

SUPPORTING INFORMATION

Evaluation of azido 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) analogues for click chemistry-mediated metabolic labelling of *Myxococcus xanthus* DZ2 lipopolysaccharide

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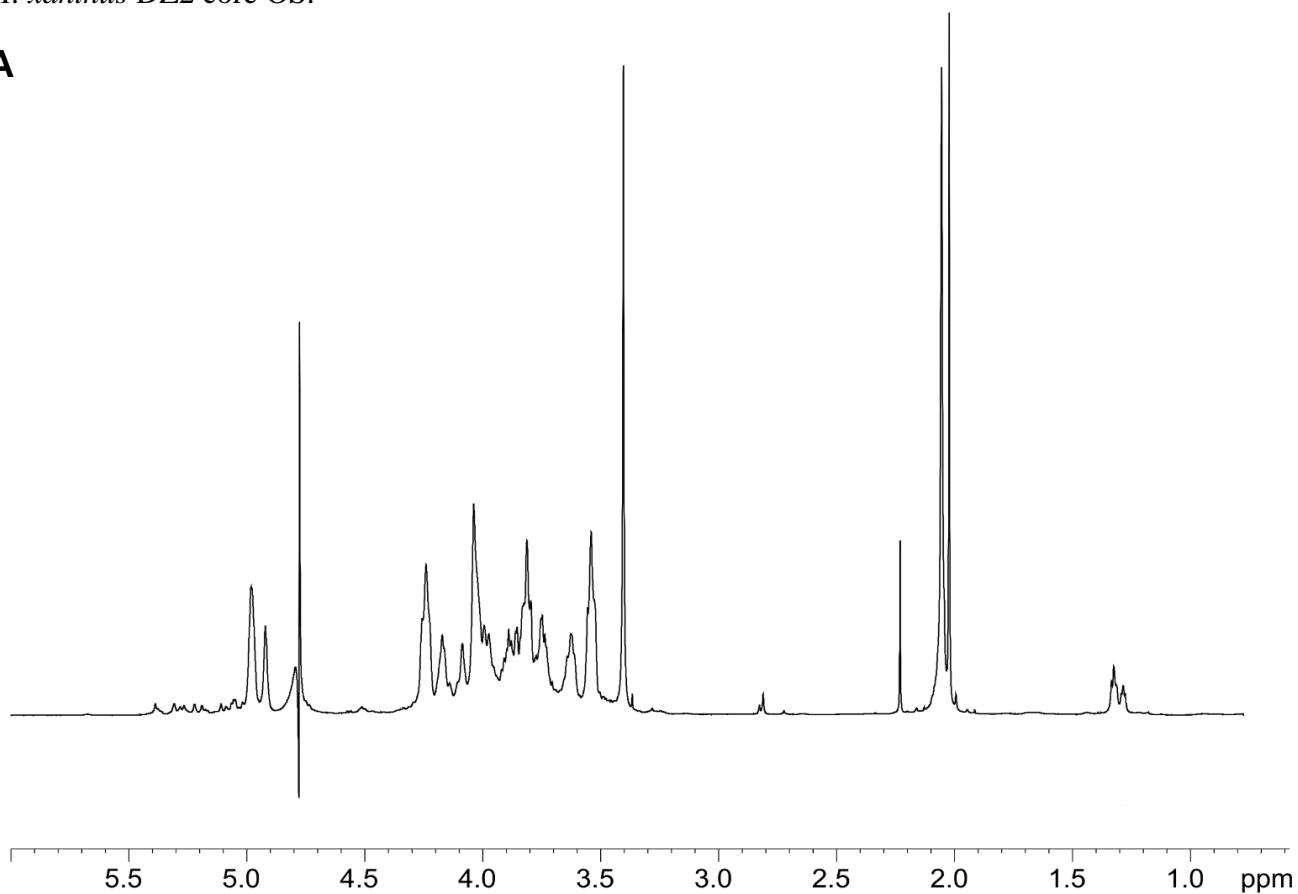
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Figure S1. (A) ^1H NMR spectrum of *M. xanthus* DZ2 O antigen. (B) Negative-mode ESI MS of the *M. xanthus* DZ2 core OS.

A



B

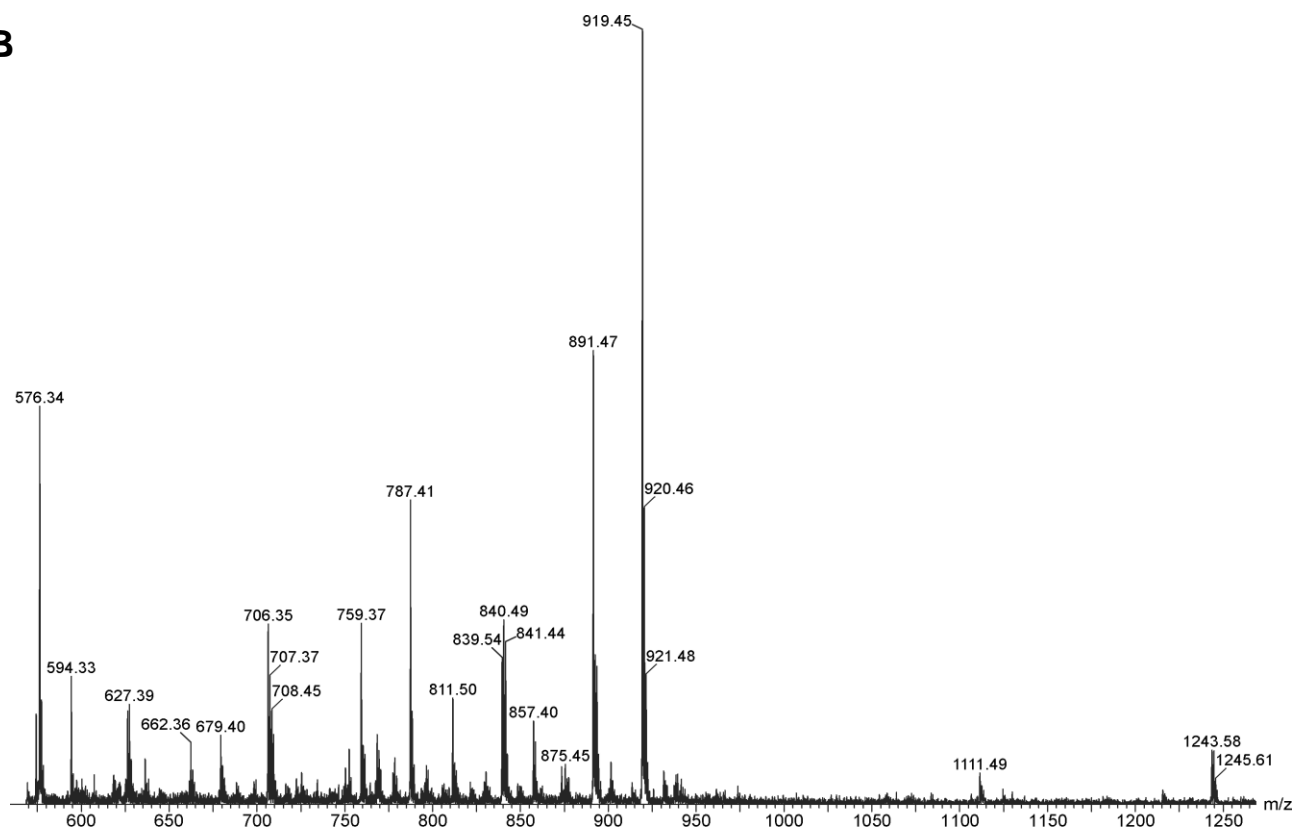
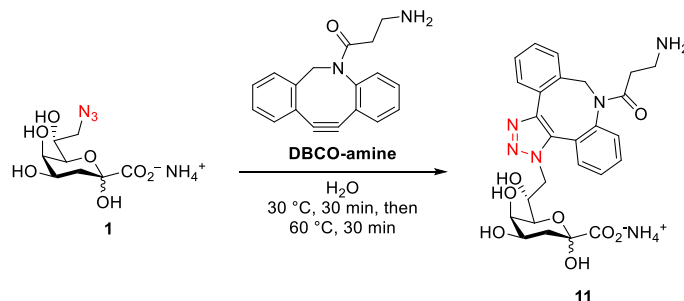
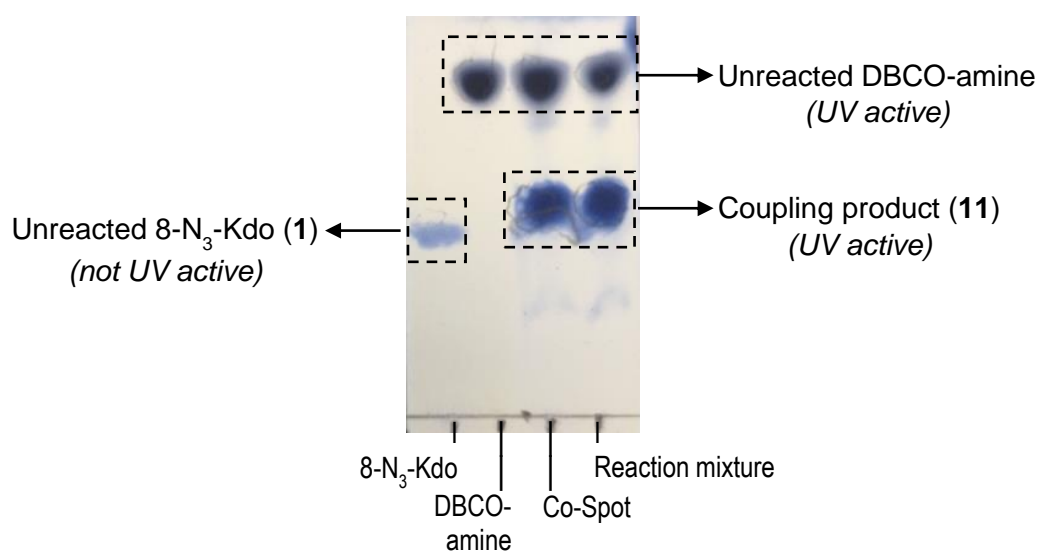


Figure S2. SPAAC reaction of 8-N₃-Kdo with DBCO-amine. (A) Reaction scheme. (B) Monitoring via TLC of published reaction conditions (i.e. 30 °C for 30 min, then 60 °C for 30 min). (C) Time-course monitoring via TLC of reaction conditions for live-cell labelling (i.e. 32 °C for 30 min).

A



B



C

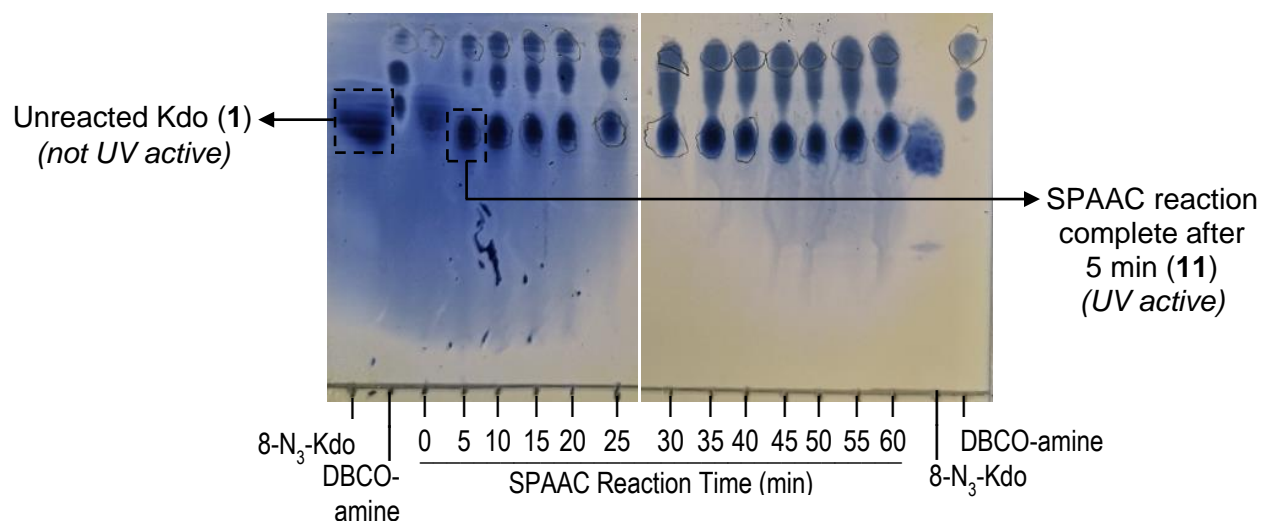
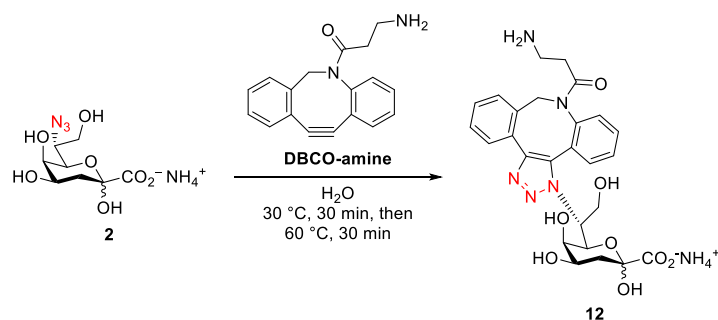
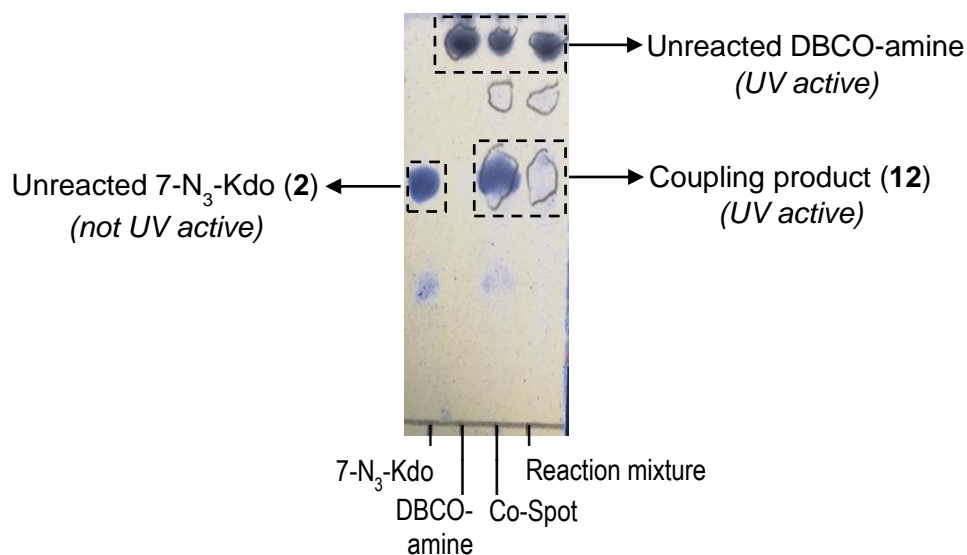


Figure S3. SPAAC reaction of 7-N₃-Kdo with DBCO-amine. (A) Reaction scheme. (B) Monitoring via TLC of published reaction conditions (i.e. 30 °C for 30 min, then 60 °C for 30 min). (C) Time-course monitoring via TLC of reaction conditions for live-cell labelling (i.e. 32 °C for 30 min).

A



B



C

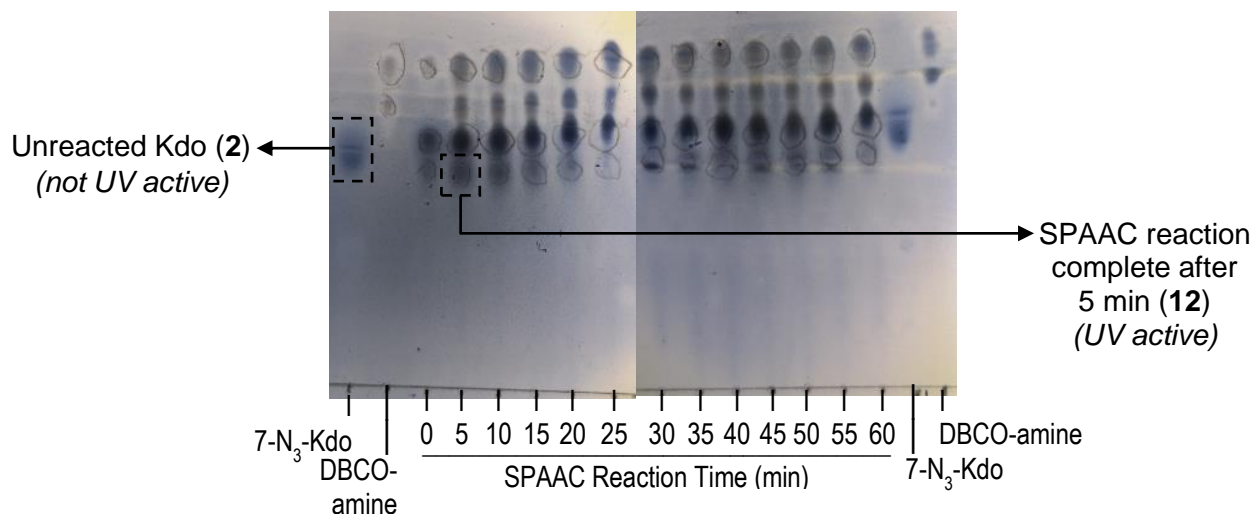


Figure S4. Binding pocket of KdsB. Depicted within the binding pocket of KdsB from *E. coli* (PDB 3K8D) is a molecule of 2-deoxy-Kdo (yellow) and a molecule of CTP (orange). Carbon 7 (*magenta*) and Carbon 8 (*blue*) are indicated, sites at which a $-N_3$ group is instead attached in the case of 7- N_3 -Kdo and 8- N_3 -Kdo, respectively.

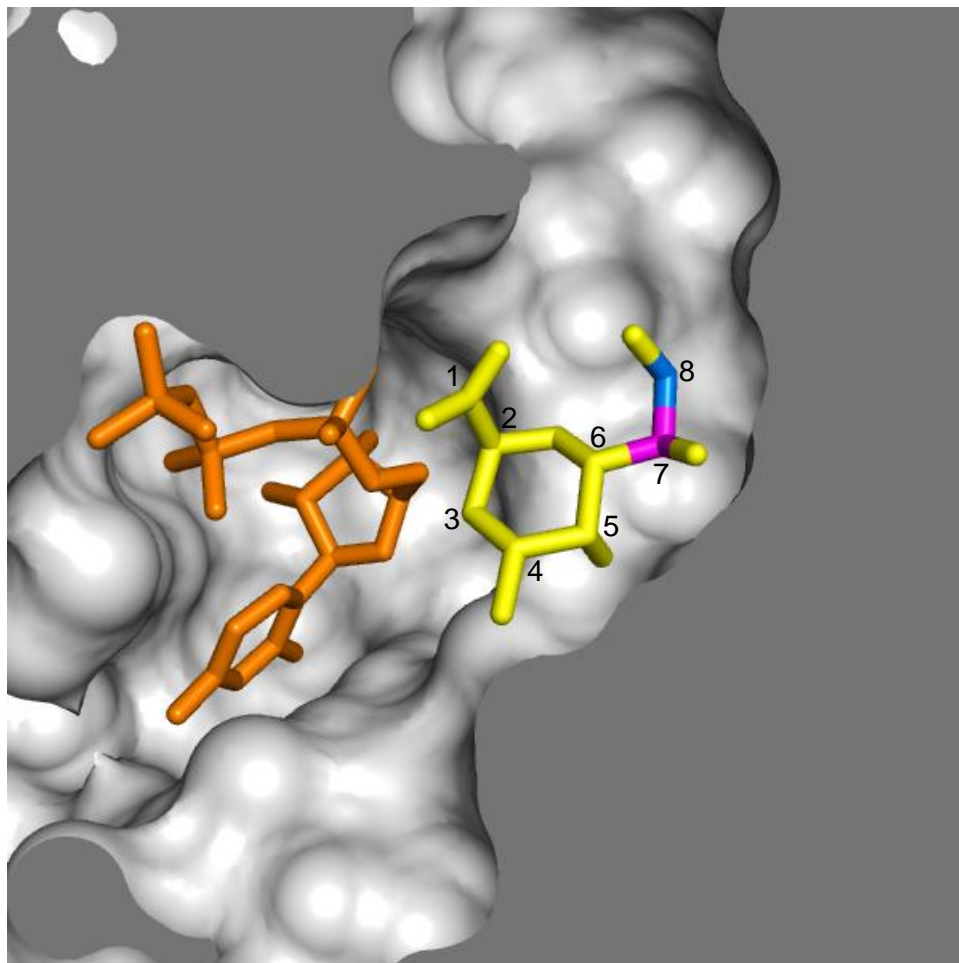


Figure S5. (continued)

Q98 D100 R164

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>Klebsiella pneumoniae_KPHS_18250
>Nitrosomonas communis_AAW31_RS15905
>Pelobacter propionicus_PPRO_RS08035
>Neisseria gonorrhoeae_HT085_RS01615
>Marinomonas mediterranea_MARME_RS16965
>Acidithiobacillus ferridurans_AFERRID_RS08600
>Anaeromyxobacter dehalogenans_A2CP1_RS21785
>Desulfolutivibrio sulfoxidireducens_GD604_RS17550
>Bradyrhizobium diazoefficiens_Bdiaspc4_RS07150
>Burkholderia pyrrocinia_ABD05_RS31445
>Sulfurospirillum halorespirans_SHALO_RS08590
>Azospirillum oryzae_HUE56_RS21610
>Geobacter metallireducens_GMET_RS06420
>Agarivorans albus_K5620_RS12215
>Shewanella putrefaciens_JEZ96_RS10345
>Rhizobium ruizarguesonis_J3P73_RS00955
>Myxococcus xanthus_MXDZ_RS0234105
>Proteus mirabilis_PMI_RS03550
>Caulobacter rhizosphaerae_G3M57_RS23705
>Pseudomonas putida_PP4_RS19565
>Escherichia coli_K-12_MG1655_b0918
>Campylobacter coli_FOC43_RS03610
>Acinetobacter soli_KCV09_RS06290
>Syntrophobacter fumaroxidans_SFUM_RS00060
>Pseudomonas aeruginosa_PA2979
>Citrobacter freundii_EGX89_RS22265
>Pasteurella dagmatis_CKV78_RS05090
>Aeromonas salmonicida_HED66_RS02635
>Bdellovibrio bacteriovorus_BD_RS03685
>Malaciobacter pacificus_APAC_RS07785
>Methylobacterium radiotolerans_MRAD2831_RS50090
>Yersinia pestis_A1122_RS19185
>Desulfarculus baarsii_DEBA_RS03280
>Aliivibrio salmonicida_VSAL_RS19830
>Pseudoalteromonas espejiana_PESP_RS09120
>Xanthomonas citri_AMD14_RS11065
>Serratia rhizosphaerae_FO014_RS01960
>Vibrio cholerae_MS6_RS08560
Consensus/90%

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...lllNlQcDbeHh1.s..lp.hh.....h.....bs*.l.p.. ph.sspHVkhlhs....p..AlYF*Rs.h.ph.c.....

Figure S5. (continued)

D235
C terminal Alpha helix ↓ (Q227-A245)

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>Klebsiella pneumoniae KPHS 18250 GDSLLRHIGLYYRAGFIRRYVSWAPSLEQIEMLEQLRVLWYGEKIHYVA VAAEVPGTGVDTPEDLERVRAEL-----R
>Nitrosomonas communis AAW31_RS15905 -TSAYKQVCHIFPRRATLLHYTQLPTTLEQLESIDMLRLLEHNIRVKMV ET-EFNSQAVDTEADLARVSKLMPEP (4) SSS
>Pelobacter propionicus PPRO_RS08035 KLLCYKHVGLIYVYRRDFLVEFAAMAPTLETSEKLEQLRALENGARIRVV ET-EFESIGVDTPDDLVKARERFRA--IQSK
>Neisseria gonorrhoeae HT085_RS01615 ETAVLRHIGLYAYRVGLQRVYAEMSVPLETIESLEQLRVLWHGYPIAVE TAKEAPAAGVDTQEDLDRVRAVFO-----TV
>Marinomonas mediterranea MARME_RS16965 DYVAYRHVGLIYVYRGLLARYAGMAVSPLEKWEKLEQLRFLHHDVNIQVA IAESLPPHGVDTIEDLETLRHLIA-----SR
>Acidithiobacillus ferridurans AFERRID_RS08600 PGIWWRHIGLYAYRNAFLQDYACWPASPLESIESLEQMRALERGVQIYAVY CAEAPAAAGVDTAADLDRVRDLF-----P
>Anaeromyxobacter dehalogenans A2CP1_RS21785 -PLARAHVGLIYAFYAAFLQETFTALAPGRLEGEAELEQLRALEHGVDIRVA DT-GYRFGTIDTPDDLERARALLA----AGA
>Desulfolutivibrio sulfoxidireducens GD604_RS17550 --THLKHMGLEYAYRRSFDDPSPSPLEACEQLEQLRFQAGIPIAMG LTDMPGSPVDTAADLEAVRAVLATR-LPAG
>Bradyrhizobium diazoefficiens Bdiaspc4_RS07150 --PRYHHIGLYAYRRAALERFVSLPPSPLELQESLEQLRAVEAGMRIDIM IVDS-VPRGVDTPPDLLETARSILS-----KS
>Burkholderia pyrrocinia ABD05_RS31445 RSVFRLRHIGLYGYANAALQALASAMPCELEQLEQLRALWLGTPIDVM RWHDAFPAGIDTPDDVARVVSLLKR (6) EPY
>Sulfurospirillum halorespirans SHALO_RS08590 --CYLGHIGLYAFRRKNALETFCALPYAPIEHIEKLEQLRALYHGHSIAMV EV-QSQSFGIDTPEDLERALAFF-----S
>Azospirillum oryzae HUE56_RS21610 --PLYHHIGLYAYRRAALERFVSLPPSVLEQRERLEQLRALNMGMRIDAA VVDA-VPLGVDTPADLERACALLSA--RKGG
>Geobacter metallireducens GMET_RS06420 RLLCYKHVGLIYVYRRDFLLAFARMAPTLELAEKLEQLRVLWYGEKIHYVA ET-SFESIGVDTPSDDLVKVEKLS---RFQI
>Agarivorans albus K5620_RS12215 -ELYSRHIGLYAYRAHFVGEYLQSPSPLEKMEKLEQLRVLWHGYKIKMA RALETPAHGVDTPEDLEKVRALF-----A
>Shewanella putrefaciens JEZ96_RS10345 -YPVFKHIGLYAYTSKVFQTFALPLGRLEDELEKLEQLRALEHGHIKIA IS-ASNSLEVDREPDIHKCEQRLA----GK
>Rhizobium ruizarguesonis J3P73_RS00955 --PLYHHIGLYAYRRAALERFVSLPPSPLEKRESLEQLRALEAGMRIDAE IVDT-VPLGVDTPADLEKARRILSA---KSN
>Myxococcus xanthus MXDZ_RS0234105 -VQRWGHIGLYGYRREVLSLAKLAPTLEDAEKLEQLRALEHGIPIRCA KV-TSHTVAVDLPGDVEKVEALMRA---RGG
>Proteus mirabilis PMI_RS03550 GEHYLRHIGLYAYRAGFIRRYITWEPSPLESIEMLEQLRVLWYGEKIHYVA KALEVPGVGDTPDDIIAARAERAL-NQEF
>Caulobacter rhizosphaerae G3M57_RS23705 --PIWRHIGLYAYRRAALEAFNAAPPSPLEKREKLEQLRAMELGLSLRAA VAAE-APISDNPSDLEAARAYSNR (5) KTA
>Pseudomonas putida PP4_RS19565 GVPYRRHIGMYAYRVGFLQDFVGVGPGCLEQTEALEQLRALWHGVRIHVE DAIEAPAVGVDTPEDLERVRRLL-----EA
>Escherichia coli K-12 MG1655_b0918 GDNFLRHIGLYYRAGFIRRYVNWQPSLEHIEMLEQLRVLWYGEKIHYVA VAQEVPGTGVDTPEDLERVRAEM-----R
>Campylobacter coli FOC43_RS03610 --SFKHIGLYAYSVKALCEFTLENSALEKAEKLEQLRALENGKKIKML QI-NTSSMGIDTKEDYERALRIYL----GK
>Acinetobacter soli KCV09_RS06290 FDHAYRHIGLYAYRVSLQEQYVVKWQGVLEKLESLEQLRVLWYGEKIHYVA VADSALPPGVDTPEDLARLNALPVS---YFD
>Syntrophobacter fumaroxidans SFUM_RS00060 -PAFLKHIGLYAYSASFQTFESRLPPGRLEGLEKLEQLRALEHGYYGIRVA LS-PVDTRGVDTPEDLEAVVPVLAQD-AVGA
>Pseudomonas aeruginosa PA2979 GVPYRRHIGLYAYRVGLADFAVWGPCWLENAESLEQLRALWHGVRIHVA DARENMLPGVDTPEDLERVRRVL-----GG
>Citrobacter freundii EGX89_RS22265 GDTFLRHIGLYYRAGFIRRYVNWQPSLEHIEMLEQLRVLWYGEKIHYVA VAKEVPGTGVDTTEDLERVRAEM-----P
>Pasteurella dagmatis CKV78_RS05090 GDHYLRHIGLYAYRAGFIKQVYVQWQTSLEQIESLEQLRVLWYGEKIHYVE LAKEVPPGVDTPEDLEKVRSLQK---NLF
>Aeromonas salmonicida HED66_RS02635 GGHYQRHIGLYAYRAGFIQRYVDWAPSLEQVEALEQLRVLWYGEKIHYVA QALQAPPVGVDTQADDLDRVRAIML-----AN
>Bdellovibrio bacteriovorus BD_RS03685 MDGCLKHIGMYAYSRLFQKQFCEAPPALIEKASLEQLRALYLGAKIKVI RVKE-ASVGVDTPEDLARLEKLLSS---QGM
>Malaciobacter pacificus APAC_RS07785 -AVYHGHIGLYYGTTKSLNEFCSLKPSLENLKLEQLRALDNNYNITMV KV-ESKSFQIDTQEDLNNALRIFQ----NNS
>Methylobacterium radiotolerans MRAD2831_RS50090 --PLWHHIGLYAYRRATLSRFVALPPGELEVRKLEQLRALEAGMRIDAA IVDD-LPLGVDTPADLERARALLQG--RRLN
>Yersinia pestis A1122_RS19185 GDCFLRHIGLYAYRAGFIRRYVNWAPSLEQIEMLEQLRVLWYGEKIHYVA VAKAVPAVGVDTQSDLDRVRAIML-----NQ
>Desulfarculus baarsii DEBA_RS03280 -VTFYKHIGLYYFRKGFLDIFAGLADGVLEAEKLEQLRVLWYGEKIHYVA IS-QFDSPEVDRPADAQRVAAALLA-----GA
>Aliivibrio salmonicida VSAL_RS19830 ANPFLRHIGLYAYRAGFINTYINWQPSVLEKIECLEQLRVLWYGEKIHYVA VAKEAPAAGVDTQEDLDRVRAIML-----AL
>Pseudoalteromonas espejana PESP_RS09120 LAPFQRHVGLEYAYRAGFIKQYIELSVSPELVLESLEQLRVLWYGEKIHYVA QAKVTPHAGVDTPEDLAKVIAYIEG---NPA
>Xanthomonas citri AMD14_RS11065 EGGWLRHIGLYAYRAGFQRFAAMPVGMLEERTESLEQLRVMAGYRIAVA VTPPEFPFGIDTADDLVRQMRVA-----SA
>Serratia rhizosphaerae FO014_RS01960 GDNLLRHIGLYAYRAGFVRRYVSWAPSLEKIELEQLRVLWYGEKIHYVA VAKAIPSVGVDTAEDLQRVDRSL-----NA
>Vibrio cholerae MS6_RS08560 VQPLLRHIGLYAYRAGFINTYLDWQPSLEKIECLEQLRVLWYGEKIHYVA VALEAPPAAGVDTPEDLEVVRRIVAE---RAQ
Consensus/90% ...hb.HlGlysap..hl..ah.b.ss.LE..E.LEQLRslb.G..I.h..s....s.sld.s..Dl..s...h.....
```

Figure S6. ¹H NMR spectrum (MeOD, 600 MHz) of compound 8.

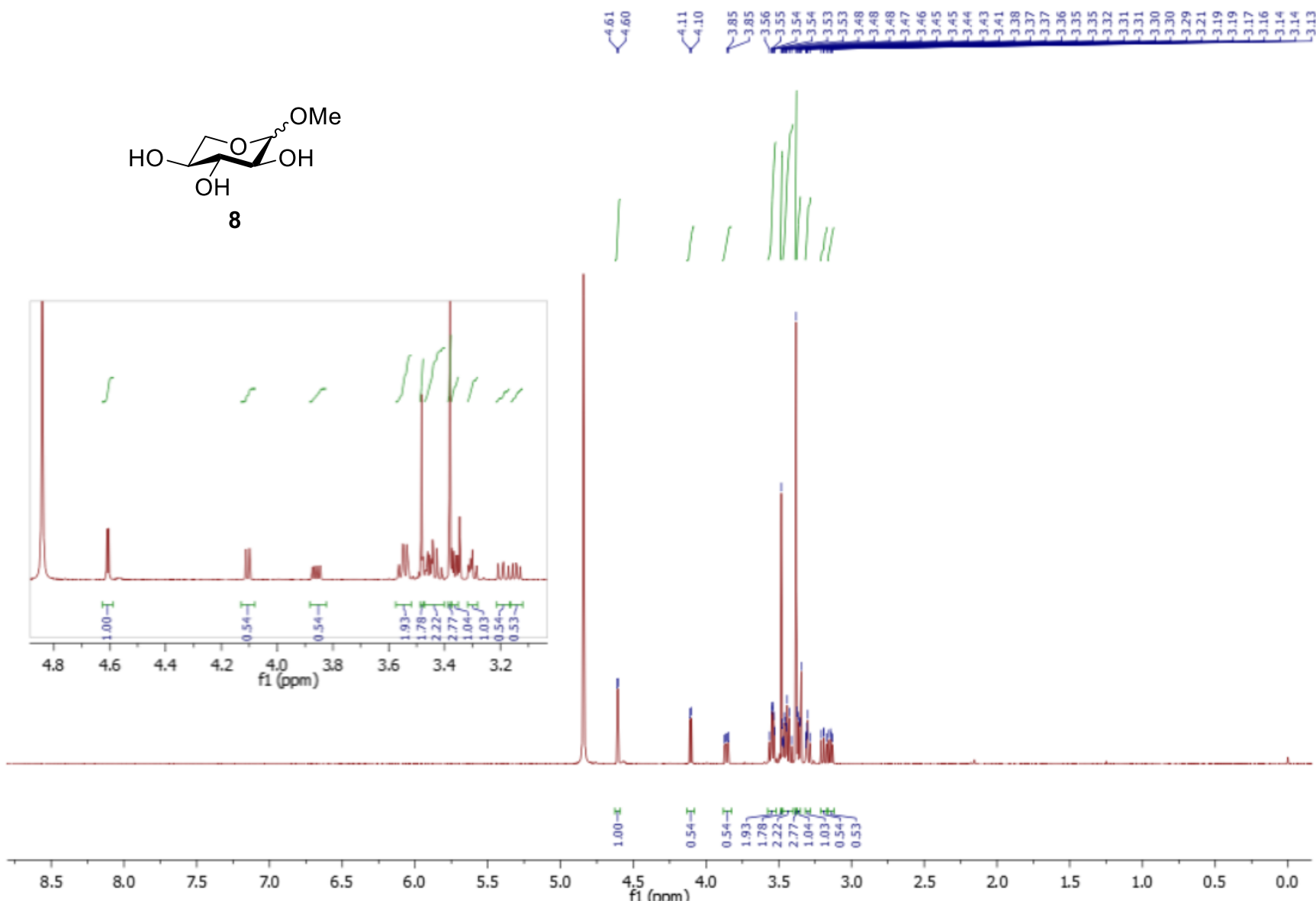


Figure S7. ^{13}C NMR spectrum (MeOD, 150 MHz) of compound **8**.

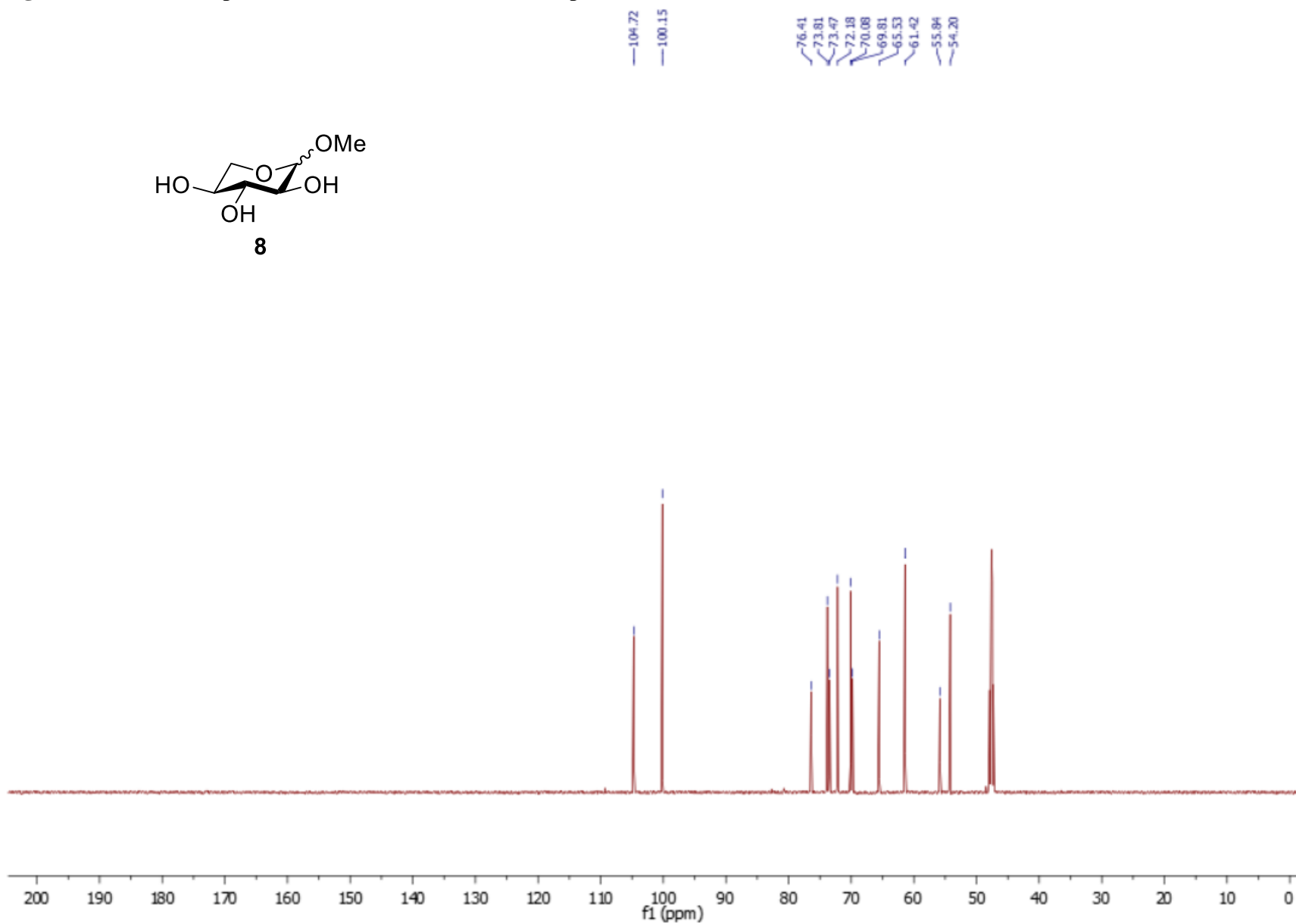


Figure S8. COSY NMR spectrum (MeOD, 600 MHz) of compound **8**.

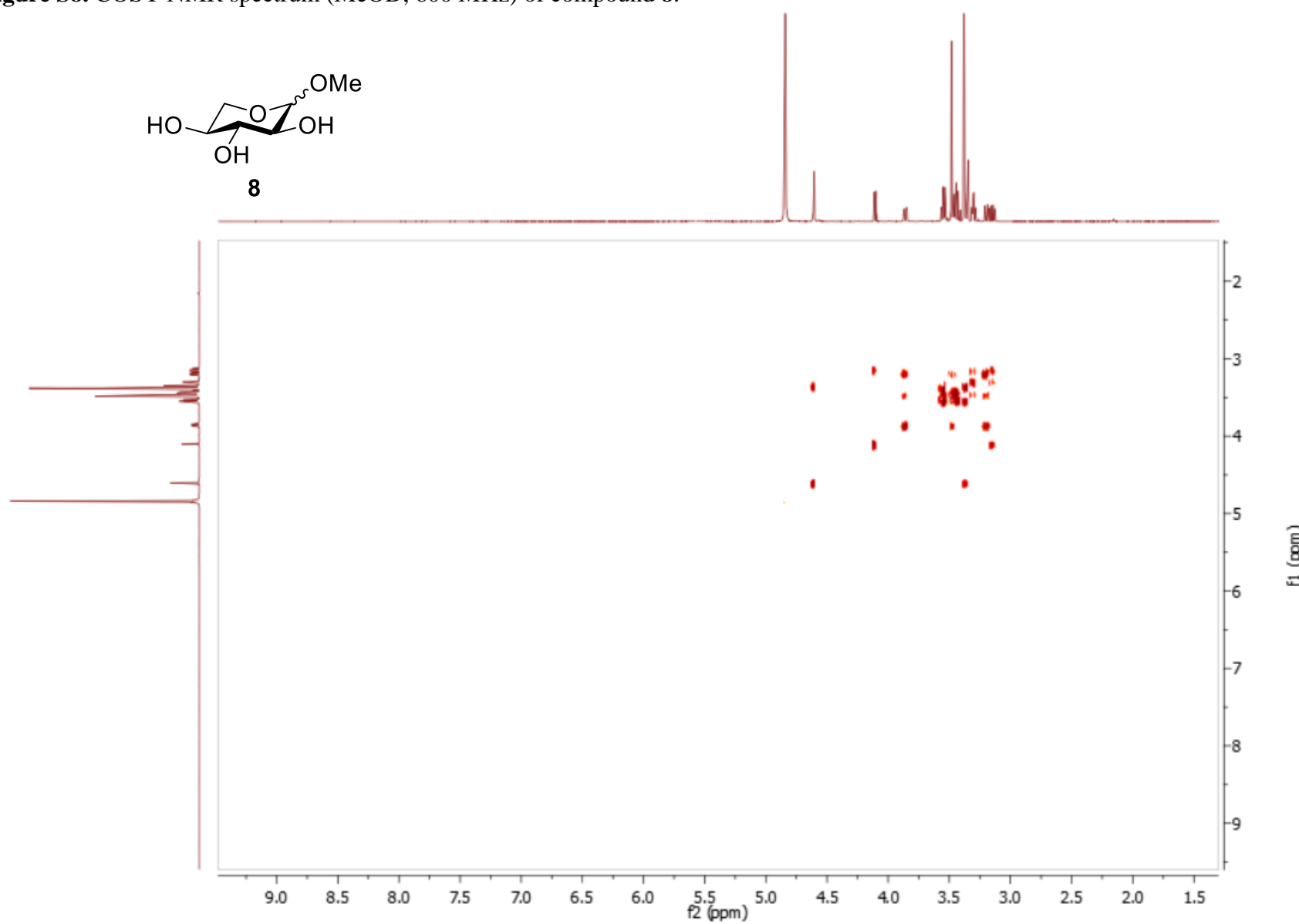


Figure S9. HSQC NMR spectrum (MeOD, 600 MHz) of compound **8**.

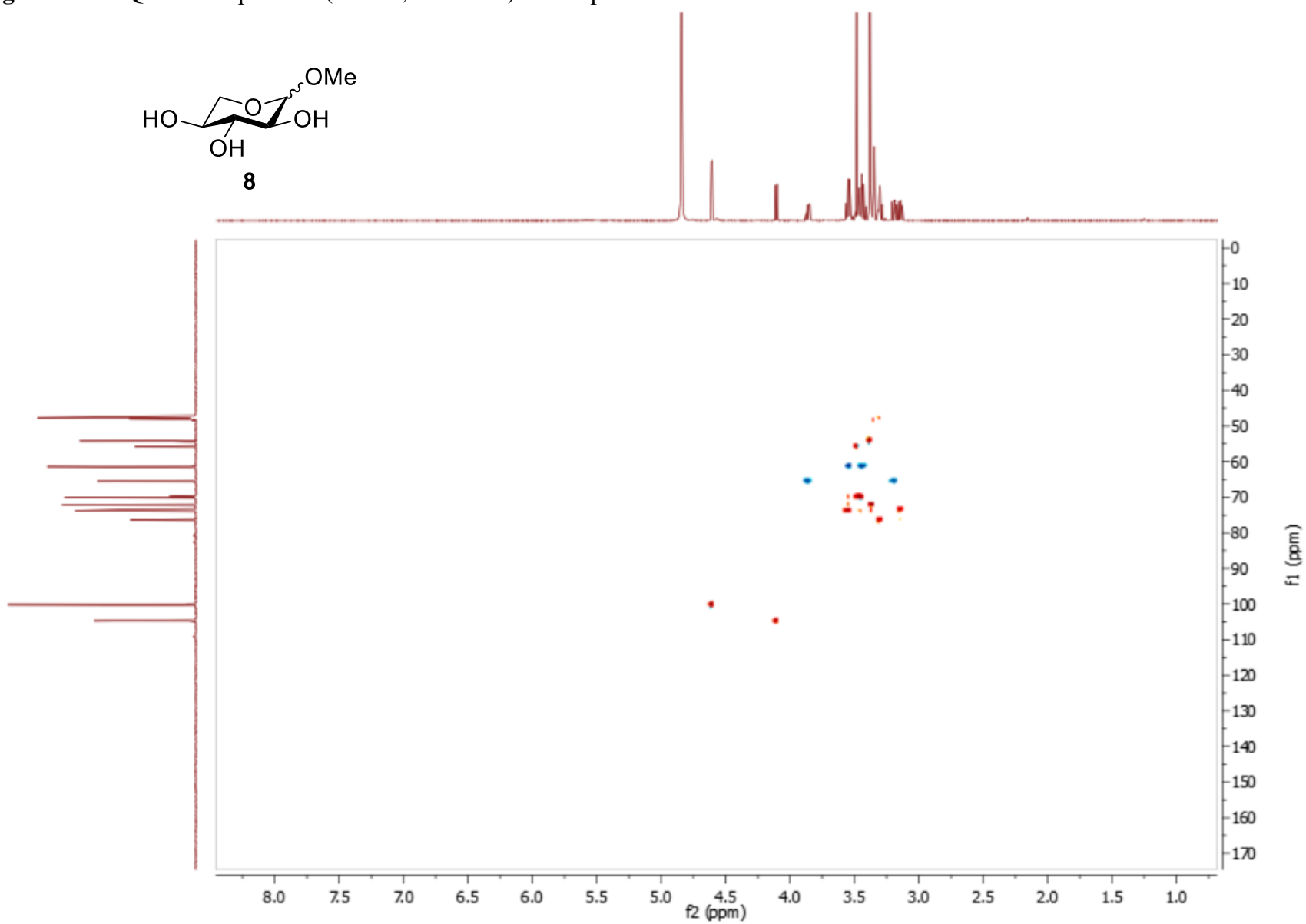


Figure S10. ¹H NMR spectrum (CDCl₃, 600 MHz) of compound **9**.

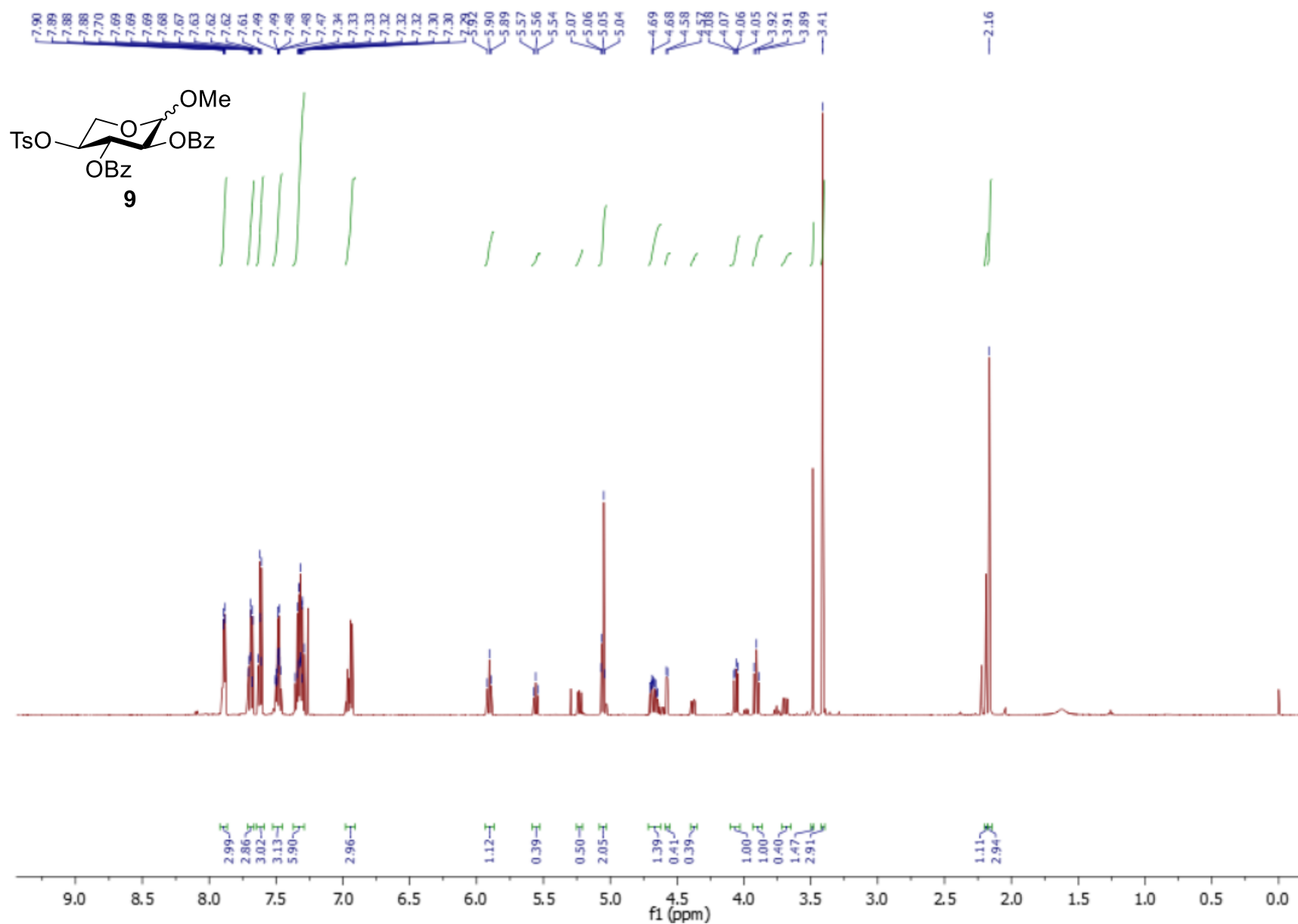


Figure S11. ^{13}C NMR spectrum (CDCl_3 , 150 MHz) of compound **9**.

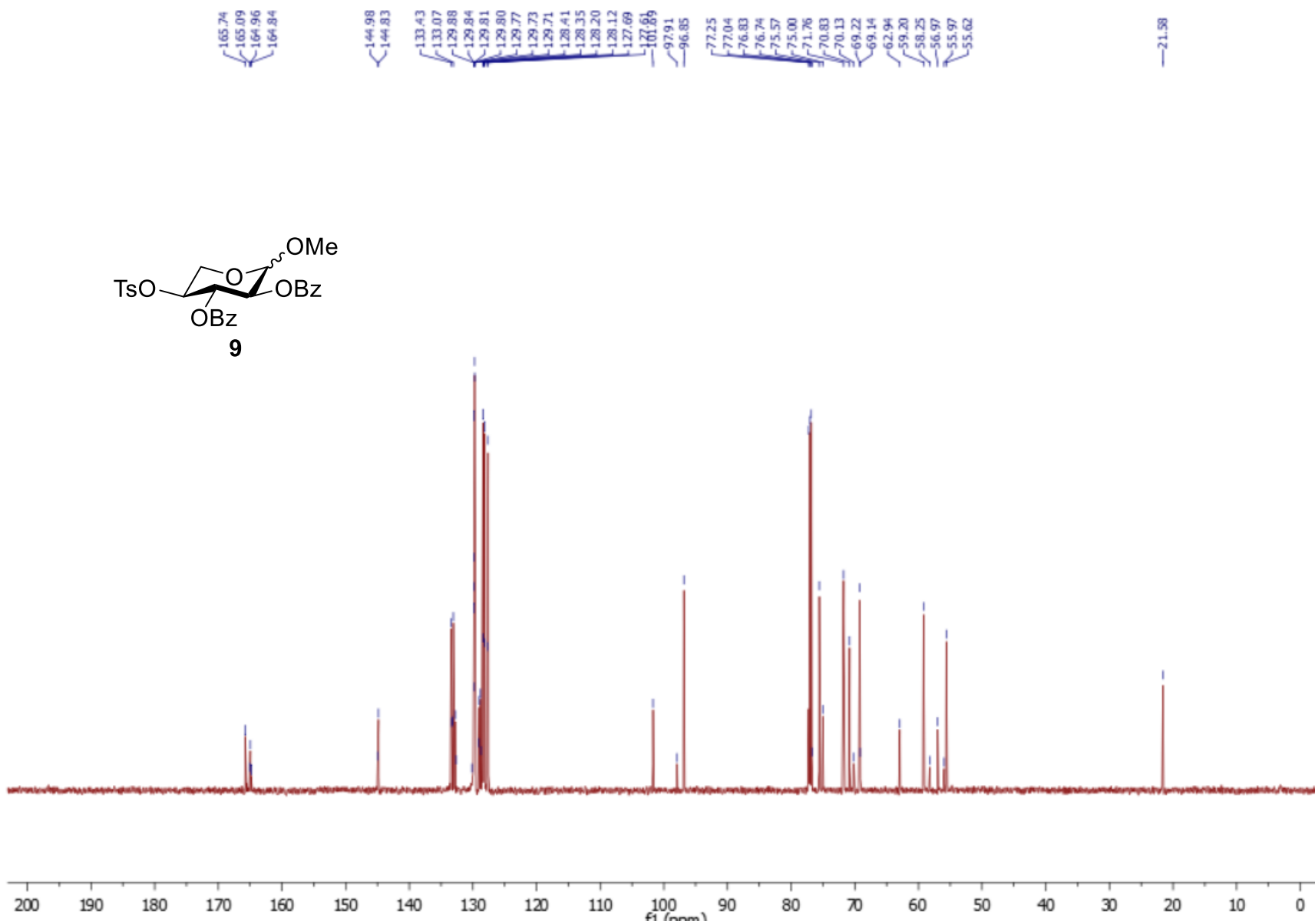


Figure S12. COSY NMR spectrum (CDCl₃, 600 MHz) of compound **9**.

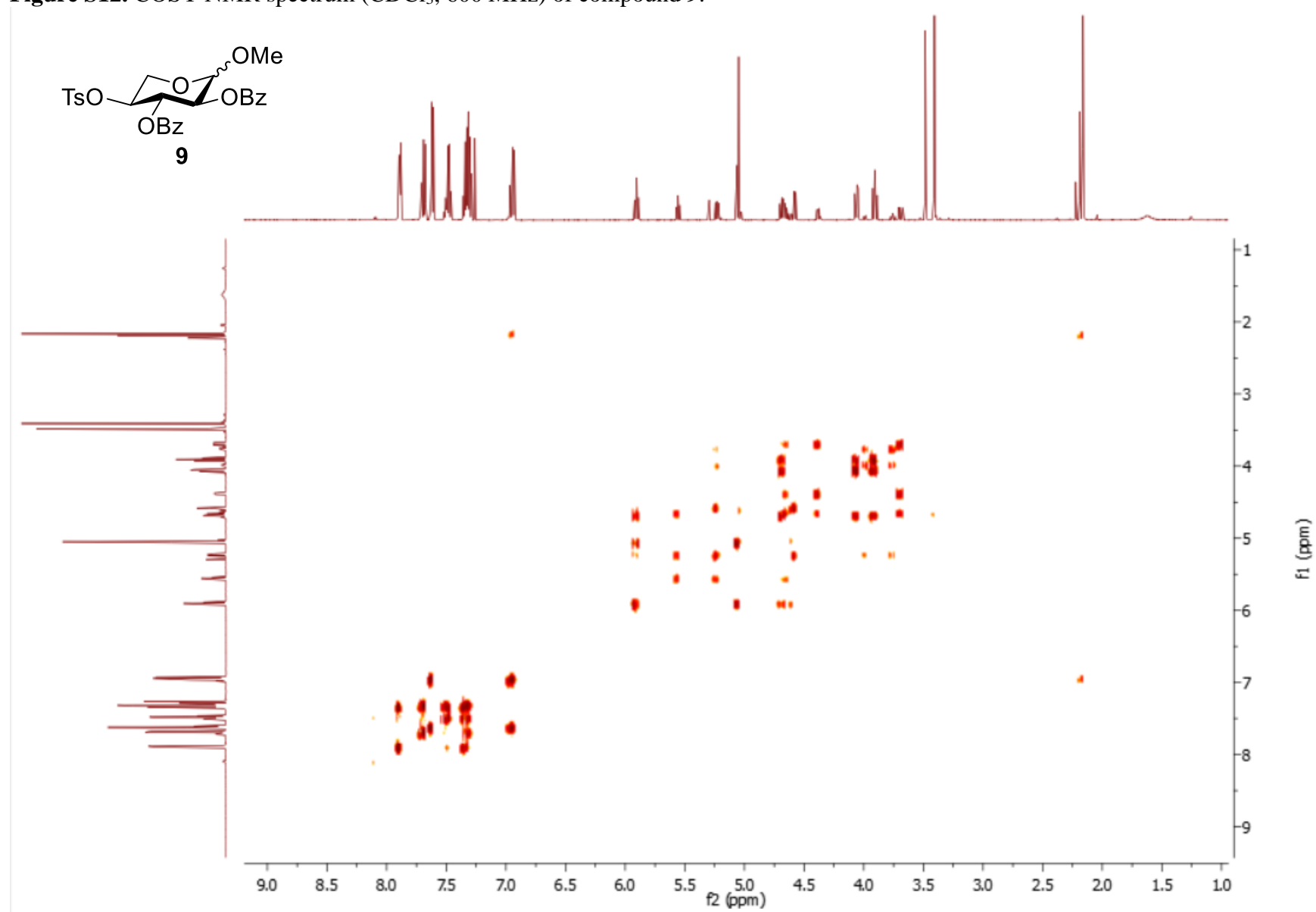


Figure S13. HSQC NMR spectrum (CDCl₃, 600 MHz) of compound **9**.

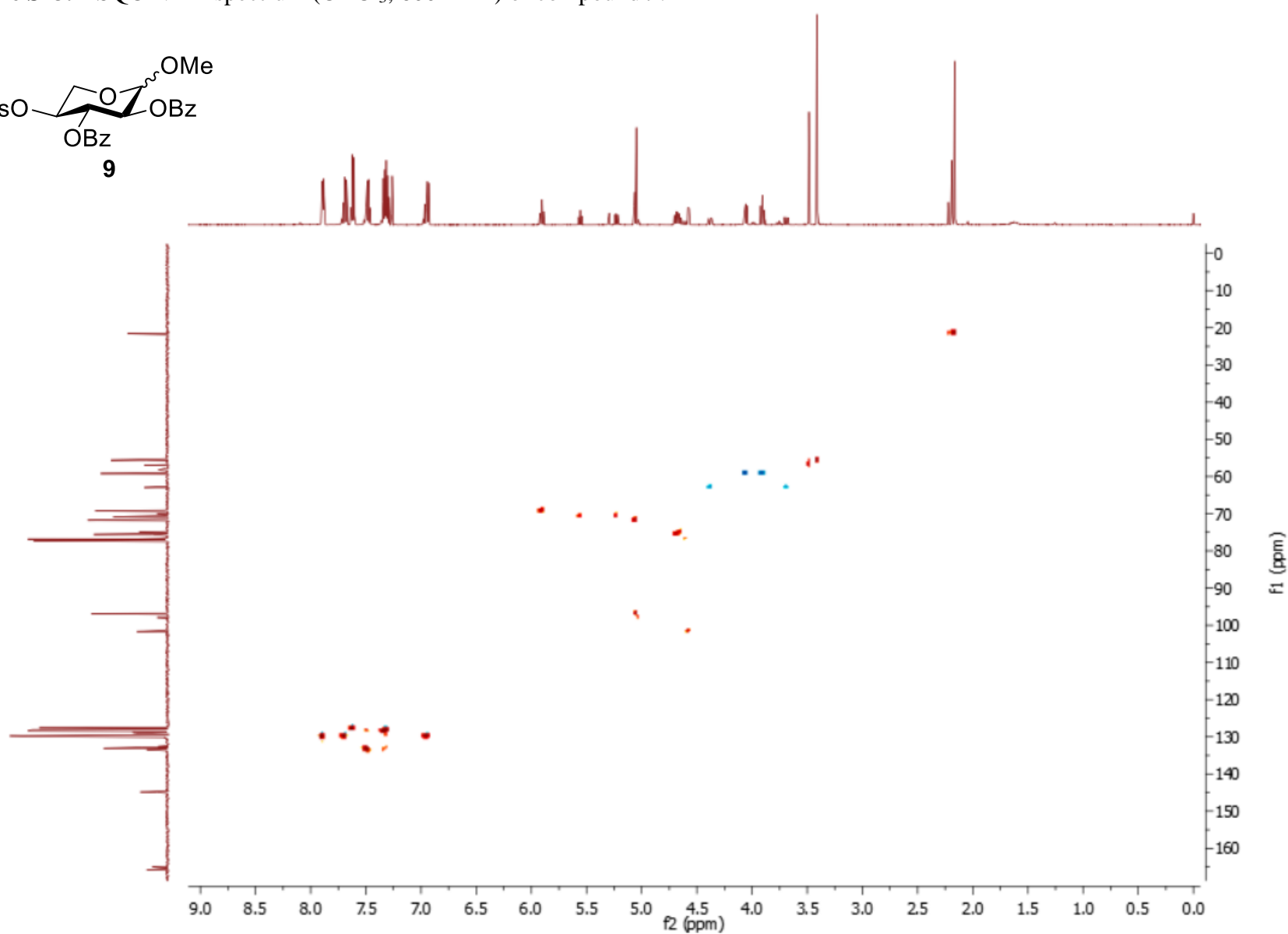
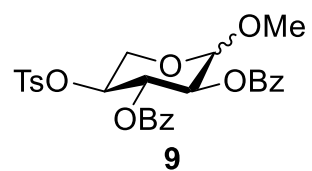


Figure S14. ^1H NMR spectrum (CDCl_3 , 600 MHz) of methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-D-arabinopyranoside (**S1**).

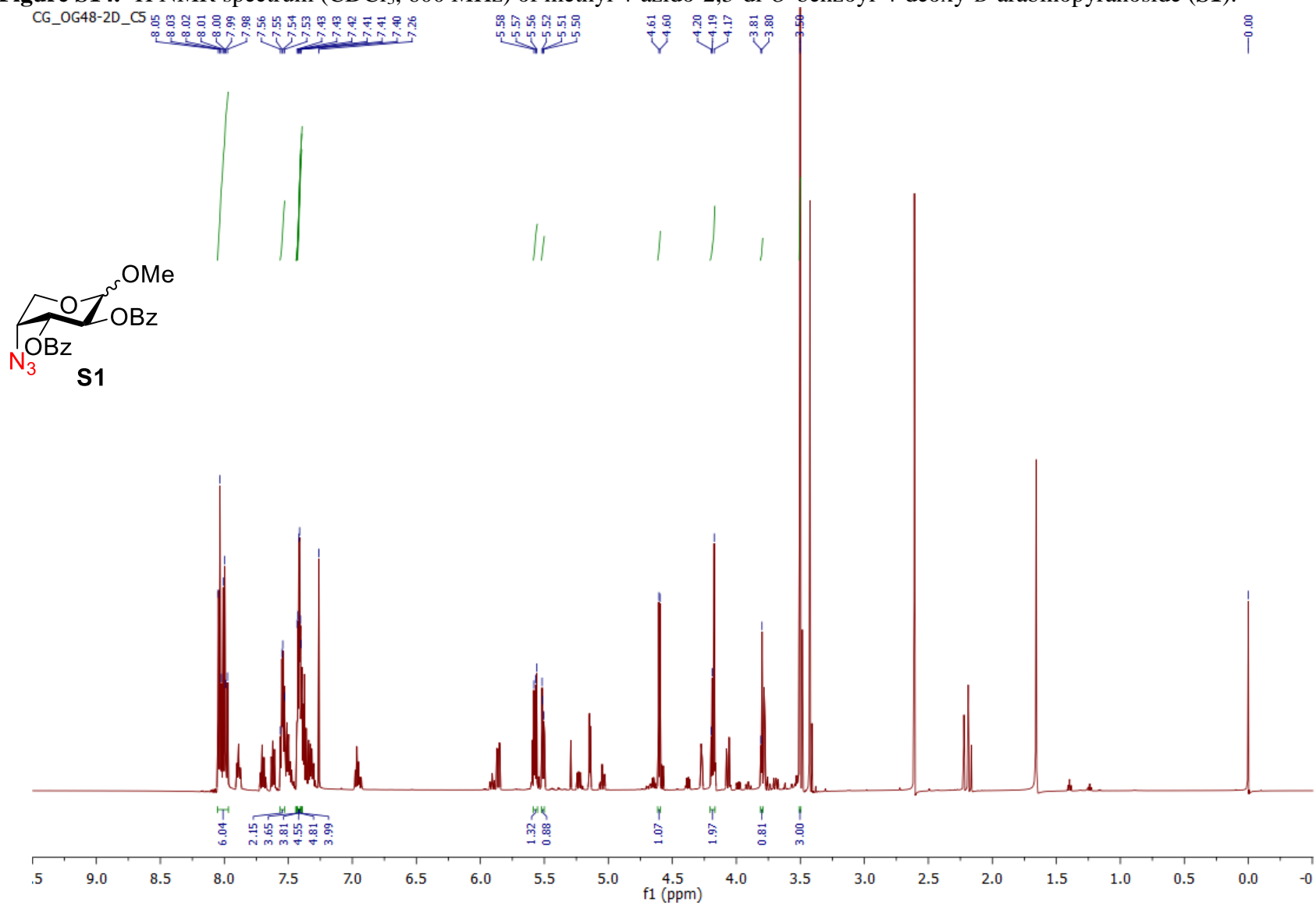


Figure S15. ^{13}C NMR spectrum (CDCl_3 , 150 MHz) of methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-D-arabinopyranoside (**S1**).

CG_OG48-2D_C5

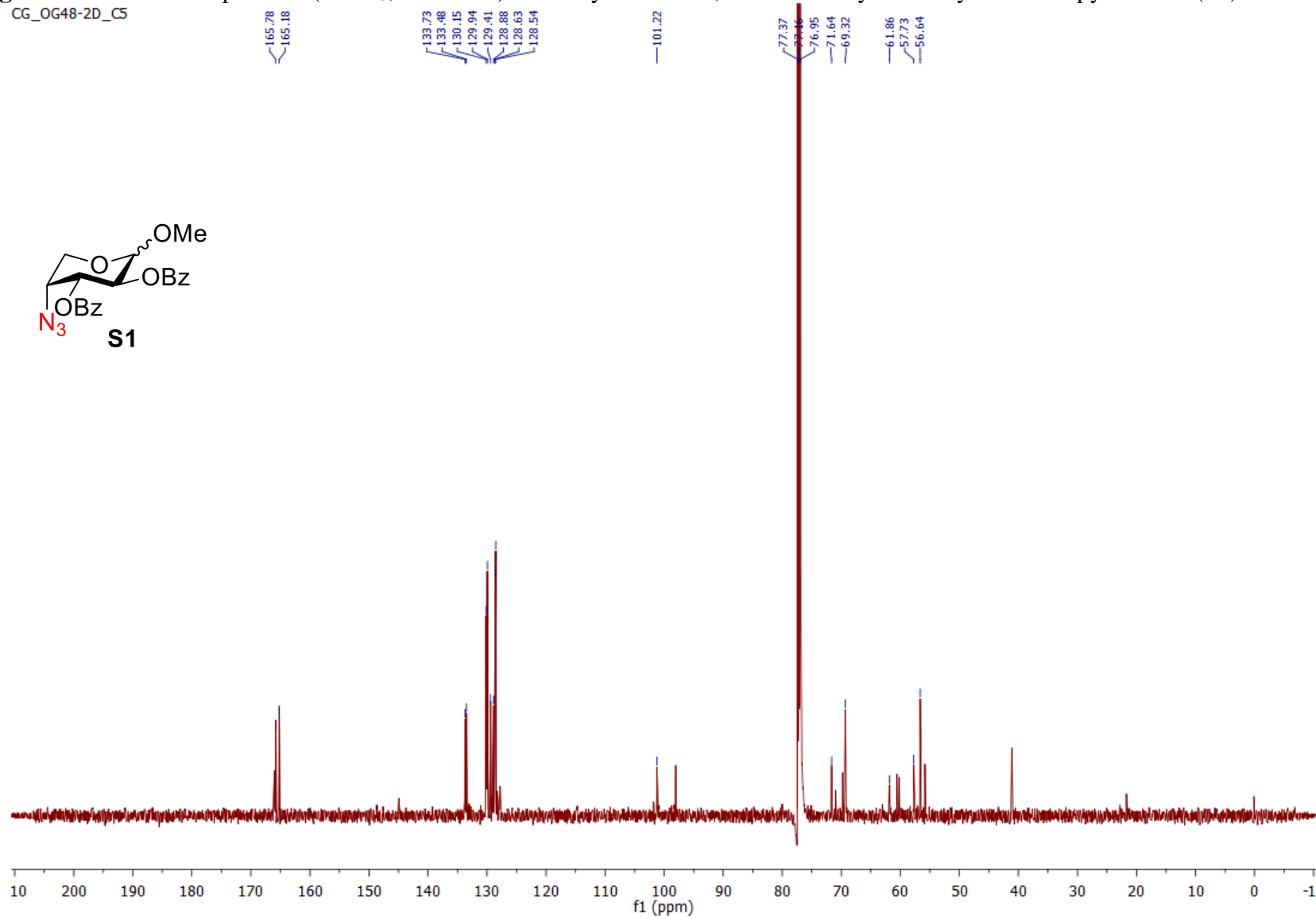


Figure S16. COSY NMR spectrum (CDCl₃, 600 MHz) of methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-D-arabinopyranoside (**S1**).

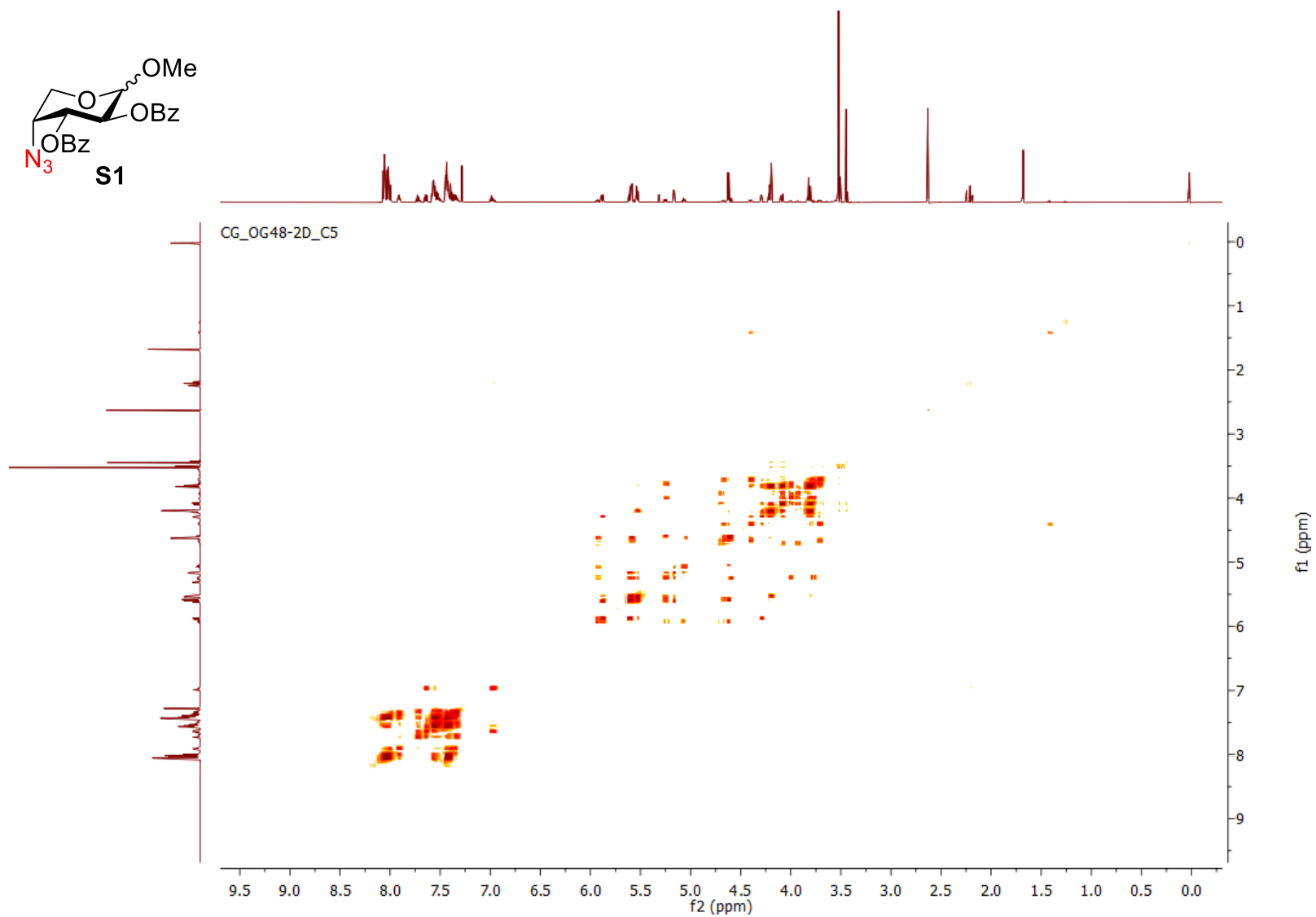


Figure S17. HSQC NMR spectrum (CDCl₃, 600 MHz) of methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-D-arabinopyranoside (**S2**).

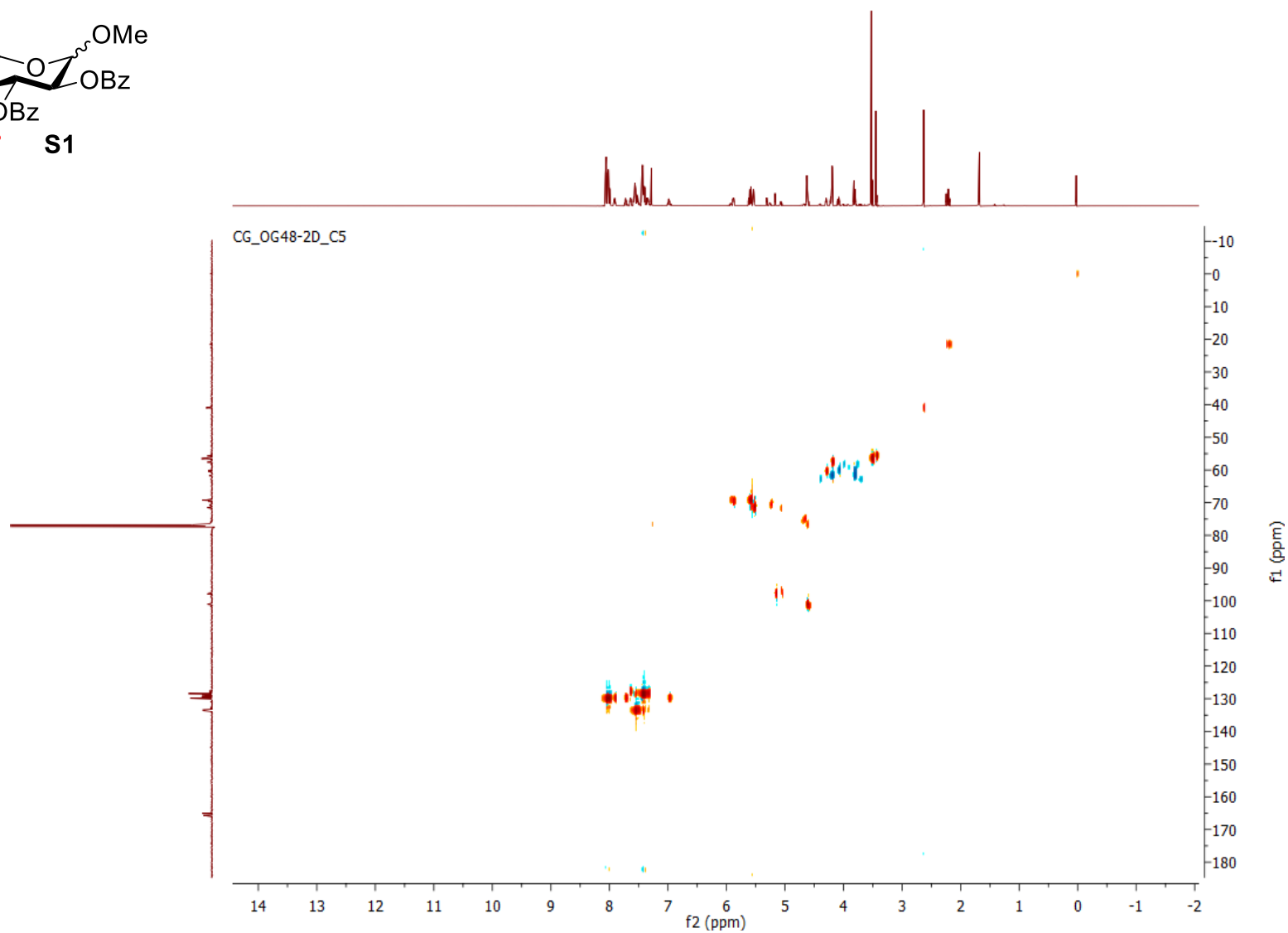
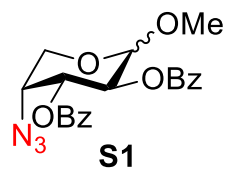


Figure S18. ^1H NMR spectrum (D_2O , 600 MHz) of compound **10**.

CG_OG50-2D_C3

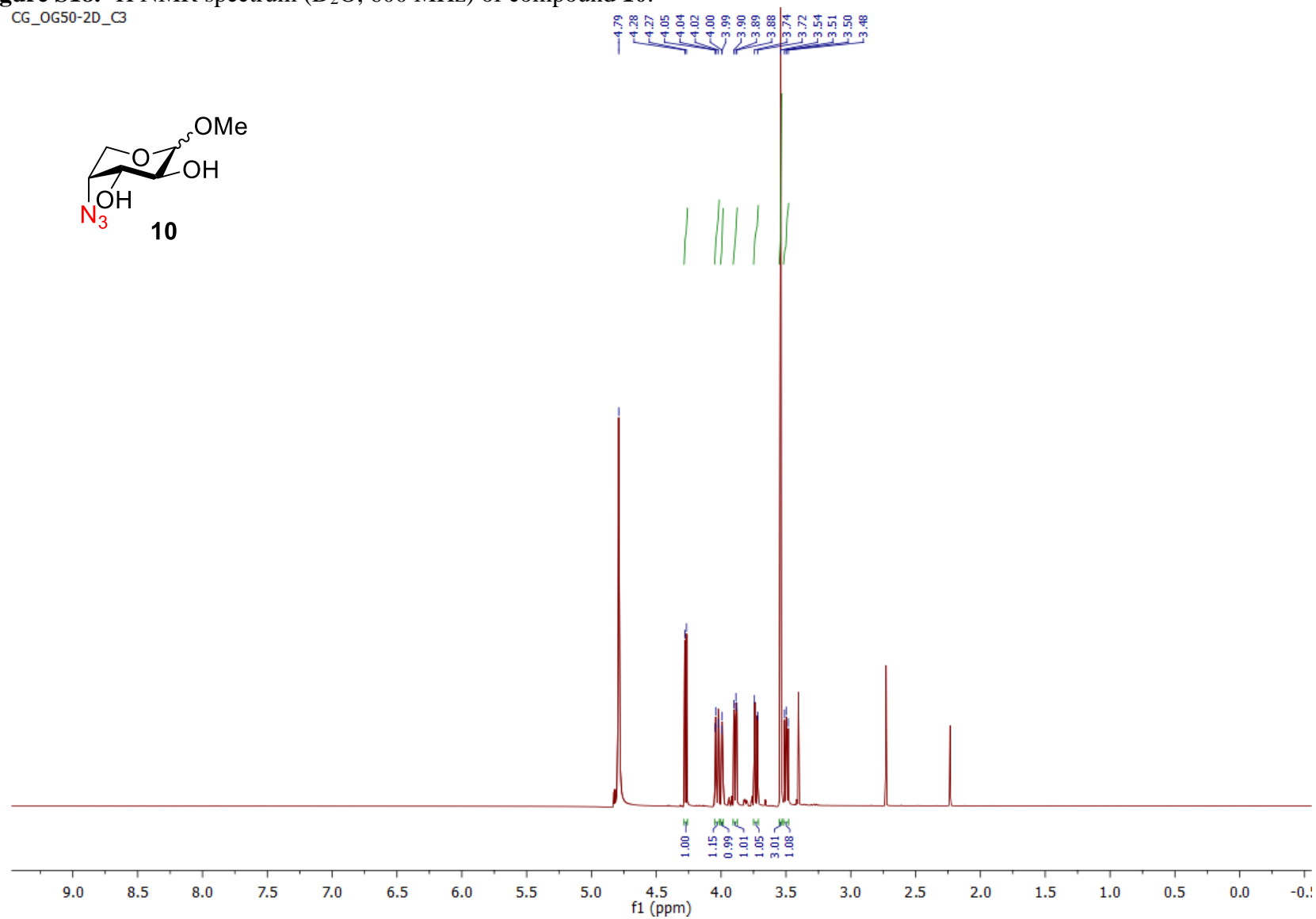


Figure S19. ^{13}C NMR spectrum (D_2O , 150 MHz) of compound **10**.

CG_0650-2D_C3

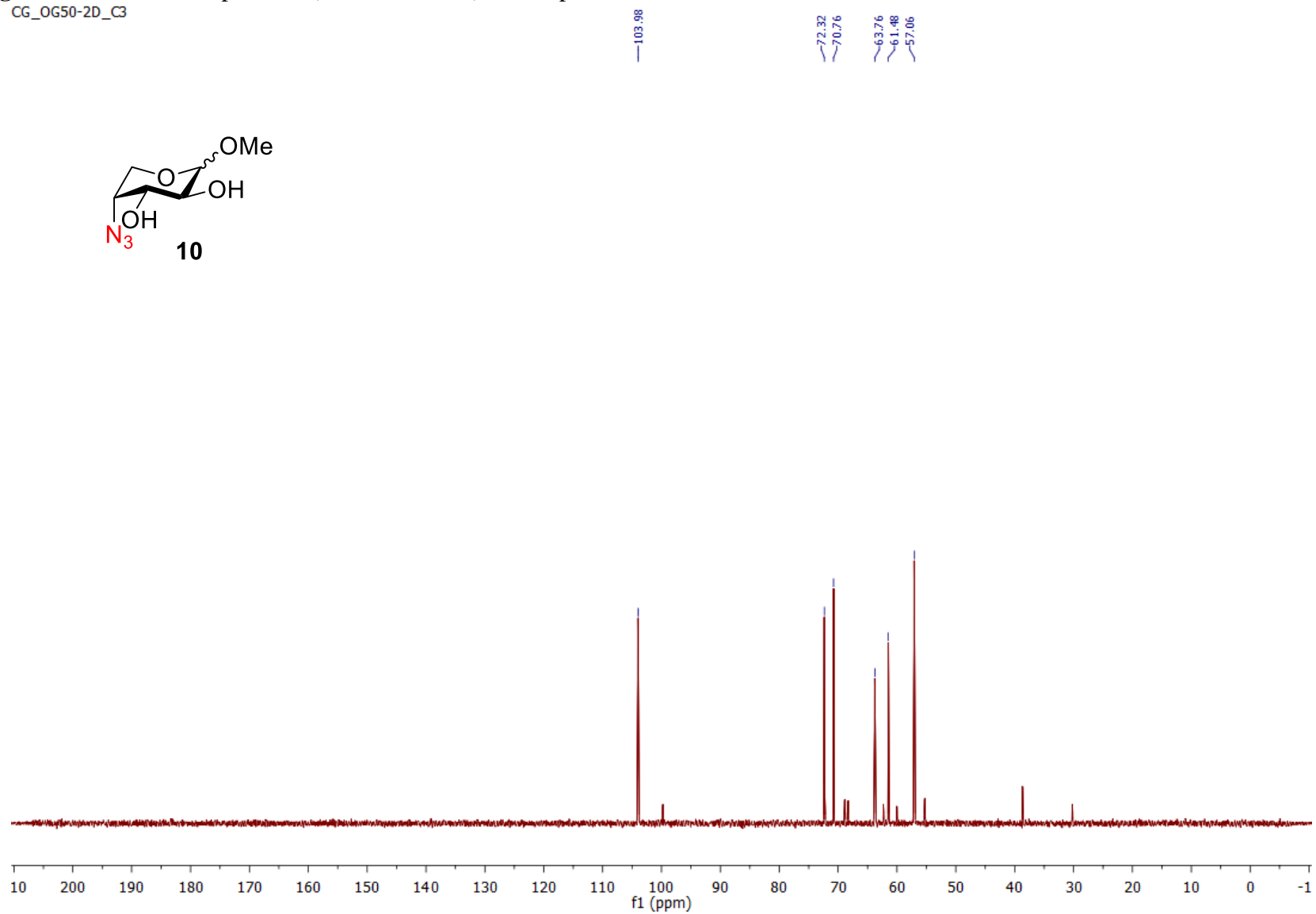


Figure S20. COSY NMR spectrum (D₂O, 600 MHz) of compound **10**.

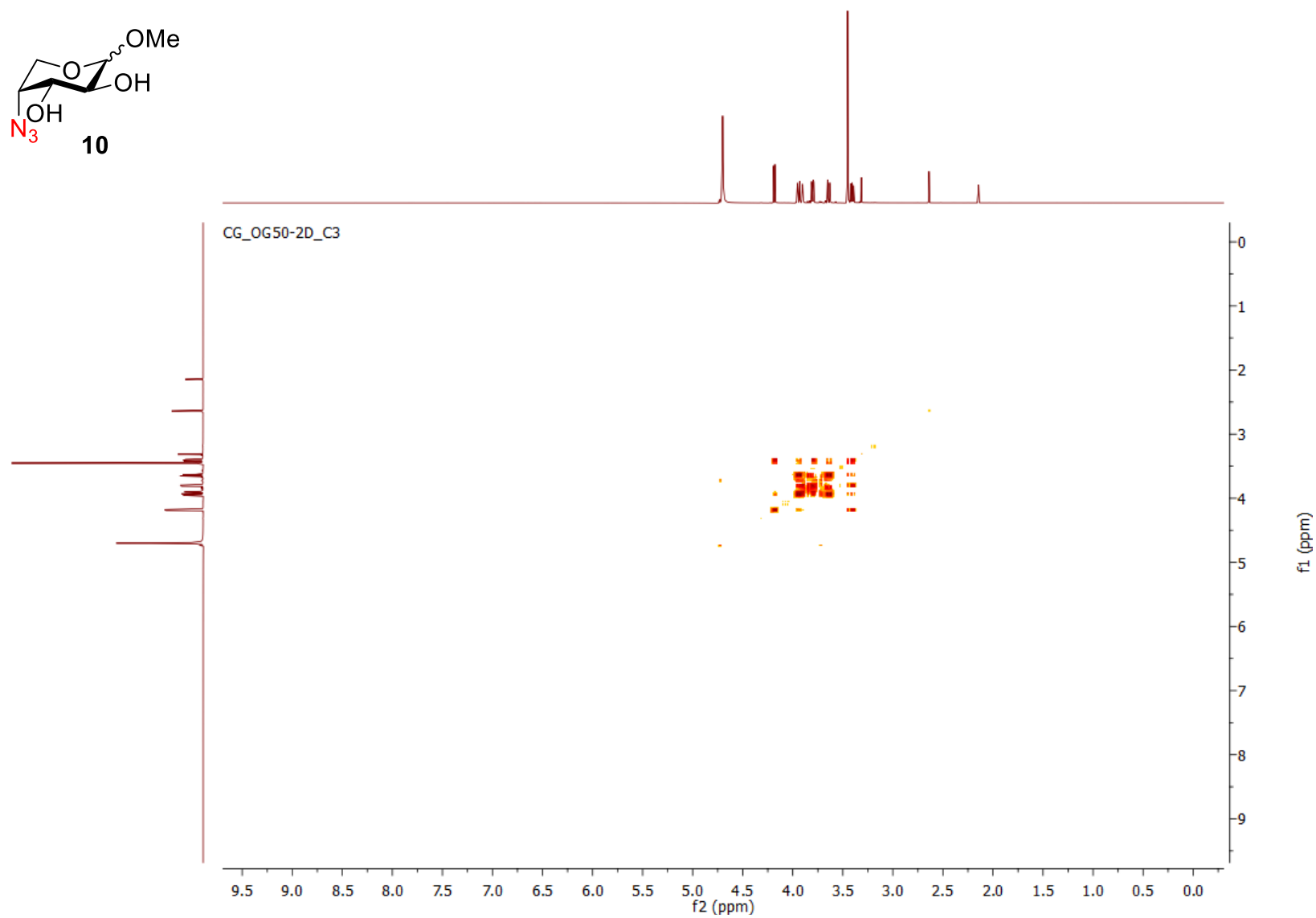
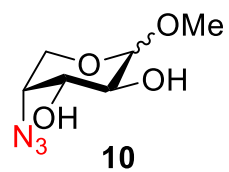


Figure S21. HSQC NMR spectrum (D₂O, 600 MHz) of compound **10**.

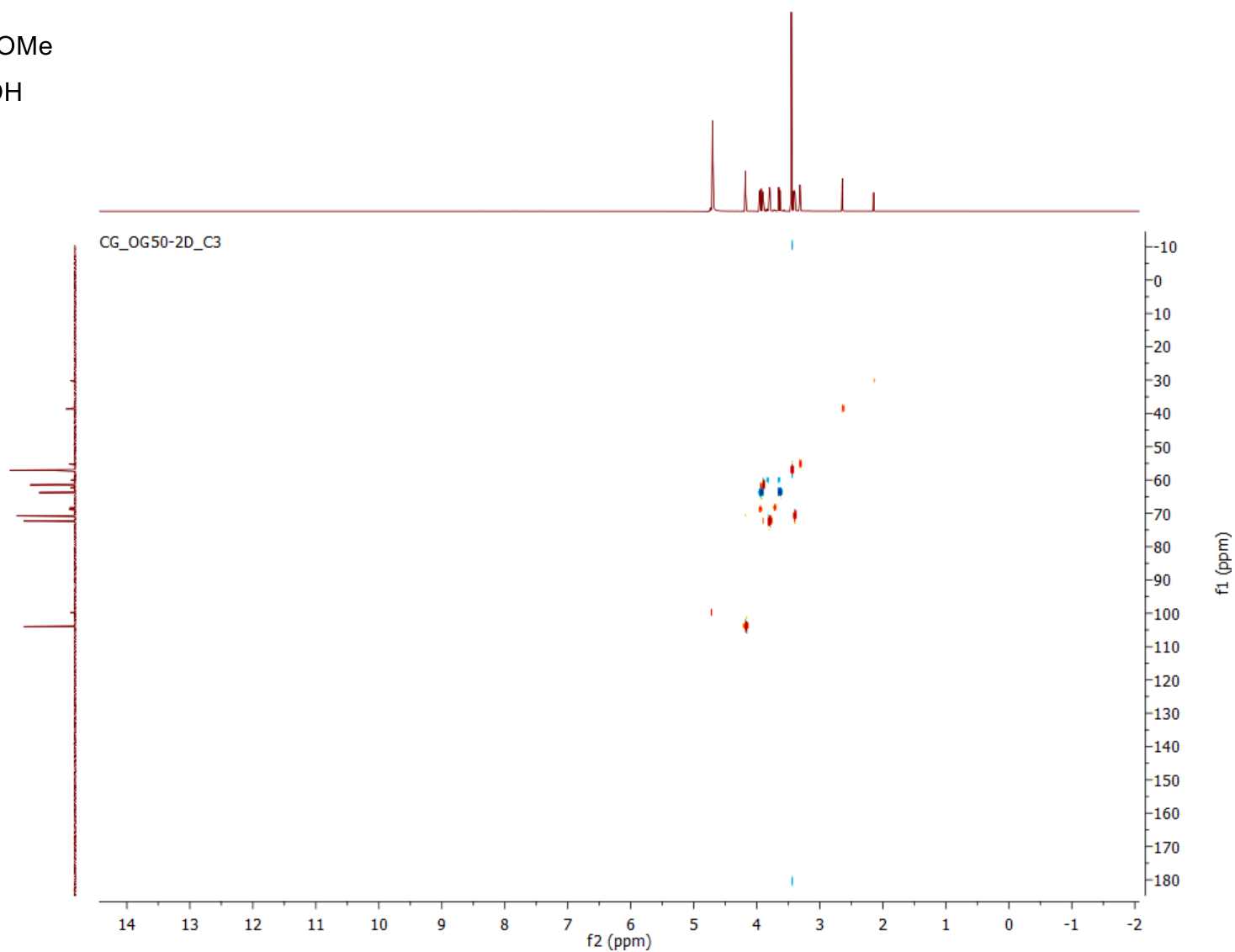
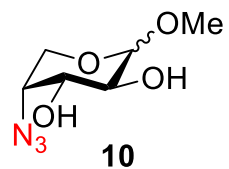


Figure S22. ^1H NMR spectrum (CDCl_3 , 600 MHz) of 1,2,3-tri-*O*-acetyl-4-azido-4-deoxy-D-arabinopyranoside (**S2**).

CG_0G38-2D_Cl

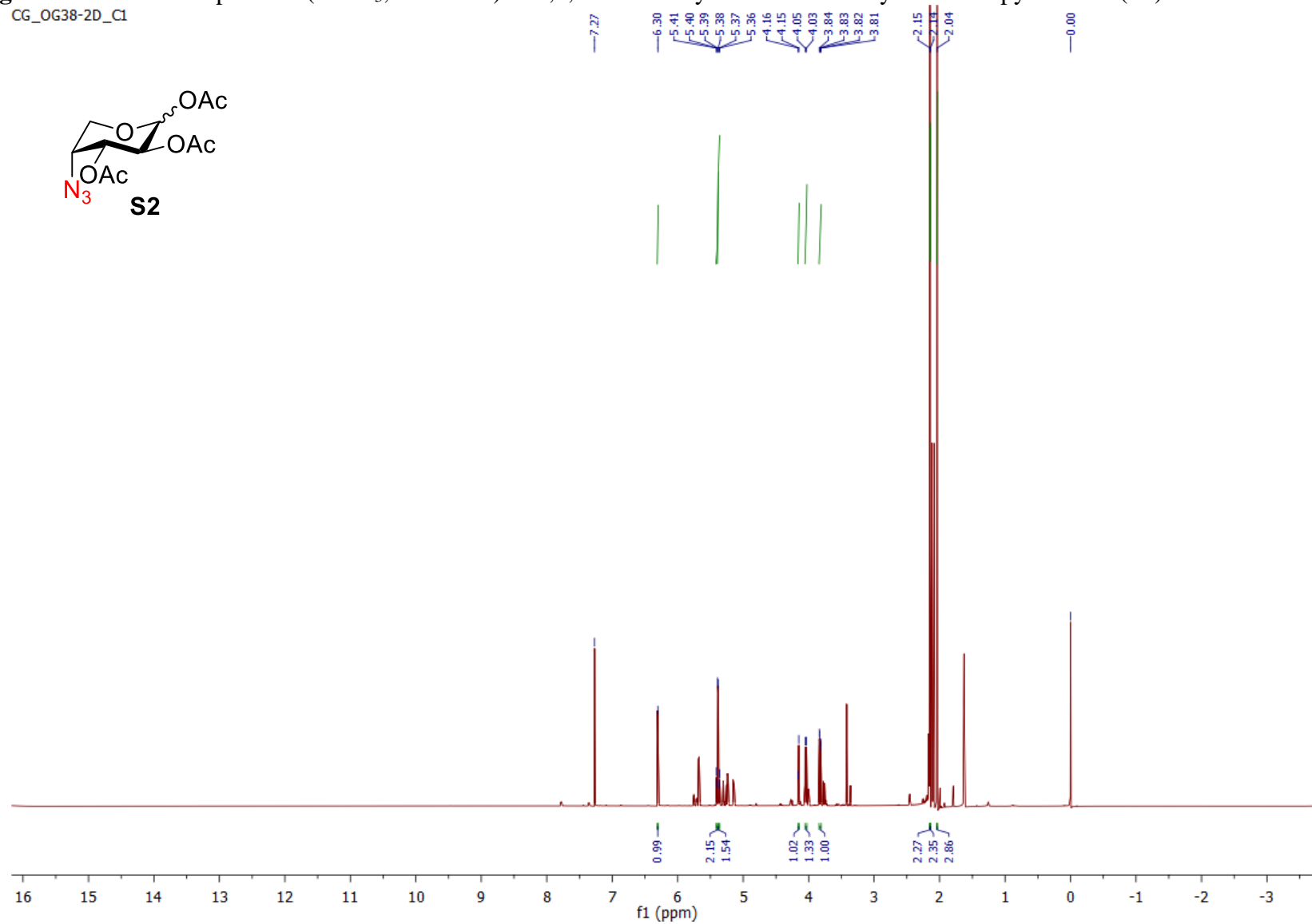


Figure S23. ^{13}C NMR spectrum (CDCl_3 , 150 MHz) of 1,2,3-tri-*O*-acetyl-4-azido-4-deoxy-D-arabinopyranoside (**S2**).

CG_OG38-2D_Cl

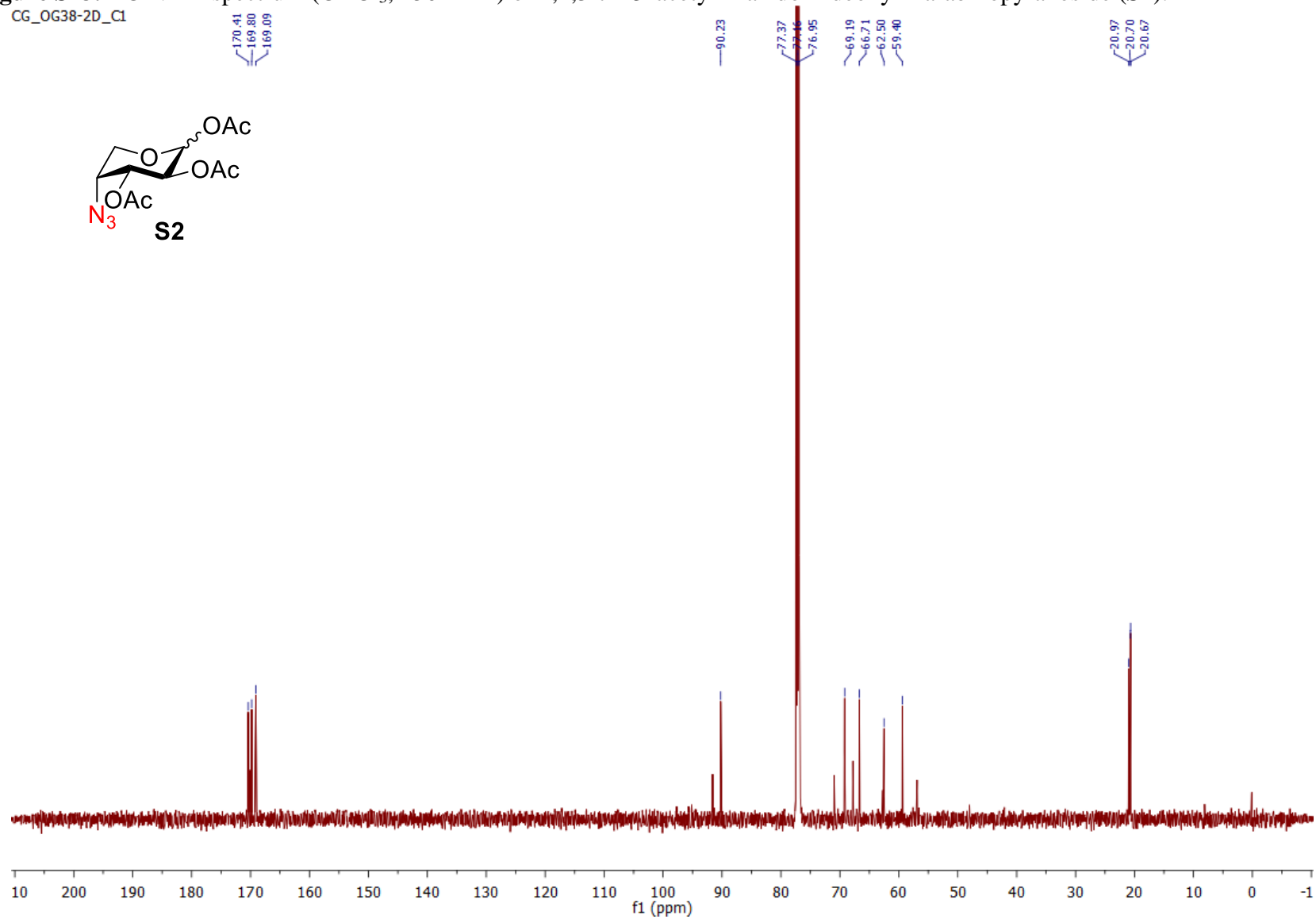


Figure S24. COSY NMR spectrum (CDCl₃, 600 MHz) of 1,2,3-tri-*O*-acetyl-4-azido-4-deoxy-D-arabinopyranoside (**S2**).

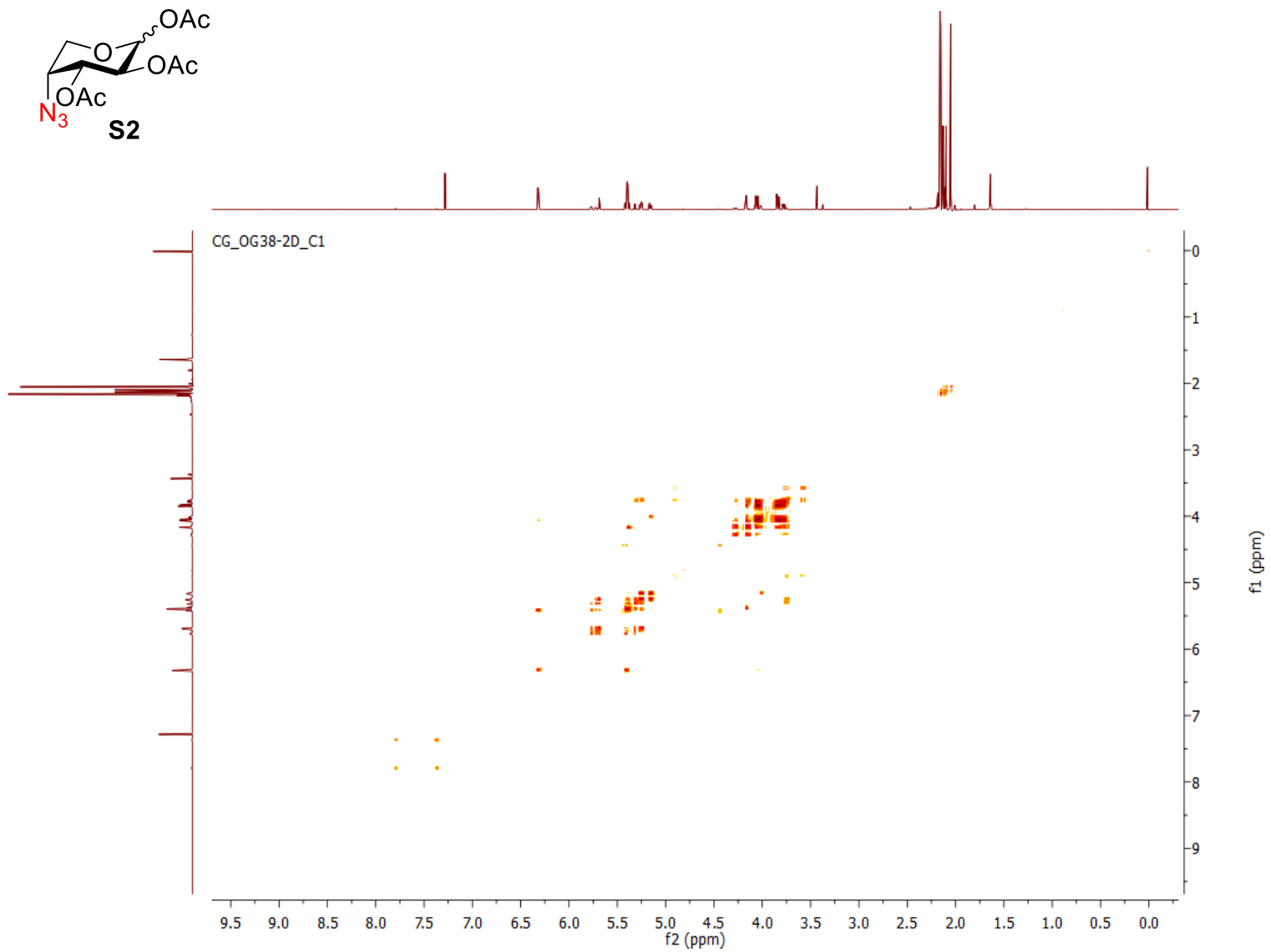


Figure S25. HSQC NMR spectrum (CDCl₃, 600 MHz) of 1,2,3-tri-*O*-acetyl-4-azido-4-deoxy-D-arabinopyranoside (**S2**).

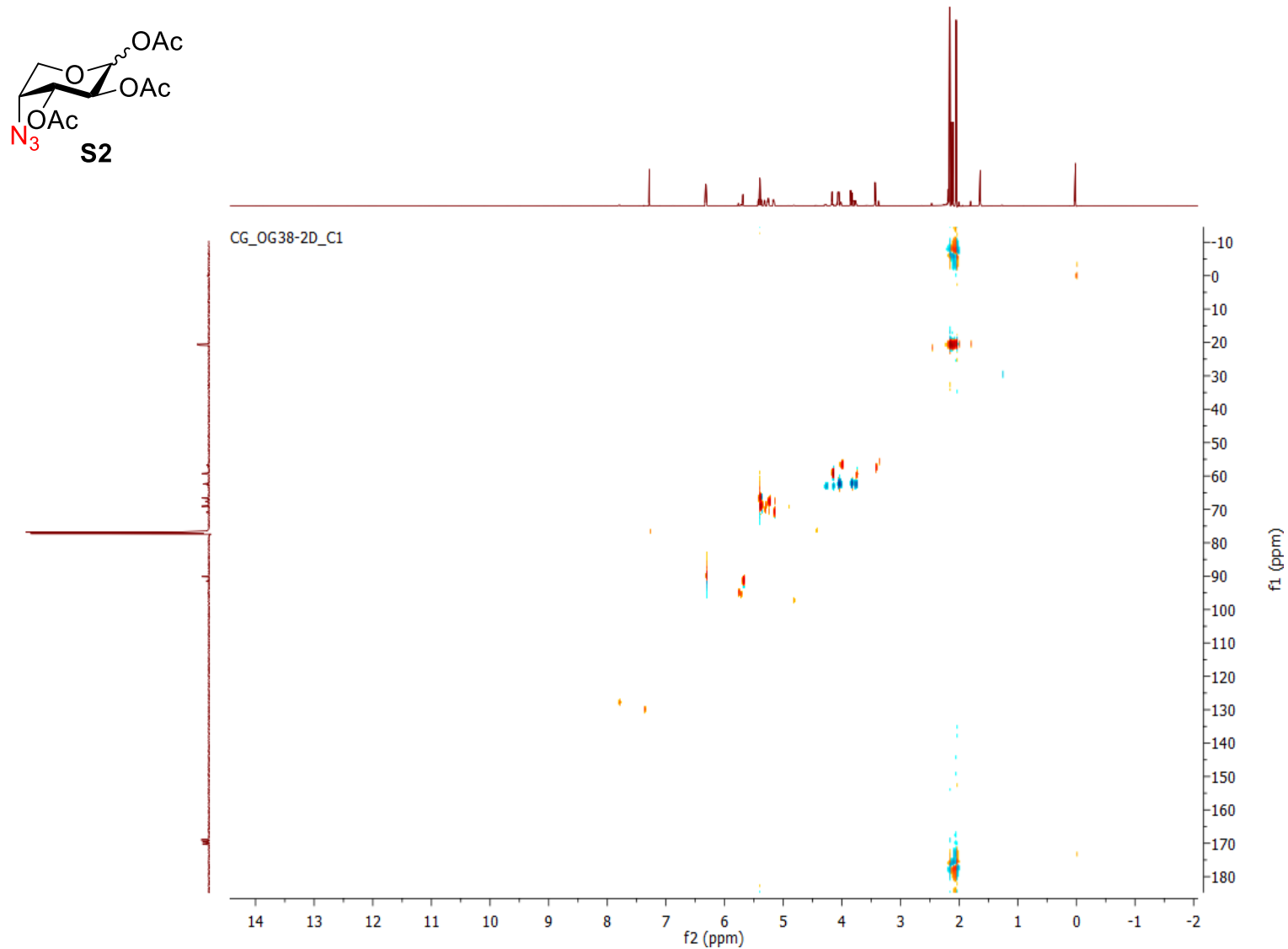


Figure S26. ¹H NMR spectrum (D₂O, 600 MHz) of compound 4

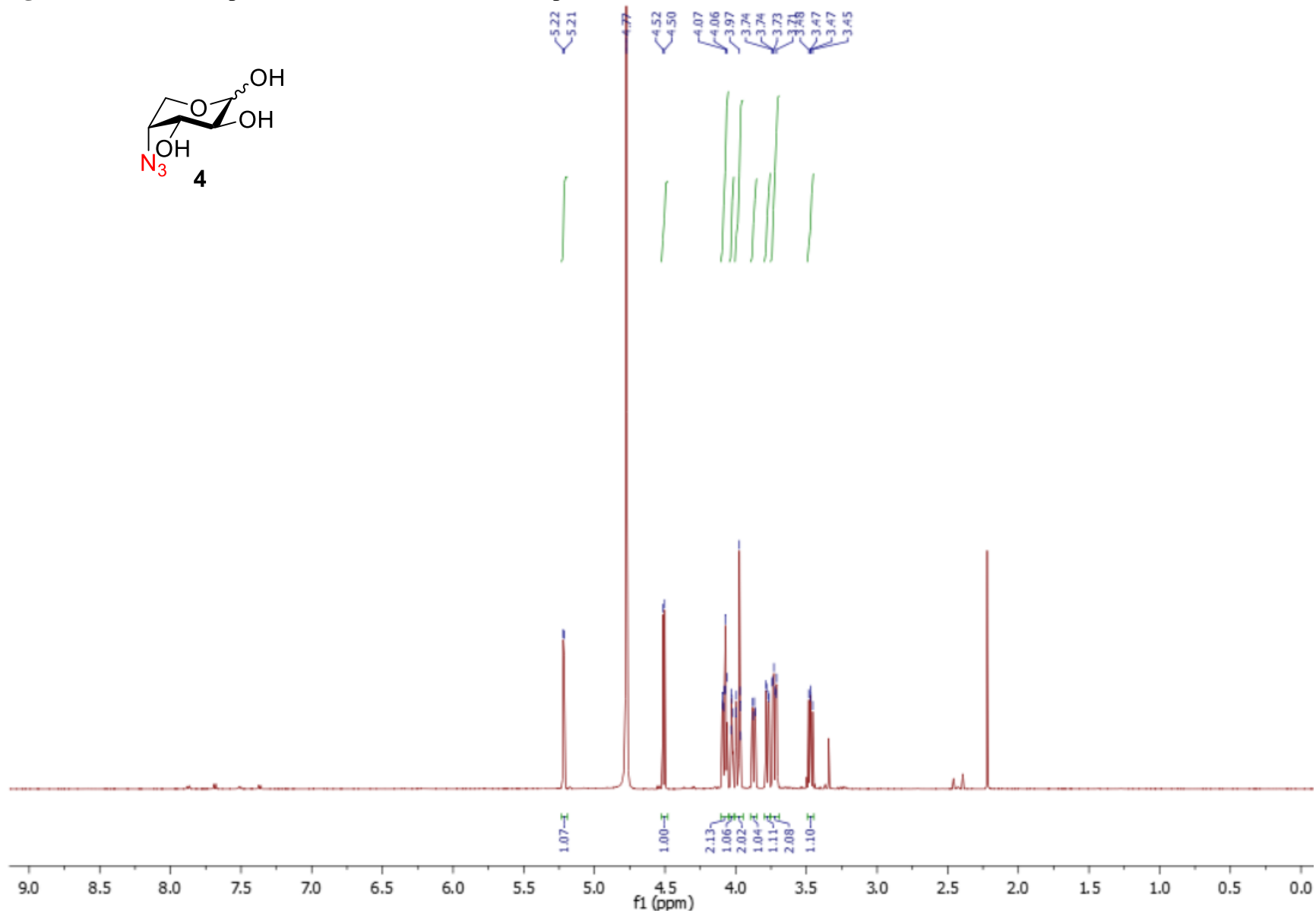


Figure S27. ^{13}C NMR spectrum (D_2O , 150 MHz) of compound **4**.

CG_OG40-2D_C2

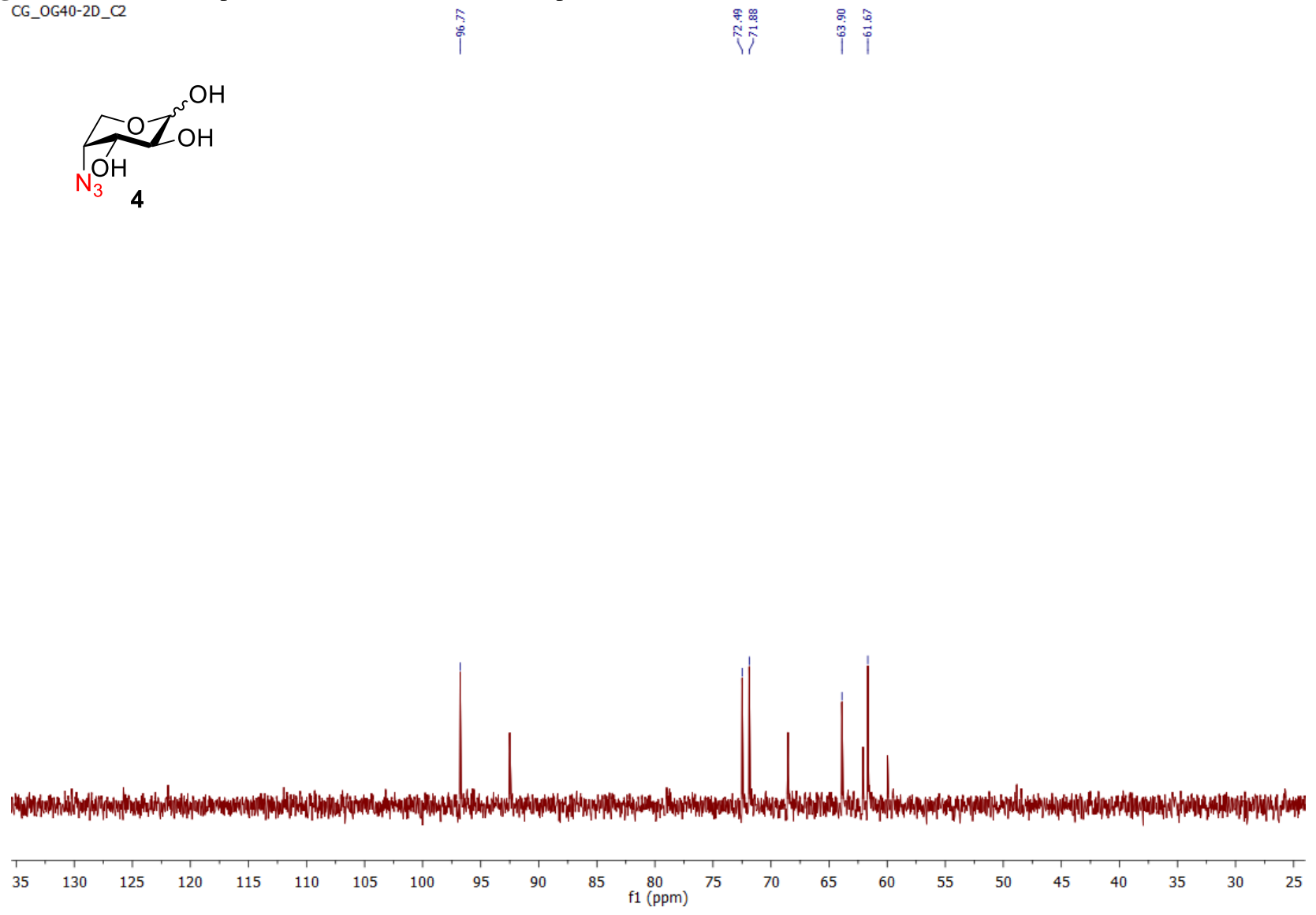
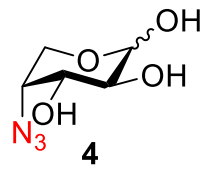


Figure S28. COSY NMR spectrum (D₂O, 600 MHz) of compound 4.

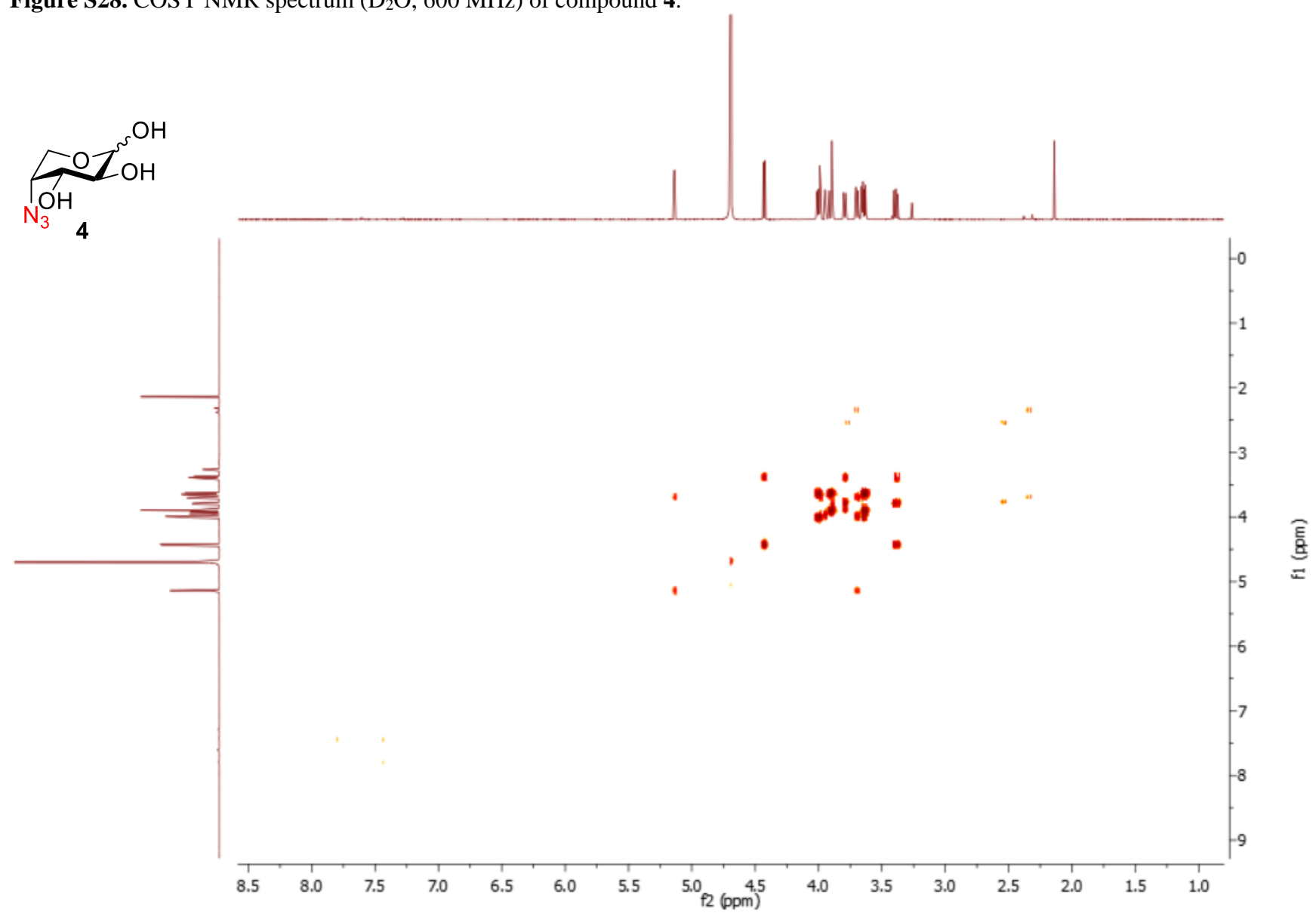
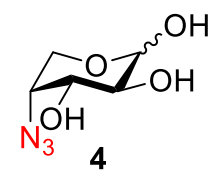


Figure S29. HSQC NMR spectrum (D₂O, 600 MHz) of compound **4**.

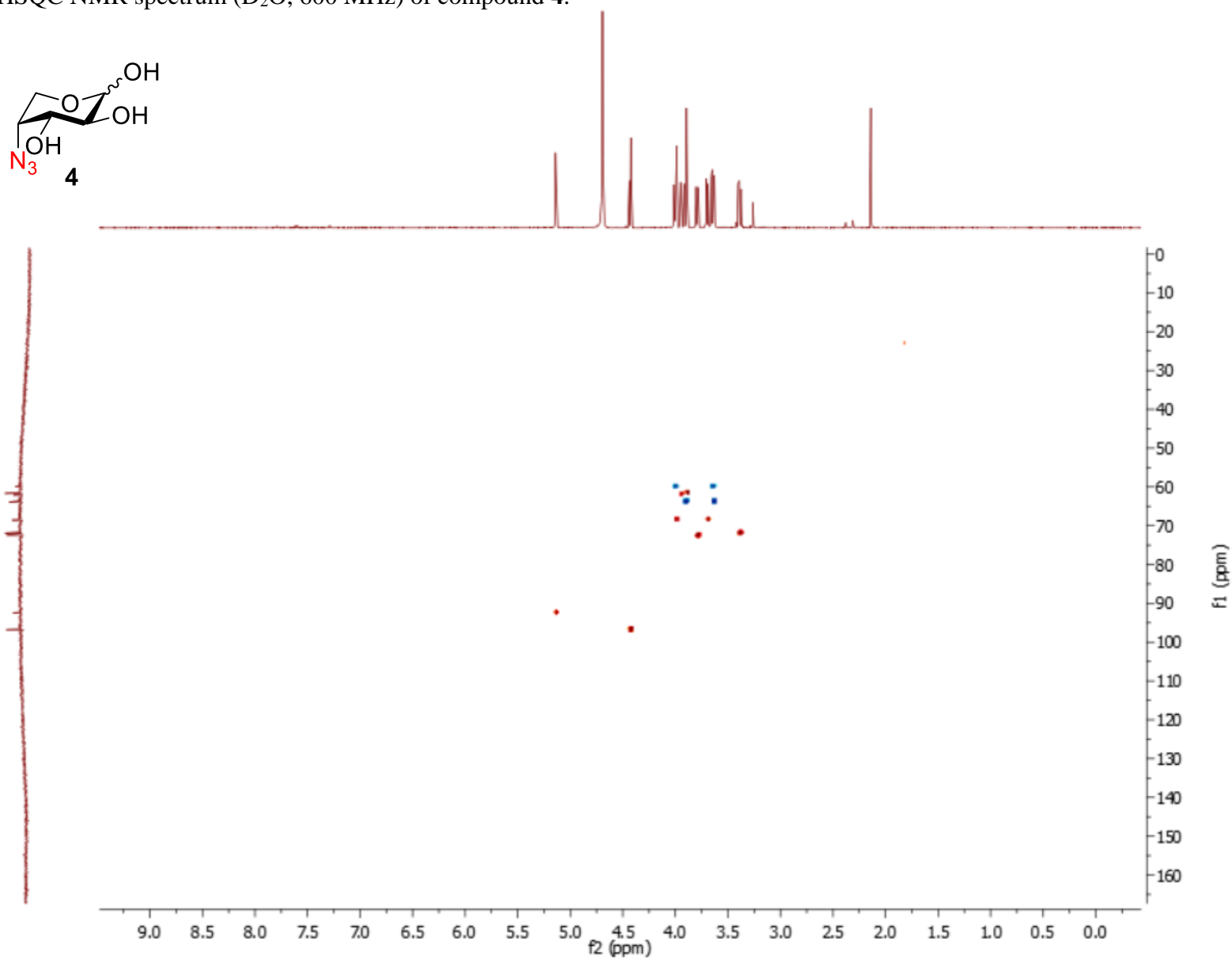


Figure S30. ^1H NMR spectrum (D_2O , 600 MHz) of compound 2.

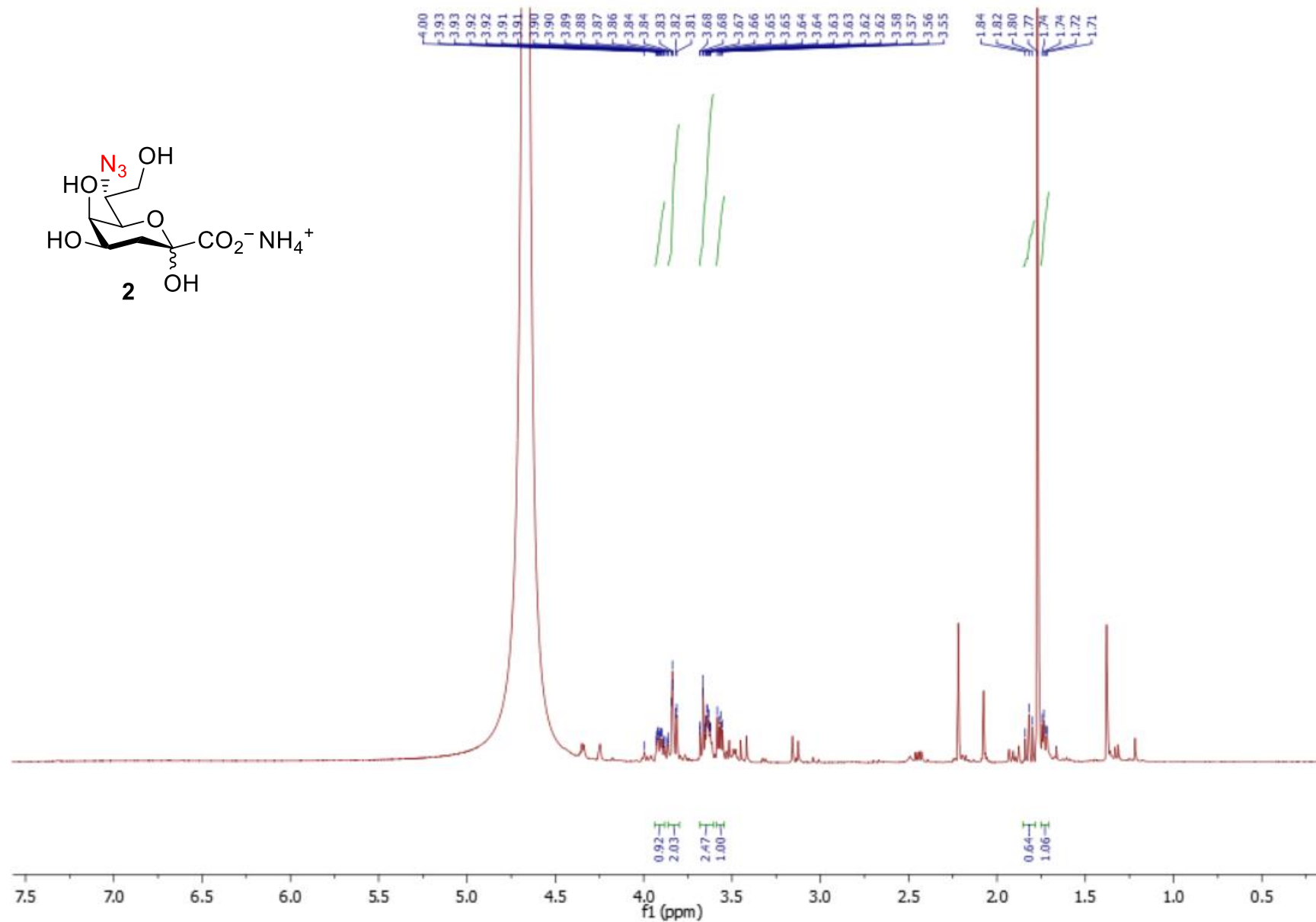


Figure S31. ^{13}C NMR spectrum (D_2O , 150 MHz) of compound **2**.

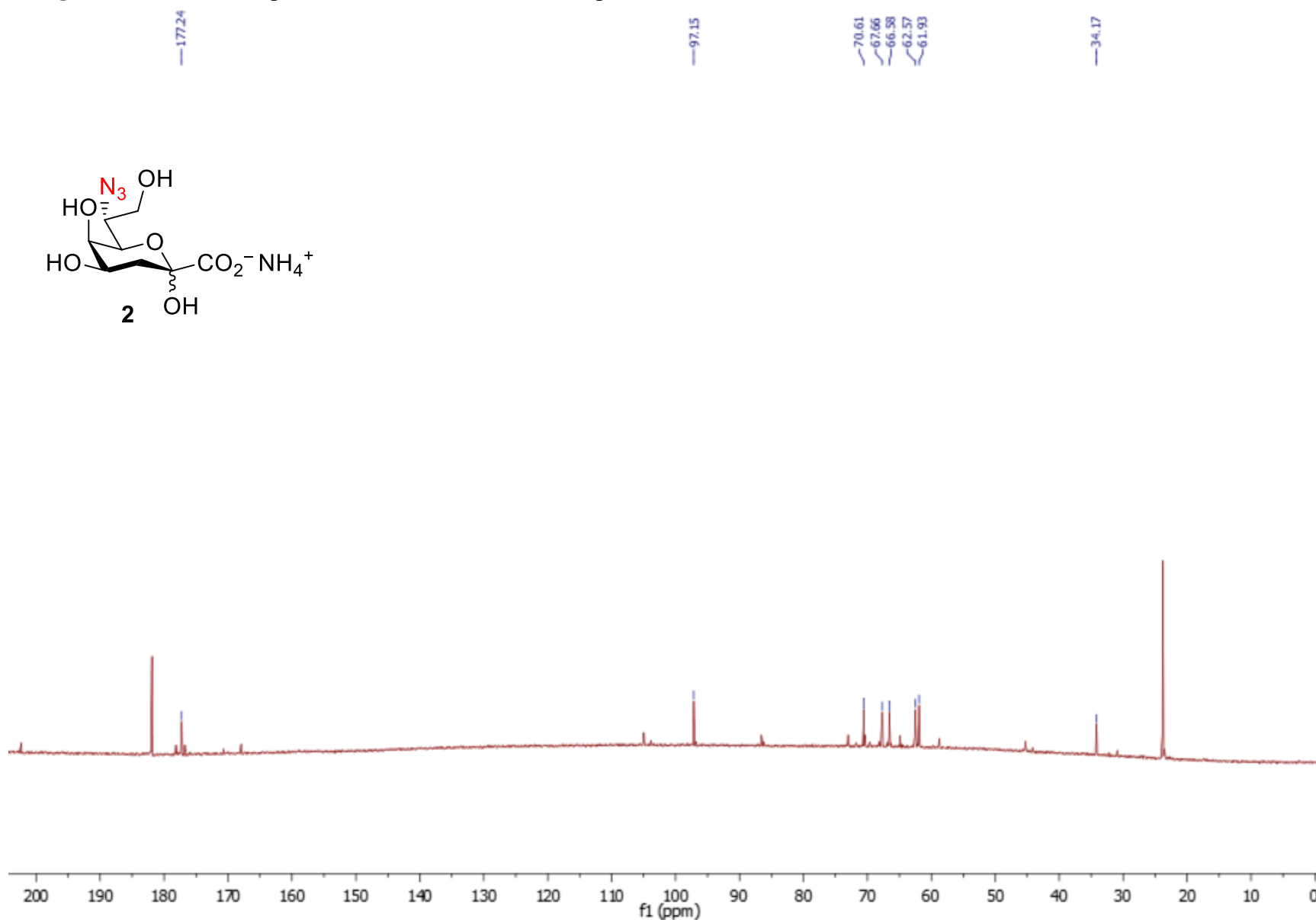


Figure S32. COSY NMR spectrum (D₂O, 600 MHz) of compound **2**.

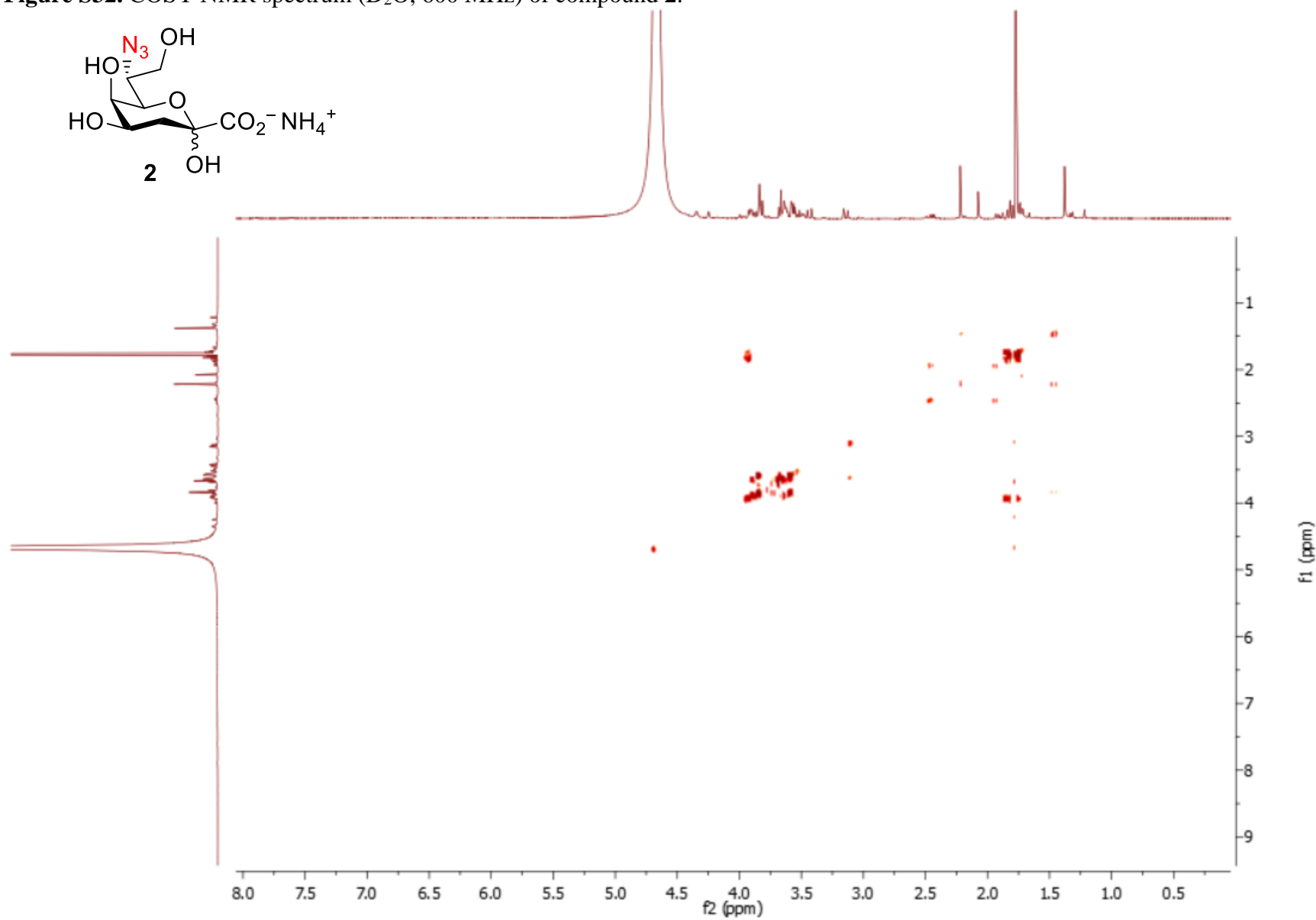
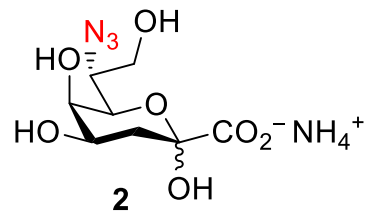


Figure S33. HSQC NMR spectrum (D₂O, 600 MHz) of compound **2**.

