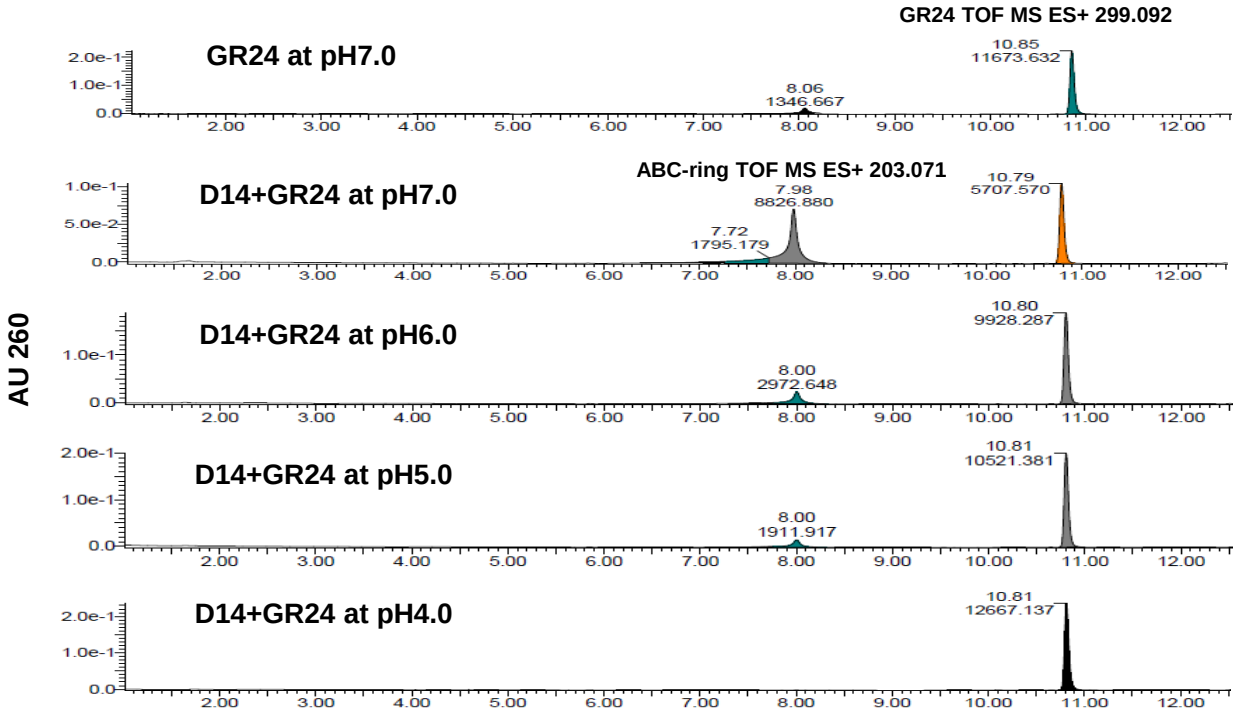
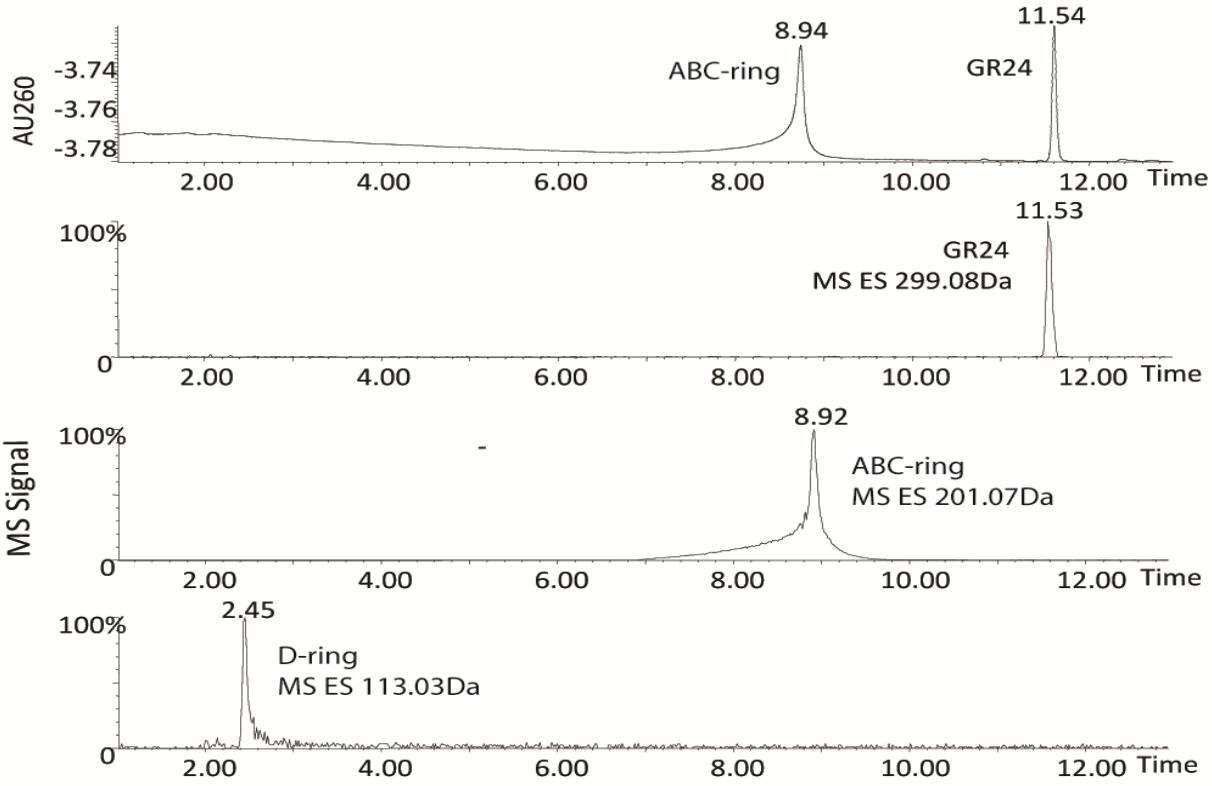

GR24 TOF MS ES+ 299.092
GR24 at pH7.0
10.85
11673.632
8.06
1346.667
ABC-ring TOF MS ES+ 203.071
D14+GR24 at pH7.0
10.79
5707.570
7.98
8826.880
7.72
1795.179
AU 260
D14+GR24 at pH6.0
10.80
9928.287
8.00
2972.648
D14+GR24 at pH5.0
10.81
10521.381
8.00
1911.917
D14+GR24 at pH4.0
10.81
12667.137


AU260
8.94
ABC-ring
11.54
GR24
Time
MS Signal
11.53
GR24
MS ES 299.08Da
8.92
ABC-ring
MS ES 201.07Da
2.45
D-ring
MS ES 113.03Da

Supplementary information, Figure S6 UPLC/MS analysis of D14-catalyzed GR24 hydrolysis. (**A**) UPLC-separation of GR24 and the ABC ring GR24 hydrolysis product as determined by UV absorbance and electrospray ionization quadrupole time-of-flight mass spectrometric analysis (ESI-Q-TOF MS). The strong pH-dependence of the reaction reflects the requirement of the triad histidine in its uncharged state in order to function as proton acceptor (pKa of the histidine imidazole ring ~ 6.0). (**B**) Identification of the ABC-ring and D-ring hydrolysis products (see Fig. S4D for chemical structures). GR24 and hydrolysis products were separated by UPLC and analyzed by ESI-Q-TOF-MS. Intact GR24 was detected in positive mode with its neutral mass at 298.08. The D-ring and ABC-ring products were detected in negative mode with their neutral masses of 114.03 and 202.07, respectively. The mass difference of the sum of D-ring plus ABC-ring and intact GR24 is 18.020, which closely matches the molecular weight of a water molecule, consistent with the hydrolysis reaction.