Biomimetic dendrimers for mineralization: Rare fibrous amorphous calcium carbonate (ACC) and branch-and-bud ACC-vaterite polymorphs

Amir Sheikhi,¹,²,³+* Søren Leth Mejlsøe,¹,² Li Na,¹,² Enzo Bomal,¹,² Theo G.M. van de Ven,¹,²,³* and Ashok Kakkar¹,²*

¹Department of Chemistry, ²Quebec Centre for Advanced Materials (QCAM), ³Pulp and Paper Research Centre, McGill University, 801 Sherbrooke St. West, Montreal, QC, H3A 0B8, Canada.

The electronic supplementary information (ESI) includes 1 figure, and full details of dendrimer synthesis.
Figure S1. Long time (~25 days) biomimetic mineralization of calcium carbonate with 10 mM CaCl₂, 10 mM NaHCO₃, and 13.3 ppm TEG-G2 (A-C) or TEG-G0 (D-I) dendrimers results in similar features as for TEG-G1: elongated ACC structures with the decorations of nacre-like vaterite (A, D, E, F), calcite spikes (A, B, G-I), and dendritic features (C).
**Dendrimer synthesis:**

![Image of dendrimer synthesis](image)

Scheme 1. Structure and synthesis of the building blocks used for the synthesis of TEG-based dendrimers.

The branching TEG entity was prepared via mono-tosylation and azide substitution of TEG to yield compound (3) (Scheme 1). The branching monomer with terminal acetylene units (6) was synthesized from easily available starting materials in a two-step high-yield synthesis, and without the need for column chromatography. For coupling using a click chemistry approach, a small molecule containing both an azido group and a phosphonate diester group was prepared (8).

**2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate, OH-TEG-Ts (2)**

This compound was prepared by adapting the procedure described in literature: 1 To a solution of tetraethylene glycol (TEG) (1) (101.88 g; 524.53 mmol) in THF (200 mL), a solution of NaOH (1.26 g; 52.45 mmol) was added in H₂O (10 mL), and the mixture was cooled to 0°C on an ice
bath. A solution of TsCl (10.00 g; 52.45 mmol) in THF (20 mL) was added dropwise over a period of 30 min. The mixture was left to stir for additional 2 h. The THF was removed under reduced pressure and DCM (50 mL) was added. The DCM phase was washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$), and the crude product concentrated \textit{in vacuo}. The product was purified using flash chromatography using EtOAc as the eluent to give 6.79 g (37\%) of the product as a colorless transparent oil. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.79 (d, $J$ = 8.3, 2H), 7.34 (d, $J$ = 8.3, 2H), 4.21 – 4.11 (m, 2H), 3.75 – 3.51 (m, 14H), 2.44 (s, 3H).

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethanol, HO-TEG-N$_3$ (3)

This compound was prepared as described in the literature$^1$ with slight modifications to temperature and the purification procedure: A mixture of 2 (6.79 g; 19.49 mmol) and NaN$_3$ (3.20 g; 49.22 mmol) in EtOH (95\%, 100 mL) was heated to 77 °C (oil bath temperature) for 20 h. The solvent was removed under reduced pressure, and H$_2$O (50 mL) was added. The product was extracted with CHCl$_3$ (3 × 50 mL). The collected organic phases were dried (K$_2$CO$_3$), filtered, and the product was concentrated \textit{in vacuo} to give 4.10 g (96\%) of the final product as a colorless oil. $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 3.79 – 3.56 (m, 14H), 3.39 (t, 2H), 2.39 (s, 1H).

Methyl 3,4,5-tris(prop-2-ynloxy)benzoate (5)

A mixture of methyl 3,4,5-trihydroxybenzoate (4) (12.82 g; 69.62 mmol), K$_2$CO$_3$ (38.55 g; 278.92 mmol) and propargyl bromide (80\% in toluene; 29.85 g reactant; 37.28 g sample; 27.80 mL; 250.46 mmol) in DMF (100 mL) was stirred for 20 h. H$_2$O (250 mL) was added, and the product was extracted with EtOAc (4 × 150 mL). The collected organic phases were washed with H$_2$O (3
× 150 mL) and brine (3 × 150 mL), dried (K₂CO₃), and concentrated under reduced pressure to give 19.05 g (92%) of the final product as a white solid material. ¹H NMR (300 MHz, CDCl₃) δ 7.47 (s, 2H), 4.81 (2×d, J = 2.4, 2.4, 6H), 3.91 (s, 3H), 2.53 (t, J = 2.4, 2H), 2.46 (t, J = 2.4, 1H). NMR data are consistent with literature.²

3,4,5-Tris(prop-2-ynyloxy)benzoic acid (6)

A mixture of 5 (4.53 g; 15.18 mmol) in THF (10 mL) was vigorously stirred with KOH(aq) (4 M, 20 mL) over night. The aqueous phase was made acidic (pH ~ 3) with HCl(aq), and the product was extracted with THF (3 × 20 mL). The collected organic phases were washed with brine (2 × 10 mL), dried (MgSO₄), filtered, and the product was concentrated in vacuo to give 3.65 g (84%) of the final product as a white solid material. ¹H NMR (200 MHz, DMSO) δ 13.06 (s, 1H), 7.38 (s, 2H), 4.89 (d, J = 2.4, 4H), 4.71 (d, J = 2.4, 2H), 3.62 (t, J = 2.4, 2H), 3.48 (t, J = 2.4, 1H). NMR data are consistent with literature.³

Diethyl (2-azidoethyl)phosphonate (8):

Sodium azide (10.60 g, 163.05 mmol) was added to a solution of 2-bromoethylphosphonate (7) (10.40 g, 42.44 mmol) in water (50 mL), and the reaction mixture was stirred for 24 h at 65°C. An extraction was performed with dichloromethane, the organic phase was dried with MgSO₄, filtered, and the residue obtained was a yellow oil (8.03 g, 0.0387 mol, 92% yield). ¹H NMR (400MHz, CDCl₃): δ = 1.18 (t, 6H), 1.91 (m, 2H), 3.38 (m, 2H), 3.97 (m, 4H) ppm. ¹³C {¹H} NMR (300MHz, CDCl₃) δ = 16.27 (d, P-CH₂-CH₃), 25.8 (d, P-CH₂-CH₃), 45.2 (d, P-CH₂-CH₂-), 61.7 (d, P-CH₂-
\( \mathrm{CH}_2 \) ppm. \(^{31}\mathrm{P}\{^1\text{H}\} \) NMR (200MHz, CDCl\(_3\)) \( \delta = 26.65 \) (P-Et) ppm. NMR data are consistent with literature.\(^4\)

**Scheme 2.** Synthesis of TEG-based dendrimers of generation 0, and the synthesis of 6-TEG-OH-G0-dendrimer (12).

Synthesis of dendrimers was conducted divergently starting from the TEG core (1) based on copper catalyzed Huisgen cycloaddition, yielding a triazole moiety, and coupling reactions between an alcohol on the TEG monomer and the carboxylic acid moiety on the AB\(_3\) branching unit. For the latter reaction, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was used as the catalyst, and neither protection nor de-protection steps were necessary. The synthesis of the TEG-type dendrimers starts with an esterification reaction involving TEG and compound 6, yielding (9) with six terminal alkynyl groups. This functional group can be used either for the
functionalization of dendrimer or for its expansion to higher generation. The functionalization is achieved by a “click” reaction with compound 8 to yield the generation 0 dendrimer with six diethyl ester phosphonic acid groups at the terminus (6-PO$_3$Et$_2$-G0-dendrimer, 10). The synthesis of the next generation dendrimer is done by reacting all the six triple bonds with six equivalents of monomer 3 yielding (12), which in turn can be coupled with six equivalents of monomer 6, yielding a G1 dendrimer with 18 alkynyl surface groups (13).

6-propyne-G0-Dendrimer (9)

To a mixture of TEG (0.81 g; 4.17 mmol) in DCM (30 mL) was in success added 2 (2.61 g; 9.18 mmol), DMAP (1.18 g; 9.66 mmol), and EDC•HCl (2.43 g; 12.68 mmol). The reaction was stirred for two days, and the solvent was removed under reduced pressure. The product was purified using flash chromatography with a mixture of EtOAc/hexanes/MeOH (1.2:1.0:0.4) to give, after removal of solvents and drying in vacuo, 2.47 g (66%) of the product as a solid, white material. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.48 (s, 4H), 4.81 (dd, $J = 2.4$, 12H), 4.50 – 4.37 (m, 4H), 3.87 – 3.74 (m, 4H), 3.73 – 3.57 (m, 8H), 2.56 (t, $J = 2.4$, 4H), 2.46 (t, $J = 2.4$, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 167.09, 151.37, 140.10, 126.70, 79.53, 78.80, 59.92, 56.90. ESI MS Calculated C$_{40}$H$_{38}$O$_{13}$: 726.73. Found: 749.22 (M$^-$Na$^+$).

6-PO$_3$Et$_2$-G0-Dendrimer (10)

A solution of 9 (0.75 g; 1.035 mmol), Na-ascorbate (0.245 g; 1.24 mmol) and 8 (1.55 g; 7.48 mmol) in THF (9 mL) was added to a solution of CuSO$_4$ (CuSO$_4$•5H$_2$O; 0.16 g; 0.64 mmol) in water (2.5 mL) and stirred for two days at 40°C. The THF was removed under reduced pressure, the product was dissolved in DCM (10 mL), and EDTA$_{aq}$ (0.20 M; 10 mL) was added and stirred
for 1 h followed by the extraction of product with DCM (4 × 10 mL). The solvent was removed under reduced pressure, and the product was re-dissolved in a small amount of DCM. Et$_2$O was dropwise added to the solution until the product came out of solution as a thick sticky brown oil. The Et$_2$O was decanted, and the precipitation procedure was repeated two additional times. All volatile components were removed in vacuo to give 1.24 g (61%) of the product as a sticky brown syrup. $^1$H NMR (200 MHz, CDCl$_3$) $\delta$ 7.94 (s,s, 6H), 7.42 (s, 4H), 5.10 (s,s, 12H), 4.75 – 4.49 (m, 12H), 4.49 – 4.36 (m, 4H), 4.20 – 3.97 (m, 24H), 3.87 – 3.75 (m, 4H), 3.73 (bs, 8H), 2.59 – 2.28 (m, 12H), 1.30 (t, $J$ = 7.1, 36H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 165.72, 151.89, 144.14, 143.39, 141.54, 125.69, 124.27, 123.73, 109.22, 70.64, 70.59, 69.12, 66.20, 64.33, 63.04, 62.16 (d, $J_{CP}$ = 6.5 Hz), 62.07, 44.65, 44.51, 27.08 (d, $J_{CP}$ = 141.1 Hz), 16.37 (d, $J_{CP}$ = 6.0 Hz). $^{31}$P NMR (81 MHz, CDCl$_3$) $\delta$ 25.63. MALDI-TOF MS Calculated C$_{76}$H$_{122}$N$_{18}$O$_3$P$_6$: 1968.69. Found: 1991.0 (M·Na$^+$).

6-PO$_3$K$_2$-G0-Dendrimer (11)

A solution of 10 (1.24 g; 0.63 mmol) and TMS-Br (2.20 mL; 2.54 g; 16.14 mmol) in DCM (10 mL) was stirred for 48 h. The volatile components were removed in vacuo and the residue was taken up in DCM. KOH$_{[aq]}$ (1M; 5 mL) was added, the DCM was removed gradually under reduced pressure, and the aqueous solution was stirred for 2 h. The pH was adjusted to 8-9. The volatile components were removed under reduced pressure to give 1.70 g (130%) of the product as a brown solid material. Yield > 100% due to residual H$_2$O. $^1$H-NMR confirmed that all ethyl groups had been removed and gave very broad signals. $^{13}$C NMR (126 MHz, D$_2$O) $\delta$ 166.94, 151.28, 142.82, 142.63, 139.84, 125.34, 125.07, 124.80, 108.88, 69.86, 69.65, 68.46, 64.92, 64.79, 61.94, 46.26, 46.05, 29.46 (d, $J_{CP}$ = 129.8 Hz). $^{31}$P NMR (81 MHz, D$_2$O) $\delta$ 18.09. MALDI-TOF MS Calculated C$_{52}$H$_{62}$K$_{12}$N$_{18}$O$_3$P$_6$, 2087.89. Found, 2052 C$_{52}$H$_{62}$K$_{12}$N$_{18}$O$_3$P$_6$ (- 1K$^+$ + 1H$^+$).
**6-TEG-OH-G0-Dendrimer (12)**

A solution of CuSO₄•5H₂O (0.28 g; 1.12 mmol) in H₂O (4 mL) was added to a mixture of 9 (1.34 g; 1.84 mmol), 3 (2.93 g; 13.36 mmol), and Na-ascorbate (0.44 g; 2.22 mmol) in THF (40 mL), and the mixture was allowed to stir at 40°C (oil bath) for 24 h. All the volatile components were removed under reduced pressure, and the residue was taken up in CH₂Cl₂ (50 mL), stirred with Na-EDTA for 2 h, and filtered. Most of the CH₂Cl₂ was removed under reduced pressure and Et₂O was added dropwise to precipitate the product, yielding a brown sticky syrup. The Et₂O was decanted, the residue was re-dissolved in CH₂Cl₂, and the precipitation procedure repeated twice. The product was left in vacuo overnight to give 2.98 g (80%) of the product as a brown sticky syrup. 

**1H NMR (300 MHz, CDCl₃)** δ 7.98 (s, 4H), 7.87 (s, 2H), 7.42 (s, 4H), 5.12 (s, 12H), 4.62 – 4.34 (m, 16H), 3.95 – 3.74 (m, 16H), 3.73 – 3.46 (m, 80H). MALDI-TOF MS Calculated C₈₈H₁₄₀N₁₈O₃₇: 2042.18. Found: 2064.50 (M+Na⁺).

**18-propyne-G1-Dendrimer (13)**

A mixture of 12 (1.63 g; 0.798 mmol), 6 (1.63 g; 5.73 mmol), DMAP (0.64 g; 5.24 mmol), and EDC•HCl (1.38 g; 5.22 mmol) in DCM (15 mL) was stirred for 24 h. The reaction mixture was filtered through a plug of silica (twice) and concentrated under reduced pressure (the material may initially form a sticky foam with a (surprisingly) large volume) to give 2.21 g (76%) of the product as a brown sticky thick syrup. 

**1H NMR (300 MHz, CDCl₃)** δ 7.91 (s, 4H), 7.83 (s, 2H), 7.48 (s, 12H), 5.18 (s, 12H), 4.77 (d,d, J = 2.4, 36H), 4.56 – 4.34 (m, 28H), 3.88 – 3.70 (m, 28H), 3.70 – 3.45 (m, 60H), 2.59 (s, 12H), 2.48 (s, 6H). 

**13C NMR (75 MHz, CDCl₃)** δ 165.76, 151.23, 143.97, 143.14, 141.68, 141.08, 125.53, 109.97, 70.53, 69.34, 66.41, 63.12, 57.10, 50.19, 59.92, 56.90. MALDI-TOF MS Calculated C₁₈₄H₂₀₆N₁₈O₆₁: 3639.69. Found: 3638.66.
As with the G0 dendrimer, compound 13 can be either functionalized with phosphonate ester to give (14) or used as the starting material for the synthesis of the G2 dendrimer with another round of “click” reaction/esterification reactions. Note that for each generation, the number of end-groups triples from 6 to 18 to 54. The diethyl esters phosphonates are first deprotected to form the free phosphonic acid by reacting with TMS-Br, before being turned into phosphonate dipotassium salt by an aqueous KOH treatment.

18-PO₃Et₂-G1-Dendrimer (14)

A solution of CuSO₄•5H₂O (0.05 g; 0.20 mmol) in H₂O (0.3 mL) was added to a mixture of 13 (0.34 g; 0.09 mmol), Na-ascorbate (0.07 g; 0.35 mmol), and 8 (0.47 g; 2.27 mmol) in THF (5 mL), and the mixture was stirred for 48 h at 40°C. All volatile components were removed in vacuo (including water), and the residue was re-dissolved in DCM (5mL) and stirred with Na-EDTA (about 0.15 g) for 1 h, stirred with Na₂SO₄ for ten minutes, filtered, and most of the DCM was removed using a stream of N₂. Et₂O (about 25 mL) was dropwise added to this solution until the product precipitated as a sticky brown syrup. The Et₂O was decanted, the residue was dissolved in DCM, and the precipitation repeated one additional time. The Et₂O was decanted, and the product was dried under reduced pressure to give 0.31 g (45%) of the product as a sticky, foamy brown material. ¹H NMR (400 MHz, CDCl₃) δ 7.92 (s,s, 24H), 7.39 (s, 16H), 5.20 (s, 48H), 4.71 – 4.38 (m, 62H), 4.15 – 4.01 (m, 72H), 3.90 – 3.47 (m, 86H), 2.59 – 2.26 (m, 36H), 1.39 (t, J = 7.0, 108H). All signals were broad. ¹³C NMR (75 MHz, CDCl₃) δ 165.85, 152.10, 152.03, 144.23, 143.53, 141.63, 125.80, 125.68, 124.53, 124.01, 109.31, 70.57, 69.39, 69.20, 66.30, 64.41, 63.13, 62.35 (d, JCP = 6.5 Hz), 50.34, 44.78, 44.64, 27.14 (d, JCP = 141.7 Hz), 17.27 (d, JCP = 6.0 Hz). ³¹P NMR (81 MHz, CDCl₃) δ 25.64.
TMS-Br (0.70 g; 0.6 mL; 4.57 mmol) was added to a solution of 14 (0.30 g; 0.04 mmol) in DCM (7 mL), and the mixture was stirred for 48 h. All the volatile components were removed under reduced pressure, and the residue was re-dissolved in DCM (2 mL). H$_2$O (2 mL) and KOH$_{aq}$ (1.40 mL; 1M) were added and the mixture was stirred for 3 h. The DMC was removed under reduced pressure. The pH was adjusted to 8-9 by the dropwise addition of KOH$_{aq}$ (1 M; only a few drops) and the mixture was stirred for additional 1 h. All the volatiles were removed in vacuo to give 0.41 g (130%) of the product as a light brown, solid material. Yield was above 100% due to residual water in product. $^{13}$C NMR (126 MHz, D$_2$O) δ 166.63, 151.26, 142.88, 142.74, 142.53, 139.92, 125.28, 124.94, 124.74, 108.64, 72.71, 72.24, 69.69, 69.60, 69.38, 68.50, 68.40, 65.02, 64.51, 62.80, 61.92, 50.03, 46.84, 46.61, 30.07 (d, $J_{CP} = 127.0$ Hz). $^{31}$P NMR (81 MHz, D$_2$O) δ 16.79. MALDI-TOF MS Calculated C$_{220}$H$_{272}$K$_{36}$N$_{72}$O$_{113}$P$_{18}$: 7721.99. Found: 7718.24 (M$^+$Na$^+$).
Scheme 4. Synthesis of dendrimers with 18 terminal alkynes (13) and 54-PO3K2-G3-dendrimer (19).
**18-TEG-OH-G1-Dendrimer (16)**

A solution of CuSO$_4$•5H$_2$O (0.13 g; 0.52 mmol) in H$_2$O (2 mL) was added to a solution of 13 (0.96 g; 0.26 mmol), 3 (1.35 g; 6.16 mmol) and Na-ascorbate (0.20 g; 1.01 mmol) in THF (10 mL), and the mixture was allowed to stir at 40°C (oil bath) for 24 h. Na-EDTA (about 0.50 g) was added and left to stir for 2 h. All the volatile components were removed under reduced pressure (including the water), and the residue was taken up in CH$_2$Cl$_2$ (50 mL) and filtered. Most of the CH$_2$Cl$_2$ was removed under reduced pressure, and Et$_2$O was added dropwise to precipitate the product, yielding a brown sticky syrup. The Et$_2$O was decanted, the residue re-dissolved in CH$_2$Cl$_2$, and the precipitation procedure repeated. The product was left *in vacuo* for two days to give 1.69 g (85%) of the product as a brown sticky syrup. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 8.16 (s, 16H), 8.03 (bs, 8H), 7.46 (s, 16H), 5.17 (s, ~48H), 4.61 – 4.30 (m, ~60H), 3.92 – 3.43 (m, ~340H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.59, 152.05, 148.42, 130.50, 109.45, 70.49, 69.31, 69.23, 61.49, 56.35, 53.43, 50.45, 50.26. MALDI-TOF MS Calculated C$_{328}$H$_{506}$N$_{72}$O$_{133}$: 7586.03. Found: 7648.08 (M$^2$-K$^+$/Na$^+$).

**54-propyn-G2-Dendrimer (17)**

A mixture of 16 (1.45 g; 0.19 mmol), 6 (1.17 g; 4.12 mmol), DMAP (0.64 g; 5.24 mmol), and EDC•HCl (1.01 g; 5.27 mmol) in DCM (30 mL) was stirred overnight. The reaction mixture was filtered through a plug of silica, and the plug was washed with additional DCM. The product was concentrated under reduced pressure and left *in vacuo* overnight to give 1.69 g (72%) of a sticky, brown foam. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.29 – 7.77 (m, 24H), 7.61 (s, 52H), 5.44 (s, J = 28.5, 48H), 4.61 (s, ~108H), 4.60 – 3.36 (m, ~400H), 2.64 (s, 54H). Very broad peaks. $^{13}$C NMR
(126 MHz, CDCl$_3$) $\delta$ 165.92, 165.76, 152.14, 151.39, 141.24, 125.81, 125.68, 110.14, 109.45, 78.82, 78.16, 76.59, 75.90, 72.64, 70.82, 70.68, 70.61, 70.47, 70.33, 69.43, 69.26, 64.57, 64.43, 63.30, 61.64, 60.44, 57.27, 50.36.

**54-PO$_3$Et$_2$-G2-Dendrimer (18)**

A solution of CuSO$_4$•5H$_2$O (0.18 g; 0.72 mmol) in H$_2$O (2 mL) was added to a mixture of 17 (1.60 g; 0.13 mmol), 8 (1.97 g; 9.51 mmol), and Na-ascorbate (0.28 g; 1.41 mmol) in THF (50 mL) and stirred at 40°C overnight. Na-EDTA (ca 0.5 g) was added and left to stir for 2 h. All solvents were removed under reduced pressure, and the residue left under reduced pressure for 7 h. The residue was taken up in DCM (50 mL) and filtered through a plug of celite with suction. The plug was washed with additional DCM. Most of the DCM was evaporated under reduced pressure, and Et$_2$O was added dropwise to precipitate the product. The Et$_2$O was decanted, the product taken up in a small amount of DCM, and the precipitation repeated twice. Following the third decantation, the product was left under reduced pressure overnight to give 1.8 g (59%) of the product as a crispy, brown foam (the product becomes very large under reduced pressure). $^1$H-NMR confirmed that there were no triple bonds left, and there were very broad peaks. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 165.71, 151.96, 125.70, 109.27, 70.51, 69.13, 64.33, 62.20, 44.76, 27.03 (d, $J_{CP} = 138.1$ Hz), 16.42 (d, $J_{CP} = 5.0$ Hz). $^{31}$P NMR (81 MHz, CDCl$_3$) $\delta$ 25.62.

**54-PO$_3$K$_2$-G2-Dendrimer (19)**

TMS-Br (2.32 g; 2.00 mL; 14.67 mmol) was added to a solution of 18 (1.60 g; 0.07 mmol) in DCM (30 mL), and the mixture was stirred overnight. All the volatile components were removed
under reduced pressure, and the residue was re-dissolved in DCM (20 mL). H₂O (10 mL) and KOH\(_{\text{aq}}\) (7 mL, 1 M) were added, and the mixture was stirred for 2 h. The DMC was removed under reduced pressure. The pH was adjusted to 8-9 by the dropwise addition of KOH\(_{\text{aq}}\) (1 M), and the mixture allowed to stirrer for additional 2 h. All the volatiles were removed \textit{in vacuo} to give 2.26 g (135%) of the product as a brown, solid material. Yield was above 100% due to residual water in product. \(^1\)H-NMR confirmed that all ethyl groups were removed. \(^{13}\)C NMR (126 MHz, D₂O) \(\delta\) 166.45, 166.23, 151.24, 150.98, 142.96, 142.68, 142.56, 139.99, 125.42, 124.98, 124.81, 108.70, 108.48, 104.99, 72.71, 72.34, 71.58, 69.76, 69.60, 69.49, 69.34, 69.26, 68.47, 65.06, 64.50, 62.80, 62.32, 61.91, 60.21, 50.06, 46.47, 46.29, 29.65 (d, \(J_{\text{CP}} = 129.6\) Hz). \(^{31}\)P NMR (81 MHz, D₂O) \(\delta\) 17.54. MALDI-TOF MS Calculated C\(_{724}\)H\(_{902}\)K\(_{108}\)N\(_{234}\)O\(_{367}\)P\(_{54}\): 24624.57. Found: 12696.36 (M\(^{2+}\)).

References