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An Efficient Thermal Elimination Pathway toward Phosphodiester Hydrogels via a Precursor Approach

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Phosphodiester hydrogels offer a wide range of fascinating properties. Not only do they exhibit excellent hemocompatibility and cellular compatibility, they also show a remarkable resistance to protein adsorption, thereby limiting the foreign body response. In this work, phosphodiester-crosslinked hydrogels are produced by a simple free-radical polymerization of a phosphotriester crosslinker. In a second step, this material is transformed to the phosphodiester, by heating it up to 60 °C in phosphate-buffered saline. Compared to earlier methods, there is no need for acids, bases, or oxidizing agents to achieve this final conversion to the phosphodiester. This method thus reduces the risk to damage or degrade any sensitive biomolecules that might be of interest to tissue engineers, such as various growth factors or other proteins. The phosphotriester crosslinker is readily synthesized out of common laboratory chemicals in multigram quantities with good yield and easy workup and purification.

1. Introduction

Hydrogels enjoy wide application in the biomedical sciences and tissue engineering.[1–4] Their structure consists of hydrophilic polymer chains, held together by physical or chemical crosslinks to prevent dissolution.[1,5] This structure allows for the rapid transfer of solutes. In addition, it possesses physical properties that are not dissimilar to those of the extracellular matrix,[1,3] which contributes to the material's cellular compatibility. Phosphodiester-based materials possess a number of additional properties that make them valuable for tissue engineers and researchers in the (bio)medical sciences.[6] The biomimetic nature of phosphodiesters tends to enhance hemocompatibility and cellular compatibility as well as resisting the adsorption of proteins.[6–13] As protein adsorption is the first method by which the organism identifies objects for expulsion by foreign body response, limiting the adsorption of these proteins is desirable.[2,11,14,15] Phosphodiesters are shelf-stable, yet efficiently degraded enzymatically.[16,17] They are fully ionized under physiological conditions, and do not cause acidification of their environment upon degradation as can occur in the case of polyester biomaterials.[18,19] Previously, our research group reported on the development of novel phosphodiester hydrogels starting from cyanoethyl-bearing phosphotriester precursors.[13] The method features a free radical polymerization process followed by a base-induced elimination step. The novel hydrogel structure has already been tested on hemo- and cell compatibility and showed very good results.[13] Although this synthetic route yields direct access to these novel promising materials, the base-treatment that is required for the deprotection of the phosphotriester prepolymer, is rather harsh. This can be troublesome for applications involving additives or comonomers that are base-sensitive, or where the formation of small amounts of acrylonitrile as an elimination product (**Figure 1**a) before being washed out may be undesirable. Possible examples where a loss of functionality may occur due to the elimination conditions or reaction with acrylonitrile are polypeptides such as fibrin or various growth factors.[2–4] For those cases, a precursor route based on a different phosphotriester prepolymer elimination method is highly desirable. In the literature, a few methods have been described to effect this transformation ranging from dealkylation by triethylamine[8] or thiophenolate,[20,21] hydrolysis under acidic conditions,[7] oxidation by dinitrogen tetroxide[22,23] to the retro-Michael condensation[13,24] method widely used in nucleoside synthesis. Thermolytic routes that do not require the addition of any chemicals and proceed in neutral media have also been described, both in polymer science[25] as in oligonucleoside synthesis.[26] That last approach was selected for this work due to its high selectivity and mild deprotection conditions.

An improved novel phosphodiester precursor route is presented that is based on the free radical polymerization of an entirely different phosphotriester crosslinker, followed by the thermal elimination (Figure 1b) of the resultant prepolymer to yield versatile bio- and hemocompatible hydrogels. The discovery and synthesis of this new monomer allowed direct access to the thermal elimination route. The use of a thermal elimination avoids the use of a base and the associated issues described

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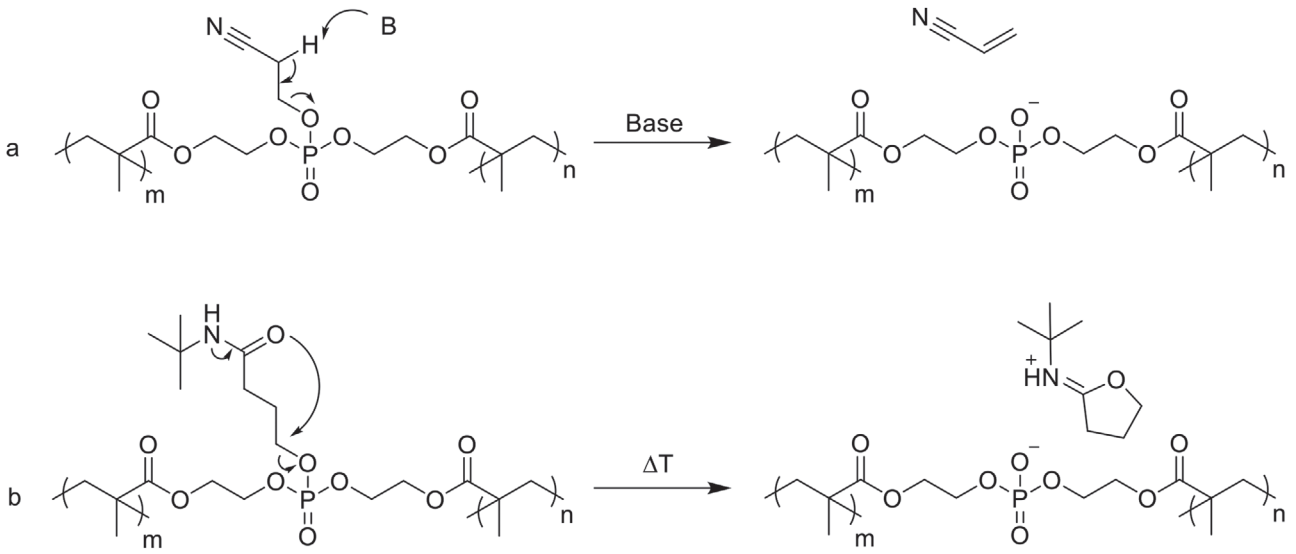

Figure 1. Base-induced versus thermal elimination mechanisms.

above. The resulting hydrogel materials are analyzed with solid-state carbon and phosphorus NMR spectroscopy.[27–30]

2. Results and Discussion

2.1. Synthesis of the Crosslinker

The novel synthetic pathway toward the phosphodiester hydrogel is shown is **Figure 2**. An established synthetic approach toward phosphates is the phosphoramidite method.[31,32] However, in this particular case, an approach utilizing phosphonates[33] seemed the more practical route. The precursor phosphoramidite is not widely available as a precursor for nucleoside chemistry; and due to the heavy amide sidechain, the purification by distillation is expected to be much more difficult compared to the cyanoethyl derivative. Furthermore, using the symmetric phosphonate **6** avoids the use of hazardous phosphorus trichloride, as well as avoiding an additional oxidation step. Phosphorus oxychloride is also a less attractive precursor due to a reduced coupling efficiency compared to the phosphonate. As illustrated in Figure 2, Bis(methacryloxyethyl) phosphonate (**6**) is conveniently prepared by transphosphonylation[34–36] (65% yield) from diphenyl phosphite (**4**). An alternative method allows for synthesis from phosphonic acid (**5**) via a mixed-anhydride approach (57% yield). Both reactions yield the same colorless oil after purification, therefore depending on availability of precursors one or the other method may

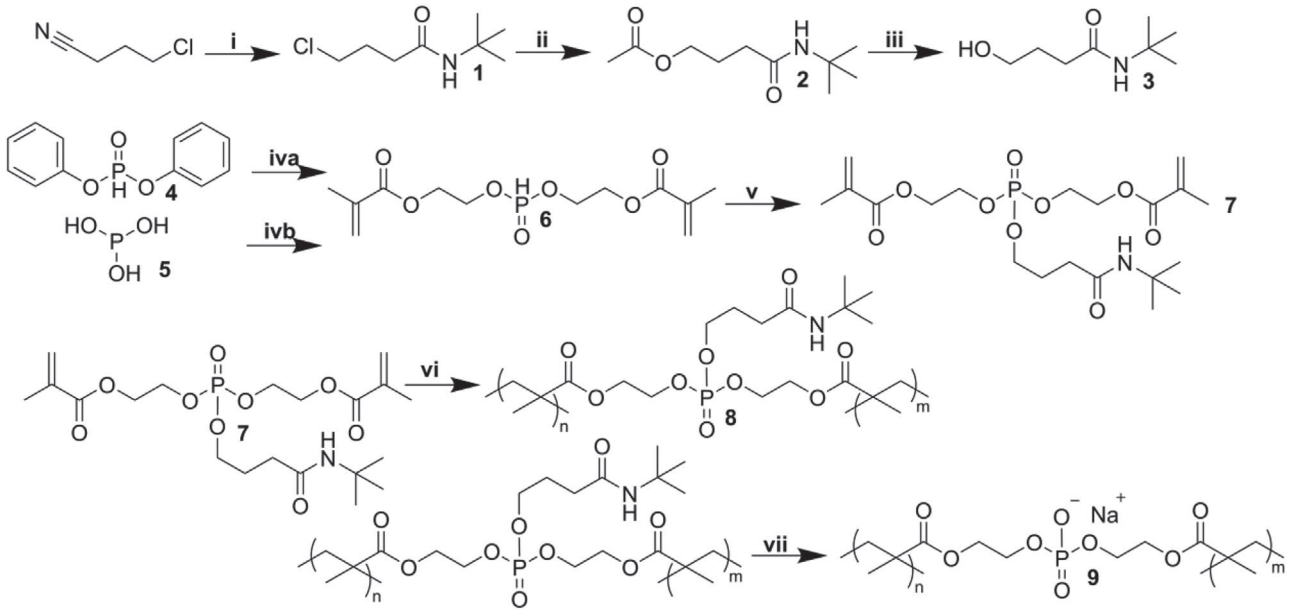

Figure 2. Synthetic approach toward phosphodiester hydrogel 9: i) tBuOAc, H_2SO_4, 42 °C; ii) KOAc, KI, DMSO, 80 °C; iii) MeOH, NaOH(aq), room temperature; iv(a)) HEMA, pyridine; iv(b)) pivaloyl chloride, HEMA, pyridine; v) 3, CCl_3Br, Et_3N, CH_2Cl_2, vi) DMSO, AIBN or VA-044, vii) T, PBS.

be preferred. The final crosslinker could be prepared via the Atherton–Todd reaction[37–39] between phosphonate **6** and *N-tert*-butyl-4-hydroxybutanamide **3**. After chromatography, the product is obtained as a colorless oil with a yield of 79%. Alcohol **3** was synthesized via a three-step synthesis, rather than by ring-opening reaction of γ-butyrolactone with *tert*-butylamine as reported in literature,[26] since γ-butyrolactone is quickly becoming a controlled substance rendering it somewhat unattractive as a synthon. The route presented here is fast, easy, and does not require forcing conditions whereas the literature procedure for the ring-openings reaction requires 3 days in a sealed vial at elevated temperatures.[26] The first step is a Ritter reaction[40] (Figure 2i) to transform 4-chlorobutyronitrile into the tert butyl amide (90% yield after recrystallization). This is then followed by transformation of the chloride moiety to the acetate;[41] which is obtained as a colorless oil (85% yield). The final step is hydrolysis of the acetate under mild conditions to yield a white crystalline powder (85% yield after recrystallization). The reactions are straightforward, furthermore workup and purification are easy. The combined yield of the final material starting from diphenyl phosphite is 51%; whereas starting from phosphonic acid it is 45%.

2.2. Polymerization

The polymerization was initially performed identically to the base-deprotected approach,[13] using AIBN in DMSO at 80 °C, but this was found to be suboptimal as there was noticeable discoloration of the gels. This is likely due to decomposition or thermal side-reactions of the crosslinker at 80 °C. AIBN was thus replaced with a lower-temperature initiator, 2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044), which polymerizes the crosslinker at 50 °C in DMSO, a temperature at which no visible discoloration occurs.

2.3. Thermal Deprotection and Analysis by Phosphorus NMR Spectroscopy

Preliminary deprotection experiments were performed under conditions as employed in the original publication of the thermal deprotection of polynucleotides,[26] which suggested a water bath at 80 °C, and PBS as the deprotection solution. For all samples, the PBS was replaced every 25 min. During these experiments, the non-eliminated ("pristine") sample was found to be very hydrophilic. Whereas the cyanoethyl-protected prepolymer shrinks as it loses DMSO, the *N-tert*-butyl-carboxypropyl protected prepolymer takes up significant amounts of water. This behavior is not uncommon for polyamides.[42] The resulting hydrogels were analyzed with solid-state phosphorus NMR. These results are displayed in **Figure 3**. The phosphotriester signals of the prepolymer can be found at −5.9 ppm, whereas those of the phosphodiester are located around −3.1 ppm. A small shoulder can be seen to emerge around 2.4 ppm, consistent with the breaking of a phosphodiester bond to form phosphomonoesters.

Given how the reaction goes to completion within 25 min, as evidenced by the shift from −5.9 to 3.1 ppm, it should be possible to perform the transformation at lower temperatures. This was investigated by performing 1 h elimination experiments at various temperatures with the buffer being refreshed after half an hour. Tested temperatures were 30, 40, 50, and 60 °C. Since previous research showed that DMSO can be washed

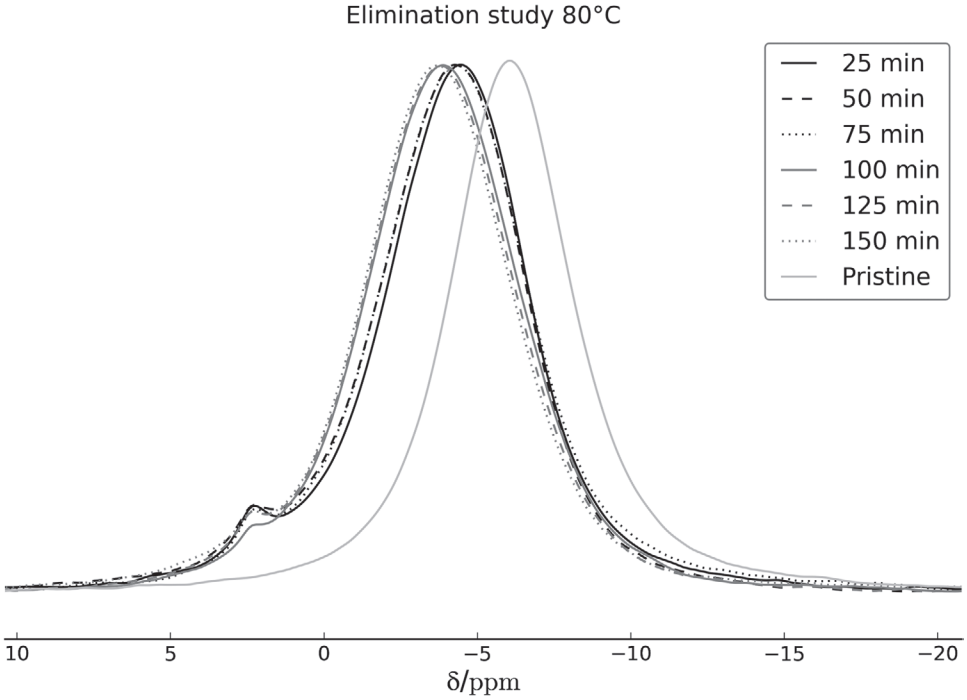

Figure 3. Solid state phosphorus NMR spectra showing that the thermal elimination goes to completion within 25 min at 80 °C in PBS buffer as evidenced by the shift from −5.9 to 3 ppm of the phosphorus NMR signal.

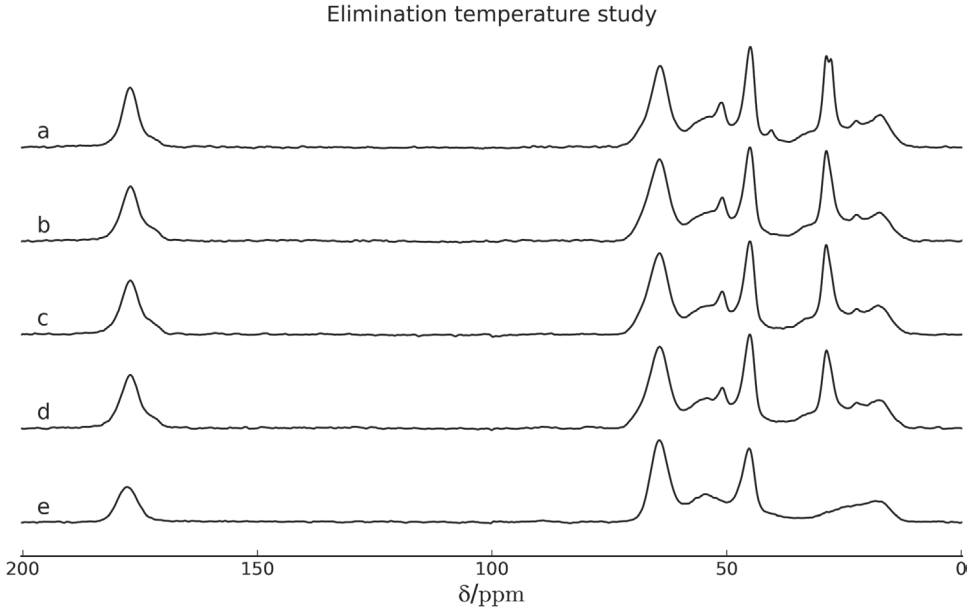

Figure 4. Solid-state carbon spectra as a function of the elimination temperature: a) Pristine, b) 30 °C, c) 40 °C, d) 50 °C, and e) 60 °C, showing that an elimination process of 2 × 30 min is inefficient below 60 °C. The samples were washed 12 times with PBS buffer after the elimination reaction.

out in 12 consecutive washing steps, the same protocol was applied here.[13] If any elimination occurs, it should be visible as a decrease of the *t*-butyl signal around 28 ppm. The results are displayed in **Figure 4**. From the disappearance of the *t*-butyl signal around 28 ppm, it can be stated that an elimination temperature of at least 60 °C is required for a reaction time of 1 h under the utilized reaction conditions.

In the next step, a set of experiments was conducted to determine whether the elimination products could be removed efficiently from the final hydrogel material. Results of the washing protocol and the resultant carbon and phosphorus spectra are comparable to earlier data on hydrogels prepared under similar conditions utilizing the base-induced precursor and elimination method.[13] The *t*-butyl signal of the prepolymer (**Figure 5** "pristine") around 28 ppm is very prominent and therefore, useful to determine the efficiency of the elimination and washing process. Other signals from the protecting group that can be used to follow the elimination reaction are the small upfield shoulder around 172 ppm associated with the amide carbonyl, the quaternary carbon signal of the *t*-butyl group around 51 ppm, and the methylene signals around 29 and 34 ppm. Figure 5 shows a series of ^{13}C-NMR spectra of a specimen obtained after several washing steps with cold PBS buffer following an elimination process of 2 × 30 min at 60 °C. The spectrum of the pristine sample clearly shows the DMSO signal around 40 ppm. It can be observed that DMSO and the elimination product can be completely removed after ≈11 washing steps.

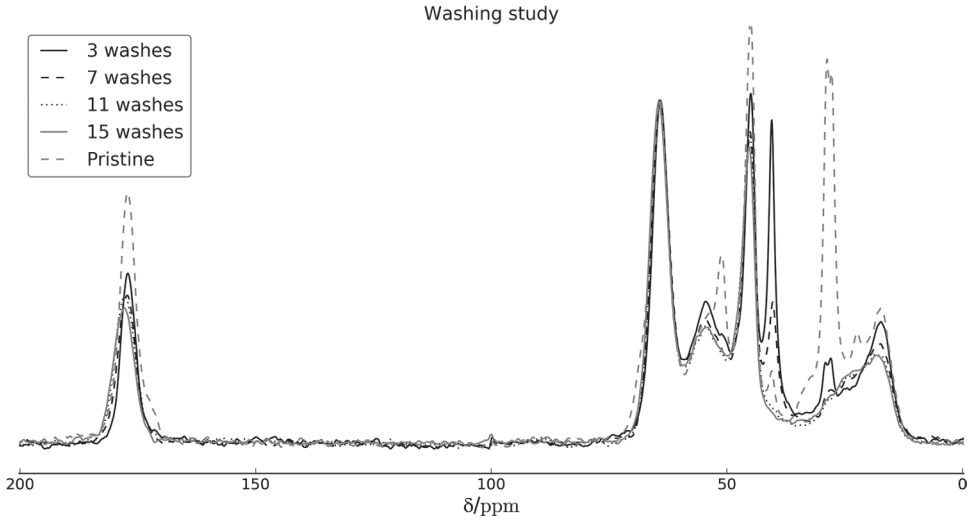

Figure 5. Solid-state carbon NMR spectra of a gel sample eliminated for 25 min followed by a variable number of washing steps. The pristine sample was not eliminated as a reference.

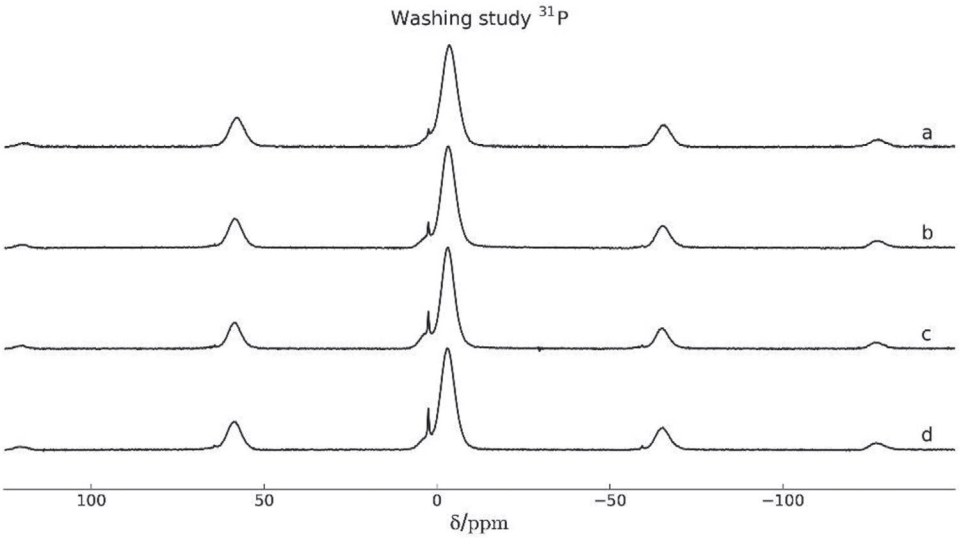

Figure 6. Solid state phosphorous spectra of an eliminated gel sample after a variable number of washing steps: a) three washes, b) seven washes, c) 11 washes, and d) 15 washes.

During the washings, some degradation of the phosphodiesters to phosphomonoesters also takes place as can be observed in the phosphorus spectra shown in **Figure 6**. The reason for this degradation is possibly a different topology of the hydrogel network due to a templating effect of the amide side chain; rendering it more strained after deprotection. If this is the case, it may be possible to avoid this degradation by tuning the polymerization conditions to yield a more randomly oriented network. The contribution of the sharper phosphomonoester signal at 2.4 ppm can be determined by peak deconvolution. Taking the peak areas of the spinning sidebands into account, the amount of phosphomonoester can be obtained accurately as shown in **Table 1**. While this amount of degradation is measurable via solid-state NMR, it is most likely to be negligible for most applications.

2.4. Dynamic Mechanical Analysis

The viscoelastic properties of the thermally deprotected hydrogel were compared to those of a base-eliminated hydrogel prepared under similar conditions. As can be seen in **Figure 7**, the thermally deprotected gel is generally easier to deform than the base-eliminated material. This may be explained by the degradation of the phosphodiester crosslinks observed in the phosphorus NMR spectra; however, differences in the hydrogel network topology, if any, may also play some role in its mechanical properties. Nonetheless both materials are gel-like at all tested frequencies consistent with a chemically crosslinked hydrogel.

Table 1. Amount of phosphomonoester present as a function of the number of washing steps.

Number of washing steps	Phosphomonoester [%]
3	0.3%
7	0.5%
11	1.1%
15	1.3%

3. Conclusion

A phosphodiester-based hydrogel system has been developed that is based on a conveniently synthesized phosphotriester crosslinker. The synthesis of this crosslinker uses common reagents, avoids harsh reaction conditions, and is easily scaled to multigram amounts. The conversion from phosphotriester to phosphodiester by elimination is performed via an efficient thermal process. The polymerization solvent and elimination products can easily be washed out. This new route provides facile access to various phosphodiester materials, whose diverse features including hemo- and cellular compatibility has been established in previous works, with the outlook of creating sophisticated and innovative hybrid biomaterials. The applicability of these materials is now widened since the phosphotriester elimination can be accomplished in neutral media in contrast to previous approaches, which required harsher reagents.

4. Experimental Section

Products were purchased from Sigma–Aldrich, Acros, or TCI. VA-044 was obtained from WAKO chemicals. IR spectra were recorded on a Bruker Tensor 27, using NaCl discs. Carbon-13 solid-state CP/MAS NMR spectra were acquired at ambient temperature on an Agilent VNMRS DirectDrive 400 MHz spectrometer (9.4 T wide bore magnet) equipped with a T3HX 3.2 mm probe dedicated for small sample volumes and high decoupling powers. Magic angle spinning (MAS) was performed at 9 kHz with ceramic zirconia rotors of 3.2 mm in diameter (22 µL rotors) and with TOSS (total suppression of spinning sidebands). The aromatic signal of hexamethylbenzene was used to determine the Hartmann–Hahn condition ($\omega_{1H} = \gamma_H B_{1H} = \gamma_C B_{13C} = \omega_{13C}$) for cross-polarization

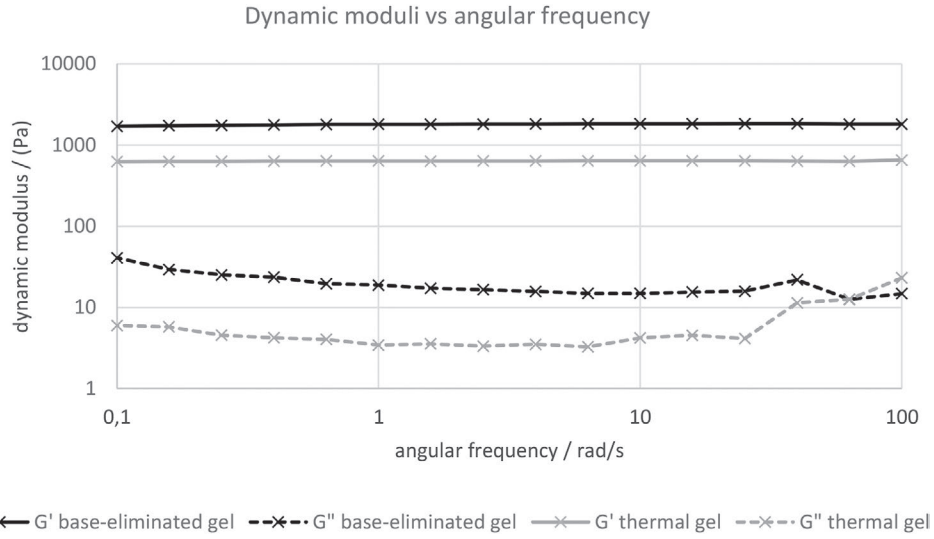

Figure 7. The storage and loss moduli of the thermal and base-deprotected gels as a function of angular frequency.

(CP), and to calibrate the carbon chemical shift scale (132.1 ppm). Acquisition parameters used were a spectral width of 50 kHz, a 90° pulse length of 2.5 µs, a spin-lock field for CP of 100 kHz, a contact time for CP of 1 ms, an acquisition time of 15 ms, a recycle delay time of 2.5 s, and 5000 accumulations. High power proton dipolar decoupling during the acquisition time was set to 100 kHz. Phosphorus-31 solid-state CP/MAS NMR spectra were acquired on the same spectrometer with the same probe. MAS was performed at 10 kHz using the same rotors. Monopotassium phosphate (KH_2PO_4) was used to calibrate the phosphorus chemical shift scale (0 ppm). Acquisition parameters used were a spectral width of 50 kHz, a 90° pulse length of 3.7 µs, a spin-lock field for CP of 100 kHz, a contact time for CP of 0.9 ms, an acquisition time of 15 ms, a recycle delay time of 10 s and 200 accumulations. High power proton dipolar decoupling during the acquisition time was set to 100 kHz. The Hartmann–Hahn condition for CP was calibrated accurately on the samples themselves. The linear viscoelastic behavior (G′ and G″) of the samples was measured using an Anton Paar MCR 702 Twindrive with parallel plate geometry.

N-tert-Butyl-4-Chlorobutanamide (1): A three-necked, flame-dried round-bottom flask equipped with a septum, magnetic stirring bar, and reflux condenser was charged with a solution of 4-chlorobutyronitrile (10.35 g, 0.1 mol) in *tert*-butyl acetate (75 mL). Concentrated sulfuric acid (5 mL) was added dropwise, and the solution was heated to 42 °C for 2 h. The reaction was quenched by slowly adding it to a solution of water (400 mL) with $KHCO_3$ (50 g). The mixture was extracted with ethyl acetate (5 × 50 mL). The combined organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. An oily solid was obtained. This solid was purified by recrystallization in refluxing pentane. After recrystallization and drying under vacuum, 8.4 g (90%) of the final product was recovered. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.41 (s, 1H), 3.58 (t, J = 6.1 Hz, 2H), 2.26 (t, J = 7.1 Hz, 2H), 2.01-2.12 (m, 2H), 1.32 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.62, 51.96, 45.25, 34.69, 29.43, 28.81. FTIR: 3319 (ν_{as} N-H), 3066 (ν_s N-H), 2966 (ν_{as} CH_3), 2927 (ν_{as} CH_2), 1645 (Amide I band ν C = O), 1546 (Amide II band δ_β NH), 1454 (δ_{as} CH3), 1419 ($\delta_{\beta s}$ CH_2-CO), 1390 (δ_s CH_3 of t-Bu), 1365 (δ_s CH_3 of t-Bu), 1299 (Amide III band ν C-N), 1261 (skel $C(CH_3)_3$), 1224 (skel $C(CH_3)_3$). MP (pentane): 62.3 °C.

N-tert-Butyl-4-Acetoxybutanamide (2): A three-necked, flame-dried flask was equipped with a reflux condenser and magnetic stirring bar. The flask was charged with potassium acetate (20 g, 0.2 mol), *N-tert*-butyl-4-chlorobutanamide (18.57 g, 0.1 mol), potassium iodide (0.4 g, 2.4 mmol), and dry DMSO (80 mL). The mixture was heated to 80 °C under an inert atmosphere and stirred overnight. The reaction was quenched by the addition of 100 mL water, and extracted with ethyl acetate (2 × 100 mL). The aqueous phase was salted, and extracted once more with ethyl acetate (100 mL). The combined organic phase was dried with $MgSO_4$ and the solvent was removed in vacuo. The resultant yellow oil was purified by column chromatography using diethyl ether as eluent. The product was obtained as 9.6 g (85%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.48 (s, 1H), 4.06 (t, J = 6.3 Hz, 2H), 2.14 (t, J = 7.3 Hz, 2H), 2.01 (s, 3H), 1.92 (m, 2H), 1.31 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.99, 171.84, 64.33, 51.97, 34.35, 29.43, 25.39, 21.63. FTIR: 3309 (ν_{as} N—H), 3076 (ν_s N—H), 1741 (ν C=O), 1649 (amide I band ν C=O), 1544 (amide II band δ_β NH), 1454 (δ_{as} CH_3), 1390 (δ_s CH_3 of *t*-Bu), 1365 (δ_s CH_3 of *t*-Bu and CH_3COO), 1242 (ν_{as} C—O—C; ester band), 1043 (ν_s C—O—C).

N-tert-Butyl-4-Hydroxybutanamide (3): A three-necked flask equipped with magnetic stirring was charged with *N-tert*-butyl-4-acetoxybutanamide (10 g, 0.05 mol) and methanol (30 mL). While stirring, an aqueous sodium hydroxide solution (1 M, 50 mL) was slowly added. The mixture was stirred for 3 h, and extracted with ethyl acetate (4 × 50 mL). The combined organic phase was dried over $MgSO_4$. After removing the solvent under reduced pressure, the resultant solid was recrystallized from refluxing diethyl ether. After drying in vacuo, 6.7 g (85%) of a white crystalline powder was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 3.74 (s, 1H), 3.62 (t, J = 5.7 Hz, 2H), 2.23 (m, 2H), 1.79 (m, 2H), 1.29 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.15, 60.23, 49.11, 32.95, 26.76, 26.23. FTIR: 3292 (b, ν O—H), 1643 (amide I band ν C=O), 1556 (amide II band δ_β NH), 1461 (δ_{as} CH_3), 1442 ($\delta_{\beta s}$ CH_2—CO), 1392 (δ_s CH_3 of *t*-Bu), 1359 (δ_s CH_3 of *t*-Bu), 1288 (skel $C(CH_3)_3$), 1224 (skel $C(CH_3)_3$), 1047 (ν pOH). MP (Et_2O): 72.4 °C.

Bis(Methacryloxyethyl) H-Phosphonate (6): A flame-dried, three-necked flask was equipped with magnetic stirring and an inert atmosphere. Phosphonic acid (3 g, 0.037 mol) was suspended in diethyl ether (80 mL). Pivaloyl chloride (9.9 ml, 0.080 mol) and pyridine (6.48 ml, 0.080 mol) were added and the reaction mixture was stirred for half an hour to allow the mixed anhydride to form. Hydroxyethyl methacrylate (9.68 mL, 0.080 mol) was added and the reaction mixture was stirred overnight. The reaction mixture was filtered and the filtrate washed with 10 mL diethyl ether. The ether phase was transferred to an extraction funnel and washed with a saturated sodium carbonate solution (20 mL). The ethereal phase was dried over magnesium sulfate, concentrated in vacuo, and subjected to column chromatography using gradient elution (Et_2O with 0–5% MeOH, TLC visualized with vanillin) to obtain bis(methacryloxyethyl) phosphonate (6.42 g, 57%) as a colorless oil. Analysis identical as described in "Bis(methacryloxyethyl) H-phosphonate (6)."

Bis(Methacryloxyethyl) H-Phosphonate (6): A flame-dried, three-necked flask was equipped with magnetic stirring and an inert atmosphere. 2-Hydroxyethyl methacrylate (30 mL, 0.25 mol) and pyridine (50 mL, 0.62 mol) were added to the flask, and it was cooled in an ice bath. Diphenyl phosphite (20 mL, 0.1 mol) is added slowly. The ice bath was removed after addition. After stirring for 8 h, the reaction mixture was diluted with 150 mL diethyl ether and washed with aqueous HCl (2 M, 3 × 50 mL) and once with brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The obtained oil was purified via column chromatography using gradient elution (Et_2O with 0–5% MeOH, TLC visualized with vanillin) to obtain bis(methacryloxyethyl) phosphonate (20 g, 65%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (dm, $^1J_{PH}$ = 714 Hz, 1H), 6.10 (dq, J = 2.0, 1.0 Hz, 2H), 5.56 (p, J = 1.6 Hz, 2H), 4.51–4.05 (m, 8H), 1.89 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.52, 136.32, 127.07, 64.21 (d, $^2J_{P\text{-}C}$ = 5.7 Hz), 63.91 (d, $^3J_{P\text{-}C}$ = 5.5 Hz), 18.89. ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.8 (d, $^1J_{PH}$ = 714 Hz), FTIR: 3105 (ν_{as} = CH_2), 2958 (ν_{as} CH_3), 2443 ($\nu_{P\text{-}H}$), 1719 (ν C = O), 1637 (ν C = C), 1452 (δ_{as} CH_3), 1404 (δ_β = CH_2), 1321 (δ_s CH_3-C =) 1298 (ν C-O-C), 1257 (ν P = O), 1166 (ν_{as} P-O-CH_2R), 1069 (ν C-O-C), 966 (ν_s P-O-CH_2R).

Bis(Methacryloxyethyl)-N-tert-Butyl-Butanamide-4-yl-Phosphate (7): A flame-dried, three-necked flask was equipped with an inert atmosphere and magnetic stirring. It was charged with dry DCM (35 mL), *N-tert*-butyl-4-hydroxybutanamide (1.56 g, 9.8 mmol), triethylamine (0.99 g, 9.8 mmol), and freshly purified bis(methacryloxyethyl) H-phosphonate (2.5 g, 8.2 mmol). The flask was placed in an ice bath, and bromotrichloromethane (0.97 mL) was added in a dropwise manner. The ice bath was removed and the solution was stirred for 3 h. Water was added and the dichloromethane phase was drawn off. The aqueous phase was washed with diethyl ether (3 × 30 mL) and the combined organic phase was dried over $MgSO_4$. Solvent was removed in vacuo, and the product was purified using column chromatography using gradient elution (Et_2O, 0–5% methanol). After purification, bis(methacryloxyethyl)-*N-tert*-butyl-butanamide-4-yl-phosphate was obtained as a colorless oil (3 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (m, 2H), 5.75 (s, 1H), 5.59 (p, J = 1.5 Hz, 2H), 4.38 (m, 4H), 4.27 (m, 4H), 4.06 (dt, J = 7.6, 5.9 Hz, 2H), 2.20 (t, J = 7.2 Hz, 2H), 1.96 (m, 2H), 1.93 (m, 6H), 1.34 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.80, 167.69, 136.42, 127.06, 68.00 (d, J = 6 Hz), 66.14 (d, J = 5.5 Hz), 63.72 (d, J = 6.9 Hz), 33.66, 29.42, 26.89 (d, J = 6.4 Hz), 18.94 ^{31}P NMR (162 MHz, $CDCl_3$) δ -5.6 ppm, FTIR: 3319 (ν_{as} N-H), 2966 (ν_{as} CH_3), 2929 (ν_{as} CH_2), 1720 (ν C = O), 1654 (Amide I band ν C = O), 1546 (Amide II band δ_β NH), 1454 (δ_{as} CH_3), 1392 (δ_s CH_3 of t-Bu), 1365 (δ_s CH_3 of t-Bu), 1321 (δ_s CH_3-C =), 1298 (amide III band ν C-N), 1269 (ν P = O), 1168 (ν_{as} C-O-C; ester band), 1066 (ν_s C-O-C), 1031 (ν P-O-C).

General Procedure for the Preparation of the Prepolymer: A solution of VA-044 (1 mg mL^{-1}) and the crosslinker (compound 7, 154 mg) in DMSO (1 mL) was placed in an autosampler vial equipped with a rubber septum. The vial was purged with an inert gas for 20 min and placed in a heating block at 50 °C overnight. The vial was shattered and the gel was retrieved.

Solid state ^{31}P-CP/MAS NMR: –5.9 ppm, ^{13}C-CP/MAS NMR: 177.1 (shoulder ≈173 ppm), 64.1, 53.5, 51, 44.8, 28.6 (shoulder ~34 ppm), 22.2, and 17.1 ppm.

General Procedure for the Deprotection toward the Ionic Hydrogel: The gel was submerged in PBS at 60 °C for 30 min. The buffer was replaced and the gel was again submerged in the warm buffer for 30 min. The gel was then placed in fresh cold buffer for at least 2 h. The buffer was replaced at least 11 more times, leaving at least 2 h between replacements. The gel could be lyophilized or used as is.

Solid state ^{31}P-CP/MAS NMR: –3.1 ppm, ^{13}C-CP/MAS NMR: 178.1, 64.3, 54.8, 45.1, and 17.3 ppm.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomaterials, hydrogels, phosphodiesters

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