Thorpe–Ingold effects in cyclizations to five-membered and six-membered rings containing planar segments. The rearrangement of N(1)-alkyl-substituted dihydroorotic acids† to hydantoinacetic acids in base

Jose Kaneti,* Anthony J. Kirby,‡ Asen H. Koedjikov§ and Ivan G. Pojarlieff

* Institute of Organic Chemistry, Bulgarian Academy of Sciences, ul. Acad. G. Bonchev block 9, Sofia 1113, Bulgaria. E-mail: ipojarli@orgchm.bas.bg

‡ University Chemical Laboratory, Cambridge, UK CB2 1EW

Received 9th January 2004, Accepted 17th February 2004
First published as an Advance Article on the web 10th March 2004

While the gem-dimethyl effect (GDME) is quantitatively similar for cyclizations to cyclopentane and cyclohexane rings and their homomorphs, in systems containing planar segments the GDME is stronger for the formation of five-membered rings. Planar pentagons have smaller angles than planar hexagons and their formation is helped by the decrease in the potential internal bond angle caused by substituents, as suggested by Thorpe and Ingold for small rings. The phenomenon is illustrated with crystal structure data on five-membered hydantoins and six-membered dihydrooracils containing four-atom planar segments. Such a Thorpe-Ingold effect explains the rearrangement in base of N-alkyl substituted dihydroorotic acids to hydantoinacetic acids where this reduction in bond angle is available for many systems but seem not to have been analyzed in detail. A convenient point of reference is the parent cycloalkane. Equilibrium constants for the formal cyclizations of alkanes and gem-dimethylalkanes calculated from thermodynamic data show very similar increases in stability for cyclopentane and cyclohexane (around 70§ for substitution at C(3)). Similarly Warren and coworkers have shown that in competitive cyclizations to tetrahydrofurans and pyrans the GDME is of equal importance. However, some systems show a significantly stronger GDME for the formation of five-membered rings. The lactonizations of o-hydroxyalkylbenzoic acids (Scheme 1) present such an example.

A second example is provided by the relative rates of acid catalyzed cyclization of hydantoin and 2-ureidopropionic acids (Scheme 2).

† The numbering of dihydroorotic acid differs from that of dihydroacids due to priority of the carboxy group. For easier comparison with the parent ring systems we use 1-alkyldihydroorotic acid to denote 3-alkyl-2,5-dioxohexahydroimidazoline-4-carboxylic acid and 1-alkyldihydantoins acid to denote (3-methyl-2,5-dioxoimidazolidin-4-yl)-acetic acid.

‡ The effect is not limited to gem-substitution.

§ The number given in Table 14 of ref. 7 is incorrect.

This difference in behaviour, of compounds resembling the cyclic alkanes on the one hand and the lactones of benzoic acid and cyclic ureides on the other, can be rationalized by the inter-
A measure of the Thorpe–Ingold effect on the formation of five-membered rings with five planar atoms is founded in the intramolecularly catalyzed hydrolysis of maleamic acids (which involves cyclization to the anhydrides). Introduction of one and two methyl groups increases the rate by factors of 30 and 1.5 × 10^4 respectively, reflecting substantial decreases in the prospective internal bond angles at the alkene carbons.

The rearrangement of 1-alkyl-substituted dihydroorotic acids to hydantoinacetic acids in base

We report a strong Thorpe–Ingold effect on the formation of five-membered rings from the rearrangement of dihydroorotic to hydantoinacetic acids in KOH solutions: which occurs on alkylation of N(1) of the dihydrouracil ring (Scheme 3).

The change in behaviour in aqueous alkali is quite spectacular. Dihydroorotic acid 1a is readily hydrolyzed to N-carbamoylaspartate diion 2a, which is stable under the conditions. The hydrolysis products 2b and 2e of the methyl and ethyl derivatives slowly cyclize, to form equilibrium amounts of the dianion of hydantoinacetic 3. And the formation of hydantoinacetic 3 is so fast for the secondary alkyl derivatives 2d and 2e that the process followed by UV appears superficially as a direct conversion of 1d (e) into 3d (e).

The rate increase in the cyclization of the dianion of N-carbamoylaspartic acid (k_{12} of Scheme 3) was found to be ca. 40 when R = H was substituted for Me—a value comparable to the maleic acid case quoted above.

Results

The alkaline hydrolysis of 1-alkyldihydroorotic acids 1 has been studied previously, to assess the effect on rates of an axial carboxylate group. (This conformational preference is the result of allylic strain: in the parent acid the COO\(^-\) group is more or less planar, respectively, reflecting substantial decreases in the prospective internal bond angles at the alkene carbons.)

### Table 1  Ring geometries in hydantoins and dihydrouracils

<table>
<thead>
<tr>
<th>Ring atom</th>
<th>N(1)</th>
<th>C(2)</th>
<th>N(3)</th>
<th>C(4)</th>
<th>C(5)</th>
<th>C(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydantoin</td>
<td>112.3 ± 1.2</td>
<td>107.6 ± 0.9</td>
<td>111.5 ± 1.0</td>
<td>106.9 ± 0.6</td>
<td>101.2 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Dihydrouracil</td>
<td>122.0 ± 1.7</td>
<td>116.7 ± 0.9</td>
<td>125.2 ± 1.6</td>
<td>115.3 ± 1.3</td>
<td>109.9 ± 1.9</td>
<td>109.7 ± 1.6</td>
</tr>
</tbody>
</table>

### Scheme 2

Scheme 3

![Diagram](image2.png)
less equally axial and equatorial.) When the study was extended to include R = isopropyl and R = cyclohexyl the reaction studied by UV appeared to show a direct conversion into the corresponding hydantoin acid. This could in principle have resulted from intramolecular attack of carboxylate anion on the C=O at position 4, perhaps helped by buttressing by the neighbouring secondary alkyl group.

However, this pathway is not consistent with the observed kinetics. In 0.1–1 M KOH dihydroorotic acids 1 exist as the dimers (Scheme 3). The rate of formation of 1 shows a first order dependence on [OH⁻]: an intramolecular reaction of the dianion of 1d would be independent of hydroxide concentration. Furthermore, a 1H NMR study of the course of the reaction with 1d revealed transient formation of the intermediate N-(1'-isopropylcarbamoyl)aspartic acid 2d. Finally, when solutions of the hydrolyzed products 2 of methyl and ethyl derivatives 1b and 1c were left to stand they slowly cyclized, forming equilibrium mixtures of 2 and 3.

The course of the rearrangement is thus that outlined in Scheme 3, involving base catalyzed nucleophilic attack of the urea NH₂ on the carboxylic acid group. In the case of 1b and 1c the two consecutive reactions are well separated in time and separate pseudo first order rate constants could be obtained from the decrease in absorbance of 1d at λmax = 240 nm and the subsequent increase in absorbance at 232 nm (λmax for 3b and 3c). The isopropyl and cyclohexyl derivatives 2b and 3b, on the other hand, cyclized much faster so that this simple treatment was no longer possible: the rate constants were obtained by curve fitting of the variation of absorbance with time to the integrated first order rate equation for two consecutive reactions.

The preferred mechanism 13 of alkaline hydrolysis of dihydouracils and hydantoins (shown in Scheme 4) is characterized by two parallel modes of decomposition of the tetrahedral intermediate T⁻, catalyzed by water and OH⁻ respectively.

As expected, in 0.1–1 M KOH the hydrolyses of the N-isopropyl and N-cyclohexyl-dihydroorotic acids 1d and 1e, and the formation of the hydantoinoacetic acids 3d and 3e are apparently first order in [OH⁻]:

\[ V = k_{3d}[I^2][OH⁻] \] and \[ V = k_{3e}[I^2][OH⁻] \].

The kinetics are revealed as being of the second order when the unproductive ionizations are accounted for,

\[ k_{cor} = k_{obs}[OH⁻] \]

The cyclizations of the N-methyl and N-ethyl-carbamoylacetic acids 2b and 2c are too slow for a pH rate profile to be measured, so rates were measured in 1 M KOH. To check the kinetic order a pH rate profile for the cyclization of the ethyl derivative 2c was obtained at 50 °C, and reaction found to be first order in [OH⁻] (see Fig. 1).

![Fig. 1 Plots of the observed pseudo-first order rates, s⁻¹, against [KOH] at 50.0 °C: open circles hydrolysis of 1c, \( k_1 = 1.25 \times 10^{3} \) dm³ mol⁻¹ s⁻¹; filled circles cyclization of 2c, \( k_3c = 3.07 \times 10^{-4} \) dm³ mol⁻¹ s⁻¹. Measuring the equilibria for cyclization of the carbamoylaspartic acids 2b and 2c also provided the rate constants (\( k_{12} \)) for hydrolysis of hydantoinoacetic acids 3b and 3c, because \( k_{obs} = k_{12} + k_{13} \).

The full set of kinetic data is shown in Table 2. Two sets of rate constants are presented for comparison. For each compound the first column presents the apparent second or first order rate constant determined in 1 M KOH. However, our earlier work 14 indicates that the observed hydrolysis rates, \( k_{12} \), of the parent dihydroorotic acid 1a and its N-methyl derivative 1b in 1 M KOH are determined mainly by \( k_1 \), the rate constant for formation of the tetrahedral intermediate. For the remaining compounds the OH⁻ catalyzed breakdown of the tetrahedral intermediate \( k_1 k_2/k_{-1} \) is rate determining at 1 M KOH. The latter mechanism is the one for which rate constants could be obtained in all cases, and these are given in parentheses in Table 2. These rate constants are readily obtained from the experimental second order rate constants and pK₅; from eqns. (1) and (2), under conditions where [OH⁻] >> \( K_w \)

\[ k_{12} k_2/k_{-1} = (k_{12} or k_{13}) K_{5}\text{pH} K_w \] (3)

The pK₅; values for 1 and 3 measured for this purpose appear in Table 3.

The data obtained for the equilibrium:

\[ K^2 = [I^2]/[2^2] \] (4)

...
The cyclization of ureido acids is a step in the “de novo” biosynthesis of pyrimidine bases. An intriguing point, recently reexamined, is that in mineral acids the opposite regioselectivity is observed: cyclization proceeds by attack of the ureido NH$_2$-group on the 1-CO$_2$H to give hydantoinic acid, the only product isolated by Nye and Mitchell. The new interpretation is that in the chemical reaction, attack on the distant carboxyl is hindered by a strong hydrogen bond between the two carboxy groups, which reduces the flexibility of the chain. Attack on the 1-CO$_2$H takes place in acid because the geninal ureido is favourably disposed. It was also postulated that this hydrogen bond is broken in the enzyme active site. From VH vicinal coupling constants assigned from specifically deuterium-labeled 2a we have estimated that the conformations with the carboxy groups gauche account for less than 60% of the neutral form in D$_2$O—not consistent with stabilization by a strong hydrogen bond. In contrast the population of conformations with the ureido and 4-CO$_2$H gauche is 90%, so that proximity in forming the six-membered ring should not be problem. Further, we showed previously that in HCl solutions the cyclization of N-carbamoylaspartic acid actually gives both products, with a kinetic regioselectivity [3a]; [1a] of no more than 10 : 1, much less than the thermodynamic preference discussed above.

At biological pH, near 7, both carboxy groups are ionized. Because carboxylate anions are poor electrophiles reaction must involve the neutral CO$_2$H group. Since the more distant carboxy group is a 30-times weaker acid more of it will be present in the neutral CO$_2$H form near pH 7, thus over-riding the kinetic selectivity. The conclusion from chemical reactivity data is thus that dihydroorotic acid is the preferred product at neutral pH, so that enzyme catalysis is not in fact working against strong intrinsic regioselectivities upon cyclization of N-carbamoylaspartic acid.

**The effect of N-substituents**

Cyclizations of ureido acids are observed even in alkaline solution in fixed molecules or highly encumbered systems like 6-ureidobenzoic acid or 2,2,3,5-tetramethylhydantoinic acid. Remarkably, just two substituents of the size of carboxymethyl and isopropyl in the 1,5-position of hydantoin are enough to make the ring stable in 1 M KOH. There is abundant evidence, for example in the acid catalyzed cyclization of ureido acids,
that substitution at N(1) in the product causes the greatest accelerations ($k_{oc}$-values obtained by comparison with the corresponding unsubstituted ureido acids); 24,25,5a

Agami and Couty26 have identified strong Thorpe–Ingold effects due to N-alkyl substitution facilitating the formation of 1,3-oxazolidin-2-ones or oxazolidin-2,5-diones compared to the N-unsubstituted compounds. AM1 calculations showed decreases in the C–NR–C angle of the open-chain reactant when R = Me, leading Agami et al.27 to the conclusion that a classical Thorpe–Ingold effect operates to favour ring-closure upon N-methyl substitution.

A very strong such effect is observed on the equilibrium hydantoinacetic acid $\rightleftharpoons$ N-carbamoylaspartic acid [$(3^2\pi)(2^2\pi)$] (Table 4), where an N-methyl shifts the equilibrium by a factor of 600. This seems too large to be simply a bond-angle effect: the relative rates of similar acid-catalyzed cyclizations (Schemes 2 and 5) show that rate increases for substitution at the nitrogen atom are much larger than that at C(5), even though significant reductions of bond angles occur at both centres (data of Scheme 6 involves no bond-angle reduction). Studies of rotational barriers around partial double bonds indicate 28 that in ureas considerable strain accumulates in the ground state upon N-substitution. This is released upon cyclization as one pair of the eclipsing groups is incorporated in the ring, as explained by Allinger 4 for the case of substituted hexanes.

In the absence of special effects on the transition state the rate increases upon cyclization or decreases upon ring-opening should be smaller than those for equilibrium; this is borne out by the data in the final 4 columns of Table 2 (which compare N–Me with N–H). The rate increase on cyclization is larger than that on ring opening, which suggests a transition state closer to the cyclic form. The hydrolyses of compounds a and b allow a comparison of six versus five-membered ring behaviour. When the apparent second order rate constants for hydantoinacetic acids 3 are compared to the first order constants for dihydroorotic acids 1 in 1 M KOH the decrease is larger for the five-membered ring. As explained above, however, the data for formation and hydrolysis of the hydantoinacetic acids refer to the doubly negatively charged transition state 4 so that it is more appropriate to compare them with the third order rate constants $k_{oc}/k_{oc}$ for hydrolysis of 1. The latter exhibit a very large decrease upon substitution of H for Me, which has been interpreted previously 22 as an “inverse” GDME on ring opening. The same trend is observed when the size of the N-substituent is increased further (Table 2).

Increasing the size of the substituent from methyl to isopropyl or cyclohexyl exhibits substantial effects on the rates of cyclization of the N-alkylcarbamoylaspartic acids to hydantoinacetic acids (see columns 4 and 5 of Table 2)

### Experimental

Unless otherwise stated IR-spectra are for Nujol mulls, using a Bruker IFS 113v instrument, and frequencies in cm$^{-1}$. $^1$H NMR spectra were recorded on a Bruker Spectrospin WM 250 instrument (chemical shifts in ppm against TMS, couplings in Hz), and UV measurements made using Unicam SP 800 UV and Carl Zeiss Jena UV VIS spectrophotometer provided with a thermostatted cell compartment, wavelengths in nm. Melting points were taken on a Koeller block.

### Materials

Inorganic reagents and buffer components were of analytical grade and were used without further purification. Potassium hydroxide and buffer solutions were prepared with CO$_2$-free distilled water. The preparation of 3-alkyl-2,6-dioxo-hexahydropyrimidine-4-carboxylic acids 1b,c, and d has been described previously. 29

### 3-Cyclohexyl-2,6-dioxohexahydropyrimidine-4-carboxylic acid 1e.

The parent 2-cyclohexylaminosuccinic acid mono-amide was prepared as previously described. 28 This was converted into the N-ethoxycarbonyl derivative by dissolving the mono-amide (0.5 g, 2.23 mmol), followed by 1 ml of ethyl chloroformate, in 10 ml of dry triethylamine. After standing for 2 hours at 50 °C the triethylamine was removed and the residue dried in vacuo at 50 °C. The residual 1 g was treated for 4 h under reflux with sodium ethoxide (7 mmol) in 5 ml of dry ethanol. The precipitate formed under cooling was filtered rapidly to avoid moisture and washed with dry ethanol. The solid was dissolved in a 3 ml of cold water and acidified with HCl (Congo Red), then the precipitate filtered and recrystallized from ethanol yielding 1e (180 mg, 34%), mp 234–235 °C. (Found: C, 54.97; H, 6.70; N, 11.73. Calc. for C$_{15}$H$_{15}$N$_5$O$_4$: C, 54.99; H, 6.71; N 11.66%). $\delta$$_H$ (DMSO-d$_6$) 1.0–1.8 (10 H, m, CH$_2$). 2.566 (1 H, d, J$_{4e,5}$ = 1.5, 4e-H), 2.851 (1 H, dd, J$_{4a,5}$ = 7.2, 4a-H), 4.052 (1 H, s, broad unresolved, 1-H), 4.220 (1 H, d, J$_{4a,5}$ = 1.5, 5-H), 10.055 (1 H, s, NH).

### (3-Alkyl-2,5-dioxo-imidazolidin-4-yl)-acetic acids 3. The

3-alkyl-2,6-dioxohexahydropyrimidine-4-carboxylic acid 1 (0.44 mmol) was dissolved in 20 ml of hydrochloric acid diluted 1:1 with water and refluxed for 2 hours. The residue was evaporated to dryness and the residue recrystallized from ethanol. Data for these compounds are summarized in Table 5.

$^1$H NMR in DMSO-d$_6$. The CH$_2$ protons and the 4-H proton formed an ABX system. The data for these protons were obtained from the analysis of this system. 1b. 2.741 (3 H, s, Me), 2.760 (2 H, octet, J$_{AB}$ 11.1 Hz, J$_{AX}$ 17.1, J$_{AX}$ 4.7, J$_{AX}$ 4.3, CH$_3$), 4.160 (1 H, t, J 4.5, 4-H), 10.73 (1 H, s, NH), 12.76 br (CO$_2$H). 1c. 1.022 (3 H, s, J 7.1, Me), 2.767 (2 H, octet, J$_{AB}$ 10.7 Hz, J$_{AB}$ 17.2, J$_{AX}$ = J$_{AX}$ = 4.5 CH$_3$), 3.040 (1 H, sextet, J 14.1, J 7.1, CH$_2$-CH$_2$), 3.415 (1 H sextet, J 14.1, J 7.1, CH$_2$-CH$_2$), 4.251 (1 H, t, J 4.5, 4-H), 10.74 (1 H, s, NH), 12.58 (1 H, s, CO$_2$H). 1d. || 1.123 (3 H, d, J 6.9, Me), 1.169 (3 H, d, J 6.9, Me), 2.765 (2 H, octet, J$_{AB}$ 7.2 Hz, J$_{AB}$ 17.0, J$_{AX}$ 4.6, J$_{AX}$ = 4.0, CH$_3$), 3.869 (1 H, septet, J 6.9, 4.199 (1 H, t, J 4.3, 4-H). 1e. 1.0–1.8 (10 H, m, (CH$_2$)$_2$), 2.822 (2 H d (J 3.4) CH$_3$), 3.494 (1 H, t unresolved, 4-H), 4.225 (1 H, br, 1-H), 10.73 (1 H, s, NH), 12.55 (1 H, br, CO$_2$H).

### Determination of $pK_a$'s for dissociation at NH

$pK_a$-values were determined spectrophotometrically in a series of buffers at 25.0 °C using the absorptions of the anions: $\lambda_{max}$ $\cong$ 234 nm for N-alkylhydantoinacetic acids and 238 nm for N-alkyldihydropyrimidinacetic acids. $pK_a$-values were measured using a Radiometer pH M 84 Research pH-meter, equipped with a GK 2401 C electrode standardized at pH 6.87, 4.01 and 9.18.

$^4$ Spectrum taken on a 400 MHz instrument.
Kinetic measurements

Reactions were followed using stopped cells, in the temperature-controlled cell holder of the spectrophotometer or in sealed vials at 25.0 °C. The reaction was initiated by adding 20 µl of a 0.05 M solution of the substrate in UV-grade methanol to 2.75 ml of the KOH solution. The ionic strength was maintained at 1 M with KCl.

(a) N-Methyl and N-ethyl derivatives. The rates of hydrolysis of dihydroorotic acids 1a, 1b and 1c, reported before,13 were followed by monitoring the decrease of the absorbance of the diamin at 238 nm. In the case of 1b and 1c, on standing the subsequent increase of absorbance with a \( \lambda_{\text{max}} \) at 234 nm showed that the formation of the respective hydantoinic acids took place at a considerably slower rate. The two processes are well separated in time and with some approximation their rates could be measured separately, justifying the previous treatment of the hydrolysis of dihydroorotic acids 1b and 1c as single reactions. The rates of cyclization of N-carbamoylaspartic acids 2b and 2c were monitored by following the increase in the absorbance at 234 nm after opening of the dihydrooracil ring. Rate constants were obtained by curve fitting to pseudo first order rate equations.

The final absorbances after cyclization were smaller than those of hydantoins 3b or 3c at the same concentration. This could be because the reaction had reached an equilibrium, or a result of hydrolysis of the ureido function in the carbamoyl aspartic acid. The latter possibility was excluded by treating the end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting end product with HCl. Because the absorbances of the hydantoins are more ready...