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A Simple Microscale Method for Determining the Relative Stereochemistry of Statine Units

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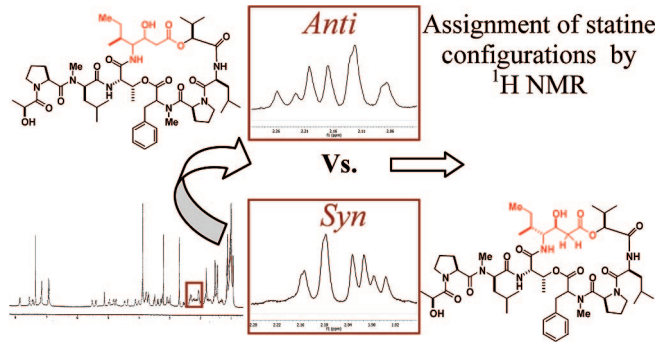
Received June 25, 2008



A simple method to determine the relative stereochemistry of statine amino acids (γ-amino-β-hydroxyacids) by using ^{1}H NMR spectroscopy is described. Configurational assignment of statine units within complex natural products is possible without degradation or derivatization as the *syn* and *anti* diastereomers can be distinguished by using a combination of chemical shift and coupling constant information derived from the α-methylene ABX system. Seventy-three examples are provided, demonstrating the scope and limitations of the methodology. These examples range in complexity from simple statine units to cyclic depsipeptides, such as tamandarin B.

Introduction

Modern structure determination of small molecules remains a challenging problem. The number of structural reassignments reported each year is testimony to the myriad of potential pitfalls.[1] While classical approaches to structure determination rely heavily on chemical degradation, most modern approaches use nondestructive techniques to provide the same connectivity information.[2] Any structure determination encompasses three discrete assignments: planar, relative, and absolute. Advances in NMR instrumentation and NMR pulse sequences have greatly simplified the assignment of the planar structure, i.e., the constitutional connectivites between the various nuclei.[3] Conversely, relative[4] and absolute stereochemical assignments[5] are becoming more challenging as the isolation of submicromolar quantities of metabolites becomes increasingly more common.[6]

Statine-containing peptides exemplify these challenges. Statine amino acids (**1**), γ-amino-β-hydroxy acids, are present in several compounds including the anticancer agents didemnin B,[7] tamandarin B,[8] and hapalosin.[9] This isostere is often associated with improved pharmacokinetic properties and increased potency.[10,11] While the planar moiety is easily

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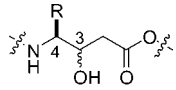
10.1021/jo8012429 CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/07/2008

identified by conventional NMR spectroscopy, there is no simple method to determine the relative configuration of the vicinal chiral centers bearing the amine and the alcohol. Traditional solutions to this problem have involved either hydrolysis and subsequent HPLC/GC comparison with synthetic standards or conversion to the oxazolidine/oxazolidinone derivative.



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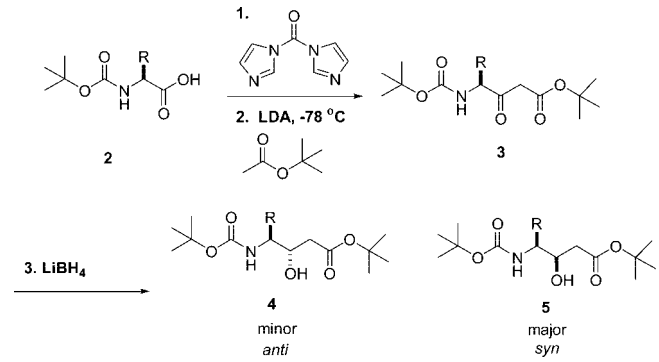
Ideally, a simple NMR-based method could be used to determine the relative configuration of the statine unit within the intact peptide. This would be analogous to the Universal NMR databases developed by Kishi for configurational assignment of polyketides, which utilizes the distinctive ^{13}C NMR chemical shift patterns of the diastereomers.[12] Several years ago we noted that it might be possible to distinguish the two diastereomers of 4-amino-3-hydroxy-5-phenylpentanoic acid using the proton signals for H_2-2,[13] but little supporting evidence was available at that time for the generality of the method.

Results and Discussion

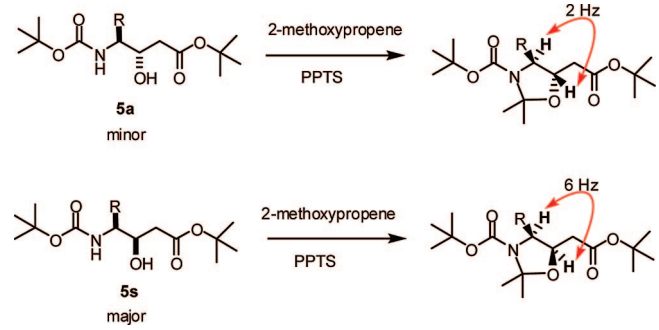
To explore these issues, we synthesized a variety of γ-amino-β-hydroxy acids derived from the proteogenic amino acids using a simple procedure (Scheme 1). The imidazole-activated Boc-amino acid was coupled with the enolate derived from *tert*-butyl acetate or allyl acetate. Subsequent reduction with lithium or sodium borohydride produced a mixture of C-3 diastereomers,[14] which were separated by normal phase chromatography to yield the protected statine units **4** and **5**. As expected, the *syn* diastereomer, formed via a chelation-controlled reduction,[14] was the major product in all cases.[15] This stereochemical outcome was confirmed by converting representative compounds **11**, **12**, **16**, **21**, and **22** to the oxazolidine derivatives (Scheme 2)[16] and analysis of the vicinal proton−proton coupling constant between the two chiral centers. The *syn* and *anti* derivatives displayed $^3J_{H4,H3}$ values of approximately 6 and 2 Hz, respectively.[17,18]

A comparison of the spectral data for the diastereomers revealed no predictive clustering of carbon or proton chemical shifts that could be used to assign the relative configuration of an unknown statine unit (Figure 1).[19] Likewise, the magnitude of the ^3J values between H-3 and H-4 did not provide a consistent means of distinguishing the respective diastereomers.[20] These results are likely due to the conformational flexibility between the two chiral centers C-3 and C-4 and are consistent with the trends observed for 1,2-diols[21] and for α,γ-dimethyl-β-hydroxycarbonyls.[22]

SCHEME 1. Synthesis of Statine Units



SCHEME 2. Conversion to the Oxazolidine Derivatives



A more detailed analysis of the NMR spectroscopic data for the diastereomeric statine derivatives revealed a diagnostic pattern associated with the methylene protons (H_2-2) though. When NMR spectra were recorded in $CDCl_3$, the ABX patterns of the α-methylene protons of the two diastereomers were essentially mirror images. For the *syn* diastereomer, the downfield proton signal of the methylene pair was consistently a doublet of doublets with a *small* vicinal coupling to H-3. Conversely, for the *anti* diastereomer, the downfield methylene proton signal displayed a *large* vicinal coupling to H-3. The upfield protons of these pairs showed the opposite pattern, i.e., the *syn* and *anti* diastereomers had large and small vicinal couplings to H-3, respectively. Figure 2 shows the AB portion of the ^1H NMR spectra of **8a** and **8b** where this trend is clearly observed. On average, the magnitude of the ^3J values between the downfield resonance of H_2-2 and H-3 was 10 Hz for the *anti* diastereomer and 2 Hz for the *syn* diastereomer (Figure 3).

Technically in a second-order ABX system, such as shown in Figure 2, only J_{AB} and the sum $J_{AX} + J_{BX}$ can be determined directly from the spectrum. In other words, exact values for J_{AX} and J_{BX}, specifically in this case $^3J_{H3,H2d}$ and $^3J_{H3,H2u}$, cannot be determined by simple subtraction of the line frequencies.[2] In practice outside a small community, this issue is largely ignored and coupling constants in second-order ABX systems are extracted as if these are first order. We have chosen to

(12) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2181–2184.
(13) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1006–1009.
(14) Harris, B. D.; Bhat, K. L.; Joullie, M. M. *Tetrahedron Lett.* **1987**, *28*, 2837–2840.
(15) In general, the *anti* diastereomer eluted first with normal phase HPLC, except for the threonine- and proline-derived statine units. While the order of elution was reversed for these two pairs of derivatives, the *syn* reduction product depicted dominates.
(16) Ori, M.; Toda, N.; Takami, K.; Tago, K.; Kogen, H. *Tetrahedron* **2005**, *61*, 2075–2104.
(17) Harris, B. D.; Joullie, M. M. *Tetrahedron* **1988**, *44*, 3489–3500.
(18) The stereochemistry of **24a** was determined by conversion to the oxazolidinone derivative through treatment with triphosgene.The stereochemistry was then assigned via analysis of the $J_{H3,H4}$ coupling constant. See: Wang, M.; Chen, Y.; Lou, L.; Tang, W.; Wang, X.; Shen, J. *Tetrahedron Lett.* **2005**, *46*, 5309–5312.
(19) A comparison of carbon and proton chemical shifts with the nature of the side chain (polar, nonpolar, aromatic, branched, etc) provided no predictive trend either.
(20) There are reports of using $^3J_{H3,H4}$ values for the relative stereochemical assignment of an Ala-Sta derivative (4-methylcarnitine: Comber, R. N.; Brouillette, W. J. *J. Org. Chem.* **1988** *53* 1121−1122), but based on our data (see the Supporting Information) this is not a general trend
(21) Higashibayashi, S.; Kishi, Y. *Tetrahedron* **2004**, *60*, 11977–11982.
(22) Heathcock, C. H.; Pirrung, M. C.; Sohn, J. E. *J. Org. Chem.* **1979**, *44*, 4294–4299.

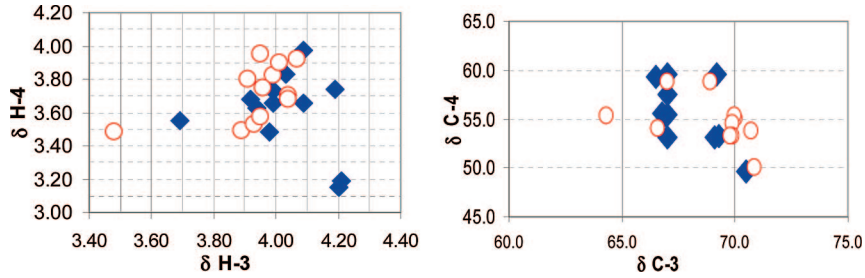


FIGURE 1. (a) Chemical shifts (ppm) for H-3 and H-4 of the *syn* (blue diamond) and *anti* (red circle) statine diastereomers. (b) Chemical shifts for C-3 and C-4 of the *syn* (blue diamond) and *anti* (red circle) statine diastereomers.

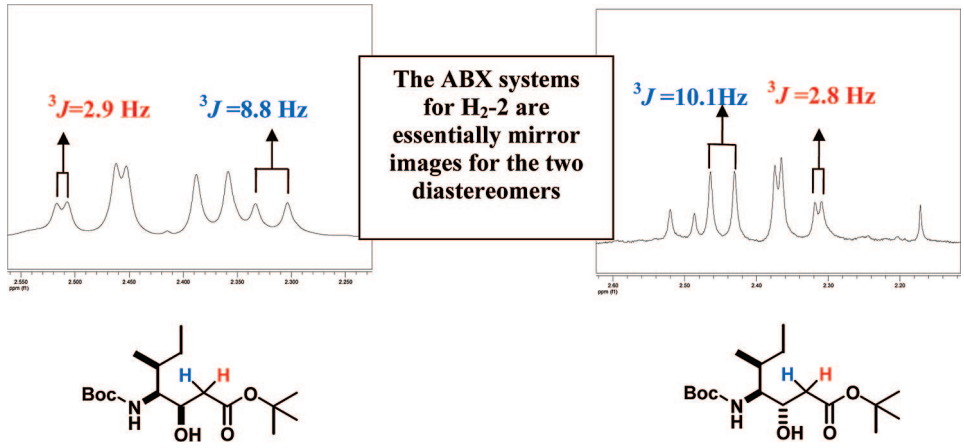


FIGURE 2. Expansions of the H_2-2 ABX systems in the ^{1}H NMR spectra of two diastereomers (*syn* and *anti*). These patterns are essentially mirror images allowing assignment of the relative configuration.

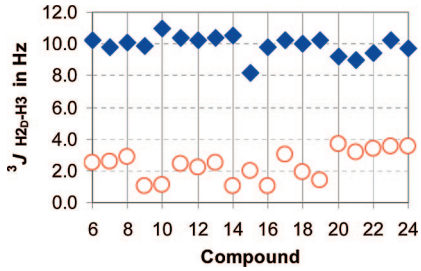


FIGURE 3. Comparison of the vicinal proton−proton coupling constants between H-2_D and H-3 for the *anti* (blue diamond) and *syn* (red circle) diastereomers for compounds **6**−**24**.

analyze all the ABX systems in this manner as it provides a simple way of designating "large" and "small" for this technique.

Table 1 shows the complete data set for 39 synthetic statine derivatives in $CDCl_3$. In all cases, the proton signals for H_2-2 were unambiguously identified by either simple inspection of the ^{1}H NMR spectra or through a combination of 1D TOCSY and selective decoupling experiments. The *anti* derivatives clearly showed the diagnostic pattern, with the downfield methylene resonance displaying a large vicinal coupling to H-3, while the upfield proton had a small vicinal coupling. These could be easily distinguished from the corresponding *syn* derivatives based on the pattern shown in Figure 2.

It is worth noting that in some cases, the proton resonances for the ABX system were strongly second order at 300 MHz. The statine units derived from Ala (**10a**,**b**) displayed such a large distortion that the magnitude of the coupling constants could not be directly extracted at 300 MHz. In general, this effect was more prevalent with the *syn* diastereomers as lysine (**16b**), glutamate (**23b**), methionine (**19b**), and proline (**24b**) derivatives all had strongly distorted ABX spin systems for H_2-2. Since second-order systems occur when $\Delta J/(\Delta\delta$ in Hz) < 6, increasing the spectrometer frequency provided one simple solution. Figure 4 shows expansions of the ABX systems for the two derivatives that displayed the largest second-order distortions at 300 MHz and the corresponding spectra at 800 MHz.[23] In both cases, the higher field strength resolves the signals into a pattern consistent with the rest of the data set. Coupling constants in these second-order systems could also be obtained by comparison of the AB systems of experimental and simulated NMR spectrum at 300 MHz (Figure 5).[24] Coupling constants and chemical shifts in Table 1 for the *anti*-Ala (**10a**), *syn*-Pro (**24b**), and *syn*-Glu-Sta (**23b**) were determined in this manner.

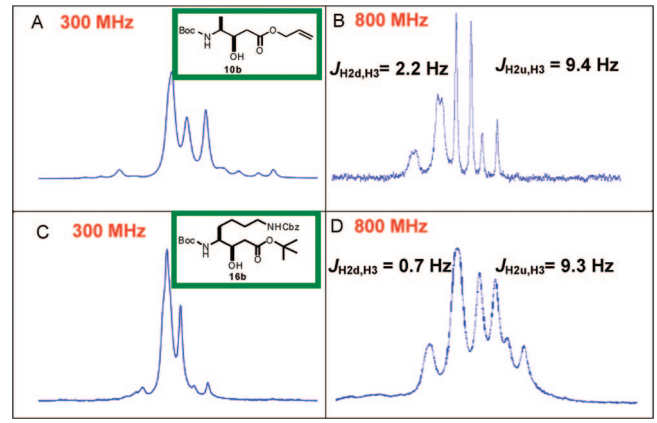


FIGURE 4. Expansion of the AB pattern for H_2-2 in the ^{1}H NMR spectra: (a) **10b** at 300 MHz, (b) **10b** at 800 MHz, (c) **16b** at 300 MHz, and (d) **16b** at 800 MHz.

(23) At 500 MHz, the ^{1}H NMR spectra for these compounds were second order.

TABLE 1. ^{1}H NMR Data for H_2-2 ABX System of Statine Derivatives

anti compd	parent AA	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})	*syn* compd	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})
6a[d]	leucine	2.47 (8.2)	2.45 (0.4)	**6b**[d]	2.29 (3.7)	2.28 (10.0)
7a	valine	2.66 (9.8)	2.54 (3.0)	**7b**	2.63 (2.6)	2.50 (8.9)
8a	isoleucine	2.48 (10.1)	2.39 (2.8)	**8b**	2.48 (2.9)	2.35 (8.8)
9a	isoleucine	2.60 (9.9)	2.31 (2.2)	**9b**	2.43 (1)[a]	2.31 (8.7)
10a	alanine	2.56 (11.0)[d]	2.53 (0.7)[d]	**10b**[b]	2.54 (2.2)	2.51 (9.4)
11a	phenylalanine	2.51 (10.4)	2.27 (2.4)	**11b**	2.51 (2.4)	2.41 (8.9)
12a	phenylalanine	2.61 (10.2)	2.41 (2.5)	**12b**	2.61 (2.2)	2.52 (8.2)
13a	tyrosine	2.51 (10.4)	2.28 (2.6)	**13b**	2.51 (2.5)	2.41 (9.0)
14a	tryptophan	2.52 (10.5)	2.25 (2.6)	**14b**	2.54 (1)[a]	2.46 (8.3)
15a	histidine	2.58 (8.2)	2.43 (4.7)	**15b**	2.55 (2.0)	2.51 (8.6)
16a	lysine	2.48 (9.8)	2.36 (3.1)	**16b**[b]	2.45 (0.7)	2.41 (9.3)
17a	cysteine	2.47(10.2)	2.26 (1)[a]	**17b**	2.48 (3.0)	2.35 (9.0)
18a[c]	aspartate	2.47 (10.0)	2.40 (2.6)	**18b**	2.53 (1.9)	2.46 (9.0)
19a	methionine	2.60 (10.2)	2.53 (3.0)	**19b**	2.58 (1.4)	2.54 (9.4)
20a	serine	2.43 (9.2)	2.31 (4.0)	**20b**	2.46 (3.7)	2.40 (8.5)
21a	threonine	2.39 (9.0)	2.28 (1.6)	**21b**	2.50 (3.2)	2.33 (8.7)
21c	threonine	2.53 (9.8)	2.43 (3.3)			
22a	arginine	2.40 (9.4)	2.30 (3.3)	**22b**	2.37 (3.4)	2.28 (8.9)
23a	glutamate	2.60 (10.2)	2.53 (2.2)	**23b**[d]	2.56 (3.5)	2.53 (8.9)
24a[d]	proline	2.36 (9.7)	2.33 (2.5)	**24b**	2.54 (3.5)	2.44 (8.5)

[a] broad proton signal with no discernible coupling to Hx that for illustrative purposes is considered approximately a 1 Hz coupling. [b] Data recorded at 800 MHz. [c] Coupling constants determined though a combination of 1D-TOCSY and selective homonuclear decoupling at 500 MHz. [d] Coupling constants and chemical shifts determined by comparison of simulated and experimental NMR spectra.

TABLE 2. Relevant Literature Examples

anti compd	parent AA	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})	*syn* compd	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})	ref
25a	leucine	2.34 (7.9)	2.27 (3.4)	**25b**	2.48 (2.9)	2.29 (10.0)	28
26a	valine	NA	NA	**26b**	2.58 (2.9)	2.47 (9.1)	29
27a	valine	NA	NA	**27b**	2.64 (2.1)	2.32 (10.6)	28
28a	isoleucine	NA	NA	**27b**	2.60 (2.7)	2.48 (9.1)	30
29a	isoleucine	NA	NA	**29b**	2.60 (2.7)	2.42 (8.7)	30
30a	phenylalanine	2.40 (9.5)	2.04 (2.4)	**30b**	2.63 (2.5)	2.22 (10.1)	28
31a	phenylalanine	2.59 (9.9)	2.37 (2.6)	**31b**	NA	NA	31
32a	phenylalanine	2.57 (10.3)	2.38 (3.0)	**32b**	NA	NA	31
33a	phenylalanine	2.54 (9.8)	2.28 (3.1)	**33b**	NA	NA	32
34a	phenylalanine	2.38 (9.0)	2.19 (4.0)	**34b**	NA	NA	32
35a	phenylalanine	2.55 (9.5)	2.32 (3.0)	**35b**	NA	NA	32
36a	isoleucine	NA	NA	**36b**	3.25 (1)[a]	2.44 (8.1)	8
37a	isoleucine	NA	NA	**37b**	3.25 (2.7)	2.48 (7.5)	8
38a	hydroxyglutamic acid	2.62 (8.8)	2.54 (5.0)	**38b**	2.74 (3.7)	2.69 (10.5)	33
39a	hydroxyglutamic acid	2.65 (9.2)	2.55 (4.6)	**39b**	NA	NA	33
40a	hydroxyglutamic acid	NA	NA	**40b**	2.62, m		33
41a	phenylalanine	NA	NA	**41b**	2.65 (3.6)	2.57 (5.6)	34
42a (DMSO)	tryptophan	NA	NA	**42b**	2.35 (3.7)	2.28 (9.1)	35
43a (DMSO)	tyrosine	NA	NA	**41b**	2.38 (3.7)	2.29 (9.5)	36
44a (MeOH)	leucine	2.36 (7.2)	2.27 (6.7)	NA	NA	NA	37

[a] Broad proton signal with no discernible coupling to Hx that for illustrative purposes is considered approximately a 1 Hz coupling.

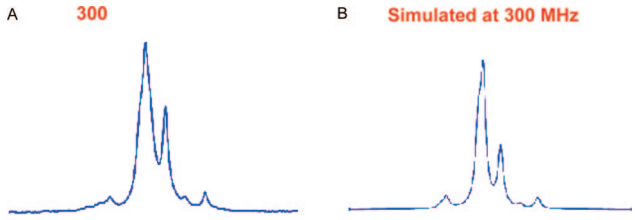


FIGURE 5. (a) 300 MHz spectrum of **16b** in $CDCl_3$ at 300 MHz. (b) Simulated spectrum at 300 MHz (δ_{Ha} 2.426, δ_{Hb} 2.385, $^2J_{AB} = -15.84$, $^3J_{AX} = 0.7$ Hz, $^3J_{BX} = 9.3$ Hz)

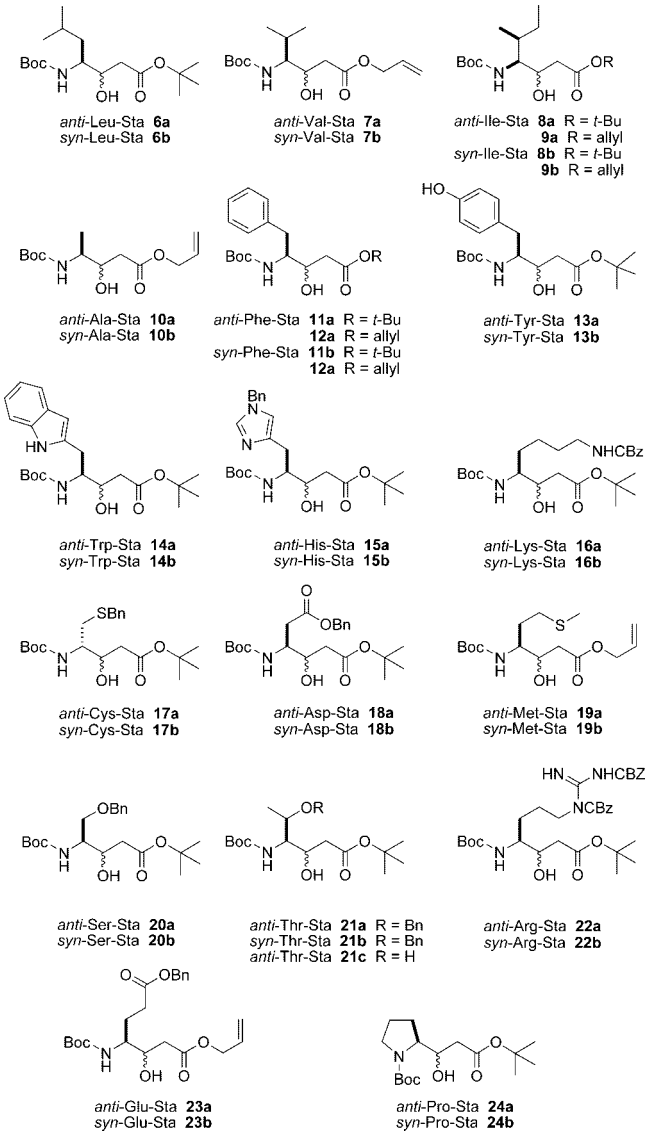
A number of statine-containing compounds have been described.[25] Table 2 summarizes some additional reports consistent with our observations. The diagnostic trend was observed with statine derivatives when the amine was protected as the *tert*-butoxycarbonyl, acetyl, and dibenzyl derivatives and with a variety of different protecting groups on the C-1 ester. These latter examples (**25a**, **25b**, **30a**, **30b**) suggest the configuration of statine units containing 3° amines can be assigned by using this technique. The generality of the trend is further exemplified by the linear di- and tripeptides **33a**, **34a**, and **35a**, which contain additional chiral centers.

(24) Spin simulations were done with MestreC 4.9.8.0 in an iterative fashion comparing with the experimental spectra until identical patterns were obtained. Experimental data were used as a constraint in these simulations when possible, i.e., δ_{H3}, $^2J_{H2u,H2d}$, $^3J_{H3,H2d} + {}^3J_{H3,H2u}$, ca. δ_{H2}.

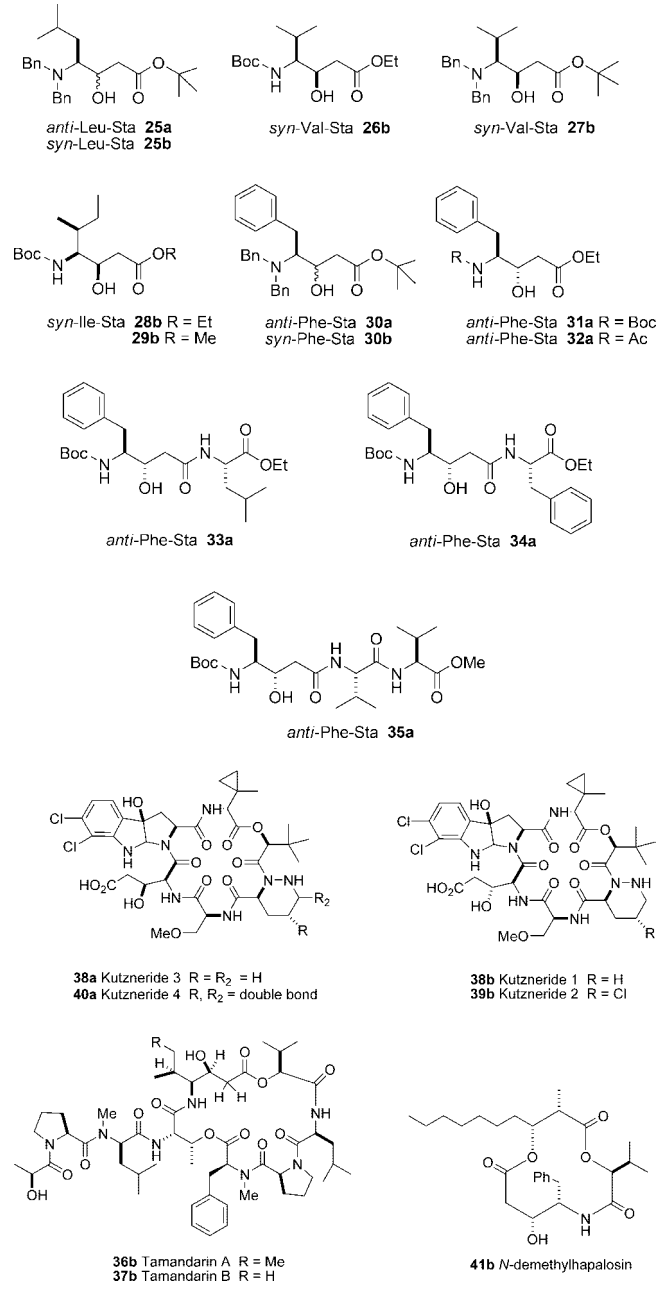
(25) A Scifinder Scholar search for the basic carbon skeleton yielded approximately 10 000 compounds, but the utility of this literature NMR data varied greatly. For example, H_2-2 was frequently reported as a multiplet spanning a considerable chemical shift range. This indicated the methylene protons were probably diastereotopic (otherwise H_2-2 would be a simple doublet), but provided no coupling constant information for comparison.

(26) Sakai, R.; Stroh, J. G.; Sullins, D. W.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 3734–3748.

(27) Zhang et al. have recently reported a new cyclic thiazolyl peptide Phillipmycin containing a statine unit, whose configuration can be correctly predicted with this method. See: Zhang, C.; et al. *J. Am. Chem. Soc.* **2008**, *130*, 12102−12110.



Natural products of significant structural complexity can also be assigned by using this approach. For example, the configuration of the statine unit within the didemnin[26]/tamandarin and hapalosin series of cyclic natural products (**36b**, **37b**, **41b**) can be correctly predicted as *syn* based on analysis of the reported NMR data. Likewise, the epimeric derivatives belonging to the Kutzneride class of compounds (**38**−**40**) can also be correctly assigned with the *syn* derivatives displaying the smaller three-bond proton−proton coupling to the downfield signal of H_2-2.[27]



Natural products (**42**−**44**) in solvents other than $CDCl_3$ show the same diagnostic patterns, and while it would be tempting to apply this methodology, our data indicate that caution must be exercised. As illustrated in Table 3 there is a clear solvent

(28) Andrés, J. M.; Pedrosa, R.; Pérez, A.; Pérez-Encabo, A. *Tetrahedron* **2001**, *57*, 8521–8530. This reference also includes two Pro-Stat derivatives in which the ABX pattern appears to be reversed. We have synthesized one of these compounds, the Pro-Stat protected as a *tert*-butyl ester, and find the NMR data to be consistent with the rest of our data set.

(29) Liang, B.; Richard, D. J.; Portonovo, P. S.; Joullie, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 4469–4474.

(30) Rinehart, K. L.; Sakai, R.; Kishore, V.; Sullins, D. W.; Li, K. M. *J. Org. Chem.* **1992**, *57*, 3007–3013.

(31) Doi, T.; Kokubo, M.; Yamamoto, K.; Takahashi, T. *J. Org. Chem.* **1998**, *63*, 428–429.

(32) Palomo, C.; Miranda, J. I.; Linden, A. *J. Org. Chem.* **1996**, *61*, 9196–9201.

(33) Broberg, A.; Menkis, A.; Vasiliauskas, R. *J. Nat. Prod.* **2006**, *69*, 97–102.

(34) Okuno, T.; Ohmori, K.; Nishiyama, S.; Yamamura, S.; Nakamura, K.; Houk, K. N.; Okamoto, K. *Tetrahedron* **1996**, *52*, 14723–14734.

(35) Ishida, K.; Murakami, M. *J. Org. Chem.* **2000**, *65*, 5898–5900, The vicinal coupling constants for the methylene proton signals (δ_H 2.35 and 2.28) in Table 1 of this reference are transposed. We thank Drs. Ishida, Murakami, and Okada for providing copies of the original ^{1}H NMR spectrum so that we could check these data. The NMR spectra is included in our Supporting Information.

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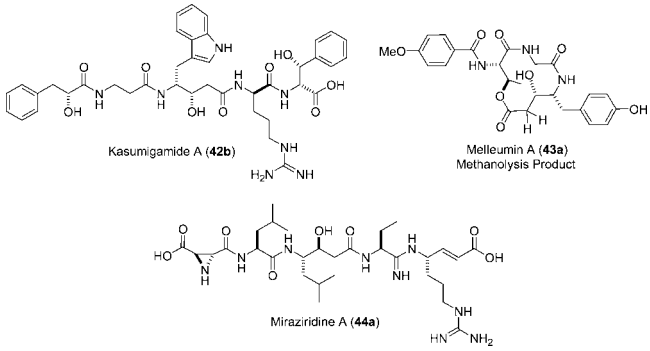
(37) Isolation: Nakao, Y.; Fujita, M.; Warabi, K.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc.* **2000**, *122*, 10462–10463. Synthesis: Konno, H.; Kubo, K.; Makabe, H.; Toshiro, E.; Hinoda, N.; Nosaka, K.; Akaji, K. *Tetrahedron* **2007**, *63*, 9502–9513.

TABLE 3. Solvent Effect

anti compd	solvent	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})	*syn* compd	δ_{Ha} (J_{AX})	δ_{Hb} (J_{BX})
6a	$CDCl_3$	2.47 (10.2)	2.34 (2.4)	**6b**	2.47 (2.5)	2.34 (8.9)
7a	DMSO-d_6	2nd order		**7b**	2.36 (2.5)	2.16 (9.6)
6a	DMSO-d_6	2.20 d (2H, 7.0)		**6b**	2.39 (3.2)	2.15 (9.3)
11a	DMSO-d_6	2.33 (4.2)	2.16 (8.8)	**11b**	2.39 (3.9)	2.16 (9.3)
6a	CD_3OD	2.27 d (2H, 6.8)		**6b**	2.39 (3.2)	2.15 (9.3)
7a	acetone-d_6	2.47 (2 H, 6.6)		**7b**	2.60 (2.6)	2.35 (9.1)
10a	acetone-d_6	2.50 (4.2)	2.43 (8.8)	**10b**	2.57 (3.5)	2.39 (9.3)

effect, which is more pronounced for the *anti* derivatives than the *syn*. In some cases, such as **6a** and **7a** in DMSO and MeOH-d_4, the methylene protons become magnetically equivalent.[38] In other cases, the diastereomers of the pairs **10a/10b** and **11a/11b** are indistinguishable in acetone-d_6 and DMSO-d_6, respectively, as in *both* cases, the downfield methylene proton displays the small vicinal proton coupling. Application of this methodology in these instances would lead to an erroneous assignment of the *anti* diastereomer as *syn*. Thus, $CDCl_3$ insoluble compounds should be converted to a soluble derivative prior to analysis.[39]



This approach is not without precedent as a similar technique was proposed by Roush et al. to assign the relative stereochemistry of β-hydroxy ketones with alkyl substituents in the γ-position.[40] Two differences should be noted. First in Roush's method, varying the solvent had no noticeable effect on the pattern in the ^{1}H NMR spectra for the two compounds examined. Second, with those substrates C-5 must be branched, e.g., analogous to **7a**, for the two diastereomers to be distinguishable.[40a] The latter is not the case for statine moieties as is illustrated by examples **6**, **10**−**20**, and **22**−**23** in Table 1. These compounds are not branched at C-5, yet are distinguishable.

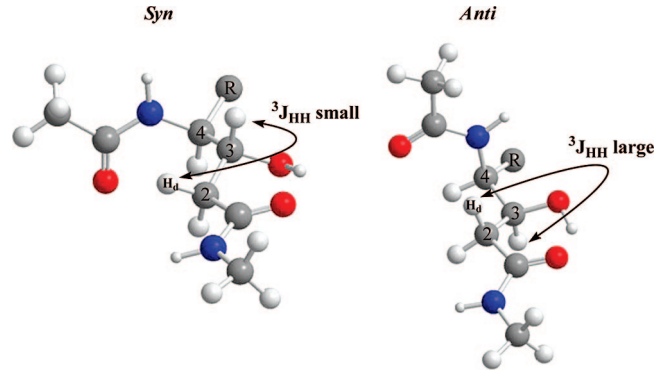


FIGURE 6. Proposed Conformational Model.

Conformational preferences for **6a** and **6b** are known based on conformational computations.[41] Analysis of these data suggests one possible explanation for the observed NMR patterns as shown in Figure 6. In both diastereomers, the carbonyls are in an eclipsed conformation with the C-2/C-3 bond coplanar to the carbonyl, but with the hydroxy C−O oxygen bond (+)-gauche and (−)-gauche to the carbonyl in the *anti* and *syn* diastereomers, respectively. Stabilization of these conformations by hydrogen bonding, analogous to that proposed in the Stiles−House model for α-alkyl-β-hydroxycarbonyls[42] or Roush's method for β-hydroxy ketones with alkyl substituents in the γ-position,[40] is suggested based on the work of Rich[41] and Nisato,[43] although not required.[44] If these conformers predominate in $CDCl_3$ then the pro-*S* methylene proton is deshielded in both diastereomers relative to the pro-*R*. This deshielding of the pro-*S* methylene proton may be due to the amide attached to C-4, in a fashion analogous to that reported by Whelton et al. for substituted cyclohexamines.[45] It should be noted that for simplicities sake, we have chosen to depict only a single rotatmer between the vicinal chiral centers C-3/C-4, although at least two rotatmers exist in solution based on coupling constant analysis. A reversal of the relative population of the rotamers around the C-3/C-4 bond may ultimately be responsible for the solvent dependency. A more detailed conformational analysis of the factors responsible for the observed NMR patterns is underway.

The trend noted here provides the simplest methodology for the relative configurational assignment of $CDCl_3$-soluble statine-containing peptides. The methodology requires no degradation or derivatization, and is amenable to micromolar concentrations of analytes given the sensitivity limits of modern NMR spectrometers. Several examples above suggest this methodology is applicable to statine units within cyclic peptides/depsipeptides, although until a more complete understanding of the underlying factors responsible is available, we would urge caution in these constrained systems. While we have not found any examples, conformational constraints in cyclic systems could induce a reversal of the ^{1}H NMR trend similar to that illustrated with solvents other than chloroform. For cyclic depsipeptides,

(38) Occasionally, H_2-2 appeared as a simple doublet in the initial proton spectra of **6**−**24** immediately after silica chromatography. After 24 h in $CDCl_3$, these proton signals were generally resolved.

(39) Because of this requirement of $CDCl_3$ solubility, data for the deprotected compounds **6**−**24** were generally not included.

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conversion to a linear derivative would be advisable. Another fundamental limitation of the technique is obtaining sufficient dispersion in the ABX NMR spin system. Potential solutions to this issue include increasing the NMR field strength, although most of our data were acquired at 300 MHz, or simulation of the observed spin system to extract coupling constants through comparison with experimental spectra. As such, this methodology should be broadly applicable to the assignment of statine units within both natural products and synthetic derivatives using ^{1}H NMR spectroscopy.

Experimental Section

General Procedure for Synthesis of β-Keto Esters.[15] One gram of the protected amino acid (1 equiv) was dissolved in 20 mL of dry THF. To this solution was added 1.1 equiv of 1,1′-carbonyldiimidazole (recrystallized from THF) with stirring under dry nitrogen at room temperature. Butyl lithium (2.2 M hexane solution, 3.3 equiv) under nitrogen was diluted with 20 mL of THF and cooled to 0 °C with an ice bath. To this was added dropwise diisopropylamine (3.6 equiv). After being stirred for 10 min at 0 °C, the solution was diluted with 70 mL of THF and cooled to −78 °C. To the LDA solution was added either *tert*-butyl acetate or allyl acetate (3.5 equiv). After 10 min, the Boc-amino acid/imidazole solution was cooled to −78 °C and cannulated into the enolate solution under nitrogen. The reaction was allowed to stir for 30 min at −78 °C then quenched with 50 mL of 10% citric acid and allowed to warm to room temperature. The aqueous solution was extracted with 200 mL of EtOAc (4 × 50 mL) and washed with 100 mL of saturated $NaHCO_3$ (2 × 50 mL) and 50 mL of brine and dried over $MgSO_4$. The solvent was evaporated and the crude material purified on silica eluting with mixtures of hexane/EtOAc.

Allyl 4(*S*)-[(*tert*-butoxycarbonyl)amino]-5-methyl-3-oxohexanoate (4b): amorphous powder; $[\alpha]^{27}_D$ +8.5 (*c* 5.4, $CHCl_3$); UV (*i*-PrOH) λ_{max} (log ε) 248 nm (8.10); IR (film) ν_{max} 3369, 1749, 1246, 1168 cm^{-1}; ^{1}H NMR ($CDCl_3$ at room temperature) δ 5.90 (ddt, J = 17.2, 10.4, 5.6 Hz, 1H), 5.33 (dd, J = 17.2, 1.5 Hz, 1H), 5.28 (dd, J = 10.4, 1.5 Hz, 1H), 5.06 (d, J = 8.3 Hz, 1H), 4.63 (d, J =5.6 Hz, 2H), 4.32 (dd, J = 8.7, 4.1 Hz, 1H), 3.57 (s, 2H), 2.24 (m, 1H), 1.48 (s, 9H), 1.01 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H); ^{13}C NMR δ 202.0, 166.2, 155.7, 131.5, 80.0, 66.0, 64.3, 47.0, 29.5, 28.2, 19.8, 16.7; HRESI-MS *m*/*z* 322.1636 [M + Na]$^+$ [calcd for $C_{15}H_{25}NO_5Na^+$ 322.1631, −3.8 ppm error].

General Procedure for Reduction.[15] To a flame-dried flask was added 0.5 g (1 equiv) of BOC-β-keto-γ-amino *tert*-butyl ester dissolved in 20 mL of dry THF then the mixture was cooled to −78 °C under nitrogen. To this solution was added 1.3 equiv of $LiBH_4$ (recrystallized from Et_2O) with stirring at −78 °C until the reaction reached completion as determined by TLC. The reaction was quenched by the addition of 1 N HCl and allowed to warm to room temperature over 15 min. This aqueous solution was extracted with 60 mL of Et_2O (3 × 20 mL) and then dried over $MgSO_4$. Purification over silica eluting with hexane/EtOAc provided a mixture of diastereomers. HPLC purification on silica provided pure diastereomers.

Allyl 4(*S*)-[(*tert*-butoxycarbonyl)amino]-5-methyl-3(*S*)-hydroxyhexanoate (7a): amorphous powder; $[\alpha]^{27}_D$ −1.7 (*c* 1.0, $CHCl_3$); UV (*i*-PrOH) λ_{max} (log ε) 248 nm (8.10); IR (film) ν_{max} 3375, 1715, 1506, 1261, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$ at room temperature) δ 5.90 (ddt, J = 17.2, 10.4 Hz, 1H), 5.33 (dq, J = 17.2, 1.5 Hz, 1H), 5.26 (dd, J =10.4, 1.5 Hz, 1H), 4.83 (d, J = 9.8 Hz, 1H), 4.60 (dt, J = 5.8, 1.5 Hz, 2H), 4.20 (br. d, J = 9.4 Hz, 1H), 3.20 (d, J = 2.6 Hz, 1H), 3.15 (td, J = 10.5, 1.7 Hz, 1H), 2.60 (dd, J = 16.9, 9.8 Hz, 1H), 2.51 (dd, J = 16.9, 3.2 Hz, 1H), 1.86 (dm, 1H), 1.43 (m, 9H), 0.99 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H); ^{13}C NMR δ 173.3, 156.4, 131.7, 118.7, 79.2, 67.0, 65.5, 59.6, 39.0, 30.3, 29.7, 28.4, 19.8, 19.5; HRESI-TOF *m*/*z* 324.1767 [M + Na]$^+$ [calcd for $C_{15}H_{27}NO_5Na^+$ 324.1787, −4.5 ppm error].

Allyl 4(*S*)-[(*tert*-butoxycarbonyl)amino]-5-methyl-3(*R*)-hydroxyhexanoate (7b): $[\alpha]^{24}_D$ +7.9 (*c* 1.0, $CHCl_3$); UV (*i*-PrOH) λ_{max} (log ε) 203 (7.56), 205 nm (5.54); IR (film) ν_{max} 3456, 3369, 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$ at room temperature) δ 5.98 (ddt, J = 17.2, 10.4, 5.8 Hz, 1H), 5.33 (ddt, J = 17.2, 2.7, 1.5 Hz, 1H), 5.24 (dd, J = 10.4, 1.1 Hz, 1H), 4.57 (dt, J = 5.8, 1.3 Hz, 2H), 4.50 (d, J = 9.6 Hz, 1H), 3.89 (td, J = 8.4, 2.9 Hz, 1H), 3.49 (m, 1H), δ 2.58 (dd, J = 16.5, 2.9 Hz, 1H), 2.46 (dd, J = 16.5, 8.8 Hz, 1H), 2.09 (m, 1H), 1.43 (m, 9H), 0.94 (d, J = 6.9 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR δ 172.9, 156.4, 131.8, 118.6, 79.6, 69.2, 65.5, 58.8, 38.3, 28.3, 27.5, 20.1, 16.3; HRESI-TOF *m*/*z* 302.1955 [M + H]$^+$ [calcd for $C_{15}H_{28}NO_5^+$ 302.1962, 2.4 ppm error].

Acknowledgment. This work was funded by grants from the Victoria S. and Bradley L. Geist Foundation (20070461), the National Science Foundation (OCE04-32479), and the National Institute of Environmental Health Sciences (P50 ES012740). Funds for the upgrades of the NMR instrumentation were provided by the CRIF program of the National Science Foundation (CH E9974921) and the Elsa Pardee Foundation. The purchase of the Agilent LC-MS was funded by grant W911NF-04-1-0344 from the Department of Defense. We thank P. Moeller, NOAA, and W. Yoshida, UH Manoa, for the 800 and 500 MHz NMR data, respectively. B. Wong and S. Yuen aided in the collection of the physical data.

Supporting Information Available: General experimental procedures for the preparation of and spectral data for compounds **6**−**24**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO8012429