Tetrahedron Vol. 43, No. 17, pp. 4043 to 4050, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00

A QUANTITATIVE C-13 NMR STUDY OF THE RELATIVE NUCLEOPHILICITIES OF THE NITROGEN ATOMS OF PHENYLHYDRAZINE IN ITS REACTION WITH CHALCONE

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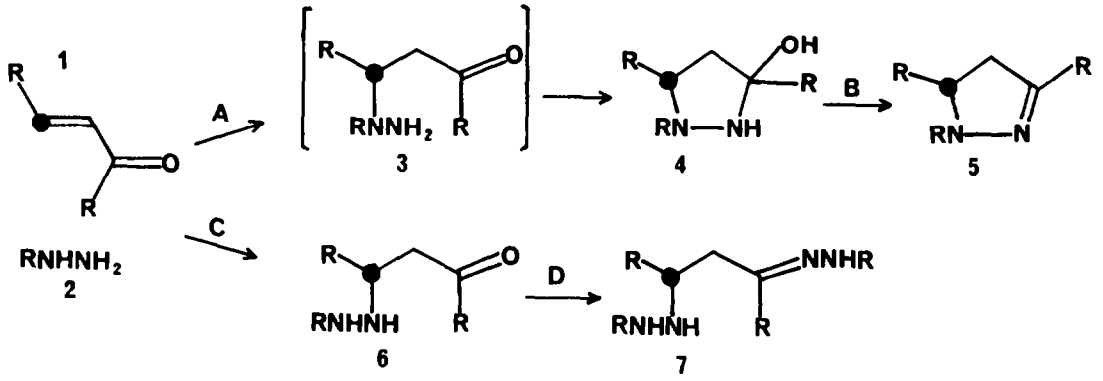
(*Received in UK 25 June* 1987)

Abstract: The reaction of chalcone with phenylhydrazine involves competitive 1,4-additions of the two nitrogen atoms in the latter. Base catalysis, both by phenylhydrazine and by 1,4-diazabicyclo[2,2,2]octane, is observed for reaction at N-2 but not for reaction at N-1.

NMR techniques are well suited for the study of complex organic reactions.[1] ^{1}H NMR has been applied extensively in quantitative work and flow techniques allow fairly fast reactions to be followed.[2] ^{13}C NMR has been used mainly in a semiquantitative manner with little quantitative work being reported.[3,4] Partly this is because of the relatively low inherent sensitivity of ^{13}C NMR but also because variable nuclear Overhauser enhancements (NOE) and long and variable longitudinal relaxation times (T_1) reduce the quantitative relability of the measurements, even if good signal to noise (S/N) can be achieved.[5] Use of compounds, selectively enriched with ^{13}C, in such experiments results in a huge increase in sensitivity (vital for good time resolution) and dramatically simplifies the spectra, a very important consideration when the reaction sequence is complex. While semiquantitative studies may often suffice to establish a reaction sequence, quantitative kinetic experiments are necessary if mechanistic information about the individual steps in the sequence is required.



Scheme 1: Products and intermediates observed by ^{13}C NMR in the reaction between **1** and **2** (R= Phenyl, ●=^{13}C, ^{13}C chemical shifts: **1** (145.9), **4** (69.1), **5** (65.5), **6** (61.6), **7** (63.6). **3** was not observed due to its rapid cyclisation.

The reaction[6] of chalcone (**1**) with phenylhydrazine (**2**) is a representative example of the most general and widely used synthetic route to 2-pyrazolines.[7] Mechanistic work, using polarography for analysis of the reaction mixtures, has been reported but it was incorrectly assumed that chalcone phenylhydrazone was an intermediate and that its cyclisation to give the product 1,3,5-triphenyl-2-pyrazoline (**5**) was fast.[8] This cyclisation is a 5-endo-trig process and is predicted to be most unfavourable.[9] A recent semiquantitative kinetic study by ^{13}C NMR, using both ^{13}C and ^{15}N labelling, has determined the reaction sequence (Scheme 1).[10] Note that the cyclisation step in this scheme is a 5-exo-trig process and therefore not expected to be unfavourable.[9] Protonation of phenylhydrazine is expected to take place at N-2 rather than N-1 and evidence has been presented which suggests that this is the case.[11] The semiquantitative study did not address the question as to how N-1 of phenylhydrazine is able to compete as a nucleophile with the presumably much more

basic N-2 and in order to answer this question a quantitative ^{13}C NMR kinetic study was undertaken. The most surprising result of this work is that base catalysis, both by phenylhydrazine and 1,4-diazabicyclo[2,2,2]octane (DABCO), was found to be much more important for 1,4-addition at N-2 (Scheme 1, process C) than for 1,4-addition at N-1 (Scheme 1, process A).

RESULTS

The reaction between **1** and **2** was studied in (1,1,1-$^{2}H_3$)methanol using (3-^{13}C)chalcone. Spectra were broad band proton decoupled and full NOE factors were assumed for the carbon nuclei of interest: this is reasonable because these carbons have directly bonded protons and are in adequately large molecules.[5] The NOE can of course be suppressed using gated decoupling but this can result in as much as a threefold loss of sensitivity,[5] increasing the time required to achieve a given S/N by up to an order of magnitude which can be critically limiting in a time-resolved experiment. A delay of 10 s between pulses was adequately long for this set of nuclei to prevent saturation. Lorentzian lineshapes were fitted to the spectral data using standard curve-fitting techniques,[12,13] allowing integrated intensities to be measured with considerably greater precision than the more commonly used numerical integration methods normally permit.[14]

Initially it was thought that the unexpectedly strong nucleophilicity of N-1 in this reaction might be a result of base catalysis of the 1,4-addition by another molecule of **2**. An experiment was performed to investigate this possibility. (3-^{13}C)Chalcone was made to react with phenylhydrazine at different concentrations such that the latter was always present in a large excess. After a few hours quantifiable amounts of **5**, **6** and **7** had accumulated although **4** had completely disappeared from the reaction mixture. The relative rates of processes A and C were computed from the integrated intensities measured for **5**, **6** and **7** (equation 1).

$$R_C/R_A=(I_6+I_7)/I_5 \qquad (1)$$

The implication of this experiment (summarised in Figure 1) is that it is process C not process A which appears to be subject to catalysis by **2**.

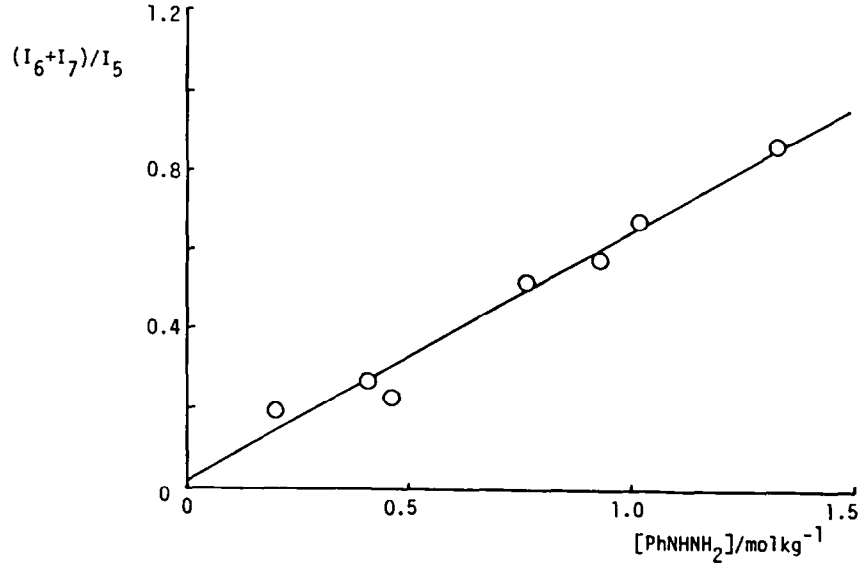


Figure 1: Relative reactivities of N-1 and N-2 as a function of phenylhydrazine concentration.

In order to confirm this result it was necessary to perform time-resolved experiments and subject the data obtained to numerical analysis. Because of the higher concentrations of reactants used, the reaction solvent was a mixture of (1,1,1-$^{2}H_3$)methanol and tetrahydrofuran, the latter

helping to prevent precipitation of the relatively insoluble product, **5**. In these experiments a narrow sweepwidth was used and only the resonances due to **4**, **5**, **6** and **7** were observed. This resulted in improved digital resolution, which is an important factor when curve-fitting spectral data, but it did mean that an intensity reference was necessary. Therefore integrated intensities were measured relative to that for the C-7 resonance of 4-bromo-(7-^{13}C)benzyl alcohol.[15] The parameter, s, relating integrated intensity to concentration (equation 2) was optimised in the curve-fitting of the intensity-time profiles along with the kinetic parameters.

Experiments with different starting concentrations[16] of the reactants and with added base catalyst (DABCO) were conducted. The reaction temperature (measured with an iodoethane chemical shift thermometer[17]) varied considerably from experiment to experiment but for a given experiment remained acceptably constant. Obviously better temperature control would be most desirable but since the kinetic parameters of greatest interest are orders rather than the actual rate constants, this is not too much of a problem. The following equations were fitted to the data employing the same techniques that were used for optimisation of spectral parameters[12,18]:

$$x_j = sI_j/I_{ref} \quad (2)$$

$$R_A = k_1 x_1 (x_2)^n \quad (3)$$

$$R_B = k_2(1+at+bt^2)x_4 \quad (4)$$

$$R_C = k_3 x_1 (x_2)^m \quad (5)$$

$$R_D = k_4 x_2 x_6 \quad (6)$$

I_j=integrated intensity for j^{th} species
I_{ref}=integrated intensity for intensity reference
x_j=molality of j^{th} species
R_p=Rate of process P
t=time

The use of a quadratic function of time to describe the kinetics of process B deserves some comment. This process was not adequately described by a single kinetic parameter but was of little interest in the context of this work. In a curve-fitting problem of this nature a poor mathematical description of one process will result in systematic errors in the optimised parameters which describe the other processes since all the parameters are obtained simultaneously from the entire data set. Equation 4 obviously has no theoretical basis but it does ensure that the mathematical description of process B is adequate for the purposes of this study.

These equations were fitted to the data and the effect, on the fit,[19] of varying n and m was investigated. The results of these calculations are given in Table 1 and they too suggest that the rate of process C is second order in phenylhydrazine concentration.

Table 1: Dependence of fit on orders of reaction for two time-resolved experiments.

Experiment*		2	3
n	m	Standard deviations (arbitary units)	
1	1	1.29	1.26
2	1	1.72	1.59
1	2	1.00	1.00

*See Table 2 for starting concentrations in these experiments.

Using n=1 and m=2 curves were fitted to the unweighted data for experiments 1-3. The fit for experiment 1 is shown in Figure 2 and the kinetic parameters are listed for all three experiments in Table 2. Note that a fit of this quality would not have been possible if the assumption of equal NOE factors was unsound.

Table 2: Optimised kinetic parameters for time-resolved NMR experiments with uncertainties in parameters are in parenthesis.

Experiment		1	2	3
Starting Concentration/molkg^{-1}	1	0.34	0.32	0.25
	2	1.36	0.67	0.29
T/K		296	304	307
10^5k_1/mol^{-1}kgs^{-1}		5.2 (0.3)	7.9 (0.8)	9.4 (0.4)
10^4k_2/s^{-1}		1.6 (0.3)	1.8 (0.8)	2.3 (0.8)
10^5a/s^{-1}		7.5*	0.5*	6.1*
10^9b/s^{-2}		-3.5*	5.1*	9.2*
10^5k_3/mol^{-2}kg^{2}s^{-1}		4.3 (0.2)	8.3 (0.7)	12.0 (1.4)
10^5k_4/mol^{-1}kgs^{-1}		0.7 (0.3)	2.0 (0.5)	7.0 (1.4)

*It was not possible to derive meaningful estimates for the uncertainties in these parameters.

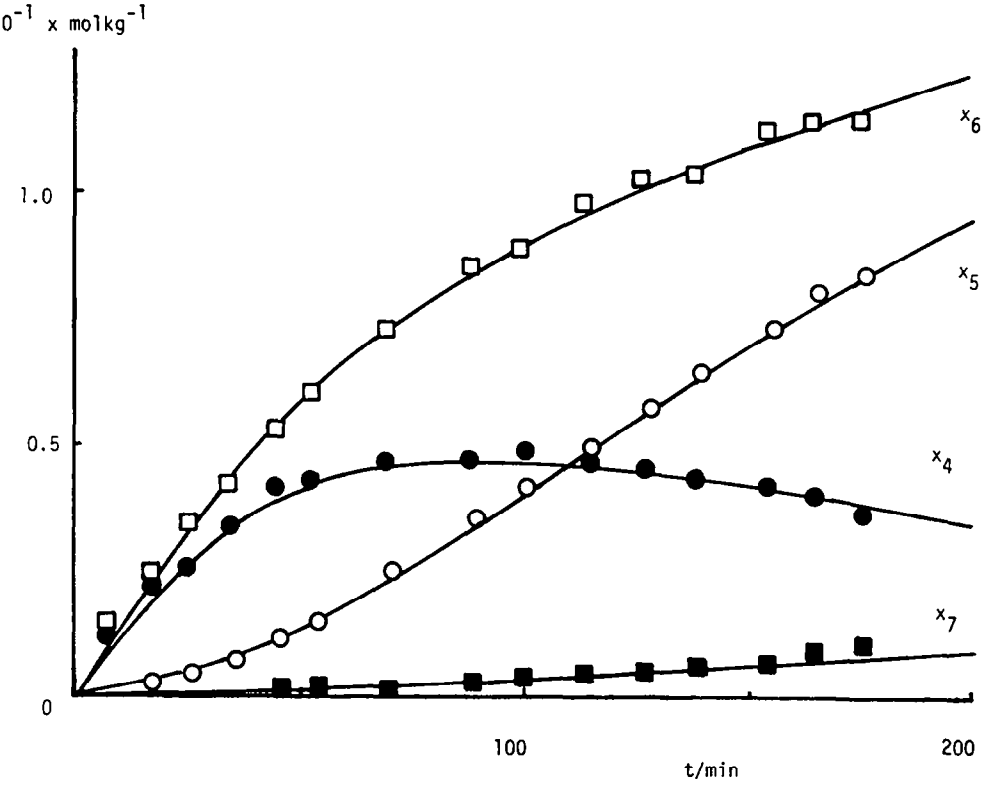


Figure 2: Concentration-time profiles for intermediates and products in the reaction between **1** and **2**. Data are for experiment 1 (Table 2).

In experiment 4 the catalytic effect of a stronger base (DABCO) than phenylhydrazine was investigated. The intermediate **4** was only observed in the early stages of this experiment so the intensities of this species and **5** were summed, thus effectively ignoring the dehydration of **4**. The fitting of the data was found to be improved by assuming that process C was reversible (equation 7) although further experiments will be necessary to unequivocally establish that this equilibrium does indeed operate at the temperature of this experiment. Equations 2, 3, 6 and 7 were fitted to the unweighted data using n=1 and the results of this experiment are shown in Figure 3 along with the fitted data from experiment 3 (Figure 4) for comparison (the starting concentrations of **1** and **2** were very similar in these two experiments). The catalytic effect of the DABCO is obvious from inspection and the optimised kinetic parameters for this system are given in Table 3.

$$R_C = k_5 x_1 x_2 - k_{-5} x_5 \qquad (7)$$

Table 3: Rate constants for DABCO catalysed reaction with uncertainties in rate constants indicated in parenthesis.

Experiment		4
Starting Concentration/molkg^{-1}	1	0.28
	2	0.33
	DABCO	0.11
T/K		300
$10^5 k_1$/mol^{-1}kgs^{-1}		6.6 (2.7)
$10^4 k_5$/mol^{-1}kgs^{-1}		9.7 (1.1)
$10^5 k_{-5}$/s^{-1}		6.9 (1.9)
$10^5 k_4$/mol^{-1}kgs^{-1}		1.8 (1.7)

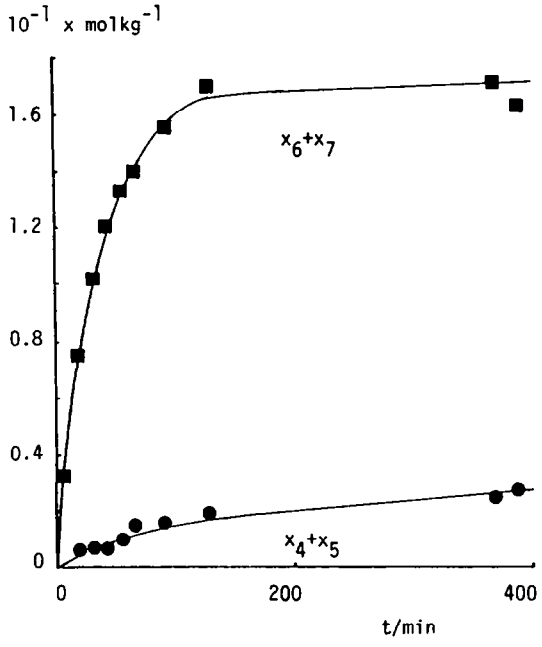


Figure 3
Concentration-time profiles for DABCO catalysed reaction between 1 and 2.

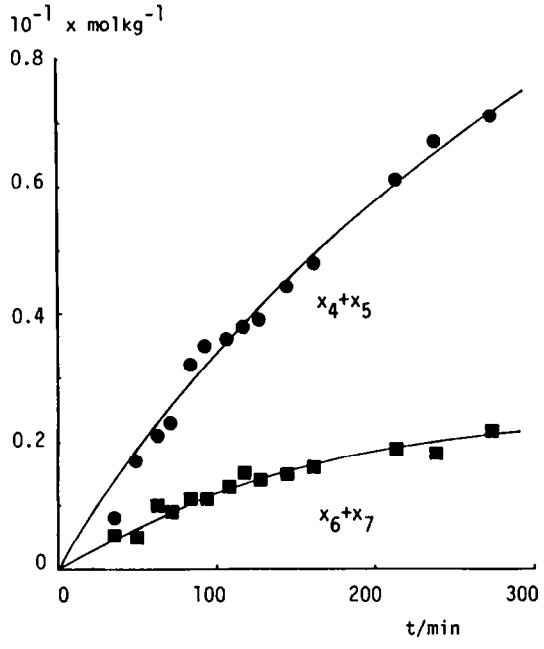


Figure 4
Concentration-time profiles for experiment 3 (Table 2).

Estimates of k_1 (6.5×10^{-5} mol^{-1}kgs^{-1}) and k_3 (6.2×10^{-5} mol^{-2}kg^{2}s^{-1}) at 300K can be derived from the results summarised in Table 2. The estimate for k_1(300K) does not differ measurably from the corresponding entry in Table 3, implying that base catalysis of process A by DABCO is relatively unimportant when compared with the uncatalysed process. The estimate for k_3(300K) can be compared with k_5 in Table 3, divided by the concentration of DABCO in experiment 4 (8.8×10^{-3} mol^{-2}kg^{2}s^{-1}). Thus at 300K, DABCO (pK_a=8.72[20]) is $10^{2.2}$ times more effective than phenylhydrazine (pK_a=5.2[21]) as a catalyst for process C.

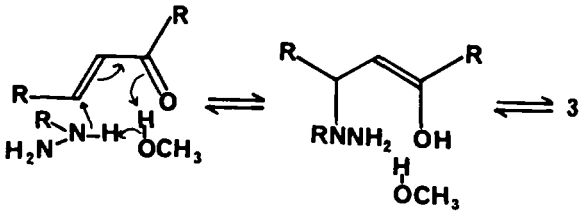
DISCUSSION

It is clear from the results presented that the 1,4-additions at N-1 and at N-2 proceed by strikingly different mechanisms. The 1,4-addition at N-1 does not appear to occur unless base catalysed while the rate of 1,4-addition at N-1 is not measurably affected by the presence of DABCO (a highly effective catalyst for the former process) in the reaction mixture. As indicated

earlier, base catalysis might have been expected to be more important for 1,4-addition at N-1 since the N-1 is less basic than N-2 and the proton of the former is expected to be more acidic than those of the latter (the pK_{HA} values for aniline and ammonia have been estimated to be 27.3[22] and 33.5[23] respectively). This makes the results all the more surprising.

One possible rationalisation of the kinetic data is that the solvent participates as catalyst for the 1,4-addition at N-1 but not for addition at N-2. However methanol (estimates[21] of pK_a range from -0.34 to -4.9) and tetrahydrofuran (pK_a=-2.08[21]) are both relatively feeble bases compared to phenylhydrazine and DABCO and it is hard to see how they could compete as base catalysts with the latter two species, even when present at rather higher concentrations. The molality of methanol in these experiments is calculated to be 21 $molkg^{-1}$ which is 190 times greater than that of DABCO in experiment 4. The figure of 190 should be compared with the difference in base strengths of methanol and DABCO which is 9 or more orders of magnitude. If 1,4-addition is catalysed by methanol then either base catalysis of this process is extremely insensitive to the base strength of the catalyst or the methanol catalysed 1,4-addition at N-1 takes place by an entirely different mechanism to the DABCO or phenylhydrazine catalysed 1,4-addition at N-2. It is true that base catalysed addition at N-1 should be less sensitive than base catalysed addition at N-2 to the basicity of the catalyst, on account of the greater acidity of the N-1 proton.[24] However the rate of 1,4-addition at N-2 is quite sensitive to the basicity of the catalyst and it seems rather improbable that the rate of addition at N-1 could be sufficiently insensitive to the catalyst basicity to account for the unimportance of the DABCO and phenylhydrazine catalysed additions at N-1.

It is more likely that catalysis by methanol takes place by a quite distinct mechanism, one in which methanol is able to make use of its relatively acidic proton as shown in Scheme 2. The efficiency of this process will depend critically on a matching of the acid-base characteristics of all three participating species which will make the process fairly specific. N-2 does not undergo 1,4-addition by this mechanism because its protons are not sufficiently acidic.



Scheme 2: Catalysis of addition at N-1 by methanol (R=Phenyl).

This work clearly illustrates the power of ^{13}C NMR as quantitative kinetic tool. More conventional methods of quantitative analysis would have been hard-pressed dealing with a system of this complexity. The results are also of significance in synthesis since they predict highest yields of pyrazoline when the reaction is carried out at low concentration.

EXPERIMENTAL

General

^{13}C NMR spectra were recorded on a Bruker WH90 Fourier transform instrument operating at 22.63 MHz in conjunction with a Nicolet B-NC 12 computer. For quantitative work broad-band proton decoupled spectra (sweepwidth, 500 Hz, 10 s between pulses) were transformed and phased by eye before being transferred to a Hewlett-Packard 9825B computer via a 16-bit parallel interface. Spectral parameters were determined by fitting[12,13] Lorentzian lineshapes, with simultaneous refinement of the phase correction, to the transferred data.

(3-^{13}C)Chalcone (86.2 atom % ^{13}C) was prepared[25] from (7-^{13}C)benzaldehyde which was obtained by the nitrogen dioxide oxidation[26] of (7-^{13}C)benzyl alcohol.[27] The product was recrystallised from

ethanol and melted at 56-57.5°C (uncorrected, lit.[28] 59°C). 4-Bromo-(7-^{13}C)benzyl alcohol (86.2 atom % ^{13}C) was prepared from 1,4-dibromobenzene in an exactly analogous manner to (7-^{13}C)benzyl alcohol. The crude product was recrystallised twice from pentane and was sublimed, the product melting at 74-76°C (uncorrected, lit.[28] 77°C).

Phenylhydrazine (Aldrich) was dried over potassium hydroxide and triply distilled at reduced pressure. The distilled phenylhydrazine was then partly frozen under nitrogen, the supernatent liquid was removed and the procedure was repeated. The purified material was stored under nitrogen, at -5°C and was used within one month. (1,1,1-$^{2}H_3$)Methanol was prepared by the distillation at reduced pressure of a mixture of ($^{2}H_4$)methanol (10.0g, Aldrich) and ethanediol (250g, Aldrich) and the subsequent redistillation of the product. Tetrahydrofuran (Aldrich, gold label grade) and DABCO (Aldrich) were used without further purification.

Investigation of catalysis by phenylhydrazine by analysis of product/byproduct ratio

Weighed samples of a stock solution of (3-^{13}C)chalcone (0.05 molkg^{-1}) in (1,1,1-$^{2}H_3$)methanol were equilibrated at 30.0°C in NMR sample tubes. A weighed sample of phenylhydrazine was added to each of the tubes and the samples were maintained at 30.0°C for 15-20 hours after which measurable quantities of the reaction products had formed. The ^{13}C NMR spectra of the samples were recorded and spectral parameters were optimised. The intermediate **4** was not detected in any of the reaction mixtures and the ratio R_C/R_A was computed, as a function of phenylhydrazine concentration, from the integrated intensities for **5**, **6** and **7** using equation 1.

Time-resolved NMR experiments

Weighed samples of (3-^{13}C)chalcone and 4-bromo-(7-^{13}C)benzyl alcohol were dissolved in a weighed amount of a mixture of (1,1,1-$^{2}H_3$)methanol (85 mol %) and tetrahydrofuran (15 mol %) and placed in an NMR sample tube. A weighed amount of phenylhydrazine was then added to the sample and the tube containing the reaction mixture was placed in the probe. The ^{13}C NMR spectrum of the reaction mixture was then recorded as a function of time, the sample remaining in the probe for the duration of the experiment. The sampling period was 400 s and it was assumed that a spectrum represented the composition of the reaction mixture at the middle of the sampling period.[29] Spectral parameters were optimised and integrated intensities were divided by the integrated intensity measured for the resonance of the intensity reference, 4-bromo-(7-^{13}C)benzyl alcohol. Equations 2-6 were then fitted to this data using standard techniques.[12,18] The experiment in which 1,4-diazabicyclo[2,2,2]octane was present in the reaction mixture, was carried out in a completely analogous mannner, fitting equations 2, 3, 6 and 7 to the data.

Acknowledgment: We thank the Government of Trinidad and Tobago for a studentship (to P.W.K.) and the Science and Engineering Research Council for financial support.

REFERENCES AND NOTES

1. NMR lines are narrow in relation to typical spectral width and chemical shifts are very sensitive to structure so it is possible to resolve signals from several species. In this respect ^{13}C NMR is superior to ^{1}H NMR since in the former case couplings are not normally observed and the chemical shifts are rather more sensitive to relatively small (e.g. stereochemical) differences in structure. NMR techniques are also non-destructive and do not perturb the reacting system chemically so transient species can be observed as they exist in the reaction mixture.
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14. With good quality data curve-fitting techniques also allow accurate deconvolution of overlapping signals and enable resonance frequencies to be measured to a precision better than the digital resolution of the spectrum.
15. The ^{13}C chemical shift corresponding to the isotopically substituted site is 64.6 ppm which lies within the group of observed resonances but not too close to any of them.
16. Molalities rather than molarities were used because solutions can be prepared on a small scale more accurately by weight than by volume.
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18. P.W. Kenny and M.J.T. Robinson, unpublished computer programs.
19. Fit was measured as a standard deviation computed from all data points without weighting. Large differences in the quality of fit for different species in a given experiment may justify some weighting of the data.
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29. This assumption breaks down when the reaction rate changes significantly during the sampling period. This will be the case either when the reaction is relatively fast or when long sampling periods are necessary to quantify species at low concentrations. Change in reaction rate during the sampling period can be easily dealt with mathematically although this does add some complexity to the implementation of the curve-fitting algorithm. For further details see P.W. Kenny, D.Phil. Thesis, Oxford, 1982, Chapter 4.