SUPPORTING INFORMATION

Programing a cyanide-free transformation of aldehydes to nitriles and one-pot synthesis of amides through tandem chemo-enzymatic cascades

Haoteng Zheng,[a] Qinjie Xiao,[a] Feiying Mao,[a] Anming Wang,*[a] Mu Li,[b] Qiuyan Wang,[c] Pengfei Zhang [a] and Xiaolin Pei *[a]

[a] *College of Material, Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou, 311121, PR China*

[b] *College of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, PR China*

[c] *School of Basic Medical Sciences, Hangzhou Normal University, Hangzhou, 311121, PR China*

*Corresponding author: Pei Xiaolin; Tel.: +86-571-28865978; Email: pxl@hznu.edu.cn (X. Pei); waming@hznu.edu.cn (A. Wang)

1. Experimental information

1.1 Chemicals and Biological agents

All aldehydes were purchased from Bidepharm (Shanghai, China) and Aladdin (Shanghai, China). Oximes and amides were purchased from Energy Chemical (Shanghai, China) and Alfa Aesar (Thermo Fisher Scientific, USA). Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Sigma-Aldrich (Merck KGaA, Germany). DNA polymerase PrimeSTAR, T4 DNA ligase, and all restriction endonucleases (*Bam*H I, *Not* I *Nde* I and *Eco*R V) were purchased from Takara Biomedical Technology (Beijing) Co., Ltd. Other analytical-grade chemicals were obtained from Sinopharm Chemical Reagent (Shanghai, China).

1.2 Media

E. coli strains were cultivated in Luria-Bertani (LB) medium with kanamycin (50 μg/mL). The composition was 10 g/L peptone, 5 g/L yeast extract, and 10 g/L NaCl. LB-agar medium was complemented with 15 g/L agar before sterilization (121 ℃ for 20 min). A modified Terrific Broth (TB) medium was used for the expression of recombinant OxdF1 and NHase1229 in 5-L fermentor. A one-liter solution consists of 20 g tryptone, 24 g yeast extract, 10 g glycerol, potassium phosphate buffer (12.52 g/L K_2HPO_4, 2.32g/L KH_2PO_4) and cobaltous chloride (0.1 mM) as required.

1.3 Recombinant enzymes expression in E. coli BL21 (DE3)

Chemical competent *E. coli* BL21 (DE3) cells (100 μL) were transformed with pROxdF1 and pROxdF1-NHase according to standard protocols. *E. coli* cells were plated on LB agar containing kanamycin (50 μg/mL) and incubated overnight at 37 ℃,

ang the positive clones were screened and confirmed by gene sequencing (QingKe Biological Technology, Hangzhou, China).

A single clone was transferred into 10 mL of LB medium and shaken overnight at 37 ℃ and 220 rpm. The preinoculum culture was inoculated into 100 mL TB medium in 500-mL shaker flasks with a 2.5% of inoculation volume. After 3 h of culture at 37 ℃ to a density of OD_{600} = 0.6, 20 μL of 0.5 M IPTG stock solution was added to induce the expression of recombinant enzymes at 18 ℃ and 220 rpm for 14 h. *E. coli* cells were harvested by centrifugation (8000 × g, 5 min, 4 ℃). The collected cells were washed and suspended in 50 mM phosphate buffer (pH 7.0) to a final concentration of 30 mg/mL (dry cell weight, CDW).

Bach fermentations were carried out in a 5-L jar fermenter (Biotech-5BG, BXBIO, Shanghai, China) with an initial 3 L of modified TB medium. The inoculum culture was inoculated into the fermenter under sterile conditions. The inoculation volume was 5%. Th culture was performed at 37 ℃ for 3 h until an OD600 of approximately 2 was obtained. The temperature was gradually decreased to 18 ℃, and 500 mM IPTG was added to induce the expression of recombinant OxdF1 and NHase1229. During the entire process, the pH was maintained at 7.0, and antifoam was added manually when necessary. The dissolved oxygen level (DO) was maintained at approximately 30% air saturation throughout fermentation, which was combined the stirring rate and manually controlled by the airflow rate.

1.4 Analytical methods

The expression of the recombinant enzymes was analyzed using Sodium dodecyl

sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 15%) with 4% stacking gel. The collected cells were resuspended in 50 mM PPB (pH7.0) to a final concentration of OD600 at approximately 10. The cell suspension was subsequently disrupted through sonication (Sonicator 400, Misonix, USA) in an ice bath. The crude cell extract was centrifuged at 4 ℃ and 10,000 × g for 20 min to separate the insoluble cell debris. Protein concentration was determined by a Bradford protein assay kit (Sangon Biotech, China).

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III 400 (1H: 400MHz) spectrometer. Proton chemical shifts (δ) are reported in ppm with respect to the solvent signal of CDCl3 Peak multiplicities were reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, m = multiplet, br = broad signal.

2. Aldoxime dehydratase from *Pseudomonas putida* F1 (OxdF1) sequence, plasmid, and expression

2.1 Base sequence (GenBank: CP000712.1)

> OxdF1

ATGGAATCTGCAATCGATAAACACCTCGTGTGCCCGCGCACGTTATCGCGCC
GGGTGCCTGATGATTACCAGCCGCCCTTCCCGATGTGGGTCGGCCGCGCTGA
TGAGCAGCTGACGCAAGTAGTGATGGCCTATCTGGGTGTGCAGTACCGGGG
AGATGGTCAGCGTGAGCGGGCCTTGCAAGCGATGCGCGAGATTCTCGGCAG
CTTTAGCTTAACCGACGGCCCGCTGACTCACGACCTGACGCACCACACCGA
CAGCAGTGGCTACGACAACCTGATGATCGTCGGCTACTGGAAAGATGCCGG

CGCTTACTGCCGCTGGTTACGCTCGCCTGAGGTGGATGGCTGGTGGAGTTCG
CCGCAACGCTTGAATGATGGCCTTGGTTACTACCGCGAAATCACCGCGCCG
CGGGCCGAGCAGTTCGAGACGCTGTATGCGTTCCAGAACGATTTGCCCGGG
GTCGGGGCGATCATGGACAACACCAGTGGCGAAATCGAGGAACACGGTTAC
TGGGGCTCGATGCGCGACCGTTTCCCGGTATCGCAAACGGACTGGATGAAC
CCGAACGGCGAACTGCGAGTCGTGGCTGGCGACCCGGCCAAAGGCGGTCGC
GTGGTGGTGCTCGGCCATGACAATATCGCCTTGATTCGCTCGGGCCAGGACT
GGGCAACGGCAGAAGCGGCTGAGCGCTCGCTGTACCTGGATGAAATCTTGC
CGACCTTGCAGGATGGTATGGATTTTCTGCGCGATAACGGCCAGCCGCTGG
GCTGTTACAGCAATCGCTTTGTACGCAATATCGACGCTGACGGCAATCTACT
CGACATGAGTTACAACATCGGCCACTGGCGTTCACTGGAGAAACTCGAACG
CTGGGCGGAATCGCACCCCACCCATCTGCGTATTTTCGTCACCTTCTTTCGG
GTAGCGGCAGGGCTCGAGAAGTTGCGGCTCTATCACGAAGTCTCCGTGTCG
GATGCAAGCAGCCAGGTGTTCGAATACATCAACTGCCACCCACACACCGGC
ATGTTGCGCGACGCAAAAGTCAGTTCCAACTAG

2.2 Amino acid sequence

>OxdF1

MESAIDKHLVCPRTLSRRVPDDYQPPFPMWVGRADEQLTQVVMAYLGVQYRG
DGQRERALQAMREILGSFSLTDGPLTHDLTHHTDSSGYDNLMIVGYWKDAGAY
CRWLRSPEVDGWWSSPQRLNDGLGYYREITAPRAEQFETLYAFQNDLPGVGAI
MDNTSGEIEEHGYWGSMRDRFPVSQTDWMNPNGELRVVAGDPAKGGRVVVL
GHDNIALIRSGQDWATAEAAERSLYLDEILPTLQDGMDFLRDNGQPLGCYSNRF
VRNIDADGNLLDMSYNIGHWRSLEKLERWAESHPTHLRIFVTFFRVAAGLEKLR
LYHEVSVSDASSQVFEYINCHPHTGMLRDAKVSSN*

3. Nitrile hydratase from *Aurantimonas manganoxydans* ATCC BAA-1229 (NHase1229) sequence, plasmid, and expression

3.1 α subunit base sequence of NHase1229 (GenBank: KP236109.1)

>NHase1229-α

ATGACGGGATCGCACGGCAGGGACGGTGATCACCACGGCCATCACCACGAC

CGTGATCACGACAACCATCTCGACCCGATGACCGCGCGGGTCATGGCGCTGG
AGACGATCCTCACCGAAAAGGGCATGGTCGACCCGGACGCCCTCGACGCCA
TCATCGACACCTACGAGACCAAGGTCGGGCCGCGCAACGGCGCCAGCGTCG
TCGCCAAGGCCTGGAGCGACCCGGACTACGCCGACTGGCTGGCGCGCGACG
CAACCGCCGCCATTGCCTCGCTTGGCTTCACCGGCCGCCAGGGCGAGCACAT
GCAGGCGGTGTTCAACACCCCGGAGCGCCACAACCTCGTCGTCTGCACCCT
GTGCTCCTGCTATCCGTGGTCAGTGCTCGGCCTGCCGCCGGTCTGGTACAAG
TCGCCGCCCTATCGCTCGCGCGCCGTCTCCGATCCGCGCGGCGTCCTGCGCG
AATTCGGCGTCGCGCTGCCGGACGGCGTCTCGGTGCGAGTCTGGGACTCCAC
CGCCGAGCTGCGCTACCTCGTCGTGCCCGAGCGCCCGGCGGGTACCGAGGG
ACTGTCCGAGGCGGCGCTGGCGGCGCTCGTCACCCGCAAGTCCATGATCGGT
ACCGAGCGTGACCTGAGCCCGCATGCCGCGCCGGAGACGGCGGCATGA

3.2 α subunit Amino acid sequence of NHase1229 (GenBank: AKD27903.1)

>NHase1229-α

MTGSHGRDGDHHGHHHDRDHDNHLDPMTARVMALETILTEKGMVDPDALDAI
IDTYETKVGPRNGASVVAKAWSDPDYADWLARDATAAIASLGFTGRQGEHMQA
VFNTPERHNLVVCTLCSCYPWSVLGLPPVWYKSPPYRSRAVSDPRGVLREFGVA
LPDGVSVRVWDSTAELRYLVVPERPAGTEGLSEAALAALVTRKSMIGTERDLSP
HAAPETAA

3.3 β subunit base sequence of NHase1229 (GenBank: KP236110.1)

>NHase1229-β

ATGAACGGCCCCCACGATCTCGGCGGTCGGCACGGCTTCGGGCCGATCGCGC
CGAAGGCAGACGAGCCGCTGTTCCATGCGCCCTGGGAGCGCCGCGCCCTCG
CCCTGACGCTCGCCGCCGGTGCGATGGGCCATTGGTCGATCGACGAAAGCCG
CGCCGCCCGTGAGGATCGCCACCCGGCCGACTATTACGGTTCGTCCTATTACG
AGATCTGGACCAAGGGCCTTGAGACGCTGCTCCTGCGCCACGGCCTCATCAG
CCATCGCGAATTGCGCGCCGGGCGGCCCCTCGACCTGACCGTGCCGCCGAAC
CGCATCCTGAAGGCCGATGCCGTCGCGCCGGCCCTTGCCAAGGGCAGTCCG
GCCAACCGCGATCCCGAAGGCAGCACGCCCGTTTTCGCGCCGGGCGACAGG

GTCCGCACGCTGAACCTGCAGCCGCGCCATCACATCCGCCTGCCCGCCTATG
CCCGCGAGAAGGCCGGCACCATCGAAACCGTTCAGGGTTTCCATGTCTTCGC
GGATGCCAGCGCCAAGGGCGACGACCATGTCGCGCACTGGCTCTACACGGT
GGTCTTCGACGCATTCACGCTGTGGGGCGGCGACGCTTCGCCCAACGACACC
GTCTCCATCGATGCCTGGGAGCCCTATCTTGCGCACGCCTGA

3.4 β subunit Amino acid sequence of NHase1229 (GenBank: AKD27904.1)

>NHase1229-β

MNGPHDLGGRHGFGPIAPKADEPLFHAPWERRALALTLAAGAMGHWSIDESR
AAREDRHPADYYGSSYYEIWTKGLETLLLRHGLISHRELRAGRPLDLTVPPNRIL
KADAVAPALAKGSPANRDPEGSTPVFAPGDRVRTLNLQPRHHIRLPAYAREKAGT
IETVQGFHVFADASAKGDDHVAHWLYTVVFDAFTLWGGDASPNDTVSIDAWEP
YLAHA

3.5 Activator subunit base sequence of NHase1229 (GenBank: KP236111.1)

>NHase1229-acc

ATGCCTGGGAGCCCTATCTTGCGCACGCCTGAGACCGGCATCGCCGCATCGC
CCGGCCTGCCACGCGATGCGGCGGGTGAACCCGTCTTCTTCGCGCCCTGGCA
GGCCAAGGCCTTCGCCATGACCGTCGCGCTGAACGAGCGCGGCATCCTTGCC
TGGACCGACTGGGCTGCCGCGCTCGGCCGCGCCTGCGCCAGCCTGCCCGCC
GCCGGCCCCTCGCCCGAAGCAACAGCGGATGCCTATTTCACCGCATGGCTCG
TCGCGCTCGAAGAAATCCTCACGGCACGGGCGCTGGTAAGCGCCAATGCCGT
CGACGCGGCGCAGGCCGTCTGGCACCGCGCCGCCGAGGCCACGCCCCACGG
CACGCCGATCCGCTTCGAGGCCGGCCTGCCGAACCCACACGACTGA

3.6 Activator amino acid sequence of NHase1229 (GenBank: AKD27905.1)

>NHase1229-acc

MPGSPILRTPETGIAASPGLPRDAAGEPVFFAPWQAKAFAMTVALNERGILAWT
DWAAALGRACASLPAAGPSPEATADAYFTAWLVALEEILTARALVSANAVDAAQ
AVWHRAAEATPHGTPIRFEAGLPNPHD

3.7 Base sequence of NHase1229 gene cluster

>NHase1229

ATGACGGGATCGCACGGCAGGGACGGTGATCACCACGGCCATCACCACGAC

CGTGATCACGACAACCATCTCGACCCGATGACCGCGCGGGTCATGGCGCTGG

AGACGATCCTCACCGAAAAGGGCATGGTCGACCCGGACGCCCTCGACGCCA

TCATCGACACCTACGAGACCAAGGTCGGGCCGCGCAACGGCGCCAGCGTCG

TCGCCAAGGCCTGGAGCGACCCGGACTACGCCGACTGGCTGGCGCGCGACG

CAACCGCCGCCATTGCCTCGCTTGGCTTCACCGGCCGCCAGGGCGAGCACAT

GCAGGCGGTGTTCAACACCCCGGAGCGCCACAACCTCGTCGTCTGCACCCT

GTGCTCCTGCTATCCGTGGTCAGTGCTCGGCCTGCCGCCGGTCTGGTACAAG

TCGCCGCCCTATCGCTCGCGCGCCGTCTCCGATCCGCGCGGCGTCCTGCGCG

AATTCGGCGTCGCGCTGCCGGACGGCGTCTCGGTGCGAGTCTGGGACTCCAC

CGCCGAGCTGCGCTACCTCGTCGTGCCCGAGCGCCCGGCGGGTACCGAGGG

ACTGTCCGAGGCGGCGCTGGCGGCGCTCGTCACCCGCAAGTCCATGATCGGT

ACCGAGCGTGACCTGAGCCCGCATGCCGCGCCGGAGACGGCGGCATGAACG

GCCCCCACGATCTCGGCGGTCGGCACGGCTTCGGGCCGATCGCGCCGAAGG

CAGACGAGCCGCTGTTCCATGCGCCCTGGGAGCGCCGCGCCCTCGCCCTGAC

GCTCGCCGCCGGTGCGATGGGCCATTGGTCGATCGACGAAAGCCGCGCCGCC

CGTGAGGATCGCCACCCGGCCGACTATTACGGTTCGTCCTATTACGAGATCTG

GACCAAGGGCCTTGAGACGCTGCTCCTGCGCCACGGCCTCATCAGCCATCGC

GAATTGCGCGCCGGGCGGCCCCTCGACCTGACCGTGCCGCCGAACCGCATCC

TGAAGGCCGATGCCGTCGCGCCGGCCCTTGCCAAGGGCAGTCCGGCCAACC

GCGATCCCGAAGGCAGCACGCCCGTTTTCGCGCCGGGCGACAGGGTCCGCA

CGCTGAACCTGCAGCCGCGCCATCACATCCGCCTGCCCGCCTATGCCCGCGA

GAAGGCCGGCACCATCGAAACCGTTCAGGGTTTCCATGTCTTCGCGGATGCC

AGCGCCAAGGGCGACGACCATGTCGCGCACTGGCTCTACACGGTGGTCTTCG

ACGCATTCACGCTGTGGGGCGGCGACGCTTCGCCCAACGACACCGTCTCCAT

CGATGCCTGGGAGCCCTATCTTGCGCACGCCTGAGACCGGCATCGCCGCATC

GCCCGGCCTGCCACGCGATGCGGCGGGTGAACCCGTCTTCTTCGCGCCCTGG

CAGGCCAAGGCCTTCGCCATGACCGTCGCGCTGAACGAGCGCGGCATCCTTG

CCTGGACCGACTGGGCTGCCGCGCTCGGCCGCGCCTGCGCCAGCCTGCCCG

CCGCCGGCCCCTCGCCCGAAGCAACAGCGGATGCCTATTTCACCGCATGGCT
CGTCGCGCTCGAAGAAATCCTCACGGCACGGGCGCTGGTAAGCGCCAATGC
CGTCGACGCGGCGCAGGCCGTCTGGCACCGCGCCGCCGAGGCCACGCCCCA
CGGCACGCCGATCCGCTTCGAGGCCGGCCTGCCGAACCCACACGACTGA

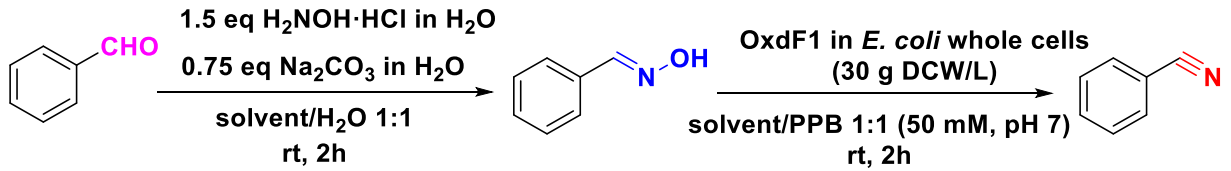
4. Tables

Table S1 Primer sequences used in this study

Primers	**Sequence** [a]	**Restriction site**
OxdF1_Up	CGC*GGATCC*GATGGAATCTGCAATCGATAAACA	*Bam*H I
OxdF1_Down	ATAAGAAT*GCGGCCGC*CTAGTTGGAACTGACTTTTGCGT	*Not* I
NHase1229_Up	GGAATTC*CATATG*ACGGGATCGCACG	*Nde* I
NHase1229_Down	CCG*GATATC*TCAGTCGTGTGGGTTCGG	*Eco*R V

[a] The restriction sites are underlined in *italics*

Table S2



Entry	Solvent	Operation between two steps	Conversion to aldoxime [a] (%)
1	*n*-hexane	Two phases separation	> 99
2	*n*-hexane	Two phases separation and three times of water washing	> 99
3	Amyl acetate	Two phases separation	76.8
4	Amyl acetate	Two phases separation and three times of water washing	> 99
5	None[b]	Adding bacteria directly as catalyst	5.4
6	None[b]	EtOAc extraction, three times of water washing, and rotary evaporation	> 99

[a] The conversions to benzonitrile were determined by HPLC analysis.

[b] 10% ethanol was used as cosolvent in the biotransformation by OxdF1.

5. Figures

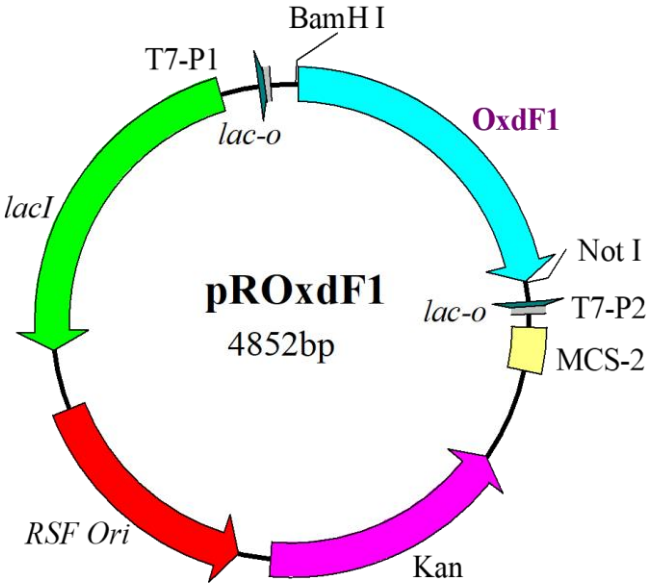


Figure S1 Recombinant plasmid pROxdF1 for expressing OxdF1

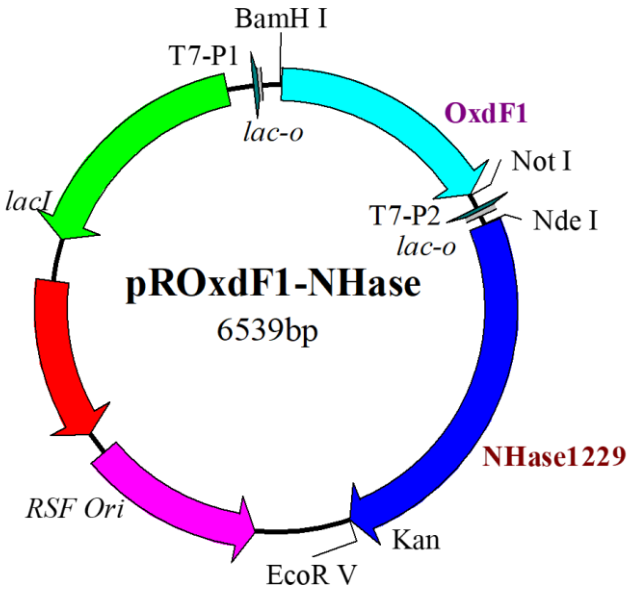


Figure S2 recombinant plasmid pROxdF1-NHase for co-expressing OxdF1 and NHase1229

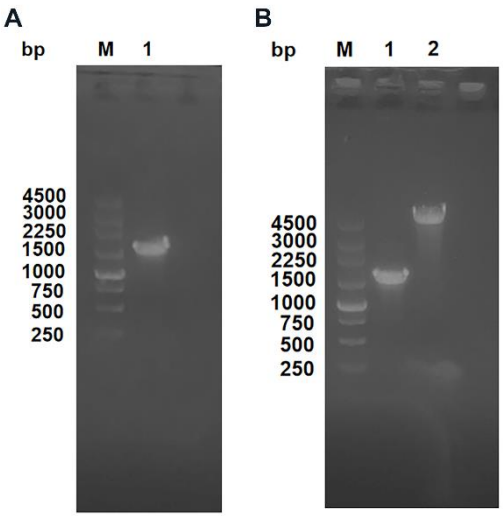


Figure S3 Agarose gel electrophoresis of gene and recombinant plasmid. A: Lane M: DNA marker, 1: NHase1229 by PCR; B: lane 1: NHase1229 digested by *Nde* I and *Eco*R V, Lane 2: recombinant plasmid pROxdF1 digested by *Nde* I and *Eco*R V.

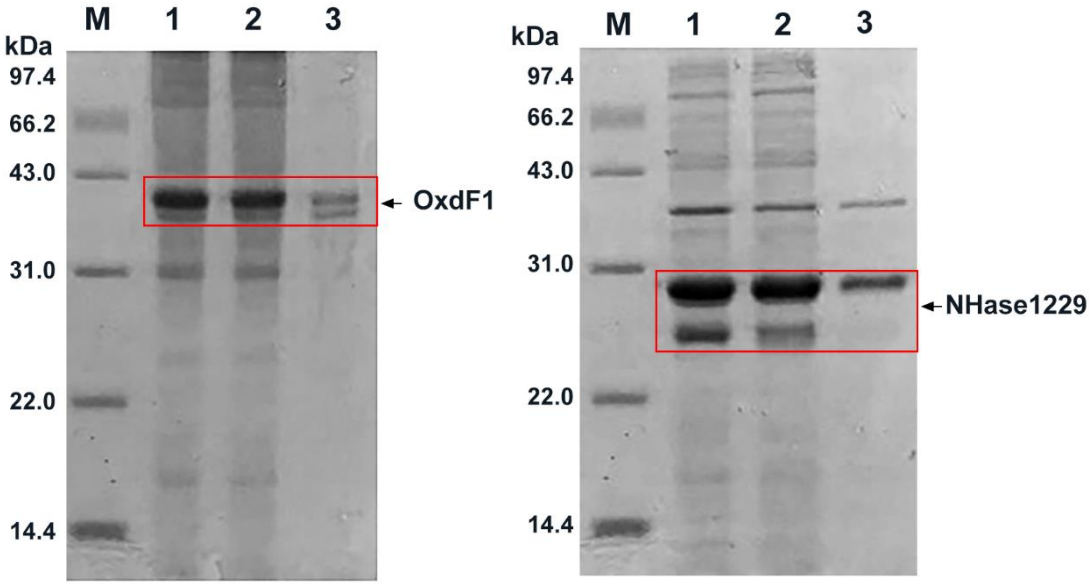


Figure S4 The expression of OxdF1 and NHase1229 in *E. coli* BL21 (DE3) harboring recombinant plasmid pROxdF1 and pENHase1229. M: protein markers, lane 1: whole region, lane 2: soluble region, lane 3: insoluble region.

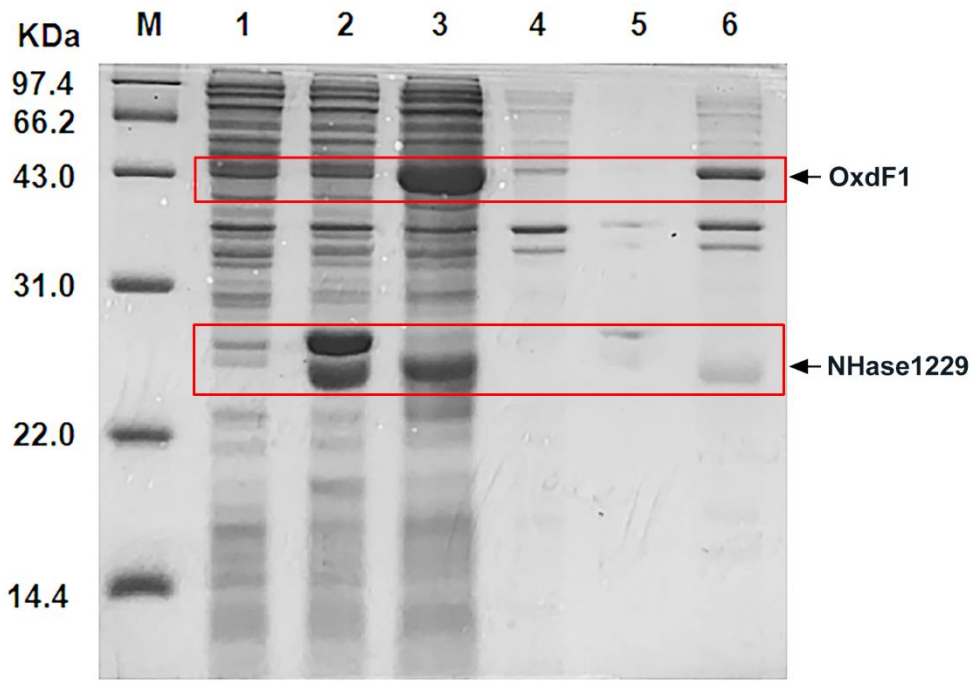


Figure S5 The expression of OxdF1 and NHase1229 in *E. coli* BL21 (DE3) harboring recombinant plasmid pROxdF1-NHase and pENHase1229. M: protein markers, lane 1: whole region, lane 2: soluble region, lane 3: insoluble region.

HPLC figures

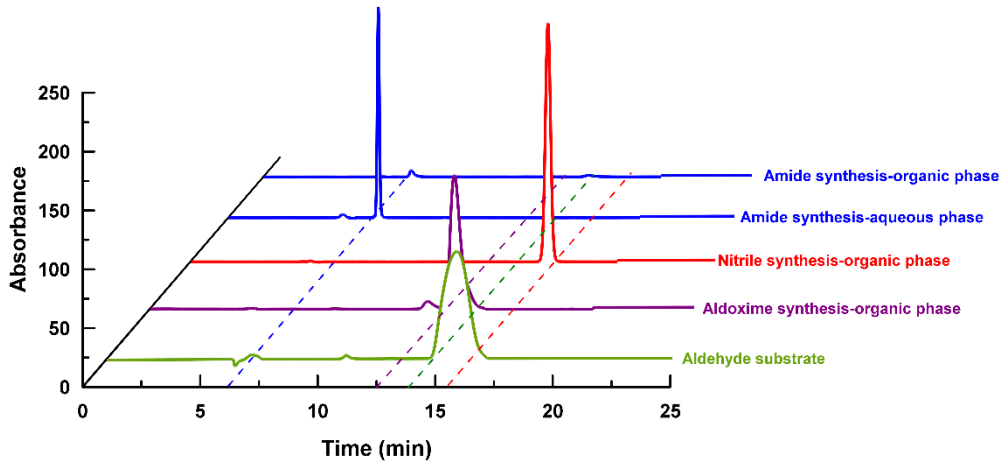


Figure S6 Reversed-phase HPLC analysis of benzaldehyde (1a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

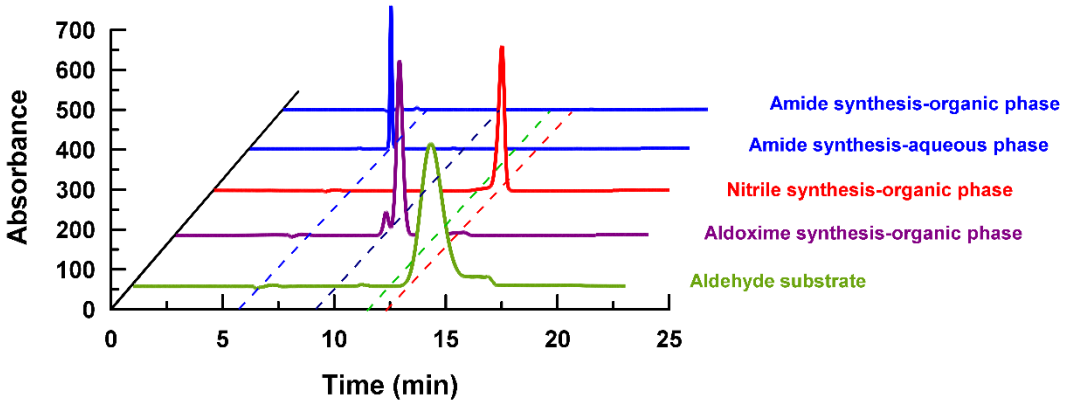


Figure S7 Reversed-phase HPLC analysis of phenylacetaldehyde (2a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

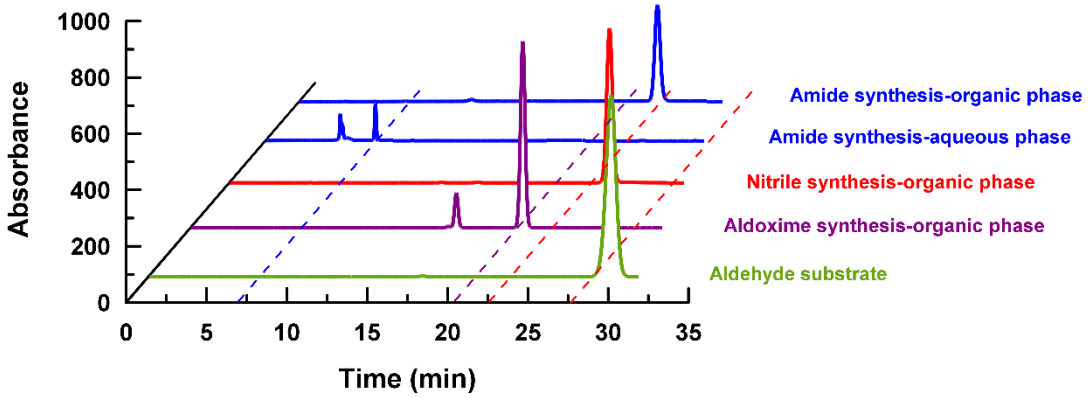


Figure S8 Reversed-phase HPLC analysis of 2-bromobenzaldehyde (3a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

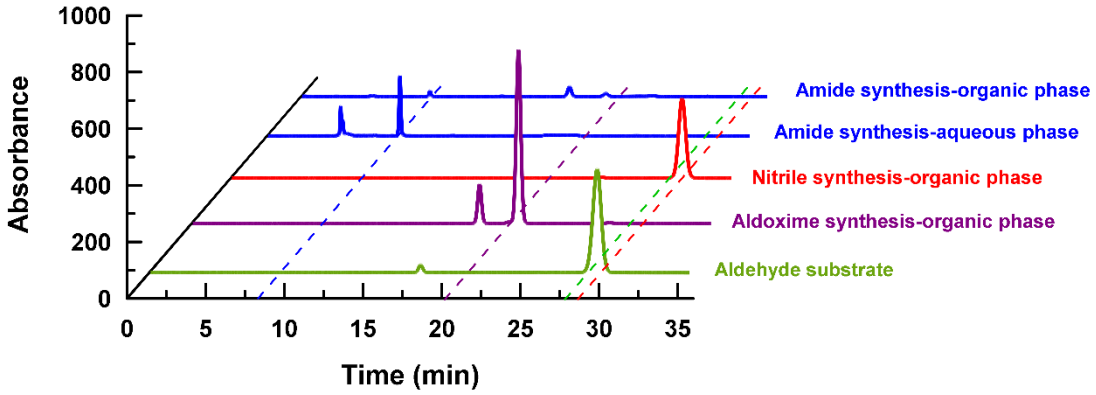


Figure S9 Reversed-phase HPLC analysis of 3-bromobenzaldehyde (4a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

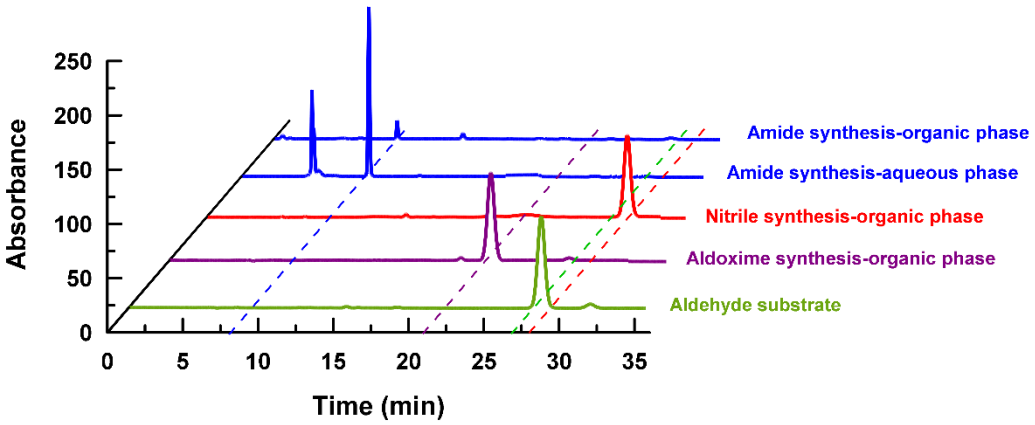


Figure S10 Reversed-phase HPLC analysis of 4-bromobenzaldehyde (5a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

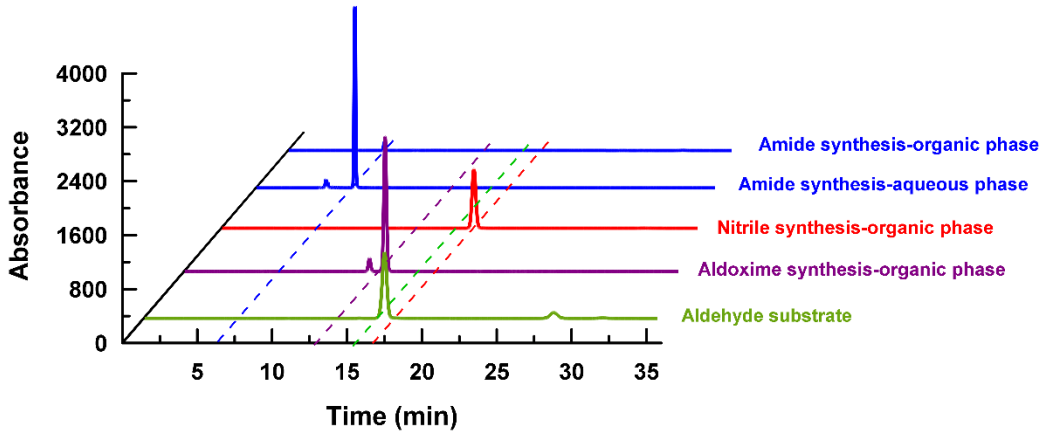


Figure S11 Reversed-phase HPLC analysis of 2-fluorobenzaldehyde (6a) and corresponding oxime, nitrile, and amide. The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

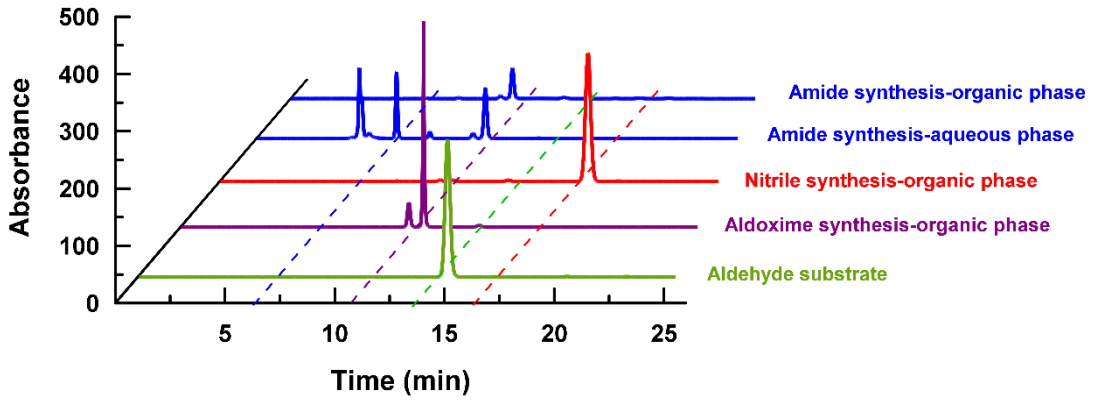


Figure S12 Reversed-phase HPLC analysis of *p*-anisaldehyde (7a) and corresponding oxime (7b), nitrile (7c), and amide (7d). The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

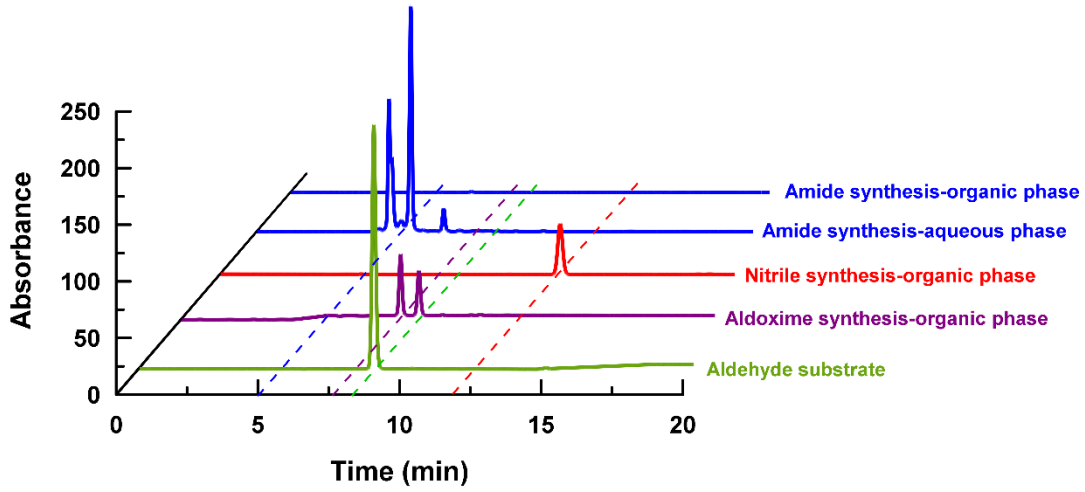


Figure S13 Reversed-phase HPLC analysis of furfural (8a) and corresponding oxime (8b), nitrile (8c), and amide (8d). The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

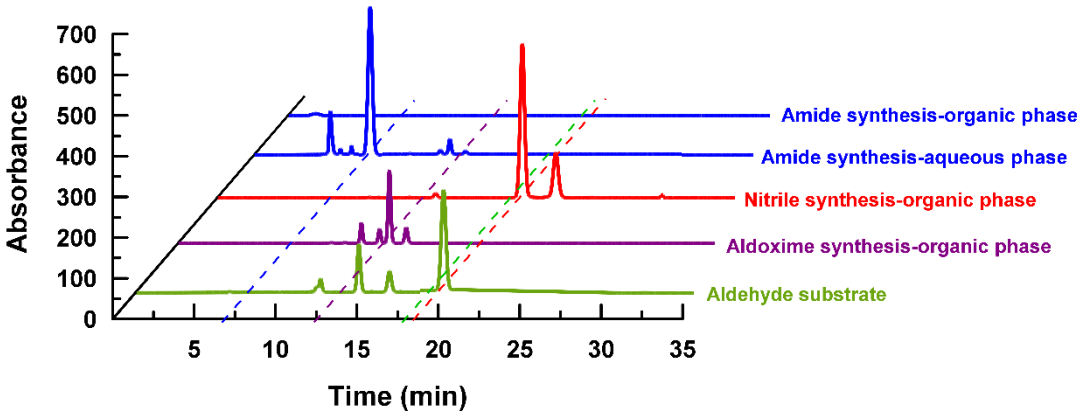


Figure S14 Reversed-phase HPLC analysis of 3-methyl-2-thenaldehyde (9a) and corresponding oxime (9b), nitrile (9c), and amide (9d). The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

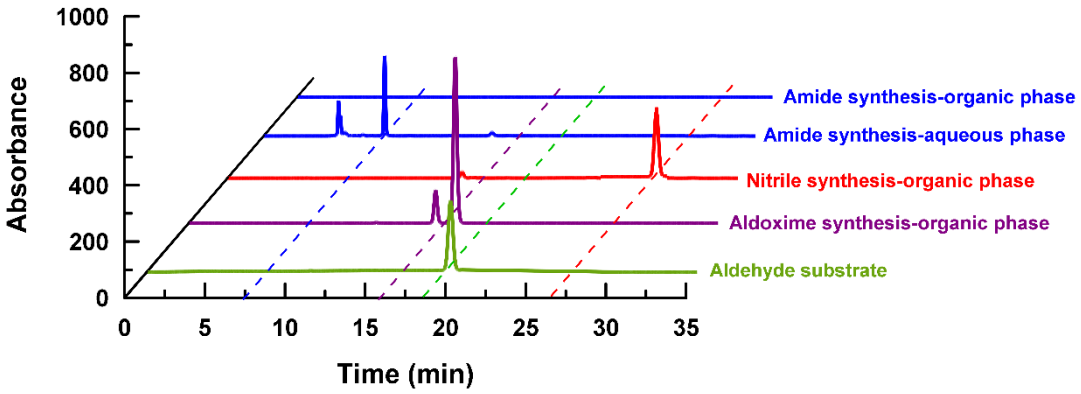


Figure S15 Reversed-phase HPLC analysis of cinnamaldehyde (10a) and corresponding oxime (10b), nitrile (10c), and amide (10d). The analysis was performed by high pressure liquid chromatography (HPLC, Agilent 1100) with a C18 reverse phase column (5 μm, 4.6 mm×250 mm). The elution solvent consisted of 0.5% trifluoroacetic acid (TFA) ddH20 and acetonitrile (40:60, v/v) at a flow rate of 0.5 mL/min. The absorbance was measured at 230 nm.

GC figures

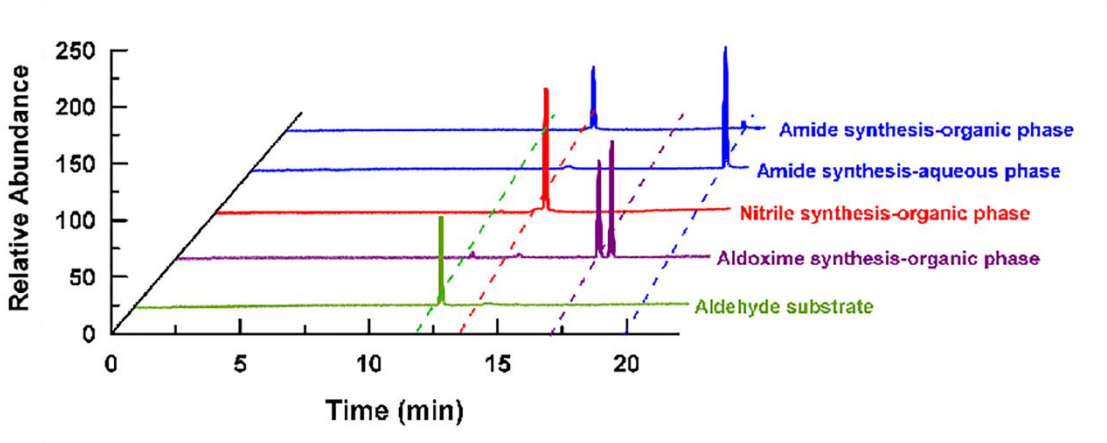


Figure S16 GC analysis of citronellal (11a) and corresponding oxime (11b), nitrile (11c), and amide (11d). The analysis was performed by gas chromatography (GC, Agilent Technologies Inc., USA) with a flame ionization detector and a HP-5 capillary column (0.1 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and held for 2 min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 2.0 ml/min as the carrier.

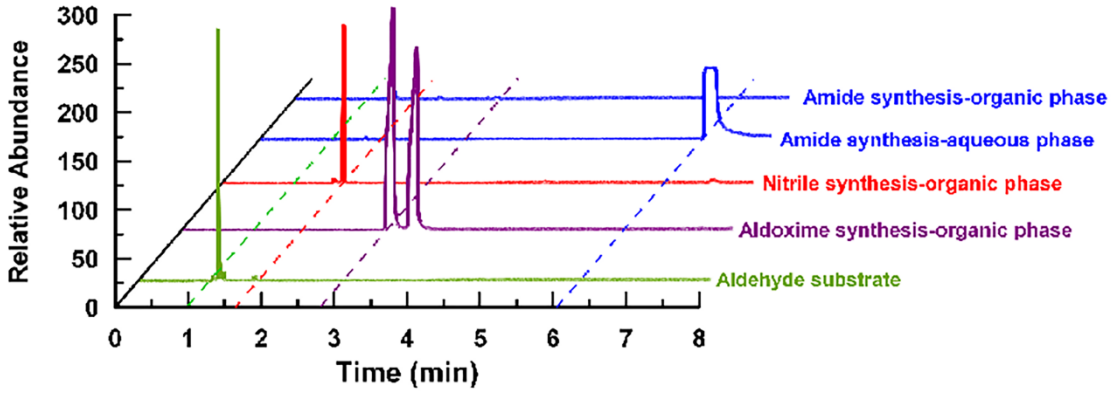


Figure S17 GC analysis of butyraldehyde (12a) and corresponding oxime (12b), nitrile (12c), and amide (12d). The analysis was performed by gas chromatography (GC, Agilent Technologies Inc., USA) with a flame ionization detector and a HP-5 capillary column (0.1 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and held for 2 min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 2.0 ml/min as the carrier.

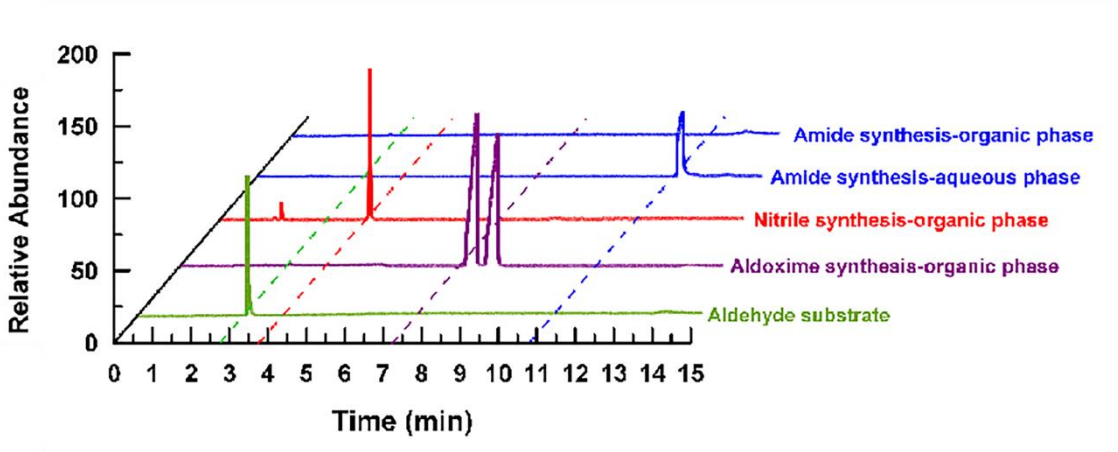


Figure S18 GC analysis of hexanal (13a) and corresponding oxime (13b), nitrile (13c), and amide (13d). The analysis was performed by gas chromatography (GC, Agilent Technologies Inc., USA) with a flame ionization detector and a HP-5 capillary column (0.1 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and held for 2 min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 2.0 ml/min as the carrier.

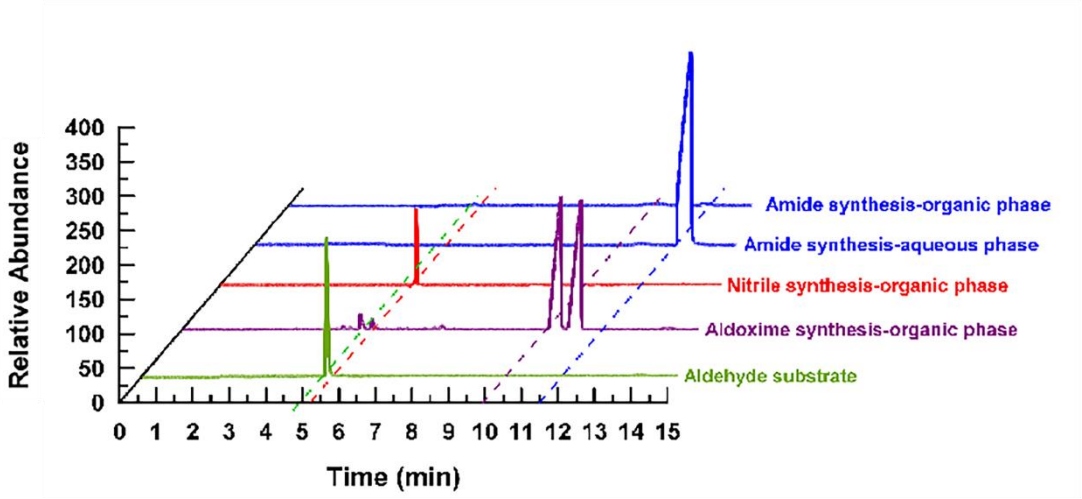


Figure S19 GC analysis of heptanal (14a) and corresponding oxime (14b), nitrile (14c), and amide (14d). The analysis was performed by gas chromatography (GC, Agilent Technologies Inc., USA) with a flame ionization detector and a HP-5 capillary column (0.1 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and held for 2 min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 2.0 ml/min as the carrier.

NMR figures

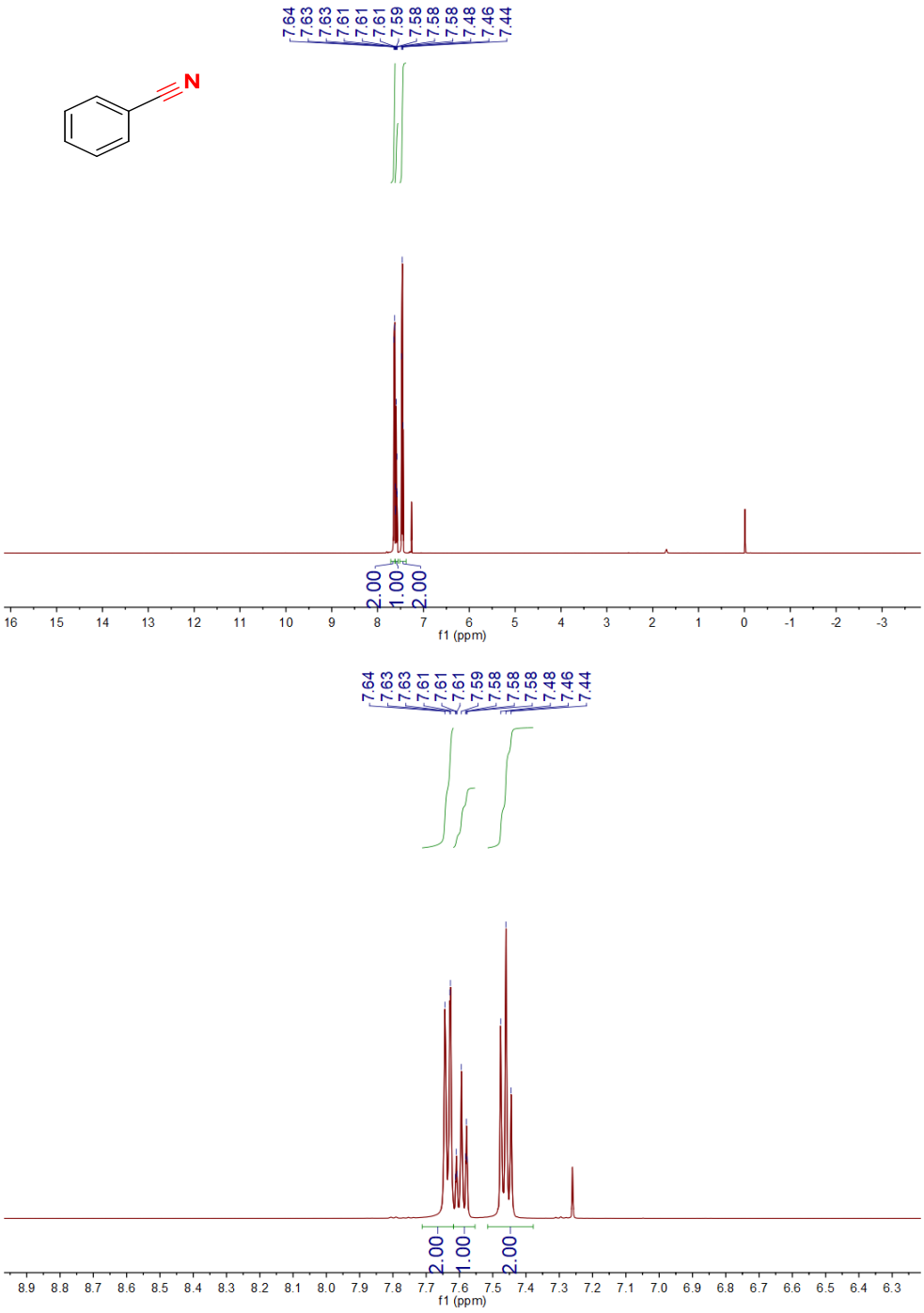


Figure S20 Following the general procedure, the product **1c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (62.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J =8.4 Hz, 2H), 7.59 (t, J =7.6 Hz, 1H), 7.46 (dd, J =7.6 Hz, 2H).

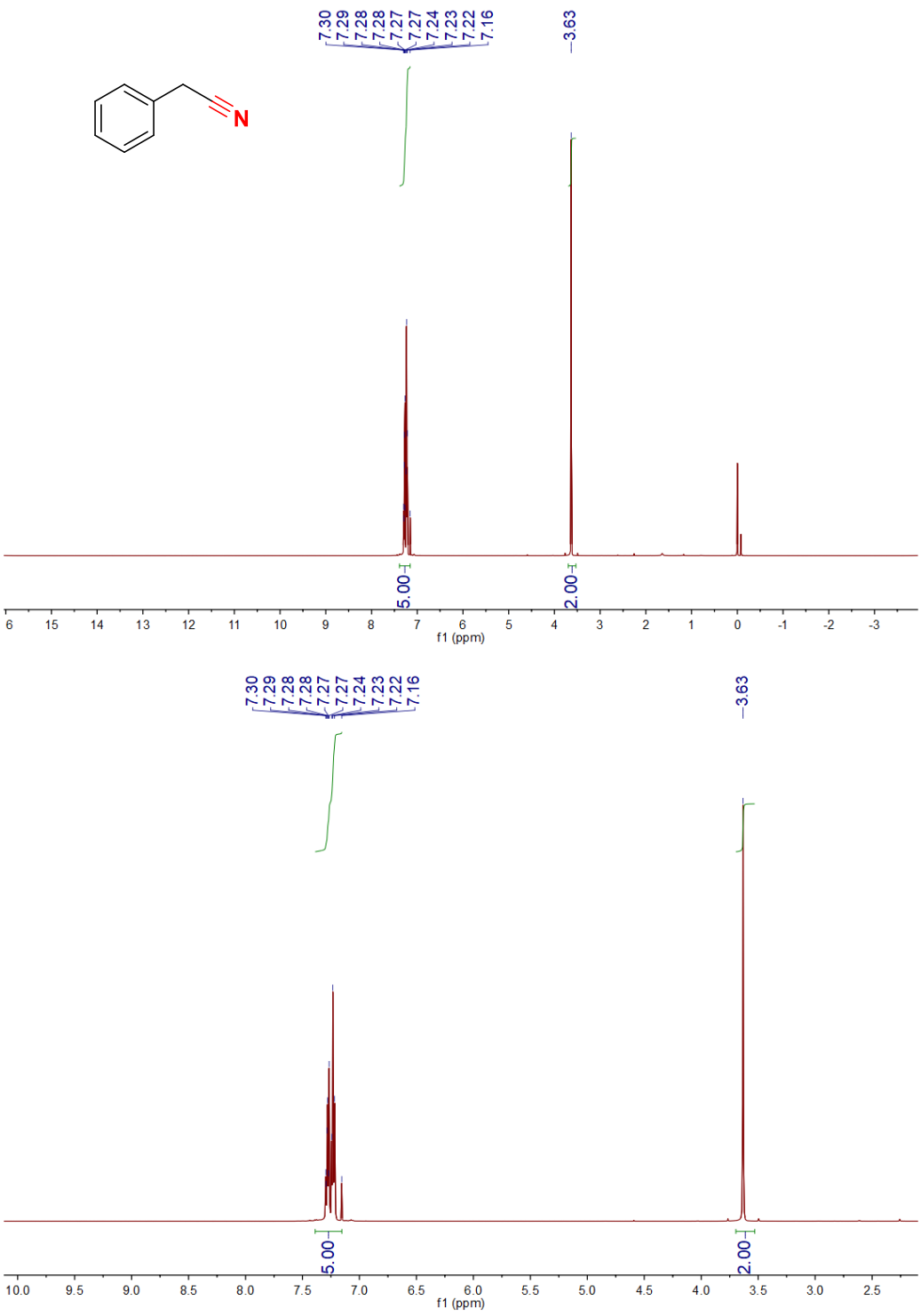


Figure S21 Following the general procedure, the product **2c** was prepared and purified by flash column chromatography (n-hexane:EA = 4:1) as a colorless oil (61.7% yield). 1H NMR (500 MHz, CDCl3) δ 7.72 – 6.68 (m, 5H), 3.63 (s, 2H).

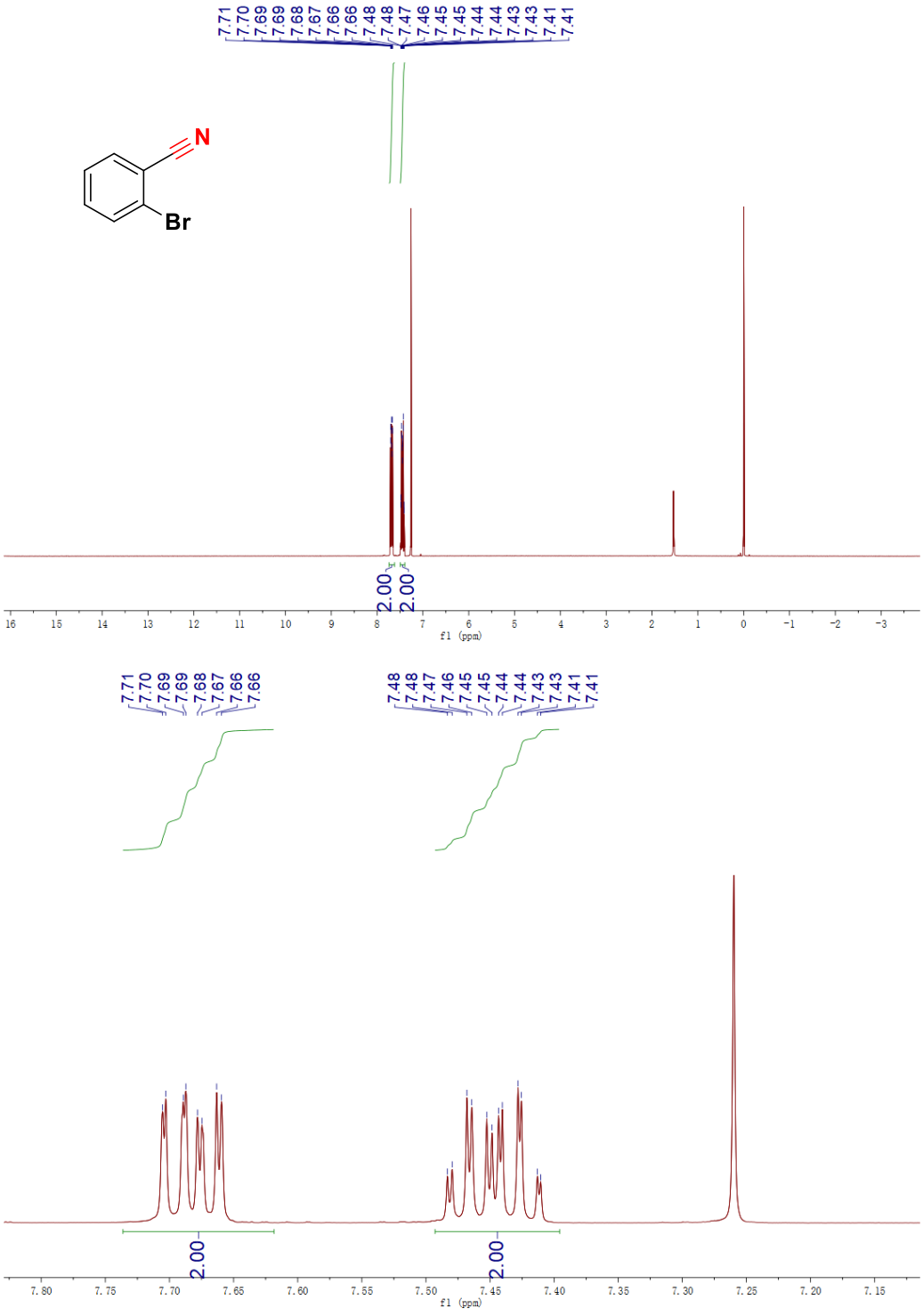


Figure S22 Following the general procedure, the product **3c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (41.2% yield). 1H NMR (500 MHz, CDCl3) δ 7.70 (dd, J = 8.0, 1.1 Hz, 1H), 7.67 (dd, J = 7.6, 1.8 Hz, 1H), 7.47 (dd, J = 7.6, 1.9 Hz, 1H), 7.43 (td, J = 7.6, 1.4 Hz, 1H).

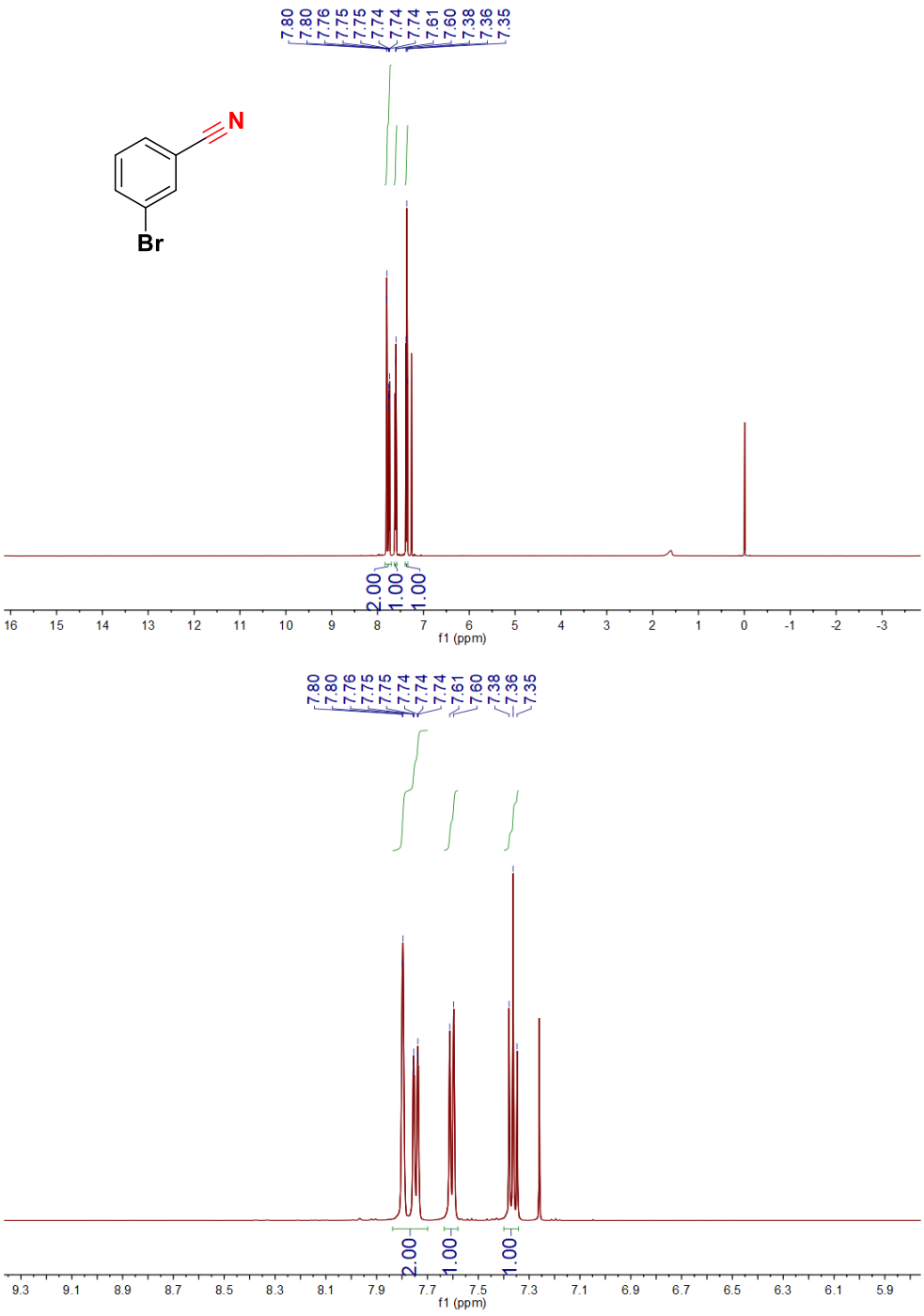


Figure S23 Following the general procedure, the product **4c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (51.4% yield). 1H NMR (500 MHz, CDCl3) δ 7.80 (s, 1H), 7.75 (d, J =8.2 Hz, 1H), 7.60 (d, J =7.8 Hz, *1*H), 7.36(dd, *J* =8.2 Hz,7.8Hz, *1*H).

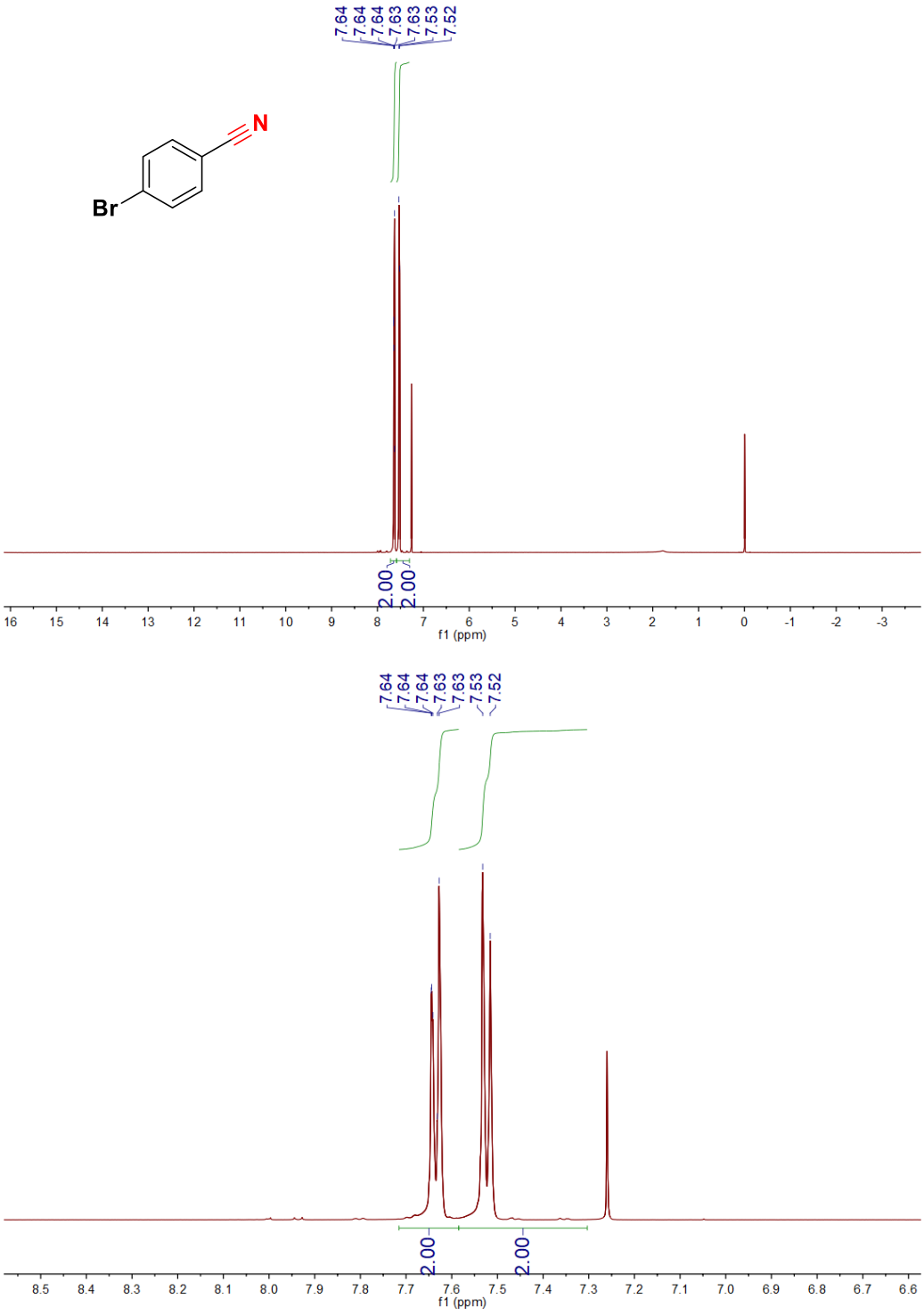


Figure S24 Following the general procedure, the product **5c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (62.7% yield). 1H NMR (500 MHz, CDCl3) δ 7.64 (d, J =8.2 Hz, 2H), 7.52 (d, J =8.2 Hz, 2H).

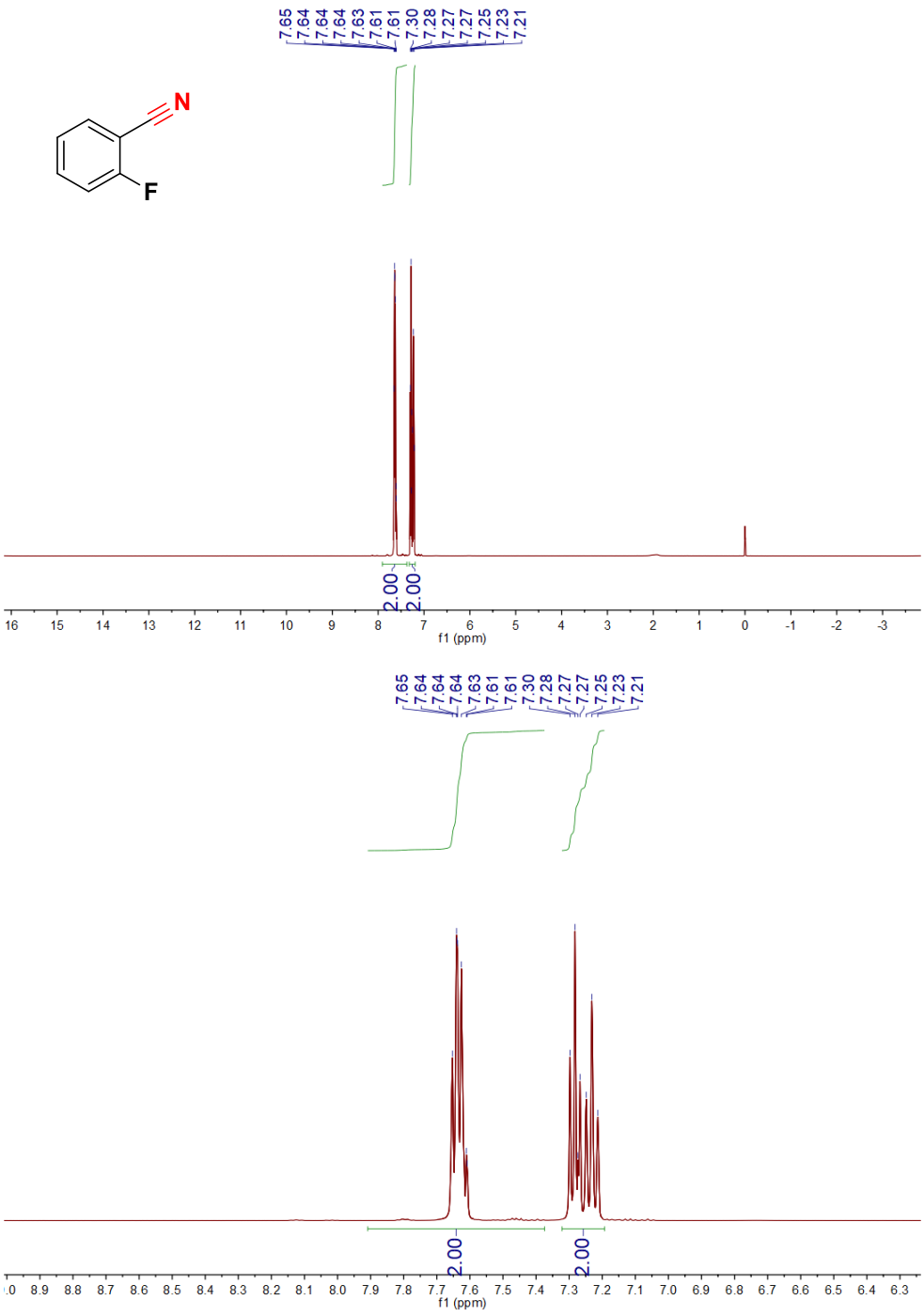


Figure S25 Following the general procedure, the product **6c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (55.7% yield). 1H NMR (500 MHz, CDCl3) δ 7.67 – 7.58 (m, 1H), 7.28 (dd, J = 9.8, 5.5 Hz, 1H), 7.23 (t, J = 8.7 Hz, 1H).

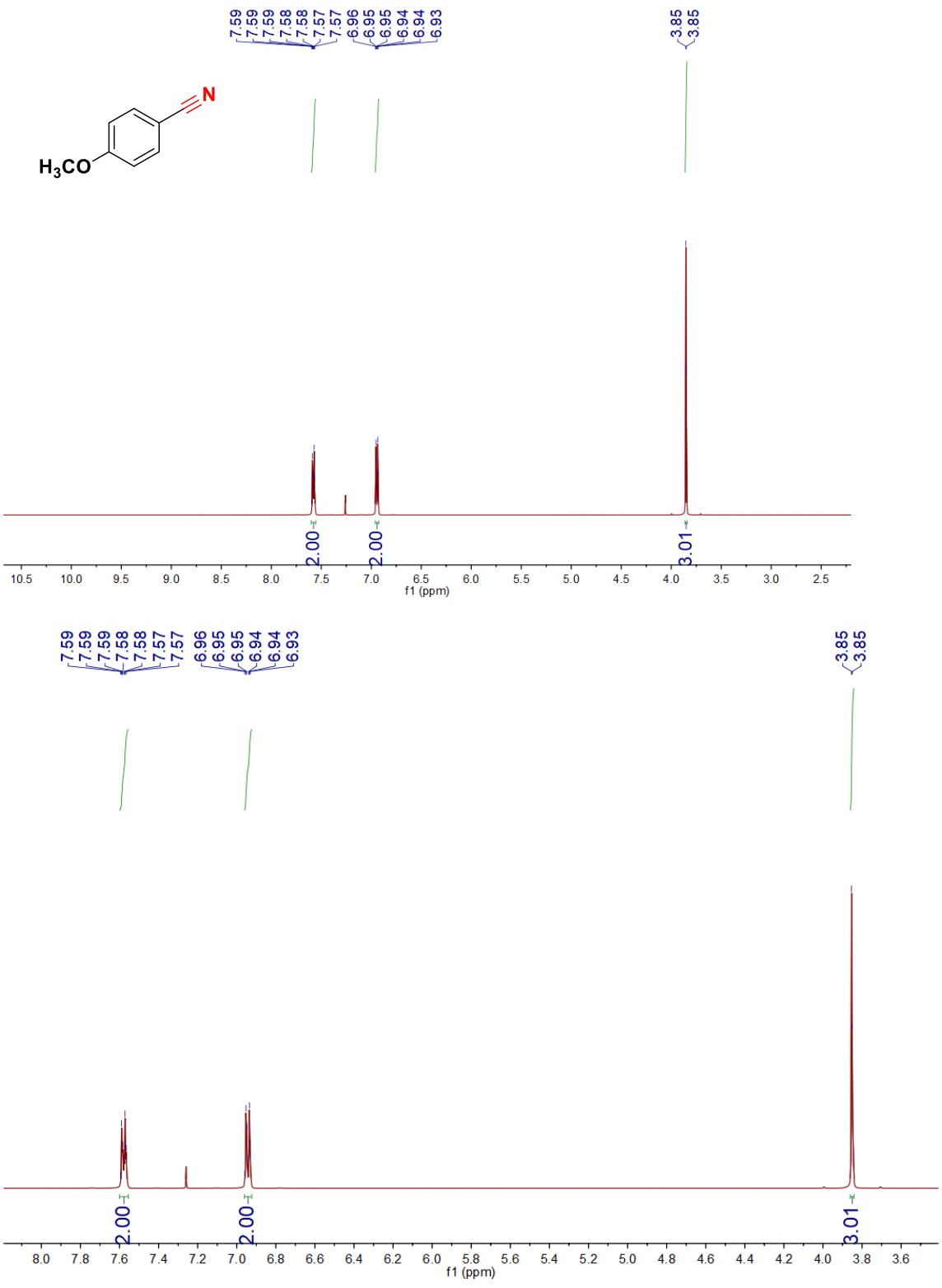
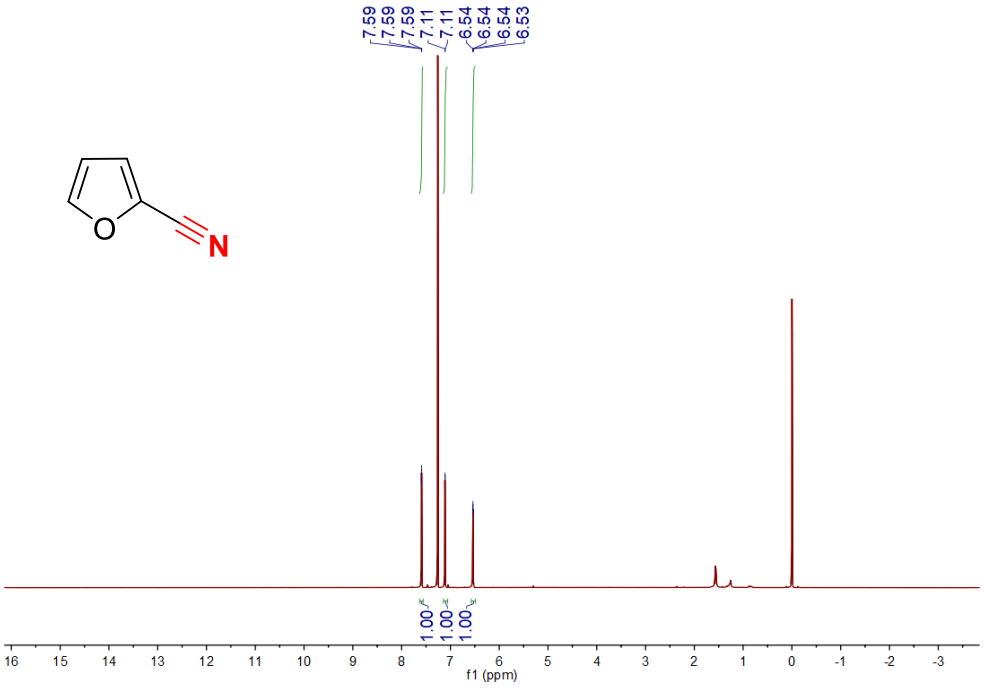
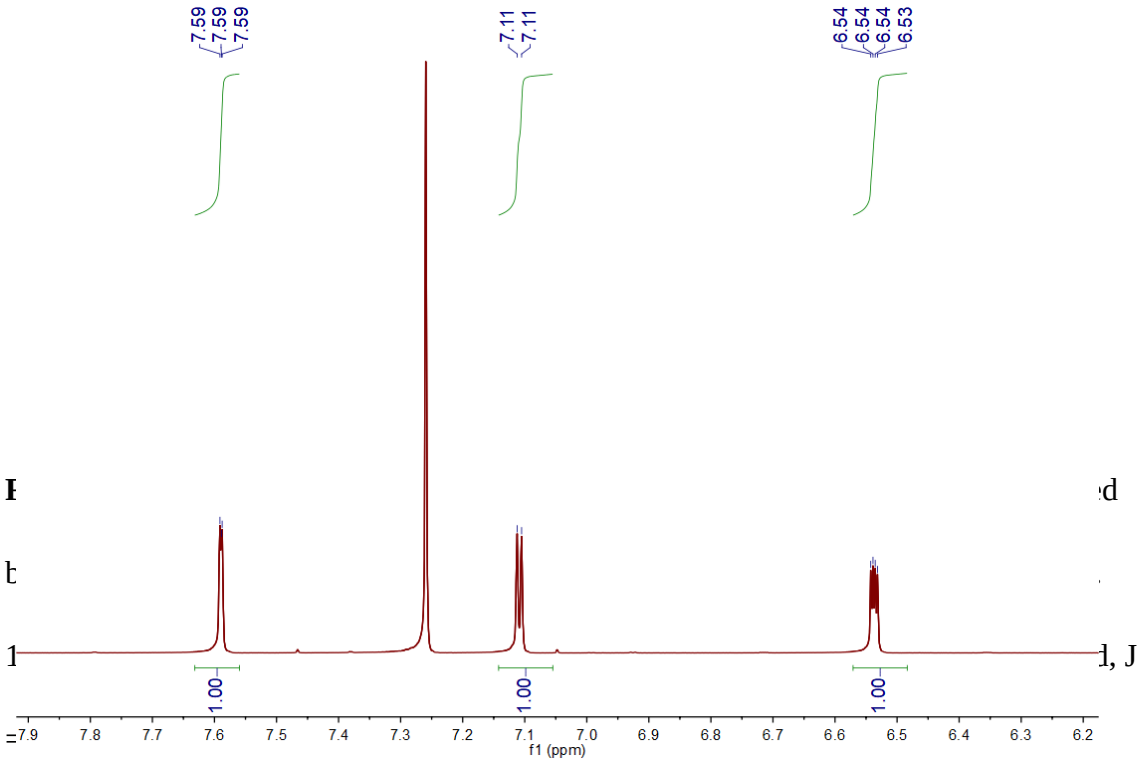


Figure S26 Following the general procedure, the product **7c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (55.7% yield). 1H NMR (500 MHz, CDCl3) δ 7.58 (m, 2H), 6.94 (d, J =8.9 Hz, 2H), 3.85(s, 3H).


7.59
7.59
7.59
7.11
7.11
6.54
6.54
6.54
6.53
O
N
1.00
1.00
1.00
f1 (ppm)


7.59
7.59
7.59
7.11
7.11
6.54
6.54
6.54
6.53
1.00
1.00
1.00
f1 (ppm)

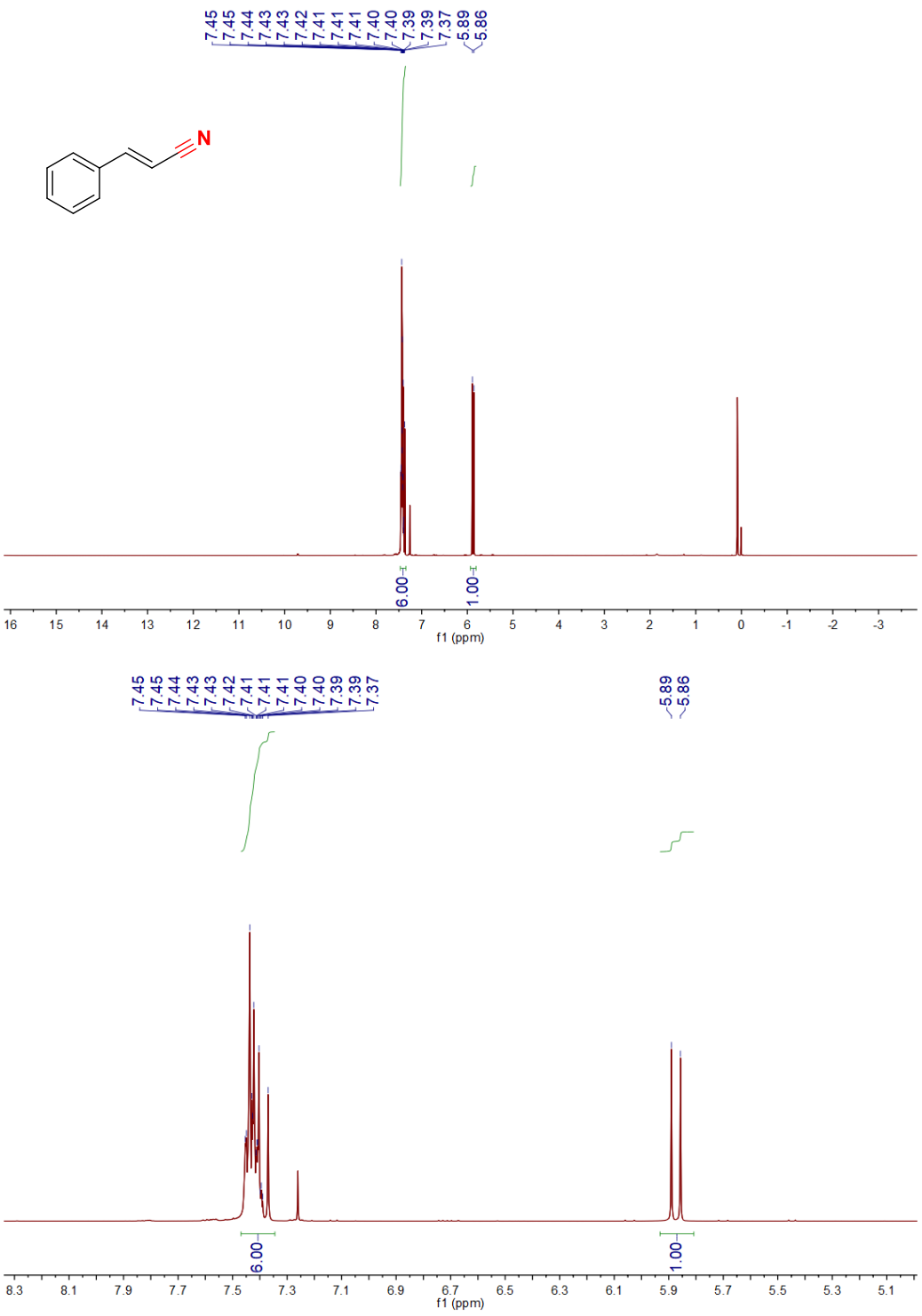


Figure S28 Following the general procedure, the product **10c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (59.3% yield). 1H NMR (500 MHz, CDCl3) δ 7.82–6.90 (m, 6H), 5.87 (d, J = 16.6 Hz, 1H).

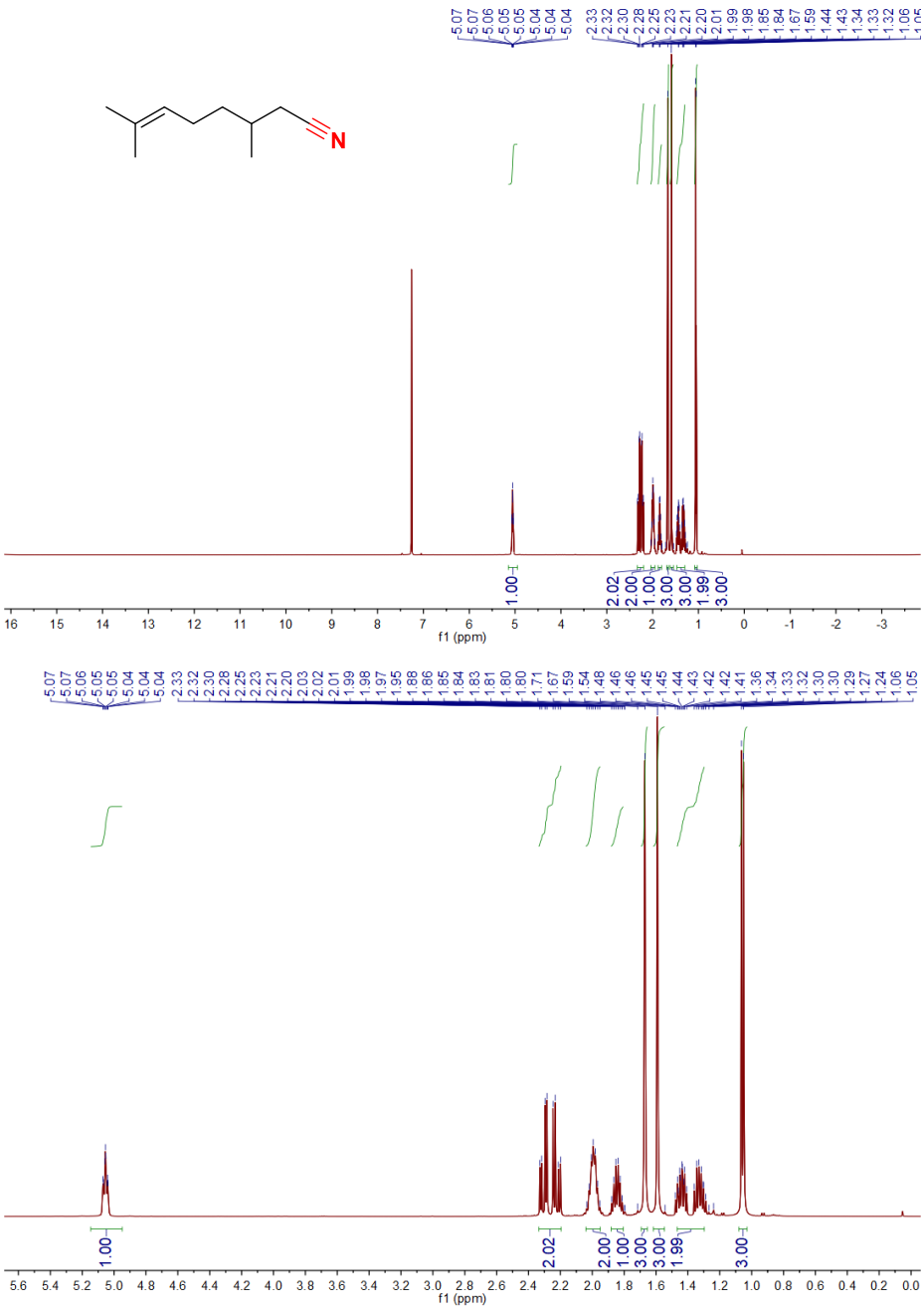


Figure S29 Following the general procedure, the product **11c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (71.3% yield). 1H NMR (500 MHz, CDCl3) δ 5.05 (ddd, J = 7.1, 4.2, 1.2 Hz, 1H), 2.26 (ddd, J = 23.6, 16.7, 6.3 Hz, 2H), 1.99 (dp, *J* = 14.5, 7.3 Hz, 2H), 1.91–1.76 (m, 1H), 1.67 (s, 3H), 1.57 (d, *J* = 23.9 Hz, 3H), 1.49–1.19 (m, 2H), 1.06 (d, *J* = 6.7 Hz, 3H).

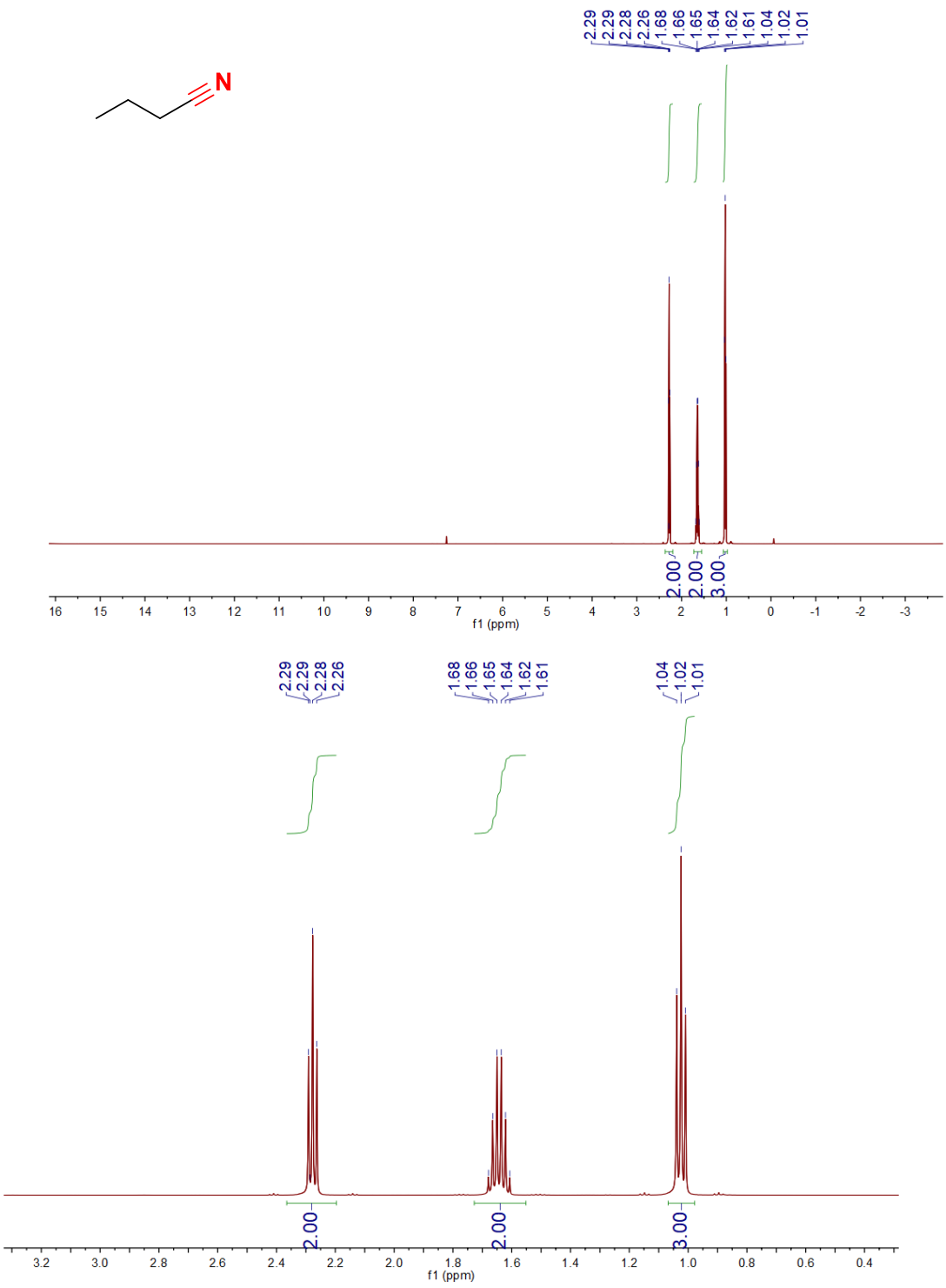


Figure S30 Following the general procedure, the product **12c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (40.4% yield). 1H NMR (500 MHz, CDCl3) δ 2.28 (t, J =7.1 Hz, 2H),1.64 (m, 2H), 1.02 (t, J =7.4 Hz, 3H).

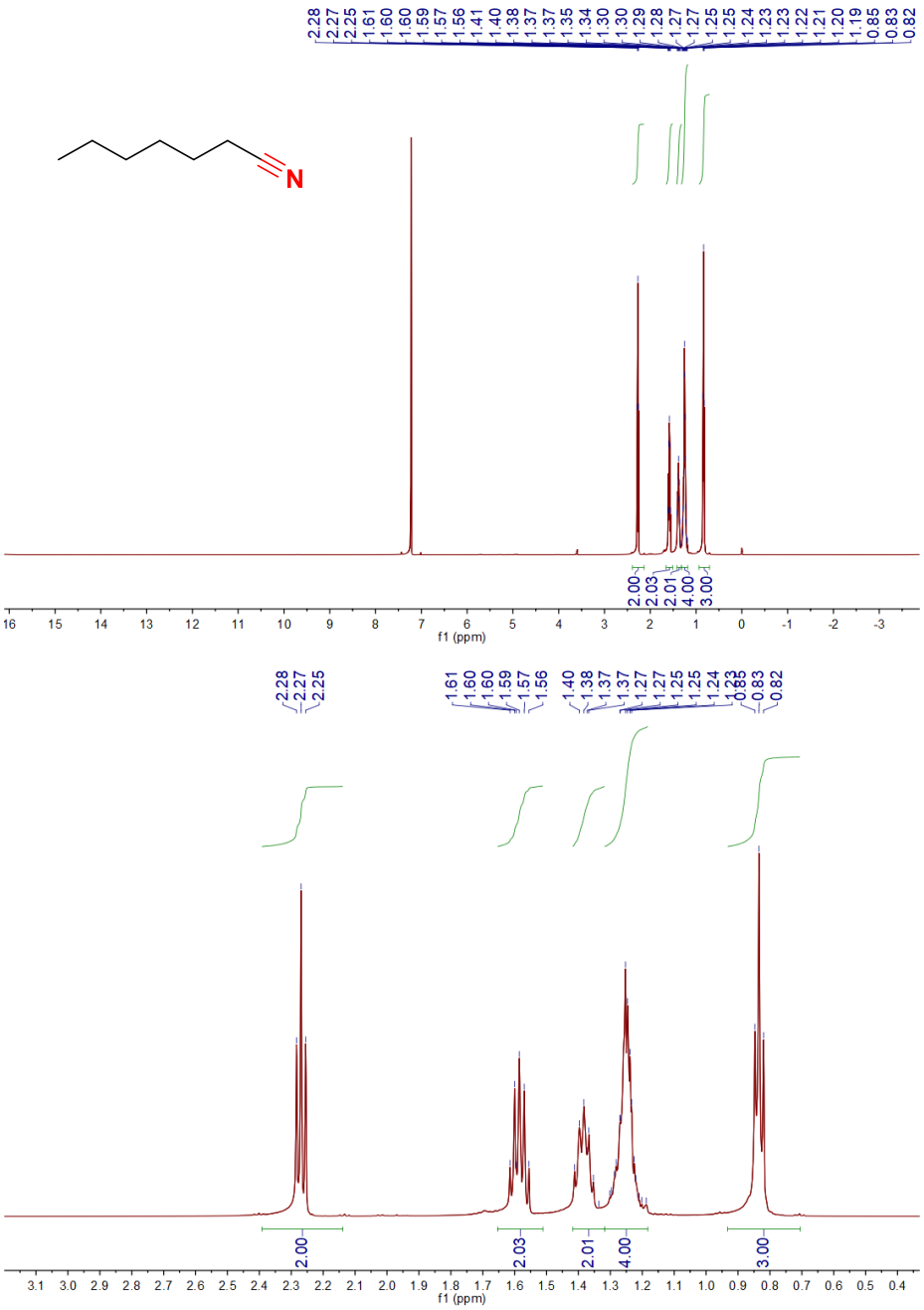


Figure S31 Following the general procedure, the product **14c** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (64.5% yield). 1H NMR (500 MHz, CDCl3) δ 2.31 (t, J = 7.1 Hz, 2H), 1.62 (dt, J = 15.1, 7.2 Hz, 2H), 1.42 (dt, J = 9.7, 7.3 Hz, 2H), 1.35 – 1.17 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H).

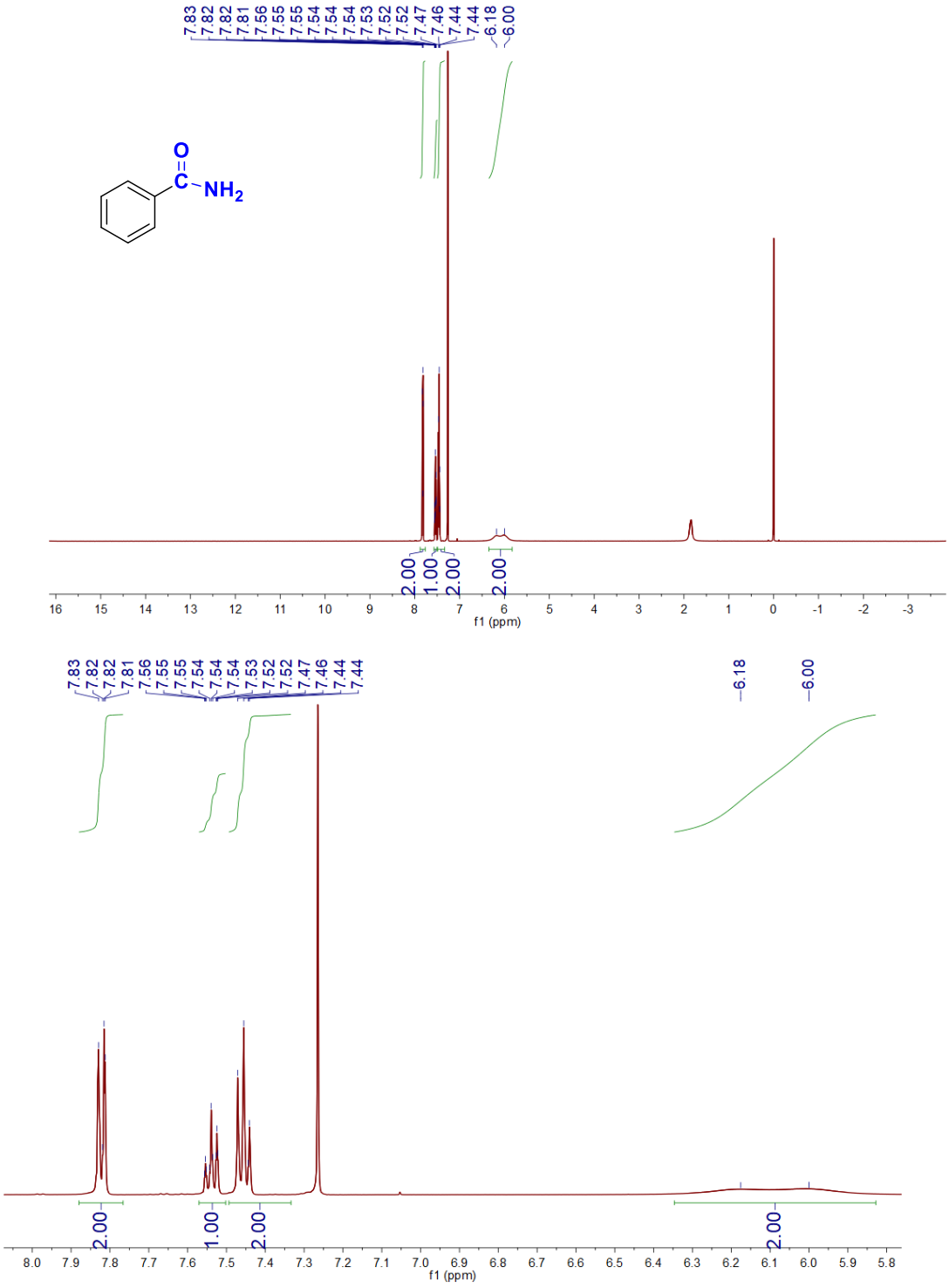


Figure S32 Following the general procedure, the product **1d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (64.1% yield). 1H NMR (500 MHz, CDCl3) δ 7.82 (dd, J = 5.2, 3.3 Hz, 2H), 7.59 – 7.50 (m, 1H), 7.45 (dd, J = 10.4, 4.8 Hz, 2H), 6.09 (d, J = 88.0 Hz, 2H).

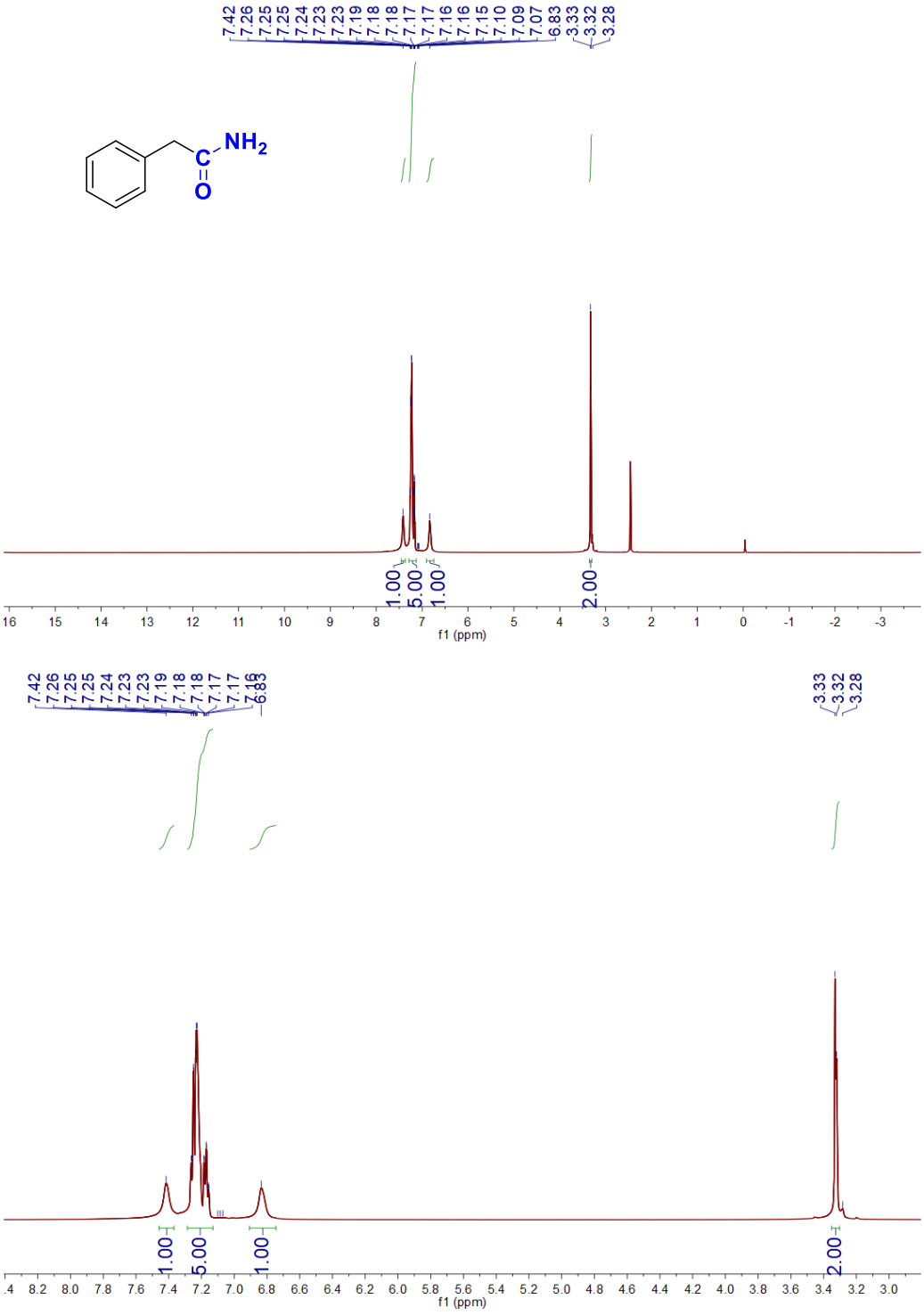


Figure S33 Following the general procedure, the product **2d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (64.1% yield). 1H NMR (500 MHz, DMSO) δ 7.42 (s, 1H), 7.16 (m, 5H), 6.83 (s, 1H), 3.37 (m, 2H).

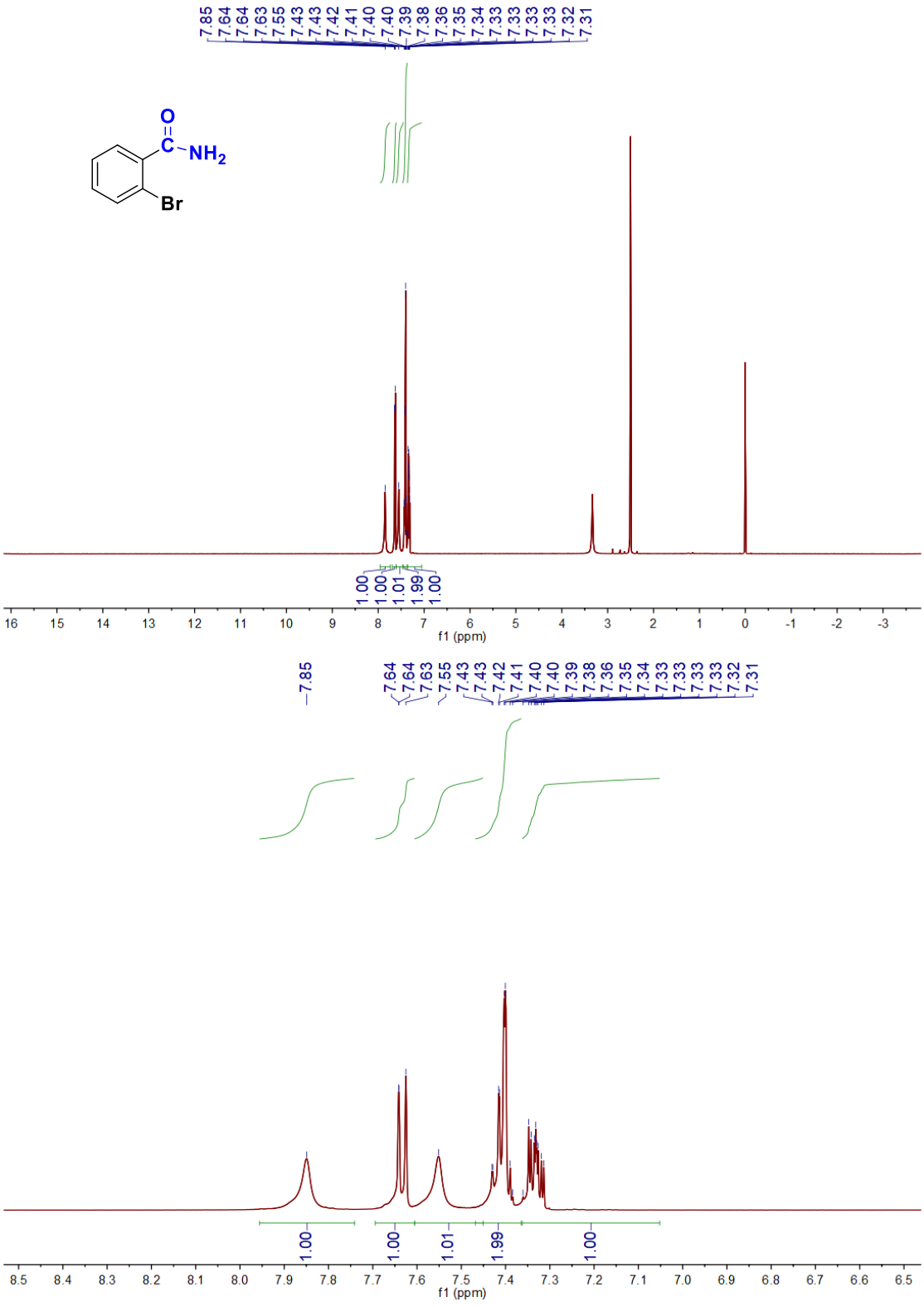


Figure S34 Following the general procedure, the product **3d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (19.4% yield). 1H NMR (500 MHz, DMSO) δ 7.85 (s, 1H), 7.64 (d, J =4.7 Hz, 1H), 7.55 (s, 1H), 7.41 (m ,2H), 7.33 (m, 1H).

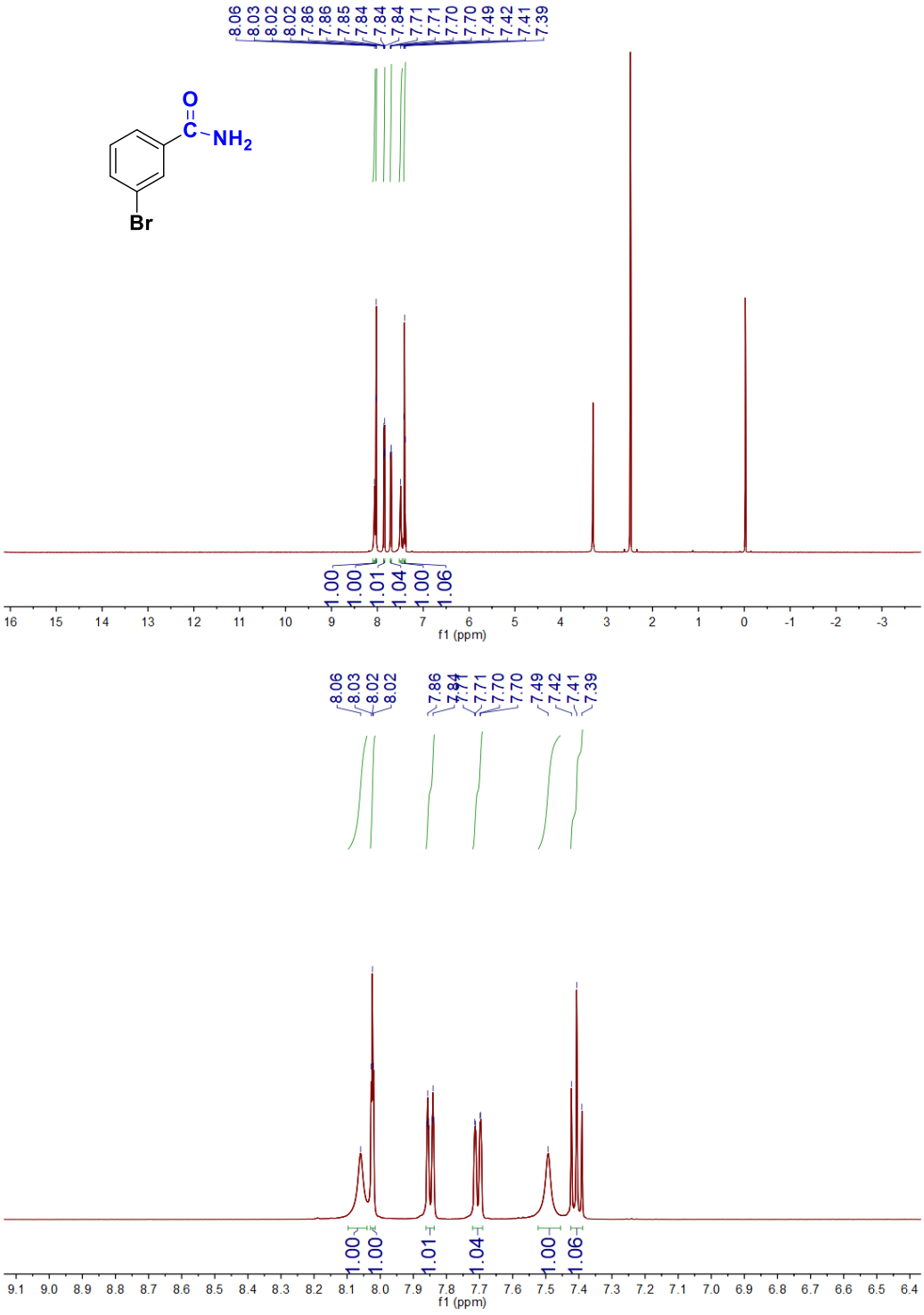


Figure S35 Following the general procedure, the product **4d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (50.7% yield). 1H NMR (500 MHz, CDCl3) δ 8.06 (s, 1H), 8.02 (m1H),7.85 (d, *J* =7.9 Hz,1H), 7.71 (d, *J* =9.0 Hz,1H), 7.49 (s, 1H), 7.41 (dd , *J* =7.9 Hz,9.0 Hz).

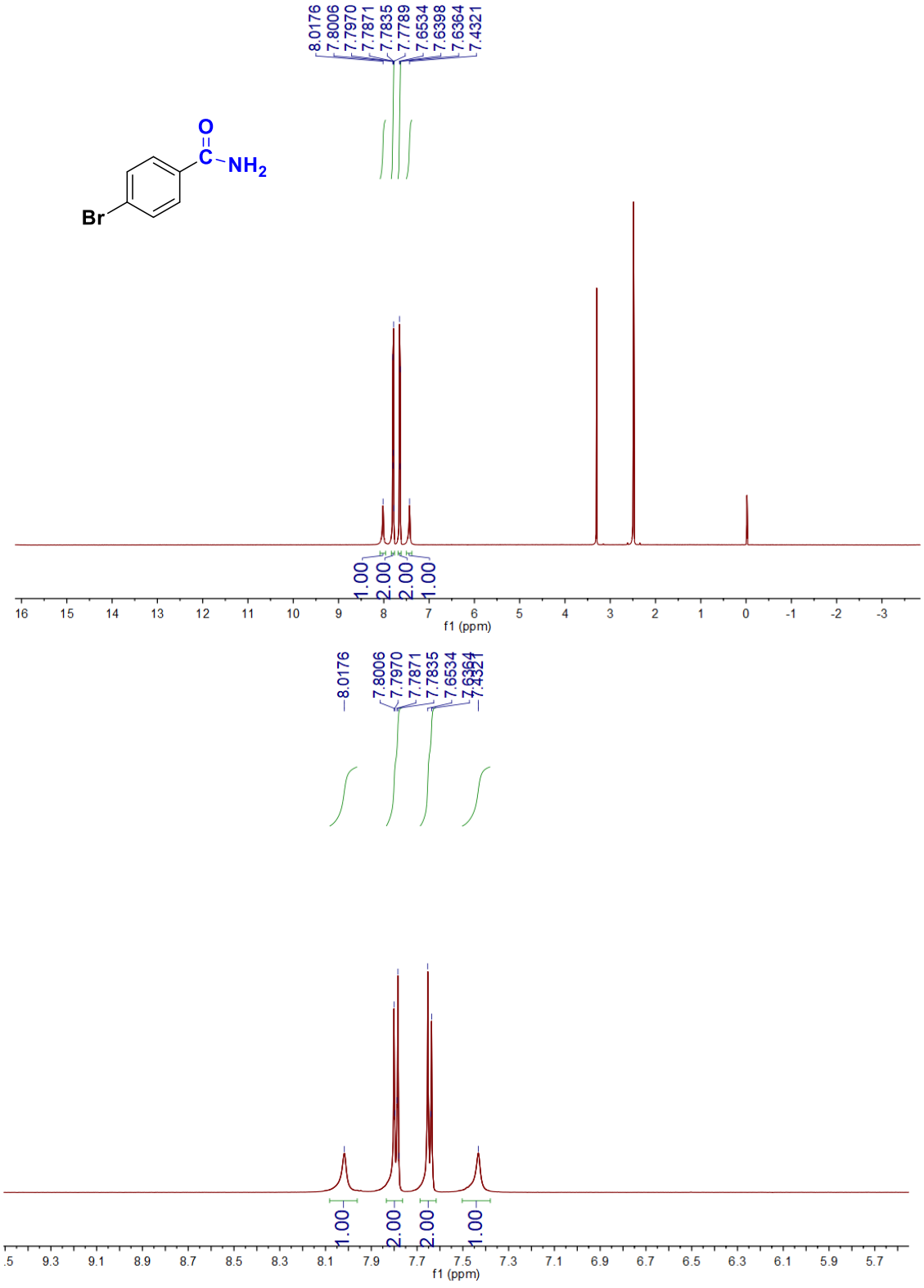


Figure S36 Following the general procedure, the product **5d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (63.0% yield). 1H NMR (500 MHz, CDCl3) δ 8.02 (s, 1H), 7.79 (d, J =8.6 Hz, 2H),7.64 (d, J =8.6 Hz, 2H), 7.43 (s, 1H).

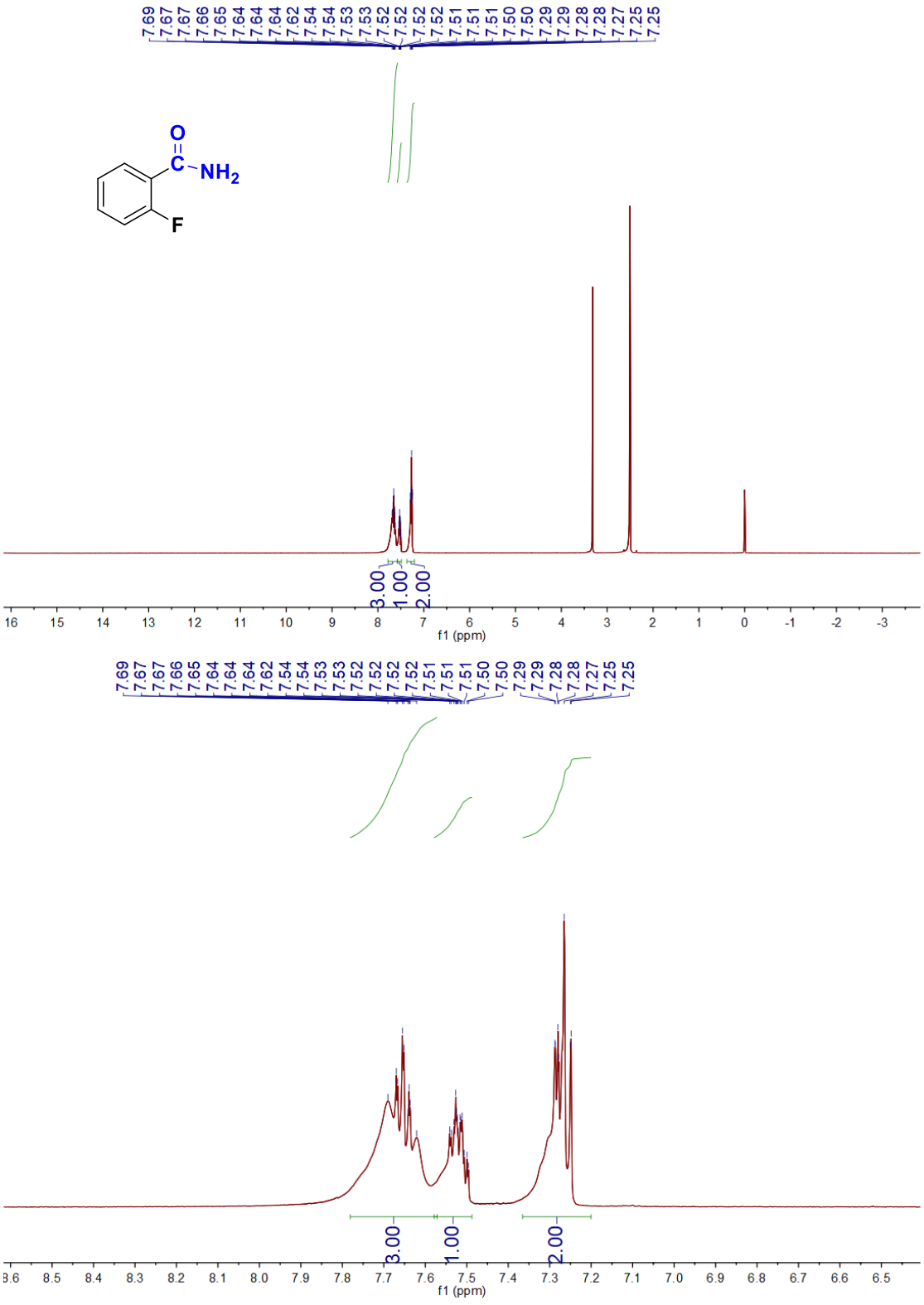


Figure S37 Following the general procedure, the product **6d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (55.4% yield). 1H NMR (500 MHz, CDCl3) δ 7.65 (m, 3H), 7.52 (m, 1H), 7.27 (m,2H).

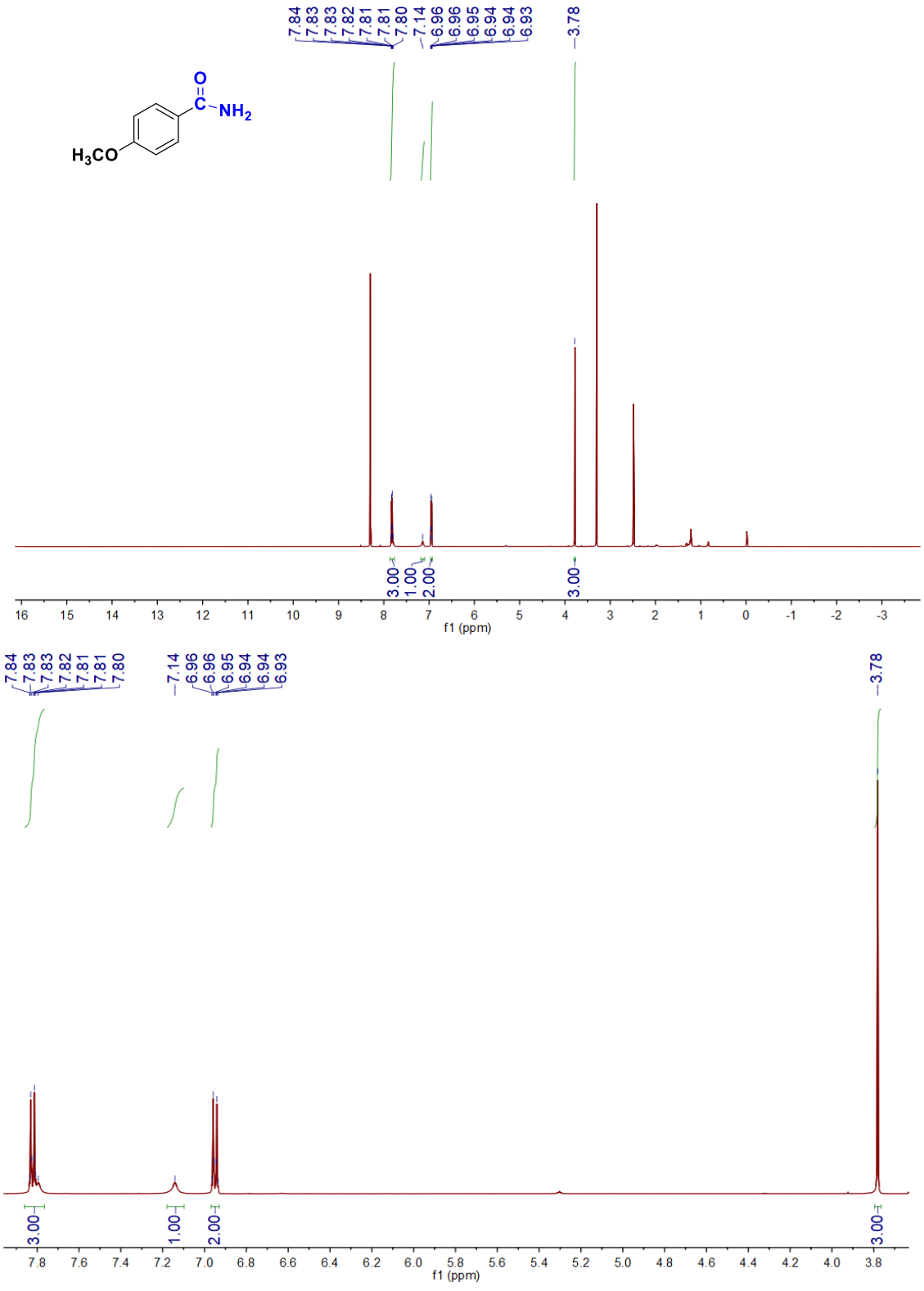


Figure S38 Following the general procedure, the product **7d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (28.1% yield). 1H NMR (500 MHz, DMSO) δ 7.89 (m, 1H), 7.14 (s, 1H), 7.03 (m, 1H), 3.78 (s, 1H).

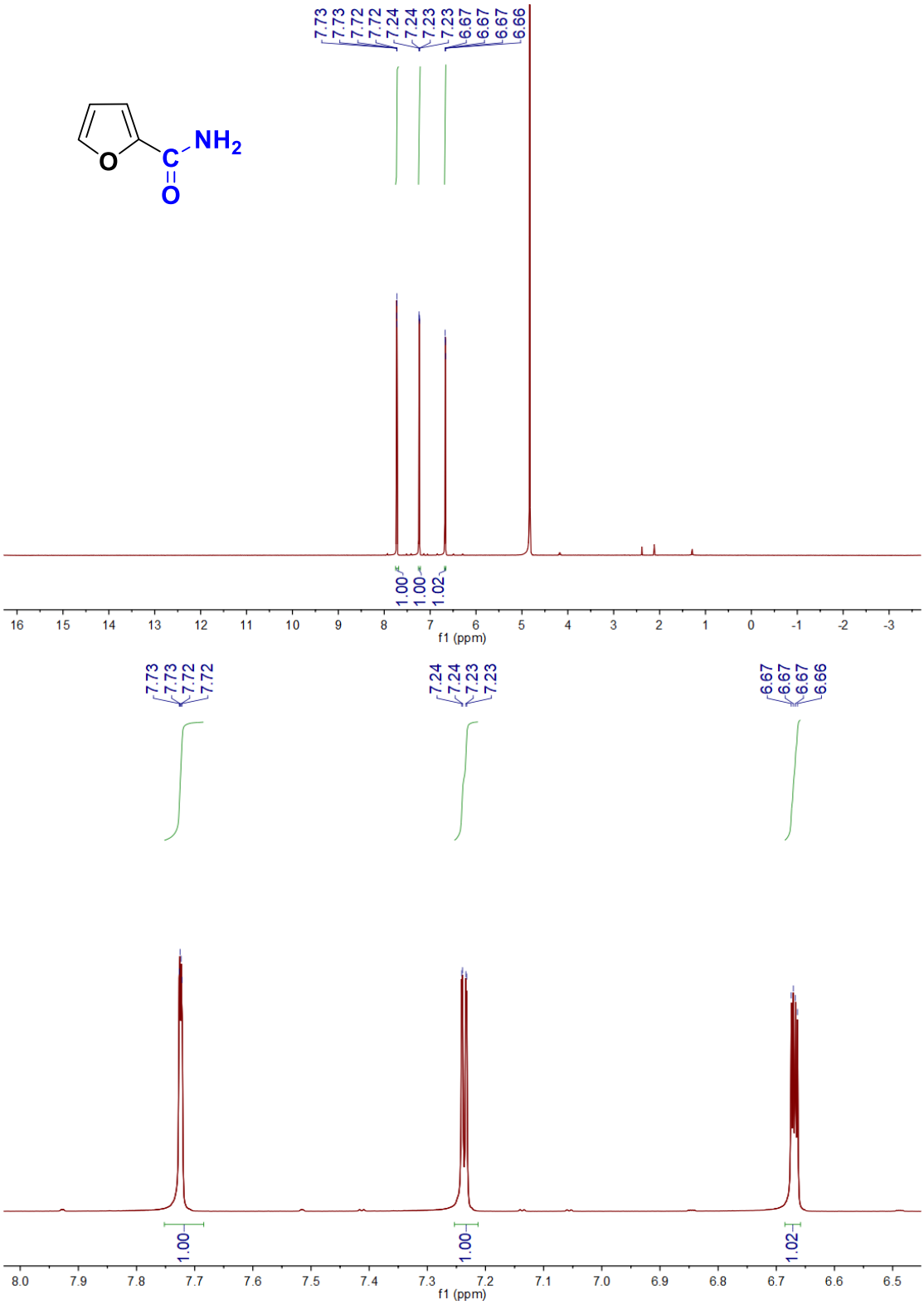


Figure S39 Following the general procedure, the product **8d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (53.9% yield). 1H NMR (500 MHz, D2O) δ 7.72 (dd, J = 1.7, 0.7 Hz, 1H), 7.24 (dd, J = 3.6, 0.7 Hz, 1H), 6.67 (dd, J = 3.6, 1.8 Hz, 1H).

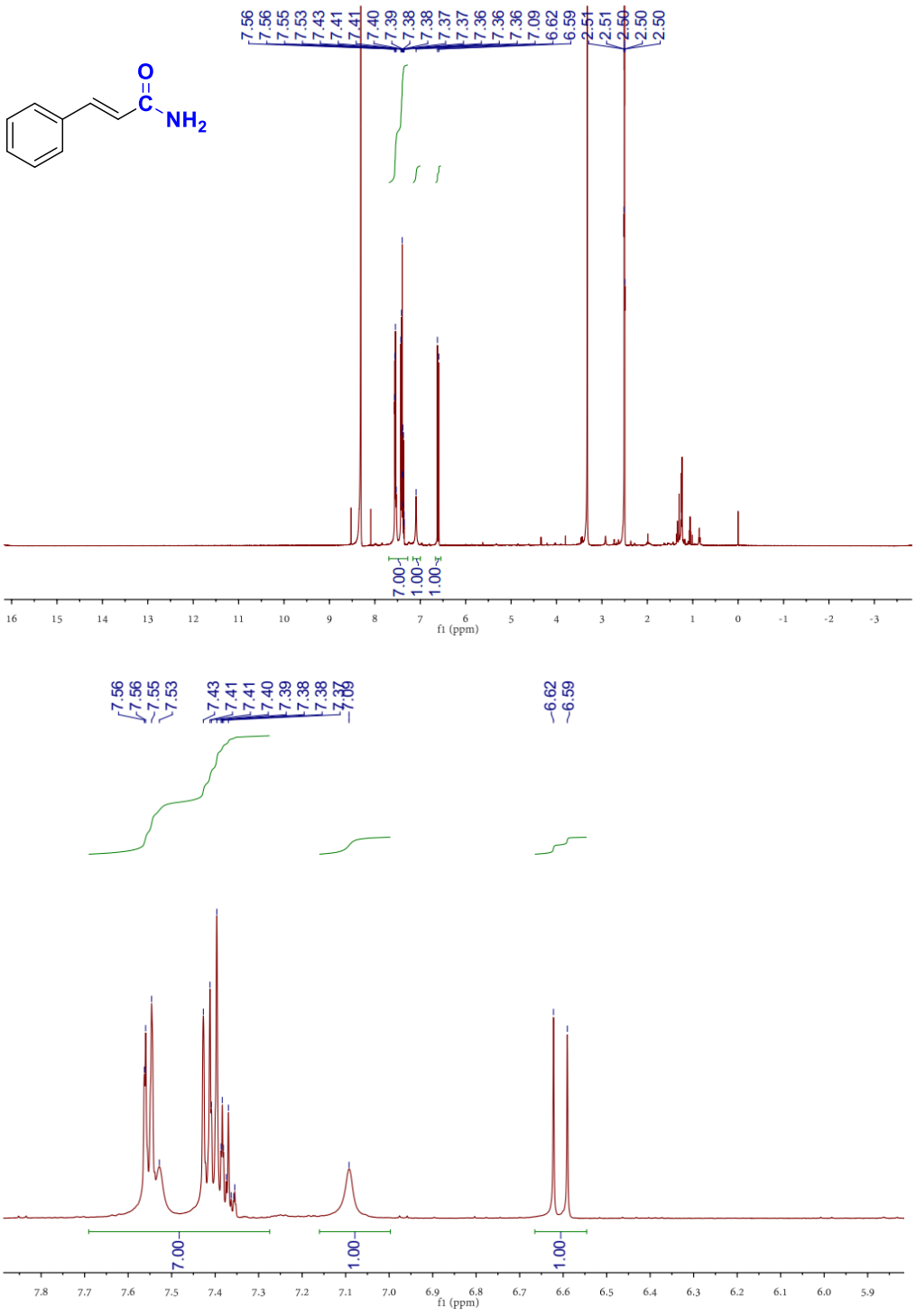


Figure S40 Following the general procedure, the product **10d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (60.3% yield). 1H NMR (500 MHz, DMSO) δ 7.43 (dddd, J = 13.0, 9.8, 6.3, 1.4 Hz, 1H), 7.09 (s, 1H), 6.61 (d, *J* = 15.9 Hz, 1H).

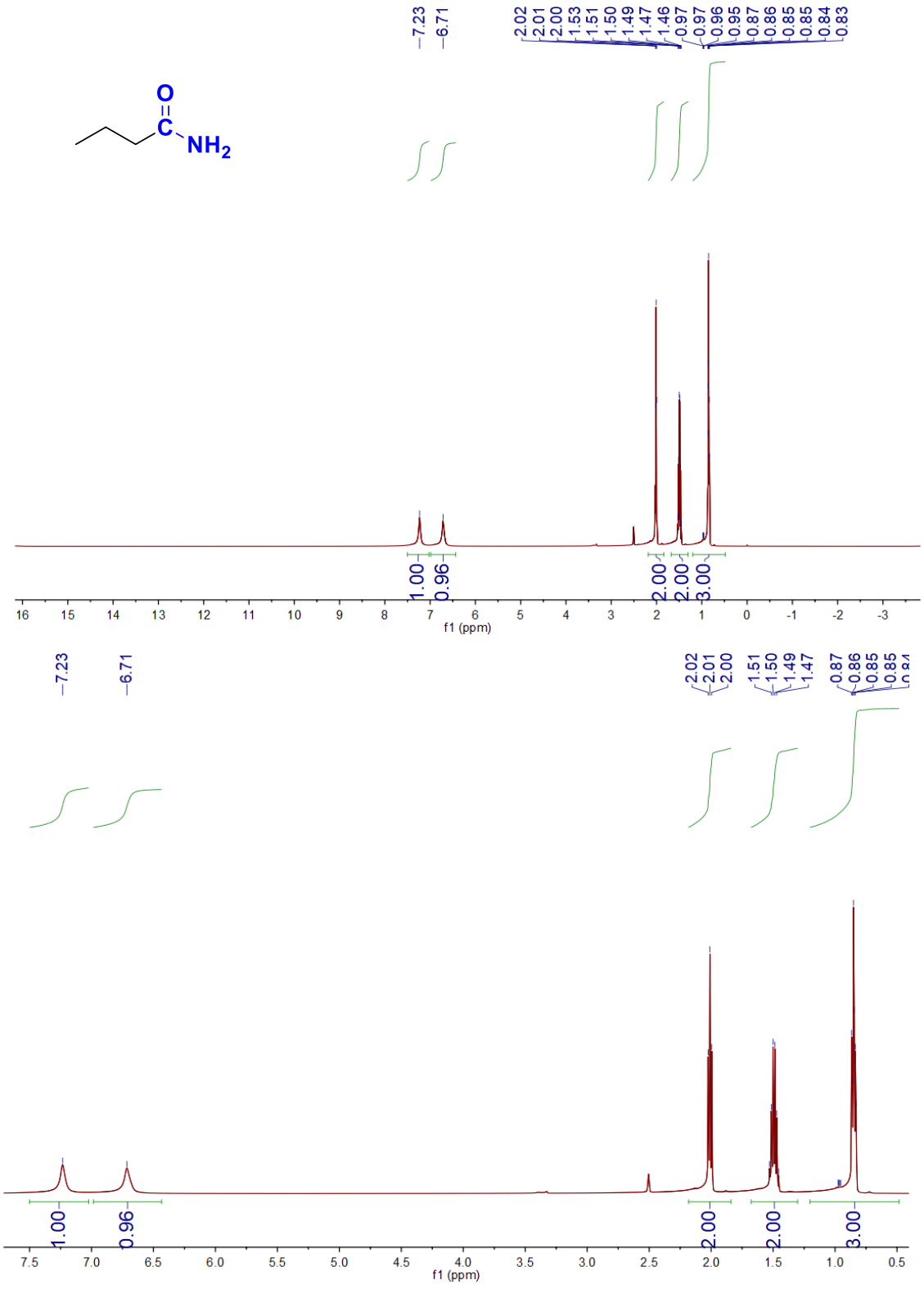


Figure S41 Following the general procedure, the product **12d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (39.2% yield). 1H NMR (500 MHz, CDCl3) δ 7.23 (s, 1H), 6.71 (s, 1H),2.01 (t, J =7.4 Hz, 2H), 1.49 (m, 2H), 0.89 (t, *J* =7.4 Hz, 3H).

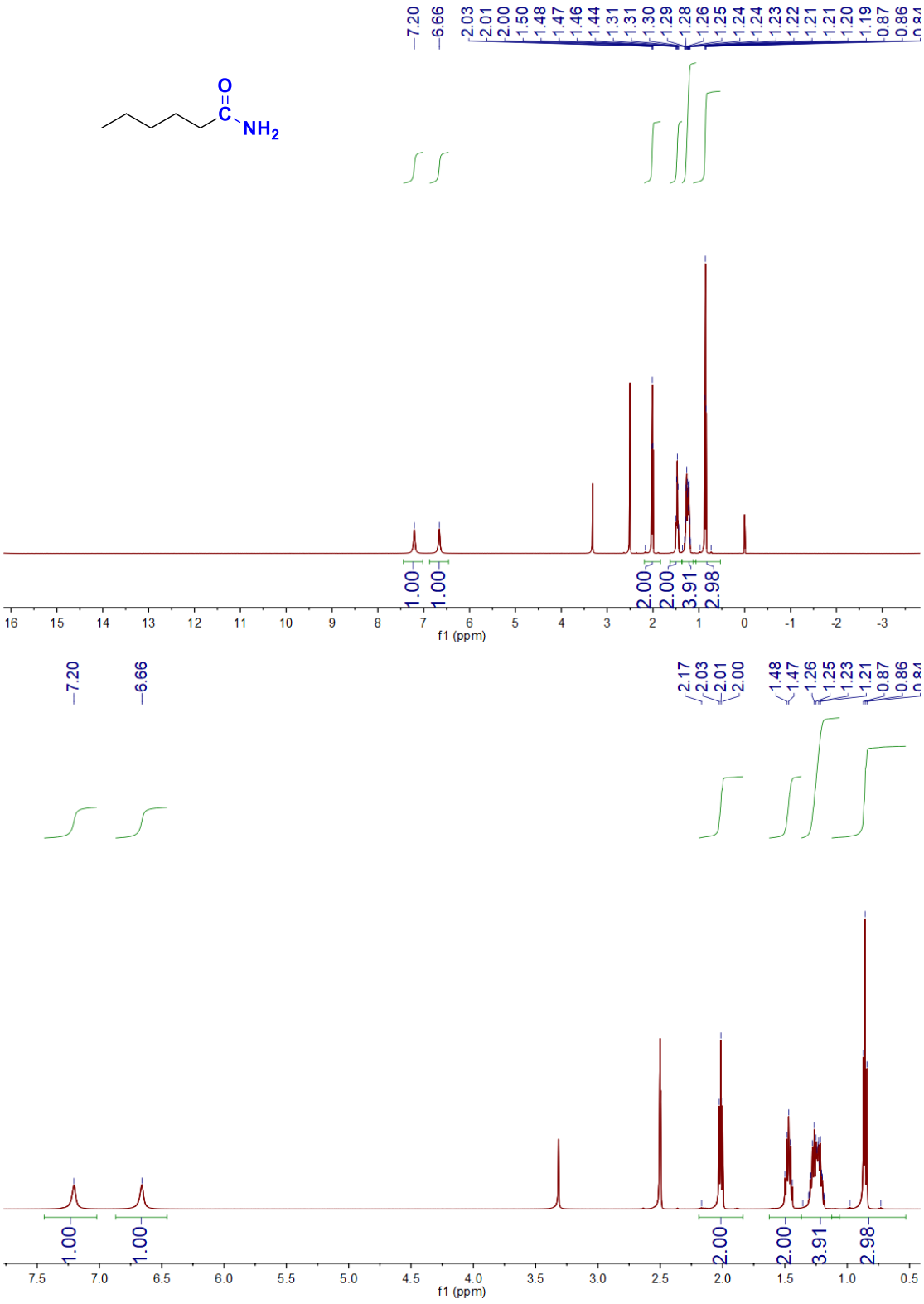


Figure S42 Following the general procedure, the product **13d** was prepared and purified by flash column chromatography (*n*-hexane:EA = 20:1) as a colorless oil (71.3% yield). 1H NMR (500 MHz, CDCl3) δ 7.20 (s, 1H), 6.66 (s, 1H), 2.01 (t, J =7.5 Hz, 2H), 1.47 (m, 2H), 1.24 (m, 4H), 0.86 (t, *J* =7.1 Hz, 3H).

GC-MS figures

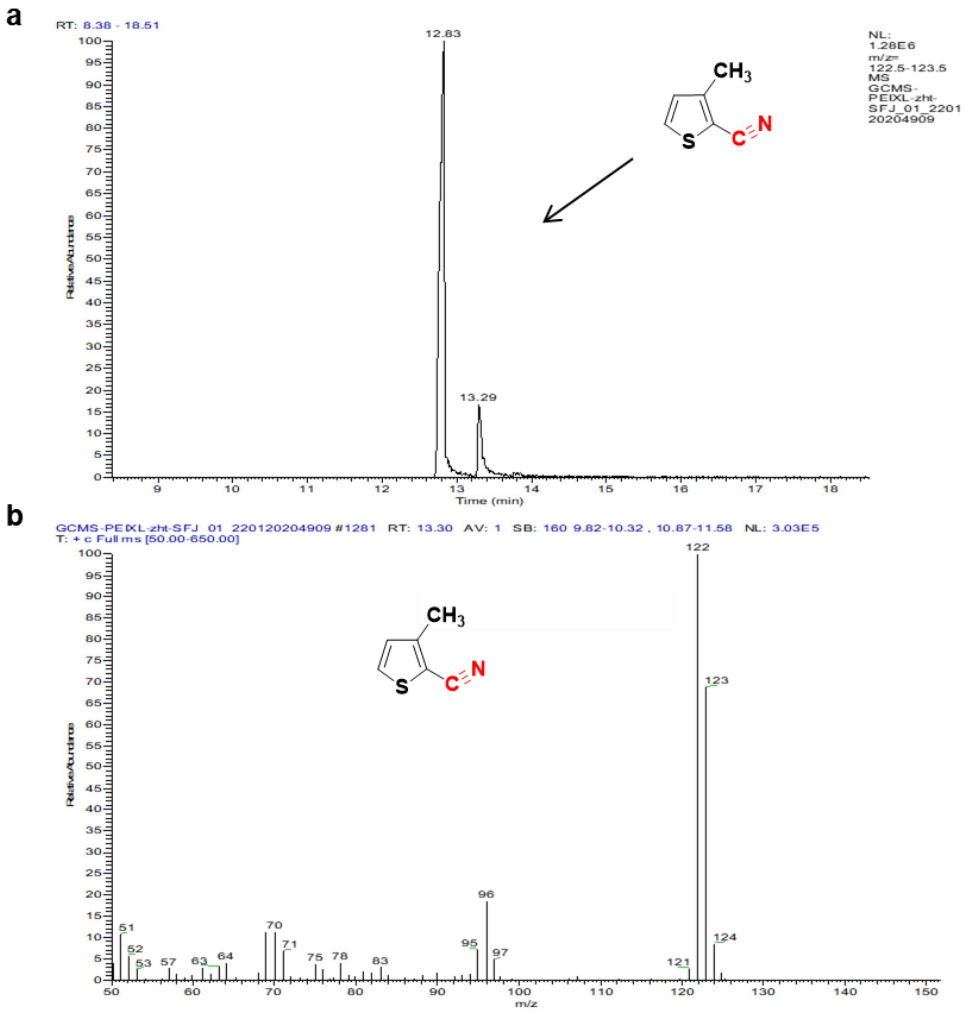


Figure S43 GC-MS analysis of 3-methylthiophene-2-carbonitrile (9c). The analysis was performed by gas chromatography mass spectrometer (Finnigan Trace DSQ GC/MS systems, Thermo Fisher, USA) with an electron impact (EI) ion source and an Xcalibur 1.4 workstation. The column was a HP-5MS capillary column (0.25 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and further held for 2min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 1.0 ml/min as the carrier. The transfer line temperature was set at 250 ℃. the MS acquisition parameters were: Ion source 200 ℃, electron impact ionization 70 eV, and solvent delay 4 min. The MS was operated at the selected ion-monitoring mode. The mass range and scan speed were set at 50 to 550 m/z and 1200 u/s, respectively. The chemical structure was deduced by the *in silico* spectra program.

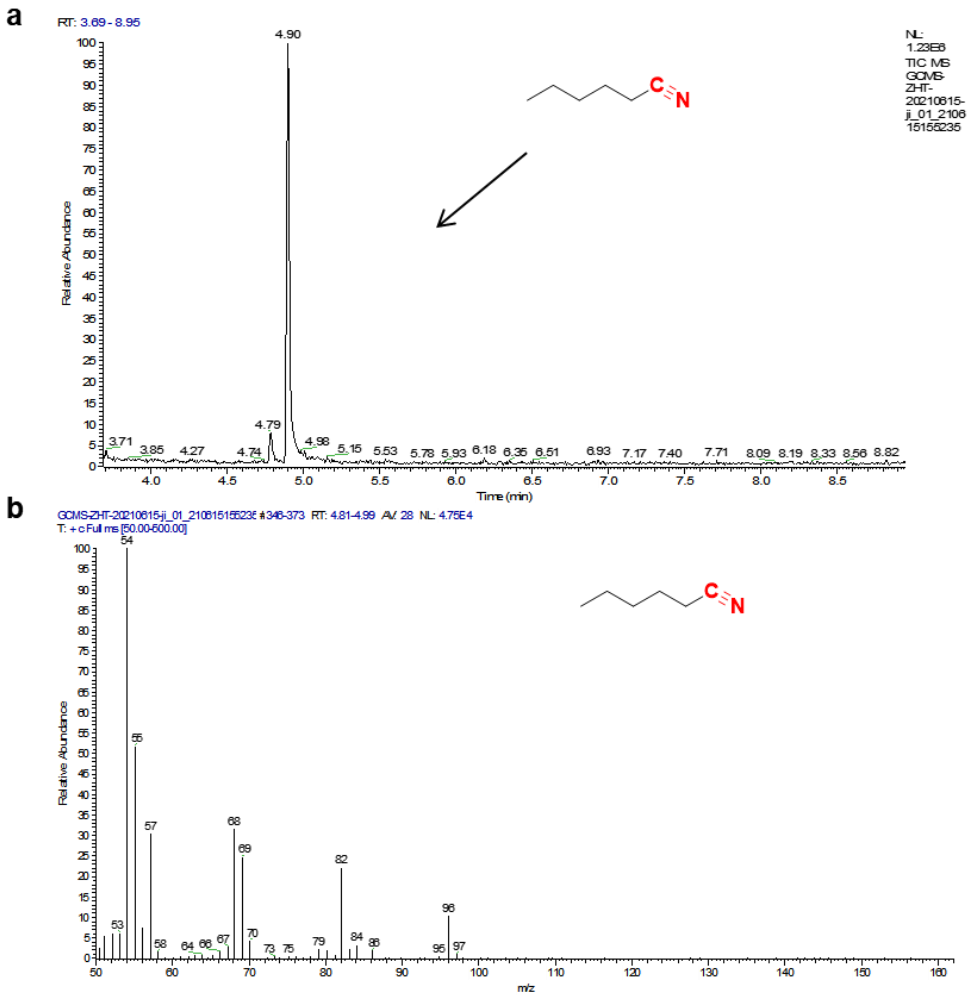


Figure S44 GC-MS analysis of hexanenitrile (13c). The analysis was performed by gas chromatography mass spectrometer (Finnigan Trace DSQ GC/MS systems, Thermo Fisher, USA) with an electron impact (EI) ion source and an Xcalibur 1.4 workstation. The column was a HP-5MS capillary column (0.25 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and further held for 2min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 1.0 ml/min as the carrier. The transfer line temperature was set at 250 ℃. the MS acquisition parameters were: Ion source 200 ℃, electron impact ionization 70 eV, and solvent delay 4 min. The MS was operated at the selected ion-monitoring mode. The mass range and scan speed were set at 50 to 550 m/z and 1200 u/s, respectively. The chemical structure was deduced by the *in silico* spectra program.

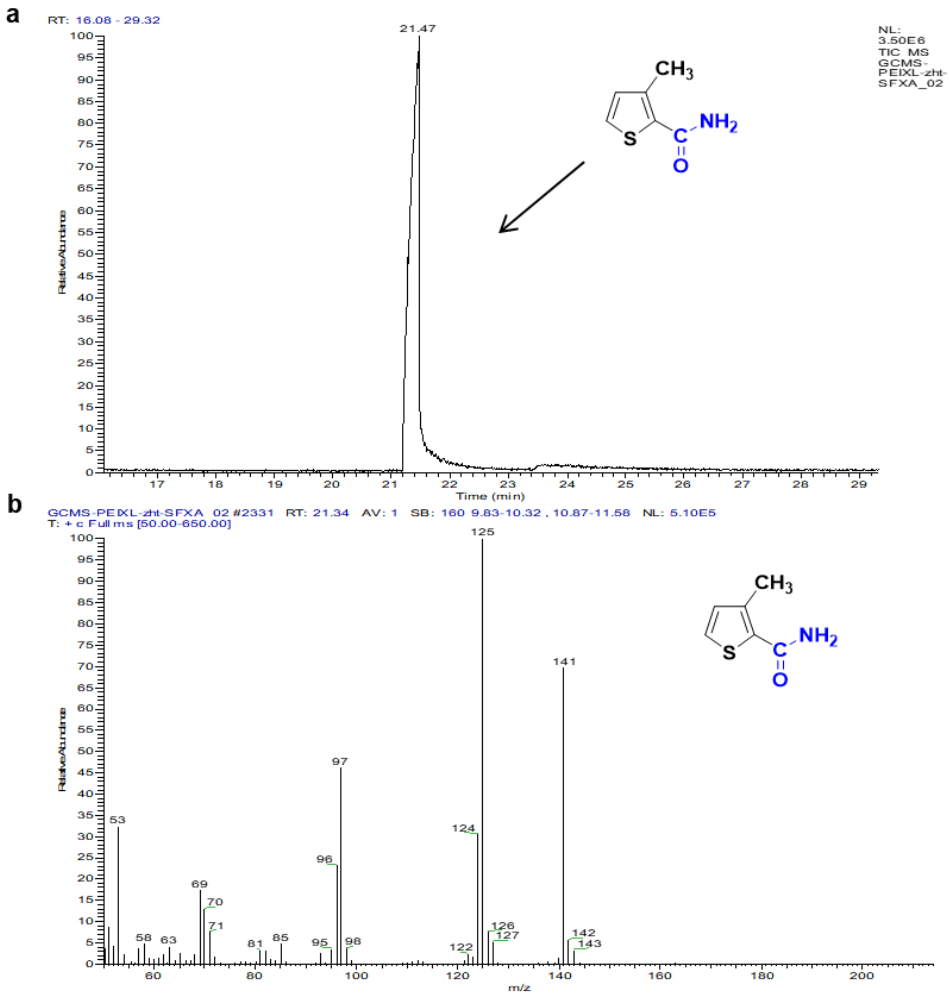


Figure S45 GC-MS analysis of 3-methylthiophene-2-carboxamide (9d). The analysis was performed by gas chromatography mass spectrometer (Finnigan Trace DSQ GC/MS systems, Thermo Fisher, USA) with an electron impact (EI) ion source and an Xcalibur 1.4 workstation. The column was a HP-5MS capillary column (0.25 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and further held for 2min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 1.0 ml/min as the carrier. The transfer line temperature was set at 250 ℃. the MS acquisition parameters were: Ion source 200 ℃, electron impact ionization 70 eV, and solvent delay 4 min. The MS was operated at the selected ion-monitoring mode. The mass range and scan speed were set at 50 to 550 m/z and 1200 u/s, respectively. The chemical structure was deduced by the *in silico* spectra program.

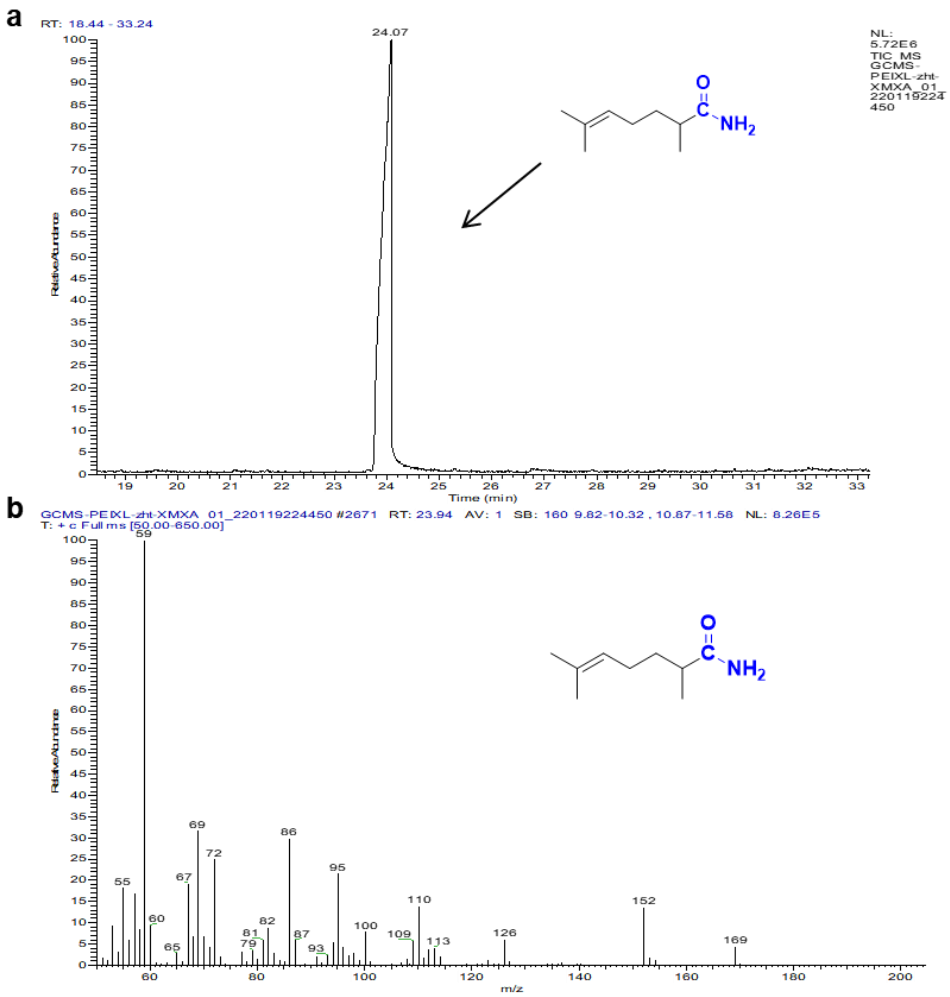


Figure S46 GC-MS analysis of citronellamide (11d). The analysis was performed by gas chromatography mass spectrometer (Finnigan Trace DSQ GC/MS systems, Thermo Fisher, USA) with an electron impact (EI) ion source and an Xcalibur 1.4 workstation. The column was a HP-5MS capillary column (0.25 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and further held for 2min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 1.0 ml/min as the carrier. The transfer line temperature was set at 250 ℃. the MS acquisition parameters were: Ion source 200 ℃, electron impact ionization 70 eV, and solvent delay 4 min. The MS was operated at the selected ion-monitoring mode. The mass range and scan speed were set at 50 to 550 m/z and 1200 u/s, respectively. The chemical structure was deduced by the *in silico* spectra program.

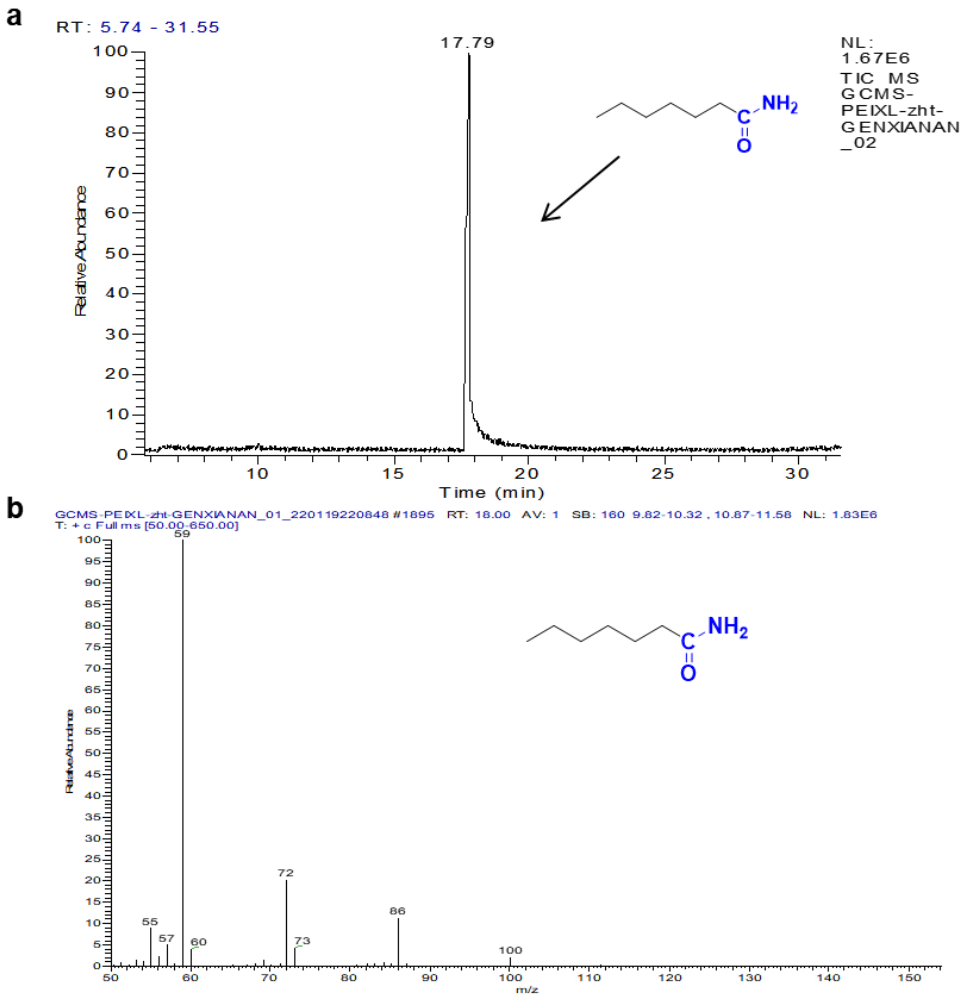


Figure S47 GC-MS analysis of heptamide (14d). The analysis was performed by gas chromatography mass spectrometer (Finnigan Trace DSQ GC/MS systems, Thermo Fisher, USA) with an electron impact (EI) ion source and an Xcalibur 1.4 workstation. The column was a HP-5MS capillary column (0.25 μm, 30 m×0.25 mm). The initial column oven temperature was regulated at 35 ℃ and further held for 2min, then heated to 200 ℃ at 5 ℃/min and heated to 280 ℃ at 20 ℃/min. Nitrogen gas was set at 1.0 ml/min as the carrier. The transfer line temperature was set at 250 ℃. the MS acquisition parameters were: Ion source 200 ℃, electron impact ionization 70 eV, and solvent delay 4 min. The MS was operated at the selected ion-monitoring mode. The mass range and scan speed were set at 50 to 550 m/z and 1200 u/s, respectively. The chemical structure was deduced by the *in silico* spectra program.

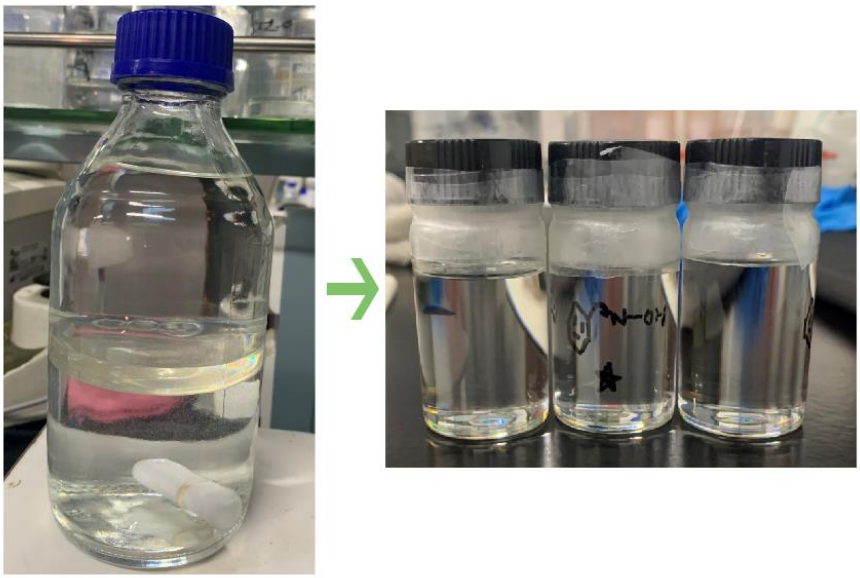


Figure S48 Chemical synthesis of benzaldoxime in *n*-hexane/H_2O biphasic system (1:1, *v/v*). Benzaldehyde (1 M, 47.7g) was dissolved in 450 mL n-hexane, NH_2OH ⊣Cl (1.5 eq, 1.5 M, 46.9 g) and sodium carbonate (0.55 eq, 0.55 M, 26.2 g) were dissolved in 450 m pure water. The both two solutions were mixed and stirred at room temperature until all benzaldehyde was completely conversed to benzaldoxime.

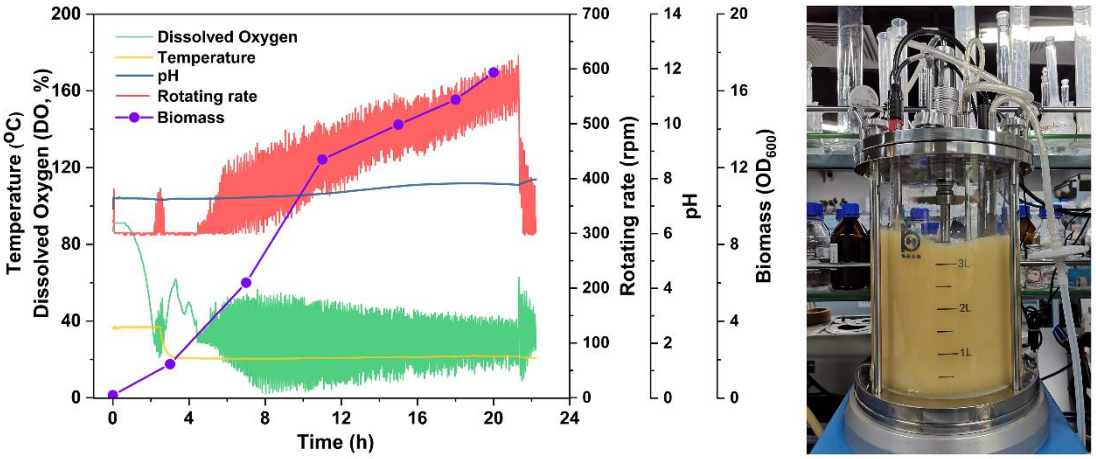


Figure S49 Time course of batch culture for expressing recombinant OxdF1 in 5-L jar fermenter.

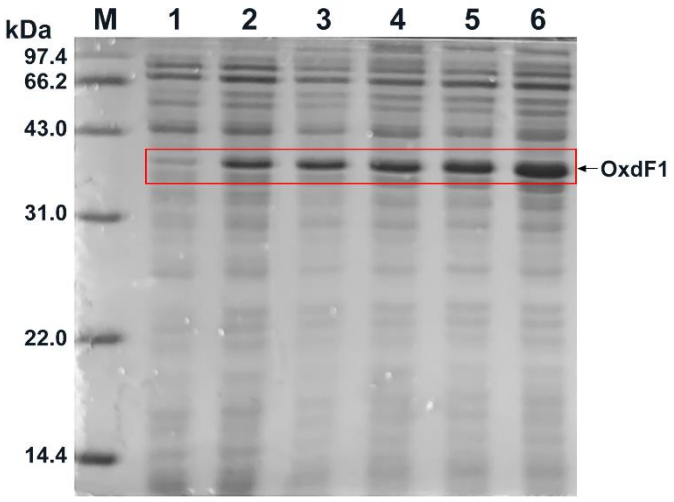


Figure S50 SDS-PAGE showing the expression of recombinant OxdF1 in a 5-fermenter at different time. M: protein molecular mass markers; Lane 1: 3 h after induction; Lane 2: 7 h; lane 3: 11 h; lane 4: 15 h; lane 5: 18 h; lane 6: 20 h.

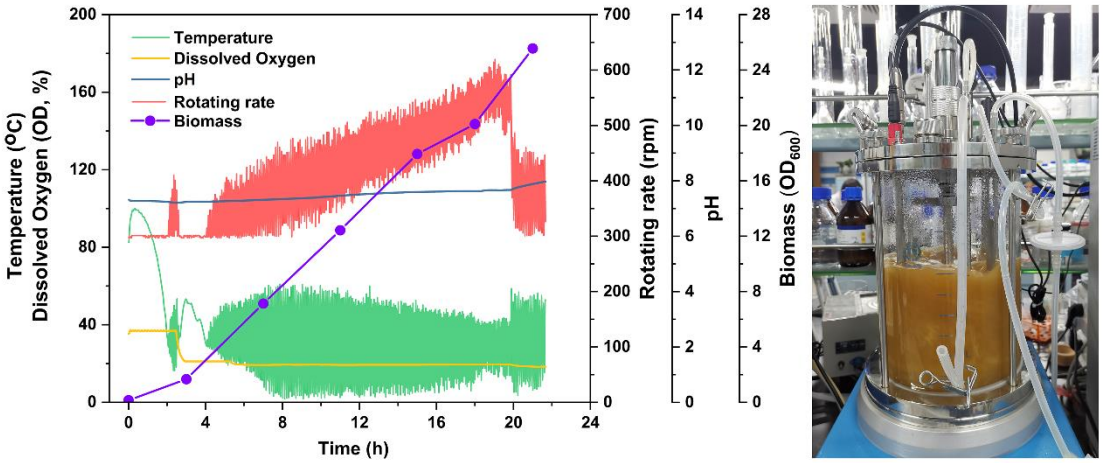


Figure S51 Time course of batch culture for expressing recombinant OxdF1 and NHase1229 in 5-L jar fermenter.

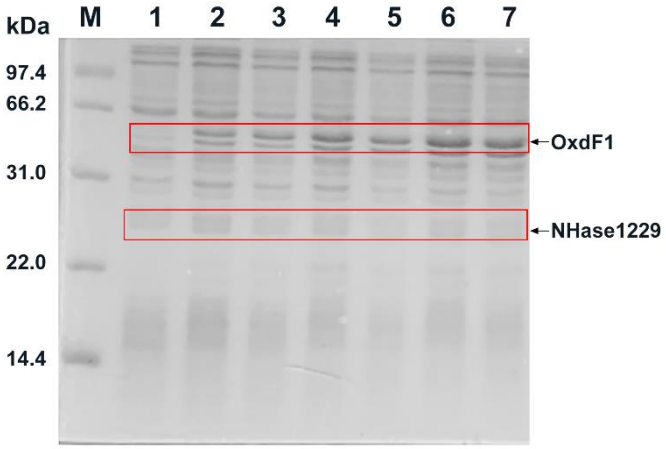


Figure S52 SDS-PAGE showing the expression of recombinant OxdF1 and NHase1229 in a 5-fermenter at different time. M: protein molecular mass markers; Lane 1: 3 h after induction; Lane 2: 7 h; lane 3: 11 h; lane 4: 15 h; lane 5: 18 h; lane 6: 20 h; lane 7: 22 h.

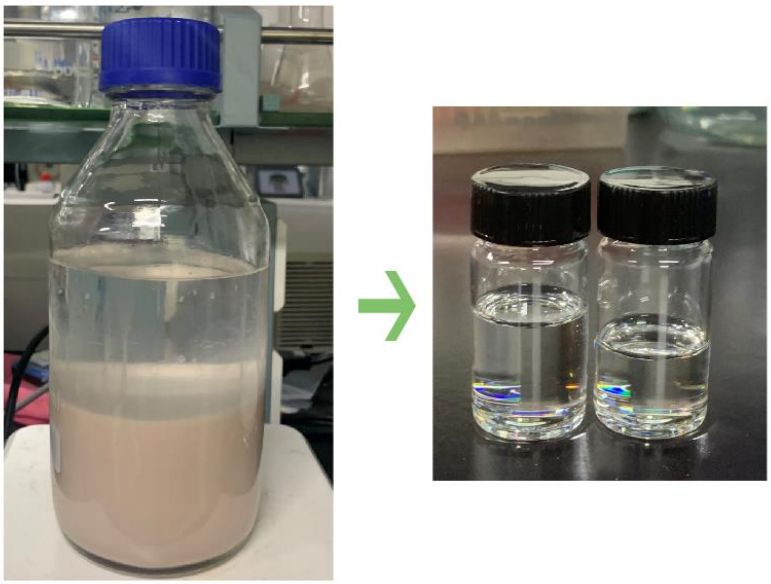


Figure S53 Biocatalytic synthesis of benzonitrile in *n*-hexane/H_2O biphasic system . The upper organic phase containing oximes was separated and A portion of the hexane phase was selected to be mixed directly with the aqueous phase medium, resulting in a benzaldehyde oxime concentration of 500 mM. And the aqueous phase containing recombinant OxdF1 *E. coli* whole cells (50 mm PPB, pH 7.0). The biphasic reaction system was stirred at room temperature until all benzaldoxime was completely conversed to benzonitrile. Add the hexane phase separated in the first step and mix with the reaction solution until the benzaldehyde oxime concentration reaches 200 mM and converted to benzonitrile.

Figure S54 Biocatalytic synthesis of benzoamide in *n*-hexane/H_2O biphasic system. The upper organic phase containing oximes was separated and a portion of the hexane phase was selected to be mixed directly with the aqueous phase medium, resulting in a benzaldehyde oxime concentration of 100 mM. And the aqueous phase containing recombinant OxdF1-NH08 *E. coli* whole cells (50 mm PPB, pH 7.0). The biphasic reaction system was stirred at room temperature until all benzaldoxime was completely conversed to benzamide. Three batches of 100 mM benzaldoxime were then added to the reaction mixture by solution dilution and converted to benzamide.