

Supporting Information

Ester-mediated peptide formation promoted by deep eutectic solvents: A facile pathway to proto-peptides

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1. Experimental Procedures

Materials

Glycine (99%), glycolic acid (99%), choline chloride (99%), L-alanine (99%), L-lysine (98%), deuterium oxide (99.8 atom % D), methyl glycolate (98%), and tetrabutylammonium iodide (98%) were purchased from Across Organics, Belgium. HPLC grade water was purchased from DUKSAN, Korea. HPLC grade acetonitrile and potassium biphthalate (99%) were purchased from J.T Baker, USA. Diglycine (98%), ammonium chloride (99%), tetrapropylammonium chloride (98%), L-aspartic acid (98%) were purchased from Sigma-Aldrich. Triglycine (99%), glycine anhydride (99%), ammonium formate (98%), L-glutamic acid (98%), tetraethylammonium bromide (98%), tetrabutylammonium bromide (98%), and D-alanine (99%), were purchased from Alfa Aesar, USA. Tetraglycine (95%), tetramethylammonium chloride (98%), tetraethylammonium chloride (98%), tetrabutylammonium chloride (98%), tetraethylammonium iodide (98%), L-valine (98%), and L-arginine (98%), were purchased from TCI, Japan. Sodium chloride (99.5%) was purchased from SHOWA, Japan. Formic acid (98%) was purchased from Fisher scientific, USA. AMBERJET™ 1000H cation exchange resin was from Dow Chemical Company, USA.

Dry-down reactions

The reaction typically started by first drying 100 μ L aqueous solution of 100 mM tetraethylammonium chloride (TEACl) at 95°C in a temperature-controlled oven for 1 day. 200 μ L aqueous solution of 50 mM glycolic acid and 50 mM glycine was added to the dry TEACl to make an aqueous mixture with a molar ratio of TEACl:g:G=1:1:1. The mixture was allowed to dry and to react at 95°C. To study the formation of decapeptides under various conditions, TEACl was replaced by different salts and the ratio of reactants was adjusted. The control reaction without TEACl was carried out in the identical dry down condition.

Oligomer characterization

Before LC-UV analysis, each sample was dissolved in 500 μ L of 50% v/v acetonitrile/water. Samples were analyzed with an Agilent 1260 Infinity II HPLC with an inline Agilent UV absorbance detector (210 nm). Oligomers were separated using a SeQuant ZIC-HILIC column (150 \times 2.1 mm, 3.5 μ m particle size, 100 Å). The flow rate was 0.4 mL min⁻¹, and the column temperature was held at 40°C. The mobile phase was (A) water with 0.5% v/v formic acid and (B) acetonitrile. The gradient method started with 3% A for 10 minutes and then ramped to 49% A over 23 minutes. The mobile phase composition was then held at 49% A for 5 minutes and then returned to 3% A in 1 minute. For the quantitative analysis of the 1gnG oligomers, the UV extinction coefficients of standard compounds (1g1G, 1g2G, 1g3G, 1g4G, and glycine diketopiperazine) were measured first. The extinction coefficients of oligomers longer than 1g4G can be estimated using the method reported by Codari *et al.*¹ and Campbell *et al.*² Assuming the extinction coefficients of the chromophores (amides and carboxylic acids) are similar and are independent of the chain length, the UV extinction coefficients of the 1gnG oligomers can be calculated based on the number of carboxylic acid and amide groups. The same LC method was used to separate the reaction mixtures of TEACl/glycolic acid/aspartic acid, TEACl/glycolic acid/glutamic acid, TEACl/glycolic acid/arginine, TEACl/glycolic acid/lysine, and the mixtures using multiple amino acids.

For the control experiment, a hydrolysis treatment was used to reveal the 1gnG oligomer series.³ Each dry mixture was first dissolved in 500 μ L of water, sealed in a vial, and then heated at 95°C for 2 days to hydrolyze ester bonds. After the hydrolysis, the samples were lyophilized for the quantitative analysis of oligomer distributions.

For the reaction mixtures of TEACl/glycolic acid/alanine and TEACl/glycolic acid, oligomers were separated by an Agilent Poroshell 120 EC-C18 reverse-phase column (100 \times 4.6 mm, 2.7 μ m particle size). The flow rate was 0.5 mL min⁻¹. The mobile phase was (A) water with 0.2% v/v formic acid and (B) acetonitrile. The gradient method started with 98% A for 2 minutes and then ramped to 88% A over 20 minutes. The mobile phase composition was then ramped to 0% A and held for 2 minutes. Finally, the gradient returned to 98% A in 1 minute. For the reaction mixture of TEACl/glycolic acid/valine, the same

reverse-phase column was used. The gradient method started with 98% A for 2 minutes, ramped to 58% A over 20 minutes, and then returned to 98% A in 1 minute.

LC-MS data were collected on a Bruker compact QqTOF mass spectrometer. All data were obtained with negative-ion mode electrospray ionization (ESI) with a capillary voltage of 3.5 kV. The dry gas flow was 9 L min⁻¹ at 250°C. Sodium formate was used for mass calibration. Samples were diluted to 0.015 mg mL⁻¹ of starting monomers by adding 50% v/v acetonitrile/water. The oligomers were separated using the same conditions in the LC-UV analysis. For the tandem MS experiment, collision energy for fragmentation was in the range of 10 to 35 eV (lab-frame). The log₂-ratio analysis was performed using MZmine 2 software and plotted in Origin 2020. The symbol color of each species indicates the ratio of peak areas in the MS chromatograms for the sample with DES and without DES (See the equation below).

$$\text{Log}_2\text{-ratio} = \log_2\left(\frac{\text{MS peak area in the DES sample}}{\text{MS peak area in the control}}\right)$$

IR data was obtained on a Thermo Scientific Nicolet 6700 FTIR Spectrometer. Samples were analyzed dry in an Attenuated Total Reflectance (ATR) sample chamber. Spectra were background-subtracted from 650 to 4000 cm⁻¹ and signal-averaged (64 scans per spectrum) with a resolution of 1.928 cm⁻¹. Data processing (normalization and subtraction) was performed using Origin 2020 software.

The NMR spectra were recorded by a Bruker Avance III 600 MHz spectrometer. Dry samples were rehydrated in 500 μL of D₂O. 49.06 mM of potassium hydrogen phthalate (KHP) was added as an internal standard to measure the conversion of glycine. All spectra were processed and plotted by MestReNova 6.1.

Synthesis of standard compounds

For the synthesis of 1g1G dimer standard compound (HO-g-G-COOH), 8 mmol of methyl glycolate, 4 mmol of glycine and 8 mmol of TEACl were mixed in a closed sample vial. The mixture was allowed to react for 4 days at 95°C to complete the reaction. The crude mixture was purified by a semi-preparative SeQuant ZIC-HILIC column (150 × 10 mm, 5 μm particle size, 200 Å). Excess solvent was then removed by a rotary evaporator. To fully remove TEACl, the product was dissolved in 2 ml of 0.1% formic acid solution, and then passed through a column packed with 10 g of AMBERJET™ 1000H cation exchange resin, swelled by 0.1% formic acid solution. Finally, the product was lyophilized before use. A similar method was used to synthesize 1g2G trimer, 1g3G tetramer, and 1g4G pentamer by replacing glycine with diglycine, triglycine and tetraglycine, respectively.

The identification of each standard compound was achieved by ¹H NMR, ¹³C NMR (Figure S11 to S18), and ESI MS in negative-ion mode. The expected [M-H]⁻ value for 1g1G standard compound was 132.0297 Da, 132.0297 Da was observed. Similarly, for 1g2G with expected m/z of 189.0511 Da, 189.0515 Da was observed. 1g3G, expected m/z of 246.0726 Da, was observed as 246.0733 Da. Finally, the expected value of 1g4G is 303.0941 Da. 303.0944 Da was detected.

Synthesis and characterization of deep eutectic solvents composed of different standard compounds

Deep eutectic solvents composed of different standard compounds were prepared by mixing TEACl with glycolic acid, 1g1G, 1g2G, 1g3G, and 1g4G, respectively. Each solid mixture was heated to 95°C for 1 hour in an oven to form a transparent liquid, and naturally cooled to room temperature. For 1g3G and 1g4G, the molar ratio of TEACl: hydrogen bond donor was 2:1 to form deep eutectic solvents when the samples were cooled down to room temperature.

Differential scanning calorimetry (DSC) experiments were conducted on a NETZSCH DSC 200 F3 Maia thermal analyzer under a nitrogen atmosphere. The weights of the samples were around 2.0 mg. The heating rate was 5°C per minute and the range of temperature was -90 to 20°C. All data were processed through the NETZSCH Measurement 4.8.4.

Table S1 Accurate mass table

Oligomers	Theo. Mass (neutral)	Theo. [M-H]⁻	Meas. [M-H]⁻	Error (ppm)
1gnG amide linked oligomers				
1g1G	133.0375	132.0297	132.0297	0.00
1g2G	190.0590	189.0511	189.0515	2.12
1g3G	247.0804	246.0726	246.0734	3.25
1g4G	304.1019	303.0941	303.0943	0.66
1g5G	361.1234	360.1155	360.1161	1.67
1g6G	418.1448	417.1370	417.1374	0.96
mg+nG mixed depsipeptides				
2g1G	191.0430	190.0357	190.0362	3.16
2g2G	248.0645	247.0572	247.0565	-2.83
2g3G	305.0859	304.0786	304.0782	-1.32
2g5G	419.1288	418.1216	418.1218	1.91
3g6G	534.1558	533.1485	533.1491	1.13
3g7G	591.1772	590.1700	590.1693	-1.19
3g8G	648.1987	647.1914	647.1897	-2.63
4g7G	649.1827	648.1755	648.1765	1.54

2. LC-MS analysis of the g/G depsipeptides

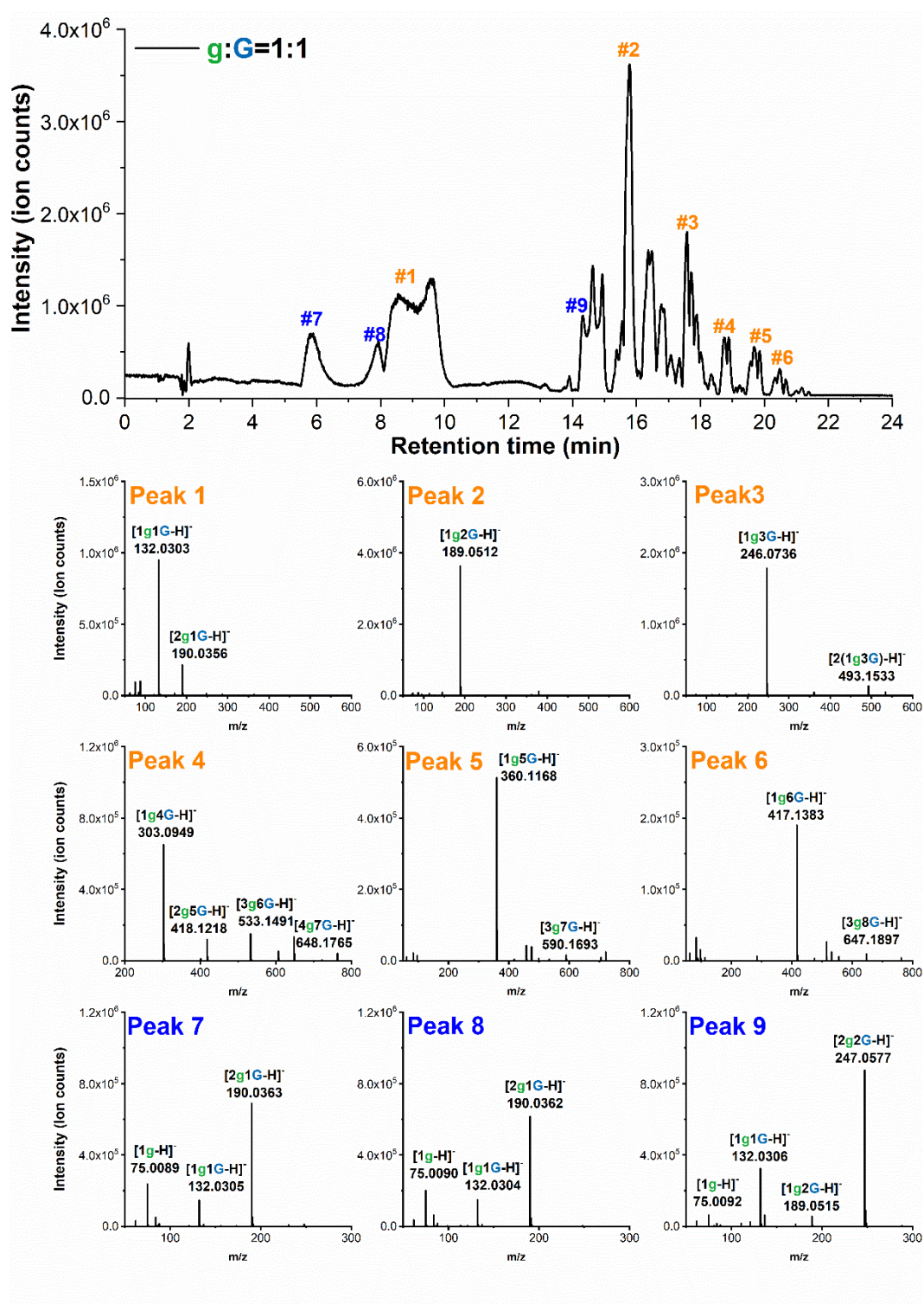


Fig. S1 LC-MS analysis of the control experiment without TEACl. The molar ratio of g and G is 1:1. The sample was prepared by heating at 95°C for 7 days.

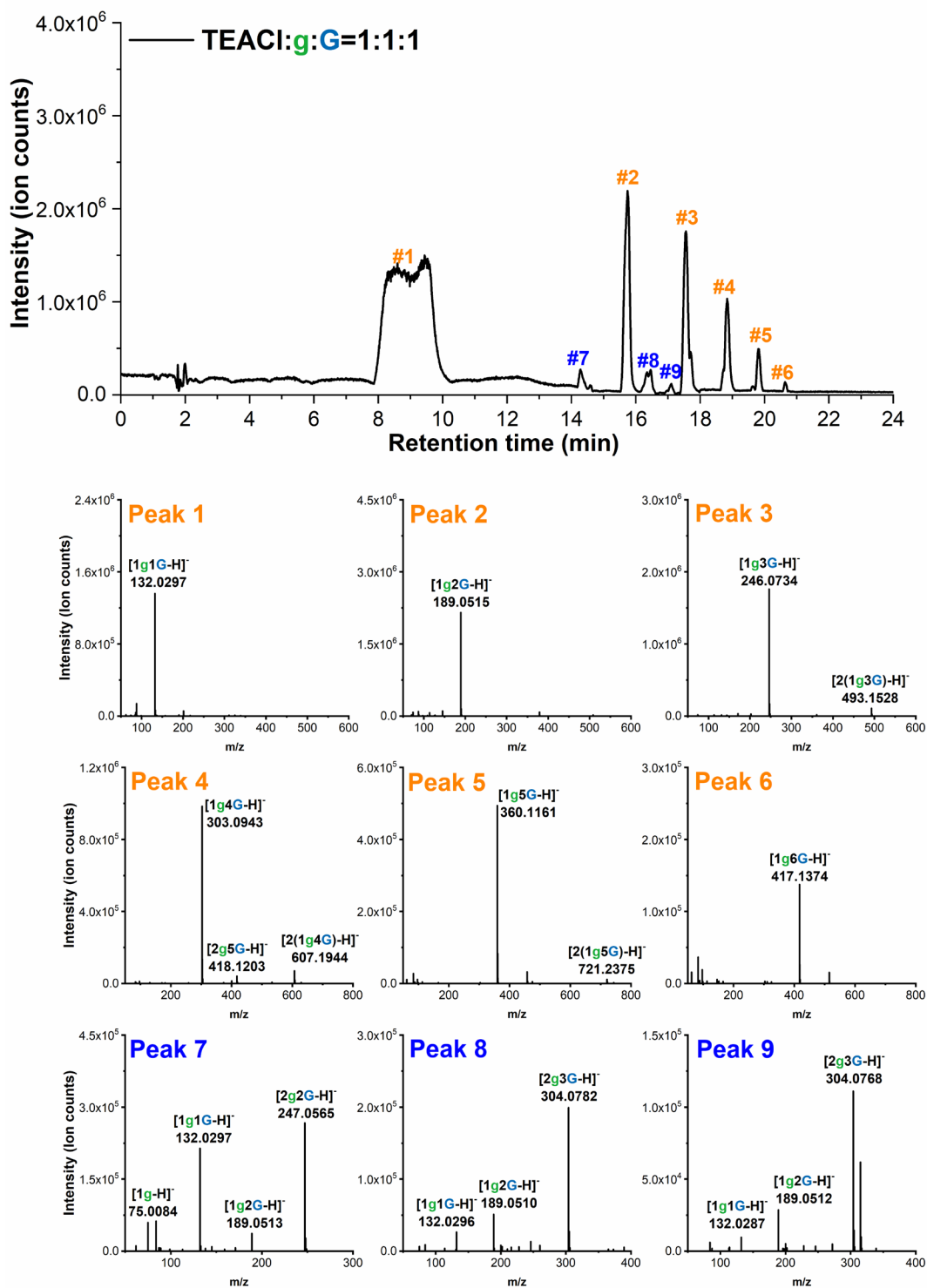


Fig. S2 LC-MS analysis of the TEACl/g/G mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 7 days.

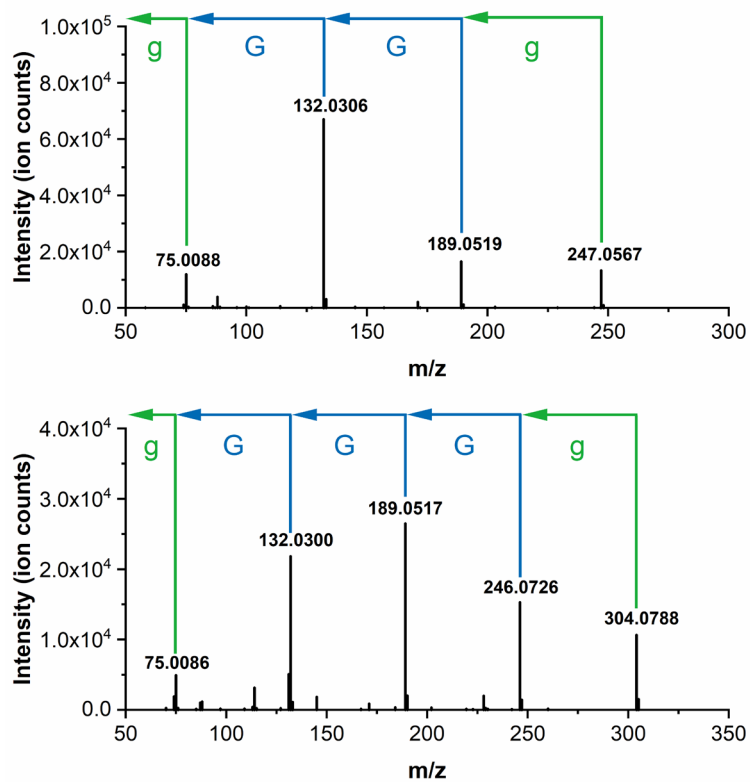


Fig. S3 Tandem MS sequencing analysis of the minor products from the mixture of TEACl/g/G (molar ratio of 1:1:1). The reaction was carried out at 95°C for 7 days. The sequence of each oligomer was determined by the α -cleavages.

3. The effects of salts on the formation of depsipeptides

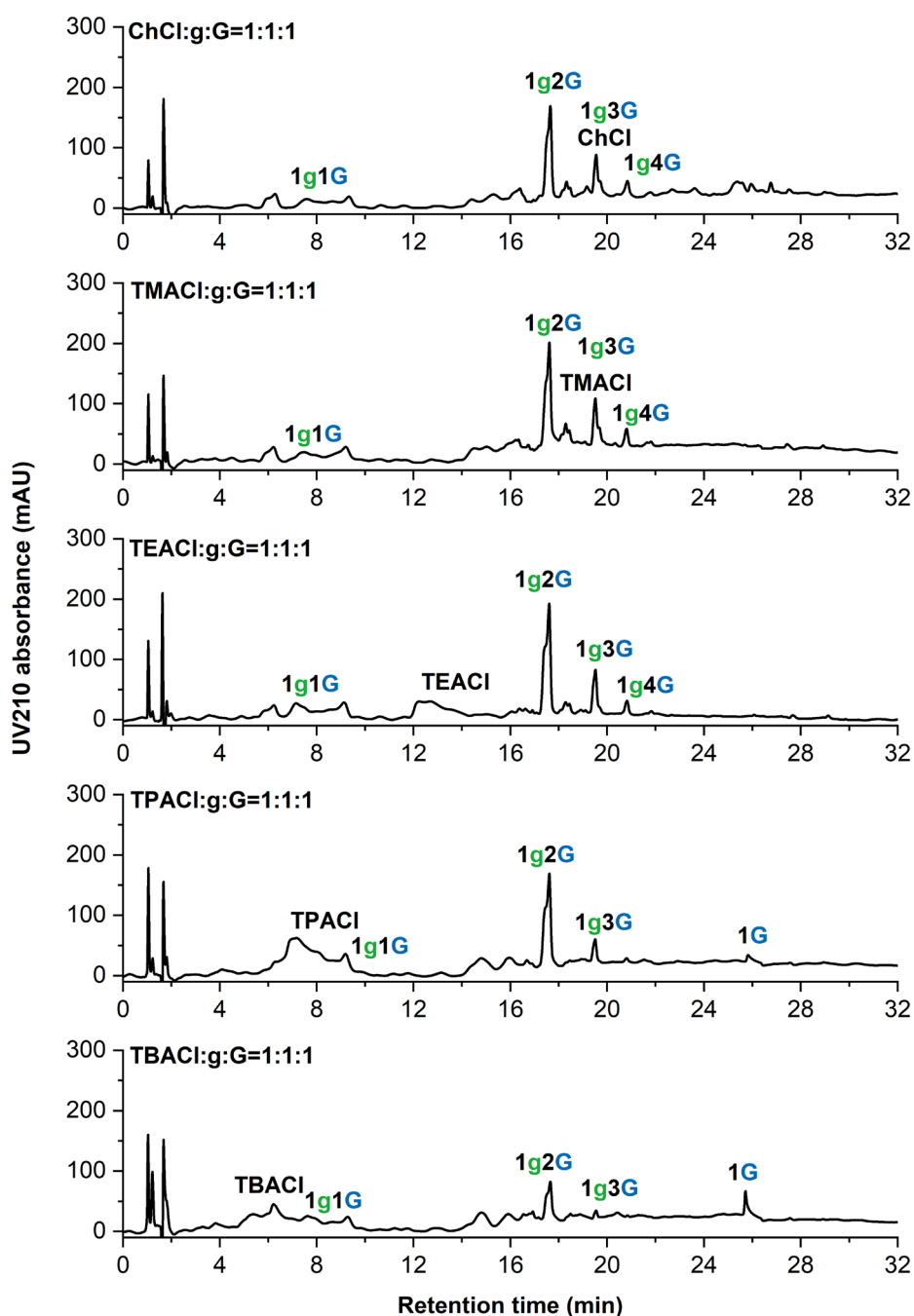


Fig. S4 LC-UV chromatograms of the reaction mixtures with different quaternary ammonium salts such as choline chloride (ChCl), tetramethyl ammonium chloride (TMACl), tetraethyl ammonium chloride (TEACl), tetrapropyl ammonium chloride (TPACl) and tetrabutyl ammonium chloride (TBACl). The reaction mixtures were prepared by drying at 95°C for 7 days. The peaks of ChCl and TMACl overlap with 1g3G. TPACl and TBACl co-elute with 1g1G.

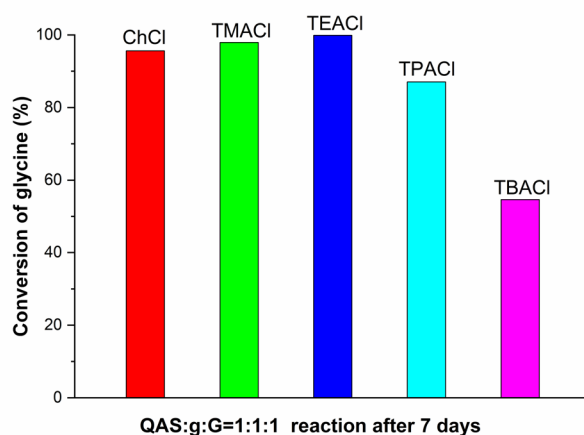


Fig. S5 Conversion of glycine with different quaternary ammonium salts (QAS). The reaction mixtures were prepared by drying at 95°C for 7 days. The conversion of glycine was determined by ^1H NMR. The conversion of glycine was similar in the DESs of ChCl, TMACl, and TEACl. On the other hand, we observed a decreased conversion when using TPACl and TBACl. The slow polymerization rate of glycine in the DESs of TPACl and TBACl may come from the poor solubility of glycine in nonpolar DESs.

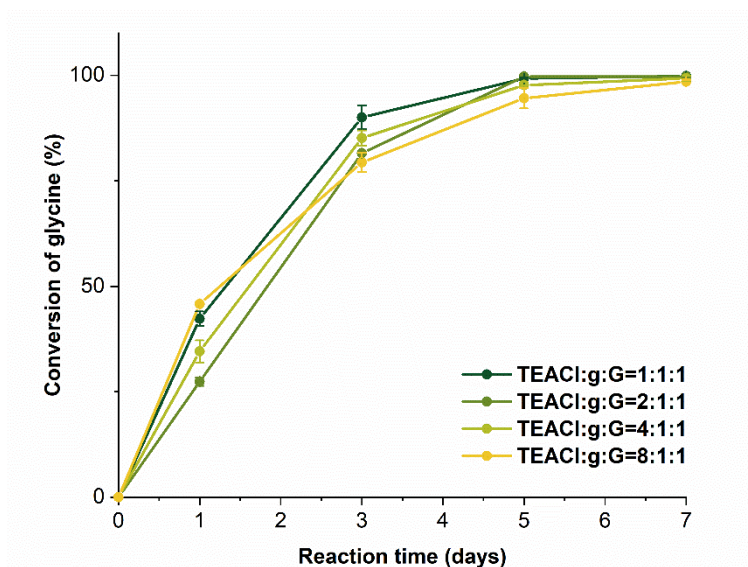


Fig. S6 Conversion of glycine with different contents of TEACl at 95°C. The conversion of glycine was determined by ^1H NMR.

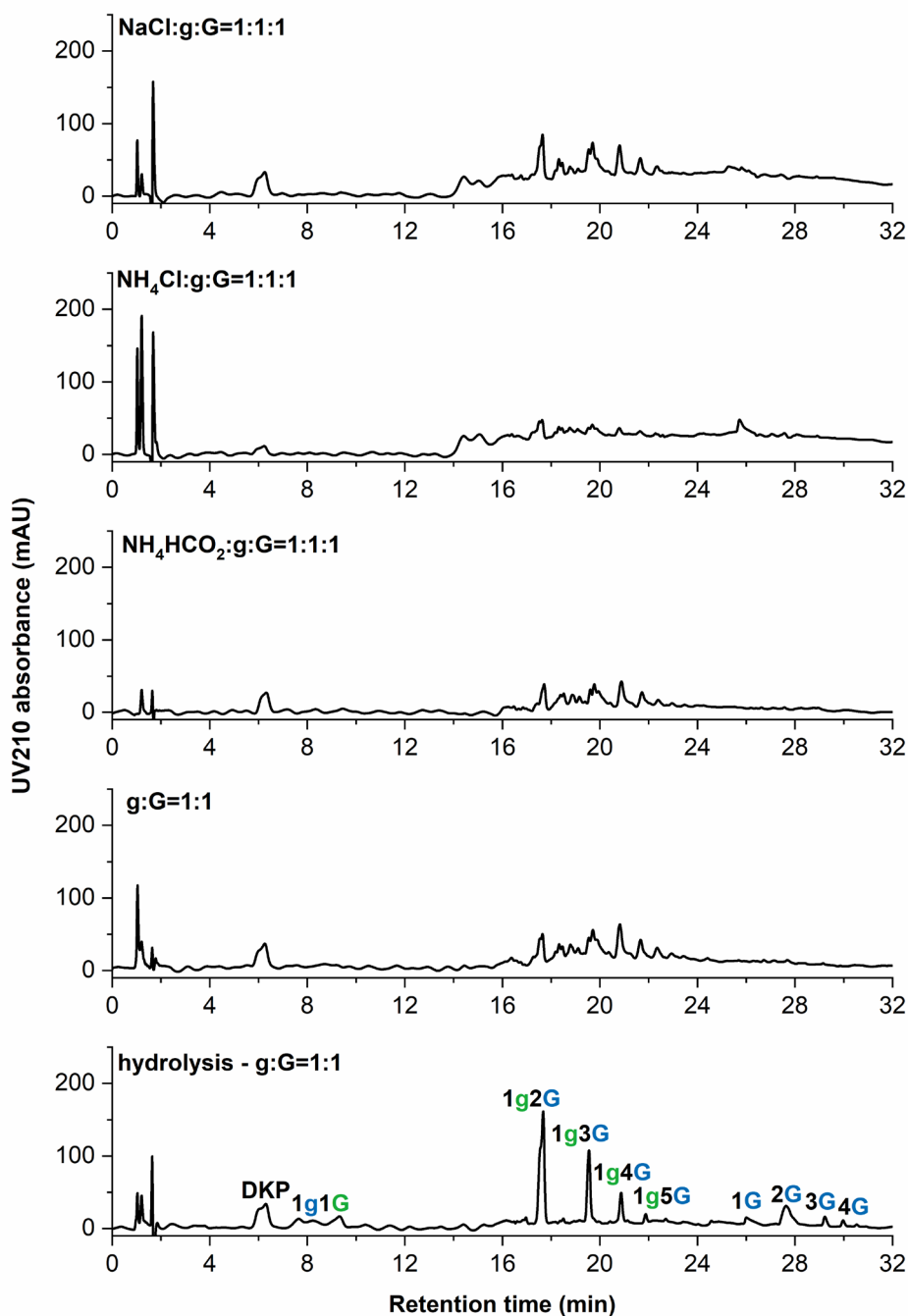


Fig. S7 LC-UV chromatograms of the reaction mixtures with different common salts. The last two chromatograms represent the control experiment without adding TEACl to form DES. The bottom chromatogram shows the 1gnG oligomer series after the samples was treated by hydrolysis of ester for 2 days at 95 °C. All reaction mixtures were prepared by drying at 95°C for 7 days.

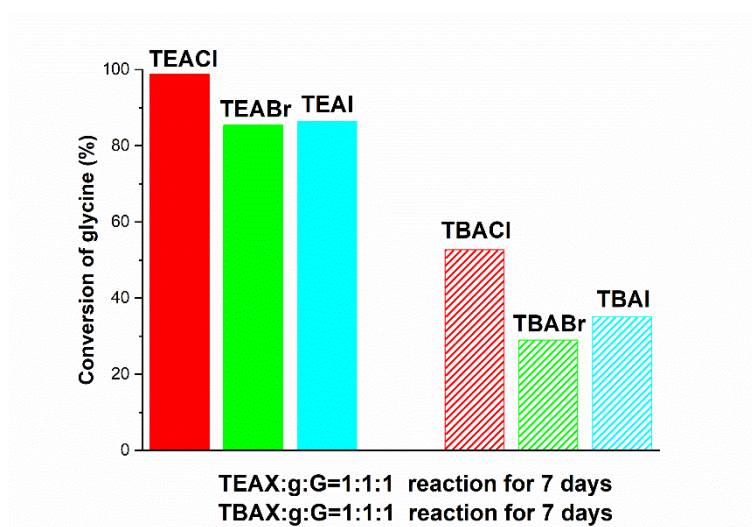


Fig. S8 Conversion of glycine using quaternary ammonium salts with different anions. The reaction mixtures were prepared by drying at 95°C for 7 days. The conversion of glycine was determined by ^1H NMR. The salts used were tetraethyl ammonium chloride (TEACl), tetraethyl ammonium bromide (TEABr), tetraethyl ammonium iodide (TEAI), tetrabutyl ammonium chloride (TBACl), tetrabutyl ammonium bromide (TBABr) and tetrabutyl ammonium iodide (TBAI). Similar to the previous results, the reaction rate of glycine decreased when the alkyl chain length of the salts increased. For the salts using bromide and iodide as anions, we observed a slight decrease in glycine conversion.

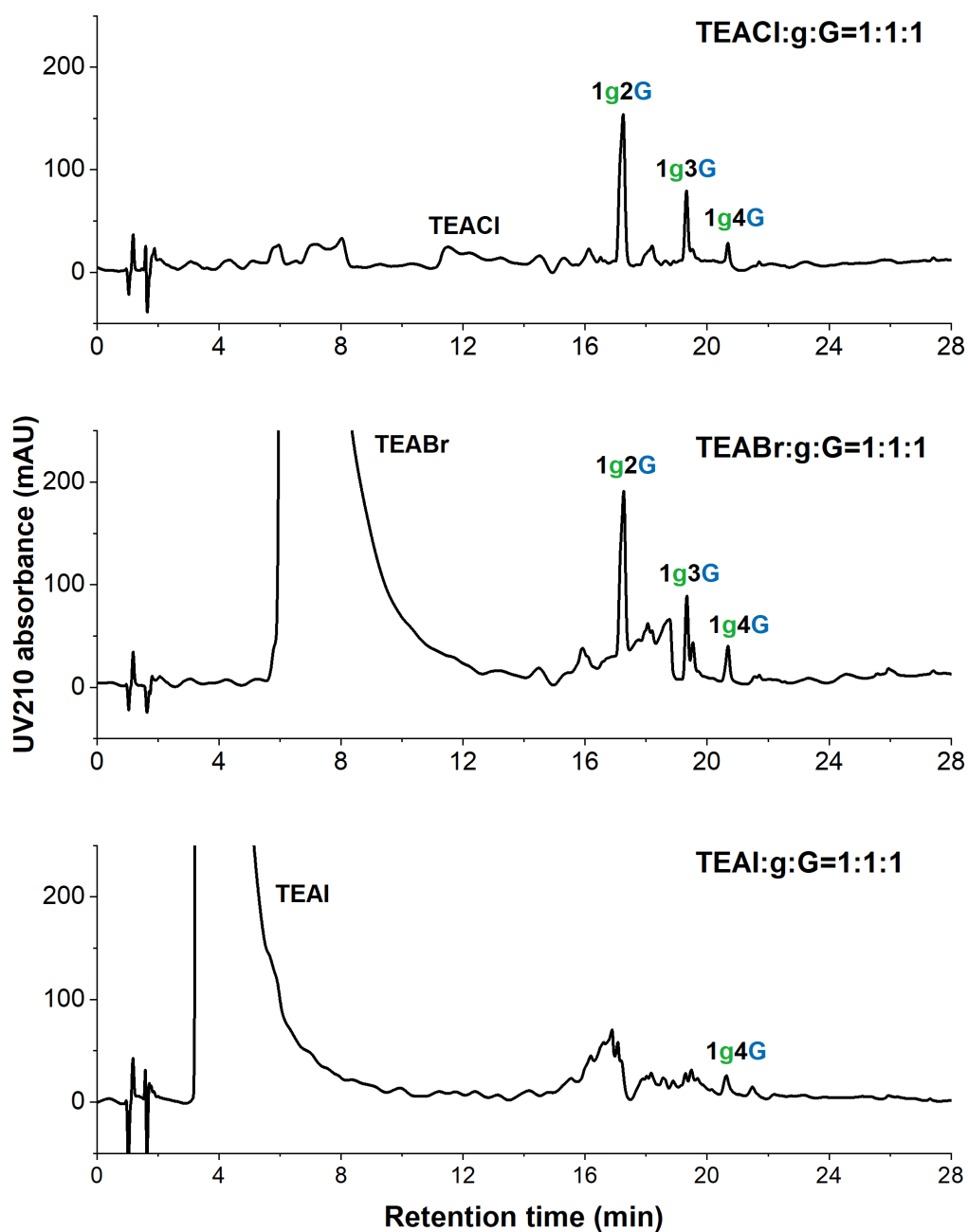


Fig. S9 LC-UV chromatograms of the reaction mixtures using quaternary ammonium salts with different anions. The reaction mixtures were prepared by drying at 95°C for 7 days. Both TEABr and TEAI showed strong UV absorbance than TEACl and the depsipeptides.

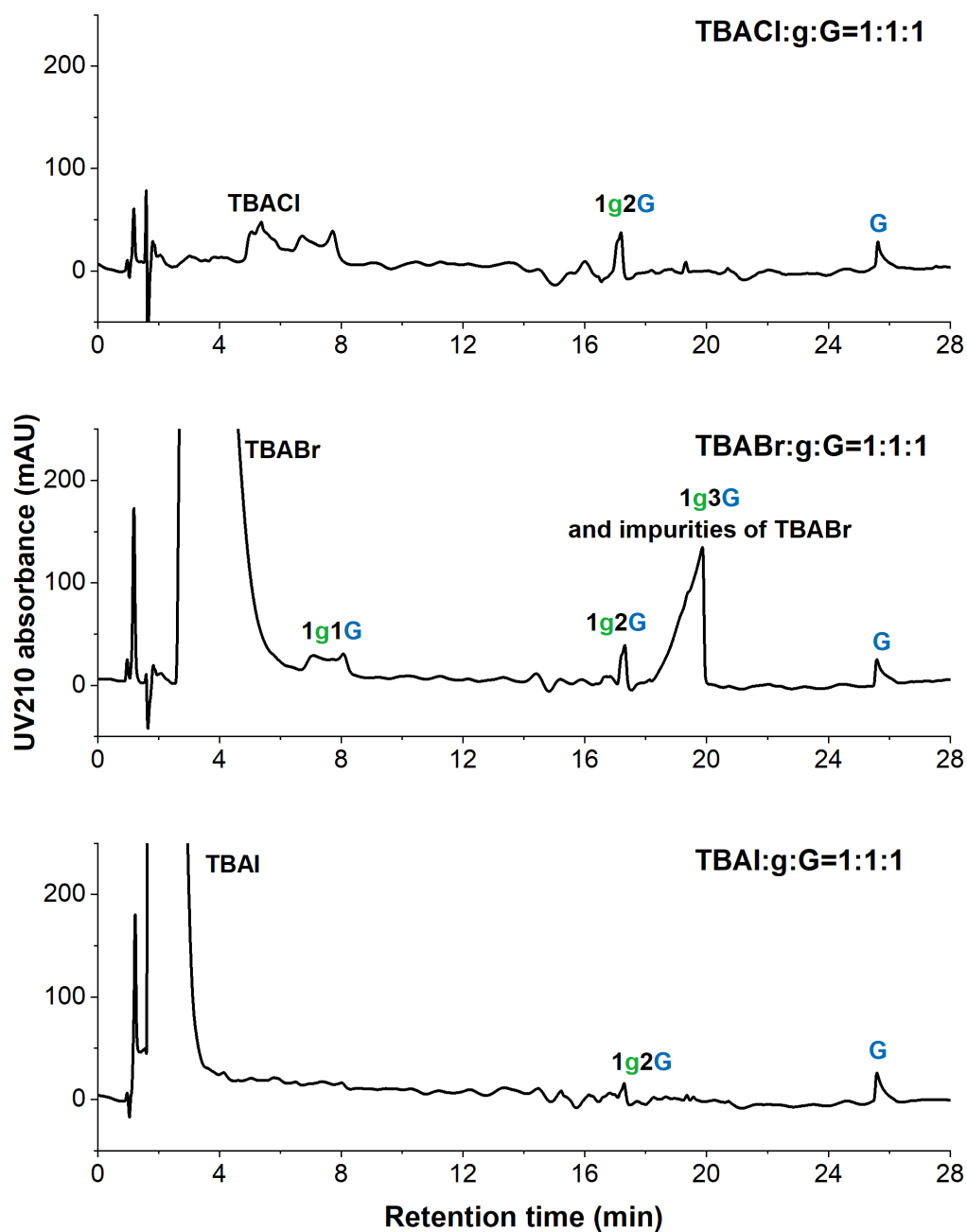


Fig. S10 LC-UV chromatograms of the reaction mixtures using quaternary ammonium salts with different anions. The reaction mixtures were prepared by drying at 95°C for 7 days. The conversion of glycine was determined by ^1H NMR. Both TBABr and TBAI showed strong UV absorbance than TBACl and the depsipeptides. In addition, we found a impurity of TBABr co-eluted with 1g3G.

4. Characterization of the 1g1G standard compounds

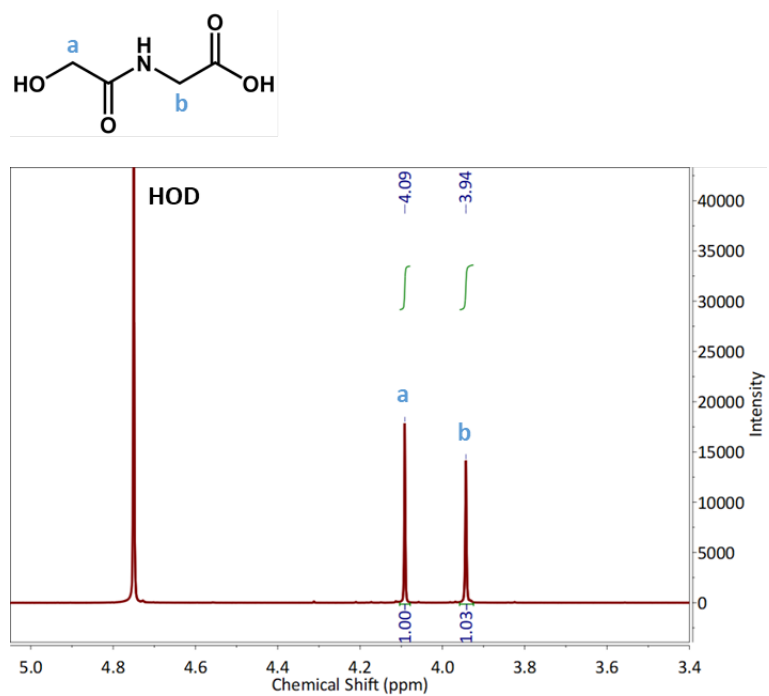


Fig. S11 ¹H NMR of 1g1G standard compound.

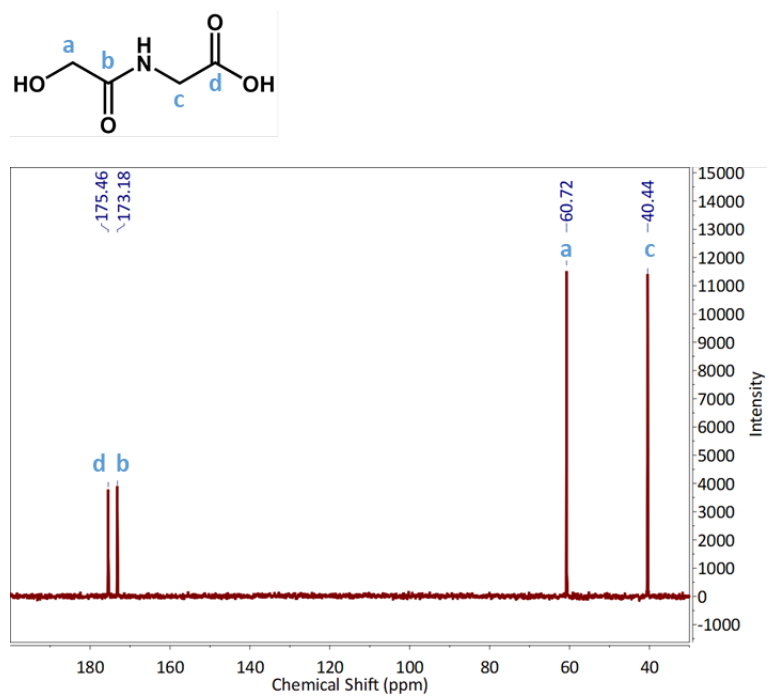


Fig. S12 ¹³C NMR of 1g1G standard compound.

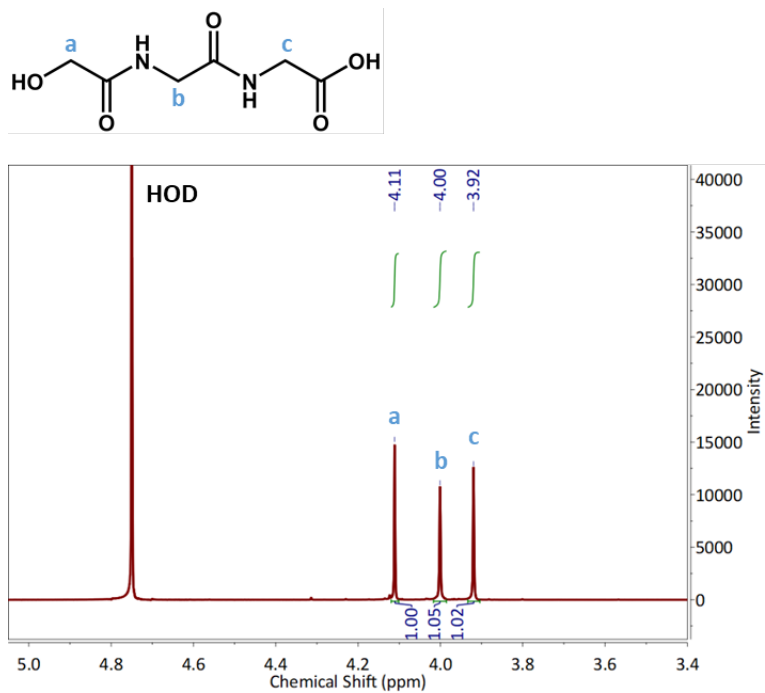


Fig. S13 ¹H NMR of 1g2G standard compound.

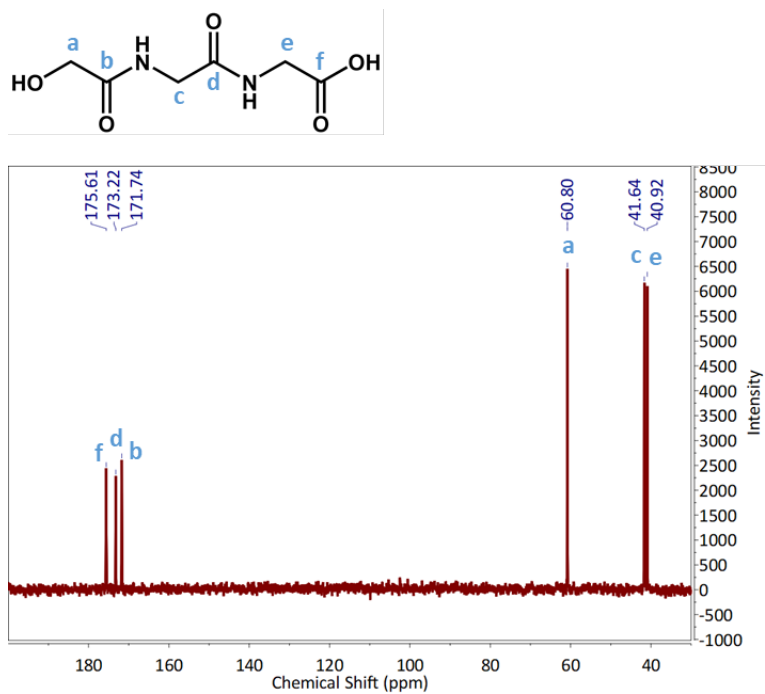


Fig. S14 ¹³C NMR of 1g2G standard compound.

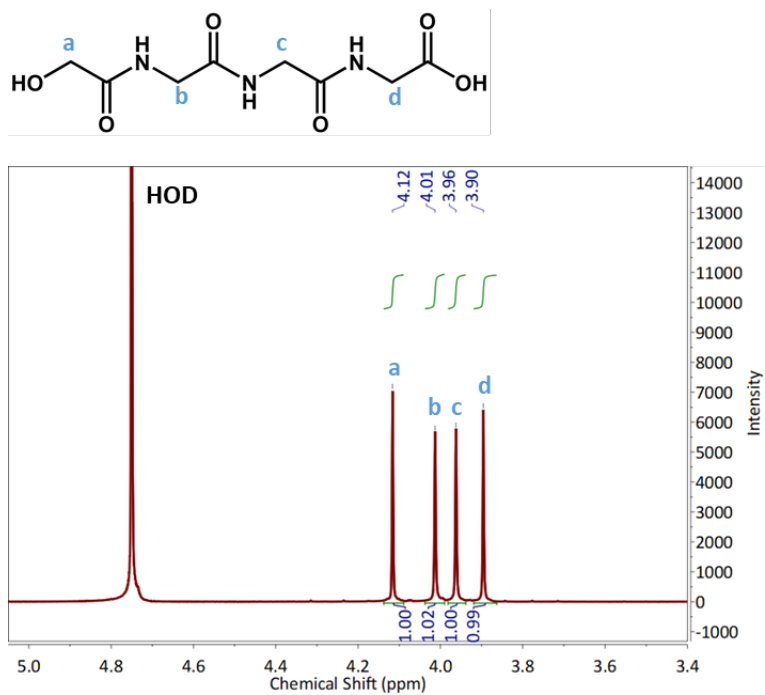


Fig. S15 ^1H NMR of 1g3G standard compound.

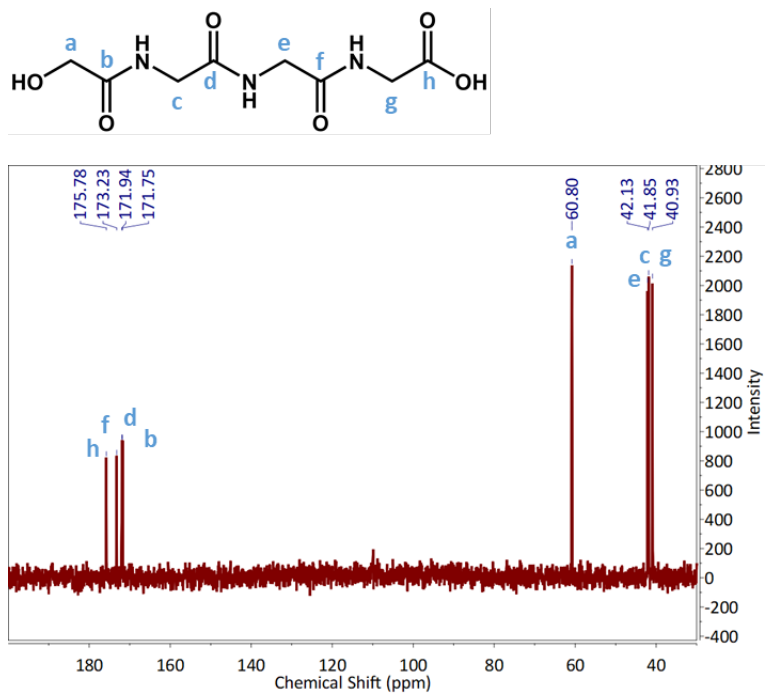


Fig. S16 ^{13}C NMR of 1g3G standard compound.

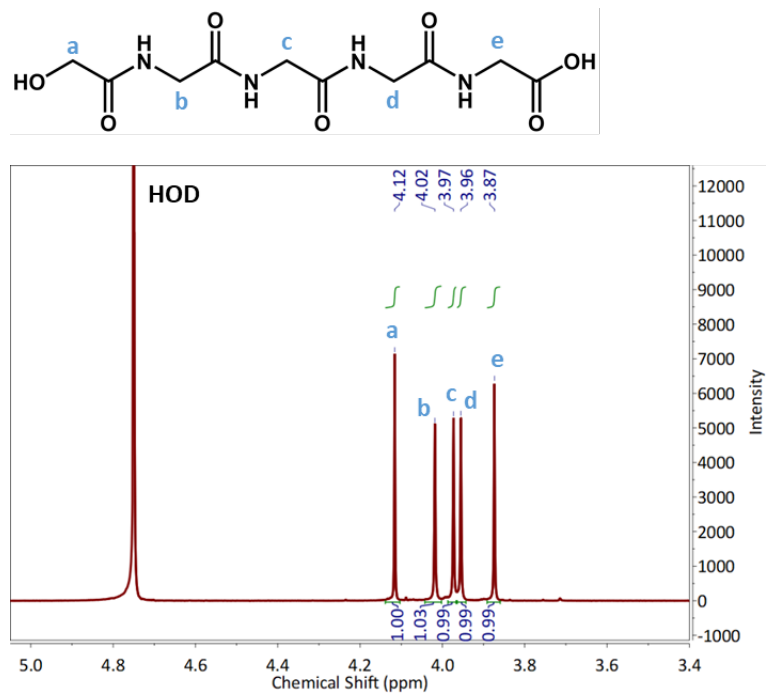


Fig. S17 ¹H NMR of 1g4G standard compound.

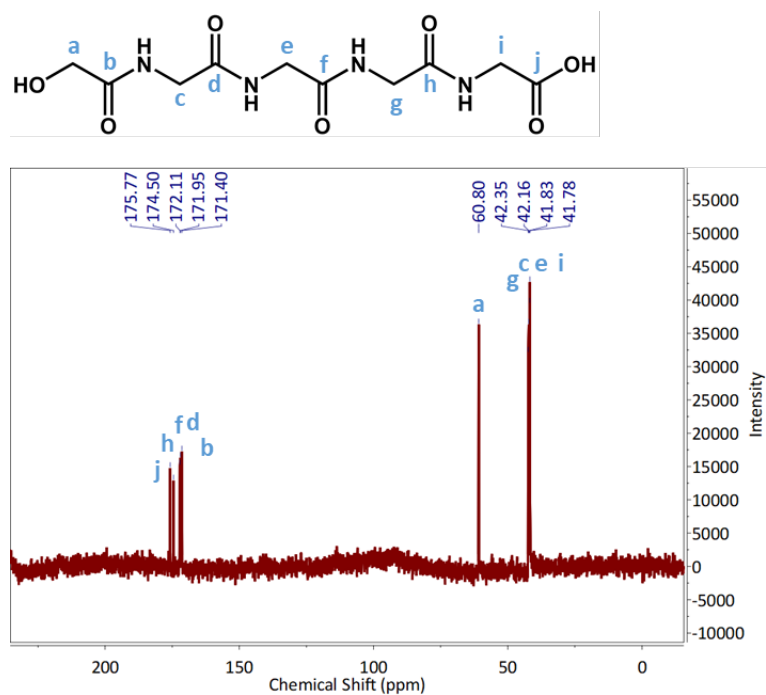


Fig. S18 ¹³C NMR of 1g4G standard compound.

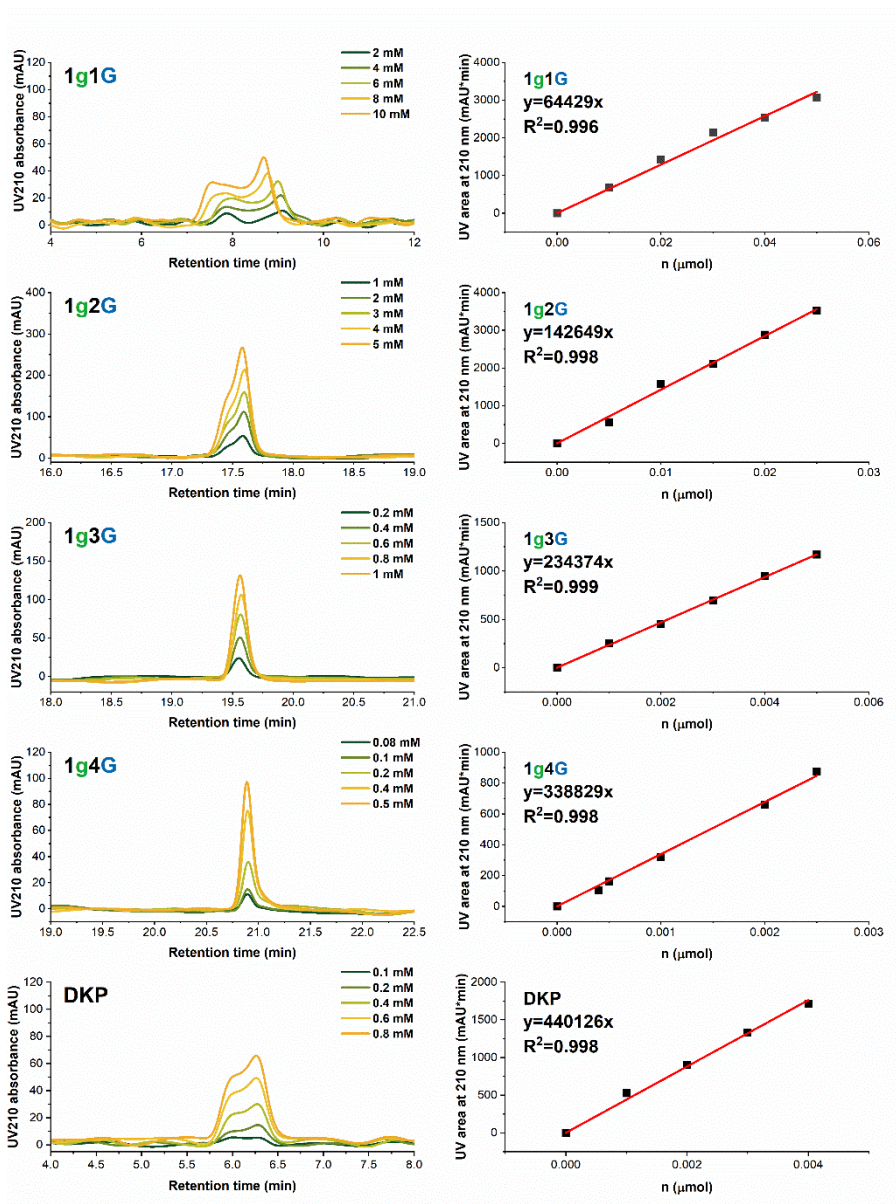


Fig. S19 UV traces and calibration curves of 1g1G, 1g2G, 1g3G, 1g4G and DKP.

Table S2 UV calibration factors at 210 nm for g/G oligomers

Compound	UV calibration factor, k [$\mu\text{mole}/(\text{mAU}\cdot\text{min})$]
1g1G	1.55×10^{-5}
1g2G	7.01×10^{-6}
1g3G	4.27×10^{-6}
1g4G	2.95×10^{-6}
1g5G	2.36×10^{-6}
1g6G	1.94×10^{-6}
DKP	2.27×10^{-6}

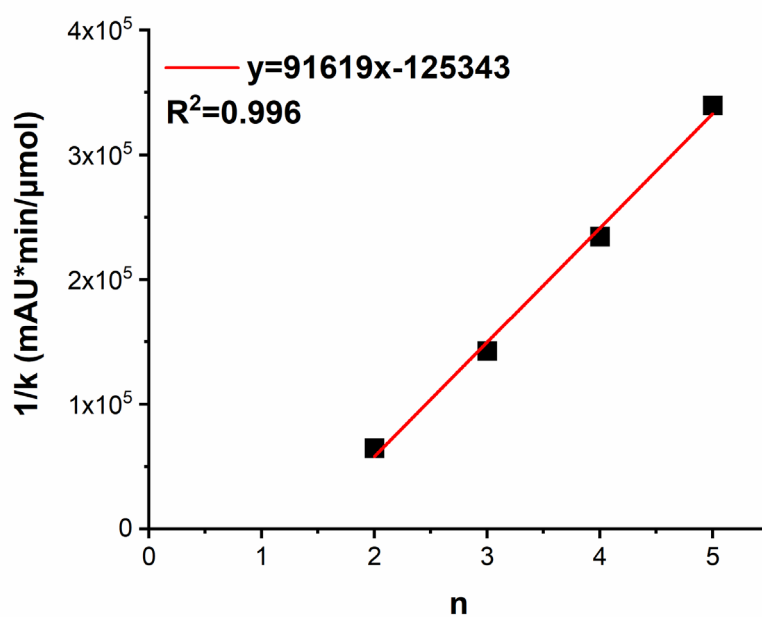


Fig. S20 Plot of UV extinction coefficients vs chain length of the 1gnG oligomers. The UV extinction coefficients were found to be proportional to the chain length of the 1gnG series.

5. Characterization of deep eutectic solvents

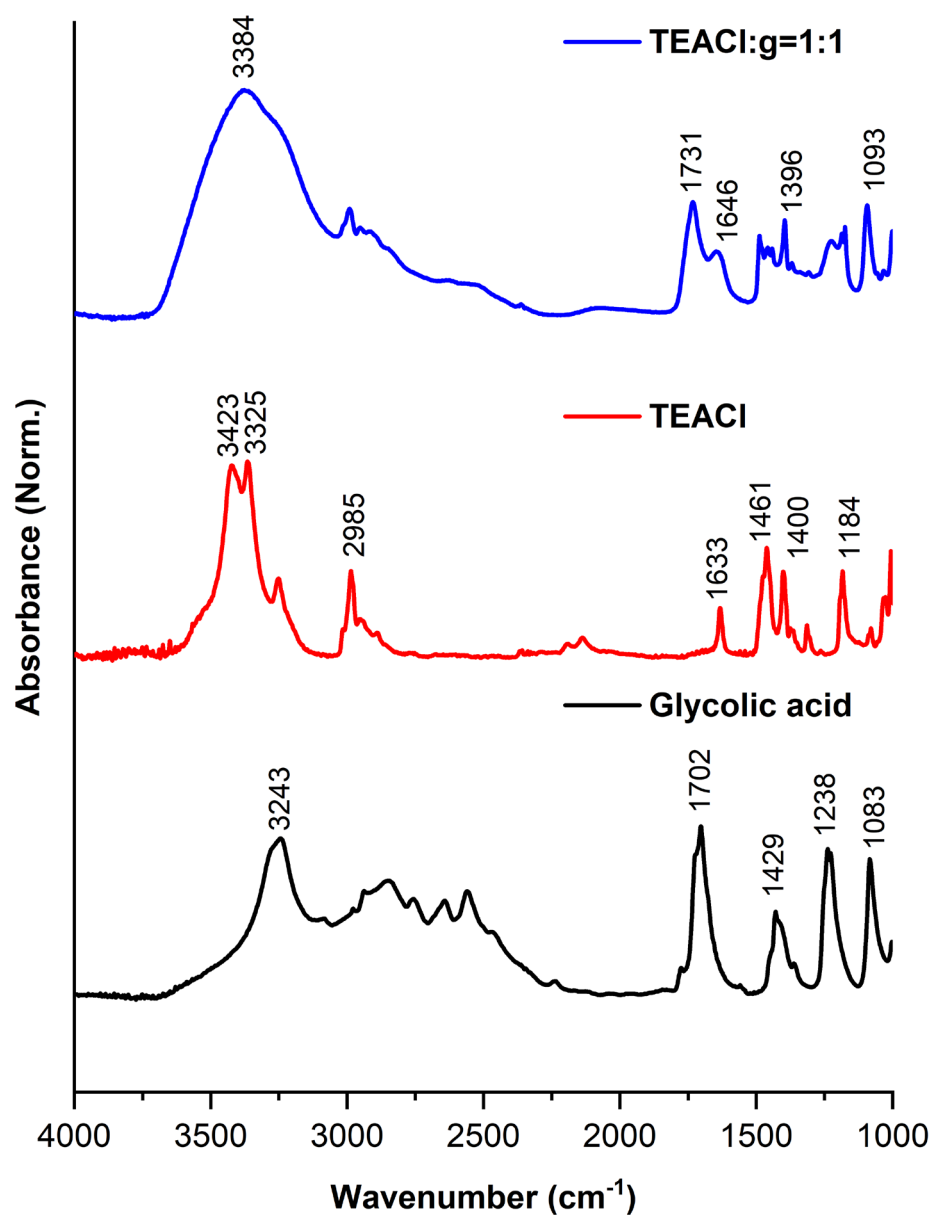


Fig. S21 FT-IR spectra of glycolic acid (black), TEACl (red), and the DES formed by 1:1 mixture of TEACl and glycolic acid (blue).

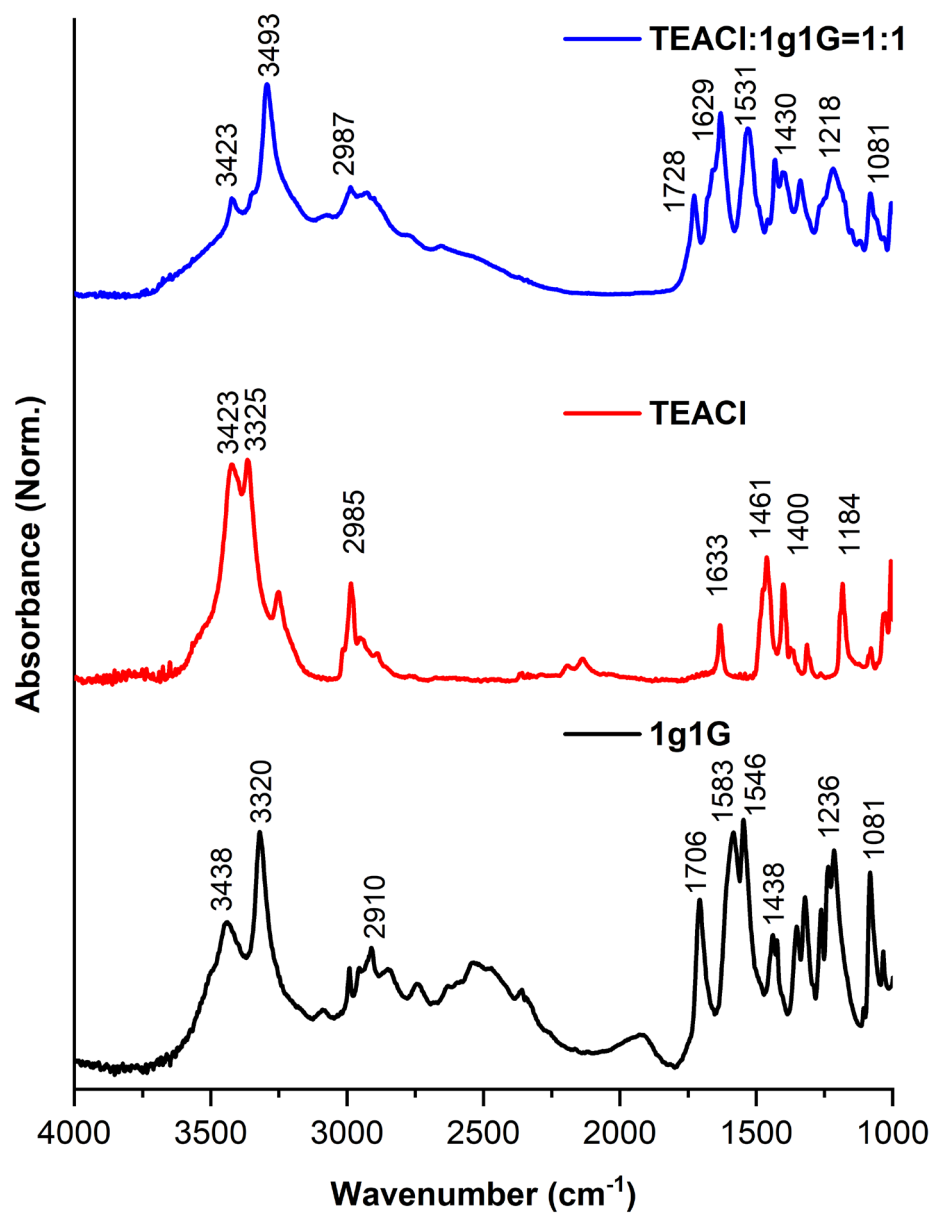


Fig. S22 FT-IR spectra of 1g1G dimer (black), TEACl (red), and the DES formed by 1:1 mixture of TEACl and 1g1G (blue).

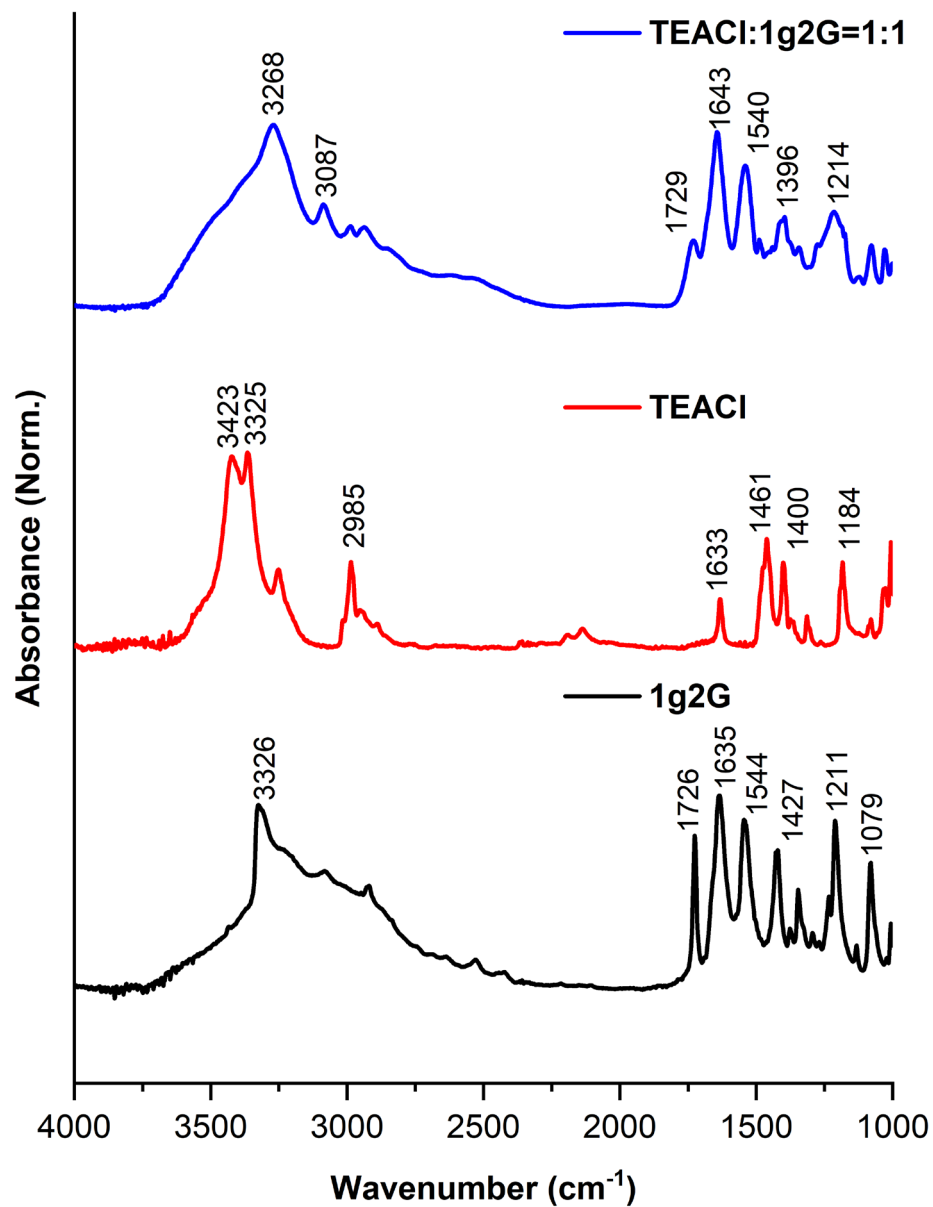


Fig. S23 FT-IR spectra of 1g2G trimer (black), TEACl (red), and the DES formed by 1:1 mixture of TEACl and 1g2G (blue).

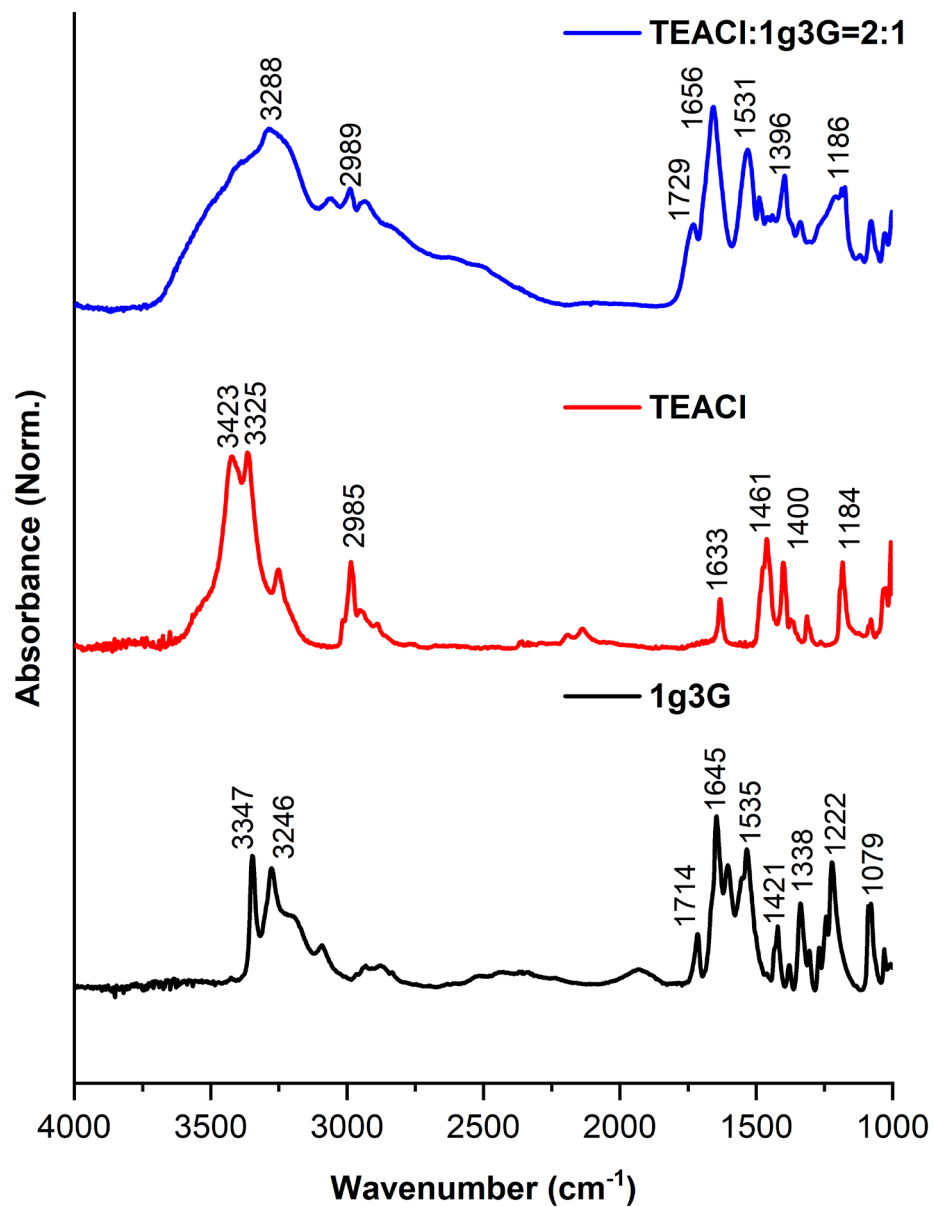


Fig. S24 FT-IR spectra of 1g3G tetramer (black), TEACl (red), and the DES formed by 2:1 mixture of TEACl and 1g3G (blue).

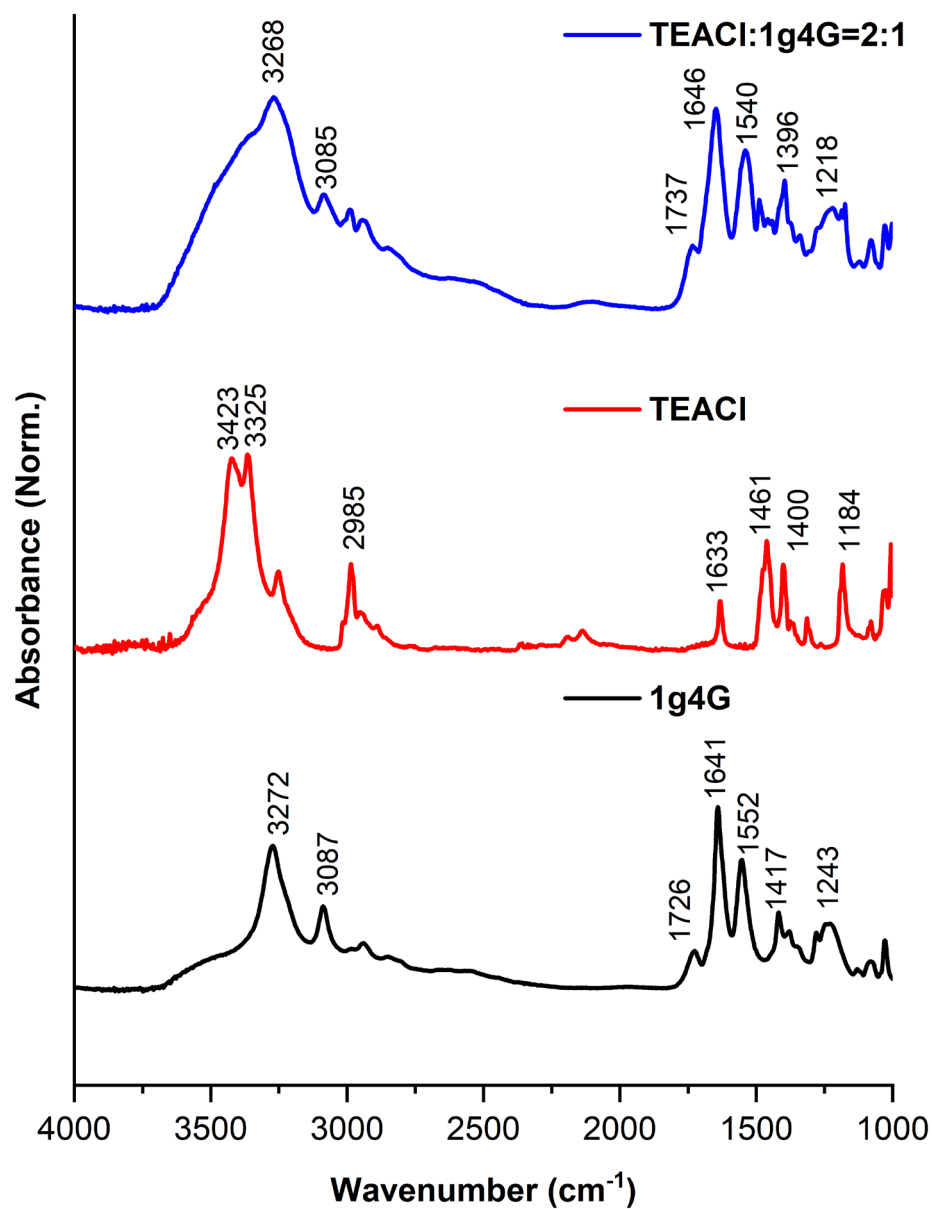


Fig. S25 FT-IR spectra of 1g4G pentamer (black), TEACl (red), and the DES formed by 2:1 mixture of TEACl and 1g4G (blue).

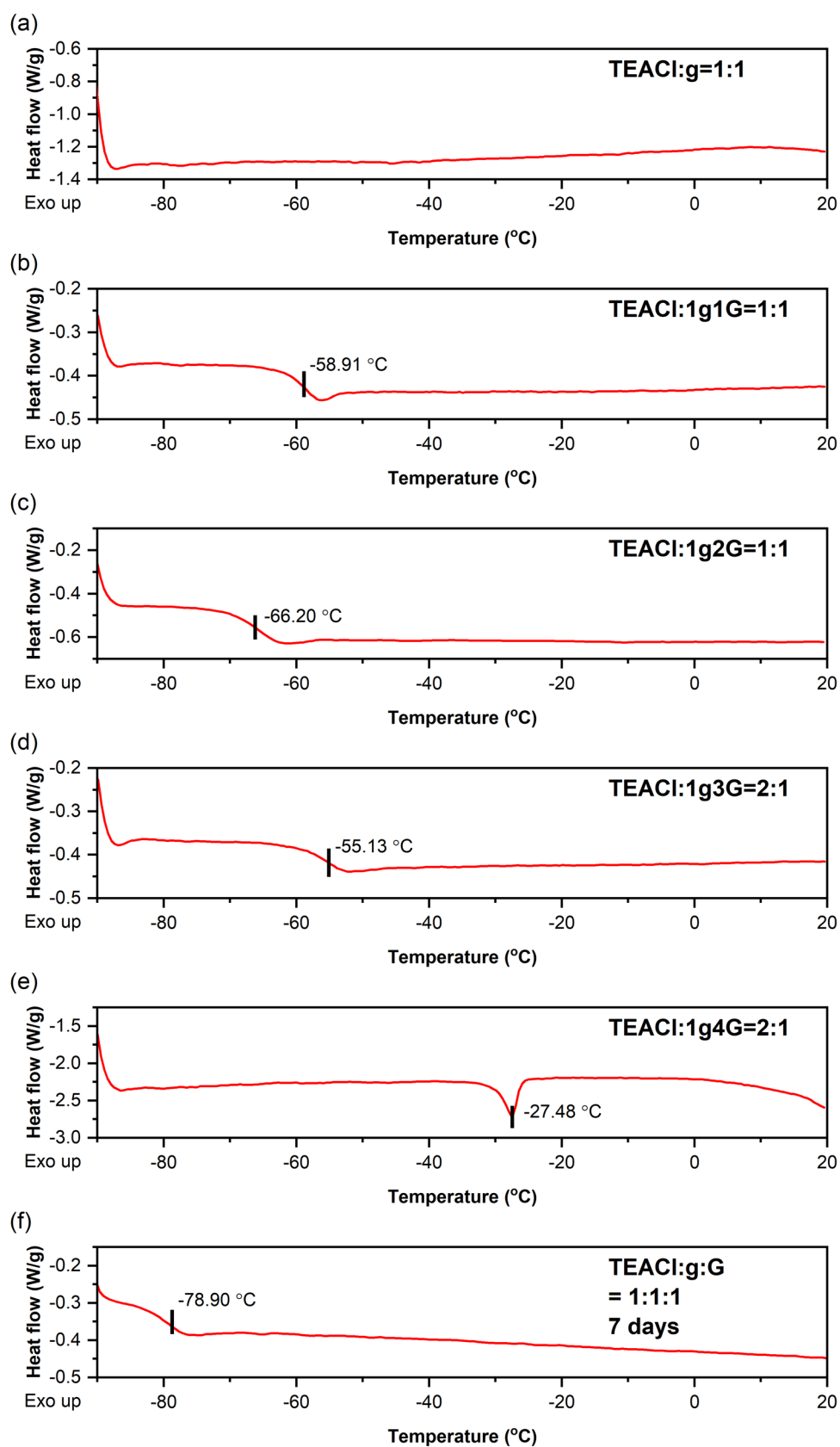


Fig. S26 DSC traces for DESs. The mixtures of TEACl with a) glycolic acid, b) 1g1G, c) 1g2G, d) 1g3G, e) 1g4G. f) The reaction mixture of TEACl/g/G.

6. Oligomer distributions of depsipeptides

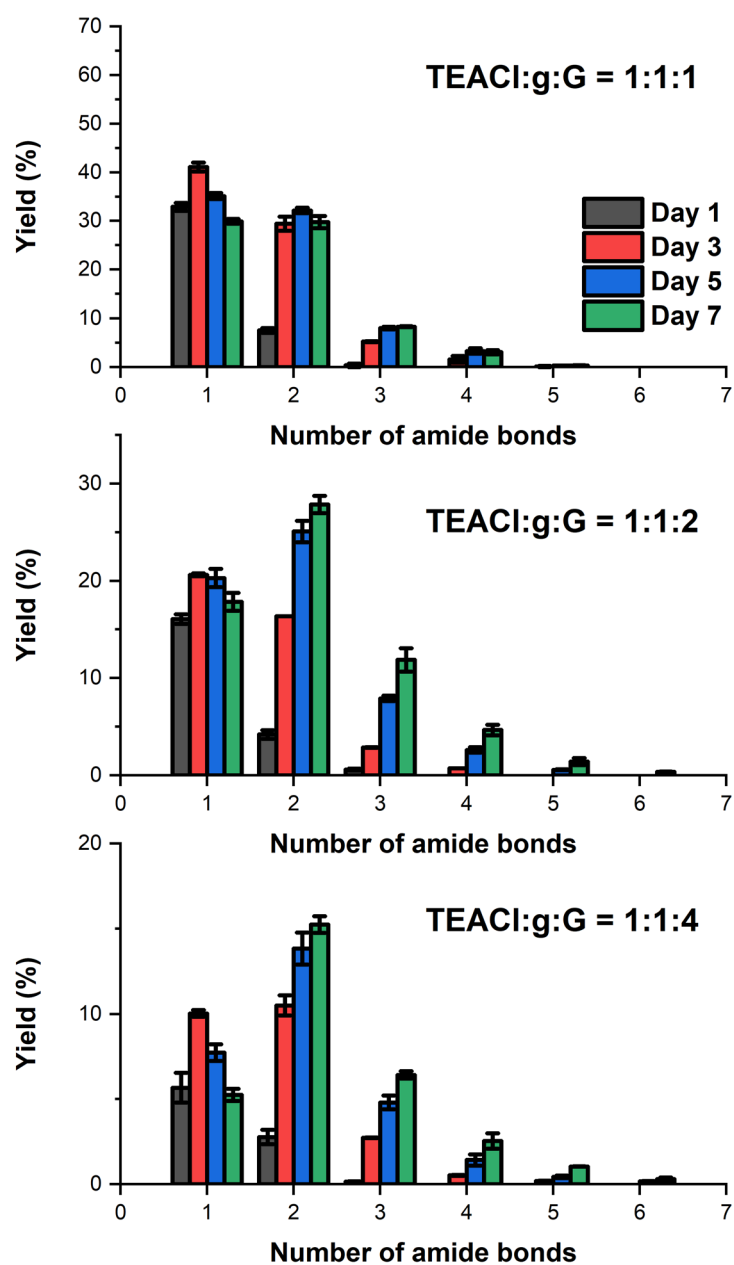


Fig. S27 Growth of the 1gnG oligomers with various contents of glycine from the mixtures of TEACl/g/G (molar ratio of 1:1:1 to 1:1:4).

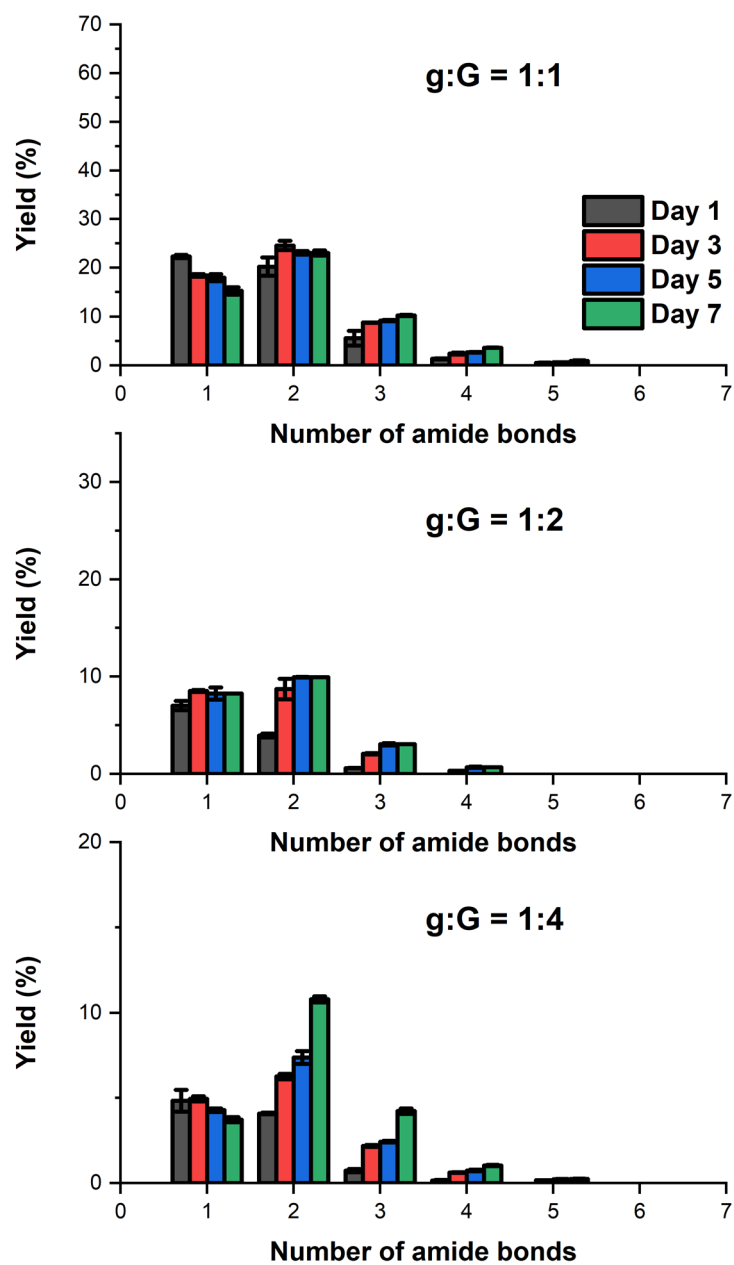


Fig. S28 Growth of the 1nG oligomers in the control experiments without TEACl. Each sample was hydrolyzed at 95°C for two days to reveal the 1nG oligomers with peptide backbones.

7. The yield of diketopiperazine

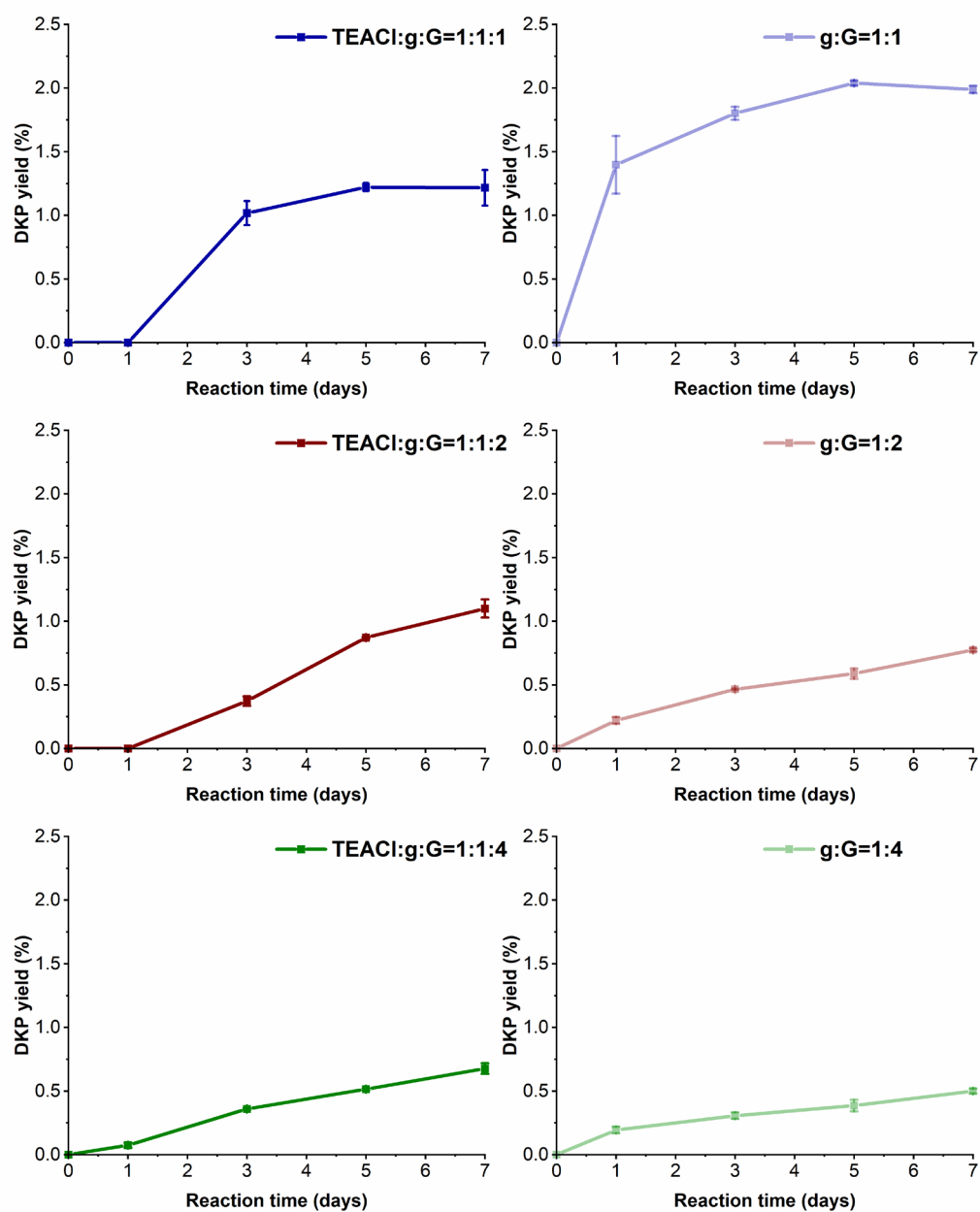


Fig. S29 Yields of glycine diketopiperazine (DKP) with various contents of glycine with or without the addition of TEACl.

8. The reaction of other amino acids in DES

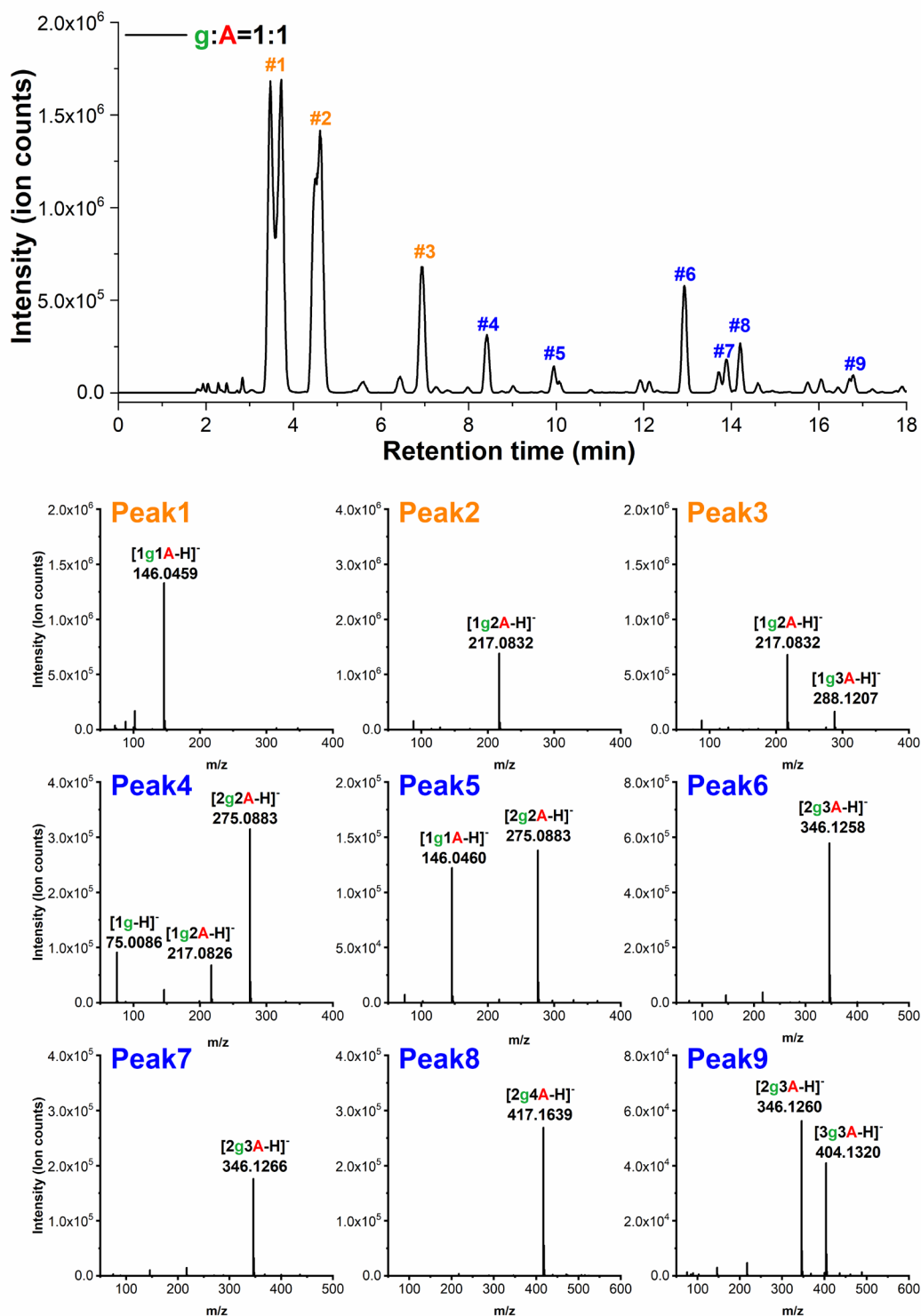


Fig. S30 LC-MS analysis of the g/L-alanine (A) mixture (molar ratio of 1:1). The sample was prepared by heating at 95°C for 5 days.

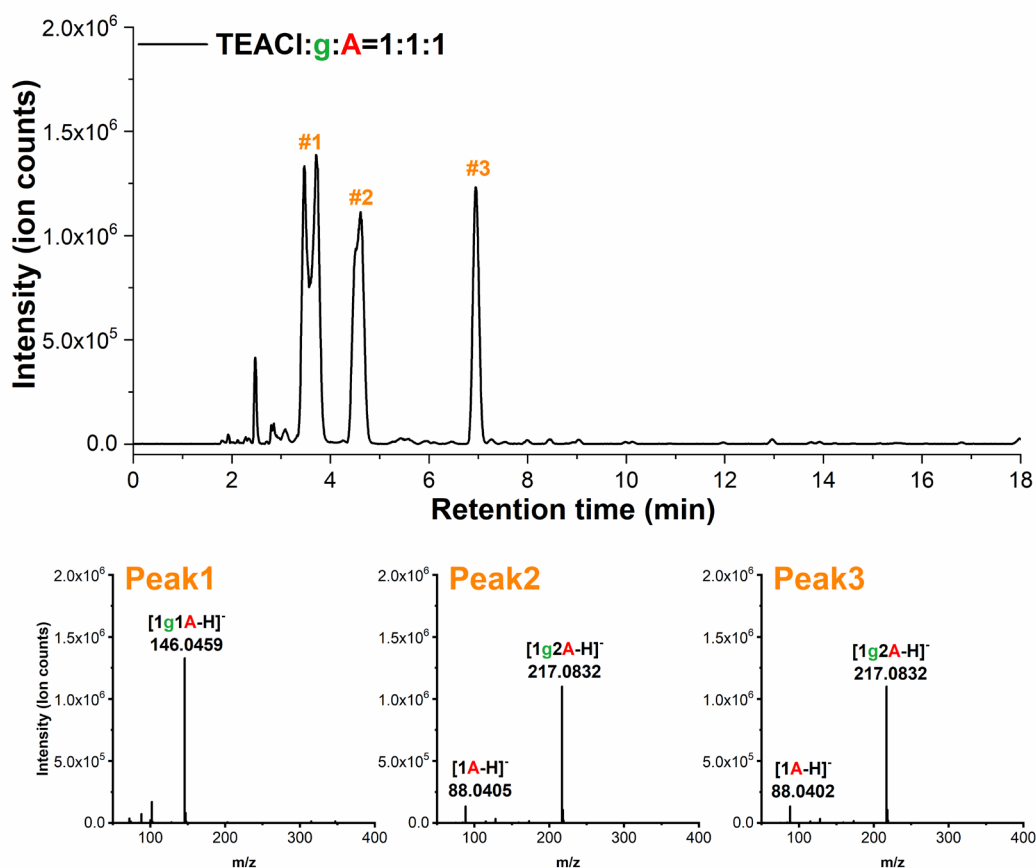


Fig. S31 LC-MS analysis of the TEACl/g/L-alanine (A) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 5 days. Although most of the samples for LC-MS analysis were synthesized for 7 days, we used a shorter reaction time for alanine to demonstrate the enhanced formation of amino acid-enriched oligomers also occurs at different stages of the reaction.

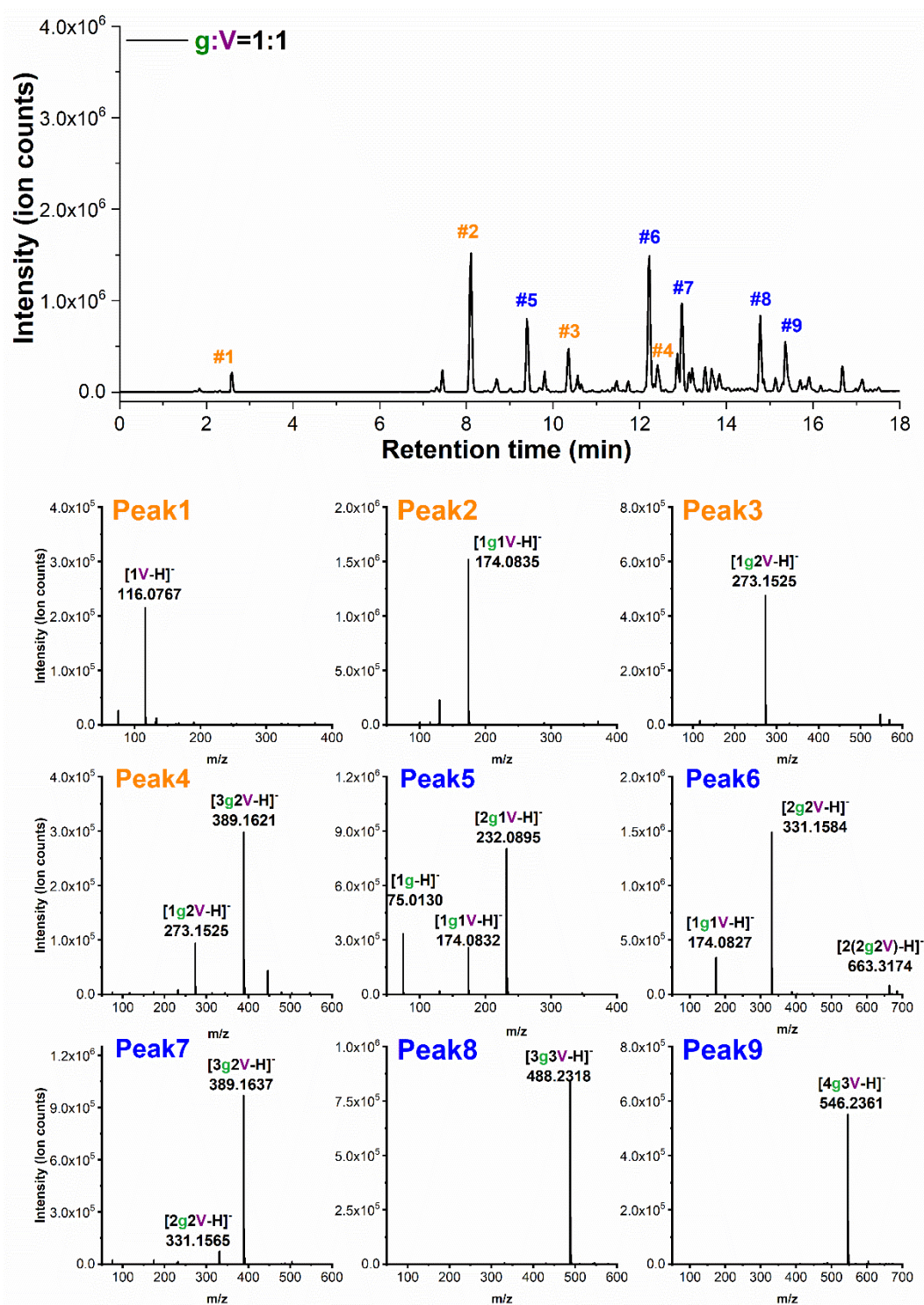


Fig. S32 LC-MS analysis of the g/L-valine (V) mixture (molar ratio of 1:1). The sample was prepared by heating at 120°C for 1 day.

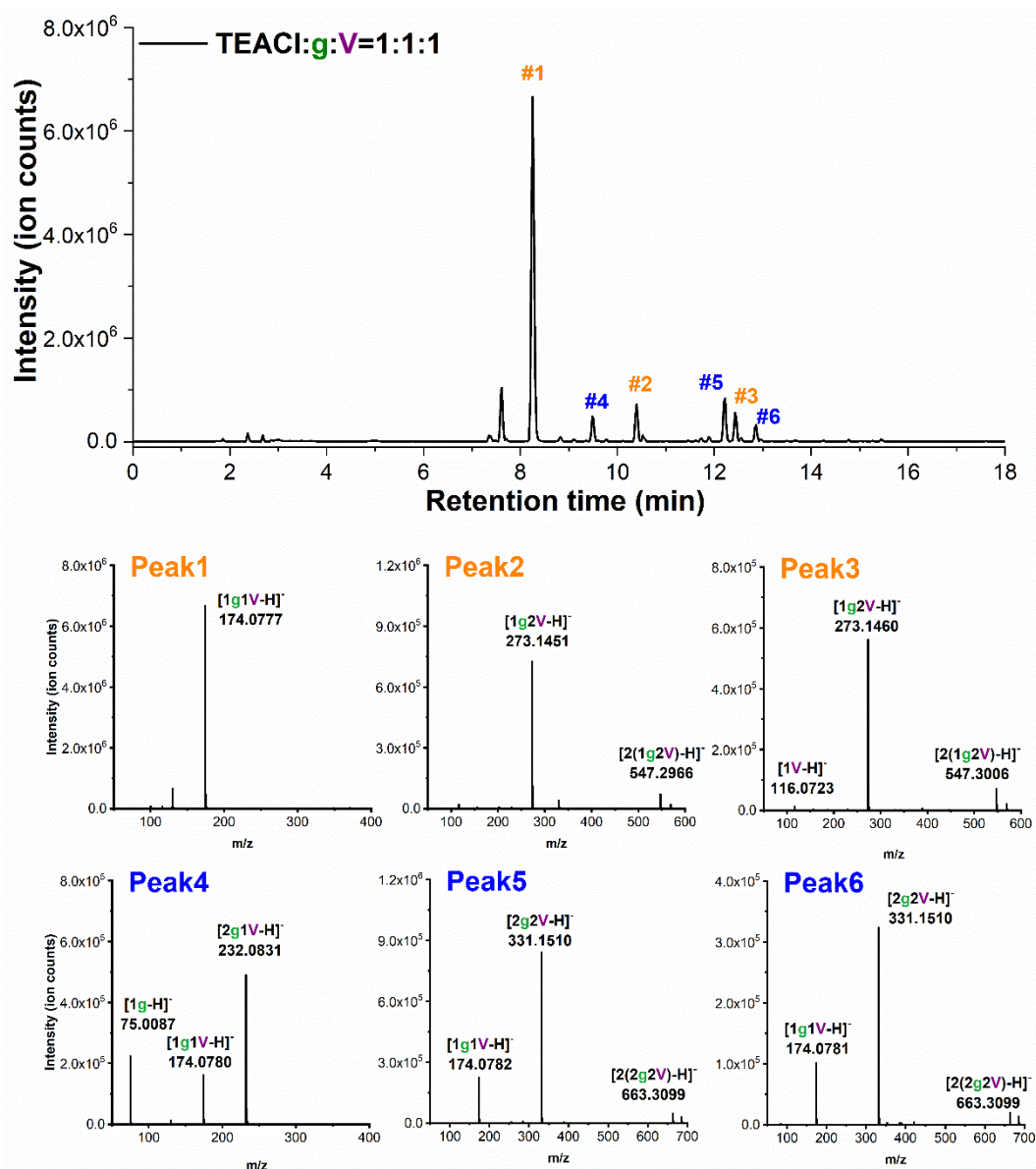


Fig. S33 LC-MS analysis of the TEACl/g/L-valine (V) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 120°C for 1 day. The polymerization of valine by the ester-amide exchange reaction is slower than simple amino acids like glycine or alanine.⁴ Therefore, the reaction temperature was increased to 120°C, so more oligomers could be observed.

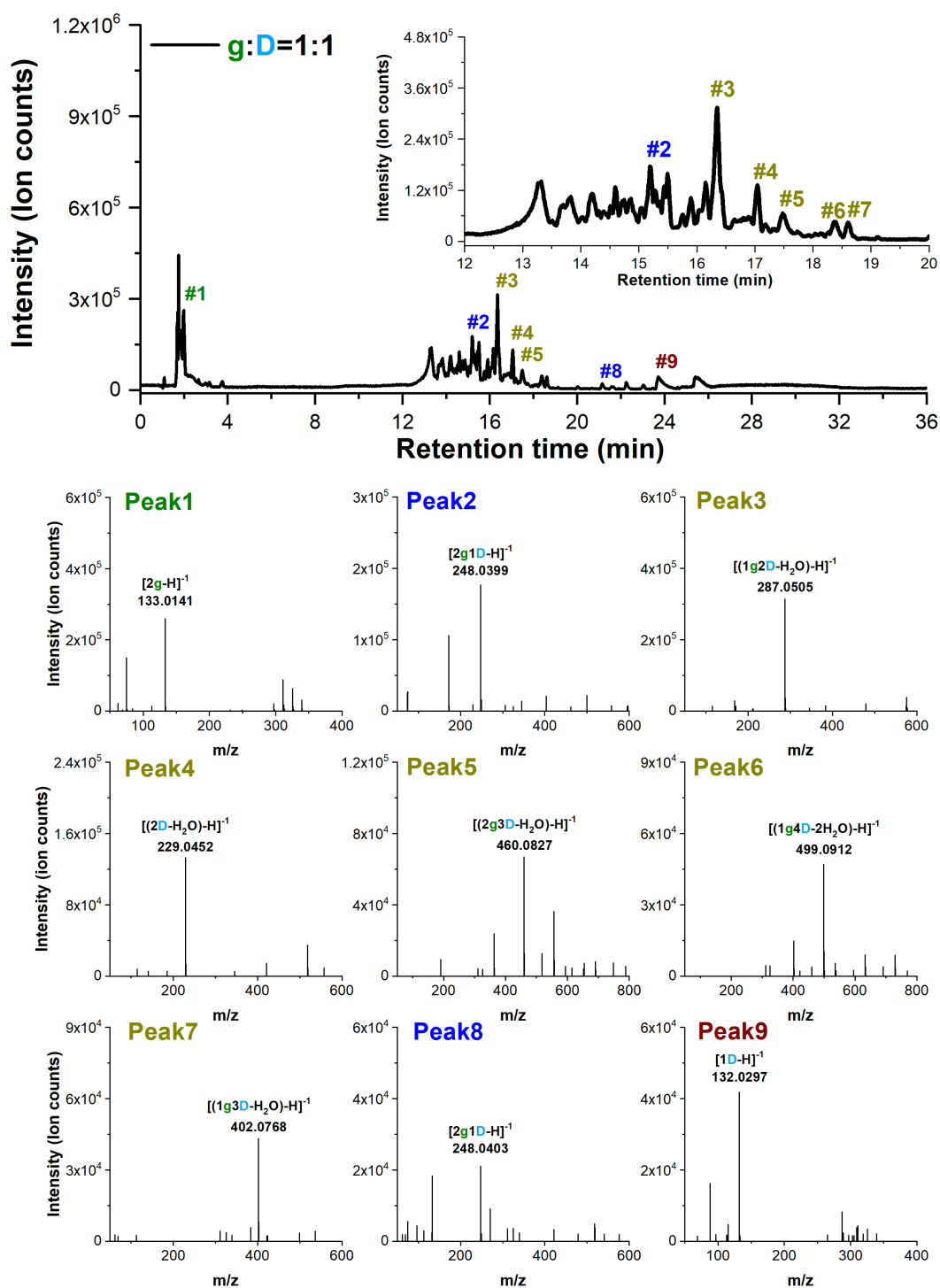


Fig. S34 LC-MS analysis of the g/L-aspartic acid (D) mixture (molar ratio of 1:1). The sample was prepared by heating at 95°C for 7 days. The loss of water molecules from the oligomers was observed, possibly due to the cyclization of aspartic acid residues through the side chains.

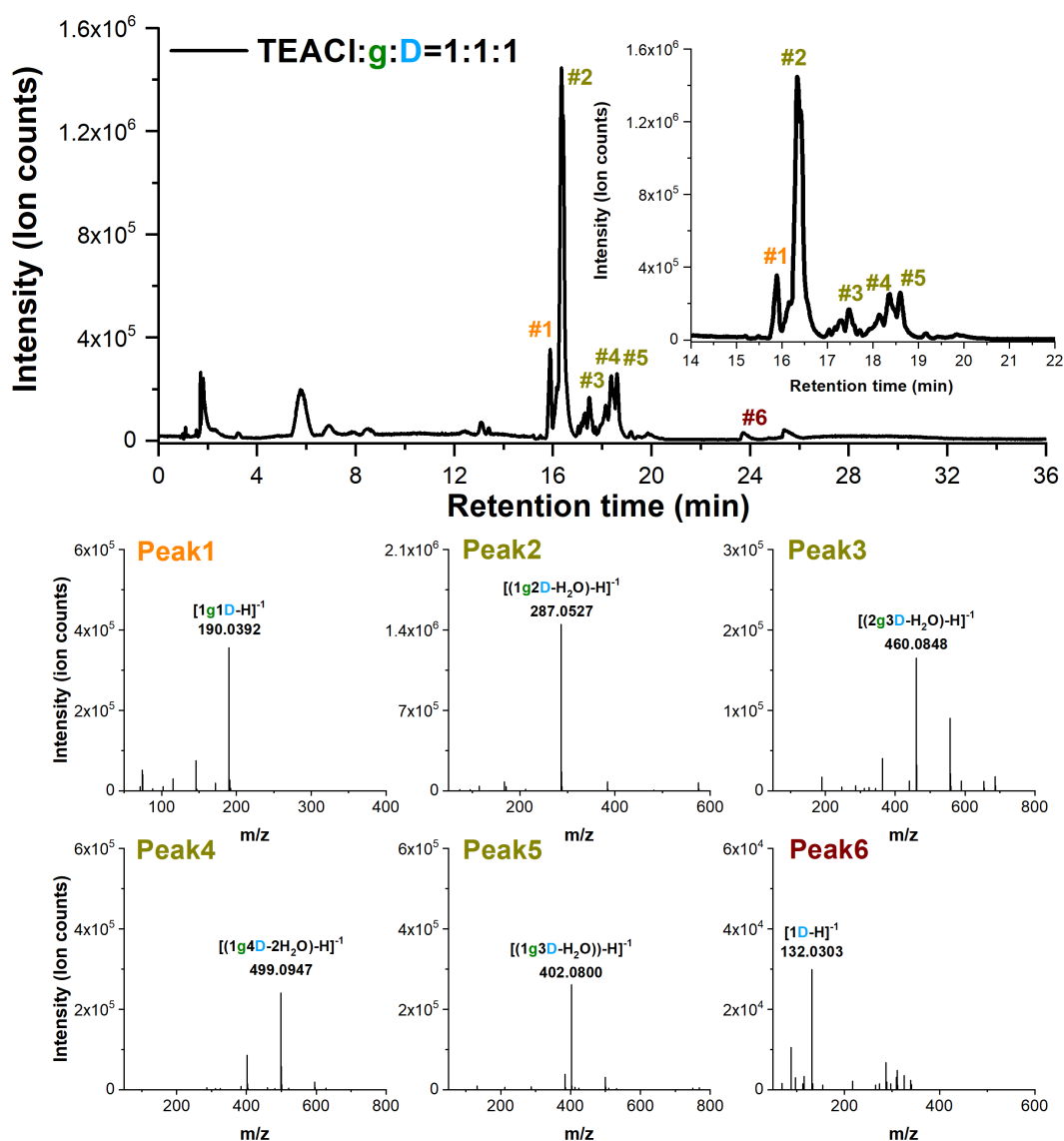


Fig. S35 LC-MS analysis of the TEACl/g/L-aspartic acid (D) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 7 days. While the control experiment without TEACl showed a complex LC-MS trace, the reaction of aspartic acid in DESs gave a simple product distribution with the main species of 1g2D (peak #2). Also, we found the loss of water molecules from the oligomers, possibly due to the cyclization of aspartic acid residues through the side chains.

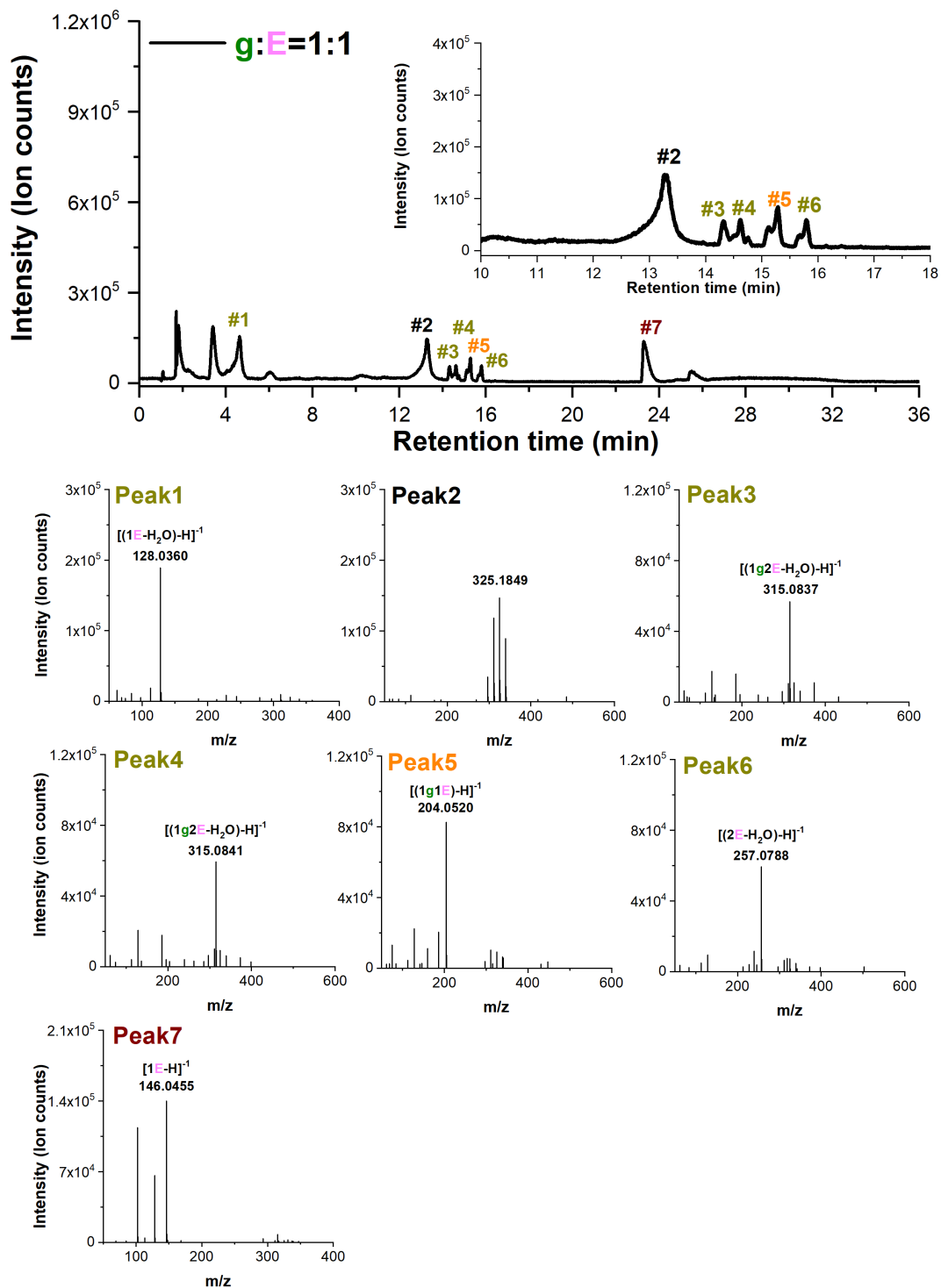


Fig. S36 LC-MS analysis of the g/L-glutamic acid (E) mixture (molar ratio of 1:1). The sample was prepared by heating at 95°C for 7 days. Peak #2 corresponds to the series of alkylbenzene sulfonates as contaminants in the instrument.

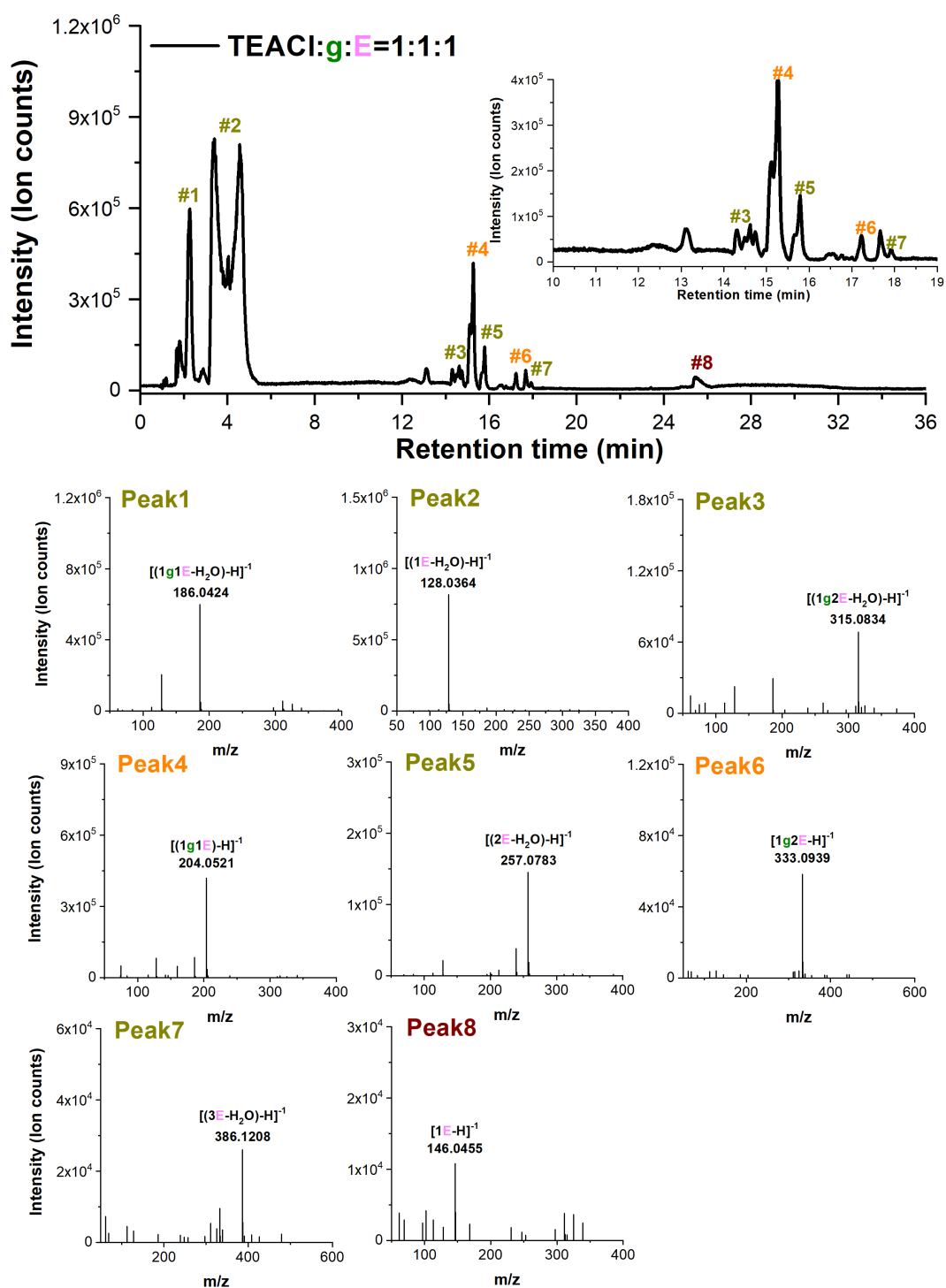


Fig. S37 LC-MS analysis of the TEACl/g/L-glutamic acid (E) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 7 days. Similar to aspartic acids, the reaction of glutamic acid in DESs gave an increased abundance of 1g1E oligomers. The loss of water molecules was also commonly observed in the oligomers of glutamic acid by the cyclization.

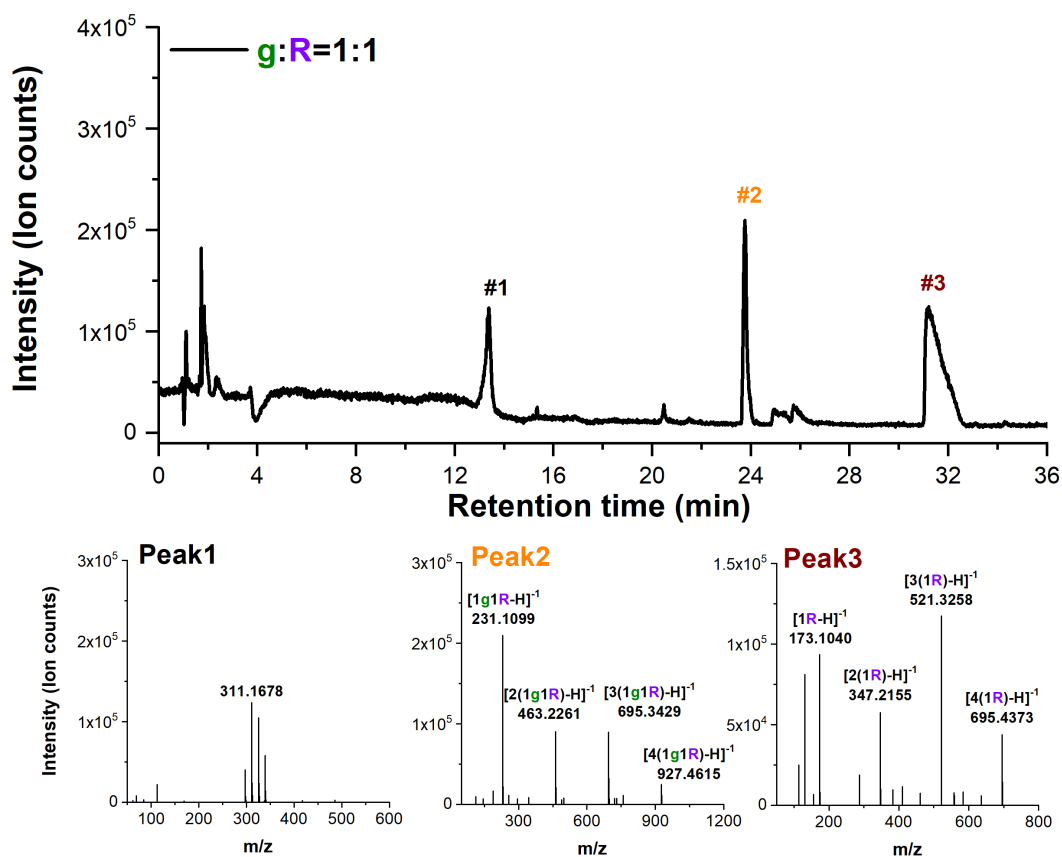


Fig. S38 LC-MS analysis of the g/L-arginine (R) mixture (molar ratio of 1:1). The sample was prepared by heating at 95°C for 7 days. Peak #1 corresponds to the series of alkylbenzene sulfonates as contaminants in the instrument.

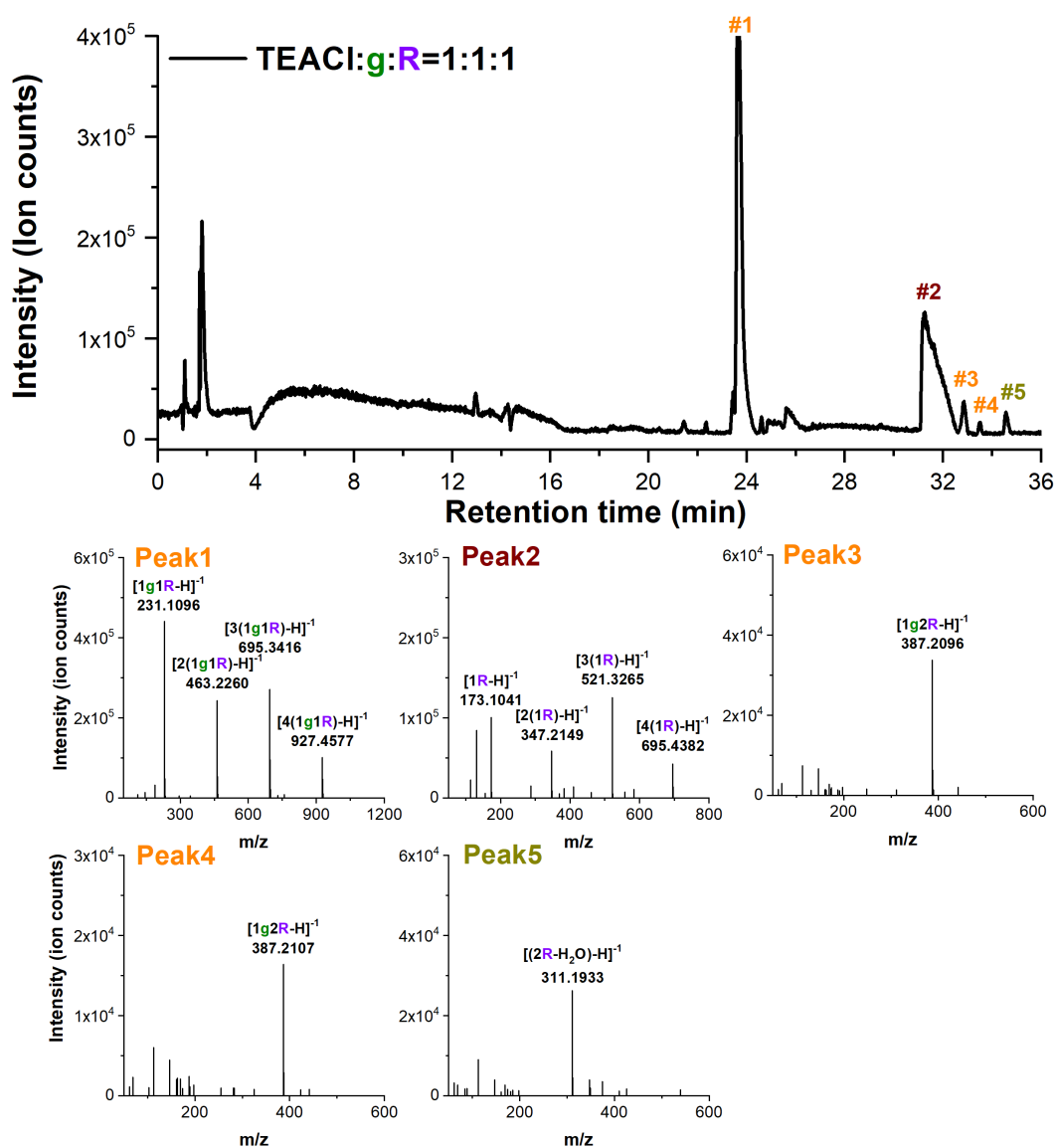


Fig. S39 LC-MS analysis of the TEACl/g/L-arginine (R) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 7 days. Comparing with the control experiment, the reaction with TEACl gave an increase of 1g2R oligomers and the abundance of arginine monomer decreased. Therefore, the reaction rate of arginine also increased in DESs.

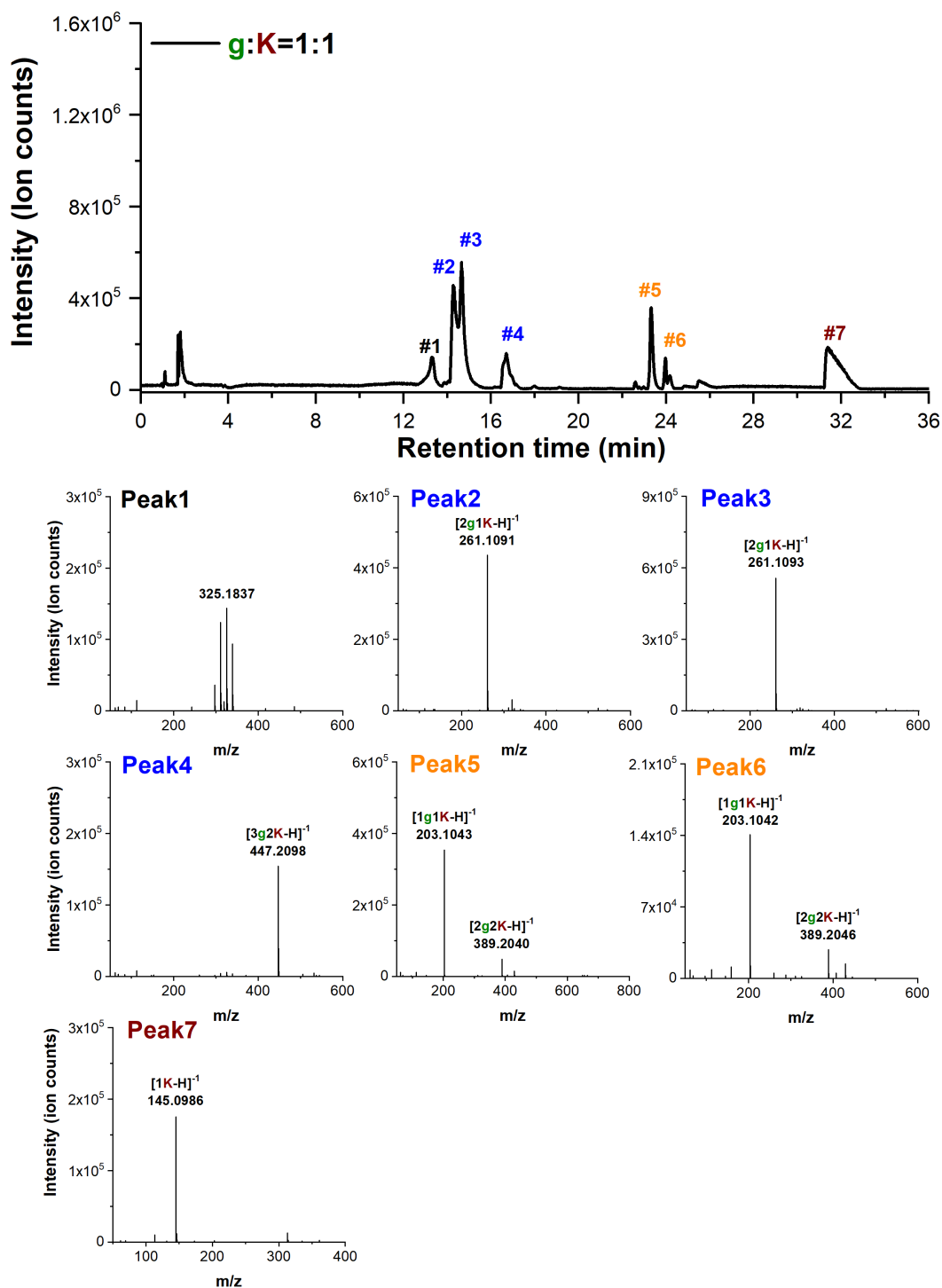


Fig. S40 LC-MS analysis of the g/L-lysine (K) mixture (molar ratio of 1:1). The sample was prepared by heating at 95°C for 7 days. Peak #1 corresponds to the series of alkylbenzene sulfonates as contaminants in the instrument.

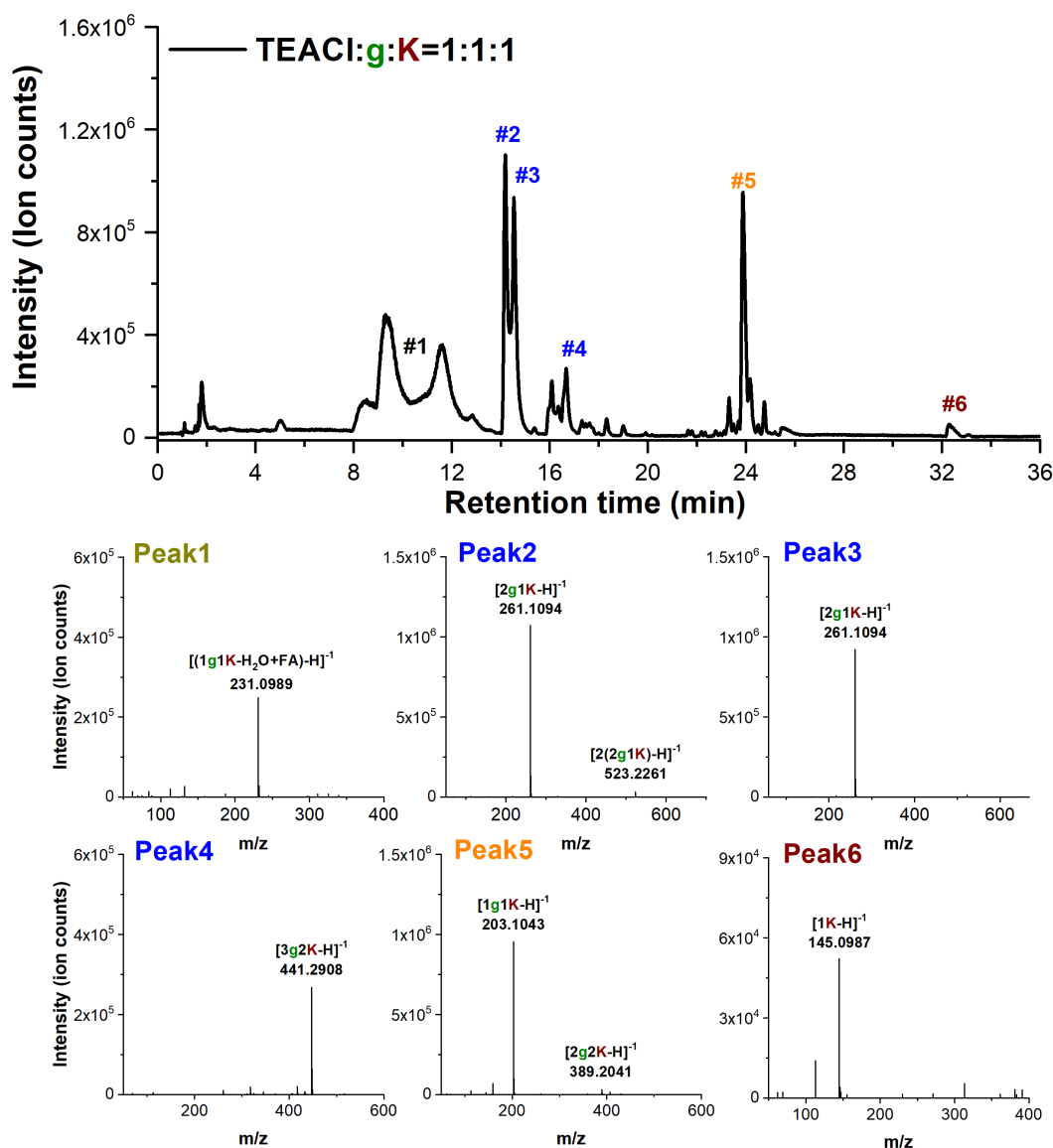


Fig. S41 LC-MS analysis of the TEACl/g/L-lysine (K) mixture (molar ratio of 1:1:1). The sample was prepared by heating at 95°C for 7 days. We tentatively assigned the species of peak #1 as 1g1K-H₂O. It could be the cyclic dimer of glycolic acid and lysine with formic acid (FA) adduct. Further studies are needed to confirm this. Comparing with the control experiment, the reaction with TEACl gave an increase of 1g1K oligomers and the abundance of lysine monomer decreased. Therefore, the reaction rate of arginine also increased in DESs. Because the side chain of lysine can also perform the ester-amide exchange reaction, the 2g1K oligomers might correspond to the amidation of α -amine and ϵ -amine by glycolic acid. A recent report has suggested the α -amine of lysine has a higher reactivity for amidation than ϵ -amine.⁵

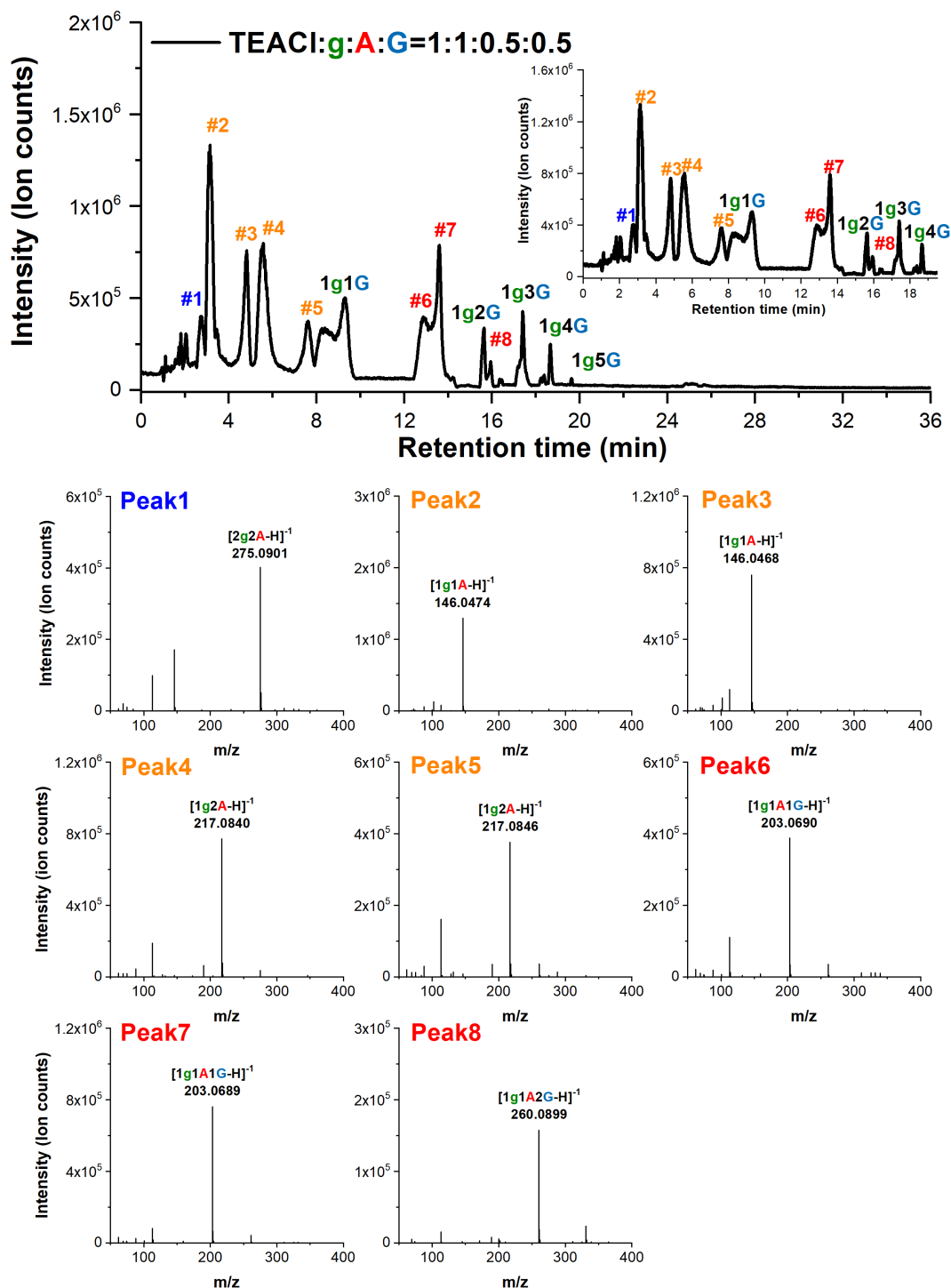


Fig. S42 LC-MS analysis of the TEACl/g/G/A mixture (molar ratio of 1:1:0.5:0.5). L-alanine was used. The sample was prepared by heating at 95°C for 7 days. Besides the series of 1gnG and 1gnA oligomers, we also observed the copolymers of glycine, alanine and glycolic acid.

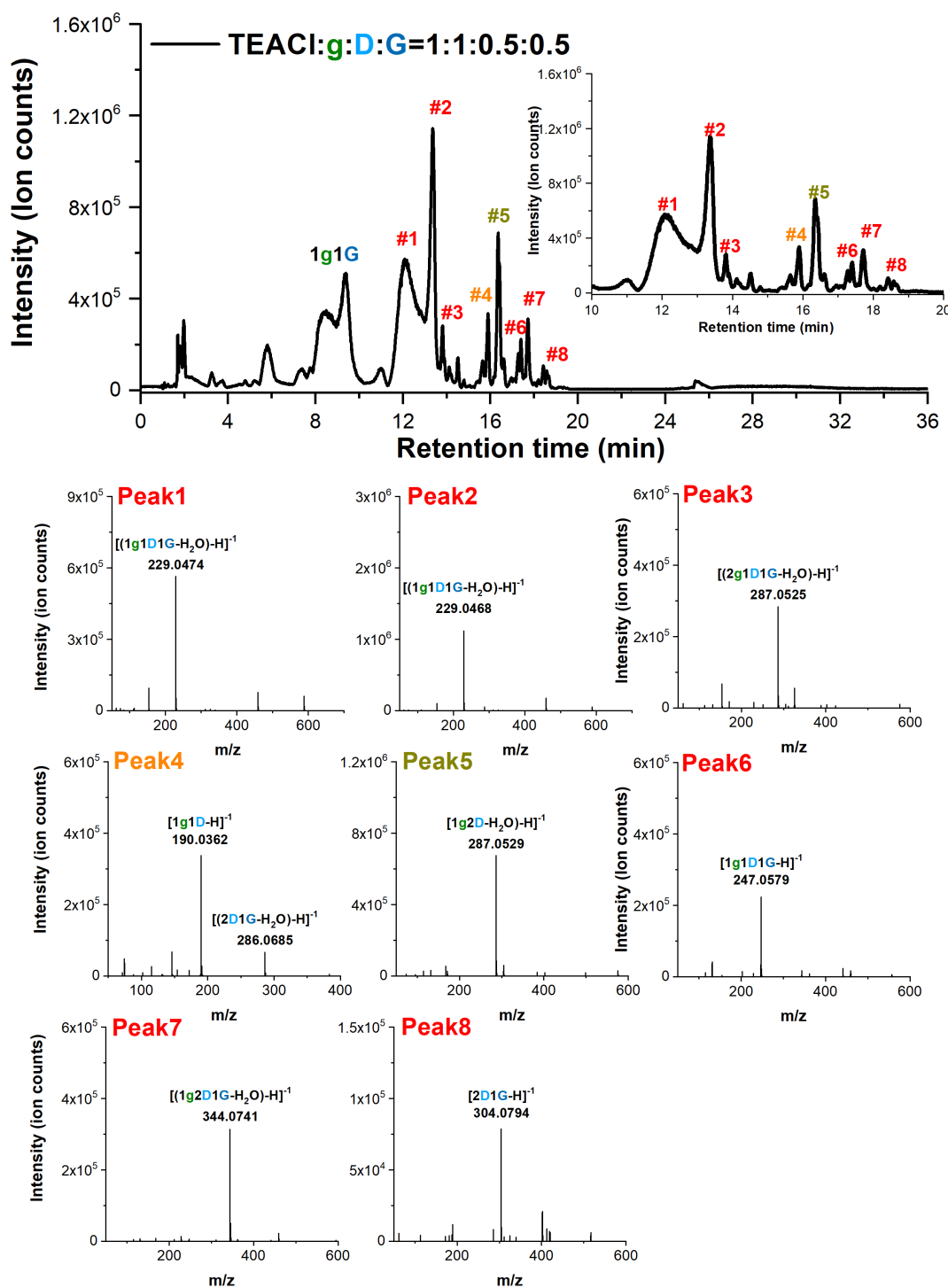


Fig. S43 LC-MS analysis of the TEACl/g/G/D mixture (molar ratio of 1:1:0.5:0.5). L-aspartic acid was used. The sample was prepared by heating at 95°C for 7 days. Besides the series of 1gnG and 1gnD oligomers, we also observed the copolymers of glycine, aspartic acid and glycolic acid. Interestingly, pure peptides like 2D1G were also formed. Since the side chain of aspartic acid can be esterified and undergo the ester-amide exchange reaction, the pure peptides are likely to be branched.

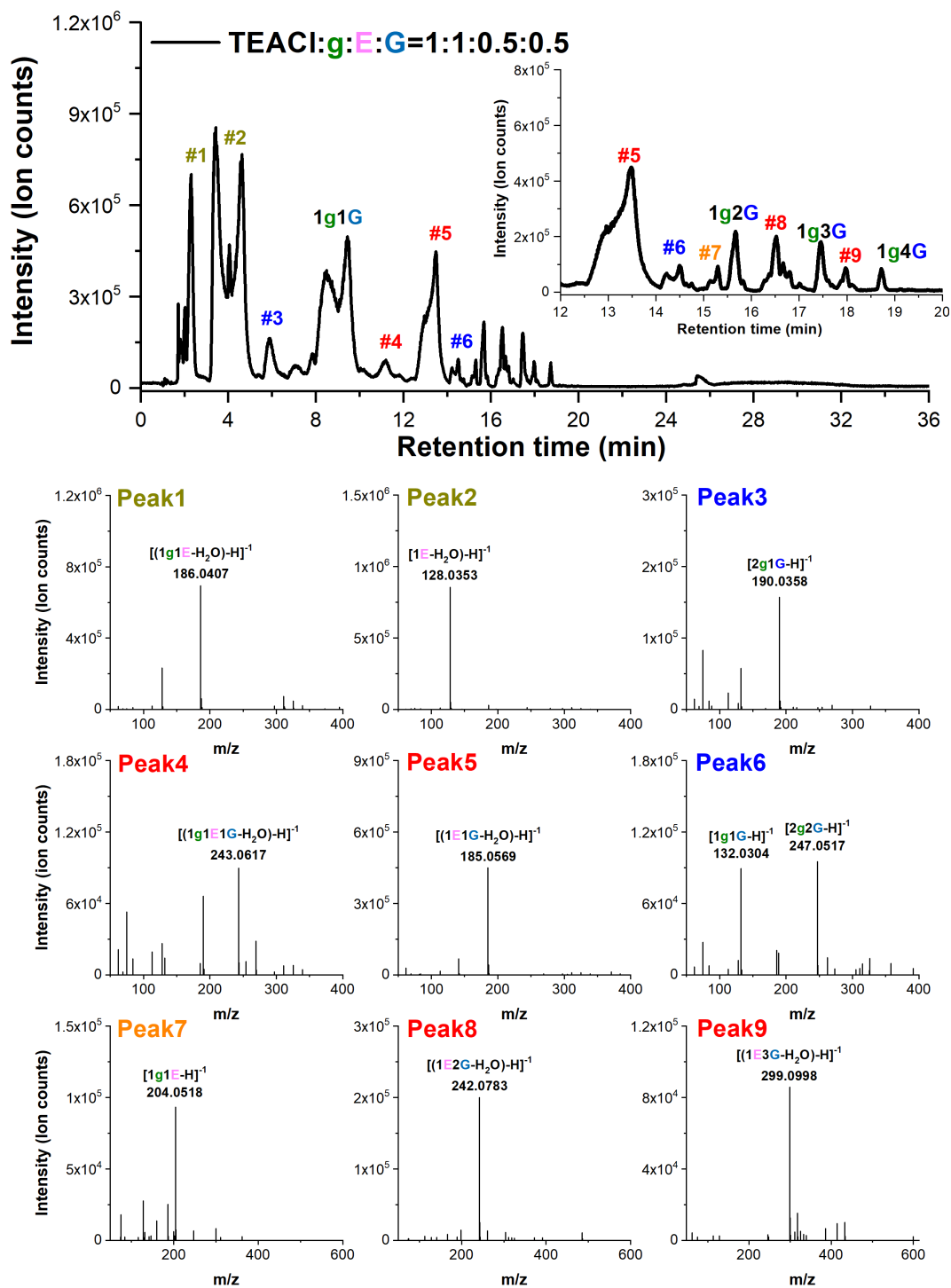


Fig. S44 LC-MS analysis of the TEACl/g/G/E mixture (molar ratio of 1:1:0.5:0.5). L-glutamic acid was used. The sample was prepared by heating at 95°C for 7 days. Besides the series of 1gnG and 1gnE oligomers, we also observed the copolymers of glycine, glutamic acid and glycolic acid. Similar to aspartic acid, we found pure peptides like 1E2G or 1E3G. These pure peptides also have the loss of water molecules. Besides branching, cyclization might also occur.

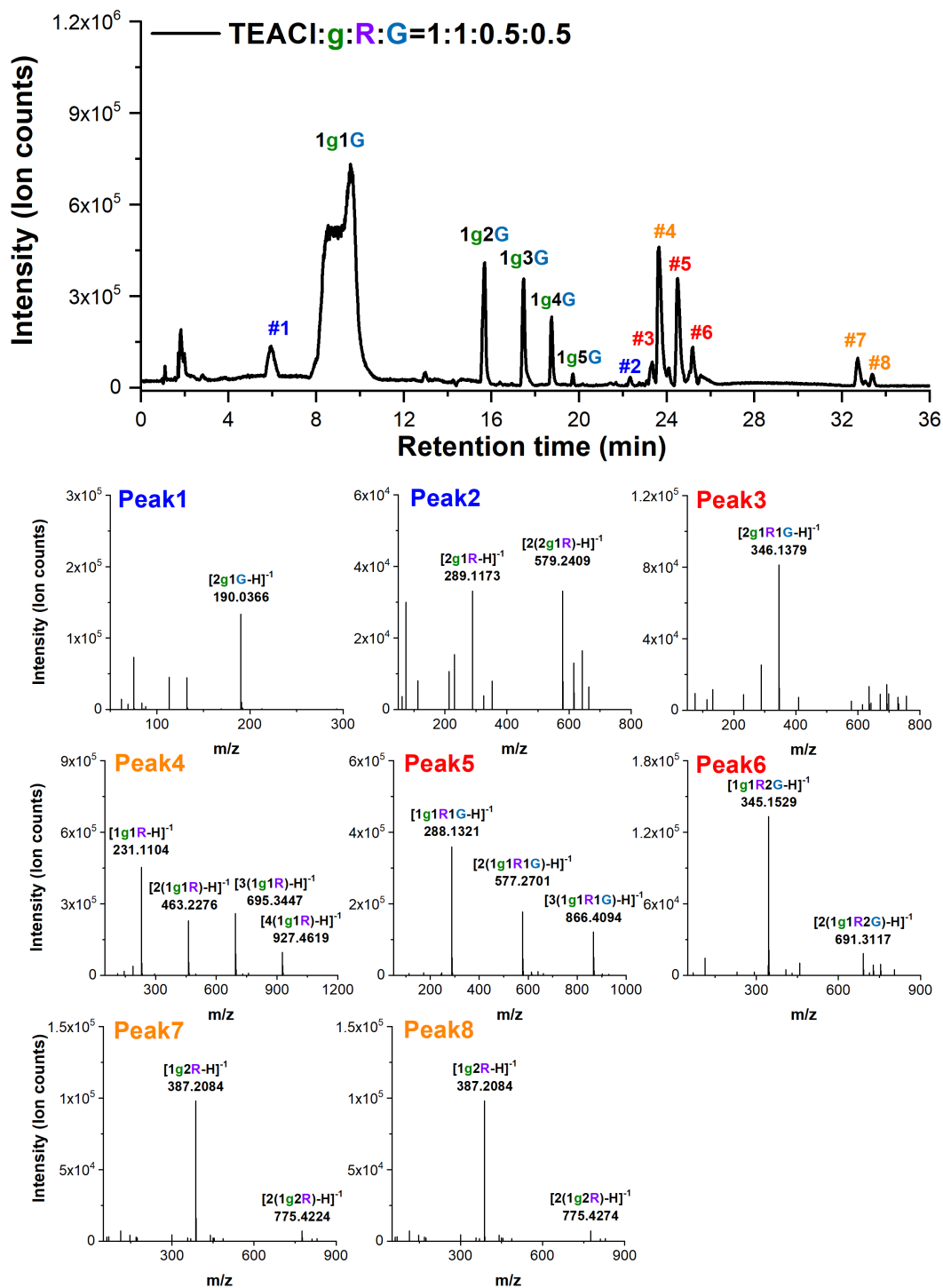


Fig. S45 LC-MS analysis of the TEACl/g/G/R mixture (molar ratio of 1:1:0.5:0.5). L-arginine was used. The sample was prepared by heating at 95°C for 7 days. Besides the series of 1gnG and 1gnR oligomers, we also observed the copolymers of glycine, arginine and glycolic acid. Glycine can be more easily polymerized than arginine and multiple glycine residues can be included in the copolymers.

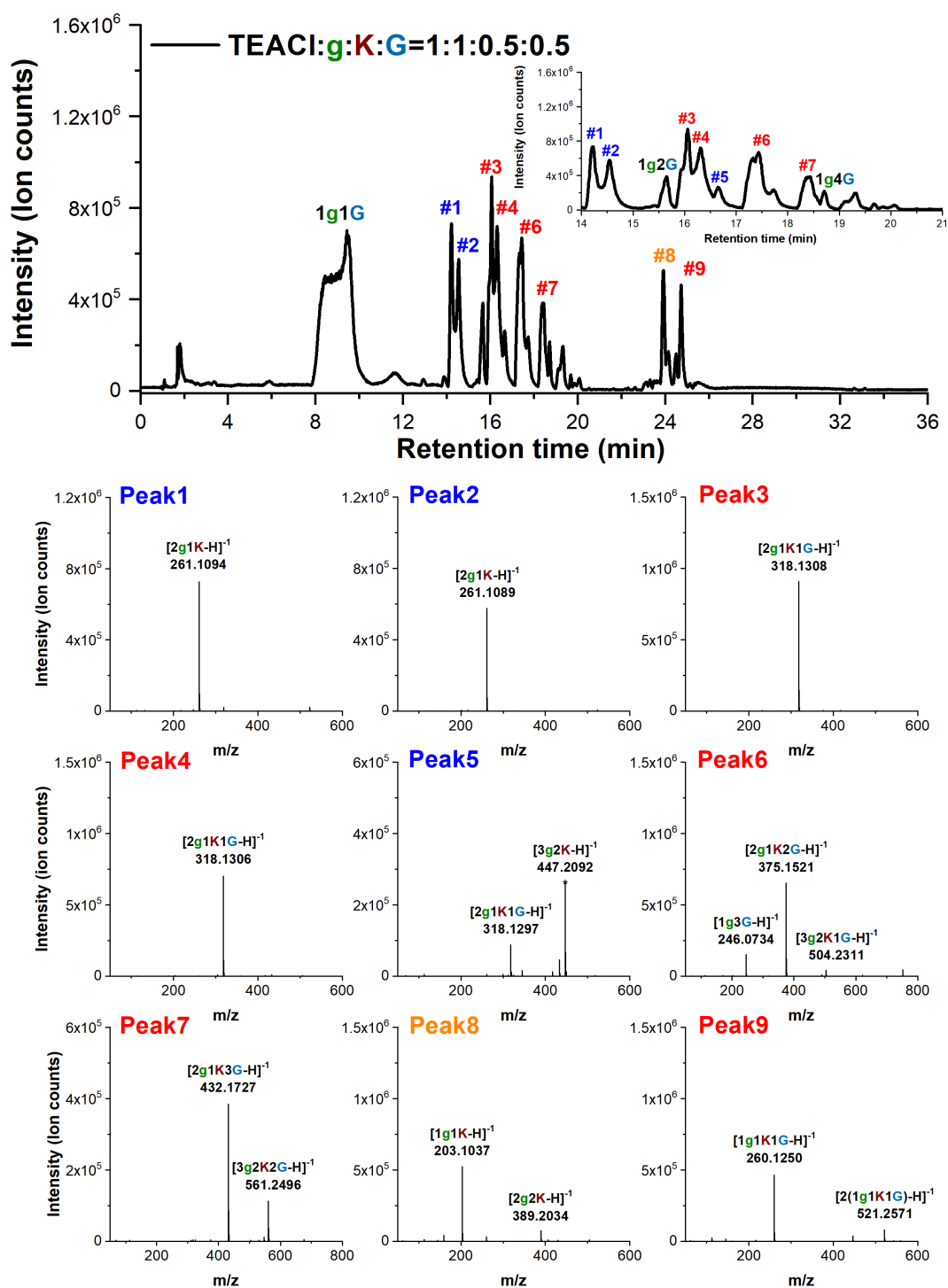


Fig. S46 LC-MS analysis of the TEACl/g/K/G mixture (molar ratio of 1:1:0.5:0.5). L-lysine was used. The sample was prepared by heating at 95°C for 7 days. Besides the series of 1gnG and 1gnK oligomers, we also observed the copolymers of glycine, lysine and glycolic acid.

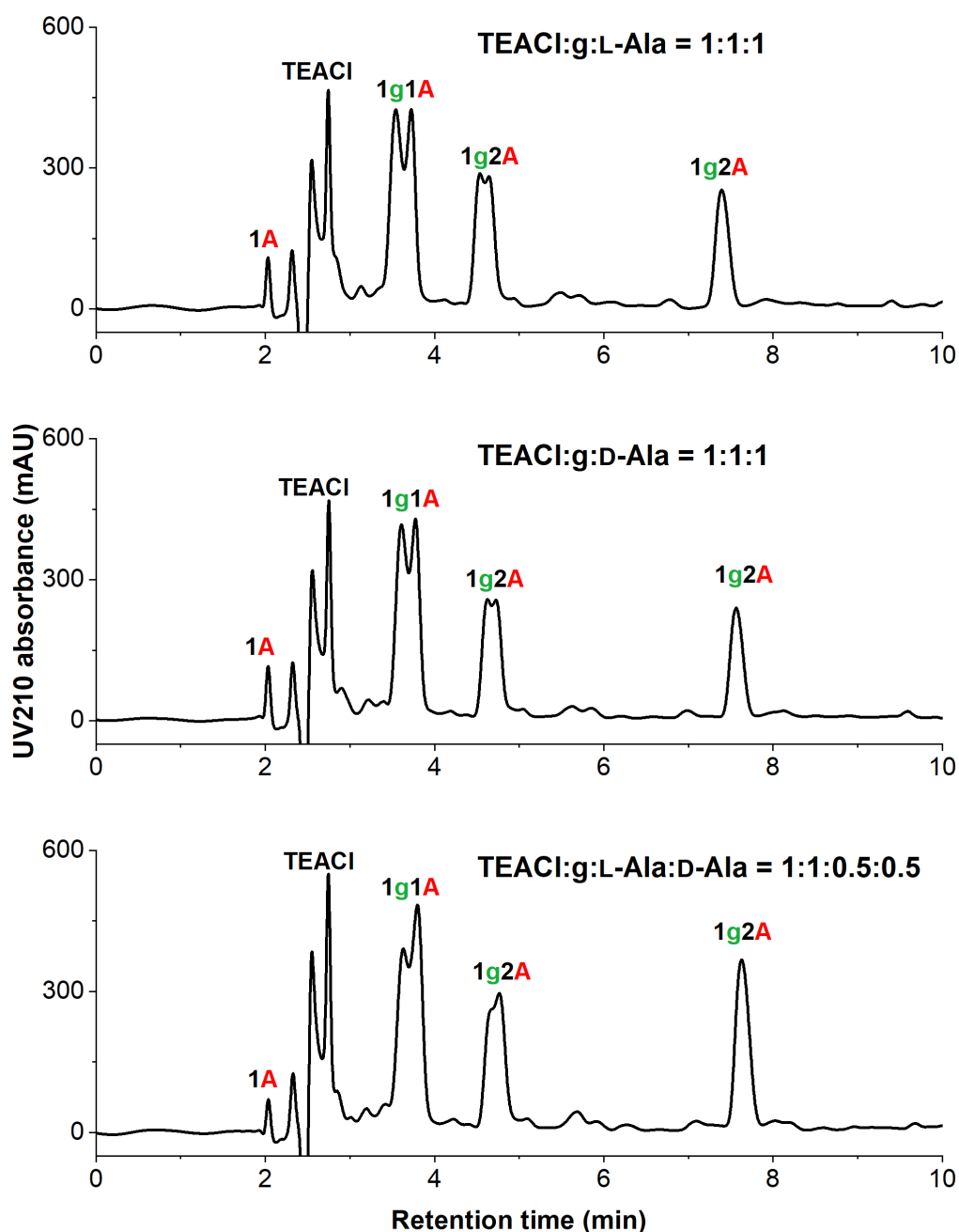


Fig. S47 LC-UV analysis of the TEACl/g/alanine (molar ratio of 1:1:1) with different enantiomers, L-alanine (top), D-alanine (middle), and a racemic mixture (bottom). The sample was prepared by heating at 95°C for 7 days. The HPLC chromatograms show the 1g2A oligomers elutes at two distinct retention times, suggesting the racemization of amino acid residues. The depsipeptides likely form oxazolone intermediates, similar to the activated amino acids in the solid-phase peptide synthesis.⁶ The subsequent ester-amide exchange produces the diastereomers of depsipeptides. In addition, when using a racemic mixture of alanine, the product distribution did not significantly change, indicating no stereochemical preference of the ester-amide exchange in DESs. The previous work using a racemic mixture of alanine with wet-dry cycles also shows no stereochemical preference.⁷

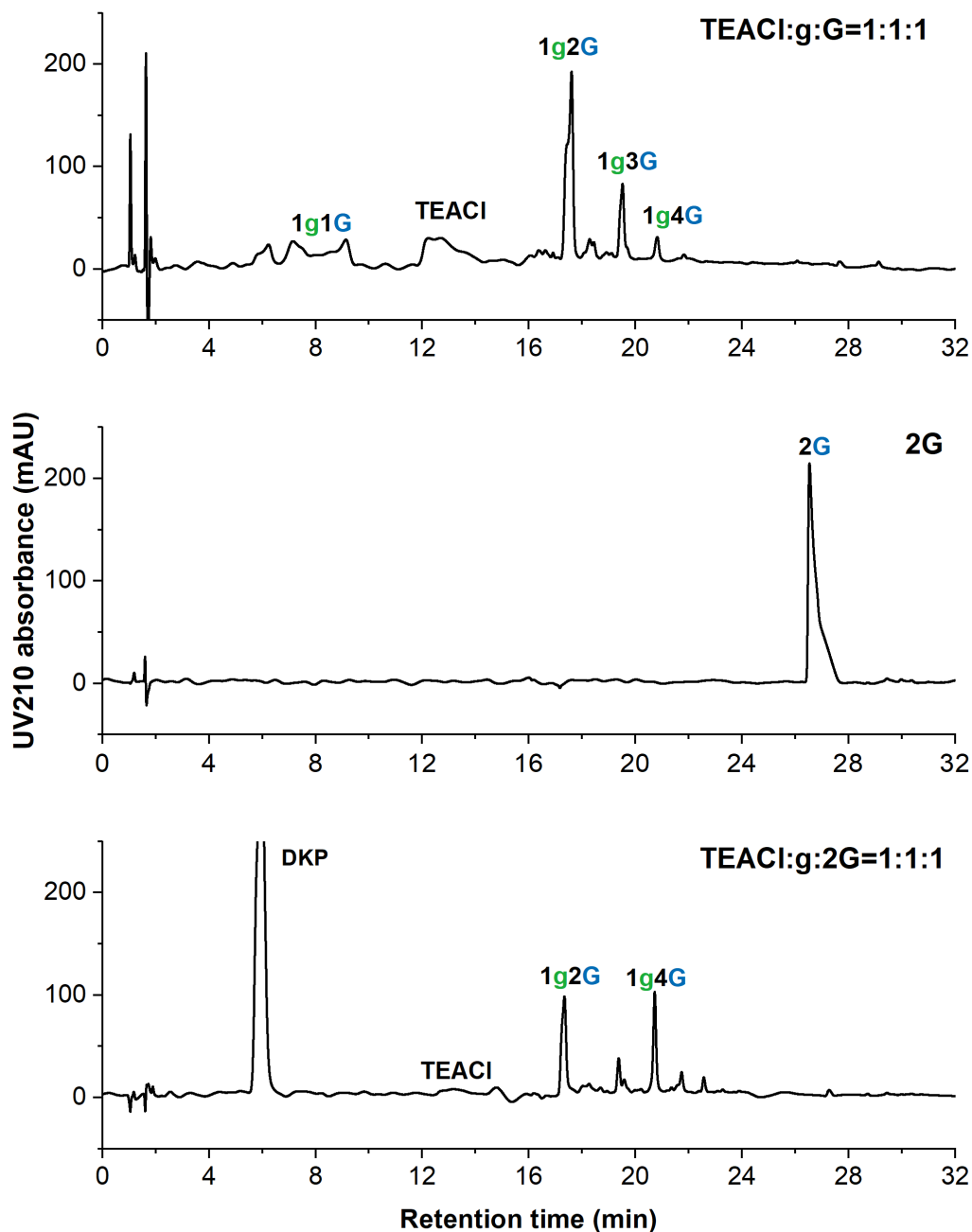


Fig. S48 LC-UV analysis of the reaction mixture using glycine dipeptide. The top figure is TEACl/g/G (molar ratio of 1:1:1). The middle is the product of using only glycine dipeptide (2G). The bottom chromatogram shows the products of TEACl/g/2G (molar ratio of 1:1:1). All samples were prepared by heating at 95°C for 7 days. While most of the samples in this work were dissolved in 500 μ L of water for the HPLC analysis, 1500 μ L of water was used for the samples containing diglycine due to the low solubility of products in water. Without glycolic acid and TEACl, the glycine dipeptide did not polymerize into other oligomers. As expected, if glycine dipeptide is added into the DESs, the major products are 1g2G and 1g4G. Therefore, long oligomers might be produced by using short peptides as the starting materials. In addition, a significant amount of glycine diketopiperazine (DKP) was produced by glycine dipeptides, suggesting the cyclization of peptides.

9. The reaction of glycolic acid or glycine with TEACl

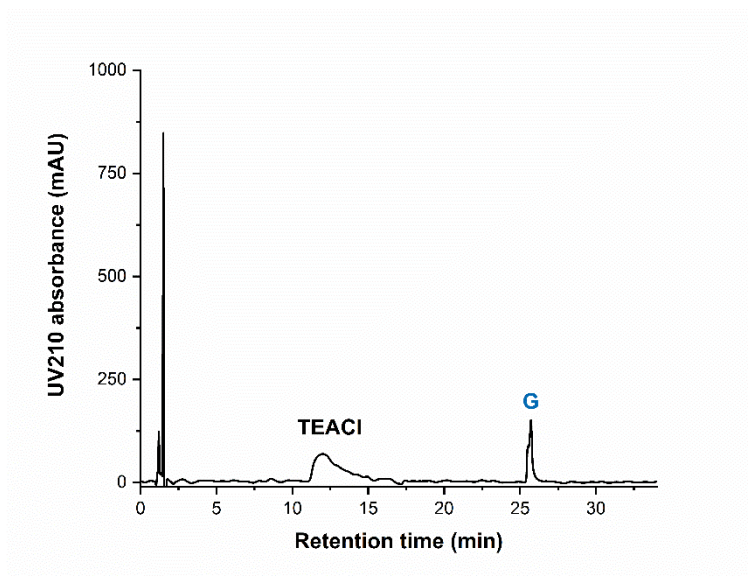


Fig. S49 LC-UV chromatogram for the reaction mixture of TEACl and glycine (molar ratio=1:1). The sample was prepared by heating at 95°C for 7 days.

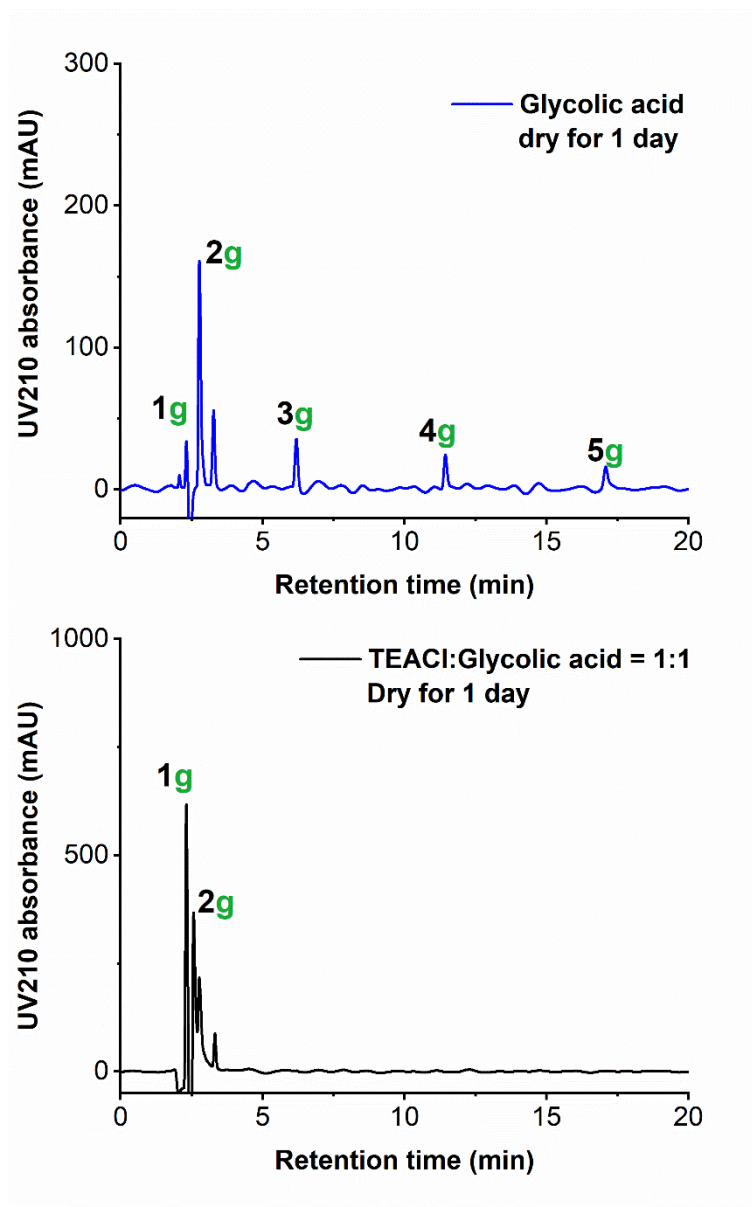
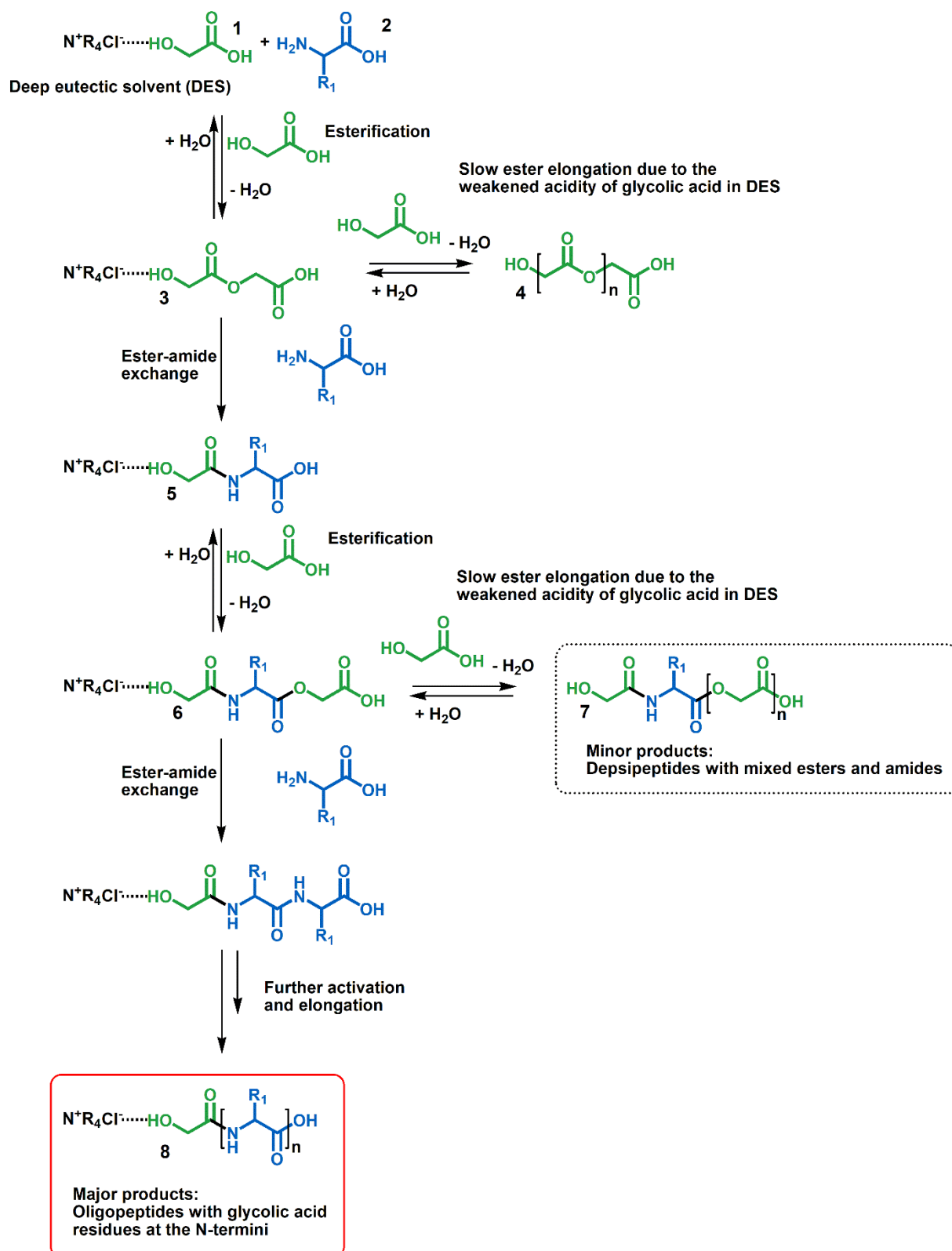


Fig. S50 LC-UV chromatograms for the polymerization of glycolic acid with or without TEACl.

10. Proposed mechanism



Scheme S1. Proposed mechanism for the reaction between glycolic acid and amino acids in DES.

Description of the proposed mechanism:

Glycolic acid (1), amino acids (2) and quaternary ammonium salts are initially mixed together and dried. Glycolic acid and quaternary ammonium salts form deep eutectic solvents to dissolve amino acids. The initial esterification of glycolic acids leads to a glycolic acid dimer (3). It has been shown a high concentration of quaternary ammonium salts in a mixture weakens the acidity of simple carboxylic acids.⁸ Therefore, the acid-catalyzed esterification rate to long oligoesters in DES is slower than that in the control experiment. Oligoesters of glycolic acid (4) and depsipeptides with mixed ester and amide backbones (6, 7) become minor products in the presence of quaternary ammonium salts. As shown in Fig. S3, we have found 1g-1G-1G-1g and 1g-1G-1G-1G-1g, suggesting the elongation of the peptide backbone still come from the esterification of the C-termini. The oligomers with glycolic acid residues on the N-termini (8) also form DES with quaternary ammonium salts. The reaction mixture in DES should be more homogeneous than the control experiment. We hypothesize the increased difference of reaction rates (esterification/exchange) and the better solubilization of the reactants in DES, give a synergic effect that leads to the high yield of amino acid-enriched oligopeptides.

References

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