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To cite this version:
Audrey Beillard, François Quintin, Jérémie Gatignol, Pascal Retailleau, Jean-Luc Renaud, et al.. Solving the Challenging Synthesis of Highly Cytotoxic Silver Complexes bearing Sterically Hindered NHC Ligands with Mechanochemistry. Dalton Transactions, Royal Society of Chemistry, 2020, 49 (36), pp.12592-12598. 10.1039/D0DT00410C . hal-02919846

HAL Id: hal-02919846
https://hal-normandie-univ.archives-ouvertes.fr/hal-02919846
Submitted on 19 Nov 2020

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Solving the Challenging Synthesis of Highly Cytotoxic Silver Complexes bearing Sterically Hindered NHC Ligands with Mechanochemistry

Audrey Beillard, François Quintin, Jérémie Gatignol, Pascal Retailleau, Jean-Luc Renaud, Sylvain Gaillard, Thomas-Xavier Métro, Frédéric Lamaty and Xavier Bantreil

Dedicated to Professor Ei-ichi Negishi on the occasion of his 85th birthday

The use of ball-mills enabled the straightforward synthesis of a variety of silver(I) complexes featuring challenging NHC ligands. Sterically hindered including electron-poor or with very low solubility imidazolium salts were grinded with silver(I) oxide to furnish heteroleptic or homoleptic complexes in high yield and short reaction times. The synthesis of heteroleptic bis-NHC silver(I) complexes was also performed for the first time in a ball-mill. The efficiency and rapidity of the mechanochemical approach enabled the generation of a library of unprecedented NHC silver complexes, which cytotoxicity on HCT116 colorectal cancer cell line was evaluated providing a rare example of medicinal mechanochemistry. The cationic silver complexes were found more potent than the neutral analogues, with IC₅₀ values down to 21 nM, 256 times more potent than cisplatin.

Introduction

N-heterocyclic carbenes (NHC) are widely used for the stabilization of transition metals, especially compared to phosphines in catalytic systems. Additionally, NHC-based complexes have found interest due to their biological properties, for example for their antibacterial, antifungal or anticancer properties. Besides, many groups have shown that NHC-gold(I) and NHC-gold(III) complexes could be valid alternatives to cisplatin due to their different mode of action. Silver(I) complexes have also been studied because they might behave similarly to gold complexes. However, NHC-silver(I) complexes have mostly been studied for their antibacterial and antimicrobial activity, and only some of them were found to be active as anticancer agents. However, most of these complexes were either neutral [AgCl(NHC)] complexes or cationic homoleptic [Ag(NHC)₂] complexes featuring N,N-dialkyl ligands. Cationic homoleptic [Ag(NHC)₂] complexes bearing N,N-diaryl NHC ligands have been less studied because their synthesis is known to be more challenging. Yet, we recently developed a mechanochemical solvent-free method that enables a rapid and highly efficient access to such complexes. Additionally, ball-milling permits to easily synthesize neutral [AgCl(NHC)] complexes, either with alkyl or aryl substituents. In this manuscript, we initially focused our attention on the mechanochemistry of less conventional silver complexes bearing sterically hindered NHC ligands including one with electron withdrawing groups, to assess if the mechanochemical approach could solve synthetic problems encountered in solution. Then, we evaluated the cytotoxicity of the corresponding complexes, as well as heteroleptic bis-NHC complexes, as anticancer agents on colorectal HCT116 cancer cell line. Of note, this represents one of the rare examples of mechanochemical synthesis of molecules for biological testing, a branch of medicinal mechanochemistry.

Results and discussion

Mechanosynthesis of heteroleptic neutral silver(I) complexes

First, the synthesis of neutral heteroleptic complexes was realized by milling highly encumbered imidazolium salts 1a-e and silver(I) oxide in slight excess in a stainless steel jar containing a 1 cm diameter stainless steel ball, using a vibratory MM400 ball-mill (Scheme 1). Gratifyingly, after 3 h at 30 Hz, full conversion was obtained in all cases. Complexes 2a and 2b, featuring ligands IPr(OMe)(1,3-bis(4-methoxy-2,6-disopropylphenyl)imidazol-2-ylidene) and H₂IPr(OMe)(1,3-bis(4-methoxy-2,6-disopropylphenyl)-4,5-dimethylimidazol-2-ylidene) could be isolated in 81% and 86% yield, respectively. In solution, the use of electron poor IPr-HCl 1c (1,3-bis(2,6-disoproplyphenyl)-4,5-dichloroimidazolium chloride) revealed problematic. Indeed, even when full conversion was obtained, isolated yield of corresponding complex 2c did not exceed
Mechanosynthesis of homoleptic cationic silver(I) complexes

We then turned our attention to unprecedented homoleptic [Ag(NHC)₂]PF₆ complexes. Imidazolium salts 1a-i were first converted into their PF₆⁻ counterpart 3a-i using either the classical method, which consists in solubilizing the chloride salt in water in the presence of KPF₆ and recover the hexafluorophosphate salt that precipitates, or our previously reported solvent-free milling approach. This latter technique enabled the reduction of the quantity of KPF₆ and water used, and was found to be particularly efficient for salt 1e that reacts poorly in water due to a reduced solubility. In the ball-mill, anion metathesis occurred in 30 min under solvent-free conditions. We first focused on the synthesis of complex 4c, featuring electron poor ClIPr ligand (Table 1). As discussed above, isolation of complexes featuring this ligand is difficult when the reaction is performed in solution. Reaction of 3c with

![Scheme 2. Mechanosynthesis of homoleptic [Ag(NHC)₂]PF₆ complexes bearing highly encumbered NHC ligands](image)

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**Table 1. Comparison of methods for the synthesis of [Ag(ClIPr)]PF₆**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>t (h)</th>
<th>Conv. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CHCl₃, reflux</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>NaOH (1.1), CH₂Cl₂, rt</td>
<td>24</td>
<td>100 (45)</td>
</tr>
<tr>
<td>3</td>
<td>NaOH (1.1), vbm, 30 Hz</td>
<td>3</td>
<td>100 (85)</td>
</tr>
</tbody>
</table>

*a Reaction conditions: 3c (0.15 mmol, 1 eq.), Ag₂O (0.075 mmol, 0.5 eq.). *b Conversion determined by 1H NMR. Isolated yield is given in brackets.*
silver(I) oxide in refluxing chloroform gave only 31% NMR conversion after 24 h, and 90% after 48 h (Table 1, entry 1). In dichloromethane at room temperature, with the addition of sodium hydroxide, it was possible to obtain full conversion of \( \text{Ag}^{\text{II}} \text{IPr}^* \text{HPF}_6 \) in 24 h (Table 1, entry 2). However, after filtration on Celite\(^\text{®}\) and evaporation, complex 4c was obtained in only 45% yield. When imidazolium salt 3c was ball-milled with NaOH and Ag₂O under solvent-free conditions at 30 Hz for 3 h, 100% conversion was also observed. More importantly, after the same treatment as when the reaction was performed in solution, homoleptic complex \( \text{Ag}^{\text{II}} \text{IPr}^* \text{HPF}_6 \) was isolated in 85% yield. The desired complex 4c may thus easily decompose in solution while the solvent-free approach gives a much faster access to the complex and in a two-fold higher isolated yield.

The mechanochemical approach was then applied to salts 3a-e in the presence of silver oxide and sodium hydroxide (Scheme 2). As for complexes 2a-e, full conversion was observed in all cases in 3 h of milling. Homoleptic complexes 4a and 4b were isolated in excellent yields. The milling method was then applied successfully to poorly soluble \( \text{IPr}^* \text{OMe} \) and corresponding \( \text{Ag}^{\text{II}} \text{IPr}^* \text{OMe} \text{PF}_6 \). An improved yield of 94% was isolated, compared to 90% for complex 2a. As for complexes 2b-d, bearing a mesityl and a 2,6-diisopropylphenyl group resulted in a slightly lower yield of 70% and 88% for 4d and 4e, respectively. On the other hand, \( \text{Ag}^{\text{II}} \text{IPr}^* \text{OMe} \text{PF}_6 \) revealed highly unstable decomposition compared to 4d, probably because of the worse σ-donation of \( \text{IPr}^* \) compared to \( \text{IPr} \).

\(^1\text{H} \) NMR analysis showed the disappearance of the characteristic CH proton of the imidazolium salt 3e, with the formation of corresponding complex 4e. Due to a quick decomposition of 4e, it was impossible to obtain \( ^{13}\text{C} \) NMR analysis of the pure compound. Nevertheless, solid-state HR-MAS (high resolution magic angle spinning) \( ^{13}\text{C} \) NMR spectroscopy on a 600 MHz spectrometer confirmed the formation of the complex with the appearance of the characteristic carbenic carbon signal of 4e at 182 ppm (Figure 1). Of note, the expected doublets for the carbenic signal appear as a broad singlet in solid-state \( ^{13}\text{C} \) NMR. The low stability of \( \text{Ag}^{\text{II}} \text{IPr}^* \text{OMe} \text{PF}_6 \) could explain the low yields when the reactions were performed in solution. Indeed, the best conversion obtained when the reaction was attempted in refluxing chloroform was 62% after 48 h, yet along with the important decomposition. Comparatively, complex 4e was isolated in 93% yield after 3 h of milling.

The synthesis of novel homoleptic complexes featuring N-alkyl, N-aryl ligands was next performed (Scheme 3). Reaction of imidazolium salt 3f, bearing a mesityl and a 2-picolyl group on the nitrogen atoms, was found to be slightly slower than with \( N,N\)-diaryl imidazolium salts 3a-e, with 79% conversion after 3 h at 30 Hz. Hence, reaction was performed using a planetary ball-mill, which is more adapted to longer reaction times. After 5 h at 450 rpm, full conversion was obtained, yielding complex 4f in 80%. With a similar ligand bearing a benzyl instead of the 2-picolyl group, reaction proceeded efficiently and 4g was isolated in 97% yield. Increasing the steric hindrance on the aromatic ring with a 2,6-dimisopropylphenyl group resulted in slightly lower yields of 70% and 88% for 4h and 4i, respectively.

Mechanosynthesis of heteroleptic cationic silver(I) complexes

Finally, heteroleptic bis-NHC silver(I) complexes were synthesized using the vibratory ball-mill, starting from \( \text{Ag}^{\text{II}} \text{IPr} \) 2f (\( \text{IPr} = 1,3\)-bis(2,6-dimisopropylphenyl)-imidazol-2-ylidene), which was prepared via mechanochemistry. To the best of our knowledge, the synthesis of heteroleptic silver(I) complexes was never attempted in a ball-mill. Reaction was thus first realized using classical IMes-HPF6 (1,3-bis(2,4,6-trimethylphenyl)imidazolium) and SIMes-HPF6 (1,3-bis(2,4,6-trimethylphenyl)imidazolium) salts in the presence of sodium hydroxide (Scheme 4).

After 1 h of milling at 30 Hz in a vibratory ball-mill, full conversions were obtained and heteroleptic complexes 5a and 5b were isolated in 90% and 91% yield, respectively. Reaction with \( \text{Cl} \text{IPr}^* \text{PF}_6 \) 3c and \( \text{Me}^* \text{IPr}^* \text{OMe}^* \text{PF}_6 \) 3b yielded corresponding...
complexes 5c and 5d in 88% and 89%, respectively. To widen the scope of attainable heteroleptic complexes using this methodology, \( N,N \)-dibenzylimidazolium hexafluorophosphate and TPT-HPF\(_6\) (1,3,4-triphenyl-1,2,4-triazolium hexafluorophosphate) were reacted efficiently to furnish 5e and 5f in excellent yields. Of note, as shown by \( ^1 \)H NMR and HRMS analyses, only traces of ligand scrambling, resulting in the formation of undesired homoleptic silver complexes, were observed.\(^{16}\)

X-ray quality crystals could be grown by slow diffusion of diethyl ether into a dichloromethane solution of complexes 2a,

<table>
<thead>
<tr>
<th>Entry</th>
<th>Complex ( \text{d}_{C-Ag} ) (Å)</th>
<th>%Vbur(^a)</th>
<th>%Vbur(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>2.080</td>
<td>40.2</td>
</tr>
<tr>
<td>2</td>
<td>2b</td>
<td>2.087</td>
<td>42.8</td>
</tr>
<tr>
<td>3</td>
<td>2e(^{14})</td>
<td>2.078</td>
<td>52.9</td>
</tr>
<tr>
<td>4</td>
<td>4a</td>
<td>2.099</td>
<td>38.7</td>
</tr>
<tr>
<td>5</td>
<td>4b</td>
<td>2.122</td>
<td>40.2</td>
</tr>
</tbody>
</table>

\(^a\) \%Vbur calculated with real C-Ag distance, sphere radius of 3.5 Å. \(^b\) Distance C-Ag normalized at 2.1 Å, sphere radius 3.5 Å.

\(^{17}\) As already witnessed with other metals, the introduction of methyl groups on the NHC backbone (2a vs 2b and 4a vs 4b), which push the aromatic moieties towards the metal center, induces an increase in \%Vbur in each case. The increase of the steric hindrance also

**Table 2.** \%Vbur for the different complexes

**Figure 2.** ORTEPs (at 50% probability level) of compounds (a) 2b, (b) 4a. Hydrogen atoms and PF\(_6\) anion are omitted for clarity.
results in a longer NHC-metal distance. In comparison, ligand IPr* was found to be extremely hindered as %Vbur calculated for 2e reaches a value of 52.9 while the %Vbur of the isopropyl analogues do not exceed the value of 42.8.

Cytotoxic activity of NHC silver(I) complexes

Since homoleptic silver(I) complexes featuring benzimidazole or imidazopyridine-based NHC ligand have already shown promising activity against colorectal HCT116 cancer cell lines compared to 5-fluorouracil or cisplatin, respectively, the unique capacity of mechanochemistry to generate rapidly a library of NHC silver complexes (2a-5f) prompted us to evaluate their cytotoxicity against this cell line. The percentage inhibition of cell proliferation was firstly measured using 10⁻⁵ and 10⁻⁶ M solutions of the complexes (Figure 3). Half growth inhibition concentration (IC₅₀) was then measured only for compounds that showed high percentage of inhibition at 10⁻⁶ M (Figure 4). As a comparison, doxorubicin and cisplatin, which are commonly used to treat cancer, were evaluated on the same cancer cell line and possess IC₅₀ of 810 nM and 5.37 μM, respectively. In addition, [AgClIIPr]PF₆ 4j complexes were also tested to evaluate the influence of substitutions of the ligand on cytotoxicity. Among the neutral heteroleptic complexes 2a-f, only 2d and 2e were found almost inactive. Such behavior could be assigned to the poor solubility of the complexes in DMSO. Complex 2a showed an IC₅₀ of 259 nM. As a comparison, 2f, which contains the classical IPr ligand, exhibited an IC₅₀ of 390 nM, thus showing the positive influence of the methoxy groups on the NHC. The introduction of methyl group on the backbone of the NHC resulted in another positive effect as 2b was found to be active at 96.8 nM. On the other hand, 2c, featuring chlorine atoms on the backbone of the NHC, displayed an IC₅₀ of 616 nM. Cationic homoleptic complexes having N,N-diaryl NHC ligands 4a-c displayed a higher cytotoxicity, with IC₅₀ values down to 24.9 nM for 4b. Once again, the positive effect of the methoxy group was demonstrated as [Ag(IPr)][PF₆] complex displayed an IC₅₀ of 140 nM (vs 35.4 nM for 4a). The addition of methyl groups on the NHC backbone results in even lower IC₅₀ value (35.4 nM for 4a vs 24.9 nM for 4b). Homoleptic complexes 4f-i, containing N-aryl, N-alkyl NHC ligands, were found to be less active. In this family, 4h was the most active, with an IC₅₀ at 293 nM. Cationic bis-NHC silver complexes 5a-f also showed promising activity. 5a, featuring an IMes ligand, was more cytotoxic than 5b that contains the analogous saturated ligand. While very similar in structure, 5d, having one IPr ligand in place of Me₄IPrOMe, was found to be slightly more active than homoleptic 4b, with the best IC₅₀ of 21 nM. 5d is thus 256 and 38 times more active than cisplatin and doxorubicin, respectively. Interestingly, 5f, containing a TPT ligand, showed an IC₅₀ of 163 nM, thus demonstrating a possible diversification of the structure of the active complexes. On the other hand, 5c and 5e, featuring a ClIPr and a N,N-dibenzyl NHC ligand, respectively, were not as active, with a % inhibition of cell proliferation below the values obtained for the other complexes. Thus, this preliminary study shows that introducing chlorine atoms on the backbone of the NHC, or using N-alkyl, N-aryl NHC ligands is detrimental to the biological activity. In the case of complexes featuring chlorine-substituted NHC ligands, the lower activity could be due to instability issues, as observed during their synthesis in solution. On the other hand, the Me₄IPrOMe ligand, either in neutral heteroleptic or cationic complexes, seems to present the best positive effect on the cytotoxicity of the silver complexes. Of note, apart from 4g, all the silver complexes tested displayed a higher cytotoxicity than doxorubicin and cisplatin.
Conclusions

In conclusion, mechanochemistry overcame solution-based chemistry, permitting to access rapidly and efficiently novel families of neutral heteroleptic and cationic homoleptic complexes featuring NHC ligands bearing sterically hindering groups, and also electron-donating or withdrawing substituents. Importantly, the use of ball-mills enabled the isolation of complexes difficult if not impossible to prepare and isolate using solution-based strategy. As most of the compounds obtained displayed a novel structure, their biological activity was evaluated. As preliminary results, the silver complexes showed high cytotoxic activity against colorectal HCT116 cancer cell line, with IC₅₀ down to 21 nM, which is 256 times better than cisplatin.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The Université de Montpellier, Centre Nationale de la Recherche Scientifique (CNRS) and Agence Nationale de la Recherche (grant no. ANR-16-CE07-0009-01) are acknowledged for funding. We thank Anthony Pinon and Marouan Lakhlil for their contribution to the design of the graphical abstract.

Notes and references

16. See Supporting Information for details.