Use of Organic Superbases and Temperature Effects for the Development of Reversible Protic Amino Acid Salts

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Abstract: Novel reversible organic salts based on amino acids in the presence of organic superbases [1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and tetramethylguanidine (TMG)] have been prepared. An optimized acid–base reaction methodology allowed the preparation of novel protic amino acid salts with improved water-solubility profiles and unexpected phase behavior. Complementary differential scanning calorimetry (DSC) and thermal ^{1}H NMR analysis indicated that a phase separation between water and amino acid salt occurs and that the process is reversible, depending upon temperature and the selected organic superbase. These studies open the possibility for tuning miscibility and ionicity of organic salts as well as development of reversible protic chiral ionic liquids or molten salts.

Key words : amino acids, green chemistry, ionic liquids, cations, protonation

Protic ionic liquids (PIL) and molten salts can be modified in order to tune some physicochemical properties such as melting point, solubility, conductivity, and polarity. Not only the pK_a (difference between acid and base components) but also the ability to form a hydrogen bond,[1] the coulombic, π–π, and permanent/induced dipolar interactions control the behavior of the PIL. An appropriate choice of acid and base is used to optimize the PIL for specific tasks in different scientific topics such as organic synthesis, analytical and biological applications, for example, fuel cells, explosives or as lubricants,[2] among others.

Organic superbases, an established class of compounds, open diverse perspectives in the field of organic synthesis relating to their versatility for the application as reagents, catalysts, or even solvents. Nitrogen and phosphorous are the key elements that control and define the pK_a of such compounds. In order to illustrate this fact, the order of basicity increases with the number of conjugated nitrogen atoms in the molecule (amine < amidine < guanidine).[3] The literature describes several examples of superbases such as the amidine (1,8-diazabicyclo[5.4.0]undec-7-ene, DBU)[4] or the guanidine (tetramethylguanidine, TMG) systems,[3,5] used in the construction of PIL. As previously noted,[4,6] increasing temperature can lead to a decrease in the ionicity of PIL by reversible proton exchange.

A different type of carbonate- and carbamate-based PIL resulting from reaction of alcohols[7] or amines[8] with CO_2 in the presence of an organic superbase are even more prone to reversibility under moderate temperatures, associated with flushing by an inert gas to remove CO_2 from the system.

From another perspective, amino acids can be used to construct chiral ionic liquids (IL), allowing their use as anionic[9] or cationic species.[10] Additionally, the choice of the side chain of amino acids can open new perspectives for the applications of their corresponding PILs as molten salts, in particular as chiral solvents, shift reagents, enantioselective catalysts,[11] in cellulose dissolution,[12] electrochemistry,[13] or as solvatochromic probes.[14] Ohno and Fukumoto reported that an amino acid based IL, whilst forming two phases when in contact with water at room temperature, can mix completely with water at lower temperatures.[12] Another example, based on the polyethylene oxide/water system, shows the same principle of weakening hydrogen bonding incrementally with temperature leading to phase separation.[15]

NMR spectroscopy can be used to evaluate the hydrogen bond strength,[1] relative degree of aggregation,[16] proton exchange,[17] and degree of ionicity,[18] in particular how these parameters vary with temperature. Such studies should be valuable for the optimization of processes such as control of miscibility between two hydrogen-bond-based solvents in extractions, tuning of ionic strength, solubilization or selective precipitation, and in potentiometric applications.

In the present work, the acid–base reaction between an amino acid (glycine, L-alanine, L-phenylalanine, and D,L-tryptophan) and an organic superbase (DBU and TMG) have been evaluated in order to obtain their respective salts. The physical, chemical, and thermal properties of these salts including the variation of phase behavior were studied for all the prepared PILs. Additionally, a detailed temperature-dependent ^{1}H NMR study was performed for two representatives in order to illustrate the importance of temperature in the proton disposition within the system.

The selected amino acid and organic superbase were combined to result in equivalent cation/anion (1:1) composition. Glycine, L-alanine, L-phenylalanine, and D,L-tryptophan were selected as representative amino acids and reacted with DBU and 1,1,3,3-tetramethylguanidine (TMG) as representative organic superbases (Scheme 1).[25–27]

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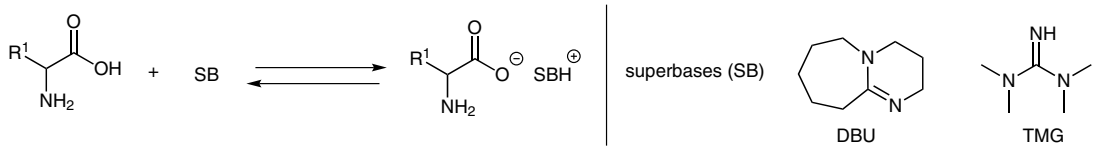

Scheme 1 Acid–base reaction between selected amino acids and organic superbases: glycine (R^1 = H), L-alanine (R^1 = Me), L-phenylalanine (R^1 = Bn), and D,L-tryptophan (R^1 = methylindolyl).

Table 1 summarizes the yields and some of the properties of the salts thus prepared. In general, high isolated yields (89–100%) were achieved except in the cases of [TMGH][Gly] and [TMGH][(L)-Ala]. In these cases a significant discrepancy between amino acid (anion) and superbase (cation) when compared with the expected 1:1 proportion was observed after solvent evaporation. In order to explain this fact it is important to consider that, when a reagent is removed from an acid–base chemical equilibrium, the correspondent equilibrium is shifted to replace that component (in this case conversion of $TMGH^+$ into TMG). TMG possess a vapor pressure of 30 Pa at 20 °C, and the acid–base reaction of TMG with the glycine and alanine appears either to be incomplete or the proton is hydrogen bonded between the amino acid and the organic superbase. In contrast, the salts [TMGH][(L)-Phe] and [TMGH][(D,L)-Trp] do not appear to behave in the same manner, and the 1:1 proportion between amino acid and superbase was retained after solvent evaporation. A possible explanation for this fact should be related with the additional π–π interactions between the L-phenylalanine and D,L-tryptophan with TMG.[19]

The loss of TMG from [TMGH][Gly] and [TMGH][(L)-Ala] raises the question of the true nature of the interaction between the amino acid and the superbase. According to MacFarlane et al.,[20] a pK_a difference of 4 is enough for a complete proton transfer. In the case of TMG, the pK_a difference between it and the NH_3^+ group of the amino acids is around 4 (slightly lower when DBU is used). In contrast to TMG, however, the 1:1 proportion was preserved in all cases where DBU was used as the organic superbase – possibly explained by high boiling point of DBU.

In this context, it is possible to propose that, in these situations, the cation and anion are not well defined; but instead proton sharing by hydrogen bonding might be occurring. This hypothesis is supported by NMR and FTIR data. There is a significant difference in chemical shift from δ = 3.57 ppm for the α-proton of zwitterionic glycine[21] to δ = 3.14 ppm for the equivalent proton in [DBUH][Gly][28] in the respective ^{1}H NMR spectra in D_2O, indicating a higher degree of shielding as a result of a hydrogen-bonding interaction between the amino acid and DBU. Additionally, the amidinium carbon in the DBU structure is more deshielded {δ = 165.91 ppm as experimental value, observed for [DBUH][Gly] in D_2O} when compared with the calculated value (δ = 157.3 ppm[22]) as another indication that DBU is protonated. Additionally, FTIR spectroscopy shows relatively low-intensity overtone bands attributed to the NH_3^+ group of the amino acid at 2623 and 2169 cm^{-1} and a strong band at 1647 cm^{-1} for protonated DBU. Furthermore, [DBUH][Gly] demonstrates a higher water affinity than the parent amino acid showing and intense broad absorption centered at 3442 cm^{-1} attributed to the presence of water. Equally, [DBUH][(L)-Ala][29] showed a similar chemical shift for the carbons adjacent to the amidinium group of DBU, again attributed to the proton interaction between the amino acid and DBU.

The [DBUH][(L)-Ala] was obtained as homogeneous solid at room temperature but it became heterogeneous at temperatures over 35 °C. The broad absorption centered at 3397 cm^{-1} in the FTIR spectrum and the high water affinity (Table 1) are indicative that a considerable quantity of water is present in the product and that, on heating, hydrogen bonding becomes weaker such that the mixture becomes heterogeneous.[12]

The ^{1}H NMR spectrum of [DBUH][(L)-Phe] is indicative that the α-protons are more shielded (δ = 3.89 vs. δ = 3.99 ppm[21]) than in parent amino acid. Regarding [TMGH][(L)-Phe], α- and β-protons of the anion are even more shielded in relation to the zwitterionic L-phenylalanine, indicating that TMG is a more effective superbase than DBU for proton transfer. These observations can be corroborated by FTIR spectra of [DBUH][(L)-Phe][30] and [TMGH][(L)-Phe].[31] Whereas in the former, the low-intensity overtone band at 2123 cm^{-1} is indicative of the existence of the NH_3^+ group of the amino acid, the virtual absence of a corresponding absorption in the TMG salt highlights the complete proton transfer between L-phenylalanine and TMG. In both cases DBU and TMG were protonated as indicated by intense bands at 1646 and 1609 cm^{-1}, respectively.

In the case of the (D,L)-tryptophan-derived salts {[DBUH][(D,L)-Trp][32] and [TMGH][(D,L)-Trp][33]} ^{1}H NMR analysis indicates that, in both compounds, the β-protons are more shielded than equivalent protons in the parent amino acid;[21] this effect being more pronounced in [(D,L)-Trp] as with the L-phenylalanine-based salts. The FTIR spectra indicated more intense overtone bands related to the NH_3^+ group in [DBUH][(D,L)-Trp] than in [TMGH][(D,L)-Trp], again as expected, and a lower proportion of water than with the glycine-, L-alanine-, or L-phenylalanine-based salts according to the water affinities presented in Table 1. In all the salts was observed an increase of aqueous solubility, comparing with parent zwitterionic amino acids.

Table 1 Acid–Base Reaction between Selected Amino Acids and Organic Superbases

Acid–base product	Yield (%)[a]	Physical state	Transition beginning of heterogeneity (°C)[b]	Water solubility (g/L)[c]
[DBUH][Gly]	89	heterogeneous mixture	–	800 (300)
[TMGH][Gly][d]	n.d.	–	–	–
[DBUH][(L)-Ala]	97	white solid	35	900 (200)
[TMGH][(L)-Ala][d]	n.d.	–	–	–
[DBUH][(L)-Phe]	98	orange paste	68	200 (40)
[$TMGH^+$][(L)-Phe^-]	100	brown paste	127	100 (40)
[$DBUH^+$][(D,L)-Trp^-]	100	yellow solid	85	7 (<6)
[$TMGH^+$][(D,L)-Trp^-]	100	brown solid	111	15 (<6)

[a] Isolated yield.
[b] Indication of initial of transition from homogeneous to heterogeneous mixture, this heterogeneity remains over a high temperature range.
[c] Water solubility: solubility of original amino acid indicated in parentheses.
[d] Cation and anion are not in stoichiometric proportions (^{1}H NMR analysis) due to loss of tetramethylguanidine by evaporation under reduced pressure.

An important point of this study is related to the observation of the onset of temperature-dependent heterogeneity instead of a defined melting point. Two different hypotheses could explain such a behavior. Normally, acid–base reactions are exothermic. Therefore, assuming that these systems follow the same principle when increasing the temperature, by Le Chatelier's principle the equilibrium will be moved towards the reagents (solid amino acid and liquid superbase). Alternatively, the presence of water in the salts and the transition to heterogeneity could be a result of the weakening of hydrogen bonds with temperature[12,15] and consequent phase separation.

To distinguish these hypotheses a differential scanning calorimetry (DSC) study was performed with [DBUH][(L)-Ala] when an endotherm was observed starting at ~ 30 °C to 120 °C. In the subsequent heating cycles the endothermic process was less pronounced. These data indicate that, in the first cycle when a considerable quantity of water was present, a phase separation between the water and the [DBUH][(L)-Ala] occurred in the initial stages. With further increase to 120 °C, water was physically lost and, in the further heating cycles the endothermic effect was lessened. To confirm the water effect, the sample was then exposed to air during 24 hours, and after that period a similar DSC study was performed. Once again the first cycle a pronounced endothermic effect was observed when heating the sample, but this diminished significantly in further cycles.

In order to investigate further the molecular interactions responsible for such behavior, a thermal ^{1}H NMR study of [DBUH][(D,L)-Trp] was performed in predried deuterated DMSO (Figure 1).

In these ^{1}H NMR thermal studies it was possible to observe a steady decrease of the area of the peaks with the increase of temperature, presumably arising because density decreases with the increase in temperature. Additionally, it was possible to observe the conservation of the 1:1 proportion, (D,L)-tryptophan/superbase for both compounds studied, with the variation of temperature.

Considering [DBUH][(D,L)-Trp] (Figure 1), when increasing the temperature from 30 to 70 °C, the broad resonance corresponding to water and possibly amidine NH and amine NH_2 protons (Figure 1, a) shifts to lower field, indicating a reinforcement of hydrogen-bond interactions or higher degree of association, in contradiction with other studies.[12,15] With further increase in temperature to 90 °C the trend is reversed, the band shifts once again to higher field, indicating that hydrogen bonding is become weaker (heterogeneity commences at 85 °C, Table 1). To explain this reversal of behavior, it is important to recall that the pK_w of water decreases with temperature until ca. 250 °C at 0.1 MPa (atmospheric pressure), and in that range, the variation of pK_w decreases with the increasing temperature.[23] In addition, the evidence that DBU is less protonated and that (D,L)-tryptophan is more neutral at lower temperatures (Figure 1, b and c) leads to the conclusion that the more pronounced increase in ionicity of water and DBU (Figure 1, b), in the initial stages of increase in temperature (30–70 °C), is more important in strengthening the hydrogen-bond interactions (the major energetic component of hydrogen bonds is electrostatic and only a small fraction is covalent[24]) than is the effect of direct increase of temperature in diminishing them.

With further increase of temperature, increasing ionicity slows down, and the effect of temperature is more pronounced in reducing directional hydrogen-bonding interactions. Considering Figure 1 (d), the displacement of the NH resonance of the secondary aromatic amine towards higher field is indicative of a decrease in hydrogen-bonding interaction with increasing temperature (the proton is more localized on the nitrogen atom). The fact that DMSO

is insensitive to variation of temperature as shown in Figure 1 (e) is indicative that any solvent interaction with [DBUH][(D,L)-Trp] is independent of temperature.

In order to verify that the system [DBUH][(D,L)-Trp] was reversible, a comparison of two ^{1}H NMR spectra at 70 °C was carried out, one recorded when increasing the temperature, the other recorded when decreasing the temperature. Both spectra are superimposable, indicating that the system is reversible.

In summary, the combination of amino acids with organic superbases has allowed the preparation of novel organic salts in high yields with improved water solubilities and unexpected temperature-dependent phase behavior. In particular, the transition to heterogeneity instead of a melting point was observed. A combination of DSC analysis with variable temperature ^{1}H NMR studies indicate that phase separation on increasing temperature results from weakening of hydrogen-bond interactions between water and the salts. ^{1}H NMR studies, [DBUH][(D,L)-Trp]/water verified that the process was reversible. Additionally, the choice of the organic superbase is the key step in order to tune the corresponding protonation or deprotonation of the amino acid.

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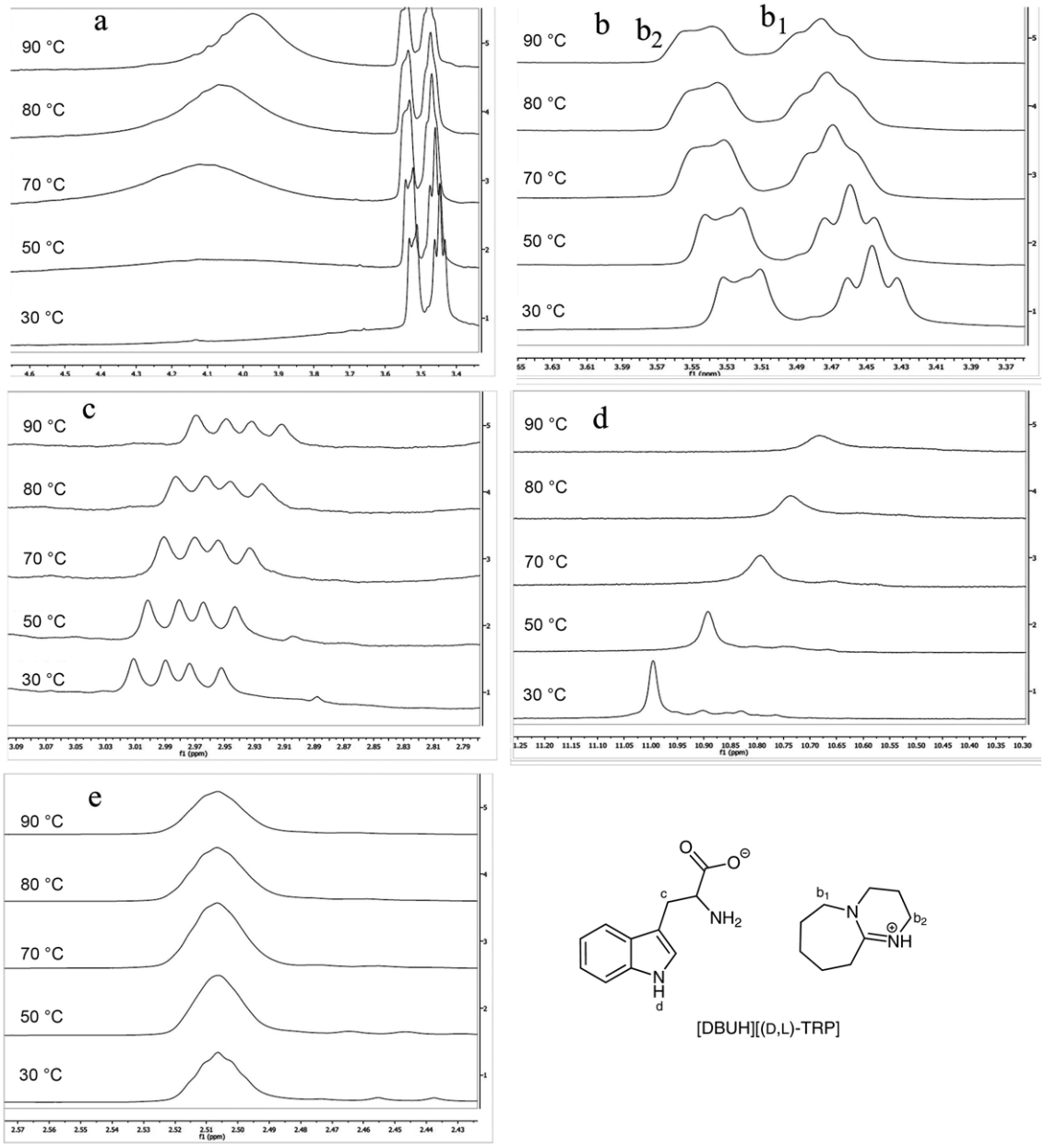

Figure 1 Chemical-shift variation with temperature in ^{1}H NMR spectra of [DBUH$^+$][(D,L)-Trp] after increasing and decreasing the temperature (30–90 °C): a) water and presumably primary amine and NH amidine hydrogen bond band; b) protons belonging to CH_2 group adjacent to the amidinium core; c) D,L-tryptophan CH_2 protons; d) tryptophan secondary amine proton; e) DMSO peak.

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(25) **Reagents and Solvents**
Commercial reagents were used as supplied: Glycine was supplied by BDH with a purity of 99%, L-alanine with a purity of 99% was provided by Alfa Aesar, L-phenylalanine purchased from Merck with a purity of >99%, (D,L)-tryptophan was supplied by Merck with a purity >99%, 1,1,3,3-tetramethylguanidine (TMG), 99%, was supplied by Sigma-Aldrich and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) ≥99%, was provided by Fluka. Solvents were also used as supplied: CH_2Cl_2 was supplied by Sigma-Aldrich, p.a. grade, MeOH was supplied by Sigma-Aldrich HPLC grade, distilled H_2O was processed by Diwer Technologies water max w2 equipment.
(26) **General Procedure for the Preparation of Amino Acid Based PIL and Ionic Mixtures**
The organic superbase (1 equiv) diluted in an organic solvent (1.5–2 mL) was added slowly to a suspension of an amino acid (1 equiv in 1.5–2 mL of solvent). The resultant mixture was stirred during a variable period of time at r.t. For workup, the solvent was evaporated using a rotary evaporator, and the resultant product was left under high vacuum for a period of 8–16 h. All the compounds prepared following this procedure were stored at 7 °C.
(27) **Solubility in Water**
To a weighed sample of a salt was added distilled H_2O, dropwise, until homogeneity. The mixture was weighed, and the proportion of compound to minimum quantity of H_2O was thus obtained.
(28) **2,3,4,6,7,8,9,10-Octahydropyrimido[1,2-*a*]azepin-1-ium 2-Aminoacetate {[DBUH][Gly]}**
Prepared using the general procedure for the preparation of amino acid based PIL and ionic mixtures. For the preparation of this specific compound, the reaction proceeds over 6 h using MeOH (3 mL) as solvent. After workup the product was obtained as a heterogeneous white liquid and solid mixture; yield 89%. ^{1}H NMR (400 MHz, D_2O): δ = 1.57–1.62 (m, 6 H), 1.90 (quint, *J* = 6 Hz, 2 H), 2.50–2.52 (m, 2 H), 3.14 (s, 2 H), 3.21 (t, *J* = 6 Hz, 2 H), 3.42 (t, *J* = 6 Hz, 2 H), 3.44–3.46 (m, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 18.87, 23.25, 25.81, 28.40, 32.75, 37.91, 43.87, 48.16, 54.10, 165.91, 179.2 ppm. IR (KBr): 3422, 3250, 3119, 2935, 2862, 2623, 2231, 2169, 1647, 1586, 1560, 1476, 1437, 1401, 1366, 1324, 1302, 1270, 1207, 1156, 1126, 1107, 1089, 1043, 1009, 996, 984, 966, 929, 888, 829, 687, 666, 635, 609 cm^{-1}. Anal. Calcd for $C_{11}H_{21}N_3O_2 \cdot 2H_2O$): C, 50.17; H, 9.57; N, 15.96. Found: C, 49.58; H, 9.27; N, 16.97.
(29) **2,3,4,6,7,8,9,10-Octahydropyrimido[1,2-*a*]azepin-1-ium (L)-2-Aminopropanoate {[DBUH][(L)-Ala]}**
The reaction proceeded during 24 h at r.t. using MeOH (4 mL) as solvent. After workup the product was obtained as a white solid; yield 97%. ^{1}H NMR (400 MHz, D_2O): δ = 1.12 (d, *J* = 8 Hz, 3 H), 1.56–1.60 (m, 6 H), 1.88 (quint, *J* = 4 Hz, 2 H), 2.48–2.51 (m, 2 H), 3.19 (t, *J* = 6 Hz, 2 H), 3.23 (m, 1 H), 3.40 (t, *J* = 6 Hz, 2 H), 3.43–3.45 (m, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 18.86, 19.88, 23.25, 25.80, 28.39, 32.73, 37.90, 48.14, 51.29, 54.08, 165.89, 183.65 ppm. IR (KBr): 3397, 3251, 3088, 3000, 2989, 2937, 2863, 2814, 2605, 2505, 2469, 2417, 2294, 2248, 2113, 1653, 1648, 1600, 1521, 1506, 1456, 1413, 1385, 1363, 1320, 1310, 1271, 1237, 1208, 1153, 1115, 1014, 997, 985, 967, 919, 888, 851, 773, 721, 648, 620 cm^{-1}.
(30) **2,3,4,6,7,8,9,10-Octahydropyrimido[1,2-*a*]azepin-1-ium (L)-2-Amino-3-phenylpropanoate {[DBUH][(L)-Phe]}**
The reaction proceeded during 75 h at r.t. using CH_2Cl_2 (4 mL) as solvent. After workup procedure the product was obtained as an orange paste; yield 98%. ^{1}H NMR (400 MHz, D_2O): δ = 1.53–1.58 (m, 6 H), 1.85 (quint, *J* = 6 Hz, 2 H), 2.46–2.47 (m, 2 H), 2.94 (dd, J_1 = 12 Hz, J_2 = 8 Hz, 1 H), 3.10 (dd, J_1 = 16 Hz, J_2 = 6 Hz, 1 H), 3.16 (t, *J* = 4 Hz, 2 H), 3.36 (t, *J* = 6 Hz, 2 H), 3.40–3.42 (m, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 18.85, 23.24, 25.80, 28.39, 32.74, 36.96, 37.90, 48.14, 54.08, 56.20, 127.49, 128.99, 129.33, 135.52, 175.16, 177.49 ppm. IR (KBr): 3426, 3107, 2935, 3258, 3033, 2908, 2883, 2123, 1646, 1560, 1496, 1457, 1410, 1323, 1307, 1207, 1162, 1107, 1075, 984, 913, 848, 746, 699 cm^{-1}. Anal. Calcd for $C_{18}H_{27}N_3O_2 \cdot 2.6H_2O$): C, 59.35; H, 8.91; N, 11.54. Found: C, 59.13; H, 8.37; N, 11.11.

(31) **Bis(dimethylamino)methaniminium (L)-2-Amino-3-phenylpropanoate {[TMGH][(L)-Phe]}**
The reaction proceeded during 75 h at r.t. using CH_2Cl_2 (4 mL) as solvent. After workup procedure the product was obtained as a beige paste; yield 100%. ^{1}H NMR (400 MHz, D_2O): δ = 2.80 (s, 12 H), 2.85 (m, 1 H), 3.00 (dd, J_1 = 12 Hz, J_2 = 4 Hz, 1 H), 3.62 (t, J = 6 Hz, 1 H), 7.15–7.27 (m, 5 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 38.32, 38.77, 56.63, 127.15, 128.81, 129.34, 136.51, 161.31, 177.81 ppm. IR (KBr): 3419, 3208, 2964, 2853, 2130, 1609, 1565, 1495, 1456, 1436, 1410, 1340, 1335, 1320, 1307, 1293, 1227, 1163, 1154, 1091, 1073, 1037, 1004, 949, 913, 876, 848, 779, 746, 699, 682, 605 cm^{-1}.

(32) **2,3,4,6,7,8,9,10-Octahydropyrimido[1,2-*a*]azepin-1-ium (D,L)-2-Amino-3-(1*H*-indol-3-yl)propanoate {[DBUH][(D,L)-Trp]}**
The reaction proceeded during 20 h at r.t. using CH_2Cl_2 (4 mL) as solvent. After workup procedure the product was obtained as a yellow solid; yield 100%. ^{1}H NMR (400 MHz, DMSO): δ = 1.56–1.63 (m, 6 H), 1.85 (quint, J = 6 Hz, 2 H), 2.67–2.70 (m, 2 H), 2.88 (dd, J_1 = 16 H, J_2 = 8 Hz, 1 H), 3.19 (t, J = 6 Hz, 2 H), 3.25 (m, 1 H), 3.42 (t, J = 6 Hz, 2 H), 3.48–3.50 (m, 2 H), 6.92 (t, J = 8 Hz, 1 H), 7.03 (t, J = 8 Hz, 1 H), 7.20 (s, 1 H), 7.32 (d, J = 8 Hz, 1 H), 7.53 (d, J = 8 Hz, 1 H), 10.80–11.00 (m, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 18.97, 23.49, 26.04, 28.31, 28.51, 31.22, 37.50, 47.79, 53.18, 54.94, 55.25, 110.46, 111.26, 118.08, 118.43, 120.71, 123.83, 127.45, 136.28, 165.25, 172.47, 172.51 ppm. IR (KBr): 3403, 3242, 3106, 3053, 2934, 2861, 2555, 2097, 1719, 1703, 1647, 1608, 1487, 1456, 1404, 1358, 1341, 1320, 1312, 1278, 1229, 1207, 1160, 1106, 1068, 1055, 1008, 995, 984, 965, 914, 900, 877, 864, 845, 834, 766, 746, 738, 720, 691, 660, 625 cm^{-1}. Analysis Calcd for $C_{20}H_{28}N_4O_2 \cdot 2.4H_2O$: C, 60.10; H, 8.27; N, 14.02. Found: C, 59.88; H, 7.95; N, 14.28.

(33) **Bis(dimethylamino)methaniminium (D,L)-2-Amino-3-(1*H*-indol-3-yl)propanoate {[TMGH][(D,L)-Trp]}**
The reaction proceeded during 23 h at r.t. using CH_2Cl_2 (4 mL) as solvent. After workup procedure the product was obtained as a brown solid; yield 100%. ^{1}H NMR (400 MHz, DMSO): δ = 2.76 (dd, J_1 = 16 Hz, J_2 = 8 Hz), 2.84 (s, 12 H), 3.21 (dd, J_1 = 16 Hz, J_2 = 4 Hz, 1 H), 3.29–3.30 (m, 1 H) 6.93 (t, J = 8 Hz, 1 H), 7.02 (t, J = 8 Hz, 1 H), 7.18 (s, 1 H), 7.32 (d, J = 8 Hz, 1 H), 7.52 (d, J = 8 Hz, 1 H), 11.01 (s, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO): δ = 29.78, 29.80, 55.79, 111.25, 111.37, 117.38, 118.44, 120.62, 123.61, 127.56, 136.29, 161.65, 173.86 ppm. IR (KBr): 3404, 3088, 3056, 2965, 2816, 2742, 2546, 2099, 1718, 1703, 1653, 1608, 1506, 1487, 1456, 1412, 1359, 1341, 1312, 1279, 1232, 1167, 1098, 1068, 1039, 1008, 988, 964, 929, 877, 865, 846, 836, 773, 747, 738, 721, 660, 625 cm^{-1}. Anal. Calcd for $C_{16}H_{25}N_5O_2 \cdot 2.2H_2O$: C, 53.52; H, 8.25; N, 19.51. Found: C, 53.94; H, 7.56; N, 18.83.

