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Natural α-amino acid based synthesis of morpholin-2-ones, prospective monomers for new-generation polymeric lipofectants

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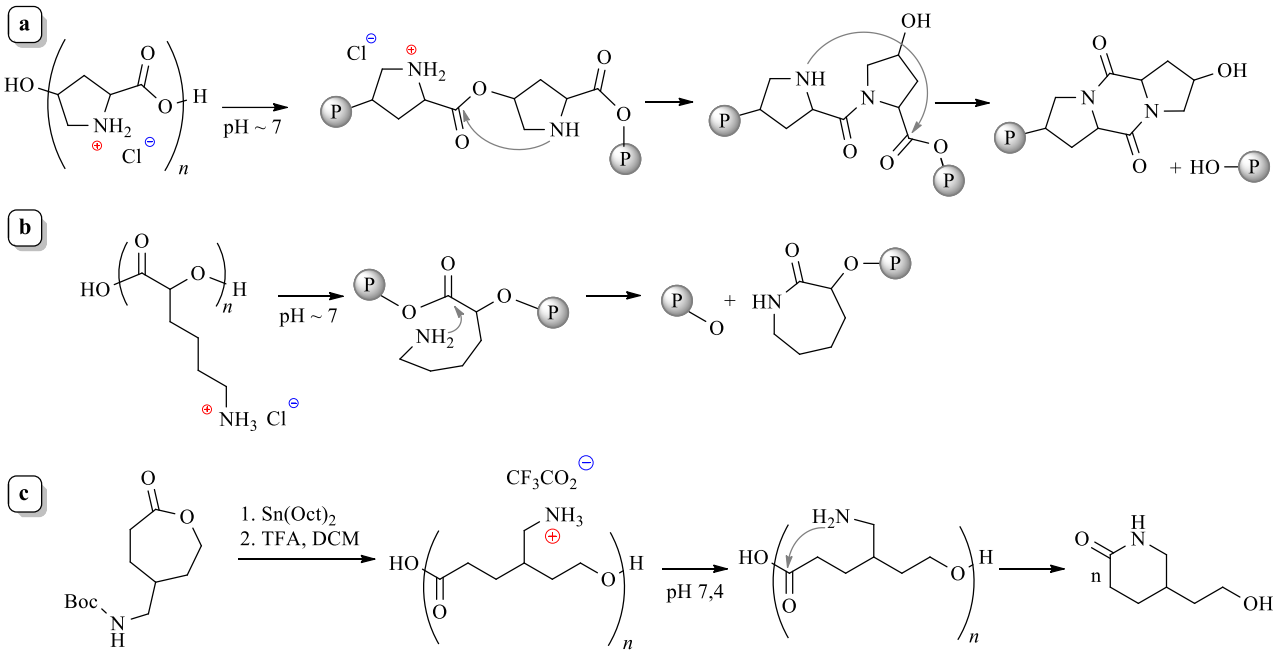
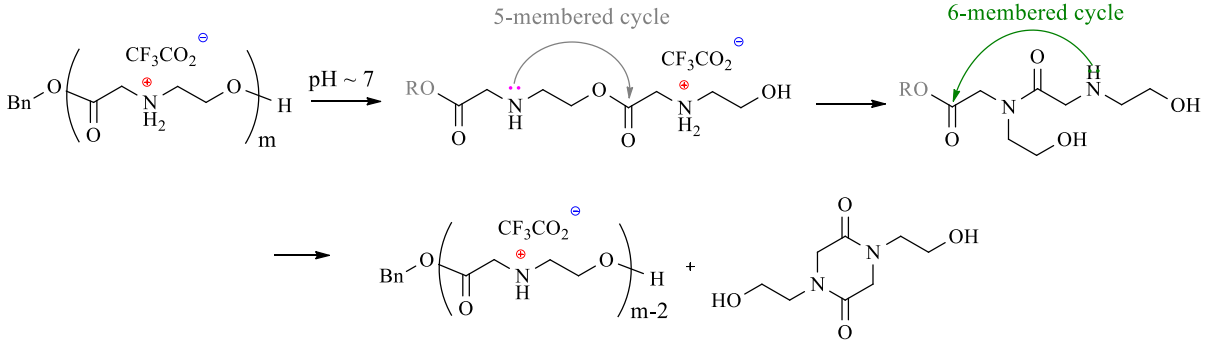
Supplementary Information

S1. Amino-functionalized polymers and mechanisms of their degradation



Scheme S1. Mechanisms of degradation of poly(4-oxy-L-proline) (*a*),[S1] poly[α-(4-aminobutyl)-L-glycolic acid] (*b*)[S2] and poly(5-*Z*-amino-δ-valerolactone) (*c*).[S3]



Scheme S2. Mechanisms of degradation of morpholin-2-one based CARTs.[S4]

S2. Synthetic procedures

S2.1. General

All synthetic experiments were conducted under an argon atmosphere. Diethyl ether (Et_2O) and triethylamine (Et_3N) were refluxed with Na/benzophenone/dibenzo-18-crown-6 and distilled prior to use. DMF, benzyl bromide, ethylene glycol, 4-(*N*,*N*-dimethylamino)pyridine and DMSO were distilled under reduced pressure. MeCN was distilled over P_2O_5. CH_2Cl_2 was distilled over CaH_2. Glycine, L-alanine, L-valine, L-leucine, L-phenylalanine, L-tyrosine, dry EtOH, methanol, sodium triacetoxyborohydride, oxalyl chloride and di-*tert*-butyl dicarbonate (Merck) were used as purchased.

$CDCl_3$ (Cambridge Isotope Laboratories, Inc., D 99.8 %) was used as purchased. DMSO-d_6 (Aldrich, ≥99.5 atom % ^2H) was distilled over CaH_2 and stored over molecular sieves. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer (400 MHz) at 20 °C. The chemical shifts are reported in ppm relative to the solvent residual peaks.

S2.2. Synthesis of 2-(benzyloxy)acetaldehyde

2-(Benzyloxy)ethanol.[S5] Sodium metal (9.66 g, 0.42 mol, 1.25 eq.) was added in small chunks with stirring to ethylene glycol (112.5 mL, 2.02 mol, 6 eq.). The mixture was heated to 110 °C, and in 10 min after Na dissolution benzyl bromide (40 mL, 0.336 mol) was added dropwise. After 4 h of stirring at 110 °C and 12 h at 20 °C water (500 mL) was added. After extraction with Et_2O (3×100 mL), the combined organic fraction was dried over $MgSO_4$, evaporated under reduced pressure, and distilled (B. p. ~115 °C at 10 Torr). The yield 47.6 g (93%).

2-(Benzyloxy)acetaldehyde.[S6] DMSO (5.68 mL, 80 mmol, 2.4 eq.) was added at –78° C to a solution of oxalyl chloride (3.43 mL, 40 mmol, 1,2 eq.) in CH_2Cl_2 (100 mL). After 45 min of stirring, a solution of 2-(benzyloxy)ethanol (4.74 mL, 33.3 mmol) in CH_2Cl_2 (10 mL) was added dropwise, and after additional 45 min Et_3N (27.8 mL, 200 mmol, 6 eq.) was added. The mixture was allowed to warm to 20 °C, washed with 1M HCl (2×20 mL), water (2×20 mL), dried over $MgSO_4$ and evaporated under reduced pressure and distilled (B. p. 60 °C at 0.15 Torr). The yield 3.88 g (77%).

S2.3. Common synthetic methodics

Synthesis of hydrochlorides of α-amino acid methyl esters. Thionyl chloride $SOCl_2$ (4.7 mL) was added at 0 °C to a suspension of α-amino acid (50 mmol) in MeOH (250 mL). The mixture was heated to reflux, stirred for 2 h, cooled to 20 °C and stirred for additional 12 h. After evaporation under reduced pressure, the residue was washed with Et_2O and recrystallized from dry EtOH.

Reductive amination of 2-(benzyloxy)acetaldehyde. α-Amino acid hydrochloride (8.79 mmol, 1.2 eq.) was suspended in CH_2Cl_2 (50 mL), the mixture was cooled to 0 °C. Triethylamine Et_3N (1.53 mL, 11.0 mmol, 1.5 eq.), 2-(benzyloxy)acetaldehyde (1.00 mL, 7.32 mmol) and sodium triacetoxyborohydride (2.33 g, 11.0 mmol, 1.5 eq.) were added subsequently in 15 min intervals. The mixture was allowed to warm to 20 °C, stirred for 12 h, washed with water (2×10 mL), 1M HCl (2×5 mL), water (2×10 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The product was used in the next step without further purification, except L-tyrosine derivative (see Section S2).

*Synthesis of N-Boc-protected amino esters **2a**–**2e***.

The product obtained at the previous stage (7.51 mmol) was dissolved in CH_2Cl_2 (25 mL). The mixture was cooled to 0 °C, and a solution of 4-(*N*,*N*-dimethylamino)pyridine (9.2 mg, 0.07 mmol, 0.01 eq.), Et_3N (1.25 mL, 9.00 mmol, 1.20 eq.) and di-*tert*-butyl dicarbonate (2.0 g, 9.16 mmol, 1.22 eq.) in CH_2Cl_2 (10 mL) were added. The mixture was allowed to warm to 20 °C, stirred for 12 h, washed with 1M HCl (2×5 mL), water (2×10 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by gradient column chromatography (silica, 16:1, 8:1, and 4:1 petroleum ether/ethyl acetate mixtures as eluents).

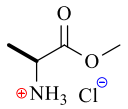
*Synthesis of morpholin-2-ones **M2**–**M6.***

Catalyst 10% Pd/C (0.5 g) was added to a solution of *N*-Boc-protected amino ester (9.5 mmol) in MeOH (25 mL), the mixture was heated to 50 °C, degassed, and the flask was filled with H_2 from the gas meter. After completion of hydrogenolysis (control by NMR), the catalyst was separated by centrifugation, the supernatant was evaporated, dissolved in toluene (100 mL). *p*-TSA (0.1 g) was added, and the mixture was refluxed with Dean–Stark head for 3 h. The mixture was cooled to 20 °C, washed with aq. $NaHCO_3$ (2×5 mL), water (2×10 mL), dried over $MgSO_4$, and evaporated under reduced pressure. Products **M2**–**M4** were purified by recrystallization from Et_2O with subsequent high-vacuum sublimation. Products **M5** and **M6** were purified by distillation.

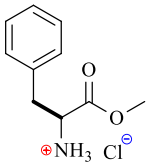
S3. Preparation details and NMR spectra

S3.1. Synthetic features and analysis data

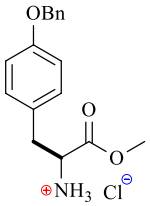
Hydrochlorides of α-amino acid methyl esters. Compounds **1a**–**1e** have been synthesized and described previously. In the present work, we present ^{1}H NMR spectra of these compounds recorded using modern equipment and anhydrous DMSO-d_6 as a solvent.



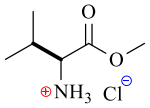
(*S*)-1-Methoxy-1-oxopropan-2-aminium chloride (**1a**). ^{1}H NMR (DMSO-d_6, 20 °C, 400 MHz) δ: 8.75 (bs, 3H); 4.01 (s, 1H); 3.70 (s, 3H); 1.40 (d, ^{3}J = 7.2 Hz, 3H).



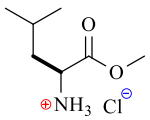
(*S*)-1-Methoxy-1-oxo-3-phenylpropan-2-aminium chloride (**1b**). ^{1}H NMR (DMSO-d_6, 20 °C, 400 MHz) δ: 8.89 (bs, 3H); 7.46–6.94 (m, 5H); 4.18 (s, 1H); 3.61 (s, 3H); 3.24 (dd, ^{2}J = 13.9 Hz, ^{3}J = 5.4 Hz, 1H); 3.09 (dd, ^{2}J = 13.9 Hz, ^{3}J = 7.7 Hz, 1H).



(*S*)-3-(4-Benzyloxyphenyl)-1-methoxy-1-oxopropan-2-aminium chloride (**1c**). ^{1}H NMR (DMSO-d_6, 20 °C, 400 MHz) δ: 8.71 (bs, 3H); 7.57–7.26 (m, 5H); 7.14 (d, ^{3}J = 8.7 Hz, 2H); 6.95 (d, ^{3}J = 8.7 Hz, 2H); 5.07 (s, 2H); 4.17 (s, 1H); 3.64 (s, 3H); 3.13 (dd, ^{2}J = 14.1 Hz, ^{3}J = 5.7 Hz, 1H); 3.04 (dd, ^{2}J = 14.1 Hz, ^{3}J = 7.1 Hz, 1H).



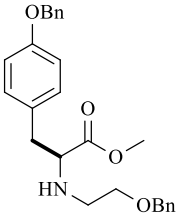
(*S*)-1-Methoxy-3-methyl-1-oxobutan-2-aminium chloride (**1d**). ^{1}H NMR (DMSO-d_6, 20 °C, 400 MHz) δ: 8.54 (bs, 3H); 3.86 (s, 1H); 3.74 (s, 3H); 2.17 (m, 1H); 0.96 (d, ^{3}J = 6.9 Hz, 3H); 0.92 (d, ^{3}J = 6.9 Hz, 3H).



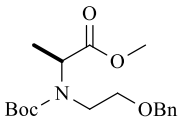
(*S*)-1-Methoxy-4-methyl-1-oxopentan-2-aminium chloride (**1e**). ^{1}H NMR (DMSO-d_6, 20 °C, 400 MHz) δ: 8.48 (bs, 3H); 3.96 (t, ^{3}J = 7.1 Hz, 1H); 3.73 (s, 3H); 1.73 (n, ^{3}J = 6.6 Hz, 1H); 1.62 (q, ^{3}J = 6.6 Hz, 2H); 0.88 (d, J = 6.6 Hz, 6H).

Reductive amination and N-Boc-protected amino esters

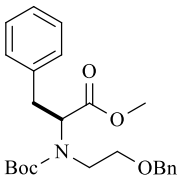
The product of reductive amination of $BnOCH_2CHO$ by **1c** was purified at the intermediate stage using column chromatography.



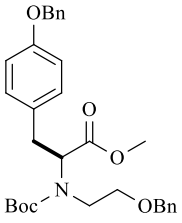
Methyl (*S*)-2-[(2-benzyloxyethyl)amino]-3-(4-benzyloxyphenyl)propanoate. ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.54–7.24 (m, 10H); 7.13 (d, ^3J = 8.6 Hz, 2H); 6.91 (d, ^3J = 8.6 Hz, 2H); 5.04 (s, 2H); 4.50 (bs, 2H); 3.66 (s, 3H); 3.57 (m, 3H); 2.96–2.91 (m, 2H); 2.87 (ddd, ^2J = 12.1 Hz, ^3J = 6.2 Hz, ^4J = 4.4 Hz, 1H); 2.72 (ddd, ^2J = 12.1 Hz, ^3J = 5.8 Hz, ^4J = 4.5 Hz, 1H); 2.00 (bs, 1H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ 174.41; 157.28; 137.89; 136.67; 129.81; 129.07; 128.20; 128.18; 128.00; 127.99; 127.97; 127.57; 127.27; 127.25; 127.22; 127.20; 127.18; 127.12; 114.44; 72.55; 69.56; 69.54; 69.21; 62.84; 51.29; 47.13; 38.32.



Methyl (*S*)-2-[*N*-(2-benzyloxyethyl)-*N*-(*tert*-butoxycarbonyl)amino]propanoate (**2a**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.42–7.15 (m, 5H); 4.50 (s, 2H); 4.58–3.90 (m, 1H); 3.67 (s, 3H); 3.66–3.25 (m, 4H); 1.46 (d, ^3J = 6.9 Hz, 3H); 1.42 (d, ^3J = 3.3 Hz, 9H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 172.53; 172.43; 154.95; 154.42; 137.80; 127.98; 127.16; 80.00; 79.87; 76.98; 76.66; 76.35; 72.83; 72.71; 69.43; 68.83; 56.47; 54.75; 51.60; 47.09; 45.33; 27.90; 15.64; 15.10.

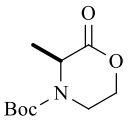


Methyl (*S*)-2-[*N*-(2-benzyloxyethyl)-*N*-(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate (**2b**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.45–6.96 (m, 10H); 4.40 (s, 3H); 4.47–4.20 (m, 1H); 3.70 (s, 3H); 3.58–2.69 (m, 6H); 1.44 (two s, 9H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 171.33; 171.29; 154.66; 154.28; 137.85; 137.79; 128.99; 128.88; 128.11; 127.96; 127.36; 127.16; 126.16; 126.04; 80.27; 79.87; 72.75; 72.67; 68.84; 68.35; 63.03; 62.98; 62.06; 51.73; 51.66; 48.28; 47.50; 36.05; 35.03; 27.94.

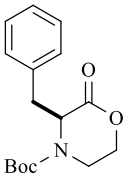


Methyl (*S*)-2-[*N*-(2-benzyloxyethyl)-*N*-(*tert*-butoxycarbonyl)amino]-3-(4-benzyloxyphenyl)propanoate (**2c**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.61–7.27 (m, 10H); 7.21–7.11 (m, 2H); 6.99–6.83 (m, 2H); 5.07 (s, 2H), 4.56–4.34 (m, 2H); 4.41–4.13 (m, 1H); 3.73 (m, 3H); 3.68–2.77 (m, 6H); 1.69–1.35 (m, 9H). ^{13}C NMR (CDCl3, 20 °C, 101 MHz) δ: 171.38, 169.81, 157.23, 157.11, 149.58, 149.29, 146.71, 146.40, 137.86, 137.55, 137.46, 136.66, 136.61, 136.57, 129.96, 129.91, 128.19, 128.03, 127.98, 127.58, 127.33, 127.16, 127.13, 114.64, 114.56, 84.68, 84.62, 72.90, 72.87, 69.59, 68.90, 68.38, 68.20, 63.63, 63.49, 63.10, 52.08, 51.66, 49.11, 48.97, 35.16, 35.07, 33.41, 27.97, 27.91, 27.07.

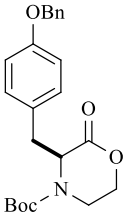
N-Boc protected morpholin-2-ones.



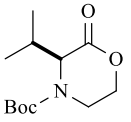
tert-Butyl (*S*)-3-methyl-2-oxomorpholine-4-carboxylate (**M2**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 4.69 (bs, 1H); 4.38 (m, 2H); 3.85 (bs, 1H); 3.42 (bm, 1H); 1.49 (d, ^{3}J = 7.1 Hz, 3H); 1.46 (s, 9H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 169.25; 152.85; 80.78; 67.28; 51.83; 37.73; 27.87; 18.12.



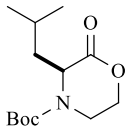
tert-Butyl (*S*)-3-benzyl-2-oxomorpholine-4-carboxylate (**M3**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.44–7.09 (m, 5H); 5.05–2.47 (group of bm, 7H); 1.47 (bs); 1.39 (bs) {9H}. ^{1}H NMR ($CDCl_3$, 55 °C, 400 MHz) δ: 7.41–7.05 (m, 5H); 4.90 (t, ^{3}J = 5.5 Hz, 1H); 4.18 (m, 1H); 3.87 (bs, 1H); 3.74 (bs, 1H); 3.27 (m, 2H); 2.97 (m, 1H); 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 168.19; 153.46; 136.45; 129.79; 128.56; 127.09; 81.17; 67.18; 58.08; 39.01; 38.55; 28.15.



tert-Butyl (*S*)-3-(4-(benzyloxy)benzyl)-2-oxomorpholine-4-carboxylate (**M4**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 7.55–7.29 (m, 5H); 7.08 (d, ^{3}J = 8.6 Hz, 2H); 6.91 (d, ^{3}J = 8.6 Hz, 2H); 5.05 (s, 2H); 4.83 (bs, 1H); 4.23 (bs, 1H); 3.99–3.55 (m, 2H); 3.25 (m, 2H); 2.93 (m, 1H); 1.40 (bs, 2H). ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 157.66; 153.10; 136.46; 130.42; 128.19; 127.60; 127.08; 114.73; 76.82; 69.60; 57.92; 29.30; 27.80; 27.00.



tert-Butyl (*S*)-3-isopropyl-2-oxomorpholine-4-carboxylate (**M5**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 4.51–4.28 (m, 3H); 3.80 (bs, 1H); 3.39 (s, 1H); 2.12 (m, 1H); 1.41 (s, 9H); 0.98 (d, ^{3}J = 6.8 Hz); 1.00 (d, ^{3}J = 6.8 Hz) {6H}. ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 167.87; 153.88; 80.86; 66.10; 65.54; 61.61; 60.64; 40.30; 38.92; 32.51; 27.78; 19.04.



tert-Butyl (*S*)-3-isobutyl-2-oxomorpholine-4-carboxylate (**M6**). ^{1}H NMR ($CDCl_3$, 20 °C, 400 MHz) δ: 4.61 (bs, 1H), 4.39–4.29 (m, 2H); 3.83 (bs, 1H); 3.36 (bs, 1H); 1.75–1.52 (m, 3H); 1.41 (s, 9H); 0.93 (d, ^{3}J = 6.3 Hz); 0.91 (d, ^{3}J = 6.3 Hz) {6H}. ^{13}C NMR ($CDCl_3$, 20 °C, 101 MHz) δ: 168.51; 153.23; 80.93; 80.51; 66.76; 66.40; 54.59; 53.85; 41.74; 38.96; 37.33; 27.82; 24.00; 21.97; 21.94.

S3.2. NMR spectra

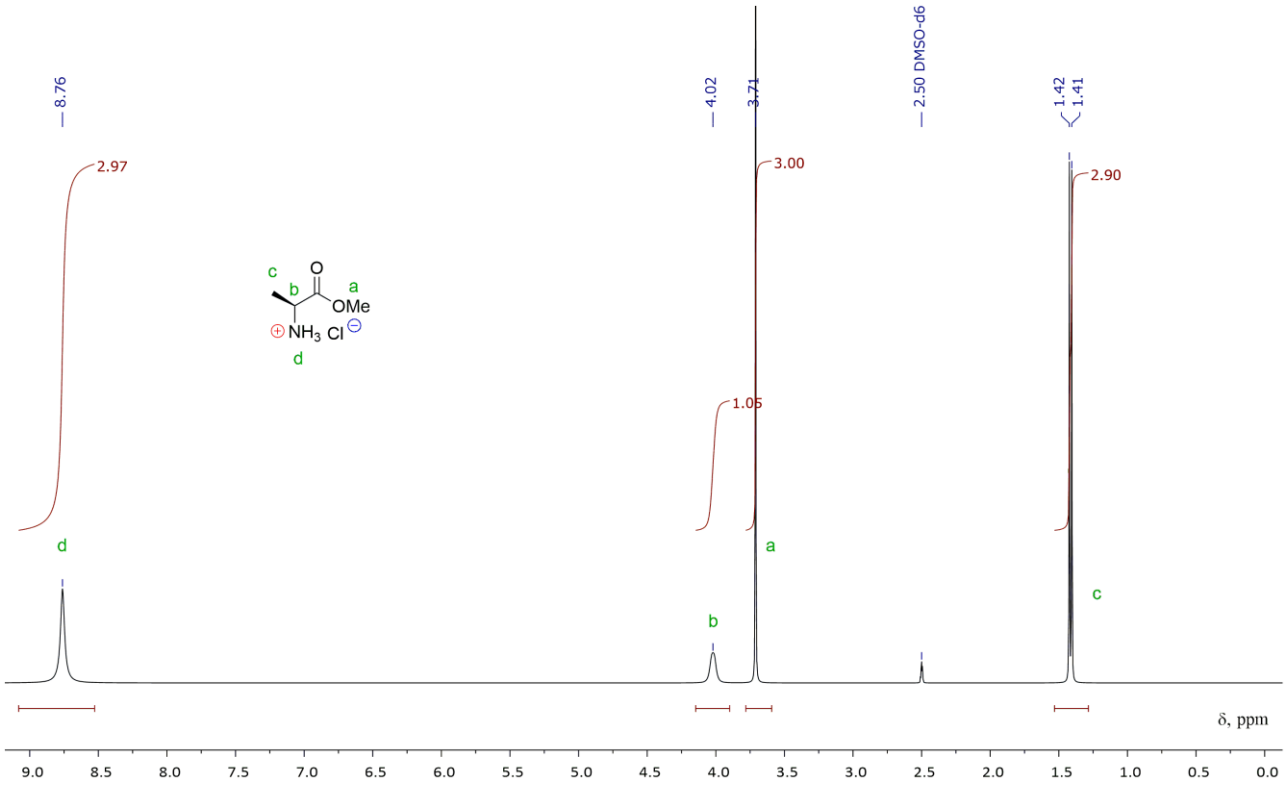


Fig. S1. ^{1}H NMR spectrum (DMSO-d$_6$, 20 °C, 400 MHz) of **1a**.

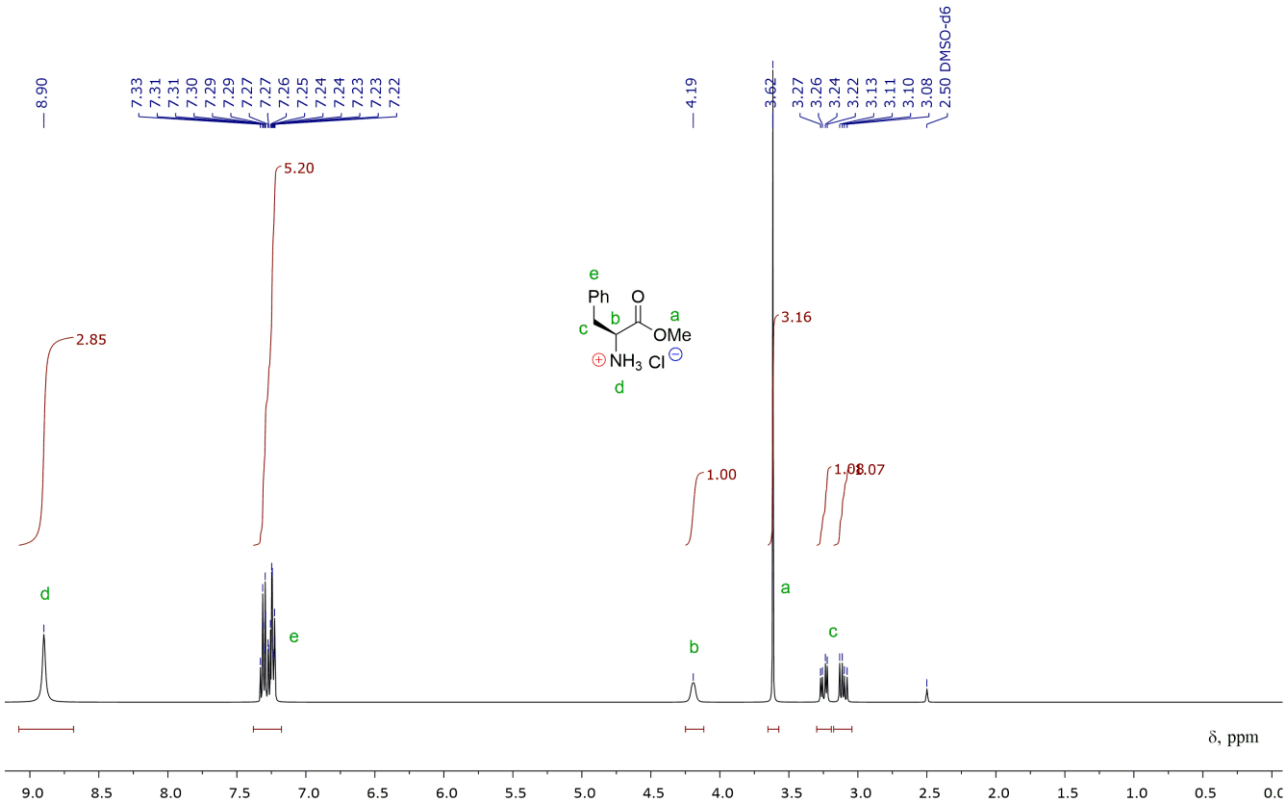


Fig. S2. ^{1}H NMR spectrum (DMSO-d$_6$, 20 °C, 400 MHz) of **1b**.

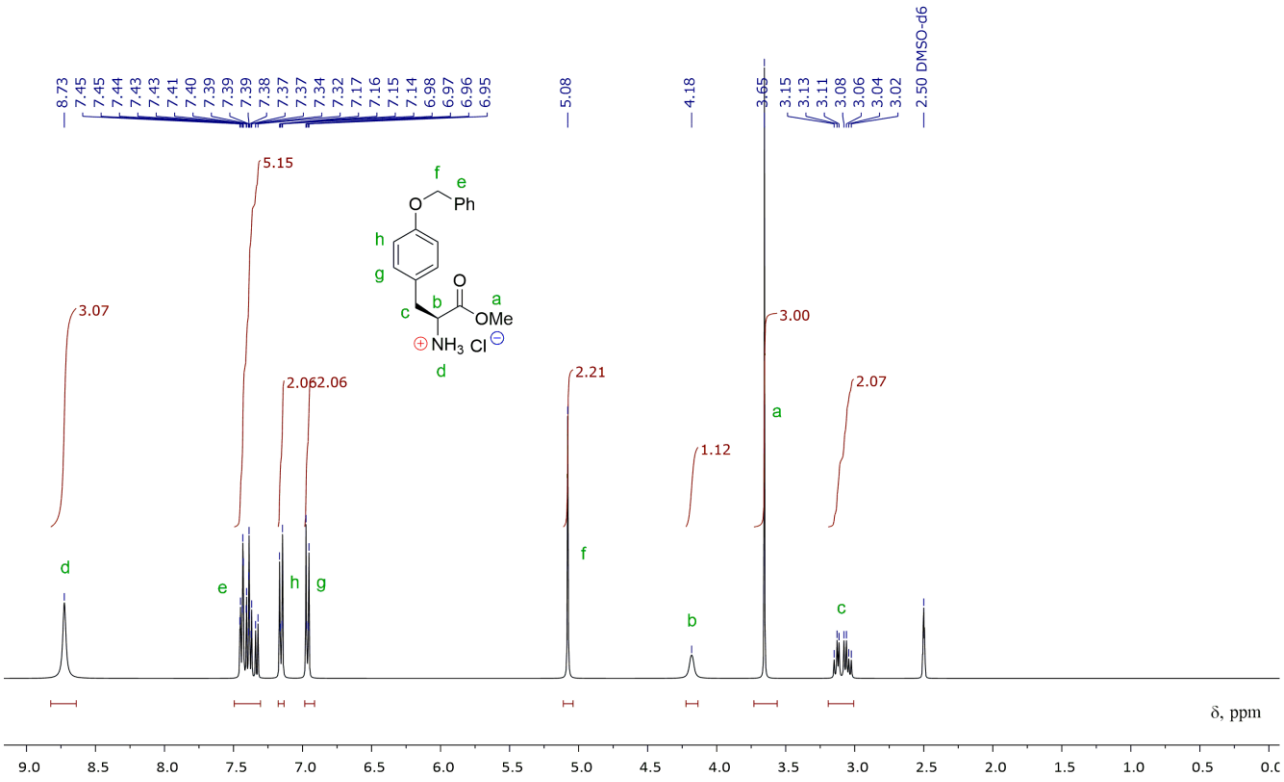


Fig. S3. ^{1}H NMR spectrum (DMSO-d$_6$, 20 °C, 400 MHz) of **1c**.

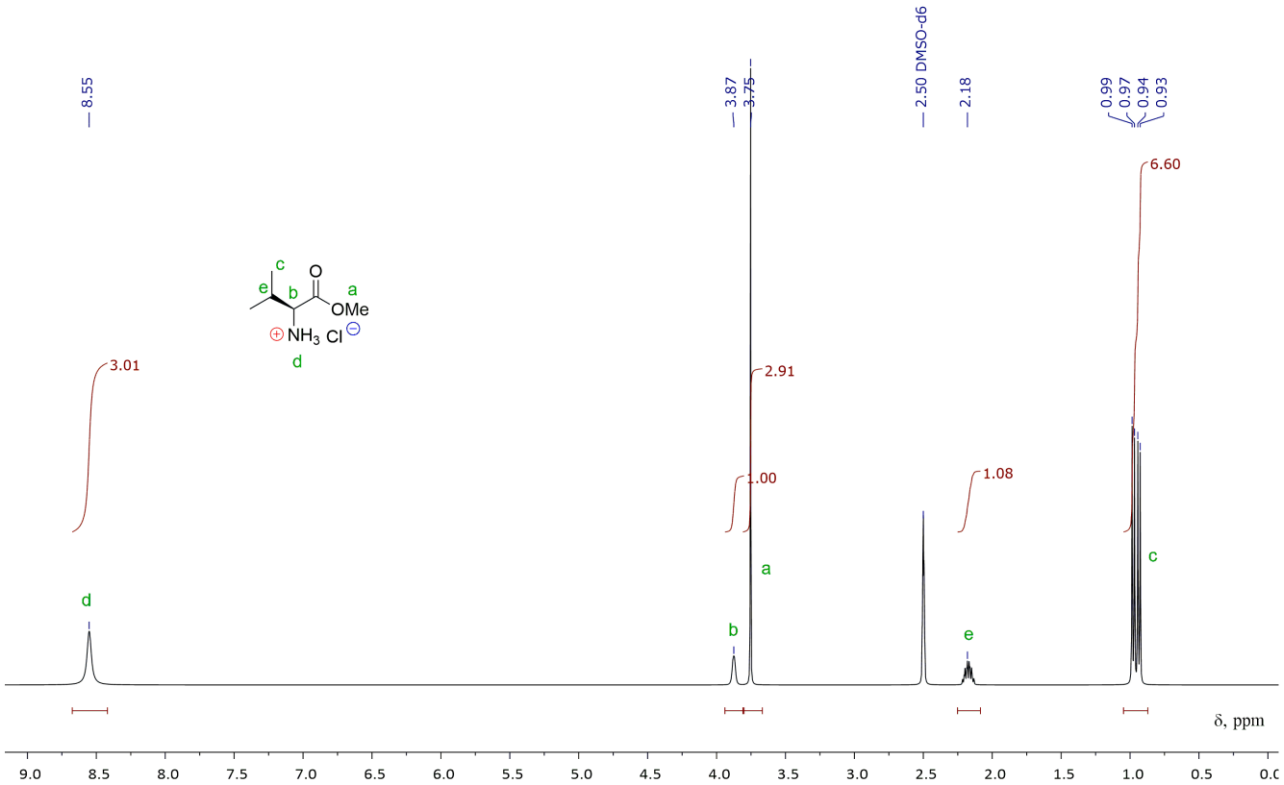


Fig. S4. ^{1}H NMR spectrum (DMSO-d$_6$, 20 °C, 400 MHz) of **1d**.

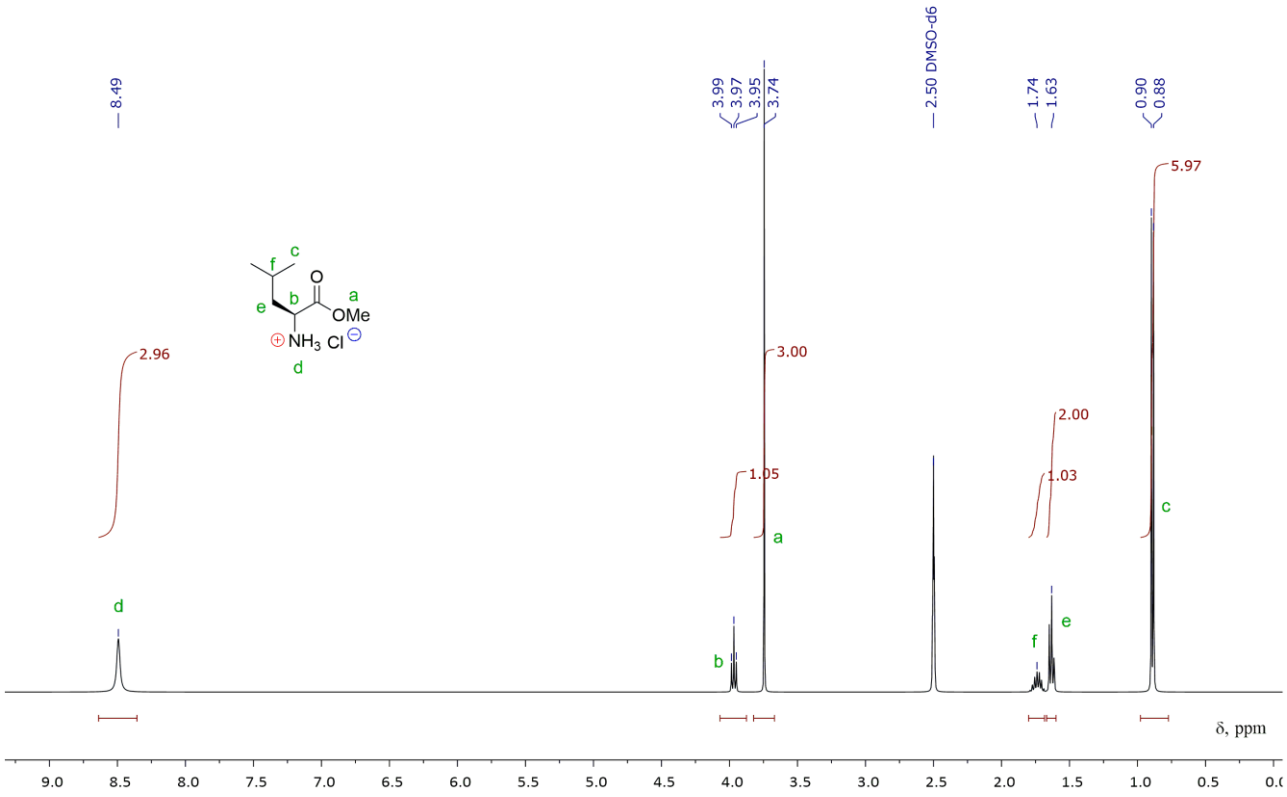


Fig. S5. ^{1}H NMR spectrum (DMSO-d$_6$, 20 °C, 400 MHz) of **1e**.

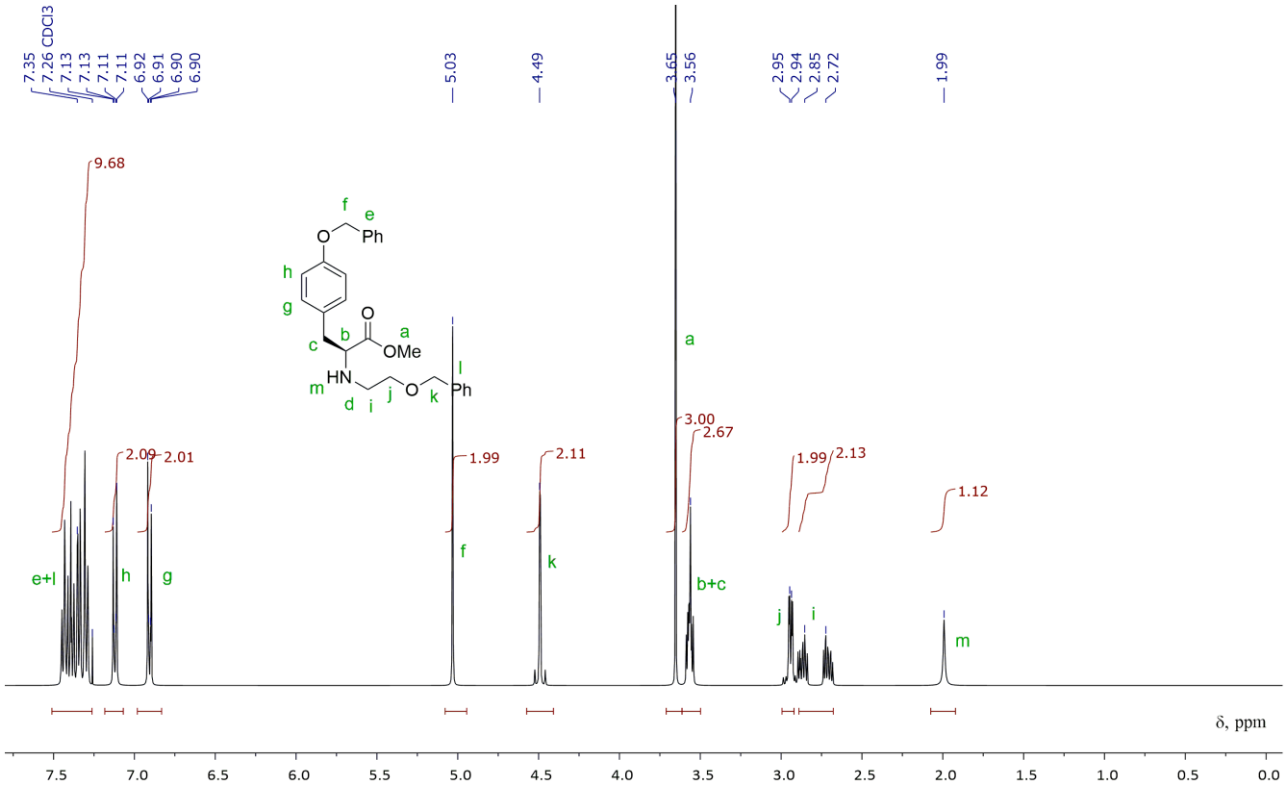


Fig. S6. ^{1}H NMR spectrum (CDCl$_3$, 20 °C, 400 MHz) of (*S*)-methyl 2-((2-(benzyloxy)ethyl)amino)-3-(4-(benzyloxy)phenyl)propanoate.

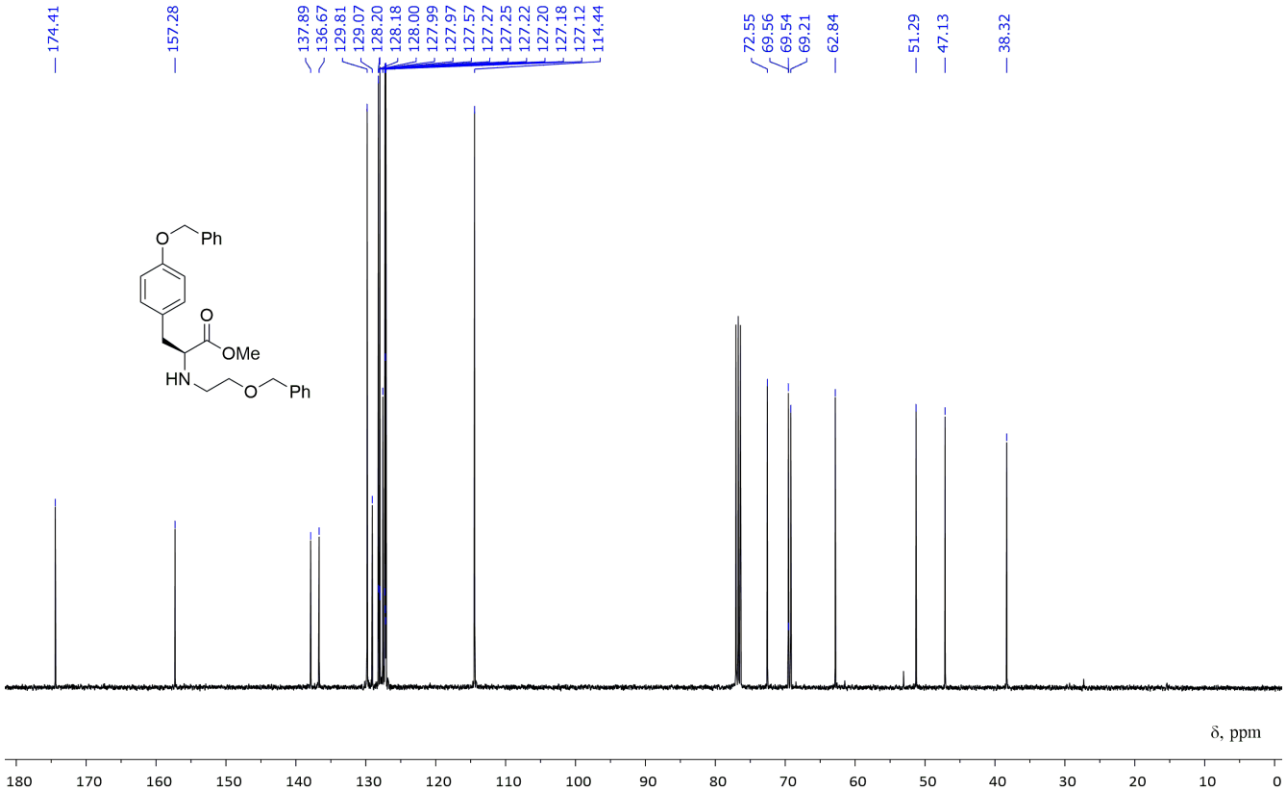


Fig. S7. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of (*S*)-methyl 2-((2-(benzyloxy)ethyl)amino)-3-(4-(benzyloxy)phenyl)propanoate.

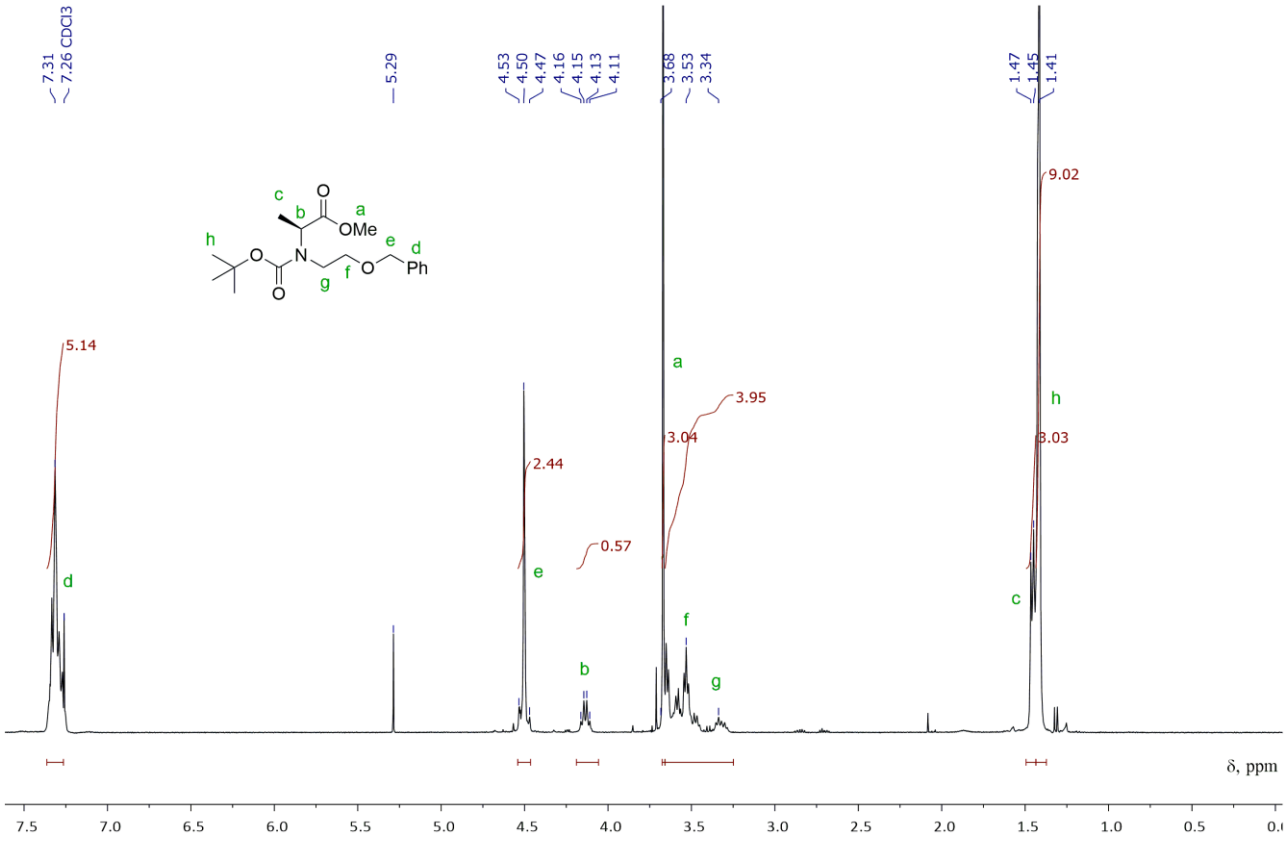


Fig. S8. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **2a**.

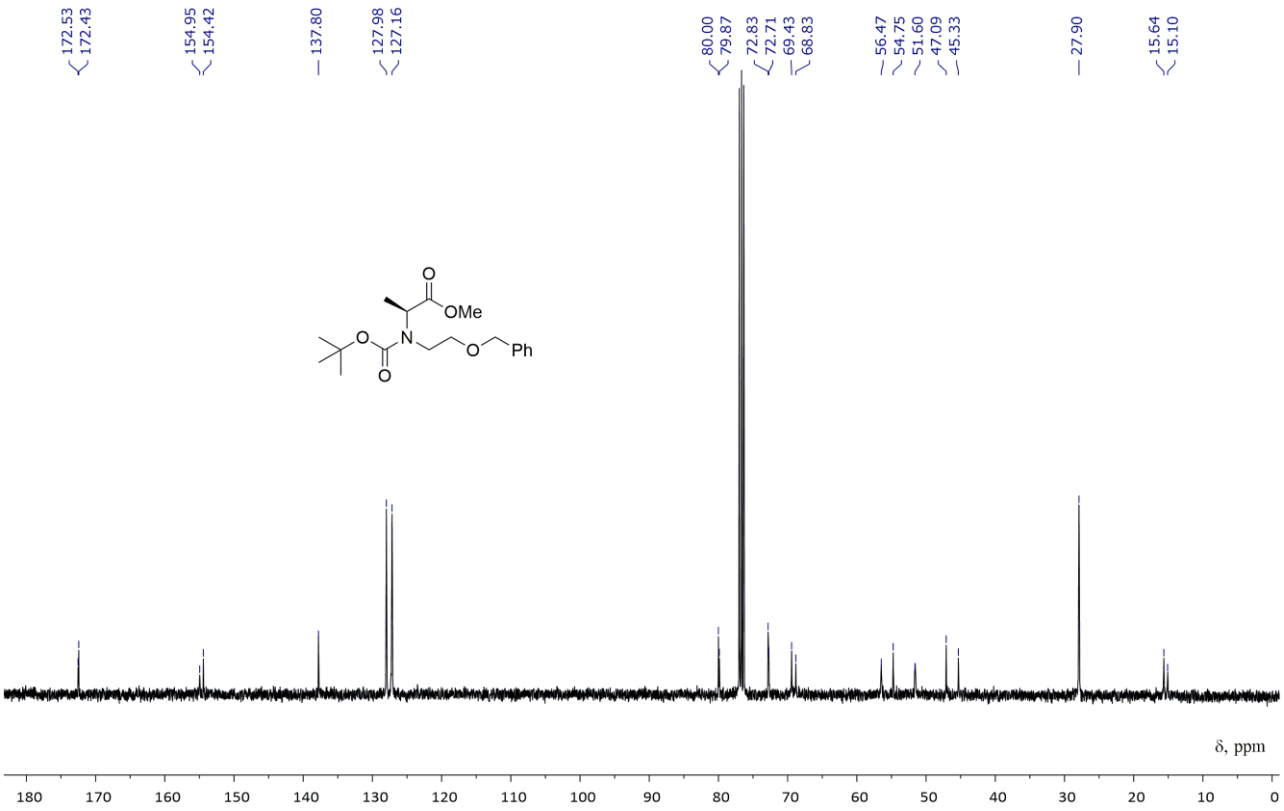


Fig. S9. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **2a**.

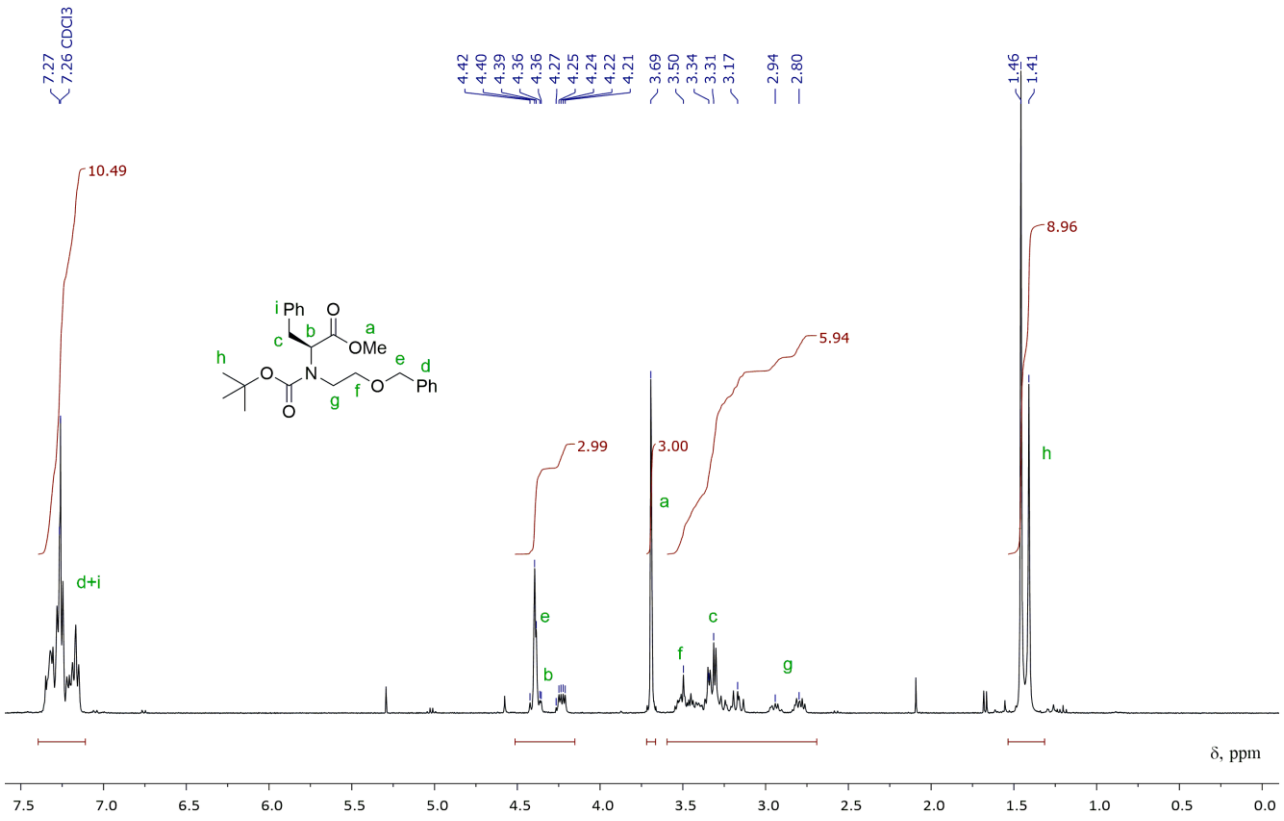


Fig. S10. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **2b**.

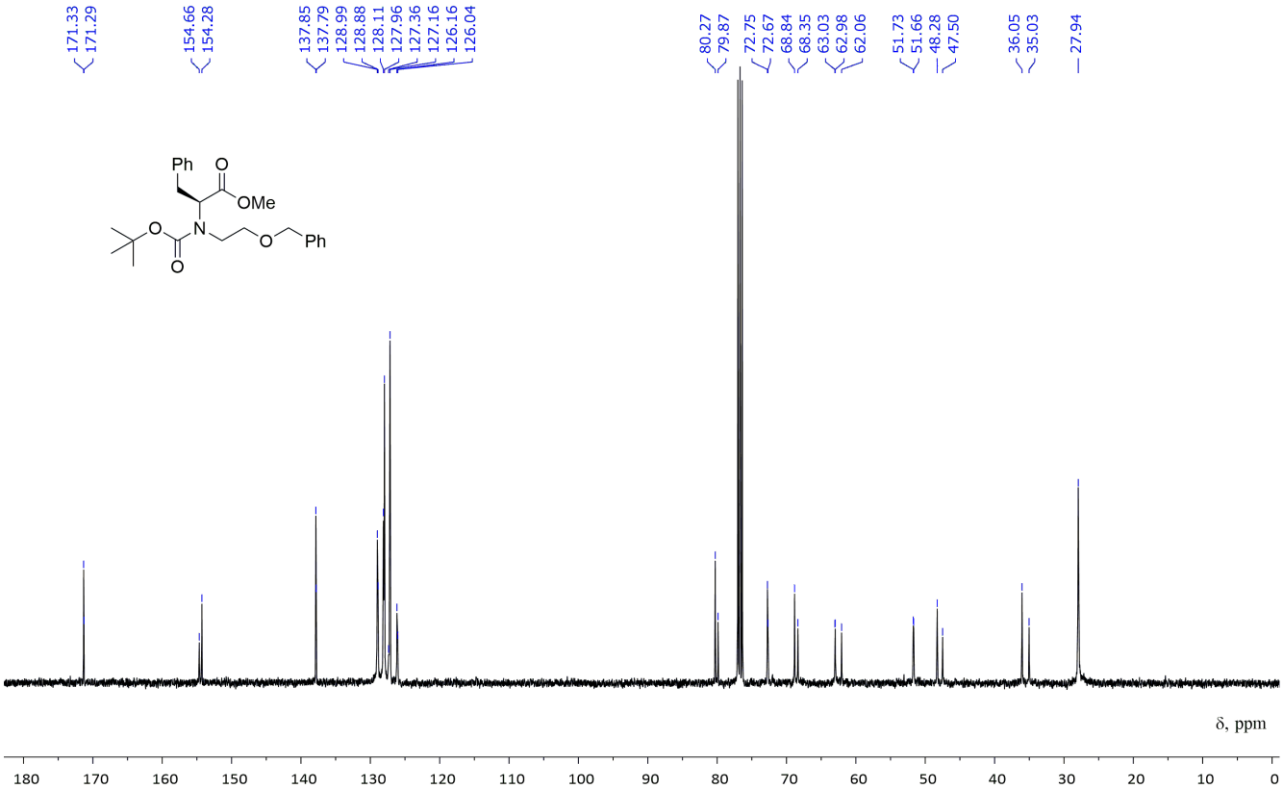


Fig. S11. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **2b**.

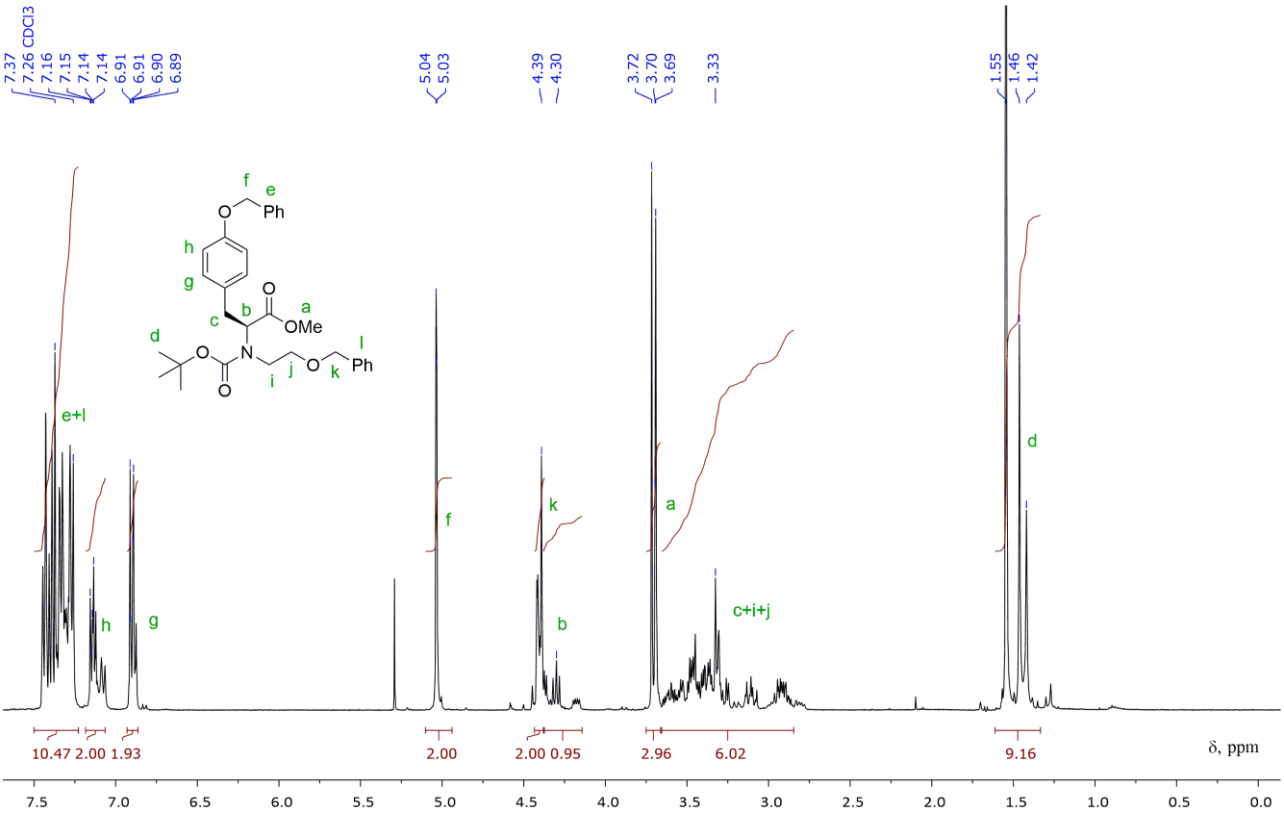


Fig. S12. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **2c**.

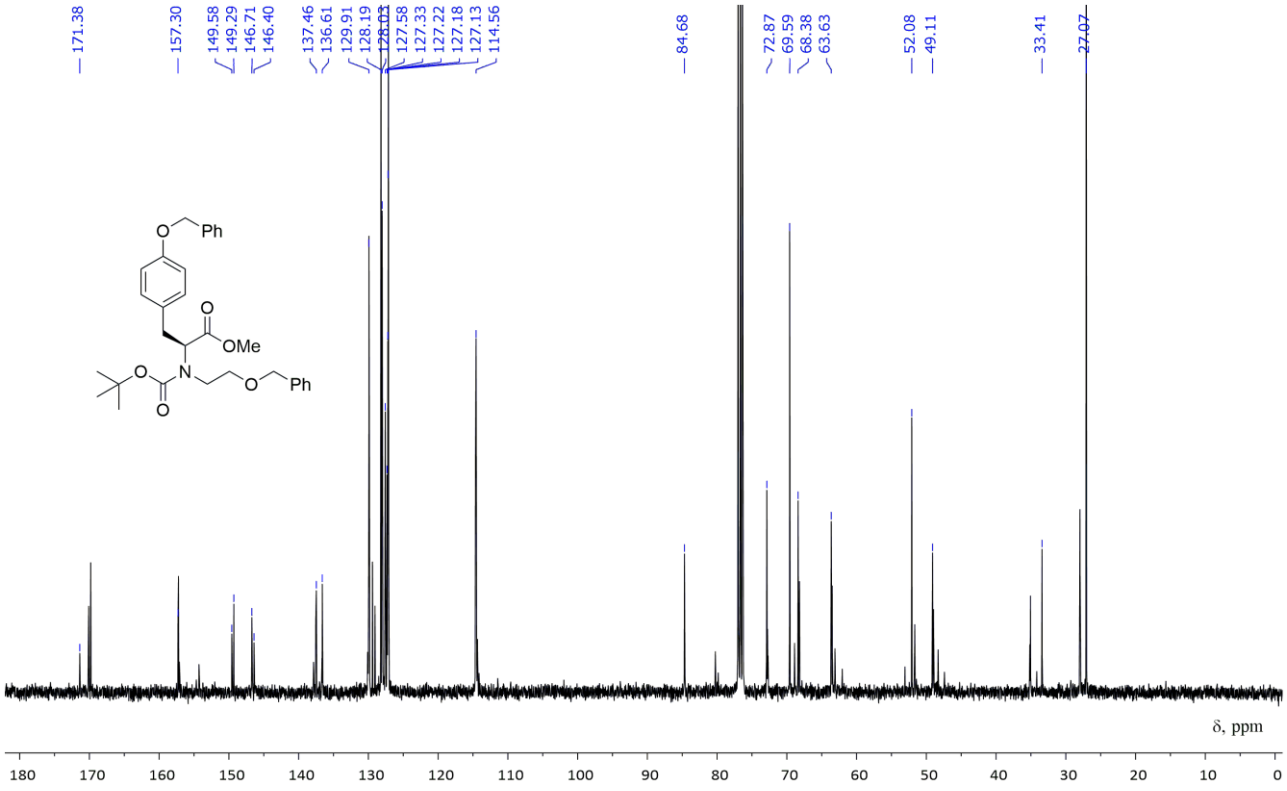


Fig. S13. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **2c**.

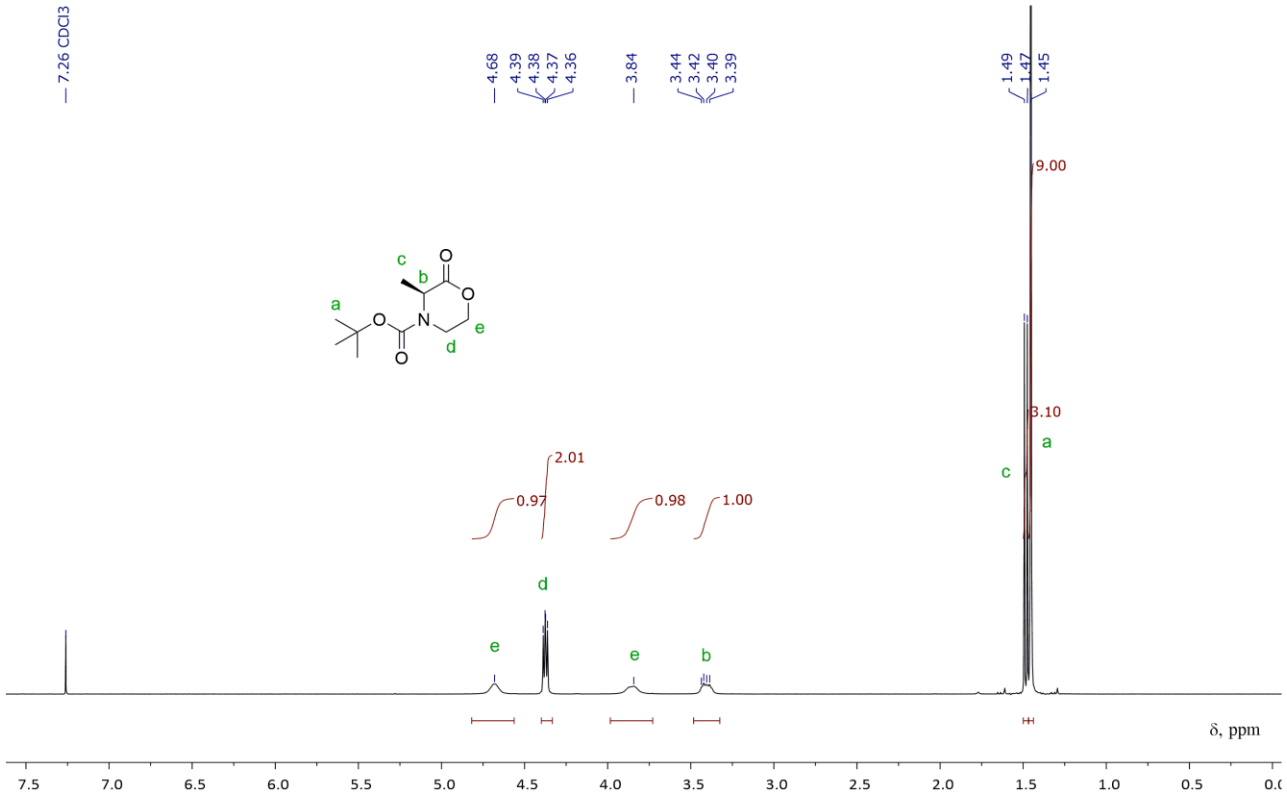


Fig. S14. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **M2**.

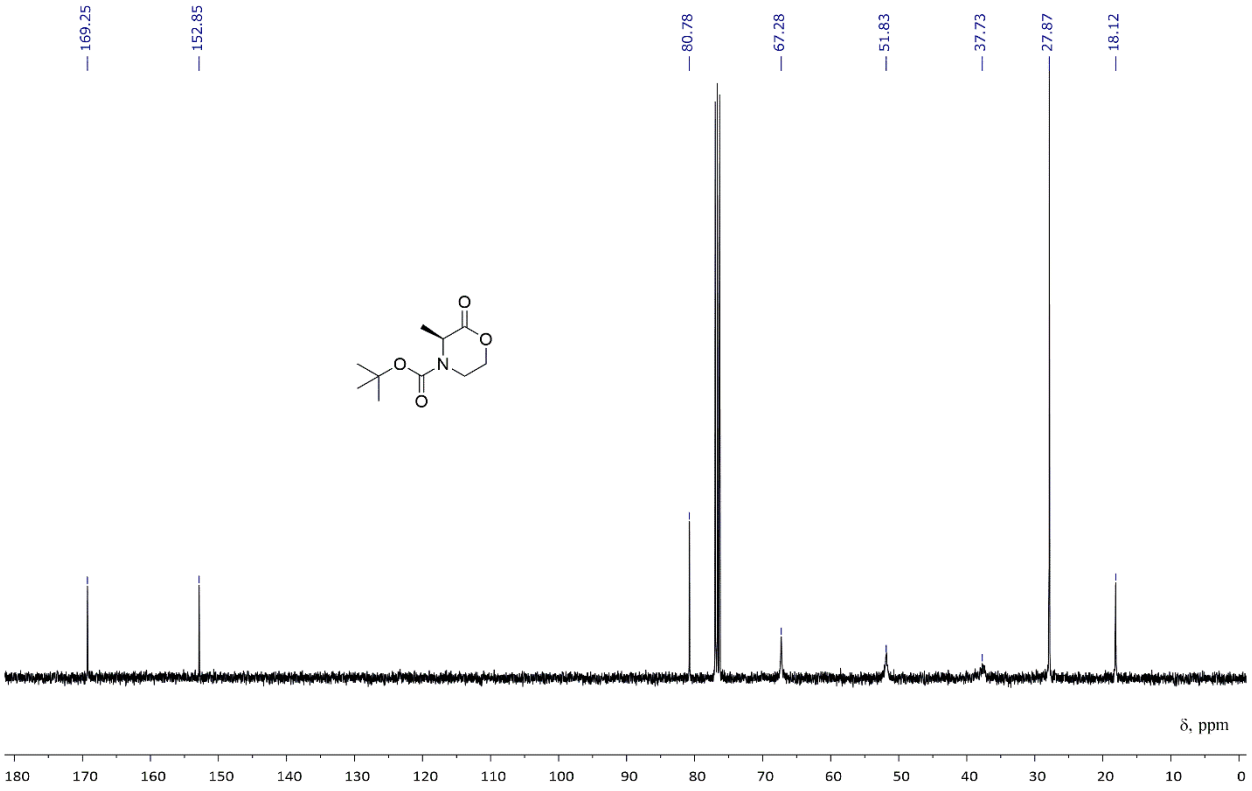


Fig. S15. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **M2**.

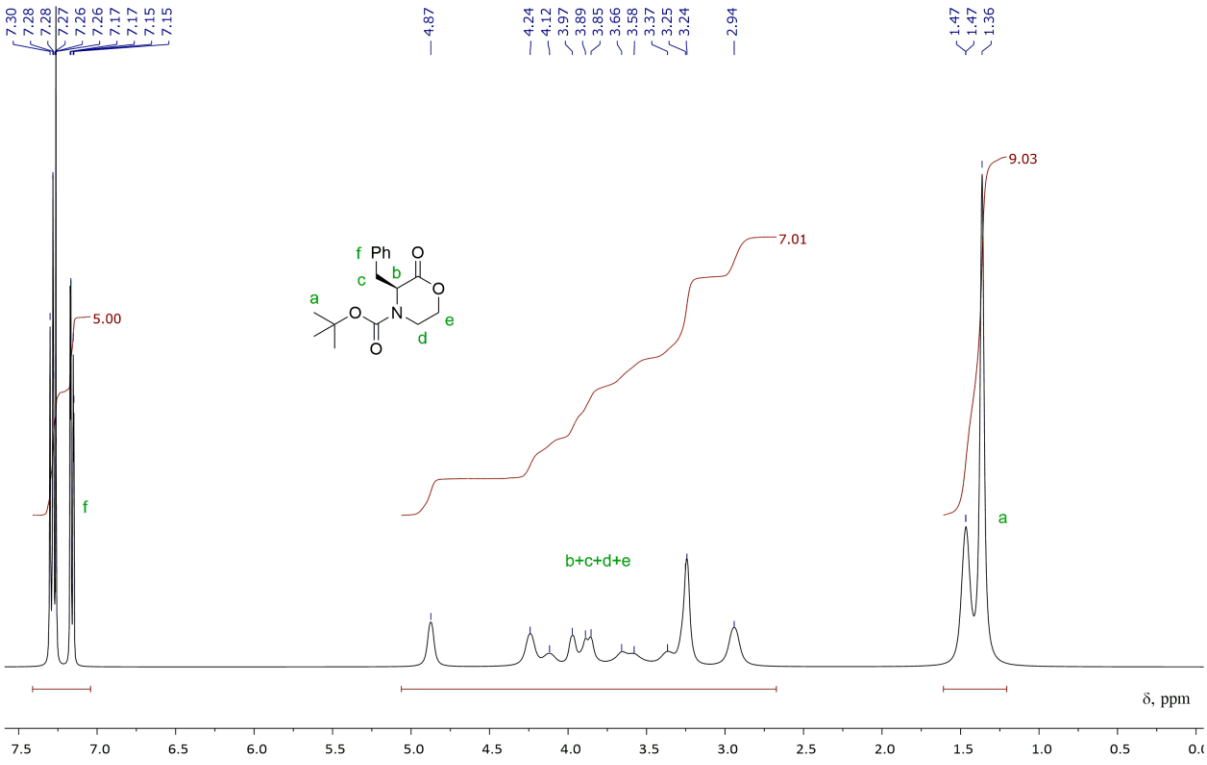


Fig. S16. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **M3**.

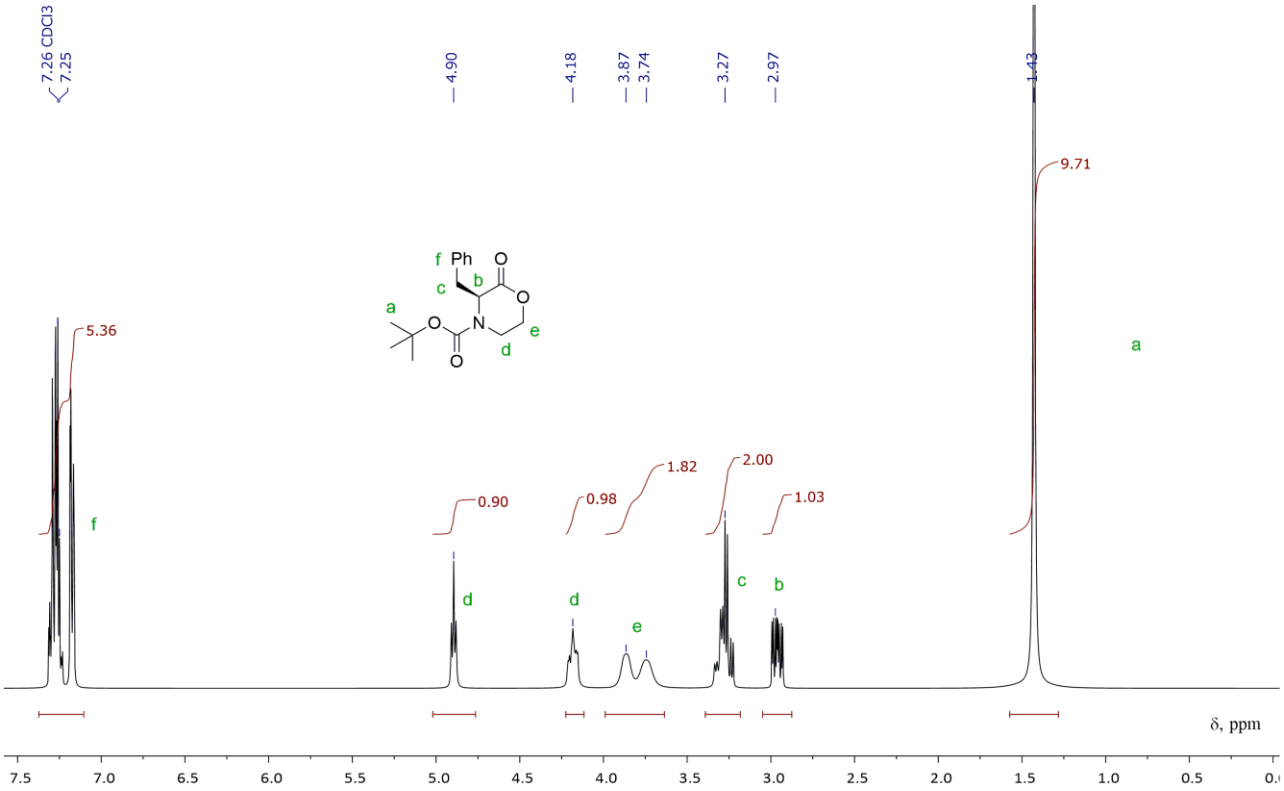


Fig. S17. ^{1}H NMR spectrum ($CDCl_3$, 55 °C, 400 MHz) of **M3**.

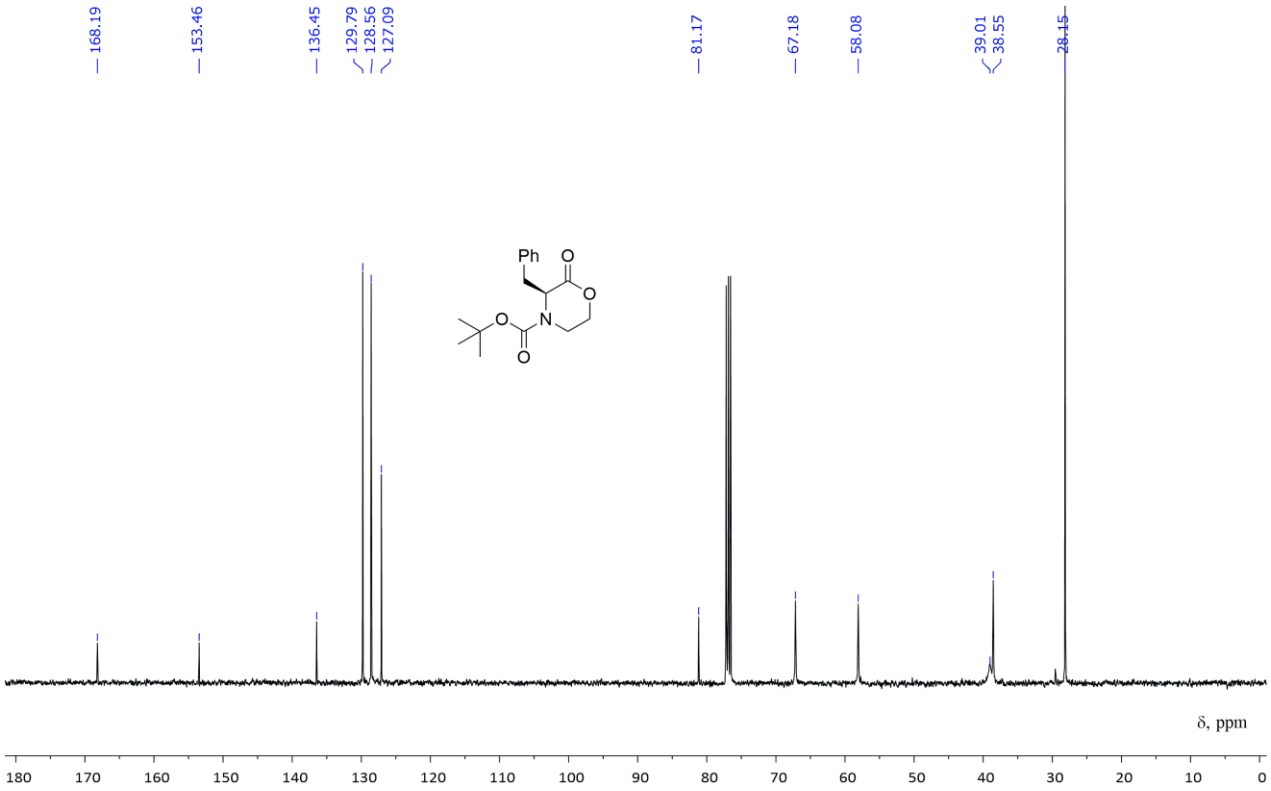


Fig. S18. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **M3**.

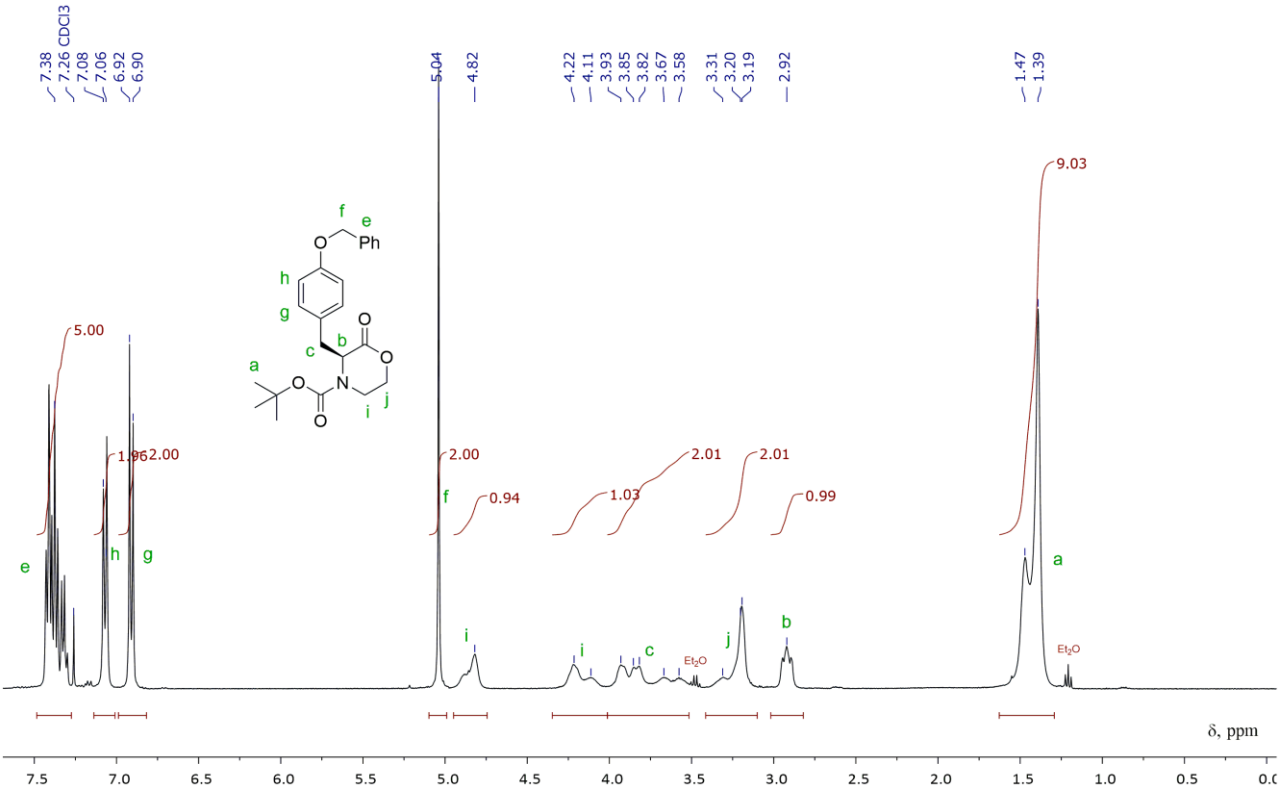


Fig. S19. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **M4**.

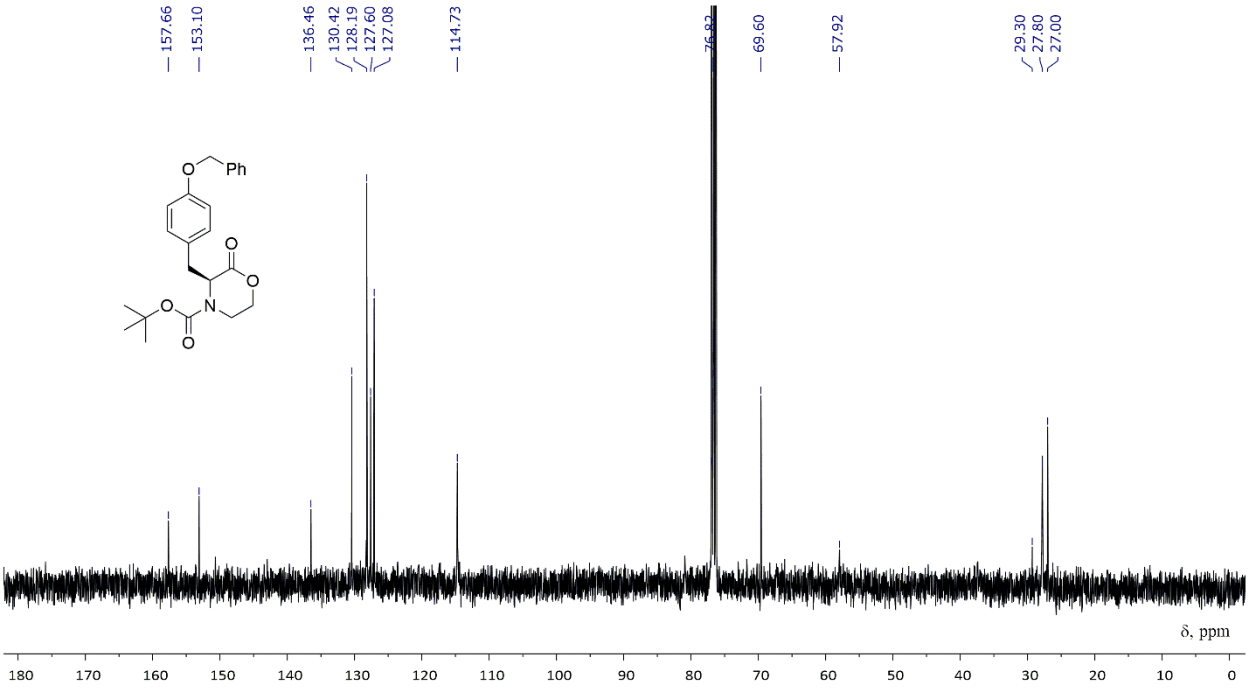


Fig. S20. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **M4**.

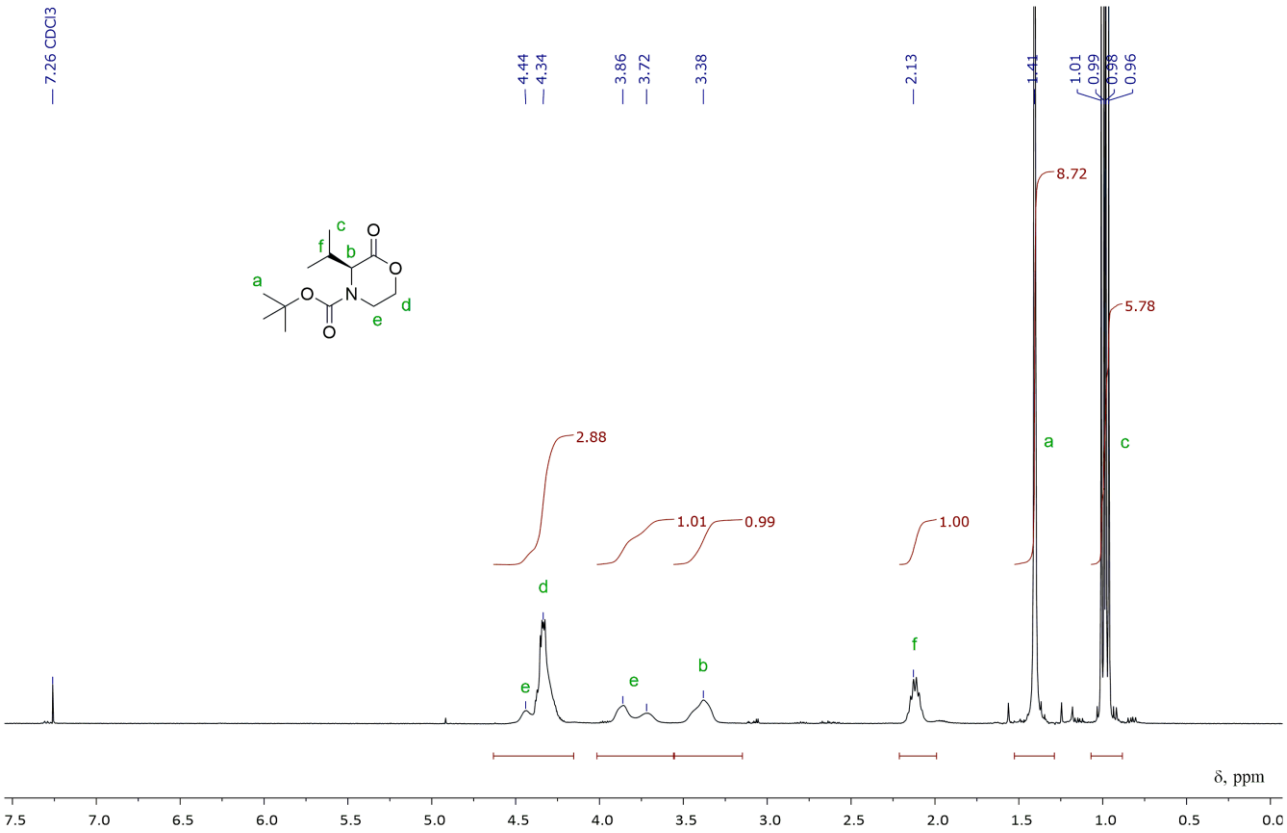


Fig. S21. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **M5**.

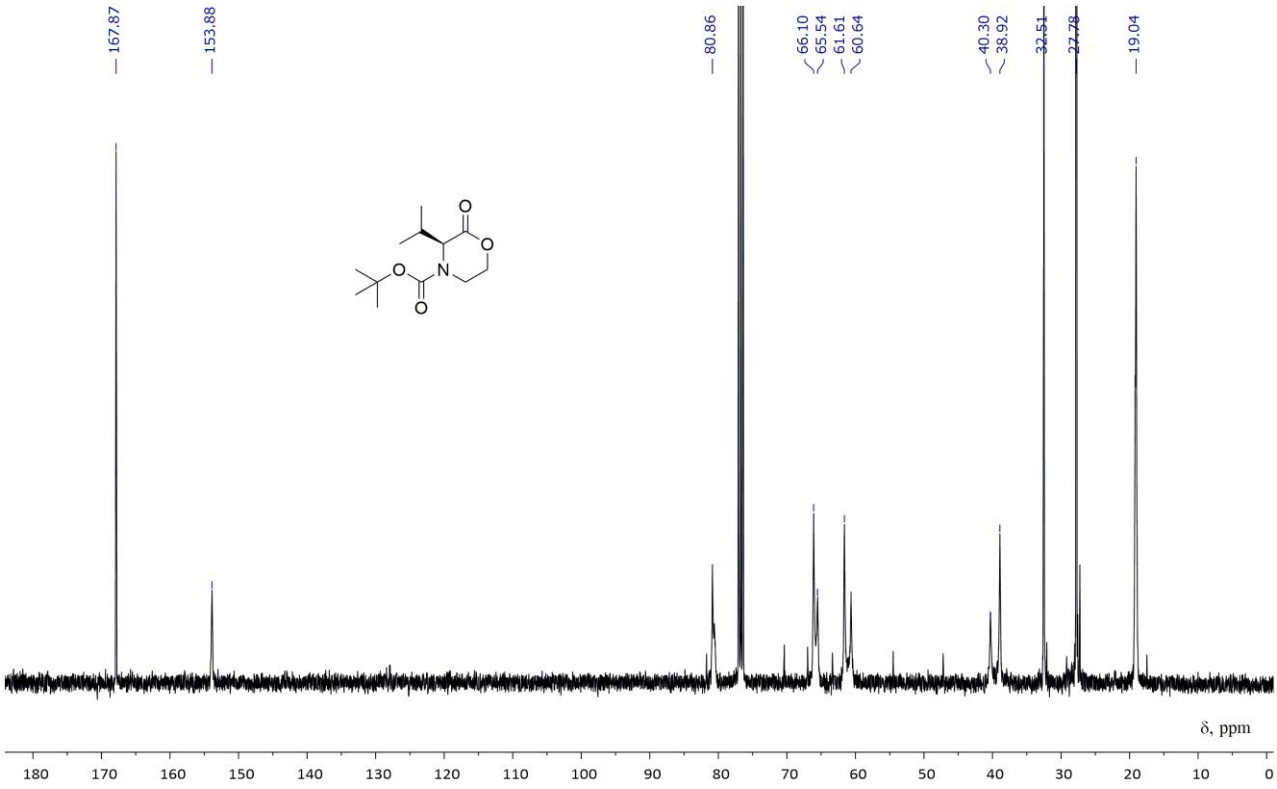


Fig. S22. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **M5**.

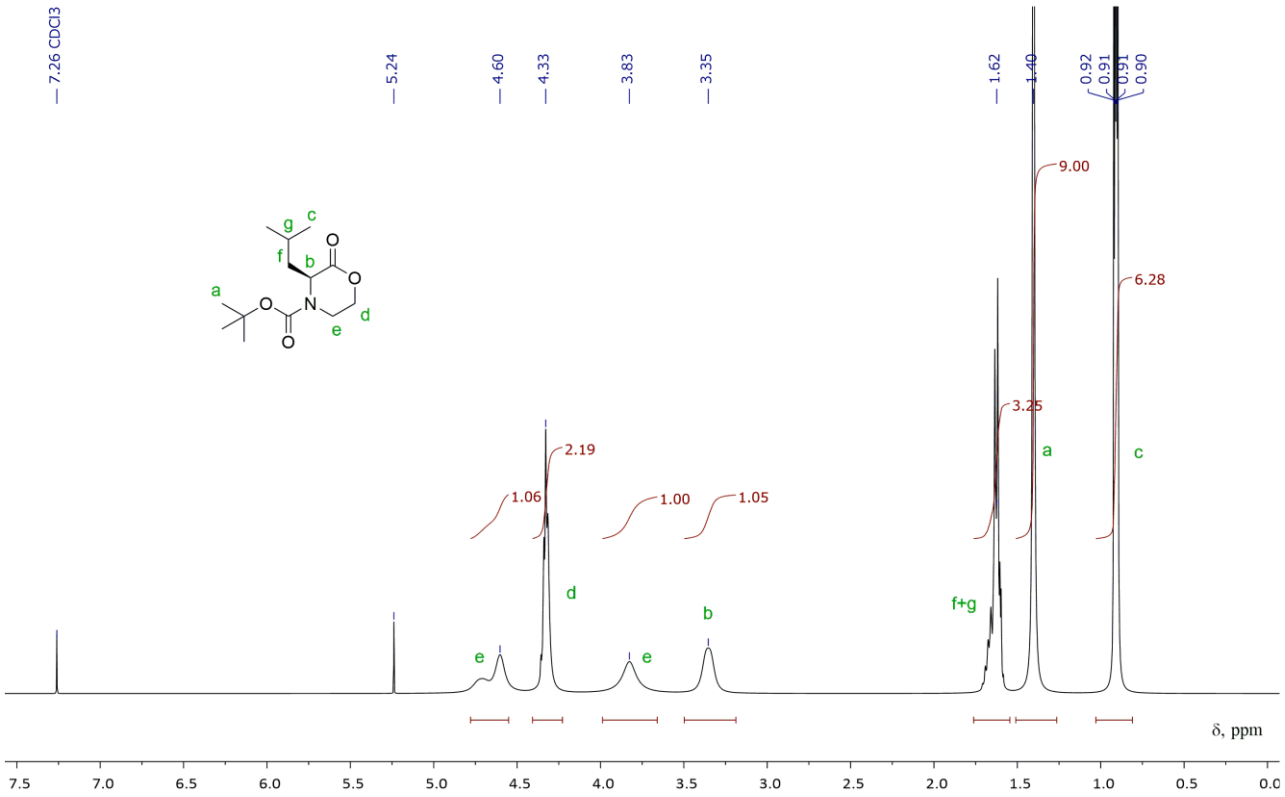


Fig. S23. ^{1}H NMR spectrum ($CDCl_3$, 20 °C, 400 MHz) of **M6**.

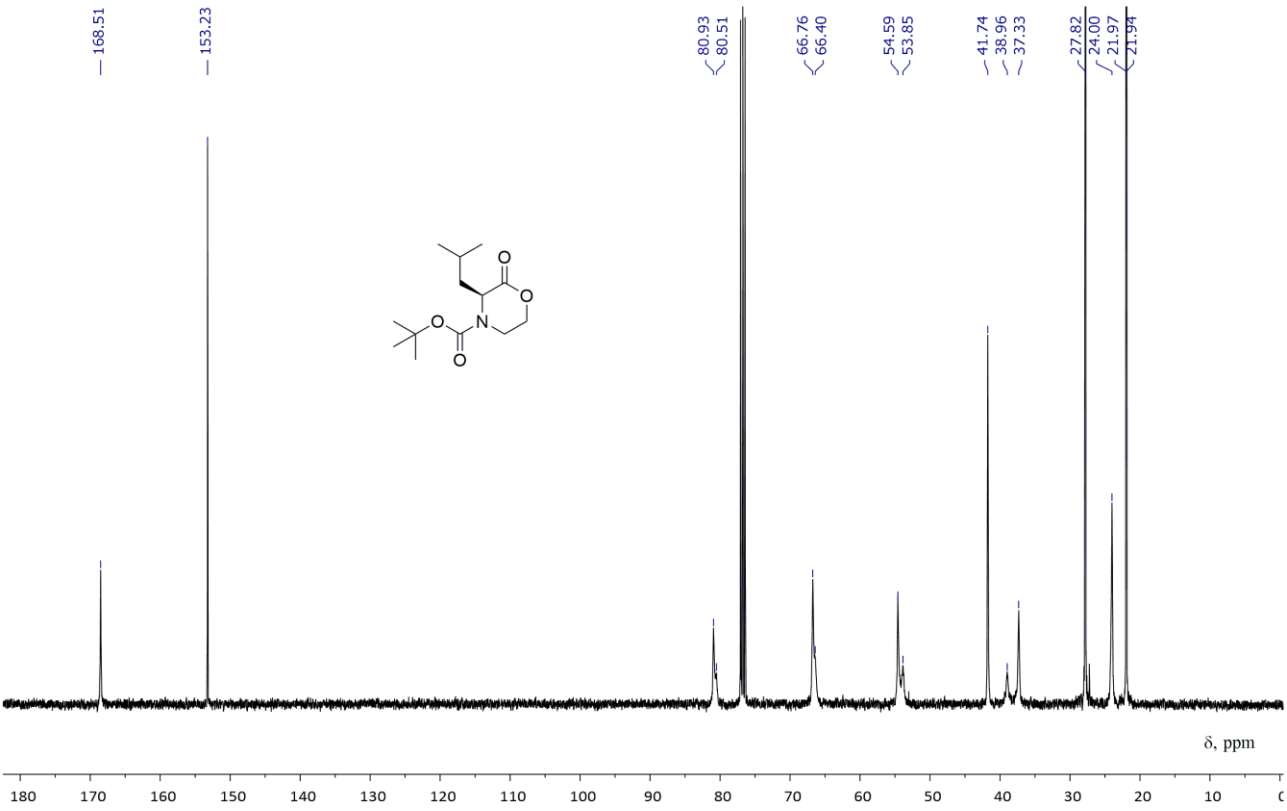


Fig. S24. ^{13}C NMR spectrum ($CDCl_3$, 20 °C, 101 MHz) of **M6**.

References

S1. Y. Lim, Y. H. Choi and J. Park, *J. Am. Chem. Soc.*, 1999, **121**, 5633.

S2. Y. Lim, C. Kim, K. Kim, S. W. Kim and J. Park, *J. Am. Chem. Soc.*, 2000, **122**, 6524.

S3. C. de Gracia Lux and A. Almutairi, *ACS Macro Lett.*, 2013, **2**, 432.

S4. E. I. Geihe, C. B. Cooley, J. R. Simon, M. K. Kiesewetter, J. A. Edward, R. P. Hickerson, R. L. Kaspar, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *Proc. Natl. Acad. Sci.*, 2012, **109**, 13171.

S5. S. Ghosh and S. Ramakrishnan, *Angew. Chem., Int. Ed.*, 2005, **44**, 5441.

S6. N. Maulucci, I. Izzo, G. Bifulco, A. Aliberti, C. De Cola, D. Comegna, C. Gaeta, A. Napolitano, C. Pizza, C. Tedesco, D. Flot and F. De Riccardis, *Chem. Commun.*, 2008, 3927.