Simple, Efficient Route to O- and S-Linked Carbohydrate Functionalized N-Carboxyanhydrides (GlycoNCAs)

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General Experimental Details

All chemicals were used as supplied unless otherwise stated. N-Boc-L-threonine (>98%), N-Boc-L-serine (>98%) and N-Boc-L-cysteine were purchased from Fluka. Acetobromo-α-D-glucose (>95%), acetobromo-α-D-galactose (>95%), iodine (>99.8%), potassium carbonate (>99%), trifloroacetic acid (>98%), sulfuric acid (95-98%) and potassium carbonate (>99.5%) were purchased from Aldrich. Triphosgene (>96%) and triethylamine (>98%) were purchased from Lancaster synthesis. THF, MeCN and DCM (>99.5%) were purchased from Fischer Scientific and dried by passage through two alumina columns using an Innovative Technology Inc. solvent purification system and stored under N₂. Anhydrous ethyl acetate (>99.8%, <0.005% H₂O) was purchased from Aldrich. Hexane (Fischer Scientific >99%) was dried over 3A molecular sieves. 3A molecular sieves (Aldrich) were

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activated in an oven at 200°C before use. Glass-backed silica plates (Biotage) were used for TLC with 5wt % phosphomolybdic acid in ethanol, developed at 80°C as the visualising agent. Column chromatography was undertaken on a Biotage SP1 flash chromatography unit using UV detectors at 200 and 236nm. Gradients were calculated the on-board software using Rf values from TLC.

NMR spectroscopy ($^1$H, $^{13}$C, COSY, NOESY, HSQC) was conducted on a Varian Inova-500 at 500Mhz. Mass spectral analyses were performed on a Micromass LCT using positive or negative electrospray mode. Infrared spectroscopy was conducted on a Nicolet Nexus FT-IR as a KBr disc. Liquid samples were analysed by direct injection of the reaction medium into a liquid cell with KBr windows. Elemental analyses were conducted on an Exeter Analytical E-440 elemental analyser.
GENERAL EXPERIMENTAL PROCEDURE

The same methodology was applied for the synthesis of each glycosylated NCA, with only the carbohydrate or N-Boc amino acid varying in each case. The full procedure for the synthesis of Thr(GluAc) NCA is detailed below.

Synthesis of \( N^\alpha-(\text{Butoxycarbonyl})-3-O-(2,3,4,6\text{-tetra-O-acetyl-}\beta-D-\text{glucopyranosyl})-L\text{-threonine} - \text{Boc} \text{Thr(GluAc)} – 2(a) \)

\( N\)-Boc-L-threonine (3.0g, 13.6mmol), potassium carbonate (1.41g, 10.2mmol) and acetobromoglucose (2.79g, 6.8mmol) were dissolved in 40 ml of dry acetonitrile under an \( N_2 \) atmosphere with stirring for 5 minutes to ensure dissolution. Iodine (2.59g, 10.2mmol) was then added in a single portion against a flow of \( N_2 \). The vessel was sealed and allowed to stir at ambient temperature with the exclusion of light for 5 hours.

To the still stirring solution, saturated sodium thiosulfate (aqueous) was added until the deep red colouration had disappeared, to leave a slightly yellow solution. The insoluble components (residual potassium carbonate) were removed by filtration and the filtrate concentrated to ¼ of its original volume under reduced pressure on a rotory evaporator. 40 mL of dichloromethane were then added, and the solution extracted with sodium bicarbonate (aqueous 5% w/v, 50 mL) followed by brine (2 x 50mL), and the organic layer dried over magnesium sulphate. Separation by column chromatography (Hexane/THF 10:1 \( \rightarrow \) 2:8) yielded the title product as a white solid. Isolated yield 2.05 g, 55%.
Synthesis of $3-O-(2,3,4,6$-tetra-$O$-acetyl-$\beta$-$D$-glucopyranosyl)-$L$-threonine acetic acid salt – Thr(GluAc) – 2(b)

BocThr(GluAc) (1g, 1.82 mmol) was dissolved in 10 mL of DCM under N$_2$. TFA (0.7 ml, 9.11 mmol) was then added dropwise and allowed to stir at ambient temperature for 90 minutes. The solution was then concentrated under reduced pressure on a rotory evaporator to give a thick syrup. The syrup was taken up in 20 mL of 10% w/v acetic acid(aq) solution and extracted 3 times with 20 mL portions of n-hexane. The aqueous solution was freeze dried, and re-precipitated from CHCl$_3$ to di-ethyl ether to afford the title product without further purification as an off-white solid: 0.90 g, 97% yield.

Synthesis of $3-O-(2,3,4,6$-tetraacetyl)$\beta$-$D$-glucopyranose)-$L$-threonine-$N$-carboxyanhydride - Thr(GluAc)NCA – 2(c)

Thr(GluAc) (100mg, 0.20 mmol) was taken up in 5 mL of EtOAc under N$_2$. Triphosgene (39mg, 0.13 mmol) was added in a single portion, against the flow of N$_2$ followed by $\alpha$-pinene (0.37mL, 2.34 mmol) and allowed to stir at ambient temperature for 20 hours then concentrated to $\frac{1}{2}$ of its original volume on a rotary evaporator under reduced pressure, with the water bath below 30°C. The concentrate was precipitated into hexane at -20°C. This was re-precipitated from ethyl acetate into hexane at -20°C three times. The white solid was obtained by centrifugation following each precipitation, and finally dried under vacuum to give a white solid. Yield 81mg, 87% yield.
CHARACTERISATION

As indicated in the main text, NCA polymerisation is often complicated by residual amino acid or acidic impurities remaining from the synthetic procedure. Here acidic impurities were removed by the HCl scavenger α-pinene. In the 1H NMR spectra of the NCAs, trace amounts of amino acid residues (appearing slightly shifted relative to the main peaks) can be seen. Additional purification is possible (by an aqueous extraction) but this should only be conducted prior to polymerisation, as further degradation reactions will occur rapidly negating any increase in purity. This purification was not undertaken, allowing comparison with other published glycoNCA synthetic procedures.

\[
\text{N}^\alpha-(\text{Butoxycarbonyl})-3-O-(2,3,4,6-tetra-O-acetyl-\beta-D-galatopyranosyl)-L-threonine - BocThr(GalAc) – 1(a)
\]

\[\text{H NMR (CDCl}_3\text{)}\ \delta_{\text{ppm}}:\ 5.73\ (1H, d, J_{1,2} = 8.26\text{Hz, }H^1),\ 5.42\ (1H, dd, J_{4,3} = 3.41\text{Hz, }J_{4,5} = 0.94, \ H^5),\ 5.33\ (1H, dd, J_{2,1} = 8.26\text{Hz, }J_{2,3} = 10.44\text{Hz, }H^3),\ 5.08\ (1H, dd, J_{3,4} = 3.41\text{Hz, }J_{3,2} = 10.44\text{Hz, }H^3),\ 4.22\ (1H, d, J_{7,16} = 9.2\text{ Hz, }H^7),\ 4.35\ (1H, m, H^8),\ 4.20\ (1H, dd, J_{6a-5} = 6.40\text{Hz, }J_{6a-6b} = 10.86\text{Hz, }H^{6a}),\ 4.07\ (1H, dd, J_{6b-5} = 6.14\text{Hz, }J_{6b-6a} = 10.89\text{Hz, }H^{6b}),\ 4.04\ (1H, td, J_{5-4} = 0.74\text{Hz, }J_{5-6a} = J_{5-6b} = 6.47\text{Hz, }H^5),\ 1.99 – 2.06\ (12H, m, H^{15}),\ 1.26\ (3H, d, J_{16-7} = 6.38\text{Hz, }H^{16}).
\]

\[\text{C NMR (CDCl}_3\text{)}\ \delta_{\text{ppm}}:\ 170.6\ (C^9),\ 169.9-170.1\ (4\times C^{14}),\ 156.0\ (C^{11}),\ 92.9\ (C^1),\ 80.1\ (C^{12}),\ 72.3,\ (C^5),\ 71.4\ (C^3),\ 67.9\ (C^2),\ 67.2\ (C^4),\ 62.3\ (C^8),\ 60.8\ (C^6),\ 59.0\ (C^7),\ 28.2\ (C^{13}),\ 20.5-20.7\ (4\times C^{15}),\ 19.9\ (C^{16}),
\]

\[\text{IR (KBr disc) cm}^{-1}:\ 3447\ (\text{br., }N-H),\ 2981\ (C-H),\ 1757\ (C=O\ Ac),\ 1718\ (C=O\ Boc).
\]

\[\text{MS (ES+)}\ m/z = 572.2\ [M+Na]^+ 100\%.
\]

**Elemental** (Found %) C, 50.48; H, 6.54; N, 2.36 (Expected %) C, 50.27; H, 6.42; N, 2.55.
\(\alpha\)-(Butoxy carbonyl)-3-\(O\)-(2,3,4,6-tetra-\(O\)-acetyl-\(\beta\)-d-glucopyranosyl)-L-threonine BocThr(GluAc) – 2(a)

\(^1H\) NMR (CDCl\(_3\)) \(\delta_{ppm}\): 5.75 (1H, d, \(J_{1-2} = 8.17\) Hz, \(H^1\)), 5.30 (1H, d, \(J_{8-7} = 9.6\) Hz, \(H^8\)), 5.26 (1H, t, \(J = 9.47\) Hz, \(H^3\)), 5.15 (1H, dd, \(J_{2-1} = 8.18\) Hz, \(J_{2-3} = 9.60\) Hz, \(H^2\)), 5.10 (1H, t, \(J = 9.70\) Hz, \(H^4\)), 4.33 (1H, m, \(H^8\)), 4.30 (1H, dd, \(J_{6b-5} = 5.02\) Hz, \(J_{6b-6a} = 12.44\) Hz, \(H^6b\)), 4.23 (1H, dd, \(J_{7-9} = 6.36\) Hz, \(J_{7-8} = 9.7\) Hz, \(H^7\)), 4.09 (1H, dd, \(J_{6a-5} = 2.19\) Hz, \(J_{6a-6b} = 12.36\) Hz, \(H^6a\)), 3.82 (1H, ddd, \(J_{5-6a} = 2.20\) Hz, \(J_{5-6b} = 4.96\) Hz, \(J_{5-4} = 10.07\) Hz, \(H^5\)), 2.01 – 2.06 (12H, m, \(H^{15}\)), 1.25 (3H, d, \(J_{16-7} = 6.39\) Hz, \(H^{16}\)).

\(^{13}C\) NMR (CDCl\(_3\)) \(\delta_{ppm}\): 170.9 (C\(^9\)), 169.4-167.0 (4xC\(^{14}\)), 156.0 (C\(^11\)), 92.4 (C\(^1\)), 80.1 (C\(^{12}\)), 72.9 (C\(^5\)), 72.5 (C\(^3\)), 70.0 (C\(^2\)), 67.8 (C\(^6\)), 67.5 (C\(^8\)), 61.3 (C\(^6\)), 58.9 (C\(^7\)), 28.2 (C\(^{16}\)), 20.5 – 20.7 (4xC\(^{15}\)), 19.9 (C\(^{16}\)).

IR (KBr disc) cm\(^{-1}\): 3447 (br., N-H), 2980 (C-H), 1757 (C=O Ac), 1718 (C=O Boc).

MS (ES+) m/z = 572.2 [M+Na\(^+\)] 100%.

Elemental (Found %) C, 50.16; H, 6.38; N, 2.35 (Expected %) C, 50.27; H, 6.42; N, 2.55.

\(\alpha\)-(Butoxy carbonyl)-3-\(O\)-(2,3,4,6-tetra-\(O\)-acetyl-\(\beta\)-d-galactopyranosyl)-L-serine - BocSer(GalAc) – 3(a)

\(^1H\) NMR (CDCl\(_3\)) \(\delta_{ppm}\): 5.73 (1H, d, \(J_{1-2} = 8.21\) Hz, \(H^1\)), 5.46 (1H, dd, \(J_{4-5} = 1.08\) Hz, \(J_{4-3} = 3.35\) Hz, \(H^4\)), 5.31 (1H, dd, \(J_{2-1} = 8.28\) Hz, \(J_{2-3} = 10.43\) Hz, \(H^2\)), 5.08 (1H, dd, \(J_{3-4} = 3.45\) Hz, \(J_{3-2} = 10.45\) Hz, \(H^3\)), 1.99 – 2.06 (12H, m, \(H^{15}\)), 1.43 (9H, s, H\(^{13}\)).

\(^{13}C\) NMR (CDCl\(_3\)) \(\delta_{ppm}\): 169.2 – 170.5 (4xC\(^{14}\) and C\(^9\)), 155.0 (C\(^{11}\)), 89.8 (C\(^1\)), 80.3 (C\(^{12}\)), 72.1 (C\(^5\)), 70.8 (C\(^3\)), 67.5 (C\(^2\)), 66.7 (C\(^8\)), 62.0 (C\(^6\)), 61.0 (C\(^6\)), 57.7 (C\(^7\)), 29.2 (C\(^{15}\)), 20.4 – 20.7 (4xC\(^{15}\)).

IR (KBr disc) cm\(^{-1}\): 3411 (br., N-H), 2980 (C-H), 1757 (C=O Ac), 1718 (C=O Boc).

MS (ES+) m/z = 558.2 (85%) [M+Na\(^+\)]\(^\dagger\), 599.2 (100%) [M+Na+CH\(_3\)CN\(^+\)].

Elemental (Found %) C, 49.12; H, 6.30; N, 2.30 (Expected %) C, 49.34; H, 6.21; N, 2.62.
$\alpha$-(Butoxycarbonyl)-3-O-(2,3,4,6-tetra-O-acetyl-$\beta$-D-glucopyranosyl)-L-serine  - (BocSer(GluAc) – 4(a))

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 5.75 (1H, d, $J_{1-2} = 8.13$Hz, H$^1$), 5.40 (1H, dd, $J_{8-7a} = 2.79$Hz, $J_{8-7b} = 6.67$Hz, H$^8$), 5.26 (1H, t, $J = 9.38$Hz, H$^3$), 5.14 (1H, dd, $J_{2-1} = 8.15$Hz, $J_{2-3} = 9.47$Hz, H$^2$), 5.11 (1H, t, $J = 9.90$Hz , H$^4$), 4.35 (1H, m, H$^{7b}$), 4.28 (1H, dd, $J_{6b-5} = 4.81$Hz, $J_{6b-6a} = 12.45$Hz, H$^{6b}$), 4.10 (1H, dd, $J_{6a-5} = 2.20$Hz, $J_{6a-6b} = 12.48$Hz, H$^{6a}$), 4.02 (1H, m, H$^{7a}$), 3.83 (1H, ddd, $J_{5-6a} = 2.15$Hz, $J_{5-6b} = 4.65$Hz, $J_{5-4} = 9.95$Hz, H$^5$), 1.99 – 2.07 (12H, m, H$^{15}$), 1.43 (9H, s, H$^{13}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 170.8 (C$^9$), 169.2 – 170.0 (4x C$^{14}$), 155.1 (C$^{11}$), 92.4 (C$^1$), 72.8 (C$^5$), 72.4 (C$^3$), 71.1 (C$^{12}$), 70.0 (C$^2$), 67.6 (C$^4$), 62.3 (C$^8$), 61.0 (C$^6$), 56.2 (C$^7$), 28.0 (C$^{13}$), 20.5 – 20.7 (4xC$^{15}$).

IR (KBr disc) cm$^{-1}$: 3447 (br., N-H), 2979 (C-H), 1753 (C=O Ac), 1717 (C=O Boc). MS (ES+) m/z = 558.2 (100%) [M+Na]$^+$. 

Elemental (Found %) C, 49.52; H, 6.25; N, 2.10 (Expected %) C, 49.34; H, 6.21; N, 2.62.

$\alpha$-(Butoxycarbonyl)-3-O-(2,3,4,6-tetra-O-acetyl-$\beta$-D-galactopyranosyl)-(1-4)-1,2,3,4,6-tetra-O-acetyl-$\beta$-D-glucopyranose)-L-serine - BocSer(LacAc) – 5(a)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 5.77 (1H, d, $J_{1-2} = 7.50$Hz, H$^1$), 5.72 (1H, d, $J_{1-2'} = 9.00$Hz, H$^{1'}$), 5.54 (1H, t, $J = 9.68$Hz, H$^3$), 5.41 (1H, t, $J = 3.50$Hz, H$^4$), 5.30 (1H, d, $J_{3-2'} = 9.52$ Hz, H$^{3'}$), 5.25 (1H, t, $J = 9.00$ Hz, H$^3$), 5.07-5.15 (2H, m, H$^{2'},H^2$), 4.24-4.31 (1H, m, H$^{6b}$), 4.01-4.17 (4H, m, H$^{6a}$,$H^{6b}$,$H^{6a}$,$H^5$), 3.88 (1H, td $J_{5-6} = 6.00$Hz, $J_{5-4} = 1.50$Hz, H$^5$), 3.72 (1H, t, $J_{5-4} = 6.00$ Hz, H$^3$), 1.96 - 2.16 (21H, m, H$^{15}$), 1.41 - 1.44 (9H, s(b), H$^{13}$).
**$^{13}$C NMR** (CDCl$_3$) $\delta$ ppm: 171.1 (C$^9$), 166.5-170.3 (4 x C$^{14}$), 157 (C$^{11}$), 94.0 (C$^{1'}$), 92.6 (C$^{1}$), 73.1 (C$^{4}$), 72.6 (C$^{2}$), 72.1 (C$^{3}$), 71.4 (C$^{5}$), 70.7 (C$^{2'}$), 70.3 (C$^{3'}$), 68.3 (C$^{2'}$), 66.9 (C$^{4'}$), 61.8 (C$^{6}$), 61.1 (C$^{6'}$), 30.2 (C$^{13}$), 20.7-20.9 (4xC$^{15}$).

**MS** (ES+) m/z = 887.7 [M+Na+CH$_3$CN]$^+$.  
**Elemental** Found (%) C 49.39, H 6.33, N 2.22, Expected (%)C 49.57; H 6.00; N 1.70  
**IR** (KBr disc) cm$^{-1}$: 3450 (br., N-H), 2970 (CH), 1755 (C=O Ac), 1717 (C=O Boc).

$N^\alpha$-(Butoxycarbonyl)-3-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-cysteine - BocCys(GluAc) – 6(a)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 6.25 (1H, d, J$_{1-2}$ = 9.11Hz, H$^1$), 5.49 (1H, d, J$_{4-3}$ = 3.50Hz, H$^4$), 5.29 (1H, t, J$_{2-1}$ = 9.00 Hz, H$^2$), 5.10 -5.20 (4H, m, H$^7$a, H$^7$b, H$^8$, H$^7$), 4.93 (1H, dd, J$_{5-4}$ = 3.5Hz, J$_{5-6}$ = 6.50Hz, H$^5$), 4.88 (1H, dd, J$_{6a-5}$ = 8.00Hz, J$_{6a-6b}$ 2.00Hz, H$^{6a}3.91$ (1H, dd, J$_{6a-6b}$ = 4.00Hz, J$_{6b-5}$, 9.50Hz, H$^{6b}$), 2.04 - 2.11 (12H, m, H$^{15}$), 1.36 - 1.38 (9H, s(b), H$^{13}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 171.5 (C$^0$), 169.5-170.5 (4 x C$^{14}$), 158 (C$^{11}$), 90.1 (C$^{1'}$), 70.1 (C$^{2'}$), 69.8 (C$^{3'}$), 68.7 (C$^{5}$), 67.7 (C$^{6}$), 67.3 (C$^{3}$), 62.2 (C$^{6'}$), 61.8 (C$^{7}$), 32.9 (C$^{13}$), 20.8 - 21.2 (4xC$^{15}$).

**MS** (ES+) m/z = 615.4 [M+Na+CH$_3$CN]$^+$.  
**IR** (KBr disc) cm$^{-1}$: 3450 (br., N-H), 2980 (C-H), 1757 (br., C=O Ac), 1715 (C=O Boc).
**N^α-(Butoxycarbonyl)-3-S-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-L-cysteine - BocCys(GalAc) – 7(a)**

**1H NMR** (CDCl₃) δ ppm: 6.04 (1H, d, J₁-₂ = 6.00Hz, H₁), 5.46 (1H, dd, J₄-₃ = 2.50Hz, J₄-₅ = 6.00Hz, H₄), 5.15-5.23 (2H, m, H₂, H₃), 4.99 (1H, dd, J₆b-₆a = 3.50Hz, J₆b-₅ = 4.50Hz, H₆b) 4.69 (1H, dd, J₆a-b = 2.50Hz, J₆a-₅ = 7.50Hz, H₆a), 4.39 (1H, d, J₄-₅ = 6.50Hz, H₄), 4.14 (1H, ddd, J₅-₆a = 3.00Hz, J₅-₆b 3.60Hz, J₅-₄ = 7.06Hz, H₅), 2.05 - 2.15 (12H, m, H₁₅), 1.42 - 1.44 (9H, s(b), H₁₃).

**13C NMR** (CDCl₃) δ ppm; 171.1 (C⁹), 169.2 -170.4 (4 x C¹⁴), 154.0 (C¹¹), 91.1 (C¹), 70.1 (C⁵), 69.6 (C³), 68.8 (C²), 68.3 (C⁴), 67.3 (C⁸), 62.2 (C⁶), 61.7 (C⁷), 33.2 (C¹³), 20.7 - 21.1 (4xC¹⁵).

**IR** (KBr disc) cm⁻¹: 3450 (br., N-H), 2985 (C-H), 1754 (br., C=O Ac), 1713 (C=O Boc).

**MS (ES⁺) m/z = 574.9 [M+Na]^⁺.**

**3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-L-threonine acetic acid salt - Thr(GalAc) – 1(b)**

**1H NMR** (CDCl₃) δ ppm: 5.96 (1H, d, J₁-₂ = 8.07Hz, H₁), 5.45 (1H, d, J₄-₃ = 2.89Hz, H₄), 5.31 (1H, dd, J₂-₁ = 8.25Hz, J₂-₃ = 10.10Hz, H₂), 5.22 (1H, dd, J₃-₂ = 10.44Hz, J₃-₄ = 3.04Hz, H₃), 4.39 (1H, q, J₇-₁ = J₇-₈ = 6.27Hz, H₇), 4.19 (1H, d, J₆b-₆a = 10.31Hz, J₆b-₅ = 5.91Hz, H₆b), (1H, dd, J₆a-b = 10.84Hz, J₆a-₅ = 5.92Hz, H₆a), 4.08 (1H, d, J₅-₆a = 6.62Hz, J₅-₆b = 7.88Hz, H₅), 3.97 (1H, m, H₈), 1.95 – 2.06 (15H, m, H₁₁ + H¹₃), 1.41 (1H, d, J₁₅-₇ = 5.88, H₁₅).

**13C NMR** (CDCl₃) δ ppm; 175.6 (C¹⁴), 179.8 – 171.0 (4xC¹²), 166.6 (C⁹), 93.7 (C¹), 71.6 (C⁵), 70.3 (C³), 67.5 (C²), 66.6 (C⁴), 65.8 (C⁷), 61.9 (C⁵), 60.6 (C⁶), 59.2 (C⁸), 19.5 – 20.9 (4xC¹¹, C¹³, C¹⁵).

**MS (ES⁺) m/z = 449.9 [M]^⁺ (100%).**
3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-threonine acetic acid salt - Thr(GluAc) – 2(b)

$^1$H NMR (CDCl$_3$) $\delta$ ppm; 6.00 (1H, d, J$_{1-2}$ = 8.12Hz, H$_1$), 5.35 (1H, t, J = 9.41Hz, H$_3$), 5.12 (2H, t, 9.24Hz, H$_2$ + H$_4$), 4.35 (1H, q, J$_{7-8}$ = J$_{7-15}$ = 6.14Hz, H$_7$), 4.25 (1H, dd, J$_{6a-6b}$ = 12.37Hz, J$_{6a-5}$ = 2.74Hz, H$_{6a}$), 4.15 – 4.22 (2H, m, H$_8$ + H$_{6b}$), 4.06 (1H, d, J(b), J = 9.95Hz, H$_5$), 1.99 – 2.14 (12H, m, H$_{11}$ + H$_{13}$), 1.49 (3H, d, J$_{15-7}$ = 6.08Hz, H$_{15}$).

MS (ES+) m/z = 449.9 [M$^+$] (100%).

3-O-((2,3,4,6-tetra-O-acetyl)-β-D-glucopyranosyl)-L-serine acetic acid salt - Ser(GluAc) – 3(b)

$^1$H NMR (CDCl$_3$) $\delta$ ppm; 5.90 (1H, d, J$_{1-2}$ = 8.58Hz, H$_1$), 5.33 (1H, td, J$_{2-3}$ = 2.00Hz, J$_2$. 1 = 8.5Hz, H$_2$), 5.29 (1H, t, J$_{3-4}$ = J$_{3-2}$ = 5.00Hz, H$_3$), 5.21 (1H, dd, J$_{4-5}$ = 3.50Hz, J$_4$-$_3$ = 7.45Hz, H$_4$), 4.22 (1H, t, J$_{8-7}$ = 4.50Hz, H$_8$), 4.02-4.14 (4H, m, H$_6a$, H$_{6b}$, H$_7b$, H$_5$), 3.86 (1H, dd, J$_{7a-7b}$ = 3.63Hz, J$_{7a-8}$ = 4.43Hz, H$_{7a}$), 1.98- 2.18 (15H, m, H$_{11}$, H$_{13}$)

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm; 166.6 - 171 (C$^9$, 4xC$^{12}$, C$^{14}$), 98.9 (C$^1$), 72.2 (C$^5$), 71.0 (C$^3$), 69.8 (C$^2$), 67.4 (C$^4$), 66.2 (C$^8$), 60.3 (C$^8$), 59.1 (C$^6$), 58.9 (C$^7$), 18.4 - 21.4 (4xC$^{11}$, C$^{13}$).

MS (ES+) m/z = 435.0[M$^+$] 80%, 458.7[M+Na$^+$] 20%.
3-O-((2,3,4,6-tetra-O-acetyl)-β-D-galactopyranosyl)-L-serine acetic acid salt - Ser(GalAc) – 4(b)

$^1$H NMR (CDCl$_3$) $\delta$ppm: 6.04 (1H, d, J$_{1-2} = 6.00$Hz, H$_1$), 5.05 (1H, dd, J$_{3-4} = 3.5$Hz, J$_{3-2} = 4.5$Hz, H$_3$), 5.46 (1H, m, H$_{6b}$), 4.91 (1H, t, J = 4.50Hz, H$_2$), 4.80 (1H, m, H$_{6a}$), 4.69 (1H, dd, J$_{7a-7b} = 2.50$Hz, J$_{7a-8} = 7.5$Hz, H$_{7a}$), 4.39 (1H, t, J$_{4-5} = 4.5$Hz, H$_4$), 4.14 (1H, ddd, J$_{5-6a} = 3.00$Hz, J$_{5-6b} = 3.60$Hz, J$_{5-4} = 7.06$Hz, H$_5$), 2.05 - 2.15 (12H, m, H$_{11}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ppm: 174.8 (C$_{14}$), 168.2-170.6 (C$_9$, 4xC$_{12}$), 99.1 (C$_1$), 72.3 (C$_4$), 71.0 (C$_3$), 69.1 (C$_2$), 65.2 (C$_8$), 60.2 (C$_6$), 59.3 (C$_6$), 59.0 (C$_7$), 20.5 - 21.2 (4xC$_{11}$).

MS (ES+): m/z = 435.1[M$^+$] 100%.

3-O-((2,3,4,6-tetra-O-acetyl)-β-D-galactopyranosyl-(1-4)-(2,3,4,6-tetra-O-acetyl)-β-D-glucopyranose)-L-serine acetic acid salt - Ser(LacAc) – 5(b)

$^1$H NMR (CDCl$_3$) $\delta$ppm: 5.77 (1H, d, J$_{1-2} = 7.50$Hz, H$_1$), 5.72 (1H, d, J$_{1'-2'} = 9.00$Hz, H$_{1'}$), 5.54 (1H, t, J = 9.68Hz, H$_4'$), 5.41 (1H, t, J = 3.5Hz, H$_{5'}$), 5.30 (1H, t, J$_{2-3} = 9.52$Hz, H$_3$), 5.25 (1H, t, J$_{2-3} = 9.00$Hz, H$_2$), 5.07-5.15 (2H, m, H$_{2'}$, H$_{2'}$), 4.24 - 4.31 (1H, m, H$_{6b}$), 4.01-4.17 (4H, m, H$_{6a}$, H$_{6b}$, H$_{6a}$, H$_{5'}$), 3.88 (1H, td, J$_{4-5} = 6.0$Hz, J$_{5-6} = 1.5$Hz, H$_5$), 3.72 (1H, t, J$_{4-5} = 6.00$Hz, H$_5$), 1.96 - 2.16 (24H, m, H$_{11}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ppm: 166.5 - 171.1 (C$_9$, 4xC$_{12}$, C$_{14}$), 94.0 (C$_1'$), 92.6 (C$_1$), 73.1 (C$_4$), 72.6 (C$_5$), 72.1 (C$_3$), 71.4 (C$_2$), 70.7 (C$_8$), 70.3 (C$_8$), 68.3 (C$_6$), 66.9 (C$_6$), 61.6 (C$_7$), 61.1 (C$_7$), 20.7-20.9 (4xC$_{11}$, C$_{13}$). MS (ES+) m/z = 723.6 (100%), [M$^+$].
3-S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-cysteine acetic acid salt - Cys(GluAc) – 6(b)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 6.12 (1H, d, J$_{1-2} = 6$Hz, H$^1$), 5.38 (1H, d, J$_{2-3} = 3.5$Hz, H$^2$), 5.29 (1H, t, J$_{4-5} = 9.0$Hz, H$^4$), 5.29 (1H, t, J$_{4-5} = 9.0$Hz, H$^4$), 5.06 (4H, m, H$^6$a, H$^6$b, H$^7$a, H$^7$b), 4.90 (1H, dd, J$_{7b-7a} = 3.5$Hz, J$_{7b-8} = 6.5$Hz, H$^7$b), 4.79 (1H, dd, J$_{1-2} = 8.0$Hz, J$_{3-4} = 2.0$Hz, H$^3$), 3.93 (1H, dd, J$_{5-6} = 4.0$Hz, J$_{4-5} = 9.5$Hz, H$^5$), 1.99 - 2.10 (15H, m, H$^{11}$, H$^{13}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 169.5 - 171.5 (4xC$^{12}$, C$^{14}$), 166.4 (C$^9$), 90.1 (C$^1$), 70.1 (C$^5$), 69.8 (C$^3$), 68.7 (C$^2$), 67.7 (C$^4$), 67.3 (C$^8$), 62.2 (C$^6$), 61.8 (C$^7$), 20.8 - 21.2 (4xC$^{11}$, C$^{13}$).

MS (ES+) m/z = 451.1 [M$^+$].

3-S-((2,3,4,6-tetra-O-acetyl)-β-D-galactopyranosyl)-L-cysteine acetic acid salt - Cys(GalAc) – 7(b)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 6.02 (1H, d, J$_{1-2} = 6.0$Hz, H$^1$), 5.33 (1H, dd, J$_{2-1} = 6.1$Hz, J$_{2-3} = 2.5$Hz, H$^2$), 5.05 (1H, dd, J$_{3-4} = 3.5$Hz, J$_{2-3} = 4.5$Hz, H$^3$), 4.61 - 4.81 (3H, m, H$^6$a, H$^6$b, H$^8$), 4.55 (1H, dd, J$_{7a-7b} = 2.5$Hz, J$_{7b-8} = 7.5$Hz, H$^7$a), 4.38 (1H, t, J$_{3-4} = 6.5$Hz, H$^4$), 4.12 (1H, ddd, J$_{5-6a} = 3.0$Hz, J$_{5-6b} = 3.6$Hz, J$_{5-4} = 7.0$Hz, H$^5$), 1.99 - 2.15 (15H, m, H$^{11}$, H$^{13}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 169.2 - 171.1 (4xC$^{12}$, C$^{14}$), 166.9 (C$^9$), 91.1 (C$^1$), 70.1 (C$^5$), 69.6 (C$^3$), 68.8 (C$^2$), 68.3 (C$^4$), 67.3 (C$^8$), 62.5 (C$^6$), 62.2 (C$^7$), 18.2 - 21.1 (4xC$^{11}$, C$^{13}$).

MS (ES+) m/z = 451.0 [M + Na$^+$].
3-\textit{O}-(2,3,4,6-tetra-\textit{O}-acetyl)-\textit{\textbeta}-D-galactopyranose)-L-threonine-\textit{N}-
carboxyanhydride - (Thr(GalAc)NCA – 1(c)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 6.16 (1H, s, H$_9$), 5.68 (1H, d, J$_{1-2} = 8.18$Hz, H$_1$), 5.43 (1H, d, J$_{4-3} = 2.69$ Hz, H$_4$), 5.29 (1H, dd, J$_{2-3} = 10.40$Hz, J$_{2-1} = 8.22$Hz, H$_2$), 5.11 (1H, dd, J$_{3-2} = 10.45$Hz, J$_{3-4} = 3.88$Hz, H$_3$), 4.80 (1H, m, H$_7$), 4.00 (1H, d, J$_{8-7} = 4.83$Hz, H$_8$), 1.96 – 2.19 (12H, m, H$_{12}$), 1.55 (3H, d, J$_{14-7} = 6.32$Hz, H$_{14}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 169.8 – 170.4 (4 x C$_{13}$), 168.0 (C$_{11}$), 157.9 (C$_{10}$), 93.3 (C$_{1}$), 75.4 (C$_{7}$), 72.2 (C$_{5}$), 70.5 (C$_{3}$), 67.9 (C$_{2}$), 66.8 (C$_{4}$), 61.1 (C$_{6}$), 60.2 (C$_{8}$), 20.5 – 21.0 (4 x C$_{12}$, C$_{14}$).

HRMS (ES$^+$) m/z = 498.1230 [M+Na]$^+$; expected 498.1218

3-\textit{O}-(2,3,4,6-tetra-\textit{O}-acetyl)-\textit{\textbeta}-D-glucopyranose)-L-threonine-\textit{N}-
carboxyanhydride - Thr(GluAc)NCA – 2(c)

$^1$H NMR (CDCl$_3$) $\delta$ ppm: 6.30 (1H, s, H$_9$), 5.72 (1H, d, J$_{1-2} = 8.14$Hz, H$_1$), 5.26 (1H, t, J = 9.41Hz, H$_3$), 5.12 (2H, m, H$_2^2 + H_2^1$), 4.76 (1H, m, H$_7$), 4.28 (1H, dd, J$_{6b-6a} = 12.58$Hz, J$_{6b-6d} = 4.52$Hz, H$_{6b}$), 4.09 (1H, dd, J$_{6a-6b} = 12.30$Hz, J$_{6a-6d} = 1.14$Hz, H$_{6a}$), 4.00 (1H, d, J$_{6-7} = 4.85$Hz, H$_5$), 3.87 (1H, dd, J$_{6-7} = 10.99$Hz, J$_{5-6} = 2.33$Hz, H$_6$), 1.98 – 2.10 (12H, m, H$_{12}$), 1.54 (3H, d, J$_{14-7} = 6.24$Hz, H$_{14}$).

$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 169.3 – 170.5 (4 x C$_{13}$), 168.8 (C$_{11}$), 157.7 (C$_{10}$), 92.8 (C$_{1}$), 75.0 (C$_{7}$), 72.9 (C$_{5}$), 72.2 (C$_{3}$), 69.9 (C$_{2}$), 67.6 (C$_{4}$), 61.3 (C$_{6}$), 59.9 (C$_{8}$), 20.5 – 21.0 (4 x C$_{12}$, C$_{14}$).

HRMS (ES$^+$) m/z = 476.1396 [M+H]$^+$; Expected 476.1399.

((2,3,4,6-tetra-\textit{O}-acetyl)-\textit{D}-galactopyranosyl)-L-serine-\textit{N}-
carboxyanhydride - Ser(GalAc)NCA – 3(c)
1H NMR (CDCl₃) δ ppm: 6.71 (1H, s, H⁹), 6.04 (1H, d, J₁,₂ = 6Hz, H¹), 5.45 (1H, dd, J₃, = 2.00Hz, J₃,₄ = 3.50Hz, H³), 5.07 (1H, dd, J₂,₃ = 3.50Hz, J₁,₂ = 4.50Hz, H²), 4.35 - 4.48 (4H, m, H⁴, H⁶ₐ, H⁶ₕ, H⁸), 4.00 (1H, ddd, J₅,₆ₐ = 3.00Hz, J₅,₆ₕ = 3.60Hz, J₅,₄ = 7.06Hz, H⁵), 2.02 - 2.14 (H¹³).

13C NMR (CDCl₃) δ ppm: 167.2-170.1 (4xC¹³), 164.7 (C¹¹), 155.8 (C¹⁰), 99.1 (C¹), 72.6 (C⁵), 72.2 (C⁴), 71.0 (C³), 69.2 (C²), 60.4 (C⁸), 59.3 (C⁶), 59.0 (C⁷), 20.5 - 21.2 (4xC¹³).

HRMS (ES+) m/z = 484.1061 [M+Na⁺]; expected 484.1062.

3-O-((2,3,4,6-tetra-O-acetyl)-β-D-glucopyranosyl)-L-serine-N-carboxyanhydride - Ser(GluAc)NCA – 4(e)

1H NMR (CDCl₃) δ ppm: 6.07 (1H, s, H⁹), 5.75 (1H, d, J₁,₂ 8.00Hz, H¹), 5.25 (1H, td, J₂,₃ = 2.00Hz, J₂,₁ = 7.7Hz, H²), 5.11 (1H, dd, J₄,₃ = 7.50Hz, J₄,₁ = 3.00Hz, H₄), 4.77 (1H, m, H⁵), 4.21 (1H, t, J₃,₄ = 4.50Hz, H³), 4.06-4.19 (4H, m, H⁶ₐ, H⁶ₕ, H⁷ₐ, H⁸), 3.70 (1H, dd, J₇ₐ,₇b = 3.50Hz, J₇ₐ,₈ = 4.00Hz, H⁷ₐ), 1.99 - 2.19 (12H, m, H¹³).

13C NMR (CDCl₃) δ ppm: 168.7 -171.0 (4xC¹³), 165.3(C¹¹), 154.1(C¹⁰), 98.6 (C¹), 72.2 (C⁵), 71.0 (C³), 69.8 (C²), 66.2, 67.4 (C⁴), 60.1 (C⁸), 59.1 (C⁶), 58.9 (C⁷), 20.7 - 21.8 (4xC¹²).

HRMS (ES+) m/z = 484.1066 [M+Na⁺]; expected 484.1062.
3-O-((2,3,4,6-tetra-O-acetyl)-β-D-galactopyranosyl-(1-4)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose)-L-serine-N-carboxyanhydride - Ser(LacAc)NCA – 5(c)

\(^1\)H NMR (CDCl\(_3\)) \(\delta_{\text{ppm}}\): 7.67 (1H, s, H\(_9\)), 5.82 (1H, d, J\(_{1-2}\) = 7.50Hz, H\(^1\)), 5.76 (1H, d, J\(_{1'-2'}\) = 9.00Hz, H\(^{1'}\)), 5.44 (1H, t, J\(_{3-4}\) = 9.68Hz, H\(_4\)), 5.22 – 5.37 (3H, m H\(_3\), H\(^3'\), H\(^4\)), 5.07-5.15 (2H, m, H\(^{2'}\), H\(^{2}\)), 4.24 - 4.31 (1H, m, H\(^{6b}\)), 4.01-4.17 (4H, m, H\(^{6'a}\), H\(^{6'b}\), H\(^{7'a}\), H\(^{7'b}\)), 3.70-3.82 (2H, m, H\(^4\), H\(^5\)), 1.97-2.15 (21H, m, H\(^{12}\)).

MS (ES+) m/z =: 749.7 [M]+.

3-((2,3,4,6-tetra-O-acetyl)-β-D-glucopyranosyl)-L-cysteine-N-carboxyanhydride - Cys(GluAc) NCA – 6(c)

\(^1\)H NMR (CDCl\(_3\)) \(\delta_{\text{ppm}}\): 6.32 (1H, d, J\(_{1,2}\) = 6Hz, H\(^{9}\)), 6.05 (1H, d, J\(_{1,2}\) = 6.2Hz), 5.38 (1H, d, J\(_{2,3}\) = 3.5Hz, H\(^2\)), 5.29 (1H, t, J\(_{3,4}\) = 9.0Hz, H\(^3\)), 5.06 (4H, m, H\(^{6a}\), H\(^{6b}\), H\(^{7b}\), H\(^{8}\)), 4.90 (1H, dd, J\(_{7a-7b}\) = 3.5Hz, J\(_{7,8}\) = 6.5Hz, H\(^{7a}\)), 4.79 (1H, dd, J\(_{4,5}\) = 8.00Hz, J\(_{3,4}\) = 2.0Hz, H\(^5\)), 3.93 (1H, dd, J\(_{5,6}\) = 4.00Hz, J\(_{4,5}\) = 9.5Hz, H\(^5\)), 2.04 - 2.11 (12H, m, H\(^{12}\)).

\(^1\)H NMR (CDCl\(_3\)) \(\delta_{\text{ppm}}\): 169.4 -171.5 (4 x C\(^{13}\)), 163.9 (C\(^{11}\)), 153.6 (C\(^{10}\)), 94.1(C\(^1\), 70.1 (C\(^5\)), 69.9 (C\(^3\)), 68.7 (C\(^2\)), 67.7 (C\(^6\)), 67.3 (C\(^8\)), 62.2 (C\(^6\)), 61.8 (C\(^7\)), 20.8 - 21.2 (4 x C\(^{12}\)).

MS (ES+) m/z = 477.2 [M]+.

3-S-((2,3,4,6-tetra-O-acetyl)-β-D-galactopyranosyl)-L-cysteine-N-carboxyanhydride - Cys(GalAc)NCA – 7Cc)

\(^1\)H NMR (CDCl\(_3\)) \(\delta_{\text{ppm}}\): 6.92 (1H, s, H\(^9\)), 6.02 (1H, d, J\(_{1,2}\) = 6Hz, H\(^1\)), 5.41 (1H, d, J\(_{2,1}\) = 3.5Hz, H\(^2\)), 5.33 (1H, dd, J\(_{6b-6a}\) =1Hz, J\(_{6b-5}\) = 2.5Hz, H\(^{6b}\)), 5.29 (1H, t, J\(_{3,4}\) = 9.0Hz, H\(^3\)), 5.05 (1H, dd, J\(_{6a-6b}\) = 3.5Hz, J\(_{6a-5}\) = 4.5Hz, H\(^{6a}\)), 4.44 (1H, dd, J\(_{7a-7b}\) = 2.5Hz, J\(_{7,8}\) = 7.5Hz, H\(^{7a}\)), 4.17- 4.29 (2H, m, H\(^4\) + H\(^5\)), 2.03 - 2.14 (12H, m, H\(^{12}\)).
$^{13}$C NMR (CDCl$_3$) $\delta$ ppm: 169.0 - 171.1 (4xC$^{13}$), 164.1 (C$^{11}$), 155.0 (C$^{10}$), 91.7 (C$^1$), 70.1 (C$^5$), 69.6 (C$^3$), 69.3 (C$^2$), 68.9 (C$^4$), 66.7 (C$^8$), 61.5 (C$^6$), 60.2 (C$^7$), 21.0 - 21.4 (4xC$^{12}$).

MS (ES$^+$) m/z = 477.4 (100%) [M]$^+$. 

Elemental (Found %) C, 45.37; H, 4.66; N, 2.11 (Expected %) C, 45.28 H, 4.86 N, 2.53.
NMR SPECTRA

In this section $^1$H and $^{13}$C spectra for all new compounds are shown. (SerGalAc NCA and SerGluAc NCA have been described previously$^3$ and are not included here. The intermediate glycosylated amino acids, 1 (b) $\rightarrow$ 7 (b), were used without purification and were thus not ‘analytically’ pure and not included here. A single example, Thr(GluAc) - 1 (b) is shown for completeness.
BocThr(GalAc) – 1(a)
BocThr(GluAc) – 2(a)
BocCys(GalAc) – 7 (a)
BocCys(GluAc) – 6(b)
ThrGal(Ac) – 1 (b)

ThrGalAc NCA – 1(c)
ThrGluAc NCA – 2(c)
SerLacAc NCA – 5(c)

* = Residual α-pinene
CysGluAc NCA – 6(c)
CysGalAc NCA – 7(c)

* = Residual α-pinene, $ = TMS

* = Residual α-pinene
References