# Supporting Information

## Bioinspired photoenzymatic cascade enabled by a bifunctional arylformamidase for efficient synthesis of L-kynurenine derivatives

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## Table of contents

# Experimental procedures

## General information

All reagents involved in this study were purchased and used without further purification. The analytical grade reagent of isopropanol (*i*PrOH), dichloromethane (DCM), methanol (MeOH), petroleum ether (PE), ethyl acetate (EA), acetonitrile (MeCN), ether, NaCl, NaOH, HCl, dimethyl sulfoxide (DMSO), ethanol (EtOH), triethylamine ($Et_3N$) and other chemical reagents were purchased from Sinopharm Chemical Reagent (Beijing, China). $^1H$ and $^{13}C$ NMR spectra were recorded on Bruker Avance spectrometer (400/600 MHz for $^1H$ and 101/151 MHz for $^{13}C$) in $CD_3OD$ or $CDCl_3$ with internal signals as reference. The reaction progress was monitored *via* thin-layer chromatography (TLC) on Leyan PLC silica gel 60 GF254 plates (0.9-1 mm thickness) with UV detection. The reaction mixtures were determined by high performance liquid chromatography (HPLC) system (Waters 2695), and stationary phase using an Agilent Eclipse XDB C18 column (4.6 × 250 mm, 5 μm).

## Plasmids construction and confirmation

According to the protein sequence of the twelve arylfomamidases mining from National Center for Biotechnology Information (NCBI) and UniProt, the genes of the twelve arylfomamidases in pET-21b (+) vector with 6 × His tag on C terminal were synthesized by Yixin Biotechnology (Shanghai, China) after codon optimization. The mutant of *AchAF* construction steps were as follows. Primers containing the corresponding mutation site respectively was designed and synthesized by Yixin Biotechnology. Then the primers were applied to PCR reaction respectively for amplification of the target gene using pET-21b (+)-*AchAF* wild type as a template. The PCR product was purified by FastPure Gel DNA Extraction Mini kit (Vazyme Biotechnology, Jiangsu, China) after agarose gel electrophoresis and ligated by 2X MultiF Seamless Assembly Mix (ABclonal Biotechnology, Hubei, China). Next, the constructed plasmids were transformed into the competent cells of *Escherichia coli* (*E. coli*) TG1, and single colony was selected for amplification, which was then extracted by 8 min FastPure Plasmid Mini kit (Vazyme Biotechnology, Jiangsu, China). The accuracy of the gene sequences was confirmed by DNA sequencing (Yixin Biotechnology (Shanghai, China)).

## Expression and purification of enzymes

The recombinant plasmid with genes encoding His-tagged enzymes employed in this study were cloned into pET-21b (+) vectors after codon optimization, and the enzymes were heterologously expressed in *E. coli* BL21 (DE3)[1-5]. The procedures are described as follows. An *E. coli* BL21 (DE3) colony transformed with the enzyme genes were inoculated in 20 mL Luria-Bertani (LB) medium containing 100 μg/mL ampicillin, and grown overnight at 37 °C. After inoculation of the seed culture into 500 mL 2×YT medium with 100 μg/mL ampicillin, the cells were grown at 37 °C in shaking speed of 135 rpm for 3 h until $OD_{600}$ reached 0.6-0.8 before the culture temperature was adjusted to 18 °C. Then the protein expression was induced after adding isopropyl β-D-1-thiogalactopyranoside (IPTG, 0.5 mM). After induction of 12 h, cells were collected by centrifugation (7000 rpm for 7 min at 4 °C) and resuspended

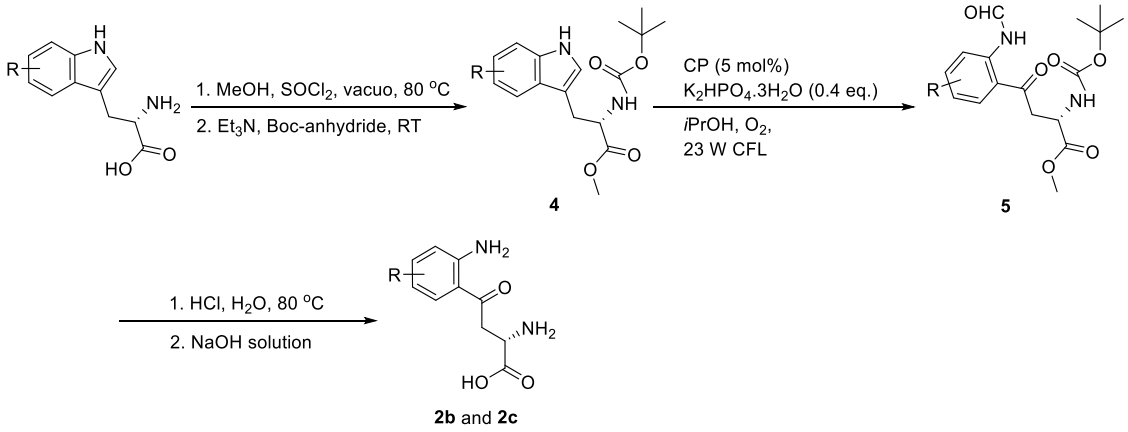
with Tris-HCl buffer (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, 10% glycerol (v/v), pH = 8). The mixture was further lysed by a high-pressure homogenizer and centrifuged in speed of 18300 rpm for 30 min at 4 °C to collect the supernatant, which was then purified by a nickel-NTA column. The protein was desalted and condensed to appropriate concentration with 25 mM Tris-HCl (pH 8.0), 150 mM NaCl buffer using an Amicon Ultra concentration tube. The final protein solution was stored at -80 °C until later use.

## General procedure for the photoenzymatic reaction

A reaction mixture containing substrate (1 μmol), *Ach*AF-V170A (0.6 mol%), and CP (0.2 mol%) in PB buffer (0.1 M, pH 8.0 containing 20 μM $ZnCl_2$) with EtOH (0.5% v/v) was prepared in a final volume of 1 mL, which was stirred at 30 °C for 24 h under $O_2$ atmosphere and the irradiation of LEDs (20 W white LED). Upon completion of the reaction, methanol (1.6 mL) was added to quench the reaction. The yields of reaction mixture were determined by HPLC[6]. The HPLC conditions were listed as follows.

| **Column** | | Eclipse XDB C18, 5 μm, 4.6 × 250 mm, Agilent. | | |
|---|---|---|---|---|
| **Mobile phase** | | MeOH | dd$H_2O$ (0.1% TFA) | MeCN |
| **Gradient** | 0 min | 5% | 95% | 0% |
| | -10 min | 14% | 86% | 0% |
| | -30 min | 14% | 46% | 40% |
| **Flow:** 1 mL/min | | **Inject volume:** 20 μL | | **Detection:** UV, 260 nm |

## General procedure for the synthesis of 2b and 2c




Step 1: carboxyl-protection of L-tryptophan derivatives[7].

L-tryptophan derivatives (0.42 mmol, 1 equiv) and a magnetic stir bar were first charged to a vacuum Schlenk tube (10 mL), 1 mL cold MeOH with thionyl chloride (1.9 equiv) was added by syringe, and the mixture was stirred on ice for 30 min before it was heated at 80 °C for 1.5 h. After the mixture cooled to ambient temperature, the volatiles were removed in vacuo and the crude product was directly used for the further reaction.

Step 2: $N^{\alpha}$-protection of L-tryptophan methyl ester derivatives[8].

A solution of L-tryptophan methyl ester derivatives (0.4 mmol, 1 equiv) and $Et_3N$ (1.1 equiv) in

anhydrous dichloromethane (DCM) (1.7 mL) was added to a solution of Boc-anhydride (12.8 equiv) in anhydrous DCM (1.6 mL). The mixture was stirred for 2 h at room temperature. The organic solvent was washed with brine and dry over $Na_2SO_4$. Then remove the solvent in vacuo. The crude was purified by chromatography on silica gel (200–300 mesh) (DCM/MeOH) to afford the corresponding products **4**.

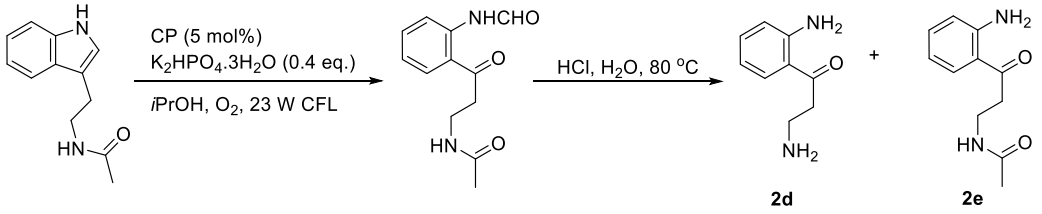
Step 3: Ring-opening catalyzed by cercosporin (CP)[9].

A sealed oven-dried Schlenk tube was charged with substrates **4** (0.1 mmol, 1 equiv), CP (5 mol%), and $K_2HPO_4$ (0.4 equiv). *i*PrOH (8 mL) was added under $O_2$ atmosphere after the tube is thoroughly flushed with $O_2$. Then the mixture was stirred at room temperature under irradiation of a 23 W compact fluorescent lamp (CFL) for 24 h. The organic solvent was removed in vacuo and the crude product was purified by chromatography on silica gel (200–300 mesh) (PE/EA or DCM/MeOH) to afford the corresponding product **5**.

Step 4: Deprotection[9].

Compound **5** (0.1 mmol, 1 equiv) and 1 mL water with 0.6 mmol HCl (6 equiv) were charged to an oven-dried Schlenk tube with a magnetic stir bar. The mixture was heated to 80 ℃ for 4 h. After the mixture cooling to ambient temperature, the reaction mixture was basified to pH 12 with 50% NaOH solution, and stirred at room temperature for 6 h. The solution was washed with 2 mL DCM, 2 mL ethyl acetate and 2 mL ether for three times, respectively. Then the pH value of the solution was adjusted to 6 with HCl. The crude was purified by semi-preparative HPLC after being dissolved in 30% (v/v) aqueous MeOH containing 0.1% TFA and 3% MeCN to afford desired product **2b** and **2c**. The separation method was 80% $H_2O$ containing 0.1% TFA and 20% MeCN, UV 260 nm. Column: Eclipse XDB C18, 5 μm, 9.4*250 mm, Agilent.

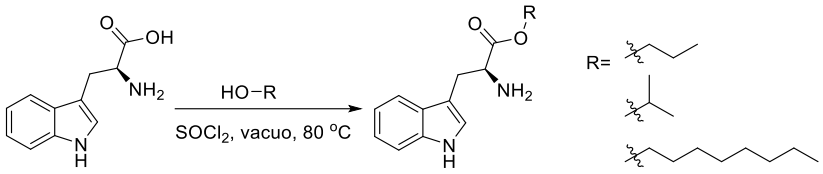
## General procedure for the synthesis of 2d and 2e




The synthesis of compound **2d** and **2e**[9] started with *N*-acetyltryptamine follows the same step 3 and step 4 as that of compound **2b** and **2c**. The crude product was purified by chromatography on silica gel (200–300 mesh) (DCM/MeOH) to afford the desired product **2e** and crude **2d**. Then the crude **2d** was purified by semi-preparative HPLC after being dissolved in water containing 1% TFA, 15% MeCN, and 30% MeOH to obtain product **2d**. The separation method was listed as follows.

| Column | | Eclipse XDB C18, 5 μm, 9.4×250 mm, Agilent. | |
|---|---|---|---|
| Mobile phase | | MeCN : MeOH=1:2 | $ddH_2O$ (0.1% TFA) |
| Gradient | 0min | 50% | 50% |
| | -15min | 100% | 0 |
| Flow: 2 mL/min | | Inject volume: 20 μL | Detection: UV, 280 nm |

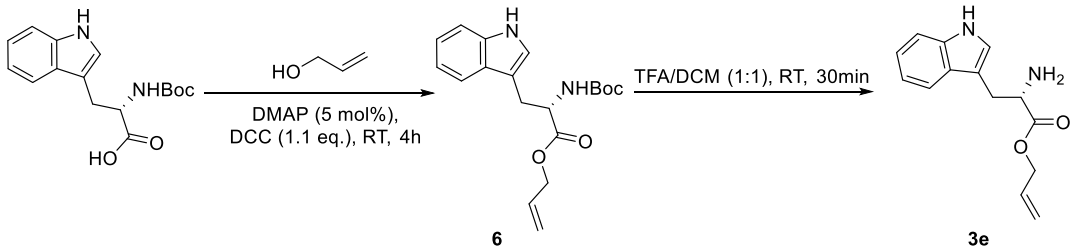
## General procedure for the synthesis of 3c, 3d, 3f




**3c**, **3d**, and **3f**

The procedure for the synthesis of **3c**, **3d**, and **3f** [7] could be referred to the first step of synthesizing **2b** and **2c**. The alcohols in the reaction were replaced with the corresponding alcohols, and the crude was further purified by chromatography on silica gel (200–300 mesh) (PE/EA or DCM/MeOH).

## General procedure for the synthesis of 3e

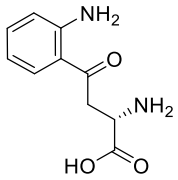



**6** **3e**

Allyl alcohol (1 equiv) was added to anhydrous DCM containing N-Boc-Trp (1 equiv), followed by a catalytic amount of DMAP (5 mol%). A solution of anhydrous DCM containing DCC (1.1 equiv) was dropwise added after the reaction being cooled to 0 ℃. The mixture was stirred for 4 h at room temperature. Precipitate was removed by filtration, and then the organic solvent was removed in vacuo. The crude was purified by chromatography on silica gel (200–300 mesh) (PE/EA) to afford the corresponding product **6**. The afforded compound **6** was dissolved in mixed solution of DCM and TFA (1:1) and stirred for 30 min. Saturated $NaHCO_3$ was added to adjust pH of the reaction mixture to 6.0, then the mixture was extracted for three times with an equal volume of DCM. The organic solvent was removed in vacuo, and the crude was purified by chromatography on silica gel (200–300 mesh) (DCM/MeOH) to give the titled compound **3e**[10].

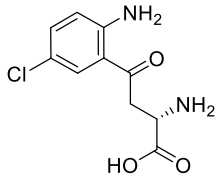
## Characterization of compounds

### L-Kyn (2a)



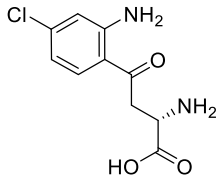
**$^1$H NMR** (400 MHz, DMSO-$d_6$ + 1% TFA) δ 7.73 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.30 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 6.80 (dd, *J* = 8.5, 1.2 Hz, 1H), 6.58 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 4.31 (dd, *J* = 5.2, 4.5 Hz, 1H), 3.67 – 3.54 (m, 2H) ppm.
The $^1$H NMR spectrum is consistent with the previous literature[9].
**HRMS** (ESI) calcd. for $[C_{10}H_{13}N_2O_3^+H]^+$ 209.0921, 209.0907 (found).

### L-5-Cl-Kyn (2b)



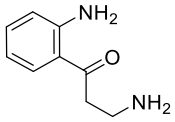
**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.62 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.69 (d, *J* = 8.9 Hz, 1H), 4.28 (dd, *J* = 6.2, 4.0 Hz, 1H), 3.66-3.54 (m, 2H) ppm.
**$^{13}$C NMR** (151 MHz, Methanol-d4) δ 196.42, 170.05, 150.49, 134.83, 129.37, 118.90, 118.82, 116.00, 48.65, 38.18 ppm.
**HRMS** (ESI) calcd. for $[C_{10}H_{12}ClN_2O_3^+H]^+$ 243.0531, 243.0540 (found).

### L-4-Cl-Kyn (2c)



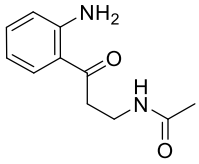
**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ 7.62 (d, *J* = 8.7 Hz, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 6.48 (dd, *J* = 8.8, 2.1 Hz, 1H), 4.25 (t, *J* = 5.0 Hz, 1H), 3.59 (d, *J* = 5.1 Hz, 1H) ppm.
**$^{13}$C NMR** (151 MHz, Methanol-$d_4$) δ 196.71, 170.34, 152.68, 140.80, 132.35, 115.89, 115.03, 114.32, 48.85, 38.28 ppm.
**HRMS** (ESI) calcd. for $[C_{10}H_{12}ClN_2O_3^+H]^+$ 243.0531, 243.0544 (found).

### Kynuramine (2d)



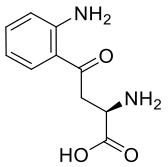
**$^1$H NMR** (400 MHz, DMSO-$d_6$ + 1% TFA) δ 7.94 (s, 2H), 7.71 (dd, $J$ = 8.2, 1.5 Hz, 1H), 7.29 (ddd, $J$ = 8.4, 6.9, 1.5 Hz, 1H), 6.82 (d, $J$ = 8.3 Hz, 1H), 6.59 (t, J = 7.4 Hz, 1H), 3.35 (t, $J$ = 6.5 Hz, 2H), 3.18 – 3.11 (m, 2H) ppm.
**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 199.02, 151.50, 134.98, 131.37, 117.65, 116.54, 115.17, 36.25, 34.91 ppm.
**HRMS** (ESI) calcd. for $[C_9H_{13}N_2O^+H]^+$ 165.1022, 165.1017 (found).

***N*-Ace-Kynuramine (2e)**



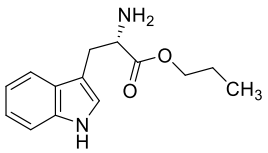
**$^1$H NMR** (600 MHz, Methanol-$d_4$) δ 7.63 (d, $J$ = 8.2 Hz, 1H), 7.14 – 7.11 (m, 1H), 6.62 (d, $J$ = 8.4 Hz, 1H), 6.49 – 6.46 (m, 1H), 3.42 (t, $J$ = 6.5 Hz, 2H), 3.05 (t, $J$ = 6.5 Hz, 2H), 1.81 (s, 3H) ppm.
**$^{13}$C NMR** (151 MHz, MeOD) δ 200.49, 171.96, 151.29, 134.06, 130.82, 117.11, 116.93, 114.78, 37.87, 34.97, 21.12 ppm.
**HRMS** (ESI) calcd. for $[C_{11}H_{15}N_2O_2^+H]^+$ 207.1128, 207.1131 (found).

**D-Kyn (2f)**



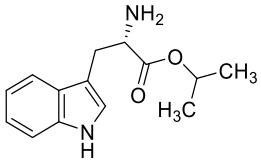
**$^1$H NMR** (400 MHz, DMSO-$d_6$ + 1% TFA) δ 7.73 (dd, $J$ = 8.5, 1.2 Hz, 1H), 7.32 – 7.29 (m, 1H), 6.81 (d, $J$ = 8.3 Hz, 1H), 6.58 (t, $J$ = 7.5 Hz, 1H), 4.31 (dd, $J$ = 5.1, 4.6 Hz, 1H), 3.66 – 3.56 (m, 2H) ppm.
**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 196.47, 170.12, 150.77, 134.39, 130.53, 116.59, 114.88, 114.20, 47.45, 38.08 ppm.
**HRMS** (ESI) calcd. for $[C_{10}H_{13}N_2O_3^+H]^+$ 209.0921, 209.0948(found).

**L-Trp-OPr (3c)**



**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 10.87 (s, 1H), 7.49 (d, $J$ = 7.9 Hz, 1H), 7.33 (d, $J$ = 8.1 Hz, 1H), 7.12 (d, $J$ = 2.4 Hz, 1H), 7.05 (t, $J$ = 7.5 Hz, 1H), 6.97 (t, $J$ = 7.4 Hz, 1H), 5.76 (s, 1H), 3.89 (t, $J$ = 6.6 Hz, 2H), 3.63 (t, $J$ = 6.4 Hz, 1H), 3.01 (dd, $J$ = 14.2, 6.4 Hz, 1H), 2.94 (dd, $J$ = 14.2, 6.5 Hz, 1H), 1.48 (q, $J$ = 7.0 Hz, 2H), 0.77 (t, $J$ = 7.4 Hz, 3H) ppm.
The $^1$H NMR spectrum is consistent with the previous literature[11].

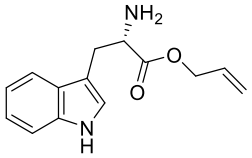
**L-Trp-O*i*Pr (3d)**



**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.85 (s, 1H), 7.50 (d, $J$ = 7.8 Hz, 1H), 7.33 (d, $J$ = 7.9 Hz, 1H), 7.11 (d, $J$ = 2.4 Hz, 1H), 7.07 – 7.03 (m, 1H), 6.96 (td, $J$ = 7.5, 7.0, 1.1 Hz, 1H), 4.79 (hept, $J$ = 6.3 Hz, 1H), 3.57 (t, $J$ = 6.5 Hz, 1H), 3.00 (dd, $J$ = 14.2, 6.5 Hz, 1H), 2.91 (dd, $J$ = 14.2, 6.5 Hz, 1H), 1.11 (d, $J$ = 6.2 Hz, 3H), 1.02 (d, $J$ = 6.2 Hz, 3H) ppm.

The $^1$H NMR spectrum is consistent with the previous literature[12].

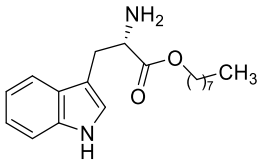
**L-Trp-OAcr (3e)**



**$^1$H NMR** (400 MHz, $CD_3OD$) δ 7.42 (d, $J$ = 8.0 Hz, 1H), 7.23 (d, $J$ = 8.0 Hz, 1H), 7.00 – 6.96 (m, 2H), 6.92 – 6.88 (m, 1H), 5.72 (ddt, $J$ = 17.3, 10.5, 5.7 Hz, 1H), 5.13 (dq, $J$ = 17.2, 1.6 Hz, 1H), 5.06 (dq, $J$ = 10.4, 1.4 Hz, 1H), 4.41 (d, $J$ = 5.7 Hz, 2H), 3.68 (t, $J$ = 6.2 Hz, 1H), 3.09 (dd, $J$ = 14.3, 5.8 Hz, 1H), 3.01 (dd, $J$ = 14.3, 6.7 Hz, 1H) ppm.

**$^{13}$C NMR** (151 MHz, $CD_3OD$) δ 174.50, 136.72, 132.01, 127.35, 123.27, 121.09, 118.41, 117.84, 117.25, 110.94, 109.13, 65.20, 54.60, 30.07 ppm.

**L-Trp-OOct (3f)**



**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 11.14 (s, 1H), 8.69 (s, 3H), 7.52 (d, $J$ = 7.9 Hz, 1H), 7.37 (d, $J$ = 8.0 Hz, 1H), 7.25 (d, $J$ = 2.4 Hz, 1H), 7.08 (t, $J$ = 7.5 Hz, 1H), 7.00 (t, $J$ = 7.4 Hz, 1H), 4.17 (dd, $J$ = 7.4, 5.6 Hz, 1H), 4.01 – 3.94 (m, 2H), 3.37 – 3.34 (m, 1H), 3.26 (dd, $J$ = 14.7, 7.4 Hz, 1H), 1.42 – 1.37 (m, 2H), 1.26 (q, $J$ = 6.9 Hz, 2H), 1.22 – 1.15 (m, 6H), 1.10 (q, $J$ = 7.3 Hz, 2H), 0.86 (t, $J$ = 7.1 Hz, 3H) ppm.

The $^1$H NMR spectrum is consistent with the previous literature[13].

**Protein sequences of arylformamidases involved in this research**

***Sc*AF (from *Saccharomyces cerevisiae*)**
MSNTVRAISPDITLFNKTLTFQEISQNTREAVIYIHGGAWNDPENTPNDFNQLANTIKSMDTEST
VCQYSIEYRLSPEITNPRNLYDAVSNITRLVKEKGLTNINMVGHSVGATFIWQILAALKDPQEK
MSEAQLQMLGLLQIVKRVFLLDGIYSLKELLVEYPEYDCFTRLAFPDGIQMYEEEPSRVMPYV
KKALSRFSIDMHLVHSYSDELLTLRQTNCLISCLQDYQLSFKLYLDDLGLHNDVYKNGKVAKY
IFDNIC

***Pa*KynB (from *Pseudomonas aeruginosa*)**
MTSLRYWDISPALDPNTPTWPGDTPFQQEWAARLDEQCPVNVGRITLSPHTGAHVDGPLHYR
ADGLPIGQVPLDIYMGPCRVIHCIGANPLVTPEHLAGQLDDLPSRVLLRTFERVPANWPEGFCAI
APATIECLAERGVRLVGIDTPSLDPQHSKTLDAHHAVGRHGMAILEGVVLDDVPAGDYELLAL
PLKFTHLDASPVRAVLRALPTAE

***Pc*AF (from *Paracidovorax citrulli*)**
MTTPRMLWDISAPVHAGSPVFPGDTPYSQQWCATIGPQCPVNVSALAMSPHVGTHADAPLHY
DPQGATIGDVPLDAFIGPCRVIHAIGRGPLVAWEHIAHALGADRPALPQRVLVRTYERMPLDRW
DAALAAYAPDTIERLADLGVVLVGIDTASIDPADSKSLDSHQVIRRRGLRVLENLVLDEVPEGD
YELIALPLKLTTADASPVRAVLRTPA

***Ach*AF (from *Achromobacter*)**
MKRLWDISPPVSTASPVFPGDTPYRQQWKWSLTPGCPVNVSEITLSPHIGAHADAPLHYQNGA
AAIGAVSLEPFLGPCRVIHAIDCGPLITTDHLAHAALNLPPRVLVRTAKHAAQDWWTDDFSAYA
PQTIEWLAERGVMLIGLDTASIDPASSKTLDSHHTILRHDMRVLENLVLDDVPEGDYELIALPLA
LVQADASPVRAVLREL

***Bc*KynB (from *Burkholderia cenocepacia*)**
MDTLWDISPPVSPATPVWPGDTPVAVERVWRMEAGSPVNVARLTLSPHTGAHCDAPLHYDAD
GAPIGAVPLDTYLGPCRVIHCIGAAPVVRPADVEAALDGVPPRVLLRTYARAAVEQWDSNFCAV
APDTVDLLAAHGVKLIGIDTPSLDPQESKTMDAHRRVRAHRMAILEGIVLDDVPPGDYELIALP
LKFATLDASPVRAVLRALPAQAS

***Bu*AF (from *Burkholderia ubonensis*)**
MDTLWDISPPVDPATPVWPGDTPVTVERVWRMEAGSPVNVARLTLSPHTGAHTDAPLHYDAD
GAPIGAVPLDAYLGRCRVIHCIGASPVVMPDDVAAALDGVPPRVLLRTYAHAPAAQWDPDFCA
VAPDTIDLLAERGVKLIGIDTPSLDPQESKTMDAHRRIRAHRMAILEGIVLDAVPPGDYELIALP
LKLATLDASPVRAVLRALPGRAD

***Ph*AF (from *Paraburkholderia hospital*)**
MDTLWDITPAVDTATPVWPGDTPVGIERVWRMEAGSPVNVARLTLSPHTGAHTDAPLHYDAD
GAAIGAVPLDAYLGRCRVIHCIGATPLVSPEHVAASLDGVPPRVLLRTYREAPVTAWDGNFCAV
APDTIDLLAAHGVKLIGIDTPSLDPQESKTMDAHHRIRAHRMAILEGIVLDAVAPGDYELIALPL
KLTTLDASPVRAVLRALPEQRPSA

***Cm*KynB (from *Cupriavidus metallidurans*)**

MPQAPQLHDGRRIWYDISPAVSPATPVWPGDTPFQHDPAWQLDEHCPVNVGRITMSPHTGAHA
DAPLHYAADGAPIGAVPLDAYLGPCRMIHCIGAAPRVEPQHIAHALAGTPPRVLLRTYAQAPQG
KWDSAFCAVAPETISLLARHGVRLIGIDTPSLDPETSKTMDAHHAVRDHQLAILEGIVLDEVPAG
DYELIALPLRLATLDASPVRAVLRELP

***Pp*AF (from *Pseudomonas proteolytica*)**

MPTRKLFDISPPITTAIATWPGDTDYQQEPVWVLDHQCPVNVGKITLSAHTGAHADAPLHYSN
SGAAIGSVPLEPYLGTCRVIHCFDSDGLVRPAQLLPHLAQAPERILLRTYRHSDPCIWDQHSTAI
AVAAIELLARHGVKLVGIDTPSVDPQHSKTLDAHHAIQRHGMAILEGLVLDEVPAGDYELIALP
LKFMALDASPVRAVLRSLPTQVPRSG

***Ds*KFase (from *Drosophila melanogaster*)**

MYNPRCKDLDRDYFPSYHTTRFQDQPEPNLAVLEHFVRVTKQHGRELTEKQGITVDHLRYGE
GRQLVDVFYSEKTTNQAPLFVFVHGGYWQEMDMSMSCSIVGPLVRRGYRVAVMDYNLCPQV
TLEQLMTQFTHFLNWIFDYTEMTKVSSLTFAGHSAGAHLLAQILMRPNVITAQRSKMVWALIF
LCGVYDLRELSNLESVNPKNILGLNERNIESVSPMLWEYTDVTVWNSTKIYVVAAEHDSTTFIE
QSRHYADVLRKKGYKASFTLFKGYDHFDIIEETAIDDSDVSRFLRNIEIE

**Tar16 (from *Saccharomonospora* sp. CNQ-490)**

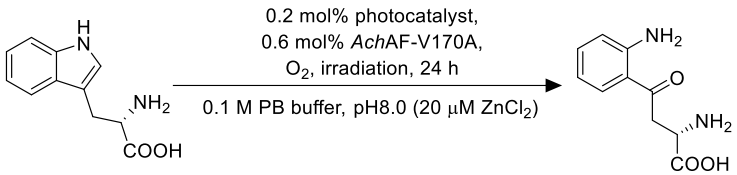
MATAVFRSYDQRELDLQYSPSSCVDDVEGYFHEYTRRSAEARREIEGFVEVRYGDEPDQVLDF
FPAKTHGSPLLVFLHGGYWQEFSRREAAFMAMDLTAQGVSVAALGYGLAPRYTLPEIVTMVSE
GVRWICRNTDGLPGSPRRVVLSGCSAGAHLVAMALLDEIGWRREGVRPTEAIAGAVLLSGVYD
LDPVRRTYVNSPLGLDVDTALACSPRHLPLTGLPPLVIARGENETTEFARQHTEFVAAVRQAGG
CVSDLVVPGRNHFDLPFDLGDPGTSLGAAVRRLFVPSVGGDAR

***Ba*KynB (from *Bacillus anthracis*)**

MKTSKWIDISQPLNNDIATWPGDTPFSYEVLWSKEESGSVNVGKLTMSIHTGTHIDAPFHFDND
GKKVLDLDIQVYVGPTRIIDVSNLESIGKKELEKFHLEGVERLLLRTSSHGKANEFPDIIPHLRA
DIAPFLSEKGIRLIGVDVPSVDPLDDKELAAHHQLFKHSIHILENVVLDHVADGDYELIALPLAL
SDADGSPVRAVIRPI

# Green chemistry metrics and EcoScale

1. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* our method.




**1a**, 1.0 eq **2a**, yield 70.5%

| reagent | eq | weight (μg) | n (μmol) | MW |
|---|---|---|---|---|
| reactant **1a** | 1.0 | 204 | 1 | 204.2 |
| catalyst CP | 0.002 | 1 | 0.002 | 534.5 |
| *Ach*AF-V170A | 0.006 | 136 | 0.006 | 22690 |
| EtOH | 0.14 | 6 | 0.14 | 46.1 |
| product **2a** | 0.705 | 147 | 0.715 | 208.2 |
| total waste | | 201 | | |

Atom Economy (%) $\frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} = \frac{208.2}{204.2+32\ (O_2)} \times 100 = \times 100$ = 88.1%

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (70.5% × 88.1%) ×100 = 62.1%

Carbon Efficiency (%) = $\frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{11} \times 100 = 90.9\%$

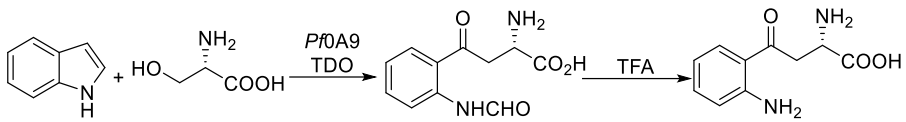
*E*-factor = $\frac{\text{total waste (μg)}}{\text{total product (μg)}} = \frac{201}{147}$ = 1.4 μg waste/ μg product

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 70.5)/2 | 15 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (< $10) = | 0 |
| 3. Safety (Solvent: EtOH, highly flammable) = | 5 |
| 4. Technical setup (Simple setup) = | 0 |
| 5. Temperature/time (Heating, > 1 h) = | 3 |
| 6. Workup and purification (Classical chromatography) = | 10 |
| Total of individual penalties = | **33** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 33 = **67**

Following scores: > 75, excellent; **> 50, acceptable**; and < 50, inadequate.

2. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* Song's method[14].




| reagent | eq | weight (μg) | n (μmol) | MW |
|---|---|---|---|---|
| reactant indole | 1.0 | 879 | 7.5 | 117.2 |
| reactant L-serine | 1.1 | 867 | 8.3 | 105.1 |
| reactant PLP | 0.1 | 185 | 0.75 | 247.1 |
| *Pf*0A9 | 0.02 | 6339 | 0.15 | 42260 |
| TDO | 0.02 | 5195 | 0.15 | 34630 |
| hemin | 0.024 | 117 | 0.18 | 651.9 |
| sodium ascorbate | 0.025 | 37 | 0.19 | 198.1 |
| TFA | 519.2 | 443989 | 3893.85 | 114.02 |
| product **2a** | 0.83 | 1296 | 6.225 | 208.2 |
| total waste | | 456312 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{117.2+105.1+32\ (O_2)} \times 100 = 81.9\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (83% × 81.9%) ×100 = 68.0%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{11} \times 100 = 90.9\%$$

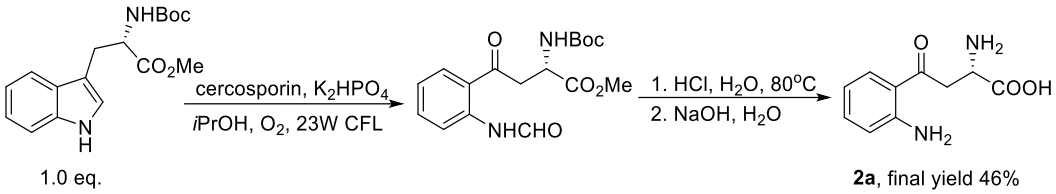
$$E\text{-factor} = \frac{\text{total waste (μg)}}{\text{total product (μg)}} = \frac{456312}{1296} = 352.1\ \mu\text{g waste/ μg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 83)/2 | 9 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (>$10, < $50) = | 3 |
| 3. Safety (Solvent: TFA, highly flammable) = | 5 |
| 4. Technical setup (Simple setup) = | 0 |
| 5. Temperature/time (Heating, > 1 h) = | 3 |
| 6. Workup and purification (Classical chromatography) = | 10 |
| Total of individual penalties = | **30** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 30 = **70**

Following scores: > 75, excellent; **> 50, acceptable**; and < 50, inadequate.

3. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* Rao's method[9].




1.0 eq. **2a**, final yield 46%

| reagent | eq | weight (mg) | n (mmol) | MW |
|---|---|---|---|---|
| reactant Trp analogue | 1.0 | 31840 | 100 | 318.4 |
| catalyst CP | 0.05 | 2673 | 5 | 534.5 |
| $K_2HPO_4 \cdot 3H_2O$ | 0.4 | 1129 | 40 | 28.2 |
| *i*PrOH | 1 | 6010 | 100 | 60.1 |
| HCl | 5.3 | 19251 | 530 | 36.5 |
| product **2a** | 0.46 | 9578 | 46 | 208.2 |
| total waste | | 51321 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{318.4+32\ (O_2)} \times 100 = 59.4\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (46% × 59.4%) ×100 = 27.3%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{17} \times 100 = 58.8\%$$

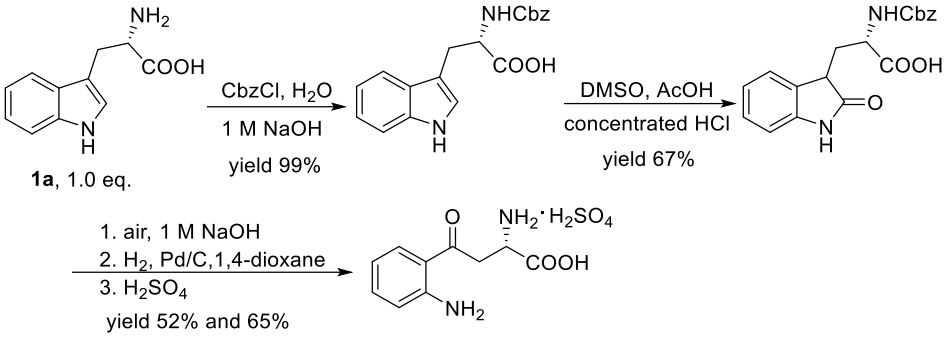
$$E\text{-factor} = \frac{\text{total waste (μg)}}{\text{total product (μg)}} = \frac{51321}{9578} = 5.4 \text{ mg waste/ mg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 46)/2 | 27 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (<$10) = | 0 |
| 3. Safety (Solvent: HCl, *i*PrOH, highly flammable) = | 5 |
| 4. Technical setup (Simple setup) = | 0 |
| 5. Temperature/time (Heating, > 1 h) = | 3 |
| 6. Workup and purification (Classical chromatography) = | 10 |
| Total of individual penalties = | **45** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 45 = **55**

Following scores: > 75, excellent; **> 50, acceptable**; and < 50, inadequate.

4. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* Martin's method[15].




**2a**, final yield 23%.

| reagent | eq | weight (mg) | n (mmol) | MW |
|---|---|---|---|---|
| Reactant **1a** | 1.0 | 10211 | 50 | 204.2 |
| Reactant CbzCl | 1.0 | 8530 | 50 | 170.6 |
| NaOH | 2.0 | 4000 | 100 | 40.0 |
| AcOH | 17.7 | 53086 | 884 | 60.1 |
| DMSO | 2.6 | 10001 | 128 | 78.1 |
| HCl | 6.1 | 11047 | 303 | 36.5 |
| NaOH | 11.4 | 22758 | 569 | 40.0 |
| 1,4-dioxane | 25.8 | 113481 | 1288 | 88.1 |
| HCl | 4.1 | 7438 | 204 | 36.5 |
| 10% Pd/C | | 341 | | |
| $H_2SO_4$ | 0.42 | 2060 | 21 | 98.1 |
| product **2a** | 0.23 | 2394 | 11.5 | 208.2 |
| total waste | | 240558 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{204.2+170.6+36.5+78.1} \times 100 = 42.5\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (23% × 42.5%) ×100 = 9.8%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{2+8+11} \times 100 = 47.6\%$$

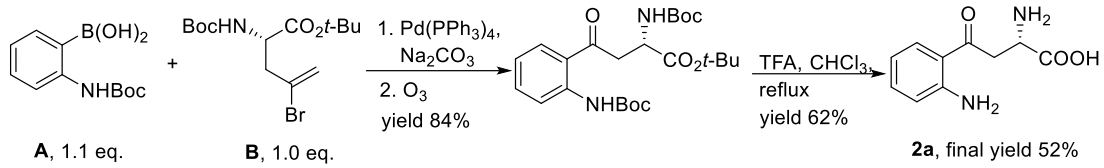
$$E\text{-factor} = \frac{\text{total waste (mg)}}{\text{total product (mg)}} = \frac{240558}{2394} = 100.5 \text{ mg waste/ mg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 23)/2 | 39 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (>$10, < $50) = | 3 |
| 3. Safety (solvent: DMSO, HCl, 1,4-dioxane, highly flammable) = | 5 |
| 4. Technical setup (Pressure equipment) = | 3 |
| 5. Temperature/time (Cooling to 0°C, room temperature, < 24 h) = | 5 |
| 6. Workup and purification (Crystallization and filtration, classical chromatography) = | 11 |
| Total of individual penalties = | **66** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 66 = **34**

Following scores: > 75, excellent; **>** 50, acceptable; and **< 50, inadequate**.

5. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* Andrews's method[16].




**A**, 1.1 eq. **B**, 1.0 eq. **2a**, final yield 52%

| reagent | eq | weight (mg) | n (mmol) | MW |
|---|---|---|---|---|
| Reactant **A** | 1.1 | 2608 | 11 | 237.06 |
| Reactant **B** | 1.0 | 3503 | 10 | 350.25 |
| $Pd(PPh_3)_4$ | 0.3 | 3467 | 3 | 1155.56 |
| $Na_2CO_3$ | 2 | 2120 | 20 | 105.99 |
| PhMe | 18.9 | 17442 | 189.3 | 92.14 |
| EtOH | 8.6 | 3976 | 86.3 | 46.07 |
| $H_2O_2$ | 1.6 | 554 | 16.3 | 34.01 |
| TFA | 109 | 124318 | 1090.32 | 114.02 |
| $CHCl_3$ | 105 | 125327 | 1049.9 | 119.37 |
| product **2a** | 0.52 | 1083 | 5.2 | 208.2 |
| total waste | | 282231 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{237.06+350.25+48.0\ (O_3)} \times 100 = 32.8\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (52% × 32.8%) ×100 = 17.1%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{10+11} \times 100 = 47.6\%$$

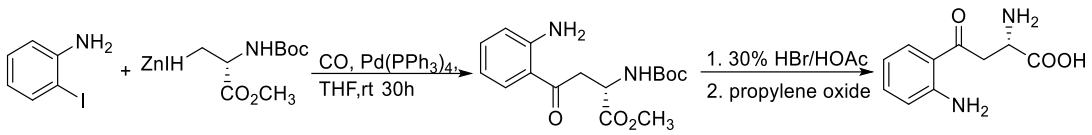
$$E\text{-factor} = \frac{\text{total waste (mg)}}{\text{total product (mg)}} = \frac{282231}{1083} = 260.7 \text{ mg waste/ mg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 52)/2 | 24 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (> $50) = | 5 |
| 3. Safety (solvent: PhMe, $CHCl_3$, TFA, extremely toxic) = | 10 |
| 4. Technical setup (Pressure equipment, $O_3$) = | 4 |
| 5. Temperature/time (Room temperature, < 24 h) = | 1 |
| 6. Workup and purification (Classical chromatography) = | 10 |
| Total of individual penalties = | **54** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 54 = **46**

Following scores: > 75, excellent; **>** 50, acceptable; and **< 50, inadequate**.

6. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* Block's method[17].




**C**, 1.0 eq. **D**, 0.75 eq. **2a**, final yield 47%

| reagent | eq | weight (mg) | n (mmol) | MW |
|---|---|---|---|---|
| Reactant **C** | 1.0 | 219 | 1 | 219.03 |
| Reactant **D** | 0.75 | 297 | 0.75 | 395.52 |
| $Pd(PPh_3)_4$ | 0.038 | 44 | 0.038 | 1155.56 |
| THF | 18.7 | 1348 | 18.7 | 72.11 |
| HBr/HOAc | | 6618 | | |
| 2-propanol | 370.3 | 22252 | 370.3 | 60.10 |
| propylene oxide | 3.12 | 156 | 3.12 | 50.08 |
| product **2a** | 0.47 | 97 | 0.47 | 208.2 |
| total waste | | 30837 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{219.03+395.52+28.01\ (CO)} \times 100 = 32.4\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (47% × 32.4%) ×100 = 15.2%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{1+6+9} \times 100 = 62.5\%$$

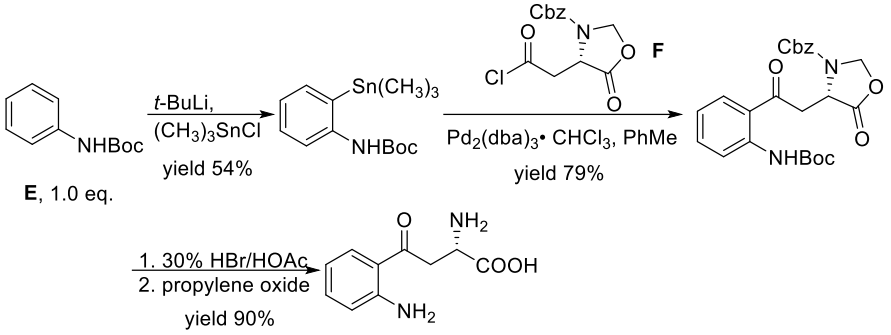
$$E\text{-factor} = \frac{\text{total waste (mg)}}{\text{total product (mg)}} = \frac{30837}{97} = 317.9 \text{ mg waste/ mg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 47)/2 | 27 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (> $50) = | 5 |
| 3. Safety (solvent: THF, propylene oxide, highly flammable) = | 5 |
| 4. Technical setup (Pressure equipment, CO) = | 4 |
| 5. Temperature/time (Room temperature, > 24 h) = | 1 |
| 6. Workup and purification (Classical chromatography, liquid-liquid extraction) = | 13 |
| Total of individual penalties = | **55** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 55 = **45**

Following scores: > 75, excellent; **>** 50, acceptable; and **< 50, inadequate**.

7. Evaluation of green chemistry metrics and EcoScale for the synthesis of **2a** *via* McDonald's method[18].




**2a**, final yield 39%

| reagent | eq | weight (mg) | n (mmol) | MW |
|---|---|---|---|---|
| Reactant **E** | 1.0 | 3003 | 15.54 | 193.25 |
| Reactant *t*-BuLi | 2.1 | 2088 | 32.6 | 64.06 |
| Reactant $(CH_3)_3SnCl$ | 1 | 3097 | 15.54 | 199.27 |
| THF | 38.6 | 43253 | 599.84 | 72.11 |
| Reactant **F** | 0.54 | 2498 | 8.39 | 297.69 |
| $Pd_2(dba)_3{\cdot}CHCl_3$ | 0.013 | 207 | 0.202 | 1023.01 |
| PhMe | 60.4 | 86484 | 938.62 | 92.14 |
| HBr/HOAc | | 85046 | | |
| 2-propanol | 254.1 | 237302 | 3948.71 | 60.10 |
| propylene oxide | 2.58 | 2008 | 40.09 | 50.08 |
| product **2a** | 0.39 | 1262 | 6.06 | 208.2 |
| total waste | | 463724 | | |

$$\text{Atom Economy (\%)} = \frac{\text{Mol. wt. of product}}{\text{Mol. wt. of all reactants}} \times 100 = \frac{208.2}{193.25+297.69+199.27+64.06} \times 100 = 27.6\%$$

Atom Efficiency (%) = (%yield of product × %atom economy) ×100 = (39% × 27.6%) ×100 = 10.8%

$$\text{Carbon Efficiency (\%)} = \frac{\text{amount of carbon in product}}{\text{amount of carbon in reactants}} \times 100 = \frac{10}{3+11+13} \times 100 = 37.0\%$$

$$\textit{E}\text{-factor} = \frac{\text{total waste (mg)}}{\text{total product (mg)}} = \frac{463724}{1262} = 367.5 \text{ mg waste/ mg product}$$

| Parameter Penalty points | Penalty points |
|---|---|
| 1. Yield (100 - %yield)/2 = (100 – 39)/2 | 31 |
| 2. Price of reaction components (To obtain 10 mmol of the end product), (> $50) = | 5 |
| 3. Safety (solvent: THF, PhMe, highly flammable) = | 5 |
| 4. Technical setup (Pressure equipment) = | 3 |
| 5. Temperature/time (Heating, > 1 h) = | 3 |
| 6. Workup and purification (Classical chromatography) = | 10 |
| Total of individual penalties = | **57** |

**EcoScale calculation** = 100 - Total of individual penalties = 100 - 57 = **43**

Following scores: > 75, excellent; **>** 50, acceptable; and **< 50, inadequate**.

# Supplementary tables

**Table S1.** Screening of enzyme loadings.

| entry[a] | *Ach*AF loading | yield of **2a** [%][b] |
|---|---|---|
| 1 | 2 mol% | 7.8 |
| 2 | 1 mol% | 12.6 |
| 3 | 0.8 mol% | 13.1 |
| 4 | 0.6 mol% | 14.9 |
| 5 | 0.4 mol% | 12.3 |
| 6 | 0.2 mol% | 7.1 |
| 7 | 0.1 mol% | 3.1 |
| 8 | 0.05 mol% | 2.8 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.05-2 mol%), DMSO (10 % v/v), Kpi buffer (0.1 M, pH 7.4, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at ambient temperature, air, 23 W CFL, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S2.** Screening of metal-free photocatalysts.

| entry[a] | non-metal PCs | yield of **2a** [%][b] |
|---|---|---|
| 1 | Cercosporin (CP) | 14.9 |
| 2 | 2,4,6-Triphenylpyrylium Tetrafluoroborate (2,4,6-TPTF) | 1.8 |
| 3 | Mes-Acr$^{+}$ClO$_4^{-}$ | 2.3 |
| 4 | Eosin Y (EY) | 2.6 |
| 5 | Perylene | 2.1 |
| 6 | Methylene Blue (MeB) | 8.9 |
| 7 | Congo Red (CR) | 1.1 |
| 8 | Methyl Orange (MO) | 1.5 |

[a] Reaction conditions: **1a** (1 μmol), non-mental PC (1 mol%), *Ach*AF (0.6 %), DMSO (10 % v/v), Kpi buffer (0.1 M, pH 7.4, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at ambient temperature, air, 23 W CFL, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S3.** Screening of reaction buffers in different pH values.

| entry[a] | buffer | pH value | yield of **2a** [%][b] |
|---|---|---|---|
| 1 | Bis-Tris | 5.5 | <2.0 |
| 2 | Bis-Tris | 6.0 | <2.0 |
| 3 | Bis-Tris | 6.5 | <2.0 |
| 4 | Bis-Tris | 7.0 | <2.0 |
| 5 | Bis-Tris | 7.4 | <2.0 |
| 6 | Tris-HCl | 7.0 | 7.4 |
| 7 | Tris-HCl | 7.4 | 5.9 |
| 8 | Tris-HCl | 8.0 | 16.3 |
| 9 | Tris-HCl | 8.5 | 16.8 |
| 10 | Tris-HCl | 9.0 | 15.9 |
| 11 | Phosphate buffer (PB) | 6.0 | 3.4 |
| 12 | Phosphate buffer (PB) | 6.5 | 4.6 |
| 13 | Phosphate buffer (PB) | 7.0 | 6.7 |
| 14 | Phosphate buffer (PB) | 7.4 | 13.5 |
| 15 | Phosphate buffer (PB) | 8.0 | 17.0 |
| 16 | Potassium phosphate buffer (Kpi) | 6.0 | 2.9 |
| 17 | Potassium phosphate buffer (Kpi) | 6.4 | 5.2 |
| 18 | Potassium phosphate buffer (Kpi) | 7.0 | 7.8 |
| 19 | Potassium phosphate buffer (Kpi) | 7.4 | 14.9 |
| 20 | Potassium phosphate buffer (Kpi) | 8.0 | 15.3 |
| 21 | $Na_2CO_3$-$NaHCO_3$ | 9.2 | 11.7 |
| 22 | $Na_2CO_3$-$NaHCO_3$ | 9.5 | 10.6 |
| 23 | $Na_2CO_3$-$NaHCO_3$ | 9.9 | 5.8 |
| 24 | $Na_2CO_3$-$NaHCO_3$ | 10.5 | 3.1 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.6 %), DMSO (10 % v/v), buffer (0.1 M, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at ambient temperature, air, 23 W CFL, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S4.** Screening of co-solvents.

| entry[a] | co-solvents | concentration (v/v) | yield of **2a** [%][b] |
|---|---|---|---|
| 1 | DMSO | 10 % | 17.0 |
| 2 | MeOH | 10 % | 17.5 |
| 3 | *i*PrOH | 10 % | 11.0 |
| 4 | MeCN | 10 % | 17.1 |
| 5 | Acetone | 10 % | 16.2 |
| 6 | DMF | 10 % | 7.5 |
| 7 | EtOH | 10 % | 20.0 |
| 8 | EtOH | 2% | 25.2 |
| 9 | EtOH | 4% | 26.0 |
| 10 | EtOH | 6% | 25.5 |
| 11 | EtOH | 8% | 22.9 |
| 12 | EtOH | 12% | 14.7 |
| 13 | EtOH | 14% | 9.7 |
| 14 | EtOH | 16% | 7.4 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.6 %), co-solvent, PB buffer (0.1 M, pH 8.0, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at ambient temperature, air, 23 W CFL, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S5.** Screening of light sources.

| entry[a] | irradiations | yield of **2a** [%][b] |
|---|---|---|
| 1 | CFL (23 W) | 26.0 |
| 2 | white LED (20 W) | 31.8 |
| 3 | blue LED (20 W) | 20.6 |
| 4 | purple LED (20 W) | 29.3 |
| 5 | green LED (20 W) | 27.4 |
| 6 | pink LED (20 W) | 29.2 |
| 7 | white LED (15 W) | 27.9 |
| 8 | white LED (30 W) | 19.9 |
| 9 | white LED (40 W) | 16.1 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.6 %), EtOH (4 % v/v), PB buffer (0.1 M, pH 8.0, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at ambient temperature, air, irradiations, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S6.** Screening of reaction temperatures.

| entry[a] | temperature | yield of **2a** [%][b] |
|---|---|---|
| 1 | 16 ℃ | 18.3 |
| 2 | 20 ℃ | 19.4 |
| 3 | 25 ℃ | 27.3 |
| 4 | 30 ℃ | 35.8 |
| 5 | 35 ℃ | 28.3 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.6 %), EtOH (4 % v/v), PB buffer (0.1 M, pH 8.0, with 20 μM $ZnCl_2$) in 0.2 mL reaction system, at specific temperature, air, 20 W white LED, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC.

**Table S7.** Screening of reaction volume.

| entry[a] | reaction volume | yield of **2a** [%][b] |
|---|---|---|
| 1 | 0.1 mL | 15.0 |
| 2 | 0.2 mL | 35.8 |
| 3 | 0.4 mL | 31.6 |
| 4 | 0.8 mL | 30.9 |
| 5 | 1.6 mL | 39.5 |
| 6 | 3.2 mL | 38.5 |
| 7[c] | 1.6 mL | 52.4 |

[a] Reaction conditions: **1a** (1 μmol), CP (1 mol%), *Ach*AF (0.6 %), 8 μL EtOH, PB buffer (0.1 M, pH 8.0, with 20 μM $ZnCl_2$) in specific volume of reaction system, 30 ℃, air, 20 W white LED, 15 h. [b] Yields of **2a** presented as average of three independent experiments were determined by HPLC. [c] $O_2$ instead of air.

**Table S8.** Regents used in this research.

| Regents | Purity (%) | Source |
|---|---|---|
| Primers | | Exsyn-bio (Wuxi, China) |
| DNA marker | | TaKaRa |
| Protein marker | | TaKaRa |
| PrimerSTAR MAX DNA Polymerase | | TaKaRa |
| Tryptone | | OXOID |
| Yeast extract | | OXOID |
| NaCl | 99.8 | Sinopharm Chemical Reagent (Shanghai, China) |
| Acetonitrile (HPLC) | 99.9 | Adamas-Beta (Shanghai, China) |
| Methanol (HPLC) | 99.9 | Adamas-Beta (Shanghai, China) |
| Trifluoroacetic acid (HPLC) | 99.5 | Energy Chemical (Shanghai, China) |
| L-Tryptophan (**1a**) | 98 | Sigma, Merck |
| D-Tryptophan | 98 | Bide Pharmatech (Shanghai, China) |
| L-Kynurenine | 98 | Bide Pharmatech (Shanghai, China) |
| D-Kynurenine | 98 | Bide Pharmatech (Shanghai, China) |
| 5-Cl-L-Tryptophan | 97 | Bide Pharmatech (Shanghai, China) |
| 6-Cl-L-Tryptophan | 98 | Bide Pharmatech (Shanghai, China) |
| Tryptamine | 99 | Bide Pharmatech (Shanghai, China) |
| *N*-Ace-Tryptamine | 98 | Bide Pharmatech (Shanghai, China) |
| L-Tryptophan-OMe | 98 | Bide Pharmatech (Shanghai, China) |
| L-Tryptophan-OEt | 97 | Bide Pharmatech (Shanghai, China) |
| L-Tryptophan-OBn | 95 | Bide Pharmatech (Shanghai, China) |
| D-Tryptophan-OMe | 98 | Bide Pharmatech (Shanghai, China) |
| D-Tryptophan-OEt | 97 | Bide Pharmatech (Shanghai, China) |
| D-Tryptophan-OBn | 97 | Bide Pharmatech (Shanghai, China) |
| L-Trp-N-Boc | 97 | Bide Pharmatech (Shanghai, China) |
| $Na_2HPO_4$ | 99 | Sinopharm Chemical Reagent (Shanghai, China) |
| $NaH_2PO_4$ | 98 | Sinopharm Chemical Reagent (Shanghai, China) |
| $ZnCl_2$ | 98 | Sinopharm Chemical Reagent (Shanghai, China) |
| $K_2HPO_4$ | 99 | Sinopharm Chemical Reagent (Shanghai, China) |
| $KH_2PO_4$ | 99 | Sinopharm Chemical Reagent (Shanghai, China) |
| FastPure Gel DNA Extraction Mini kit | | Vazyme (Nanjing, China) |
| 2× MultiF Seamless Assembly Mix | | ABclonal (Wuhan, China) |

**Table S9**. Primers used in this study

| Name | Sequence (5'-3') |
|---|---|
| *Ach*AF-F18A-F | GTCGCACCTGGTGACACGCC |
| *Ach*AF-F18A-R | AGGTGCGACGGGACTGGC |
| *Ach*AF-F18W-F | CGTCTGGCCTGGTGACACGC |
| *Ach*AF-F18W-R | AGGCCAGACGGGACTGGC |
| *Ach*AF-D21A-F | CTGGTGCAACGCCCTACCGC |
| *Ach*AF-D21A-R | CGTTGCACCAGGAAAGACGGGAC |
| *Ach*AF-S46A-F | CTGGCTCCACACATTGGGGCG |
| *Ach*AF-S46A-R | TGGAGCCAGTGTAATTTCGGACACATTTACG |
| *Ach*AF-Y59A-F | TACACGCACAGAACGGCGCG |
| *Ach*AF-Y59A-R | CTGTGCGTGTAAGGGGGCATCC |
| *Ach*AF-I149A-F | TAGCGCTGATCCAGCCAGTAGCAAA |
| *Ach*AF-I149A-R | ATCAGCGCTAGCTGTGTCTAACCCAA |
| *Ach*AF-L157A-F | AACGGCAGACAGCCATCACACAA |
| *Ach*AF-L157A-R | GTCTGCCGTTTTGCTACTGGCTG |
| *Ach*AF-V170A-F | TGCGCGCTCTTGAAAACTTAGTATTGGAT |
| *Ach*AF-V170A-R | CAAGAGCGCGCATATCATGGCG |
| *Ach*AF-A198V-F | GATGTAAGCCCTGTCCGCGCG |
| *Ach*AF-A198V-R | GCTTACATCGGCCTGGACAAGTGC |
| *Ach*AF-F18Y-F | GTCTATCCTGGTGACACGC |
| *Ach*AF-F18Y-R | AGGATAGACGGGACTGGC |
| *Ach*AF-F18H-F | GTCCATCCTGGTGACACG |
| *Ach*AF-F18H-R | AGGATGGACGGGACTGG |
| *Ach*AF-D21E-F | CCTGGTGAAACGCCCTAC |
| *Ach*AF-D21E-R | GGGCGTTTCACCAGGAAAG |
| *Ach*AF-S46D-F | ACACTGGACCCACACATTG |
| *Ach*AF-S46D-R | GTGTGGGTCCAGTGTAATTTCG |
| *Ach*AF-S46E-F | ACACTGGAACCACACATTGG |
| *Ach*AF-S46E-R | GGTTCCAGTGTAATTTCGGACAC |
| *Ach*AF-L157V-F | AAAACGGTGGACAGCCATCA |
| *Ach*AF-L157V-R | GGCTGTCCACCGTTTTGCTAC |
| *Ach*AF-L157I-F | AAAACGATTGACAGCCATCACA |
| *Ach*AF-L157I-R | GGCTGTCAATCGTTTTGCTACT |
| *Ach*AF-V170G-F | GCGCGGCCTTGAAAACT |
| *Ach*AF-V170G-R | CAAGGCCGCGCATATCAT |
| *Ach*AF-V170M-F | ATGCGCATGCTTGAAAACTT |
| *Ach*AF-V170M-R | TTCAAGCATGCGCATATCATG |
| *Ach*AF-V170S-F | ATGCGCTCACTTGAAAACTTAG |
| *Ach*AF-V170S-R | TTCAAGTGAGCGCATATCATG |
| *Ach*AF-A198G-F | CGATGGTAGCCCTGTCC |
| *Ach*AF-A198G-R | GCTGGTATCGGCCTGG |

# Supplementary figures

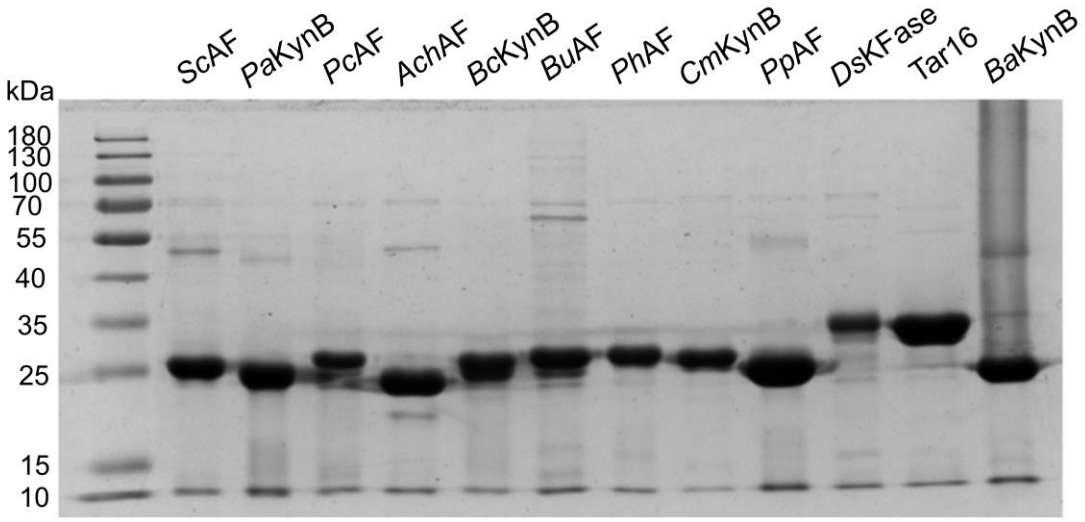



**Figure S1.** SDS-PAGE of arylformamidases expressed in *E. coli*. Lane 1: marker.

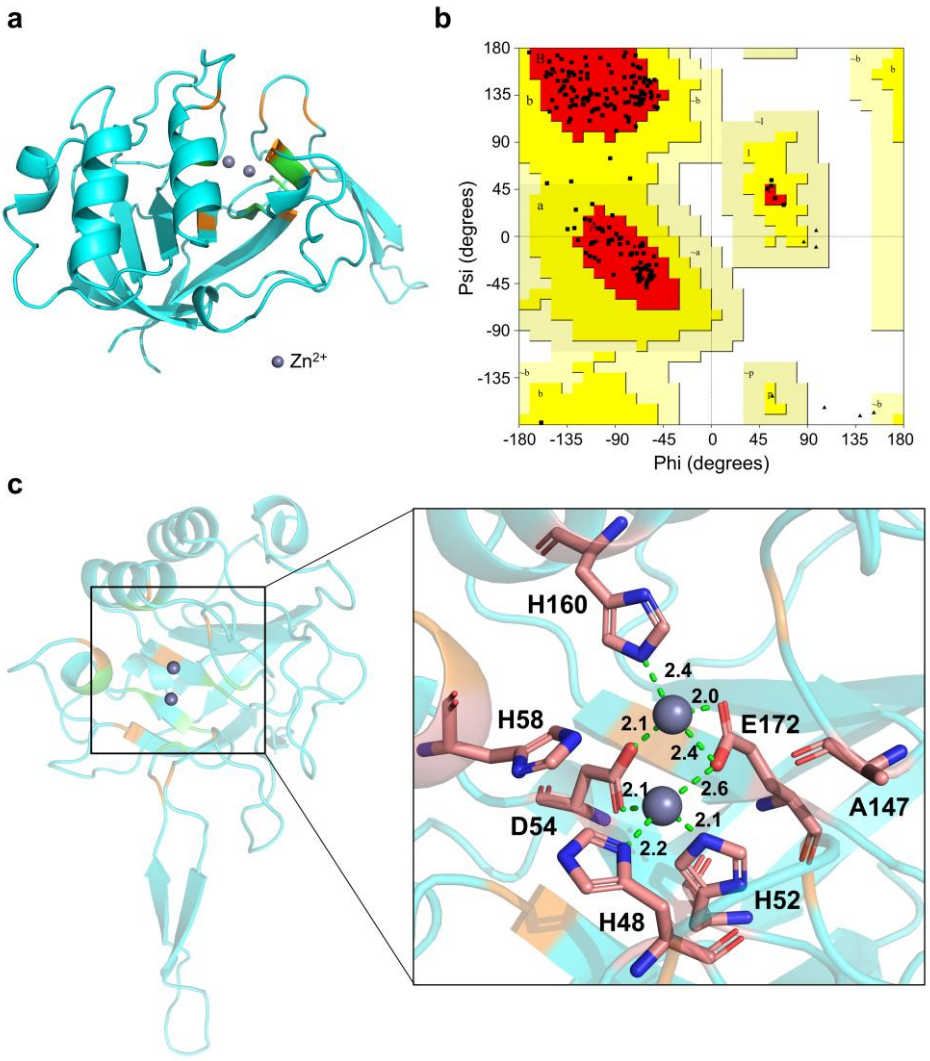



**Figure S2. *Ach*AF structure predicted by AlphaFold3. a)** Cartoon representation of the predicted WT *Ach*AF structure containing two coordinated metal ions $Zn^{2+}$. **b)** The rationality of the Ramachandran plot is 92.7%. **c)** Cartoon representation of the amino acids that coordinate with the two $Zn^{2+}$.

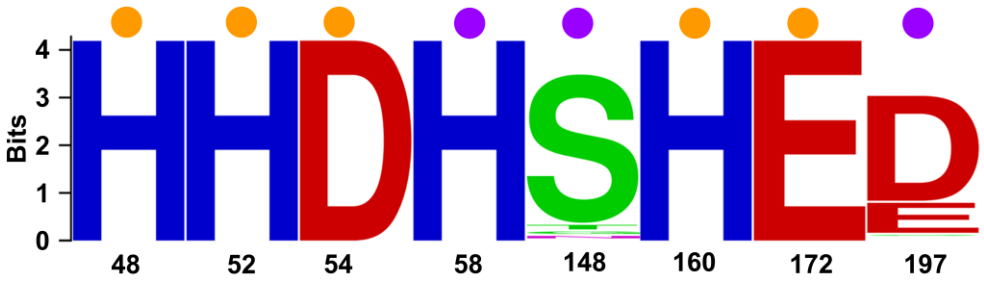



**Figure S3. Conservation analysis of amino acids coordinating with zinc cations (marked by orange dots) and catalytic triads (marked by purple dots) for *Ach*AF and its 150 homologous proteins.**

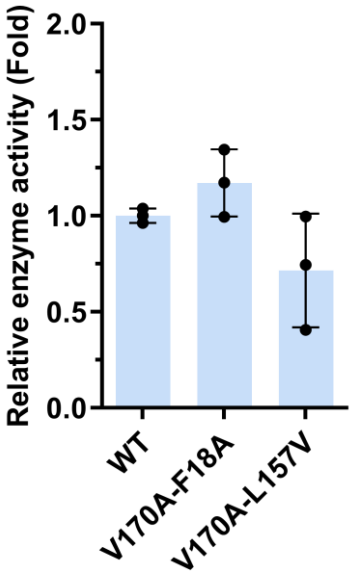



**Figure S4. Relative enzyme activities of combinatorial mutagenesis of V170A, F18A and L157V.**

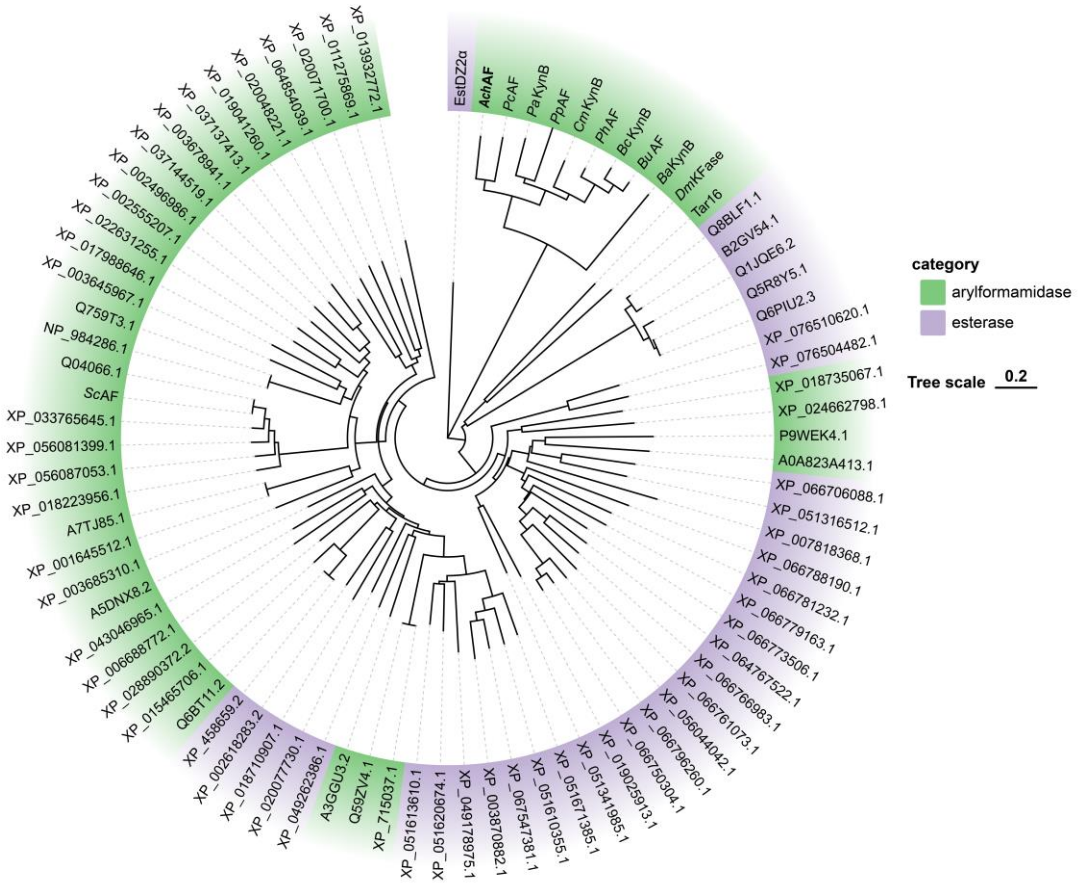



**Figure S5. Phylogenetic tree analysis of kynurenine formamidases and esterases for Homologous proteins of *Ach*AF[19].**

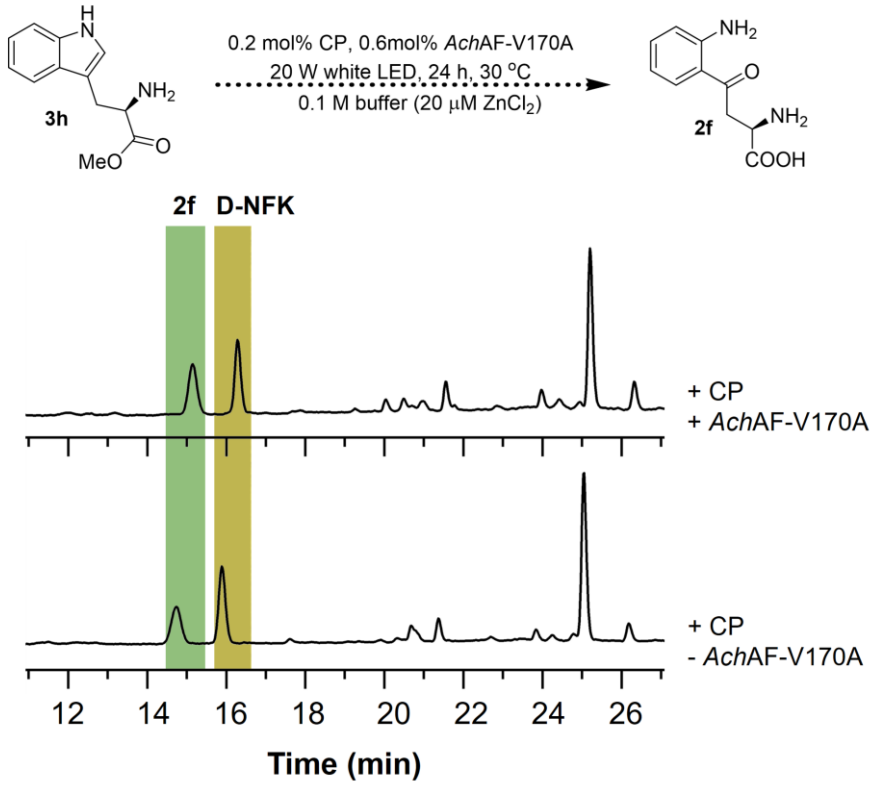



**Figure S6. Synthetic analysis of D-Kyn (2f) from D-Trp-OMe.** Reaction conditions: **3h** (1 μmol), CP (0.2 mol%), *Ach*AF-V170A (0.6 mol%), EtOH (0.5% v/v), PB buffer (0.1 M, pH 8.0 containing 20 μM $ZnCl_2$) in 1.6 mL reaction system irradiated by 20 W white LED at 30 ℃ in $O_2$ for 24 h. For the reaction with *Ach*AF-V170A, the yield of **2f** was 17.2%, while for the reaction without *Ach*AF-V170A, the yield of **2f** was still 16.9%.

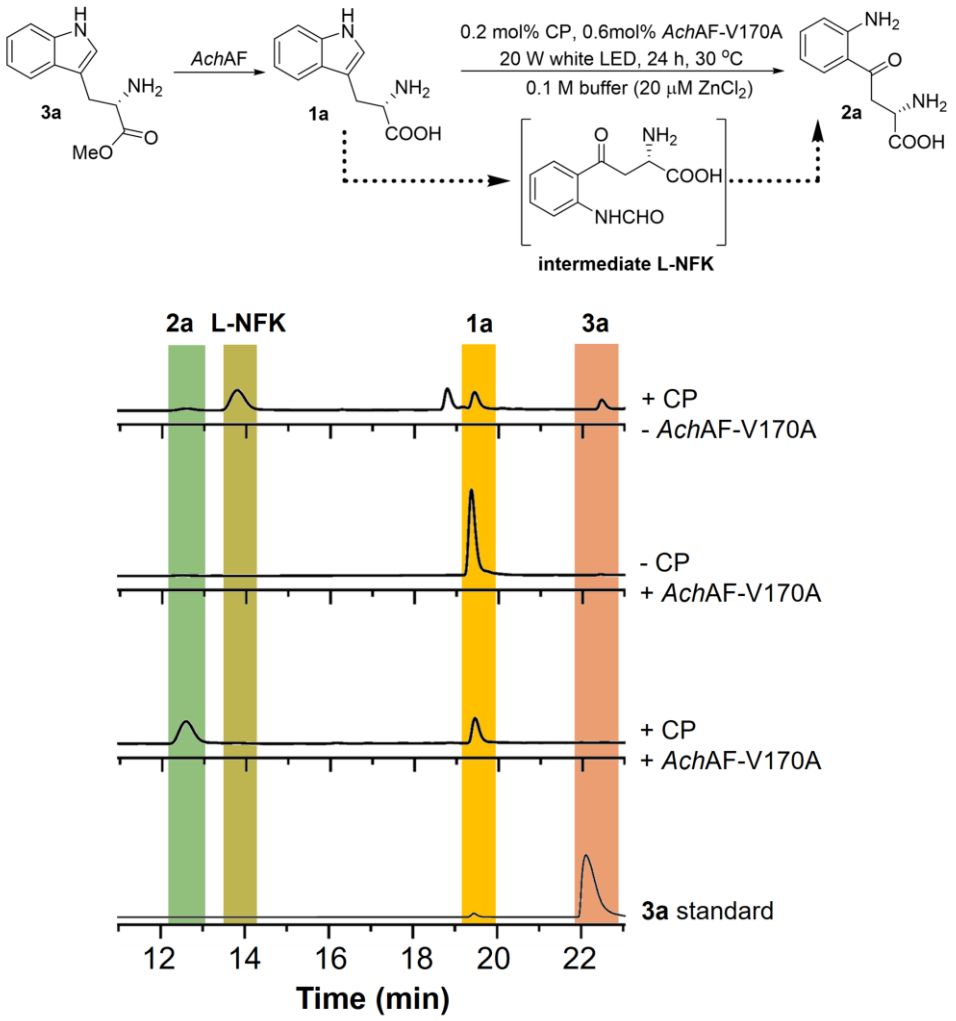



**Figure S7. Synthetic analysis of L-Kyn from tryptophan esters.** Reaction conditions: **3a** (1 μmol), CP (0.2 mol%), *Ach*AF-V170A (0.6 mol%), EtOH (0.5% v/v), PB buffer (0.1 M, pH 8.0 containing 20 μM $ZnCl_2$) in 1.6 mL reaction system irradiated by 20 W white LED at 30 ℃ in $O_2$ for 24 h.

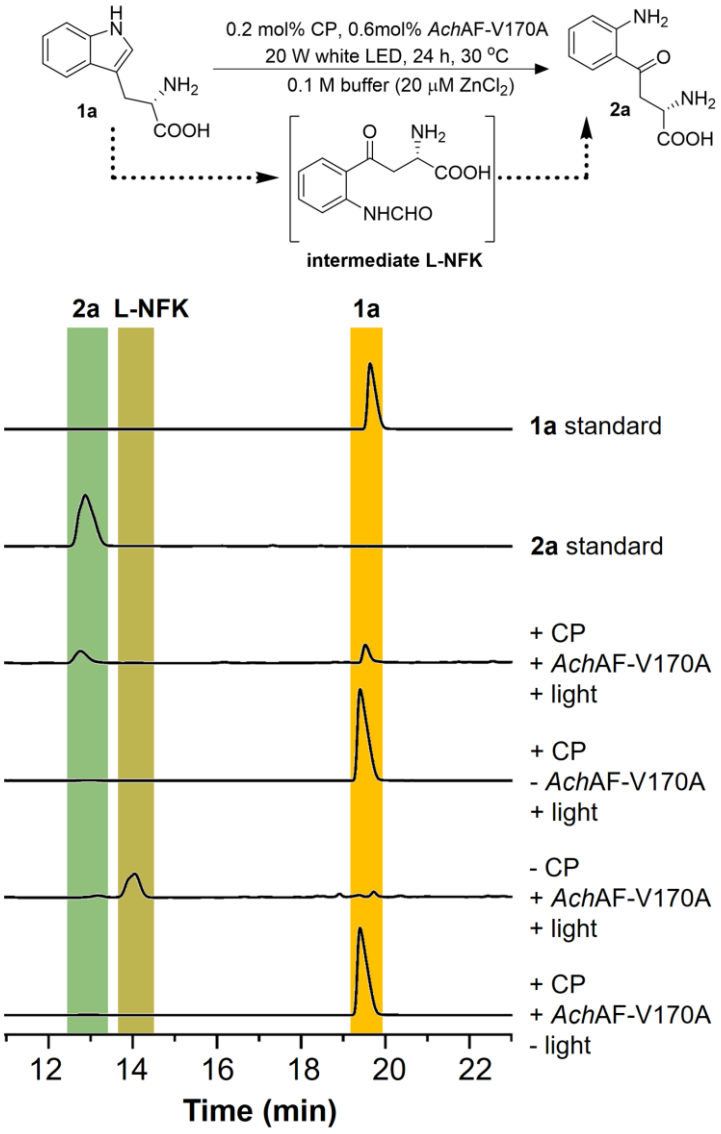



**Figure S8. Control experiments of the model reaction for mechanism investigation.**

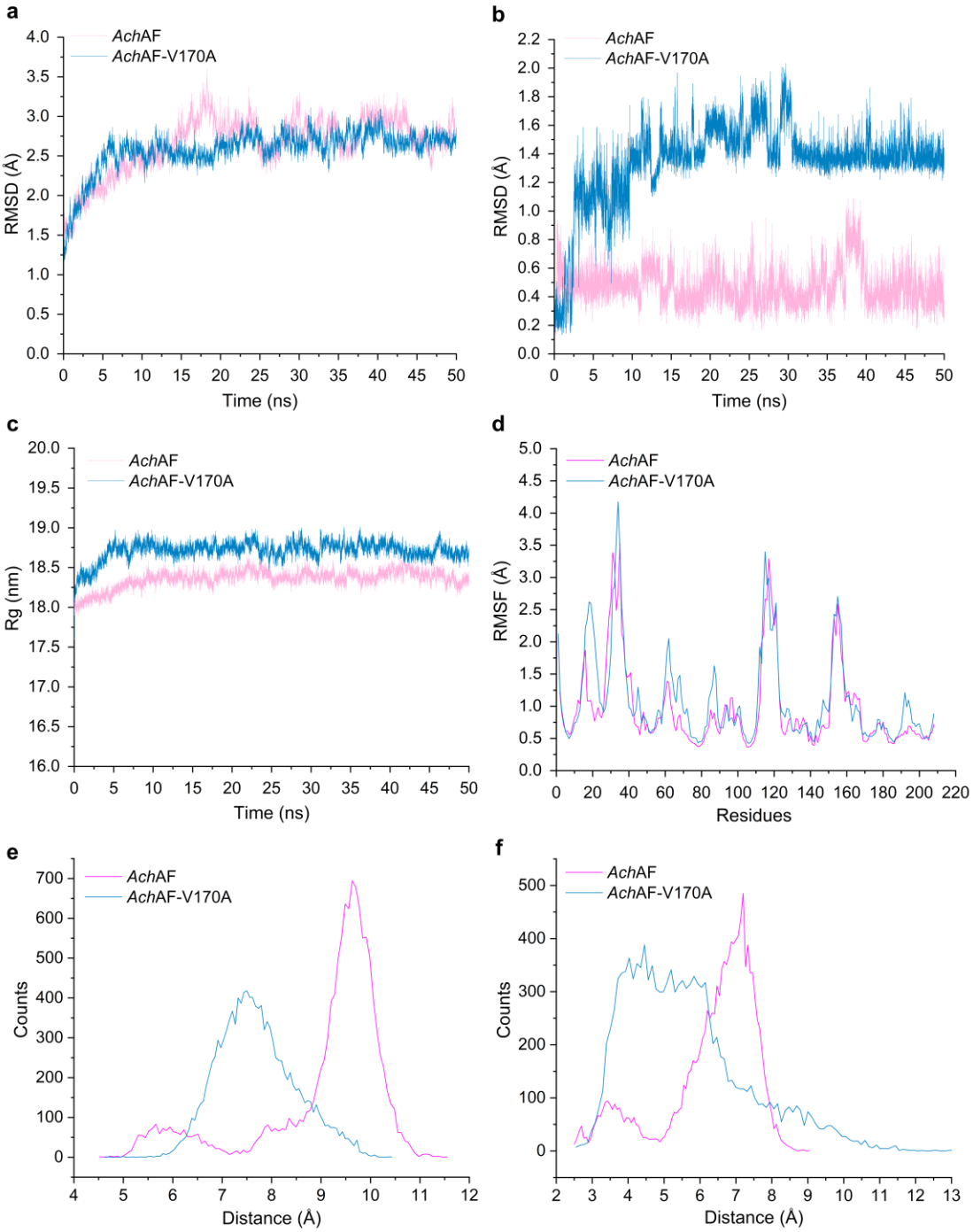



**Figure S9. Molecular dynamics simulations for mutational effects on *Ach*AF. a)** Root mean square deviation (RMSD) of *Ach*AF and *Ach*AF-V170A; **b)** Root mean square deviation (RMSD) of substrate L-NFK in *Ach*AF and *Ach*AF-V170A; **c)** Radius of gyration (Rg) of *Ach*AF and *Ach*AF-V170A; **d)** Root mean square fluctuation (RMSF) of *Ach*AF and *Ach*AF-V170A; **e)** Distance between proton of H58 in *Ach*AF and *Ach*AF-V170 and N atom of the formyl group in L-NFK; **f)** Distance between proton of S148 in *Ach*AF and *Ach*AF-V170 and O atom of the formyl group in L-NFK.

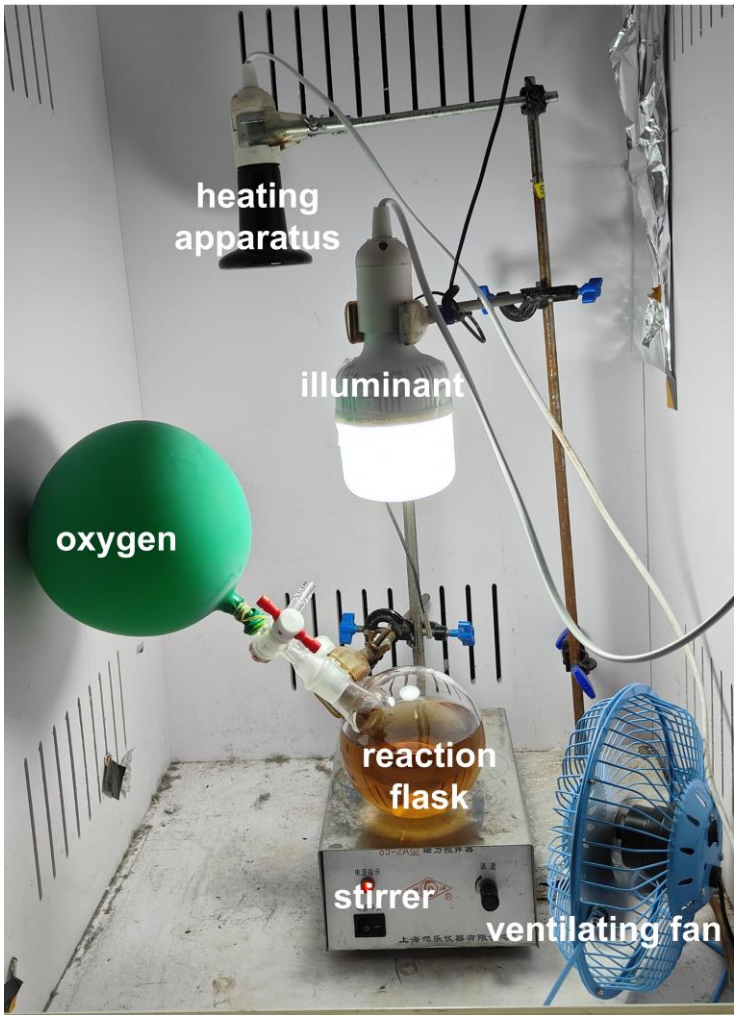



**Figure S10. Photoenzymatic reaction set up for the scale up synthesis.**

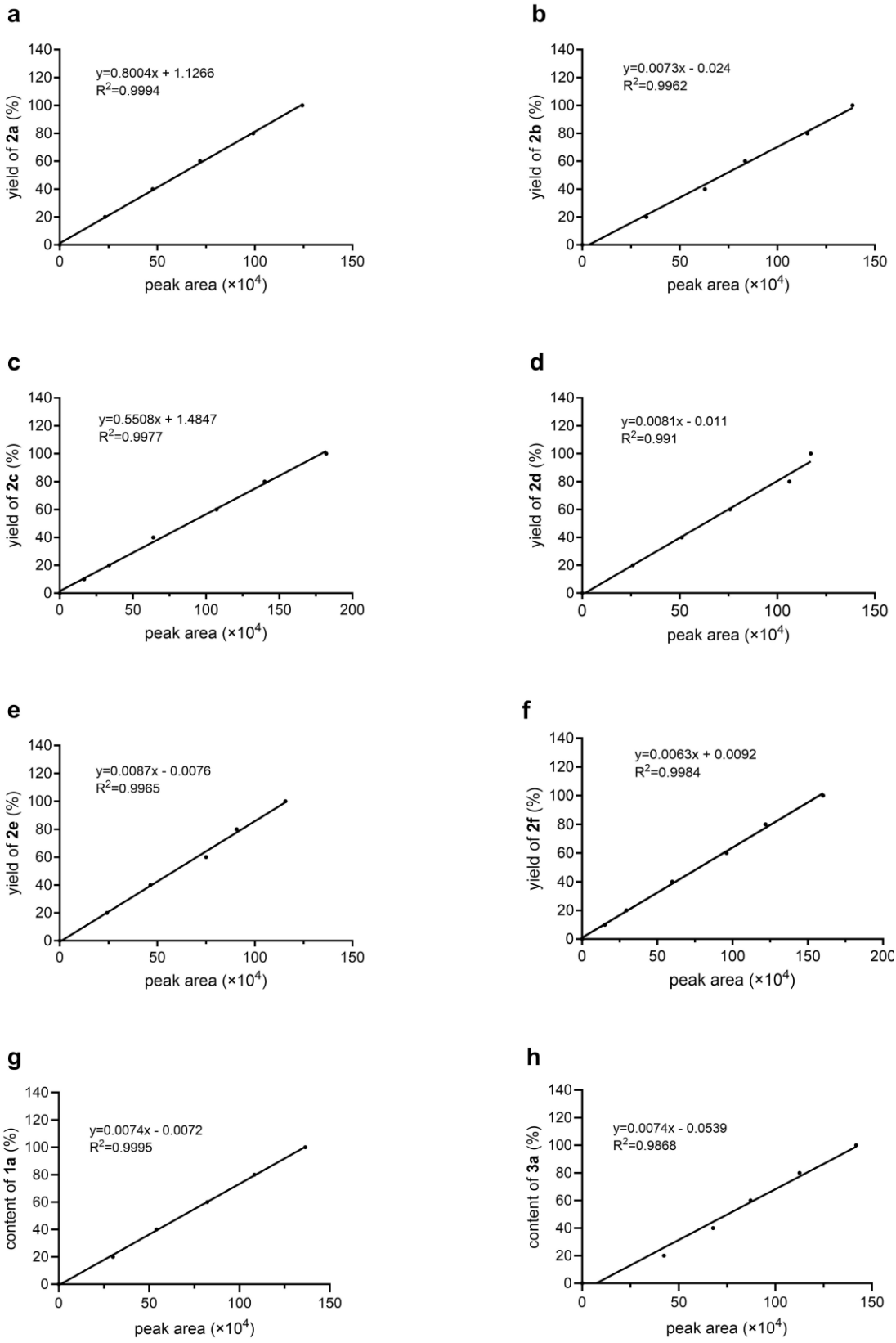



**Figure S11. Standard curves for desired products and substrates.**

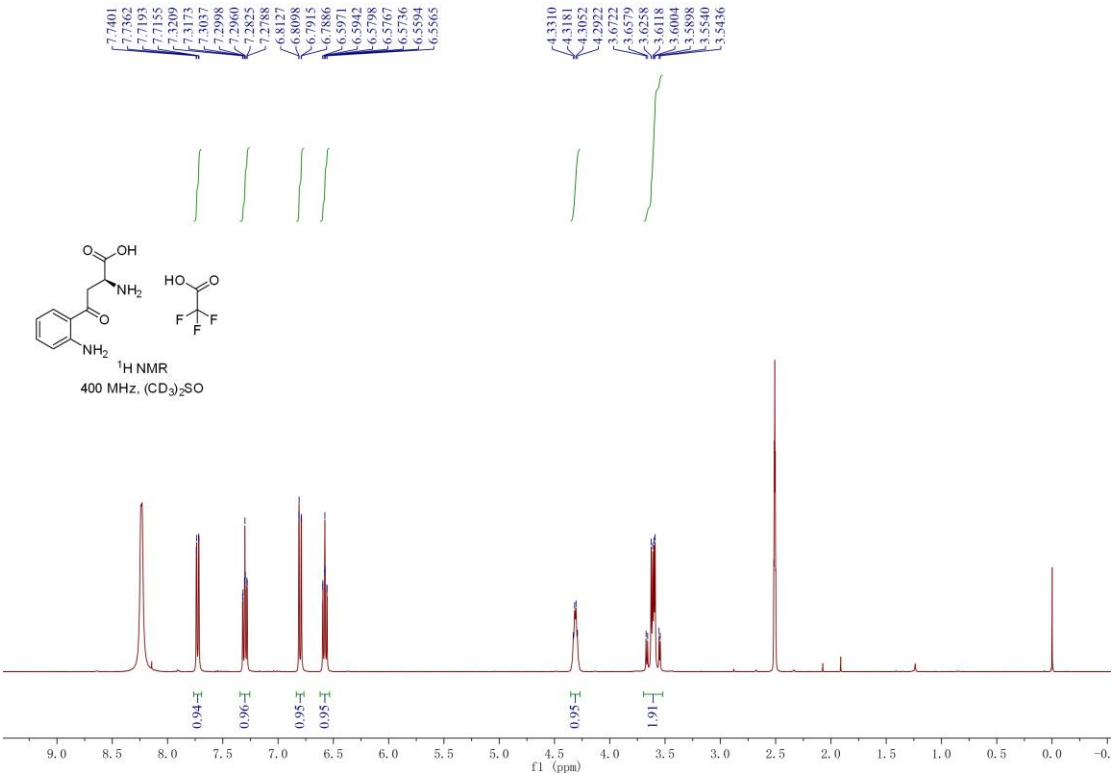



**Figure S12. $^{1}$H NMR spectra of 2a in $(CD_3)_2SO$ with 10% (v/v) TFA.**

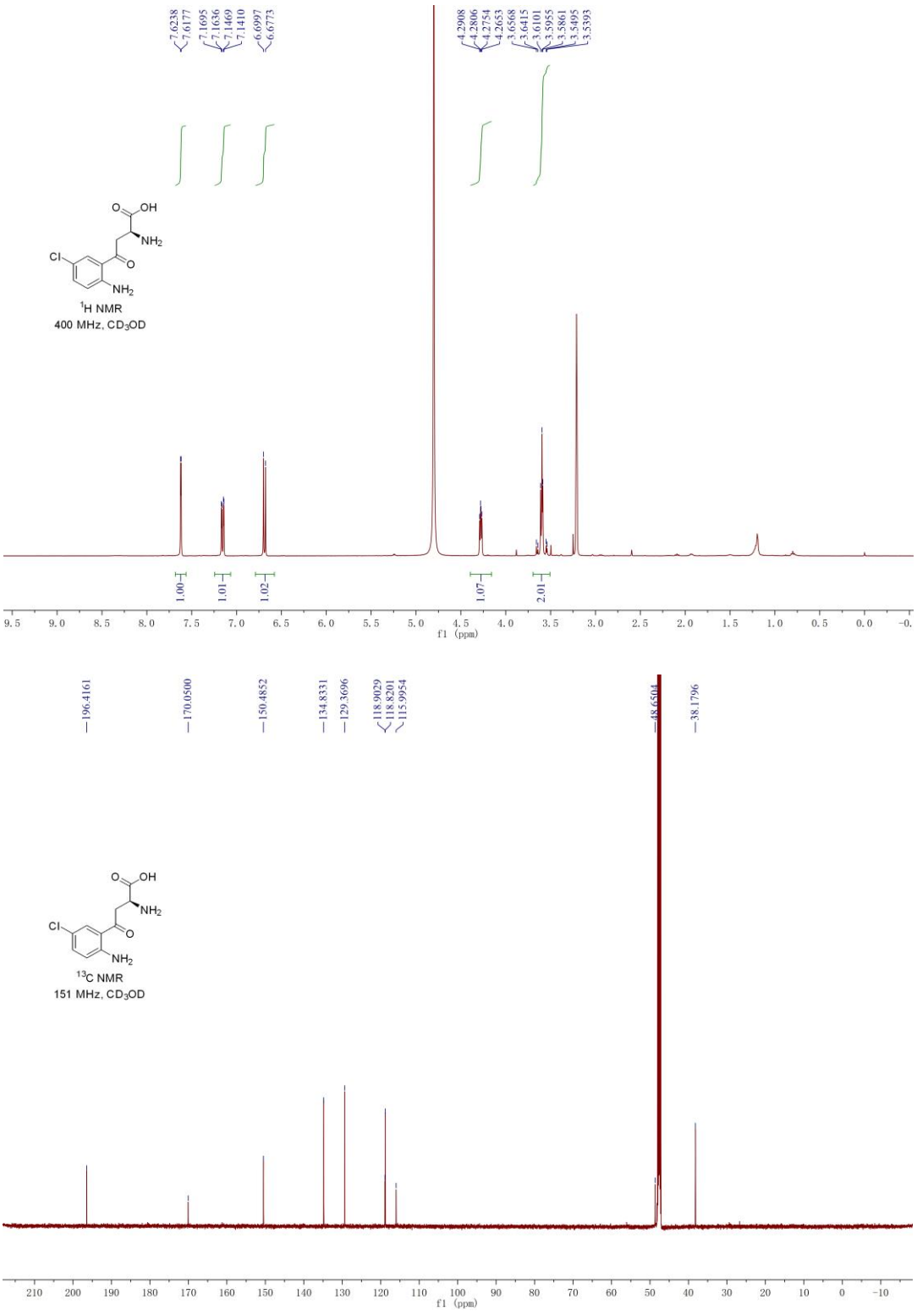



**Figure S13. $^{1}$H NMR and $^{13}$C NMR spectra of 2b in $CD_3OD$.**

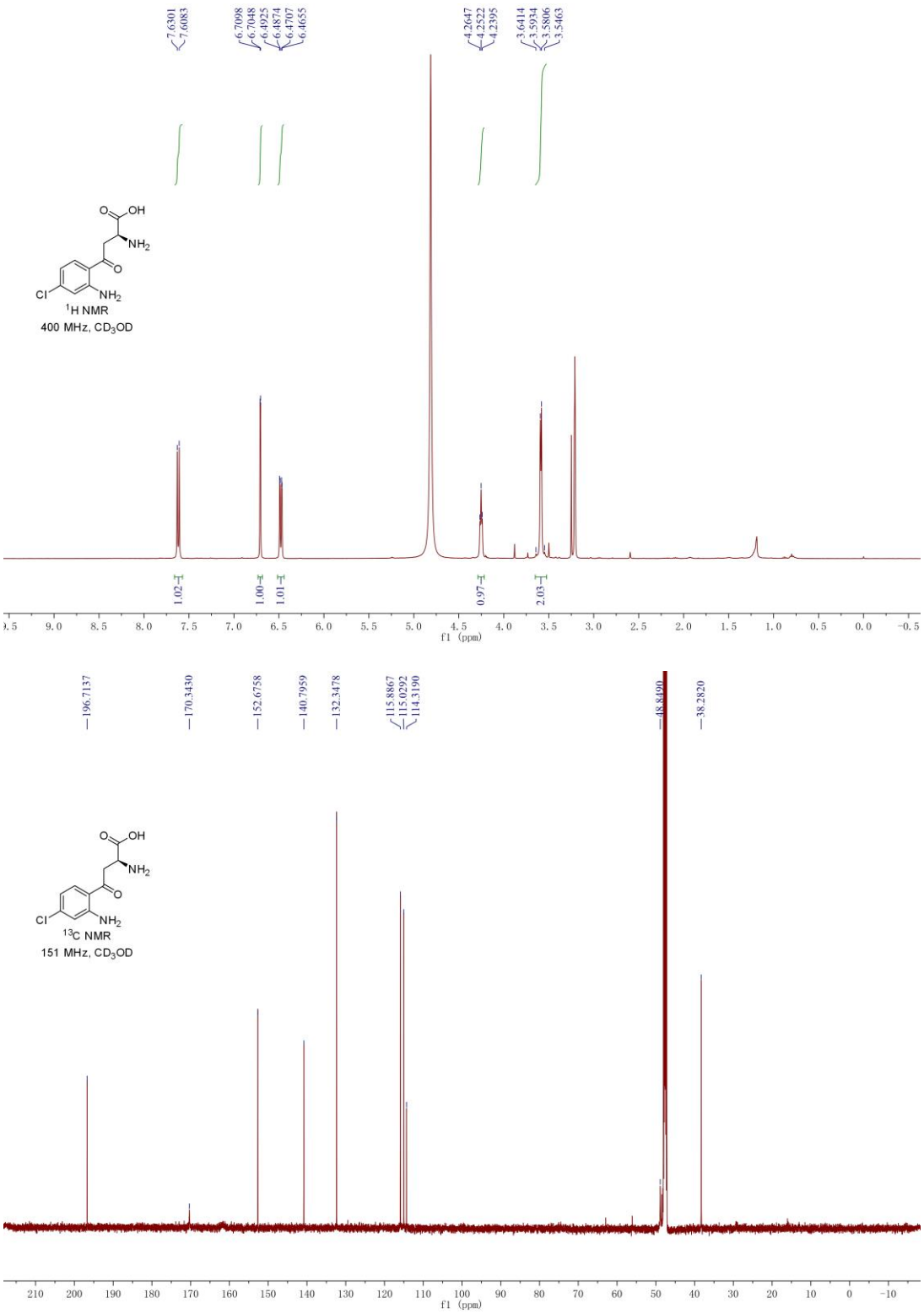



**Figure S14. $^{1}$H NMR and $^{13}$C NMR spectra of 2c in $CD_3OD$.**

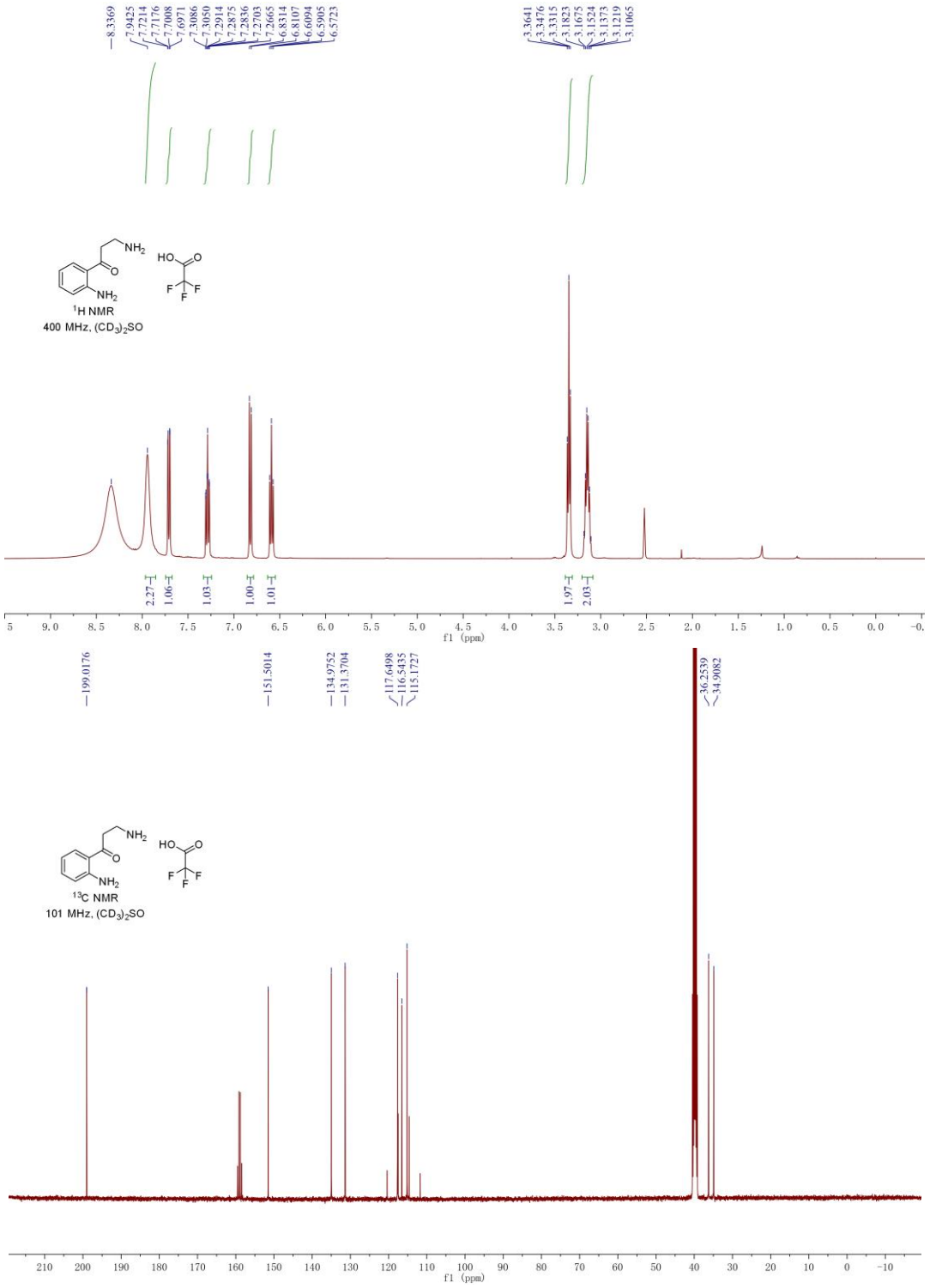



**Figure S15. $^{1}$H NMR and $^{13}$C NMR spectra of 2d in $(CD_3)_2SO$ with 10% (v/v) TFA.**

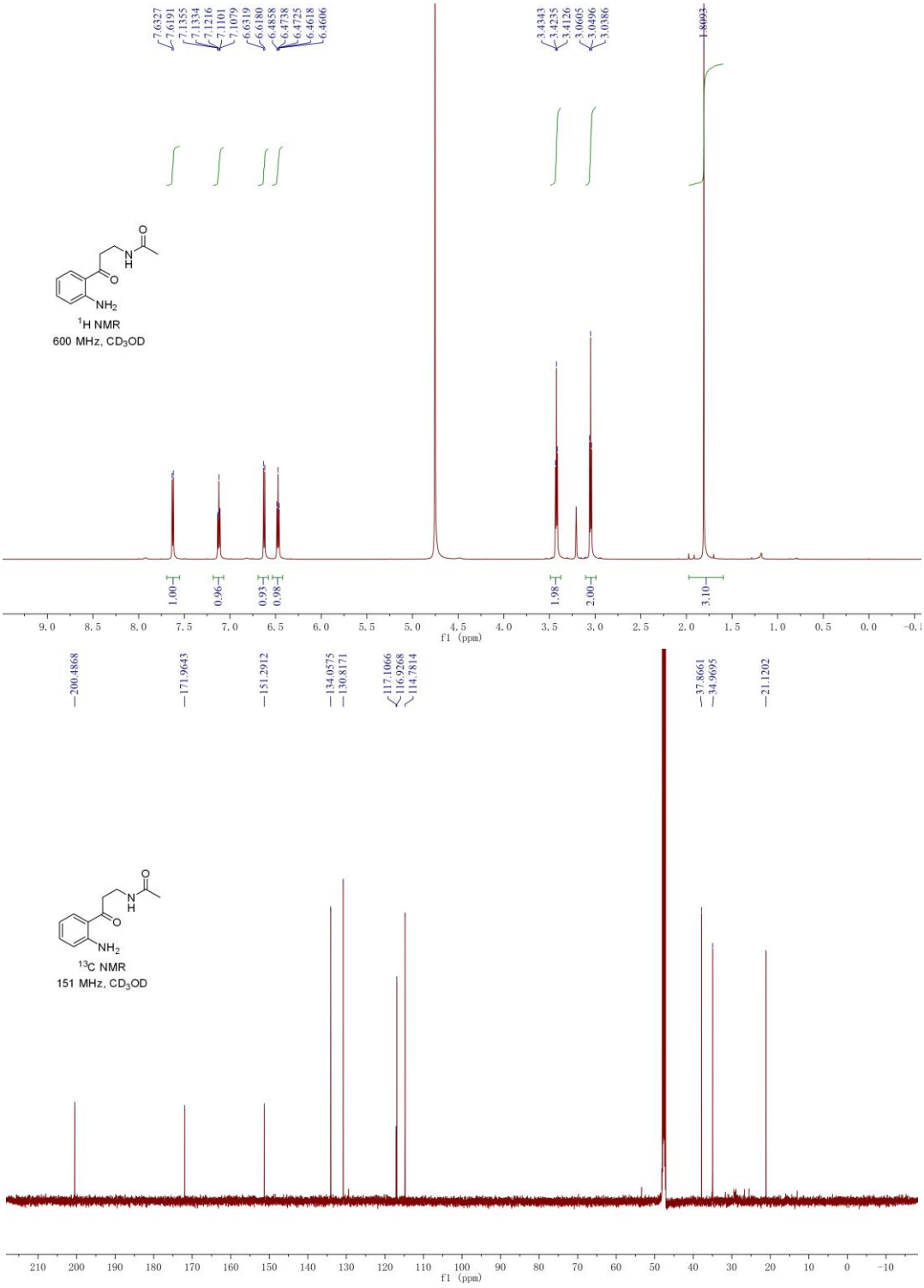



**Figure S16. $^1$H NMR and $^{13}$C NMR spectra of 2e in CD$_3$OD.**

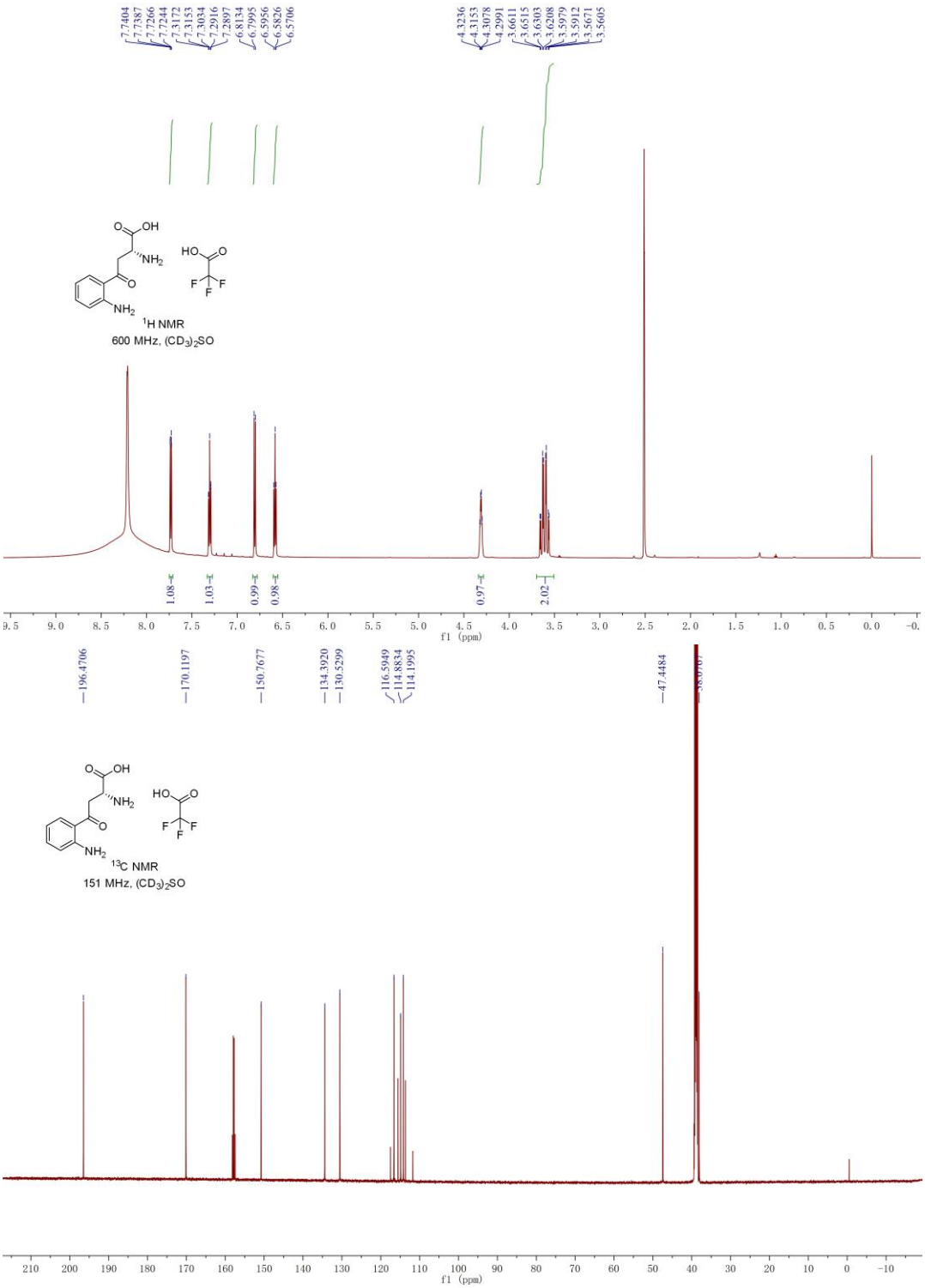



**Figure S17. $^{1}$H NMR and $^{13}$C NMR spectra of 2f in $(CD_3)_2SO$ with 10% (v/v) TFA.**

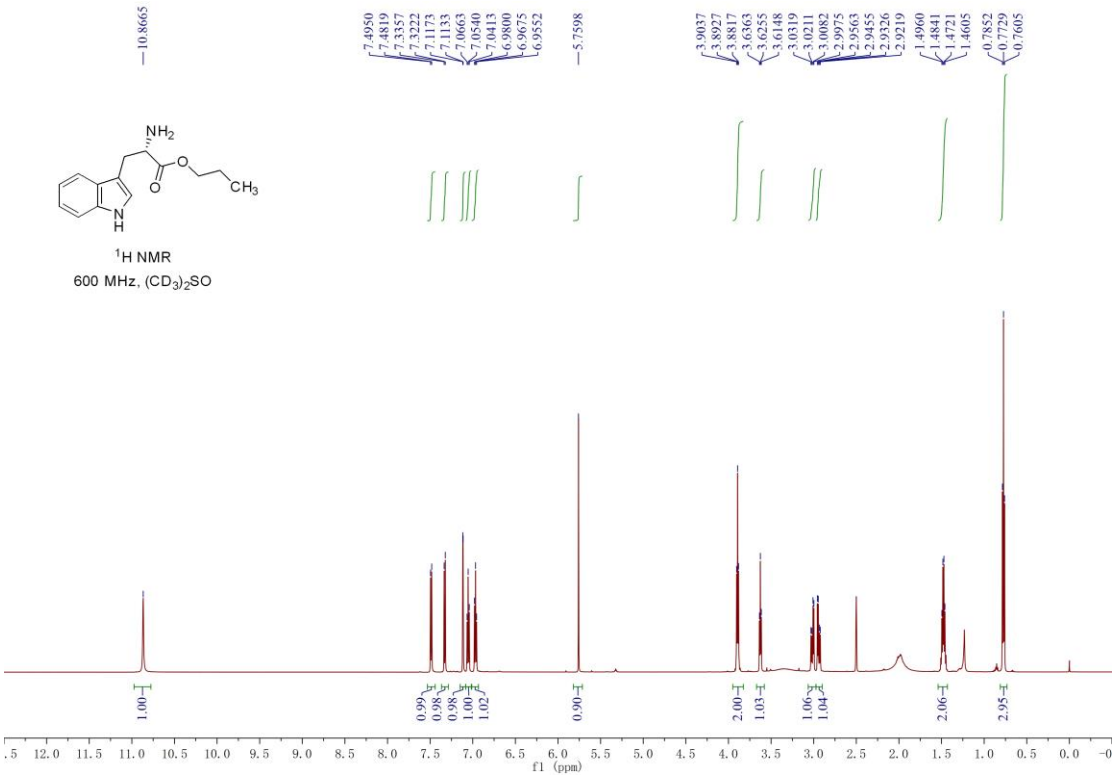



**Figure S18. $^{1}$H NMR spectra of 3c in $(CD_3)_2SO$.**

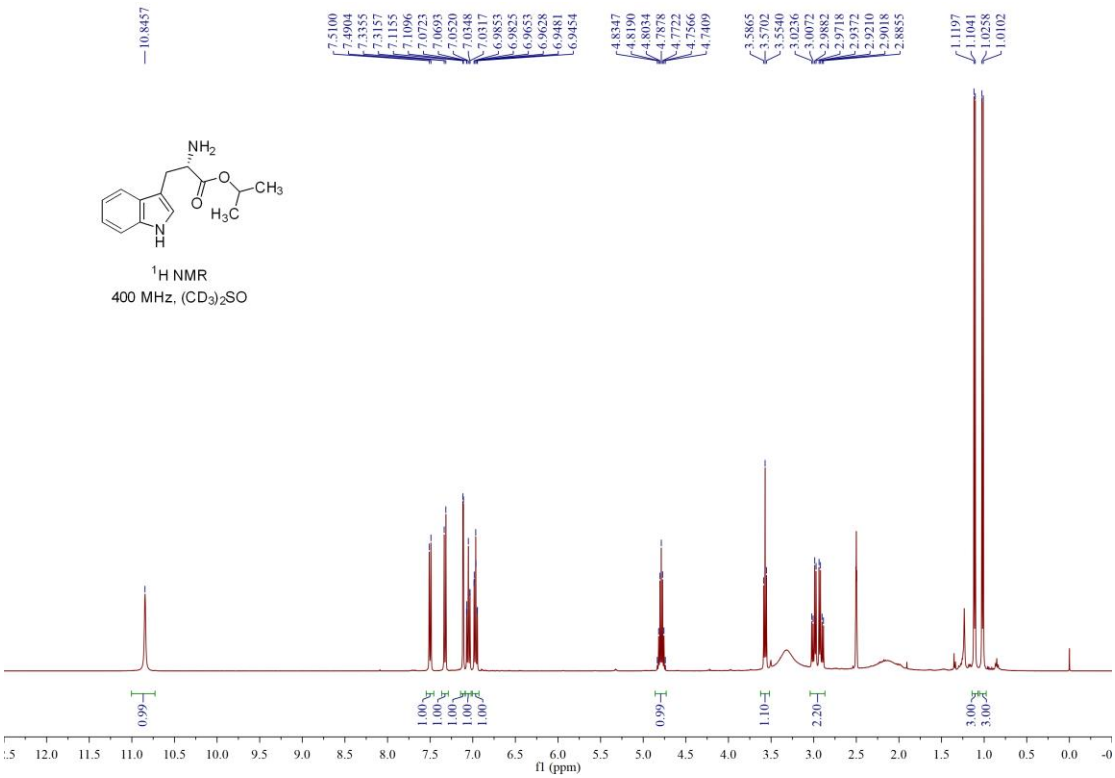



**Figure S19. $^{1}$H NMR spectra of 3d in $(CD_3)_2SO$.**

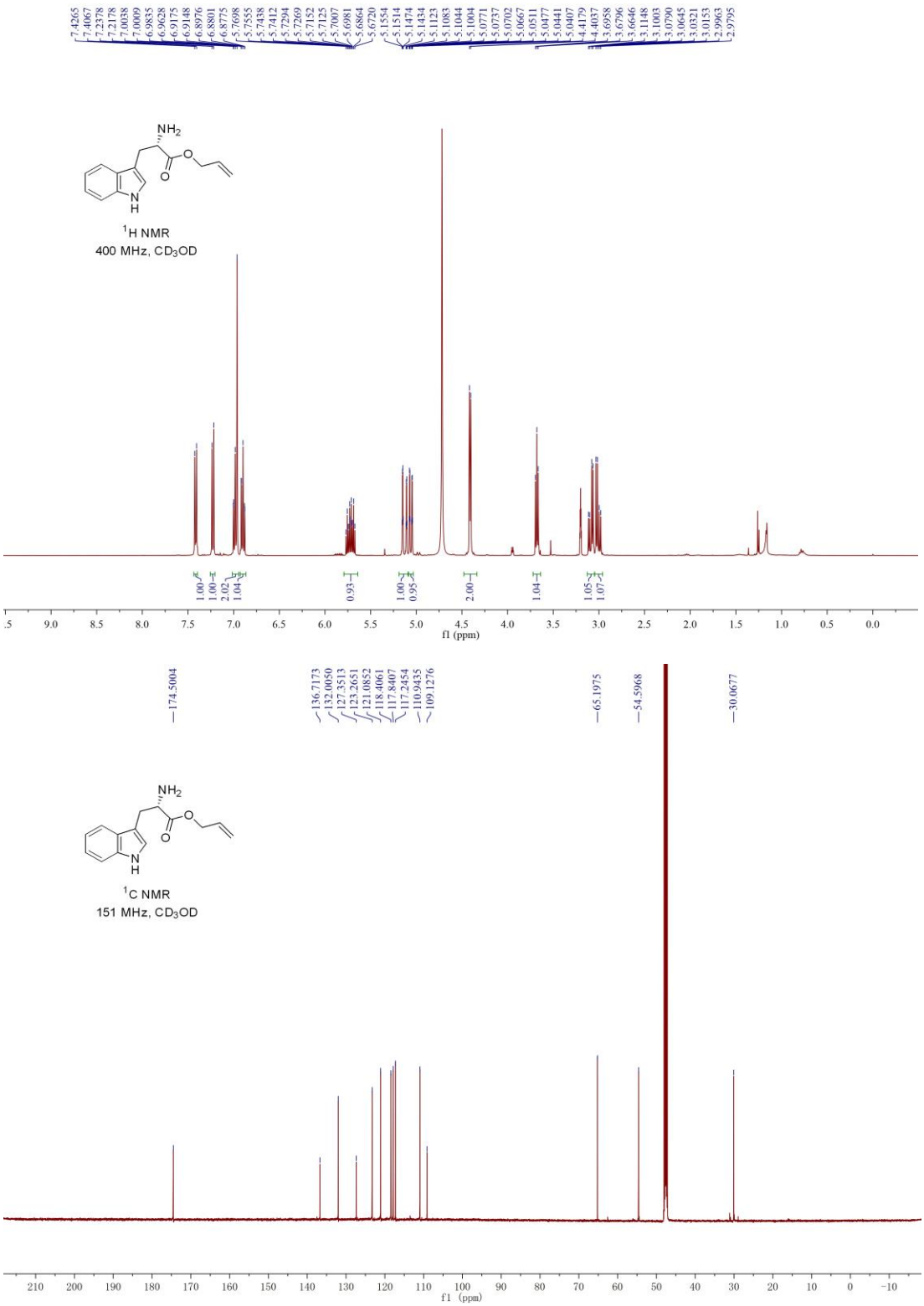



**Figure S20. $^{1}$H NMR and $^{13}$C NMR spectra of 3e in $CD_3OD$.**

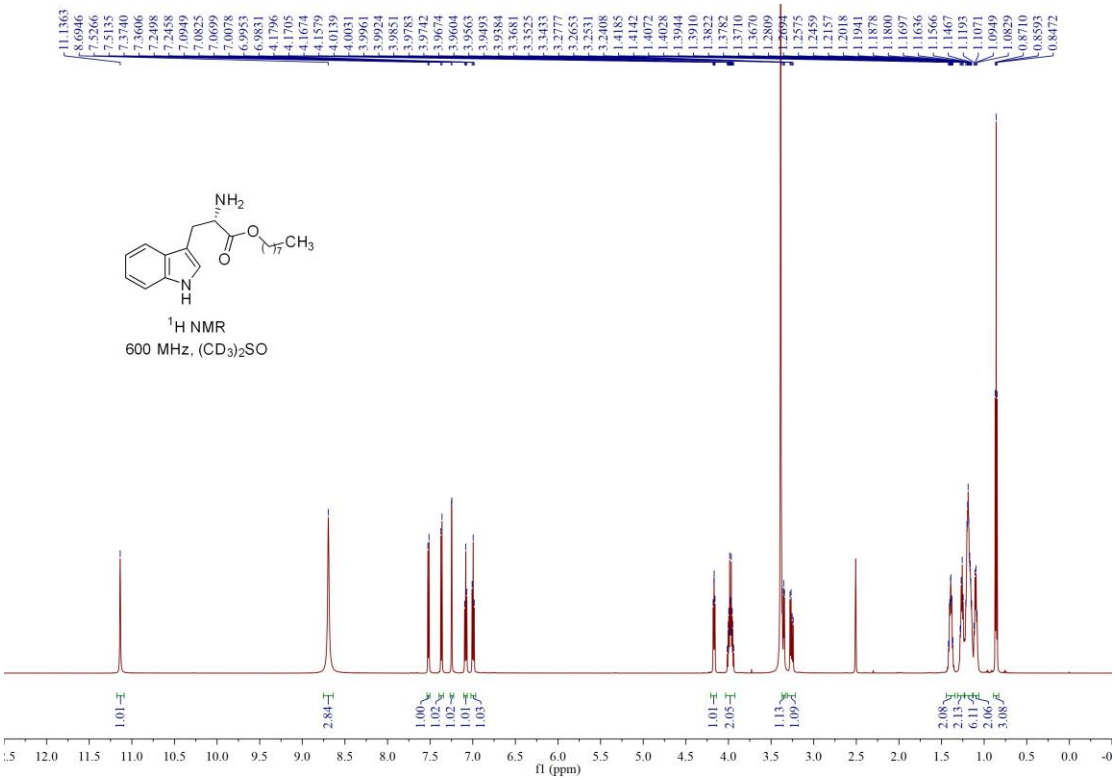



**Figure S21. $^{1}$H NMR spectra of 3f in $(CD_3)_2SO$.**

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