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Supporting Information

Terminal Alkenes as Versatile Chemical Reporter Groups for Metabolic Oligosaccharide Engineering

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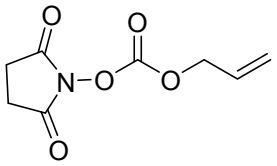
Content

General Methods

LC-MS analyses were conducted on a LCMS2020 instrument from *Shimadzu* (pumps LC-20 AD, autosampler SIL-20AT HAT, column oven CTO-20AC, UV-Vis detector SPD-20A, controller CBM-20, ESI detector and software LCMS-solution) with an EC 125/4 Nucleodur C18, 3 µM column (*Machery-Nagel*). A binary gradient of acetonitrile (with 0.1 % formic acid) in water (with 0.1 % formic acid) was used at a flow rate of 0.4 mL min^{-1}. Semi-preparative high performance liquid chromatography (HPLC) was conducted on a LC-20A prominence system (pumps LC-20AT, auto sampler SIL-20A, column oven CTO-20AC, diode array detector SPD-M20A, ELSD-LT II detector, controller CBM-20A and software LC-solution) from *Shimadzu*. For reversed-phase HPLC a Kinetex 5U C18 100A Axia column from *Phenomenex* (250 × 21.2 mm, flow 9 mL min^{-1}) was used as stationary phase and a gradient of acetonitrile (with 0.1 % formic acid) in water (with 0.1 % formic acid) was used as mobile phase.

Allyl succinimidyl carbonate 8

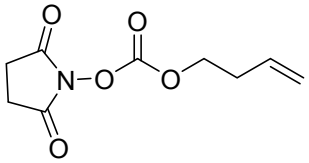


$C_8H_9NO_5$
199.16 g/mol

Disuccinimidyl carbonate (6 g, 25 mmol) was added to a solution of allyl alcohol (1 g, 17.2 mmol) and triethylamine (7.1 mL, 52.2 mmol) in acetonitrile (40 mL). The reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated and the crude product was purified by FC (CH_2Cl_2). The product was obtained as an oil (1.96 g, 57 %).

R_f=0.22 (CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$): δ=5.96 (ddt, *J*=17.2, 10.4, 5.9 Hz, 1H; CH), 5.56 – 5.30 (m, 2H; CHC$\underline{H}_2$), 4.79 (dt, *J*=5.9, 1.3 Hz, 2H; CH_2), 2.84 (s, 4H, 2x $COCH_2$) ppm. **^{13}C NMR:** (101 MHz, $CDCl_3$): δ=168.5, 151.4 (2x CO), 129.8 (CH), 120.8 (C$\underline{C}H_2$), 71.3 (CH_2), 25.5 (CO$\underline{C}H_2$) ppm. **HRMS**: *m/z* calcd for $C_8H_9NO_5$: 222.03729 [*M* + Na]$^+$, found: 222.03638.

But-3-en-1-yl succinimidyl carbonate 9

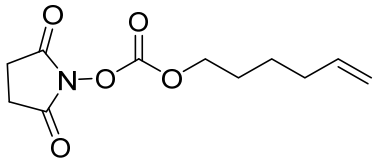


$C_9H_{11}NO_5$
213.19 g/mol

Disuccinimidyl carbonate (11.5 g, 46 mmol) was added to a solution of but-3-en-1-ol (2 g, 27 mmol) and triethylamine (11.36 g, 81 mmol) in acetonitrile (100 mL). The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the crude product was purified by FC (CH_2Cl_2). Succinimidyl but-3-en-1-yl carbonate was obtained as yellow oil (4.5 g, 78.5 %).

R_f=0.27 (petroleum ether/ethyl acetate 3:1). **^{1}H NMR:** (400 MHz, $CDCl_3$): δ=5.79 (ddt, *J*=17.1, 10.2, 6.8 Hz, 1H, C$\underline{H}$CH_2), 5.24 – 5.07 (m, 2H, CHC$\underline{H}_2$), 4.36 (t, *J*=6.8 Hz, 2H, OC$\underline{H}_2$), 2.83 (s, 4H, 2x $COCH_2$), 2.51 (qt, *J*=6.7, 1.3 Hz, 2H, $OCH_2C\underline{H}_2$) ppm. **^{13}C NMR:** (101 MHz, $CDCl_3$): δ=168.7 (CO), 132.4 (C$\underline{H}$CH_2), 118.7 (CH$\underline{C}H_2$), 70.4 (O$\underline{C}H_2$), 32.9 ($OCH_2\underline{C}H_2$), 25.6 (CO$\underline{C}H_2$) ppm. **HRMS**: *m/z* calcd for $C_9H_{11}NO_5$: 236.05294 [*M* + Na]$^+$, found: 236.05244.

Hex-5-en-1-yl succinimidyl carbonate 11

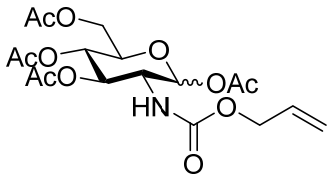


$C_{11}H_{15}NO_5$
241.24 g/mol

Disuccinimidyl carbonate (8.8 g, 34 mmol) was added to a solution of hex-5-en-1-ol (2 g, 20 mmol) and triethylamine (8.4 g, 60 mmol) in acetonitrile (100 mL). The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the crude product was purified by FC (petroleum ether/ethyl acetate 4:1). Succinimidyl hex-5-en-1-yl carbonate was obtained as colorless oil (3.3 g, 68 %).

R_f=0.28 (petroleum ether/ethyl acetate 3:1). **^{1}H NMR:** (400 MHz, $CDCl_3$): δ=5.79 (ddt, *J*=16.9, 10.2, 6.6 Hz, 1H, C<u>H</u>CH_2), 5.10 – 4.87 (m, 2H, CHC<u>H$_2$</u>), 4.33 (t, *J*=6.6 Hz, 2H, OC<u>H$_2$</u>), 2.83 (s, 4H, 2x $COCH_2$), 2.20 – 1.96 (m, 2H, OCH_2CH_2C<u>H$_2$</u>), 1.88 – 1.68 (m, 2H, OCH_2C<u>H$_2$</u>), 1.60 – 1.38 (m, 2H, $OCH_2CH_2CH_2C$<u>H$_2$</u>) ppm. **^{13}C NMR:** (101 MHz, $CDCl_3$): δ=168.8, 151.7 (2x CO), 138.0 (<u>C</u>HCH_2), 115.4 (CH<u>C</u>H_2), 71.6 (OC<u>H</u>$_2$), 33.2 (OCH_2CH_2<u>C</u>H_2), 27.9 (OCH_2<u>C</u>H_2), 25.6 (CO<u>C</u>H_2), 24.8 ($OCH_2CH_2CH_2$<u>C</u>H_2) ppm. **HRMS**: *m/z* calcd for $C_{11}H_{15}NO_5$: 264.08424 [*M* + Na]$^+$, found: 264.08385.

1,3,4,6-Tetra-*O*-acetyl-2-(allyloxycarbonylamino)-2-deoxy-D-glucopyranose (Ac_4GlcNAloc) 23

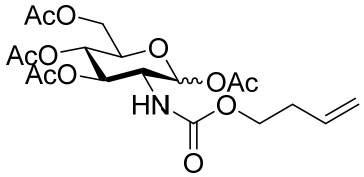


$C_{18}H_{25}NO_{11}$
431.39 g/mol

Glucosamine hydrochloride (663 mg, 3.07 mmol) and allyl succinimidyl carbonate **8** (640 mg, 3.2 mmol) were reacted in MeOH (25 mL) according to the general procedure. The crude product was purified by FC (petroleum ether/ethyl acetate 30-70 % in 15 min). To remove remaining *N*-hydroxysuccinimide (NHS), the combined product fractions were evaporated, redissolved in CH_2Cl_2 and extracted three times with 1 N NaOH. Ac_4ManNAloc **23** was obtained as mixture of anomers (0.81 g, 61 %, α/β 3:1).

R_f=0.29 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.20 (d, *J*=3.7 Hz, 1H, H-1), 5.88 (ddt, *J*=16.5, 11.1, 5.7 Hz, 1H, C<u>H</u>CH_2), 5.33 – 5.06 (m, 4H, CHC<u>H$_2$</u>, H-3, H-4), 4.82 (d, *J*=9.5 Hz, 1H, NH), 4.55 (qd, *J*=13.2, 5.6 Hz, 2H, CH_2), 4.31 – 4.23 (m, 1H, H-6a), 4.18 (td, *J*=10.2, 3.7 Hz, 1H, H-2), 4.06 (dd, *J*=12.4, 2.4 Hz, 1H, H-6b), 3.99 (ddd, *J*=9.9, 4.2, 2.4 Hz, 1H, H-5), 2.18 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.03 (s, 3H, CH_3) ppm. **β-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.88 (ddt, *J*=16.5, 11.1, 5.7 Hz, 1H, C<u>H</u>CH_2), 5.69 (d, *J*=8.7 Hz, 1H, H-1), 5.33 – 5.06 (m, 4H, CHC<u>H$_2$</u>, H-3, H-4), 4.82 (d, *J*=9.5 Hz, 1H, NH), 4.55 (qd, *J*=13.2, 5.6 Hz, 2H, CH_2), 4.31 – 4.23 (m, 1H, H-6a), 4.11 (dd, *J*=12.4, 2.2 Hz, 1H, H-6b), 3.92 (q, *J*=9.5 Hz, 1H, H-2), 3.80 (ddd, *J*=9.6, 4.7, 2.3 Hz, 1H, H-5), 2.11 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3) ppm. **α/β-Anomers:** ^{13}C NMR (101 MHz, $CDCl_3$): δ=171.4, 170.8, 169.3, 168.8, 155.5 (5x CO), 132.5 (<u>C</u>HCH_2), 118.3 (CH<u>C</u>H_2), 92.7 (C-1β), 90.9 (C-1α), 73.0 (C-5β), 72.4 (C-3β or C-4β), 70.8 (C-3α or C-4α), 69.8 (C-5α), 68.0 (C-3β or C-4β), 67.8 (C-3α or C-4α), 66.3 (CH_2α), 66.0 (CH_2β), 61.8 (C-6β), 61.7 (C-6α), 55.2 (C-2β), 53.0 (C-2α), 21.1 , 21.0, 20.9, 20.8, 20.8, 20.8, 20.7, 20.7 (8x CH_3) ppm; **HRMS**: *m/z* calcd for $C_{18}H_{25}NO_{11}$: 454.13198 [*M* + Na]$^+$, found: 454.13027.

1,3,4,6-Tetra-*O*-acetyl-2-(but-3-en-1-yl-oxycarbonylamino)-2-deoxy-D-glucopyranose (Ac_4GlcNBeoc) 24

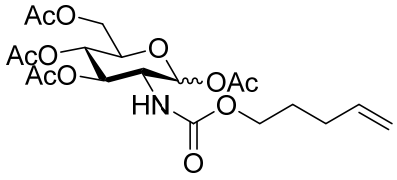


$C_{19}H_{27}NO_{11}$
445.42 g/mol

Glucosamine hydrochloride (780 mg, 3.61 mmol) and but-3-en-1-yl succinimidyl carbonate **9** (800 mg, 3.77 mmol) were reacted in MeOH (20 mL) according to the general procedure. The crude product was purified by FC (petroleum ether/ethyl acetate 30-70 % in 15 min). To remove remaining *N*-hydroxysuccinimide (NHS), the combined product fractions were evaporated, redissolved in CH_2Cl_2 and extracted three times with 1 N NaOH. Ac_4GlcNBeoc **24** was obtained as mixture of anomers (1 g, 62 %, α/β 3:1).

R_f=0.48 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.18 (d, *J*=3.7 Hz, 1H, H-1), 5.75 (dt, *J*=14.3, 5.4 Hz, 1H, C<u>H</u>CH_2), 5.25 – 5.13 (m, 2H, H-3, H-4), 5.12 – 5.01 (m, 2H, CHC<u>H</u>$_2$), 4.78 (d, *J*=9.5 Hz, 1H, NH), 4.26 (ddd, *J*=13.5, 9.1, 4.3 Hz, 1H, H-6a), 4.20 – 4.13 (m, 1H, H-2), 4.13 – 4.01 (m, 3H, H-6b, C<u>H</u>$_2$OCO), 3.98 (ddd, *J*=9.8, 4.2, 2.4 Hz, 1H, H-5), 2.34 (tt, *J*=9.0, 6.4, 5.2 Hz, 2H, C<u>H</u>$_2$CHCH_2), 2.17 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (d, *J*=1.1 Hz, 3H, CH_3) ppm. **β-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.75 (dt, *J*=14.3, 5.4 Hz, 1H, C<u>H</u>CH_2), 5.70 (s, 1H, H-1), 5.25 – 5.13 (m, 2H, H-3, H-4), 5.12 – 5.01 (m, 2H, CHC<u>H</u>$_2$), 4.84 (d, *J*=9.7 Hz, 1H, NH), 4.26 (ddd, *J*=13.5, 9.1, 4.3 Hz, 1H, H-6a), 4.13 – 4.01 (m, 3H, H-6b, C<u>H</u>$_2$OCO), 3.89 (d, *J*=9.9 Hz, 1H, H-2), 3.79 (ddd, *J*=9.8, 4.6, 2.2 Hz, 1H, H-5), 2.34 (tt, *J*=9.0, 6.4, 5.2 Hz, 2H, C<u>H</u>$_2$CHCH_2), 2.10 (s, 3H, CH3), 2.07 (s, 3H, CH_3), 2.02 (d, *J*=1.1 Hz, 3H, CH_3), 2.02 (s, 3H, CH_3) ppm. **α/β-Anomers:** ^{13}C NMR (101 MHz, $CDCl_3$): δ=171.4, 170.8, 170.7, 169.5, 169.4, 169.3, 168.8, 155.9, 155.8 (5x COα,β), 134.0 (<u>C</u>HCH_2), 117.4 (CH<u>C</u>H_2), 92.6 (C-1β), 90.9 (C-1α), 72.9, 72.4, 70.7, 69.8, 68.1, 67.8 (C-3α,β, C-4α,β, C-5α,β), 64.7 (<u>C</u>H_2OCO), 61.8 (C-6β), 61.7 (C-6α), 55.0 (C-2β), 52.9 (C-2α), 33.4 (<u>C</u>H_2CHCH_2), 21.0 (CH_3α), 21.0, 20.8 (2x CH_3β), 20.8, 20.8 (2x CH_3α), 20.7, 20.7 (2x CH_3β), 20.7 (CH_3α) ppm; **HRMS**: *m/z* calcd for $C_{19}H_{27}NO_{11}$: 468.14763 [*M* + Na]$^+$, found: 468.14524.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(pent-4-en-1-yl-oxycarbonylamino)-D-glucopyranose (Ac_4GlcNPeoc) 25



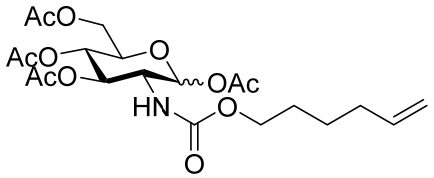
$C_{20}H_{29}NO_{11}$
459.44 g/mol

Glucosamine hydrochloride (700 mg, 3.23 mmol) and pent-4-en-1-yl succinimidyl carbonate **10**[1] (770 mg, 3.43 mmol) were reacted in MeOH (25 mL) according to the general procedure. The crude product was purified by FC (petroleum ether/ethyl acetate 30-70 % in 15 min). To remove remaining *N*-hydroxysuccinimide (NHS), the combined product fractions were evaporated, redissolved in CH_2Cl_2 and extracted three times with 1 N NaOH. Ac_4GlcNPeoc **25** was obtained as mixture of anomers (0.9 g, 61 %, α/β 4:1).

[1] A. Niederwieser, A.-K. Späte, L. D. Nguyen, C. Jüngst, W. Reutter, V. Wittmann, *Angew. Chem., Int. Ed.* **2013**, *52*, 4265-4268.

R_f=0.31 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (d, *J*=3.7 Hz, 1H, H-1), 5.78 (ddt, *J*=16.9, 10.1, 6.6 Hz, 1H, C<u>H</u>CH_2), 5.29 – 5.11 (m, 2H, H-3, H-4), 5.07 – 4.91 (m, 2H, CHC<u>H</u>$_2$), 4.73 (d, *J*=9.5 Hz, 1H, NH), 4.27 (ddd, *J*=12.8, 9.0, 4.4 Hz, 1H, H-6a), 4.18 (td, *J*=9.9, 3.6 Hz, 1H, H-2), 4.14 – 4.02 (m, 3H, H-6b, C<u>H</u>$_2$OCO), 3.99 (ddd, *J*=9.6, 4.2, 2.4 Hz, 1H, H-5), 2.19 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.10 – 2.06 (m, 2H, C<u>H</u>$_2$CHCH$_2$), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.76 – 1.63 (m, 2H, C<u>H</u>$_2$CH$_2$CHCH$_2$) ppm. **β-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.78 (ddt, *J*=16.9, 10.1, 6.6 Hz, 1H, C<u>H</u>CH_2), 5.69 (d, *J*=8.7 Hz, 1H, H-1), 5.29 – 5.11 (m, 2H, H-3, H-4), 5.07 – 4.91 (m, 2H, CHC<u>H</u>$_2$), 4.62 (s, 1H, NH), 4.27 (ddd, *J*=12.8, 9.0, 4.4 Hz, 1H, H-6a), 4.14 – 4.02 (m, 3H, H-6b, CH_2OCO), 3.90 (d, *J*=10.1 Hz, 1H, C-2), 3.80 (ddd, *J*=9.6, 4.6, 2.3 Hz, 1H, C-5), 2.12 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.10 – 2.06 (m, 2H, C<u>H</u>$_2$CHCH$_2$), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.76 – 1.63 (m, 2H, C<u>H</u>$_2$CH$_2$CHCH$_2$) ppm. **α/β-Anomers:** ^{13}C NMR (101 MHz, $CDCl_3$): δ=171.4, 170.8, 169.3, 168.8, 155.9 (5x CO), 137.5 (<u>C</u>HCH$_2$), 115.5 (CH<u>C</u>H$_2$), 92.8 (C-1β), 91.0 (C-1α), 73.0, 72.5, 70.8, 69.8, 68.0, 67.8 (C-3α,β, C-4α,β, C-5α,β), 65.2 (<u>C</u>H$_2$OCO), 61.8 (C-6β), 61.7 (C-6α), 53.0 (C-2α,β), 30.0 (<u>C</u>H$_2$CHCH$_2$), 28.2 (<u>C</u>H$_2$CH$_2$CHCH$_2$), 21.1 (CH_3α), 21.0, 20.9 (2x CH_3β), 20.8, 20.8 (2x CH_3α), 20.7, 20.7 (2x CH_3β), 20.7 (CH_3α) ppm; **HRMS**: *m/z* calcd for $C_{20}H_{29}NO_{11}$: 482.16328 [*M* + Na]$^+$, found: 482.16164.

1,3,4,6-Tetra-*O*-acetyl-2-desoxy-2-(hex-5-en-1-yl-oxycarbonylamino)-D-glucopyranose (Ac$_4$GlcNHeoc) 26

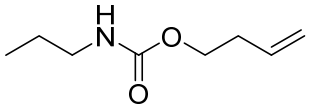


$C_{21}H_{31}NO_{11}$
473.47 g/mol

Glucosamine hydrochloride (1 g, 4.33 mmol) and hex-5-en-1-yl succinimidyl carbonate **11** (1.1 g, 4.58 mmol) were reacted in MeOH (25 mL) according to the general procedure. The crude product was purified by FC (petroleum ether/ethyl acetate 30-70 % in 15 min). To remove remaining *N*-hydroxysuccinimide (NHS), the combined product fractions were evaporated, redissolved in CH_2Cl_2 and extracted three times with 1 N NaOH. Ac$_4$GlcNHeoc **26** was obtained as mixture of anomers (1.63 g, 80 %, α/β 3:1).

R_f=0.37 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.18 (d, *J*=3.7 Hz, 1H, H-1), 5.77 (dddd, *J*=13.4, 10.5, 8.4, 5.9 Hz, 1H, C<u>H</u>CH_2), 5.28 – 5.06 (m, 2H, H-3, H-4), 5.07 – 4.88 (m, 2H, CHC<u>H</u>$_2$), 4.74 (d, *J*=9.7 Hz, 1H, NH), 4.26 (ddd, *J*=13.1, 9.3, 4.4 Hz, 1H, H-6a), 4.21 – 4.13 (m, 1H, H-2), 4.13 – 3.95 (m, 4H, H-5, H-6b, CH_2OCO), 2.17 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.07 – 2.04 (m, 2H, C<u>H</u>$_2$CHCH$_2$), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.69 – 1.51 (m, 2H, C<u>H</u>$_2$CH$_2$OCO), 1.50 – 1.31 (m, 2H, C<u>H</u>$_2$CH$_2$CHCH$_2$) ppm. **β-Anomer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=5.77 (dddd, *J*=13.4, 10.5, 8.4, 5.9 Hz, 1H, C<u>H</u>CH_2), 5.68 (d, *J*=8.7 Hz, 1H, H-1), 5.28 – 5.06 (m, 2H, H-3, H-4), 5.07 – 4.88 (m, 2H, CHC<u>H</u>$_2$), 4.74 (d, *J*=9.7 Hz, 1H, NH), 4.26 (ddd, *J*=13.1, 9.3, 4.4 Hz, 1H, H-6a), 4.13 – 3.95 (m, 3H, H-6b, CH_2OCO), 3.90 (d, *J*=9.6 Hz, 1H, H-2), 3.79 (ddd, *J*=9.6, 4.6, 2.2 Hz, 1H, H-5), 2.11 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.07 – 2.04 (m, 2H, C<u>H</u>$_2$CHCH$_2$), 2.02 (s, 6H, 2x CH_3), 1.69 – 1.51 (m, 2H, C<u>H</u>$_2$CH$_2$OCO), 1.50 – 1.31 (m, 2H, C<u>H</u>$_2$CH$_2$CHCH$_2$) ppm. **α/β-Anomers:** ^{13}C NMR (101 MHz, $CDCl_3$): δ=171.4, 170.8, 170.7, 169.5, 169.3, 168.8, 156.1, 156.0 (5x COα,β), 138.4 (<u>C</u>HCH$_2$), 115.0 (CH<u>C</u>H$_2$), 92.7 (C-1β), 91.0 (C-1α), 73.0, 72.5, 70.8, 69.8, 68.0, 67.8 (C-3α,β, C-4α,β, C-5α,β), 65.6 (<u>C</u>H$_2$OCO), 61.8 (C-6β), 61.7 (C-6α), 55.0 (C-2β), 52.9 (C-2α), 33.4 (<u>C</u>H$_2$CHCH$_2$), 28.5 (<u>C</u>H$_2$CH$_2$OCO β), 28.4 (<u>C</u>H$_2$CH$_2$OCOα), 25.1 (<u>C</u>H$_2$CH$_2$CHCH$_2$), 21.0 (CH_3α), 21.0, 20.8 (2x CH_3β), 20.8, 20.8 (2x CH_3α), 20.7, 20.7 (2x CH_3β), 20.7 (CH_3α) ppm; **HRMS**: *m/z* calcd for $C_{21}H_{31}NO_{11}$: 496.17893 [*M* + Na]$^+$, found: 496.17678.

But-3-en-1-yl propylcarbamate 17

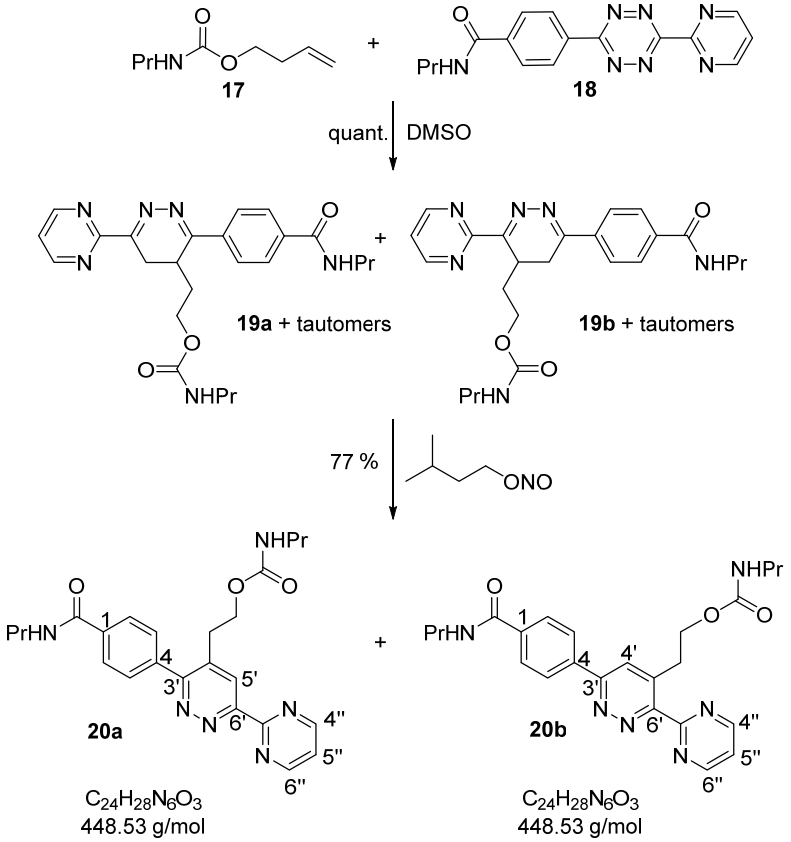


$C_8H_{15}NO_2$
157.21 g/mol

A mixture of but-3-en-1-ol (1 g, 13 mmol) and *n*-propyl isocyanate (1.58 mL, 16.6 mmol) in THF (13 mL) was refluxed for 3.5 h and then stirred for 72 h at room temperature. The solvent was evaporated and the crude product was purified by FC (petroleum ether/ethyl acetate 1:9). Carbamate **17** was obtained as colorless oil (2.54 g, 96 %).

R_f=0.86 (petroleum ether/ethyl acetate 1:1). **^{1}H NMR:** (400 MHz, $CDCl_3$): δ=5.78 (ddt, *J*=17.0, 9.9, 6.7 Hz, 1H, C<u>H</u>CH_2), 5.18 – 4.95 (m, 2H, CH<u>CH_2</u>), 4.69 (s, 1H, NH), 4.09 (t, *J*=6.7 Hz, 2H,O<u>CH_2</u>), 3.12 (q, *J*=6.8 Hz, 2H, CH_3CH_2<u>CH_2</u>), 2.36 (q, *J*=6.8 Hz, 2H, OCH_2<u>CH_2</u>), 1.50 (h, *J*=7.3 Hz, 2H, CH_3<u>CH_2</u>), 0.90 (td, *J*=7.4, 1.0 Hz, 3H, <u>CH_3</u>) ppm. **^{13}C NMR:** (101 MHz, $CDCl_3$): δ=156.7 (NHCO), 134.5 (<u>C</u>HCH_2), 117.1 (CH<u>C</u>H_2), 63.9 (O<u>C</u>H_2), 42.8 (CH_3CH_2<u>C</u>H_2), 33.7 (OCH_2<u>C</u>H_2), 23.4 (CH_3<u>C</u>H_2), 11.3 (<u>C</u>H_3) ppm.

DAinv reaction in preparative scale



Tetrazine **18** (90 mg, 0.28 mmol) was dissolved in DMSO (10 mL) and a solution of but-3-en-1-yl propyl carbamate **17** (50.7 mg, 0.32 mmol) in DMSO (0.8 mL) was added. The reaction was stirred overnight and solvents were removed. The residue was dissolved in glacial acetic acid (7 mL) and amyl nitrite (40.3 mg, 0.34 mmol) was added. After stirring at rt for 48 h, the solvents were removed and the residue was purified by FC (5 % MeOH in CH_2Cl_2) to afford a mixture of isomers **20a** and **20b** in 77 %. Isomers could be separated by HPLC (30-60 % MeCN in H_2O containing 0.1 % formic acid in 30 min, R_t **20a** = 13.5 min, R_t **20b** = 15 min).

R_f=0.88 (CH_2Cl_2/MeOH 10:1). **20a:** ^{1}H NMR (400 MHz, $CDCl_3$): δ=9.00 (d, *J*=4.9 Hz, 2H, H-4'' and H-6''), 8.56 (s, 1H, H-5'), 7.92 (d, *J*=7.9 Hz, 2H, H-2 and H-6 or H-2 and H-3), 7.70 (d, *J*=7.9 Hz, 2H, H-2 and H-6 or H-2 and H-3), 7.43 (t, *J*=4.9 Hz, 1H, H-5''), 6.20 (s, 1H, NHaryl), 4.58 (s, 1H, NHalkyl), 4.29 (t, *J*=6.5 Hz, 2H, $C\underline{H}_2CH_2OCO$ or $CH_2C\underline{H}_2OCO$), 3.48 (q, *J*=6.7 Hz, 2H, $CCONHC\underline{H}_2$), 3.08 (p, *J*=6.3 Hz, 4H, $C\underline{H}_2CH_2OCO$ or $CH_2C\underline{H}_2OCO$ and $OCONHC\underline{H}_2$), 1.69 (q, *J*=7.3 Hz, 2H, $CCONHCH_2C\underline{H}_2$), 1.46 (q, *J*=7.2 Hz, 2H, $OCONHCH_2C\underline{H}_2$), 1.02 (t, *J*=7.4 Hz, 3H, $CCONHCH_2CH_2C\underline{H}_3$), 0.87 (t, *J*=7.4 Hz, 3H, $OCONHCH_2CH_2C\underline{H}_3$) ppm. **20b:** ^{1}H NMR (600 MHz, $CDCl_3$): δ=8.98 (d, *J*=4.9 Hz, 2H, H-4'' and H-6''), 8.25 (d, *J*=8.2 Hz, 2H, H-2 and H-6 or H-2 and H-3), 7.94 (d, *J*=8.4 Hz, 2H, H-2 and H-6 or H-2 and H-3), 7.89 (s, 1H, H-4'), 7.44 (t, *J*=4.9 Hz, 1H, H-5''), 6.19 (s, 1H, NHaryl), 4.61 (s, 1H, NHalkyl), 4.37 (t, *J*=6.5 Hz, 2H, $C\underline{H}_2CH_2OCO$ or $CH_2C\underline{H}_2OCO$), 3.48 (q, *J*=6.7 Hz, 2H, $CCONHC\underline{H}_2$), 3.33 (t, *J*=6.4 Hz, 2H, $C\underline{H}_2CH_2OCO$ or $CH_2C\underline{H}_2OCO$), 3.08 (q, *J*=6.8 Hz, 2H, $OCONHC\underline{H}_2$), 1.69 (q, *J*=7.3 Hz, 2H, $CCONHCH_2C\underline{H}_2$), 1.47 (q, *J*=7.3 Hz, 2H, $OCONHCH_2C\underline{H}_2$), 1.03 (t, *J*=7.4 Hz, 3H, $CCONHCH_2CH_2C\underline{H}_3$), 0.88 (t, *J*=7.4 Hz, 3H, $OCONHCH_2CH_2C\underline{H}_3$) ppm. **20a/b:** ^{13}C NMR (101 MHz, $CDCl_3$): δ=167.1, 162.0, 161.9 (C_{quart}), 157.9 (C-4'' and C-6''), 139.5, 136.8, 135.6 (C_{quart}), 129.6, 127.4, 127.2 (C-2, C-3, C-5, C-6, C-5'), 121.2 (C-5''), 62.7 ($\underline{C}H_2CH_2OCO$ or $CH_2\underline{C}H_2OCO$), 42.7 ($CCONH\underline{C}H_2$), 41.9 ($OCONH\underline{C}H_2$), 31.6 ($\underline{C}H_2CH_2OCO$ or $CH_2\underline{C}H_2OCO$), 23.1, 22.9 ($CCONHCH_2\underline{C}H$, $OCONHCH_2\underline{C}H_2$), 11.5, 11.1 ($OCONHCH_2CH_2\underline{C}H_3$, $CCONHCH_2CH_2\underline{C}H_3$) ppm.

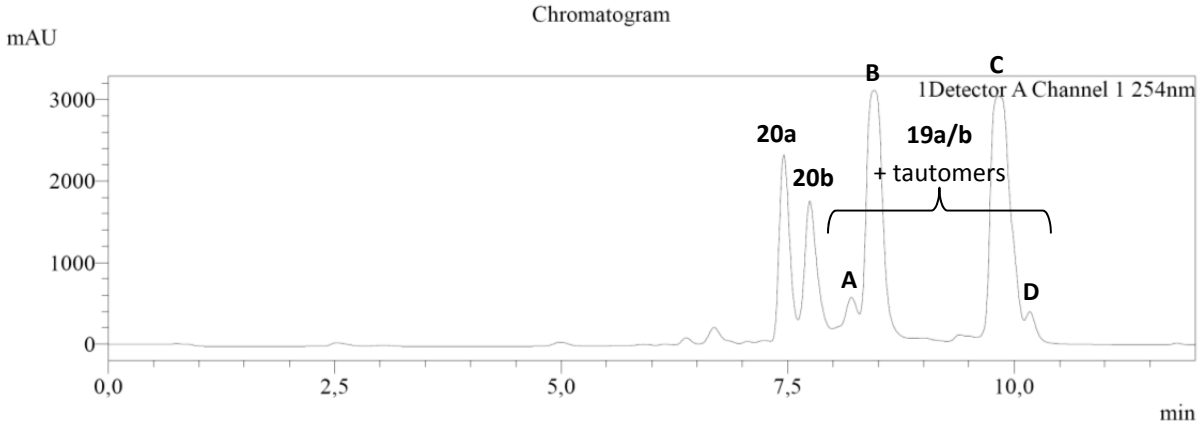


Figure S1: LC-MS analysis of crude reaction mixture of reaction of **17** with **18**. The four peaks A, B, C, D correspond to **19a/b** and the tautomeres. The first two peaks correspond to in situ-formed oxidation products (**20a/b**). Conditions: Binary gradient of CH_3CN in H_2O with 0.1 % formic acid (20-90 % in 10 min).

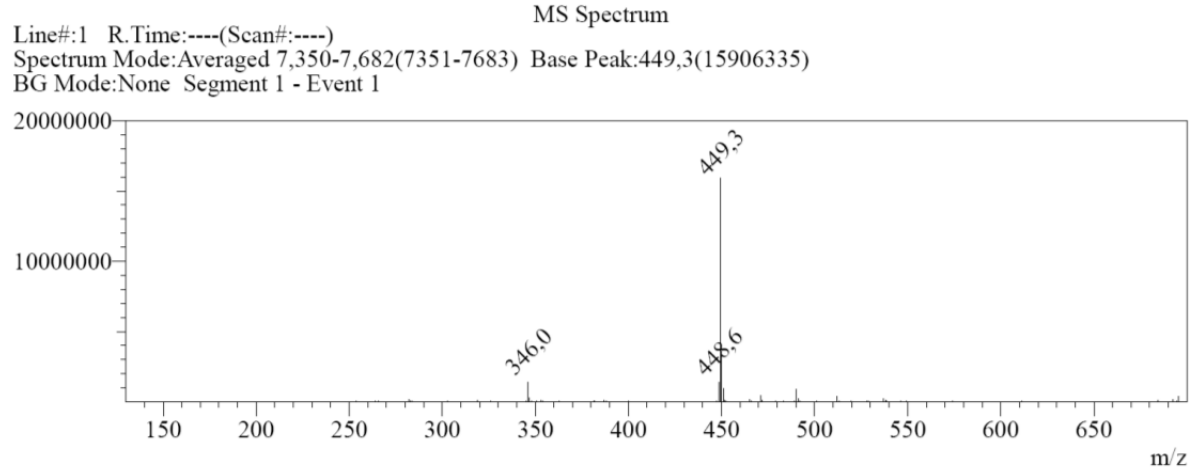


Figure S2: Mass spectrum of the first peak (**20a**) of the chromatogram shown in Figure S1.

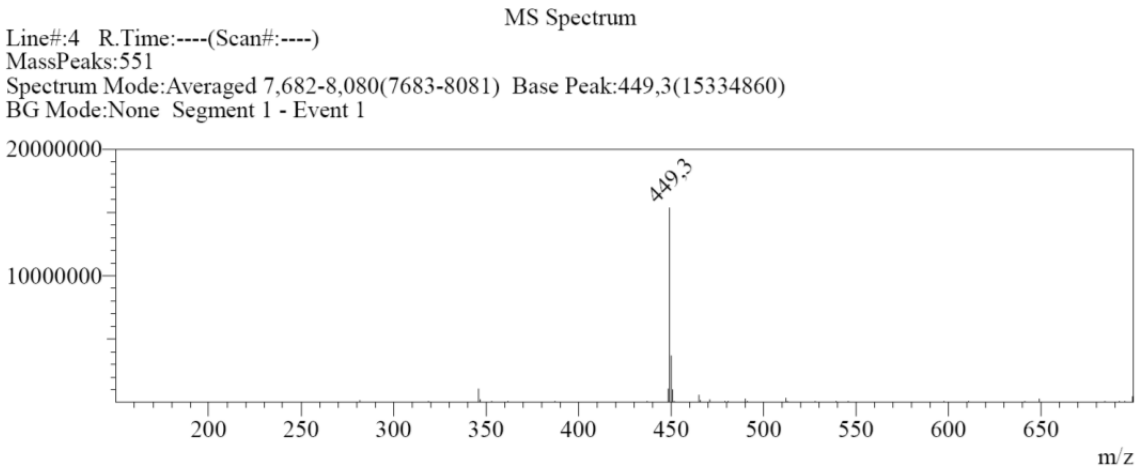


Figure S3: Mass spectrum of the second peak (**20b**) of the chromatogram shown in Figure S1.

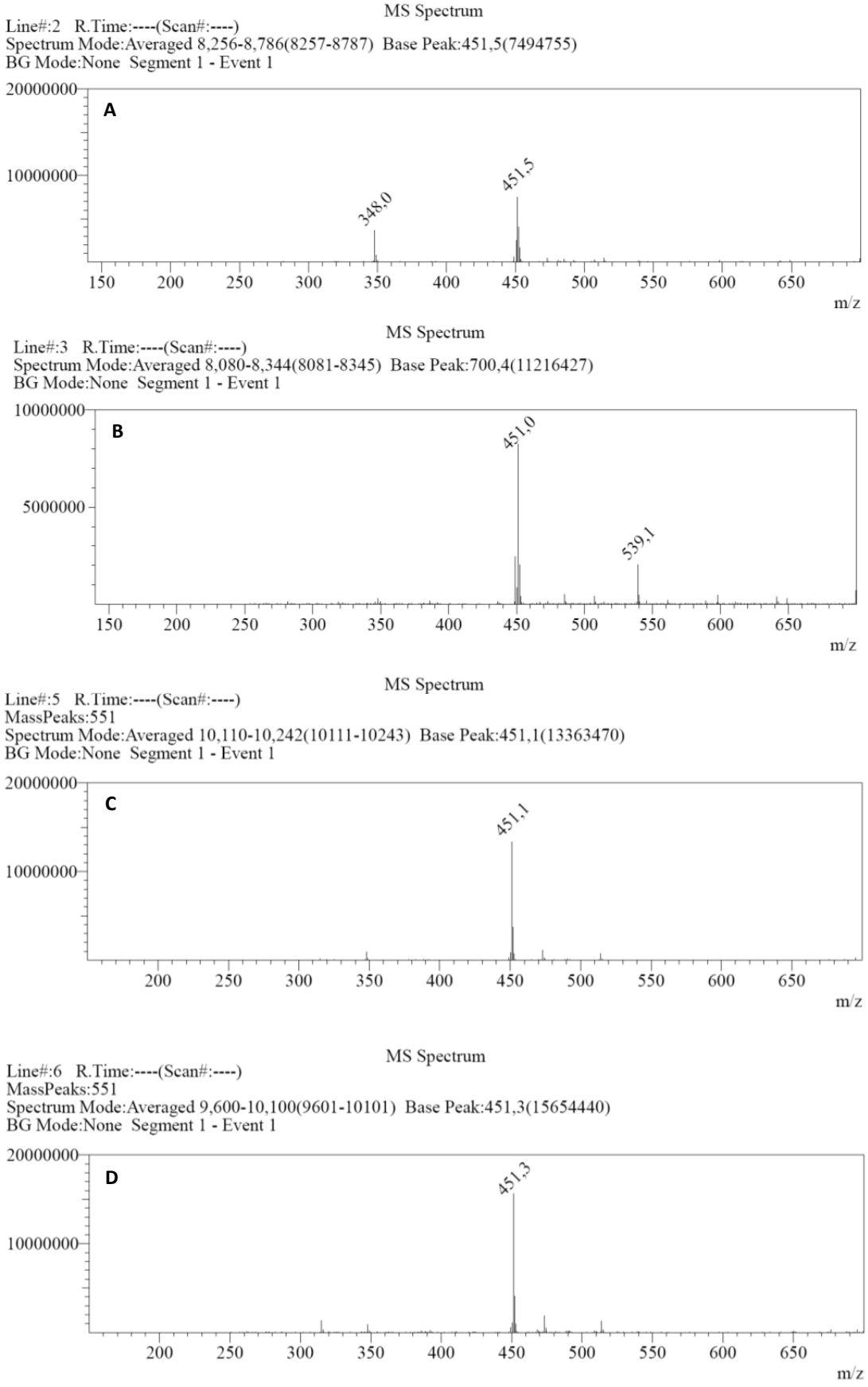
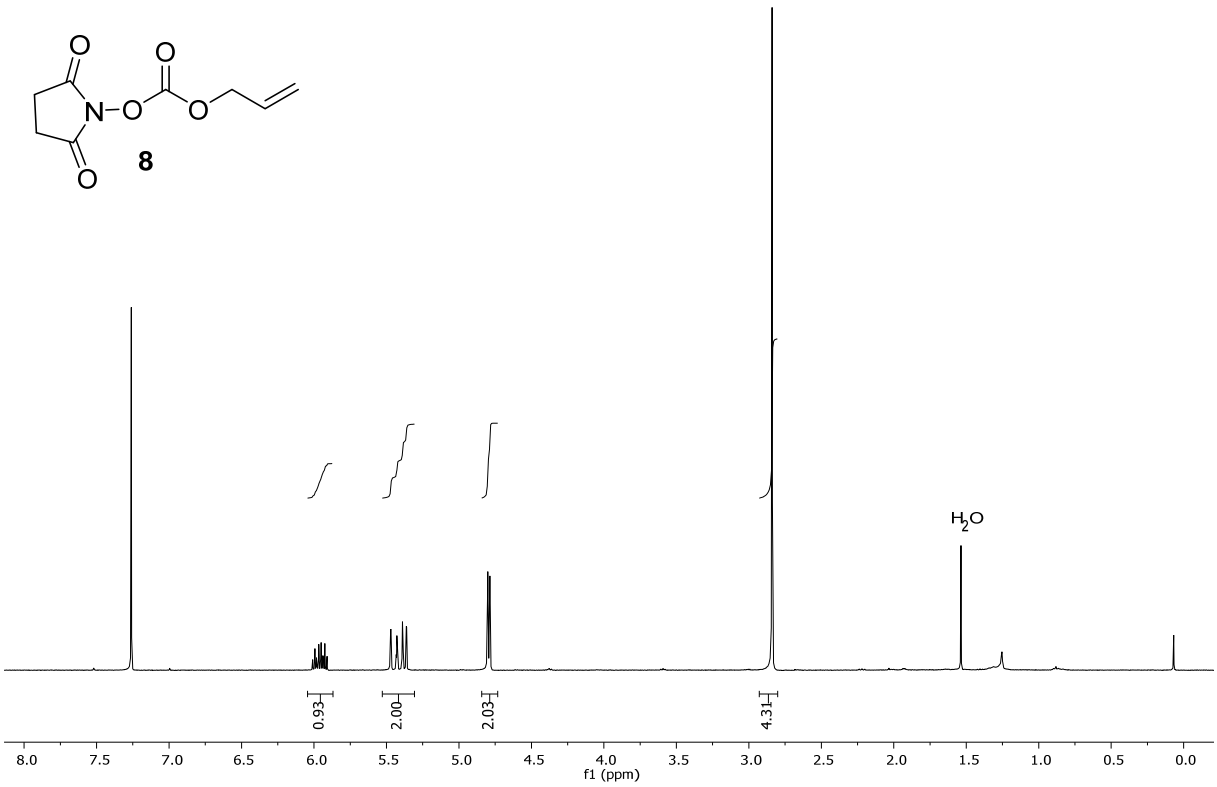
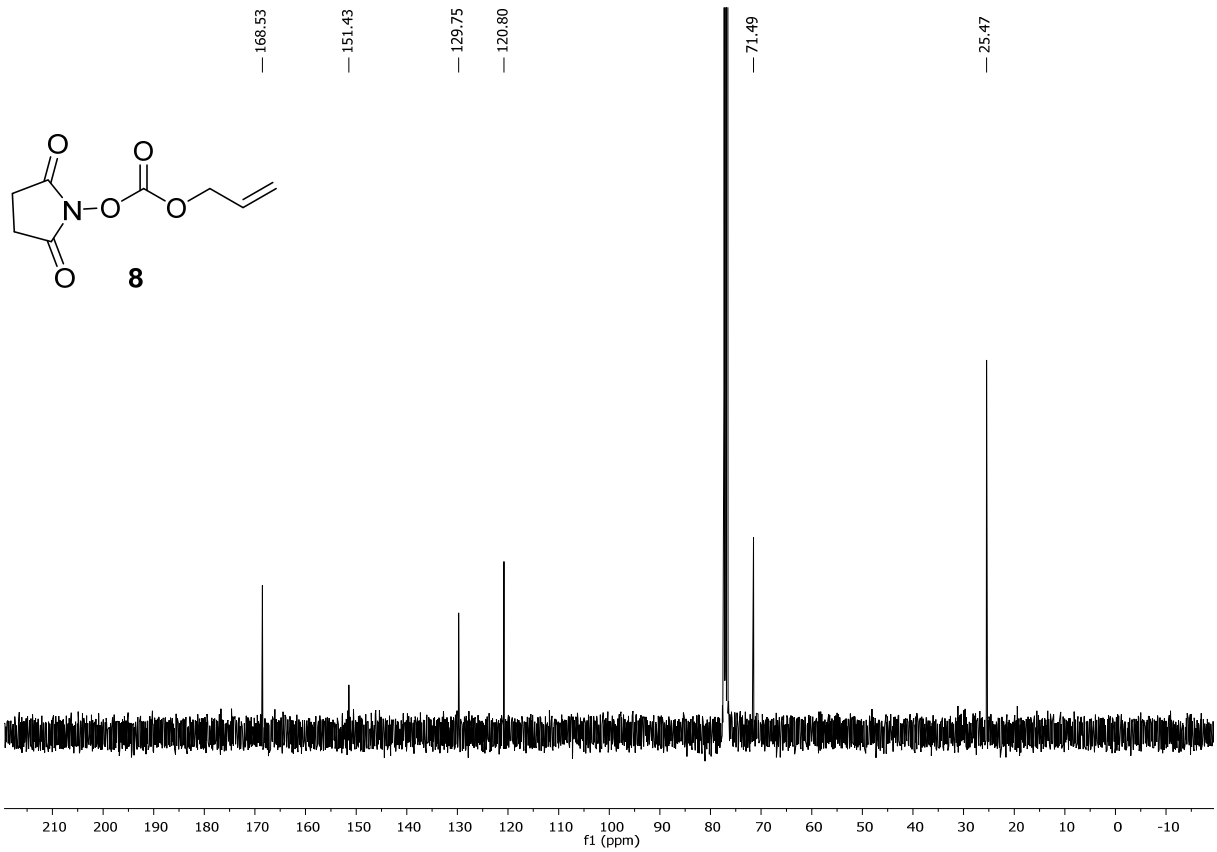


Figure S4: Mass spectra of the peaks A–D of the chromatogram shown in Figure S1 corresponding to **19a/b** and tautomers.

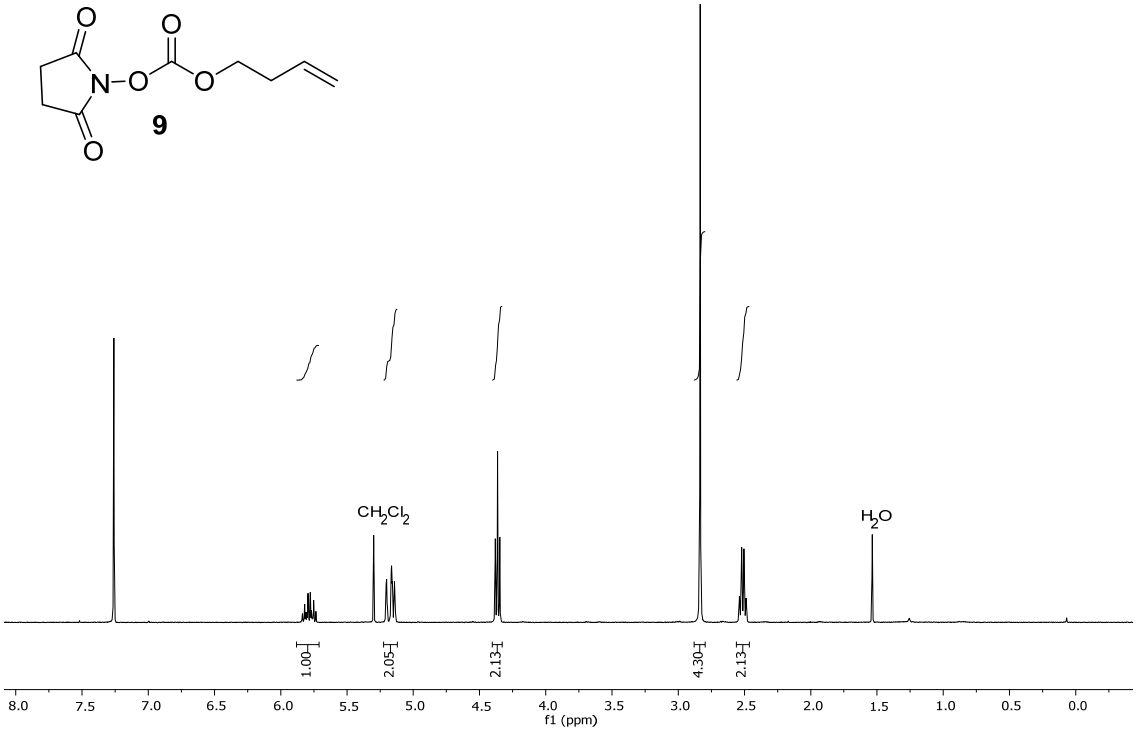
NMR Spectra



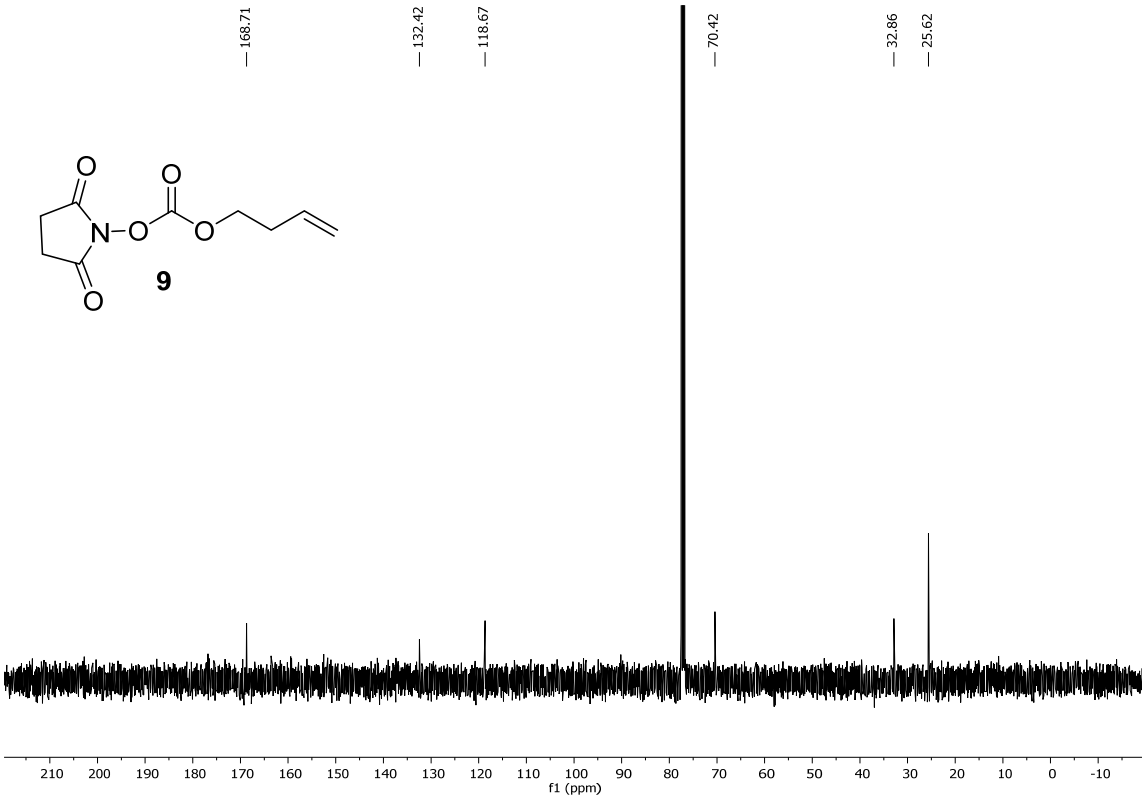
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of allyl succinimidyl carbonate **8**.



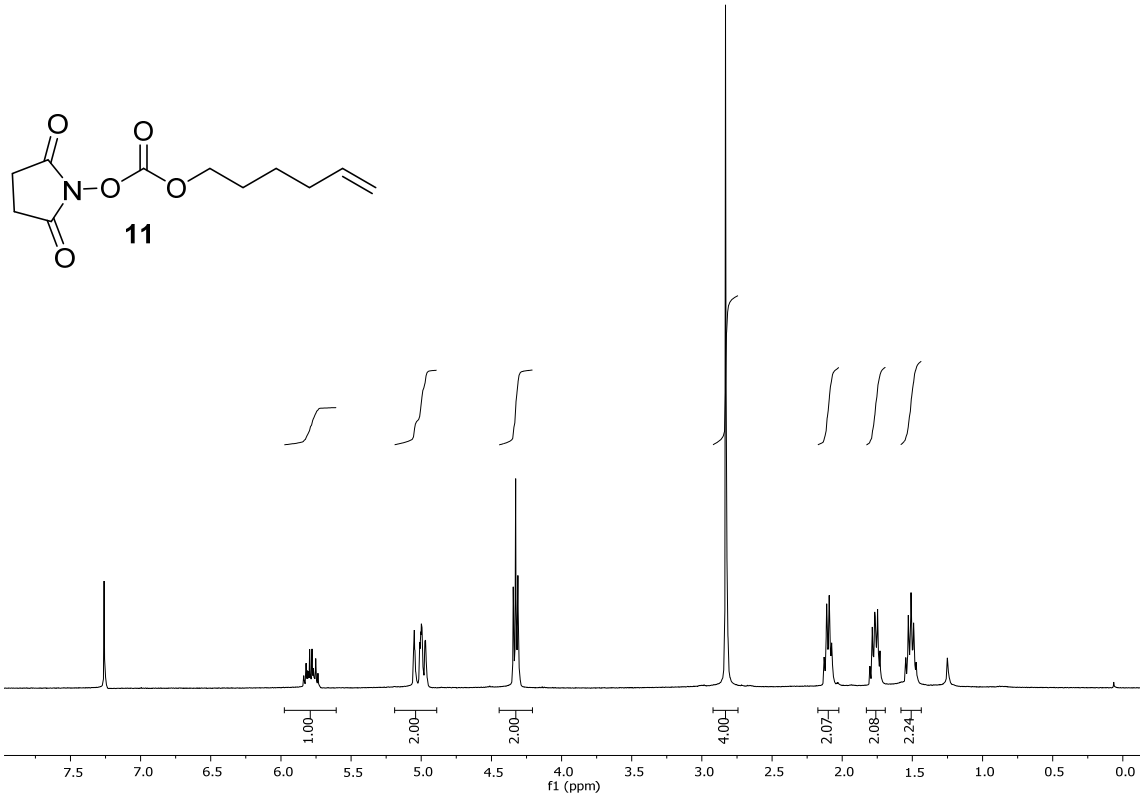
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of allyl succinimidyl carbonate **8**.



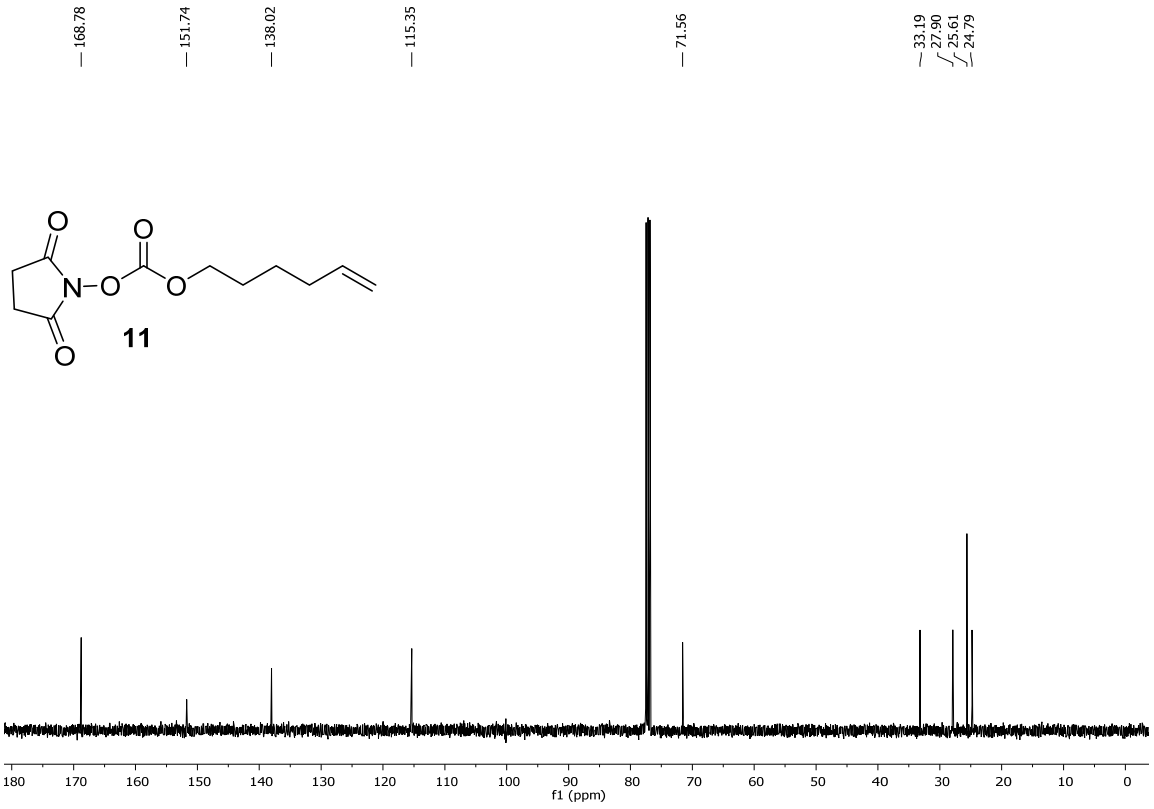
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of but-3-en-1-yl succinimidyl carbonate **9**.



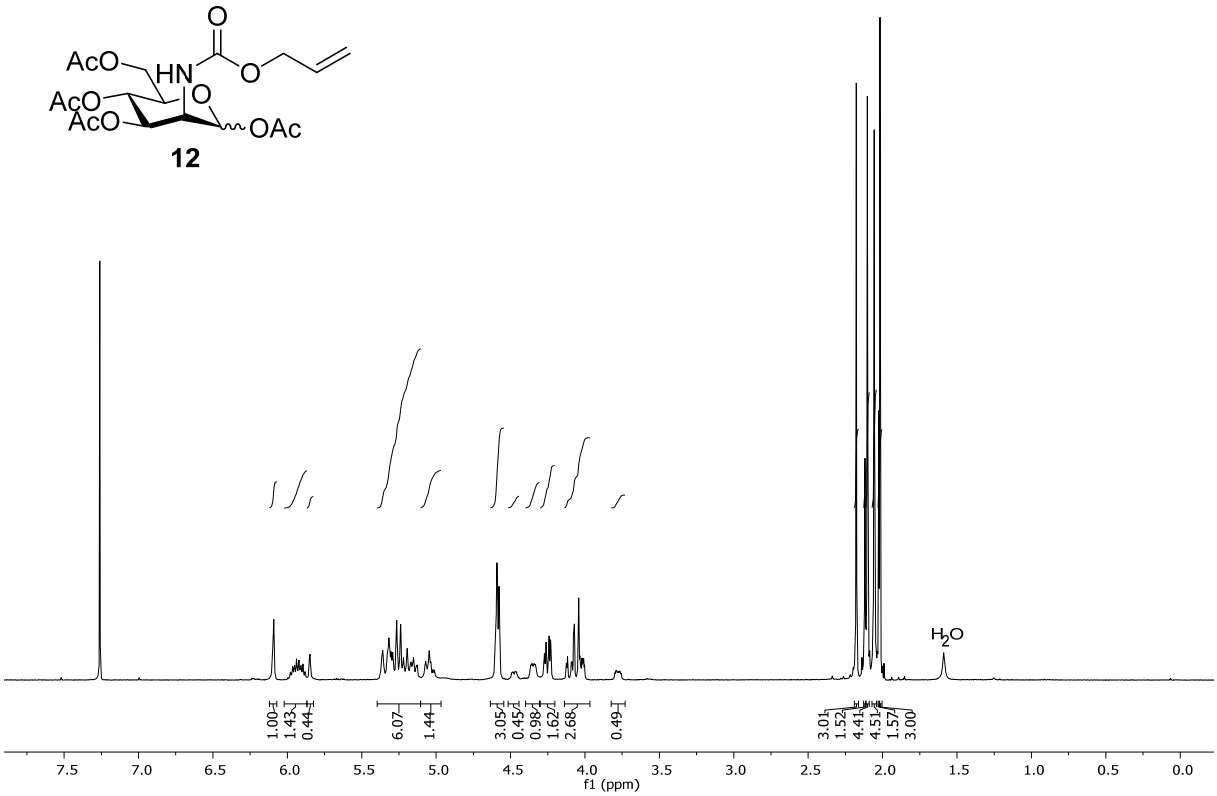
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of but-3-en-1-yl succinimidyl carbonate**9**.



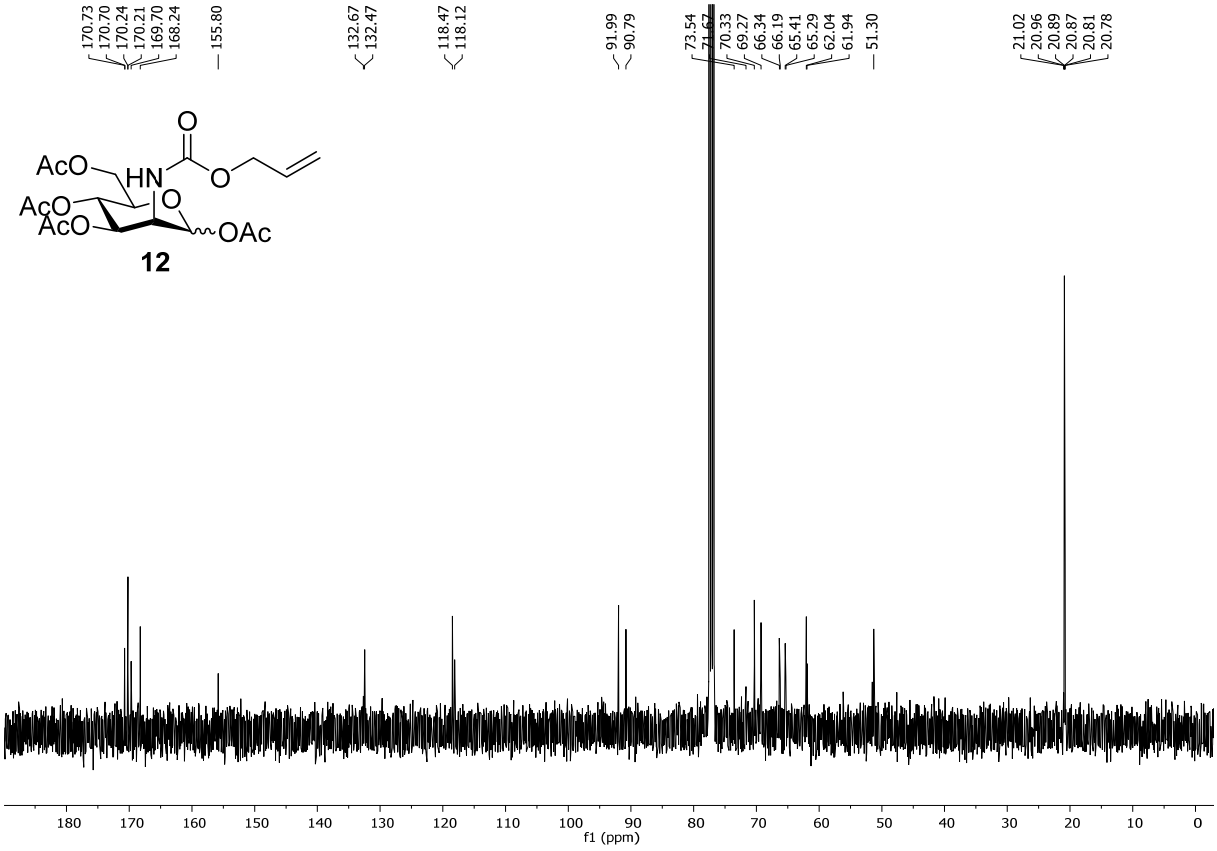
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of hex-5-en-1-yl succinimidyl carbonate **11**.



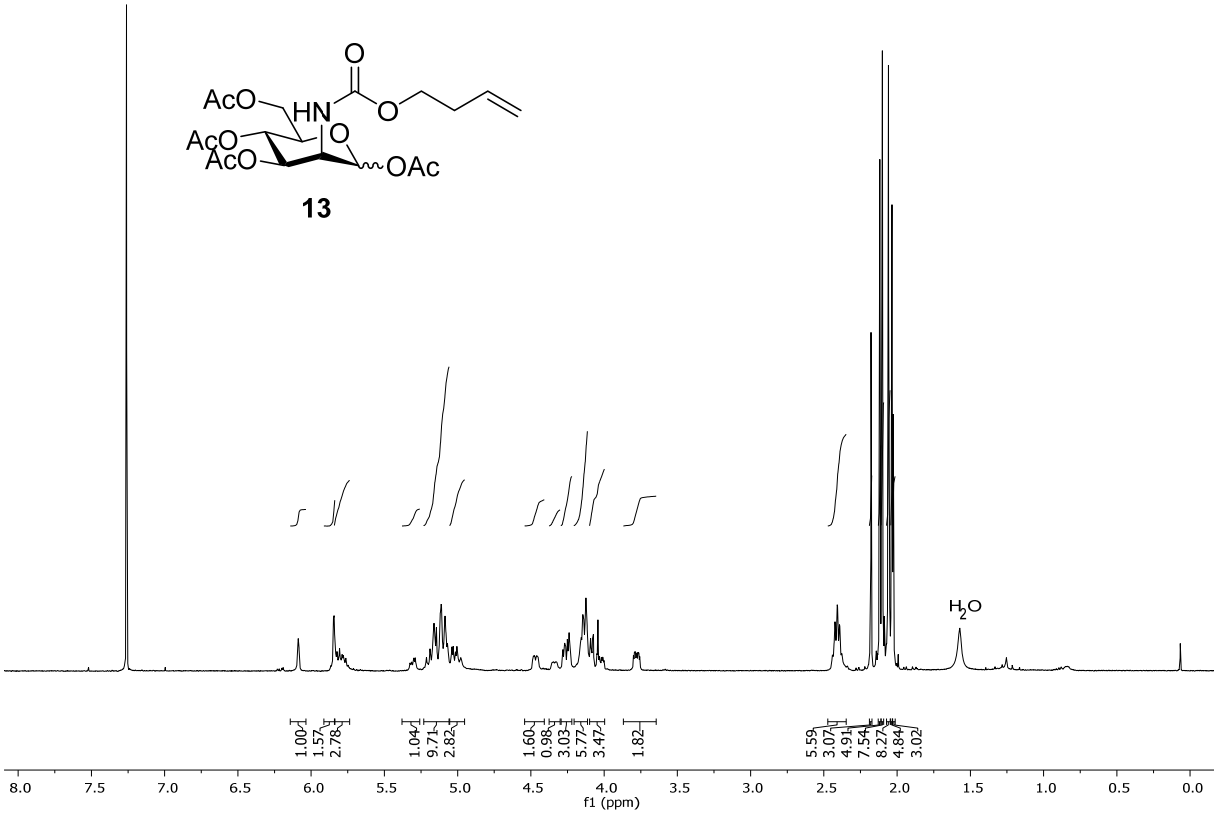
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of hex-5-en-1-yl succinimidyl carbonate **11**.



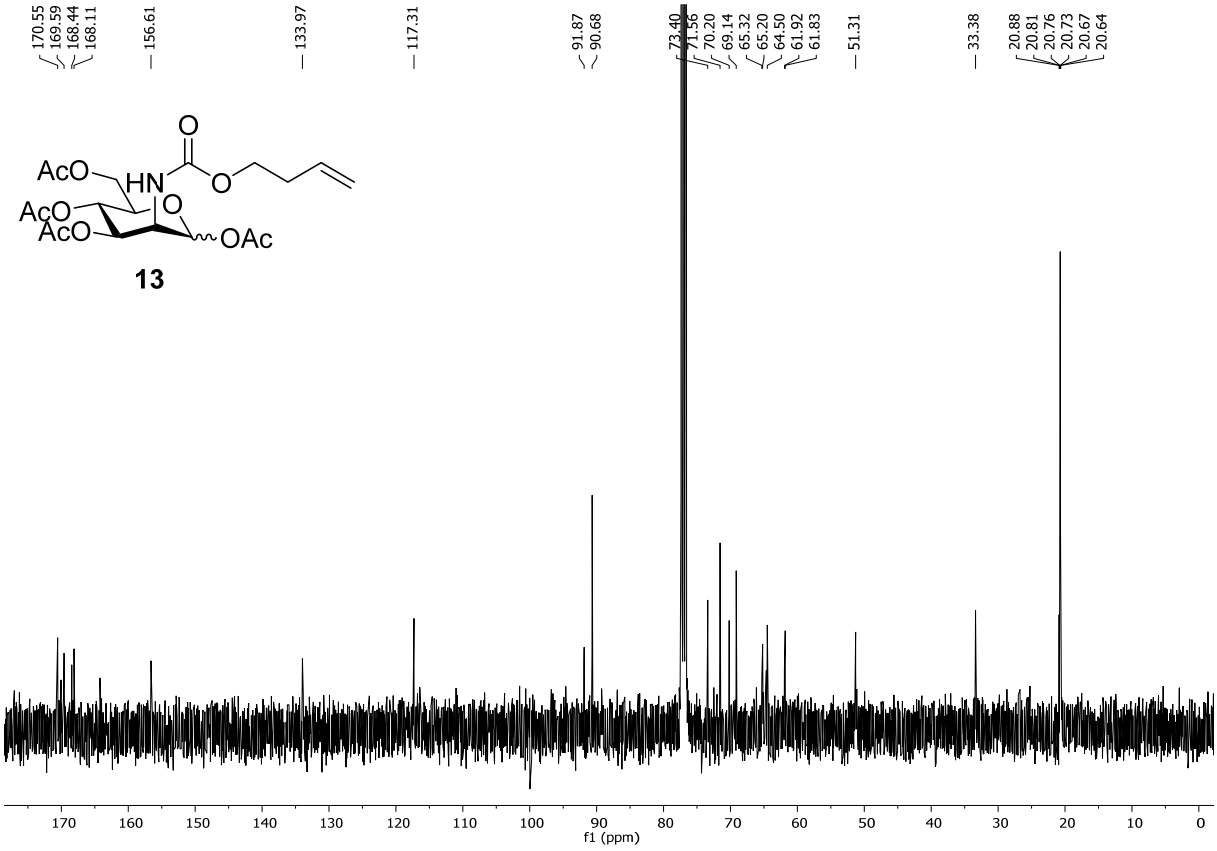
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4ManNAloc **12**.



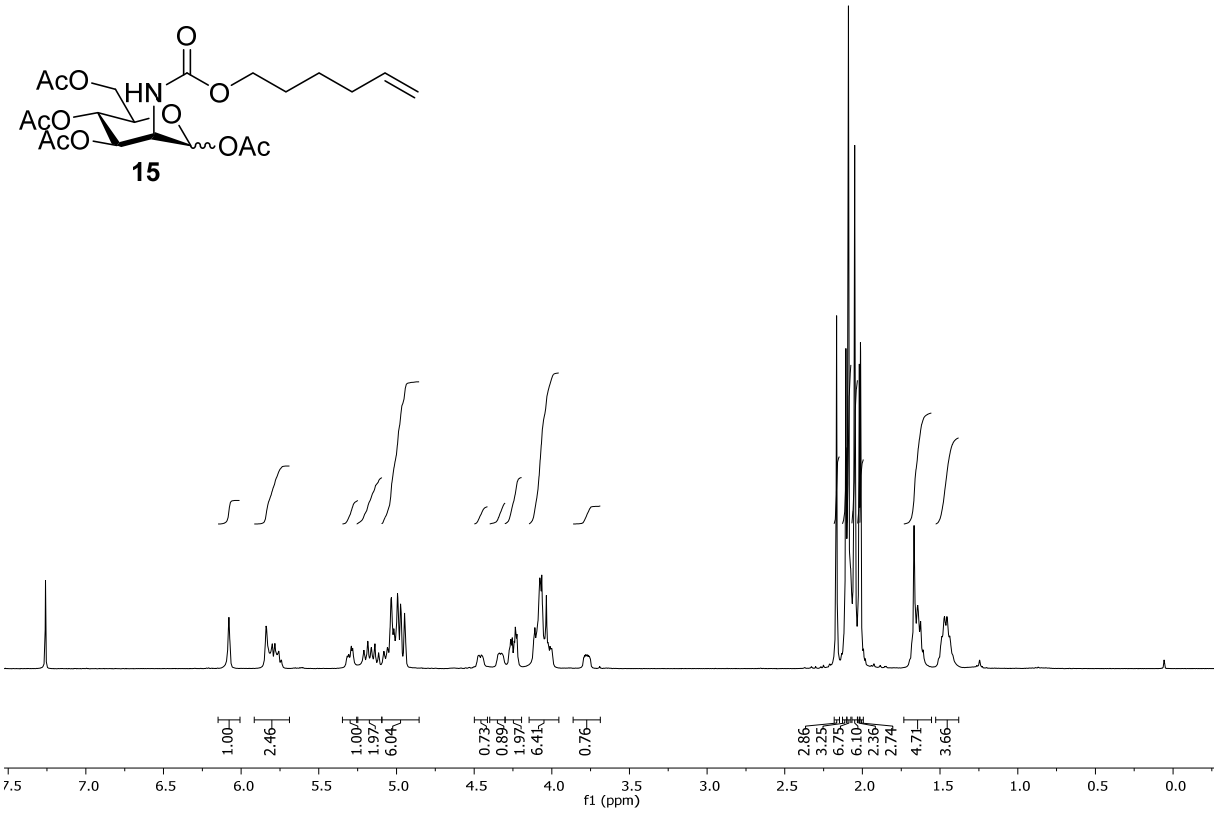
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4ManNAloc **12**.



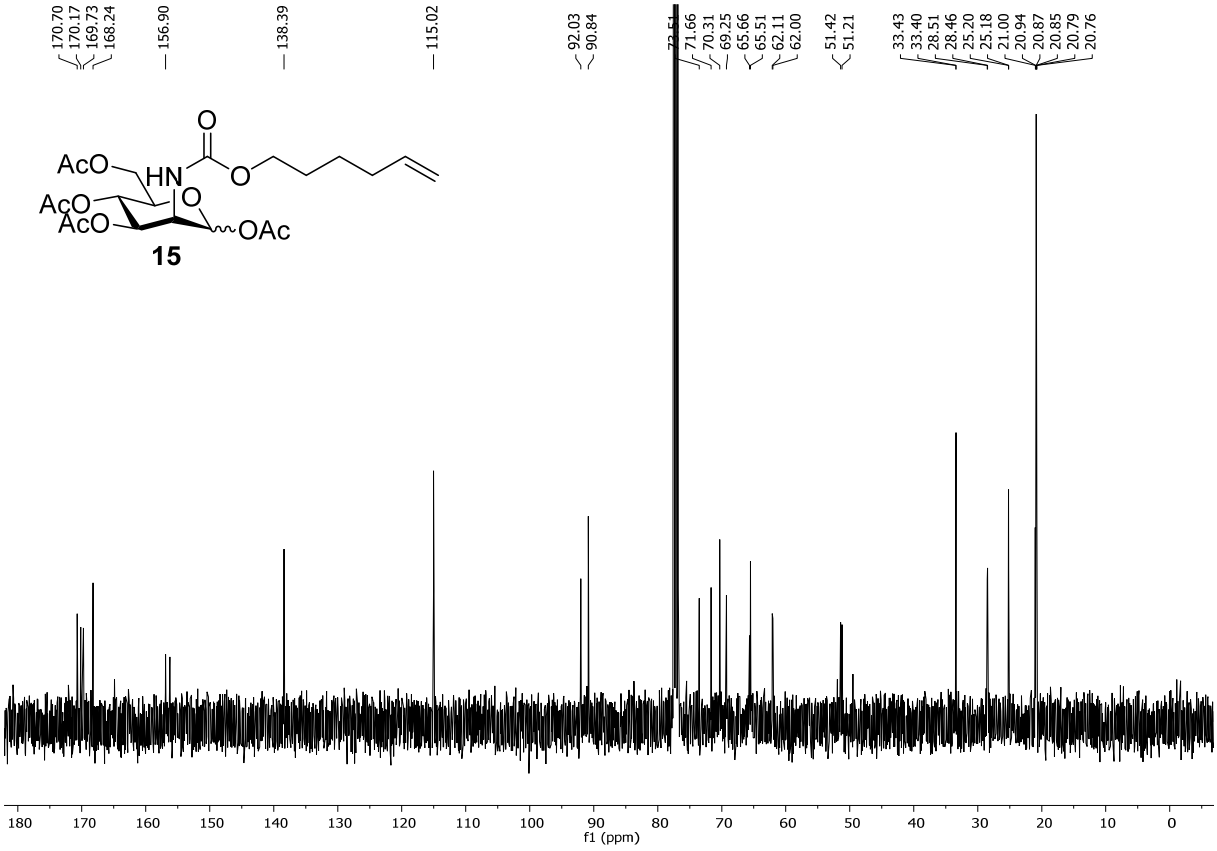
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4ManNBeoc **13**.



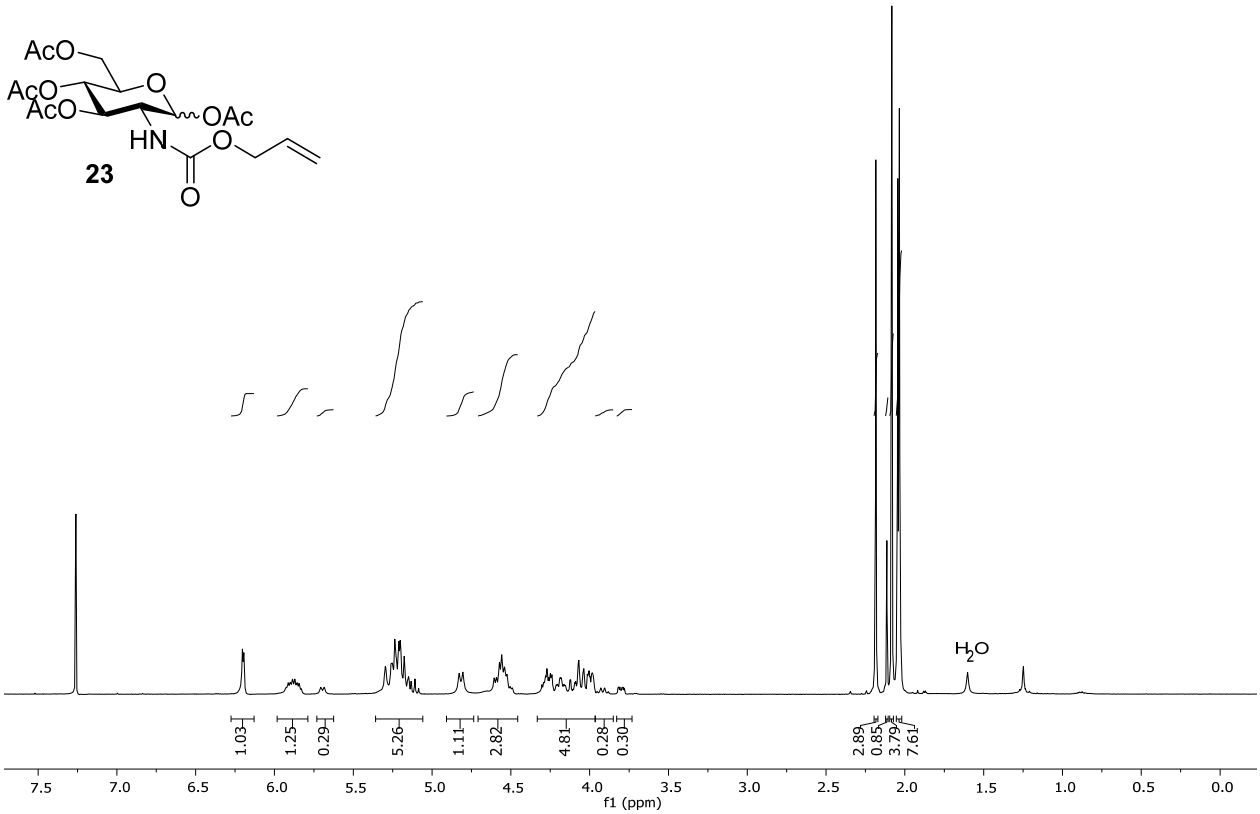
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4ManNBeoc **13**.



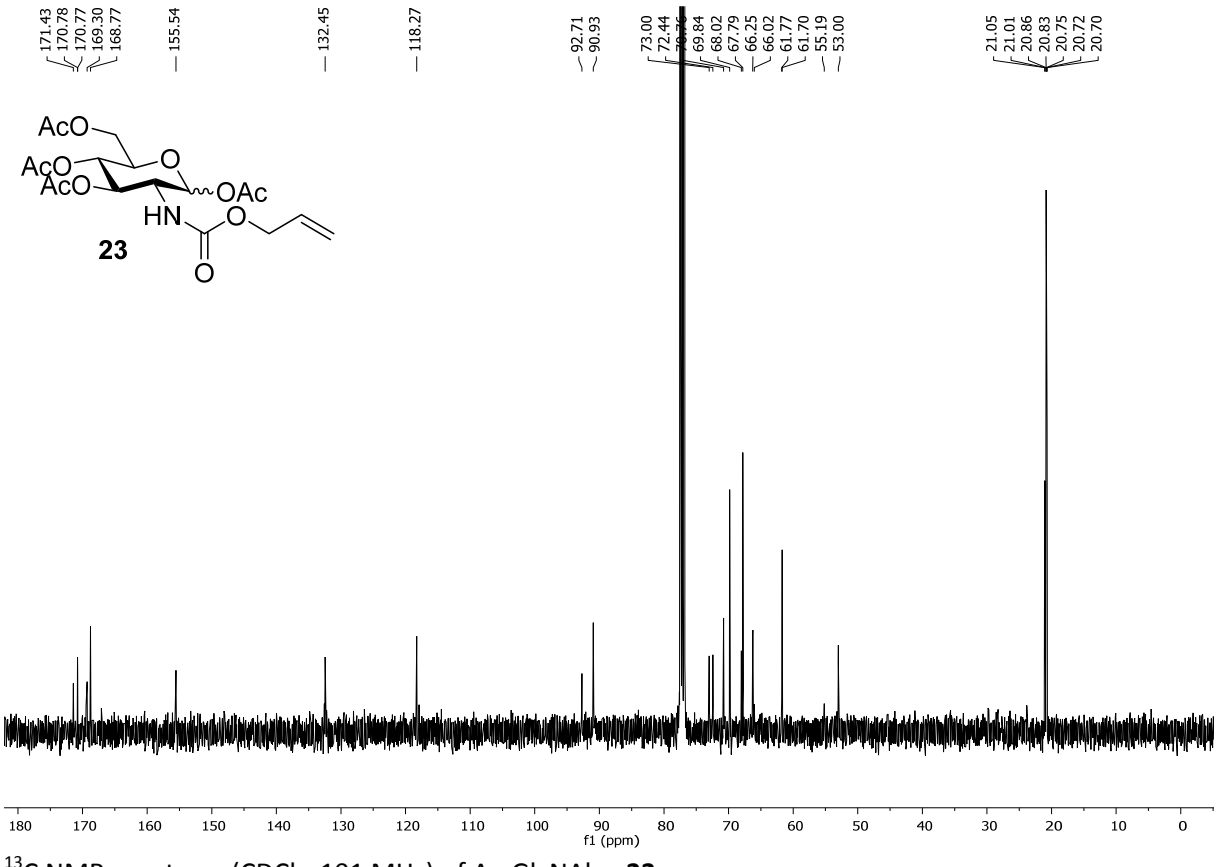
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4ManNHeoc **15**.



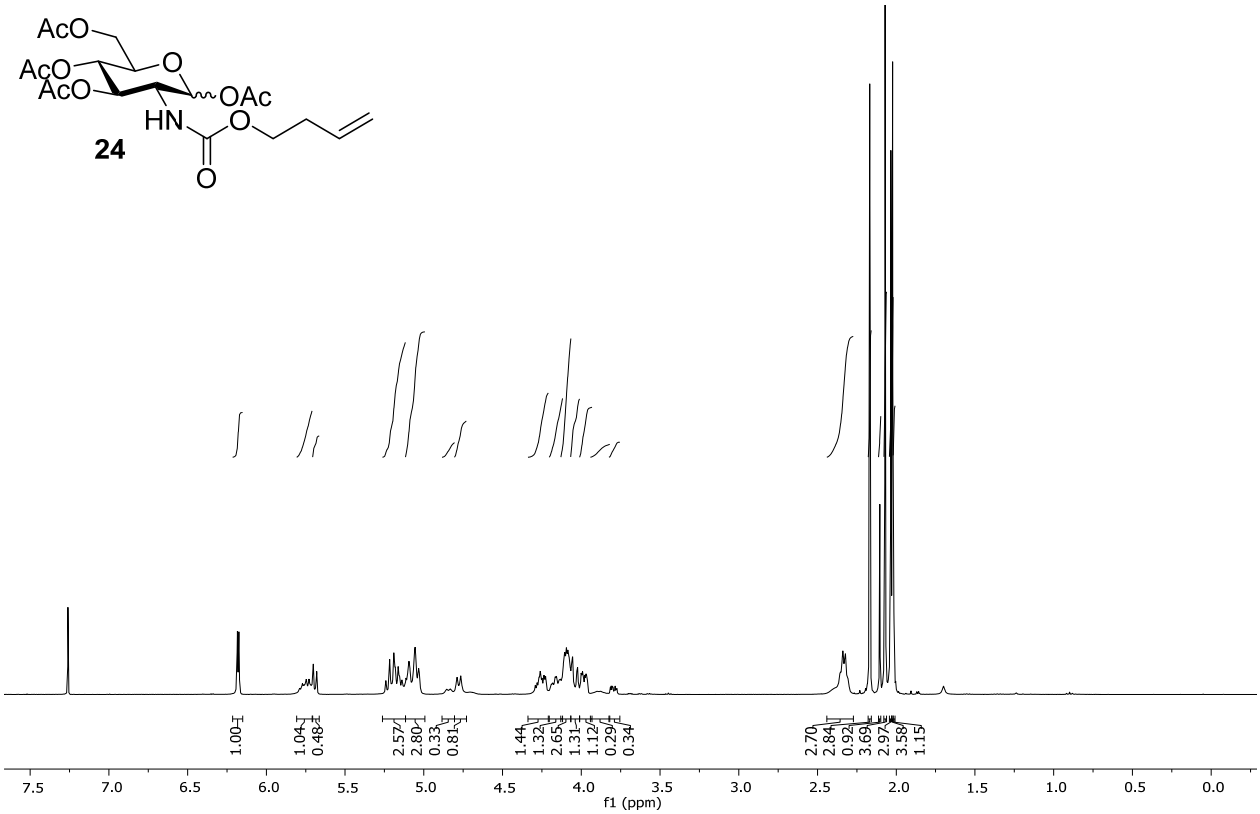
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4ManNHeoc **15**.



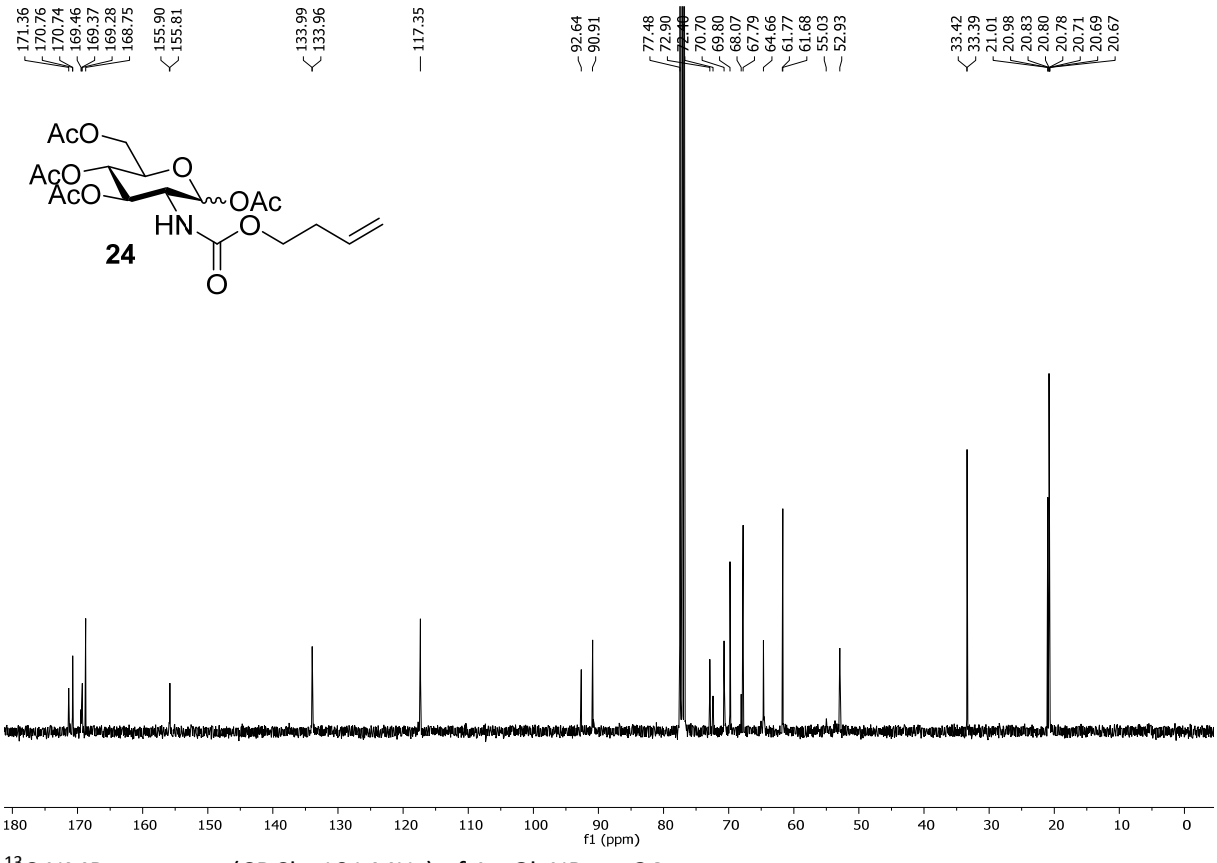
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4GlcNAloc **23**.



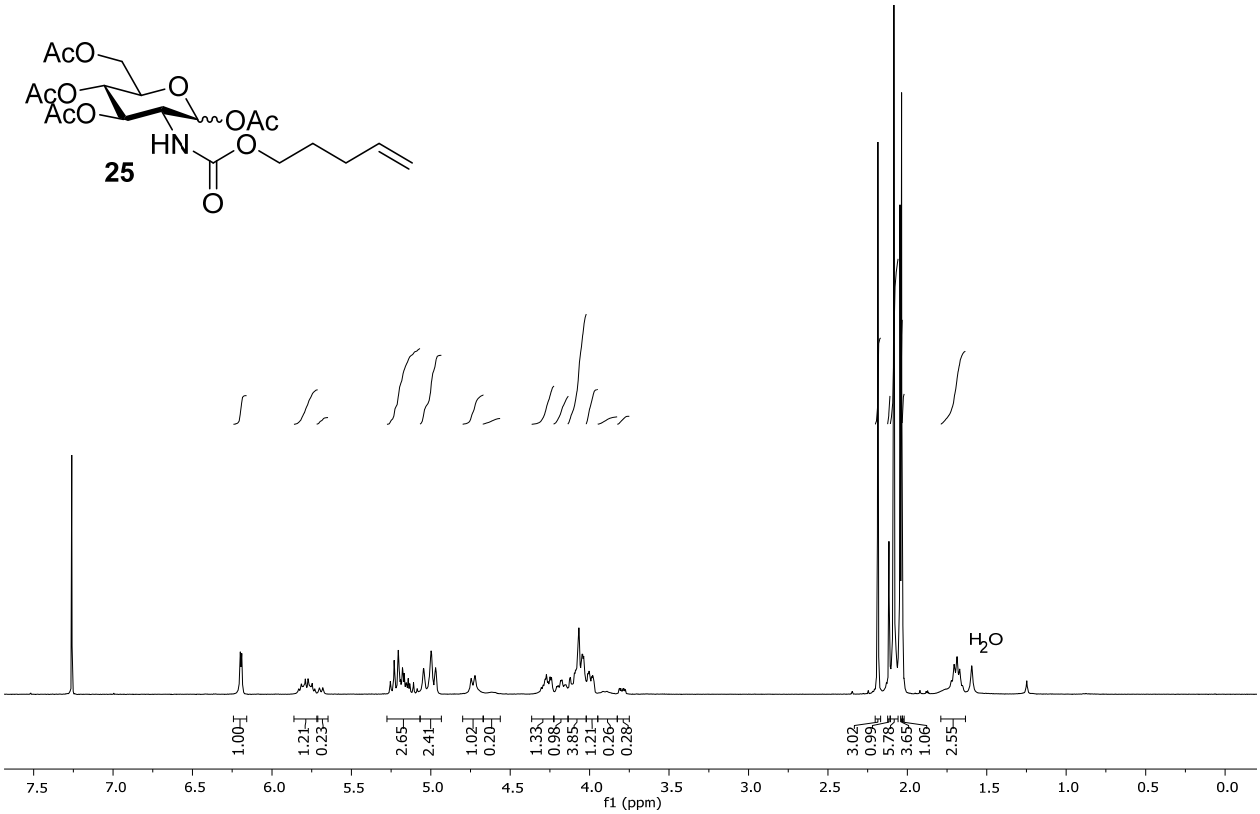
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4GlcNAloc **23**.



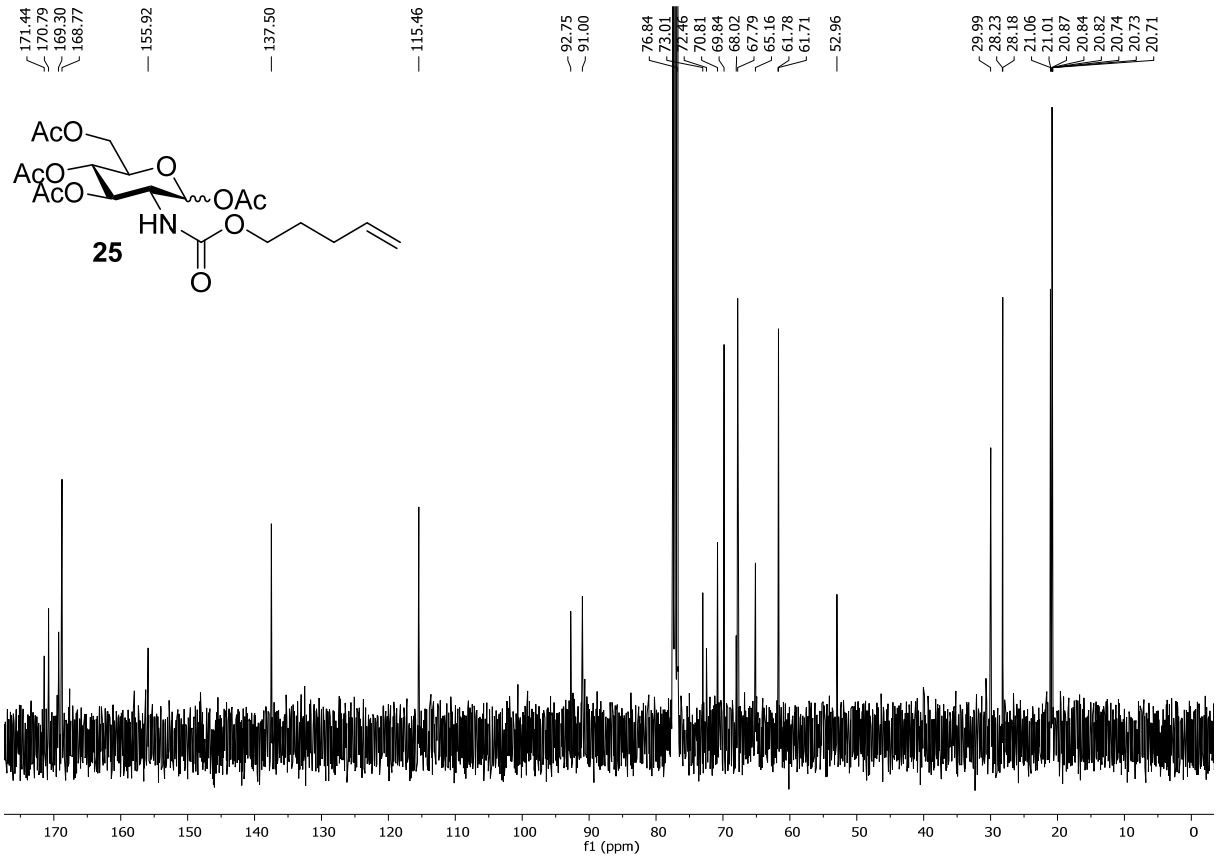
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4GlcNBeoc **24**.



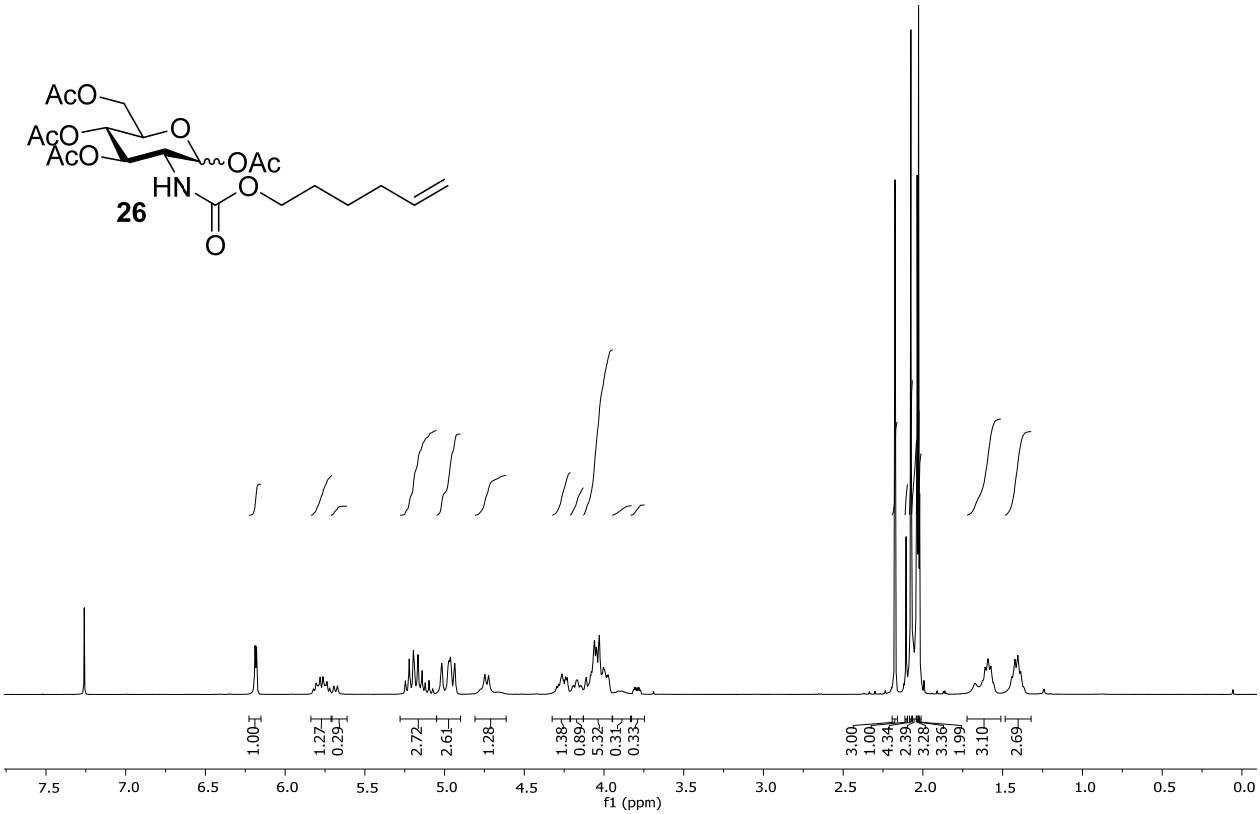
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4GlcNBeoc **24**.



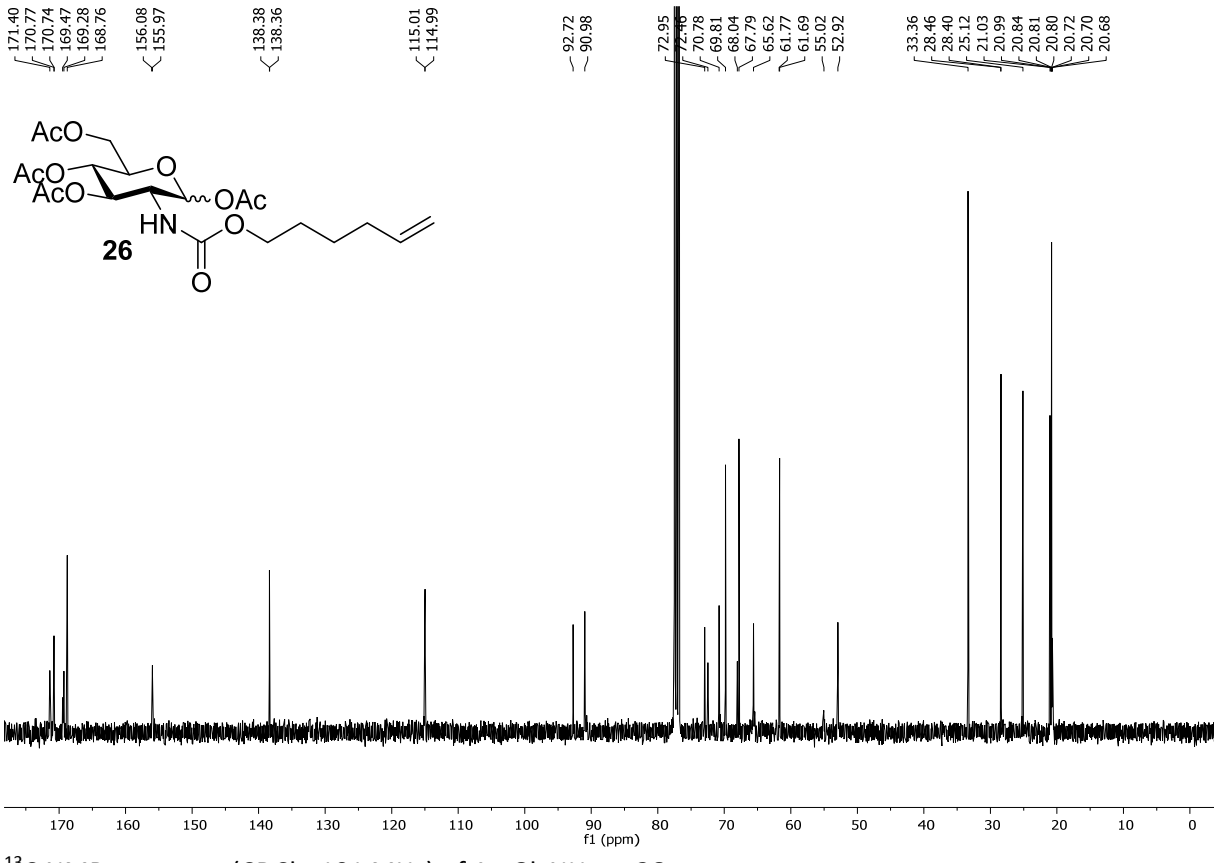
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4GlcNPeoc **25**.



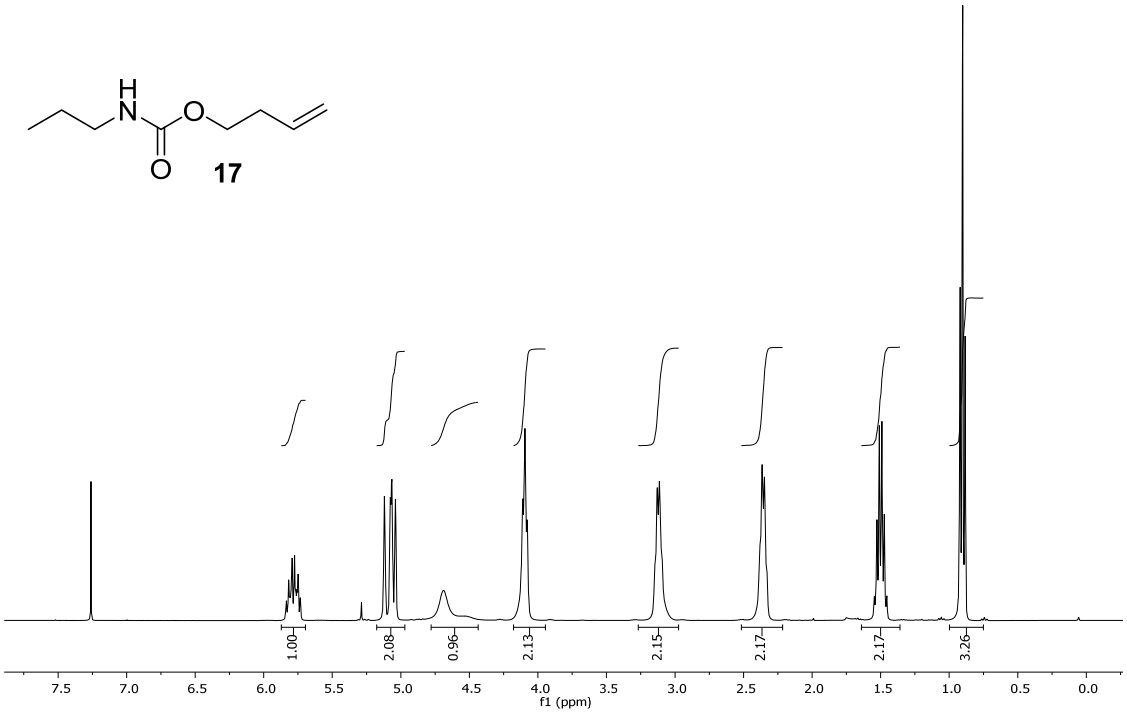
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4GlcNPeoc **25**.



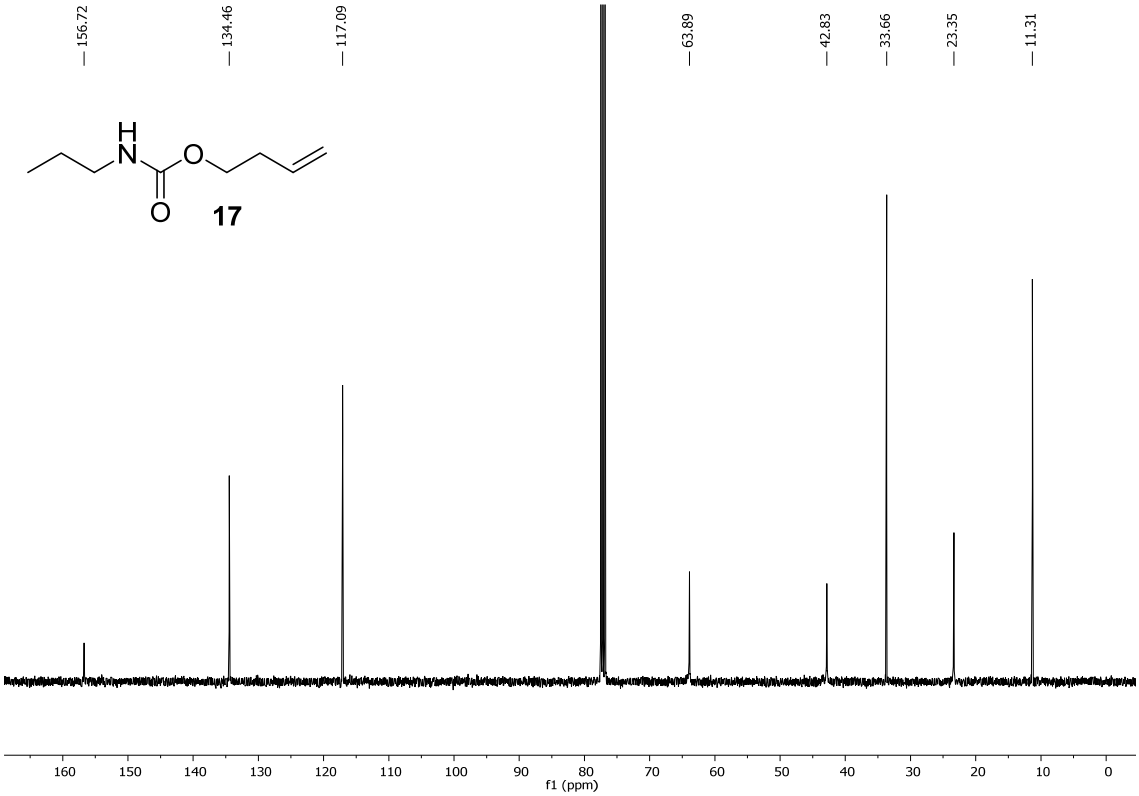
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of Ac_4GlcNHeoc **26**.



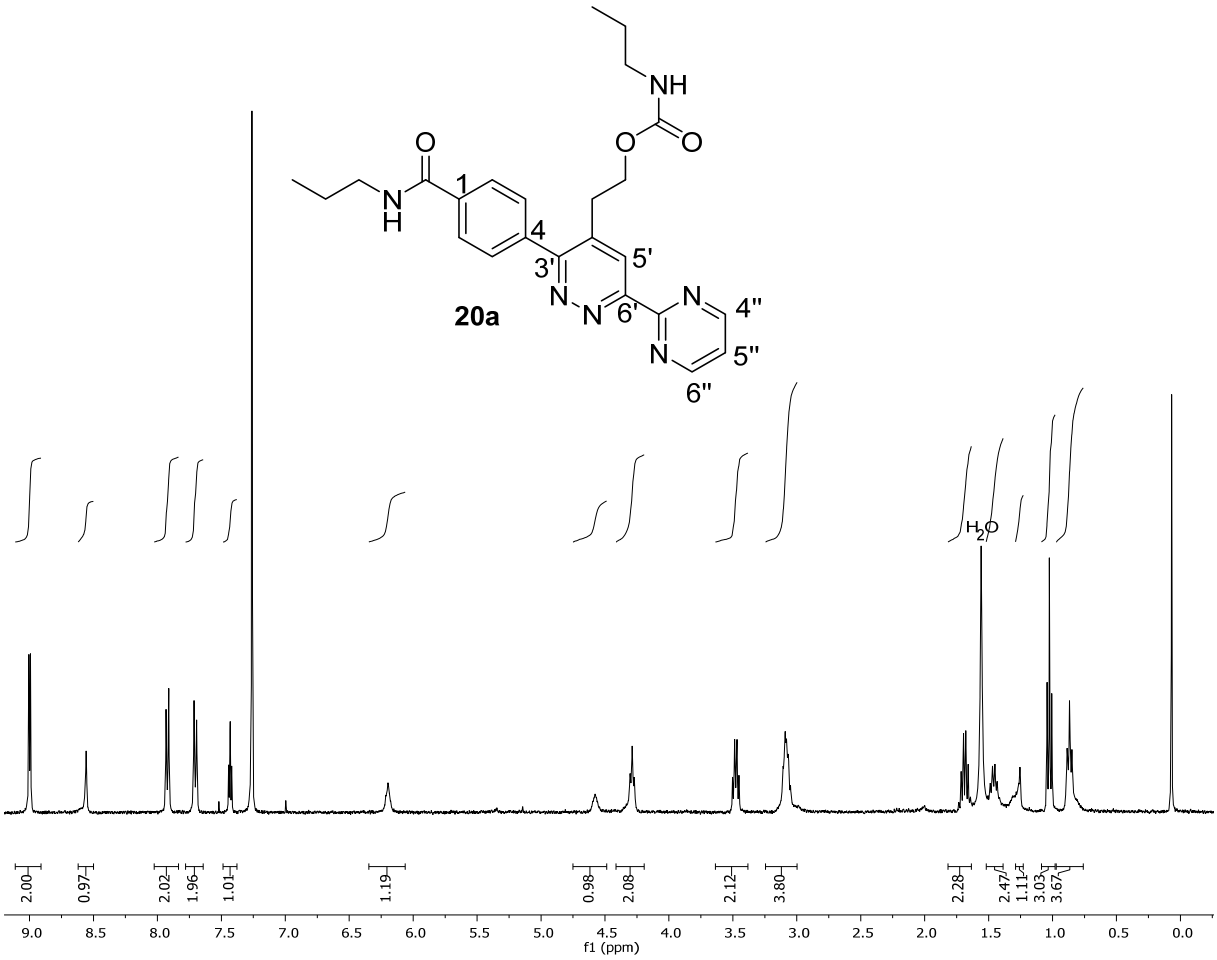
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of Ac_4GlcNHeoc **26**.



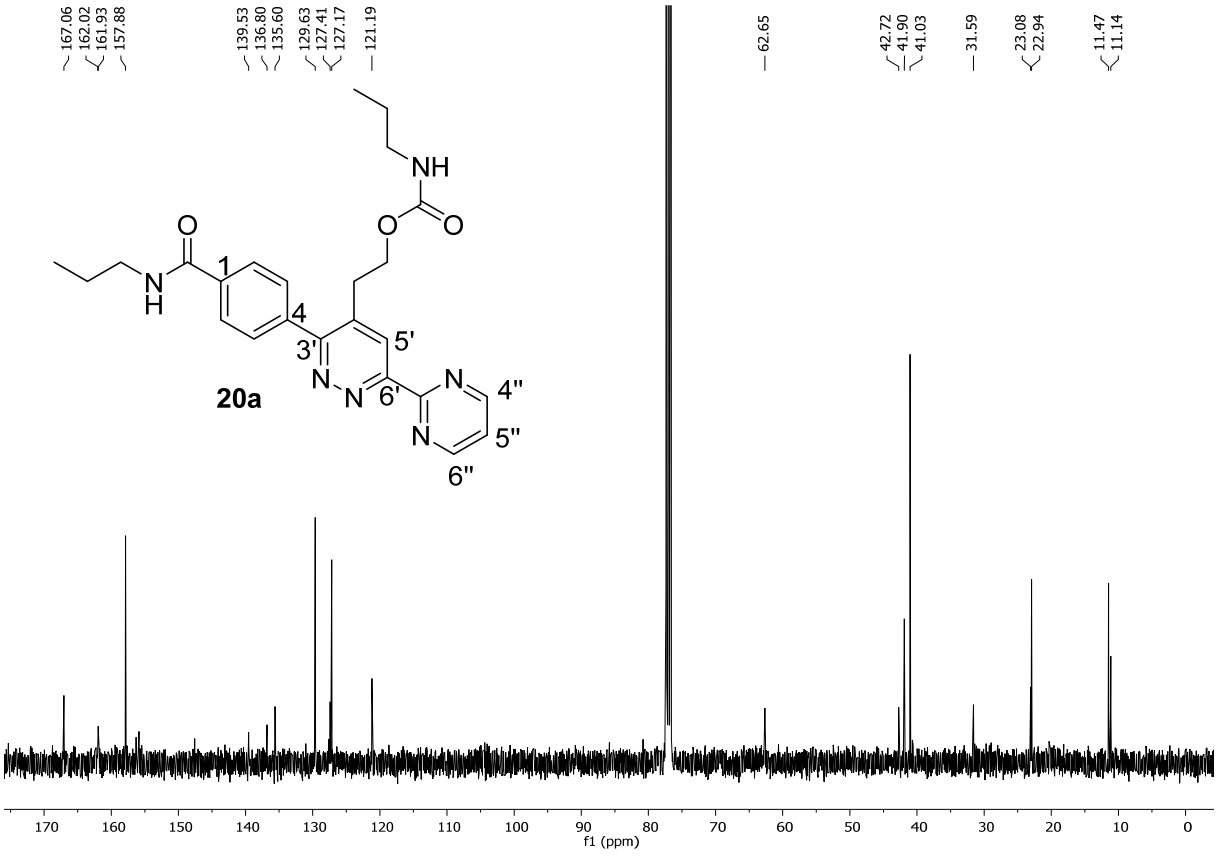
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of but-3-en-1-yl propylcarbamate **17**.



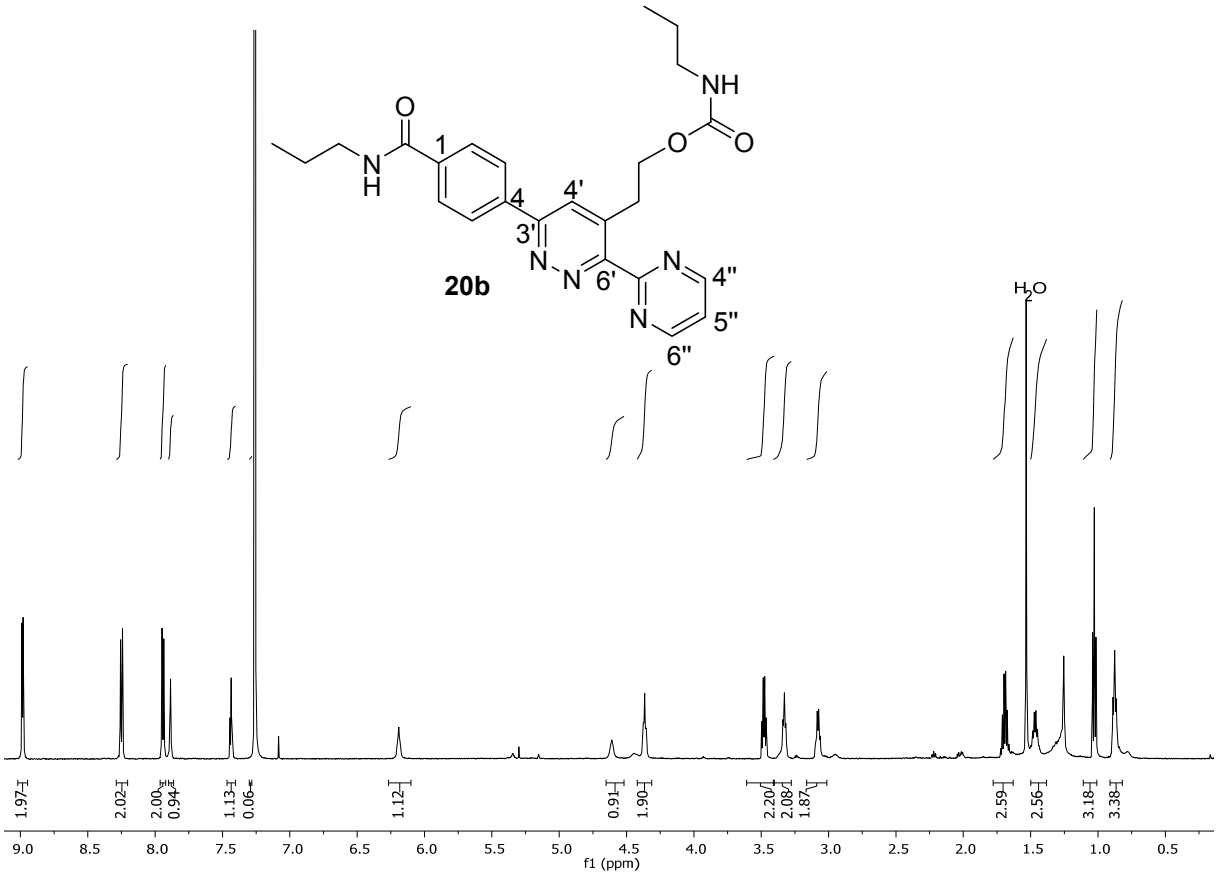
^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of but-3-en-1-yl propylcarbamate **17**.



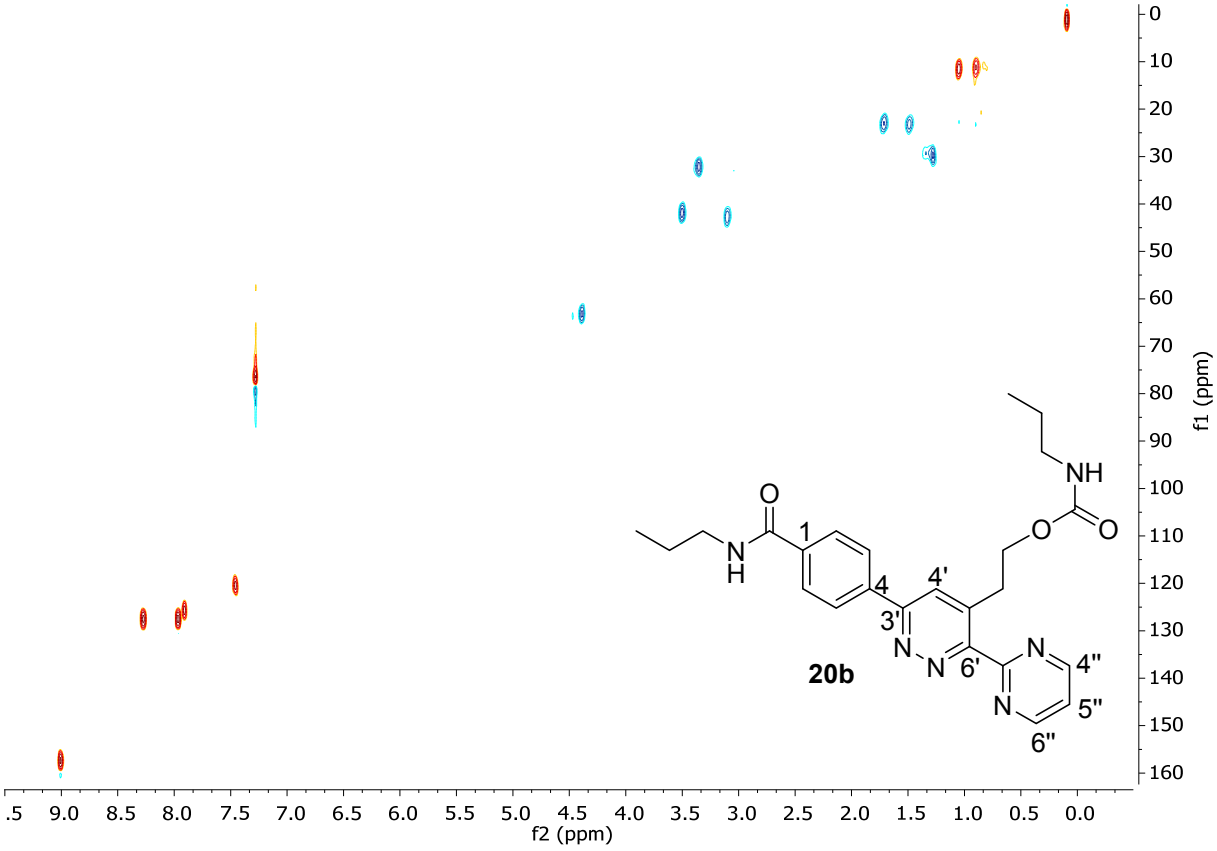
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of pyridazine **20a**.



^{13}C NMR spectrum ($CDCl_3$, 101 MHz) of pyridazine **20a**.



^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of pyridazine **20b**.



HSQC spectrum ($CDCl_3$, 101 MHz) of pyridazine **20b**.