Supporting information to:

The herringbone helix: a noncanonical folding pattern in mixed aliphatic–aromatic amide sequences

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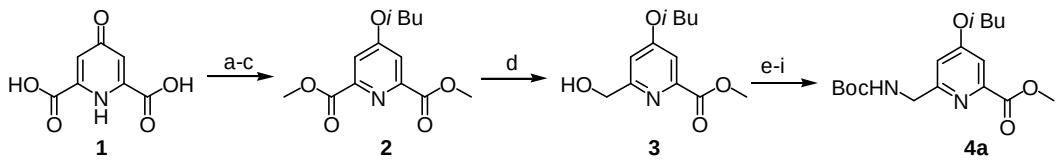
Description of syntheses. The synthesis of protected "P" monomer **4a** is depicted in Scheme S1. A key step is the desymmetrization of dimethyl 2,6-pyridinedicarboxylate **2**. The monoreduction of **2** using an optimized stoichiometry of $NaBH_4$ (2 equiv) yields the ester-alcohol in 77% yield. The hydroxyl group can then be converted into a Boc protected amine using conventional methods. Thus, reaction with $SOCl_2$ yielded the corresponding chloride, which was substituted with sodium azide. The benzylic azide was reduced via a Staudinger reaction and the obtained amine was protected with a Boc group to give **4a**.

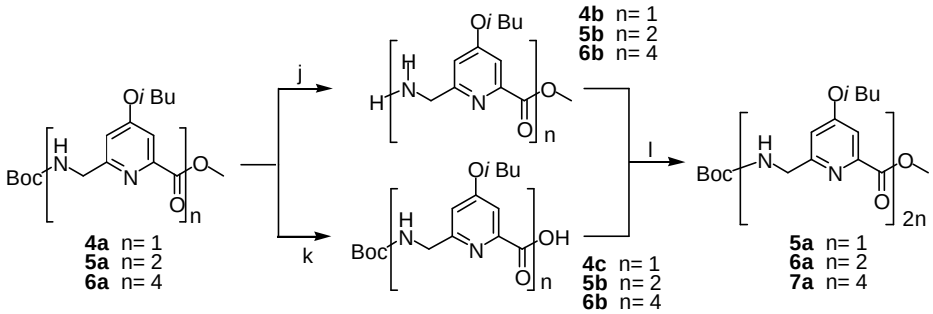
The elongation of the oligomer Bo-$(P)_n$-OMe was achieved through a convergent strategy consisting in doubling the oligomer length at each cycle (Scheme S2). Before each coupling step, part of the Boc ester protected starting material was saponified to give access to the corresponding acid, and part was treated with trifluoroacetic acid to cleave the Boc group and produce the corresponding amine. The coupling steps were handled using classical peptide synthesis conditions.

The synthesis of Boc-$(PQ)_n$-OMe oligomers was achieved in a similar way (Scheme 3). For the preparation of the Boc-$(PQ)_1$-OCH_3 dimer **9a** from pyridine acid **4b** and quinoline amine **6**, PyBOP was preferred to HBTU as a coupling agent because of the low reactivity of the aromatic amine.

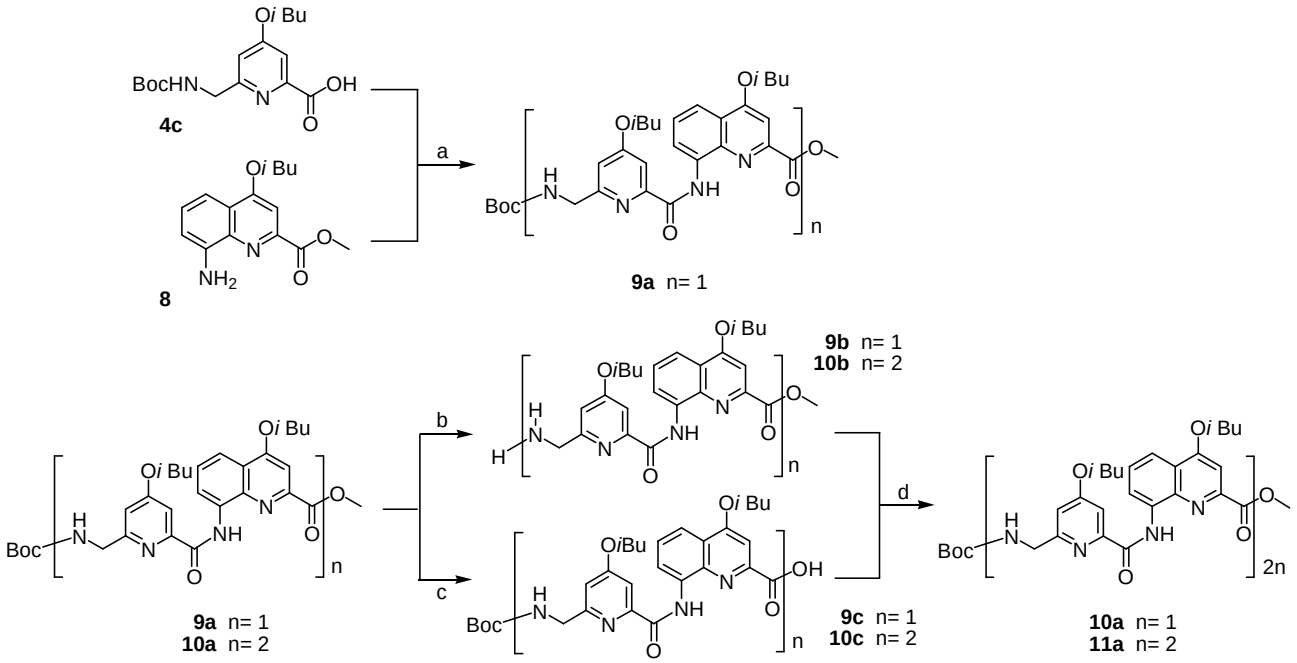


1 **2** **3** **4a**

Scheme S1: synthesis of BocNH-P-OCH_3. a) HCl, MeOH, dimethoxypropane, 60°C, 12h; b) K_2CO_3, DMF, 120°C 1.5h; c) *i*BuI, DMF, 70°C, 3h; d) $NaBH_4$, DCM, MeOH, r.t. 2h; e) $SOCl_2$, toluene, r.t., 3h; f) NaN_3, DMF, r.t., 3h; g) PPh_3, THF, r.t., 3h; h) H_2O, r.t., 5h; i) BocOboc, DMAP, toluene, r.t., 12h.



Scheme S2: synthesis of BocNH-P_8-OCH_3. j) TFA, DCM, r.t., 6h. k) LiOH, THF, MeOH, r.t., 16h. l) HBTU, HOBt, DIEA, DMF, r.t., 16h.



Scheme S3: synthesis of BocNH-$(PQ)_4$-OCH_3: a) PyBop, DIEA, DCM, r.t., 3h[1,2]. b) TFA, DCM, r.t., 6h. c) KOH, THF, MeOH, r.t., 16h. d) HBTU, HOBt, DIEA, DMF, r.t., 16h.

NMR solution studies.

Reconstitution of the sequence of Boc-$(PQ)_4$-OCH_3. Solution studies were performed in toluene-d8 at 75°C where sharp peaks are observed and where the signals spread over a large chemical shift range despite the repetitive nature of sthe sequence, consistent with folding phenomena (Figure S2). The spin systems of the different residues were partially identified from COSY experiments: strong correlations between H5, H6, H7 protons of the quinoline rings were observed and strong correlations between H3 and H5 were also observed for the pyridine rings (Figure S3). However, these experiments do not allow to distinguish H5 from H7 protons on a given residue and to associate them with the corresponding H3 of the same residue. The whole spin systems were unambigously identified from HMBC experiments and required the assignment of ^{13}C NMR signals corresponding to the aromatic backbone of the oligomer. Long range correlations between protons and carbons, namely H6-C10 (^3J), C10-H3 (^3J), H3-C4 (^2J), and C4-H5 (^3J) allowed a complete assignment of all spin systems (Figure S4).

The whole sequence was then reconstituted on the basis of 2D HMBC, HSQC and NOESY experiments. As shown in Figure S5, the strong proton-carbon correlations H3p-COp, COp-NHq, NHq-C7 (where p and q stand for pyridine and quinoline, respectively) allowed to hop from a pyridine residue to a quinoline residue. In the same way, proton-carbon correlations H3q-COq, COq-NHp, NHp-CH_2 allowed to hop from a quinoline residue to the CH_2 group of a pyridine residue. Finally, NOE correlations between this CH_2 protons and the neighbour H5 proton allowed to connect each CH_2 group to the correct pyridine spin system (Figure S6a)

All the experiments have allowed to assign the signals of all aromatic, amide and methylene protons and carbons. The final sequence is presented in the figure. The NOE correlations have been then fully assigned.

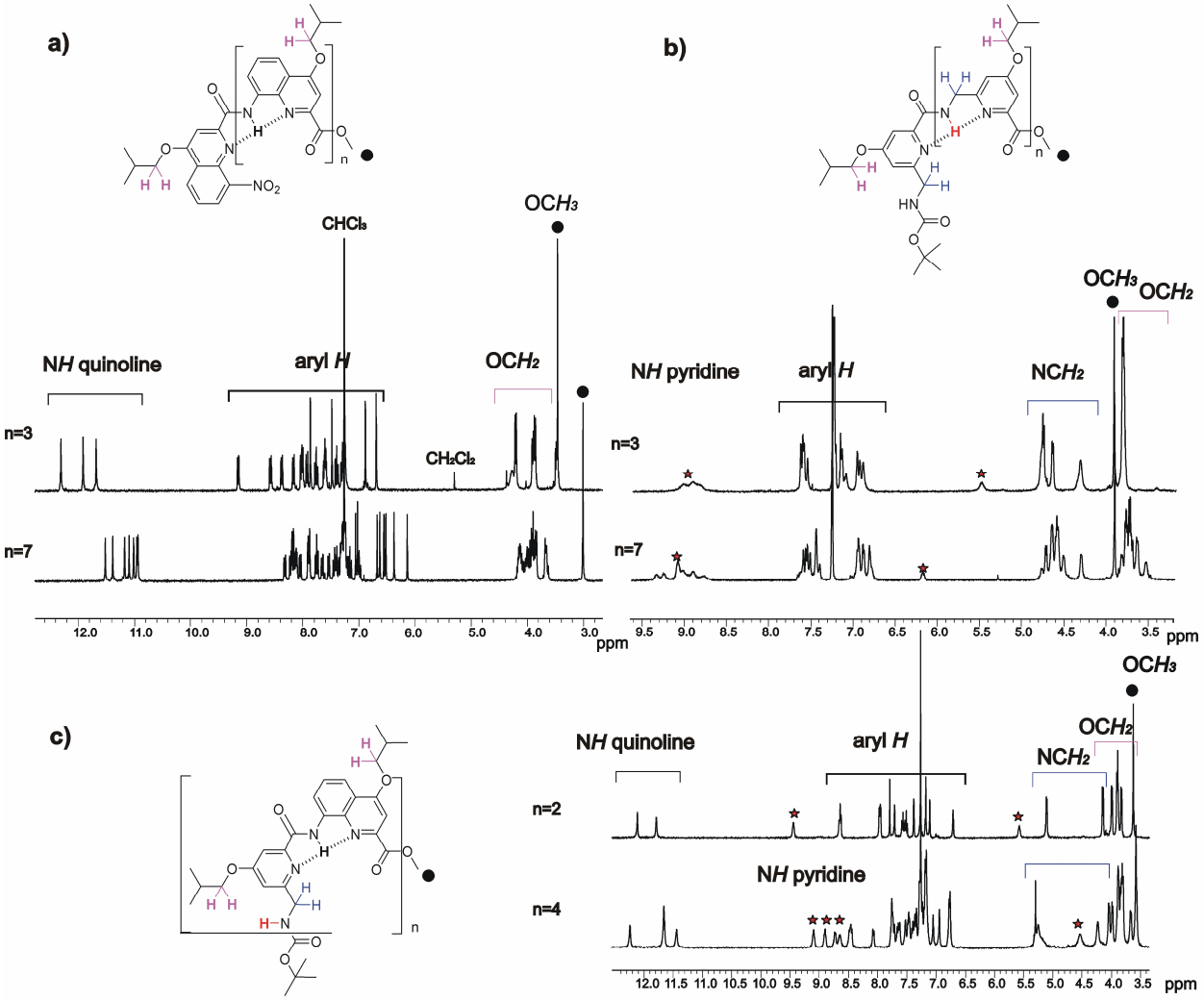


Figure S1: Part of 400 MHz ^{1}H NMR spectra of various oligomers in $CDCl_3$ at 25°C: a) O_2N-Q_n-OCH_3 in $CDCl_3$ at 25°C; b) Boc-P_n-OCH_3; c) Boc-$(PQ)_n$-OCH_3. The spectra illustrate the extent to which NMR signals spread over a large chemical shift for O_2N-Q_n-OCH_3 and Boc-$(PQ)_n$-OCH_3 oligomers but not for Boc-P_n-OCH_3 oligomers.

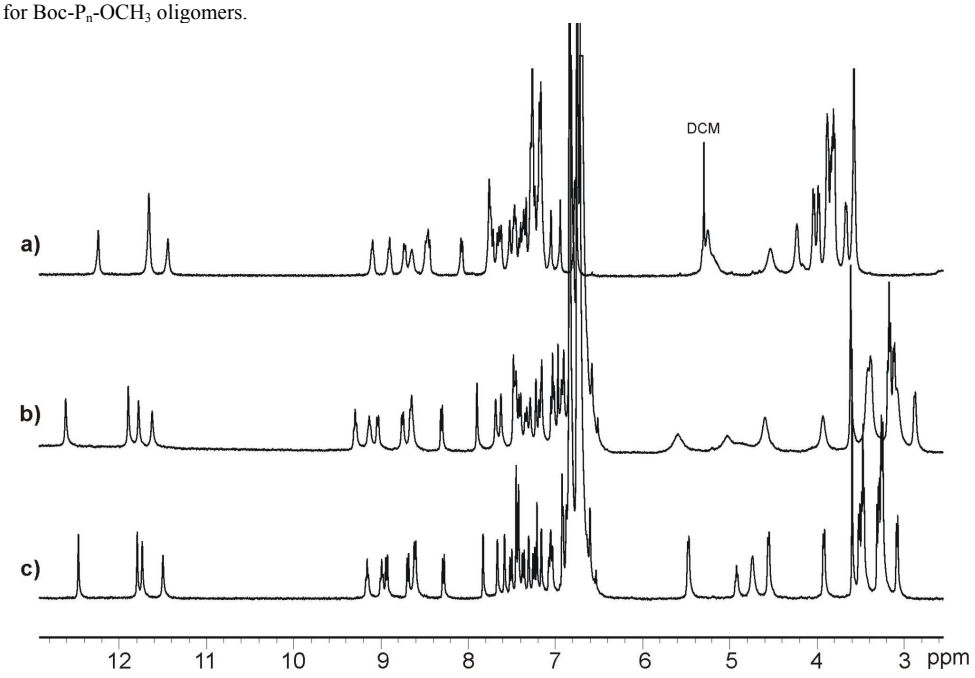


Figure S2: Part of the 400 MHz ^{1}H NMR spectra of oligomer Boc-$(PQ)_4$-OCH_3: a) in $CDCl_3$ at 25°C; b) in toluene-d_8 at 25°C. c) in toluene-d_8 at 75°C.

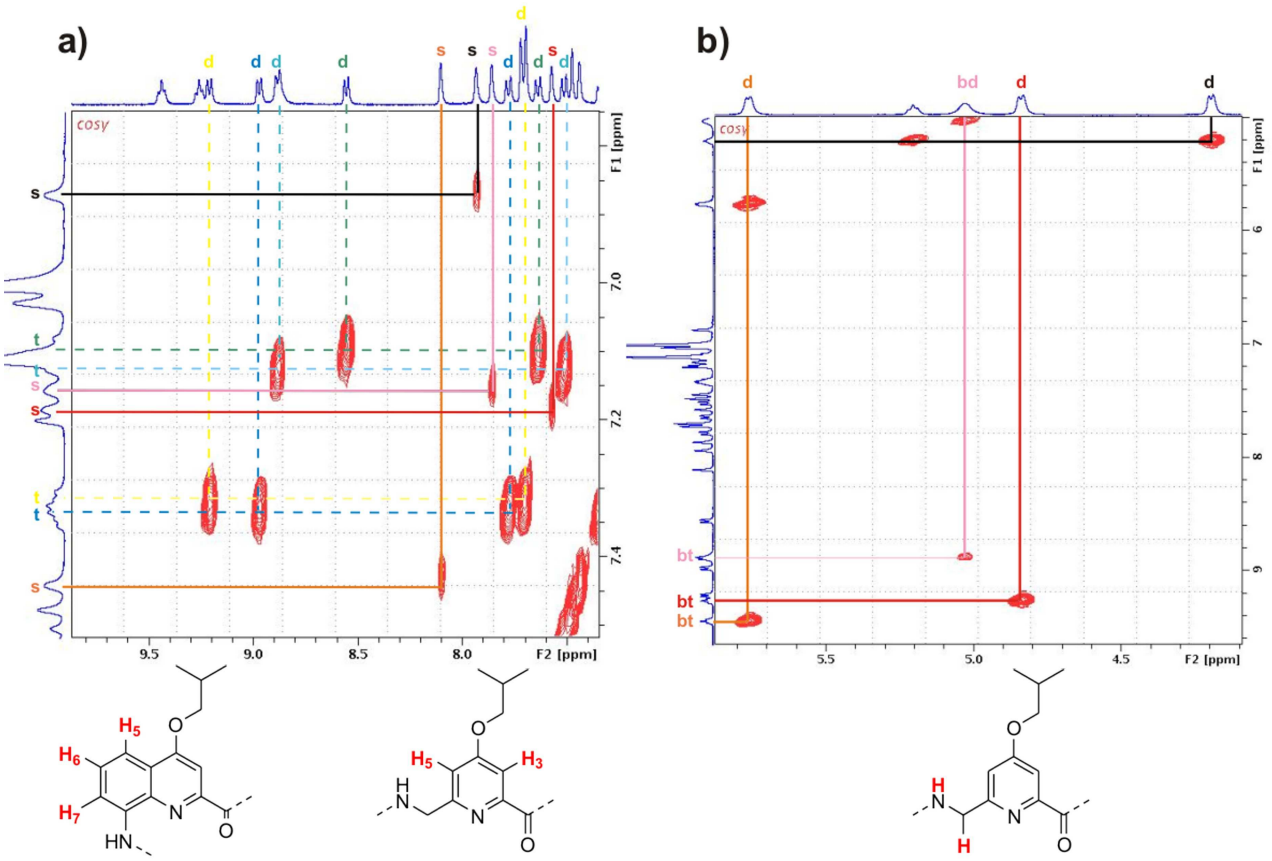


Figure S3: Part of the 400 MHz COSY plot of Boc-$(PQ)_4$-OCH_3. at 75°C in toluene-d_8 showing cross-peaks between: a) protons H5, H6, H7 of quinoline rings as well as between H3 and H5 of pyridine rings; and b) between NH and CH_2 of pyridine rings. See Figure S6 for the color code.

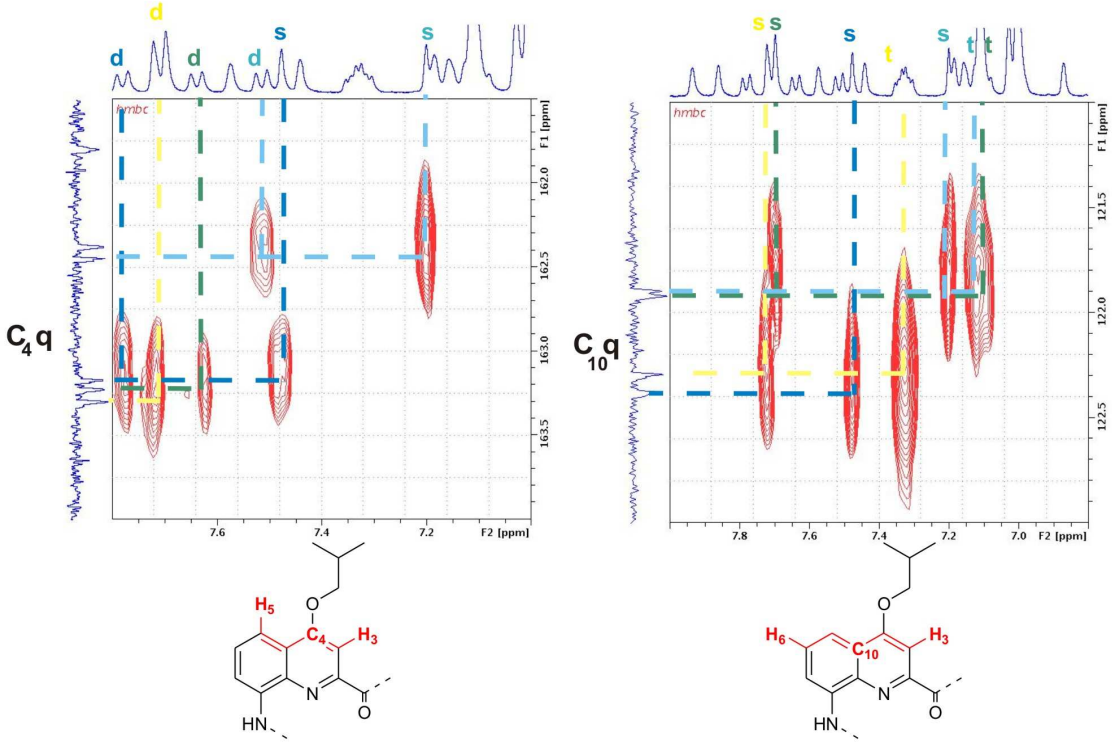


Figure S4: Part of the 400 MHz HMBC plot of Boc-$(PQ)_4$-OCH_3. at 75°C in toluene-d_8 showing cross-peaks between protons H3, H5 and carbon C4 (left) and cross-peaks between H3 and H6 and carbon C10 of the quinoline rings. See Figure S6 for the color code.

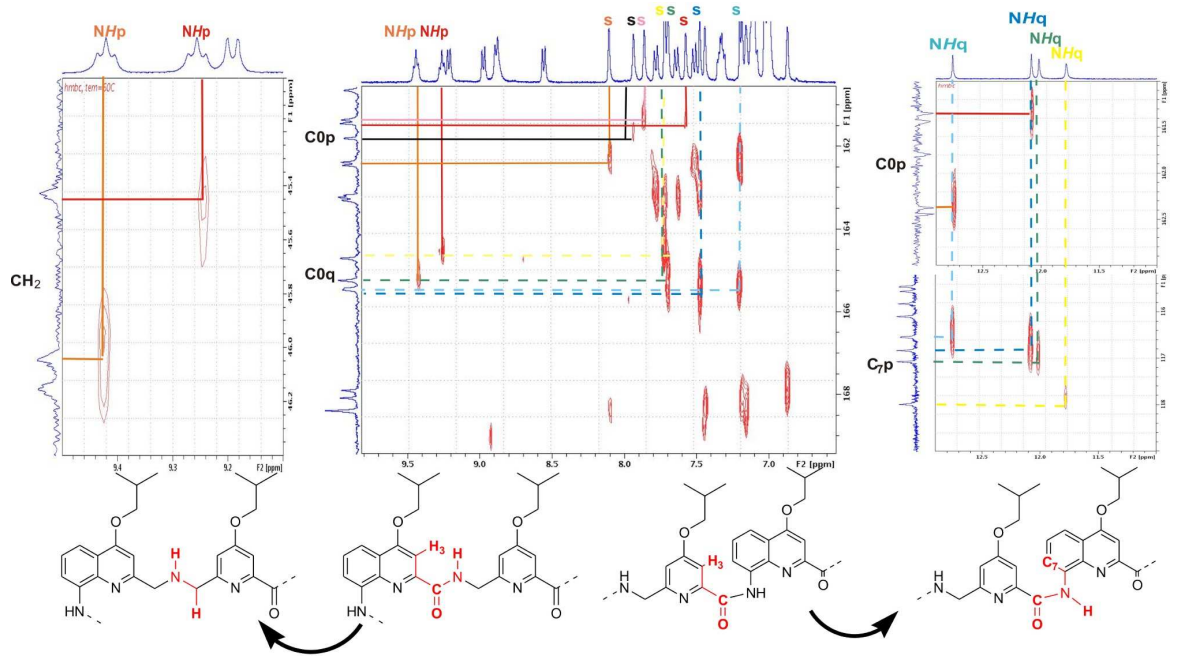


Figure S5: Part of the 400 MHz HMBC plot of Boc-$(PQ)_4$-OCH_3. at 75°C in toluene-d_8 showing, cross-peaks between protons H3 and CO, and NHp and CO (center); between and NHp and CH_2 (left); and between NHq and COp, and NHq and C7q (right). See Figure S6 for the color code.

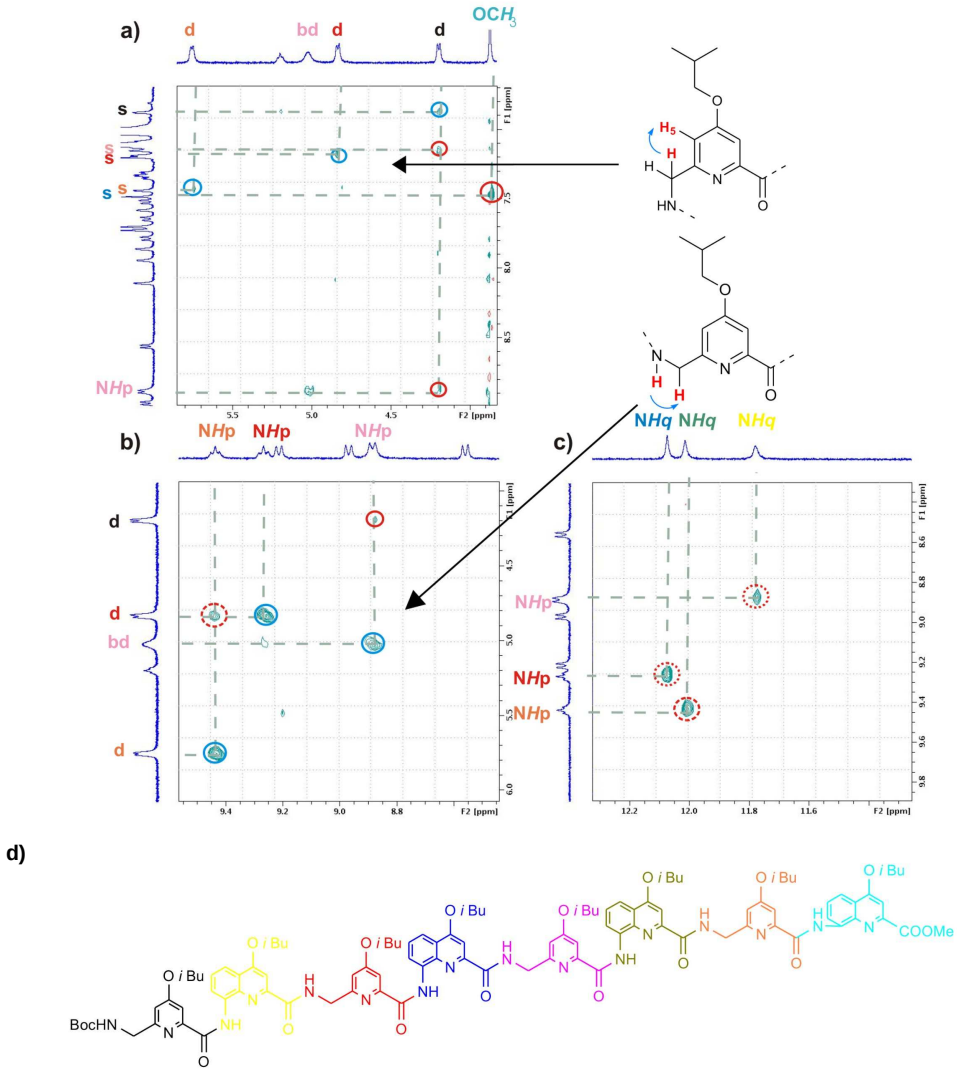


Figure S6: Part of the 400 MHz NOESY plots of Boc-$(PQ)_4$-OCH_3. at 75°C in toluene-d_8 showing cross-peaks between: a) CH_2 and H5 of pyridine rings; b) NH and CH_2 of pyridines; c) NHq and NHp. Blue circles show the NOE correlations that were used to reconstitute the sequence. Red solid-lines circles show correlations consistent with a herringbone helix only, red dotted-lines circles show correlations consistent with a canonical helix only and red dashed-lines show correlations compatible with both types of helices; d) Final color-coded sequence. The same color code was used in Figures S3, S4 and S5.

Table S1. Plausible assignment of observed NOE correlations to possible conformers of Boc-$(PQ)_4$-OMe. The distances compatible with the observed correlations are shown in bold blue.

Observed NOE correlations	Corresponding atomic distance (Å) in the folded conformations			
	Canonical helix	Non canonical helices		
		II in Fig. 2c (solid state structure, PQ unit flipped at the C terminus)	I in Fig. 2c	III in Fig. 2c (PQ units flipped at both C and N termini)
NH-3/NH-4	**3.1**	5.6	5.6	5.6
NH-6/NH-7	**2.9**	**3.0**	**3.0**	**3.0**
NH-2/NH-5	**4.3**	6.6	6.6	7.3
H3-4/OCH_3	7.8	9.5	**5.9**	9.5
CH_2-1/H5-5	8.2	**3.1**	3.1	6.0
CH_2-1/NH-5	6.1	**3.9**	**3.9**	5.1
CH_2-3/NH-7	6.5	**3.9**	**3.9**	**3.9**

Experimental section

General Procedures and Materials. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. THF was distilled from Na/benzophenone while MeOH was distilled from Mg/I_2 and CH_2Cl_2, diisopropylethylamine (DIEA) and DMF were distilled from CaH_2 prior to use. Chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.0), DMSO-d_3 (δ 2.50, 39.4), or CD_3OD (δ 3.31, 49.1). All coupling constants are reported in Hz. Silica gel chromatography was performed using Merck Kieselgel Si 60. Electronic impact mass spectra were obtained in the positive ion mode and matrix assisted laser desorption ionization time of flight (MALDI) mass spectra were obtained in positive ion mode using α-cyanohydroxycinnamic acid as a matrix.

General procedures, Boc-P_n-OCH_3 serie.

Saponification. The methyl ester (2.74 mmol 1 equiv.) was dissolved in a mixture THF/water 1:1 vol/vol (10:10 mL). LiOH (1.5 equiv.) was added and the solution was stirred at room temperature for 16 h. The solution was neutralized using excess AcOH. The solvents were evaporated, the crude mixture was diluted in DCM and washed once with water. The organic phase was dried over Na_2SO_4, filtered and evaporated to provide a solid characterized by ^{1}H NMR and used without further purification.

Boc cleavage. The Boc protected amine (1.51 mmol, 1 equiv.) was dissolved in DCM (26 mL). TFA was added dropwise (16 equiv.) and the solution was stirred at room temperature for 6h. Toluene was then added and the solvents were evaporated under vacuum.

Peptide couplings. To a solution of the acid (1.51 mmol, 1 equiv.), HBTU (1.5 equiv.), HOBt (1 equiv.) in DMF (30 mL) was added DIEA (5 equiv.) under a nitrogen atmosphere. The solution was stirred 0.5 h at room temperature. The amine (1 equiv.) dissolved in DMF (10 mL) was added and the reaction mixture was stirred at room temperature for 16 h. The solution was diluted with toluene and washed once with a 1 M citric acid solution and twice with water. The organic phase was dried over Na_2SO_4, filtered and evaporated to provide a solid.

General procedures, Boc-$(PQ)_n$-OCH_3 serie.

Saponification. The methyl ester (0.17 mmol, 1 equiv.) was dissolved in a mixture THF/MeOH 3:1 vol/vol (3:1 mL). KOH (3 equiv.) was added and the solution was stirred at room temperature for 16 h. The solvents were evaporated, AcOH (3 equiv.) and water (2 mL) was added and the precipitate was filtered to provide a yellow solid characterized by ^{1}H NMR and used without further purification.

Boc cleavage. The Boc protected amine (0.17 mmol, 1 equiv.) was dissolved in DCM (1.5 mL). TFA was added dropwise (8 equiv.) and the solution was stirred at room temperature for 6h. Toluene was then added and the solvents were evaporated under vacuum.

Peptide couplings. To a solution of the acid (0.30 mmol 1 equiv.), HBTU (1.5 equiv.), HOBt (1 equiv.) in DMF (7 mL) was added DIEA (5 equiv.) under a nitrogen atmosphere. The solution was stirred 0.5 h at room temperature. The amine (1 equiv.) dissolved in DMF (1 mL) was added and the reaction mixture was stirred at room temperature for 16 h. The solution was diluted with toluene and washed once with a 1 M citric acid solution and twice with water. The organic phase was dried over Na_2SO_4, filtered and evaporated. Cold MeOH was added and the precipitate was filtered to provide a yellow solid.

Dimethyl 4-isobutoxy-2,6-pyridinedicarboxylate 2. To a solution of dimethyl 4-hydroxy-2,6-pyridinedicarboxylate[3] (1.03 g, 4.9 mmol, 1.0 equiv.) in DMF (15 mL) was added K_2CO_3 (2.03 g, 14.7 mmol, 3 equiv.) under a nitrogen atmosphere. The reaction mixture was stirred at 120°C for 1.5 hour, then cooled down to 70°C. *i*BuI (840 µL, 7.3 mmol, 1.5 equiv.) was added and the reaction mixture was stirred at 70°C for 3 hours. It was then cooled down to room temperature, diluted with toluene (50 mL) and washed three times with water (50 mL). The organic phase was dried with Na_2SO_4, filtered, and evaporated. The product (1.01g, 77 % yield) was used without purification. ^{1}H NMR ($CDCl_3$, 400 MHz)*:* δ= 7.80 (2H, s), 4.01 (6H, s), 3.90 (2H, d, *J* = 6.4), 2.16 (1H, m), 0.93 (6H, d, *J* = 6.8).

Methyl 6-(hydroxymethyl)-4-isobutoxy-2-pyridine carboxylate 3. To a solution of diester **2** (3.73 g, 14 mmol, 1.0 equiv.) in DCM (38 mL) and MeOH (75 mL) was added $NaBH_4$ (1.06 g, 28 mmol, 2 equiv.) under a nitrogen atmosphere at 0°C. The reaction mixture was stirred at 0°C for 0.5 hour then at room temperature for 2 hours. The reaction mixture was neutralized with 1N HCl solution (10 mL). The solvents were removed and the crude product was diluted in DCM and washed three times with water. The organic phase was dried with Na_2SO_4, filtered, and evaporated. The product was purified by silica gel chromatography using DCM/MeOH 98:2 vol/vol to provide 2.57 g (77 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.55 (1H, d, *J* = 2.0), 7.02 (1H, d, *J* = 2.0), 4.79 (2H, s), 3.98 (3H, s), 3.83 (2H, d, *J* = 6.8), 2.12 (1H, m), 1.03 (6H, d, *J* = 6.4); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 166.7, 165.6, 162.3, 148.3, 110.9, 109.5, 74.6, 64.7, 52.8, 27.9, 19.0; IR (NaCl), *ν (cm^{-1})* 3388, 3034, 2961, 2921, 2872, 1729, 1601, 1444, 1335, 1267, 1243, 1122, 1044, 892, 867, 791, 738; MS (EI): m/z = 240 $[M+H]^+$, 239 $[M]^+$.

Methyl 6-chloromethyl-4-isobutoxy-2-pyridinecarboxylate. To a solution alcohol **3** (1.0 g, 4.2 mmol, 1.0 equiv.) in anhydrous toluene (15 mL) under a nitrogen atmosphere was added $SOCl_2$ (1.4 mL, 18.9 mmol, 4.5 equiv.). The reaction mixture was stirred at room

temperature for three hours. The solvents were removed to yield the product (1.06 g , quant. yield) which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.63 (1H, d, *J* = 2.0), 7.28 (1H, d, *J* = 2.0), 4.85 (2H, s), 4.02 (3H, s), 3.90 (2H, d, *J* = 6.8), 2.15 (1H, m), 1.06 (6H, d, *J* = 6.8); MS (EI): m/z= 261 $[M(Cl^{37})+2H]^+$, 260 $[M(Cl^{37})+H]^+$), 258 $[M(Cl^{35})+H]^+$.

Methyl 6-azidomethyl-4-isobutoxy-2-pyridinecarboxylate. To a solution of methyl 6-chloromethyl-4-isobutoxy-2-pyridine-carboxylate (1.06 g, 4.11 mmol, 1.0 equiv.) in DMF (15 mL) was added NaN_3 (321 mg, 4.93 mmol, 1.2 equiv.) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for three hours. DMF was removed and DCM (10 mL) was added. Undissolved salts were filtered off and the DCM was evaporated to yield the product (1.08 g, 98%) which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.50 (1H, d, *J* = 2.4), 7.27 (1H, d, *J* = 2.4), 4,54 (2H, s), 3.93 (2H, d, *J* = 6.8), 3.87 (3H, s), 2.05 (1H, m), 0.99 (6H, d, *J* = 6.4); MS (EI): m/z = 265 $[M+2H]^+$, 264 $[M+H]^+$.

Methyl 6-{[(*tert*-butoxycarbonyl)amino]methyl}-4-isobutoxy-2-pyridinecarboxylate 4a. To a solution of Methyl 6-azidomethyl-4-isobutoxy-2-pyridinecarboxylate (2.26 g, 8.58 mmol, 1.0 equiv.) in anhydrous THF (30 mL) was added PPh_3 (2.25 g, 8.58 mmol, 1.0 equiv.) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for three hours. Then water (2.80 mL) was added and the reaction mixture was heated to reflux for 5 hours. Toluene (60 mL) was added and the solvents were reduced by evaporation down to a volume 30 mL. $(Boc)_2O$ (2.8 g, 12.87 mmol, 1.5 equiv.) and DMAP (200 mg, 1.64 mmol, 0.2 equiv.) were added and the reaction mixture was stirred at room temperature for 16 hours. All volatiles were removed, the crude material was diluted in DCM, washed three times with water, dried over Na_2SO_4, filtered and evaporated. The product was purified by silica gel chromatography using Et_2O/cyclohexane/DCM 6:3:1 vol/vol/vol to yield **4a** (2.18 g, 75 % yield).^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.54 (1H, d, *J* = 2.0), 6.98 (1H, d, *J* = 2.0), 4.45 (2H, d, *J* = 6.0), 3.98 (3H, s), 3.82 (2H, d, *J* = 6.8), 2.11 (1H, m), 1.46 (9H, s), 1.03 (6H, d, *J* = 6.8); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 166.7, 165.7, 160.1, 155.9, 148.9, 111.0, 110.9, 79.6, 74.7, 52.9, 46.0, 28.3, 28.0, 19.0; IR (NaCl), *ν (cm^{-1})* 3390, 3105, 2967, 2873, 1716, 1688, 1598, 1471, 1436, 1419, 1367, 1332, 1286, 1269, 1252, 1171, 1110, 1045, 894, 865, 791, 765; MS (EI): m/z = 339 $[M+H]^+$, 338 $[M]^+$

Boc-P_2-OCH_3 5a. Boc-P_1-OCH_3 **4a** (511 mg, 1.51 mmol, 1 equiv.) was saponified to the corresponding acid **4c** in quantitative yield using the general procedure with LiOH (95 mg, 2,27 mmol, 1.5 equiv.) in THF (5.5 mL), water (5.5 mL). Boc-P_1-OCH_3 **4a** (510 mg, 1.51 mmol, 1 equiv.) was separately submitted to Boc cleavage to produce the corresponding amine **4b** in quantitative yield using the general procedure with TFA (931 µL, 12.08 mmol, 12 equiv.), DCM (5 mL). The acid 4**c** (490 mg, 1.51 mmol, 1 equiv.) and the amine **4b** (360 mg, 1.51 mmol, 1 equiv.) were coupled following the general coupling procedure with DIEA (1.31 mL, 7.55 mmol, 5 equiv.), HBTU (860 mg, 2.27 mmol, 1.5 equiv.), HOBt (204 mg, 1.51 mmol, 1.0 equiv.), in DMF (40 mL). The crude product was purified by silica gel chromatography using Et_2O/cyclohexane/DCM 60:30:10 vol/vol/vol to provide 707 mg (86 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) : δ= 8.90 (1H, bs), 7.63 (1H, d, *J* = 2.0), 7.57 (1H, d, *J* = 2.0), 7.06 (1H, d, *J* = 2.0), 6.90 (1H, d, *J* = 2.0), 4.79 (2H, d, *J* = 6.0), 4.39 (2H, d, *J* = 5.2), 4.01 (3H, s), 3.83 (4H, m), 2.11 (2H, m), 1.45 (9H, s), 1.02 (12H, d, *J* = 6.4); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 167.2, 166.0, 164.7, 159.8, 158.7, 156.3, 151.2, 149.2, 112.1, 111.3, 111.0, 107.1, 75.0, 75. 0, 55.9, 53.3, 45.2, 28.7, 28.3, 19.5; IR (NaCl), *ν (cm^{-1})* 3385, 3307, 3109, 3068, 2953, 2874, 1716, 1685, 1602, 1576, 1527, 1473, 1445, 1356, 1335, 1270, 1248, 1207, 1173, 1140, 1115, 1041, 895, 866, 790, 767.; MS (EI): m/z = 545 $[M+H]^+$, 544 $[M]^+$.

Boc-P_4-OCH_3 6a. Boc-P_2-OCH_3 **5a** (301 mg, 0.55 mmol, 1 equiv.) was saponified to the corresponding acid **5c** in quantitative yield using the general procedure with LiOH (36 mg, 0.83 mmol, 1.5 equiv.), THF (3 mL), water (3 mL). Boc-P_2-OCH_3 **5a** (301 mg, 0.55 mmol, 1 equiv.) was separately submitted to Boc cleavage to produce the corresponding amine **5b** in quantitative yield using the general procedure with TFA (1.02 mL, 8.8 mmol, 16 equiv.), DCM (5 mL). The acid **5c** (292 mg, 0.55 mmol, 1 equiv.) and the amine **5b** (253 mg, 0.55 mmol, 1 equiv.) were coupled following the general coupling procedure with DIEA (476 µL, 2.75 mmol, 5 equiv.), HBTU (325 mg, 0.86 mmol, 1.5 equiv.), HOBT (77 mg, 0.55 mmol, 1.0 equiv.), DMF (15 mL). The product was purified by silica gel chromatography using toluene/EtOAc 60:40 vol/vol to provide the desired product **6a** (402 mg, 73 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) : δ= 9.03 (1H, bs), 8.94 (1H, bs), 8.86 (1H, bs), 7.80 (1H, d, *J* = 6.8), 7.69 (1H, d, *J* = 7.6), 7.58 (2H, s), 7.52 (1H, s), 7.06 (1H, s), 6.96 (1H, s), 6.88 (1H, s), 5.58 (1H, bs), 4.75 (4H, s), 4.61 (2H, d, *J* = 4.8), 4.28 (2H, d, *J* = 4.0), 3.87 (3H, s), 3.78 (8H, d, *J* = 5.6), 2.07 (4H, m), 1.37 (9H, s), 0.99 (24H, d, *J* = 5,6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 166.9, 166.9, 166.5, 165.2, 164.3, 164.2, 159.2, 158.3, 157.6, 157.5, 150.4, 150.3, 148.3, 128.5, 127.7, 124.8, 111.5, 111.4, 111.2, 110.6, 110.5, 110.2, 110.1, 74.5, 74.3, 52.4, 45.1, 44.3, 44.1, 44.0, 27.8, 27.5, 18.6; IR (NaCl), *ν (cm^{-1})* 3351, 3094, 3053, 2962, 2916, 2875, 1718, 1668, 1600, 1568, 1520, 1445, 1361, 1328, 1251, 1174, 1141, 1041, 877, 844, 790, 736; MS (maldi): m/z = 957.35 $[M+H]^+$, 974.34 $[M+Na]^+$.

Boc-P_8-OCH_3 7a. Boc-P_6-OCH_3 **6a** (100 mg, 0.10 mmol, 1 equiv.) saponified to the corresponding acid **6c** in quantitative yield using the general procedure with LiOH (20 mg, 0.45 mmol, 4.5 equiv.), THF (1 mL), water (1 mL). Boc-P_6-OCH_3 **6a** (100 mg, 0.10 mmol, 1 equiv.) was separately submitted to Boc cleavage to produce the corresponding amine **6b** in quantitative yield using the general procedure with TFA (130 µL, 1.6 mmol, 16 equiv.), DCM (1 mL). .The acid **6c** (99 mg, 0.10 mmol, 1 equiv.) and the amine **6b** (90 mg, 0.10 mmol, 1 equiv.) were coupled following the general coupling procedure with DIEA (91 µL, 0.5 mmol, 5 equiv.), HBTU (60 mg, 0.15 mmol, 1.5 equiv.), HOBt (14 mg, 0.10 mmol, 1 equiv.), DMF (3 mL). The crude was submitted to silica gel chromatography using toluene/EtOAc 60:40 vol/vol to provide 121 mg of the product (65 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) : δ= 9.34 (1H, bs), 9.26 (1H, bs), 9.09 (2H, bs), 9.04 (1H, bs), 8.91 (1H, bs), 8.79 (1H, bs), 7.60 (1H, d, *J* = 2.0), 7.57 (1H, d, *J* = 2.0), 7.56 (1H, d, *J* = 2.4), 7.52 (1H, d, *J* = 1.6), 7.45 (3H, bs), 7.41 (1H, s), 6.96 (1H, s), 6.95 (2H, d, *J* = 2.0), 6.94 (1H, s), 6.90 (1H, s), 6.88 (1H, s), 6.82 (1H, s), 6.79 (1H, s), 6.19 (1H, bs), 4.78 (2H, d, *J* = 4.0), 4.72 (2H, d, *J* = 5.6), 4.65 (4H, d, *J* = 2.0), 4.60 (4H, m), 4.52 (2H, d, *J* = 3.2), 4.31 (2H, d, *J* = 3.6), 3.91 (3H, s), 3.83 (2H, d, *J* = 4.8), 3.79-3.70 (10H, m), 3.64 (2H, d, *J* = 5.2), 3.54 (2H, d, *J* = 4.8), 2.05 (8H, m), 1.18 (9H, s), 1.03-0.94 (42H, m), 0.87 (6H, d, *J* = 6.0); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 167.3 ; 167.1, 167.1, 167.0, 166.7, 165.6, 165.0, 164.7, 164.4, 164.2, 164.1, 159.7, 158.5, 158.3, 158.2, 157.7, 157.0, 156.8, 156.0, 151.1, 151.1, 150.9, 150.9, 150.9, 150.8, 150.4, 148.7, 111.7, 111.4, 111.4, 111.2, 111.1, 110.9, 110.9, 110.6, 107.5, 107.3, 107.2, 107.1, 107.0, 106.9, 106.8, 79.1, 74.8, 74.7, 74.6, 52.8, 45.6, 45.0, 44.8, 44.6, 44.0, 43.9, 28.1, 28.0, 27.9, 27.8, 19.0, 19.0, 18.9; IR (NaCl), *ν (cm^{-1})* 3339, 3077, 3044, 2962, 2916, 2875, 1704, 1671, 1601, 1568, 1527, 1444, 1357, 1327, 1263,

1163, 1138, 1039, 871, 790, 737; MS (maldi): m/z = 1803.94 $[M+Na]^+$.

Boc-$(PQ)_1$-OCH_3 9a. To a solution of Boc-P_1-COOH **4b** (486 mg, 1.50 mmol, 1 equiv.) and PyBOP (781 mg, 1.50 mmol, 1 equiv.) in DCM (15 mL) was added DIEA (520 µL, 3 mmol, 2 equiv.) followed by H_2N-Q-OCH_3 **8**[1,2] (452 mg, 1.65 mmol, 1.1 equiv.) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h, then washed twice with a saturated $NaHCO_3$ solution and once with a citric solution (1 M). The organic phase was dried over Na_2SO_4, filtered, and evaporated. Precipitation from cold MeOH (5 mL) yielded the pure product (696 mg , 80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 12.61 (1H, s), 8.96 (1H, d, *J* = 7.2), 7.96 (1H, d, *J* = 8.4), 7.73 (1H, d, *J* = 2.0), 7.65 (1H, t, *J* = 8.0 and *J* = 8.0), 7.60 (1H, s) 6.98 (1H, d, *J* = 2.0), 6.09 (1H, s), 4.58 (2H, d, *J* = 5.6), 4.11 (3H, s), 4.07 (2H, d, *J* = 6.8), 3.89 (2H, d, *J* = 6.4), 2.31 (1H, m), 2.14 (1H, m), 1.37 (9H, s), 1.16 (6H, d, *J* = 6.4), 1.05 (6H, d, *J* = 6,8); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 167.8, 166.1, 163.3, 162.6, 159.0, 156.7, 151.7, 147.2, 139.7, 135.2, 128.7, 122.5, 117.6, 116.1, 111.2, 107.3, 101.5, 79.7, 75.4, 70.9, 53.4, 45.9, 28.6, 28.5, 28.3, 19.5, 19.4; IR (NaCl), *ν (cm^{-1})* 3340, 3300, 3081, 2963, 2932, 2874, 1721, 1682, 1602, 1574, 1531, 1470, 1441, 1421, 1384, 1360, 1333, 1265, 1177, 1110, 1043, 994, 966, 913, 867, 818, 761, 723 ; MS (maldi): m/z = 581.16 $[M+H]^+$, 603.15 $[M+Na]^+$, 619.11 $[M+K]^+$.

BocNH-$(PQ)_2$-OCH_3 10a. Boc-$(PQ)_1$-OCH_3 **9a** (100 mg, 0.17 mmol, 1 equiv.) was saponified to the corresponding acid **9c** in quantitative yield using the general procedure with KOH (32 mg, 0.57 mmol, 3 equiv.), THF (3 mL), MeOH (1 mL). Boc-$(PQ)_1$-OCH_3 **9a** (100 mg, 0.17mmol, 1 equiv.) was separately submitted to Boc cleavage to produce the corresponding amine **9b** in quantitative yield using the general procedure with TFA (104 µL, 1.36 mmol, 8 equiv.), DCM (1.5 mL). The acid **9c** (86 mg, 0.17 mmol, 1 equiv.) and the amine **9b** (82 mg, 0.17 mmol, 1 equiv.) were coupled following the general coupling procedure with DIEA (147 µL, 0.85 mmol, 5 equiv.), HBTU (97 mg, 0.26 mmol, 1.5 equiv.), HOBt (23 mg, 0.17 mmol, 1 equiv.), DMF (4.5 mL). The crude product was purified by silica gel chromatography using EtOAc/cyclohexane 70:30 vol/vol to provide 104 mg (65% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 12.14 (1H, s), 11.79 (1H, s), 9.52 (1H, s), 8.65 (2H, s), 7.96 (2H, d, *J* = 8.4), 7.79 (1H, s), 7.71 (1H, s), 7.57 (1H, t, *J* = 7.6 and *J* = 7.6), 7.52 (1H, t, *J* = 7.6 and *J* = 7.6), 7.42 (1H, s), 7.21 (1H, s), 7.12 (1H, d, *J* = 2.0), 6.75 (1H, s), 5.64 (1H, s), 5.11 (2H, d, *J* = 4.8), 4.14 (2H, d, *J* = 6.8), 3.99 (2H, d, *J* = 6.4), 3.94 (2H, d, *J* = 3.2), 3.89 (2H, d, *J* = 6,4), 3.84 (2H, d, *J* = 6.4), 3.66 (3H, s), 2.33 (2H, m), 2.14 (2H, m), 1.21 (9H, s), 1.19 (6H, d, *J* = 6.8), 1.18 (6H, d, *J* = 6.8), 1.05 (12H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 167.6, 167.1, 165.2, 164.7, 163.2, 162.7, 161.9, 161.4, 158.8, 157.6, 155.7, 151.6, 150.8, 149.7, 146.4, 139.3, 138.3, 134.5, 134.0, 127.9, 127.3, 122.0, 117.4, 117.0, 116.2, 115.9, 110.7, 109.6, 107.5, 107.0, 101.0, 99.1, 79.2, 75.3, 75.0, 74.8, 74.6, 52.5, 45.3, 44.9, 28.2, 28.0, 19.3, 19.2, 19.0; IR (NaCl), *ν (cm^{-1})* 3395, 3319, 3082, 2962, 2931, 2874, 1720, 1683, 1602, 1572, 1531, 1470, 1421, 1385, 1358, 1330, 1267, 1173, 1114, 1045, 993, 967, 916, 867, 818, 760, 724; MS (maldi): m/z = 1029.20 $[M+H]^+$, 1051.22 $[M+Na]^+$.

BocNH-$(PQ)_4$-OCH_3 11a. Boc-$(PQ)_2$-OCH_3 **10a** (72 mg, 0.07 mmol, 1 equiv.) was saponified to the corresponding acid **10c** in quantitative yield using the general procedure with KOH (12 mg, 0.21 mmol, 3 equiv.), THF (1.5 mL), MeOH (0.5 mL). Boc-$(PQ)_2$-OCH_3 **10a** (72 mg, 0.07 mmol, 1 equiv.) was separately submitted to Boc cleavage to produce the corresponding amine **9b** in quantitative yield using the general procedure with TFA (44 µL, 0.56 mmol, 8 equiv.), DCM. The acid 10c (71 mg, 0.07 mmol, 1 equiv.) and the amine **10b** (65 mg, 0.07 mmol, 1 equiv.) were coupled following the general coupling procedure with DIEA (61 µL, 0.35 mmol, 5 equiv.), HBTU (40 mg, 0.105 mmol, 1.5 equiv.), HOBt (10 mg, 0.07 mmol, 1 equiv.), DMF (2 mL). The crude product was purified by silica gel chromatography using EtOAc/cyclohexane 70:30 vol/vol to provide 103 mg (77% yield). X-ray quality single crystals were obtained by slow diffusion of of MeOH into a DMSO-$CHCl_3$ solution. ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 12.24 (1H, s), 11.66 (2H, s), 11.44 (1H, s), 9.09 (1H, bs), 8.90 (1H, bs), 8.72 (1H, d, *J* = 7.2), 8.64 (1H, bs), 8.45 (2H, m), 8.07 (1H, d, *J* = 7.2), 7.76-7.23 (10H, m), 7.18 (5H, m), 7.05 (1H, s), 6.94 (1H, s), 6.77 (1H, s), 6.76 (3H, m), 5.24 (1H, bs), 4.53 (2H, bs), 4.23 (2H, bs), 4.03 (2H, d, *J* = 6.0), 3.97 (2H, d, *J* = 6.0), 3.88-3.81 (12H, m), 3.66 (2H, d, *J* = 6.0), 3.57 (5H, m), 2.32 (3H, m), 2.20 (1H, m), 2.06 (3H, m), 1.90 (1H, m), 1.27 (9H, s), 1.25-0.88 (48H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ= 167.7, 167.4, 167.3, 164.8, 164.7,164.1, 162.9, 162.8, 162.1, 161.9, 161.4, 161.0, 159.3, 158.5, 155.6, 151.7, 151.4, 151.3, 151.2, 148.6, 148.5, 146.3, 138.5, 138.0, 137.6, 137.5, 134.3, 133.8, 133.5, 133.1, 128.1, 127.6, 127.3, 127.1, 126.7, 125.6, 121.7, 121.6, 121.3, 121.2, 117.5, 116.5, 116.4, 116.3, 116.2, 116.0, 115.7, 115.6, 110.7, 109.9, 109.6, 107.3, 107.0, 106.8, 106.5, 100.6, 99.0, 98.4, 79.4, 75.1, 74.7, 74.6, 52.5, 45.9, 45.3, 45.1, 44.4, 28.3, 28.2, 28.1, 28.0, 27.9, 27.3, 19.7, 19.4, 19.3, 19.2, 19.1, 19.0. IR (NaCl), *ν (cm^{-1})* 3386, 3307, 3055, 2960, 2934, 2874, 1715, 1681, 1601, 1566, 1531, 1469, 1422, 1385, 1356, 1330, 1265, 1176, 1117, 1046, 994, 969, 916, 868, 819, 736, 705; MS (maldi): m/z = 1925.38 $[M+H]^+$, 1947.49 $[M+Na]^+$, 1963.39 $[M+K]^+$.

NMR solution studies. Spectra were recorded with a Bruker Avance 400 NB US NMR spectrometer by means of a 5 mm direct QNP ^{1}H/X probe with gradient capabilities. The temperature was maintained at 348K for the structure determination. ^{1}H, ^{13}C, correlated spectroscopy (DQF-COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser spectroscopy (NOESY), spectra were used for sequence-specific assignements of the BocNH-$(PQ)_4$-OMe. Data processing was performed with TOP SPIN 2.0 sofware.

The COSY: acquisition with 2048 (t_2) × 256 (t_1) data points; relaxation delay of 2s; sweep width of 6000 Hz in both dimensions, QF mode in t_1 and 24 scans per increment. Processing was done after a sine-bell multiplication in both dimensions, and Fourier transformed in 1k × 1k real data points.

The HMQC acquisition was performed with 2048 (t_2) × 512 (t_1) data points in QF mode in t_1; a relaxation delay of 1.5 s and 20 scans per increment; and a sweep width of 5600 Hz for the proton dimension and 25000 Hz for the carbon dimension. Processing was done after a qsine multiplication in both dimensions (ssb = 2), and Fourier transformed in 2k × 1k real data points.

The HMBC acquisition was performed with 2048 (t_2) × 512 (t_1) data points in QF mode in t_1; a relaxation delay of 1.5 s and 32 scans per increment ; and a sweep width of 5600 Hz for the proton dimension and 25000 Hz for the carbon dimension. Processing was done after a qsine multiplication in both dimensions (ssb = 2), and Fourier transformed in 2k × 1k real data points.

The NOESY acquisition was performed with 2048 (t_2) × 512 (t_1) data points in States-TPPI mode; a relaxation delay of 2s and 24 scans per increment; and a sweep width of 5600 Hz in both dimensions; and a mixing time of 300 ms. Processing was done after a qsine multiplication in both dimensions (ssb = 2), and Fourier transformed in 1k × 1k real data points.

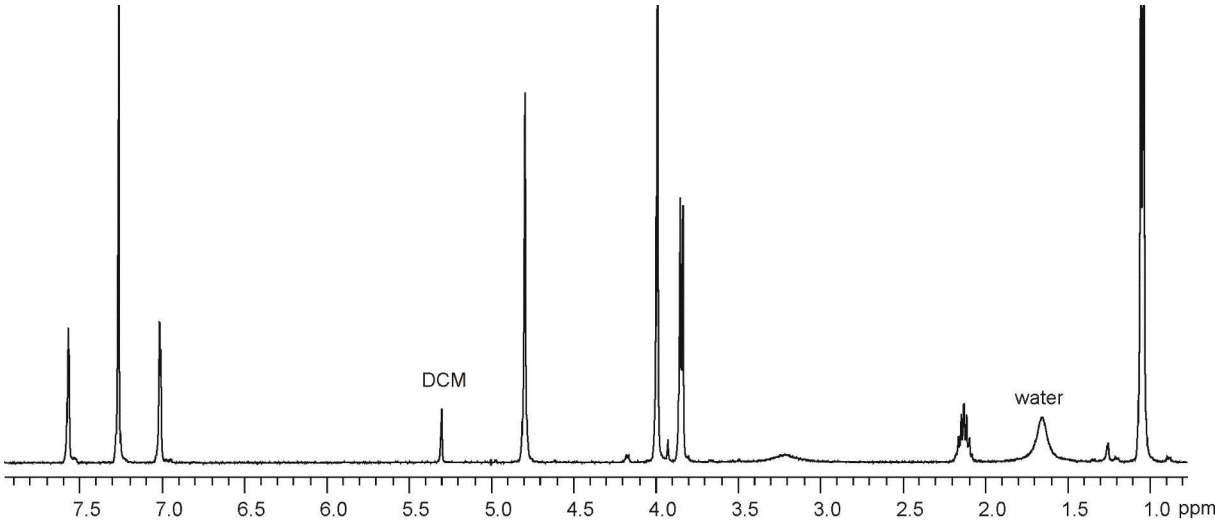
X-ray crystallography. A single crystal of **11a** was mounted on a Rigaku R-Axis Rapid diffractometer equipped with a MM007 micro focus rotating anode generator with monochromatized Cu-Kα radiation (1.54178 Å). The data

collection, unit cell refinement, and data reduction were performed using the CrystalClear software package. The positions of non-H atoms were determined by the program SHELXD, and the position of the H atoms were deduced from coordinates of the non-H atoms and confirmed by Fourier synthesis. H atoms were included for structure factor calculations but not refined.

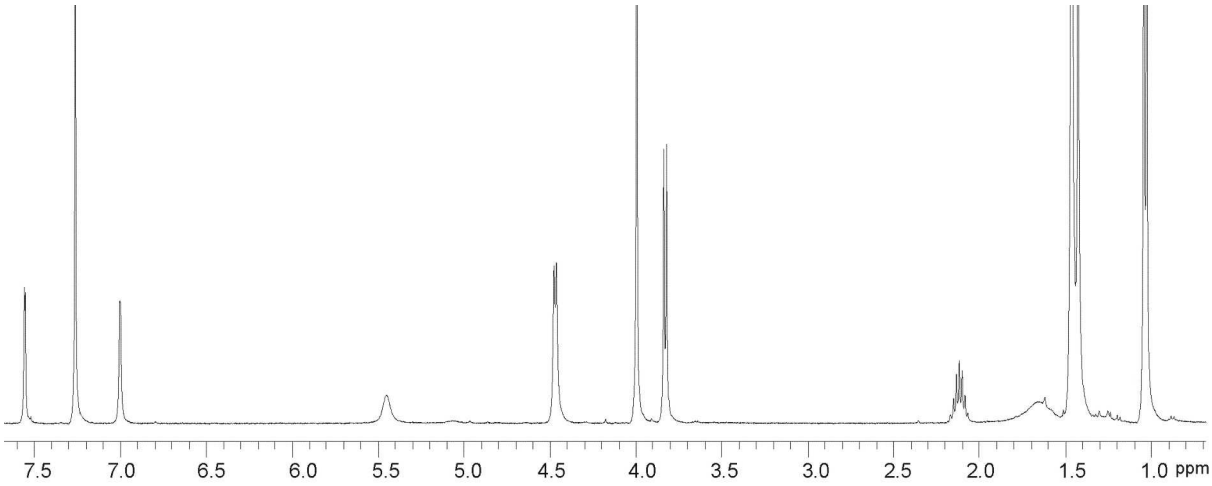
A summary of crystallographic for **11a** (BocNH-$(PQ)_4$-OCH_3) data is as follows. Formula (asymmetric unit): $C_{106}H_{124}N_{16}O_{19}(H_2O)_3(CH_3OH)_9$, Crystal dimensions (mm): 0.3 × 0.3 × 0.5; crystal aspect: Colorless prism; Cryst. System: triclinic; Space group *P*-1; $Z = 2$; Cell parameters: a = 16.1046 (10) Å, b = 20.9270 (13) Å, c = 22.4266 (12) Å, α = 116.022 (4)°, β = 99.677 (4)°, γ = 99.965 (4)°; T = 183(2) K; V = 6430.3 (7) Å^3; FW = 2253.26 g.mol^{-1}; ρ = 1.164 g.cm^{-3}; λ = 1.5418 Å (Cu(K_α)); $6.52 \leq \theta \leq 71.95$; refl. measured = 89008; refl. unique = 22080; GOF = 1.091; R_1 ($I>2\sigma(I)$) = 0.0946; wR_2 (all data) = 0.3243.

NMR spectra

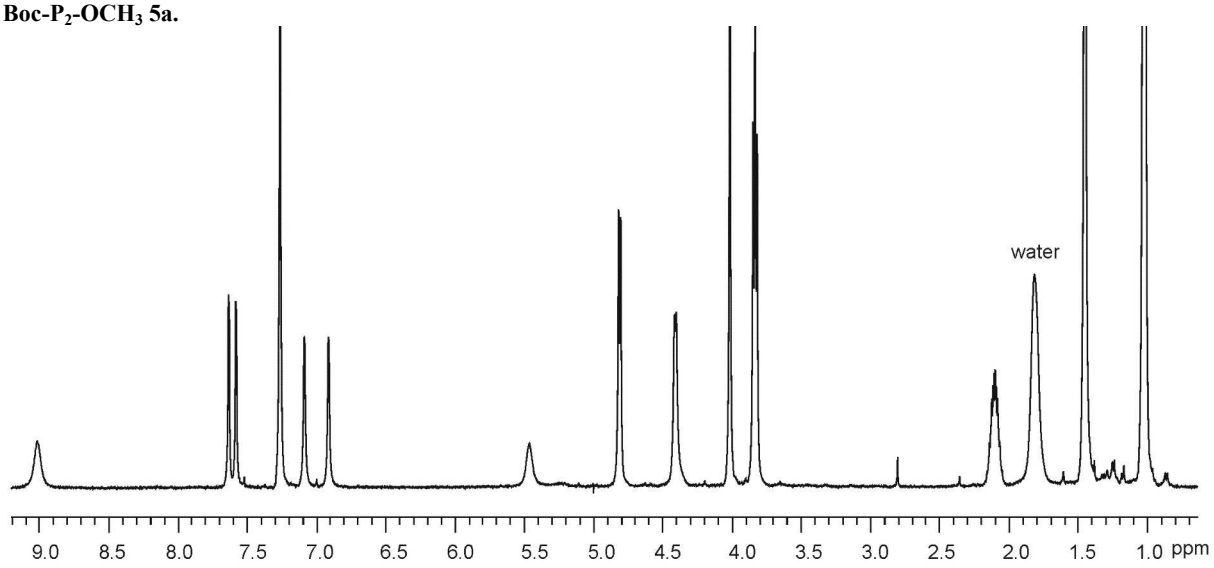
Methyl 6-(hydroxymethyl)-4-isobutoxy-2-pyridine carboxylate 3.



Methyl 6-{[(*tert*-butoxycarbonyl)amino]methyl}-4-isobutoxy-2-pyridinecarboxylate 4a.



Boc-P_2-OCH_3 5a.



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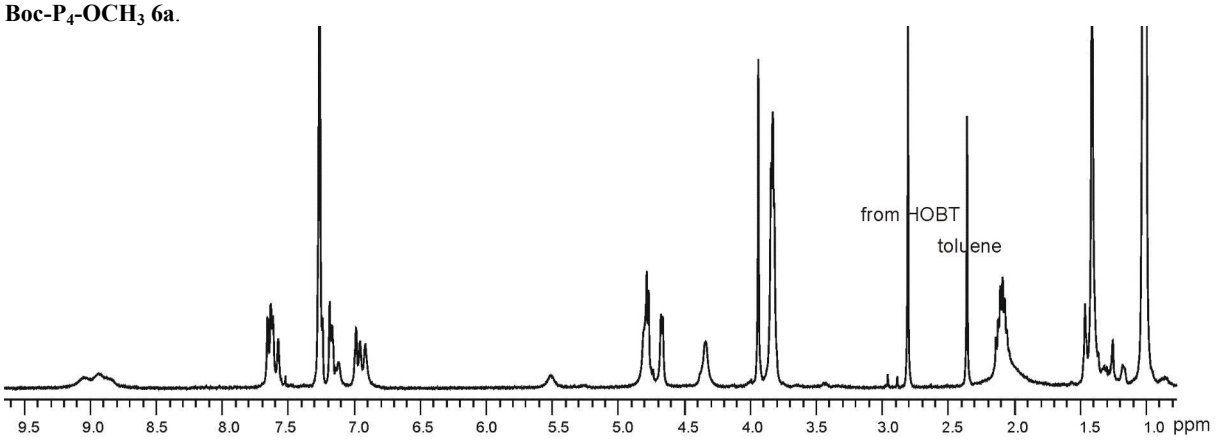
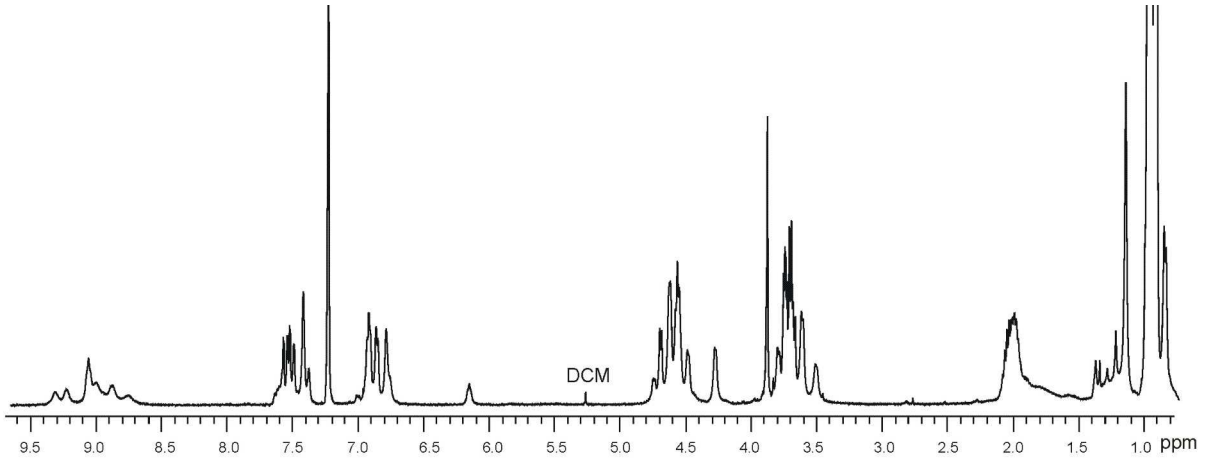
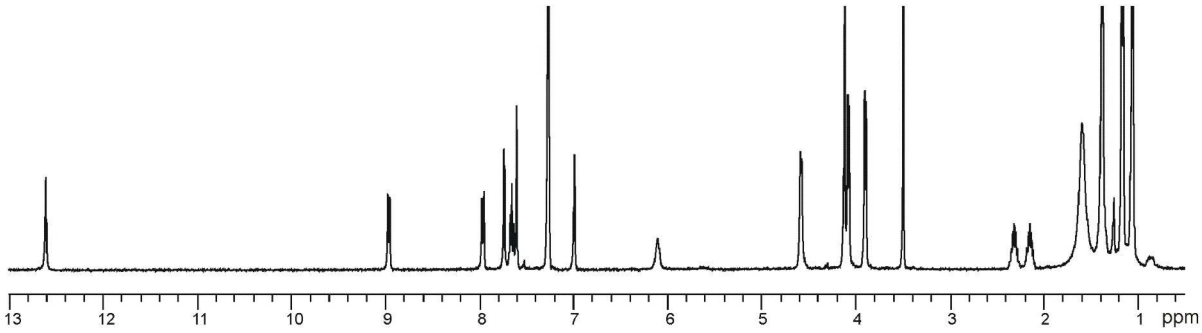


Boc-P_8-OCH_3 7a.



Boc-(PQ)$_1$-OCH$_3$ 9a



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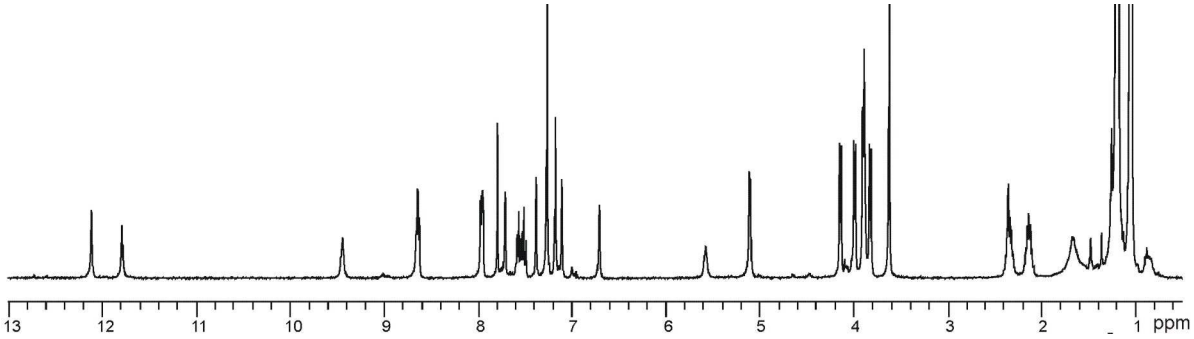
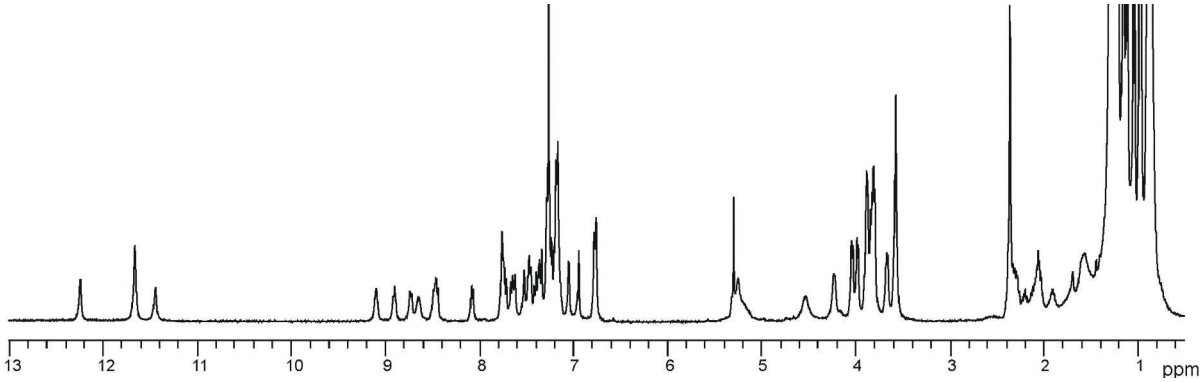


Boc-(PQ)$_2$-OCH$_3$ 11a



Complete reference 6a of the manuscript:
Gong, B.; Zeng, H.; Zhu, J.; Yuan, L.; Han, Y.; Cheng, S.; Furukawa, M; Parra, R. D.; Kovalevsky, A. Y.; Mills, J. L.; Skrzypczak-Jankun, E.; Martinovic, S.; Smith, R. D.; Zheng, C.; Szyperski, T.; Cheng Zeng, X. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11583–11588

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