A Versatile Reagent to Synthesize Diverse Ionic Liquids Ranging from Small Molecules and Dendrimers to Functionalized Proteins

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Experimental section

Material. All solvents were dried and freshly distilled prior to use (CH_2Cl_2 with CaH_2). All chemicals were purchased from Aldrich, Acros, Sigma or CHEM IMPEX as highest purity grade and used without further purification. All reactions were performed under nitrogen atmosphere. NMR spectra were recorded on a Varian INOVA spectrometer (for ^1H and ^{13}C at 400 and 100.6 MHz, respectively). Chemical ionization mass spectra were obtained on a Hewlett-Packard HP 5988A spectrometer using NH_3. Fast atom bombardment mass spectra (FABMS) were obtained on a JEOL JMS-SX102A spectrometer using a 3-nitrobenzyl alcohol matrix. MALDI experiments were performed on a Kratos Shimadzu Axima CFR Plus Maldi TOF. Elemental analysis was obtained from Atlantic Microlab, Inc. Microwave reactions were performed with a CEM Discover microwave.

TEA = triethylamine, DCC = dicyclohexylcarbodiimide, DMF = *N*,*N*-dimethylformamide, DCU = 1,3-dicyclohexylurea, HOBT = hydroxybenzotriazol, NHS = N-hydroxysuccinimide. Carbonate buffer was at pH = 8.0.

Tri-n-butyl(carboxypropyl)phosphonium chloride 3

In a glove box, 1 eq. of tributylphosphine (1.57 g, 7.76 mmol) was mixed with 1eq. of 4-chlorobutyric acid (952 mg, 7.76 mmol) in a microwave tube. The tube was then microwave irradiated for 15 minutes at 60 °C with a power setting of 250W. Compound **3** was obtained in 99% yield. ^1H NMR ($CDCl_3$): δ 0.95 (t, 9H), 1.53 (m, 12H), 1.90 (m, 4H), 2.29-2.58 (m, 6H), 2.69 (t, 2H). ^{13}C ($CDCl_3$): δ 14,07 (CH_3), 17.92-27.97 (CH_2), 35.09 (CH_2-CO), 174.65 (COOH). ^{31}P ($CDCl_3$): 21.42

Tri-n-butyl[carboxy(succinimidyl)propyl)phosphonium chloride 2

At 0 °C, compound **3** was dissolved in dry DCM then 1.1 eq of NHS (525 mg, 4.56 mmol), 1.1 eq of DCC (939 mg, 4.56 mmol) and a catalytic amount of HOBT were successively added. The mixture was allowed to stir at room temperature for 10 hrs. DCU was filtered and the filtrate was added drop-wise to a cold ether solution. The oily residue obtained was collected, dissolved in DCM and precipitated in ether. This procedure was repeated three times to afford compound **2** (1.50 g, 87%). ^1H NMR ($CDCl_3$): δ 0.95 (t, 9H), 1.53 (m, 12H), 2.11 (m, 2H), 2.39 (m, 6H), 2.66 (m, 4H), 2.87 (s, 2H), 2.94 (t, 2H). ^{13}C ($CDCl_3$): δ 14,07 (CH3), 17.92-27.97 (CH2), 35.09 (CH2-CO), 174.65 (COOH). ^{31}P ($CDCl_3$): 21.40. HRMS: 386.243 *m/z* $(MCl)^-$ (theory: 386.246 *m/z* (MCl^-)). Elemental analysis: theory: C, 62.15; H, 9.65; N, 3.62; O, 16.56; P, 8.01 found C, 62.34; H, 9.15; N, 3.58; O, 16.99

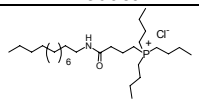
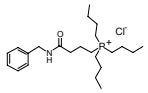
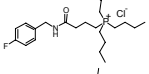
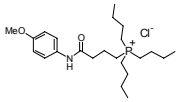
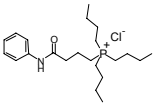
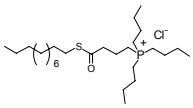
General procedure for the synthesis of compounds **4-9:**

The nucleophile was dissolved in dry DCM, 1 eq of TEA and 1.2 eq of compound **2** were successively added. The mixture was allowed to stir for 12 hrs; the yields were then determined by ^{1}H NMR ($CDCl_3$) by integrating respective peaks for the starting material and product. We used the alpha methylenic protons for compounds **4**, **5**, **6** and **9**, the methoxy protons for compound **7,** and the aromatic protons for compound **8**.

Detailed procedure for compound **10**:

1eq (100 μL) of [G3]-PAMAM (20% in methanol) was dissolved in 5 mL of carbonate buffer (pH 8), and 48 eq.(1.5eq./amine function) of compound **2** were added. The mixture was stirred for 2 days. The modified dendrimer was then purified on sephadex (Sephadex G25 M, phosphate buffer pH 8). MALDI mass spectrometry analysis using a CHCA matrix was performed. One drop of the product was dissolved in 0.5 mL of methanol and analyzed on a MALDI-TOF plate in comparison with the starting [G3]-PAMAM and indicated that 32 of the 32 amine functions were coupled with compound **2**. The mass spectrometry data was consistent with previous reports on PAMAM (*Macromolecules* **2007**, *40*, 5599-5605).

Table 1. Mass Spectrometry (**4-9:** positive ionization mode, **10:** MALDI-TOF)

Product	#	MS
	**4**	Theo : 456.433 Exp : 456.426
	**5**	Theo : 378.558 Exp : 378.289
	**6**	Theo : 396.283 Exp : 396.269
	**7**	Theo : 394.287 Exp : 394.284
	**8**	Theo : 364.276 Exp : 364.276
	**9**	Theo : 459.378 Exp : 459.359
[G3]-PAMAM-(NHC(O)-IL)$_{32}$	**10**	Theo : 16733 Exp : 16816

Detailed for procedure for lysozyme modification:

1eq (100 mg) of lysozyme chicken egg white was dissolved in 8 mL of carbonate buffer (pH 8), and 3 eq. (3eq./amine function of lysozyme) of compound **2** was added every two hours over a six hour period. The mixture was stirred for 2 days. The modified protein was then purified by dialysis (3400 Mw cutoff) for 24 hours. MALDI mass spectrometry analysis using a CHCA matrix was performed. One drop of the product was dissolved in 0.5 mL of methanol and analyzed on a MALDI-TOF plate in comparison with the native lysozyme. 7 of the 7 amine (6-lysines and terminal amine) functions were coupled with compound **2**.

Enzyme Assay.

Lysozyme enzyme activity was measured with the Molecular Probes Enzchek® Lysozyme Assay Kit per manufacturer's guidelines, using enzyme concentrations ranging from 0.005 nM to 500 nM. Green fluorescence was detected on a Molecular Devices SpectraMax M5 fluorescent plate reader, at an excitation of 494 nm and an emission of 518 nm. This is a well-characterized fluorescence-based assay that involves *Micrococcus lysodeikticus* cell walls which have been extensively modified with fluorescein such that the fluorescence is quenched. Cleavage of the β-(1-4)-glucosidic linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine of the cell wall by lysozyme afforded an increase in fluorescence and thus a measure of enzymatic activity