Supplementary Information for:

Quantitative helix handedness bias through a single H vs. CH_3 stereochemical differentiation

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Contents

List of Abbreviations

AcOH acetic acid

CD circular dichroism

CyHex Cyclohexane

DCM dichloromethane

DIAD diisopropyl azodicarboxylate

DIPEA *N*,*N*-diisopropylethylamine

DMF *N*,*N*-dimethylformamide

DMSO dimethyl sulfoxide

EI electron ionization

ESI electrospray ionization

EtOAc ethylacetate

Fmoc fluorenylmethoxycarbonyl

HMBC heteronuclear multiple bond correlation

HMQC heteronuclear multiple quantum correlation

HPLC high performance liquid chromatography

HRMS high resolution mass spectrometry

MeOH methanol

MW molecular weight

NMR nuclear magnetic resonance

RP reversed phase

SPFS solid phase foldamer synthesis

TEA triethylamine

TFA trifluoroacetic acid

THF tetrahydrofuran

TIPS triisopropyl silane

TLC thin layer chromatography

TMSP 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt

UV/Vis ultraviolet–visible

1 Supplementary Figures

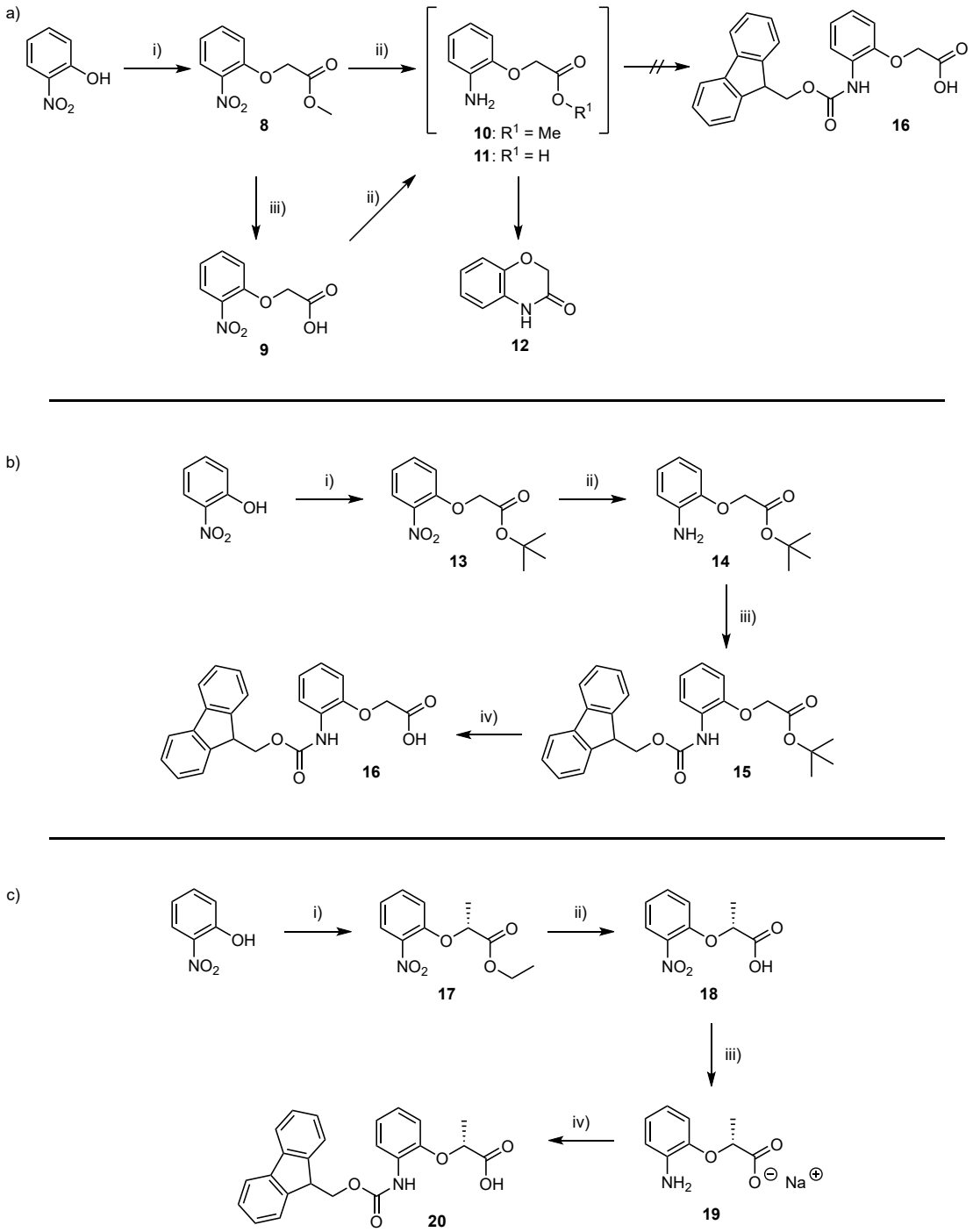


Fig. S1 (a) Failed attempts to synthesize Fmoc-$\mathbf{B}^{\mathbf{Gly}}$-OH: i) methyl bromoacetate, K_2CO_3, acetone. ii) H_2, Pd/C, EtOAc or THF. iii) LiOH, H_2O, THF. (b) Synthesis route to Fmoc-$\mathbf{B}^{\mathbf{Gly}}$-OH: i) *tert*-butyl bromoacetate, K_2CO_3, acetone. ii) H_2, Pd/C, ethanol. iii) Fmoc-Cl, DIPEA, DCM. iv) TIPS, TFA, DCM. (c) Synthesis route to Fmoc-$\mathbf{B}^{\mathbf{Rme}}$-OH: i) (-)-ethyl L-lactate, PPh_3, DIAD, THF. ii) LiOH, H_2O, THF. iii) H_2, Pd/C, Na_2CO_3, MeOH. iv) Fmoc-Cl, $NaHCO_3$, H_2O, 1,4-dioxane. For detailed synthetic procedures see section 3.3

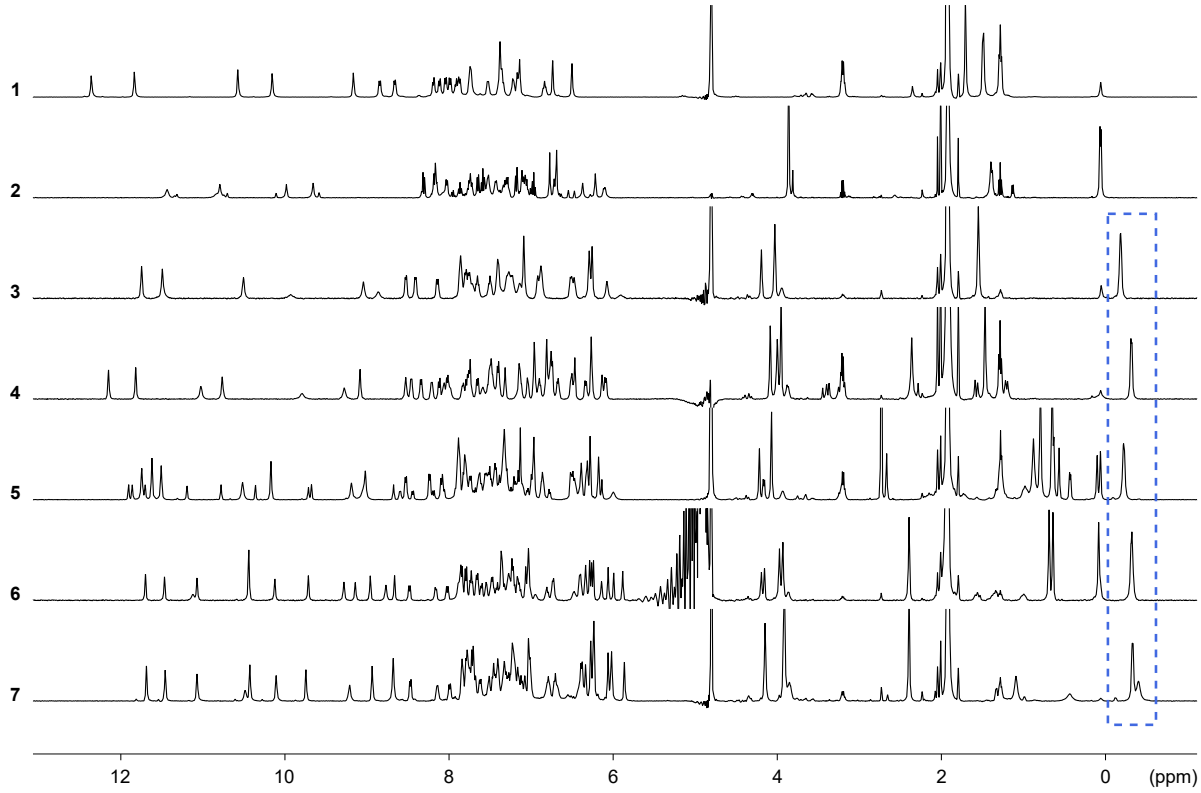


Fig. S2 ^{1}H NMR spectra of oligomers **1**–**7** in 12 mM NH_4OAc buffer with water suppression (**1**: 0.13 mM, **2**: 0.5 mM, **3**: 0.39 mM, **4**: 0.1 mM, **5**: 0.37 mM, **6**: 0.54 mM, **7**: 0.32 mM). The chiral CH_3 groups of $\mathbf{B^{Rme}}$ in oligomers **3**–**7** are highlighted with a dashed rectangle.

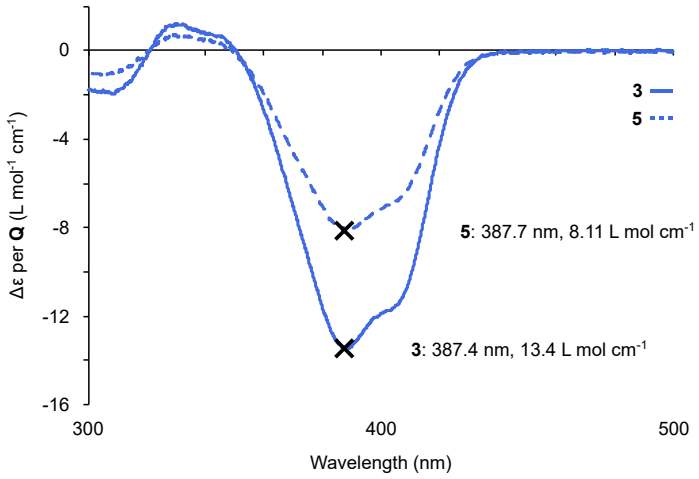


Fig. S3 CD spectra of oligomers **3** and **5**. Maxima are marked with crosses and their respective x and y values are displayed.

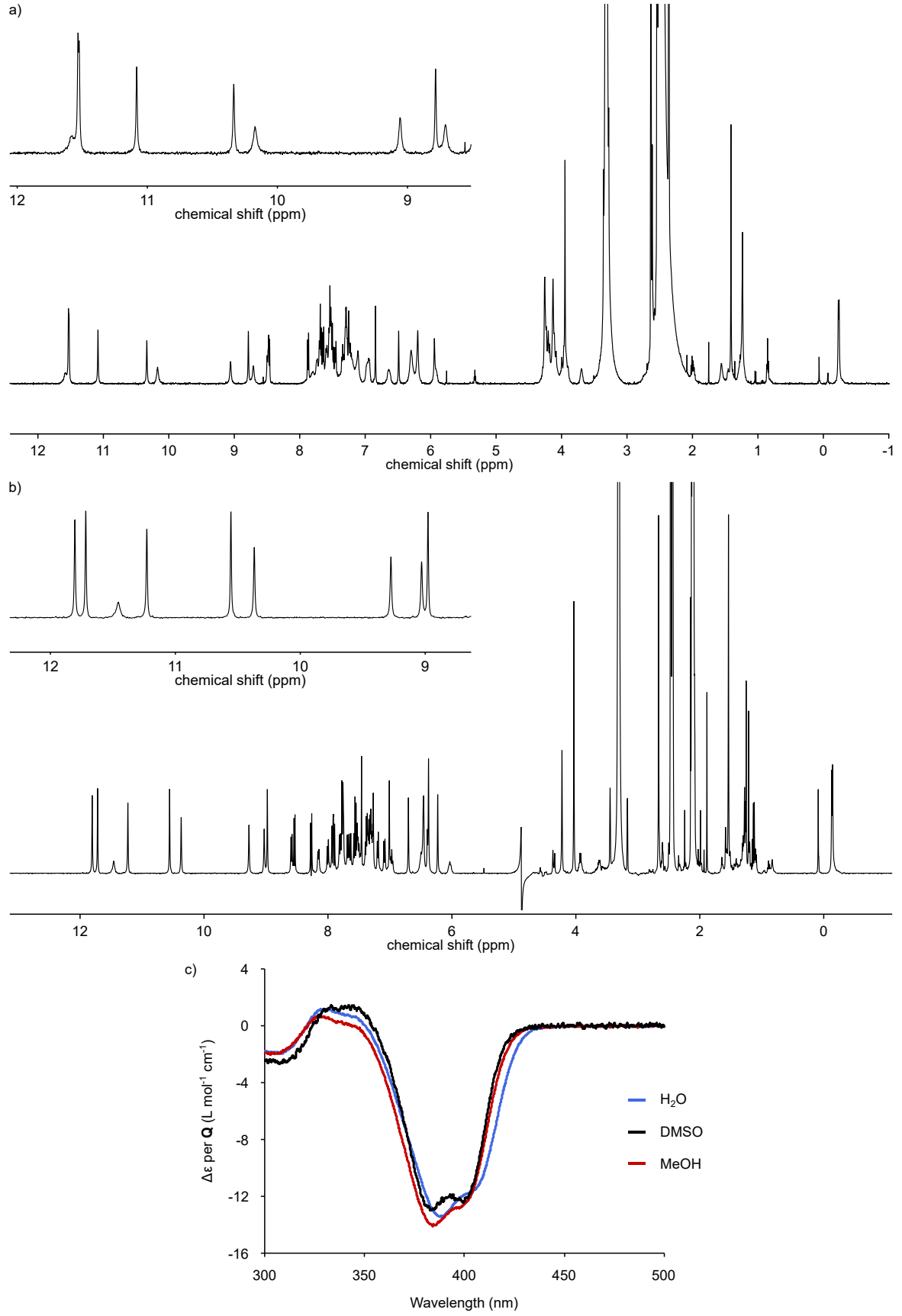


Fig. S4 ^{1}H NMR spectra of oligomer **3**: a) 0.4 mM (500 MHz, DMSO-d_6) and b) 0.4 mM (500 MHz, MeOH-d_3, OH-suppression). A zoom of the amide region is shown above the specta, respectively. c) CD spectra of oligomer **3**: 22.6 µM in H_2O (blue), 6.85 µM in DMSO (black) and 19.3 µM in MeOH (red).

2 Chiral derivatisation experiment

The chiral monomer Fmoc-$\mathbf{B^{Rme}}$-OH was coupled to the chiral amine **21** to test if racemization occurred during its synthesis (Fig. S5). Four different products can be expected for this reaction and their ratios are determined by the optical purity of the starting materials. Therefore, knowing the enantiomeric purity of **21** ($\geq$99%), the enantiomeric purity of **20** can be estimated.

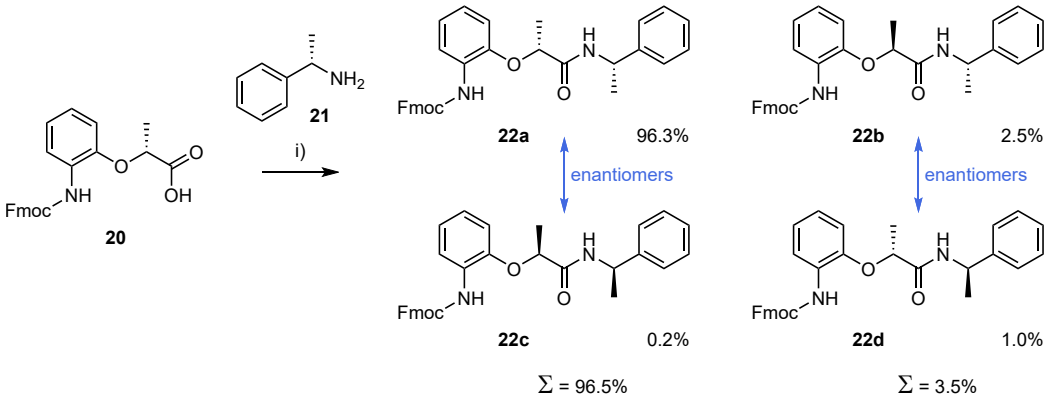


Fig. S5 Reaction scheme for the coupling of monomer **20** to the chiral amine **21** (enantiomeric purity: $\geq$99%). i) Ghosez's reagent, THF. The expected ratios of products **22a**–**d**, based on the enantiomeric purities given by the supplier, are shown next to the respective structures.

HPLC analysis of the chiral derivatisation crude shows that two diastereomeric products were formed with a ratio of 98/2, even exceeding the expected ratio of 96.5/3.5 (Fig. S6). This shows that the initial enantiomeric ratio of the chiral precursor of **20** (given by the supplier as $\geq$97.5/2.5) was preserved throughout its synthesis.

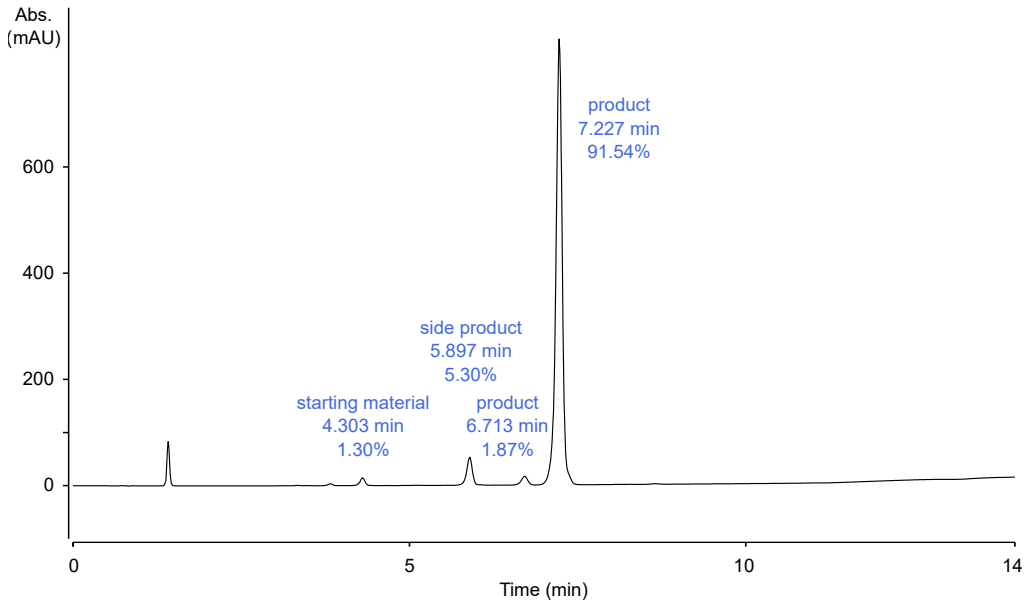


Fig. S6 HPLC analysis (C18, 55–80B, 25 °C; A: H_2O + 0.1% TFA, B: acetonitrile + 0.1% TFA) of the chiral derivatisation of **20**. Peaks were assigned by isolation via semi-prep HPLC (C18, 55–80B, 25 °C; A: H_2O + 0.1% TFA, B: acetonitrile + 0.1% TFA) followed by mass analysis. Assignments, retention times and relative areas are given next to the respective peaks.

3 Materials and Methods

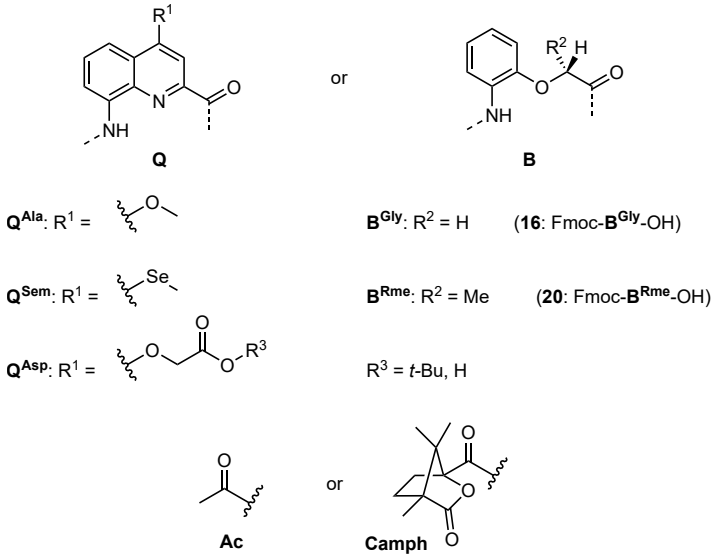


Fig. S7 Summary of monomers and N-terminal groups used in SPFS. Fmoc-$\mathbf{Q}^{\mathbf{Ala}}$-OH and Fmoc-$\mathbf{Q}^{\mathbf{Asp}}$(*t*-Bu)-OH have been described previously.[1,2] The synthesis of Fmoc-$\mathbf{Q}^{\mathbf{Sem}}$-OH will be published elsewhere. Fmoc-$\mathbf{B}^{\mathbf{Gly}}$-OH (**16**) and Fmoc-$\mathbf{B}^{\mathbf{Rme}}$-OH (**20**) are described here (section 3.3).

3.1 General

Commercial reagents (Suppliers: Abcr, Fisher Scientific, Merck, Sigma-Aldrich, TCI or VWR) were used without further purification unless otherwise stated. Wang resin LL (100–200 mesh) was purchased from Novabiochem. Peptide grade *N*,*N*-dimethylformamide (DMF) was purchased from Carlo Erba. Anhydrous chloroform, triethylamine (TEA) and *N*,*N*-diisopropylethylamine (DIPEA) were obtained via distillation over CaH_2 prior to use. Anhydrous tetrahydrofuran (THF) and dichloromethane (DCM) were obtained via an MBRAUN SPS-800 solvent purification system. Ultrapure water was obtained via a Sartorius arium® pro VF ultrapure water system. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60-F254 plates and observed under UV light. Column chromatography purifications were carried out on Merck GEDURAN Si60 (40–63 µm). Nuclear magnetic resonance (NMR) spectra were recorded on an Avance III HD 400 MHz Bruker BioSpin spectrometer or an Avance III HD 500 MHz Bruker BioSpin spectrometer equipped with a broad band observe 5-mm BB-H&F-D CryProbe™ Prodigy. Measurements were performed at 25 °C unless stated otherwise. Water suppression was performed with excitation sculpting. Processing was done with MestReNova (v.12.0.0-20080) NMR processing software from Mestrelab Research. Chemical shifts are reported in ppm and calibrated via residual solvent signals or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) when water suppression was applied.[3,4] Signal multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet, and m, multiplet. Signals were assigned using ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC spectra. Electrospray ionization (ESI) mass spectra were recorded on Bruker microTOF II and Thermo Finnigan LTQ FT Ultra spectrometers. Electron ionization (EI) mass spectra were recorded on a Thermo Q Exactive GC Orbitrap or a Finnigan MAT 95 sector mass spectrometer. Analytical and semi-preparative reversed phase (RP) high performance liquid chromatography (HPLC) was performed on a

Thermo Fisher Scientific Ultimate 3000 HPLC System using Macherey-Nagel Nucleodur C18 Gravity columns (4 × 100 mm, 5 µm and 10 × 250 mm, 5 µm) and Macherey-Nagel Nucleodur C8 Gravity columns (4 × 50 mm, 5 µm and 10 × 100 mm, 5 µm). UV absorbance was monitored at 300 nm if not stated otherwise. Simple ultraviolet–visible (UV/Vis) absorbance measurements were done with a Thermo Fisher Scientific Nanodrop One instrument using a 1 cm quartz cuvette. Circular dichroism (CD) spectra were measured on a Jasco J-810 spectrometer. Measurements were performed at 20 °C if not stated otherwise. Microwave-assisted solid phase foldamer synthesis (SPFS) was performed via a CEM® Discover Bio manual microwave peptide synthesizer. The temperature within the reactor vessel was monitored with an optical fiber probe.

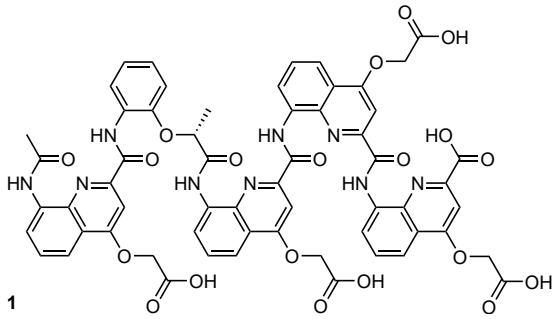
3.2 Solid phase synthesis procedures

Oligomers were synthesized according to previously reported SPFS protocols,[2,5] hereafter referred to as standard method. Fmoc acid building blocks were activated *in situ* by generating the respective acid chlorides prior to coupling.

Capping: The resin (1.0 equiv.) was washed with DCM (3×) and incubated in acetic anhydride in DCM (50% v/v) for 10 min. Then, the resin was washed with DCM (2×), DMF (3×) and kept suspended in DMF (if stored longer than 24 h, it was kept at 4 °C).

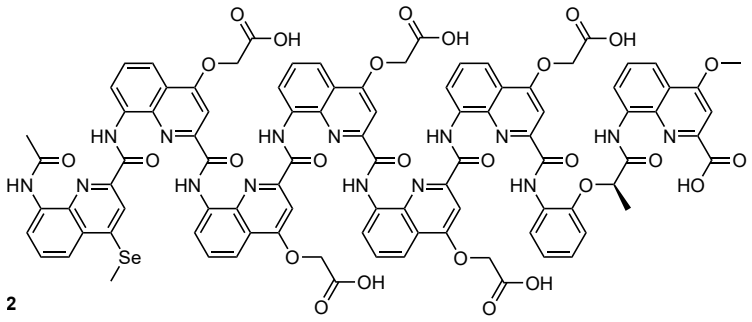
Acetylation: In the microwave vessel: after the resin (1.0 equiv.) was washed with anhydrous THF (4×), DIPEA (10.0 equiv.) and acetyl chloride (5.0 equiv.) in anhydrous THF (1 mL per 100 mg resin; not less than 2 mL) were added and the suspension was heated to 50 °C for 15 min (25 W, ramp to 50 °C over 5 min, hold at 50 °C for 15 min). The resin was washed with anhydrous THF (3×) and the coupling step was repeated once. Then, the resin was washed again with anhydrous THF (1×) and DMF (5×), and kept suspended in DMF (if stored longer than 24 h, it was kept at 4 °C).

Camphanylation: In the microwave vessel: after the resin (1.0 equiv.) was washed with anhydrous THF (4×), DIPEA (10.0 equiv.) and camphanic chloride (5.0 equiv.) in anhydrous THF (1 mL per 100 mg resin; not less than 2 mL) were added and the suspension was heated to 50 °C for 15 min (25 W, ramp to 50 °C over 5 min, hold at 50 °C for 15 min). The resin was washed with anhydrous THF (3×) and the coupling step was repeated once. Then, the resin was washed again with anhydrous THF (1×) and DMF (5×), and kept suspended in DMF (if stored longer than 24 h, it was kept at 4 °C).



1

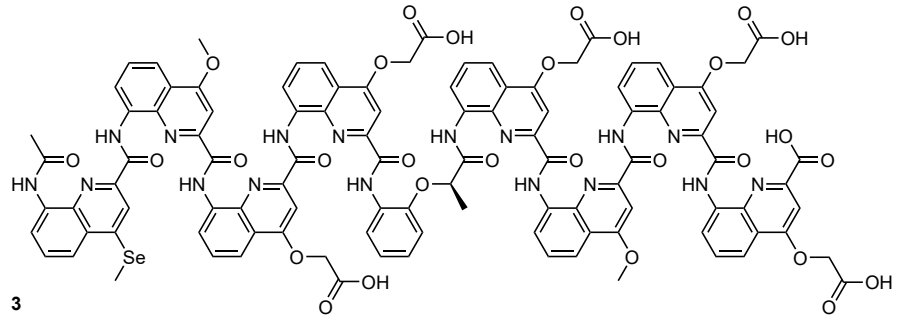
Compound 1: Oligomer **1** was synthesized on Wang resin (0.37 mmol g^{-1}, 9.25 µmol scale) according to the standard method. Loading of the first monomer: 0.21 mmol g^{-1} (56%). Unreacted amines were capped using the general capping method after each coupling step. The final acetyl group was installed via the general acetylation method. After purification by semi-prep HPLC (C8, 0–30B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (0.937 mg, 0.781 µmol, 8.4%; HPLC-purity: 98.6%). **^{1}H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 12.35$ (s, 1H), 11.82 (s, 1H), 10.56 (s, 1H), 10.14 (s, 1H), 9.15 (s, 1H), 8.83 (d, $J = 8.79$ Hz, 1H), 8.65 (d, $J = 8.55$ Hz, 1H), 8.18 (d, $J = 9.16$ Hz, 1H), 8.10 (d, $J = 9.47$ Hz, 1H), 8.03 (d, $J = 9.24$ Hz, 1H), 7.97 (d, $J = 9.32$ Hz, 1H), 7.94–7.82 (m, 2H), 7.78–7.66 (m, 2H), 7.51 (d, $J = 8.97$ Hz, 1H), 7.42–7.27 (m, 4H), 7.20 (d, $J = 8.08$ Hz, 1H), 7.18–7.07 (m, 2H), 6.82 (t, $J = 8.64$ Hz, 1H), 6.72 (s, 1H), 6.49 (s, 1H), 3.19 (q, $J = 8.26$ Hz, 2H), 1.69 (s, 4H), 1.48 (d, $J = 6.62$ Hz, 4H), 1.27 (t, $J = 8.06$ Hz, 4H). **HRMS** (ESI^-) *m/z* calcd. for $C_{59}H_{44}N_9O_{20}$: 1198.2708 $(M\text{-}H)^-$; found: 1198.3269.



2

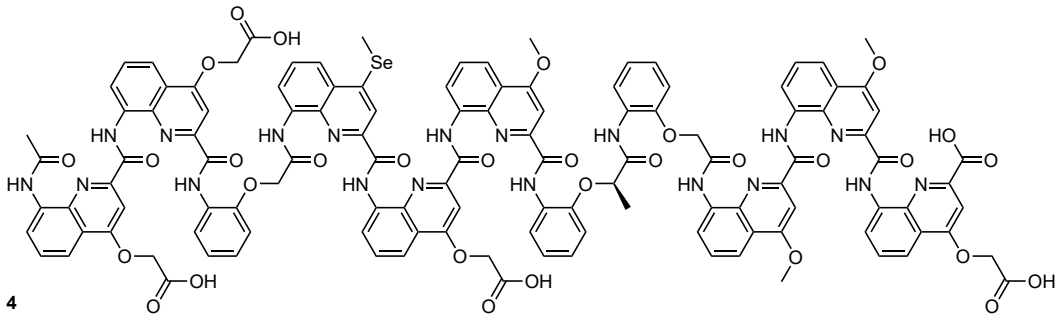
Compound 2: Oligomer **2** was synthesized on Wang resin (0.44 mmol g^{-1}, 17.6 µmol scale) according to the standard method. Loading of the first monomer: 0.44 mmol g^{-1} (100%). The final acetyl group was installed via the general acetylation method. After purification by semi-prep HPLC (C8, 0–20B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (17.2 mg, 9.03 µmol, 51%; HPLC-purity: 97.3%). **^{1}H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 11.44$ (s, 1H), 11.35 (s, 1H), 11.32 (s, 1H), 10.93–10.73 (m, 1H), 10.70 (s, 1H), 10.11 (s, 1H), 9.98 (s, 1H), 9.66 (s, 1H), 9.58 (s, 1H), 8.37–8.25 (m, 2H), 8.25–8.12 (m, 3H), 8.12–7.98 (m, 2H), 7.95 (t, $J = 8.56$ Hz, 1H), 7.87 (t, $J = 8.96$ Hz, 1H), 7.81 (d, $J = 9.15$ Hz, 1H), 7.79–7.69 (m, 2H), 7.65 (d, $J = 8.37$ Hz, 1H), 7.59 (t, $J = 9.10$ Hz, 1H), 7.53 (d, $J = 8.32$ Hz, 1H), 7.48–7.39 (m, 1H), 7.37 (d, $J = 8.52$ Hz, 1H), 7.36–7.24 (m, 2H), 7.19 (s, 1H), 7.17 (s, 1H), 7.15–7.02 (m, 3H), 7.01 (s, 1H), 6.97 (t, $J = 8.54$ Hz, 1H), 6.77 (s, 2H), 6.72 (s, 1H), 6.69 (s, 2H), 6.64 (d, $J = 9.48$ Hz, 1H), 6.55 (s, 1H), 6.47 (s, 1H), 6.37 (s, 1H), 6.28 (s, 1H), 6.22 (s, 1H), 6.16–6.02 (m, 1H), 3.86

(s, 5H), 3.81 (s, 1H), 1.39 (d, $J = 9.70$ Hz, 5H), 1.13 (d, $J = 7.20$ Hz, 1H), 0.06 (d, $J = 6.60$ Hz, 6H). **HRMS** (ESI$^-$) *m/z* calcd. for $C_{93}H_{68}N_{15}O_{27}Se$: 1906.3580 (M-H)$^-$; found: 1906.4064.



3

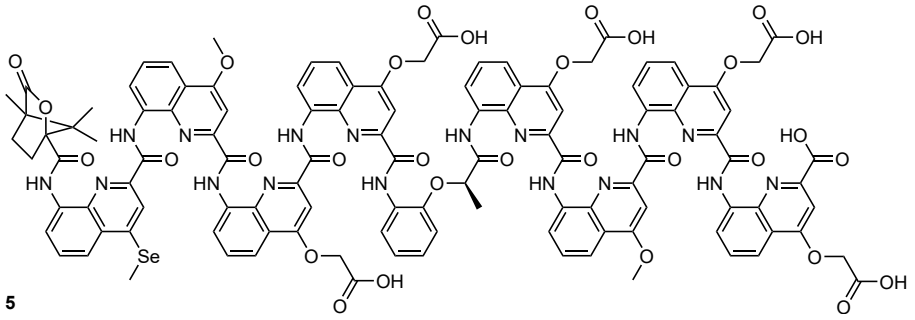
Compound 3: Oligomer **3** was synthesized on Wang resin (0.37 mmol g^{-1}, 22.0 µmol scale) according to the standard method. Loading of the first monomer: 0.34 mmol g^{-1} (92%). Unreacted amines were capped using the general capping method after each coupling step. The final acetyl group was installed via the general acetylation method. After purification by semi-prep HPLC (C18, 0–50B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (18.1 mg, 8.58 µmol, 39%; HPLC-purity: 99.2%). ^{1}**H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 11.75$ (s, 1H), 11.49 (s, 1H), 10.51 (s, 1H), 9.93 (s, 1H), 9.04 (s, 1H), 8.86 (s, 1H), 8.53 (d, $J = 8.53$ Hz, 1H), 8.41 (d, $J = 8.35$ Hz, 1H), 8.14 (d, $J = 9.79$ Hz, 1H), 7.86 (s, 2H), 7.80 (d, $J = 8.75$ Hz, 1H), 7.78–7.68 (m, 1H), 7.66 (d, $J = 9.21$ Hz, 1H), 7.50 (s, 1H), 7.40 (s, 1H), 7.34–7.18 (m, 2H), 7.14 (s, 1H), 7.09 (s, 2H), 6.98–6.79 (m, 3H), 6.58–6.41 (m, 2H), 6.29 (s, 2H), 6.26 (s, 1H), 6.08 (s, 1H), 5.91 (s, 1H), 4.19 (s, 1H), 4.03 (s, 2H), 3.95 (s, 1H), 1.55 (s, 3H), -0.18 (s, 3H). **HRMS** (ESI$^-$) *m/z* calcd. for $C_{104}H_{76}N_{17}O_{29}Se$: 2106.4166 (M-H)$^-$; found: 2106.4162.



4

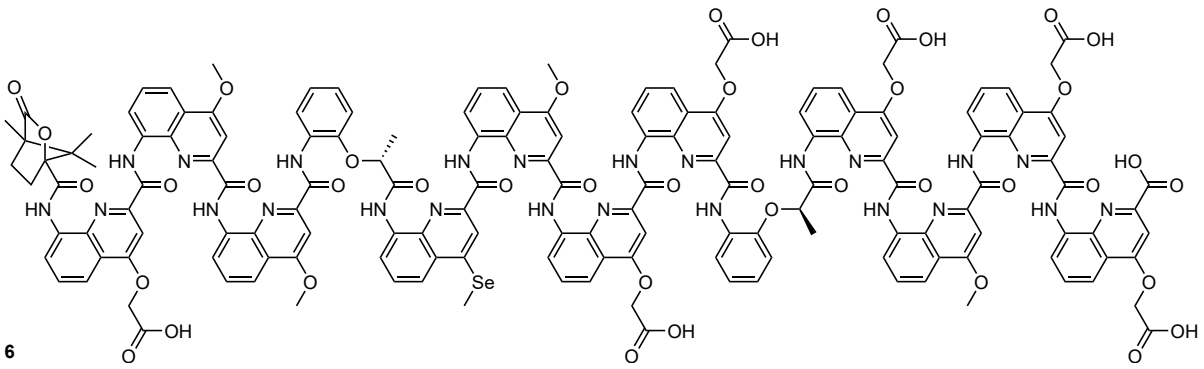
Compound 4: Oligomer **4** was synthesized on Wang resin (0.37 mmol g^{-1}, 18.5 µmol scale) according to the standard method. Loading of the first monomer: 0.33 mmol g^{-1} (89%). Unreacted amines were capped using the general capping method after each coupling step. The final acetyl group was installed via the general acetylation method. After purification by semi-prep HPLC (C18, 10–20B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (9.75 mg, 4.13 µmol, 22%; HPLC-purity: 99.9%). ^{1}**H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 12.15$ (s, 1H), 11.82 (s, 1H), 11.02 (s, 1H), 10.76 (s, 1H), 9.79 (s, 1H), 9.28 (s, 1H), 9.08 (s, 1H), 8.53 (s, 1H), 8.46 (d, $J = 8.29$ Hz, 1H), 8.34 (d, $J = 8.25$ Hz, 1H), 8.21 (d, $J = 8.27$ Hz, 1H), 8.12 (d, $J = 9.13$ Hz, 1H), 8.06 (s, 1H), 8.05–7.93 (m, 1H), 7.82 (s,

1H), 7.81–7.69 (m, 2H), 7.65 (d, $J = 9.10\,\mathrm{Hz}$, 1H), 7.59 (s, 1H), 7.56–7.44 (m, 2H), 7.40 (d, $J = 8.47\,\mathrm{Hz}$, 1H), 7.32 (s, 1H), 7.20–7.09 (m, 2H), 7.05 (s, 1H), 6.96 (s, 2H), 6.89 (t, $J = 7.97\,\mathrm{Hz}$, 1H), 6.81 (s, 1H), 6.79–6.72 (m, 3H), 6.68 (d, $J = 7.64\,\mathrm{Hz}$, 1H), 6.50 (s, 1H), 6.47 (s, 1H), 6.33 (d, $J = 9.08\,\mathrm{Hz}$, 1H), 6.27 (s, 2H), 6.13 (s, 1H), 6.09 (d, $J = 9.00\,\mathrm{Hz}$, 1H), 4.09 (s, 2H), 4.00 (s, 2H), 3.95 (s, 2H), 3.87 (d, $J = 7.59\,\mathrm{Hz}$, 1H), 3.38 (d, $J = 15.15\,\mathrm{Hz}$, 1H), 3.31–3.11 (m, 3H), 2.36 (s, 3H), 1.57 (d, $J = 15.40\,\mathrm{Hz}$, 1H), 1.47 (s, 3H), 1.28 (t, $J = 7.92\,\mathrm{Hz}$, 3H), 1.21 (d, $J = 13.39\,\mathrm{Hz}$, 1H), -0.32 (d, $J = 6.97\,\mathrm{Hz}$, 3H). **HRMS** (ESI$^-$) *m/z* calcd. for $C_{119}H_{90}N_{19}O_{31}Se$: 2360.5221 (M-H)$^-$; found: 2360.5251.



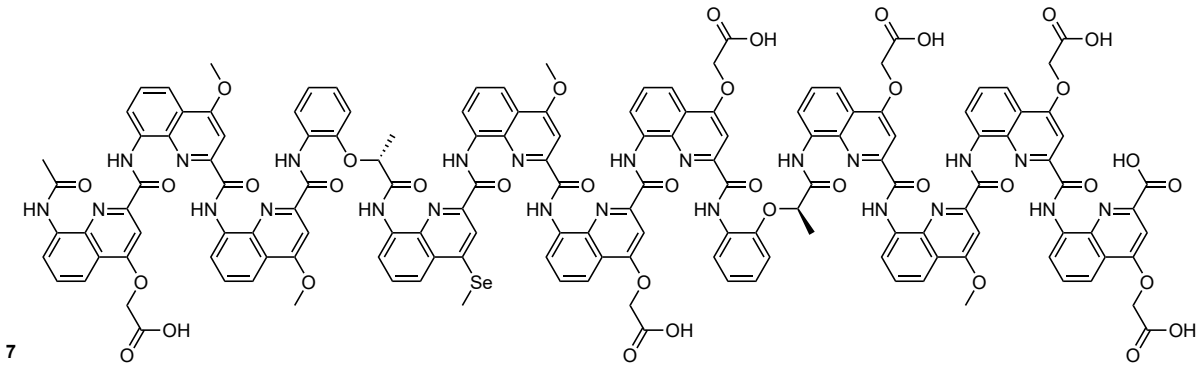
5

Compound 5: Oligomer **5** was synthesized on Wang resin (0.37 mmol g^{-1}, 22.0 µmol scale) according to the standard method. Loading of the first monomer: 0.34 mmol g^{-1} (92%). Unreacted amines were capped using the general capping method after each coupling step. The final camphanyl group was installed via the general camphanylation method. After purification by semi-prep HPLC (C18, 0–50B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (12.7 mg, 5.64 µmol, 26%; HPLC-purity: 98.0%). ^{1}**H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 11.90$ (s, 1H), 11.86 (s, 1H), 11.74 (s, 4H), 11.70 (s, 1H), 11.62 (s, 3H), 11.51 (s, 3H), 11.19 (s, 1H), 10.78 (s, 1H), 10.52 (s, 3H), 10.36 (s, 1H), 10.17 (s, 3H), 9.71 (s, 1H), 9.68 (s, 1H), 9.19 (s, 3H), 9.02 (s, 4H), 8.68 (s, 1H), 8.60 (d, $J = 8.31\,\mathrm{Hz}$, 1H), 8.53 (d, $J = 8.36\,\mathrm{Hz}$, 3H), 8.44 (d, $J = 8.41\,\mathrm{Hz}$, 1H), 8.24 (d, $J = 8.22\,\mathrm{Hz}$, 4H), 8.19 (d, $J = 9.22\,\mathrm{Hz}$, 1H), 8.14–8.00 (m, 4H), 7.97–7.85 (m, 11H), 7.85–7.77 (m, 7H), 7.74 (d, $J = 8.99\,\mathrm{Hz}$, 2H), 7.69 (d, $J = 8.76\,\mathrm{Hz}$, 1H), 7.67–7.59 (m, 3H), 7.59–7.48 (m, 3H), 7.43 (d, $J = 8.01\,\mathrm{Hz}$, 3H), 7.41–7.26 (m, 12H), 7.24 (d, $J = 8.56\,\mathrm{Hz}$, 1H), 7.22–7.08 (m, 7H), 7.05 (d, $J = 8.11\,\mathrm{Hz}$, 1H), 7.02–6.91 (m, 8H), 6.86 (t, $J = 8.11\,\mathrm{Hz}$, 4H), 6.77 (d, $J = 8.26\,\mathrm{Hz}$, 1H), 6.58–6.42 (m, 7H), 6.39 (s, 3H), 6.32 (d, $J = 8.23\,\mathrm{Hz}$, 4H), 6.28 (s, 4H), 6.18 (s, 4H), 6.14 (s, 1H), 6.00 (s, 2H), 4.22 (s, 4H), 4.16 (d, $J = 8.38\,\mathrm{Hz}$, 2H), 4.07 (s, 6H), 3.93 (s, 2H), 2.73 (s, 13H), 2.67 (s, 3H), 0.98 (s, 1H), 0.88 (s, 10H), 0.79 (s, 12H), 0.64 (d, $J = 10.55\,\mathrm{Hz}$, 16H), 0.57 (s, 3H), 0.43 (d, $J = 7.18\,\mathrm{Hz}$, 3H), 0.10 (s, 4H), 0.06 (s, 4H), -0.22 (d, $J = 7.00\,\mathrm{Hz}$, 9H). **HRMS** (ESI$^-$) *m/z* calcd. for $C_{112}H_{86}N_{17}O_{31}Se$: 2244.4846 (M-H)$^-$; found: 2244.5082.



6

Compound 6: Oligomer **6** was synthesized on Wang resin (0.37 mmol g^{-1}, 11.0 µmol scale) according to the standard method. Loading of the first monomer: 0.34 mmol g^{-1} (92%). Unreacted amines were capped using the general capping method after each coupling step. The final camphanyl group was installed via the general camphanylation method. After purification by semi-prep HPLC (C18, 10–35B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (4.65 mg, 1.52 µmol, 14%; HPLC-purity: 96.6%). **^{1}H NMR** (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): δ = 11.70 (s, 1H), 11.47 (s, 1H), 11.12 (s, 1H), 11.07 (s, 1H), 10.44 (s, 2H), 10.12 (s, 1H), 9.71 (s, 1H), 9.28 (s, 1H), 9.14 (s, 1H), 8.96 (s, 1H), 8.77 (s, 1H), 8.66 (s, 1H), 8.48 (d, J = 8.48 Hz, 1H), 8.16 (d, J = 8.20 Hz, 1H), 8.02 (d, J = 9.25 Hz, 1H), 7.93–7.82 (m, 2H), 7.79 (d, J = 8.93 Hz, 1H), 7.73 (t, J = 7.95 Hz, 1H), 7.66 (d, J = 9.17 Hz, 1H), 7.60 (d, J = 8.11 Hz, 1H), 7.55 (s, 1H), 7.48 (d, J = 7.48 Hz, 1H), 7.42 (d, J = 8.32 Hz, 1H), 7.40–7.31 (m, 2H), 7.27 (s, 1H), 7.25–7.19 (m, 1H), 7.19–7.09 (m, 1H), 7.07 (s, 1H), 7.06–6.98 (m, 3H), 6.95 (s, 1H), 6.80 (t, J = 8.58 Hz, 1H), 6.73 (s, 1H), 6.48 (s, 1H), 6.40 (d, J = 8.41 Hz, 2H), 6.34 (s, 1H), 6.29 (s, 1H), 6.26 (s, 1H), 6.24 (s, 1H), 6.14 (s, 1H), 6.06 (s, 1H), 5.99 (s, 1H), 5.88 (s, 1H), 4.17 (d, J = 18.58 Hz, 3H), 3.95 (d, J = 20.88 Hz, 5H), 2.39 (s, 3H), 0.69 (s, 4H), 0.64 (s, 3H), 0.07 (s, 4H), -0.32 (d, J = 7.74 Hz, 5H). **HRMS** (ESI^-) *m/z* calcd. for $C_{155}H_{118}N_{24}O_{41}Se$: 1525.3531 $(M\text{-}2H)^{2-}$; found: 1525.3591.

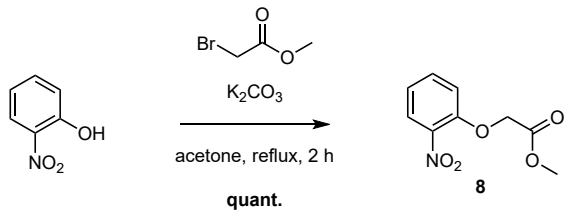


7

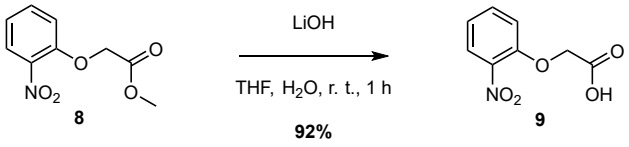
Compound 7: Oligomer **7** was synthesized on Wang resin (0.37 mmol g^{-1}, 11.0 µmol scale) according to the standard method. Loading of the first monomer: 0.34 mmol g^{-1} (92%). Unreacted amines were capped using the general capping method after each coupling step. The final acetyl group was installed via the general acetylation method. After purification by semi-prep HPLC (C18, 10–35B, 25 °C; A: 13 mmol NH_4OAc buffer pH 8.5, B: acetonitrile), the title compound was obtained as a white solid (8.33 mg, 2.86 µmol, 26%; HPLC-purity: 97.6%).

^{1}H NMR (500 MHz, 12 mmol NH_4OAc buffer pH 8.5 in H_2O/D_2O 9:1): $\delta = 11.69$ (s, 1H), 11.46 (s, 1H), 11.07 (s, 1H), 10.48 (s, 1H), 10.43 (s, 1H), 10.11 (s, 1H), 9.74 (s, 1H), 9.21 (s, 1H), 8.94 (s, 1H), 8.68 (s, 2H), 8.47 (d, $J = 8.38$ Hz, 1H), 8.14 (d, $J = 8.11$ Hz, 1H), 7.99 (d, $J = 9.29$ Hz, 1H), 7.84 (t, $J = 6.87$ Hz, 1H), 7.81–7.74 (m, 1H), 7.71 (d, $J = 9.28$ Hz, 1H), 7.68 (s, 1H), 7.62 (d, $J = 9.15$ Hz, 1H), 7.51 (s, 1H), 7.47 (t, $J = 8.76$ Hz, 1H), 7.40 (d, $J = 6.59$ Hz, 1H), 7.37–7.30 (m, 1H), 7.28 (d, $J = 7.44$ Hz, 1H), 7.26–7.18 (m, 3H), 7.16 (d, $J = 7.14$ Hz, 1H), 7.12 (d, $J = 8.01$ Hz, 1H), 7.08 (s, 1H), 7.05–6.95 (m, 3H), 6.78 (t, $J = 8.29$ Hz, 1H), 6.75–6.65 (m, 1H), 6.46–6.36 (m, 2H), 6.34 (s, 1H), 6.27 (s, 2H), 6.24 (s, 2H), 6.06 (s, 1H), 6.02 (s, 1H), 5.86 (s, 1H), 4.15 (s, 3H), 3.92 (s, 4H), 3.85 (s, 1H), 2.39 (s, 3H), 1.09 (s, 2H), -0.33 (d, $J = 7.07$ Hz, 3H), -0.40 (s, 2H). **HRMS** (ESI$^-$) *m/z* calcd. for $C_{147}H_{109}N_{24}O_{39}Se$: 2913.6454 $(M\text{-}H)^-$; found: 2913.6924.

3.3 Monomer synthesis procedures

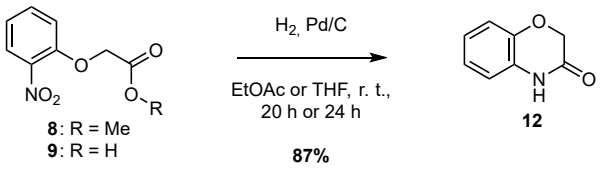


Compound 8: Compound **8** was prepared according to previously described methods.[6] Analytical data is in line with the literature. ($C_9H_9NO_5$, MW $= 211.17\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 9:1) $= 0.10$. ^{1}**H NMR** (500 MHz, $CDCl_3$): $\delta = 7.88$ (dd, $J = 8.11, 1.71$ Hz, 1H, C3-H), 7.52 (ddd, $J = 8.29, 7.44, 1.69$ Hz, 1H, C5-H), 7.11 (ddd, $J = 8.33, 7.49, 1.16$ Hz, 1H, C4-H), 6.99 (dd, $J = 8.43, 1.11$ Hz, 1H, C6-H), 4.79 (s, 2H, C7-H), 3.81 (s, 3H, C9-H). ^{13}**C NMR** (126 MHz, $CDCl_3$): $\delta = 168.4$ (C8), 151.4 (C1), 140.6 (C2), 134.2 (C5), 126.1 (C3), 122.0 (C4), 115.3 (C6), 66.7 (C7), 52.7 (C9). **HRMS** (ESI$^+$) *m/z* calcd. for $C_9H_{10}NO_5$: 212.0553 $(M+H)^+$; found: 212.0556.



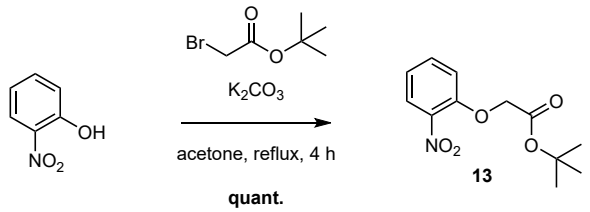
Compound 9: Compound **8** (8.21 g, 38.9 mmol, 1.0 equiv.) was dissolved in THF (120 mL). Then, LiOH (1.86 g, 77.8 mmol, 2.0 equiv.) in H_2O (30 mL) was added and the reaction mixture was stirred at room temperature for 1 h. After acidifying the solution to pH 1 using 1 M $HCl_{\,(aq.)}$, it was extracted with DCM (3×), dried over $MgSO_4$, and solvents were removed *in vacuo* (50 °C). The title compound was obtained as an off white solid (7.02 g, 35.6 mmol, 92%). ($C_8H_7NO_5$, MW $= 197.15\,\mathrm{g\,mol^{-1}}$). ^{1}**H NMR** (500 MHz, DMSO-d_6): $\delta = 13.21$ (s, 1H, O9-H), 7.87 (dd, $J = 8.04, 1.71$ Hz, 1H, C3-H), 7.62 (ddd, $J = 8.87, 7.38, 1.71$ Hz, 1H, C5-H), 7.26 (dd, $J = 8.55, 1.07$ Hz, 1H, C6-H), 7.13 (ddd, $J = 8.27, 7.43, 1.10$ Hz, 1H, C4-H), 4.91 (s, 2H, C7-H). ^{13}**C NMR** (101 MHz, DMSO-d_6): $\delta =$ 169.3 (C8), 150.4 (C1), 139.7 (C2), 134.1 (C5), 124.9 (C3), 121.1 (C4), 115.0 (C6), 65.3 (C7). **HRMS** (ESI$^-$) *m/z*

calcd. for $C_8H_6NO_5$: 196.0251 $(M\text{-}H)^-$; found: 196.0250. (Modified literature procedure;[6] analytical data is in line with the literature).



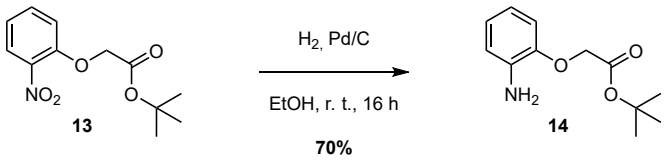
Compound 12: From the ester: Compound **8** (6.85 g, 32.4 mmol, 1.0 equiv.) was dissolved in EtOAc (350 mL). The solution was quickly degassed by vacuum N_2 cycles (3×), then Pd/C (685 mg, 10% m/m to **9a**) was added and the N_2 atmosphere was replaced by H_2. After stirring for 20 h the reaction mixture was filtered over celite©, washed with EtOAc and solvents were removed *in vacuo* (50 °C). The title compound was obtained as a light brown solid (4.22 g, 28.3 mmol, 87%).

From the acid: Compound **9** (7.02 g, 35.6 mmol, 1.0 equiv.) was dissolved in THF (600 mL). The solution was quickly degassed by vacuum N_2 cycles (3×), then Pd/C (702 mg, 10% m/m to **9e**) were added and the N_2 atmosphere was replaced by H_2. After stirring for 24 h the reaction mixture was filtered over celite©, washed with THF and solvents were removed *in vacuo* (50 °C). The crude product was not further purified. ($C_8H_7NO_2$, MW $= 149.15\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 7:3) $= 0.30$. ^{1}**H NMR** (500 MHz, $CDCl_3$): $\delta = 8.46$ (s, 1H, C9-H), 7.06–6.89 (m, 3H, C3-H, C4-H, C6-H), 6.87–6.78 (m, 1H, C5-H), 4.63 (s, 2H, C7-H). ^{13}**C NMR** (126 MHz, $CDCl_3$): $\delta = 166.0$ (C8), 143.8 (C1), 126.2 (C2), 124.4 (C3), 122.9 (C6), 117.0 (C4), 116.1 (C5), 67.4 (C7). **HRMS** (ESI$^-$) *m/z* calcd. for $C_8H_6NO_2$: 148.0404 $(M\text{-}H)^-$; found: 148.0403. (Known compound; analytical data is in line with the literature).[7]

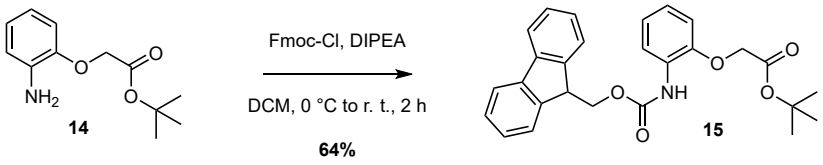


Compound 13: 2-nitrophenol (3.0 g, 21.6 mmol, 1.0 equiv.), *tert*-butyl bromoacetate (3.50 mL, 23.7 mmol, 1.1 equiv.) and K_2CO_3 (3.28 g, 23.7 mmol, 1.1 equiv.) were suspended in anhydrous acetone (60 mL) under an N_2 atmosphere. Then, the reaction mixture was refluxed for 4 h (bath temperature 75 °C). After cooling to room temperature the mixture was filtered, washed with acetone and solvents were removed *in vacuo* (50 °C). Without further purification, the title compound was obtained as a brown solid (5.46 g, 21.6 mmol, quant.). ($C_{12}H_{15}NO_5$, MW $= 253.25\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 9:1) $= 0.21$. ^{1}**H NMR** (500 MHz, $CDCl_3$): $\delta = 7.86$ (dd, J = 8.09 Hz, 1.72 Hz, 1H, C3-H), 7.51 (ddd, $J = 8.43, 7.45 1.77$ Hz, 1H, C5-H), 7.08 (ddd, $J = 8.36, 7.44, 1.16$ Hz, 1H, C4-H), 6.96 (dd, $J = 8.47, 1.14$ Hz, 1H, C6-H), 4.67 (s, 2H, C9-H), 1.46 (s, 9H, C10-H, C11-H, C12-H). ^{13}**C NMR** (126 MHz, $CDCl_3$): $\delta = 166.9$ (C8), 151.5 (C1), 140.4 (C2), 134.0 (C5), 126.0 (C3), 121.5 (C4), 114.9 (C6), 83.2 (C9), 66.8 (C7), 28.1 (C10, C11, C12). **HRMS** (EI$^+$) *m/z* calcd. for $C_{12}H_{15}NO_5$: 253.0945 $(M)^+$; found:

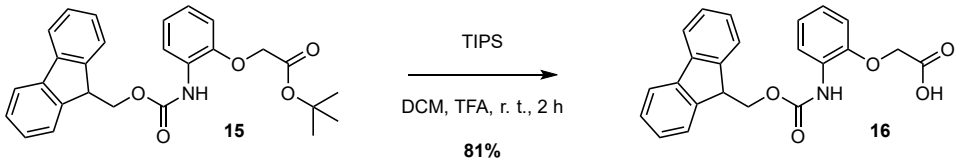
253.0955. (Known compound; analytical data is in line with the literature).[8]



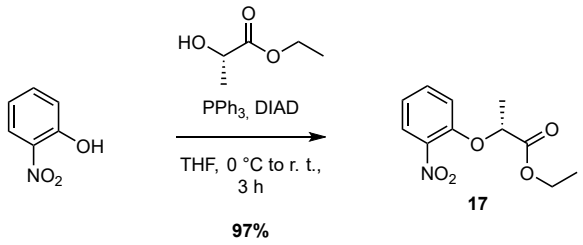
Compound 14: Compound **13** (5.46 g, 21.6 mmol, 1.0 equiv.) was dissolved in ethanol (350 mL). The solution was quickly degassed by vacuum N_2 cycles (3×), then Pd/C (546 mg, 10% m/m to **9b**) were added and the N_2 atmosphere was replaced by H_2. After stirring for 16 h the reaction mixture was filtered over celite©, washed with ethanol and solvents were removed *in vacuo* (50 °C) (product was found to be unstable, especially at elevated temperatures). The title compound was obtained as a brown solid (4.60 g, contaminated with 30% cyclic side product). The crude product was directly used in the next step without further purification. ($C_{12}H_{17}NO_3$, MW = $223.27\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 7:3) = 0.59. ^{1}**H NMR** (500 MHz, $CDCl_3$): δ = 8.24 (s, 1H), 7.03–6.91 (m, 1H), 6.87–6.77 (m, 1H), 6.77–6.63 (m, 3H), 4.62 (s, 1H), 4.53 (s, 2H), 1.49 (s, 9H).



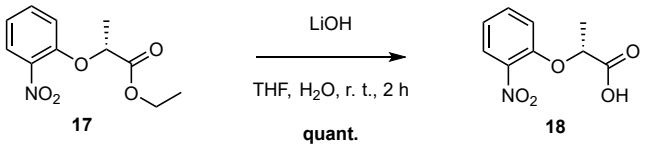
Compound 15: Compound **14** (4.60 g, contaminated with 30% lactam, 15.1 mmol, 1.0 equiv.) was dissolved in anhydrous DCM (100 mL) under an N_2 atmosphere. Then, DIPEA (3.94 mL, 22.6 mmol, 1.5 equiv.) was added and the solution was cooled to 0 °C. After the addition of Fmoc-Cl (5.08 g, 19.6 mmol, 1.3 equiv.) at 0 °C the reaction mixture was stirred at room temperature for 2 h. The mixture was acidified by adding citric acid $_{(aq.)}$ (1 M, 15.1 mL, 15.1 mmol, 1.0 equiv.), extracted with DCM (2×), the organic phases were dried over $MgSO_4$ and solvents were removed *in vacuo* (50 °C). After purification by column chromatography (SiO_2, CyHex/EtOAc 95:5) the title compound was obtained as a colorless oil (4.32 g, 9.70 mmol, 64%). ($C_{27}H_{27}NO_5$, MW = $445.52\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 9:1) = 0.31. ^{1}**H NMR** (500 MHz, $CDCl_3$): δ = 8.13 (s, 1H, C3-H), 7.97 (s, 1H, N13-H), 7.78 (d, J = 7.70 Hz, 2H, C20-H, C21-H), 7.70 (d, J = 7.46 Hz, 2H, C-17-H, C24-H), 7.41 (t, J = 7.54 Hz, 2H, C19-H, C22-H) 7.33 (t, J = 7.41 Hz, 2H, C18-H, C23-H), 7.03 (t, J = 8.32 Hz, 1H, C4-H), 6.98 (td, J = 7.72, 7.53, 1.76 Hz, 1H, C5-H), 6.83 (d, J = 8.06 Hz, 1H, C6-H), 4.60 (s, 2H, C7-H), 4.49 (d, J = 7.20 Hz, 2H, C15-H), 4.32 (t, J = 7.24 Hz, 1H, C16-H), 1.51 (s, 9H, C10-H, C11-H, C12-H). ^{13}**C NMR** (126 MHz, $CDCl_3$): δ = 168.5 (C8), 153.6 (C14), 147.1 (C2), 144.5 (C1), 144.1 (C16a, C24a), 141.5 (C20a, C20b), 129.2 (C4), 127.9 (C19, C22), 127.3 (C18, C23), 125.4 (C17, C24), 123.0 (C5), 120.1 (C20, C21), 119.2 (C3), 113.7 (C6), 83.0 (C9), 68.0 (C7), 67.2 (C15), 47.3 (C16), 28.2 (C10, C11, C12). **HRMS** (ESI$^+$) *m/z* calcd. for $C_{27}H_{27}NO_5Na$: 468.1781 $(M+Na)^+$; found: 468.1790.



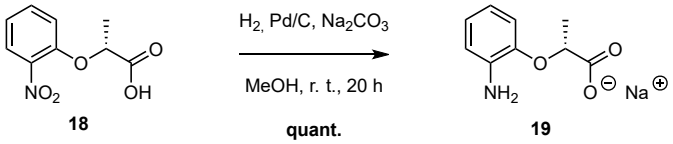
Compound 16: Compound **15** (4.32 g, 9.70 mmol, 1.0 equiv.) and triisopropyl silane (500 µL, 5% v/v) were dissolved in DCM (10 mL). After the addition of TFA (10 mL) the reaction mixture was vigorously stirred under an N_2 atmosphere for 2 h. The solution was extracted with DCM (3×) (some precipitate appeared, so the organic phases could not be dried) and solvents were removed *in vacuo* (50 °C). The crude product was triturated with Et_2O, filtered and washed with cold Et_2O to yield the title compound as a white solid (3.06 g, 7.87 mmol, 81%). ($C_{23}H_{19}NO_5$, MW $= 389.41\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 8:2 + 1% AcOH) $= 0.20$. ^{1}**H NMR** (500 MHz, DMSO-d_6): $\delta =$ 13.10 (s, 1H, O9-H), 8.67 (s, 1H, N10-H), 7.91 (d, $J = 7.63$ Hz, 2H, C17-H, C18-H), 7.75 (d, $J = 7.51$ Hz, 2H, C14-H, C21-H), 7.64 (s, 1H, C3-H), 7.43 (t, $J = 7.49$ Hz, 2H, C16-H, C19-H), 7.34 (td, $J = 7.44, 1.18$ Hz, 2H, C15-H, C20-H) 7.03 (ddd, $J = 8.73, 7.20, 1.65$ Hz, 1H, C5-H), 6.98 (dd, $J = 8.26, 1.51$ Hz, 1H, C6-H), 6.93 (t, $J = 7.70$ Hz, 1H, C4-H), 4.73 (s, 2H, C7-H), 4.42 (d, $J = 7.10$ Hz, 2H, C12-H), 4.31 (t, J = 7.01 Hz, 1H, C13-H). ^{13}**C NMR** (126 MHz, DMSO-d_6): $\delta = 170.5$ (C8), 153.6 (C11), 148.59 (C2), 148.58 (C1), 143.8 (C13a, C21a), 140.7 (C17a, C17b), 127.7 (C16, C19), 127.1 (C15, C20), 125.3 (C14, C21), 124.1 (C5), 121.4 (C4), 120.2 (C17, C18), 113.5 (C6), 66.11 (C12), 66.06 (C7), 46.6 (C13). **HRMS** (ESI$^+$) *m/z* calcd. for $C_{23}H_{20}NO_5$: 390.1336 $(M+H)^+$; found: 390.1330.



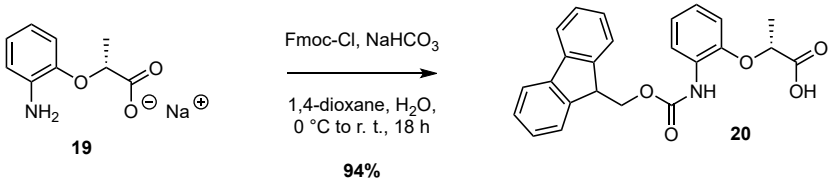
Compound 17: 2-nitrophenol (6.00 g, 43.1 mmol, 1.0 equiv.), (-)-ethyl L-lactate (4.93 mL, 43.1 mmol, 1.0 equiv.) and PPh_3 (13.6 g, 51.8 mmol, 1.2 equiv.) were dissolved in anhydrous THF (80 mL) under an N_2 atmosphere. Then, DIAD (10.2 mL, 51.8 mmol, 1.2 equiv.) was slowly added at 0 °C and the resulting mixture was stirred at room temperature for 3 h. Solvents were evaporated *in vacuo* (50 °C), and the residue was purified by column chromatography (SiO_2, CyHex/EtOAc 9:1) yielding the title compound as a white solid (10.0 g, 41.8 mmol, 97%). ($C_{11}H_{13}NO_5$, MW $= 239.23\,\mathrm{g\,mol^{-1}}$). $\mathbf{R_f}$ (CyHex/EtOAc 8:2) $= 0.32$. ^{1}**H NMR** (500 MHz, $CDCl_3$): $\delta = 7.83$ (dd, $J =$ 8.09, 1.71 Hz, 1H, C3-H), 7.48 (ddd, $J = 8.48, 7.48, 1.75$ Hz, 1H, C5-H), 7.07 (ddd, $J = 8.18, 7.45, 1.12$ Hz, 1H, C4-H), 6.96 (dd, $J = 8.40, 1.12$ Hz, 1H, C6-H), 4.84 (q, $J = 6.84$ Hz, 1H, C7-H), 4.21 (qd, $J = 7.16, 3.98$ Hz, 2H, C10-H), 1.69 (d, $J = 6.80$ Hz, 3H, C8-H), 1.24 (t, $J = 7.31$ Hz, 3H, C11-H). ^{13}**C NMR** (101 MHz, $CDCl_3$): $\delta = 171.1$ (C9), 151.2 (C1), 140.9 (C2), 133.9 (C5), 125.8 (C3), 121.6 (C4), 116.0 (C6), 74.8 (C7), 61.8 (C10), 18.5 (C8), 14.2 (C11). **HRMS** (ESI$^+$) *m/z* calcd. for $C_{11}H_{14}NO_5$: 240.0866 $(M+H)^+$; found: 240.0866.



Compound 18: Compound **17** (6.00 g, 25.1 mmol, 1.0 equiv.) was dissolved in THF (100 mL). After the addition of LiOH (1.20 g, 50.2 mmol, 2.0 equiv.) in H_2O (20 mL), the mixture was stirred at room temperature for 2 h. Then, the solution was acidified by adding HCl $_{(aq.)}$ (1 M, 62.7 mL, 62.7 mmol, 2.5 equiv.) and extracted with DCM (3×). Drying over $MgSO_4$ and removing the solvents *in vacuo* (50 °C) yielded the title compound as a white solid (5.35 g, 25.1 mmol, quant.). ($C_9H_9NO_5$, MW $= 211.17\,\mathrm{g\,mol^{-1}}$). ^{1}**H NMR** (500 MHz, DMSO-d_6): δ = 13.24 (s, 1H, O10-H), 7.85 (dd, $J = 8.06, 1.68$ Hz, 1H, C3-H), 7.61 (ddd, $J = 8.61, 7.38, 1.71$ Hz, 1H, C5-H), 7.19 (dd, $J = 8.62, 1.09$ Hz, 1H, C6-H), 7.12 (ddd, $J = 8.36, 7.40, 1.09$ Hz, 1H, C4-H), 5.11 (q, $J = 6.83$ Hz, 1H, C7-H), 1.52 (d, $J = 6.80$ Hz, 3H, C8-H). ^{13}**C NMR** (126 MHz, DMSO-d_6): δ = 172.0 (C9), 150.1 (C1), 140.0 (C2), 134.1 (C5), 124.9 (C3), 121.0 (C4), 115.5 (C6), 72.8 (C7), 18.0 (C8). **HRMS** (ESI$^-$) *m/z* calcd. for $C_9H_8NO_5$: 210.0408 $(M\text{-}H)^-$; found: 210.0408. (Modified literature procedure;[6] analytical data is in line with the literature).



Compound 19: Compound **18** (4.85 g, 23.0 mmol, 1.0 equiv.) and Na_2CO_3 (2.43 g, 23.0 mmol, 1.0 equiv.) were suspended in methanol (400 mL). The solution was quickly degassed by vacuum N_2 cycles (3×), then Pd/C (485 mg, 10% m/m to **6c**) were added and the N_2 atmosphere was replaced by H_2. After stirring for 20 h the reaction mixture was filtered over celite©, washed with methanol and solvents were removed *in vacuo* (50 °C). The crude product was used in the next step without further purification. ($C_9H_{10}NO_3Na$, MW $= 203.17\,\mathrm{g\,mol^{-1}}$). ^{1}**H NMR** (500 MHz, DMSO-d_6): δ = 6.65 (dd, $J = 7.94, 1.30$ Hz, 1H, C6-H), 6.62–6.57 (m, 1H, C4-H), 6.56 (dd, $J = 7.73, 2.01$ Hz, 1H, C3-H), 6.39 (ddd, $J = 7.84, 6.94, 2.02$ Hz, 1H, C5-H), 4.91 (s, 2H, N11-H), 4.05 (q, $J = 6.72$ Hz, 1H, C7-H), 1.36 (d, $J = 6.71$ Hz, 3H, C8-H). ^{13}**C NMR** (126 MHz, DMSO-d_6): δ = 175.2 (C9), 147.2 (C1), 139.9 (C2), 121.3 (C4), 116.3 (C5), 115.7 (C6), 114.4 (C3), 78.4 (C7), 20.2 (C8). **HRMS** (ESI$^+$) *m/z* calcd. for $C_9H_{12}NO_3$: 182.0812 $(M\text{+}H)^+$; found: 182.0809.



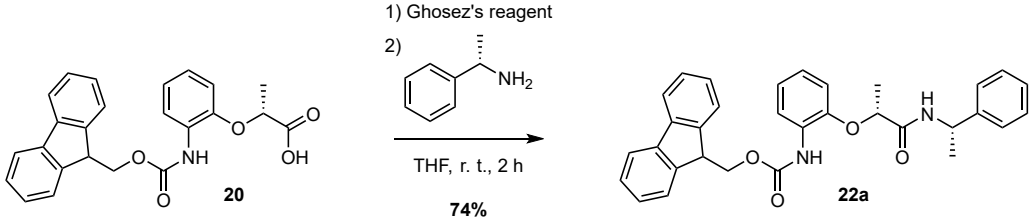
Compound 20: Compound **19** (4.16 g, 23.0 mmol, 1.0 equiv.) and $NaHCO_3$ (9.64 g, 115 mmol, 5.0 equiv.) were dissolved in H_2O (300 mL). Then, Fmoc-Cl (7.72 g, 29.8 mmol, 1.3 equiv.) in 1,4-dioxane (300 mL) was added at 0 °C over 1 h. After stirring at 0 °C for 1 h, the reaction mixture was stirred at room temperature for an additional

16 h. The mixture was acidified below pH 4 using $HCl_{(aq.)}$ (1 M), extracted with DCM (3×), the organic phases were dried over $MgSO_4$ and solvents were removed *in vacuo* (50 °C). The residue was purified by column chromatography (SiO_2, CyHex/EtOAc 7:3 + 1% DIPEA → CyHex/EtOAc 7:3 → CyHex/EtOAc 7:3 + 1% AcOH → EtOAc + 1% AcOH) to yield the title compound as a white solid (8.73 g, 21.6 mmol, 94%). ($C_{24}H_{21}NO_5$, MW = $403.43\,g\,mol^{-1}$). $\mathbf{R}_f$ (CyHex/EtOAc 8:2 + 1% AcOH) = 0.10. **^{1}H NMR** (500 MHz, DMSO-d_6): δ = 13.16 (s, 1H, O10-H), 8.72 (s, 1H, N11-H), 7.91 (d, J = 7.53 Hz, 2H, C18-H, C19-H), 7.74 (dd, J = 7.69, 3.35 Hz, 2H, C15-H, C22-H), 7.61 (s, 1H, C3-H), 7.43 (t, J = 7.44 Hz, 2H, C17-H, C20-H), 7.34 (t, J = 7.41 Hz, 2H, C16-H, C21-H), 7.07–6.98 (m, 1H, C5-H), 6.99–6.89 (m, 2H, C4-H, C6-H), 4.81 (q, J = 6.77 Hz, 1H, C7-H), 4.49–4.35 (m, 2H, C13-H), 4.32 (t, J = 7.07 Hz, 1H, C14-H), 1.56 (d, J = 6.75 Hz, 3H, C8-H). **^{13}C NMR** (126 MHz, DMSO-d_6): δ = 173.5 (C9), 153.5 (C12), 148.4 (C1), 143.8 (C14a, C22a), 140.7 (C18a, C18b), 128.1 (C2), 127.7 (C17, C20), 127.1 (C16, C21), 125.29 (C15, C22), 125.27 (C3), 124.1 (C5), 121.6 (C4), 120.2 (C18, C19), 114.6 (C6), 74.0 (C7), 66.2 (C13), 46.5 (C14), 18.5 (C8). **HRMS** (ESI^-) *m/z* calcd. for $C_{24}H_{22}NO_5$: 404.1492 $(M\text{-}H)^-$; found: 404.1491.

The synthesis of $\mathbf{B}^{\mathbf{Rme}}$ (**20**) and its (*S*)-enantiomer $\mathbf{B}^{\mathbf{Sme}}$ was repeated with the protocol reported above. This time precursors with an enantiomeric purity given by the supplier as ≥99% were used. The enantiomeric ratio is retained during synthesis as shown by chiral derivatisation (section 2). Therefore, an enantiomeric purity of ≥99% is expected for the final Fmoc-monomers as well. Their specific rotation was measured and is shown in table S1.

Table S1 Specific rotation values of chiral **B** monomers.

$\mathbf{B}^{\mathbf{Rme}}$	$[\alpha]_D^{20} = -26.9$ (c 2.24, MeOH)
$\mathbf{B}^{\mathbf{Sme}}$	$[\alpha]_D^{20} = +26.8$ (c 0.56, MeOH)



Compound 22a: Compound **8** (100 mg, 0.248 mmol, 1.0 equiv.) was dissolved in anhydrous THF (2 mL). Then, 1-Chlor-*N*,*N*,2-trimethyl-1-propenylamin (Ghosez's reagent) (66.0 µL, 0.496 mmol, 2.0 equiv.) was added, and the reaction mixture was stirred at room temperature for 1.5 h. Solvents were evaporated at room temperature under high vacuum for 4 h, and the residue was redissolved in anhydrous THF (2 mL). After the addition of (*S*)-1-phenyl-ethylamin (96.0 µL, 7.44 µmol, 3.0 equiv.), the mixture was stirred at room temperature for another 30 min. The mixture was diluted with $HCl_{(aq.)}$ (1 M), extracted with DCM (2×), and the organic phases were dried over $MgSO_4$. Evaporating the solvents *in vacuo* (50 °C) yielded the crude product as a white solid (93.0 mg, 184 µmol, 74%). ($C_{32}H_{30}N_2O_4$, MW = $506.60\,g\,mol^{-1}$). $\mathbf{R}_f$ (CyHex/EtOAc 8:2) = 0.20. **^{1}H NMR** (500 MHz, DMSO-d_6): δ = 8.98 (s, 1H, C19-H), 8.51 (d, J = 8.26 Hz, 1H, C10-H), 7.91 (d, J = 7.57 Hz, 2H, C26-H, C27-H),

7.72 (dd, $J = 7.71, 3.70$ Hz, 2H, C23-H, C30-H), 7.47 (s, 1H, C3-H), 7.43 (t, $J = 7.49$ Hz, 2H, C25-H, C28-H), 7.32 (t, $J = 8.75$ Hz, 1H, C24-H, C29-H), 7.25 (d, $J = 4.30$ Hz, 3H, C14-H, C15-H, C17-H, C18-H), 7.18 (h, $J = 4.41, 4.34$ Hz, 1H, C16-H), 7.06 (t, $J = 7.76$ Hz, 1H, C5-H), 6.95 (d, $J = 8.53$ Hz, 1H, C6-H), 6.93–6.87 (m, 1H, C4-H), 4.92 (p, $J = 7.23$ Hz, 1H, C11-H), 4.80 (q, $J = 6.60$ Hz, 1H, C7-H), 4.44 (d, $J = 7.63$ Hz, 2H, C21-H), 4.32 (t, $J = 6.91$ Hz, 1H, C22-H), 1.44 (d, $J = 6.59$ Hz, 3H, C8-H), 1.24 (d, $J = 7.00$ Hz, 3H, C12-H). ^{13}**C NMR** (101 MHz, DMSO-d_6): δ = 170.4 (C9), 154.0 (C20), 148.5 (C1), 144.1 (C13), 143.7 (C22a, C30a), 140.8 (C26a, C26b), 128.2 (C15, C17), 127.7 (C25, C28), 127.4 (C2), 127.1 (C24, C29), 126.6 (C16), 125.7 (C14, C18), 125.1 (C23, C30), 124.8 (C5), 123.2 (C3), 121.3 (C4), 120.2 (C26, C27), 113.7 (C6), 74.5 (C7), 66.0 (C21), 47.4 (C11), 46.6 (C22), 22.0 (C12), 18.7 (C8).

4 X-ray Crystallography

After preparative HPLC, the ammonium salt of compound **7** was dissolved in pure water to final concentration of 3.5 mM. Crystallization trials were performed at 20 °C in standard aqueous sitting drop vapour diffusion method and subsequently optimized by hanging drop vapour diffusion method at 20 °C. Hexagonal crystals (Fig. S8) were obtained by mixing 1 µL of **7** and 2 µL of crystallization reagent 5% v/v 2-propanol, 50 mM TRIS buffer, pH 7.5, 10 mM Magnesium chloride; equilibrated against 500 µL of crystallization reagent in the reservoir. For data collection, a single crystal was fished using a MiTeGen microloop, cryo-protected in a solution composed of 33% v/v Glycerol, 3.3% 2-propanol, 33% TRIS buffer, pH 7.5, 6.6 mM Magnesium chloride and flash frozen in liquid nitrogen.

Synchrotron data was collected on beam line P14 operated by EMBL Hamburg at the Petra III storage ring (DESY, Hamburg, Germany) using 0.9808 Å wavelength. During data collection, the crystal was cooled to 100 K. The crystal was exposed for 0.008 s and 0.1° oscillation per frame and a rotation pass of 360° was measured using an EIGER 16M detector. Data were processed using xia2[9] with DIALS[10] for integration and using Pointless /Aimless[11,12] for scaling and merging respectively. The crystal belonged to the space group $P3_121$ (or $P3_221$) with unit cell parameters $a = b = 79.06$ Å and $c = 39.86$ Å, $V = 212\,837$ Å^3 and 4 molecules in the asymmetric unit.

The structure was solved by Molecular Replacement (MR) method using *PHASER*[13] from the *CCP4* suite[14]. The model was built in *Maestro* (Version 11.5.011) in the following way: First, a **Q** helix was built, and was minimized using the parameters shown in Table S2. Then, respective **Q** units were converted to **B** units and side chains were added. Finally, the structure was minimized again using the same parameters. The side chain atoms were omitted from the model to remove flexibility and to allow minimum number of clashes.[15] The best MR solution (Fig. S9) had log likelihood gain (LLG) of 284 and translation function Z-score (TFZ) of 7.4 in $P3_121$ space group.

Geometric restraints for maximum likelihood restrained refinement was generated using *PRODRG*[16]. Model building and restrained refinement were performed in *Coot*[17] and *Refmac5*[18] respectively. Thermal displacement parameters were refined isotropically. Fig. S10 shows the sigma weighted $2F_o$-F_c electron density map superimposed on one of the four helices. The final model was refined to a resolution of 2.86 Å, with R_{work} and R_{free} factors of 24.72% and 30.37% respectively. Statistics of data collection and refinement can be found in Table S3, with statistics for

the highest-resolution shell shown in parentheses.

The final cif file was checked using IUCr's checkcif algorithm. Due to large volume fractions of disordered solvent molecules, weak diffraction intensity and poor resolution, a number of A- and B-level remain in the checkcif file. These alerts are inherent to the data and refinement procedures and illustrate the limited practicality of the checkcif tool for medium-size molecule crystallography. Atomic coordinates and structure factors for **7** was deposited in the Cambridge Crystallographic Data Centre (CCDC) with accession code 2070816. The data is available free of charge upon request (www.ccdc.cam.ac.uk/).

Table S2 Parameters used to build the model of **7**.

Forcefield	OPLS3
Solvent	Water
Charges from	Force Field
Cutoff	Extended
Constraints	0
Method	TNCG
Max. Iterations	2500
Converge on	Gradient
Converge Threshold	0.05
Minimization Mode	Minimization of non-conformers

Table S3 Crystallographic data collection and refinement statistics for **7**

Data collection	
Space group	$P3_121$
Unit cell	
a, b, c (Å)	79.06, 79.06, 39.86
α, β, γ (°)	90.0, 90.0, 120.0
Resolution (Å)	39.53 – 2.86 (2.93 – 2.86)
R_{meas}	0.260 (1.944)
CC half	0.992 (0.921)
I / σ	4.8 (1.1)
Completeness (%)	100 (100)
Reflections (total)	68739
Reflections (unique)	3481
Redundancy	19.7 (20.8)
Refinement	
Resolution (Å)	39.53 – 2.86
R_{work} / R_{free} (%)	24.72 / 30.37
No. of non-H atoms	852
Overall B-factor (Å^3)	52.30
R.m.s. deviations	
Bond lengths (Å)	0.019
Bond angles (°)	3.139
CCDC entry	2070816*

*As key information were lost during the .pdb file to .cif file conversion process, the entire .pdb file was inserted into the 'comments' section of .cif file available from the Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/getstructures/.

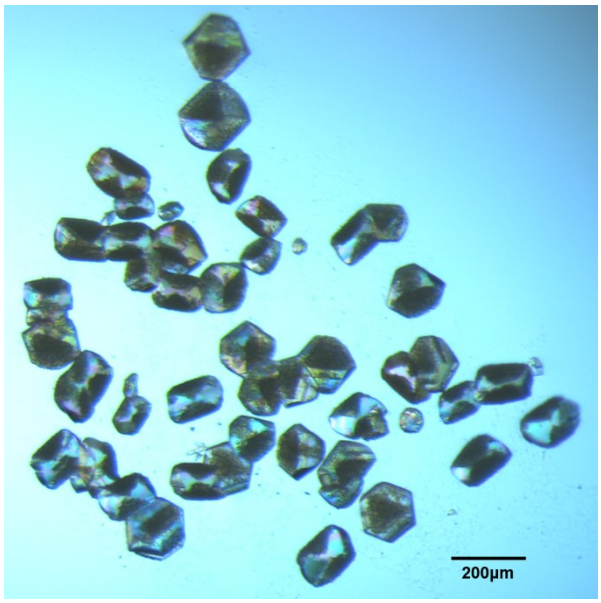


Fig. S8 Crystals of **7** observed under crossed polarizing microscope.

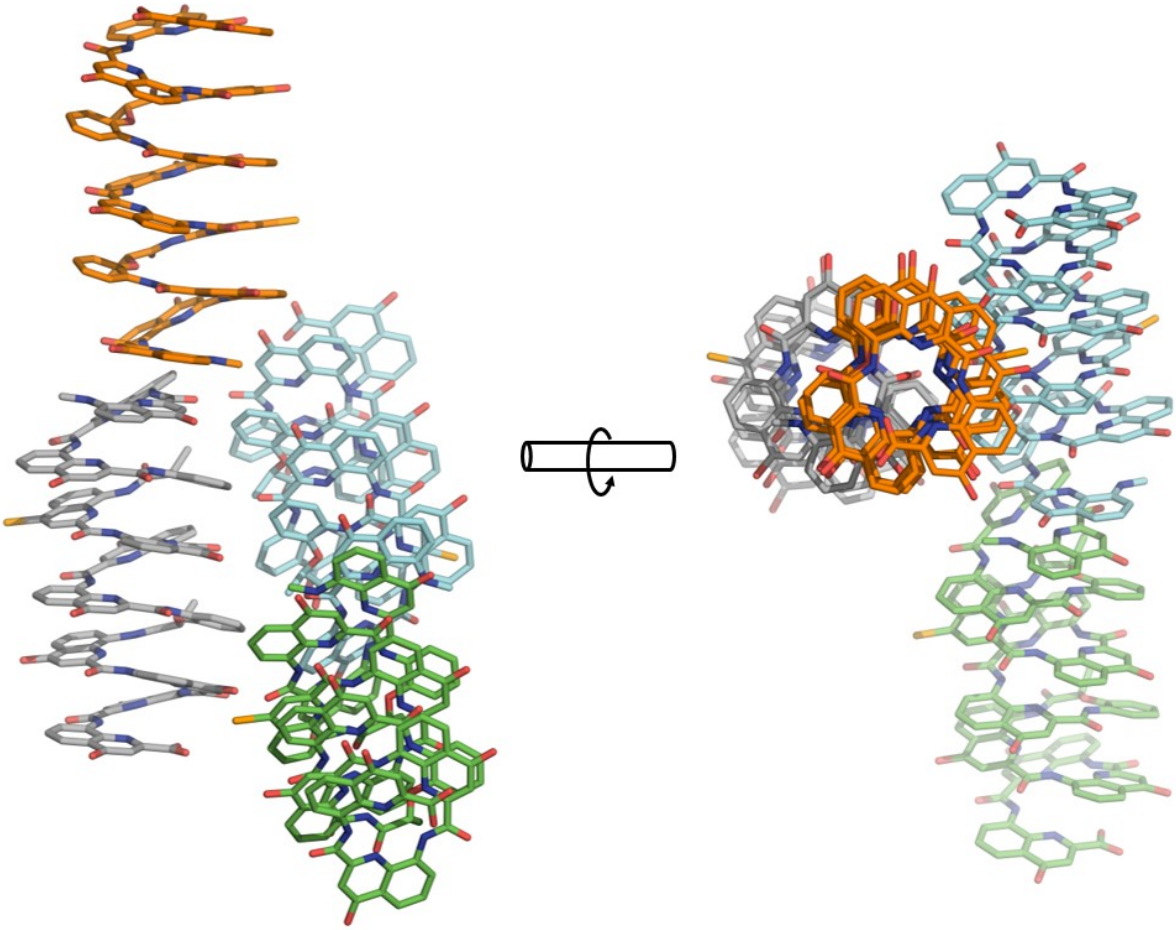


Fig. S9 The crystallographic asymmetric unit of **7** consisted of four independent molecules.

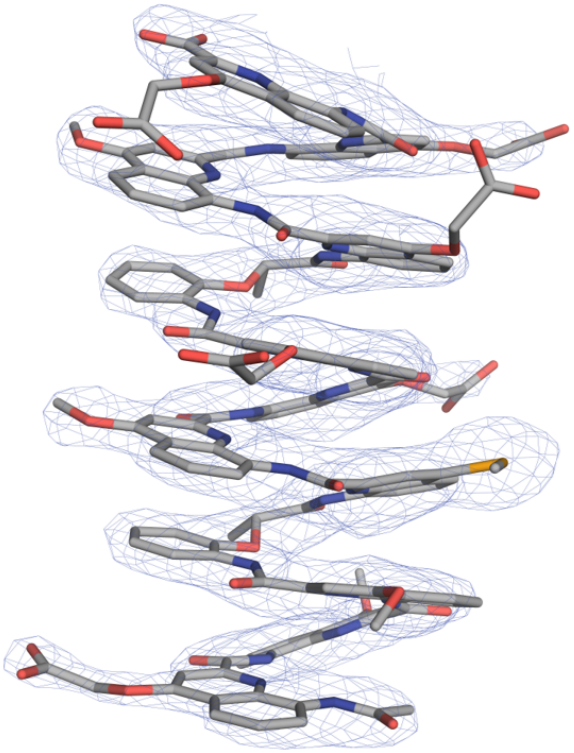


Fig. S10 Sigma weighted $2F_o$-F_c electron density map (contoured at 1.6 σ, carved within 2.0 Å) superimposed on one **7** helix.

5 Spectra and Chromatograms

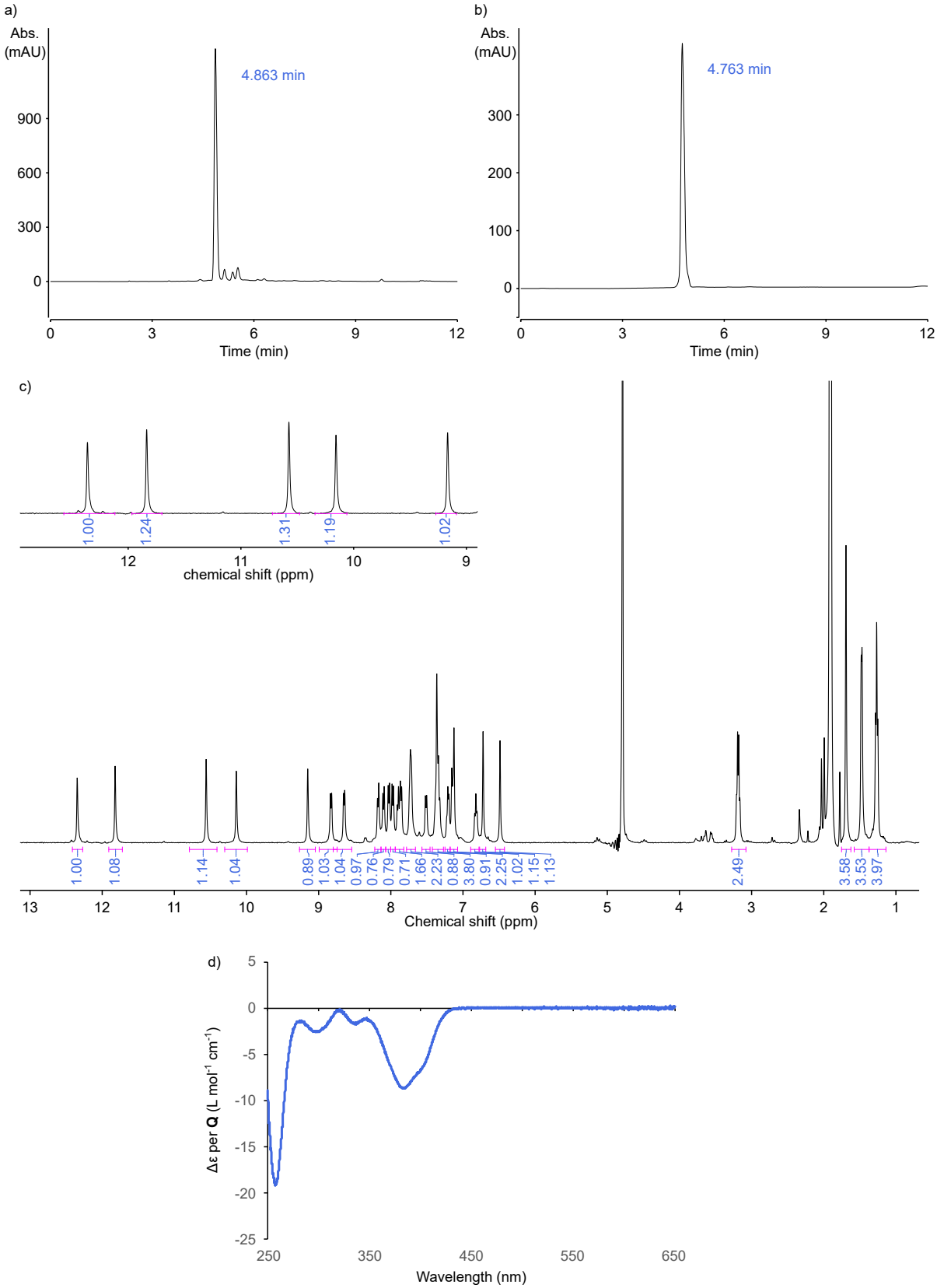


Fig. S11 Analytical data of compound **1**. HPLC chromatograms after cleavage from the resin (a) and after purification (b) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (41.2 µM in 13 mM NH_4OAc buffer pH 8.5).

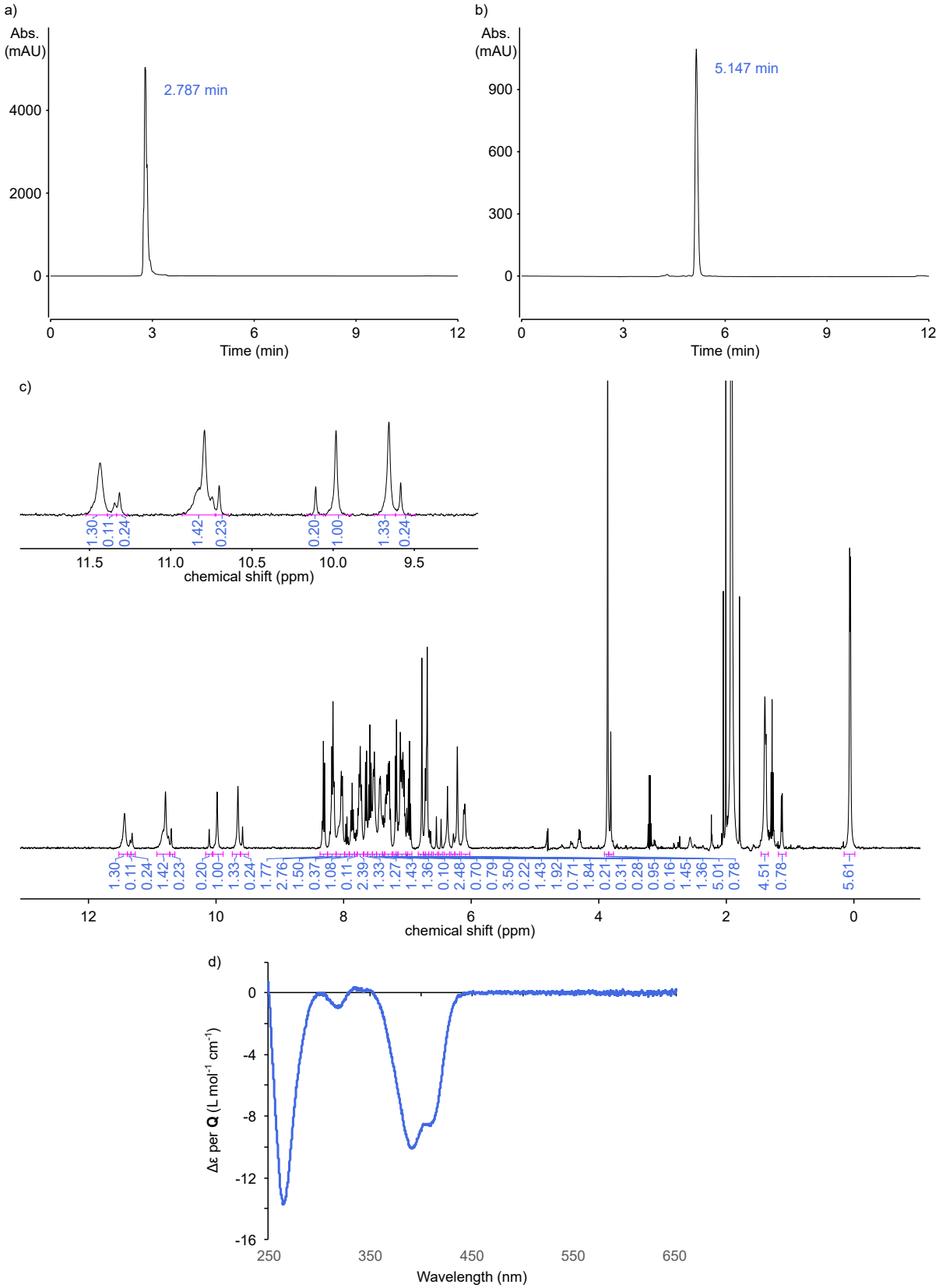


Fig. S12 Analytical data of compound **2**. HPLC chromatograms after cleavage from the resin (a) (C8, 0–60B, 50 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile) and after purification (b) (C8, 0–40B, 50 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (15.4 µM in 13 mM NH_4OAc buffer pH 8.5).

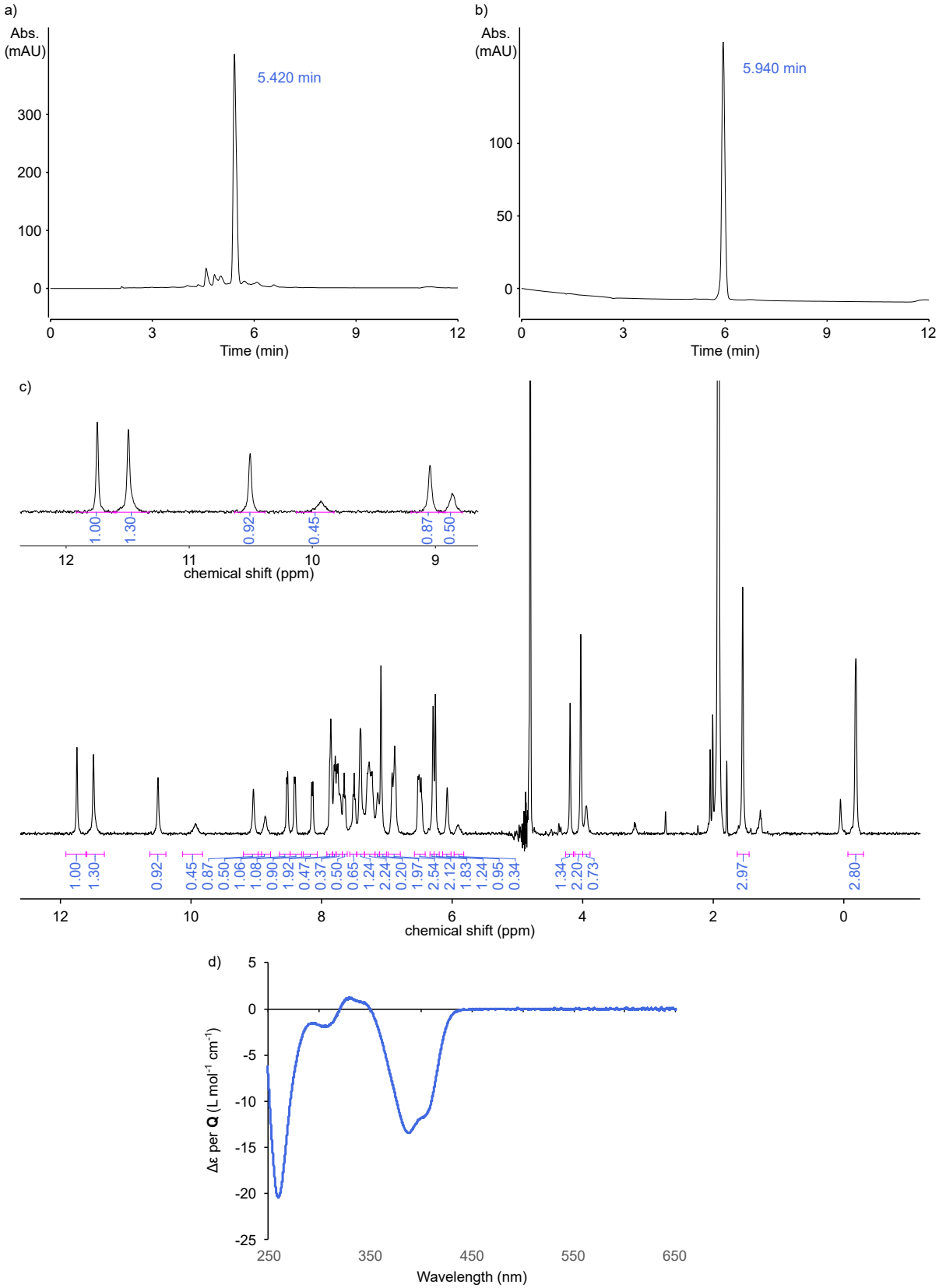


Fig. S13 Analytical data of compound **3**. HPLC chromatograms after cleavage from the resin (a) and after purification (b) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (22.6 µM in 13 mM NH_4OAc buffer pH 8.5).

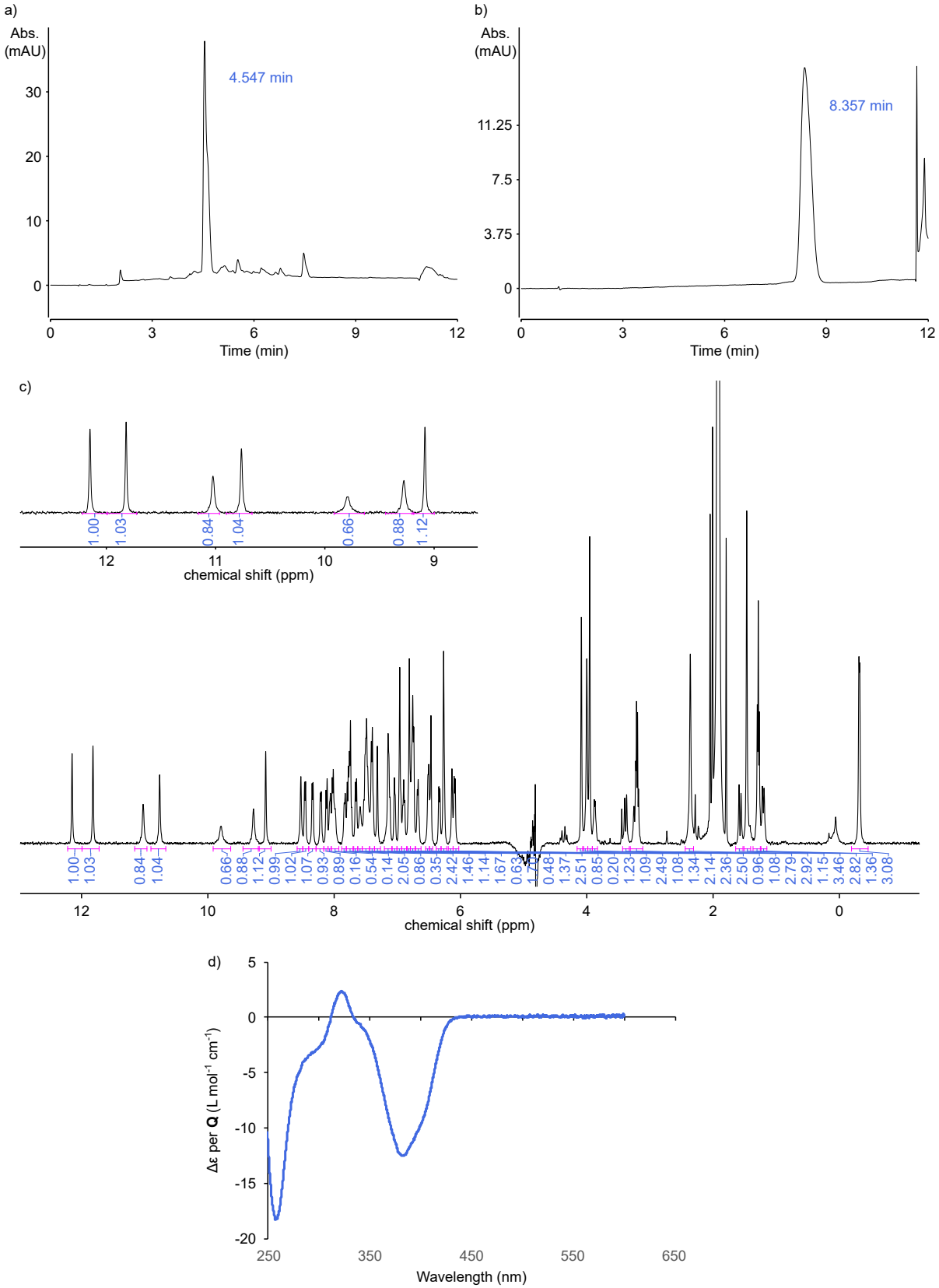


Fig. S14 Analytical data of compound **4**. HPLC chromatograms after cleavage from the resin (a) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile) and after purification (b) (C18, 10–20B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (10.2 µM in 13 mM NH_4OAc buffer pH 8.5).

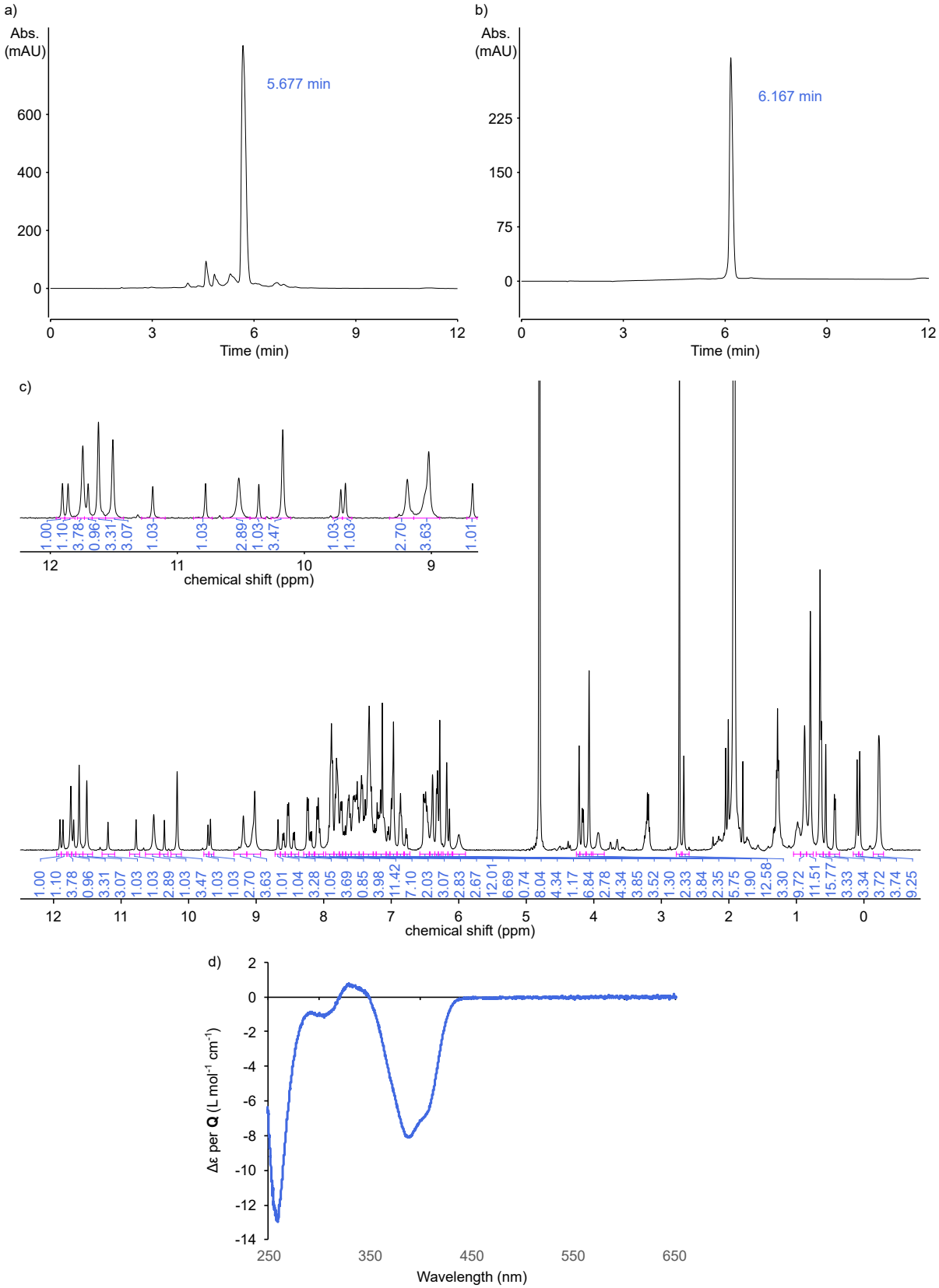


Fig. S15 Analytical data of compound **5**. HPLC chromatograms after cleavage from the resin (a) and after purification (b) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (31.1 µM in 13 mM NH_4OAc buffer pH 8.5).

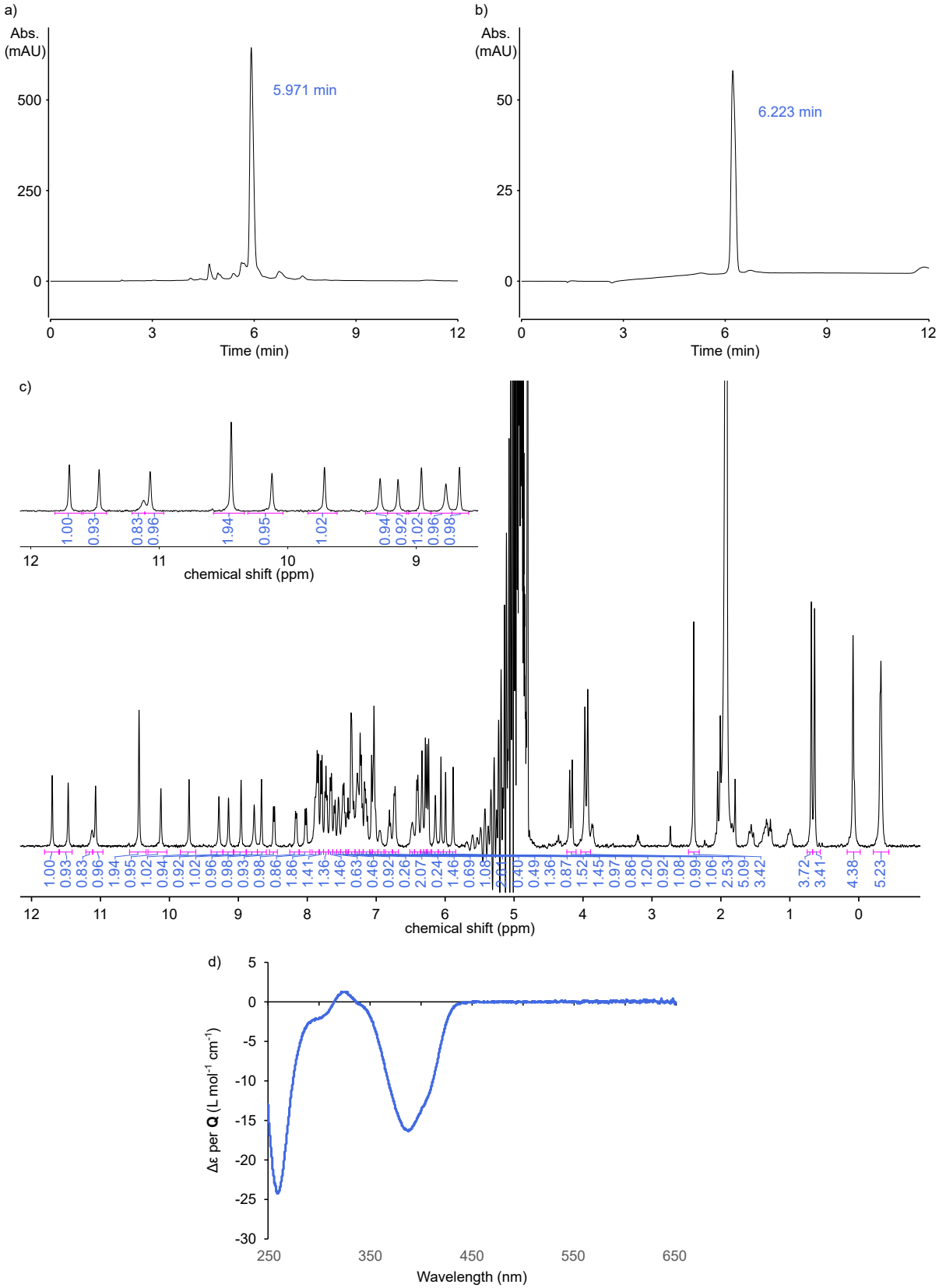


Fig. S16 Analytical data of compound **6**. HPLC chromatograms after cleavage from the resin (a) and after purification (b) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (8.73 µM in 13 mM NH_4OAc buffer pH 8.5).

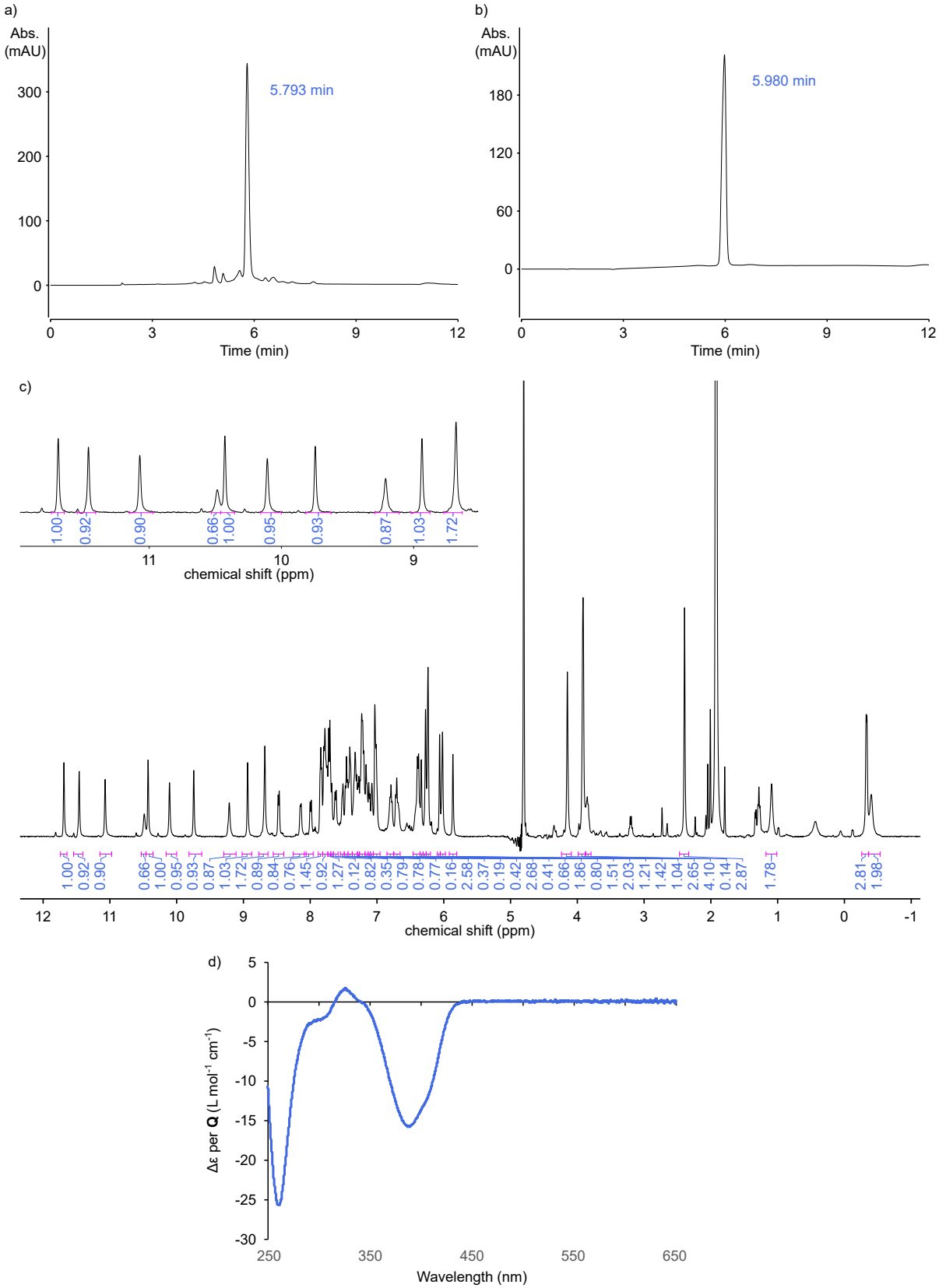


Fig. S17 Analytical data of compound **7**. HPLC chromatograms after cleavage from the resin (a) and after purification (b) (C18, 0–60B, 25 °C; A: 13 mM NH_4OAc buffer pH 8.5, B: acetonitrile). c) ^1H NMR (500 MHz, 0.13 mM in 12 mM NH_4OAc buffer pH 8.5 H_2O/D_2O 9:1, H_2O suppression). d) CD spectrum (9.04 µM in 12 mM NH_4OAc buffer pH 8.5).

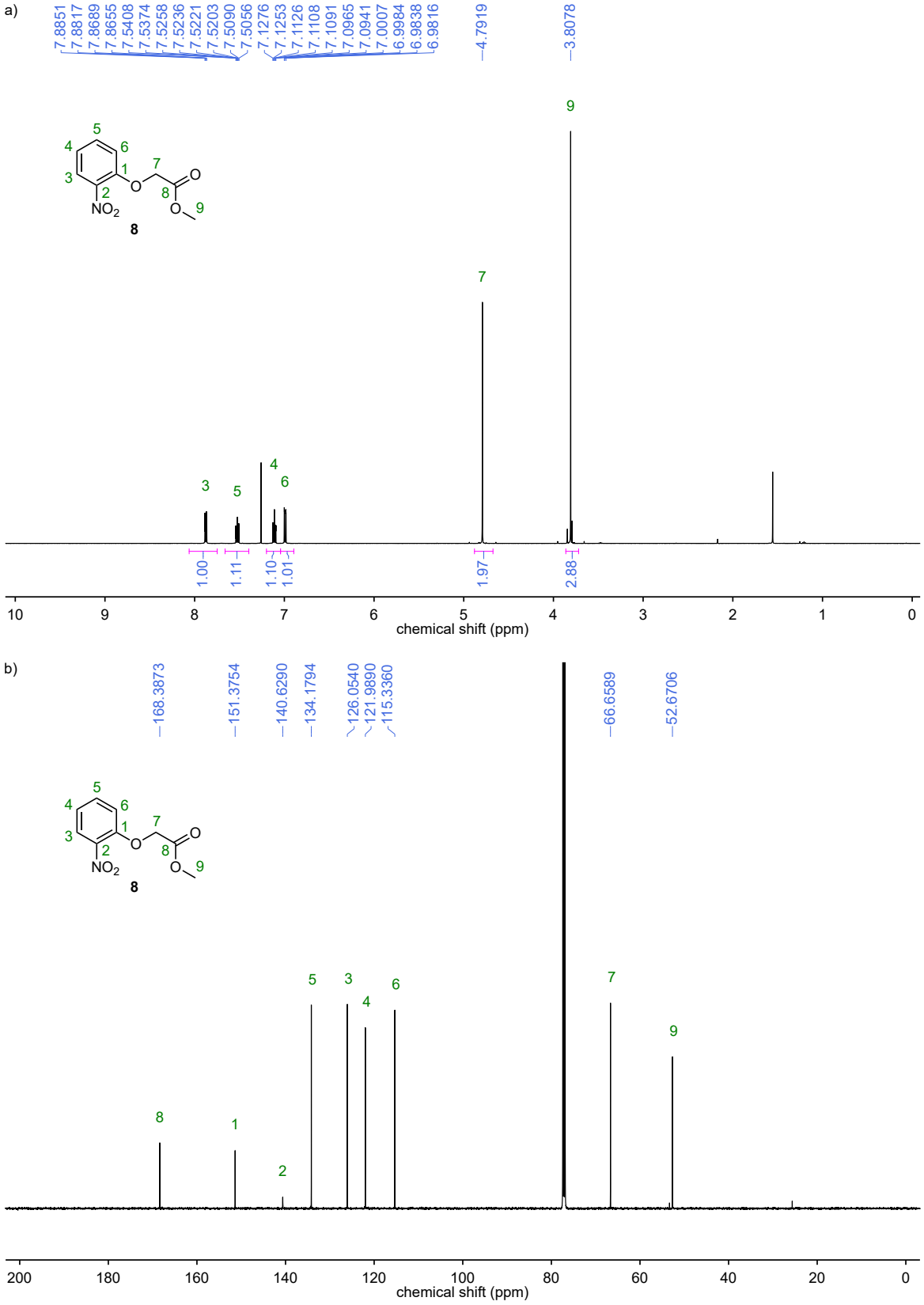


Fig. S18 NMR spectra of compound **8**. a) ^{1}H NMR (500 MHz, $CDCl_3$). b) ^{13}C NMR (126 MHz, $CDCl_3$).

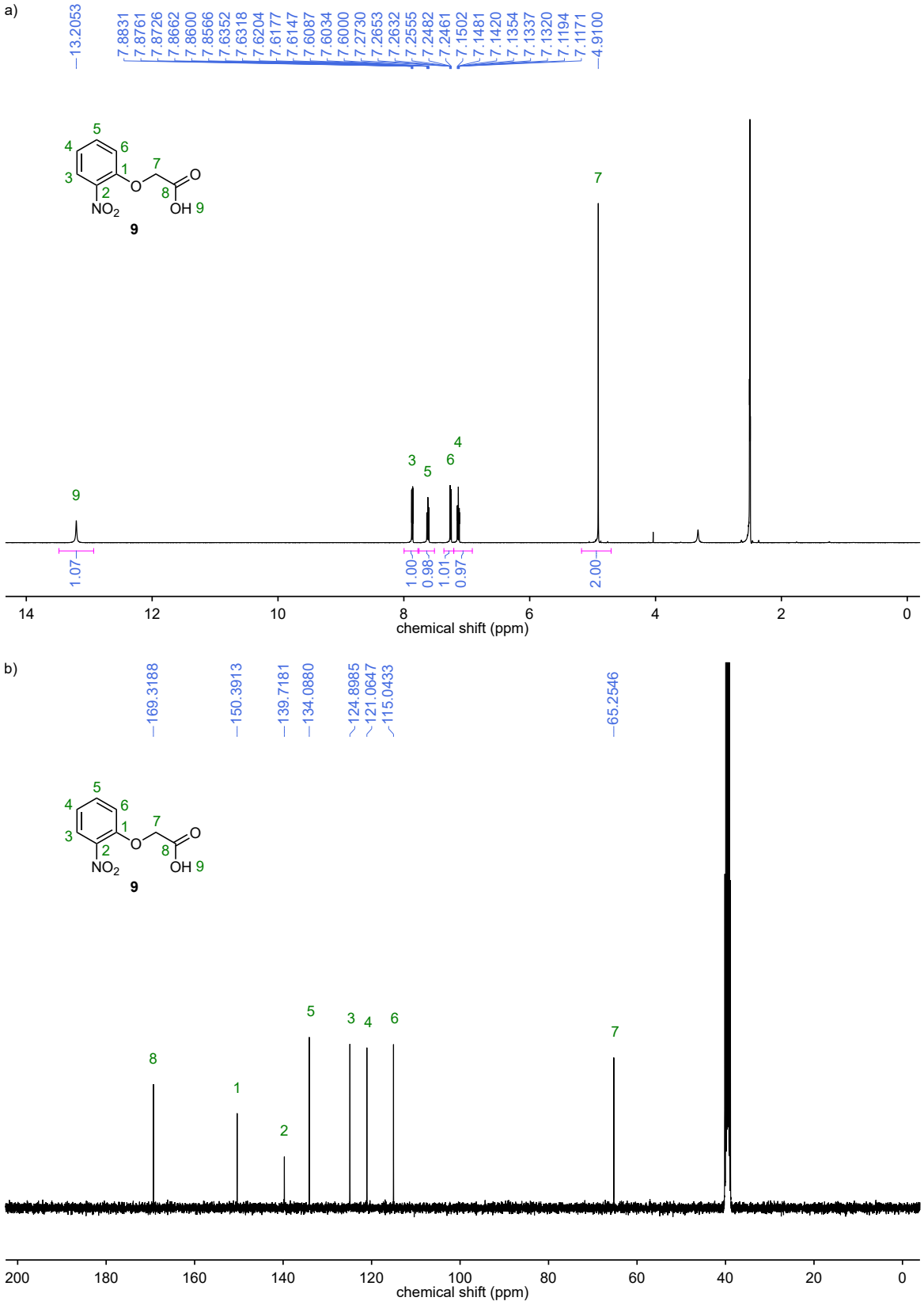


Fig. S19 NMR spectra of compound **9**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (101 MHz, DMSO-d_6).

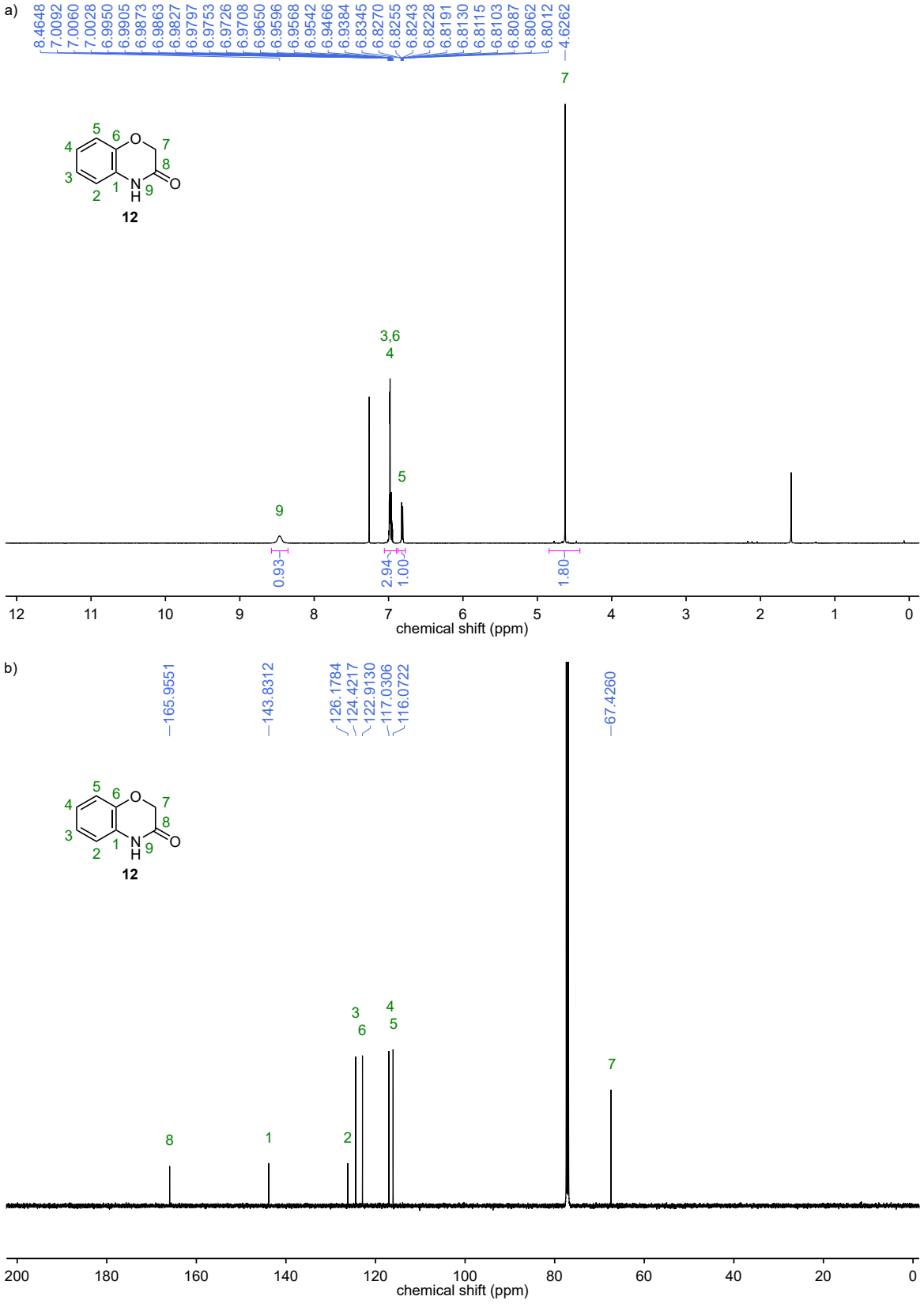


Fig. S20 NMR spectra of compound **12**. a) ^{1}H NMR (500 MHz, $CDCl_3$). b) ^{13}C NMR (126 MHz, $CDCl_3$).

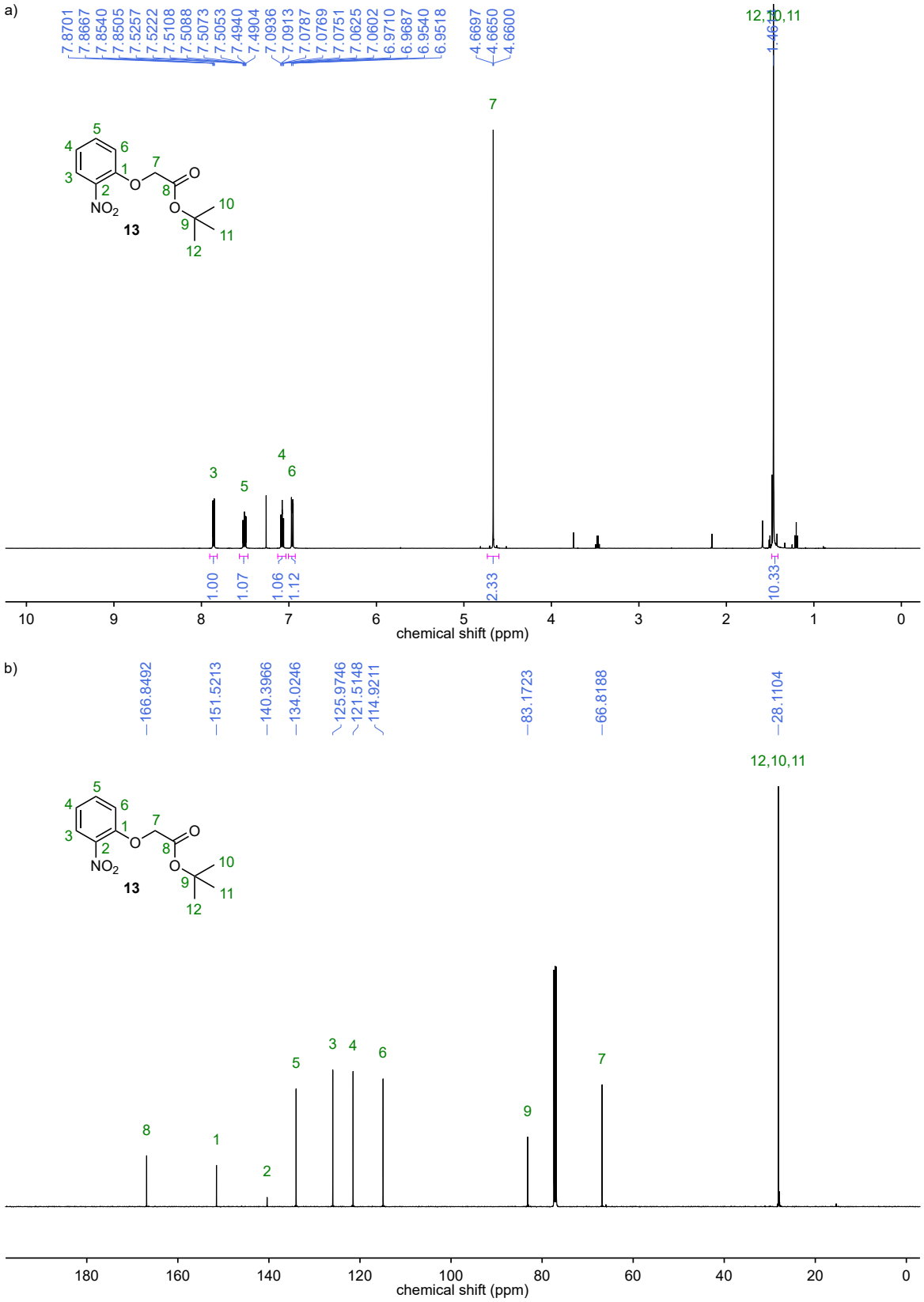


Fig. S21 NMR spectra of compound **13**. a) ^{1}H NMR (500 MHz, $CDCl_3$). b) ^{13}C NMR (126 MHz, $CDCl_3$).

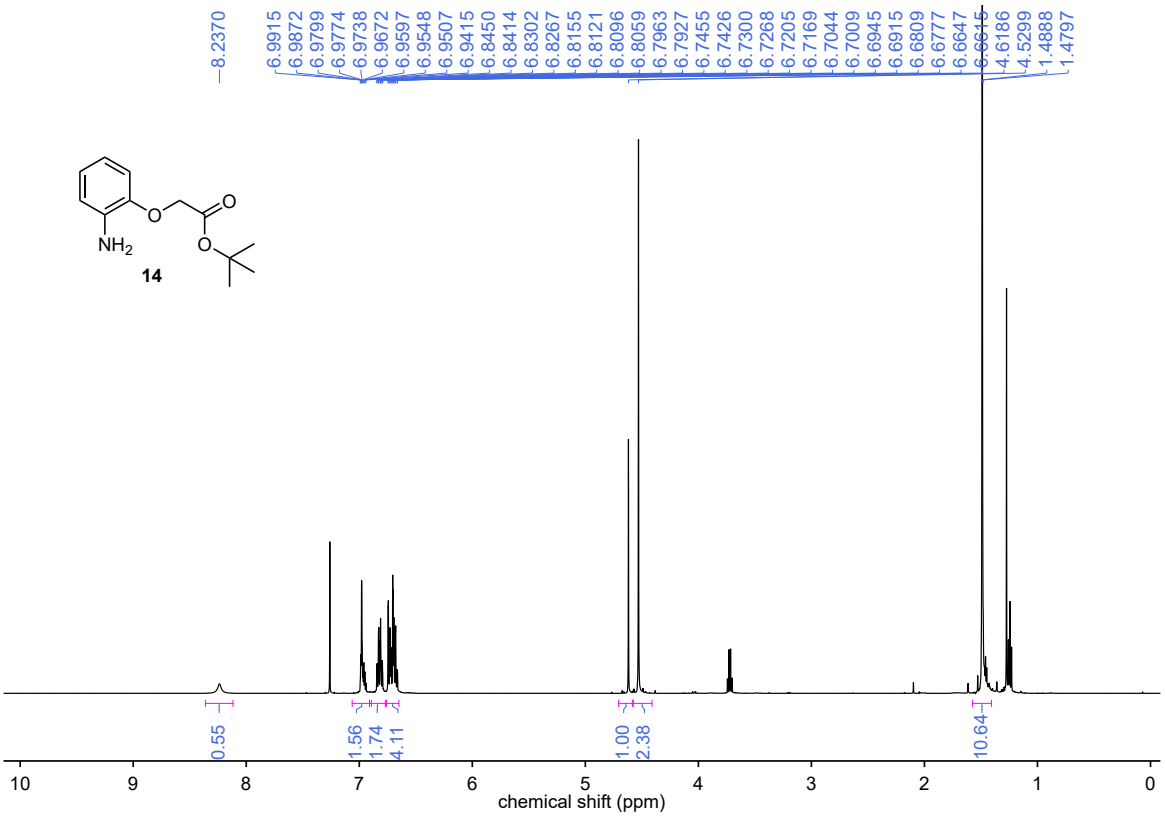


Fig. S22 ^{1}H NMR spectrum of compound **14** (500 MHz, $CDCl_3$).

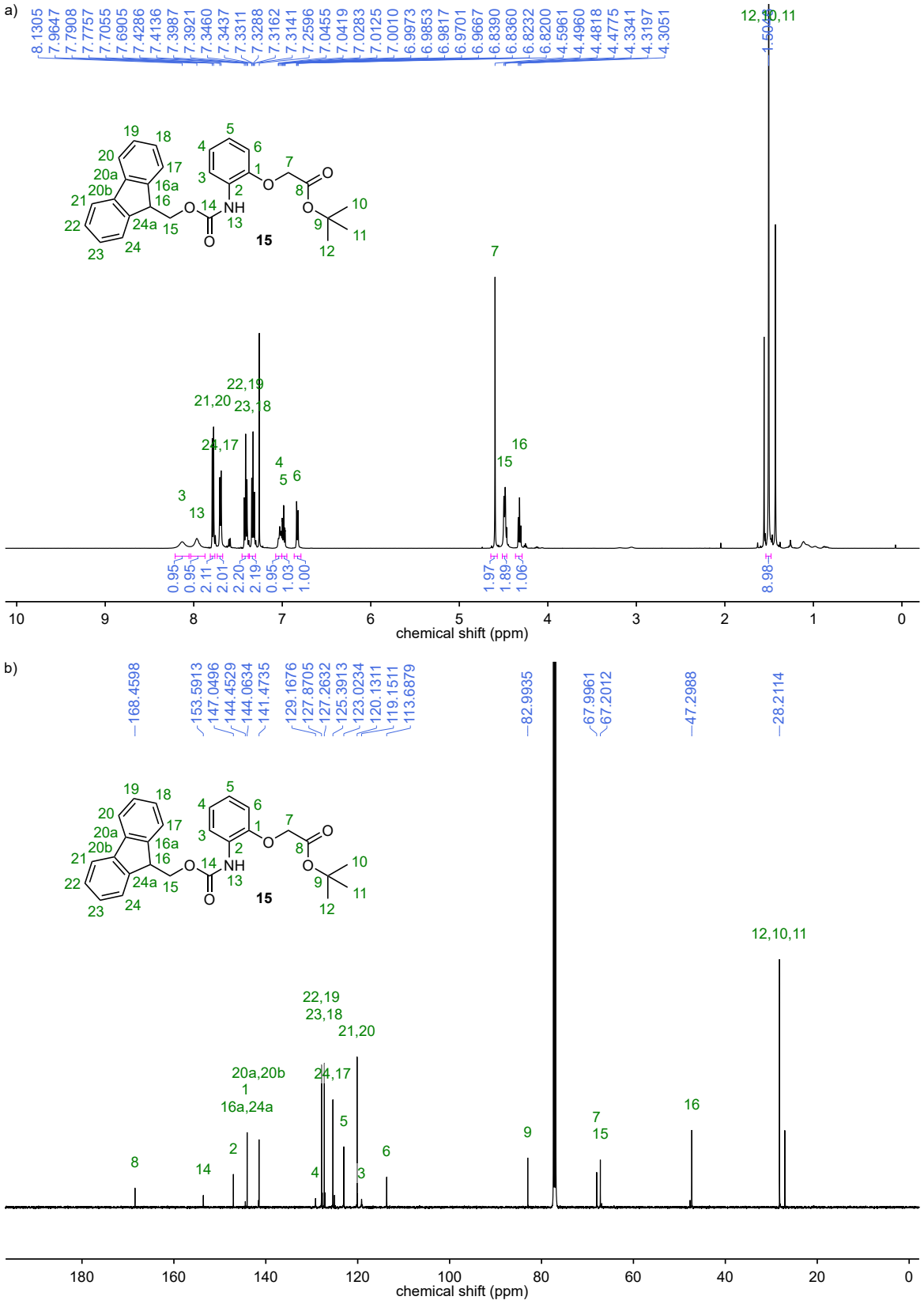


Fig. S23 NMR spectra of compound **15**. a) ^{1}H NMR (500 MHz, $CDCl_3$). b) ^{13}C NMR (126 MHz, $CDCl_3$).

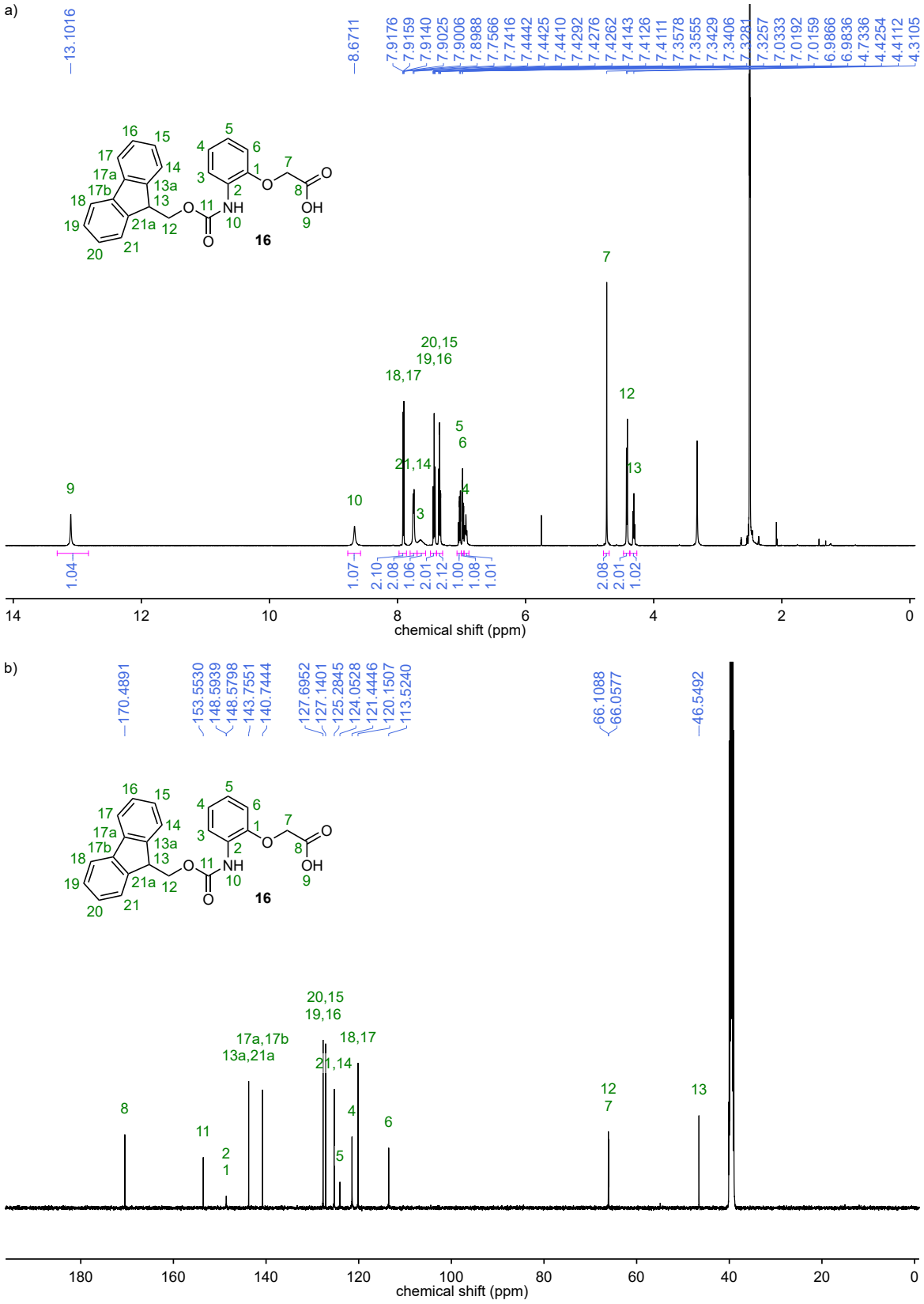


Fig. S24 NMR spectra of compound **16**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (126 MHz, DMSO-d_6).

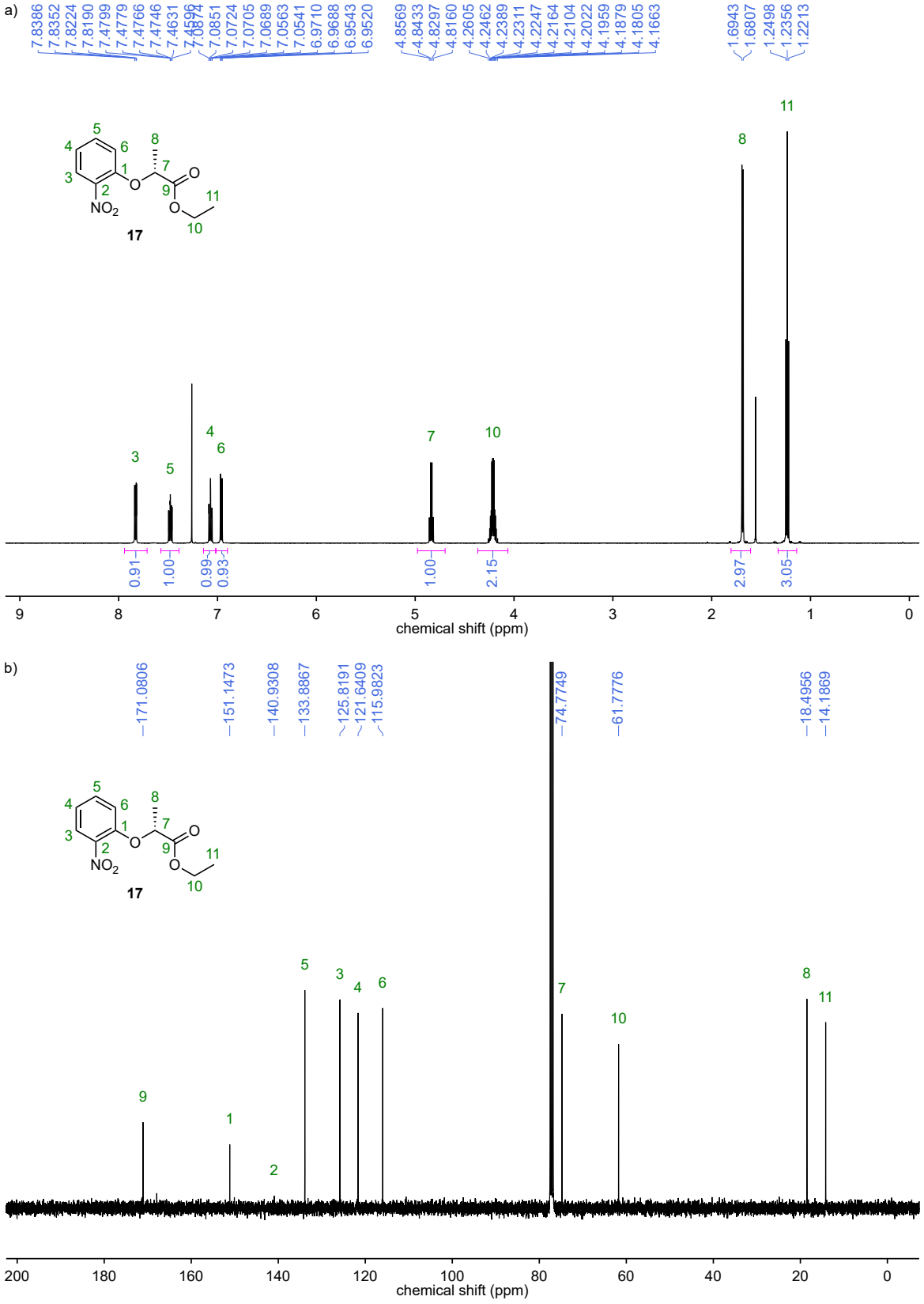


Fig. S25 NMR spectra of compound **17**. a) ^{1}H NMR (500 MHz, $CDCl_3$). b) ^{13}C NMR (101 MHz, $CDCl_3$).

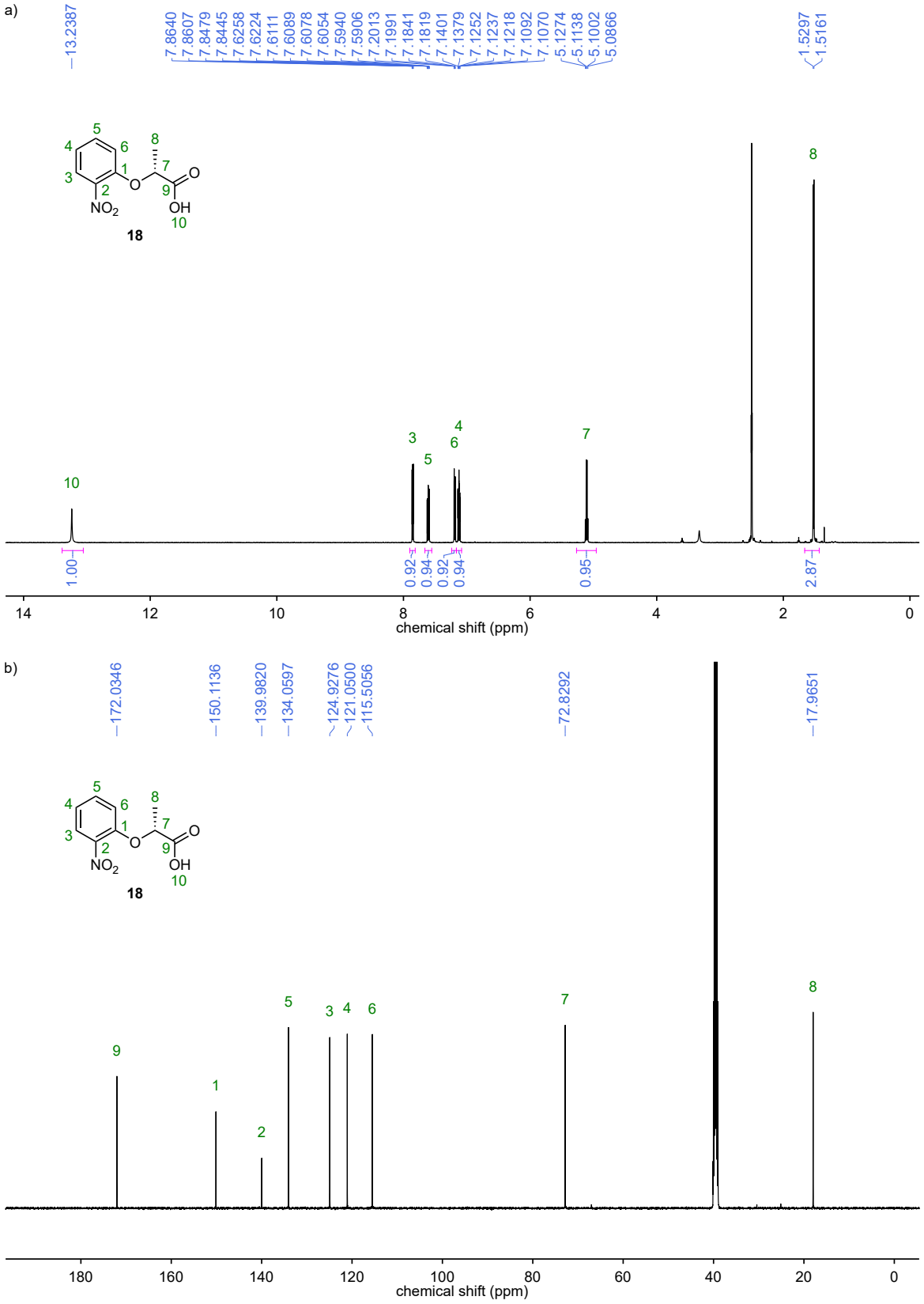


Fig. S26 NMR spectra of compound **18**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (126 MHz, DMSO-d_6).

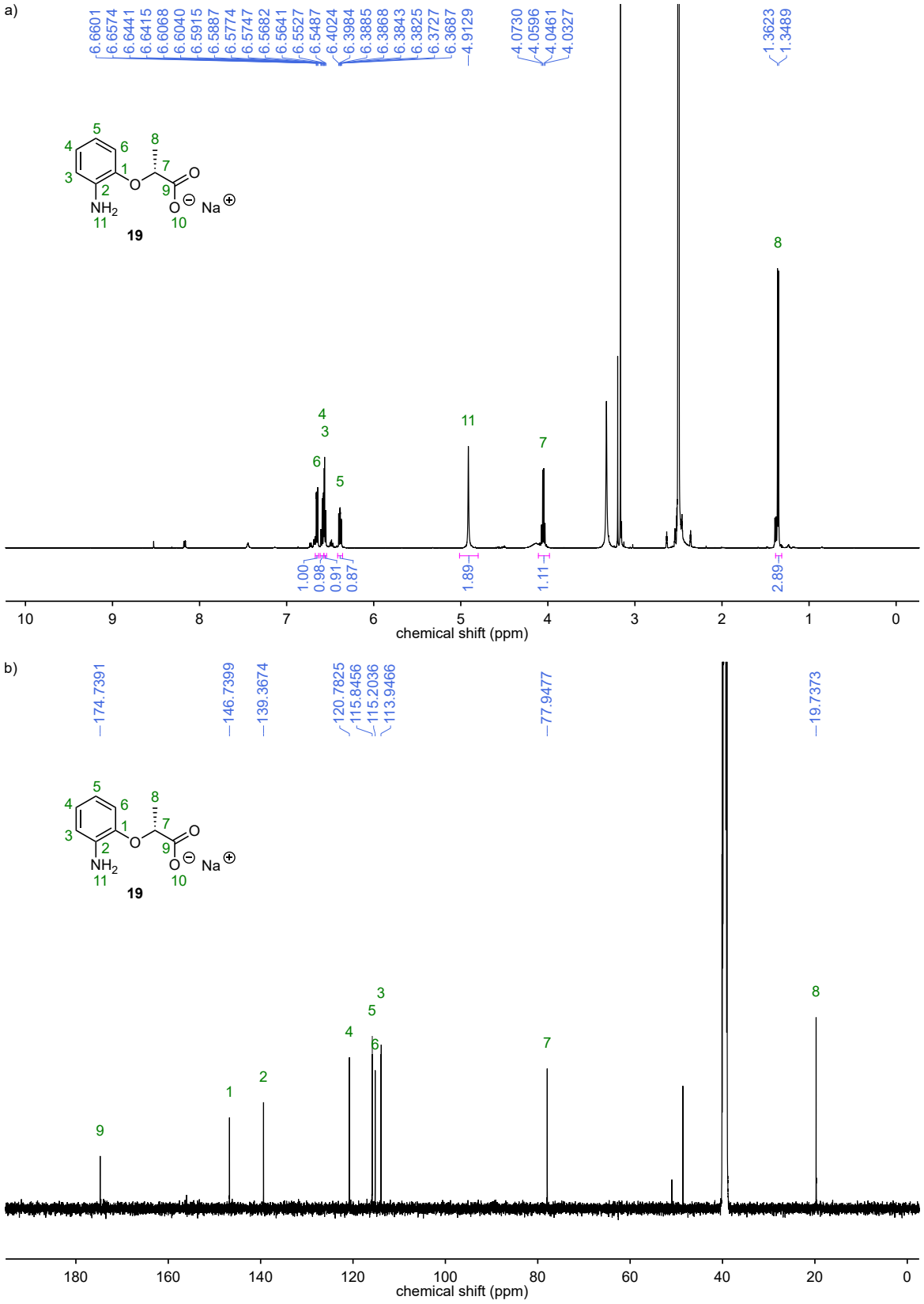


Fig. S27 NMR spectra of compound **19**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (126 MHz, DMSO-d_6).

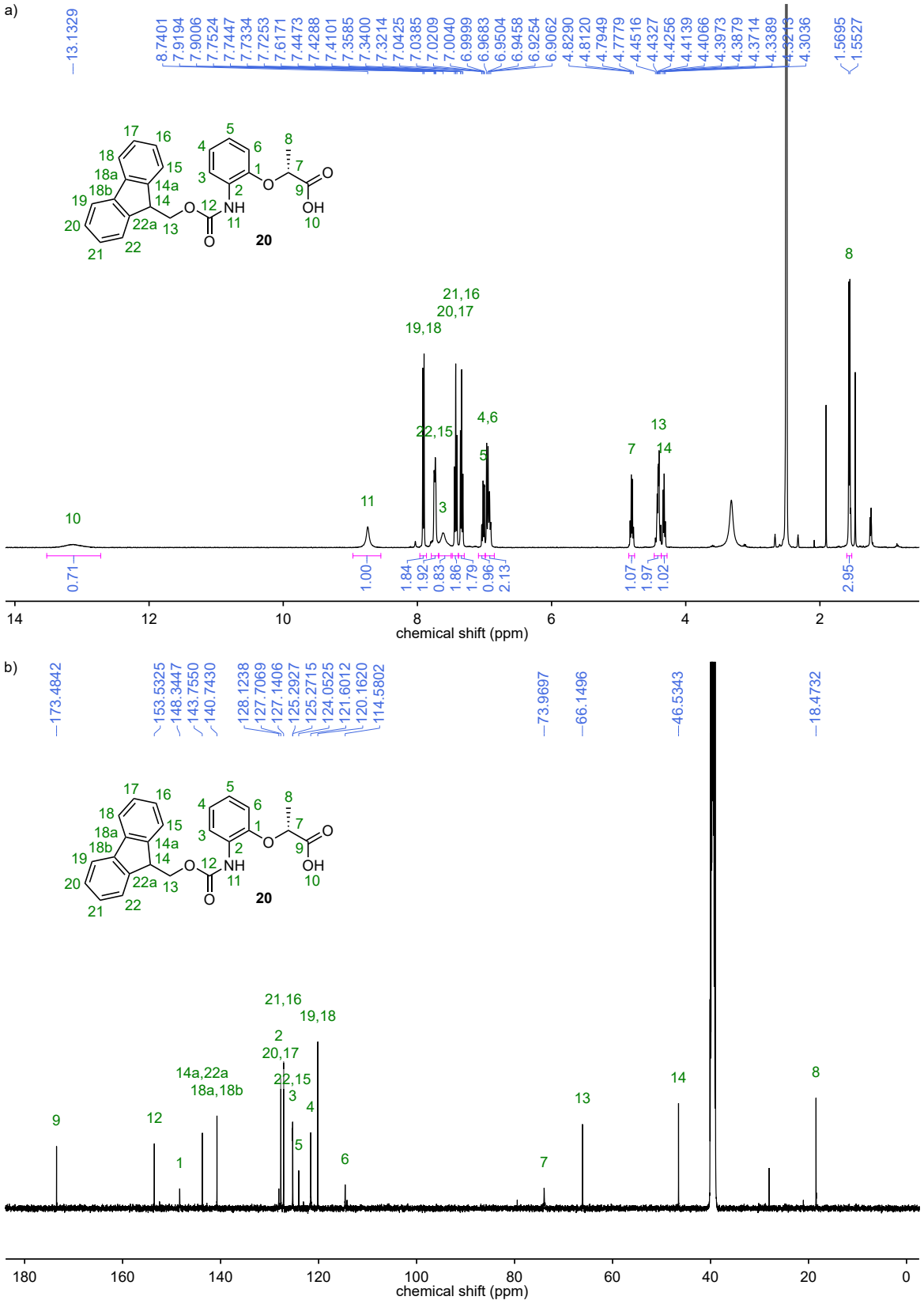


Fig. S28 NMR spectra of compound **20**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (126 MHz, DMSO-d_6).

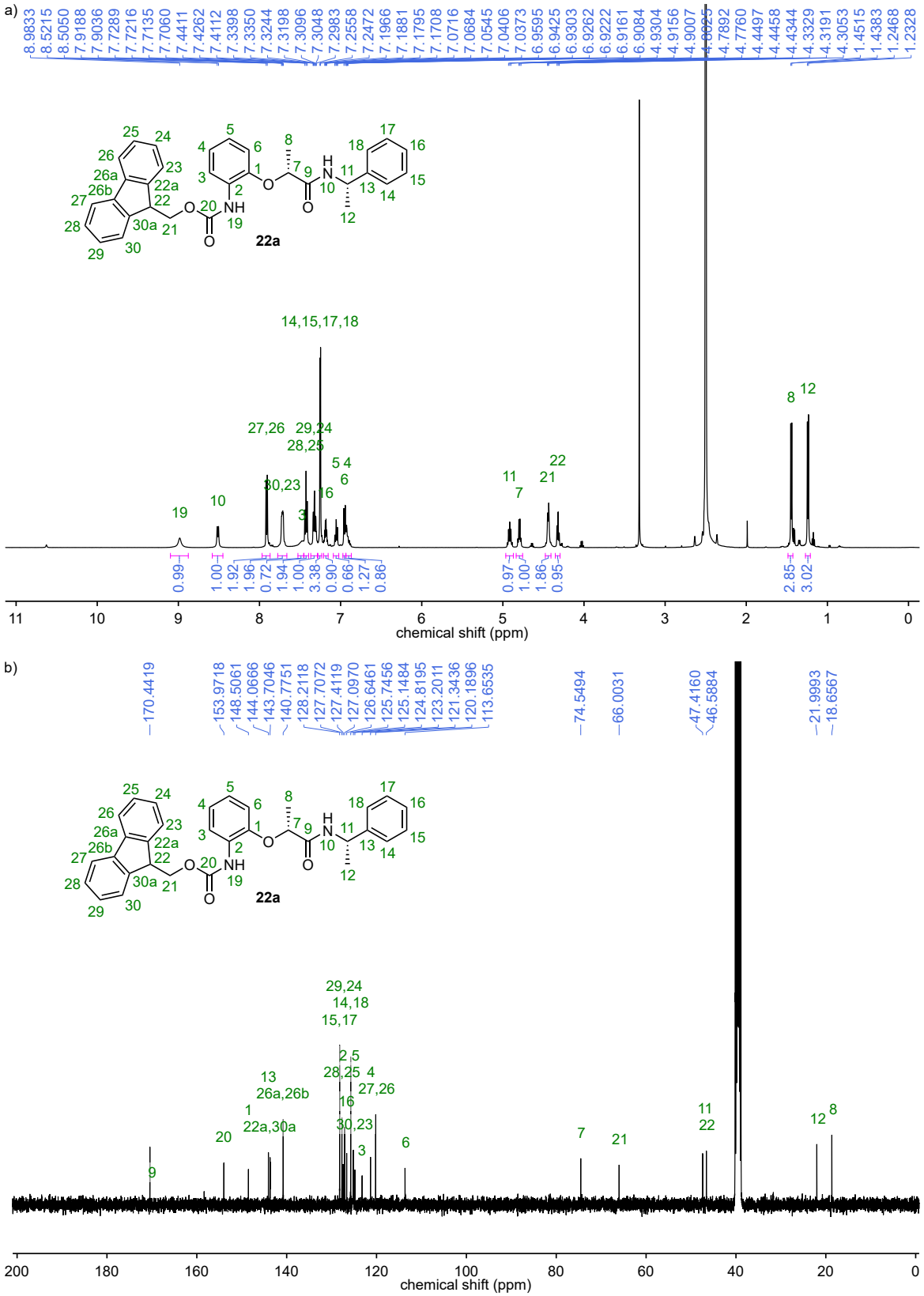


Fig. S29 NMR spectra of compound **22**. a) ^{1}H NMR (500 MHz, DMSO-d_6). b) ^{13}C NMR (101 MHz, DMSO-d_6).

6 Literature

(1) J. Buratto, C. Colombo, M. Stupfel, S. J. Dawson, C. Dolain, B. Langlois d'Estaintot, L. Fischer, T. Granier, M. Laguerre, B. Gallois and I. Huc, *Angew. Chem. Int. Ed.*, 2014, **53**, 883–887.

(2) B. Baptiste, C. Douat-Casassus, K. Laxmi-Reddy, F. Godde and I. Huc, *J. Org. Chem.*, 2010, **75**, 7175–7185.

(3) H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512–7515.

(4) L. Pohl and M. Eckle, *Angew. Chem. Int. Ed.*, 1969, **8**, 381–381.

(5) X. Hu, S. J. Dawson, Y. Nagaoka, A. Tanatani and I. Huc, *J. Org. Chem.*, 2016, **81**, 1137–1150.

(6) M. Akazome, Y. Ishii, T. Nireki and K. Ogura, *Tetrahedron Lett.*, 2008, **49**, 4430–4433.

(7) Z. Xu, K. Li, R. Zhai, T. Liang, X. Gui and R. Zhang, *RSC Adv.*, 2017, **7**, 51972–51977.

(8) O. Tour, S. R. Adams, R. A. Kerr, R. M. Meijer, T. J. Sejnowski, R. W. Tsien and R. Y. Tsien, *Nat. Chem. Biol.*, 2007, **3**, 423–431.

(9) G. Winter, *J. Appl. Crystallogr.*, 2010, **43**, 186–190.

(10) G. Winter, D. G. Waterman, J. M. Parkhurst, A. S. Brewster, R. J. Gildea, M. Gerstel, L. Fuentes-Montero, M. Vollmar, T. Michels-Clark, I. D. Young, N. K. Sauter and G. Evans, *Acta Crystallogr. D*, 2018, **74**, 85–97.

(11) P. Evans, *Acta Crystallogr. D*, 2006, **62**, 72–82.

(12) P. R. Evans and G. N. Murshudov, *Acta Crystallogr. D*, 2013, **69**, 1204–1214.

(13) A. J. Mccoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J. Appl. Crystallogr.*, 2007, **40**, 658–674.

(14) M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. Mccoy, S. J. Mcnicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson, *Acta Crystallogr. D*, 2011, **67**, 235–242.

(15) A. J. Mccoy, *Acta Crystallogr. D*, 2007, **63**, 32–41.

(16) A. W. Schüttelkopf and D. M. F. Van Aalten, *Acta Crystallogr. D*, 2004, **60**, 1355–1363.

(17) P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr. D*, 2010, **66**, 486–501.

(18) G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long and A. A. Vagin, *Acta Crystallogr. D*, 2011, **67**, 355–367.