A novel NMR method for screening soluble compound libraries

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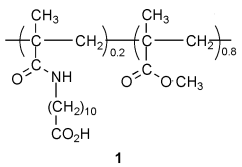
The use of high resolution diffusion-ordered NMR spectroscopy (HR-DOSY) to detect association between a soluble polymer and different components of small libraries of soluble compounds is illustrated for libraries binding respectively to weakly acidic and weakly basic polymers.

In recent years there has been great interest in combinatorial chemistry,[1–3] most commonly involving multi-step polymer-supported syntheses to give *polymer-supported libraries* containing from tens to millions of polymer-supported species. An alternative approach is to synthesise *soluble libraries*[2,4,5] which, to simplify the procedures, may be synthesised with the assistance of polymer-supported reactants or scavengers. Screening of libraries is commonly conducted for biological activity, but may also, for example, be for catalytic activity[6,7] or recognition ability.[8,9]

We report here an NMR method for screening soluble libraries of up to 20–30 components for recognition properties. One possible application would be in refining the design of host molecules for host–guest chemical sensors. The method is based on High Resolution Diffusion-Ordered SpectroscopY (HR-DOSY), a form of multi-dimensional spectroscopy in which signals are dispersed in an extra dimension according to the diffusion coefficient.[10–12]

Diffusion coefficients of organic compounds in common solvents are typically of the order of 10^{-9} m^2 s^{-1}; macromolecules diffuse much more slowly. The basis of the method proposed is to link the species to be recognised (*e.g.* a 'guest') to a suitable soluble polymer. When this polymer is added to a soluble library (of potential 'hosts'), the rates of diffusion of compounds in the library which, on the NMR timescale, bind rapidly and reversibly to the functionalised polymer, will decrease by an amount dependent on the binding constant. The method is related to the DECODES (DOSY-TOCSY) method proposed by Lin *et al*,[13–16] but it differs in the use of a polymer-bound ligand, maximising sensitivity to binding, and in the use of HR-DOSY, which can improve the diffusion resolution and allows the extraction of isolated subspectra for individual components.

As a trial of the method we investigated the interactions between the polymer-supported weak acid **1** and a simulated



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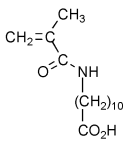

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library containing 11 commercially available natural products or related compounds (see Table 1). Polymer **1** was prepared by a free radical initiated copolymerisation of monomer **2**[17] and methyl methacrylate (mole ratio, 1:5), to give a copolymer soluble in CD_3OD with no ^{1}H-NMR signals above 4.3 ppm. The polymer had a number average molecular weight M_n of 16500 and a weight average molecular weight M_w of 61500 by gel permeation chromatography (GPC) relative to polystyrene standards. Because the diffusion coefficient of the polymer is small compared with those of the members of the soluble library, such a polydispersity does not affect the estimation of binding constants significantly. A solution in CD_3OD (2.9 mg ml^{-1}) showed a diffusion coefficient by HR-DOSY of 1.0×10^{-10} m^2 s^{-1}. The DOSY spectra of the simulated library alone, and in the presence of the polymer **1**, are shown in Figs. 1 and 2. It is only necessary for there to be one well-resolved characteristic signal for a given component for the diffusion coefficient to be clearly identified. The diffusion coefficients D_f and D_m of the components in the free mixture and with the polymer present are summarised in Table 1.

It is clear from the spectra of Figs. 1 and 2 that the rate of diffusion of hydroquinine **3**, (arrowed signals) is affected strongly by the presence of the polymer, whereas any effect on the other components is much smaller. The small increase in solution viscosity caused by the polymer may be corrected for by calculating estimated diffusion coefficients $D'_m = D_m D_f(\text{ref})/D_m(\text{ref})$, where D_f(ref) and D_m(ref) are the respective diffusion coefficients for a reference compound (in this case residual OH in the solvent) not significantly affected by binding to the polymer. In Table 1, values of D'_m were calculated using the measured MeOH OH diffusion coefficients for the two samples; D'_p, the corrected diffusion coefficient for the dilute free polymer, remained at 1.0×10^{-10} m^2 s^{-1}. Using a simple two-site model, the bound fraction F of a given component is given by $F = (D_f - D'_m)/(D_f - D'_p)$ from which the association constant K between the polymer and that component may be calculated by $K = F/(1 - F)(c_p - Fc)$, where c and c_p are the total concentrations of the component and of the functional polymer repeat unit.

The values of F in Table 1 show clear association between hydroquinine **3** and the polymer; the apparent association constant K is approximately 11 M^{-1}. The spectrum of the

Table 1 Summary of DOSY measurements for library A[a]

Compound	c/mM	$D_f/10^{-10}$ m^2 s^{-1}	$D_m/10^{-10}$ m^2 s^{-1}[b]	$D'_m/10^{-10}$ m^2 s^{-1}[c]	F
Cholest-5-en-3-one	7.8	8.1	7.3	7.8	0.04
(*R*)-(+)-Citronellal	32.4	13.2	12.0	12.7	0.04
(*S*)-(–)-Citronellol[d]	32.0	9.5	8.8	9.3	0.03
Hydroquinine (**3**)	15.6	6.3	3.7	4.0	0.44
Methyl nicotinate	27.7	14.0	12.6	13.3	0.05
N-Methylnicotinamide	24.3	10.3	9.6	10.1	0.01
(1*S*)-(–)-β-Pinene	29.4	13.9	13.1	13.9	0.00
1,6-Dehydopregnenolone acetate	11.8	8.1	7.2	7.7	0.05
Progesterone	15.6	8.3	7.8	8.2	0.01
o-Vanillin	18.1	12.9	12.1	12.9	0.00
Estrone	9.3	7.8	7.0	7.4	0.05
Methanol (solvent)		18.9	17.8	(18.9)	(0.00)

[a] Experiments were carried out in CD_3OD at 20 °C nominal temperature on a Varian Unity 400 spectrometer using the BPPSTE pulse sequence,[12] with gradient levels from 1 to 20 G cm^{-1} and lasting approximately 30 min. Data were analysed as described previously,[11–12] but using explicit correction for field gradient non-uniformity.[19] [b] The concentration c_p of functional repeat units of the polymer was 77.5 mM. [c] Diffusion coefficients were corrected for the difference in viscosity between the solutions of the free library and the library with the polymer present (see text). [d] The signal at 5.1 ppm for which diffusion data are reported contains components from citronellol, citronellal, and higher molecular weight impurities.

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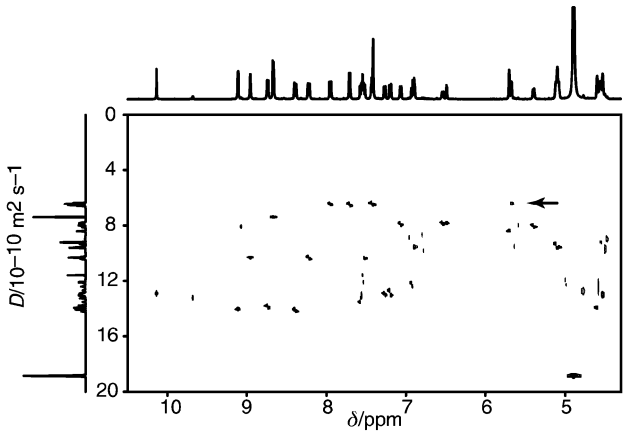


Fig. 1 HR-DOSY spectrum of the library of Table 1 in free CD_3OD solution, with (top) the normal ^1H spectrum and (side) the integral projection onto the diffusion axis.

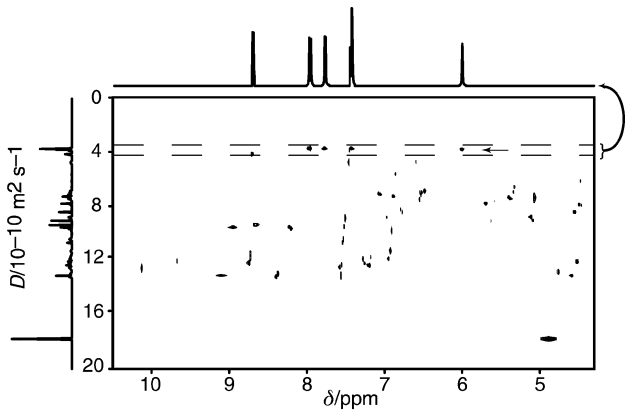
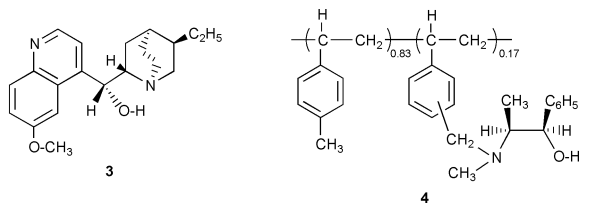


Fig. 2 HR-DOSY spectrum of the library of Table 1 and polymer **1** in CD_3OD, with (top) the integral projection onto the chemical shift axis of the region between the dotted lines, showing the subspectrum of the bound species, and (side) the integral projection onto the diffusion axis.



shifted hydroquinine **3** is shown on the top scale in Fig. 2. The changes in chemical shift for **3** between free solution and the polymer mixture show that the quinuclidine moiety in **3** protonates, causing association between the cation and the polyanionic polymer. No detectable change in either diffusion or shift is seen for methyl nicotinate and *N*-methylnicotinamide, both weaker bases than **3**.

In a second trial, the previously-described polymer **4**,[18] with M_n = 4900 and M_w = 9200 by GPC, was used to detect interactions with the β-amino-alcohol unit of (1*R*,2*S*)-*N*-benzylephedrine moieties. Polymer **4** showed a diffusion coefficient of 1.3×10^{-10} m^2 s^{-1} in $CDCl_3$ solution (2.6 mg ml^{-1}). Its interactions with a simulated library of 7 compounds were investigated, with the results summarised in Table 2. Here the only component to show significant binding is α-methoxyphenylacetic acid, which comes close to saturating the polymer binding sites and has an apparent association constant of several thousand M^{-1}. The specific nature of the binding is evidenced by the splitting of the α-proton and methyl signals of the racemic acid on binding to the chiral polymer.

Table 2 Summary of DOSY measurements for library B[a]

Compound	*c*/mM	$D_f/10^{-10}$ m^2 s^{-1}	$D_m/10^{-10}$ m^2 s^{-1}[b]	*F*
Cholest-5-en-3-one	21.3	7.5	7.4	0.02
(*R*)-(+)-Citronellal	21.4	12.2	12.4	−0.02
(*S*)-(–)-Citronellol	19.2	11.3	11.0	0.03
(1*S*)-(–)-β-Pinene	23.5	14.1	14.8	−0.06
1,6-Dehydopregnenolone acetate	21.6	7.8	7.8	0.00
Progesterone	20.0	7.9	8.5	−0.10
(±)-α-Methoxyphenylacetic acid	19.8	9.5	3.1	0.81

[a] Experimental conditions were as for Table 1. [b] The concentration c_p of functional repeat units of the polymer was 17.3 mM. No correction was made for changes in viscosity.

We are currently using the above method to explore interactions of amines, amides and peptides with functional macrocycles.

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