Supporting Information for

Biosynthetic Tailoring of Microcin E492m: Post-Translational Modification Affords an Antibacterial Siderophore-Peptide Conjugate

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Substrate Preparations.

Preparation of Ent and Glycosylated Ents. Enterobactin was synthesized as described in the literature.[1-3] IroB-catalyzed reactions were performed to obtain multi-milligram quantities of MGE and DGE for use in enzymatic assays. [4] A 15 mL solution containing Ent (15.6 mg, 23 μmol, dissolved in 500 μL DMSO), UDP-Glc (124 mg, 1.64 mmol), 5 mM $MgCl_2$, 2.5 mM TCEP and 75 mM Tris-HCl buffered at pH 8 was incubated at room temperature for 3.5 h, quenched by addition of 7 mL of 2.5 N HCl in MeOH, vortexed and filtered through a 0.2 μm membrane. Preparative HPLC using a gradient of 0 to 40% B (solvent A, 0.2% TFA/H_2O; solvent B, MeCN) over 40 min and a flow-rate of 10 mL/min afforded pure MGE (30.9 mg, 37.1 μmol) and DGE (14.3 mg, 14.4 μmol). Product purity was verified by analytical HPLC and identity by co-elution with known standards and MS. LRMS $[M+H]^+$ *m/z* calc 832.2 (MGE), 994.3 (DGE); found, 832. 1, 994.2.

Large-Scale Enzymatic Preparation of lin-Ent. Ent (20 mg, 30 μmol) was dissolved in 400 μL of DMSO and diluted with 15 mL of 75 mM Hepes pH 7.5 buffer. A portion (135 μL of 71 μM stock) of MceD was added and the solution was mixed and incubated at room temperature for 27 min, and quenched with 5 mL of 2.5 N HCl in MeOH. The mixture was vortexed and filtered through a 0.2 μm membrane. Preparative HPLC using a solvent gradient of 15 to 60% B (solvent A, 0.1% TFA/H_2O; solvent B, MeCN) allowed for separation of the reaction components. The compounds were collected and lyophilized to dryness, which afforded white powders. The identity of lin-Ent was verified by MS and co-elution with lin-Ent prepared from a IroD-catlayzed reaction.[5] LRMS: $[M+H]^+$ *m/z* calc, 688.2; found, 688.3.

Preparation of Ferric Ent, MGE and DGE. The ferric complexes of Ent, MGE and DGE were prepared from $FeCl_3$ and siderophore in the presence of

base by modification of existing protocols.[5,6] For $[Fe(Ent)]^{3-}$: A 50.3-mL portion of Ent (10.5 mM in DMSO) was diluted with 49.7 μL of MeOH (5.28 mM final concentration) and aqueous $FeCl_3$ (5.28 mM, 110 μL) was added. The solution turned purple and was mixed with a pipette. A 7-mL aliquot of 1 M K_2CO_3 was added and the solution was mixed and turned wine-colored. The reaction was loaded onto a Sephadex LH-20 column packed with MeOH and eluted with MeOH. The $[Fe(Ent)]^{3-}$ containing fractions were concentrated to dryness and the resulting dark-red residue was dissolved in 150 μL of DMSO. The product identity was verified and stock concentration determined by UV-vis. For $[Fe(MGE)]^{3-}$: As described for $[Fe(Ent)]^{3-}$ except that the 5.28 mM MGE solution was prepared in 1:1 MeOH/H_2O. For $[Fe(DGE)]^{3-}$: As described for $[Fe(Ent)]^{3-}$ except that the DGE solution was prepared in MeOH (1.33 mM) and the $FeCl_3$ solution was also 1.33 mM.

References

(1) Ramirez, R. J. A.; Karamanukyan, L.; Ortiz, S.; Guiterrez, C. G. *Tetrahedron Lett.* **1997,** *38*, 749-752.
(2) Gardner, R. A.; Kinkade, R.; Wang, C.; Phastiel, O. I. *J. Org. Chem.* **2004**, *69*, 3530-3537.
(3) Deleuze, H.; Maillard, B. *J. Organomet. Chem.* **1995**, *490*, C14-C17.
(4) Fischbach, M. A.; Lin, H.; Liu, D. R.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2005,** *102*, 571-576.
(5) Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T. *J. Am. Chem. Soc.* **2005**, *127*, 11075-11084.
(6) Abergel, R. J.; Warner, J. A.; Shuh, D. K.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 8920-8931.

Table S1. Mass Data (*m*/*z*) for Hydrolysis Products from MceD-Catalyzed Reactions

Substrate	Product	Calc $[M+H]^+$	Found $[M+H]^+$	Calc $[M-H]^-$	Found $[M-H]^-$
Ent		670.2	670.2		
	lin-Ent	688.2	688.3		
	DHB-Ser Dimer	465.1	465.1		
	DHB-Ser Monomer			240.1	n.d.[a]
MGE		832.2	832.1		
	lin-MGE	850.2	850.1		
	Glc-Dimer	627.2	627.1		
	Glc-Monomer	404.1	n.d.		
	DHB-Ser			240.1	n.d.
DGE		994.3	994.1		
	lin-DGE	1012.3	1012.2		
	Glc-Dimer	627.2	627.3		
	Glc-Monomer	404.1	n.d.		
	DHB-Ser Monomer			240.1	n.d.

[a] n.d. = not determined

Table S2. NOE Correlations for C_{10}-C6'-MGE (left) and C_{10}-C4'-MGE (right)

Group	Atom	Nuc	Shift
C6			
Glc	H1	1H	3.987
Glc	H2	1H	3.462
Glc	H3	1H	3.575
Glc	H4	1H	3.715
Glc	H5	1H	3.510
Glc	H61	1H	4.454
Glc	H62	1H	4.244
DHB1	H4	1H	7.021
DHB1	H6	1H	7.156
DHB1	H9	1H	4.957
DHB1	HN	1H	8.945
DHB2	H4	1H	6.912
DHB2	H5	1H	6.612
DHB2	H6	1H	6.970
DHB2	H9	1H	4.898
DHB2	HN	1H	8.692
DHB3	H4	1H	6.908
DHB3	H5	1H	6.610
DHB3	H6	1H	6.956
DHB3	H9	1H	5.041
DHB3	HN	1H	8.733
S1	HA	1H	4.114
S1	HN	1H	8.366
S1	QB	1H	3.853
A2	HA	1H	4.404
A2	HN	1H	8.709
A2	QB	1H	1.369
T3	HA	1H	4.196
T3	HB	1H	4.181
T3	QG2	1H	1.141
T3	HN	1H	8.150
S4	HA	1H	4.317
S4	HN	1H	8.168
S4	QB	1H	3.917
S5	HN	1H	7.796
S5	QB	1H	3.781
S6	HN	1H	8.189
S6	QB	1H	3.938
G7	HN	1H	8.324
G7	QA	1H	4.373
S8	HA	1H	4.489
S8	HN	1H	8.283
S8	QB	1H	3.960
G9	HN	1H	8.265
G9	QA	1H	4.428
S10	HA	1H	4.470
S10	HN	1H	7.072
S10	QB	1H	3.949

Group	Atom	Nuc	Shift
C4			
Glc	H1	1H	4.077
Glc	H2	1H	3.474
Glc	H3	1H	3.590
Glc	H4	1H	4.960
Glc	H5	1H	3.545
Glc	Q6	1H	3.983
DHB1	H4	1H	7.033
DHB1	H6	1H	7.184
DHB1	H9	1H	4.999
DHB1	HN	1H	9.027
DHB2	H4	1H	6.933
DHB2	H5	1H	6.647
DHB2	H6	1H	7.000
DHB2	H9	1H	4.890
DHB2	HN	1H	8.805
DHB3	H4	1H	6.933
DHB3	H5	1H	6.645
DHB3	H6	1H	7.002
DHB3	H9	1H	4.915
DHB3	HN	1H	8.778
S1	HA	1H	4.117
S1	HN	1H	8.410
S1	QB	1H	3.953
A2	HA	1H	4.390
A2	HN	1H	8.713
A2	QB	1H	1.371
T3	HA	1H	4.324
T3	HB	1H	4.188
T3	QG2	1H	1.142
T3	HN	1H	8.154
S4	HA	1H	4.632
S4	HN	1H	8.238
S4	QB	1H	3.968
S5	HA	1H	4.452
S5	HN	1H	8.280
S5	QB	1H	3.804
S6	HA	1H	4.463
S6	HB2	1H	3.858
S6	HB3	1H	3.790
S6	HN	1H	8.375
G7	HN	1H	7.132
S8	HA	1H	4.420
S8	HN	1H	8.313
S8	QB	1H	3.825
G9	HN	1H	8.203
G9	QA	1H	4.405
S10	HA	1H	4.001
S10	HN	1H	7.067
S10	QB	1H	3.496

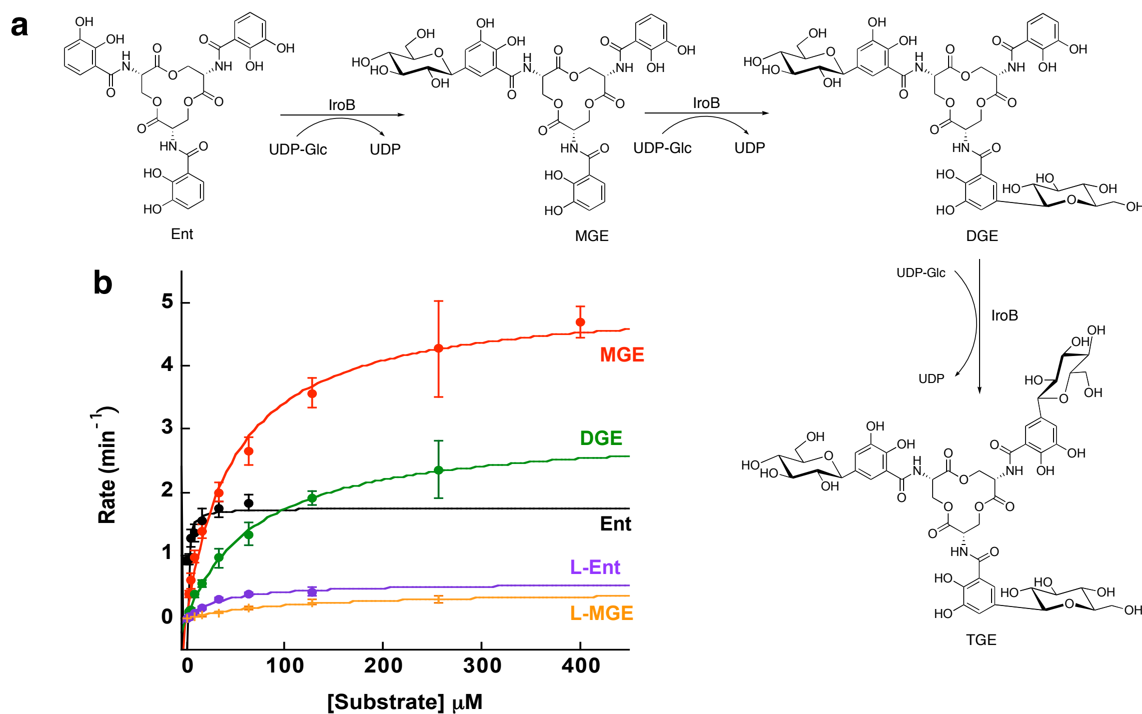


Figure S1. C-Glycosylation of Ent by IroB to give MGE, DGE and TGE. (b) Kinetic data for IroB-catalyzed C-glycosylation. Corresponding k_{cat} and K_m values are given in Table 1 of the main text.

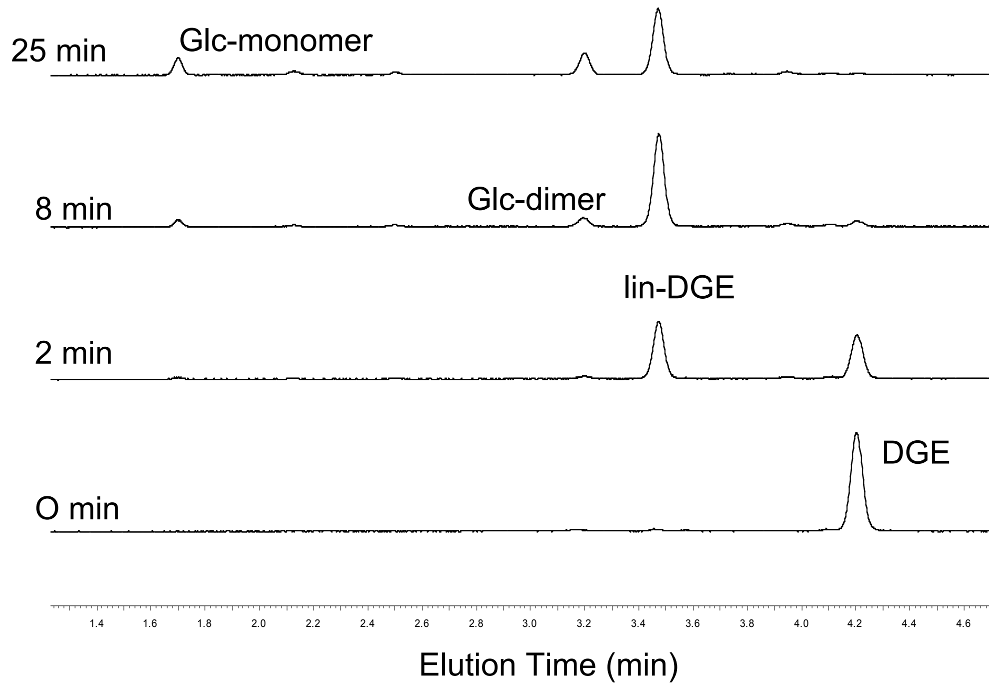


Figure S2. HPLC analysis of the MceD-catalyzed hydrolysis of DGE (75 mM Hepes, pH 7.5). The concentration of DGE was 32 μM and 20 nM MceD was employed. MS values for the products are listed in Table S1.

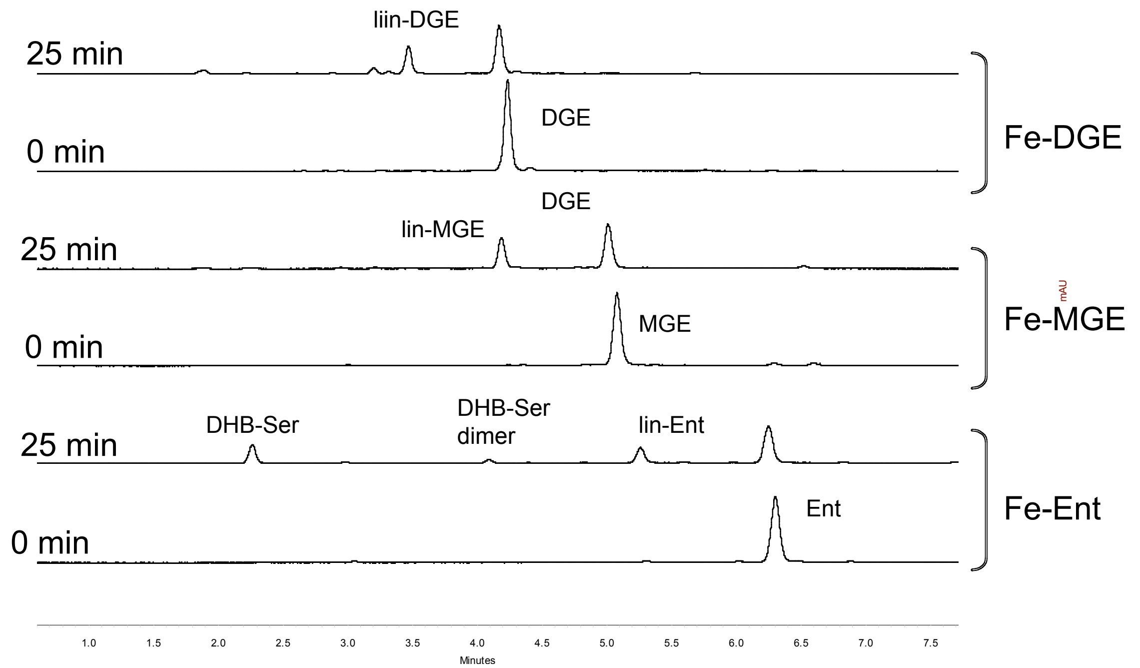


Figure S3. MceD-catalyzed hydrolysis of $[Fe(Ent)]^{3-}$, $[Fe(MGE)]^{3-}$ and $[Fe(DGE)]^{3-}$ (75 mM Hepes, pH 7.5). The MceD concentration was 20 nM and the substrate concentrations were 32 μM.

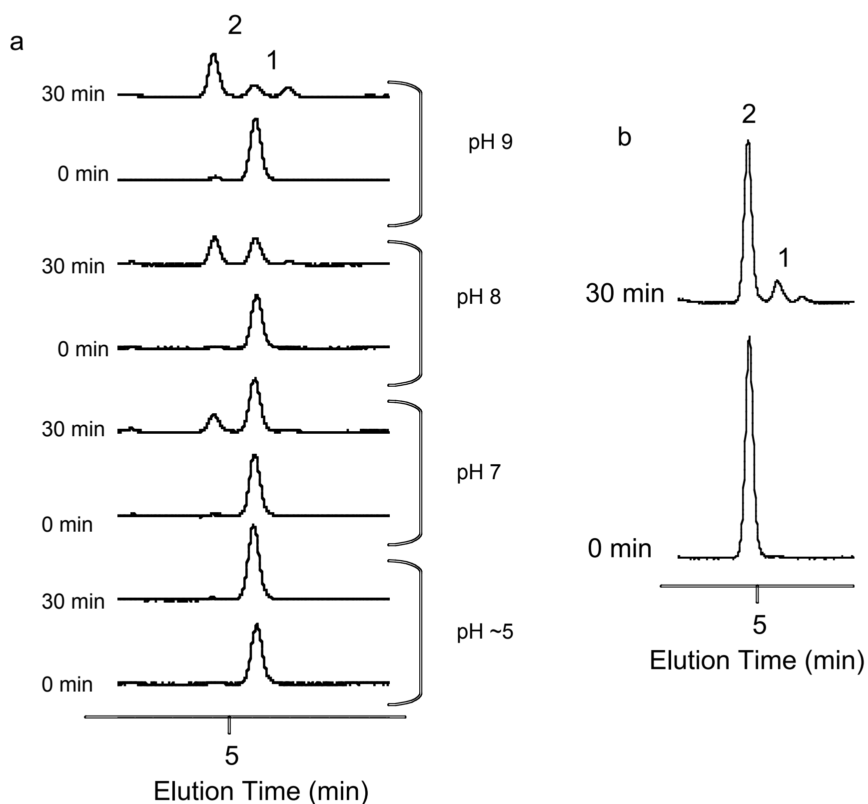
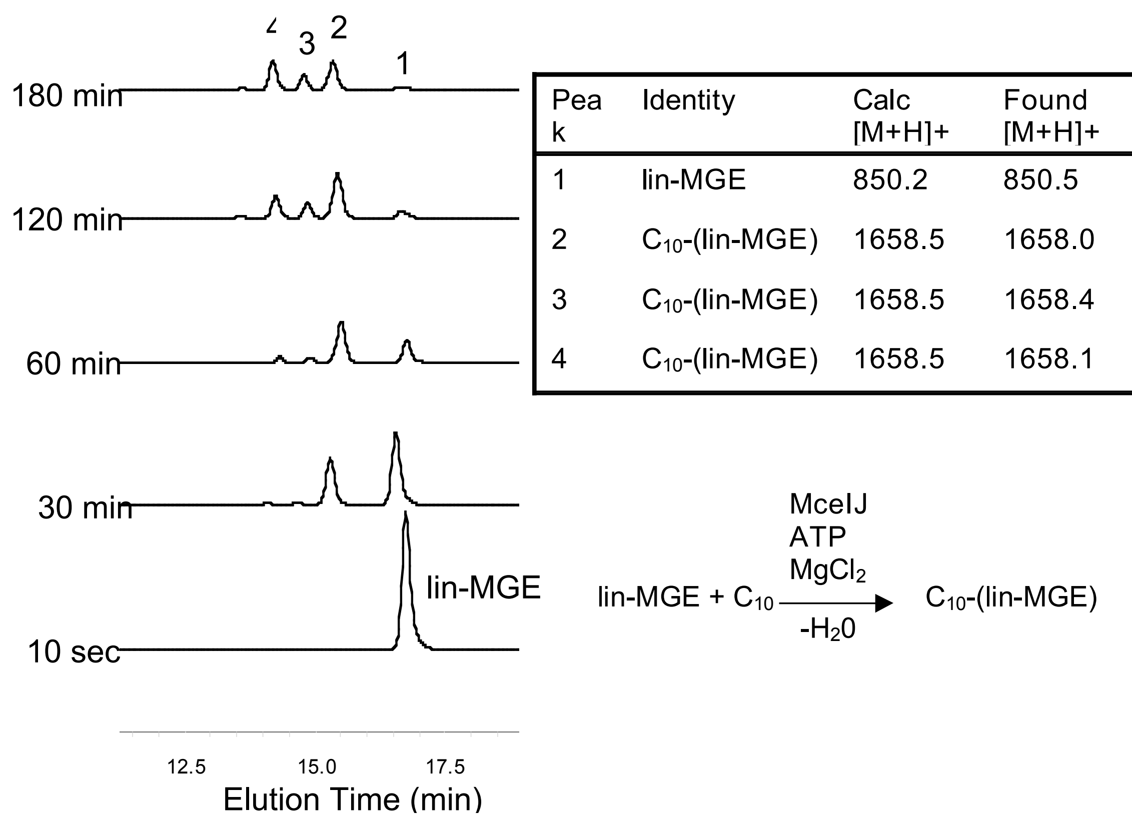
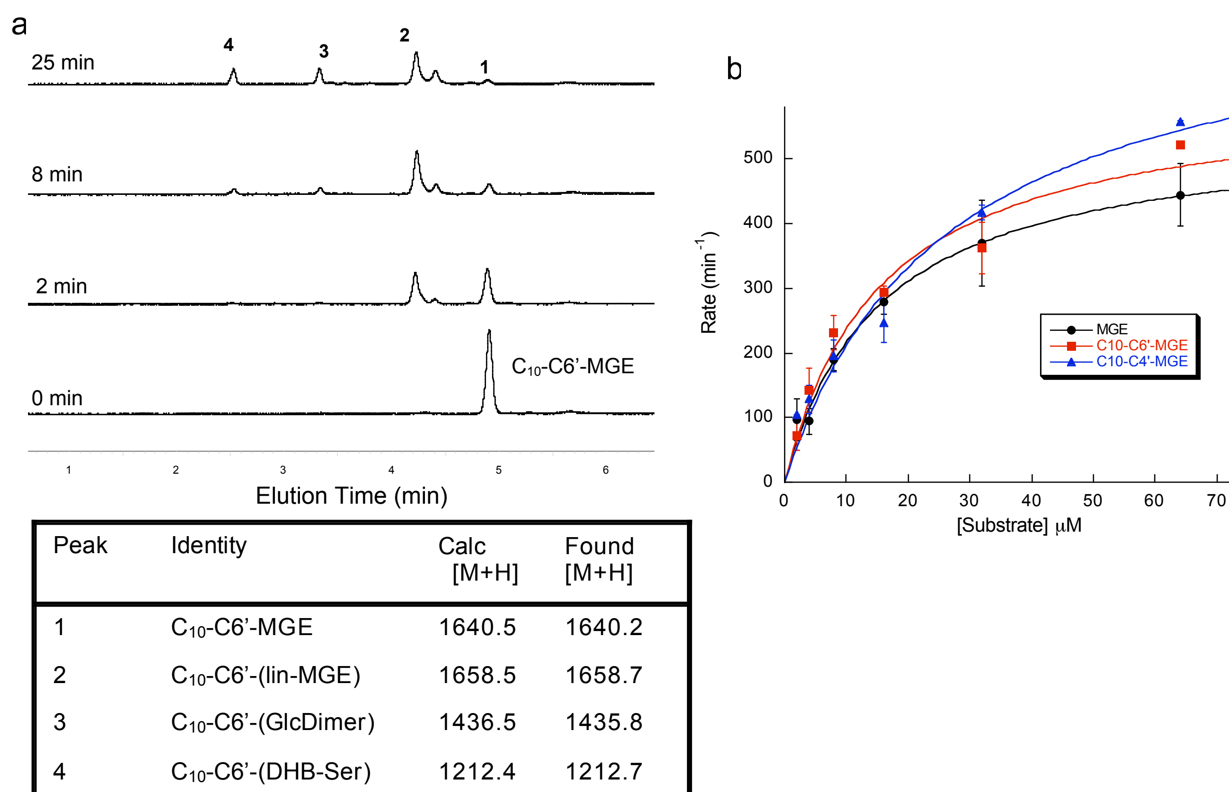


Figure S4. (a) Effect of pH on the migration of C_{10} from the C4′ to the C6′ position of the glucose moiety of MGE. Peak **1** is C_{10}-C4′-MGE. Peak **2** is C_{10}-C6′-MGE. Solutions of 50 μM C_{10}-C6′-MGE were prepared and incubated at room temperature for 30 min in aqueous solution at pH ~5 (H_2O), pH 7 (75 mM Tris-HCl), pH 8 (75 mM Tris-HCl) and pH 9 (10 mM CHES). Migration to the C6′ position is accelerated by the presence of base. Some background hydrolysis of the ester bond between C_{10} and MGE is observed at pH 9. (b) Incubation of 50 μM C_{10}-C6′-MGE at room temperature in pH 8 buffer (75 mM Tris-HCl). Some formation (~10%) of C_{10}-C4′-MGE is observed during this timeframe.



Peak	Identity	Calc [M+H]+	Found [M+H]+
1	lin-MGE	850.2	850.5
2	C_{10}-(lin-MGE)	1658.5	1658.0
3	C_{10}-(lin-MGE)	1658.5	1658.4
4	C_{10}-(lin-MGE)	1658.5	1658.1

Figure S5. MceIJ-catalyzed attachment of lin-MGE to the C_{10} peptide (2 μM MceIJ, 100 μM lin-MGE, 500 μM C_{10}, 5 mM ATP, 5 mM $MgCl_2$, 75 mM Tris-HCl pH 8).

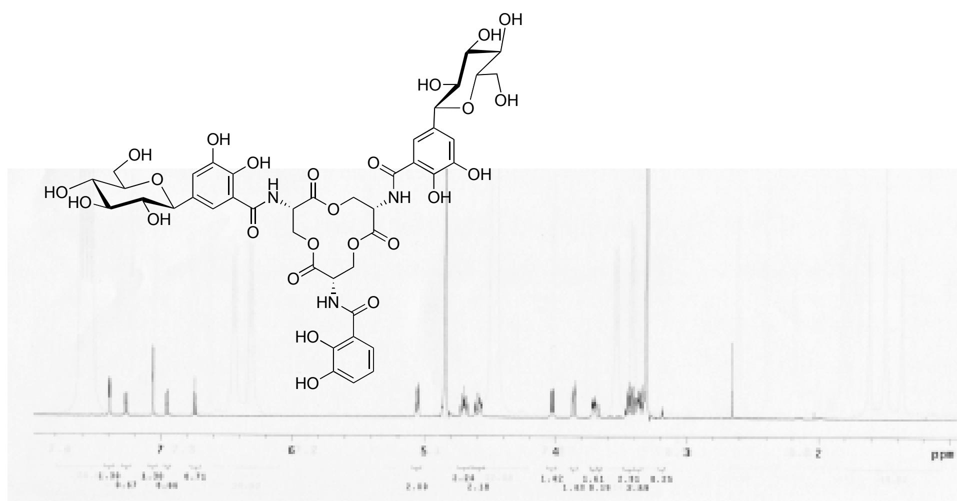


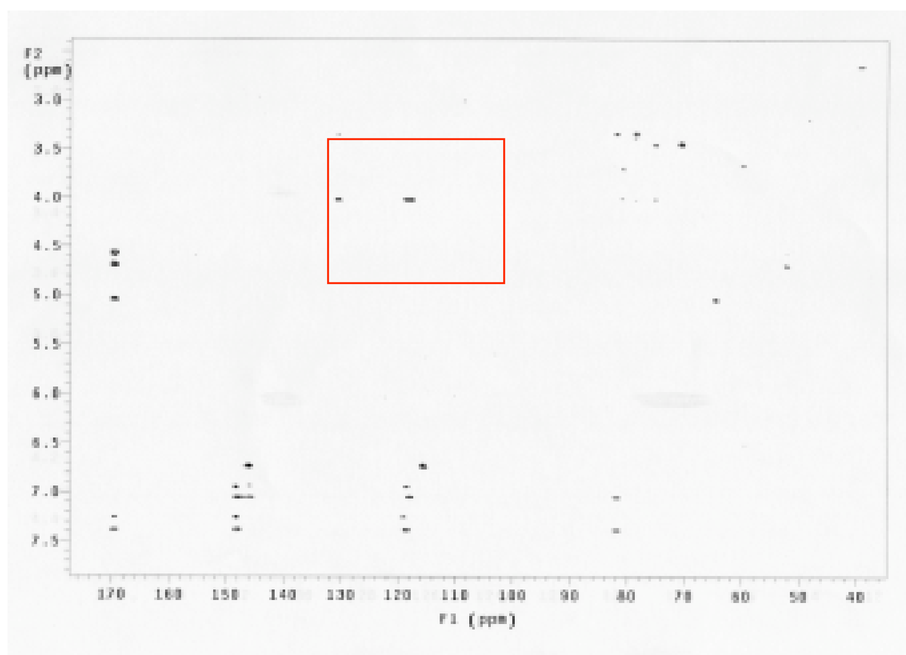
Peak	Identity	Calc [M+H]	Found [M+H]
1	C_{10}-C6'-MGE	1640.5	1640.2
2	C_{10}-C6'-(lin-MGE)	1658.5	1658.7
3	C_{10}-C6'-(GlcDimer)	1436.5	1435.8
4	C_{10}-C6'-(DHB-Ser)	1212.4	1212.7

Figure S6. (a) MceD-catalyzed hydrolysis of the MGE moiety in C_{10}-C6′-MGE (20 nM MceD, 32 μM C_{10}-C6′-MGE, 75 mM Hepes pH 7.5). (b) Kinetic traces for the MceD-catalyzed hydrolysis of C_{10}-C6′-MGE, C_{10}-C4′-MGE and MGE. Corresponding kinetic parameters are given in Table 2 of the main text.

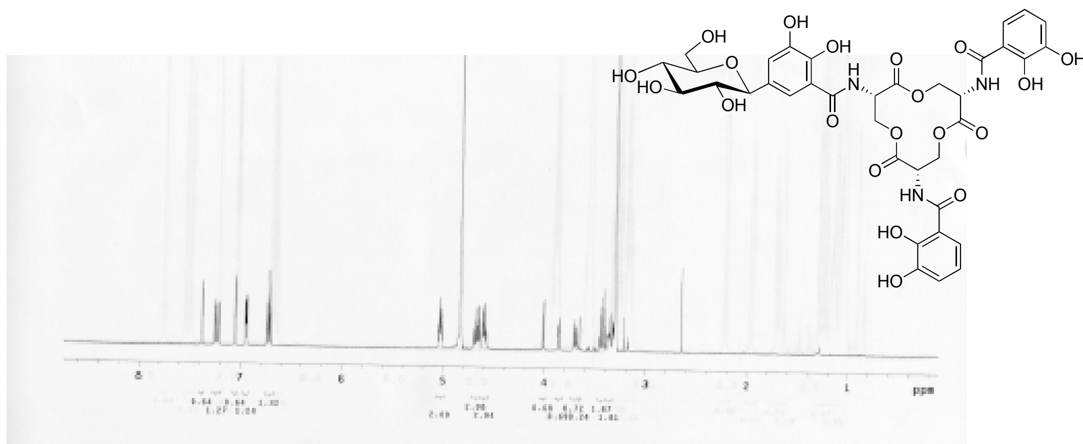
Representative NMR Spectra

^{1}H NMR and HBMC of DGE isolated from MceC-catalyzed glycosylation of Ent.

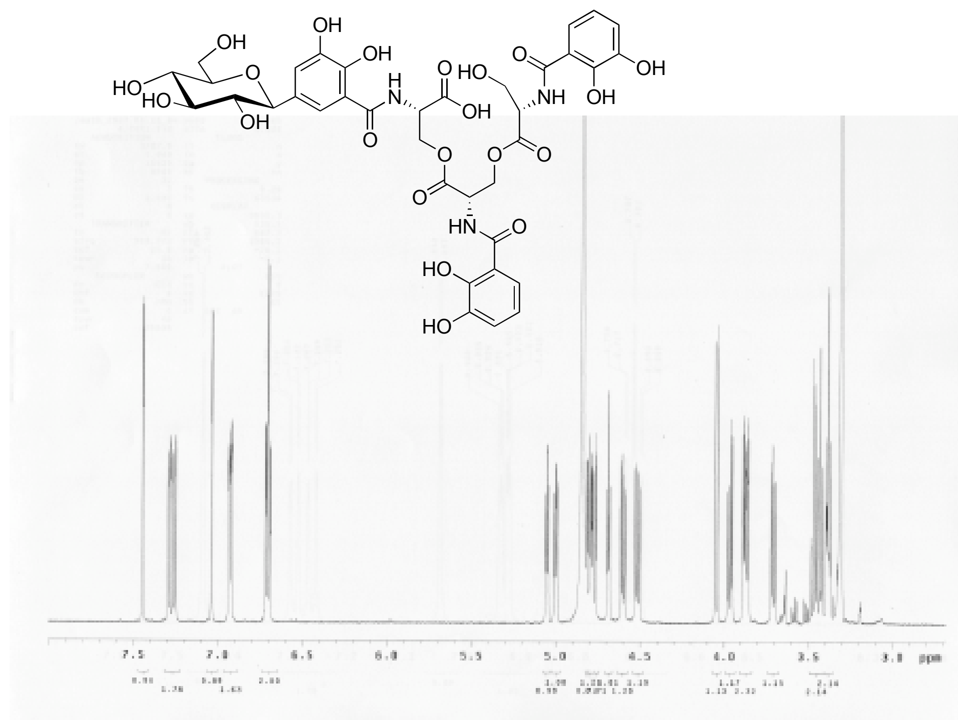




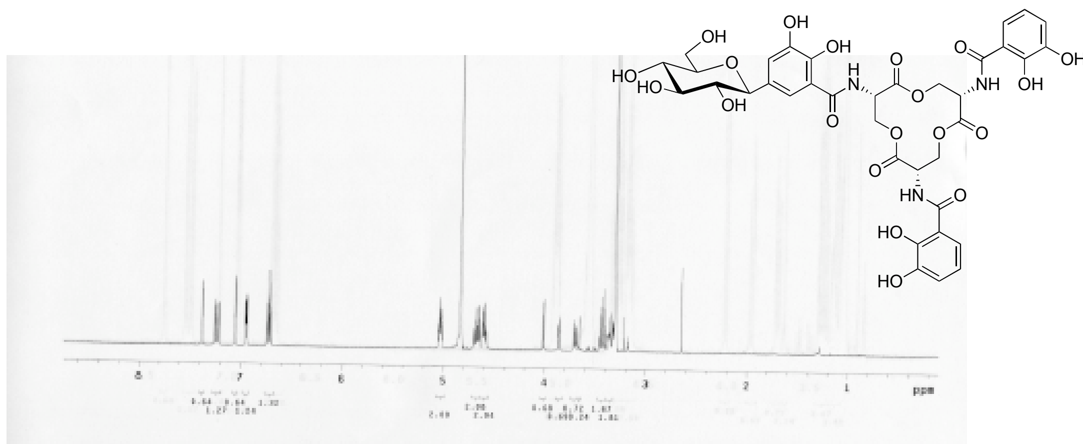
^{1}H NMR of MGE isolated from MceC-catalyzed glycosylation of Ent.



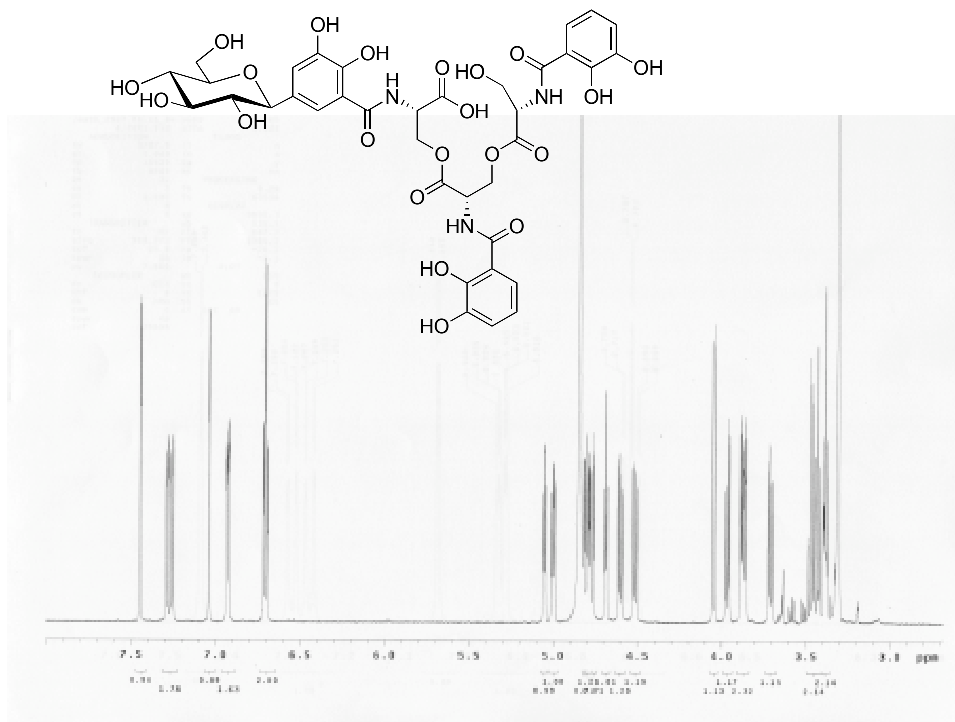
^{1}H NMR of linear MGE isolated from MceD-catalyzed hydrolysis of MGE.



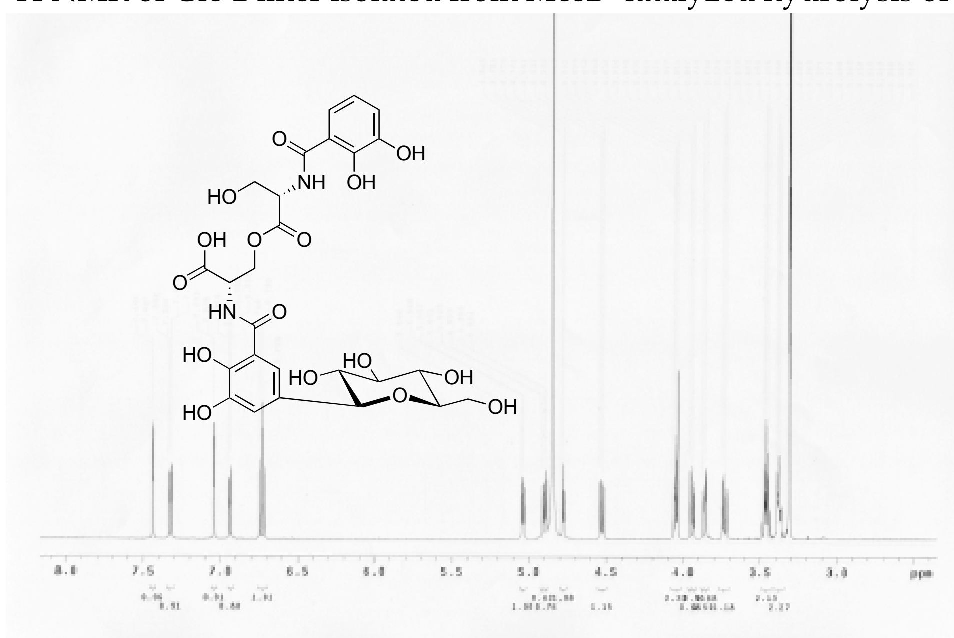
^{1}H NMR of MGE isolated from MceC-catalyzed glycosylation of Ent.



^{1}H NMR of linear MGE isolated from MceD-catalyzed hydrolysis of MGE.



^{1}H NMR of Glc-Dimer isolated from MceD-catalyzed hydrolysis of MGE.



^{1}H NMR of linear DGE from MceD-catalyzed hydrolysis of DGE.