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Validation of the Distal Effect of Electron-Withdrawing Groups on the Stability of Peptide Enolates and Its Exploitation in the Controlled Stereochemical Inversion of Amino Acid Derivatives

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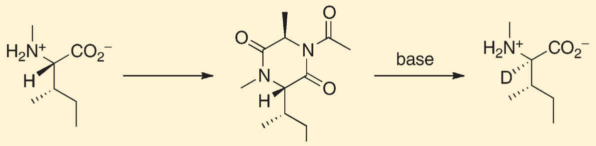
Supporting Information

ABSTRACT: Theoretical studies had predicted that *N*-electron-withdrawing substituents, hydrogen bonding, and protonation at amide nitrogen selectively increase the acidity of a distal proton adjacent to the amide carbonyl to the extent that the α-carbonyl acidity of some *N*-substituted amides exceeds that of typical ketones. Now, in the present work, competitive, base-catalyzed hydrogen−deuterium exchange experiments, with diisopropyl ketone and a series of *N*-substituted acetamides and diketopiperazines, have established that there is a strong correlation between the calculated acidities and the experimental rates of deprotonation in these systems. The results show that the rates of exchange of the distal protons of *N*-acylated and *N*-sulfonylated amides are more than 4 orders of magnitude faster than those of the *N*-methylated analogues and that the acylated and sulfonylated amides are much more reactive in this regard than diisopropyl ketone. The magnitude and regioselectivity of the distal effect is sufficient for practical applications and has been exploited in the manipulation of *N*-acetyl α,α′-disubstituted diketopiperazines for the controlled α-deuteration and stereochemical inversion of *N*-methylamino acids, and in the production of α-deuterated (2*R*,3*S*)-*N*-methylalloisoleucine from the nondeuterated (2*S*,3*S*)-isoleucine diastereomer.



INTRODUCTION

The acidity of α-carbonyl protons of amino acids and peptides and their derivatives is of interest because the corresponding enolates are key intermediates in the biochemical[1−6] and synthetic transformations[7−11] of compounds of these types. In this context, Amyes and Richard and co-workers developed convenient ^{1}H NMR spectroscopic methods to provide reliable estimates of the aqueous pK_as of carbon acids and applied these to investigate the stability and ease of formation of amino acid[12−15] and peptide[16] enolates. More recently, we developed computational procedures for accurately predicting general gas phase and aqueous acidities.[17−19] A proton-exchange scheme that combines high-level ab initio gas phase energies with solvation energies from a continuum solvent model was found to perform particularly well in predicting the aqueous pK_as of a range of α-carbonyl carbon acids. This approach was recently used to determine the effect of *N*-electron-withdrawing substituents and protonation and hydrogen bonding at amide nitrogen on the carbon acidity of a series of acetamides, diketopiperazines, and linear dipeptides.[20] While the effect was found to generally increase the gas phase acidity of both a CH proton adjacent to the amide carbonyl (H_{dist}) as well as one proximal to the amide nitrogen (H_{prox}), the magnitude of the effect at the position more remote or distal from the nitrogen (H_{dist}) is substantially larger. This effect is also seen in solution where, for example, computed aqueous pK_a values show that *N*-acetylation of glycine anhydride results in a six-unit reduction in the pK_a of the distal α-CH proton (H_{dist}) but no increase in the acidity of H_{prox} (Figure 1).

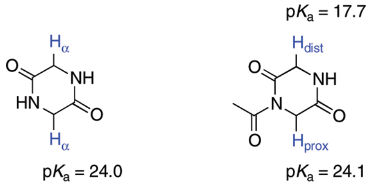


Figure 1. *N*-Acetylation of glycine anhydride results in a six-unit decrease in the calculated pK_a of the distal α-CH proton (H_{dist}).

The differential enhancement in acidity is a result of the *N*-electron-withdrawing groups, hydrogen bonding and protonation, providing a combination of inductive and resonance stabilization to a distal enolate, whereas a proximal enolate is stabilized only by an inductive effect.[20] The magnitude of the distal effect is such that the α-carbonyl acidities of some *N*-substituted amides were predicted to exceed those of typical ketones.

In the present work, we have studied competitive hydrogen−deuterium exchange reactions between diisopropyl ketone (**1**) and a series of *N*-substituted acetamides and diketopiperazines to establish that there is a strong correlation between the calculated pK_as and the experimental rates of deprotonation in these systems. The results show that the rates of exchange of the distal protons (H_{dist}) of *N*-acylated and *N*-sulfonylated amides

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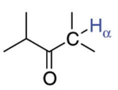
are more than 4 orders of magnitude faster than those of the *N*-methylated analogues and that the acylated and sulfonylated amides are much more reactive in this regard than diisopropyl ketone (**1**). We have also studied reactions of *cis*- and *trans*-α,α′-disubstituted diketopiperazines to establish that the rates and regioselectivity of their epimerization also correlate with the calculated pK_as and the experimental rates of hydrogen–deuterium exchange. These results have been exploited in the controlled stereochemical inversion of (*S*)-*N*-methylamino acids to give their α-deuterated (*R*)-enantiomers and in the production of α-deuterated (2*R*,3*S*)-*N*-methylalloisoleucine [2-^{2}H-(2*R*,3*S*)-**29**] from the nondeuterated (2*S*,3*S*)-isoleucine diastereomer [(2*S*,3*S*)-**29**].

RESULTS AND DISCUSSION

In the first phase of this investigation, hydrogen–deuterium exchange reactions were carried out with the *N*-substituted acetamides **2**–**4**, the diketopiperazines **5** and **6**, and diisopropyl ketone (**1**). The acetamides **2**–**4** and the ketone **1** had been investigated in the computational study,[20] while the diketopiperazines **5** and **6** were selected as *N*-methylated analogues of compounds studied in the previous work. Fully substituted amides were used to avoid any complications associated with exchange of N–H protons. Compounds **1**–**6** were treated individually and as mixtures with triethylamine and diazabicycloundec-7-ene (DBU), in acetone-d_6. Under these conditions, the labeled acetone is the source of the deuterium for the base-catalyzed hydrogen–deuterium exchange. The reactions were monitored using ^{1}H NMR spectroscopy for loss of proton resonances, as illustrated for *N*-acetyl-*N*-methylacetamide (**3**) in the Supporting Information. Disappearance of the resonances followed first order kinetics and the rate constants (k_{ex}) for exchange of the various types of protons of compounds **1**–**6**, measured in this way and normalized for the concentration of the base, are shown in Table 1. From these rate constants, the reactivity of the various types of protons relative to that of the α-carbonyl protons of diisopropyl ketone (**1**) (k_{rel}), adjusted for the number of protons of each given type, was calculated for the DBU-catalyzed reactions, and those values are also included in Table 1. While this statistical adjustment seems to be the most appropriate method for comparing the exchange rate constants, it only alters k_{rel} by at most a factor of 3, and therefore does not significantly impact on the conclusions of the study based on rate enhancements of more than 4 orders of magnitude. The computed aqueous pK_as for compounds **1**–**6** are also shown in Table 1 for comparison. The calculations were performed using a variation of the previous method where B3LYP/6-31+G(d) instead of BMK/6-31+G(d) geometries and frequencies were used for gas-phase free energy calculations. Both approaches perform similarly in the calculation of gas-phase acidities with a test set of carbon acids.[17,18]



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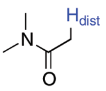

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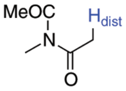

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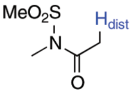

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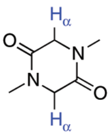

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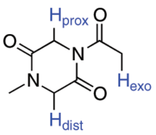

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Table 1. Computed Aqueous pK_a Values and Experimental Hydrogen–Deuterium Exchange Rate Constants (k_{ex}, M^{-1} s^{-1}) and Relative Exchange Rates (k_{rel})

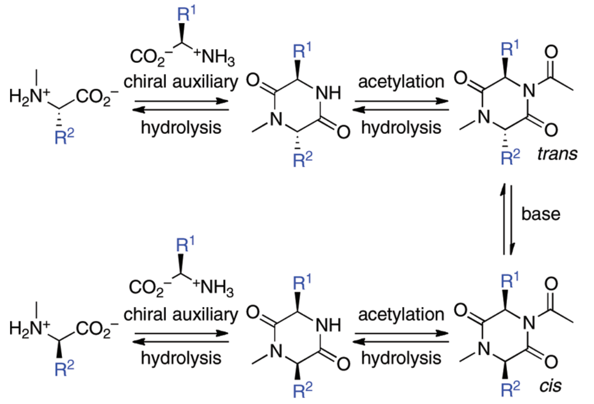
compd	proton	pK_a[a]	$-\log(k_{ex})$ (DBU)[b]	$-\log(k_{ex})$ (NEt_3)[c]	k_{rel} (DBU)[d]
1	H_α	21.0	5.1	ND[e,f,g]	1
2	H_{dist}	30.9 (29.7)[h]	ND[e]	ND[e,f]	<0.001[i]
3	H_{dist}	20.8 (20.8)[h]	2.6	6.2[f]	105
4	H_{dist}	19.7 (20.6)[h]	3.2	6.0[f]	55
5	H_α	26.6	5.7	ND[e,g]	0.10
6	H_{prox}	25.8	ND[e,j]	ND[e,g]	<0.15[i]
6	H_{dist}	18.4	1.3	4.0[g]	6300
6	H_{exo}	22.1	3.4	5.6[g]	35

[a] Calculated at 298 K. [b] DBU as the base at 323 K, the elevated temperature being necessary to obtain practical rate constants. [c] Triethylamine as the base. [d] Relative reactivity per chemically equivalent proton of the designated type. [e] Hydrogen–deuterium exchange not detected. [f] At 323 K. [g] At 308 K. [h] Data from ref 20. [i] Based on estimated limits of detection. [j] Reaction time limited in practical terms by competing deacetylation of the substrate **6**.

Under the conditions of these experiments, triethylamine catalyzes measurable rates of hydrogen–deuterium exchange of protons with computed pK_as of up to 22, whereas the stronger base, DBU, induces faster exchange and substitution of less acidic protons. Comparison of the results in Table 1 shows that the calculated effect of electron-withdrawing substituents on amide nitrogen to increase the acidity of distal hydrogens adjacent to the amide carbonyl is reflected in increased rates of hydrogen–deuterium exchange of the corresponding protons in the condensed phase experiments. Replacement of a methyl group of the acetamide **2** with acetyl and methanesulfonyl substituents in compounds **3** and **4**, respectively, decreases the calculated pK_a by approximately 10 units, in each case, and increases the DBU-catalyzed hydrogen–deuterium exchange rate by at least 5 × 10^4 times. Analogous effects are seen with the diketopiperazines **5** and **6**, where the acetylation reduces the calculated pK_a of the distal proton (H_{dist}) by 8.2 units and increases the corresponding DBU-catalyzed exchange rate by more than 63000 times. The protons of the acetyl group of the diketopiperazine **6** (H_{exo}) are in a similar environment to H_{dist} of the *N*-acetylacetamide **3** (i.e., adjacent to the carbonyl of an amide substituted with an electron-withdrawing acyl group) and, accordingly, they have similar pK_as and undergo hydrogen–deuterium exchange at similar rates. Whereas the calculated carbon acidity of the parent amides **2** and **5** is much less than that of diisopropyl ketone (**1**) and that of the acetylated and sulfonylated analogues **3**, **4**, and **6** is more, the rates of the DBU-catalyzed exchange reactions of the parent amides **2** and **5** are lower than those of diisopropyl ketone (**1**), with those of the amides **3**, **4**, and **6** substituted with electron-withdrawing groups being much higher. The results confirm that acetylation and sulfonylation of amide nitrogen increases the acidity of the distal α-carbonyl protons, making them more acidic than the α-protons of a ketone.

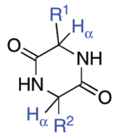
The predicted acidity and observed reactivity of the diketopiperazine **6** indicated that the regioselectivity of the distal effect of an *N*-acetyl group might be sufficient for exploitation, in the manipulation of α,α′-disubstituted diketopiperazines for the stereocontrolled preparation of *N*-methylamino acids. Peptides containing *N*-methylamino acids exhibit increased proteolytic stability[21−24] and membrane permeability[25,26] as well as having altered conformational characteristics.[27] *N*-Methylamino acids are interesting targets for stereocontrolled synthesis, not only because of their properties and those of their peptides, but also because they are less readily available than the nonmethylated, proteinogenic analogues from which their synthesis via *N*-methylation is often complicated by loss of α-stereochemistry.[27] Accordingly, in one approach that is illustrated in Scheme 1, it was envisaged that a homochiral nonmethylated amino acid would be used as a chiral auxiliary by being attached to an *N*-methylamino acid in a diketopiperazine. Acetylation would then facilitate base-catalyzed epimerization of the *N*-methylamino acid residue, the enantiomers of which would be obtained by separation of the *cis*- and *trans*-diketopiperazines and hydrolysis of the individual species. The efficiency of the method is dependent on the equilibrium ratio of the *cis*- and *trans*-*N*-acetyldiketopiperazines. Thus, for the controlled stereochemical inversion of an *N*-methylamino acid according to the procedure illustrated clockwise in Scheme 1, a high *cis*/*trans* ratio is preferable, and vice versa for the anticlockwise process. The main criterion for selecting which amino acid to use as the chiral auxiliary is its impact on the *cis*/*trans* ratio. If one enantiomer of the chiral auxiliary gives a ratio less than 1:1, the ratio for the other enantiomer would be proportionately higher so, in principle, interconversion of the (*S*)- and (*R*)-enantiomers of *N*-methylamino acids could be performed in either direction.

Scheme 1

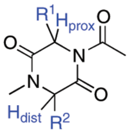


To examine the validity of the hypothesis represented by Scheme 1, it was necessary to first investigate *cis*/*trans* ratios (*K*) in α,α′-disubstituted diketopiperazines and to confirm the effect of *N*-acetylation on acidity in these systems. Results for compounds **7**−**26** are summarized in Table 2. The calculated *K* values establish that α,α′-disubstituted *N*-acetyl-*N*′-methyldiketopiperazines, where both substituents are Me or CH_2CHMe_2 (compounds **11**, **13**, **19**, and **21**), typically show a strong preference for the *cis*-isomer. This relates to their preferred

Table 2. Computed Gas and Aqueous Phase *Cis*/*Trans* Ratios (*K*) and Aqueous pK_a Values of Diketopiperazines[a]



7 $R^1 = R^2 = Me$
8 $R^1 = R^2 = CHMe_2$
9 $R^1 = R^2 = CH_2CHMe_2$
10 $R^1 = R^2 = CMe_3$

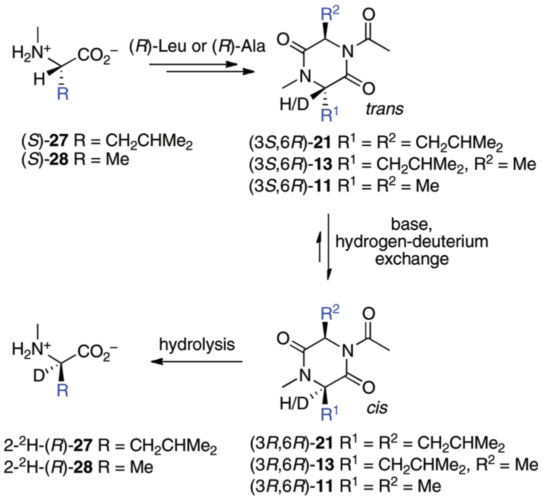


11 $R^1 = R^2 = Me$
12 $R^1 = Me, R^2 = CHMe_2$
13 $R^1 = Me, R^2 = CH_2CHMe_2$
14 $R^1 = Me, R^2 = CMe_3$
15 $R^1 = CHMe_2, R^2 = Me$
16 $R^1 = R^2 = CHMe_2$
17 $R^1 = CHMe_2, R^2 = CH_2CHMe_2$
18 $R^1 = CHMe_2, R^2 = CMe_3$
19 $R^1 = CH_2CHMe_2, R^2 = Me$
20 $R^1 = CH_2CHMe_2, R^2 = CHMe_2$
21 $R^1 = R^2 = CH_2CHMe_2$
22 $R^1 = CH_2CHMe_2, R^2 = CMe_3$
23 $R^1 = CMe_3, R^2 = Me$
24 $R^1 = CMe_3, R^2 = CHMe_2$
25 $R^1 = CMe_3, R^2 = CH_2CHMe_2$
26 $R^1 = R^2 = CMe_3$

compd	K_{gas}	K_{aq}	pK_a (H_{prox})[b]	pK_a ($H_α$)[b]	pK_a (H_{dist})[b]
7	0.6	0.6		26.1	
8	0.1	0.5		26.1	
9	0.7	0.7		26.1	
10	0.01	0.1		26.4	
11	13	7	28.6		18.4
12	3	6	27.5		18.4
13	33	26	28.2		18.2
14	0.3	0.7	26.1		19.5
15	5	11	29.6		17.3
16	0.4	6	28.9		17.4
17	11	41	29.4		17.2
18	0.01	0.1	27.1		16.5
19	13	8	28.6		17.6
20	4	7	27.6		17.4
21	350	210	28.4		17.1
22	0.4	1	26.6		17.5
23	0.4	3	28.8		16.5
24	0.001	0.007	27.0		14.9
25	0.2	1.7	28.4		16.0
26	0.0002	0.0001	24.3		13.3

[a] At 298 K. Gas-phase calculations were performed at the G3MP2(+)//B3 level, and aqueous solvation free energies were computed using the CPCM-UAKS continuum model at the B3LYP/6-31+G(d) level. [b] Refers to the *cis*-isomer.

Scheme 2



conformations. Consistent with NMR data of related compounds,[28,29] B3LYP/6-31+G(d) calculations of such species in the present work show that they adopt a boat conformation in which the substituents of the *cis*-isomer are both in axial positions, relatively free of steric interactions. By comparison, one of the α-substituents in the *trans*-diastereomer occupies an equatorial position, with resulting unfavorable interactions with the adjacent amide carbonyl and the nearby amide nitrogen substituent. With secondary $(CHMe_2)$ and, more particularly, tertiary (CMe_3) alkyl substituents, their increased steric bulk destabilizes the *cis*-boat conformation, to the extent that the *trans*-isomer is strongly preferred for compounds **18**, **24**, and **26**. The trends are apparent from both the gas- and solution-phase data and for the *N*-unsubstituted compounds **7**−**10** as well as the *N,N*′-disubstituted species **11**−**26**, although the *N*-methyl and acetyl substituents of compounds **11**−**26** amplify the effects. The distal effect of an *N*-acetyl group is not only retained with the α, α′-disubstituted diketopiperazines **11**−**26**, where the pK_a of H_{dist} ranges from 13.3−19.5 and is from 6.6−12.4 units more acidic than H_{prox} in the same compound, generally the difference between the acidity of H_{dist} and H_{prox} is actually 2−3 units larger with the α,α′-disubstituted diketopiperazines **11**−**26** than with the α,α′-unsubstituted analogue **6**. This is presumably due to steric interactions between the α-substituents and the *N*-acetyl group that destabilize the proximal enolates.

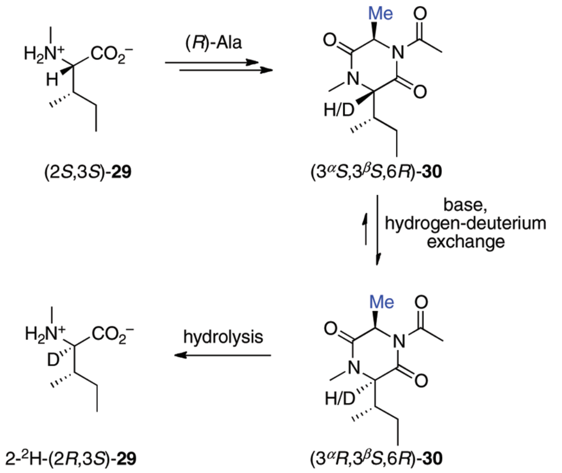
On the basis of the above considerations, we chose to verify Scheme 1 by carrying out the inversion of (*S*)-*N*-methylleucine [(*S*)-**27**] and (*S*)-*N*-methylalanine [(*S*)-**28**] to produce the corresponding α-deuterated (*R*)-enantiomers 2-^{2}H-(*R*)-**27** and 2-^{2}H-(*R*)-**28** (Scheme 2). As discussed above, the calculations show that amino acids of this type with a methyl or primary alkyl side chain are most suitably matched with chiral auxiliaries also having a methyl or primary alkyl side chain, in order to obtain high *cis/trans* ratios from equilibration of the corresponding diketopiperazines. In such cases the *cis*-diastereomer is thermodynamically more stable, so the process was expected to work most efficiently by first preparing the less stable *trans*-isomer, allowing α-deuteration and epimerization to produce the *cis*-form, and then isolating the inverted amino acid from that species. The (*R*)-enantiomers of leucine and alanine were therefore selected as the chiral auxiliaries.

In the event, the (*S*)-*N*-methylamino acids (*S*)-**27** and (*S*)-**28** were coupled with (*R*)-leucine and (*R*)-alanine to give dipeptides that were cyclized. The cyclized products were acetylated to give the diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11**, each as a single diastereomer. The epimerization reactions were carried out under the conditions used for the hydrogen−deuterium exchange experiments, with DBU as the base in acetone-d_6 as the deuterium source. The reactions were monitored using ^{1}H NMR spectroscopy which showed conversion of the *trans*-diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11** to equilibrium mixtures with their *cis*-diastereomers (3*R*,6*R*)-**21**, **13**, and **11**, in which the latter dominated, and highly regioselective but slower deuteration at C-3 of both diastereomers (directly analogous epimerization without deuteration was observed when unlabeled acetone was used instead of acetone-d_6). At equilibrium after deuteration, the *cis/trans* ratios of the diketopiperazines **21**, **13**, and **11** were determined by ^{1}H NMR spectroscopy to be 16, 20, and 7, respectively, in general accord with the corresponding calculated values. At 323 K, the rate constants for conversion of the undeuterated *trans*-diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11** to their *cis*-isomers (3*R*,6*R*)-**21**, **13**, and **11** were determined to be 0.46, 0.26, and 0.26 $M^{-1}\ s^{-1}$, respectively, while those for C-3 deuteration of the *cis*-diketopiperazines (3*R*,6*R*)-**21**, **13**, and **11** were recorded as 0.023, 0.034, and 0.059 $M^{-1}\ s^{-1}$. It was impractical to delineate other rate constants due to competing processes. The observation that epimerization was faster than deuteration indicates that DBU abstracts a proton from the diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11** and delivers it to the opposite face of the corresponding enolates faster than hydrogen−deuterium exchange with the solvent. That exchange presumably involves DBU-$^2H^+$ being formed by deuterium extraction from the acetone-d_6 and then delivering deuterium to the diketopiperazine anions, with DBU-$^1H^+$ neutralizing the acetone enolate to complete the process.

To complete the syntheses, the deuterated *cis*-diketopiperazines 3-^{2}H-(3*R*,6*R*)-**21**, **13**, and **11** were separated from their *trans*-isomers 3-^{2}H-(3*S*,6*R*)-**21**, **13**, and **11** through chromatography and hydrolyzed using 6 M hydrochloric acid. Analysis of the products using HPLC, after derivatization with (*S*)-N^{α}-(2,4-dinitro-5-fluorophenyl)alaninamide or -leucinamide[30] and by comparison with known standards, showed that α-deuterated (*R*)-*N*-methylleucine [2-^{2}H-(*R*)-**27**] was obtained in 99% and 95% enantiomeric excess from the diketopiperazines 3-^{2}H-(3*R*,6*R*)-**21** and 3-^{2}H-(3*R*,6*R*)-**13**, respectively, while α-deuterated (*R*)-*N*-methylalanine [2-^{2}H-(*R*)-**28**] was produced in 98% enantiomeric excess from the diketopiperazine 3-^{2}H-(3*R*,6*R*)-**11**. This establishes the stereochemical integrity of the *trans*-diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11** and their *cis*-isomers (3*R*,6*R*)-**21**, **13**, and **11** and shows that epimerization is limited to the C-3 position, thus confirming that the distal effect of the acetyl group to increase acidity in these the systems is highly regioselective and has practical utility.

While a variety of other applications of this chemistry may be anticipated, including stereocontrolled synthesis of *N*-methylamino acids by α-alkylation of sarcosine derivatives, one is illustrated in Scheme 3. Coupling (2*S*,3*S*)-*N*-methylisoleucine [(2*S*,3*S*)-**29**] with (*R*)-alanine gave a dipeptide that was cyclized, and the cyclized product was acetylated to give the *trans*-diketopiperazine $(3^{\alpha}S,3^{\beta}S,6R)$-**30**. Treatment with DBU in acetone-d_6 afforded, at equilibrium, a 5:1 mixture of the C-3 deuterated *cis*- and *trans*-diketopiperazines 3-^{2}H-$(3^{\alpha}R,3^{\beta}S,6R)$-**30** and 3-^{2}H-$(3^{\alpha}S,3^{\beta}S,6R)$-**30**. Interestingly, in this case, there was no epimerization without deuteration. At 323 K, the rate constant for conversion of the *trans*-diketopiperazine $(3^{\alpha}S,3^{\beta}S,6R)$-**30** to the deuterated

Scheme 3



isomer 3-^{2}H-(3$^{\alpha}$*R*,3$^{\beta}$*S*,6*R*)-**30** was determined to be 0.0019 M^{-1} s^{-1}, much lower than the corresponding rate constants for reactions of the diketopiperazines (3*S*,6*R*)-**21**, **13**, and **11**. This slower reaction of the diketopiperazine (3$^{\alpha}$*S*,3$^{\beta}$*S*,6*R*)-**30** can be attributed to increased steric bulk around the reaction center and provides more opportunity for the protonated DBU to dissociate from the diketopiperazine anion, and DBU-$^{2}H^{+}$ to then deliver deuterium to the anion's opposite face. The deuterated *cis*-diketopiperazine 3-^{2}H-(3$^{\alpha}$*R*,3$^{\beta}$*S*,6*R*)-**30** was separated from its isomer 3-^{2}H-(3$^{\alpha}$*S*,3$^{\beta}$*S*,6*R*)-**30** through chromatography and hydrolyzed using 6 M hydrochloric acid. Analysis of the product by HPLC, as described above, showed that α-deuterated (2*R*,3*S*)-*N*-methylalloisoleucine [2-^{2}H-(2*R*,3*S*)-**29**] was produced as a single diastereomer in 97% enantiomeric excess (in this and the other cases described above the upper limit on the enantiomeric excess is primarily determined by the optical integrity of the starting materials). Numerous methods are available for the diastereoselective synthesis of amino acids having chiral centers at C-2 and C-3.[27,31,32] Where these produce the opposite diastereomer to that which is needed, the approach illustrated above provides a way to access the required material through controlled stereochemical inversion regiospecifically at C-2.

The stereocontrolled synthesis of *N*-methylamino acids as their α-deuterated forms was a matter of convenience in this study, since the conditions for epimerization allowed for deuterium incorporation. Nevertheless, it illustrates another benefit of the general synthetic methods, as lack of stereochemical integrity is a common problem with other routes for the preparation of α-deuterated amino acids and their derivatives. There is no reason to expect that the methodology is limited to the synthesis of deuterated amino acids, or to the stereocontrolled syntheses exemplified above but, in any event, the results as presented confirm that acetylation and sulfonylation of amide nitrogen increases the acidity of the distal α-carbonyl protons, making them more acidic than the α-protons of a ketone. They also show that the magnitude and regioselectivity of the distal effect is sufficient to be employed in asymmetric synthesis.

■ EXPERIMENTAL SECTION

Theoretical Procedures. Standard ab initio molecular orbital theory and density functional theory calculations were performed with Gaussian 03[33] and Gaussian 09.[34] Systematic conformer searches were carried out at the B3LYP/6-31+G(d) level, and the resulting global minimum structures and harmonic frequencies were also determined at this level. The scale factors[35] for B3LYP/6-31G(d) vibrational frequencies have been used with electronic energies obtained at various levels of theory for the free energy calculations. Gas-phase electronic energies were calculated using the G3MP2(+) composite procedure. This approach is a variant of the G3MP2[36] method in which calculations with the 6-31G(d) basis set have been replaced with the 6-31+G(d) basis set. For the larger compounds in Table 2, where this approach was not feasible, the energies were instead calculated using an ONIOM approximation[37,38] (see the Supporting Information for further details).

The condensed-phase calculations were carried out via a thermodynamic cycle that combines the gas-phase free energies with solvation free energies from continuum models to yield the solution free energies. In calculating the aqueous p*K*$_a$s, an isodesmic proton-exchange scheme was further employed to maximize error cancellation in the gas and solvation calculation, as previously described.[20] MeCOMe (experimental methyl p*K*$_a$ = 19.2),[39] NH_2COMe (experimental methyl p*K*$_a$ = 28.4),[40] and $MeCONHCH_2CONH_2$ (experimental methylene p*K*$_a$ = 23.9)[16] were used as reference carbon acids for the p*K*$_a$ calculations for diisopropyl ketone (**1**), the acetamides **2**−**4**, and the diketopiperazines **5**−**26**, respectively. Based on earlier assessment studies, these predicted constants have an uncertainty of 1−2 units.[17,18]

Solvation calculations were performed using the conductor-polarizable continuum model (CPCM),[41,42] applied at the B3LYP/6-31+G(d) level of theory with the UAKS cavities. All solvation calculations were carried out on solution phase optimized geometries by relaxing the gas-phase structure in the presence of solvent. Unless otherwise noted, the energy correction term (accounting for geometry changes going from gas into solution)[43] to the solvation free energy is neglected because geometrical changes going from gas to solution were generally small.

General Experimental Procedures. (*S*)-*N*-Methylleucine [(*S*)-**27**] and (*S*)-*N*-methylalanine [(*S*)-**28**] were coupled to (*R*)-leucine and (*R*)-alanine by GLBiochem Ltd. to form the dipeptides (*R*)-leucyl-(*S*)-*N*-methylleucine, (*R*)-alanyl-(*S*)-*N*-methylleucine, and (*R*)-alanyl-(*S*)-*N*-methylalanine. Compounds **3**, **4**, and **6** were prepared by acetylation of methylacetamide and methylmethanesulfonamide and by cyclization and acetylation of glycylsarcosine, respectively, using literature methods.[44,45]

Hydrogen−Deuterium Exchange Reactions of Compounds 1−6. Compounds **1**−**6** were each dissolved in acetone-*d*$_6$ (ca. 0.08 M) and Et_3N or DBU was added. The amount of base added was varied between 0.004−0.8 M according to the CH acidity of the compound and the strength of the base, in order to obtain practical reaction rates. The solutions were incubated at either 308 or 323 K and periodically monitored via ^{1}H NMR spectroscopy. Reactions of mixtures of compounds **1**−**6** were carried out in an identical fashion in order to confirm relative reaction rates. The spectrum of compound **2** did not change under these conditions, even after 56 days. Exchange of the methine protons of diisopropyl ketone (**1**) was observed by loss of the doublet resonance at δ 1.02 ppm (*J* = 5.4 Hz), corresponding to the methyl protons, and appearance of a triplet resonance at δ 1.01 ppm (*J* = 0.9 Hz). For H_{dist} of compounds **3** and **4**, exchange resulted in loss of the singlet resonances at δ 2.34 and 2.35 ppm, respectively, with initial appearance of multiplet resonances upfield, before further exchange. Similarly, for sarcosine anhydride (**5**), the singlet resonance at δ 3.93 ppm, corresponding to the methylene protons, decreased in intensity with time, and was initially replaced by an upfield triplet at δ 3.91 (*J* = 2.4 Hz) before further deuteration. For compound **6**, exchange of H_{dist} and H_{exo} was seen from the loss of singlet resonances at δ 4.22 and 2.47 ppm. The signal corresponding to H_{prox} at δ 4.27 ppm did not change unless a much higher concentration of base was used, resulting in competing deacetylation of the substrate **6**. The ^{1}H NMR spectra of compounds

1−**6** before and after hydrogen−deuterium exchange are provided in the Supporting Information.

From the percentage decrease in intensity of ^{1}H NMR signals as a function of time (R), hydrogen−deuterium exchange rate constants were determined according to the following rate law:

$$R = k_{ex}[\text{base}][\text{substrate}] \qquad (1)$$

Normalization for the concentration of base was used to calculate rate constants (k_{ex}) that are shown in Table 1 along with relative exchange rates (k_{rel}). Where no exchange was observed, the upper limits on k_{rel} are based on a predicted limit of detection of 5% exchange, corresponding to exchange rate constants of $<1.2 \times 10^{-8}$ and $<1.2 \times 10^{-6}$ M^{-1} s^{-1} for H_{dist} of compound **2** and H_{prox} of compound **6**, respectively.

Preparation of α,α′-Disubstituted Diketopiperazines. *(3S,6R)-1-Acetyl-3,6-di(2-methylprop-1-yl)-4-methylpiperazine-2,5-dione [(3S,6R)-**21**].* A suspension of (R)-leucyl-(S)-N-methylleucine (0.40 g, 1.5 mmol) in toluene (10 mL) was heated at reflux under nitrogen for 14 h, before it was cooled and concentrated under reduced pressure, to give a pale yellow waxy residue. This residue was dissolved in acetic anhydride (5 mL), and the solution was heated at reflux under nitrogen for 3 h before it was cooled and concentrated under reduced pressure. The crude product was chromatographed on silica (EtOAc/DCM) to give the title compound (3S,6R)-**21** as a pale yellow solid (0.36 g, 82%): mp 87−88 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.04 (dd, J = 7.6, 5.4 Hz, 1H), 4.05 (dd, J = 6.0, 4.1 Hz, 1H), 2.97 (s, 3H), 2.47 (s, 3H), 1.93−2.05 (m, 2H), 1.55−1.77 (m, 4H), 0.97 (d, J = 5.7 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.1, 170.5, 167.4, 60.4, 54.9, 43.2, 39.9, 31.4, 26.7, 24.9, 24.8, 23.3, 23.2, 22.4, 22.0; HRMS (ESI$^+$) (m/z) [M + Na$^+$] calcd for $C_{15}H_{26}N_2O_3Na$ 305.1841, found 305.1841 (0.0 ppm, 0.0 mDa).

*(3S,6R)-1-Acetyl-4,6-dimethyl-3-(2-methylprop-1-yl)piperazine-2,5-dione [(3S,6R)-**13**].* The title compound (3S,6R)-**13** was prepared from (R)-alanyl-(S)-N-methylleucine, as described above for the preparation of compound (3S,6R)-**21**, and obtained as a pale yellow solid: mp 101−102 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.93 (q, J = 6.9 Hz, 1H), 4.03 (dd, J = 6.5, 3.9 Hz, 1H), 2.98 (s, 3H), 2.48 (s, 3H), 1.95 (m, 2H), 1.75 (m, 1H), 1.48 (d, J = 6.9 Hz, 3H), 0.96 (d, J = 6.6 Hz, 2H), 0.93 (d, J = 6.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.5, 169.8, 167.7, 60.9, 53.0, 40.5, 31.8, 26.9, 24.8, 23.4, 22.4, 20.6; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_{12}H_{21}N_2O_3$ 241.1550, found 241.1552 (−0.8 ppm, −0.2 mDa).

*(3S,6R)-1-Acetyl-3,4,6-trimethylpiperazine-2,5-dione [(3S,6R)-**11**].* The title compound (3S,6R)-**11** was prepared from (R)-alanyl-(S)-N-methylalanine, as described above for the preparation of compound (3S,6R)-**21**, and obtained as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.02 (q, J = 7.1 Hz, 1H), 4.13 (q, J = 7.0 Hz, 1H), 2.98 (s, 3H), 2.50 (s, 3H), 1.63 (d, J = 6.9 Hz, 3H), 1.43 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.5, 169.7, 168.3, 56.4, 52.7, 30.3, 27.2, 18.4, 16.7; HRMS (ESI$^+$) (m/z) [M + Na$^+$] calcd for $C_9H_{14}N_2O_3Na$ 221.0903, found 221.0902 (0.5 ppm, 0.1 mDa).

*($3^{\alpha}S,3^{\beta}S$,6R)-1-Acetyl-4,6-dimethyl-3-(2-methylprop-1-yl)piperazine-2,5-dione [($3^{\alpha}S,3^{\beta}S$,6R)-**30**].* (R)-Alanyl-(2S,3S)-N-methylisoleucine methyl ester was obtained by coupling (2S,3S)-N-methylisoleucine methyl ester with Boc-(R)-alanine using the BOP coupling reagent, followed by treatment with TFA, according to standard procedures.[46] The title compound ($3^{\alpha}S,3^{\beta}S$,6R)-**30** was then prepared as described above for the preparation of compound (3S,6R)-**21** and obtained as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.83 (q, J = 6.9 Hz, 1H), 3.97 (d, J = 3.3 Hz, 1H), 2.96 (s, 3H), 2.44 (s, 3H), 2.08 (m, 1H), 1.62 (m, 1H), 1.50 (d, J = 6.6 Hz, 3H), 1.46 (m, 1H), 1.00 (t, J = 7.5 Hz, 3H), 0.84 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 170.9, 168.2, 167.4, 66.4, 53.3, 38.5, 32.7, 26.7, 26.6, 21.6, 13.6, 12.4; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_{12}H_{21}N_2O_3$ 241.1552, found 241.1552 (0.0 ppm, 0.0 mDa).

Deuteration and Stereochemical Inversion of α,α′-Disubstituted Diketopiperazines. *3-^{2}H-(3R,6R)-1-Acetyl-3,6-di(2-methylprop-1-yl)-4-methylpiperazine-2,5-dione [3-^{2}H-(3R,6R)-(**21**)].* Compound (3S,6R)-**21** (60 mg, 0.21 mmol) was dissolved in acetone-d_6 (0.8 mL) and DBU (1 μL, 7 μmol), and the mixture was maintained at 323 K. ^{1}H NMR spectroscopy showed that the starting material (3S,6R)-**21** [δ 4.90 (dd, J = 8.1, 5.4 Hz, 1H), 4.31 (dd, J = 6.6, 3.6 Hz, 1H), 2.96 (s, 3H), 2.42 (s, 3H), 2.04 (m, 2H), 1.83 (m, 1H), 1.70 (m, 2H), 1.54 (m, 1H), 0.96 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 7.2 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H)] initially converted to a mixture with the nondeuterated *cis*-diastereomer (3R,6R)-**21** [δ 4.89 (dd, J = 9.3, 5.7 Hz, 1H), 4.14 (dd, J = 8.7, 5.1 Hz, 1H), 2.95 (s, 3H), 2.46 (s, 3H), 1.47−2.00 (m, 6H), 1.04 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H)] and then to a 16:1 mixture of the deuterated analogues 3-^{2}H-(3R,6R)-**21** [δ 4.89 (dd, J = 9.3, 5.7 Hz, 1H), 2.95 (s, 3H), 2.46 (s, 3H), 1.47−2.00 (m, 6H), 1.04 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H)] and 3-^{2}H-(3S,6R)-**21**. Addition of HCl (5 μL, 2 M, 10 μmol) followed by concentration of the solution and chromatography of the residue on silica gel (EtOAc/DCM) yielded the α-deuterated *cis*-diketopiperazine 3-^{2}H-(3R,6R)-**21** as a colorless oil (45 mg, 75%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.03 (dd, J = 9.2, 6.0 Hz, 1H), 2.97 (s, 3H), 2.53 (s, 3H), 1.78−2.00 (m, 3H), 1.47−1.69 (m, 3H), 1.04 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.7, 170.0, 166.7, 61.8 (t, J = 86 Hz), 54.9, 44.9, 43.8, 32.6, 27.6, 25.7, 25.4, 23.3, 23.0, 22.0, 21.8; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_{15}H_{26}DN_2O_3$ 284.2084, found 284.2086 (0.7 ppm, 0.2 mDa).

*3-^{2}H-(3R,6R)-1-Acetyl-4,6-dimethyl-3-(2-methylprop-1-yl)piperazine-2,5-dione [3-^{2}H-(3R,6R)-**13**].* The title compound 3-^{2}H-(3R,6R)-**13** was prepared from its nondeuterated *trans*-diastereomer (3S,6R)-**13**, as described above for the preparation of compound 3-^{2}H-(3R,6R)-**21**, and obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.96 (q, J = 7.2 Hz, 1H), 2.97 (s, 3H), 2.54 (s, 3H), 1.62−1.96 (m, 3H), 1.52 (d, J = 7.2 Hz, 3H), 1.04 (d, J = 6.3 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.9, 169.0, 167.6, 61.8 (t, J = 84 Hz), 53.0, 43.3, 32.7, 27.8, 25.4, 23.0, 21.9, 20.2; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_{12}H_{20}DN_2O_3$ 242.1615, found 242.1613 (−0.8 ppm, −0.2 mDa).

*3-^{2}H-(3R,6R)-1-Acetyl-3,4,6-trimethylpiperazine-2,5-dione [3-^{2}H-(3R,6R)-**11**].* The title compound 3-^{2}H-(3R,6R)-**11** was prepared from its nondeuterated *trans*-diastereomer (3S,6R)-**11**, as described above for the preparation of compound 3-^{2}H-(3R,6R)-**21**, and obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.97 (q, J = 7.2 Hz, 1H), 2.97 (s, 3H), 2.54 (s, 3H), 1.58 (apparent s, 3H), 1.51 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.8, 169.9, 167.1, 59.2 (t, J = 86.4 Hz), 52.8, 31.9, 27.6, 20.4, 18.6; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_9H_{14}DN_2O_3$ 200.1145, found 200.1138 (−3.5 ppm, −0.7 mDa).

*3-^{2}H-($3^{\alpha}R,3^{\beta}S$,6R)-1-Acetyl-4,6-dimethyl-3-(2-methylprop-1-yl)piperazine-2,5-dione [3-^{2}H-($3^{\alpha}R,3^{\beta}S$,6R)-**30**].* The title compound 3-^{2}H-($3^{\alpha}R,3^{\beta}S$,6R)-**30** was prepared from its nondeuterated diastereomer ($3^{\alpha}S,3^{\beta}S$,6R)-**30**, as described above for the preparation of compound 3-^{2}H-(3R,6R)-**21**, and obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.96 (q, J = 7.1 Hz, 1H), 3.00 (s, 3H), 2.54 (s, 3H), 2.00 (m, 1H), 1.70 (m, 1H), 1.55 (d, J = 7.2 Hz, 3H), 1.38 (m, 1H), 1.03 (d, J = 6.9 Hz, 3H), 0.98 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 172.1, 168.0, 167.7, 68.4 (t, J = 86 Hz), 53.2, 39.5, 35.0, 28.1, 26.6, 19.8, 16.1, 11.8; HRMS (ESI$^+$) (m/z) [M + H$^+$] calcd for $C_{12}H_{20}DN_2O_3$ 242.1615, found 242.1613 (−0.8 ppm, −0.2 mDa).

Diketopiperazine Hydrolysis and Stereochemical Analysis of the Product *N*-Methylamino Acids. The deuterated diketopiperazines 3-^{2}H-(3R,6R)-**21**, 3-^{2}H-(3R,6R)-**13**, 3-^{2}H-(3R,6R)-**11**, and 3-^{2}H-($3^{\alpha}R,3^{\beta}S$,6R)-**30** were each heated at reflux in 6 M HCl for 12 h. Evaporation of the solutions afforded crude hydrolysates that were analyzed directly using Marfey's method,[30] with (S)-N^{α}-(2,4-dinitro-5-fluorophenyl)alaninamide as the derivatizing agent in the cases of 2-^{2}H-(R)-N-methylleucine [2-^{2}H-(R)-**27**] and 2-^{2}H-(2R,3S)-N-methylalloisoleucine

[2-^{2}H-(2*R*,3*S*)-**29**] and with (*S*)-*N*$^{\alpha}$-(2,4-dinitro-5-fluorophenyl)leucinamide in the case of 2-^{2}H-(*R*)-*N*-methylalanine [2-^{2}H-(*R*)-**28**]. HPLC analysis of the derivatives was performed on a C18 column (4.6 × 250 mm), eluting with an isocratic (40:60) mixture of 1% TFA/acetonitrile and 1% TFA/water, monitoring at 350 nm. Peaks were assigned by comparison with those of authentic samples.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Full computational results, including Gaussian archive entries for B3LYP/6-31+G(d)-optimized geometries, ^{1}H NMR and ^{13}C NMR spectra of all new compounds, ^{1}H NMR spectra of hydrogen−deuterium exchange and epimerization reactions, and details of the derivation of kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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