Article

Characterization of Spatially Addressable Libraries: Stereoisomer Analysis of Tetrahydro-β-carbolines as an Example

Cesar C. Cheng, and Yen-Ho Chu

J. Comb. Chem., **1999**, 1 (6), 461-466• DOI: 10.1021/cc980026a • Publication Date (Web): 15 September 1999

Articles

Characterization of Spatially Addressable Libraries: Stereoisomer Analysis of Tetrahydro-β-carbolines as an Example

Cesar C. Cheng and Yen-Ho Chu*,†

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received October 13, 1998

Combinatorial chemistry approaches have facilitated the process of lead discovery and optimization for new drugs, catalysts, and materials. A successful combinatorial program typically includes high throughput library synthesis, characterization, and screening. One of many challenges in this program is to develop high throughput characterization methods to address issues concerning reaction stereoselectivity and racemization during the library synthesis. Using the core structure of tetrahydro-β-carboline as an example, we demonstrate the usefulness of capillary electrophoresis in enantiomeric separation of stereoisomers generated by parallel synthesis and rapid quantitative measurement of the isomer ratios, in addition to assessing possible racemization during reactions for its overall potential in library characterization.

Combinatorial chemistry approaches have facilitated the process of lead discovery and optimization for new drugs, catalysts, and materials.[1] A successful combinatorial program often includes high throughput library synthesis, characterization, and screening. In terms of library synthesis, the use of either parallel or split synthesis can generate large numbers of small organic compounds.[2] For library characterization, HPLC and mass spectrometry are currently the methods of choice to address the purity and chemical integrity of the compounds from libraries synthesized in, for example, 96-microtiter racks.[3] In high throughput screening, cell-based activity assays or biochemical assays (e.g. scintillation proximity assay and fluorescence polarization) have increased the speed of the lead discovery process.[4] The challenge remains for developing high throughput characterization methods to address many important issues, including reaction stereoselectivity and possible racemization involved during the library synthesis. Using the core structure of tetrahydro-β-carboline as an example[5] and capillary electrophoresis (CE) as the characterization method,[6] we report here our initial efforts in the analysis of stereoisomers present in libraries generated by parallel synthesis.

Tetrahydro-β-carboline is a useful building block in the synthesis of various indole and isoquinoline alkaloids.[5] Natural and synthetic products having the carboline structure often exhibit important medicinal properties. For example, demethoxyfumitremorgin C and cyclotryprostatins have been reported as mammalian cell cycle inhibitors.[7] Using the Pictet−Spengler reaction (Scheme 1), substituted tetrahydro-β-carbolines can be directly prepared from tryptophan reaction with various aldehydes and ketones.[5] The reaction requires acid catalysts (e.g. trifluoroacetic acid) and typically produces mixtures of stereoisomers in moderate to excellent yields.[5] Therefore, an efficient analytical method is needed to monitor the reaction stereoselectivity and quantitative measurement of the relative amounts of the stereoisomers formed under various reaction conditions. Methods frequently employed for stereoisomer separations include HPLC,[8] TLC,[9] GC,[10] and more recently, CE.[11] CE is a high resolution separation technique that measures the electrophoretic mobility of a charged species in the presence of an electric field gradient.[6] The CE separation is typically carried out in a fused silica capillary with a small inner diameter (20−100 μm) that allows efficient dissipation of the joule heat generated during the electrophoresis, thus permitting the use of high voltages (30 kV) to result in fast separation with high resolution.[6] Compared with HPLC in chiral separation, CE offers the advantages of low cost (e.g. the chiral selector is used as an additive and does not require immobilization as compared to chiral stationary phase in HPLC), high efficiency (∼10^6 theoretical plates), rapid method development and optimization, and easy application to chiral separation studies. In this article, CE was applied in the enantiomeric separation of tetrahydro-β-carboline stereoisomers and rapid quantitative measurement of the isomer ratios, in addition to assessing racemization during reactions.

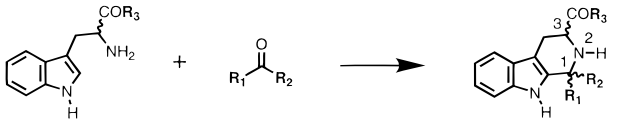
Tetrahydro-β-carboline analogues (**1**−**10**) used in this study were prepared using the Pictet−Spengler reaction (Scheme 1). Butyraldehyde, benzaldehyde, and acetone were chosen as representative aliphatic aldehydes, aromatic alde-

* To whom correspondence should be addressed.

† Present address: Dr. Yen-Ho Chu, Deaprtment of Chemistry, National Chung-Cheng University, Chia-Yi, Taiwan 621, Republic of China. Tel: +886 5 242 8148. Fax: +886 5 272 1040. E-mail: cheyhc@ccunix.ccu.edu.tw.

10.1021/cc980026a CCC: $18.00
Published on Web 09/15/1999

Scheme 1. Pictet−Spengler Reaction between Tryptophans and Aldehydes or Ketones[a]



	Tryptophan	R_1	R_2	R_3
1	*S*-form	CH_3	CH_3	OH
2	*R*-form	CH_3	CH_3	OH
3	*S*-form	C_3H_7	H	OH
4	*R*-form	C_3H_7	H	OH
5	*S*-form	C_6H_5	H	OH
6	*R*-form	C_6H_5	H	OH
7	*S*-form	CH_3	C_2H_5	(*S*)-tyrosine
8	*S*-form	CH_3	C_2H_5	(*R*)-tyrosine
9	*R*-form	CH_3	C_2H_5	(*S*)-tyrosine
10	*R*-form	CH_3	C_2H_5	(*R*)-tyrosine

[a] Note (*R* or *S*, *R* or *S*)-compound correspond to C1, C3, and tyrosine stereochemistries, respectively.

hydes, and ketones, respectively. The aldehydes and ketones were individually coupled with tryptophan of both *R* and *S* forms to generate tetrahydro-β-carboline enantiomers in the case of acetone and diastereomeric mixtures in the case of the aldehydes. Cyclodextrin, a widely used chiral selector for small organic (mostly aromatic) compounds, was employed as additive in the electrophoresis buffer to resolve the tetrahydro-β-carboline compounds using micellar electrokinetic chromatography (MEKC).[11] MEKC is a useful application of CE where the mobile "chromatographic" phase consists of a micelle and chiral selector such as cyclodextrin in the electrophoresis buffer. The chiral separation is achieved due to differential partitioning of enantiomers between the micelle and the chiral selector (because of different binding affinities of the enantiomers to the chiral selector), resulting in differential migration.[11] Using MEKC, γ-cyclodextrin in a tris−glycine buffer containing SDS readily furnished the separation of compounds **1**−**6** (Figure 1). Peak assignments in the separation were achieved by injecting mixtures of isomers at various concentrations. Also, the stereochemistry of diastereomeric *cis*- and *trans*-1,3-disubstituted tetrahydro-β-carbolines was determined by ^{13}C NMR following the method developed by Cook and co-workers.[12] The chemical signals corresponding to the carbon atoms 1 and 3 in the trans isomers are shifted upfield relative to the cis isomers, presumably due, in part, to the unfavorable 1,3-diaxial interactions present in the trans isomers but which are absent in the cis isomers.[12] Integration of the peaks in electropherograms can readily afford the ratio of cis and trans diastereomers for each compound synthesized at ambient temperature (e.g. 77:23 for **6**). Under the conditions of the electrophoresis run, enantiomers (*R*,*R*)-**6** and (*S*,*S*)-**5**, (*R*,*S*)-**5** and (*S*,*R*)-**6**, (*R*,*S*)-**3** and (*S*,*R*)-**4** were separated (Figure 1).

To further demonstrate the potential of CE in stereoisomer analysis, tetrahydro-β-carboline dipeptide analogues containing three chiral centers (compounds **7**−**10**) were synthesized. Tryptophan and tyrosine amino acids of both *R* and *S* forms were coupled, each followed by the Pictet−Spengler reaction with butanone to generate four diastereomeric mixtures (**7**−**10**). Tyrosine was chosen for its aromatic ring feature and butanone for its clean reaction and facile workup. Figure 2 shows that, using β-cyclodextrin in MEKC, all eight ($2^3 = 8$) stereoisomers of the carboline dipeptide analogues (**7**−**10**) were successfully separated. Each pair of enantiomers were resolved within 30 min, with baseline separation for compounds **8** and **9** utilizing an increased concentration of β-cyclodextrin (see the inset in Figure 2). Again, comparison and integration of the peaks can readily yield the ratio of cis and trans isomers for each of the dipeptide analogues. Results of Figures 1 and 2 clearly demonstrated that CE is well suited for rapid diastereomer analysis.[13] Moreover, because of the stereoisomer separation evident for compounds **5**−**10** (Figures 1, 2), this MEKC can be directly applied for assessing racemization during library synthesis.

The Pictet−Spengler reaction has recently been exploited for library generation of tetrahydro-β-carbolines on solid support.[14] In high throughput synthesis of tetrahydro-β-carbolines, obtaining an optimized reaction condition for every library compound may not be straightforward due to the wide range of chemical reactivity of the aldehydes and ketones. The generalized reaction condition is difficult to obtain for those aldehydes and, in particular, aryl ketones (e.g. acetophenone, benzophenone) that react sluggishly at ambient temperature. Although the Pictet−Spengler reaction can be accelerated at elevated temperatures, racemization under these heated conditions have been reported.[15,16] Both ^1H and ^{13}C NMR are currently utilized for stereochemical assignments of the diastereomers,[5] and without the aid of chiral shift reagents, they yield no information about the presence or absence of enantiomers as a result of reaction racemization. CE can be readily employed to monitor racemization, if any, during reactions and is likely to facilitate the rapid optimization of experimental conditions for library synthesis (Figures 1 and 2). Figure 3 shows that the reaction of (*S*)-tryptophan with benzaldehyde at refluxing temperature resulted in 30% racemization with the reaction completed in 3 h (only 50% completion observed at ambient temperature) (see Experimental Section for details). Our preliminary studies of the tetrahydro-β-carboline library indicated that racemization may occur before the completion of the Pictet−Spengler reaction at elevated temperatures. Additional racemization study and further improvement in reaction stereoselectivities of the tetrahydro-β-carbolines and other small organic compounds are in progress and will be reported in due course.

In summary, we have demonstrated the usefulness of CE in characterizing the stereoisomers of substituted tetrahydro-β-carbolines, determining isomer ratios, and assessing possible reaction racemization during library synthesis. CE should be applicable, at least in principle, to the stereoisomer characterization of other libraries of small organic molecules such as pyrrolidines, prolines, β-lactams, hydantoins, and isoxazolines.[17] With CE as a useful tool for library characterization, the development of a stereospecific route to molecular diversity would therefore increase the integrity of material available for study and offer greater potency of compounds in drug development.[18]

Experimental Section

(*R*)- and (*S*)-Tryptophan, (*R*)- and (*S*)-tyrosine, and butyraldehyde were obtained from either Sigma or Aldrich Chemical Co. and were used as received. Commercially available solvents including acetone, DMF, dichloromethane, methyl alcohol, toluene, and diethyl ether were of HPLC

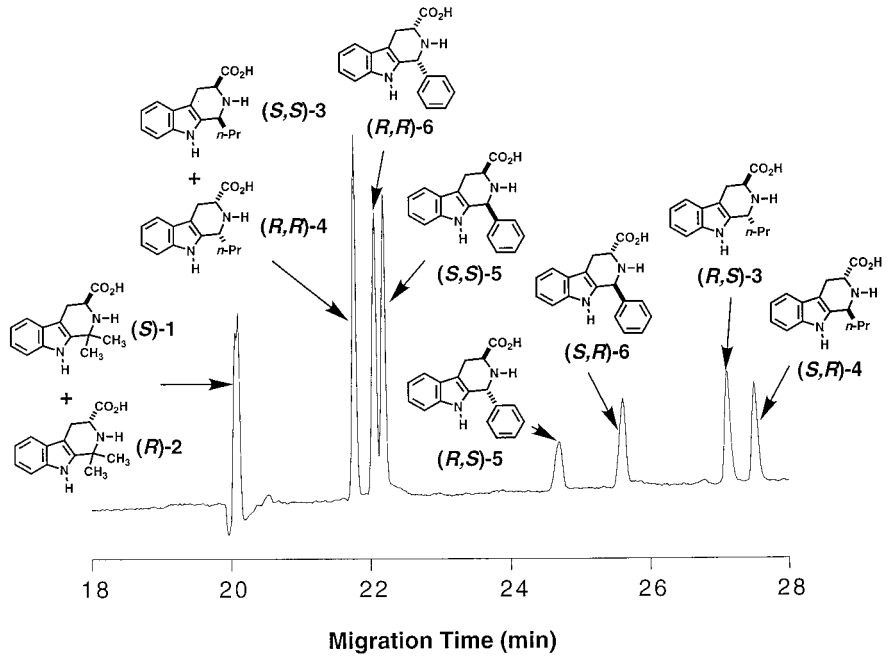


Figure 1. Stereoisomer separation of substituted tetrahydro-β-carbolines **1**−**6** using capillary electrophoresis in the presence of γ-cyclodextrin. Integration of the peaks can readily yield the ratio of cis and trans isomers for each of the compounds. Buffer used: 214 mM glycine, 25 mM tris base, 100 mM sodium dodecyl sulfate, 50 mM γ-cyclodextrin (pH 8.64); capillary: uncoated fused silica, 97 cm total length, 90 cm effective length, 50 μm inner diameter; CE condition: 30 kV, 26 mamp, 280 nm, 25 °C.

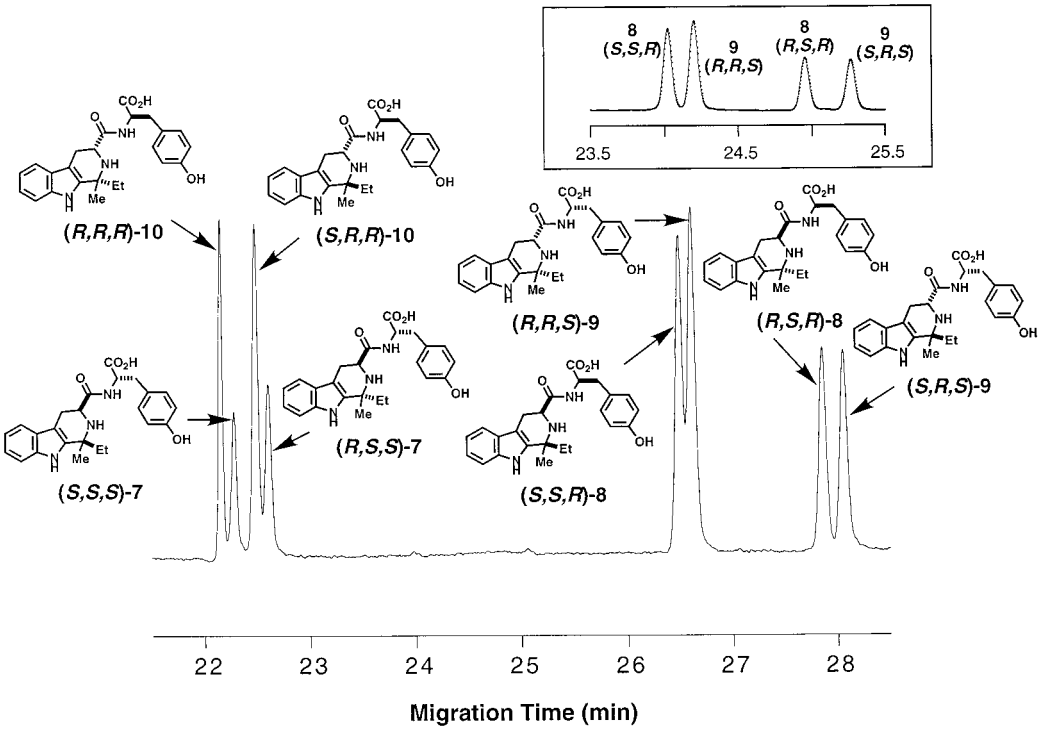


Figure 2. Stereoisomer separation of substituted tetrahydro-β-carboline dipeptide analogues **7**−**10**. All eight possible stereoisomers were separated using β-cyclodextrin. Buffer used: 192 mM glycine, 25 mM tris base, 100 mM sodium dodecyl sulfate, 8.8 mM β-cyclodextrin (pH 8.56); capillary: uncoated fused silica, 97 cm total length, 90 cm effective length, 50 μm inner diameter; CE condition: 30 kV, 26 mamp, 280 nm, 25 °C. Inset: Baseline separation of enantiomers **8** and **9** under increased concentration (26.4 mM) of β-cyclodextrin.

grade and were obtained from Tedia Co. Butanone was obtained from MCB Reagents and trifluoroacetic acid from Oakwood Products, Inc. Sodium phosphate, *p*-toluenesulfonic acid, sodium chloride, magnesium sulfate, benzaldehyde, and triethylamine were from J. T. Baker. Benzyl alcohol was purchased from Mallinckrodt. Sodium dodecyl sulfate was obtained from Bio-Rad Laboratories. Both β- and γ-cyclodextrins used in this work were gifts from the American Maize-Products Co.

Melting points were determined on a Mel-Temp II and are uncorrected. NMR spectra were recorded on a Bruker AC200 at 200 MHz (^{1}H) and 50.3 MHz (^{13}C) unless otherwise stated. Chemical shifts were measured in ppm on the δ scale downfield from tetramethylsilane as an internal

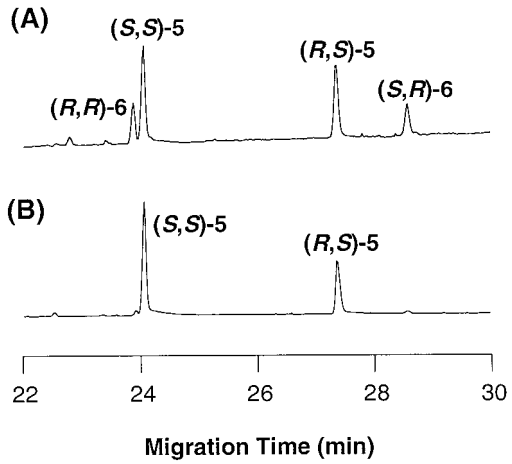


Figure 3. Racemization study of the Pictet−Spengler reaction of (*S*)-tryptophan with benzaldehyde. The reactions were carried out in dioxane at refluxing temperature (A) and room temperature (B) for 3 h. Although completed at 3 h, the heated reaction gave racemized products, whereas the room temperature reaction in 3 h was incomplete (50% conversion) with products (*S*,*S*)-**5** and (*R*,*S*)-**5** essentially racemization-free. Only Pictet−Spengler products are shown in the figure (migration time of tryptophan, 18.1 min).

standard. Where appropriate, ^{1}H NMR spectral data in brackets referred to diastereoisomeric signals that were resolved, but not specifically referencing to either the cis or trans diastereoisomer. ^{13}C NMR spectral data included the signals for both cis and trans diastereoisomers. Analytical TLC was performed on Whatman aluminum sheet silica gel F_{254} plates (thickness 250 mm). Compounds were visualized with a UV hand lamp. Unless otherwise indicated, all reactions were carried out under an atmosphere of dry nitrogen or argon.

General Procedure for the Synthesis of 1,2,3,4-Tetrahydro-β-carboline Analogues (1−6) Using the Pictet−Spengler Reaction. In a typical reaction, (*R*)- or (*S*)-tryptophan (5 mmol) was dissolved in dry dichloromethane (50 mL) and trifluoroacetic acid (5%, v/v). The aldehyde or ketone (20 mmol) was added in one portion to the stirred mixture at room temperature. The reaction was allowed to proceed until tryptophan was completely consumed as monitored by TLC using the ninhydrin test (2−4 h for butyraldehyde and benzaldehyde; 24−36 h for acetone). Upon completion of the reaction, the reaction mixture was concentrated to dryness under reduced pressure, and the product was afforded by precipitation with diethyl ether. In most cases, the product was slightly soluble in diethyl ether, resulting in the less than quantitative yield. Products were obtained in excellent purity as indicated by NMR and capillary electrophoresis; therefore, no further purification was performed and no attempts were made to maximize the reaction yield.

(*S*)-Tryptophan and Acetone (1): white solid (0.76 g, 61.9% yield), mp 212−216 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.70 (s, 6H), [1.82 (s, 6H)], 3.01 (dd, J = 11.8, 15.9 Hz, 1H), 3.32 (dd, J = 5.0, 15.9 Hz, 1H), 4.64 (dd, J = 4.8, 11.3 Hz, 1H), 6.99−7.17 (m, 4H), 7.37 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 11.40 (s, 1H); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 22.33, 25.02, 25.31, 51.59, 56.40, 103.19, 111.42, 118.10, 119.13, 121.86, 125.47, 134.86, 136.34, 170.17; EI-HRMS: $[M + H]^+$ calcd = 244.1212, found = 244.1231.

(*R*)-Tryptophan and Acetone (2): white solid (1.22 g, 99.8% yield), mp 212−216 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.70 (s, 6H), [1.81 (s, 6H)], 3.00 (dd, J = 11.8, 15.9 Hz, 1H), 3.32 (dd, J = 5.0, 15.9 Hz, 1H), 4.64 (dd, J = 4.8, 11.5 Hz, 1H), 6.99−7.17 (m, 4H), 7.36 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 11.37 (s, 1H); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 22.70, 25.39, 25.67, 51.88, 56.66, 103.54, 111.70, 118.49, 119.36, 122.13, 125.80, 135.27, 136.61, 170.68; EI-HRMS: $[M + H]^+$ calcd = 244.1212, found = 244.1225.

(*S*)-Tryptophan and Butyraldehyde (3): yellow solid (0.88 g, 68.3% yield; 50:50 diastereomeric mixture), mp 98−102 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.96 (t, J = 7.3 Hz, 3H), [0.99 (t, J = 7.2 Hz, 3H)], 1.51−1.63 (m, 2H), 1.88−2.10 (m, 2H), [2.17−2.25 (m, 2H)], 2.96−3.18 (m, 1H), 3.25−3.35 (m, 1H), 4.41 (dd, J = 4.8, 12.0 Hz, 1H), 4.61−4.73 (m, 1H), 7.00−7.16 (m, 2H), 7.38 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 7.7 Hz, 1H), 11.15 (s, 1H), [11.19 (s, 1H)]; ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 13.92, 18.06, 18.54, 21.91, 22.45, 33.22, 34.48, 51.22, 52.18, 53.38, 55.39, 104.20, 105.26, 111.69, 118.26, 119.34, 122.04, 125.82, 130.20, 130.31, 136.58, 136.76, 170.352, 170.46; EI-HRMS: $[M + H]^+$ calcd = 258.1368, found = 258.1375.

(*R*)-Tryptophan and Butyraldehyde (4): yellow solid (1.05 g, 81.2% yield; 46:54 diastereomeric mixture), mp 98−102 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.97 (t, J = 8.0 Hz, 3H), [1.00 (t, J = 7.8 Hz, 3H)], 1.52−1.61 (m, 2H), 1.90−2.00 (m, 2H), [2.16−2.24 (m, 2H)], 2.92−3.16 (m, 1H), 3.21−3.34 (m, 1H), 4.26 (dd, J = 4.9, 11.9 Hz, 1H), 4.48 (br t, 1H), [4.67 (br t, 1H)], 7.01−7.17 (m, 2H), 7.38 (d, J = 7.9 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 11.08 (s, 1H), [11.13 (s, 1H)]; ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 13.93, 18.04, 18.49, 21.98, 22.56, 33.26, 34.40, 51.00, 52.08, 53.24, 55.83, 104.47, 105.54, 111.61, 118.19, 119.24, 121.91, 125.83, 130.30, 130.42, 136.50, 136.70, 170.48, 170.62; EI-HRMS: $[M + H]^+$ calcd = 258.1368, found = 258.1349.

(*S*)-Tryptophan and Benzaldehyde (5): light yellow solid (1.15 g, 78.8% yield; 23:77 diastereomeric mixture), mp 128−132 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.14−3.54 (m, 2H), 4.37 (dd, J = 5.8, 7.7 Hz, 1H), [4.65 (dd, J = 5.0, 11.6 Hz, 1H)], 5.92 (s, 1H), [6.01 (s, 1H)], 7.00−7.61 (m, 9H), 10.85 (s, 1H), [11.07 (s, 1H)]; ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 22.23, 55.70, 57.82, 106.29, 107.12, 111.89, 118.43, 119.41, 122.24, 125.64, 125.75, 128.12, 128.84, 129.08, 130.21, 130.65, 134.07, 134.55, 136.85, 137.09, 170.06, 170.20; EI-HRMS: $[M + H]^+$ calcd = 292.1212, found = 292.1237.

(*R*)-Tryptophan and Benzaldehyde (6): light yellow solid (1.07 g, 73.4% yield; 32:68 diastereomeric mixture), mp 128−132 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.16−3.55 (m, 2H), 4.32−4.42 (m, 1H), [4.65 (dd, J = 4.8, 11.4 Hz, 1H)], 5.93 (s, 1H), [6.03 (s, 1H)], 7.05−7.67 (m, 9H), 10.87 (s, 1H), [11.09 (s, 1H)]; ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 22.22, 55.52, 57.66, 106.08, 106.93, 111.68, 118.21, 119.22, 122.04, 125.45, 125.54, 127.91, 128.62, 128.87,

130.00, 130.43, 133.85, 134.33, 136.75, 136.90, 169.82, 169.98; EI-HRMS: $[M + H]^+$ calcd = 292.1212, found = 292.1198.

Synthesis of (*R*)- and (*S*)-Tyrosine Benzyl Ester *p*-Toluenesulfonate Salt [(*R*)- or (*S*)-Tyr-OBzl·Tos]. Tyrosine (55.2 mmol), *p*-toluenesulfonic acid (60.7 mmol), and benzyl alcohol (0.2 mol) were added to benzene (50 mL) and refluxed azeotropically using a Dean−Stark apparatus for 15 h or until no additional water was observed. The reaction mixture was a cloudy suspension and became a clear solution as the reaction proceeded. The desired product was precipitated by diethyl ether (300 mL) and collected by vacuum filtration. The product was obtained as a white solid (21.57 g, 88.1% yield): ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.40 (d, J = 7.2 Hz, 3H), 2.28 (s, 3H), 4.18 (m, 1H), 5.23 (s, 2H), 7.11 (d, J = 6.4 Hz, 2H), 7.37 (m, 5H), 7.49 (d, J = 6.4 Hz, 2H), 8.33 (br s, 3H); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 15.7, 20.8, 48.0, 67.0, 128.0, 128.0, 128.1, 128.1, 128.5, 135.2, 137.9, 145.3, 169.8.

General Procedure for the Synthesis of 1,2,3,4-Tetrahydro-β-carboline Dipeptide Analogues (7−10) Using the Pictet−Spengler Reaction. In a typical reaction, (*R*)- or (*S*)-tryptophan (2.5 mmol) was dissolved in 4 N NaOH solution (2 mL) at 0 °C. To this solution, benzyl chloroformate (2.8 mmol) was added dropwise over 15 min with vigorous stirring. The reaction was carried at 0 °C for 2 h and room temperature for additional 2 h. Water (10 mL) was added and the reaction mixture acidified slowly with concentrated HCl. The white precipitate was collected by vacuum filtration and dried by lyophilization to afford Cbz-(*R*)- or (*S*)-tryptophan (2.5 mmol, 99.8% yield). Cbz-(*R*)- or (*S*)-tryptophan (2.5 mmol) and (*R*)- or (*S*)-Tyr-OBzl·Tos (2.5 mmol) was dissolved in DMF (2 mL) and triethylamine (1 mL) at 0 °C. To this solution, EDC (3.8 mmol) was added as a solid in one portion and was completely dissolved over a 45 min period. The reaction mixture was allowed to proceed at 0 °C for 2 h and at room temperature for an additional 8 h. Water (2 mL) was added, and the reaction mixture was extracted with ethyl acetate (3×). The organic layer was washed with 10% $NaHCO_3$ (2×), 5% citric acid solution (2×), saturated NaCl solution (1×), and dried over anhydrous $MgSO_4$. The organic layer was evaporated in vacuo to yield a viscous oil. The oil was dissolved in methanol:dichloromethane (1:1, v/v, 25 mL) with a palladium catalyst (40 mg, 20% $Pd(OH)_2$/C). A steady stream of hydrogen was bubbled into the reaction mixture for 12 h. The catalyst was filtered by vacuum filtration, and the filtrate was concentrated in vacuo to yield a white solid as the tryptophan tyrosine dipeptide. The white solid was dissolved in dry dichloromethane (10 mL) and trifluoroacetic acid (5%, v/v). To the solution, 2-butanone (10 mmol) was added in one portion to the stirred mixture at room temperature. The reaction was allowed to proceed until the dipeptide was completely consumed as monitored by TLC using the ninhydrin test (typically 48 h). Upon completion of the reaction, the mixture was concentrated to dryness under reduced pressure, and the product was afforded by precipitation with diethyl ether (typical isolated yields: 55−75%).

(*S*)-Tryptophan-(*S*)-tyrosine (7): off-white solid (0.72 g, 68.3% yield; 42:58 diastereomeric mixture), mp 150−160 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.98 (t, J = 7.4 Hz, 3H), [1.05 (t, J = 7.2 Hz, 3H)], 1.61 (s, 3H), [1.75 (s, 3H)], 1.91−2.32 (m, 2H), 2.86−3.21 (m, 3H), 3.36 (dd, J = 4.1, 15.5 Hz, 1H), 4.34−4.53 (m, 2H), 6.72 (d, J = 8.4 Hz, 2H), 6.96−7.24 (m, 4H), 7.35−7.47 (m, 2H), 8.89 (d, J = 7.6 Hz, 1H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 7.34, 8.88, 21.95, 22.24, 23.37, 30.91, 31.79, 35.66, 51.41, 52.28, 54.41, 54.58, 58.98, 59.33, 103.38, 103.68, 111.51, 115.14, 117.92, 119.19, 121.88, 125.35, 125.39, 127.18, 127.25, 130.14, 133.37, 135.16, 136.29, 136.33, 156.18, 168.23, 168.34, 172.33, 172.43; EI-HRMS: $[M + H]^+$ calcd = 421.2002, found = 421.1998.

(*S*)-Tryptophan-(*R*)-tyrosine (8): off-white solid (0.79 g, 75.3% yield; 45:55 diastereomeric mixture), mp 150−155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.93−1.09 (m, 3H), 1.59 (s, 3H), [1.71 (s, 3H)], 1.91−2.56 (m, 3H), 2.73−3.04 (m, 2H), 3.17 (dd, J = 3.7, 13.5 Hz, 1H), 4.21−4.71 (m, 2H), 6.79 (d, J = 7.9 Hz, 2H), 6.94−7.18 (m, 4H), 7.34−7.44 (m, 2H), 8.85−8.92 (m, 1H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 7.20, 8.81, 21.70, 21.99, 23.44, 30.77, 31.68, 36.49, 51.11, 52.04, 53.16, 58.75, 59.22, 103.25, 103.57, 111.45, 114.93, 117.94, 119.32, 122.01, 125.19, 127.05, 130.42, 132.85, 134.77, 136.11, 156.06, 167.66, 167.75, 167.83, 172.48, 172.51; EI-HRMS: $[M + H]^+$ calcd = 421.2002, found = 421.2012.

(*R*)-Tryptophan-(*S*)-tyrosine (9): off-white solid (0.76 g, 72.4% yield; 49:51 diastereomeric mixture), mp 150−155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.97 (t, J = 5.9 Hz, 3H), [1.03 (t, J = 5.7 Hz, 3H)], 1.59 (s, 3H), [1.71 (s, 3H)], 1.91−2.29 (m, 2H), 2.29−2.56 (m, 1H), 2.73−3.04 (m, 2H), 3.17 (dd, J = 3.9, 13.6 Hz, 1H), 4.23−4.77 (m, 2H), 6.79 (d, J = 8.2 Hz, 2H), 6.97−7.18 (m, 4H), 7.34−7.45 (m, 2H), 8.84−8.91 (m, 1H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 7.25, 8.90, 21.83, 22.08, 23.57, 30.82, 31.78, 36.62, 51.28, 52.20, 53.31, 58.81, 59.26, 103.31, 103.66, 111.50, 114.99, 117.91, 119.23, 121.90, 125.30, 127.00, 130.41, 133.17, 135.10, 136.26, 136.29, 156.32, 167.81, 167.90, 172.55, 172.60; EI-HRMS: $[M + H]^+$ calcd = 421.2002, found = 421.2018.

(*R*)-Tryptophan-(*R*)-tyrosine (10): off-white solid (0.69 g, 65.3% yield; 47:53 diastereomeric mixture), mp 150−160 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.97 (t, J = 7.3 Hz, 3H), [1.04 (t, J = 7.1 Hz, 3H)], 1.58 (s, 3H), [1.73 (s, 3H)], 1.91−2.29 (m, 2H), 2.83−3.19 (m, 3H), 3.34 (dd, J = 4.4, 15.7 Hz, 1H), 4.29−4.51 (m, 1H), 6.70 (d, J = 8.4 Hz, 2H), 6.94−7.18 (m, 4H), 7.34−7.47 (m, 2H), 8.86 (d, J = 7.5 Hz, 1H); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 7.55, 8.98, 22.21, 22.47, 23.43, 30.01, 31.01, 35.65, 51.48, 52.35, 54.42, 54.56, 59.01, 59.35, 103.42, 103.72, 111.55, 115.18, 118.00, 119.27, 121.98, 125.39, 127.28, 130.21, 133.37, 135.20, 136.32, 136.36, 156.20, 168.31, 168.37, 172.37, 172.47; EI-HRMS: $[M + H]^+$ calcd = 421.2002, found = 421.1990.

CE Instrumentation and Buffer. Capillary electrophoresis was performed on a Beckman P/ACE 5510 with System Gold software. Uncoated fused silica capillaries (Polymicro Technologies, Phoenix, AZ) of 97 cm total length/90 cm effective length/50 μm i.d. were used for electrophoresis.

The running buffer used for enantiomeric separation of compounds **1**−**6** consisted of 214 mM glycine, 25 mM tris base, 100 mM sodium dodecyl sulfate, and 50 mM γ-cyclodextrin (pH 8.64). The running buffer used for compounds **7**−**10** consisted of 192 mM glycine, 25 mM tris base, 100 mM sodium dodecyl sulfate, and 8.8 mM β-cyclodextrin (Inset. 26.4 mM β-cyclodextrin) (pH 8.56). Capillaries were flushed with water and running buffer for 15 min each prior to electrophoresis run.

Procedure for Racemization Study. (*S*)-Tryptophan (0.12 mmol) was dissolved in dioxane (5 mL) and trifluoroacetic acid (1%, v/v). Benzaldehyde (0.48 mmol) was added in one portion to the stirred mixture at reflux. After 3 h, the volume of the reaction mixture was reduced with a stream of nitrogen to obtain an oil. The oil was dissolved in buffer and used for CE analysis without further workup. The corresponding reaction carried out at room temperature was performed under identical conditions.

References and Notes

(1) Lam, K. S. Application of Combinatorial Library Methods in Cancer Research and Drug Discovery. *Anti-Cancer Drug Design* **1997**, *12*, 145−167. Senkan, S. M. High-Throughput Screening of Solid-State Catalyst Libraries. *Nature* **1998**, *394*, 350−353. Berg, T.; Vandersteen, A. M.; Janda, K. D. High-Throughput Synthesis and Direct Screening for the Discovery of Novel Hydrolytic Meta Complexes. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1221−1224. Porte, A. M.; Reibenspies, J.; Burgess, K. Design and Optimization of New Phosphine Oxazoline Ligands via High-Throughput Catalyst Screening. *J. Am. Chem. Soc.* **1998**, *120*, 9180−9187.

(2) Bunin, B. A. *The Combinatorial Index*; Academic Press: San Diego, 1998.

(3) Kyranos, J. N.; Hogan, J. C., Jr., High-Throughput Characterization of Combinatorial Libraries Generated by Parallel Synthesis. *Anal. Chem.* **1998**, *70*, 389A−395A.

(4) Nakayama, G. R.; Nova, M. P.; Parandoosh, Z. A Scintillating Microplate Assay for the Assessment of Protein Kinase Activity. *J. Biomol. Screening* **1998**, *3*, 43−48. Seethala, R.; Menzel, R. A Homogeneous, Fluorescence Polarization Assay for Src-Family Tyrosine Kinases. *Anal. Biochem.* **1997**, *253*, 210−218. Collins, L. A.; Torrero, M. N.; Frnazblau, S. G. Microplate Alamar Blue Assay versus BACTEC 460 System for High-Throughput Screening of Compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1998**, *42*, 344−347.

(5) Cox, E. D.; Cook, J. M. The Pictet−Spengler Condensation: A New Direction for an Old Reaction. *Chem. Rev.* **1995**, *95*, 1797−1842.

(6) Landers, J. P. *Handbook of Capillary Electrophoresis*; CRC Press: Boca Raton, FL, 1994.

(7) Toyota, M.; Ihara, M. Recent Progress in the Chemistry of Non-Monoterpenoid Indole Alkaloids. *Nat. Prod. Rep*. **1998**, *15*, 327−340.

(8) Krstulovic, A. M. *Chiral Separations by HPLC*; Halsted Press: New York, 1989. Hancock, W. S. *CRC Handbook of HPLC for the Separation of Amino Acids, Peptides, and Proteins;* CRC Press: Boca Raton, FL, 1984.

(9) Schreier, P. *Analysis of Chiral Organic Molecules: Methodology and Applications*; Walter de Gruyter: New York, 1995.

(10) König, W. A. *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*; John Wiley & Sons: New York, 1992. Jack, D. B. *Drug Analysis by Gas Chromatography*; Academic Press: Orlando, FL, 1984.

(11) Otsuka, K.; Terabe, S. Chiral Separation by Capillary Electrophoresis and Electrokinetic Chromatography. In *Capillary Electrophoresis Technology*; Guzman, N. A., Ed.; Marcel Dekker: New York, 1993; pp 617−629. Fanali, S.; Cristalli, M.; Vespalec, R.; Bocek, P. Chiral Separations in Capillary Electrophoresis. In *Advances in Electrophoresis*; Crambach, A., Dunn, M. J., Radola, B. J., Eds.; VCH Publishers: New York, 1994; pp 1−86. Chankvetadze, B.; Endresz, G.; Blaschke, G. Charged Cyclodextrin Derivatives as Chiral Selectors in Capillary Electrophoresis. *Chem. Soc. Rev.* **1996**, 141−153. Zhang, L.; Jin, S.; Zhou, X.; Gu, J.-L.; Fu, R.-N. Separation of Diastereomers of a 1,3-Disubstituted Tetrahydro-β-Carboline by Capillary Electrophoresis with β-Cyclodextrin. *Chromatographia* **1996**, *42*, 385−388. Chu, Y.-H.; Cheng, C. C. Affinity Capillary Electrophoresis in Molecular Recognition. *Cell. Mol. Life Sci*. **1998**, *54*, 663−683.

(12) Sandrin, J.; Soerens, D.; Cook, J. M. ^{13}C NMR of 1, 3-Disubstituted-1,2,3,4-Tetrahydro-β-Carbolines. *Heterocycles* **1976**, *4*, 1249−1255.

(13) The throughput of diastereomers characterization can be readily improved (by shortening the electrophoresis time from 30 to 5 min) using a 27-cm capillary, since two products generated from every Pictet−Spengler reaction at ambient temperature are diastereomers and well separated in CE (for example, in Figure 1, 4.1 and 2.5 min differences in migration time for compounds (*R/S, R*)-**4** and (*R/S, R*)-**6**, respectively).

(14) Fantauzzi, P. P.; Yager, K. M. Synthesis of Diverse Tetrahydro-β-Carboline-3-Carboxamides and -2,3-Bis-Lactams on a Versatile 4-Hydroxythiophenol-Linked Solid Support. *Tetrahedron Lett.* **1998**, *39*, 1291−1294. Mayer, J. P.; Bankaitis-Davis, D.; Zhang, J.; Beaton, G.; Bjergarde, K.; Anderson, C. M.; Goodman, B. A.; Herrera, C. J. Application of the Pictet−Spengler Reaction in Combinatorial Chemistry. *Tetrahedron Lett.* **1996**, *37*, 5633−5636. Yang, L.; Guo, L. Pictet−Spengler Reaction on Solid Support. *Tetrahedron Lett.* **1996**, *37*, 5041−5044. Mohan, R.; Chou, Y.-L.; Morrissey, M. Pictet−Spengler Reaction on Solid Support: Synthesis of 1,2,3,4-Tetrahydro-β-Carboline Libraries. *Tetrahedron Lett.* **1996**, *37*, 3963−3966. Kaljuste, K.; Unden, A. Solid-Phase Synthesis of 1,2,3,4-Tetrahydro-β-Carbolines: Implications for Combinatorial Chemistry. *Tetrahedron Lett.* **1995**, *36*, 9211−9214.

(15) Bailey, P. D.; Hollinshead, S. P.; McLay, N. R.; Morgan, K.; Palmer, S. J.; Prince, S. N.; Reynolds, C. D.; Wood, S. D. Diastereo- and Enantio-Selectivity in the Pictet−Spengler Reaction. *J. Chem. Soc., Perkin Trans.* **1993**, *1*, 431−439.

(16) Bailey, P. D.; Moore, M. H.; Morgan, K. M.; Smith, D. I.; Vernon, J. M. Enhancing the Yield and Diastereoselectivity of the Pictet−Spengler Reaction: A Highly Efficient Route to *Cis*-1,3-Disubstituted Tetrahydro-β-Carbolines. *Tetrahedron Lett.* **1994**, *35*, 3587−3588. Bailey, P. D. On the Stereochemistry of the Pictet−Spengler Reaction. *Tetrahedron Lett.* **1987**, *28*, 5181−5184.

(17) Tsuge, O.; Kanemasa, S. Recent Advances in Azomethine Ylide Chemistry. *Adv. Heterocycl. Chem.* **1988**, *45*, 231−349. Murphy, M. M.; Schullek, J. R.; Gordon, E. M.; Gallop, M. A. Combinatorial Organic Synthesis of Highly Functionalized Pyrrolidines: Identification of a Potent Angiotensin Converting Enzyme Inhibitor from a Mercaptoacyl Proline Library. *J. Am. Chem. Soc.* **1995**, *117*, 7029−7030. Hamper, B. C.; Dukesherer, D. R.; South, M. S. Solid-Phase Synthesis of Proline Analogues via a Three Component 1,3-Dipolar Cycloaddition. *Tetrahedron Lett.* **1996**, *37*, 3671−3674. Ruhland, B.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. Solid-Supported Combinatorial Synthesis of Structurally Diverse β-Lactams. *J. Am. Chem. Soc.* **1996**, *118*, 253−254. Park, K.-H.; Olmstead, M. M.; Kurth, M. J. Diastereoselective Solid-Phase Synthesis of Novel Hydantoin- and Isoxazoline-Containing Heterocycles. *J. Org. Chem.* **1998**, *63*, 6579−6585.

(18) An enantiospecific Pictet−Spengler reaction was recently reported: Yamada, H.; Kawate, T.; Matsumizu, M.; Nishida, A.; Yamaguchi, K.; Nakagawa, M. Chiral Lewis Acid-Mediated Enantioselective Pictet−Spengler Reaction of N_b-Hydroxytryptamine with Aldehydes. *J. Org. Chem.* **1998**, *63*, 6348−6354.

CC980026A