Supplementary Material (ESI) for Green Chemistry

Supporting information for

Toxicity evaluation on human colon carcinoma cells (CaCo-2) of ionic liquids based on guanidinium, ammonium, phosphonium, pyridinium and pyrrolidinium cation

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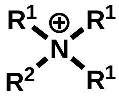
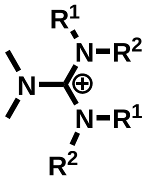
Index:

Cations

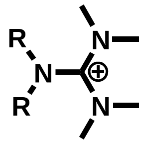
$R^1=CH_3$, $R^2=n\text{-}C_4H_9$	[C_4MIM]
$R^1=CH_3$, $R^2=n\text{-}C_8H_{17}$	[C_8MIM]
$R^1=CH_3$, $R^2=n\text{-}C_{10}H_{22}$	[C_{10}MIM]
$R^1=CH_3$, $R^2=(CH_2)_2OH$	[C_2OHMIM]
$R^1=CH_3$, $R^2=(CH_2)_2O(CH_2)_2OMe$	[C_5O_2MIM]
$R^1=CH_3$, $R^2=CH_2Ph$	[BzMIM]
$R^1=CH_3$, $R^2=C_{10}H_{20}CO_2H$	[$C_{10}O_2$HMIM]
$R^1=CH_3$, $R^2=C_{10}H_{20}CO_2HEt$	[$C_{10}O_2$EtMIM]
$R^1=CH_2Ph$, $R^2=CH_2Ph$	[BzIMBz]



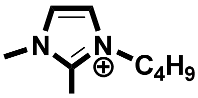
$R^1=CH_3$, $R^2=(CH_2)_2OH$	[Choline]
$R^1=CH_3$, $R^2=Ph$	[PhTMA]
$R^1=n\text{-}C_2H_5$, $R^2=CH_2Ph$	[BzTEA]
$R^1=n\text{-}C_8H_{17}$, $R^2=CH_3$	[Aliquat]



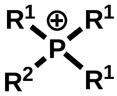
$R^1=R^2=n\text{-}C_4H_9$	[(di-b)$_2$dmg]
$R^1=R^2=n\text{-}C_6H_{13}$	[(di-h)$_2$dmg]
$R^1=R^2=n\text{-}C_8H_{17}$	[(di-o)$_2$dmg]
$R^1=CH_3$, $R^2=n\text{-}C_4H_9$	[(mb)$_2$dmg]
$R^1=CH_3$, $R^2=n\text{-}C_4H_{13}$	[(mh)$_2$dmg]
$R^1=n\text{-}C_2H_5$, $R^2=n\text{-}C_4H_9$	[(eb)$_2$dmg]
$R^1=n\text{-}C_2H_5,CH_3$ $R^2=n\text{-}C_4H_9$	[(eb)(mb)dmg]
$R^1=n\text{-}C_2H_5$, $n\text{-}C_4H_9$ $R^2=n\text{-}C_4H_9$	[(di-b)(eb)dmg]
$R^1=R^2=CH_2CH_2OCH_3$	[(C_3O)$_4$dmg]



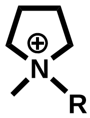
$R=n\text{-}C_4H_9$	[(di-b)tmg]
$R=n\text{-}C_7H_{15}$	[(di-hept)tmg]
$R=n\text{-}C_{12}H_{21}$	[(di-dodec)tmg]



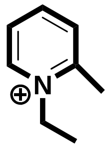
[BDMIM]



$R^1=n\text{-}C_6H_{13}$, $R^2=n\text{-}C_{14}H_{29}$ [$P_{6,6,6,14}$]



R = n-C_4H_9 [C_4MPyr]

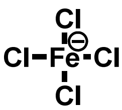


[2-MEPy]

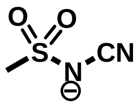
Anions

[Cl]
[Br]
[I]

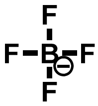
[$FeCl_4$]



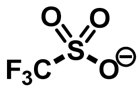
[CMS]



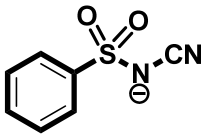
[BF_4]



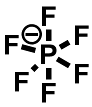
[TfO]



[CBS]



[PF_6]



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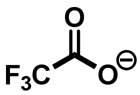
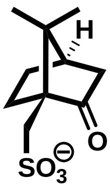
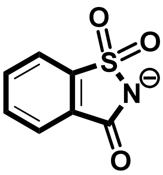


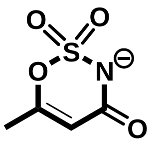
[CSA]



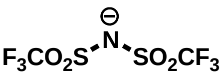
[SAC]



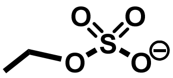
[ACS]



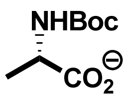
[NTf_2]



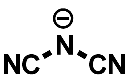
[$EtOSO_3$]



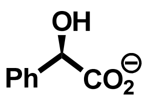
[Boc Ala]



[DCA]



[Mand]



[Boc Thr]

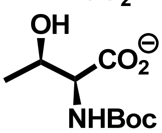


Figure 1 – Structure of the Ionic Liquids (ILs) studied

Experimental

Materials

Commercially supplied reagents were used as supplied. RPMI Medium 1640, Fetal bovine serum and L-Glutamine 200mM (100x) and trypsin-EDTA solution were from GIBCO and the cell proliferation reagent MTT was purchased from Sigma. NMR spectra were recorded on a Bruker AMX 400 Spectrometer.

Cell culture

Human colon carcinoma CaCo-2 (ATTC, USA) were cultured in 175 cm^2 flasks in RPMI Medium 1640, supplemented with 10% fetal bovine serum (FBS) and 2mM L-Glutamine at 37ºC in a humidified atmosphere of 5% CO_2.

Viability cell assay

Viability assay was conducted on confluent CaCo-2 cells which have the characteristic of differentiating in human intestinal epithelium [2]. Stock solutions of the ionic liquids were prepared in 100% DMSO (except for [PhTMA] [Cl] which was prepared in water) and were posterior diluted with the cell culture medium to attain the following concentrations: 750, 2250, 4500, 7500, 15000 μM. Cells were incubated with the ionic liquid solutions for 4 hours and then medium was removed and substituted by fresh medium with 3-(4, 5- Dimethylthiazolyl-2) -2, 5- diphenyltetrazolium bromide (MTT) 0.5 mg/ml. Plate returned to the incubator for an additional period of 3-4 hours and reaction was stopped with 150 μl DMSO per well. The amount of product was measured at two wavelengths of 570 nm and 690 nm in a plate reader spectrophotometer. Incubations were done in triplicate, as the controls, which consisted of cells unexposed to the ionic liquids. The ratio between the absorbance of sample treated cells and the absorbance of control cells was used to determine the cell viability and this parameter was plotted in function of the base 10 logarithm of the ionic liquid concentration (μM); each experimental point represents the average of the three replicates. The function that best fitted to the experimental points was used to represent graphically the ionic liquid toxicity curves. EC_{50} values represent the concentration at which the ionic liquid induced 50% decrease of cells viability.

In order to give a combined overview of the toxicity data, we provided below all the toxicity data obtained from the laboratory including the ones prior reported. [1, 3] From figures 2 to 20, we present the dose curve responses obtained for each specified ionic liquid, that was left in contact with confluent CaCo-2 cells [2]. Concentrations up to 6000 μM [1, 3] or 15000 μM are showed in case of ionic liquids studied previously or in this experimental study, respectively. In figure 22, it is presented the result obtained for a single concentration of 6000 μM (maximum concentration used in the experimental study [1]). Since the respective ionic liquids did not demonstrate to be significantly toxic, their dose curve responses were not produced at the time. In figure 23, it is showed the viability obtained for a 4000 μM concentration of [(di-h)$_2$dmg] cation with [Boc Thr], [Boc Ala] (configuration D and L), [Mand] (configuration D and L) and [CSA] (configuration R and S), as anions. As they were already very toxic at this concentration, since they induced more than 50% cell death, they were assumed as toxic and their dose curve responses were not created. Moreover, they all caused turbidity in the cell culture medium.

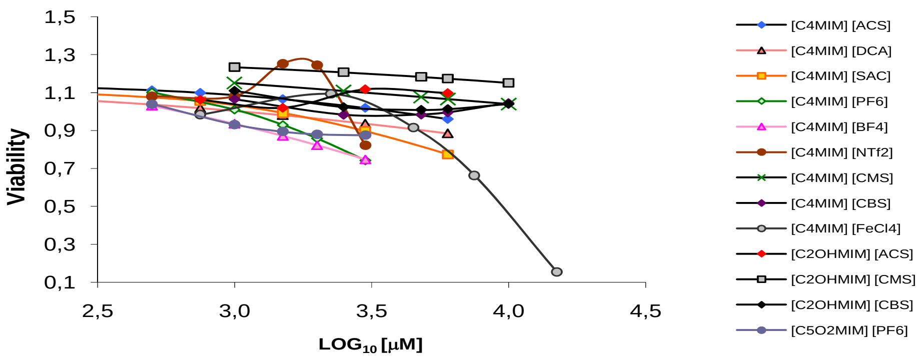


Figure 2 – Dose confluent CaCo-2 viability curves obtained in response to different concentrations of several studied anions with [C$_4$MIM], [C$_2$OHMIM] and [C$_5$OHMIM] cations.

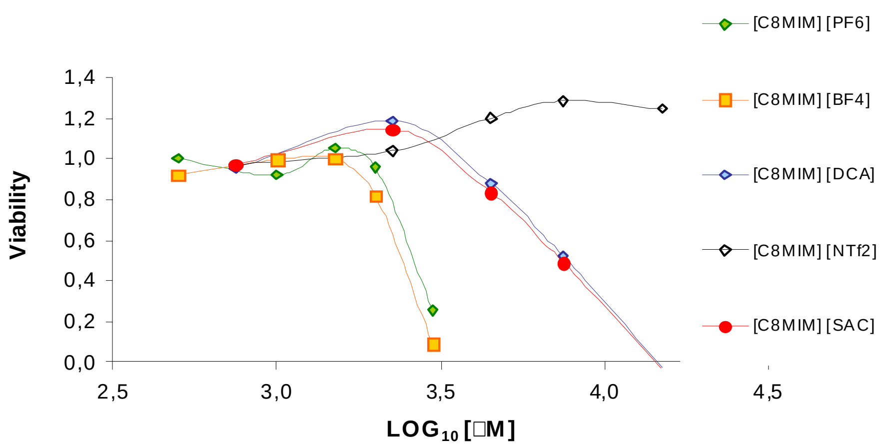


Figure 3 – Dose confluent CaCo-2 viability curves obtained in response to different concentrations of several studied anions with [C$_8$MIM] cation.

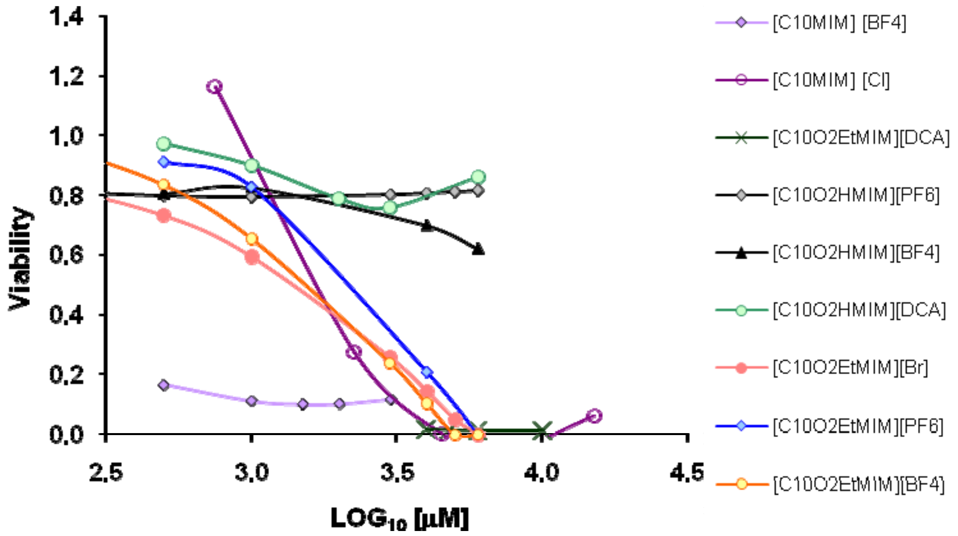


Figure 4 – Dose confluent CaCo-2 viability curves obtained after exposure of the cells to different concentrations of [C_{10}MIM], [$C_{10}O_2$HMIM] and [$C_{10}O_2$EtMIM] ionic liquids.

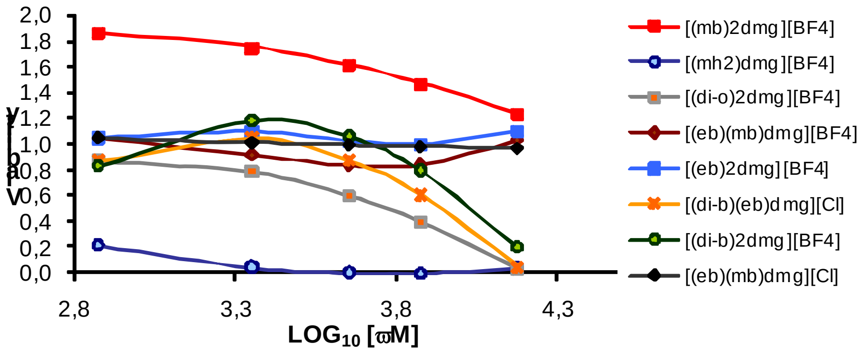


Figure 5 – Dose confluent CaCo-2 viability curves in response to different concentrations of dimethyl-guanidinium [dmg] with alkyl chain of variable lengths.

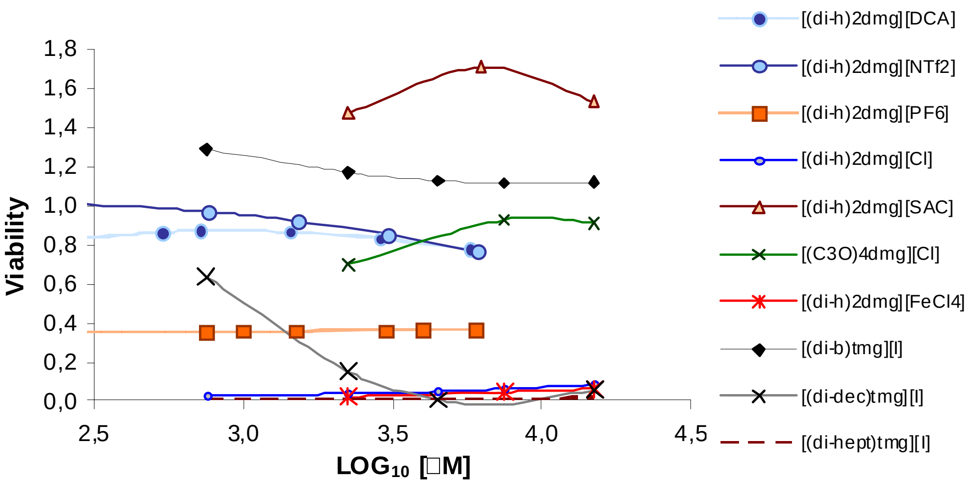


Figure 6 – Dependence of confluent CaCo-2 cell viability with different concentrations of dimethyl-guanidinium in combination with the anions DCA, NTf_2, PF_6, Cl, SAC and $FeCl_4$; and effect of the ether functionality. Effect of the tetramethyl-guanidinium cation with alkyl chain of different lengths. [(di-h)$_2$dmg] [Cl/SAC/$FeCl_4$] and [(di-dodec)tmg] [I] were seen to have low solubility in aqueous medium. [(di-h)$_2$dmg] [DCA/PF_6/NTf_2] mediated effects are possibly influenced by the same problem [1].

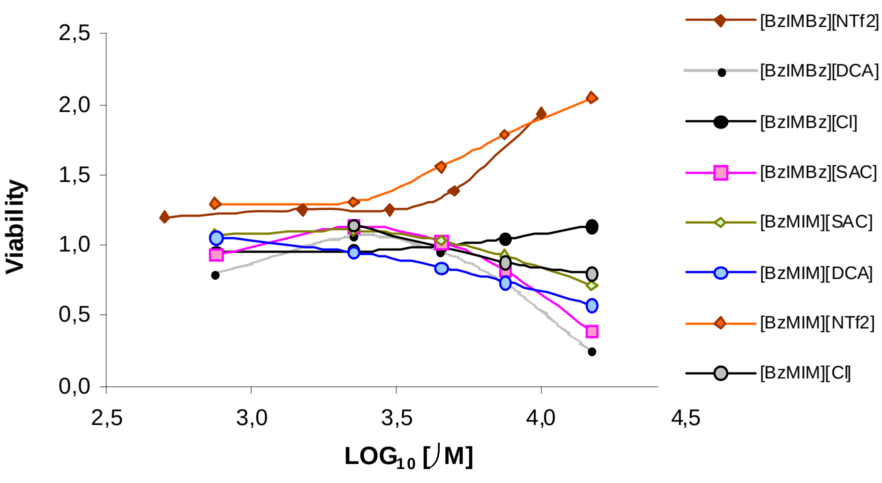


Figure 7 – Dose confluent CaCo-2 viability curves in response to different doses of [BzIMBz] [NTf_2/DCA/Cl/SAC] and [BzMIM] [NTf_2/DCA/Cl/SAC] ionic liquids.

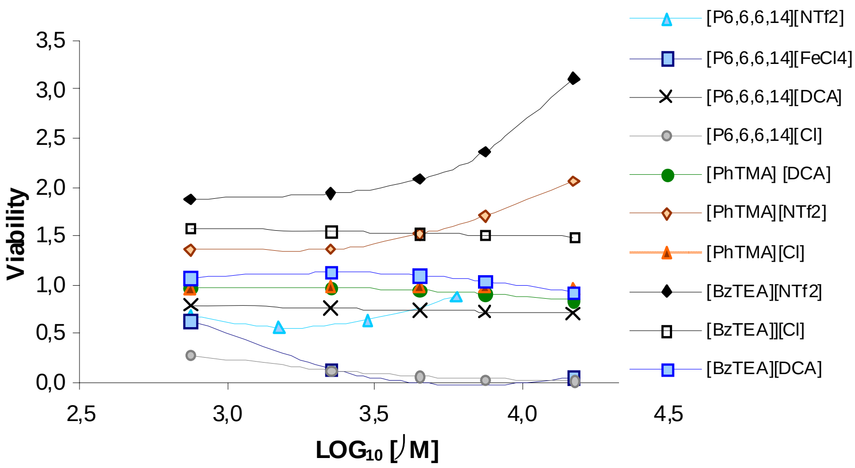


Figure 8 – Dose confluent CaCo-2 viability curves in response to different concentrations of [P6,6,6,14] [NTf_2/DCA/Cl/$FeCl_4$] and [PhTMA] [NTf_2/DCA/Cl] and [BzTEA] [NTf_2/DCA/Cl] ionic liquids. [P6,6,614] [$FeCl_4$] showed lack of solubility in aqueous medium and [P6,6,6,14] [NTf_2/DCA] were likely poorly soluble in aqueous medium [1].

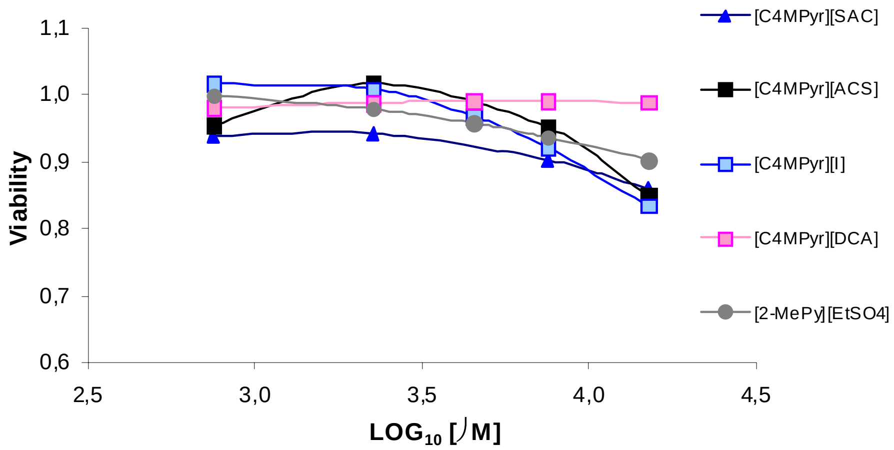


Figure 9 – Confluent CaCo-2 viability in dependence of several anions combined with [C_4MPyr] and [2-MEPy] [EtSO4] ionic liquids.

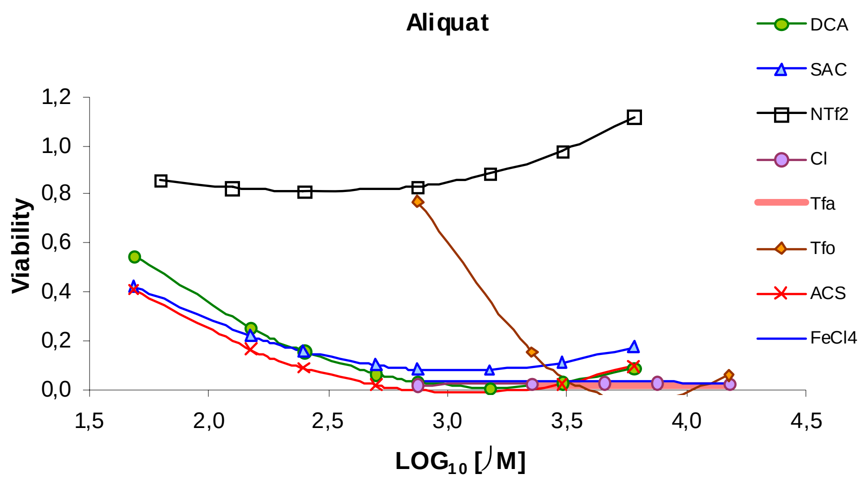


Figure 10 – Confluent CaCo-2 viability in dependence of several anions and [Aliquat] cation. [Aliquat] [Cl/Tfa/Tfo/FeCl$_4$] ionic liquids were seen to form precipitate in aqueous medium after some time upon mixture and the same possibly occurred previously for [Aliquat] [ACS/SAC/DCA] [1].

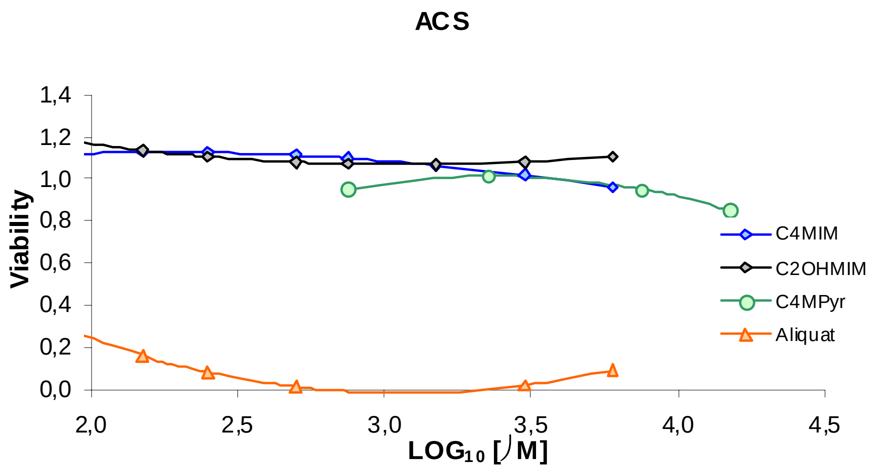


Figure 11 – Confluent CaCo-2 cells viability in the presence of [C$_4$MIM], [C$_2$OHMIM], [C$_4$MPyr] and [Aliquat] cation with Acesulfame (ACS) as anion. [Aliquat] [ACS] was likely poorly soluble in aqueous medium [1].

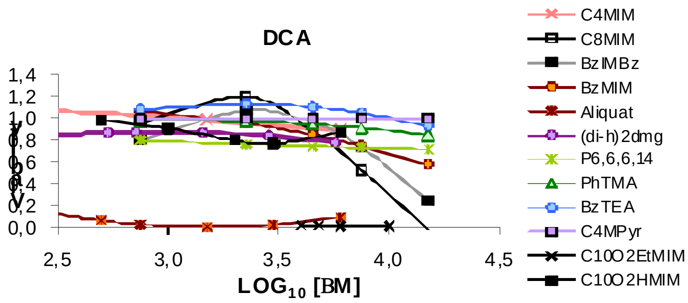


Figure 12 – Confluent CaCo-2 cells viability in the presence of several cations with dycianoamide (DCA) anion. [Aliquat] [SAC] and [P6,6,6,14] [DCA] were likely poorly soluble in aqueous medium [1].

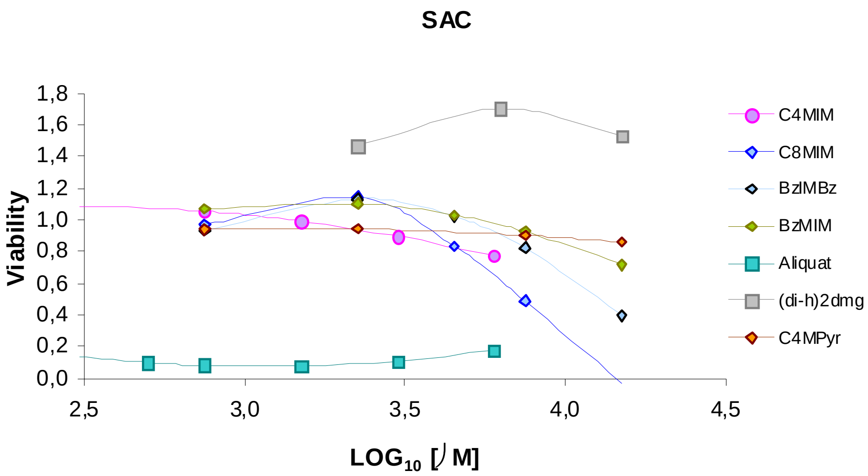


Figure 13 – Confluent CaCo-2 cells viability in the presence of several cations with Saccharine (SAC) anion. [Aliquat] [SAC] and [$(di\text{-}h)_2$dmg] [SAC] were likely poorly soluble in aqueous medium [1].

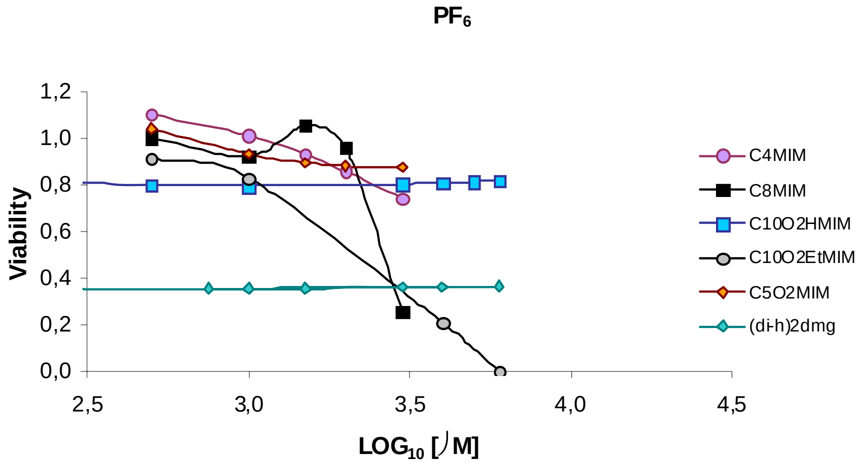


Figure 14 – Confluent CaCo-2 cells viability in the presence of several cations with hexafluorophosphate (PF_6) anion. [(di-h)$_2$dmg] [PF_6] was likely poorly soluble in aqueous medium [1].

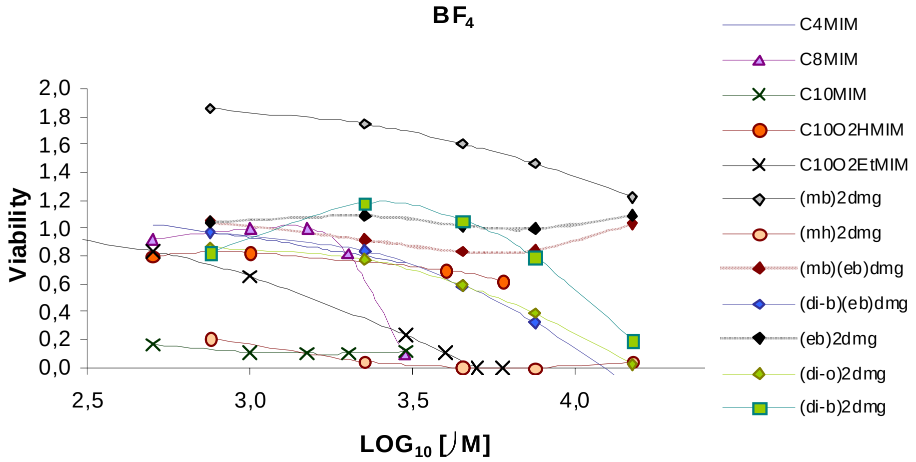


Figure 15 – Confluent CaCo-2 cells viability in the presence of several c[illegible]afluoroborate (BF_4) anion. [(di-o)$_2$dmg] [BF_4] was poorly soluble in aqueous medium.

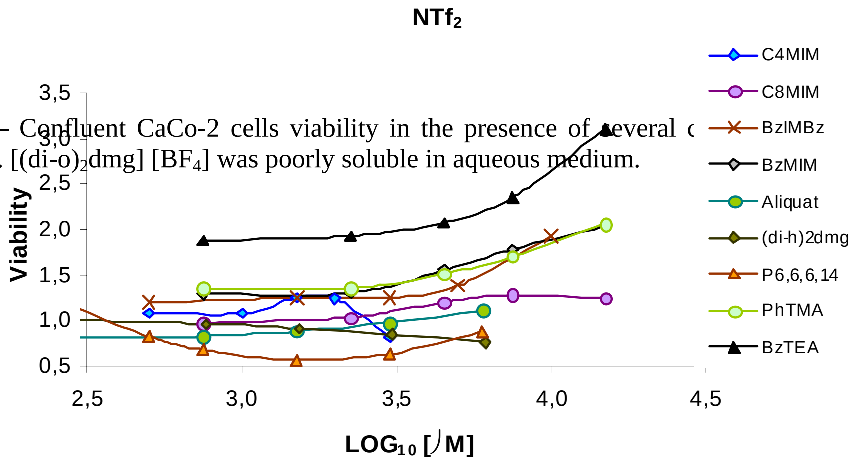


Figure 16 – Confluent CaCo-2 cells viability in the presence of several cations with bis(trifluoromethanesulfonyl)-imide (NTf_2) anion. [Aliquat] [NTf_2] and [P6,6,6,14] [NTf_2] were likely poorly soluble in aqueous medium [1].

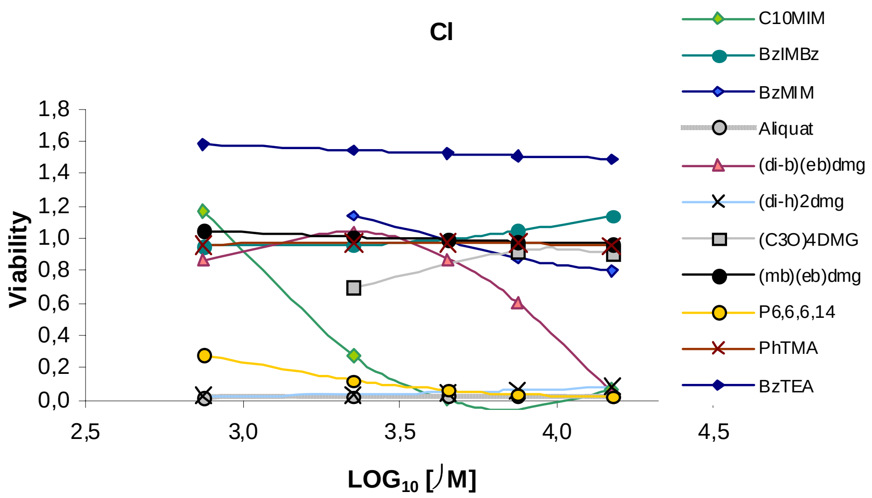


Figure 17 – Viability of confluent CaCo-2 cells exposed to several classes of cations with halogen (Cl) as anion. [Aliquat] [Cl] and [(di-h)$_2$dmg] [Cl] showed lack of solubility in aqueous medium.

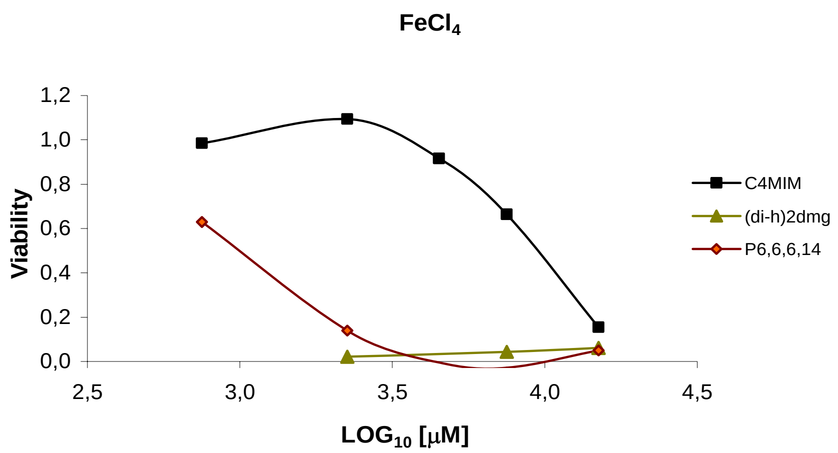


Figure 18 – Viability of confluent CaCo-2 cells exposed [C$_4$MIM], [(di-h)$_2$dmg] and [P6,6,6,14], with FeCl$_4$ as the counter ion. [(di-h)$_2$dmg] [FeCl$_4$] and [P6,6,6,14] [FeCl$_4$] were not very soluble in aqueous medium.

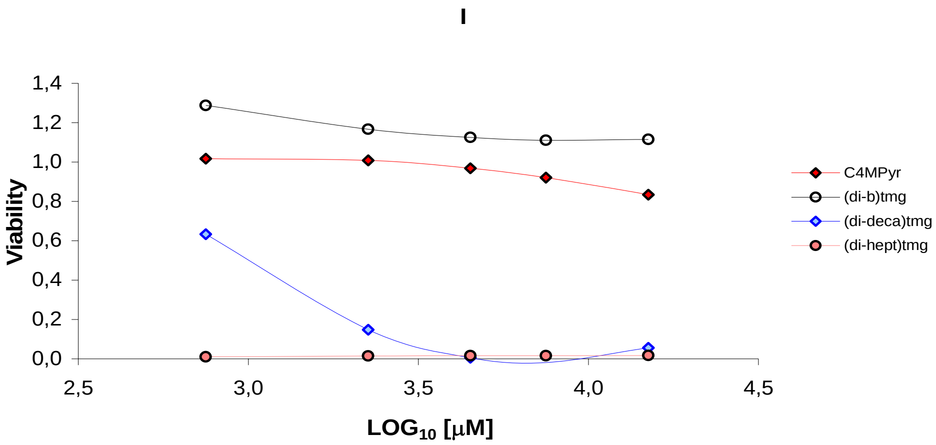


Figure 19 – Viability of confluent CaCo-2 cells exposed to tetramethyl-guanidinium [tmg] and [C_4MPyr] ionic liquids when the counter ion is iodine (I).

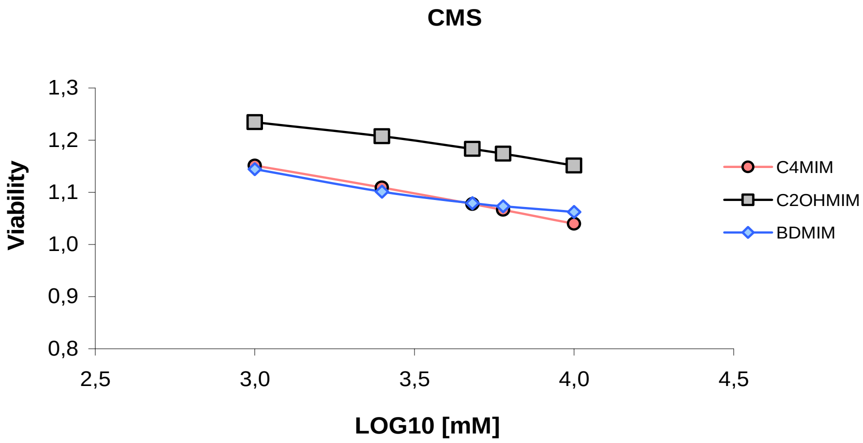


Figure 20 – Viability of confluent CaCo-2 cells exposed to [C_4MIM], [C_2OHMIM] and [BDMIM] combined with N-cyanomethanesulfonamide (CMS) as anion.

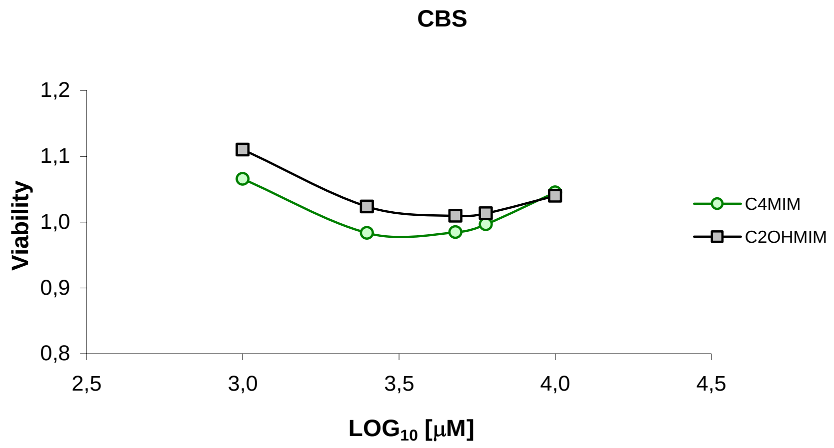


Figure 21 – Viability of confluent CaCo-2 cells exposed to [C_4MIM] and [C_2OHMIM] combined with N-cyanobenzenesulfonamide (CBS) as anion.

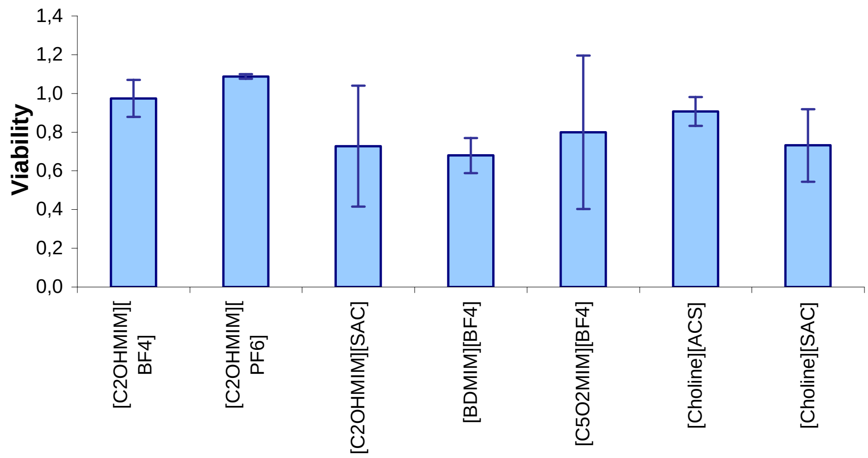


Figure 22 – Viability of confluent CaCo-2 cells exposed to some ionic liquids at 6000 μM concentration.

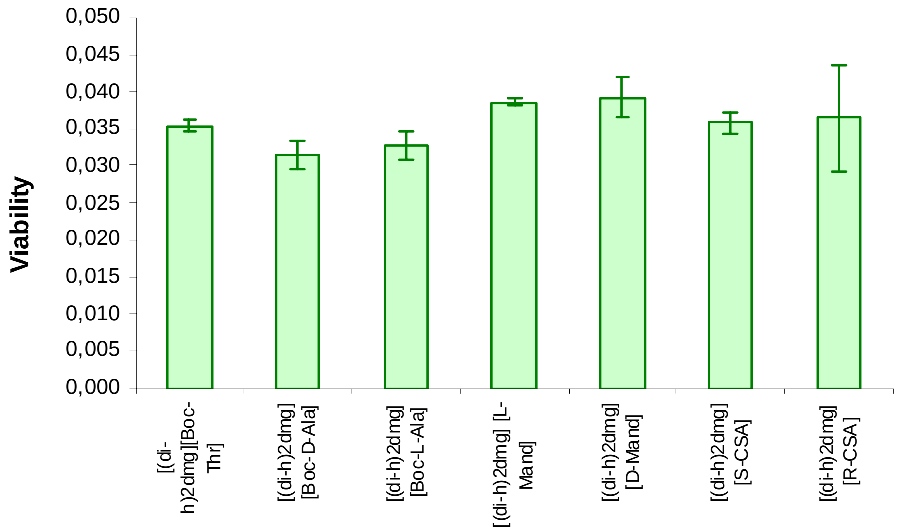


Figure 23 – Viability of confluent CaCo-2 cells exposed to chiral ionic liquids [(di-h)$_2$dmg] [Boc Ala/ Mand/ CSA] and [(di-h)$_2$dmg] [Boc Thr] at concentration of 4000 μM concentration. These ionic liquids did not present a good solubility in the aqueous medium.

Table 1 – Summary of the toxicity of the ionic liquids tested.

Cations/ Anions	[BF$_4$]/ [PF$_6$]	[DCA]/ [NTf$_2$]	[ACS]/ [SAC]	[CMS]/ [CBS]	[Cl]/ [FeCL$_4$]/ [Br]	[Glu]/ [I]/ [EtSO$_4$]	[TFA]/ [TfO]	[R/S-CSA] / [D/L-Mand]/ [Boc-D/L Ala]/ [Boc-Thr]
[C$_4$MIM]	NT/NT	NT/NT	NT/NT	NT/NT	-/T/-	-/-/-	-/-	-/-/-/-
[C$_2$OHMIM]	NT/NT	-/-	NT/NT	NT/NT	-/-/-	-/-/-	-/-	-/-/-/-
[C$_5$O$_2$MIM]	NT/NT	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[BDMIM]	NT/-	-/-	-/-	NT/-	-/-/-	-/-/-	-/-	-/-/-/-
[C$_8$MIM]	T/T	T/NT	-/T	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[C$_{10}$MIM]	T/-	-/-	-/-	-/-	T/-/-	-/-/-	-/-	-/-/-/-
[C$_{10}$O$_2$EtMIM]	T/T	T/-	-/-	-/-	-/-/T	-/-/-	-/-	-/-/-/-
[C$_{10}$O$_2$HMIM]	NT/NT	NT/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[BzIMBz]	-/-	T/T[2]	-/T	-/-	NT/-/-	-/-/-	-/-	-/-/-/-
[BzMIM]	-/-	NT/T[2]	-/NT	-/-	NT/-/-	-/-/-	-/-	-/-/-/-
[Aliquat]	-/-	T[1]/NT[1]	T[1]/ T[1]	-/-	T[1]/T[1]/-	-/-/-	T[1]/ T[1]	
[(di-h)$_2$dmg]	-/ T[1]	NT[1]/NT[1]	-/T [1,2]	-/-	T[1]/T[1]-	-/-/-	-/-	T[1]/T[1]/T[1]/T[1]
[(mh)$_2$dmg]	T/-	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[(mb)$_2$dmg]	T[2]/-	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-

[(eb)(mb)dmg]	NT/-	-/-	-/-	-/-	NT /-/-	-/-/-	-/-	-/-/-/-
[(di-b)(eb)dmg]	-/-	-/-	-/-	-/-	T/-/-	-/-/-	-/-	-/-/-/-
[$(eb)_2$dmg]	NT/-	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[$(di\text{-}o)_2$dmg]	T[1]/-	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[$(di\text{-}b)_2$dmg]	T/-	-/-	-/-	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[$(C_3O)_4$dmg]	-/-	-/-	-/-	-/-	NT/-/-	-/-/-	-/-	-/-/-/-
[$P_{6,6,6,14}$]	-/-	NT/NT	-/-	-/-	T[1]/ T[1]/-	-/-/-	-/-	-/-/-/-
[PhTMA]	-/-	NT/T[2]	-/-	-/-	NT/-/-	-/-/-	-/-	-/-/-/-
[BzTEA]	-/-	NT/T[2]	-/-	-/-	T[2]/-/-	-/-/-	-/-	-/-/-/-
[C_4MPyr]	-/-	NT/-	NT/NT	-/-	-/-/-	NT/NT/-	-/-	-/-/-/-
[2-MEPy]	-/-	-/-	-/-	-/-	-/-/-	-/NT/-	-/-	-/-/-/-
[Choline]	-/-	-/-	NT/NT	-/-	-/-/-	-/-/-	-/-	-/-/-/-
[(di-b)tmg]	-/-	-/-	-/-	-/-	-/-/-	-/NT/-	-/-	-/-/-/-
[(di-hept)tmg]	-/-	-/-	-/-	-/-	-/-/-	-/T/-	-/-	-/-/-/-
[(di-deca)tmg]	-/-	-/-	-/-	-/-	-/-/-	-/T[1]/-	-/-	-/-/-/-

Note: Not Toxic (NT) means that cell viability varied within 30% maximum; Toxic (T) means that cell viability decreased/increased more than 30%

[1]- Low solubility in water.

[2] –cell viability increased unexpectedly and values were considerably higher compared to control cells and therefore ionic liquids were considered toxic.

ILs synthesis

ILs listed in Table 2 were prepared following the reported procedures, or commercially obtained.

Table 2 – Commercial resources and reported procedures for some ILs used on the toxicology studies.

Ionic Liquids: Cations	Ionic Liquids: Anions	References
[BMIM]	[DCA]/[SAC]/[ACS]/[PF_6]/[BF_4]/[NTf_2]/[CMS]/[CBS]/[$FeCl_4$]	4/5/5/6/6/4/3/3/7
[C_8MIM]	[DCA]/[SAC]/[PF_6]/[BF_4]/[NTf_2]	8/8/4/4/8
[C_{10}MIM]	[Cl]/[BF_4]	4
[C_2OHMIM]	[SAC]/[ACS]/[PF_6]/[BF_4]/[CMS]/[CBS]	3/3/6/6/3/3
[C_5O_2MIM]	[PF_6]/[BF_4]	6
[BDMIM]	[BF_4]	4
[BzMIM]	[Cl]/ [DCA]/[SAC]/[NTf_2]	4/8/8/8
[BzIMBz]	[Cl]/ [DCA]/[SAC]/[NTf_2]	4/8/8/8
[$C_{10}O_2$HMIM]	[DCA]/[PF_6]/[BF_4]	3
[$C_{10}O_2$EtMIM]	[Br]/[DCA]/[PF_6]/[BF_4]	9/3/9/9
[Aliquat]	[Cl]/[DCA]/[SAC]/[ACS]/[NTf_2]/[TFA]/[TfO]	10/11/8/3/8/8/8
[Choline]	[SAC]/[ACS]	3
[PhTMA]	[Cl]/[DCA]/[NTf_2]	10/8/8
[BzTEA]	[Cl]/[DCA]/[NTf_2]	10/8/8
[$P_{6,6,6,14}$]	[Cl]/[DCA]/[NTf_2]/[$FeCl_4$]	12/12/3/13
[$(di\text{-}b)_2$dmg]	[BF_4]	14
[$(di\text{-}h)_2$dmg]	[Cl]/[DCA]/[SAC]/[PF_6]/[NTf_2]/[CSA]/[Mand]/[Boc-Ala]	14/8/8/14/14/15/15/15
[$(di\text{-}o)_2$dmg]	[BF_4]	14
[$(mb)_2$dmg]	[BF_4]	14
[$(eb)_2$dmg]	[BF_4]	14
[(di-b)tmg]	[I]	16
[C_4MPyr]	[I]/[DCA]	17
[2-MEPy]	[$EtOSO_3$]	4

[$(C_3O)_4$dmg][Cl], **[$(mh)_2$dmg][BF_4], [(di-b)(eb)dmg][Cl], [(eb)(mb)dmg][Cl], [(eb)(mb)dmg][BF_4], [$(di\text{-}h)_2$dmg][Boc-Thr]** were prepared following reported procedure[14, 15]. For [(di-b)(eb)dmg][Cl], [(eb)(mb)dmg][Cl] was used a 1:1 mixture of each secondary amine.

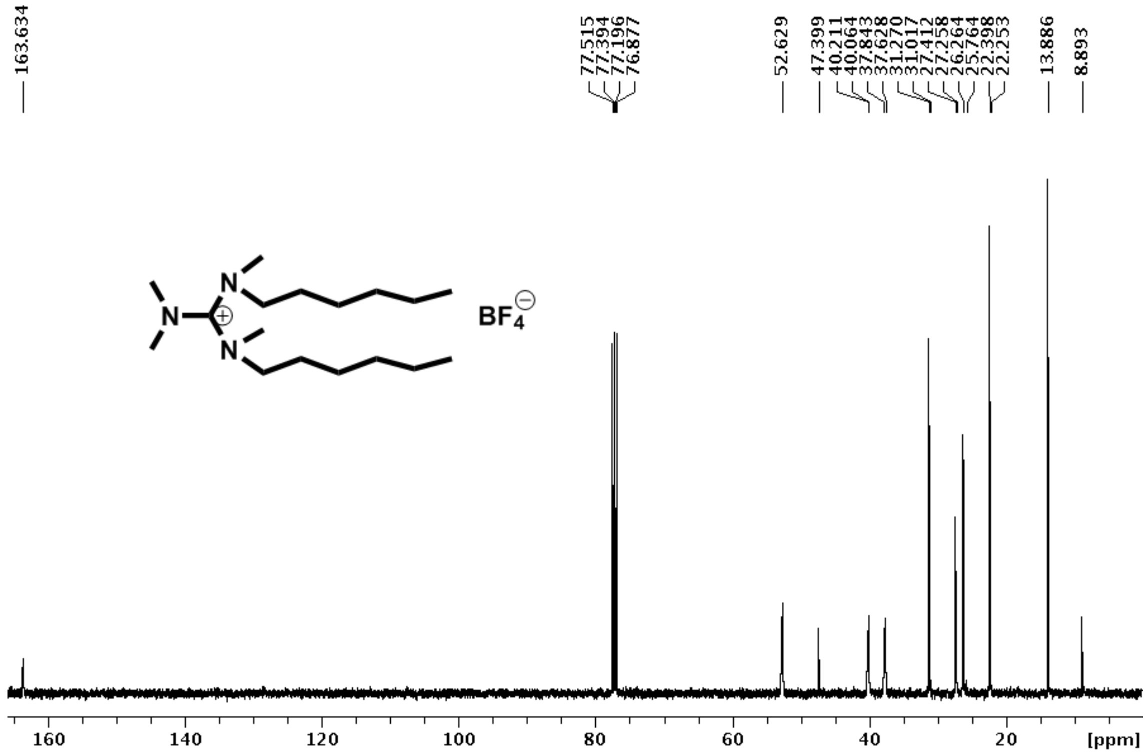


Figure 24 – [(mh)$_2$dmg][BF_4] ^{13}C-NMR (400MHz, CDCl3).

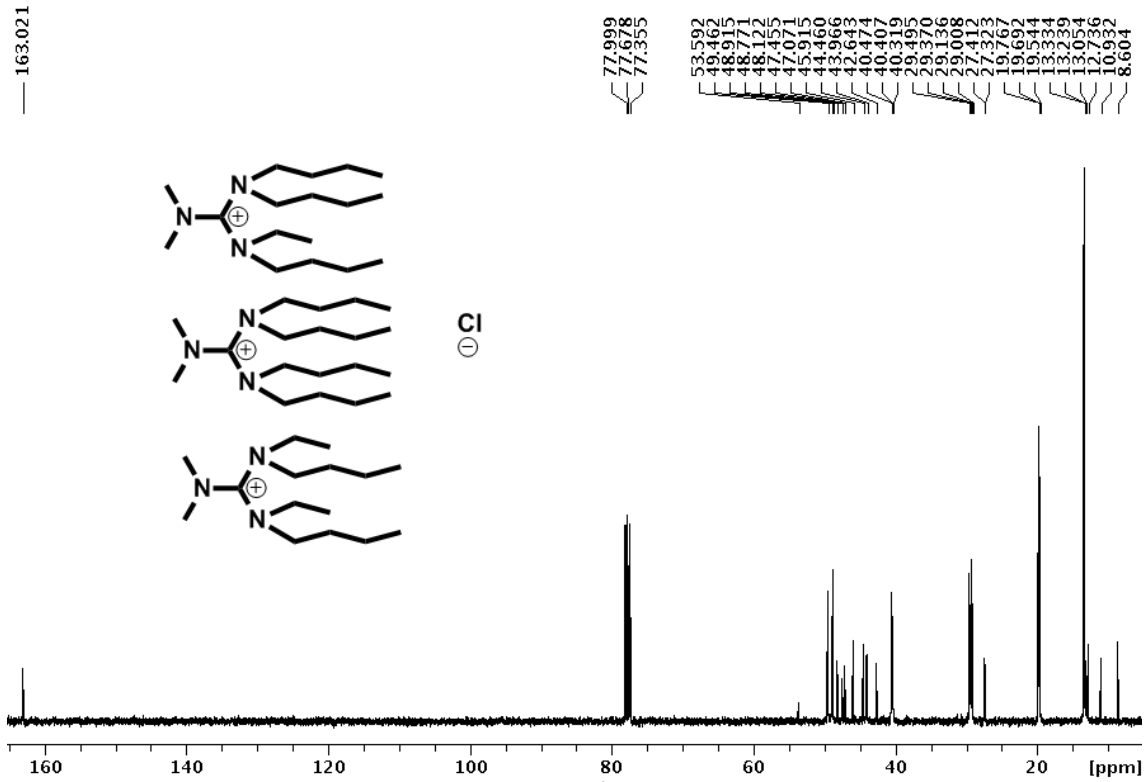


Figure 25 – [(di-b)(eb)dmg][Cl] ^{13}C-NMR (400MHz, CDCl3).

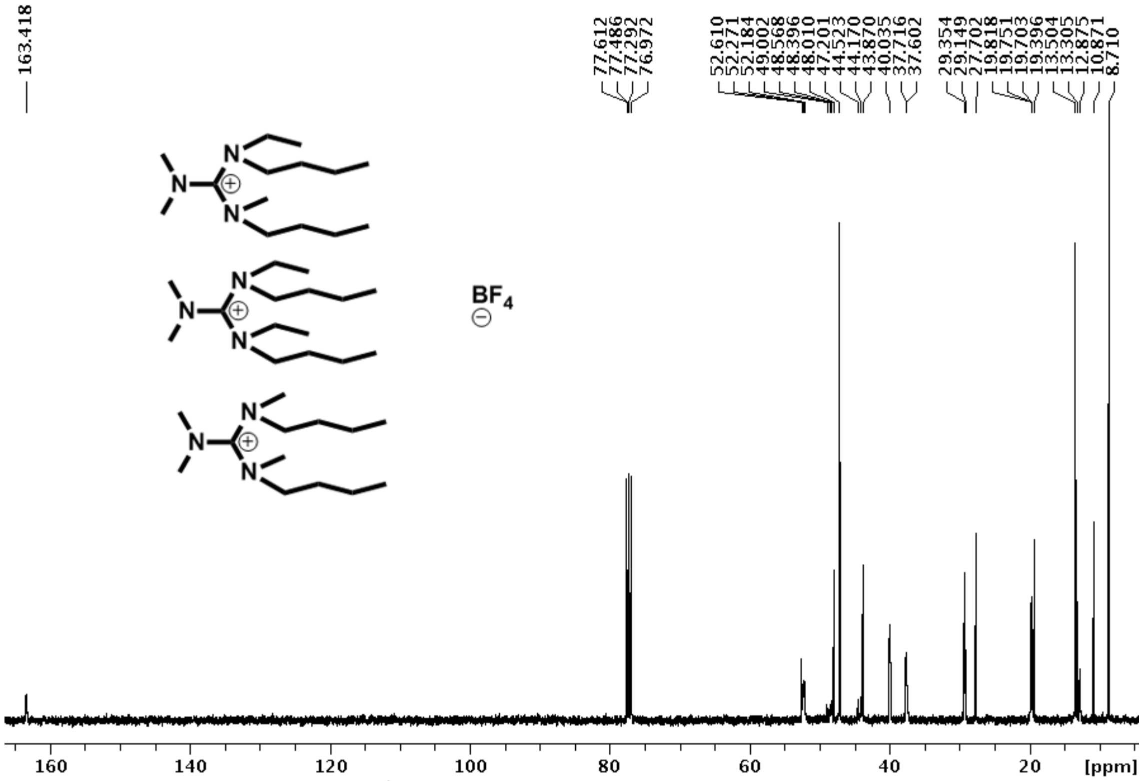


Figure 26 – [(eb)(mb)dmg][BF_4] ^{13}C-NMR (400MHz, CDCl3). Prepared by using a 1:1 mixture of each secondary amine.

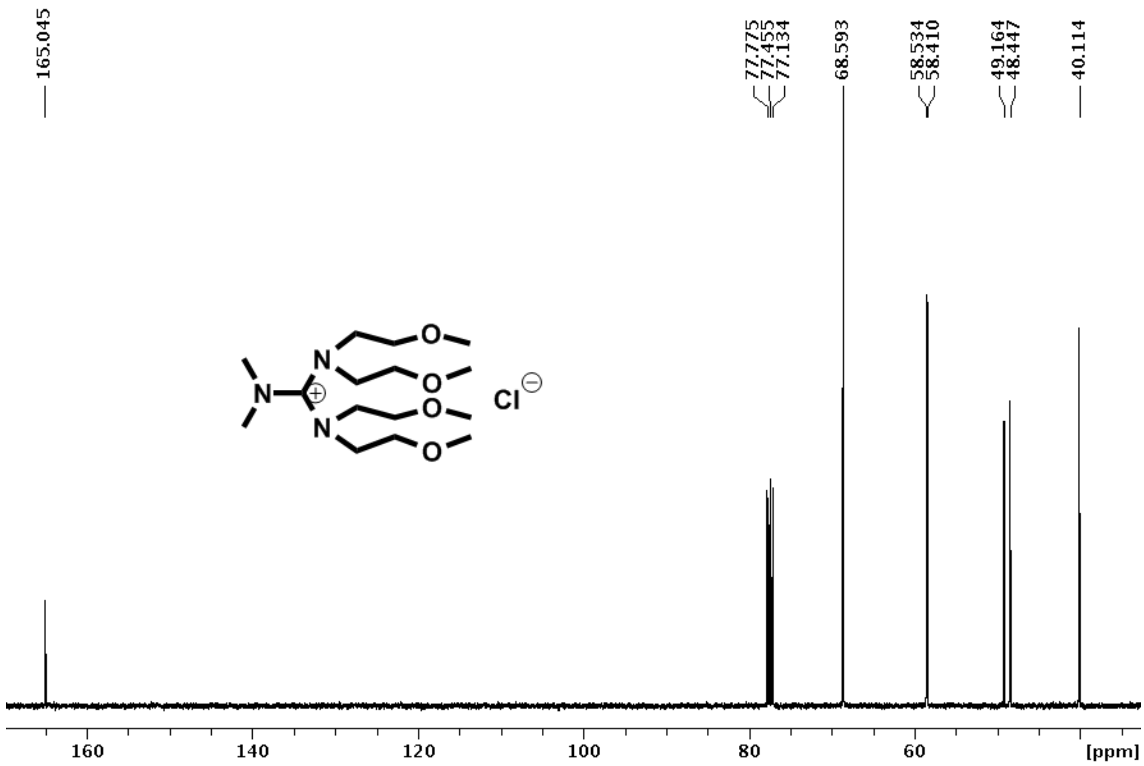


Figure 27 – [$(C_3O)_4$dmg][Cl] ^{13}C-NMR (400MHz, CDCl3).

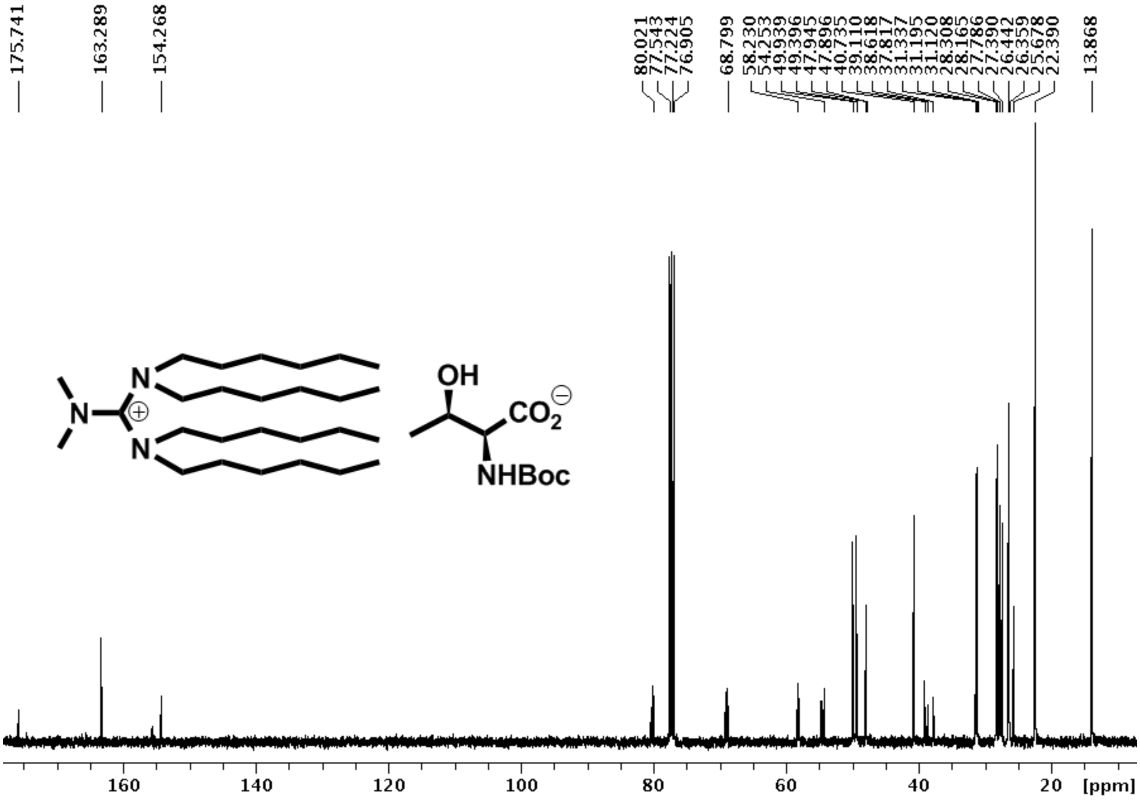


Figure 28 – [(di-h)$_2$dmg][Boc-Thr] ^{13}C-NMR (400MHz, CDCl3).

[C$_4$MPyr][SAC] To a solution of [C$_4$MPyr][I][17] (1.04g, 3.8 mmol) in dichloromethane (10 mL) was added sodium saccharin (1.31 g, 1.5 equiv.) and the mixture stirred at room temperature for 24 hours. The sodium chloride salt was removed by filtration and the organic phase evaporated under vacuum. The residual solid was then purified by passing through a column with silica and activated carbon, and the solvent removed under vacuum. The residue was stirred under vacuum (<1 mmHg) at 60ºC overnight (0.93 g, 75%).

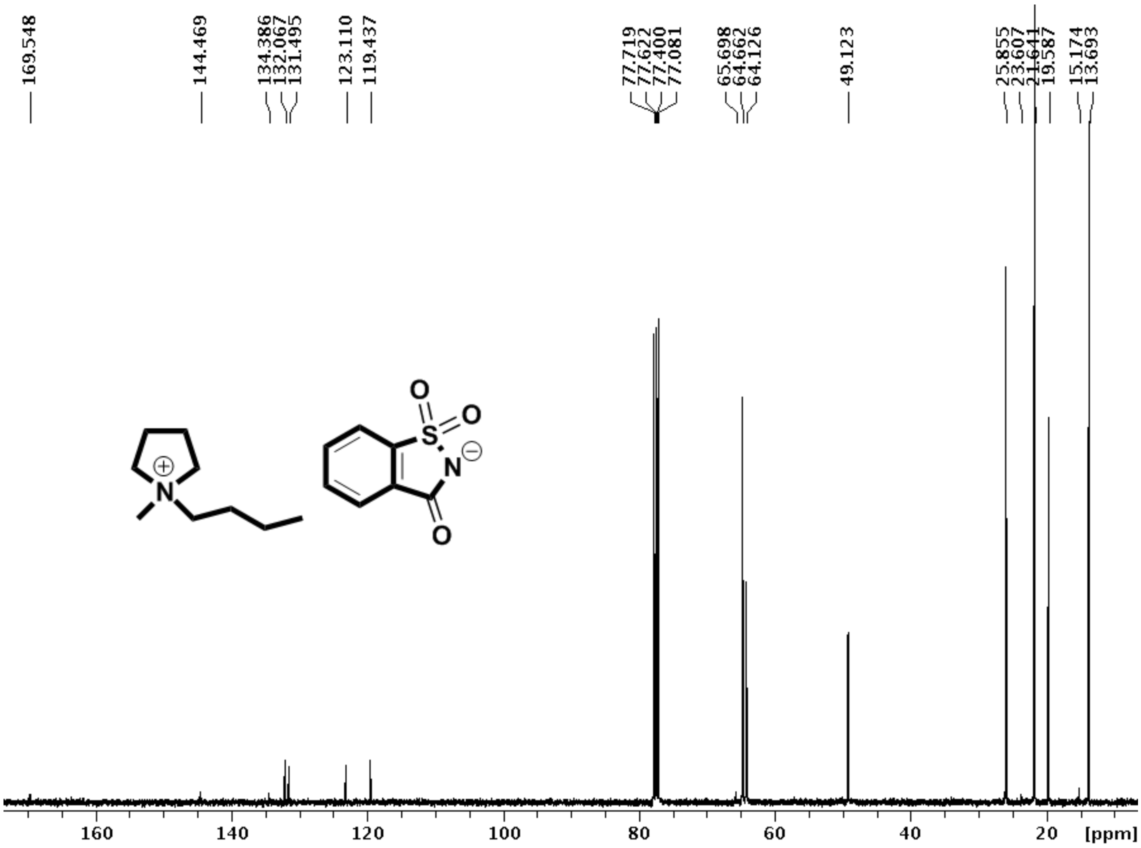


Figure 29 – [C_4MPyr][SAC] ^{13}C-NMR (400MHz, CDCl3).

[C_4MPyr][ACS] To a solution of [C_4MPyr][I][17] (1g, 3.7 mmol) in dichloromethane (10 mL) was added potassion acesulfame (1.16 g, 1.5 equiv.) and the mixture stirred at room temperature for 24 hours. The potassium chloride salt was removed by filtration and the organic phase evaporated under vacuum. The residual solid was then purified by passing through a column with silica and activated carbon, and the solvent removed under vacuum. The residue was stirred under vacuum (<1 mmHg) at 60ºC overnight (0.76 g, 70%).

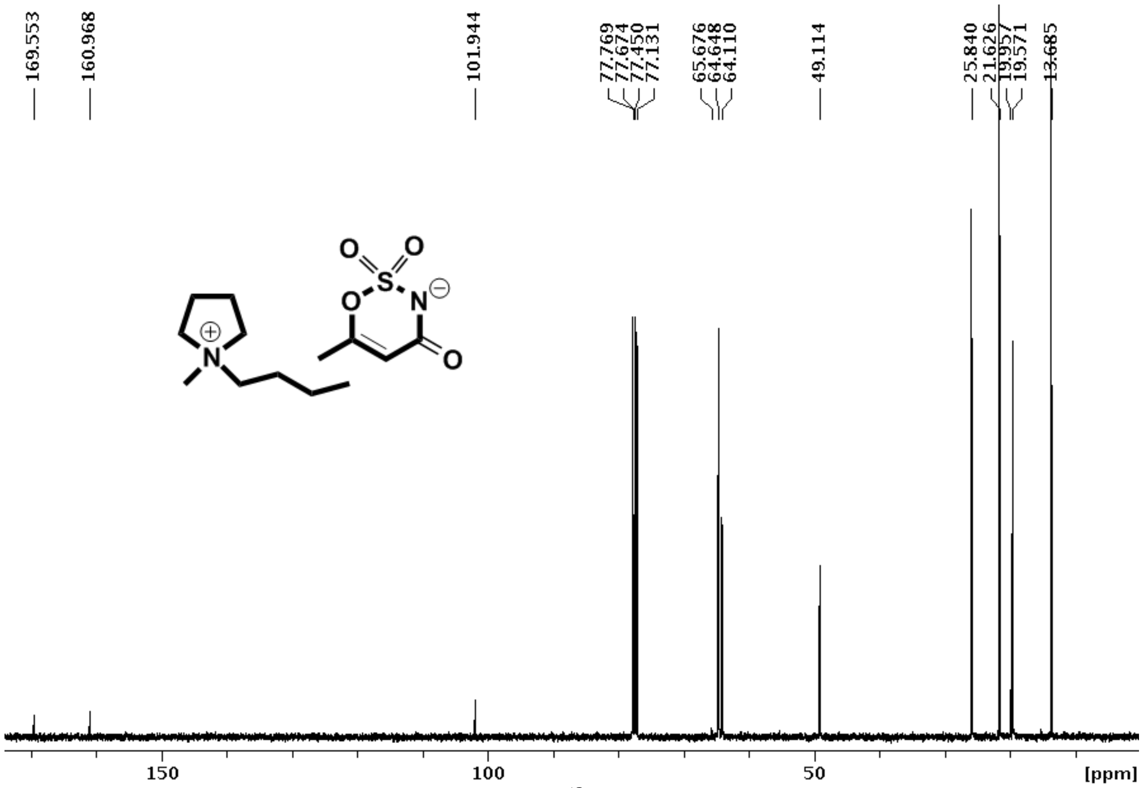


Figure 30 – [C_4MPyr][ACS] ^{13}C-NMR (400MHz, CDCl3).

[(di-hept)tmg][I] To a solution of N,N,N',N'-tetramethylguanidinium (2.754 g, 0.024 mol) in dichloromethane (20 mL) was added iodoheptane (15.7 ml, 4 equiv.) and NaOH (1.92 g, 2 equiv.). The mixture was stirred at room temperature for 6 days. After filtration, the organic phase was evaporated under vacuum. The residual solid (15.44 g) was stirred under vacuum (<1 mmHg) at 60ºC overnight.

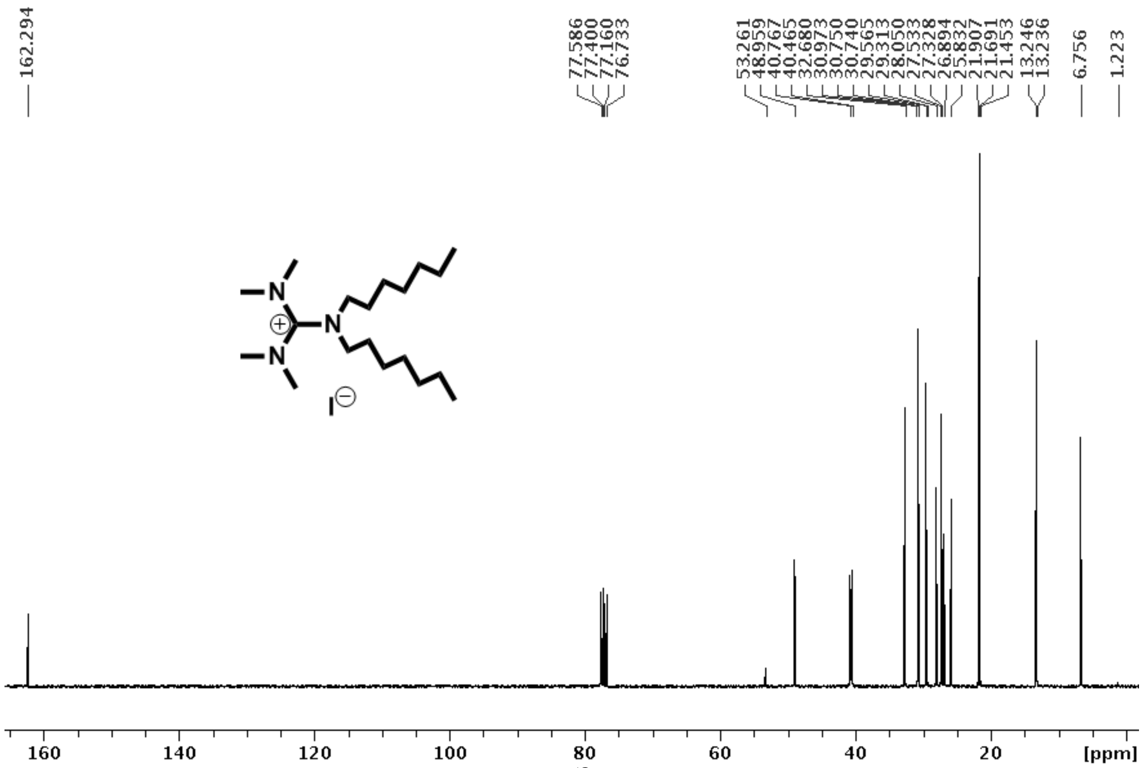


Figure 31 – [(di-hept)tmg][I] ^{13}C-NMR (400MHz, CDCl3).

[(di-dodec)tmg][I] To a solution of N,N,N',N'-tetramethylguanidinium (1.836 g, 0.016 mol) in dichloromethane (20 mL) was added iodododecane (15.8 ml, 4 equiv.) and NaOH (1.28 g, 2 equiv.). The mixture was stirred at room temperature for 6 days. After filtration, the organic phase was evaporated under vacuum. The residual solid (20.43 g, 45.7%) was stirred under vacuum (<1 mmHg) overnight.

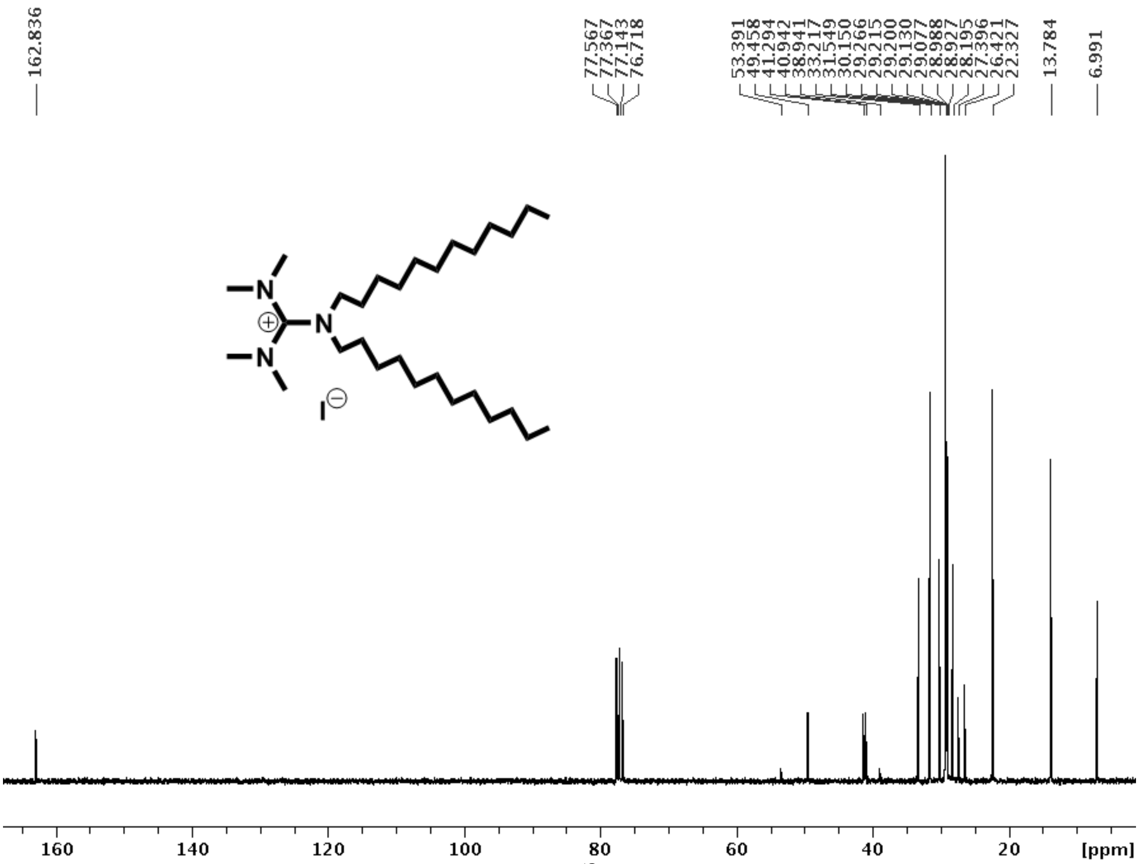


Figure 32 – [(di-dodec)tmg][I] ^{13}C-NMR (400MHz, CDCl3).

[(di-h)$_2$dmg][FeCl$_4$] To a solution of [(di-h)$_2$dmg][Cl][14] (0.5 g; 1.08 mmol) in 10 ml of dichloromethane (to ensure efficient mixing) was added Iron (III) chloride hexahydrate (0.35 g, 1.2 equivs.). The solution was stirred at room temperature for 2 days. After that two layers have been formed. The organic layer was washed several times with distilled water (6×10 ml) and the solvent evaporated under reduce pressure. [(di-h)$_2$dmg][FeCl$_4$] was obtained as viscous dark brown oil (89.7%).

[Aliquat][FeCl$_4$] To a solution of [Aliquat][Cl] (0.5 g; 1.15 mmol) in 10 ml of dichloromethane (to ensure efficient mixing) was added Iron (III) chloride hexahydrate (0.37 g, 1.2 equivs.). The solution was stirred at room temperature for 2 days. After that two layers have been formed. The organic layer was washed several times with distilled water (6×10 ml) and the solvent evaporated under reduce pressure. [Aliquat][FeCl$_4$] was obtained as viscous dark brown oil (79.7%).

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