JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Nanomole-Scale Assignment of Configuration for Primary Amines Using a Kinetic Resolution Strategy

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Supporting Information

ABSTRACT: The absolute configurations of primary amines were assigned using a kinetic resolution strategy with Mioskowski's enantioselective **1**-(*R*,*R*) and **2**-(*S*,*S*) acylating agents. A simple mnemonic was developed to determine the configuration. A pseudoenantiomeric pair of reagents, **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3, was prepared and used to assay primary amines on a micromolar scale. The ESI-MS readout of the resulting acetamide products reproduced the selectivity factors from kinetic experiments. The method can be used on mixtures of amines and was validated with amine samples as small as 50 nmol.

Isolation of natural products has reached the point where nanomole-scale structure assignments are becoming feasible.[1] Except for special cases (e.g., α-amino acids)[2] the assignment of absolute configuration on nanomole samples is an unsolved problem. We report a new strategy for the assignment of primary amines that is effective on micromole-down to nanomole-scale samples and can be applied to mixtures of amines.

Amines are often found in natural products, compounds of medicinal interest, and synthetic intermediates.[3] Direct methods to assign the absolute configuration of amines usually involve derivatization and NMR analysis of the resulting amides,[4] but other methods have been developed.[2,5] We have developed a new method[6] inspired by Horeau's strategy for determining the configuration of optically pure alcohols using a strategy based on kinetic resolution.[7] The method uses Mioskowski's enantioselective acetylating reagents **1**-(*R*,*R*) (Figure 1) and **2**-(*S*,*S*).[8] It is easy to implement, shows a broad scope for different primary amines, and is extraordinarily sensitive.

Initial studies to establish the feasibility of the method are presented in Figure 1. (*R*)-1-(1-Naphthyl)ethylamine (**3**) was treated with reagent **1**-(*R*,*R*) or **2**-(*S*,*S*) in an NMR tube in $CDCl_3$, and the conversion to acetamide **4** was followed by integrating the NMR signal of the H atom adjacent to the amine or amide. The rates of the reaction with the two enantiomeric reagents were determined, and the **2**-(*S*,*S*) reagent was preferred by a factor of 2.6. This preference is consistent with reports by Mioskowski using the reagents **1**-(*R*,*R*) and **2**-(*S*,*S*) for kinetic resolutions with similar primary amines in $CHCl_3$.[8] The reaction followed pseudo-first-order kinetics under these conditions.

The NMR analysis is straightforward but requires milligram samples of substrate and significant instrument time. Analyzing

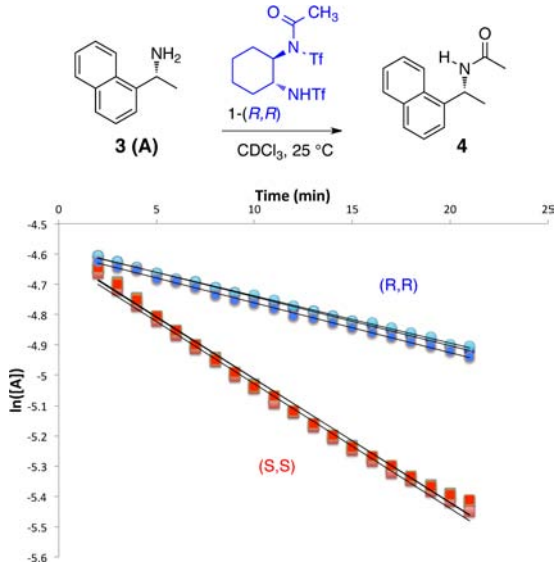


Figure 1. The rate of acetylation of amine **3** with the Mioskowski reagents **1**-(*R*,*R*) and **2**-(*S*,*S*) was found to favor the **2**-(*S*,*S*) reagent by a factor of 2.6. The ratio of starting material to product was monitored by ^{1}H NMR integration, and the experiments were done in triplicate. Each reaction used a 3-fold excess of the acylation reagent and was followed to ca. 50% conversion.

the conversion at fixed times would simplify this procedure, although the inherent sensitivity of NMR spectroscopy still limits this method.[6] In contrast, mass spectrometry (MS) is exquisitely sensitive and allows for the rapid detection of species with different masses. Therefore, we envisioned a more powerful MS-based approach using deuterium-labeled pseudoenantiomers to identify the relative rates of acetylation with reagents **1** and **2** (Figure 2). Amine **5** would react with reagent **1**-(*R*,*R*) to produce unlabeled acetamide **6**-(M), whereas reagent **2**-(*S*,*S*)-d_3 would afford deuterated acetamide **6**-(M +3). To maintain pseudo-first-order kinetics, the reagents **1** and **2** should be used in excess. This kinetic regime would allow a direct measurement of the selectivity factor by comparison of the relative ratios of **6**-$(M)^+$ and **6**-$(M+3)^+$ peaks in the MS analysis.[9] MS methods have been used previously to estimate the enantiomeric excess of compounds,[10] but rarely have they been used to assign absolute configurations.[7d] Unlike the NMR kinetics in Figure 1, this MS method uses a single rapid experiment with a simple analysis.

Received: October 28, 2012
Published: December 4, 2012

   dx.doi.org/10.1021/ja310620c | J. Am. Chem. Soc. 2012, 134, 20318−20321

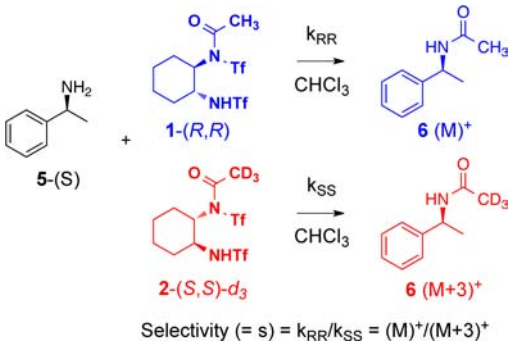


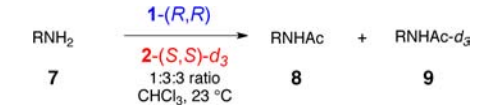
Figure 2. The selectivity for the enantioselective acylation reaction can be determined by using the pseudoenantiomeric Mioskowski reagents **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3 and analyzing the acylated product **6** by ESI-MS.

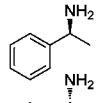
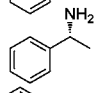
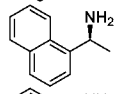
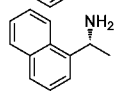
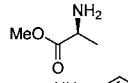
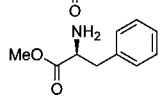
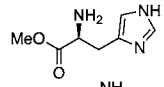
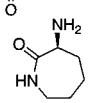
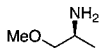
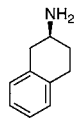
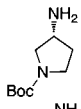
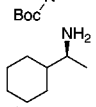
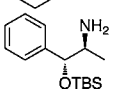
The deuterated reagent **2**-(*S*,*S*)-d_3 was prepared using a modified version[11] of Mioskowski's procedure.[8a] A 1:3:3 ratio of amine, **1**-(*R*,*R*), and **2**-(*S*,*S*)-d_3 gave reproducible kinetics and was used in the experiments. The two acylation reagents are stable crystalline solids and do not exchange acetyl groups. A mixture of the solid reagents was stable indefinitely, and it was dissolved in chloroform to produce the stock solutions used in these experiments. In the standard procedure, a solution containing 0.5 μmol of amine was combined with the mixture of reagents **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3 in a total volume of 50 μL of $CHCl_3$ and allowed to stand for 60 min, at which point the reaction was quenched with methanol. The mixture was then analyzed directly by electrospray ionization (ESI) MS.[12] The results are shown in Tables 1 and 2.

Despite the modest selectivities of the reagents, clear differences in the MS peak intensities, coupled with small standard deviations, allowed for the confident assignment of the absolute configurations of a variety of amines (Table 1). The selectivity factors ranged from 1.12 to 6.6 for the examples shown. Multiple trials were run for each amine, and the number of trials and the standard deviation are listed for each entry in the table. In most cases, the standard deviation was very small (<0.1). In a few cases, the standard deviation was larger (entries 6 and 8), but these examples corresponded to unusually high selectivity and did not present any ambiguity in the analysis. Enantiomeric pairs showed the expected complementary reactivities (entries 1 and 2 and entries 3 and 4).[13] Perhaps the largest source of error was deviation of the **1**:**2** ratio in the stock solutions.[14] The results for amino alcohol substrates are gathered in Table 2. The range of selectivities was similar to that for the nonprotic substrates in Table 1.

A mnemonic for assigning the absolute configuration of the amine from the observed selectivity with the reagents **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3 is presented in Figure 3. In each case, the "large" group is drawn to the left and the "small" group is drawn to the right. When **1**-(*R*,*R*) reacts faster, the amine is forward, and when **2**-(*S*,*S*)-d_3 reacts faster, the amine is back. The amines in Table 1 are oriented to match the mnemonic in Figure 3 (with the large group to the left and the small group to the right). The group assignments are empirical. Aromatic rings and carbonyls, both of which contain sp^2 carbons, behave as large groups. Alcohols (Table 2) behave as small groups, even when they carry substituents (Table 2, entry 6). Protected alcohols (Table 1, entries 9, 11, and 14) behave as bulky substituents and show a small preference for the large side. Aliphatic groups show only very modest preferences (Table 1, entry 13).

Table 1. Determination of the Absolute Configurations of Chiral Primary Amines by Enantioselective Protio/Deuteroacetylation and ESI-MS Analysis[a]



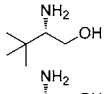
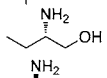
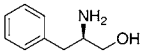
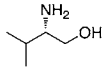
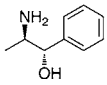
entry	amine	M+ intensity[b]	:	(M+3)+ intensity[b]	# trials/σ[c]
1		**2.60**	:	1	5 / 0.08
2		1	:	**2.68**	5 / 0.03
3		**3.13**	:	1	5 / 0.03
4		1	:	**3.38**	5 / 0.08
5		**3.67**	:	1	3 / 0.04
6		**4.16**	:	1	3 / 0.12
7		**1.28**	:	1	3 / 0.05
8		**6.62**	:	1	3 / 0.16
9		**1.37**	:	1	3 / 0.02
10		**1.36**	:	1	3 / 0.02
11		**1.21**	:	1	5 / 0.02
12		1	:	**1.18**	3 / 0.01
13		**1.13**	:	1	3 / 0.02
14		**1.12**	:	1	3 / 0.01

[a]The amine (0.01 M) and the acylating agents **1** and **2** were combined in a 1:3:3 molar ratio in 50 μL of $CHCl_3$. After 60 min, the mixture was diluted with MeOH and analyzed by ESI-MS. [b]The sodium peaks (M+23) were analyzed in all cases. [c]The standard deviation (σ) for the multiple runs is included.

Remote substituents influence the selectivity in a modest but predictable fashion (Table 1, entries 10 and 12). These examples all follow the mnemonic in Figure 3, but caution should be exercised in interpreting very small selectivities; we consider any selectivity less than 1.2:1 to be inconclusive. In most cases, the assignments of the small and large groups are obvious and, combined with the relative reactivity observed with the reagents **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3, lead to direct and

dx.doi.org/10.1021/ja310620c | J. Am. Chem. Soc. 2012, 134, 20318−20321

Table 2. Absolute Configurations of α-Amino Alcohols[a]

entry	amine	M^+ intensity[b] : $(M+3)^+$ intensity[b]	# trials/σ[c]
1		1 : **5.44**	3 / 0.25
2		1 : **1.96**	5 / 0.04
3		**1.36** : 1	5 / 0.03
4		**2.30** : 1	3 / 0.02
5		1 : **2.87**	3 / 0.02
6		**1.22** : 1	3 / 0.02

[a−c]See the corresponding footnotes in Table 1.

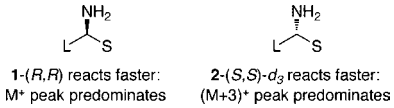


Figure 3. Mnemonic for assigning the absolute configuration of a primary amine on the basis of its relative reactivity with the acylating agents **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3. Adjacent alcohols behave as small groups, and aryls and esters behave as large groups.

unambiguous assignments of the absolute configuration for primary amines.

The origins of the selectivity in the acylation reaction have not been defined, and the analysis is complicated by a large solvent effect observed for these reagents.[8] The rate-determining step is likely the formation of the tetrahedral intermediate, but there may be exceptions to this proposal.[8b] The structure of the favored neutral intermediate formed by the reaction of (*R*)-α-methylbenzylamine with **2**-(*S*,*S*) is reproduced in Figure 4.[15] The faster-reacting diastereomeric pair, the

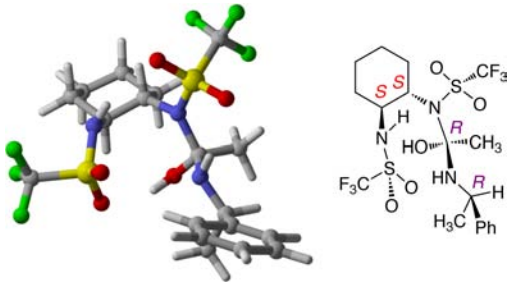
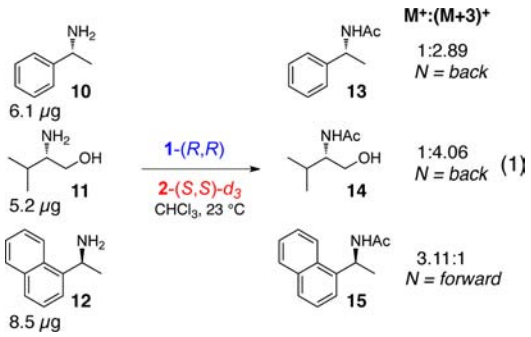


Figure 4. The most stable tetrahedral intermediate for the acylation of (*R*)-α-methylbenzylamine with **1**-(*R*,*R*) or **2**-(*S*,*S*) is shown. The **2**-(*S*,*S*) reagent and the *R* amine are the observed faster-reacting pair. Calculations were performed at the B3LYP/6-31G(d) level.[15]

R amine and the **2**-(*S*,*S*) reagent, corresponds to the lower-energy intermediate. The special role of hydroxyl groups as "small" groups may result from hydrogen bonding with hydrogen-bond acceptors in the transition state. To characterize the important intermediates and transitions states in this process more effectively, we will continue to investigate the reaction by computational and experimental methods.

The standard experiment uses 0.5 μmol (ca. 75 μg) of amine for each determination, but the method itself can be much more sensitive (eq 1). To demonstrate the potential of this



approach, a mixture containing 50 nmol (<10 μg) of each of the three amines **10**−**12** was analyzed using the standard protocol with **1**-(*R*,*R*) and **2**-(*S*,*S*)-d_3 (1.5 μmol each) in 50 μL of solution. ESI-MS analysis allowed the selectivity and thus the absolute configuration to be defined for all three amines in a single experiment.

We have developed a new method for assigning the absolute configurations of primary amines using kinetic resolution reagents. The method can be completed in less than 2 h, uses no exotic equipment beyond a standard ESI-MS instrument, and is sensitive enough to analyze nanomole samples. The method can be applied to simple mixtures of amines without prior separation. It will be useful in natural product assignments, medicinal chemistry, and synthetic chemistry. The development of kinetic resolution strategies for the determination of absolute configurations remains an active area of research in our laboratory.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparation of deuterated reagent **2**-(*S*,*S*)-d_3, the standard procedure for the configuration assignment of primary amines, representative data and analysis, and coordinates for intermediate in Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Support was provided by NSF Award CHE 1152449. Input from Dr. Viengkham Malathong and Dr. Noel Powell is gratefully acknowledged.

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(9) To compensate for having less than 100% deuterium incorporation in **2**-(*S*,*S*)-d_3, the intensities of the $(M+3)^+$ peaks were corrected by a percentage determined by reacting the substrate amine with only **2**-(*S*,*S*)-d_3 and calculating the ratio of the intensities of the resultant $(M+3)^+$ and $(M+2)^+$ peaks. See the Supporting Information for more details.

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(11) The deuterated reagent **2**-(*S*,*S*)-d_3 was prepared by acylation of the corresponding bistriflamide with CD_3COCl in the presence of pyridine. Details are provided in the Supporting Information section.

(12) The sodium-bound peaks in the mass spectrum were analyzed and found to generate more reliable ratios than the protonated peaks. For the amino alcohols, the mixture was diluted with 50 ppm NaOAc in methanol to ensure complete conversion to sodium-containing peaks in the mass spectrum.

(13) Acylation of (*S*)-α-methylbenzylamine with a 1:1 mixture of **2**-(*S*,*S*) and **2**-(*S*,*S*)-d_3 led to a H/D ratio of 1.00:1.01, which is indicative of a very small deuterium isotope ratio in the acylation reaction.

(14) Stock solutions were prepared by weighing 25.0 mg of **1** and **2** on a balance, with an uncertainty of ca. ±0.5 mg.

(15) Models were prepared from (*R*)-α-methylbenzylamine and reagent **1** or **2**, and both aminol configurations were explored. Conformational searches for all four diastereomers were performed, and the structures were subsequently optimized at the HF/3-21G and B3LYP/6-31G(d) levels. The structure shown in Figure 4 is the lowest-energy intermediate identified in the search.