Electronic Supplementary Information (ESI)

Chitosan nanofiber-catalyzed highly selective Knoevenagel condensation in aqueous methanol

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1. General information

Transmission electron microscopy (TEM) observation of CsNFs and chitosan powders was performed using a JEM-2100HCKM microscope (JEOL, Tokyo, Japan) at the Ultramicroscopy Research Center Kyushu University. X-Ray diffraction (XRD) patterns were recorded using a SmartLab (Rigaku, Tokyo, Japan) instrument at the Center of Advanced Instrumental Analysis, Kyushu University. Infrared spectra were recorded on an FT/IR-620 spectrometer (JASCO, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. ^{1}H NMR spectra were recorded on an ECZ-400 spectrometer (400 MHz; JEOL, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. Chemical shifts are reported in ppm, with tetramethylsilane (TMS) as an internal standard (TMS; 0.00 ppm). Data are reported as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration. ^{13}C NMR spectra were recorded on the ECZ-400 spectrometer (100.5 Hz) with complete proton decoupling. Chemical shifts are reported in ppm relative to the solvent resonance as internal standard ($CDCl_3$; 77.0 ppm). Electrospray ionization mass spectroscopy (ESI-MS) analysis was performed on a JMS-T100CS spectrometer (JEOL, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University.

2. Characterization of chitosan samples

2.1 Transmission electron microscopy (TEM) analysis

Samples were mounted on a glow-discharged carbon-coated Cu grid, negatively stained with sodium phosphotungstate (1 wt%), washed with deionized water, and air-dried. The TEM apparatus was operated at an accelerating voltage of 200 kV in bright-field mode.

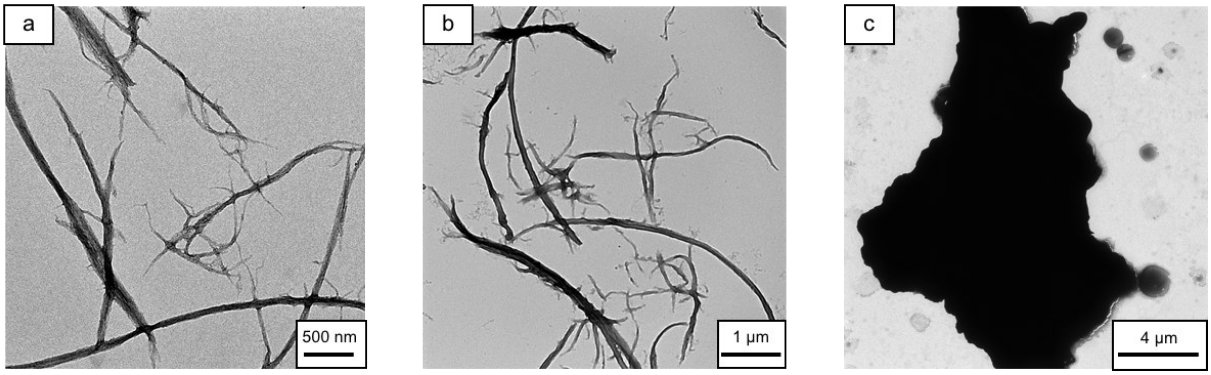


Figure S1. TEM images of (a) chitosan nanofibers (CsNFs), (b) CsNFs after the fifth run of recycling test, and (c) chitosan powder.

2.2 X-Ray diffraction (XRD) analysis

Freeze-dried samples were pressed at 750 MPa for approx. 1 min to make disk pellets for measurement. The XRD apparatus was operated at 40 kV and 20 mA with Ni-filtered Cu Kα radiation (λ = 0.15418 nm) in reflection mode. Scattered radiation was detected from 5° to 40° at a scan rate of 2° min^{-1} with 0.01° intervals.

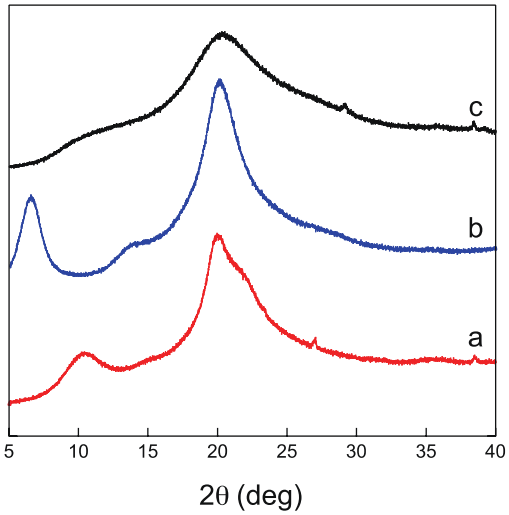


Figure S2. XRD patterns of (a) CsNFs, (b) CsNFs after the fifth run of recycling test, and (c) chitosan powder.

2.3 Fourier transform infrared (FTIR) spectroscopy

Each freeze-dried sample (approx. 2 mg) was mixed and ground with 200 mg of KBr. The resulting mixtures were pressed into transparent pellets and analyzed in the spectral range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} for each sample.

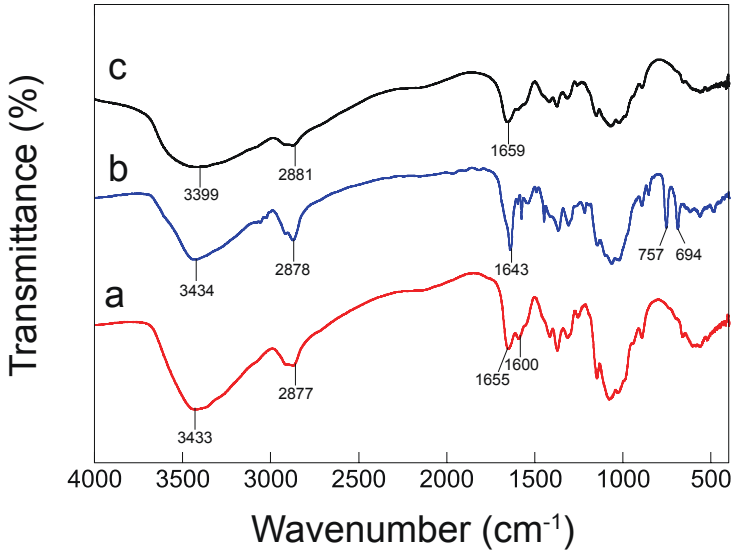


Figure S3. FTIR spectra of (a) CsNFs, (b) CsNFs after the fifth run of recycling test, and (c) chitosan powder.

Each spectrum exhibited a broad band around 3400 cm^{-1}, which corresponds to O−H stretching band overlapping N−H stretching one. The absorption bands around 2880 cm^{-1} are assigned to C−H stretching vibration. The amide I peaks of chitosan samples were found around 1650 cm^{-1}. For CsNFs after the recycling test, two characteristic peaks at 757 cm^{-1} and 694 cm^{-1} were detected, possibly originating from C−H bending of monosubstituted aromatic compound, which implies the imine (C=N, overlapping around 1650 cm^{-1} region) formation between benzaldehyde and CsNFs.

2.4 Quantification of amino group content

The total amino group contents of chitosan samples and other catalysts were determined by electrical conductivity titration.[1] A dried chitosan sample (approx. 50 mg) was suspended in pure water (100 mL), followed by the addition of 1.0 M aq. HCl to obtain a pH value in the range of 2.5–3.0. A 0.1 M aq. NaOH solution was added at a rate of 0.2 mL min^{-1} using a syringe driver to obtain a pH value of 11. The total amino group contents of the samples were determined from the conductivity and pH curves.

Table S1. Amino group contents determined by electrical conductivity titration.

Sample name	NH_2 content (mmol g^{-1})
CsNF	4.35
Chitosan powder	6.82
Polyallylamine	11.2
3-Aminopropyl silica	0.36

The accessible amino group content of each chitosan sample was determined by reacting the chitosan catalysts with salicylaldehyde.[2] Excess salicylaldehyde was reacted with the accessible amino groups of the chitosan samples to irreversibly form stable imines, with the remaining unreacted salicylaldehyde quantified to determine the amount of reacted amino groups. A dried chitosan sample (approx. 80 mg) was added to a methanol solution (6.0 mL) containing salicylaldehyde (0.17 mol L^{-1}), and the resultant mixture was stirred at room temperature for 1 h. The concentration of salicylaldehyde remaining in the supernatant was analyzed by supercritical fluid chromatography (ACQUITY UPC^2 system; Nihon Waters, Tokyo, Japan) equipped with a Chiralpak OD-3 column (DAICEL, Osaka, Japan) using nitrobenzene as an internal standard.

[1] T. Saito and A. Isogai, *Biomacromolecules*, 2004, **5**, 1983–1989.

[2] K. R. Reddy, K. Rajgopal, C. U. Maheswari and M. Lakshmi Kantam, *New J. Chem.*, 2006, **30**, 1549–1552.

3. Preparation of chitosan hydrogel beads[3]

Chitosan powder (0.64 g, FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in 0.1 M HCl (40 mL) at room temperature for 1 h. The as-prepared viscous solution was added dropwise to 0.1 M NaOH aqueous solution (600 mL) at room temperature using a dropping funnel, resulting in immediate coagulation of the droplets into beads. The distance between the dropping funnel tip and solution surface was set at 10 mm to ensure almost uniform beads with spherical geometry. The obtained beads were matured in this solution for 1 h without stirring before being collected using a Buchner funnel without filter paper and washed with ample deionized water until the pH value of the filtrate reached approx. 7.0. The hydrogel chitosan beads were gently placed on filter paper to remove the excess water before use in catalytic experiments.

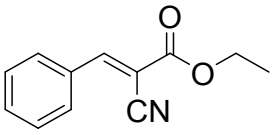
[3] D. Kühbeck, G. Saidulu, K. R. Reddy and D. D. Díaz, *Green Chem.*, 2012, **14**, 378–392.

4. Characterization of Knoevenagel products

Cyanoacetates **2a**, **2b**, and **2d** were commercially available. Cyanoacetates **2c**, **2e**, **2f**, and **2g** were prepared in accordance with a literature method[4] and purified by silica gel column chromatography. Knoevenagel products **3a**–**3g** were fully characterized by ^{1}H and ^{13}C NMR, FT-IR, and ESI-MS analyses.

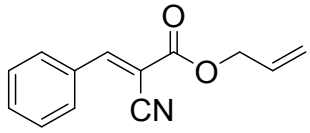
[4] G. B. Dharma Rao, B. Anjaneyulu and M. P. Kaushik, *RSC Adv*., 2014, **4**, 43321–43325.

Ethyl (*E*)-2-cyano-3-phenylacrylate (3a)



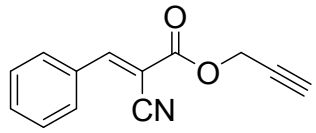
White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.01-7.98 (m, 2H), 7.57-7.49 (m, 3H), 4.39 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 161.9, 154.5, 132.9, 131.0, 130.6, 128.9, 115.1, 102.6, 62.3, 13.7; IR (KBr) 3431, 3069, 3031, 2982, 2901, 2457, 2346, 2224, 1977, 1952, 1883, 1731, 1607, 1574, 1497, 1465, 1445, 1387, 1367, 1302, 1256, 1201, 1090, 1011, 973, 920, 888, 850, 771, 686, 617, 588, 580, 526, 485, 457, 403; ESI-MS Calculated for $C_{12}H_{11}NO_2$ $[M+Na]^+$ 224.0682, Found 224.0753.

Allyl (*E*)-2-cyano-3-phenylacrylate (3b)



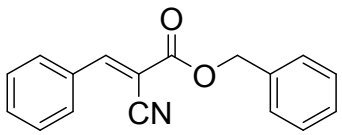
Pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.02-7.99 (m, 2H), 7.59-7.49 (m, 3H), 6.06-5.96 (m, 1H), 5.45 (dq, J = 17.3, 1.4 Hz, 1H), 5.34 (dq, J = 10.5, 1.4 Hz, 1H), 4.82 (td, J = 3.4, 1.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 161.9, 155.1, 133.3, 131.2, 131.0, 130.9, 129.1, 119.0, 115.2, 102.5, 66.8; IR (KBr) 2224, 1733, 1648, 1607, 1575, 1542, 1508, 1497, 1450, 1362, 1321, 1263, 1203, 1189, 1091, 1000, 943, 769, 687, 580, 480, 419; ESI-MS Calculated for $C_{13}H_{11}NO_2$ $[M+Na]^+$ 236.0682, Found 236.0757.

Prop-2-yn-1-yl (*E*)-2-cyano-3-phenylacrylate (3c)



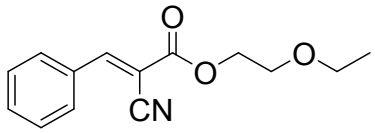
White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.98-8.02 (m, 2H), 7.50-7.61 (m, 3H), 4.92 (d, J = 2.3 Hz, 2H), 2.56 (t, J = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$); 161.8, 155.9, 133.6, 131.2, 129.3, 115.0, 101.9, 76.5, 75.9, 53.7; IR (KBr) 3855, 3735, 3676, 3649, 3282, 3059, 3028, 2360, 2342, 2226, 2127, 1731, 1609, 1574, 1541, 1495, 1448, 1378, 1322, 1278, 1259, 1205, 1189, 1092, 1016, 979, 931, 831, 786, 768, 686, 668, 583, 547, 520, 480, 419; ESI-MS Calculated for $C_{13}H_9NO_2$ $[M+Na]^+$ 234,0525, Found 234.0562.

Benzyl (*E*)-2-cyano-3-phenylacrylate (3d)



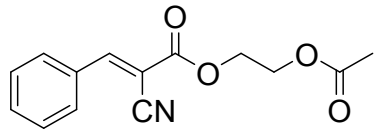
White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.01-7.98 (m, 2H), 7.59-7.33 (m, 8H), 5.36 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 162.2, 155.3, 134.8, 133.3, 131.3, 131.0, 129.2, 128.6, 128.5, 128.1, 115.2, 102.6, 68.0; IR (KBr) 3088, 3067, 3032, 2958, 2936, 2885, 2346, 2222, 1964, 1892, 1845, 1820, 1731, 1609, 1574, 1498, 1450, 1375, 1324, 1278, 1235, 1209, 1164, 1107, 1080, 1028, 1001, 989, 925, 911, 889, 845, 768, 739, 697, 686, 618, 584, 529, 481, 456; ESI-MS Calculated for $C_{17}H_{13}NO_2$ $[M+Na]^+$ 286.0838, Found 286.0773.

2-Ethoxyethyl (*E*)-2-cyano-3-phenylacrylate (3e)



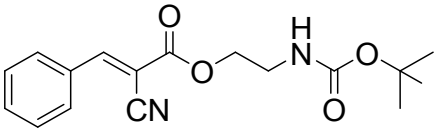
White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.02-7.99 (m, 2H), 7.59-7.49 (m, 3H), 4.48-4.46 (m, 2H), 3.77-3.75 (m, 2H), 3.59 (q, J = 7.0 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 162.4, 155.1, 133.2, 131.3, 131.0, 129.2, 115.2, 102.7, 67.8, 66.7, 66.7, 15.0; IR (KBr) 3735, 3448, 3064, 2971, 2929, 2874, 2820, 2346, 2223, 1975, 1825, 1733, 1607, 1576, 1542, 1494, 1448, 1422, 1374, 1356, 1323, 1288, 1261, 1203, 1190, 1117, 1091, 1053, 1016, 1001, 981, 956, 935, 900, 850, 825, 789, 769, 756, 743, 688, 590, 581, 539, 514, 484, 443, 418, 404; ESI-MS Calculated for $C_{14}H_{15}NO_3$ $[M+Na]^+$ 268.0944, Found 268.0923.

2-Acetoxyethyl (*E*)-2-cyano-3-phenylacrylate (3f)



White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.02-8.00 (m, 2H), 7.60-7.50 (m, 3H), 4.54-4.52 (m, 2H), 4.41-4.39 (m, 2H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.7, 162.2, 155.5, 133.4, 131.2, 131.1, 129.2, 115.1, 102.3, 64.0, 61.6, 20.7; IR (KBr) 3455, 3064, 3031, 2962, 2882, 2224, 2057, 1998, 1977, 1889, 1732, 1608, 1574, 1545, 1497, 1449, 1395, 1377, 1361, 1326, 1271, 1231, 1205, 1156, 1126, 1098, 1057, 1026, 1002, 964, 941, 897, 836, 820, 784, 769, 756, 741, 689, 635, 616, 601, 583, 524, 510, 479, 427; ESI-MS Calculated for $C_{14}H_{13}NO_4$ $[M+Na]^+$ 282.0737, Found 282.0772.

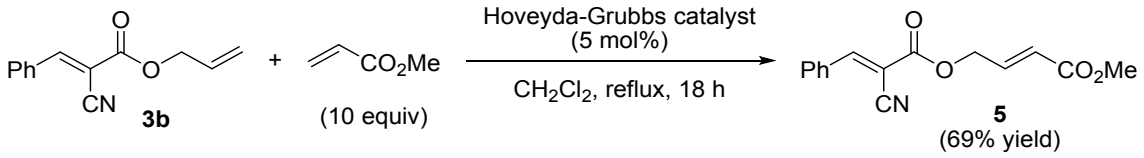
2-((Tert-butoxycarbonyl) amino) ethyl (*E*)-2-cyano-3-phenylacrylate (3g)



White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.01-7.99 (m, 2H), 7.60-7.50 (m, 3H), 4.88 (s, 1H), 4.38 (t, J = 5.0 Hz, 2H), 3.53 (d, J = 5.0 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 162.3, 155.7, 155.5, 133.4, 131.2, 131.0, 129.2, 115.2, 102.3, 79.6, 65.9, 39.3, 28.2; IR (KBr) 3408, 3091, 3040, 3001, 2979, 2893, 2226, 1958, 1909, 1885, 1715, 1611, 1574, 1519, 1451, 1423, 1391, 1368, 1318, 1249, 1206, 1173, 1093, 1074, 1042, 1001, 957, 914, 899, 862, 830, 768, 749, 714, 683, 582, 529, 478, 432, 418; ESI-MS Calculated for $C_{17}H_{20}N_2O_4$ $[M+Na]^+$ 339.1315, Found 339.1261.

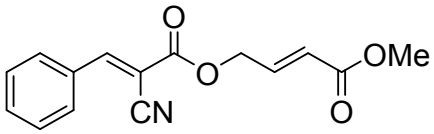
5. Derivatization of Knoevenagel product 2b[5]

The derivatization of Knoevenagel product **3b** was performed by alkene metathesis using the Hoveyda–Grubbs catalyst. Briefly, **3b** (0.34 mmol, 73 mg) and Hoveyda–Grubbs catalyst (0.017 mmol, 10.7 mg, 5 mol%) were weighed in a dried two-necked flask equipped with a condenser. After the flask was degassed and purged with nitrogen, dichloromethane (5 mL) and methyl acrylate (3.4 mmol, 305.9 μL) were added sequentially. The resulting mixture was refluxed for 18 h under a nitrogen atmosphere. The reaction was quenched by adding aq. NH_4Cl, and then extracted three times with ethyl acetate. The combined organic layer was dried over Na_2SO_4, concentrated, and the residue was purified by column chromatography to afford product **5** in 69% yield. Direct alkene metathesis of Knoevenagel product **3b** synthesized under green conditions was possible, although this reaction uses a halogenated solvent.



Scheme S1. Derivatization of Knoevenagel product **3b** by alkene metathesis.

[5] Y. Ge, W. Sun, B. Pei, J. Ding, Y. Jiang and T. P. Loh, *Org. Lett*., 2018, **20**, 2774–2777.



Methyl (*E*)-4-(((*E*)-2-cyano-3-phenylacryloyl)oxy)but-2-enoate (5)

White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.01-8.03 (m, 2H), 7.51-7.61 (m, 3H), 7.02 (dt, *J* = 15.7, 4.7 Hz, 1H), 6.17 (dt, *J* = 16.0, 1.8 Hz, 1H), 4.99 (q, *J* = 2.3 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 166.0, 161.9, 156.0, 139.9, 133.7, 131.2, 129.3, 128.6, 122.6, 115.1, 102.0, 64.3, 51.8; IR (KBr) 3035, 2953, 2361, 2224, 1719, 1670, 1598, 1574, 1500, 1455, 1435, 1378, 1317, 1267, 1209, 1111, 1060, 1022, 977, 919, 885, 840, 772, 686, 593, 517, 433, 418; ESI-MS Calculated for $C_{15}H_{13}NO_4$ $[M+Na]^+$ 294.0737, Found 294.0742.

6. Purity assay of Knoevenagel product 3a

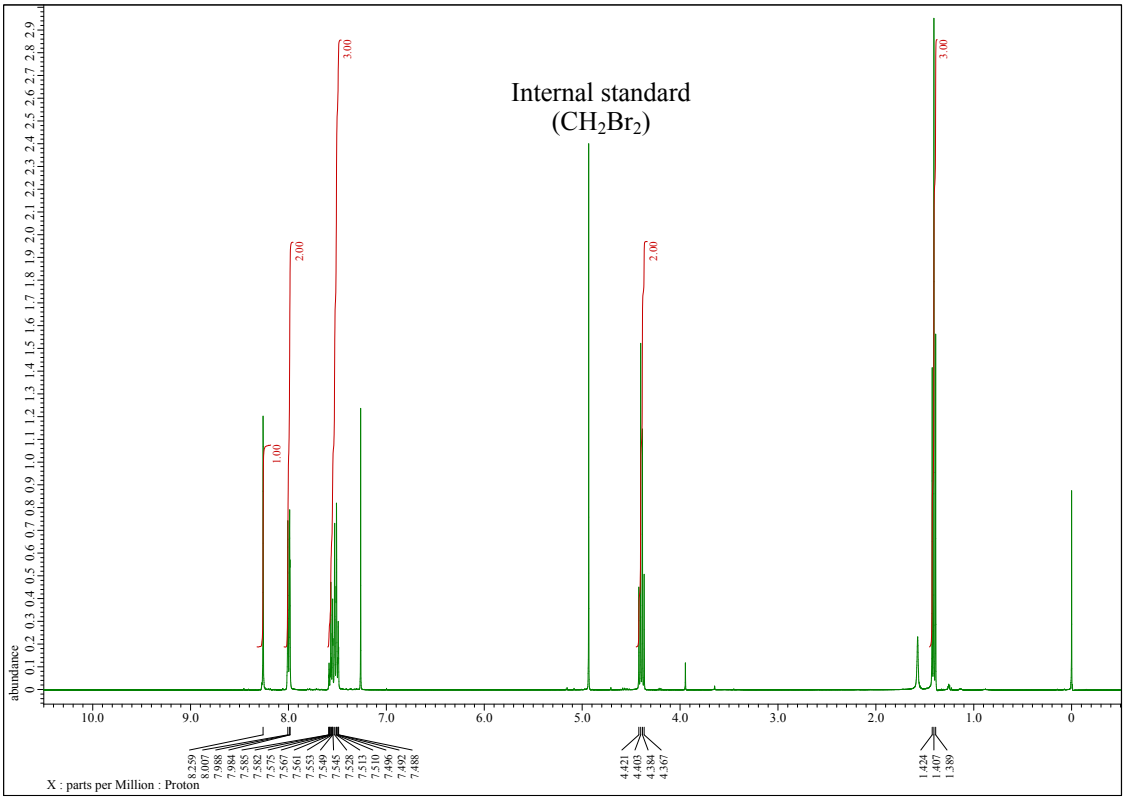


Figure S4. ^{1}H NMR spectrum of crude reaction product (Table 1, entry 1).

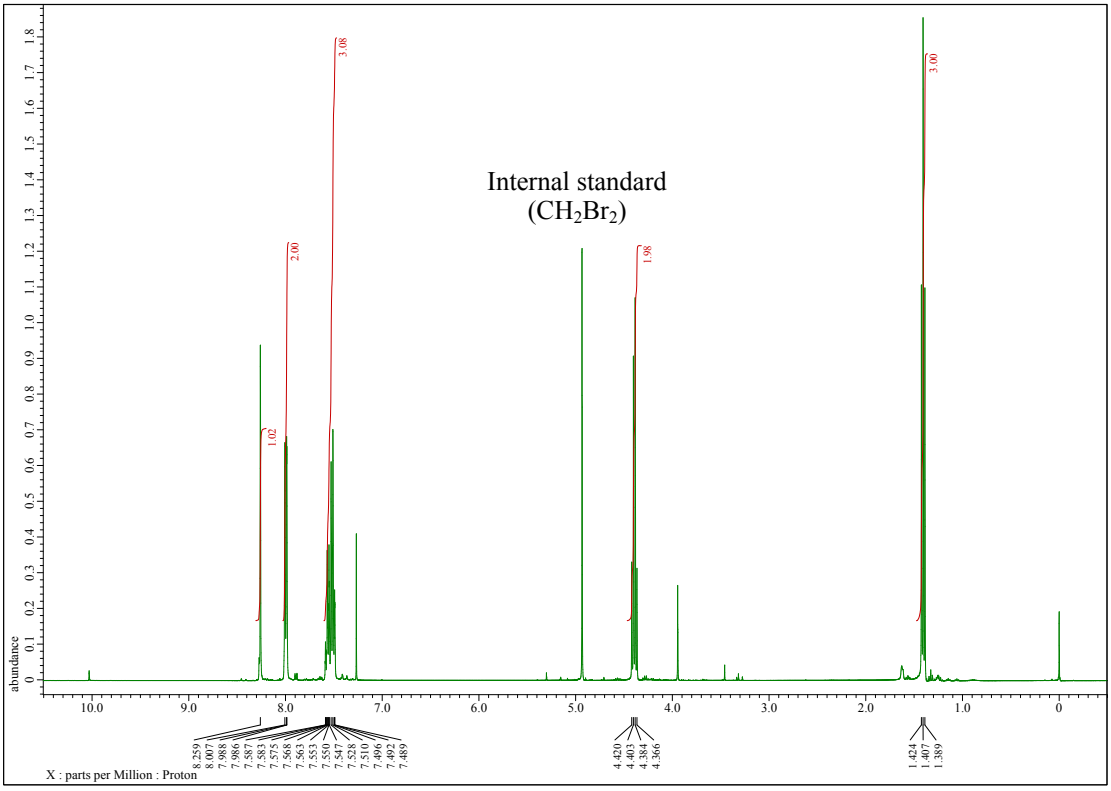
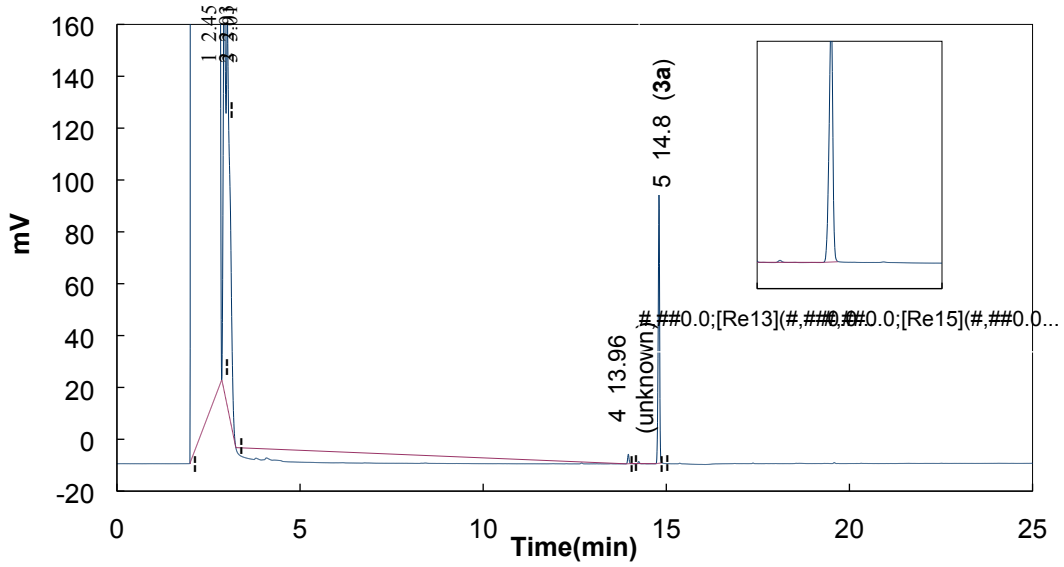


Figure S5. ^{1}H NMR spectrum of crude reaction product of a gram-scale reaction (Scheme 2).

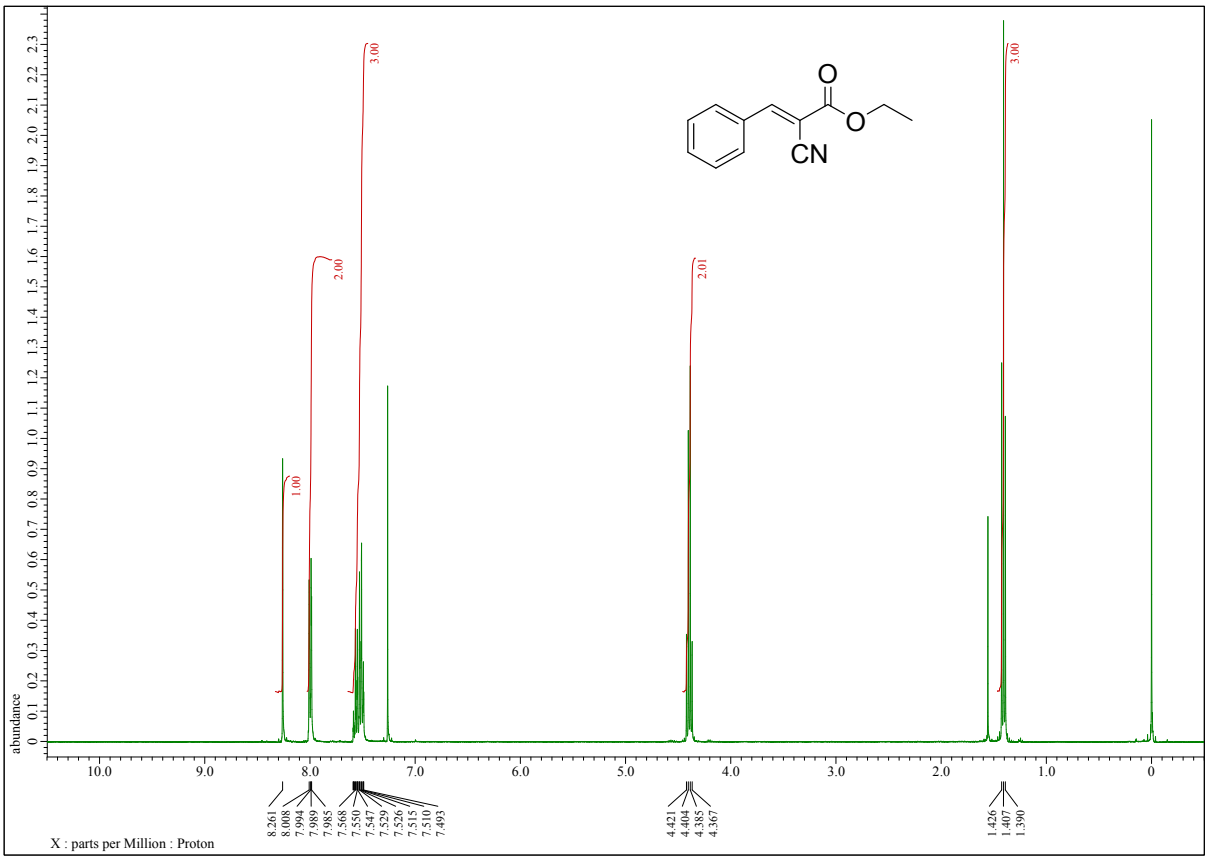


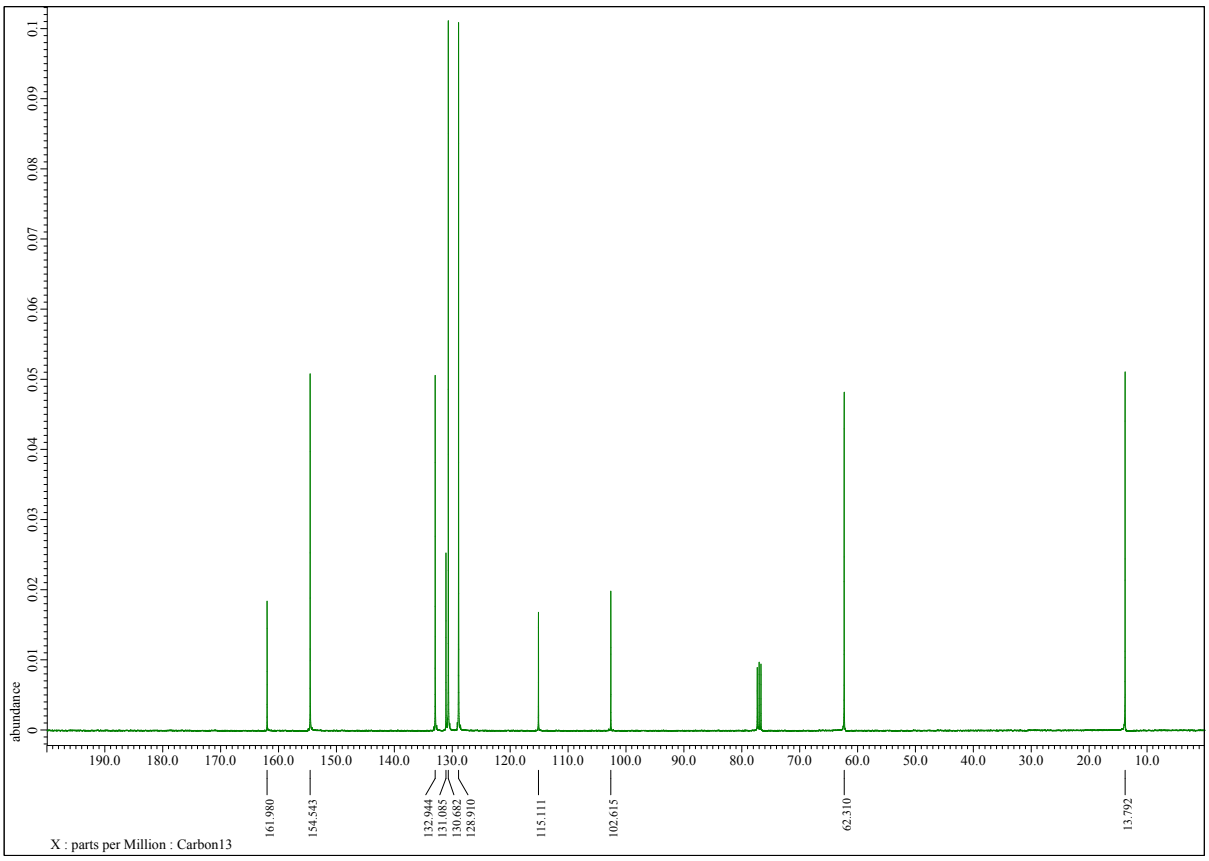
	Peak area ratio
Unknown	3.26%
3a	96.74%

Figure S6. GC-FID spectrum of crude reaction product of a gram-scale reaction (Scheme 2).

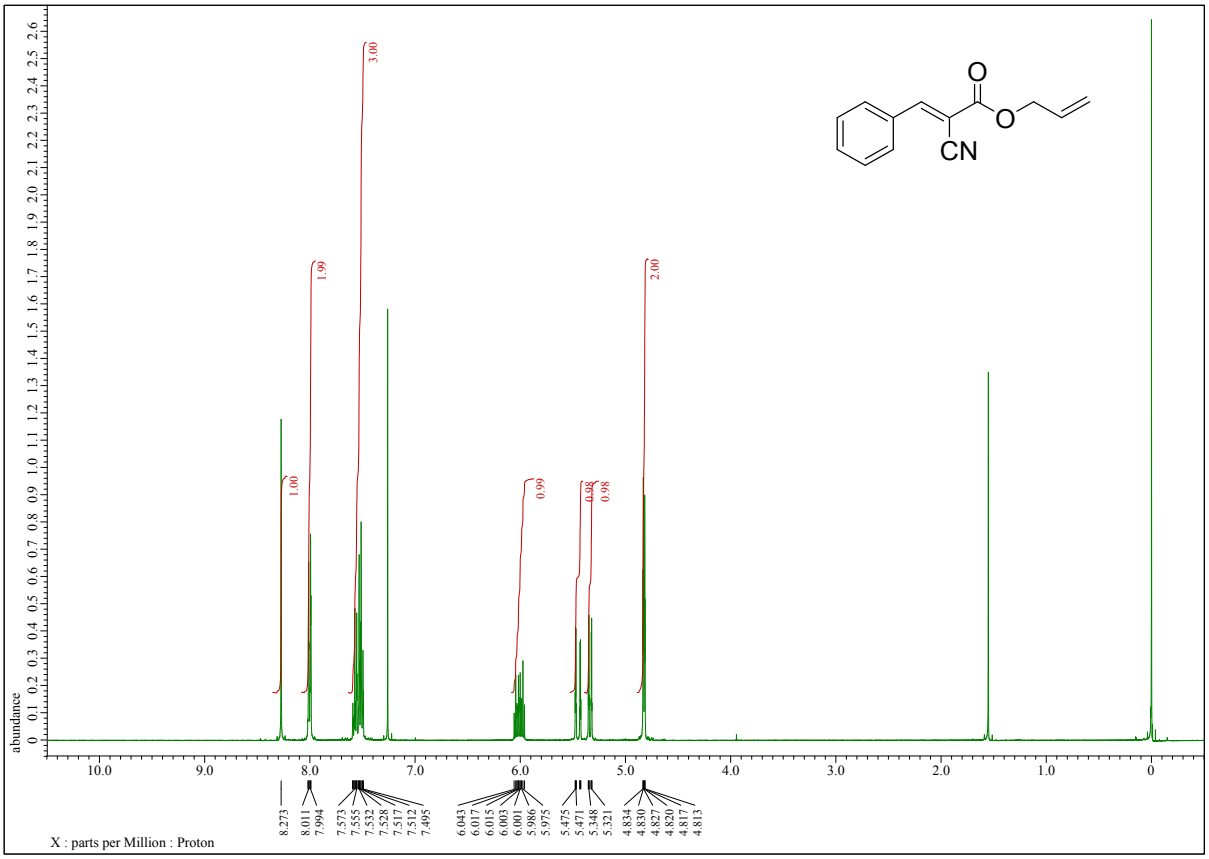
7. ^{1}H and ^{13}C NMR spectra

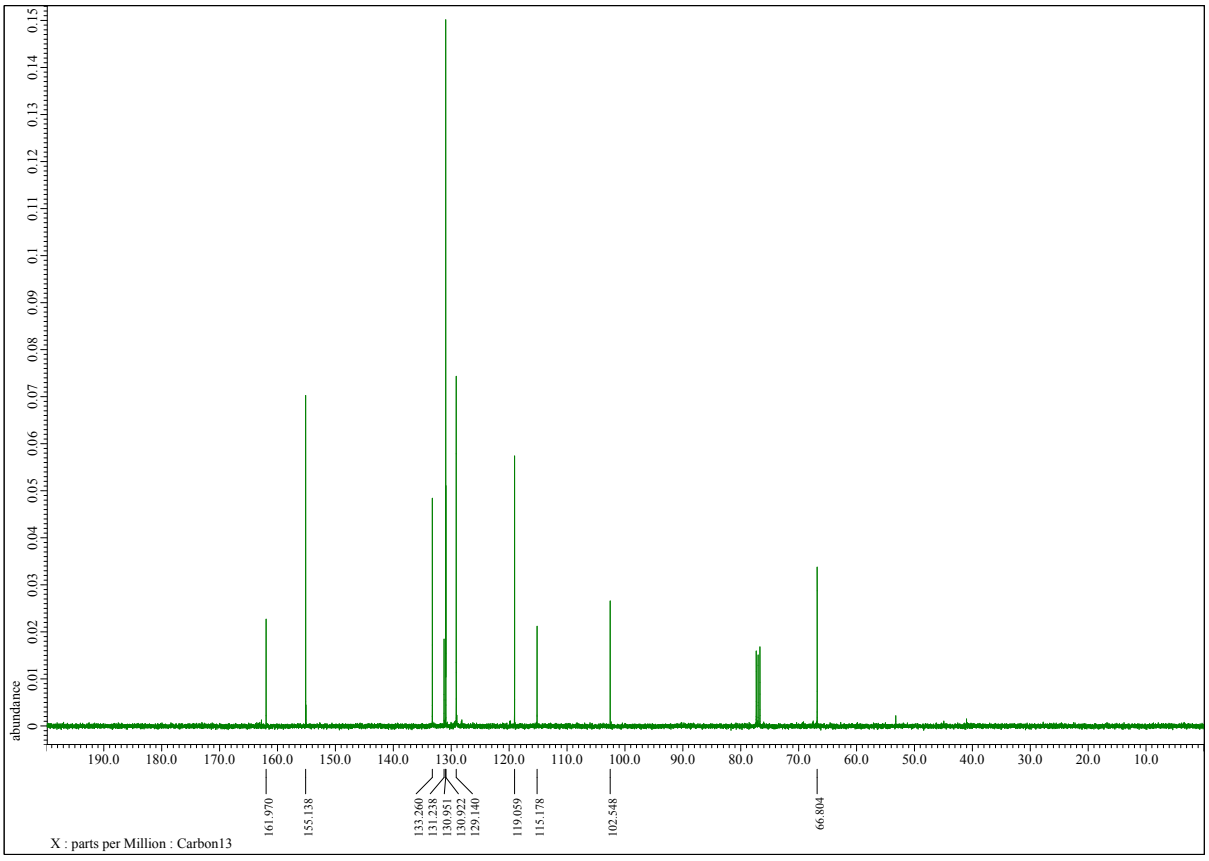
Ethyl (*E*)-2-cyano-3-phenylacrylate (**3a**)



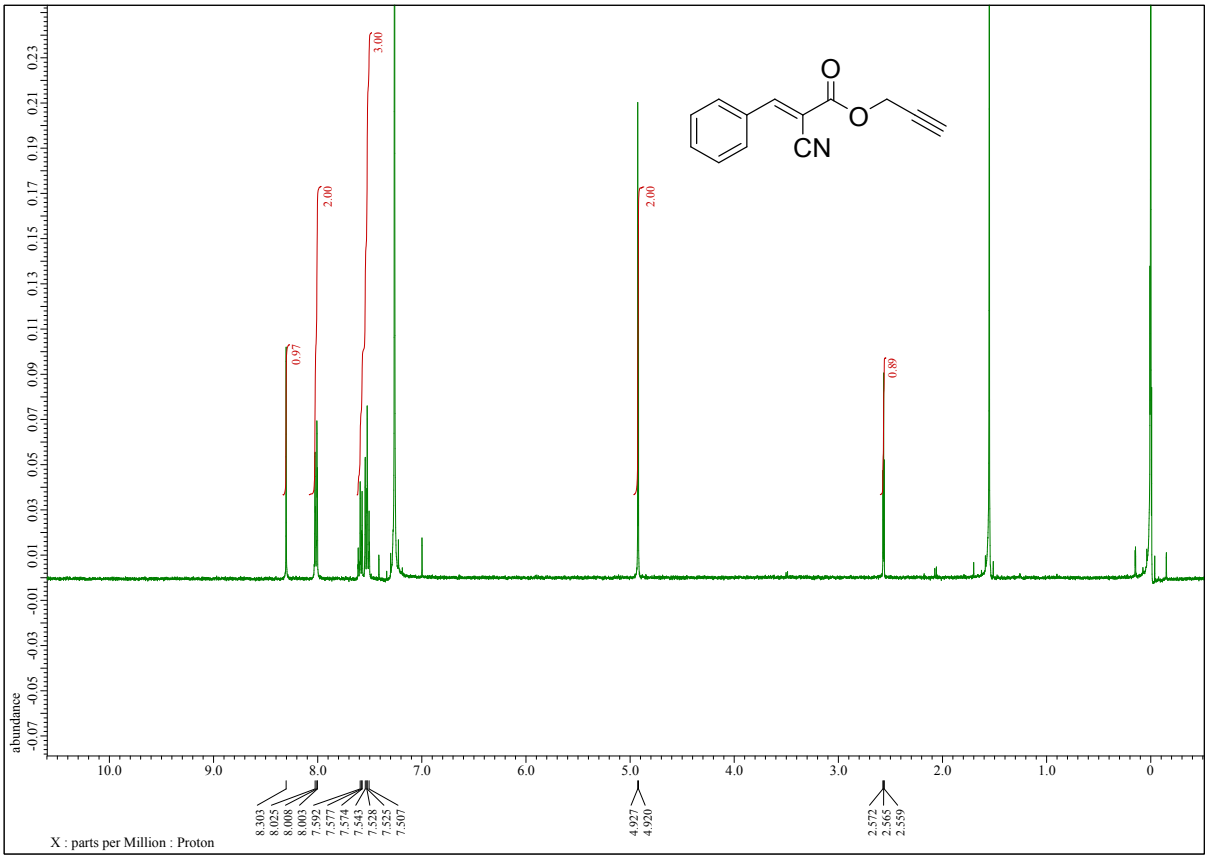


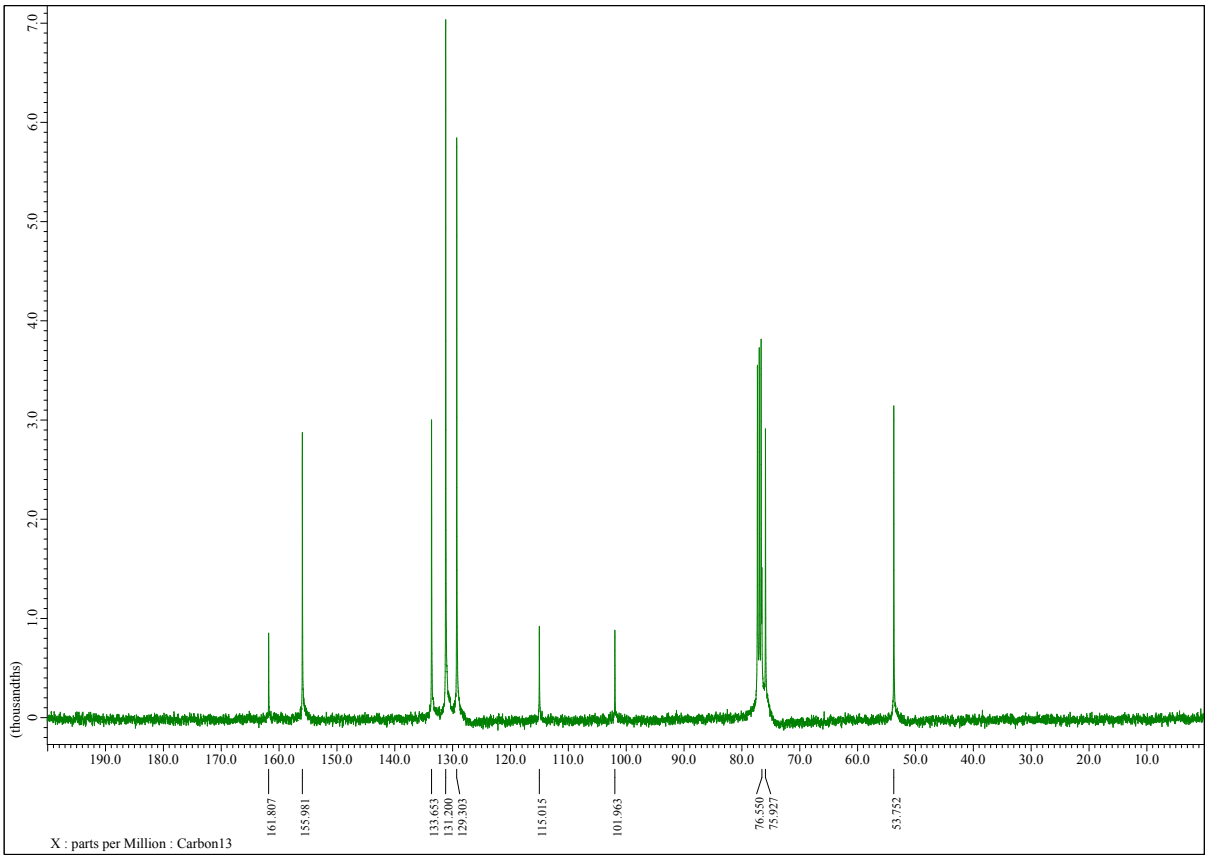
Allyl (*E*)-2-cyano-3-phenylacrylate (**3b**)



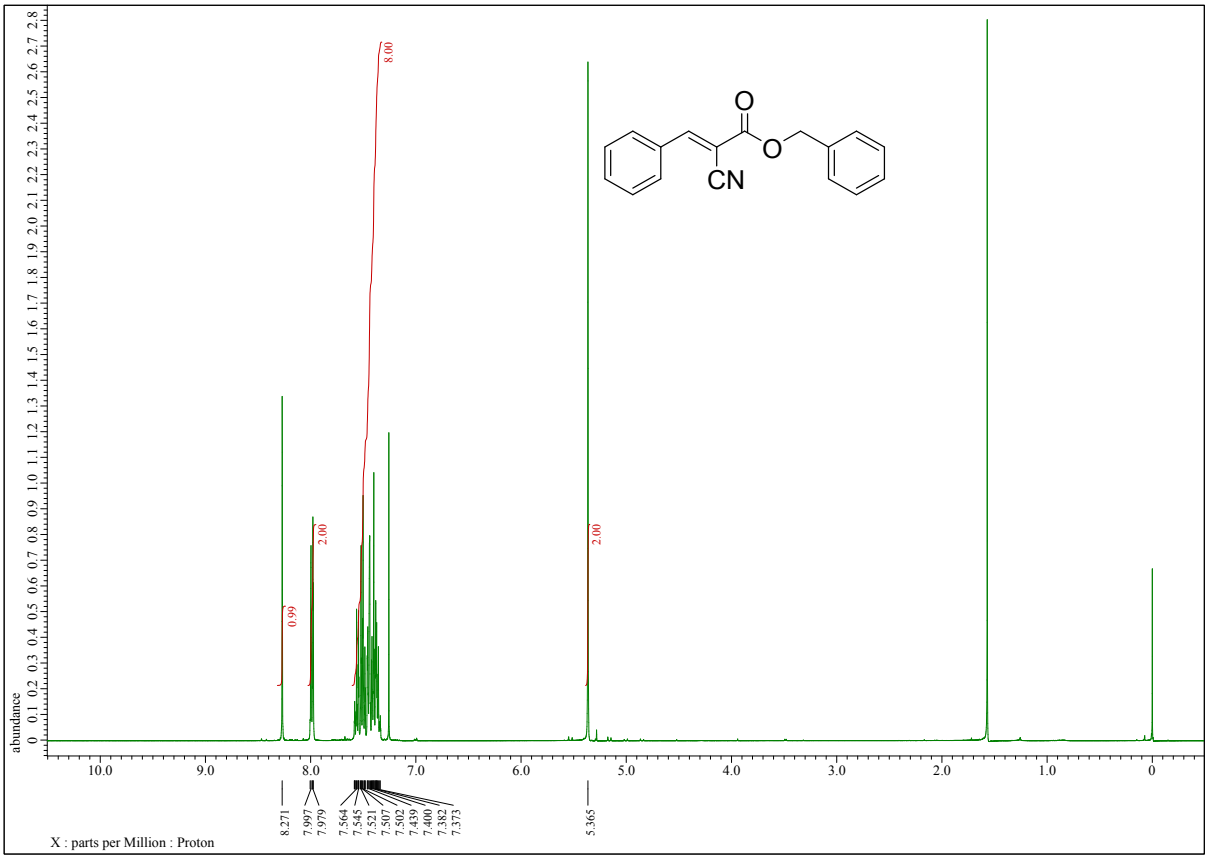


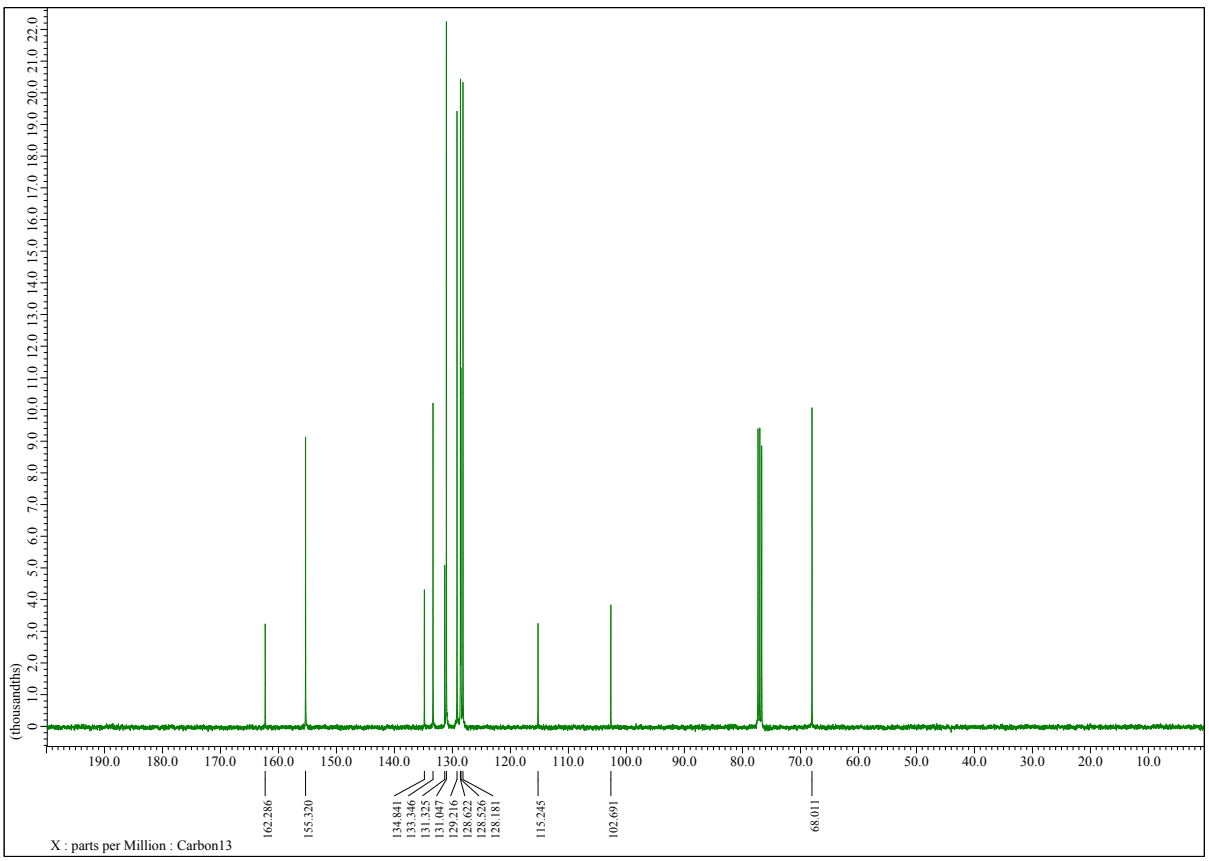
Prop-2-yn-1-yl (*E*)-2-cyano-3-phenylacrylate (**3c**)



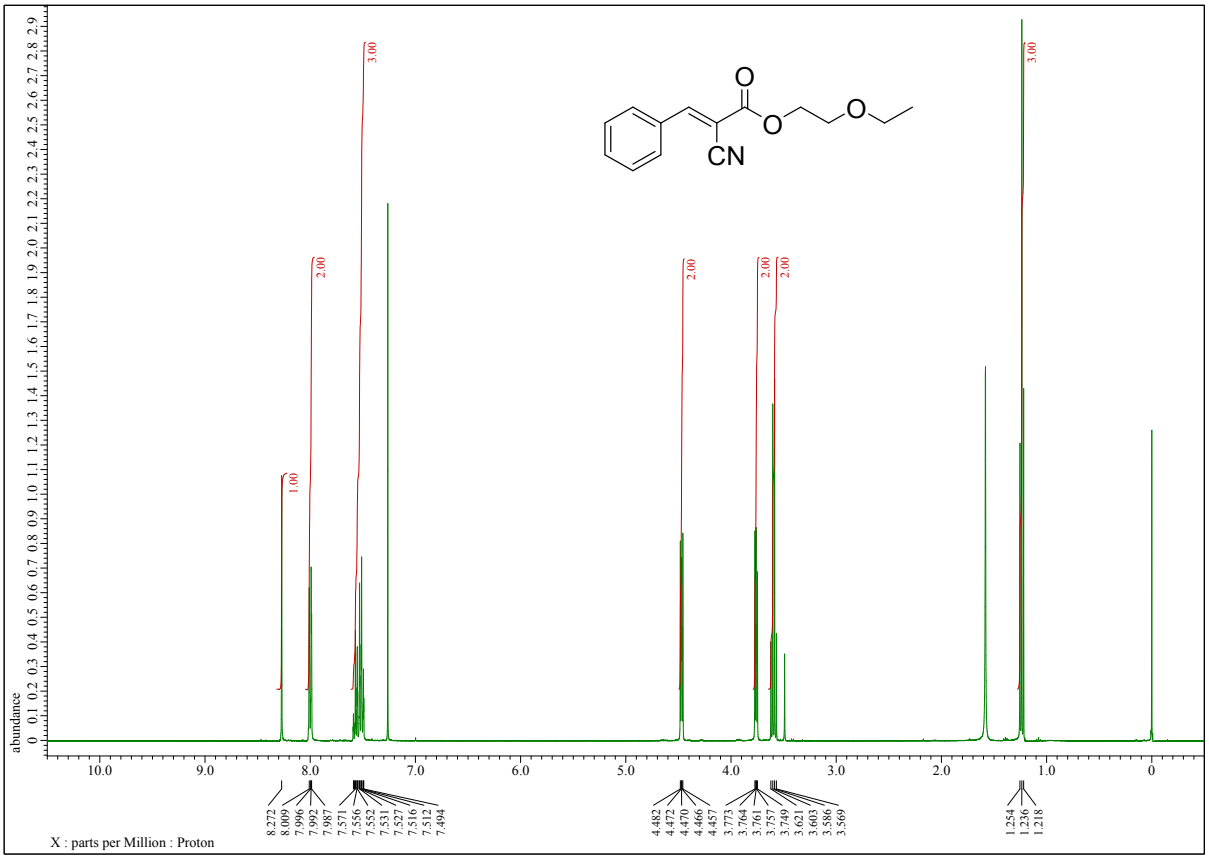


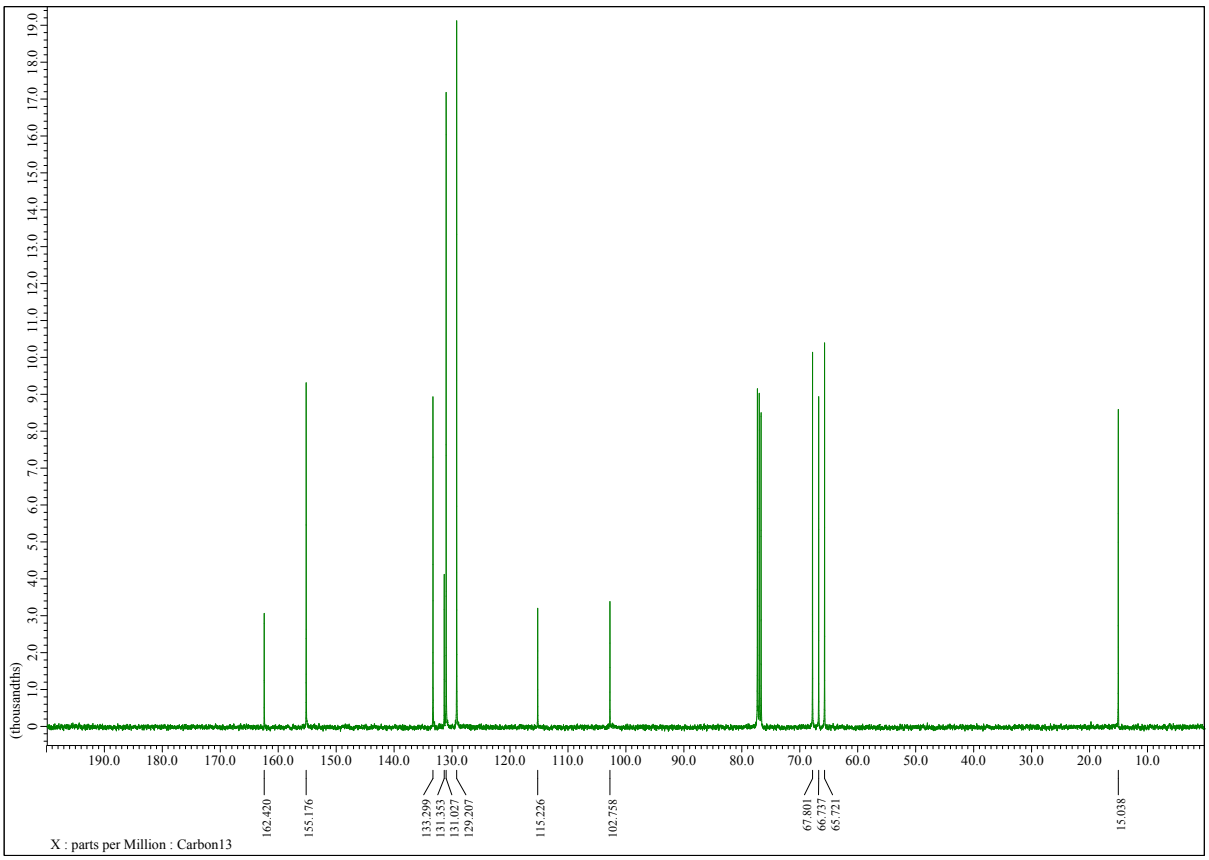
Benzyl (*E*)-2-cyano-3-phenylacrylate (**3d**)



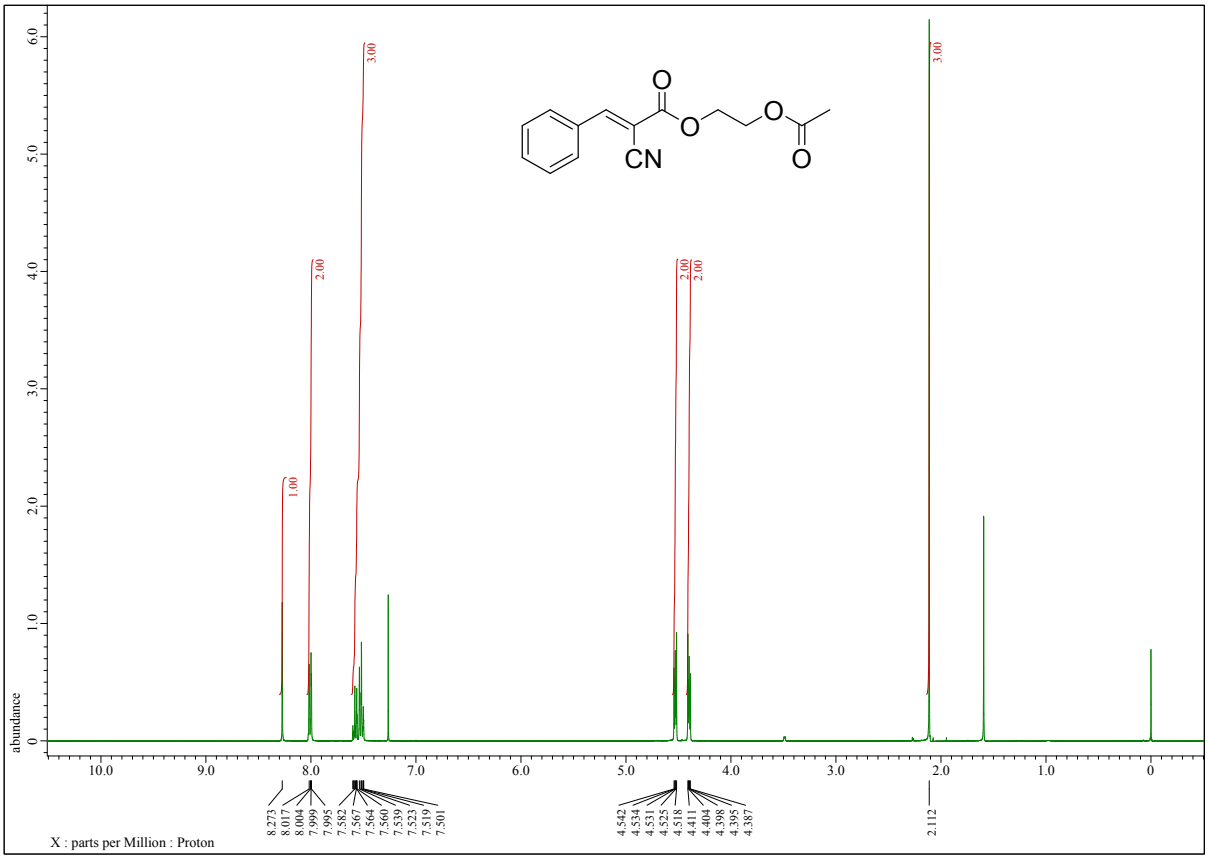


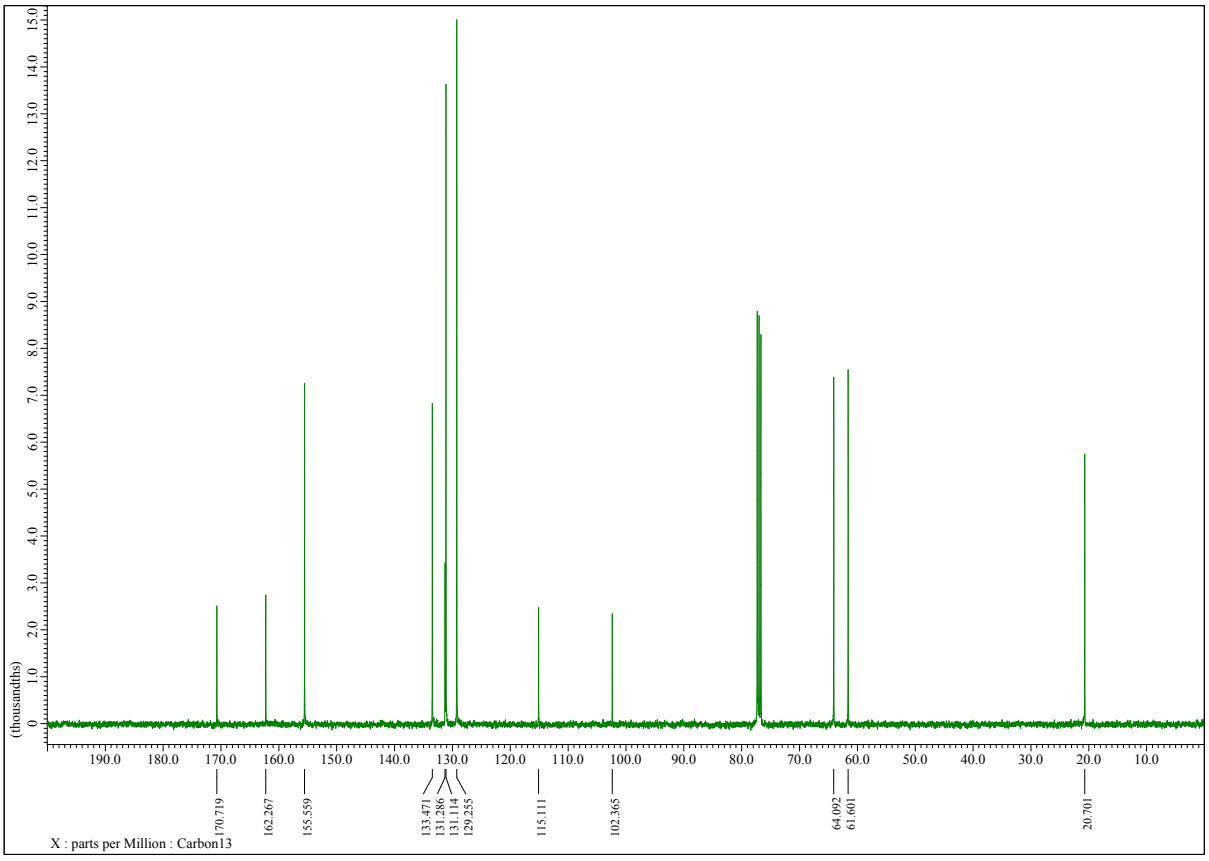
2-Ethoxyethyl (*E*)-2-cyano-3-phenylacrylate (**3e**)



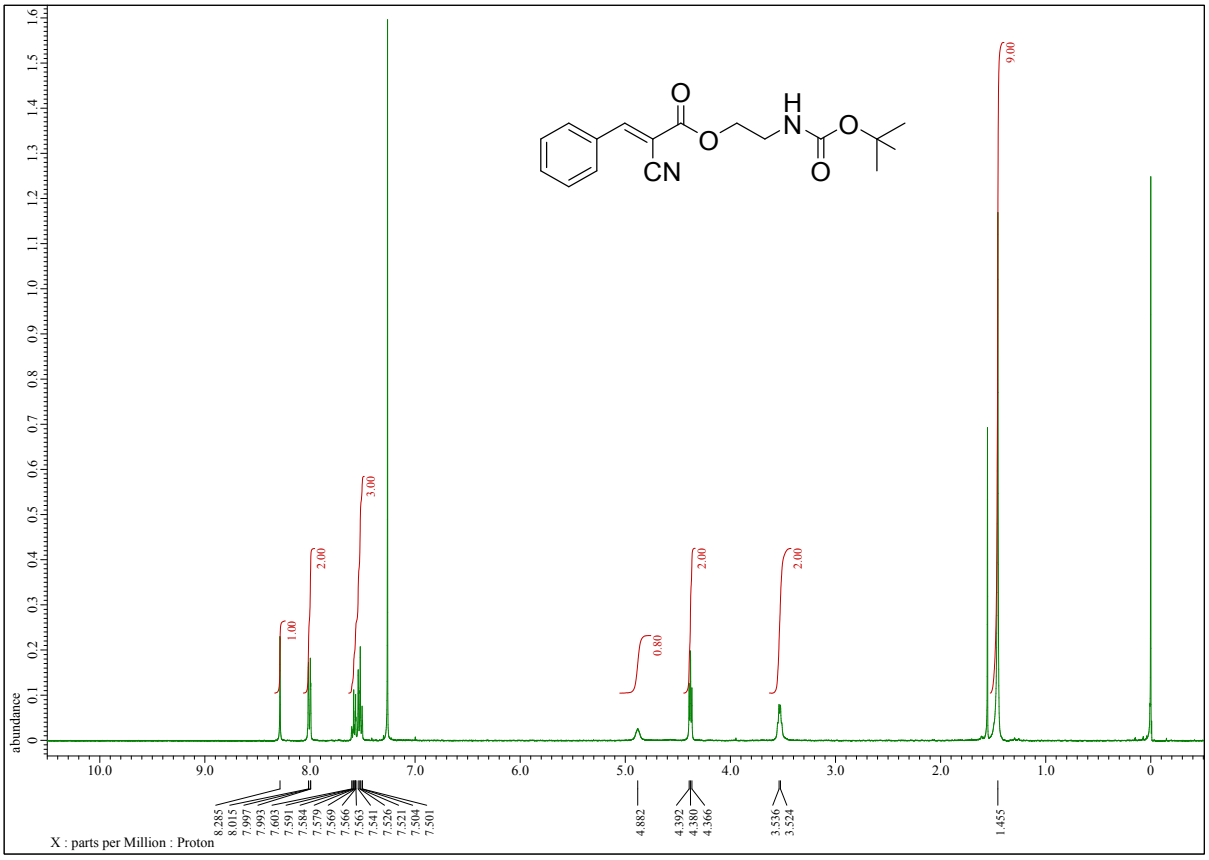


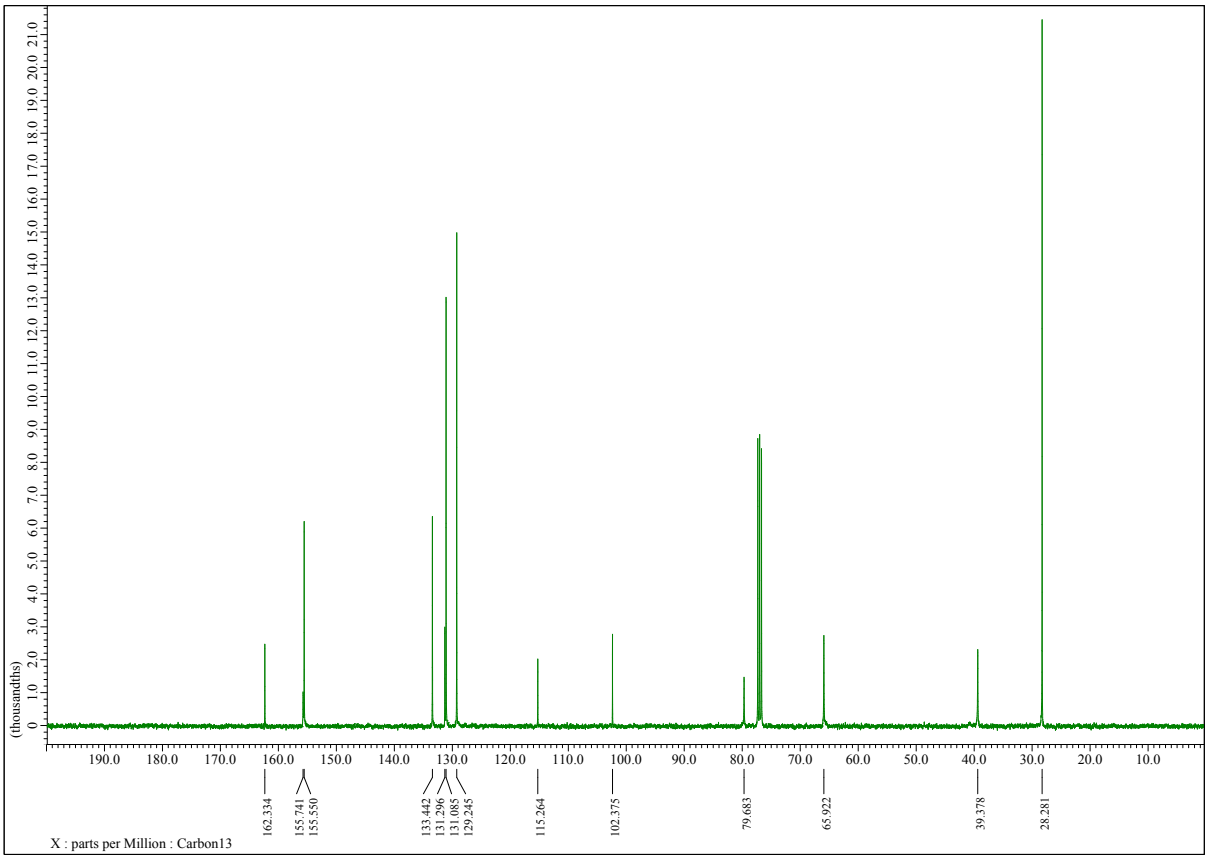
2-Acetoxyethyl (*E*)-2-cyano-3-phenylacrylate (**3f**)





2-((*Tert*-butoxycarbonyl)amino)ethyl (*E*)-2-cyano-3-phenylacrylate (**3g**)





Methyl (*E*)-4-(((*E*)-2-cyano-3-phenylacryloyl)oxy)but-2-enoate (**5**)