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Synthesis, structural analysis and application of a series of solid-state fluorochromes—aryl hydrazones of 4-hydrazino-*N*-hexyl-1,8-naphthalimide

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ABSTRACT

The development of red solid-state fluorochromes is important for different applications. The influence of the electronic effects of substituents on the chemical shifts in the ^{1}H NMR spectra and solid-state fluorescent properties of aryl hydrazones of 4-hydrazino-*N*-hexyl-1,8-naphthalimide is evaluated. The main fragmentation pathway of hydrazones is determined using electrospray ionization mass spectrometry and high resolution MS/MS. A possible application of these fluorochromes for the in situ imaging of enzyme activities is presented.

1. Introduction

1,8-Naphthalimides have been widely studied due to their photophysical properties. These are one of the most important building blocks for the synthesis of efficient fluorescent materials showing strong fluorescence, large quantum yields and sizable Stokes shifts. Naphthalimides are widely used as colourants in the polymer industry,[1,2] laser dyes,[1,3,4] chemosensors[5,6] and fluorescence probes for biomedical purposes, including fluorescence cell makers[7] and anti-cancer agents.[8] Efficient solid-state emission of organic materials is essential for optoelectronic devices, and the molecular design and development of novel fluorochromes that emit visible light in the solid state with high efficiency are strongly desired for different applications.[9] Though many organic compounds show red fluorescence in solution, fewer compounds fluoresce in the solid state due to intermolecular interactions in the condensed states.[10] Gan and co-workers[11,12] have synthesized several aryl hydrazones of 4-hydrazino-*N*-butyl-1,8-naphthalimide and studied their electronic and fluorescence spectra for application as materials for organic light-emitting diodes (OLEDs). The authors have mentioned that materials for green and blue OLEDs are available, whereas red fluorescent materials of high efficiency are not common. Photophysical properties of the other synthesized hydrazones of 1,8-naphthalimide have been investigated,[13,14] including those covalently bound to single-wall carbon nanotubes[15] and components of copolymers.[16–18] Naphthalimide hydrazones based on vanillin[19] and salicylaldehyde[20] were designed and synthesized as highly selective fluorescent probes for Ag^+ and Cu^{2+}, respectively.

Correlation analysis, the study of the relationships between the parameters known as substituent constants and various directly measurable quantities, plays an important role in physical organic chemistry for the description of the electronic properties of molecules. Free energy relationships have been observed for a number of spectroscopic properties,[21] including mass spectrometry.[21,22] Correlation analyses have also been applied to demonstrate that dependencies exist between substituent effect and chemical shifts in nuclear magnetic resonance spectroscopy.[23,24] So, the established relationships are of substantial value for the prediction of NMR properties.[25]

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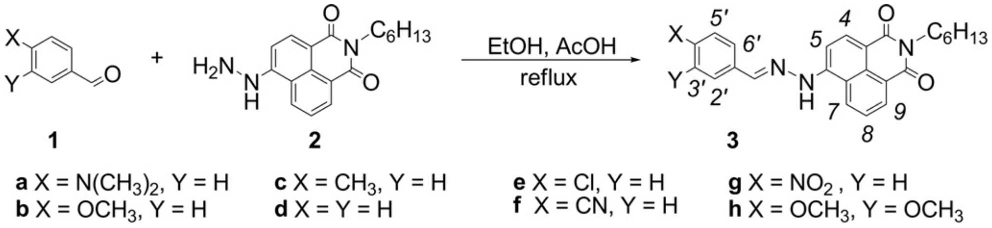
Fluorescence-based imaging of enzyme activity in vitro and in vivo promises many applications from elucidation of the enzyme function and mechanism, to better disease detection and monitoring.[26–28] The possibility for application of 1,8-naphthalimide hydrazones for fluorescent histochemical localization of enzymes—markers for malignant or other diseases has been explored. Members of our group have developed methods for fluorescent histochemical localization of dipeptidyl peptidase IV, tripeptidyl peptidase I[29] and gamma-glutamyl transpeptidase.[30] The enzyme hydrolyzes the corresponding synthetic substrate to yield free 4-hydrazino-*N*-hexyl-1,8-naphthalimide, which couples simultaneously with piperonal and gives a water-insoluble fluorescent hydrazone precipitating on the sites of enzyme activity and marking them.

In the present work, a series of substituted aryl hydrazones of 4-hydrazino-*N*-hexyl-1,8-naphthalimide were synthesized and characterized by nuclear magnetic resonance and mass spectrometry. The effects of substituents at the benzene ring on the ^{1}H NMR chemical shifts have been studied using models, based on mono and dual substituent parameter relationship. Electrospray ionization mass spectrometry (ESI-MS) and high resolution MS/MS have been used in order to explore the general and specific fragmentation of the compounds studied. The substituent effect on the solid-state fluorescence properties has been rationalized using Hammett type correlation, and the possibilities for application of the series of hydrazones to develop new techniques for fluorescent imaging of enzyme activity have been explored.

2. Results and discussion

2.1. Synthesis

The most common method for the preparation of *N*-substituted hydrazones is the reaction of aldehydes or ketones with *N*-alkyl or *N*-aryl hydrazines.[31] Usually the reaction proceeds in ethanol solution under reflux for a few hours. *N*-Aryl hydrazones and especially those substituted with electron-withdrawing groups are stable to acids and can be prepared under acidic conditions. In a previous work[29] we described the synthesis of the piperonal hydrazone of 4-hydrazino-*N*-hexyl-1,8-naphthalimide in the presence of acetic acid. In the present study this approach is successfully applied for the preparation of a series of substituted aryl hydrazones of *N*-hexyl-1,8-naphthalimide (Scheme 1).



Scheme 1. Synthesis of hydrazones **3a**–**h**.

The reactions between aldehydes **1a**–**1h** and 4-hydrazino-1,8-naphthalimide (**2**) were carried out under reflux in ethanol solution in the presence of acetic acid for several minutes. The reaction time depends on the electronic effects of the substituents. The reaction between *p*-nitrobenzaldehyde and **2** was carried out for 5 min, whereas the interaction with *p*-dimethylaminobenzaldehyde was completed in 15 min. Hydrazones **3b**–**3h** precipitated on cooling; compound **3g** crystallized partially during the reaction. For the isolation of hydrazone **3a** a few drops of water were added to the solution and the resulting solid was filtered. The crude crystalline compounds were purified by column chromatography.

2.2. Effect of phenyl substituents on the proton chemical shift

According to the principle of substituent-induced changes in the chemical shift (SCS), the variations in the ^{1}H NMR spectra of hydrazones **3a**–**3g** were correlated using different linear free energy relationship (LFER) models, based on the mono and dual substituent parameters (σ_p, σ_p^+, F, R and R^+). The protons chemical shifts (δ_H), which correlate with the Hammett type substituent constants are shown in Table 1. Good linear correlation (n=7, r=0.9865) between the chemical shift of the amine hydrogen (N*H*) and σ_p substituent constants was observed for this series (Fig. 1). The slope ρ is 0.34±0.02. A similar dependence (ρ=0.65±0.03) was observed in the series of *N*-phenylhydrazones of *p*-substituted benzaldehydes.[32] In these compounds the sensitivity to the electronic effects of substituents is double that of the established for *N*-hexyl-1,8-naphthalimide hydrazones (**3a**–**3g**).

Table 1
NMR chemical shifts (δ_H) for two types of hydrogen atoms in the series of *p*-substituted hydrazones **3a**–**3g** and Hammett substituent constants correlating with the respective chemical shifts

Compound		δ_H[a] (ppm)		Hammett substituent constants[b]		
	X	N*H*	H-5	σ_p	σ_p^+	R
3a	N(CH$_3$)$_2$	11.25	7.65	−0.83	−1.70	−0.98
3b	OCH$_3$	11.39	7.70	−0.27	−0.78	−0.56
3c	CH$_3$	11.46	7.74	−0.17	−0.31	−0.18
3d	H	11.52	7.77	0.00	0.00	0.00
3e	Cl	11.54	7.73	0.23	0.11	−0.19
3f	CN	11.72	7.78	0.66	0.66	0.15
3g	NO$_2$	11.82	7.84	0.78	0.79	0.13

[a] Solvent—CD$_3$SOCD$_3$.
[b] Taken from Ref. 33.

The observed LFER in the two series of hydrazones shows that shielding/deshielding of the N*H* hydrogen atoms is determined from the nitrogen atom shielding/deshielding caused by the electronic effect of the aromatic ring substituents. The excellent linear correlation (r=0.9957) obtained between δ_H and M06/6-31+G(d) computed Hirshfeld charges on the amine nitrogen for *N*-methyl analogues of **3a**–**3g**[14] also supports this assumption (Fig. S1 in the Supplementary data).

Hammett correlations of ^{15}N NMR chemical shifts of the amine and imine type nitrogen atoms (δ_N) with σ_p constants in the series of *N*-phenyl[34] and *N*-alkyl-*N*-(2-hydroxycyclohexyl)[35] hydrazones of *p*-substituted benzaldehydes have also been determined. In both cases ρ is negative. This indicates that electron-withdrawing (EW) substituents cause deshielding of the nitrogen atoms, whereas electron-donating (ED) substituents cause shielding effects. The resonance structure **4** has the main contribution[34–36] to the electron density distribution in hydrazones (Scheme 2). Electron-withdrawing substituents increase the stability of this structure[35,37,38] by field and resonance (form **5**) effects. In contrast, ED substituents increase the stability of resonance forms **6** and **7** (Scheme 2). To estimate the influence of the field and resonance