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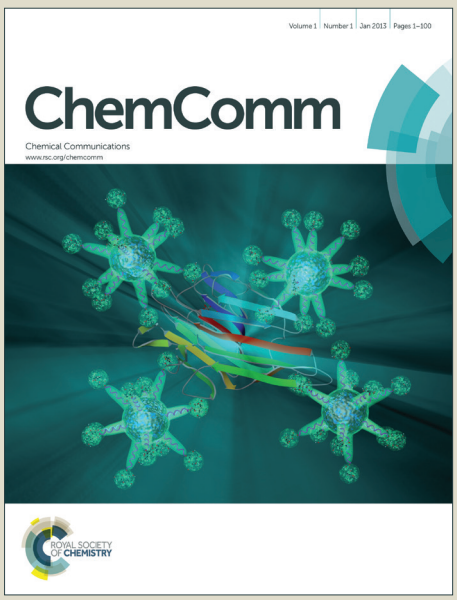

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A novel example of double 6-*exo-trig* heterocyclization: nitrile conversion to new anticancer active (HeLa cells) primary amine ionic liquids†

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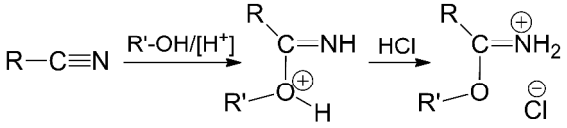
Conversion of nitrile under mild conditions leads to a new class of primary amines, including room temperature ionic liquids, acting as efficient anticancer agents.

The chemistry of primary amines has been well developed as a result of their wide-ranging application as solvents, reagents, pharmaceuticals, agrochemicals, etc. However, preparation of these from cyano compounds is not trivial. Nitriles can be reduced to amines using Raney nickel (hydrogen), rhodium catalysts (hydrogen), diborane or lithium aluminum hydride via nitrogen-hydrogen bond forming reaction. However, some syntheses are complicated by the use, for example, of hazardous reagents such as hydrazine as well as the danger of catalysts releasing hydrogen from any water present. Thus the development of eco-friendly synthetic routes and reagents would be of considerable benefit. This could be realized through quaternization reactions currently widely used in the synthesis of ionic liquids (ILs) - indispensable "tools" of modern green chemistry.[1]

Molecules containing bicyclic rings of an aliphatic character with a nitrogen atom in common occur in many of the natural alkaloids. The 1-aza-bicyclo-[1.3.3]-nonane ring system, also called the isogranatanine skeleton, was synthesized first by McElvain and R. Adams via Dieckmann condensation in 1923.[2] Heteroatom analogues of isogranatanine (4,6-dioxa-1-azabicyclo[3.3.1]nonane) are of particular interest both from a scientific point of view as well as for industrial organic syntheses. So far only a few patents which are related to the biologically active heterocyclic isogranatanine motifs are known.[3, 4]

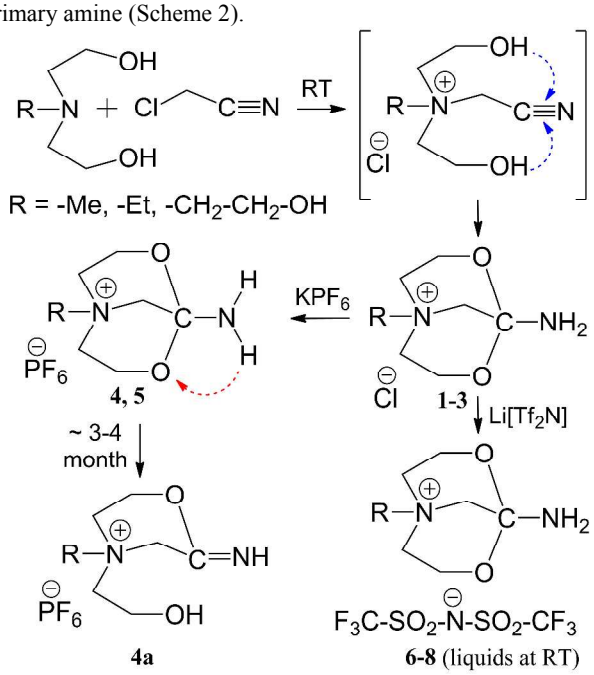
Here we present a simple, quick and low cost method of converting carbonitriles into primary amine cyclic ether quaternized salts, including room temperature ionic liquids. The potential of these as anticancer agents has been assessed using ovarian HeLa cell lines.

Pinner salts[5, 6] are produced through treatment of a nitrile with an alcohol under acidic conditions (with gaseous HCl or 4N HCl) to give the hydrochloride of an imino ester or an alkyl imidate (Scheme 1):



Scheme 1. The classical Pinner reaction (nitrile alcoholysis).

In our case, the quaternization reactions (ESI) were carried out in acetonitrile (or acetone) at room temperature by first a S_N2 reaction of N-substituted diethanolamine with 2-chloroacetonitrile (1:1). According to Baldwin's rules, the 6-*exo-trig* heterocyclization is the favoured process here which leads to a double ring closure and the conversion of a nitrile group into a primary amine (Scheme 2).



Scheme 2. Preparation of substituted 1-amino-2,8-dioxa-5-azoniabiciclo[3.3.1]nonanium salts.

Compounds **4-8** could be obtained from metathesis reactions in water of chloride-containing quaternized salts (**1-3**) with KPF_6 (**4, 5**) or $Li[Tf_2N]$ (**6-8**) (ESI). In the case of the reaction between 1-amino-5-(2-hydroxyethyl)-2,8-dioxa-5-azoniabicyclo[3.3.1]nonanium chloride (**3**) with KPF_6, the expected precipitate (hexafluorophosphate salt) was not observed. Also attempts to obtain the target products with other N-substituted diethanolamines (R = *t*-butyl, *n*-butyl, phenyl) or 3-chloropropionitrile were unsuccessful. Most probably due to steric hindrance of the side chain with the bis-heterocycle or mild N-alkylation condition for these cases.

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The structures of compounds **1, 3, 4** and **4a** were confirmed by means of single crystal X-ray diffraction (ESI). Suitable crystals were obtained by re-crystallization from water (**1** and **3**) or acetone (**4** and **4a**). In the case of compound **4**, the crystal-to-crystal transformation to the half-closed structure (**4a**) was observed (Figure 1).

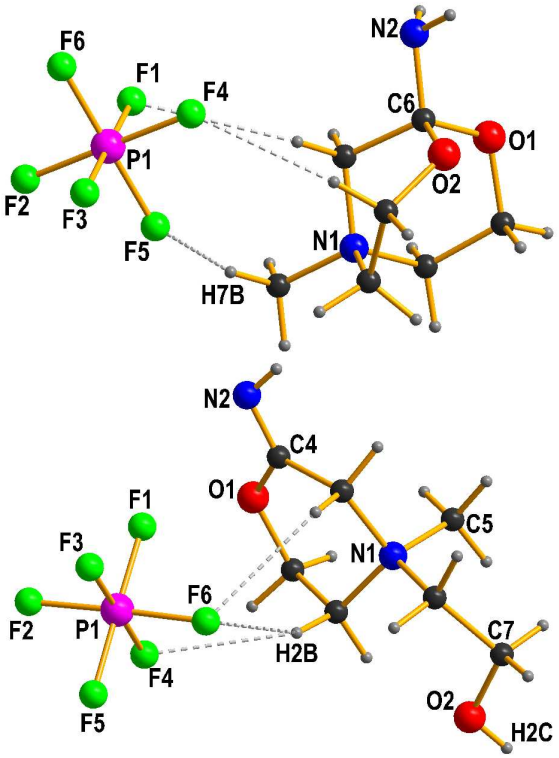

Fig. 1. Structures of the compound **4** (up) and its 'half-closed' form **4a** (bottom).

So-called dynamic structural transformation based on flexible frameworks is recognized as a fascinating phenomenon. Responding to the "guest impact" (mobility/interactions of molecules, external influence, etc.) can be categorized in terms of three main types.[7] The first, ''recoverable collapsing'', has the property that by elimination of guest molecules a network collapses and regenerates under the initial conditions. The second, ''guest-induced transformation'', has the property that structural changes in the network are induced by the simultaneous exchange of guest molecules. The last, ''guest-induced reformation'', has the property that removal of guest molecules causes a structural change in the network to give a new one. In our case, we have an unusual ''self-induced reformation'' and critical softness of this framework probably results from a combination of weak hydrogen bonds and 'mobility' of one hydrogen atom.

To gain insights into the mechanism of the transformation from compound **4** to compound **4a**, the electronic states of the these compounds were calculated. To do this, an *ab initio* method (RHF, basis-6-311 G^{++}(d,p)) with full geometry optimization was used (ESI). The symmetrical structure of the compound **4** (C_s-point group) was taken for the calculations. The vibrational frequency calculations in the frameworks of geometry optimisation showed all stationary points as minima or transition states (TS). Intrinsic reaction coordinate (IRC) calculations were also performed for the TS. As we can see from results presented in Table S1, the **4→4a** transition happens without considerable energy losses (ΔE_{tot}=0.0023 a.u.). The structure **4** optimisation has shown the presence of symmetric intra-molecular hydrogen bonds between oxygen and hydrogen of the amino groups' atoms ($R_{O2...H}$=2.30 Å). It should be noted that the hydrogen bond N2-H...O2 is strongly polarised (q^{N2}=-0.41, q^{H}=0.43, q^{O2}= -0.21). All this can cause the hydrogen atom tunnel transition from 'N2' to 'O2' with the covalent bond 'H2C'-'O2' appearance and thus compound **4a** formation. The analysis of the frontier orbitals shows that the electron density redistribution happens on the orbitals of those atoms that participate in the corresponding transformation (ESI).

The majority of toxicological studies on available ionic liquids have focused on choline derivatives, alkyl(pyridinium, phosphonium, ammonium)[8] and, especially, alkylimidazolium ionic liquids[9] but it has never been investigated with aza-oxo heterocyclic ionic liquids. For evaluation of their biological properties, compounds **1-8** have been tested against Human Ovarian Cancer cells (HeLa cell line)[10] and quantified using sulforhodamine B (SRB) assay (ESI). All these compounds showed an efficient cytotoxic behavior against HeLa when compared with Vincristine, a standard anticancer drug. Highest activity of **1-8** was found at the concentration of 10 μM and corresponding results are presented in terms of percent inhibition (See Table 1 and Figure 2).

Table 1. Melting point (or glass transition point, ˚C) and cytotoxicity of compounds **1-8** on the HeLa and Vero cell lines at 10 μM concentration.

Compound	$T_{m.p.}$ (˚C)	HeLa, IC_{50} ± SEM (μM)[b]	Vero, %age cytotoxicity (10μM)
[Me]Cl (**1**)	171	2.37± 0.51	**10.36±3.31**
[Et]Cl (**2**)	170	1.86 ± 0.12	16.50±5.21
[2HE]Cl (**3**)	168	1.57 ± 0.21	22.71±3.57
[Me]PF_6 (**4**)	85	1.48 ± 0.15	18.86±4.74
[Et]PF_6 (**5**)	174	1.30 ± 0.34	24.86±7.30
[Me]Tf_2N (**6**)	LRT[b]	1.63 ± 0.17	21.41±7.28
[Et]Tf_2N (**7**)	LRT[b]	1.09 ± 0.37	20.06±5.05
[2HE]Tf_2N (**8**)	LRT[b]	**0.97 ± 0.07**	27.83±5.69
Vincristine (VCT)	220[c]	1.02 ± 0.06	12.90±1.52

[a] Values are the mean ± SEM of three experiments; [b] liquid at room temperature (LRT); [c] from http://www.drugbank.ca/drugs/DB00541.

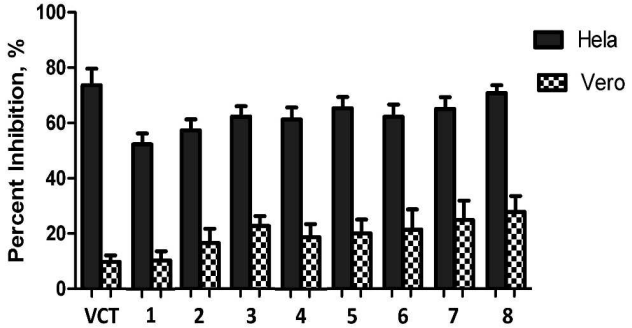

Fig. 2. Cytotoxic potential of standard anticancer drug (Vincristine, VCT) and (1-amino-5-(alkyl)-2,8-dioxa-5-azoniabicyclo[3.3.1]nonanium salts **1-8** against Human Ovarian Cancer cells (HeLa cell line) at 10 μM concentration.

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From the cytotoxicity values presented in Table 1 and Figure 2, it appears that all tested substances possess low cytotoxicity at vero cells and good anticancer activity against HeLa cells. Vero cells are normal epithelial kidney cells extracted from African green monkey and used here as control to determine the safety and selectivity index (SI) of anticancer agents (eqn 1):

$$Selectivity\ index\ (SI) = \frac{Percent\ inhibition\ of\ cancerous\ cells\ (HeLa)}{Percent\ inhibition\ of\ normal\ cells\ (vero)} \quad (1)$$

The results obtained from SI data are helpful in determining a therapeutic window of anticancer agent and this information may be useful for in-vivo studies.[11] Compound [Me]Cl (**1**) displayed the most efficient inhibition activity toward HeLa with $SI_{10\mu M} \geq 5.0$, while the [2HE]Tf_2N (**8**) showed the lowest limit of efficiency within the substituted 1-amino-2,8-dioxa-5-azoniabiciclo[3.3.1]nonanium salts ($SI_{10\mu M} \geq 2.5$).

In conclusion, we have found an acid-free, eco-friendly route to a new class of primary amines, 1-amino-5-(alkyl)-2,8-dioxa-5-azoniabicyclo[3.3.1]nonanium salts. These include bis(trifluoromethane)sulfonimides derivatives (**6**-**8**) which are room temperature ionic liquids. The unusual crystal-to-crystal transformation **4**→**4a** (''self-induced reformation'') was observed. The corresponding *ab initio* calculations show that the hydrogen atom tunnel transition between nitrogen and oxygen atoms can take place without considerable energy losses for whole transformation. All compounds were tested against Human Ovarian Cancer cells (HeLa cell line) and showed an efficient cytotoxic behavior with pronounced selectivity indexes (2.5 ÷ 5.1) which provide opportunities for the development of new safe and efficient anticancer agents.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Crystallographic data for **1, 3, 4**, **4a** (CCDC no. 953755-953758) in CIF format. The synthetic protocols, selected thermal TG/DSC analysis and details of HOMO-LUMO calculations. See DOI: 10.xxxx/b000000x/

‡ Crystal data for **1** (CCDC 953755): $C_7H_{15}N_2O_2Cl$, M_r = 194.66, orthorhombic, space group *Pbca* (no. 61), a = 12.708(3)Å, b = 10.363(2)Å, c = 13.781(3)Å, V = 1814.8(6)Å^3, Z = 8. T = 298(2) K, D_c = 1.425 g·cm^{-3}, $F(000)$ = 832, R_1 = 0.0511 (117 parameters), wR_2 = 0.0677 [$I \geq 2\sigma(I)$], GOF = 1.128 for all 1812 data.

Crystal data for **3** (CCDC 953756): $C_8H_{17}N_2O_3Cl$, M_r = 224.69, orthorhombic, space group *Pbca* (no. 61), a = 12.111(2)Å, b = 12.998(3)Å, c = 12.998(3)Å, V = 2045.9(7)Å^3, Z = 8. T = 298(2) K, D_c = 1.459 g·cm^{-3}, $F(000)$ = 960, R_1 = 0.0874 (136 parameters), wR_2 = 0.1049 [$I \geq 2\sigma(I)$], GOF = 1.006 for all 2057 data.

Crystal data for **4** (CCDC 953757): $C_7H_{15}N_2O_2F_6P$, M_r = 304.18, monoclinic, space group $P2_1/n$, a = 8.8041(4)Å, b = 12.8542(6)Å, c = 10.0647(8)Å, β = 90.020(5)°, V = 1139.02(12)Å^3, Z = 4. T = 180(2) K, D_c = 1.774 g·cm^{-3}, $F(000)$ = 624, R_1 = 0.0317 (169 parameters), wR_2 = 0.0351 [$I \geq 2\sigma(I)$], GOF = 1.061 for all 1998 data.

Crystal data for **4a** (CCDC 953758): $C_7H_{15}N_2O_2F_6P$, M_r = 304.18, monoclinic, space group $P2_1$ (no. 4), a = 6.3381(13)Å, b = 8.1858(16)Å, c = 11.115(2)Å, β = 100.48(3)°, V = 567.1(2)Å^3, Z = 2. T = 180(2) K, D_c = 1.782 g·cm^{-3}, $F(000)$ = 312, R_1 = 0.0354 (163 parameters), wR_2 = 0.0405 [$I \geq 2\sigma(I)$], GOF = 1.088 for all 1745 data.

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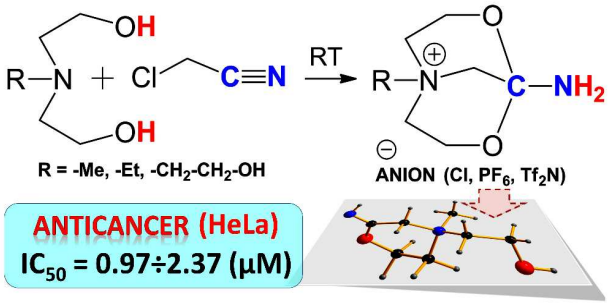

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