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Structurally Defined Imidazolium-Type Ionic Oligomers as Soluble/Solid Support for Peptide Synthesis

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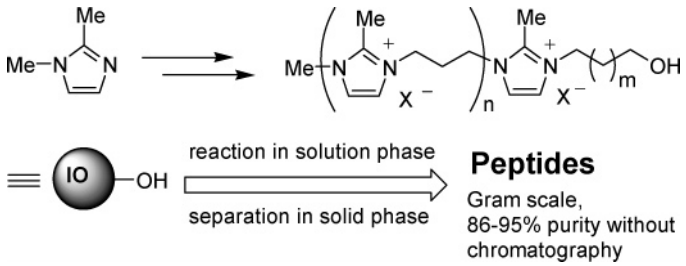
ABSTRACT



Structurally defined ionic liquid-type imidazolium oligomers have been synthesized in multigram scales. These imidazolium salts have been applied to synthesize peptides efficiently in gram scale. The assembly of oligopeptides was conducted in homogeneous solution phase without the need of much excess reagents and capping as in the case of solid-phase peptide synthesis. Importantly, this approach made efficient peptide block couplings possible.

Ionic liquids (ILs) have been used in diverse areas such as organic catalysis,[1] electrochemical devices,[2] and analytic chemistry[3] because of their characteristic properties such as non-flammability, high thermal and chemical stability, lack of measurable vapor pressure, high ionic conductivity[4] and electrochemical stability.[5] Recently, ILs have been used as soluble supports[6] for catalysis,[7] reagents, and synthesis for small molecules[8] and bio-oligomers such as peptides, oligosaccharides, and oligonucleotides.[9] The IL-supported synthesis may supplement the solid-phase synthesis[10] or other solution-phase methodologies such as soluble-polymer supported synthesis[11] or fluorous phase synthesis.[12] The ionic liquids used are usually imidazolium salts with one unit charge.

While the IL-supported synthesis possesses the advantages common to the solution-phase synthesis in that the reactions can be conducted in homogeneous phase, the product separation step by phase separation is not as conveneient as the solid-phase synthesis where simple filtration and washing

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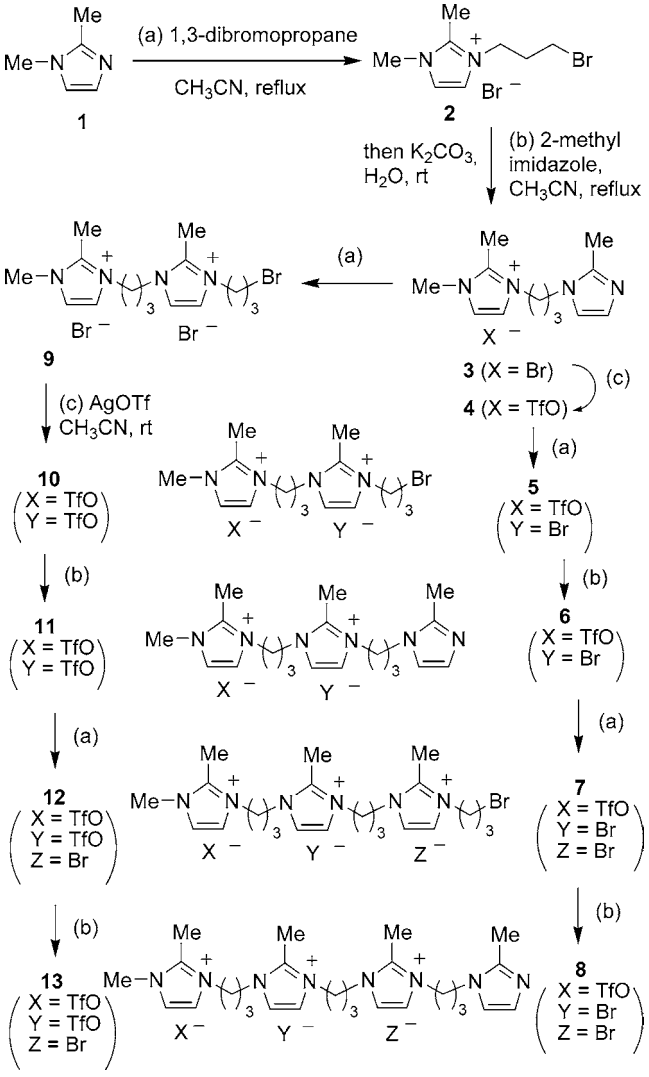
are used for the purification of solid-bound species. The IL-bound species usually separated out from the solution phase as viscous liquid, making its purification tedious. Another problem is that the phase tag role played by the IL moiety is reduced when large oligomers are bound to the ILs, thus overwhelming the characteristics of the ILs and modifying the solubility, an issue also common to most soluble phase tags. It would be useful to develop new ionic tags which can combine the benefits of homogeneous reactions of soluble support and the ease of separation of solid support. Herein, we report the synthesis of novel structurally defined imidazolium-type ionic oligomers (IO) with multiple unit charges and their successful application to the peptide synthesis.

The synthesis of the imidazolium-type ionic oligomers is shown in Scheme 1, starting from the commercially available compound **1**, 1,2-dimethylimidazole, via either one of two routes (**3** to **8** vs **3** to **13**). Further treatment of compound **8** or **13** with 1,3-dibromopropane and then 2-methylimidazole can extend the imidazolium oligomer chain. Each of the steps in the synthesis gave high yields with ease of purification by phase separation, leading readily to good overall yields in multigram quantities (see the Supporting Information). The difference between these two synthetic routes was that the products were obtained with different anions as confirmed by mass spectrometry in the negative ion mode. This rendered them having different solubilities and different purification procedures. For example, when compound **3** was treated with 1,3-dibromopropane in CH_3CN under refluxing conditions, the expected product **9** precipitated out in the reaction mixture because this compound had very low solubility in CH_3CN. The product was obtained in 98% yield and had a melting point of up to 201 °C, while compound **5**, having the same cationic connecting sequence as **9** but with different anions, was well soluble in CH_3CN and had a melting point of 76 °C.

The thermal stability of the imdazolium oligomers was measured by thermogravimetric analysis (TGA) (Supporting Information).[13] Their onset decomposing temperatures (ODTs) show that imidazolium oligomers have good thermal stability, with ODTs at more than 212 °C. From the data, we can draw the general conclusion that the molecules with more ionic units have higher ODTs and anion exchange from bromide to triflate renders the oligomer more thermally stable. The low residue percentages from TGA measurements (Supporting Information), around 1% for all samples, show that the oligomeric compounds contained only a small amount of inorganic salts (less than 2%), indicating that the inorganic salts had been removed efficiently during workup procedures.

Scheme 1. Synthesis of Imidazolium Ionic Oligomers



In addition, the experimental results showed that molecules with multiple imidazolium units were generally soluble in water and alcohols and polar solvents such as DMF, DMSO, CH_3CN, and CH_3NO_2, less soluble in CH_2Cl_2 or ethyl acetate, and insoluble in alkanes, THF, Et_2O, and $CHCl_3$. Their solubility in organic solvents can be tuned by anion exchanges. The imidazolium oligomers with bromide anions have less solubility in CH_3CN and stronger moisture affinity than those with triflate anions and the same cationic structures. Furthermore, compounds with more imidazolium units have less moisture affinity that those with fewer imidazolium units.

With these molecules in hand, peptide synthesis was chosen as application target to evaluate their potential as novel ionic tags for organic synthesis. Thus far, solid-phase peptide synthesis (SPPS) with polymer resins or glass beads as support is used extensively because the process has been automated. However, there are drawbacks assocated with SPPS[14] because the reactions are performed in the hetero-

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(13) The thermogravimetric analysis (TGA) was performed on a TGA Q500 from TA instruments. The running method used was ramped from 25 to 550 °C, at 20 deg/min, under nitrogen, switching to air and then ramped at 20 deg/min to 700 °C. The type of pan used was platinum.

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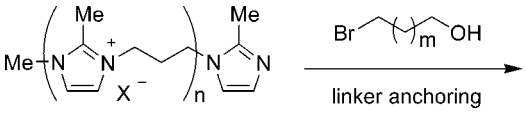
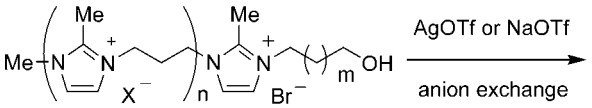
geneous phase, which generally requires a large excess of reagents to drive the reaction to completion. Another issue is that, during the course of SPPS, the growing peptide chain can fold over itself or aggregate, leading to low yield or truncated fragments. Finally, the low loading level of the supports also makes it expensive to do large-scale reactions. Because of these limitations, in the large-scale manufacture of peptide theraputics by chemical synthesis, classical solution-phase synthesis is still employed over SPPS in the majority of cases.[15]

The use of ionic oligomers as support for peptide synthesis started with the hydroxyalkyl compounds **20**−**23** synthesized according to Scheme 2. These compounds are generally

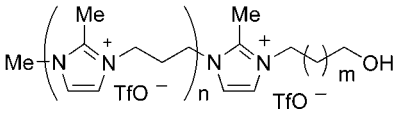
Scheme 2. Synthesis of Imidazolium-Type Ionic Oligomers for Peptide Synthesis Supports



1, n = 0; **3,** n = 1, X = Br; **11,** n = 2, X = TfO, TfO;
8, n = 3, X = TfO, Br, Br; **13,** n = 3, X = TfO, TfO, Br



14, n = 0, m = 1; **15,** n = 1, m = 1, X = Br;
16, n = 1, m = 4, X = Br; **17,** n = 2, m = 1, X = TfO, TfO;
18, n = 3, m = 1, X = TfO, Br, Br; **19,** n = 3, m = 1, X = TfO, TfO, Br



20, n = 0, m = 1
21, n = 1, m = 4
22, n = 2, m = 1
23, n = 3, m = 1

soluble in H_2O, alcohols, DMSO, CH_3CN, and CH_3NO_2, less soluble in THF and ethyl acetate, and insoluble in alkanes, Et_2O, and $CHCl_3$. The thermal stability of these imidazolium salts was also measured by TGA (see the Supporting Information), and the results showed good thermal stability.

The ionic oligomer-supported peptide synthesis is shown in Scheme 3a. The peptide sequence, which was chosen from a segment of the natural peptide Mucin4 (MUC4),[16] is mostly composed of serine and threonine because these two amino acids play a significant role in binding glycals in glycoproteins.[17] The first amino acid was covalently bound to the imidazolium salt supports **20**−**23** through an esterification reaction between them, using 1,3-dicyclohexylcarbodiimide (DCC) as the coupling reagent and 4-dimethylaminopyridine (DMAP) as the catalyst in CH_3CN at room temperature. The white precipitate, dicyclohexylurea, generated in the reaction process, was filtered off, and the excess reagents and other byproducts were washed away by Et_2O or the mixed solvents

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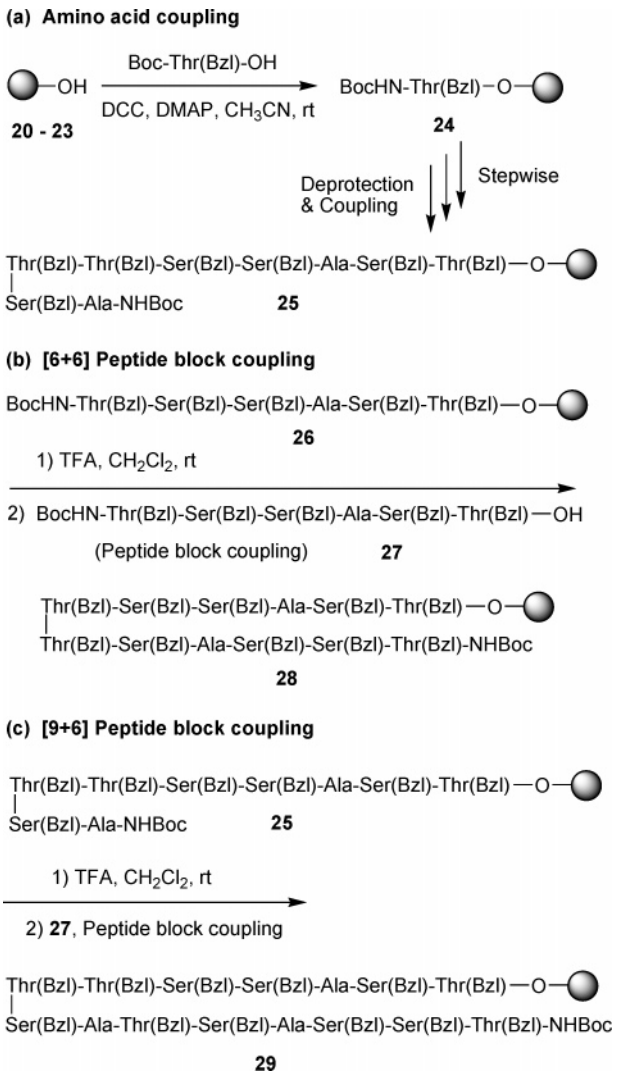
(17) Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 836.

Scheme 3. Imidazolium Oligomers−Supported Amino Acid Assemblings and Peptide Block Couplings



of diethyl ether and ethyl acetate (v/v = 1:1). The dimer (**21**) and oligomer (**22** or **23**)-supported products were obtained as solid, in contrast to the imidazolium monomer (**20**)-attached product that was obtained as a thick oil.

The removal of the acid-labile Boc protection group on the supported amino acids was realized by treating the compounds with trifluoroacetic acid (TFA) in CH_2Cl_2. The byproduct derived from the Boc group was washed away by Et_2O. The amino acid coupling was conducted by use of the combination of HBTU and HOBt in quantitative yield, without needing the capping technique. This generic coupling protocol is slightly different from the procedures previously reported by our group in which PyBOP was used as a coupling reagent because the tripiperidinophosphine oxide, which was generated from PyBOP,[9a] was very difficult to wash away during the purification procedures. Hünig's base, DIPEA, was used to release the unprotonated amine and acted as acid scavenger. The purification procedures of the products generated from ionic dimer (**21**) or oligomer (**22** or **23**)-supported amino acid couplings were quite simple.

When the reaction was completed, the reaction mixture was concentrated by rotary evaporation under reduced pressure and then Et_2O was added to precipitate the product and the liquid phase was separated and removed by filtration or centrifugation and decantation. The solid phase was washed with water 3−4 times and then Et_2O twice and obtained as a powder after drying. For the ionic monomer (**20**)-supported amino acid coupling, the product was obtained as a viscous oily product layer when the less polar Et_2O was added. Scratching the oily product layer with a spatula was necessary to allow the byproducts and excess reagents to disperse into the washing solvents. This process was more difficult to perform especially for the larger scale reactions. The product for each step was clean according to the easy characterization by solution NMR and MS, in contrast to the solid-phase synthesis. Nonapeptides **25** were obtained readily in high overall yields.

It is worthy to emphasize that the chemical assembly of oligopeptides in Scheme 3 was carried out in the homogeneous solution phase without the need of much excess reagent; generally, 2−2.5 equiv of amino acids relative to the support-bound substrates was used. Importantly, we found that the peptide block coupling could be done by use of these ionic oligomers as supports.[18] The ionic trimer (**22**) was used to investigate the supported peptide block couplings of [6+6] and [9+6] as illustrated in Scheme 3b,c. The protected hexapeptide **27**, obtained by cleavage from the supported precursor **26**, was coupled in the same generic coupling protocol to give the [6+6] adduct **28** in 95% yield. Similarly, hexapeptide **27** was coupled to the supported nonapeptide **25** to give the [9+6] adduct **29** in 99% yield. The products were also purified under the standard conditions described above.

The cleavage of peptides from the ionic oligomer supports was carried out through hydrolysis of the ester bond under aqueous basic conditions. The ionic oligomer-supported peptides were hydrolyzed by aqueous solution of LiOH or NaOH, with addition of some THF or CH_3CN, to generate the corresponding products. The purification of the peptides after cleavage from the supports was done through washing with water without the need of chromatography because the ionic supports were soluble in water while the protected peptides were insoluble in water. Moreover, the ionic supports were easily recovered in 80−90% yields by removing the water and then washing and phase separation with organic solvents. The recovered ionic supports had been reused, giving the same results as the original supports. The peptides thus obtained were pure according to NMR and MS analysis, and by HPLC[19] have a purity in the range of 86−95% without racimization occurring.[9a] Their ESI-mass spectra showed a single peak due to the molecular ions, indicating the absence of deletion sequences. These results are quite encouraging and better than that from solid-phase peptide synthesis, which generally gives protected peptides of 70−80% purity after cleavage from the supports.

In conclusion, structurally defined imidazolium ionic oligomers in multigram scales have been synthesized. These ionic oligomers have been applied to efficiently synthesize peptides in the gram scale. The quantitative chemical assembly of amino acids was conducted in homogeneous solution phase without the need for much excess reagent and capping technique. Importantly, this approach made possible efficient peptide block couplings. We believe that this new approach has the potential to be used for the large-scale synthesis of peptide theraputics and likely also for other biopolymer syntheses.

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Supporting Information Available: Experimental details, characterization data of compounds, copies of NMR spectra of ionic oligomers, ionic-oligomer-supported peptides, and cleaved protected peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) For discussion of segment coupling in peptide synthesis, see: (a) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065. (b) Kates, S. A.; Albericio, F., Eds. *Solid-Phase Synthesis, A Practical Guide*; Marcel Dekker: New York, 2000; pp 377−418.

(19) HPLC was equipped with reversed phase C_{18} column (Agilent Zorbax Extend-C18, 4.6 × 250 mm^2). The elution was in a linear gradient fashion from 50% to 100% of B in A over 20 min, followed by a linear gradient elution from 100% to 50% of B in A over 10 min, where A was H_2O/0.05% TFA (v/v) and B was CH_3CN/0.05% TFA (v/v), at a flow rate of 0.8 mL/min. UV absorbance was detected at 210 nm.