Solid-phase synthesis of protected α-amino phosphonic acid oligomers

Yoshitaka Ishibashi and Masato Kitamura*

Contribution from the Research Center for Materials Science and the Department of Chemistry, Nagoya University

Note: All of Figures S1–S11 for the supporting information were attached at the end.

(1) General

Instruments. Nuclear magnetic resonance (NMR) spectra were recorded on JNM-ECA-500 and JNM-ECA-600 spectrometers (500 MHz for ^{1}H, 126 MHz for ^{13}C, and 202 MHz for ^{31}P; 600 MHz for ^{1}H, 151 MHz for ^{13}C, and 243 MHz for ^{31}P) unless otherwise specified. The chemical shifts are expressed in parts per million (ppm) downfield from tetramethylsilane or in ppm relative to $CHCl_3$ (δ 7.26 in ^{1}H NMR and δ 77.0 in ^{13}C NMR), CH_3OD (δ 3.31 in ^{1}H NMR and δ 49.0 in ^{13}C NMR), DMSO (δ 2.50 in ^{1}H NMR and δ 39.52 in ^{13}C NMR), CH_3CN (δ 1.94 in ^{1}H NMR), and H_3PO_4 (δ 0.00 in ^{31}P NMR). Signal patterns of ^{1}H NMR are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. All of spectra were measured at 25 °C with samples of 5–50 mM. High performance liquid chromatography analyses were performed on a JASCO PU-980 instrument equipped with an SCL-10A system controller, a DG-980-50 degasser, and a JASCO UV-970 UV detector. HRMS was measured by ESI-TOF-MS QSTAR (PE Biosystems). MALDI-TOF-MS spectra were taken on a PerSeptive Biosystems Voyger system. A BURRELL SCIENTIFIC wrist-action laboratory shaker was used for solid-phase synthesis.

Materials.

Gas: Argon (Ar) gas was purified by being passed through a column of the BASF R3-11 catalyst at 80 °C and then through a column of granular calcium sulfate.

Solvents: Solvents for the synthesis of monomer, the condensation, and the detachment were dried at the reflux temperature in the presence of appropriate drying agents (2.5 g/L) under Ar stream for 6 h and distilled into Schlenk flasks: methanol (CH_3OH) from Mg; *N*,*N*-dimethylformamide (DMF), acetonitrile (CH_3CN),

dichloromethane (CH_2Cl_2), and chloroform ($CHCl_3$) from CaH_2; acetone from Molecular Sieves 4A (MS 4A); ether from benzophenone ketyl. CD_3CN, CD_3OD, DMSO-d_6, and $CDCl_3$ were purchased from Cambridge Isotope Laboratory Inc., and, for NMR experiments, CD_3CN and $CDCl_3$ were purified by distillation over CaH_2, and CD_3OD over Mg. $CDCl_3$ for the general NMR measurement was dried over MS 4A. Distilled water purchased from nakarai tesque (NT) was used without further purification. First grade solvents purchased from NT were used for extraction and partition.

Silica gel: Analytical thin-layer chromatography (TLC) was performed using Merck 5715 indicating plates precoated with silica gel 60F254 (layer thickness, 0.25 mm). The product spots were visualized with a solution of PMA or *o*-anisaldehyde. Flash column chromatography was performed using Daiko AP 300 or NT Silica Gel 60 (spherical, neutral).

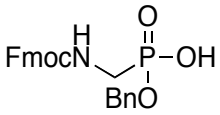
Reagents and chemicals: The reagents were purchased from companies and used without further purification unless otherwise specified. These are listed next in an alphabetical order neglecting number suffix. Kishida: trifluoroacetic acid (TFA). NT: triethylamine (TEA), 1-hydroxybenzotriazole (HOBt), *N*,*N*-diisopropylethylamine (DIEA), hydrochloric acid (aq 12 *M*), piperidine, pyridine (Py), sodium iodide (NaI), sodium sulfate (Na_2SO_4), sodium thiosulfate ($Na_2S_2O_3$), and thionyl chloride ($SOCl_2$). Tokyo Kasei Institute (TCI): benzyl alcohol, 1-hydroxy-7-azabenzotriazole (HOAt), and diisopropylcarbodiimide (DIC). Wako: sodium bicarbonate ($NaHCO_3$). Novabiochem: TentaGel (0.26 mmol/g). $SOCl_2$ was distilled before use. *N*-Fmoc-1-aminomethylphosphonic acid (Fmoc-GlyP(OH)-OH)[1,2] and $[RuCp(CH_3CN)_3]PF_6$[3] were synthesized according to the

literatures.

General synthetic operation: Condensation, Fmoc removal, and detachment reactions were carried out under Ar atmosphere by use of a general Schlenk technique unless otherwise specified. Solvents for the catalytic detachment were degassed before use. A Schlenk with Teflon J. Young valve and an NMR tube with the valve were specified by "Young-type Schlenk" and "Young-type NMR tube," respectively. Schlenks and general round bottom flasks were dried at ca. 250 °C by use of heat gun under a reduced pressure just before use, and silicon grease was used for connecting to a reflux condenser and a glass stopper. A Teflon-coated magnetic bar was used for stirring the reaction mixture. Liquid reagents were introduced by use of syringe via septum rubber. After introduction, the septum was replaced with a Young valve or a glass stopper. Cannulation was performed by use of Teflon tube or stainless tube through a septum rubber under a slightly positive pressure of Ar. Reactions at 0 °C was carried out by use of ice bath. Room temperature (rt) ranges from 25 °C to 28 °C. Solvents after general workup process were removed by means of a rotary evaporator. Concentration of a reaction mixture in a Schlenk tube or a flask was performed by connecting to a vacuum-Ar line via a cold trap. Organic extraction from aqueous layer by a general partition-based workup was dried over Na_2SO_4 (ca. 5% for the total extractions) for ca. 15 min, filtered, and concentrated under a reduced pressure (dryness/filtration/concentration process). Purification of a crude product by silica gel flush column chromatography was expressed simply by "chromatography." "Aqueous" and "saturated" were abbreviated "aq" and "sat," respectively.

(2) Synthesis of Fmoc-GlyP(OBn)-OH (**1**)

Fmoc-GlyP(OH)-OH[1] was converted to dichloridate and allowed to react with benzyl alcohol. Debenzylation of the resulting diester using NaI in acetone afforded *N*-9-fluorenylmethoxycarbonyl-*O*-benzyl-aminomethylphosphonate (Fmoc-GlyP(OBn)-OH, **1**) in 58% overall yield.



A 20-mL Young-type Schlenk was charged with Fmoc-GlyP(OH)-OH (1.50 g, 4.50 mmol), and then $SOCl_2$ (5.0 mL) was introduced at 0 °C. The resulting colorless solution was stirred at rt for 2 h. Concentration of the mixture at rt under a reduced pressure gave dichloro *N*-(9-fluorenylmethoxycarbonyl)-1-aminomethylphosphonate (Fmoc-GlyP(Cl)-Cl) as a white solid (1.7 g, quant), which was used for the next reaction without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.23 (t, *J* = 6.20 Hz, 1H, C*H*CH_2OC(O)N), 4.30 (d, *J* = 6.20 Hz, 2H, CHC*H*$_2$OC(O)N), 4.52 (d, *J* = 6.89 Hz, 2H, FmocNCH_2P(O)Cl_2), 5.61 (brt, *J* = 6.89 Hz, 1H, FmocNH), 7.32 (t, *J* = 7.57 Hz, 2H, aromatic), 7.41 (t, *J* = 7.57 Hz, 2H, aromatic), 7.58 (d, *J* = 7.57 Hz, 2H, aromatic), 7.77 (d, *J* = 7.57 Hz, 2H, aromatic); ^{13}C NMR (151 MHz, $CDCl_3$) δ 47.2, 50.8 (d, *J* = 115.6 Hz), 67.5 (d, *J* = 101.1 Hz), 120.2, 125.1, 127.3, 128.0, 141.5, 143.5, 155.7; ^{31}P{^{1}H} NMR (215 MHz, $CDCl_3$) δ 44.0, 44.3 (95:5 ratio).

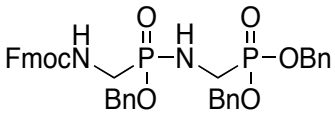
The crude Fmoc-GlyP(Cl)-Cl (1.7 g, 4.5 mmol) was dissolved in CH_2Cl_2 (10 mL), and the mixture was cooled to 0 °C. To this solution were slowly added benzyl alcohol (1.03 mL, 10.0 mmol) and TEA (1.40 mL, 10.0 mmol). After 8-h stirring at rt, CH_2Cl_2 (20 mL) was added. The whole mixture was washed with water (10 mL x 2) and brine (10 mL). Dryness/filtration/concentration process gave a colorless oil, which was purified by chromatography (40 g, 1:1 hexane–ethyl acetate then 9:1 CH_2Cl_2–CH_3OH) afforded

N-9-fluorenylmethoxycarbonyl-*O*-dibenzyl-aminomethylphosphonate (Fmoc-GlyP(OBn)-OBn) (1.90 g, 82% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 (d, *J* = 11.0 Hz, 2H, FmocNCH_2P(O)(OBn)$_2$), 4.16 (t, *J* = 6.85 Hz, 1H, C*H*CH_2OC(O)N), 4.36 (d, *J* = 7.57 Hz, 2H, CHCH_2OC(O)N), 4.93–5.15, 5.47, 5.80 (m, brs, brs, 4H, P(O)(OCH_2Ph)$_2$), 7.25–7.44 (m, 14H, aromatic), 7.55 (d, *J* = 7.57, 2H, aromatic), 7.75 (d, *J* = 7.57 Hz, 2H, aromatic), 10.5 (br, 1H, FmocNH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 37.1 (d, *J* = 157.5 Hz), 47.1, 67.4, 68.1 (d, *J* = 5.78 Hz), 120.1, 125.1, 127.1, 127.8, 128.2, 128.6, 128.8, 135.9, 141.4, 143.8, 156.2; ^{31}P{^{1}H} NMR (202 MHz, $CDCl_3$) δ 23.5, 24.3 (96:4 ratio).

Fmoc-GlyP(OBn)-OBn (1.90 g, 3.70 mmol), acetone (20 mL), and NaI (1.60 g, 10.7 mmol) were placed in a 30-mL round-bottom flask, and a reflux condenser was equipped. The yellow solution was refluxed for 2 days and then cooled to rt. After concentration to ca. 5 mL at rt under a reduced pressure, water (50 mL) was added, and the mixture was washed with ethyl acetate (15 mL). The aq layer was acidified to be pH 2 by addition of 5 *M* aq HCl (5 mL) and extracted with ethyl acetate (20 mL x 5). The combined organic layers were washed with sat aq $Na_2S_2O_3$ (5 mL). Dryness/filtration/concentration process afforded a yellow solid (1.3 g), which was recrystallized from ether–$CHCl_3$ to give Fmoc-GlyP(OBn)-OH (**1**) (1.11 g, 71% yield) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.49 (dd, *J* = 6, 10 Hz, 2H, FmocNCH_2P(O)(OBn)(OH)), 4.22 (t, *J* = 6.89 Hz, 1H, C*H*CH_2OC(O)N), 4.29 (d, *J* = 7.57 Hz, 2H, CHCH_2OC(O)N), 5.01 (d, *J* = 6.89 Hz, 2H, P(O)(OCH_2Ph)), 7.25–7.37 (m, 5H, aromatic), 7.41 (t, *J* = 7.57 Hz, 4H, aromatic), 7.74 (d, *J* = 7.57 Hz, 2H, aromatic), 7.79 (t, *J* = 6.20 Hz, 1H, FmocNH), 7.88 (d, *J* = 7.57 Hz, 2H, aromatic); ^{13}C NMR (151 MHz, DMSO-d_6) δ 37.1 (d, *J* = 154.6 Hz), 46.7, 66.0, 66.2 (d, *J* = 5.78 Hz),

120.2, 125.4, 127.2, 127.5, 127.7, 127.9, 128.4, 137.3, 140.8, 143.9, 156.3; $^{31}P\{^{1}H\}$ NMR (243 MHz, DMSO-d_6) δ 21.0, 21.4 (1:9 ratio); $^{31}P\{^{1}H\}$ NMR (243 MHz, CD_3CN) δ 21.7. HRMS exact mass calcd for $C_{23}H_{22}NO_5P$ + Na^+ 446.1133, found 446.1102. The NMR spectra were shown in Figure S1.

(3) Condensation of Fmoc-GlyP(OBn)-OH (**1**) with H-GlyP(OBn)-OBn (**2**)



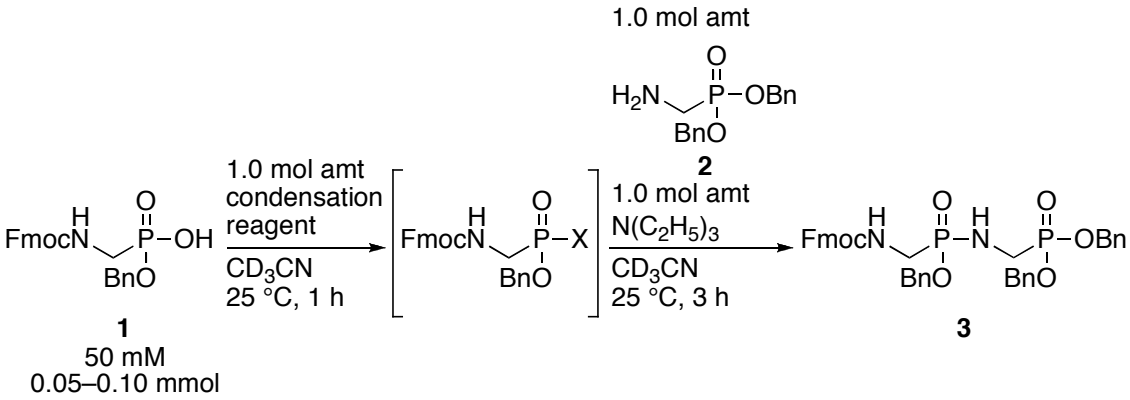
Fmoc-(GlyP(OBn)$_2$-OBn (**3**): *O*-Dibenzyl aminomethylphosphonate (H-GlyP(OBn)-OBn, **2**) was prepared by Fmoc removal from Fmoc-GlyP(OBn)-OBn as follow. A 50-mL round bottom flask was charged with Fmoc-GlyP(OBn)-OBn (800 mg, 1.56 mmol) and CH_2Cl_2 (9.0 mL). To this solution was added piperidine (1.0 mL, 10.1 mmol) at rt. After 1-h stirring at rt, the reaction mixture was concentrated. The $^{31}P\{^{1}H\}$ NMR analysis showed quantitative conversion to **2**. The residue was purified by chromatography (30 g, ethyl acetate then 9:1 CH_2Cl_2–CH_3OH, tailing), giving H-GlyP(OBn)-OBn (**2**) (314 mg, 69% yield) as a colorless oil. ^{1}H NMR (600 MHz, CD_3CN) δ 1.34 (brs, 2H, NH_2), 2.97 (d, J = 10.3 Hz, 2H, $H_2NCH_2P(O)(OBn)_2$), 5.01 (dd, J = 8.26, 11.7 Hz, 2H, $P(O)(OCHHPh)_2$), 5.05 (dd, J = 8.26, 12.1 Hz, $P(O)(OCHHPh)_2$), 7.32–7.40 (m, 10H, aromatic); ^{13}C NMR (151 MHz, CD_3CN) δ 38.6 (d, J = 150.3 Hz), 68.0 (d, J = 7.22 Hz), 118.3, 128.8, 129.5 138.0; $^{31}P\{^{1}H\}$ NMR (243 MHz, CD_3CN) δ 29.3; HRMS exact mass calcd for $C_{15}H_{18}NO_3P$ 291.1024, found 291.1024. The NMR spectra were shown in Figure S2.

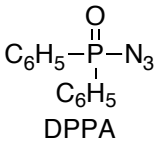
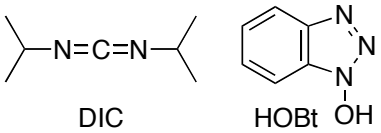
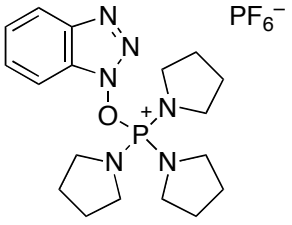
The progress of condensation between **1** and **2** was monitored by $^{31}P\{^{1}H\}$ NMR. The spectrum change was shown in Figure S3. A 5-mm Young-type NMR tube was charged with **1** (21.2 mg, 50 μmol) and CD_3CN (0.50 mL), and then DIC (9.4

μL, 60 μmol) was added. After 10 min at rt, the complete conversion of **1** was determined by $^{31}P\{^{1}H\}$-NMR analysis (Figure S3 (**c**), δ 16.7). The signal was assigned to the pyrophosphonate anhydride **4**. The chemical shift was close to the value reported for (Fmoc-GlyP(OC_2H_5))O (δ 16.3 and several signals in $CDCl_3$).[1] To this colorless solution was added HOAt (8.2 mg, 60 μmol) at rt. The NMR analysis after 30 min at rt indicated that the ^{31}P signal at δ 16.7 completely disappeared to converge with the signal at δ 30.7 (Figure S3 (**d**)), which was assigned to the HOAt ester **5**. To the reaction mixture was added a CD_3CN solution (0.25 mL) of **2** (14.6 mg, 50 μmol) and DIEA (9.0 μL, 52 μmol) in another 5-mm Young-type NMR tube. The ^{31}P signal at δ 30.7 disappeared after 1 h at rt to give new signals assignable to Fmoc-(GlyP(OBn))$_2$-OBn (**3**) (Figure S3 (**e**): δ 26.2 (50%), d, J = 21.8 Hz, $CH_2P(O)(OBn)_2$; δ 27.3 (4%); δ 28.0 (46%), d, J = 21.8 Hz, $CH_2P(O)(OBn)N$). Signal at δ 27.3 would be assigned to the rotamer of Fmoc moiety. The ^{31}P-NMR analysis indicated near-quantitative formation of **3**. The reaction mixture was concentrated, and the residue was chromatographed on silica gel (3 g; 9:1 ethyl acetate–CH_3OH) to give **3** as a colorless oil (32 mg, 93% isolated yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.19–3.36 (m, 2H, $P(O)NCH_2P(O)(OBn)_2$), 3.54 (ddd, J = 5.51, 10.33, 15.84 Hz, 1H, FmocNC*H*HP(O)N), 3.70 (ddd, J = 6.89, 9.98, 16.18 Hz, 1H, FmocNCH*H*P(O)N), 4.18 (t, J = 7.57 Hz, 1H, C*H*CH_2OC(O)N), 4.33 (dd, J = 6.89, 10.33 Hz, 1H, CHC*H*HOC(O)N), 4.37 (dd, J = 7.57, 10.33 Hz, 1H, CHCH*H*OC(O)N), 4.91–5.07 (m, 6H, P(O)(OCH_2Ph)$_2$ and NP(O)(OCH_2Ph)), 6.18 (brt, J = 6.20 Hz, 1H, FmocNH), 7.24–7.33 (m, 17H, aromatic), 7.37 (t, J = 7.57 Hz, 2H, aromatic), 7.60 (brd, J = 6.89 Hz, 2H, aromatic), 7.74 (d, J = 7.57, 2H, aromatic); ^{13}C NMR (151 MHz, $CDCl_3$) δ 37.0 (d, J = 160.4 Hz), 38.2 (d, J = 145.9 Hz), 47.2, 66.3 (d, J = 5.78 Hz),

67.3, 68.2 (d, J = 5.78 Hz), 68.3 (d, J = 7.22 Hz) 120.1, 125.2, 127.2, 127.8, 128.0, 128.3, 128.5, 128.7, 128.8, 128.9, 135.9, 136.1, 141.4, 143.9, 156.6; $^{31}P\{^{1}H\}$ NMR (243 MHz, $CDCl_3$) δ 25.8, 27.0, 28.0 (50:4:46 ratio); $^{31}P\{^{1}H\}$ NMR (243 MHz, CD_3CN) δ 25.9, 27.0, 28.0 (50:4:46 ratio); HRMS exact mass calcd for $C_{38}H_{38}N_2O_7P_2$ + Na^+ 719.2052, found 719.2007. The NMR spectra were shown in Figure S4.

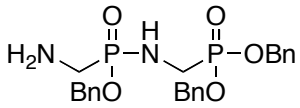
The other routes that have been explored are shown below.



condensation reagent	X	% yield of **3**[a]
 DPPA	N_3	<20
$SOCl_2$	Cl	80
 DIC HOBt	OBt	<5
 PyBOP	OBt	<5

[a] Determined by ^{31}P-NMR analysis.

(4) Fmoc removal from Fmoc-(GlyP(OBn))$_2$-OBn (**3**)

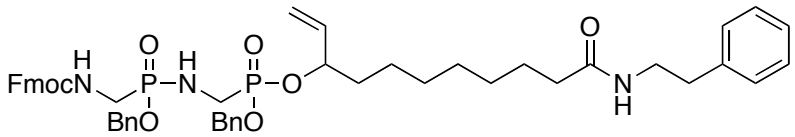


H-(GlyP(OBn))$_2$-OBn: A 5-mm Young-type NMR tube was charged with Fmoc-(GlyP(OBn))$_2$-OBn (**3**) (30 mg, 43 μmol) and $CDCl_3$ (0.70 mL), and then piperidine (22 μL, 0.22 mmol) was added. After 1 h at rt, the ^{31}P signals at 25.8, 27.0, and 28.0 (50:4:46) of **3** completely disappeared and the new signals at δ 25.6 and 32.3 appeared, indicating quantitative Fmoc removal (Figure S5). The mixture was concentrated and the residue was purified by chromatography (4 g, ethyl acetate then 9:1 CH_2Cl_2–CH_3OH, tailing) to give H-(GlyP(OBn))$_2$-OBn (17 mg, 83% yield) as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$) δ 1.57 (brs, 2H, NH_2), 2.76 (brm, 1H, P(O)NH), 2.91 (d, J = 8.95, 2H, $H_2NCH_2P(O)N$), 3.20–3.34 (m, 2H, $P(O)NCH_2P(O)(OBn)_2$), 4.89 (dd, *J* = 7.57, 11.71 Hz, 1H, P(O)(OC*H*HPh)), 4.95–5.09 (m, 3H, P(O)(OCH*H*Ph) and P(O)(OCH_2Ph)$_2$), 7.28–7.36 (m, 15H, aromatic); ^{13}C NMR (151 MHz, $CDCl_3$) δ 37.1 (d, *J* = 159.0 Hz), 39.4 (d, *J* = 138.7 Hz), 65.8 (d, *J* = 7.22 Hz), 68.2 (d, *J* = 5.78 Hz), 128.1, 128.4, 128.6, 128.8, 128.9, 135.9, 136.4; $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) δ 25.6, 32.3 (49:51 ratio); HRMS exact mass calcd for $C_{23}H_{28}N_2O_5P_2 + Na^+$ 497.1371, found 497.1375. The NMR spectra were shown in Figure S6.

(5) Detachment of Fmoc-(GlyP(OBn))$_2$-OH/DIEA (**7c**) from Fmoc-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ (**7a**)

The compound **7a** was prepared in three steps from Fmoc-GlyP(OBn)-OH (**1**) and a model linker **8a**: (1) condensation between **1** and **8a** giving Fmoc-GlyP(OBn)-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$, (2) Fmoc removal, and (3) condensation with **1**. The conditions were not optimized. The compound **7a**

was then subjected to RuCp/$P(C_6H_5)_3$-catalyzed deallylation reaction.

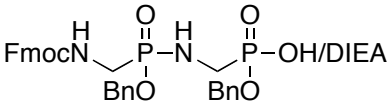


Fmoc-(GlyP(OBn))$_2$-O CH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ (**7a**): A 50-mL Young-type Schlenk was charged with **1** (1.75 g, 4.13 mmol) and CH_3CN (15 mL). To this solution were added DIC (1.55 mL, 9.9 mmol) and HOAt (1.35 g, 9.9 mmol). After 15-min stirring at rt, a CH_3CN solution (10 mL) of model linker HOCH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ (**8a**) (1.25 g, 4.12 mmol) was introduced, and the colorless solution was stirred at rt for 3 h. The mixture was partitioned between ethyl acetate (150 mL) and water (80 mL), and the aq layer was extracted with ethyl acetate (50 mL x 2). The combined organic layers were washed with water (50 mL) and brine (30 mL x 2). Dryness/filtration/concentration followed by chromatography (250 g, 1:2 hexane-ethyl acetate) afforded Fmoc-GlyP(OBn)-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ as a colorless solid (2.02 g, 69% yield). $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) δ 22.2, 23.0, 23.2. This compound (1.00 g, 1.41 mmol) and CH_2Cl_2 (10 mL) were placed in a 5-mL Young-type Schlenk, and then piperidine (1.0 mL, 10.1 mmol) was added. After 1 h at rt, the mixture was concentrated to give an oil, which was purified by short chromatography (15 g, ethyl acetate, then 10:1 CH_2Cl_2–CH_3OH) to give H-GlyP(OBn)-O CH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ (550 mg, 80% yield) as a colorless oil. $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) δ 28.0, 28.4.

Fmoc-GlyP(OBn)-OH (**1**) (627 mg, 1.48 mmol) and CH_3CN (5.0 mL) were placed in a 20-mL Young-type Schlenk. To this was added DIC (0.31 mL, 2.0 mmol) and HOAt (272 mg, 2.0 mmol). After 30-min stirring at rt, the solution was added at 0 °C to an CH_3CN solution (5.0 mL) containing the N terminal free H-GlyP(OBn)-O

$CH(CH{=}CH_2)(CH_2)_7CONH(CH_2)_2C_6H_5$ (720 mg, 1.48 mmol) and DIEA (340 μL, 2.0 mmol). After 8-h stirring at rt, the whole mixture was partitioned between ethyl acetate (20 mL) and water (20 mL). The aq layer was extracted with ethyl acetate (20 mL x 2), and the combined organic layers were washed with water (20 mL) and brine (20 mL). Dryness/filtration/concentration followed by chromatography (80 g, ethyl acetate, then 9:1 ethyl acetate–CH_3OH) afforded Fmoc-$(GlyP(OBn))_2$-$OCH(CH{=}CH_2)(CH_2)_7CONH(CH_2)_2C_6H_5$ (**7a**) as a colorless solid (1.26 g, 95% yield). Full assignment of the NMR signals was difficult because of the existence of theoretically four diastereomers. Only the major signals or all of the digital data are listed. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.15–1.34 (m, 11H, $(CH_2)_6$), 1.47–1.73 (m, 3H, $(CH_2)_6$), 2.02–2.12 (m, 2H, $CH_2C(O)N$), 2.79 (t, J = 6.90 Hz, 2H, $CH_2CH_2C_6H_5$), 2.79 (overlapped br signal, 1H, P(O)NH), 3.27 (m, 2H, $P(O)NCH_2P(O)O_2$), 3.48 (dt, J = 6.20, 6.90 Hz, 2H, $C(O)NHCH_2$), 3.57 (m, 1H, $FmocNCHHP(O)N$), 3.71 (m, 1H, $FmocNCHHP(O)N$), 4.18 (t, J = 6.90, 1H, $CHCH_2OC(O)N$), 4.29–4.4 (m, 2H, $CHCH_2OC(O)N$), 4.72–4.85 (m, 1H, $CH_2{=}CHCHO$), 4.94–5.31 (m, 6H, $CH_2{=}CHCHO$, P(O)(OCH_2Ph)(OLinker) and NP(O)(OCH_2Ph)), 5.60 (brm, 1H, C(O)NH (major)), 5.71–5.83 (m, 1H, $CH_2{=}CHCHO$), 6.28 (brm, 1H, FmocNH (major)), 7.14–7.40 (m, 19H, aromatic), 7.59 (d, J = 7.57, 2H, aromatic), 7.74 (d, J = 7.57, 2H, aromatic); ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.5, 25.5, 28.7, 28.8, 28.9, 29.0, 35.97, 35.98, 38.5, 40.4, 46.9, 65.9, 67.0, 67.7, 68.0, 79.1, 119.8, 125.1, 126.2, 127.5, 127.6, 127.9, 128.2, 128.4, 128.6, 135.8, 136.8, 138.9, 141.0, 156.5, 173.0; ^{31}P{^{1}H} NMR (243 MHz, $CDCl_3$) δ 24.5, 24.6, 25.7, 24.9, 27.0, 27.8; ^{31}P{^{1}H} NMR (243 MHz, CD_3OD) δ 24.9, 25.0, 25.1, 25.2, 28.7, 29.5, 29.6; HRMS exact mass calcd for $C_{50}H_{60}N_3O_8P_2 + Na^+$ 914.3675, found 914.3654. The NMR spectra were

shown in Figure S7.



Fmoc-(GlyP(OBn))$_2$-OH/DIEA (**7c**): Catalytic deallylation was carried out in an NMR tube, and the detachment of **7c** from Fmoc-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH(CH$_2$)$_2$C$_6$H$_5$ (**7a**) was monitored by ^{31}P{^{1}H} NMR. The spectrum change was shown in Figure S8. A 5-mm Young-type NMR tube containing **7a** (22.3 mg, 25 μmol), DIEA (4.35 μL, 25 μmol), and CD$_3$OD (0.25 mL) was degassed by three freeze–thaw cycles. To this was added a degassed 1.0 mM CD$_3$OD solution of [RuCp(P(C$_6$H$_5$)$_3$)(CH$_3$CN)$_2$]PF$_6$ (0.25 mL, 0.25 μmol). After 3 h at rt, the ^{31}P signals of **7a** disappered to converge with a different set of signals (δ 18.9 (d, *J* = 26.3 Hz, OP(O)O), 29.9 (d, *J* = 26.3 Hz, NP(O)O) (Figure S8 (**b**)), which were assigned to Fmoc-(GlyP(OBn))$_2$-OH/DIEA (**7c**). The spectrum change in the reaction without DIEA was shown in Figure S8 (**d–f**).

In a similar way, a double-scale detachment reaction was carried out (**7a** (44.6 mg, 50 μmol), DIEA (9 μL, 52 μmol), [CpRu(P(C$_6$H$_5$)$_3$)(CH$_3$CN)$_2$]PF$_6$ (1.0 mM CH$_3$OH solution, 0.5 mL, 0.5 μmol), CH$_3$OH (1 mL), rt, 2 h). The mixture was concentrated, and then partitioned between water (2 mL) and ether (2 mL). The aq layer was washed with ether (2 mL x 2), and freeze-dried to give a white solid (36 mg). ^{1}H NMR (600 MHz, CD$_3$OD) δ 1.30–1.33 (brd and brt, *J* = 6.89 Hz, 15H, C*H*$_3$CH$_2$N(CH(C*H*$_3$)$_2$)$_2$), 3.04 (brt, *J* = 5.51 Hz, 1H, P(O)NH), 3.12–3.18 (m, 4H, CH$_3$C*H*$_2$N(C*H*(CH$_3$)$_2$)$_2$), 3.53–3.70 (m, 4H, FmocNHC*H*$_2$P(O)N and P(O)NC*H*$_2$P(O)(OBn)(OH)), 4.15 (t, *J* = 6.89 Hz, 1H, C*H*CH$_2$OC(O)N), 4.24–4.32 (two dd (higher order), 2H, CHC*H*$_2$OC(O)N), 4.95–5.04 (m, 4H, P(O)(OCH$_2$Ph) x 2), 7.16–7.31 (m, 8H, aromatic), 7.32 (d, *J* = 6.89 Hz, 2H, aromatic), 7.37 (t, *J* = 7.57 Hz, 2H, aromatic), 7.41 (d, *J* = 7.57 Hz, 2H, aromatic), 7.65 (d, *J* = 7.57 Hz, 2H, aromatic),

7.78 (d, J = 7.57 Hz, 2H, aromatic); ^{13}C NMR (151 MHz, CD_3OD) δ 13.1, 17.3, 18.7, 23.0, 23.7, 38.5 (d, J = 150.3 Hz), 38.7 (d, J = 145.9 Hz), 43.7, 45.6, 48.3, 55.7, 67.0 (d, J = 5.78 Hz), 67.4 (d, J = 4.33 Hz), 68.3, 120.9, 126.3, 128.6, 128.6, 128.7, 129.2, 129.4, 129.5, 129.8, 137.9, 139.9, 142.5, 145.3, 158.6; ^{31}P{^{1}H} NMR (243 MHz, CD_3OD) δ 18.9 (d, J = 26.3 Hz, OP(O)O), 29.9 (d, J = 26.3 Hz, NP(O)O); HRMS exact mass calcd for $C_{39}H_{50}N_3O_7P_2$ 736.3202, found 736.3225. The signals at δ 17,3 (br), 18.7 (br), 23.0, 23.7, 43.7, 45.6, and 48.3 in the ^{13}C NMR spectrum are ambiguous. The NMR spectra were shown in Figure S9. Concentration of the organic layer afforded a crude oil (16 mg). The ^{1}H- NMR analysis showed that 11-methoxy-*N*-phenethylundec-9-enamide and 9-methoxy-*N*-phenethylundec-10-enamide (**8b**) was formed in ca. 1:3 ratio (δ 5.67 (dt, J = 7.6 and 15.2 Hz, 1H, CH_2CH=$CHCH_2$) and δ 5.60 (ddd, J = 7.6, 7.6, and 16.5 Hz, 1H, CH_2CH=CH_2)).

(6) Solid-Phase Synthesis of Fmoc-(GlyP(OBn))$_6$-OH/DIEA

Fmoc-(GlyP(OBn))$_6$-OH/DIEA was prepared on TentaGel-attached Jones branched allyl linker **8c** (HOCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel) by (1) attachment of Fmoc-GlyP(OBn)-OH (**1**) on **8c** using DIC/HOAt/DIEA method, (2) Fmoc removal, (3) chain elongation with **1** using DIC/HOAt/DIEA method, (4) four repetition of (2)–(3) process, and finally (5) detachment of Fmoc-(GlyP(OBn))$_6$-OH/DIEA from Fmoc-(GlyP(OBn))$_6$-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel.

HOCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel: TentaGel-NH_2 (500 mg, 0.13 mmol) and DMF (3.0 mL) were placed in a 20-mL Young-type Schlenk, and the mixture was stood for 15 min for swelling. To this was added

9-((4-methoxyphenyl)diphenylmethoxy)-10-undecenoic acid[4] (160 mg, 0.338 mmol), DIC (120 μL, 0.776 mmol), and HOBt (110 mg, 0.814 mmol). The mixture was shaken for 10 h at rt. After confirming the conversion is >99% by Kaiser test (both of beads and solution showed yellow color),[5] the resin was filtered off and washed with DMF (5 mL x 3), CH_3OH (5 mL x 3), and CH_2Cl_2 (5 mL x 3). The resin was dried under a reduced pressure for 1 h, and swelled in CH_2Cl_2 (5 mL), To this was added TFA (50 μL, 0.67 mmol). After 1-h shaking at rt, the resin was collected by filtration and washed with DMF (5 mL x 3), CH_3OH (5 mL x 3), and CH_2Cl_2 (5 mL x 3). Dryness under a reduced pressure gave $HOCH(CH{=}CH_2)(CH_2)_7CONH$-TentaGel, which was used for the next reaction.

Fmoc-GlyP(OBn)-$OCH(CH{=}CH_2)(CH_2)_7CONH$-TentaGel: A 20-mL Young-type Schlenk was charged with $HOCH(CH{=}CH_2)(CH_2)_7CONH$-TentaGel obtained above, CH_3CN (6.0 mL), and DIEA (62 μL, 0.36 mmol), and the mixture was stood for 15 min for swelling. Another 5-mL Young-type Schlenk was charged with Fmoc-GlyP(OBn)-OH (**1**) (83 mg, 0.20 mmol) and CH_3CN (3.0 mL). DIC (47 μL, 0.30 mmol) and HOAt (41 mg, 0.30 mmol) were introduced, and the solution was stirred for 30 min at rt. This solution was cannulated to the swelled resin mixture at 0 °C. After 10-h shaking at rt, the resin was collected by filtration and washed with DMF (5 mL x 3), CH_3OH (5 mL x 3), CH_2Cl_2 (5 mL x 3). The resin was dried under reduced pressure for 1 h to give Fmoc-GlyP(OBn)-$OCH(CH{=}CH_2)(CH_2)_7CONH$-TentaGel. $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) δ 22.6, 23.4, 24.6 (4:85:11).

The resin (7.2 mg) was placed in a meth cylinder (20.0 mL), and CH_2Cl_2 containing 10% of piperidine was introduced so that the total volume becomes 20.0 mL.

After shaking for 30 min, the solution part was transferred to a quartz cell (1 cm x 1 cm x 2 cm) and measured the UV absorbance at 273 nm to be 1.19. According to Lambert-Beer rule, the concentration of the released Fmoc moiety (molar extinction coefficient, $\varepsilon = 12300$),[6] was determined to be 0.91 mM, indicating the formation of Fmoc-GlyP(OBn)-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel as well as MMTr removal is nearly quantitative.

H-GlyP(OBn)-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel: A 20-mL Young-type Schlenk was charged with Fmoc-GlyP(OBn)-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel obtained in the previous process, and swelled with CH_2Cl_2 (4.0 mL) for 15 min. To this was added piperidine (1.0 mL), and the mixture was shaken for 1 h at rt. Filtration/washing (DMF (5 mL x 3), CH_3OH (5 mL x 3), CH_2Cl_2 (5 mL x 3)/drying process afforded GlyP(OBn)-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel. $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) δ 28.0, 28.3, 29.4 (44:44:12). The signal at δ 29.4 could not be assigned. N-Allylation or –benzylation may occur.

Chain elongation to Fmoc-(GlyP(OBn))$_6$-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel: The DIC/HOAt/DIEA condensation/Fmoc removal process was the same as that for the synthesis of H-GlyP(OBn)-OCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel from HOCH(CH=CH_2)(CH_2)$_7$CONH-TentaGel (see above). The conversions in all steps were determined by the ^{31}P NMR analysis on the basis of the signal/noise (S/N) ratio. Fmoc UV analysis was not performed unless otherwise specified. Listed below are $^{31}P\{^1H\}$ NMR (243 MHz, $CDCl_3$) data, the signal area ratio, S/N ratio, and % convn. The ^{31}P NMR spectra change was shown in Fig. 3 in the manuscript.

Fmoc-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 29 (NP(O)O); ca. 1:1; 45:1; >97% convn. Weak signals which would be assigned to the rotamers were also observed. The conversion was confirmed by the UV analysis of the Fmoc removal reaction mixture obtained 5.5 mg of regin (273 nm; absorbance, 0.940).

H-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 33 (NP(O)O); ca. 1:1; 78:1; >98% convn.

Fmoc-(GlyP(OBn))$_3$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 28.5, 29 (terminal NP(O)O), 30 (internal NP(O)O); ca. 1:1:1; 40:1; >97% convn.

H-(GlyP(OBn))$_3$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 30 (internal NP(O)O), 33 (terminal NP(O)O); ca. 1:1:1; 97:1; >98% convn.

Fmoc-(GlyP(OBn))$_4$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 28.5, 29.5 (terminal NP(O)O), 30 (internal NP(O)O); ca. 1:1:2; 67:1; >98% convn.

H-(GlyP(OBn))$_4$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 30 (internal NP(O)O), 33 (terminal NP(O)O); ca. 1:2:1; 104:1; >99% convn.

Fmoc-(GlyP(OBn))$_5$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25.5 (OP(O)O), 28.5, 30 (terminal NP(O)O), 30.5 (internal NP(O)O); ca. 1:1:3; 60:1; >98% convn.

H-(GlyP(OBn))$_5$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 30.5 (internal NP(O)O), 32 (terminal NP(O)O); ca. 1:3:1; 44:1; >97% convn.

Fmoc-(GlyP(OBn))$_6$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel: δ 25 (OP(O)O), 28, 29 (terminal NP(O)O), 31 (internal NP(O)O); ca. 1:1:4; 51:1; >98%

convn.

Detachment of Fmoc-(GlyP(OBn))$_6$-OH/DIEA: A 20-mL Young-type Schlenk was charged with Fmoc-(GlyP(OBn))$_6$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONH-TentaGel (100 mg, 26 μmol), 1:1 CH_3OH–CH_2Cl_2 (3.0 mL), and DIEA (22 μL, 126 μmol). After 15 min, 100 μL of a yellow solution of [RuCp(P(C_6H_5)$_3$)(CH_3CN)$_2$]PF_6 (1.7 mg, 2.6 μmol) in CH_3OH (1 mL) was added to the swelled resin-containing Schlenk, and the whole mixture was shaken for 24 h at rt. The resin was removed by filtration and washed with CH_3OH (2 mL x 5) and CH_2Cl_2 (2 mL x 5). The filtrate was concentrated to give an oil (15 mg). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30 (m, 15H, DIEA), 2.98–3.20 (brm, 2H, CH_2PO_2), 3.24–3.50 (m, 10H, NP(O)CH_2), 4.11 (m, 1H, OCH_2CH), 4.23 (m, 2H, OCH_2CH), 4.93–5.07 (m, 12H, $C_6H_5CH_2$), 7.17–7.42 (m, 32H, aromatic), 7.56 (m, 2H, aromatic), 7.65 (m, 2H, aromatic), 7.77 (d, J = 6.89 Hz, 2H, aromatic); ^{31}P{^{1}H} NMR (202 MHz, $CDCl_3$) δ 19 (m, OP(O)O), 29.5 (m, terminal NP(O)O), 31.5 (m, internal NP(O)O) (ca. 1:1:4 ratio); HPLC analysis (column, Develosil ODS-UG; eluent, a 9:1 CH_3OH–H_2O; flow rate, 1 mL/min; detection, 254-nm light; t_R of hexamer, 3.3 min; MALDI-TOF-MS m/z (Fmoc-(GlyP(OBn))$_6$-O^- Na^+) obsd 1360.5, calcd 1360.3. The NMR spectra were shown in Figure S10 and the HPLC chart and the MS spectrum were shown in Figure S11. The ^{31}P NMR spectra change was shown in Fig. 3 in the manuscript.

Notes and references

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2 α-Amino phosphonic acid abbreviation follows the IUPAC rule determined for

usual α-amino acids by the addition of "P" in the end of three character: e.g. GlyP for α-aminomethyl phosphonic acid, and H-GlyP(OH)-OH represents $H_2NCH_2P(O)(OH)_2$.

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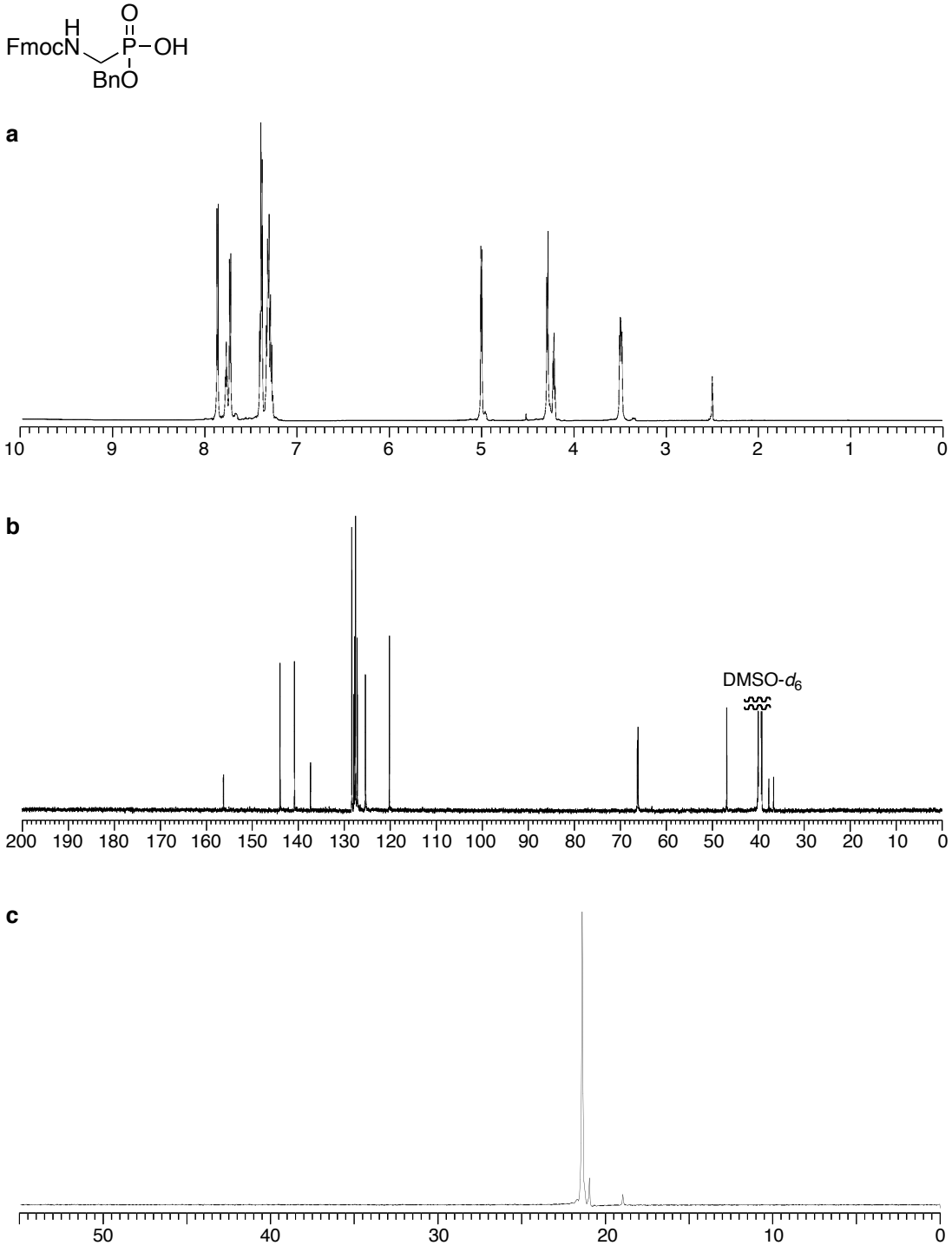

Figure S1. NMR spectra of Fmoc-GlyP(OBn)-OH (**1**). **a**: ^{1}H NMR (DMSO-d_6). **b**: ^{13}C NMR (DMSO-d_6). **c**: ^{31}P NMR (DMSO-d_6).

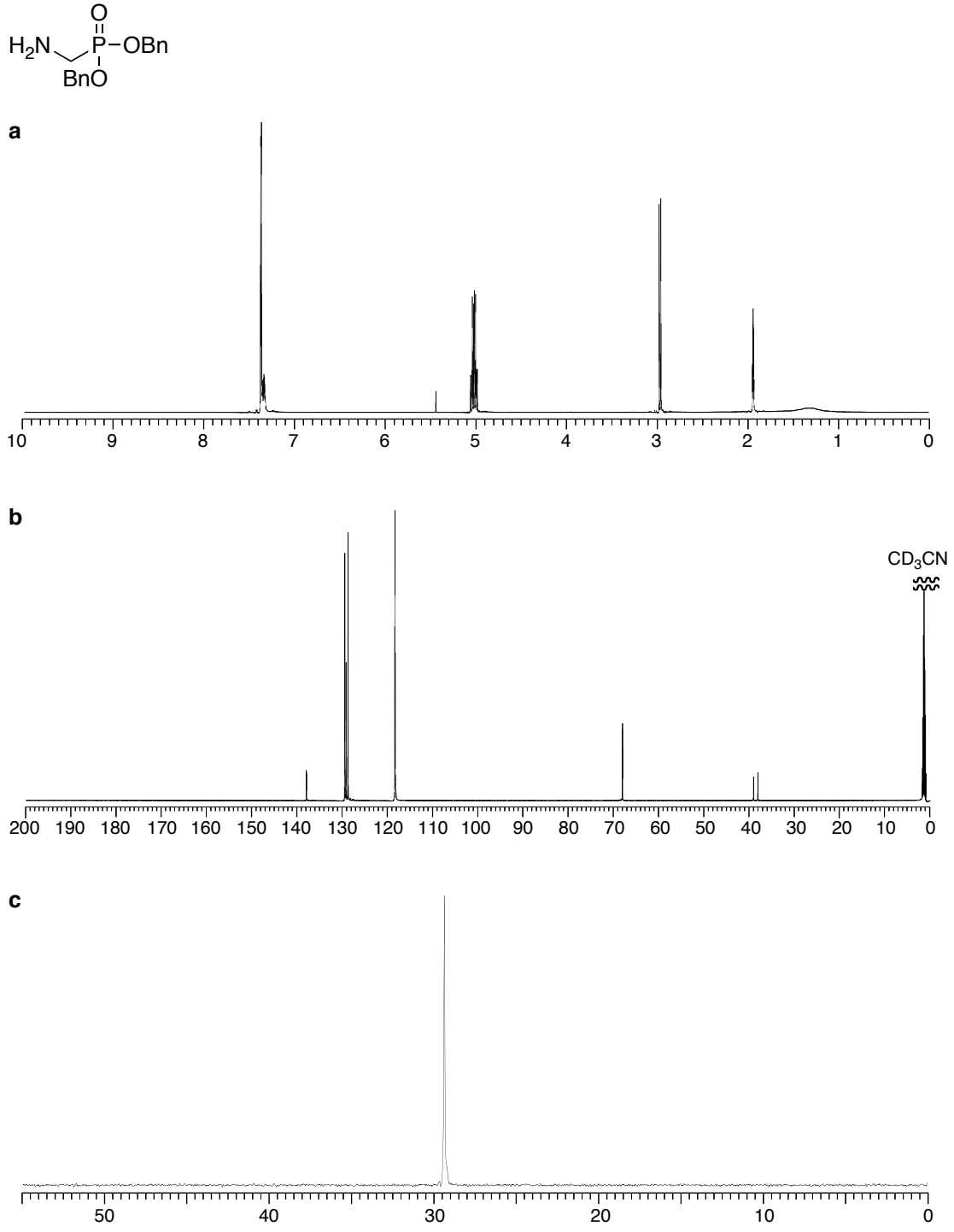

Figure S2. NMR spectra of H-GlyP(OBn)-OBn (**2**). **a**: ^{1}H NMR (CD_3CN). **b**: ^{13}C NMR (CD_3CN). **c**: ^{31}P NMR (CD_3CN).

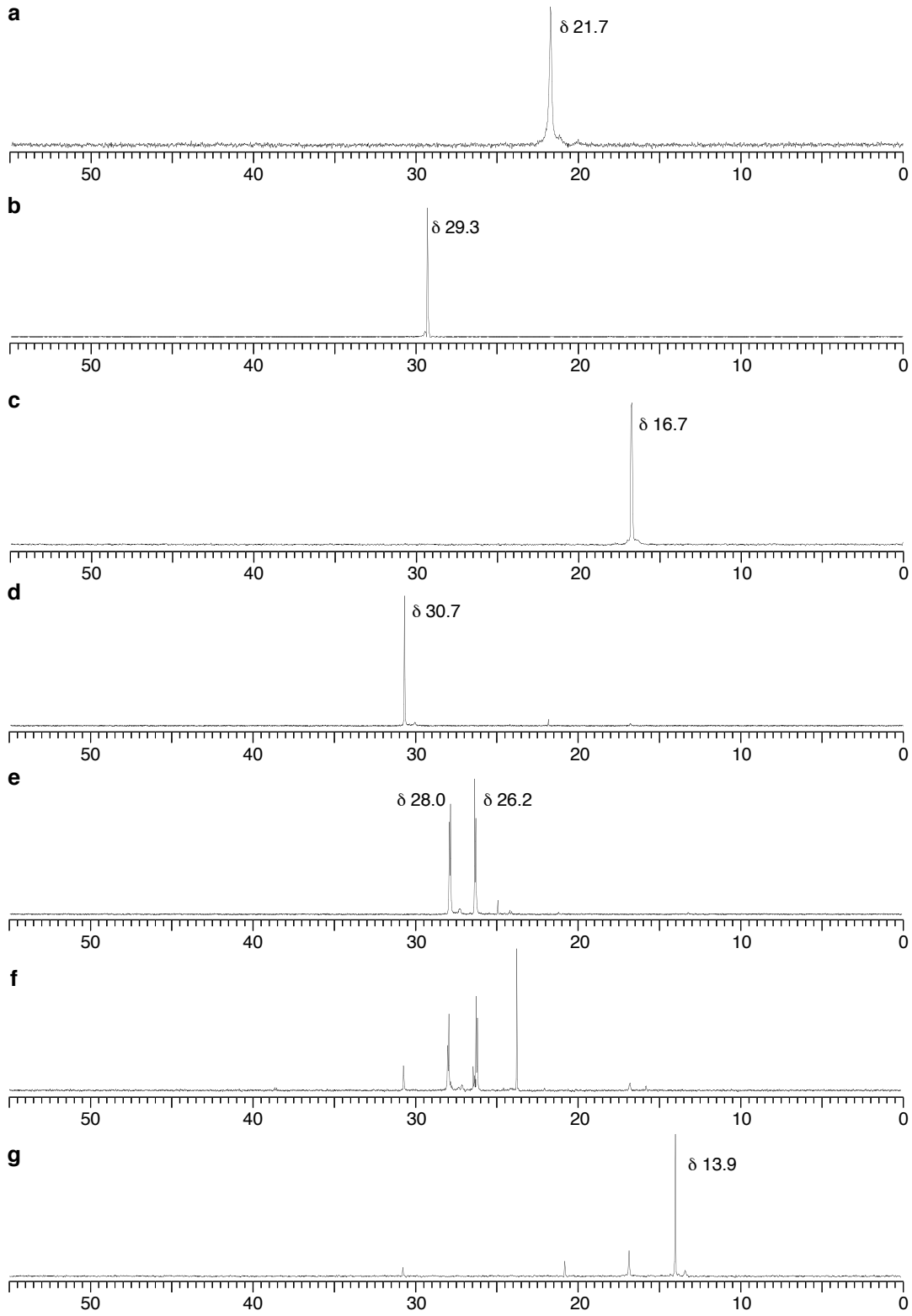

Figure S3. ^{31}P NMR spectra change in condensation of Fmoc-GlyP(OBn)-OH (**1**) with H-GlyP(OBn)-OBn (**2**) using DIC/HOAt/DIEA method in CD_3CN. **a**: Fmoc-GlyP(OBn)-OH. **b**: ^{1}H-GlyP(OBn)-OBn (**2**). **c**: 10 min after addition of 1.5 equivalent of DIC at the stage **a**. **d**: 30 min after addition of 1 equivalent of HOAt at the stage **c**. **e**: Addition of 1 equivalent of **2** and DIEA at the stage **d**. **f**: Addition of **2** at the stage **d**. **g**: 30 min after addition of 1 equivalent of HOAt and DIEA at the stage **c**.

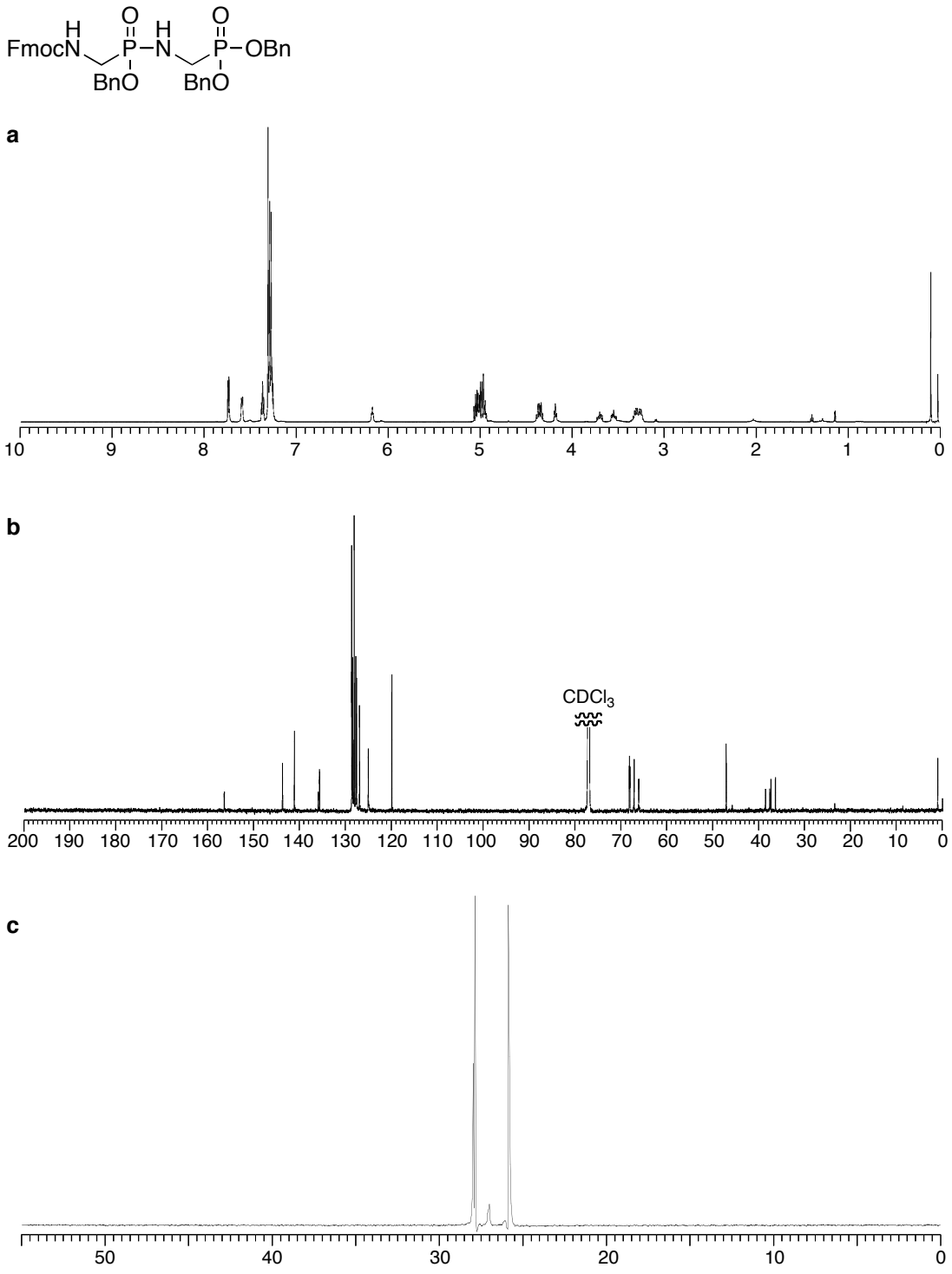

Figure S4. NMR spectra of Fmoc-(GlyP(OBn))$_2$-OBn (**3**). **a**: ^{1}H NMR ($CDCl_3$). **b**: ^{13}C NMR ($CDCl_3$). **c**: ^{31}P NMR ($CDCl_3$).

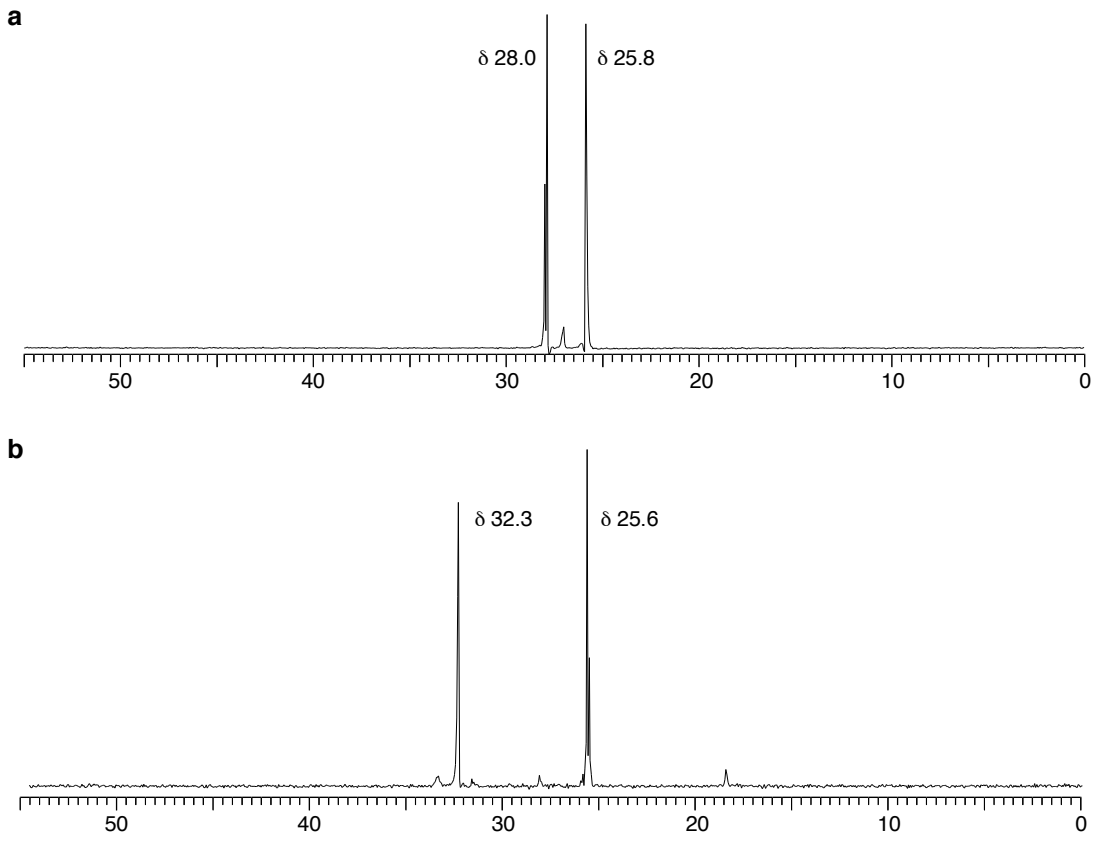

Figure S5. ^{31}P NMR spectra change in Fmoc removal from Fmoc-(GlyP(OBn))$_2$-OBn (**3**) using piperidine in $CDCl_3$. **a**: Fmoc-(GlyP(OBn))$_2$-OBn (**3**). **b**: 1 h after addition of piperidine.

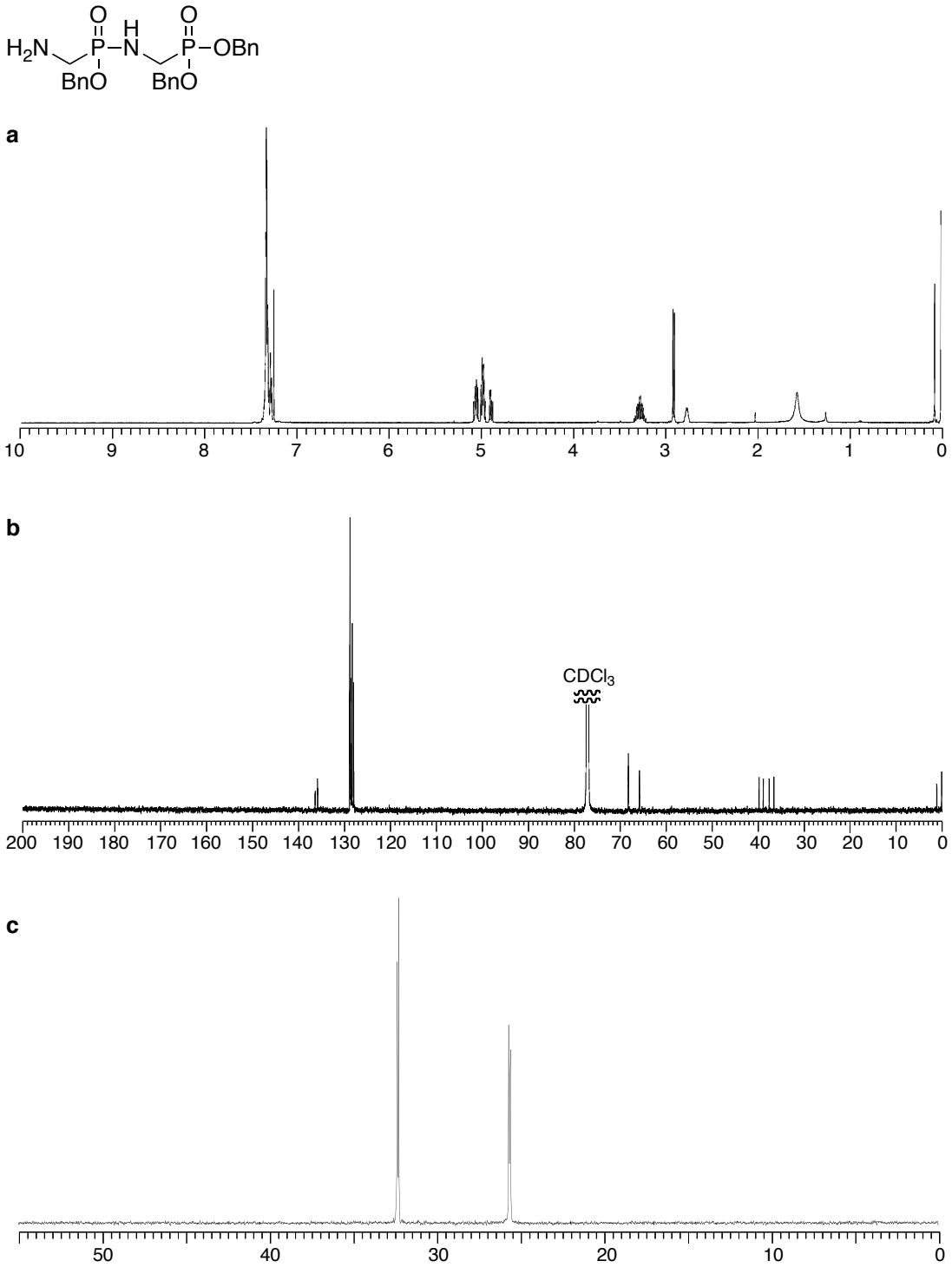

Figure S6. NMR spectra of H-(GlyP(OBn))$_2$-OBn. **a**: ^{1}H NMR ($CDCl_3$). **b**: ^{13}C NMR ($CDCl_3$). **c**: ^{31}P NMR ($CDCl_3$).

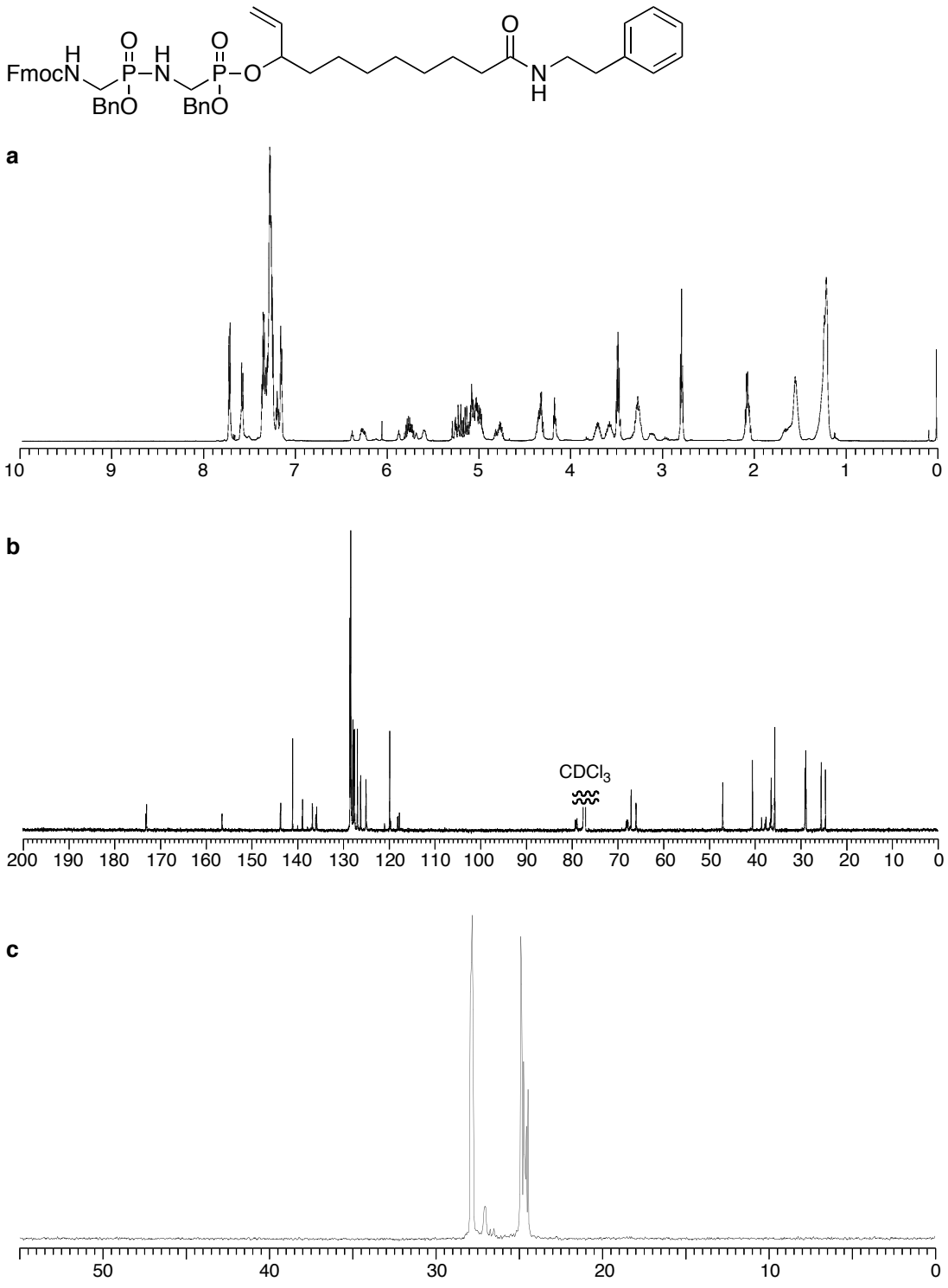

Figure S7. NMR spectra of Fmoc-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONHCH$_2$CH$_2$C$_6$H$_5$ (**7a**). **a**: ^{1}H NMR ($CDCl_3$). **b**: ^{13}C NMR ($CDCl_3$). **c**: ^{31}P NMR ($CDCl_3$).

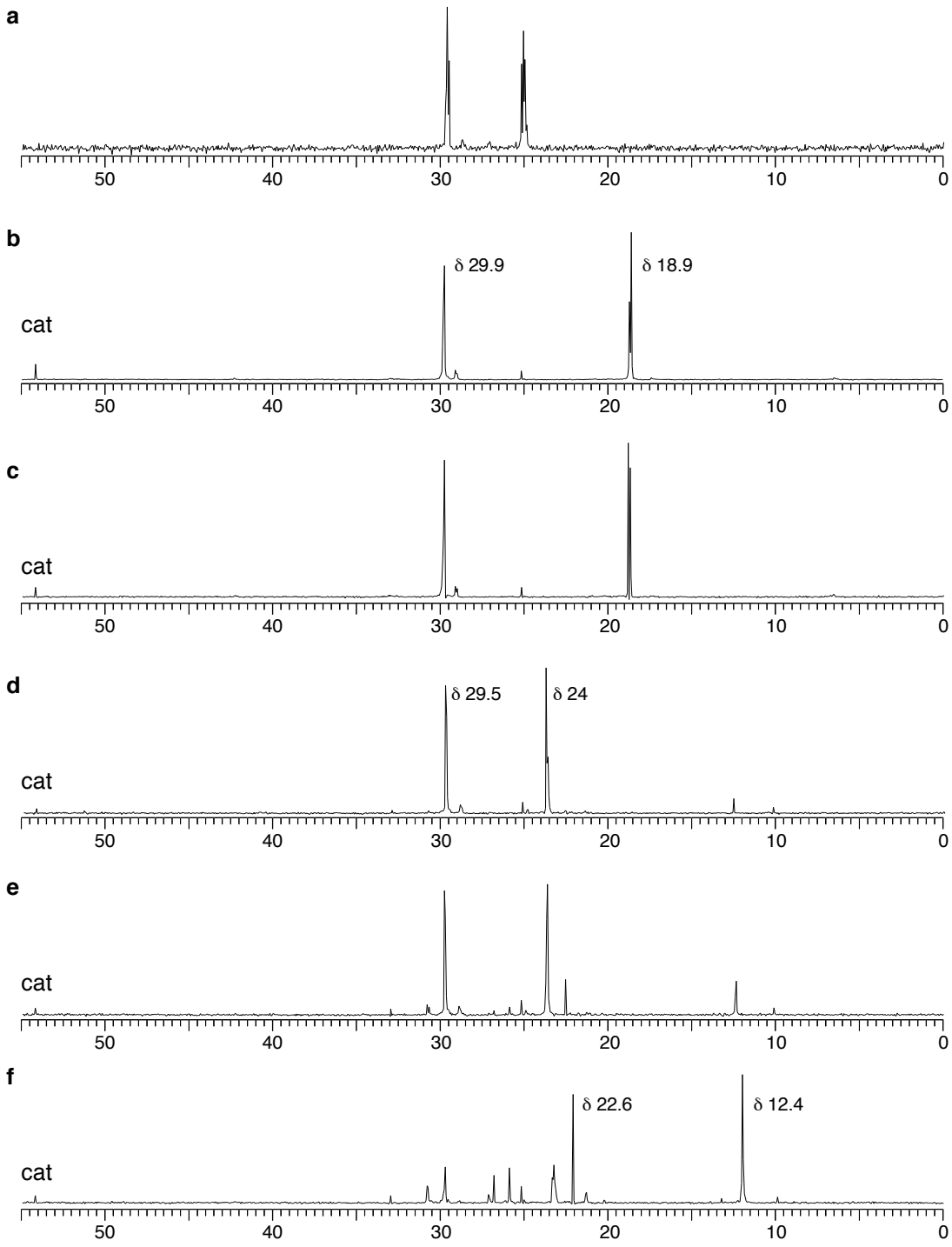

Figure S8. ^{31}P NMR spectra change in detachment of Fmoc-(GlyP(OBn))$_2$-OH/DIEA (**7c**) from Fmoc-(GlyP(OBn))$_2$-OCH(CH=CH$_2$)(CH$_2$)$_7$CONHCH$_2$CH$_2$C$_6$H$_5$ (**7a**) using [RuCp(P(C$_6$H$_5$)$_3$)(CH$_3$CN)$_2$]PF$_6$ in CD$_3$OD. **a**: **7a**. **b**: 3 h after addition of CpRu complex to the stage **a** in the presence of DIEA. **c**: 45 h after the stage **b**. **d**: 3 h after addition of CpRu complex to the stage **a** in the absence of DIEA. **e**: 5 h after the stage **d**. **f**: 45 h after the stage **d**.

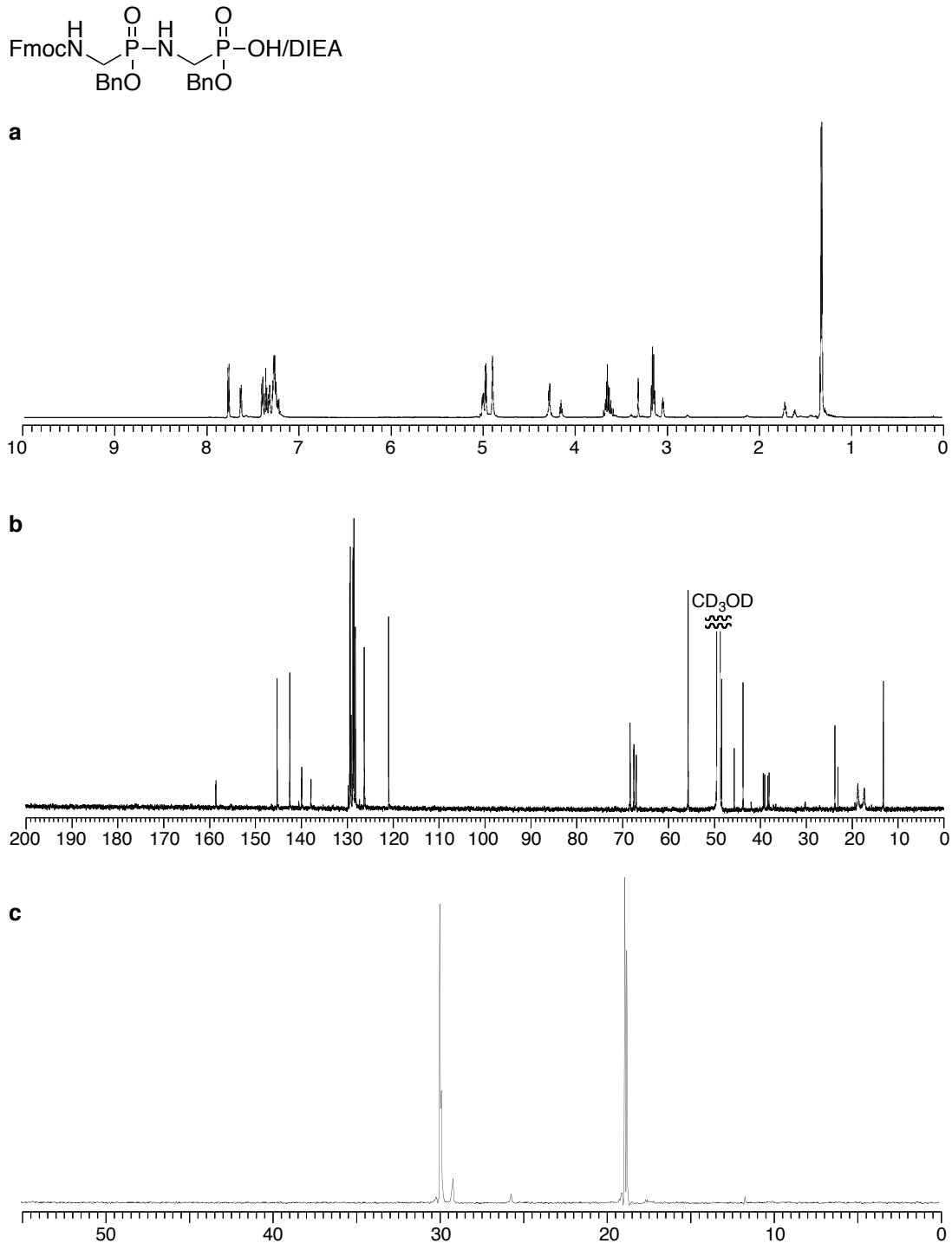

Figure S9. NMR spectra of Fmoc-(GlyP(OBn))$_2$-OH/DIEA (**7c**). **a**: ^{1}H NMR ($CDCl_3$). **b**: ^{13}C NMR ($CDCl_3$). **c**: ^{31}P NMR ($CDCl_3$).

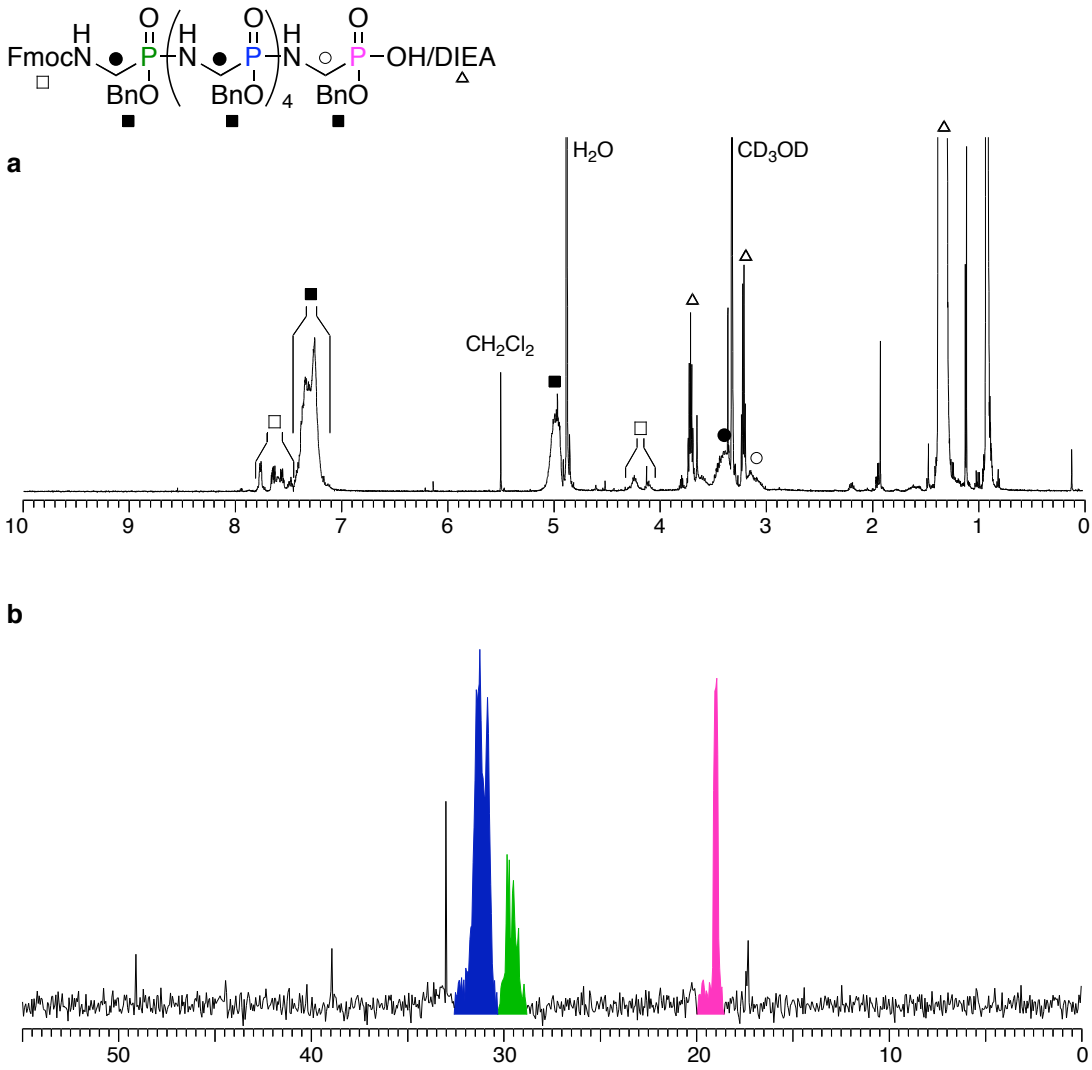

Figure S10. NMR spectra of crude Fmoc-$(GlyP(OBn))_6$-OH/DIEA detached from TentaGel. **a**: ^{1}H NMR ($CDCl_3$). **b**: ^{13}C NMR ($CDCl_3$). **c**: ^{31}P NMR ($CDCl_3$).

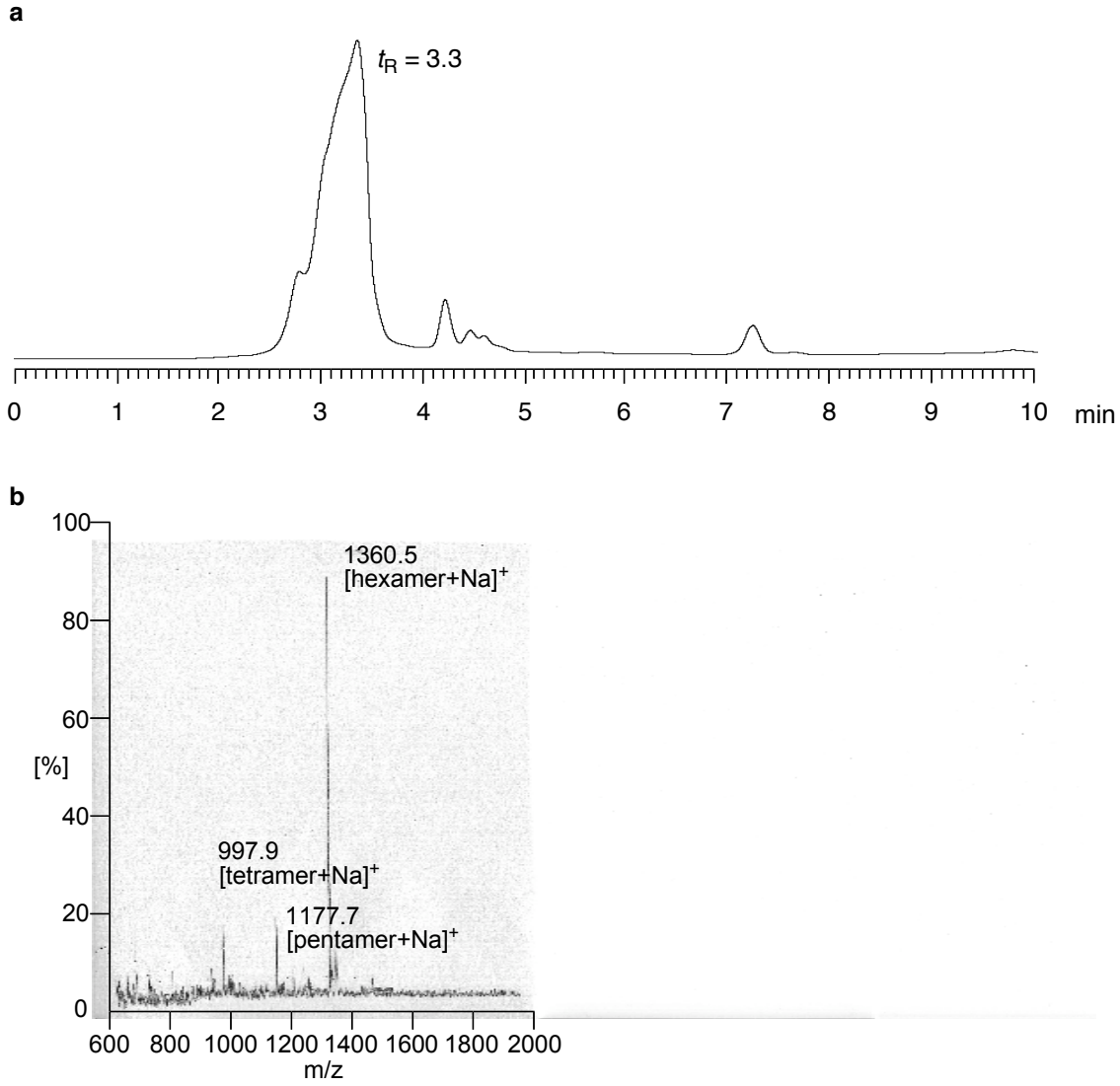

Figure S11. HPLC anslysis (**a**) and MALDI-TOF-MS spectrum (**b**) of crude Fmoc-(GlyP(OBn))$_6$-OH/DIEA detached from TentaGel.