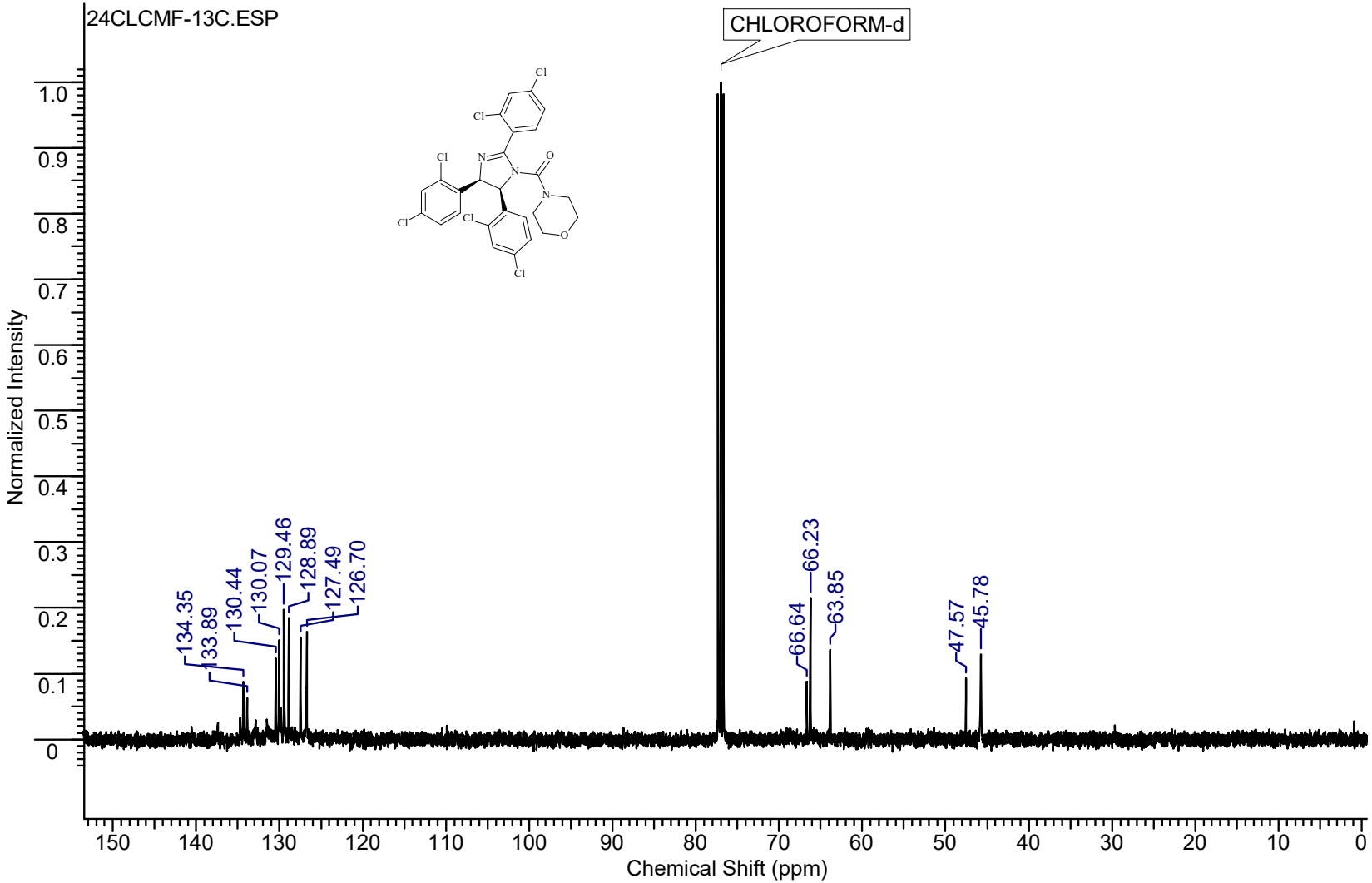
13C NMR *Cis-N,N*-diethyl-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazole-1-carboxamide (2y).



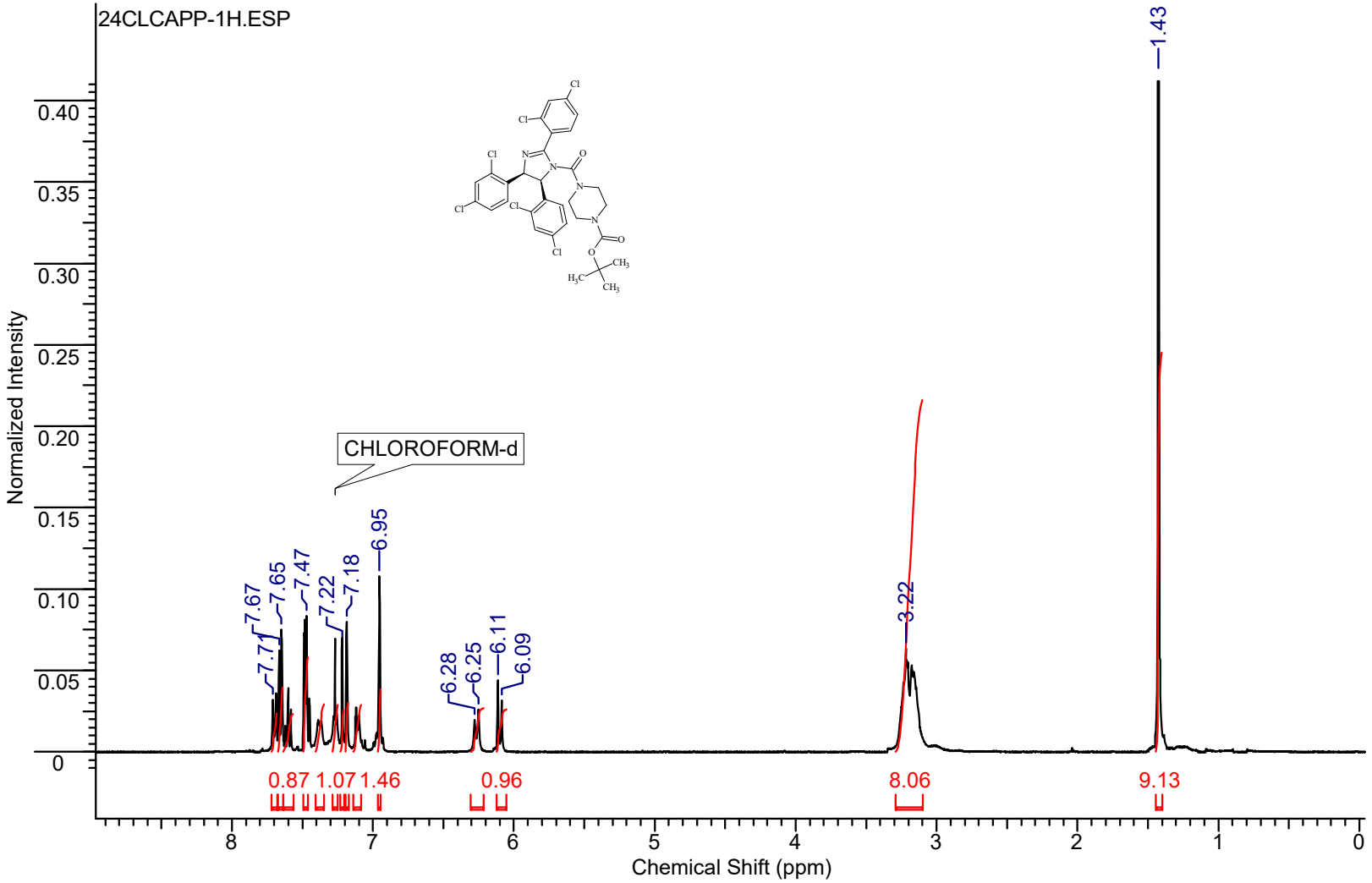
1H NMR 4-{[*Cis*-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2z).



13C NMR 4-{[*Cis*-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazol-1-yl]carbonyl}morpholine (2z).



1H NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2aa).



13C NMR *tert*-Butyl 4-(*cis*-2,4,5-*tris*(2,4-dichlorophenyl)-4,5-dihydro-*1H*-imidazole-1-carbonyl)piperazine-1-carboxylate (2aa).