

Characterization of the different compounds

Figure S1. ^1H -NMR spectrum of 5 α -cholestan-3 β -ol **1**.

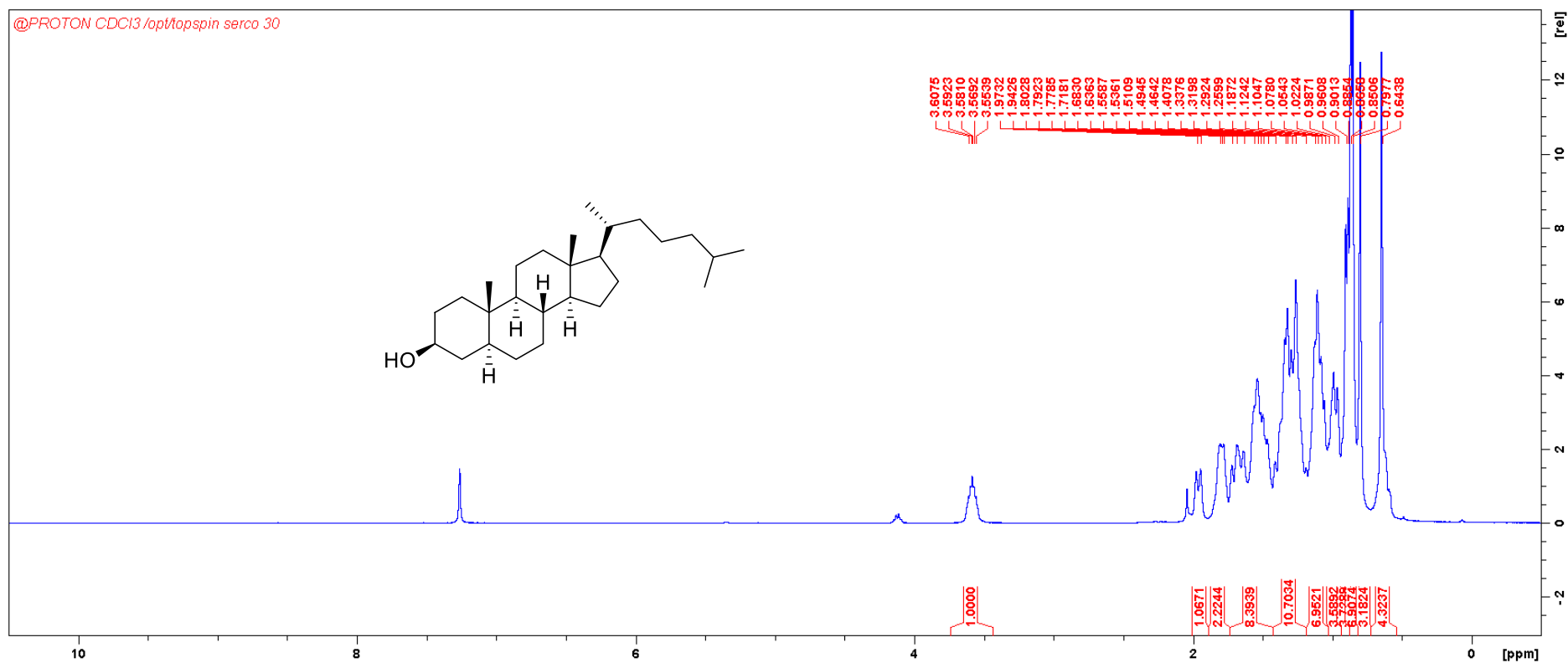


Figure S2. ^{13}C -NMR spectrum of 5 α -cholestan-3 β -ol **1**.

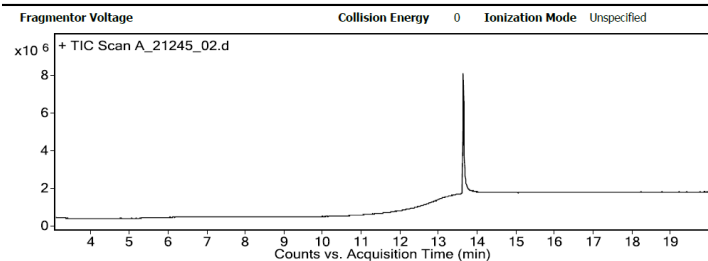


Figure S3. GC-MS analysis of 5 α -cholestan-3 β -ol **1**.

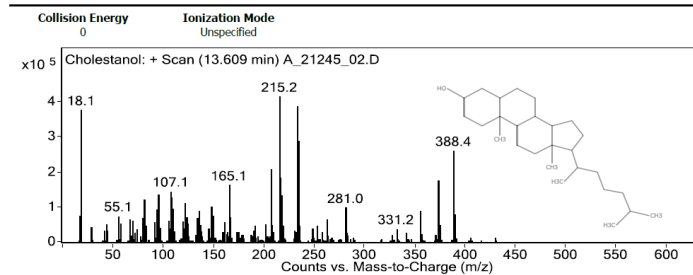
Qualitative Analysis Report

Data Filename	A_21245_02.D	Sample Name	befl002HF4
Sample Type		Position	3
Instrument Name		User Name	
Acq Method	AD_EI_CH2Cl2_300C.M	Acquired Time	9/2/2021 11:40:09 AM
IRM Calibration Status	Not Applicable	DA Method	AD_Method.m
Comment			
Expected Barcode		Sample Amount	
Dual Inj Vol	2	TuneName	etune20210902.u
TunePath	D:\MassHunter\GCMS\1\5977	MSFirmwareVersion	6.00.21
Acquisition Time #2	2021-09-02 09:40:09Z	OperatorName	
RunCompletedFlag	True	Acquisition SW Version	MassHunter GC/MS Acquisition 8.07.01.1805 12-Mar-2014 Copyright © 1989-2014 Agilent Technologies, Inc.

User Chromatograms

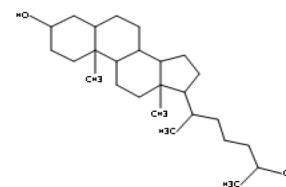


User Spectra



Spectrum Structure
Cholestanol

Qualitative Analysis Report



NIST Score : 85.2%

--- End Of Report ---

Figure S4. ^1H -NMR spectrum of 3 α ,5 α -chloroacetylamincholestane **5**.

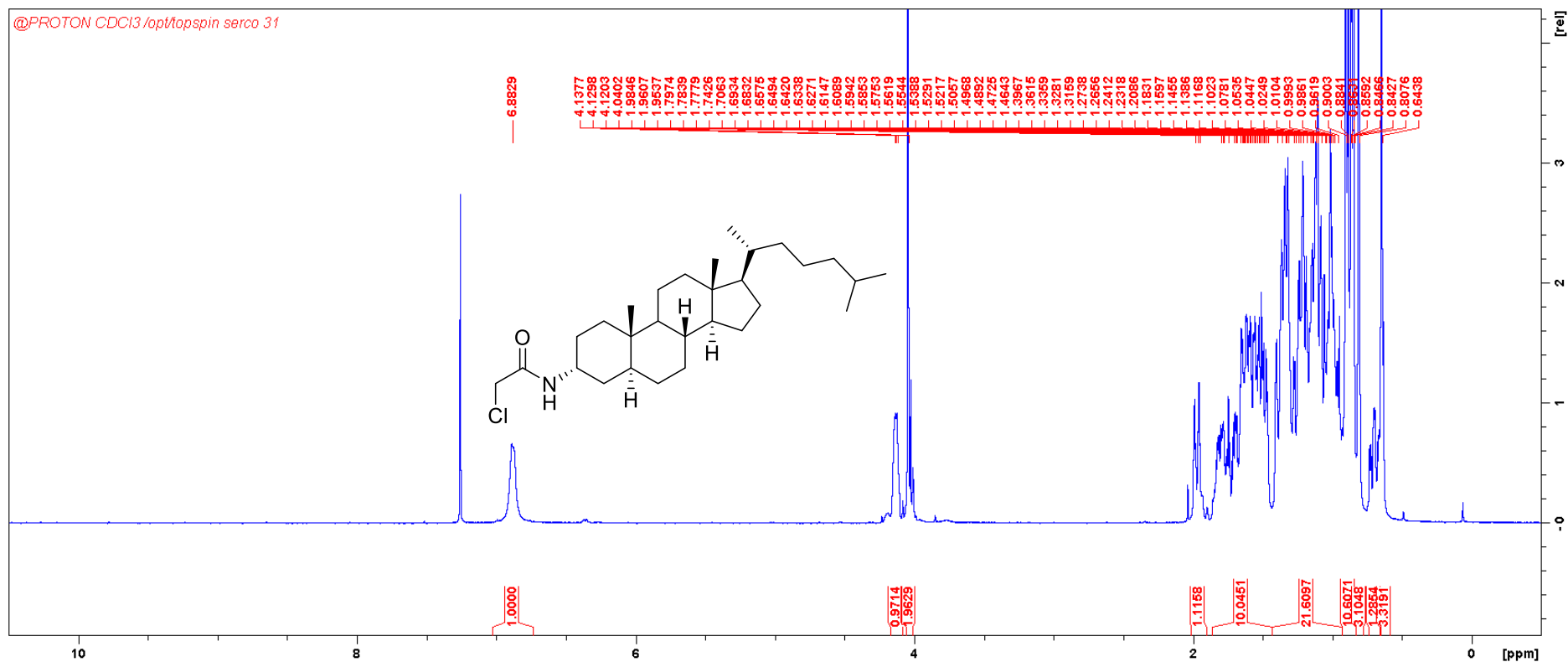


Figure S5. ^{13}C -NMR spectrum of 3 α ,5 α -chloroacetylamincholestane **5**.

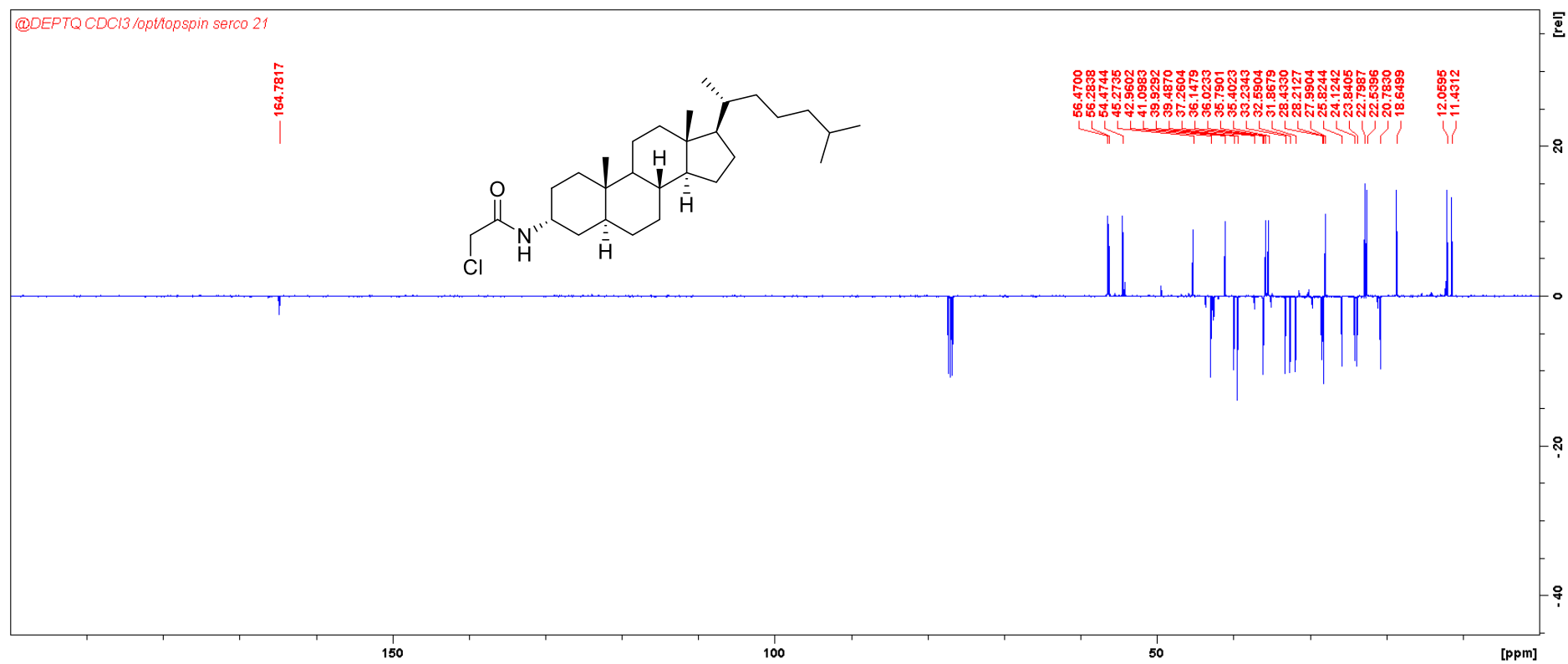


Figure S6. X-ray analysis of 3 α ,5 α -chloroacetylamincholestane **5**

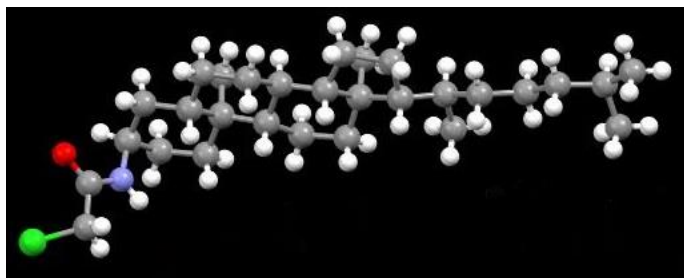


Figure S7. ^1H -NMR spectrum of 3 α ,5 α -piperidinoacetylamincholestane **6**.

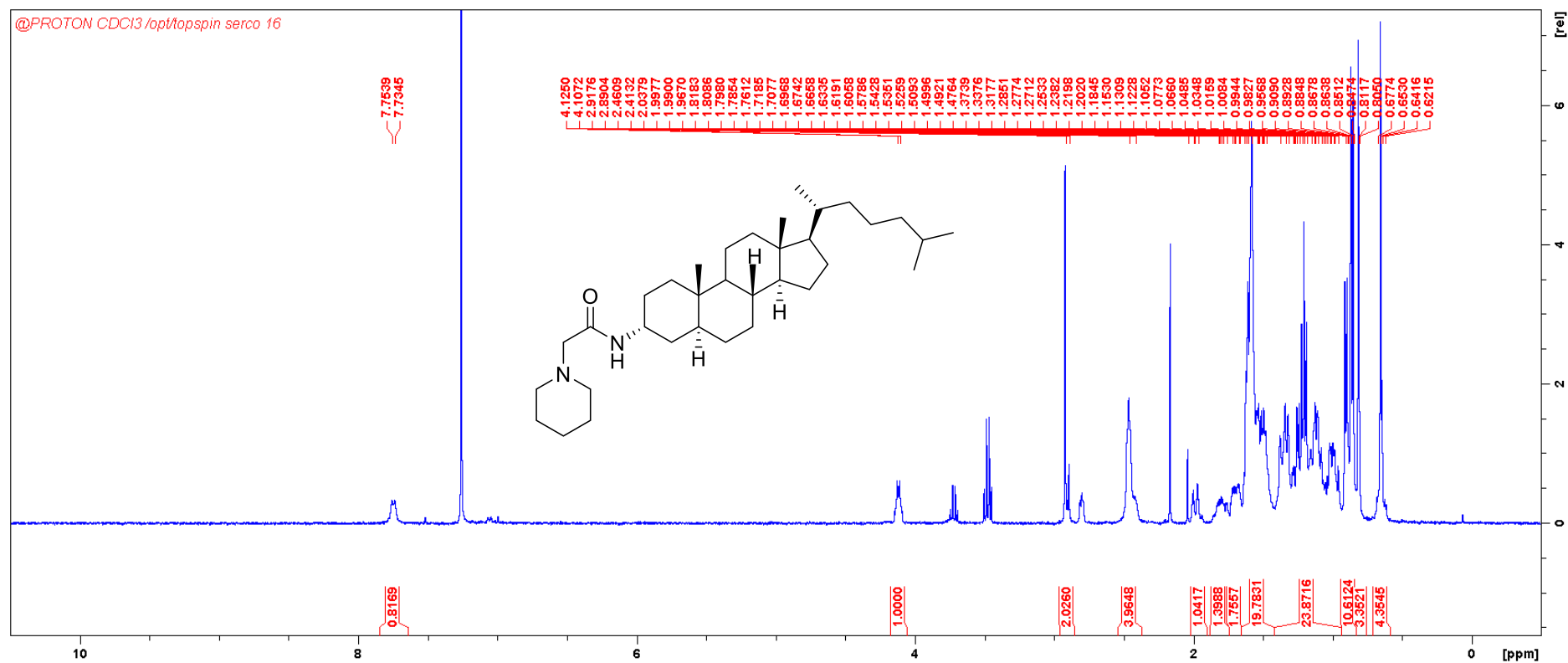


Figure S8. ^{13}C -NMR spectrum of 3 α ,5 α -piperidinoacetylamincholestane **6**.

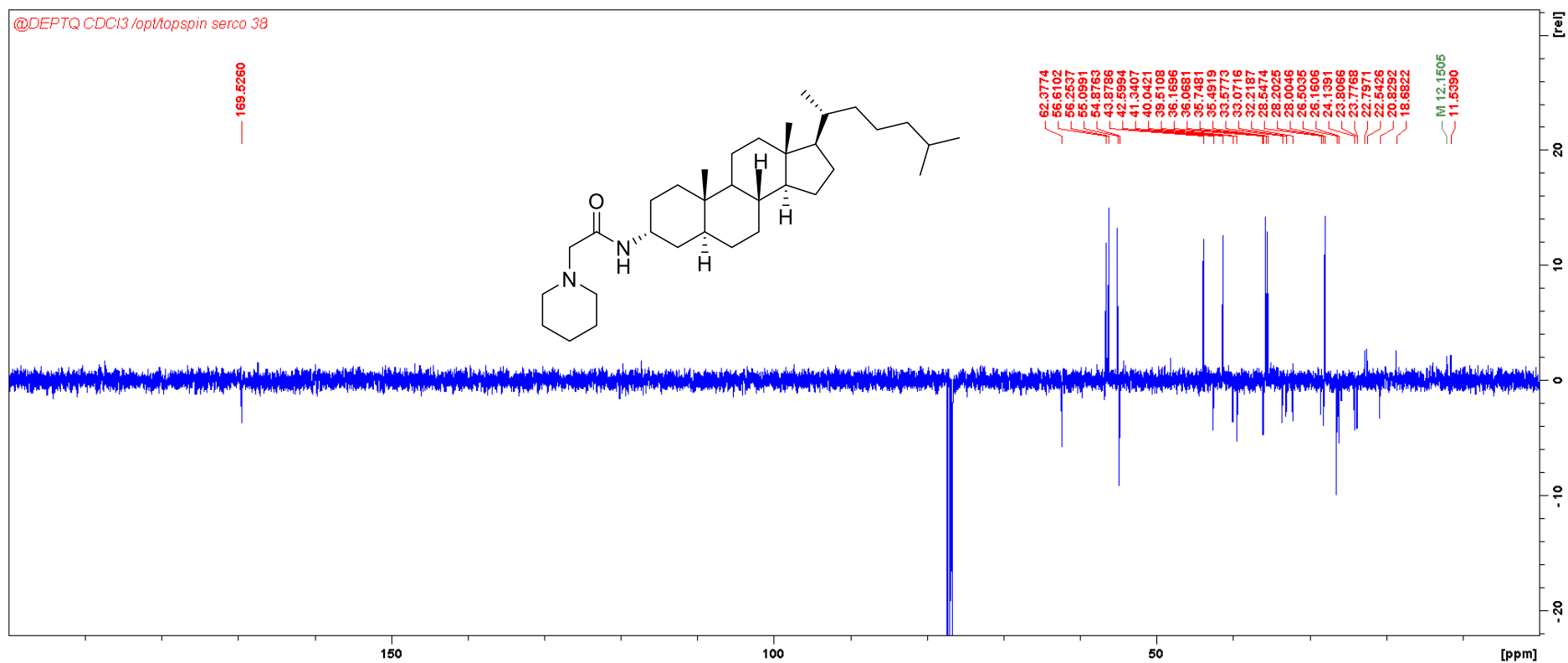


Figure S9. ^1H -NMR spectrum of IMC-48.

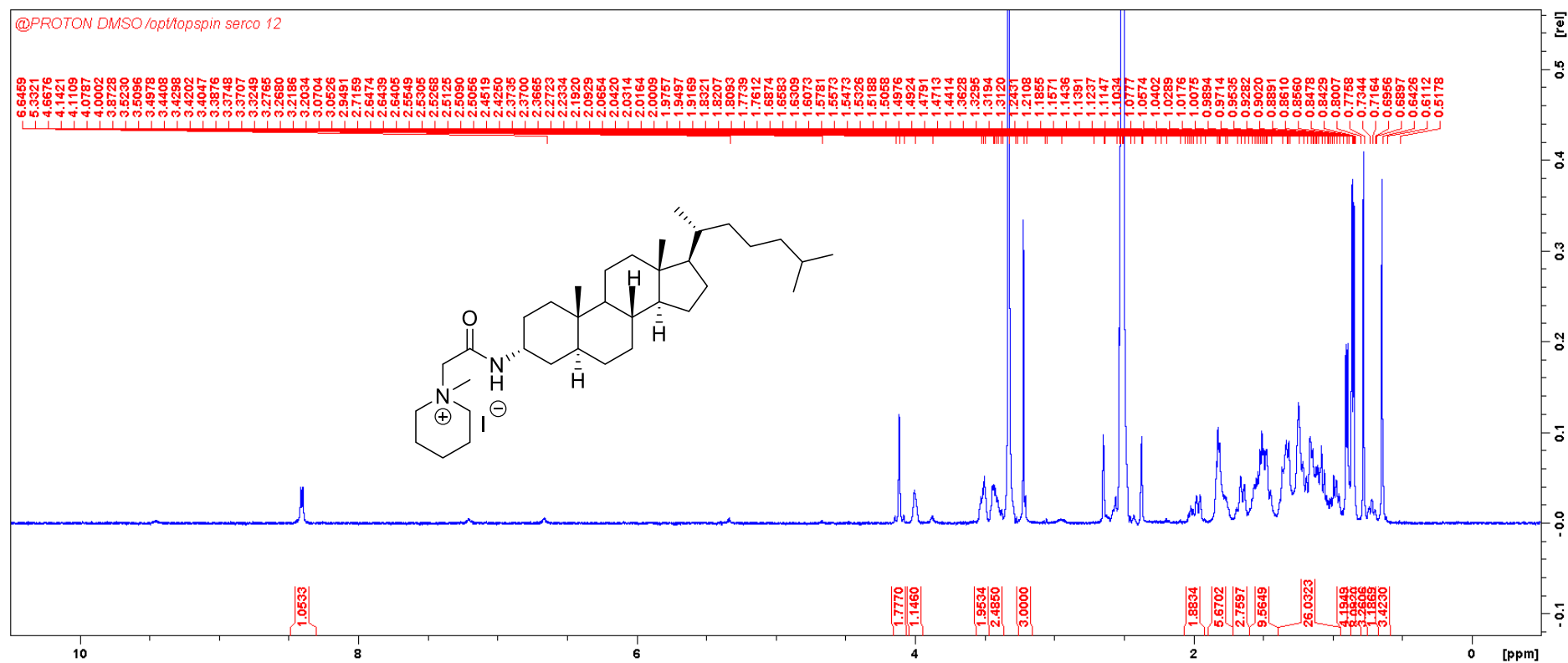


Figure S10. ^{13}C -NMR spectrum of IMC-48

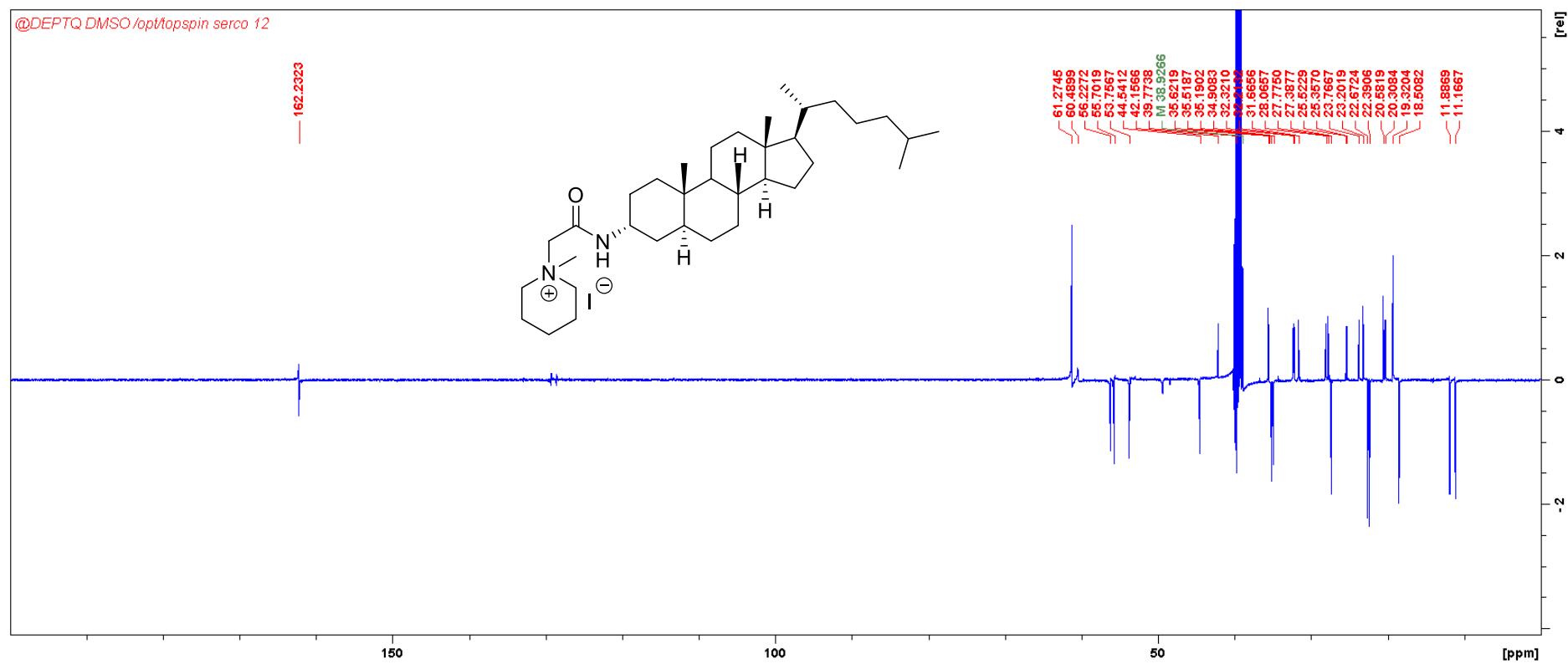


Figure S11. HPLC-MS analysis of **IMC-48**

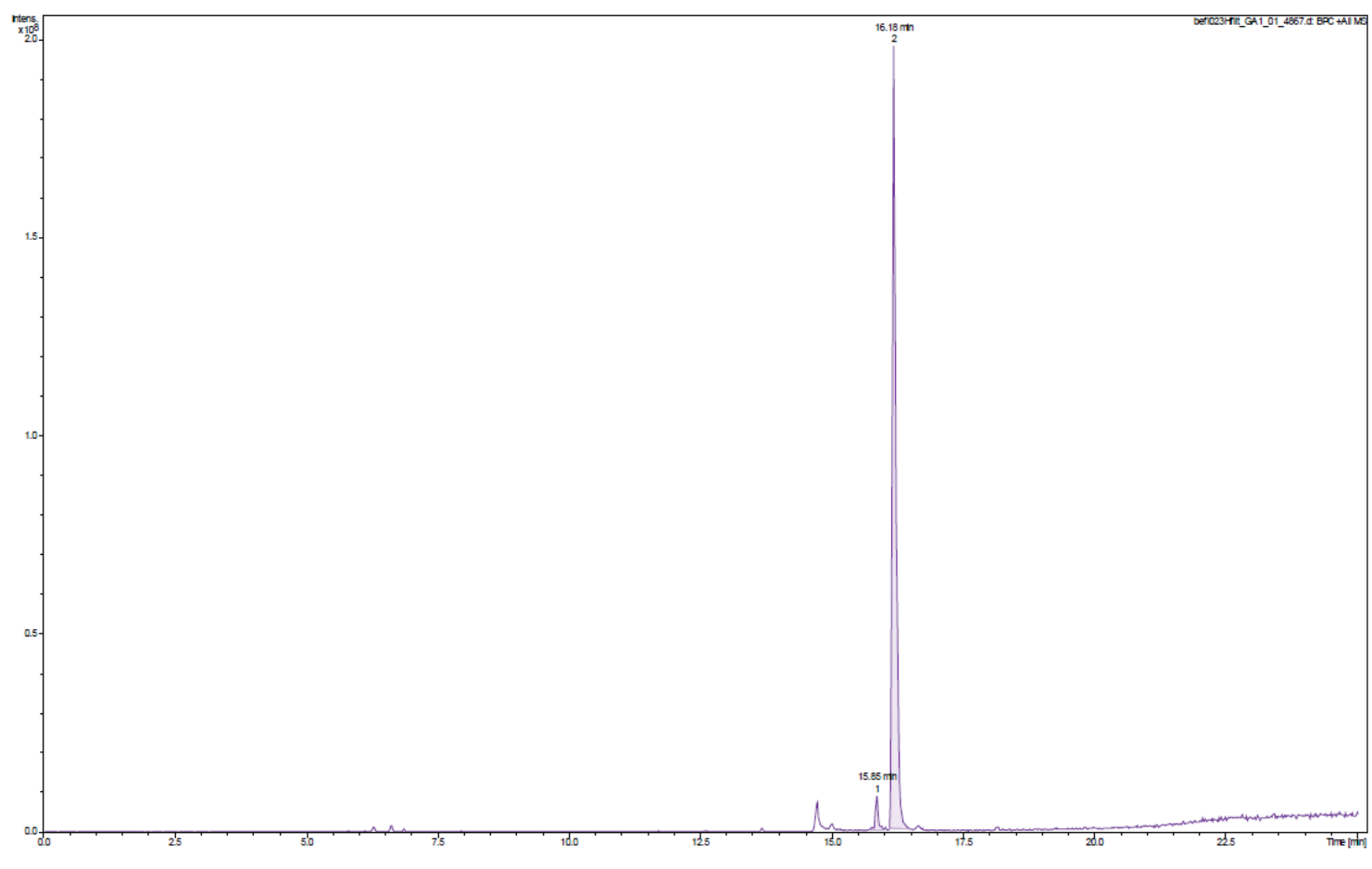


Figure S12. HPLC-MS analysis of IMC-48

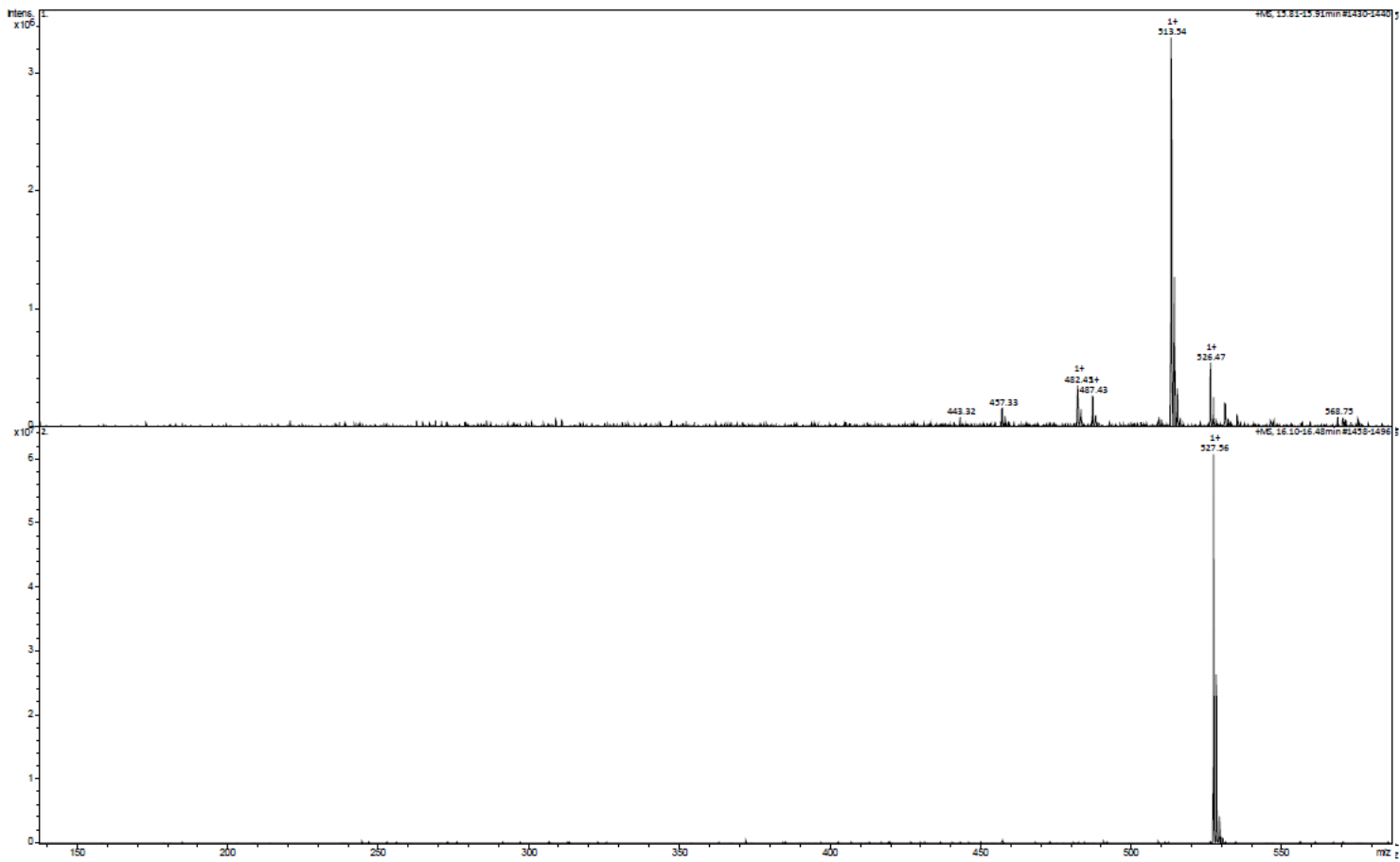
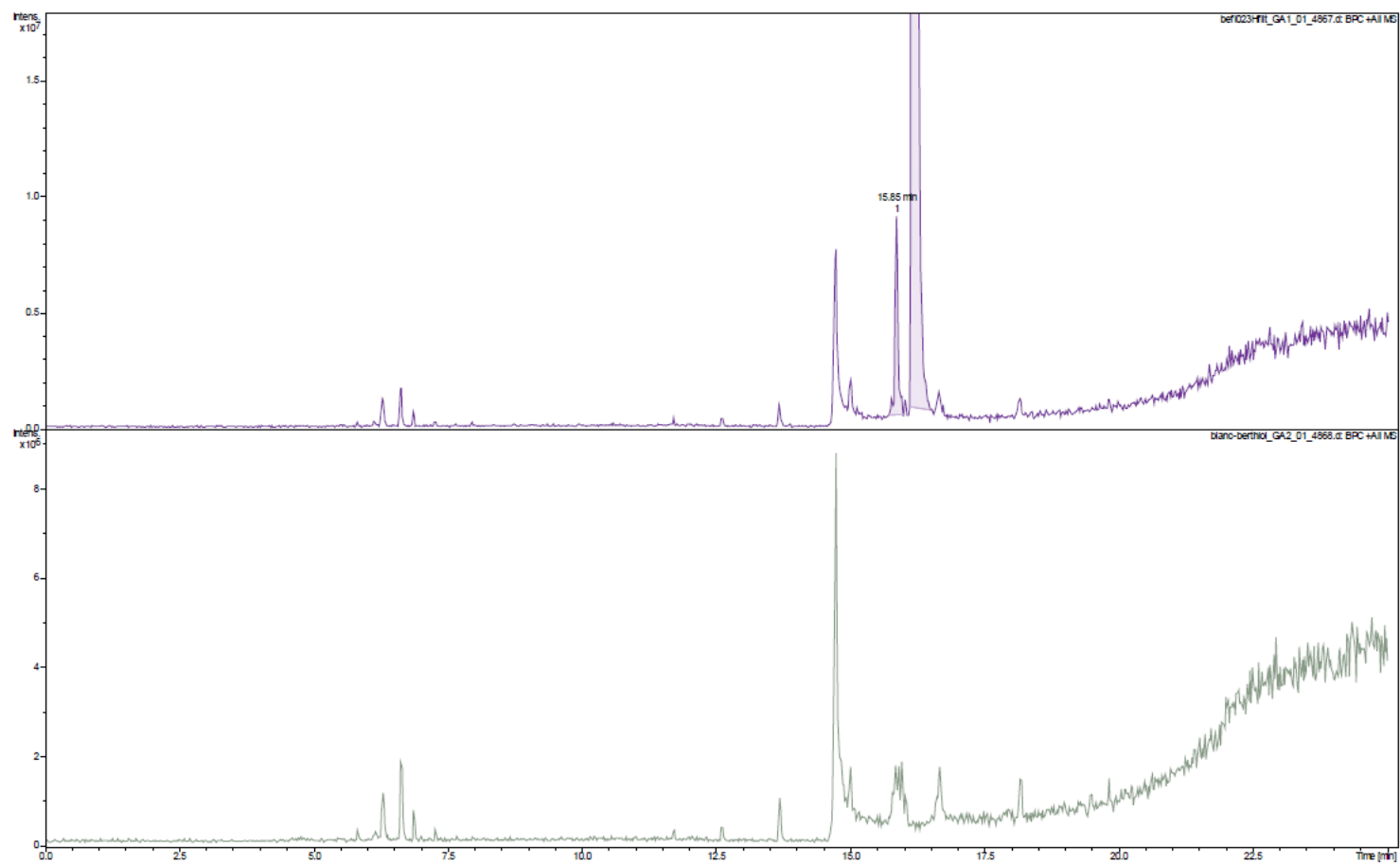
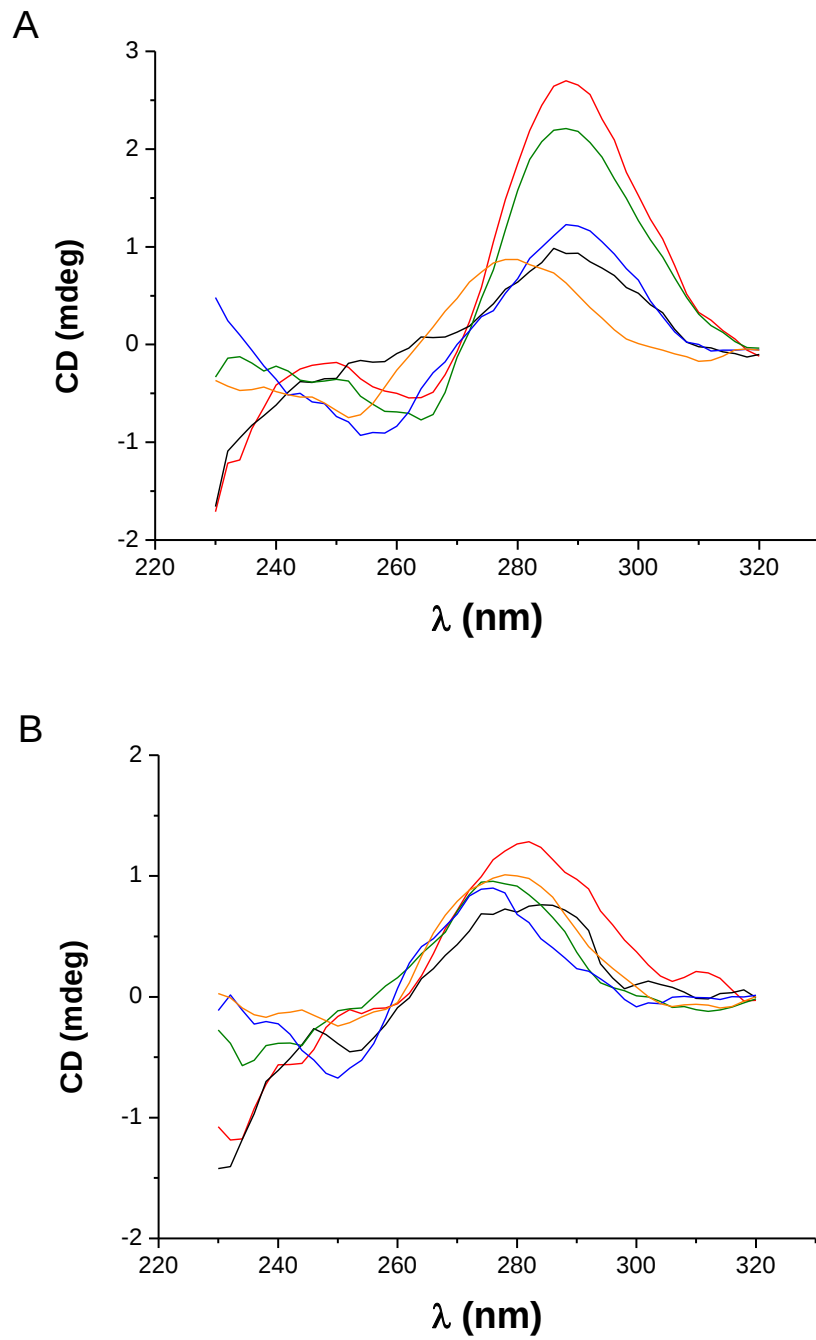


Figure S13. HPLC-MS of **IMC-48** (background down).



CD spectra.

Figure S14. CD analyses of the i-motif sequences at pH 5.5 (A) and 7.5 (B). *HRAS* (black), *bcl-2* (red), *HTelo-C* (blue), *C-myc* (green) and *DAP-Mut* (orange). Buffer: 50 mM TrisHCl, 50 mM KCl, 2 % DMSO at pH = 5.5 and 7.5



BLI analysis

Figure S15. Langmuir isotherm with the plateau value at the equilibrium at pH 5.5 (A) or pH 7.5 (B) for *HRAS* (black), *bcl-2* (red), *HTelo-C* (blue), *c-myc* (violet), HP GC (green), DAP-Mut (orange) and ssDNA (cyan). Concentration range: 0.1, 0.5, 1, 5, 10, 25, 50 and 100 μM .

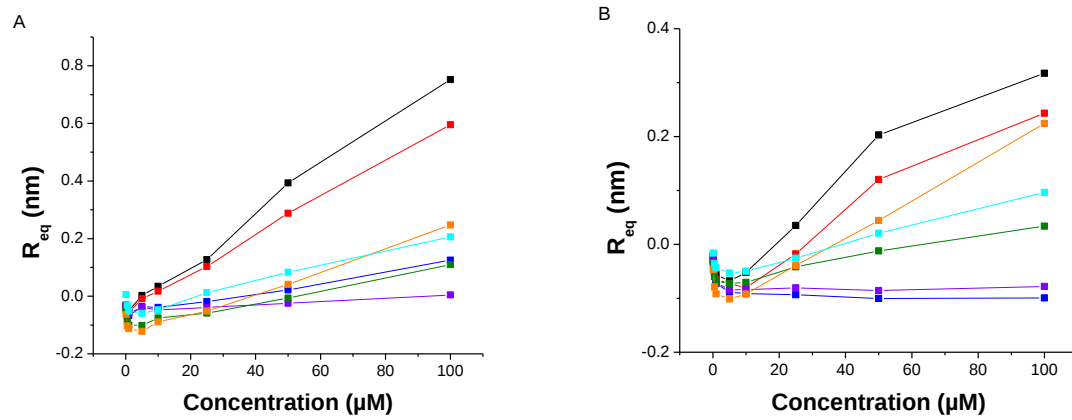
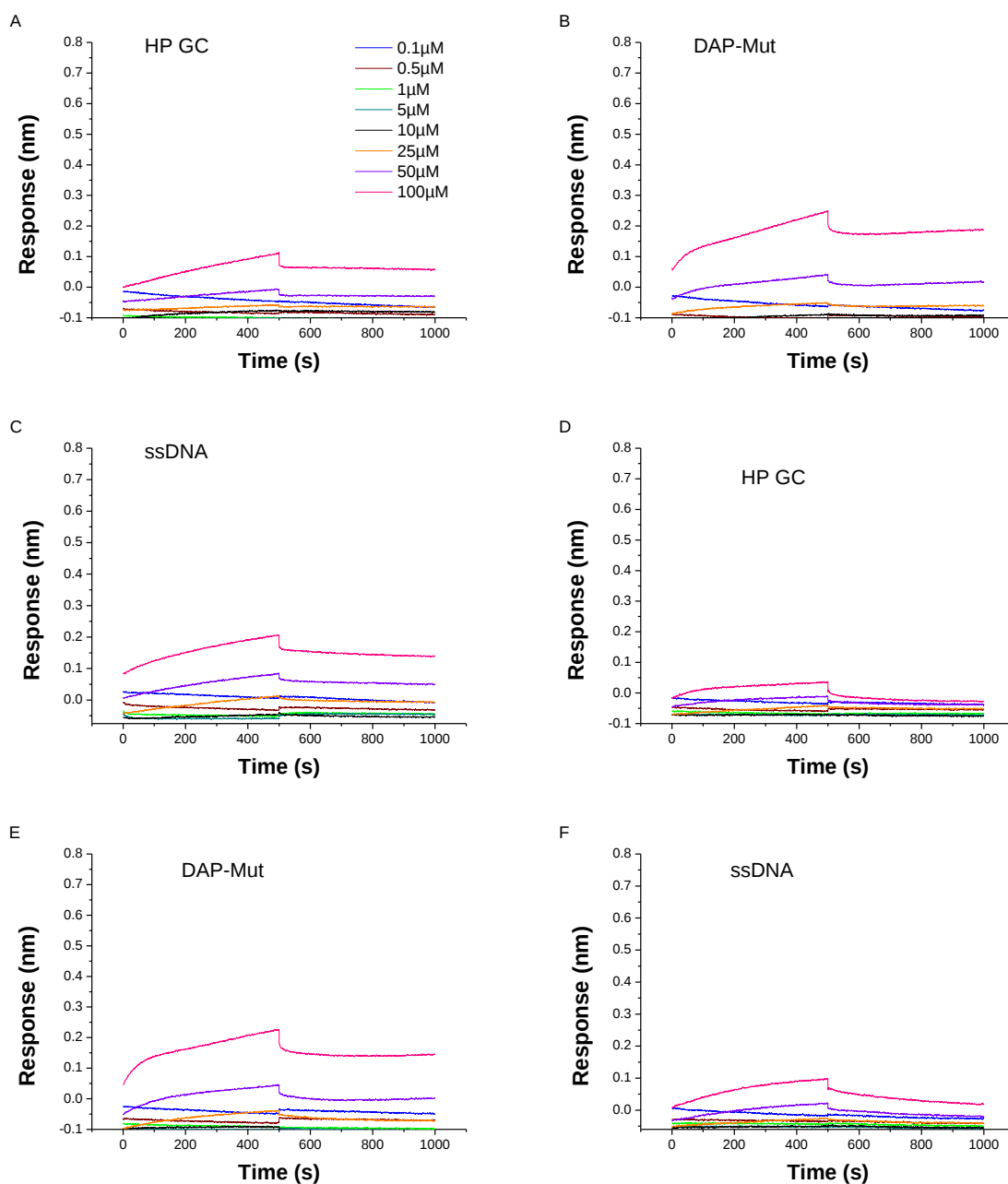


Figure S16. Sensorgrams recorded for the recognition of **IMC-48** with the control sequences: A) *HP GC* at pH 5.5, B) *DAP-Mut* at pH 5.5, C) *ssDNA* at pH 5.5, D) *HP GC* at pH 7.5, E) *DAP-Mut* at pH 7.5 and F) *ssDNA* at pH 7.5. Concentration range: 0.1 (blue), 0.5 (brown), 1 (green), 5 (dark cyan), 10 (black), 25 (orange), 50 (violet) and 100 μM (pink).



CD study

Figure S17. Circular dichroism spectra of A) *HRAS*, B) *bcl-2*, C) *HTelo-C* and D) *c-myc* at pH=7.2 in the absence (blue points) and in the presence of 20 μ M of **IMC-48** (green points) and at pH=5.5 in the absence (red points) and in the presence of 20 μ M of **IMC-48** (black points).

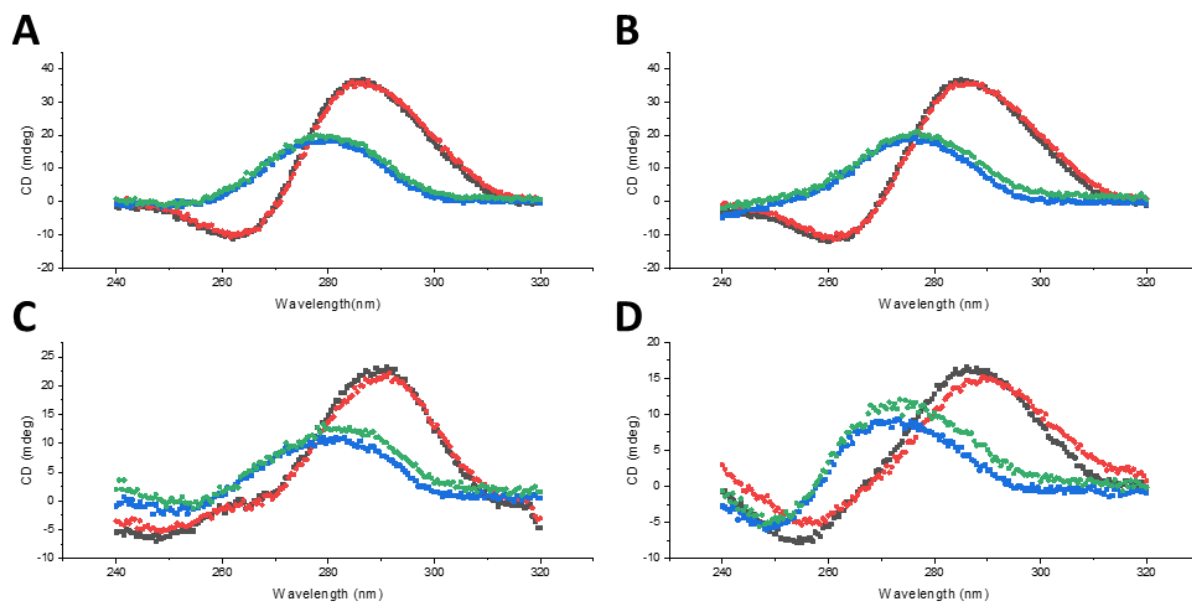


Figure S18. Representative thermal denaturation profiles of A) *HRAS*, B) *bcl-2*, C) *HTelo-C* and D) *c-myc* in the absence (black points) and in the presence of 20 μ M of **IMC-48** (red points). Left panels: heating (denaturing) ramps, right panels: cooling (renaturing) ramps. The arrows indicate the denaturing/renaturing processes.

