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Single Nucleotide-Catalyzed Biomimetic Reductive Amination

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Abstract: We have successfully developed a single nucleotide (adenosine 5′-diphosphate)-catalyzed enantioselective direct reductive amination of aldehydes and ketones using a Hantzsch ester as reducing agent. The process is a simple, efficient and a real mimic of the NADH reduction approach for the synthesis of structurally diverse amines. This reaction is the first report demonstrating the ability of a single nucleotide as catalyst and one of the most genuine biomimetic reactions of organic chemistry.

Keywords: biomimetic process; Hantzsch esters; nucleotides; reductive amination

Introduction

Nucleic acids as catalyst were merely a theoretical possibility until the discovery of catalytic RNAs (ribozymes) in the early 1980s.[1] Today, a variety of natural ribozymes,[2] many artificial ribozymes as well as deoxyribozymes have been identified through *in-vitro* selection from pools of random sequences[3] and were found to catalyze many chemical reactions such as, phosphodiester cleavage, ligation,[4] RNA polymerization,[5] redox reactions,[6] carbon-carbon bond formation (Diels–Alder reaction),[7,8] enantioselective Michael reactions.[9] The catalytic activity of nucleic acid place DNA and RNA[10] as the primordial macromolecules in the emergence of life at the earth. Thus, "RNA world" and "DNA world" hypothesis for the origin of life was postulated. Since the basic units of these nucleic acids are the nucleotides, we argued whether a single nucleotide could be an efficient catalyst in organic reactions. The literature survey reveals that there are no reports on the application of single nucleotides as catalyst in organic reactions.

A variety of enzyme cofactors such as coenzyme A, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) serving a wide range of biochemical functions, include adenosine as part of their structure. The adenosine portion's participation in the primary function is not yet very clear but the removal of adenosine generally results in a drastic reduction of cofactor activities. Based on these finding we selected adenosine nucleotides as catalysts to demonstrate the catalysis by single nucleotides. For these studies, we have selected reductive amination, which is a distinguished and vital reaction in biosynthesis.

It has long been established that nature has perfected the reduction of ketimines as an *in vivo* chemical tool for the synthesis of essential biomonomers. As a preeminent example, transferase enzymes in combination with a dinucleotide (NADH), selectively reduce pyruvate-derived ketimines, thereby ensuring the formation of naturally occurring amino acids.[11] This finding prompted us to explore whether the conceptual blueprints of biochemical amination could be translated to a reduction of ketimines wherein enzymes and cofactors (NADH) are replaced by single nucleotide catalysts analogues.

This single nucleotide-catalyzed transfer hydrogenation approach is one of the closest biomimetic reactions of organic chemistry. It is also of great interest to investigate the catalytic efficiency of nucleotides in organic synthesis.

Nucleotide adenosine diphosphate (ADP) in combination with a Hantzsch ester is the most genuine catalytic model mimicking the naturally occurring reducing agent NAD(P)H (Figure 1). In this paper, for the first time, we are reporting the use of a single nucleotide (ADP) as a catalyst for direct asymmetric reductive amination.

Imine reduction is one of the most useful methods for the preparation of amines and related functional compounds.[12–17] Recently some fascinating reports on asymmetric imine reduction using substituted chiral binaphthyl hydrogen phosphate as catalyst were reported by List (**5h**),[18] and Rueping (**5g**).[19] Extensive

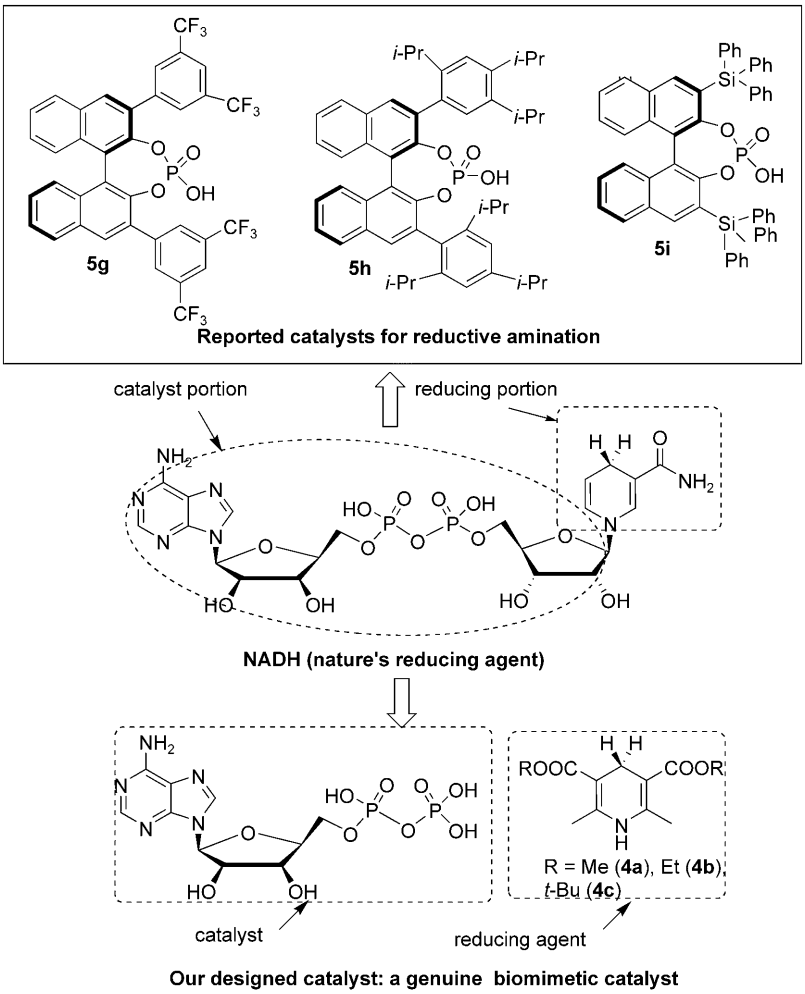
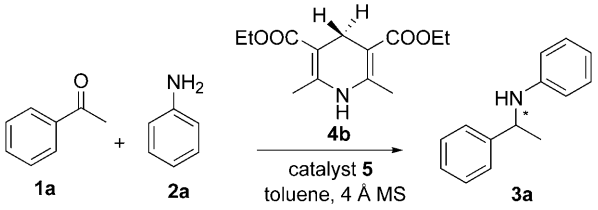


Figure 1. Biomimetic catalyst design.

studies on enantioselective imine reduction have been done by Sun,[20] and Kočovský[21] using trichlorosilane and a Lewis base organocatalyst. However, only MacMillan et al. (**5i**)[22] have reported the synthesis of enantioselective amines *via* reductive amination using an *ortho*-triphenylsilyl variant of the Terada–Akiyama catalyst. This prompted us to develop a nucleotide-catalyzed reductive amination instead of a conventional imine reduction.



Scheme 1. Nucleotide-catalyzed biomimetic reductive amination.

Results and Discussion

Initial evaluation of the proposed reductive amination was performed with acetophenone **1a**, aniline **2a**, ethyl Hantzsch ester **4b**, and a number of organocatalysts (Scheme 1, Table 1). While adenine **5a** and adenosine **5b** did not induce reductive amination to any significant extent, adenosine 3′-monophosphate **5c**, adenosine 5′-monophosphate **5d**, ADP **5e** and ATP **5f** did provide the desired amine product in excellent yields (Figure 2). ADP **5e** was found to be the best for achieving both the high yields and enantioselectivity. Introduction of 4 Å molecular sieve increased the yield of **3a** to a significant extent. This reveals that water, generated in the initial condensation step, has a deleterious impact on both iminium formation and the hydride reduction step. As such, the introduction of 4 Å molecular sieves was found to be critical to achieve a useful reaction rate and selectivity.

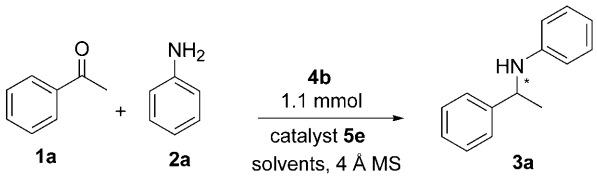
We observed that 2 mol% of ADP was sufficient to push the reaction forward and further increasing the amount of catalyst (2 to 5 mol%) did not improve the

Table 1. Screening of organocatalysts for the reductive amination of acetophenone with aniline and ethyl Hantzsch ester.[a]

Entry	Catalyst	Additive	Time [h]	*ee* [%][b]	Yield of **3a** [%][c]1
1	none	none	72	–	0
2	**5a**	none	72	–	<5
3	**5b**	none	72	–	<5
4	**5c**[d]	none	48	40	60
5	**5d**[e]	none	48	44	55
6	**5e**[f]	none	48	60	78
7	**5f**[e]	none	48	57	70
8	**5e**	4 Å MS	48	81	95
9	**5e**[f]	4 Å MS	48	62	95

[a] *Reaction conditions:* acetophenone (1 mmol), aniline (1 mmol), ethyl Hantzsch ester (1.1 mmol), catalyst 2 mol%, toluene (5 mL), 30 °C, N_2 atomosphere.
[b] Enantiomeric exess was determined by chiral HPLC.
[c] Isolated yield.
[d] Free acids.
[e] Sodium salt.
[f] 5 mol% catalyst.

Table 2. Screening of solvents.



Entry[a]	X [mol%]	Solvent	Time [h]	Conversion [%][b]	*ee* [%][c]
1	5	$CHCl_3$	72	47	56
2	5	DCM	72	51	59
3	5	dioxane	72	62	72
4	5	THF	48	63	74
5	5	xylene	48	85	79
6	5	AcOEt	48	69	66
7	5	benzene	48	79	75
8	5	Et_2O	48	76	71
9	5	toluene	48	97	64
10	2	toluene	48	96	82
11[d]	2	toluene	48	59	66
12	1	toluene	48	89	63

[a] *Reaction conditions:* acetophenone (1 mmol), aniline (1 mmol), 1.10 mmol of **4b**.
[b] Determined by ^{1}H NMR.
[c] Determined by chiral HPLC analysis.
[d] Reaction was carried out at 0 °C.

yield or selectivity to any significant extent. Yield and *ee* were significantly decrease by reducing the amount of the catalyst to 1 mol% (Table 2, entry 12). With 2 mol% of **5e** as the catalyst at room temperature, various solvents have been examined for this reaction, as summarized in Table 2. The asymmetric transfer hydrogenations in several solvents such as benzene, dichloromethane (DCM), ethyl acetate, THF, MeOH, $CHCl_3$, dioxane, Et_2O, toluene and xylene have been evaluated. Toluene was chosen as the optimal solvent since the reaction in toluene led to complete conversion in 48 h (Table 2, entry 10). The *ee* has been decreased by lowering the temperature to 0 °C (Table 2, entry 11).

We further investigated the suitability of methyl, ethyl and *tert*-butyl Hantzsch esters as reducing agent in the ADP-catalyzed reductive amination of acetophenone with aniline. Methyl Hantszsch ester **4a**, as reducing agent resulted in a poor yield of **3a**. This could be attributed to the poor solubility of methyl Hantzsch ester **4a**. Butyl Hantzsch ester **4c** resulted in reasonably good yields and enantioselectivity but the reaction took a longer time to complete (3 days). Ethyl Hantzsch ester **4b** was found to be the best for achieving both the high yields and enantioselectivity; thus, further experiments were carried out using **4b** as reducing agent (Table 3).

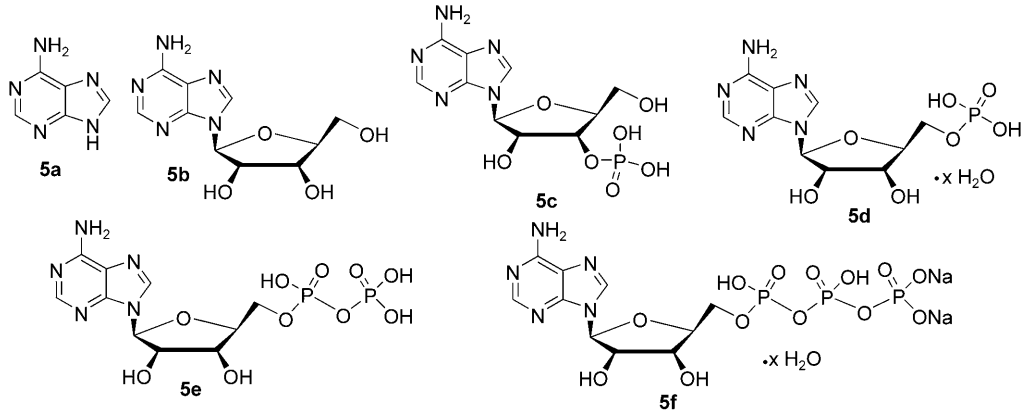


Figure 2. Nucleotides and precursors screened for catalytic activity.

Table 3. Investigation of Hantzsch esters.

Entry[a]	Hantzsch Ester	Time [h]	Conversion [%][b]	*ee* [%][c]
1	**4a**	68	65	63
2	**4b**	48	95	81
3	**4c**	74	77	69

[a] *Reaction conditions:* acetophenone (1 mmol), aniline (1 mmol), 2 mol% of **5e** in toluene at room temperature.
[b] Determined by ^{1}H NMR.
[c] Determined by chiral HPLC analysis.

The structure-selectivity relationship suggests that the 3′-nucleotide **5c** should induce greater enantioselectivity than the remaining 5′-nucleotides **5d**, **5e** and **5f** as the phosphoric acid group is directly attached to the chiral carbon in 3′-nucleotide **5c** but the experimental results are against it. The nucleotides with di-/triphosphate groups (**5e** and **5f**) are showing somewhat better enantioselectvity than nucleotides with monophosphate groups (**5c** and **5d**).

Recently Goodman and co-worker have reported on computational studies[23] of the Hantzsch ester hydrogenation of imines catalyzed by chiral BINOL-phosphoric acid; based on this report we have proposed a transition state of ADP-bound imine and Hantzsch ester structure, in which the phosphate group is attached with both the Schiff base and the Hantzsch ester, respectively, and the transition state might be further stabilized *via pi–pi* interactions between adenine and theSchiff base. The *Re* face of the imine is sterically more crowed in comparison to the *Si* face. Thus, the transfer hydrogenation with Hantzsch ester occurs predominantly from the less hindered *Si* face of the imine. We have achieved good enantiomeric excess in the reductive amination of ketones (*ee* 75–89%). These experimental results are well explained by the transition state (Figure 3). In order to find an exact picture of the transition state, we attempted to perform a single crystal X-ray analysis of imine salts of ADP but we could not succeed in getting a good crystal of imine salt of ADP to date.

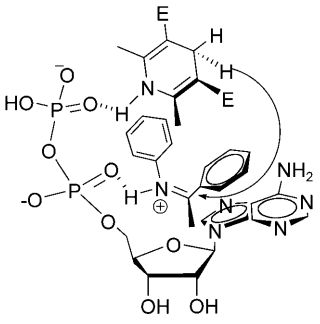


Figure 3. Plausible transition state for the catalyst-bound imine and Hantzsch ester structure.

Having established the optimal conditions for the reductive amination of ketones, we next examined the scope of the ketone and amine components in this nucleotide-catalyzed asymmetric reductive amination. However, presently this reductive amination is restricted to a smaller substrate spectrum. As revealed in Figure 4, a variety of substituted acetophenone derivatives can be successfully coupled with a number of primary amines including electron-rich as well as electron-deficient aryl ketone systems. The products were obtained generally in good enantioselectivity and excellent yields. However, reductive aminations with secondary amines were unsuccessful.

In order to examine the versatility of the single nucleotide-catalyzed reductive amination, we carried out the ADP-catalyzed reductive amination of aldehydes (Scheme 2, Table 4). The reactions were carried out in dry toluene under a nitrogen atomosphere. Ethyl Hantzsch ester **4b** was taken as reducing agent.

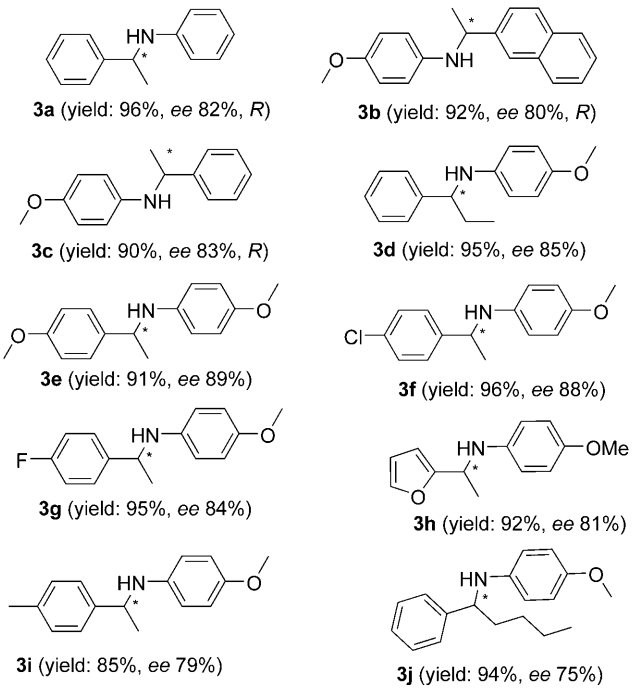
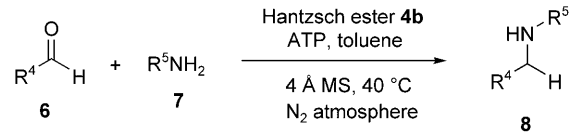


Figure 4. Scope of ADP-catalyzed reductive amination of ketones.



Scheme 2. Biomimetic reductive amination of aldehydes.

Table 4. ADP-catalyzed reductive amination of aldehydes.[a]

Entry	R^4	R^5	Product	Yield [%][b]
1	C_6H_5	4-NO_2-C_6H_4	**8a**	91
2	$C_6H_4CH{=}CH$	C_6H_5	**8b**	93
3	C_6H_5	C_6H_5	**8c**	94
4	2-furyl	C_6H_5	**8d**	95
5	4-OCH_3-C_6H_4	4-NO_2-C_6H_4	**8e**	92
6	C_6H_5	$C_6H_5CH_2$	**8f**	92
7	4-Cl-C_6H_4	C_6H_5	**8g**	94
8	3-OCH_3-C_6H_4	C_6H_5	**8h**	93
9	*n*-pentyl	C_6H_5	**8i**	91
10	3-methylbutyl	4-F-C_6H_4	**8j**	96

[a] *Reaction conditions:* aldehyde (1 mmol), amine (1 mmol), ethyl Hantzsch ester (1.1 mmol), ADP (2 mol%), toluene (5 m), 30 °C, N_2 atomosphere.
[b] Isolated yield.

Conclusions

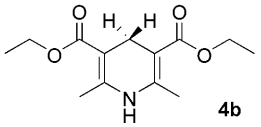
In conclusion, for the first time we have demonstrated that a single nucleotide (adenosine 5′-diphosphate) is able to catalyze the direct asymmetric reductive amination of carbonyl compounds using a Hantzsch ester as transfer hydrogenating agent, resulting in structurally diverse amines. The process is a real mimic of NADH reduction in biological system in terms of selection of the hydrogen transfer agent as well as catalyst. Besides this, one major advantage of using nucleotides as catalyst is that they are commercially available. This fascinating single nucleotide catalysis have an immense impact in many fields of science such as chemistry, biochemistry and even in prebiotic studies, especially the RNA world and DNA world hypothesis for understanding the origin of life.

Experimental Section

General Information

Unless otherwise specified all the reagents and catalysts were purchased from Sigma–Aldrich and were used without further any purification. The common and HPLC grade solvents were purchased from Ranbaxy. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Molecular sieves (4 Å) were activated by flame under vacuum and stored at 180 °C. Chromatographic purification of products was accomplished using flash chromatography on 230–400 mesh silica gel. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates visualized under UV light, iodine or $KMnO_4$ staining. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-300 spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (*J*) are in Hz. IR spectra were recorded on an FT-IR spectrophotometer type Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra (ESI MS) were obtained on a Micromass Quattro II instrument. High-performance liquid chromatography (HPLC) was performed on Hewlett–Packard 1100 Series chromatographs using a Daicel Chiracel OD-H column (25 cm) and equivalent guard column (5 cm) and HPLC data for compounds **3a**–**3i** are provided in the Supporting Information. The absolute configurations were determined by comparing the sign of optical rotation with values form the literature. Optical rotations were measured on a Rudolf Autopol III polarimeter, and $[\alpha]_D^t$ values are reported in $10^{-1}\,dg\,cm^2\,g^{-1}$; concentration (*c*) is in g/100 mL.

Typical Experimental Procedure for the synthesis of Hantzsch Ester (4b)



4b

In a 50-mL round-bottom flask, 37% aqueous formaldehyde (0.81 mL, 10 mmol), ethyl acetoacetate (2.60 g, 20 mmol), ammonium acetate (0.77 g, 10 mmol), PTSA (0.038 g, 20 mol%) and 15 mL methanol were charged. The resulting mixture was stirred at room temperature (4 h) until the reaction was complete (TLC). After the completion of reaction, the solvent was evaporated to yield a crude product. Pure ethyl Hantzsch ester **4b** was obtained by crystallization of the crude product from methanol; yield: 2.38 g (94%). ESI-MS: $m/z = 254$ $(M+H)^+$; ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 1.25$ (t, $J = 7.1$ Hz, 6H), 2.12 (s, 6H), 4.15 (q, $J = 7.1$ Hz, 4H), 3.19 (s, 2H), 5.20 (bs, 1H).

Typical Experimental Procedure for Reductive Amination of Aldehydes/Ketones

A solution of acetophenone **1a** (0.120 g, 1.00 mmol) and aniline **2a** (0.093 g, 1.00 mmol) in 5 mL of dry toluene was treated with the Hantzsch ester **4b** (0.280 g, 1.10 mmol), adenosine 5′-diphosphate **5e** (0.0086 g, 0.02 mmol) and 4 Å molecular sieve (1.0 g). The mixture was stirred 2 days at 30 °C under nitrogen. After filtration over celite, the solvent was evaporated and the residue purified by flash chromatography on silica gel (hexane/ethyl acetate = 95:5) to give **3a**; yield: 96%. The enantiomeric excess was measured by using HPLC with a chiral column. The absolute configurations determined by comparing the sign of optical rotation with values from the literature.

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