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

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Abstract

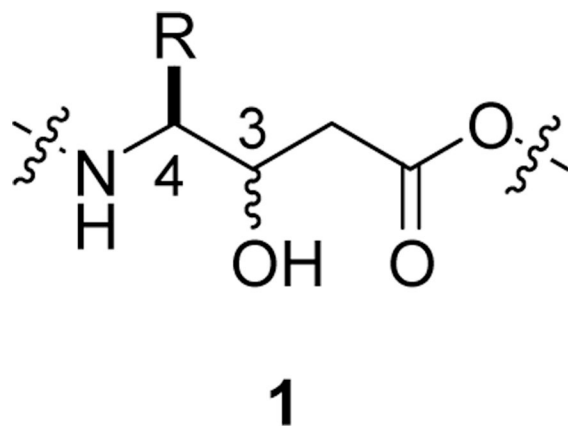
A simple method to determine the relative stereochemistry of statine amino acids (γ -amino- β -hydroxyacids) using ^1H NMR spectroscopy is described. Configurational assignment of statine units within complex natural products is possible without degradation or derivatization as the *syn* and *anti* diastereomers can be distinguished 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 non-destructive 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 connectivities between the various nuclei.³ Conversely, relative⁴ and absolute stereochemical assignments⁵ 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 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 *t*-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 $^3J_{\text{H}_4,\text{H}_3}$ values of approximately 6 and 2 Hz, respectively.^{17,18}

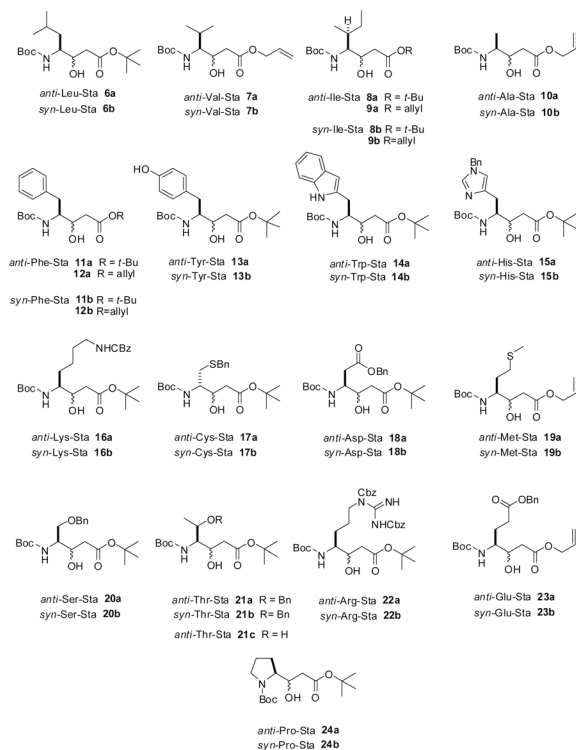
A comparison of the spectral data for the diastereomers revealed no predictive clustering of carbon or proton chemical shifts that could be used to assign the relative configuration of an unknown statine unit (Figure 1).¹⁹ Likewise, the magnitude of the 3J values between H-3 and H-4 did not provide a 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-diols²¹ and for α,γ -dimethyl- β -hydroxycarbonyls.²²

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 H₂-2 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 $^3J_{\text{H}_3, \text{H}_{2d}}$ and $^3J_{\text{H}_3, \text{H}_{2u}}$, 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 analyze all the ABX systems in this manner as it provides a simple way of designating “large” and “small” for this technique.

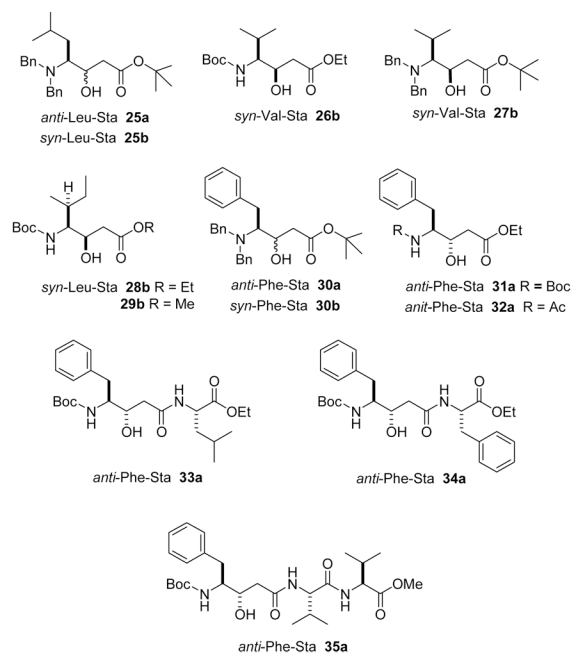
Table 1 shows the complete data set for 48 synthetic statine derivatives in CDCl_3 . In all cases, the proton signals for H₂-2 were unambiguously identified by either simple inspection of the ^1H NMR spectra or through a combination of 1D TOCSY and selective decoupling experiments. The *anti* derivatives 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.

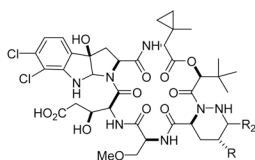


It is worth noting that in some cases, the proton resonances for the ABX system were strongly second order at 300 MHz. The statine units derived from Ala (**10a–b**) displayed such a large distortion that magnitude of the coupling constants could not be directly extracted at 300 MHz. In general, this effect was more prevalent with the *syn* diastereomers as lysine (**16b**), glutamate (**17b**), methionine (**19b**), and proline (**24b**) derivatives all had strongly distorted ABX spin systems for H₂-2. Since second order systems occur when $\Delta J/(\Delta\delta \text{ in Hz}) < 6$, 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

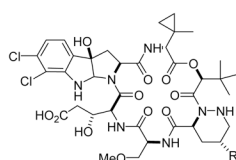
and the corresponding spectra at 800 MHz.²³ In both cases, the higher field strength resolves the signals into a pattern consistent with the rest of the data set. Coupling constants in these second order systems could also be obtained by comparison of the AB systems of experimental and simulated NMR 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.

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 using this technique. The generality of the trend is further exemplified by the linear di- and tripeptides **33a**, **34a**, **35a**, which contain additional chiral centers. Natural products of significant structural complexity can also be assigned using this approach. For example, the configuration of the statine unit within the didemnin²⁶/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 H₂-2.²⁷

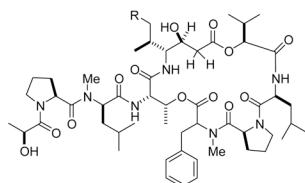




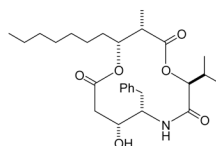
38a Kutzneride 3 R = H
40a Kutzneride 4 R, R₂ = double bond



38b Kutzneride 1 R = H
39b Kutzneride 2 R = Cl

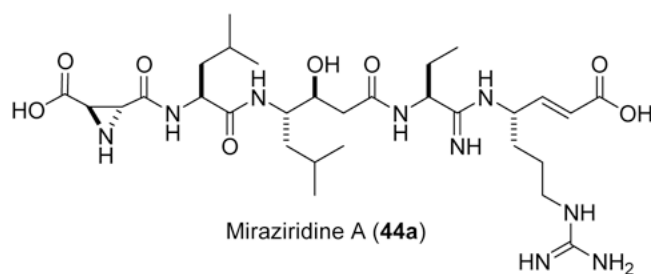
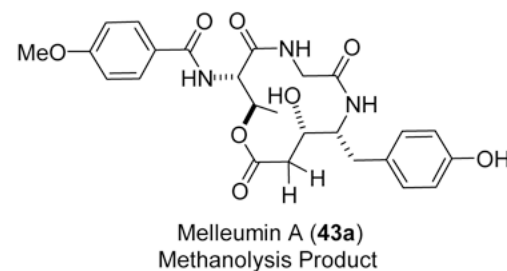
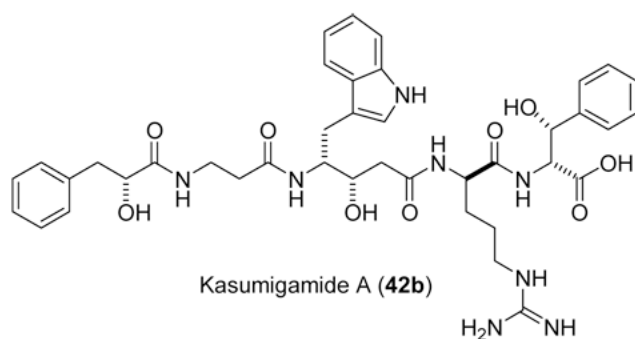


36b Tamandarin A R = Me
37b Tamandarin B R = H



(41b) N-demethylhapalosin

Natural products (**42–44**) in solvents other than CDCl₃ show the same diagnostic patterns, and while it would be tempting to apply this methodology, our data indicates that caution must be exercised. As illustrated in Table 3 there is a clear solvent 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, CDCl₃ 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 ¹H 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 C-5, yet are distinguishable.

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 -hydroxycarbonyls⁴² or Roush's method for β -hydroxy ketones with alkyl substituents in the γ -position⁴⁰ is suggested based on the work of Rich⁴¹ and Nisato,⁴³ although not required.⁴⁴ If these conformers predominate in CDCl₃ 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 rotamer between the vicinal chiral centers C-3/C-4, although at least two rotamers 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 CDCl₃ 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 ¹H NMR trend similar to that illustrated using solvents other than chloroform. For cyclic depsipeptides, 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 was 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 ¹H NMR spectroscopy.

Experimental Section

General Procedure for Synthesis of β -keto esters.¹⁵

One gram of the protected amino acid (1 eq.) was dissolved in 20 mL of dry THF. To this solution was added 1.1 eq. of 1,1'-carbonyldiimidazole (recrystallized from THF) with stirring under dry nitrogen at room temperature. Butyl lithium (2.2M hexane solution, 3.3 equivalents) 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 equivalents). After stirring for ten minutes at 0°C, the solution was diluted with 70 mL of THF and cooled to –78°C. To the LDA solution was added either *t*-butyl acetate or allyl acetate (3.5 equivalents). After ten minutes, 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 minutes 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 EtOAc (4x 50 mL) and washed with 100 mL saturated NaHCO₃ (2x50 mL), 50 mL brine, and dried over MgSO₄. The solvent was evaporated and the crude material purified on silica eluting with mixtures of hexane/EtOAc).

Alllyl 4(S)-[(*tert*-butoxycarbonyl)-amino]-5-methyl-3-oxohexanoate (4b)—

amorphous powder: $[\alpha]_D^{27} = +8.5$ (*c* 5.4, CHCl₃); UV (*i*-PrOH) λ_{\max} (log ϵ) 248 (8.10) nm; IR (film) ν_{\max} : 3369, 1749, 1246, 1168 cm⁻¹; ¹H NMR (CDCl₃ at room temperature) δ 5.90 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), δ 5.33 (dd, *J* = 17.2, 1.5 Hz, 1H), δ 5.28 (dd, *J* = 10.4, 1.5 Hz, 1H), δ 5.06 (d, *J* = 8.3 Hz, 1H), δ 4.63 (d, *J* = 5.6 Hz, 2H), δ 4.32 (dd, *J* = 8.7, 4.1 Hz, 1H), δ 3.57 (s, 2H), δ 2.24 (m, 1H), δ 1.48 (s, 9H), δ 1.01 (d, *J* = 6.8 Hz, 3H), δ 0.82 (d, *J* = 6.8 Hz, 3H); ¹³C 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]⁺ [Calc. for C₁₅H₂₅NO₅Na⁺, 322.1631, -3.8 ppm error].

General procedure for Reduction.¹⁵

To a flame dried flask was added 0.5 g (1 eq.) of BOC- β -keto- γ -amino *t*-butyl ester dissolved in 20 mL dry THF and cooled to -78°C under nitrogen. To this solution was added 1.3 eq. of LiBH₄ (recrystallized from Et₂O) which was stirred at -78°C until the reaction reached completion by 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 Et₂O (3x20 mL) and then dried over MgSO₄. Purification over silica eluting with hexane/EtOAc provided a mixture of diastereomers. HPLC purification on silica provided pure diastereomers.

Alllyl 4(S)-[(*tert*-butoxycarbonyl)-amino]-5-methyl-3(S)-hydroxyhexanoate (7a)

—amorphous powder: $[\alpha]_D^{27} = -1.7$ (*c* 1.0, CHCl₃); UV (*i*-PrOH) λ_{\max} (log ϵ) 248 (8.10) nm; IR (film) ν_{\max} : 3375, 1715, 1506, 1261, 1172 cm⁻¹; ¹H NMR (CDCl₃ at room temperature) δ 5.90 (ddt, *J* = 17.2, 10.4 Hz, 1H), δ 5.33 (dq, *J* = 17.2, 1.5 Hz, 1H), δ 5.26 (dd, *J* = 10.4, 1.5 Hz, 1H), δ 4.83 (d, *J* = 9.8 Hz, 1H), δ 4.60 (dt, *J* = 5.8, 1.5 Hz, 2H), δ 4.20 (br. d, *J* = 9.4 Hz, 1H), δ 3.20 (d, *J* = 2.6 Hz, 1H), δ 3.15 (td, *J* = 10.5, 1.7 Hz, 1H), δ 2.60 (dd, *J* = 16.9, 9.8 Hz, 1H), δ 2.51 (dd, *J* = 16.9, 3.2 Hz, 1H), δ 1.86 (dm, 1H), δ 1.43 (m, 9H), δ 0.99 (d, *J* = 6.7 Hz, 3H), δ 0.96 (d, *J* = 6.8 Hz, 3H); ¹³C 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]⁺ [Calc. for C₁₅H₂₇NO₅Na⁺, 324.1787, -4.5 ppm error].

Alllyl 4(S)-[(*tert*-butoxycarbonyl)-amino]-5-methyl-3(R)-hydroxyhexanoate (7b)

— $[\alpha]_D^{24} = +7.9$ (*c* 1.0, CHCl₃); UV (*i*-PrOH) λ_{\max} (log ϵ) 203 nm (7.56), 205 nm (5.54); IR (Film) ν_{\max} : 3456, 3369, 1696 cm⁻¹; ¹H NMR (CDCl₃ at room temperature) δ 5.98 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), δ 5.33 (ddt, *J* = 17.2, 2.7, 1.5 Hz, 1H), δ 5.24 (dd, *J* = 10.4, 1.1 Hz, 1H), δ 4.57 (dt, *J* = 5.8, 1.3 Hz, 2H), δ 4.50 (d, *J* = 9.6 Hz, 1H), δ 3.89 (td, *J* = 8.4, 2.9 Hz, 1H), δ 3.49 (m, 1H), δ 2.58 (dd, *J* = 16.5, 2.9 Hz, 1H), δ 2.46 (dd, *J* = 16.5, 8.8 Hz, 1H), δ 2.09 (m, 1H), δ 1.43 (m, 9H), δ 0.94 (d, *J* = 6.9 Hz, 3H), δ 0.88 (d, *J* = 6.8 Hz, 3H); ¹³C 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]⁺ [Calc. for C₁₅H₂₈NO₅⁺, 302.1962, 2.4 ppm error].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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References and Notes

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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, H₂-2 was frequently reported as a multiplet spanning a considerable chemical shift range. This indicated the methylene protons

were probably diastereotopic (otherwise H₂-2 would be a simple doublet), but provided no coupling constant information for comparison.

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39. Because of this requirement of CDCl₃ solubility, data for the deprotected compounds **6–24** was generally not included.
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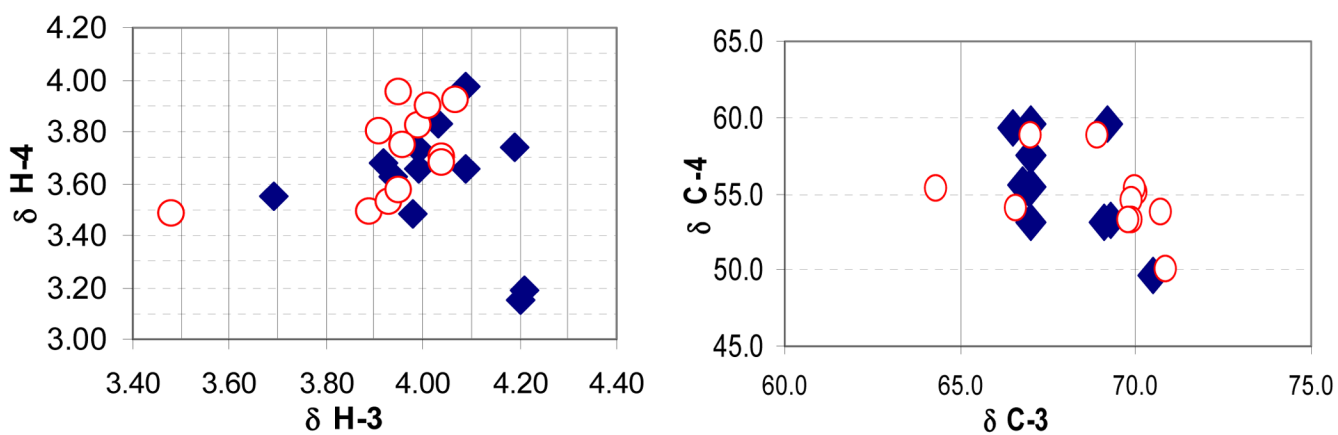


Figure 1.

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.

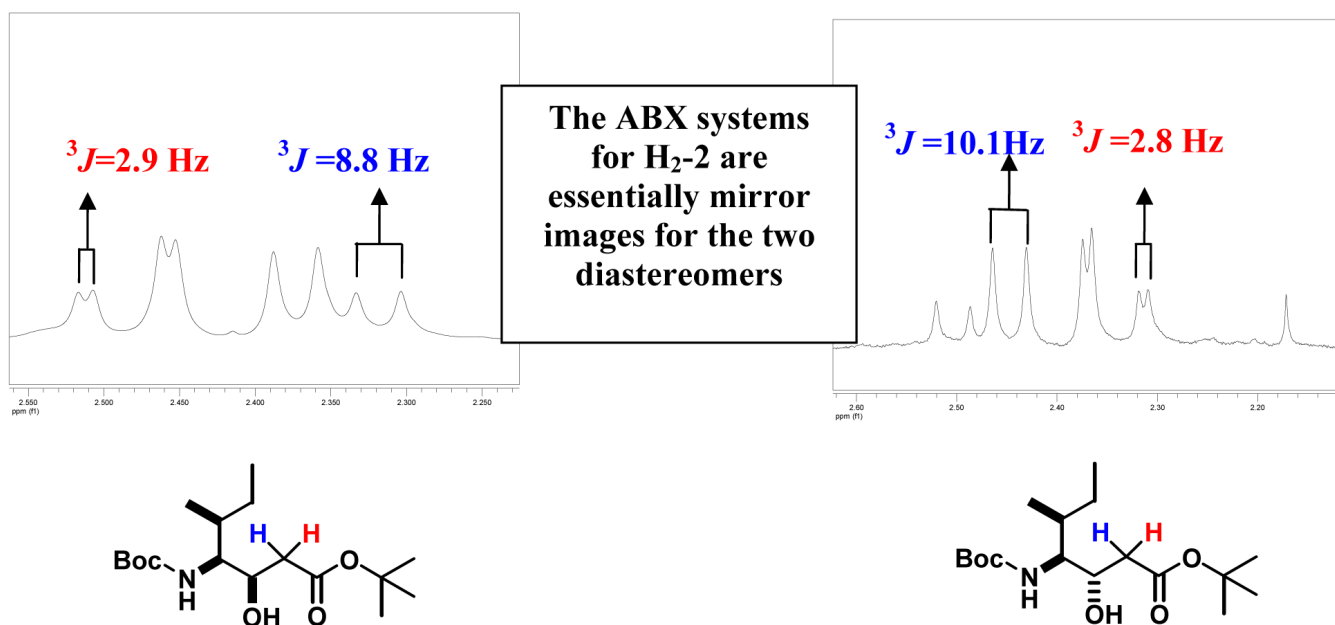


Figure 2.

Expansions of the H₂-2 ABX systems in the ¹H NMR spectra of two diastereomers (*syn* & *anti*). These patterns are essentially mirror images allowing assignment of the relative configuration.

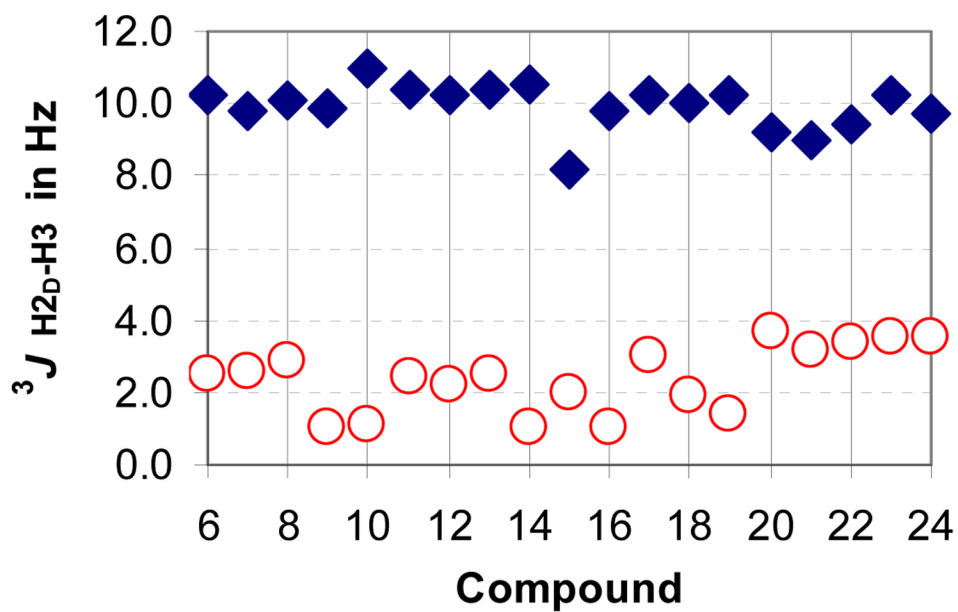


Figure 3. Comparison of the vicinal proton-proton coupling constants between H-2_D and H-3 for the *anti* (blue diamond) and *syn* (red circle) diastereomers for compounds **6–24**

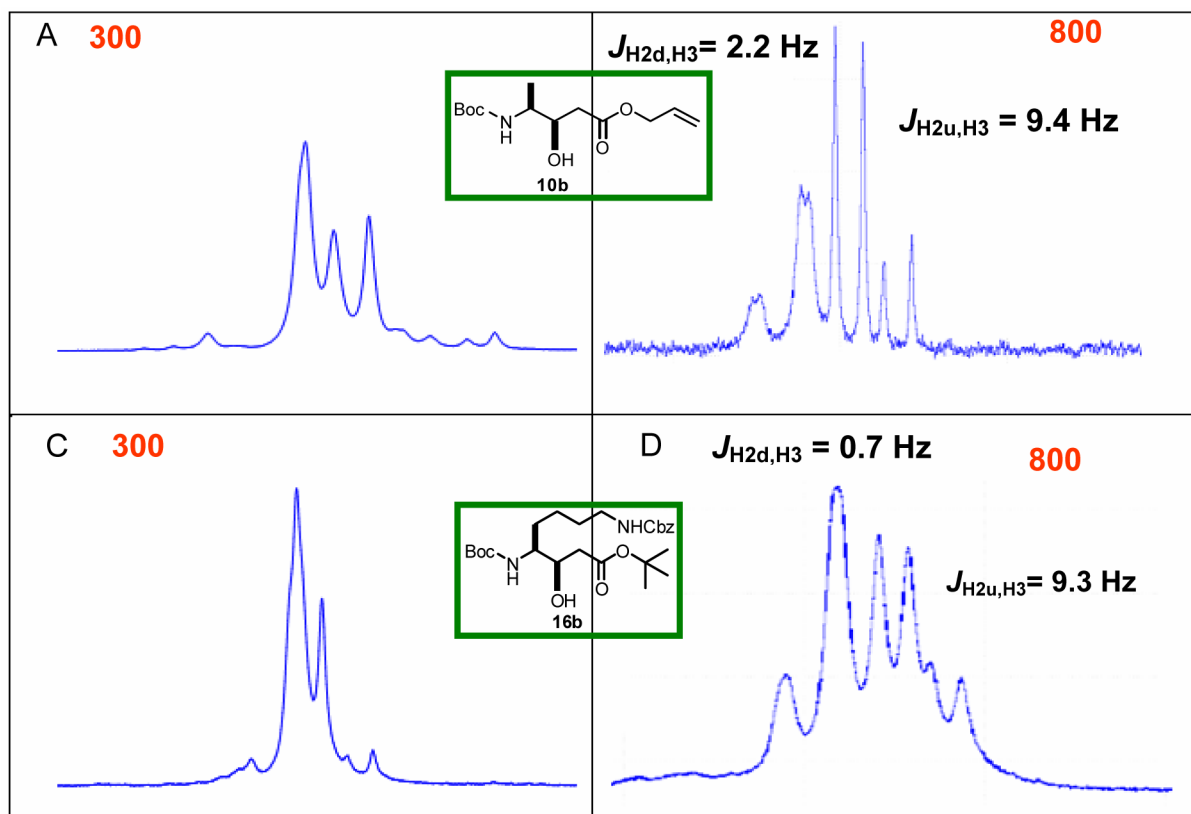


Figure 4. Expansion of the AB pattern for H₂-2 in the ¹H NMR spectra a) **10b** at 300 MHz b) **10b** at 800 MHz c) **16b** at 300 MHz d) **16b** at 800 MHz

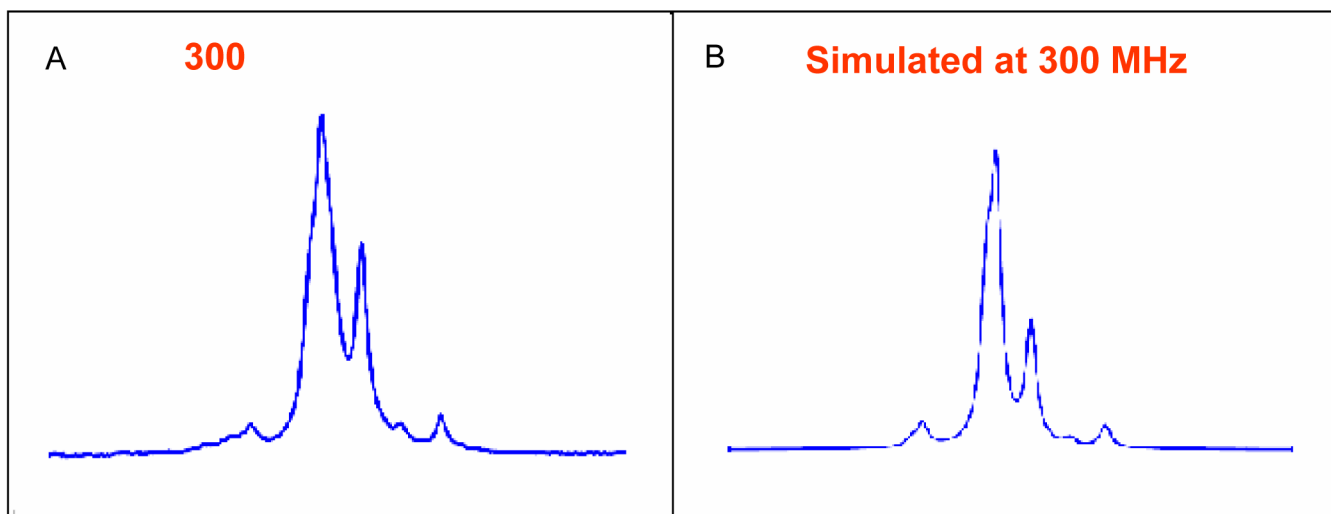
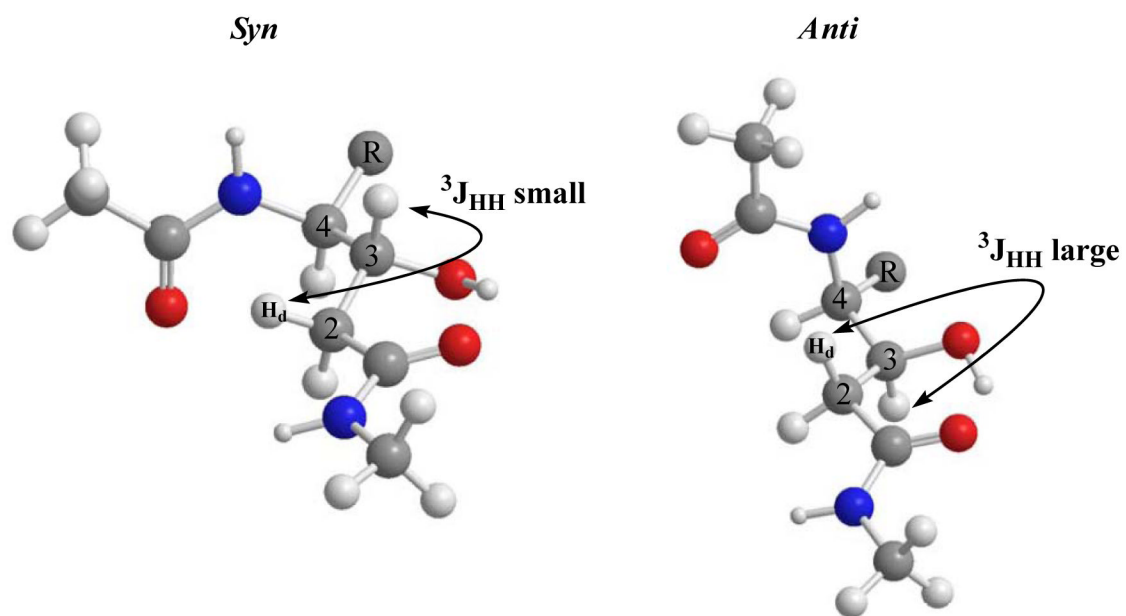
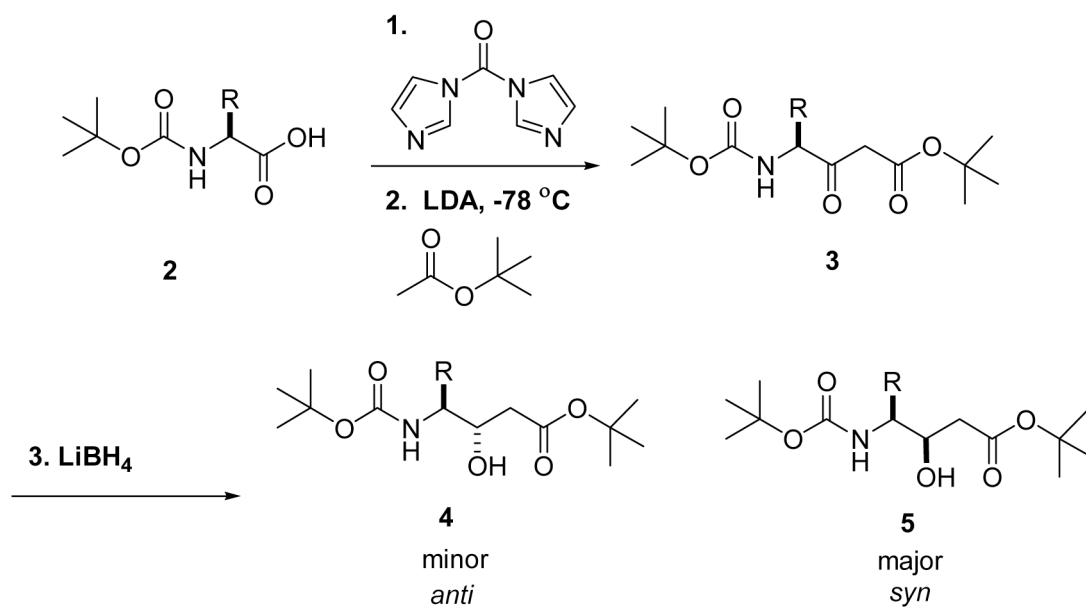


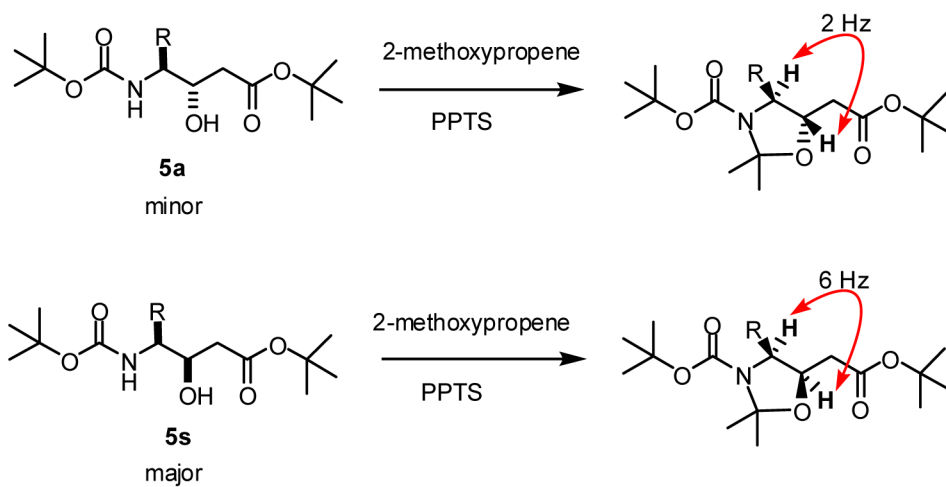
Figure 5.

a) 300 MHz Spectrum of **16b** in CDCl₃ 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)





Scheme 1.
Synthesis of Statine Units.



Scheme 2.
Conversion to the Oxazolidinone Derivatives.

Table 1

¹H NMR data for H₂-2 ABX system of Statine Derivatives

Anti Compound	Parent AA	δ_{H_a} (J_{AX})	δ_{H_b} (J_{BX})	Syn Compound	δ_{H_a} (J_{AX})	δ_{H_b} (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) ^d	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) ^d	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) ^d	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 discernable coupling to H_x that for illustrative purposes is considered approximately a 1 Hz coupling.^b Data recorded at 800 MHz.^c Coupling constants determined through 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 Compound	Parent AA	δ_{H_a} (J_{AX})	δ_{H_b} (J_{BX})	Syn Compound	δ_{H_a} (J_{AX})	δ_{H_b} (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) ^d	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	NA	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

^d broad proton signal with no discernable coupling to H_x that for illustrative purposes is considered approximately a 1 Hz coupling.

Table 3

Solvent Effect

Anti Compound	Solvent	δ_{H_a} (J_{AX})	δ_{H_b} (J_{BX})	Syn Compound	δ_{H_a} (J_{AX})	δ_{H_b} (J_{BX})
6a	CDCl ₃	2.47 (10.2)	2.34 (2.4)	6b	2.47 (2.5)	2.34 (8.9)
7a	DMSO- <i>d</i> ₆	2 nd order	-----	7b	2.36 (2.5)	2.16 (9.6)
6a	DMSO- <i>d</i> ₆	2.20 d (2H, 7.0)	-----	6b	2.39 (3.2)	2.15 (9.3)
11a	DMSO- <i>d</i> ₆	2.33 (4.2)	2.16 (8.8)	11b	2.39 (3.9)	2.16 (9.3)
6a	CD ₃ OD	2.27 d (2H, 6.8)	-----	6b	2.39 (3.2)	2.15 (9.3)
7a	Acetone- <i>d</i> ₆	2.47 (2 H, 6.6)	-----	7b	2.60 (2.6)	2.35 (9.1)
10a	Acetone- <i>d</i> ₆	2.50 (4.2)	2.43 (8.8)	10b	2.57 (3.5)	2.39 (9.3)