Supporting Information

Dienophile-Modified Mannosamine Derivatives for Metabolic Labeling of Sialic Acids: A Comparative Study

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Supporting Information

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Synthesis of Ac₄ManNBtl (11)

Scheme S1. Synthesis of Ac₄ManNBtl (11).
Determination of Second-Order Rate Constants

A)

\[
\text{ManNPeoc (8): } X = \text{O, } n = 3 \\
\text{ManNBtl (12): } X = \text{CH}_2, \ n = 0 \\
\text{ManNBeac (18): } X = \text{NH, } n = 2 \\
\]

\[
\text{ManNPeoc} + \text{Tz-PEG-OH (1)} \rightarrow \text{DAinv products} \\
\text{ManNBtl} + \text{Tz-PEG-OH (1)} \rightarrow \text{DAinv products} \\
\text{ManNBeac} + \text{Tz-PEG-OH (1)} \rightarrow \text{DAinv products} \\
\]

B)

\[
\begin{align*}
1/c [M^{-1}] & \quad t [h] \\
\hline
0 & 200 \\
200 & 400 \\
400 & 600 \\
600 & 800 \\
800 & 1000 \\
1000 & 1200 \\
1200 & 1400 \\
1400 & 1600 \\
1600 & 1800 \\
1800 & 2000 \\
\end{align*}
\]

Figure S1. A) Reaction of ManNPeoc (8), ManNBtl (12), or ManNBeac (18) with Tz-PEG-OH (1) to determine the second-order rate constant of the DAinv reaction. The reaction was followed by observation of the absorbance of the tetrazine at \( \lambda = 522 \text{ nm} \). B) Plot of the inverse concentration \( c \) of the tetrazine against the reaction time \( t \). The slope of the lines (with \( t \) in seconds) obtained by linear regression equals the second-order rate constant \( k_2 \).
Figure S2. Analysis of DMB-NeuSAlloc (31) by RP-HPLC (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). $R_t = 22.5$ min.
Figure S3. Analysis of DMB-Neu5Beoc (32) by RP-HPLC with a fluorescence detector (excitation 372 nm, emission 456 nm). A) 10% – 30% B in 40 min: $R_t = 24.9$ min, B) 10% – 25% B in 40 min: $R_t = 36.3$ min.
Figure S4. Analysis of DMB-Neu5Peoc (33) by RP-HPLC (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). $R_t = 34.5$ min.

Figure S5. Analysis of DMB-Neu5Heoc (34) by RP-HPLC (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). $R_t = 41.0$ min.
**Figure S6.** Analysis of DMB-Neu5Btl (35) by RP-HPLC (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). \( R_t = 29.3 \) min.

**Figure S7.** Analysis of DMB-Neu5Ptl (36) by RP-HPLC (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). \( R_t = 48.1 \) min.
Figure S8. Analysis of DMB-Neu5HxI (37) by RP-HPLC with a fluorescence detector (excitation 372 nm, emission 456 nm). A) 10 % – 18 % B in 85 min: \( R_t = 75.5 \) min, B) 10 % – 25 % B in 40 min: \( R_t = 37.9 \) min.
Figure S9. Analysis of DMB-Neu5Beac (38) by RP-HPLC (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm). \( R_t \approx 24.1 \text{ min.} \)
Analysis of DMB-Labeled Sialic Acids Released from Cells

Figure S10. RP-HPLC analysis (10 % – 30 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control). A) Complete chromatogram, B) and C) enlarged regions.
**Figure S11.** RP-HPLC analysis (10% – 18% B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control).

**Figure S12.** RP-HPLC analysis (10% – 25% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown without added sugar (solvent control).
**Figure S13.** RP-HPLC analysis (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNAloc (3).

**Figure S14.** RP-HPLC analysis (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNBeoc (5).

**Figure S15.** RP-HPLC analysis (10% – 25% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNBeoc (5).
**Figure S16.** RP-HPLC analysis (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with AcᵣManNPėoc (7). A) Complete chromatogram, B) enlarged region.
Figure S17. RP-HPLC analysis (10% – 30% B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac$_4$ManNHeoc (9). A) Complete chromatogram, B) enlarged region.

Figure S18. RP-HPLC analysis (10% – 18% B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac$_6$ManNBtl (11).
**Figure S19.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNptl (13).

**Figure S20.** RP-HPLC analysis (10 % – 18 % B in 85 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNHxl (15).

**Figure S21.** RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac₄ManNHxl (15).
Figure S22. RP-HPLC analysis (10 % – 25 % B in 40 min) with a fluorescence detector (excitation 372 nm, emission 456 nm) of DMB-labeled sialic acids released from cells grown with Ac4ManNBeac (17).
NMR Spectra

\[ \text{AcO} - \text{HN} \quad 3' \quad 4' \]

\[ \text{AcO} - \text{OAc} \]

11 Ac\(_2\)ManNB\(_3\)

\[ \text{1H-NMR spectrum (CDCl}_3, \ 400 \text{ MHz) of compound 11 (enriched } \alpha\text{-anomer).} \]

\[ \text{13C NMR spectrum (CDCl}_3, \ 101 \text{ MHz) of compound 11 (enriched } \alpha\text{-anomer).} \]

S18
$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of the separated α-anomer of compound 17.

$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of the separated α-anomer of compound 17.
$^1$H NMR spectrum (D$_2$O, 400 MHz) of compound 19.

$^{13}$C-NMR spectrum (D$_2$O, 101 MHz) of compound 19.
\(^{1}\text{H} \text{NMR spectrum (D}_2\text{O, 600 MHz) of compound 20.}\)
\(^1\)H NMR spectrum (D\(_2\)O, 600 MHz) of compound 21.

\(^1\)C NMR spectrum (D\(_2\)O, 151 MHz) of compound 21.
\( ^1\)H NMR spectrum (D\(_2\)O, 600 MHz) of compound 22.

\( ^{13}\)C NMR spectrum (D\(_2\)O, 151 MHz) of compound 22.
$^1$H NMR spectrum (D$_2$O, 400 MHz) of compound 23.

$^{13}$C NMR spectrum (D$_2$O, 101 MHz) of compound 23.
$^1$H NMR spectrum (D$_2$O, 600 MHz) of compound 24.

$^{13}$C NMR spectrum (D$_2$O, 151 MHz) of compound 24.
$^1$H NMR spectrum (D$_2$O, 400 MHz) of compound 25.

$^{13}$C NMR spectrum (D$_2$O, 151 MHz) of compound 25.
$^1$H NMR spectrum (D$_2$O, 400 MHz) of compound 26.

$^1$H-NMR spectrum (CDCl$_3$, 400 MHz) of compound 28.
$^{13}$C-NMR spectrum (CDCl$_3$, 101 MHz) of compound 28.

$^1$H NMR spectrum (CDCl$_3$, 400 MHz) of compound 30.
$^{13}$C NMR spectrum (CDCl$_3$, 101 MHz) of compound 30.