Enzymatic Hydrolysis of Trilactone Siderophores: Where Chiral Recognition Occurs in Enterobactin and Bacillibactin Iron Transport

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Supporting Information

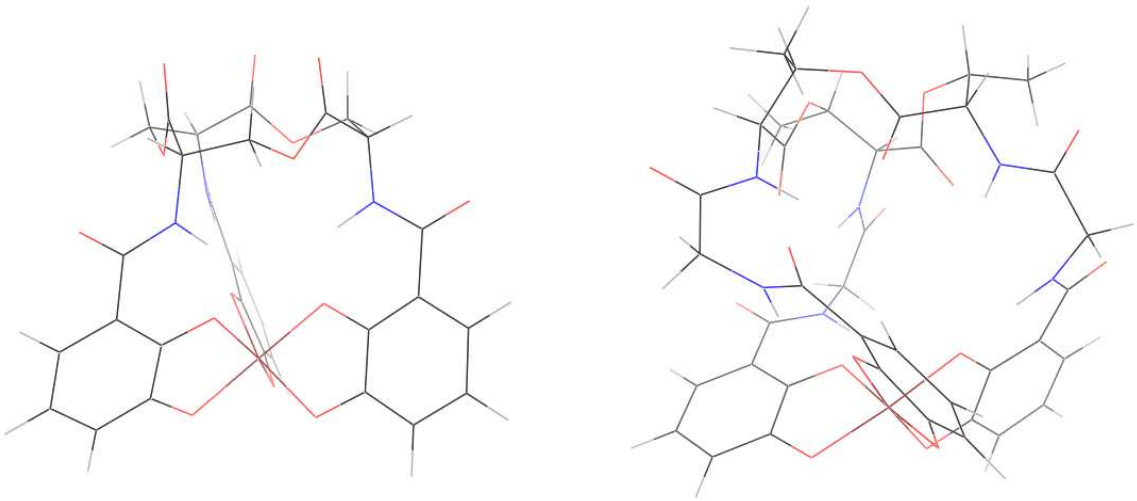


Figure S1. Previously published (Bluhm M.E., *et al.*, *Inorg. Chem.* **2002**, *41*, 5475-5478) energy minimized structures of $[Fe^{III}(Ent)]^{3-}$ (left) and $[Fe^{III}(BB)]^{3-}$ (right). The trilactone rings are in a chair conformation, with the amide functionalities in axial ($[Fe^{III}(Ent)]^{3-}$) or equatorial ($[Fe^{III}(BB)]^{3-}$) positions.

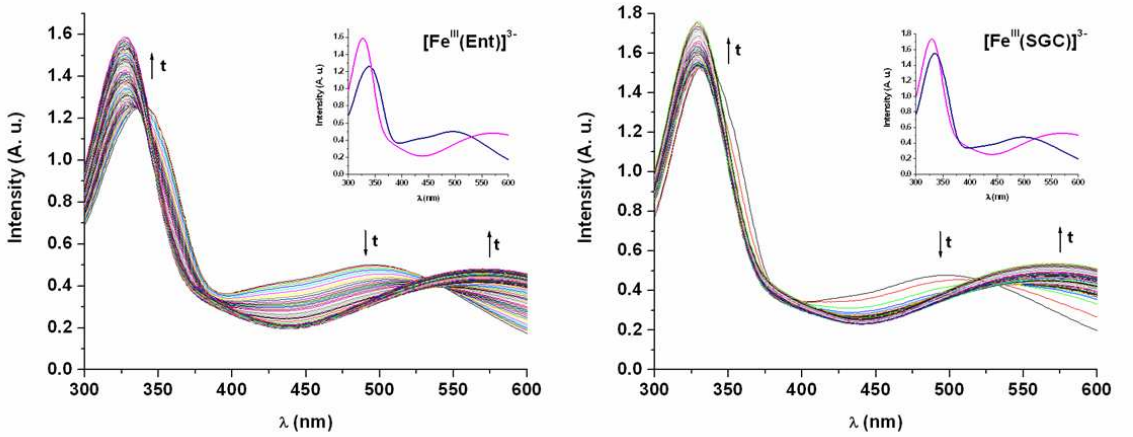


Figure S2. Bes-catalyzed hydrolysis of $[Fe^{III}(Ent)]^{3-}$ (left) and $[Fe^{III}(SGC)]^{3-}$ (right) to $[Fe^{III}(2,3\text{-}DHBS)_3]^{3-}$ and $[Fe^{III}(2,3\text{-}DHBGS)_3]^{3-}$, respectively, followed by UV-vis spectroscopy ([Fe(L)] = 100 μM, [Bes] = 1 μM, 75mM HEPES, pH 7.5, 25 °C, 1 cm cell). The insets show the spectra at t = 0 h (blue) and t = 12 h (pink).

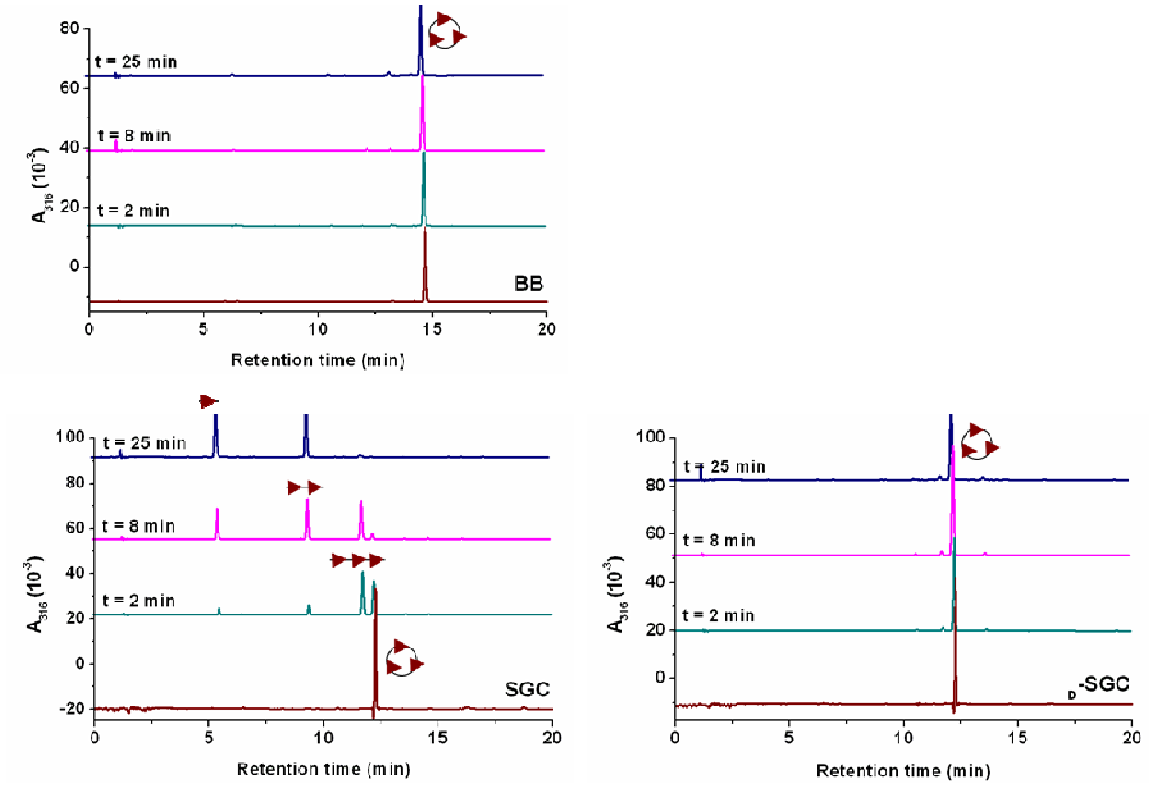


Figure S3. Reaction time course of Fes-catalyzed hydrolysis of SGC (bottom left). The presence of Fes in solution did not affect BB (top) and $_D$-SGC (bottom right). Reaction aliquots were quenched at different time points and analyzed by HPLC. The assignment of the hydrolysis products is based on the corresponding mass spectrometry data, and the schematic representations of the hydrolysis products are shown consistently with similar previous studies.

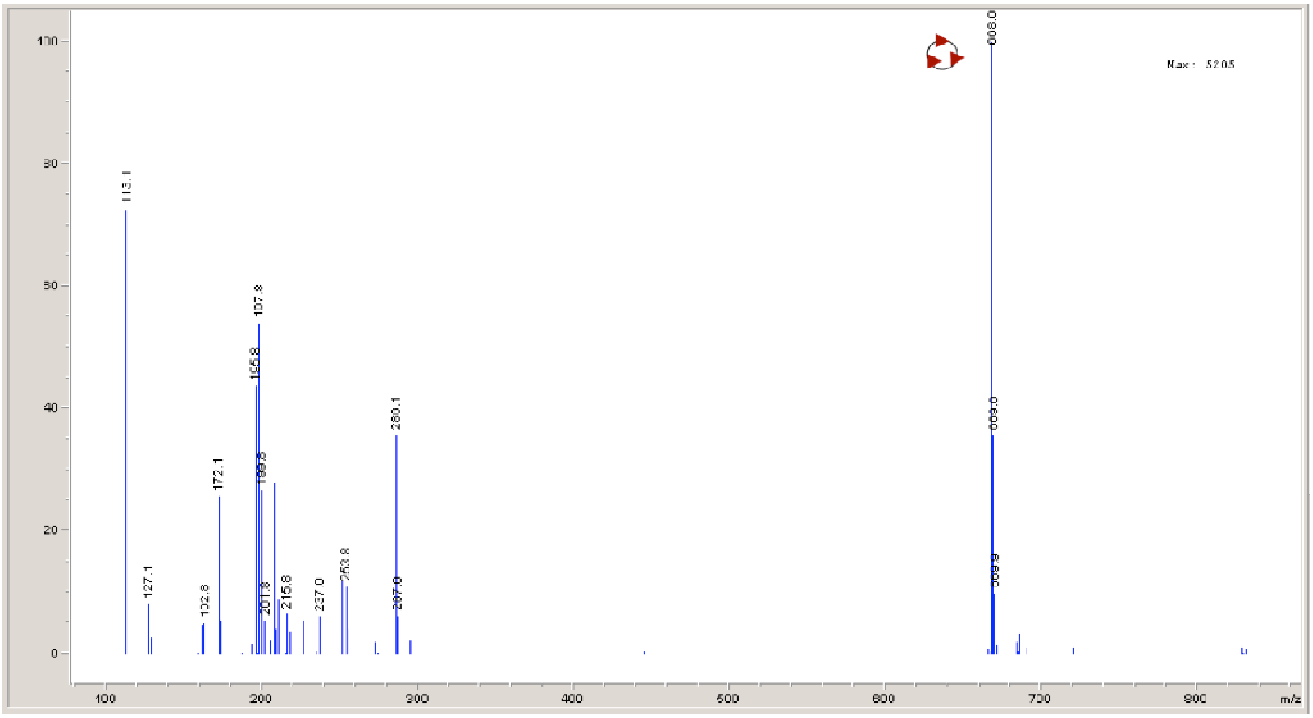


Figure S4 - A. Example of a mass spectrum of Ent from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 668.0 $(M-H)^-$.

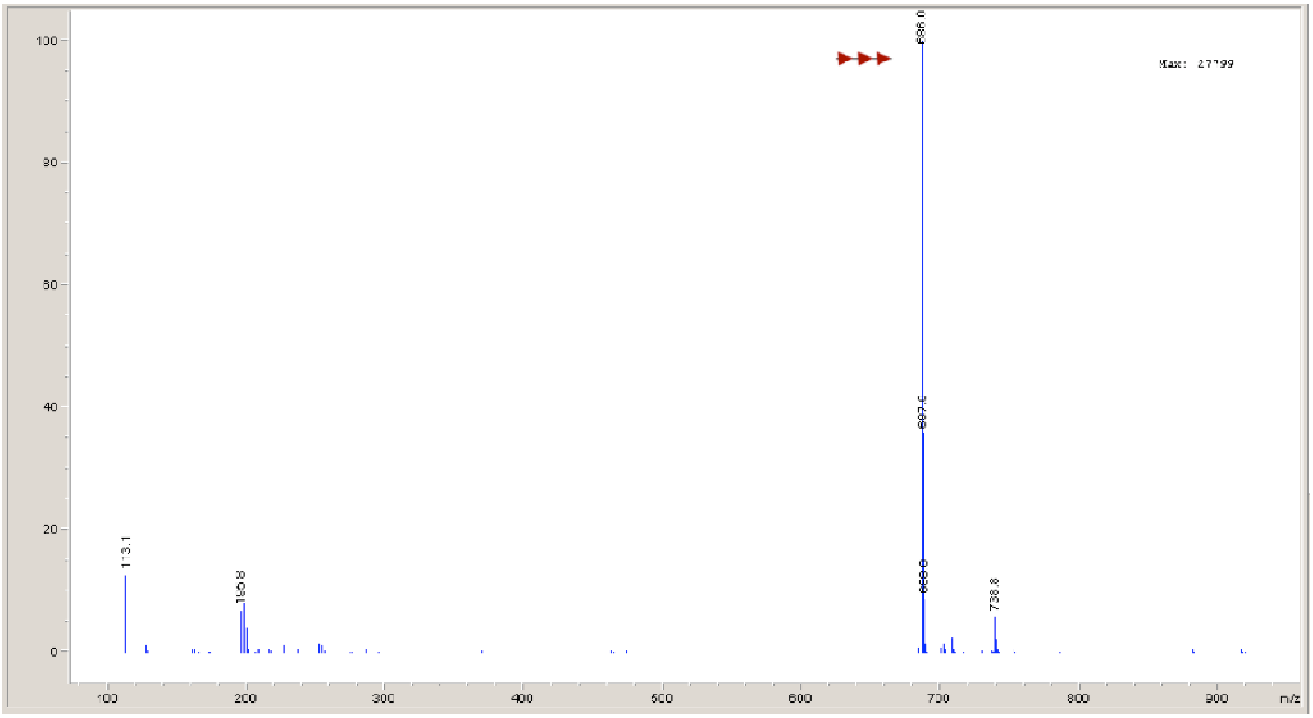


Figure S4 - B. Example of a mass spectrum of the Ent trimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 686.0 $(M-H)^-$.

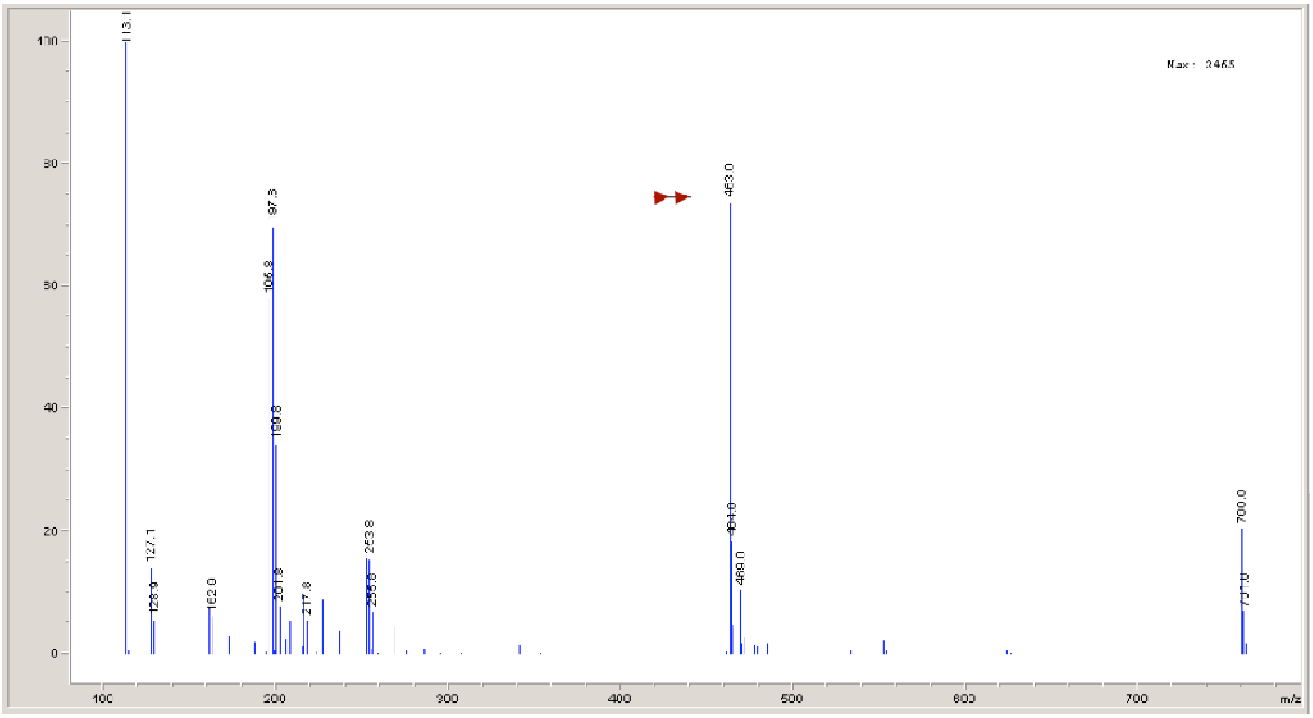


Figure S4 - C. Example of a mass spectrum of the Ent dimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 463.0 $(M-H)^-$.

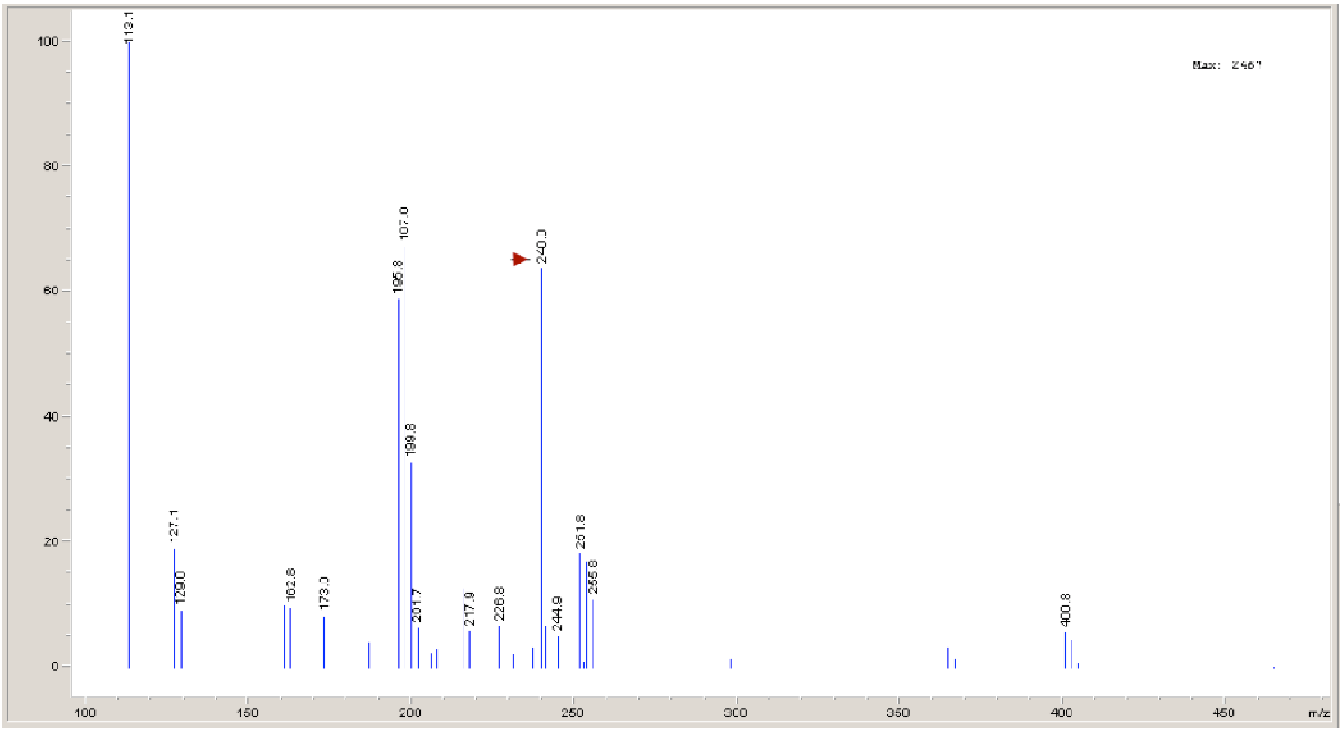


Figure S4 - D. Example of a mass spectrum of the Ent monomer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 240.0 $(M-H)^-$.

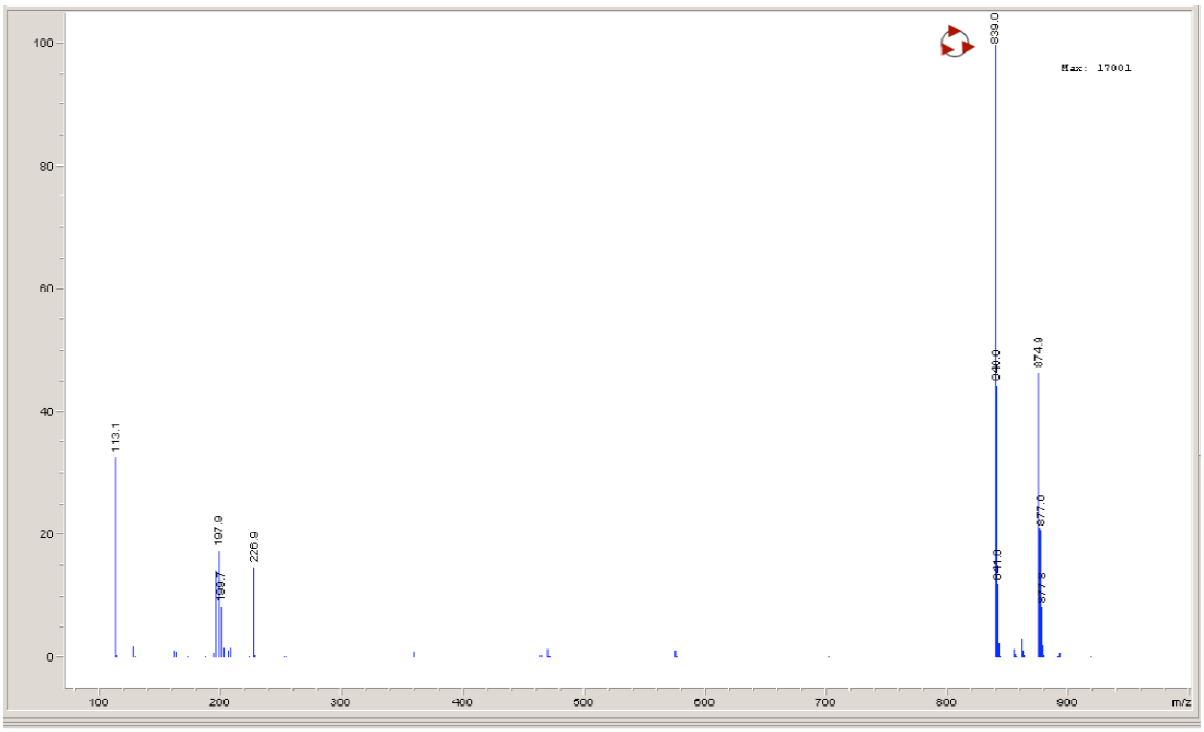


Figure S5 - A. Example of a mass spectrum of SGC from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 839.0 $(M-H)^-$.

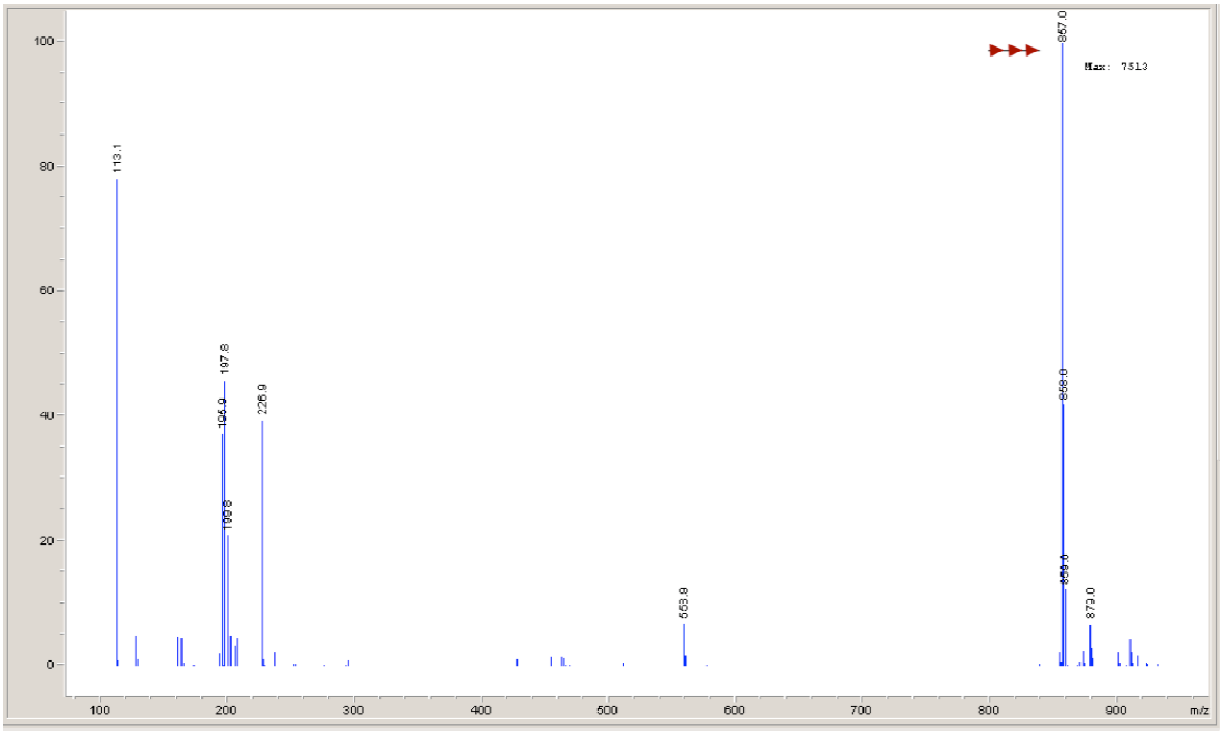


Figure S5 - B. Example of a mass spectrum of the SGC trimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 857.0 $(M-H)^-$.

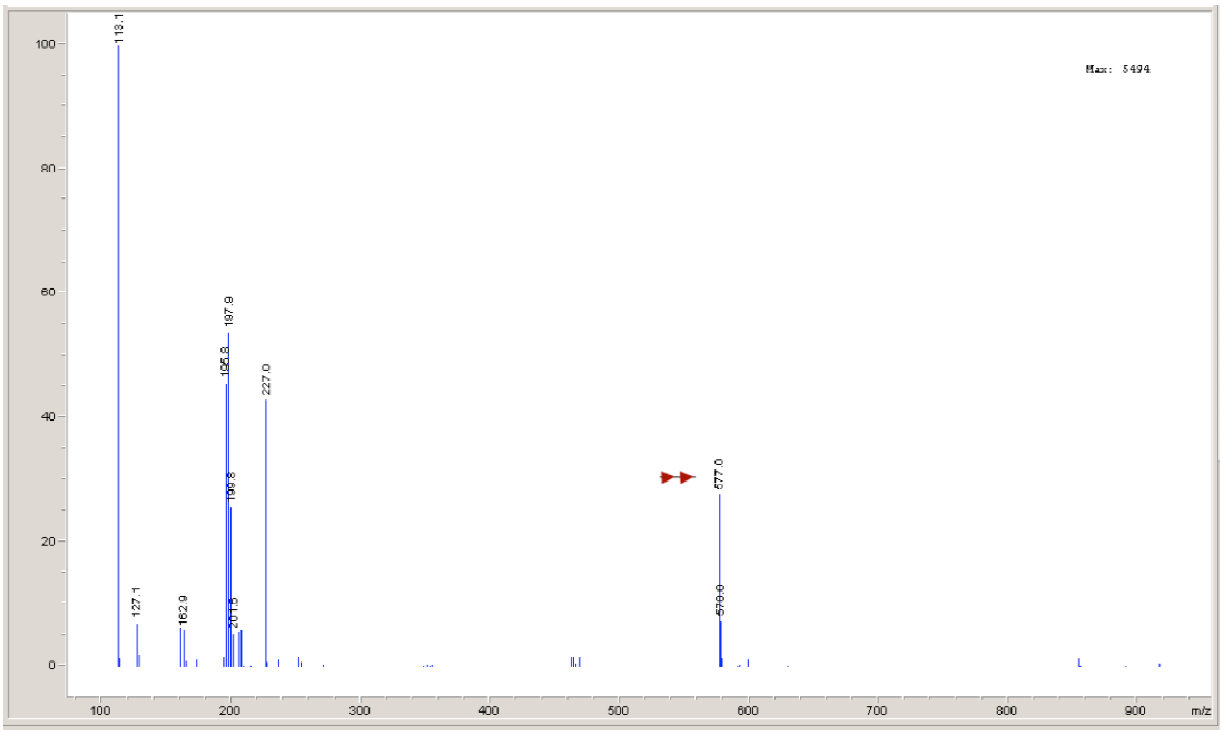


Figure S5 - C. Example of a mass spectrum of the SGC dimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 577.0 $(M-H)^-$.

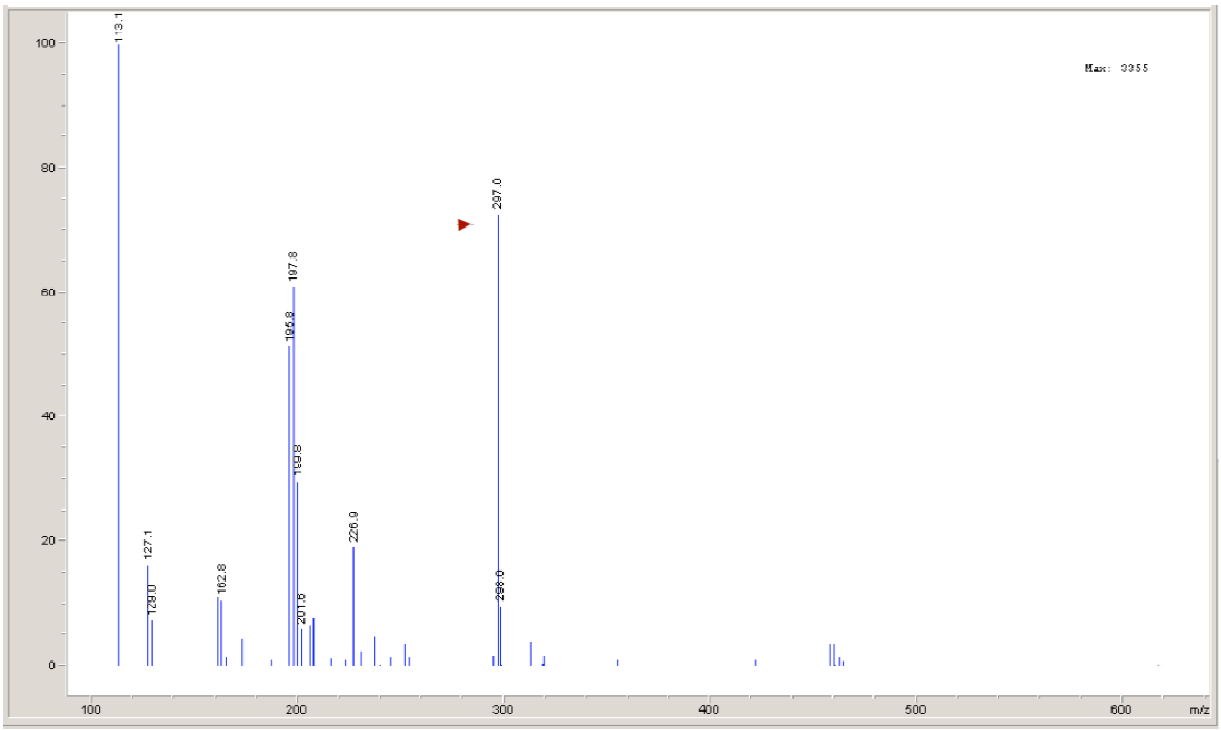


Figure S5 - D. Example of a mass spectrum of the SGC monomer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 297.0 $(M-H)^-$.

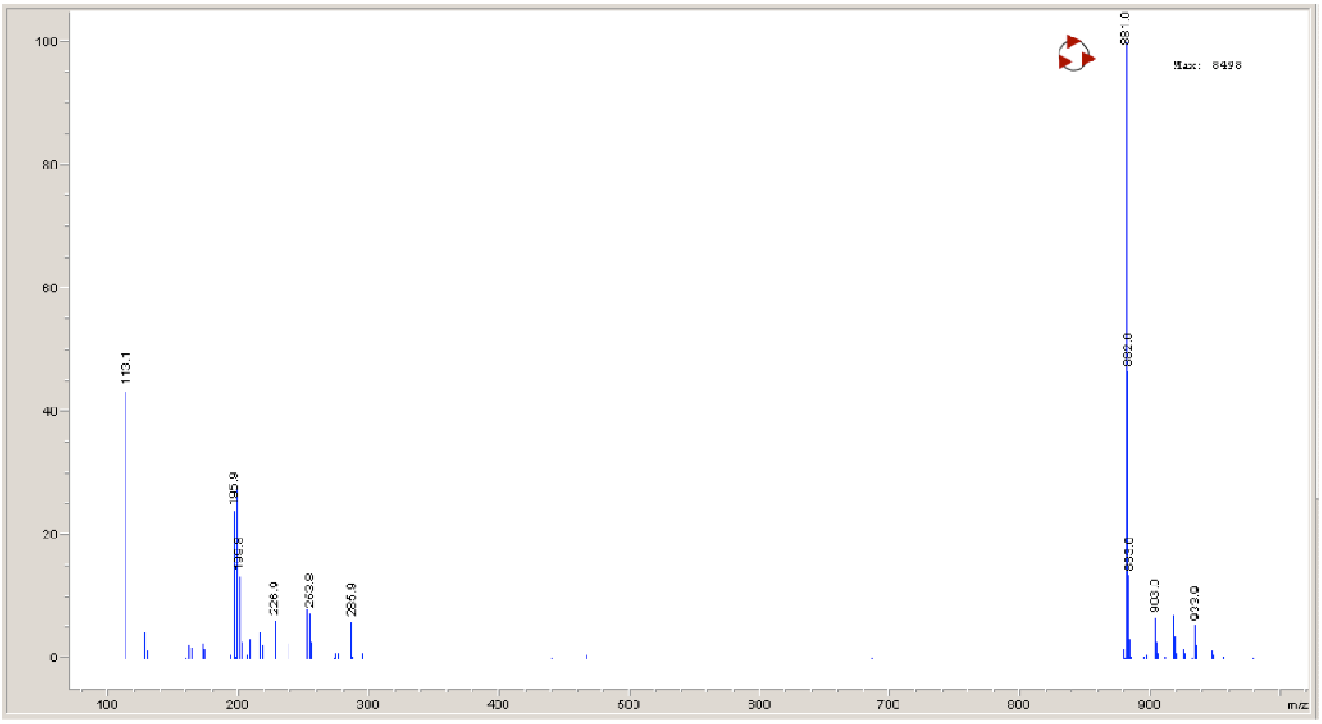


Figure S6 - A. Example of a mass spectrum of BB from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 881.0 $(M-H)^-$.

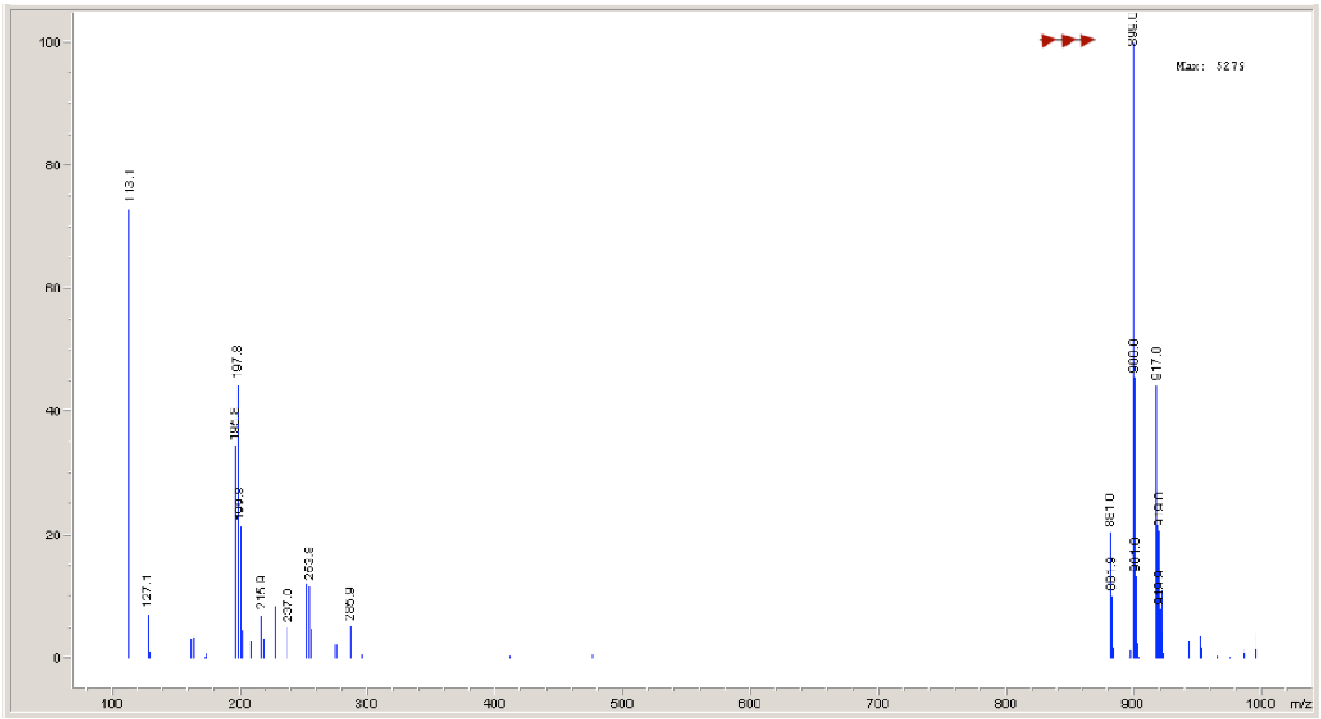


Figure S6 - B. Example of a mass spectrum of the BB trimer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 899.0 $(M-H)^-$.

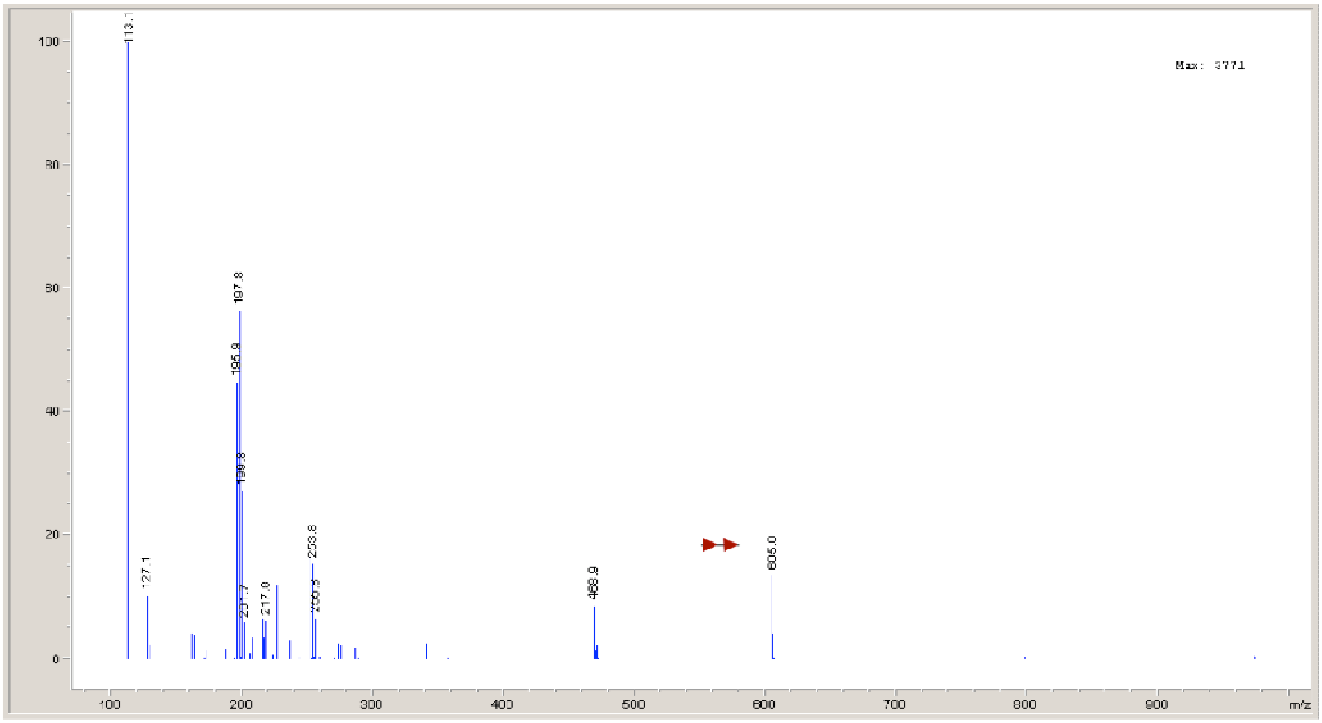


Figure S6 - C. Example of a mass spectrum of the BB dimer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 605.0 $(M-H)^-$.

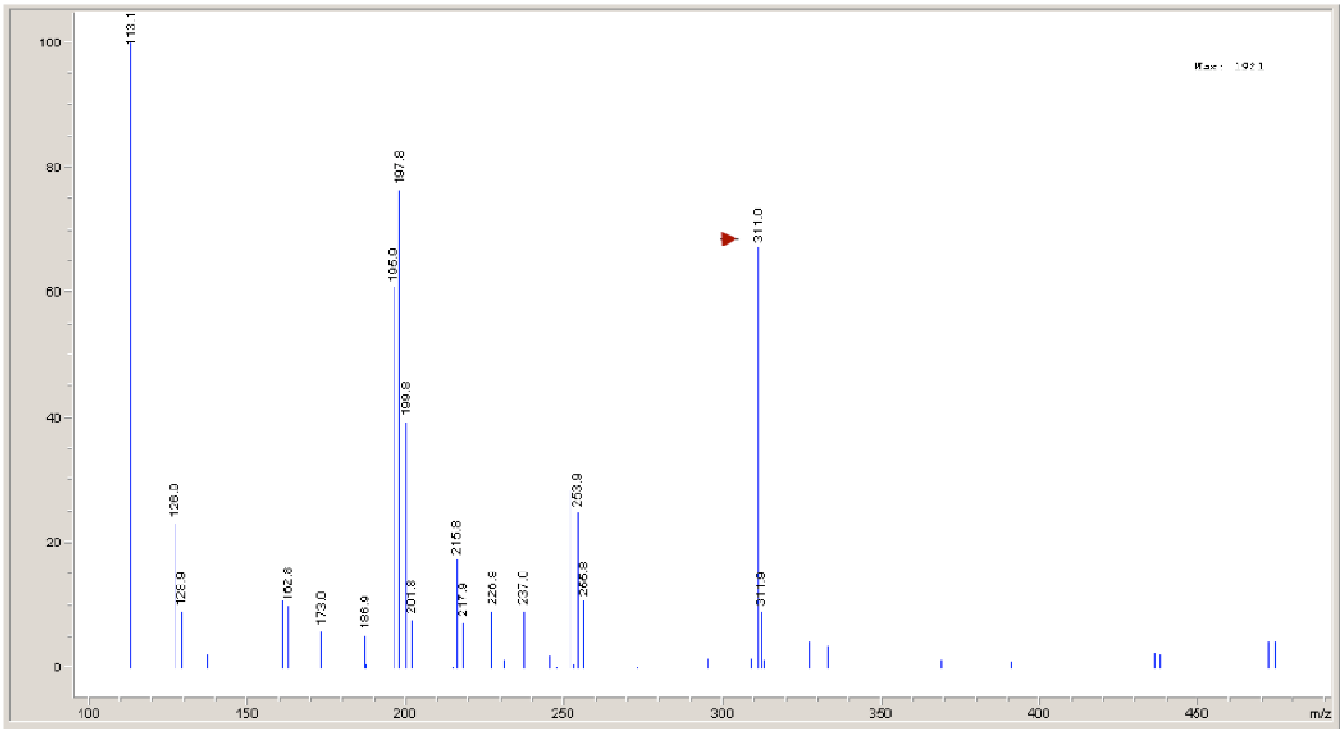


Figure S6 - D. Example of a mass spectrum of the BB monomer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 311.0 $(M-H)^-$.

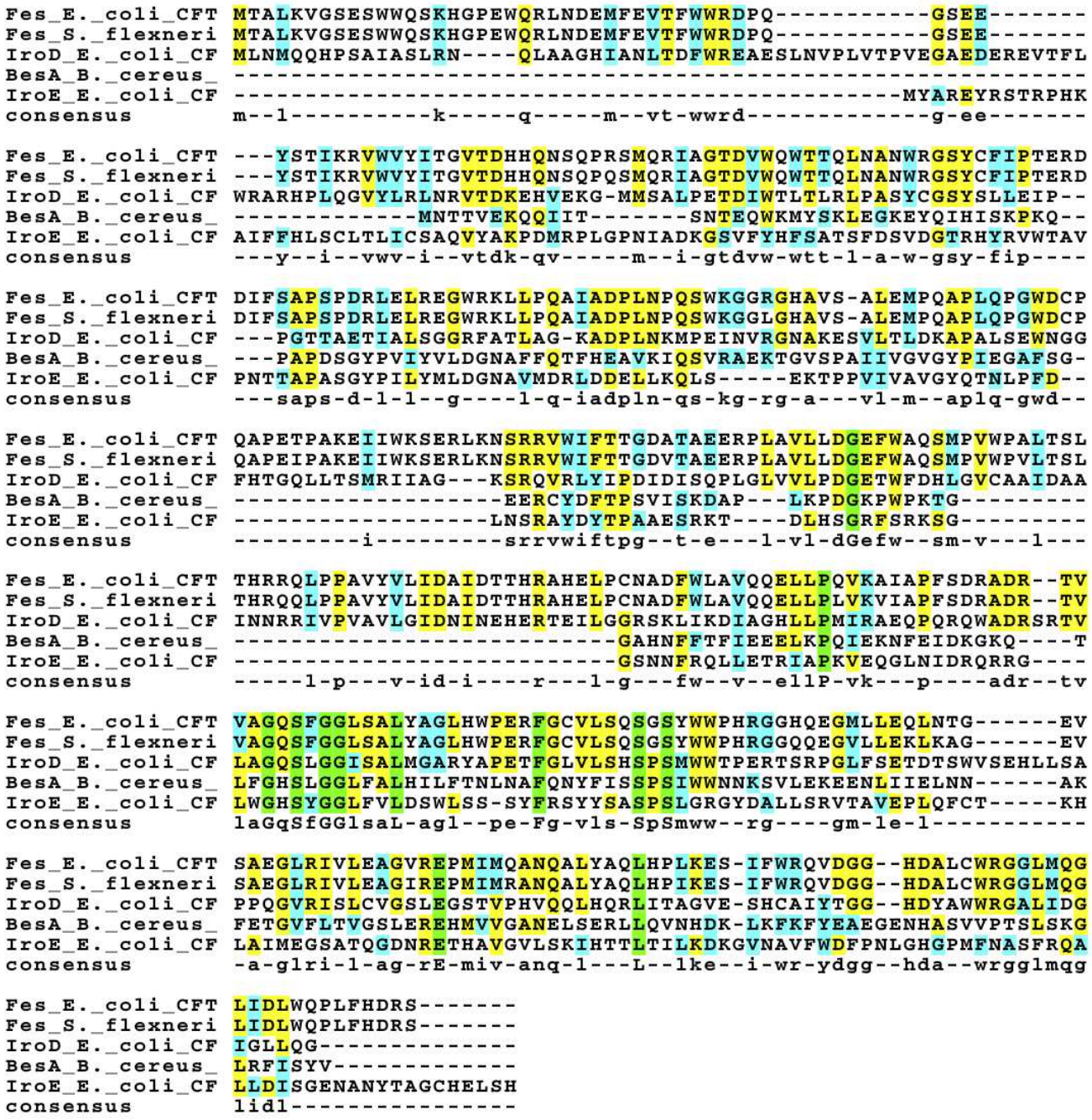


Figure S7 - A. Alignment (CLUSTALW) of esterase sequences from *E. coli* (Fes, IroD, and IroE), *S. flexneri* (Fes), and *B. cereus* (BesA). All proteins contain a conserved GxSxG serine esterase motif.

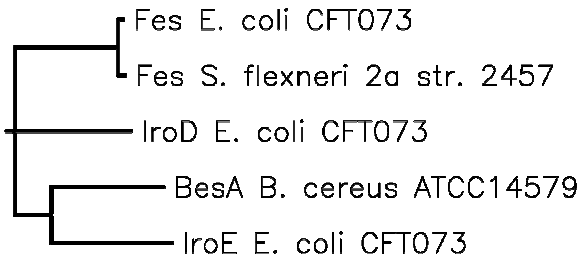


Figure S7 - B. Dendrogram of selected esterases from *E. coli*, *S. flexneri*, and *B. cereus*.

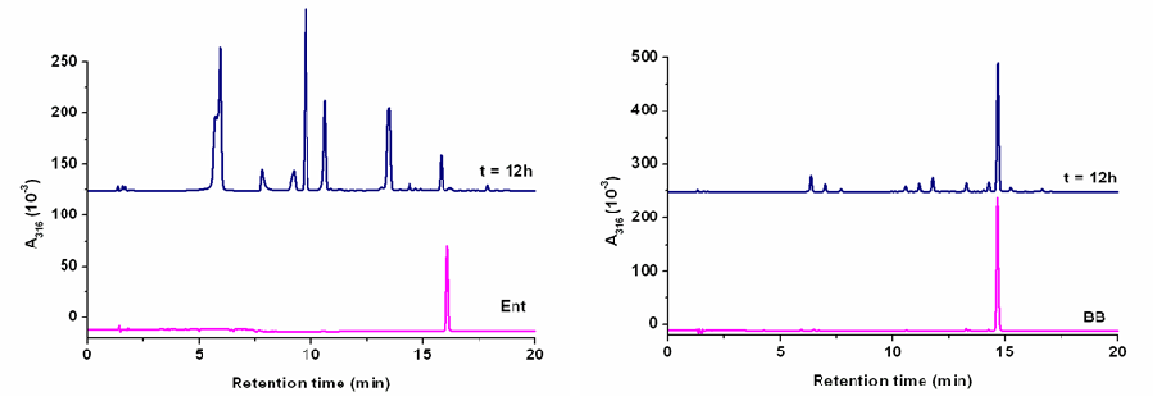


Figure S8. HPLC traces of acid-hydrolyzed solutions of Ent (left) and BB (right).

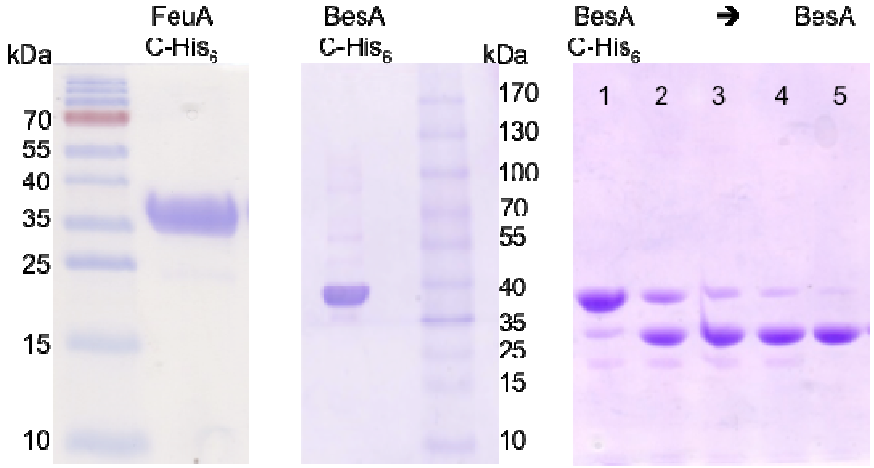
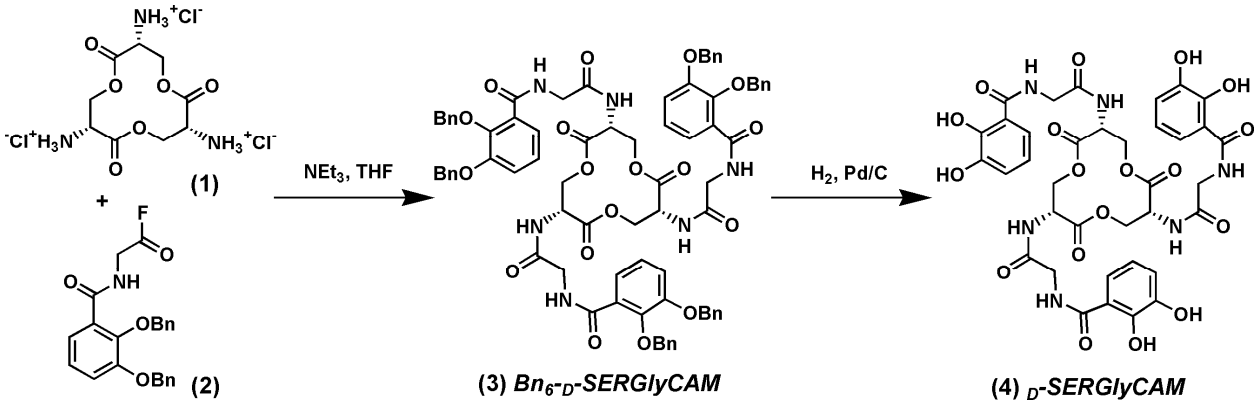


Figure S9. SDS-PAGE analysis of purified FeuA-His_6 (left) and BesA-His_6 (center); His-tag cleavage on BesA (right; lanes 1 to 4: incubation of BesA-His_6 with TEV protease at 5 min, 1h, 2h, and 3h; lane 5: BesA after Ni-agarose purification).



Scheme S1. Synthesis of $_{D}$-SERGlyCAM.