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Bone Collagen Cross-Links: A Convergent Synthesis of (+)-Deoxypyrroloine

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A convergent total synthesis of (+)-deoxypyrrololine (Dpl, **4**), a putative cross-link of bone collagen, is described starting from a commercially available L-glutamic acid derivative, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**16**). Condensation of aldehyde (*S*)-(−)-**17** with nitro compound (*S*)-(−)-**27**, both of which were prepared from a common precursor (*S*)-**16**, gave the α-hydroxynitro compound **28**, which upon acetylation afforded α-acetoxynitro compound **14** in good yield. Subsequent condensation and cyclization of α-acetoxynitro compound **14** with benzyl isocyanoacetate (**15**) in the presence of DBU in THF gave the key pyrrole intermediate (*S*,*S*)-(−)-**12** in 57% yield. N-Alkylation of pyrrole (*S*,*S*)-(−)-**12** with iodide (*S*)-(−)-**13** using *t*-BuOK in THF afforded the 2-benzyloxycarbonyl-1,3,4-substituted pyrrole derivative (−)-**29** in 42% yield. Removal of the protective groups in (−)-**29** followed by hydrogenolysis and decarboxylation afforded the cross-link (+)-Dpl (**4**) in good overall yield. The synthesis of an analogue (*S*)-(+)-**24** and formation of a novel tetrahydroindole derivative (−)-**31** are also described.

Introduction

Bone is a complex and highly specialized form of connective tissue, which serves several functions including support of the body, protection of internal organs, and as a reservoir for minerals. In order for bones to respond and adapt to the mechanical stress as well as to maintain serum mineral metabolism, they undergo constant remodeling. The bone remodeling process, which is also called bone turnover, begins with resorption of old bone by osteoclasts, followed by the formation of new bone by osteoblasts.[1] Any alterations or imbalance in the remodeling process results in various metabolic bone diseases such as osteoporosis. Osteoporosis affects more than 25 million of the aged population in the U.S., particularly postmenopausal women,[2] and accounts for nearly 1.3 million fractures annually. The diagnosis of osteoporosis involves analysis of bone based on histomorphometry and densitometric measurements.[3] Efforts for prevention of this bone disease as well as to develop an effective antiresorptive therapy have increased the search for reliable and noninvasive biochemical markers of bone resorption.[4] The traditional markers of bone resorption, e.g., urinary calcium[5] and hydroxyproline,[6] lack the desired clinical sensitivity and specificity for diagnosis of osteoporosis. In recent years, the pyridinium cross-links (+)-pyridinoline (Pyd, **1**)[7] and (+)-deoxypyridinoline (Dpd, **2**)[8] (Figure 1) have gained much attention because of their potential clinical utility in the diagnosis of osteoporosis[9,10] and other metabolic bone diseases.[11] In addition to the presence of these pyridinium cross-links (**1** and **2**), Scott et al.[12] in 1981 postulated the existence of pyrrole cross-links, pyrrololine (Pyl, **3**) and deoxypyrrololine (Dpl, **4**)[13] (Figure 1), in various tissues. Subsequent studies by several other groups have provided

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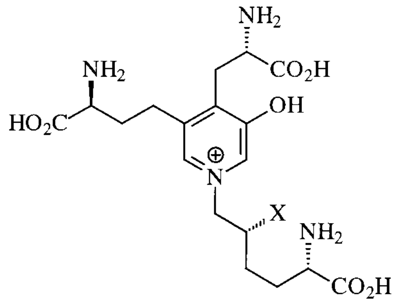
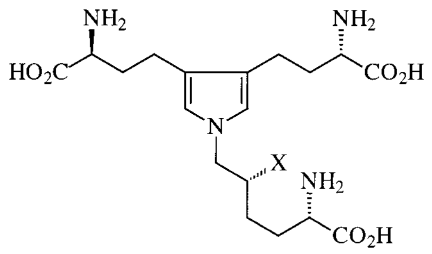


(+)-**1**: X = OH, Pyridinoline (Pyd)
(+)-**2**: X = H, Deoxypyridinoline (Dpd)



3: X = OH, Pyrrololine (Pyl)
(+)-**4**: X = H, Deoxypyrrololine (Dpl)

Figure 1. Structure of bone collagen cross-links (**1**−**4**).

further convincing evidence for the existence of pyrrole cross-links.[14,15] Unfortunately, attempts to isolate the cross-links **3** and **4** have not been successful so far.[4a,d,14f] It was proposed that the pyrrole cross-links **3** and **4** are also formed from the natural (2*S*,5*R*)-hydroxylysine and (*S*)-lysine residues present in collagen by a lysyl oxidase mediated enzymatic process, in an analogous fashion to the pyridinium cross-links **1**, **2** as shown in Figure 2.[4d,14c,d,f] Thus, the reaction of keto-amine (hydroxylysino-5-oxonorleucine, **5**) with aldehyde **6** leads to the formation of the hydroxylated Schiff base adduct **7**, which then undergoes a series of transformations such as Amadori rearrangement, tautomerization, and ring closure to produce the unsaturated ketone **8**. Subsequent enolization and oxidation of ketone **8** would lead to pyridinium cross-links **1** and **2**. Similarly, the reaction of keto-amine **5** with the aldehyde **9** would produce Schiff base adduct **10**, which upon ring closure ultimately leads to the formation of pyrrole cross-links **3** and **4**. The common basis for formation of cross-links (**1**, **2** and **3**, **4**) is the key reaction of a keto-amine **5** with a free telopeptidyl aldehyde (**6** or **9**). Aldehydes **6** and **9** are generated by lysyl oxidase from the hydroxylysine and lysine residues, respectively, present in the telopeptide domains of the collagen molecules. Thus, when the aldehyde is hydroxylated (i.e., **6**), the pyridinium cross-links **1** and **2** are generated, and the pyrrole cross-links **3** and **4** are formed from the unhydroxylated aldehyde (i.e., **9**). We have been interested in measuring the pyridinium cross-links **1** and **2** by immunoassay[16] and other techniques[17] as markers for diagnosis of osteoporosis and other metabolic bone diseases. Because the pyrrole cross-links (**3** and **4**) are also excreted in urine during the process of bone degradation along with pyridinium cross-links (**1** and **2**), it is essential to consider the presence of cross-links **3** and **4** in the development of any assays for osteoporosis [e.g., measurement of Pyd (**1**) or Dpd (**2**)]. Additionally, the pyrrole cross-links **3** and **4** themselves may potentially be useful as markers in diagnosis and therapy management of osteoporosis as well as to study the metabolism of bones. In this paper, we detail the synthesis of pyrrole cross-link (+)-deoxypyrroloine (Dpl, **4**) starting from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**16**).[18]

Results and Discussion

Intrigued by the novel structural features of pyrrole cross-links of bone collagen (**3**, **4**) and their potential applications, studies directed toward the total synthesis of this class of 1,3,4-trisubstituted pyrrole amino acids seemed to be appropriate. The ultimate goal of our effort was to devise an efficient total synthesis of (+)-deoxypyrroloine (**4**), thereby providing material for preliminary evaluation, and also to prepare analogues useful for the diagnosis and therapy management of osteoporosis. The basic synthetic strategy for construction of the cross-link (+)-Dpl (**4**) involved (Figure 3) the introduction of a lysine side chain via N-alkylation of pyrrole derivative (*S*,*S*)-(−)-**12** with iodide (*S*)-(−)-**13**, followed by hydrolysis of the protective groups and removal of 2-carboxybenzyl ester. The Boc and *tert*-butyl groups were chosen as protective groups for amine and carboxylic acid functionalities, respectively, as they are not only stable for the reaction conditions during the synthetic pathway but also could be hydrolyzed simultaneously using trifluoroacetic acid. Additionally, we decided to protect the amino functionalities as bis-Boc to avoid any interference in the critical synthetic steps such as N-alkylation. The foundation for the synthesis of key intermediate 2-benzyloxycarbonyl-3,4-substituted pyrrole derivative (*S*,*S*)-(−)-**12** was based on our prior work in the area of 2-carboxy-3,4-substituted pyrroles and their derivatives.[19] Thus, the key synthon, (*S*,*S*)-(−)-**12**, was envisioned to arise from

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[13] The names for pyrrole cross-links pyrroloine (Pyl, **3**) and deoxypyrroloine (Dpl, **4**) were assigned in analogy to the pyridinium cross-links pyridinoline (Pyd, **1**) and deoxypyridinoline (Dpd, **2**).

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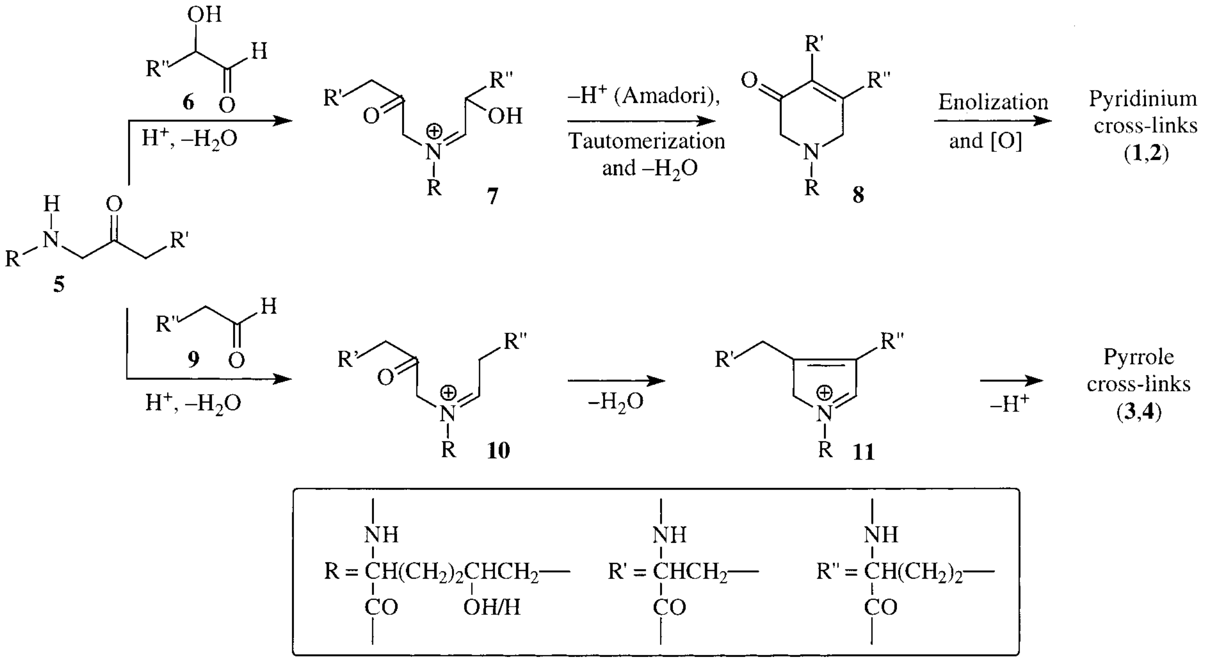

Figure 2. Formation of pyridinium and pyrrole cross-links (**1**, **2** and **3**, **4**) via condensation of keto-amine (**5**) with aldehydes (**6** or **9**).

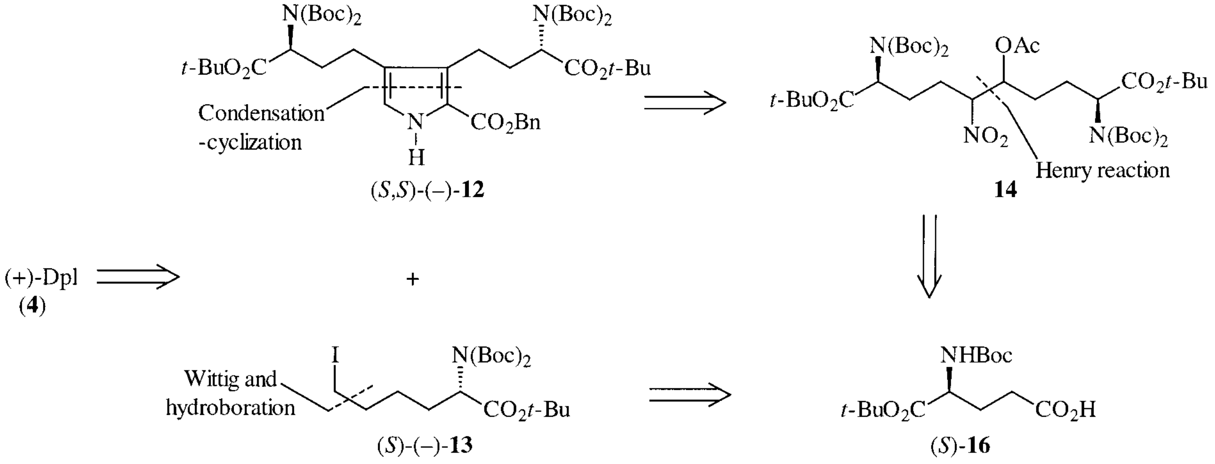

Figure 3. Retrosynthetic analysis of (+)-deoxypyrrololine (Dpl, **4**).

α-acetoxynitro compound **14** and benzyl isocyanoacetate (**15**) via a base-promoted condensation and cyclization process.[20] The α-acetoxynitro compound **14** could be derived from a common chiral starting material, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**16**), which is a commercially available L-glutamic acid derivative. To validate this proposed synthetic strategy, particularly the decarboxylation of 1,3,4-substituted 2-carboxypyrrole derivatives [i.e., (−)-**29**], 3,4-diethylpyrrole derivative (*S*)-(+)-**24** (Scheme 1), a simplified analogue of (+)-Dpl (**4**), was first targeted. Analogue (*S*)-(+)-**24** has the desired lysine chain at the pyrrole nitrogen, which is present in the native (+)-Dpl (**4**) and the ethyl groups at 3 and 4 positions, and thus offers the simplicity for optimization of reaction conditions for crucial steps including the N-alkylation.

Preparation of Analogue (*S*)-(+)-24. The synthesis of analogue (*S*)-(+)-**24** began with the preparation of (*S*)-(−)-*tert*-butyl-2-[bis(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**13**) from the L-glutamic acid derivative (*S*)-**16** (Scheme 1). Thus, (*S*)-**16** was converted to the known aldehyde (*S*)-(−)-**17** in three steps on a 20 g scale by following our previously developed procedure.[16b] The aldehyde (*S*)-(−)-**17** was then extended by a methylene to the corresponding olefin (*S*)-(−)-**18** via a Wittig reaction,[16b] which upon hydroboration afforded the alcohol (*S*)-(−)-**19** in excellent yield (87%). The hydroxyl group in (*S*)-(−)-**19** was then converted to the desired iodide (*S*)-(−)-**13** in THF using I_2, PPh_3, and imidazole in 92% yield. The next step in the synthesis of analogue (*S*)-(+)-**24** was the alkylation of known 2-benzyloxycarbonyl-3,4-diethylpyrrole (**21**)[20b] with iodide (*S*)-(−)-**13**. Thus, ester **21** was treated with 1.0 equiv of potassium *tert*-butoxide in THF, followed by reaction with 2.0 equiv of iodide (*S*)-(−)-**13** in the presence of 18-crown-6,[21] at room temperature. Purification of the crude compound by preparative HPLC afforded the N-alkylated pyrrole derivative (*S*)-(−)-**22** in 66% yield. The synthesis of analogue (+)-**24** required hydrolysis of benzyl ester,

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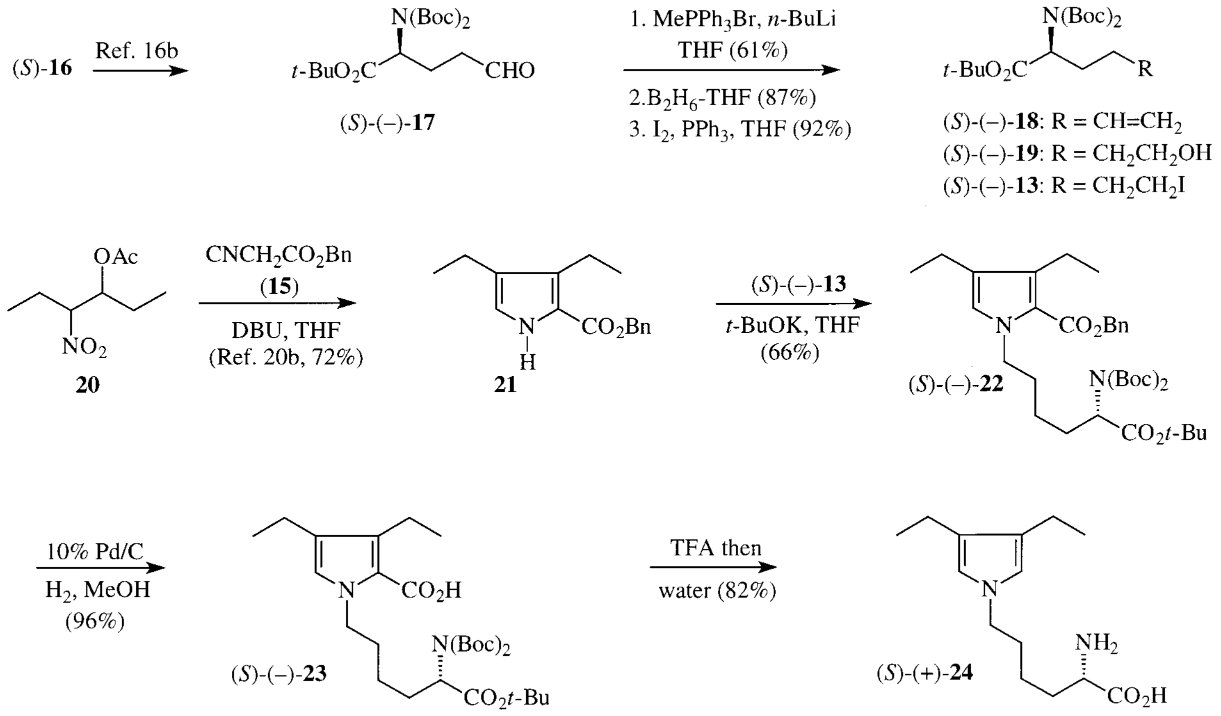
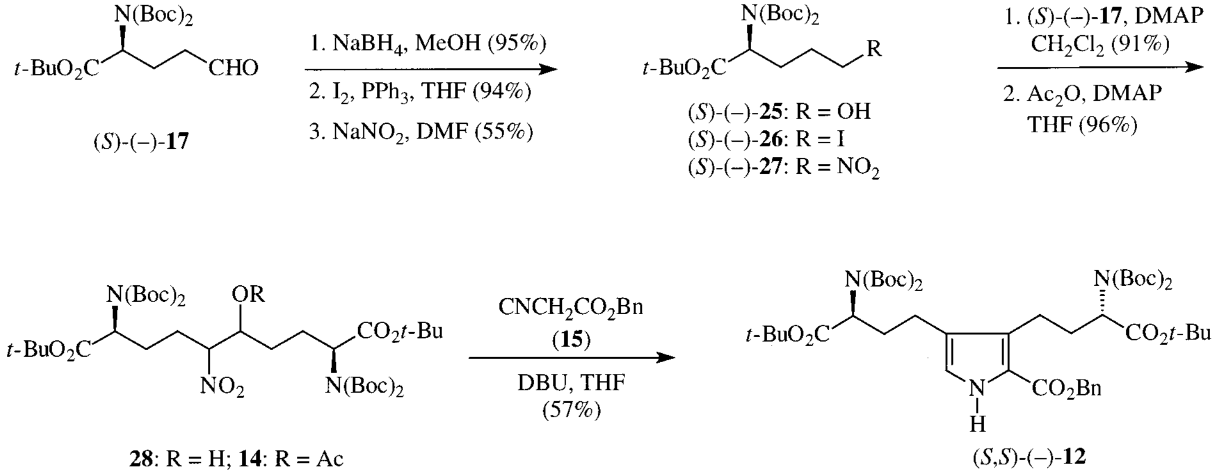
Scheme 1. Preparation of Analog (*S*)-(+)-24



Scheme 2. Synthesis of Key Intermediate (*S*,*S*)-(−)-12



unmasking the protective groups (i.e., Boc and *tert*-butyl), and removal of the carboxyl group at the 2-position of the pyrrole ring via decarboxylation. We planned to achieve the last two transformations [i.e., hydrolysis of protective groups (Boc and *tert*-butyl) and decarboxylation] simultaneously using trifluoroacetic acid.[22] Accordingly, the benzyl ester in (*S*)-(−)-**22** was first cleaved by hydrogenolysis in methanol to afford acid (*S*)-(−)-**23** in almost quantitative yield. Treatment of acid (*S*)-(−)-**23** with trifluoroacetic acid at room temperature and purification of the crude product by preparative reversed-phase HPLC afforded the 3,4-dimethylpyrrole derivative (*S*)-(+)-**24** in 82% yield as its TFA salt.

Synthesis of Key 2,3,4-Trisubstituted Pyrrole Intermediate (*S*,*S*)-(−)-12. Having successfully completed the preparation of analogue (*S*)-(+)-**24**, we then proceeded to the synthesis of (+)-Dpl (**4**) (Schemes 2−4). Our first goal in the synthesis of (+)-Dpl (**4**) was to prepare the required α-acetoxynitro compound **14** needed for construction of the key pyrrole synthon (*S*,*S*)-(−)-**12**. Accordingly, the aldehyde (*S*)-(−)-**17** was reduced with $NaBH_4$ in MeOH to the corresponding alcohol (*S*)-(−)-**25** in 95% yield, which was subsequently transformed to the iodide (*S*)-(−)-**26**, using I_2, PPh_3, and imidazole, in excellent yield (94%). Treatment of iodide (*S*)-(−)-**26** with sodium nitrite in DMF[23] afforded the corresponding nitro compound (*S*)-(−)-**27** in 55% yield, a key component required for condensation with aldehyde (*S*)-(−)-**17**. Treatment of nitro compound (*S*)-(−)-**27** with aldehyde (*S*)-(−)-**17** in the presence of DMAP in CH_2Cl_2 allowed smooth condensation (Henry reaction) to give the α-hydroxynitro compound **28** in 91% yield as a mixture of

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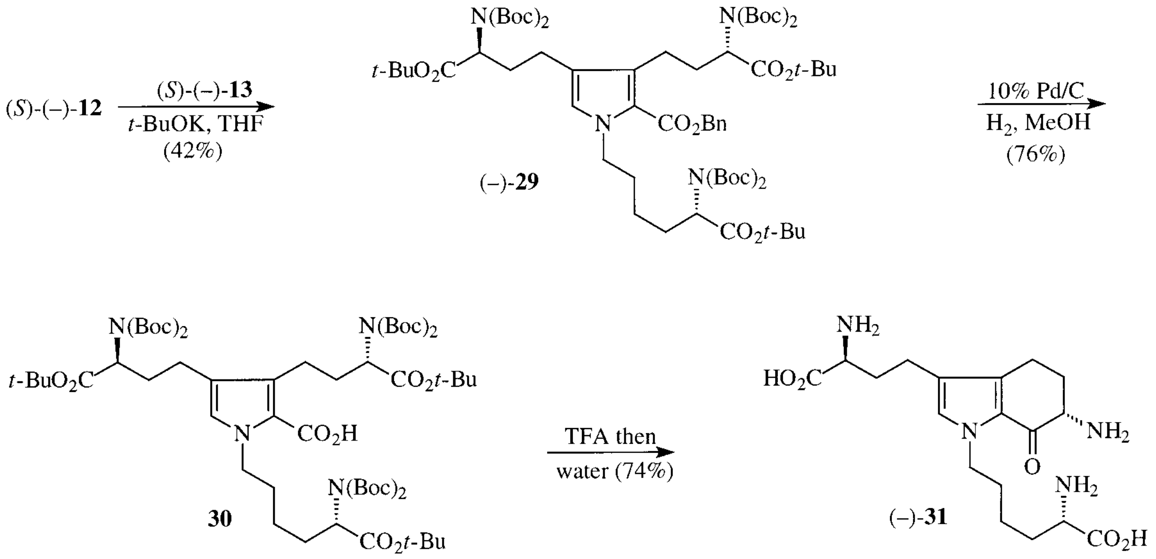
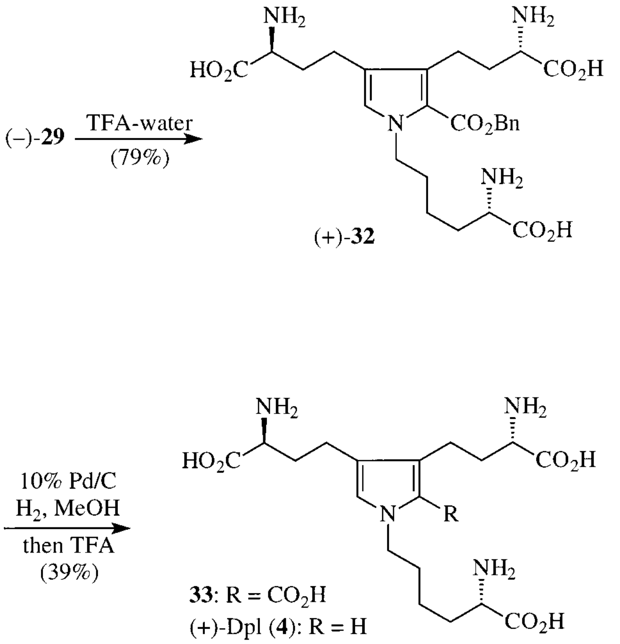
Scheme 3. Isolation of a Novel Tetrahydroindole Derivative (−)-31



Scheme 4. Completion of the Synthesis of (+)-Dpl (4)



diastereomers. Because the two newly generated chiral centers at the NO_2 and OH groups in α-hydroxynitro compound **28** would be eventually eliminated during the pyrrole ring formation, the diastereomeric mixture of **28**, as such, was converted to the corresponding acetate derivative (**14**) by treatment with acetic anhydride in THF in almost quantitative yield (96%). Finally, the reaction of α-acetoxynitro compound **14** with benzyl isocyanoacetate (**15**) in the presence of DBU in THF at 0 °C to room temperature underwent smooth condensation, followed by cyclization. The crude compound from this reaction was purified by silica gel column chromatography to afford the key intermediate, 2-benzyloxycarbonyl-3,4-substituted pyrrole derivative (*S*,*S*)-(−)-**12,** in 57% yield.

Isolation of a Novel Tetrahydroindole Derivative (−)-31 and Completion of the Synthesis of (+)-Dpl (4). The next key step in the synthesis of (+)-**4** was the installation of a lysine chain at the pyrrole nitrogen in pyrrole (*S*,*S*)-(−)-**12** via alkylation (Scheme 3) by following the conditions developed for analogue (+)-**24**. Thus, treatment of 2-benzyloxycarbonyl-3,4-substituted pyrrole derivative (*S*,*S*)-(−)-**12** with 1.0 equiv of potassium *tert*-butoxide in THF and subsequent reaction with 2.0 equiv of iodide (*S*)-(−)-**13** in the presence of 18-crown-6 afforded the N-alkylated pyrrole derivative (−)-**29** in 42% yield. The derivative (−)-**29** has the required three amino acid side chains at the 1,3,4-positions on the pyrrole ring. Synthesis of (+)-Dpl (**4**) required unmasking the protective groups and removing the benzyloxycarbonyl group at the 2-position of the pyrrole ring. Thus, hydrogenolysis of benzyl ester (−)-**29** using 10% Pd/C in ethanol afforded the acid **30** in 76% yield. The acid **30** was then treated with trifluoroacetic acid, followed by water, to hydrolyze the protective groups (i.e., Boc and *t*-Bu ester) and simultaneously remove the acid group at the 2-position via decarboxylation. However, purification of the crude compound by preparative reversed-phase HPLC afforded a product which did not correspond to the expected collagen cross-link, (+)-Dpl (**4**). In the ^{1}H NMR, only one pyrrole proton (δ 7.08) was present, indicating that all other positions on the ring were substituted. The ^{13}C NMR spectrum indicated the presence of three carbonyl groups (δ 182.1, 172.4, and 172.2), one of which had a distinct chemical shift (δ 182.1). The ESI-mass spectrum showed $(M + H)^+$ at 381, not the expected $(M + H)^+$ at 399 for (+)-Dpl (**4**). It became evident that the isolated product from the TFA−water hydrolysis reaction was a novel (−)-(2*S*)-2-amino-6-{(6*S*)-6-amino-3[(4*S*)-3-aminocarboxypropyl]-7-oxo-4,5,6,7-tetrahydro-1*H*-indol-1-yl}-hexanoic acid (**31**) but not the desired (+)-Dpl (**4**). Tetrahydroindole (−)-**31** was isolated as the sole product from the TFA−water reaction in 74% yield and presumed to be formed via an acid-catalyzed decarboxylation−acylation process.[24] The formation of (−)-**31** led us to modify our original synthetic plan to achieve the total synthesis of (+)-Dpl (**4**). Thus, we thought that hydrolysis of the protective groups (i.e., Boc and *tert*-butyl) in acid **30** prior to the decarboxylation process could circumvent

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[24] For the importance of the tetrahydroindole structural unit, see: (a) Joshi, K. C.; Chand, P. *Pharmazie* **1982**, *37*, 1−13. (b) De Kimpe, N.; Keppens, M. *Tetrahedron* **1996**, *52*, 3705−3718 and references therein.

the formation of tetrahydroindole derivative (−)-**31**. Accordingly, compound (−)-**29** was first treated with trifluoroacetic acid and water to hydrolyze the Boc and *tert*-butyl ester protective groups (Scheme 4). Purification of the crude compound by preparative reversed-phase HPLC afforded the benzyl ester (+)-**32** in 79% yield. The benzyl group in (+)-**32** was then removed by hydrogenolysis over 10% Pd/C in MeOH to give the corresponding acid **33**, which without purification was treated with trifluoroacetic acid to initiate the decarboxylation. Concentration of the crude reaction mixture followed by preparative reversed-phase HPLC purification and lyophilization afforded the cross-link (+)-deoxypyrrololine (Dpl, **4**) in 39% yield as a pale pink powder. Under these conditions during the transformation of compound (+)-**32** to (+)-Dpl (**4**), we did not observe tetrahydroindole **31**.

In summary, a general and convergent total synthesis of a novel pyrrole cross-link of collagen, (+)-deoxypyrrololine (Dpl, **4**), is described, utilizing a commercially available L-glutamic acid derivative, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**16**) as a common source for all three chiral centers.

Experimental Section

General Methods and Materials. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz), and the chemical shifts (δ) were reported. Electrospray ionization mass spectrometry (ESI-MS) was carried on a Perkin-Elmer (Norwalk, CT) Sciex API 100 benchtop system employing a Turbo IonSpray ion source, and HRMS were obtained on a Nermang 3010 MS-50 or a JEOL SX102-A. Thin-layer chromatography was performed on precoated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and visualized with UV light, KMnO$_4$ solution [KMnO$_4$ (1.0 g) and NaOH (8.0 g) in water (200 mL)], phosphomolybdic acid reagent (20 wt % solution in ethanol), or ninhydrin reagent (0.2% ninhydrin in ethanol). Column chromatography was performed on silica gel, Merck grade 60 (230−400 mesh). Anhydrous solvents were freshly distilled (THF from a purple solution of sodium and benzophenone and CH$_2$Cl$_2$ from CaH$_2$) under nitrogen. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and used without purification, except where noted. All of the solvents employed were of HPLC grade purchased from EM Science (Gibbstown, NJ) and used as received. Analytical reversed-phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10 μm (8 × 100 mm) column (solvents ratio v/v reported) unless otherwise stated. Preparative reversed-phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10 μm (40 × 100 mm) column (solvents ratio v/v reported) unless otherwise stated. Optical rotations were measured on an Autopol III polarimeter from Rudolph Research (Flanders, NJ). IUPAC names of all new compounds were obtained using the ACD/Ilab Web service version 3.5 at http://www.acdlabs.com/ilab.

(*S*)-(−)-*tert*-Butyl-2-[bis-(*tert*-butoxycarbonyl)amino]-5-oxopentanoate (**17**) was prepared from a commercially available (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**16**) in three steps on a 20 g scale following our previously developed procedure.[16b] 2-Benzyl 3,4-diethyl-1*H*-pyrrole-2-carboxylate (**21**) was prepared from 1-ethyl-2-nitrobutyl acetate (**20**) and benzyl isocyanoacetate (**15**) according to the known procedure on a 8.6 g scale in 72% yield.[20b]

(*S*)-(−)-*tert*-Butyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-hexenoate (18). *n*-BuLi (2.5 M solution in hexanes, 12.6 mL, 31.4 mmol, 2.0 equiv) was added dropwise to a suspension of methyl triphenylphosphonium bromide (11.2 g, 31.4 mmol, 2.0 equiv) in THF (230 mL) at room temperature under nitrogen. After the mixture was stirred for 30 min, the resulting orange ylide solution was cooled to 0 °C in an ice bath, and a solution of (*S*)-(−)-**17** (6.07 g, 15.7 mmol) in THF (60 mL) was added via a double-ended needle. The mixture was stirred for 45 min at 0 °C and then quenched with a saturated aqueous NH$_4$Cl solution (60 mL). The mixture was diluted with water (300 mL) and extracted with EtOAc (3 × 300 mL). The combined organic layers were dried (Na$_2$SO$_4$), and the solvent was removed on a rotary evaporator. The crude compound was purified by silica gel column chromatography (10% EtOAc in hexanes) to afford 3.66 g of (*S*)-(−)-**18** in 61% yield as a colorless viscous oil.[16b] R_f: 0.62 (15% EtOAc in hexanes). $[\alpha]^{23}_{D}$: −16.9 (*c* 1.48, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 70:30, 2.0 mL/min at 225 nm, t_R 4.90 min, 98%. ^{1}H NMR (CDCl$_3$): δ 5.88−5.74 (m, 1 H), 5.07−4.95 (m, 2 H), 4.75−4.70 (m, 1 H), 2.22−2.06 (m, 3 H), 2.00−1.89 (m, 1 H), 1.50 (s, 18 H), 1.44 (s, 9 H). ^{13}C NMR (CDCl$_3$): δ 170.0, 152.6, 137.6, 115.4, 82.7, 81.1, 58.3, 30.4, 28.6, 27.9, 29.8. ESI-MS (*m*/*z*): 386 (M + H)$^+$, 788 (2 × M + NH$_4$)$^+$.

(*S*)-(−)-*tert*-Butyl-2-[bis(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (19). (*S*)-(−)-**18** (3.611 g, 9.37 mmol) in THF (50 mL) was cooled to 0 °C in an ice bath, and a solution of borane−THF complex (1.0 M solution in THF, 12.2 mL, 12.2 mmol, 1.3 equiv) was added under nitrogen. The cooling bath was removed, and the mixture was allowed to warm to room temperature and stirred for 17 h. The reaction mixture was then cooled to 0 °C, and 1.0 N aqueous NaOH (14.0 mL, 14.0 mmol, 1.5 equiv) was added followed by 30% aqueous H$_2$O$_2$ (11.7 mL, 103.0 mmol, 11.0 equiv) and stirred for 30 min. The mixture was diluted with a 20% aqueous NaCl solution (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 3.281 g of (*S*)-(−)-**19** in 87% yield as a colorless viscous oil. R_f: 0.30 (40% EtOAc in hexanes). Analytical RP HPLC: MeCN/0.05% aqueous trifluoroacetic acid 60:40, 2.0 mL/min at 215 nm, t_R 6.19 min, 99.5%. $[\alpha]^{23}_{D}$: −16.2 (*c* 1.46, MeOH). ^{1}H NMR (CDCl$_3$): δ 4.70 (dd, 1 H, J = 9.3, 5.0 Hz), 3.64 (q, 2 H, J = 12.3, 6.3 Hz), 2.13−2.01 (m, 1 H), 1.93−1.80 (m, 1 H), 1.66−1.38 (m, 5 H), 1.50 (s, 18 H), 1.44 (s, 9 H). ^{13}C NMR (CDCl$_3$): δ 169.9, 152.5, 82.7, 81.1, 62.5, 58.7, 32.2, 28.8, 28.0, 27.9, 22.5. ESI-MS (*m*/*z*): 404 (M + H)$^+$, 442 (M + K)$^+$. HRMS (FAB, *m*/*z*): calcd for $C_{20}H_{38}NO_7$, 404.2648 (M + H)$^+$; observed, 404.2658.

(*S*)-(−)-*tert*-Butyl-2-[bis(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (13). Triphenylphosphine (3.12 g, 11.9 mmol, 1.5 equiv), imidazole (0.865 g, 12.7 mmol, 1.6 equiv), and iodine (3.02 g, 11.9 mmol, 1.5 equiv) were added sequentially to a solution of (*S*)-(−)-**19** (3.197 g, 7.92 mmol) in THF (80 mL) at room temperature under nitrogen. After the mixture was stirred for 1 h, it was diluted with EtOAc (80 mL) and water (80 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 80 mL). The combined organic layers were dried (Na$_2$SO$_4$), and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (10% EtOAc in hexanes) to afford 3.741 g of (*S*)-(−)-**13** in 92% yield as a pale yellow viscous oil. R_f: 0.46 (15% EtOAc in hexanes). $[\alpha]^{23}_{D}$: −22.1 (*c* 1.73, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 80:20, 2.0 mL/min at 215 nm, t_R 5.26 min, 99.5%. ^{1}H NMR (CDCl$_3$): δ 4.72 (dd, 1 H, J = 9.0, 5.1 Hz), 3.28−3.13 (m, 2 H), 2.22−2.09 (m, 1 H), 2.04−1.81 (m, 3 H), 1.51 (s, 18 H), 1.45 (s, 9 H). ^{13}C NMR (CDCl$_3$): δ 169.4, 152.3, 82.9, 81.4, 57.8, 30.4, 30.2, 28.0, 27.9, 5.8. ESI-MS (*m*/*z*): 500 (M + H)$^+$. HRMS (FAB, *m*/*z*): calcd for $C_{20}H_{37}NO_6I$, 514.1666 (M + H)$^+$; observed, 514.1663.

(−)-Benzyl-1-[(5*S*)-5-[bis(*tert*-butoxycarbonyl)amino]-6-(*tert*-butoxy)-6-oxohexyl]-3,4-diethyl-1*H*-pyrrole-2-carboxylate (22). A solution of 18-crown-6 (0.073 g, 0.276 mmol, 0.1 equiv) in THF (11.0 mL) and a solution of *t*-BuOK (1.0 M solution in THF, 2.76 mL, 2.76 mmol, 1.0 equiv) were added sequentially to a solution of ester **21** (0.709 g, 2.76 mmol) in THF (11.0 mL) at room temperature under nitrogen. To this mixture was added a solution of (*S*)-(−)-iodide **13** (2.832 g, 5.52

mmol, 2.0 equiv) in THF (11.0 mL) via a double-ended needle, and the reaction mixture was stirred for 7 h. The reaction mixture was then diluted with 20% aqueous NaCl solution (20 mL) and EtOAc (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to give 3.01 g of product, which was further purified by preparative reversed-phase HPLC (Waters, C18, RCM, Symmetry, 7.0 μm, 40 × 100 mm; MeCN/0.1 aqueous trifluoroacetic acid/93:7, 45 mL/min at 225 nm). Lyophilyzation of the product afforded 1.14 g of (−)-**22** in 66% yield as a pale yellow viscous oil. R_f: 0.64 (20% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −2.3 (*c* 0.53, MeOH). Analytical RP HPLC (Waters, C18, RCM, Symmetry, 7.0 μm, 8 × 100 mm): MeCN/0.1% aqueous trifluoroacetic acid 95:5, 2.0 mL/min at 225 nm, t_R 8.14 min, >99%. ^1H NMR ($CDCl_3$): δ 7.43−7.26 (m, 5 H), 6.57 (s, 1 H), 5.27 (s, 2 H), 4.67 (dd, 1 H, J = 9.3, 5.1 Hz), 4.22−4.14 (m, 2 H), 2.68 (q, 2 H, J = 7.5 Hz), 2.38 (q, 2 H, J = 7.2 Hz), 2.10−1.24 (m, 6 H), 1.49 (s, 18 H), 1.44 (s, 9 H), 1.15 (t, 3 H, J = 7.5 Hz), 1.04 (t, 3 H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 169.8, 161.4, 152.5, 136.5, 134.8, 128.5, 128.3, 128.0, 126.0, 124.2, 117.6, 82.7, 81.1, 65.4, 58.8, 49.5, 31.5, 29.0, 28.0, 27.9, 23.9, 19.1, 17.8, 15.7, 14.9. ESI-MS (*m*/*z*): 643 $(M + H)^+$, 660 $(M + NH_4)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{36}H_{54}N_2O_8$, 642.3880 $(M)^+$; observed, 642.3884.

(−)-1-[(5*S*)-5-[Bis(*tert*-butoxycarbonyl)amino]-6-(*tert*-butoxy)-6-oxohexyl]-3,4-diethyl-1*H*-pyrrole-2-carboxylic Acid (23). A solution of benzyl ester (−)-**22** (0.268 g, 0.074 mmol) in MeOH (19.0 mL) was treated with 10% Pd/C (0.092 g), and the mixture was stirred under H_2 atmosphere, using a balloon, at room temperature. After the mixture was stirred for 1 h, it was filtered through a pipet (fitted with cotton and Celite) and washed with MeOH (2.0 mL). The filtrate was concentrated on a rotary evaporator to afford 0.221 g of (*S*)-(−)-**23** in 96% yield as a pale pink gummy material. R_f: 0.29 (20% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −6.9 (*c* 0.45, MeOH). Analytical RP HPLC (Waters, C18, RCM, Symmetry, 7.0 μm, 8 × 100 mm): MeCN/0.1% aqueous trifluoroacetic acid 95:5, 2.0 mL/min at 225 nm, t_R 3.46 min, 98%. ^1H NMR ($CDCl_3$): δ 6.62 (s, 1 H), 4.69 (dd, 1 H, J = 9.3, 5.1 Hz), 4.24−4.18 (m, 2 H), 2.75 (q, 2 H, J = 7.5 Hz), 2.41 (q, 2 H, J = 7.5 Hz), 2.12−1.26 (m, 6 H), 1.49 (s, 18 H), 1.44 (s, 9 H), 1.23 (t, 3 H, J = 7.5 Hz), 1.17 (t, 3 H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 169.8, 164.8, 152.5, 136.6, 127.0, 124.5, 116.8, 82.7, 81.2, 58.8, 49.4, 31.3, 29.0, 28.0, 27.9, 23.6, 19.0, 17.7, 15.6, 14.8. ESI-MS (*m*/*z*): 575 $(M + Na)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{29}H_{48}N_2O_8$, 552.3411 $(M)^+$; observed, 552.3404.

(+)-6-(3,4-Diethyl-1*H*-pyrrol-1-yl)-L-norleucine (24). Trifluoroacetic acid (10.0 mL) was added to (*S*)-(−)-**23** (0.217 g, 0.393 mmol) at room temperature. After the mixture was stirred for 2 h, water (0.5 mL) was added, and the stirring was continued at room temperature for an additional 2 h. The reaction mixture was then concentrated on a rotary evaporator (<35 °C bath temperature), and the residue was dissolved in MeCN/0.1% aqueous trifluoroacetic acid (50:50, 15 mL) and purified by preparative RP HPLC (Waters, C18, RCM, Symmetry, 7.0 μm, 40 × 100 mm; MeCN/0.1% aqueous trifluoroacetic acid 35:65, 45 mL/min at 225 nm). The product was collected and lyophilized to afford 0.117 g of analogue (*S*)-(+)-**24** as its TFA salt in 82% yield (pale pink gummy material). $[\alpha]^{23}{}_D$: +3.96 (*c* 0.63, MeOH). Analytical RP HPLC (Waters, C18, RCM, Symmetry, 7.0 μm, 8 × 100 mm): MeCN/0.1% aqueous trifluoroacetic acid 30:70, 2.0 mL/min at 225 nm, t_R 8.54 min, >99%. ^1H NMR (CD_3OD): δ 6.37 (s, 2 H), 3.82−3.76 (m, 3 H), 2.36 (q, 4 H, J = 7.8 Hz), 1.98−1.50 (m, 4 H), 1.48−1.36 (m, 2 H), 1.13 (t, 6 H, J = 7.2 Hz). ^{13}C NMR (CD_3OD): δ 172.4, 124.8, 54.4, 32.3, 31.4, 23.4, 19.5, 15.4. ESI-MS (*m*/*z*): 253 $(M + H)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{14}H_{25}N_2O_2$, 253.1916 $(M + H)^+$; observed, 253.1915.

(*S*)-(−)-*tert*-Butyl-2-[bis-(*tert*-butoxycarbonyl)amino]-5-hydroxypentanoate (25). Sodium borohydride (0.798 g, 21.1 mmol, 1.0 equiv) was added to a solution of aldehyde (*S*)-(−)-**17** (8.177 g, 21.1 mmol) in MeOH (100 mL) at 0 °C under nitrogen. The mixture was stirred for 45 min, quenched with water (200 mL), and extracted with EtOAc (3 × 200 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The crude compound was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 7.82 g of (*S*)-(−)-**25** in 95% yield as a colorless viscous oil. R_f: 0.31 (40% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −14.9 (*c* 1.28, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 60:40, 2.0 mL/min at 215 nm, t_R 5.51 min, 99.3%. ^1H NMR ($CDCl_3$): δ 4.74 (dd, 1 H, J = 9.0, 5.4 Hz), 3.66 (dt, 2 H, J = 12.3, 6.6 Hz), 2.22−2.11 (m, 1 H), 1.95−1.83 (m, 1 H), 1.67−1.50 (m, 3 H), 1.50 (s, 18 H), 1.44 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 169.8, 152.5, 82.8, 81.2, 62.2, 58.6, 29.5, 28.0, 27.9, 25.7. ESI-MS (*m*/*z*): 390 $(M + H)^+$, 412 $(M + Na)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{19}H_{36}NO_7$, 390.2492 $(M + H)^+$; observed, 390.2498.

(*S*)-(−)-*tert*-Butyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-iodopentanoate (26). Triphenylphosphine (3.78 g, 14.4 mmol, 1.5 equiv), imidazole (1.04 g, 15.3 mmol, 1.6 equiv), and iodine (3.65 g, 14.4 mmol, 1.5 equiv) were added sequentially to a solution of (*S*)-(−)-**25** (3.734 g, 9.59 mmol) in THF (100 mL) at room temperature under nitrogen. After the mixture was stirred for 1.5 h, the solvent was removed on a rotary evaporator to dryness, and the crude product was purified by silica gel column chromatography (10% EtOAc in hexanes) to afford 4.498 g of (*S*)-(−)-**26** in 94% yield as colorless viscous oil. R_f: 0.51 (20% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −17.8 (*c* 1.39, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 80:20, 2.0 mL/min at 215 nm, t_R 5.26 min, 99.5%. ^1H NMR ($CDCl_3$): δ 4.72 (dd, 1 H, J = 9.0, 5.1 Hz), 3.28−3.13 (m, 2 H), 2.22−2.09 (m, 1 H), 2.04−1.81 (m, 3 H), 1.51 (s, 18 H), 1.45 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 169.4, 152.3, 82.9, 81.4, 57.8, 30.4, 30.2, 28.0, 27.9, 5.8. ESI-MS (*m*/*z*): 500 $(M + H)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{19}H_{35}NO_6I$, 500.1509 $(M + H)^+$; observed, 500.1511.

(*S*)-(−)-*tert*-Butyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-nitropentanoate (27). Sodium nitrite ($NaNO_2$, 1.95 g, 28.3 mmol, 2.0 equiv) was added to a solution of (*S*)-(−)-**26** (7.053 g, 14.1 mmol) in DMF (70 mL) at room temperature under nitrogen. After the reaction mixture was stirred for 40 min, it was diluted with 20% aqueous NaCl solution (200 mL) and EtOAc (200 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 200 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 3.263 g of (*S*)-(−)-**27** in 55% yield as a colorless viscous oil. R_f: 0.35 (20% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −21.8 (*c* 1.97, MeOH). Analytical RP HPLC: MeCN/0.05% aqueous trifluoroacetic acid 70:30, 2.0 mL/min at 215 nm, t_R 5.57 min, 99.7%. ^1H NMR ($CDCl_3$): δ 4.75−4.70 (m, 1 H), 4.44−4.38 (m, 2 H), 2.18−1.91 (m, 4 H), 1.51 (s, 18 H), 1.44 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 169.0, 152.4, 83.2, 81.6, 74.9, 57.9, 28.0, 27.8, 26.0, 24.3. ESI-MS (*m*/*z*): 419 $(M + H)^+$, 436 $(M + NH_4)^+$, 854 $(2 \times M + NH_4)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{19}H_{35}N_2O_8$, 419.2393 $(M + H)^+$; observed, 419.2405.

1,10-Di-*tert*-butyl-2,9-bis[bis(*tert*-butoxycarbonyl)amino]-2,3,4,5,7,8,9-heptadeoxy-5-nitro-D-*threo*-decarate (28). A mixture of (*S*)-(−)-*tert*-butyl-2-[bis(*tert*-butoxycarbonyl)-amino]-5-nitropentanoate (**27**, 1.907 g, 4.56 mmol) and (*S*)-(−)-*tert*-butyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-oxopentanoate (**17**, 1.887 g, 4.87 mmol, 1.1 equiv) were dissolved in CH_2Cl_2 (6.0 mL), and 4-(dimethylamino)pyridine (DMAP, 2.23 g, 18.2 mmol, 4.0 equiv) was added at room temperature under nitrogen. After the reaction mixture was stirred for 2 days, an additional amount of CH_2Cl_2 (6.0 mL) was added, and the mixture was stirred for 6 days. The crude reaction mixture was then directly purified by silica gel column chromatography (20−30% EtOAc in hexanes) to afford 2.67 g of α-hydroxynitro compound **28** in 73% yield as a mixture of diastereomers (on the basis of the recovered nitro compound (*S*)-(−)-**27**, the yield of **28** was 91%). R_f: 0.48 (30% EtOAc in hexanes). Analytical RP HPLC: MeCN/0.05% aqueous trifluoroacetic acid 80:20, 2.0 mL/min at 215 nm, t_R 6.87 min, >99%. ^1H NMR ($CDCl_3$): δ 4.75−4.66 (m, 2 H), 4.56−4.36 (m, 1 H), 4.08−3.84 (m, 1 H), 2.92−1.80 (m, 9 H), 1.50 (s, 36 H), 1.43 (s, 18 H). ESI-MS (*m/z*): 823 (M + NH_4)$^+$, 828 (M + Na)$^+$. HRMS (FAB, *m/z*): calcd for $C_{38}H_{67}N_3O_{15}Na$, 828.4464 (M + Na)$^+$; observed, 828.4467. Also, 0.383 g of (*S*)-(−)-**27** (yield: 20%) and 0.333 g of (*S*)-(−)-**17** in (yield: 18%) of both the starting materials were recovered, which were recycled.

1,10-Di-*tert*-butyl-6-*O*-acetyl-2,9-bis[bis(*tert*-butoxycarbonyl)amino]-2,3,4,5,7,8,9-heptadeoxy-5-nitro-D-*threo*-decarate (14). Acetic anhydride (0.463 mL, 4.91 mmol, 1.5 equiv) and 4-(dimethylamino)pyridine (0.040 g, 0.327 mmol, 0.1 equiv) were added sequentially to a solution of α-hydroxynitro compound **28** (2.638 g, 3.27 mmol) in THF (25 mL) at room temperature under nitrogen. After the mixture was stirred for 2 h, it was diluted with water (70 mL) and EtOAc (70 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (70 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 2.67 g of α-acetoxynitro compound **14** in 96% yield as a mixture of diastereomers. R_f: 0.23 (20% EtOAc in hexanes). Analytical RP HPLC: MeCN/0.05% aqueous trifluoroacetic acid 85:15, 2.0 mL/min at 215 nm, t_R 6.02 min, 99%. ^{1}H NMR ($CDCl_3$): δ 5.28−5.16 (m, 1 H), 4.74−4.60 (m, 3 H), 2.20−1.60 (m, 11 H), 1.50 (s, 36 H), 1.43 (s, 18 H). ESI-MS (*m/z*): 865 (M + NH_4)$^+$, 870 (M + Na)$^+$. HRMS (FAB, *m/z*): calcd for $C_{40}H_{69}N_3O_{16}Na$, 870.4570 (M + Na)$^+$; observed, 870.4586.

(−)-Benzyl-3,4-bis[(3*S*)-3-[bis(*tert*-butoxycarbonyl)amino]-4-(*tert*-butoxy)-4-oxobutyl]-1*H*-pyrrole-2-carboxylate (12). The diastereomeric mixture of α-acetoxynitro compound **14** (4.457 g, 5.26 mmol) was dissolved in THF (40 mL) and cooled in an ice bath (0−5 °C), and a solution of benzyl isocyanoacetate (**15**, 1.20 g, 6.84 mmol, 1.3 equiv) in THF (10 mL) was added via a double-ended needle under nitrogen. To this mixture was added via a syringe 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 2.0 mL, 13.0 mmol, 2.5 equiv), and the mixture was stirred for 30 min. The cooling bath was then removed, the reaction mixture was allowed to warm to room temperature, and the stirring was continued for an additional 5 h. The mixture was partitioned between a 20% aqueous NaCl solution (130 mL) and EtOAc (130 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 130 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 2.75 g of (*S*,*S*)-(−)-benzyl ester (**12**) in 57% yield. R_f: 0.64 (30% EtOAc in hexanes). ESI-MS (*m/z*): 934 (M + NH_4)$^+$. A 0.081 g portion of (*S*,*S*)-(−)-benzyl ester (**12**) was dissolved in MeCN/water (85:15, 10 mL) and purified by preparative RP HPLC (MeCN/0.05% aqueous trifluoroacetic acid 85:15; 2.0 mL/min at 215 nm). The compound was collected, and the solvent was removed on a rotary evaporator to about 100 mL and lyophilized to afford 0.047 g of analytically pure (*S*,*S*)-(−)-benzyl ester (**12**). Analytical RP HPLC: MeCN/0.05% aqueous trifluoroacetic acid 85:15, 2.0 mL/min at 215 nm, t_R 8.91 min, 97%. $[\alpha]^{23}{}_D$: −14.9 (*c* 1.37, MeOH). ^{1}H NMR ($CDCl_3$): δ 8.77 (br s, 1 H), 7.42−7.29 (m, 5 H), 6.70 (d, 1 H, *J* = 3.0 Hz), 5.28 (q, 2 H, *J* = 30.9, 12.0 Hz), 4.74−4.68 (m, 2 H), 2.88−2.68 (m, 2 H), 2.50−2.40 (m, 2 H), 2.38−2.23 (m, 2 H), 2.32−1.92 (m, 2 H), 1.48 (s, 36 H), 1.43 (s, 9 H), 1.42 (s, 9 H). ESI-MS (*m/z*): 934 (M + NH_4)$^+$. HRMS (FAB, *m/z*): calcd for $C_{48}H_{73}N_3O_{14}Na$, 938.4978 (M + Na)$^+$; observed, 938.4985.

(−)-Benzyl-3,4-bis[(3*S*)-3-[bis(*tert*-butoxycarbonyl)amino]-4-(*tert*-butoxy)-4-oxobutyl]-1-[(5*S*)-5-[bis(*tert*-butoxycarbonyl)amino]-6-(*tert*-butoxy)-6-oxohexyl]-1*H*-pyrrole-2-carboxylate (29). A solution of 18-crown-6 (0.020 g, 0.074 mmol, 0.1 equiv) in THF (3.0 mL) and a solution of *t*-BuOK (1.0 M solution in THF, 0.740 mL, 0.74 mmol, 1.0 equiv) were added sequentially to a solution of (*S*,*S*)-(−)-benzyl ester **12** (0.677 g, 0.79 mmol) in THF (3.0 mL) at room temperature under nitrogen. To this mixture was added a solution of (*S*)-(−)-iodide **13** (0.760 g, 1.48 mmol, 2.0 equiv) in THF (3.0 mL) via a double-ended needle, and the mixture was stirred for 7 h. The reaction mixture was then diluted with 20% aqueous NaCl solution (20 mL) and EtOAc (20 m). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 0.404 g of (−)-**29** in 42% yield. R_f: 0.42 (20% EtOAc in hexanes). $[\alpha]^{23}{}_D$: −6.8 (*c* 2.0, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 95:5, 2.0 mL/min at 215 nm, t_R 8.7 min, 97%. ^{1}H NMR ($CDCl_3$): δ 7.43−7.26 (m, 5 H), 6.58 (s, 1 H), 5.26 (q, 2 H, *J* = 48.3, 12.3 Hz), 4.72−4.61 (m, 3 H), 4.11 (t, 2 H, *J* = 8.1 Hz), 2.82−2.60 (m, 2 H), 2.44−1.24 (m, 12 H), 1.49 (s, 18 H), 1.48 (s, 18 H), 1.47 (s, 18 H), 1.44 (s, 9 H), 1.43 (s, 9 H), 1.42 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 169.8, 169.7, 169.6, 161.0, 152.5, 152.4, 136.6, 132.2, 128.5, 127.9, 126.5, 121.4, 118.2, 82.7, 82.5, 82.4, 81.1, 81.0, 77.2, 65.3, 59.3, 58.8, 58.3, 49.7, 31.7, 31.1, 29.2, 29.1, 28.0, 27.9, 23.8, 23.1, 21.3. ESI-MS (*m/z*): 1319 (M + NH_4)$^+$. HRMS (FAB, *m/z*): calcd for $C_{68}H_{109}N_4O_{20}Na$, 1323.7427 (M + Na)$^+$; observed, 1323.7449.

3,4-Bis[(3*S*)-3-[bis(*tert*-butoxycarbonyl)amino]-4-(*tert*-butoxy)-4-oxobutyl]-1-[(5*S*)-5-[bis(*tert*-butoxycarbonyl)-amino]-6-(*tert*-butoxy)-6-oxohexyl]-1*H*-pyrrole-2-carboxylic Acid (30). 10% Pd/C (0.020 g) was added to a solution of benzyl ester (−)-**29** (0.096 g, 0.074 mmol) in EtOH (4.0 mL), and the reaction mixture was stirred under H_2 atmosphere using a balloon at room temperature. After the mixture was stirred for 1 h, it was filtered through a pipet (fitted with cotton and Celite) and washed with MeOH (2.0 mL). The filtrate was concentrated on a rotary evaporator, and the crude product was purified by silica gel column chromatography (30% EtOAc in hexanes) to give 0.068 g of acid **30** in 76% yield. Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 19:81; 2.0 mL/min at 215 nm, t_R 4.89 min, 99%. ^{1}H NMR (CD_3OD): δ 6.65 (s, 1 H), 4.80−4.65 (m, 3 H), 4.21−4.16 (m, 2 H), 2.80−2.72 (m, 2 H), 2.46−2.38 (m, 2 H), 2.36−1.68 (m, 10 H), 1.49 (s, 54 H), 1.43 (s, 27 H). ESI-MS (*m/z*): 1229 (M + NH_4)$^+$.

(−)-(2*S*)-2-Amino-6-{(6*S*)-6-amino-3-[(4*S*))-3-aminocarboxypropyl]-7-oxo-4,5,6,7-tetrahydro-1*H*-indol-1-yl}-hexanoic Acid (31). Trifluoroacetic acid (2.0 mL) was added to acid **30** (0.058 g, 0.048 mmol) at room temperature. After the mixture was stirred for 2 h, water (0.1 mL) was added, and the stirring was continued at room temperature for an additional 4 h. The reaction mixture was concentrated on a rotary evaporator (<35 °C bath temperature). The residue was dissolved in MeCN/0.05% aqueous trifluoroacetic acid (10:90, 8 mL) and purified by preparative RP HPLC (MeCN/0.05% aqueous trifluoroacetic acid 5:95, 40 mL/min at 215 nm). The product was collected and lyophilized to afford 0.025 g of tetrahydroindole derivative (−)-**31** as its TFA salt in 74% yield. $[\alpha]^{23}{}_D$: −7.7 (*c* 0.31, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 5:95, 2.0 mL/min at 215 nm, t_R 3.55 min, 98%. ^{1}H NMR (CD_3OD): δ 7.08 (s, 1 H), 4.36−4.20 (m, 2 H), 4.14−4.06 (m, 1 H), 3.92−3.82 (m, 2 H), 2.98−2.77 (m, 2 H), 2.50−2.42 (m, 3 H), 2.20−2.04, (m, 3 H), 1.96−1.74 (m, 4 H), 1.54−1.34 (m, 2 H). ^{13}C NMR (CD_3OD): δ 182.1, 172.4, 172.2, 138.2, 132.7, 125.1, 120.9, 56.4, 54.3, 53.7, 32.3, 31.7, 31.2, 30.8, 22.9, 21.2, 21.1. ESI-MS (*m/z*): 381 (M + H)$^+$. HRMS (FAB, *m/z*): calcd for $C_{18}H_{28}N_4O_5$, 381.2138 (M + H)$^+$; observed, 381.2137.

(+)-Benzyl Ester (32). Compound (−)-**29** (0.0383 g, 0.294 mmol) was dissolved in a mixture of TFA/water (95:5, 10 mL) at room temperature and stirred for 2 h. The mixture was concentrated on a rotary evaporator (<40 °C bath temperature), and the residual TFA was removed azeotropically using toluene (2 × 10 mL). The residue was dissolved in MeCN/water (20:80, 30 mL) and purified by preparative RP HPLC (MeCN/0.1% aqueous trifluoroacetic acid 19:81, 40 mL/min at 215 nm). The product was collected and concentrated on a rotary evaporator (<40 °C bath temperature) to about 75 mL and lyophilized to afford 0.23 g of (+)-**32** in 79% yield as its TFA salt. $[\alpha]^{23}{}_D$: +17.9 (*c* 1.32, MeOH). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 19:81, 2.0 mL/min at 215 nm, t_R 4.27 min, >99%. ^{1}H NMR (CD_3OD): δ 7.48−7.34 (m, 5 H), 6.86 (s, 1 H), 5.31 (s, 2 H), 4.19 (t, 2 H, *J* = 7.5 Hz), 3.92 (t, 1 H, *J* = 6.0 Hz), 3.84 (t, 1 H, *J* = 6.6 Hz), 3.72 (dist t, 1 H, $J = 6.6$ Hz), 3.98−2.89 (m, 1 H), 2.89−2.76 (m, 1 H), 2.66−2.54 (m, 2 H), 2.20−1.98 (m, 4 H), 1.86−1.74 (m, 2 H), 1.74−1.62 (m, 2 H), 1.40−1.28 (m, 2 H). ^{13}C NMR (CD_3OD): δ 172.2, 172.1, 170.0, 162.6, 137.7, 132.3, 129.8, 129.7, 129.5, 128.7, 122.1, 119.7, 67.2, 54.0, 53.9, 53.8, 50.5, 32.9, 32.8, 32.4, 31.2, 23.1, 22.4, 21.1. ESI-MS (*m*/*z*): 533 $(M + H)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{26}H_{37}N_4O_8$, 533.2611 $(M + H)^+$; observed, 533.2615.

(+)-Deoxypyrrololine (Dpl, 4). 10% Pd/C (0.019 g) was added to a solution of benzyl ester (+)-**32** (0.086 g, 0.087 mmol) in MeOH (4.0 mL) and stirred under H_2 atmosphere using a balloon at room temperature. After the mixture was stirred for 1 h, it was filtered through a pipet (fitted with cotton and Celite powder) and washed with MeOH (2.0 mL). The filtrate was concentrated on a rotary evaporator (<40 °C bath temperature) and dried on a vacuum pump (0.5 mm/Hg) to give 0.069 g of crude acid **33**. Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 6:94, 2.0 mL/min at 215 nm, t_R 7.23 min, 70%. ESI-MS (*m*/*z*): 443 $(M + H)^+$. A small amount of the desired decarboxylated compound, (+)-Dpl (**4**), was also found to be present in the crude acid **33**. Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 6:94, 2.0 mL/min at 215 nm, t_R 4.77 min, 30%. ESI-MS (*m*/*z*): 399 $(M + H)^+$. To this crude mixture of acid **33** and (+)-Dpl (**4**) (0.055 g) was added trifluoroacetic acid (2.0 mL) at room temperature, and the mixture was stirred for 15 min. The reaction mixture was then concentrated on a rotary evaporator (<35 °C bath temperature), and the residual trifluorocaetic acid was removed azeotropically using a mixture of toluene and MeOH (1:1, 2 × 4 mL). The resulting residue was dissolved in MeCN/water (10:90, 10 mL) and purified by preparative reversed-phase HPLC (MeCN/0.1% aqueous trifluoroacetic acid 5:95, 40 mL/min at 215 nm). The product was collected and lyophilized to afford 0.021 g of (+)-Dpl (**4**) TFA salt in 39% yield as a pale pink powder. $[\alpha]^{23}_D$: +20.6 (*c* 0.17, H_2O). Analytical RP HPLC: MeCN/0.1% aqueous trifluoroacetic acid 4:96, 2.0 mL/min at 215 nm, t_R 5.54 min, 96%. ^{1}H NMR (CD_3OD): δ 6.51 (s, 2 H), 3.92−3.78 (m, 5 H), 2.64−2.52 (m, 4 H), 2.20−1.98 (m, 4 H), 1.94−1.82 (m, 2 H), 1.82−1.70 (m, 2 H), 1.48−1.38 (m, 2 H). ^{13}C NMR (D_2O + 2.0 μL of MeCN): δ 173.7, 120.0, 119.7, 54.1, 54.0, 49.0, 31.6, 30.8, 30.1, 22.1, 20.7, 1.5. ESI-MS (*m*/*z*): 399 $(M + H)^+$. HRMS (FAB, *m*/*z*): calcd for $C_{18}H_{31}N_4O_6$, 399.2244 $(M + H)^+$; observed, 399.2253.

Supporting Information Available: Copies of ^{1}H NMR spectra of (*S*,*S*)-(−)-**12**, (*S*)-(−)-**13**, **14**, (*S*)-(−)-**19**, (*S*)-(−)-**22**, (*S*)-(−)-**23**, (*S*)-(−)-**25**, (*S*)-(−)-**26**, (*S*)-(−)-**27**, **28**, (−)-**29**, **30**, and (+)-**32** and the ^{1}H NMR and ^{13}C NMR spectra of (+)-Dpl (**4**), (*S*)-(+)-**24**, and (−)-**31**. This material is published free of charge via the Internet at http://pubs.acs.org.

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