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Quantification of the hydride donor abilities of NADH, NADPH, and BH_3CN^- in water†

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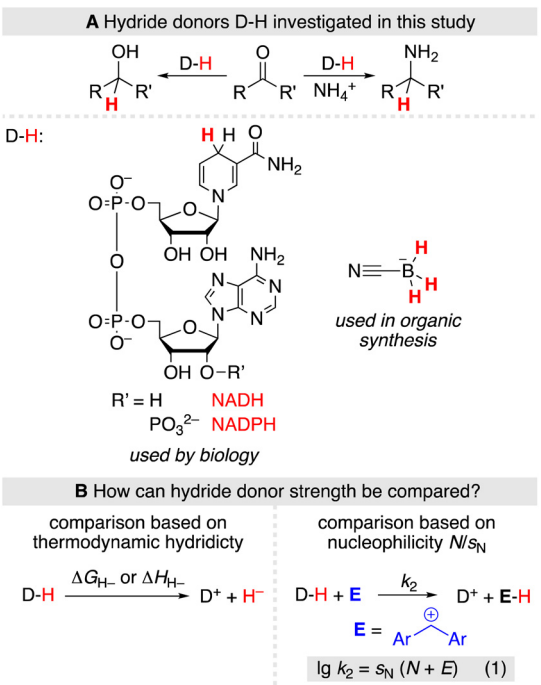
The nucleophilic reactivities of the hydride donors NADH, NADPH, and BH_3CN^- in water were quantified using kinetic measurements with benzhydrylium ions as reference electrophiles. All three hydride donors were found to possess almost identical nucleophilic reactivities, providing a potential explanation for why they are involved in similar transformations in biochemistry and organic synthesis, respectively.

NADH, NADPH, and BH_3CN^- are the hydride donors of choice for reduction and reductive amination reactions in aqueous solutions in biochemistry and organic chemistry, respectively (Scheme 1A).[1,2] However, despite their similar reactivity profiles, no study has yet directly compared their hydride donor abilities experimentally.

The strength of hydride donors can be compared based on thermodynamic hydricities (ΔG_{H-} and ΔH_{H-}), which are derived from equilibrium or calorimetric measurements and combined within thermodynamic cycles (Scheme 1B, left).[3] Alternatively, hydride donor strength can be quantified based on the nucleophilicity parameters popularized by Mayr and coworkers using a linear-free energy relationship (Scheme 1B, right, eqn (1))[4] which has been applied to characterize a variety of hydride donors.[5] Eqn (1) allows to predict absolute rate constants k_2 from the solvent-dependent nucleophilicity parameter N and susceptibility s_N as well as the solvent-independent electrophilicity parameter E.

The choice of methods for an experimental comparison of the hydride donor strength of NAD(P)H and BH_3CN^- is delicate due to the chemically vastly different nature of both species. Thermochemical methods have been extensively applied to compare the hydride donor strength of various NADH analogues, typically in acetonitrile solutions.[3,6] However, due to the instability of BH_2CN,[7] the oxidation product of BH_3CN^-, equilibrium studies are hampered. The only tentative thermochemical comparison of the hydride donor strength of BH_3CN^- with NADPH thus comes from quantum-chemically calculated enthalpic hydricities considering acetonitrile solvation by an implicit solvation model (ΔH_{H-} (NADPH) = 77.1 kcal mol^{-1}, ΔH_{H-} (BH_3CN^-) = 75.3 kcal mol^{-1}).[3,8]

In contrast, the application of kinetic methods for determining hydride donor strength has fewer experimental limit-



Scheme 1 (A) Reduction and reductive amination reactions enabled by NAD(P)H and BH_3CN^-. (B) Methods for quantification of hydride donor strength.

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ations. As such, they do not have specific solvent requirements, nor are they limited by the stability of the products. However, using kinetic methods, the nucleophilicity parameter N for the BH_3CN^- anion in water was previously only approximated from literature data of its reaction with two triarylcarbenium ions,[5a,9] using an estimated s_N parameter. Likewise, some dihydropyridines have previously been studied by Mayr and Richter in organic solvents and aqueous mixtures,[5b] but the nucleophilic reactivities of NADH and NADPH itself remain unknown. In this work, we now set out to determine the nucleophilic reactivity of NADH, NADPH, and BH_3CN^- in aqueous solution using a consistent set of benzhydrylium ions as reference electrophiles.[10]

Initially, the reaction products were investigated to verify that benzhydrylium ions **E** react with both NADH and BH_3CN^- in the desired hydride transfer and not a side reaction (Scheme 2). When mixing equimolar amounts of the benzhydrylium ion **E6** with NADH or analogously $NaBH_3CN$ in a D_2O/CD_3CN mixture, rapid decolorization of the carbocation was observed. ^1H and $^1H,^{13}C$-HSQC NMR analysis of the reaction products with both reductants indicated clean hydride transfer to yield the diarylmethane **E6**-H (see the ESI for the spectroscopic analysis†). In the case of NADH, oxidation to NAD^+ occurred, whereas in the case of $NaBH_3CN$, oxidation to boric acid and borate was observed, which are formed due to the hydrolysis of BH_2CN (see the ESI pp. S4–S7†).[7]

Having established the identity of the reactions of **E** with both hydride donors, kinetic studies were next performed. Nucleophilicity parameters were determined using a consistent set of amino-substituted benzhydrylium ions **E** as reference electrophiles (Fig. 1).[10] UV/Vis spectroscopy was used to study the kinetics of the reactions of hydride donors with the colored benzhydrylium ions **E1–E6** in an aqueous buffered solution at pH 7 and 20 °C. The disappearance of the color of the benzhydrylium ions **E** is due to three competing reactions (Fig. 1A): the reaction with either hydroxide or water, as well as the actual reaction of interest with the hydride donor. Due to the large excess of the hydride donor over the electrophiles (>10 equiv.), pseudo-first order conditions resulted, from which the pseudo-first order rate constants k_{obs} were determined by non-linear fitting (Fig. 1B). As the kinetics were measured in 0.05 M phosphate buffer at pH 7, the concentration of hydroxide and water are identical for all measurements.[11]

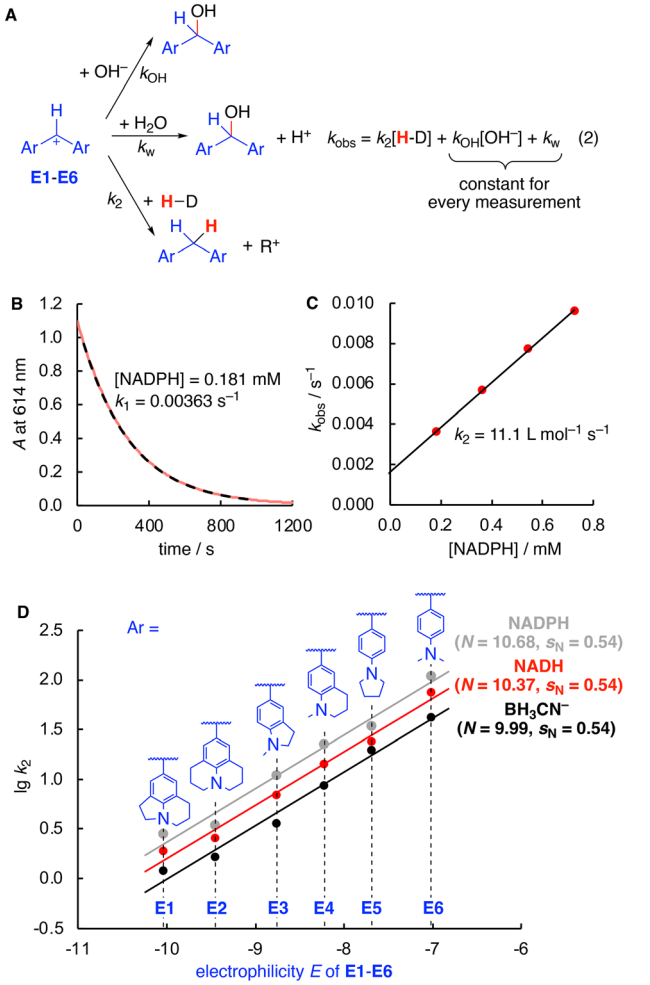
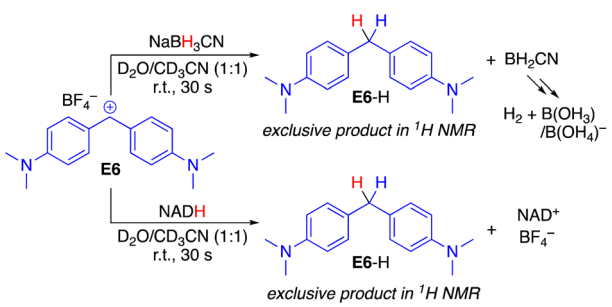


Fig. 1 (A) Competing reactions of hydride donors H-R with benzhydrylium ions **E** and kinetic analysis. (B) UV/Vis trace (red) for the reaction of **E3** with NADH and fitted (dashed) curve. (C) Correlation of k_{obs} *vs.* [NADPH] to determine the second order rate constant k_2 for the reaction of **E3** with NADPH. (D) Determination of nucleophilicity parameters N and s_N from the correlation of lg k_2 with the electrophilicity E of **E1–E6**.

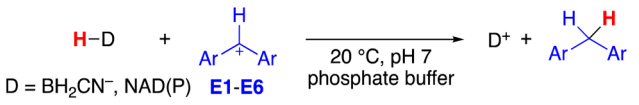


Scheme 2 Product analysis of the reaction of **E6** with NADH and $NaBH_3CN$.

Accordingly, a correlation of k_{obs} with the nucleophile concentration was found to be linear with the slope corresponding to k_2 and the intercept with the ordinate to $k_{OH}[OH^-] + k_w$ (Fig. 1A, eqn (2) and Fig. 1C). Finally, the correlations of lg k_2 (Table 1) and the electrophilicity parameters E of **E1–E6** afforded linear relationships, the slope of which corresponds to s_N. The nucleophilicity N is obtained from the intercept with the abscissa, as according to eqn (1) at lg k_2 = 0 the electrophilicity E equals the $-N$ (Fig. 1D).

As evident from both the comparison of the absolute rate constants in Table 1 as well as the nucleophilicity parameters in Fig. 1D, NADH, NADPH, and BH_3CN^- possess a very similar nucleophilic reactivity for hydride transfer toward carbenium ions with rate constants between BH_3CN^- and NADPH differing by factors of about 2.5 in all cases, except **E3** where

Table 1 Second-order rate constants k_2 for the reactions of **E1–E6** with $NaBH_3CN$, NADPH, and NADH at 20 °C in 0.05 M phosphate buffer (pH 7)



	E1	E2	E3	E4	E5	E6
E^a	−10.04	−9.45	−8.76	−8.22	−7.69	−7.02
	k_2/L mol^{-1} s^{-1},^{b}					
BH_3CN^-	1.20	1.66	3.59	8.58	19.6	42.1
NADH	1.89	2.57	6.96	14.4	24.0	75.3
NADPH	2.82	3.44	11.1	22.8	34.6	109

[a] Electrophilicity E from ref. 4. [b] For the errors of the individual rate constants, see the ESI.†

the difference is around a factor of 3. Despite large methodological differences (experimental *vs.* computational, water *vs.* MeCN, kinetic *vs.* thermochemical values), the observed similarity in kinetic hydride donor strength in water is in line with the similar enthalpic hydricities obtained computationally in MeCN.[3] Notably, NADPH is kinetically a slightly better hydride donor compared to NADH by approx. a factor of 1.5 in rate constants. This small but significant difference might be attributed to conformational differences of both species in solution due to the presence or absence of the phosphate group that can be involved in intramolecular hydrogen bonding.[12]

Having established the nucleophilic reactivity of NADH, NADPH and BH_3CN^-, a comparison with other previously investigated hydride donors now becomes possible (Fig. 2). The nucleophilic reactivities of NADH and NADPH are approximately one *N*-unit lower than that of the frequently employed analogue *N*-benzyl-1,4-dihydronicotineamide (BDNA, N = 11.36, s_N = 0.66), which was previously studied in a water-acetonitrile mixture due to its limited solubility in pure water.[5b] The close similarity of the nucleophilic reactivities of BDNA and NADH, which differ only by the presence of a benzyl group

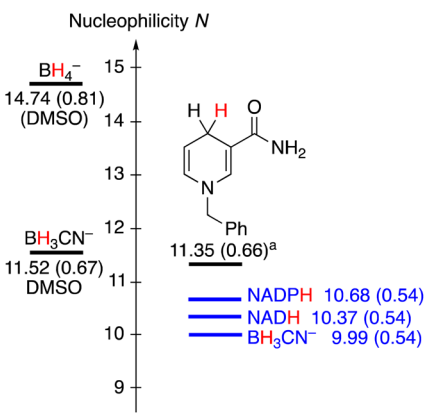


Fig. 2 Comparison of the nucleophilicity parameters N of different hydride donors (s_N parameters in brackets). [a] Studied in 90% water/10% acetonitrile.

versus an adenine dinucleotide, illustrates that substitution at nitrogen does not largely alter the reactivity of the dihydronicotinamide. Due to their rapid hydrolysis, a comparison of the reactivity of BH_3CN^- with other borohydrides is difficult. However, previously the nucleophilicity of BH_3CN^- was reported in DMSO, where it was found to be around 1.5 *N* units higher.

On the one hand, the very similar nucleophilicity of NAD(P)H and BH_3CN^- that we have observed in our experiments is surprising given the large reactivity range of hydride donors previously characterized in organic solvents.[5a,13] On the other hand, this observation might quantitatively rationalize why both NAD(P)H and BH_3CN^- display similar reactivity in the contexts of biochemistry and organic synthesis, respectively, enabling reductions of C=O and C=N functionalities.[1,2,14] Future research will show if the close similarity in the nucleophilicity of NADH and BH_3CN^- found toward reference carbenium ions like **E** still holds with neutral electrophiles like carbonyl compounds, where solvation effects on the electrophile as well as acid/base catalysis might become relevant.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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