Supporting Information

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Terminal Alkenes as Versatile Chemical Reporter Groups for Metabolic Oligosaccharide Engineering

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Content

Synthetic procedures ........................................ S3
NMR spectra .......................................................... S11
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

\[ \text{C}_9\text{H}_9\text{NO}_5 \]

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 (CH₂Cl₂). The product was obtained as an oil (1.96 g, 57%).

R_f=0.22 (CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ=5.96 (ddt, J=17.2, 10.4, 5.9 Hz, 1H; CH), 5.56 – 5.30 (m, 2H; CH₃), 4.79 (dt, J=5.9, 1.3 Hz, 2H; CH₂), 2.84 (s, 4H, 2x COCH₂) ppm. ¹³C NMR: (101 MHz, CDCl₃): δ=168.5, 151.4 (2x CO), 129.8 (CH), 120.8 (CH₂), 71.3 (CH₃), 25.5 (COCH₂) ppm. HRMS: m/z calcd for C₉H₉NO₅: 222.03729 [M + Na]^+, found: 222.03638.

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

\[ \text{C}_9\text{H}_{11}\text{NO}_5 \]

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 (CH₂Cl₂). 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). ¹H NMR: (400 MHz, CDCl₃): δ=5.79 (ddt, J=17.1, 10.2, 6.8 Hz, 1H, CH₃), 5.24 – 5.07 (m, 2H, CH₂), 4.36 (t, J=6.8 Hz, 2H, OCH₂), 2.83 (s, 4H, 2x COCH₂), 2.51 (qt, J=6.7, 1.3 Hz, 2H, OCH₂CH₂) ppm. ¹³C NMR: (101 MHz, CDCl₃): δ=168.7 (CO), 132.4 (CH₂), 118.7 (CH₂), 70.4 (OCH₂), 32.9 (OCH₂CH₂), 25.6 (COCH₂) ppm. HRMS: m/z calcd for C₉H₁₁NO₅: 236.05294 [M + Na]^+, found: 236.05244.
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 %).

Rf = 0.28 (petroleum ether/ethyl acetate 3:1). ¹H NMR: (400 MHz, CDCl₃): δ = 5.79 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H, CHCH₂), 5.10 – 4.87 (m, 2H, CHCH₂), 4.33 (t, J = 6.6 Hz, 2H, OCH₂), 2.83 (s, 4H, 2x COCH₂), 2.20 – 1.96 (m, 2H, OCH₂CH₂CH₂), 1.88 – 1.68 (m, 2H, OCH₂CH₂), 1.60 – 1.38 (m, 2H, OCH₂CH₂CH₂CH₂) ppm. ¹³C NMR: (101 MHz, CDCl₃): δ = 168.8, 151.7 (2x CO), 138.0 (CHCH₂), 115.4 (CHCH₂), 71.6 (OCH₂), 33.2 (OCH₂CH₂CH₂), 27.9 (CH₃), 25.6 (COCH₂), 24.8 (OCH₂CH₂CH₂CH₂) ppm. HRMS: m/z calcld for C₁₇H₁₉NO₅: 264.0842 [M + Na]+, found: 264.08385.

**1,3,4,6-Tetra-O-acetyl-2-(allyloxy-carbonylamino)-2-deoxy-D-glucopyranose (Ac₄GlcNAloc) 23**

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 CH₂Cl₂ and extracted three times with 1 N NaOH. Ac₄ManNAloc 23 was obtained as mixture of anomers (0.81 g, 61 %, α/β 3:1).

Rf = 0.29 (petroleum ether/ethyl acetate 1:1). α-Anomer: ¹H NMR (400 MHz, CDCl₃): δ = 6.20 (d, J = 3.7 Hz, 1H, H-1), 5.88 (ddt, J = 16.5, 11.1, 5.7 Hz, 1H, CHCH₂), 5.33 – 5.06 (m, 4H, CHCH₂, H-3, H-4), 4.82 (d, J = 9.5 Hz, 1H, NH), 4.55 (qd, J = 13.2, 5.6 Hz, 2H, CH₂), 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, CH₃), 2.08 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.03 (s, 3H, CH₃) ppm. β-Anomer: ¹H NMR (400 MHz, CDCl₃): δ = 5.88 (ddt, J = 16.5, 11.1, 5.7 Hz, 1H, CHCH₂), 5.69 (d, J = 8.7 Hz, 1H, H-1), 5.33 – 5.06 (m, 4H, CHCH₂, H-3, H-4), 4.82 (d, J = 9.5 Hz, 1H, NH), 4.55 (qd, J = 13.2, 5.6 Hz, 2H, CH₂), 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, CH₃), 2.08 (s, 3H, CH₃), 2.04 (s, 3H, CH₃), 2.03 (s, 3H, CH₃) ppm. α/β-Anomers: ¹³C NMR (101 MHz, CDCl₃): δ = 171.4, 170.8, 169.3, 168.8, 155.5 (5x CO), 132.5 (CHCH₂), 118.3 (CHCH₂), 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 (CH₂α), 66.0 (CH₂β), 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, 20.7, 20.7 (8x CH₃) ppm; HRMS: m/z calcld for C₁₉H₂₅NO₁₇: 454.13198 [M + Na]+, found: 454.13027.
1,3,4,6-Tetra-O-acetyl-2-(but-3-en-1-yl-oxy carbonylamino)-2-deoxy-D-glucopyranose (Ac₄GlcNBec) 24

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\text{C}_{19}\text{H}_{27}\text{NO}_{11}
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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 CH₂Cl₂ and extracted three times with 1 N NaOH. Ac₄GlcNBec 24 was obtained as mixture of anomers (1 g, 62%, α/β 3:1).

Rᵣ=0.48 (petroleum ether/ethyl acetate 1:1). α-Anomer: 1H NMR (400 MHz, CDCl₃): δ=6.18 (d, J=3.7 Hz, 1H, H-1), 5.75 (dt, J=14.3, 5.4 Hz, 1H, CH₂CH₂), 5.25 – 5.13 (m, 2H, H-3, H-4), 5.12 – 5.01 (m, 2H, CH₂CH₂), 4.78 (d, J=9.5 Hz, 1H, NH), 4.26 (ddd, J=13.3, 9.1, 4.3 Hz, 1H, H-6a), 4.20 – 4.13 (m, 1H, H-2), 4.13 – 4.01 (m, 3H, H-6b, CH₂OOC), 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, CH₂CH₂), 2.17 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 2.03 (s, 3H, CH₃), 2.02 (d, J=1.1 Hz, 3H, CH₃), ppm. β-Anomer: 1H NMR (400 MHz, CDCl₃): δ=5.75 (dt, J=14.3, 5.4 Hz, 1H, CH₂CH₂), 5.70 (s, 1H, H-1), 5.25 – 5.13 (m, 2H, H-3, H-4), 5.12 – 5.01 (m, 2H, CH₂CH₂), 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, CH₂OOC), 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, CH₂CH₂), 2.10 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 2.02 (d, J=1.1 Hz, 3H, CH₃), ppm. α/β-Anomers: 13C NMR (101 MHz, CDCl₃): δ=171.4, 170.8, 170.7, 169.5, 169.4, 169.3, 168.8, 155.9, 155.8 (5x COα,β), 134.0 (CH₂CH₂), 117.4 (CH₂CH₂), 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 (CH₂OOC), 61.8 (C-6β), 61.7 (C-6α), 55.0 (C-2β), 52.9 (C-2α), 33.4 (CH₂CH₂), 21.0 (CH₃), 21.0, 20.8 (2x CH₂β), 20.8, 20.8 (2x CH₃α), 20.7, 20.7 (2x CH₃β), 20.7 (CH₃α) ppm; HRMS: m/z calcd for C₁₉H₂₇NO₁₁: 468.14763 [M + Na]⁺, found: 468.14524.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(pent-4-en-1-yl-oxy carbonylamino)-D-glucopyranose (Ac₄GlcNPec) 25

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\text{C}_{20}\text{H}_{29}\text{NO}_{11}
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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 CH₂Cl₂ and extracted three times with 1 N NaOH. Ac₄GlcNPec 25 was obtained as mixture of anomers (0.9 g, 61%, α/β 4:1).

**Rf**=0.31 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=6.19 (d, J=3.7 Hz, 1H, H-1), 5.78 (ddt, J=16.9, 10.1, 6.6 Hz, 1H, CH$_2$(CH$_3$), 5.29 – 5.11 (m, 2H, H-3, H-4), 5.07 – 4.91 (m, 2H, CH$_2$(CH$_3$)), 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, CH$_2$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, CH$_2$(CH$_3$CH$_2$)), 2.05 (s, 3H, CH$_3$), 2.04 (s, 3H, CH$_3$), 1.76 – 1.63 (m, 2H, CH$_3$(CH$_2$CH$_2$) ppm. **β-Anomer:** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=5.78 (ddt, J=16.9, 10.1, 6.6 Hz, 1H, CH$_2$(CH$_3$)), 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, CH$_2$(CH$_3$)), 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$_2$OCO), 3.90 (d, J=10.1 Hz, 1H, C-2), 3.80 (dd, J=9.6, 4.2, 2.3 Hz, 1H, C-5), 2.12 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$), 2.10 – 2.06 (m, 2H, CH$_3$(CH$_2$CH$_2$)), 2.04 (s, 3H, CH$_3$), 2.03 (s, 3H, CH$_3$), 1.76 – 1.63 (m, 2H, CH$_2$(CH$_3$CH$_2$)) ppm. **α/β-Anomers:** $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$=171.4, 170.8, 169.3, 168.8, 155.9 (5x CO), 137.5 (CH$_2$(CH$_3$)), 115.5 (CH$_2$(CH$_3$)), 92.8 (C-1β), 91.0 (C-1α), 73.0, 72.5, 70.8, 69.8, 68.0, 67.8 (C-3αβ, C-4αβ, C-5αβ), 65.2 (CH$_2$OCO), 61.8 (C-6β), 61.7 (C-6α), 53.0 (C-2αβ), 30.0 (CH$_2$(CH$_2$CH$_3$)), 28.2 (CH$_3$(CH$_2$CH$_2$)), 21.1 (CH$_3$α), 21.0, 20.9 (2x CH$_3$β), 20.8, 20.8 (2x CH$_3$α), 20.7, 20.7 (2x CH$_3$β), 20.7 (CH$_3$α) ppm; HRMS: m/z calcd for C$_{26}$H$_{35}$NO$_{11}$: 482.16328 [M + Na]$^+$, found: 482.16164.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(hex-5-en-1-yl-oxy carbonylamino)-D-glucopyranose (Ac$_4$GlcNHec) 26

![Structure Image]

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$_2$Cl$_2$ and extracted three times with 1 N NaOH. Ac$_4$GlcNHec 26 was obtained as mixture of anomers (1.63 g, 80 %, α/β 3:1).

**Rf**=0.37 (petroleum ether/ethyl acetate 1:1). **α-Anomer:** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=6.18 (d, J=3.7 Hz, 1H, H-1), 5.77 (dddt, J=13.4, 10.5, 8.4, 5.9 Hz, 1H, CH$_2$(CH$_3$), 5.28 – 5.06 (m, 2H, H-3, H-4), 5.07 – 4.88 (m, 2H, CH$_2$(CH$_3$)), 4.74 (d, J=9.7 Hz, 1H, NH), 4.26 (dd, 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$_2$OCO), 2.17 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$), 2.07 – 2.04 (m, 2H, CH$_3$(CH$_2$CH$_3$)), 2.04 (s, 3H, CH$_3$), 2.03 (s, 3H, CH$_3$), 1.69 – 1.51 (m, 2H, CH$_2$(CH$_2$OCO)), 1.50 – 1.31 (m, 2H, CH$_3$(CH$_2$CH$_2$)) ppm. **β-Anomer:** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=5.77 (dddt, J=13.4, 10.5, 8.4, 5.9 Hz, 1H, CH$_2$(CH$_3$), 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, CH$_2$(CH$_3$)), 4.74 (d, J=9.7 Hz, 1H, NH), 4.26 (dd, J=13.1, 9.3, 4.4 Hz, 1H, H-6a), 4.13 – 3.95 (m, 3H, H-6b, CH$_2$OCO), 3.90 (d, J=9.6 Hz, 1H, H-2), 3.79 (dd, 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, CH$_3$(CH$_2$CH$_3$)), 2.02 (s, 6H, 2x CH$_3$), 1.69 – 1.51 (m, 2H, CH$_2$(CH$_2$OCO)), 1.50 – 1.31 (m, 2H, CH$_3$(CH$_2$CH$_2$)) ppm. **α/β-Anomers:** $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$=171.4, 170.8, 170.7, 169.5, 169.3, 168.8, 156.1, 156.0 (5x COαβ), 138.4 (CH$_3$(CH$_2$)), 115.0 (CH$_2$(CH$_3$)), 92.7 (C-1β), 91.0 (C-1α), 73.0, 72.5, 70.8, 69.8, 68.0, 67.8 (C-3αβ, C-4αβ, C-5αβ), 65.6 (CH$_2$OCO), 61.8 (C-6β), 61.7 (C-6α), 55.0 (C-2β), 52.9 (C-2α), 33.4 (CH$_2$(CH$_2$OCO)), 28.5 (CH$_2$OCOβ), 28.4 (CH$_2$(CH$_2$OCA)), 25.1 (CH$_2$(CH$_2$CH$_2$)), 21.0 (CH$_3$α), 21.0, 20.8 (2x CH$_3$β), 20.8, 20.8 (2x CH$_3$α), 20.7, 20.7 (2x CH$_3$β), 20.7 (CH$_3$α) ppm; HRMS: m/z calcd for C$_{24}$H$_{32}$NO$_{11}$: 496.17893 [M + Na]$^+$, found: 496.17678.
But-3-en-1-yl propylcarbamate 17

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). Carbnamate 17 was obtained as colorless oil (2.54 g, 96%).

Rf=0.86 (petroleum ether/ethyl acetate 1:1). $^1$H NMR: (400 MHz, CDCl$_3$): $\delta$=5.78 (ddt, $J$=17.0, 9.9, 6.7 Hz, 1H, CHCH$_2$), 5.18 – 4.95 (m, 2H, CHCH$_2$), 4.69 (s, 1H, NH), 4.09 (t, $J$=6.7 Hz, 2H, OCH$_2$), 3.12 (q, $J$=6.8 Hz, 2H, CH$_3$CH$_2$CH$_2$), 2.36 (q, $J$=6.8 Hz, 2H, OCH$_2$CH$_2$), 1.50 (h, $J$=7.3 Hz, 2H, CH$_3$CH$_2$), 0.90 (td, $J$=7.4, 1.0 Hz, 3H, CH$_3$) ppm. $^{13}$C NMR: (101 MHz, CDCl$_3$): $\delta$=156.7 (NHCO), 134.5 (CHCH$_2$), 117.1 (CHCH$_2$), 63.9 (OCH$_2$), 42.8 (CH$_3$CH$_2$CH$_2$), 33.7 (OCH$_2$CH$_2$), 23.4 (CH$_3$CH$_2$), 11.3 (CH$_3$) 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$_2$Cl$_2$) to afford a mixture of isomers 20a and 20b in 77%. Isomers could be separated by HPLC (30-60% MeCN in H$_2$O containing 0.1% formic acid in 30 min, R$_t$ 20a = 13.5 min, R$_t$ 20b = 15 min).
$R_f=0.88$ (CH$_2$Cl$_2$/MeOH 10:1). **20a**: $^1$H NMR (400 MHz, CDCl$_3$): $\delta=9.00$ (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, CH$_2$CH$_2$OCO or CH$_2$CH$_2$OCO), 3.48 (q, $J=6.7$ Hz, 2H, CCONHCH$_2$), 3.08 (p, $J=6.3$ Hz, 4H, CH$_3$CH$_2$OCO or CH$_2$CH$_2$OCO and OCONHCH$_2$), 1.69 (q, $J=7.3$ Hz, 2H, CCONHCH$_2$CH$_2$), 1.46 (q, $J=7.2$ Hz, 2H, OCONHCH$_2$CH$_3$), 1.02 (t, $J=7.4$ Hz, 3H, OCONHCH$_2$CH$_2$), 0.87 (t, $J=7.4$ Hz, 3H, OCONHCH$_2$CH$_2$)$_2$ ppm. **20b**: $^1$H NMR (600 MHz, CDCl$_3$): $\delta=8.98$ (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, CH$_2$CH$_2$OCO or CH$_2$CH$_2$OCO), 3.48 (q, $J=6.7$ Hz, 2H, CCONHCH$_2$), 3.33 (t, $J=6.4$ Hz, 2H, CH$_3$CH$_2$OCO or CH$_2$CH$_2$OCO), 3.08 (q, $J=6.8$ Hz, 2H, OCONHCH$_2$), 1.69 (q, $J=7.3$ Hz, 2H, CCONHCH$_2$CH$_2$), 1.47 (q, $J=7.3$ Hz, 2H, OCONHCH$_2$CH$_3$), 1.03 (t, $J=7.4$ Hz, 3H, OCONHCH$_2$CH$_2$), 0.88 (t, $J=7.4$ Hz, 3H, OCONHCH$_2$CH$_2$)$_2$ ppm. **20a/b**: $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta=167.1, 162.0, 161.9$ (C$_{\text{quart}}$), 157.9 (C-4" and C-6"), 139.5, 136.8, 135.6 (C$_{\text{quat}}$), 129.6, 127.4, 127.2 (C-2, C-3, C-5, C-6, C-5'), 121.2 (C-5''), 62.7 (CH$_2$CH$_2$OCO or CH$_2$CH$_2$OCO), 42.7 (CCONHCH$_2$), 41.9 (OCONHCH$_2$), 31.6 (CH$_3$CH$_2$OCO or CH$_2$CH$_2$OCO), 23.1, 22.9 (CCONHCH$_2$CH, OCONHCH$_2$CH$_2$), 11.5, 11.1 (OCONHCH$_2$CH$_2$CH$_2$, CCONHCH$_2$CH$_2$)$_2$ 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 tautomers. The first two peaks correspond to in situ-formed oxidation products (20a/b). Conditions: Binary gradient of CH$_3$CN in H$_2$O 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

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\text{\textsuperscript{1}H NMR spectrum (CDCl}_3, 400 MHz) of allyl succinimidyl carbonate 8.}
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\text{\textsuperscript{13}C NMR spectrum (CDCl}_3, 101 MHz) of allyl succinimidyl carbonate 8.}
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$^1$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$_4$ManNAloc 12.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$ManNAloc 12.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$ManNBeoc 13.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$ManNBeoc 13.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$ManNHeoc 15.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$ManNHeoc 15.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$GlcNAloc 23.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$GlcNAloc 23.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$GlcNBeoc 24.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$GlcNBeoc 24.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$GlcNP$\text{e}c$ 25.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$GlcNP$\text{e}c$ 25.
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of Ac$_4$GlcNHeoc 26.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of Ac$_4$GlcNHeoc 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.