

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

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Supporting Information

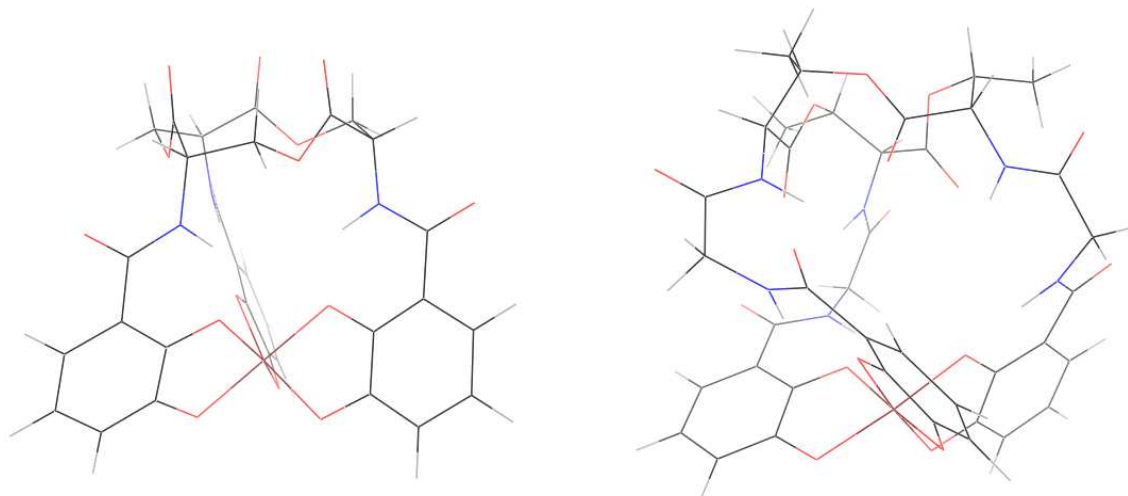


Figure S1. Previously published (Bluhm M.E., *et al.*, *Inorg. Chem.* **2002**, *41*, 5475-5478) energy minimized structures of $[\text{Fe}^{\text{III}}(\text{Ent})]^{3-}$ (left) and $[\text{Fe}^{\text{III}}(\text{BB})]^{3-}$ (right). The tris(2-aminoethyl)amine rings are in a chair conformation, with the amide functionalities in axial ($[\text{Fe}^{\text{III}}(\text{Ent})]^{3-}$) or equatorial ($[\text{Fe}^{\text{III}}(\text{BB})]^{3-}$) positions.

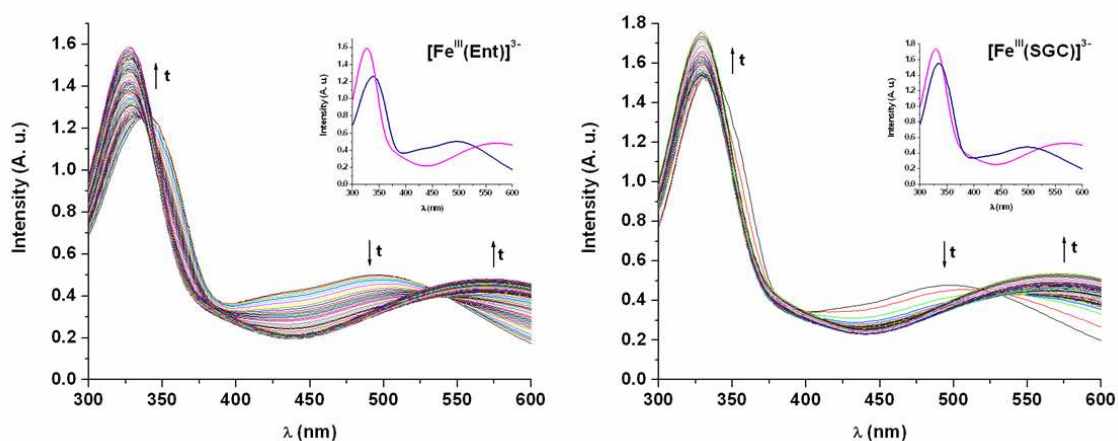


Figure S2. Bes-catalyzed hydrolysis of $[\text{Fe}^{\text{III}}(\text{Ent})]^{3-}$ (left) and $[\text{Fe}^{\text{III}}(\text{SGC})]^{3-}$ (right) to $[\text{Fe}^{\text{III}}(2,3\text{-DHBS})_3]^{3-}$ and $[\text{Fe}^{\text{III}}(2,3\text{-DHBGS})_3]^{3-}$, respectively, followed by UV-vis spectroscopy ($[\text{Fe}(\text{L})] = 100 \mu\text{M}$, $[\text{Bes}] = 1 \mu\text{M}$, 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).

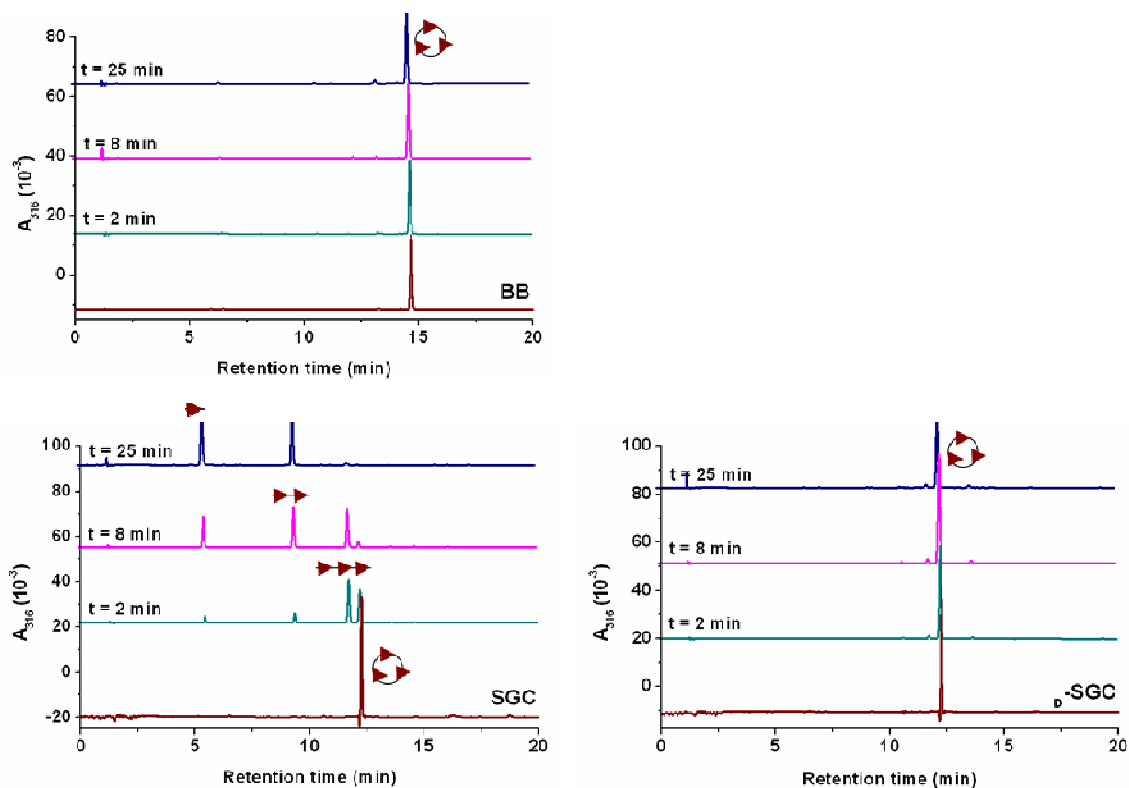


Figure S3. Reaction time course of Fes-catalyzed hydrolysis of SGC (bottom left). The presence of Fes in solution did not affect BB (top) and D -SGC (bottom right). Reaction aliquots were quenched at different time points and analyzed by HPLC. The assignment of the hydrolysis products is based on the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

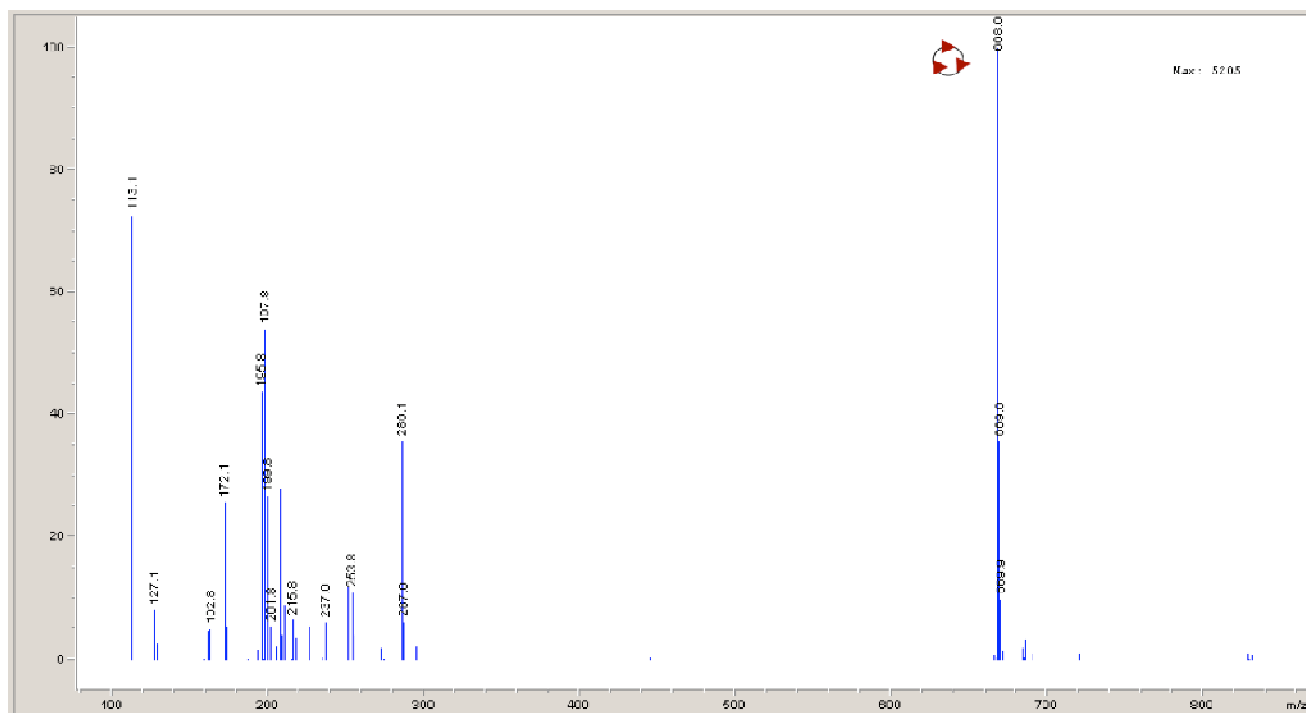


Figure S4 - A. Example of a mass spectrum of Ent from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 668.0 (M-H)⁻.

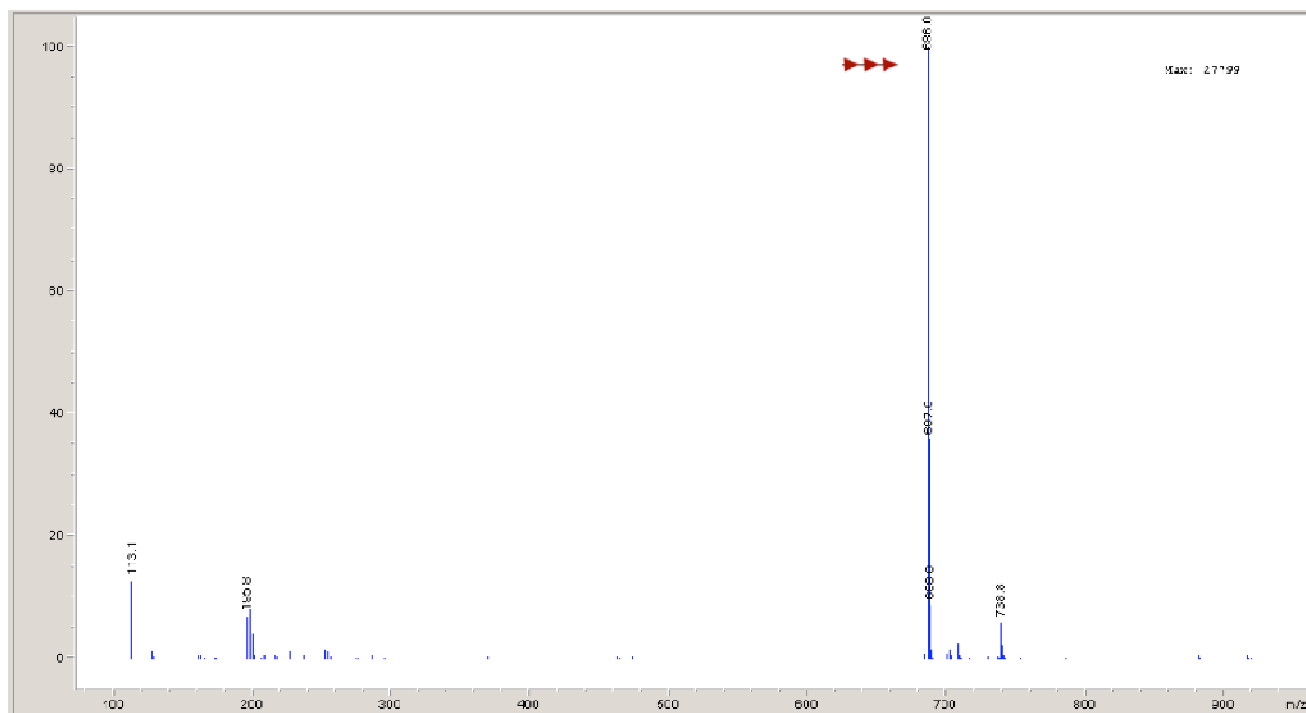


Figure S4 - B. Example of a mass spectrum of the Ent trimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 686.0 (M-H)⁻.

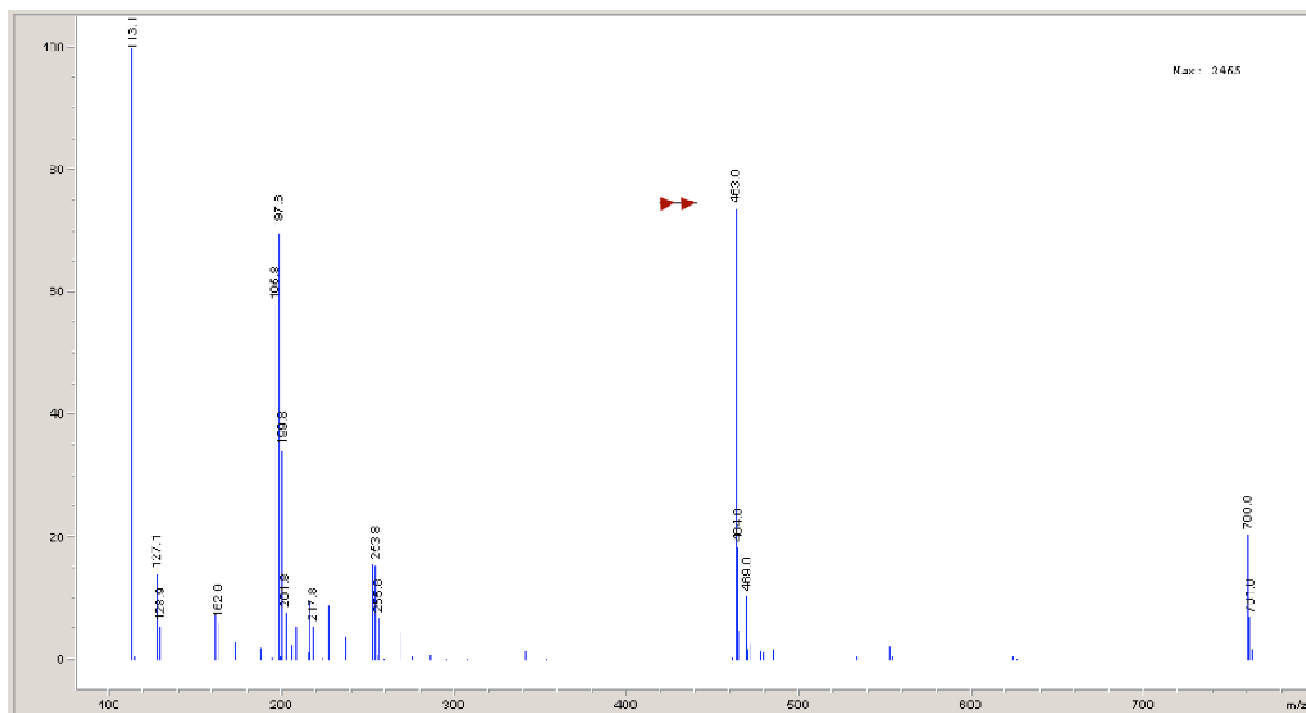


Figure S4 - C. Example of a mass spectrum of the Ent dimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 463.0 (M-H)⁻.

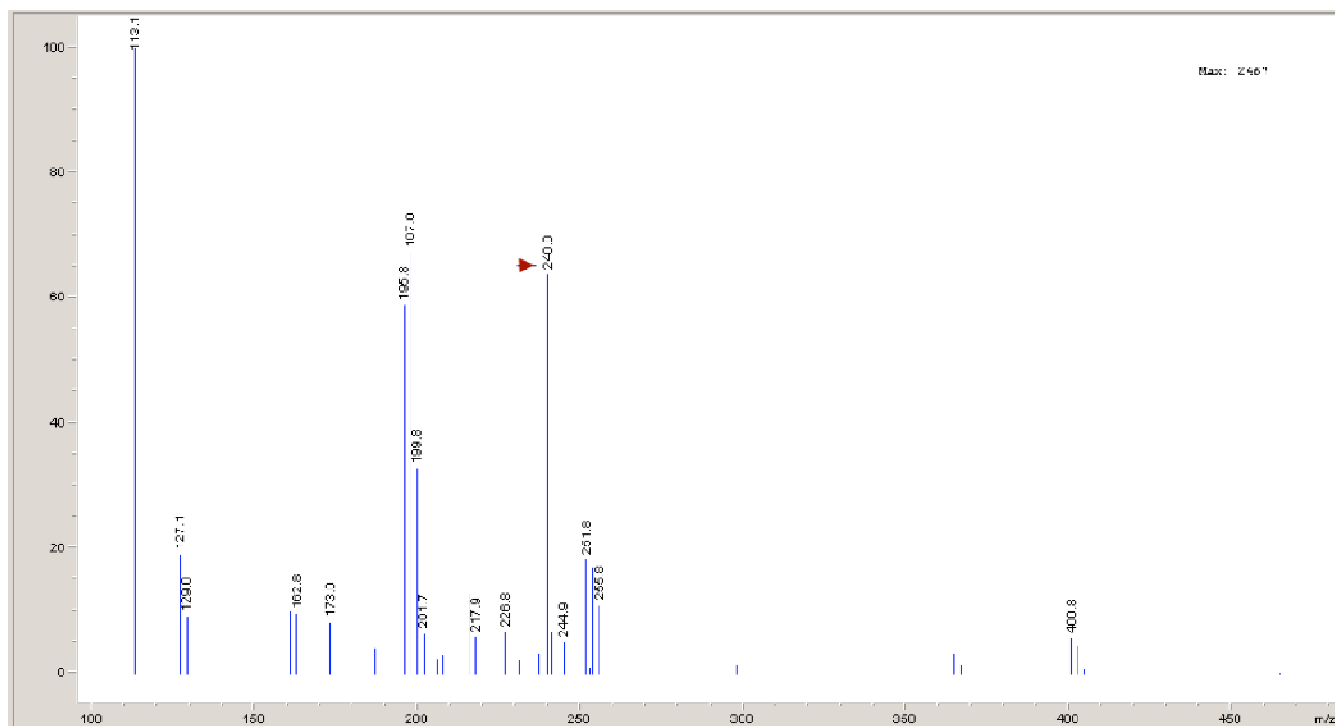


Figure S4 - D. Example of a mass spectrum of the Ent monomer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 240.0 (M-H)⁻.

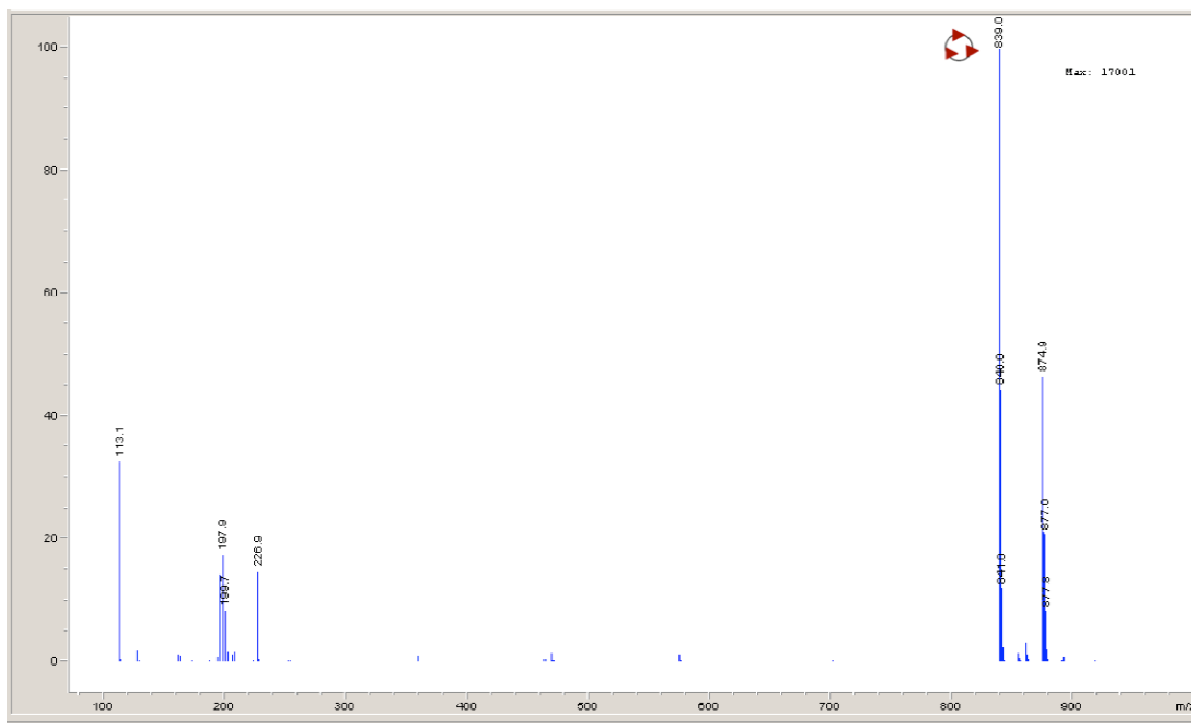


Figure S5 - A. Example of a mass spectrum of SGC from the FeS-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 839.0 (M-H)⁻.

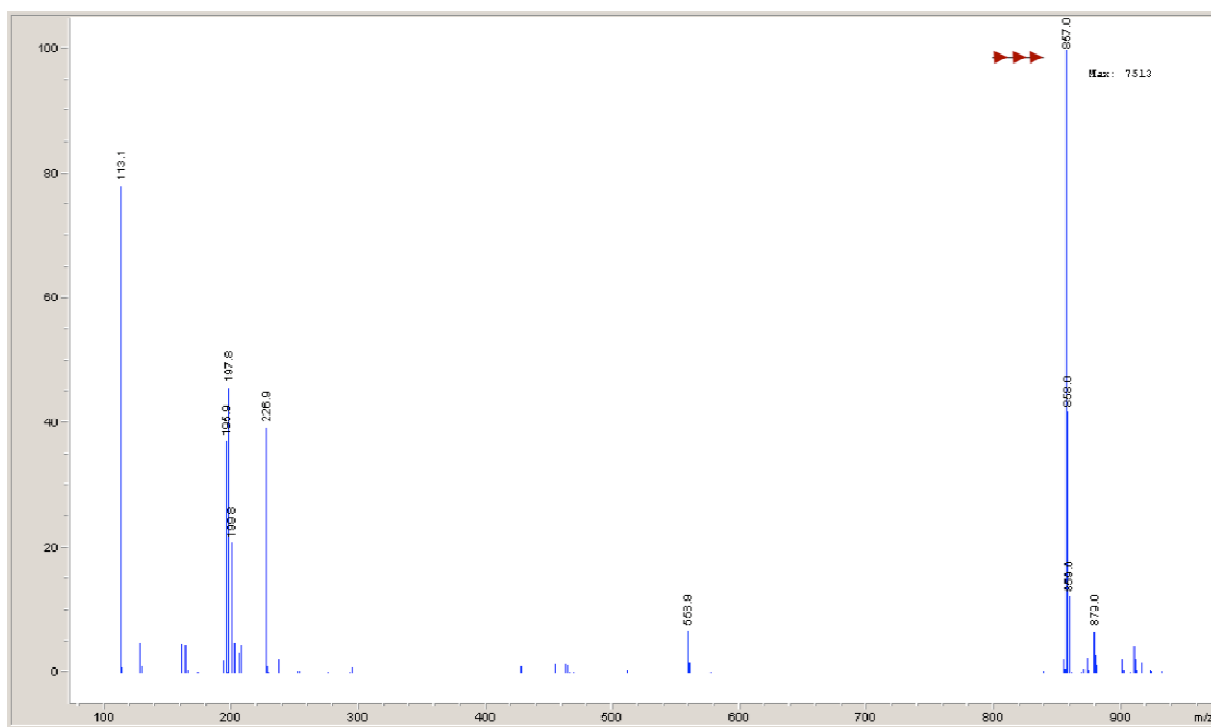


Figure S5 - B. Example of a mass spectrum of the SGC trimer derivative from the FeS-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 857.0 (M-H)⁻.

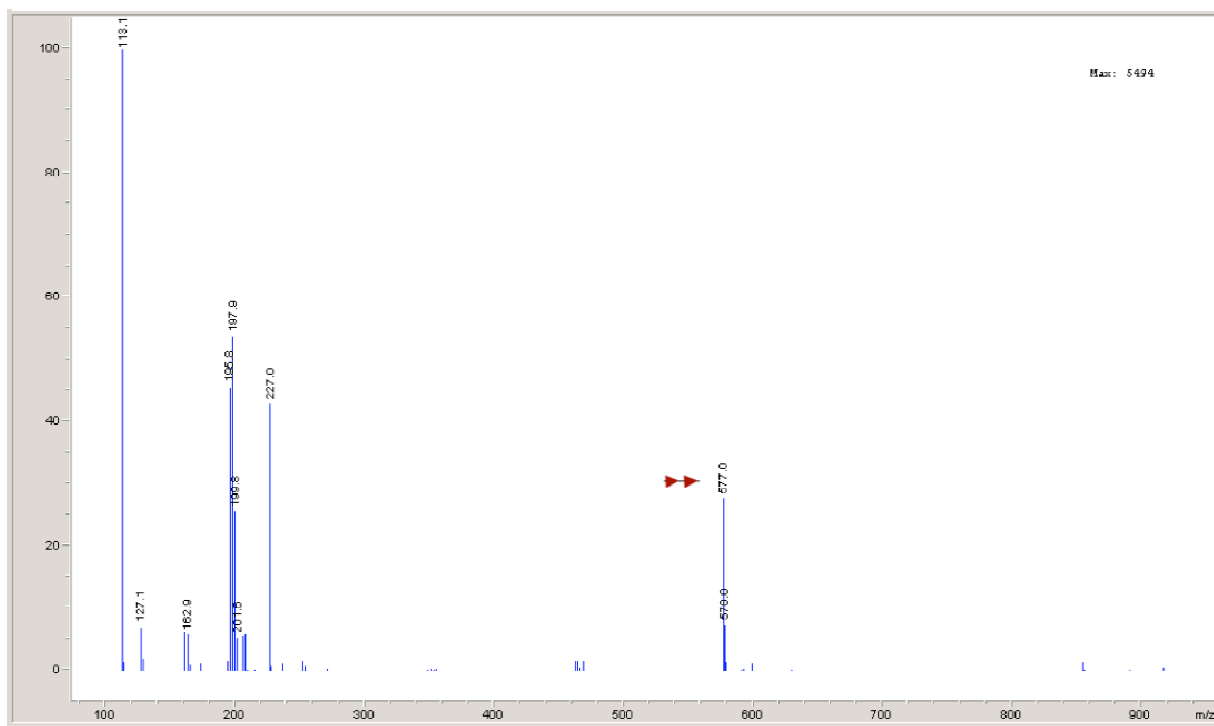


Figure S5 - C. Example of a mass spectrum of the SGC dimer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 577.0 (M-H)⁻.

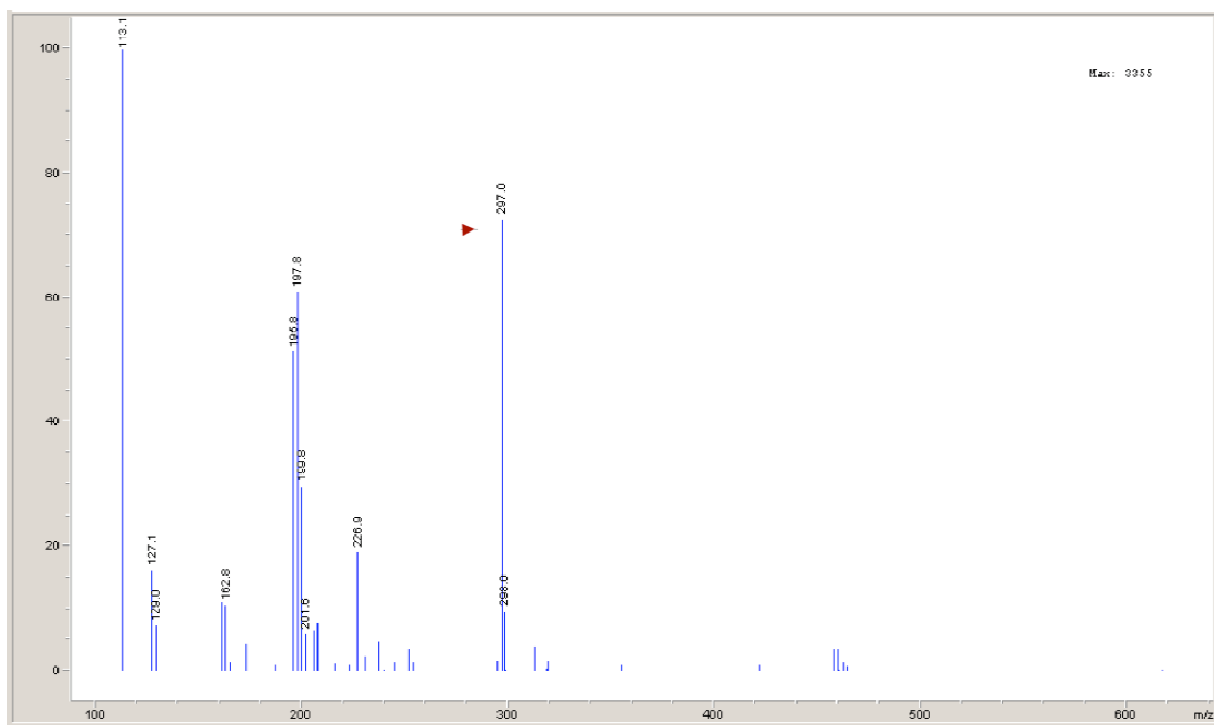


Figure S5 - D. Example of a mass spectrum of the SGC monomer derivative from the Fes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 297.0 (M-H)⁻.

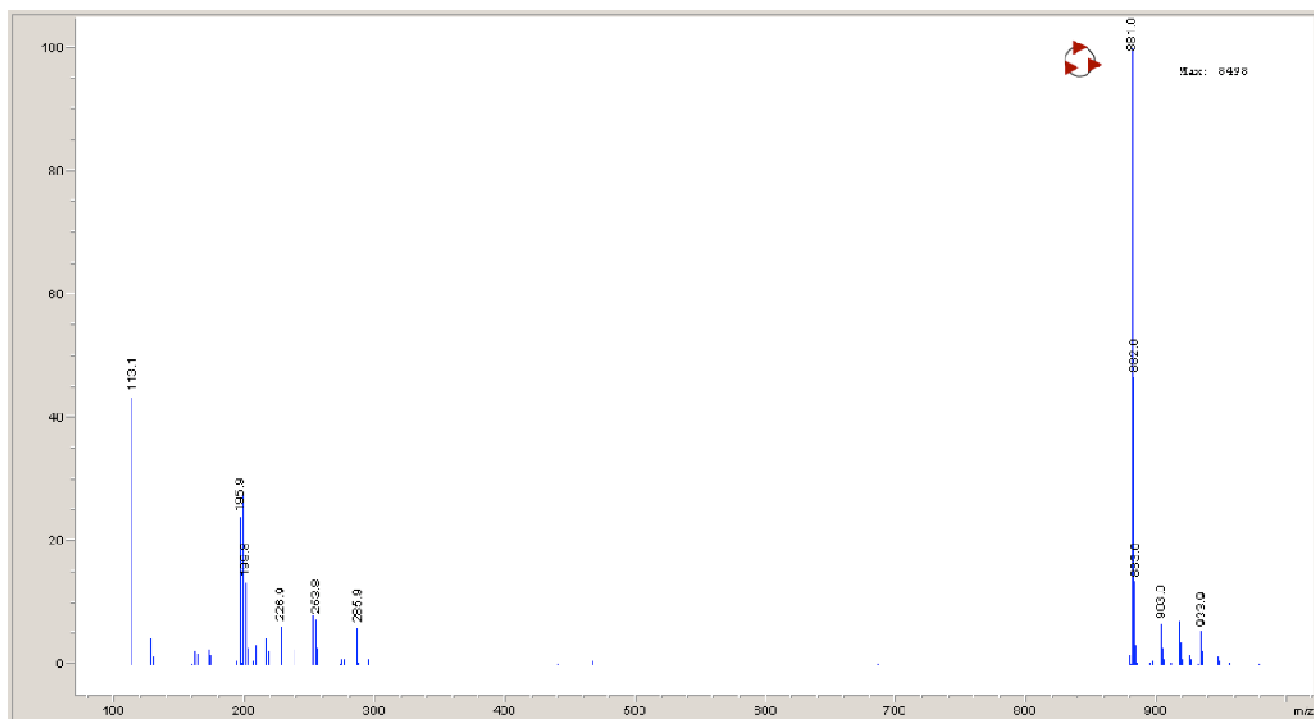


Figure S6 - A. Example of a mass spectrum of BB from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 881.0 (M-H)⁻.

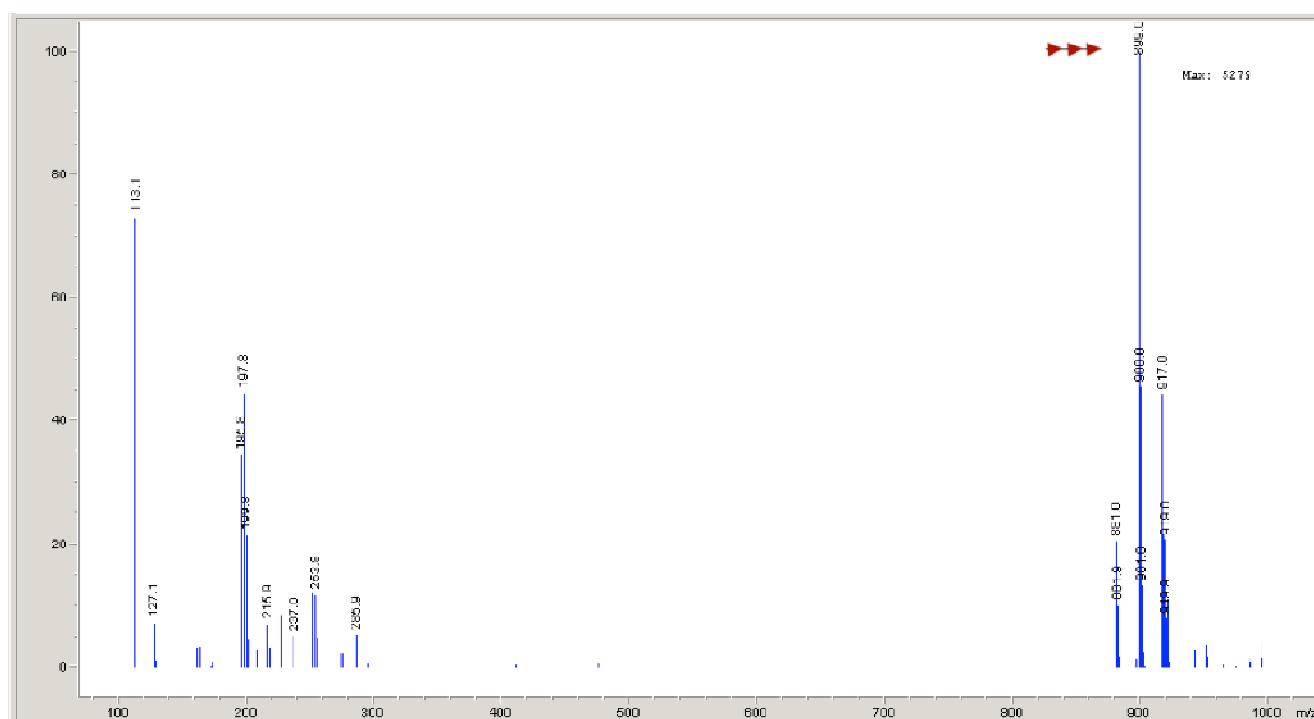


Figure S6 - B. Example of a mass spectrum of the BB trimer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 899.0 (M-H)⁻.

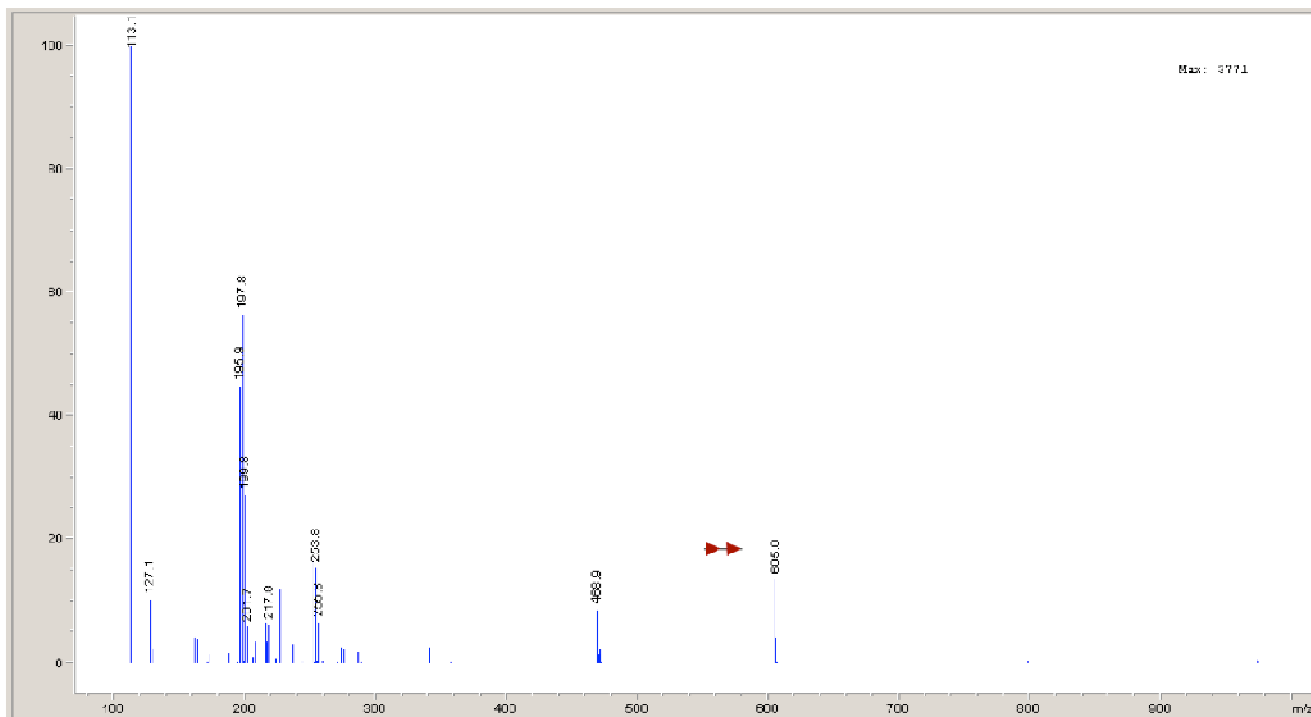


Figure S6 - C. Example of a mass spectrum of the BB dimer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 605.0 (M-H)⁻.

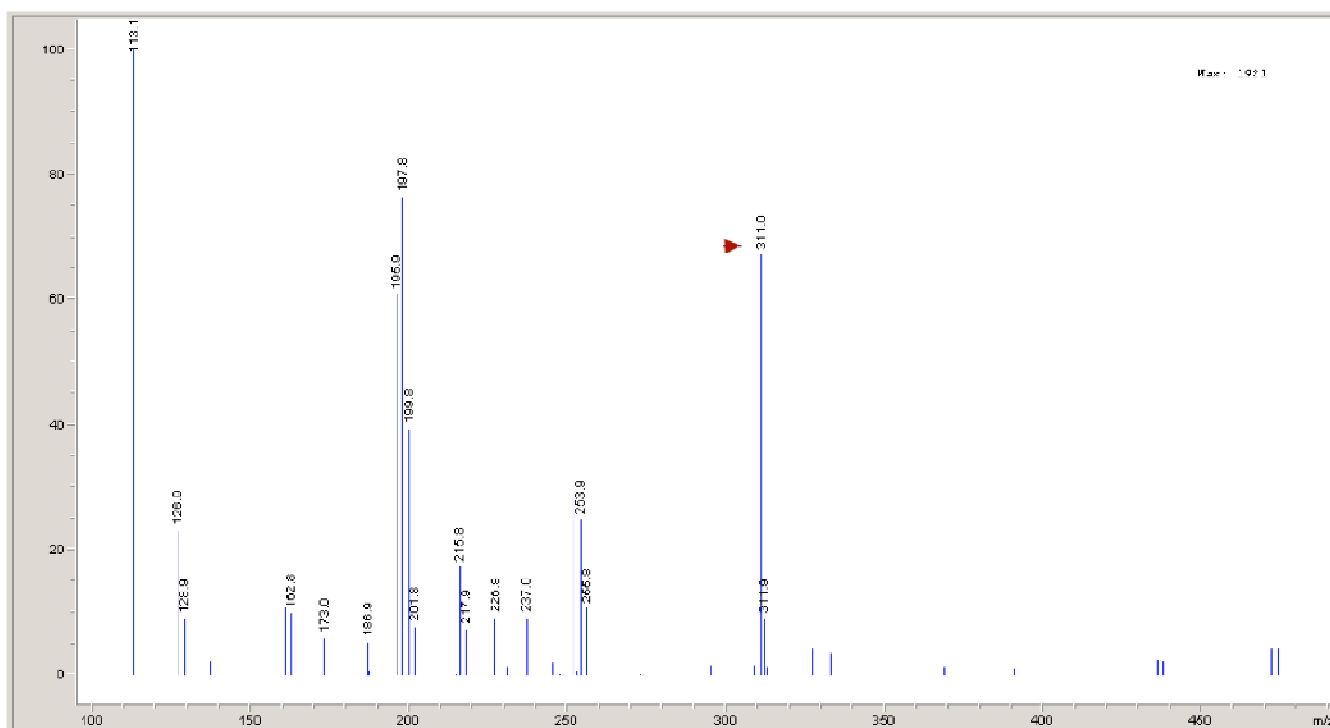


Figure S6 - D. Example of a mass spectrum of the BB monomer derivative from the Bes-catalyzed hydrolysis experiments; (-)-ESIMS: m/z 311.0 (M-H)⁻.

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Fes_E._coli_CFT MTALKVGSSESWWQSKHGPEWQRLNDEMFEVTFWWRDPQ-----GSEE-----
Fes_S._flexneri MTALKVGSSESWWQSKHGPEWQRLNDEMFEVTFWWRDPQ-----GSEE-----
IroD_E._coli_CF MLNMQQHPSAIASLRN---QLAAGHIANLTDFFWREAESLNVPLVTPVEGAEDEREVTFLL
BesA_B._cereus_ -----
IroE_E._coli_CF -----MYAREYRSTRPHK
consensus      m--l-----k-----q-----m--vt-wwr-----g-ee-----

Fes_E._coli_CFT ---YSTIKRVVWYITGVTDHHSQSPRSMQRIAGTDVWQWTTQLNANWRGSGYCFIPTERD
Fes_S._flexneri ---YSTIKRVVWYITGVTDHHSQSPQSMQRIAGTDVWQWTTQLNANWRGSGYCFIPTERD
IroD_E._coli_CF WRARHPLQGVYLRNLNRVTDKEHVEKG-MMSALPETDIWTLTLRLPASVYCGSYSLLEIP--
BesA_B._cereus_ -----MNTTVEKQQIIT-----SNTEQWKMYSKLEGKEYQIHIKPKQ--
IroE_E._coli_CF AIFFHLSCLTLLICSAQVYAKPDMRPLGPNIAADKGSVVFYHFSATSFDSDVDGTRHYRVWTVAV
consensus      ---y--i--vwv-i--vtdk-qv-----m--i-gtdvw-wtt-l-a-w-gsy-fip----

Fes_E._coli_CFT DIFSAPSPDRLELREGWRKLLPQAIADPLNPQSWKGGRGHAVS-ALEMPQAPLQPGWDCP
Fes_S._flexneri DIFSAPSPDRLELREGWRKLLPQAIADPLNPQSWKGGGLGHAVS-ALEMPQAPLQPGWDCP
IroD_E._coli_CF ---PGTTAETIALSGGRFATLAG-KADPLNKMPEINVRGNAKESVLTLDKAPALSEWNGG
BesA_B._cereus_ ---PAPDSGYPVIIYVLDGNAFFQTFHEAVKIQSVRAEKTGVSPAIIVGVGYPIEGAFSG-
IroE_E._coli_CF PNTTAPASGPILYMLDGNVMDRLDDELLKQLS-----EKTPPVIVAVGYQTNLFPD--
consensus      ---saps-d-l-l-g-----l-q-iadpln-qs-kg-rg-a---vl-m--aplq-gwd--

Fes_E._coli_CFT QAPETPAKEIIWKSERLKNSSRRVWIFFTGDATAEERPLAVLLDGEFWAQSMPVWPALTSLSL
Fes_S._flexneri QAPEIPAKEIIWKSERLKNSSRRVWIFFTGDVTAEEERPLAVLLDGEFWAQSMPVWPVLTSL
IroD_E._coli_CF FHTGQLLTSMRIIAG---KSRQVRLYIPDIDISQPLGLVVLDPGETWFDHLGVCAAIDAA
BesA_B._cereus_ -----EERCYDFTPSVISKDAP---LKPDKGKPPKPTG-----
IroE_E._coli_CF -----LNSRAYDYTPAAESRKT---DLHSGRFRSRKSG-----
consensus      -----i-----srrvwiftgp--t-e--l-vl-dgef--sm-v--l---

Fes_E._coli_CFT THRRQLPPAVYVLIDAIIDTTHRAHELPCNADFWLAVQQEELLPQVKAIAPFSADRADR--TV
Fes_S._flexneri THRRQLPPAVYVLIDAIIDTTHRAHELPCNADFWLAVQQEELLPLVKVIAPFSADRADR--TV
IroD_E._coli_CF INNRRIVPVAVLGDININEHERTEILGGRSKLIKDIAGHLLPMIRAEQPPQRQWADRSTRV
BesA_B._cereus_ -----GAHNFFTFIEEELKPKQIEKNFEIDKKGKQ----T
IroE_E._coli_CF -----GSNNFRQLLETRIAPKVEQGLNIDRQRRG----
consensus      -----l-p---v-id-i---r---l-g---fw--v--ellp-vk---p---adr--tv

Fes_E._coli_CFT VAGQSFGGLSALYAGLHWPERFGCVLSQSGSYWVPHRGGHQEGMLLEQLNTG-----EV
Fes_S._flexneri VAGQSFGGLSALYAGLHWPERFGCVLSQSGSYWVPHRGGQEGVLLLEKLGKAG-----EV
IroD_E._coli_CF LAGQSLGGISALMGARYAPETFGVLVLSHSPSMWWTPERTSRPGLFSETDTSWVSEHLLSA
BesA_B._cereus_ LFGHSLGGLFALHILFTNLNAFQNYFISSPSIWNNKSVLEKEENLIIELNN-----AK
IroE_E._coli_CF LWGHSYGGLFVLDLSS-SYFRSYYSSASPSLGRGYDALLSRVTAVEPLQFCT-----KH
consensus      laGqSfGGLsaL-agl--pe-Fg-vls-SpSmww--rg---gm-le-l-----

Fes_E._coli_CFT SAEGLRIVLEAGVREPMIMQANQALYAQLHPLKES-IFWRQVDGG--HDALCWRGGLMQG
Fes_S._flexneri SAEGLRIVLEAGIREPMIMRANQALYAQLHPIKES-IFWRQVDGG--HDALCWRGGLMQG
IroD_E._coli_CF PPQGVRIISLCVGSLEGSTVPHVQQLHQRLITAGVE-SHCAIYTG--HDYAWWRGALIDG
BesA_B._cereus_ FETGVFLTVGSLEREHMVVGANELSERLLQVNHDK-LKFKFYEAEGENHASVVPSTLSKG
IroE_E._coli_CF LAIMEGSATQGDNRETHAVGVLSKIHTTLTILKDKGVNAVFWDFPNLGHGPMFNASFRQA
consensus      -a-glri-l-ag-re-miv-anq-l---l--lke--i-wr-ydgg--hda--wrgglmqg

Fes_E._coli_CFT LIDLWQPLFHDRS-----
Fes_S._flexneri LIDLWQPLFHDRS-----
IroD_E._coli_CF IGLLQG-----
BesA_B._cereus_ LRFISYV-----
IroE_E._coli_CF LLDISGENANYTAGCHELSH
consensus      lidl-----

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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.

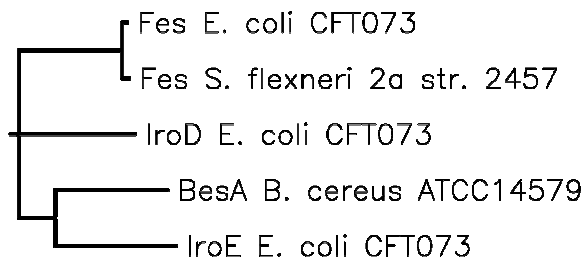


Figure S7 - B. Dendrogram of selected esterases from *E. coli*, *S. flexneri*, and *B. cereus*.

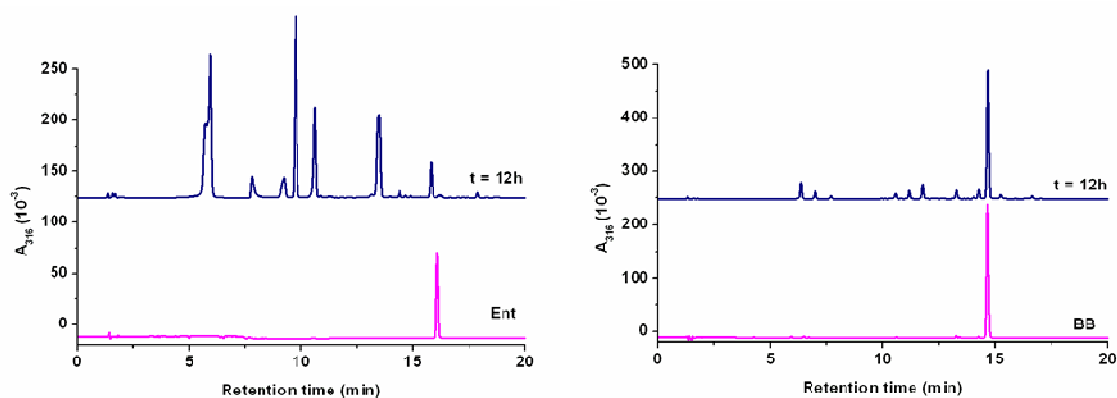


Figure S8. HPLC traces of acid-hydrolyzed solutions of Ent (left) and BB (right).

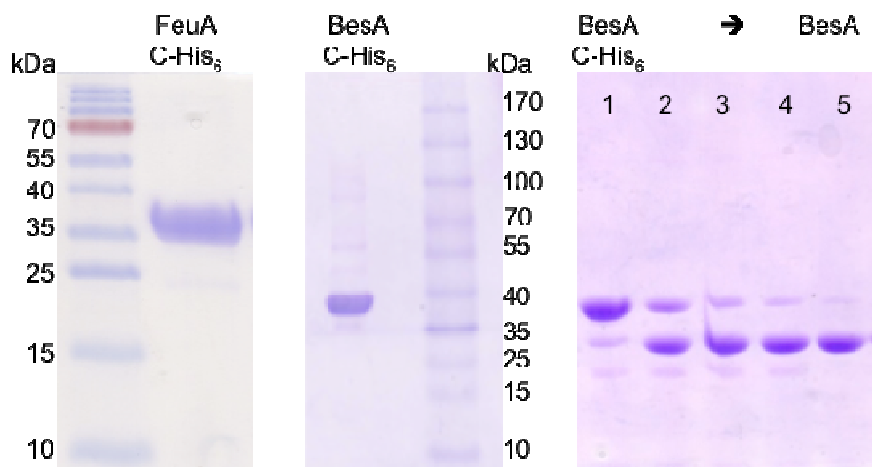
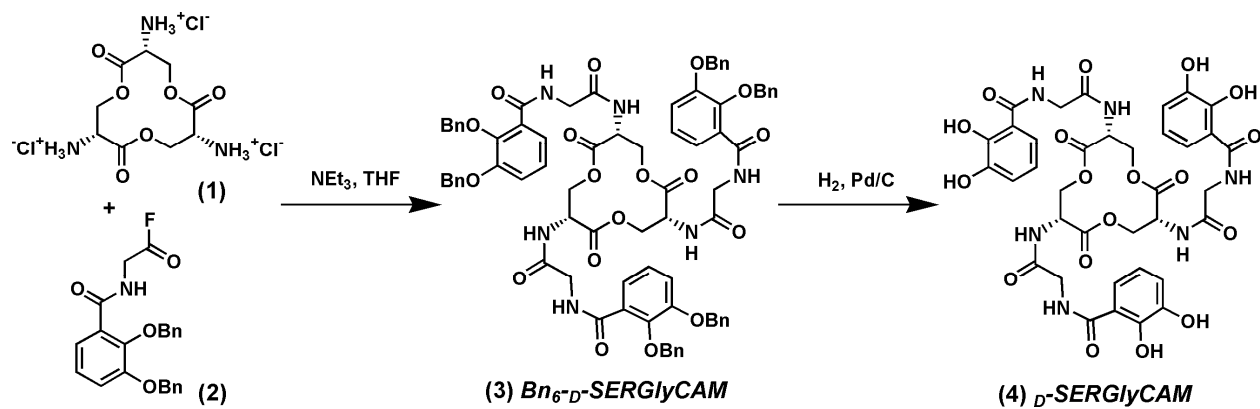


Figure S9. SDS-PAGE analysis of purified FeuA-His₆ (left) and BesA-His₆ (center); His-tag cleavage on BesA (right; lanes 1 to 4: incubation of BesA-His₆ with TEV protease at 5 min, 1h, 2h, and 3h; lane 5: BesA after Ni-agarose purification).



Scheme S1. Synthesis of *D*-SERGlyCAM.