Supporting Information

Dual-Strategy Guided Bioinspired Catalysts for Efficient Synthesis of Pyrazole Derivatives Via Hydrazide–Ketone Condensation

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1 Materials and Instrumentation

Reagents

All chemicals and enzymes were purchased from commercial suppliers (MERCK, Sigma Aldrich, Aladdin, Energy Chemical, Sinopharm Chemical Reagent Co., Ltd., etc) and were used as received unless otherwise noted.

Reagents	Purity	Source
3-methylpentane-2,4-dione	AR	Merck & Co. Inc
3-chloropentane-2,4-dione	AR	Merck & Co. Inc
4-methylbenzohydrazide	AR	Merck & Co. Inc
2-methylbenzohydrazide	AR	Merck & Co. Inc
3-methylbenzohydrazide	AR	Merck & Co. Inc
4-methoxybenzohydrazide	AR	Merck & Co. Inc
4-chlorobenzohydrazide	AR	Merck & Co. Inc
4-bromobenzohydrazide	AR	Merck & Co. Inc
4-hydroxybenzohydrazide	AR	Merck & Co. Inc
hexanehydrazide	AR	Merck & Co. Inc
Benzoyl hydrazine	AR	Energy Chemical Co., Ltd
Acetylacetone	AR	Energy Chemical Co., Ltd
Benzaldehyde	AR	Energy Chemical Co., Ltd
Acetamide	AR	Sinopharm Chemical Reagent Co., Ltd
heptane-3,5-dione	AR	Shanghai Aladdin Biochemical Technology Co., Ltd

Reagents	Purity	Source
hexane-2,4-dione	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
4-fluorobenzohydrazide	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
P-Toluenesulfonamide	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
Serine	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
Histidine	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
Aspartic acid	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
Asparagine	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
Boc-Asparagine	AR	Shanghai Aladdin Biochemical Technology Co., Ltd
CAL-B	-	Sigma-Aldrich (Japan) Co., Ltd
SGP	-	Sigma-Aldrich (Japan) Co., Ltd
PURO	-	BGI Genomics Co., Ltd.
PBS	-	Shanghai Yuanye Biotechnology Co., Ltd
Water	AR	Sinopharm Chemical Reagent Co., Ltd
EtOH	AR	Sinopharm Chemical Reagent Co., Ltd
MeOH	AR	Sinopharm Chemical Reagent Co., Ltd
NPA	AR	Sinopharm Chemical Reagent Co., Ltd
NBA	AR	Sinopharm Chemical Reagent Co., Ltd
ACN	AR	Sinopharm Chemical Reagent Co., Ltd
GVL	AR	Sinopharm Chemical Reagent Co., Ltd
DMSO	AR	Sinopharm Chemical Reagent Co., Ltd

Reagents	Purity	Source
DMF	AR	Sinopharm Chemical Reagent Co., Ltd
IPA	AR	Sinopharm Chemical Reagent Co., Ltd
EA	AR	Sinopharm Chemical Reagent Co., Ltd
THF	AR	Sinopharm Chemical Reagent Co., Ltd
DCM	AR	Sinopharm Chemical Reagent Co., Ltd
NMP	AR	Sinopharm Chemical Reagent Co., Ltd

Monitoring

The reaction was monitored using thin-layer chromatography (TLC) on precoated aluminum sheets (0.2 mm silica gel 60 F254, Merck®), and visualized under UV light (254 nm).

Purification of reaction product

The separation of the products was carried out using silica gel column chromatography. The mobile phase consisted of petroleum ether/ethyl acetate or methanol/dichloromethane, with an equal proportion of triethylamine (0.1%) incorporated into the mobile phase. Purification of the compounds was achieved by employing solvents with appropriate polarity ratios. The instrument used was a Biotage system, and the silica gel employed was from Sweden AB with a mesh size of 200-300.

Mass spectrometry

The mass spectrometry was recorded using electrospray ionization (ESI) or electron shock (EI) methods in Thermo Fisher's Ultimate3000 UHPLC liquid phase and Bruker's Compact Q-TOF mass

UV-Vis spectrometry

The UV-Vis spectrometry was recorded using a HPLC with temperature control (Agilent 1260 Infinity II), and the concentration of the reaction solution was determined by the external standard method.

NMR spectrometry

NMR spectroscopy ^{1}H and ^{13}C NMR spectra were recorded at room temperature using Bruker models AVANCE-III 600 MHz. Chemical shifts were reported in ppm with the solvent signal ($^{1}H/^{13}C$: deuterated chloroform $CDCl_3$ 7.26/77.2 ppm, dimethylsulfoxide DMSO-d6 2.49/39.5 ppm or methanol CD3OD-d4 3.3/49.0 ppm and tetramethylsilane TMS 0.00 ppm) as an internal standard using reportedshifts.

2 Constructs and Expression of Natural Enzymes

2.1 Plasmid information of PURO

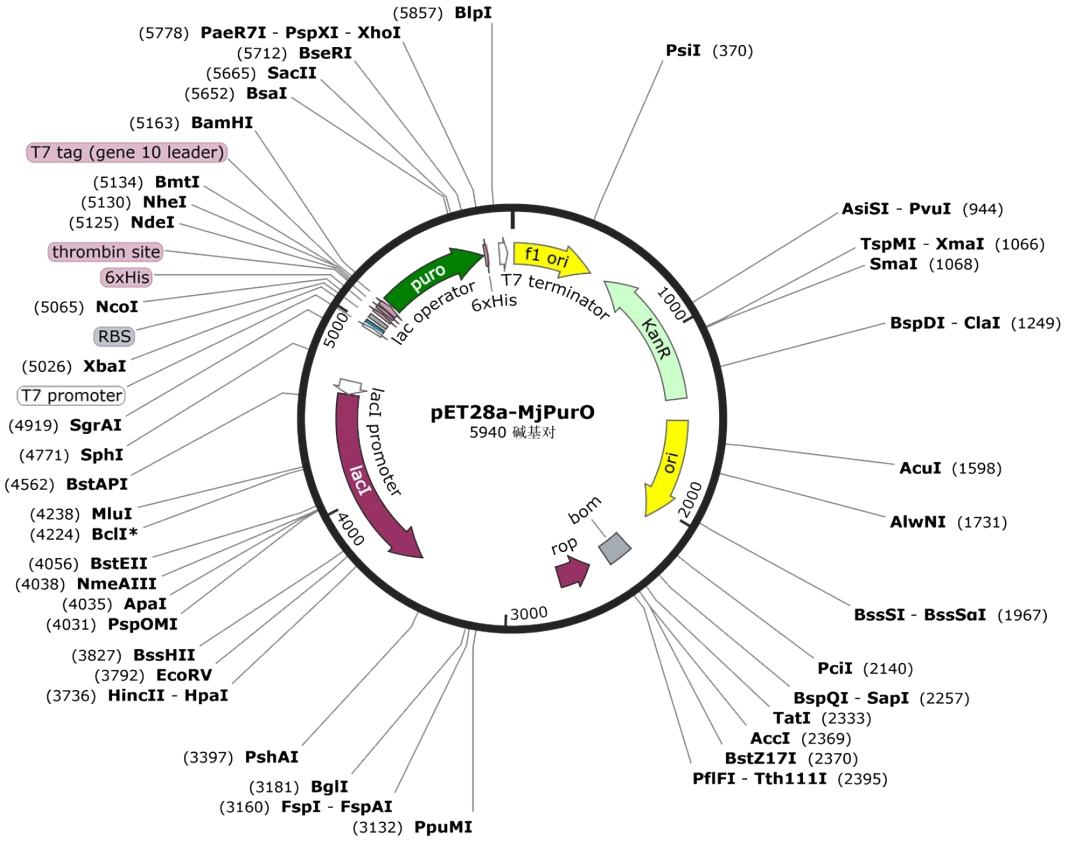

Figure S1. PURO enzyme plasmid information, by BGI Genomics Co., Ltd. Provided.

Plasmid information of PURO:

ATGTATATTGGCCGCTTTCTGGTGGTGGGCAAAACCAAAGAAGGCAAACC

GTTTGCGGCGTATCGCGTGAGCAGCCGCAGCTTTCCTAATCGCGAAGCGA

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TGCATTTCGGCAAGCGCGATGCGCTGGCGTATGTGTTAGCGGTGATGGAT

TATGAAAAAGACGATTACAAGACCCCGCGCATTGCGGCGATTCTGGATGA

AAACGAATGCTATATGGGCTATGTGGCGCATGATGATATTCGCGTGAAAA

AAGTGGAACTGAAAGATGGCAAAGGCTATTATCTGGGCGTGTATAATGCG

TGCAAAATTGATGAAAACCAGATCATCGACATTAAGGGCGAGACCGCGG

AAGAAATTGCGGATTATATTCTGAACTATGAGGAGTTCGAGCACCCGGTG

GCGTGCGCGGTTGCAGTTATTGATAAAGATGGCATTAAAATCGCGACCAA

GGGCAAATAA

2.2 Protein constructs and production of PURO

The PURO gene was successfully cloned into the pET28a expression vector and expressed in E. coli BL21 cells. This cloning and expression work was performed by BGI Genomics Co., Ltd.

Single colonies were inoculated into sterile tubes containing 10 mL LB medium supplemented with 10 μL antibiotic (1:1000 volume). Cultures were incubated overnight at 37°C with shaking at 200 rpm for activation. A 100 μL aliquot from the overnight culture was transferred to fresh 10 mL LB medium containing 10 μL antibiotic. This secondary culture was incubated at 37°C with shaking at 200 rpm for 4-5 hours. The activated culture was inoculated into 250 mL LB medium at a 1:100 dilution ratio. Antibiotic supplementation (250 μL kanamycin, 0.1% v/v) was added to the main culture. Cultures were grown at 37°C with shaking at 200 rpm for 3-4 hours. Optical density measurements were initiated when visible turbidity developed (OD_{600} monitoring). Exponential growth phase was confirmed at OD_{600} = 0.5-0.7. Protein expression was induced by adding 25 μL IPTG, followed by overnight incubation at 30°C with shaking at 200 rpm. Cultures were centrifuged at 8000 rpm for 10 minutes to harvest bacterial cells (pellet). Cell pellets were resuspended in lysis buffer (25 mM HEPES pH 7.5, 5 mM imidazole, 0.5 M NaCl). Cells were lysed via high-pressure homogenization, with soluble protein retained in the supernatant. The lysate was incubated with 2.5 mL Ni-NTA resin on ice for 1 hour with gentle agitation. The mixture was loaded onto a chromatography column, allowed to settle for 2-3 minutes, and the flow-through was collected. The column was washed twice with 15 mL washing

buffer (25 mM HEPES pH 7.5, 150 mM imidazole, 0.3 M NaCl). Target protein was eluted using 7 mL elution buffer (25 mM HEPES pH 7.5, 150 mM imidazole, 0.3 M NaCl), with the eluate collected in 2 mL fractions. This process was repeated twice. Eluted fractions (14 mL total) were concentrated to ~500 µL using a 30 kDa molecular weight cutoff ultrafiltration device at 4°C and 5000 rpm. Final protein concentration was determined using standard spectroscopic methods.

2.3 Structural information of natural enzymes

The pocket information for PURO, CAL-B, and SGP was predicted using CASTpFold.

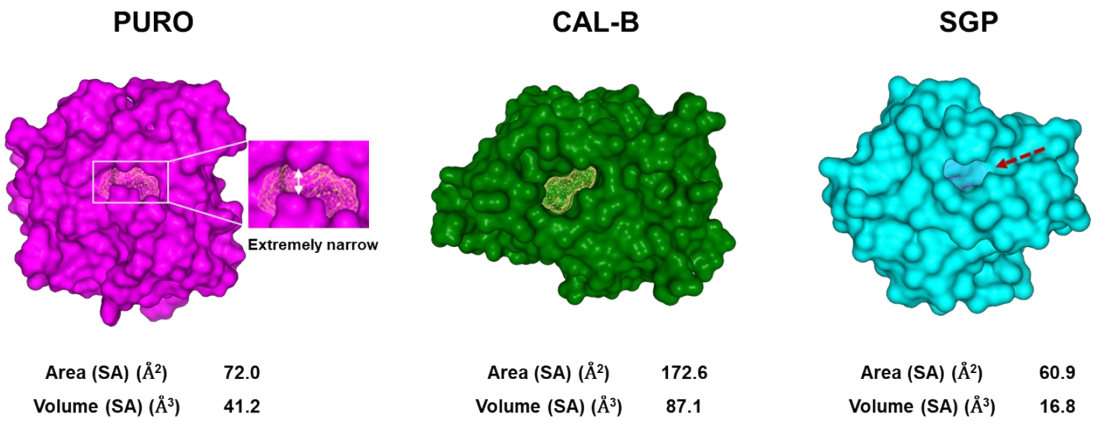


Figure S2. Pocket information of PURO, CAL-B and SGP enzymes.

3 Synthesis and Characterization of Catalysts

3.1 General procedure for the synthesis of catalysts

All hydrolase mimics are synthesized by solid-phase synthesis technology. According to the Amino acid connection sequence in hydrolase mimics, they are numbered as amino acid-1,2,3....

Specific synthesis Procedure: Weigh 10.0 g of dried Rink-amide resin (100-200 mesh, 7 mmol/g amino loading) into a reaction vessel. Swell the resin in 50 mL N,N-dimethylformamide (DMF) for 1 hour, then filter and dry under vacuum. Add 30 mL of 20% piperidine/DMF solution and stir at room temperature for 20 minutes. Filter and dry under vacuum, then repeat this deprotection cycle three additional times. Wash the resin alternately with 40 mL DMF and 40 mL isopropanol (IPA) three times each, filtering and drying under vacuum after each wash. Set aside the prepared resin. In a reaction vessel, combine 15.4 mmol each of Fmoc-Amino acid-1, HATU, and HOBT with 40 mL DMF. Add the prepared resin and 23.1 mmol triethylamine, then seal the vessel and stir at room temperature. Monitor reaction completion using ninhydrin colorimetric test. Upon completion, wash the resin alternately with 40 mL DMF and 40 mL IPA three times each, filtering and drying under vacuum after each wash. Subsequently add 15.4 mmol each of Fmoc-Amino acid-2, Fmoc-Amino acid-3 or Fmoc-Amino acid-4, etc, repeating the coupling and washing procedures sequentially until full peptide chain assembly is achieved. Remove the terminal Fmoc protection group using the above procedure, yielding the protected peptide resin Rink-NH-Catalysts after filtration and drying. Transfer the protected peptide resin (120 mL reaction scale) to a cleavage mixture containing TFA (84.5%), water (5%), thioanisole

(3%), triisopropylsilane (TIPS, 5%), and 1,2-ethanedithiol (EDT, 2.5%). Seal and stir at room temperature for 2.5 hours. Filter the resin, remove TFA under reduced pressure, then precipitate the crude product by adding cold diethyl ether. Collect the precipitate via centrifugation, redissolve in water, and lyophilize to obtain the catalysts.

The synthesized catalyst was desalinated by ion exchange (with chloride ions replacing trifluoroacetate ions) and then purified by HPLC to obtain the final catalyst. For the detailed process, please refer to 3.2 General Procedure for the Purification of catalysts.

Whether the hydrolase mimics are successfully synthesized is determined by the results of HPLC, MS, ^{1}H, and ^{13}C Spectrum.

Table S1. The sequence of hydrolase mimics.

Hydrolase mimics	Sequence
HM1	NH_2-Glu-Tyr-Arg
HM2	NH_2-Asp-His-Ser
HM3	NH_2-Asp-His-Ser-Gly
HM4	NH_2-Asp-Ser-His
HM5	NH_2-Ser-Asp-His
HM6	NH_2-Asp-His
HM7	NH_2-Asp-Ser
HM8	NH_2-Asp-Ser-Gly
HM9	NH_2-Gly-Asp-His-Ser-Gly

3.2 General procedure for the Purification and determination of catalysts

Desalting Procedure

a. The molar amount of CF_3COO^- was calculated based on the mass of the hydrolase mimic. Anion exchange resin (Type 717, chloride form, exchange capacity 3 mmol/g) was weighed and soaked in ultrapure water overnight. A NaOH (1 M) solution was prepared.

b. The fully soaked resin was removed and rinsed repeatedly with ultrapure water (5-minutes each time) until the washings became colorless.

c. The resin was subsequently washed three times with NaOH solution (5-minutes each time).

d. Following this, the resin was thoroughly rinsed with ultrapure water (5-minutes each time) until neutral pH was achieved.

e. The hydrolase mimic and resin were dissolved in a water-acetonitrile mixture, followed by 1 hour of stirring.

f. After desalting completion, the supernatant was collected and freeze-dried. The resin was then rinsed repeatedly with the same solvent mixture (10-minutes each time) before final freeze-drying.

Purification Procedure

Further purification of the hydrolase mimic was achieved through high-performance liquid chromatography (HPLC). The chromatography column employed was a Daisogel C18-10μ-120A (30*250mm) with the following mobile phase composition:

Solvent A: Water (0.1% formic acid)

Solvent B: Acetonitrile (0.1% formic acid)

Table S2. HPLC method for purifying catalysts.

Time(min)	Flow velocity(mL/min)	A%	B%
0	17	90	10
25	17	50	50
30	17	10	90
40	17	5	95
50	17	0	100

Concentration detection procedure

The catalyst concentration was determined by HPLC(Agilent 1260 Infinity Ⅱ). Chromatographic column uses Agilent ZORBAX StableBond SB-C18, 4.6x250 mm, 5um.

Solvent A: Water (0.1% formic acid)

Solvent B: Acetonitrile (0.1% formic acid)

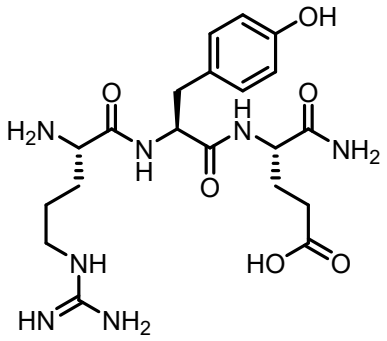
Wavelength: 220 nm

Table S3. HPLC method for detecting catalysts.

Time(min)	Flow velocity(mL/min)	A%	B%
0	1	95	5
25	1	70	30
25.1	1	0	100
30	1	Stop	

3.3 Characterization of catalysts

HM1 (NH_2-Glu-Tyr-Arg)

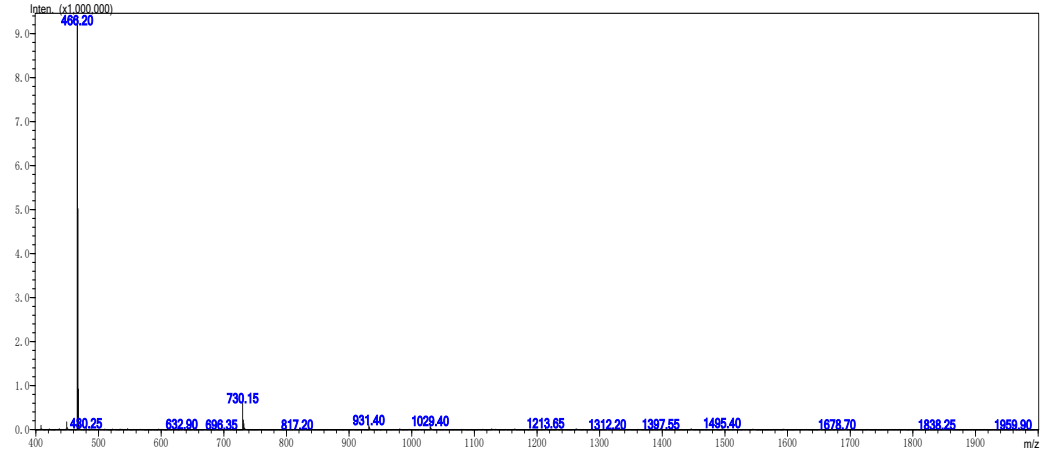


MS (ESI+): Calculated for $C_{13}H_{20}N_6O_6$ $[M+H]^+$ 466.23, found 466.20(1+).

^{1}H NMR (600 MHz, DMSO) δ 8.57 (d, J = 7.7 Hz, 1H), 8.21 (d, J = 8.1 Hz, 1H), 8.11 (d, J = 5.8 Hz, 3H), 7.68 (t, J = 5.9 Hz, 1H), 7.26 (s, 1H), 7.12 (s, 1H), 7.09 (s, 1H), 7.07 (s, 1H), 6.66 (d, J = 8.5 Hz, 2H), 4.51 (ddd, J = 9.6, 7.7, 4.5 Hz, 1H), 4.18 (td, J = 8.3, 5.3 Hz, 1H), 3.77 (q, J = 6.1 Hz, 1H), 3.10 (q, J = 7.0 Hz, 2H), 2.96 (dd, J = 14.3, 4.7 Hz, 1H), 2.70 (dd, J = 14.2, 9.4 Hz, 1H), 2.25 – 2.19 (m, 2H), 1.94 – 1.88 (m, 1H), 1.79 – 1.74 (m, 1H), 1.71 (p, J = 6.3 Hz, 2H), 1.52 (dt, J = 14.2, 6.9 Hz, 2H).

^{13}C NMR (151 MHz, DMSO) δ 177.4, 174.8, 173.7, 170.9, 157.3, 155.9, 130.0, 127.9, 114.9, 54.6, 53.4, 53.3, 35.9, 33.5, 31.4, 27.9, 24.7, 22.6.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **95.39 %**.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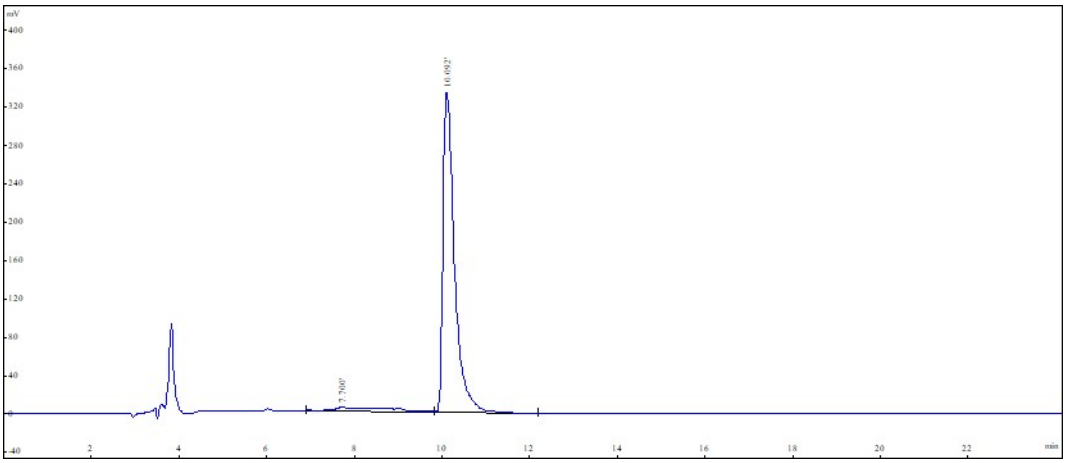
m/z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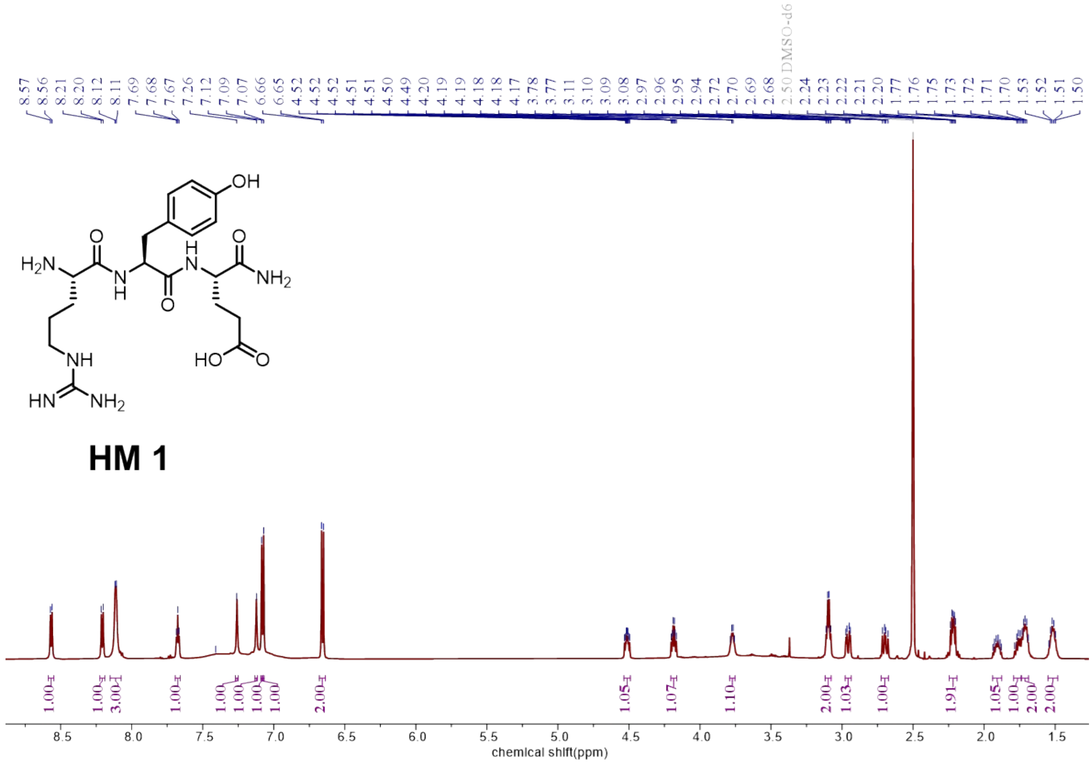
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N
H
H
N
O
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O
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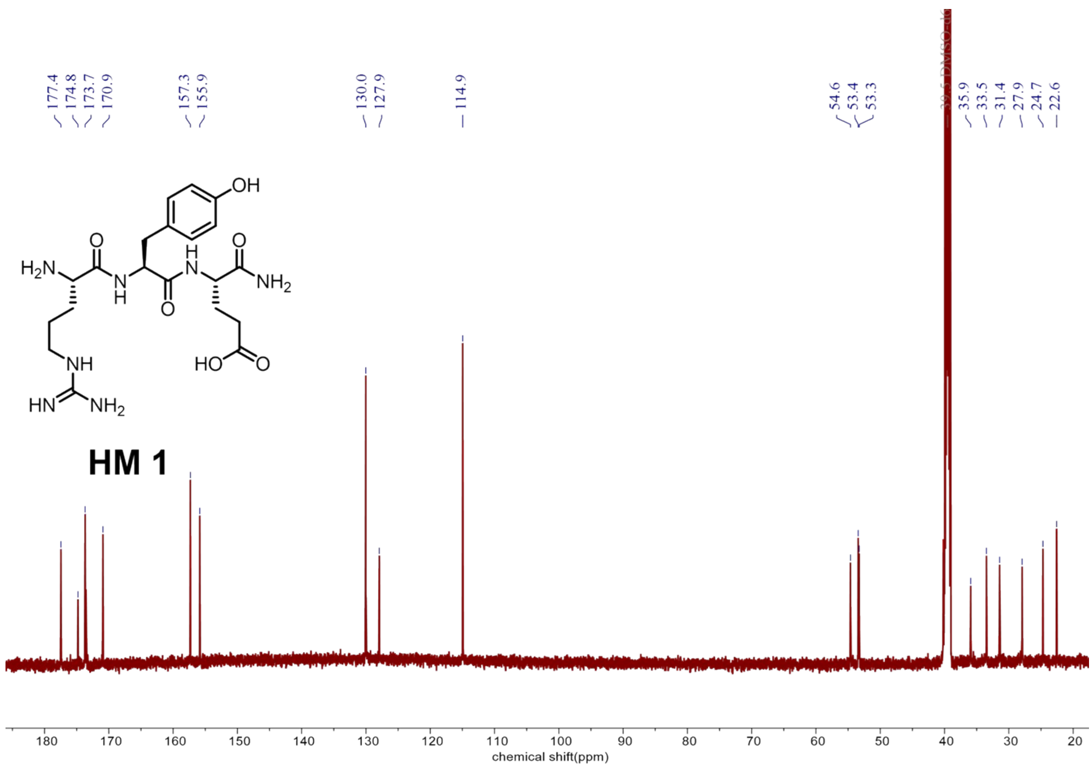
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OH
O
H₂N
N
H
H
N
O
NH₂
O
NH
HN
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chemical shift(ppm)

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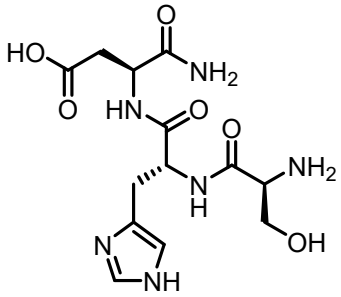
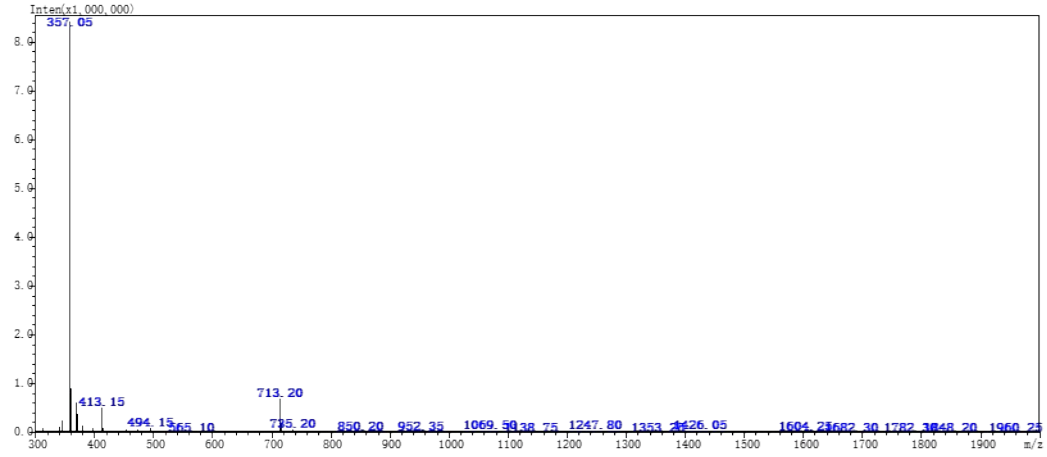


MS (ESI+): Calculated for $C_{13}H_{20}N_6O_6$ $[M+H]^+$ 357.14, found 357.05(1+).

^{1}H NMR (600 MHz, DMSO) δ 8.92 (s, 1H), 8.75 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 7.4 Hz, 1H), 8.16 (s, 2H), 7.48 (s, 1H), 7.36 (s, 1H), 7.26 (s, 1H), 4.66 – 4.62 (m, 1H), 4.48 (td, J = 8.0, 5.2 Hz, 1H), 3.92 – 3.88 (m, 1H), 3.73 (dd, J = 11.4, 4.4 Hz, 1H), 3.67 (dd, J = 11.4, 6.3 Hz, 1H), 3.11 (dd, J = 15.6, 5.5 Hz, 1H), 3.02 (dd, J = 15.5, 6.9 Hz, 1H), 2.69 (dd, J = 16.7, 5.2 Hz, 1H), 1.91 (s, 2H).

^{13}C NMR (151 MHz, DMSO) δ 172.7, 171.6, 169.1, 167.0, 133.9, 128.9, 117.2, 60.3, 54.2, 51.8, 49.6, 36.1, 27.2.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **96.61%**.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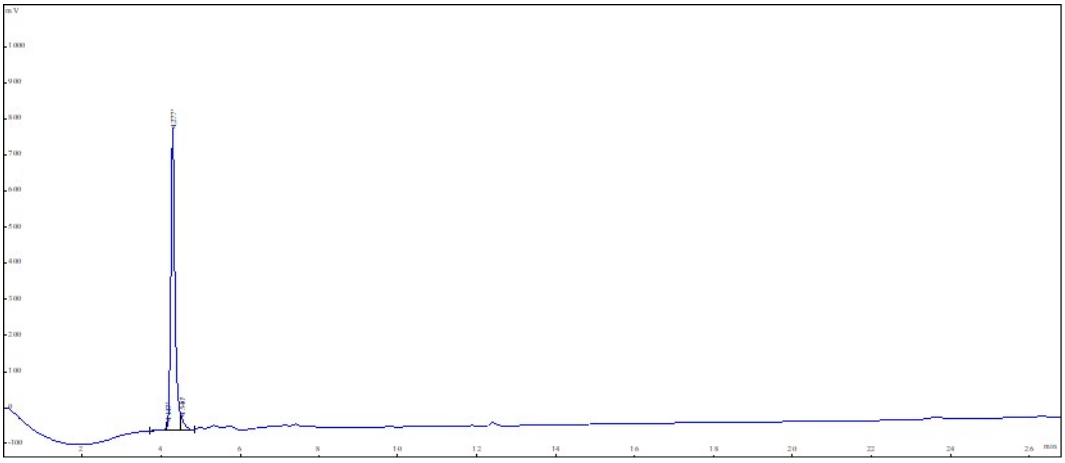

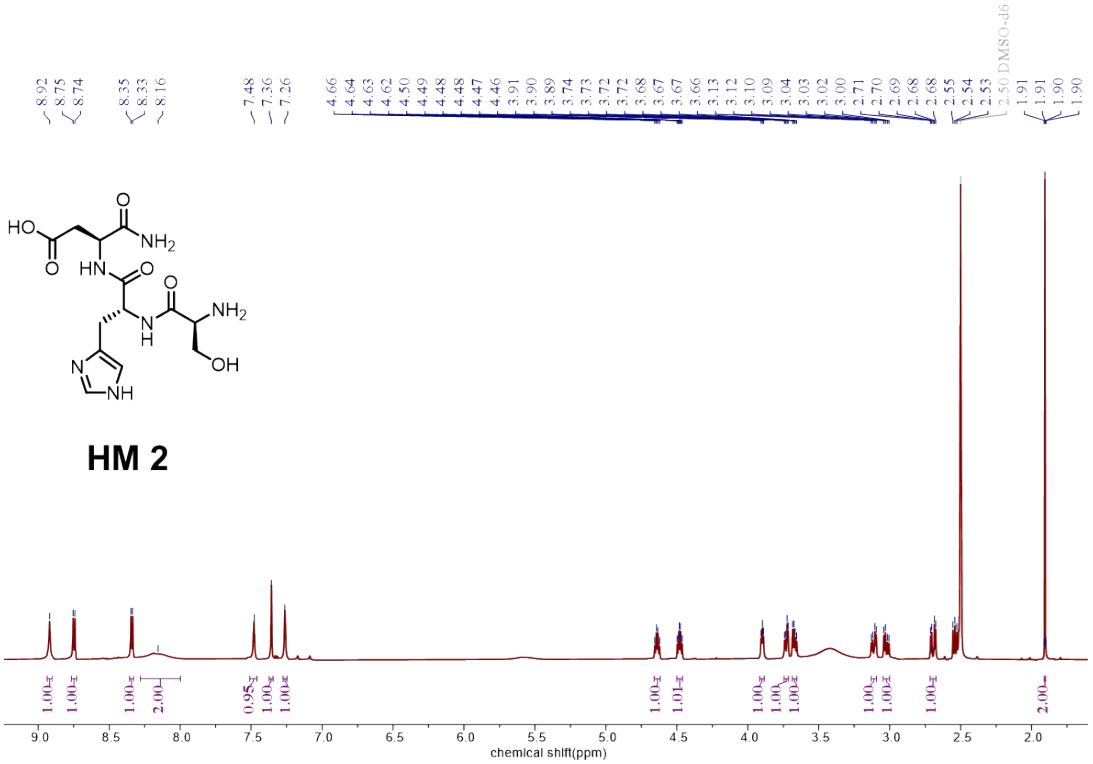
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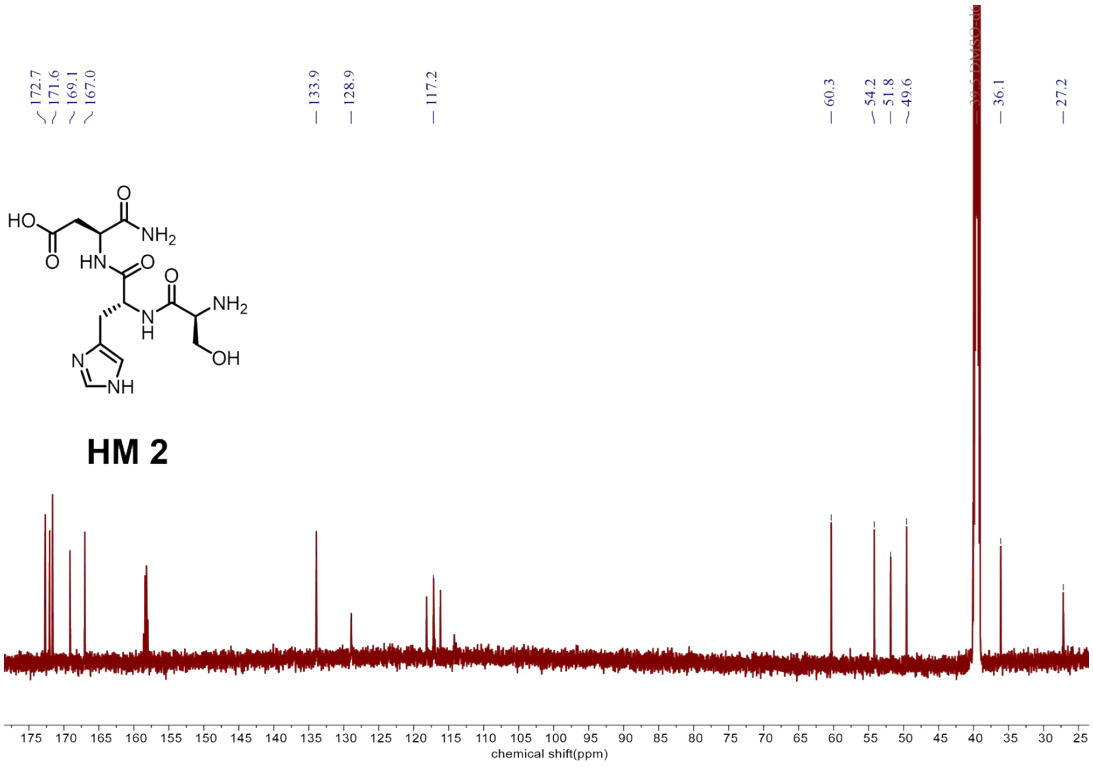
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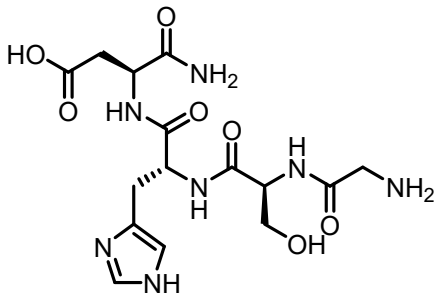
chemical shift(ppm)

HM3 (NH_2-Asp-His-Ser-Gly)

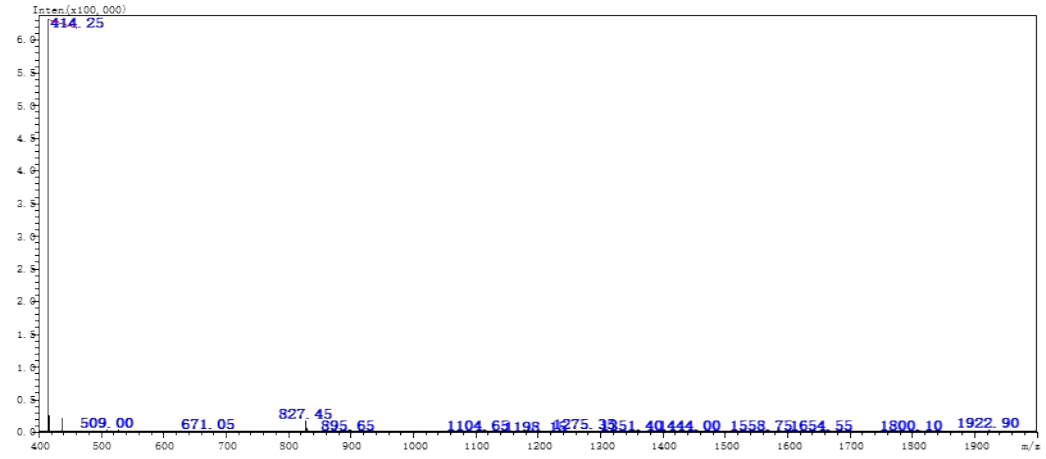


MS (ESI+): Calculated for $C_{15}H_{23}N_7O_7$ $[M+H]^+$ 414.16, found 414.25(1+).

^{1}H NMR (600 MHz, DMSO) δ 8.94 (s, 1H), 8.60 (d, J = 7.5 Hz, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.03 (s, 2H), 7.35 (s, 1H), 7.32 (s, 1H), 7.26 (s, 1H), 5.29 (s, 1H), 4.59 – 4.53 (m, 1H), 4.50 – 4.44 (m, 1H), 4.39 (q, J = 5.9 Hz, 1H), 3.60 (d, J = 10.5 Hz, 3H), 3.56 – 3.54 (m, 1H), 3.14 (dd, J = 15.4, 5.3 Hz, 1H), 2.98 (dd, J = 15.4, 7.9 Hz, 1H), 2.69 (dd, J = 16.6, 5.4 Hz, 1H), 2.55 – 2.51 (m, 1H).

^{13}C NMR (151 MHz, DMSO) δ 173.6, 172.2, 170.6, 170.3, 168.9, 134.8, 61.3, 56.3, 53.9, 50.5, 41.4, 40.1, 37.9.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **95.02 %**.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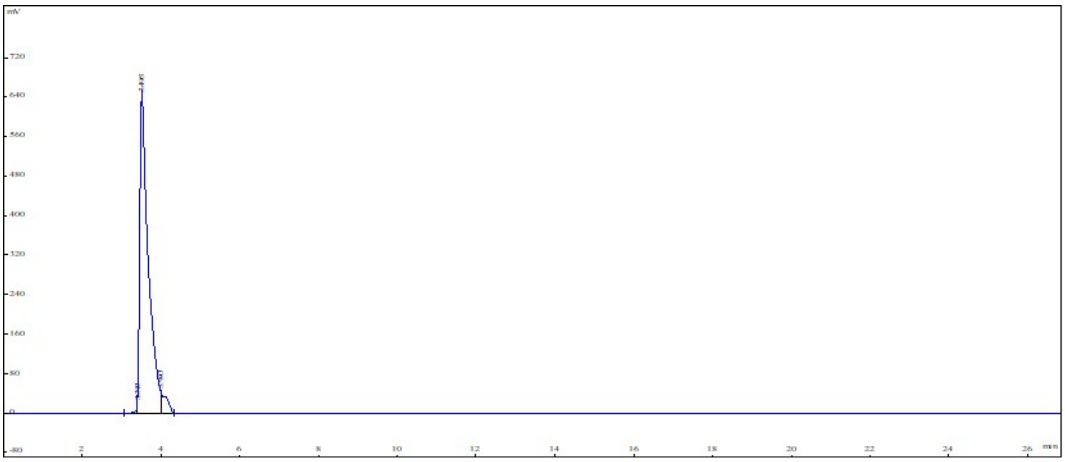
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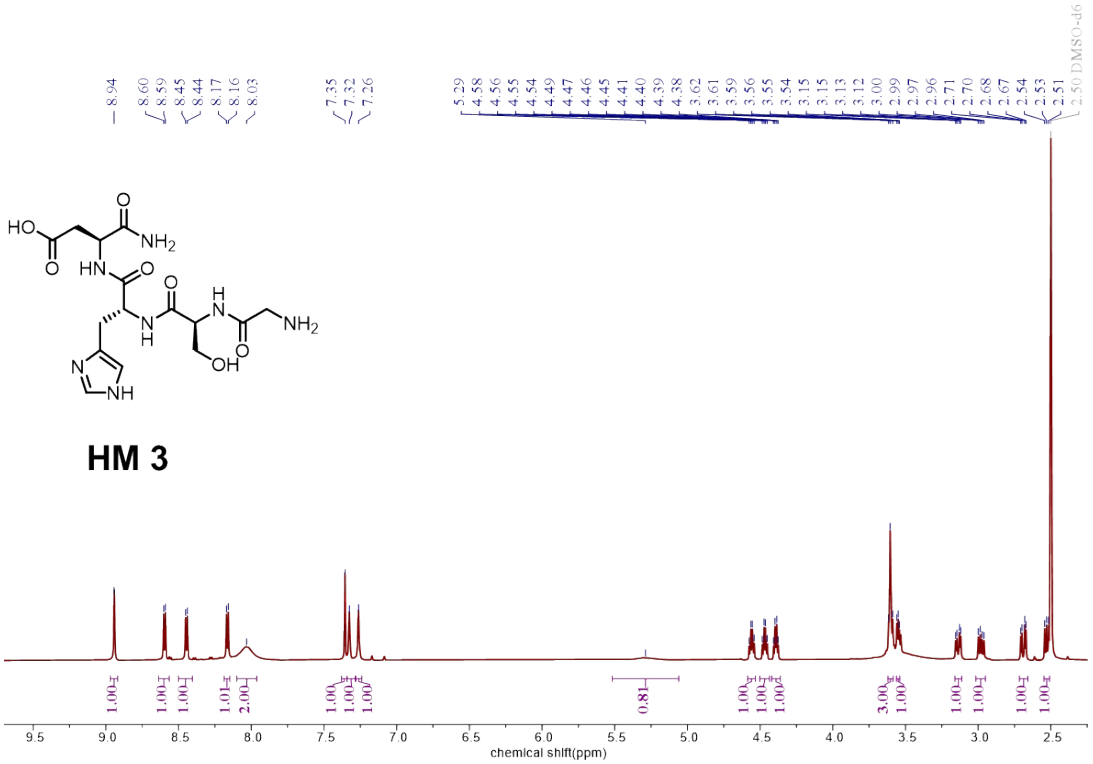
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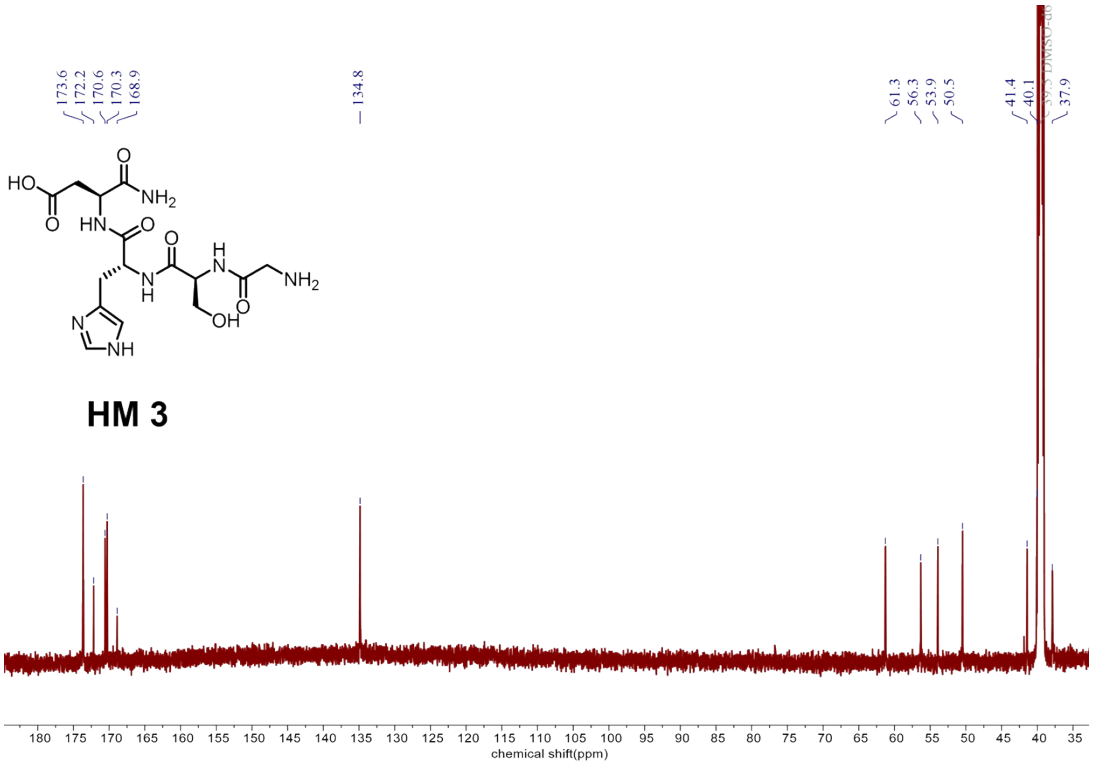
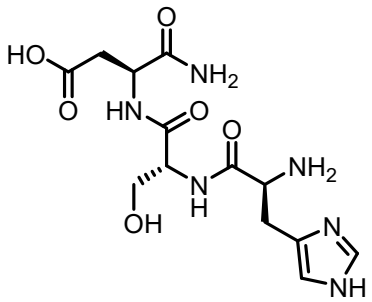

HM 3
chemical shift(ppm)

HM4 (NH_2-Asp-Ser-His)

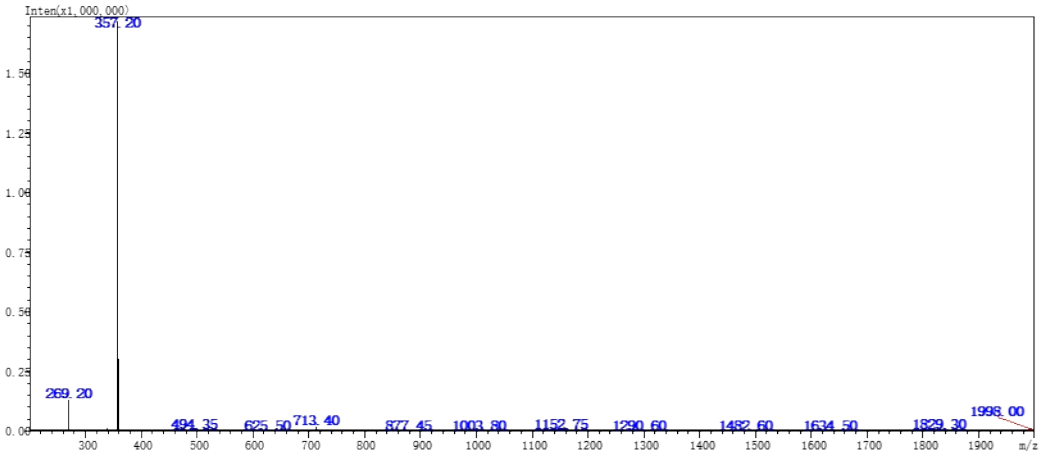


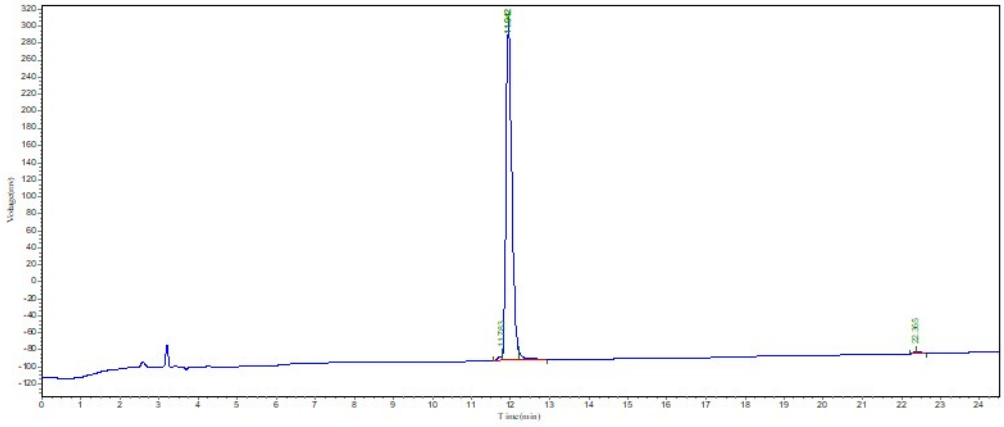
MS (ESI+): Calculated for $C_{13}H_{20}N_6O_6$ $[M+H]^+$ 357.14, found 357.20(1+).

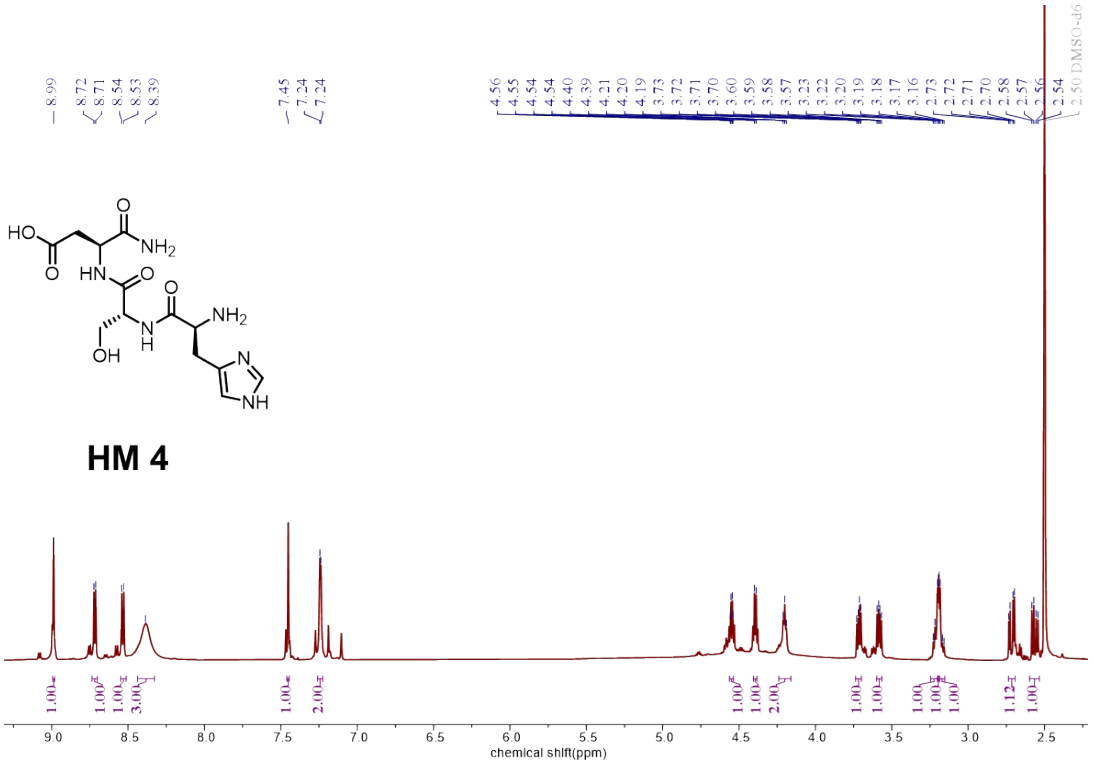
^{1}H NMR (600 MHz, DMSO) δ 8.99 (s, 1H), 8.72 (d, J = 7.2 Hz, 1H), 8.53 (d, J = 8.1 Hz, 1H), 8.39 (s, 3H), 7.45 (s, 1H), 7.24 (d, J = 3.8 Hz, 2H), 4.56 – 4.54 (m, 1H), 4.39 (d, J = 7.2 Hz, 1H), 4.20 (t, J = 6.6 Hz, 2H), 3.72 (dd, J = 10.8, 5.8 Hz, 1H), 3.58 (dd, J = 10.8, 6.1 Hz, 1H), 3.25 – 3.20 (m, 1H), 3.19 (s, 1H), 3.19 – 3.15 (m, 1H), 2.72 (dd, J = 16.7, 5.4 Hz, 1H), 2.56 (dd, J = 16.7, 7.7 Hz, 1H).

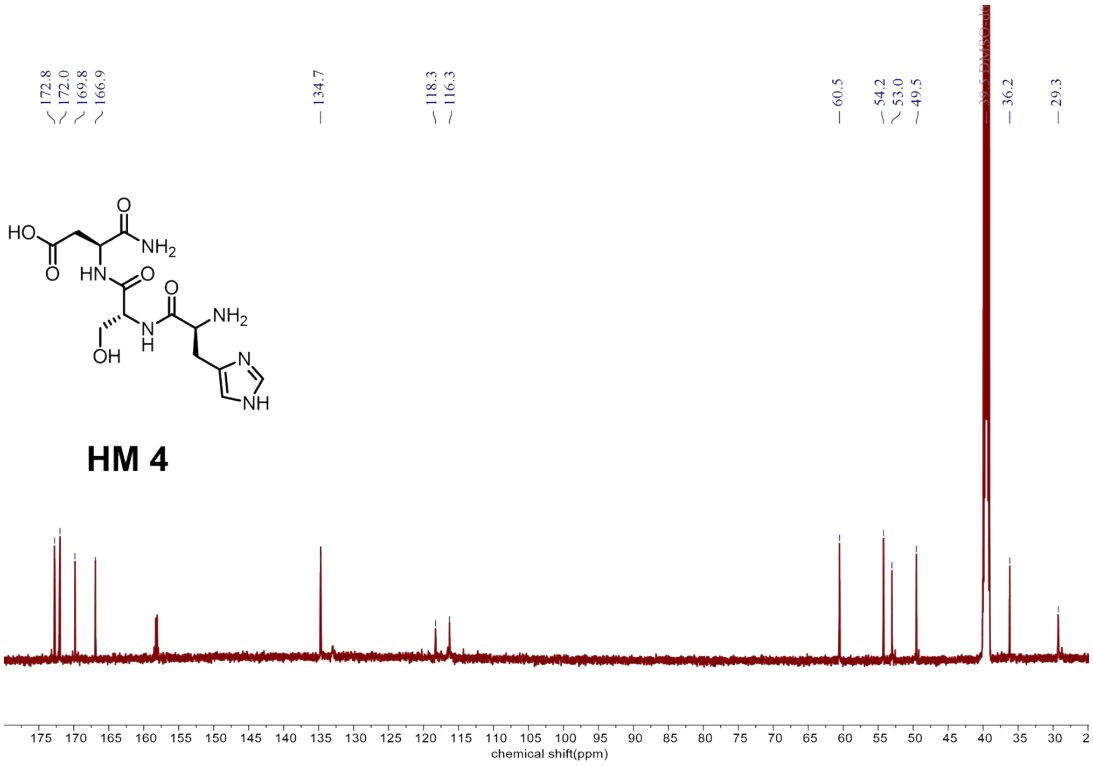
^{13}C NMR (151 MHz, DMSO) δ 172.8, 172.0, 169.8, 166.9, 134.7, 118.3, 116.3, 60.5, 54.2, 53.0, 49.5, 36.2, 29.3.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **97.02 %**.

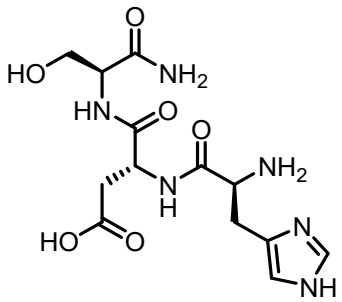

Inten(x1,000,000)
357.20
269.20
494.35
625.50
713.40
877.45
1003.80
1152.75
1290.60
1482.60
1634.50
1829.30
1998.00
m/z


Voltage(mV)
T ime(min)


8.99
8.72
8.71
8.54
8.53
8.39
7.45
7.24
7.24
4.56
4.55
4.54
4.54
4.40
4.39
4.21
4.20
4.19
3.73
3.72
3.71
3.70
3.60
3.59
3.58
3.57
3.23
3.22
3.20
3.19
3.18
3.17
3.16
2.73
2.72
2.71
2.70
2.58
2.57
2.56
2.54
2.50 DMSO-d6
HO
O
NH2
HN
O
O
N
H
NH2
OH
N
NH
HM 4
chemical shift(ppm)


172.8
172.0
169.8
166.9
134.7
118.3
116.3
60.5
54.2
53.0
49.5
39.5 DMSO-d6
36.2
29.3
HO
O
NH2
HN
O
O
N
H
NH2
OH
N
NH
HM 4
chemical shift(ppm)

HM5 (NH_2-Ser-Asp-His)

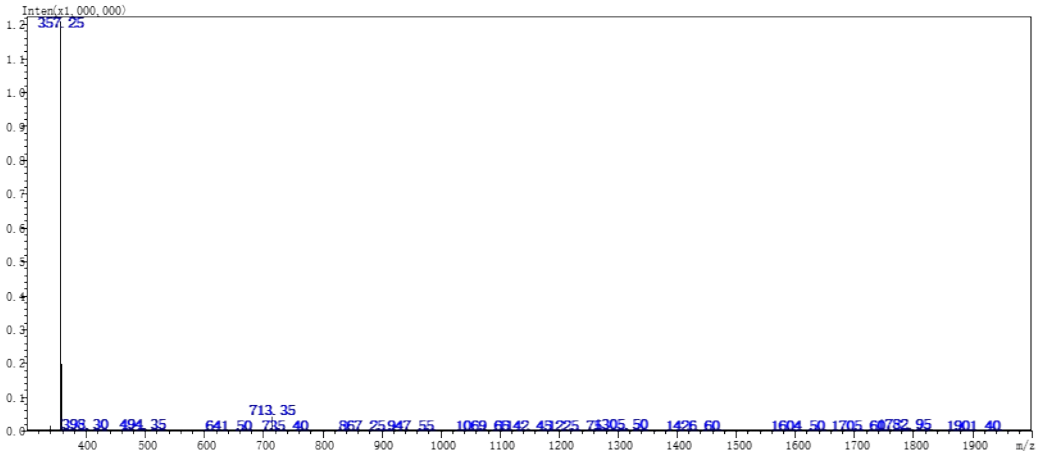


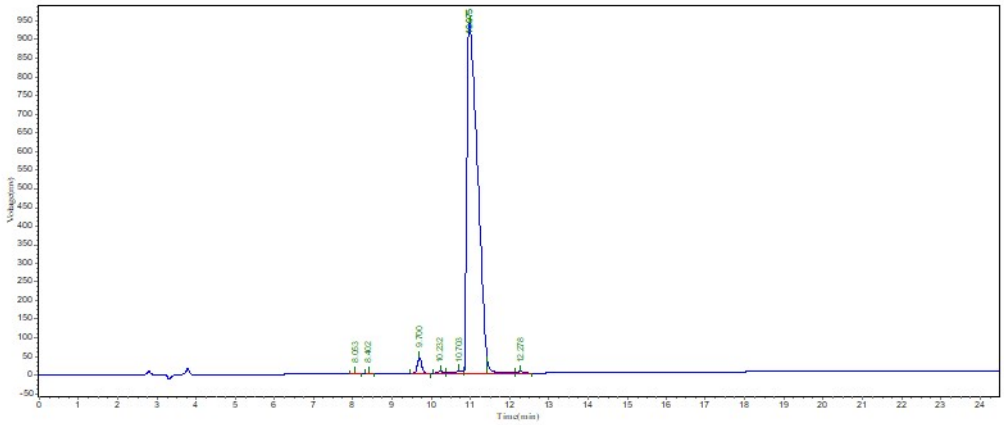
MS (ESI+): Calculated for $C_{13}H_{20}N_6O_6$ $[M+H]^+$ 357.14, found 357.25(1+).

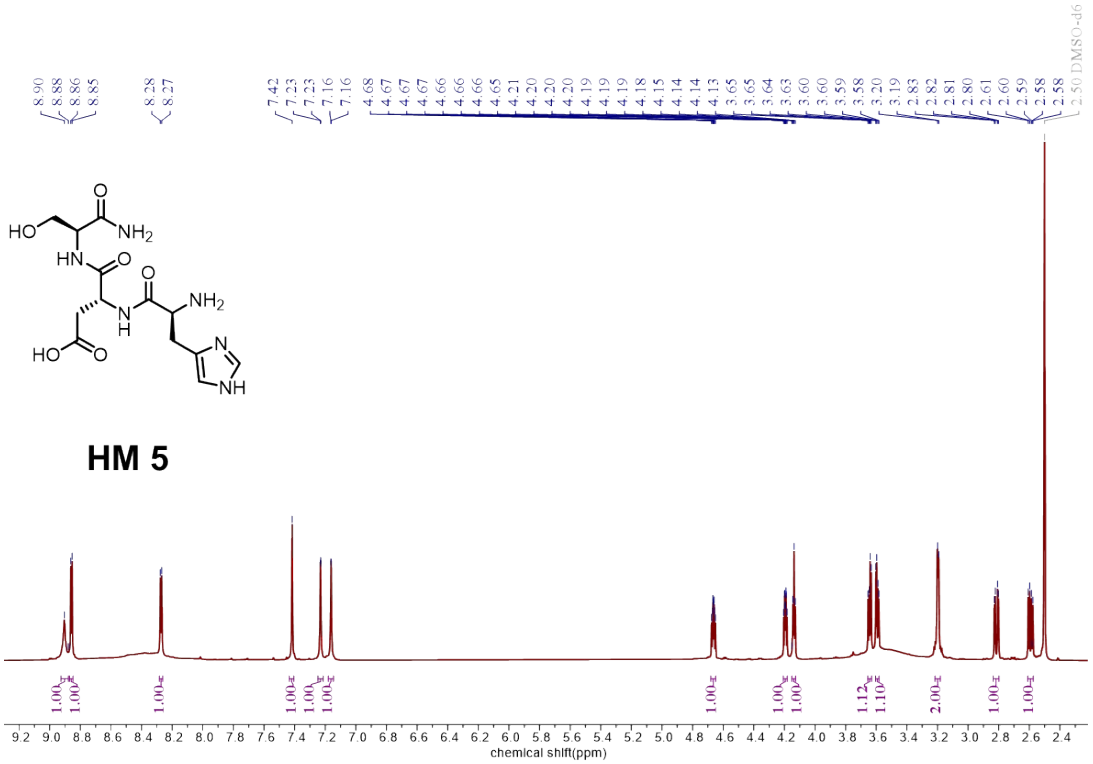
^{1}H NMR (800 MHz, DMSO) δ 8.90 (s, 1H), 8.86 (d, J = 7.0 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.42 (s, 1H), 7.23 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 2.0 Hz, 1H), 4.66 (ddd, J = 8.3, 7.0, 5.2 Hz, 1H), 4.19 (ddd, J = 7.8, 5.7, 4.8 Hz, 1H), 4.14 (t, J = 6.3 Hz, 1H), 3.64 (dd, J = 10.9, 5.8 Hz, 1H), 3.59 (dd, J = 11.0, 4.8 Hz, 1H), 3.20 (d, J = 6.4 Hz, 2H), 2.81 (dd, J = 17.1, 5.2 Hz, 1H), 2.59 (dd, J = 17.1, 8.2 Hz, 1H).

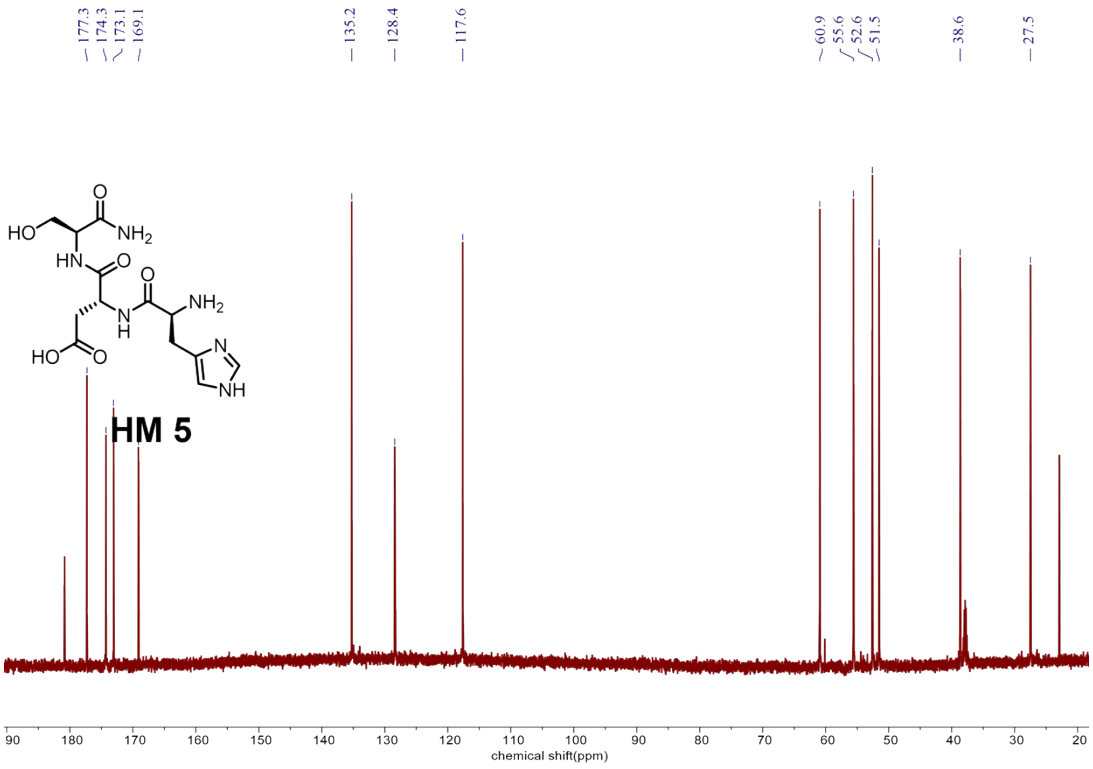
^{13}C NMR (151 MHz, D_2O) δ 177.3, 174.3, 173.1, 169.1, 135.2, 128.4, 117.6, 60.9, 55.6, 52.6, 51.5, 38.6, 27.5.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **96.76 %**.

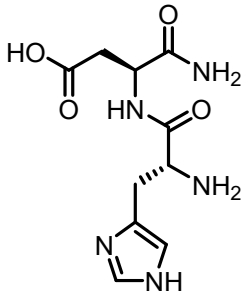

Inten(x1,000,000)
357.25
713.35
m/z


8.063
8.402
9.700
10.232
10.703
12.278
Time(min)


HM 5
chemical shift(ppm)


HM 5
chemical shift(ppm)

HM6 (NH_2-Asp-His)

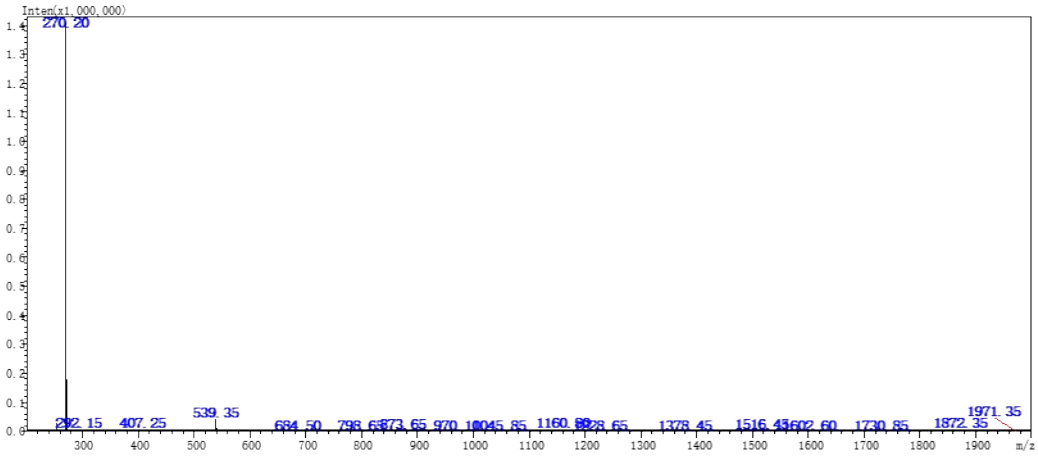


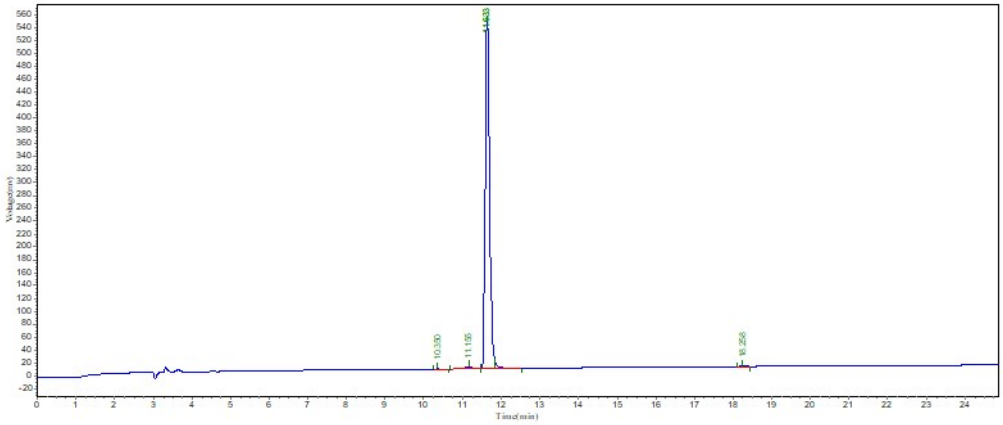
MS (ESI+): Calculated for $C_{10}H_{15}N_5O_4$ $[M+H]^+$ 270.11, found 270.20(1+).

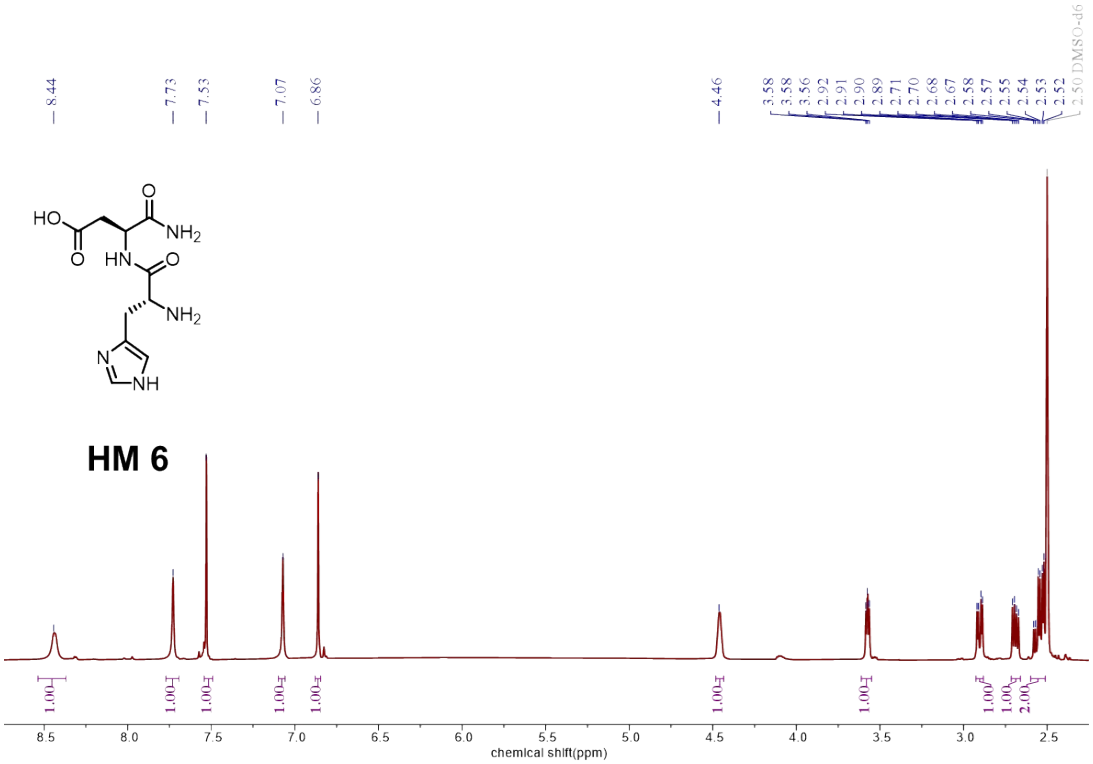
^{1}H NMR (600 MHz, DMSO) δ 8.44 (s, 1H), 7.73 (s, 1H), 7.53 (s, 1H), 7.07 (s, 1H), 6.86 (s, 1H), 4.46 (s, 1H), 3.61 – 3.55 (m, 1H), 2.90 (dd, *J* = 14.6, 5.0 Hz, 1H), 2.69 (dd, *J* = 14.5, 7.4 Hz, 1H), 2.60 – 2.51 (m, 2H).

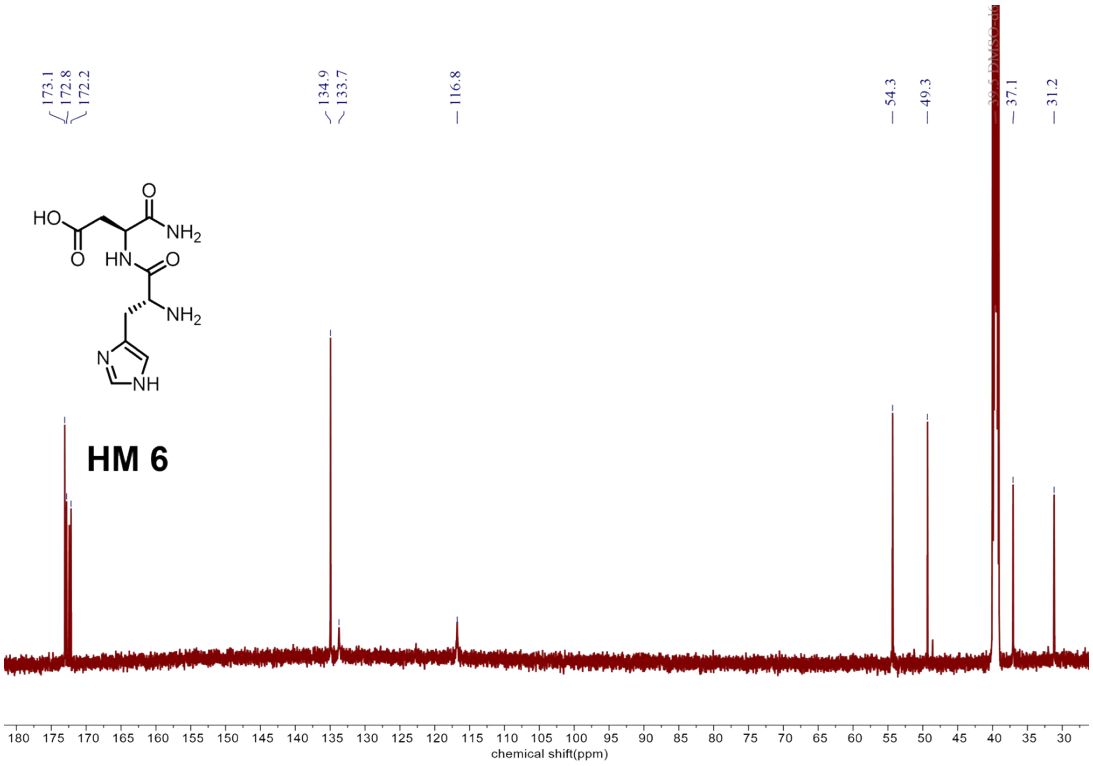
^{13}C NMR (151 MHz, DMSO) δ 173.1, 172.8, 172.2, 134.9, 133.7, 116.8, 54.3, 49.3, 37.1, 31.2.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **97.73 %**.

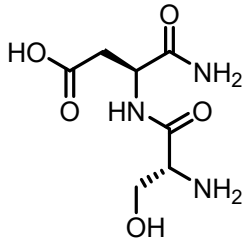

Inten(x1,000,000)
270.20
292.15
407.25
539.35
684.50
798.65
873.65
970.10
1045.85
1160.80
1328.65
1378.45
1516.45
1602.60
1730.85
1872.35
1971.35
m/z


10.330
11.155
11.433
18.258
Time(min)


8.44
7.73
7.53
7.07
6.86
4.46
3.58
3.58
3.56
2.92
2.91
2.90
2.89
2.71
2.70
2.68
2.67
2.58
2.57
2.55
2.54
2.53
2.52
2.50 DMSO-d6
HO
O
O
HN
NH2
O
NH2
N
NH
HM 6
1.00
1.00
1.00
1.00
1.00
1.00
1.00
1.00
1.00
2.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
chemical shift(ppm)


173.1
172.8
172.2
134.9
133.7
116.8
54.3
49.3
37.1
31.2
HO
O
O
HN
NH2
O
NH2
N
NH
HM 6
180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
chemical shift(ppm)

HM7 (NH_2-Asp-Ser)

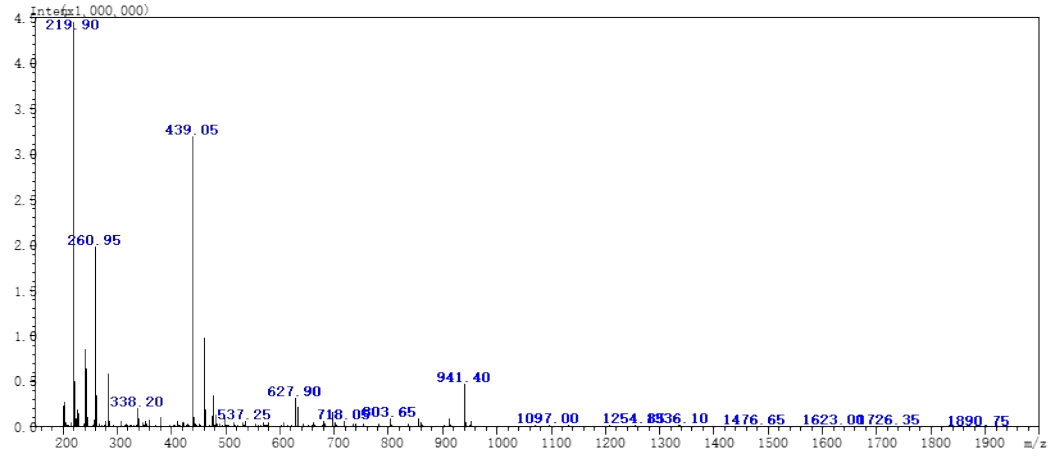


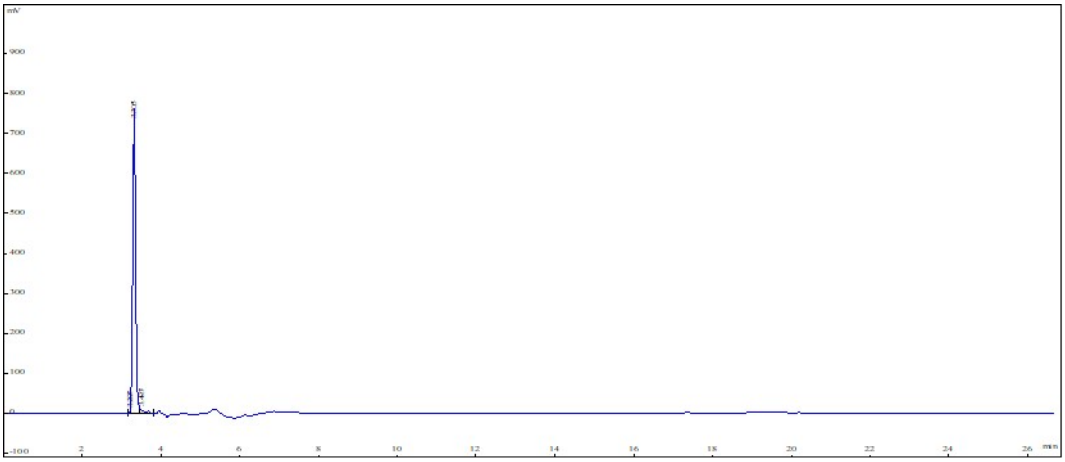
MS (ESI+): Calculated for $C_7H_{13}N_3O_5$ $[M+H]^+$ 220.08, found 219.90(1+).

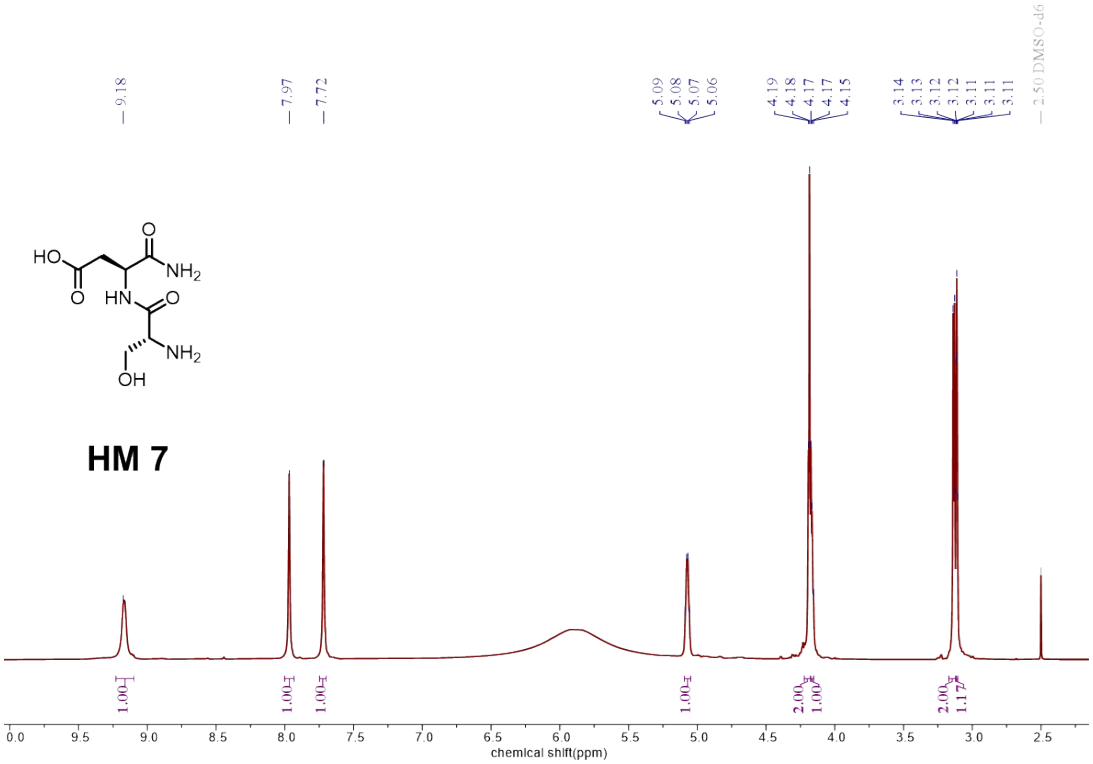
^{1}H NMR (600 MHz, DMSO) δ 9.18 (s, 1H), 7.97 (s, 1H), 7.72 (s, 1H), 5.07 (q, *J* = 6.1 Hz, 1H), 4.19 (d, *J* = 4.5 Hz, 2H), 4.17 (d, *J* = 4.0 Hz, 1H), 3.13 (d, *J* = 6.2 Hz, 2H), 3.12 – 3.11 (m, 1H).

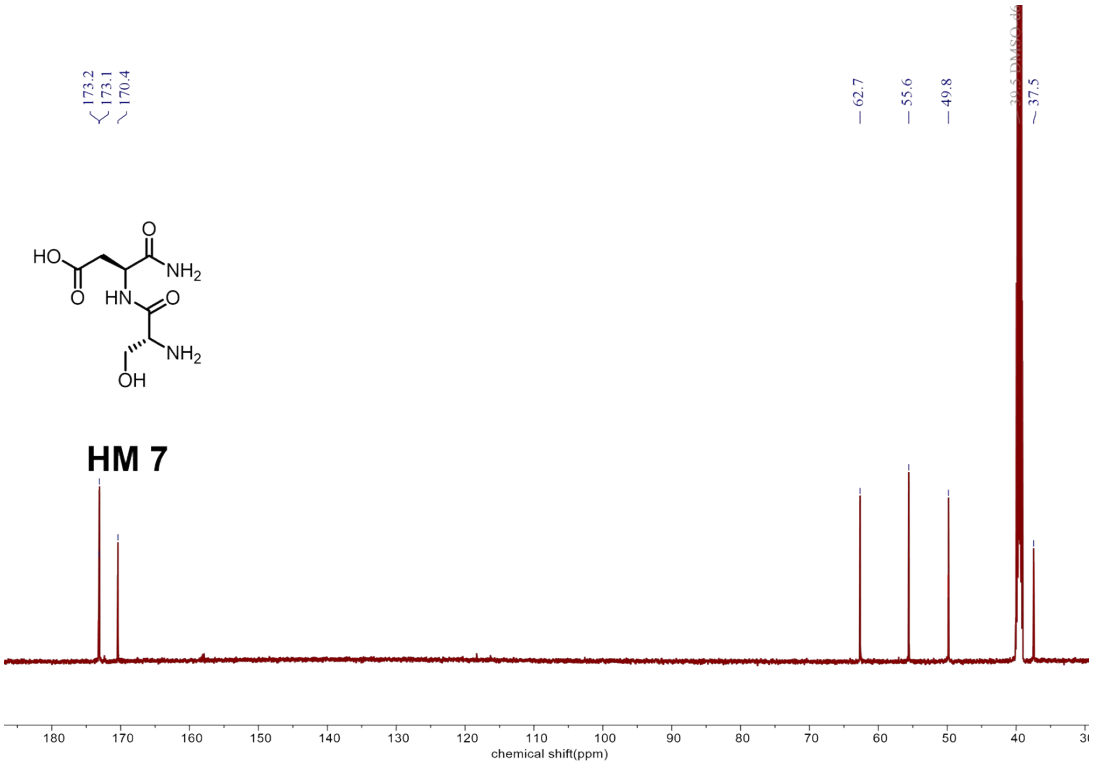
^{13}C NMR (151 MHz, DMSO) δ 173.2, 173.1, 170.4, 62.7, 55.6, 49.8, 37.5.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **96.73 %**.

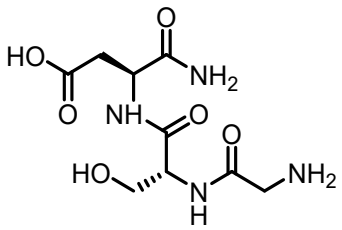

Intens.
x1,000,000)
219.90
439.05
260.95
338.20
627.90
537.25
941.40
1097.00
1476.65
1623.00
1726.35
1890.75
m/z


mV
min


9.18
7.97
7.72
5.09
5.08
5.07
5.06
4.19
4.18
4.17
4.17
4.15
3.14
3.13
3.12
3.12
3.11
3.11
3.11
2.50 DMSO-d6
HO
O
O
NH2
HN
O
NH2
OH
HM 7
1.00
1.00
1.00
1.00
2.00
1.00
2.00
1.17
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
chemical shift(ppm)


173.2
173.1
170.4
62.7
55.6
49.8
39.5 DMSO-d6
37.5
HO
O
O
NH2
HN
O
NH2
OH
HM 7
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
chemical shift(ppm)

HM8 (NH_2-Asp-Ser-Gly)

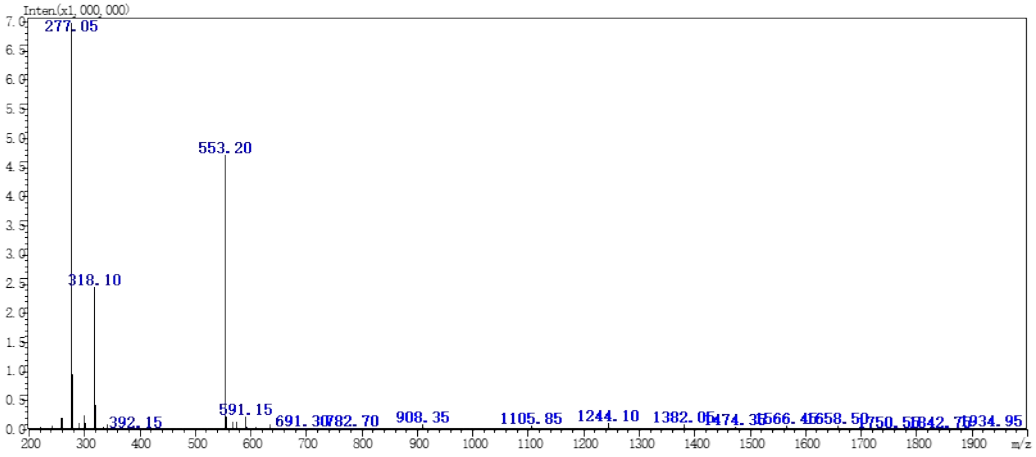


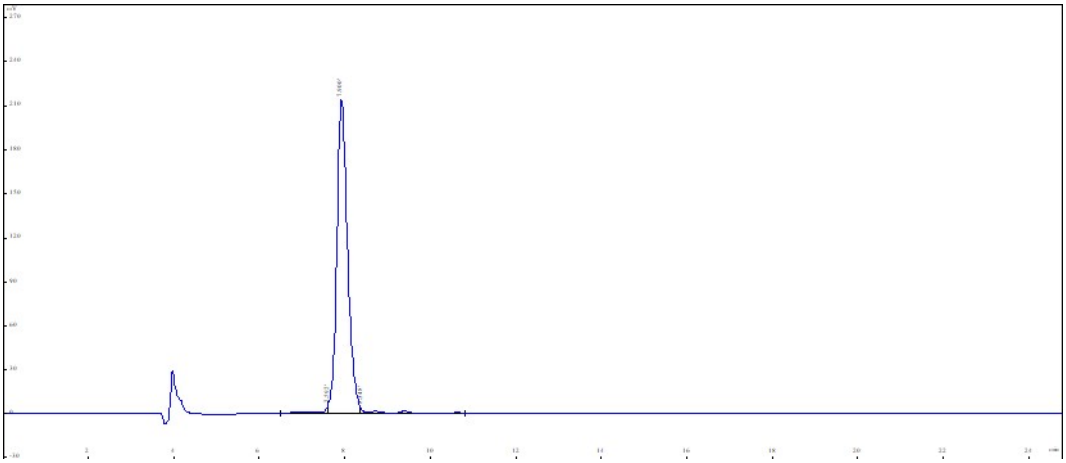
MS (ESI+): Calculated for $C_9H_{16}N_4O_6$ $[M+H]^+$ 277.10, found 277.05 (1+).

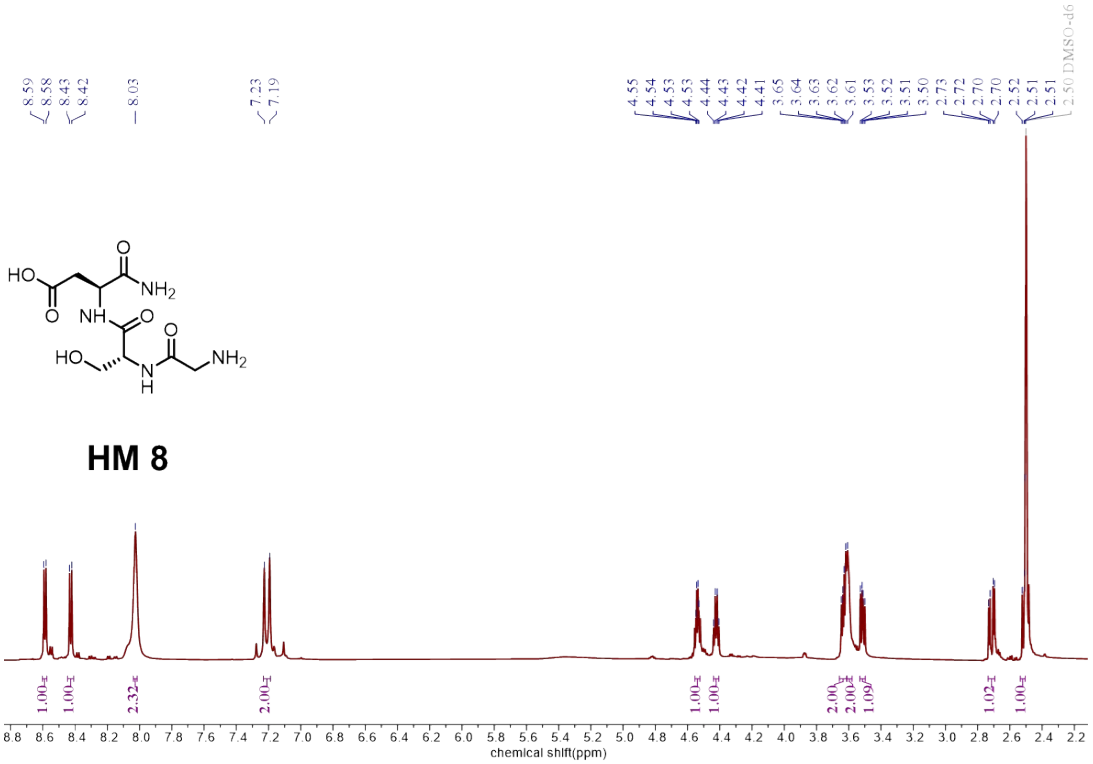
^{1}H NMR (600 MHz, DMSO) δ 8.59 (d, J = 7.9 Hz, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.03 (s, 2H), 7.21 (d, J = 20.2 Hz, 2H), 4.55 – 4.52 (m, 1H), 4.44 – 4.41 (m, 1H), 3.63 (dd, J = 10.5, 5.8 Hz, 2H), 3.61 (s, 2H), 3.51 (dd, J = 10.5, 6.6 Hz, 1H), 2.71 (dd, J = 16.6, 5.2 Hz, 1H), 2.51 (d, J = 10.5 Hz, 1H).

^{13}C NMR (151 MHz, DMSO) δ 173.1, 172.8, 169.4, 169.4, 61.5, 55.2, 50.1, 42.0, 36.9.

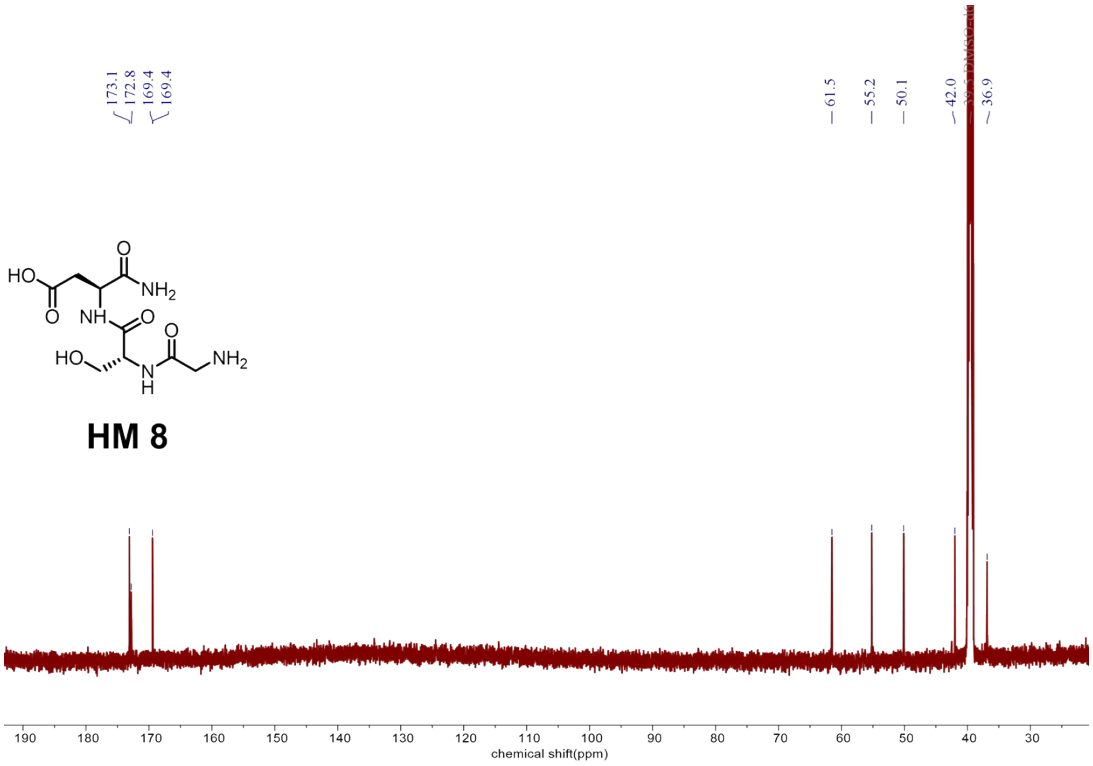
The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **95.32 %**.


Inten(x1,000,000)
277.05
553.20
318.10
392.15
591.15
691.30
782.70
908.35
1105.85
1244.10
1382.05
1934.95
m/z



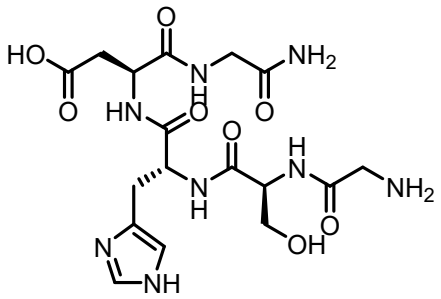

8.59
8.58
8.43
8.42
8.03
7.23
7.19
4.55
4.54
4.53
4.53
4.44
4.43
4.42
4.41
3.65
3.64
3.63
3.62
3.61
3.53
3.52
3.51
3.50
2.73
2.72
2.70
2.70
2.52
2.51
2.51
2.50 DMSO-d6
HO
O
NH2
O
NH
O
HO
N
H
O
NH2
1.00
1.00
2.32
2.00
1.00
1.00
2.00
2.00
1.09
1.02
1.00
chemical shift(ppm)

HM 8


173.1
172.8
169.4
169.4
61.5
55.2
50.1
42.0
36.9
HO
O
NH2
O
NH
O
HO
N
H
O
NH2
chemical shift(ppm)

HM 8

HM9 (NH_2-Gly-Asp-His-Ser-Gly)

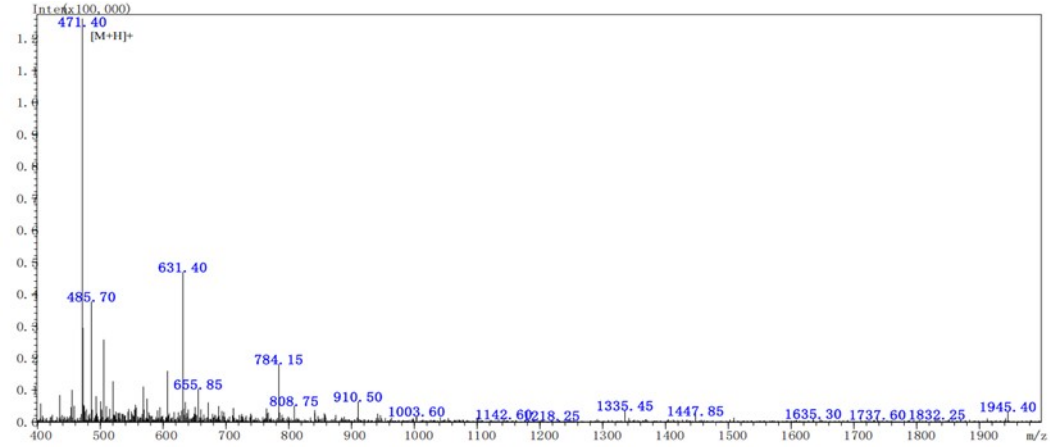


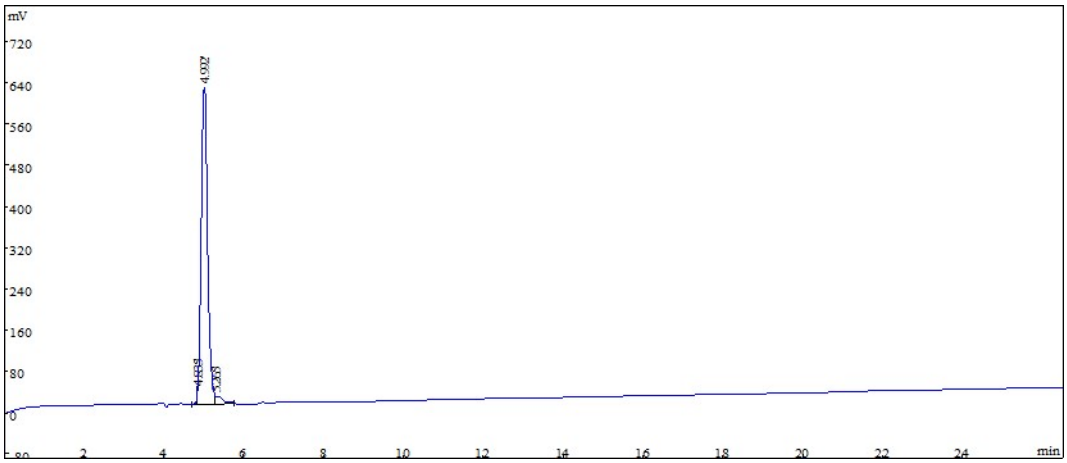
MS (ESI+): Calculated for $C_{17}H_{26}N_8O_8$ $[M+H]^+$ 471.18, found 471.40(1+).

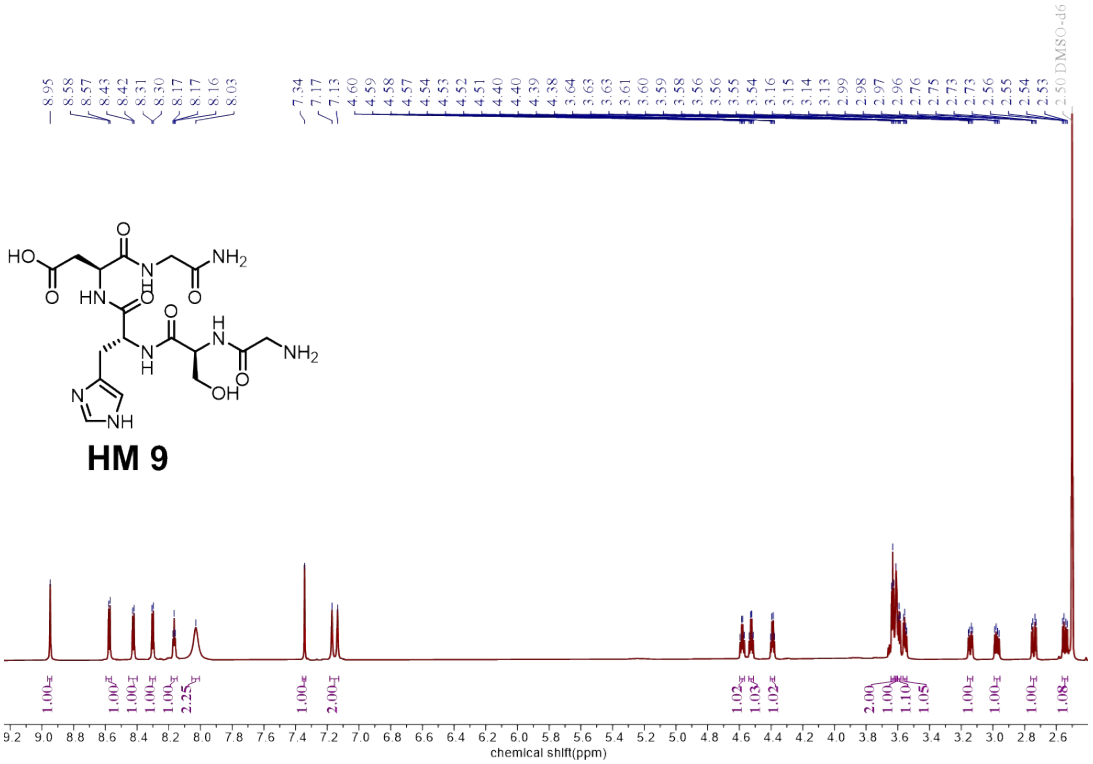
^{1}H NMR (800 MHz, DMSO) δ 8.95 (s, 1H), 8.57 (d, J = 7.6 Hz, 1H), 8.42 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 7.4 Hz, 1H), 8.17 (t, J = 5.9 Hz, 1H), 8.03 (s, 2H), 7.34 (s, 1H), 7.15 (d, J = 28.4 Hz, 2H), 4.60 – 4.57 (m, 1H), 4.54 – 4.51 (m, 1H), 4.40 – 4.38 (m, 1H), 3.63 (t, J = 5.7 Hz, 2H), 3.61 (s, 1H), 3.59 (t, J = 5.4 Hz, 1H), 3.55 (dd, J = 10.8, 5.7 Hz, 1H), 3.14 (dd, J = 15.4, 5.4 Hz, 1H), 2.97 (dd, J = 15.4, 7.9 Hz, 1H), 2.74 (dd, J = 16.8, 6.0 Hz, 1H), 2.55 (dd, J = 16.8, 7.4 Hz, 1H).

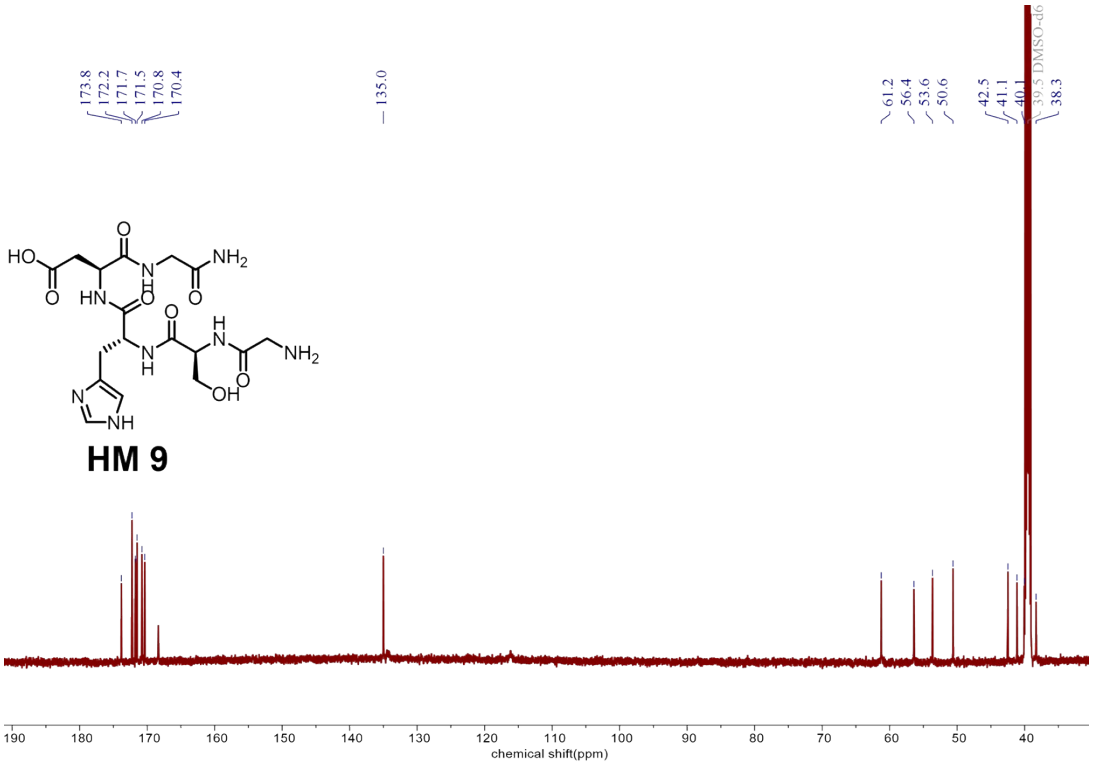
^{13}C NMR (201 MHz, DMSO) δ 173.8, 172.2, 171.7, 171.5, 170.8, 170.4, 135.0, 61.2, 56.4, 53.6, 50.6, 42.5, 41.1, 40.1, 38.3.

The purity of the hydrolase mimic was determined via HPLC, with the peak area accounting for **96.01 %**.


Inten(x100,000)
471.40
[M+H]+
631.40
485.70
784.15
655.85
808.75
910.50
1003.60
1142.60
1218.25
1335.45
1447.85
1635.30
1737.60
1832.25
1945.40
m/z


mV
4.992
4.433
5.303
min


HM 9
chemical shift(ppm)

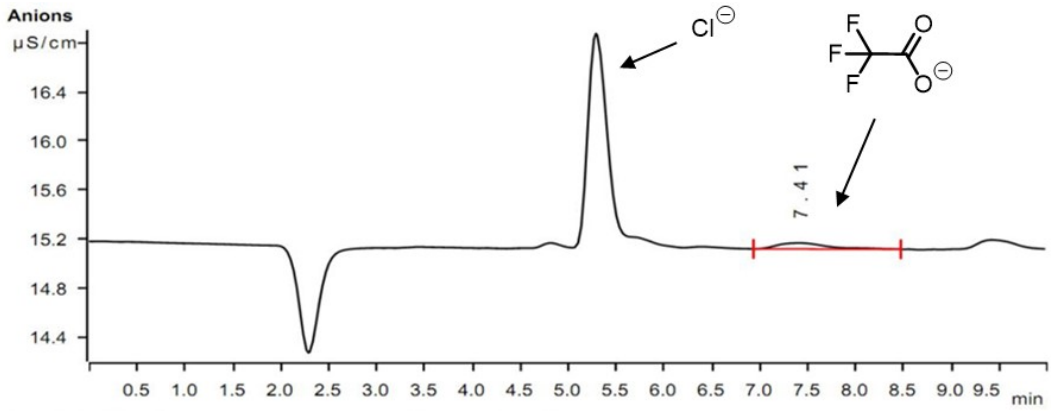

HM 9
chemical shift(ppm)

3.4 The content and Influence of CF_3COO^-

IEC

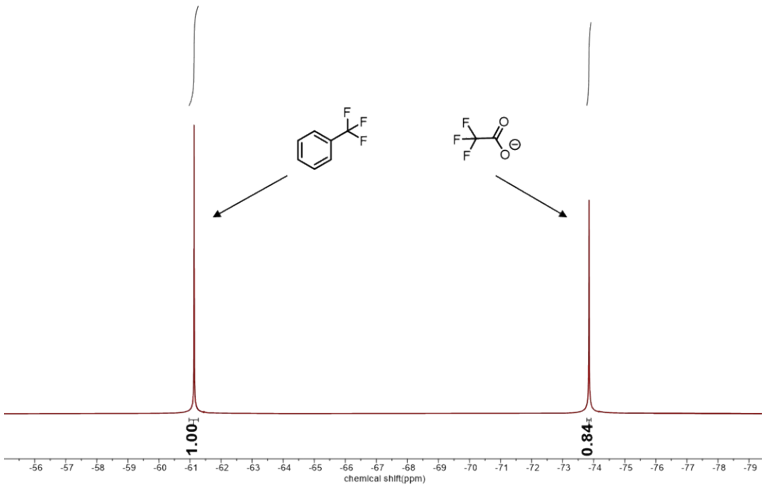
This work was conducted by Jiangsu Coastal Chemical Analysis & Technological Service Ltd. or GL Biochem (Shanghai) Ltd. Drug R&D Center, which also provided the relevant data.

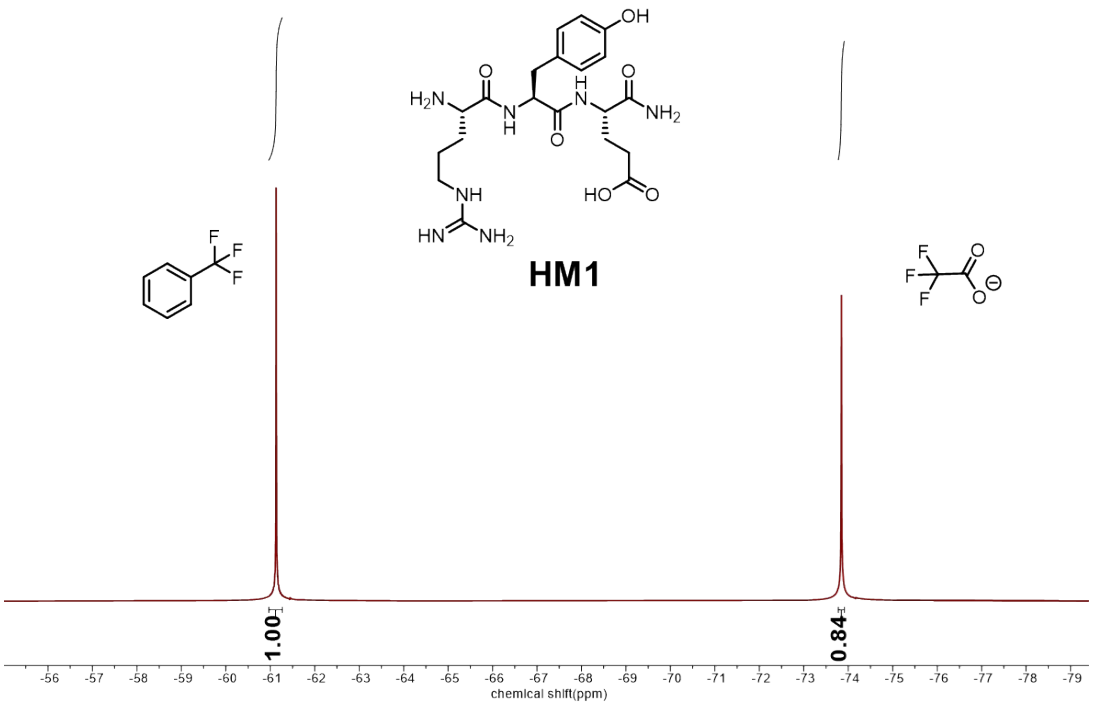
For a detailed report, please refer to the attached material: Detection of CF_3COO^- content.



^{19}F NMR

According to the content determined by IEC, add an equal amount of the internal standard of trifluorotoluene to the hydrolase mimic using DMSO-d6 as the solvent. ^{19}F NMR spectra were recorded at room temperature using Bruker models AVANCE-III 753 MHz. The content of TFA was quantified through comparative analysis of its NMR peak area ratio relative to that of compound trifluorotoluene.

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0.84
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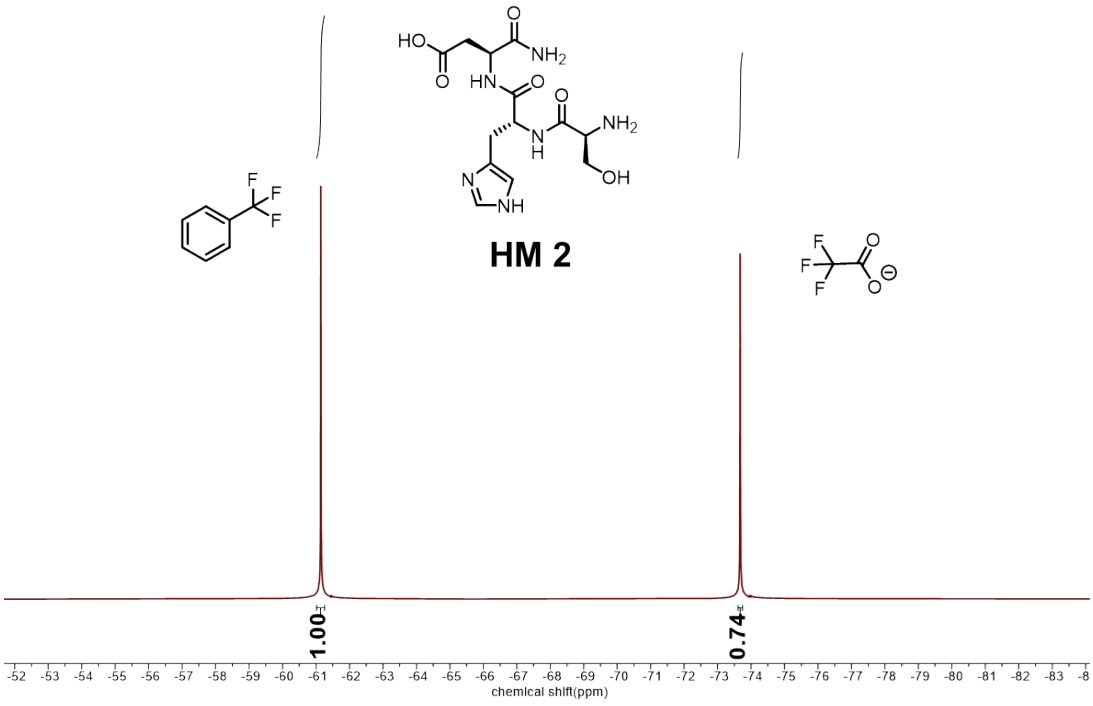
chemical shift(ppm)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0.74
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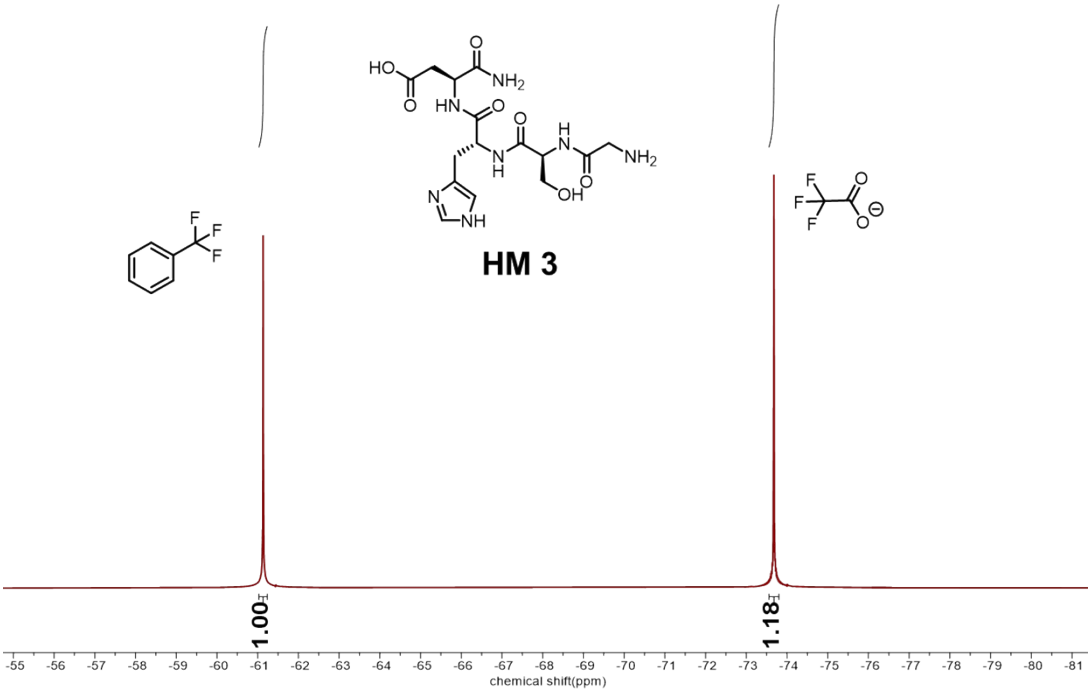
chemical shift(ppm)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1.18
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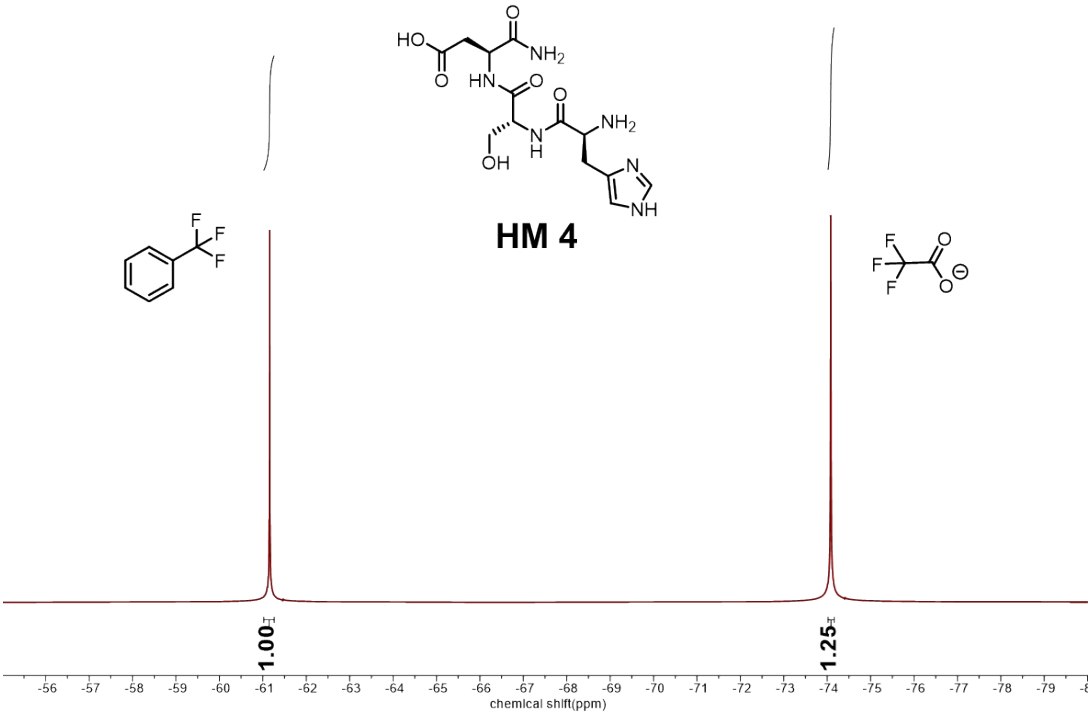
chemical shift(ppm)


HM 4
1.00
1.25
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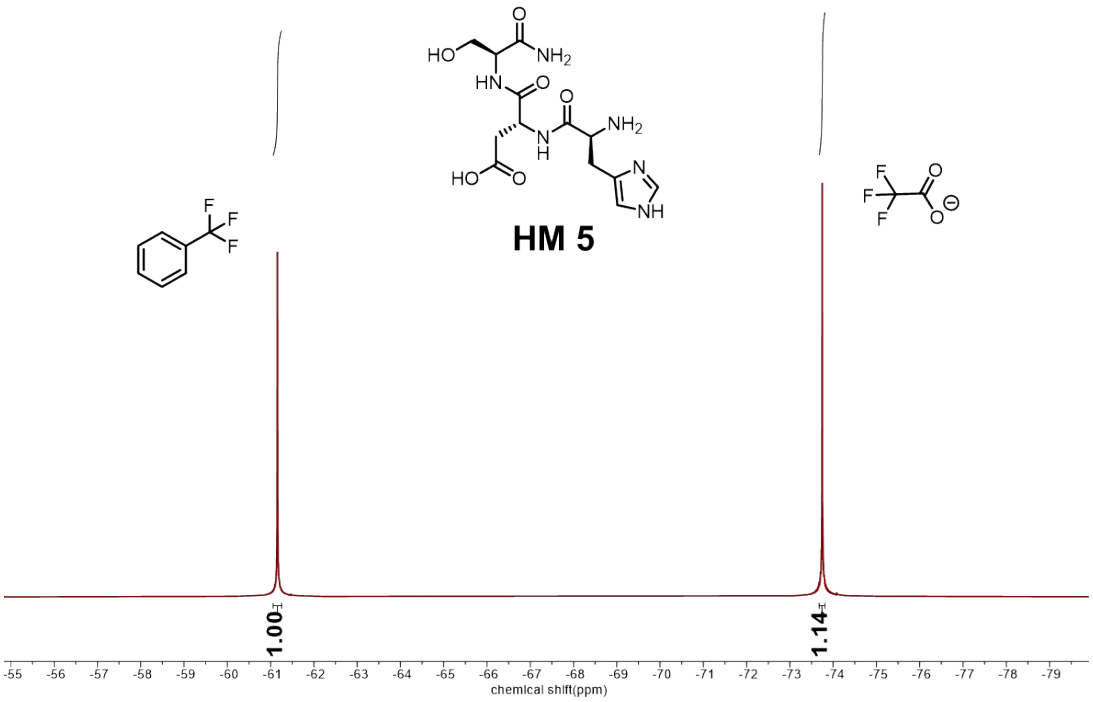
chemical shift(ppm)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1.14
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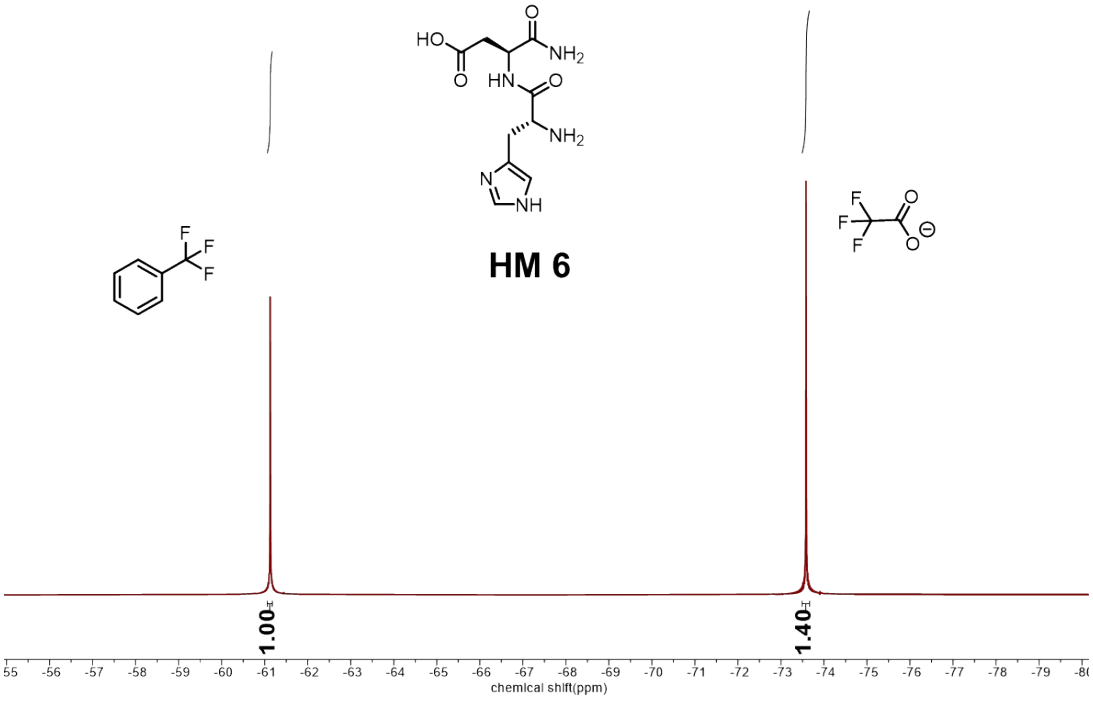
chemical shift(ppm)


HM 6
1.00
1.40
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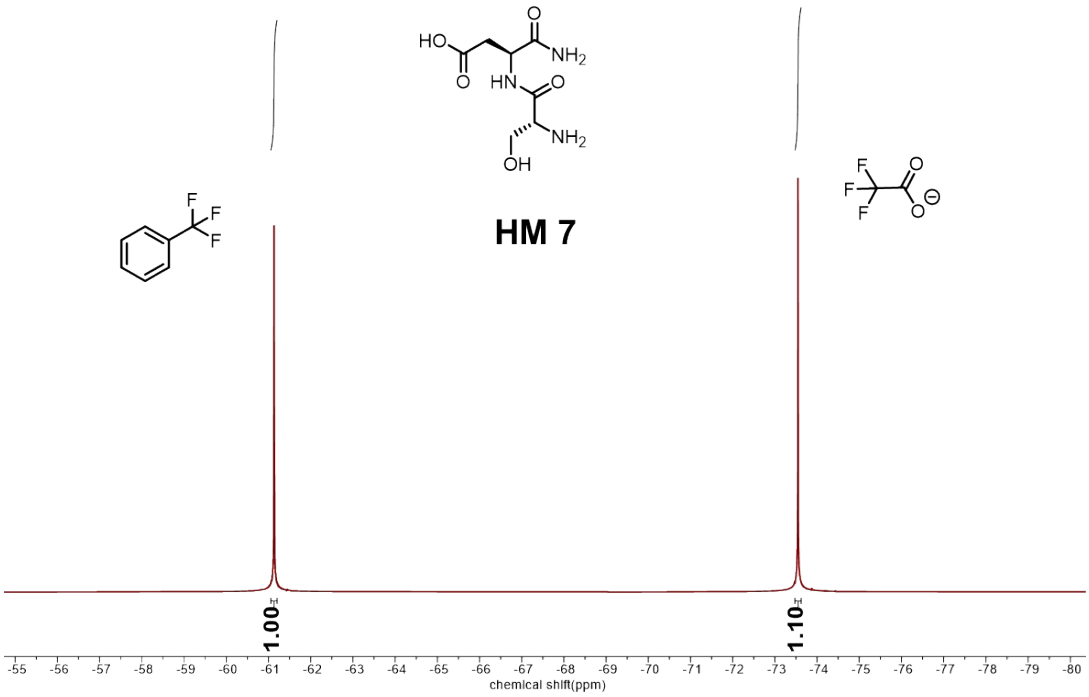
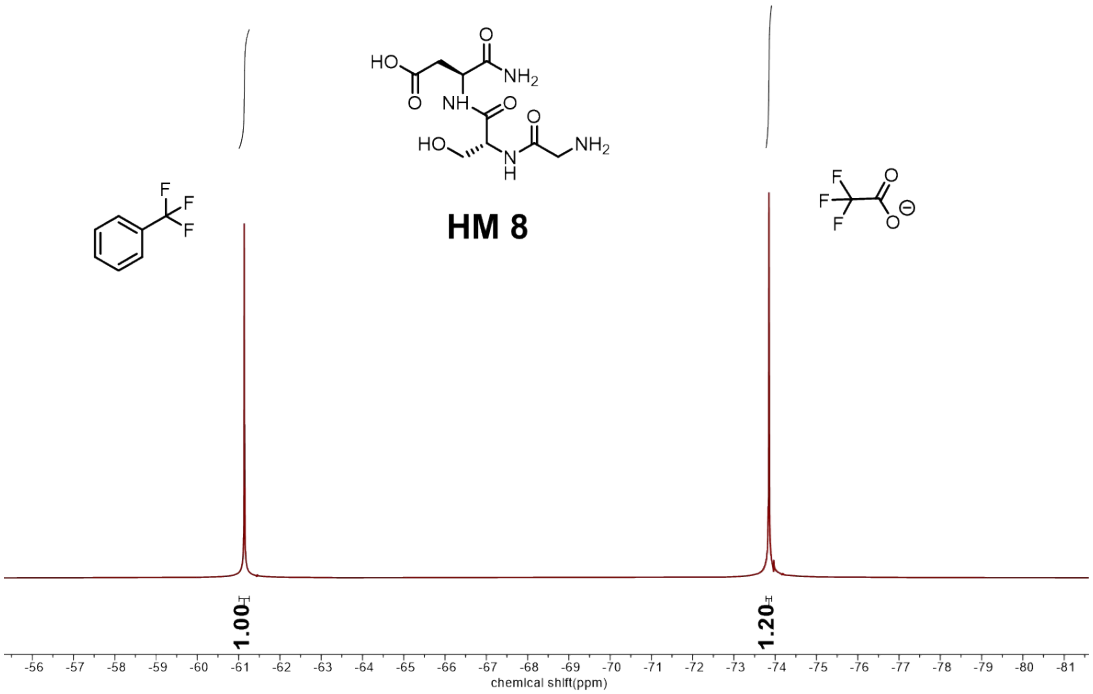
chemical shift(ppm)


HM 7
1.00
1.10
-55 -56 -57 -58 -59 -60 -61 -62 -63 -64 -65 -66 -67 -68 -69 -70 -71 -72 -73 -74 -75 -76 -77 -78 -79 -80
chemical shift(ppm)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1.20
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chemical shift(ppm)

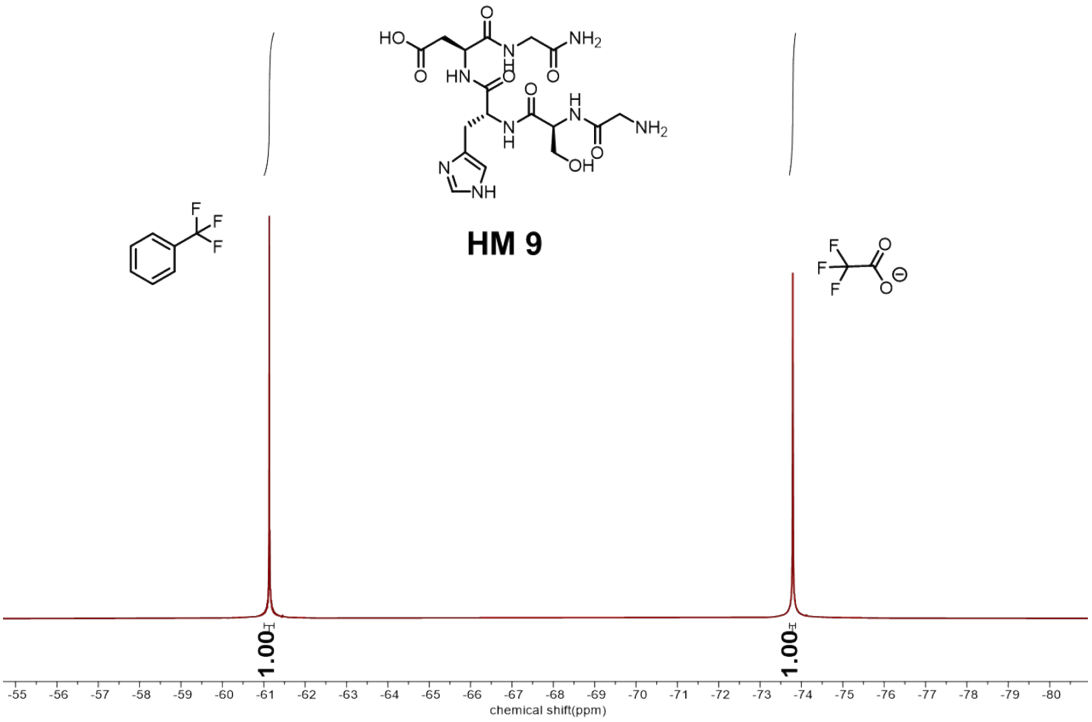

HM 9
1.00
1.00
-55 -56 -57 -58 -59 -60 -61 -62 -63 -64 -65 -66 -67 -68 -69 -70 -71 -72 -73 -74 -75 -76 -77 -78 -79 -80
chemical shift(ppm)

Table S4. The Content and Influence of CF_3COO^-.

Catalysts	Yield / %[a]	The content of CF_3COO^- (IEC) / mol%	The content of CF_3COO^-(^{19}F NMR) / mol%
HM1	39	0.03	0.025
HM2	35	0.541	0.649
HM3	44	0.696	0.515
HM4	45	0.467	0.584
HM5	57	0.610	0.695
HM6	33	0.795	1.113
HM7	33	0.606	0.667
HM8	50	0.488	0.576
HM9	65	0.688	0.688

[a] Reaction conditions: to a 5 mL glass vial, add 1a (0.4 - 0.42 mmol), 1b (0.4 mmol), water (the volume of the supplementary reaction system was 1 mL), and catalyst (1 mol%). Allow the mixture to react for 4 hours at temperatures of 25 ℃. Two parallel sets. Yields were determined by HPLC.

Table S5. The effect of PBS on the CF_3COO^- in hydrolase mimics.

Entry	Solvent	Catalyst	Catalyst loading / mol%	Yield / %
1	Water	Free	-	20
2	PBS	Free	-	0
3	Water	HM9[a]	1	91
4	Water	HM9[b]	1	99
5	Water	TFA[a]	0.01	21
6	Water	TFA[b]	0.01	22
7	Water	TFA[a]	0.1	26
8	Water	TFA[b]	0.1	32
9	Water	TFA[a]	1	99
10	Water	TFA[b]	1	99

Reaction conditions: to a 5 mL glass vial, add 1a (0.4 - 0.42 mmol), 1b (0.4 mmol), water (The volume of the supplementary reaction system was 1 mL), and Catalyst. Allow the mixture to react for 2 hours at temperatures of 40 ℃ [a] Catalyst was dissolved in PBS (0.01M, Ph=8.0) at a final concentration of 0.25 M. [b] Catalyst was dissolved in Water at a final concentration of 0.25 M.

Table S6. The influence of PBS on the spontaneous reaction.

Entry	Solvent (Water : PBS)	Catalyst	Yield / %
1	9:1	Free	1
2	9:1	HM9	13
3	8:2	Free	0
4	8:2	HM9	11
5	7:3	Free	0
6	7:3	HM9	5

Reaction conditions: to a 5 mL glass vial, add 1a (0.4 - 0.42 mmol), 1b (0.4 mmol), solvent, and catalyst (1 mol%). Allow the mixture to react for 1 hours at room temperature.

Table S7. The influence of temperature on reactions.

Entry	Temperature / ℃	Yield[a] / %	Yield[b] / %
1	40	11	11
2	50	23	27
3	60	43	46
4	70	65	67
5	80	99	99
6	90	93	99
7	100	85	85

Reaction conditions: to a 5 mL glass vial, add 1a (0.4 - 0.42 mmol), 1b (0.4 mmol), Solvent (Water : PBS = 8:2), and Catalyst (1 mol%). Allow the mixture to react for 2 hours at different temperatures. [a] HM5 is used as a catalyst. [b] HM9 is used as a catalyst.

4 Activity Studies

4.1 Detection method of reaction

To evaluate the catalytic activity of hydrolase mimics in catalyzing dehydration condensation reactions, the reaction involving the two-step dehydration condensation of 1a and 1b to form 5 was chosen as a model reaction. The yield was determined by analyzing the UV spectroscopic signal of product 5.

A series of gradient solutions of product 5 were prepared for liquid-phase detection, and a standard curve relating the concentration of 5 to its peak area was established. The concentration of the reaction product under the presence of different catalysts was calculated using the equation, and the final yield was determined accordingly:

$$\mathbf{y = 18.986x - 23.098}$$

$$\mathbf{R^2 = 0.9997}$$

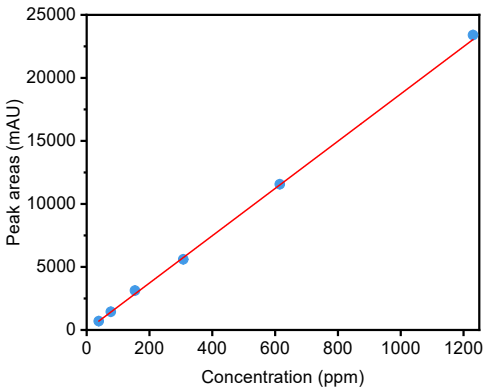


Figure S3. Standard curve for product 5.

The reaction was determined by HPLC(Agilent 1260 InfinityII). Chromatographic column uses Agilent ZORBAX StableBond SB-C18, 4.6x250 mm, 5um.

Solvent A: Water (0.1% formic acid)

Solvent B: Acetonitrile (0.1% formic acid)

Wavelength: 252 nm

Table S8. HPLC method for reaction.

Time(min)	Flow velocity(mL/min)	A%	B%
0	1	50	50
20	0	Stop	

4.2 General procedure for the activity verification of catalysts

The catalyst was dissolved in PBS (0.01M, pH=8.0) at a final concentration of 0.25 M to slow down the influence of CF_3COO^- on the reaction system.

The substrates and catalyst were combined in a 5 mL transparent glass vial, and the reaction system was adjusted to the final volume of 1 mL using solvent. Control the temperature and time by the metal bath magnetic stirrer. Perform the reaction in parallel in two separate sets. For the sake of simple analysis, the molecular weights of the three enzymes were all calculated at 20,000 g/mol.

The yield of Product 5 was determined by HPLC, while the yields of other products were determined by mass.

4.3 Catalyst recycling

General procedure: Upon completion of the reaction, the mixture was subjected to multiple extractions with an equal volume of dimethyl carbonate until no fluorescence was observed under a 254 nm UV lamp, indicating the complete transfer of the substrate and product into the organic phase. Any residual dimethyl carbonate remaining in the reaction system was subsequently removed by rotary evaporation. Finally, the volume of the reaction system was adjusted to 1 mL by the addition of water. Then the next round of reactions will be initiated.

Table S9. Recycling capacity of the catalyst.

Cycle	Yield / %	Activity retention / %
1	92	100
2	91	99
3	91	99
4	89	97
5	84	91

4.4 Gram-scale reaction

Table S10. Gram-scale reaction.

Entry	Scale	Yield / %
1	gram-scale	73[a]
2	gram-scale	96[b]
3	ten-gram scale	64[a]
4	ten-gram scale	79[b]

Reaction conditions: to a glass vial, add 1a (5 or 50 mmol), 1b (5 or 50 mmol), Solvent (replenish the reaction volume with water to 12.5 or 125 mL), and Catalyst (HM9 with 1 mol%) at 40 ℃ [a] reaction for 2 hours. [b] reaction for 4 hours.

4.5 Reproducibility for separation yield of the products

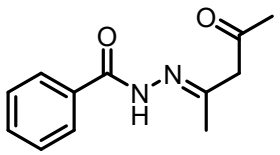
Table S11. Reproducibility for separation yield of the products.

Products	Initial Yield / %	Reproducible Yield / %
6	99	99
10	83	87
19	99	99
20	92	90

5 Substrate Scope

5.1 Characterization of products

(E)-N'-(4-oxopentan-2-ylidene)benzohydrazide (3)

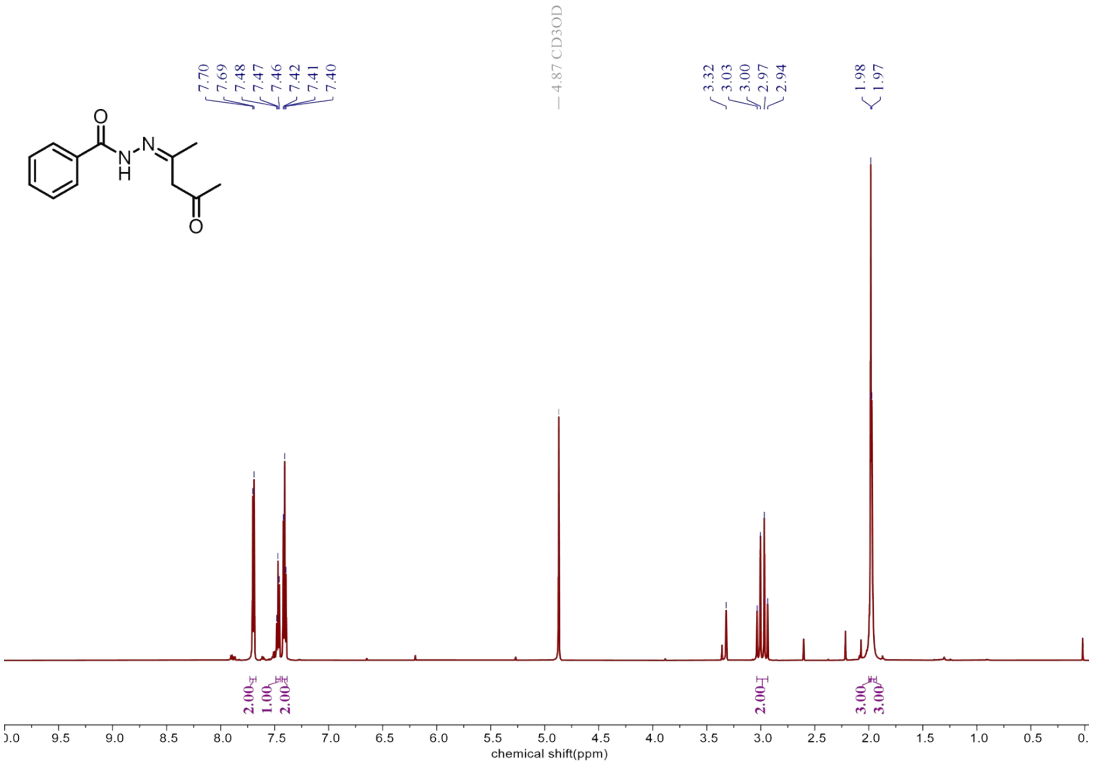


MS (ESI+): Calculated for $C_{12}H_{14}N_2O_2$ $[M+H]^+$ 219.1055, found 219.1089 (1+).

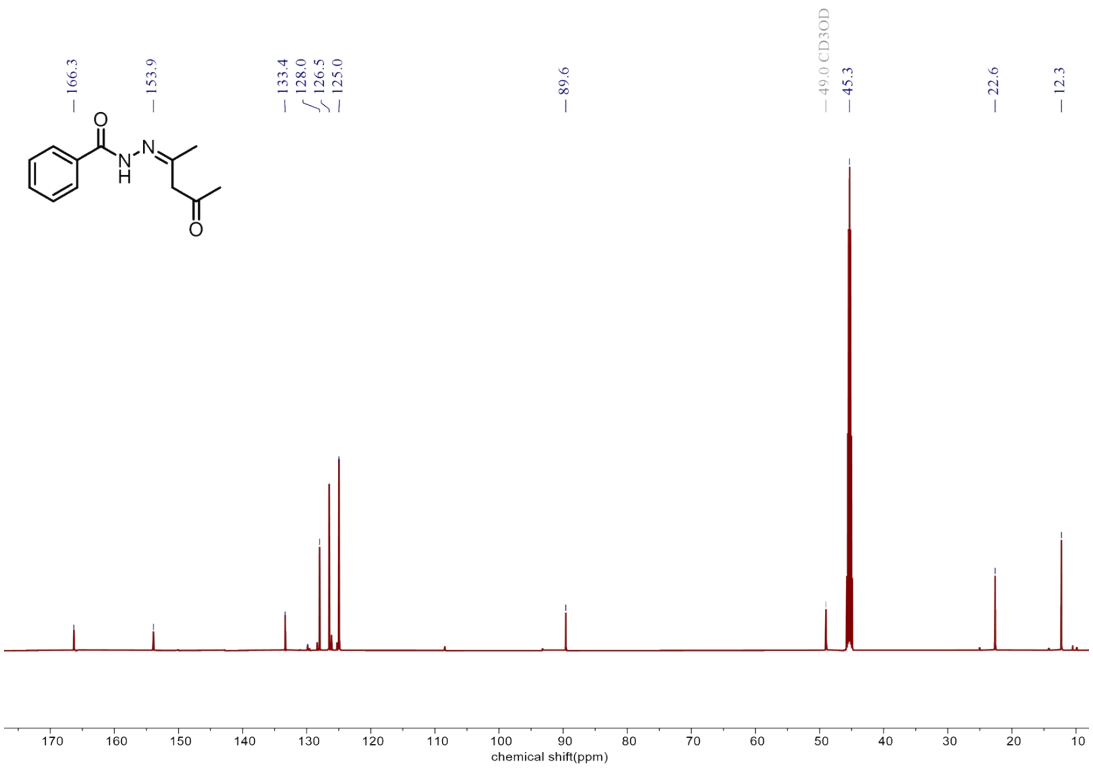
^{1}H NMR (600 MHz, MeOD) δ 7.70 (d, *J* = 7.1 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 3.04 – 2.94 (m, 2H), 1.98 (d, *J* = 5.8 Hz, 6H).

^{13}C NMR (151 MHz, MeOD) δ 166.3, 153.9, 133.4, 128.0, 126.5, 125.0, 89.6, 45.3, 22.6, 12.3.

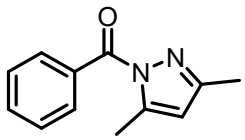
^{1}H NMR (600 MHz, MeOD) 3



^{13}C NMR (151 MHz, MeOD) 3



(3,5-dimethyl-1H-pyrazol-1-yl)(phenyl) methanone (5)

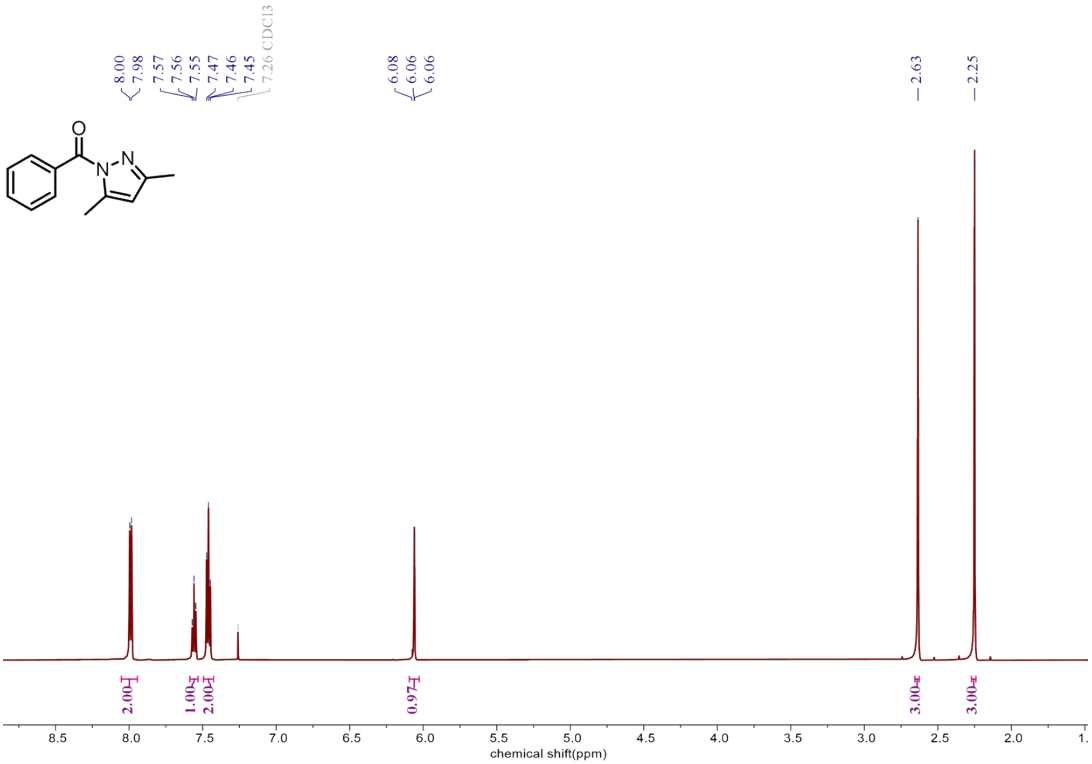


MS (ESI+): Calculated for $C_{12}H_{12}N_2O$ $[M+H]^+$ 201.0950, found 201.1156 (1+).

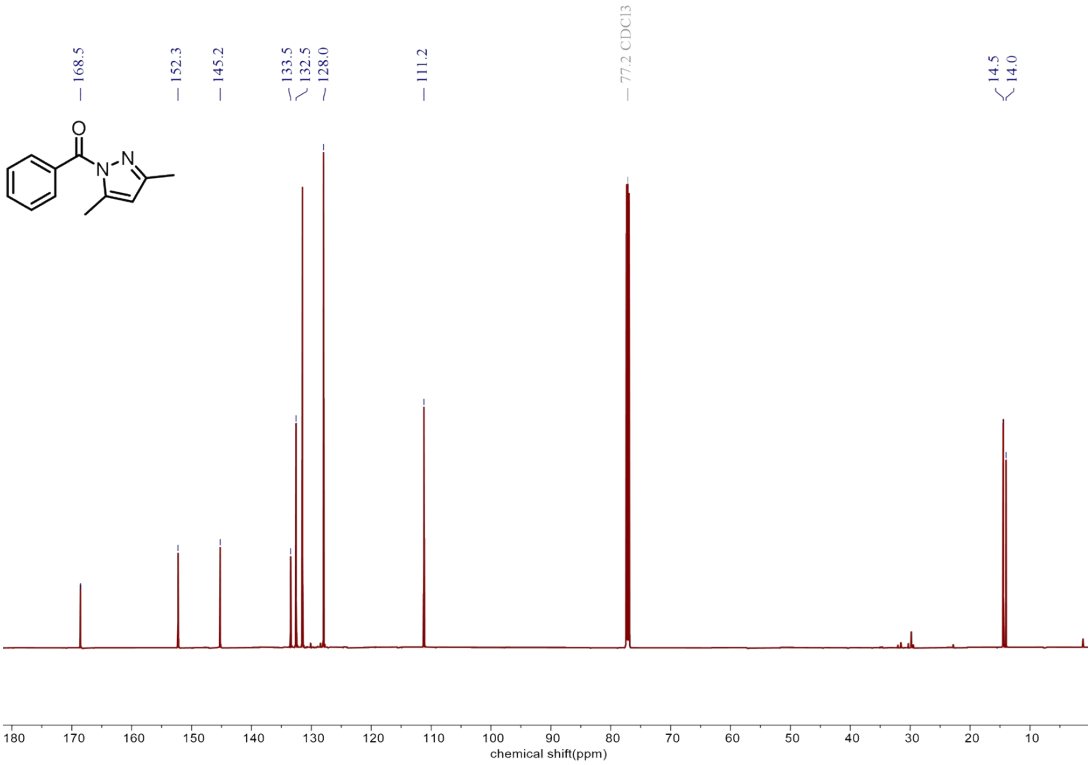
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (s, 2H), 7.54 (s, 1H), 7.47 (s, 2H), 6.06 (s, 1H), 2.64 (s, 3H), 2.23 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 168.5, 152.3, 145.2, 133.5, 132.5, 128.0, 111.2, 14.5, 14.0.

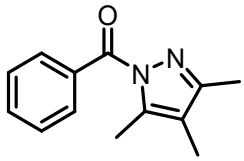
^{1}H NMR (600 MHz, $CDCl_3$) 5



^{13}C NMR (151 MHz, $CDCl_3$) 5



phenyl(3,4,5-trimethyl-1H-pyrazol-1-yl)methanone (6)

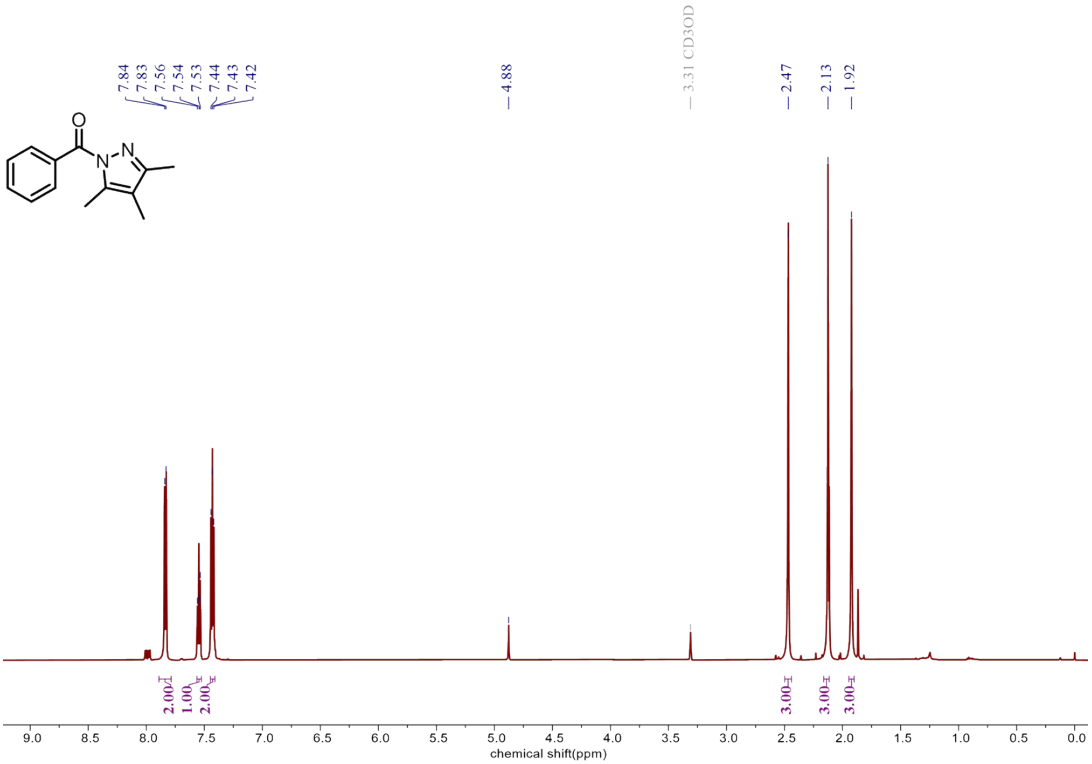


MS (ESI+): Calculated for $C_{13}H_{14}N_2O$ $[M+H]^+$ 215.1106, found 215.1165 (1+).

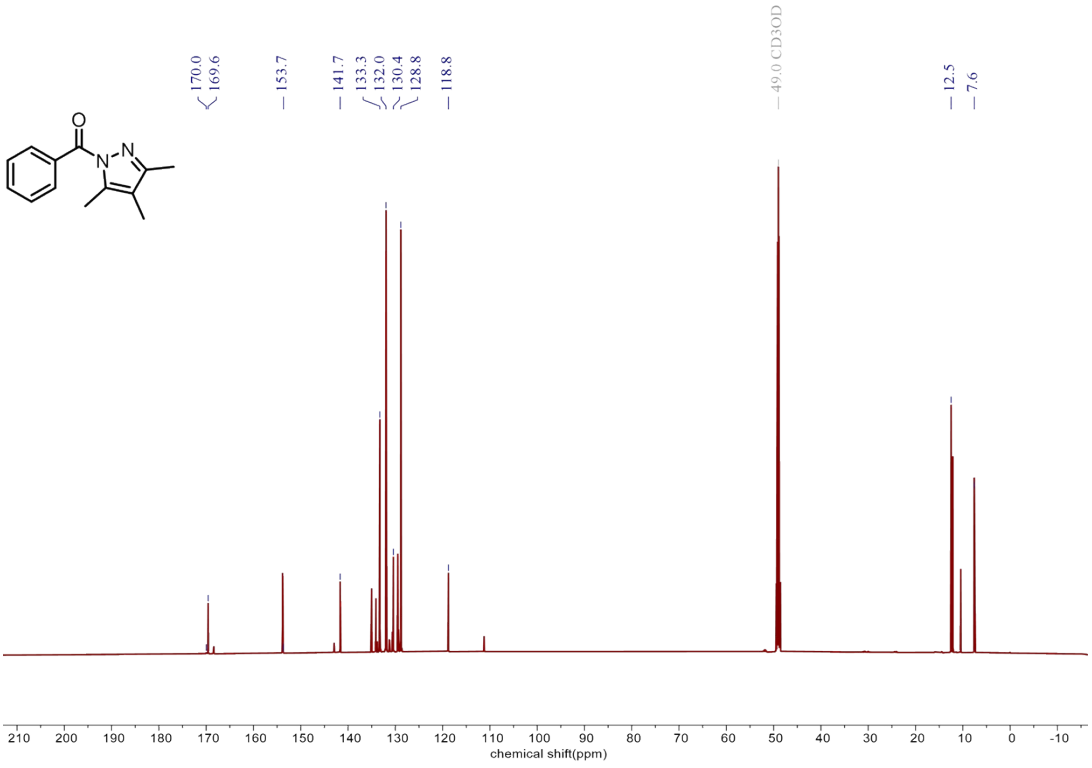
^{1}H NMR (600 MHz, MeOD) δ 7.84 (d, J = 7.2 Hz, 2H), 7.56 – 7.53 (m, 1H), 7.45 – 7.41 (m, 2H), 2.47 (s, 3H), 2.13 (s, 3H), 1.92 (s, 3H).

^{13}C NMR (151 MHz, MeOD) δ 169.6, 141.7, 133.3, 132.0, 130.4, 128.8, 118.8, 12.5, 7.6.

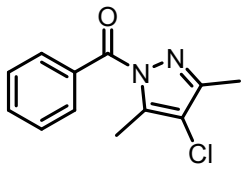
^{1}H NMR (600 MHz, MeOD) 6



^{13}C NMR (151 MHz, MeOD) 6



(4-chloro-3,5-dimethyl-1H-pyrazol-1-yl)(phenyl)methanone (7)

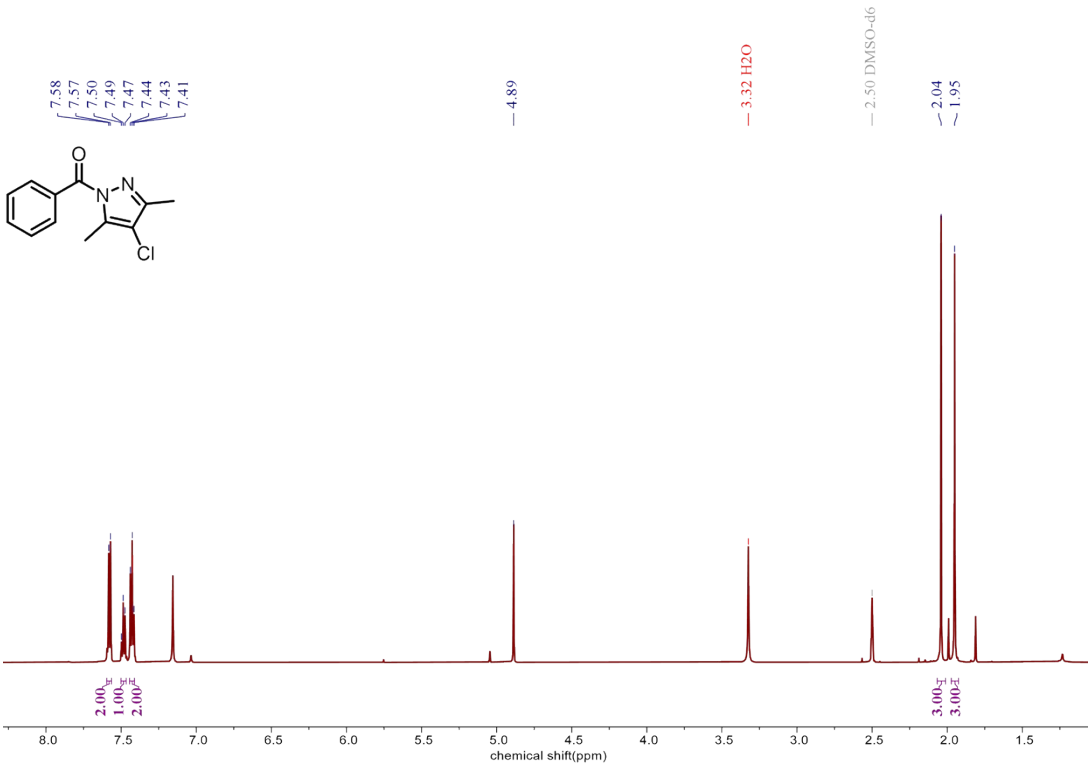


MS (ESI+): Calculated for $C_{12}H_{11}ClN_2O$ $[M+H]^+$ 235.0560, found 235.0619 (1+).

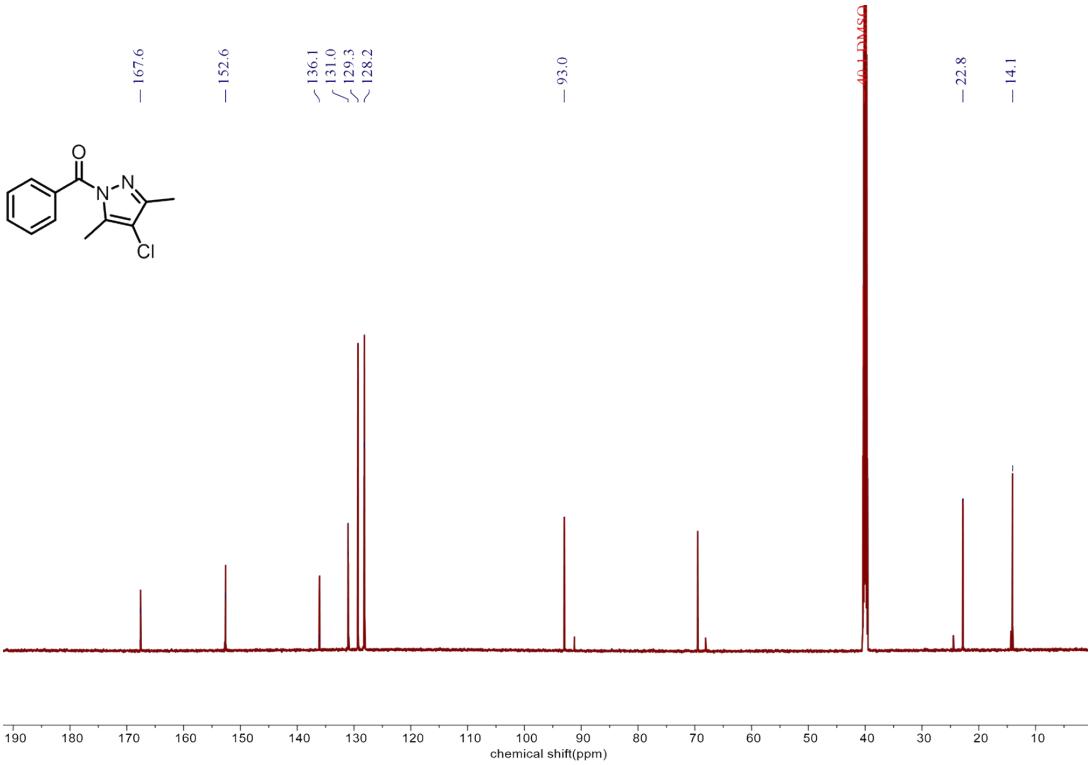
^{1}H NMR (600 MHz, DMSO) δ 7.58 (d, *J* = 7.0 Hz, 2H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.3 Hz, 2H), 2.04 (s, 3H), 1.95 (s, 3H).

^{13}C NMR (151 MHz, DMSO) δ 167.6, 152.6, 131.0, 129.3, 128.2, 93.0, 22.8, 14.1.

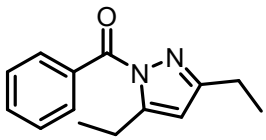
^{1}H NMR (600 MHz, DMSO) 7



^{13}C NMR (151 MHz, DMSO) 7



(3,5-diethyl-1H-pyrazol-1-yl)(phenyl)methanone (8)

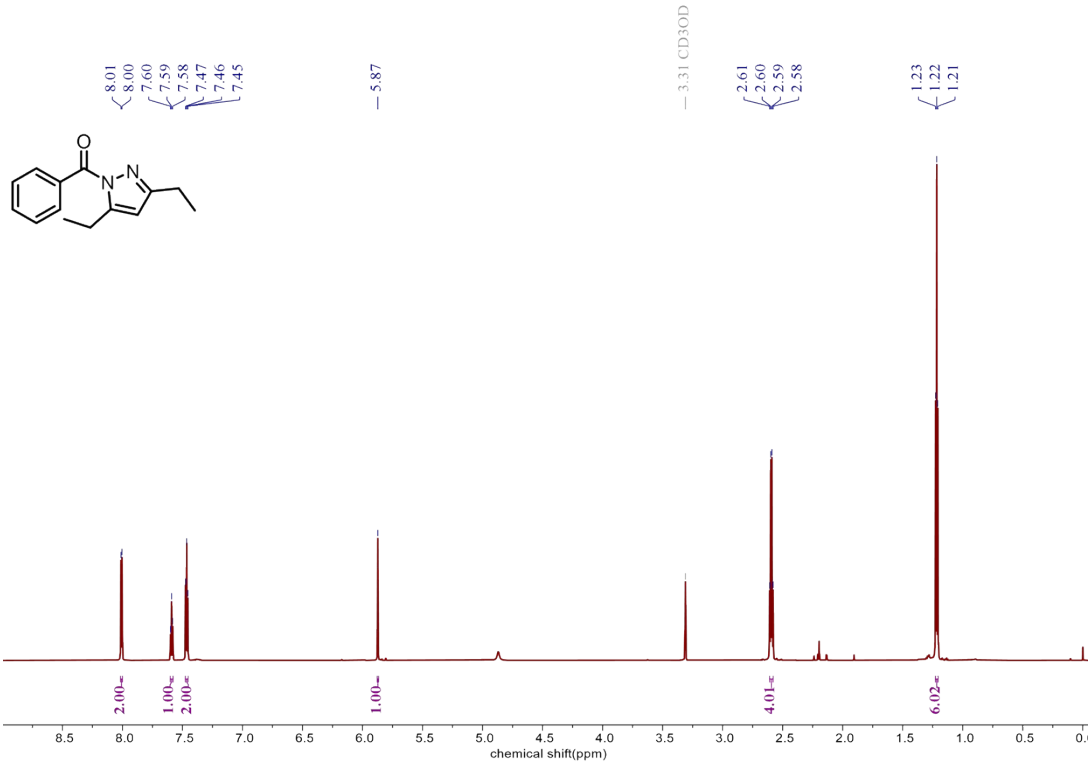


MS (ESI+): Calculated for $C_{14}H_{16}N_2O$ $[M+H]^+$ 229.1263, found 229.1213 (1+).

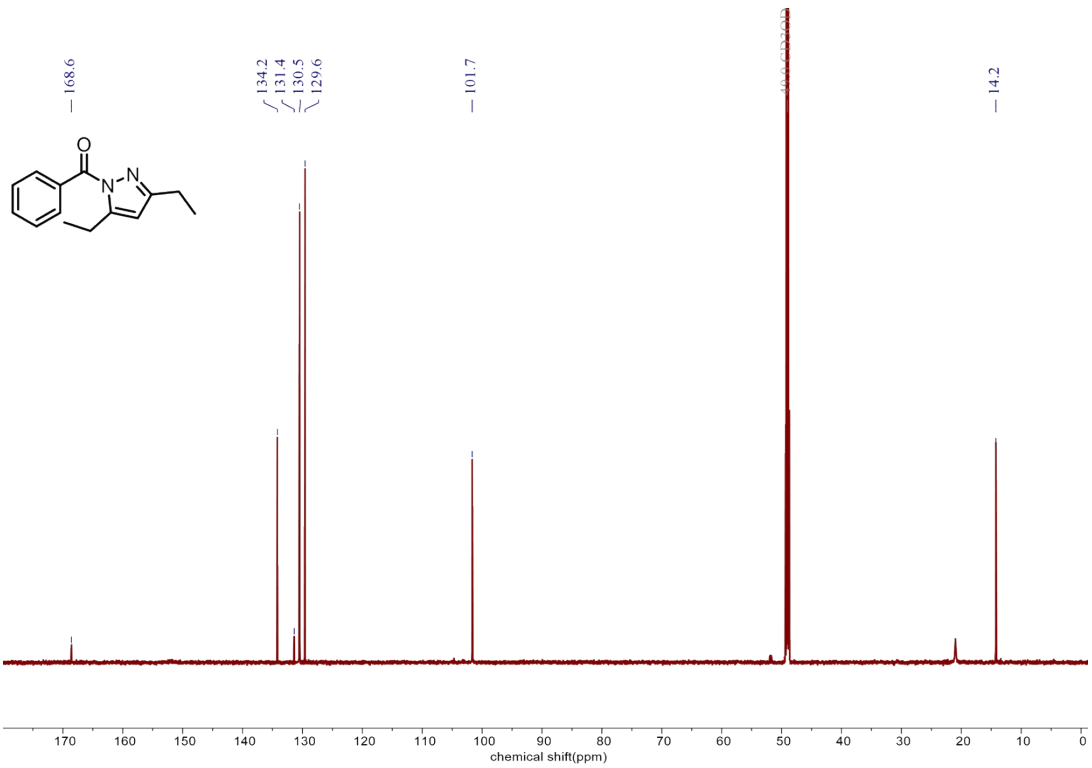
^{1}H NMR (800 MHz, MeOD) δ 8.01 (d, *J* = 7.0 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 2H), 5.87 (s, 1H), 2.59 (q, *J* = 7.7 Hz, 4H), 1.22 (t, *J* = 7.6 Hz, 6H).

^{13}C NMR (201 MHz, MeOD) δ 134.2, 131.4, 130.5, 129.6, 101.7, 14.2.

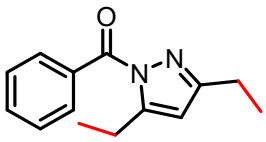
^{1}H NMR (800 MHz, MeOD) 8



^{13}C NMR (201 MHz, MeOD) 8



(3 or 5-ethyl-5 or 3-methyl-1H-pyrazol-1-yl)(phenyl)methanone (9)

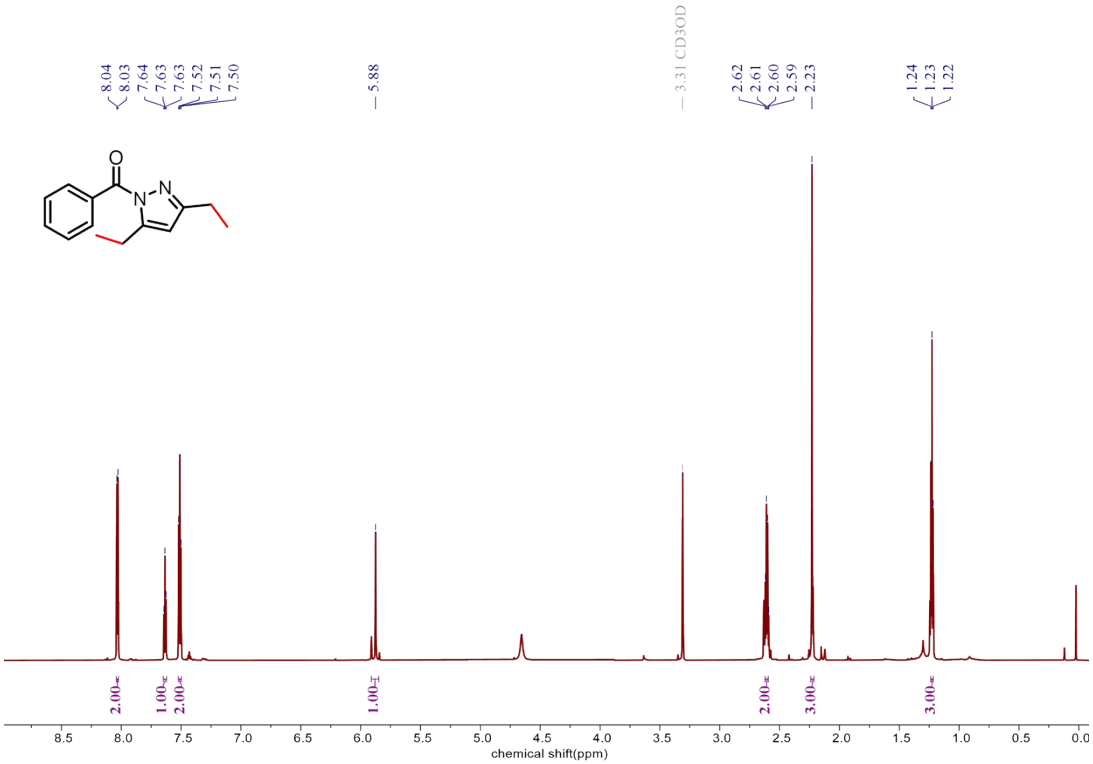


MS (ESI+): Calculated for $C_{13}H_{14}N_2O$ $[M+H]^+$ 215.1106, found 215.1172(1+).

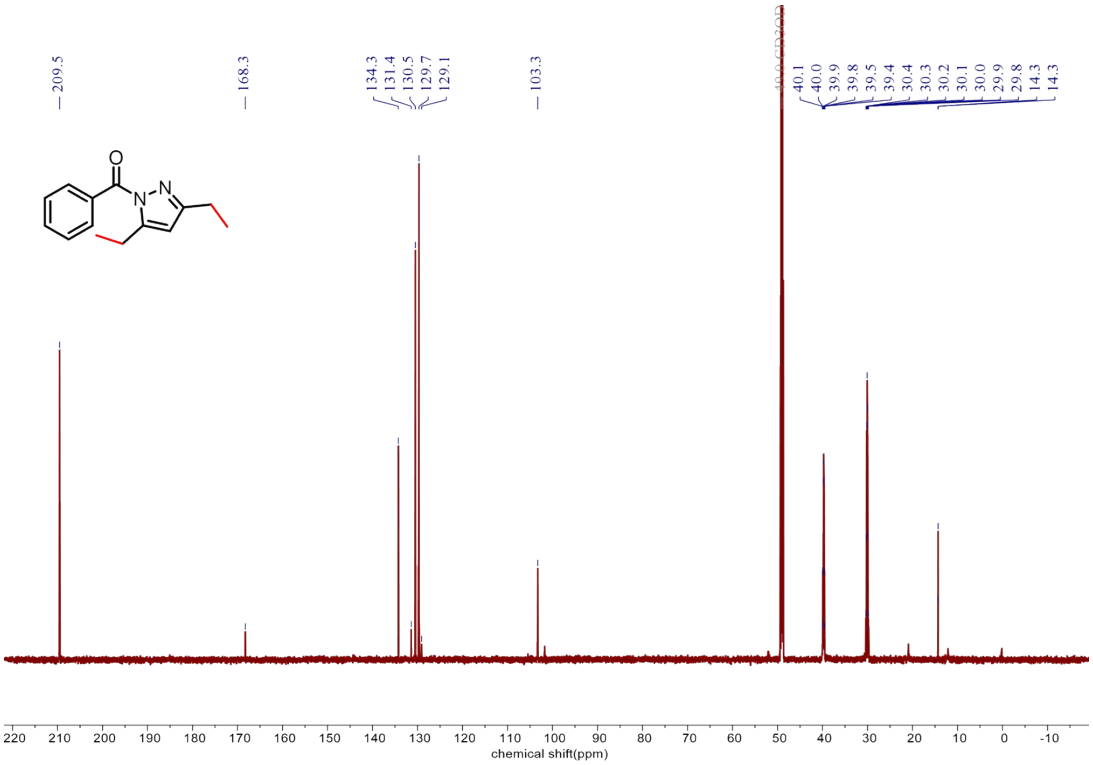
^{1}H NMR (800 MHz, MeOD) δ 8.03 (d, *J* = 7.0 Hz, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.52 – 7.50 (m, 2H), 5.88 (s, 1H), 2.62 – 2.59 (m, 2H), 2.23 (s, 3H), 1.23 (t, *J* = 7.7 Hz, 3H).

^{13}C NMR (201 MHz, MeOD) δ 209.5, 168.3, 134.3, 131.4, 130.5, 129.7, 129.1, 103.3, 40.1, 40.0, 39.9, 39.8, 39.5, 39.4, 30.4, 30.3, 30.2, 30.1, 30.0, 29.9, 29.8, 14.3, 14.3.

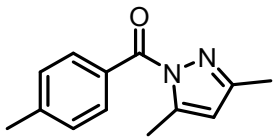
^{1}H NMR (800 MHz, MeOD) 9



^{13}C NMR (201 MHz, MeOD) 9



(3,5-dimethyl-1H-pyrazol-1-yl)(p-tolyl)methanone (10)

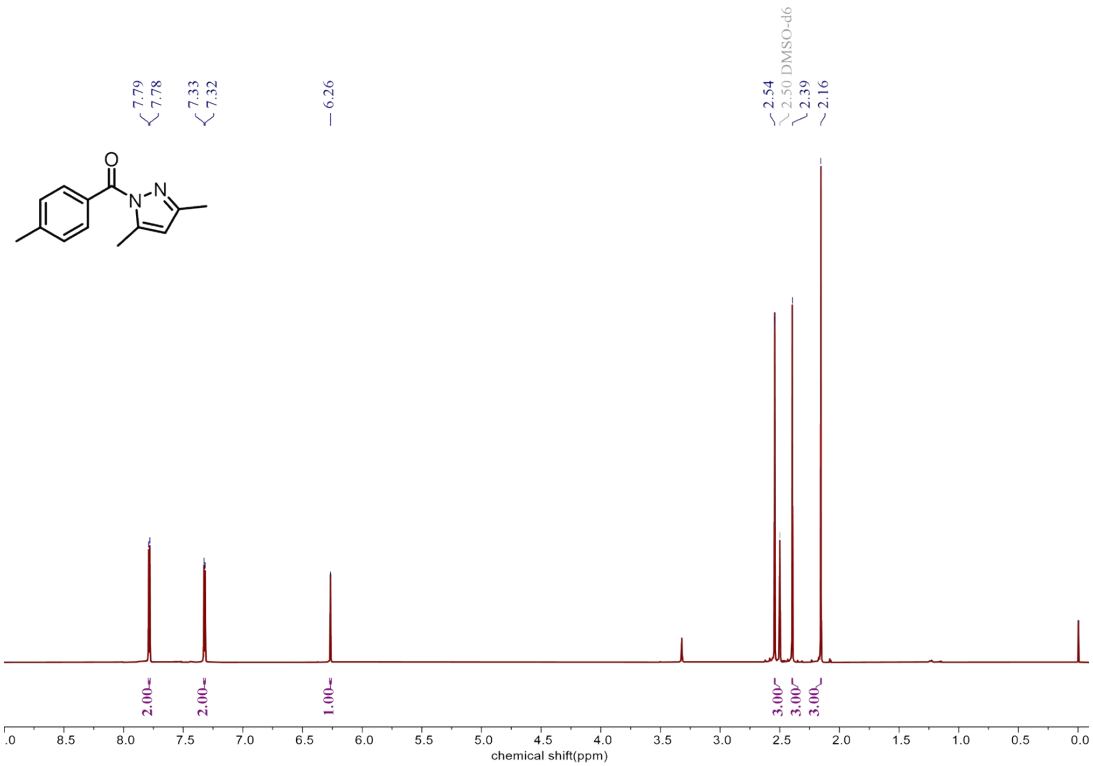


MS (ESI+): Calculated for $C_{13}H_{14}N_2O$ $[M+H]^+$ 215.1106, found 215.1160(1+).

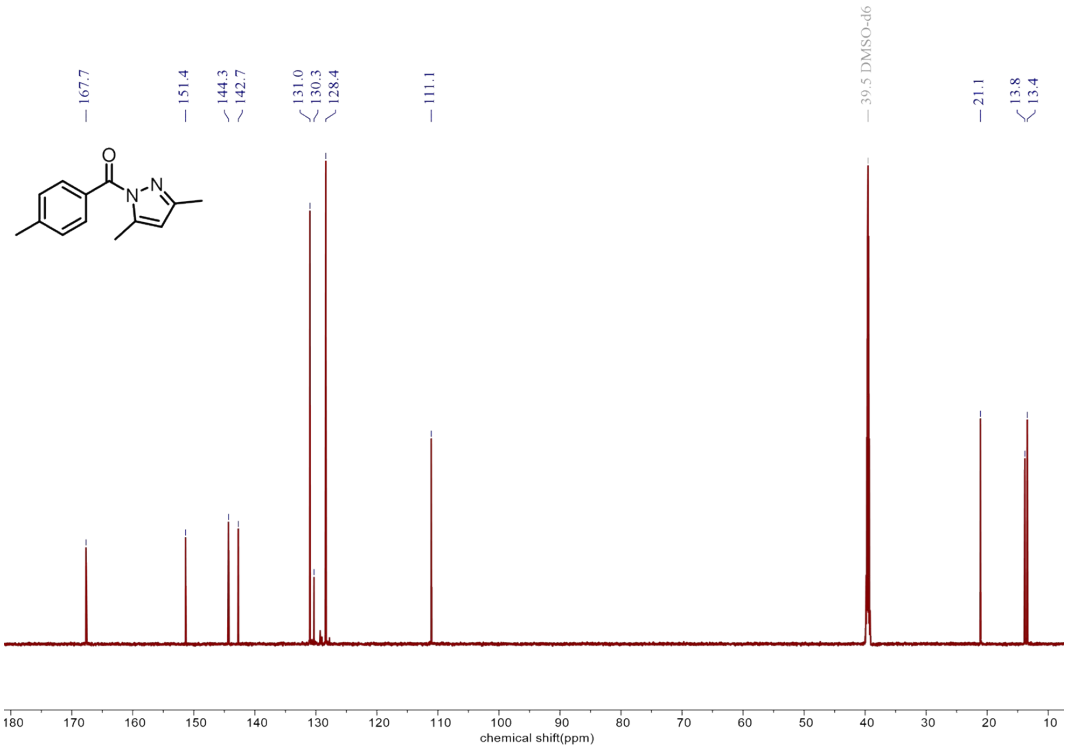
^{1}H NMR (800 MHz, DMSO) δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 6.26 (s, 1H), 2.54 (s, 3H), 2.39 (s, 3H), 2.16 (s, 3H).

^{13}C NMR (201 MHz, DMSO) δ 167.7, 151.4, 144.3, 142.7, 131.0, 130.3, 128.4, 111.1, 21.1, 13.8, 13.4.

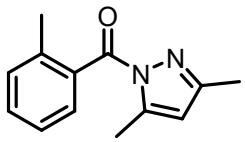
^{1}H NMR (800 MHz, DMSO 10



^{13}C NMR (201 MHz, DMSO) 10



(3,5-dimethyl-1H-pyrazol-1-yl)(o-tolyl)methanone (11)

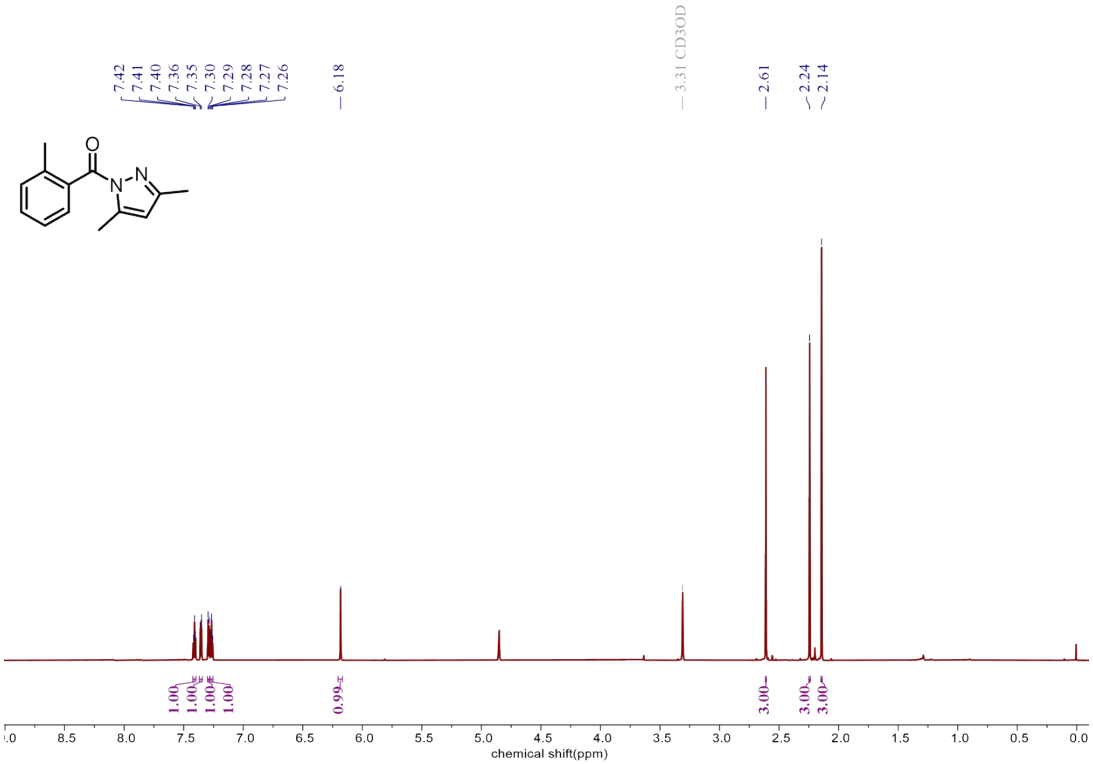


MS (ESI+): Calculated for $C_{13}H_{14}N_2O$ $[M+H]^+$ 215.1106, found 215.1149(1+).

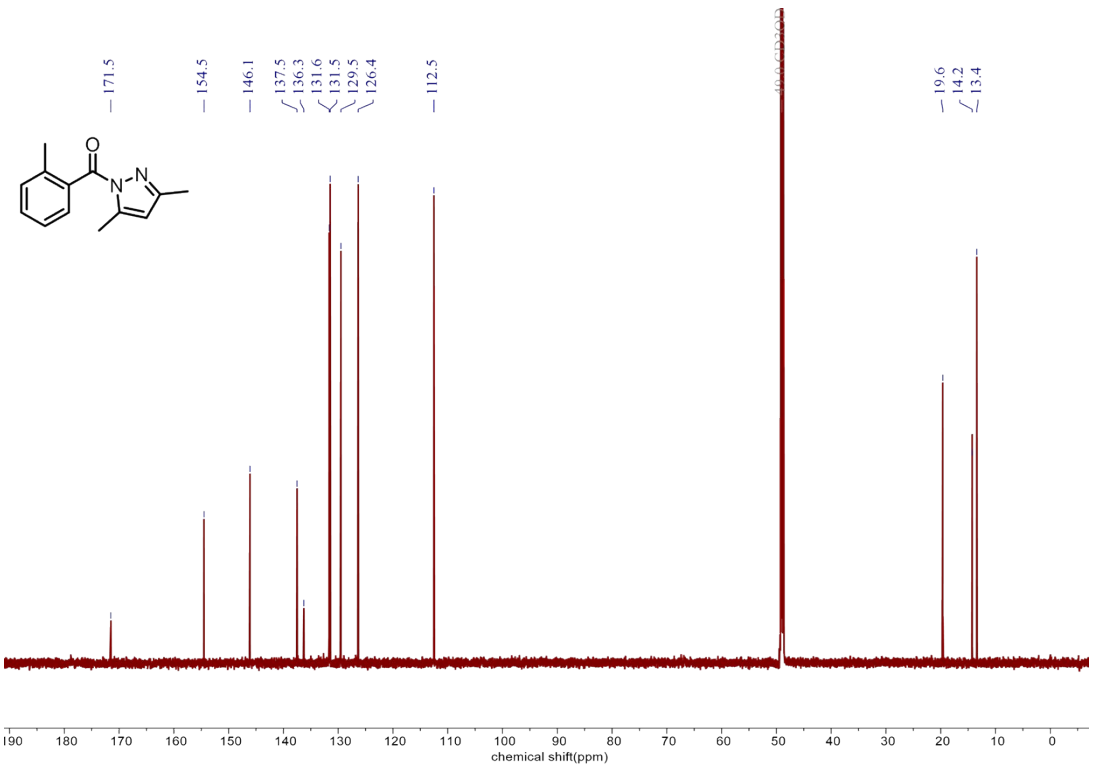
^{1}H NMR (800 MHz, MeOD) δ 7.41 (t, J = 6.8 Hz, 1H), 7.36 (d, J = 7.6 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 6.18 (s, 1H), 2.61 (s, 3H), 2.24 (s, 3H), 2.14 (s, 3H).

^{13}C NMR (201 MHz, MeOD) δ 171.5, 154.5, 146.1, 137.5, 136.3, 131.6, 131.5, 129.5, 126.4, 112.5, 19.6, 14.2, 13.4.

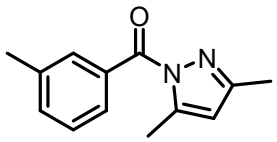
^{1}H NMR (800 MHz, MeOD) 11



^{13}C NMR (201 MHz, MeOD) 11



(3,5-dimethyl-1H-pyrazol-1-yl)(m-tolyl)methanone (12)

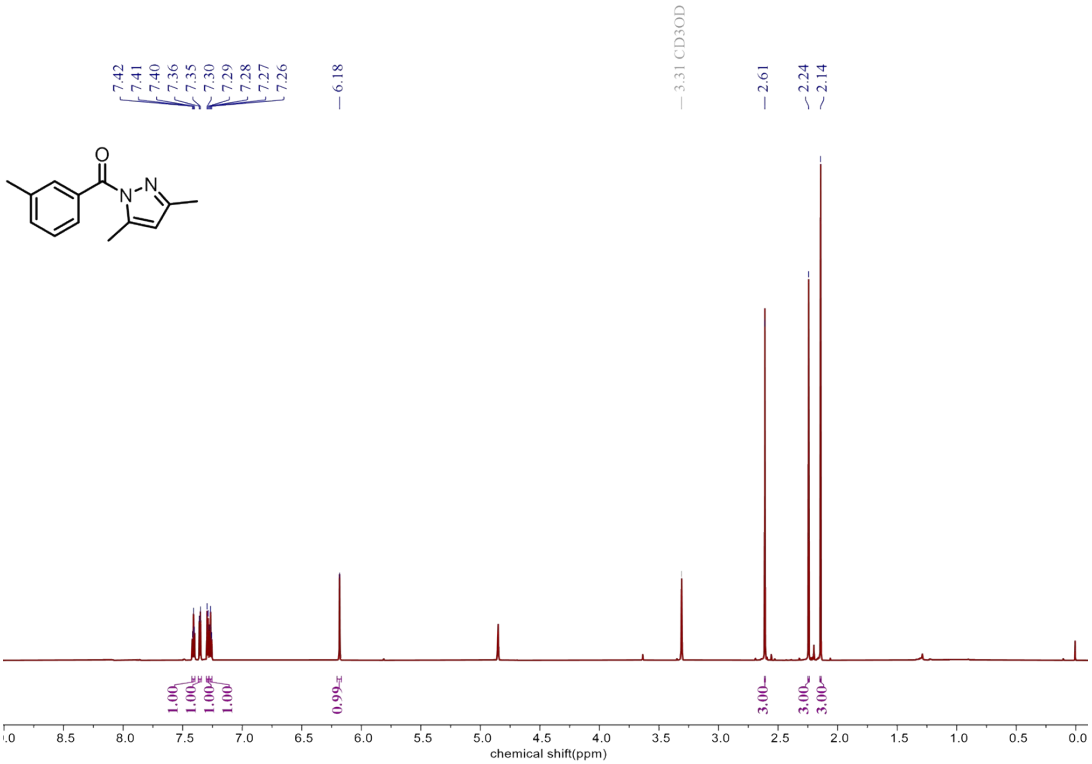


MS (ESI+): Calculated for $C_{13}H_{14}N_2O$ $[M+H]^+$ 215.1106, found 215.1169(1+).

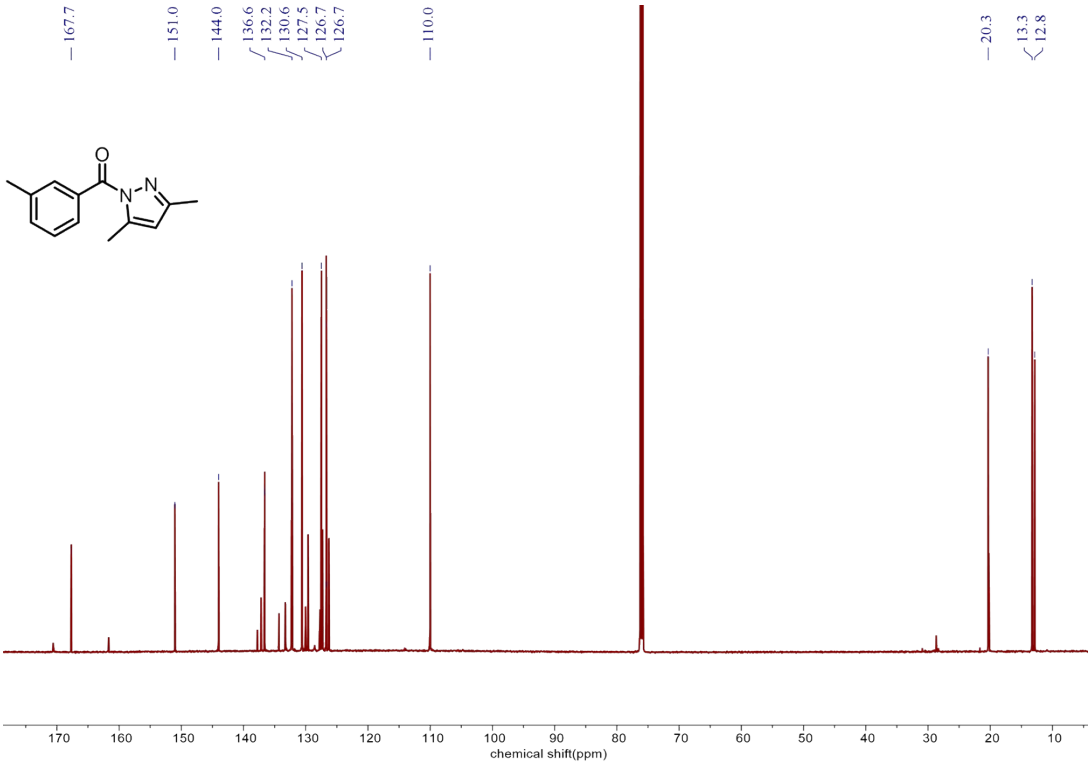
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (d, J = 28.0 Hz, 1H), 7.69 (s, 1H), 7.29 – 7.27 (m, 1H), 7.25 (d, J = 7.6 Hz, 1H), 5.97 (s, 1H), 2.54 (s, 3H), 2.33 (s, 3H), 2.17 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 167.7, 151.0, 144.0, 136.6, 132.2, 130.6, 127.5, 126.7, 126.7, 110.0, 20.3, 13.3, 12.8.

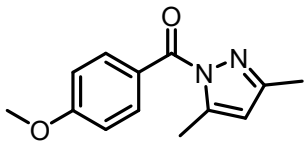
^{1}H NMR (600 MHz, $CDCl_3$) 12



^{13}C NMR (151 MHz, $CDCl_3$) 12



(3,5-dimethyl-1H-pyrazol-1-yl)(4methoxyphenyl)methanone (13)

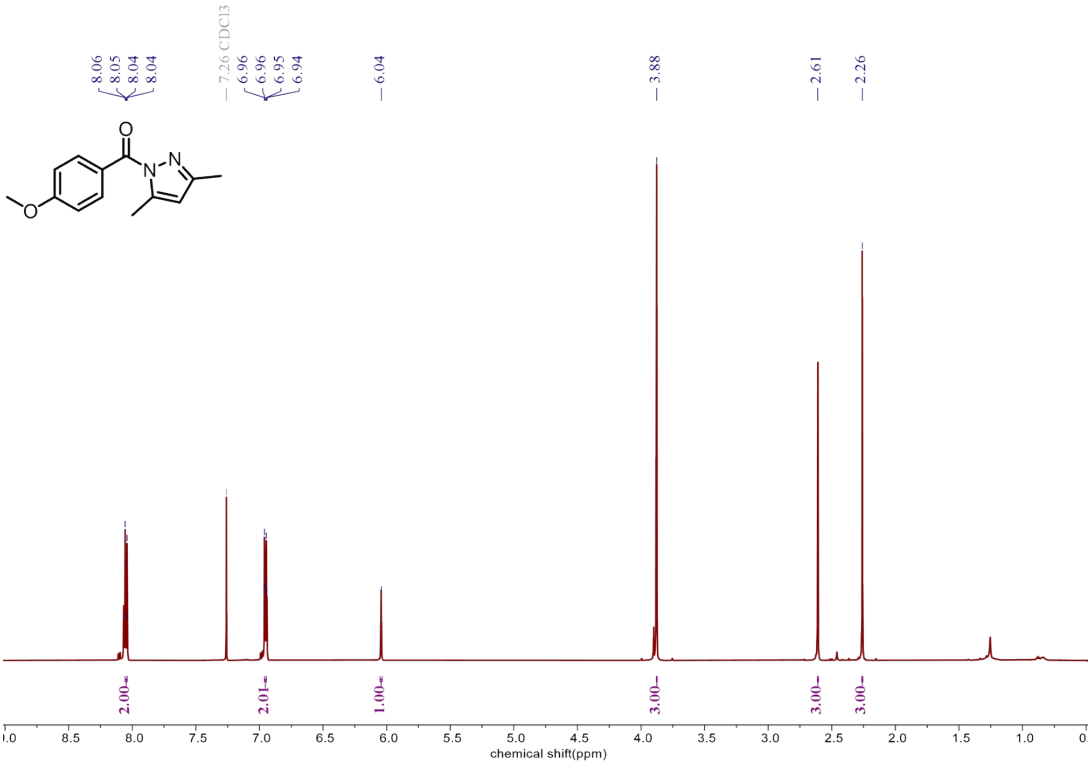


MS (ESI+): Calculated for $C_{13}H_{14}N_2O_2$ $[M+H]^+$ 231.1055, found 231.1116(1+).

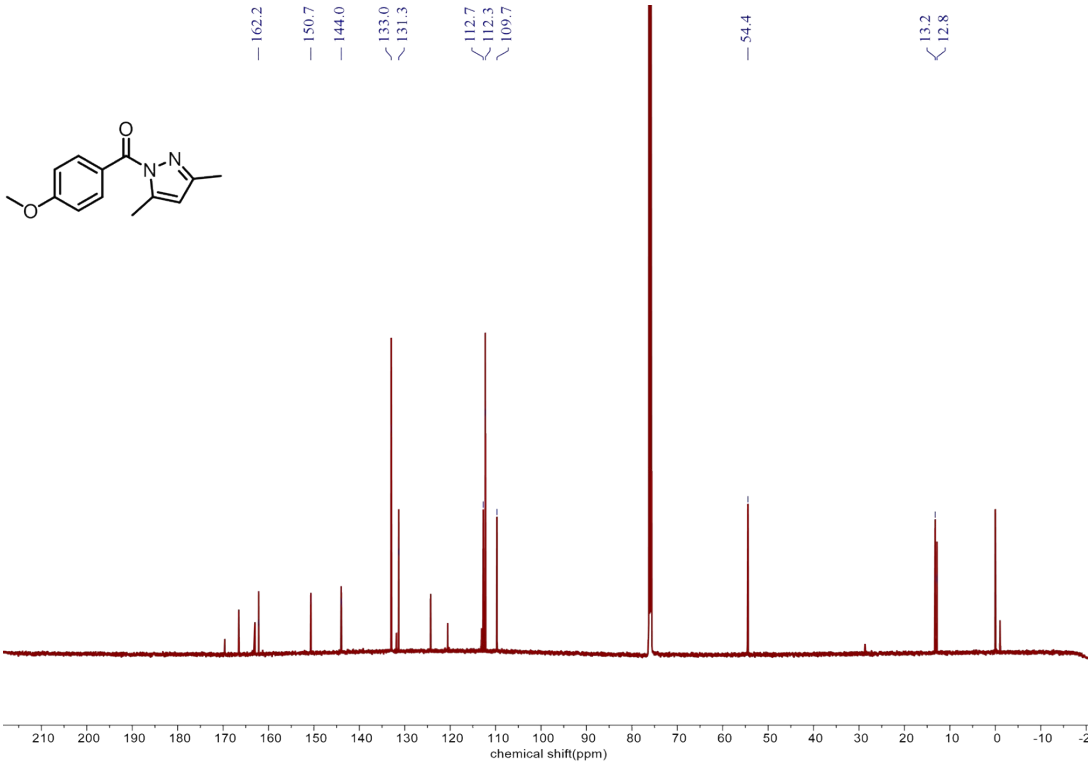
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 6.04 (s, 1H), 3.88 (s, 3H), 2.61 (s, 3H), 2.26 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 162.2, 150.7, 144.0, 133.0, 131.3, 112.7, 112.3, 109.7, 54.4, 13.2, 12.8.

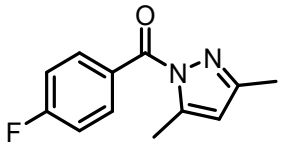
^{1}H NMR (600 MHz, $CDCl_3$) 13



^{13}C NMR (151 MHz, $CDCl_3$) 13



(3,5-dimethyl-1H-pyrazol-1-yl)(4-fluorophenyl)methanone (14)

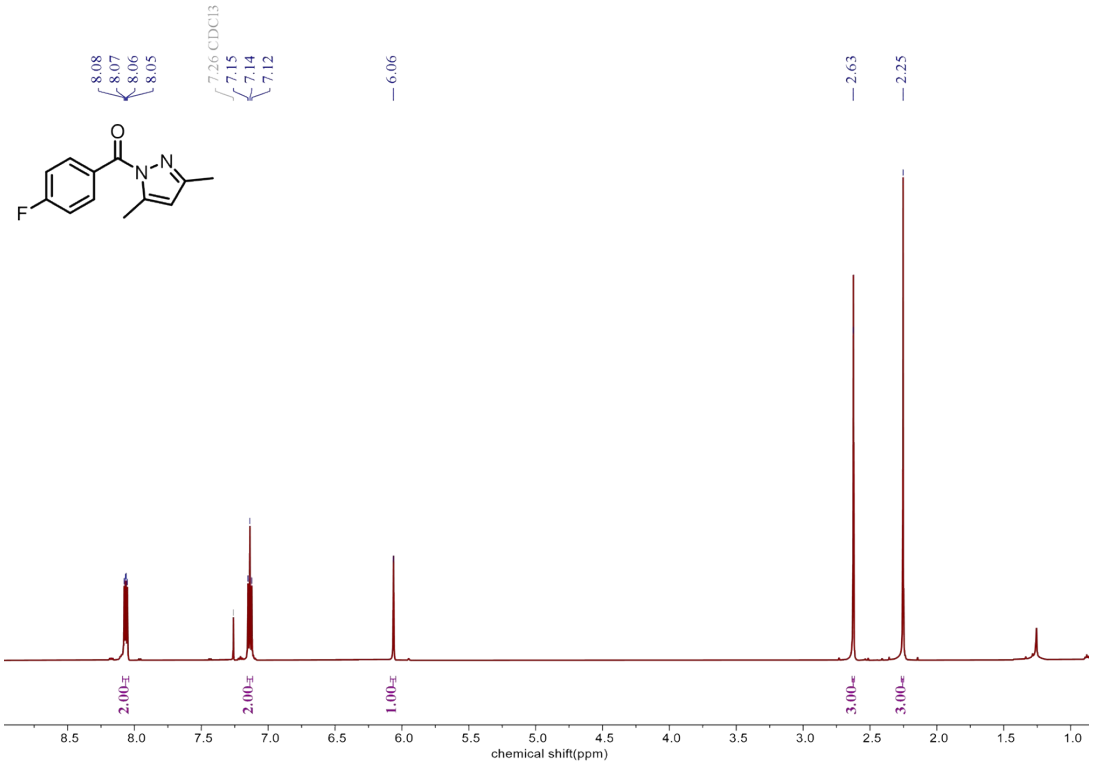


MS (ESI+): Calculated for $C_{12}H_{11}FN_2O$ $[M+H]^+$ 219.0855, found 219.0913(1+).

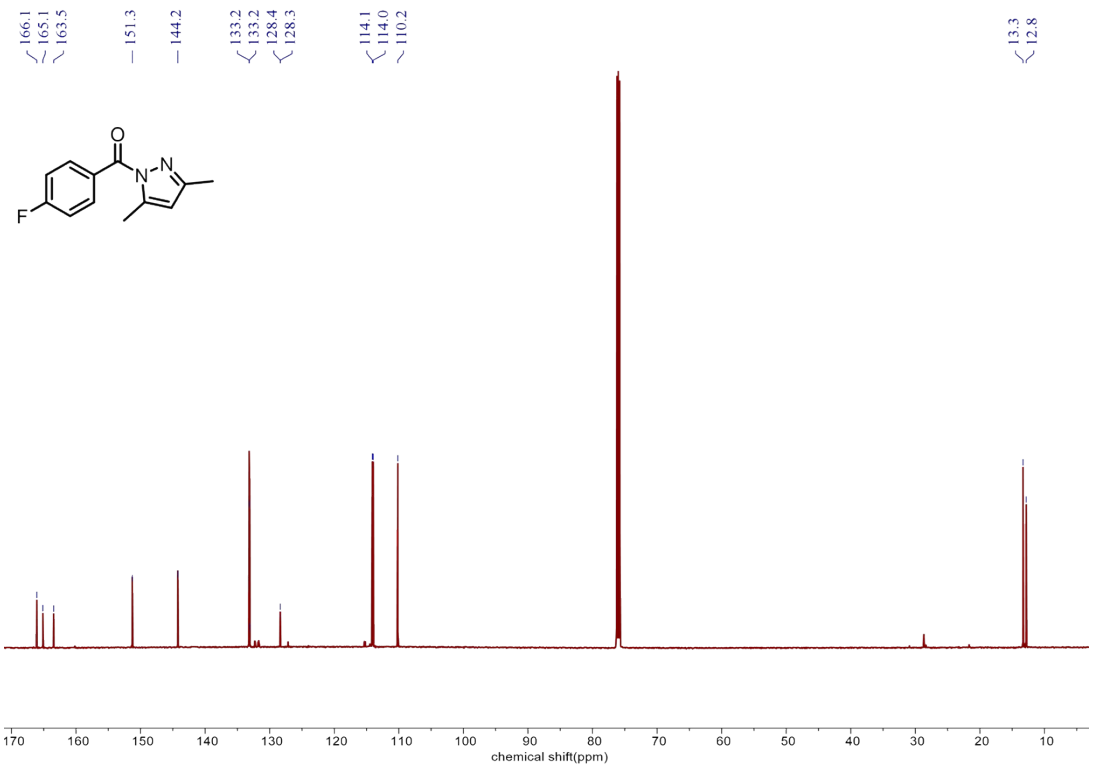
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (dd, J = 9.0, 5.4 Hz, 2H), 7.14 (t, J = 8.7 Hz, 2H), 6.06 (s, 1H), 2.63 (s, 3H), 2.25 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 165.1, 163.5, 151.3, 144.2, 133.2, 133.2, 128.4, 128.3, 114.1, 114.0, 110.2, 13.3, 12.8.

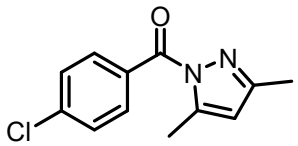
^{1}H NMR (600 MHz, $CDCl_3$) 14



^{13}C NMR (151 MHz, $CDCl_3$) 14



(4-chlorophenyl)(3,5-dimethyl-1H-pyrazol-1-yl)methanone (15)

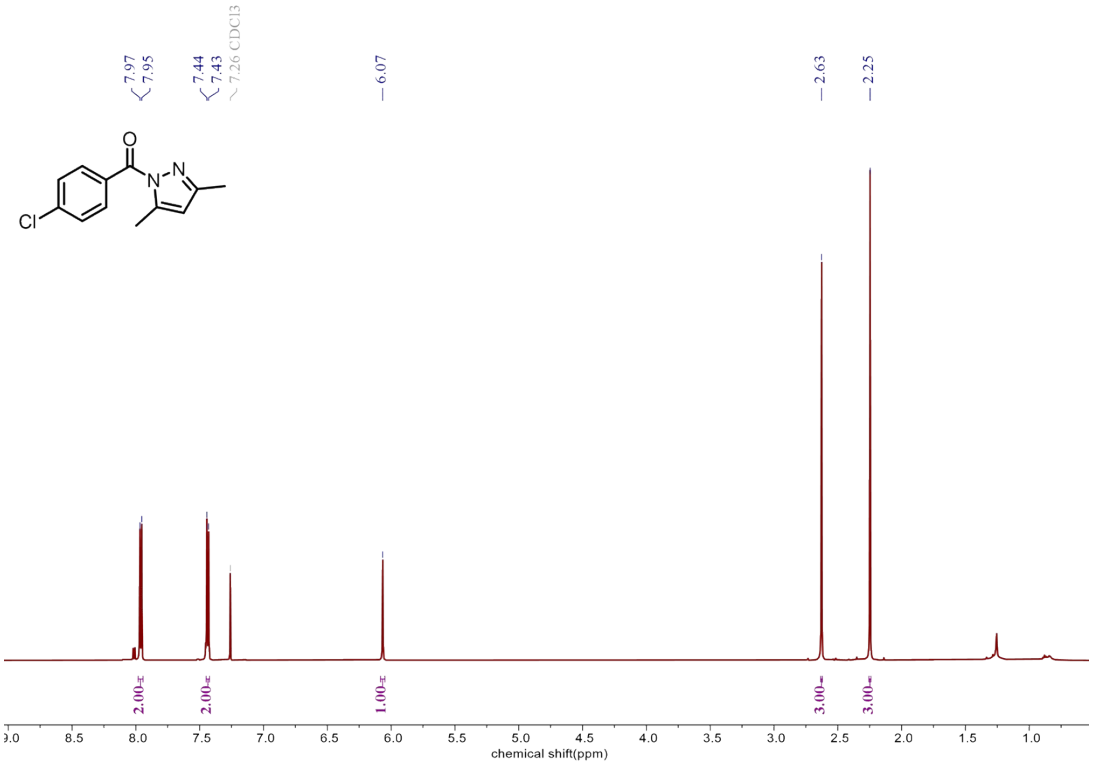


MS (ESI+): Calculated for $C_{12}H_{11}ClN_2O$ $[M+H]^+$ 235.0560, found 235.0618(1+).

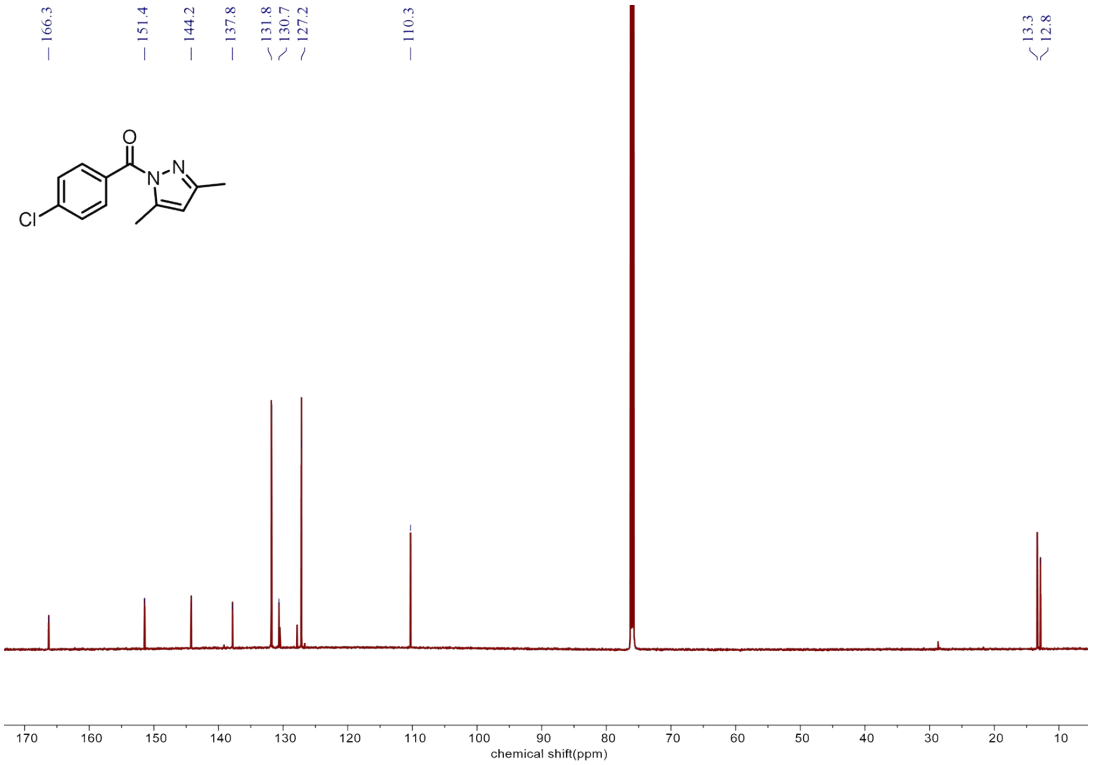
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 6.07 (s, 1H), 2.63 (s, 3H), 2.25 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 166.3, 151.4, 144.2, 137.8, 131.8, 130.7, 127.2, 110.3, 13.3, 12.8.

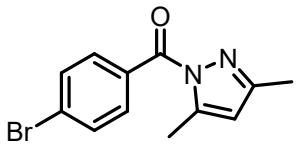
^{1}H NMR (600 MHz, $CDCl_3$) 15



^{13}C NMR (151 MHz, $CDCl_3$) 15



(4-bromophenyl)(3,5-dimethyl-1H-pyrazol-1-yl)methanone (16)

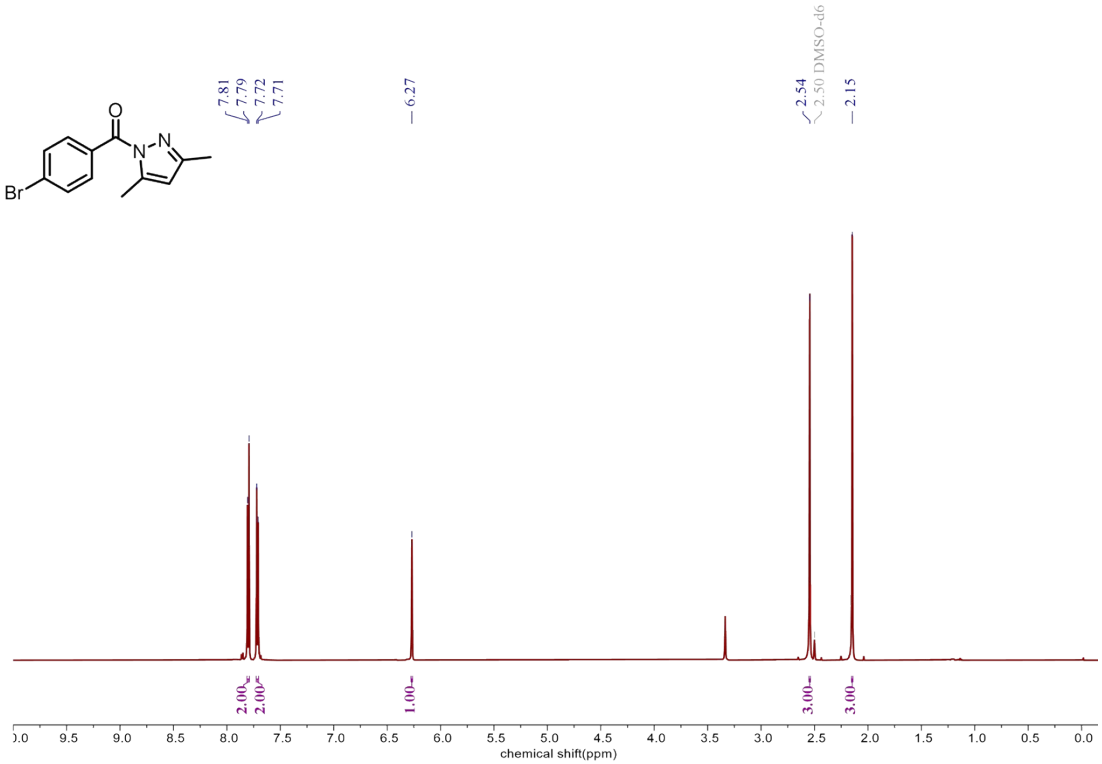


MS (ESI+): Calculated for $C_{12}H_{11}BrN_2O$ $[M+H]^+$ 279.0055, found 279.0107(1+).

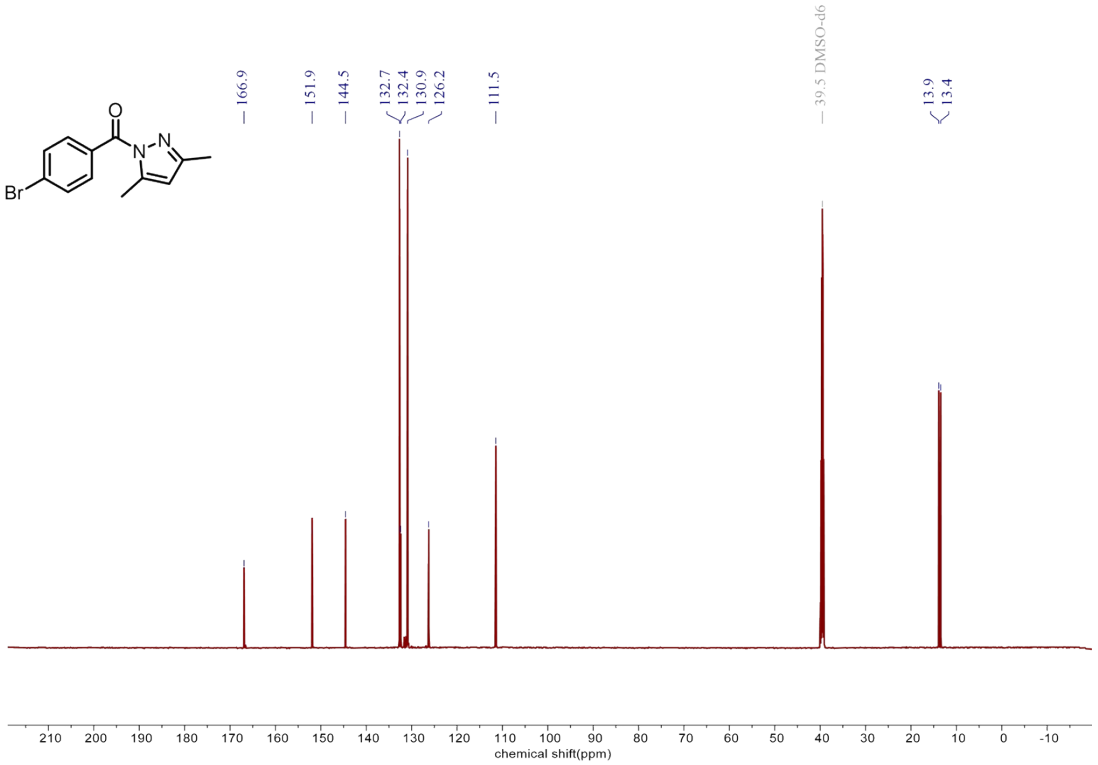
^{1}H NMR (600 MHz, DMSO) δ 7.80 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H), 6.27 (s, 1H), 2.54 (s, 3H), 2.15 (s, 3H).

^{13}C NMR (151 MHz, DMSO) δ 166.9, 151.9, 144.5, 132.7, 132.4, 130.9, 126.2, 111.5, 13.9, 13.4.

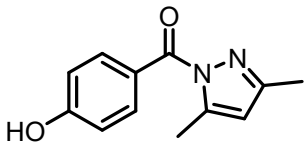
^{1}H NMR (600 MHz, DMSO) 16



^{13}C NMR (151 MHz, DMSO) 16



(3,5-dimethyl-1H-pyrazol-1-yl)(4-hydroxyphenyl)methanone (17)

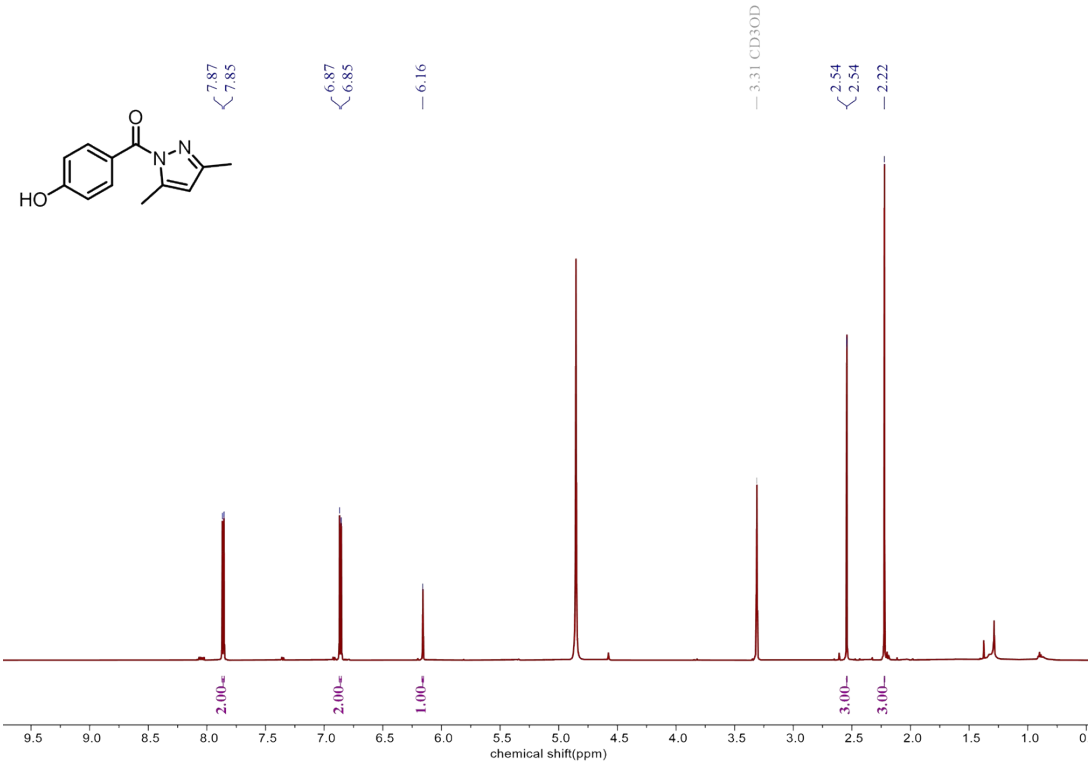


MS (ESI+): Calculated for $C_{12}H_{12}N_2O_2$ $[M+H]^+$ 217.0899, found 217.0961(1+).

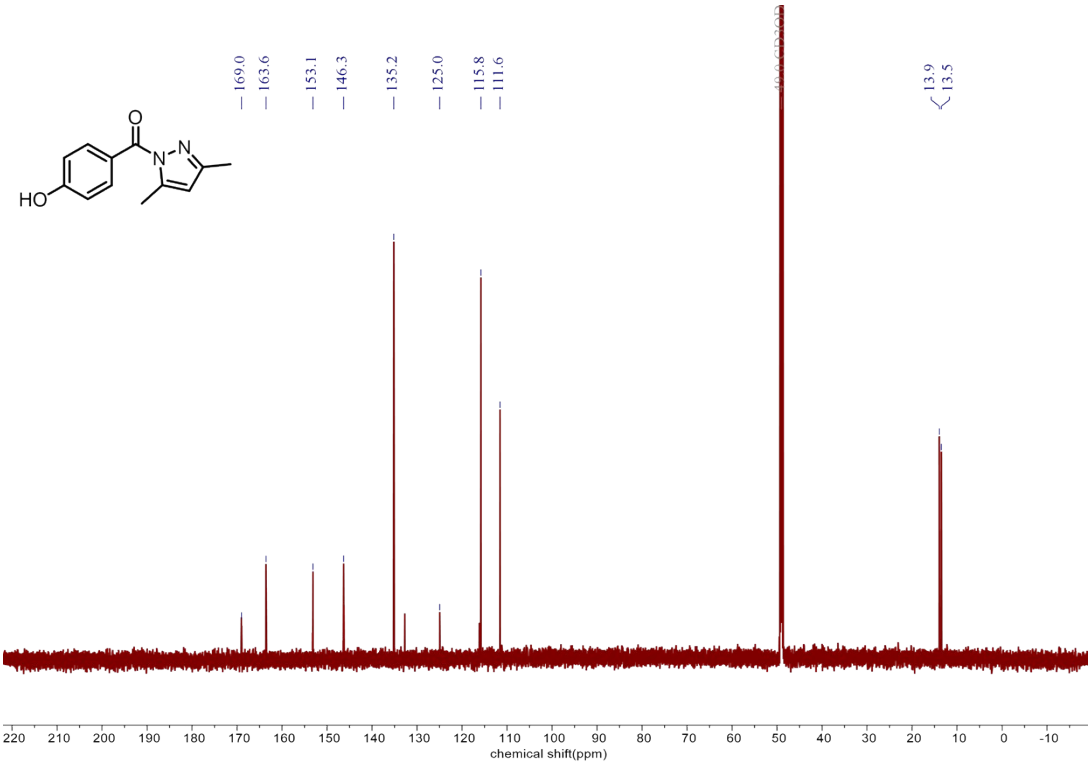
^{1}H NMR (600 MHz, MeOD) δ 7.86 (d, J = 8.9 Hz, 2H), 6.86 (d, J = 8.9 Hz, 2H), 6.16 (s, 1H), 2.54 (d, J = 1.1 Hz, 3H), 2.22 (s, 3H).

^{13}C NMR (201 MHz, MeOD) δ 169.0, 163.6, 153.1, 146.3, 135.2, 125.0, 115.8, 111.6, 13.9, 13.5.

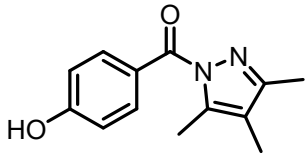
^{1}H NMR (600 MHz, MeOD) 17



^{13}C NMR (201 MHz, MeOD) 17



(4-hydroxyphenyl)(3,4,5-trimethyl-1H-pyrazol-1-yl)methanone (18)

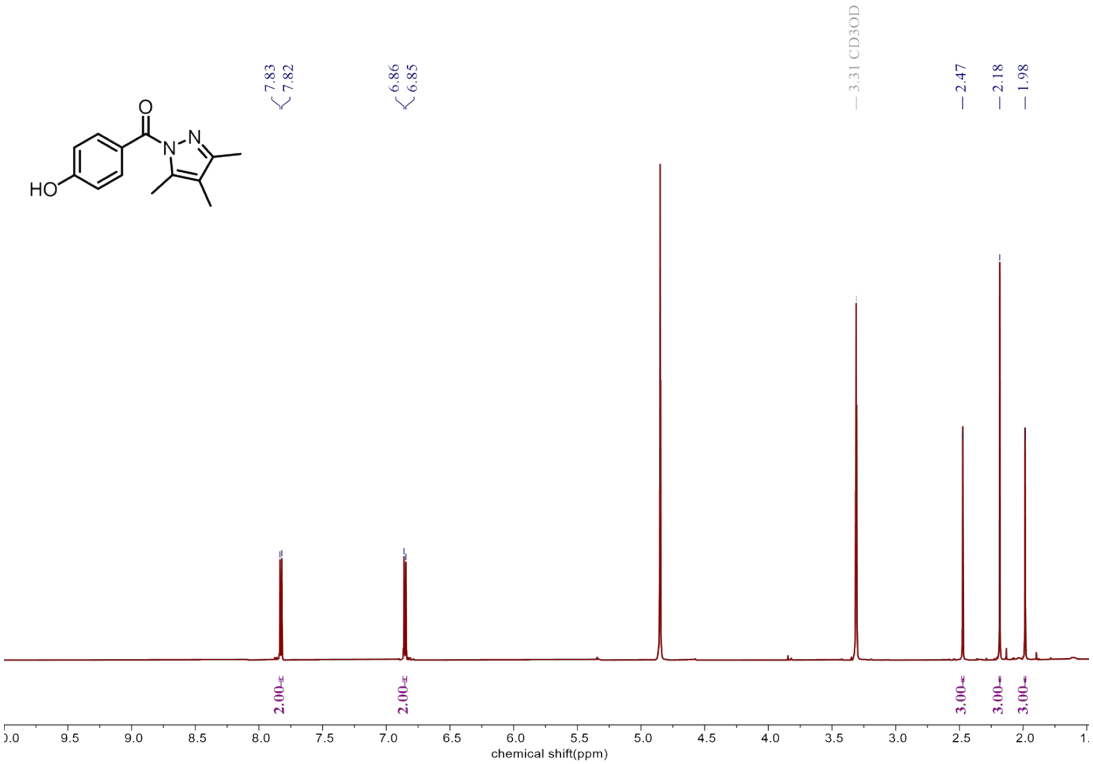


MS (ESI+): Calculated for $C_{13}H_{14}N_2O_2$ $[M+H]^+$ 231.1055, found 231.1088(1+).

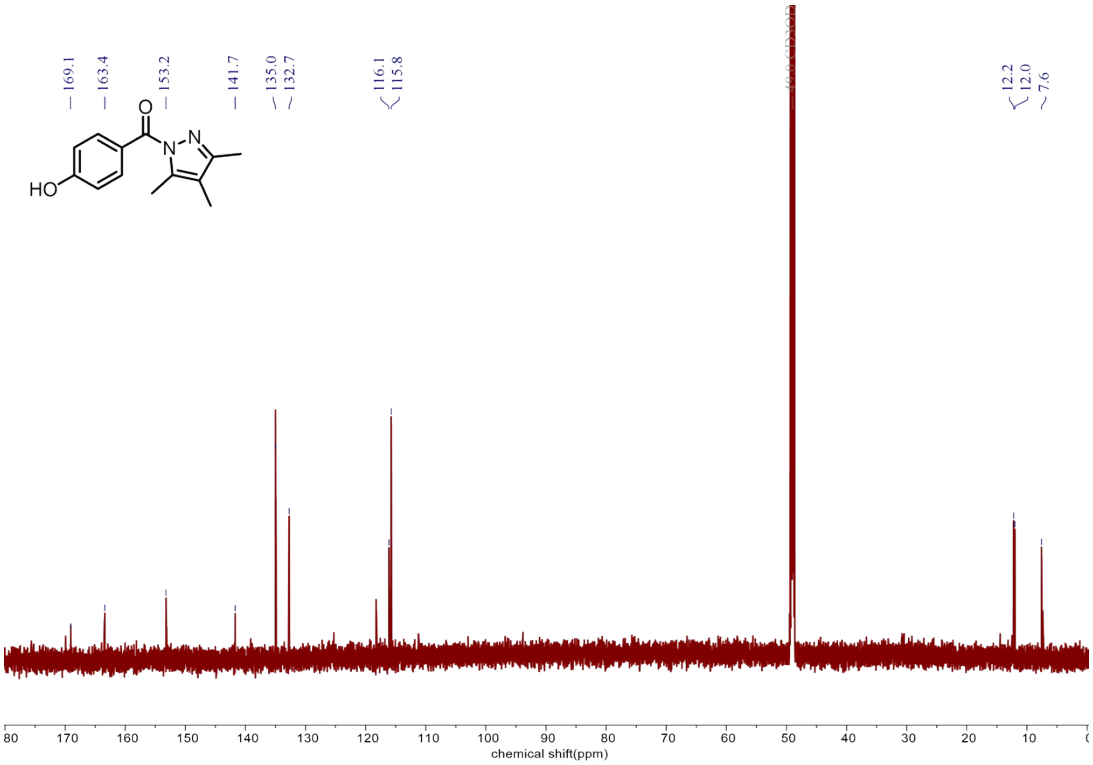
^{1}H NMR (600 MHz, MeOD) δ 7.83 (d, J = 8.9 Hz, 2H), 6.85 (d, J = 8.9 Hz, 2H), 2.47 (s, 3H), 2.18 (s, 3H), 1.98 (s, 3H).

^{13}C NMR (201 MHz, MeOD) δ 169.1, 163.4, 153.2, 141.7, 135.0, 132.7, 116.1, 115.8, 12.2, 12.0, 7.6.

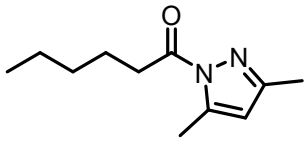
^{1}H NMR (600 MHz, MeOD) 18



^{13}C NMR (201 MHz, MeOD) 18



1-(3,5-dimethyl-1H-pyrazol-1-yl)hexan-1-one (19)

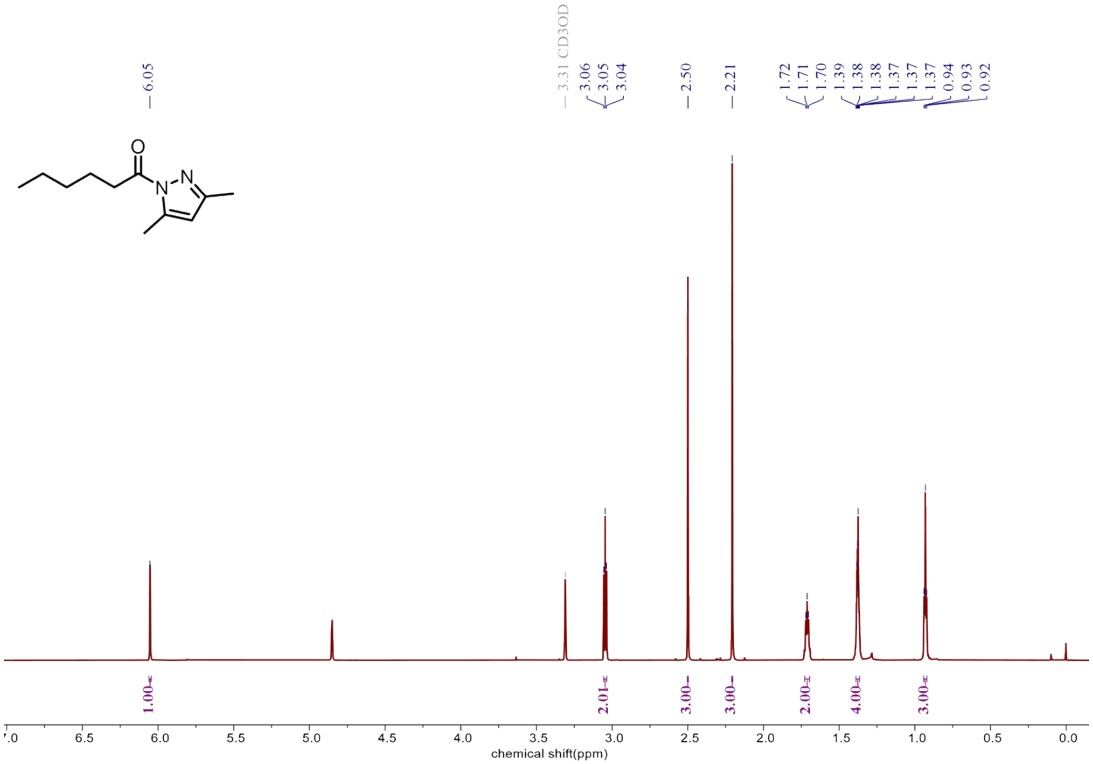


MS (ESI+): Calculated for $C_{11}H_{18}N_2O$ $[M+H]^+$ 195.1419, found 195.1474 (1+).

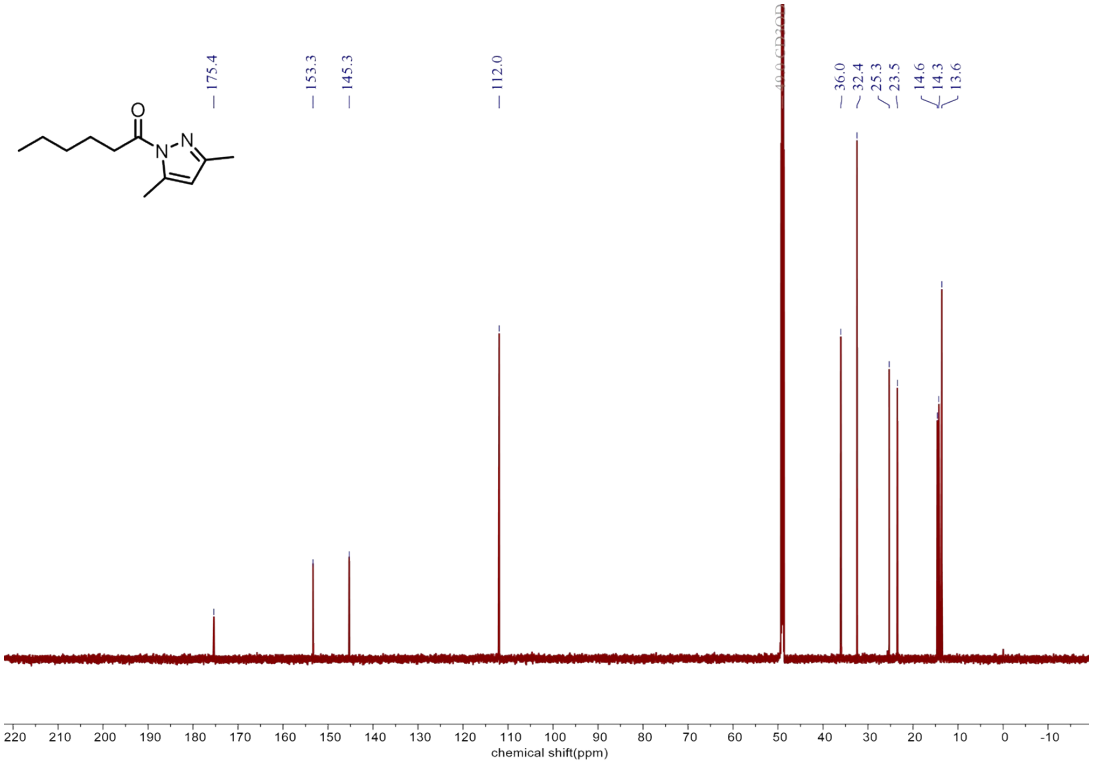
^{1}H NMR (800 MHz, MeOD) δ 6.05 (s, 1H), 3.05 (t, *J* = 7.5 Hz, 2H), 2.50 (s, 3H), 2.21 (s, 3H), 1.71 (t, *J* = 7.4 Hz, 2H), 1.38 (h, *J* = 3.7 Hz, 4H), 0.93(m, 3H).

^{13}C NMR (201 MHz, MeOD) δ 175.4, 153.3, 145.3, 112.0, 36.0, 32.4, 25.3, 23.5, 14.6, 14.3, 13.6.

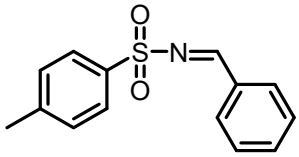
^{1}H NMR (800 MHz, MeOD) 19



^{13}C NMR (201 MHz, MeOD) 19



N-benzylidene-4-methylbenzenesulfonamide (20)

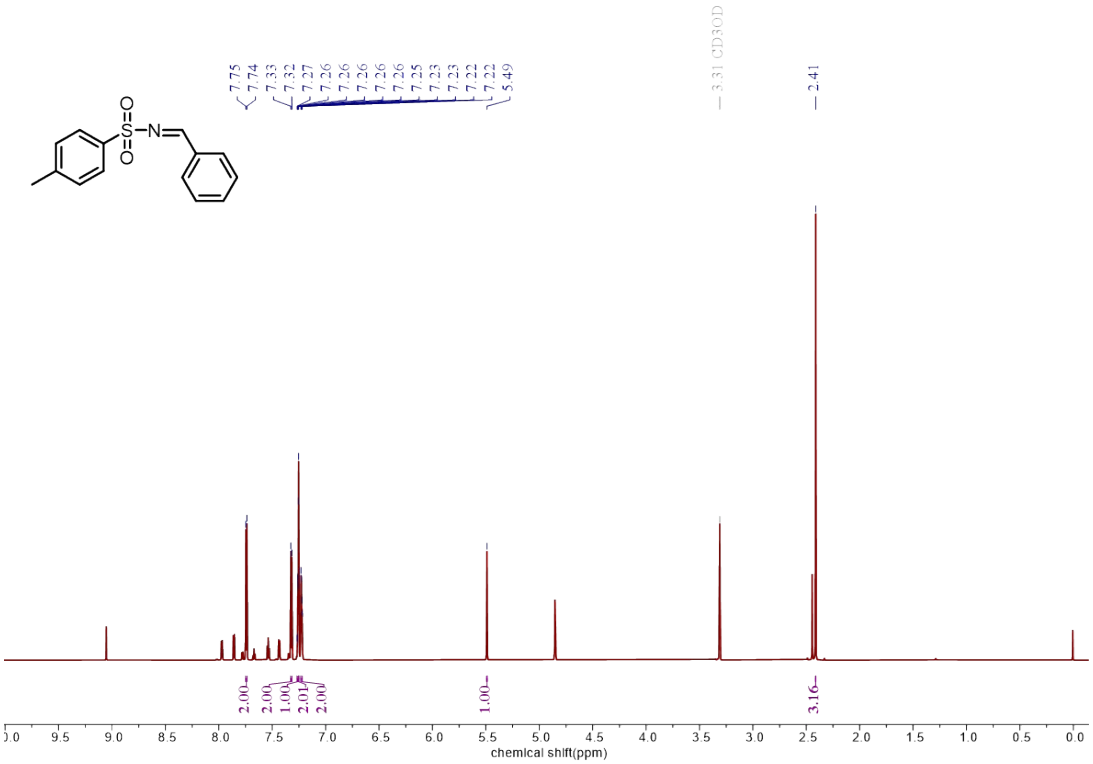


MS (ESI+): Calculated for $C_{14}H_{13}NO_2S$ $[M+H]^+$ 260.0677, found 260.2105 (1+).

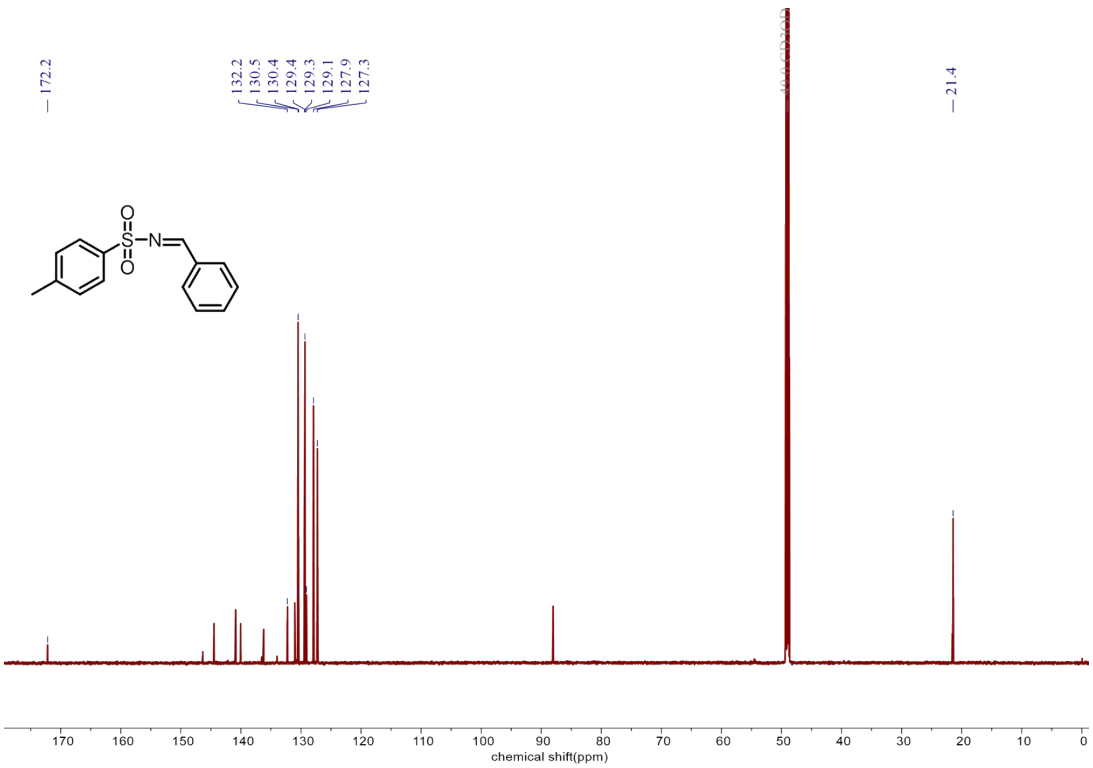
^{1}H NMR (800 MHz, MeOD) δ 7.74 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.27 – 7.26 (m, 1H), 7.25 (d, *J* = 2.4 Hz, 2H), 7.22 (dd, *J* = 6.8, 3.0 Hz, 2H), 5.49 (s, 1H), 2.41 (s, 3H).

^{13}C NMR (201 MHz, MeOD) δ 172.2, 132.2, 130.5, 130.4, 129.4, 129.3, 129.1, 127.9, 127.3, 21.4.

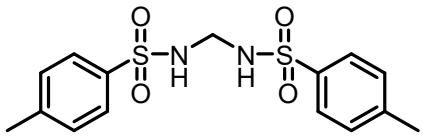
^{1}H NMR (800 MHz, MeOD) 20



^{13}C NMR (201 MHz, MeOD) 20



N,N'-methylenebis(4-methylbenzenesulfonamide) (21)

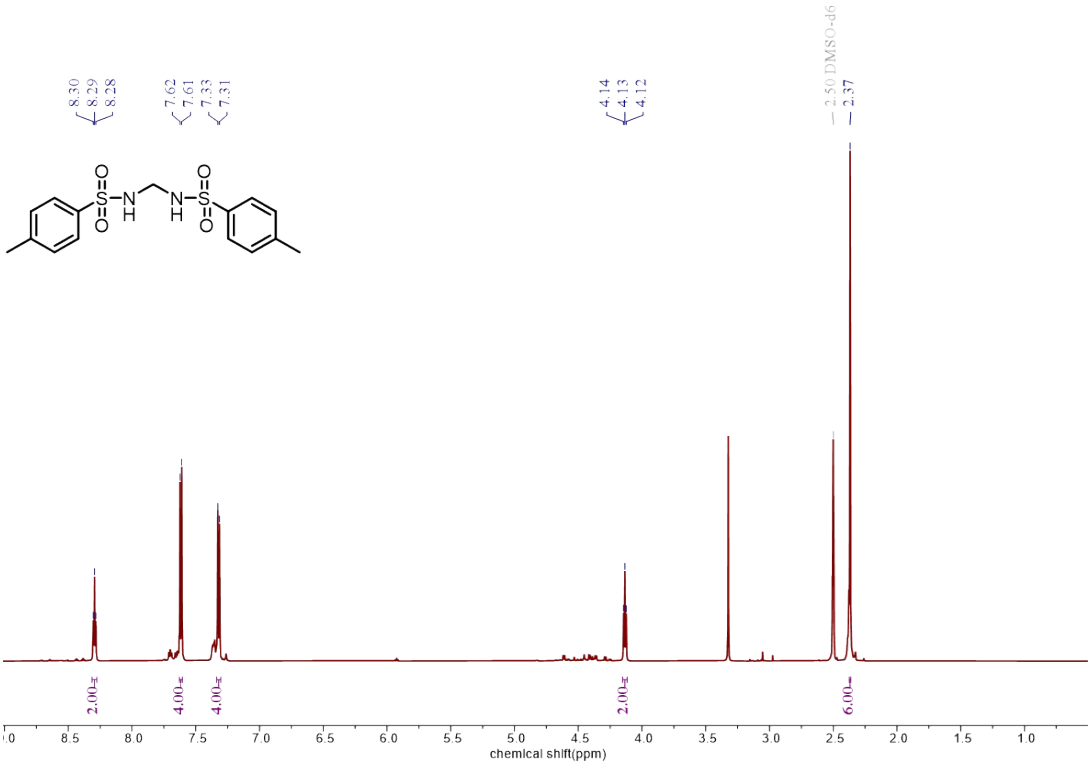


MS (ESI+): Calculated for $C_{15}H_{18}N_2O_4S_2$ $[M+H]^+$ 355.0708, found 355.0762 (1+).

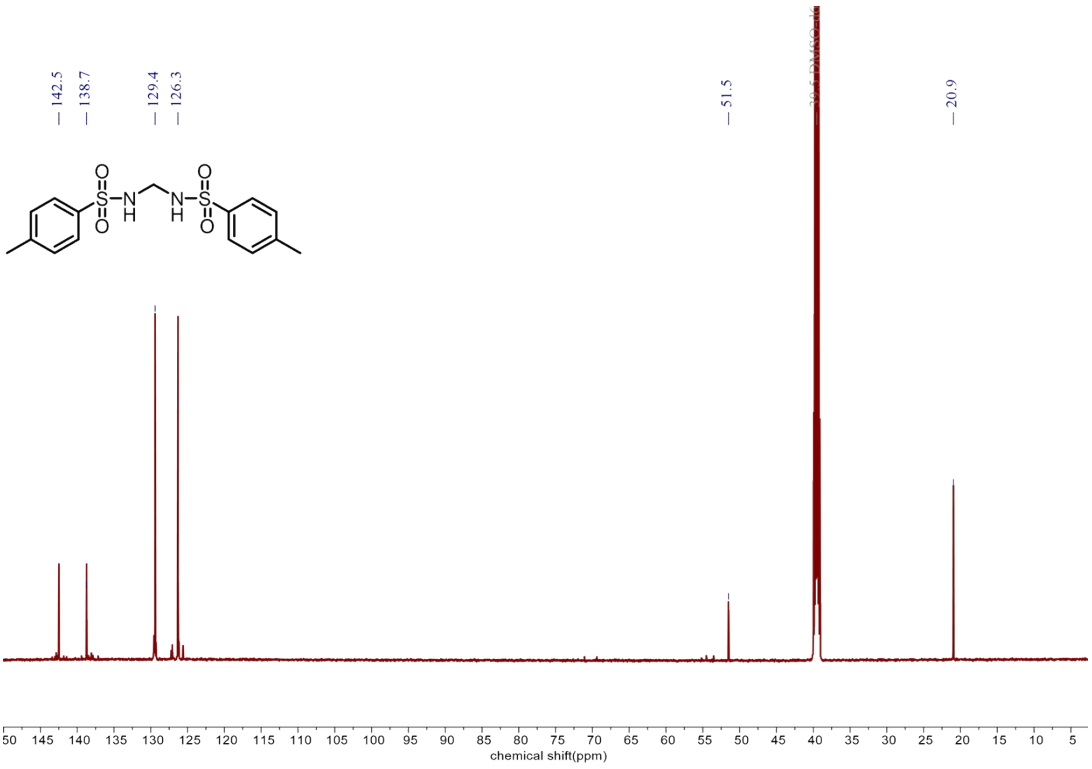
^{1}H NMR (600 MHz, DMSO) δ 8.29 (t, J = 6.3 Hz, 2H), 7.62 (d, J = 8.3 Hz, 4H), 7.32 (d, J = 7.8 Hz, 4H), 4.13 (t, J = 6.3 Hz, 2H), 2.37 (s, 6H).

^{13}C NMR (151 MHz, DMSO) δ 142.5, 138.7, 129.4, 126.3, 51.5, 20.9.

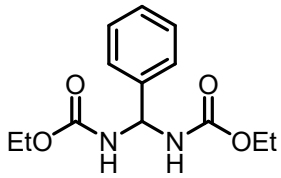
^{1}H NMR (600 MHz, DMSO) 21



^{13}C NMR (151 MHz, DMSO) 21



Diethyl (phenylmethylene)dicarbamate (22)

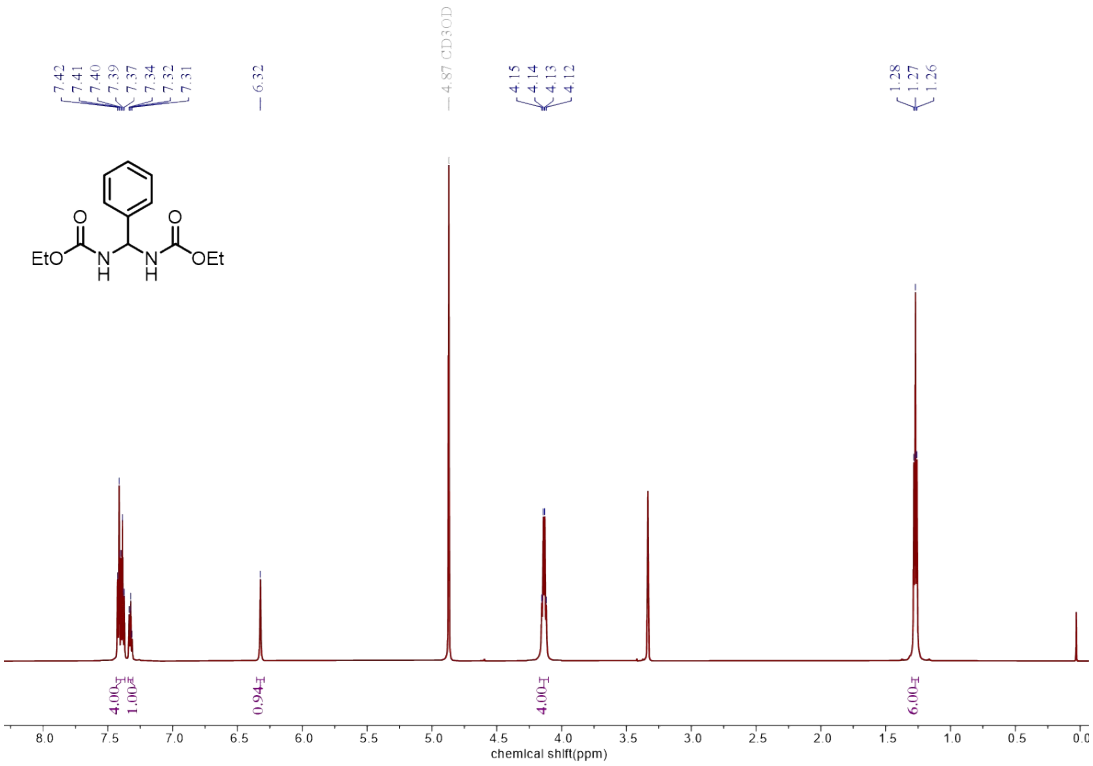


MS (ESI+): Calculated for $C_{13}H_{18}N_2O_4$ $[M+H]^+$ 267.1267, found 267.1284 (1+).

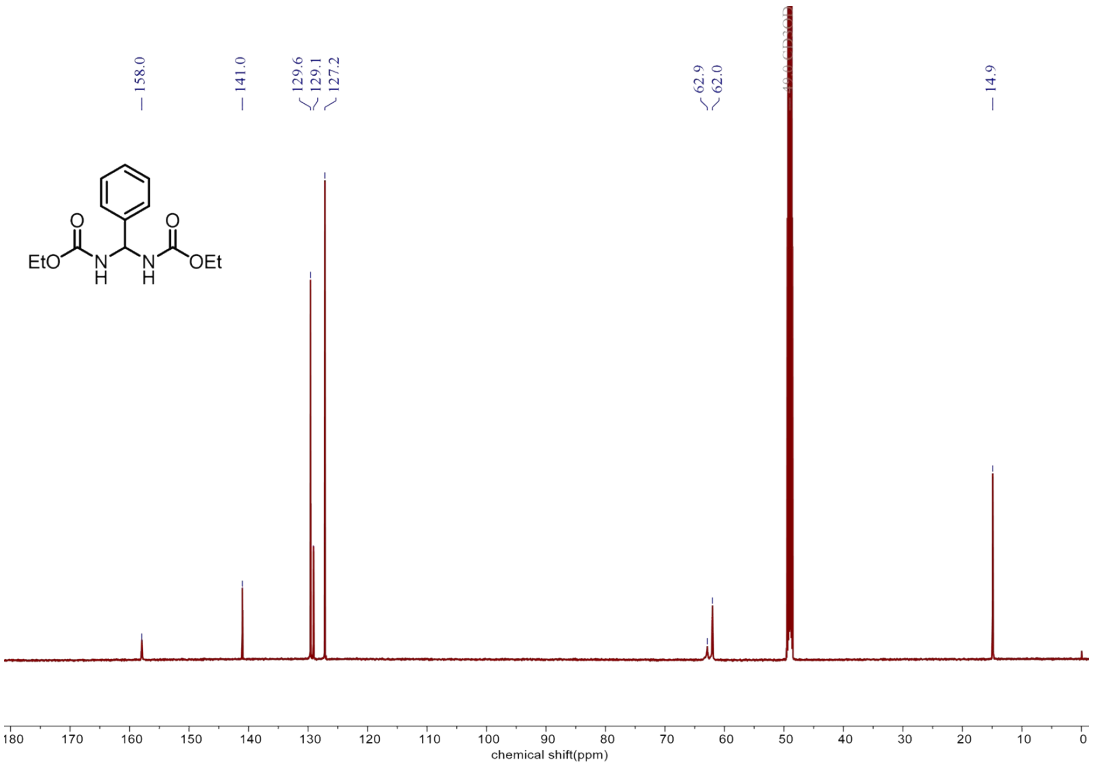
^{1}H NMR (600 MHz, MeOD) δ 7.40 (dt, *J* = 15.3, 7.5 Hz, 4H), 7.32 (t, *J* = 7.2 Hz, 1H), 6.32 (s, 1H), 4.14 (q, *J* = 7.2 Hz, 4H), 1.27 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (151 MHz, MeOD) δ 158.0, 141.0, 129.6, 129.1, 127.2, 62.9, 62.0, 14.9.

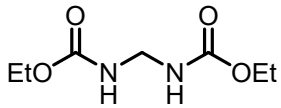
^{1}H NMR (600 MHz, MeOD) 22



^{13}C NMR (151 MHz, MeOD) 22



Diethyl methylenedicarbamate (23)

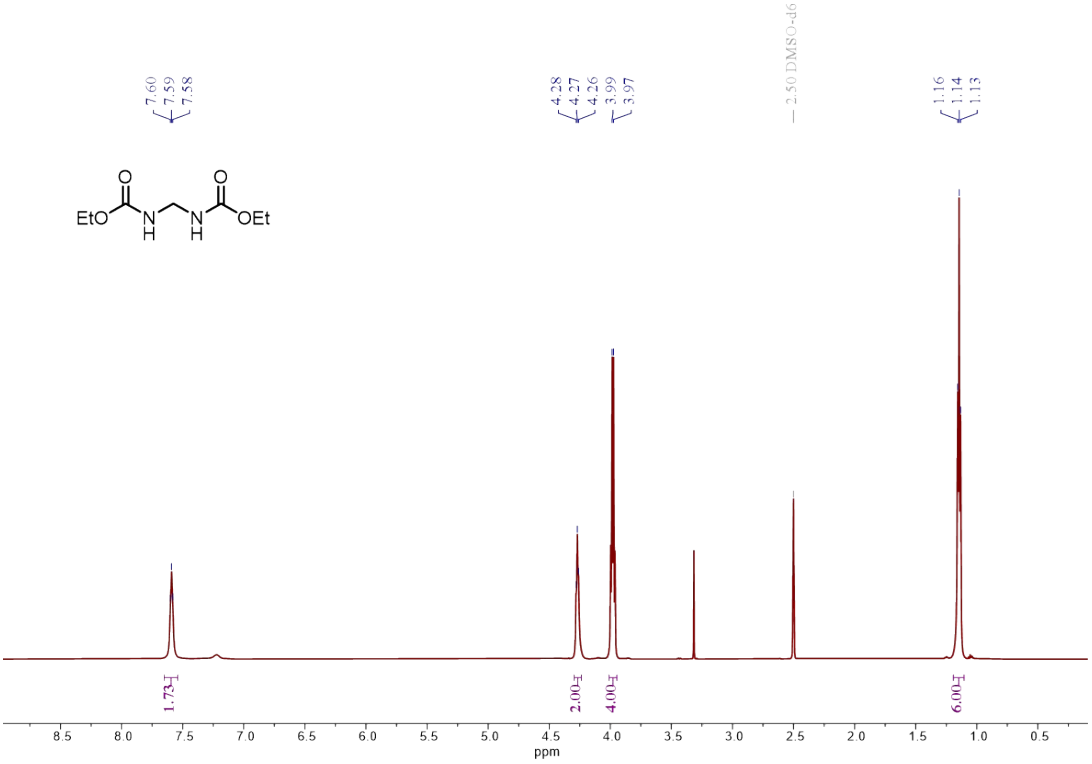


MS (ESI+): Calculated for $C_7H_{14}N_2O_4$ $[M+H]^+$ 191.0954, found 191.0831 (1+),213.0628 (1+).

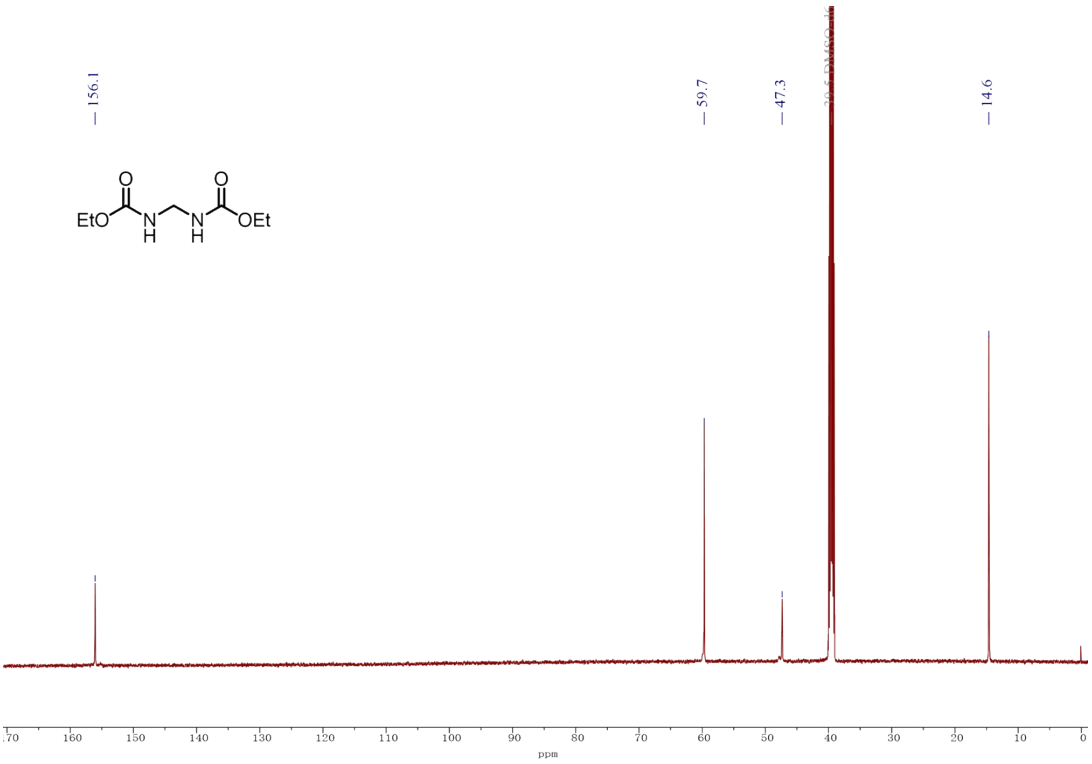
^{1}H NMR (600 MHz, DMSO) δ 7.59 (t, J = 6.0 Hz, 2H), 4.27 (t, J = 6.1 Hz, 2H), 3.98 (d, J = 7.1 Hz, 4H), 1.14 (t, J = 7.1 Hz, 6H).

^{13}C NMR (151 MHz, DMSO) δ 156.1, 59.7, 47.3, 14.6.

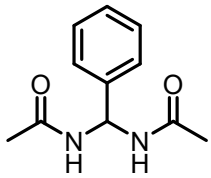
^{1}H NMR (600 MHz, DMSO) 23



^{13}C NMR (151 MHz, DMSO) 23



N,N'-(phenylmethylene)diacetamide (24)

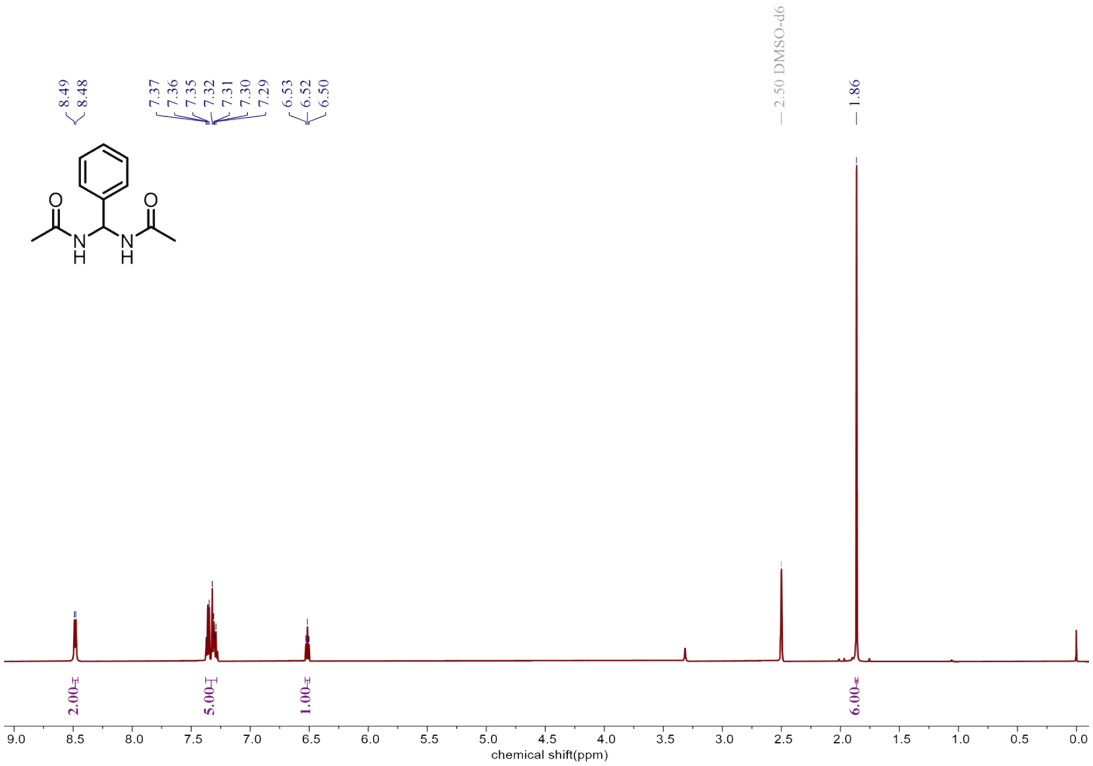


MS (ESI+): Calculated for $C_{11}H_{14}N_2O_2$ $[M+H]^+$ 207.1055, found 207.0748 (1+).

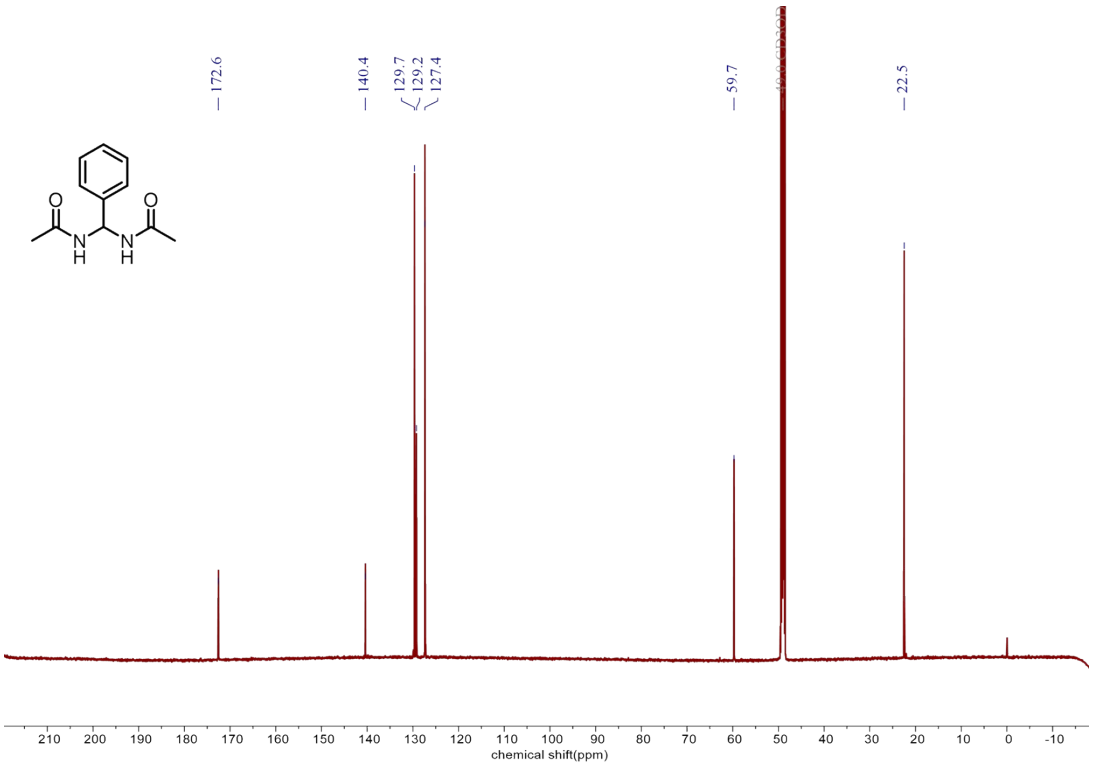
^{1}H NMR (600 MHz, DMSO) δ 8.48 (d, J = 8.0 Hz, 2H), 7.38 – 7.28 (m, 5H), 6.52 (t, J = 7.9 Hz, 1H), 1.86 (s, 6H).

^{13}C NMR (151 MHz, MeOD) δ 172.6, 140.4, 129.7, 129.2, 127.4, 59.7, 22.5.

^{1}H NMR (600 MHz, MeOD) 24



^{13}C NMR (151 MHz, MeOD) 24



5.2 Nature and melting point of the products

Table S12. Nature and melting point of the products.

products	nature	melting point / ℃
5	solid	230-233
6	solid	234-236
7	solid	54-55
8	oil	-
9	solid	185
10	solid	240
11	solid	210
12	solid	225
13	solid	177
14	solid	110
15	solid	138
16	solid	150
17	solid	182-183
18	solid	-
19	solid	-
20	solid	111-112
21	solid	140-143
22	solid	172-173
23	solid	127-130

products	nature	melting point / ℃
24	solid	239-240

6 Kinetic Correlation

To investigate whether the hydrolase mimics truly exhibit higher catalytic activity, a comparison was made by determining the Michaelis-Menten equations for both the enzyme (CAL-B) and the hydrolase mimics (HM5, HM9).

The kinetic experiments were conducted in a pure water system at a temperature of 40 degrees Celsius, with a catalyst amount of 0.4 μmol. Unless otherwise specified, the reaction time was 1 hour.

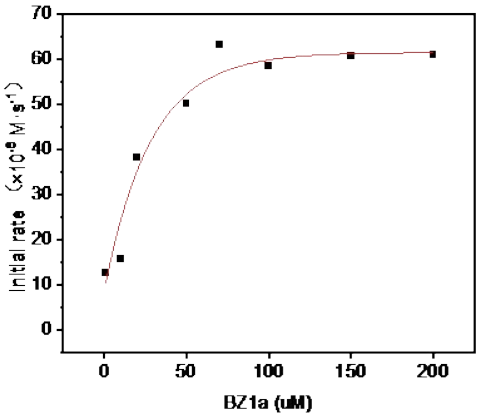


Figure S4. The Michaelis-Menten equation fitting curve for the formation of 5 through dehydration condensation of 3(BZ1a) was catalyzed by CAL-B.

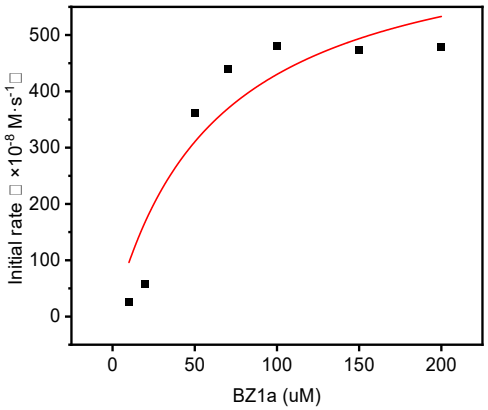


Figure S5. The Michaelis-Menten equation fitting curve for the formation of 5 through dehydration condensation of 3(BZ1a) was catalyzed by HM5.

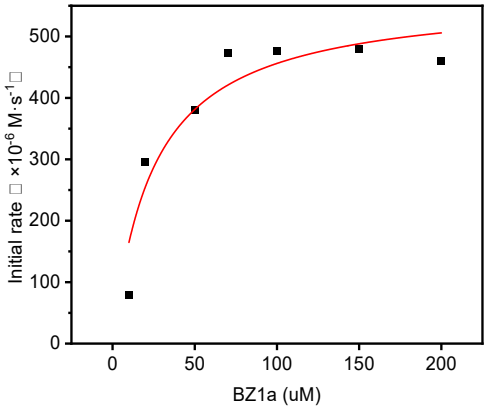


Figure S6. The Michaelis-Menten equation fitting curve for the formation of 5 through dehydration condensation of 3(BZ1a) was catalyzed by HM9.

7 Estimation of the Green Chemistry Metrics

Table S13. Formulas and value for green chemistry metrics.

Metrics	Formula	Value
E-factor	$\frac{\sum Mass\ of\ waste\ (s)}{Mass\ of\ product}$	0.18
RME	$\frac{Mass\ of\ \ product}{\sum Mass\ of\ reactants\ (s)} \times 100\%$	84.2 %
AE	$\frac{Molecular\ weight\ of\ product}{\sum Molecular\ weight\ of\ reactant(s)} \times 100\%$	84.7 %
OE	$\frac{REM}{AE} \times 100\%$	99.4 %

8 Computational Studies

8.1 Electrostatic calculations

The molecular structure of the catalyst was initially constructed using Chem 3D and pre-optimized with its built-in MM2 force field. Subsequently, geometry optimization was carried out at the B3LYP/6-31G(d,p) level of theory with an implicit solvation model (water, SMD) using Gaussian 09. Frequency calculations confirmed the absence of imaginary frequencies, ensuring the structure corresponded to a true minimum on the potential energy surface.

The electrostatic potential (ESP) was then evaluated based on the optimized electron density. The formatted checkpoint file (.fchk) from the Gaussian calculation was processed using Multiwfn (version 3.8) to compute the extremum points of the ESP and to generate the ESP surface map. The resulting data were visualized using VMD (Visual Molecular Dynamics) to illustrate the spatial distribution of electrostatic potential around the catalyst.

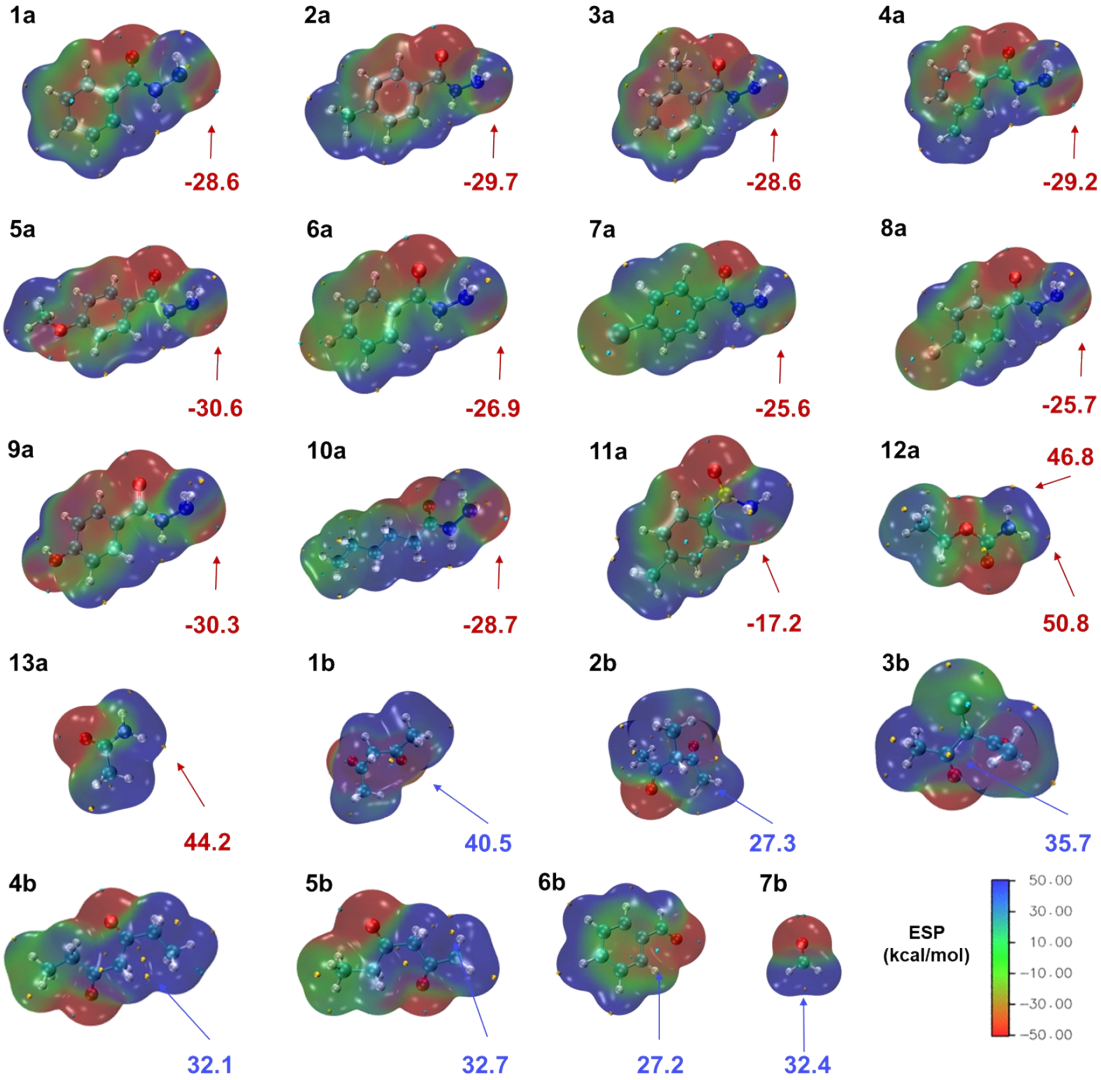


Figure S7. The stable conformation and surface electrostatic potential of the substrate.

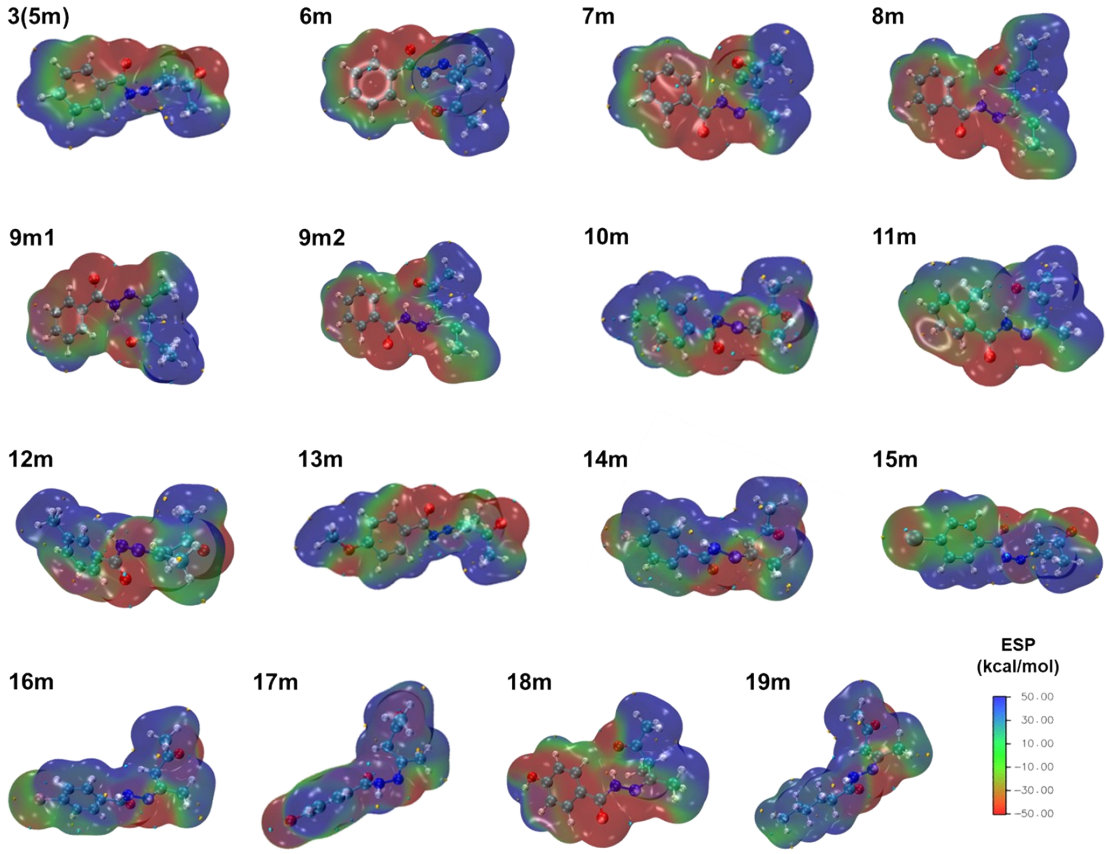


Figure S8. molecular electrostatic potential of substrates.

Table S14. The electrostatic potential energy of important compounds.

Compound	N atom (kcal/mol)	C atom (kcal/mol)
3	-9.0	-1.5
6m	-17.9	NaN
7m	-18.3	NaN
8m	-16.7	NaN
9m-1	-16.7	NaN
9m-2	-16.5	NaN
10m	-10.6	-2.0
11m	-19.8	NaN
12m	-9.1	-1.9
13m	-11.6	-2.2
14m	-7.2	-0.9
15m	-5.8	-0.9
16m	-5.8	-1.0
17m	-11.7	-2.0
18m	-20.1	NaN
19m	-6.2	-1.6

Note: The N atom refers to the specific nitrogen atom that will participate in the subsequent condensation step. The C atom refers to the specific carbonyl carbon atom that will participate in the subsequent condensation step.

8.2 Molecular dynamics simulations

The force field parameters for the catalysts and small molecule ligands used in the simulations were generated based on the GAFF (General Amber Force Field) using AmberTools22 and ACPYPE. Following the docking of the catalyst with the ligand, the complex was placed in a cubic box measuring 8 nm × 8 nm × 8 nm, and counterions were added to maintain overall charge neutrality. All simulations were performed using the Gromacs-2025.2 software package. Periodic boundary conditions were applied throughout to maintain a constant number of particles. After system construction, energy minimization was conducted for 50,000 steps. Subsequently, the minimized system was equilibrated under an NVT ensemble for 100 ps. The cutoff distance for non-bonded interactions was set to 12 Å. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) summation method. The temperature was maintained at 313 K using the V-rescale thermostat with a coupling constant of 0.2 ps. Pressure was maintained at 1 bar using the C-rescale barostat. Hydrogen bonds were constrained using the LINCS algorithm. The integration time step was set to 2 fs. Finally, a production simulation of 100 ns was performed for the entire model system.

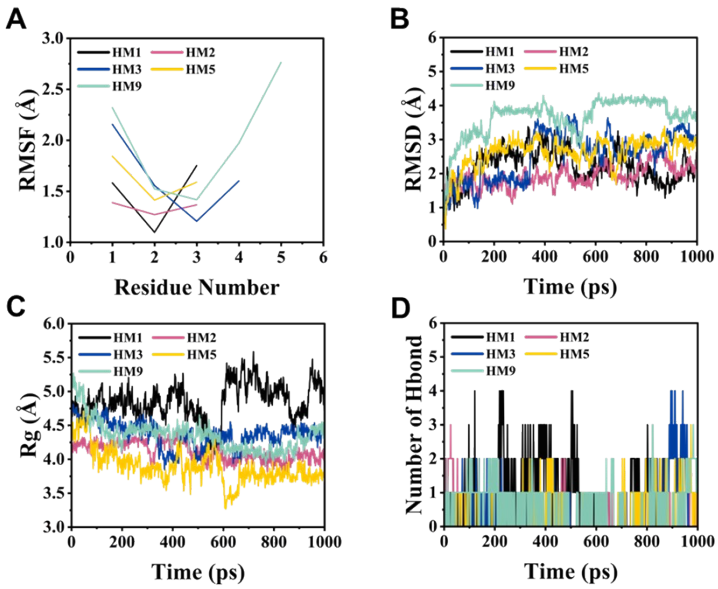


Figure S9. A) RMSF value of each residue in hydrolase mimics. B) RMSD value during the dynamic interaction process. C) Rg value during the dynamic interaction process. D) The number of hydrogen bonds during the dynamic interaction process.

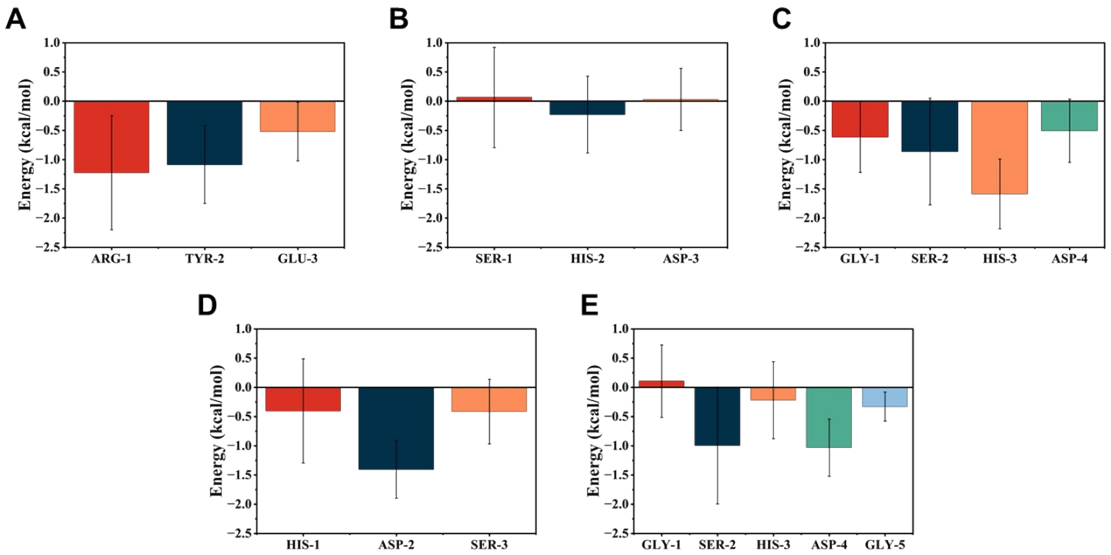


Figure S10. The force field decomposition between different residues of hydrolase mimics. A) HM1. B) HM2. C) HM3. D) HM5. E) HM9.

Table S15. Force field decomposition for HM1.

Energy Component	Average (kcal/mol)	SD (kcal/mol)	SEM (kcal/mol)
VDWAALS	-8.79	2.88	0.20
EEL	-4.12	5.45	0.38
EGB	7.85	5.23	0.37
ESURF	-1.33	0.44	0.03
DELTA G gas	-12.91	7.31	0.52
DELTA G solv	6.51	4.85	0.34
DELTA TOTAL	-6.40	2.78	0.20

Table S16. Force field decomposition for HM2.

Energy Component	Average (kcal/mol)	SD (kcal/mol)	SEM (kcal/mol)
VDWAALS	-2.32	2.07	0.15
EEL	-0.84	2.61	0.18
EGB	2.52	2.59	0.18
ESURF	-0.46	0.40	0.03
DELTA G gas	-3.16	3.40	0.24
DELTA G solv	2.05	2.33	0.16
DELTA TOTAL	-1.10	1.55	0.11

Table S17. Force field decomposition for HM3.

Energy Component	Average (kcal/mol)	SD (kcal/mol)	SEM (kcal/mol)
VDWAALS	-10.47	1.79	0.13
EEL	-14.72	8.20	0.58
EGB	18.54	6.64	0.47
ESURF	-1.53	0.20	0.01
DELTA G gas	-25.19	8.87	0.62
DELTA G solv	17.01	6.49	0.46
DELTA TOTAL	-8.18	2.62	0.18

Table S18. Force field decomposition for HM5.

Energy Component	Average (kcal/mol)	SD (kcal/mol)	SEM (kcal/mol)
VDWAALS	-6.72	1.50	0.11
EEL	-9.53	3.66	0.26
EGB	12.68	3.32	0.23
ESURF	-0.98	0.16	0.01
DELTA G gas	-16.25	4.50	0.32
DELTA G solv	11.70	3.22	0.23
DELTA TOTAL	-4.55	1.78	0.13

Table S19. Force field decomposition for HM9.

Energy Component	Average (kcal/mol)	SD (kcal/mol)	SEM (kcal/mol)
VDWAALS	-10.15	0.87	2.09
EEL	-5.94	5.75	0.41
EGB	11.05	5.33	0.38
ESURF	-1.55	0.16	0.01
DELTA G gas	-16.09	7.19	0.51
DELTA G solv	9.49	5.27	0.37
DELTA TOTAL	-6.60	2.38	0.17

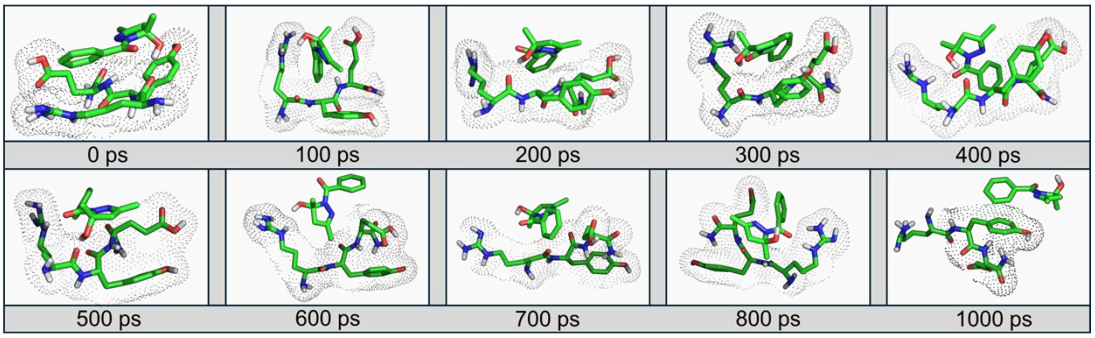


Figure S11. The dynamic process of the interaction between HM1 and the substrate.

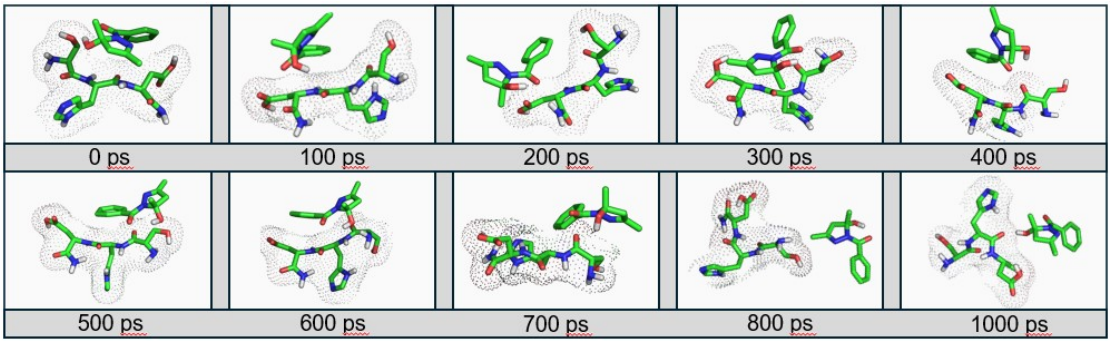


Figure S12. The dynamic process of the interaction between HM2 and the substrate.

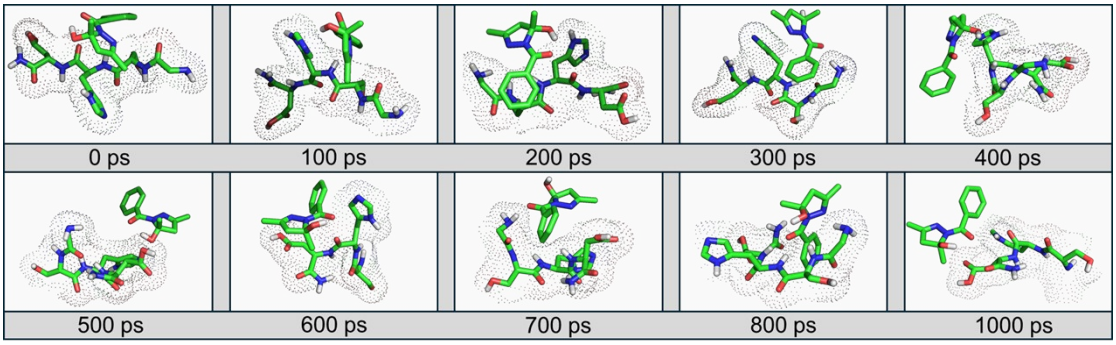


Figure S13. The dynamic process of the interaction between HM3 and the substrate.

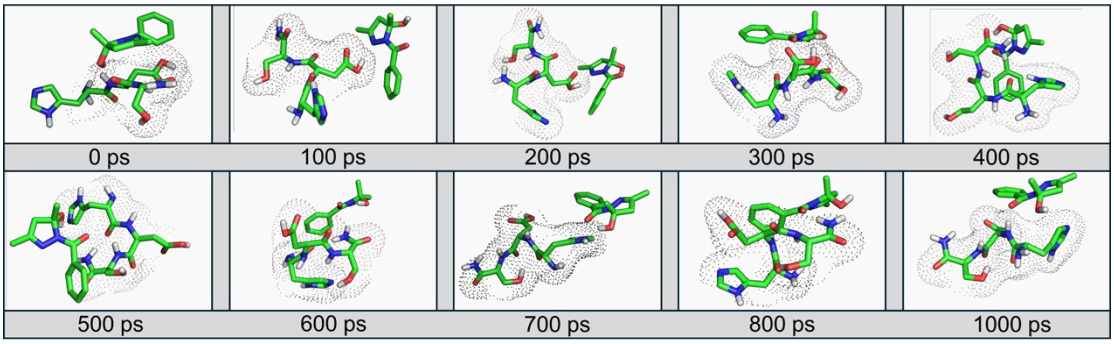


Figure S14. The dynamic process of the interaction between HM5 and the substrate.

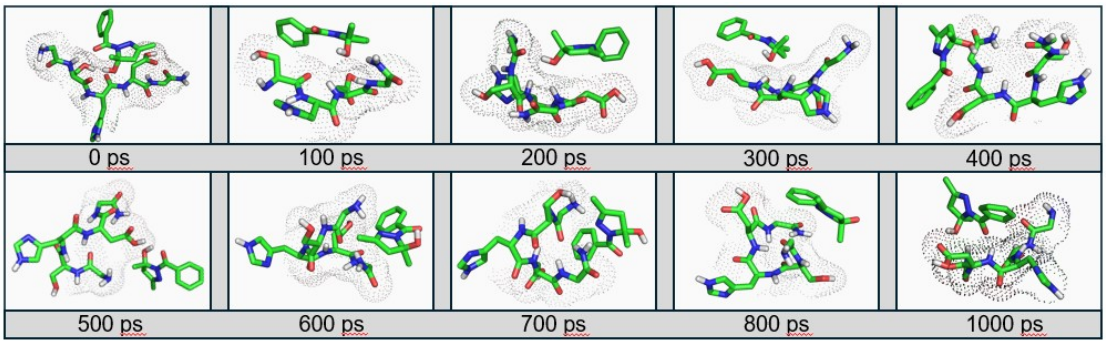


Figure S15. The dynamic process of the interaction between HM9 and the substrate.

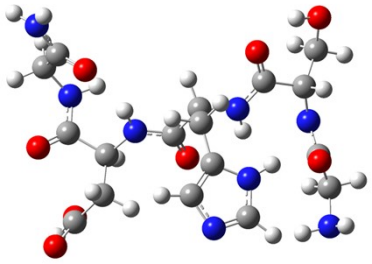
8.3 Transition-State calculations

The B3LYP density functional method was utilized in this study to perform all computational tasks. Geometry optimizations and harmonic frequency calculations were conducted using the 6-31G(d) basis set for all elements. Vibrational frequency analyses were carried out at the same level of theory for all optimized structures to determine whether the stationary points corresponded to local minima or transition states. Additionally, intrinsic reaction coordinate (IRC) calculations were performed to verify that each transition state correctly connected the corresponding reactants and products. Single-point energy calculations were conducted employing the def2-TZVP basis set to achieve more accurate energy corrections. All DFT computations were performed using the Gaussian 09 software package.

Table S20. The final energy.

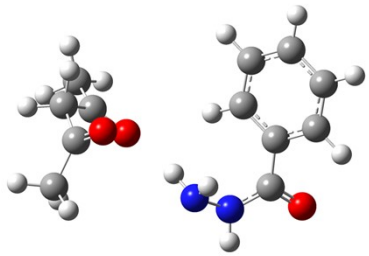
Entry	Substrates	E(kcal/mol)
1	HM9	-1703.641182
2	H_2O	-76.449122
3	1	-802.142308
4	TS1-2	-802.076902
5	TS1-2-w	-878.556802
6	2-w	-878.58334
7	2	-802.063710
8	TS2-3	-801.985112
9	TS2-3-HM	-2505.764823
10	3-HM	-2505.796759
11	3	-725.697819
12	TS3-4	-725.626245
13	TS3-4-w	-802.102087
14	4-w	-802.14707
15	4	-725.693624
16	TS4-5	-725.614772
17	TS4-5-HM	-2429.310992
18	5-HM	-2429.359397
19	5	-649.264509

HM9



H	-4.468076	-3.47173	-2.610752
C	0.45849	0.934222	0.498448
C	0.508072	0.450194	1.979459
N	1.777268	0.924553	-0.10733
C	0.906736	-0.979371	2.168835
H	1.190805	1.12707	2.50469
H	-0.479945	0.592133	2.426725
C	-0.438935	0.034557	-0.362717
H	0.075838	1.959765	0.494787
C	2.683737	1.89235	0.102816
H	1.959923	0.175866	-0.766296
N	2.164314	-1.487865	1.898583
C	0.186349	-2.062137	2.625214
N	-1.766228	0.161445	-0.142326
O	0.0414	-0.76154	-1.173523
C	4.078616	1.705271	-0.512556
O	2.453157	2.900711	0.791754
C	2.138807	-2.816841	2.190421
H	2.968094	-1.000486	1.505557
N	0.957952	-3.20558	2.63854
H	-0.845549	-2.068103	2.95122
C	-2.747684	-0.66533	-0.846375
H	-2.127619	0.863845	0.503428
C	4.488434	2.97619	-1.291184
N	4.225624	0.512327	-1.332227
H	4.755485	1.606487	0.34284
H	3.007111	-3.447339	2.057038
C	-2.895479	-2.048909	-0.196702
C	-4.08317	0.098119	-0.836722
H	-2.403804	-0.786777	-1.878576
O	4.600302	4.093814	-0.434221
H	3.766559	3.153405	-2.105445
H	5.468248	2.802583	-1.746847
C	4.577412	-0.688581	-0.798021
H	4.22043	0.613265	-2.339682
H	-3.217722	-1.957111	0.841278
N	-4.012126	1.378604	-1.310147
O	-5.116978	-0.407922	-0.412566
H	3.805465	4.063221	0.133206
C	4.952261	-1.790857	-1.795861
O	4.611982	-0.878192	0.423352
C	-4.913505	2.375389	-0.775956
H	-3.081733	1.715202	-1.524577
N	4.968586	-3.135457	-1.240937
H	4.271902	-1.753811	-2.653727
H	5.949723	-1.544992	-2.183546
C	-4.452537	2.8166	0.629986
H	-5.916205	1.947172	-0.707703
H	-4.959151	3.238201	-1.445417
H	4.026847	-3.378759	-0.938938
H	5.521097	-3.119439	-0.385339
N	-5.167894	3.815851	1.178174
O	-3.498447	2.279397	1.201335
H	-4.933196	4.142213	2.104446
H	-1.909677	-2.527345	-0.202484
C	-3.856659	-2.983987	-0.895398
O	-3.847973	-2.821638	-2.236641
O	-4.527154	-3.83099	-0.342055
H	-5.953295	4.23793	0.706189

1



C	2.549362	-0.160526	-0.132069
C	1.602044	0.835388	-0.377603
C	1.834181	2.138549	0.048003
C	3.000137	2.45239	0.738838
C	3.946159	1.462186	0.987985
C	3.728168	0.164986	0.542599
H	0.69518	0.605591	-0.920684
H	1.102403	2.90864	-0.159994
H	3.173495	3.465861	1.077753
H	4.856083	1.702163	1.522997
H	4.468903	-0.604328	0.716958
C	2.395888	-1.5585	-0.649397
O	3.319722	-2.119765	-1.238012
N	1.212881	-2.203378	-0.465233
H	1.202998	-3.162092	-0.783492
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C	-3.273135	-0.191734	1.254507
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H	-4.995341	-1.444742	1.296114
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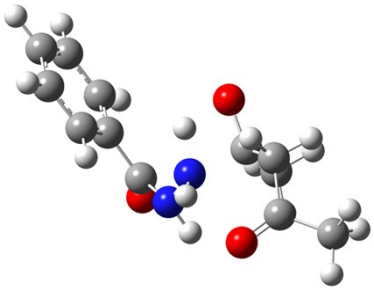
TS1-2



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C	-3.245172	0.715114	0.583167
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H	-3.379269	-2.720712	-1.211489
H	-5.131653	-2.110131	0.440333
H	-5.047559	0.097403	1.579827
H	-3.206594	1.687966	1.061499
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N	0.681489	-0.185278	-0.446860
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C	1.501579	-0.062106	0.939042
C	1.843903	1.385501	1.276690
H	2.519050	1.850980	0.549845
H	0.933672	1.983323	1.358635
H	2.338710	1.394652	2.252070
O	0.536604	-0.636536	1.696323
H	0.061046	-0.760716	0.396091
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H	3.290445	-0.963168	1.725105
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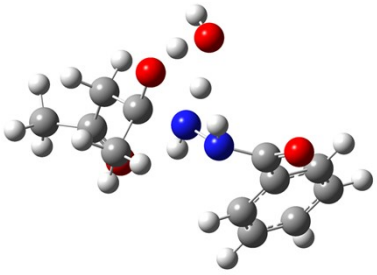
TS1-2-w



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C	3.839718	2.018189	0.286843
C	4.165545	0.80494	0.9003
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H	2.518267	3.024071	-1.089843
H	4.430657	2.905544	0.491809
H	5.00774	0.748578	1.582711
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C	1.565221	-1.527026	-0.517426
O	2.123485	-2.612324	-0.63466
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H	-3.233149	3.776424	0.325401
O	-1.022096	-1.88317	2.102272
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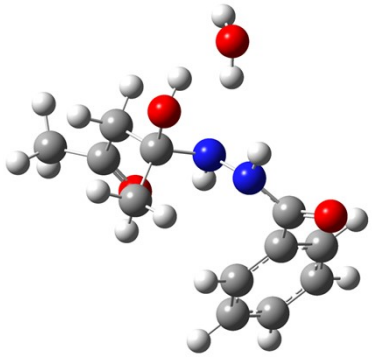
2-w



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C	3.919752	0.760345	1.099165
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H	2.655392	2.677984	-1.395751
H	4.327622	2.761462	0.428792
H	4.653246	0.79078	1.89461
H	3.32435	-1.260139	1.52066
C	1.47958	-1.719804	-0.353561
O	2.095986	-2.773184	-0.522091
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H	-0.301013	-2.624523	-0.461111
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H	-3.858512	2.852829	1.227042
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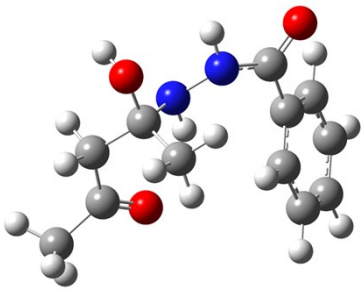
2



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H	-3.638031	-0.815197	-1.445746
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H	1.177328	-1.509384	2.065973
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O	2.595629	-2.255991	-0.012648
C	2.97583	0.089993	-0.467626
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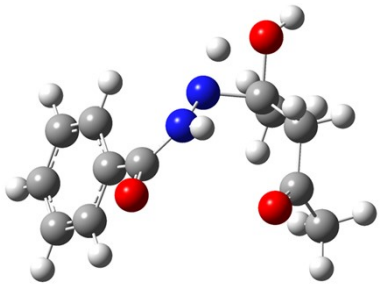
C	3.635768	2.581463	-0.478723
H	4.249652	2.540774	0.428824
H	4.301345	2.365921	-1.321084
H	3.206151	3.578449	-0.582892
H	2.695359	-2.399955	-0.966784

TS2-3



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C	2.687490	-1.243154	0.312431
H	1.739754	1.754155	-0.984125
H	3.776697	2.471159	0.225090
H	5.096768	0.822844	1.534726
H	4.395070	-1.564296	1.581313
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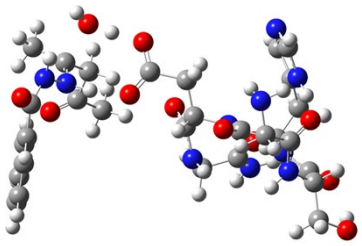
TS2-3-HM



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H	5.602028	3.967899	-0.882252
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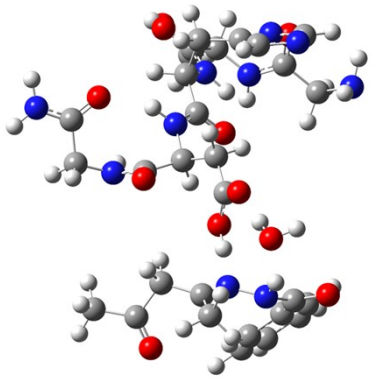
3-HM



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H	5.024088	-2.254675	4.083837
H	3.30074	-4.003311	4.460723
H	2.405637	-5.295744	2.532986
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O	5.021249	-4.015457	-1.358258
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C	6.015506	0.31459	-0.448479
C	7.36038	0.240228	-1.108131
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H	5.797227	2.451331	-0.555415
H	4.481943	1.647987	0.283696
C	6.234095	1.917157	1.482165
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H	6.527139	4.037493	1.425153
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C	0.20374	1.232964	-0.168855
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C	-7.141687	-0.080085	2.937112
N	-5.953816	-1.782268	1.586994
H	-7.457415	-0.555328	0.86425
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C	0.661599	0.525832	-1.451818
C	1.015817	2.515303	0.084427
H	0.329998	0.552502	0.679187
O	-7.985448	1.05027	2.860318
H	-6.293184	0.085385	3.621636
H	-7.730722	-0.913104	3.333352
C	-5.971754	-2.630953	0.523907
H	-5.613672	-2.125356	2.476789

H	0.50659	1.163192	-2.323689
N	0.591548	3.258942	1.150298
O	1.955406	2.844109	-0.633939
H	-7.52103	1.679863	2.274982
C	-5.476174	-4.057413	0.788538
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C	0.755086	4.695545	1.115509
H	-0.259191	2.938647	1.595753
N	-5.154364	-4.822499	-0.406217
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H	-6.268552	-4.572211	1.347819
C	-0.313204	5.33247	0.200523
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H	-5.938276	-4.747011	-1.052292
N	-0.325409	6.677814	0.185815
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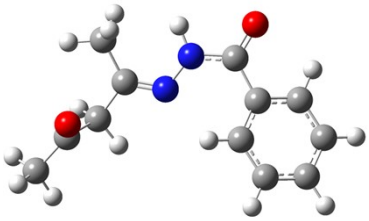
H	-0.986176	7.160101	-0.40613
H	0.043268	-0.369815	-1.57914
C	2.111203	0.087932	-1.448152
O	2.552728	-0.250034	-0.242559
O	2.772923	0.017052	-2.47739
H	0.321554	7.228594	0.729526
H	4.397964	-0.545332	-3.196030

3



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C	1.569484	1.538165	0.188194
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H	-0.173445	2.544044	0.220434
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C	-1.865388	0.801904	-0.414903
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H	-2.343473	2.401494	0.958085
H	-2.184257	2.917272	-0.729356
C	-2.704633	-0.342254	-0.917797
H	-3.293548	-0.036213	-1.793600
H	-2.055606	-1.159782	-1.251748
C	-3.665880	-0.902131	0.131998
O	-3.644812	-0.515524	1.289158
C	-4.634874	-1.959285	-0.347716
H	-5.339834	-1.515619	-1.060239
H	-4.106625	-2.758196	-0.878338
H	-5.186052	-2.372972	0.497671