Figure S1. Alignment of amino sequences of *C. albicans* Eno1 with *S. cerevisiae* Eno1 and human Eno1. The identity of amino acid sequences of *C. albicans* Eno1 vs. *S. cerevisiae* Eno1 and *C. albicans* Eno1 vs. human Eno1 were 76.66% and 61.98%, respectively.

Figure S2. Comparative analysis of the structure of *C. albicans* Eno1 (blue) with *S. cerevisiae* Eno1 (purple) (A) and human Eno1 (green) (B).
Figure S3. The multiple alignment analysis between *C. albicans* Enol1 and Enol1 with known PLG-binding sites (marked by a blue dotted line) from different kinds of micro-organisms. Ca *C. albicans*; Tm *Taenia multiceps*; Ts *Taenia solium*; Tp *Taenia pisiformis*; Lm *Leishmania mexicana*; Sp *Streptococcus pneumoniae*; Mt *Mycobacterium tuberculosis*. 
| No. | Protein Name | Score (>700) | Coverage | Unique Peptides (>10) | Peptides | Peptides PSMs | Area | AAs | MW [kDa] | calc. pI |
|-----|--------------|--------------|----------|-----------------------|----------|---------------|------|-----|---------|-------|
| 1   | Acc1         | 11917.41     | 62.57    | 134                   | 134      | 356           | 2.004E9 | 2271 | 253.3   | 6.40  |
| 2   | Pyc2         | 9655.40      | 70.43    | 83                    | 83       | 300           | 2.469E9 | 1177 | 129.7   | 6.38  |
| 3   | Pdc11        | 2772.71      | 58.55    | 28                    | 28       | 89            | 9.656E8 | 567  | 62.4    | 5.58  |
| 4   | Adh1         | 2448.85      | 71.63    | 23                    | 26       | 77            | 1.303E9 | 349  | 36.8    | 6.05  |
| 5   | Tdh3         | 2225.91      | 80.00    | 26                    | 26       | 61            | 1.289E9 | 335  | 35.8    | 7.12  |
| 6   | Tef1         | 2047.35      | 52.62    | 27                    | 27       | 72            | 1.610E9 | 458  | 36.8    | 6.05  |
| 7   | Cdc19        | 1635.60      | 53.77    | 25                    | 25       | 44            | 2.747E8 | 504  | 55.4    | 6.99  |
| 8   | Pgi1         | 1457.30      | 64.99    | 29                    | 29       | 45            | 2.834E8 | 417  | 45.2    | 6.48  |
| 9   | Hsp70        | 1358.66      | 49.09    | 11                    | 26       | 35            | 1.370E8 | 656  | 70.3    | 5.17  |
| 10  | Enol1        | 1340.09      | 55.68    | 24                    | 24       | 31            | 1.630E8 | 440  | 47.2    | 5.81  |
| 11  | Cef3         | 1338.31      | 38.76    | 31                    | 32       | 42            | 8.969E7 | 1050 | 116.9   | 5.69  |
| 12  | Eft2         | 1272.14      | 43.94    | 34                    | 34       | 43            | 2.736E8 | 842  | 93.3    | 6.47  |
| 13  | Ssb1         | 1136.15      | 40.46    | 20                    | 20       | 25            | 1.511E8 | 613  | 66.4    | 5.38  |
| 14  | Gnd1         | 1044.79      | 50.30    | 22                    | 22       | 28            | 1.174E8 | 495  | 54.3    | 6.09  |
| 15  | Gad1         | 1014.33      | 42.25    | 17                    | 17       | 23            | 4.677E7 | 568  | 63.9    | 6.55  |
| 16  | Fba1         | 932.76       | 54.60    | 18                    | 18       | 25            | 2.427E8 | 359  | 39.2    | 6.06  |
| 17  | Pma1         | 923.09       | 27.15    | 19                    | 19       | 25            | 9.121E7 | 895  | 97.6    | 4.98  |
| 18  | Ald5         | 914.26       | 42.89    | 15                    | 15       | 25            | 9.248E7 | 499  | 53.9    | 5.80  |
| 19  | Rpl4B        | 902.12       | 46.83    | 13                    | 13       | 21            | 2.579E8 | 363  | 39.2    | 10.74 |
| 20  | Atpl         | 872.31       | 37.18    | 19                    | 19       | 23            | 7.710E7 | 546  | 58.9    | 9.04  |
| 21  | Tal1         | 857.20       | 55.11    | 16                    | 16       | 20            | 4.658E7 | 323  | 35.7    | 5.00  |
| 22  | Pfk1         | 791.39       | 27.36    | 21                    | 23       | 28            | 3.724E7 | 987  | 108.5   | 6.62  |
| 23  | Mdh1-1       | 784.22       | 59.34    | 12                    | 12       | 16            | 4.501E7 | 332  | 34.7    | 6.10  |
| 24  | Xyl2         | 747.14       | 46.11    | 13                    | 13       | 18            | 3.800E7 | 360  | 38.7    | 5.97  |
| 25  | Gph1         | 746.44       | 29.56    | 21                    | 21       | 21            | 5.112E7 | 900  | 102.8   | 6.02  |
| 26  | Fas1         | 746.39       | 13.45    | 22                    | 22       | 23            | 1.893E7 | 2037 | 227.7   | 6.05  |
| 27  | Atp2         | 746.10       | 43.65    | 13                    | 13       | 17            | 5.699E7 | 504  | 53.9    | 5.19  |
| 28  | Hsp90        | 716.07       | 30.27    | 18                    | 18       | 20            | 2.850E7 | 707  | 80.8    | 4.88  |
| 29  | Pck1         | 714.67       | 39.60    | 17                    | 17       | 19            | 6.744E7 | 553  | 60.9    | 6.48  |
Table S1B. Potential targets of BE obtained by a competition experiment of BE and biotin-N3 labeled label-BE

| No. | Protein Name | Score (>700) | Coverage | Unique Peptides (>10) | Peptides | PSMs | Area | AAs | MW [kDa] | calc. pI |
|-----|--------------|--------------|----------|-----------------------|----------|------|------|-----|---------|---------|
| 1   | Adh1         | 9185.48      | 93.70    | 40                    | 46       | 333  | 1.759E9 | 349 | 36.8    | 6.05    |
| 2   | Pgk1         | 3507.30      | 93.53    | 52                    | 53       | 111  | 2.451E8 | 417 | 45.2    | 6.48    |
| 3   | Pyc2         | 1648.51      | 46.13    | 44                    | 44       | 53   | 1.685E7 | 1177 | 129.7   | 6.38    |
| 4   | Pda1         | 1422.99      | 66.58    | 27                    | 27       | 44   | 1.466E7 | 401 | 44.1    | 8.13    |
| 5   | Tuf1         | 1228.34      | 80.52    | 25                    | 25       | 43   | 3.428E7 | 426 | 46.7    | 5.97    |
| 6   | Erg10        | 1209.81      | 68.16    | 26                    | 26       | 32   | 1.873E7 | 402 | 41.9    | 6.90    |
| 7   | Acc1         | 1182.52      | 21.71    | 38                    | 38       | 41   | 3.081E6 | 2271 | 253.3   | 6.40    |
| 8   | Pdc11        | 1128.91      | 55.56    | 24                    | 24       | 34   | 4.586E7 | 567 | 62.4    | 5.58    |
| 9   | Gcv1         | 1054.49      | 64.72    | 20                    | 20       | 32   | 4.142E7 | 394 | 43.7    | 7.61    |
| 10  | Act1         | 969.20       | 67.82    | 22                    | 22       | 32   | 2.520E7 | 376 | 41.7    | 5.69    |
| 11  | Sam2         | 964.53       | 62.60    | 23                    | 23       | 33   | 5.856E7 | 385 | 42.2    | 5.97    |
| 12  | Gnd1         | 951.14       | 54.14    | 21                    | 21       | 30   | 8.268E6 | 495 | 54.3    | 6.09    |
| 13  | Eno1         | 918.49       | 46.82    | 20                    | 20       | 23   | 5.501E6 | 440 | 47.2    | 5.81    |
| 14  | Idp1         | 857.95       | 51.73    | 21                    | 21       | 27   | 5.010E6 | 433 | 48.5    | 6.96    |
| 15  | Tef1         | 771.26       | 58.30    | 23                    | 23       | 28   | 2.103E7 | 458 | 50.0    | 9.03    |
| 16  | Gln1         | 711.93       | 57.91    | 19                    | 19       | 25   | 2.682E7 | 373 | 41.7    | 6.07    |

Table S1C. LC-MS/MS analysis showed that Eno1 was the dominant protein in brightness changed band

| No. | Protein Name | Score | Coverage | Unique Peptides | Peptides | PSMs | Area | AAs | MW [kDa] | calc. pI |
|-----|--------------|-------|----------|-----------------|----------|------|------|-----|---------|---------|
| 1   | Eno1         | 27983.96 | 80.00    | 41              | 41       | 695  | 6.362E10 | 440 | 47.2    | 5.81    |
| 2   | Fdh3         | 57.30  | 3.14     | 1               | 1        | 1    | 1.549E7  | 255 | 27.4    | 5.69    |
| 3   | Zcf22        | 40.44  | 1.34     | 1               | 1        | 1    | 9.970E8  | 448 | 50.6    | 5.96    |
| 4   | Utp20        | 39.55  | 1.25     | 1               | 1        | 2    | 1.388E8  | 1197 | 136.6   | 5.66    |
| 5   | Tuf1         | 38.46  | 3.52     | 1               | 1        | 1    | 1.033E8  | 426 | 46.7    | 5.97    |
| 6   | Jem1         | 37.08  | 1.42     | 1               | 1        | 1    | 4.165E7  | 636 | 74.1    | 8.75    |
|   |    |   |   |   |   |    |   |   |   |
|---|---|---|---|---|---|----|---|---|---|
| 7 | tO19.110 | 30.17 | 2.19 | 1 | 1 | 1 | 9.736E7 | 547 | 62.9 | 5.06 |
| 8 | Rad54   | 29.95 | 1.06 | 1 | 1 | 1 | 1.963E9 | 848 | 95.6 | 8.95 |
| 9 | Oct1    | 29.88 | 0.89 | 1 | 1 | 1 | 1.427E7 | 783 | 89.3 | 6.44 |
| 10| Atp1    | 29.77 | 1.90 | 1 | 1 | 1 | 5.950E6 | 368 | 39.7 | 9.42 |
| No. | Strain   | genotype                          | Source or reference     |
|-----|----------|-----------------------------------|-------------------------|
| 1   | SC5314   | Wild type                         | Eukaryot Cell. 2005 Feb;4(2):298-309. |
| 2   | SN152    | *his1* /*his1* *arg4* /*arg4* *leu2* /*leu2* | Eukaryot Cell. 2005 Feb;4(2):298-309. |
| 3   | ENO1/eno1 | *ENO1* /*eno1* ::*HIS1* *arg4* /*arg4* *leu2* /*leu2* | This study               |
| 4   | eno1/eno1 | eno1 ::*HIS1* /eno1 ::*ARG4* *leu2* /*leu2* | This study               |
| 5   | BL21(DE3) | F- ompT hsdSB(rB- mB-) gal dcm (DE3) | TIANGEN, China           |

**C. albicans**

**E. Coli**
Table S2B. Primers used in this study

| No. | Primer name | Primer sequence (5' to 3') |
|-----|-------------|-----------------------------|
| 1   | ENO1up      | GGCCATATGATGTCTTACGCCACTAAAATC |
| 2   | ENO1down    | CCGCTCGAGCAATTGAGAAGCCTTTTTG |
| 3   | ENO1 P1     | CGGGAAGTTGTGTTCCATTC         |
| 4   | ENO1 P3     | eacggegegeccagacgTGTTGATATTTCGATTTTT |
| 5   | ENO1 P4     | gtcagegegcgeccctgcGTGTTGCCTCTGATTAAATTA  |
| 6   | ENO1 P6     | TCAGATTAGCCCCCATTTGGC        |
| 7   | Universal p | ccgctgctaggcgcgccgtgACCAGTGTGATGGATATCTGC |
| 8   | Universal p | gcagggatgcggccgctgacAGCTCGGATCCACTAGTACG |
| 9   | ENO1 Uche   | GTGATCCACACTAATTATA          |
| 10  | ENO1 Dche   | ATTAGATACGTTGGTGGTTC         |
| 11  | HIS1 Left   | AACACACTGCAAAATCTGG          |
| 12  | HIS1 Right  | AACACACTGCAAAATCTGG          |
| 13  | ARG4 Left   | ACACAGAGATACCTTGTACT         |
| 14  | ARG4 Right  | ACGGAGTACCACATACGATG         |
Table S3. Interaction of fluconazole (FLC) and BE probes against isolates of *Candida albicans* by MIC (µg/mL) of checkerboard microdilution assay.

| Compounds | Molecular structure | Individual action MIC (µg/mL) | Interaction MIC(µg/mL) | FICI | Mode of interaction |
|------------|---------------------|-------------------------------|------------------------|------|--------------------|
| BE         | ![Molecular structure](image) | 32 | 1 | 1 | 0.039 | Synergism |
| BE-1       | ![Molecular structure](image) | >64 | >64 | 1 | 1.01 | Indifference |
| BE-2       | ![Molecular structure](image) | >64 | 1 | 4 | 0.039 | Synergism |
| BE-3       | ![Molecular structure](image) | >64 | 1 | 4 | 0.039 | Synergism |
| BE-4       | ![Molecular structure](image) | >64 | >64 | 1 | 1.01 | Indifference |
| BE-5       | ![Molecular structure](image) | >64 | 0.25 | 8 | 0.0645 | Synergism |
| BE-6       | ![Molecular structure](image) | >64 | >64 | 1 | 1.01 | Indifference |
### Table S4. Structure and characterization of compounds were identified by NMR

| No. | Name     | Structural formula | Character      | ESI-MS   | NMR                                      |
|-----|----------|--------------------|----------------|----------|------------------------------------------|
| 1   | TPD-6a   | ![Structural formula](image1.png) | Yellow liquid  | 244.05   | $^1$H-NMR(300MHz,CDCl$_3$): δ 10.45(s,1H), 7.8(d,J=8.1Hz,1H), 6.85(d,J=8.1Hz,1H), 6.70(s,1H), 3.95(s,3H). $^1$H-NMR(300MHz,DMSO-d$_6$): δ 11.15(s,1H), 10.28(s,1H), 7.72(d,J=6.4Hz,1H), 6.87(s,1H, 6.71(d,J=6.4Hz,1H). |
| 2   | TPD-7a   | ![Structural formula](image2.png) | White solid    | 230.03   | $^1$H-NMR(300MHz,CDCl$_3$): δ 10.44(s,1H), 6.92(s,1H), 6.87(d,J=8.1Hz,1H), 4.85(d,J=2.1Hz,2H), 2.63(t,J=6.4Hz,1H). |
| 3   | TPD-8a   | ![Structural formula](image3.png) | Yellow liquid  | 268.05   | $^1$H-NMR(300MHz,CDCl$_3$): δ 7.39(d,J=7.8Hz,1H), 6.83(d,J=9.0Hz,2H), 4.71(s,J=2.4Hz,2H), 4.52(s,J=2.4Hz,2.5J=2.0Hz,1H). |
| 4   | TPD-9a   | ![Structural formula](image4.png) | Light yellow liquid | 270.06   | $^1$H-NMR(300MHz,CDCl$_3$): δ 7.39(d,J=8.1Hz,1H), 6.83(s,1H), 6.79(d,J=8.1Hz,1H), 4.80(d,J=2.4Hz,2H), 4.52(s,J=2.4Hz,2.5J=2.0Hz,1H). |
| 5   | TPD-10a  | ![Structural formula](image5.png) | White solid    | 331.98   | $^1$H-NMR(300MHz,CDCl$_3$): δ 7.87-7.85(m,2H), 7.55-7.50(m,4H), 6.66(s,1H), 2.45(s,3H), 2.35(s,3H), 2.34(s,3H). |
| 6   | BE'      | ![Structural formula](image6.png) | Grayish white solid | 396.08   | $^1$H-NMR(600MHz,CDCl$_3$): δ 7.2 Hz, 2H), 7.73(d,J=7.8Hz,1H), 7.62-7.50(m,3H), 7.04(d,J=7.8Hz,1H), 6.97(s,1H), 6.82(s,2H), 6.74(s,1H), 6.64(s,2H), 6.24(s,2H), 5.52(s,2H), 5.02(s,2H), 4.51(s,J=2.4Hz,2H), 4.45(s,J=2.4Hz,2.5J=2.0Hz,1H). |
| 7   | BE-1     | ![Structural formula](image7.png) | Yellow solid   | 521.12   | $^1$H-NMR(600MHz,DMSO-d$_6$): δ 12.58(s,1H), 8.83(s,1H), 8.11(d,J=8.4Hz,2H), 7.69(d,J=8.4Hz,1H), 7.60(m,3H), 7.04(d,J=5.4Hz,2H), 7.01(s,1H), 6.98(s,1H), 5.26(s,2H), 5.01(d,J=2.4Hz,2H). |
| 8   | label-BE | ![Structural formula](image8.png) | Yellow solid   | 522.1    |  $^1$H-NMR(600MHz,DMSO-d$_6$): δ 12.58(s,1H), 8.83(s,1H), 8.11(d,J=8.4Hz,2H), 7.69(d,J=8.4Hz,1H), 7.60(m,3H), 7.04(d,J=5.4Hz,2H), 7.01(s,1H), 6.98(s,1H), 5.26(s,2H), 5.01(d,J=2.4Hz,2H). |
9  B  Purple powder  329.3  
\[
\begin{align*}
\delta: & \\
& 13.44 \text{ (s, 2H)}, 12.38 \text{ (s, 1H)}, \\
& 8.49 \text{ (d, 1H, } J = 1.7 \text{ Hz)}, 8.20 \\
& \text{(dd, 1H, } J = 7.9, 1.7 \text{ Hz)}, 7.52 \\
& \text{(d, 1H, } J = 7.9 \text{ Hz)}, 6.80 \text{ (d, 1H, } J = 9.2 \text{ Hz)}, 6.20 \text{ (dd, 1H,} \\
& \text{CD3OD-}d_6, 300 \text{ MHz)} \\
\end{align*}
\]

10  C  Purple powder  430.4  
\[
\begin{align*}
\delta: & \\
& 8.73 \text{ (s, 1H)}, 8.18 \text{ (d, 1H, } J = 7.5 \text{ Hz)}, 7.28 \text{ (d, 1H, } J = 7.5 \text{ Hz)}, 7.25 \text{ (d, 2H, } J = 9.3 \text{ Hz),} \\
& 6.98 \text{ (dd, 2H, } J = 9.3, 1.7 \text{ Hz)}, 6.83 \\
& \text{(DMSO-d}_6, 300 \text{ MHz)} \\
\end{align*}
\]

11  TAMRA-N3  Purple powder  512.5  
\[
\begin{align*}
\delta: & \\
& 8.85 \text{ (t, 1H, } J = 5.4 \text{ Hz)}, 8.45 \text{ (s, 1H)}, 8.20 \text{ (d, 1H, } J = 8.1 \text{ Hz),} \\
& 7.30 \text{ (d, 1H, } J = 7.8 \text{ Hz),} 6.51 \\
& \text{(CDCl}_3, 300 \text{ MHz)} \\
\end{align*}
\]

12  D  Colorless liquid  86.1  
\[
\begin{align*}
\delta: & \\
& 3.30 \text{ (CDCl}_3, 300 \text{ MHz)} \\
\end{align*}
\]

13  E  White solid  341.3  
\[
\begin{align*}
\delta: & \\
& 6.42 \text{ (s, 1H),} 6.36 \text{ (s, 1H),} 4.32-4.27 \\
& \text{(m, 1H),} 4.16-4.11 \text{ (m, 1H),} 3.12-3.06 \text{ (m, 1H),} 2.85-2.78 \\
& \text{(m, 5H),} 2.66 \text{ (t, 2H, } J = 7.3 \text{ Hz),} 2.57 \text{ (d, 1H, } J = 11.4 \text{ Hz),} \\
& 1.68-1.58 \text{ (m, 3H),} 1.54-1.35 \text{ (DMSO-d}_6, 300 \text{ MHz)} \\
\end{align*}
\]

14  biotin-N3  White solid  312.3  
\[
\begin{align*}
\delta: & \\
& 4.13-4.09 \text{ (m, 1H),} 3.31 \\
& \text{(DMSO-d}_6, 300 \text{ MHz)} \\
\end{align*}
\]
Text S1

**Synthesis of label-BE**

Weigh m-bromoanisole (20 g, 106 mM) into a 500 ml three-neck flask, add 300 ml of dry tetrahydrofuran (THF) for dissolution, protect with nitrogen, and slowly add n-hexane solution of n-butyl lithium (64 ml, 2.5 M in hexane, 1.5 eq) at -78 °C. After addition, stir for a reaction for 10 min, and then slowly drop F₃COOMe (18.2 g, 140 mM, 1.3 eq) at -78 °C. After reaction for 2 h, the reaction is complete by thin-layer chromatography (TLC). Dissolve 23.6 ml concentrated hydrochloric acid in 56.2 ml methanol, then add the solution to the reaction solution to quench the reaction. Raise the temperature to 0 °C, add 140 ml ethyl acetate (EA) to stir, and then use water (2 × 30 ml), dry with anhydrous sodium sulfate, spin dry the solvent to obtain the crude product of colorless liquid compound TPD-1 (17.5 g, yield 81%), which is directly used in the next reaction.

Weigh TPD-1 (17.5 g, 86 mM) into a 250 ml round bottom flask, add 62 ml of absolute ethanol to dissolve, then add hydroxylamine hydrochloride (7.7 g, 112 mM, 1.3 eq), 125 ml of pyridine, reflux at 85 °C for 10 h, detect the complete reaction by TLC. Spin the solvent dry under reduced pressure, add 100 ml EA, and then use water (3 × 100 ml) wash, 1 M HCl (2 × 100 ml) wash with water (1 × 100 ml), dried with anhydrous sodium sulfate, and spin dry the solvent to obtain a colorless oily compound TPD-2 (17.5 g, yield 93%).

Dissolve TPD-2 (17.5 g, 80 mM) in pyridine (200 ml), add p-toluenesulfonyl chloride (22.9 g, 120 mM, 1.5 eq) in batches, reflux at 125 °C for 2 h, cool to room temperature, spin dry the solvent under reduced pressure, add 200 ml water, and then EA (3 × 100 ml) extraction, 1 M HCl (2 × 100 ml) washing water (2 × 100 ml) was washed, dried with anhydrous sodium sulfate, spin dry the solvent, recrystallized (Petroleum ether (PE): EA = 3:1) and purified to obtain solid white TPD-3 (21.9 g, yield 73%).
Dissolve TPD-3 (21.9 g, 58.7 mM) in CH₂Cl₂ (200ml), pass about 50 ml of liquid ammonia at - 78 °C, raise the temperature to - 50 °C for a reaction for 10h, open it at room temperature overnight, make the excess ammonia absorb into water or discharge into the atmospheric environment, filter out impurities, and use water for filtrate (2 × 100 ml), dry with anhydrous sodium sulfate, spin dry the solvent to obtain the crude product of light yellow liquid compound TPD-4 (8.6 g, yield 67%).

Weigh TPD-4 (9.0 g, 41.3 mM) in a 100 ml round bottom flask, add methanol (50 ml) to dissolve it, then add triethylamine (8.4 g, 82.6 mM, 2 eq), slowly add I₂ (10.5 g, 41.3 mM) under ice bath conditions, react at room temperature for 3 h, TLC test the reaction is complete, add 20 ml of 10% citric acid solution, and then add sodium thiosulfate until the color of the reaction solution becomes lighter and EA (3 × 50 ml) extraction, water (3 × 50 ml), dry with anhydrous sodium sulfate, spin dry the solvent to obtain yellow liquid TPD-5 (8.0 g, yield 90%).

Place TPD-5 (8.0 g, 37 mM) in a 500 ml round bottom flask, add 200 ml CH₂Cl₂ to dissolve it, slowly add TiCl₄ (10 g, 55.5 mM, 1.5 eq) at 0 °C, then add Cl₂CHOMe (6.0 g, 55.5 mM, 1.5 eq), after adding, rise to room temperature for a reaction for 1 h, TLC test the reaction completely, add 250 ml water at 0 °C to quench the reaction, and then successively use water (2 × 100 ml) wash, saturated NaHCO₃ (2 × 100 ml) washing water (2 × 100 ml) washing, anhydrous sodium sulfate drying, spin dry the solvent, silica gel column chromatography (PE: EA = 100:1) to obtain yellow oily compounds TPD-6a (2.7 g, yield 30%).

Weigh TPD-6a (1.45 g, 6 mM) in a 50 ml round bottom flask, add 20 ml CH₂Cl₂ to dissolve, protect with nitrogen, and slowly drop BBr₃ solution (1.65 ml, 4 M in CH₂Cl₂) at - 20 °C. The drop was raised to 0 °C for 1 h, the reaction was complete by TLC detection, 20 ml water was added, and CH₂Cl₂ (3 × 20 ml), combine the organic layers, and use saturated sodium chloride (3 × 20 ml), dry with anhydrous sodium sulfate, spin dry the solvent to obtain solid white TPD-7a (1.1 g, yield 80%).
Weigh and dissolve TPD-7a (0.5 g, 2.17 mM) in 10 ml N,N-Dimethylformamide, successively add K$_2$CO$_3$ (0.6 g, 4.34 mM, 2 eq), bromopropargyne (0.258 g, 2.17 mmol, 1 eq), react at room temperature for 2 h, complete the reaction by TLC, add 10 ml water, and then EA (3 × 10 ml), and the organic layer is successively extracted with saturated sodium bicarbonate (2 × 10 ml) wash, saturated sodium chloride (2 × 10 ml), dried with anhydrous sodium sulfate, evaporated and dried the solvent to obtain yellow liquid TPD-8a (0.57 mg, yield 98%).

Weigh TPD-8a (0.26 g, 1 mmol) into a 25 ml round bottom flask, add 6 ml THF and 0.2 ml water, then add NaBH$_4$ (20 mg, 0.5 mmol, 0.5 eq) into the reaction solution, react at room temperature for 2 h, complete the reaction by TLC, add 6 ml water, and then EA (3 × 6 ml) and the organic layer was extracted with saturated sodium bicarbonate (2 × 6 ml) washing, saturated sodium chloride (2 × 6 ml), dried with anhydrous sodium sulfate, evaporated and dried the solvent to obtain light yellow liquid TPD-9a (0.23 mg, yield 85%).

Weigh TPD-9a (0.17 g, 0.63 mmol) in a 25 ml round bottom flask, add 2 ml CH$_2$Cl$_2$ for dissolution, protect with nitrogen, and drip PBr$_3$ (0.062 g, 0.23 mmol, 0.36 eq) at 0 °C, react at room temperature for 1 h, complete the reaction by TLC, add 2 ml water, CH$_2$Cl$_2$ (3 × 2 ml) extraction, saturated sodium bicarbonate (3 × 2 ml) wash, saturated sodium chloride (3 × 2 ml), dried with anhydrous sodium sulfate, evaporated and dried the solvent to obtain solid white TPD-10a (0.18 g, yield 88%).

Weigh BE (1.35 g, 5 mmol) in 25 ml round bottom flask, add acetic anhydride (10 ml), sodium acetate (41 mg, 0.5 mmol, 0.1 eq), 80 °C for 2 h, thin-layer chromatography (TLC) detection reaction is complete, pour the reaction liquid to 50 ml ice water, filter, wash the cake with a small amount of cold ethanol, and drain the filter residue to obtain the gray-white solid BE’ (1.63 g, yield 82%). Take 25 ml of dried acetone into 50 ml round bottom flask, add BE’ (277 mg, 0.7 mmol), TPD-10a (700 mg, 2.1 mmol, 3 eq), K$_2$CO$_3$ (386 mg, 2.8 mmol, 4 eq), KI (11.62 mg, 0.07 mmol, 0.1 eq), 60 °C, and react for 8 hours under nitrogen protection condition. After TLC detection, filter the hot
mixed solution and dry the filtrate. The crude product was washed with a small amount of cold EA and vacuum dried to obtain solid white BE-0 (310 mg, yield 73%). BE-0 (100 mg, 0.165 mmol) was dissolved with 2 ml methanol, 2 ml ammonia was added, heated, and reflux for 18 h, TLC detection reaction was complete, and the reaction was completely reduced to room temperature, 2 ml of water were added and EA (3 × 5ml) extraction, and washing by water (2 × 5 ml), drying the solvent, the crude product was obtained the yellow solid BE-1 (70 mg, yield 81%) was obtained by silica gel column chromatography (DCM: MeOH = 20:1). BE-0 (100 mg, 0.165 mmol) was dissolved with 5 ml ethanol, 0.3ml of concentrated hydrochloric acid was added, nitrogen was protected, and the reaction of TLC was complete for 10 hours. The yellow solid was obtained by vacuum concentration, filtered, washed with cold ethanol (5 ml), and the yellow solid label-BE (76 mg, yield 88%) was obtained by recrystallization of dichloromethane isopropyl ether system. The compounds were identified by NMR (Bruker DRX-600) (Table S6).

**Synthesis of TAMRA-N3**

Synthesis of TAMRA-N3 was performed as described previously. Weigh and dissolve sodium azide (1.95 g, 30 mmol, 3 eq) in H2O (8 ml), slowly add chloropropylamine hydrochloride (1.29 g, 10 mmol), add it, react at 80 °C for 10 h, and detect that the reaction is complete; Cool the reaction liquid to 0 °C, add solid KOH (3.28 g), add ether (8 ml), separate the organic layer, and use ether (3 × 10 ml) for the water layer extraction, combined organic layer, anhydrous MgSO4, drying, filtration, spin-drying, crude product A (980 mg) was obtained. Weigh 3 - (dimethylamino) phenol (1.37 g, 10 mmol) into a round bottom flask, add anhydrous toluene (30 ml) to dissolve it, raise the temperature to 60 °C, add trimellitic anhydride (2.30 g, 12 mmol, 1.2 eq), add it, reflux at 120 °C for 24 hours. Cool the reaction liquid to room temperature, filter, and use toluene (3× 5 ml) for the filter cake washing; Dissolve the filter cake in MeOH (30 ml), reflux for 10 min, add formic acid (10 ml) and then spin dry the solvent. The obtained solid is dissolved in MeOH (20 ml). After refluxing for 2 h, cool the solution and place
it at 4 °C overnight; After filtration, the filter cake was washed with MeOH (5 ml) to obtain compound B (2.5 g, 76%). Compound B (1.3 g, 3.9 5 mmol) and 3 - (dimethylamino) phenol (0.65 g, 4.74 mmol) were added to dry DMF (50 ml), polyphosphoric acid (2.6 g) was added, heated to 120 °C for 8 hours; Cool the reaction solution to room temperature, pour it into 200 ml of distilled water, stir it overnight; After filtration, the filter cake was washed with ice water to obtain grayish-brown solid compound C (1.55 g, 91.1%). Dissolve compound C (0.5 g, 1.16 mmol) in DMF (30 ml), add PyBoP (0.605 g, 1.16 mmol, 1 eq), DIEA (0.30 g, 2.32 mmol, 2 eq), stir for 5 min, dissolve compound A (0.118 g, 1.16 mmol, 1 eq) in DMF (5 ml), add it into the reaction solution, stir at room temperature for 2 h; EA (50 ml) in the reaction solution was diluted with 5% NaHCO₃ (1 × 10 ml) wash, saturated NaCl (1 × 10 ml), dried with anhydrous Na₂SO₄, filtered, and evaporated the filtrate through the column to obtain grayish-brown solid compound TAMRA-N3 (450 mg, 75.6%). TAMRA-N3 was identified by NMR (Bruker DRX-600) (Table S6).

**Synthesis of biotin-N3**

Biotin-N3 was synthesized by the following method. Weigh and dissolve sodium azide (1.904 g, 29.3 mmol, 3 eq) in H₂O (8 ml), slowly add bromoethylamine hydrobromate (2 g, 9.76 mmol), add it, raise the temperature of the reaction solution to 75 °C, stir for 21 h, and detect that the reaction is complete; Cool the reaction liquid to 0 °C, add solid KOH (3.2 g), add ether (8 ml), separate the organic layer, and use ether (3× 10 ml) for the water layer extraction, combined organic layer, anhydrous MgSO₄, drying, filtration, spin-drying, directly used in the next step. Crude product D (783 mg) was obtained. Weigh biotin (1.5 g, 6.14 mmol) into a round bottom flask, add DMF (50 ml) to dissolve, add n-hydroxysuccinamide (0.85 g, 7.37 mmol, 1.2 eq), EDC · HCl (1.41 g, 7.37 mmol, 1.2 eq), add it, stir at room temperature for 21 h, and the reaction is complete; Evaporate the solvent, add CH₂Cl₂ (200 ml), and use saturated NaHCO₃ (2 × 10 ml) for the organic phase wash, dried with anhydrous MgSO₄, filtered and spin-dried to obtain the white solid compound E (1.15 g, 55%). Weigh compound D (241 mg, 2.8 mmol, 1.4 eq) into
a round bottom flask, add DMF (30 ml) to dissolve, add Et₃N (0.3 ml, 2 mmol, 1 EQ),
dissolve compound E (700 mg, 2 mmol) in DMF (20 ml), stir at room temperature for
24 hours. The solvent was evaporated dry, and the white solid compound biotin-N₃ (477
mg, 75%) was obtained by silica gel column chromatography (Table S6).