Synthesis of chemical tools to label the mycomembrane of corynebacteria using a modified Iron (III) chloride-mediated protection of trehalose

Supporting Information

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I. Abbreviations in the manuscript and supporting information

AG	Arabinogalactan
Ag85	Antigen 85 complex
AGM	Arabinogalactan-linked mycolate
Alk	Alkyne
Az	Azide
BHI	Brain Heart Infusion
Bodipy	4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene
CDG	Cephalosporinase-dependent green
CmpL	Corynebacterial membrane proteins large
CR110-CCH	5/6-Carboxyrhodamine 110-PEG_4-alkyne
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
Dabcyl	4-(Dimethylaminoazo)benzene-4-carboxylic acid
DCM	Dichloromethane
DMN	4-*N*,*N*-Dimethylamino-1,8-naphatlimide
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
Fbp	Fibronectin binding protein
FI	Fluorescein
FITC	Fluorescein isothiocyanate
FRET	Fluorescence resonance energy transfer
MA	Mycolic acid
MFI	Mean fluorescence intensity
MM	Mycomembrane
MmpL	Mycobacterial membrane proteins large
Myt	Mycoloyl transferase
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
PG	Peptidoglycan
Pks	Polyketide synthase
QTF	Quencher trehalose fluorophore
RND	Resistance nodulation division
SPAAC	Strain-promoted alkyne-azide cycloaddition
TBAF	*n*-Tetrabutylammonium fluoride
$TBAN_3$	*n*-Tetrabutylammonium azide
TCO	*trans*-Cyclooctene
TDM	Trehalose dimycolate
Tf	Trifluoromethanesulfonyl
TGTA	Tris[(1-glucosyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
THF	Tetrahydrofuran
TMM	Trehalose monomycolate
TMS	Trimethylsilyl
TMR	Tetramethylrhodamine
Tre	Trehalose

II. Methods

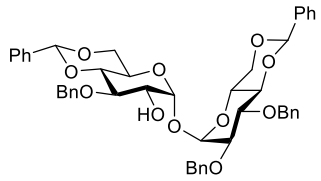
1. Chemical Synthesis

General methods

All air sensitive reactions were carried out in oven-dried glassware under a slight positive pressure of argon. Solvents were dried by standard methods. THF was distilled from sodium benzophenone ketyl. All chemical reagents and dry solvents were of analytical grade, purchased from chemical suppliers, and used without further purifications. TLC (Silica Gel 60 F_{254}) were visualized under UV (254 nm) and by staining either in 5% ethanolic sulfuric acid or orcinol or phosphomolybdic acid. Silica gel SDS 60 ACC 35-70 μm was used for column chromatography. NMR spectra were recorded on Bruker DRX 300, AV 360 or Advance 300 or 500 spectrometers. Chemical shifts (in ppm) were determined relative to residual protonated solvent as an internal reference. Abbreviations of multiplicity were as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), at (apparent triplet), m (multiplet), b (broad). Coupling constants in hertz (Hz) were measured from one-dimensional spectra. High-resolution mass spectra (positive or negative mode ESI) were recorded on a Bruker Daltonics micrOTOF-QII spectrometer or on a Waters LCT Premier XE (ToF). Optical rotations were measured on an Anton Paar MCP 150 polarimeter or with an Anton Paar MCP 300 polarimeter in a 10-cm cell at 20 °C and 589 nm (*c* in g / 100 mL). IR-FT spectra were recorded on a Perkin Elmer Spectrum 100 spectrometer. Characteristic absorptions are reported in cm^{-1}.

Synthesis of probes I, II and III

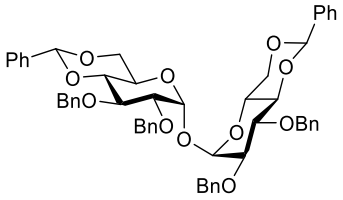
2',3,3'-Tri-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-α,α-D-trehalose (1)



To a solution of per-*O*-silylated α,α-D-trehalose **2**[i,ii] (200 mg, 0.217 mmol, 1.0 equiv) in dry CH_2Cl_2 (180 µL) at 0 °C were added benzaldehyde (220 µL, 2.174 mmol, 10.0 equiv) and a solution of $FeCl_3$•$6H_2O$ (45 µL of a 242 mM solution in CH_3CN, 5.0 mol%, dropwise). Triethylsilane (140 µL, 0.870 mmol, 4.0 equiv) was then added and the reaction mixture was warmed to room temperature and stirred overnight. Then, the reaction mixture was diluted with EtOAc and quenched by addition of an ice-cold saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted three times with EtOAc, and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 85:15 to 70:30) to give the expected product **1** (68 mg, 40%). The di- and tetra-*O*-benzylated derivatives (i.e. compounds **3** and **5** respectively) were also obtained as by-products, see table 1 of the main text. For larger scales (i. e. 0.5 - 2.0 g of starting material), a TBAF treatment could be required, and the yield of expected compound **1** can decrease to 30-36%.

M = 788.89 g/mol. Chemical formula: $C_{47}H_{48}O_{11}$
Rf = 0.65 (cyclohexane/EtOAc 6/4). IR (cm^{-1}): 3478, 3064, 3032, 2955, 2867, 1496, 1453, 1370, 1250, 1154, 1105, 1071, 1010, 984, 874, 841, 749, 696. ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 7.55-7.10 (m, 25H, H_{Ar}), 5.58 (s, 2H, 2CH-Ph), 5.19 (d, 1H, *J* = 3.8 Hz, H_1), 5.15 (d, 1H, *J* = 3.8 Hz, $H_{1'}$), 5.02 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.99 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.87 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.85 (d, 1H, *J* = 11.8 Hz, CH_2-Ph), 4.76 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.74 (d, 1H, *J* = 11.8 Hz, CH_2-Ph), 4.29 (dd, 1H, *J* = 10.0 Hz, *J* = 4.8 Hz, $H_{6'a}$), 4.27 (td, 1H, *J* = 10.0 Hz, *J* = 5.0 Hz, H_5), 4.16 (dd, 1H, *J* = 10.0 Hz, *J* = 5.0 Hz, H_{6a}), 4.14 (td, 1H, *J* = 10.0 Hz, *J* = 4.8 Hz, $H_{5'}$), 4.09 (at, 1H, *J* = 9.2 Hz, $H_{3'}$), 3.98 (at, 1H, *J* = 9.2 Hz, H_3), 3.78 (dd, 1H, *J* = 9.2 Hz, *J* = 3.8 Hz, H_2), 3.75-3.64 (m, 4H, H_{6b}, $H_{6b'}$, H_4, $H_{4'}$), 3.65 (dd, 1H, *J* = 9.2 Hz, *J* = 3.8 Hz, $H_{2'}$). ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 138.8, 138.5, 138.0, 137.5, 137.4 (5C, 5 Cq-Ar), 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 128.0, 127.9, 127.8, 127.6, 126.2, 126.0 (25C, 25 CH-Ar), 101.3, 101.2 (2C, 2 CH-Ph), 95.5 (C_1), 94.7 ($C_{1'}$), 82.3, 82.3 (2C, C_4, $C_{4'}$), 78.8, 78.7, 78.7 (3C, C_3, $C_{3'}$, $C_{2'}$), 75.3, 75.0, 73.9 (3C, 3 CH_2-Ph), 71.8 (C_2), 69.0, 68.9 (2C, C_6, $C_{6'}$), 63.3 (C_5), 63.1 ($C_{5'}$). HRMS (ESI^+): calcd for $C_{47}H_{48}NaO_{11}$ $[M+Na]^+$ 811.3089, found 811.3082. $[\alpha]_D^{20}$: + 61 (*c* 1.0, $CHCl_3$).

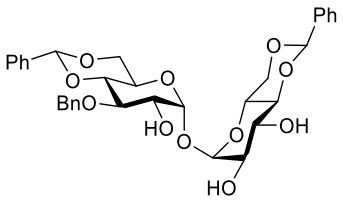
2,2',3,3'-Tetra-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-α,α-D-trehalose (5)



Compound **5** was obtained in 16 % yield as a by-product during the synthesis of **1**.

$C_{54}H_{54}O_{11}$; M = 879.02 g.mol^{-1}; R_f = 0.70 (cyclohexane/ethyl acetate: 7/3); ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 7.56-7.28 (m, 15H, H_{ar}), 5.58 (s, 1H, CH-Ph), 5.14 (d, 1H, *J* = 3.9 Hz, H_1), 4.99 (d, 1H, *J* = 11.0 Hz, CH_2-Ph), 4.88 (d, 1H, *J* = 11.0 Hz, CH_2-Ph), 4.87 (d, 1H, *J* = 12,0 Hz, CH_2-Ph), 4.76 (d, 1H, *J* = 12.0 Hz, CH_2-Ph), 4.31 (td, 1H, *J* = 4.8 Hz, *J* = 10.0 Hz, H_5), 4.17 (at, 1H, *J* = 9.3 Hz, H_3), 4.15 (dd, 1H, *J* = 10.0 Hz, *J* = 4.8 Hz, H_{6a}), 3.69 (at, 1H, *J* = 10.0 Hz, H_{6b}), 3.67 (dd, 1H, *J* = 10.0 Hz, *J* = 9.3 Hz, H_4), 3.65 (dd, 1H, *J* = 9.3 Hz, *J* = 3.9 Hz, H_2); ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 138.9, 138.2, 137.6 (3C, Cq-Ar), 129.0, 128.6, 128.4, 128.3, 128.1, 127.9, 127.7, 126.3 (15C, CH-Ar), 101.3 (CH-Ph), 95.1 (C_1), 82.4 (C_4), 78.8 (2C, C_2, C_3), 75.2 (CH_2-Ph), 73.9 (CH_2-Ph), 69.1 (C_6), 63.0, (C_5); ESI HRMS for $C_{54}H_{55}O_{11}$ $[M+H]^+$: Calcd, 879.3739, Found 879.3706; $[\alpha]_D^{20}$ = +47 (*c* 0.75, $CHCl_3$).

3-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-α,α-D-trehalose (4)

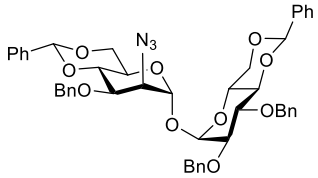


To a solution of per-*O*-silylated α,α-D-trehalose **2**[i,ii] (100 mg, 0.109 mmol, 1.0 equiv) in dry CH_2Cl_2 (180 µL) at 0 °C were added benzaldehyde (35 µL, 0.354 mmol, 3.2 equiv) and a solution of $FeCl_3$•$6H_2O$ (45 µL of a 136 mM solution in CH_3CN, 5.0 mol%, dropwise). Triethylsilane (20 µL, 0.125 mmol, 1.1 equiv) was then added and the reaction mixture was warmed to room temperature and stirred for 2 h. Then, the reaction mixture was diluted with EtOAc and quenched by addition of an ice-cold saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted three times with EtOAc, and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 60:40) to give compound **4** (24 mg, 35%). The di-*O*-benzylated derivative **3** was also obtained as by-product, see table 1 of the main text. NMR data were in agreement with those reported.[iii]

M = 608.63 g.mol^{-1}. Chemical formula: $C_{33}H_{36}O_{11}$
R_f = 0.14 (cyclohexane/ethyl acetate: 6/4); ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm): 7.52-7.28 (m, 15H, H_{ar}), 5.58 (s, 1H, CH-Ph), 5.50 (s, 1H, CH-Ph), 5.19 (d, 1H *J* = 4.0 Hz, H_1), 5.13 (d, 1H, *J* = 4.0 Hz, $H_{1'}$), 5.01 (d, 1H, *J* = 11.5 Hz, CH_2-Ph), 4.75 (d, 1H, *J* = 11.5 Hz, CH_2-Ph), 4.31

(dd, 1H, *J* = 5.0 Hz, *J* = 10.4 Hz, H_{6b}), 4.27 (dd, 1H, *J* = 5.0 Hz, *J* = 10.4 Hz, 1H, $H_{6'b}$), 4.14-3.98 (m, 3H, H_5, $H_{5'}$, $H_{3'}$), 3.93 (t, 1H, *J* = 9.0 Hz, H_3), 3.81-3.64 (m, 5H, H_{6a}, H_2, $H_{6'a}$, $H_{2'}$, H_4), 3.47 (t, 1H, *J* = 9.4 Hz, 1H, $H_{4'}$), 3.20 (s, 1H, OH), 2.55 (d, 1H *J* = 5.8 Hz, OH), 2.46 (d, 1H, *J* = 5.4 Hz, OH); ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm): 138.6, 137.5, 137.3 (3C, Cq-Ar), 129.4, 129.2, 128.7, 128.5, 128.2, 128.1, 126.5, 126.3 (15C, CH-Ar), 102.1, 101.6 (CH-Ph), 95.2, 95.1 (C_1, $C_{1'}$), 82.4 (C_4), 81.3 ($C_{4'}$), 78.9 (C_3), 75.2 (CH_2-Ph), 72.6 ($C_{2'}$), 71.9 (C_2), 71.5 ($C_{3'}$), 69.1, 69.0 (C_6, $C_{6'}$), 63.7, 63.2 (C_5, $C_{5'}$); ESI HRMS for $C_{33}H_{36}NaO_{11}$ $[M+Na]^+$: Calcd 631.2150, Found 631.2144; $[\alpha]_D^{24}$= + 75 (*c* 1.6, $CHCl_3$).

2-Azido-2',3,3'-tri-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-2-deoxy-2-epi-α,α-D-trehalose (7)

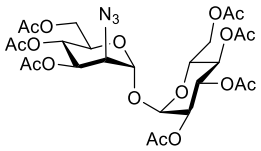


To a solution of compound **1** (500 mg, 0.63 mmol, 1.0 equiv) and anhydrous pyridine (0.5 mL, 6.1 mmol, 10.0 equiv) in anhydrous CH_2Cl_2 (10 mL, 0.06 M) at 0 °C under an argon atmosphere was added triflic anhydride (250.0 µL, 1.4 mmol, 2.2 equiv). The reaction mixture was stirred at room temperature under an argon atmosphere for 3 hours then cooled to 0 °C. The reaction was quenched by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with CH_2Cl_2, and the combined organic layer was dried over anhydrous sodium sulfate and co-evaporated with toluene three times under reduced pressure. The crude material was purified by flash chromatography over silica gel (cyclohexane/EtOAc, 7:3) to give the crude triflate **6** as a white powder. This compound was dissolved in toluene (12.0 mL, 0.06 M) at room temperature under an argon atmosphere and treated with tetrabutylammonium azide (600 mg, 2.1 mmol, 4.0 equiv). The reaction mixture was stirred overnight at 50 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc and quenched by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with EtOAc, and the combined organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (cyclohexane/EtOAc, 7:3) to give compound **7** (506 mg, 0.62 mmol, 98%) as a white powder.

M = 813.90 g/mol. Chemical formula: $C_{47}H_{47}N_3O_{10}$
Rf = 0.7 (heptane/EtOAc, 7:3). IR (cm^{-1}): 3064, 3032, 2931, 2867, 2108, 1496, 1453, 1371, 1250, 1129, 1105, 1088, 998, 916, 737. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.56-7.25 (m, 25H, 25 H_{ar}), 5.63 (s, 1H, CH-Ph), 5.58 (s, 1H, CH-Ph), 5.09 (d, 1H, *J* = 3.8 Hz, $H_{1'}$), 4.99 (d, 1H, *J* = 1.5 Hz, H_1), 4.97 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.94 (d, 1H, *J* = 12.0 Hz, CH_2-Ph), 4.86 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.83 (d, 1H, *J* = 11.9 Hz, CH_2-Ph), 4.73 (d, 1H, *J* = 12.0 Hz, CH_2-Ph), 4.72 (d, 1H, *J* = 11.9 Hz, CH_2-Ph), 4.29 (dd, 1H, *J* = 9.0 Hz, *J* = 3.0 Hz, $H_{6'a}$), 4.22 (dd, 1H, *J* = 9.0 Hz, *J* = 3.6 Hz, H_3), 4.20-4.10 (m, 3H, H_4, H_5, H_{6a}), 3.99 (at, 1H, *J* = 9.5 Hz, $H_{3'}$), 3.86 (dd, 1H, *J* = 3.6 Hz, *J* = 1.5 Hz, H_2), 3.79 (at, 1H, *J* = 9.7 Hz, H_{6b}), 3.78-3.70 (m, 2H,

$H_{5'}$, $H_{6b'}$), 3.66 (at, 1H, *J* = 9.5 Hz, $H_{4'}$), 3.61 (dd, 1H, *J* = 9.5 Hz, *J* = 3.8 Hz, $H_{2'}$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 138.7, 138.1, 137.9, 137.7, 137.5 (5C, 5 Cq-Ar), 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 126.4, 126.2 (25C, 25 CH-Ar), 101.8, 101.5 (2 C, CH-Ph), 95.4 (C_1), 94.4 ($C_{1'}$), 82.3 ($C_{4'}$), 79.1 (C_4**)**, 78.5 ($C_{2'}$), 78.4 ($C_{3'}$), 75.9 (C_3), 75.4 (CH_2-Ph), 74.4 (CH_2-Ph), 74.1 (CH_2-Ph), 69.1 ($C_{6'}$), 68.7 (C_6), 64.6 (C_5), 63.8 ($C_{5'}$), 63.0 (C_2). HRMS (ESI^+): calcd for $C_{47}H_{47}N_3NaO_{10}$ $[M+Na]^+$ 836.3154, found 836.3192. $[\alpha]_D^{20}$: + 57 (*c* 0.4, $CHCl_3$).

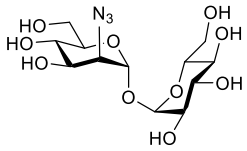
2',3,3',4,4',6,6'-Hepta-*O*-acetyl-2-azido-2-deoxy-2-epi-α,α-D-trehalose (8)



A dry 100 mL round bottom flask was outfitted with a stir bar and a rubber septum. The assembly was put in a drier and cooled under vacuum. The flask was charged with anhydrous iron (III) chloride (542 mg, 3.34 mmol, 21.0 equiv), three cycles of vacuum – argon were done. Iron (III) chloride was suspended in dry CH_2Cl_2 (20.0 mL, 0.17 M) and sonicate during 10 min. A solution of compound **7** (130 mg, 0.16 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (10 mL, 0.016 M) was added. The suspension was stirred at 0 °C and anhydride acetic (600 µL, 648 mg, 6.35 mmol, 40.0 equiv) was added. The reaction mixture was stirred under an argon atmosphere for 48 hours. Then, the reaction was quenched by addition of ice-cold saturated aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2, and the combined organic layers were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product diluted in CH_3OH (≈ 15 mL) was passed rapidly through a short pad of resin (Dowex 50WX8 hydrogen form), concentrated and then purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc, 100:0 to 80:20) to give compound **8** (64 mg, 0.097 mmol, 61%) as a white powder after lyophilization.

M = 661.57 g/mol. Chemical formula: $C_{26}H_{35}N_3O_{17}$
Rf = 0.4 (heptane/EtOAc 7:3). IR (cm^{-1}): 2960, 2111, 1743, 1433, 1368, 1214, 1143, 1034, 1010, 913, 754. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 5.42 (dd, 1H, *J* = 10.3 Hz, *J* = 9.4 Hz, $H_{3'}$), 5.35 (dd, 1H, *J* = 9.7 Hz, *J* = 3.8 Hz, H_3), 5.31 (d, 1H, *J* = 3.9 Hz, $H_{1'}$), 5.26 (dd, 1H, *J* = 9.8 Hz, *J* = 9.7 Hz, H_4), 5.07 (d, 1H, *J* = 1.8 Hz, H_1**)**, 5.06 (dd, 1H, *J* = 10.0 Hz, *J* = 9.4 Hz, $H_{4'}$), 4.99 (dd, 1H, *J* = 10.3 Hz, *J* = 3.9 Hz, $H_{2'}$), 4.24 (dd, 1H, *J* = 12.4 Hz, *J* = 4.8 Hz, $H_{6a'}$), 4.14 (dd, 1H, *J* = 12.3 Hz, *J* = 6.1 Hz, H_{6a}), 4.10-4.02 (m, 3H, H_2, $H_{6b'}$, H_{6b}), 4.01 (ddd, 1H, *J* = 10.0 Hz, *J* = 4.8 Hz, *J* = 2.4 Hz, $H_{5'}$), 3.85 (ddd, 1H, *J* = 9.8 Hz, *J* = 6.1 Hz, *J* = 2.3 Hz, H_5), 2.09-1.99 (6 s, 21H, 7 CH_3). ^{13}C NMR ($CDCl_3$¸75 MHz) δ (ppm): 170.8, 170.7, 170.1, 170.1, 169.8, 169.7 (7C, 7 C=O), 94.2 (C_1), 92.1 ($C_{1'}$), 71.0 (C_3), 70.0 (2 C, $C_{3'}$, $C_{2'}$), 69.8 (C_5), 68.6 (2C, $C_{5'}$, $C_{4'}$), 66.0, (C_4), 62.3, 62.0 (2C, C_6, $C_{6'}$), 61.2 (C_2), 20.8, 20.8, 20.7 (7C, 7 CH_3). HRMS (ESI^+): calcd for $C_{26}H_{35}N_3NaO_{17}$ $[M+Na]^+$ 684.1859, found 684.1852. $[\alpha]_D^{20}$: + 149 (*c* 1.0, $CHCl_3$).

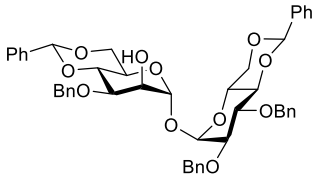
2-azido-2-deoxy-2-epi-α,α-D-trehalose (I) (2-epi-TreAz)



To a solution of compound **8** (46.5 mg, 0.07 mmol, 1.0 equiv) in anhydrous CH_3OH (10 mL, 0.007 M) was added anhydrous K_2CO_3 (5 mg, 0.035 mmol, 0.5 equiv). The reaction mixture was stirred at room temperature under an argon atmosphere overnight. The reaction was quenched by addition of CH_3OH (10.0 mL) and Dowex® H^+ resin. After 10 min, the resin was filtered and the reaction mixture was concentrated under vacuum to give compound **I** (26.1 mg, 0.07 mmol, 99%) as a white powder after lyophilization.

M = 367.31 g/mol. Chemical formula: $C_{12}H_{21}N_3O_{10}$
Rf = 0.4 (EtOAc/CH_3OH 6:4). IR (cm^{-1}): 3353, 2936, 2116, 1567, 1407, 1267, 1145, 1078, 1051, 1033, 1001. ^1H NMR (CD_3OD, 500 MHz) δ (ppm): 5.14-5.11 (m, 2H, H_1, $H_{1'}$), 4.11 (dd, 1H, *J* = 9.4 Hz, *J* = 3.9 Hz, H_3), 3.89 (dd, 1H, *J* = 3.9 Hz, *J* = 1.7 Hz, H_2), 3.86-3.75 (m, 3H, H_{6a}, $H_{6a'}$, H_5), 3.69-3.57 (m, 5H, $H_{6b'}$, H_{6b}, $H_{3'}$, H_4, $H_{5'}$), 3.48 (dd, 1H, *J* = 9.8 Hz, *J* = 3.8 Hz, $H_{2'}$), 3.28 (at, 1H, *J* = 9.5 Hz, $H_{4'}$). ^{13}C NMR (CD_3OD, 125 MHz) δ (ppm): 95.5, 95.0 (2C, $C_{1'}$, C_1), 75.1, 74.8, 74.7 (3C, $C_{5'}$, C_5, $C_{3'}$), 73.0, ($C_{2'}$), 72.4 (C_3), 72.0 ($C_{4'}$), 68.9 (C_4), 65.7 (C_2), 62.9, 62.9 (2C, C_6, $C_{6'}$). HRMS (ESI+): calcd for $C_{12}H_{21}N_3NaO_{10}$ $[M+Na]^+$ 390.1119, found 390.1133, calcd for $C_{14}H_{24}N_4NaO_{10}$ $[M+Na+CH_3CN]^+$ 431.1385, found 431.1395. $[\alpha]_D^{20}$: + 157 (*c* 0.5, CH_3OH).

2',3,3'-tri-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-2-epi-α,α-D-trehalose (9)

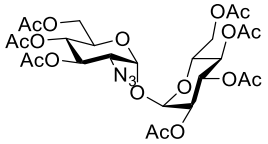


To a solution of compound **1** (500 mg, 0.63 mmol, 1.0 equiv) and anhydrous pyridine (0.5 mL, 6.1 mmol, 10.0 equiv) in anhydrous CH_2Cl_2 (10 mL, 0.03 M) at 0 °C under an argon atmosphere was added triflic anhydride (Tf_2O, 250.0 µL, 1.4 mmol, 2.2 equiv). The reaction mixture was stirred at room temperature under an argon atmosphere for 3 hours then cooled to 0 °C. The reaction was quenched by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with CH_2Cl_2, and the combined organic layer was dried over anhydrous sodium sulfate and co-evaporated with toluene three times under reduced pressure. The crude material was purified by flash chromatography over silica gel (cyclohexane/EtOAc, 7:3) to give the crude triflate **6** as a white powder. This compound was dissolved in dimethylformamide (8.0 mL, 0.07 M) at room temperature under an argon atmosphere and treated with sodium nitrite (750 mg, 10.8 mmol, 18.0 equiv). The reaction mixture was stirred overnight at 65 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc and quenched by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with EtOAc, and the combined organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by

flash chromatography over silica gel (cyclohexane/EtOAc, 8:2) to give compound **9** (231 mg, 0.29 mmol, 46%) as a white powder.

M = 788.89 g/mol Chemical formula: $C_{47}H_{48}O_{11}$
Rf = 0.4 (cyclohexane/EtOAc 6:4). IR (cm^{-1}): 3448, 3064, 3032, 2920, 2867, 1497, 1454, 1372, 1214, 1088, 998749, 697. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.55-7.26 (m, 25H, 25 H_{ar}), 5.63 (s, 1H, CH-Ph), 5.60 (s, 1H, CH-Ph), 5.19-5.16 (m, 2H, H_1, $H_{1'}$), 4.97 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.91 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.86 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.83 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.75 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.74 (d, 1H, *J* = 11.1 Hz, CH_2-Ph), 4.29 (dd, 1H, *J* = 10.1 Hz, *J* = 4.7 Hz, $H_{6a'}$), 4.22 (dt, 1H, *J* = 9.5 Hz, *J* = 4.8 Hz, H_5), 4.21-4.11 (m, 2H, H_{6a}, H_4), 4.07-4.01 (m, 3H, H_2, $H_{3'}$, H_3), 3.87-3.80 (m, 2H, H_{6b}, $H_{5'}$), 3.74 (at, 1H, *J* = 10.1 Hz, $H_{6b'}$), 3.68 (at, 1H, *J* = 9.3 Hz, $H_{4'}$), 3.64 (dd, 1H, *J* = 9.3 Hz, *J* = 3.8 Hz, $H_{2'}$), 2.72 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 138.8, 138.2, 138.0, 137.9, 137.5 (5C, 5 Cq-Ar), 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.2, 128.2, 128.1, 127.9, 126.4, 126.1 (25C, 25 CH-Ar), 101.9 (CH-Ph), 101.4 (CH-Ph), 96.1 (C_1), 94.1 ($C_{1'}$), 82.4 ($C_{4'}$), 78.9 (C_4**)**, 78.7, 78.6 (2C, $C_{2'}$,$C_{3'}$), 75.8 (C_3), 75.5, 74.2, 73.6 (3C, 3 CH_2-Ph), 70.3 (C_2), 69.2, 68.9 (2C, $C_{6'}$, C_6), 63.9 (C_5), 63.6 ($C_{5'}$). HRMS (ESI+): calcd for $C_{47}H_{49}O_{11}$, calcd for $C_{47}H_{48}NaO_{11}$ $[M+Na]^+$ 811.3089, found 811.3116. $[\alpha]_D^{20}$: + 42 (*c* 1.0, $CHCl_3$).

2',3,3',4,4',6,6'-Hepta-*O*-acetyl-2-azido-2-deoxy-α,α-D-trehalose (12)

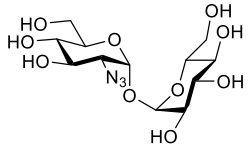


To a solution of compound **9** (318 mg, 0.40 mmol, 1.0 equiv) and anhydrous pyridine (0.5 mL, 6.18 mmol, 15.0 equiv) in anhydrous CH_2Cl_2 (2.6 mL, 0.15 M) at 0 °C, under an argon atmosphere was added triflic anhydride (Tf_2O, 148 µL, 0.88 mmol, 2.2 equiv). The reaction mixture was stirred at room temperature under an argon atmosphere for 3 hours then cooled to 0 °C. The reaction was quenched by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with CH_2Cl_2, and the combined organic layer was dried over anhydrous sodium sulfate and co-evaporated with toluene three times under reduced pressure. The crude material was purified by flash chromatography over silica gel (cyclohexane/EtOAc, 7:3) to give the crude triflate **10** as a white powder. This compound was dissolved in toluene (10.0 mL, 0.04 M) at room temperature under an argon atmosphere and treated with tetrabutylammonium azide (455 mg, 1.6 mmol, 4.0 equiv). The reaction mixture was stirred overnight at 50 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc and neutralized by addition of ice-cold saturated aqueous $NaHCO_3$. The reaction mixture was extracted three times with EtOAc, and the combined organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (heptane/EtOAc, 8:2) to give a mixture of compound **11** with the by-product of dehydration with a ratio of 2:1 (215 mg).
A dry 100 mL round bottom flask was outfitted with a stir bar and a rubber septum. The assembly was put in a drier and cooled under vacuum. The flask was charged with anhydrous

iron (III) chloride (834 mg, 5.1 mmol, 21.0 equiv), three cycles of vacuum – argon were done. Iron (III) chloride was suspended in dry CH_2Cl_2 (30.0 mL, 0.17 M) and sonicate during 10 min. A solution of compound **11** with the by-product of dehydration with a ratio of 2:1 (200 mg) in anhydrous CH_2Cl_2 (18 mL, 0.014 M) was added. The suspension was stirred at 0 °C and anhydride acetic (1 mL, 1.09 g, 8.5 mmol) was added. The reaction mixture was stirred under an argon atmosphere for 48 hours. Then, the reaction was quenched by addition of ice-cold saturated aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2, and the combined organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product diluted in CH_3OH (≈ 15 mL) was passed rapidly through a short pad of resin (Dowex 50WX8 hydrogen form), concentrated and then purified by flash chromatography on silica gel (heptane/EtOAc, 90:10 to 60:40) to give compound **12** (66.2 mg, 0.1 mmol, 25%) as a white powder after lyophilisation.

M = 661.57 g/mol. Chemical formula: $C_{26}H_{35}N_3O_{17}$
Rf = 0.3 (heptane/EtOAc, 7:3). IR (cm^{-1}): 2954, 2923, 2113, 1749, 1434, 1369, 1221, 1141, 1038, 1017. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.45 (at, 1H, *J* = 10.0 Hz, $H_{3'}$), 5.43 (at, 1H, *J* = 10.0 Hz, H_3), 5.27 (d, 1H, *J* = 4.0 Hz, $H_{1'}$), 5.17 (d, 1H, *J* = 4.0 Hz, H_1)**,** 5.09 (at, 1H, *J* = 10.0 Hz, $H_{4'}$), 5.00 (dd, 1H, *J* = 10.0 Hz, *J* = 4.0 Hz, $H_{2'}$), 4.99 (at, 1H, *J* = 10.0 Hz, H_4), 4.27 (ddd, 1H, *J* = 10.0 Hz, *J* = 4.0 Hz, *J* = 1.8 Hz, $H_{5'}$), 4.25-4.19 (m, 2H, $H_{6a'}$,H_{6a}), 4.10 (dd, 1H, *J* = 12.2 Hz, *J* = 1.8 Hz, $H_{6b'}$), 4.00 (ddd, 1H, *J* = 10.2 Hz, *J* = 5.5 Hz, *J* = 2.1 Hz, H_5), 3.98 (dd, 1H, *J* = 12.2 Hz, *J* = 2.1 Hz, H_{6b}), 3.71 (dd, 1H, *J* = 10.4 Hz, *J* = 3.8 Hz, H_2), 2.08, 2.07, 2.06, 2.05, 2.04, 2.03, 2.00 (7s, 21H, 7 CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 170.8, 170.7, 170.0, 170.0, 169.9, 169.9, 169.9 (7C, 7 C=O), 93.6 (C_1), 92.6 ($C_{1'}$), 71.5 (C_3), 70.2, 70.2 (2C, $C_{2'}$, $C_{3'}$), 68.8, 68.7, 68.5, 68.4 (4C, C_5, $C_{5'}$, C_4, $C_{4'}$), 62.0, 61.9 (2C, C_6, $C_{6'}$), 61.3 (C_2), 20.9, 20.9, 20.8, 20.8, 20.8 (7C, 7 CH_3). HRMS (ESI^+): calcd for $C_{26}H_{35}N_3NaO_{17}$ $[M+Na]^+$ 684.1859, found 684.1891. $[\alpha]_D^{20}$: + 75 (*c* 0.7, $CHCl_3$).

2-Azido-2-deoxy-α,α-D-trehalose (II) (2-TreAz)

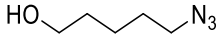


To a solution of compound **12** (69.1 mg, 0.10 mmol, 1.0 equiv) in anhydrous CH_3OH (10 mL, 0.01 M) was added anhydrous K_2CO_3 (10 mg, 0.072 mmol, 0.5 equiv). The reaction mixture was stirred overnight at room temperature under an argon atmosphere. The reaction was quenched by addition of CH_3OH (10.0 mL) and Dowex® H^+ resin. After 10 min, the resin was filtered and the reaction mixture was concentrated under vacuum to give compound **II** (37.2 mg, 0.10 mmol, 99%) as a white powder after lyophilisation. NMR data were in agreement with those reported.[iv]

M = 367.31 g/mol. Chemical formula: $C_{12}H_{21}N_3O_{10}$
Rf = 0.4 (EtOAc/CH_3OH, 6:4). IR (cm^{-1}): 3352, 2929, 2111, 1643, 1568, 1406, 1317, 1259, 1146, 1050, 995. ^{1}H NMR (CD_3OD, 500 MHz) δ (ppm): 5.21 (d, 1H, *J* = 3.6 Hz, H_1), 5.12 (d, 1H, *J* = 3.8 Hz, $H_{1'}$), 3.99 (dd, 1H, *J* = 10.0 Hz, *J* = 9.0 Hz, H_3), 3.85 (ddd, 1H, *J* = 10.0 Hz, *J*

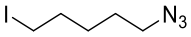
= 5.2 Hz, *J* = 2.2 Hz, H_5), 3.84-3.78 (m, 3H, $H_{5'}$, $H_{6a'}$, H_{6a}), 3.76 (at, 1H *J* = 9.5 Hz, $H_{3'}$), 3.74-3.68 (m, 2H, $H_{6b'}$, H_{6b}), 3.49 (dd, 1H, *J* = 9.5 Hz, *J* = 3.8 Hz, $H_{2'}$), 3.40 (dd, 1H, *J* = 10.0 Hz, *J* = 9.0 Hz, H_4), 3.36 (at, 1H, *J* = 9.5 Hz, $H_{4'}$), 3.27 (dd, 1H, *J* = 10.0 Hz, *J* = 3.6 Hz, H_2). ^{13}C NMR (CD_3OD, 125MHz) δ (ppm): 95.3 ($C_{1'}$), 94.2 (C_1), 74.8 ($C_{3'}$), 74.5, 74.2 (2C, $C_{5'}$, C_5), 73.2 ($C_{2'}$), 72.8 (C_3), 72.2 (C_4), 71.7 ($C_{4'}$), 64.8 (C_2), 62.7, 62.5 (2C, C_6, $C_{6'}$). HRMS (ESI^+): calcd for $C_{12}H_{21}N_3NaO_{10}$ $[M+Na]^+$ 390.1119, found 390.1133. $[\alpha]_D^{20}$: + 91 (*c* 0.7, CH_3OH).

5-Azidopentan-1-ol (S1)[v]



To a solution of commercially available 5-bromopentan-1-ol (725 µL, 5.99 mmol) in H_2O (17 mL), were added NaN_3 (778 mg, 11.96 mmol, 2 equiv) and the reaction mixture was stirred at reflux for 18 hours. After cooling to room temperature, the reaction mixture was extracted three times with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (diethyl ether/pentane, 40:60) to give the desired product **S1** (400 mg, 52%) as a colourless oil. NMR data were in agreement with those reported.[iii]
M = 129.2 g/mol. Chemical formula: $C_5H_{11}N_3O$
Rf = 0.35 (cyclohexane/EtOAc, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.66 (t, 2H, *J* = 6.4 Hz), 3.29 (t, 2H, *J* = 6.8 Hz), 1.70-1.54 (m, 4H), 1.52-1.41 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 62.8, 51.5, 32.3, 28.8, 23.2.

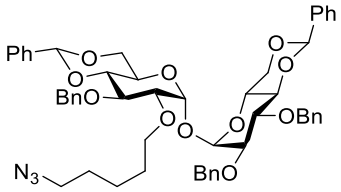
1-Azido-5-iodopentane (S2)



A solution of compound **S1** (320 mg, 2.48 mmol) in CH_3CN/THF (1/1, 3 mL) was cooled to 0 °C. Imidazole (202 mg, 2.97 mmol, 1.2 equiv), PPh_3 (845 mg, 3.22 mmol, 1.3 equiv) and iodine (880 mg, 3.47 mmol, 1.4 equiv) were then added and the reaction mixture was stirred at room temperature for 30 min. A saturated aqueous solution of $Na_2S_2O_3$ was added and the reaction mixture was extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (cyclohexane/EtOAc, 95:5) to give the desired product **S2** (539 mg, 91%) as a colourless oil.

M = 239.1 g/mol. Chemical formula: $C_5H_{10}IN_3$
Rf = 0.85 (cyclohexane/EtOAc, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.29 (t, 2H, *J* = 6.7 Hz), 3.19 (t, 2H, *J* = 6.8 Hz), 1.86 (quint, 2H, *J* = 6.8 Hz), 1.62 (quint, 2H, *J* = 6.8 Hz), 1.51 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 51.3, 33.1, 28.0, 27.8, 6.4.

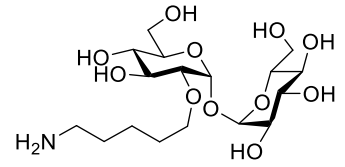
2-*O*-5-Azidopentyl-2',3,3'-tri-*O*-benzyl-4,6;4',6'-di-*O*-benzylidene-α,α-D-trehalose (13)



To a solution of compound **1** (400 mg, 0.507 mmol) in dry DMF (2 mL) at 0 °C was added NaH (45.6 mg, 1.52 mmol, 3 equiv). Then, a solution of compound **S2** (364 mg, 1.52 mmol, 3 equiv) in DMF (1.2 mL) was added at 0 °C and the reaction mixture was stirred at room temperature for 18 hours. The reaction was diluted with CH_3OH (2 mL), and a saturated aqueous solution of NaCl and Et_2O was added. The reaction mixture was extracted three times with Et_2O. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (cyclohexane/EtOAc, 95:5 to 80:20) to give the desired product **13** (395.7 mg, 87%) as a colourless oil.

M = 900.0 g/mol. Chemical formula: $C_{52}H_{57}N_3O_{11}$
Rf = 0.57 (cyclohexane/EtOAc, 7:3). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.56-7.24 (m, 25H, H_{Ar}), 5.57 (s, 1H, CH-Ph), 5.56 (s, 1H, CH-Ph), 5.18 (d, 1H, *J* = 3.7 Hz, H_1), 5.12 (d, 1H, *J* = 3.7 Hz, $H_{1'}$), 4.97 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.93 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.87 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.84 (d, 1H, *J* = 12.0 Hz, CH_2-Ph), 4.81 (d, 1H, *J* = 11.4 Hz, CH_2-Ph), 4.73 (d, 1H, *J* = 12.0 Hz, CH_2-Ph), 4.31-4.11 (m, 4H, H_5, $H_{5'}$, H_{6a}, $H_{6a'}$), 4.13 (at, 1H, *J* = 9.2 Hz, $H_{3'}$), 4.04 (at, 1H, *J* = 9.2 Hz, H_3), 3.73-3.58 (m, 7H, CH_2-O, $H_{2'}$, H_4, $H_{4'}$, H_{6b}, $H_{6b'}$), 3.47 (dd, 1H, *J* = 9.2 Hz, *J* = 3.7 Hz, H_2), 3.10 (t, 2H, *J* = 6.8 Hz, CH_2-N_3), 1.66-1.38 (m, 6H, 3 CH_2). ^{13}C NMR (CD_3OD, 75 MHz) δ (ppm): 139.0, 138.9, 138.2, 137.7, 137.6 (5C, Cq-Ar), 129.1, 128.7, 128.5, 128.4, 128.3, 128.1, 127.9, 127.7, 126.3, 126.1 (25C, CH-Ar), 101.4, 101.3 (2C, CH-Ph), 95.0 ($C_{1'}$), 94.6 (C_1), 82.5 (2C, C_4, $C_{4'}$), 80.1 (C_2), 78.9 ($C_{2'}$), 78.8 ($C_{3'}$), 78.7 (C_3), 75.5, 75.4, 74.0 (3C, CH_2-Ph), 72.0 (CH_2-O), 69.3, 69.2 (2C, C_6, $C_{6'}$), 63.1, 63.0 (2C, C_5, $C_{5'}$), 51.3 (CH_2-N_3), 29.9, 28.9, 23.6 (3C, 3 CH_2). HRMS (ESI): calcd for $C_{52}H_{57}NaN_3O_{11}$ $[M+Na]^+$ 922.3885, found 922.3868. $[\alpha]_D^{20}$: + 52 (*c* 1.1, $CHCl_3$).

2-*O*-(5-Aminopentyl)-α,α-D-trehalose (14)

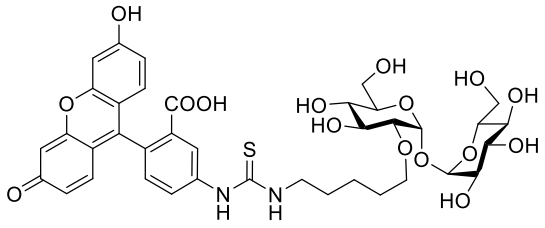


To a solution of compound **13** (67 mg, 0.07 mmol) in dioxane/H_2O (2:1, 3 mL) were added 70 mg of $Pd(OH)_2$. The reaction mixture was purged with dihydrogen and vigorously stirred at 40 °C for 3h30 under 1 atm of dihydrogen. The reaction mixture was filtered, washed three times with CH_3OH and concentrated under reduced pressure to give derivative **14** (31.8 mg, quantitative) as a syrup, which was engaged in the next step without further purification.

M = 427.4 g/mol. Chemical formula $C_{17}H_{33}NO_{11}$
Rf = 0.1 (EtOAc/CH_3OH/H_2O, 3:3:1). ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm): 5.30 (d, 1H, *J* = 3.5 Hz, H_1), 5.11 (d, 1H, *J* = 3.8 Hz, $H_{1'}$), 3.89-3.64 (m, 9H, H_3, $H_{3'}$, H_5, $H_{5'}$, H_{6a}, $H_{6a'}$, H_{6b},

$H_{6b'}$, 1H of CH_2-O), 3.59 (m, 1H, 1H of CH_2-O), 3.48 (dd, 1H, *J* = 9.8 Hz, *J* = 3.8 Hz, $H_{2'}$), 3.37-3.30 (m, 2H, H_4, $H_{4'}$), 3.23 (dd, 1H, *J* = 9.7 Hz, *J* = 3.5 Hz, H_2), 2.94 (t, 2H, *J* = 7.5 Hz, CH_2-N), 1.75-1.58 (m, 4H, 2 CH_2), 1.50 (m, 2H, CH_2). ^{13}C NMR (CD_3OD, 90 MHz) δ (ppm): 95.4 ($C_{1'}$), 92.9 (C_1), 81.3 (C_2),74.5, 73.9, 73.8, 73.6 (4C, C_3, $C_{3'}$, C_5, $C_{5'}$), 73.1 ($C_{2'}$), 71.9, 71.8, (2C, C_4, $C_{4'}$), 71.7 (CH_2-O) 62.8, 62.5 (2C, C_6, $C_{6'}$), 40.7 (CH_2-N), 30.4, 28.3, 24.1 (3C, 3 CH_2). HRMS (ESI): calcd for $C_{17}H_{34}NO_{11}$ $[M+H]^+$ 428.2126, found 428.2112. $[\alpha]_D^{20}$: + 144 (*c* 1.05, CH_3OH).

2-*O*-(5-[(Fluorescein-5-yl)thioureido]pentyl)-*α*,*α*-D-trehalose (III) (2-FIC5Tre)



To a solution of compound **14** (10.0 mg, 0.023 mmol) in 400 µL of $NaHCO_3$ buffer (75 mM, pH = 9) and CH_3CN (200 µL) was added fluorescein isothiocyanate (11.0 mg, 0.028 mmol, 1.2 equiv). The reaction mixture was stirred at 60 °C for 4 hours. The crude mixture was purified by HPLC with a C18 column (10% CH_3CN in H_2O to 100% CH_3CN, 2 mL/min) to give derivative **III** (12.6 mg, 66%) as an orange solid.

M = 816.8 g/mol. Chemical formula $C_{38}H_{44}N_2O_{16}S$
Rf = 0.9 (EtOAc/iPrOH/H_2O, 2:2:1). ^{1}H NMR (D_2O, 400 MHz) δ (ppm): 7.69 (m, 1H, H_{ar}), 7.52 (m, 1H, H_{ar}), 7.31-7.17 (m, 3H, H_{ar}), 6.70-6.60 (m, 4H, H_{ar}), 5.38 (m, 1H, H_1), 5.18 (d, 1H, *J* = 3.8 Hz, $H_{1'}$), 3.88-3.80 (m, 6H, H_3, $H_{3'}$, H_5, $H_{5'}$, H_{6a}, H_{6b},), 3.79-3.70 (m, 3H, $H_{6a'}$, $H_{6b'}$, 1H of CH_2-O), 3.68-3.56 (m, 3H, 1H of CH_2-O, CH_2-N), 3.63 (dd, 1H, $H_{2'}$, , *J* = 10.0 Hz , *J* = 3.8 Hz), 3.51-3.39 (m, 3H, H_2, H_4, $H_{4'}$), 1.79-1.58 (m, 4H, 2 CH_2), 1.42 (m, 2H, CH_2). ^{13}C NMR (D_2O, 100 MHz) δ (ppm): 180.8, 179.5, 159.9 (Cq), 133.2, 127.2, 126.2, 123.8 (CH_{ar}), 115.3 (Cq), 105.2 (CH_{ar}) 95.3 ($C_{1'}$), 92.9 (C_1), 80.8 (C_2),74.2, 74.1, 73.7, 73.5 (4C, C_3, $C_{3'}$, C_5, $C_{5'}$), 73.2 (CH_2-O), 72.8 ($C_{2'}$), 71.6, 71.2, (2C, C_4, $C_{4'}$), 62.3 (C_6), 62.0 ($C_{6'}$), 46.5 (CH_2-N), 30.5 (2C, 2 CH_2), 24.3 (CH_2). HRMS (ESI): calcd for $C_{38}H_{45}N_2O_{16}S$ $[M+H]^+$ 817.2484, found 817.2464.

2. Labeling of bacteria with trehalose-based probes

Bacterial strains, growth conditions and reagents

Dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich. Phosphate-buffered saline solution (PBS), Gene Frame seals, Microscope Slides and Microscope Coverslips were purchased from Thermo Fisher Scientific. 5/6-Carboxyrhodamine 110-PEG_4-Alkyne (CR110-CCH) was purchased from Jena Bioscience. Tris((1-(β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl)methyl)amine (TGTA) was synthesized according to a reported procedure.[vi]
Solutions stocks of organic and inorganic compounds were prepared as followed: sodium ascorbate: 50 mM in PBS 1X; TGTA: 40 mM in PBS 1X; Copper sulfate ($CuSO_4$): 20 mM in H_2O; CR110-CCH: 2 mM in DMSO. Stock solutions of trehalose analogues were prepared in milliQ water. *Corynebacterium glutamicum* ATCC13032 were inoculated into BHI growth medium and cultured at 30 °C under agitation (180 rpm).

General procedure for labeling *C. glutamicum*

C. glutamicum was inoculated (1:100) in BHI medium (final volume 900 µL). Bacteria were cultured 8 h with probe **I** or **II** (250 µM); or were cultured until mid-log phase growth (4 h, OD ≈ 6) at which point probe **III** (100 µM) was added (control experiments were treated with probe vehicle i.e. water) and the growth was pursued for 4 h at 30 °C.
Then, 400 µL of these suspensions were transferred in a 1.5 mL microtube and the cells were harvested by centrifugation at 3200 rpm for 5 min. The pellets were washed three times with PBS buffer (200 µL, 3200 rpm, 5 min, rt). The pellets of bacteria cultured with probe **III** were kept at 4 °C between 2 and 4 hours in the dark before being analyzed. The pellets of bacteria cultured with probe **I** or **II** were re-suspended in 200 µL of the "click" solution (0.05 mM CR110-CCH, 2.5 mM sodium ascorbate, 2.0 mM TGTA and 1.0 mM copper sulfate pentahydrate in PBS/DMSO (98:2) buffer) or in 200 µL of PBS/DMSO (97:3) buffer, and transferred in a 2.0 mL microtube for a better agitation. This suspension was vigorously shaken (550 rpm) in the dark for 30 min at room temperature, then transferred back in a 1.5 mL microtube and the cells were harvested by centrifugation (3200 rpm, 5 min, rt). Bacterial pellets were washed one times with PBS/DMSO (97:3) buffer (200 µL, 3200 rpm, 5 min, rt) and two times with PBS buffer (200 µL, 3200 rpm, 5 min, rt). The pellets were re-suspended in PBS buffer (200 µL) and kept at 4 °C between 1 and 3 hours in the dark before being analyzed.

Flow cytometry quantification

Fluorescence of the samples (diluted 100 times in PBS buffer) were analyzed using a Cytoflex flow cytometer (Beckman-Coulter). A minimum of 20000 events were counted for each data set (10 µL/min). Data were analyzed using CytExpert software (Beckman-Coulter). Representative histograms for *C. glutamicum* experiments labeling with probes **I**, **II** or **III** at 250 µM or 100 µM, followed by reaction with alkyne functionalized carboxyrhodamine 110 (CR110-CCH) using CuAAC.

General procedures for confocal imaging of labeled bacteria

Samples of 2 µL were deposited on agar (1%) pads placed on glass slides and then covered by coverslips. Cell morphology was observed with bright field light. Fluorescence microscopy experiments were performed on a confocal straight Leica SP8 microscope (DM 6000), using a 63x PLAN APO oil immersion lens (Leica), an argon laser and a GaAsP Hybrid detector (Hamamatsu). CR110 were excited at 488 nm and collected with a 500-550 nm band pass filter. The experiments were imaged with identical exposure setting. The microscope was operated with LAS-X program. Images were visualized and montages were constructed with ImageJ using Fiji without further processing.

3. *C. glutamicum* cell growth assays

C. glutamicum strain was grown in BHI medium overnight at 30 °C. The culture was then diluted in BHI medium to a final absorbance at 600 nm of 0.9. Sample aliquots (9 µL) of this diluted cell culture and 181 µL of BHI medium were placed in wells of a microtiter plate. The probe solution (10 µL) at various concentrations was then added to the wells and the plate was incubated at 30 °C for 16 h in a microtiter plate reader (MultiskanEX system microplate reader from Labsystem). Optical density readings (600 nm) were taken at 30 min intervals. The plate was shaken for 30 s before each reading.

III. Supplementary figures (S1-S2)

Figure S1. Growth curves of *C. glutamicum* treated with 2-TreAz **II** (A), 2-epi-TreAz **I** (B) and 2-FIC5Tre **III** (C) at 7 different concentrations. Experiments were performed in duplicate and data represent an average of two independent assays with error bars indicating standard deviations.

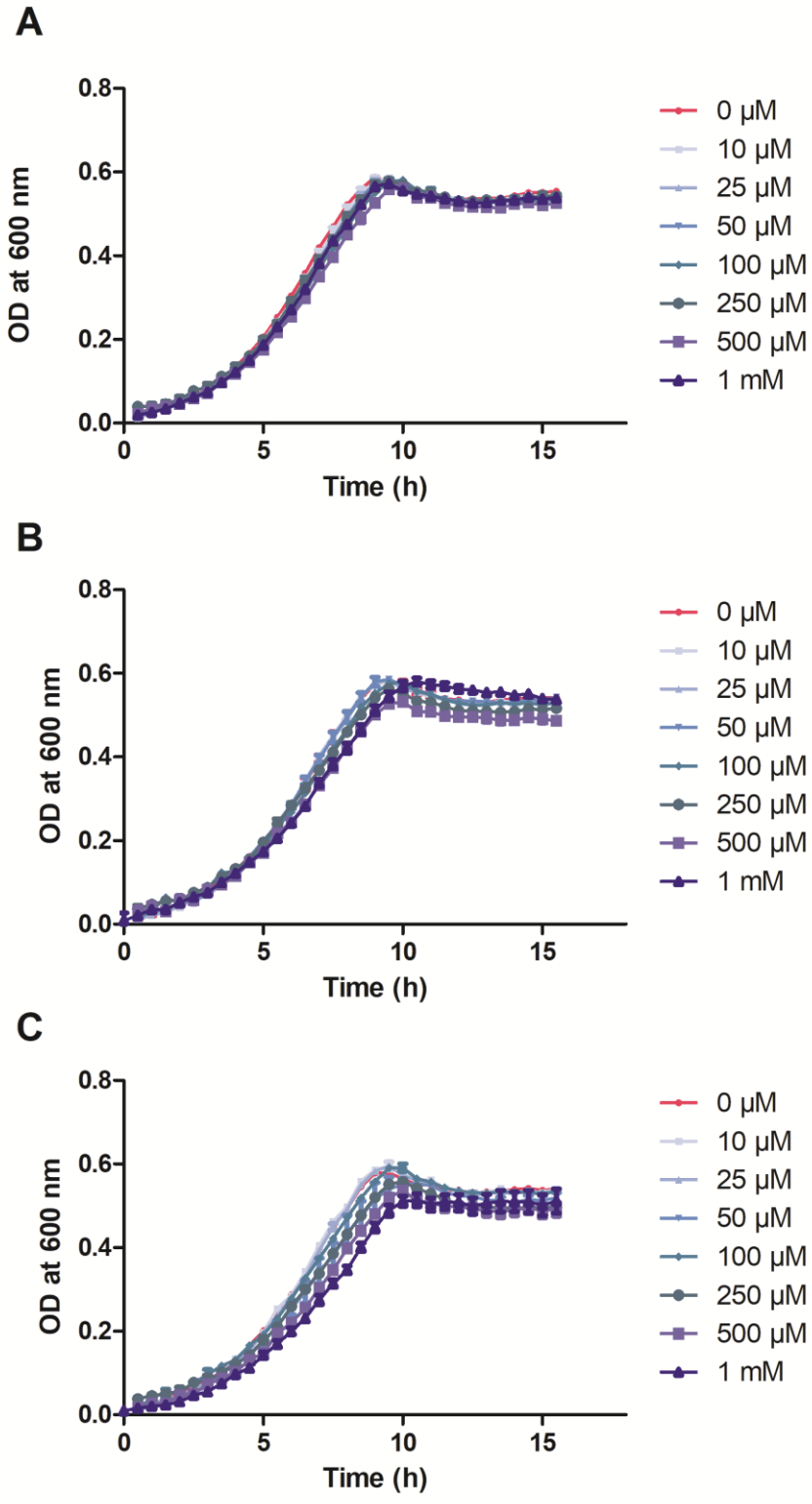
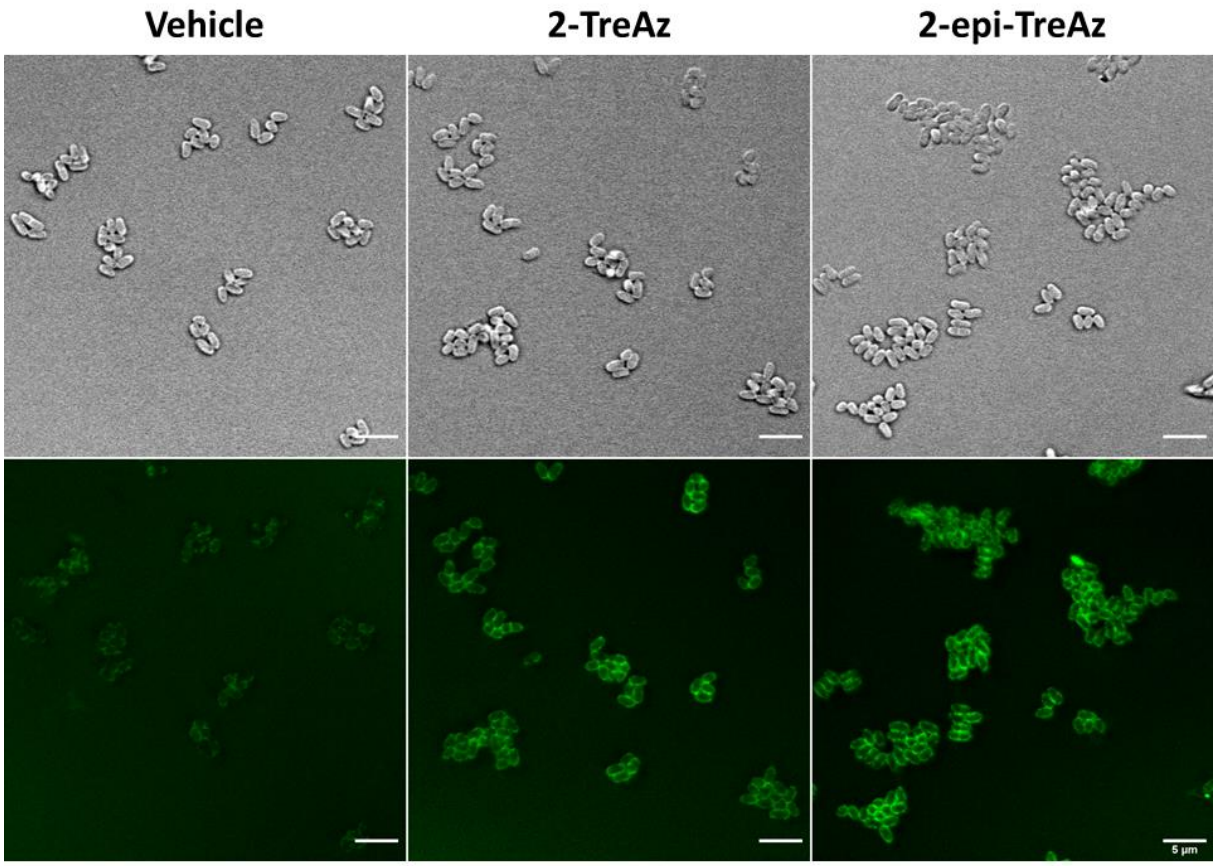
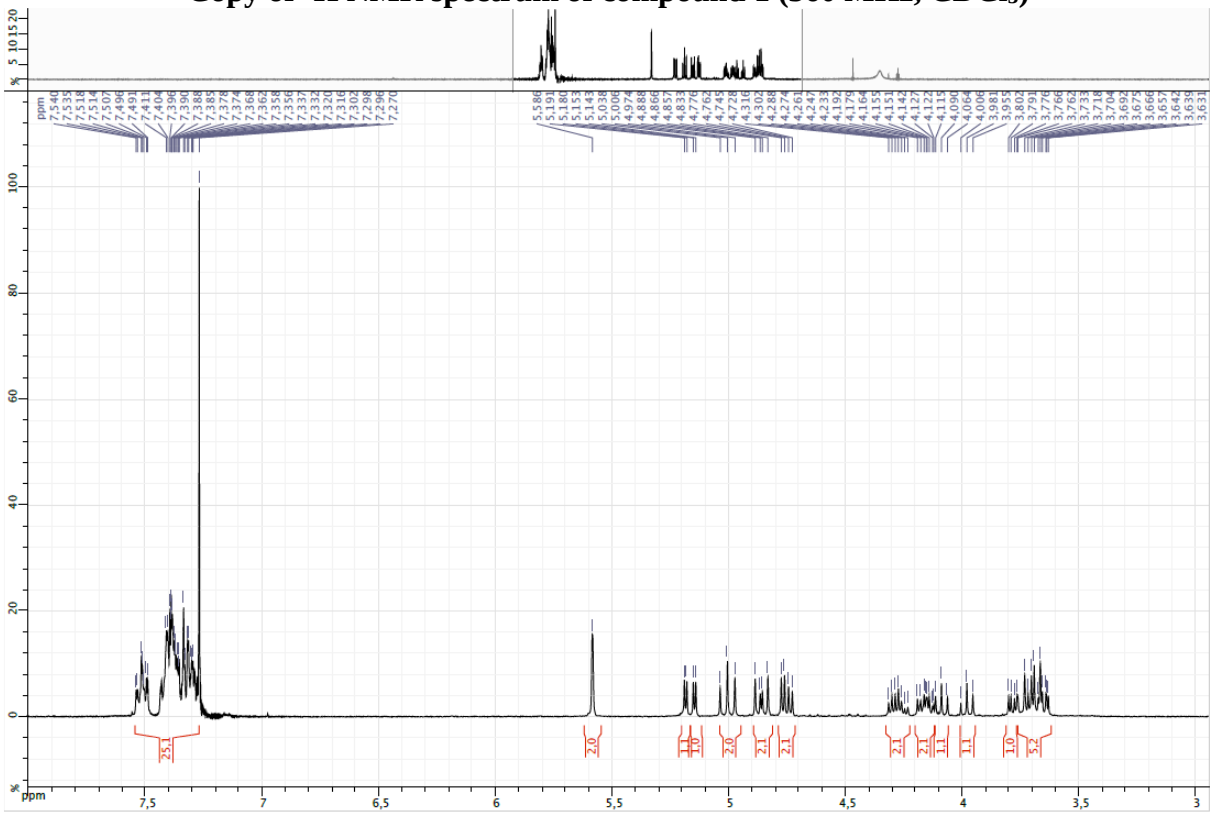


Figure S2. *C. glutamicum* incubated with with 250 μM of 2-TreAz **II** or 2-epi-TreAz **I** (or untreated) for 8 h, then reacted with CR110-CCH and analyzed by fluorescence microscopy with bright field images on top. Scale bars = 5 μm.

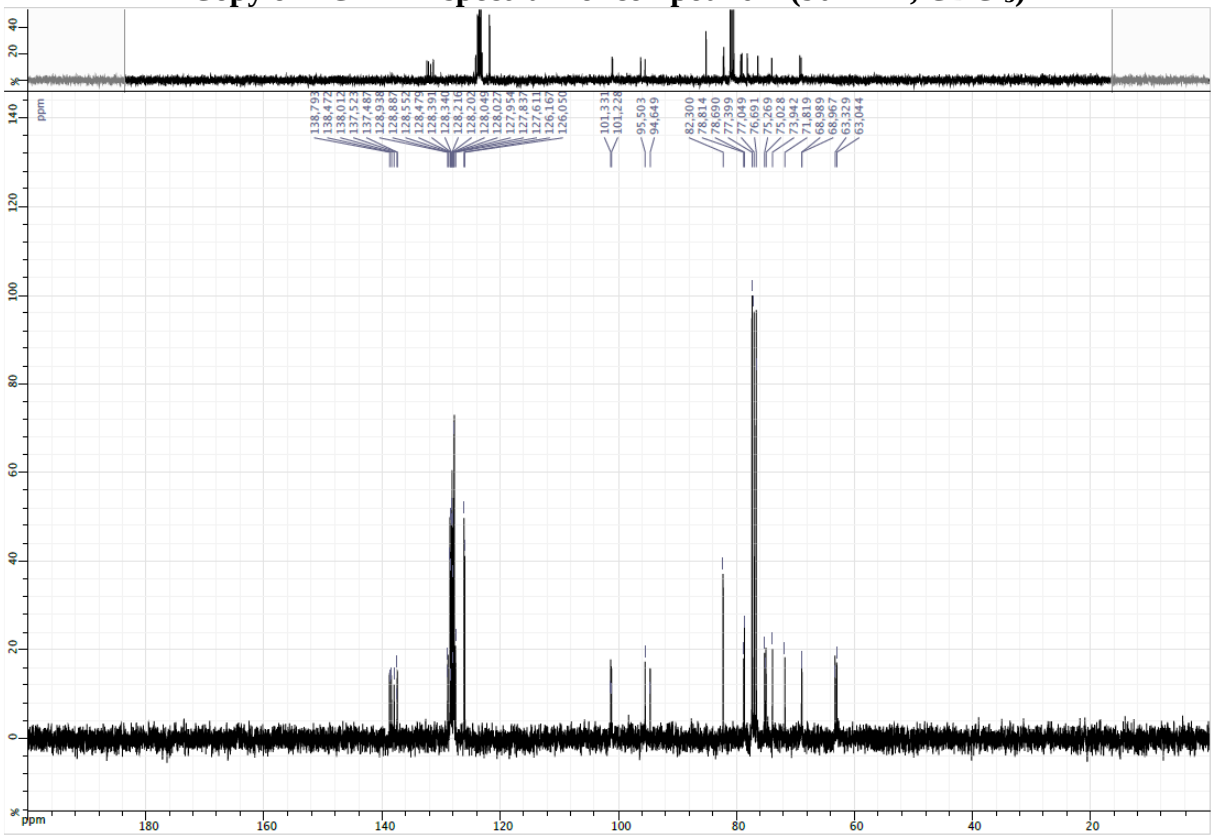


IV. NMR spectra

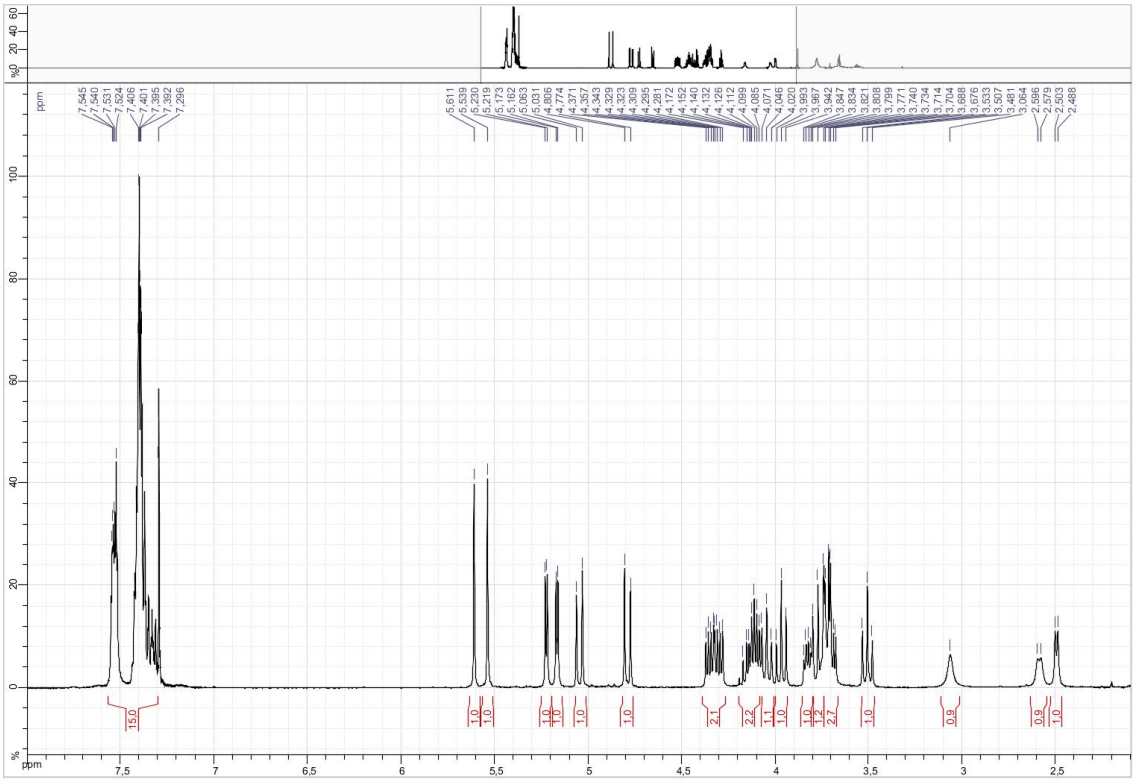
Copy of ^{1}H NMR spectrum of compound 1 (360 MHz, $CDCl_3$)



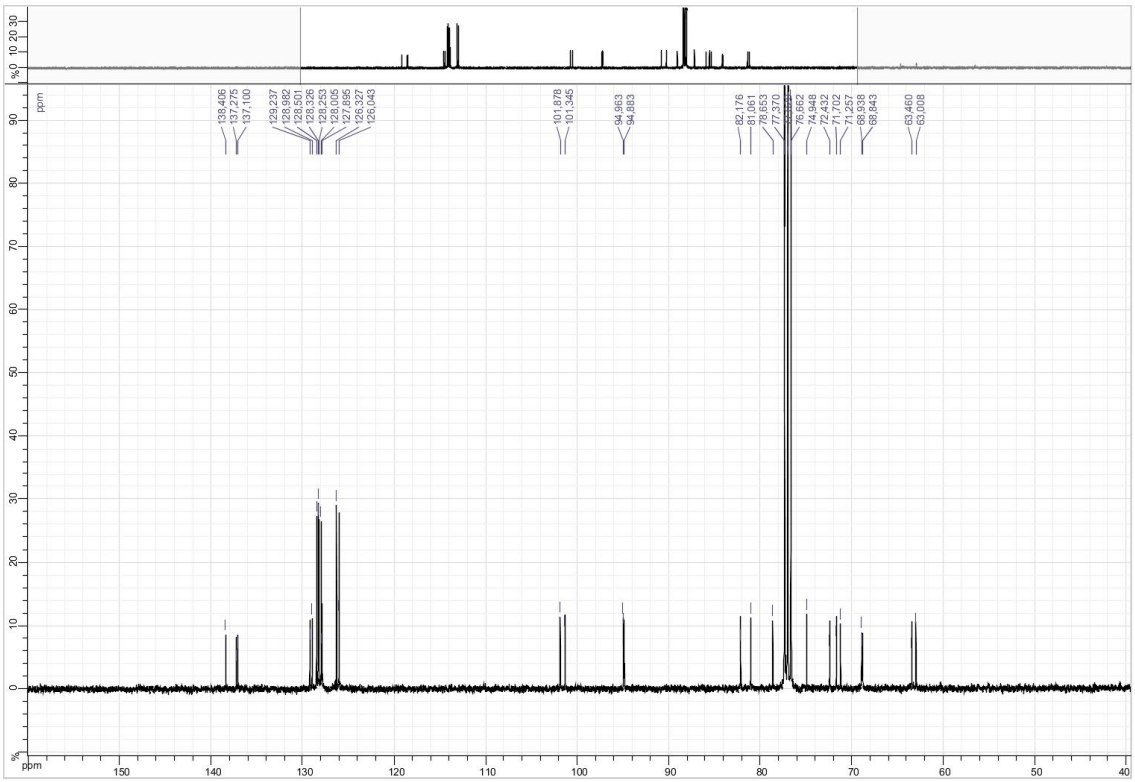
Copy of ^{13}C NMR spectrum of compound 1 (90 MHz, $CDCl_3$)



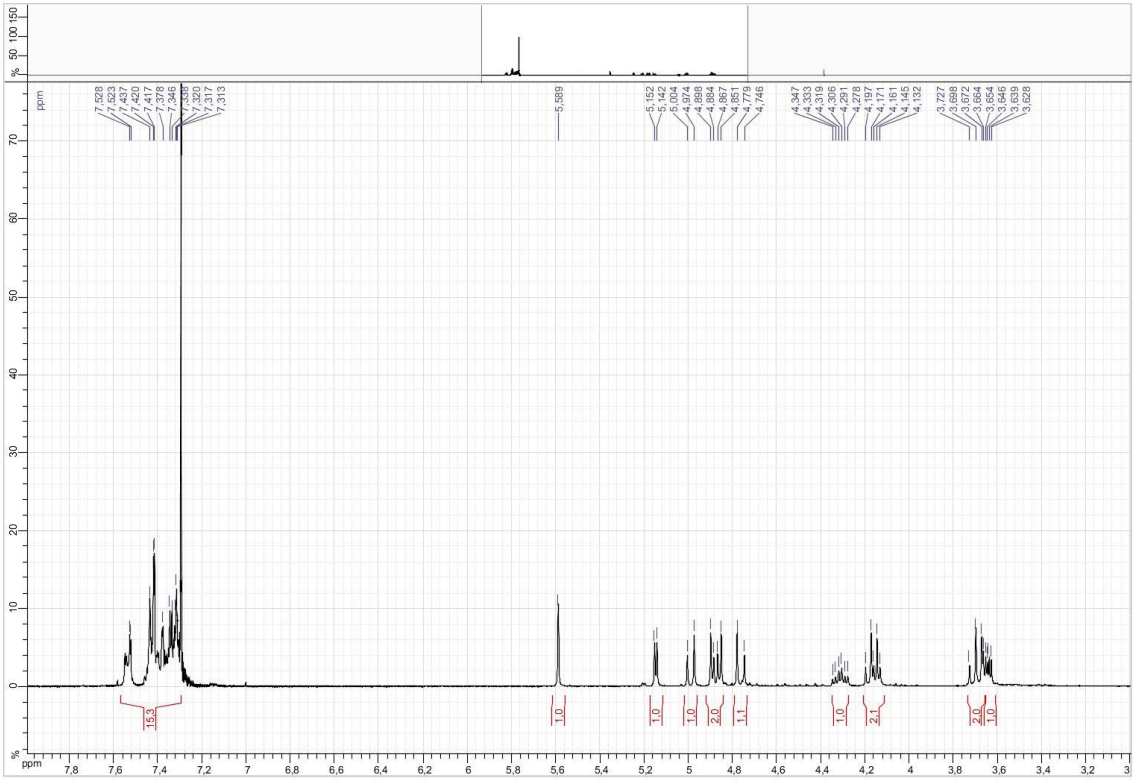
Copy of ^{1}H NMR spectrum of compound 4 (360 MHz, $CDCl_3$)



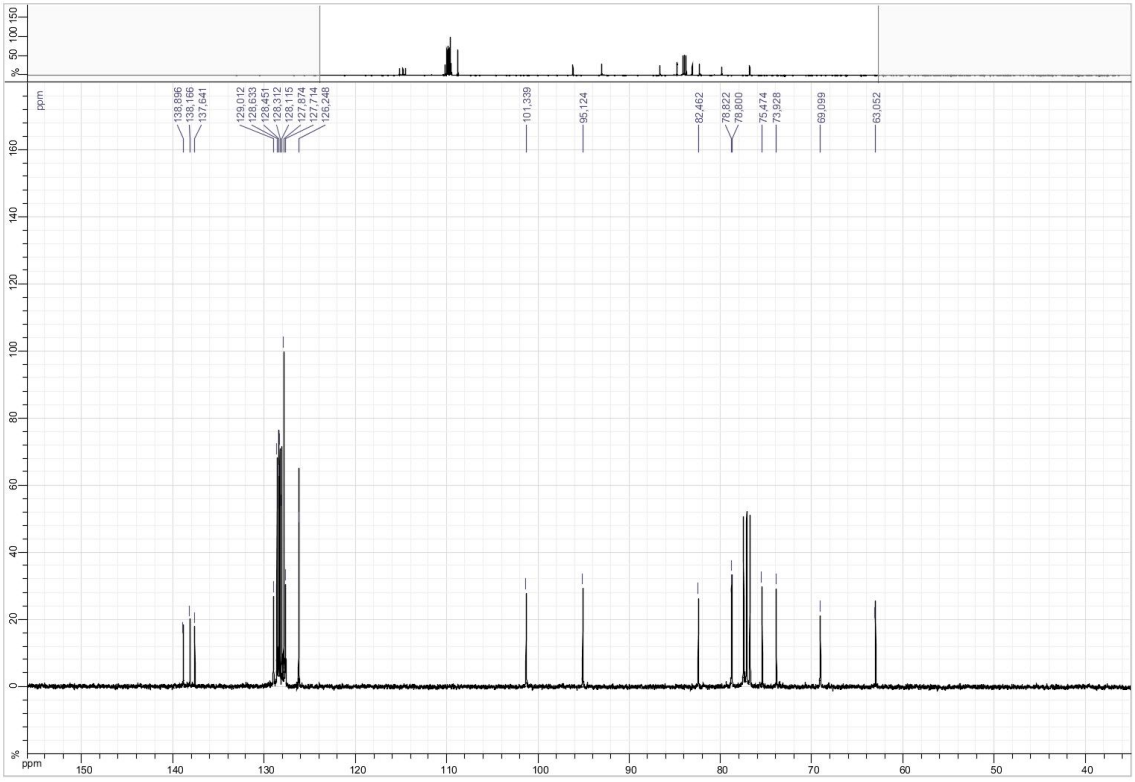
Copy of ^{13}C NMR spectrum of compound 4 (90 MHz, $CDCl_3$)



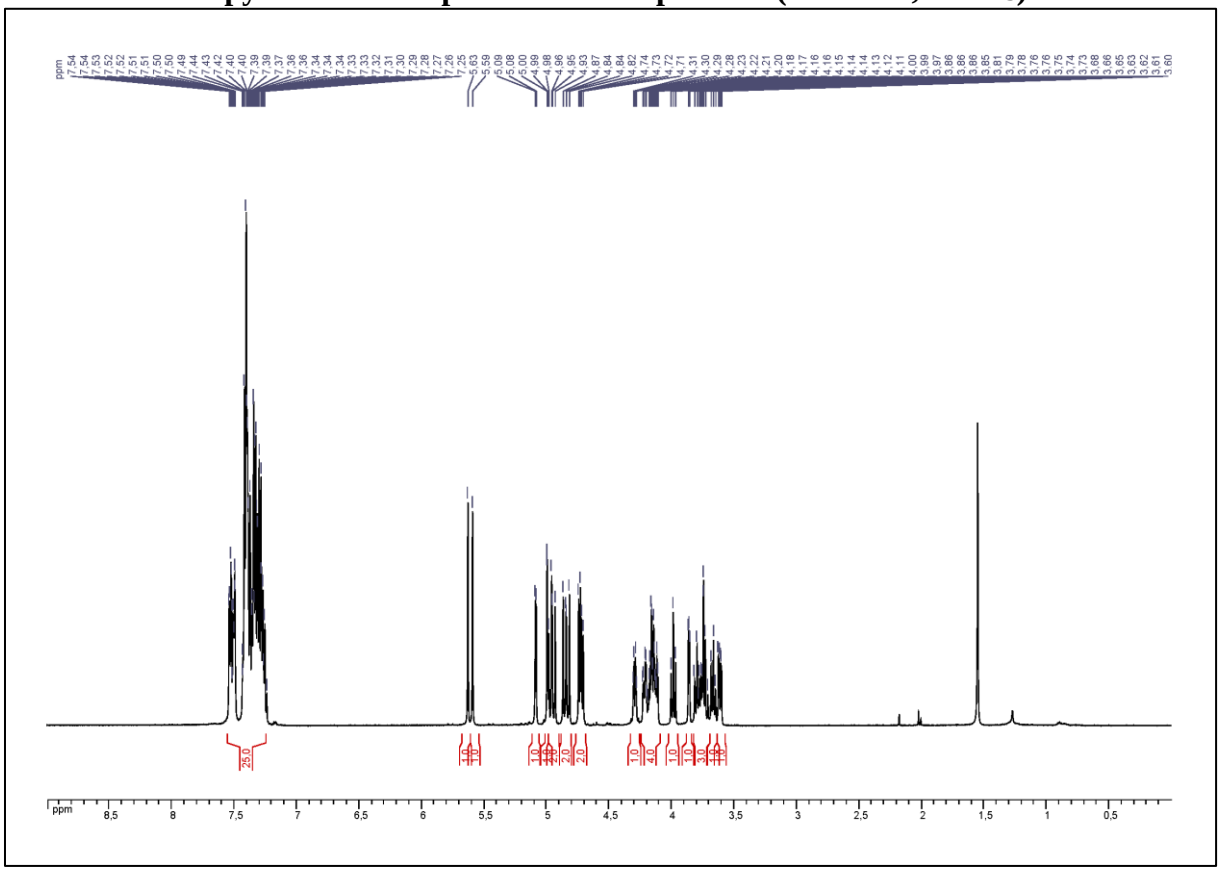
Copy of ^{1}H NMR spectrum of compound 5 (360 MHz, $CDCl_3$)



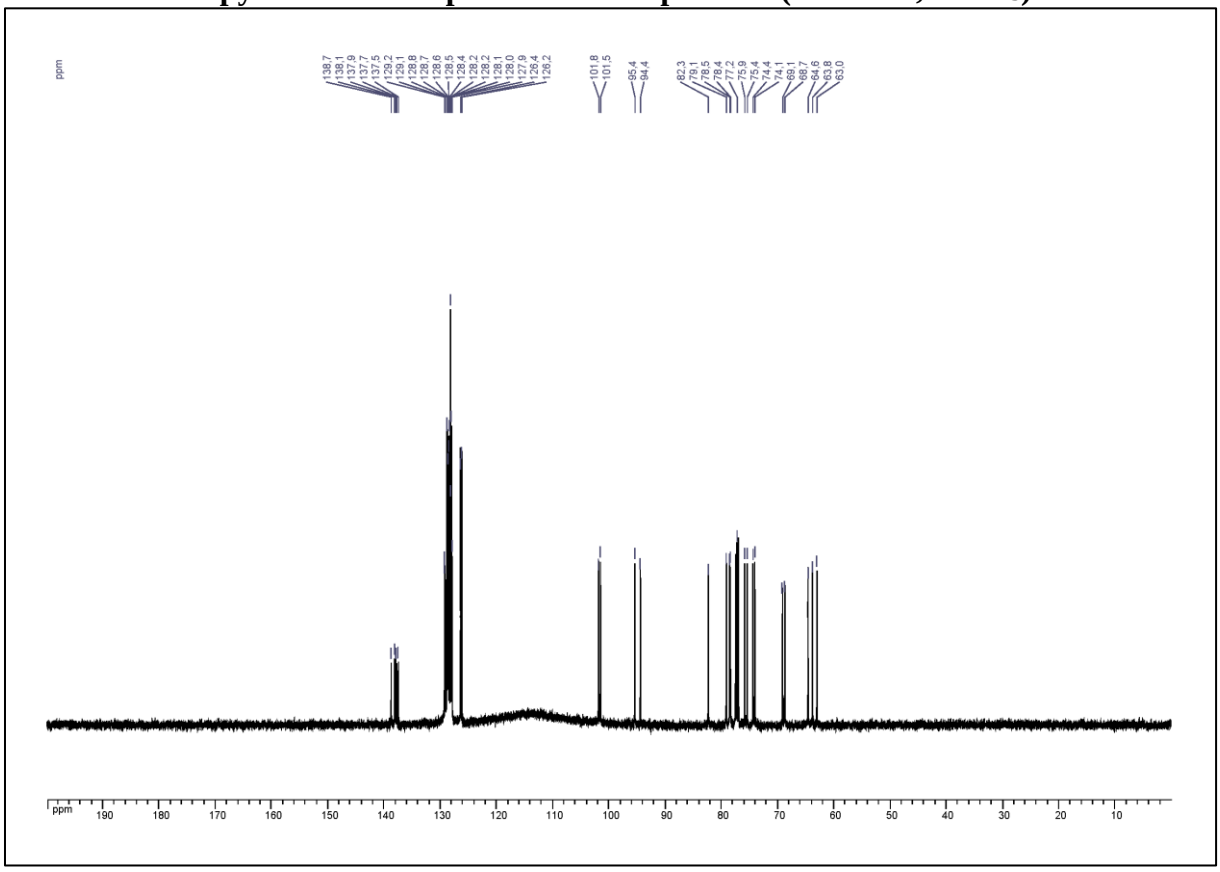
Copy of ^{13}C NMR spectrum of compound 5 (90 MHz, $CDCl_3$)



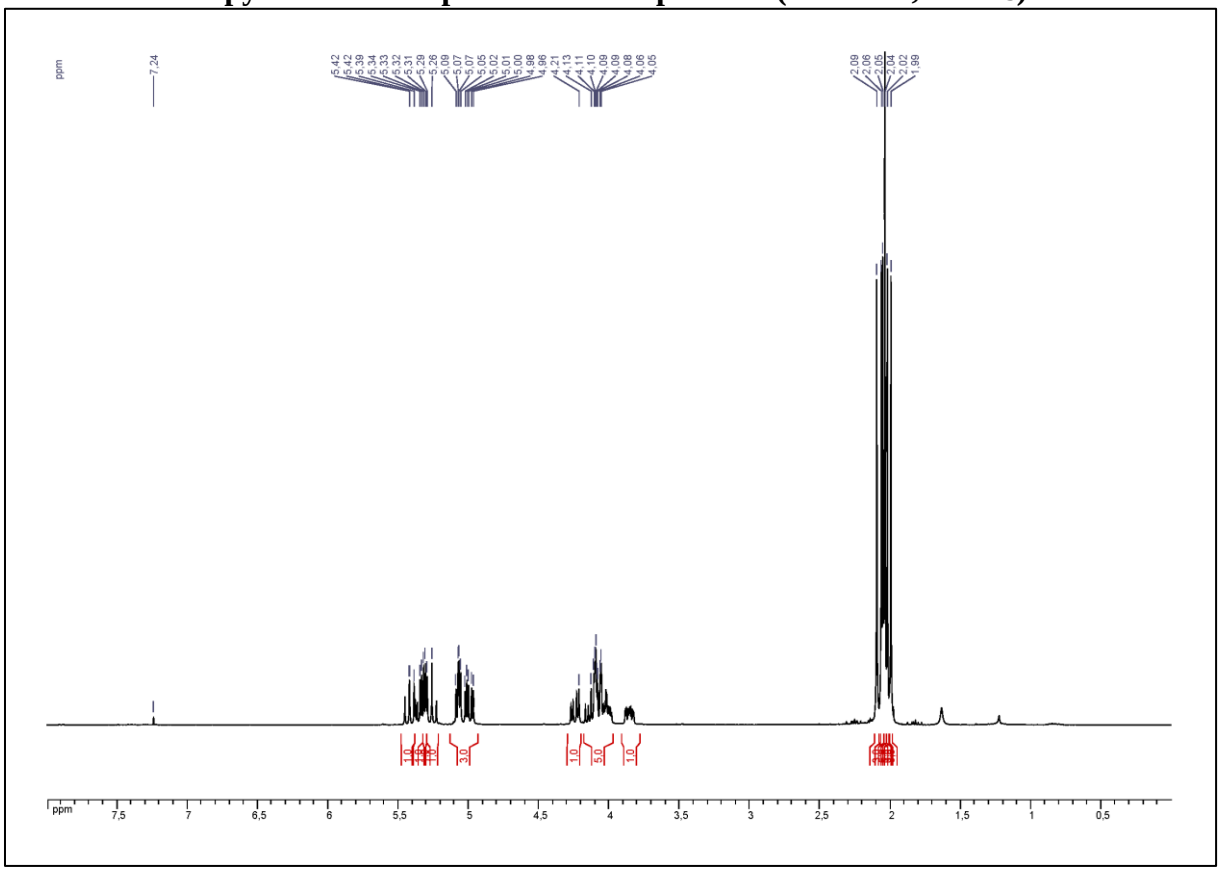
Copy of ^{1}H NMR spectrum of compound 7 (500 MHz, $CDCl_3$)



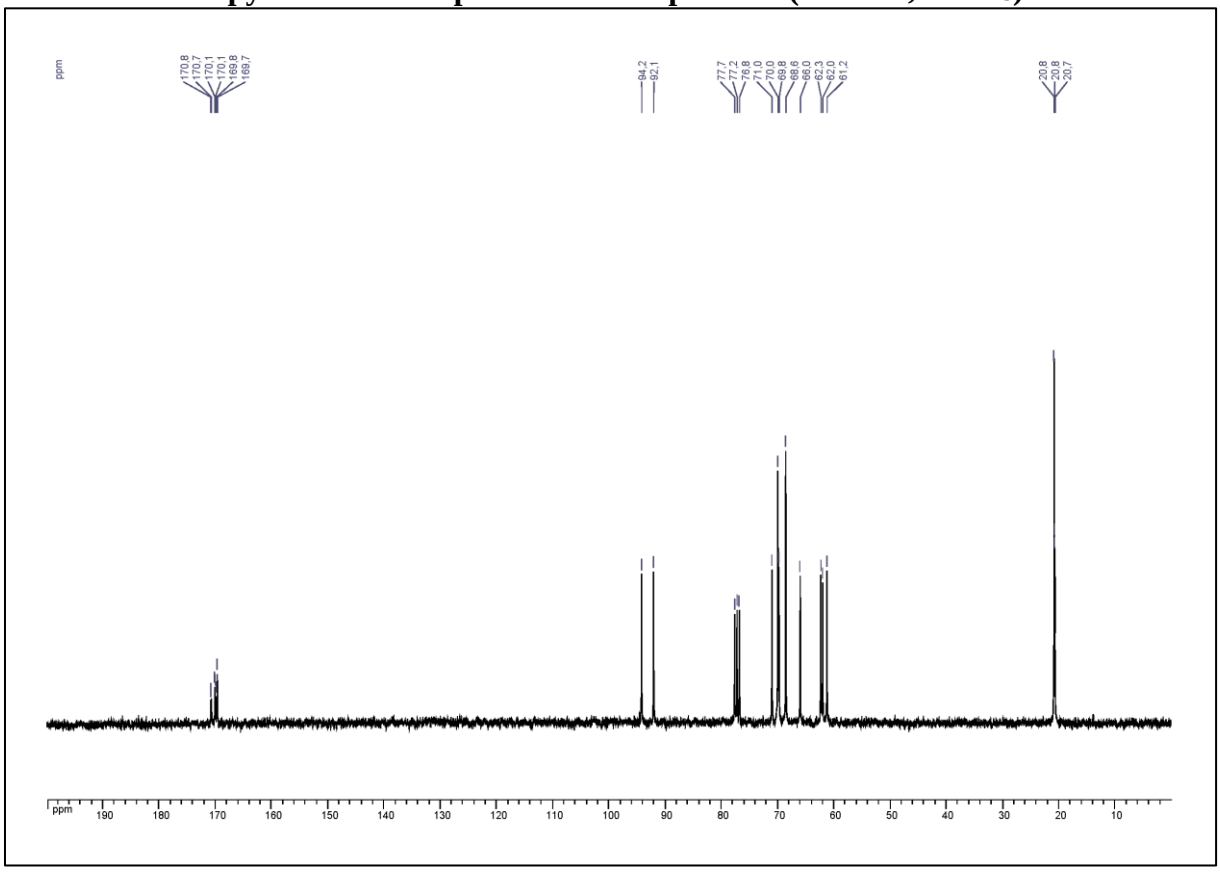
Copy of ^{13}C NMR spectrum of compound 7 (125 MHz, $CDCl_3$)



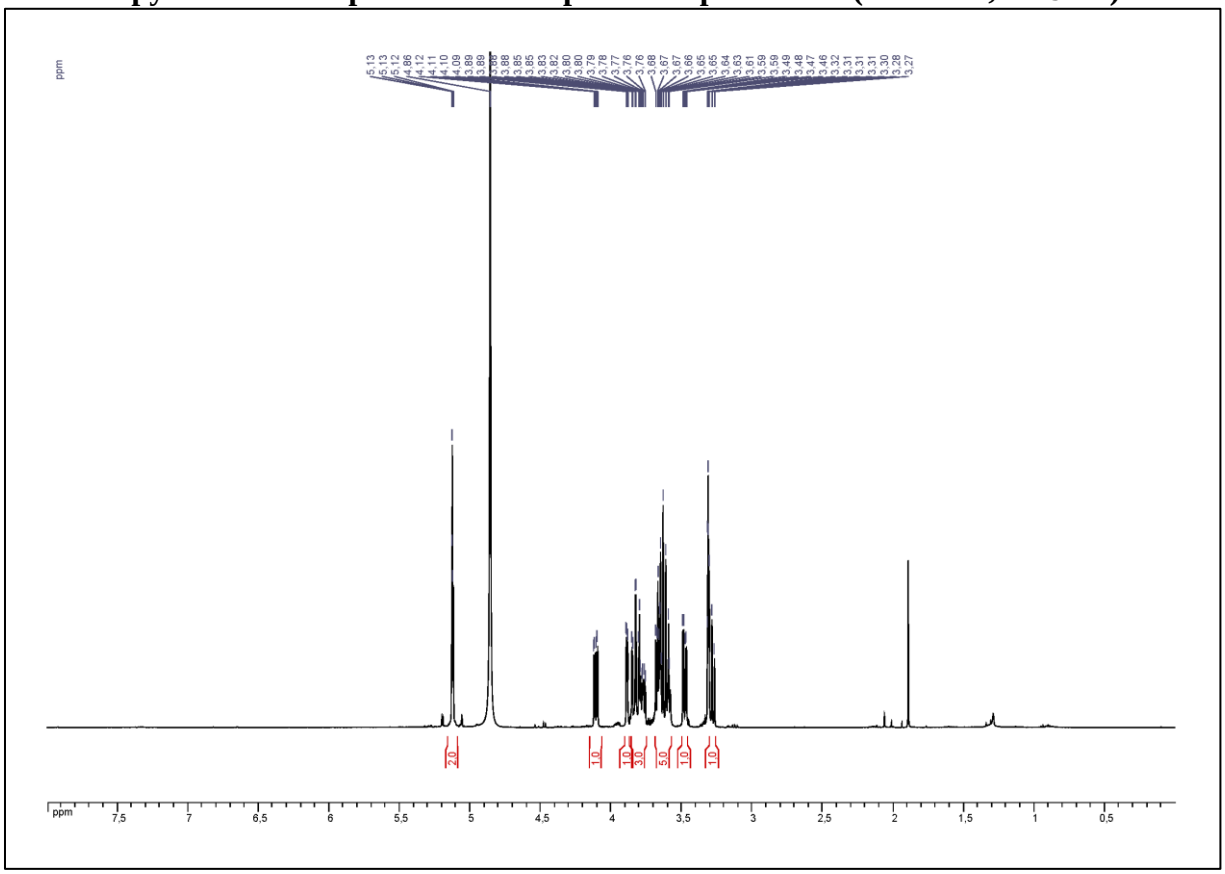
Copy of ^{1}H NMR spectrum of compound 8 (300 MHz, $CDCl_3$)



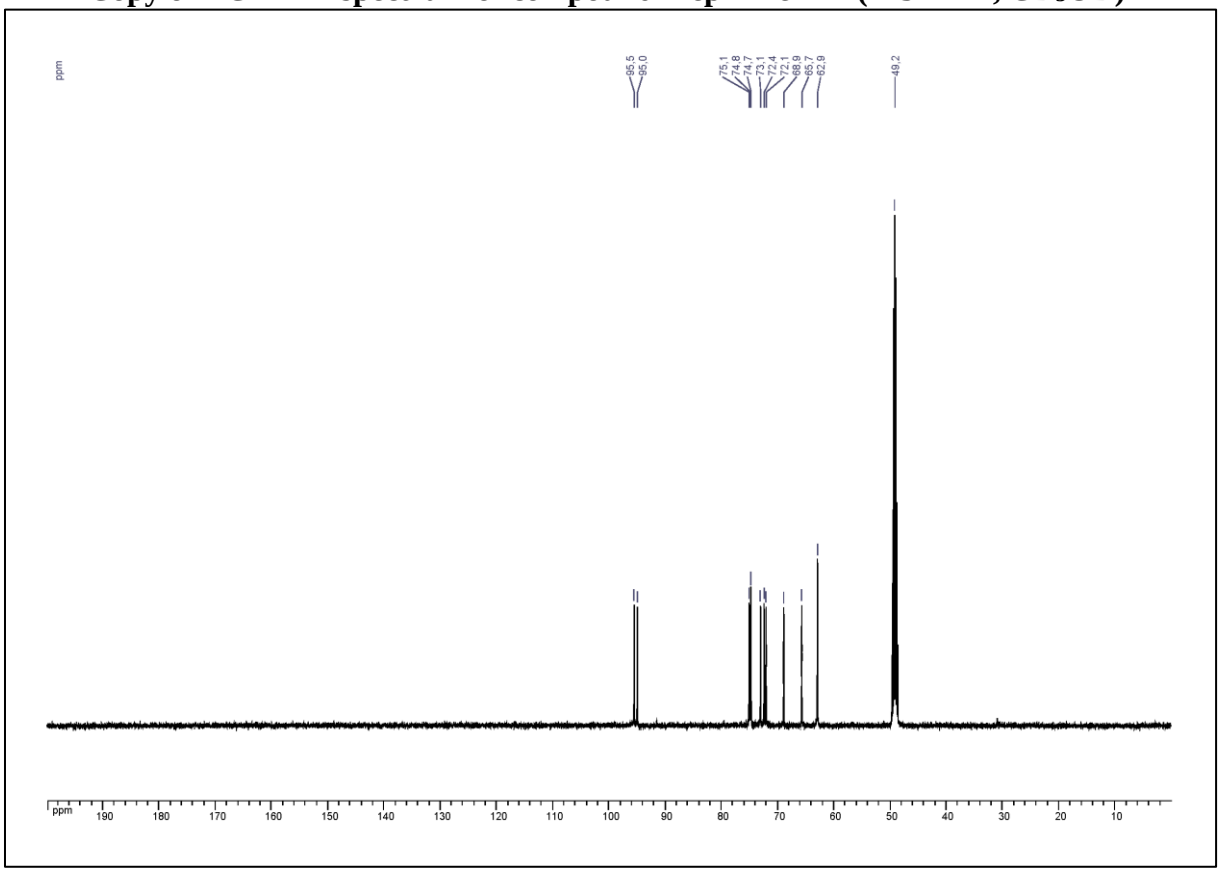
Copy of ^{13}C NMR spectrum of compound 8 (75 MHz, $CDCl_3$)



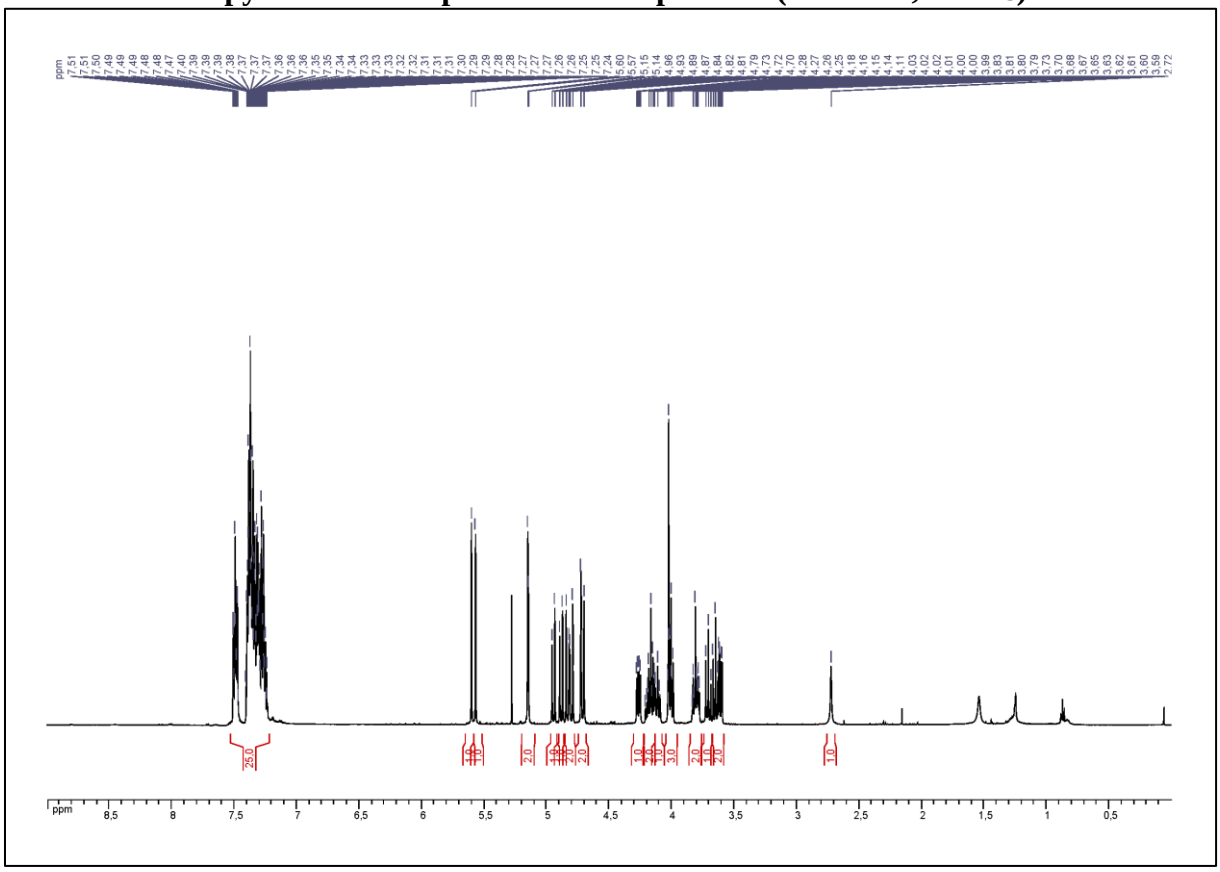
Copy of ^{1}H NMR spectrum of compound 2-epi-TreAz I (500 MHz, CD_3OD)



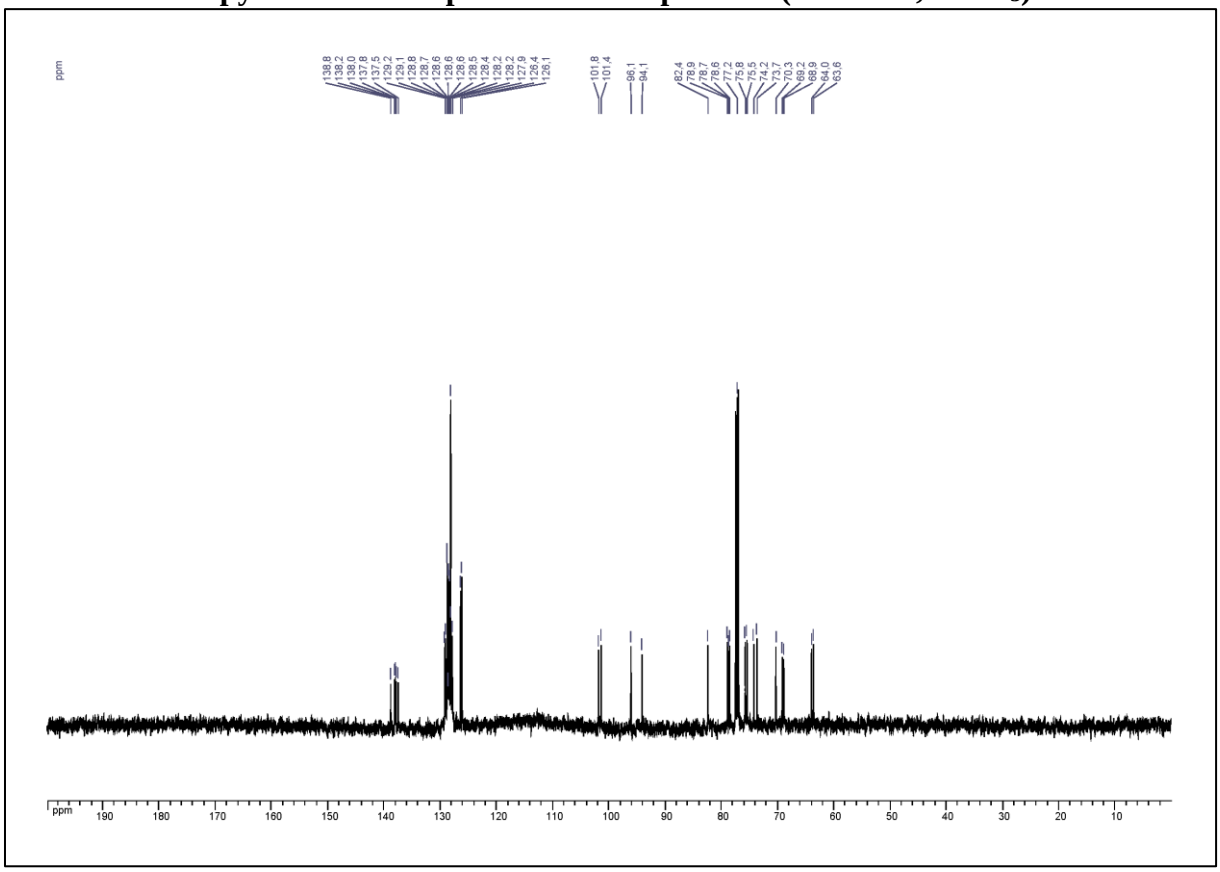
Copy of ^{13}C NMR spectrum of compound 2-epi-TreAz I (125 MHz, CD_3OD)



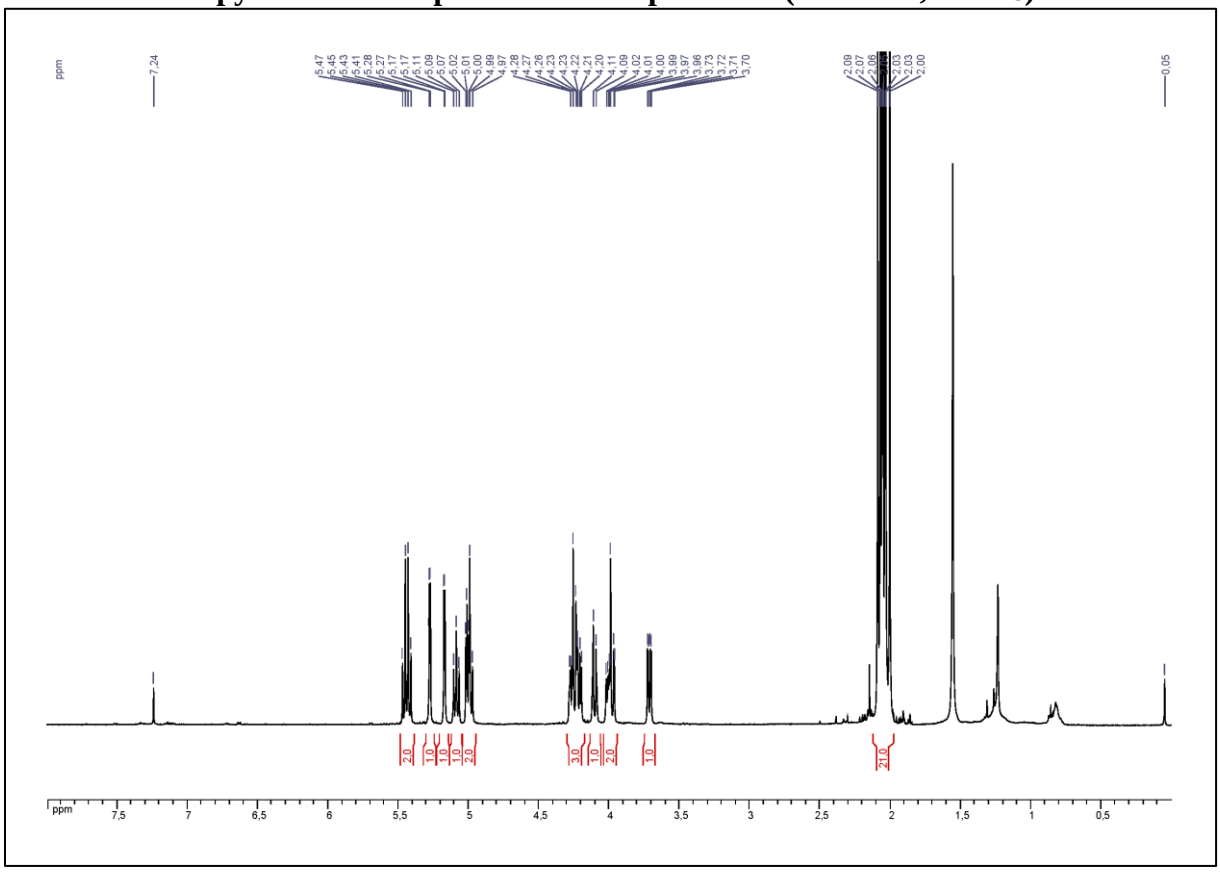
Copy of ^{1}H NMR spectrum of compound 9 (500 MHz, $CDCl_3$)



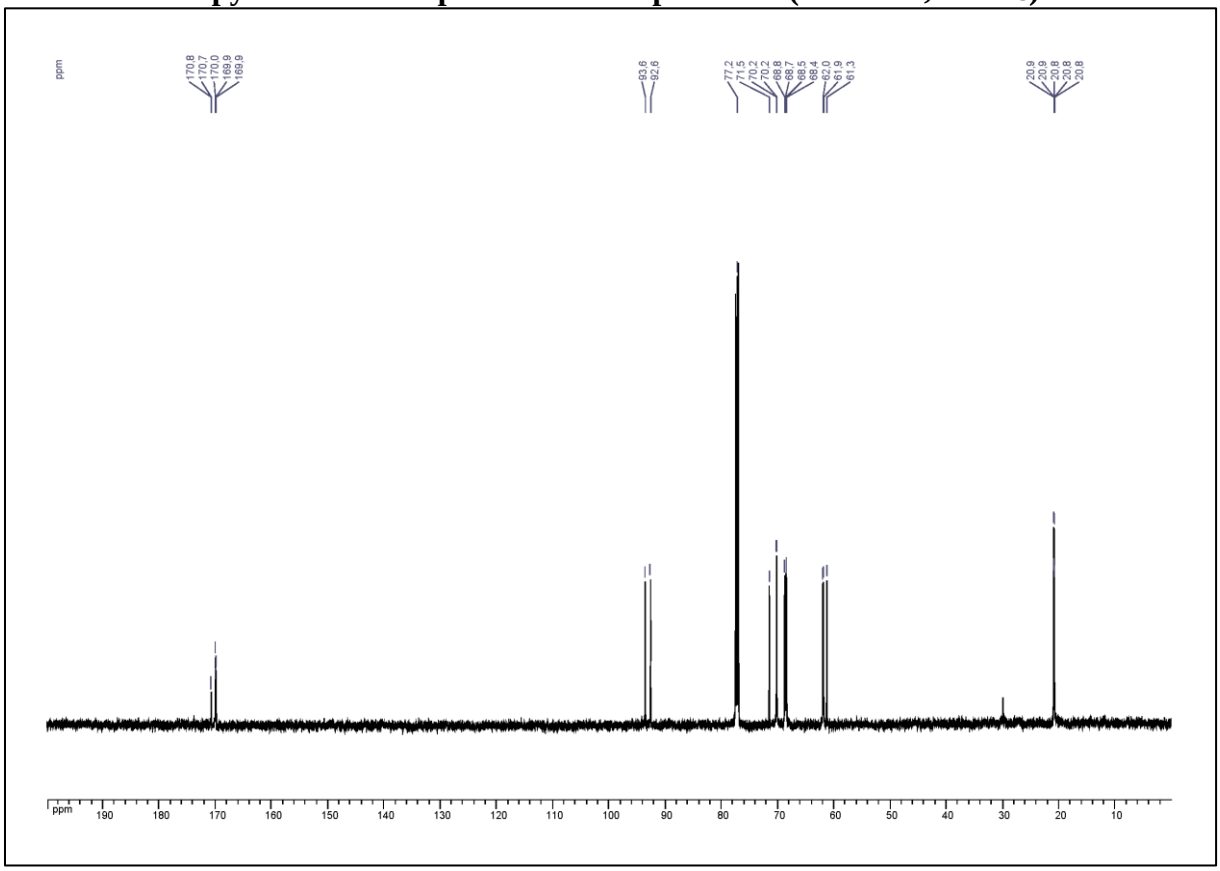
Copy of ^{13}C NMR spectrum of compound 9 (125 MHz, $CDCl_3$)



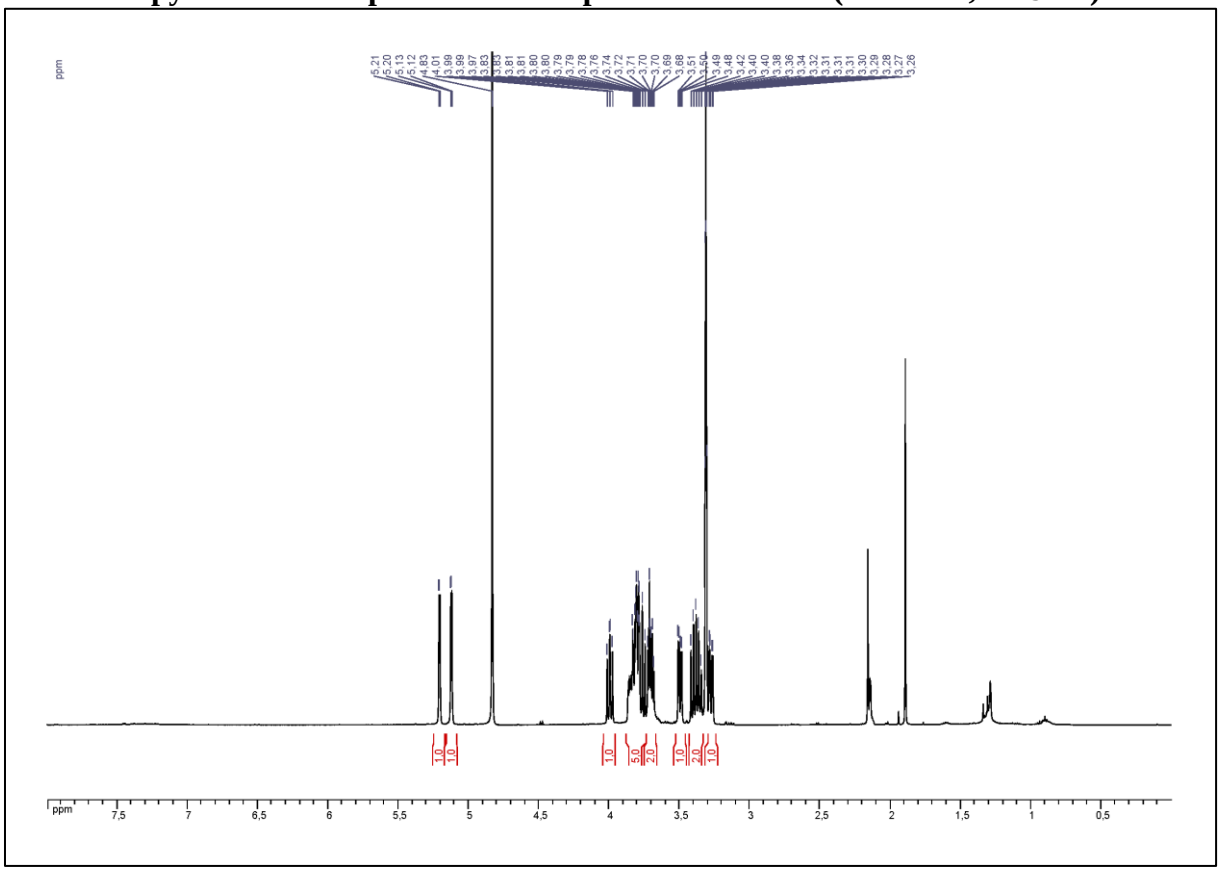
Copy of ^{1}H NMR spectrum of compound 12 (500 MHz, $CDCl_3$)



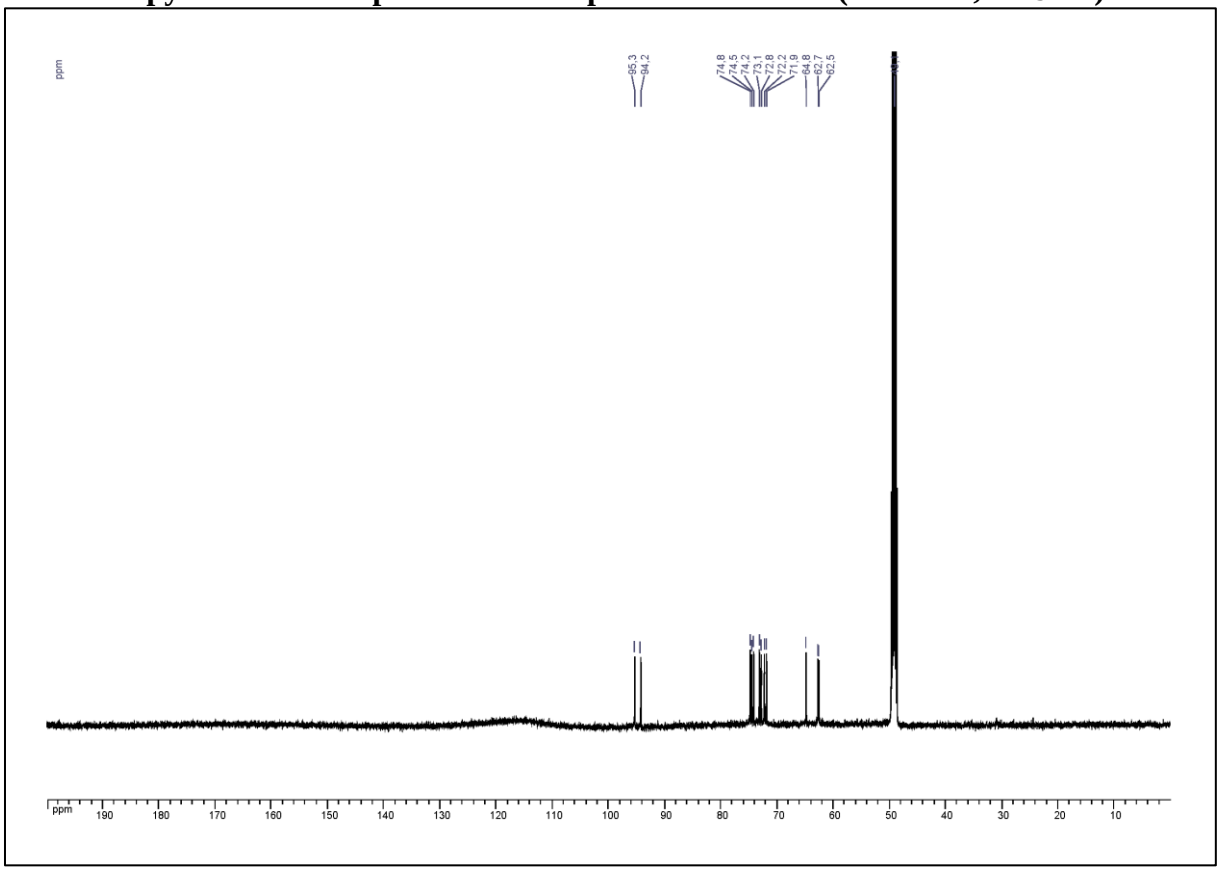
Copy of ^{13}C NMR spectrum of compound 12 (125 MHz, $CDCl_3$)



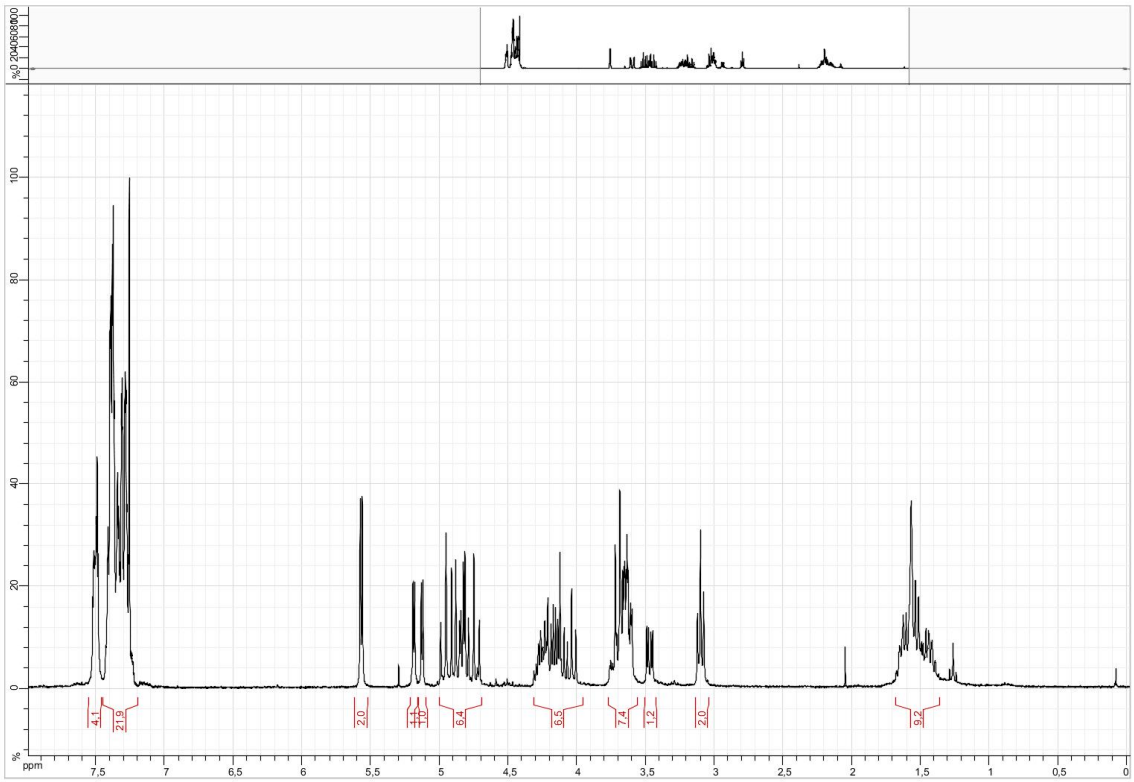
Copy of ^{1}H NMR spectrum of compound 2-TreAz II (500 MHz, CD_3OD)



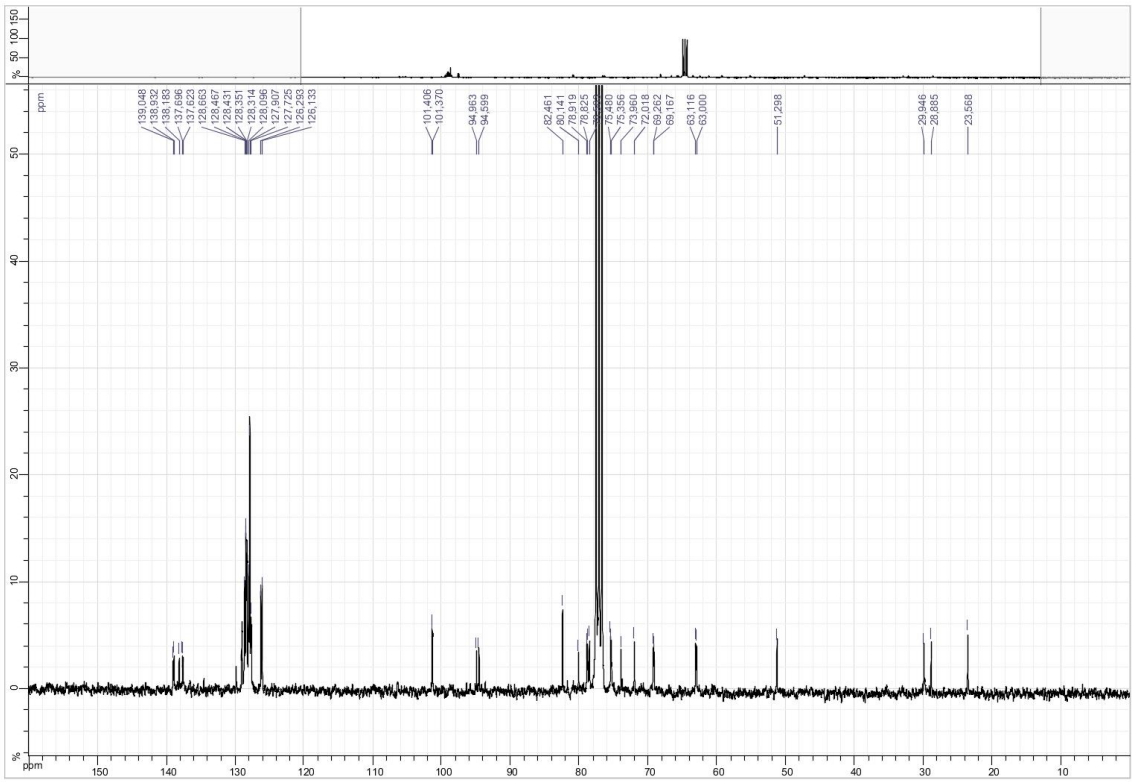
Copy of ^{13}C NMR spectrum of compound 2-TreAz II (125 MHz, CD_3OD)



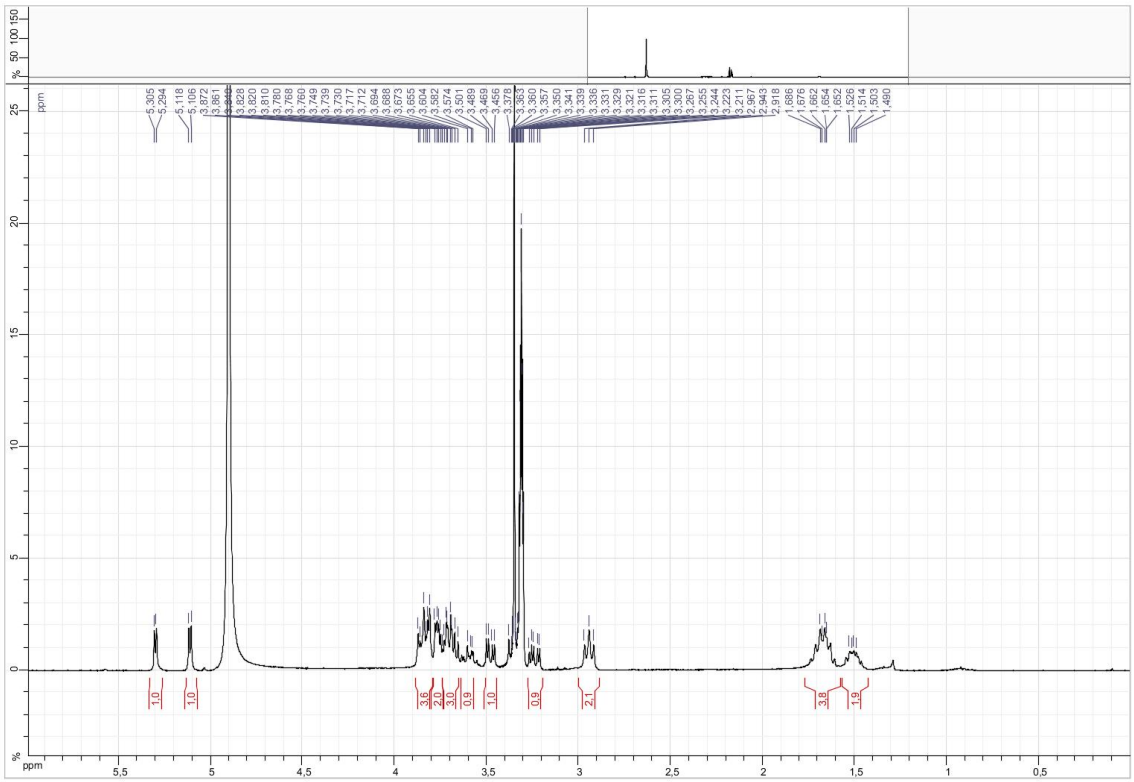
Copy of ^{1}H NMR spectrum of compound 13 (300 MHz, $CDCl_3$)



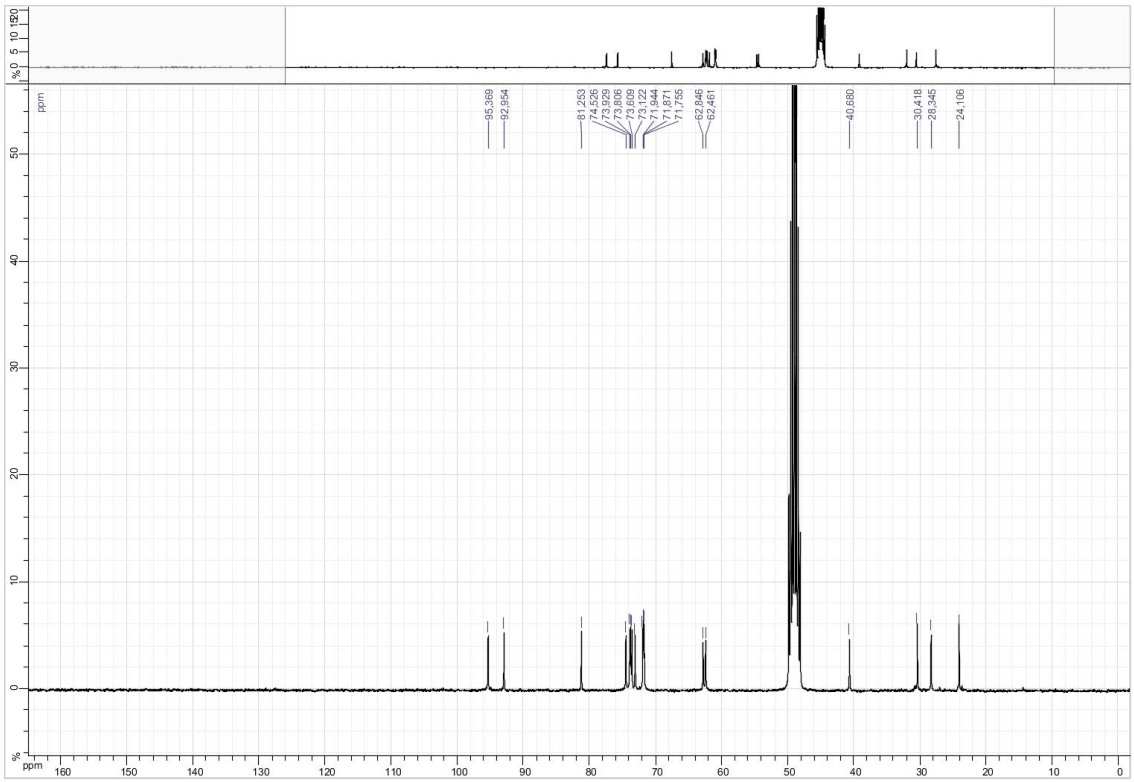
Copy of ^{13}C NMR spectrum of compound 13 (75 MHz, $CDCl_3$)



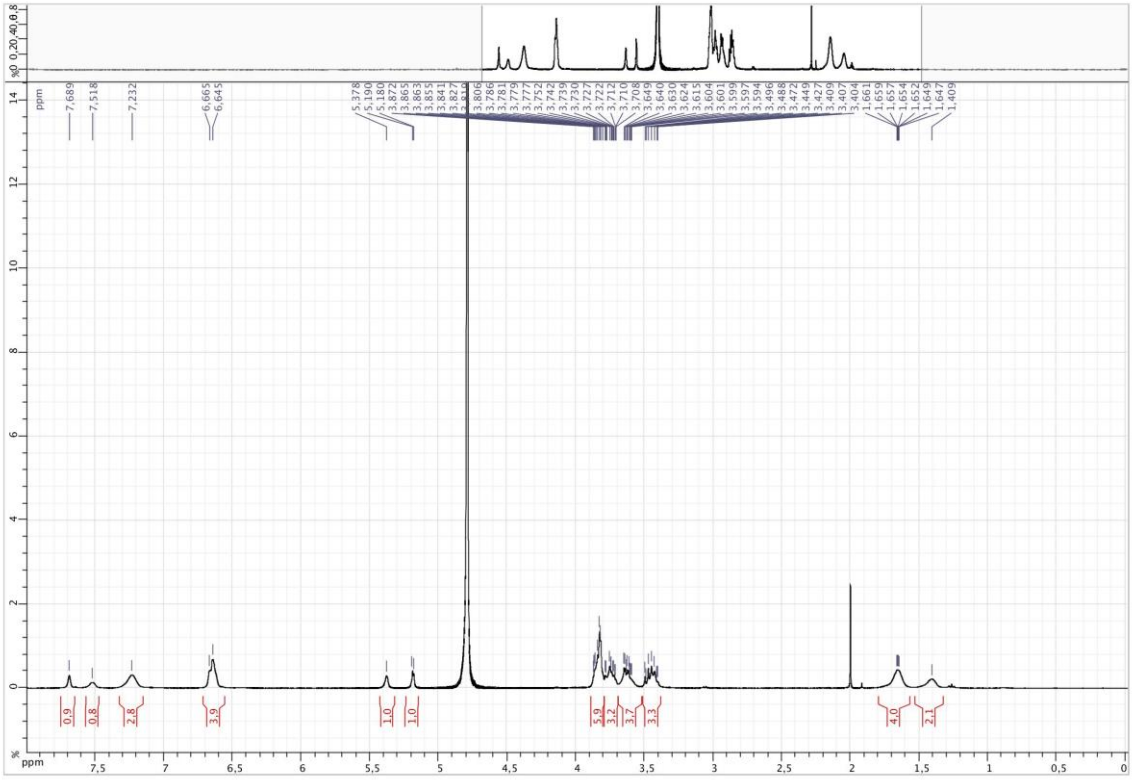
Copy of ^{1}H NMR spectrum of compound 14 (300 MHz, CD_3OD)



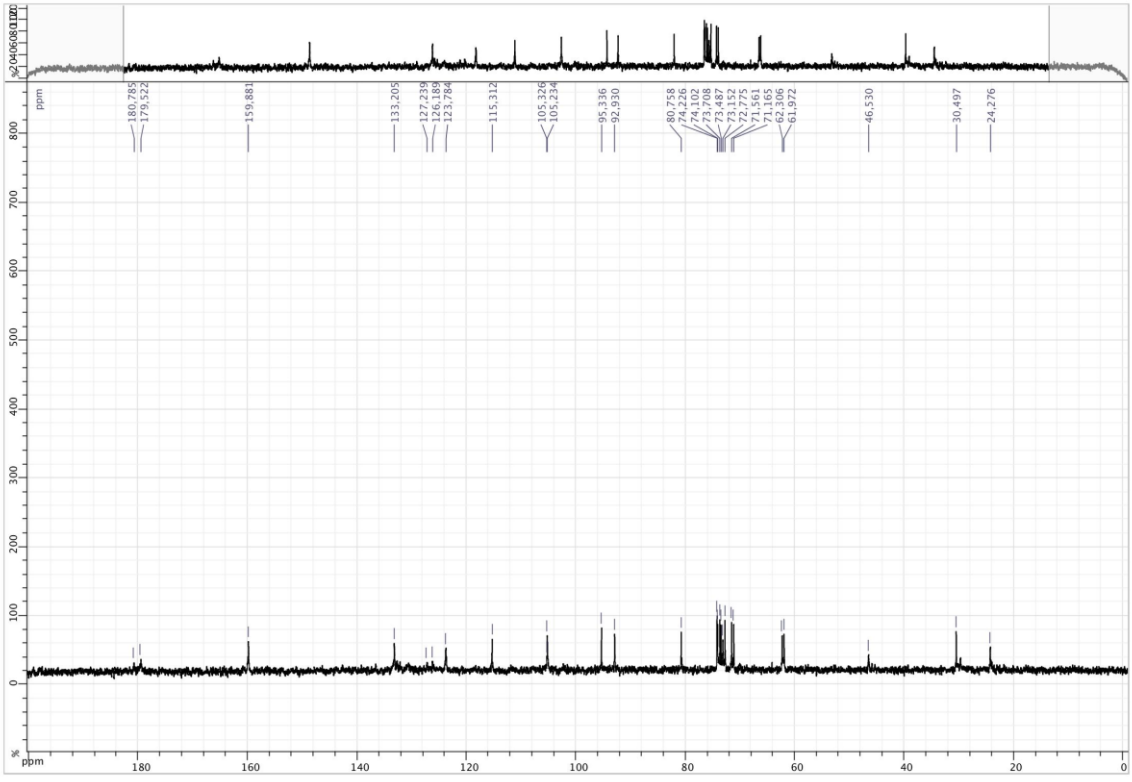
Copy of ^{13}C NMR spectrum of compound 14 (75 MHz, CD_3OD)



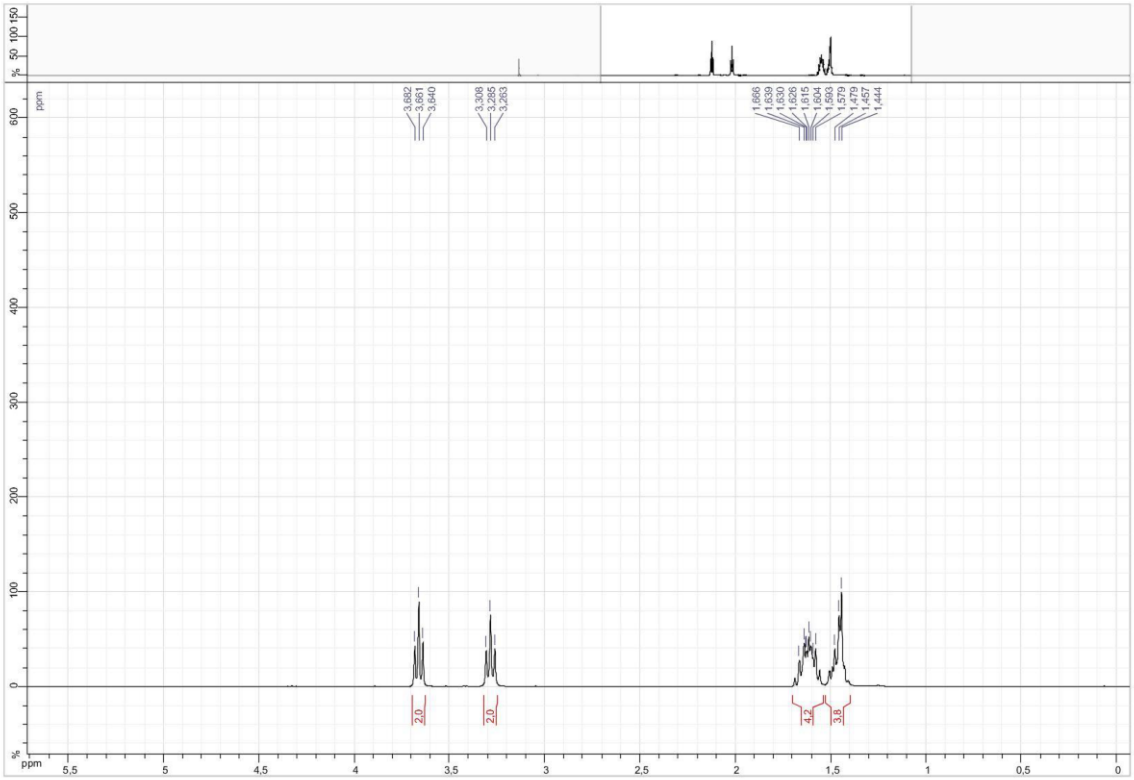
Copy of ^{1}H NMR spectrum of compound 2-FIC5Tre III (400 MHz, D_2O)



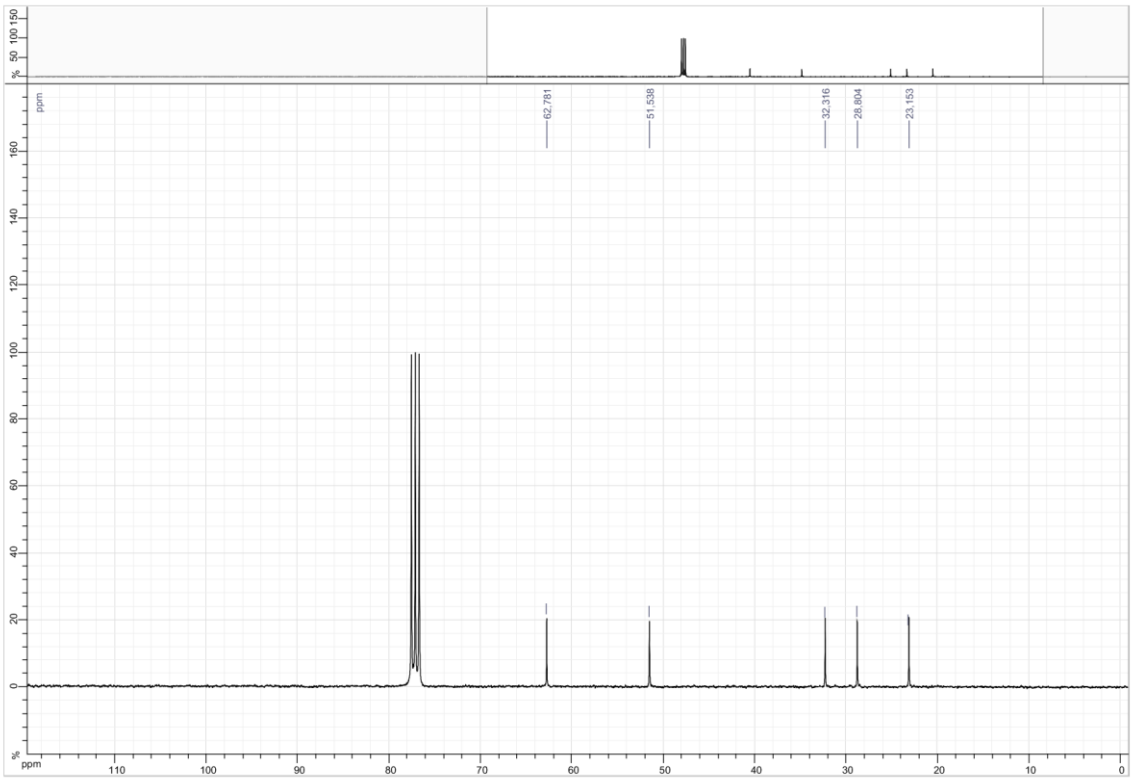
Copy of ^{13}C NMR spectrum of compound 2-FIC5Tre III (100 MHz, D_2O)



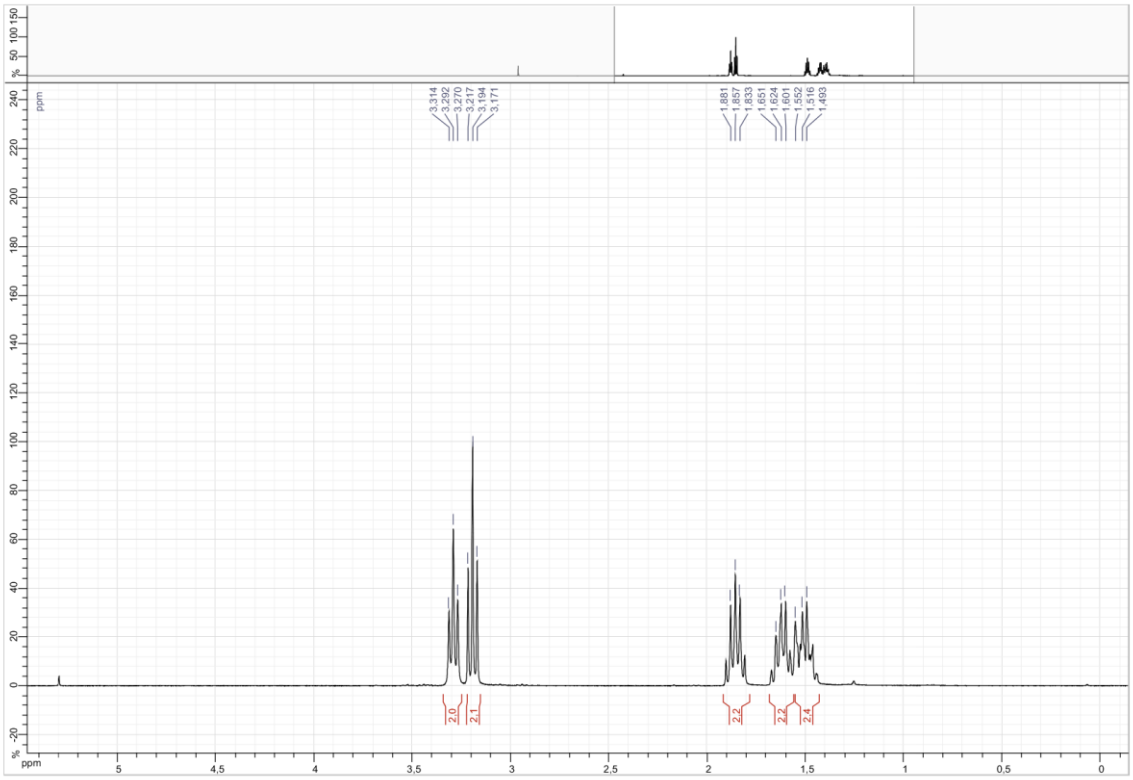
Copy of ^{1}H NMR spectrum of compound S1 (300 MHz, $CDCl_3$)



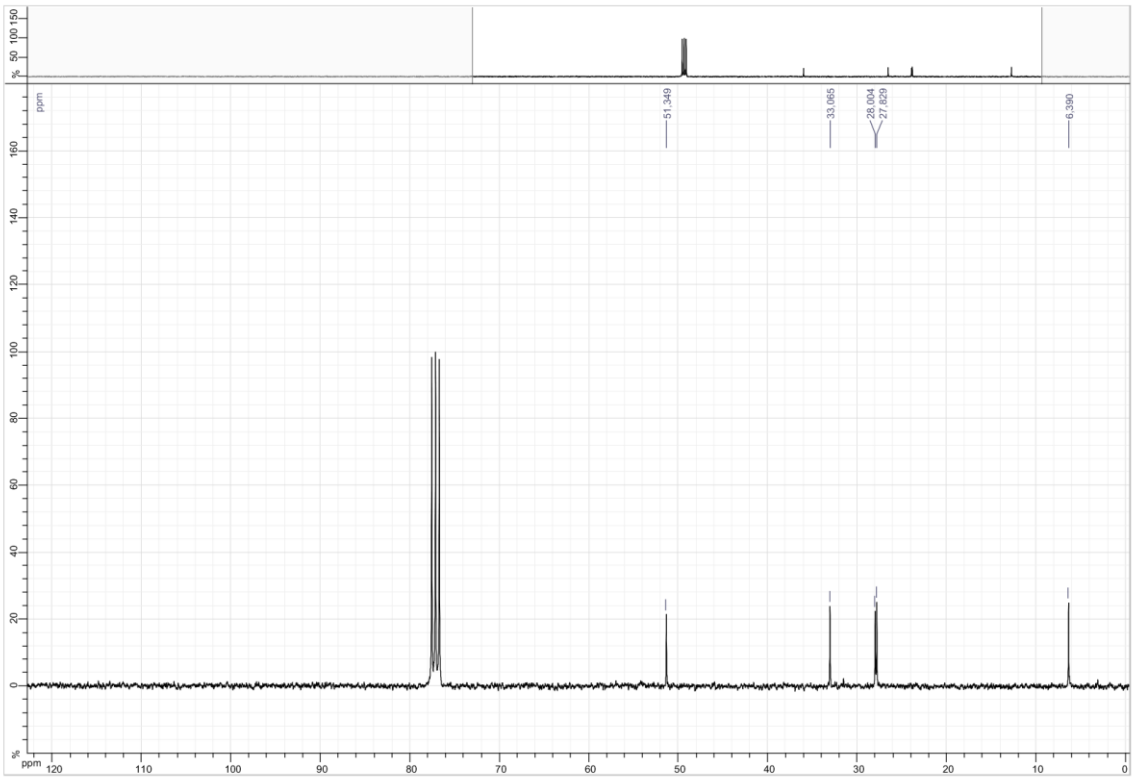
Copy of ^{13}C NMR spectrum of compound S1 (75 MHz, $CDCl_3$)



Copy of ^{1}H NMR spectrum of compound S2 (300 MHz, $CDCl_3$)



Copy of ^{13}C NMR spectrum of compound S2 (75 MHz, $CDCl_3$)



V. References in the supporting information

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[ii] Y. Bourdreux, A. Lemétais, D. Urban and J.-M. Beau, *Chem. Commun.*, 2011, **47**, 2146-2148.

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[v] K. Antien, A. Lacambra, F. P. Cossío, S. Massip, D. Deffieux, L. Pouységu, P. A. Peixoto and S. Quideau, *Chem. Eur. J.*, 2019, **25**, 11574–11580.

[vi] A. Baron, Y. Blériot, M. Sollogoub and B. Vauzeilles, *Org. Biomol. Chem*., 2008, **6**, 1898-1901.