Multivalent *C2*-alkyl trihydroxypiperidine architectures modulate β-Glucocerebrosidase activity

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Table of contents

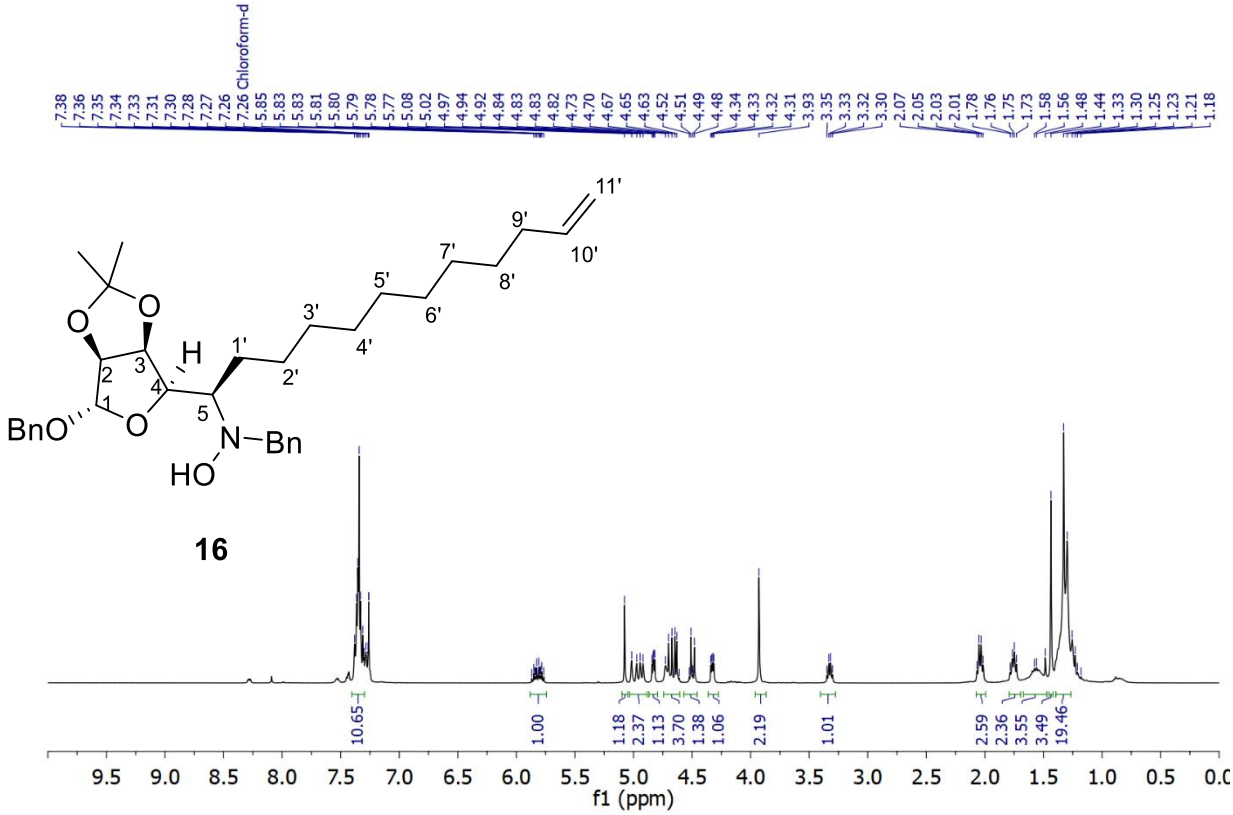


Figure S01. ^{1}H-NMR spectrum in $CDCl_3$ of compound **16**

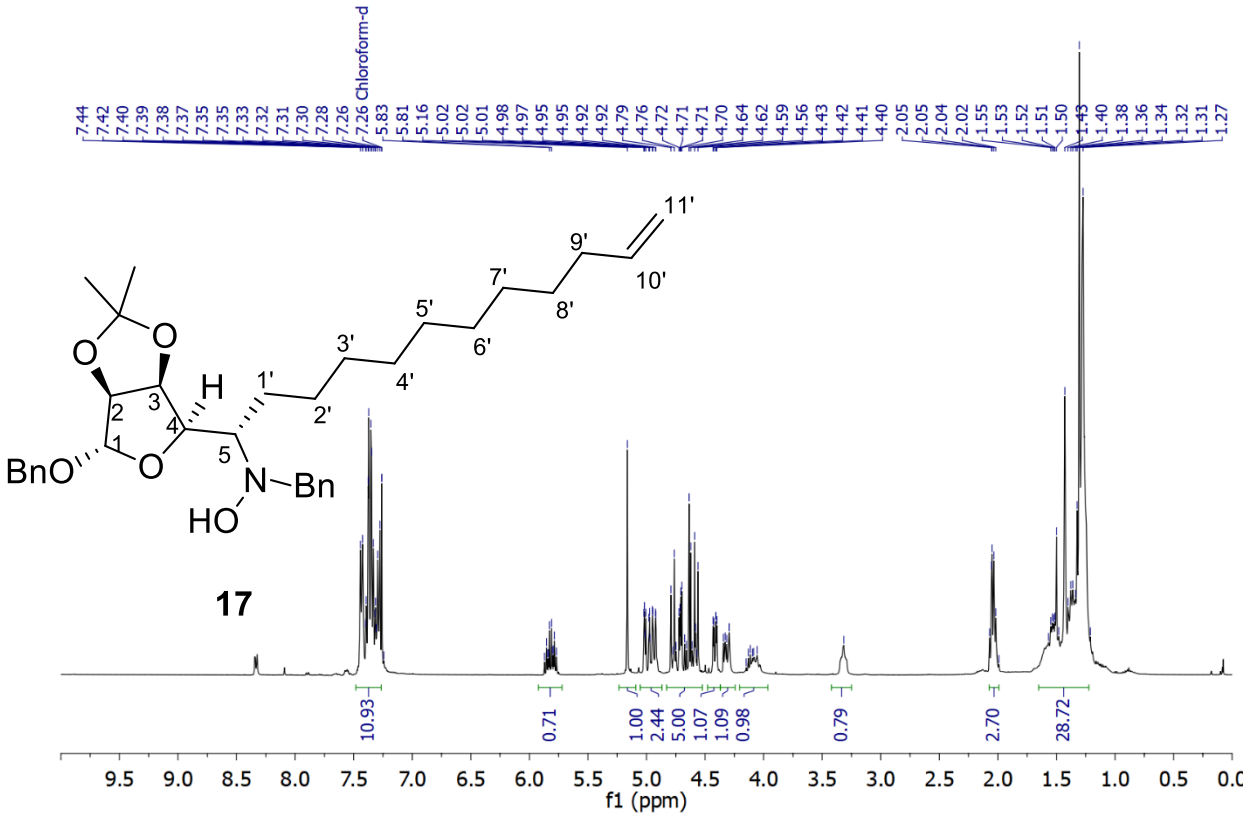


Figure S02. ^{1}H-NMR spectrum in $CDCl_3$ of compound **17**

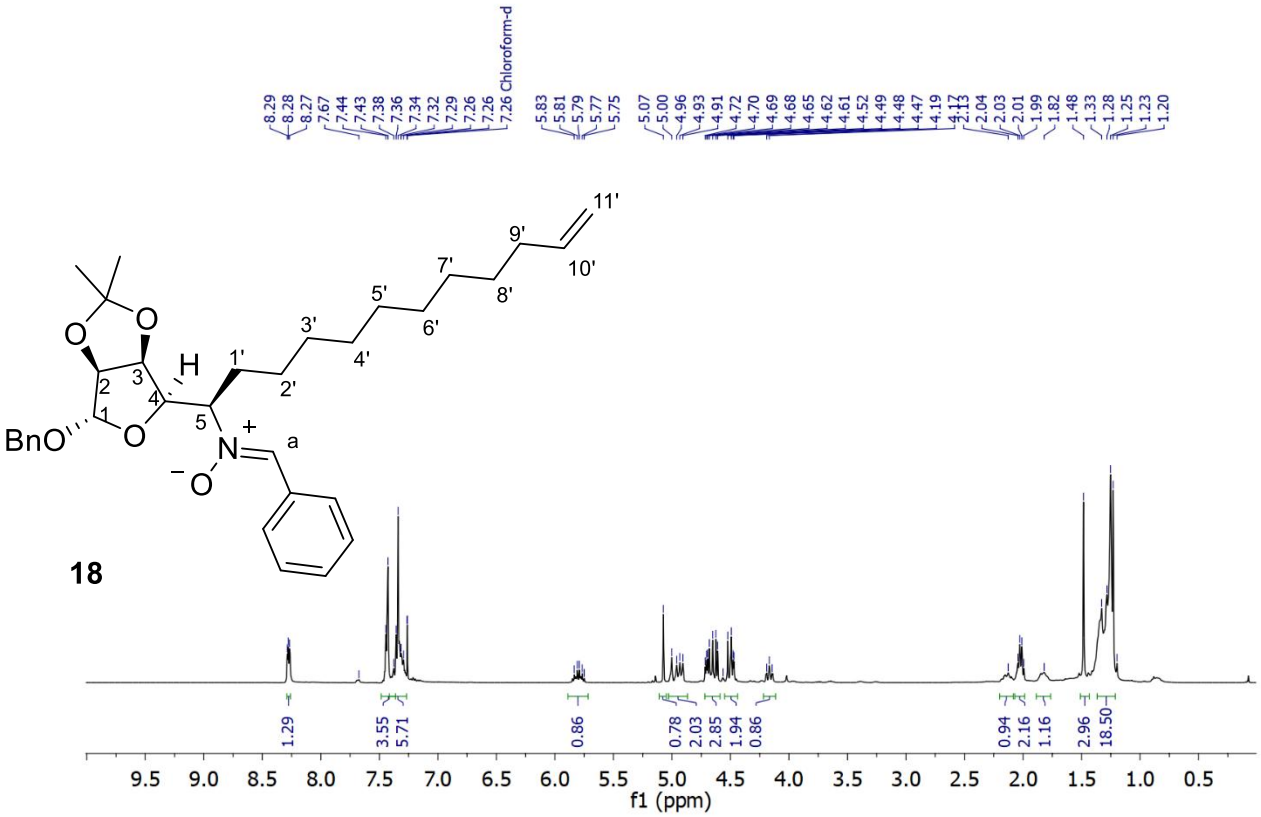


Figure S03. ^{1}H-NMR spectrum in $CDCl_3$ of compound **18**

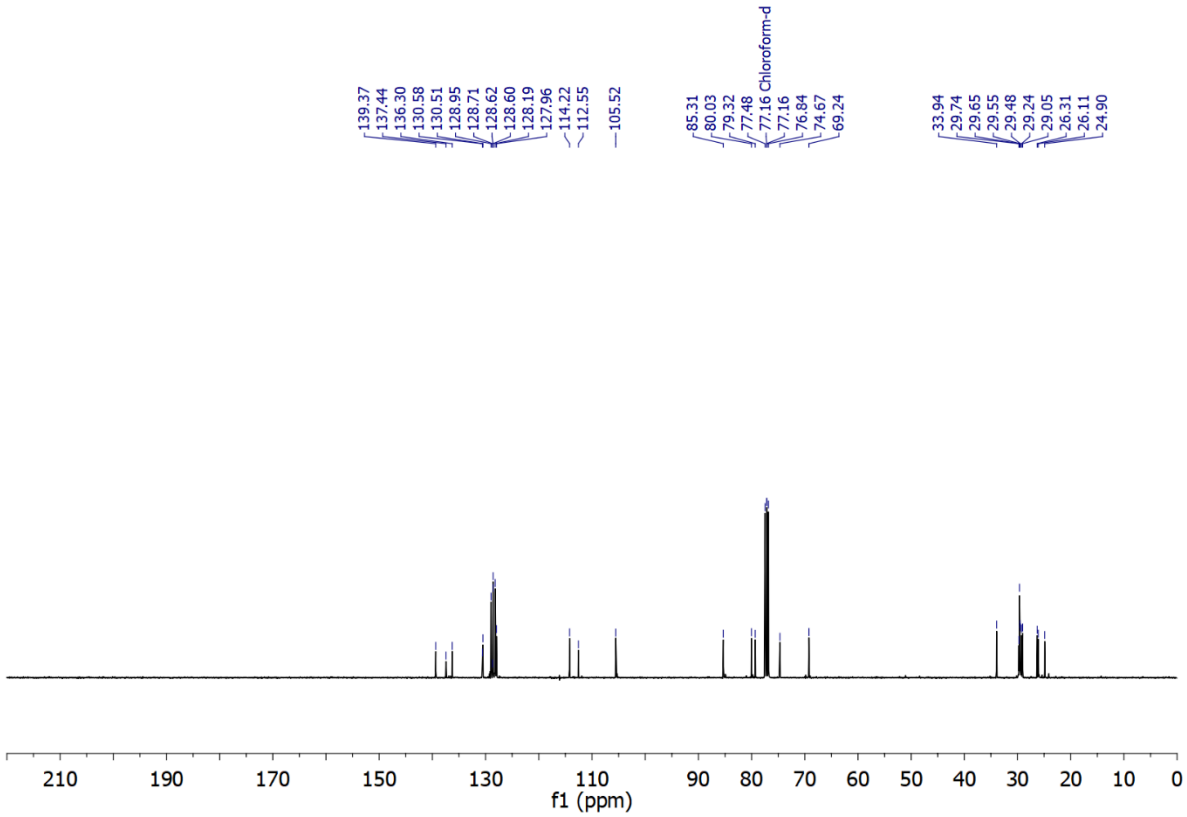


Figure S04. ^{13}C-NMR spectrum in $CDCl_3$ of compound **18**

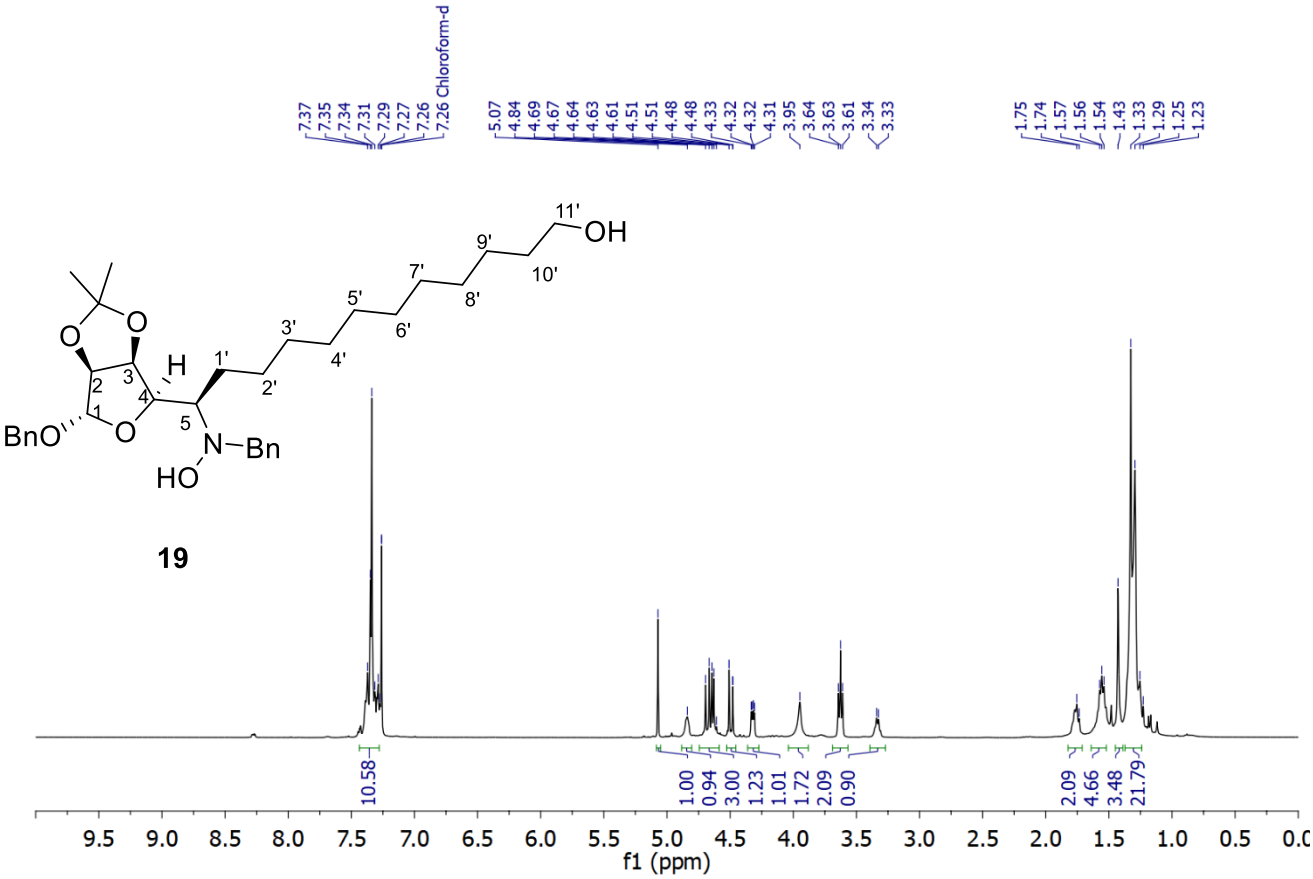


Figure S05. ^{1}H-NMR spectrum in $CDCl_3$ of compound **19**

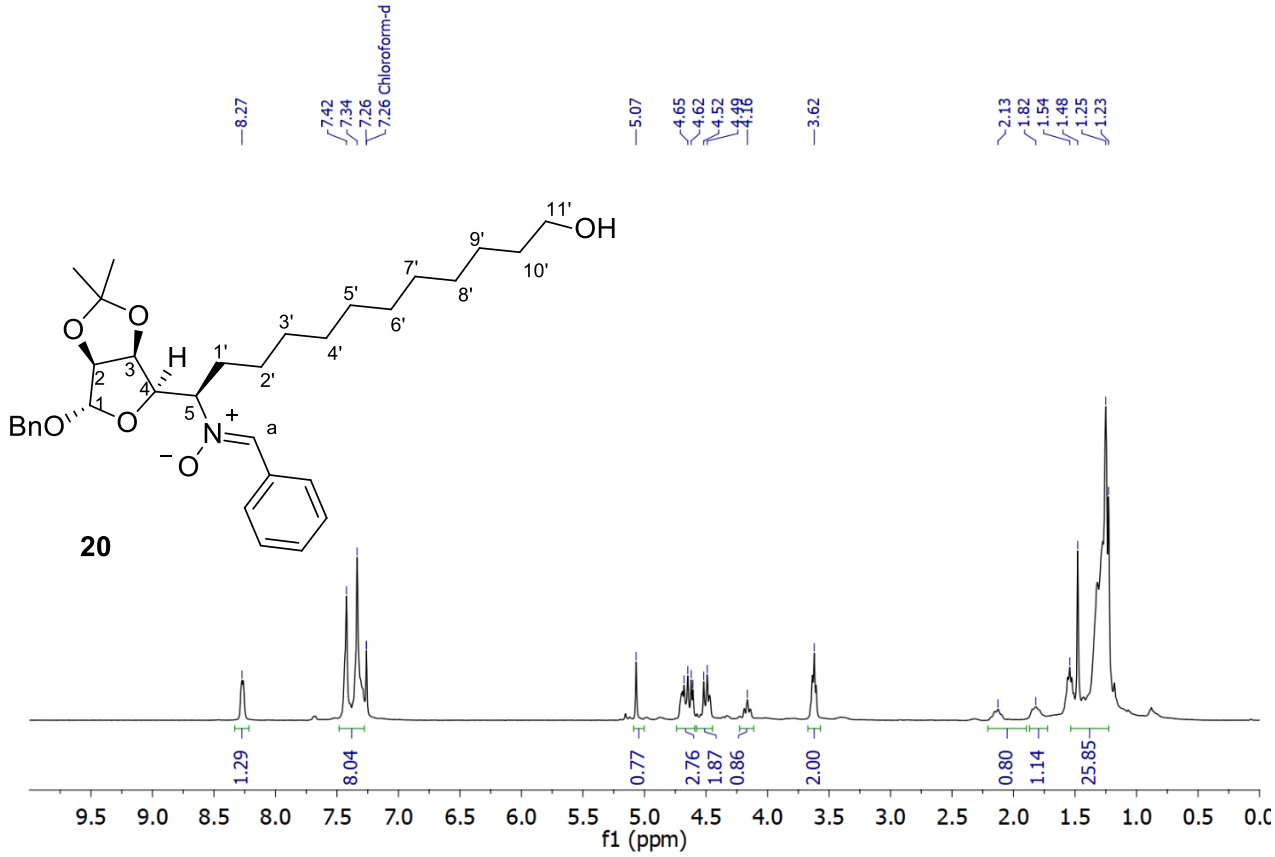


Figure S06. ^{1}H-NMR spectrum in $CDCl_3$ of compound **20**

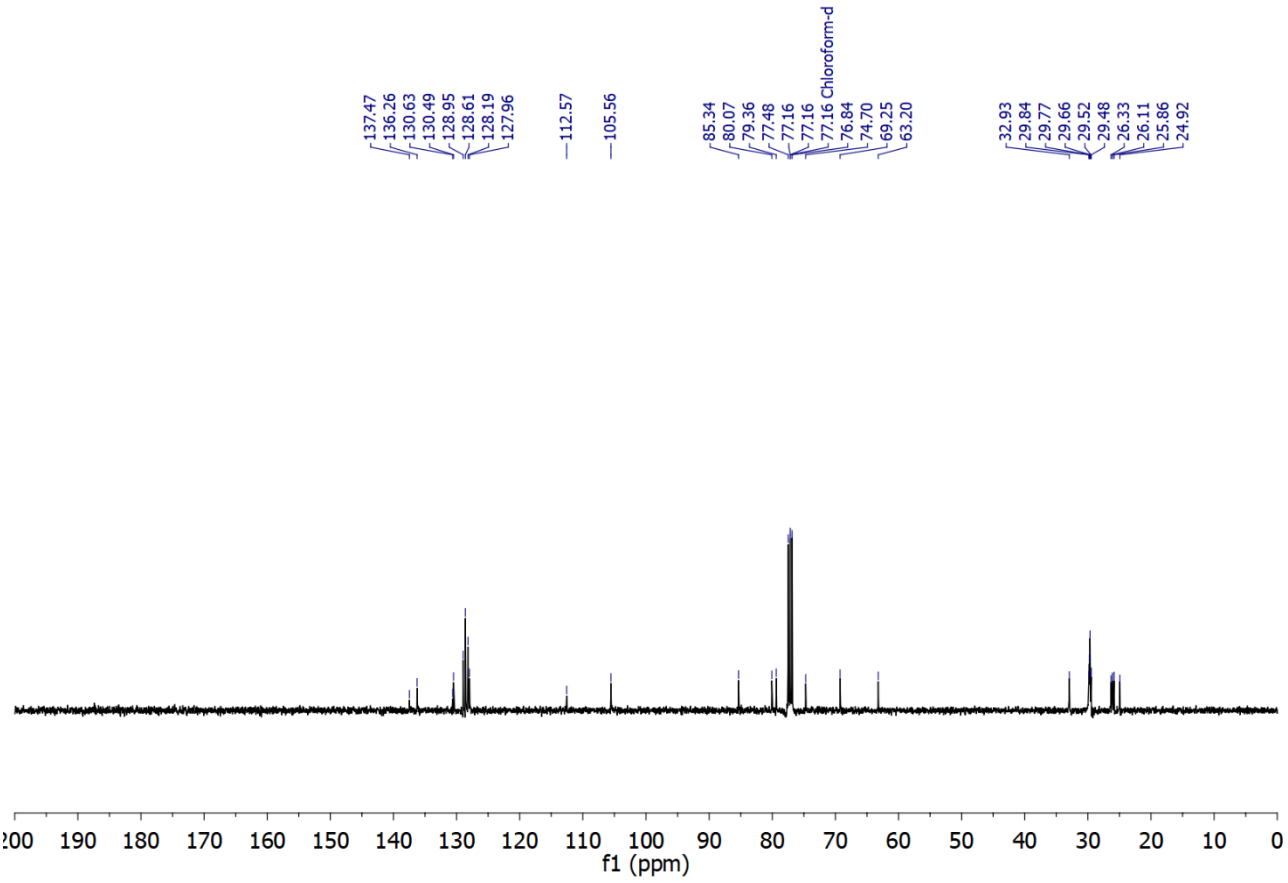
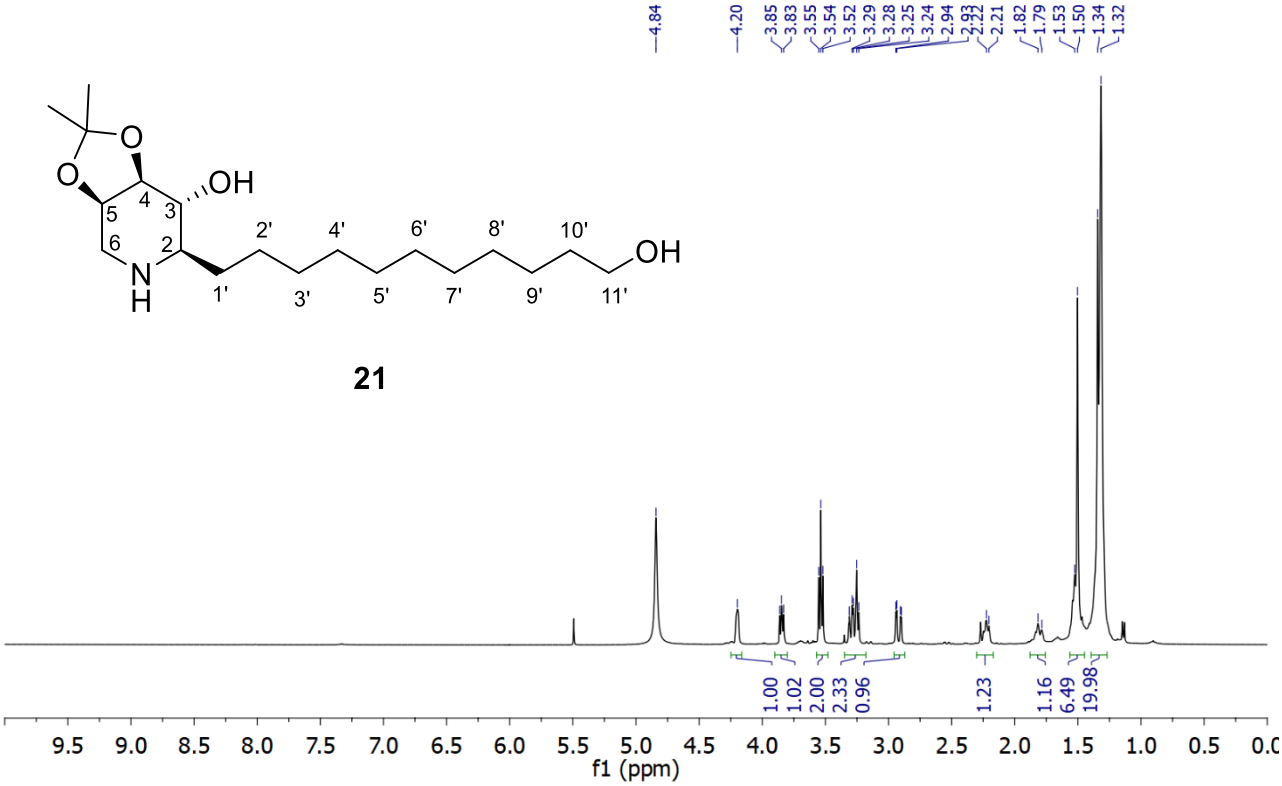


Figure S07. ^{13}C-NMR spectrum in $CDCl_3$ of compound **20**



21

Figure S08. ^{1}H-NMR spectrum in CD_3OD of compound **21**

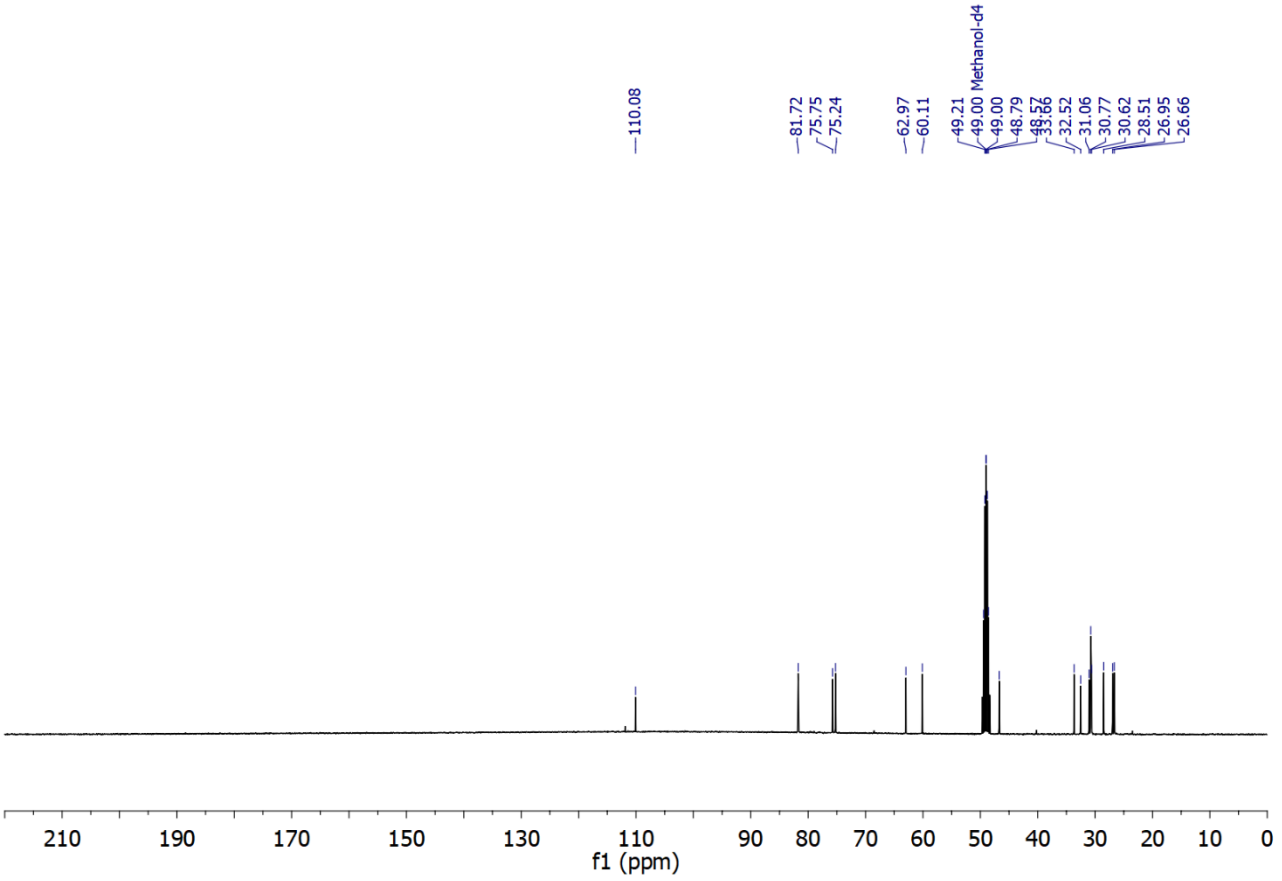


Figure S09. ^{13}C-NMR spectrum in CD_3OD of compound **21**

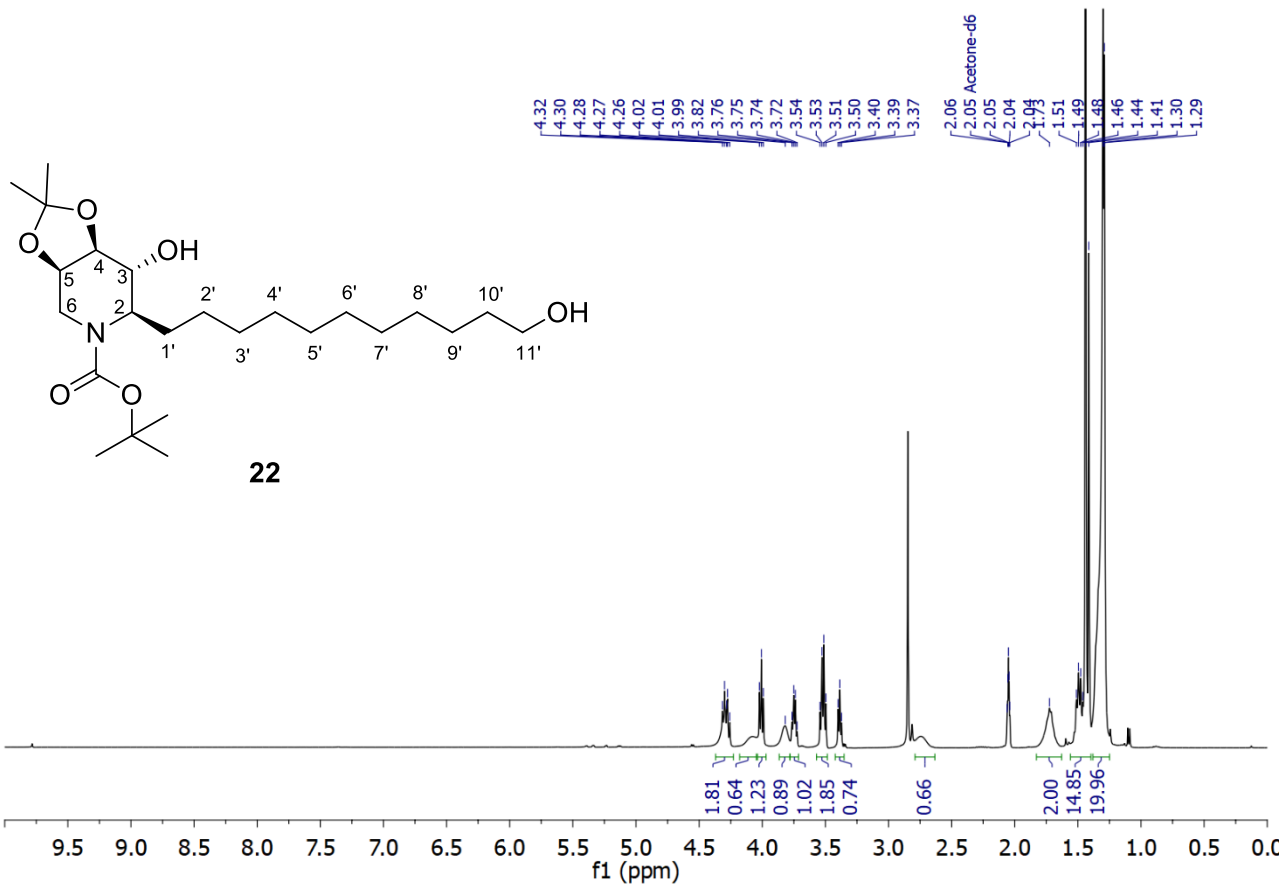


Figure S10. ^{1}H-NMR spectrum in acetone-d6 of compound **22**

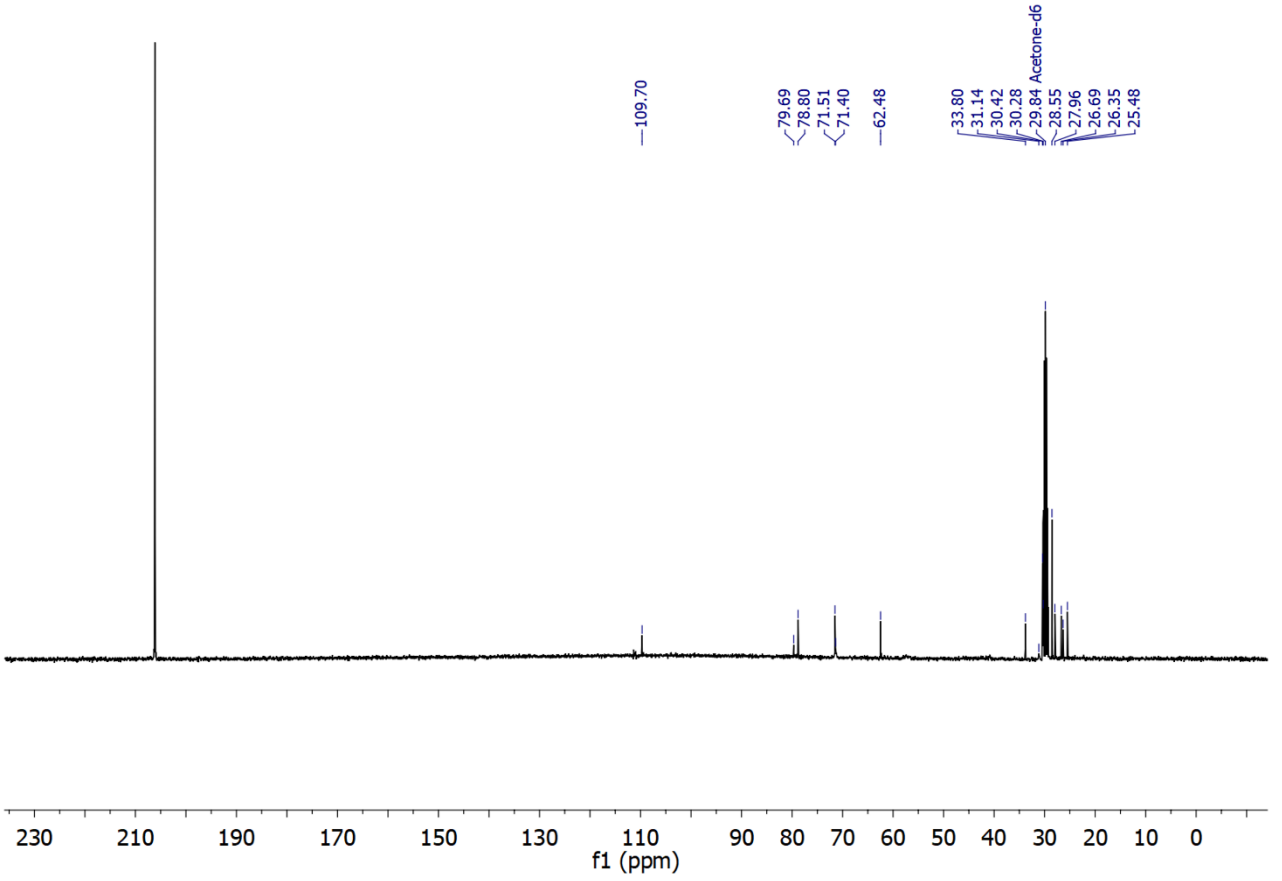


Figure S11. ^{13}C-NMR spectrum in acetone-d6 of compound **22**

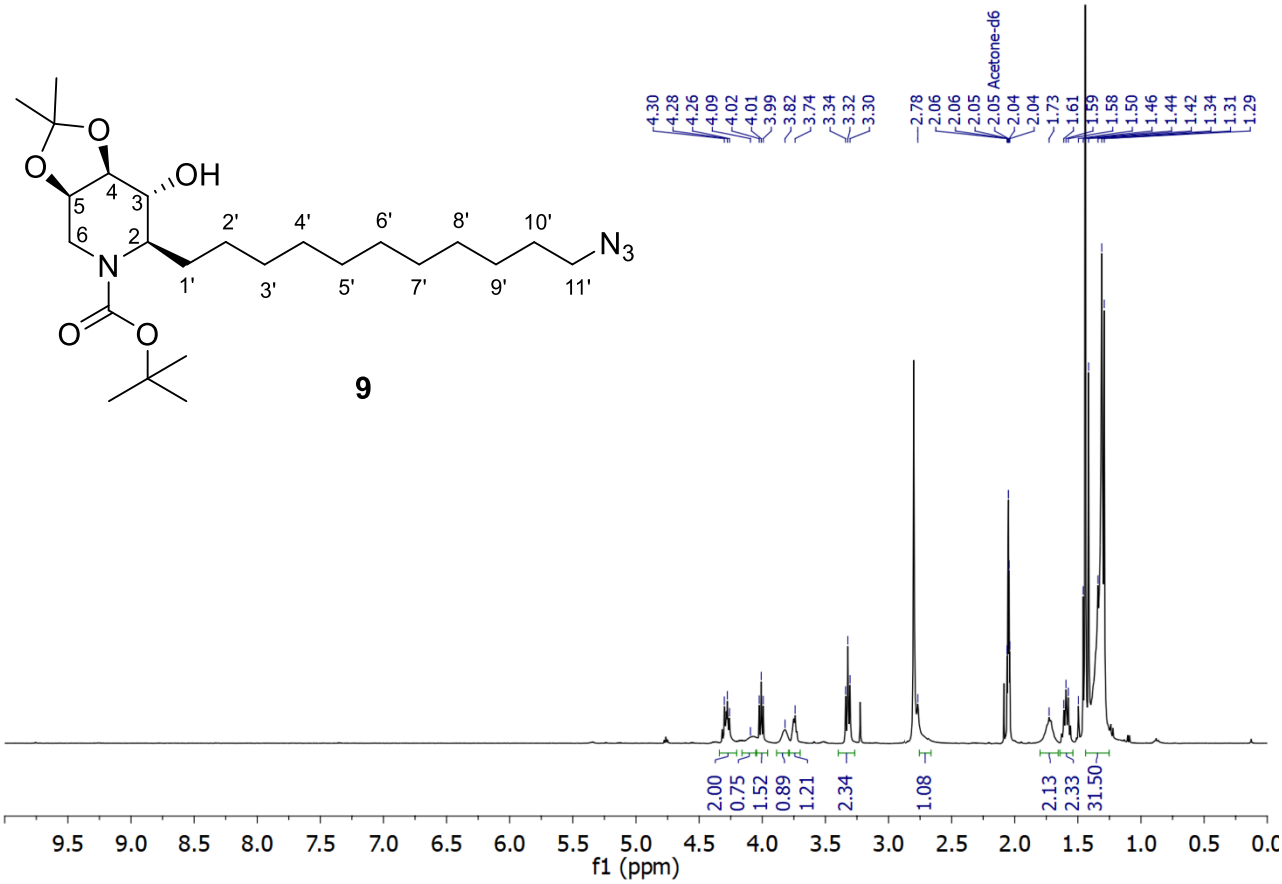


Figure S12. ^{1}H-NMR spectrum in acetone-d6 of compound **9**

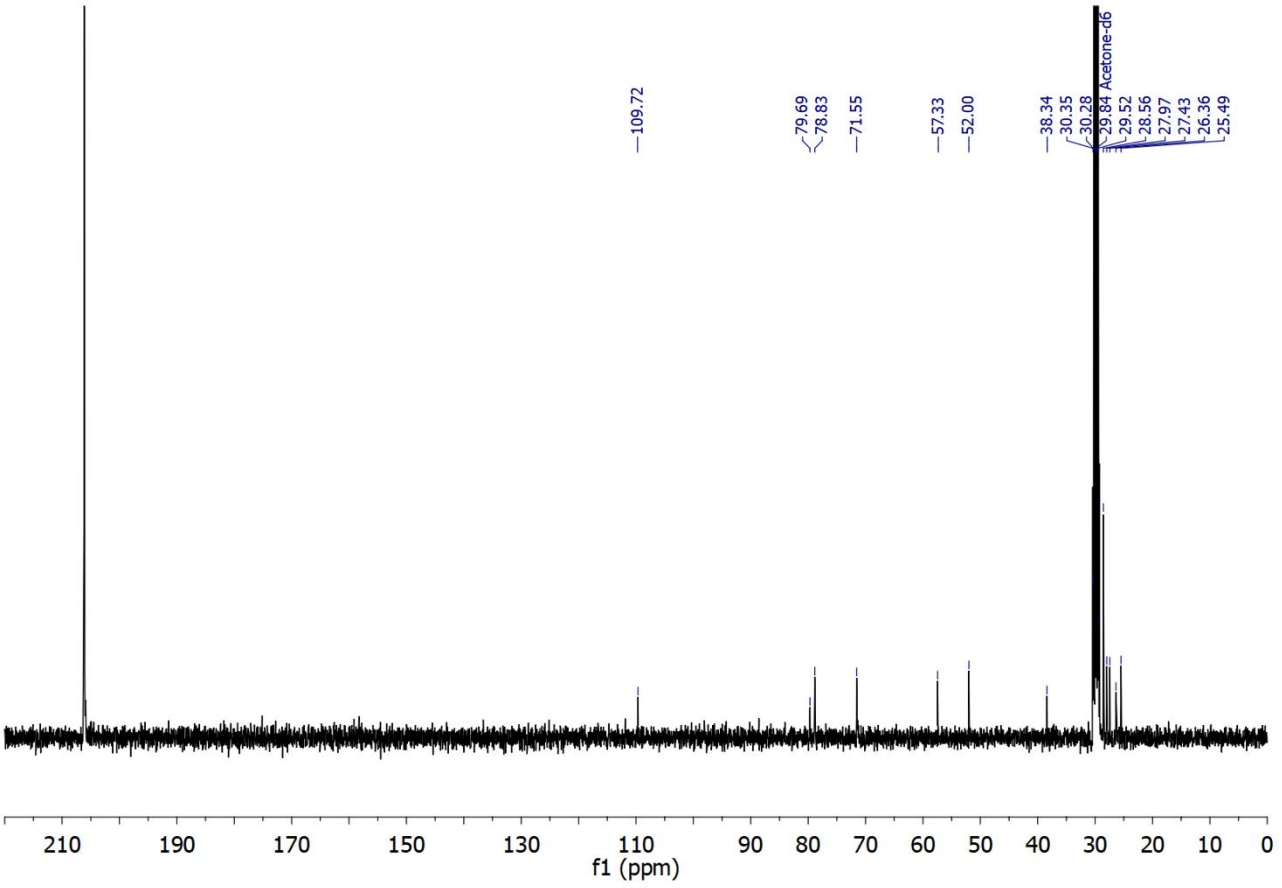


Figure S13. ^{13}C-NMR spectrum in acetone-d6 of compound **9**

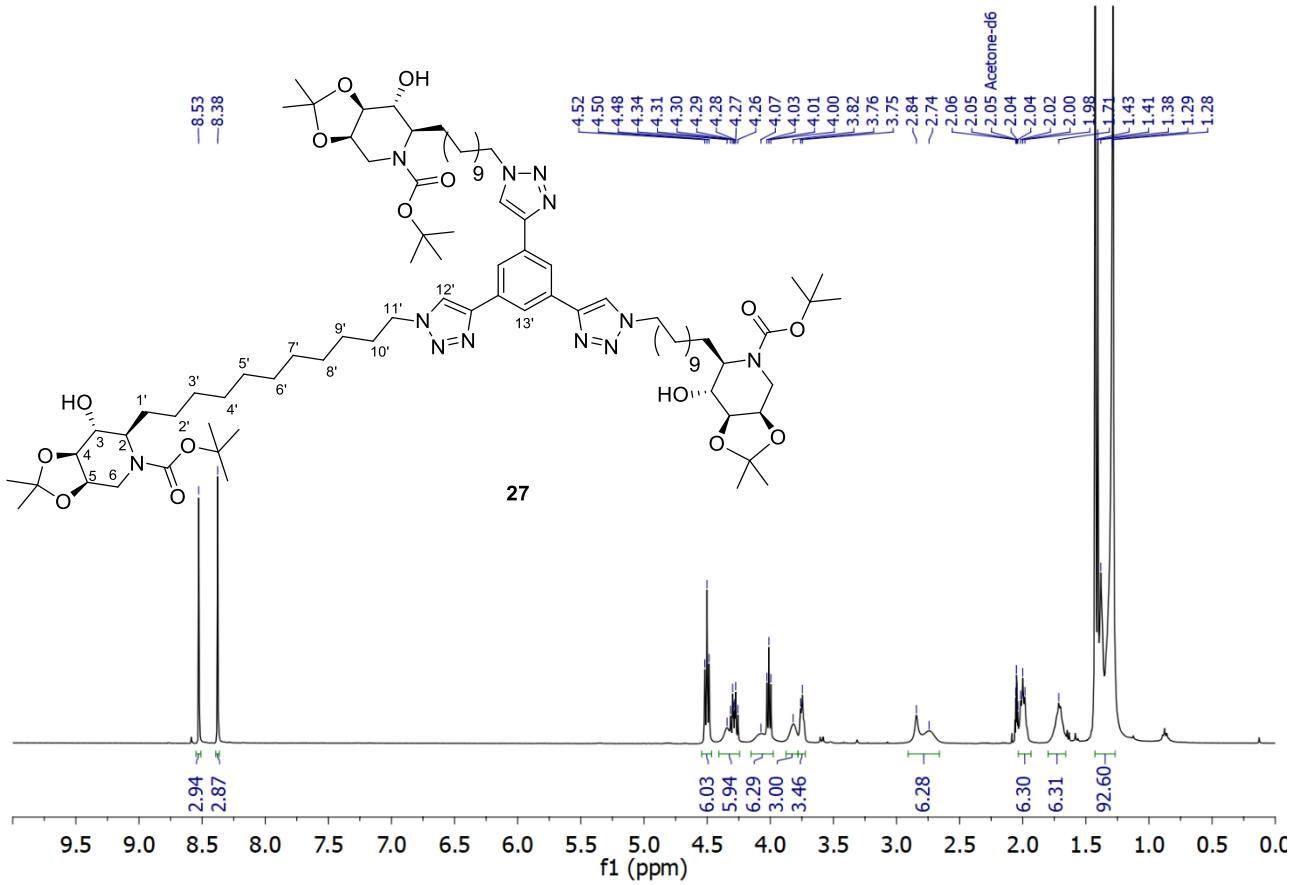


Figure S14. ^{1}H-NMR spectrum in acetone-d6 of compound **27**

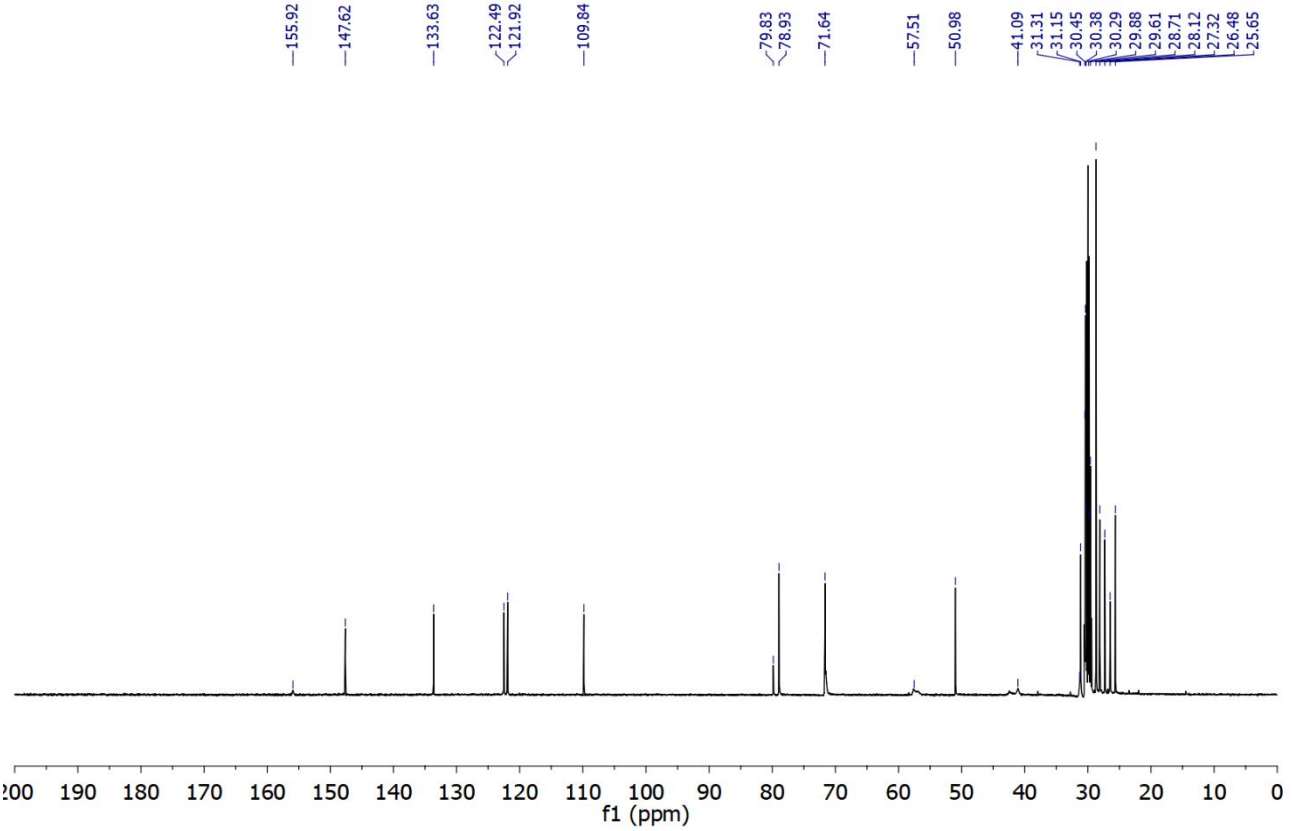


Figure S15. ^{13}C-NMR spectrum in acetone-d6 of compound **27**

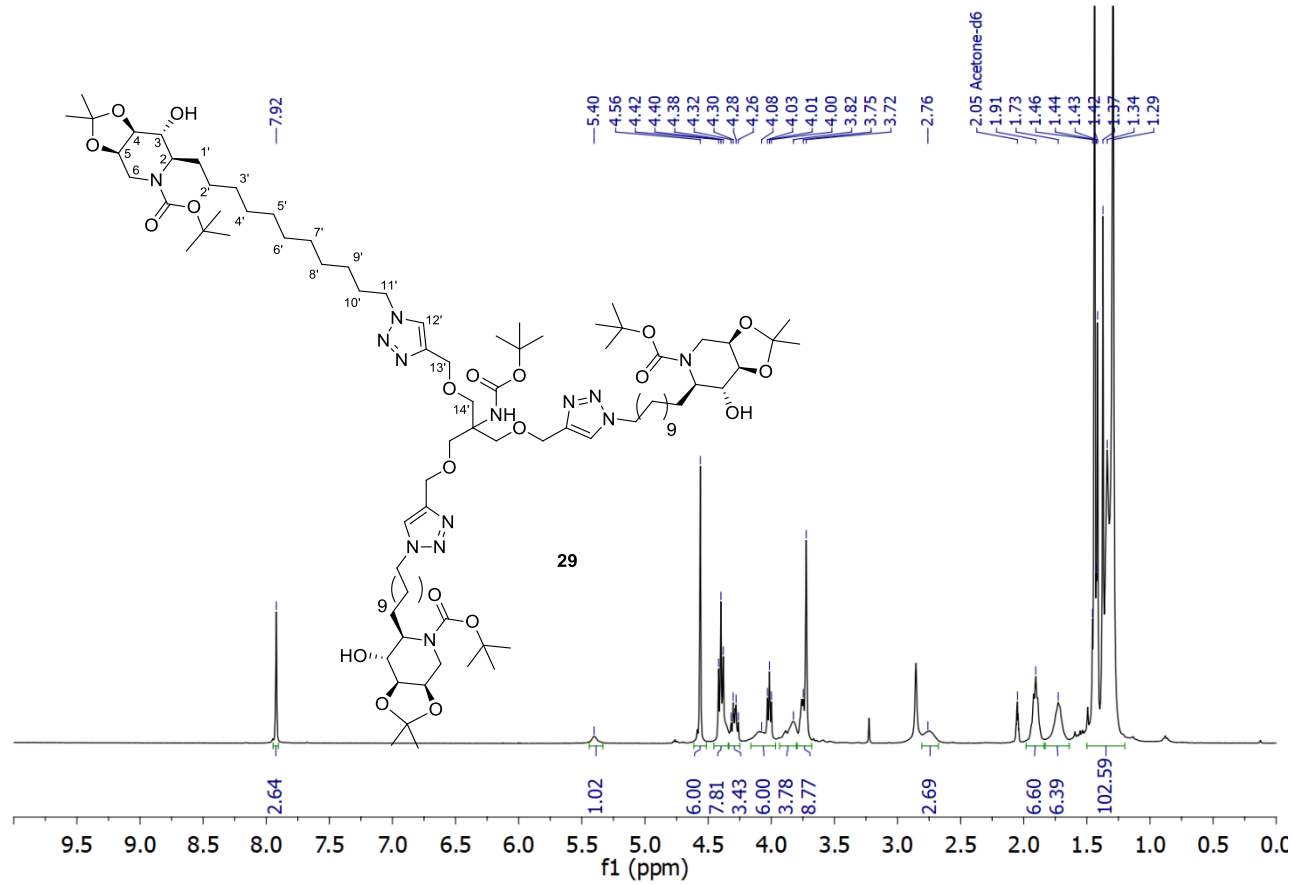


Figure S16. ^{1}H-NMR spectrum in acetone-d6 of compound **29**

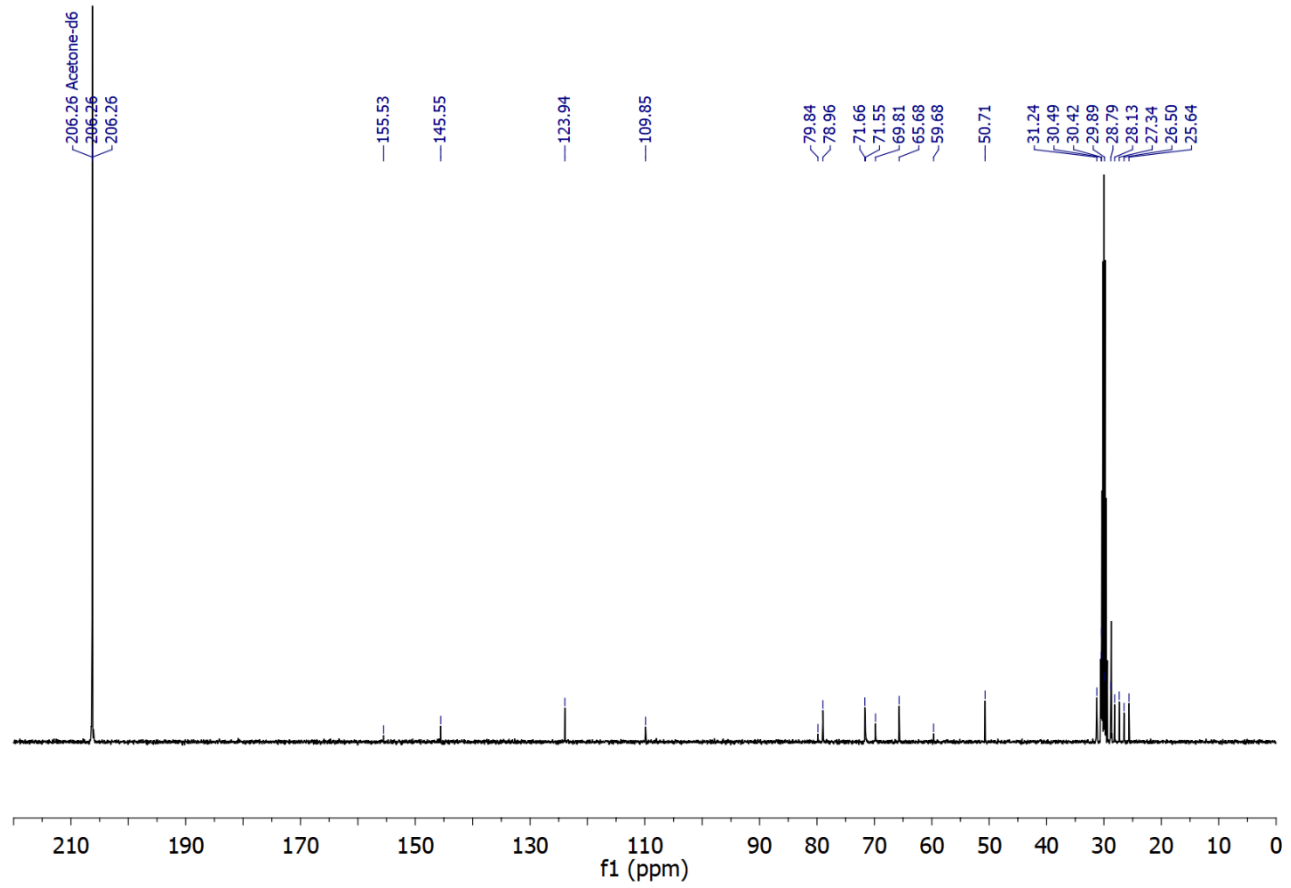


Figure S17. ^{13}C-NMR spectrum in acetone-d6 of compound **29**

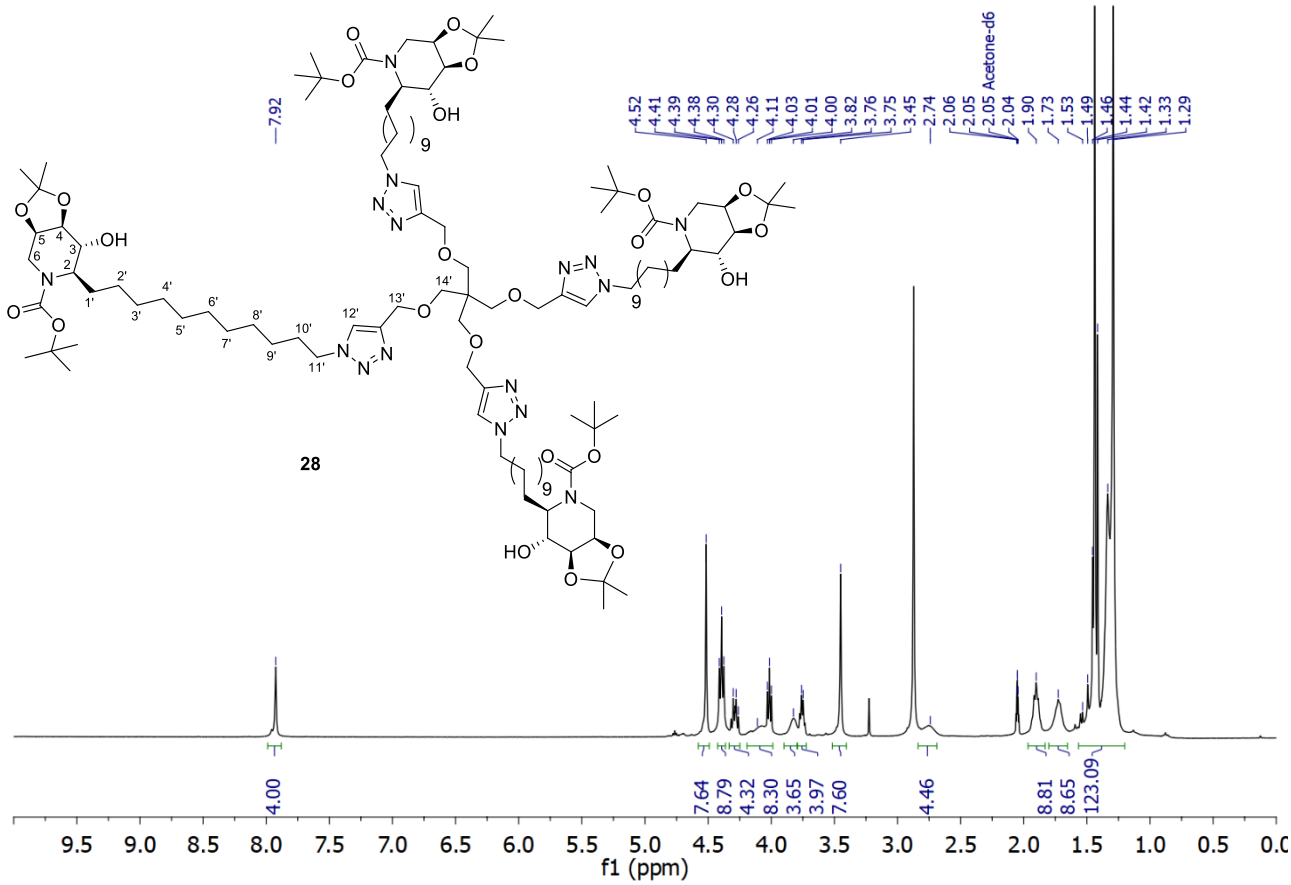


Figure S18. ^{1}H-NMR spectrum in acetone-d6 of compound **28**

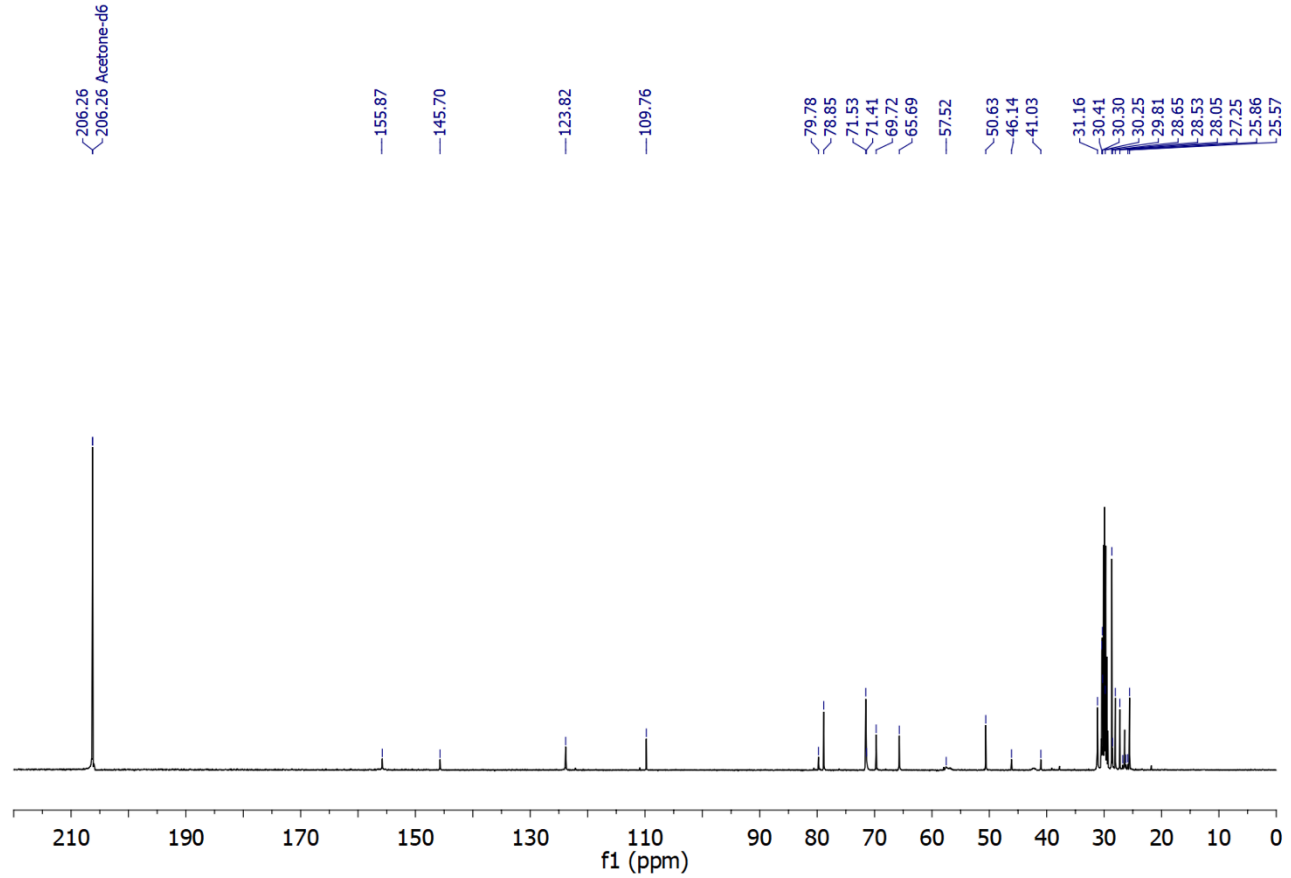


Figure S19. ^{13}C-NMR spectrum in acetone-d6 of compound **28**

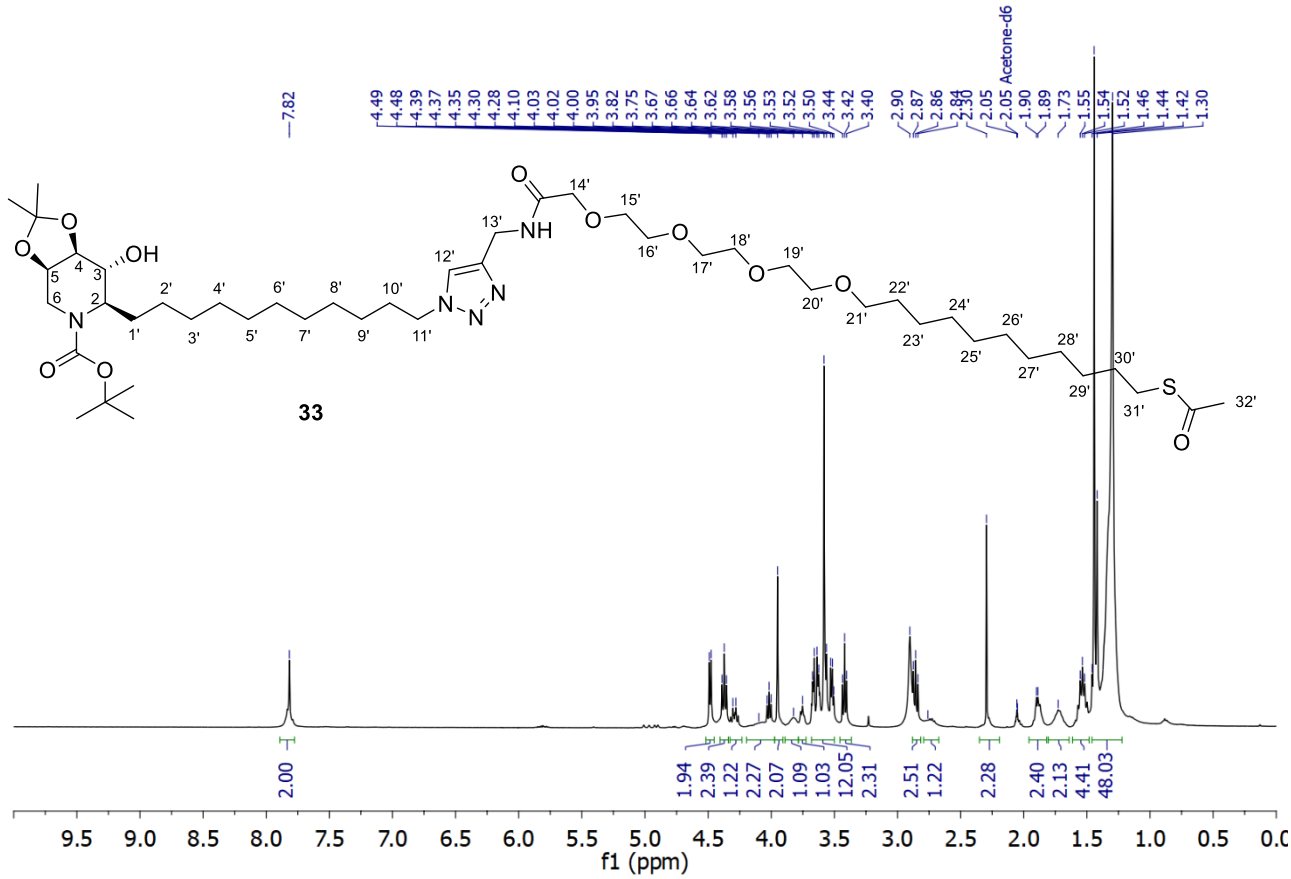


Figure S20. ^{1}H-NMR spectrum in acetone-d6 of compound **33**

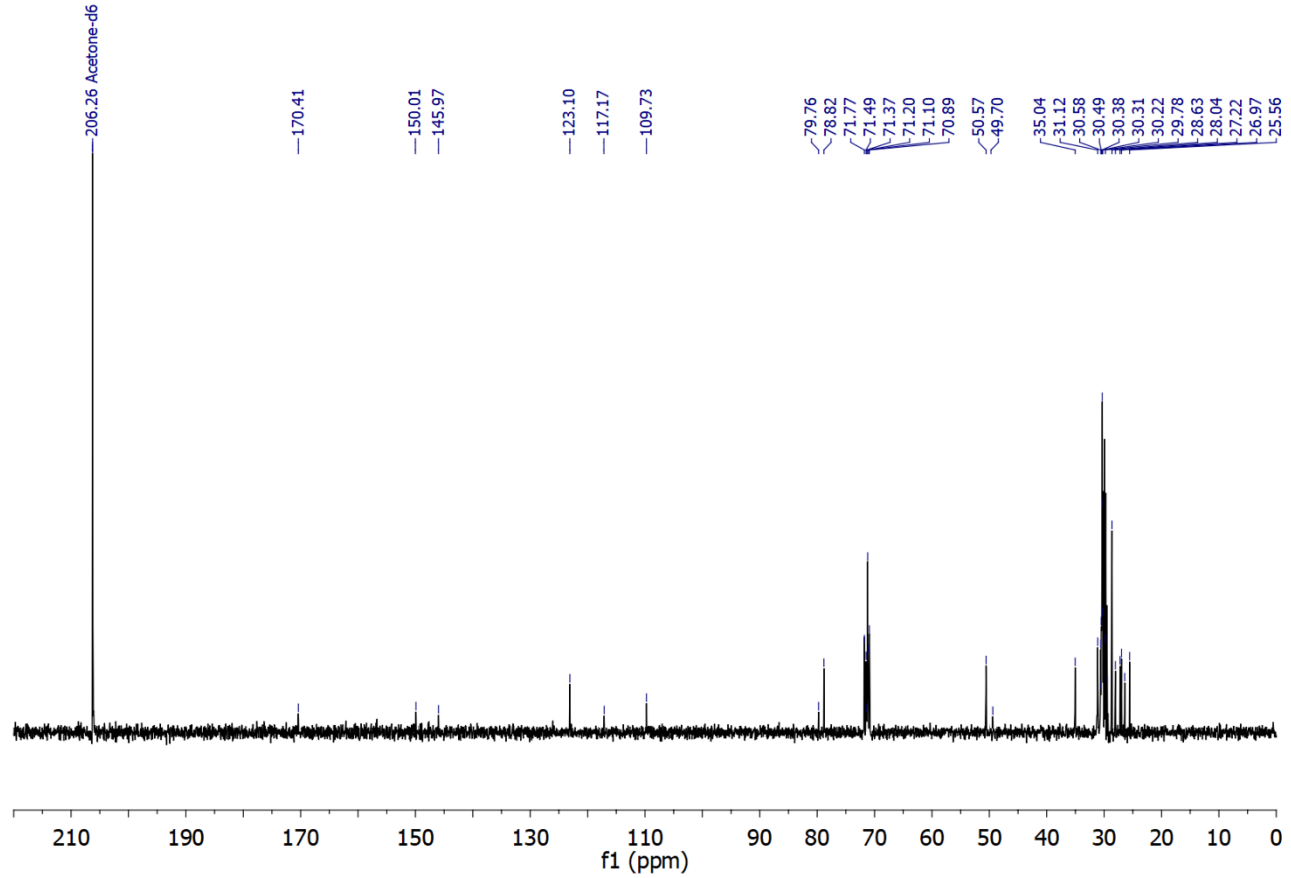


Figure S21. ^{13}C-NMR spectrum in acetone-d6 of compound **33**

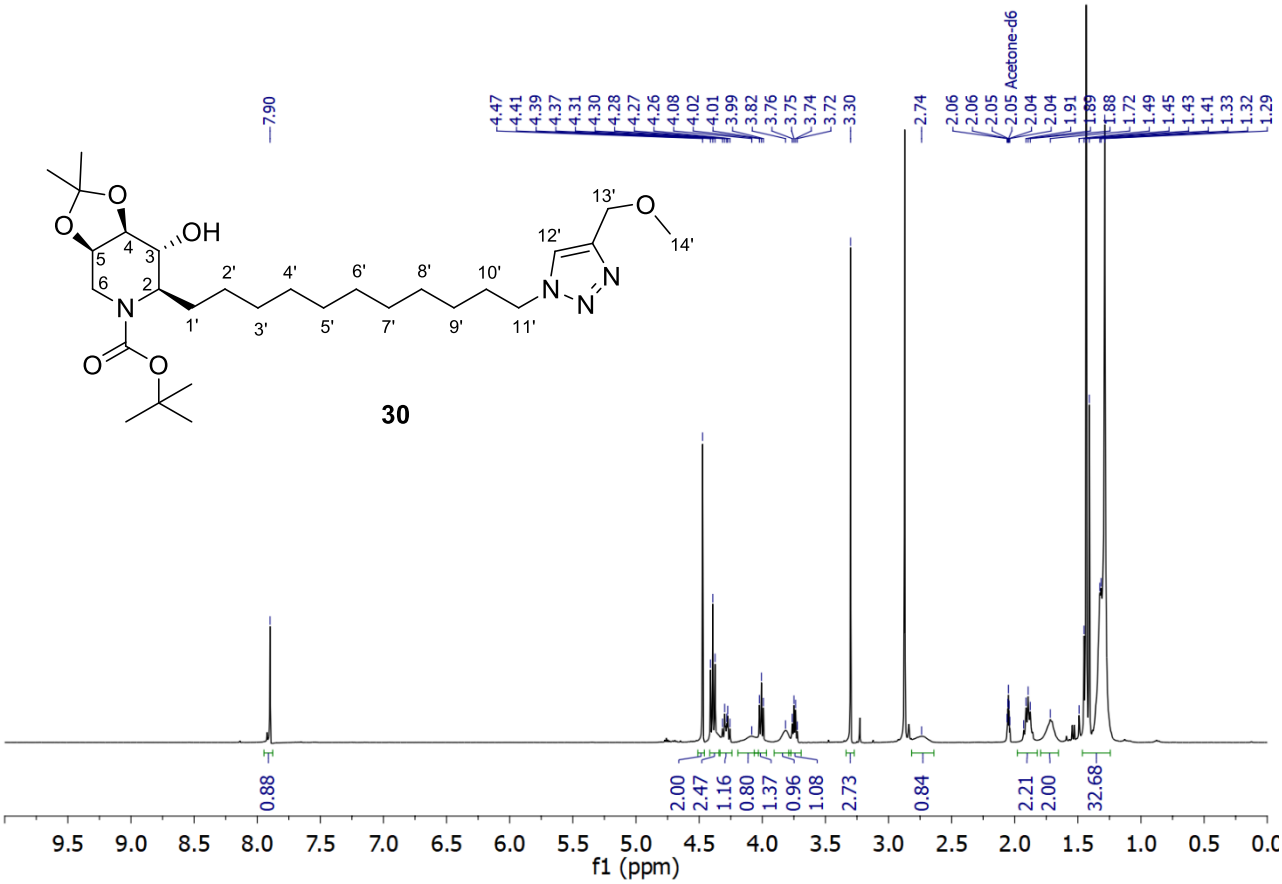


Figure S22. ^{1}H-NMR spectrum in acetone-d6 of compound **30**

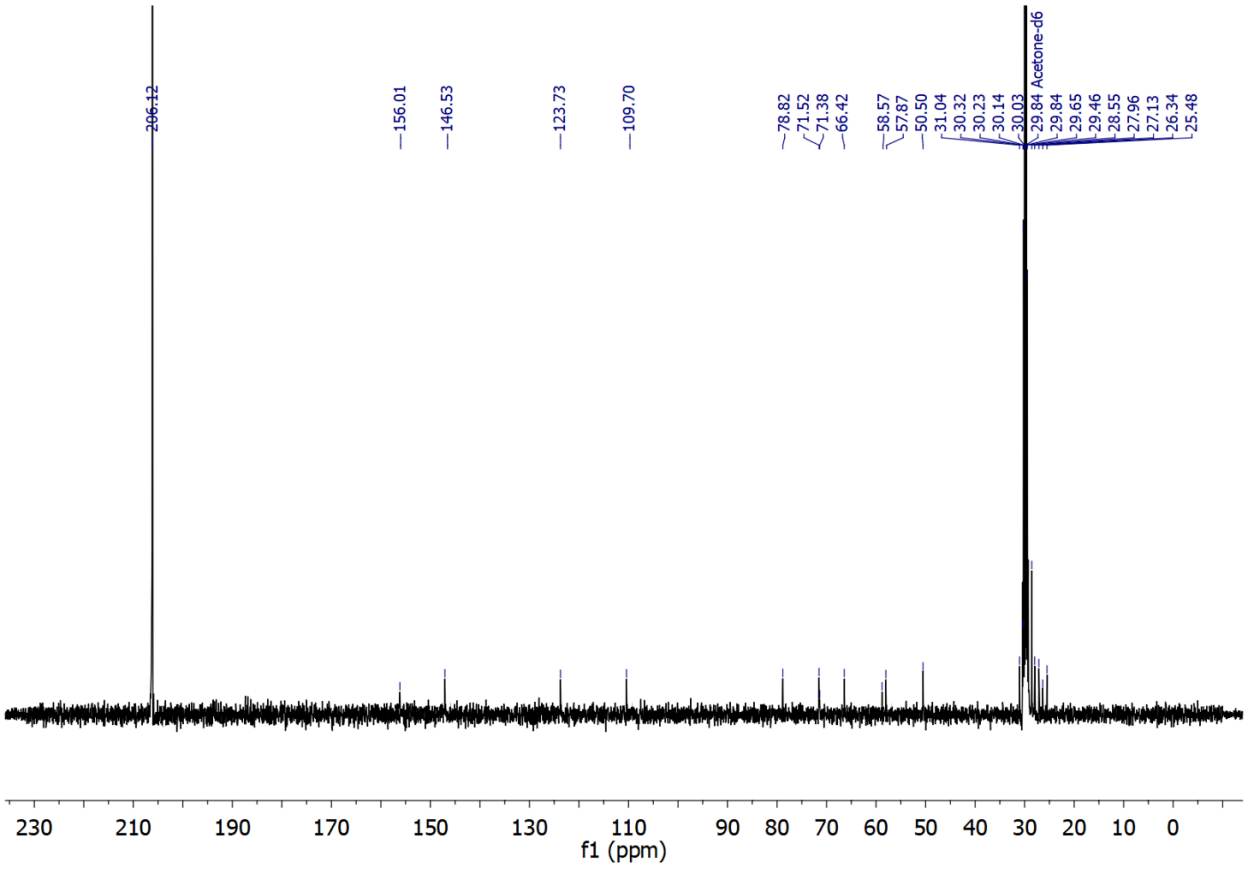


Figure S23. ^{13}C-NMR spectrum in acetone-d6 of compound **30**

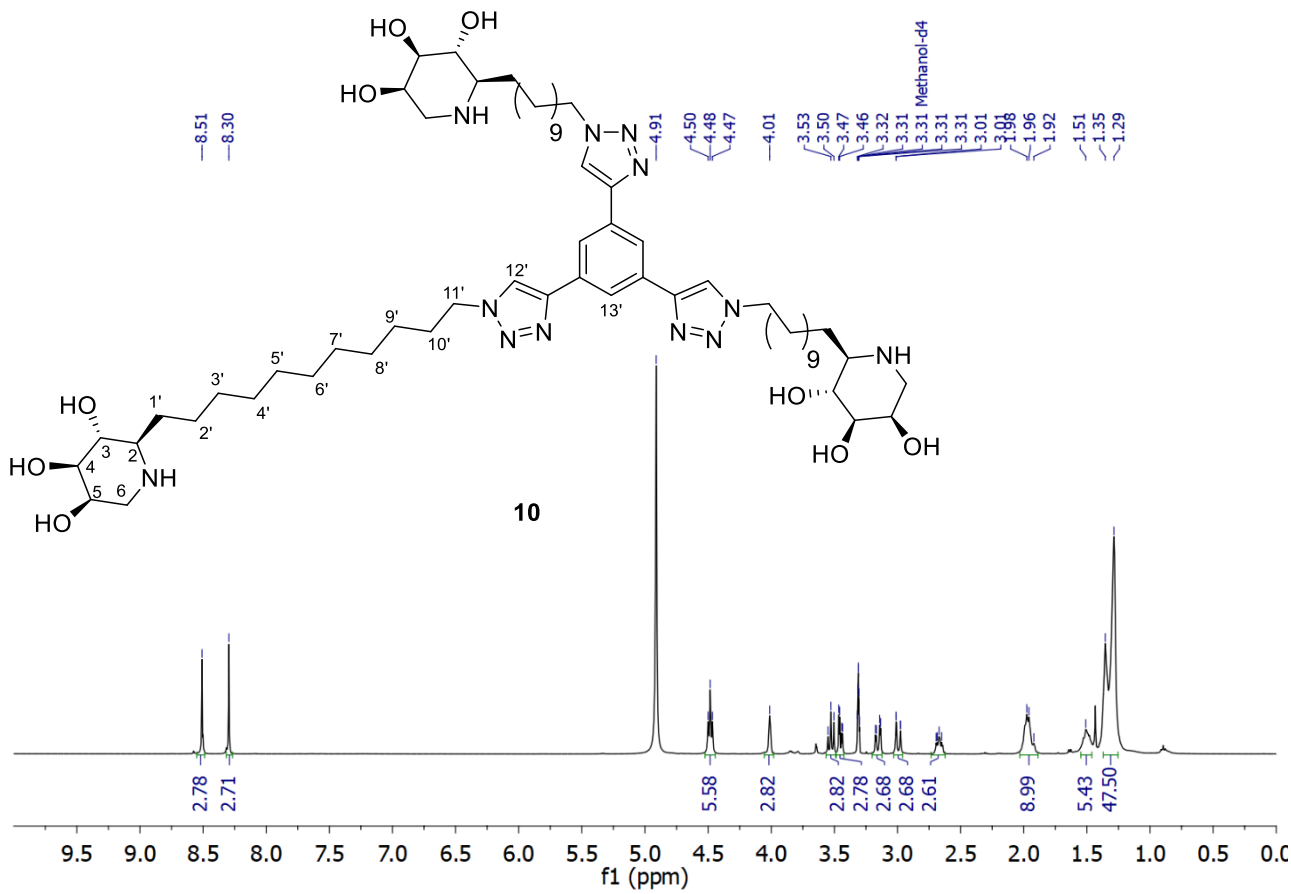


Figure S24. ^{1}H-NMR spectrum in CD_3OD of compound **10**

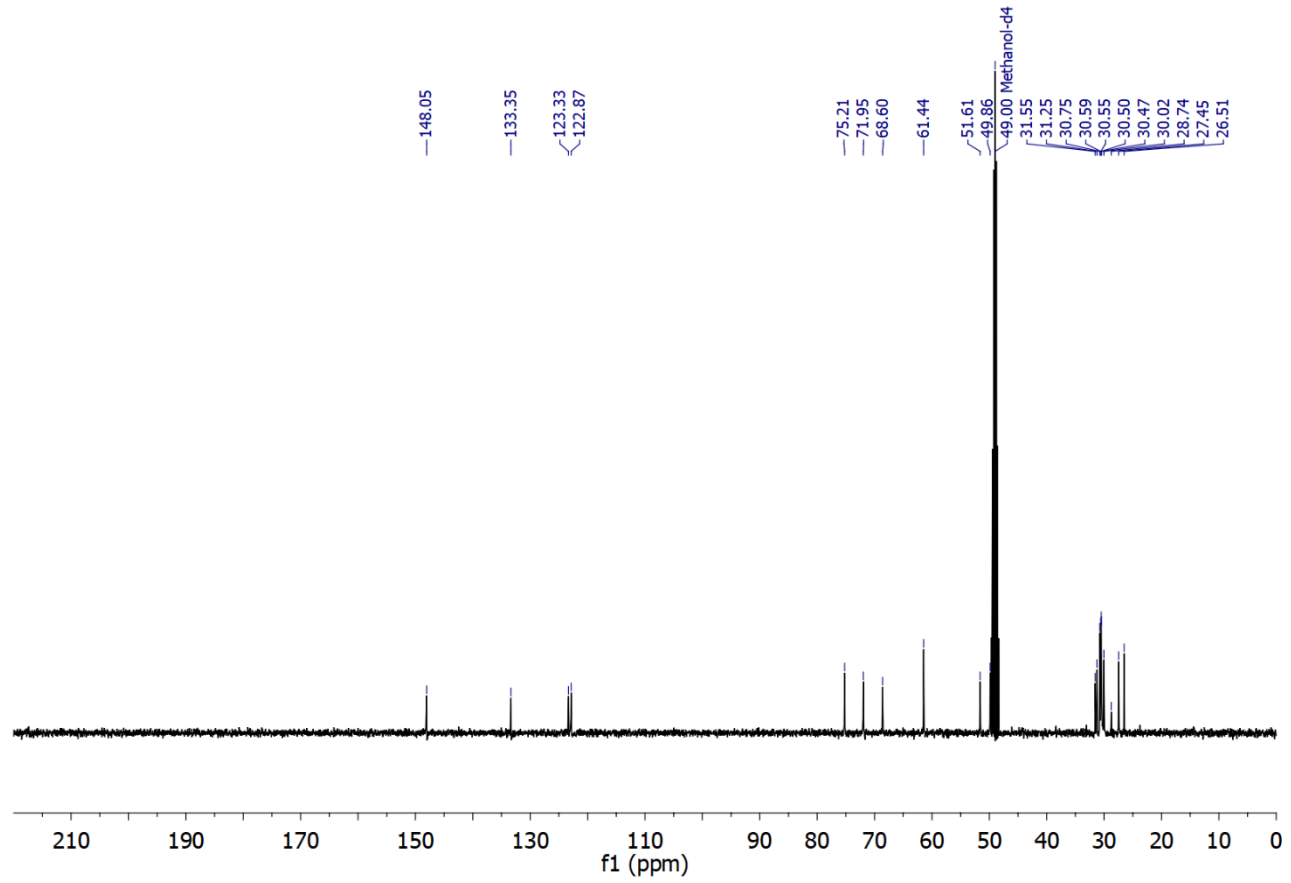


Figure S25. ^{13}C-NMR spectrum in CD_3OD of compound **10**

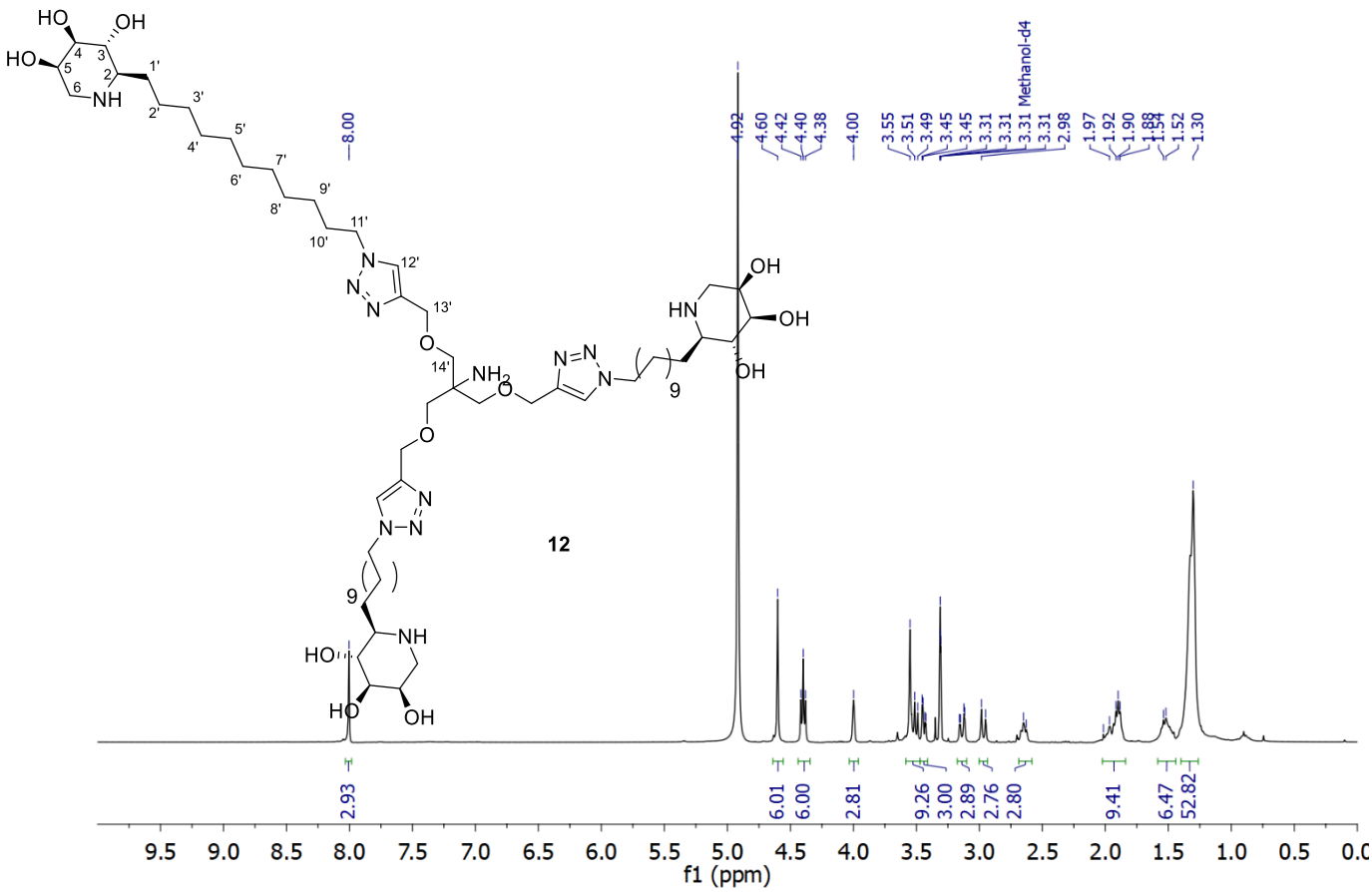


Figure S26. ^{1}H-NMR spectrum in CD_3OD of compound **12**

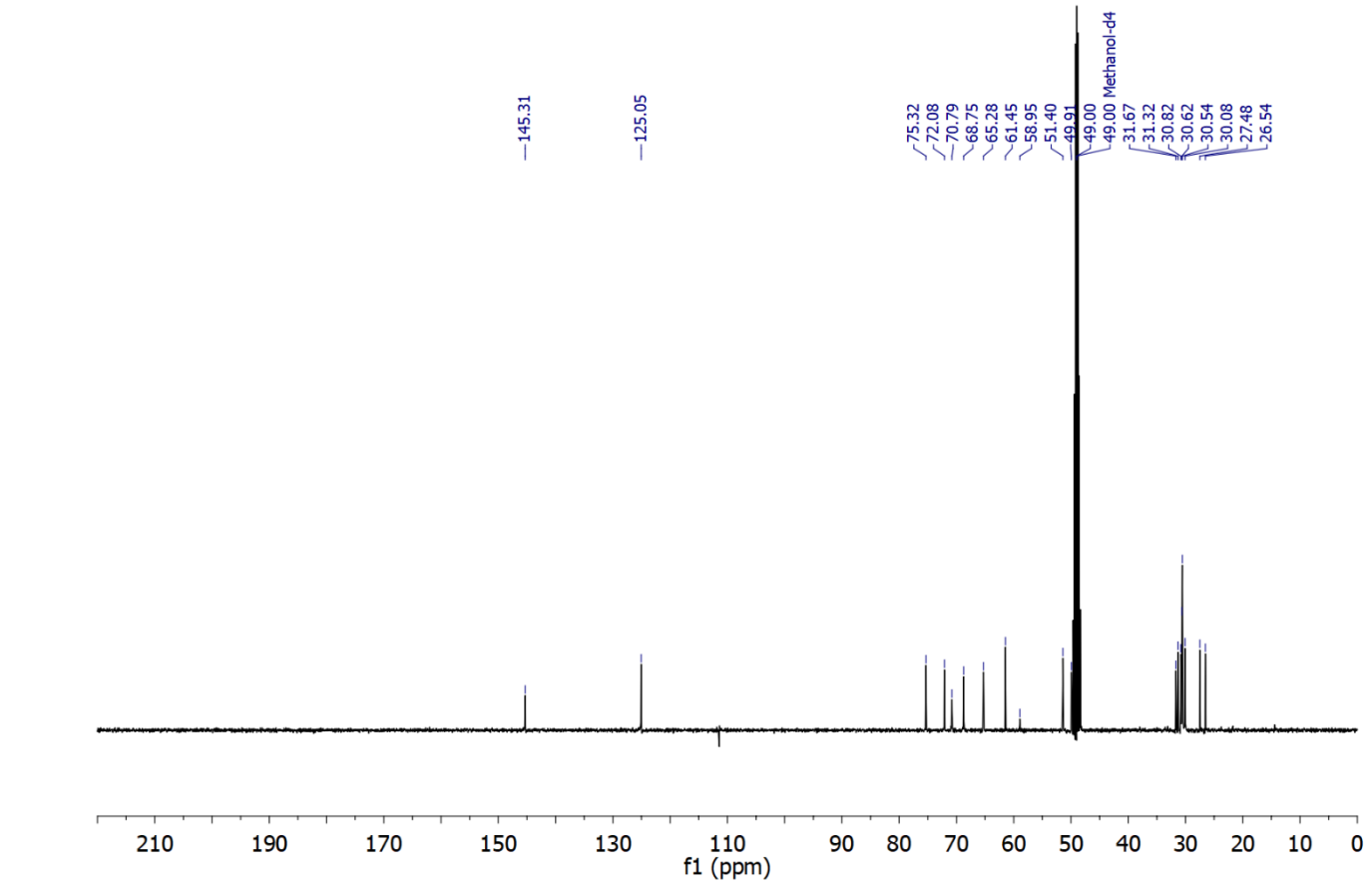


Figure S27. ^{13}C-NMR spectrum in CD_3OD of compound **12**

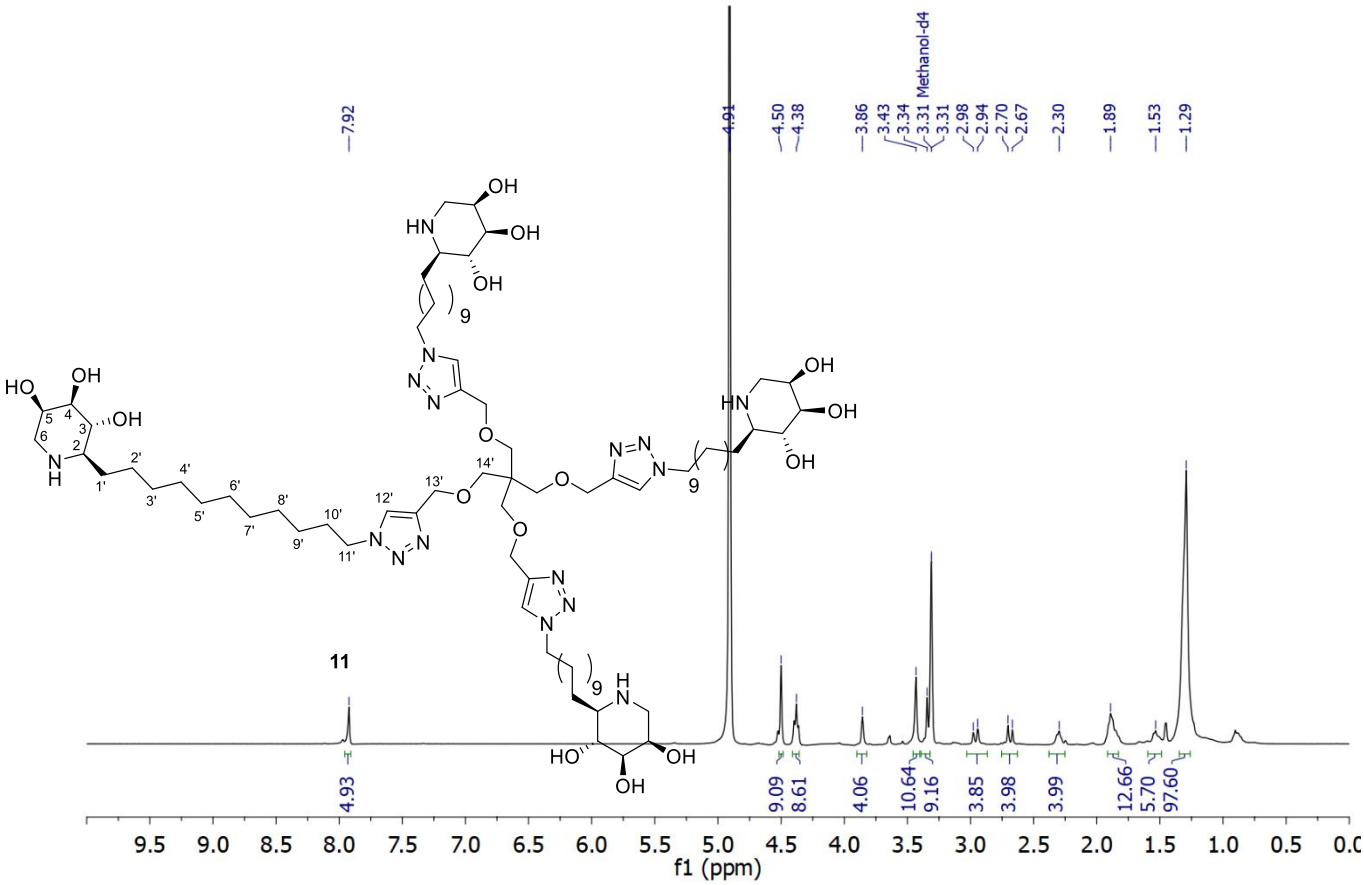


Figure S28. ^{1}H-NMR spectrum in CD_3OD of compound **11**

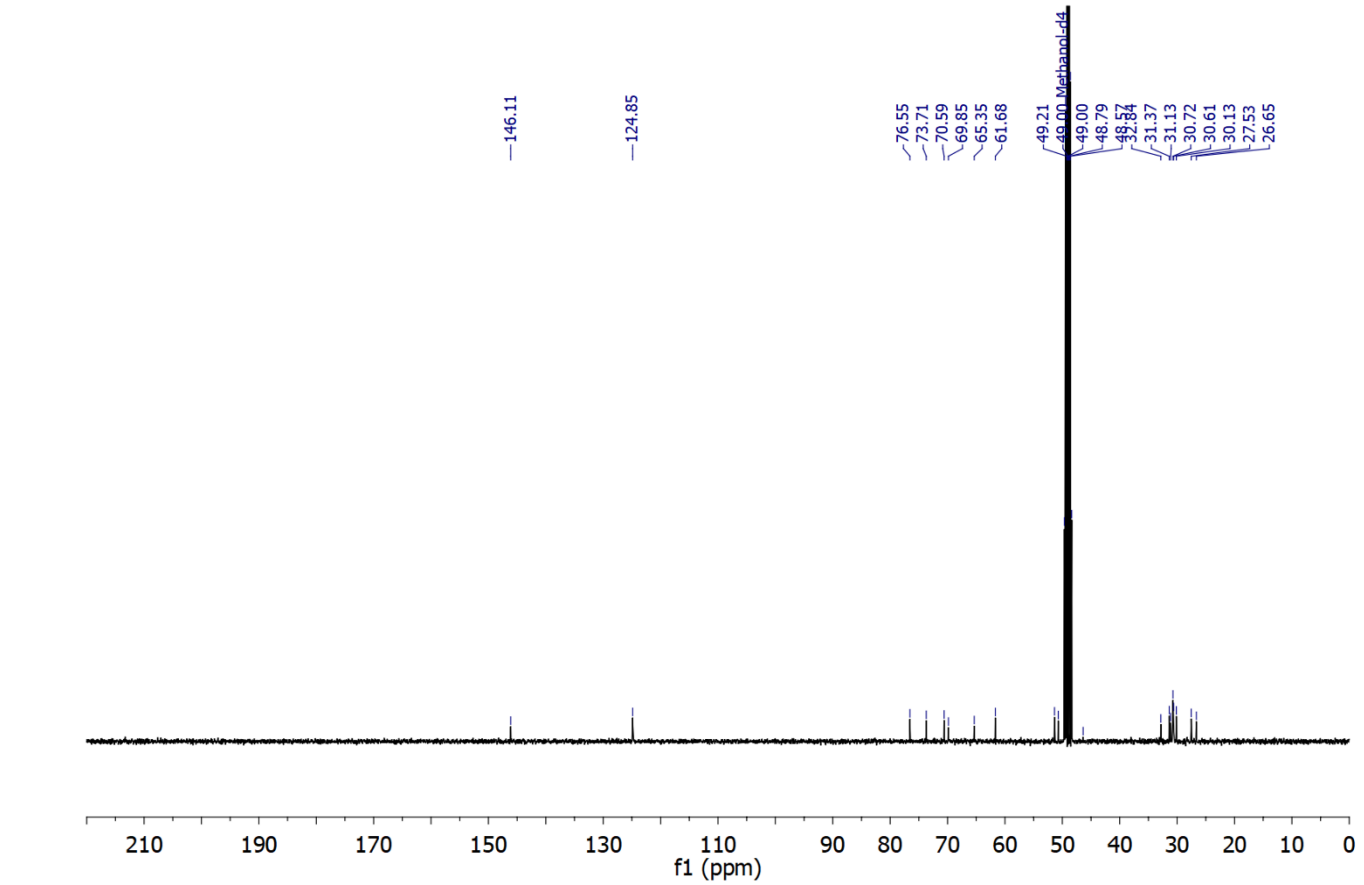


Figure S29. ^{13}C-NMR spectrum in CD_3OD of compound **11**

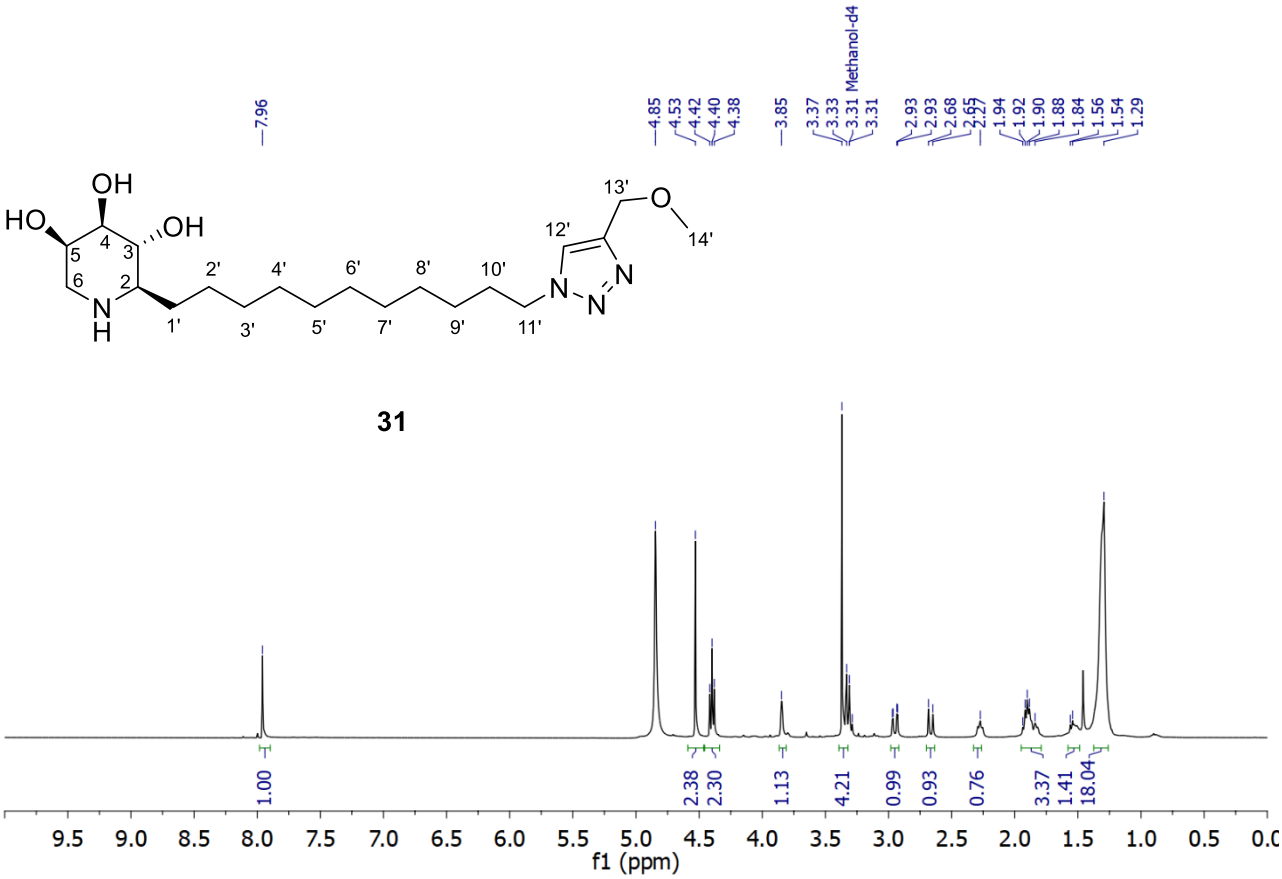


Figure S30. ^{1}H-NMR spectrum in CD_3OD of compound **31**

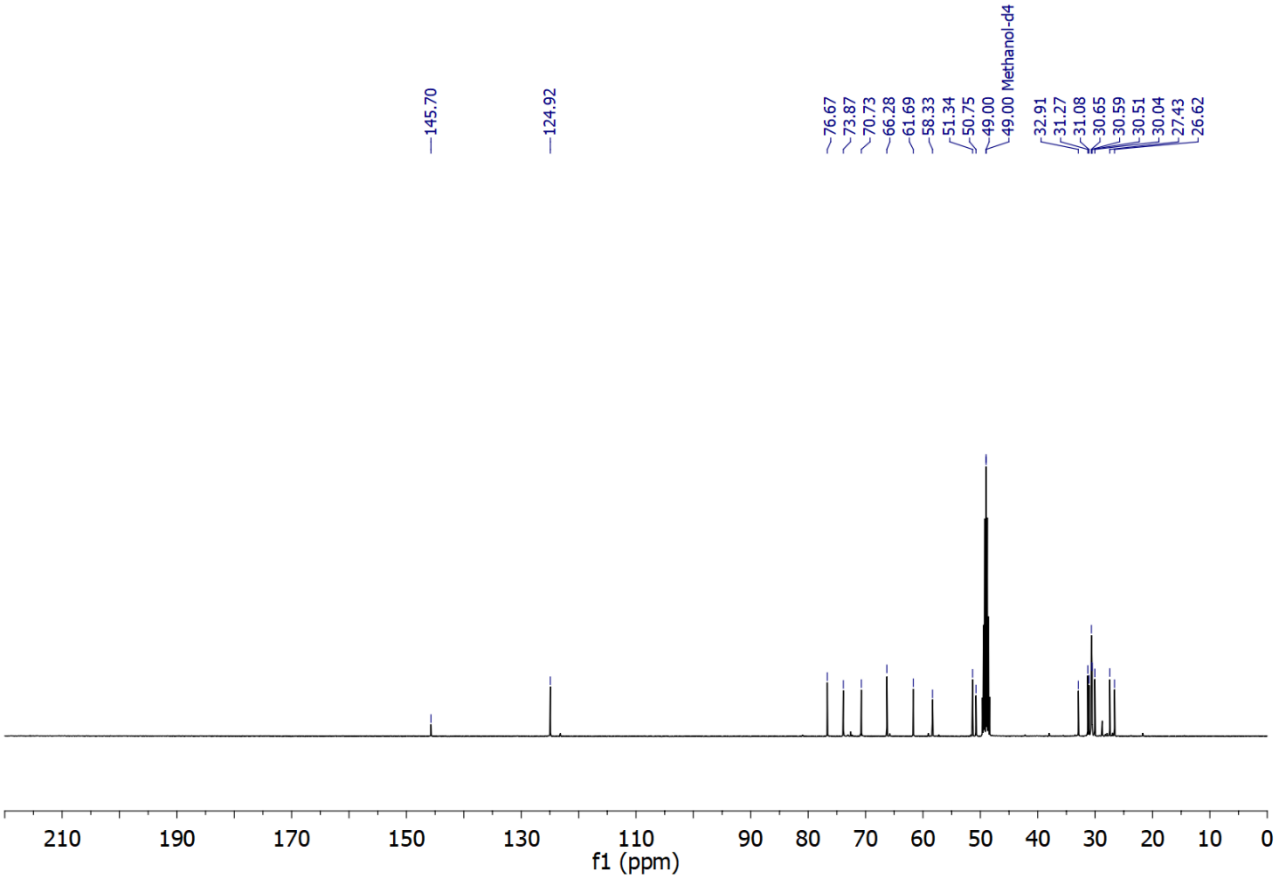


Figure S31. ^{13}C-NMR spectrum in CD_3OD of compound **31**

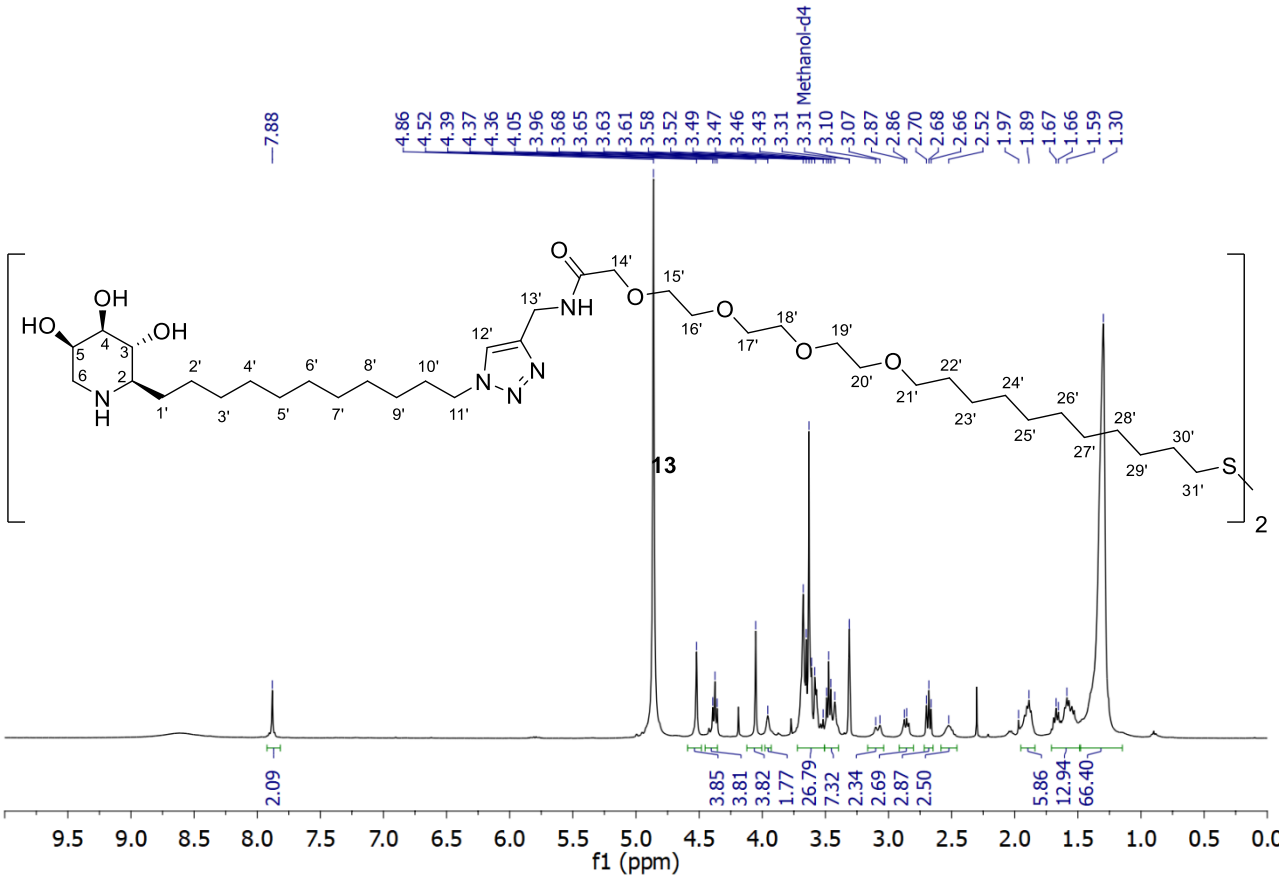


Figure S32. ^{1}H-NMR spectrum in CD_3OD of compound **13**

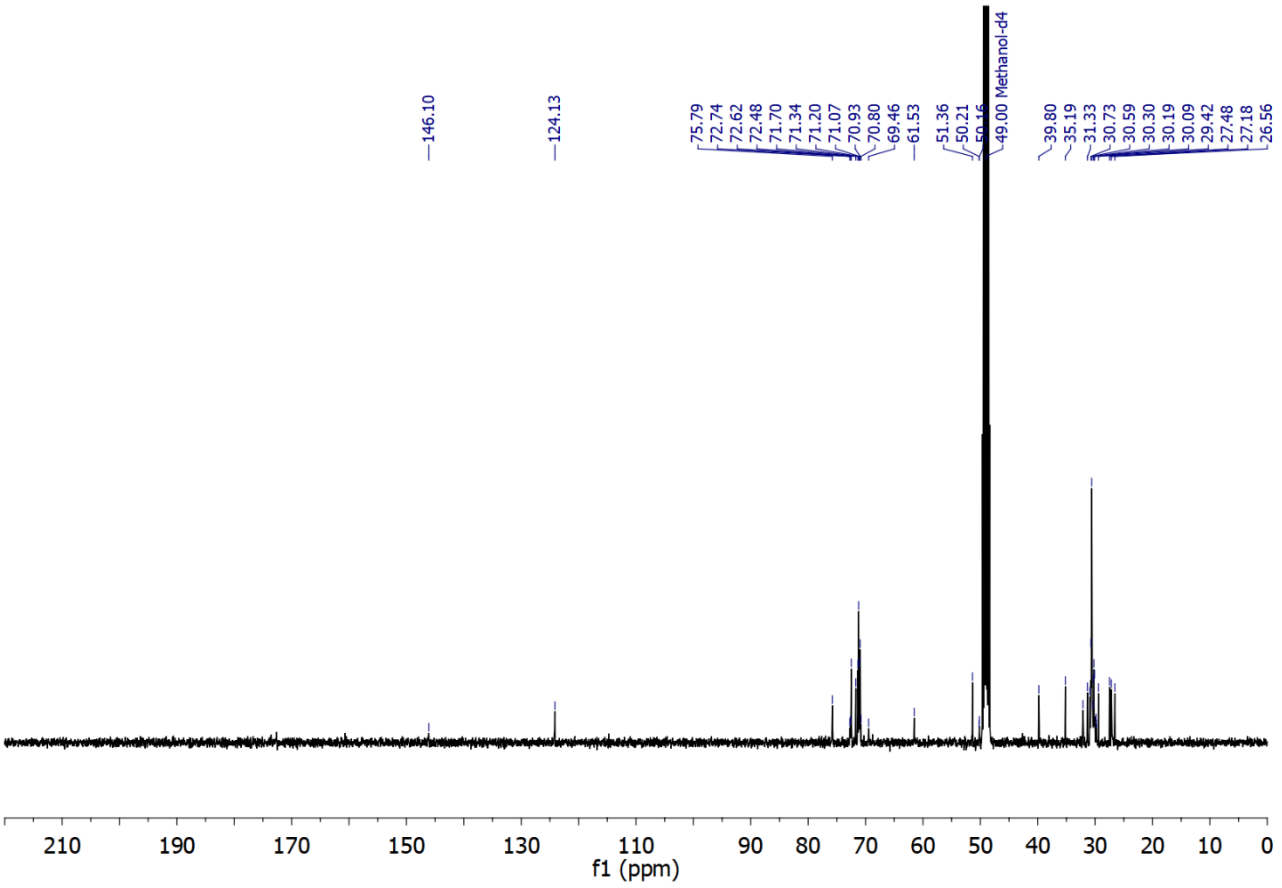
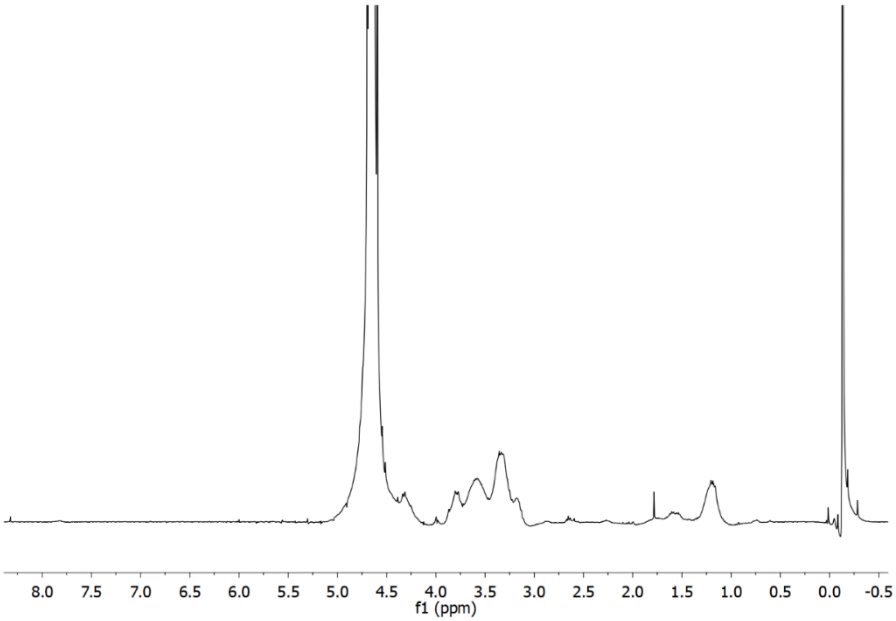
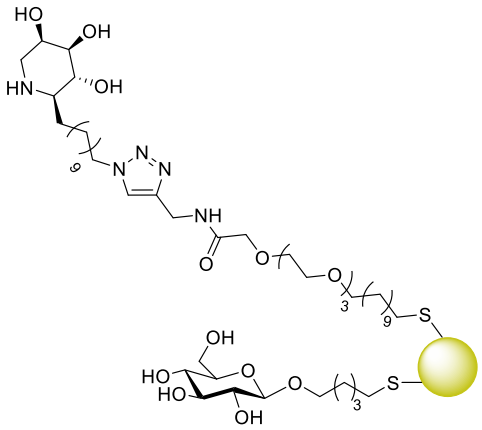


Figure S33. ^{13}C-NMR spectrum in CD_3OD of compound **13**





GNP-1: 20% iminosugar

Figure S34. ^{1}H-NMR spectrum in D_2O of **GNP-1**

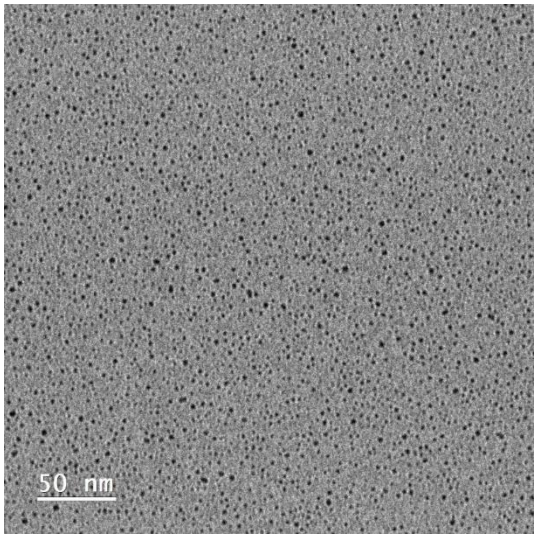


Figure S35. TEM of **GNP-1**

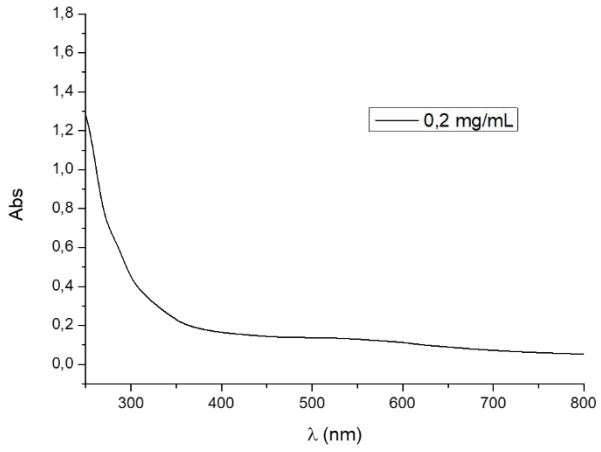
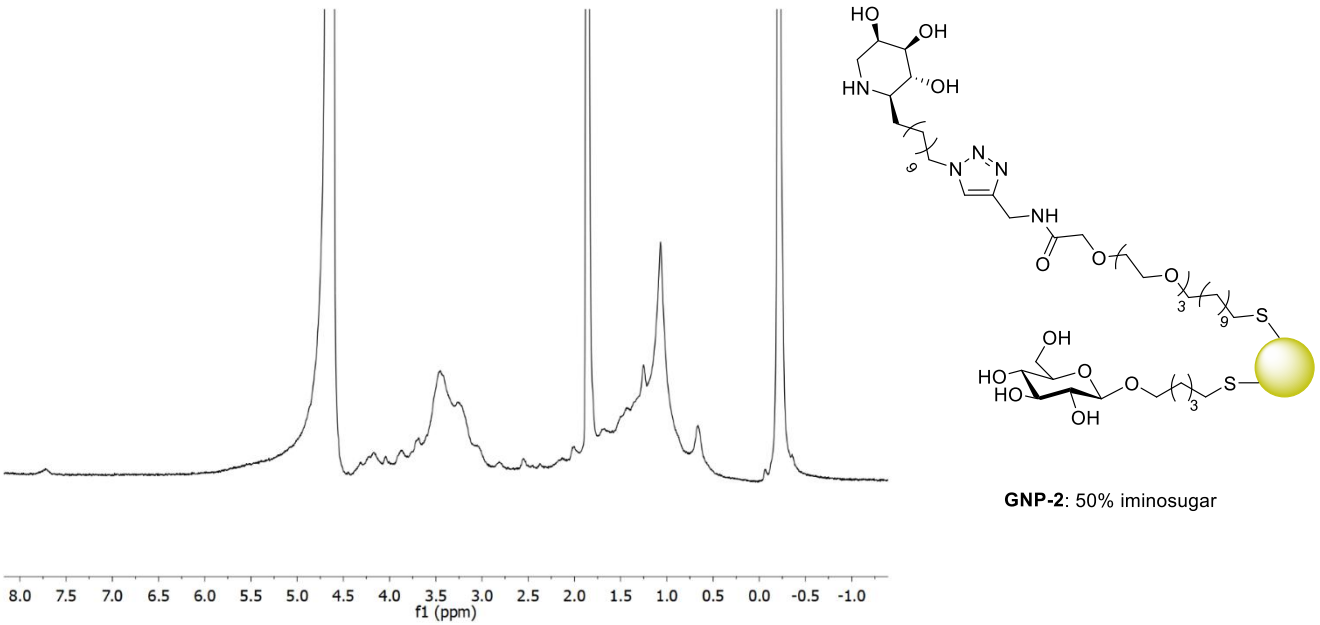


Figure S36. UV-Vis spectrum of **GNP-1**



GNP-2: 50% iminosugar

Figure S37. ^{1}H-NMR spectrum in D_2O and acetic acid-d4 of compound **GNP-2**

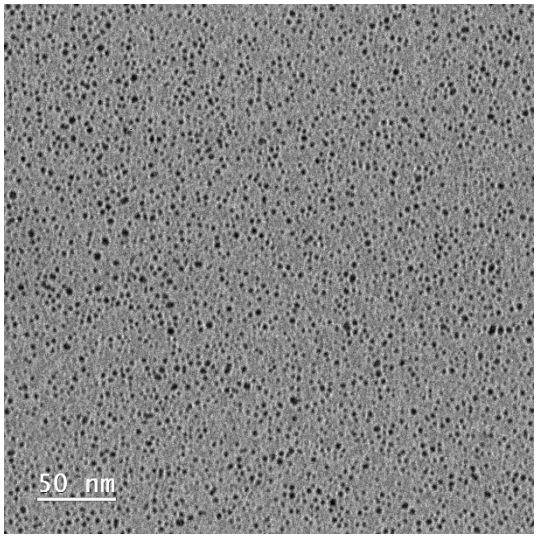


Figure S38. TEM of **GNP-2**

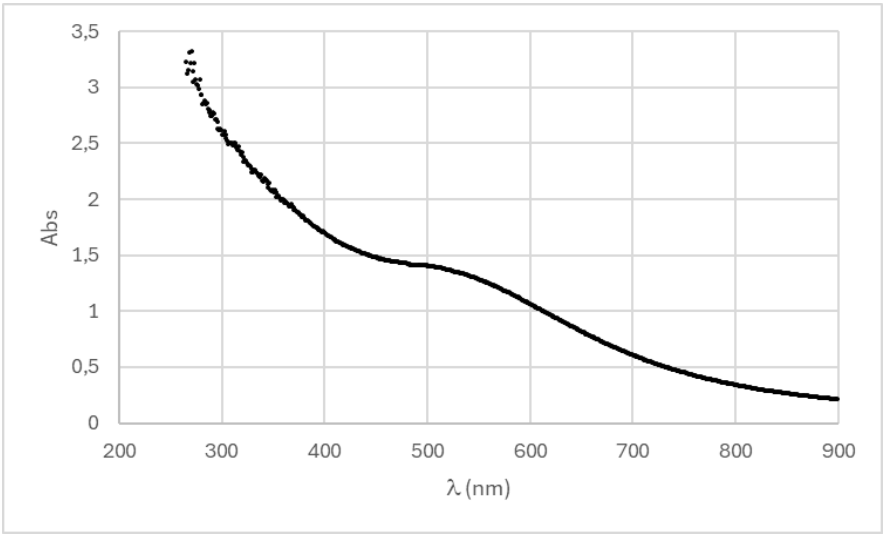


Figure S39. UV-Vis spectrum of **GNP-2**

Biochemical Characterization

-Inhibitory activity towards human GCase from leukocytes isolated from healthy donors

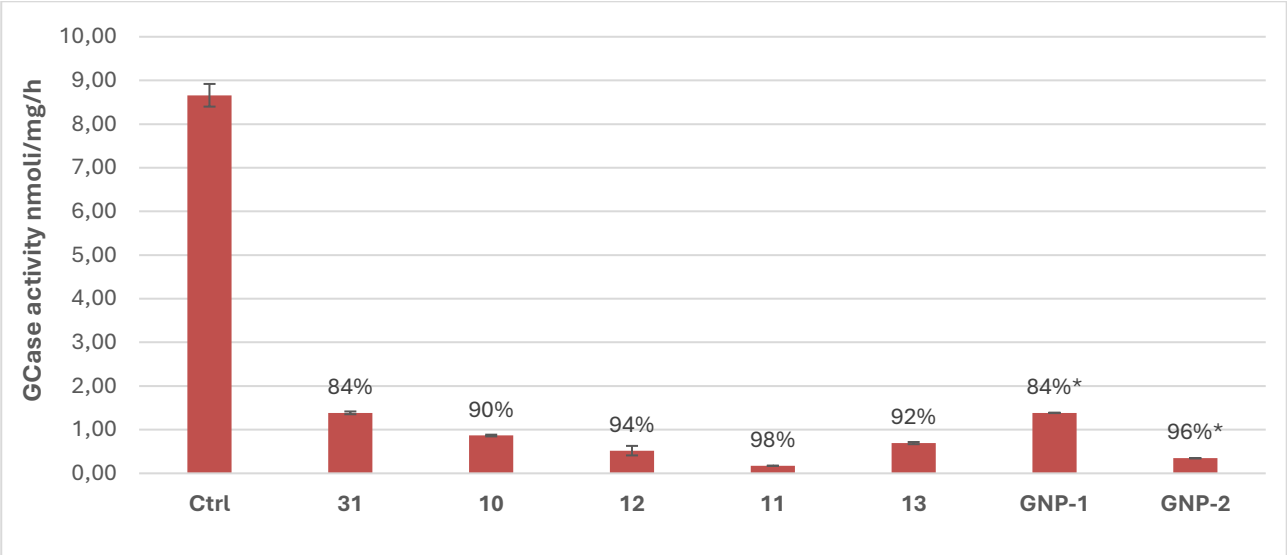
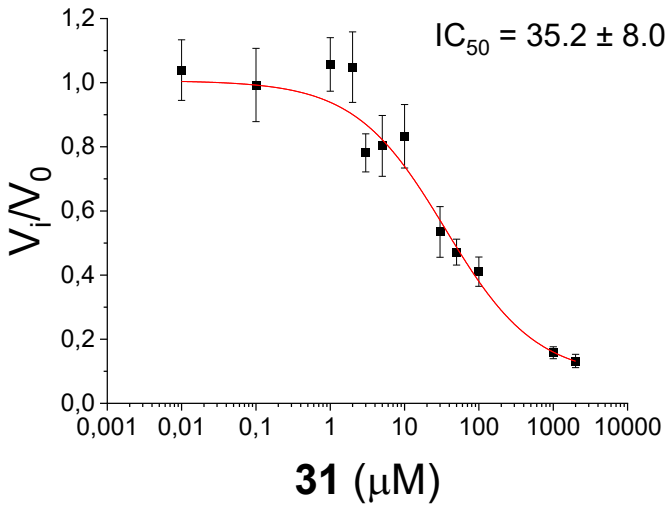
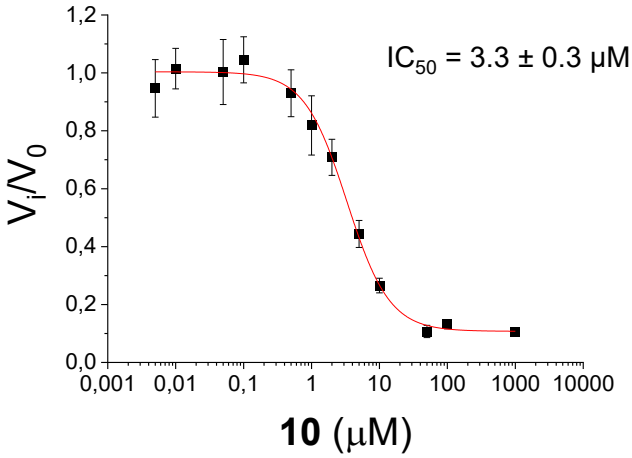


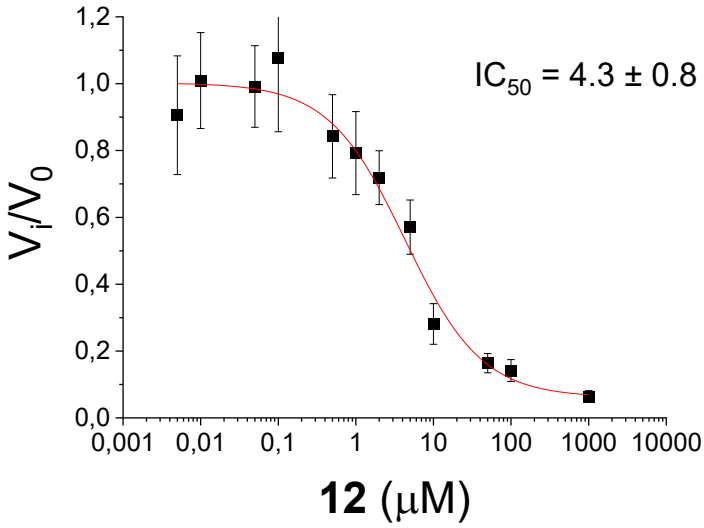
Figure S40. Activity of GCase in the presence of compounds **10-13** and **31** at 1 mM and AuNPs **GNP-1** and **GNP-2** at 0.4 mg/mL*. The corresponding calculated percentage of inhibition is indicated above each bar.

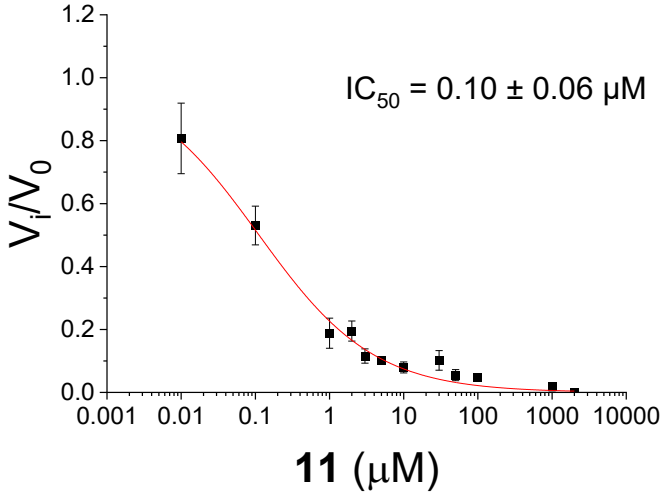
-The IC_{50} values of inhibitors against GCase were determined by measuring the initial hydrolysis rate with 4-methylumbelliferyl-β-D- glucopyranoside (3.33 mM). Data obtained were fitted to the following equation using the Origin Microcal program.

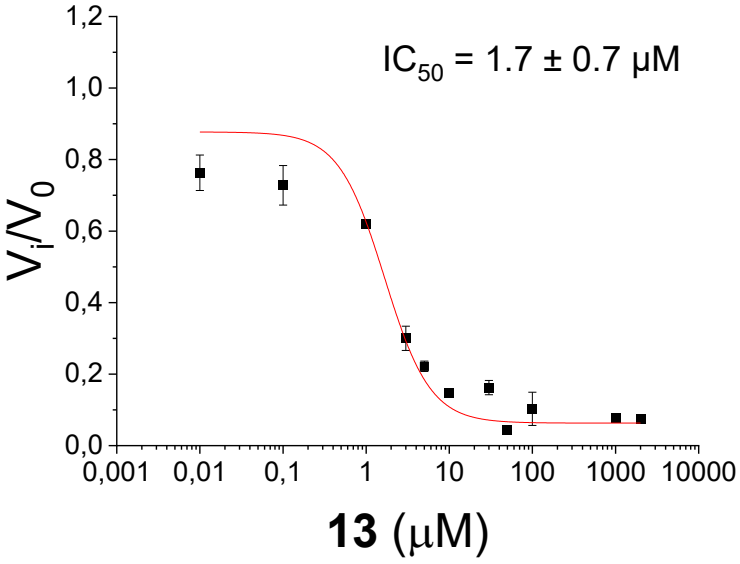
$$\frac{Vi}{Vo} = \frac{Max - Min}{1 + \left(\frac{x}{IC_{50}}\right)^{slope}} + Min$$

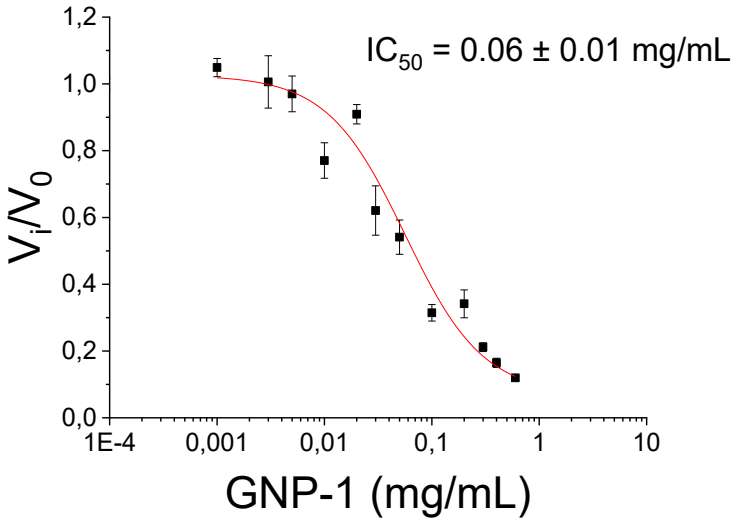


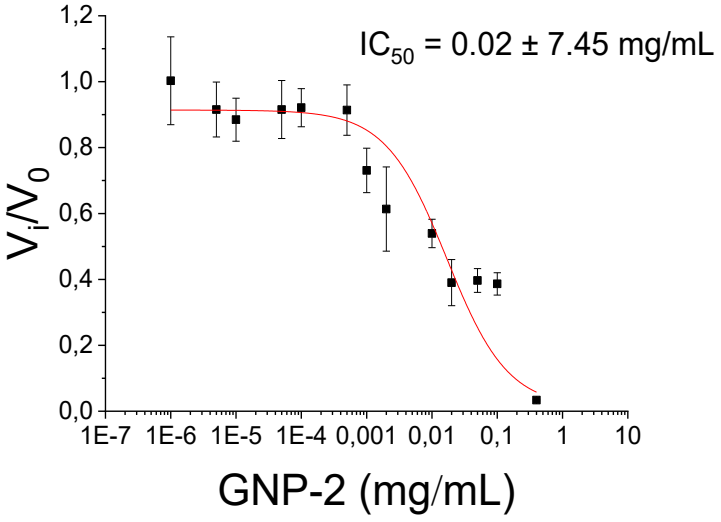

IC50 = 3.3 ± 0.3 μM
Vi/V0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,001 0,01 0,1 1 10 100 1000 10000
10 (μM)


IC50 = 4.3 ± 0.8 μM
Vi/V0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,001 0,01 0,1 1 10 100 1000 10000
12 (μM)


IC50 = 0.10 ± 0.06 μM
Vi/V0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.001 0.01 0.1 1 10 100 1000 10000
11 (μM)


IC50 = 1.7 ± 0.7 µM
Vi/V0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,001
0,01
0,1
1
10
100
1000
10000
13 (µM)


IC50 = 0.06 ± 0.01 mg/mL
Vi/V0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1E-4
0,001
0,01
0,1
1
10
GNP-1 (mg/mL)


IC50 = 0.02 ± 7.45 mg/mL
Vi/V0
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1E-7
1E-6
1E-5
1E-4
0,001
0,01
0,1
1
10
GNP-2 (mg/mL)

Chaperoning activity assays

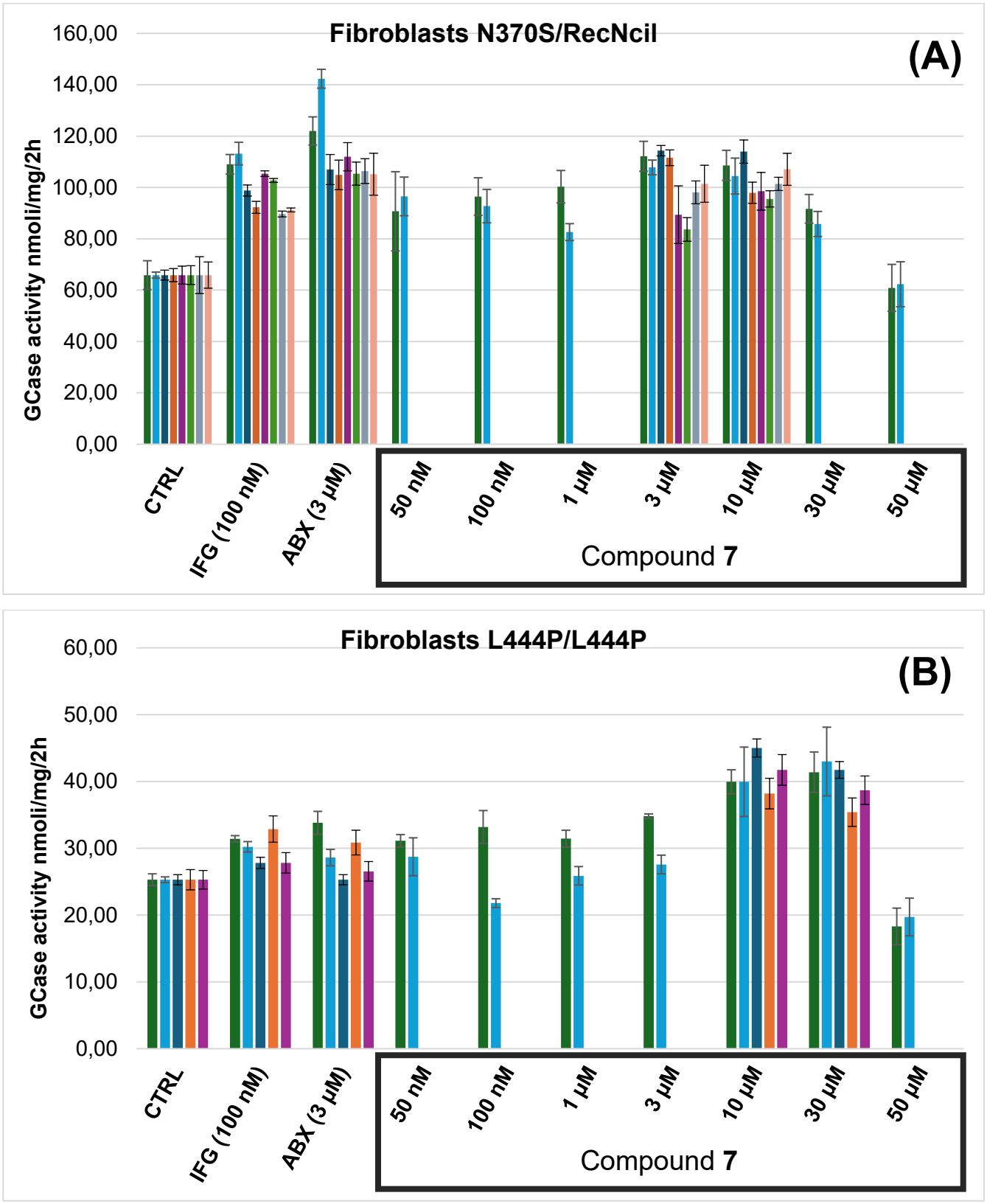


Figure S41. Fibroblasts derived from GD patients bearing A) N370S/RecNcil and B) L444P/L444P mutations were incubated without (control, CTRL) or with 7 different nominal concentrations (50 nM - 50 µM) of compound **7** using isofagomine (IFG; 100 nM) and ambroxol (ABX; 3 µM) as control. After 4 days, the GCase activity was determined in intact cell as describe. The GCase enzyme activity data in CTRL fibroblasts were expressed as mean ± standard deviation (n=8), while in treated fibroblasts the data were expressed as mean ± standard deviation (n=4). Different colors represent independent experiments.

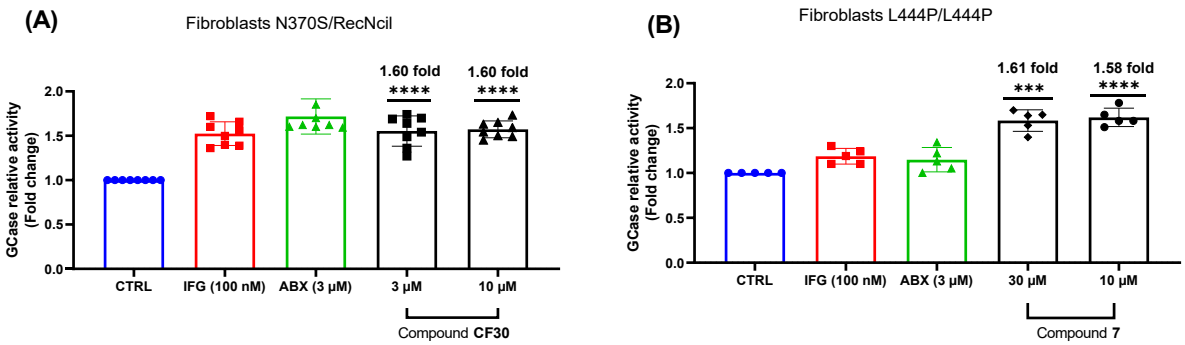


Figure S42. **GCase relative activity** is expressed as the ratio of GCase activity measured in human fibroblasts derived from GD patients carrying the N370S/RecNcil (**A**) and L444P/L444P (**B**) mutations after 4 days of incubation with **7**, to the GCase activity of the corresponding untreated control. Isofagomine (IFG, 100 nM) and ambroxol (ABX, 3 µM) were used as reference compounds. Statistical significance was determined using Student's t-test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Each data point represents the mean of octuplicate measurements for control (Ctrl) samples and triplicate measurements for compound-treated samples, obtained from N = 2 independent experiments.

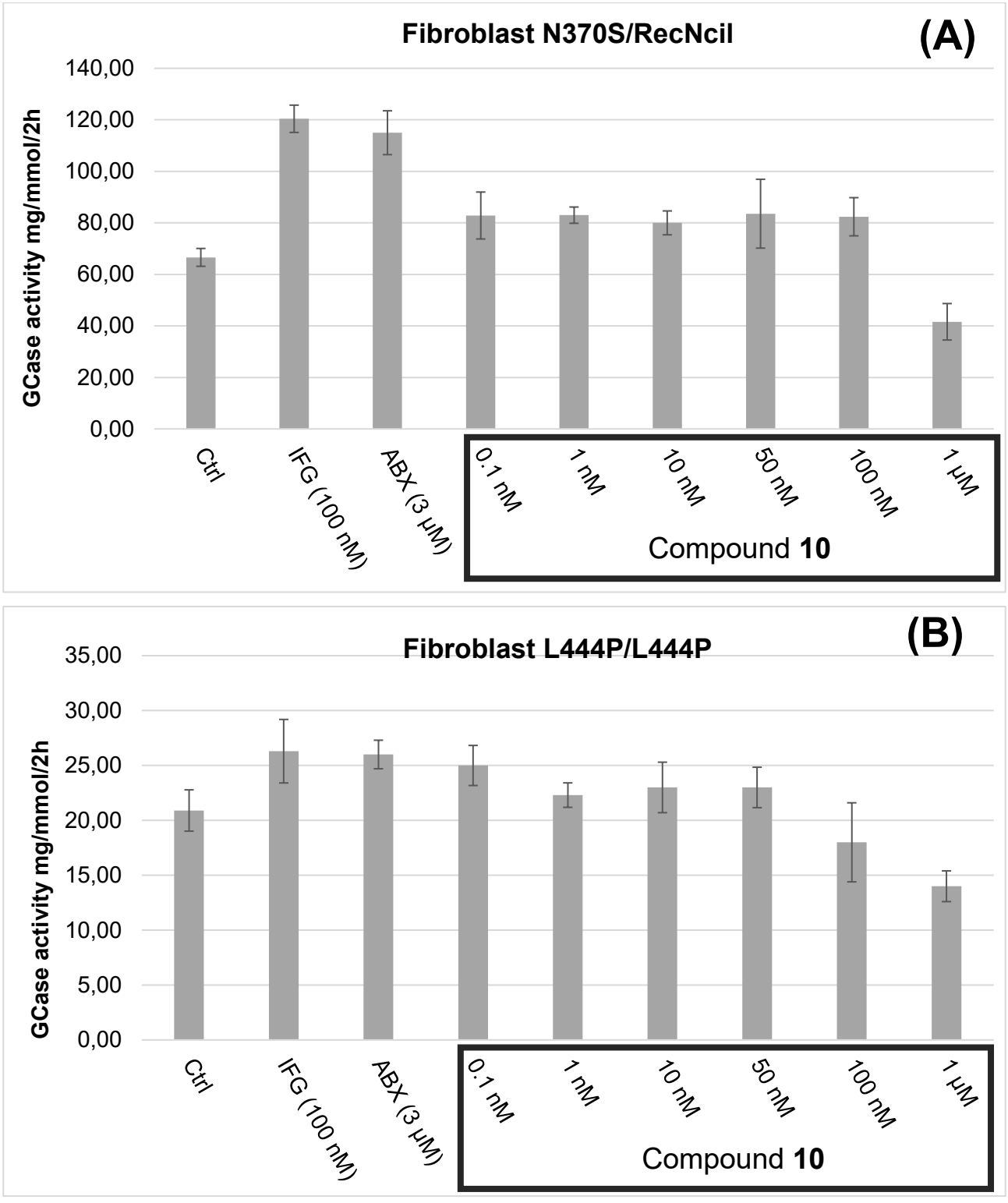


Figure S43. Fibroblasts derived from GD patients bearing A) N370S/RecNcil and B) L444P/L444P mutations were incubated without (control, CTRL) or with 6 different nominal concentrations (0.1 nM - 1 µM) of compound **10** using isofagomine (IFG; 100 nM) and ambroxol (ABX; 3 µM) as control. After 4 days, the GCase activity was determined in intact cell as describe. The GCase enzyme activity data in CTRL fibroblasts were expressed as mean ± standard deviation (n=8), while in treated fibroblasts the data were expressed as mean ± standard deviation (n=5).

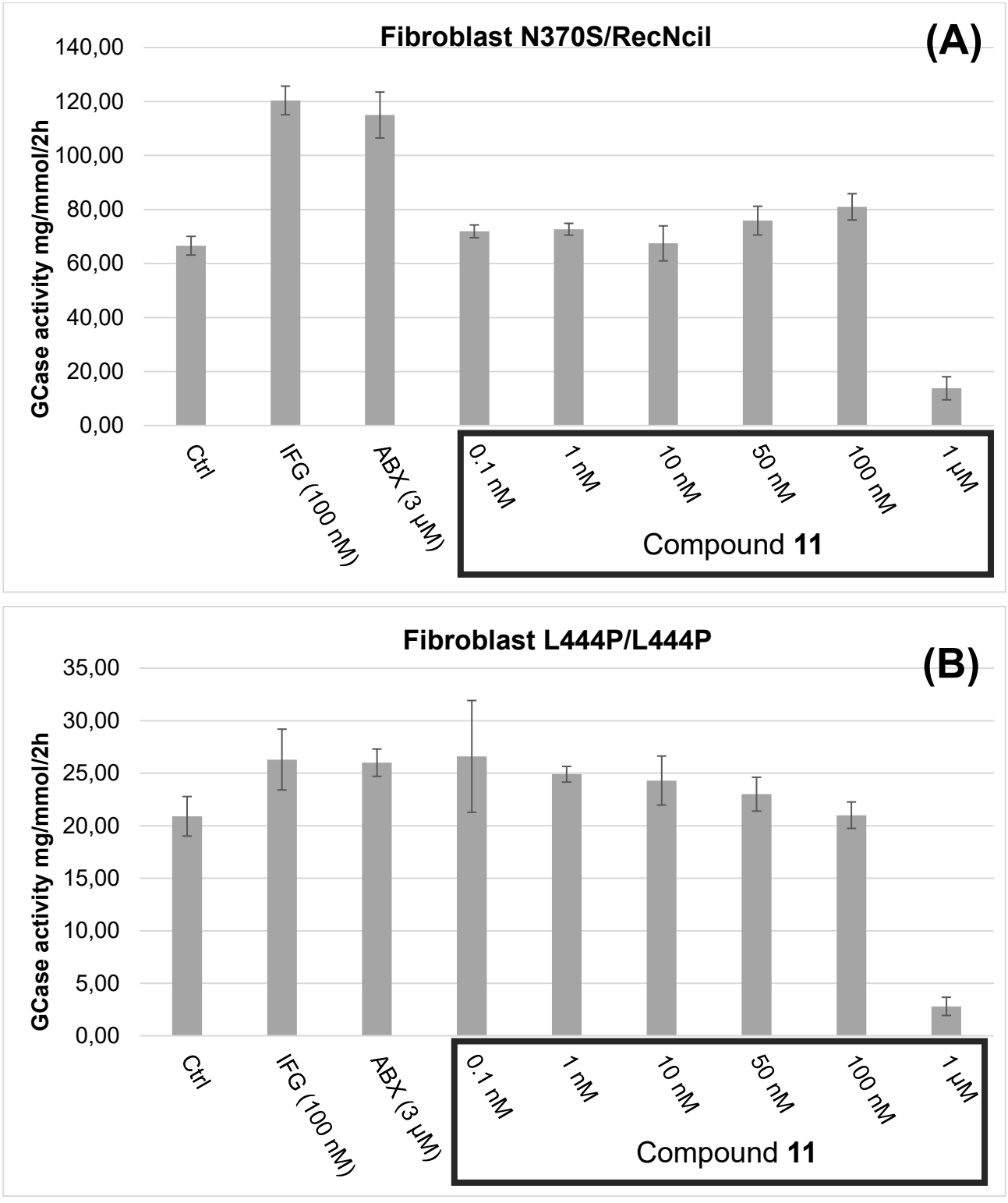
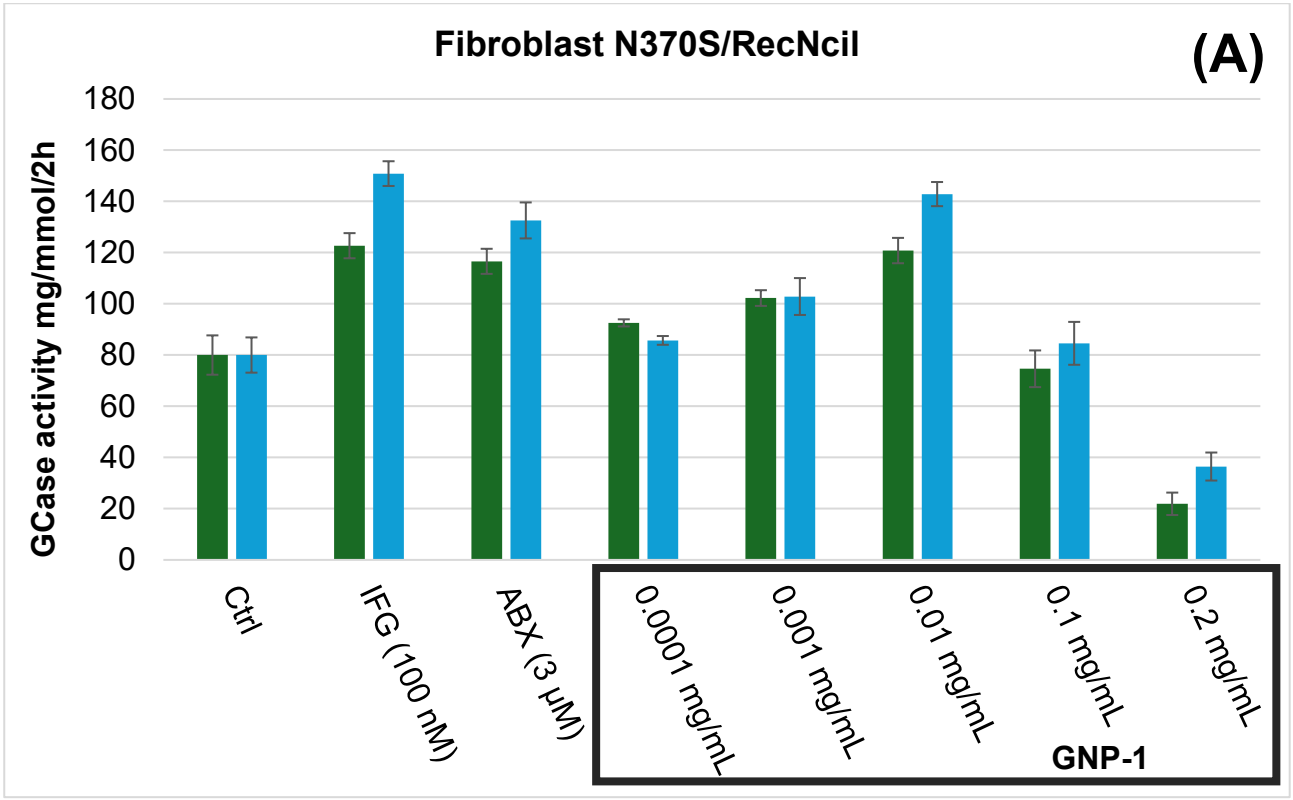


Figure S44. Fibroblasts derived from GD patients bearing A) N370S/RecNcil and B) L444P/L444P mutations were incubated without (control, CTRL) or with 6 different nominal concentrations (0.1 nM - 1 µM) of compound **11** using isofagomine (IFG; 100 nM) and ambroxol (ABX; 3 µM) as control. After 4 days, the GCase activity was determined in intact cell as describe. The GCase enzyme activity data in CTRL fibroblasts were expressed as mean ± standard deviation (n=8), while in treated fibroblasts the data were expressed as mean ± standard deviation (n=5).



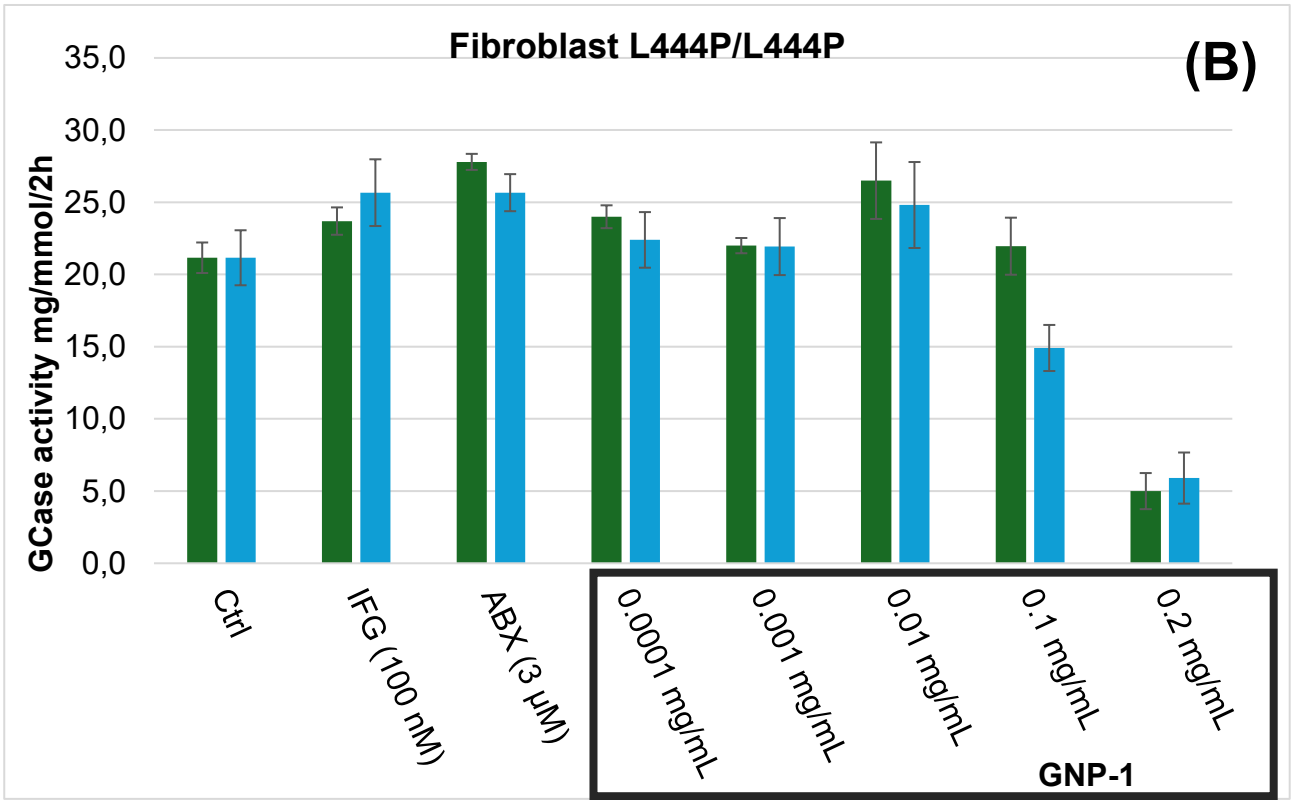


Figure S45. Fibroblasts derived from GD patients bearing A) N370S/RecNcil and B) L444P/L444P mutations were incubated without (control, CTRL) or with 5 different nominal concentrations (0.1 µM/mL – 0.2 mg/mL) of compound AuNPs **GNP-1** using isofagomine (IFG; 100 nM) and ambroxol (ABX; 3 µM) as control. After 4 days, the GCase activity was determined in intact cell as describe. The GCase enzyme activity data in CTRL fibroblasts were expressed as mean ± standard deviation (n=8), while in treated fibroblasts the data were expressed as mean ± standard deviation (n=3). Different colors represent independent experiments.

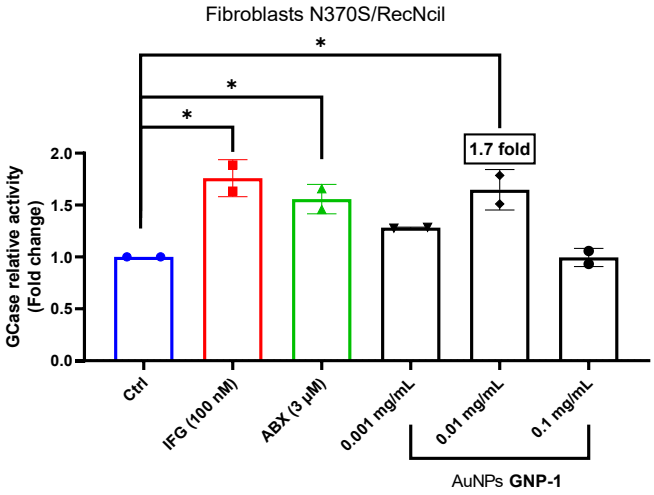


Figure S46. **GCase relative activity** is expressed as the ratio of GCase activity measured in human fibroblasts derived from GD patients carrying the N370S/RecNciI mutations after 4 days of incubation with AuNPs **GNP-1**, to the GCase activity of the corresponding untreated control. Isofagomine (IFG, 100 nM) and ambroxol (ABX, 3 µM) were used as reference compounds. Statistical significance was determined using Student's t-test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Each data point represents the mean of octuplicate measurements for control (Ctrl) samples and triplicate measurements for compound-treated samples, obtained from N = 2 independent experiments.