**Supplemental Table**

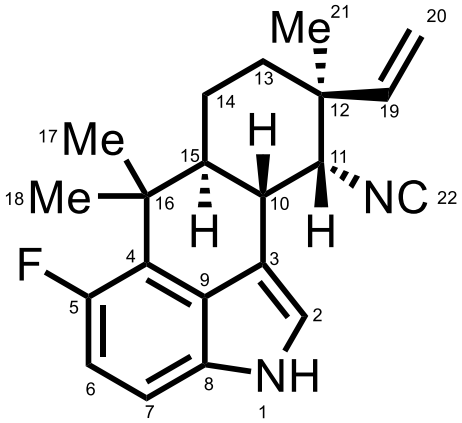
Table S1: Assigned numeral representation for the synthetic substrates, prenyltransferase and the Stig cyclases involved in the biosynthesis of natural and unnatural hapalindole/fischerindole are shown.

| Synthesis/ Designated Protein | Substrate | Product | Assigned Number |
|---|---|---|---|
| **Natural hapalindole/fischerindole system** | | | |
| Synthesized | geranyl pyrophosphate (GPP) | | **1** |
| Synthesized | *cis*-indole isonitrile | | **2** |
| **Enzymes** | | | |
| Prenyltransferase (FamD2) | **1** and **2** | *cis*-indole isonitrile-GPP (3GC) | **3** |
| **Sig cyclases** | | | |
| FamC1 | **3** | 12-*epi*-hapalindole U | **4** |
| FamC1 and FamC4 | **3** | Hapalindole U | **5** |
| FamC3 and FamC4 | **3** | Hapalindole H | **6** |
| FisC | **3** | 12-*epi*-fischerindole U | **7** |
| **Unnatural hapalindole/fischerindole system** | | | |
| Synthesized | 5-fluoro-*cis*-indole isonitrile | | **9** |
| Synthesized | 6-fluoro-*cis*-indole isonitrile | | **10** |
| **Enzymes** | | | |
| FamC1 | **1** and **9** | 5-fluoro-*12-epi*-hapalindole U | **15** |
| FamC1 | **1** and **10** | 6-fluoro-*12-epi*-hapalindole U | **16** |
| FisC | **1** and **9** | 5-fluoro-*12-epi*-fischerindole U | **17** |
| FisC | **1** and **10** | 6-fluoro-*12-epi*-fischerindole U | **18** |

Table S2: Proteomics analysis of FamD2 expression in the TT-assay containing exogenous plasmids FamD2, and the substrates 1 and 2 during *cis*-indole isonitrile-GPP intermediate 3 synthesis. The sequence highlighted in yellow showed the coverage of the synthesized protein. Note: Please see attached Excel Sheet 1

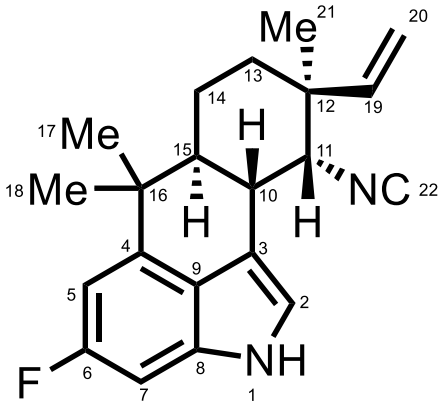
| FamD2 | | |
|---|---|---|
| Total peptide | 25 | |
| Unique peptide | 25 | |
| Total PSM | 906 | |
| Coverage (%) | 72.49 | |
| Annotated Peptide Sequence | Sequence Positions | PSM FamD2 |
| [-].MNDVNR.[I] | FamD2[1-6] | 1 |
| [R].IRTDIINVAK.[T] | FamD2 [7-16] | 30 |
| [R].TDIINVAK.[T] | FamD2 [9-16] | 11 |
| [K].TFGAEYSEK.[V] | FamD2 [17-25] | 14 |
| [K].VLDEVFQVFGEQFADNSFMIR.[T] | FamD2 [26-46] | 155 |
| [R].TSNKQPDK.[L] | FamD2 [47-54] | 10 |
| [R].TSNKQPDKLGCYFR.[Y] | FamD2 [47-60] | 2 |
| [K].QPDKLGCYFR.[Y] | FamD2 [51-60] | 7 |
| [K].LGCYFR.[Y] | FamD2 [55-60] | 16 |
| [R].YHEEDESQLGLAWDIAR.[K] | FamD2 [61-77] | 335 |
| [R].KSGLLSDQGRPVDQLIPEICETFPIMADGVDFDVK.[H] | FamD2 [78-112] | 45 |
| [K].SGLLSDQGRPVDQLIPEICETFPIMADGVDFDVK.[H] | FamD2 [79-112] | 28 |
| [K].IWQSIK.[G] | FamD2 [118-123] | 12 |
| [K].GVVPVQDAFK.[L] | FamD2 [124-133] | 22 |
| [K].LSLPASVTTHSDFLK.[N] | FamD2 [134-148] | 42 |
| [K].HHTSEYYK.[N] | FamD2 [178-185] | 1 |
| [R].LCFYLPFLNR.[E] | FamD2 [224-233] | 5 |
| [R].EAVPQNLLNPLLK.[K] | FamD2 [234-246] | 42 |
| [R].EAVPQNLLNPLLKK.[Y] | FamD2 [234-247] | 14 |
| [K].KYINEAPALVDNPGFILGWSFGPQGGK.[G] | FamD2 [247-273] | 10 |
| [K].YINEAPALVDNPGFILGWSFGPQGGK.[G] | FamD2 [248-273] | 16 |
| [K].VDVDYHGR.[T] | FamD2 [279-286] | 3 |
| [R].TVPLFMK.[V] | FamD2 [287-293] | 64 |
| [R].TVPLFMKVHSQPLPK.[A] | FamD2 [287-301] | 1 |
| [K].VHSQPLPK.[A] | FamD2 [294-301] | 6 |
| FamD2<br>MNDVNRIRTDIINVAKTFGAEYSEKVLDEVFQVFGEQFADNSFMIRTSNKQPDKLGCYFRYHEEDESQLGLAWDIARKSGLLSDQGRPVDQLIPEICETFPIMADGVDFDVKHGLAKIWQSIKGVVPVQDAFKLSLPASVTTHSDFLKNHHLDALYAFGIDYHHSSVNLYFDTYHPKHHTSEYYKNLLQDLQFQPPSDELLELLTNNGEIALTFNFASPRIERLCFYLPFLNREAVPQNLLNPLLKKYINEAPALVDNPGFILGWSFGPQGGKGTYTKVDVDYHGRTVPLFMKVHSQPLPKAADFALAQ, 309 residues | | |

**Table S3: NMR characterization of 5-fluoro-12-*epi*-hapalindole U (15)**
$[\alpha]_D^{25}$=+7.7 (c=0.07, $CH_2Cl_2$)



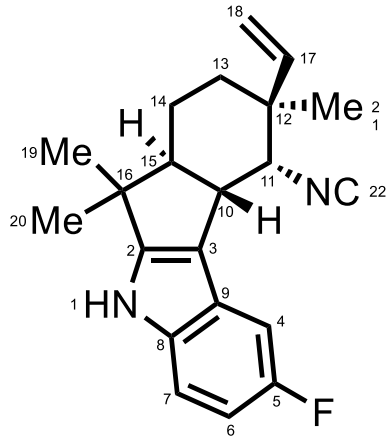

| Position | $^{13}$C shift (ppm) (Fluorine coupling) $C_6D_6$ | $^{1}$H shift (ppm), multi (J) $C_6D_6$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | | 6.55, bs | 2 | |
| 2 | 118.03 | 6.35, s | 1 | 3, 4, 8, 9 |
| 3 | 113.75 | | | |
| 4 | 124.13, d, (13.1) | | | |
| 5 | 154.29, d, (236.7) | | | |
| 6 | 112.75, d, (29.2) | 7.01, dd, (12.9, 4.4) | 6 | 4,5,8 |
| 7 | 109.71, d, (10.1) | 6.69, dd, (3.0, 8.7) | 7 | 4,5,9 |
| 8 | 130.96 | | | |
| 9 | 127.08, d, (8.6) | | | |
| 10 | 34.58 | 2.90, d, (11.5) | 15 | |
| 11 | 63.35 | 3.90, s | 10 | 10,12,13,15 |
| 12 | 39.73 | | | |
| 13 | 31.27 | 1.66, d, (3.7) & 1.44, m | 14 | 12,14,15,21 |
| 14 | 21.17 | 1.48, d, (3.7) & 1.27, m | 13,15 | 13, 15,21 |
| 15 | 43.98 | 1.98, td, (12.0, 3.4) | 10,14 | 10,11,13,14,16,17,18 |
| 16 | 37.27 | | | |
| 17 | 23.07 | 1.11, s | 18 | 9, 15, 16, 18 |
| 18 | 25.05 | 1.63, s | 17 | 9, 15, 16, 17 |
| 19 | 142.17 | 5.39, dd, (17.7, 11.0) | 20 | 11,12,13,21 |
| 20 | 114.47 | (cis) 4.91, d, (11.0) (trans), 4.84, d, (17.7) | 19 | 12, 19,21 |
| 21 | 28.35 | 1.18, s | | 11, 12, 13, 19 |
| 22 | 160.84 | | | |

**Table S4: NMR characterization of 6-fluoro-12-*epi*-hapalindole U (16)**
$[\alpha]_D^{25}$=+12.9 (c=0.04, $CH_2Cl_2$)



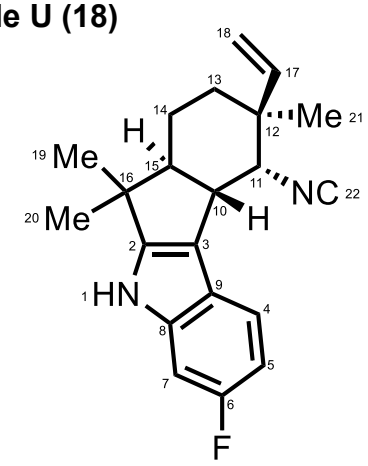

| Position | $^{13}C$ shift (ppm) (Fluorine coupling) $C_6D_6$ | $^{1}H$ shift (ppm), multi (J) $C_6D_6$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | | 6.51, bs | | |
| 2 | 116.62, d, (3.2) | 6.29, s | | 3, 8, 9 |
| 3 | 113.12 | | | |
| 4 | 142.37, d, (8.7) | | | |
| 5 | 102.96, d, (25.9) | 6.93, dd, (11.0, 1.8) | | 6,7,9 |
| 6 | 162.10, d, (236.5) | | | |
| 7 | 95.36, d, (27.4) | 6.69, d, (9.8) | | 5,6,9 |
| 8 | 134.02 | | | |
| 9 | 122.36 | | | |
| 10 | 34.88 | 2.95, d, (11.5) | 15 | 3,15 |
| 11 | 63.45 | 3.88, s | 10 | 10,12,13,15 |
| 12 | 39.81 | | | |
| 13 | 31.08 | 1.66, m & 1.43, m | 14 | 11,15 |
| 14 | 21.66 | 1.41, d, (3.7) & 1.26, m | 13,15 | 19 |
| 15 | 43.57 | 1.93, td, (11.9, 3.5) | 10,14 | |
| 16 | 37.44 | | | |
| 17 | 24.26 | 1.14, s | 18 | 4, 15, 16, 18 |
| 18 | 24.96 | 0.89, s | 17 | 4, 15, 16, 17, 19 |
| 19 | 142.55 | 5.40, dd, (17.7, 11.0) | 20 | 11, 12, 13 |
| 20 | 114.49 | (cis) 4.92, d, (11.0) (trans), 4.85, d, (17.7) | 19 | 12,19 |
| 21 | 28.27 | 1.17, s | | 11, 12, 13, 19 |
| 22 | 160.88 | | | |

**Table S5: NMR characterization of 5-fluoro-12-*epi*-fischerindole U (17)**
$[\alpha]_D^{25}$=+12.0 (c=0.15, $CH_2Cl_2$)




| Position | $^{13}C$ shift (ppm) (Fluorine coupling) $C_6D_6$ | $^{1}H$ shift (ppm), multi (J) $C_6D_6$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | | 6.43, bs | | 2,3,8,9 |
| 2 | 153.90 | | | |
| 3 | 115.16 | | | |
| 4 | 103.93, d, (23.1) | 7.27, dd (9.4,2.5) | 6 | 3,5,6,8 |
| 5 | 159.42, d, (233.5) | | | |
| 6 | 109.07, d, (26.3) | 6.99, td, (9.0, 2.6) | 7 | 4,5,8 |
| 7 | 112.55, d, (10.1) | 6.81, dd, (8.8, 4.4) | 6 | 4,5,9 |
| 8 | 136.53 | | | |
| 9 | 124.63, d, (10.1) | | | |
| 10 | 42.37 | 2.88, m | 11,15 | 3,9,14,15 |
| 11 | 62.21 | 3.95, d (3.0) | 11 | 3,12,13,15,17,22 |
| 12 | 41.04 | | | |
| 13 | 31.82 | 1.55, m | 14 | 11,12,14,15,17 |
| 14 | 20.81 | 1.34, ddd (13.1, 6.8, 4.0) & 1.23, m | 13,15 | 12,13,15 |
| 15 | 55.21 | 2.36, ddd (13.3, 10.6, 3.3) | 10, 14 | 10,14,16,19 |
| 16 | 40.18 | | | |
| 17 | 143.02 | 5.31, dd (17.6, 11.0) | 18 | 11,13,12,21 |
| 18 | 114.16 | (cis) 4.87, d (11.1) (trans) 4.84, d (17.7) | 17 | 12,17,21 |
| 19 | 24.93 | 1.01, s | | 2,15,16,20 |
| 20 | 20.78 | 0.74, s | | 2,15,16,19 |
| 21 | 28.15 | 1.13, s | | 11,1213,17 |
| 22 | 161.39 | | | |

**Table S6: NMR characterization 6-Fluoro-12-*epi*-Fischerindole U (18)**
$[\alpha]_D^{25}$=+10.8 (c=0.16, $CH_2Cl_2$)




| Position | $^{13}C$ shift (ppm), (Fluorine coupling) $C_6D_6$ | $^{1}H$ shift (ppm), multi (J) $C_6D_6$ | COSY | HMBC |
|---|---|---|---|---|
| 1 | | 6.38, bs | | |
| 2 | 152.16, d, (3.5) | | | |
| 3 | 114.88 | | | |
| 4 | 119.18, d, (10.1) | 7.20, dd, (8.6, 5.3) | 5 | 3,8,9 |
| 5 | 108.67, d, (24.2) | 6.99, td, (9.1, 2.4) | 4 | 6,7,9 |
| 6 | 159.78, d, (235.6) | | | |
| 7 | 98.80, d, (25.9) | 6.85, dd, (9.8, 2.3) | | 5,6,8,9 |
| 8 | 139.95, d, (12.0) | | | |
| 9 | 121.08 | | | |
| 10 | 42.51 | 2.89, m | 11, 15 | 15 |
| 11 | 62.44 | 4.03, s | 10 | 10,12,13,15,22 |
| 12 | 41.03 | | | |
| 13 | 31.87 | 1.56, m | 14 | 11,12,14,15,21 |
| 14 | 20.82 | 1.35, m & 1.24, m | 13 | 10,13,15 |
| 15 | 55.21 | 2.36, ddd, (13.4, 10.6, 3.2) | 10, 14 | 14,19 |
| 16 | 40.25 | | | |
| 17 | 143.08 | 5.34, dd, (17.5, 11.0) | 18 | 11,12,13 |
| 18 | 114.16 | Cis 4.89, d, (11.0) Trans 4.87, d, (17.5) | 17 | 12,17 |
| 19 | 25.07 | 1.02, s | 20 | 2,15,16,20 |
| 20 | 20.70 | 0.74, s | 19 | 2,15,16,19 |
| 21 | 28.17 | 1.15, s | | 11,12,13,17 |
| 22 | 161.26 | | | |

**Table S7: Amino acid sequences of cyclases**
**Protein Sequences**

**FamC1**
MKRKLIVAVVFLIFICLGINTPAHATSAVSIPINNAGFENPFMDVVDDYTIDTPPGWTTYDPNNLVPEKRTT
WTSNNGVGYVGPGTQFYNQLAPEGRNIGYIYLSQNPGSGVAGFEQILDATLEPDTKYTLTVDVGNLAGTF
KGLSFAGFPGYRVELLAGDTVLAADHNNLFIKEGEFKTSTVTYTSTAKDLHLGQKLGIRLVNLLQDKFSGL
DFDNVRLTTEPTET

**FamC2**
MKRNLIVAAIVLLIYICSGINTPANAAVTTSIPIKNPGFEEPILKVEGDYTIDAPPGWTTYNPNGLIPEKRTKW
TSNNGVGHVGPNYGQLFYNQQLPEGKNIGFVYLAQKTGSGIAGFEQTLDAVLEPNTSYKLIVDIGNFGGMF
KGVSFAGFPGYRVELLAGDTVLAADHNNLYIKDGEFKTSTVTFTSAANNPYLGQKLGIRLINLLQGKFSGL
DFDNVRLITETVDT

**FamC3**
MKLKSIVAVVFLIFICLGINTPANATGAVSIPIKNAGFEDPFLEVKDYYTVNTPPGWSTYDPNGLIPEQPTVQ
TSYVGVTNATPSSAFYDQKVPEGRNMGSVYLAHEPGSGIAGLEQTLDTVLESNKNYTLLVDIGNSADGYK
DISLADFPGYRVELLAGDKVIAVDHNSVYIKEGEFKTSMIKFTAKPDSPYLGQKLGIRLINSLQTLSGNIDFD
NVRLSVESAVI

**FisC**
MKRNFIIAAIVLLVYICFGISISANAASAVSIPIKNAGFEEPSLTVEDYYTIDTPPGWITYDPNGLVPAKRTRIT
SNNGVGYTGPNSAYYNHKAPEGRNVAYVYLAQEIGSGIAGLEQTLDAVLKPNTKYTLTVDIGNSGGSFQG
FPLDGFPGYRVELLAGDTVLAADQNNLYIKEKDFKTTTVTFIATPESPYLGQHLGIRLINPLQGKFSGVDFD
NVRLTAEPAET

**FimC5**
MKRNFIIAAIVLLVYIFSGINVFANAASAVCIPIKNAGFEEPILQIEDDYTIDTPPGWITYDPGGLVPAKRTRIT
SNNGVGYTGSNSEFYNHKAPEGRNVAFVYLAQEIGSGIAGLEQTLDAVLKPNTKYTLTVDIGNSGGSFQGK
TLDGFPGYRIELLAGDTVLAADHNTLYIKEKDFKSTTVTFTATPESPYLGQHLGIRLINPLQGKFSGVDFDN
VRLTAEPAET