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# Accepted Article

**Title:** Iridium catalyzed imine reduction by hydride transfer using coenzyme NADH

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# Cp*Ir catalyzed imine reductions utilizing the biomimetic 1,4-NAD(P)H cofactor and *N*-benzyl-1,4-dihydronicotinamide as the hydride transfer agent

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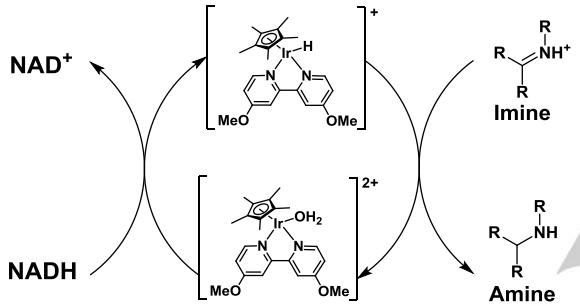
**Abstract:** *Interactions between synthetic organometallic complexes and metabolic cofactors proved to be a newly emerging topic in bio-organometallic chemistry. Thus, the first* cationic Cp*Ir-*catalyzed imine reduction in neutral buffered aqueous medium is reported. The reaction proceeds via a hydride transfer from NADH as the hydride source at room temperature and under air. Cationic Cp*Ir complexes proved to be the most efficient catalysts for this transformation. We also highlighted that the choice of the proton source was essential. The method was subsequently applied on cyclic and non-cyclic imines. Eventually, the concept was extended to one example of reductive alkylation of an amine.*

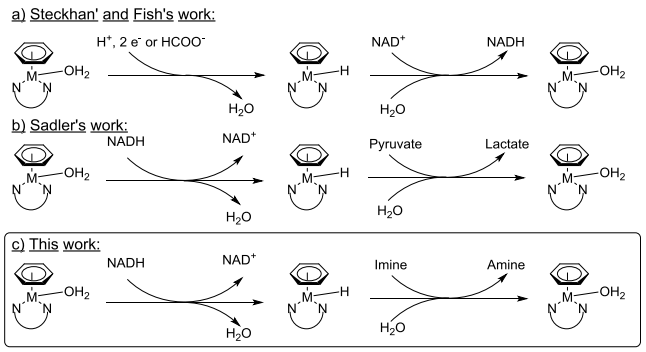



NAD(P)H is widely used by the metabolism as a cofactor for its role as an important electron carrier in many biological pathways, including glycolysis, fermentation and Krebs cycle and is known to have a key role in the metabolism of every living cell, especially cancer cells due to lower oxygen tension.[1]

Interactions between organometallics and NAD cofactors in the context of NADH regeneration has been a key topic in bio organometallic chemistry for over 3 decades and has been extensively studied.[2] The rhodium (III) complex $[Cp^*RhCl(bpy)]^+$, in combination with HCOONa as a hydride source, was first employed by Steckhan in the context of NADH regeneration (Scheme 1a).[3] Later, Fish explained the 1,4 regioselectivity of the hydride transfer from the metal center to $NAD^+$ by the coordination of the $NAD^+$ amide moiety to the metal.[4] The concept was later applied by Sadler in living cells, where he reported that Noyori type ruthenium hydride complexes could reduce $NAD^+$ to NADH in human ovarian cancer cells in the presence of formate anion, leading to cell death through a novel and original reductive stress mechanism.[5]

Fish also brought experimental evidences of the reversibility of that hydride transfer from the NADH analogue *N*-benzyl-1,4-dihydronicotinamide (**Bn-NADH**) to $[Cp^*Rh(bpy)(D_2O)](OTf)_2$ as the hydride acceptor in $D_2O$/THF-d during deuterium incorporation studies.[4c] Then, in 2012, Sadler reported the direct observation and characterization by $^1$H-NMR of a ruthenium or iridium hydride obtained directly by treatment of the corresponding aqua complexes by NADH (Scheme 1b).[6] Studies about the properties and stability of those hydrides were reported by Fukuzumi and Miller.[7] Those hydrides could further reduce biologically relevant substrates, such as pyruvate to lactate. This system was also applied to quinones by Sadler and Fukuzumi.[8] Those metal hydride are already known as versatile tools for reduction of carbonyls and imines in water,[9] and more complex functional groups such as dihydroisoquinolines.[10]

Even if those hydride transfer have already found applications within biological medium, we noticed that to date, few examples involving NAD(P)H cofactors and organometallic complexes have been published so far. Ariga applied the concept of the reduction of quinones by NADH and a cationic Cp*Ir complex reported by Sadler to quench the fluorescence of a rhodamine and used it as a probe of the tricarboxylic acids cycle.[11] More recently, Do showed that it was possible to use NADH and sub-stoichiometric amounts of a cationic Cp*Ir complex to reduce cytotoxic aldehydes in the water medium to the corresponding alcohols in water, proving that the complex acts as a catalyst.[12] However, there has been no report of catalytic hydride transfer reduction of imines involving NAD(P)H and a metal complex to the best of our knowledge. Imines are more challenging substrates than aldehydes due to their reduced electrophilic character and their hydrolytic behavior in water at acidic pH. Herein, we report the cationic Cp*Ir promoted catalytic transfer reduction of a set of imines in water using NADH or one of its analogues as the hydride source (Scheme 1c).




**Scheme 1** a) Steckhan and Fish: electrochemical or reduction of a $d^6$ complex to the hydride and reduction of $NAD^+$ to 1,4-NADH, b) Sadler: Oxidation of NADH to $NAD^+$ and formation of the hydride and subsequent reduction of pyruvate to lactate, c) This work: oxidation of NADH to NAD+ and reduction of an imine using the in situ generated hydride.

In the first phase of our investigation, we decided to optimize the reduction of a water stable and water soluble model imine using a stoichiometric amount of NADH (Scheme 2a). Recent studies

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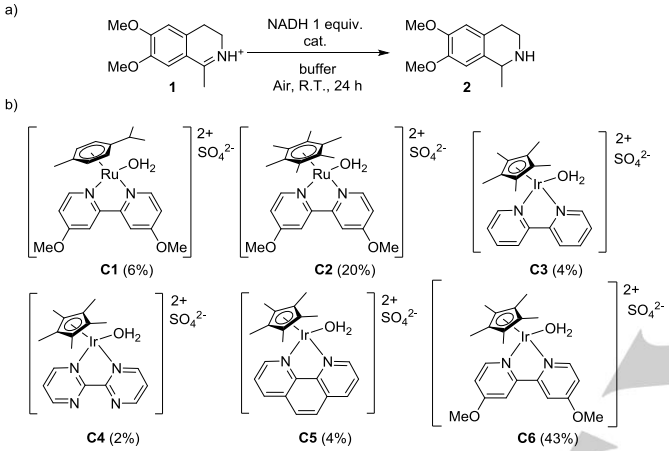
by Pikho, Yu and Vidal drew our attention to dihydroisoquinolines as good model substrates for this reduction.[10a] We therefore oriented our screening towards water soluble catalysts able to react with NADH and form a hydride stable in neutral buffered conditions and also able to reduce imines in water. We started our investigations by screening ruthenium (**C1** and **C2**) and cationic Cp*Ir (**C3**-**C6**) catalysts using a 50 mM phosphate buffer at pH 7 as medium (Scheme 2b). Despite the numerous reports by Sadler on hydride transfers from NADH to Ru complexes, **C1** and **C2**, even at high catalytic loading, did not show any good reactivity for the reduction of the imine **1**.[6] After switching to cationic Cp*Ir complexes with lower catalytic loading (2 mol%), we noticed that an electron rich bipyridine (**C6**) was vital for an efficient reduction of our model substrate. We therefore obtained a yield of 43% using 2 mol% of **C6**.




**Scheme 2** a) Model reaction studied: reduction of 6,7-dimethoxy-1-methyltetrahydroisoquinoline, b) Structure of the catalysts **C1**-**C6** used for this study and yield (%) for the model reaction. Conditions: substrate at 15 mM in 50 mM pH 7 phosphate buffer, **C1**-**C2** 10 mol% and **C3**-**C6** 2 mol%, NMR yields versus dimethyl terephthalate as internal standard.

Since the obtained amine **2** is present in its protonated form at pH 7, we expect that at least 2 $H^+$ from the medium should be consumed in the overall process. The formed imine can also trap the Cp*Ir aqua complex by coordination to the metal center, preventing thus the catalyst from performing another catalytic cycle. We believe that protonation of the formed amine can impede this inhibition mechanism by preventing the coordination of the amine to the iridium. Therefore, we investigated the impact of the acid. Preliminary studies rapidly showed a different behavior for the acids depending on their pKa (Figure 1). Our screening among acids ranging from pKa of -0.3 to 10 revealed three different phenomena (See Supporting information Table 2). Firstly, for the strong acids (pKa <4), we observed a decrease of the yield after the addition of 1 equivalent and no more conversion for $NaHSO_4$ and trifluoroacetic acid (TFA) when 3 equivalents are used. Secondly, the medium acids (pKa between 4 and 8-9) afforded a maximum of yield using 2 equivalents and only a slight decrease when a third equivalent is used. Finally, the weak acids have little to no effect compared to the situation in absence of acid. We hence suspected that the pKa threshold of 4 corresponds to the pKa of the protonation of the hydride.[13] When the acid is too strong, the hydride is reprotonated faster than it can reduce the imine. To further confirm this hypothesis, a yield of 52% was obtained when 2.5 equivalents of TFA acid were added over 5 hours, avoiding thus the contact between the acid and the hydride Then, the second threshold corresponds to the activation of the imine in solution by protonation. When the acid is too weak (PhOH), there is no effect as no activation of the substrate is possible. Our screening showed that $KH_2PO_4$ was the optimal acid for our purpose. We were pleased to obtain 60% yield (TON = 30) with the cationic Cp*Ir complex **C6** using either 2.5 equivalents of $KH_2PO_4$. Alternatively, a constant pH was reached by working with a 100 mM pH 7 phosphate buffer to obtain the same yield of 60% (Table 1 Entry a). The pH was found to change from 7.0 to 7.4 during the reaction. While performing the reaction in 100 mM buffers with pH ranging from 4 to 10, the same trend was observed. The reaction was found to exhibit an optimum for neutral pH buffers (See supporting information Table 4).

Some minor improvements of the yield were noted when an excess of NADH was used (72%), even though this option was not further investigated for economic reasons. NADPH exhibited a similar reactivity, providing 62% of yield (Table 1, Entry a). Sodium formate also gave us a yield of 99% while 10 equivalents are used (Table 1, Entry c). The formate turns out to be an efficient hydride source for our reaction. This result is especially interesting as it shows that it can be used as a NADH substitute in biological applications, as living cells can tolerate millimolar concentrations of formate.[5]

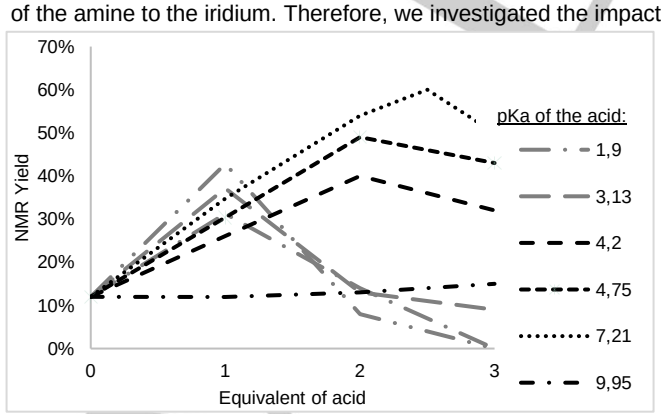
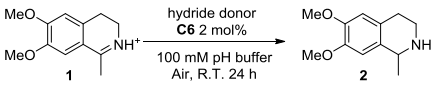



**Figure 1** Impact of the use of acids used at 0, 1, 2 and 3 equivalents. pKa: TFA (-0.3), $NaHSO_4$ (1.99), citric acid (3.13), PhCOOH (4.2), AcOH (4.75), $KH_2PO_4$ (7.21), phenol (9.95)




**Scheme 3** Study of various hydride donors

**Table 1** Yield obtained for the various hydride donors of this study

| Entry | Hydride source | Equiv. | Yield (%) |
|---|---|---|---|
| **a** | NADH | 1 | 60[a] |
| | | 2 | 72[a] |
| **b** | NADPH | 1 | 61[a] |
| **c** | HCOONa | 10 | 99[b] |
| **d** | Bn-NADH | 1 | 83[b] |
| | | 2 | 99[b] |

Conditions: Substrate 15 mM in 100 mM phosphate buffer under air. [a] NMR yield using dimethyl terephthalate, [b] isolated yield

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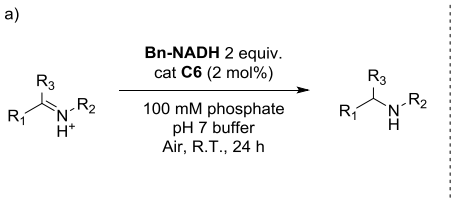
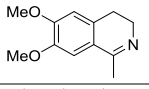
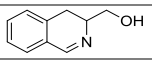
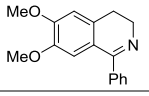
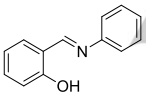
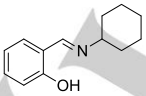
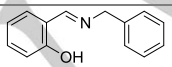
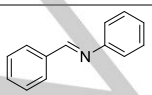
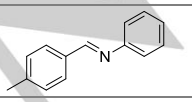
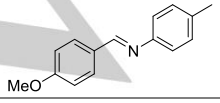
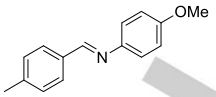
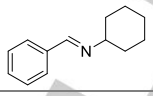
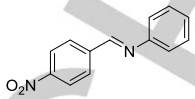
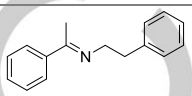
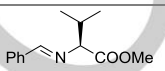
After a successful validation of the methodology with NADH, we decided to apply the concept on a panel of imines (Scheme 4, Table 2). We hereby chose a NADH analogue bearing the same redox active dihydropyridine as an economical alternative to NADH. Indeed, many NAD analogues were previously reported.[14] Among those, we chose the *N*-benzylated analogue of NADH (**Bn-NADH**) for its acceptable aqueous solubility and since its interactions with metal complexes were studied in the literature.[4] It was important to note that 2 equivalents of **Bn-NADH** were needed to get full conversion (Table 1, Entry d). We verified that the *N*-benzyl-1,4-dideuteronicotinamide (**Bn-NADH-d2**, 79% deuterated) led to a deuterium incorporation of 72 %, indicating thus that the hydride is coming from the **Bn-NADH-d2** and that the Ir-D attacks the imine. This catalytic system shows excellent yields on cyclic imines (Table 2, Entries a–c), good yields on salicylideneaniline derivatives (Table 2, Entries d–f) and on *N*-benzylidene derivatives (Table 2, Entries g–l). Some selected examples allow to compare the results of **Bn-NADH** with NADH (Table 2, Entries a, d, g, n).




**Scheme 4** a) Study of the scope of the reaction on a set of cyclic and acyclic imines; b) NADH analogue used for the application of the concept

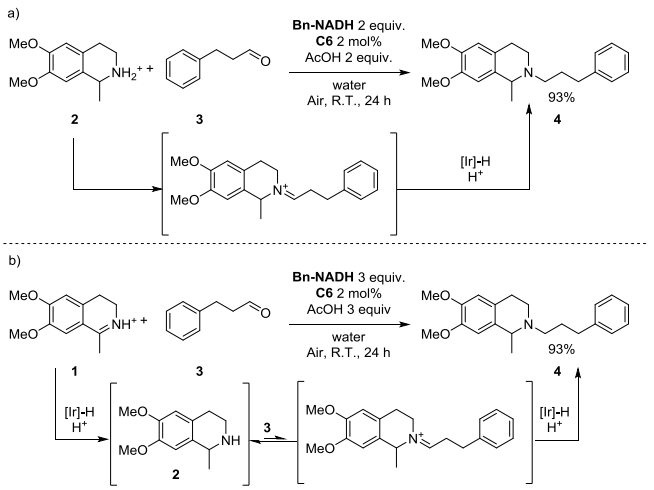
**Table 2** Reduction of cyclic and non-cyclic amines using **Bn-NADH** and NADH

| Entry | Substrate | Yield (%) |
|---|---|---|
| **a** |  | 99<br>60[a] |
| **b** |  | 87<br>58[a] |
| **c** |  | 99 |
| **d** |  | 72<br>62[a] |
| **e** |  | 99 |
| **f** |  | 99 |
| **g** |  | 97<br>40[a] |
| **h** |  | 78 |
| **i** |  | 82 |
| **j** |  | 81 |
| **k** |  | 99 |
| **l** |  | 91 |
| **m** |  | 60 |
| **n** |  | 88<br>63[a] |

Conditions: Substrate 15 mM in 100 mM phosphate buffer under air, 2 equiv. of **Bn-NADH**, isolated yields after flash column chromatography, [a] 1 equiv. NADH, NMR yield using dimethyl terephthalate as internal standard.

To further demonstrate the potential of this method in more complex cases, we chose the water soluble model amine **2** to perform the reductive alkylation by the aldehyde **3** (Scheme 5a). We studied the influence of the acid (AcOH in our case), the equivalents of **Bn-NADH**, the impact of the use of a buffer and the number of equivalents of aldehyde **3** (See supporting information Table 5). It turned out that using an excess of **3** provides a better yield compared to a stoichiometric quantity. The best conditions were found with 2 equivalents of **Bn-NADH**, 3 equivalents of aldehyde **3** and 2 equivalents of AcOH. An excess of aldehyde ensures a full complete conversion of the amine. The product **4** could even be isolated with a 93% yield.

We eventually extended this concept to a more complex sequence, involving the one pot reduction of the imine **1** followed by the reductive alkylation of the *in situ* generated amine **2** by the aldehyde **3** and we obtained the desired product **4** with a yield of 56% (Scheme 5b). The selectivity for the reductive alkylation was explained by the higher reactivity of the iminium compared to the aldehyde, even if only small amounts of iminium are present in solution due to equilibrium.




**Scheme 5** a) *Reductive alkylation of the amine **2** by the aldehyde 3, b)* One pot imine reduction and reductive alkylation

In this study, we report a novel and efficient imine reduction with good yields using NADH and the NADH mimic **Bn-NADH** as a hydride source and catalytic amounts of the cationic Cp*Ir catalyst **C6**. HCOONa was shown in one example to be another hydride source for the reaction we studied. This method was further applied to the reductive alkylation of a secondary amine. We finally showed the applicability of the method for a one pot reduction of an imine and the reductive alkylation of the amine generated *in situ*.

We expect that this method would find numerous applications in homogeneous *in cellulo* catalysis in the field of bioorganometallic chemistry.[15] This methodology could be employed as a tool for protein labelling by reductive alkylation in their native environment without the use of an external hydride source like HCOONa.[16] It could also be applied for targeted decaging of exogenous probes[17] or drugs[18] in cellular medium.

## Acknowledgements

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**Keywords:** hydride transfer • bioorthogonal chemistry • biomimetic • iridium catalysis • reductive alkylation • NADH

[1] (a) W. Kaelin; C. Thompson. *Nat. Cell Biol.* **2010,** *465*, 562-564; (b) A. Chiarugi; C. Dolle; R. Felici; M. Ziegler. *Nat. Rev. Cancer* **2012,** *12*, 741-752.

[2] (a) F. Hollmann; I. Arends; K. Buehler. *ChemCatChem* **2010,** *2*, 762-782; (b) T. Quinto; V. Köhler; T. R. Ward. *Top. Catal.* **2013,** *57*, 321-331.

[3] R. Ruppert; S. Herrmann; E. Steckhan. *J. Chem. Soc., Chem. Commun.* **1988,** *17*, 1150-1151.

[4] (a) H. C. Lo; O. Buriez; J. B. Kerr; R. H. Fish. *Angew. Chem., Int. Ed.* **1999,** *38*, 1429-1432; (b) H. C. Lo; R. H. Fish. *Angew. Chem., Int. Ed.* **2002,** *41*, 478-481; (c) H. C. Lo; C. Leiva; O. Buriez; J. B. Kerr; M. M. Olmstead; R. H. Fish. *Inorg. Chem.* **2001,** *40*, 6705-6716.

[5] J. J. Soldevila-Barreda; I. Romero-Canelon; A. Habtemariam; P. J. Sadler. *Nat. Commun.* **2015,** *6*, 6582.

[6] S. Betanzos-Lara; Z. Liu; A. Habtemariam; A. M. Pizarro; B. Qamar; P. J. Sadler. *Angew. Chem., Int. Ed.* **2012,** *51*, 3897-3900.

[7] (a) Y. Maenaka; T. Suenobu; S. Fukuzumi. *J. Am. Chem. Soc.* **2012,** *134*, 367; (b) C. L. Pitman; K. R. Brereton; A. J. M. Miller. *J. Am. Chem. Soc.* **2016,** *138*, 2252-2260.

[8] (a) Z. Liu; R. J. Deeth; J. S. Butler; A. Habtemariam; M. E. Newton; P. J. Sadler. *Angew. Chem., Int. Ed.* **2013,** *52*, 4194-4197; (b) T. Suenobu; S. Shibata; S. Fukuzumi. *Inorg. Chem.* **2016,** *55*, 7747-7754.

[9] (a) X. Wu; J. Xiao, Hydrogenation and Transfer Hydrogenation in Water. In *Metal-Catalyzed Reactions in Water*, Wiley-VCH Verlag GmbH & Co. KGaA: 2013; pp 173-242; (b) D. Wang; D. Astruc. *Chem. Rev.* **2015,** *115*, 6621; (c) J. S. Willemsen; J. C. van Hest; F. P. Rutjes. *Chem. Commun. (Cambridge, U. K.)* **2013,** *49*, 3143-3145; (d) J. r. m. Canivet; G. S?ss-Fink. *Green Chem.* **2007,** *9*, 391.

[10] (a) L. Evanno; J. Ormala; P. M. Pihko. *Chem. Eur. J.* **2009,** *15*, 12963-12967; (b) Y. M. Wilson; M. Dürrenberger; E. S. Nogueira; T. R. Ward. *J. Am. Chem. Soc.* **2014,** *136*, 8928; (c) N. Pannetier; J.-B. Sortais; J.-T. Issenhuth; L. Barloy; C. Sirlin; A. Holuigue; L. Lefort; L. Panella; J. G. de Vries; M. Pfeffer. *Adv. Synth. Catal.* **2011,** *353*, 2844-2852; (d) C. Wang; C. Li; X. Wu; A. Pettman; J. Xiao. *Angew. Chem., Int. Ed.* **2009,** *48*, 6524-6528; (e) Z. Wu; M. Perez; M. Scalone; T. Ayad; V. Ratovelomanana-Vidal. *Angew. Chem., Int. Ed.* **2013,** *52*, 4925-4928; (f) J. Wu; F. Wang; Y. Ma; X. Cui; L. Cun; J. Zhu; J. Deng; B. Yu. *Chem. Commun. (Cambridge, U. K.)* **2006,** *16*, 1766-1768.

[11] H. Komatsu; Y. Shindo; K. Oka; J. P. Hill; K. Ariga. *Angew. Chem., Int. Ed.* **2014,** *53*, 3993-3995.

[12] A. H. Ngo; M. Ibañez; L. H. Do. *ACS Catalysis* **2016,** *6*, 2637-2641.

[13] T. Abura; S. Ogo; Y. Watanabe; S. Fukuzumi. *J. Am. Chem. Soc.* **2003,** *125*, 4149.

[14] (a) L. Zhang; J. Yuan; Y. Xu; Y. H. P. Zhang; X. H. Qian. *Chem. Comm.* **2016,** *52*, 6471-6474; (b) C. E. Paul; I. W. C. E. Arends; F. Hollmann. *ACS Catalysis* **2014,** *4*, 788-797; (c) T. Knaus; C. E. Paul; C. W. Levy; S. de Vries; F. G. Mutti; F. Hollmann; N. S. Scrutton. *J. Am. Chem. Soc.* **2016,** *138*, 1033-1039.

[15] (a) P. K. Sasmal; C. N. Streu; E. Meggers. *Chem. Comm.* **2013,** *49*, 1581-1587; (b) J. J. Soldevila-Barreda; P. J. Sadler. *Curr. Opin. Chem. Biol.* **2015** *25*, 172.

[16] J. M. McFarland; M. B. Francis. *J. Am. Chem. Soc.* **2005,** *127*, 13490-13491.

[17] (a) C. Streu; E. Meggers. *Angew. Chem., Int. Ed.* **2006,** *45*, 5645-5648; (b) R. M. Yusop; A. Unciti-Broceta; E. M. Johansson; R. M. Sanchez-Martin; M. Bradley. *Nat. Chem.* **2011,** *3*, 239-243; (c) M. I. Sanchez; C. Penas; M. E. Vazquez; J. L. Mascarenas. *Chem. Sci.* **2014,** *5*, 1901-1907; (d) M. Tomas-Gamasa; M. Martinez-Calvo; J. R. Couceiro; J. L. Mascarenas. *Nat. Commun.* **2016,** *7*, 12538; (e) J. Li; J. Yu; J. Zhao; J. Wang; S. Zheng; S. Lin; L. Chen; M. Yang; S. Jia; X. Zhang; P. R. Chen. *Nat. Chem.* **2014,** *6*, 352.

[18] (a) J. T. Weiss; J. C. Dawson; C. Fraser; W. Rybski; C. Torres-Sanchez; M. Bradley; E. E. Patton; N. O. Carragher; A. Unciti-Broceta. *J. Med. Chem.* **2014,** *57*, 5395-5404; (b) J. T. Weiss; J. C. Dawson; K. G. Macleod; W. Rybski; C. Fraser; C. Torres-Sánchez; E. E. Patton; M. Bradley; N. O. Carragher; A. Unciti-Broceta. *Nat. Commun.* **2014,** *5*, 3277.

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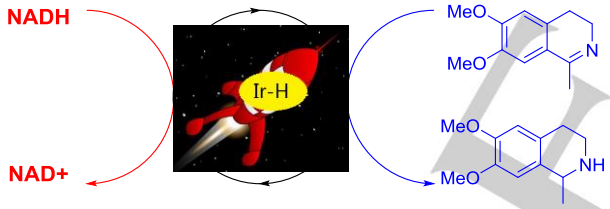


*Mathieu Soetens, Fleur Drouet, Olivier Riant**

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**Cp*Ir catalyzed imine reductions utilizing the biomimetic 1,4-NAD(P)H cofactor and *N*-benzyl-1,4-dihydronicotinamide as the hydride transfer agent**

The first cationic Cp*Ir-catalyzed imine reduction in neutral buffered aqueous medium under air at room temperature is reported. The reaction proceeds *via* a hydride transfer from NADH as the hydride source. Cp*Ir(N,N) complexes proved to be the most efficient catalysts for this transformation. The method was subsequently applied to the reduction of cyclic and non-cyclic imines. Eventually, the concept was extended