Supplemental Figure S1: Total ion chromatogram (TIC), extracted ion chromatogram (EIC) and comparative MS data of Figure 3 and Figure 4.

Panel 1: TIC (A) and EIC of hapalindole product $(m/z \ 305)$ (B) and *cis*-indole isonitrile 2 $(m/z \ 169)$ (C) compared with the standard 2 (D) for the *in vitro* TT-assay performed in the absence of cyclase and FamD2 plasmids (for Figure 3 A). **Panel 2:** MS data of the product (top) and the standard 2 (bottom) are shown.







B. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile **2** (m/z 169) (B) and *cis*-indole isonitrile-GPP intermediate **3** (m/z 305) (C) compared with the standard **3** (D) for the *in vitro* TT-assay performed in the presence of FamD2 plasmid alone (for Figure 3 B). **Panel 2:** MS data of the product (top) and the standard **3** (bottom) are shown.



C. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile **2** (m/z 169) (B), *cis*-indole isonitrile-GPP intermediate **3** (m/z 305) (C) and 12-*epi*-hapalindole U **4** (m/z 305) (D) compared with the standard **4** (E) for the *in vitro* TT-assay performed in the presence of FamD2 and FamC1 plasmids (for Figure 3 C). **Panel 2:** MS data of the product (top) and the standard **4** (bottom) are shown.



Panel 2

x10⁵ +ESI Scan (rt: 9.568 min) Frag=140.0V NEB1.d 4-305.2021 3. 328.2670 278.1903 2-169.0762 186.2215 230.1400 214.9167 250.1606 140.0684 348.2431 1. 0 x10⁵ +ESI Scan (rt: 9.543 min) Frag=140.0V 12-epi-hapU_std-R.d



D. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile **2** (m/z 169) (B), *cis*-indole isonitrile-GPP intermediate **3** (m/z 305) and hapalindole H **6** (m/z 305) product (C) were compared with the standard **6** (D) for the *in vitro* TT-assay performed in the presence of FamD2 and FamC2 plus FamC3 plasmids in the presence of lower 5 mM calcium (for Figure 3E). **Panel 2:** MS data of the product (top) and the standard **6** (bottom) are shown.



x10⁴ +ESIScan (rt: 10.993 min) Frag=140.0V NEB3.d



E. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile **2** (m/z 169) (B), and *cis*-indole isonitrile-GPP intermediate **3** (m/z 305) and hapalindole H **6** (m/z 305) (C) compared with the standard **6** (D) for the *in vitro* TT-assay performed in the presence of FamD2 and FamC2 plus FamC3 plasmids in the presence of higher 20 mM calcium (for Figure 3 E). **Panel 2:** MS data of the product (top) and the standard **6** (bottom) are shown.





F. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile 2 (m/z 169) (B) and hapalindole U 5 (m/z 305) (C) for the *in vitro* TT-assay performed in the presence of FamD2 and FamC1 plus FamC4 plasmids in the presence of lower 5 mM calcium concentration. Note that lower concentration of calcium did not show distinct product. **Panel 2:** TIC (A) and EIC of *cis*-indole isonitrile 2 (m/z 169) (B), and hapalindole U 5 and 12-*epi*-hapalindole U 4 (m/z 305) (C) compared with the standard 5 (D) and 4 (E) for the *in vitro* TT-assay performed in the presence of FamD2 and FamC2 plus FamC3 plasmids in the presence of higher 20 mM calcium concentration. **Panel 3:** MS data of the product (top) and the standard 5 (bottom) are shown.









G. **Panel 1:** TIC (A) and EIC of *cis*-indole isonitrile **2** $(m/z \ 169)$ (B) and 12-*epi*-fischerindole U **7** $(m/z \ 305)$ (C) compared with the standard **7** (D) for the *in vitro* TT-assay performed in the presence of FamD2 and FisC plasmids (for Figure 4C). **Panel 2:** MS data of the product (top) and the standard **7** (bottom) are shown.







Supplemental Figure S2: Total ion chromatogram (TIC), extracted ion chromatogram (EIC) and comparative MS data of Figure 6 (A-C).

TIC (A) and EIC (m/z 187.02) of fluorinated *cis*-indole isonitrile substrate **9** (Panel 1 and 3) and **10** (Panel 2 and 4), and the fluorinated hapalindole products **15**, **16** (Panel 1-2) and fischerindole products **17**, **18** (Panel 3-4) were compared with the standard **15**, **16**, **17** and **18** for the *in vitro* TT-assay performed in the presence FamD2 with FamC1 (Panel 1-2) or FisC (Panel 3-4) plasmids (for Figure 6). The '*' represents 5F-/6F-12-*epi*-hapalindole-GPP (Panel 1-2) and 5F-/6F-12-*epi*-fisherindiole-GPP (Panel 3-4). Panel 1, 2, 3 and 4 represents the Figure 6 A, 6B, 6C and 6D.







Figure S3: Spectra of 5-fluoro-12-epi-hapalindole U (15)

¹H, ¹³C, COSY, HSQC, HMBC NMR spectra and HRMS of 5-F-12-*epi*-Hapalindole U **15** in C_6D_6 at 600 MHZ and 125 MHz respectively.









x10 ⁴	+ Scan (rt: 7.20-7.42 min, 28 scans) 5_Fluoro_3_25_19.d	1
2.5-	101,0714	
2-		
1.5-	121.0509 340.2600	
1-	323.1922	
0.5-	136.1119 166.0984 194.1176 227.1754 300.1764 371.1013 411.0937 475.3252 475.3252	
0	90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490 Counts vs. Mass-to-Charge (m/z)	

Calc. [M+H]⁺ 323.1918 Obsv. 323.1922

Figure S4: Spectra of 6-fluoro-12-*epi*-hapalindole U (16)

¹H, ¹³C, COSY, HSQC, HMBC NMR spectra and HRMS of 6-F-12-*epi*-Hapalindole U **16** in C₆D₆ at 600 MHz for ¹H and COSY, 800 MHz for HSQC and HMBC, and 201 MHz respectively.









Calc. [M+H]⁺ 323.1918 Obsv. 323.1920



Figure S5: Spectra of 5-fluoro-12-*epi*-fischerindole U (17)

¹H, ¹³C, COSY, HSQC, HMBC NMR spectra and HRMS of 5-F-12-*epi*-fischerindole U **17** in C₆D₆ at 800 MHZ and 201 MHz respectively.







Calc. [M+H]⁺ 323.1918 Obsv. 323.1934



Figure S6: Spectra of 6-fluoro-12-*epi*-fischerindole U (18)

¹H, ¹³C, COSY, HSQC, HMBC NMR spectra and HRMS of 6-F-12-*epi*-Fischerindole U **18** in C_6D_6 at 600 MHZ and 125 MHz respectively.







Calc. [M+H]⁺ 323.1918 Obsv. 323.1925