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Supporting Information

Real-Time NMR Studies of Oxyamine Ligations of Reducing Carbohydrates under Equilibrium Conditions

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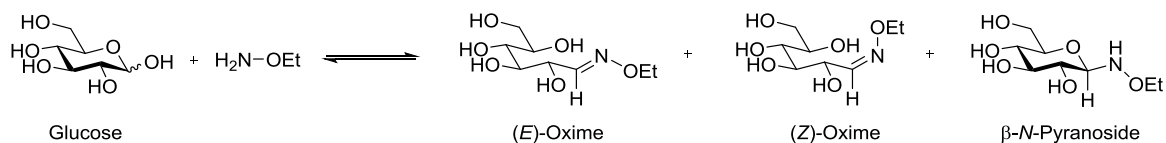
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General

All NMR spectra were recorded in 500 mM deuterated acetate buffer using a Bruker Avance III 600 instrument. ^1H chemical shifts are referenced to the TSP signal ($\delta_{\text{H}} = 0.00$ ppm). ^{13}C chemical shifts are referenced to $[\text{D}_4]\text{acetic acid}$ ($\delta_{\text{C}} = 21.0$ ppm). The multiplicity of pseudo triplets is abbreviated "t". $^1J_{\text{C1-H1}}$ coupling constants were determined from non-decoupled HSQC NMR spectra. In Figures S1B–S7B, S9B–S11B, S13B–S21B, S23B–S29B, and S31B–S33B exponential curve fitting is shown for the combined yields.

Reaction of Glc with *O*-Ethylhydroxylamine (11)

Table S1. Characteristic NMR data of products obtained by the reaction of glucose with *O*-ethylhydroxylamine (11).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
(E)-Oxime	H-1	7.53	d, 6.7	C-1	150.5
	H-2	4.40	"t", 6.9	C-2	70.2
	H-3	3.95	dd, 7.1, 1.4	C-3	71.3
	H-4	3.58	dd, 8.4, 1.4	C-4	70.6
(Z)-Oxime	H-1	6.90	d, 6.3	C-1	151.6
	H-2	4.94	"t", 6.2	C-2	65.9
	H-3	3.95	dd, 6.2, 2.6	C-3	71.2
	H-4	3.58	m	C-4	70.6
β-N-Pyranoside	H-1	4.27	d, 9.2	C-1	89.6
	H-2	3.36	"t", 9.1	C-2	69.6
	H-3	3.52	"t", 9.1	C-3	77.2
	H-6a	3.90	dd, 12.4, 2.0	C-6	60.4

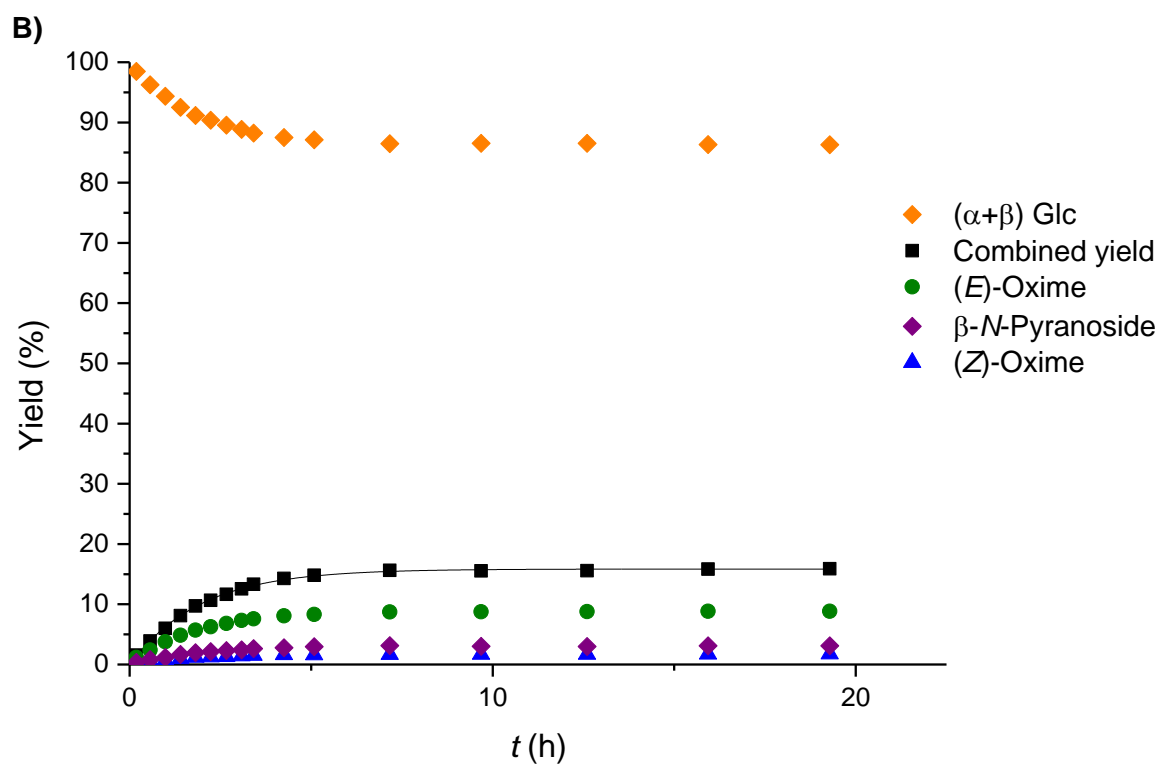
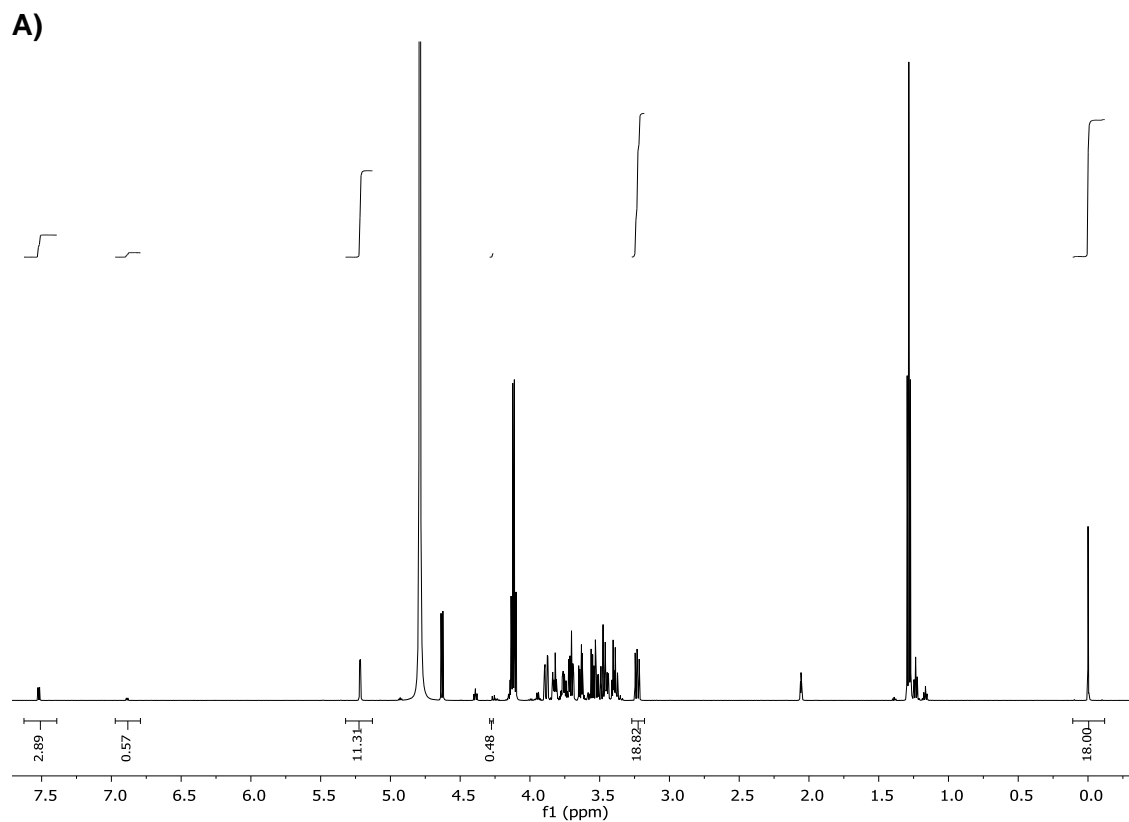


Figure S1. Ligation reaction of glucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 3. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

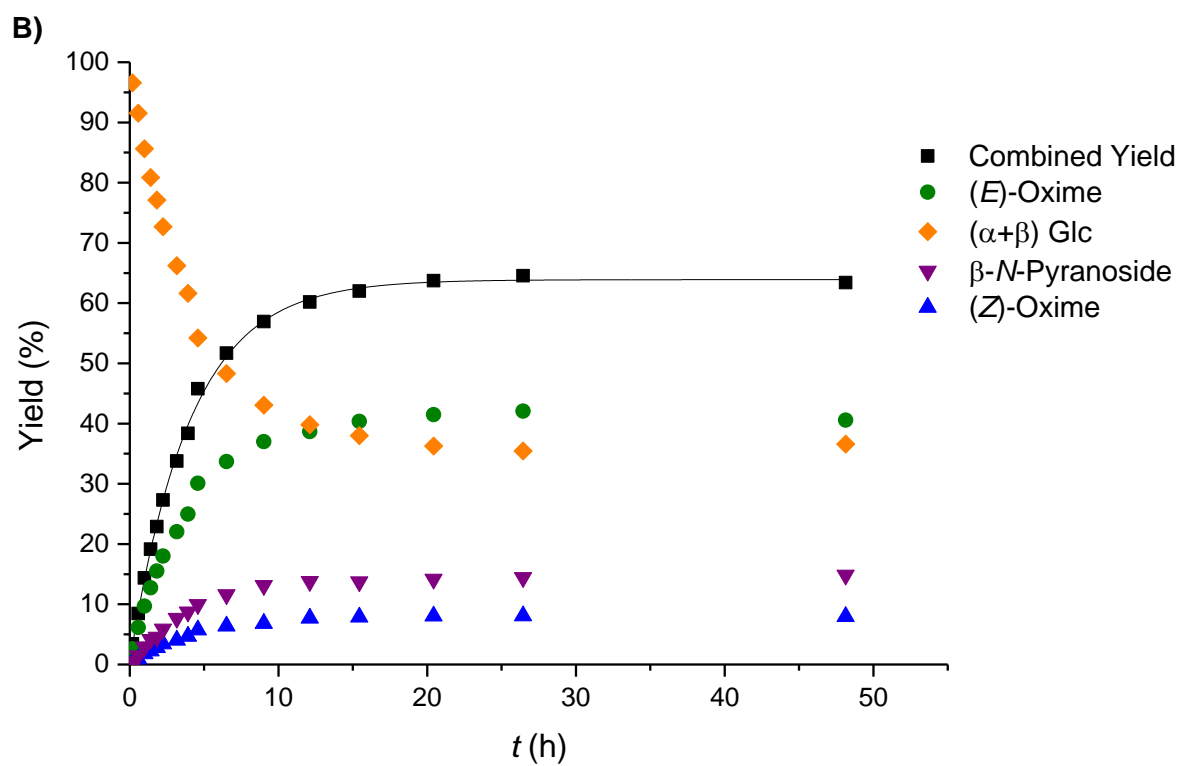
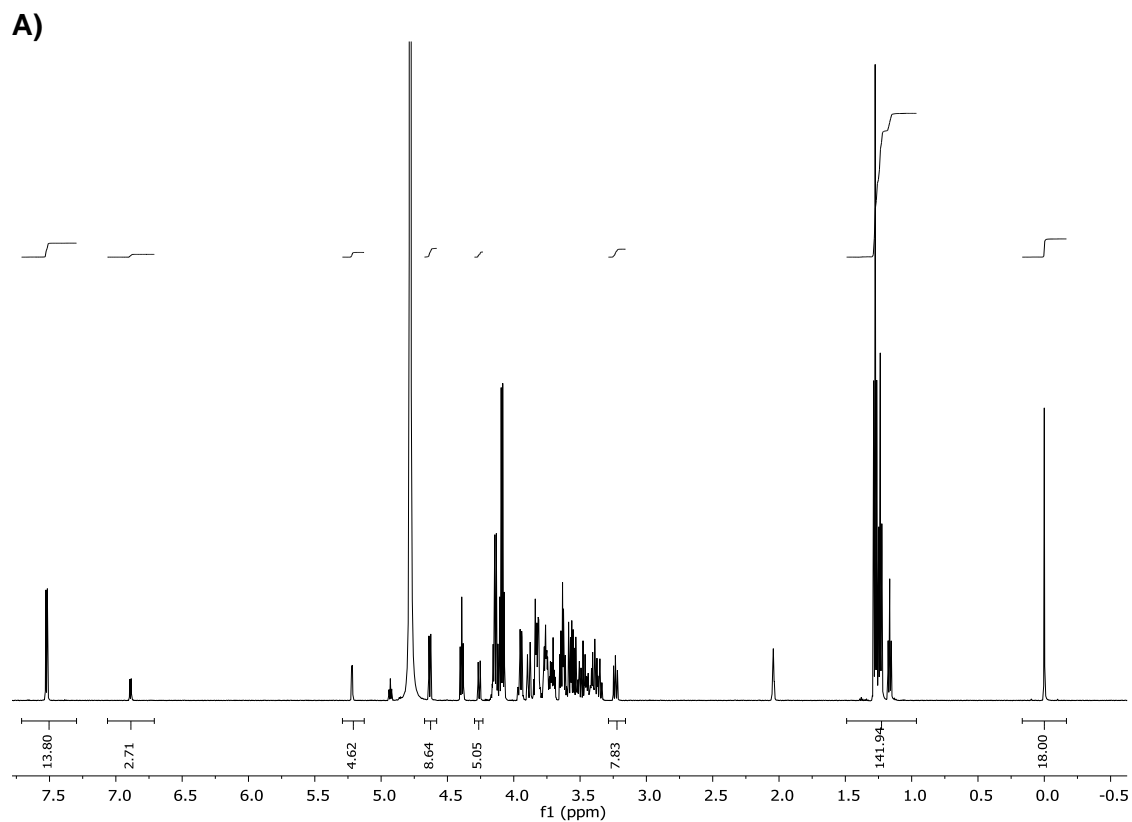


Figure S2. Ligation reaction of glucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 4. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

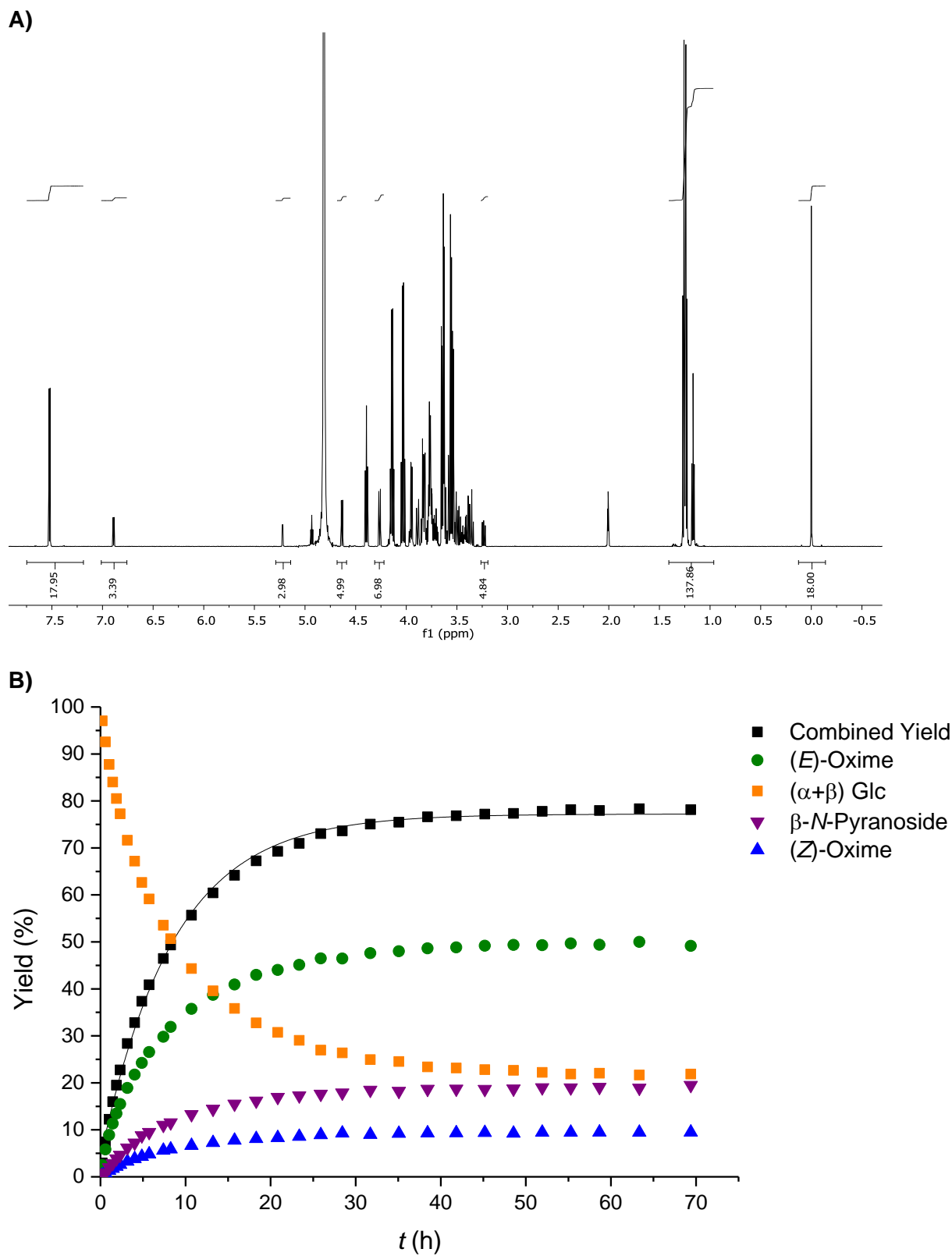


Figure S3. Ligation reaction of glucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 4.5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

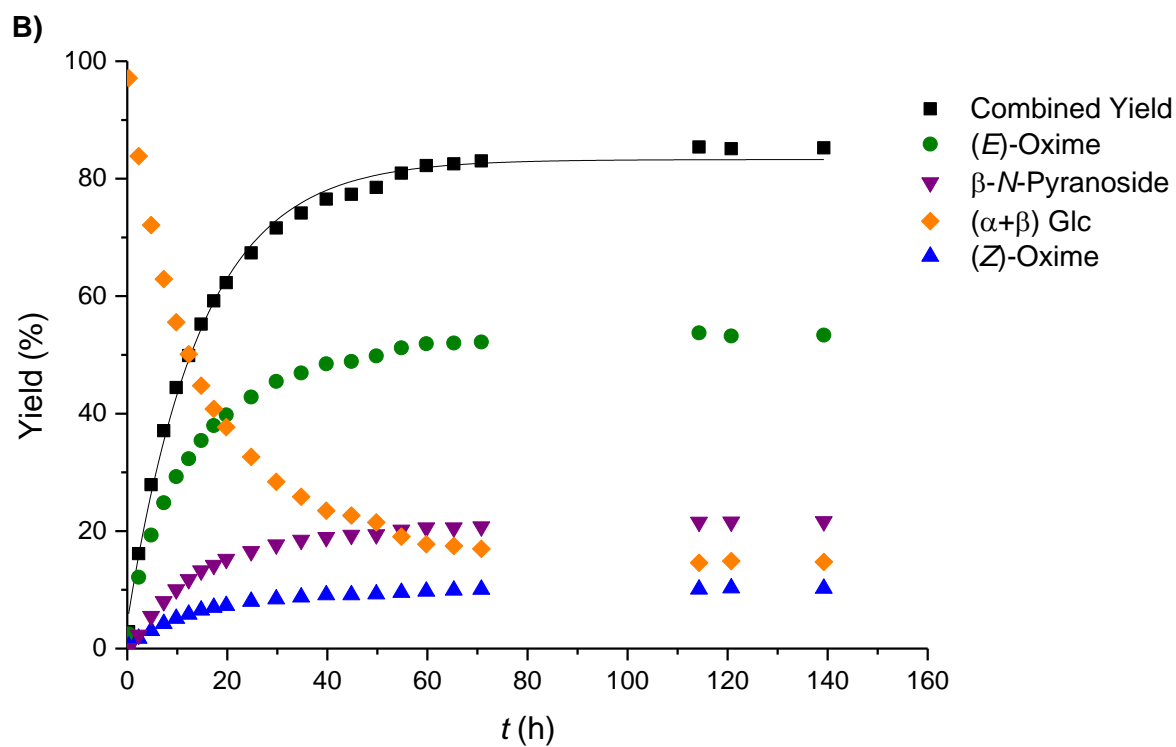
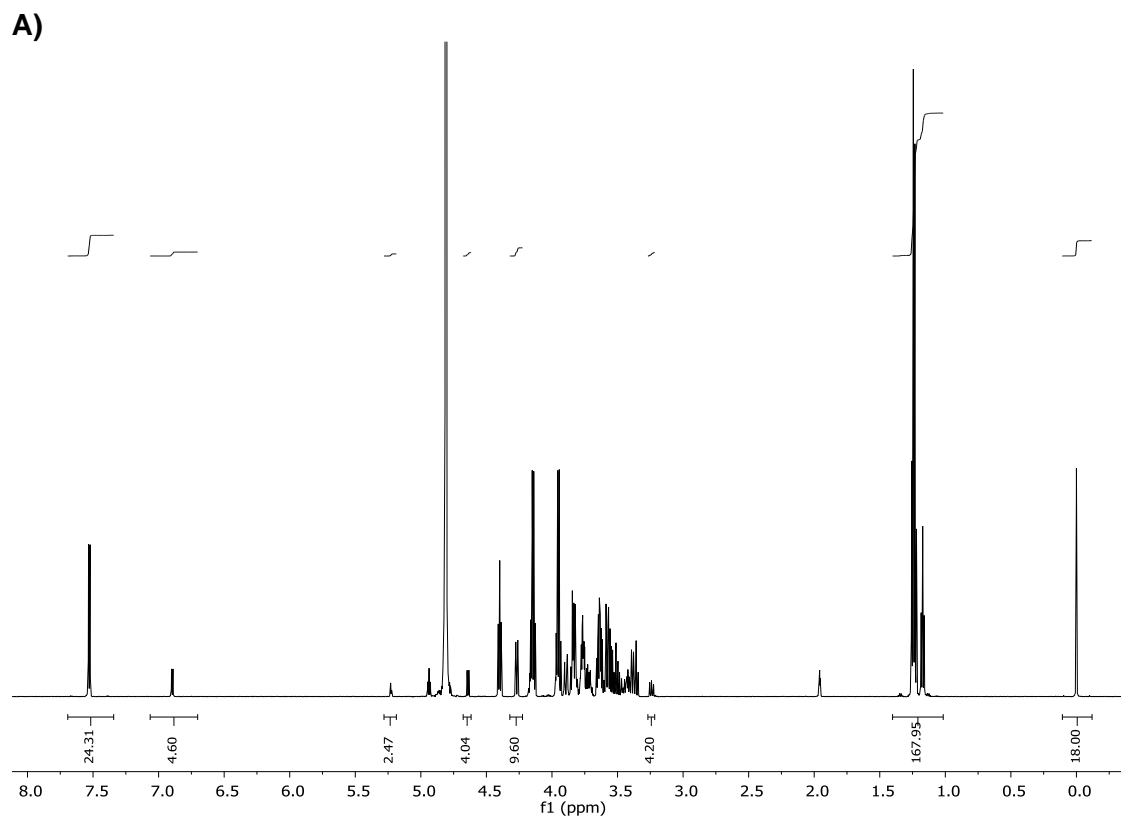


Figure S4. Ligation reaction of glucose with 1.2 equiv of O-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

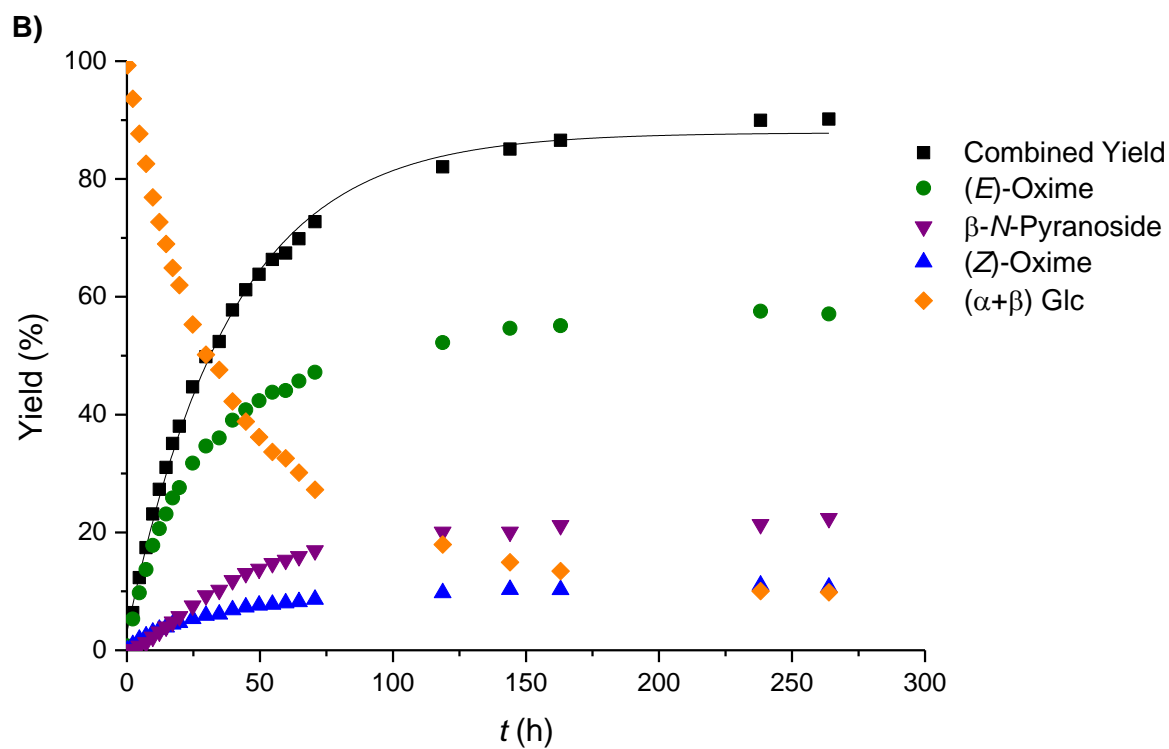
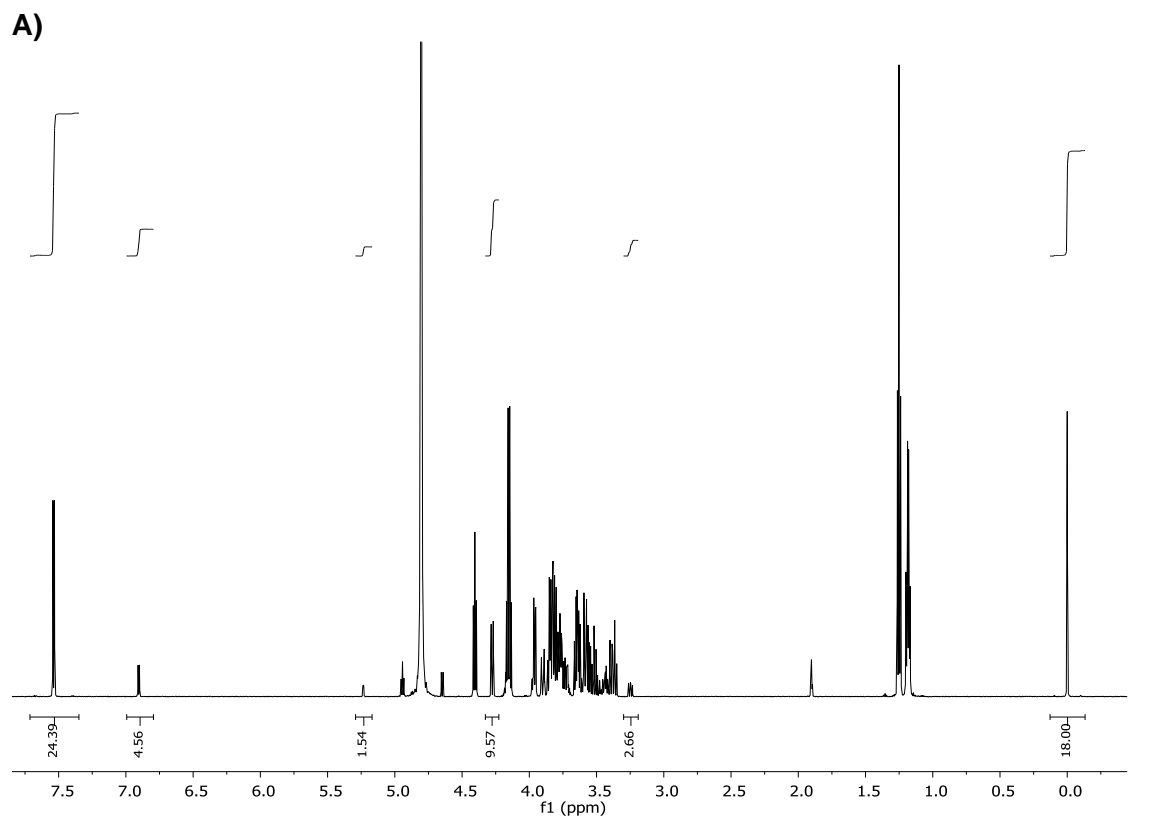


Figure S5. Ligation reaction of glucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 6. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

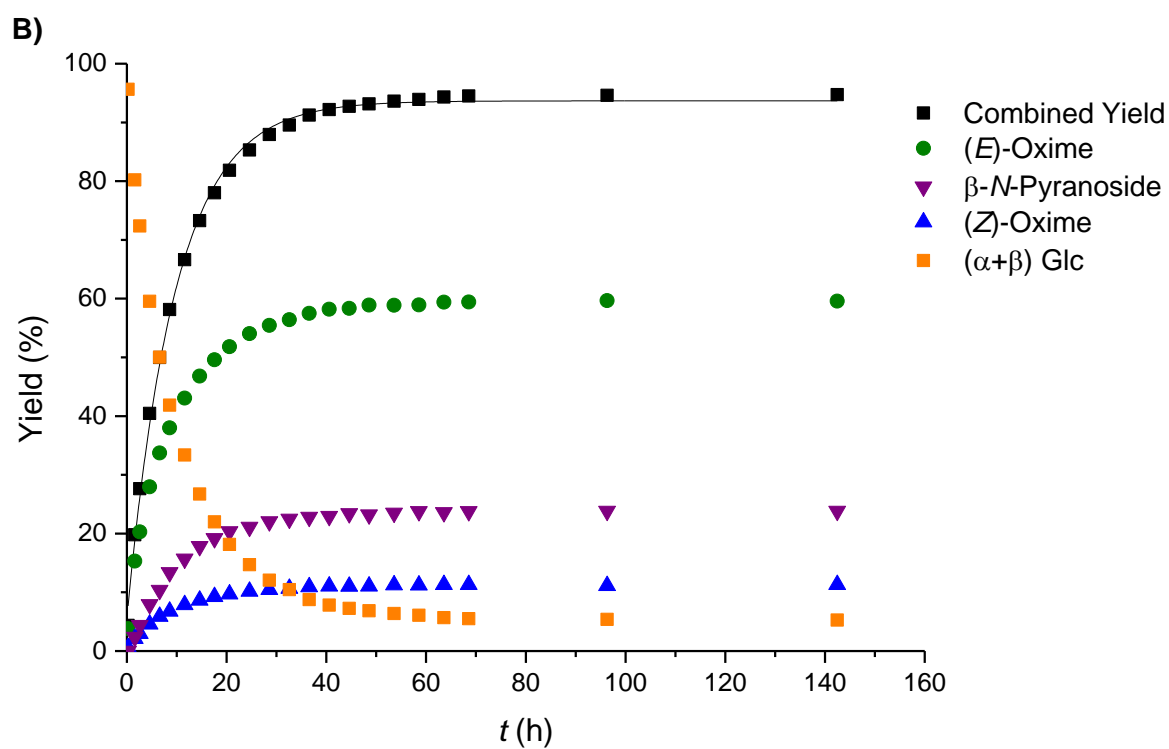
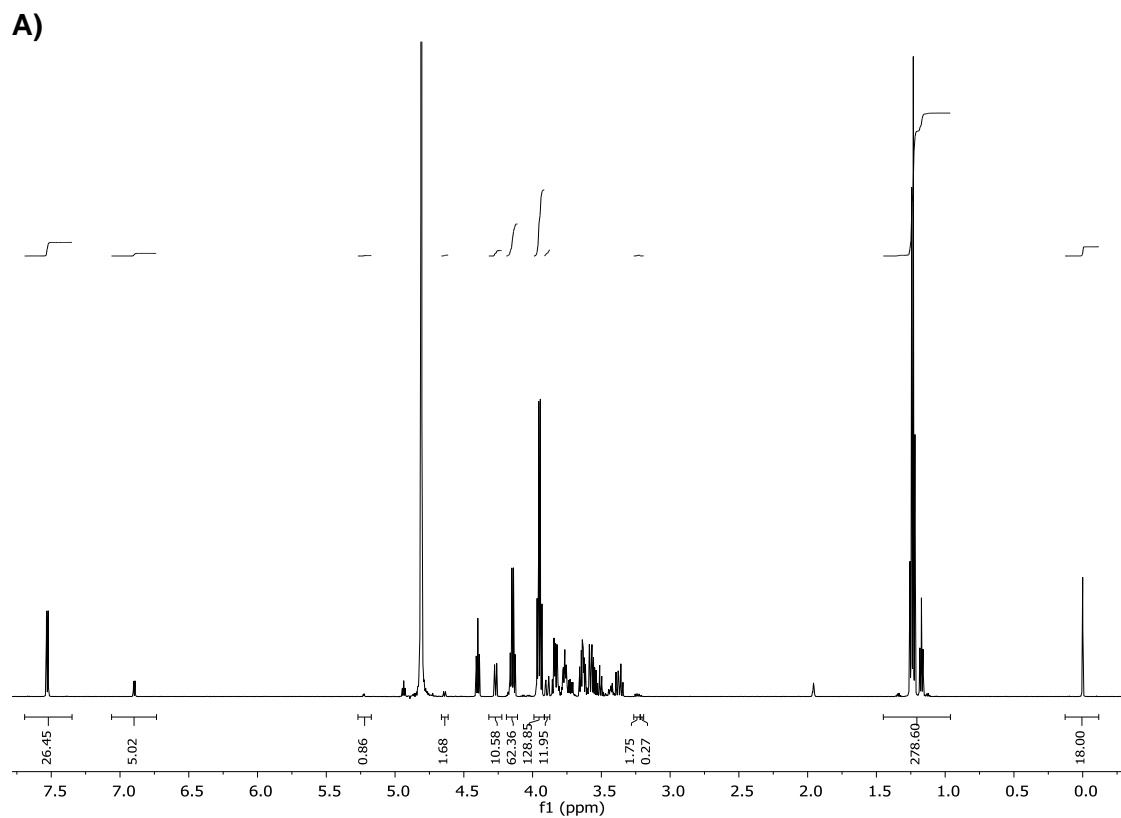


Figure S6. Ligation reaction of glucose with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

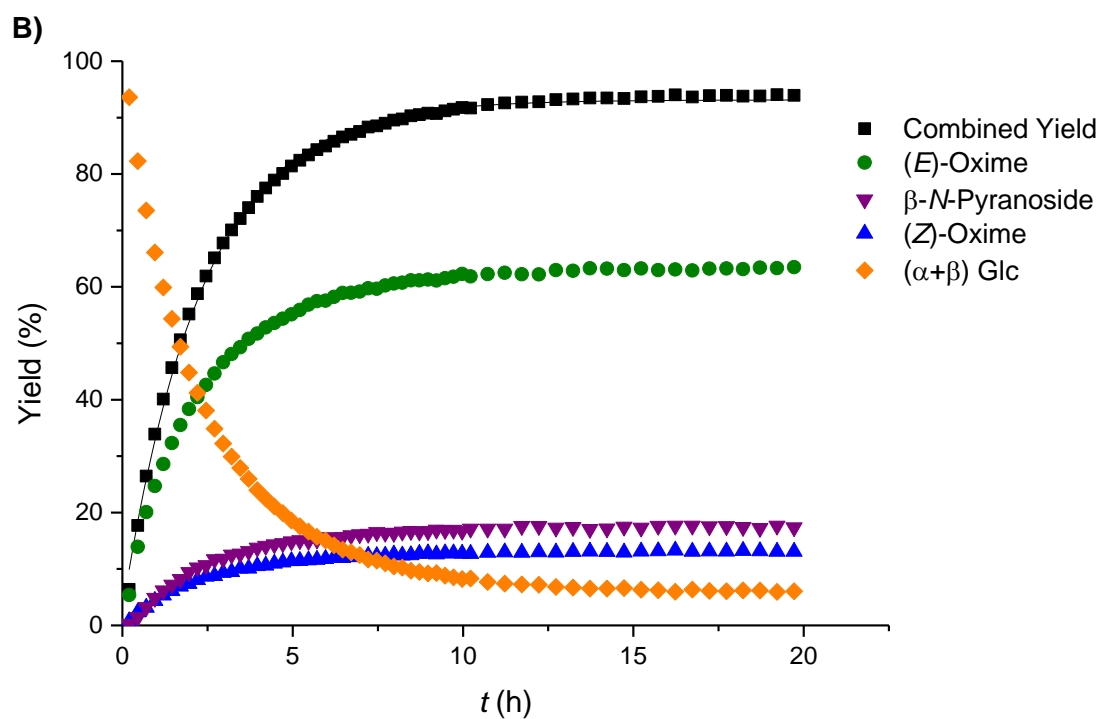
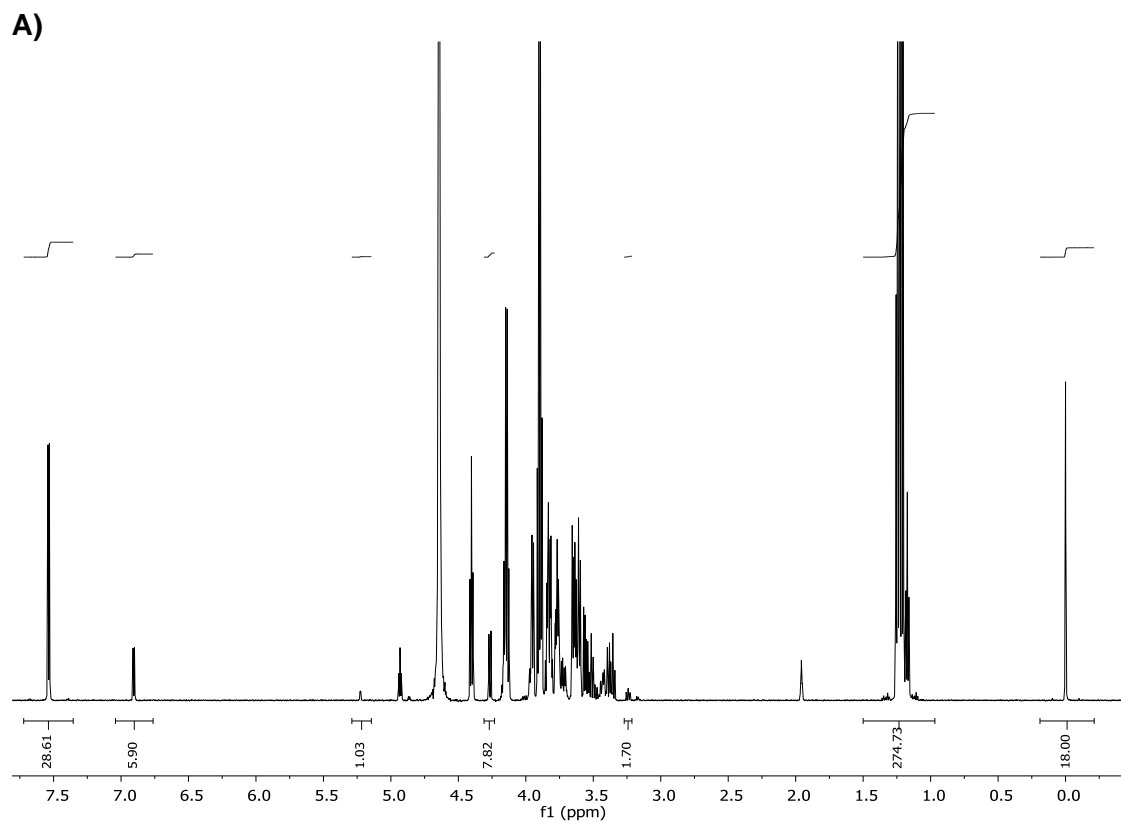


Figure S7. Ligation reaction of glucose with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 39 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

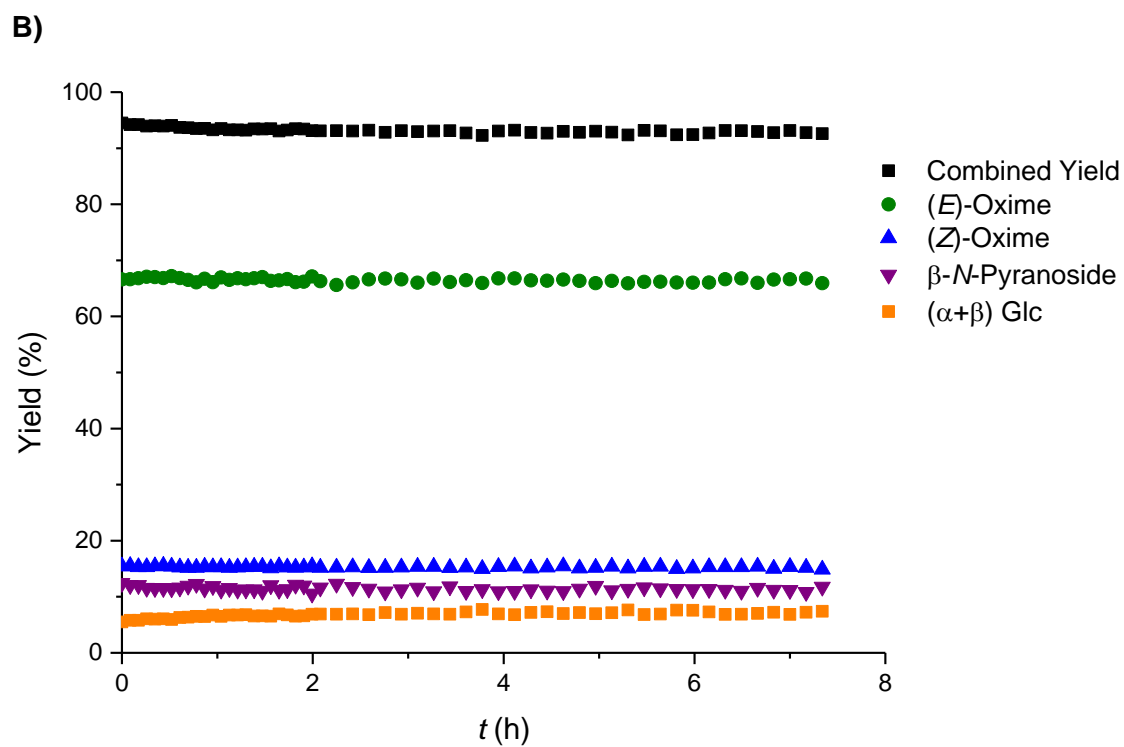
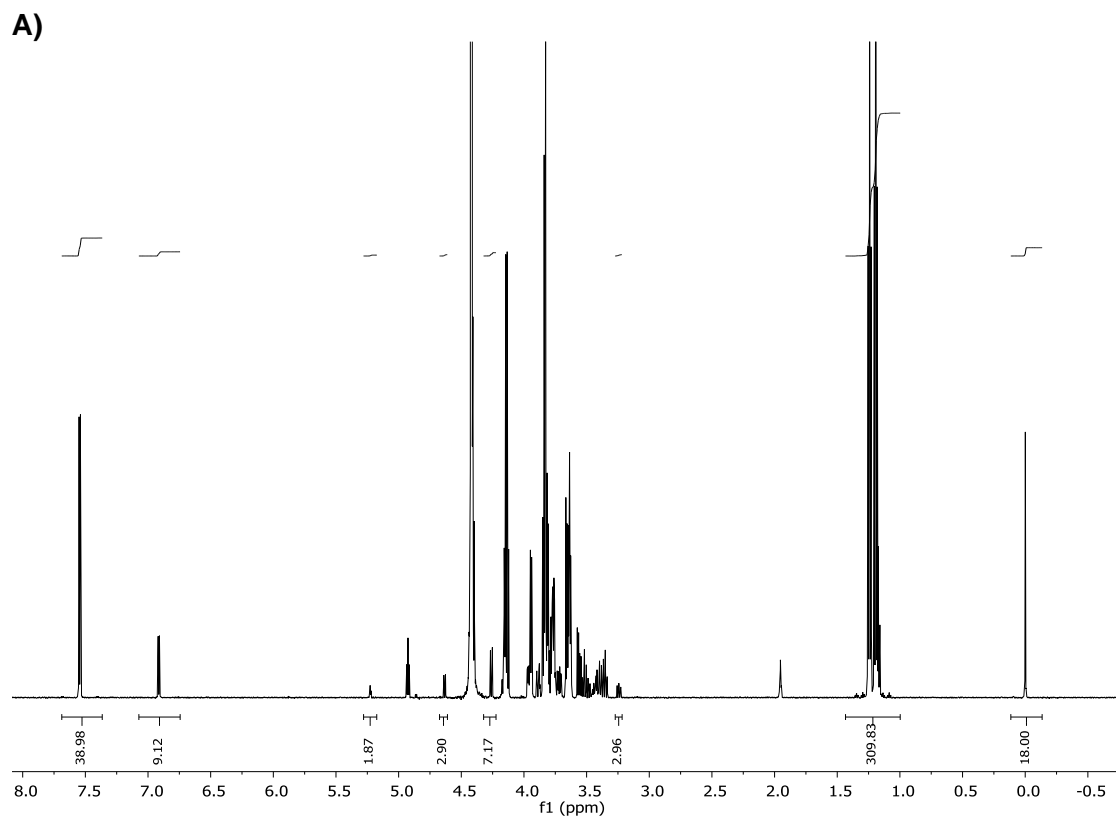


Figure S8. Ligation reaction of glucose with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at pH 5. Re-equilibration at 60 °C after the sample had been equilibrated at 39 °C. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of GlcNAc with *O*-Ethylhydroxylamine (11)

Table S2. Characteristic NMR data of products obtained by the reaction of GlcNAc with *O*-ethylhydroxylamine (11)

The reaction scheme shows the equilibrium between GlcNAc (N-acetylglucosamine) and *O*-ethylhydroxylamine (11) to form three products: (*E*)-Oxime, (*Z*)-Oxime, and β -*N*-Pyranoside. The structures are shown in their respective chair conformations.

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
<i>(E)</i> -Oxime	H-1	7.49	d, 6.2	C-1	148.8
	H-2	4.71	"t", 6.7	C-2	51.5
	H-3	4.08	dd, 7.4, 1.7	C-3	69.5
<i>(Z)</i> -Oxime	H-1	6.83	d, 6.4	C-1	149.0
	H-2	5.07	"t", 6.6	C-2	48.2
	H-3	4.13	m	C-3	69.7
β - <i>N</i> -Pyranoside	H-1	4.33	d, 9.8	C-1	88.2
	H-2	3.87	m	C-2	70.2

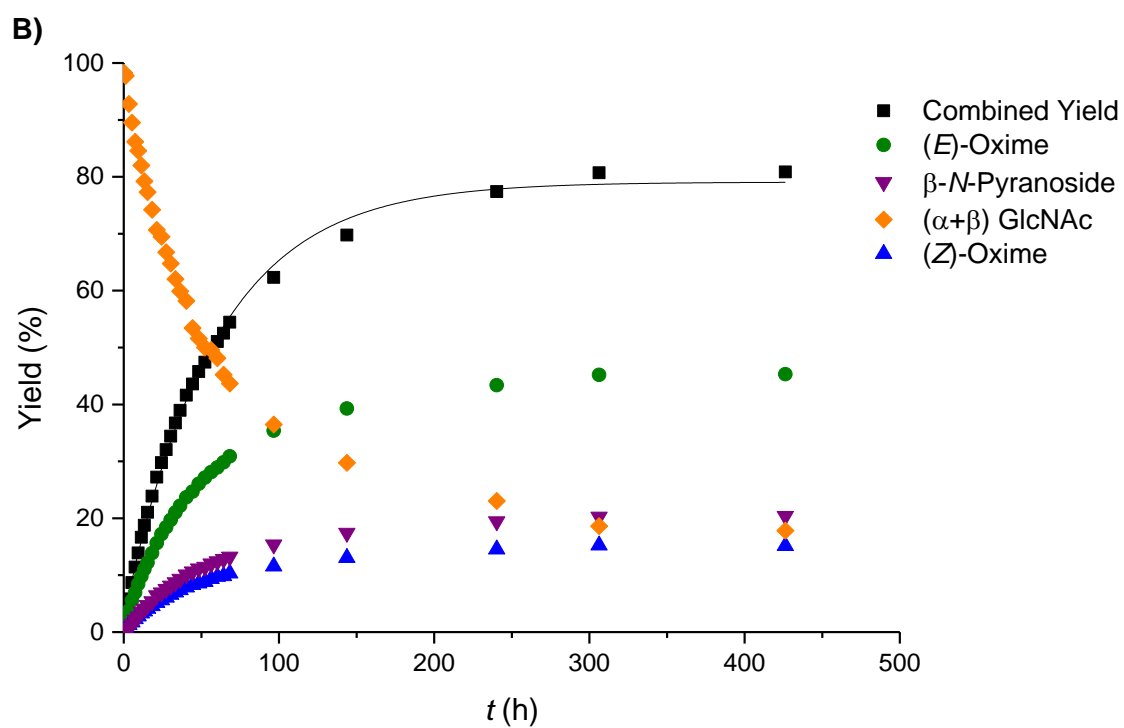
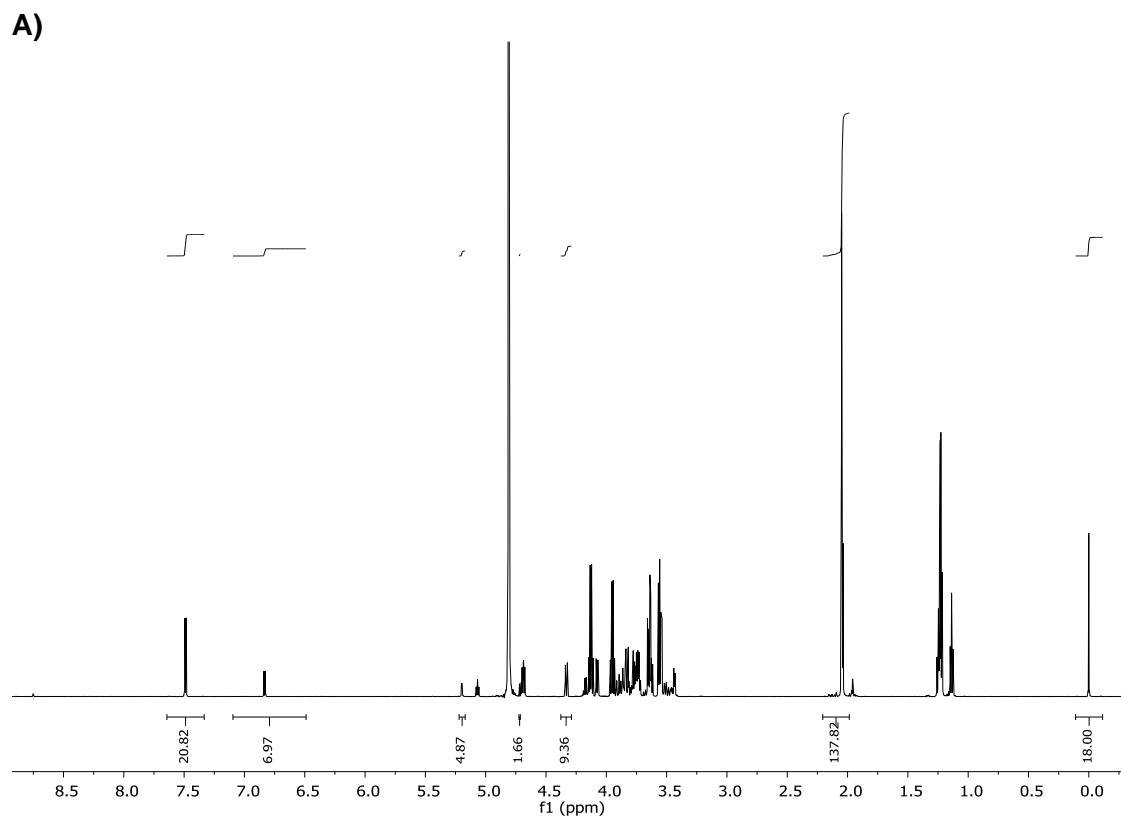


Figure S9. Ligation reaction of GlcNAc with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

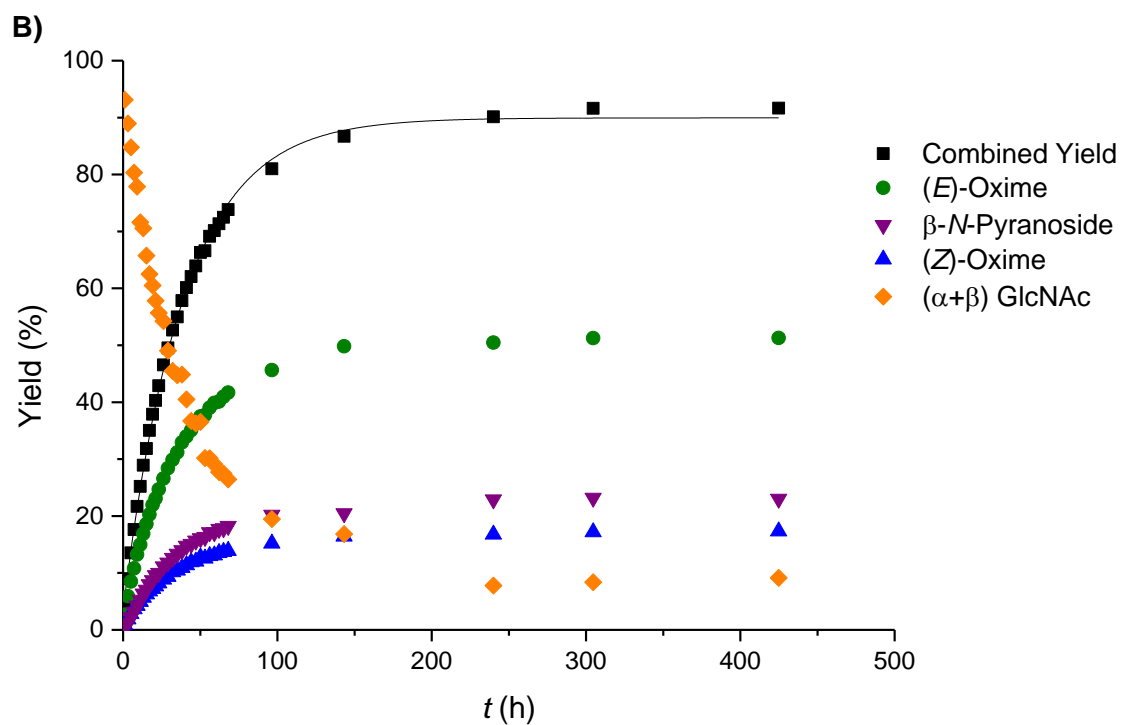
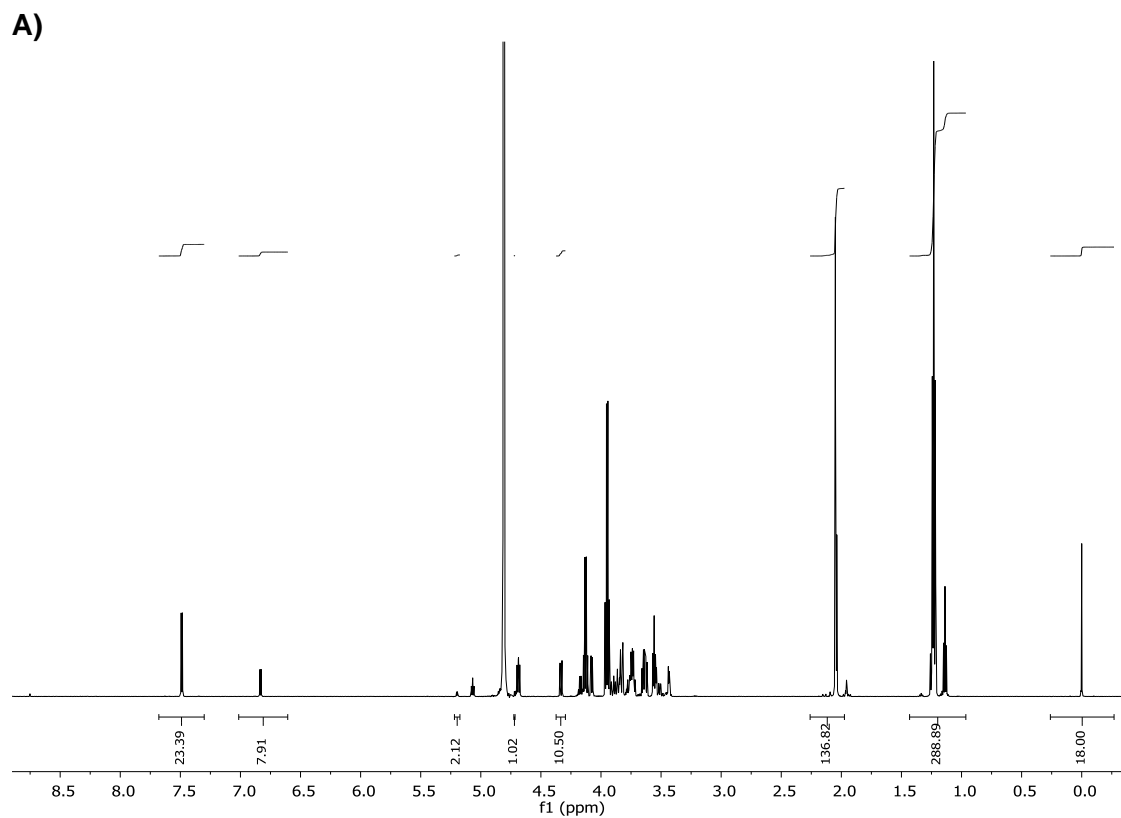


Figure S10. Ligation reaction of GlcNAc with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

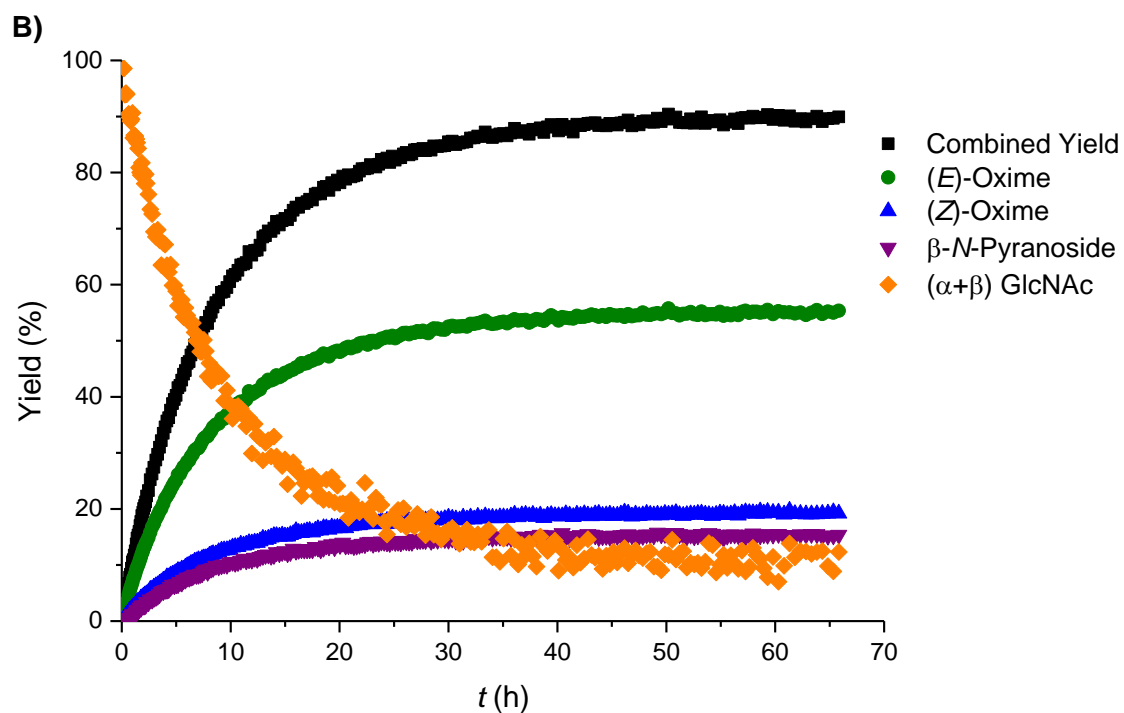
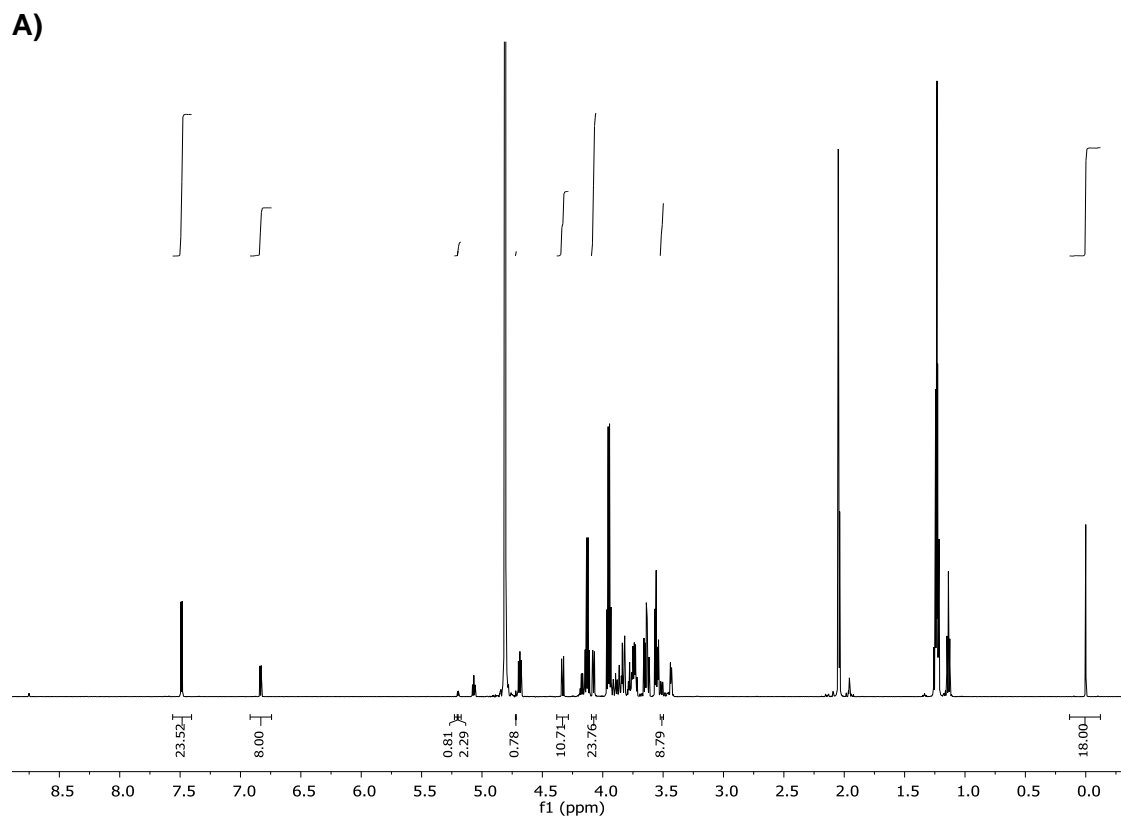


Figure S11. Ligation reaction of GlcNAc with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 39 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

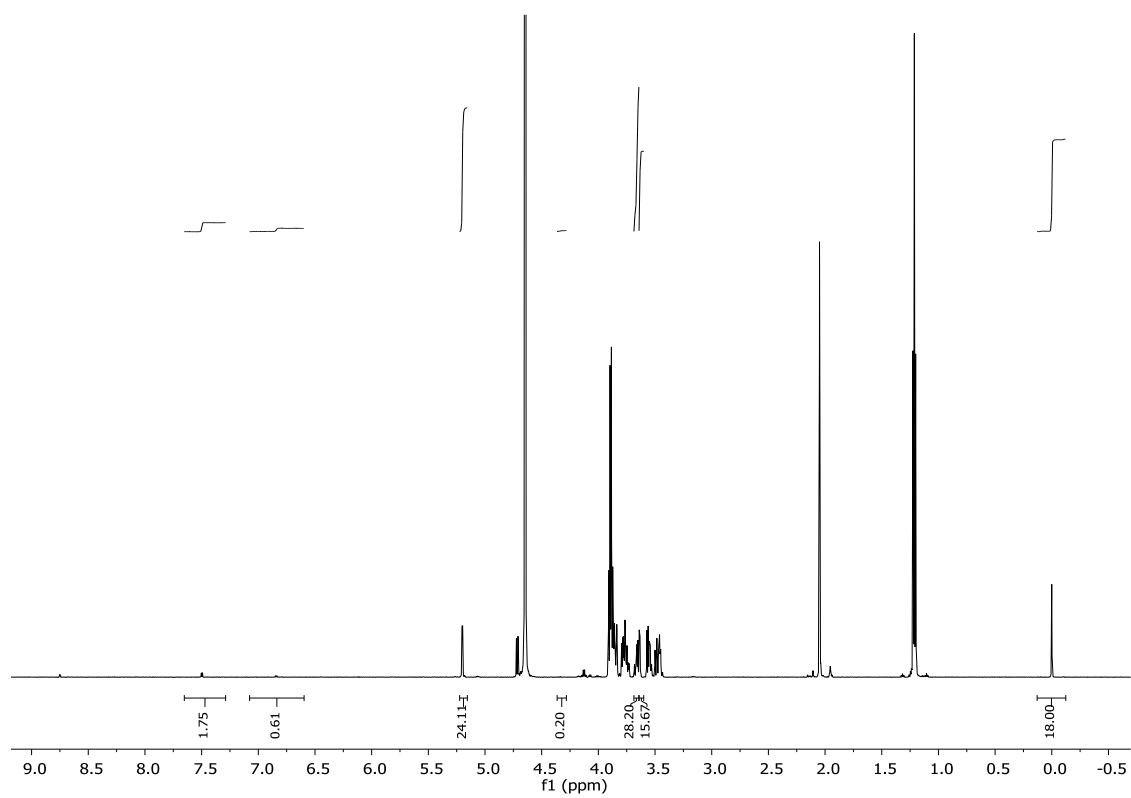
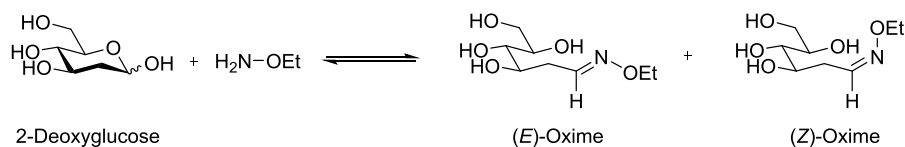


Figure S12. Ligation reaction of GlcNAc with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 21 °C and pH 5. ¹H NMR spectrum (600 MHz) after equilibration.

Reaction of 2-Deoxyglucose with *O*-Ethylhydroxylamine (11)

Table S3. Characteristic NMR data of products obtained by the reaction of 2-deoxyglucose with *O*-ethylhydroxylamine (11).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
(E)-Oxime	H-1	7.58	"t", 6.5	C-1	151.1
	H-2a	2.49-2.44	m	C-2	33.1
	H-2b	2.64-2.51	m		
	H-3	4.11	m	C-3	68.9
	H-4	3.46	dd, 8.5, 1.9	C-4	63.4
	H-5	3.81-3.72	m	C-5	72.0
	H-6a	3.86-3.82	dd, 11.8, 1.8	C-6	64.9
	H-6b	3.68-3.62	m		
(Z)-Oxime	H-1	6.99	"t", 5.5	C-1	150.7
	H-2a	2.74-2.66	m	C-2	29.7
	H-2b	2.64-2.51	m		
	H-3	4.14	m	C-3	69.2
	H-4	3.44	dd, 8.4, 2.1	C-4	63.7
	H-5	3.81-3.72	m	C-5	72.5
	H-6a	3.86-3.82	m	C-6	64.0
	H-6b	3.68-3.62	m		

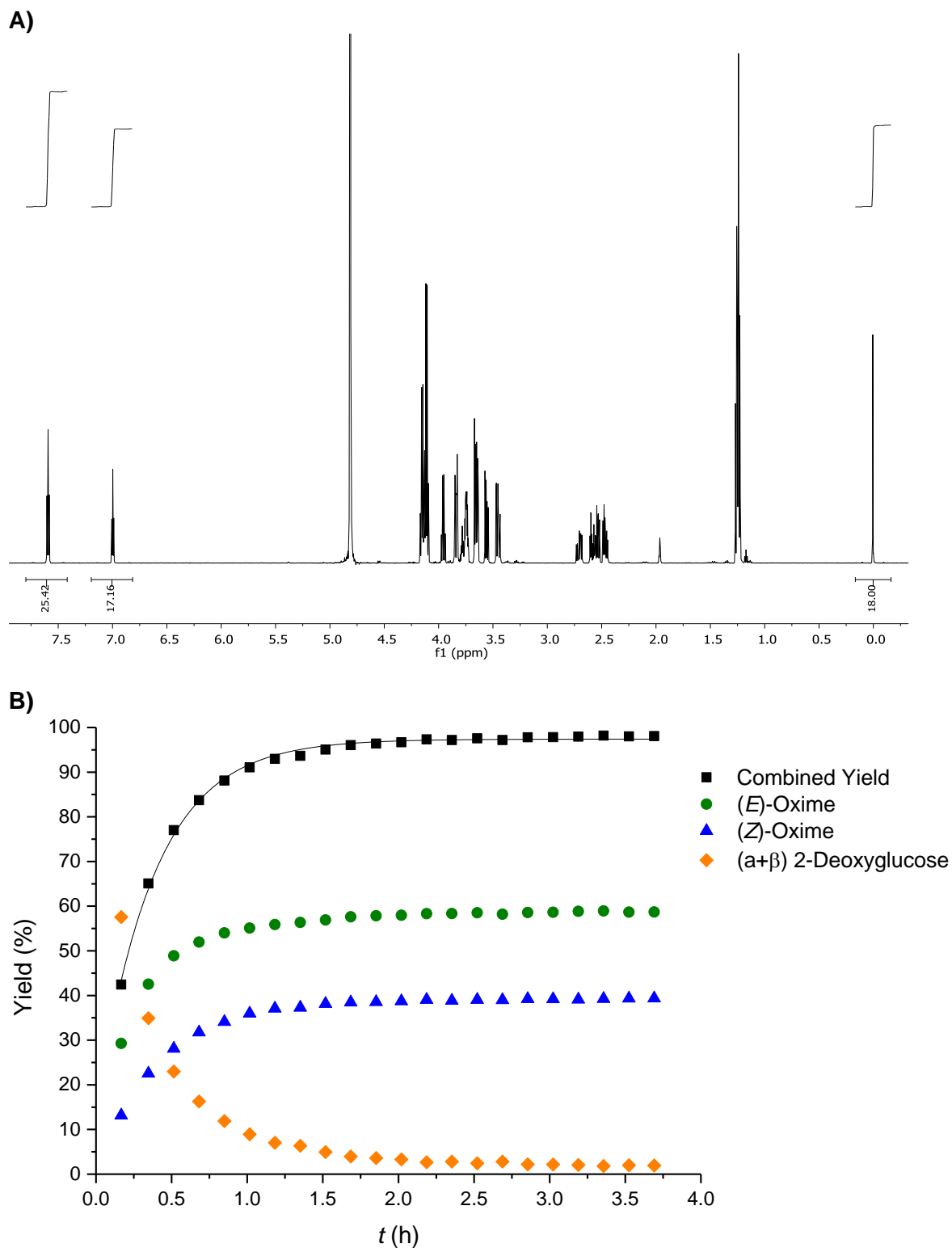


Figure S13. Ligation reaction of 2-deoxyglucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of Mannose with *O*-Ethylhydroxylamine (11)

Table S4. Characteristic NMR data of products obtained by the reaction of mannose with *O*-ethylhydroxylamine (11).

C1=CC=C(C=C1)O + H2N-OEt \rightleftharpoons C1=CC=C(C=C1)O + C1=CC=C(C=C1)O + C1=CC=C(C=C1)O

Mannose (E)-Oxime (Z)-Oxime α -N-Pyranoside

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
(E)-Oxime	H-1	7.59	d, 6.9	C-1	151.8
	H-2	4.29	dd, 8.1, 6.9	C-2	69.6
	H-3	3.93	m	C-3	71.4
	H-4	3.78-3.74	m	C-4	70.2
	H-5	3.78-3.74	m	C-5	71.8
	H-6a	3.86	dd, 12.0, 2.4	C-6	64.2
	H-6b	3.67	dd, 12.0, 5.7		
(Z)-Oxime	H-1	6.98	d, 6.8	C-1	151.7
	H-2	4.91	dd, 7.6, 6.9	C-2	63.5
	H-3	3.93	m	C-3	70.6
α -N-Pyranoside	H-1	4.58	d, 0.7	C-1	87.1

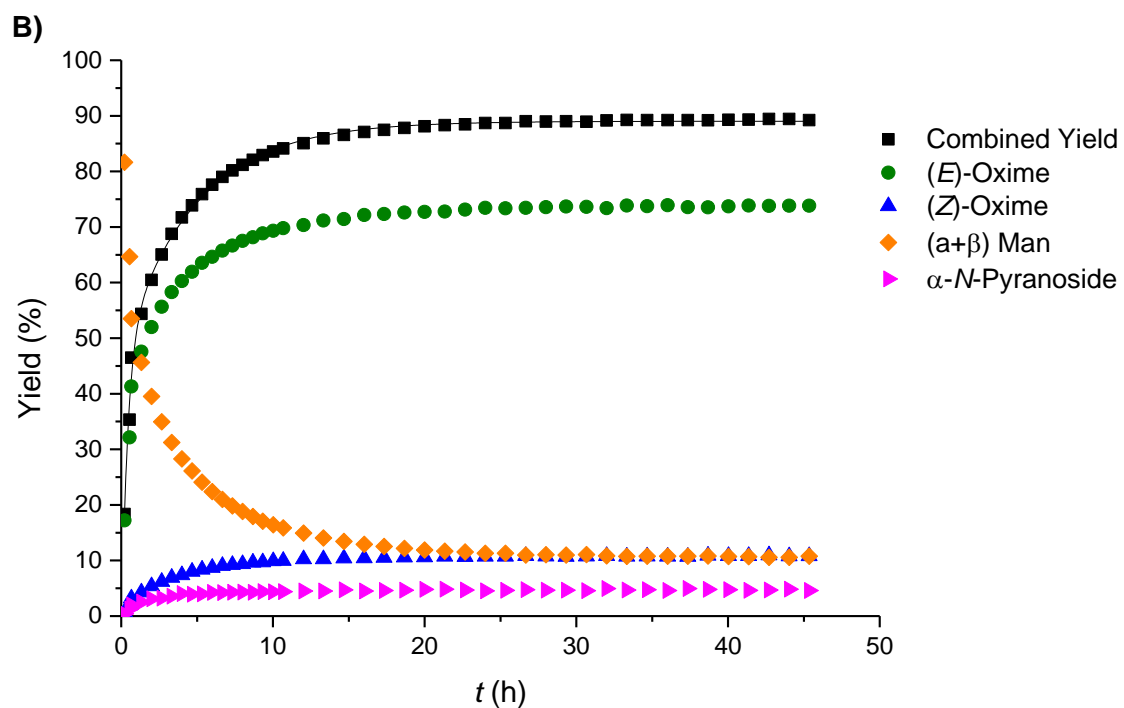
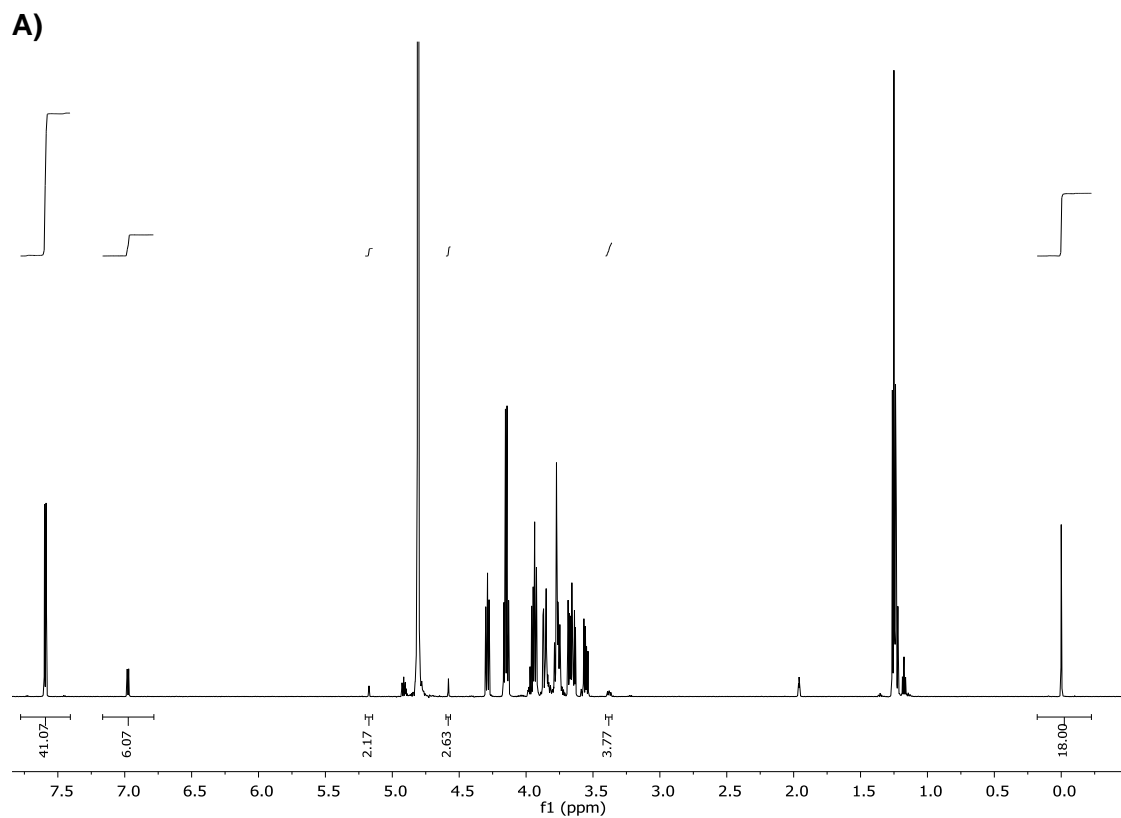


Figure S14. Ligation reaction of mannose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

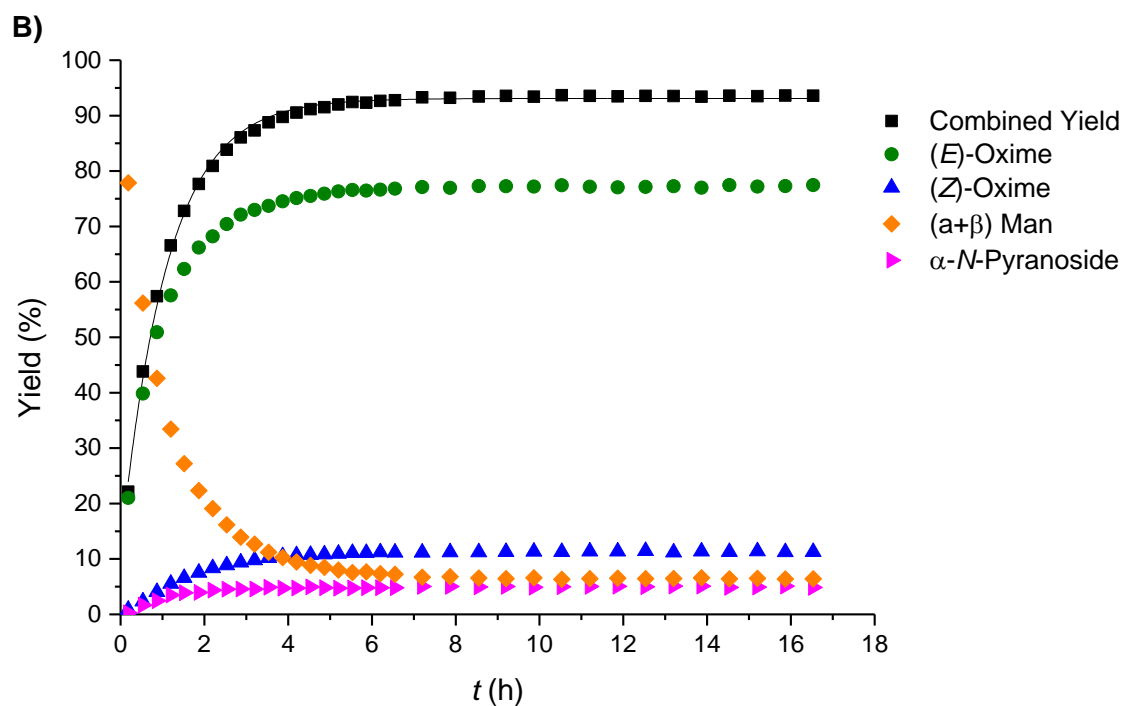
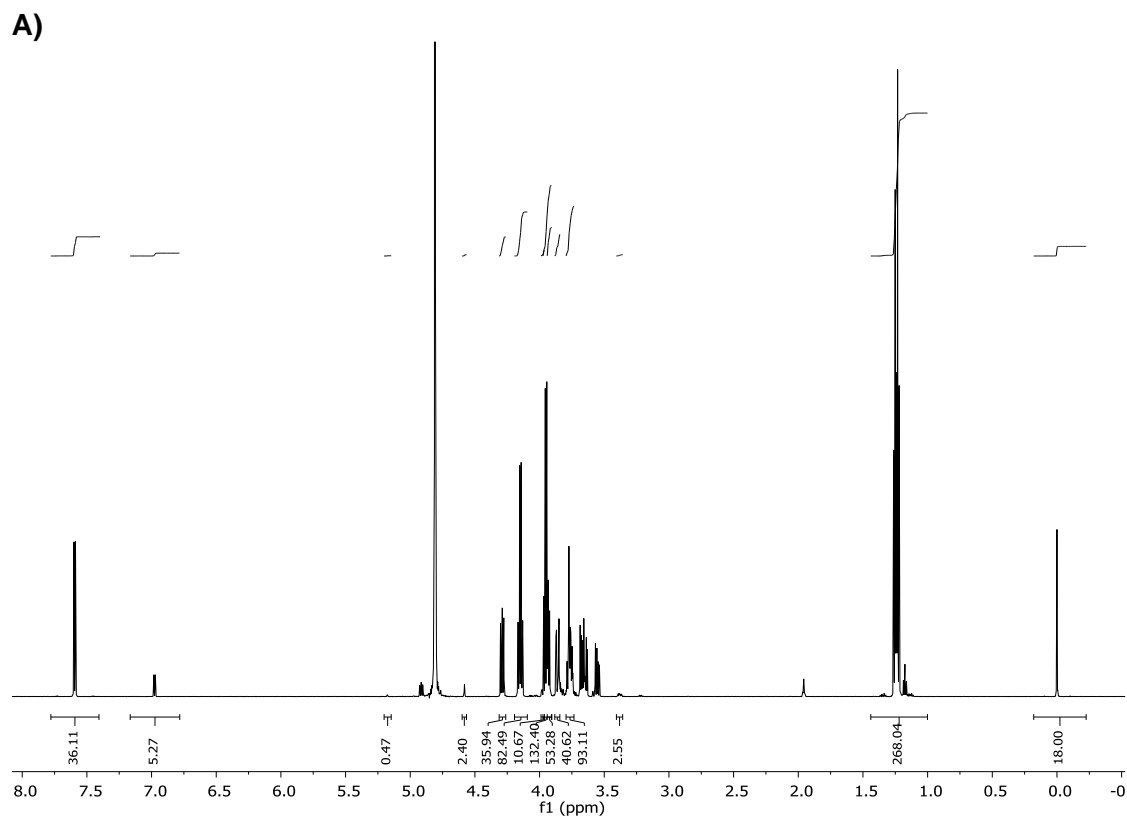


Figure S15. Ligation reaction of mannose with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

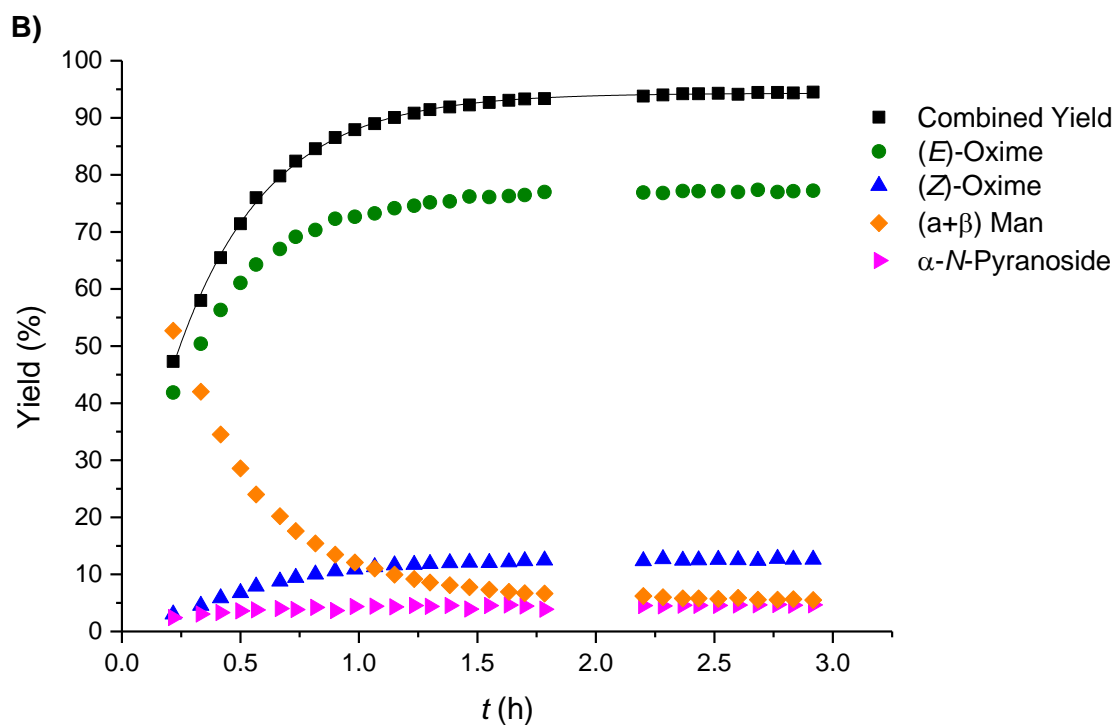
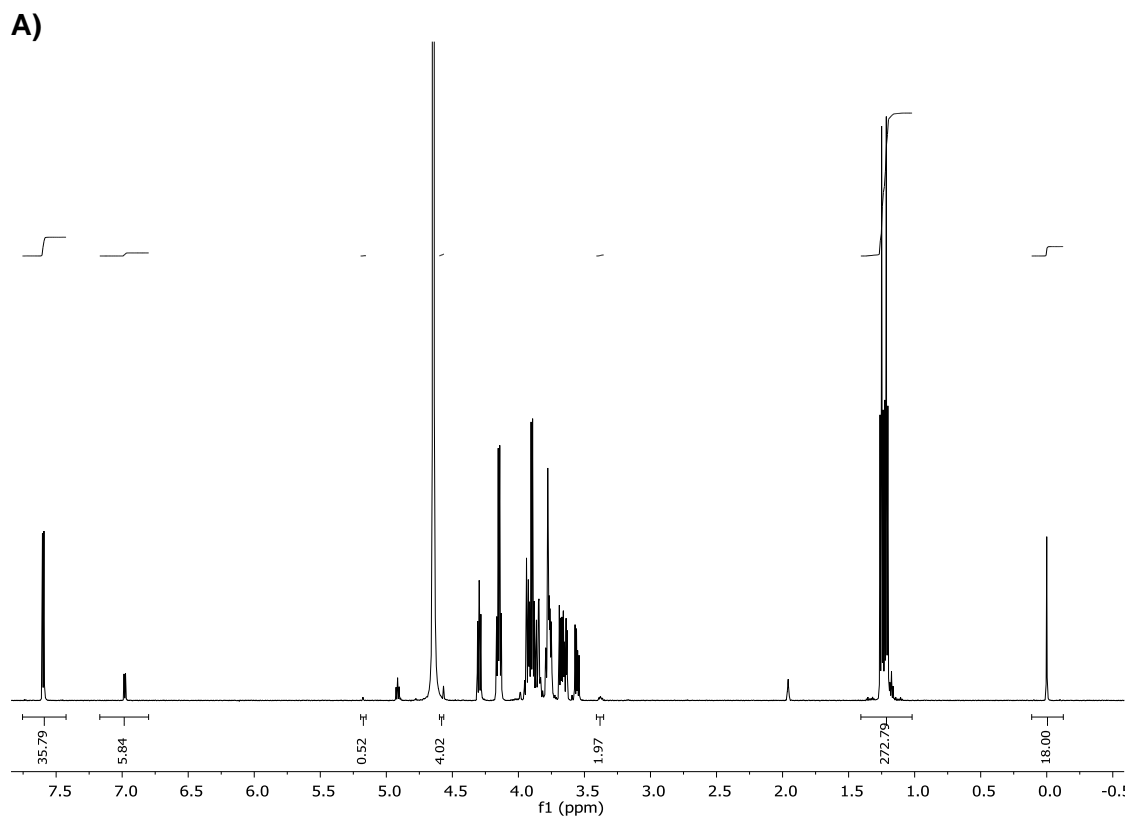
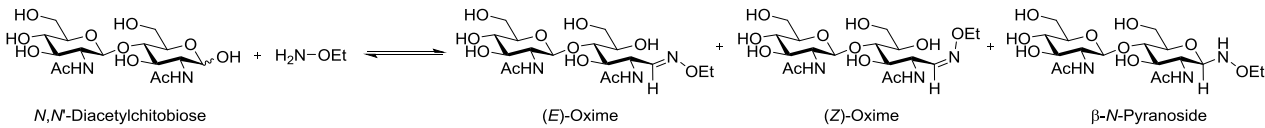


Figure S16. Ligation reaction of mannose with 2.0 equiv of *O*-ethylhydroxylamine (**11**) at 39 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of *N,N'*-Diacetylchitobiose with *O*-Ethylhydroxylamine (11)

Table S5. Characteristic NMR data of products obtained by the reaction of *N,N'*-diacetylchitobiose with *O*-ethylhydroxylamine (11).



The reaction scheme shows *N,N'*-Diacetylchitobiose reacting with $\text{H}_2\text{N-OEt}$ to form three products: *(E)*-Oxime, *(Z)*-Oxime, and β -*N*-Pyranoside.

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
<i>(E)</i> -Oxime	H-1	7.60	d, 5.3	C-1	149.6
	H-2	4.93	dd, 7.5, 5.3	C-2	50.6
	H-3	4.07	dd, 7.5, 2.1	C-3	68.7
<i>(Z)</i> -Oxime	H-1	6.95	d, 5.6	C-1	149.5
	H-2	5.11	"t", 5.9	C-2	47.9
	H-3	4.16	m	C-3	68.2
β - <i>N</i> -Pyranoside	H-1	4.33	d, 9.8	C-1	88.1
	H-2	3.88	m	C-2	69.1

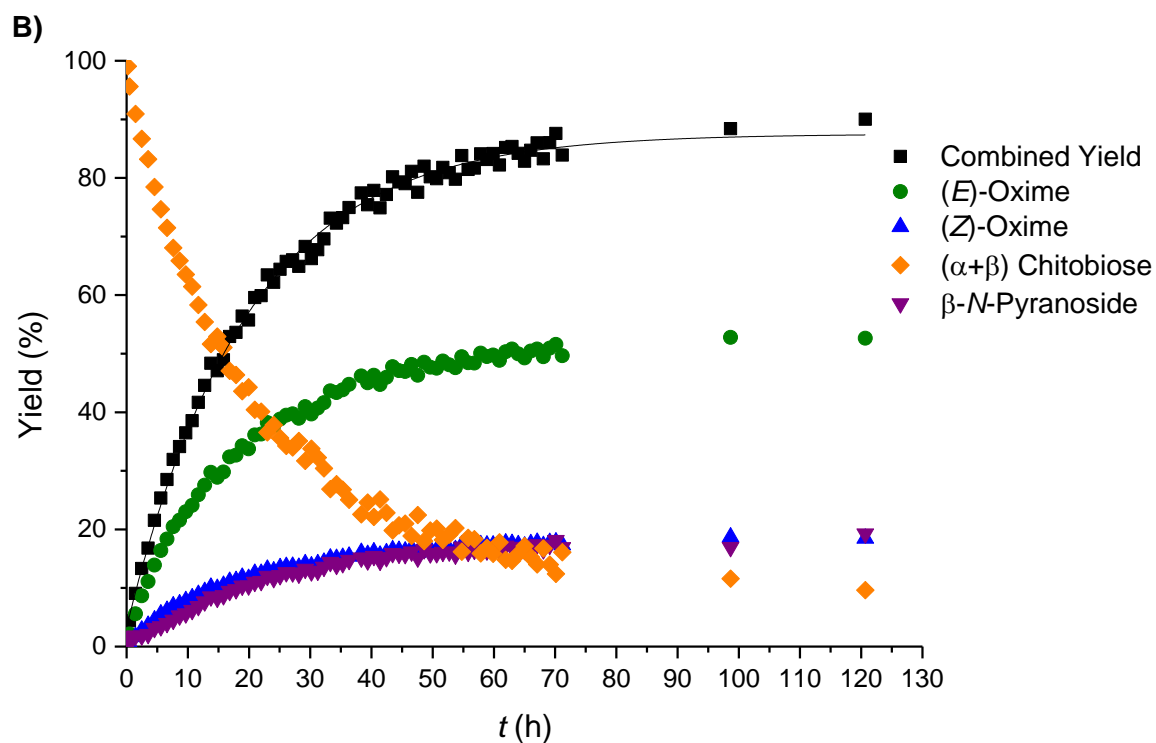
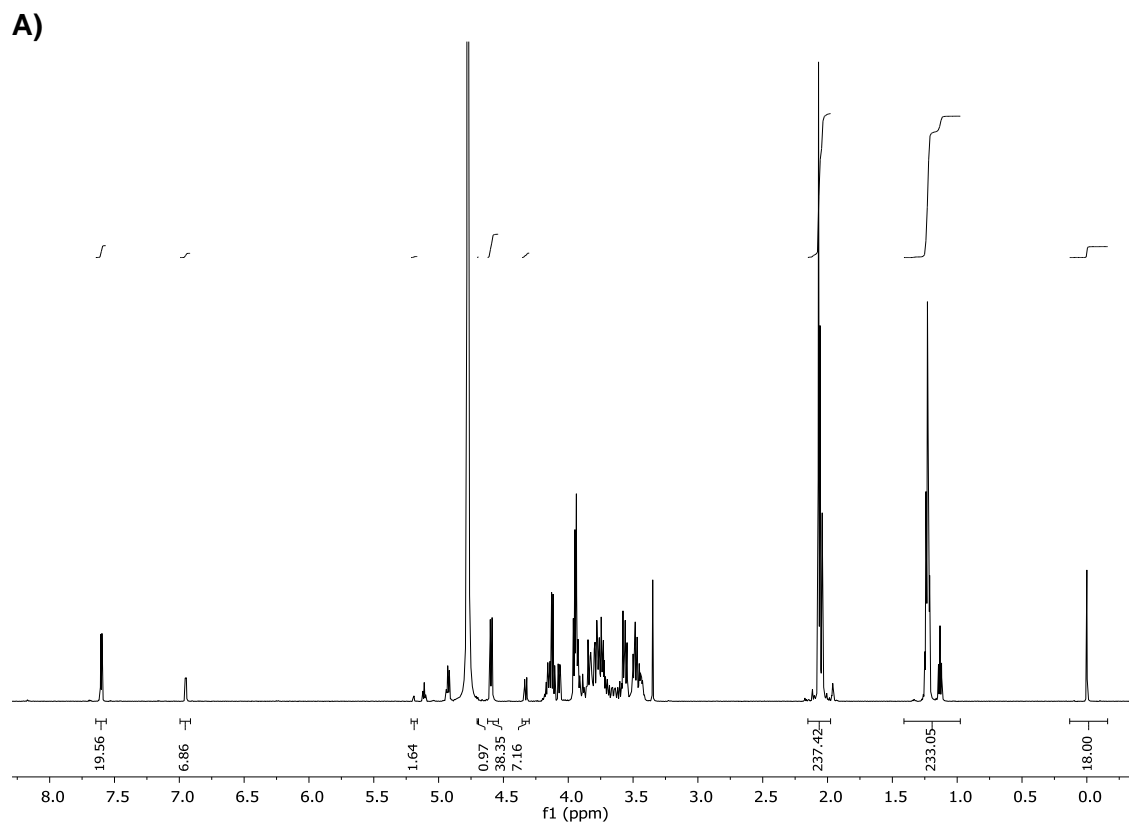


Figure S17. Ligation reaction of *N,N'*-diacetylchitobiose with 2.0 equiv of *O*-ethylhydroxylamine (11) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of Glucose with *O*-Benzylhydroxylamine (18)

Table S6. Characteristic NMR data of products obtained by the reaction of glucose with *O*-benzylhydroxylamine (18).

Glucose
(*E*)-Oxime
(*Z*)-Oxime
 β -*N*-Pyranoside

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
<i>(E)</i> -Oxime	H-1	7.58	d, 6.8	C-1	152.8
	H-2	4.37	dd, 7.2, 6.8	C-2	70.9
	H-3	3.92	dd, 7.2, 1.7	C-3	71.5
	H-4	3.49	dd, 8.5, 1.8	C-4	70.7
	H-5	3.72	ddd, 8.9, 6.2, 2.7	C-5	71.7
	H-6a	3.79	dd, 12.3, 2.7	C-6	63.5
	H-6b	3.58	dd, 12.3, 6.3		
<i>(Z)</i> -Oxime	H-1	6.90	d, 6.1	C-1	153.8
	H-2	4.97	"t", 6.1	C-2	67.2
	H-3	3.96	dd, 6.0, 2.6	C-3	71.1
	H-4	3.60-3.52	m	C-4	71.7
β - <i>N</i> -Pyranoside	H-1	4.27	d, 9.1	C-1	91.2
	H-2	3.44-3.35	m	C-2	-
	H-3	3.48	m	C-3	-
	H-4	3.76-3.69	m	C-4	-

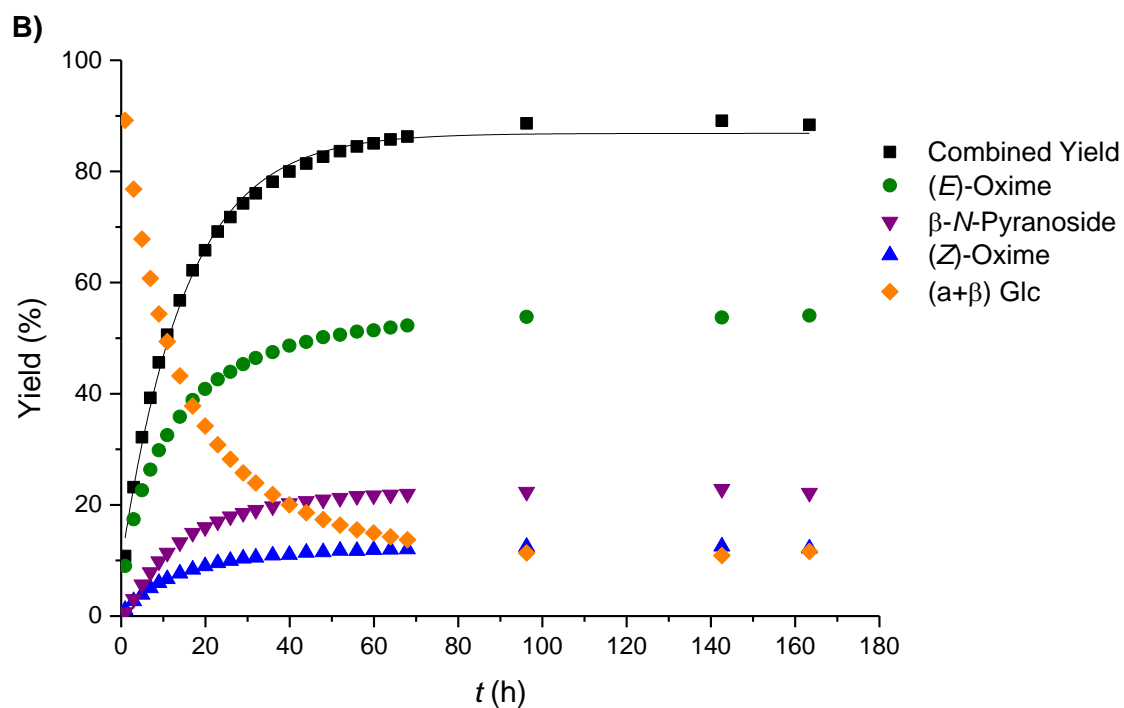
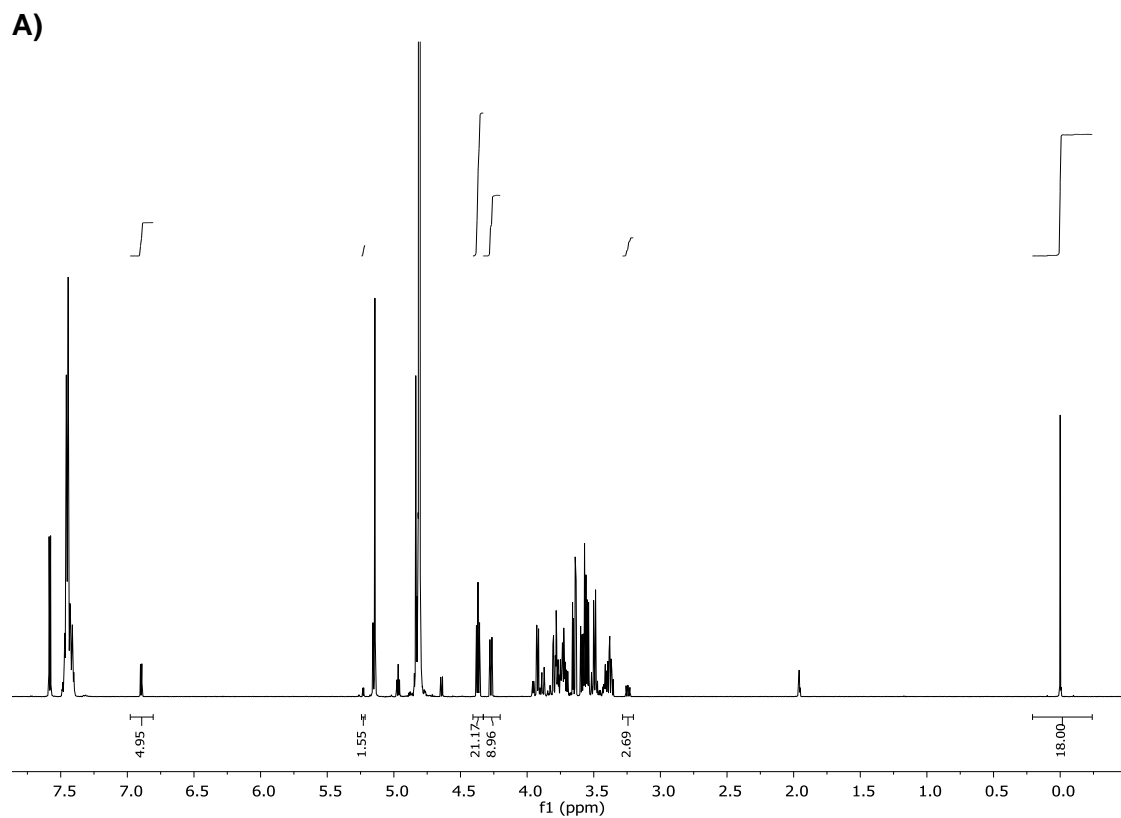


Figure S18. Ligation reaction of glucose with 1.2 equiv of *O*-benzylhydroxylamine (**18**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of GlcNAc with *O*-Benzylhydroxylamine (18)

Table S7. Characteristic NMR data of products obtained by the reaction of GlcNAc with *O*-benzylhydroxylamine (18).

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
(E)-Oxime	H-1	7.53	d, 6.0	C-1	152.8
	H-2	4.66-4.64	dd, 7.3, 6.4	C-2	51.7
	H-3	4.04	dd, 7.6, 1.8	C-3	69.1
	H-4	3.48-3.44	m	C-4	75.7
(Z)-Oxime	H-1	6.84	d, 6.3	C-1	153.8
	H-2	5.11	"t", 6.7	C-2	48.4
	H-3	4.11	dd, 7.0, 2.5	C-3	68.5
	H-4	3.45	m	C-4	73.9
β - <i>N</i> -Pyranoside	H-1	4.26	d, 9.9	C-1	90.1
	H-2	3.82	m	C-2	-

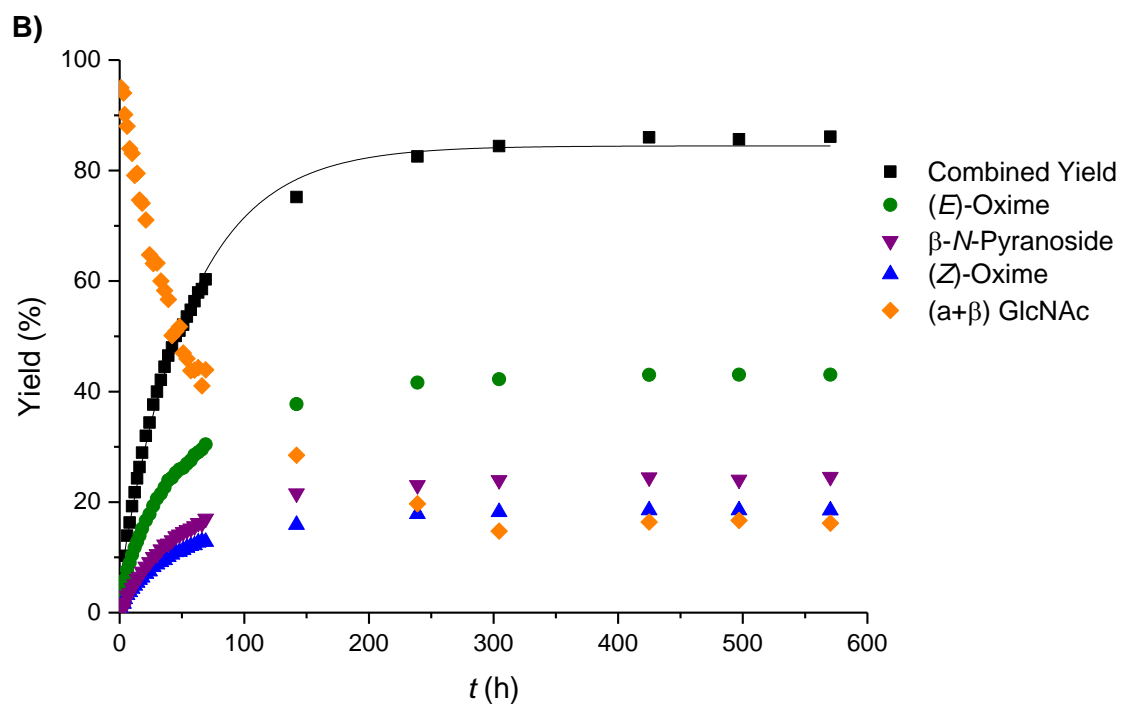
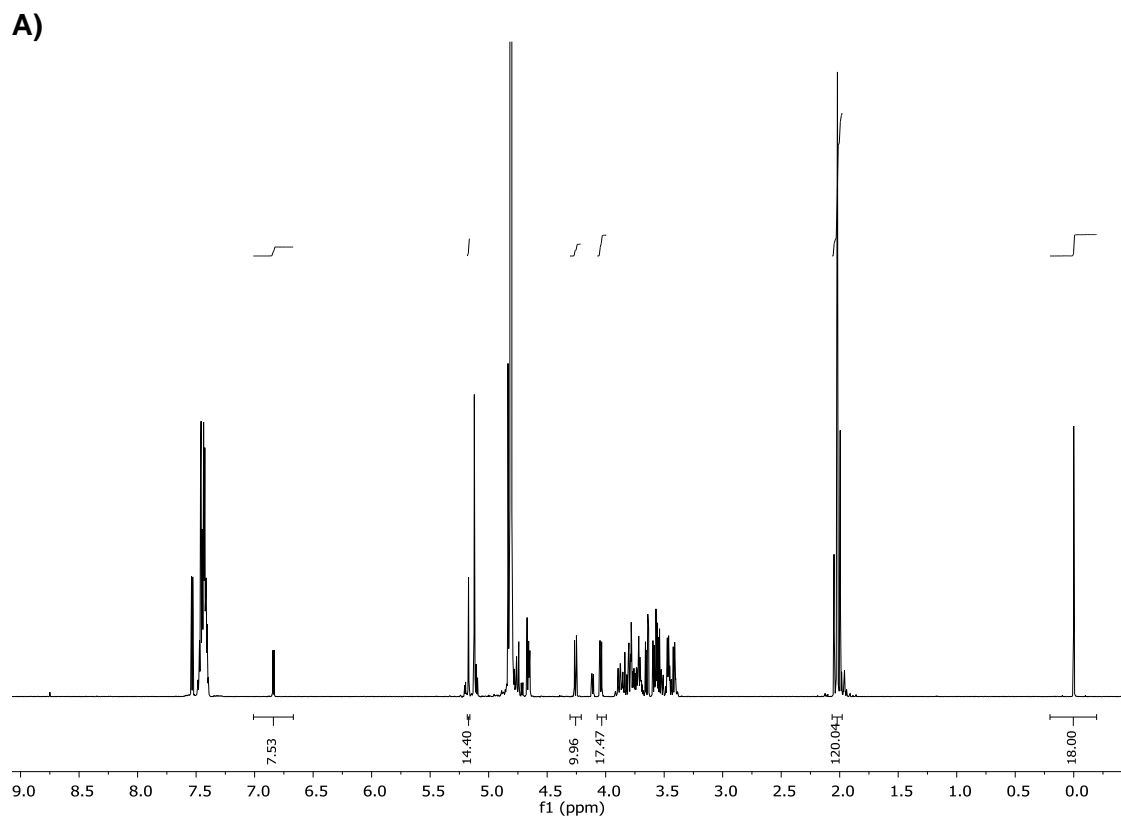
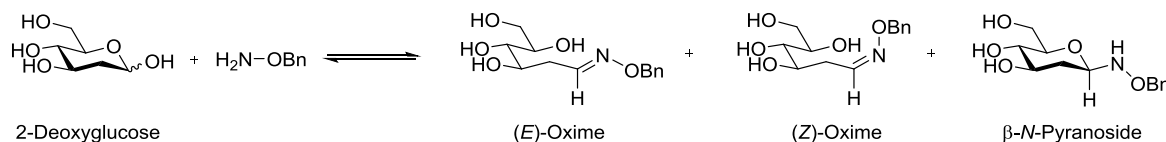


Figure S19. Ligation reaction of GlcNAc with 1.2 equiv of *O*-benzylhydroxylamine (**18**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of 2-Deoxyglucose with *O*-Benzylhydroxylamine (18)

Table S8. Characteristic NMR data of products obtained by the reaction of 2-deoxyglucose with *O*-benzylhydroxylamine (18). Determined $^1J_{C1-H1}$: 162.8 Hz (*E*)-Oxime, 175.4 Hz (*Z*)-Oxime



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
<i>E</i> -Oxime	H-1	7.64	"t", 6.4	C-1	151.2
	H-2a	2.51	ddd, 14.7, 8.5, 6,6	C-2	32.7
	H-2b	2.44	ddd, 14.7, 6.3, 5,1		
	H-3	4.08	ddd, 8.5, 5.1, 2.0	C-3	66.8
	H-4	3.43-3.39	m	C-4	71.6
	H-5	3.74-3.70	m	C-5	70.3
	H-6a	3.81-3.79	m	C-6	62.3
	H-6b	3.66-3.54	m		
<i>Z</i> -Oxime	H-1	7.00	"t", 5.5	C-1	151.7
	H-2a	2.61	d"t", 16.1, 5.0	C-2	29.5
	H-2b	2.72	ddd, 16.1, 9.0, 5.5		
	H-3	4.13	ddd, 9.0, 5.0, 2.2	C-3	66.5
	H-4	3.43-3.39	m	C-4	72.0
	H-5	3.74-3.70	m	C-5	70.3
	H-6a	3.83-3.81	m	C-6	62.3
	H-6b	3.66-3.54	m		
β- <i>N</i> -Pyranoside	H-1	4.51	dd, 11.1, 2.0	C-1	-

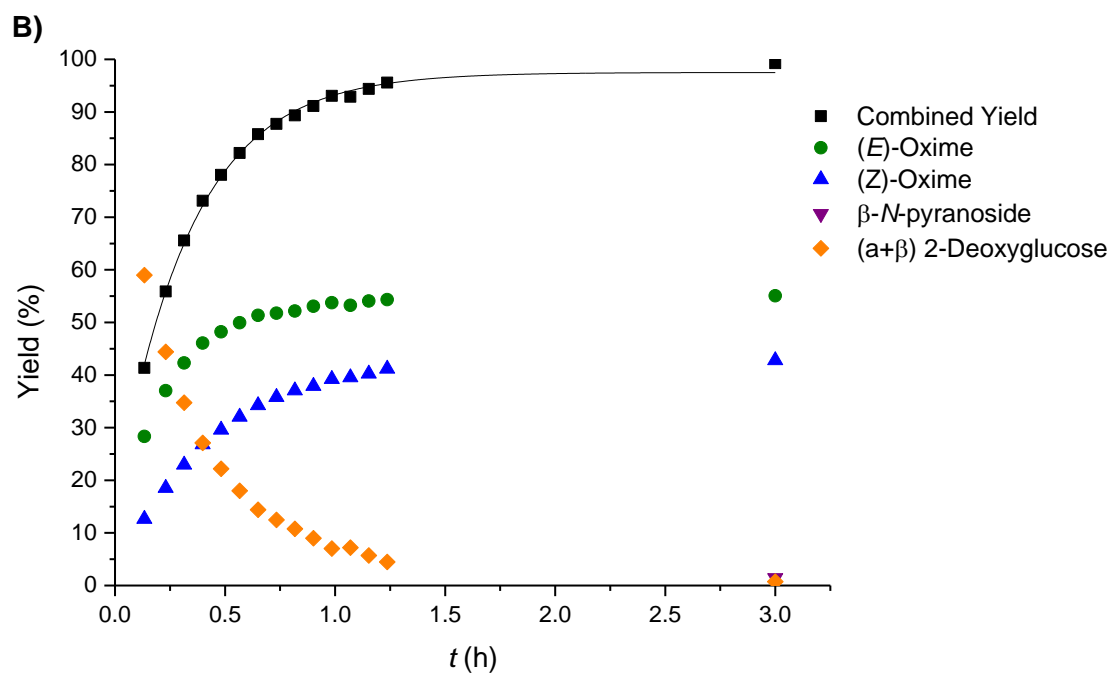
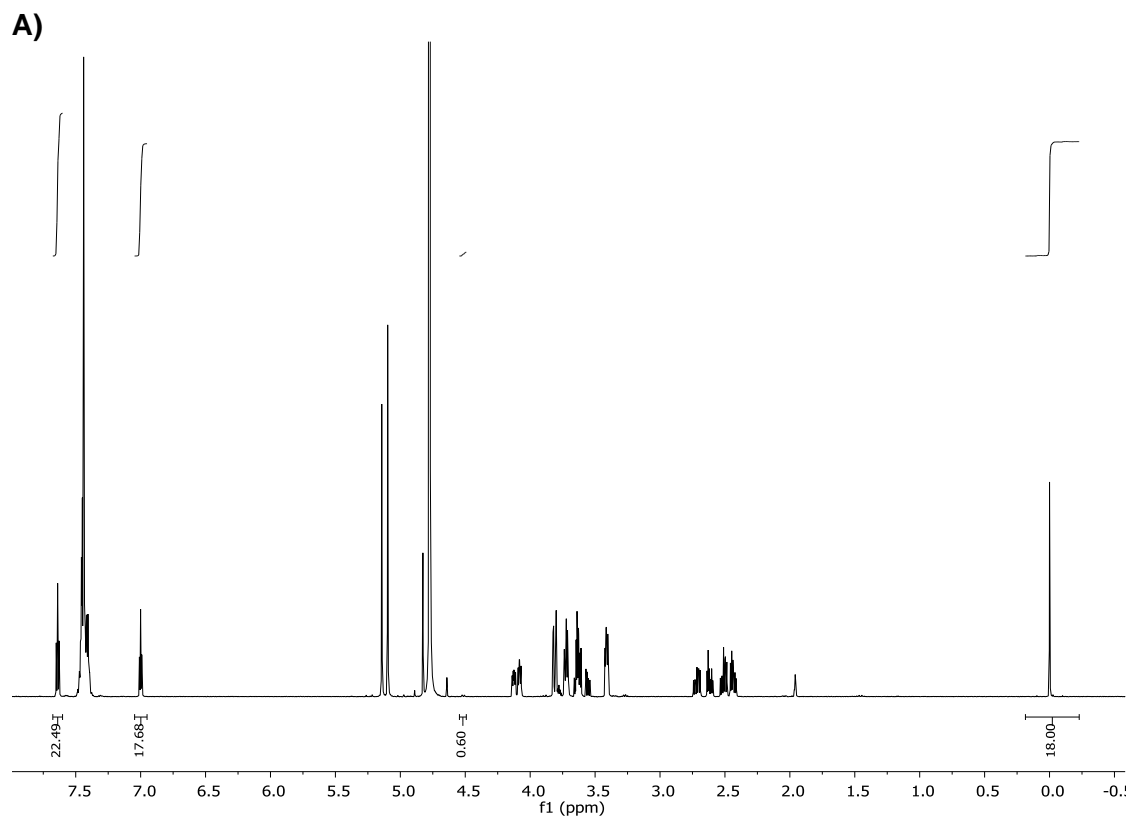


Figure S20. Ligation reaction of 2-deoxyglucose with 1.2 equiv of *O*-benzylhydroxylamine (**18**) at 27 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of Mannose with *O*-Benzylhydroxylamine (**18**)

Table S9 Characteristic NMR data of products obtained by the reaction of mannose with *O*-Benzylhydroxylamine (**18**).

Reaction scheme: Mannose + H₂N-OBn ⇌ (*E*)-Oxime + (*Z*)-Oxime + α-*N*-Pyranoside

Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
<i>(E)</i> -Oxime	H-1	7.64	d, 6.9	C-1	153.9
	H-2	4.27	dd, 8.1, 6.9	C-2	69.3
	H-3	3.90	dd, 8.1, 1.0	C-3	71.3
	H-4	3.69	m	C-4	67.4
	H-5	3.77-3.71	m	C-5	71.3
	H-6a	3.84	dd, 12.0, 2.3	C-6	63.8
	H-6b	3.67-3.62	m		
<i>(Z)</i> -Oxime	H-1	6.98	d, 6.7	C-1	153.8
	H-2	4.95	dd, 7.6, 6.8	C-2	77.9
	H-3	3.95-.3.92	m	C-3	71.0
α - <i>N</i> -Pyranoside	H-1	4.51	d, 1.0	C-1	88.5

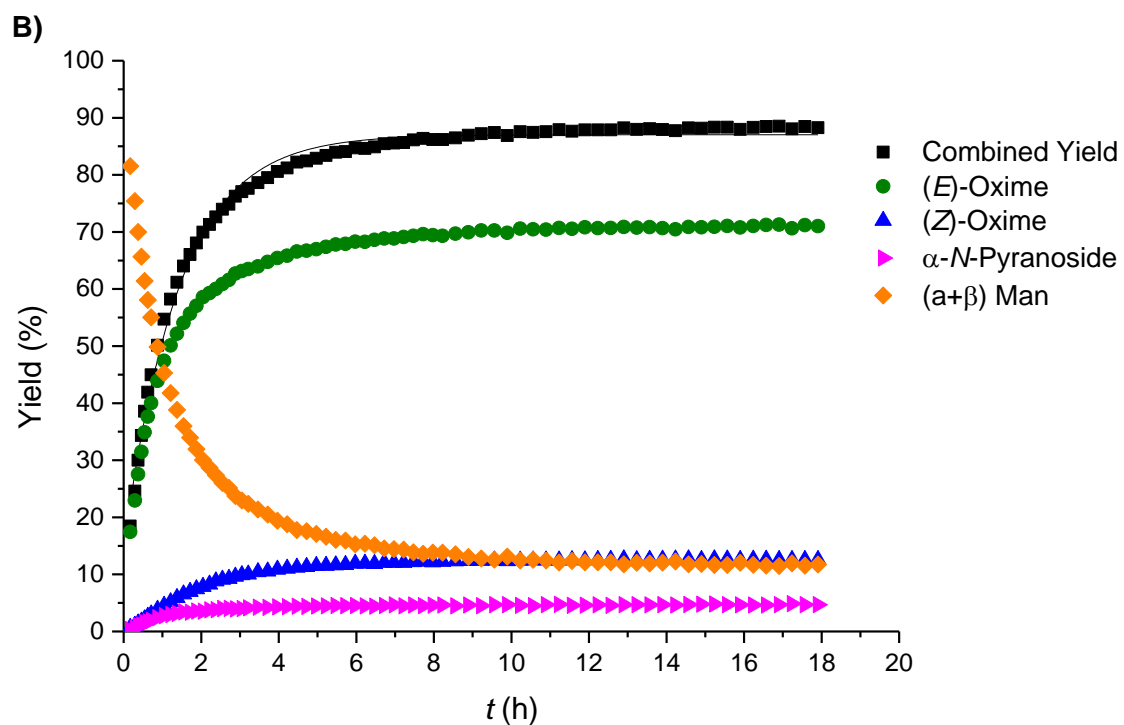
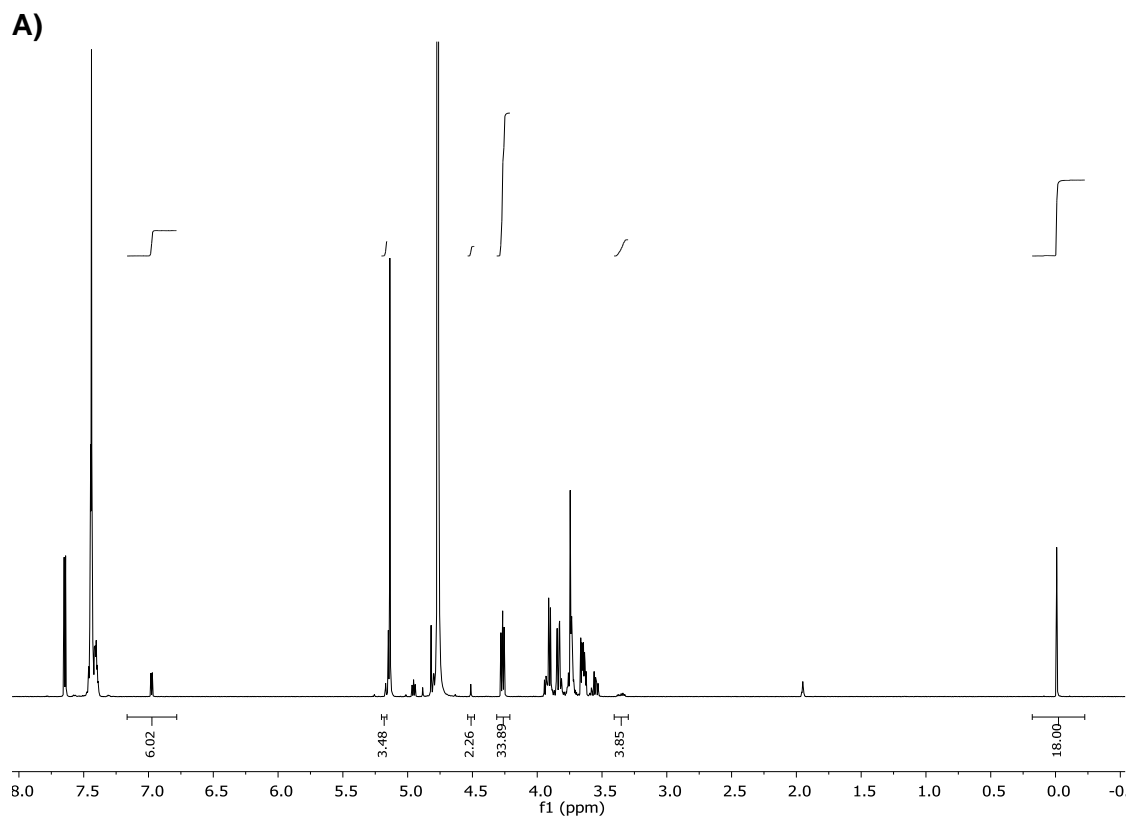
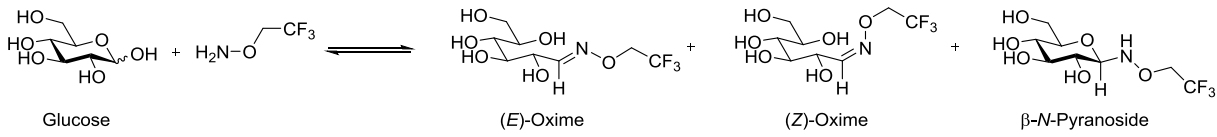


Figure S21. Ligation reaction of mannose with 1.0 equiv of *O*-benzylhydroxylamine (**18**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of Glucose with 2,2,2-Trifluoroethoxyamine (19)

Table S10. Characteristic NMR data of products obtained by the reaction of glucose with 2,2,2-trifluoroethoxyamine (19).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
(E)-Oxime	H-1	7.68	d, 6.4	C-1	154.2
	H-2	4.44	dd, 6.9, 6.4	C-2	71.2
	H-3	3.97	dd, 7.0, 1.8	C-3	72.1
	H-4	3.59	dd, 8.5, 1.8	C-4	71.1
	H-5	3.79-3.75	m	C-5	72.0
	H-6a	3.85-3.81	m	C-6	63.9
	H-6b	3.67-3.59	m		
(Z)-Oxime	H-1	7.01	d, 6.2	C-1	155.7
	H-2	5.00	"t", 5.8	C-2	67.4
	H-3	4.00	dd, 5.8, 2.8	C-3	71.4
	H-6a	3.82-3.79	m	C-6	63.9
β -N-Pyranoside	H-1	4.34	d, 9.1	C-1	91.5
	H-2	3.38	dd, 9.1, 8.0	C-2	78.2
	H-3	3.51	"t", 9.1	C-3	77.9
	H-4	3.41-3.35	m	C-4	70.5
	H-5	3.48-3.41	m	C-5	78.4
	H-6a	3.92-3.87	m	C-6	61.8
	H-6b	3.72	m		

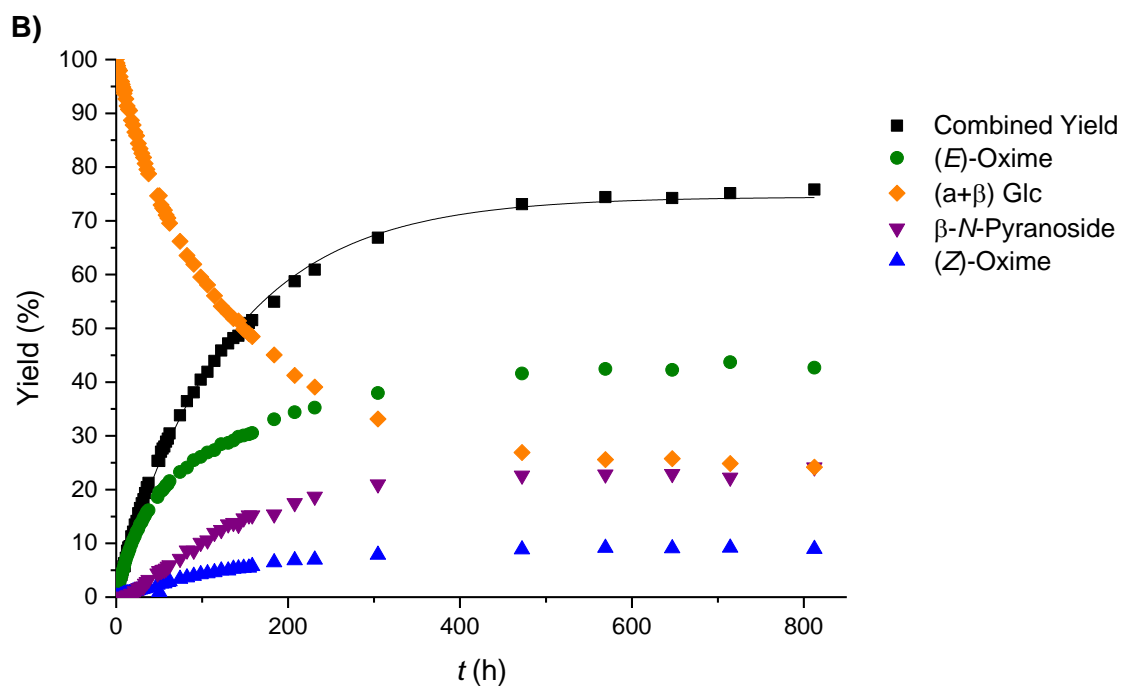
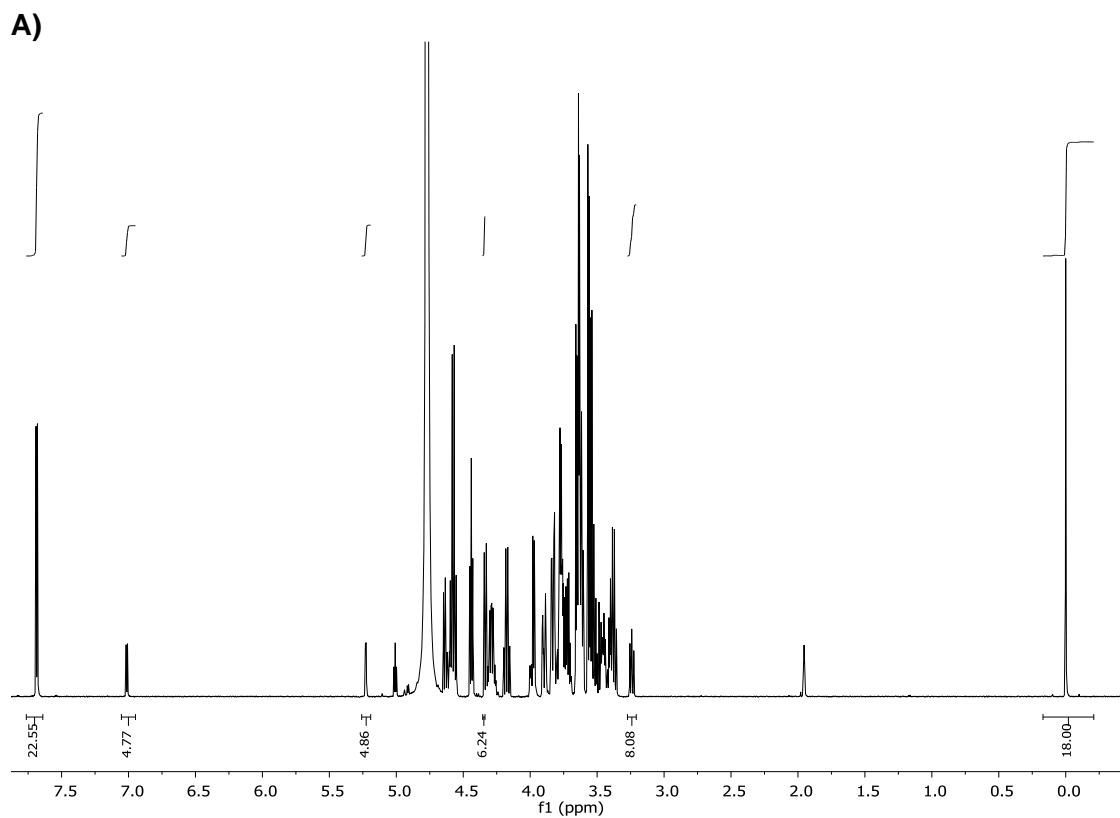
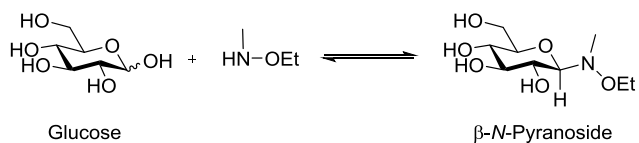


Figure S22. Ligation reaction of glucose with 1.2 equiv of 2,2,2-trifluoroethoxyamine (**19**) at 27 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of Glucose with *O*-Ethyl-*N*-Methylhydroxylamine (20)

Table S11. Characteristic NMR data of product obtained by the reaction of glucose with *O*-ethyl-*N*-methylhydroxylamine (20).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
β - <i>N</i> -Pyranoside	H-1	4.14-4.08	d, 8.6	C-1	91.9
	H-2	3.55-3.47	m	C-2	68.7
	H-3	3.42-3.35	m	C-3	75.9
	H-4	3.96-3.89	m	C-4	68.2

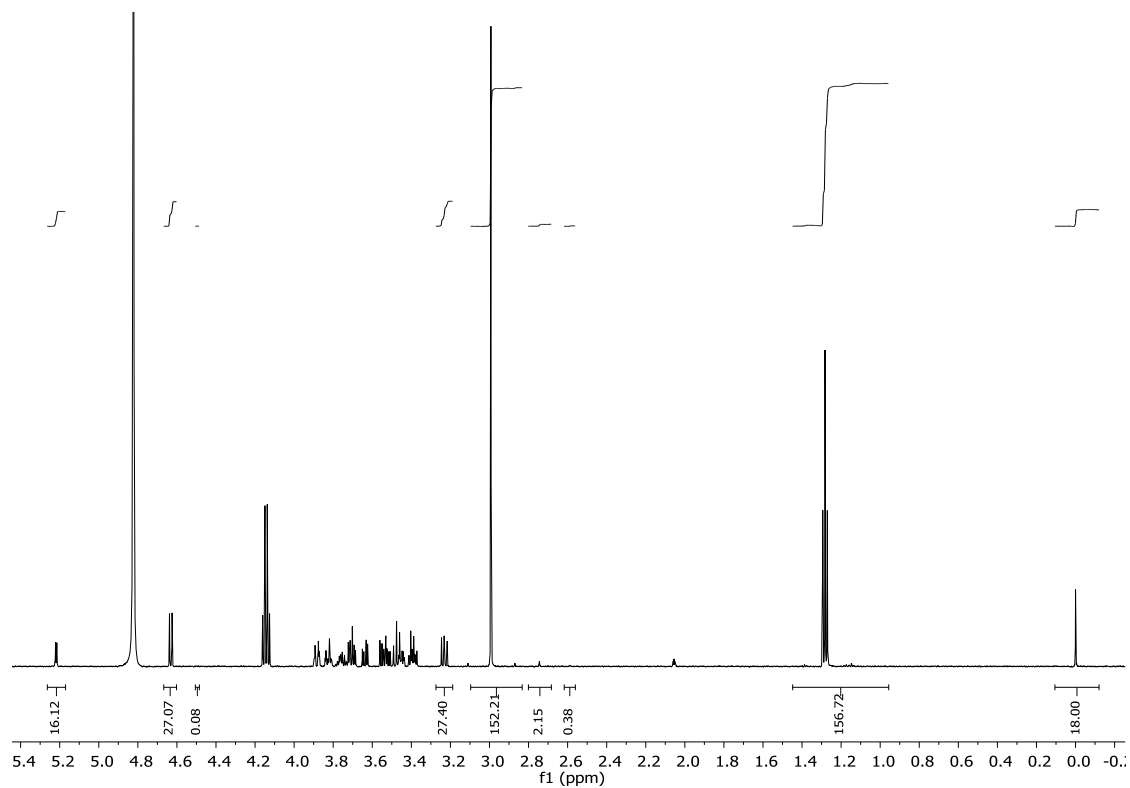


Figure S23. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 3. ¹H NMR spectrum (600 MHz) after equilibration.

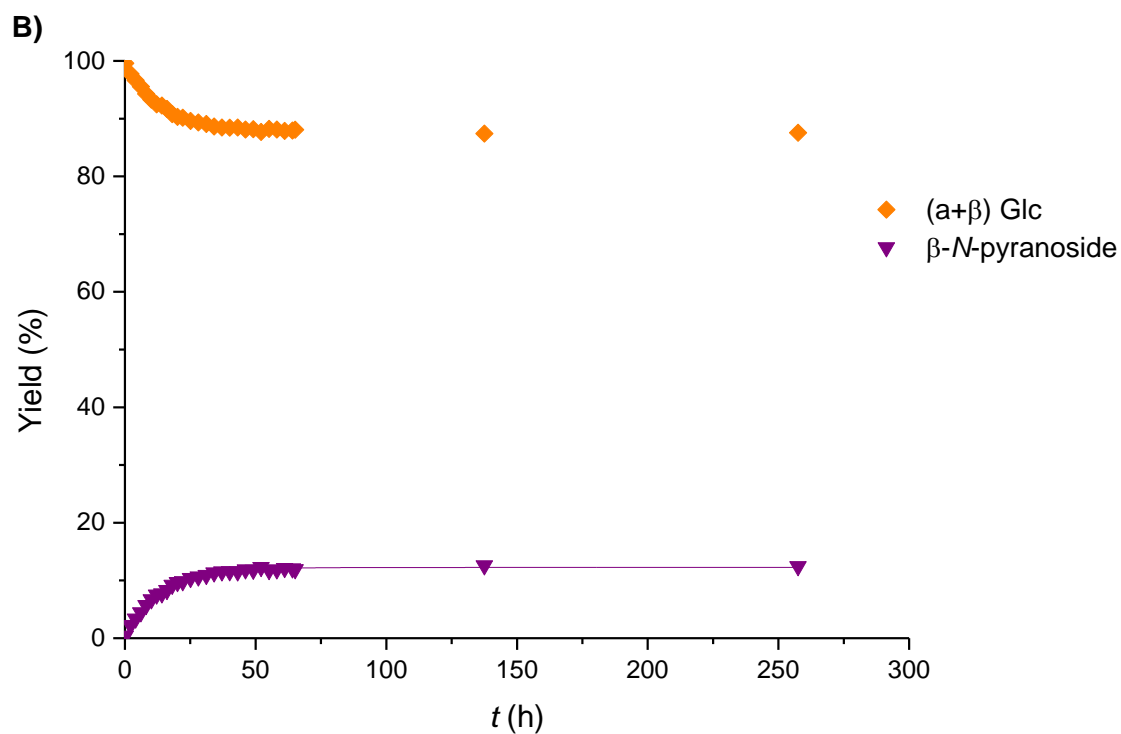
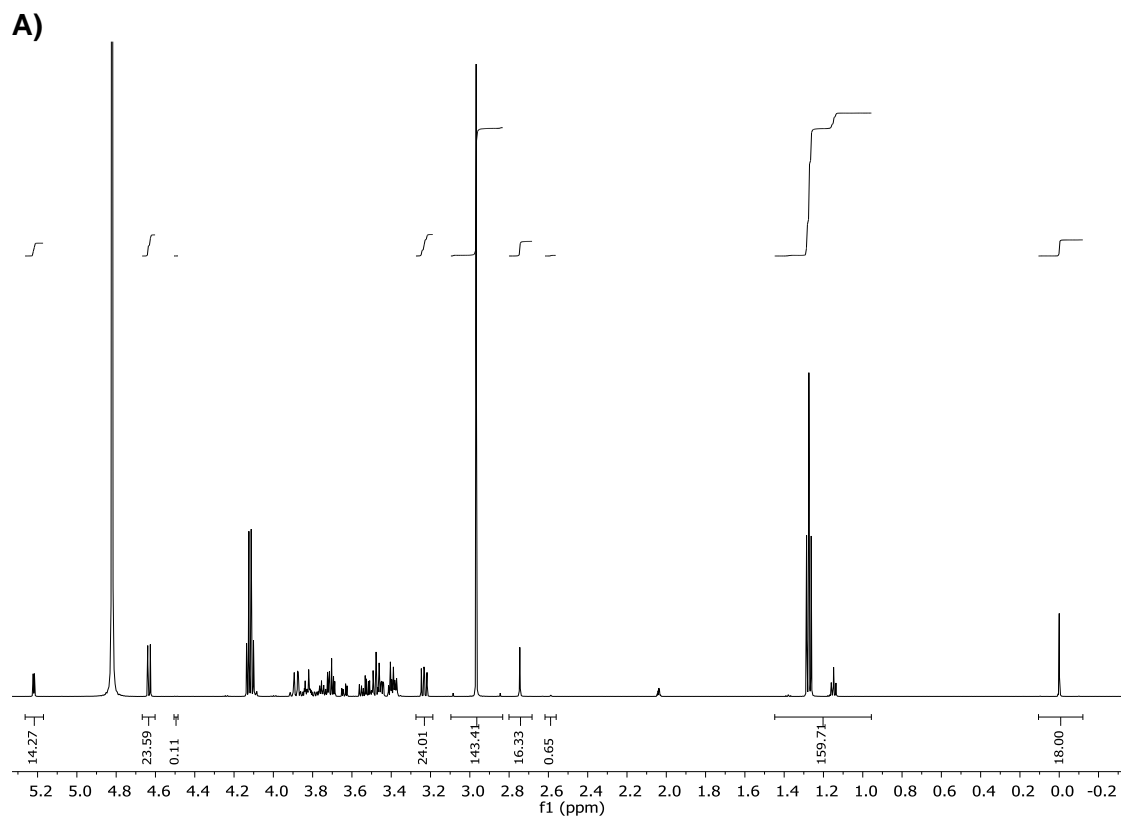


Figure S24. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 4. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

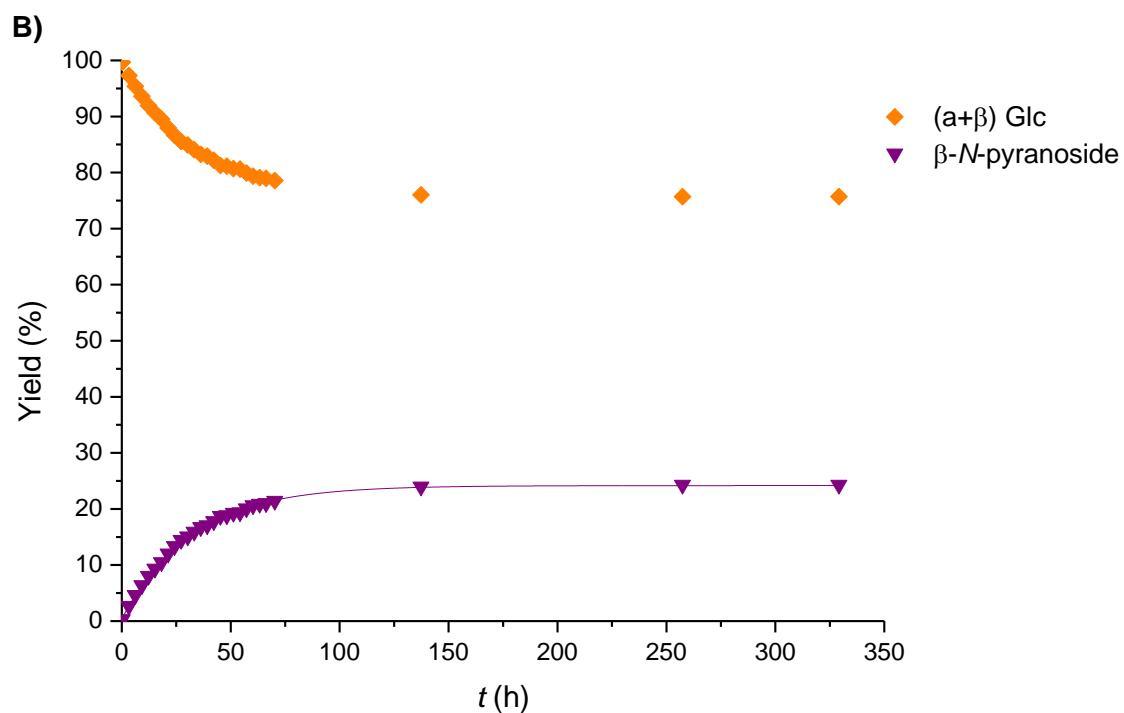
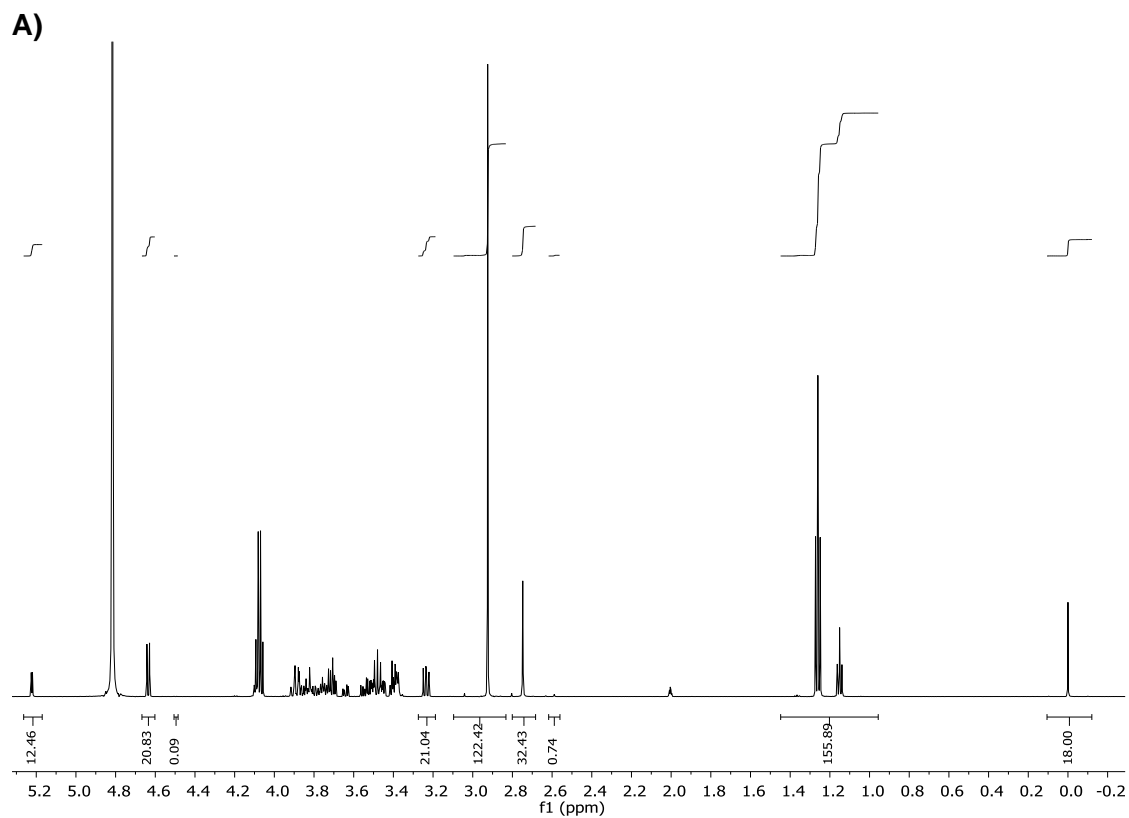


Figure S25. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 4.5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

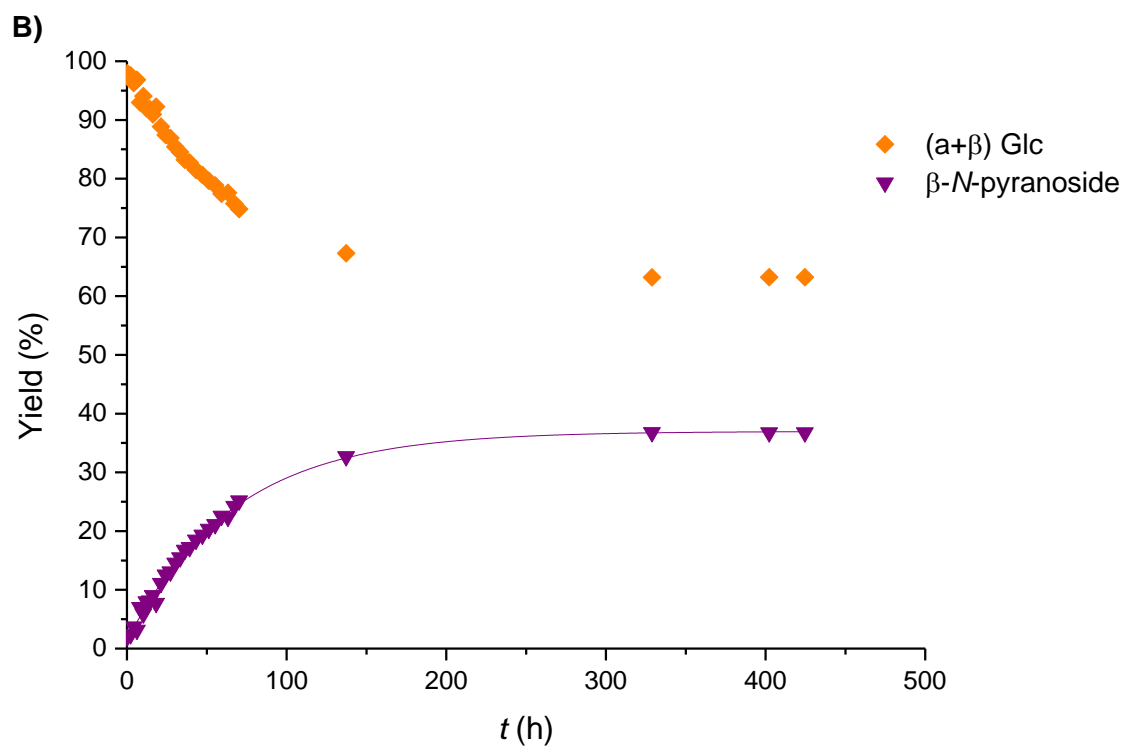
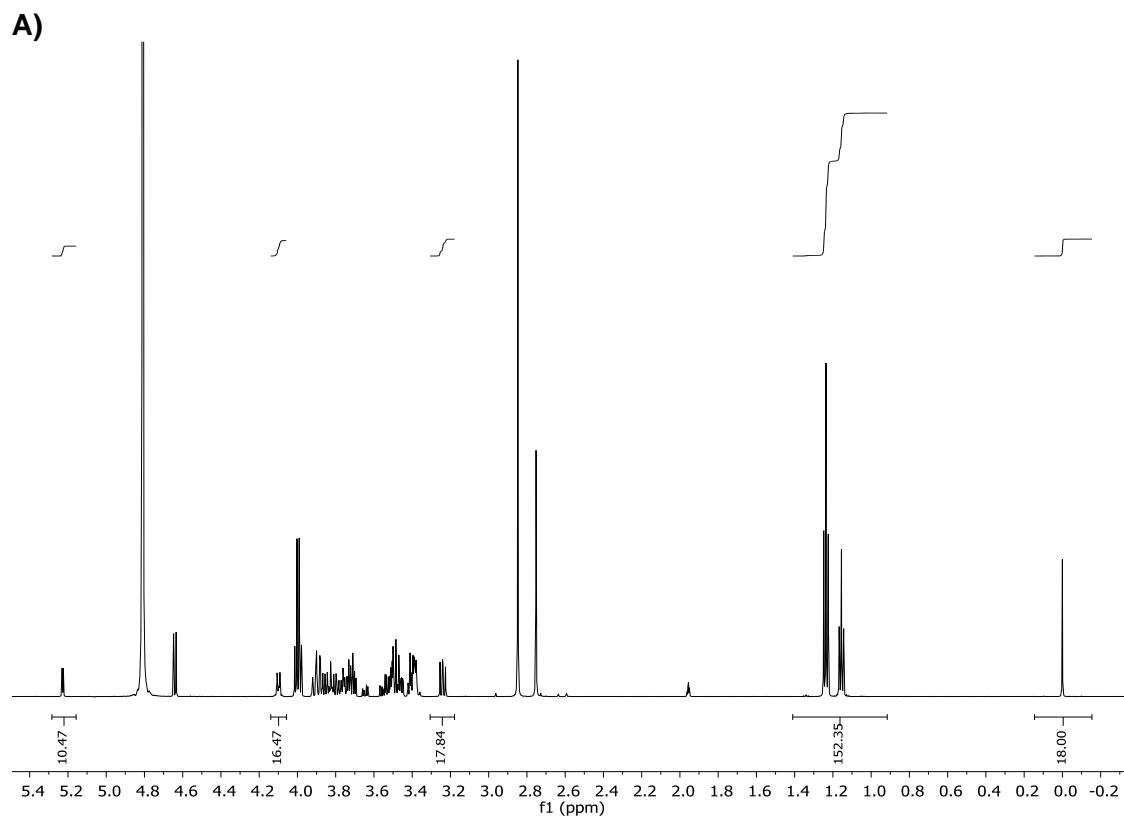


Figure S26. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

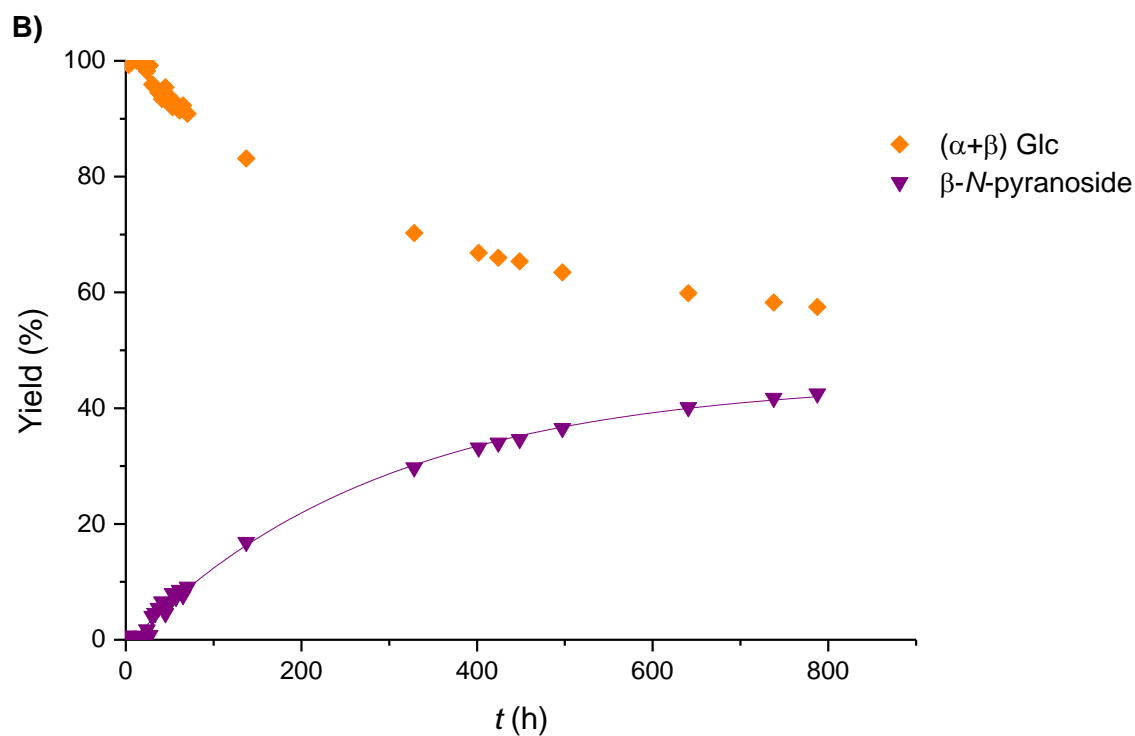
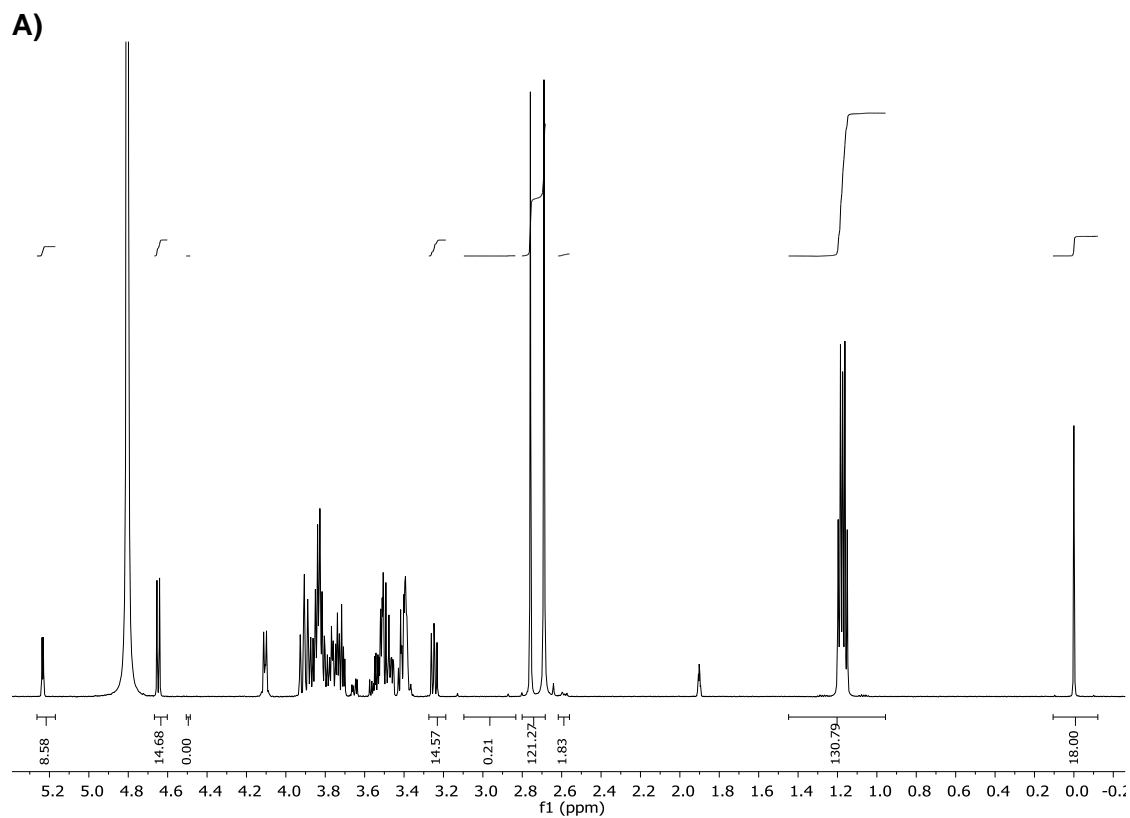


Figure S27. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 6. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

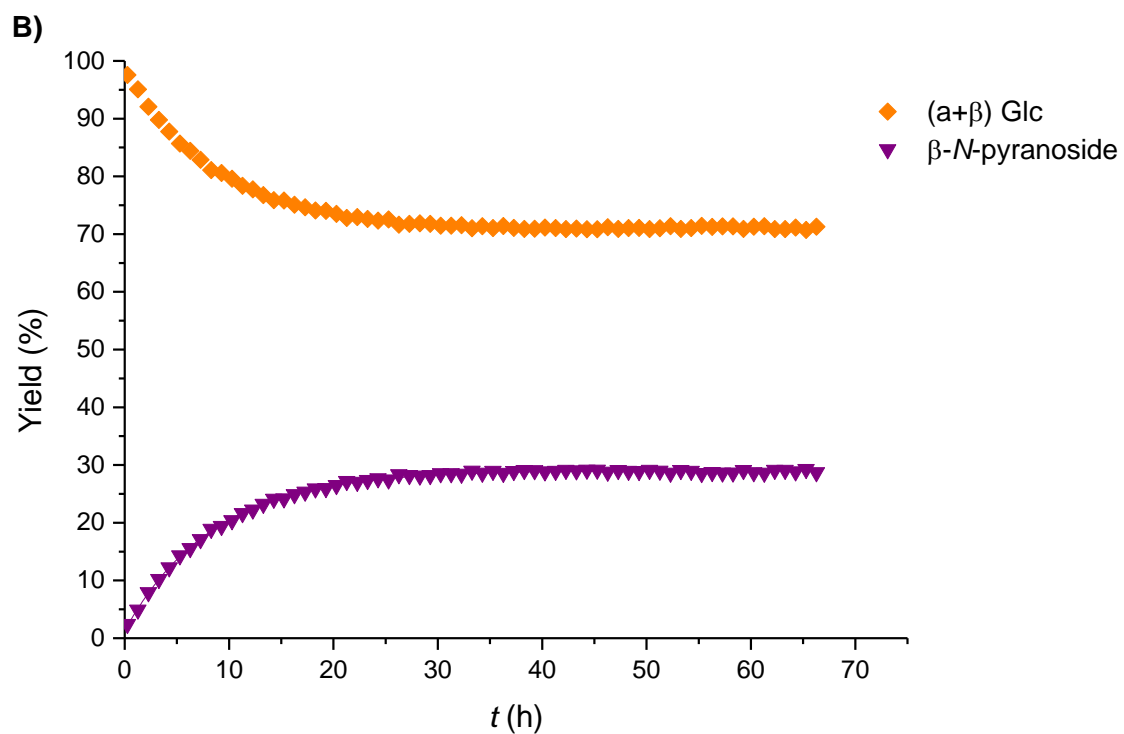
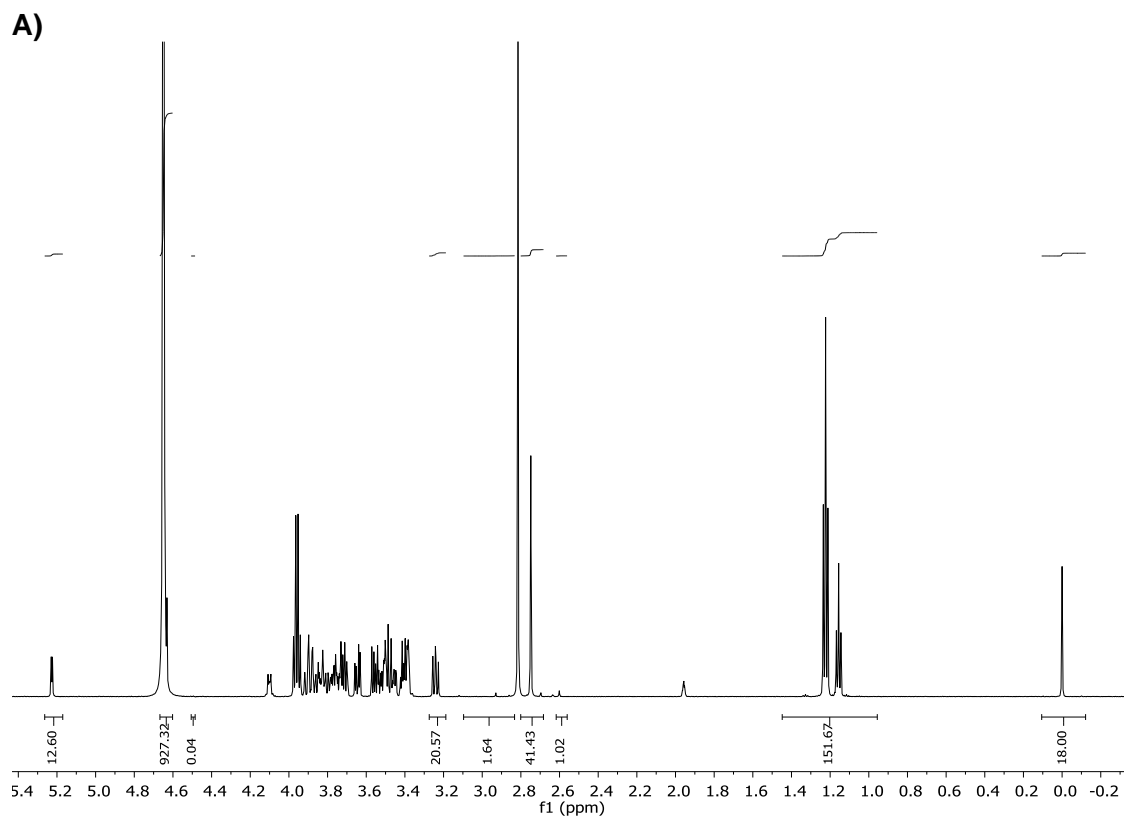


Figure S28. Ligation reaction of glucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

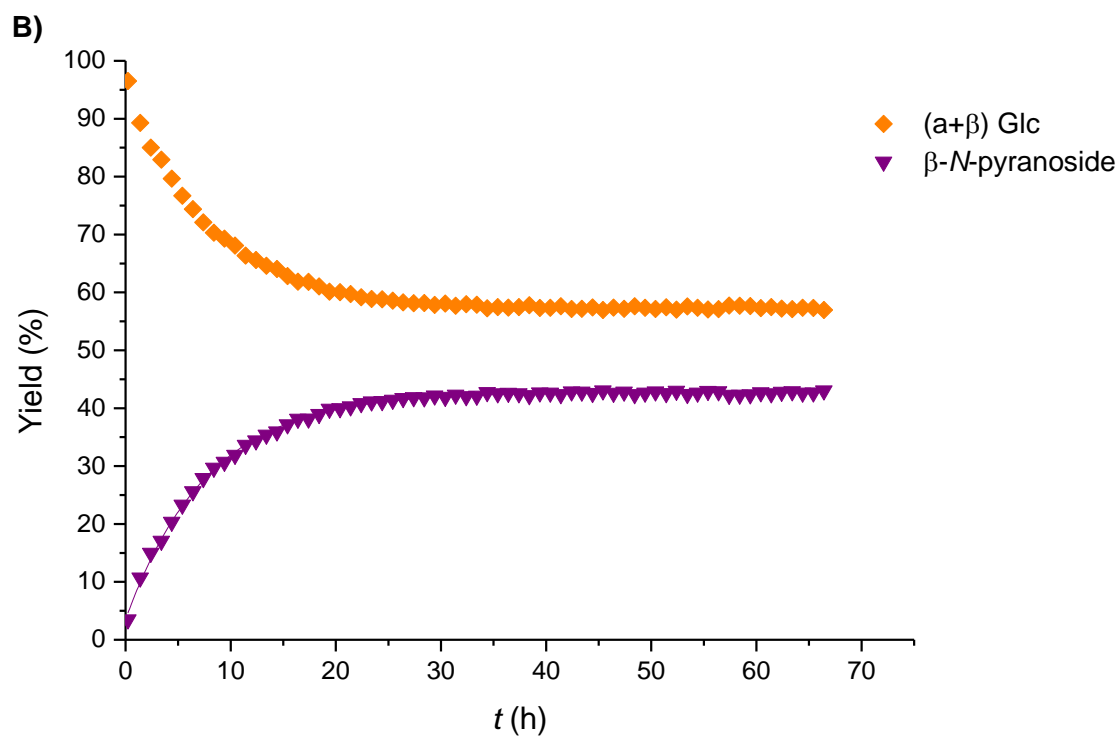
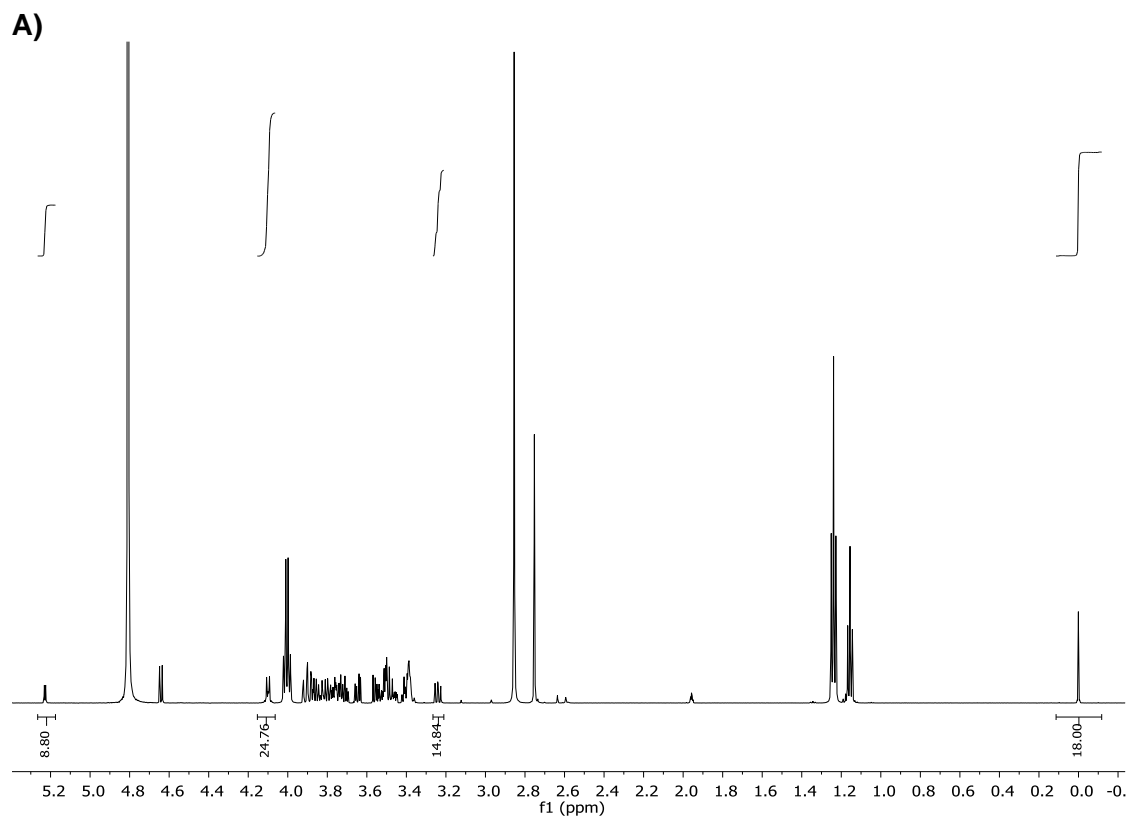


Figure S29. Ligation reaction of glucose with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

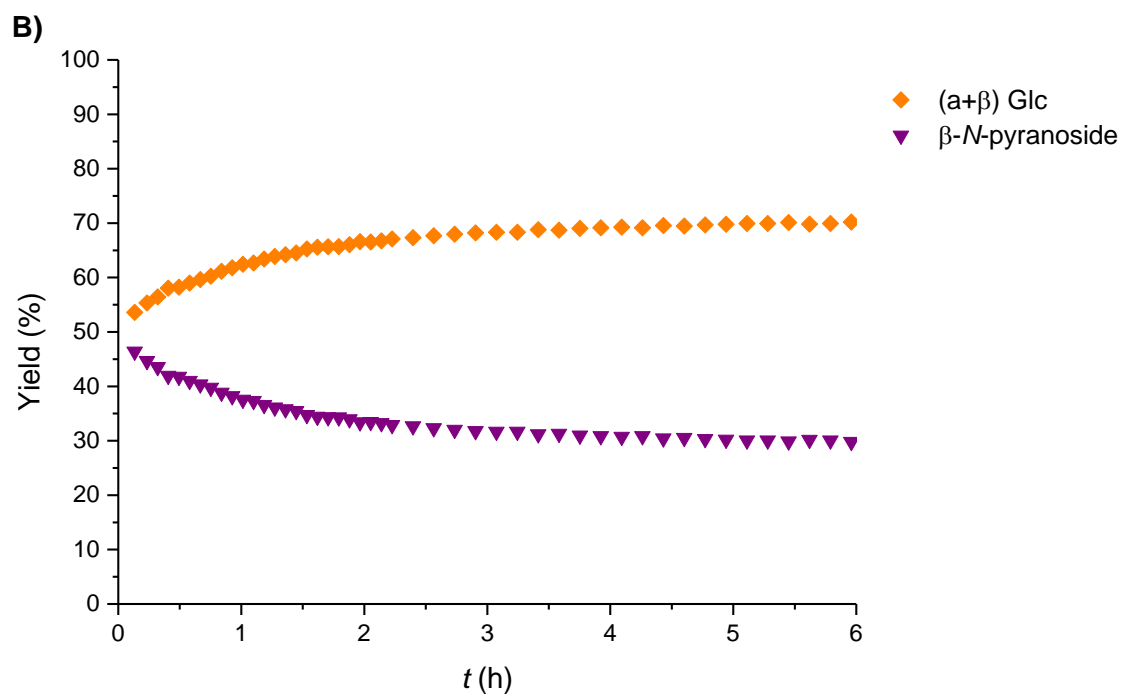
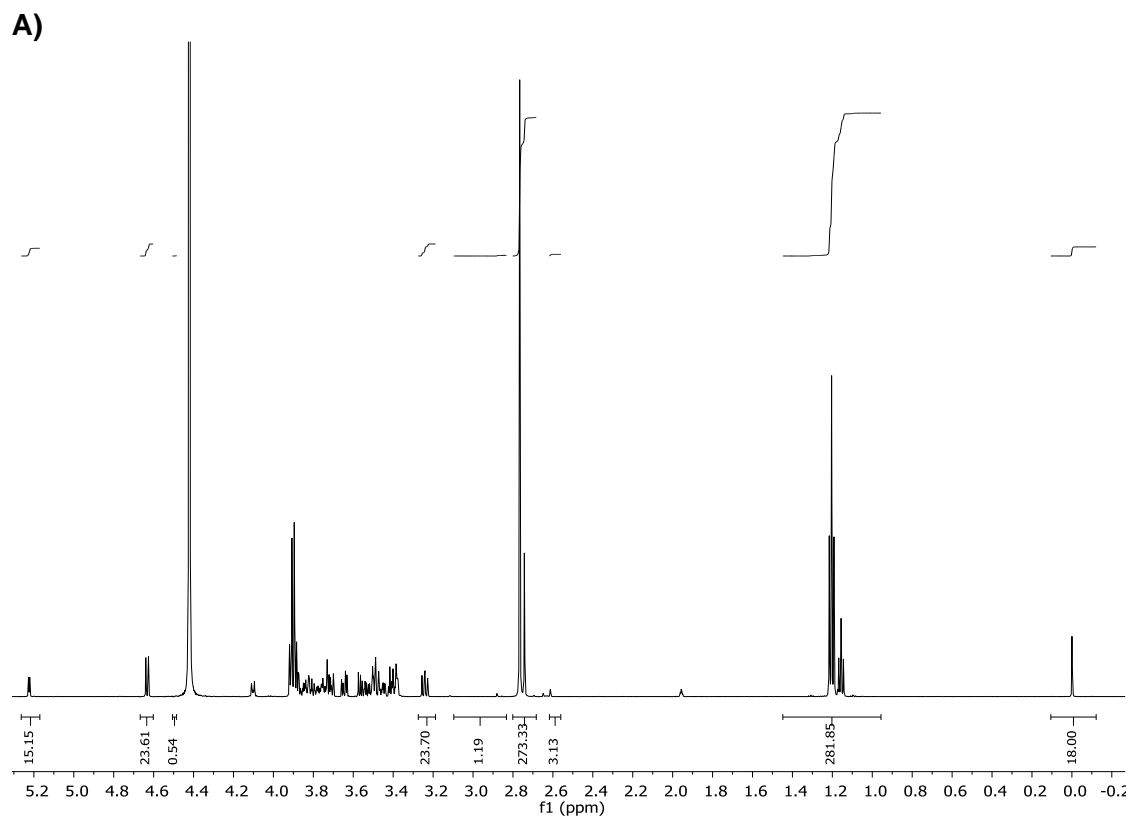


Figure S30. Ligation reaction of glucose with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at pH 5. Re-equilibration at 60 °C after the sample had been equilibrated at 39 °C. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

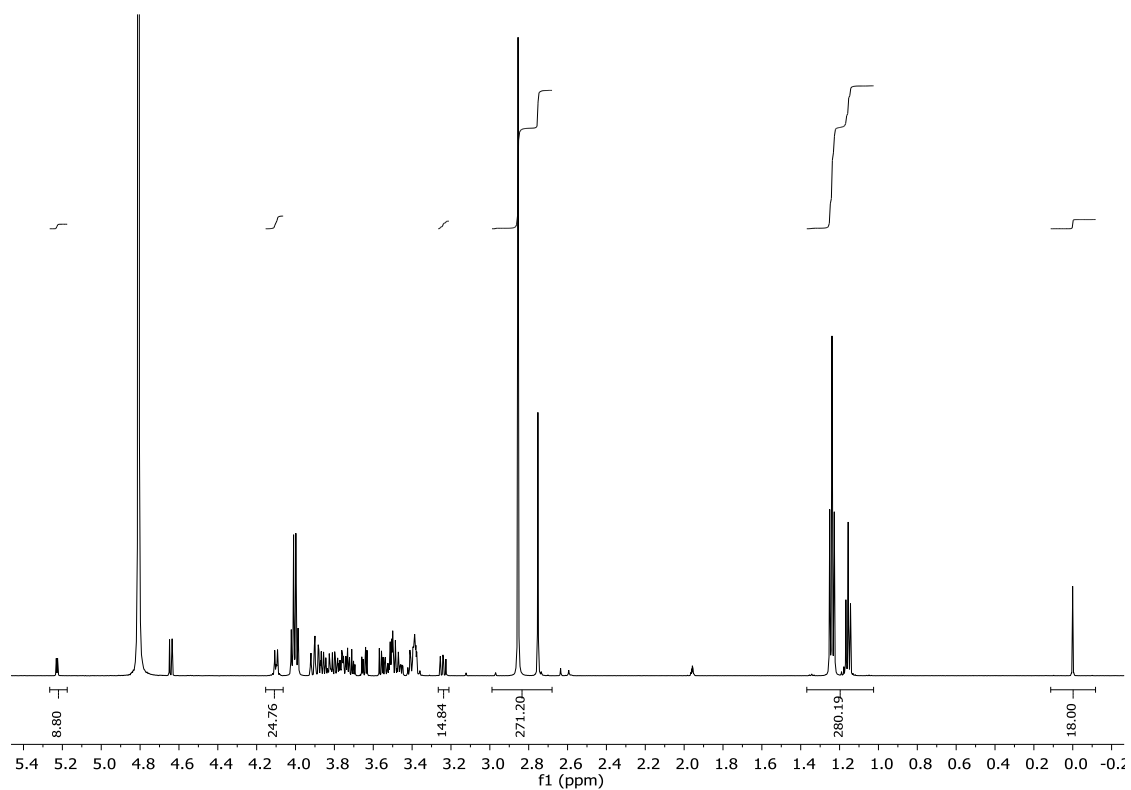
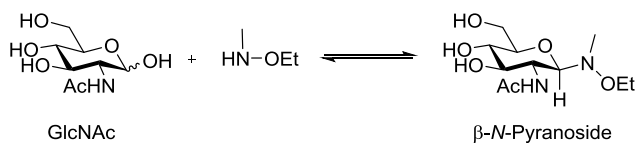


Figure S31. Ligation reaction of glucose with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 21 °C and pH 5. ¹H NMR spectrum (600 MHz) after equilibration.

Reaction of GlcNAc with *O*-Ethyl-*N*-Methylhydroxylamine (**20**)

Table S12. Characteristic NMR data of product obtained by the reaction of GlcNAc with *O*-ethyl-*N*-methylhydroxylamine (**20**).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	δ (C) (ppm)
β - <i>N</i> -Pyranoside	H-1	4.20	d, 9.7	C-1	90.4
	H-2	3.98	"t", 9.7	C-2	51.2
	H-3	3.53	dd, 10.0, 8.9	C-3	74.3
	H-4	3.44	m	C-4	68.5
	H-5	3.40	ddd, 9.8, 5.3, 2.1	C-5	76.5
	H-6	3.81-3.72	m	C-6	59.6

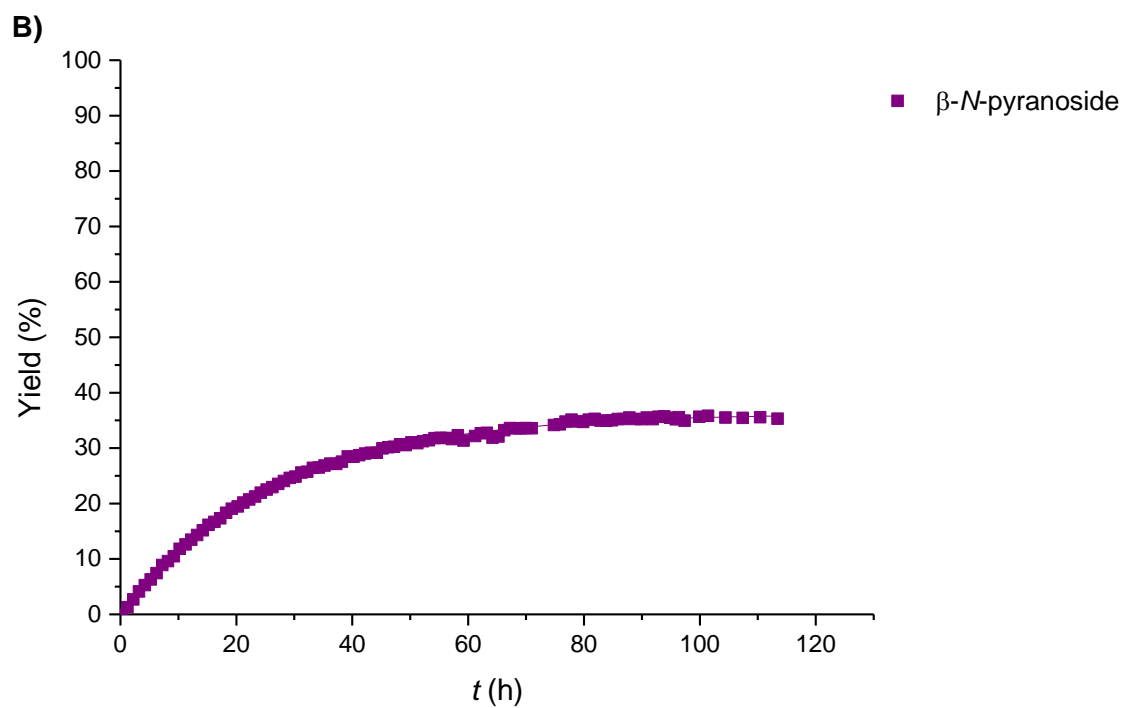
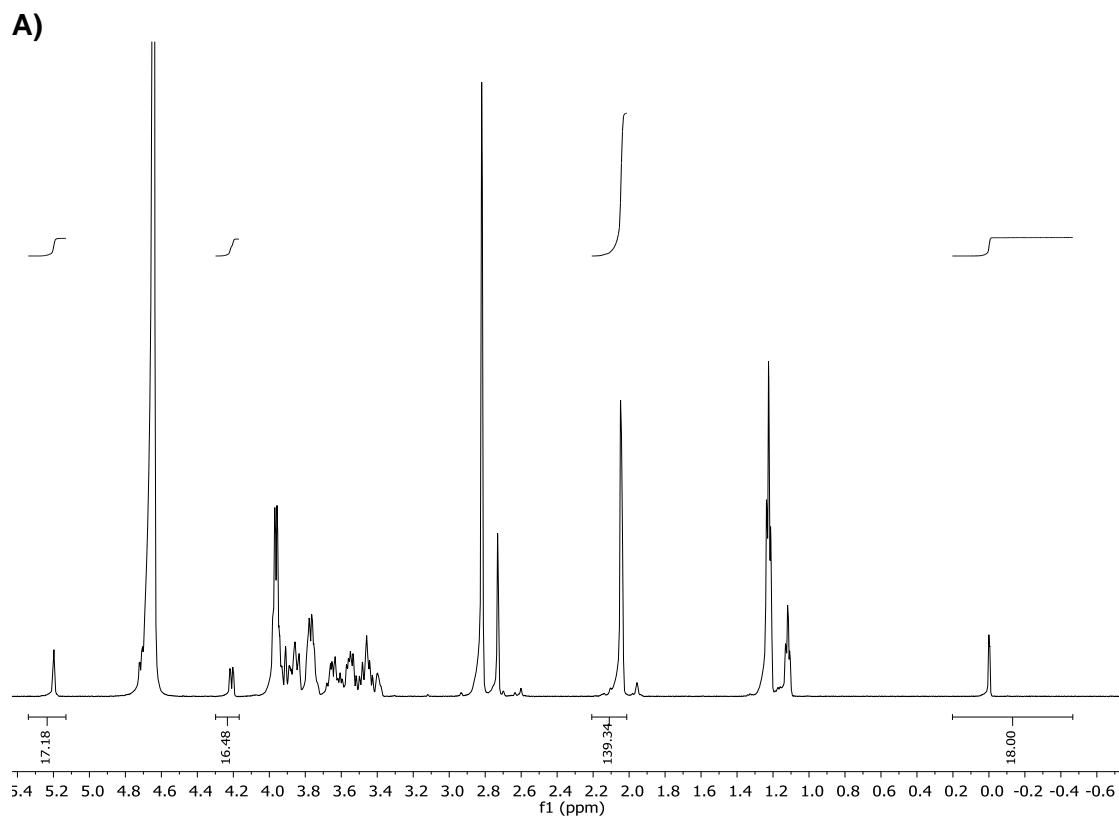
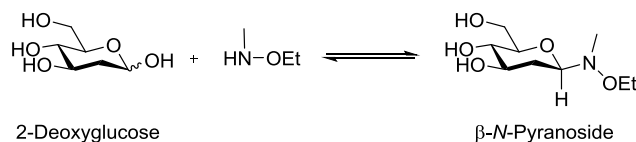


Figure S32. Ligation reaction of GlcNAc with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ^1H NMR spectrum (600 MHz) after equilibration. B) Individual yield of product over time.

Reaction of 2-Deoxyglucose with *O*-Ethyl-*N*-Methylhydroxylamine (**20**)

Table S13. Characteristic NMR data of product obtained by the reaction of 2-deoxyglucose with *O*-ethyl-*N*-methylhydroxylamine (**20**).



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
β - <i>N</i> -Pyranoside	H-1	4.34	dd, 11.0, 1.8	C-1	89.6
	H-2a	1.75-1.67	m	C-2	35.5
	H-2b	2.16-2.10	m		
	H-3	3.78-3.72	m	C-3	70.8
	H-4	3.78-3.72	m	C-4	70.4
	H-5	3.34	ddd, 9.7, 5.6, 2.2	C-5	77.1
	H-6a	3.78-3.72	m	C-6	-
	H-6b	3.78-3.72	m		

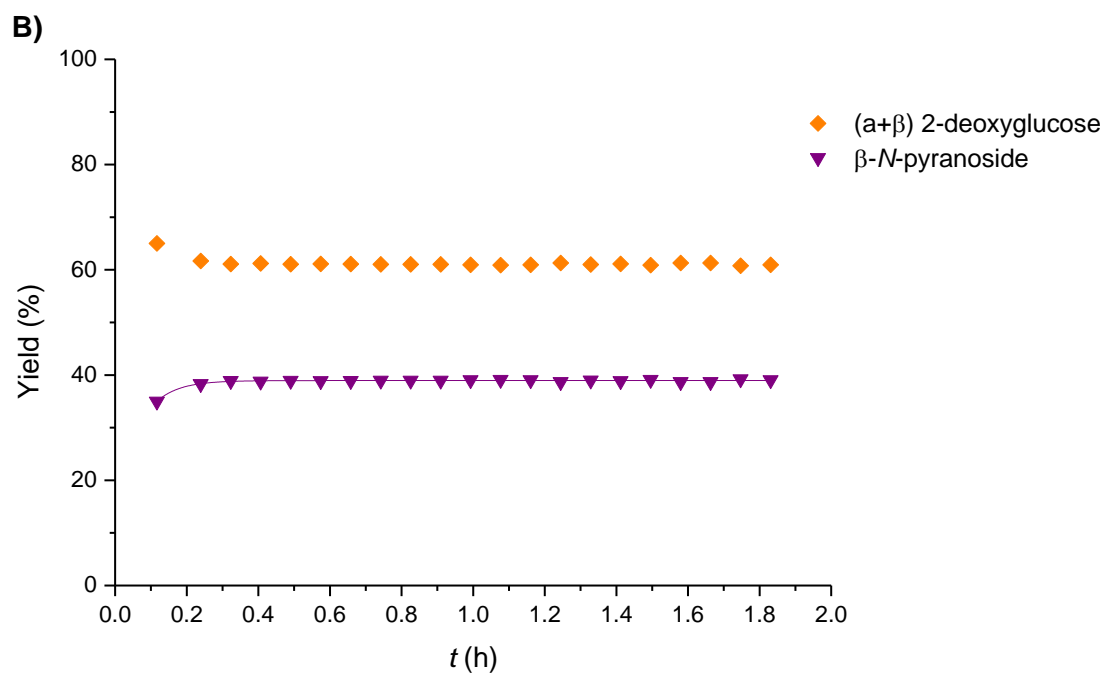
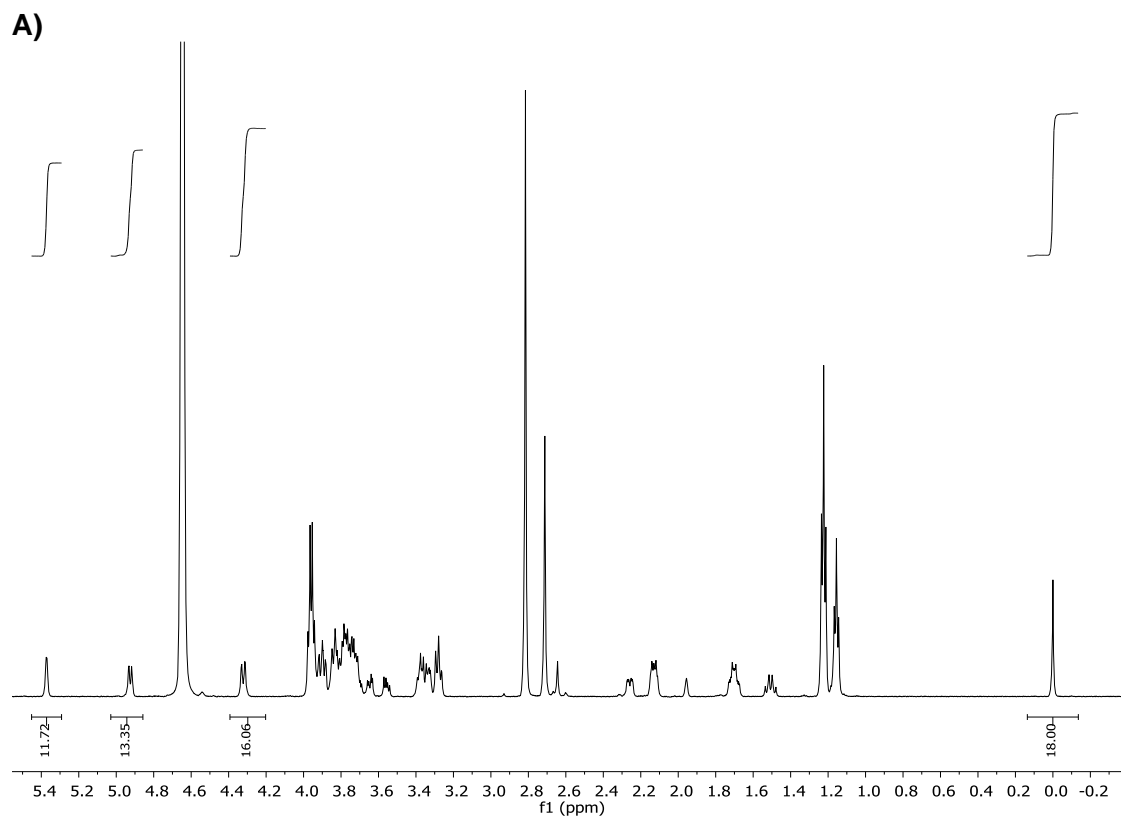
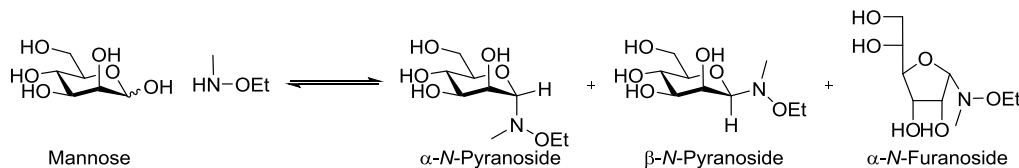


Figure S33. Ligation reaction of 2-deoxyglucose with 1.2 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and product over time.

Reaction of Mannose with *O*-Ethyl-*N*-Methylhydroxylamine (20)

Table S14. Characteristic NMR data of products obtained by the reaction of mannose with *O*-ethyl-*N*-methylhydroxylamine (20). Determined $^1J_{C1-H1}$: 158.3 Hz (α -*N*-mannofuranoside), 163.0 Hz (α -*N*-mannopyranoside), 149.3 Hz (β -*N*-mannopyranoside)



Product	Proton	δ (H) (ppm)	Multiplicity, $^3J_{H-H}$ (Hz)	Carbon	δ (C) (ppm)
α - <i>N</i> -Furanoside	H-1	4.52	d, 7.1	C-1	97.3
	H-2	4.39	dd, 7.1, 4.3	C-2	72.1
	H-3	4.29	dd, 4.3, 2.4	C-3	71.0
	H-4	4.02-3.97	m	C-4	79.6
	H-5	3.68-3.62	m	C-5	-
α - <i>N</i> -Pyranoside	H-1	4.16	d, 2.2	C-1	93.4
	H-2	4.21	dd, 3.4, 2.2	C-2	68.0
	H-3	3.92-3.87	m	C-3	70.5
	H-4	-	-	C-4	66.4
	H-5	-	-	C-5	75.1
β - <i>N</i> -Pyranoside	H-1	4.17	d, 0.9	C-1	92.2
	H-2	4.11	dd, 2.9, 0.9	C-2	69.1
	H-3	3.68-3.62	m	C-3	73.5
	H-4	-	-	C-4	66.3
	H-5	3.41-3.36	m	C-5	77.8
	H-6a	3.78-3.70	m	C-6	-

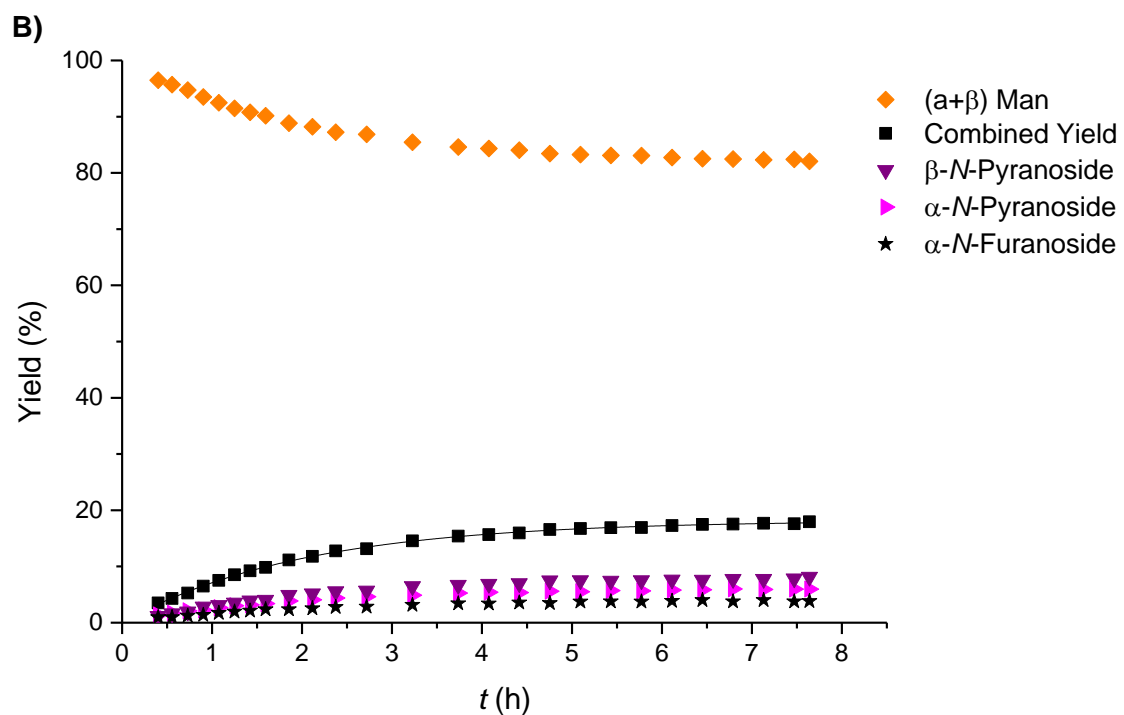
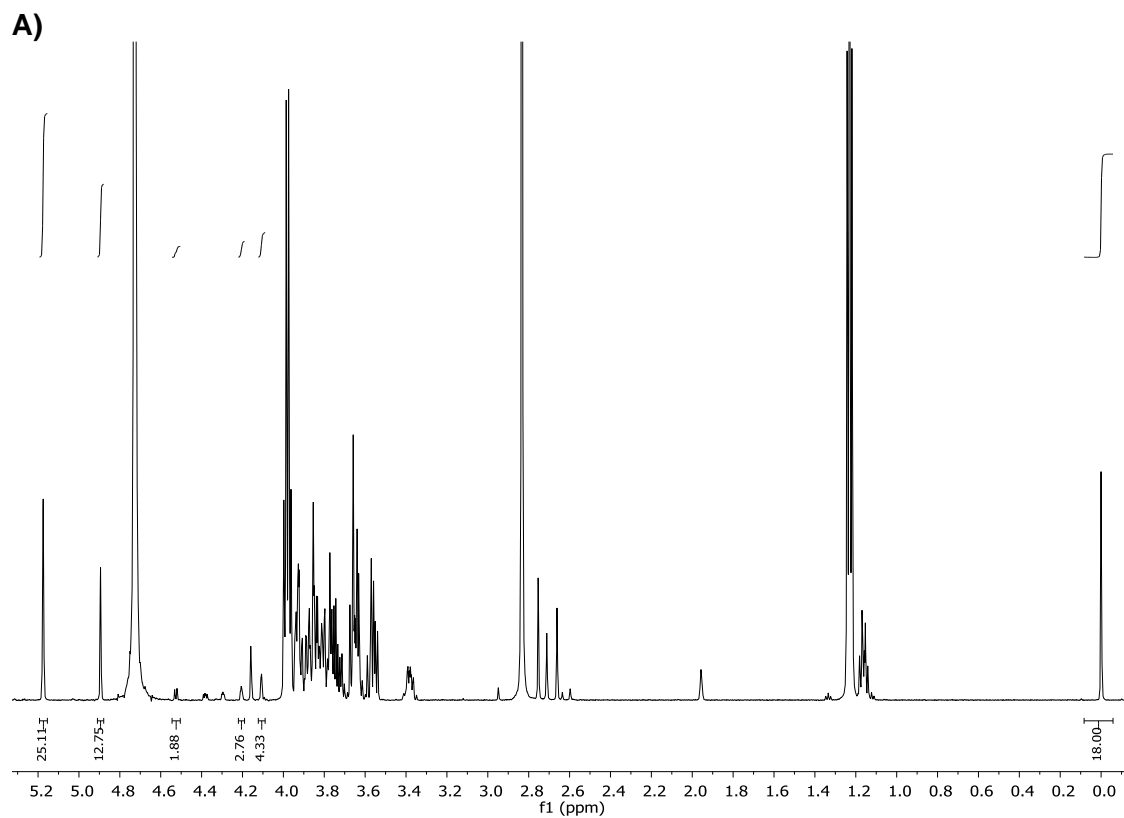
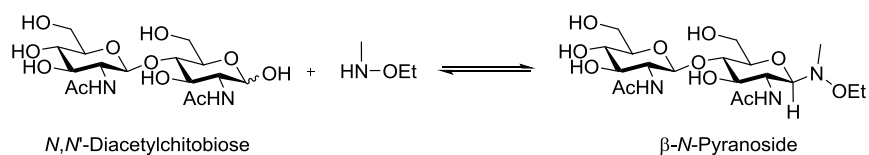


Figure S34. Ligation reaction of mannose with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yields of starting material and products over time.

Reaction of *N,N'*-Diacetylchitobiose with *O*-Ethyl-*N*-Methylhydroxylamine (**20**)

Table S15. Characteristic NMR data of product obtained by the reaction of *N,N'*-diacetylchitobiose with *O*-ethyl-*N*-methylhydroxylamine (**20**).



Product	Proton	$\delta(\text{H})$ (ppm)	Multiplicity, $^3J_{\text{H-H}}$ (Hz)	Carbon	$\delta(\text{C})$ (ppm)
$\beta\text{-}N\text{-Pyranoside}$	H-1	4.19	d, 9.7	C-1	90.9
	H-2	4.07-3.98	m	C-2	51.3
	H-3	3.71-3.55	m	C-3	73.0
	H-4	3.55-3.42	m	C-4	68.9

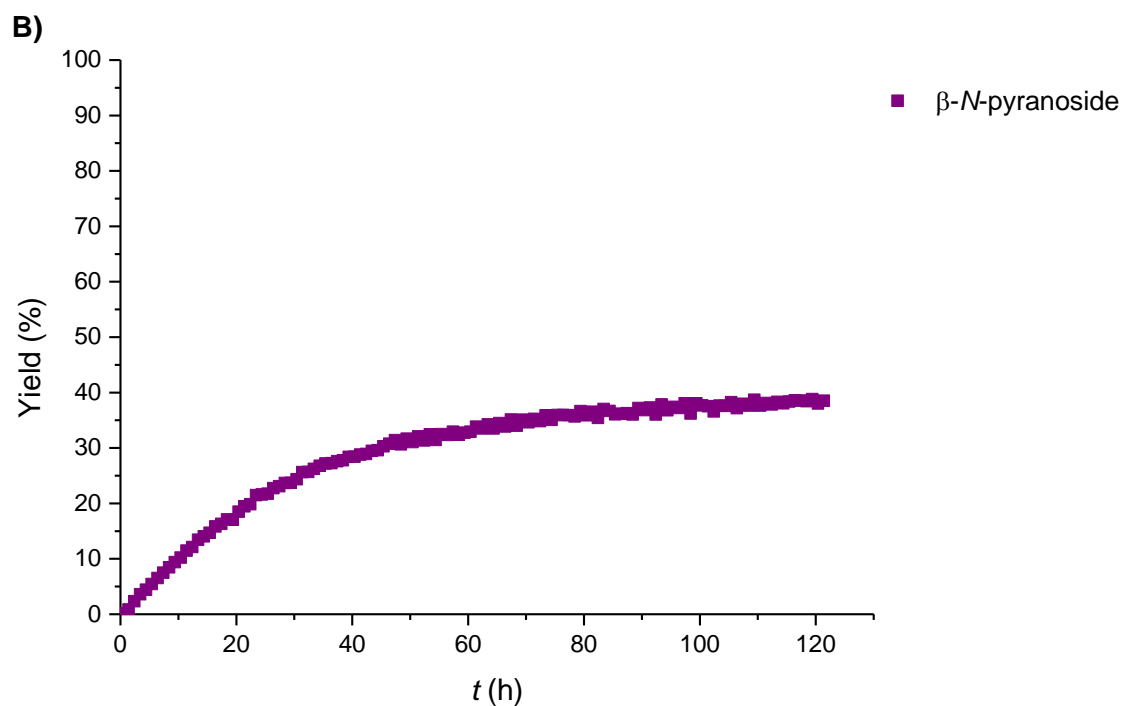
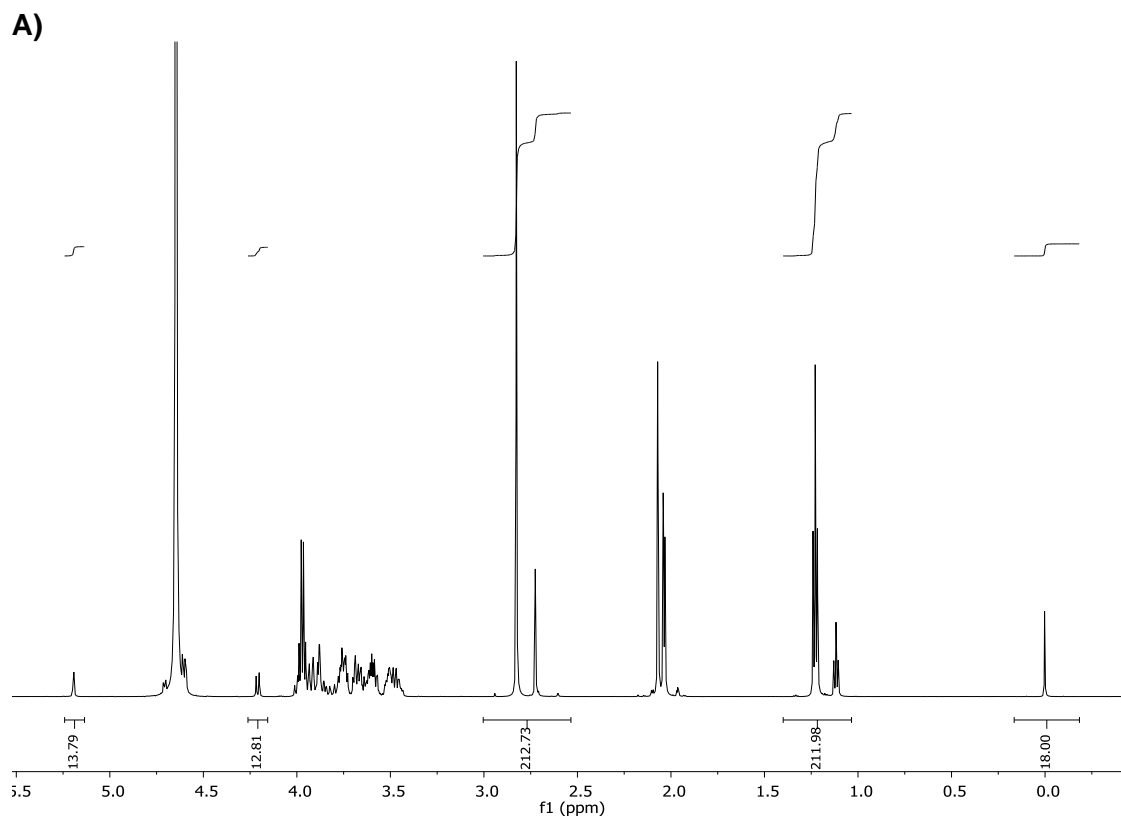
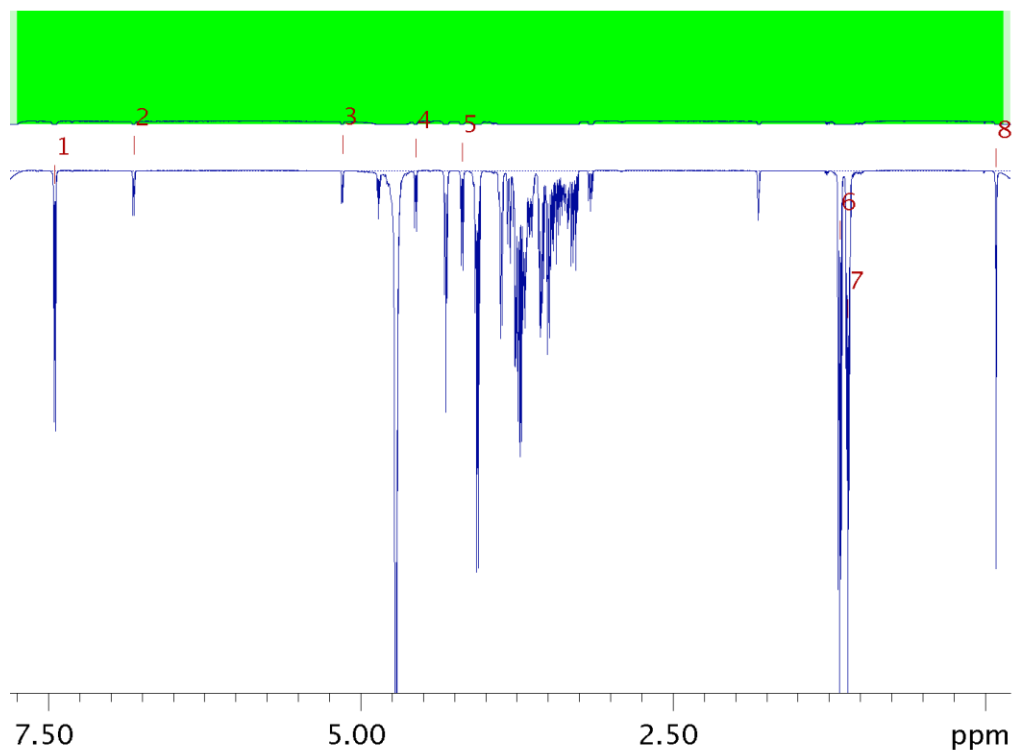


Figure S35. Ligation reaction of *N,N'*-diacetylchitobiose with 2.0 equiv of *O*-ethyl-*N*-methylhydroxylamine (**20**) at 39 °C and pH 5. A) ¹H NMR spectrum (600 MHz) after equilibration. B) Individual yield of product over time.

Determination of T1 times

A)



B)

Fitted function:	$f(t) = I_0 * [1 - 2 * \exp(-t/T1)]$
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using arbitrary y uncertainties
Confidence level:	95%
Used peaks:	
Used integrals:	peak intensities
Used Mixing time:	all values (including replicates) used

Peak name	F2 [ppm]	I ₀	error	T1 [s]	error
1	7.447	1.47e+06	7.523e+04	2.35	0.2206
2	6.814	4.35e+05	1.344e+04	3.19	0.1587
3	5.144	6.39e+05	1.031e+04	2.84	0.07728
4	4.557	2.75e+05	2.858e+04	1.28	0.2853
5	4.185	4.03e+05	3.701e+04	0.947	0.1910
6	1.162	3.48e+07	4.075e+05	2.30	0.04960
7	1.097	1.41e+07	1.948e+05	3.90	0.08061
8	-0.089	1.60e+07	7.490e+04	3.39	0.02500

Figure S36. Determination of T1 times of compounds present during the reaction of glucose with 1.2 equiv of *O*-ethylhydroxylamine (**11**) at 27 °C and pH 6. A) ¹H NMR spectrum (600 MHz) after equilibration B) Calculation of T1 times from the data obtained in Figure A.