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Rotamers or Diastereomers? An Overlooked NMR Solution

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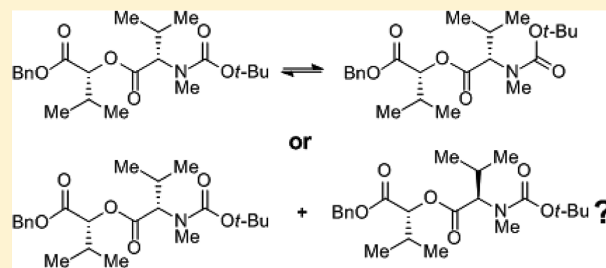
Supporting Information

ABSTRACT: The existence of rotamers in a solution of analyte complicates ^{1}H NMR analysis, especially when the presence of diastereomers is also possible. Organic chemists have often responded to this problem by conducting variable-temperature (VT) NMR experiments, changing NMR solvents, or adding complexing agents. Here, with specific examples, we illustrate the use of simple yet widely overlooked chemical-exchange NMR experiments which allow the nonintrusive rapid distinguishment of rapidly equilibrating small molecules such as rotamers from nonequilibrating diastereomers.



The appearance by ^{1}H NMR spectroscopy of equilibrating species such as rotamers due to protecting groups complicates the analysis of reaction products. Equilibrating species such as rotamers are most often distinguished from nonequilibrating diastereomers by techniques such as variable-temperature (VT) NMR, solvent switching,[1,2] or the introduction of a complexing agent.[3−5] These techniques are generally inconvenient, especially when the analyzed substrate is precious and must be recovered. When only qualitative information about the identity of constituents in a sample is required, chemical-exchange NMR experiments serve as far simpler alternatives. Chemical-exchange NMR experiments such as saturation transfer have been used in organometallic chemistry to identify isomers due to ligand movement and in biochemistry to study protein receptor−ligand interactions but are often ignored or forgotten by synthetic chemists. In this paper, we illustrate the use of 1D selective chemical-exchange NMR experiments to distinguish rotamers from diastereomers in circumstances where the possibilities of both isomers exist.

To make clear the problem we wish to address, consider the HATU[6] coupling of (*R*)-*α*-hydroxyvaline[7] (**1**) and (*R*)-*N*-Me-Boc-Val-OH[8] (**2**) to give depsipeptide **3** (Figure 1). Upon inspection of the ^{1}H NMR spectrum ($CDCl_3$) of the crude reaction mixture, two peaks at 4.59 and 4.28 ppm of equal intensities corresponding to the H_A proton in product **3** are observed.

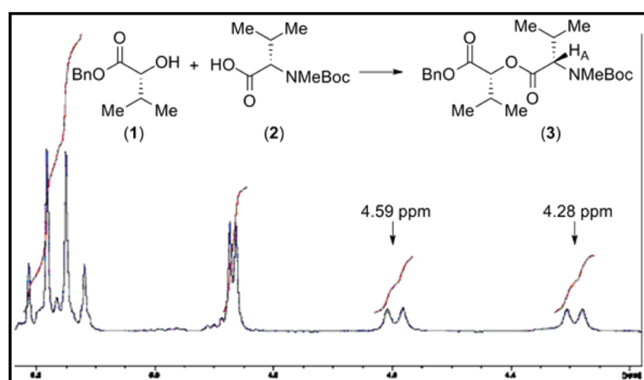


Figure 1. ^{1}H NMR spectrum of the crude mixture resulting from the coupling of alcohol **1** and acid **2** under standard coupling conditions to give depsipeptide **3** (see the Experimental Section) (5.2−4.1 ppm region only).

Do these two peaks imply the existence of two rotamers due to the protecting group (**3** and **3′**, Figure 2) or is the product of the reaction a complete mixture of diastereomers due to the epimerization of **2** after activation, as is very common in peptide coupling due to oxazolone or ketene formation?[9−12] Usually, the rate of carbamate rotameric exchange is sufficiently fast and the rate of epimerization in neutral chloroform is negligible so that we can reduce the question of rotamers vs diastereomers to a question of detecting the chemical exchange.[13] If the proton responsible for the peak at 4.59 ppm and the proton responsible for the peak at 4.28 ppm behave spectroscopically as if they are under chemical exchange, then the two compounds in solution are not diastereomers, whereas if the two protons are not under chemical exchange, the two compounds are likely to be diastereomers. It is possible to answer the chemical-exchange question with a 1D-selective chemical-exchange NMR experiment without the need for further work.

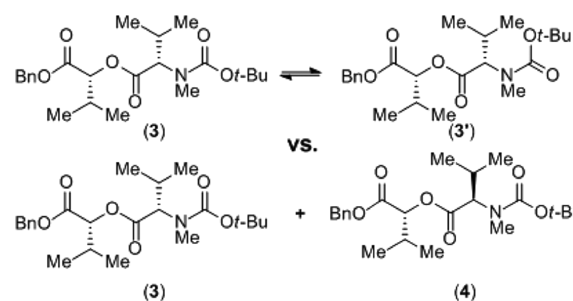


Figure 2. At first glance, the molecules present in the NMR sample may be either rotamers **3** and **3′** or diastereomers **3** and **4**.

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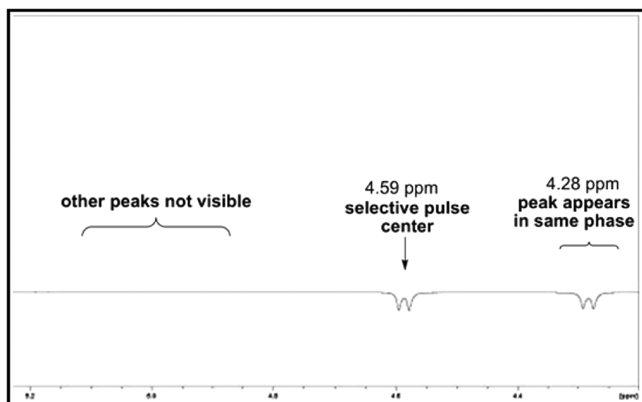

Figure 3. 1D gradient NOE spectrum of crude compound **3** (see Figure 1) with an initial selective pulse at 4.59 ppm creates a peak at 4.59 ppm as well as a new peak of the *same* phase at 4.28 ppm due to rotameric chemical exchange. In contrast, normal NOE enhancements appear in the *opposite* phase from the selective pulse peak.

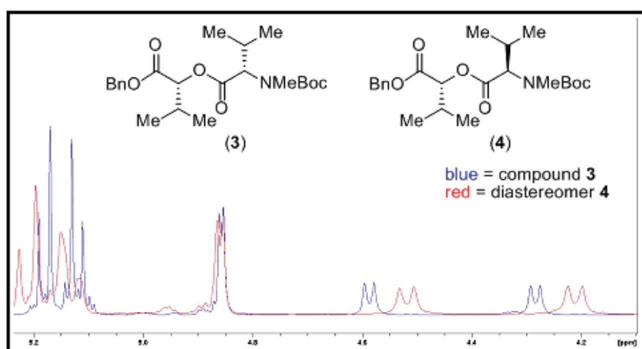

Figure 4. Direct comparison of an NMR sample of **3** with an NMR sample of synthetic **4** supports the conclusion about the identity of the two compounds in the described reaction mixture as rotamers.

A convenient method to conduct a 1D-selective chemical-exchange experiment is to simply use the pulse sequence of the commonly used 1D NOE difference experiment or its modern counterpart, the 1D gradient NOE experiment.[14,15] In a standard 1D NOE difference experiment, a selected resonance is first treated with a long saturating pulse before a nonselective 90° pulse is applied, leading to the disappearance of peaks in the targeted frequency region and a slight enhancement in intensity of peaks (in the small molecule fast-tumbling regime) corresponding to protons connected to those in the targeted frequency region through space via the nuclear Overhauser effect. The subtraction of a previously acquired standard ^{1}H NMR spectrum from the 1D NOE spectrum results in the NOE difference spectrum, which will show a negative peak at the site of irradiation and a positive peak at the sites of enhancement.[16] If the targeted peak at the site of irradiation corresponds to a proton under significant chemical exchange with another proton on the saturation time scale, the peak corresponding to the second proton will also appear diminished due to saturation transfer, resulting in a second negative peak in the difference spectrum. The selective refocusing of a frequency in a 1D gradient NOE experiment produces the same results through inversion transfer. For our work therefore, irradiation of the peak at 4.59 ppm using either a 1D NOE difference or 1D gradient NOE experiment will result in a spectrum which shows two negative peaks at 4.59 ppm and 4.28 ppm (see Figure 3), implying chemical exchange and thus the existence of rotamers. Comparison of the spectra of an intentionally prepared diastereomer **4** with **3** (Figure 4) supports this conclusion.

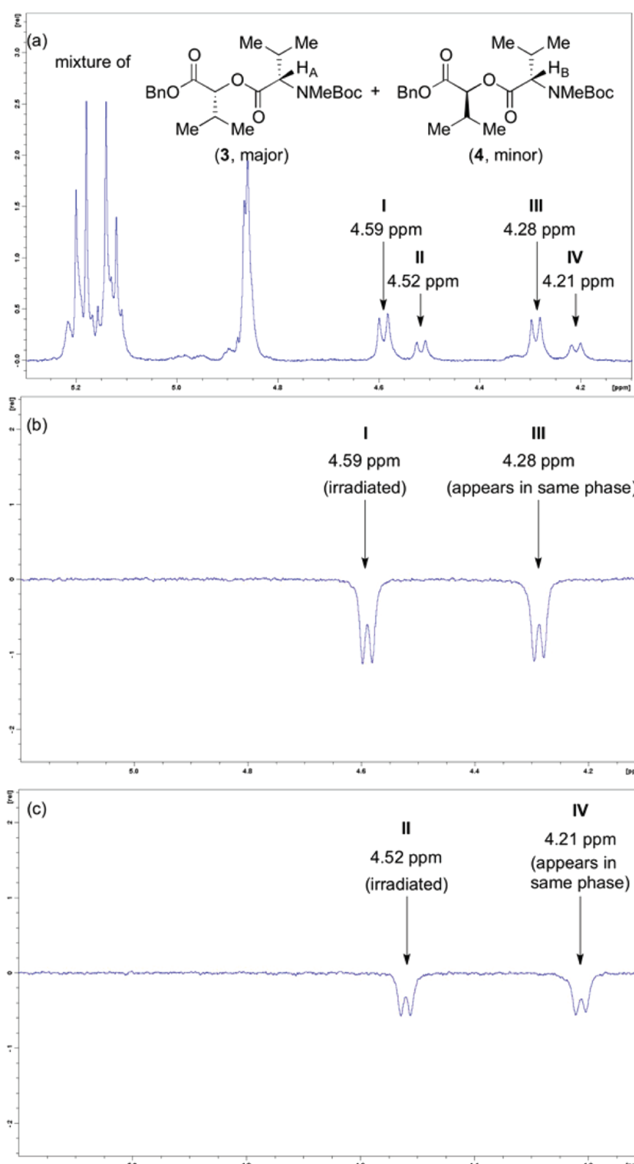

Figure 5. (a) ^{1}H NMR spectrum of a prepared sample deliberately containing both **3** and **4**. Four NMR resonances (**I**, **II**, **III**, and **IV**) are observed corresponding to protons H_A and H_B in **3**, **4**, and their respective rotamers. (b) 1D gradient NOE spectrum after selective excitation of the resonance at 4.59 ppm (**I**) produces a single downfield resonance in the same phase at 4.28 ppm (**III**), indicating that resonances **I** and **III** belong to two rotamers of the same diastereomer (**3**) and that resonances due to one diastereomer do not transfer spin information via chemical exchange to the other. (c) 1D gradient NOE spectrum after selective excitation of the diastereomeric peak at 4.52 ppm (**II**) also produces a single downfield peak in the same phase (**IV**), indicating that resonances **II** and **IV** belong to two rotamers of the same diastereomer (**4**). Only the 5.2−4.1 ppm region is shown for clarity.

When a sample containing both diastereomers **3** and **4** (Figure 5) is subject to the same experiment with a selective pulse at 4.59 ppm, a negative peak again only appears at 4.28 ppm, confirming that saturation/inversion transfer does not take place between diastereomers. Thus, chemical-exchange experiments can also be used to distinguish sets of rotamers in the presence of diastereomers

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so long as the excited peak is well resolved from the other peaks.

In a second experiment to illustrate the precision of the method, the ^{1}H NMR spectrum of the purified product mixture resulting from the coupling of depsipeptides **5** and **6** to give tetradepsipeptide **7** (Figure 6) shows an even more complicated

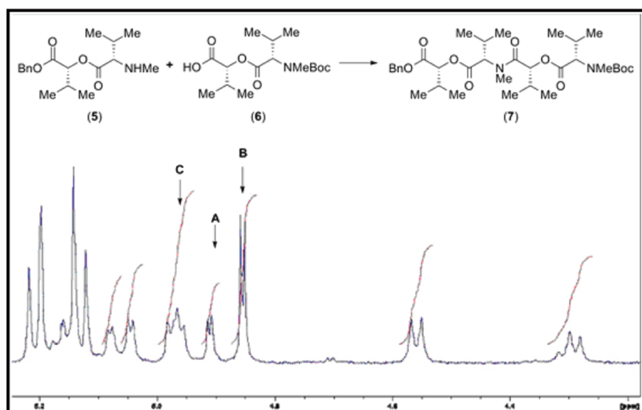

Figure 6. ^{1}H NMR spectrum of the column-chromatographed product mixture (**7**) resulting from the coupling of **5** and **6** (only 5.3−4.2 ppm region of interest shown). Resonances **A**−**C** are highlighted for clarity in the following discussion. Assuming resonance **B** corresponds to a proton in **7** or a combination of its rotamers due to its strong intensity, is resonance **A**, which is of low intensity relative to other peaks, due to the presence of an impurity or does it due to a proton in one of the many possible rotamers of **7**?

^{1}H NMR spectrum, even though the column fractions appear homogeneous by TLC. The question is again is: is peak **A** due to a rotamer or due to a diastereomer or some other impurity?

The question as before is one of chemical exchange and can be easily addressed by a 1D-selective chemical-exchange experiment. Selective irradiation of peak **B** at 4.85 ppm (Figure 7)

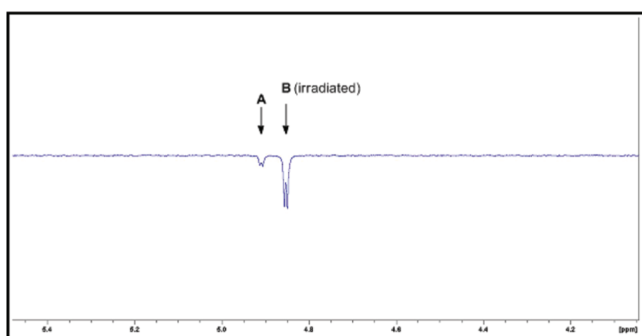

Figure 7. Selective irradiation of peak **B** (see Figure 6) at 4.85 ppm in a 1D gradient NOE experiment provides a spectrum (shown) revealing two negative peaks at frequencies 4.85 ppm (**B**) and 4.91 ppm (**A**).

results in the simultaneous appearance of an inverted peak **A** at 4.91 ppm. Because the saturating pulse does not have a perfect square frequency profile, however, there is the risk that the appearance of peak 4.91 ppm is actually due to overlap of an irradiation tail with the peak and not chemical exchange. The selectivity of the pulse, however, can be checked by irradiation of peak **A** and observing its effect at the location symmetrical to the location of peak **B**. Indeed, saturation of the peak at 4.91 ppm (Figure 8) does not result in the appearance of the peak **C** to the left (upfield) at 4.97 ppm but does result in the appearance of the

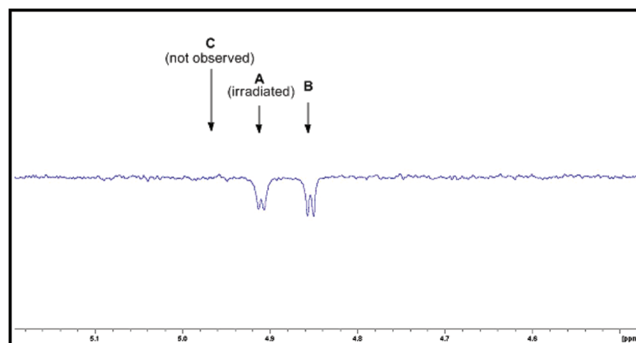

Figure 8. Selective irradiation of peak **A** (see Figure 6) at 4.91 ppm in a 1D gradient NOE experiment provides a spectrum (shown) revealing two negative peaks at frequencies 4.91 (**A**) and 4.85 ppm (**B**), but notably no peak (**C**) at 4.97 ppm.

peak **B** to the right (downfield) at 4.85 ppm, confirming that the appearance of peak 4.91 ppm with the first irradiation at 4.85 ppm was not due to broadness of the irradiation window. Thus, the selective chemical-exchange NMR technique can be used to identify peaks due to rotamers even in spectra in which peaks in question are separated by as little as 0.06 ppm at 600 MHz, as long as a proper control experiment is conducted.

In principle, selective-excitation chemical-exchange NMR experiments should be applicable to any scenario in which the simultaneous possibility of interconverting compounds and the appearance of new diastereomers or other nonexchanging impurities makes simple ^{1}H NMR analysis inconclusive as long as the exchange is slow on the chemical shift time scale such that the relevant peaks are distinct and the exchange is sufficiently fast that there is significant inversion or saturation transfer during the selective irradiation/mixing time.

While experiments to probe chemical exchange such as saturation-transfer NMR sequences have been known and applied for many years,[17] they have been largely overlooked[18] by organic chemists in common problems such as distinguishing rotamers from diastereomers. Here, we have illustrated that selective chemical-exchange NMR experiments are trivial, useful methods specifically for distinguishing rapidly equilibrating rotamers from nonequilibrating diastereomers. The techniques described can also be generally applied to other problems involving chemical exchange. We believe application of these methods avoids the use of traditional VT-NMR methods and greatly speeds up accurate product analyses.

■ EXPERIMENTAL SECTION

General Methods. Unless otherwise stated, all reactions were conducted under anhydrous conditions under an atmosphere of argon. ^{1}H NMR spectra were collected on a 600 MHz NMR spectrometer using the deuterated solvent as an internal deuterium lock. Chemical shift data are given in units δ calibrated with residual protic solvent (e.g., $CHCl_3$ at 7.26 ppm). The multiplicity of a signal is indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; etc. Coupling constants (J) are recorded to the nearest 0.1 Hz. ^{13}C NMR spectra were collected on a 150 MHz spectrometer with broadband proton decoupling using the deuterated solvent as an internal deuterium lock. Chemical shift data are given in units δ calibrated with residual protic solvent (e.g., 77.23 ppm for $^{13}CHCl_3$). The 1D gradient NOE spectra in the manuscript were obtained on a 600 MHz spectrometer using a 100 ms Gaussian selective pulse and a 1.2 s mixing time with a standard 1D gradient NOE pulse sequence.[14] Only selected absorbances (ν_{max}) are reported in the IR spectra. Optical rotations were measured with the sample temperature maintained at 25 °C. $[\alpha]_D$ is reported in units of 10^{-1} deg g^{-1} cm^2. Concentration is quoted in units of 0.01 g cm^{-3}.

Synthesis of (*S*)-*N*-Butoxycarbonyl-*N*-methylvaline (1*R*)-2-Methyl-1-[(phenylmethoxy)carbonyl]propyl Ester 3. To a solution of phenylmethyl (2*R*)-2-hydroxy-3-methylbutanoate **1** (2.0 g, 9.6 mmol), *N*-methyl-*N*-Boc-valine **2** (2.3 g, 1.05 equiv), and 4-dimethylaminopyridine (2.35 g, 2.0 equiv) in DCM (29 mL, 0.33M) at 0 °C was added solid HATU (4.0 g, 1.1 equiv) in several small portions. The reaction mixture was allowed to stir for 4 h before dilution with diethyl ether (100 mL) and water (50 mL). The organic layer was separated and the solution washed sequentially with 1 M aq HCl (100 mL), H_2O (50 mL), and half-satd aq Na_2CO_3 solution (2 × 100 mL, mixing the layers vigorously for 10 min). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford depsipeptide **3** as a clean oil sufficiently pure for the next steps (2.83 g, 70% yield) and immediately subjected to the NMR experiments described in the main text. An analytical sample for complete characterization was obtained by subjection of the product to silica gel flash column chromatography (3% EtOAc in 40−60 petroleum ether). ^{1}H NMR (600 MHz, $CDCl_3$): (mixture of rotamers) 7.37−7.28 (m, 5H), 5.18 (d, 1H, *J* = 12.0 Hz), 5.12 (d, 1H, *J* = 12.0 Hz), 4.86 (br s, 1H), 4.59 (d, 0.5H, *J* = 10.6 Hz), 4.28 (d, 0.5H, *J* = 10.6 Hz), 2.88 (s, 1.5H), 2.77 (s, 1.5H), 2.25 (br m, 1H), 2.18 (br m, 1H), 1.46 (s, 9H), 1.02−0.87 (m, 24H). ^{13}C NMR (150 MHz, $CDCl_3$): (mixture of rotamers) 171.0, 170.7, 169.2, 169.0, 156.3, 155.6, 135.3, 135.3, 128.5, 128.4, 128.3, 80.1, 79.8 66.9, 66.8, 64.7, 63.0, 30.6, 30.0, 28.3, 27.6, 27.4, 19.9, 19.1, 18.8, 18.8, 18.8, 17.0. IR (neat, cm^{-1}): 2968.9, 2935.1, 2878.2, 1740.9, 1694.5, 1455.8, 1390.2, 1366.6, 1307.4, 1258.6, 1182.8, 1146.6, 1125.4, 1024.5, 751.8, 697.1. $[\alpha]^{26.5}{}_D$: −33.3 (*c* = 1.28, $CHCl_3$). HRMS (ESI-TOF) ([M + Na$^+$]): calcd for $C_{23}H_{35}O_6NNa$ 444.2356, found 444.2356.

Synthesis of (*R*)-*N*-Butoxycarbonyl-*N*-methylvaline (1*S*)-2-Methyl-1-[(phenylmethoxy)carbonyl]propyl Ester 4. To a solution of phenylmethyl (2*S*)-2-hydroxy-3-methyl-butanoate(100 mg, 0.48 mmol), *N*-methyl-*N*-Boc-valine **2** (122 mg, 1.1 equiv), and 4-dimethylaminopyridine (152 mg, 2.6 equiv) in DCM (1.5 mL, 0.33 M) at 0 °C was added solid HATU (220 mg, 1.2 equiv) all at once. The reaction mixture was allowed to stir for 4 h before dilution with diethyl ether (10 mL) and water (5 mL). The organic layer was separated, and the solution washed sequentially with 1 M aq HCl (10 mL), H_2O (5 mL), and half-satd aq Na_2CO_3 solution (2 × 10 mL, mixing the layers vigorously for 10 min). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The product was subjected to silica gel flash column chromatography (3% EtOAc in 40−60 petroleum ether) to afford depsipeptide **4** as a clean oil (158 mg, 78% yield). ^{1}H NMR (600 MHz, $CDCl_3$): (mixture of rotamers) 7.35−7.27 (m, 5H), 5.19 (d, 1H, *J* = 12.6 Hz), 5.12 (d, 1H, *J* = 12.6 Hz), 4.85 (app. S, 1H), 4.51 (d, 0.5 H, *J* = 9.9 Hz), 4.20 (d, 0.5H, *J* = 9.9 Hz), 2.83 (s, 1.5H), 2.79 (s, 1.5H), 2.30−2.12 (m, 2H), 1.44 (s, 9H), 1.06−0.82 (m, 12H). ^{13}C NMR (150 MHz, $CDCl_3$): (mixture of rotamers) 171.1, 170.6, 169.2, 156.0, 155.4, 135.3, 128.5, 128.4, 128.3, 80.2, 79.8, 77.0, 66.9, 66.8, 64.8, 63.2, 30.7, 30.5, 30.1, 28.3, 27.6, 19.8, 19.6, 18.8, 18.8, 18.8, 18.7, 17.0. IR (neat, cm^{-1}): 2968.3, 2926.2, 2878.1, 1741.7, 1695.2, 1455.8, 1390.0, 1366.6, 1294.3, 1258.8, 1181.4, 1145.3, 1124.7, 1024.5, 935.6, 880.6, 750.5, 697.3. $[\alpha]^{26.5}{}_D$: −70.9 (*c* = 1.63, $CHCl_3$). HRMS (ESI-TOF) ([M + Na$^+$]): calcd for $C_{23}H_{35}O_6NNa$ 444.2356, found 444.2364.

Synthesis of Acid 5. To a solution of depsipeptide **3** (1.4 g, 3.3 mmol) in THF (6.5 mL, 0.5 M) was carefully added Pd/C under Ar. The solution was then purged with H_2 gas and allowed to stir under H_2 (1 atm, balloon) overnight. The reaction mixture was then purged with Ar, filtered over a pad of Celite over silica with EtOAc (100 mL), and concentrated in vacuo to afford acid **4** which was sufficiently pure for the next step (1.08 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$): (mixture of rotamers) 5.01 (br s, 0.66H), 4.89 (br s, 0.34H), 4.28 (d, 0.34H, *J* = 9.5 Hz), 4.17 (d, 0.66H, *J* = 10.0 Hz), 2.91 (s, 2H), 2.86 (s, 1H), 2.30 (br m, 2H), 1.46 (s, 9H), 1.08−0.90 (br m, 12H). ^{13}C NMR (150 MHz, $CDCl_3$): 173.96, 170.87, 170.65, 156.63, 155.85, 80.49, 80.33, 76.46, 66.13, 64.66, 63.49, 31.13, 30.07, 29.99, 29.95, 29.83, 28.30, 27.52, 27.45, 19.89, 19.80, 19.09, 18.87, 18.79, 17.19, 16.96, 16.88, 16.09. IR (neat, cm^{-1}): 2969.7, 2935.7, 2878.6, 1741.5, 1696.4, 1657.4, 1657.4, 1469.9, 1448.7, 1392.2, 1368.6, 1309.7, 1198.5, 1149.8, 1126.6, 1022.3, 752.9. $[\alpha]^{26.5}{}_D$: −49.0 (*c* = 4.03, $CHCl_3$). HRMS (ESI-TOF) ([M + H$^+$]): calcd for $C_{16}H_{30}O_6N$ 332.2068, found 332.2066.

Synthesis of Amine 6. To depsipeptide **3** (440 mg, 1.05 mmol) was added 2 mL of a solution of 4 M HCl in dioxane. The reaction was allowed to stir overnight at room temperature before the mixture was concentrated in vacuo. The liquid salt was taken up in Et_2O (25 mL) and washed with half-saturated aq Na_2CO_3 (2 × 25 mL). The aqueous layers were back-extracted with Et_2O (2 × 25 mL) and the combined organic layers dried over $MgSO_4$, filtered, and concentrated in vacuo to provide amine **6** which was sufficiently pure for the next step (212 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.39−7.28 (m, 5H), 5.21 (d, 1H, *J* = 12.0 Hz), 5.15 (d, 1H, *J* = 12.0 Hz), 4.91 (d, 1H, *J* = 4.4 Hz), 3.01 (d, 1H, *J* = 6.0 Hz), 2.35 (s, 3H), 2.27 (m, 1H), 1.94 (m, 1H), 1.53 (s, 1H), 1.02−0.92 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.9, 169.5, 135.3, 128.5, 128.4, 76.9, 69.2, 66.9, 35.2, 31.5, 30.0, 19.2, 18.9, 18.6, 17.2. IR (neat, cm^{-1}): 2965.8, 2936.8, 2878.3, 1734.1, 1498.8, 1465.8, 1456.1, 1266.9, 1178.7, 1164.0, 1126.1, 1019.5, 749.0, 696.9. $[\alpha]^{25.5}{}_D$: +28.2 (*c* = 2.15, $CHCl_3$). HRMS (ESI-TOF) ([M + H$^+$]): calcd for $C_{18}H_{28}O_4N$ 322.2013, found 322.2012.

Synthesis of Didepsipeptide 7. To a solution of acid **5** (500 mg, 1.5 mmol) in DCM (5 mL) at 0 °C was added Ghosez's reagent (240 μL, 1.2 equiv). The solution was allowed to stir for 15 min before a solution of amine **6** (480 mg, 1 equiv) and diisopropylethylamine (690 μL, 2.6 equiv) in DCM (5 mL) was added. The solution was allowed to warm to room temperature overnight. The reaction mixture was diluted with Et_2O (50 mL), upon which *N*,*N*-dimethylisobutylamide crystallized out of solution. The mixture was washed with 1 M aq HCl solution (25 mL), H_2O (25 mL), and half-saturated aq Na_2CO_3 solution (25 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to provide a clean crude product which was further purified by flash column chromatography (3% to 5% to 10% EtOAc/40−60 petroleum ether) to provide didepsipeptide **7** which was homogeneous by TLC analysis and subjected to the NMR experiments described in the main text (404 mg, 43%). ^{1}H NMR (600 MHz, $CDCl_3$): (mixture of rotamers) 7.34−7.27 (m, 5H), 5.22−3.98 (m, 4H), 3.01−2.76 (m, 6H), 2.42−2.02 (m, 4H), 1.44 (s, 9H), 1.06−0.76 (m, 24H). ^{13}C NMR (150 MHz, $CDCl_3$): (mixture of rotamers) 171.0, 170.8, 170.6, 170.3, 170.2, 169.8, 169.6, 169.6, 169.4, 169.2, 168.6, 156.4, 155.7, 135.4, 135.1, 128.6, 128.5, 128.4, 128.3, 80.0, 79.7, 77.5, 75.9, 75.5, 75.0, 74.9, 67.1, 66.8, 65.7, 64.9, 64.7, 63.3, 63.0, 61.7, 61.6, 31.8, 31.7, 31.0, 30.7, 30.3, 30.0, 29.9, 29.6, 29.4, 29.4, 28.6, 28.3, 28.3, 28.0, 27.7, 27.3, 27.3, 20.1, 19.9, 19.6, 19.5, 19.4, 19.3, 19.1, 19.0, 18.9, 18.9, 18.8, 18.6, 17.1, 17.0, 16.9, 16.6, 16.4. IR (neat, cm^{-1}): 2968.1, 2935.9, 2877.3, 1738.7, 1694.1, 1668.7, 1468.4, 1390.5, 1367.3, 1238.5, 1184.3, 1147.7, 1126.5, 1020.4, 881.3, 751.9, 697.7, 665.3. $[\alpha]^{26.5}{}_D$: −45.7 (*c* = 2.0, $CHCl_3$). HRMS (ESI-TOF) ([M + H$^+$]): calcd for $C_{34}H_{55}O_9N_2$ 635.3902, found 635.3905.

■ ASSOCIATED CONTENT

Supporting Information

Full ^{1}H and ^{13}C spectra of new compounds. 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.

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