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Bone collagen cross-links: an efficient one-pot synthesis of (+)-pyridinoline and (+)-deoxypyridinoline

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Abstract

A one-pot reaction of (2*S*,5*R*)-(–)-*tert*-butyl-[(2-*tert*-butoxycarbonyl)amino]-5-hydroxy-6-aminohexanoate **2b** or (*S*)-(–)-*tert*-butyl-[(2-*tert*-butoxycarbonyl)amino]-6-aminohexanoate **2c** with (*S*)-(–)-*tert*-butyl-6-bromo-[bis-(2-*tert*-butoxycarbonyl)amino]-5-oxohexanoate **5** in the presence of K_2CO_3 in MeCN–MeOH followed by hydrolysis gave bone collagen cross-links, (+)-Pyd **1b** or (+)-Dpd **1c**, in 42–48% yield, respectively.

1. Introduction

Bone is a complex and highly specialized form of connective tissue, which serves several functions, including support of body, protection of internal organs and as a reservoir for minerals.[1] In order for bones to respond and adapt to the mechanical stress and maintain serum mineral metabolism, they undergo constant remodeling. The bone remodeling process, which is called bone turnover, begins with the resorption of old bone by osteoclasts, followed by the formation of new bone by osteoblasts. Any alteration or imbalance in the remodeling process results in metabolic bone diseases. Collagen, a family of structurally related proteins synthesized by osteoblasts, constitutes approximately 95% of the bone.[1] Inter- and intramolecular cross-links such as pyridinoline (Pyd, **1b**)[2] and deoxypyridinoline (Dpd, **1c**)[3] (Fig. 1) are formed from the adjacent lysine and hydroxylysines present in collagen fibrils by a lysyl oxidase mediated enzymatic process.[4] During the process of bone resorption these cross-links **1b**,**1c** are released into the serum and excreted in urine.[5] Thus, the cross-links Pyd **1b** and Dpd **1c** are found to be clinically useful for diagnosis of osteoporosis,[6] and other metabolic bone diseases, e.g. cancer,[7] Paget's disease[8] and hyperparathyrodism.[9] Therefore, both **1b** and **1c** are essential for use as calibrators, controls, clinical reference standards, and for immunoassay development (synthesis of immunogens, tracers,

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affinity material, etc.). Currently, the cross-links **1b**,**1c** are isolated in a very low yield and at a high cost from bones (e.g. sheep, ox, turkey) by 6–9 M HCl hydrolysis at 110°C, a process that could affect the integrity of the stereocenters.[10] Therefore, Pyd **1b** and Dpd **1c** became attractive synthetic targets due to their novel structural features and for practical applications. Waelchli et al.[11] reported the synthesis of cross links **1b**,**1c** in an unspecified diastereomeric purity, which involved the construction of a substituted pyridine ring from amino acid components utilizing aldol chemistry. Subsequently, we have accomplished the first enantiospecific synthesis of (+)-Pyd **1b**[12] starting from L-glutamic acid. The synthesis of (+)-Dpd **1c** utilizing the aldol strategy,[12–14] and alternatively starting from vitamin B_6,[15] has also been reported. In this paper we describe an efficient one-pot synthesis of (+)-Pyd **1b** and (+)-Dpd **1c** from *tert*-butyl-(*S*)-(–)-[(2-*tert*-butoxycarbonyl)amino]-5-(*R*)-hydroxy-6-aminohexanoate **2b** or *tert*-butyl-(*S*)-(–)-[(2-*tert*-butoxycarbonyl)amino]-6-aminohexanoate **2c**,[16] respectively. Also, the synthesis of (+)-analog **1a** from (*R*)-(–)-1-amino-2-propanol **2a** is presented.

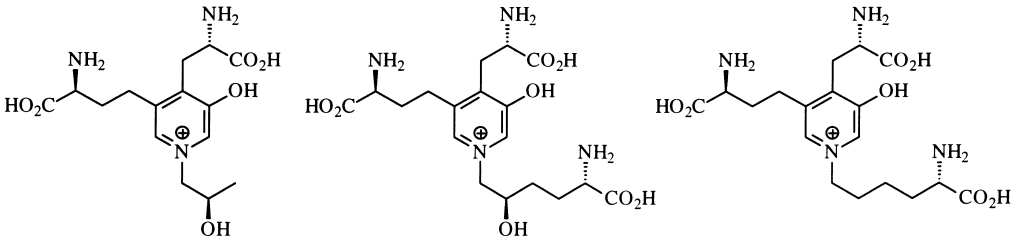
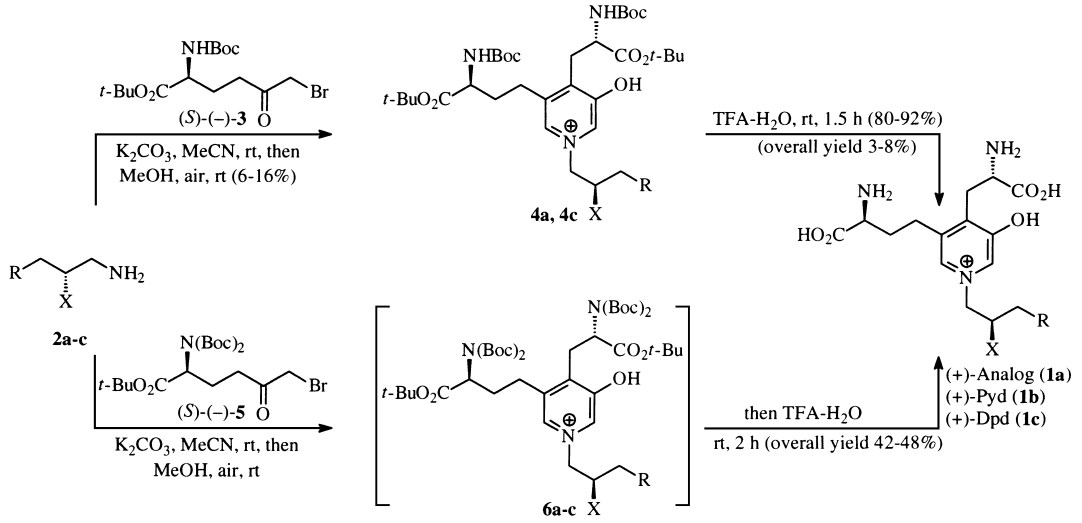


Figure 1.

2. Results and discussion

In this strategy, we envisioned that the bone collagen cross-links (+)-Pyd **1b** and (+)-Dpd **1c** (Scheme 1) could be assembled convergently and efficiently from (*S*)-(–)-amine (**2b** or **2c**) and 2 equivalents of (*S*)-bromide (**4** or **6**) via a bis-alkylation and concomitant cyclization–aromatization protocol under basic conditions. Subsequent hydrolysis of the protective groups (Boc and *t*-butyl ester) should yield the cross-links (**1b** or **1c**) possibly in one-pot. In order to explore the possibility of this one-pot synthetic strategy and to optimize the reaction conditions, we decided to carry the initial model studies on a commercially available substrate, (*R*)-(–)-1-amino-2-propanol (**2a**, X = OH, R = H) to prepare the (+)-analog **1a**. Thus, (*R*)-(–)-**2a** was treated (Table 1, entry 1) with 2.0 equivalents of (*S*)-(–)-*tert*-butyl-6-bromo-[(2-*tert*-butoxycarbonyl)amino]-5-oxohexanoate **3**[12] in the presence of potassium carbonate (5.0 equivalents) in MeCN and the progress of the reaction was monitored by TLC, HPLC and ESI-MS. After stirring the mixture for 18 h, methanol and an additional amount of potassium carbonate (10.0 equivalents) were added and the reaction mixture was exposed to air. Purification of the intermediate compound **4a** by silica gel column chromatography afforded the pyridinium compound **4a** (X = OH, R = H) in only 6% yield for three transformations (e.g. bis-alkylation, cyclization and aromatization). Under similar conditions, the reaction of (*S*)-(–)-*tert*-butyl-[(2-*tert*-butoxycarbonyl)amino]-6-aminohexanoate **2c** with (*S*)-(–)-**4** (entry 2) gave 16% yield of the pyridinium intermediate **4c**. Carrying out the reaction of (*S*)-(–)-**2c** with excess of (*S*)-(–)-**3** (4.0 equivalents) improved the yield of **4c** only marginally (25%),

but a number of unidentified side products were also observed. Hydrolysis of **4a** or **4c** with TFA–water afforded **1a** or **1c** in 80–92% yield as reported previously,[12a] and therefore, the overall yield of **1a** or **1c** from (*S*)-(–)-**2a** or (*S*)-(–)-**2c**, respectively, was only 3–8%. We therefore considered using the fully protected (*S*)-(–)-*tert*-butyl-6-bromo-[bis-(2-*tert*-butoxycarbonyl)amino]-5-oxohexanoate **5**. Thus, the reaction of (*R*)-(–)-amine **2a** (entry 3) with 2 equivalents of (*S*)-(–)-bromide **5** gave pyridinium intermediate **6a** with a clean HPLC profile, which, without purification was hydrolyzed using TFA–water. Purification of the crude product by preparative reversed phase HPLC afforded the (+)-analog **1a** in 47% overall yield for a one-pot operation. Similarly, (2*S*,5*R*)-(–)-*tert*-butyl-6-amino-2-[(*tert*-butoxycarbonyl)amino)-5-hydroxyhexanoate **2b**, which was prepared as shown in Scheme 3, was treated (entry 4) with (*S*)-(–)-bromide **5** in the presence of potassium carbonate and the resulting pyridinium intermediate **6b** was hydrolyzed with TFA–water. Purification of the crude product by HPLC afforded (+)-Pyd **1b** in 48% yield.[12a] This one-pot procedure for the preparation of (+)-Pyd **1b** represents a significant improvement in convenience, efficiency and economy when compared to the reported multistep synthesis.[12a] The versatility of this one-pot procedure was further demonstrated in the synthesis of (+)-Dpd **1c**, which was obtained by treating (entry 5) the (*S*)-(–)-amine **2c**[17] with (*S*)-(–)-bromide **5** in the presence of potassium carbonate followed by hydrolysis and purification by HPLC, in good yield (42%).[12a]



a: X=OH, R= H; **b**: X = OH, R=CH_2-[(*S*)-CH(NHBoc)]CO_2*t*-Bu; **c**: X = H, R=CH_2-[(*S*)-CH(NHBoc)]CO_2*t*-Bu

Scheme 1.

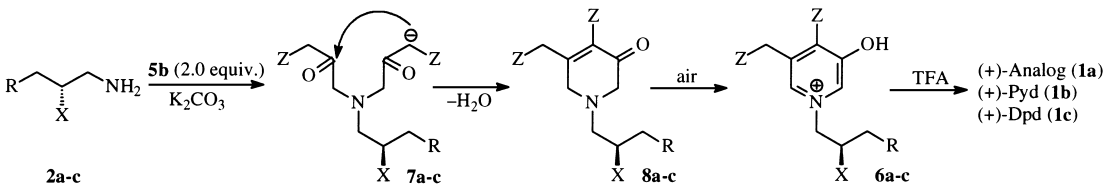
The transformations involved in this novel synthetic pathway to 3-hydroxy-1,4,5-trisubstituted pyridinium compounds are shown in Scheme 2. The initial bis-alkylation of (*S*)-(–)-amine **2** with (*S*)-(–)-bromide **5** in the presence of potassium carbonate in MeCN, affords the bis-keto compound **7**. Under these basic conditions, the aldol cyclization is then initiated, followed by the loss of water to produce the keto compound **8**. The subsequent air oxidation of intermediate **8** results in the formation of pyridinium compound **6**, which was then hydrolyzed with TFA to give the analog **1a** or cross-links **1b**–**c**. While it was possible to isolate the intermediates,[12a] e.g. bis-keto compound **7** and pyridinium compound **6**, analysis of the reaction mixture by TLC, HPLC

Table 1
One-pot synthesis of bone collagen cross-links **1b–c** and their analog **1a**

Entry	Amine (**2**) R=	X=	Bromide (**3**,**5**)	Intermediate (**4**,**6**) Yield[a]	Pyridinium Compound (**1a-c**) Overall Yield[a] (%)	$[\alpha]^{23}_D$
1.	**2a** H	OH	(*S*)-(–)-**3**	**4a** 6	Analog (**1a**) <3	
2	**2c** $CH_2CH(NHBoc)CO_2t$-Bu	H	(*S*)-(–)-**3**	**4c** 16	Dpd (**1c**) 8	
3.	**2a** H	OH	(*S*)-(–)-**5**	**6a**[b]	Analog (**1a**) 47	+14.8
5	**2b** $CH_2CH(NHBoc)CO_2t$-Bu	OH	(*S*)-(–)-**5**	**6b**[b]	Pyd (**1b**) 48	+17.0
4	**2c** $CH_2CH(NHBoc)CO_2t$-Bu	H	(*S*)-(–)-**5**	**6c**[b]	Dpd (**1c**) 42	+37.6

a) Isolated yield by preparative reversed phased HPLC. b) The pyridinium intermediate was not isolated.

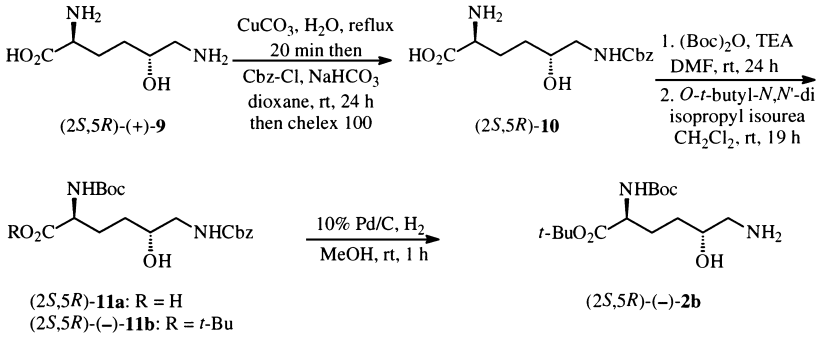
and ESI-MS indicated the presence of multiple intermediates (e.g. **7**, **8** and **6**). We therefore found that allowing the reaction to proceed to completion in one-pot was the most convenient and efficient way to achieve the total synthesis of these bone collagen cross-links **1b–c** in good yield.



a: X = H, R = Me; **b**: X = OH, R = CH_2-[(*S*)-CH(NHBoc)]CO_2t-Bu; **c**: X = H, R = CH_2-[(*S*)-CH(NHBoc)CO_2t-Bu
Z = CH_2-[(*S*)-CH(NHBoc)]CO_2t-Bu

Scheme 2.

The (2*S*,5*R*)-(–)-*tert*-butyl-6-amino-2-[(*tert*-butoxycarbonyl)amino)-5-hydroxyhexanoate **2b**, which is required for the synthesis of (+)-Pyd **1b** was previously prepared by us from (*S*)-(–)-*tert*-butyl-6-bromo-[(2-*tert*-butoxycarbonyl)amino]-5-oxohexanoate **3**.[12a] The synthesis of (2*S*,5*R*)-(–)-**2b** from (*S*)-(–)-**3** involved key transformations, e.g. reduction of the keto functionality, resolution of the resulting diastereomeric hydroxy compound using a thyroxine derivative, and subsequent conversion of bromide to the amine. While this procedure gave (2*S*,5*R*)-(–)-**2b**, which was needed for confirmation of stereochemistry, it involved resolution and a number of steps.[12a] Therefore, an alternative procedure was developed (Scheme 3) for synthesis of (2*S*,5*R*)-(–)-**2b** starting from a commercially available (2*S*,5*R*)-(+)-hydroxylysine **9**. Thus, the terminal ε-amino group in (2*S*,5*R*)-(+)-**9** was first selectively protected as its benzyloxycarbonyl (Cbz) by transformation of the amino acid terminal into a cupric chelate,[18] which was disassociated with Chelex 100 to give (2*S*,5*R*)-**10**.



Scheme 3.

The α-amine and the carboxylic acid groups in crude (2*S*,5*R*)-**10** were then protected as Boc and *tert*-butyl esters, respectively, to give (2*S*,5*R*)-(–)-**11b** in good overall yield. Finally, the removal of the Cbz group in (2*S*,5*R*)-(–)-**11b** was accomplished by hydrogenolysis to afford the desired amine (2*S*,5*R*)-(–)-**2b** in almost quantitative yield.

In summary, a highly efficient synthesis of bone collagen cross-links, (+)-pyridinoline (Pyd, **1b**) and (+)-deoxypyridinoline (Dpd, **1c**) was developed from (2*S*,5*R*)-(–)-*tert*-butyl-([(2-*tert*-butoxycarbonyl)amino]-5-hydroxy-6-aminohexanoate **2b** and (*S*)-(–)-*tert*-butyl-[(2-*tert*-butoxycarbonyl)-amino]-6-aminohexanoate **2c** respectively in 42–48% yield.

3. Experimental

3.1. 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) reported in ppm relative to TMS, and coupling constants (J) reported in hertz. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Perkin–Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing a Turbo Ionspray ion source and HRMS was performed on a Nermang 3010 MS-50, JEOL SX102-A mass spectrometer. Thin layer chromatography was performed on pre-coated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and visualized with UV light and/or using a $KMnO_4$ reagent [$KMnO_4$ (1.0 g), NaOH (8.0 g) in water (200 mL)] or phosphomolybdic acid reagent (20 wt% solution in ethanol) or ninhydrin reagent (0.2% in ethanol). Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). THF was freshly distilled from a purple solution of sodium and benzophenone. CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen. (2*S*,5*R*)-(–)-Hydroxylysine dihydrochloride was purchased from Fluka Chemie AG and all other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO). All the solvents employed were of HPLC grade purchased from EM Science (Gibbstown, NJ) and used as received. Analytical reverse phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (8×100 mm) column (solvents ratio v/v reported). Optical rotations were measured on an Autopol III polarimeter from Rudolph Research, Flanders, NJ.

(*S*)-(–)-*tert*-Butyl-6-bromo-[(2-*tert*-butoxycarbonyl)amino]-5-oxohexanoate {**3**, $[\alpha]_D^{23} = -0.88$ (*c* 0.88, $CHCl_3$)}[12a] and (*S*)-(–)-1-*tert*-butyl-5-methyl-2-[bis-(*tert*-butoxycarbonyl)amino]pentanedioate,[12b] which was needed for the preparation of (*S*)-(–)-bromide **5**, were prepared from a

commercially available (4*S*)-5-*tert*-butoxy-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid according to our previously reported procedure. (*S*)-(–)-*tert*-Butyl-6-amino-[(2-*tert*-butoxycarbonyl)amino]hexanoate **2b** was prepared in two steps from a commercially available (*S*)-(–)-6-amino-[(2-*tert*-butoxycarbonyl)amino]hexanoic acid according to the known procedure.[17]

3.2. (S)-(–)-tert-Butyl-6-bromo-2-[bis-(tert-butoxycarbonyl)amino]-5-oxohexanoate **5**

LiOH (monohydrate, 1.04 g, 24.7 mmol, 3.0 equiv.) and water (180 mL) were added sequentially to a solution of (*S*)-(–)-1-*tert*-butyl-5-methyl-2-[bis-(*tert*-butoxycarbonyl)amino]pentanedioate[12b] (3.442 g, 8.24 mmol) in THF (180 mL) at room temperature. After stirring the mixture for 2 h, the pH of the solution was adjusted to 7.0 using 1.0 M HCl and most of the THF was removed on a rotary evaporator. The pH of the resulting aqueous solution was adjusted to 3.0 using 1.0 M HCl and extracted with EtOAc (2×200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated on a rotary evaporator to afford 3.33 g of (*S*)-5-*tert*-butoxy-4-[bis-(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid in almost quantitative yield. Analytical RP HPLC: MeCN:0.1% aq. trifluoroacetic acid, 70:30, 2.0 mL/min at 215 nm, t_R: 2.83 min, 97%; ^{1}H NMR ($CDCl_3$): δ 4.82–4.76 (m, 1H), 2.52–2.36 (m, 3H), 2.24–2.10 (m, 1H), 1.50 (s, 18H), 1.45 (s, 9H); ESI-MS (*m*/*z*): 404 $(M+H)^+$; 426 $(M+Na)^+$.

Isobutylchloroformate (1.07 mL, 8.22 mmol, 1.0 equiv.) was added to a 0°C cooled mixture of the above prepared (*S*)-5-*tert*-butoxy-4-[bis-(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (3.316 g, 8.22 mmol) and 4-*N*-methylmorpholine (NMM, 0.994 mL, 9.04 mmol, 1.1 equiv.) in THF (35 mL) under nitrogen. The mixture was stirred for 40 min and quickly filtered through a Celite bed (about 5 mm thickness). The filtrate was added to freshly generated ethereal-diazomethane [generated from *N*-nitroso-*N*-methylurea (8.47 g, 82.2 mmol, 10.0 equiv.), KOH (18.5 g, 0.329 mol, 40.0 equiv.), water (35 mL) and ether (35 mL)] via a double ended needle at 0°C over a 10 min period. The mixture was then allowed to warm to about 20°C and stirred for a total of 3 h. The solvent was carefully removed on a rotary evaporator (< 35°C bath temperature) and dried on a vacuum pump. The resulting red oily residue was dissolved in ether (35 mL), cooled to –20°C and 48% aq. HBr (0.93 mL, 8.22 mmol, 1.0 equiv.) was added under nitrogen. After stirring the reaction mixture for 30 min at –20°C, it was then diluted with ether (70 mL), washed with water (3×20 mL) and dried (Na_2SO_4). Solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (15% EtOAc in hexanes) to afford 2.145 g of (*S*)-(–)-*tert*-butyl-6-bromo-2-[bis-(*tert*-butoxycarbonyl)amino]-5-oxohexanoate **5** in 54% yield as a colorless viscous oil. R_f: 0.35 (20% EtOAc in hexanes); $[\alpha]_D^{23} = -23.5$ (*c* 1.55, MeOH); ^{1}H NMR ($CDCl_3$): δ 4.70 (dd, 1H, $J = 9.3$, 5.1 Hz), 3.90 (s, 2H), 2.84–2.64 (m, 2H), 2.50–2.36 (m, 1H), 2.18–2.04 (m, 1H), 1.47 (s, 18H), 1.45 (s, 9H); ESI-MS (*m*/*z*): 502 and 504 $(M+Na)^+$; HRMS (FAB, *m*/*z*); calcd for $C_{20}H_{35}BrNO_7$: 480.1597 $(M+H)^+$; observed: 480.1596.

3.3. (2S,5R)-(–)-tert-Butyl-6-[(benzyloxycarbonyl)amino]-2-[(tert-butoxycarbonyl)amino]-5-hydroxyhexanoate **11b**

(2*S*,5*R*)-(–)-Hydroxylysine dihydrochloride (3.00 g, 11.8 mmol) was dissolved in water (30 mL) and heated to 80–90°C in an oil bath. To this mixture, copper(II) carbonate (2.61 g, 11.8 mmol, 1.0 equiv.) was added slowly over a 3 min period and the resulting pale blue mixture was gently refluxed for 20 min. The warm reaction mixture was filtered using vacuum and washed with hot

water (3×10 mL). The combined aqueous filtrates were cooled to room temperature. $NaHCO_3$ (3.37 g, 40.1 mmol, 3.4 equiv.) was added to the mixture followed by a solution of benzyl chloroformate (2.36 mL, 16.5 mmol, 1.4 equiv.) in 1,4-dioxane (30 mL) dropwise over 3 min period at room temperature. The reaction mixture was stirred for 24 h, filtered and washed with water (3×10 mL). The solid filter cake was suspended in MeOH (50 mL) and stirred for 20 h at room temperature. The mixture was then diluted with water (60 mL) and freshly prepared chelex 100 [80 g, prepared by washing the commercial chelex 100 (80 g) with 1.0 M acetic acid (6×50 mL), and water (15×50 mL)] was added. The mixture was heated to 80–90°C for 4 h, filtered and washed with a mixture of hot MeOH:water (1:1 ratio, 10×60 mL). The filtrates were combined and concentrated on a rotary evaporator to afford to 1.728 g of (2*S*,5*R*)-2-amino-6-[(benzyloxycarbonyl)amino]-5-hydroxyhexanoic acid **10** in 49% yield as a viscous oil, which was taken to the next step.

Triethylamine (0.571 mL, 4.1 mmol, 1.5 equiv.) and a solution of $(Boc)_2O$ (0.655 g, 3.0 mmol, 1.1 equiv.) in DMF (10 mL) were added sequentially to a solution of (2*S*,5*R*)-2-amino-6-[(benzyloxycarbonyl)amino)-5-hydroxyhexanoic acid **10** (0.809 g, 2.73 mmol) dissolved in DMF (30 mL) at room temperature under nitrogen. The reaction mixture was stirred for 24 h, diluted with 20% aq. NaCl solution and the pH was adjusted to 3.0 using 1.0 M HCl. The mixture was extracted with EtOAc (3×30 mL) and the combined organic layers were dried (Na_2SO_4). Solvent was removed on a rotary evaporator and the crude compound was purified by silica gel column chromatography (15% MeOH in CH_2Cl_2) to afford 0.825 g of (2*S*,5*R*)-6-[(benzyloxycarbonyl)-amino]-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoic acid **11a** as a viscous oil in 76% yield. Analytical RP HPLC: MeCN:0.1% aq. trifluoroacetic acid/40:60, 2.0 mL/min at 215 nm; t_R: 3.32 min, 95%; ^1H NMR (CD_3OD): δ 7.38–7.26 (m, 5H), 5.07 (s, 2H), 4.07–4.02 (m, 1H), 3.64–3.56 (m, 1H), 3.32–3.04 (m, 2H), 2.04–1.40 (m, 4H), 1.43 (s, 9H); ESI-MS (*m*/*z*): 397 $(M+H)^+$, 414 $(M+NH_4)^+$.

A solution of *O*-*tert*-butyl-*N*,*N*-diisopropylisourea (2.94 g, 14.7 mmol, 7.0 equiv.) in CH_2Cl_2 (10 mL) was added to the above-prepared (2*S*,5*R*)-6-[(benzyloxycarbonyl)amino]-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoic acid **11a** (0.972 g, 2.45 mmol) dissolved in CH_2Cl_2 (20 mL) at room temperature under nitrogen and the mixture was stirred for 19 h. The reaction mixture was filtered and the filtrate was concentrated on a rotary evaporator. Purification of the crude compound by silica gel column chromatography (50% EtOAc in hexanes) afforded 0.576 g of (2*S*,5*R*)-(–)-*tert*-butyl-6-[(benzyloxycarbonyl)amino]-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-hexanoate **11b** in 52% yield as a viscous oil. R_f: 0.25 (50% EtOAc in hexanes). Analytical RP HPLC: MeCN:0.1% aq. trifluoroacetic acid/50:50, 2.0 mL/min at 215 nm; t_R: 6.45 min, 98.3%; $[\alpha]_D^{23} = -11.9$ (*c* 1.33, MeOH); ^1H NMR ($CDCl_3$): δ 7.37–7.34 (m, 5H), 5.30–5.18 (m, 2H), 5.11 (s, 2H), 4.28–4.18 (m, 1H), 3.82–3.72 (m, 1H), 3.44–3.34 (m, 1H), 3.28–3.22 (m, 1H), 3.12–3.10 (m, 1H), 2.02–1.44 (m, 4H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 171.7, 157.0, 155.8, 136.4, 128.4, 128.0, 82.0, 79.9, 70.5, 66.7, 53.4, 46.9, 29.9, 29.6, 28.2, 27.9; ESI-MS (*m*/*z*): 453 $(M+H)^+$, 470 $(M+NH_4)^+$; HRMS (FAB, *m*/*z*): calcd $C_{23}H_{37}N_2O_7$: 453.2601 $(M+H)^+$; observed: 453.2607.

3.4. (2S,5R)-(–)-tert-Butyl-6-amino-2-[(tert-butoxycarbonyl)amino]-5-hydroxyhexanoate **2b**

Pd/C (10%, wet, Degussa type E101 NE/W, 0.054 g) was added to a solution of (2*S*,5*R*)-(–)-*tert*-butyl-6-[(benzyloxycarbonyl)amino]-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoate **11b** (0.541 g, 1.20 mmol) dissolved in methanol (30.0 mL) and stirred under a hydrogen atmosphere using a balloon at room temperature. After stirring the mixture for 1 h, it was filtered and the

filtrate was concentrated on a rotary evaporator to afford 0.377 g of the amine (2*S*,5*R*)-(–)-**2b** in 99% yield as a colorless viscous oil. Analytical RP HPLC: MeCN:0.1% aq. trifluoroacetic acid/ 30:70, 2.0 mL/min at 225 nm; t_R: 2.82 min, 95%; $[\alpha]_D^{23} = -16.6$ (*c* 1.59, MeOH), lit.[12] $[\alpha]_D^{23} = -18.9$ (*c* 1.46, MeOH); ^{1}H NMR (CD_3OD): δ 3.98–3.92 (m, 1H), 3.78–3.68 (m, 1H), 2.76–2.33 (m, 2H), 2.00–1.40 (m, 4H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (CD_3OD): δ 173.7, 158.1, 82.4, 80.4, 72.2, 56.0, 47.8, 32.3, 29.1, 28.7, 28.3; ESI-MS (*m*/*z*): 319 $(M+H)^+$; HRMS (FAB, *m*/*z*): calcd $C_{15}H_{31}N_2O_5$: 319.2233 $(M+H)^+$; observed: 319.2243.

3.5. General one-pot procedure for the synthesis of collagen cross-links: e.g. (+)-pyridinoline (Pyd **1b**)

Anhydrous K_2CO_3 (0.207 g, 1.5 mmol, 5.0 equiv.) was added to a mixture of (2*S*,5*R*)-(–)-amine **2b** (0.096 g, 0.3 mmol) and (*S*)-(–)-bromide **5** (0.288 g, 0.6 mmol, 2.0 equiv.), which were dissolved in dry MeCN (3.0 mL), at room temperature under nitrogen. After stirring the mixture for 19 h, MeOH (3.0 mL) and an additional amount of anhydrous K_2CO_3 (0.415 g, 3.0 mmol, 10.0 equiv.) were added to the reaction. The rubber septum was removed to expose the reaction to an open atmosphere and the mixture was stirred at room temperature for 24 h. The mixture was then concentrated on a rotary evaporator under vacuum, and the residue was treated with a mixture of trifluoroacetic acid:water (10 mL, 95:5 ratio) at room temperature. After stirring the mixture for 2 h, it was concentrated on a rotary evaporator, and the residue was dissolved in 0.1% TFA–MeCN:0.1% TFA–H_2O (20 mL, 1:99 ratio). The crude product was purified by preparative reversed phase HPLC [Waters, C18, RCM, μBondpak, 10.0 μm, 3×(40×100 mm) using 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 20 mL/min at 215 nm]. Concentration of the product to about a 10 mL volume and lyophilization afforded a gummy material which was redissolved in water (80 mL). Lyophilization of the product afforded 0.126 g of (+)-Pyd **1b** as its TFA salt in 48% yield.[12a] Analytical RP HPLC: 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 1.0 mL/min at 215 nm, t_R: 4.13 min, 99%, $[\alpha]_D^{23} = +17.0$ (*c* 0.69, MeOH), lit.[12a] $[\alpha]_D^{20} = +39.17$ (*c* 0.24, MeOH); ^{1}H NMR (CD_3OD): δ 8.32 (s, 1H), 8.26 (d, 1H, $J = 5.1$ Hz), 4.68–4.60 (m, 1H), 4.35–4.24 (m, 1H), 4.21–4.33 (m, 1H), 4.05–3.90 (m, 3H), 3.46–3.37 (m, 2H), 3.14–2.92 (m, 2H), 2.38–1.92 (m, 4H), 1.84–1.76 (m, 2H); ESI-MS (*m*/*z*): 429 $(M)^+$; HRMS (FAB, *m*/*z*): calcd $C_{18}H_{29}N_4O_8$: 429.1985 $(M)^+$; observed: 429.1980.

3.6. (+)-Analog **1a**

The reaction of (*S*)-(–)-1-amino-2-propanol **2a** (0.033 g, 0.44 mmol) and (*S*)-(–)-bromide **5** (0.423 g, 0.88 mmol, 2.0 equiv.) was carried out by following the procedure described for (+)-Pyd **1b**. The residue was dissolved in 0.1% TFA–MeCN:0.1% TFA–H_2O (14 mL, 1:99 ratio) and purified by preparative reversed phase HPLC [Waters, C18, RCM, μBondpak, 10.0 μm, 3×(40×100 mm), using 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 20 mL/min at 215 nm]. Concentration of the product to about a 70 mL volume, followed by lyophilization afforded 0.164 g of (+)-analog **1a** as its TFA salt in 47% yield. Analytical RP HPLC: 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 1.0 mL/min at 215 nm, t_R: 5.46 min, 98.7%, $[\alpha]_D^{23} = +14.8$ (*c* 0.86, H_2O); ^{1}H NMR (CD_3OD): δ 8.33 (d, 1H, $J = 1.2$ Hz), 8.25 (d, 1H, $J = 1.2$ Hz), 4.60 (dd, 1H, $J = 12.9$, 2.4 Hz), 4.30–3.96 (m, 4H), 3.46–3.40 (m, 2H), 3.18–2.60 (m, 2H), 2.38–2.13 (m, 2H), 1.29 (d, 3H, $J = 6.0$ Hz); ESI-MS (*m*/*z*): 342 $(M)^+$; HRMS (FAB, *m*/*z*): calcd $C_{15}H_{24}N_3O_6$: 342.1665 $(M)^+$; observed: 342.1656.

3.7. (+)-Deoxypyridinoline (Dpd **1c**)

The reaction of (*S*)-(–)-amine **2c** (0.060 g, 0.198 mmol) and (*S*)-(–)-bromide **5** (0.190 g, 0.396 mmol, 2.0 equiv.) was carried out by following the procedure described for (+)-Pyd **1b**. The residue was dissolved in 0.1% TFA–MeCN:0.1% TFA–H_2O (20 mL, 1:99 ratio). The crude product was purified by preparative reversed phase HPLC [Waters, C18, RCM, μBondpak, 10.0 μm, 3×(40×100 mm), using 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 20 mL/min at 215 nm]. Concentration of the product to about 120 mL volume, and lyophilization afforded 0.072 g of (+)-Dpd **1c** as its TFA salt in 42% yield.[12a] Analytical RP HPLC: 0.1% TFA–MeCN:0.1% TFA–H_2O, 1:99, 1.0 mL/min at 215 nm, t_R: 4.64 min, 99.7%; $[\alpha]_D^{23} = +36.2$ (*c* 0.535, MeOH), lit.[12a] $[\alpha]_D^{23} = +31.6$ (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD): δ 8.34 (s, 1H), 8.29 (s, 1H), 4.53 (t, 2H, $J = 6.9$ Hz), 4.21 (t, 1H, $J = 6.9$ Hz), 3.97–3.89 (m, 2H), 3.44–3.38 (m, 2H), 3.16–2.92 (m, 2H), 2.36–1.88 (m, 6H), 1.62–1.42 (m, 2H); ESI-MS (*m*/*z*): 413 $(M)^+$; HRMS (FAB, *m*/*z*): calcd $C_{18}H_{28}N_4O_7$: 413.2036 $(M)^+$; observed: 413.2034.

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