Review

Ionic Liquids: New Targets and Media for #-Amino Acid and Peptide Chemistry

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Ionic Liquids: New Targets and Media for α-Amino Acid and Peptide Chemistry

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1. Introduction

Owing to their unique properties, ionic liquids nowadays are very fascinating for chemists in various fields. Besides their potential for green chemistry, mainly due to their low vapor pressure, reexamination of organic synthesis in these new media led to a shining series of convincing examples of increases in chemical yields, chemo-, regio-, and stereoselectivity, as well as recycling of catalysts. Most of these studies have been recently reviewed by prominent scientists in the field.[1−7] Beyond this impressive series of successes it can be noted that most organic syntheses can be performed at least with equal efficiency in ionic solvents as in molecular solvents. Nevertheless, a new question emerges: in which chemistry could ionic liquids afford more than an alternative reaction medium? In other words, could ionic solvents be used for doing what is either impossible or extremely difficult in molecular solvents? In this context, two new fields emerge: first, the field of ionic liquids and chirality, including the search for new chiral ionic liquids (CIL) and their use in various applications,[8−12] and second, the synthesis and behavior of biomolecules in ionic liquids.[13−19]

Herein we wish to review the cross-fertilizing topics of α-amino acid/peptide chemistry and that of ionic liquids. Indeed, we assume that these new media could be particularly suitable for amino acid and peptide synthesis or transformations since the polarity of ionic solvents is ideal for such species. Also, it is now well established that enzyme catalysis is remarkably efficient in such media.[20] Reciprocally, amino acids represent a powerful class of starting materials for construction of new ionic liquids, especially chiral ones, because of their availability in both enantiomeric forms at a reasonable cost. The scope of the review is thus limited to the chemistry of α-amino acids and their simple derivatives such as amino alcohols, amino esters, *N*-protected amino acids, and small peptides in relation to ionic liquids.

Most of the literature summarized below has only been published during the last few years, emphasizing the novelty of these approaches and the interest of chemists in this new research field. This review has the ambition to give an exhaustive overview of published literature up to 2007. "Amino acids for ionic liquids and ionic solvents for peptide chemistry": a new emergent paradigm?

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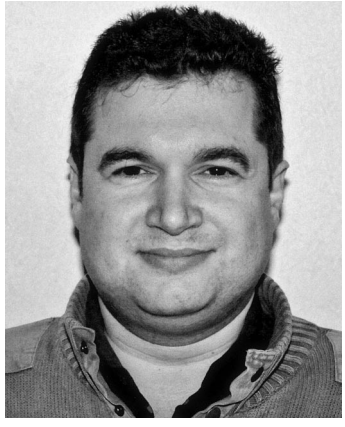
Annie-Claude Gaumont studied chemistry at the University of Rennes (France) and joined the research group of Dr. J. M. Denis, graduating with a Ph.D. in 1991. She had postdoctoral experience as a research associate with Professor J. M. Brown at the University of Oxford. From 1992 to 2000 she was appointed by CNRS at the University of Rennes. Since 2001 she has been Professor in Organic Chemistry at the University of Caen. Her research interests concern the design of phosphorus and sulfur ligands, development of new catalytic reactions for C–P and C−S bond formation, chemistry of phosphine−borane complexes, development of new media such as ionic liquids, and synthesis of analogues of biomolecules bearing a sulfur or phosphorus atom.



Frédéric Guillen obtained his Ph.D. degree in 1999 from Paris XI University (Orsay, France) working with Professor Jean-Claude Fiaud. He moved to Geneva as a postdoctoral fellow in Professor Alexandre Alexakis group and then back to Orsay with Professor Yves Langlois. He his currently Maître de Conférence at Rouen University. His research interests focus on asymmetric synthesis and catalysis and ionic liquids.

2. Ionic Liquids from Amino Acid Chemistry

There are two main strategies to prepare chiral ionic liquids: asymmetric synthesis or use of a chiral starting material. For both economy and facility, using substrates derived from the chiral pool is the most convenient approach.[10,12,25] Numerous groups have selected this methodology to construct either the chiral anion,[26] the chiral cation, or both in the ionic liquid (Figure 1). Amino acid derivatives (36%) have been preferred for this purpose over amines and alkaloids (12%), terpenes (16%), and hydroxy acid (12%) derivatives (Figure 2).

The CILs can be constructed without modification of the amino acid residue, with modification of the side chain and preservation of the amino acid moiety, with alteration of one function (acidic or basic), or with polyfunctional modification of both amine and acid functions (Figure 3).

Jocelyne Levillain was born in Saint-Lô (France) in 1963. She studied chemistry at the University of Caen and in 1994 completed her Ph.D. thesis under the supervision of Dr. M. Vazeux. After a post-doctoral stay at the Royal College of Surgeon in Ireland with Professors K. Nolan and D. Fitzgerald, she returned to Caen University as "Maître de Conférences" in J. L. Ripoll's group, working on flash vacuum thermolysis. In 2001 she joined the group of Professor A.-C. Gaumont, where she is now involved in the chemistry of chiral ionic liquids.

Catherine Malhiac was born in 1962 in Toulouse. She received her Ph.D. degree in 1993 in the laboratory of organic synthesis (Professor J.-C. Combret, Rouen University). In 1997 she joined the laboratory of organic and macromolecular chemistry of Le Havre University, where she works in the "Interactions and interface in polymeric systems" group. Her research interests now focus on polysaccharides interactions studies, aroma compounds, and chromatographic analysis.

2.1. Without Transformation of the Amino Acid Moiety

Amino acids can be straightforwardly used to design chiral anions or chiral cations by deprotonation of the carboxylic acid or protonation of the amino group using a suitable Bronstedt base or acid, respectively. The required properties for the CILs, such as the viscosity or the melting point, can be fine tuned by the choice of the organic or inorganic acid or base as well as by protection of the other functions in the amino acid derivative. Obviously, the chirality arising from the starting material is kept in the ionic liquid.

2.1.1. Amino Acid as Cationic Part

Kou's group prepared a series of chiral ILs from natural amino acids by a simple atom economic reaction. Protonation by a strong acid (HCl, HNO_3, HBF_4, CF_3COOH, H_2SO_4) of amino acids **1**−**6** enables access to the corresponding ionic structures **7**−**12** (Scheme 1). The thermal stabilities of the ILs **7**−**12** generally range from ca. 160 to 240 °C, as

Jean-Christophe Plaquevent was born in 1953 in Normandy, France. In 1977 he joined the CNRS at Rouen University, where he completed his thesis in 1980 in the laboratory of L. and P. Duhamel. He had postdoctoral experiences as a research associate with Professor A. Eschenmoser at ETH Zürich and at Rouen University where he studied molecular biology with Dr, H. Vaudry. He recently moved to Toulouse, where he is still employed by CNRS. His research interests focus on asymmetric synthesis, pharmaceutical compounds, amino acid and peptide chemistry, and ionic liquid methodologies.

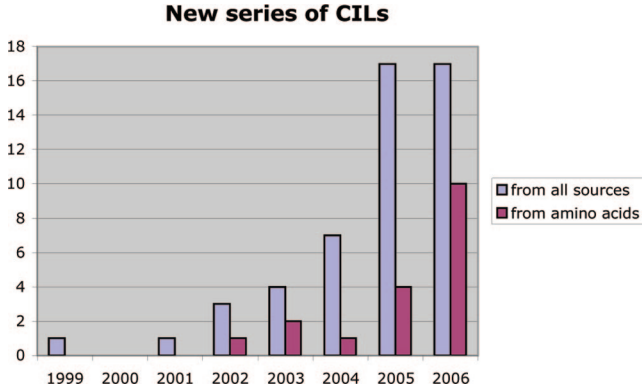


Figure 1. New series of CILs published from 1999 to 2006 (blue, chirality arising from all sources; pink, from amino acids).

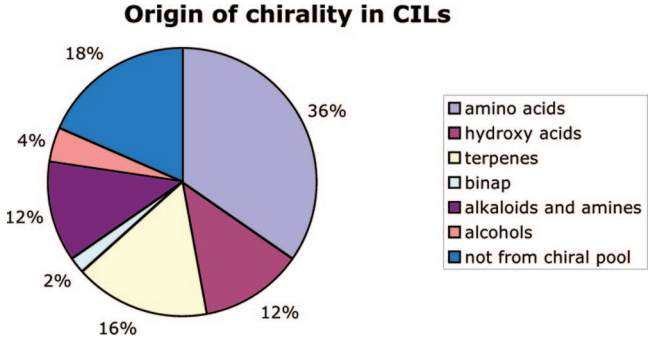


Figure 2. Source of chirality for CILs published from 1999 to 2006.

determined by thermogravimetric analysis (TGA) experiments (Table 1). The authors report physical properties such as optical rotations and densities (from 1.02 to 1.86 $g \cdot cm^{-1}$ at 20 °C) but give no yield except for compound **12b** (100%). Most of the salts have high melting points as determined by differential thermal analysis (DTA), resulting from strong hydrogen bonding due to the carboxylic group.[27]

To minimize hydrogen bonding and decrease the melting point, Kou prepared a variety of alkyl amino acid ester salts from the hydrochloride parents (Scheme 2). All salts have melting points lower than 105 °C; for seven of them the melting point is below 0 °C (Table 2).

Salts **27**−**40** are heat stable over 139 °C, except for salts **29g** and **30g** which decompose at 77 and 82 °C, respectively. Optical rotation is given for each salt, and their density ranges from 1.15 to 1.72 $g \cdot cm^{-1}$ as determined at 20 °C. High viscosities are observed in particular for compound **30a** and **32** (Table 2). It is worth noting the acidity of compound **29a** for which a 1 M aqueous solution has a pH value of 3.5, showing weak Bronsted acidity.

These new salts are miscible with water, ethanol, and acetone and immiscible with ethyl acetate, ether, benzene, and hexane. Except for salt **31** and proline derivatives **38**−**40**, all are immiscible with chloroform.

Esterification of the acidic function proved to increase the biodegradability of the CILs. Among the anions, the less toxic [NO_3] anion and the saccharinate anion, which is a well-known food additive, can be used in pharmaceutical applications. Accordingly, these new ILs **41**−**47** having one of these two anions associated to a cationic amino acid ester [AAE] were termed "fully green" ionic liquids.[28]

Salts **41**−**47** have high viscosities due to the presence of the saccharinate anion (Table 3). The thermal stabilities determined for 20 ionic liquids [AAE][NO_3] and [AAE][sac] range from 150 to 230 °C (under N_2). Each new chiral IL has a solid−solid transition temperature. One-half of them have negative melting points as determined by DSC, and all [AAE][sac] are liquid at room temperature with mp below 8 °C (Table 3).

2.1.2. Amino Acid as Anionic Part

Ohno et al. prepared a library of 20 ionic liquids from imidazolium bromide salt **48**. Use of an anion-exchange resin gave access to the imidazolium hydroxide salt that was used to neutralize a variety of amino acids. Among the cations, [emim] showed the best results in anion exchange (Scheme 3, Table 4).[29]

These CILs are liquid at room temperature. Glass-transition temperatures ranging from −65 to 6 °C were measured. Except for [emim][Cys], which decomposes at 173 °C, the new CILs are stable up to 200 °C. Solubility and miscibility of the new ILs depend on the side chain of the amino acid. Nevertheless, all of them are immiscible with ether and miscible with methanol or acetonitrile. When they contain an acidic side chain (Glu, Asp) they are immiscible with chloroform. They can dissolve their parent amino acid (as an example [emim][Val] dissolves 2% of (*S*)-valine at 25 °C). Conductivity studies showed in most cases a linear correlation with T_g measurements. However, ILs with anions containing functionalized side chains derived from Arg, His, Trp, Asn, Asp, Gln, and Glu have low ionic conductivities.

To extend the methodology, alanine salts with different cations (tetraalkylammonium, pyrrolidinium, tetraalkylphosphonium) were also investigated and compared to [emim] salt **51**.[30] No yield was given for the synthesis. Thermal properties of salts **70**−**73** were fully studied. They all showed a glass transition at temperatures ranging from −70 to −40 °C (Table 5).

Interestingly, salt **73** with a phosphonium cation displays a decomposition temperature 120 °C higher than the corresponding ammonium salts. The authors therefore extended their study to a library of amino acid ionic liquids having a tetrabutylphosphonium cation [TBP] (Scheme 4) and compared the effect of the phosphonium and ammonium groups on the physicochem-

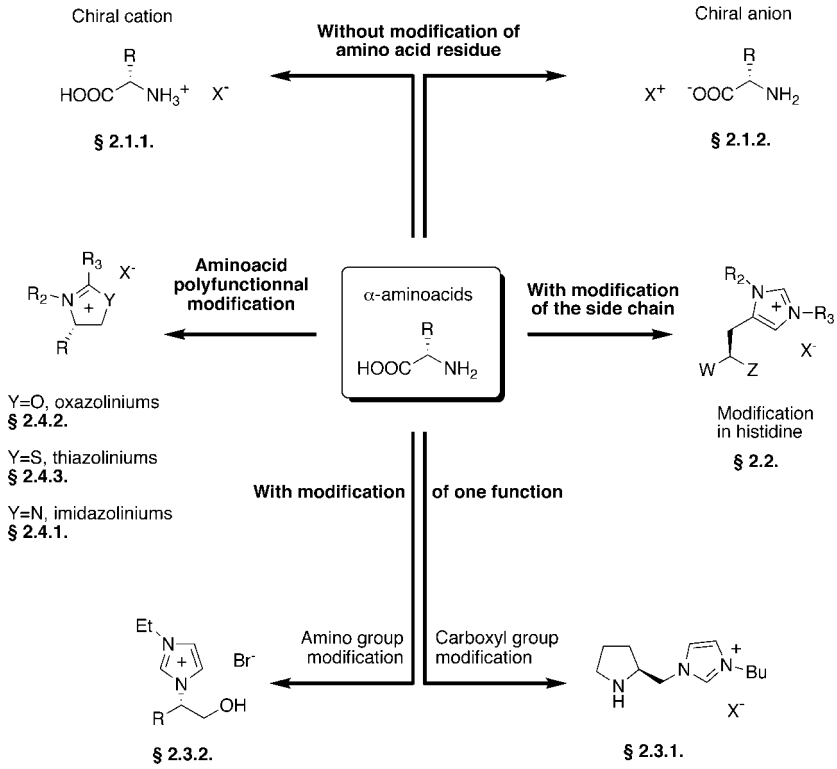


Figure 3. Main strategies for the synthesis of CILs starting from amino acids.

Scheme 1

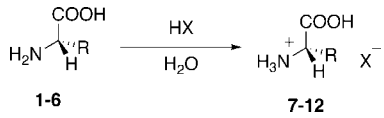


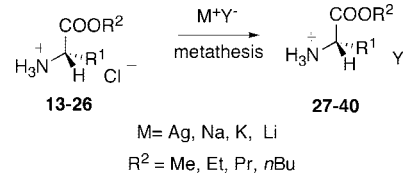
Table 1. Melting Point (T_m), Decomposition Temperature (T_{dec}), and Density of Compounds 7−12

AA	compd	X	T_m/°C[a]	T_{dec}/°C[b]	d_4^{20}/g·cm^{-1} (±5%)
1Gly	**7a**	Cl	186	195	1.40
1Gly	**7b**	NO_3	111	192	1.22
1Gly	**7c**	BF_4	116	220	1.51
1Gly	**7d**	PF_6	101	157	1.37
2Ala	**8b**	NO_3	159	168	1.26
2Ala	**8c**	BF_4	78	241	1.44
2Ala	**8d**	PF_6	*d*	176	1.33
2Ala	**8e**	TFA	82	119	1.02
2Ala	**8f**	1/2SO_4	141	193	1.55
3Val	**9**	NO_3	134	169	1.06
4 Ile	**10**	NO_3	105	167	1.05
5Thr	**11**	NO_3	*d*	147	1.86
6Pro	**12b**[c]	NO_3	−18[d]	138	1.38
6Pro	**12c**	BF_4	76	236	1.63
6Pro	**12d**	PF_6	*d*	168	1.56
6Pro	**12e**	TFA	78	192	1.48
6Pro	**12f**	1/2SO_4	92	206	1.58

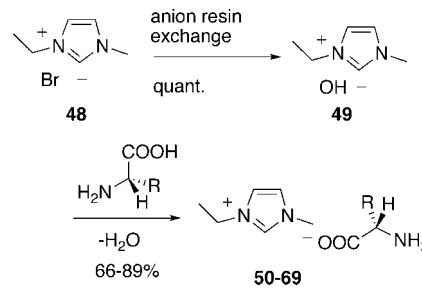
[a] Determined by DTA. [b] Determined by TGA. [c] Physical properties of **12b** were described in ref 28. (Glass-transition temperature T_g = −45 °C, Viscosity = 5140 ± 3% cP at 30 °C.) [d] No melting point observed.

ical properties in the [TBP][AA] and [emim][AA] ILs, respectively.[30] As expected, thermal decomposition of phosphonium ILs occurred at higher temperature (292 °C for [TBP][Leu] **77** compared to 220 °C for [emim][Leu] **55**). [TBP][AA] ILs present a linear relationship between viscosity and T_g as already observed in [emim][AA] ILs (Table 6). These phosphonium salts display lower viscosities and are liquid at room temperature, showing lower glass-transition temperatures than their ammonium congeners. All [TBP] ILs were shown to be halogen free.

Scheme 2



Scheme 3



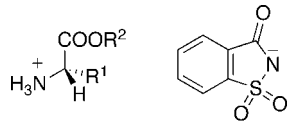
Ohno's group then modified the structure of the amino acid anion to gain access to hydrophobic ionic liquids.[31,32] Introduction of a trifluoromethanesulfonyl group on the amino group together with esterification of the acid moiety afforded the expected hydrophobic amino acid derivatives (Scheme 5). Compounds based on leucine, valine, and alanine without functionalization on the side chain were prepared and combined with [TBP][OH] or [bmim][OH]. The new CILs thus prepared are thermally stable up to 237 °C. Five of them are liquid at room temperature with glass-transition temperatures ranging from −69 to −37 °C (Table 7). The [bmim] salts are soluble in water, while the phosphoniums are less hydrophilic. Hydrophobicity strongly depends on the structure of the amino acid derivatives. While alanine salt is slightly miscible with

Table 2. Thermal Properties (T_m, T_g, T_{dec}), Density, Viscosity, and Yields of Compounds 27−40

precursor	AA	R^2	compd	Y	T_m/°C[a]	T_g/°C[a]	T_{dec}/°C[b]	d_4^{20}/g·cm^{-1} (±5%)	viscosity/ cP(°C)	yield[c] (%)
13	Gly	Me	**27**	NO_3	44	−26	178	1.50	92 (70 °C)	88
14	Gly	Et	**28**	NO_3	49	−10	182	1.67		
15	Ala	Me	**29a**	NO_3	61	−34	186	1.26		93
15	Ala	Me	**29b**	BF_4	18[d]	−48[e]	230	1.53	96 (30 °C)	
15	Ala	Me	**29c**	PF_6	*d*	−35	209	1.36		
15	Ala	Me	**29d**	NTf_2	−17[d]	−61[e]	150	1.55		
15	Ala	Me	**29e**	SCN	62	−38	139	1.27	103 (80 °C)	
15	Ala	Me	**29f**	AcO	*d*	−23	172			
15	Ala	Me	**29g**	Lac	38	−24	77	1.20		
16	Ala	Et	**30a**	NO_3	−17[d]	−45[e]	187	1.29	2030 (30 °C)	
16	Ala	Et	**30g**	Lac	75	−29	82	1.19		
17	Val	Me	**31**	NO_3	74	−33	195	1.31		84
18	Leu	Me	**32**	NO_3	75	−31	210	1.15	1550 (80 °C)	87
19	Ile	Me	**33**	NO_3	−14[d]	−36[e]	172	1.35		79
20	Phe	Me	**34**	NO_3	92	−32	224	1.16		83
21	Thr	Me	**35**	NO_3	−12[d]	−32[e]	156	1.72		85
22	Ser	Me	**36**	NO_3	105	−30	179	1.44		89
23	Pro	Me	**37a**	NO_3	−16[d]	−67[e]	159	1.53	186 (30 °C)	88
23	Pro	Me	**37b**	BF_4	*d*	−20	234	1.45		
23	Pro	Me	**37c**	PF_6	*d*	−22	221	1.47		
23	Pro	Me	**37g**	Lac	*d*	−20	140	1.22		
24	Pro	Et	**38**	NO_3	−17[d]	−50[e]	183	1.57		90
25	Pro	Pr	**39**	NO_3	6[c,d]	−71[c,e]	208[c]		398 (30 °C)[c]	87
26	Pro	*n*Bu	**40**	NO_3	−11[d]	−70[c,e]	163[c]		275 (30 °C)[c]	89

[a] Determined by DSC (differential scanning calorimetry). [b] Determined by TGA. [c] Data cited from ref 28. [d] No melting point or solid−liquid transition temperature observed. [e] Solid−solid transition temperature.

Table 3. Thermal Properties (T_m, T_g, T_{dec}), Viscosities, and Yields of ILs 41−47



[AEE][sac]

41-47

compd	AA	R^2	T_m/°C[a]	T_g/°C[b]	T_{dec}/°C	viscosity /cP(80 °C)	yield (%)
41	Ala	Me	−14	−27	184	1380	89
42	Val	Me	−16	−29	185	3040	79
43	Leu	Me	7	−1	223	1050	87
44	Ile	Me	7	−8	177	14500	78
45	Thr	Me	−1	−9	200	55 900	69
46	Pro	Me	−19	−29	190	3320	92
47	Pro	Et	8	−42	212	3020[c]	88

[a] No melting transition or solid−liquid transition observed; determined by DSC. [b] Solid−solid transition temperature; determined by DSC. [c] Measured at 50 °C.

Table 4. Glass Transition (T_g), Ionic Conductivity (σ_i), and Yield in [emim][AA]

compd	AA	T_g/°C[a]	σ_i (S/cm) at 25 °C	yield (%)
50	Gly	−65	5.7×10^{-4}	82
51	Ala	−57	6.4×10^{-4}	86
52	Met	−57	2.4×10^{-4}	78
53	Val	−52	8.8×10^{-5}	79
54	Ile	−52	6.9×10^{-5}	82
55	Leu	−51	8.1×10^{-5}	80
56	Ser	−49	6.5×10^{-4}	79
57	Lys	−47	7.8×10^{-5}	78
58	Thr	−40	1.0×10^{-4}	84
59	Phe	−36	6.0×10^{-5}	83
60	Trp	−31	9.1×10^{-9}	82
61	His	−24	1.0×10^{-7}	78
62	Tyr	−23	4.0×10^{-8}	70
63	Cys	−19	3.5×10^{-5}	77
64	Arg	−18	9.0×10^{-7}	74
65	Asn	−16	1.1×10^{-6}	89
66	Gln	−12	1.7×10^{-7}	66
67	Asp	5	1.7×10^{-9}	89
68	Glu	6	5.0×10^{-7}	80
69	Pro	−48	1.6×10^{-4}	83

[a] Determined by DSC.

Table 5. Thermal Properties (T_m, T_g, T_{dec}) of Alanine Derivatives 51 and 70−73

compd	cation	T_m/°C[a]	T_g/°C[a]	T_{dec}/°C
51	ethylmethylimidazolium	nd	−57[b]	212
70	*N*-hexyl, *N*,*N*,*N*-triethylammonium	nd	−40	150
71	*N*,*N*,*N*,*N*-tetrabutylammonium	76	nd	162
72	*N*-butyl, *N*-methylpyrrolidinium	77	−64	176
73	tetrabutylphosphonium	nd	−70	286

[a] Determined by DSC. nd: not detected. [b] Data cited from ref 29.

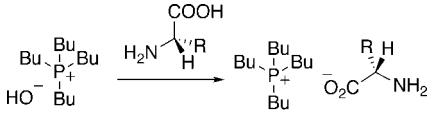
water (ionic phase containing 5.1 wt % water), for leucine salt a water content of only 2.9 wt % was measured by Karl Fischer titration.

Following the same concept, Maschmeyer et al. prepared a series of 15 CILs starting from [TBA][OH] and natural amino acids (Scheme 6).[33]

Apart from [TBA][Asn] **100**, which has a melting point of 42−44 °C, all new salts are liquid at room temperature. They are miscible with water, acetonitrile, acetone, dichloromethane, and chloroform and immiscible with ether, hexane, and toluene. No decomposition was observed after 24 h at 110 °C. NMR analysis showed an upfield shift from 0.07 to 0.66 ppm for the α-CH proton of the TBA carboxylate salt compared to the parent amino acid (Table 8).

N-protected alanine and hydroxyproline were combined with an original guanidinium cation (Scheme 7).[34] The guanidinium salts **112**−**113** were prepared by simple anion exchange and are thermally stable up to 220 °C; they are liquid at room temperature. Glass-transition temperature and viscosity at 25 °C were determined for **112** (T_g = −37.5 °C, η = 749 cP) and **113** (T_g = −32.7 °C, η= 4913 cP).

Scheme 4



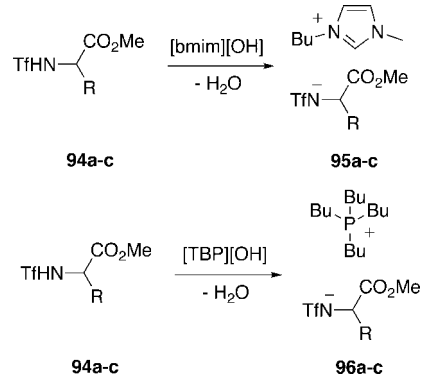
[TBP][OH] **74** [TBP] [amino acid] **75-93**

Table 6. Thermal Properties (T_m, T_g, T_{dec}) and Viscosities of Phosphonium Amino Acid Ionic Liquids 75−93

compd	amino acid	T_m/°C[a]	T_g/°C[a]	T_{dec}/°C	viscosity/ cP(25 °C)
75	Met	nd	−63.5	217	371
76	Gly	13.6	−63.7	293	415
77	Leu	30.0	−63.4	292	389
78	Ile	nd	−60.7	294	605
79	Ser	nd	−59.9	243	902
80	Val	26.0	−59.1	286	423
81	Lys	nd	−58.8	277	779
82	Pro	25.4	−57.5	314	851
83	Thr	nd	−56.1	223	965
84	Phe	8.1	−53.1	288	927
85	Arg	30.7	−36.0	286	*b*
86	Trp	nd	−25.6	316	*b*
87	Gln	nd	−25.0	311	*b*
88	Glu	101.7	−23.3	319	*b*
89	Cys	nd	−20.7	190	3029
90	Asp	nd	−7.6	246	7437(50°C)
91	Tyr	nd	−6.5	294	*b*
92	Asn	83.0	−3.9	224	*b*
93	His	85.9	nd	299	*b*

[a] Determined by DSC. nd: not detected. [b] Solid or glass at 25 °C.

Scheme 5



94a-c **95a-c**

94a-c **96a-c**

Table 7. Thermal Properties (T_m, T_g, T_{dec}) and Viscosities of Salts 95−96

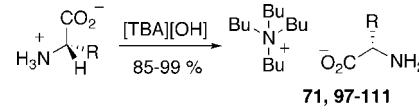
compd	cation	anion derived from	T_m/°C[a]	T_g/°C[a]	T_{dec}/°C	viscosity/ cP(25 °C)
95a	bmim	Ala	nd	−62.8	260	520
95b	bmim	Val	nd	−42.4	253	3080
95c	bmim	Leu	nd	−37.5	237	4180
96a	TBP	Ala	nd	−54.0	253	700
96b	TBP	Val	61.2	−69.2	277	nd
96c	TBP	Leu	13.8	nd	264	2130

[a] Determined by DSC. nd: not detected.

2.2. With Modification of the Side Chain and Preservation of the Amino Acid Moiety

Even though direct reaction with a suitable acid or base is the simplest and fastest way to obtain an ionic liquid from an amino acid, the resulting materials suffer from an obvious sensitivity to pH conditions. Therefore, more sophisticated structures involving nonprotonated cations and weakly basic anions, but retaining the structural integrity of the amino acid, have been devised.

Scheme 6



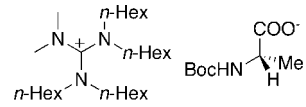
71, 97-111

Table 8. Yields and Δδ for the α-CH in ^{1}H NMR

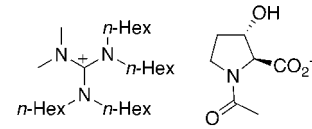
compd	AA	yield (%)	Δδ [a] in ppm (Hz)
71	(*S*)-Ala	98	0.45 (135 Hz)
98	(*S*)-His	85	0.49 (147 Hz)
99	(*S*)-Met	86	0.53 (159 Hz)
100	(*S*)-Asn	74	0.44 (132 Hz)
101	*N*-Ac-(*R*)-Cys	99	0.36 (108 Hz)
102	(*S*)-HGlu	96	0.07 (21 Hz)
103	(*R*)-HGlu	99	0.07 (21 Hz)
104	0.5 (*S*)-Glu	94	0.53−0.63 (159−189 Hz)
105	0.5 (*R*)-Glu	97	0.53−0.63 (159−189 Hz)
106	Gly	93	0.32−0.38 (90 Hz)
107	(*S*)-Phe	95	0.53 (159 Hz)
108	(*S*)-Pro	97	0.66 (198 Hz)
109	(*S*)-Ser	88	0.5 (150 Hz)
110	(*S*)-Thr	91	0.5 (150 Hz)
111	(*S*)-Val	85	0.55 (165 Hz)

[a] δ of α-CH (CIL)- δ of α-CH (starting amino acid)

Scheme 7



112



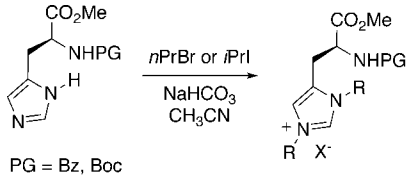
113

For this purpose, the most convergent starting material for synthesis of an amino acid based imidazolium IL is (*S*)-histidine which bears a 4-substituted imidazole ring. Alkylation of both imidazole nitrogen atoms, while leaving the amino and carboxyl groups untouched, provides a chiral bifunctional imidazolium salt whose anion can be subsequently exchanged if needed to adjust the physical and chemical properties of the salt.

Hannig et al. reported the alkylation of bis-protected PG-His-OMe (PG, Bz or Boc) with *n*-propyl bromide to give the corresponding "symmetrical" imidazolium bromide (Scheme 8).[35] Except for **119** (mp = 198 °C), these salts are liquids with melting points in the 40−55 °C range (Table 9). They have been used as chiral carbene precursors in palladium complexes.[35]

Introduction of two different alkyl groups can also be performed by selective protection of one ring nitrogen. Simultaneous protection of the amino group and the *N*-2 ring nitrogen atom via a cyclic urea allowed selective alkylation of O-protected histidine at the *N*-1 position: opening of the cyclic urea by an alcohol followed by a subsequent alkylation gave the fully protected unsymmetrical imidazolium salt.[36] The halide anion was then exchanged with PF_6^-, BF_4^-, or NTf_2^- to give the desired salts **122−124** either as liquids or waxy, low-melting solids (Scheme 9).

Scheme 8



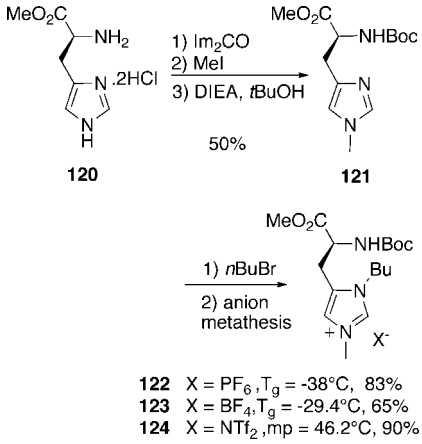
116 PG = Bz, X = I, R = *i*Pr 95%
117 PG = Bz, X = Br, R = *n*Pr 72%
118 PG = Boc, X = I, R = *i*Pr 69%
119 PG = Boc, X = Br, R = *n*Pr 92%

Table 9. Melting Points and Yields of Salts 116−119

compd	PG	X	R	T_m/°C[a]	yield (%)
116	Bz	I	*i*Pr	55	95
117	Bz	Br	*n*Pr	39	72
118	Boc	I	*i*Pr	55	69
119	Boc	Br	*n*Pr	198	92

[a] Determined by DSC.

Scheme 9



122 X = PF_6, T_g = -38°C, 83%
123 X = BF_4, T_g = -29.4°C, 65%
124 X = NTf_2, mp = 46.2°C, 90%

The 1-butyl-3-methyl-substituted histidine-derived salt can be obtained by reversing the order of the alkylation steps. Interestingly, the melting point of the salt having the "reversed" substitution pattern (with X = NTf_2) is much lower (−37 °C) than that for the isomeric 1-methyl-3-butyl-substituted one (46 °C).[37] Both residual functions can then be selectively deprotected, and the ionic amino acid can undergo peptide-coupling reactions at both the N- and C-terminal positions (see section 3.3.3).

2.3. With Modification of Only One Function

In order to gain access to more structurally diverse ionic liquids, one can use either the amino or the carboxyl function of the amino acid as precursor to build the anionic or cationic part of the salt.

2.3.1. Modification of the Carboxyl Group

Cheng's group prepared pyrrolidine chiral ionic liquids in 45% yield starting from (*S*)-proline.[38] Reduction with $LiAlH_4$ in tetrahydrofuran followed by *N*-Boc protection and tosylation in pyridine led to tosyl intermediate **125** in 68% yield. Substitution by the imidazole anion led to the pyrrolidine imidazole **126** in 83% yield. Then, alkylation by *n*BuBr and metathesis by $NaPF_6$ or $NaBF_4$ afforded the new pyrrolidine imidazolium ionic liquids **128**−**129** (Scheme 10).

Similar procedures were used to prepare pyrrolidine salts with a modified imidazolium core using 2-methylimidazole and/or bromoethanol (Scheme 11).

Most of these salts are viscous liquids at room temperature. They are moderately miscible with polar solvents (chloroform, dichloromethane, methanol) and immiscible with diethyl ether, ethyl acetate, and hexane. They were successfully employed as chiral additives in the asymmetric Michael reaction between cyclohexanone and *trans*-*β*-nitrostyrene (25−100% yield with ees between 77% and 99%). The bromide and tetrafluoroborate salts performed the reaction much better than (*S*)-proline itself in ionic liquid in terms of yields, enantioselectivity, and syn/anti diastereoselectivity. The ionic liquid moiety acts as a phase tag for recycling the catalyst and an efficient chiral inductor group to ensure high selectivity.

Luo et al. prepared chiral imidazolium salts **139**−**143** starting from natural amino acids (Ala, Val, Leu, Ile, Pro).[39] Reduction of the amino acid with $NaBH_4/I_2$ followed by bromination with PBr_3 led to chiral compounds **135**−**138** with yields higher than 76%. Alkylation with methylimidazole and then neutralization with NaOH gave the bromide salts **139a**−**143a** in excellent yields (over 92%) (Scheme 12). Anion exchange with $AgBF_4$ gave access to room-temperature ionic liquids **139b**−**143b** that have no melting point but a glass-transition temperature ranging from −49 to −35 °C (Table 10). If anion exchange is performed with KPF_6, the new salts **139c**−**143c** have melting points ranging from 6 to 73 °C. These new ILs are thermally stable up to 210 °C.

2.3.2. Modification of the Amino Group

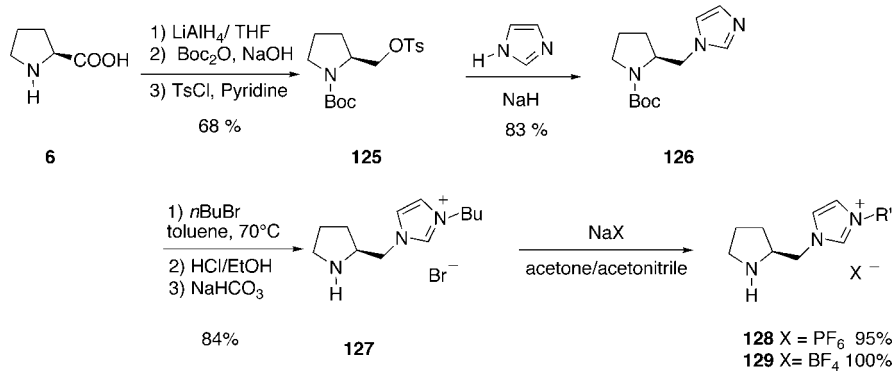
In 2003 Bao's group reported the synthesis of chiral ionic liquids derived from natural amino acids (valine, alanine, leucine) by modification of the amino group.[40] Condensation of amino acids with aldehydes under basic conditions enabled formation of the imidazole ring. Esterification followed by reduction led to the corresponding alcohol, which was then easily alkylated using ethyl bromide (Scheme 13).

The salts have low melting points ranging from 5 to 16 °C, thermal stability up to 180 °C, and good chemical stability toward water. They are miscible with water, methanol, and acetone and immiscible with ether and 1,1,1-trichloroethane.

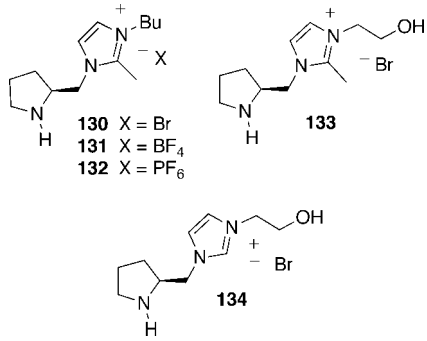
A very similar methodology was used for the synthesis of chiral di-imidazolium molecular tweezers, starting from (*S*)-alanine and (*S*)-phenylalanine (Scheme 14).[41] UV spectrometric titration experiments showed that these chiral di-imidazolium receptors exhibit excellent enantioselective recognition toward (*S*)- and (*R*)-amino acid derivatives in water or acetonitrile.

Chirality on the side chain of the C2 atom of the imidazolium core was first proposed by Li and Headley's team.[42] Condensation of a natural chiral amino alcohol with 1-methyl-2-imidazolecarboxaldehyde **156** is followed by two steps: reduction of the imine by sodium borohydride and then alkylation by bromobutane. Then, imidazolium salts **160**−**164** are transformed into ionic liquids **165**−**179** by anion exchange with KBF_4 in MeOH-H_2O, KPF_6 in H_2O, or $(CF_3SO_2)_2NLi$ in H_2O (Scheme 15). Although no melting point or glass temperature was reported, 4 salts among the 15 are reported to be solid (R = *t*Bu, X = Br or BF_4; R = *i*Bu, X = BF_4), while the others are viscous oils (Table 11).

Scheme 10



Scheme 11



The amino alcohols **157**−**159** when treated by tosylchloride led to a bis-tosylate intermediate that underwent a ring-closure reaction on heating.[43] Exchange of the tosylate anion in **180**−**182** by treatment with $(CF_3SO_2)_2NLi$ in H_2O gave salts **183**−**185** that are liquid at room temperature, while treatment with KPF_6 gave salts with high melting points (>168 °C) (Table 11, Scheme 15).

Ou et al. reported the use of *N*-methylimidazole as a starting material to react with 1-chloro-2,4-dinitrobenzene. A yield of 96% in 1-(2,4-dinitrophenyl)-3-methylimidazolium chloride **189** was achieved. Then (*S*)-amino alcohols were engaged with **189** in a Zincke-type reaction. The authors claimed that chiral imidazolium ionic liquids **190**−**198** were obtained in good yields.[44a] However, it was recently demonstrated by Génisson and co-workers that this study is erroneous.[44b] Indeed, it was demonstrated that only SnAr occurs instead of the expected Zincke process (Scheme 16). Thus, the original paper was recently retracted.

Isocyanate derivatives of amino acids were also used as precursors of a new family of chiral imidazolium ionic liquids.[45] Treatment of aminopropylimidazole with isocyanates **200**−**202** afforded the urea derivatives, which were then methylated with excellent overall yields (>95%). Anion exchange was done by treatment of the iodide salt with potassium tetrafluoroborate in methanol/water and potassium hexafluorophosphate or $(CF_3SO_2)_2NLi$ in water (Scheme 17, Table 12). Most of these ILs are described as yellow oils.

An interesting approach to a new class of reversible ILs was presented by the Weiss group.[46] Exposure to 1 atm of CO_2 gas for less than 15 min of an equimolar mixture of substituted acetamidine and various amino acid methyl esters led to amidinium carbamates with excellent conversion. The produced ILs returned to their initial state when exposed to nitrogen atmosphere (Scheme 18). Except for tyrosine derivatives, all of these compounds were liquid at room temperature. Synthesis of compounds **214** was performed under dry conditions, but the system tolerates the presence of 10% water. However, acid treatment led to rapid loss of CO_2 from the carbamic acid. The amidinium carbamates are thermally stable up to 50 °C under CO_2 atmosphere, but loss of CO_2 gas was important above room temperature under nitrogen or air. The polarity of one compound was determined using a solvatochromic dye (DAPNE) as a probe.

2.4. With Modification of Both Functions

The last option to design ionic liquids from chiral amino acids is to use both the amino and the carboxylic functions to build the cationic moiety. Several groups have selected this strategy, allowing synthesis of salts having imidazolinium, oxazolinium, or thiazolinium cations. The strategy is similar whatever the cation: the two functions of the amino acid precursors are engaged in a series of reactions to form the 4,5-hydrogenated five-membered heterocycles. Alkylation of the ring followed by metathesis leads to the new chiral ionic liquid (Scheme 19).

2.4.1. Imidazoliniums

Imidazolinium-based ILs were prepared from natural amino acid *N*-Boc-(*S*)-valine **215**.[47] Reaction between the protected amino acid and *t*Bu-aniline afforded the amide, which after deprotection and reduction gave the corresponding diamine **216**. The imidazoline cycle was then obtained after formation of the hydrochloride derivative followed by condensation with triethylorthoformate. After alkylation and anion exchange, five imidazolinium ionic liquids **218**−**222** were prepared (Scheme 20). Anion exchange with PF_6 anion led to solid salts, while NTf_2 anion gave a liquid one. Dichloromethane solutions of the salts were washed several times with neutral or acidic water without degradation.

Diastereomeric interactions with racemic Mosher acid salt were proved by ^1H and ^{19}F NMR spectroscopy. Both a hydroxyl group and a bulky group (*t*Bu) are crucial for good separation of the diastereomeric methoxy and CF_3 group NMR signals.

2.4.2. Oxazoliniums

Wasserscheid et al. prepared oxazolinium salts from (*S*)-valinol. Condensation of (*S*)-valinol with propionic acid in xylene led to the oxazoline **223**.[48] After alkylation by an alkyl bromide and then metathesis with HPF_6 the new oxazolinium salts **224**−**225** were obtained in ca. 90% yield (Scheme 21). These new salts were reported to be unstable in aqueous medium, leading to ring opening.

Scheme 12

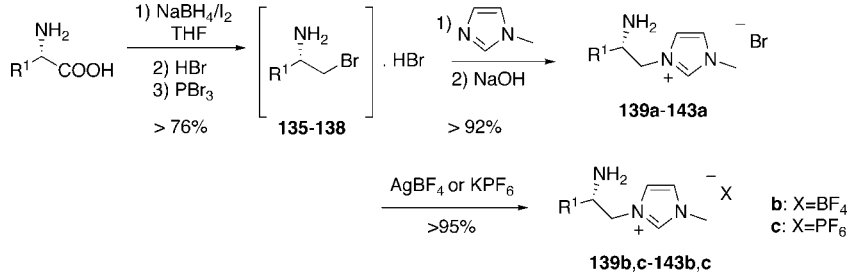


Table 10. Thermal Properties of Salts 139a−143c

compd	derived from	anion	T_m or T_g/°C[a]	T_{dec}/°C[b]
139a	Ala	Br	131 (T_m)	226
139b	Ala	BF_4	−46 (T_g)	261
139c	Ala	PF_6	6 (T_m)	218
140a	Val	Br	145 (T_m)	270
140b	Val	BF_4	−49 (T_g)	281
140c	Val	PF_6	38 (T_m)	287
141a	Leu	Br	134 (T_m)	267
141b	Leu	BF_4	−47 (T_g)	291
141c	Leu	PF_6	69 (T_m)	287
142a	Ile	Br	135 (T_m)	268
142b	Ile	BF_4	−35 (T_g)	285
142c	Ile	PF_6	73 (T_m)	281
143a	Pro	Br	141 (T_m)	257
143b	Pro	BF_4	−45 (T_g)	291
143c	Pro	PF_6	67 (T_m)	274

[a] Determined by DSC. [b] Determined by TGA.

2.4.3. Thiazoliniums

Since 2003 thiazolinium salts were prepared by some of us.[37,49−51] The sulfur atom was introduced via a dithioester reagent. The methyl isopropyldithioformiate was condensed on the appropriate chiral amino alcohol ((*R*)-2-aminobutanol **226**, (*S*)-phenylalaninol **227**) to give the hydroxythioamide intermediate. Mesylation under basic conditions gave access to thiazolines **228** and **229**, respectively. Classical alkylation with different alkyl iodides followed by anion exchange (BF_4, PF_6, NTf_2) led to a variety of thiazolinium salts **235−241** (Scheme 22).

The new salts display interesting physical properties like thermal stability (higher than 170 °C), melting points ranging from 42 to 175 °C for iodide, PF_6, and BF_4 anions, and glass-transition temperatures ranging from −68 to −40 °C for NTf_2 derivatives as measured by DSC (Table 13). No ring opening was observed upon treatment with water (neutral, basic, or acidic) unlike what was observed with oxygen analogs (vide supra). The BF_4, PF_6, and NTf_2 thiazolinium salts remain intact after these treatments. However, with the iodide salt, partial dealkylation was observed (10−20%) under these conditions.

These salts were found to generate diastereomeric interactions in ^1H and ^{19}F NMR with various racemic Mosher acid salts. The best splitting of the two diastereomeric signals was observed with salt **232** and the racemic Mosher acid potassium salt.

All examples reported in section 2 show the prominence of amino acids in the preparation of chiral ionic liquids. However, the opposite is also true. Numerous ionic liquids, because of their unique properties, are the media of choice for the chemistry involving amino acids and their derivatives. These aspects are discussed in the following part of the review.

3. Ionic Liquids for Amino Acid Chemistry

Ionic liquids, which are very polar aprotic solvents, are excellent solvents for dissolving very polar molecules such as amino acids and small peptides among other biomolecules that are beyond the scope of this review. Most of these alternative solvents are immiscible with water, unlike polar molecular solvents that are able to dissolve unprotected amino acids. Thus, various reactions involving amino acid derivatives have been performed successfully in ionic liquid media.

3.1. Catalysis by Means of Amino Acids in ILs

Homogeneous catalysis is probably the field of organic synthesis that has been the most studied in ionic liquids, mainly because of the potential of these new solvents for recycling of the catalyst/solvent system.

3.1.1. Organocatalysis

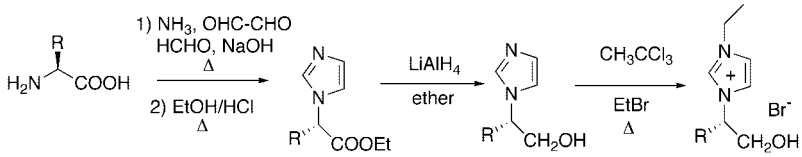
Amino acids such as proline are among the cheapest and most widely used organocatalysts. Since they are readily soluble in ionic liquids, their use as homogeneous catalysts in ionic liquids has been studied since 2002 in various reactions[52] such as aldolizations, Mannich reactions, conjugate additions, aminoxylations, and Knoevenagel reactions.

3.1.1.1. Aldolizations. The proline-catalyzed aldolization reaction in ionic liquids was first reported in 2002 independently by Loh et al. and Toma et al.[53,54] Reaction of benzaldehyde with an excess of propanone in the presence of 30 mol % proline in several imidazolium-based ionic liquids gave the expected aldol products in moderate yield (30−59%) and good ee (58−78%), similar to those obtained in molecular solvents (Table 14, entries 1−4). Up to three recyclings were carried out without significant loss of yield or ee (Table 14, entries 4−7).

In most cases an elimination product was also observed: when [omim][Cl] is used as solvent, the enone is the major product (Table 14, entry 2). The proline-[bmim][PF_6] system was also successfully evaluated with other aldehydes (Table 14, entries 9−20). The amount of catalyst could be as low as 1% with only a small decrease of the yield. Interestingly, cyclohexanone and cyclopentanone behave quite differently in this reaction: whereas reaction of cyclohexanone with *p*-trifluoromethylbenzaldehyde in the presence of proline in [bmim][PF_6] gives almost pure anti diastereomer with high enantiopurity (de > 20:1, ee 93%), cyclopentanone gives a 1:1 syn/anti mixture (anti 32% ee; syn 86% ee).

Reaction of aldehydes with 1-chloropropanone in [emim][OTf] in the presence of proline regioselectively produces chlorohydrins that can be converted to the corresponding epoxides by reaction with NEt_3 in [emim][OTf] (Scheme 23).[55]

Scheme 13



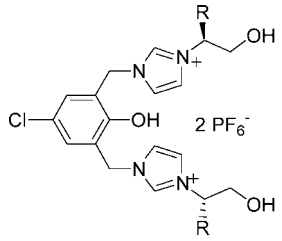
2 R = Me
3 R = *i*Pr
144 R = *i*Bu

145 R = Me 70%
146 R = *i*Pr 68 %
147 R = *i*Bu 65%

148 R = Me 59%
149 R = *i*Pr 60 %
150 R = *i*Bu 57%

151 R = Me 80%, mp = 5-6°C
152 R = *i*Pr 82%, mp =11-12°C
153 R = *i*Bu 81% mp = 15-16°C

Scheme 14



155 R = Me
154 R = Bn

The ionic liquid [bmim][PF_6] also proved to be a good solvent for the proline-catalyzed asymmetric aldol reaction of propionaldehyde (Scheme 24).[56]

Less than 5% of catalyst was required, and the aldol product was obtained with a good diastereoselectivity (anti/syn 5:1) and complete enantioselectivity for the major diastereomer. The ionic liquid/catalyst system could be recycled up to 4 times without loss of either yield or selectivity. An improved solvent system in which DMF was added was used successfully for direct cross aldol reaction of aldehydes with complete enantioselectivity and good to excellent diastereoselectivity depending on the substrates (Table 15).

[Bmim][PF_6] was also successfully used for the synthesis of pyranose analogues via two successive aldol reactions (Scheme 25). Surprisingly, although diastereoselectivity (19.1) is excellent, enantioselectivity is rather low (49%).

The proline-derived (*S*)-*N*-((1*S*,2*S*)-2-hydroxy-1,2-diphenylethyl)-pyrrolidine-2-carboxamide **242** was also used as a catalyst for the asymmetric aldol reaction in [bmim][BF_4] (Table 16).[57]

Excellent yields and ees were obtained in the reaction of various (mostly aromatic) aldehydes with propanone at 0 °C. In all cases, yields and ee were equivalent to or greater than those obtained at −25 °C in excess propanone as solvent (Table 16). The catalytic system was reused twice without any loss in yield or ee.

Similarly, (4*S*)-phenoxy-(*S*)-proline **243** catalyzes the aldol reaction between propanone and various substituted benzaldehydes (Table 17).[58]

As low as 1 mol % catalyst was used to obtain both excellent yield and ee with *o*-nitrobenzaldehyde (entry 1). The catalyst/IL system was reused up to 3 times without significant loss in yield and ee using 5 mol % catalyst.

In order to improve ease of use and recycling of the catalyst, some supported ionic liquids have also been used as immobilization media for proline.[59,60] Reaction of tris-(methoxy)silyl-substituted ionic liquids having various cations and anions with pretreated silica gel gave a range of covalently bonded supported ionic liquids (Scheme 26) to which proline was subsequently added.

The supported catalysts **244**−**246** were assessed in the aldol reaction of benzaldehyde with propanone. The best results, in terms of both yield (55%) and enantioselectivity (70%), were obtained with the pyridinium-based supported IL having the tetrafluoroborate anion. It should be noted that for all these supported catalysts crotonization is the major issue with an enone/aldol ratio ranging from 17/83 to 46/54. Using electron-poor substituted benzaldehydes the authors were able to reduce the amount of crotonization product while enhancing the yield. Up to five recyclings of the catalytic system with only a slight decrease in yield and ee were carried out with a modified system including an added unsupported IL.

Miao and co-workers reported the synthesis of ionic liquid supported proline catalysts and used them in asymmetric aldol reaction.[61] The presence of the carboxylic acid function is essential for the activity and selectivity of the catalyst: proline-derived catalyst **247** performed poorly in this reaction, whereas hydroxyproline-derived **248** gave better yield and ee than free proline (Scheme 27). Use of catalyst **248** was studied on several aromatic aldehydes in neat acetone and DMSO: whereas free proline usually performs better in DMSO than in acetone, there is only a slight difference for these two solvents when the reaction is catalyzed by **248**. Use of DMSO can therefore be avoided in this reaction. As **248** is not soluble in dichloromethane, it can easily be recycled after reaction by concentration of the reacting mixture followed by extraction with dichloromethane. The catalyst was reused 3 times with comparable yield and ee values. Lombardo and co-workers[62] used the related catalysts **249** and **250** (Scheme 27) in various ionic solvents in the aldol reaction of *p*-nitrobenzaldehyde with acetone. The reaction performed well in each tested IL (62−85% yield, 80−85% ee); however, a recycling experiment showed a drop in yield on the third cycle.

Also starting from 4-hydroxyproline, Wang and co-workers synthesized CIL **251** (Scheme 27) in which the catalytically active proline moiety is linked to the imidazolium part via an ether function:[63] use of 10 mol % of **251** promoted the aldol reaction of various aromatic aldehydes with acetone in [bmim][BF_4] with satisfactory yields (53−94%) and ees (65−93%). After washing and drying, the catalyst/IL system was recycled 6 times with only minor decreases in product yield and constant ee.

3.1.1.2. Mannich Reactions. In 2003 Barbas et al. used [bmim][BF_4] and [bmim][PF_6] as solvent for the proline-catalyzed Mannich reaction of *N*-PMP-protected α-imino ethyl glyoxylate with various aldehydes and ketones (Table 18).[64]

The Mannich adducts were obtained in very good yield and excellent diastereomeric and enantiomeric ratio (de > 19:1 except for hexanal (13:1) and 3-methylbutanal (5:1); ee > 93%, typically > 99%). The catalytic system (IL + proline) was reused 4 times with cyclohexanone without any loss of de and ee, although with a slow fall of the yield. The three-component Mannich reaction was also investigated in

Scheme 15

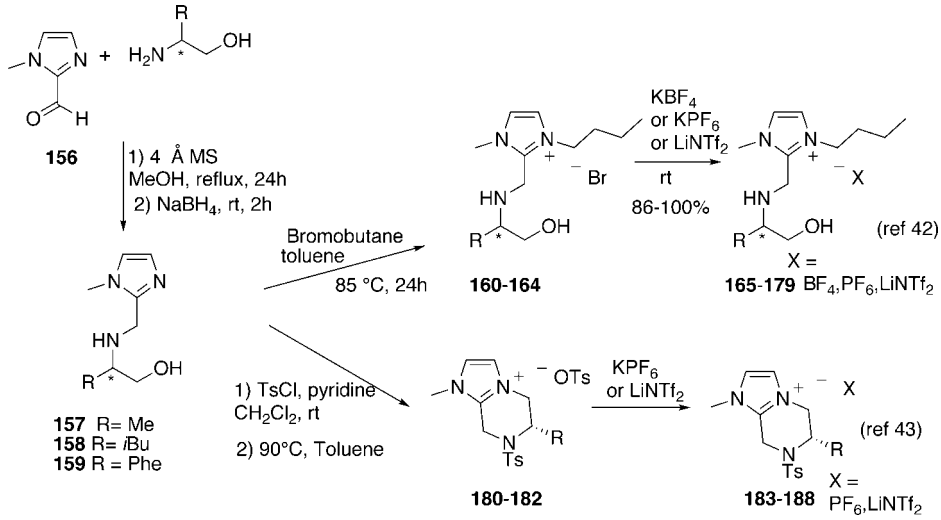


Table 11. Yield of Salts 160−188

compd	derived from	anion	yield (%)
160[a]	(*S*)-Val	Br	82
161[a]	(*S*)-Leu	Br	77
162[a]	(*R*)-Leu	Br	80
163[a]	(*S*)-2-amino-3,3-dimethylbutanoic acid	Br	90
164[a]	(*S*)-Phe	Br	75
165[a]	(*S*)-Val	BF_4	92
166[a]	(*S*)-Val	PF_6	86
167[a]	(*S*)-Val	NTf_2	95
168[a]	(*S*)-Leu	BF_4	91
169[a]	(*S*)-Leu	PF_6	87
170[a]	(*S*)-Leu	NTf_2	93
171[a]	(*R*)-Leu	BF_4	91
172[a]	(*R*)-Leu	PF_6	95
173[a]	(*R*)-Leu	NTf_2	93
174[a]	(*S*)-2-amino-3,3-dimethylbutanoic acid	BF_4	95
175[a]	(*S*)-2-amino-3,3-dimethylbutanoic acid	PF_6	100
176[a]	(*S*)-2-amino-3,3-dimethylbutanoic acid	NTf_2	98
177[a]	(*S*)-Phe	BF_4	95
178[a]	(*S*)-Phe	PF_6	94
179[a]	(*S*)-Phe	NTf_2	92
180[b]	(*S*)-Val	OTs	91
181[b]	(*S*)-Leu	OTs	92
182[b]	(S)-Phe	OTs	90
183[b]	(*S*)-Val	NTf_2	95
184[b]	(*S*)-Leu	NTf_2	92
185[b]	(*S*)-Phe	NTf_2	92
186[b]	(*S*)-Val	PF_6	96
187[b]	(*S*)-Leu	PF_6	95
188[b]	(*S*)-Phe	PF_6	95

[a] Reference 42. [b] Reference 43.

ionic liquids (Table 19). Results similar to those obtained in molecular solvents were reported.

3.1.1.3. Michael Reactions. In 2004 Kotrusz and co-workers reported the use of [bmim][PF_6] as solvent in the organocatalyzed Michael addition of aldehydes and ketones to β-nitrostyrene (Table 20).[65]

Among the various catalysts studied, proline showed the best activity (Table 20, entries 1−3). The reaction was usually run at room temperature, but the less reactive substrates required heating to 70−80 °C for the reaction to reach completion (3,3-dimethylbutanal was found to be unreactive even upon heating, entry 5). Study of the catalyst recycling revealed that the yield and de dropped from the first reuse. Enantiomeric excesses ranged from 3% to 60%, as determined by HPLC measurement of "selected samples".

Addition of cyclohexanone on β-nitrostyrene catalyzed by proline was also studied in various ILs.[66,67] The best results were obtained in (methoxyethyl)-methylimidazolium mesylate ([moemim][OMs]) with 75% yield, 90% de (syn), and 75% ee for catalyst loading of 40%. In these conditions reuse of the catalyst/IL system led to the same yield but a decreased ee. The study of this reaction with other aldehydes and ketones revealed that IL and catalyst loading should be optimized for each substrate.[66] An excellent yield of 98% was obtained in [hmim][Cl] at the cost of a lower ee (40%): in this case, recycling of the catalyst/IL system was quite efficient (up to five reuses without loss of ee or yield).[67]

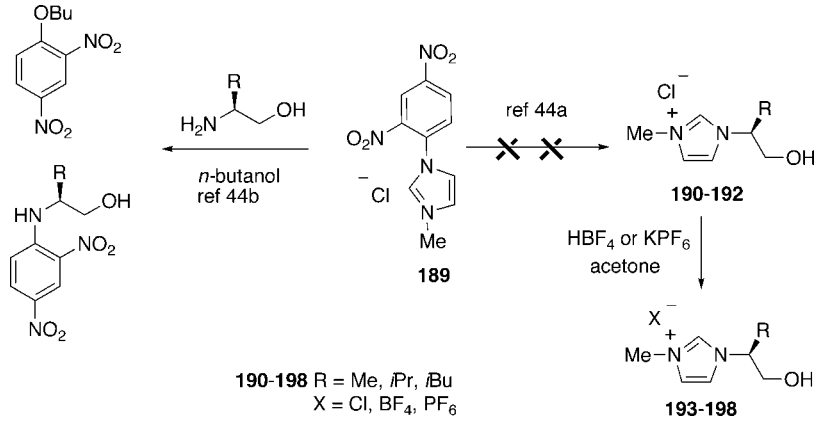
Kitazume and co-workers used a stoichiometric amount of proline to promote addition of ketones to 2-trifluoromethyl acrylic acid phenethyl ester in various ionic liquids (Scheme 28).[68] Although the yields were quite satisfactory, the diastereomeric ratio (when applicable) was close to 1:1 and no enantiomeric excess was reported.

Michael reaction of active methylene coumpounds with enones could also be catalyzed by proline in [bmim][PF_6] at room temperature (Scheme 29).[69] Although the yields are rather good in some cases (i.e., 88% for addition of dibenzoylmethane to methylvinylketone), almost no selectivity was observed in this reaction (ee < 6%).

3.1.1.4. Additions to DEAD. Toma and co-workers performed the enantioselective addition of aldehydes to diethylazadicarboxylate (DEAD) in ionic liquids, catalyzed by proline or proline derivatives. The initial products were directly converted into the configurationally stable oxazolidinones in order to prevent racemization (Table 21).[70]

[Bmim][BF_4] proved to be the best solvent in this reaction (Table 21, entries 1−9). Among the catalysts tested in this study, (*S*)-proline gave the best results, *trans*-4-hydroxy-(*S*)-proline **252** (entry 10) and (*S*)-thiazolidine-2-carboxylic acid **253** (entry 11), affording slightly better ee but a much lower yield even after a prolonged reaction time. Use of serine required increased catalyst loading and higher temperatures and gave disappointing results (entry 12). However, this example is the first one reporting the use of an amino acid other than proline (or a proline analogue or derivative) as organocatalyst in an ionic liquid. With (*S*)-proline as catalyst, the reaction was completed in 1−18 h depending on the aldehyde structure with ee ranging from 70% to 89% (entries 1, 13−16). The reaction rate and enantioselectivity were quite dependent on the structure of the aldehyde: the best results

Scheme 16



Scheme 17

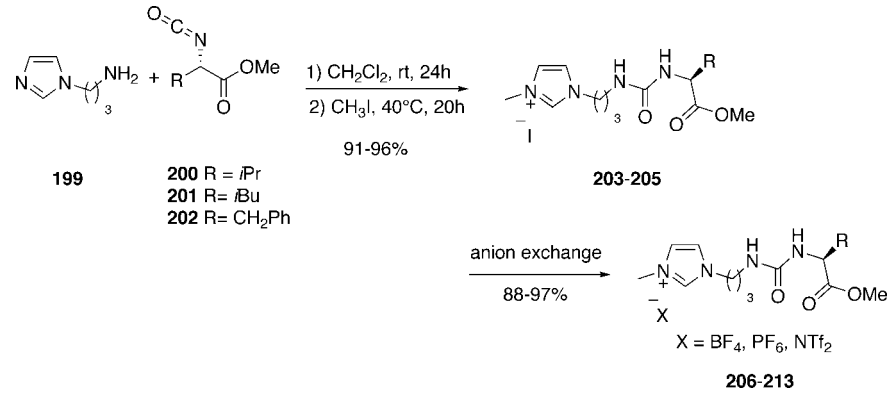


Table 12. Yields of Salts 203−213

compd	derived from	X	yield (%)
203	Val	I	95
204	Leu	I	96
205	Phe	I	99
206	Val	BF_4	92
207	Phe	BF_4	92
208	Val	PF_6	94
209	Leu	PF_6	95
210	Phe	PF_6	95
211	Val	NTf_2	96
212	Leu	NTf_2	97
213	Phe	NTf_2	97

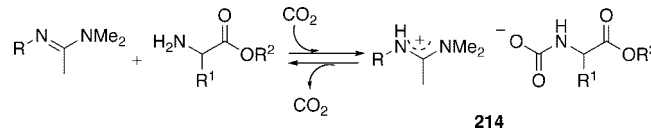
Table 13. Thermal Properties (T_m and T_g) and Yields of Salts 230−241

compd	R^1	R^2	anion	yield	T_m or T_g/°C[a]	ref
230	(*R*) Et	Bu	I	66	137[b]	46
231	(*R*) Et	$C_{12}H_{25}$	I	30	63[b]	46
232	(*S*) Bn	Bu	I	64	172[b]	48
233	(*S*) Bn	Et	I	66	175[b]	48
234	(*S*) Bn	$C_{12}H_{25}$	I	24	106[b]	48
235	(*R*) Et	Bu	PF_6	60	136[b]	46
236	(*R*) Et	Bu	NTf_2	54	−68[c]	46
237	(*R*) Et	Bu	BF_4	60	111[b]	46
238	(*R*) Et	$C_{12}H_{25}$	PF_6	25	42[b]	46
239	(*R*) Et	$C_{12}H_{25}$	NTf_2	25	−67[c]	46
240	(*S*) Bn	Bu	PF_6	44	115[b]	48
241	(*S*) Bn	Bu	NTf_2	46	−40[c]	48

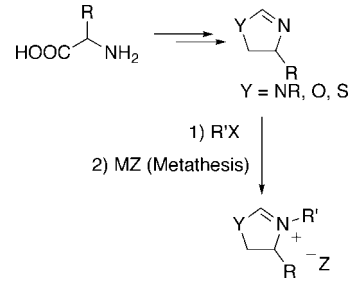
[a] Determined by DSC. [b] T_m. [c] T_g.

were obtained when R was an aliphatic chain (except if R = *t*Bu, entry 15), whereas phenylacetaldehyde gave no measurable ee (entry 17). Although the recycling of the IL/catalyst system was possible, the yield and ee of the reaction dropped from the first reuse. Preliminary studies on ketones showed a much lower reaction rate.

Scheme 18



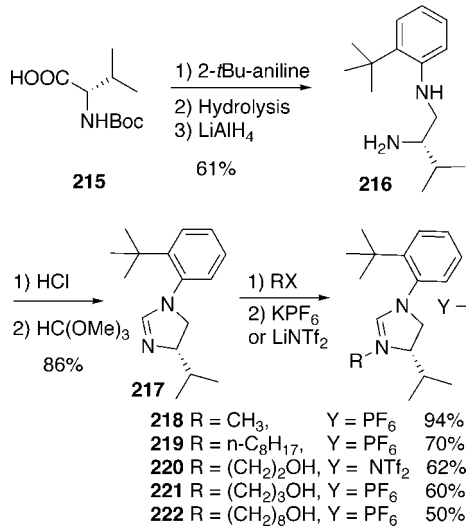
Scheme 19



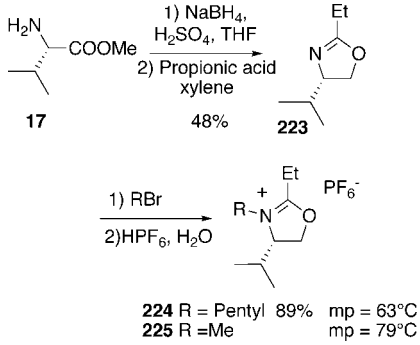
3.1.1.5. Aminoxylation Reactions. Proline has also been shown to catalyze the α-aminoxylation of aldehydes and ketones with nitrosobenzene.[71,72] This reaction can be efficiently carried out in [bmim][BF_4] with 5 mol % of proline (Scheme 30).[71] The catalyst/IL system can be reused up to 5 times without loss of yield or ee.[72]

3.1.1.6. Knoevenagel Reactions. The proline-catalyzed Knoevenagel reaction has also been studied in ionic solvents.[73] Reaction of diethylmalonate with several aromatic aldehydes in [bmim][BF_4], [bmim][PF_6], and [emim][BF_4] gives average to good yields of the desired olefins (Table 22).

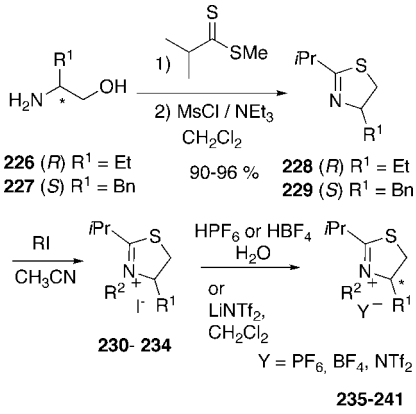
Scheme 20



Scheme 21



Scheme 22



The yields obtained are usually higher than those observed in molecular solvents; moreover, up to three recyclings were done without loss of activity of the catalytic system.

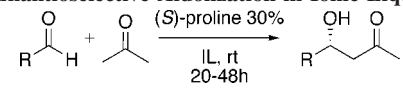
3.1.2. Organometallic Catalysis

In addition to their use as organocatalysts, amino acids can also serve as ligands for organometallic catalysis.

The coupling of various thiols with vinyl bromide, catalyzed by CuBr/Proline, was performed in an ionic liquid.[74] Yields are higher in [bmim][BF_4] than in molecular solvents (DMF, DMSO) with the added advantage of shorter reaction times. The IL/catalytic system can be easily recycled with a slow decrease of yield at each reuse. Other amino acids like glycine, alanine, histidine, or lysine gave satisfying but lower yields than proline (Scheme 31).

Analogous conditions can be used for the Ullman-type coupling of vinyl bromide with imidazoles (Scheme 32).[75] Better yields are obtained in ionic liquids than in polar molecular solvents: among them, [bmim][BF_4] and [BuPy][BF_4] perform best. The IL/CuBr/proline system can be reused three times with little effect on the rate or yield of the reaction.

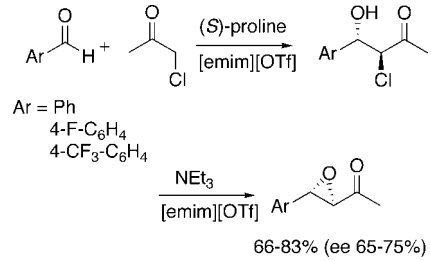
Table 14. Enantioselective Aldolization in Ionic Liquids



entry	R	IL	yield/%	ee/%	ST/EP/AP[a]	ref
1	Ph	[hmim][BF_4]	50	58	6/34/60	53
2	Ph	[omim][Cl]	30	78	0/55/45	53
3	Ph	[omim][BF_4]	59	71	11/16/73	53
4	Ph	[bmim][PF_6]	58[b]	71[b]	13/12/75[b]	53
5	Ph	[bmim][PF_6]	56[c]	71[c]	19/10/71[c]	53
6	Ph	[bmim][PF_6]	53[d]	69[d]	17/16/67[d]	53
7	Ph	[bmim][PF_6]	52[e]	67[e]	11/23/66[e]	53
8	Ph	[bmim][PF_6]	55	76		54
9	2-naphthyl	[bmim][PF_6]	60	69	15/14/71	53
10	4-Br-C_6H_4	[bmim][PF_6]	72	67	6/8/86	53
11	4-F-C_6H_4	[bmim][PF_6]	90	68		54
12	4-NO_2-C_6H_4	[bmim][PF_6]	21[f]	48[f]		54
13	4-OMe-C_6H_4	[bmim][PF_6]	69	50		54
14	4-CF_3-C_6H_4	[bmim][PF_6]	91	73		54
15	4-CF_3-C_6H_4	[bmim][PF_6]	74[g]	75[g]		54
16	2-Br-C_6H_4	[bmim][PF_6]	61	63		54
17	2-OMe-C_6H_4	[bmim][PF_6]	69	68		54
18	2-NO_2-C_6H_4	[bmim][PF_6]	93	82		54
19	3-NO_2-C_6H_4	[bmim][PF_6]	94	70		54
20	cyclohexyl	[bmim][PF_6]	65	89	8/11/81	53

[a] ST: starting material. EP: elimination product. AP: aldolization product (relative ratio determined by NMR analysis of the crude mixture). [b] First run. [c] Second run. [d] Third run. [e] Fourth run. [f] An improved procedure gave 68% yield (65% ee). [g] Using 1% (*S*)-proline.

Scheme 23



Scheme 24

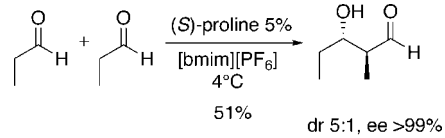
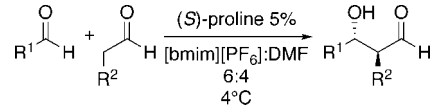


Table 15. Enantioselective Aldolization in [bmim][PF_6]



entry	R^1	R^2	yield/%	dr	ee/%
1	Me	Me	73	4:1	99
2	*i*Pr	Me	76	>19:1	>99
3	*i*Bu	Me	69	3:1	99
4	*c*-hexyl	Me	77	>19:1	>99
5	*i*Pr	*n*Bu	69	>19:1	>99

Scheme 25

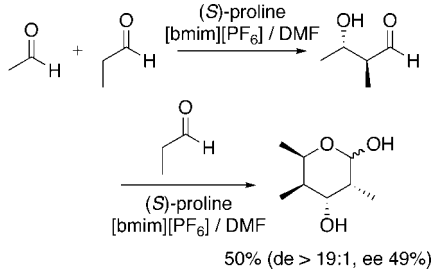
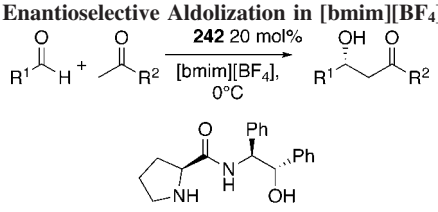


Table 16. Enantioselective Aldolization in [bmim][BF_4]

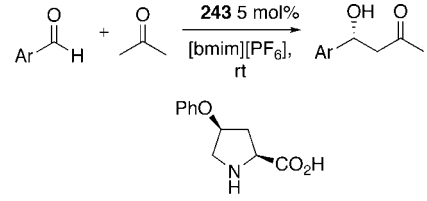


242

entry	R^1	R^2	yield[a]/%	ee[a]/%
1	Ph	Me	50 (51)	92[b] (83)
2	4-NO_2-C_6H_4	Me	82 (66)	94 (93)
3	4-Br-C_6H_4	Me	75 (77)	91 (90)
4	4-Cl-C_6H_4	Me	76 (75)	95[b] (93)
5	4-CN-C_6H_4	Me	67 (63)	93 (88)
6	4-Me-C_6H_4	Me	50 (48)	92[b] (84)
7	β-naphthyl	Me	85 (93)	91 (94)
8	3-NO_2-C_6H_4	Me	82 (63)	96[b] (87)
9	2-NO_2-C_6H_4	Me	84 (52)	96[b] (78)
10	2-Cl-C_6H_4	Me	84 (83)	91 (85)
11	*c*-hexyl	Me	70 (85)	99 (97)
12	*t*-Bu	Me	46 (51)	99 (>99)
13	4-NO_2-C_6H_4	Et	62 (32)	>99 (90)

[a] Values in parentheses are obtained in acetone. [b] Reaction performed at −25 °C.

Table 17. Enantioselective Aldolization Catalyzed by 243 in Ionic Liquids



243

entry	Ar	yield[a]/%	ee[a]/%
1	2-NO_2-C_6H_4	93 (90)	86 (78)
2	3-NO_2-C_6H_4	91 (75)	74 (71)
3	4-NO_2-C_6H_4	80 (75)	75 (76)
4	2-Cl-C_6H_4	76 (79)	76 (63)
5	3-Cl-C_6H_4	92 (79)	72 (74)
6	4-Cl-C_6H_4	73	62
7	4-Br-C_6H_4	74	54
8	Ph	61	49
9	4-Me-C_6H_4	56	47

[a] Values in parentheses are obtained in acetone.

These coupling conditions can also be extended to the coupling of vinyl bromides with sulfinic acid salts.[76] No improvement of the yield or reaction rate compared to molecular solvents was observed, but the IL/catalyst system enabled recycling and reuse three times.

Very recently, the same group reported the use of amino acid-derived ionic liquids to perform coupling of *Z*-vinyl-bromides with thiols or diphenyl diselenide without any added base or "free" amino acid (Table 23).[77]

The stereoselectivities toward the *Z* isomer obtained with ionic liquids **254**−**255** are higher than those observed with the [bmim][BF_4]/amino acid/K_2CO_3 system (compare entries 1 and 2 with entries 5 and 6), and the best yields are observed with dimethylglycine-derived **255** (entry 6). Moreover, the **255**/CuI system was successfully reused 3 times.

3.2. Transformations of Amino Acids in Ionic Media

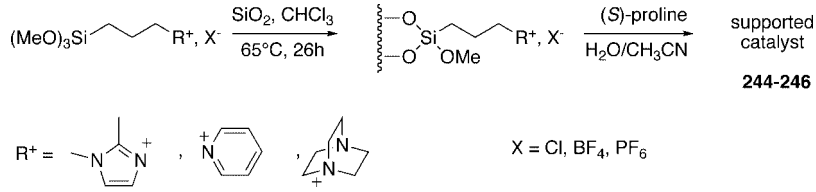
Enzymatic and chemical transformations of amino acid derivatives are among the most important reactions in organic synthesis. Great improvements (yield, ee, stability, and recycling of the catalyst) have been reported for a number of these reactions when performed in ionic liquids.

3.2.1. Enzymatic Resolutions and Transformations of Amino Acids

An exciting development in the use of ionic liquids is their application in the field of enantioselective biocatalysis.[78] This is mainly due to the improvement in enzyme catalytic performances (activity, stability, and in some cases enantioselectivity), which is observed by switching the reaction media from molecular solvents to ILs or systems containing ILs. Several factors seem to be responsible for the enzyme activity and stability including ionic character, polarity, hydrogen bonding, basicity, and anion nucleophilicity. However, there is no simple correlation between one parameter and the enzyme activity. Therefore, the current understanding of the IL effect on enzyme activity is still in its infancy. What can be said is that in hydrophobic ILs enzymes have shown very high stabilities in a number of applications. This can be explained by the fact that the hydrophobic solvents have a lesser tendency to take away the essential water from the enzyme surface. Another explanation to interpret the enzyme stability is based on the observation that ILs may form so-called organized "nanostructures" (hydrogen-bonded polymeric supramolecules, just like water) with polar and nonpolar regions. On the other hand, when hydrophilic ILs are dissolved in aqueous media they dissociate into anions and cations, ions that are known to stabilize enzymes (Hofmeister series). In many cases, kosmotropic (strongly hydrated species) anions and chaotropic (weakly hydrated species) cations stabilize proteins while chaotropic anions and kosmotropic cations destabilize them. Therefore, ions also make an individual contribution in the stabilization and level of activity of the enzyme.

One of the simplest and most efficient methods of synthesizing enantiomerically pure α-amino acids is the enzymatic resolution of racemic parents. The kinetic resolution of amino acids was influenced by the concentration, character, and polarity of the solvent; a systematic study was undertaken with variations in ionic liquid structure and concentration (Scheme 33). High ees (94.8%) and good yields (48%) in (*S*)-homophenylalanine isomer **257** were obtained using the *N*-acetyl-protected (*R*,*S*)-amino acid ester **256** and *BL*-alcalase, a commercially available endoproteinase of the serine type obtained from *Bacillus licheniforms*, when the reaction was performed in a 15% concentration of [EtPy][BF_4] in water.[79] Increasing the amount of ionic liquid beyond 20% decreases the performance of the enzyme. When using [emim][BF_4], lower yield (32%) and enantioselectivity (89%) were obtained.

Scheme 26



Scheme 27

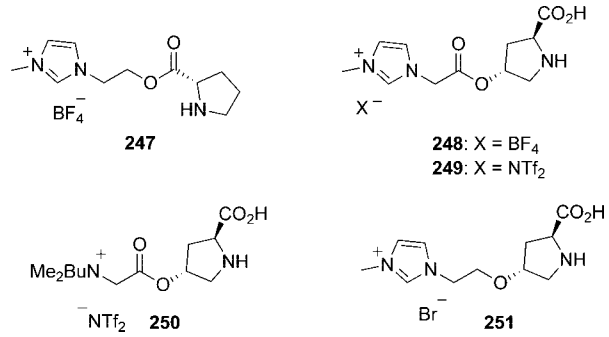
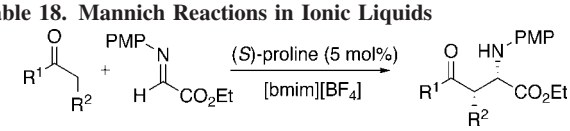
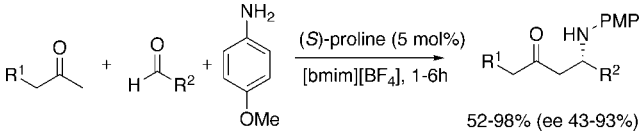


Table 18. Mannich Reactions in Ionic Liquids



entry	R^1	R^2	yield/%	dr	ee/%
1	H	*n*-Bu	87	13:1	>99
2	H	*n*-Pent	96	>19:1	>99
3	H	*i*-Pr	90	5:1	93
4	Me	Me	77	>19:1	>99
5	$-(CH_2)_2-$	$-(CH_2)_2-$	99	>19:1	>99
6	Me	H	80		97

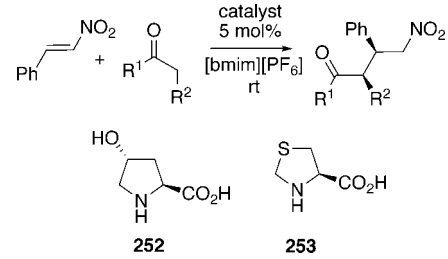
Table 19. Three-Component Mannich Reactions in Ionic Liquids



entry	R^1	R^2	yield/%	ee/%
1	H	4-NO_2-C_6H_4	54	95
2	H	1-naphthyl	63	82
3	allyl	4-NO_2-C_6H_4	60	87
4	allyl	1-naphthyl	52	93
5	H	*i*-Bu	80	43
6	H	CH_2-OBn	98	93

[EtPy][TFA] also proved to be a good substitute for molecular solvent in the kinetic resolution of *N*-acetyl amino acid esters by *Novo* alcalase, another protease also obtained from *Bacillus licheniforms* (Scheme 34).[80] Enhanced synthetic activity and enantioselectivity (86−97%) were observed in the presence of this ionic liquid in comparison with acetonitrile (Table 24). As already observed, the concentration of the ionic liquid in the solvent strongly influences the ee and yield, the best results being obtained when a 15% solution (v/v) of IL in water was used. In particular, production of two amino acids ((*S*)-serine and (*S*)-4-chlorophenylalanine) was not achievable in acetonitrile−water using *Novo* alcalase, while excellent ee and conversion were obtained in [EtPy][TFA]/water.

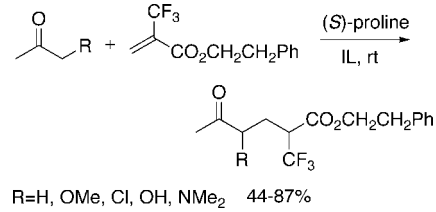
Table 20. Michael Reactions in Ionic Liquids[a]



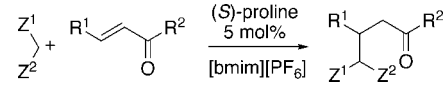
entry	R^1	R^2	catalyst	yield/% (syn/anti)	dr (syn/anti)
1	H	*i*Pr	(*S*)-proline	83	9:1
2	H	*i*Pr	**252**	0 (16)[b]	(10:1)[b]
3	H	*i*Pr	**253**	0 (51)[b]	(4:3)[b]
4	H	Et	(*S*)-proline	58	3:1
5	H	*t*Bu	(*S*)-proline	0 (0)[b]	nd
6	Me	H	(*S*)-proline	35 (77)[b]	nd
7	Me	*i*Pr	(*S*)-proline	0 (23)[b]	nd
8	$-(CH_2)_2-$	$-(CH_2)_2-$	(*S*)-proline	0 (53)[c]	(4:3)[c]
9	$-(CH_2)_3-$	$-(CH_2)_3-$	(*S*)-proline	69	3:2
10	$-(CH_2)_4-$	$-(CH_2)_4-$	(*S*)-proline	4 (87)[b]	nd (5:1)[b]

[a] nd: not determined. [b] Reaction performed at 80 °C. [c] Reaction performed at 70 °C.

Scheme 28



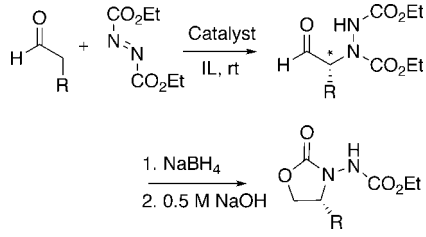
Scheme 29



Porcine pancreas lipase (PPL) in [EtPy][TFA] also displayed high activity and selectivity in the kinetic resolution of various *N*-acetyl amino acids (Scheme 35).[81,82] As already mentioned, the IL concentration is critical and 15% sol (v/v) IL in water gave the best results. Under these conditions, higher ees (73−98%) and better conversions (28−41%) were obtained if compared with the conventional acetonitrile/water medium (Table 25). These results demonstrate that ILs can be good substitutes to molecular solvents, increasing the activity and selectivity of the enzymes.

Kinetic resolution processes for producing (*S*)-*N*-(2-ethyl-6-methylphenyl)alanine **278**, an important precursor in the synthesis of herbicides such as metolachlor, from racemic methyl ester **277** using the CAL-B-catalyzed hydrolysis was studied in buffer with alkyl-guanidinium-based ionic liquids as additive (Scheme 36).[83] Optimum conditions using [ETOMG][BF_4]/buffer in a 1/1 ratio afforded enhanced enantioselectivity (ee = 92.3% at 38.4% conversion) toward

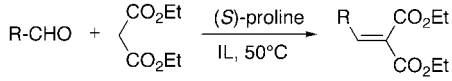
Table 21. Addition to DEAD in Ionic Liquids



entry	R	IL	cat.	time/min	yield/%	ee/%
1	*i*Pr	[bmim][BF_4]	(*S*)-proline	65	85	84
2	*i*Pr	[bmim][PF_6]	(*S*)-proline	65	68	79
3	*i*Pr	[hmim][BF_4]	(*S*)-proline	65	88	36
4	*i*Pr	[hmim][PF_6]	(*S*)-proline	65	44	81
5	*i*Pr	[bbim][PF_6]	(*S*)-proline	65	65	83
6	*i*Pr	[C_{10}mim][PF_6]	(*S*)-proline	65	48	70
7	*i*Pr	[bmim][$C_8H_{17}SO_4$]	(*S*)-proline	65	46	17
8	*i*Pr	AMMOENG^{TM} 100[a]	(*S*)-proline	65	44	11
9	*i*Pr	CYPHOSIL 101[b]	(*S*)-proline	65	45	nd
10	*i*Pr	[bmim][BF_4]	**252**	380	27	94
11	*i*Pr	[bmim][BF_4]	**253**	480	17	92
12	*i*Pr	[bmim][BF_4]	(*S*)-serine	420	<4	nd
13	Me	[bmim][BF_4]	(*S*)-proline	100	63	89
14	Et	[bmim][BF_4]	(*S*)-proline	70	63	83
15	*t*Bu	[bmim][BF_4]	(*S*)-proline	360	34	70
16	Bn	[bmim][BF_4]	(*S*)-proline	200	43	76
17	Ph	[bmim][BF_4]	(*S*)-proline	120	42	≤1

[a] Polyethylene glycol substituted ammonium methyl sulfate (Solvent Innovation). [b] Tetradecyl(trihexyl)phosphonium chloride (Cytec).

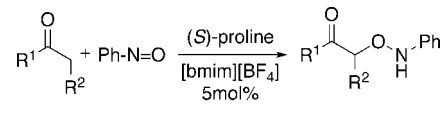
Table 22. Knoevenagel Reactions in Ionic Liquids



entry	R	IL	*T*/°C	conv./%
1	Ph	[emim][BF_4]	15	46[a]
2	Ph	[bmim][BF_4]	15	51[a]
3	Ph	[bmim][PF_6]	15	25[a]
4	Ph	[bmim][BF_4]	35	89[b]
5	Ph	[bmim][BF_4]	50	93[b]
6	Ph	[bmim][BF_4]	65	65[b]
7	Ph	[bmim][BF_4]	80	81[b]
8	*i*-Pr	[bmim][BF_4]	50	97[b]
9	4-NO_2-C_6H_4	[bmim][BF_4]	50	27[b]
10	4-OH-C_6H_4	[bmim][BF_4]	50	63[b]
11	4-Cl-C_6H_4	[bmim][BF_4]	50	45[b]
12	4-OMe-C_6H_4	[bmim][BF_4]	50	60[b]
13	furan-2-yl	[bmim][BF_4]	50	100[c]

[a] After 24 h. [b] After 12 h [c] after 6 h

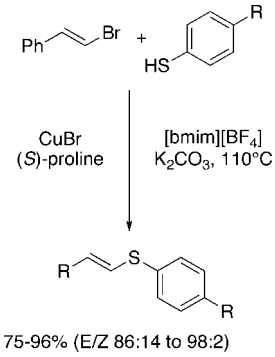
Scheme 30



the (*S*)-enantiomer **278** compared to the one obtained in pure buffer (78.7% at 45.3% conversion). Recycling of the ionic liquid was performed in four consecutive runs without loss of activity or enantioselectivity.

Zhao et al. explored the effect of kosmotropicity on the enantioselectivity in the enzymatic hydrolysis of phenylalanine methyl ester in aqueous solutions of various ILs with the aim to develop an empirical guideline in designing ILs for specific enzymatic applications.[84] *Bacillus licheniforms* was selected for the study. Both the buffer concentration and

Scheme 31



Scheme 32

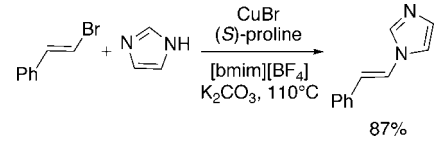
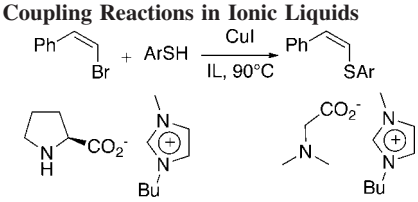


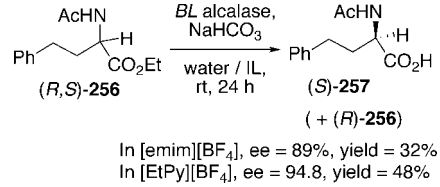
Table 23. Coupling Reactions in Ionic Liquids



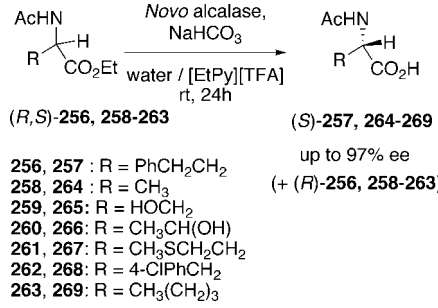
entry	conditions[a]	yield/%	*Z*:*E*
1	[bmim][BF_4]/(*S*)-proline/K_2CO_3	80	93:7
2	[bmim][BF_4]/*N*,*N*-dimethylglycine/K_2CO_3	87	93:7
3	DMF/*N*,*N*-dimethylglycine/K_2CO_3	56	85:15
4	1,4-dioxane/*N*,*N*-dimethylglycine/K_2CO_3	*b*	
5	**254**	60	98:2
6	**255**	90	98:2

[a] R = Ar = Ph, 16 h [b] Very low amount of coupling product observed.

Scheme 33



Scheme 34



the IL concentration considerably modify the enzymatic hydrolysis rate of phenylalanine methyl ester. As already mentioned, higher conversions were obtained with lower concentrations in IL (0.5M). The protease enantioselectivity was greatly enhanced using IL solutions containing kosmo-

Table 24. Enzymatic Resolution of *N*-Acetyl Amino Acids in [EtPy][TFA] IL[a]

	acetonitrile		[EtPy][TFA]	
compd	ee (%)	yield (%)	ee (%)	yield (%)
257	95	35	93	38
264	63	31	86	33
265	NA	NA	90	35
266	92	15	97	36
267	83	30	89	29
268	NA	NA	96	39
269	18	32	88	30

[a] NA: no activity.

Scheme 35

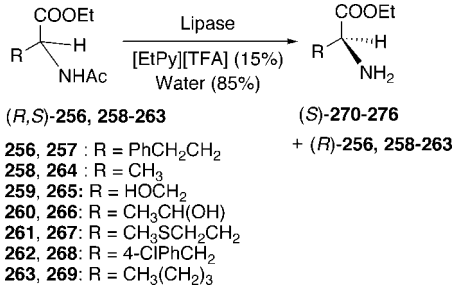
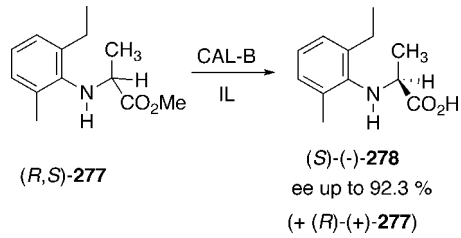


Table 25. Lipase Resolution of *N*-Acetyl Amino Acids in Ionic Liquids

	acetonitrile		[EtPy][TFA]	
compd	ee (%)	yield (%)	ee (%)	yield (%)
270	92	33	95	39
271	63	26	81	30
272	35	21	78	28
273	36	19	89	24
274	62	31	86	29
275	95	26	98	41
276	18	32	73	30

Scheme 36

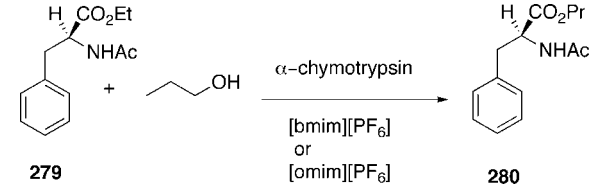


tropic anions (PO_4^{3-} > citrate^{3-} > CH_3COO^- > $EtSO_4^-$ > CF_3COO^-, Br^- > OTs^- > BF_4^-) and chaotropic cations (emim > bmim> hmim), which stabilize the enzyme. The results follow the Hofmeister series.

The activity of α-chymotrypsin in imidazolium-based ionic liquids was studied using the transesterification reaction of *N*-acetyl-L-phenylalanine ethyl ester **279** with 1-propanol.[85] Activities were better in [omim] than in [bmim] (Scheme 37).

Enzymatic resolution of phenylalanine methyl ester was also conducted as a model reaction for examining protease activity and enantioselectivity in aqueous solutions of ionic liquids carrying anions derived from amino acids.[86] The [emim] cation was selected for the study as it is a chaotropic cation, which stabilizes the enzyme while 5-aminopentanoic acid [5-APA] was selected as the anion. High enantiomeric excess (92%) and yield (97.2%) of (*S*)-phenylalanine were obtained in low concentrations of IL (0.5 M). These values

Scheme 37



are comparable to those obtained in pure water. At higher IL concentrations both ee and yield decrease. Destabilization of the enzyme was proposed to rationalize this result. In various other amino acid-based ILs a moderate to high enzyme activity and enantioselectivity were observed. Interestingly, slightly higher ees were obtained in (*R*)-amino acid-based ILs than in those based on (*S*)-amino acids. The higher kosmotropicity of the (*R*)-amino acids compared to that of the (*S*)-isomer was proposed by the authors to explain this result. Therefore (*R*)-amino acid anions are stronger enzyme stabilizers than their (*S*)-isomers.

A study of the influence of the concentration (0−4M, 60% IL, v/v) of the [emim][AcO] ionic liquid on the enzymatic resolution of (*R*,*S*)-phenylalanine methyl ester (activity and enantioselectivity) was performed by Zhao.[87] Better results were obtained with this IL compared to acetonitrile and ILs having chaotropic anions such as [emim][OTs]. The enantiomeric excess in favor of the (*S*)-derivative proved to be almost independent of the IL concentration. However, lower yields were obtained at high IL concentration. Among two lipases and three proteases, lyophilized *Bacillus licheniforms* exhibited the best activity (yield > 99) and enantioselectivity (ee = 96.3). High enantioselectivity was also obtained with several other amino acid methyl esters (methionine, 4-chlorophenylalanine, phenylglycine, *p*-hydroxyphenylglycine) in 2.0 M [emim][AcO].

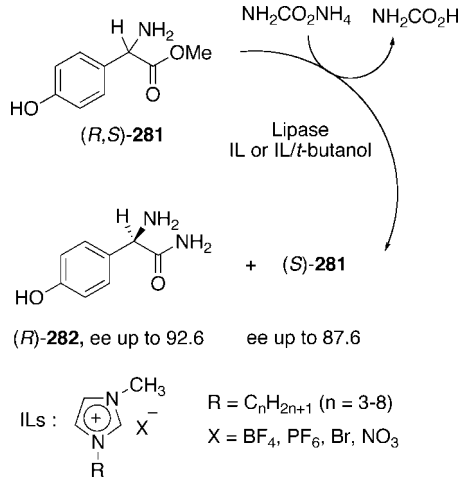
Zong et al. studied the lipase-catalyzed ammonolysis of (*R*,*S*)-*p*-hydroxyphenylglycine methyl ester **281** ((*R*,*S*)-HPGME) with ammonium carbamate as the ammonia donor in nine different ionic liquids (Table 26, Scheme 38) and compared the results to those obtained in four organic solvents (butanol, THF, 1,2 dichloroethane, *tert*-amylalcohol).[88] The results obtained clearly demonstrate that use of ILs as a cosolvent can markedly boost the activity, enantioselectivity, and stability of *Candida antartica* lipase B (CAL-B) immobilized on an acrylic resin (Novozim 435). Both the cation and the anion have a significant effect on the reaction. No ammonolysis activity toward (*R*,*S*)-HPGME **281** was observed in two ILs, [bmim][Br] and [bmim][NO_3], presumably because of the high nucleophilicity of the anions. In other ILs an enhancement in the enantioselectivity was always observed compared to the different molecular solvents tested. The reaction became faster and more enantioselective with elongation of the alkyl chain on the cation, [C_6mim][BF_4] giving the highest ee (94.1% for the product (*R*)- **282** and 86.3% for the substrate (*S*)- **281**) for a high conversion (Table 26, entries 1−4). However, whatever the IL used a lower activity of the enzyme was noticed compared to that observed in pure butanol. A good compromise between activity and selectivity was obtained using mixtures of BuOH and ILs (Table 26, entries 14−16). Of the cosolvent mixture assayed, 20% (v/v) [hmim][BF_4]/BuOH (Table 26, entry 16) gave both the highest initial rate and enantioselectivity (92.6% ee for the product (*R*)- **282**, 87.6% for the substrate (*S*)- **281** for a 46.8% yield). The high thermal

Table 26. Lipase-Catalyzed Ammonolysis of (*R*,*S*)-*p*-Hydroxyphenylglycine Methyl Ester 281 in ILs[a]

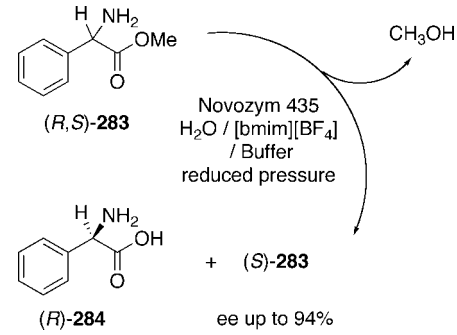
entry	lipase	medium	initial rate ($mM \cdot min^{-1} \cdot mg^{-1}$)	time/h	yield[b] (%)	ee_p (%)	ee_s (%)	*E*
1	CAL-B	$[C_3mim][BF_4]$	0.38	48	33.2	88.2	48.4	25
2	CAL-B	$[C_4$ mim$][BF_4]$	0.57	48	42.4	90.6	72.5	43
3	CAL-B	$[C_5$ mim$][BF_4]$	0.63	48	43.8	92.4	78.7	59
4	CAL-B	$[C_6$ mim$][BF_4]$	0.78	48	46.5	94.1	86.3	67
5	CAL-B	$[C_7$ mim$][BF_4]$	0.45	48	38.3	94.7	61.5	68
6	CAL-B	$[C_8$ mim$][BF_4]$	0.31	68	26.6	95.4	35.7	63
7	CAL-B	$[C_4$ mim$][PF_6]$	0.19	86	23.5	94.8	30.1	50
8	CAL-B	$[C_4$ mim$][Br]$	nr	48	nr	nr	nr	nr
9	CAL-B	$[C_4$ mim$][NO_3]$	nr	48	nr	nr	nr	nr
10	CAL-B	*t*-BuOH	1.83	4	38.6	71.4	57.5	12
11	CAL-B	*t*-AmOH	1.74	4	36.1	85.7	54.5	22
12	CAL-B	DCE[c]	0.54	40	35.2	76.5	50.4	13
13	CAL-B	THF	0.32	60	24.3	70.7	28.2	8
14	CAL-B	30% (v/v) $[C_5mim][BF_4]$-*t*-BuOH	2.66	4	45.5	89.9	82.7	48
15	CAL-B	15% (v/v) $[C_7mim][BF_4]$-*t*-BuOH	2.42	4	42.7	91.7	74.7	52
16	CAL-B	20% (v/v) $[C_6mim][BF_4]$-*t*-BuOH	3.18	4	46.8	92.6	87.6	63
17	CAL-B	50% (v/v) *t*-AmOH-*t*-BuOH	2.45	4	44.4	83.9	78.5	27
18	CAL-B	10% (v/v) DCE[c]-*t*-BuOH	2.24	4	40.3	79.8	65.0	19
19	CAL-B	10% (v/v) DCE[c]-*t*-BuOH	1.56	4	34.0	78.2	47.7	15
20	CCL	20% (v/v) $[C_6mim][BF_4]$-*t*-BuOH	2.17	6	31.3	28.6	35.7	3

[a] CAL-B: *Candida antartica* lipase B. CCL: *Candida cylindrace* lipase. nr = no reaction. [b] Yield of the product on a mole basis. [c] DCE = 1,2-dichloroethane.

Scheme 38



Scheme 39



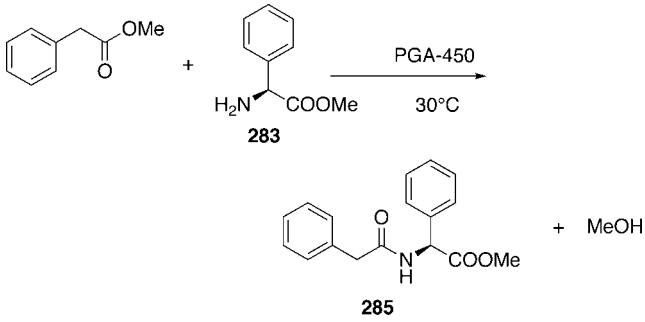
stability of the enzyme in the IL−butanol solution was also demonstrated.

Wu et al.[89−91] studied the papain-mediated asymmetric hydrolysis of four racemic amino acid esters ((*R*,*S*)-phenylglycine methyl ester, (*R*,*S*)-4-chlorophenylalanine ethyl ester, (*R*,*S*)-methionine ethyl ester, (*R*,*S*)-β-phenylalanine methyl ester) in different media containing various concentrations (from 0 to 99% (v/v)) of methyl imidazolium-based ionic liquids having anions such as BF_4^-, Cl^-, Br^-, and HSO_4^{2-}. No activity or low enantioselectivities were obtained with ionic liquids having halides or hydrogenosulfate anions. A clear enhancement of the enantioselectivity was observed when $[bmim][BF_4]$ was used as a cosolvent (optimum concentration 12.5% (v/v)). This was explained by the deceleration in the nonenzymatic hydrolysis of the amino acid in IL compared to phosphate buffer.

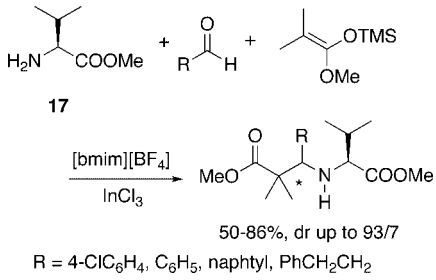
Fischer et al. studied the catalytic activity and stability of (*R*)-amino acid oxidase (DAAO EC 1.4.3.3) from *Trigonopsis variabilis* immobilized on a macroporous carrier in different water-soluble and water-insoluble ionic liquids as well as in organic solvents.[92] Note that DAAO serves as the first enzyme in a two-step enzymatic process for industrial production of 7-aminocephalospranic acid (7-ACA). A higher stability of DAAO in the water-insoluble ILs was reported. From these preliminary tests the most promising IL seems to be $[bmim][MMPO_4]$. The biotransformations of enantiopure and racemic phenylalanine and ceph-C were performed in the presence of ILs. The (*R*)-phenylalanine conversion using DAAO without addition of catalase was lower in the presence of $[bmim][BF_4]$ (40%) than in pure buffer (70% after 240 min compared to 100% in 80 min, respectively). This lower activity was explained by the reduced enzyme stability in the IL. The biotransformation of (*R*,*S*)-phenylalanine in the presence of $[mmim][MMPO_4]$ and $[bmim][BF_4]$ with addition of a free catalase resulted in conversion rates similar to those reported in pure aqueous medium.

ILs can also serve as excellent cosolvents to buffer in place of traditional organic solvent for immobilized *Candida antartica* lipase B (Novozim 435) mediated hydrolysis of (*R*,*S*)-phenylglycine methyl ester (PGME) **283** to enantiopure (*R*)-phenylglycine **284** (Scheme 39).[93] Markedly enhanced activity and enantioselectivity were obtained in the system containing $[bmim][BF_4]$ compared to several organic solvents. The best medium is obtained by mixing a phosphate buffer and $[bmim][BF_4]$ in a 80/20 (v/v) ratio. Performing the hydrolysis under low pressure allows methanol to be removed and affords higher activity and enantioselectivity.

Scheme 40



Scheme 41



The activity of immobilized penicillin G amidase (PGA-450) in ionic liquids in a reaction between methyl phenylacetate and L-PhGlyOMe **283** was studied at controlled hydration ($a_w > 0.6$).[94] Reactions carried out in [bmim][PF_6] were comparable to that obtained in toluene (complete in 3 h). In [bmim][BF_4] the reaction was complete after 24 h. The authors had shown good synthetic activity of PGA-450 in these ionic liquids, and no hydrolysis of the product occurred. Ionic liquid with sulfate anion such as CH_3OSO_3 requires more than 20% water, creating a cosolvent aqueous mixture that denatures the IL and leads to no synthetic activity of the enzyme.

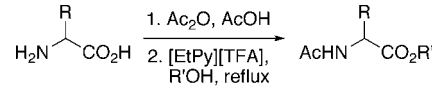
All this information clearly shows the enhanced stability and activity of enzymes when diluted in ionic solvents. However, not only can enzymatic transformations of amino acids benefit from the numerous advantages of ILs but also the chemical transformations of these species in ILs proved fruitful.

3.2.2. Chemical Transformations of Amino Acids

Different approaches that relate transformations of both functional moieties of amino acids have already been reported. Regarding amino group substitution, Loh et al. described the indium-catalyzed asymmetric three-component Mannich reaction in ionic liquids (Scheme 41).[95] The chiral amino acid used was (*S*)-valine methyl ester **17**. Different ionic liquids were tested: [bmim][BF_4], [hmim][BF_4], [omim][BF_4], and [omim][Cl]. Reactions proceeded with high diastereoselectivity in [BF_4] ionic liquids. Shorter chain lengths on the cationic part of the ionic liquid afforded better yields and lowered the amount of aldol side product. All attempts to recycle the ionic liquid/$InCl_3$ system failed. With $In(OTf)_3$ in [hmim][BF_4] the heterogenized catalyst was reused three times with a significant decrease in yield for the third cycle.

Malhotra investigated for the first time the use of ionic liquid [EtPy][TFA] as a catalyst for esterification of amino acids, including unnatural compounds.[96] After N-acetylation, the N-protected amino acid was dissolved in anhydrous ethyl or isopropyl alcohol followed by adding the ionic liquid. Yields were generally good, especially for ethyl esters (see Table 27).

Table 27. Esterification of Amino Acids with [EtPy][TFA] as Catalyst



R	ethyl ester yield (%)	*i*-propyl ester yield (%)
CH_2OH (serine)	25	12
n-Bu (norleucine)	70	72
Cl (2-chloroglycine)	93	52
Ph (phenylglycine)	72	78
$PhCH_2CH_2$ (homophenylalanine)	77	70
4-Cl-C_6H_4-CH_2	89	87
indolylmethyl (tryptophan)	95	54

Obviously, these promising results highlight the potential of ionic liquids for amino acid chemistry. One of the main topics of this chemistry consists of peptide coupling, and the first results from this research area are reported below.

3.3. Peptide Synthesis in Ionic Media

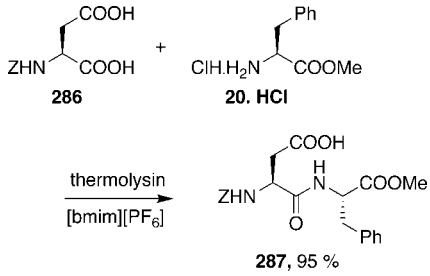
In a recent book review, Professor S. Kent, from Chicago University, recently stated:[97]"... *At the beginning of the 21st century, the field of synthetic peptide chemistry is in the early stages of a renaissance. Several factors contribute to this rebirth: the discovery of new classes of diverse and potent peptide natural products, such as the conotoxins and the cyclotides; a resurgence of interest in peptides for use as human therapeutics; and the use of synthetic peptides in the total chemical synthesis and semi-synthesis of proteins... Some of the most important challenges currently facing the worldwide biomedical research community, such as vancomycin resistance in pathogenic bacteria, will place extreme demands on innovative synthetic peptide science.*" This stimulating text highlights at least two major points: first, the still growing importance of peptide synthesis and, second, the rebirth of fundamental research in this field. Regarding this last point, one can wonder why peptide synthesis still suffers from severe drawbacks, such as low atom economy (protection/deprotection steps), high-cost coupling reagents, and tedious processes. A series of recent reviews summarizes the current approaches used in peptide synthesis, which will thus not be discussed herein.[98−101] Indeed, future studies will probably focus on use of simplified procedures, cheap reagents, their recycling, and possibly the absence of protecting groups (see some examples in the Conclusions and Prospectives). In this context, ionic solvents are increasingly tested for peptide chemistry both for synthetic and analytical purposes. Some of these very recent studies highlight a real potential as well as new opportunities.

3.3.1. Enzymatic Peptide Synthesis

Much information is available now that clearly shows the greater stability and activity of enzymes when diluted in ionic solvents.[8,14] A rather obvious consequence was to incite chemists to examine construction of peptide bonds in ionic media by means of proteolytic enzymes.[102]

The first successful study reported deals with the synthesis of Z-protected aspartame **287**, which was obtained in a thermolysin-catalyzed reaction of Z-aspartate

Scheme 42



286 and phenylalanine methyl ester hydrochloride **20**·HCl in [bmim][PF_6] (Scheme 42). The authors observed a competitive rate with respect to the same reaction in molecular solvents as well as a remarkable stability of the enzyme.[103] Recycling of the ionic liquid was successfully performed.

More recently, the protease-catalyzed synthesis of peptides from natural amino acids was achieved in ionic liquids. Various ionic liquids have been tested under different reaction conditions, and an effective, simple, and practical method has been devised. A comparative study with molecular solvents finds the approach simple and more effective in ILs. This methodology could potentially be used on a large scale.[104]

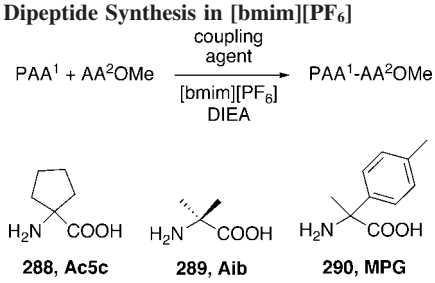
In 2007 α-chymotrypsin-catalyzed peptide synthesis was examined in ILs. The model reaction was the synthesis of a fragment of Leu-enkephalin, i.e., ZTyrGlyGlyOEt. Six different methylimidazolium hexafluorophosphates and tetrafluoroborates were screened, and it was shown that the water content of the reaction medium had a great influence on the enzyme activity and product yield. In optimized conditions several di- and tripeptides were successfully obtained in 70−75% yield.[105]

3.3.2. Chemical Peptide Synthesis

Although efficient peptide coupling has already been demonstrated by means of enzyme catalysis (vide supra), no reports about controlled chemical peptide coupling in ionic liquids were available in the literature till 2004. Indeed, one can expect this approach to be of real potential since amino acid derivatives are easily dissolved in ionic medium, and coupling could be favored because of anhydrous conditions as well as stabilization of charged coupling reagents and reaction intermediates. Trulove et al., in a congress abstract,[106] examined the incubation of amino acid mixtures in [bmim][PF_6] in the absence of any coupling reagent. After aqueous extraction peptides (as unidentified and unquantified mixtures) were obtained when the reaction temperature was greater than 100 °C. Although not useful from a preparative point of view, this kind of study is obviously relevant to prebiotic chemistry since the origin of peptide bond construction still remains obscure.

In 2004 we considered peptide coupling in ionic media by looking at modern coupling agents such as HATU and BOP as reagents of choice for this approach since they present strong structural similarities with ionic solvents.[107,108] We expected them to be both easily dissolved in ionic liquids and stabilized by the solvent in order to produce slow and selective reactions. We first coupled quaternary α-amino acids **288**−**290** which are much more difficult to couple than their tertiary proteinogenic congeners. After optimization of reaction conditions and extraction process, good yields were obtained in most cases, which compared favorably with yields in molecular solvents such as dichloromethane or THF (Table 28). The main advantage was the higher purity of the crude dipeptides obtained when the reaction was performed in an ionic liquid. A more selective chemical pathway, due to the stabilization effect of the ionic liquid on both the coupling reagent and the charged intermediates, is assumed to explain this behavior.

Table 28. Dipeptide Synthesis in [bmim][PF_6]



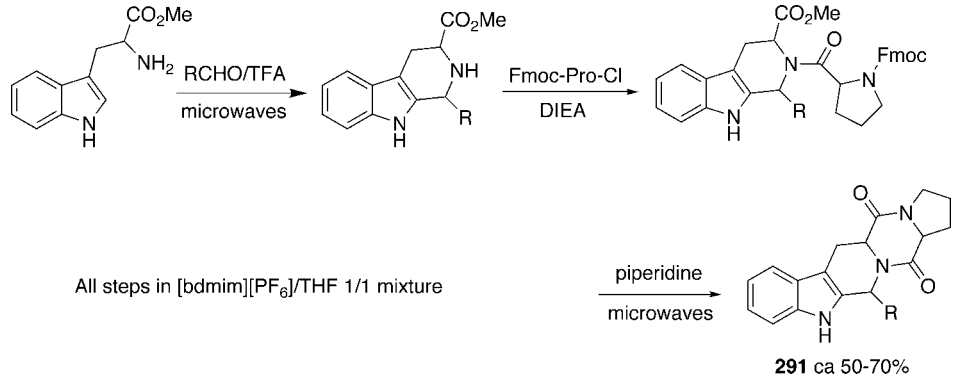
dipeptide	coupling agent	yield (%)
ZGly-GlyOMe	HATU	93
ZGly-MPGOMe	HATU	99
ZGly-MPBrGOMe	HATU	99
ZGly-Ac5cOMe	HATU	93
BocAib-Ac5cOMe	HATU	43
BocPhe-PheOMe	HATU	99
BocPhe-GlyOMe	HATU	96
BocPhe-Ac5cOMe	HATU	99
BocMPG-GlyOMe	BOP	82
BocMPG-MPGOMe	HATU	45
BocAib-MPGOMe	HATU	52
BocMPG-AibOMe	HATU	49

In addition, tetra-, octa-, and cycloocta-peptides were formed with ca. 80−85% yield. As mentioned before, one interesting aspect of this method is the high purity of the crude peptides obtained. This was assumed to be due, at least in part, to the ability of ionic solvents to retain the residues of the activation agent in solution. In addition to the benefit for the purification procedure, this could indicate the feasibility of recycling the activation agent. Our ongoing studies focus on examining the direct coupling of amino acid salts, which can be dissolved in ionic solvents. If successful, this approach would avoid protection/deprotection steps (for discussion, see Conclusions and Perspectives).

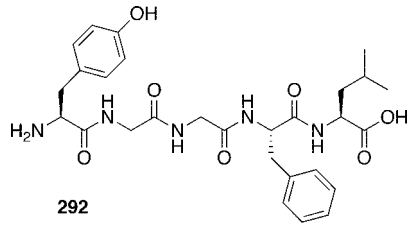
Shortly thereafter two research groups reported new results that confirmed the interest of chemical peptide coupling in ionic liquids and highlighted the main conclusions of our studies. Yen and Chu showed that diketopiperazine **291** was efficiently obtained in [bdmim][PF_6] using low-power microwave irradiation (Scheme 43). The key reaction steps were a Schotten−Baumann acylation and a Pictet−Spengler cyclization.[109]

Another interesting contribution was reported by Sega et al. in 2005.[110] During a related study on the synthesis of various amides via either the Schotten−Baumann procedure, the Weinreib amidation of esters, or the classical coupling with EDC and HBTU, the authors published an example of peptide bond construction with 90% yield (obtained peptide Cbz-Phe-Ser-OMe). A simple ether extraction afforded the desired product, and serine was coupled without protection of the side chain. A series of simple amino amides was also produced followed by direct reduction to amines in the same flask.

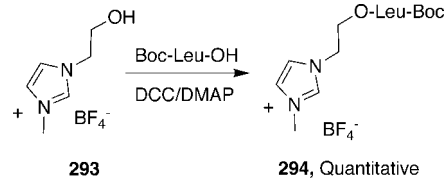
Scheme 43



Scheme 44



Scheme 45



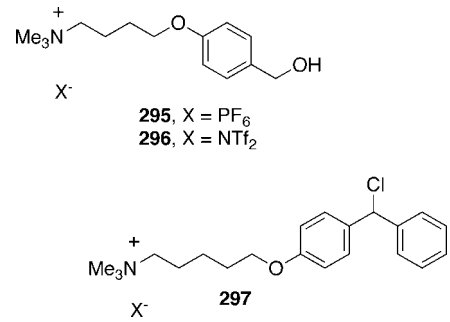
3.3.3. Supported (or Immobilized) Peptide Synthesis

Almost simultaneously with the demonstration that ionic liquids were particularly convenient solvents for peptide coupling, a series of reports proposed new insight into supported peptide synthesis. Indeed, one can imagine strategies in which peptide elongation could be performed on immobilized species using ionic liquid moieties as soluble support. This would allow both homogeneous reactions as well as high loading. Although these approaches do not strictly belong to the field of synthesis in ionic liquid media, they seem to be of major interest in the context of this review since they highlight the strong potential of task-specific ILs used for tagging the elongating peptide chain. For a first example, Miao and Chan developed this strategy for the synthesis of Leu[5]-enkephalin **292** (Scheme 44).[111]

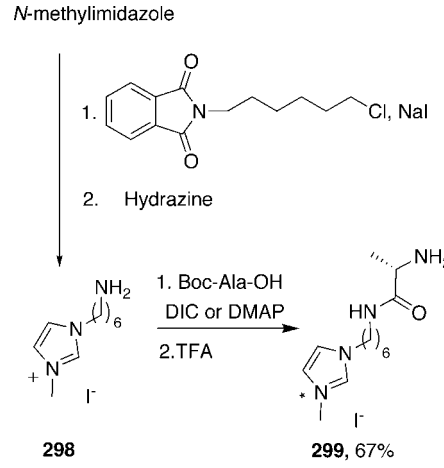
The authors showed that after the first reactant was anchored to an ionic liquid support the excess reagents and byproducts were, as expected, easily removed by simple washing (Scheme 45). Also, synthetic performance compared well with other methods, and no racemization occurred. Thus, the ionic support was compatible with peptide synthesis methodologies. More recently, the same group described structurally defined imidazolium-type ionic oligomers as soluble/solid support to synthesize peptides efficiently in gram scale.[112]

In a patent, Vaultier and co-workers highlighted the interest of such a strategy for peptide synthesis.[113] Indeed, this group developed a general strategy for construction of various structures using onium salts as tools for immobilization in

Scheme 46



Scheme 47

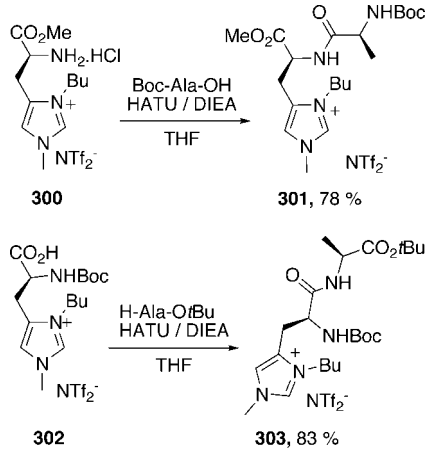


either organic, aqueous, or ionic media (Scheme 46). One of the most promising approaches used these TSIL in peptide synthesis.

A related approach was reported by Lavastre in 2006.[114,115] In this case, the functionalized ionic liquid **298** carried an amino group instead of the hydroxyl function. Amide coupling with Boc-(*S*)-Ala was successful on this support and deprotection carried out as usual (Scheme 47). The same conclusions were drawn as in Chan's study.

Finally, although in a different context, our group examined the related construction of dipeptides starting from our previously reported histidinium salts (vide supra, section 2.2). The issues that we wanted to address were the following: (1) Can histidinium salts be coupled under standard peptide synthesis methods on both terminal positions? (2) Do racemization and epimerization occur using these new species? (3) Are ionic liquid dipeptides stable and easy to purify, opening a new class of functionalized chiral ionic liquids? Fortunately, peptide coupling was efficient, not

Scheme 48



racemizing, and the required targets were obtained in a straightforward manner (Scheme 48).[36]

The promising results reported in section 3.3, although preliminary, seem to indicate a strong potential for future insights in peptide synthesis. As discussed before, the current methodologies are powerful and efficient but should progress in terms of atom economy and cost of reagents and protecting groups. Ionic liquids provide new media and tools such as devoted TSILs, which will surely afford some improvements in peptide synthesis.

3.4. ILs and Analysis, Chromatography, and Mass Spectrometry of Amino Acids and Peptides

In addition to the use of ionic liquids as solvents for organic and bioorganic synthesis reactions, their properties also make them good candidates as solvents for chemical analyses. Two main applications were investigated: their potential for separation of amino acids and proteins and use as MALDI matrices for peptide and protein mass spectrometry analysis.

3.4.1. Extraction and Chromatography

Amino acids generally exhibit a low solubility in IL. Adding a crown ether, dibenzo-18-crown-6, at 25.6 mM to the [bmim][PF_6] liquid phase at pH 1 proved to increase the solubility of some aromatic amino acids (Trp, Phe, His) in the IL media by forming a complex with the cationic form of the amino acids.[116] Although still moderate, extraction of amino acids is more efficient in the IL phase in the presence of a crown ether.

A detailed study of amino acid extraction from aqueous solutions by [bmim][PF_6] in the presence of dicyclohexyl-18-crown-6 ether was reported.[117] In acidic conditions extraction is nearly quantitative even for hydrophobic amino acids such as glycine and without the need of adding any hydrophobic counteranion. The dependence of amino acid extraction on crown ether concentration confirmed the binding of the crown ether with the ammonium group of the cationic form of the amino acid. Extractions of amino acids from pharmaceutical products were also performed with good efficiency.

Factors controlling recovery of amino acids from aqueous solutions by several imidazolium-based ILs were studied.[118] The hydrophobicity of amino acids is an important factor governing their partition in IL. An acidic pH is also required. Nevertheless, no relationship was noted between the polarity of the IL (alkyl chain length) and the partition coefficient of the amino acid. Extraction of amino acids from the aqueous phase is more efficient when a [BF_4] IL is used instead of a [PF_6] IL. Then, recovery of the amino acid is acceptable, especially in the case of tryptophan.

Since more conventional hydrophobic ionic liquids dissolved very low amounts of proteins, *N*-butyl-*N*-methyl pyrrolidium and choline saccharinate dihydrogen phosphate (20% water) were used to dissolve significant amounts of cytochrome *c*.[14] ATF-FTIR spectra showed that the secondary structure of the protein is retained even at high temperatures (110 °C) in this IL, while working with more classical ionic liquids led to structural changes for *Candida antartica* lipase B.[119]

As numerous ILs have very low solubility in water, they can be used as liquid membranes. Their potential for this purpose was evaluated for the transport of amino acids and amino esters between aqueous phases.[120] Two different liquid membranes were used in this work. A commercial polyvinylidene fluoride membrane was impregnated with the ionic liquid [omim][PF_6]. Then, the supported liquid membrane was used to separate a glass diffusion cell with two compartments, and the transport of amino acids was investigated. Using a U-shaped tube the feed and receiving aqueous phase can be directly separated by an ionic liquid phase, and this bulk liquid membrane was also studied. With amino acids, due to their high solubility in water and polar character, no transport was noted even after a long phase contact. On the other hand, amino esters (proline benzyl ester, phenylalanine methyl ester, phenylglycine methyl ester) were transported after an induction time. The mechanism established by the authors consisted of two steps. First, during the lag time, which corresponds to formation of water microenvironments inside the ionic liquid, the solute transport is governed by the solute affinity for the ionic liquid, and some selectivity can be observed. After this period, no selectivity is observed and the solute transport through water microenvironments controlled the solute transfer.

Finally, ILs can be used for chromatographic applications: two main fields have been investigated. On one hand, ILs can be used as additives for liquid chromatography to reduce the surface acidity of silica-based stationary phases, and, on the other hand, they can serve as stationary phase or additive of mobile phase for enantioselective separation.

Silica-based stationary phases are most frequent in liquid chromatography. However, free silanol groups often disturb the separation of basic solutes such as organic bases. Imidazolium tetrafluoroborate ionic liquids have been used as additives in the mobile phase to block those negative effects induced by the presence of silanol groups.[121]

[Bmim][BF_4] has also been used as a mobile phase additive in reversed phase chromatography for separation of amino and nucleic acids.[122] In the case of two amino acids (tryptophan and phenylalanine derivatives), the resolution increased with the concentration of the ionic liquid in the mobile phase.

Yuan and co-workers tested the ability of a chiral IL ((*R*)-*N*,*N*,*N*-trimethyl-2-aminobutanol-bis(trifluoromethane-sulfon)-imidate as chiral selector for chromatography.[123] The IL was used as a chiral buffer additive in high-performance capillary electrophoresis (HPCE) for the enantioselective resolution of several solutes. Separation of four different AAs (tryptophan, α-phenylglycine, phenylalanine, and tyrosine) was

achieved with satisfactory to excellent results ($R = 1.00$, 1.30, 3.70, and 6.00, respectively). As chiral additive of mobile phase in reversed high-performance liquid chromatography the tested IL enabled the chiral separation of different compounds with correct resolution (e.g., phenylalanine $\alpha = 1.11$). Results obtained with the chiral IL as stationary phase are acceptable. For example, a value near 1 is measured for the separation factor α in the case of valine (or leucine) trifluoroacetyl propanoyl ester analysis. This work represents the first application of a chiral IL for enantioselective chromatography and shows that chiral ILs can be efficient selectors for different chromatographic techniques.

The behavior of peptides and computer-assisted optimization of peptide separations in a normal-phase thin layer chromatography system with and without addition of ionic liquid in the eluent was also reported.[124] Baczek et al. tested the behavior of nine homologous peptides in normal TLC with and without IL in the eluent and also performed computed optimization of the separation. In most cases, a quadratic relationship between the retention parameter and organic concentration in the mobile phase was simulated without IL in the mobile phase, whereas a third degree polynomial dependence occurred in the presence of ILs. Simulation for nine homologous peptides using a third degree polynomial model allowed moderate separation. Experiments confirmed that the predicted separations were good enough. The best results were calculated and obtained with 46% (v/v) acetonitrile/water and 1.5% of IL [emim][BF_4]. The conditions of separation must be optimized to provide better performance. Addition of α-cyano-4-hydroxycinnamic acid (CHCA), a classical matrix for MALDI MS, to the mobile phase (20−40% acetonitrile, 1.5% IL) still allowed a satisfactory separation, compatible with further MALDI MS identification. The results summarized in this part show the growing importance of ILs for extraction and analytical separation of AA and peptides. The other analytical use of ILs concerns their use as a matrix for mass spectrometry analysis.

3.4.2. Mass Spectrometry

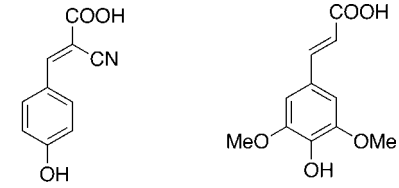
Matrix-assisted laser desorption/ionization (MALDI) is a powerful analytical tool, very useful for mass spectrometry analysis of high-weight biomolecules.

In spite of a low shot-to-shot reproducibility, due to a heterogeneous dispersion of the solute throughout the matrix, solid matrices (e.g., α-cyano-4-hydrocinnamic acid or 2,5-dihydrobenzoic acid) are mostly used as MALDI matrices. Many works thus concern employment of new matrices limiting the lack of homogeneity by favoring dispersion of the sample in the matrix. The low volatility, great ability to dissolve complex biomolecules, and high thermal stability of ionic liquids make them good candidates as MALDI matrix.

Armstrong and co-workers synthesized and tested new and conventional ionic liquids for peptide or protein analysis.[125] All conventional ionic liquids produced no MALDI signal and are not suitable for use as a MALDI matrix. Thus, nonvolatile amine salts of α-cyano-4-hydrocinnamic acid (α-CHCA) and sinapinic acid (SA) were synthesized and tested for protein analysis (Scheme 49).

The results obtained show that the ionic matrix must have an acidic proton in addition to UV absorbance. In this case, the [$M + H^+$] signal is produced with a similar or higher intensity than that obtained for solid matrix analogues, therefore reducing the limit of detection. For example, the pyridinium salt of α-cyano-4-hydrocinnamic acid has been successfully used for ionization of human insulin, and the signal obtained for the MALDI spectra is twice as intense as that obtained with an equivalent solid matrix. Moreover, dispersion of the solute throughout the ionic matrices is homogeneous; so, the spot-to-spot reproducibility is good, and the mixtures proved to be stable in a vacuum.

Scheme 49



Zabet-Moghaddam et al. improved the ionic liquid matrices (ILM) for analysis of proteins digests.[126] The ILM chosen was a pyridinium salt of α-cyano-4-hydrocinnamic acid, and the pyridine/matrix ratio was tested by comparing the MALDI signal intensities of model peptides. The best results were obtained with a matrix to pyridine molar ratio of 2:1. This matrix was then tested for identification of six standard proteins and in in vivo experiments after trypic digests. In all cases identification was unambiguous. Thus, the mixture of pyridine/α-cyano-4-hydrocinnamic acid in a 2:1 ratio seems to be a good matrix for identification and characterization of proteins by peptide mass-fingerprint analysis.

Jones et al. proved that ionic liquid matrices (nonvolatile amine salts of α-cyano-4-hydrocinnamic acid CHCA or 2,5-dihydrobenzoic acid DHB) increased the fragmentation observed with MALDI FTMS analysis of peptides compared with those observed when using MALDI TOF analysis and thus supplied complementary information.[127]

Tholey and co-workers showed that it is possible to analyze conventional ionic liquid (e.g., [bmim][PF_6]) by laser desorption/ionization (LDI) MS in positive- and negative-ion mode and also by matrix-assisted laser desorption/ionization.[128] In addition, amino acids, peptides, and proteins could be characterized in solution in ionic liquid after addition of solid matrix. Water-immiscible ionic liquid is better for AA and peptide analysis, while water-miscible ionic liquid is required for protein analysis. Quantification of AA in solution with large amounts of IL is achieved in the presence of isotope-labeled internal standard. Thus, analysis of chemical or enzymatic reactions in ionic liquid media can be performed by MALDI MS. In this paper the authors discussed a more theoretical approach of ion formation in the presence of ionic liquids in LDI and MALDI analysis.

Ionic liquids can also be successfully used as MALDI matrices for characterization of peptides and proteins.[129] To have a rapid and quantitative method which does not require the use of an internal standard for analysis of peptides and proteins is essential particularly for development of new biocatalysts, investigation of substrate specificity, and development of enzyme inhibitors. Tholey et al. showed that using ILMs as MALDI matrices allowed rapid and quantitative measurements, suitable for screening processes.

Li and Gross completed the previous studies by quantitative analysis with several peptides (Melittin, bradykinin, and

bovine insulin) having different molecular weights but similar hydrophobicity.[130] Calibration curves with good regression coefficients and low standard deviations were achieved without standard calibration. The slopes of these calibration curves correlate with the inverse of the peptide molecular mass.

Finally, the previously described ionic liquid matrices, easily prepared with an equivalent of the usual acid matrices and amine bases, also allowed low molecular mass compound analysis by MALDI MS.[131] Qualitative and quantitative MALDI MS analyses of amino acids have been successfully carried out with these ionic liquid matrices.

In conclusion, through these applications it has been shown that a new kind of IL, easily prepared with a standard solid acid MALDI matrix in the presence of an amine base, is a very efficient matrix system for MALDI MS analysis of proteins and peptides and more generally for high molecular mass compounds. These new ionic liquid matrices (ILM) facilitated sample preparation and sample procedures and gave better and more reproducible results compared with classical solid matrix. Thus, ILM exhibits great potential for the still not fully exploited MALDI MS analysis.

4. Conclusions and Perspectives

As has been seen, ionic liquids and amino acid derivatives are complementary to each other. Amino acids are key substrates for the synthesis of ILs,[132] and ILs are key media for chemical and biochemical modifications of amino acid derivatives. The outstanding series of new approaches summarized in this review highlight the role that could be played by ionic liquids for amino acid and peptide chemistry in the near future. Among the various applications we consider the following points as major scientific challenges.

(1) Synthesis and applications of novel chiral ionic liquids derived from amino acids using simple and straightforward methodologies. Use of these CILs in chirality transfer should be successful since the chemistry of chiral ionic media has recently proven its high efficiency;[133−138] indeed, seminal successes regarding asymmetric induction promoted by CILs used as solvents have been disclosed during the last years. For example, Afonso et al. described guanidinium salts with a chiral counteranion and their use in enantioselective dihydroxylation with high asymmetric induction (up to 85% ee).[134] Malhotra described copper-catalyzed enantioselective addition of diethylzinc to enones in the presence of CILs with a high asymmetric induction (ca. 76% ee).[135] Finally, twoconceptually rich approaches from Leitner et al. led to bifunctional CILs in the series of ammonium dimalatoborates and their use in aza-Baylis−Hilmann reaction with ee as high as 84%[136] as well as the use of simple ionic liquid obtained by protonation of amino acid as exclusive source of chiral information in a homogeneous transition-metal-catalyzed reaction using tropoisomeric ligands with ee up to 69%.[137] For the first time in the continuing history of asymmetric synthesis this fascinating series of results showed that, in contrast to popular opinion, application of chiral solvents (or chiral reaction media) in enantioselective synthesis can be successful! The growing interest for CILs in asymmetric synthesis is also based on the use of immobilized chiral catalysts that are designed for a specific task and belong to the class of TSILs (task-specific ionic liquids). Among a wide family of structures, special notice can be given to the derivatives of proline that were described in 2006[38] and their impressive results in Michael additions to nitroolefins. A fundamentally new concept in chirality transfer was also recently described by Schulz and Wasserscheid in which the ion-pairing effect is the asymmetric driving force for enantioselective reactions such as asymmetric hydrogenations.[138]

(2) New insights into peptide synthesis, which need further development in terms of atom economy. In this context, use of a novel family of solvents in which amino acids and their salts are directly soluble could open the way to strategies avoiding (or at least limiting) the use of costly protecting groups. Several approaches aiming to use unprotected amino acids have recently been reported.[107,108,139−144] In addition, synthesis of new coupling reagents as well as their recycling will certainly be a promising research area in the near future. As outlined before, some structural similarities exist between the coupling reagents (HATU, BOP, etc.,...) and ionic liquids. For example, a novel thiazolium salt was shown to be an excellent coupling reagent for hindered amino acids.[145]

(3) New processes in synthesis, separation, analysis, and spectrometry of amino acids and derivatives, including peptides and proteins.[146] For example, we are currently investigating the use of chiral ionic liquids for liquid−liquid resolution procedures and crystallization-induced transformations of racemic amino acids. Novel physicochemical behavior could also arise, which could have an impact on reaction and separation processes: for example, lower critical solution temperature (LCST) changes of a mixture of water and ionic liquids derived from amino acids was recently reported by Fukumoto and Ohno.[147] Also, the reversible folding−unfolding and long period stabilization against aggregation and hydrolysis of peptide solutions was reported in 2007.[148]

Obviously, these suggestions are not the only topics to be promoted by the "ionic liquid/amino acid" approach. Certainly, the most exciting aspect that we would like to highlight is use of ionic solvents, ionic media, and ionic tools for this unique chemistry of amino acids and derivatives, which offers the unprecedented possibility to use neither aqueous nor organic conditions, giving access to fundamentally new concepts in the field.

5. Acknowledgments

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