Multivalent Inhibition of the Aspergillus fumigatus KDNase

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Electronic Supplementary Information (ESI)

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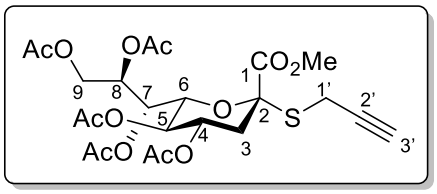
1. Materials

All reagents were purchased from Acros Organics, Alfa Aesar, Carbosynth or Aldrich and were used without further purification. Reactions requiring anhydrous conditions were performed under argon. Column chromatography was conducted on silica gel Kieselgel SI60 (40−63 μm) from Merck, or on Silica cartridge from Interchim and eluted via a puriFlash 430 with an UV and ELSD detection. Thin layer chromatography (TLC): Merck Silica gel 60 F254 analytical plates, detection either with UV (254 nm) or dipped in a solution of cerium molybdate, potassium permanganate, ninhydrin, vanillin and subsequently heated. Dialysis membrane tubing with a molar mass cutoff (MWCO) of 1.0 kDa (Spectra/por® 7 Standard RC Trial kit, Repligen Corp.) was purchased from ThermoFisher Scientific (Rancho Dominguez, CA, USA) and thoroughly rinsed with nanopure water before use. NMR spectra were recorded on a Bruker Avance 400 or Bruker Avance 300 spectrometer using tetramethylsilane, 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as a reference for ^{1}H and ^{13}C nuclei. NMR spectra were assigned on the basis of the following 1D and 2D experiments: ^{1}H, ^{13}C, DEPT-135, COSY, HSCQ, HMBC. Coupling constants (*J*) are reported in Hz and peak multiplicities are noted according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, br = broad signal. Low resolution mass spectrometry (MS) was recorded on a Thermo Finnigan DSQII quadripolar spectrometer (coupled with a Trac Ultra GC apparatus) for Chemical Ionization (CI), on a Thermo Finnigan LCQ Advantage spectrometer for ElectroSpray Ionization (ESI). High resolution mass spectra (HR-MS) were recorded on a Waters Xevo GL-XS Qtof spectrometer coupled to an Acquity H-class LC apparatus. The ionization sources were performed with the available methods (ESI+, ESI-, ASAP+, ASAP-). A tolerance of 5 ppm was applied between calculated and experimental values. Attenuated Total Reflectance (ATR) Fourier Transform Infra-Red (FT-IR) spectra were obtained using a Bruker Tensor 27 mountain equipped with the Specac® ATR diamond accessory. Spectra were obtained at regular time intervals in the MIR region of 4000-500 cm^{-1} with a resolution of 4 cm^{-1} (64 scans) and analyzed using OPUS software.

2. Chemistry

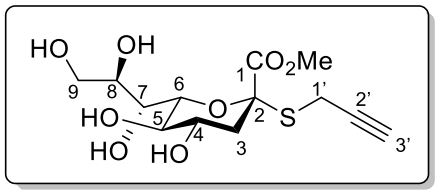
2.1. Synthesis of compounds 6 and 8



Compound 6. Compound **5**[1,2] (955 mg, 1.87 mmol, 1.0 equiv.) was diluted in dry acetone (14 mL) and a solution of KSAc (318 mg, 2.81 mmol, 1.5 equiv.) in acetone (6 mL) was added at 0 °C. The reaction mixture was stirred for 2,5h then filtered through a Celite® pad, washed with $CHCl_3$ and the filtrate was concentrated under *vacuum*. The residue was dissolved in DCM (50 mL) and the solution was washed with 1M HCl (30 mL) and water (2 x 25 mL), dried over $MgSO_4$, filtered and concentrated under *vacuum*. The resulting crude was diluted in MeOH following by the addition at 0 °C of NaSMe (144 mg, 2.06 mmol, 1.1 equiv.) and K_2CO_3 (77 mg, 0.56 mmol, 0.3 equiv.). The reactional mixture was stirred for 30 min at 0 °C then neutralized with H^+ resin until reaching neutral pH. Next, the solution was filtered, washed with MeOH and concentrated under *vacuum*. The crude residue was diluted in dry acetonitrile (15 mL), cooled to 0 °C and K_2CO_3 (77 mg, 0.56 mmol, 0.3 equiv.) and propargyl bromide (0.5 mL, 5.32 mmol, 2.8 equiv.) was added. The mixture was stirred until complete consumption of the starting material (around 1h), then the solvent was removed under reduced pressure at 45 °C. The crude product was purified by chromatography [EtOAc/Cyclohexane (7:3)] to afford **6** as a white solid (457 mg, 0.86 mmol, 47% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.47 – 5.39 (m, 1H, H_8), 5.33 (dd, 1H, $^3J_{7,8}$ = 9.2 Hz, $^3J_{6,7}$ = 2.4 Hz, H_7), 4.96 – 4.83 (m, 1H, H_4, H_5), 4.26 (dd, 1H, $^2J_{9a,9b}$ = 12.5 Hz, $^3J_{8,9a}$ = 2.6 Hz, H_{9a}), 4.12 (dd, 2H, $^2J_{9b,9a}$ = 12.5 Hz, $^3J_{9b,8}$ = 2.6 Hz, H_{9b}), 3.94 – 3.88 (m, 1H, H_6), 3.83 (s, 3H, $CO_2C\underline{H}_3$), 3.52 – 3.36 (m, 2H, $H_{1'}$), 2.80 (dd, 1H, $^2J_{3a,3b}$ = 12.6 Hz, $^3J_{3b,4}$ = 4.4 Hz, H_{3a}), 2.19 (s, 3H, $COC\underline{H}_3$), 2.17 (t, 1H, $^3J_{3',1'}$ = 2.7 Hz, $H_{3'}$), 2.09, 2.03, 2.00, 1.99 (s, 15H, 5 x $COC\underline{H}_3$) (Figure S11); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.8, 170.2, 170.0, 169.9 (5 x $\underline{C}OCH_3$), 167.8 ($\underline{C}O_2CH_3$), 82.8 (C_2), 79.6 ($C_{2'}$), 73.2 (C_6), 71.2 ($C_{3'}$), 69.9 (C_4), 67.8 (C_8, C_5), 66.5 (C_7), 62.2 (C_9), 53.3 ($CO_2\underline{C}H_3$), 21.3, 20.9, 20.8, 17.2 (5 x $CO\underline{C}H_3$) (Figure S12); HRMS (ESI^+) calculated for $C_{23}H_{30}O_{13}SNa$ [M+Na] 569.1305, found 569.1310.

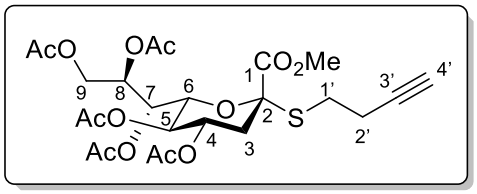
[1] J. C. Telford, J. H. F. Yeung, G. Xu, M. J. Kiefel, A. G. Watts, S. Hader, J. Chan, A. J. Bennet, M. M. Moore and G. L. Taylor, *J. Biol. Chem.*, 2011, **286**, 10783–10792.

[2] A. Nejatie, C. Colombo, B. Hakak-Zargar and A. J. Bennet, *Eur. J. Org. Chem.*, **2022**.



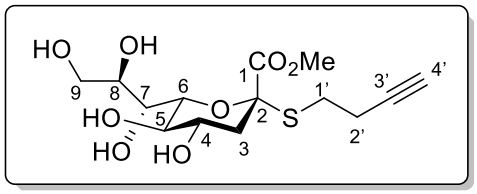
Compound 8. Compound **6** (210 mg, 0.38 mmol, 1.0 equiv.) was dissolved in MeOH (20 mL) and the solution was cooled in an ice-bath. K_2CO_3 (266 mg, 1.92 mmol, 5.0 equiv.) was added, the reactional mixture was stirred at room temperature until complete consumption of starting material. Then, acidic resin was added and the mixture was stirred until the pH was neutral. The resin was removed by filtration and solvent was removed under *vacuum* to afford product **8** as a white powder (110 mg, 85%). ^{1}H NMR (400 MHz, D_2O) δ 4.04 – 3.94 (m, 1H, H_8), 3.96 (s, 3H, $CO_2C\underline{H}_3$), 3.96 – 3.88 (m, 2H, H_7, H_{9a}), 3.76 (dd, $^3J_{9b,9a}$ = 11.9 Hz, $^3J_{9b,8}$ = 6.0 Hz, 1H, H_{9b}), 3.72 – 3.59 (m, 4H, H_4, H_5, H_6, $H_{1'a}$), 3.52 (dd, $^2J_{1'b,1'a}$ = 17.6 Hz, $^4J_{1'b,3'}$ = 2.7 Hz, 1H, $H_{1'b}$), 2.83 (dd, $^2J_{3a,3b}$ = 12.9 Hz, $^3J_{3a,4}$ = 4.4 Hz, 1H, H_{3a}), 2.72 (t, $^4J_{3',1'}$ = 2.6 Hz, 1H, $H_{3'}$), 1.88 (dd, $^2J_{3b,3a}$ = 12.9 Hz, $^3J_{3b,4}$ = 11.5 Hz, 1H, H_{3b}) (Figure S13); ^{13}C NMR (101 MHz, D_2O) δ 171.0 ($\underline{C}O_2CH_3$), 83.0 (C_2), 81.0 ($C_{2'}$), 76.2, 71.6, 71.0 (C_8), 69.6, 69.4, 67.9 (C_7), 63.1 (C_9), 53.7 ($CO_2\underline{C}H_3$), 39.3 (C_3), 16.6 ($C_{1'}$); HRMS (ESI+) calculated for $C_{13}H_{20}O_8NaS$ [M+Na^+] 359.0777, found 359.0773.

2.2. Synthesis of compounds 7 and 9



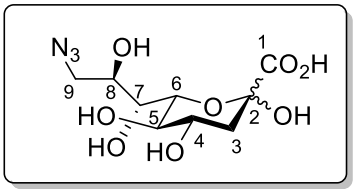
Compound 7. Compound **5** (226 mg, 0.45 mmol, 1.0 equiv.) is dissolved in dry acetone (20 ml), cooled at 0 °C and potassium thioacetate (77 mg, 0.67 mmol, 1.5 equiv.) was added. The reaction mixture was stirred for 2h30 then filtered through a Celite® pad, washed with $CHCl_3$ and the filtrate was concentrated under reduced pressure at 45 °C. The residue was dissolved in DCM (50 mL), washed with 1M HCl and H_2O (2 times), dried over $MgSO_4$ and evaporated under *vacuum*. The crude product was dissolved in MeOH (5 mL) and cooled at 0 °C following by the addition of NaSMe (35 mg, 0.50 mmol, 1.1 equiv.) and K_2CO_3 (13 mg, 0.14 mmol, 0.3 equiv.). The mixture was stirred 30 min then H^+ resin was added, stirred until reaching neutral pH, filtered and concentrated under *vacuum*. The crude residue was dissolved in dry acetonitrile (10 mL), K_2CO_3 (13 mg, 0.14 mmol, 0.3 equiv.) and butyne bromide (179 mg, 1.35 mmol, 3.0 equiv.) was added at 0°C. The reaction mixture was stirred until complete consumption of starting material (1h) and the solvent was removed under *vacuum*. The crude mixture was purified by column chromatography [EtOAc/Cyclohexane (7:3)] to afford **7** as a white solid (457 mg, 0.86 mmol, 46% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 – 5.27 (m, 2H, H_7, H_8), 5.03 – 4.78 (m,

2H, H_4, H_5), 4.25 (dd, $^3J_{9a,9b}$ = 12.6 Hz, $^3J_{9a,8}$ = 2.0 Hz, 1H, H_{9a}), 4.13 (dd, $^3J_{9b,9a}$ = 12.6 Hz, $^3J_{9b,8}$ = 2.0 Hz, 1H, H_{9b}), 3.93 – 3.83 (m, 1H, H_6), 3.81 (s, 3H, $CO_2C\underline{H}_3$), 2.99 – 2.68 (m, 1H, H_{3a}, $H_{2'a}$), 2.58 – 2.40 (m, 2H, $H_{2'}$), 2.17, 2.09 (s, 6H, 2 x $COC\underline{H}_3$), 2.05 – 1.95 (m, 11H, $COC\underline{H}_3$, H_{3b}, $H_{4'}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.3, 170.2, 170.0, 170.0 (5 x $\underline{C}OCH_3$), 168.4 (C_1), 83.0 (C_2), 82.3 ($C_{3'}$), 73.2 (C_6), 70.0 (C_4), 69.8 ($C_{4'}$), 67.9 (C_8), 67.8 (C_5), 66.5 (C_7), 62.0 (C_9), 53.2 ($CO_2\underline{C}H_3$), 37.6 (C_3), 28.2 (C_1'), 21.3, 20.9, 20.9, 20.8 (5 x $CO\underline{C}H_3$), 19.9 (C_2'); HRMS (ESI+) calculated for $C_{24}H_{32}O_{13}NaS$ [M+Na^+] 583.1461, found 583.1463.



Compound 9. Compound **8** (67 mg, 0.12 mmol, 1.0 equiv.) was dissolved in MeOH (10 mL) and the solution was cooled in an ice-bath. K_2CO_3 (83 mg, 0.6 mmol, 5.0 equiv.) was added and the reactional mixture was stirred at room temperature until complete consumption of starting material. Then, acidic resin was added and the mixture was stirred until reaching neutral pH. The resin was removed by filtration, washed with MeOH and the filtrate was concentrated under *vacuum* to afford **9** as a white powder (125 mg, 99%). ^{1}H NMR (400 MHz, D_2O) δ 3.97 – 3.90 (m, 6H, H_7, H_8, H_{9a}, $CO_2C\underline{H}_3$), 3.81 – 3.72 (m, 1H, H_{9b}), 3.71 – 3.60 (m, 3H, H_4, H_5, H_6), 3.05 – 2.86 (m, 2H, $H_{1'}$), 2.83 (dd, $^2J_{3a,3b}$ = 12.9 Hz, $^3J_{3a,4}$ = 4.3 Hz, 1H, H_{3a}), 2.63 – 2.55 (m, 2H, $H_{2'}$), 2.50 (t, $^3J_{4',2'}$ = 2.6 Hz, 1H, $H_{4'}$), 1.89 (dd, $^2J_{3b,3a}$ = 12.9 Hz, $^3J_{3b,4}$ = 10.9 Hz, 1H, H_{3b}); ^{13}C NMR (101 MHz, D_2O) δ 171.2 ($\underline{C}O_2CH_3$), 83.7 (C_2), 76.2 ($C_{3'}$), 71.1, 70.7, 69.7, 69.5, 67.9, 63.1 (C_9), 53.6 ($CO_2\underline{C}H_3$), 39.7 (C_3), 28.1 ($C_{1'}$), 18.9 ($C_{2'}$); HRMS (ESI+) calculated for $C_{14}H_{22}O_8NaS$ [M+Na^+] 373.0933, found 373.0928.

2.3. Synthesis of compound 12



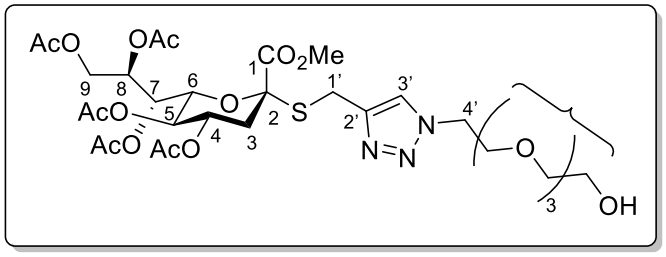
Compound 12. To a solution of **11**[3] (431 mg, 2.1 mmol, 1.0 equiv.) and sodium pyruvate (250 mg, 2.27 mmol, 1.1 equiv.) in water (2.5 mL) was added Neu5Ac aldolase from *E. coli* (5.0 mg). The reactional mixture was stirred overnight at 37 °C. The solvent was then removed under reduced pressure at 45 °C and the resulting syrup was dissolved in acetic acid (2.1 mL) and H_2O (0.3 mL). The crude solution was then added dropwise in an Erlenmeyer of acetone (24 mL) at 0 °C. The formed precipitate was filtered, washed successively with acetone (11 mL), ethanol (13.0 mL) and methanol (13 mL) and concentrated

[3] J. L. C. Liu, G. J. Shen, Y. Ichikawa, J. F. Rutan, G. Zapata, W. F. Vann and C. H. Wong, *J. Am. Chem. Soc.*, 1992, **114**, 3901–3910

under *vacuum* to afford **12** as white powder (230 mg, 38%). ^{1}H NMR (400 MHz, D_2O) δ 4.09 – 3.96 (m, 3H, H_4, H_6, H_8), 3.92 (dd, J = 9.0, 1.3 Hz, 1H, H_7), 3.79 – 3.61 (m, 2H, H_5, H_{9a}), 3.57 (dd, $^{2}J_{9a,9b}$ = 13.2 Hz, $^{3}J_{9a,8}$ = 6.2 Hz, 1H, H_{9a}), 2.23 (dd, $^{2}J_{3a,3b}$ = 13.0 Hz, $^{2}J_{3a,4}$ = 5.1 Hz, 1H, H_{3a}), 1.85 (dd, $^{2}J_{3b,3a}$ = 13.0 Hz, $^{2}J_{3b,4}$ = 11.7 Hz, 1H, H_{3b}); ^{13}C NMR (101 MHz, D_2O) δ 176.8 ($\underline{C}O_2H$), 96.5 (C_2), 71.4, 70.4 (C_5), 69.5, 69.2, 68.8 (C_7), 54.0 (C_9), 39.2 (C_3); HRMS (ESI-) calculated for $C_9H_{14}N_3O_8$ [M-H$^+$] 292.0784, found 292.0781.

Adaptation from D. C. M. Kong, M. von Itzstein, *Carbohydr. Res.*, 1998, 305, 323-329.

2.4. Synthesis of compound 1



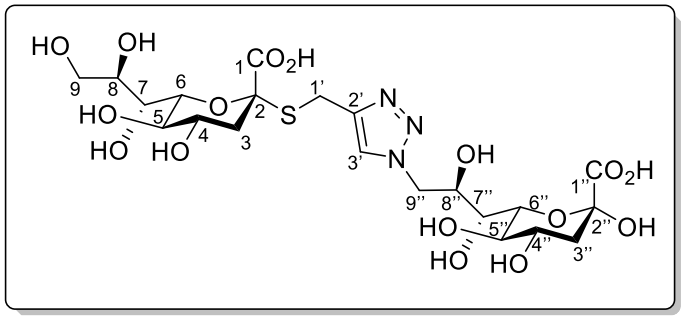
Compound 10. In microwave reactor, compound **6** (59 mg, 0.11 mmol, 1.2 equiv.), azido-tetraethyleneglycol (N3-TEG) (20 mg, 0.09 mmol, 1.0 equiv.), $CuSO_4.5H_2O$ (6 mg, 0.023 mmol, 0.25 equiv.) and sodium ascorbate (9 mg, 0.045 mmol, 0.5 equiv.) were dissolved in 1,4-dioxane (1.2 mL) and water (0.3 mL). The reactor was sealed and the reactional mixture was allowed to stir for 1h at 70 °C under microwave (100W). Copper was chelated with Chelex resin during 20 min after cooling at room temperature, filtered, washed with 1,4-dioxane and concentrated under reduced pressure at 45 °C. The product **10** was isolated by flash chromatography (2% to 5% MeOH/DCM) as a colorless oil (33 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (br. s, 1H, $H_{3'}$), 5.44 (ddd, $^{3}J_{8,7}$ = 9.2 Hz, $^{3}J_{8,9b}$ = 4.8 Hz, $^{3}J_{8,9a}$ = 2.4 Hz, 1H, H_8), 5.34 (dd, $^{3}J_{7,8}$ = 9.2 Hz, $^{3}J_{7,6}$ = 2.2 Hz, 1H, H_7), 4.88 (m, 2H, H_4, H_5), 4.51 (t, $^{3}J_{5',6'}$ = 5.3 Hz, 2H, $H_{4'}$), 4.28 (dd, $^{2}J_{9a,9b}$ = 12.5 Hz, $^{3}J_{9a,8}$ = 2.5 Hz, 1H, H_{9a}), 4.11 (dd, $^{2}J_{9b,9a}$ = 12.5 Hz, $^{3}J_{9b,8}$ = 4.8 Hz, 1H, H_{9b}), 3.91 (dd, $^{3}J_{6,5}$ = 9.9 Hz, $^{3}J_{6,7}$ = 2.2 Hz, 1H, H_6), 3.87 (t, $^{3}J_{6',5'}$ = 5.1 Hz, 2H, $H_{5'}$), 3.77 – 3.50 (m, 16H, $CO_2C\underline{H}_3$, H_{PEG}), 2.80 (dd, $^{2}J_{3a,3b}$ = 12.7 Hz, $^{3}J_{3,4}$ = 3.1 Hz, 1H, H_{3a}), 2.19, 2.11, 2.02 (3 x s, 9H, 3 x $COC\underline{H}_3$), 1.99 (m, 7H, 2 x $COC\underline{H}_3$, H_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.2, 170.1, 169.9, 169.8 (5 x C=O), 168.0 ($\underline{C}O_2CH_3$), 82.9 (C_2), 73.0 (C_6), 72.5, 70.6, 70.5, 70.4, 70.3 (C_{PEG}), 69.9 (C_4), 69.5 ($C_{5'}$), 67.8 (C_5), 67.7 (C_8), 66.5 (C_7), 62.1 (C_9), 61.7 (C_{PEG}), 53.1 ($CO_2\underline{C}H_3$), 50.3 ($C_{4'}$), 37.3 (C_3), 21.2, 20.8, 20.7, 20.7, 20.6 (5 x $CO\underline{C}H_3$); HRMS (ESI$^+$) calculated for $C_{31}H_{48}N_3O_{17}S$ [M+H$^+$] 766,2704, found 766.2719.

Compound 1. Compound **10** (33 mg, 0.043 mmol, 1.0 equiv.) was dissolved in methanol (1.6 mL) and MeOH (0.4 mL). After stirring for 15 min, LiOH (7 mg, 0.30 mmol, 7.0 equiv.) was added, and the reaction mixture was stirred for 24h at room temperature. The mixture was neutralized by adding acidic resin, stirred until reaching neutral pH, filtered and washed with MeOH. The solvent was finally removed under *vacuum* to afford compound **1** as white solid (22 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4,69 (t, $^{3}J_{4',5'}$ = 4,0 Hz, 2H, $H_{4'}$), 4.15 – 3.43 (m, 27H, H_4, H_5, H_6, H_7, H_8, H_9, H_{PEG}), 2,74 (dd, $^{2}J_{3a,3b}$ = 12,7 Hz, $^{3}J_{3a,4}$ = 4,7 Hz, 1H, H_{3a}), 1.72 (t, $^{2}J_{3b,3a}$ = $^{3}J_{3b,4}$ = 11,5 Hz, 1H, H_{3a}); ^{13}C NMR (75 MHz, D_2O) δ 174.0 ($\underline{C}O_2H$), 144.8, 124.5, 86.2, 75.7, 72.1, 71.6, 70.6, 69.6, 69.6, 69.4, 68.7, 67.6, 62.5, 60.3, 50.0, 40.3 (C_3), 23.5 ($C_{1'}$); HRMS (ESI-) calculated for $C_{20}H_{34}N_3O_{12}S$ [M-H$^+$] 540.1863, found 540.1863.

2.5. Synthesis of compound 2

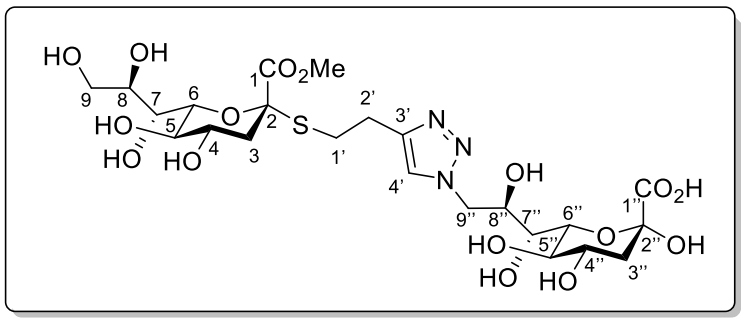
Compound 13. Compound **8** (32 mg, 0.095 mmol, 1.0 equiv.), compound **12** (32 mg, 0.095 mmol, 1.0 equiv.) were dissolved in 1,4-dioxane (8.0 mL) and water (2.0 mL). $CuSO_4.5H_2O$ (14 mg, 0.057 mmol, 0.6 equiv.) and sodium ascorbate (23 mg, 0.11 mmol, 1.2 equiv.) presolubilized in a minimum amount of water were added and the reactional mixture was allowed to stir for 24h at 60 °C. Copper was chelated with Chelex® resin during 30 min after cooling at room temperature, filtered, washed with 1,4-dioxane/water and concentrated under reduced pressure at 45 °C. The product **13** was isolated by chromatography [$CHCl_3$/MeOH/H_2O (3:4:1)] as white powder (13 mg, 46%). ^{1}H NMR (400 MHz, D_2O) δ 8.00 (s, 1H, $H_{3''}$), 4.86 (dd, $^{2}J_{9'a,9'b}$ = 14.4 Hz, $^{3}J_{9'a,8}$ = 2.8 Hz, 1H, $H_{9'a}$), 4.56 (dd, $^{2}J_{9'b,9'a}$ = 14.4 Hz, $^{3}J_{9'b,8}$ = 8.3 Hz, 1H, $H_{9'b}$), 4.24 – 3.62 (m, 17H, H_4, H_5, H_6, H_7, H_8, H_9, $H_{4'}$, $H_{5'}$, $H_{6'}$, $H_{7'}$, $H_{8'}$, $H_{1''}$, CO_2CH_3), 2.82 (dd, $^{2}J_{3a,3b}$ = 12.5 Hz, $^{3}J_{3a,4}$ = 3.8 Hz, 1H, H_{3a}), 2.23 (dd, $^{2}J_{3'a,3'b}$ = 13.1 Hz, $^{3}J_{3'a,4}$ = 5.0 Hz, 1H, $H_{3'a}$), 1.95 – 1.81 (m, 2H, H_{3b}, $H_{3'b}$); ^{13}C NMR (101 MHz, D_2O) δ 176.8 ($\underline{C}O_2CH_3$), 171.0 ($\underline{C}O_2H$), 125.1 ($C_{3''}$), 96.5 (C_2), 83.1 (C2'), 76.1, 71.4,

71.0, 70.3, 69.6, 69.5, 69.4, 69.3, 69.1, 67.9, 63.1, 53.7 ($C_{9'}$), 53.5 ($CO_2\underline{C}H_3$), 39.5 ($C_{9'}$), 39.2 (C9), 23.2 ($C_{1''}$). HRMS (ESI-) calculated for $C_{22}H_{34}N_3O_{16}S$ [M-H^+] 628.1660, found 628.1658.



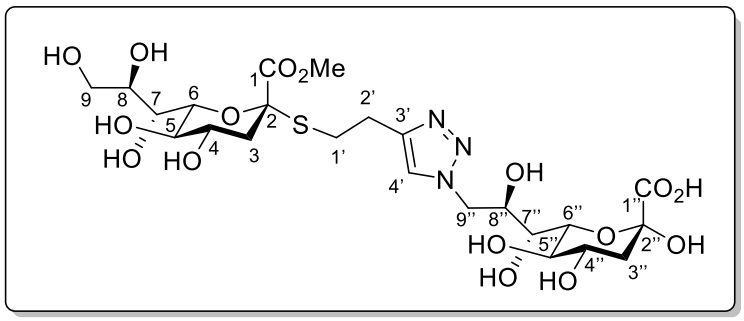
Compound 2. Compound **13** (13 mg, 0.021 mmol, 1.0 equiv.) was dissolved in water (5 mL). After stirring for 15 min, LiOH (3 mg, 0.12 mmol, 6.0 equiv.) was added, and the reactional mixture was stirred for 24h at room temperature. The mixture was neutralized by adding acidic resin, stirred until reaching neutral pH, filtered and washed with H_2O and MeOH. The solvent was finally freeze-drying to afford the final compound **2** as white powder (13 mg, 99%). ^{1}H NMR (400 MHz, D_2O) δ 7.92 (s, 1H, $H_{3''}$), 4.78 (dd, $^2J_{9',a,9',b}$ = 14.5 Hz, $^3J_{9',a,8}$ = 2.9 Hz, 1H, $H_{9',a}$), 4.47 (dd, $^2J_{9',b,9',a}$ = 14.5 Hz, $^3J_{9',b,8}$ = 8.1 Hz, 1H, $H_{9',b}$), 4.08 (dd, $^3J_{8',9',b}$ = 8.4 Hz, $^3J_{8',9',a}$ = 2.8 Hz, 1H, $H_{8'}$), 3.99 – 3.91 (m, 2H, $H_{4'}$, $H_{6'}$), 3.85 – 3.73 (m, 4H, $H_{7'}$, H_{9a}), 3.74 – 3.67 (m, 1H), 3.70 – 3.46 (m, 6H, H_4, H_{9b}, $H_{5'}$), 2.70 (dd, $^2J_{3a,3b}$ = 12.9 Hz, $^3J_{3a,4}$ = 4.7 Hz, 1H, H_{3a}), 2.23 (dd, $^2J_{3',a,3b}$ = 13.1 Hz, $^3J_{3',a,4}$ = 5.1 Hz, 1H, $H_{3',a}$), 1.87 – 1.67 (m, 2H, H_3, $H_{3'}$); ^{13}C NMR (101 MHz, D_2O) δ 173.3 ($\underline{C}O_2Me$), 172.4 ($\underline{C}O_2H$), 125.4 ($C_{3''}$), 95.3 ($C_{2'}$), 83.8 (C_2), 76.1, 71.6, 71.4, 70.0, 69.9, 69.5, 69.2 (C_8), 68.9, 68.6, 67.8, 62.9 (C_9), 53.8 ($C_{9'}$), 39.7 (C_3), 38.6 ($C_{3'}$), 23.2 ($C_{1''}$); HRMS (ESI-) calculated for $C_{21}H_{32}N_3O_{16}S$ [M-H^+] 614.1503, found 614.1508.

2.6. Synthesis of compound 3



Compound 14. Compound **9** (42 mg, 0.12 mmol, 1.0 equiv.), compound **12** (40 mg, 0.12 mmol, 1.0 equiv.) were dissolved in 1,4-dioxane (8.0 mL) and water (2.0 mL). $CuSO_4.5H_2O$ (18 mg, 0.072 mmol, 0.6 equiv.) and sodium ascorbate (29 mg, 0.14 mmol, 1.2 equiv.) presolubilized in a minimum amount of water were dissolved in dioxane (4.0 mL) and water (1.0 mL). The reactional mixture was allowed to stir for 24h at 60 °C. Copper was chelated with Chelex® resin during 30 min after cooling at room temperature, filtered, washed with 1,4-dioxane/water and concentrated under reduced pressure at 45 °C. The product **14** was isolated by chromatography [$CHCl_3$:MeOH:H_2O (3:4:1)] as white solid (34 mg, 44%). ^{1}H NMR (400 MHz, D_2O) δ 7.85 (s,

1H, $H_{4''}$), 4.77 (dd, $^2J_{9',a,9',b}$ = 14.4 Hz, $^3J_{9',a,8'}$ = 2.8 Hz, 1H, $H_{9',a}$), 4.47 (dd, $^2J_{9',b,9',a}$ = 14.4 Hz, $^3J_{9',b,8'}$ = 8.1 Hz, 1H, $H_{9',b}$), 4.08 (td, $^3J_{8',,9',b}$ = 8.4 Hz, $^3J_{8',,9',a}$ = 2.8 Hz, 1H, $H_{8'}$), 4.01 – 3.86 (m, 3H, $H_{4'}$, $H_{6'}$), 3.86 – 3.79 (s, 6H, H_8, H_{9a}, $CO_2C\underline{H}_3$), 3.79 – 3.72 (m, 1H, $H_{7'}$), 3.72 – 3.62 (m, 1H, H_{9b}), 3.62 – 3.50 (m, 3H, H_4, $H_{5'}$), 3.09 – 2.90 (m, 4H, $H_{1''}$, $H_{2''}$), 2.80 – 2.62 (m, 1H, H_{3a}), 2.14 (dd, J = 13.1, 4.9 Hz, 1H, $H_{3',a}$), 1.82 – 1.72 (m, 2H, H_{3b}, $H_{3',b}$); ^{13}C NMR (101 MHz, D_2O) δ 176.8 ($\underline{C}O_2CH_3$), 171.2 ($\underline{C}O_2H$), 124.6 ($C_{4''}$), 96.5 ($C_{2'}$), 83.7 (C_2), 76.2 ($C_{5'}$), 71.4, 71.1, 70.4, 69.7, 69.5, 69.3 (C_8'), 69.3, 69.1, 67.9, 63.1, 53.6 ($C_{9'}$, $CO_2\underline{C}H_3$), 39.7 (C3), 39.2 (C3'), 28.7 (C2"), 24.9 (C1"). HRMS (ESI-) calculated for $C_{23}H_{36}N_3O_{16}S$ [M-H^+] 642.1816, found 614.1819.



Compound 3. Compound **14** (34 mg, 0.053 mmol, 1.0 equiv.) was dissolved in water (5 mL). After stirring for 15 min, LiOH (8 mg, 0.32 mmol, 6.0 equiv.) was added, and the reactional mixture was stirred for 24h at room temperature. The mixture was neutralized by adding acidic resin, stirred until reaching neutral pH, filtered and washed with water and MeOH. The solvent was finally removed under vacuum to afford the final compound **3** as white powder (26 mg, 79%). ^{1}H NMR (400 MHz, D_2O) δ 7.98 (s, 1H, $H_{4'}$), 4.81 (dd, $^2J_{9',a,9',b}$ = 14.3 Hz, $^3J_{9',a,8'}$ = 2.9 Hz, 1H, $H_{9',a}$), 4.51 (dd, $^2J_{9',b,9',a}$ = 14.4, $^3J_{9',b,8'}$ = 8.1 Hz, 1H, $H_{9',b}$), 4.09 (ddd, $^3J_{8',,9',b}$ = 8.4 Hz, $^3J_{8',,9',a}$ = 2.8 Hz, 1H, $H_{8'}$), 4.01 – 3.90 (m, 2H, $H_{4'}$), 3.87 – 3.77 (m, 5H, H_{9a}, $H_{7'}$), 3.69 – 3.45 (m, 3H, H_4, H_{9a}), 3.12 – 2.93 (m, 4H, $H_{1'}$, $H_{2'}$), 2.68 (dd, $^2J_{3a,3b}$ = 12.9 Hz, $^3J_{3a,4}$ = 4.6 Hz, 1H, H_{3a}), 2.23 (dd, $^2J_{3',a,3',b}$ = 13.1 Hz, $^3J_{3',a,4}$ = 5.1 Hz, 1H), 1.80 (dd, $^2J_{3',b,3',a}$ = 13.1 Hz, $^3J_{3',b,4}$ = 11.7 Hz, 1H, $H_{3',b}$), 1.73 (dd, $^2J_{3b,3a}$ = 12.9 Hz, $^3J_{3b,4}$ = 11.4 Hz, 1H, H_{3b}); ^{13}C NMR (101 MHz, D_2O) δ 173.3, 172.7 (2 x $\underline{C}O_2H$), 125.7 ($C_{4'}$), 95.3 ($C_{2'}$), 84.2 (C_2), 76.1, 71.7, 71.6, 70.0, 70.0, 69.5, 69.1 ($C_{8'}$), 69.0, 68.6, 67.8, 62.9 (C_9), 54.3 ($C_{9'}$), 39.9 (C_3), 38.6 ($C_{3'}$), 28.5 ($C_{1'}$), 24.6 ($C_{2'}$); HRMS (ESI-) calculated for $C_{22}H_{34}N_3O_{16}S$ [M-H^+] 628.1660, found 628.1685.

2.7. Synthesis of compound 4

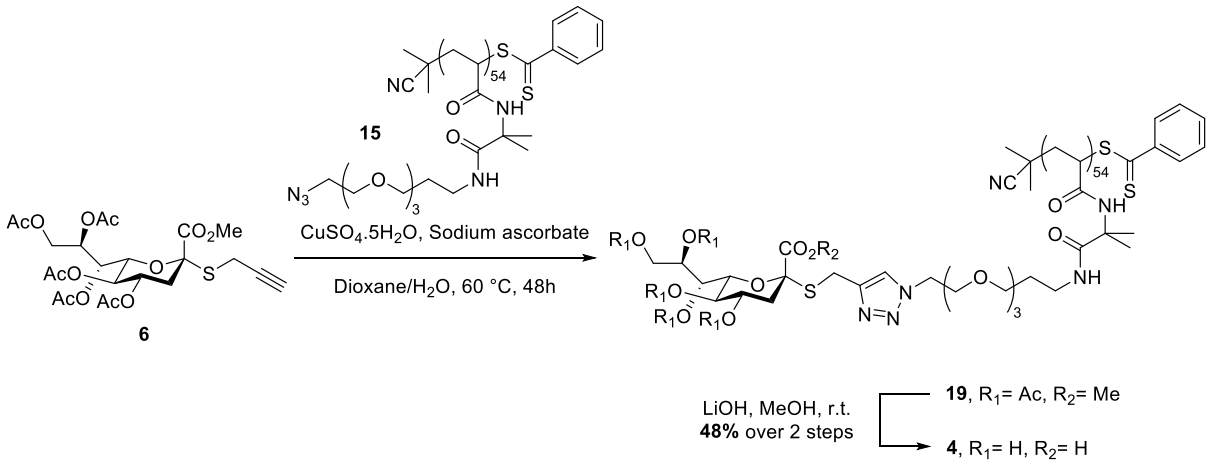
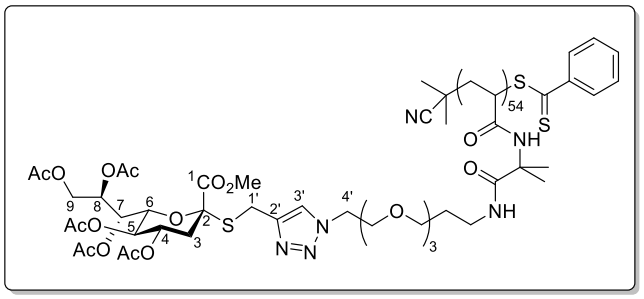


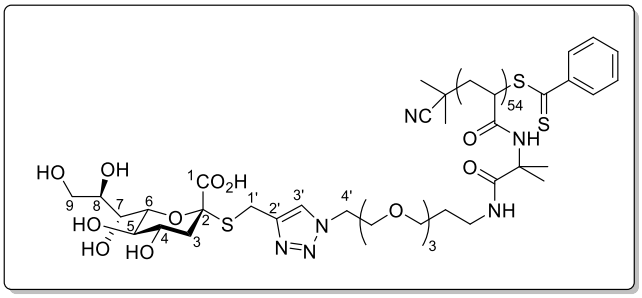
Figure S1. Synthesis pathway of compound **4**.



Compound 16. In a sealed tube, described polymer **15**[4] (15 mg, 0.041 mmol of N_3), compound **6** (34 mg, 0.062 mmol, 1.5 equiv./N_3) were dissolved in 1,4-dioxane (2.4 mL), then a solution of $CuSO_4.5H_2O$ (3 mg, 0.010 mmol, 0.25 equiv./N_3) in water (0.1 mL) was added, the mixture was bubbled with Argon for 15 min, then sodium ascorbate (4 mg, 0.021 mmol, 0.5 equiv./N_3) in water (0.5 mL) was added and the mixture was bubbling with Argon for a further 10 min. The tube was sealed and the reactional mixture was stirred at 60 °C for 24h. Copper was chelated with Chelex resin under agitation (1h) at 60 °C, filtered, washed with dioxane and concentrated under vacuum. The crude product **16** was directly engaged in the deprotection step. **FTIR** (cm^{-1}): 3379 (ν O-H, ν N-H), 2930 (ν C-H alkane), 1737 (ν COOH), 1647 (ν C=O amide), 1537 (δ N-H amide), 1453 (δ C-H alkene).

In some experiments, there were still azide functions (FT-IR: 2100 – 2200 cm^{-1}) that did not react, if so, the reaction was relaunched with compound ***8*** *(0.75 equiv.), $CuSO_4.5H_2O$ (0.25 equiv.) and sodium ascorbate (0.5 equiv.)*

[4] C. Assailly, C. Bridot, A. Saumonneau, P. Lottin, B. Roubinet, E. Krammer, F. François, F. Vena, L. Landemarre, D. Alvarez Dorta, D. Deniaud, C. Grandjean, C. Tellier, S. Pascual, V. Montembault, L. Fontaine, F. Daligault, J. Bouckaert and S. G. Gouin, *Chem. – Eur. J.*, 2021, chem.202004672.



Compound 4. Crude product **16** was dissolved in methanol (5 mL), then LiOH was added and the solution was stirred for 10 min before adding water (1.3 mL). The reactional mixture was stirred for 18h at room temperature. H^+ resin was added, the mixture was stirred until reaching neutral pH, filtered, washed with methanol and water. The solvent was removed under reduced pressure at 45 °C and the crude product was dissolved in water (2.0 mL) and methanol (0.5 mL) prior passing through a SEC column (PD-10 Sephadex G-25 resin) with pure H_2O. The collected fraction was finally freeze-dried to afford product **4** as a grey cotton (14 mg, 48% over 2 steps). ^{1}H NMR (400 MHz, D_2O) δ 7.94 (bs, nH, nH, $H_{3'}$), 4.57 (bs, nH, $H_{4'}$), 4.16 – 3.27 (m, $H_{Carb.}$, H_{PEG}), 2.71 (m, 1H, H_3), 1.99 – 1.08 (m, CH_3, CH polymer backbone). FT-IR (cm^{-1}): 3357 (ν O-H, ν N-H), 2922 (ν C-H alkane), 1728 (ν C=O acid), 1634 (ν C=O amide), 1543 (δ N-H amide), 1437 (δ C-H alkene).

3. Biology

3.1. Production of sialidase AfKDNase

*Af*KDNase was produced by the "Glycochemistry and Bioconjugation" team from Capacités https://capacites.fr/en/our-expertises/. The gene coding for *Af*KDNase, deleted from the base pairs corresponding to the signal peptide (AA 1 to 20 according to the Uniprot sequence Q4WQS0), was optimized for codon usage in *E. coli*., synthesized, and then introduced into the protein expression vector pET28a(+) between *NheI* and *NotI* restrictions sites by Genscript Biotech (Netherlands) . This construction enables the expression of an N-terminal His_6-tagged protein for IMAC purification. The recombinant plasmid was used to transform *Escherichia coli* BL21 Tuner™ DE(3). A saturated preculture was obtained in LB containing kanamycine (25 µg/mL) from a single freshly transformed *E. coli* colony (BL21 Tuner (DE3), Novagen). This preculture was used for inoculating the culture at 37 °C which was induced by IPTG (0.5 mM) in exponential phase and shaked at 200 rpm at 20 °C during 18h. The culture was centrifugated (6000g, 10 min, RT) and the pellet was flash-freezed in liquid nitrogen and resuspended in phosphate lysis buffer (50 mM, 300mM NaCl, pH 8.0, 10 mM imidazole – 10 ml/g of wet pellet). Lysozyme (from chicken egg white - Fluka) was added (1 mg/ml) and the

mixture was incubated at RT for 30 min. 1 mM of PMSF (Sigma) and 5 µg/ml of DNAse I (Thermofischer Scientific) were added just before 3 cycles of sonication in an ice-bath (5s ON / 1s OFF, 70% during 30s). The lysate was clarified by centrifugation (18000g, 30 min, 4 °C), filtered (0.45 µm) and then loaded for 30 min at 4 °C to Ni-NTA agarose beads (500 µL for 500 mL of culture; Macherey Nagel) previously equilibrated with Phosphate-NaCl buffer containing 10 mM Imidazole. The purification was carried out by gravity according to the supplier recommendations. The eluted fractions of interest were pooled, concentrated and conserved in 50 mM Tris-HCl, 100 mM NaCl pH 8.0 buffer by using centrifugal devices 30K MWCO (Pall). 50 µM and 100 µM protein samples were frozen in liquid nitrogen and then stored at -80°C.

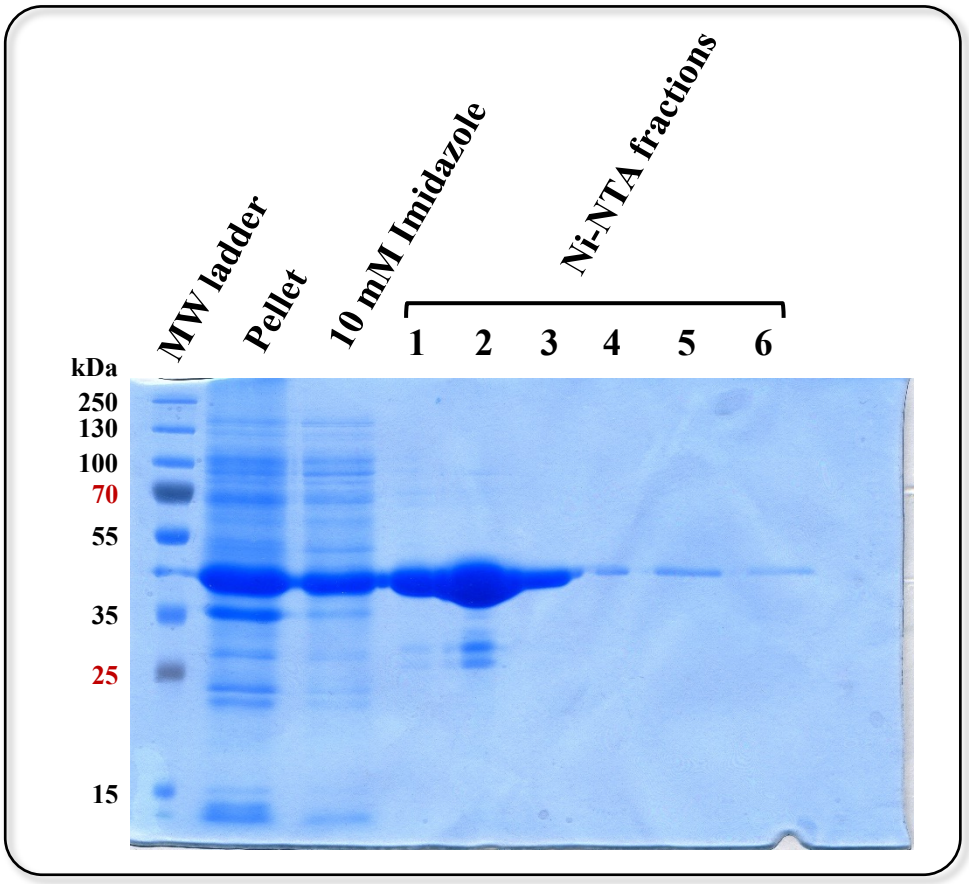


Figure S2. A stained polyacrylamide gel summarizing the purification of AfKDNase. A Coomassie-stained SDS polyacrylamide gel analyzing the purification of the AfKDNase A is shown. Lane 1 is a molecular weight ladder, with 11 bands of known size (ranging from 15 to 250 kDa). Lanes 2 to 9 illustrate the enzyme expression and purification procedure.

3.2. Enzyme Kinetics

Michaelis-Menten constant K_m was determined using synthetic 4-méthylumbelliféryl-deaminoneuraminic acid (MU-KDN). In 96-Well plates, 50 µL of *Af*KDNase in acetate buffer, pH 4.0 were incubated at 37 °C for 15 min, then 50 µL of MU-KDN in acetate buffer, pH 4.0 were added. The release of 4-MU was monitored for 15 min using microplate reader with a temperature controller at 37 °C and excitation and emission wavelengths of 365 nm and 450 nm, respectively. Relative fluorescence units (RFU) were converted into the concentration of the product 4-MU according to the 4-MU standard curve (data not show). K_m was determined from 10 initial rate measurements using a substrate concentration range of 2.4 µM to 5.0 mM with an enzyme concentration fixed at 3.1 nM. The rate *versus* substrate concentration data were fitted to Michaelis-Menten equation using GraphPad (GraphPad Software Inc., San Diego, CA).

3.3. Inhibitory assay

Each inhibitory assay was performed in acetate buffer, pH 4.0 with a final enzyme concentration at 3.1 nM in presence of various inhibitor concentrations. 10 µL of the solution of inhibitor in buffer was added to 40 µL of *Af*KDNase in buffer (final enzyme concentration = 3.1 nM). The solution was incubated for 15 min at 37 °C then 50 µL of MU-KDN in buffer was added (final substrate concentration = 500 µM). The release of MU was monitored for 15 min using microplate reader with a temperature controller at 37 °C and excitation and emission wavelengths of 365 nm and 450 nm, respectively. Relative fluorescence units (RFU) were converted into the concentration of the product 4-MU according to the 4-MU standard curve (data not show). The rate of each reaction (mM/min or µM/min) allowed to draw the Dixon plot ($1/v_0$ *vs.* [I]) where the x-intercept corresponds to the compounds IC_{50}. For each compound, IC_{50} was calculated as a mean of 3 triplicate experiments.

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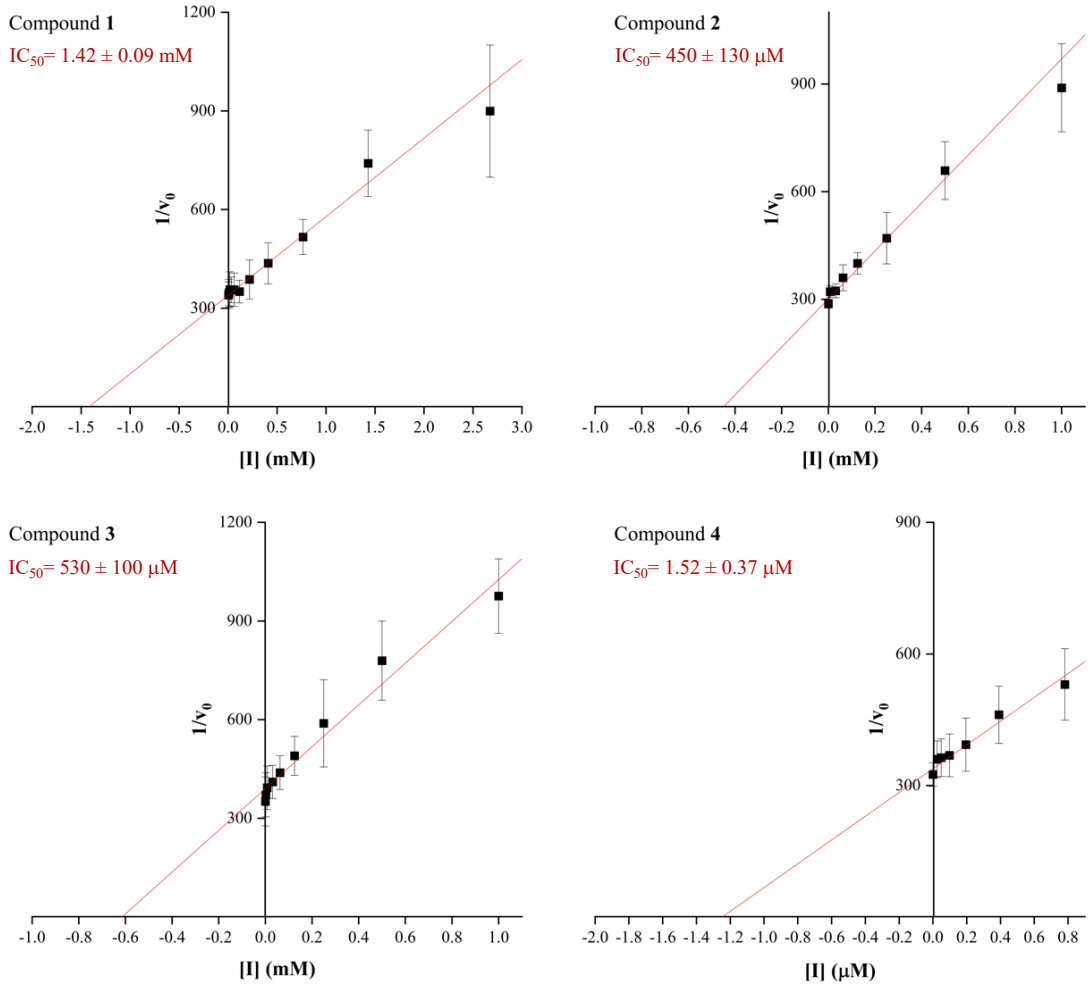

Figure S3. Determination of IC_{50} for compound **1**, **2**, **3** and **4** using Dixon graphical method.

3.4. Determination of the inhibition constants for 4

Study was performed in acetate buffer, pH 4.0 with a final enzyme concentration at 3.1 nM. For each assay, 50 µL of a solution of *Af*KDNase in buffer were incubated at 37 °C for 15 min with several concentrations of inhibitor ([I]= 0, 0.1, 0.2, 0.4 and 0.8 µM). 50 µL of MU-KDN in buffer was added to obtain final concentrations of $2K_m$, K_m, $K_m/2$ and $K_m/4$ for each inhibitor. The release of MU was monitored for 15 min using microplate reader with a temperature controller at 37 °C and excitation and emission wavelengths of 365 nm and 450 nm, respectively. The mode of the inhibition was examined using Lineweaver-Burk plotting (Figure 2). Finally, K_i was determined from 3 independent experiments using nonlinear regression (R^2= 0.9832) with the Mixed Model Inhibition from GrapPad Prism® 8.0.1.

3.5. Antifungal activity

10^2 - 10^3 *A. fumigatus* conidia per well of a sterile µ-slide 8 Well (Ibidi®) were incubated in Sabouraud at 37°C with different concentrations of **4**. After 15 hours, fungal growth was analyzed macroscopically. The antifungal activity was further quantified using two independent methods i) measuring the metabolic activity of *A. fumigatus* and ii) assessing, in the supernatant, the level of galactomannan, a polysaccharide released during *A. fumigatus* growth (Figure 3b). 10^4 *A. fumigatus* conidia per well of a sterile 96-well black-bottom plate (Greiner bio one®) were incubated in Sabouraud with different concentrations of **4** (Figure 3) or KDN2en (Figure S4) in presence of 0.001% Rezasurin and incubated for 15 hours, at 37°C. Fluorescence was measured and metabolic activity expressed in arbitrary units (A.U.). Resazurin (Sigma-Aldrich) conversion to fluorescent resorufin was used as a reporter of metabolic activity of *A. fumigatus* treated or not by a range of concentration of **4** or KDN2en (ref 35). Galactomannan antigen quantification was determined by using a kit, Platelia Aspergillus EIA (Bio-Rad) (ref 36).

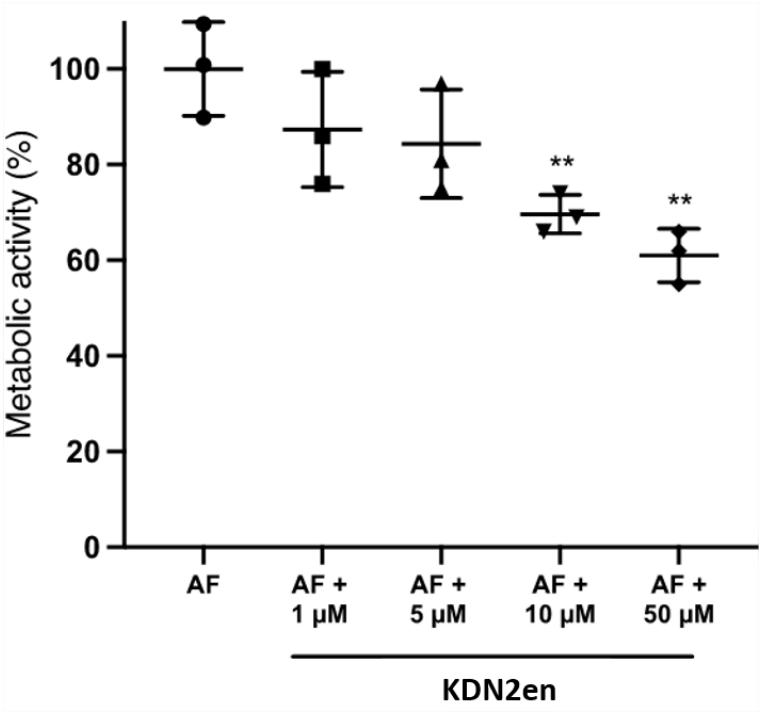

Figure S4. Metabolic activity of inhibitor Kdn2en.

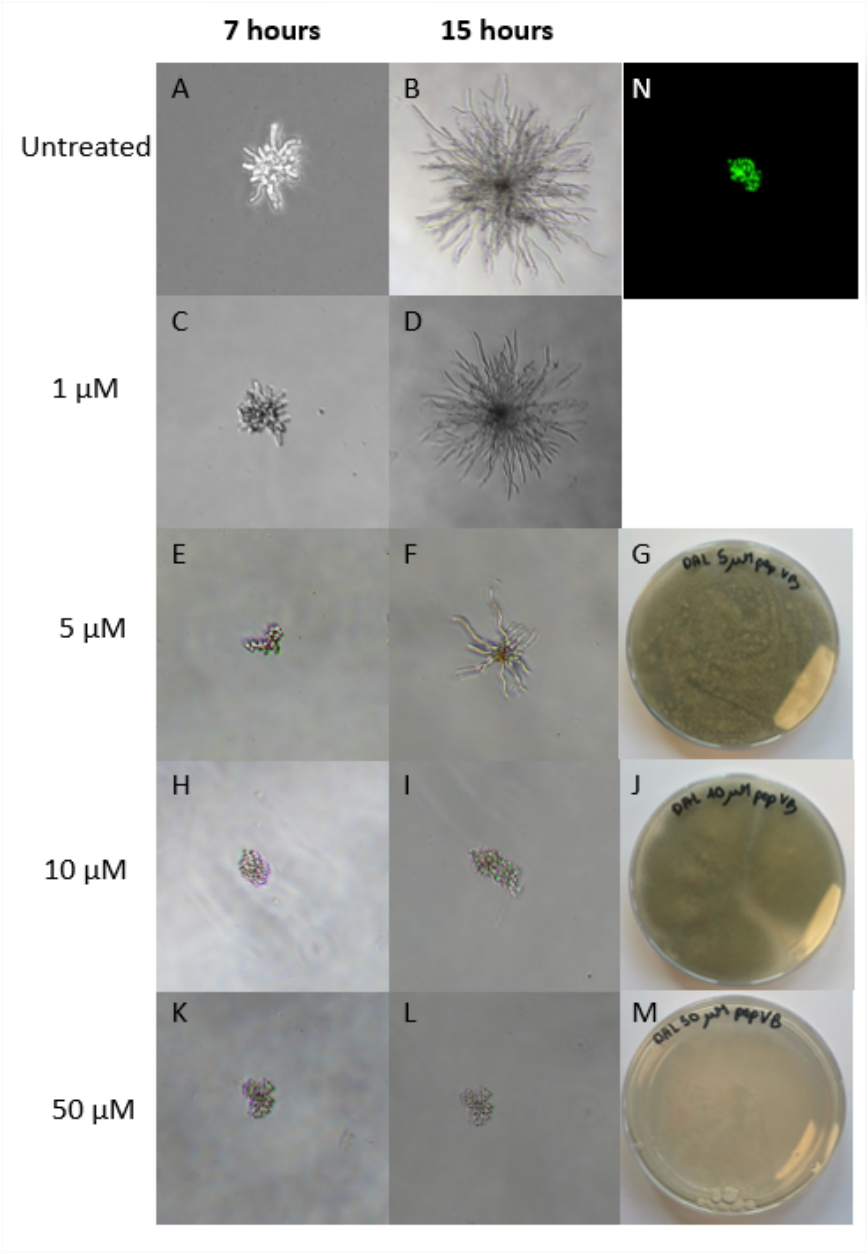

Figure S5. We conducted a microscopic analysis of *A. fumigatus* conidia treated with various concentrations of poly-KDA (1, 5, 10 and 50 µM) and compared them to untreated controls. After 7 hours, germinated conidia were observed in both the untreated sample and the sample treated with 1 µM **4** (A and C). However, no germination occurred when conidia were incubated with 5, 10 and 50 µM (E, H and K). After 15 hours, the filament morphology of the fungus treated with 1 µM **4** was identical to that of the untreated fungus (B and D). In contrast, treatment with 5 µM **4** resulted in the formation of only a few filaments, although their morphology remained similar to that of untreated filaments (F). No filament formation was observed with 10 or 50 µM **4** (I and L). Furthermore, when treated conidia were transplanted onto malt, only those treated with 10 µM **4** were able to grow (G, J, and M). These findings indicate that **4** can inhibit filament formation or kill conidia of *A. fumigatus*, depending on the concentration used.

We stained with Sytox-Green, which is a cell-impermeant fluorogenic nucleic acid stain, the fungus morphotypes treated or not with **4**. Sytox-Green easily penetrates damaged cell membranes but cannot penetrate live cell membranes. Under our experimental conditions, we

did not observe any stained conidia or filaments. To validate our observations, we incubated *A. fumigatus* conidia treated with Amphotericin B with Sytox-Green. In this positive control, we observed fluorescent fungi, indicating cell wall permeabilization (N). These results demonstrate that **4** did not induce permeability in either the conidia or the filaments.

4. NMR spectra

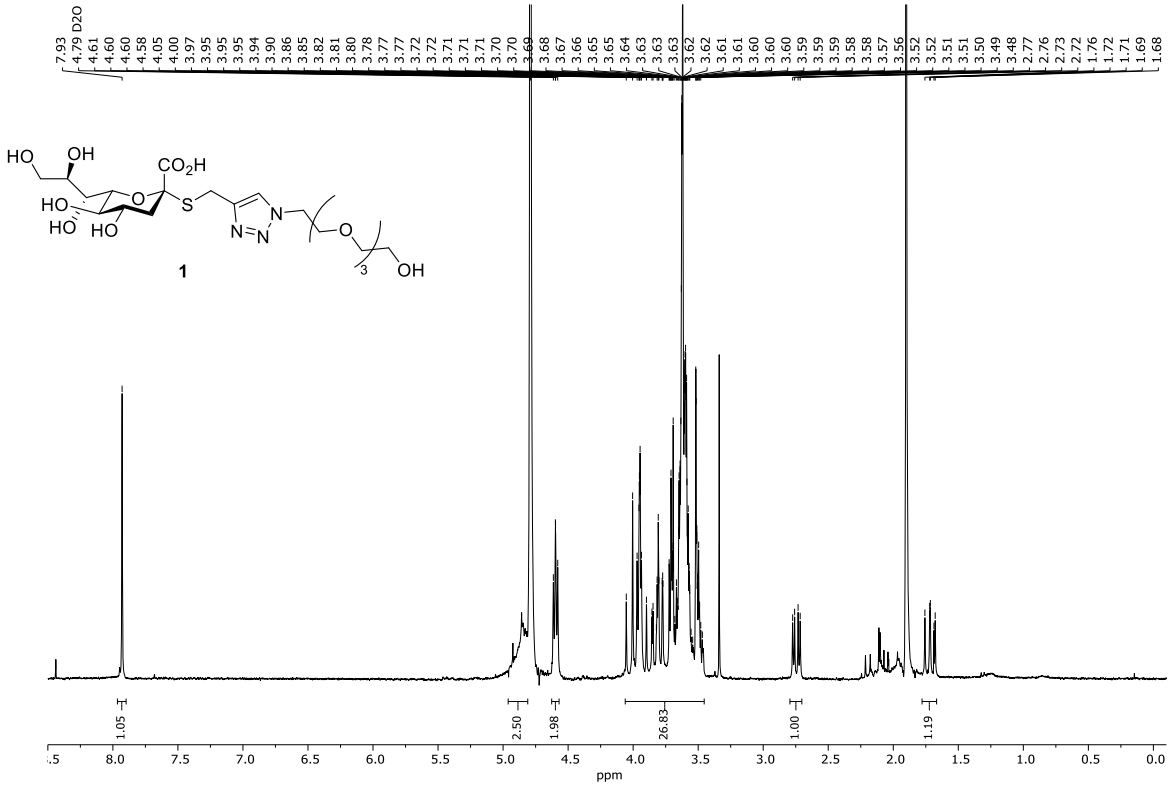

Figure S6. ^{1}H NMR spectrum of compound **1**.

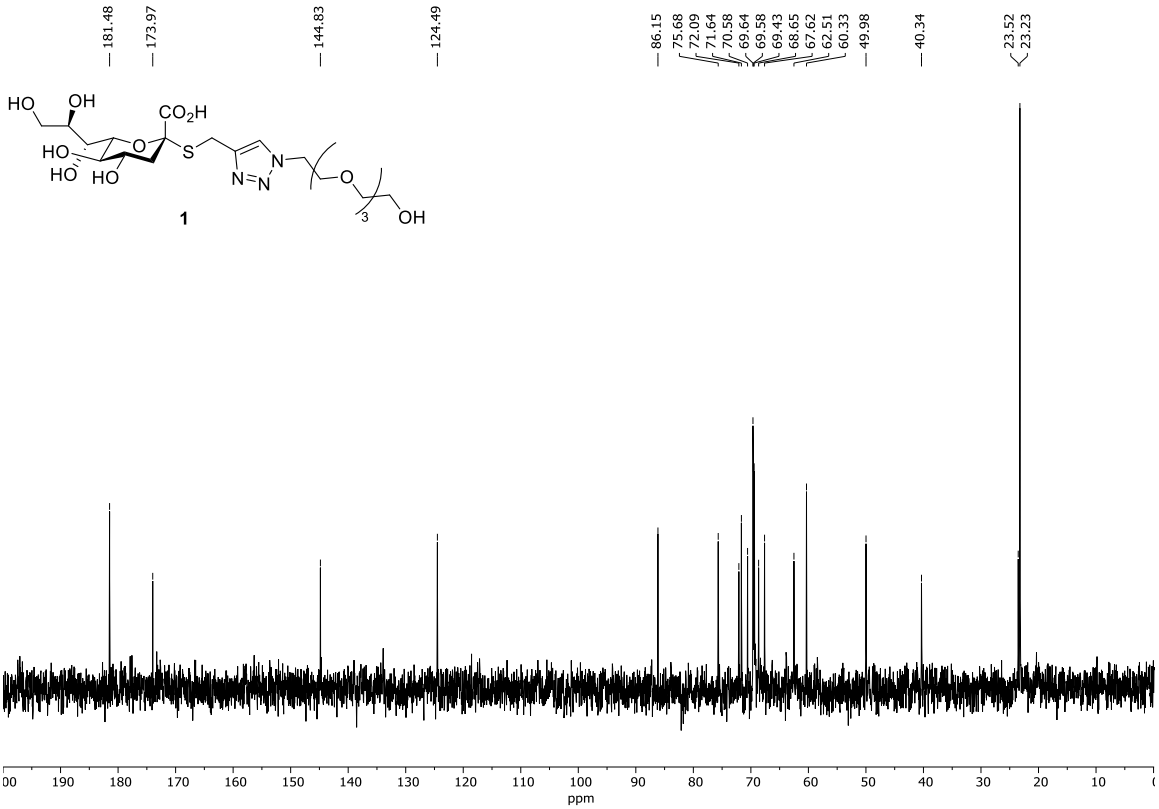

Figure S7. ^{13}C NMR spectrum of compound **1**.

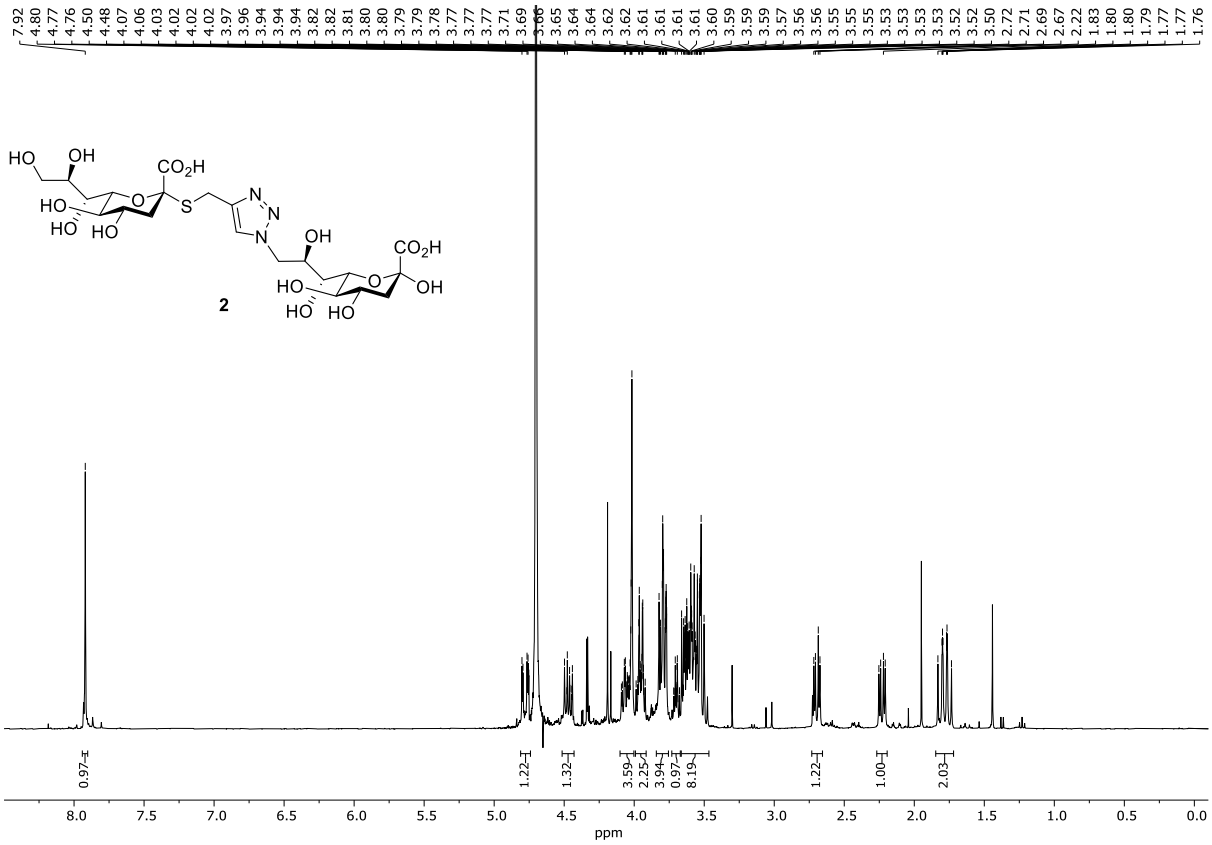

Figure S8. ^{1}H NMR spectrum of compound **2**.

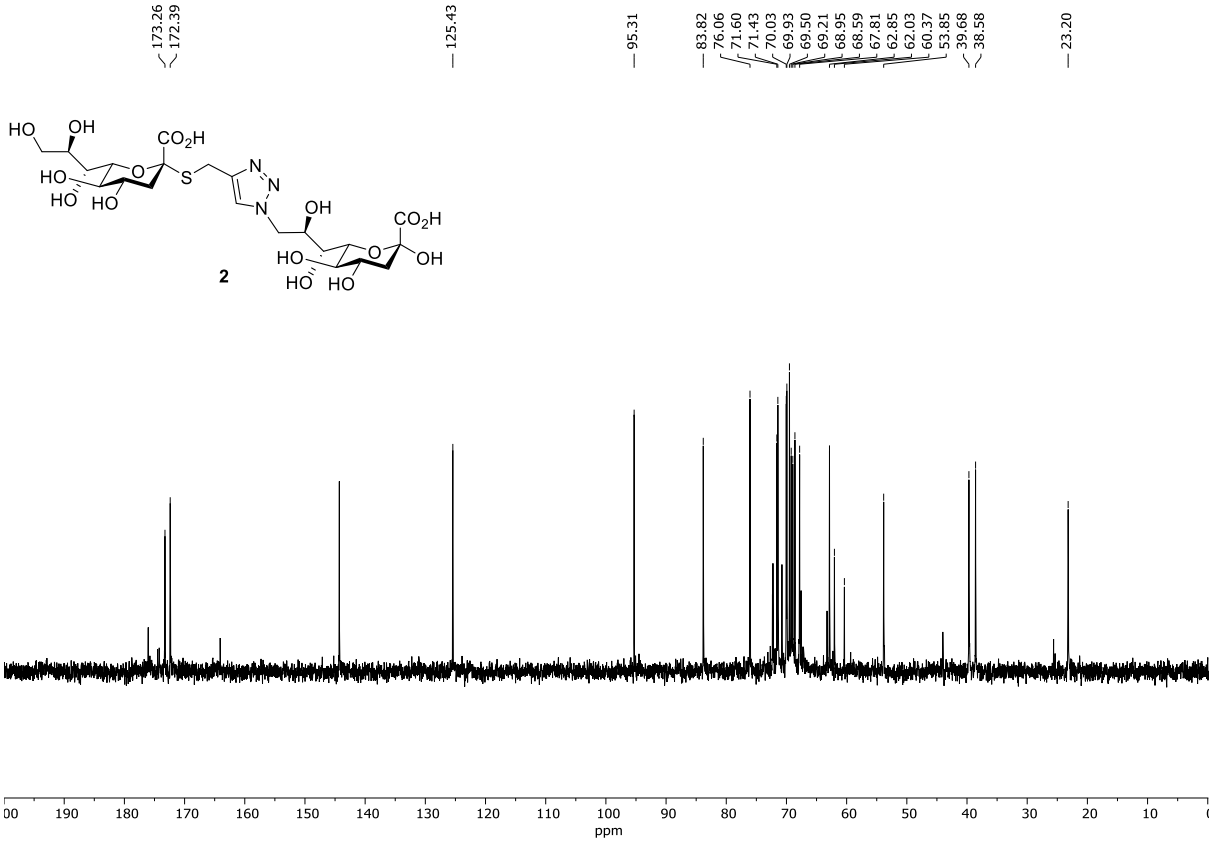

Figure S9. ^{13}C NMR spectrum of compound **2**.

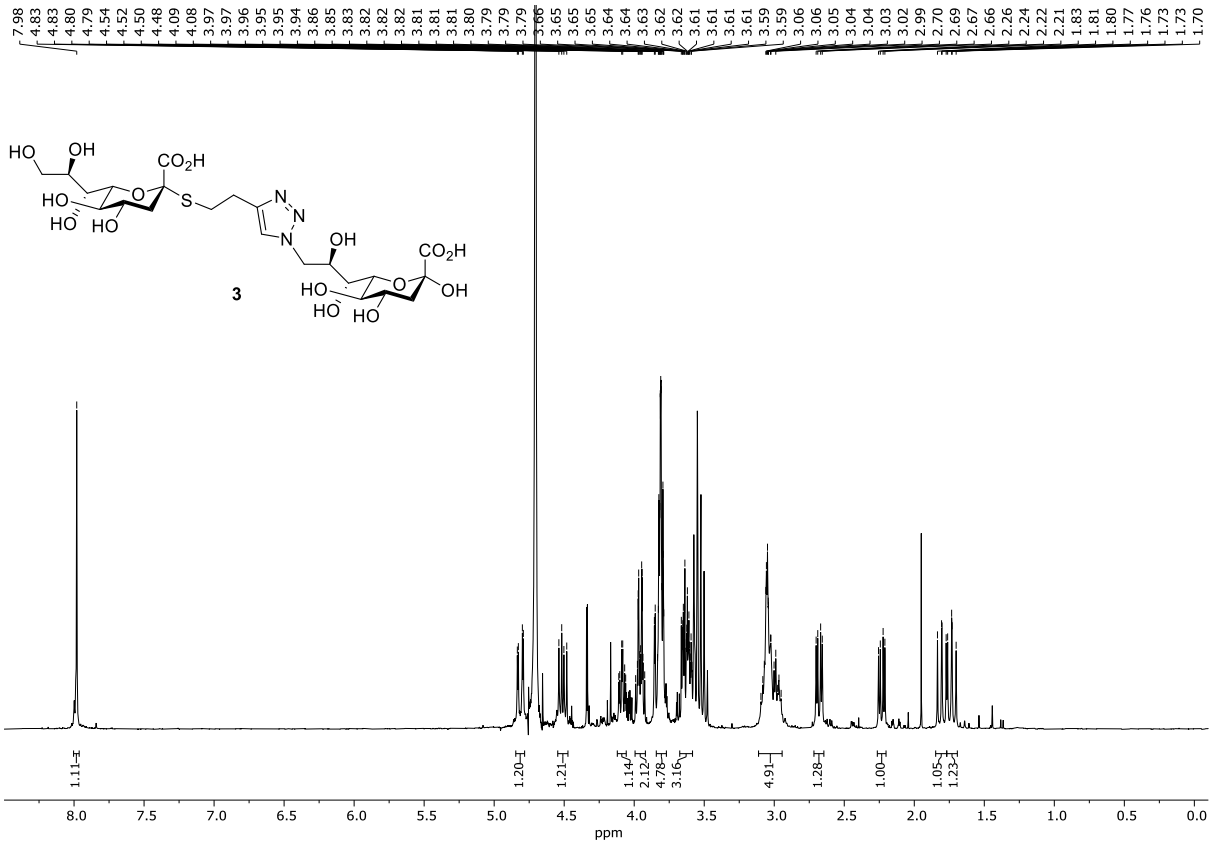

Figure S10. ^{1}H NMR spectrum of compound **3**.

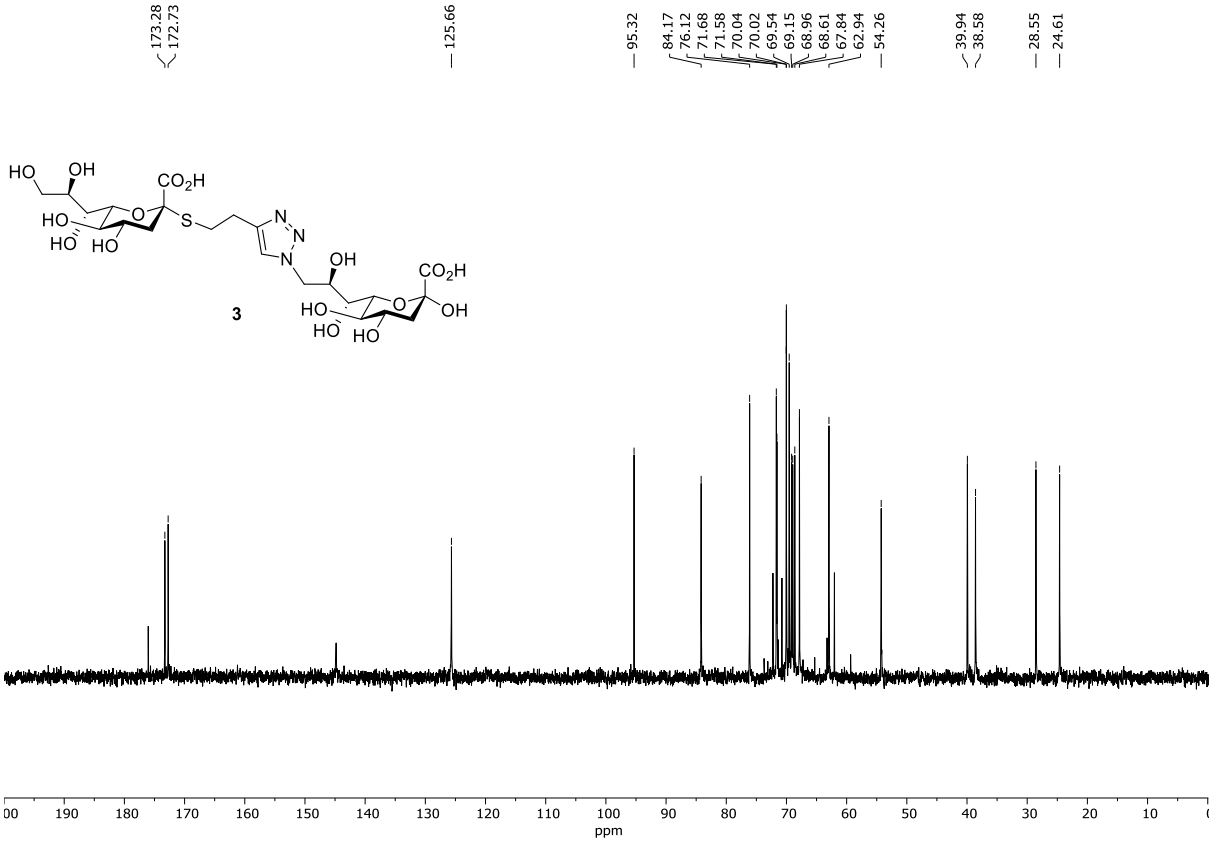

Figure S11. ^{13}C NMR spectrum of compound **3**.

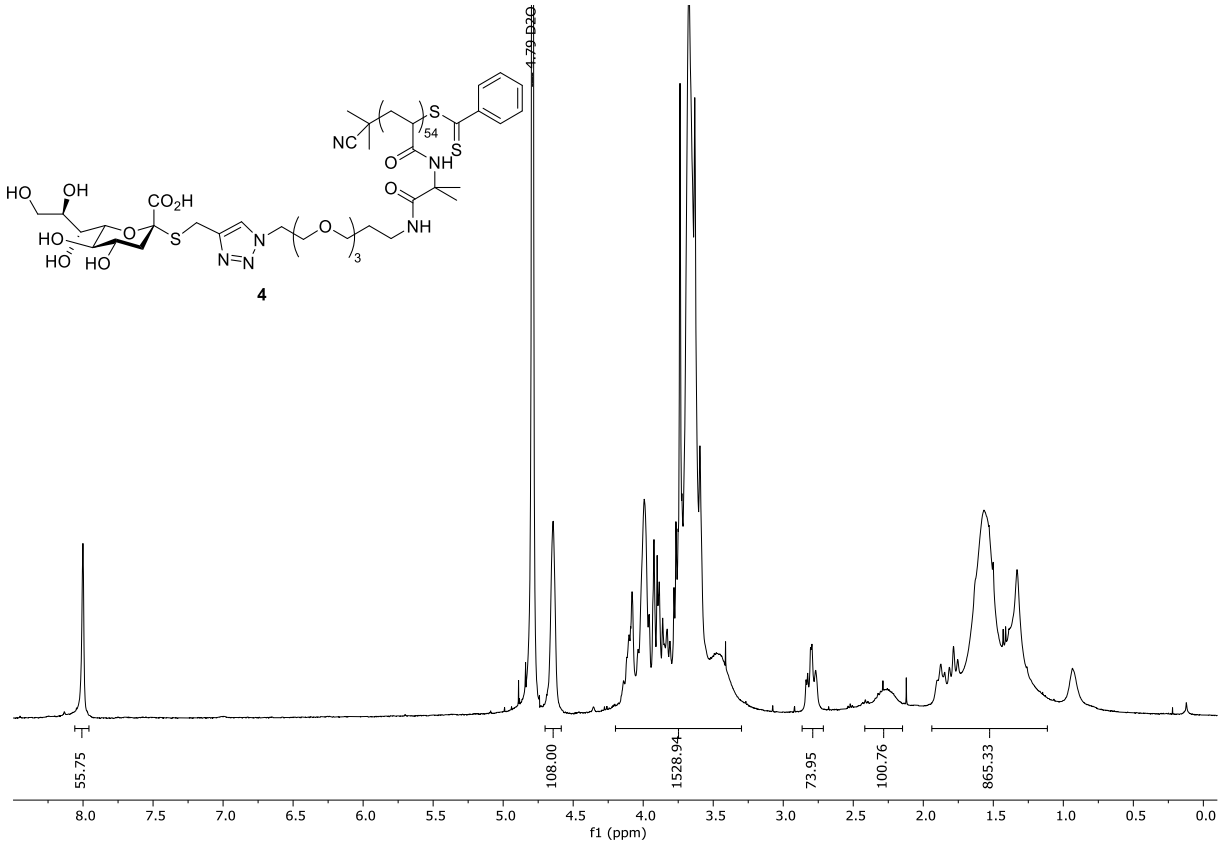

Figure S12. ^{1}H NMR spectrum of compound **4**.

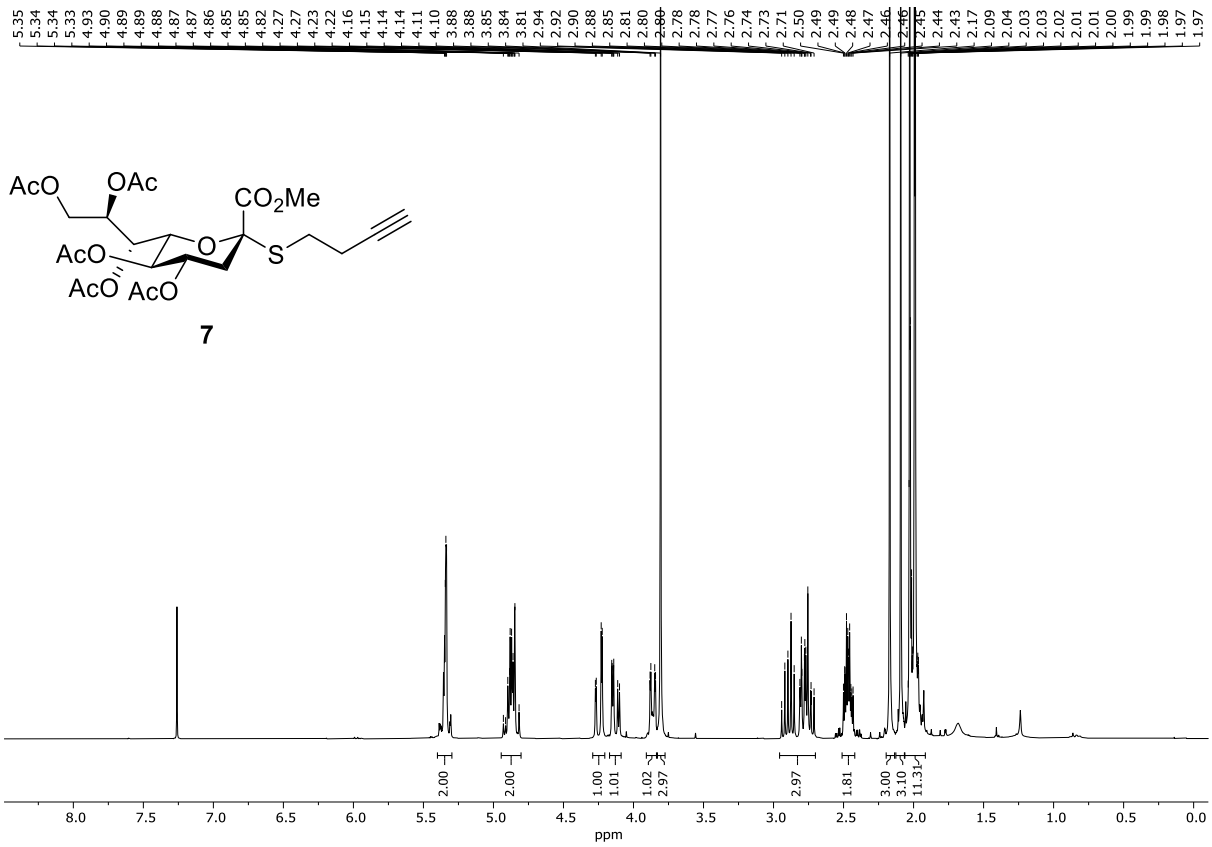

Figure S13. ^{1}H NMR spectrum of compound **7**.

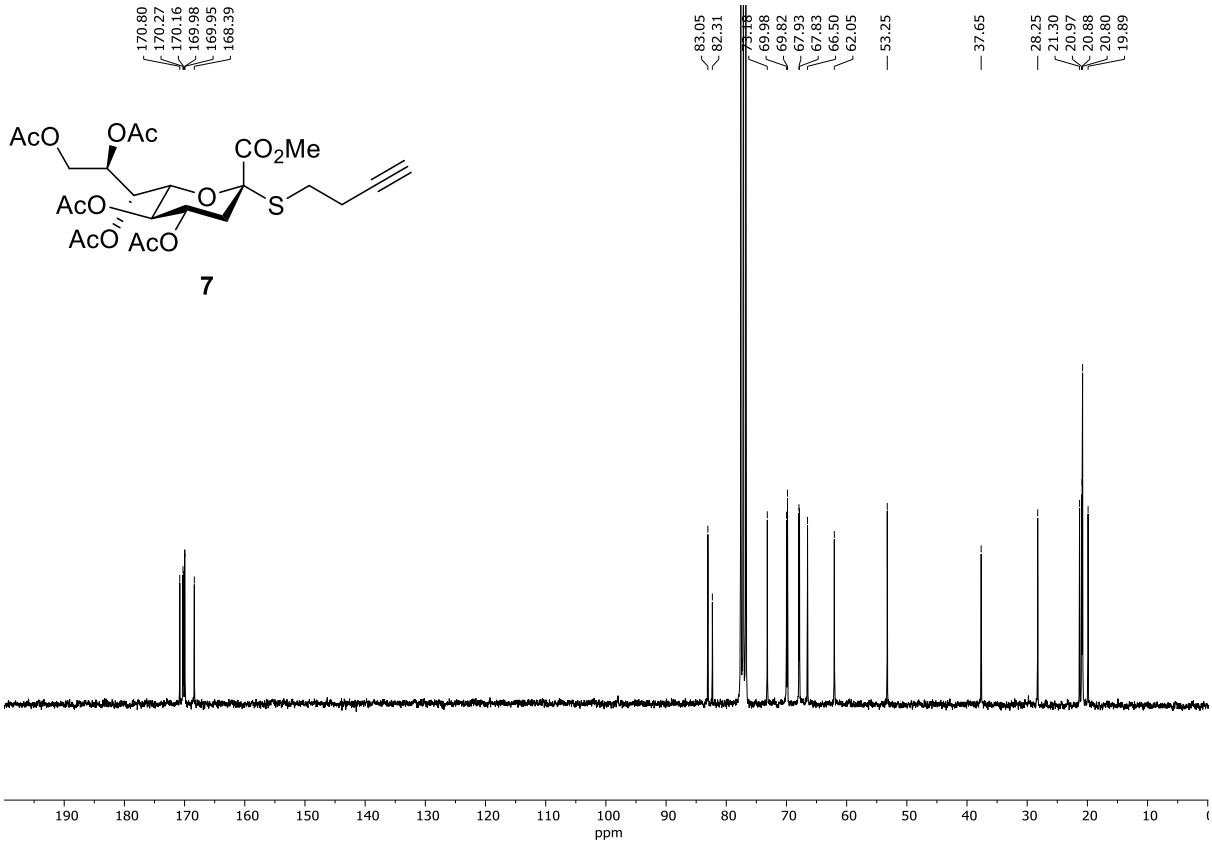

Figure S14. ^{13}C NMR spectrum of compound **7**.

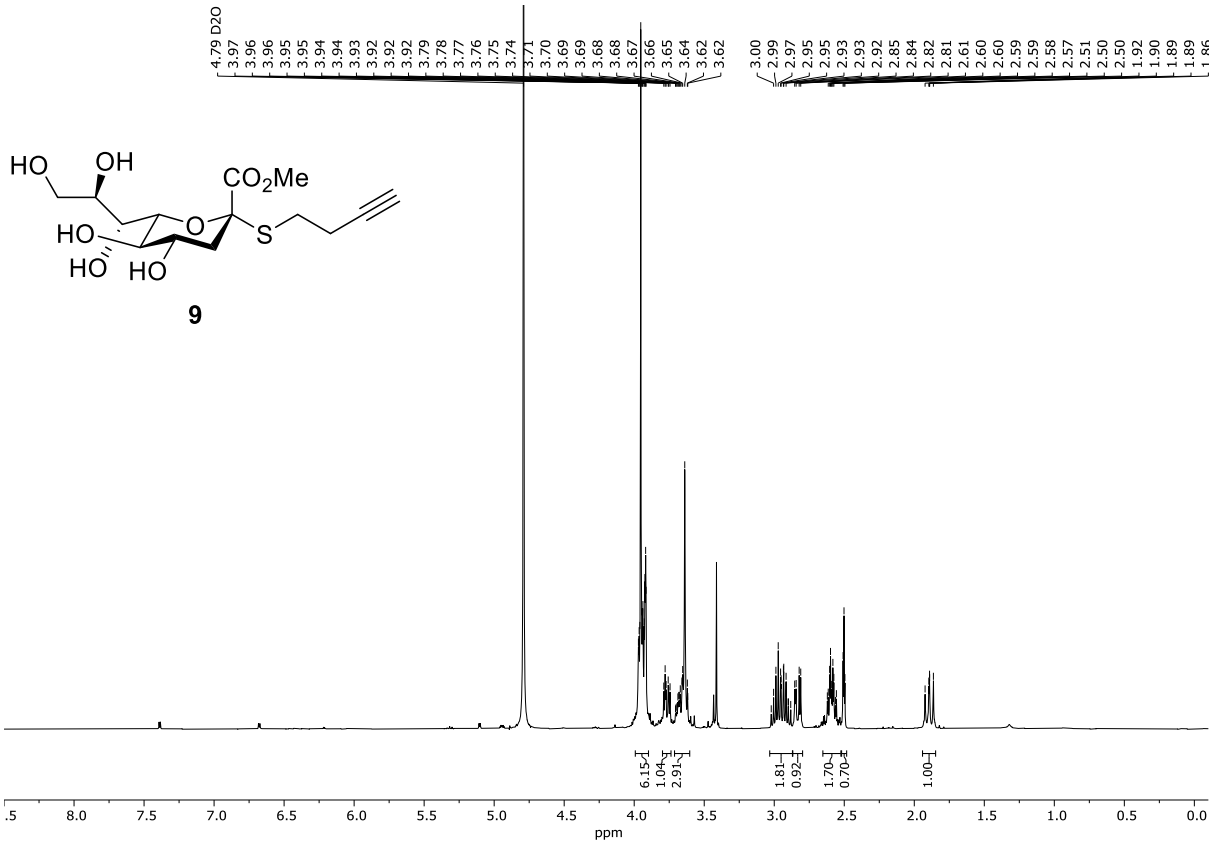

Figure S15. ^{1}H NMR spectrum of compound **9**.

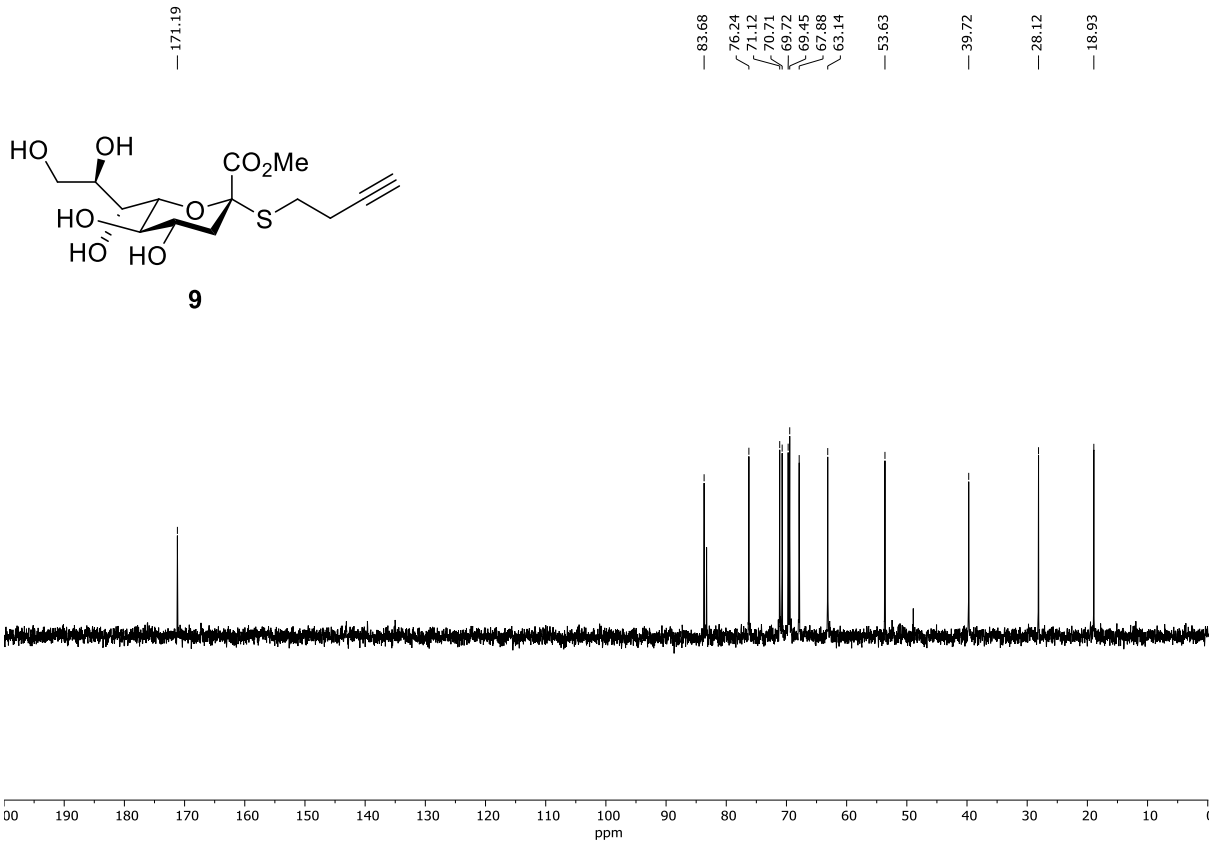

Figure S16. ^{13}C NMR spectrum of compound **9**.

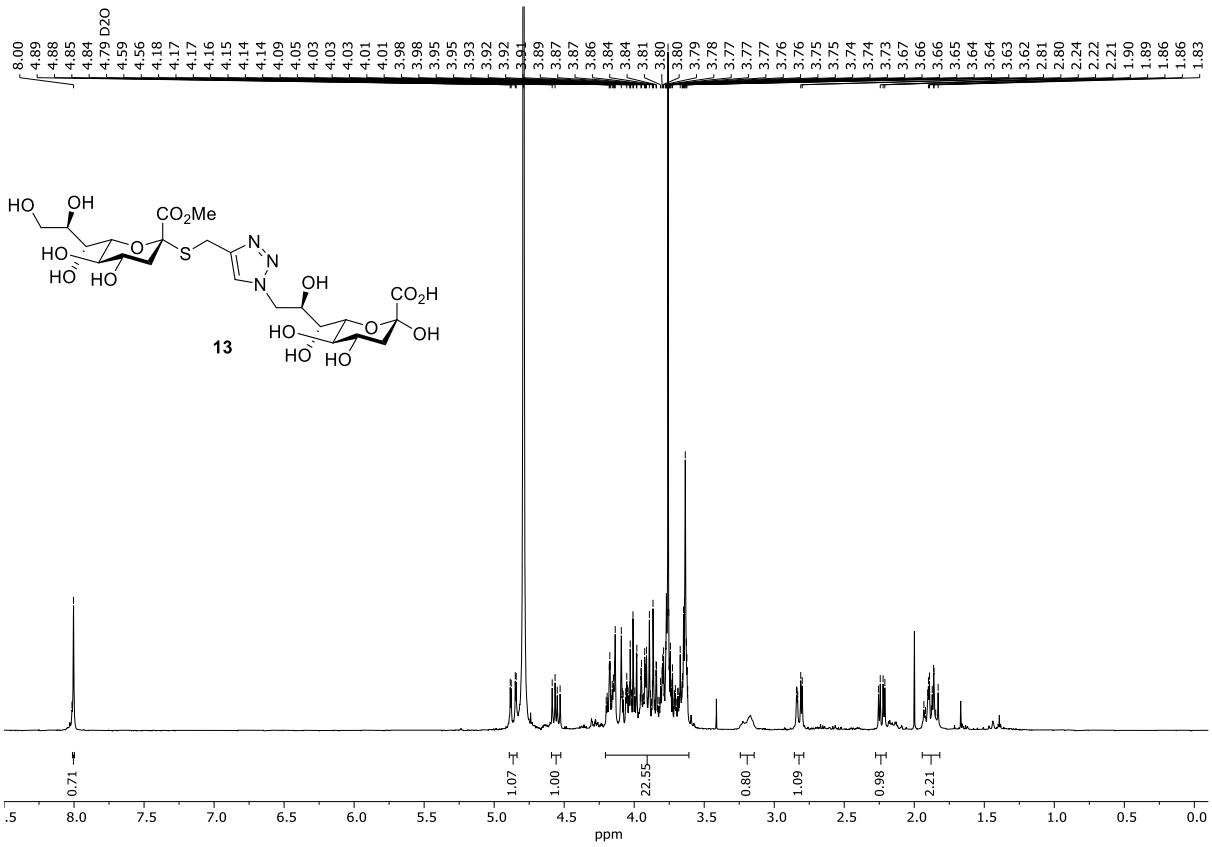

Figure S17. ^{1}H NMR spectrum of compound **13**.

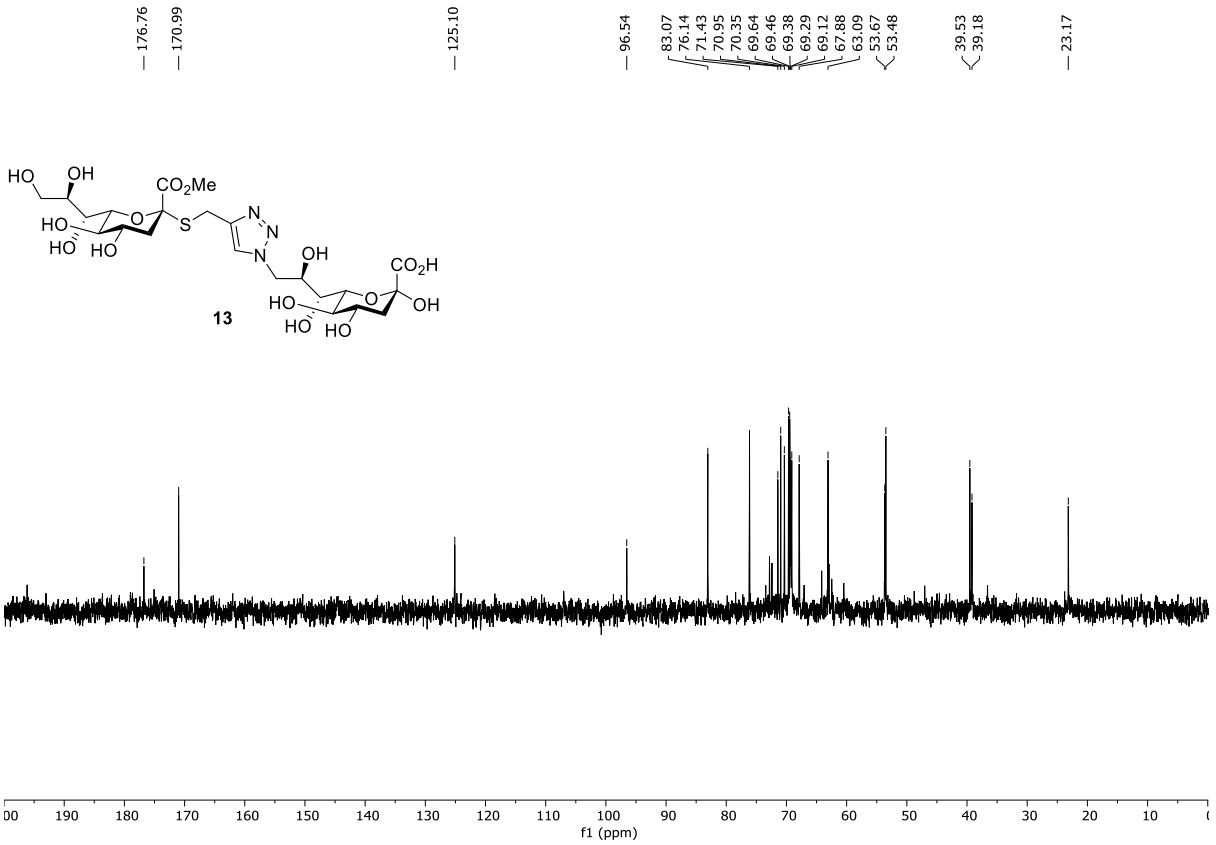

Figure S18. ^{13}C NMR spectrum of compound **13**.

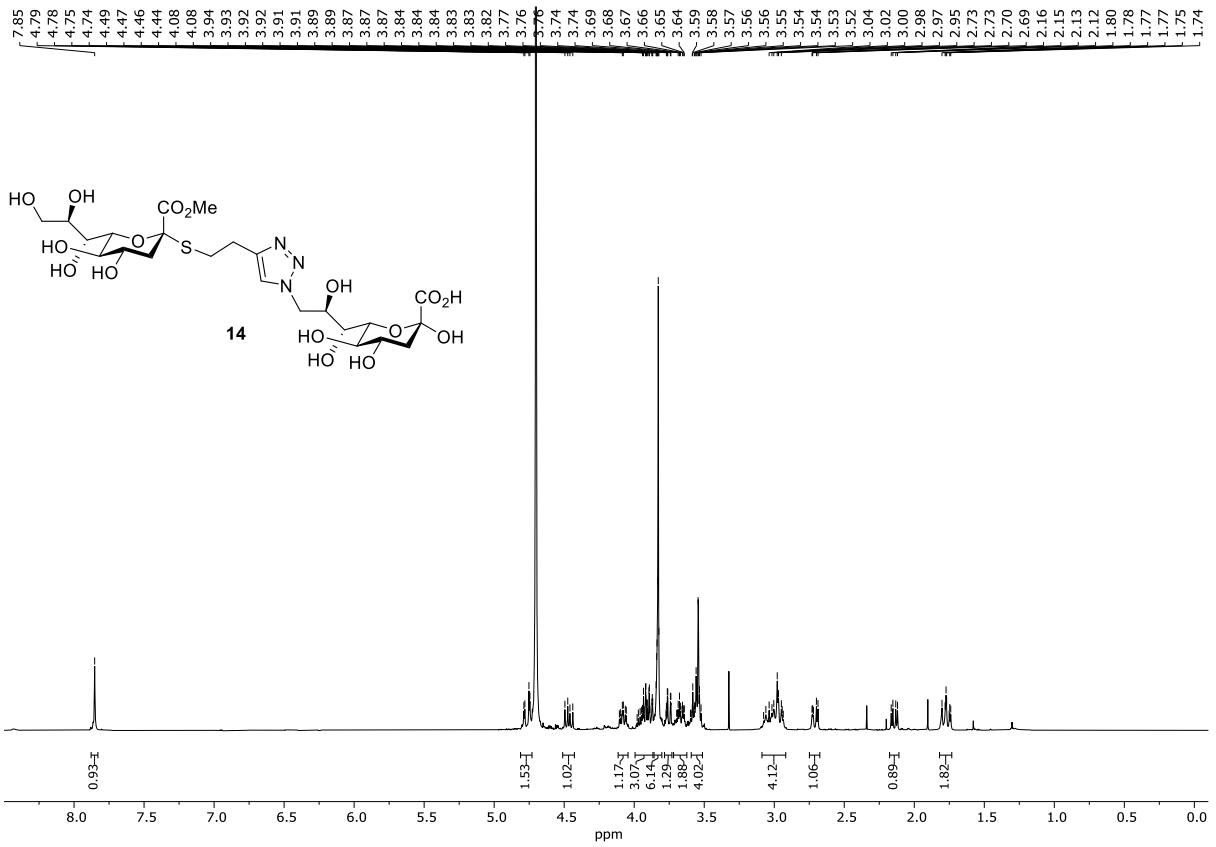

Figure S19. ^{1}H NMR spectrum of compound **14**.

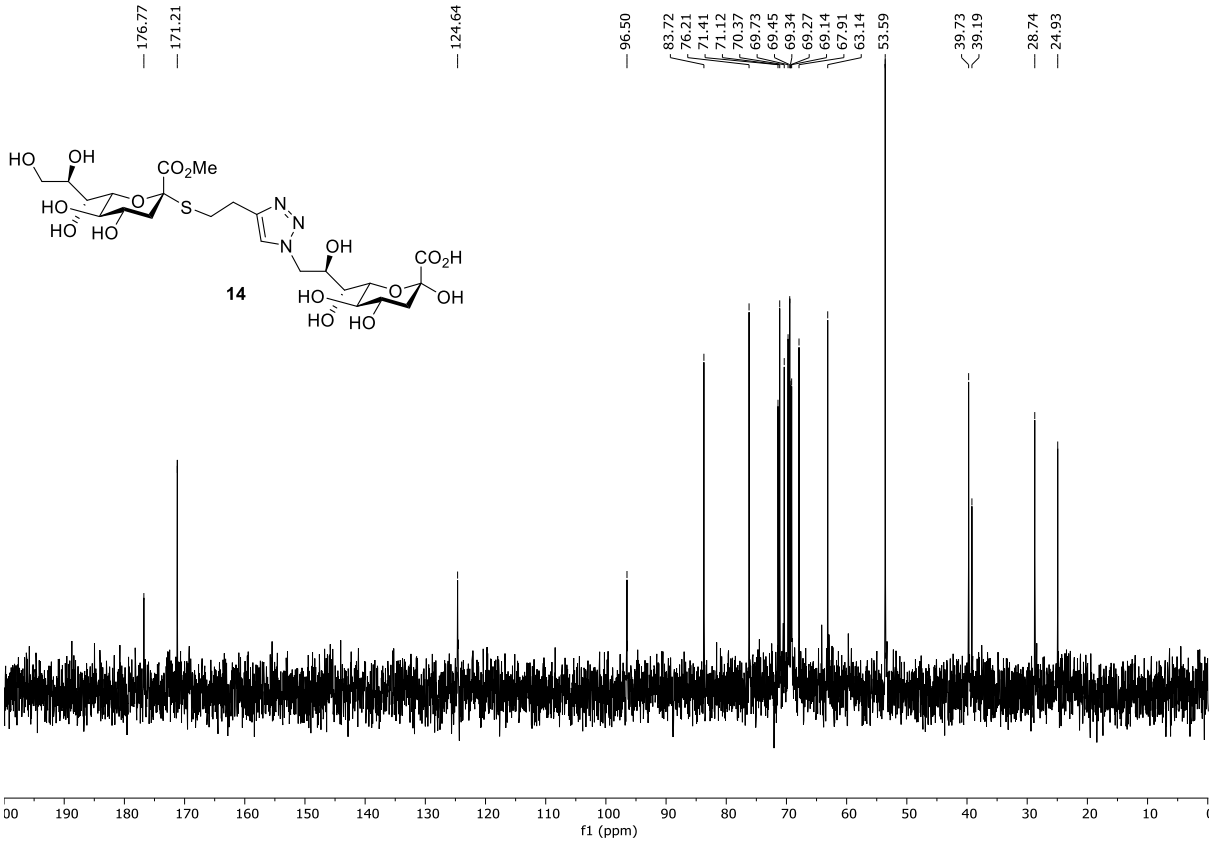

Figure S20. ^{13}C NMR spectrum of compound **14**.

5. FT-IR spectra

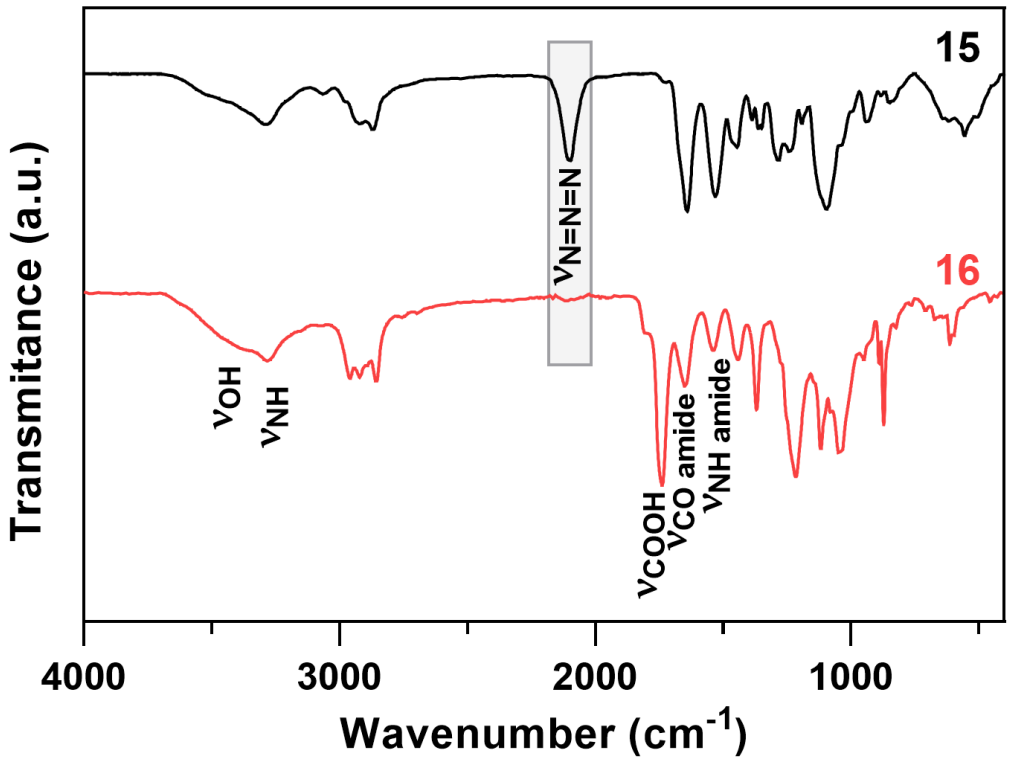

Figure S21. FT-IR spectra of polymer **15** and glycopolymer **16**. Grey rectangle corresponds to the zone of the characteristic vibration band due to the azide.