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Optimizing the reversibility of hydrazone formation for dynamic combinatorial chemistry

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Received (in Cambridge, UK) 22nd November 2002, Accepted 7th March 2003
First published as an Advance Article on the web 12th March 2003

Hydrazones from hydrazines bearing electron withdrawing groups, and aromatic or aliphatic aldehydes form and hydrolyse rapidly in water at neutral pH.

For the purpose of synthetic chemistry, reversible reactions generally inspire scepticism and are often left aside when they cannot be improved to limit product instability. Yet, interest for reversible reactions has been rising during the past few years, motivated by the development of dynamic combinatorial chemistry (DCC). This technique takes advantage of the continuous variation of the proportions of products formed *reversibly* from a set of precursors.[1] For example, the presence of a target substance in a dynamic mixture may result in an increase of the mole fraction of the products which bind to it, thus facilitating their identification.

For pharmaceutical applications of DCC, dynamic libraries are directly mixed with biological targets.[2] Thus, useful reactions should be reversible under physiological conditions and should not interfere with biological functional groups.[1,3] Several reactions have been proposed for this purpose, but very few fulfil these strict requirements. Disulfide exchange[3b,3d,4] for example may lead to cross-reaction with cysteine residues of the target. Olefin metathesis is promising but has not yet been optimized in water in the presence of biological targets.[5] Examples of boronate ester formation[5,6] and coordination to transition metals[7] have also been presented. In this communication, we show that one of the most typical reversible reactions, namely hydrazone formation, can be optimized for DCC in neutral water when using a particular class of hydrazines.

Imines from aliphatic amines and aromatic or aliphatic aldehydes form and hydrolyse rapidly, but are of low stability in neutral water. At pH 8 or below, most aliphatic amines are predominantly protonated and the equilibrium is shifted in the direction of hydrolysis to the point that the imines are hardly detectable. This problem has usually been circumvented by reducing this small percentage of imine with $NaBH_3CN$ and assuming that the proportion of amine products reflect the proportion of imines.[3a,3e,8] On the other hand, hydrazones, acyl hydrazones, semicarbazones and oximes formed by hydrazines, hydrazides, semicarbazides or hydroxylamines are stable even at low pH. However, they prove to be kinetically inert under neutral conditions. The mesomeric effects which lead to their high stability decrease the electrophilicity of the imine, slowing down hydrolysis and transimination.[9] Only under acidic conditions (at pH 4 or below) or at high temperatures do hydrolysis and transimination reach significant rates.[10]

We speculated that hydrazine derivatives bearing electron withdrawing (EWD) groups may form hydrazones sufficiently activated to be hydrolysed at neutral pH (Fig. 1).† Compounds **1–6**, which bear EWD groups of various strengths were selected to test this hypothesis. Several methods give access to such compounds and in principle, a series of similar hydrazines could easily be prepared. Some are available from commercial sources (**3**, **4**). Others can be synthesised by acylation of hydrazine precursors (**1**, **2**),[11] by aromatic nucleophilic substitution (**5**),[12] or by amination of endocyclic nitrogens (**6**).[13] The effect of the electron withdrawing substituents is illustrated by the low basicity of most of these amino groups (Table 1).

When these compounds are mixed with a model aliphatic aldehyde (isobutyraldehyde) or a model aromatic aldehyde (4-carboxy-benzaldehyde) in water under close to neutral conditions ($6 < pH < 8$), hydrazone formation takes place within minutes. The ^1H NMR spectra show a rapid decrease of the signals of the aldehyde and, when present, of its hydrate,‡ with the concomitant appearance of the signals of the hydrazones. The hemiaminals are sometimes observed but they remain minor species. At high hydrazine concentration, an equilibrium with the aminal is observed. Integration of these signals at various concentrations allows the calculation of the equilibrium constants of hydrazone formation reported in Table 1. These values range over almost three orders of magnitude and do not correlate with the pK_{BH+}'s of the hydrazines.† In most cases, the constant of hydrazone formation of a given hydrazine is several times higher with the aromatic aldehyde than with the aliphatic aldehyde. Within each series, the most stable hydrazone is formed from the amine bearing a single EWD group (**1**), followed by the imine formed from the amine bearing one

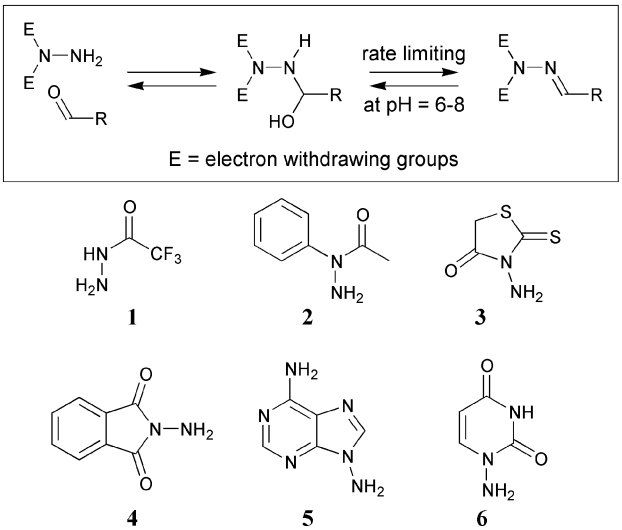

Fig. 1 Structures of amines **1–6**.

Table 1 Values of the pK_{BH+}'s of **1–6** and of their equilibrium constants of hydrazone formation with an aliphatic and an aromatic aldehyde.§

	pK_{BH+}[a]	K/L mol^{-1}[b] Isobutyraldehyde	K/L mol^{-1}[b] 4-Carboxybenzaldehyde
1	5.6	4700	29000
2	3.0	1500	9900
3	6.0	440	2500
4	4.1	470	870
5	3.9[c]	164	—[d]
6	1.9	33	44

[a] In H_2O. [b] In 9 : 1 H_2O : D_2O at pH 7 (20 mM phosphate buffer). The effect of 10% D_2O on the pH was neglected. [c] This pK_{BH+} is probably that of the amine in position 6, and not that of the amine in position 9. [d] This value could not be measured due to the precipitation of the product.

DOI: 10.1039/b211645f

carbonyl EWD group and one phenyl ring (**2**). Amines having two (thio)-carbonyl EWD groups either directly connected (**3**, **4**), or conjugated (**5**, **6**) give rise to significantly less stable hydrazones.

The reversibility of these condensations is easily demonstrated upon diluting a solution of hydrazone and observing its hydrolysis into the original hydrazine and aldehyde. Equilibria are reached within minutes at pH 6 and in less than an hour at pH 8. An illustration of this reversibility and of the potential of these reactions for applications in DCC is given in Fig. 2. On the 400 MHz ^{1}H NMR spectrum of a mixture of isobutyraldehyde and of hydrazines **2–6** (Fig. 2a), the signals of most $CH(CH_3)_2$ protons do not overlap and can be assigned to the five hydrazones, the aldehyde and its hydrate according to their chemical shifts when only one amine is present. Minor signals presumably arise from the 15 possible aminals and 5 hemiaminals. Upon addition of an excess of **1** (Fig. 2b) the equilibria are shifted in favour of the hydrazone, the hemiaminal and the aminal this hydrazide forms with isobutyraldehyde.

The compatibility of hydrazones formed by **1–6** with biological functions, and in particular with N-terminal amino groups and lysine residues of peptides and proteins was demonstrated by showing that the equilibrium in Fig. 2a is unaffected by the addition of 10 equivalents (with respect to isobutyraldehyde) of lysine hydrochloride.

In conclusion, a delicate combination of hydrazine nitrogens and EWD substituents allows the rapid formation and hydrolysis of some hydrazone derivatives under neutral conditions. That equilibrium constants vary significantly with the nature of the substituents may strongly bias dynamic combinatorial mixtures in favour of the most stable products. To some extent, this may be compensated by tuning the initial proportion of amines accordingly, as in the experiments depicted in Fig. 2. Alternatively, one may prefer to build libraries from hydrazines having similar reactivities, and to keep the groups by which they differ at positions remote from the reactive site. In this respect, the examples presented here are aimed at widening the class of compounds useful for DCC applications and do not represent an ensemble of readily compatible hydrazines. Using these findings, we are currently developing a DCC of nucleic acids involving aminobases such as 1-aminouracil **6** and 9-aminoadenine **5**.

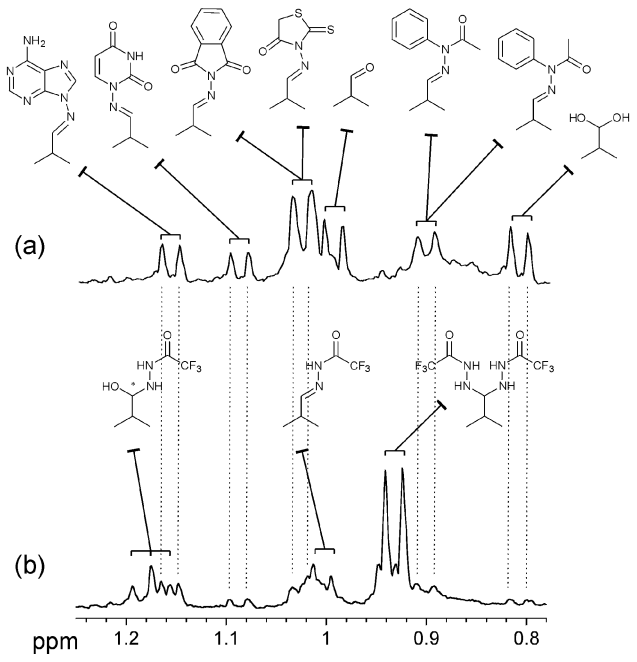

Fig. 2 Part of 400 MHz ^{1}H NMR spectra of mixtures of hydrazines and isobutyraldehyde in $H_2O:D_2O$ at pH 6 (20 mM phosphate buffer§) showing the resonances of the $CH(CH_3)_2$ protons. The upper spectrum is obtained upon mixing hydrazines **2** (0.33 mM), **3** (1 mM), **4** (1 mM), **5** (2.5 mM), **6** (12.5 mM) and isobutyraldehyde (1 mM). The lower spectrum is obtained upon adding **1** (20 mM) to the above mixture.

This work was supported by the CNRS and by the Ecole Polytechnique (predoctoral fellowship to RN). We thank Dr C. Crean for proof reading the manuscript.

Notes and references

† The hydrolysis of hydrazones is rate limited by the hydration step above pH 6 (Fig. 1). Studies on semicarbazones, acethydrazones and p-toluenesulfonylhydrazones[14] show that this rate increases with the EWD strength of nitrogen substituents, presumably because of an enhanced electrophilicity of the hydrazone. Conversely, hydrazone formation is rate limited by the dehydration step above pH 6. At pH 6–7, this step is acid catalysed and is not affected by the EWD strength of hydrazine substituents. Above pH 8, this step is faster for strongly EWD groups which promote a base catalysed pathway. The amination/deamination steps are also expected to depend upon the strength of EWD groups. These steps are not rate determining but they directly influence the overall equilibrium constant, which thus cannot be predicted in an simple way.

§ We observed that high phosphate buffer concentration promotes aldehyde Cannizzaro disproportionation.

‡ Isobutyraldehyde is 45% hydrated in neutral water; the hydrate of 4-carboxy-benzaldehyde was not detected.

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