Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

Concise asymmetric synthesis of new enantiomeric *C*-alkyl pyrrolidines acting as pharmacological chaperones against Gaucher disease

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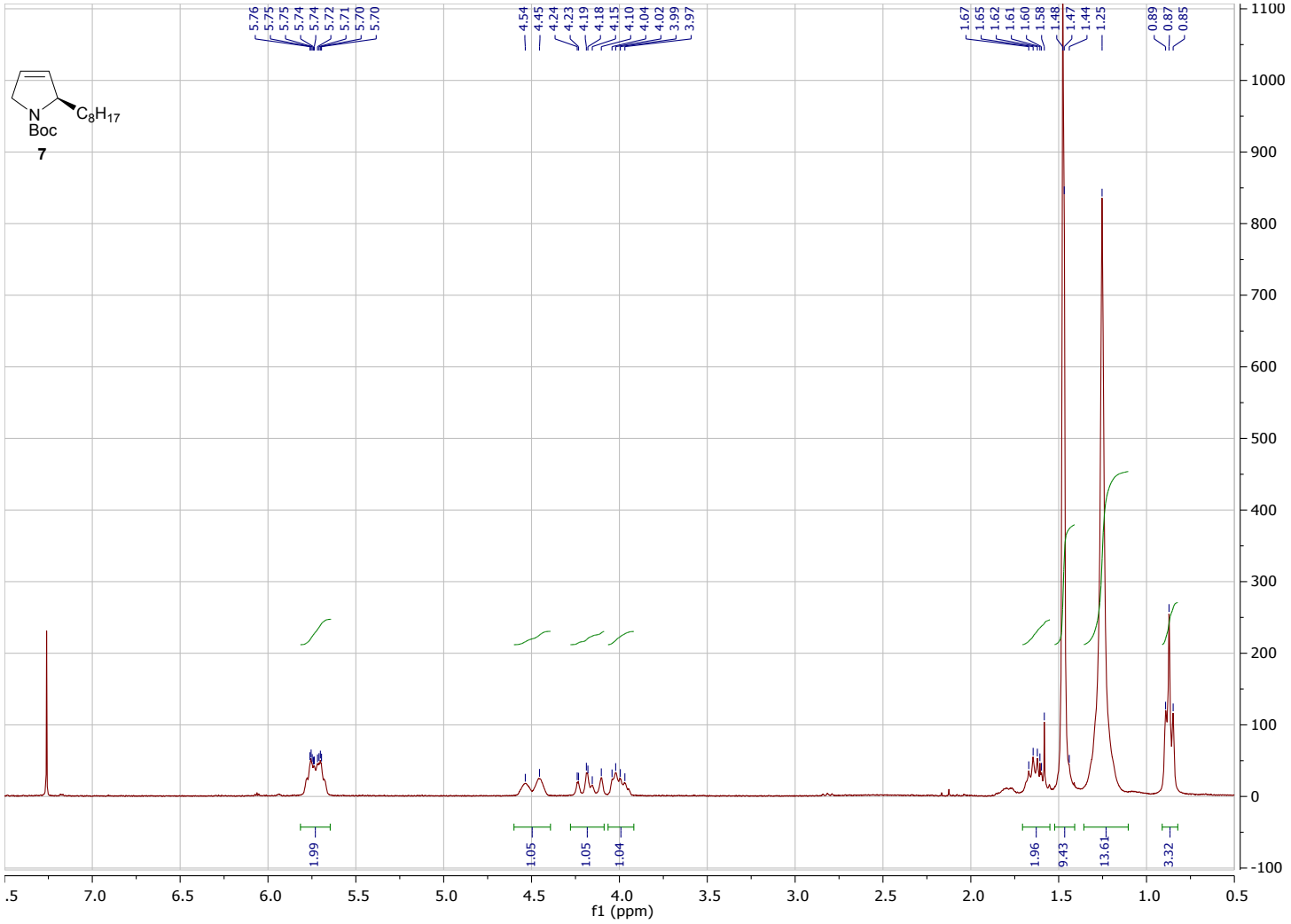
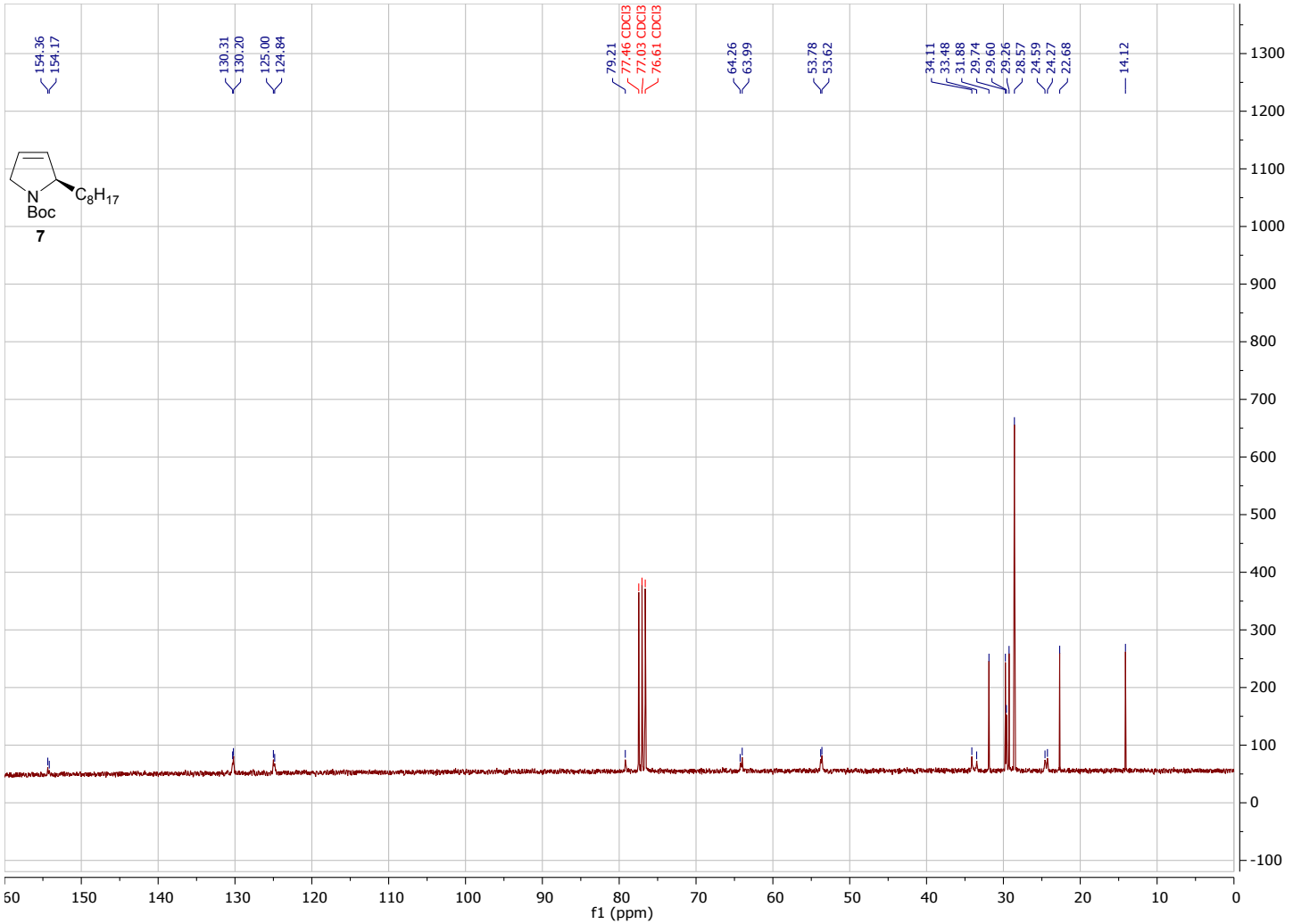
^{1}H and ^{13}C NMR spectra of new compounds

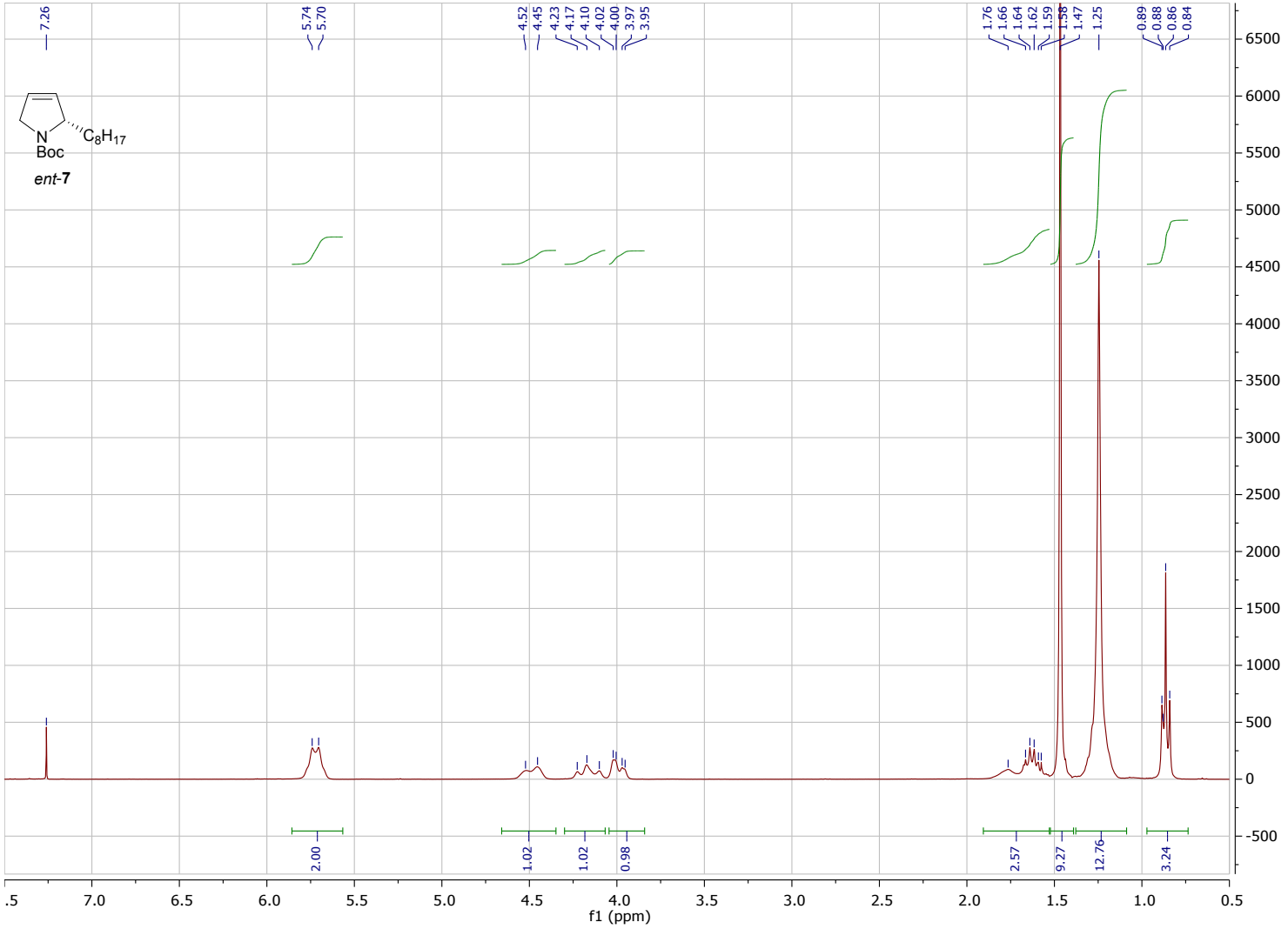
Compound **7**



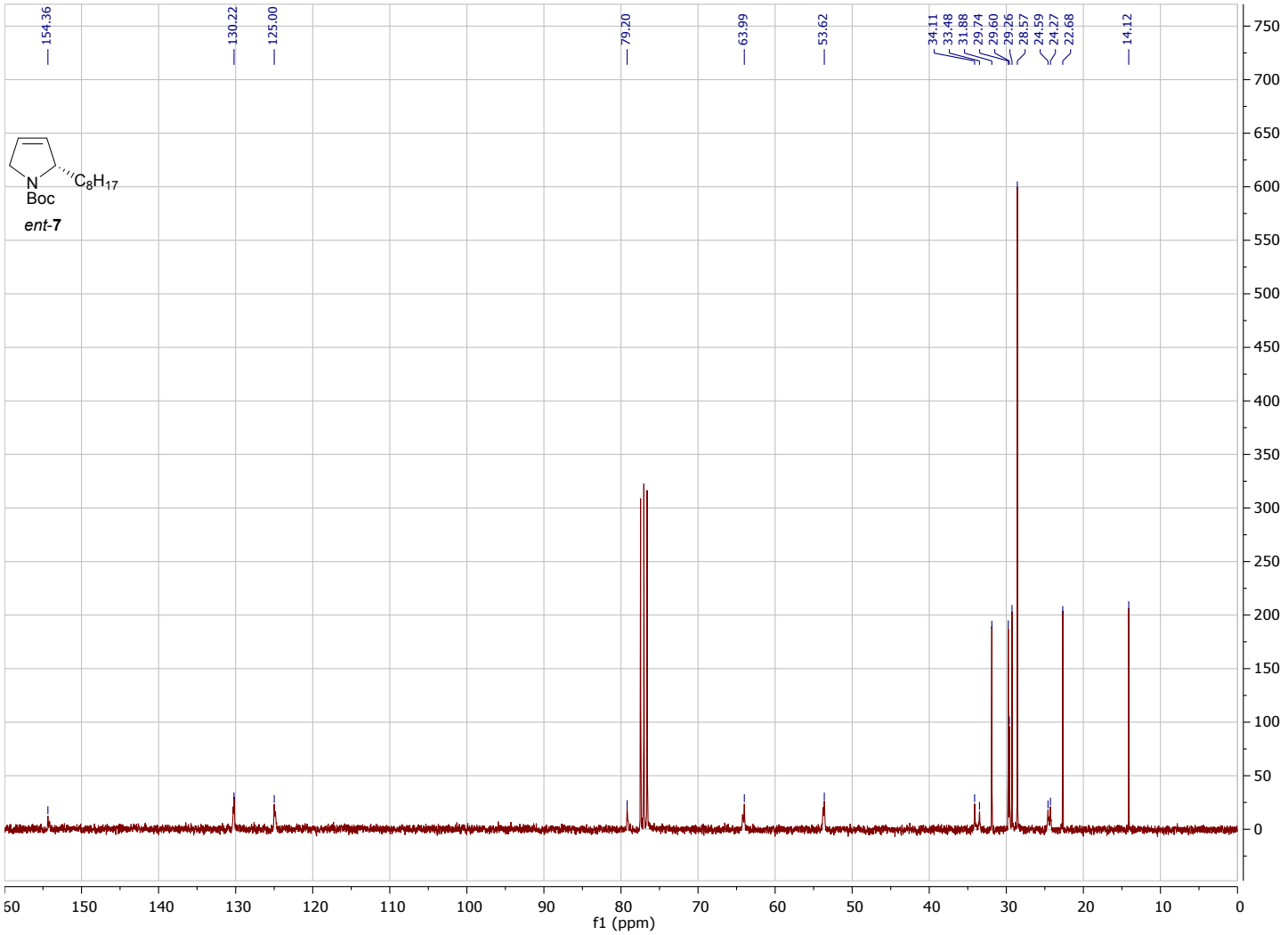
Compound **7**


154.36
154.17
130.31
130.20
125.00
124.84
79.21
77.46 CDCl3
77.03 CDCl3
76.61 CDCl3
64.26
63.99
53.78
53.62
34.11
33.48
31.88
29.74
29.60
29.26
28.57
24.59
24.27
22.68
14.12
N
Boc
C_8H_{17}
7
f1 (ppm)

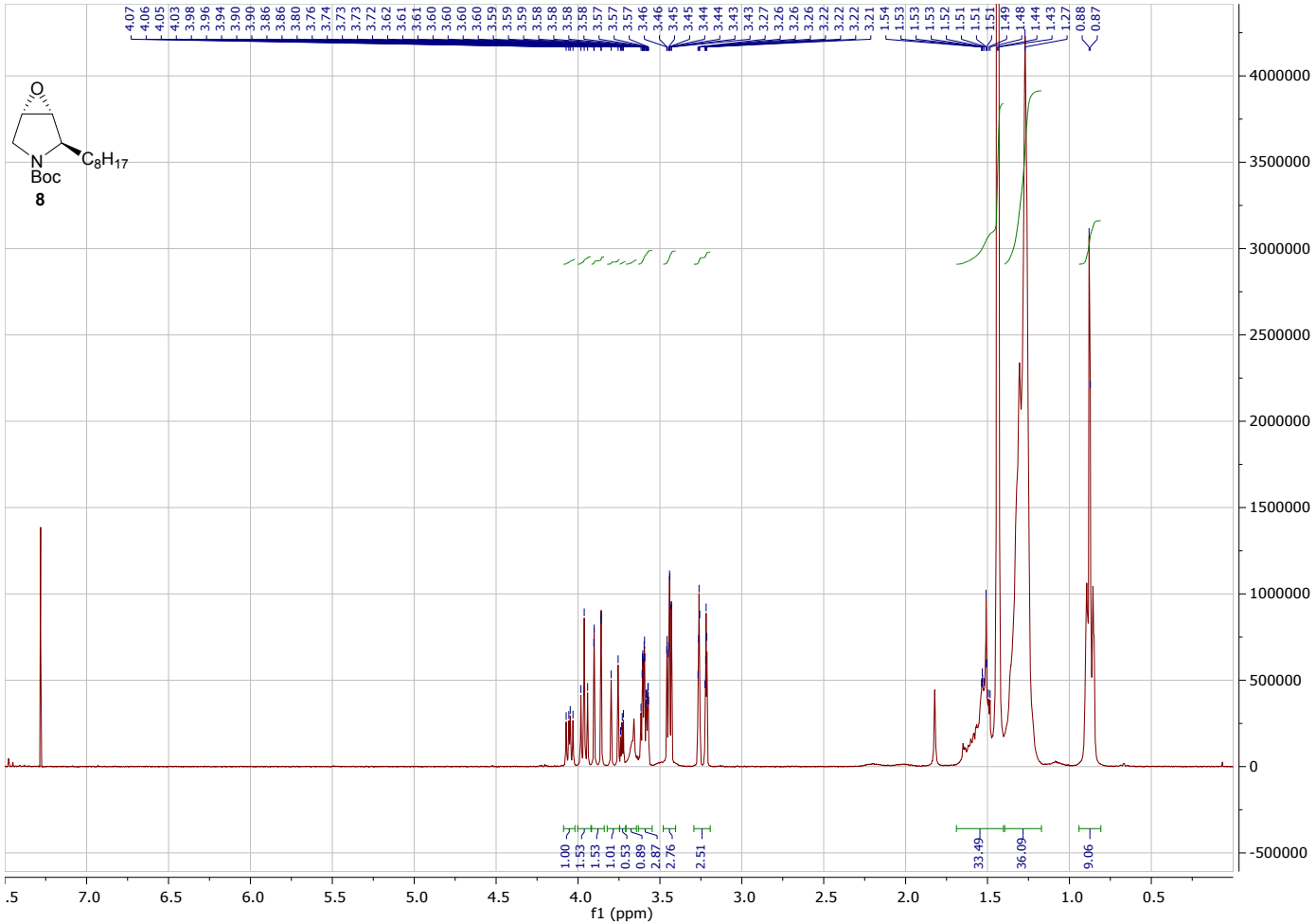
Compound *ent*-**7**



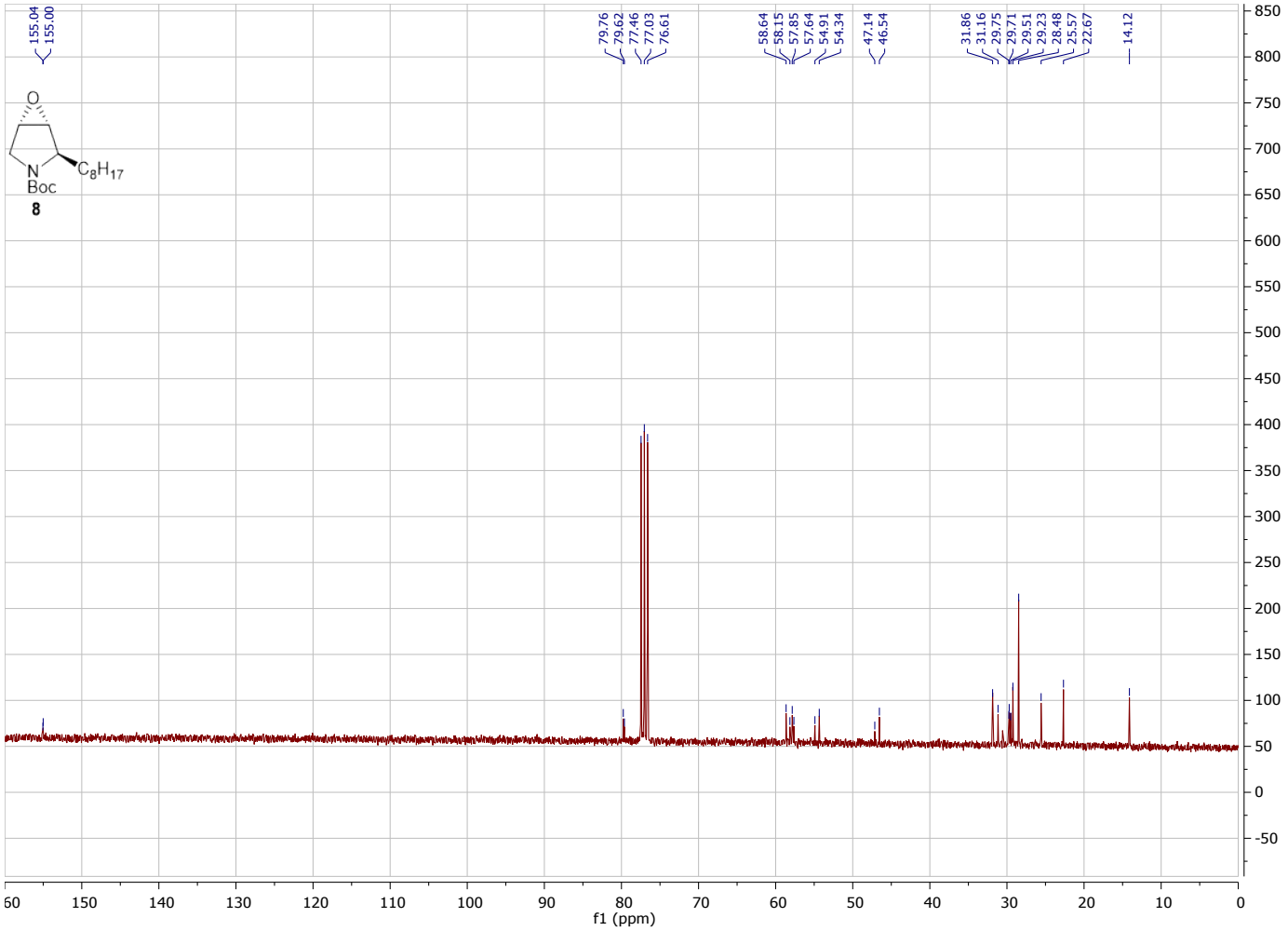
Compound *ent*-**7**



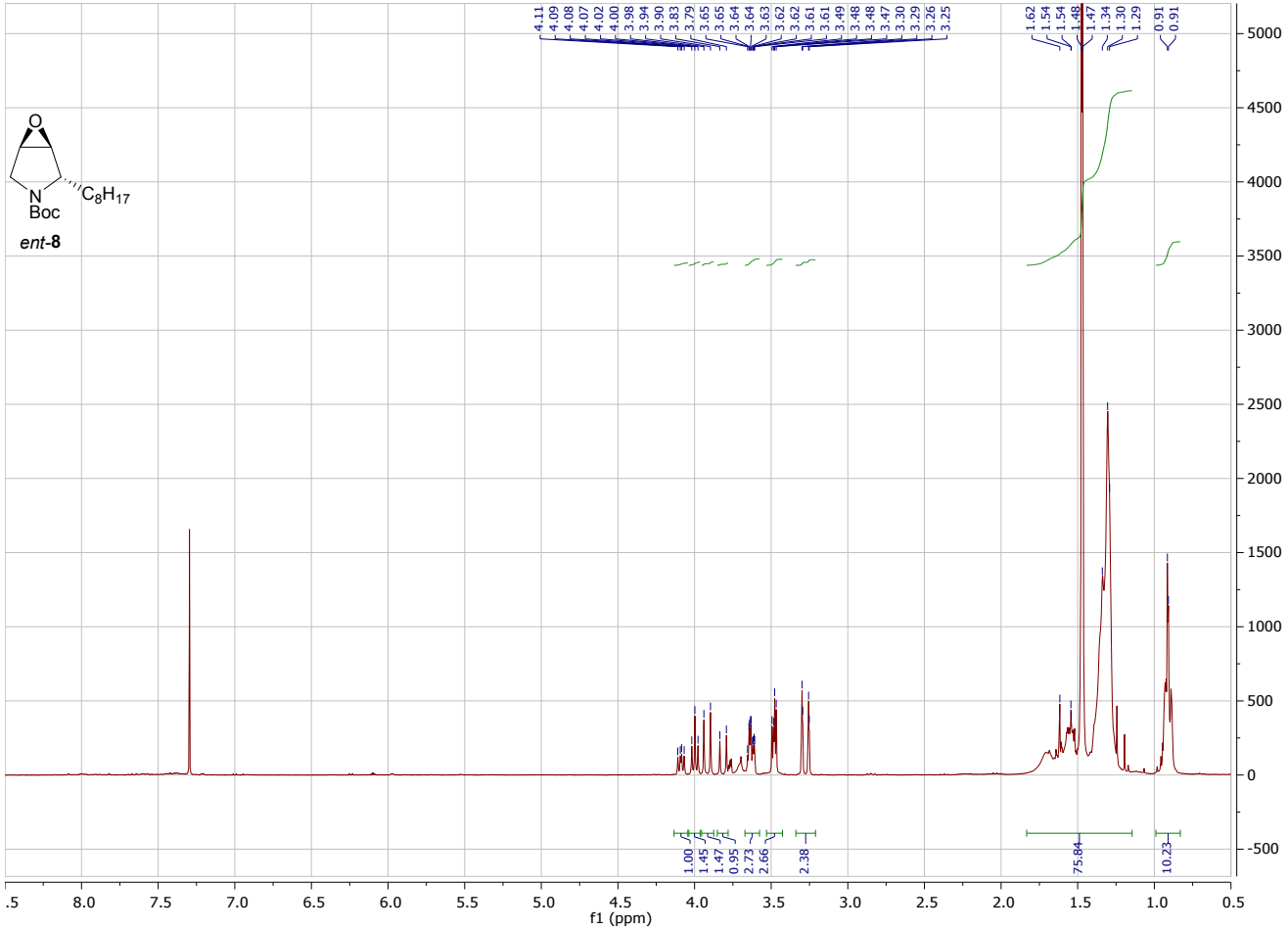
Compound **8**



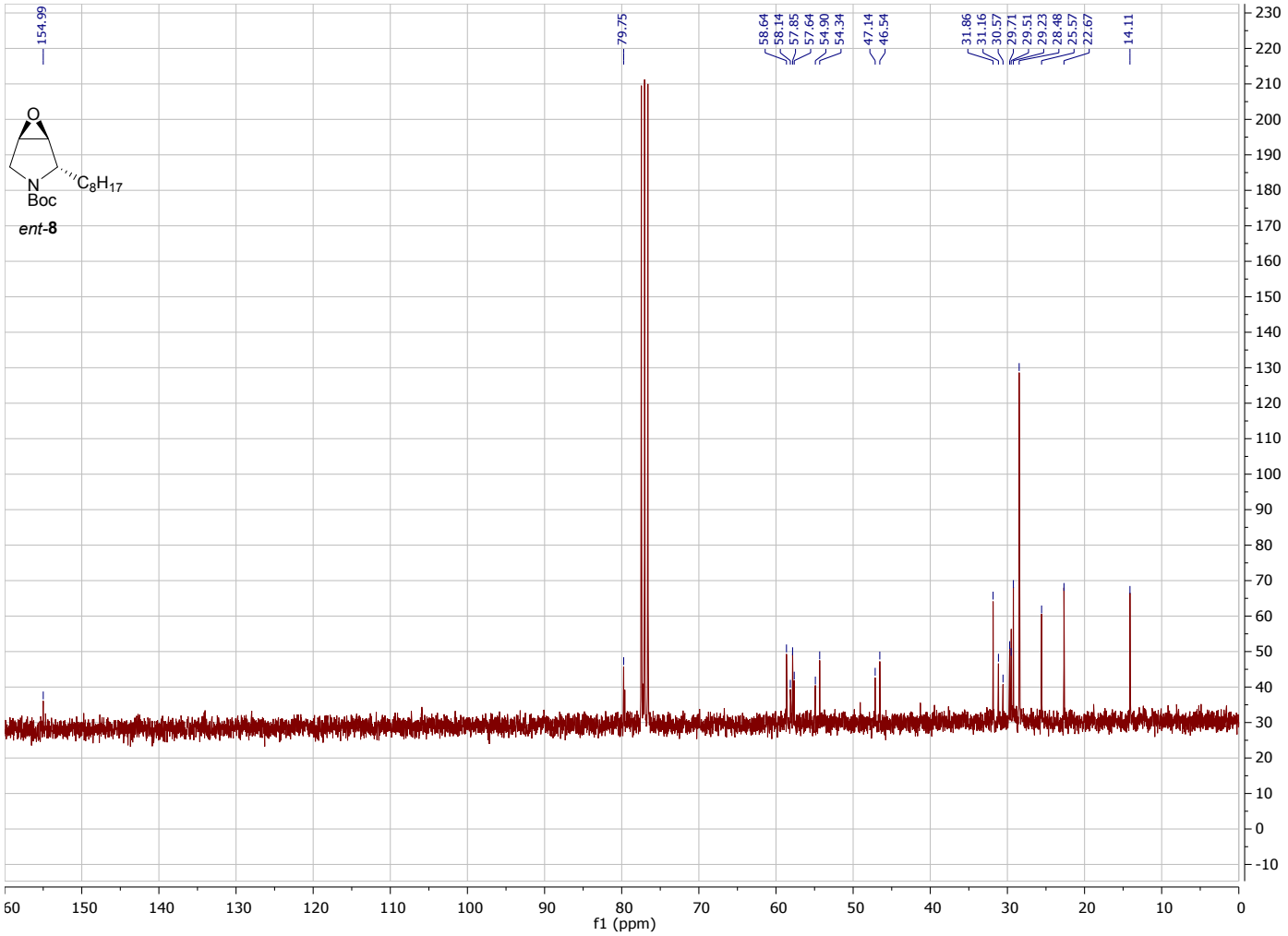
Compound **8**


155.04
155.00
79.76
79.62
77.46
77.03
76.61
58.64
58.15
57.85
57.64
54.91
54.34
47.14
46.54
31.86
31.16
29.75
29.71
29.51
29.23
28.48
25.57
22.67
14.12
O
N
Boc
C_8H_{17}
8
f1 (ppm)

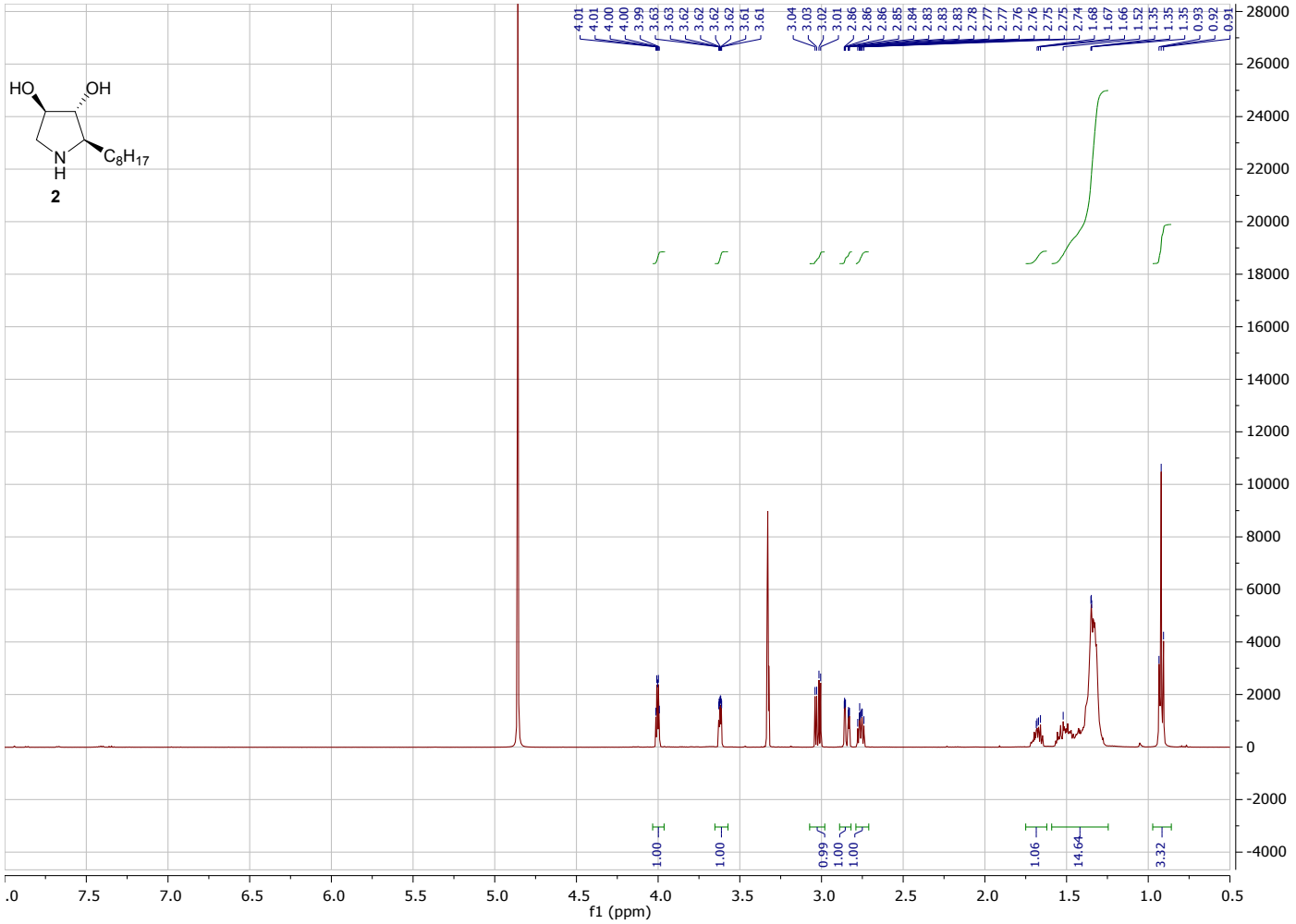
Compound *ent*-**8**



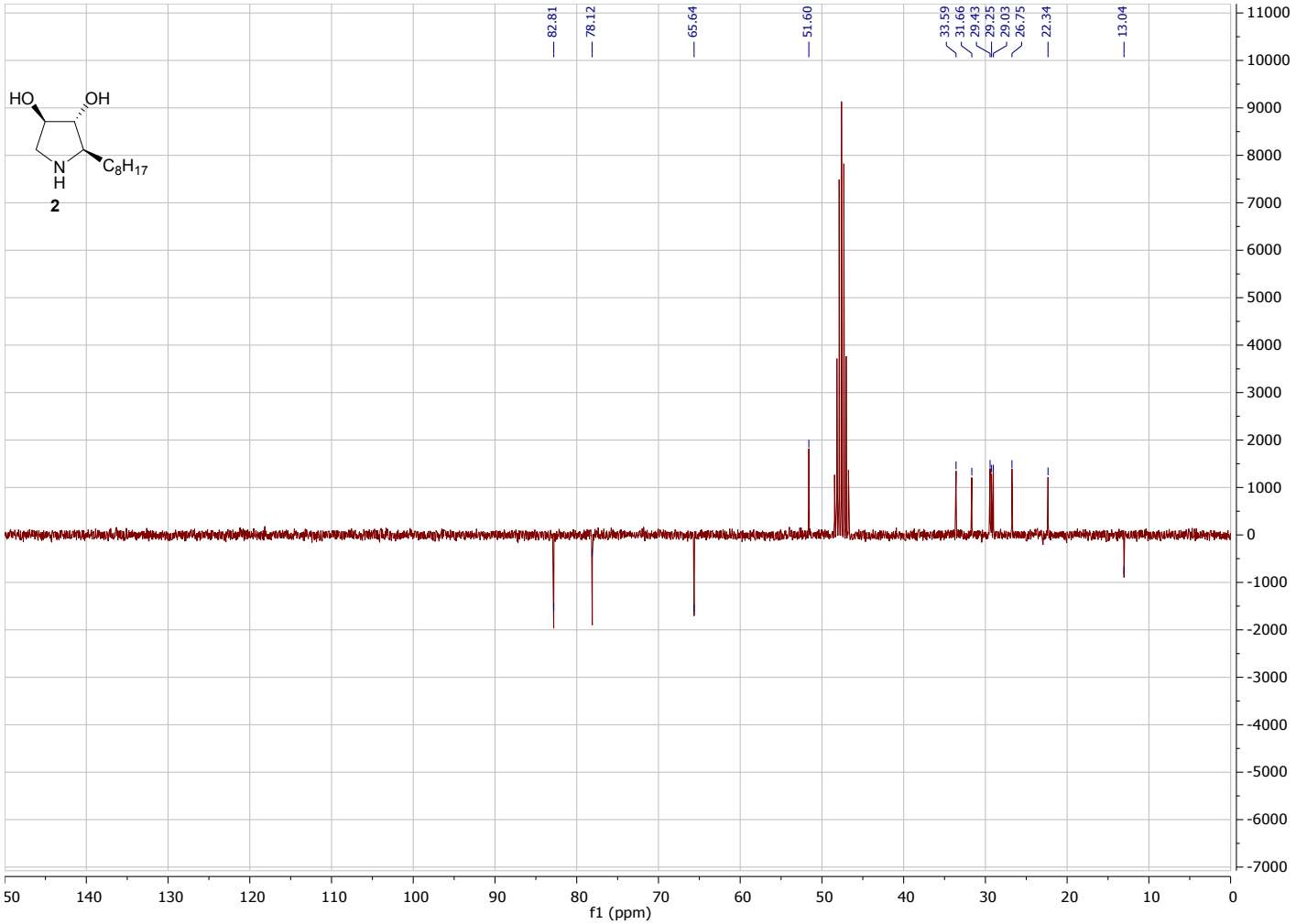
Compound *ent*-**8**



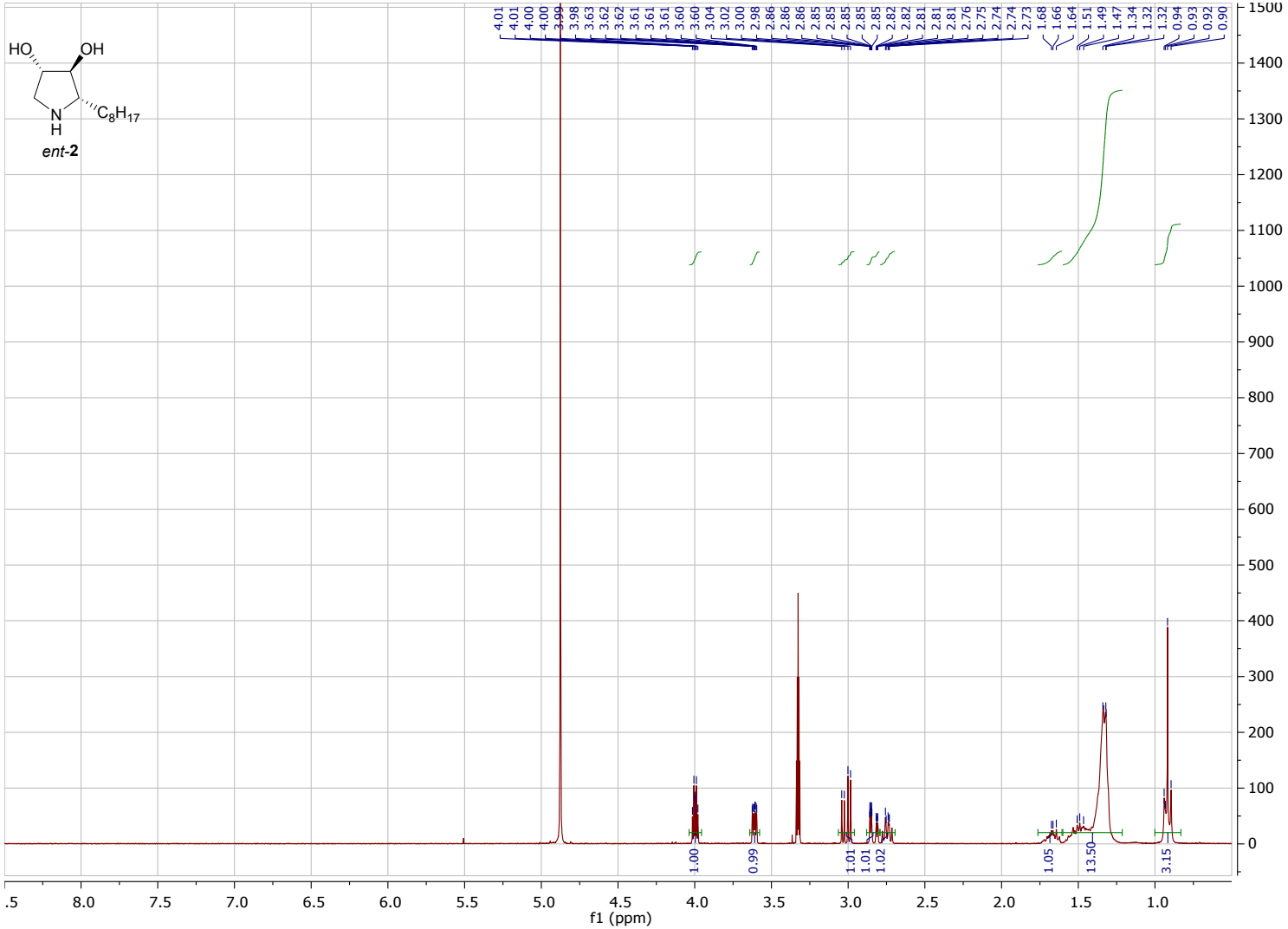
Compound **2**



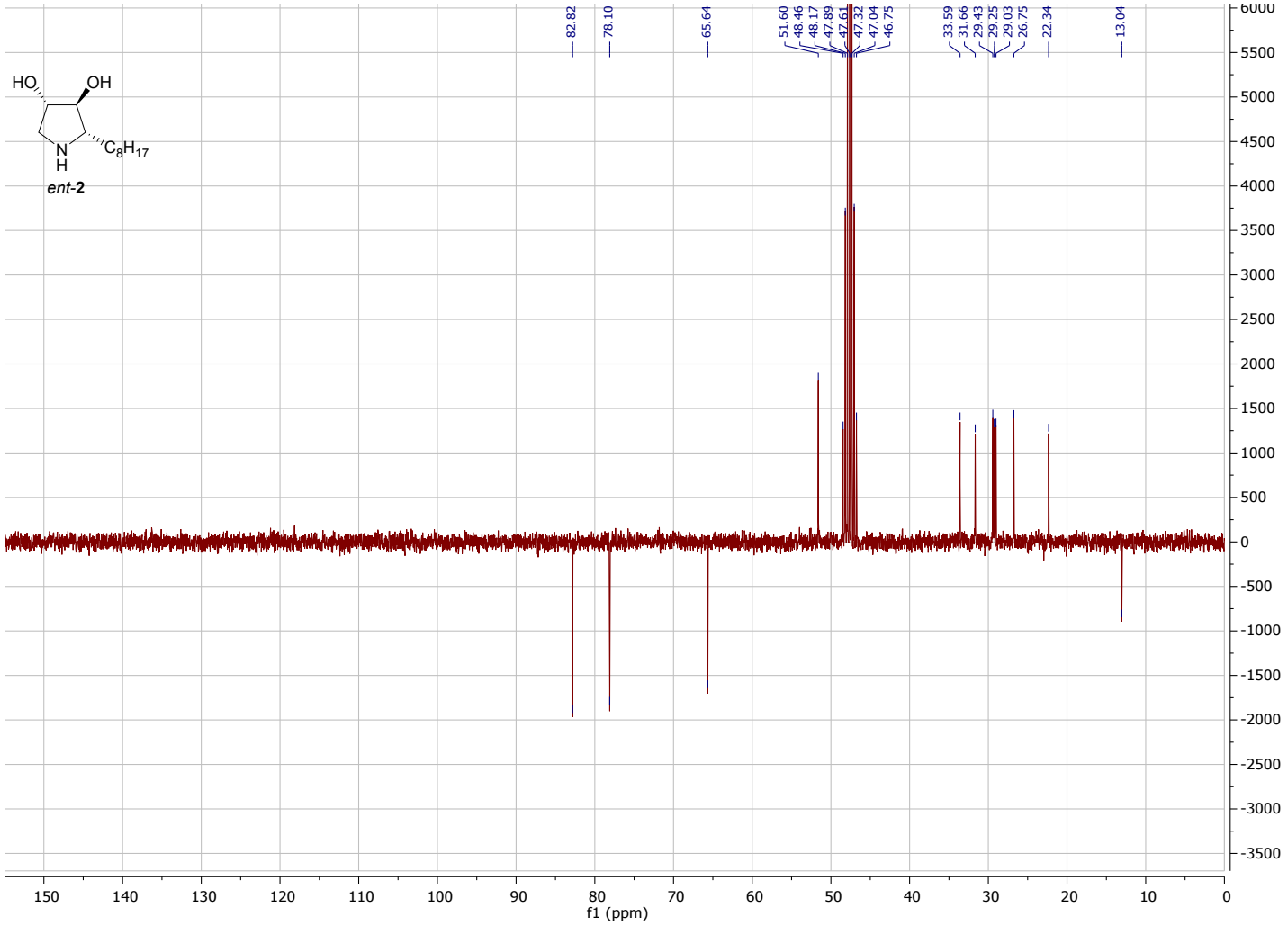
Compound **2**


82.81
78.12
65.64
51.60
33.59
31.66
29.43
29.25
29.03
26.75
22.34
13.04
HO
OH
N
H
C8H17
2
f1 (ppm)

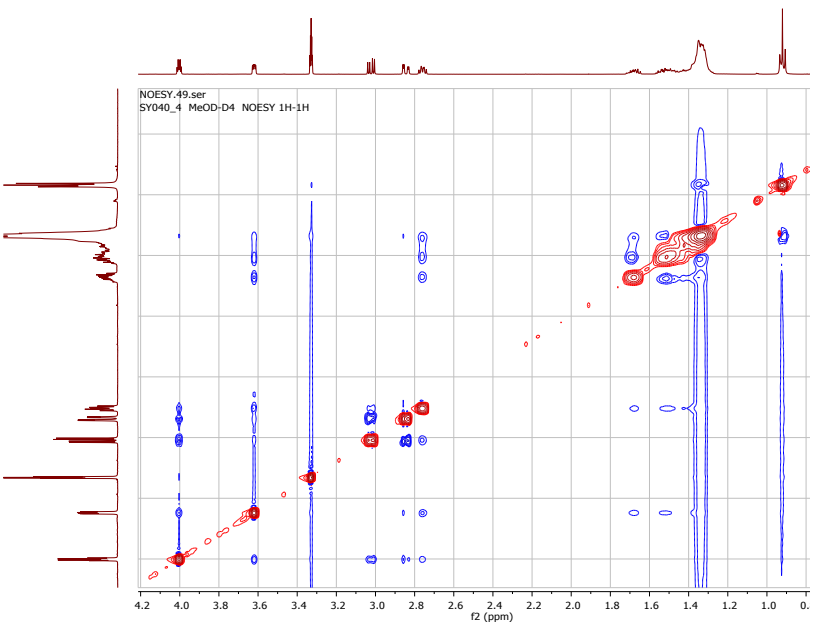
Compound *ent*-**2**

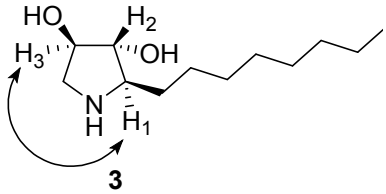


Compound *ent*-**2**



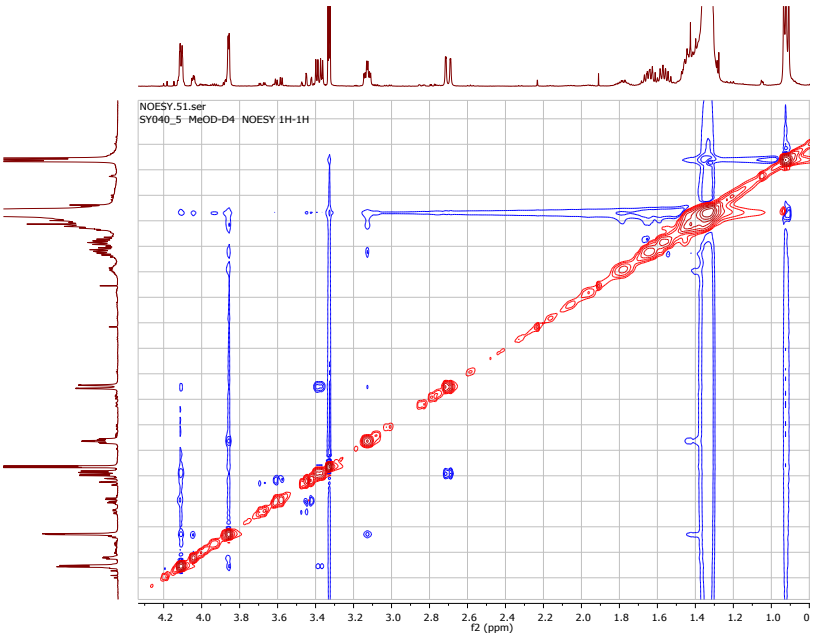
NOESY NMR analyses of compounds 2 and 3 to determine relative configuration of C1, C2, C3 carbons

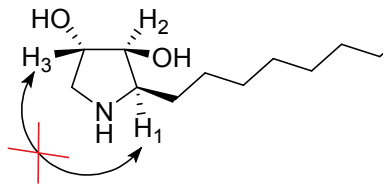




3

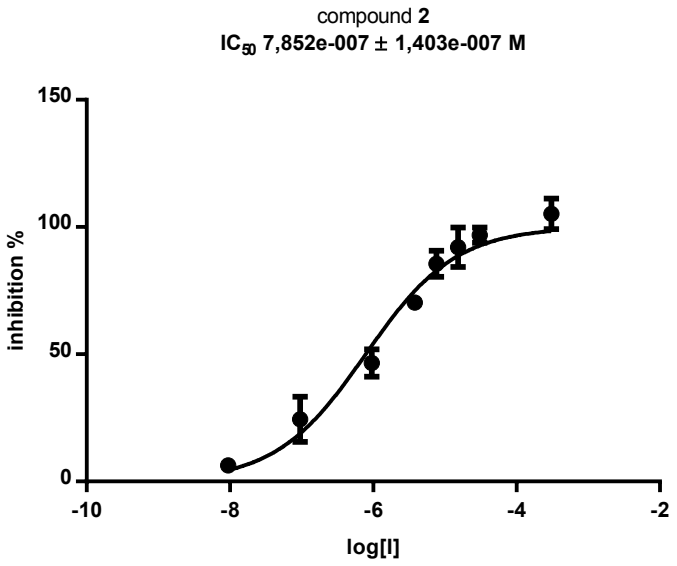
correlation between H3 (4.02 ppm) and H1 (2.75 ppm)

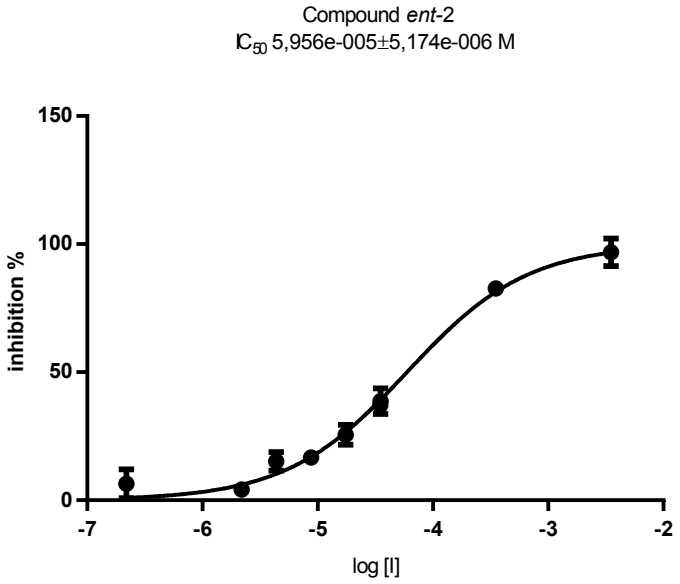




no correlation between H3 (4.08 ppm) and H1 (3.08 ppm)

Dose-response curves for compounds 2 and *ent*-2 *versus* β-GCase

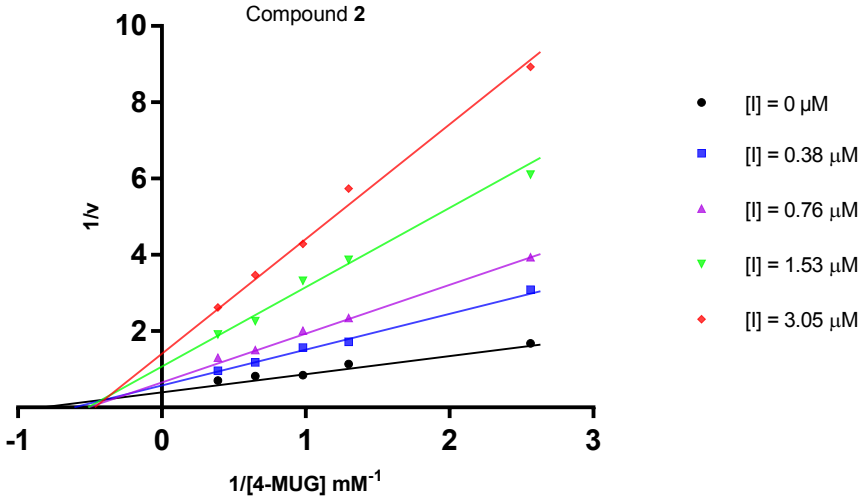




Lineweaver-Burk plots for compounds 2 and *ent*-2 versus β-GCase

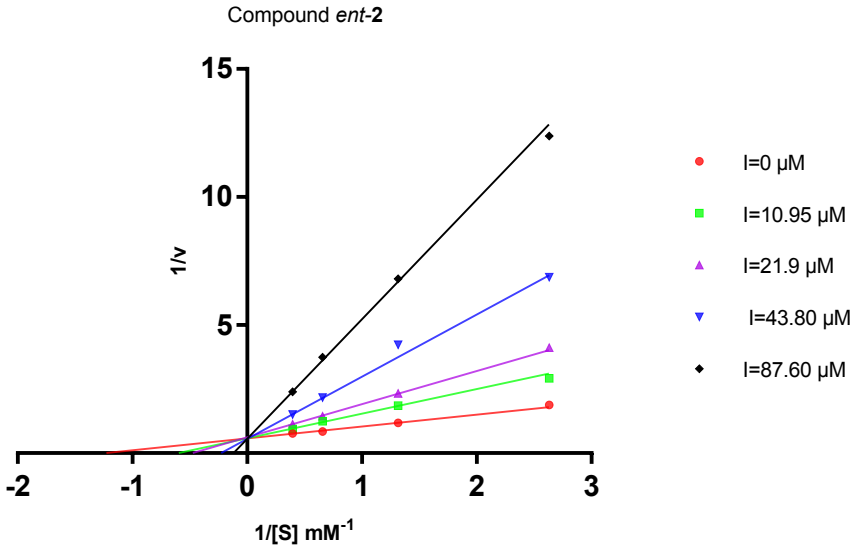
Compound 2

Non-competitive inhibition with Ki=0.40 µM



Compound *ent*-2

Competitive inhibition with Ki=6.87 µM



Best poses of compound 2 obtained with GPU docking protocol

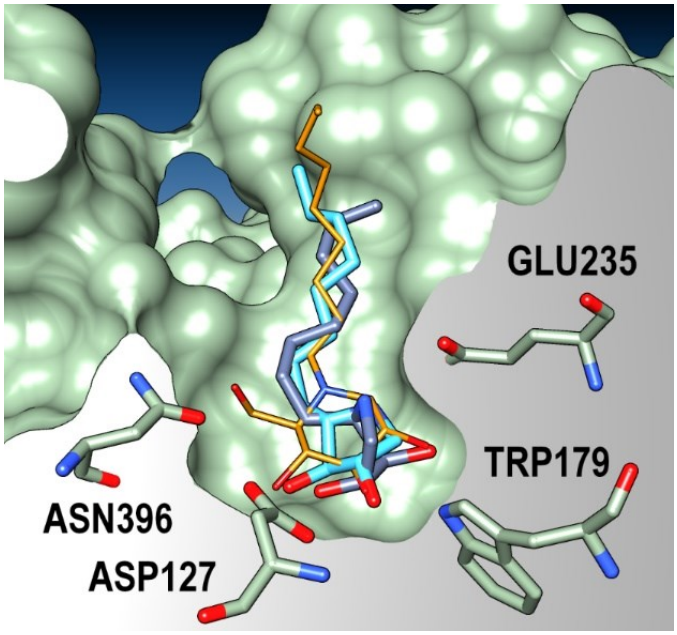
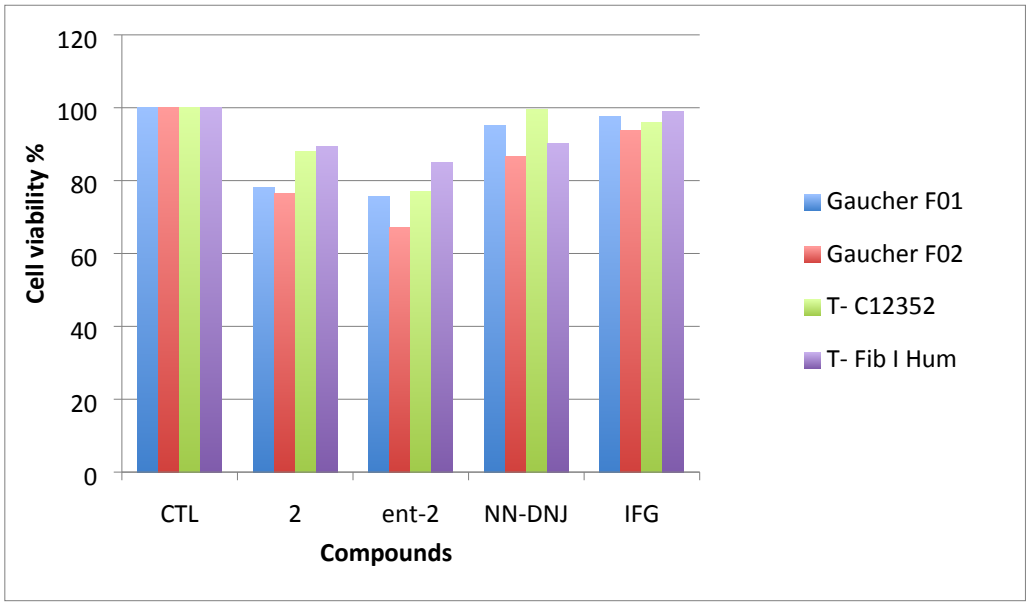


Figure 1. Comparison of NN-DNJ (orange) with best poses (P1-OPT docking protocol) of *ent*-**2** (cyan) and **2** (dark blue) compounds showing the discrepancies of conformations of the heterocycle.

MTT Test for compounds 2, *ent*-2, NN-DNJ, IFG at 30 µM



Cultured primary skin fibroblasts from control individuals and patients affected with Gaucher

Cultured primary skin fibroblasts from control individuals and patients affected with Gaucher disease (carrying the N370S mutation) were obtained from the Laboratoire de Biochimie Métabolique, CRB, IFB, CHU Toulouse, France and CBC Biotec biobank BB-0033-00046 (3809 F01 and 1541 F02 cell lines), Hospices Civils de Lyon, France. Cells were immortalized after tranfection with a plasmid encoding the SV40 large T antigen. Cells were routinely cultured in DMEM medium supplemented with 10% inactivated foetal calf serum. After incubation for 72 h in the presence of the chemical compounds or vehicle (ethanol) only, cell viability was evaluated using the MTT test as reported earlier.[1] Briefly, cells were plated in 24-well plates (30,000 cells/well) and at 50% confluency cells were incubated with fresh medium supplemented with 30 µM of compounds. After 72 h, 500 µg of MTT was added to each well. After 2 h of incubation at 37 ◦C, supernatants were discarded, and the remaining material was dissolved by 1 mL of DMSO. After 30 min, absorbance was measured at 560 nm by using a microplate reader. Background values were subtracted from all other values and viability was expressed as percentage compared with untreated controls.

[1] Wenger, D.A.; Williams, C. Techniques in Diagnostic Human Biochemical Genetics: A Laboratory Manual; Hommes, F.A., Ed.; Wiley-Liss Inc.: New York, NY, USA, 1991; pp. 587–617.