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Collagen Cross-links: Synthesis of Pyridinoline, Deoxypyridinoline and Their Analogues

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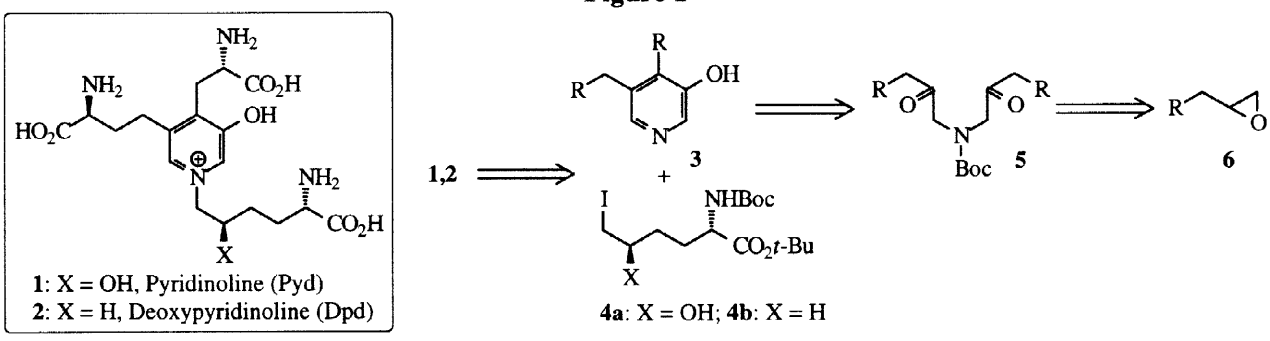
Summary: *An efficient chiral synthesis of (S,S)-(-)-**3g**, a key intermediate for the preparation of collagen cross-links pyridinoline (Pyd, **1**) and deoxypyridinoline (Dpd, **2**) was achieved from (4S)-5-(tert-butoxy)-4-[(tert-butoxycarbonyl)amino]-5-oxopentanoic acid (**21b**). Quaternization of (S,S)-(-)-**3g** with iodide (2S,5R)-(+)-**4a** followed by hydrolysis provided a first chiral synthesis of natural (+)-Pyd (**1**). 1-(2S)-(+)-Pyd (**1**) was also synthesized from (S,S)-(-)-**3g** and iodide (2S,5S)-(+)-**4a**. Similarly, quaternization of (S,S)-(-)-**3g** with iodide (2S)-(-)-**4b**, which was prepared from (2S)-(-)-6-amino-2-[(tert-butoxycarbonyl)amino]hexanoic acid (**31**) in three steps, followed by hydrolysis afforded natural (+)-Dpd (**2**) in 5.3% overall yield. Also, the synthesis of racemic Dpd [(±)-**2**] and a variety of its analogues is presented.*

Keywords: collagen, pyridinium cross-links, osteoporosis, pyridinoline, deoxypyridinoline.

Introduction

Collagen is a family of structurally related proteins present in the extracellular matrix of connective tissue (e.g., tendon, skin, ligament and bone) which provides a scaffold for provision of shape and form.[1] During the maturation of collagen, inter and intramolecular covalent cross-links (Figure 1) pyridinoline (Pyd, **1**)[2] and deoxypyridinoline (Dpd, **2**)[3] are formed from the adjacent lysines and hydroxylysines by a lysyl oxidase mediated enzymatic process.[4,5] The pyridinium cross-links (**1**,**2**) play an important role in the physiological and pathological changes of collagen and are found to be essential for maintaining the structure of collagen fibril network.[1] However, during the process of bone resorption, cross-links (**1**,**2**) are released into the serum and excreted in urine either in free form or linked to different peptide fragments of collagen.[1] Therefore, markedly elevated levels of cross-links (**1**,**2**) are observed in the urine of patients with diseases such as osteoporosis,[6]

Figure 1



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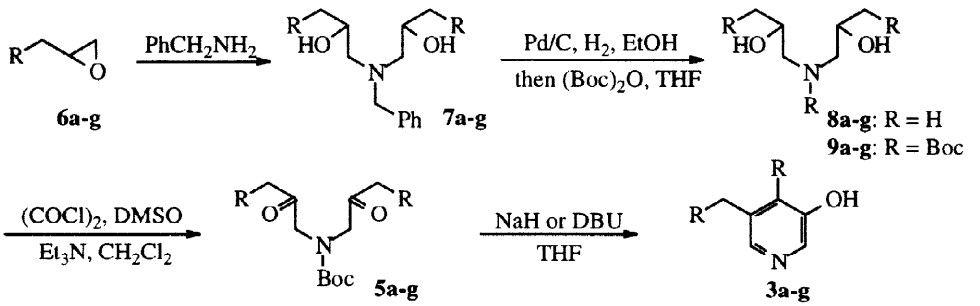
Paget's disease,[7] primary hyperparathyroidism,[8] malignant hypercalcemia[9] and cancer.[10] Since Pyd (**1**) and Dpd (**2**) are two important and representative cross-links of collagen status, estimation of their levels in urine has been shown to be useful biochemical markers in the diagnosis and management of osteoporosis and other metabolic diseases.[11] Unfortunately, Pyd (**1**) and Dpd (**2**) are mainly isolated in a very low yield from natural sources such as animal bones (ex., sheep, ox) by acid hydrolysis followed by several purification steps.[12] Thus, the cost of these materials (**1**,**2**) is extremely high.[13] Therefore, development of a general synthetic method for collagen cross-links (**1**,**2**) as well as for their analogues which are needed as clinical assay calibrators, controls and reference standards, is critically important. The cross-links (**1**,**2**) and their analogues are also imperative as components for development of immunoassays for osteoporosis and to study bone metabolism. Recently, Waelchli *et al.*[14] communicated a synthesis of a diastereomeric mixture of pyridinoline (**1**) from (2*S*)-2,5-dihydro-3,6-diethoxy-2-isopropylpyrazine, unfortunately with an undefined stereochemistry at 1-(2)-OH chiral center. Additionally, the ten step synthesis reported for (2*S*)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) utilizing (2*S*)-2,5-dihydro-3,6-diethoxy-2-isopropylpyrazine, which is essential for preparation of deoxypyridinoline (**2**), presents serious limitations in making this important biological material (**2**).[14] In this paper, we describe our efforts in detail, on the first total synthesis of natural (+)-pyridinoline (**1**) from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**21b**). The use of a novel chiral reagent, *O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine (**24**), for separation of (2*S*,5*R*)-(+)-*tert*-butyl-2-[(*tert*-butoxycar bonyl)amino]-5-hydroxy-6-iodohexanoate (**4a**) and the synthesis of 1-(2*S*)-(+)-pyridinoline (**1**) is also presented. Additionally, development of an efficient three step synthesis of (2*S*)-(-)-*tert*-butyl-2-[(*tert*-butoxycarbonyl) amino]-6-iodohexanoate (**4b**) from (2*S*)-(-)-6-amino-2-[(*tert*-butoxycarbonyl)amino]hexanoic acid (**31**) and the preparation of natural (+)-deoxypyridinoline (**2**) is described. The racemic synthesis of deoxypyridinoline [(±)-**2**] and the preparation of a variety of 3-hydroxy-4,5-substituted pyridine analogues (**3a-g**) is also presented.

Preparation of 3-Hydroxy-4,5-substituted pyridine Derivatives (3)

The strategy for the synthesis of collagen cross-links (**1**,**2**) and their analogues involves (Figure 1) the construction of appropriately functionalized 3-hydroxy-4,5-substituted pyridine derivatives (**3**), followed by the quaternization of nitrogen with an iodo compound (**4a** or **4b**), and subsequent hydrolysis of protective groups. However, there are only a limited number of methods available for preparation of 3-hydroxy-4,5-substituted pyridines (**3**) which involve elaboration of the other heterocyclic systems such as furans, pyrones[15] and oxazoles.[16] Although the oxazoles are frequently used as dienes for preparation of 3-hydroxy-4,5-substituted pyridines, the formation of regeoisomers at 4,5-positions of the pyridine ring with unsymmetrical dienophiles, is undesirable.[15b,16] Therefore, the required 3-hydroxy-4,5-substituted pyridine derivatives (**3**) were prepared from aza-diketo compounds (**5**) via a cyclization-aromatization reaction.[14] The aza-diketones (**5**), the key synthons for the cyclization reaction, were prepared from the epoxides (**6**) as shown in Scheme 1. Accordingly, the epoxides (**6a-g**) were reacted with benzylamine[17] and the crude product was purified by silica gel column chromatography to afford the *N*-benzyl compounds (**7a-g**) in 51-89% yield. The *N*-benzyl group in **7a-g** was cleaved by hydrogenolysis over 10% Pd/C in ethanol and the resulting crude amines (**8a-g**) were treated with di-*tert*-butyldicarbonate in THF at room temperature. After removal of solvent, the crude products were purified

by silica gel column chromatography to afford Boc compounds (**9a-g**). Finally, oxidation of the two hydroxyl groups in **9a-g** using oxalyl chloride and DMSO[18] in CH_2Cl_2, followed by silica gel column chromatography afforded the aza-diketones (**5a-g**), the key synthons for the cyclization-aromatization reaction, in excellent yield (63-97%).

Scheme 1



R = a) CH_2CH_3; b) CH_2CH_2Ph; c) $CH_2(CH_2)_2OTHP$; d) $CH_2(CH_2)_6CN$; e) CH_2CH_2NHBoc; f) $CH_2CH_2CO_2t$-Bu; g) (±)-$CH_2CH(NHBoc)CO_2t$-Bu

Typically, the cyclization-aromatization reaction was carried out by adding a THF solution of aza-diketones (**5a-e**) to a cooled (0-5 °C) solution of NaH (4.0 equiv.) in THF (20 mL/1.0 mmol of **5**) under nitrogen. The mixture was stirred at room temperature and progress of the reaction was monitored by TLC (40-80% EtOAc in hexane). After the reaction was complete, the mixture was cooled to 0 °C, quenched with methanol and 3-hydroxy-4,5-substituted pyridines (**3a-e**) were isolated by silica gel column chromatography.

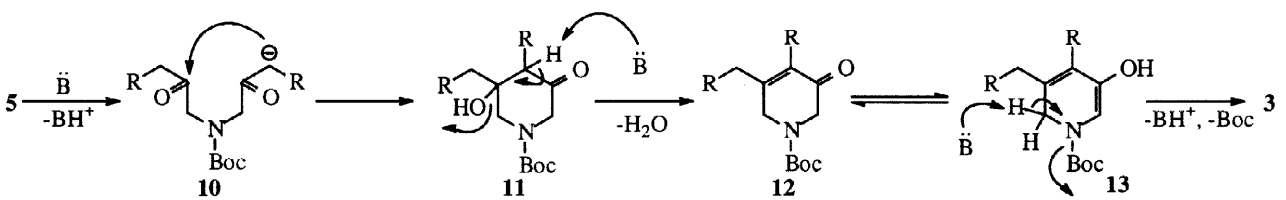
Table: Preparation of 3-hydroxy-4,5-substituted pyridine derivatives (**3a-g**) from aza-diketo compounds (**5a-g**)

Entry	Aza-diketones (**5**) R=		Base[a]/Solvent/ Temp (°C)/Time (h)	Pyridine derivatives (**3**) yield (%)[b]		mp (°C)
1.	**5a**	CH_2CH_3	*t*-BuOK/THF/rt to 60/5	**3a**	45	143-5
2.	**5a**	CH_2CH_3	NaH/THF/0-rt/24	**3a**	49	143-4
3.	**5b**	CH_2CH_2Ph	*t*-BuOK/THF/rt to 60/5.5	**3b**	35	oil
4.	**5b**	CH_2CH_2Ph	NaH/THF/0-rt/24	**3b**	45	oil
5.	**5c**	$CH_2(CH_2)_2OTHP$	NaH/THF/0-rt/23	**3c**	38	oil
6.	**5d**	$CH_2(CH_2)_6CN$	NaH/THF/0-rt/48	**3d**	44	oil
7.	**5e**	CH_2CH_2NHBoc	NaH/THF/0-rt/22	**3e**	44	gum
8.	**5f**	$CH_2(CH_2)_2CO_2t$-Bu	DBU/THF/rt/92	**3f**	62	gum
9.	**5g**	(±)-$CH_2CH(NHBoc)CO_2t$-Bu	DBU/THF/rt/92	(±)-**3g**	58	86-9

a) 4.0 equiv. of NaH or *tert*-BuOK or 6.5 equiv. of DBU was used. b) Isolated yield.

The cyclization-aromatization reaction was studied under different conditions and a variety of 3-hydroxy 4,5-substituted pyridine derivatives (**3a-g**) were prepared in 38-62% yield (table). The pyridine analogues **3a-g** are important reagents designed for testing of *anti*-Dpd antibodies and for applications in the development of clinical assay for osteoporosis. The pyridine derivatives **3a-e** were obtained in 38-49% yield using sodium hydride in THF, while the use of potassium *tert*-butoxide in THF (entry 1 and 3) did not produce noticeable difference in yield. The non-nucleophillic bases, e.g., diazabicyclo[5.4.0]-undec-7-ene (DBU) in THF at room temperature gave the pyridine derivatives (e.g., **3a** from **5a**; **3c** from **5c**) only in <5% yield. Additionally, the reaction of **5a** or **5c** with DBU in THF at higher temperature led to the decomposition of starting aza-diketo compound (**5**). The substrates containing activating groups such as phenyl and nitrile, (e.g., **5b**, **5d**) also afforded the expected pyridine derivatives (**3b**, **3d**) in 44-45% yield (entries 4, 6). The aza-diketo compounds (**5f-g**) gave pyridine derivatives (**3f-g**, entries 8 and 9) in good yields (58-62%) using DBU in THF at room temperature, but the use of NaH in these cases (**5f-g**) produced **3f-g** in low yield (< 10%) with a number of side products. The cyclization-aromatization reaction of **5a** with NaOMe in THF allowed us to isolate the conjugated keto compound **12a** (R = CH_2CH_3) in 48% yield along with the aromatized pyridine compound **3a** in only a 10% yield. The keto compound **12a** (R = CH_2CH_3) upon further treatment with *tert*-BuOK in THF produced the final product of the cyclization-aromatization reaction **3a** in 30% yield, suggesting that **12a** is an intermediate in the reaction. We therefore propose that the reaction of (**5a-g**) is proceeding (Scheme 3) through an initial base catalyzed cyclization of aza-diketone (**5**), followed by an aromatization with the elimination of Boc group, and thus leading to the formation of 3-hydroxy-4,5-substituted pyridines (**3a-g**). Thus, utilizing 2.0 equiv. of epoxide (**6a-g**), the method allows the introduction of a variety of substituents at the designated 4 and 5-positions of pyridine with placement of a hydroxyl group at the 3-position. More importantly, the substituents at the 4 and 5-positions of pyridine ring are differentiated by one methylene, which is a characteristic feature of the pyridinium cross-links (**1,2**).

Scheme 3

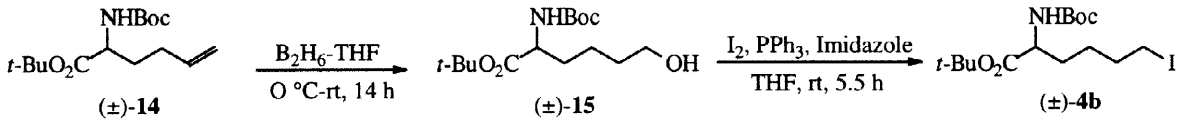


Synthesis of Racemic Deoxypyridinoline [(±)-2]

Before attempting the chiral synthesis of cross-links (**1,2**), we first decided to prepare racemic deoxypyridinoline [(±)-**2**] from (±)-**3g** in order to optimize the reaction conditions as well as to evaluate its applications for assay development. Thus, the (±)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) required for quaternization of the pyridine nitrogen[19] in (±)-**3g** was prepared from (±)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hexenoate (**14**) in two steps as shown in Scheme 4. Accordingly, the olefin (±)-**14**,[20] which was prepared for the synthesis of epoxide (±)-**6g**, upon hydroboration-oxidation reaction afforded the corresponding hydroxy compound (±)-**15** in 45% yield. The hydroxy compound (±)-**15** was converted to the

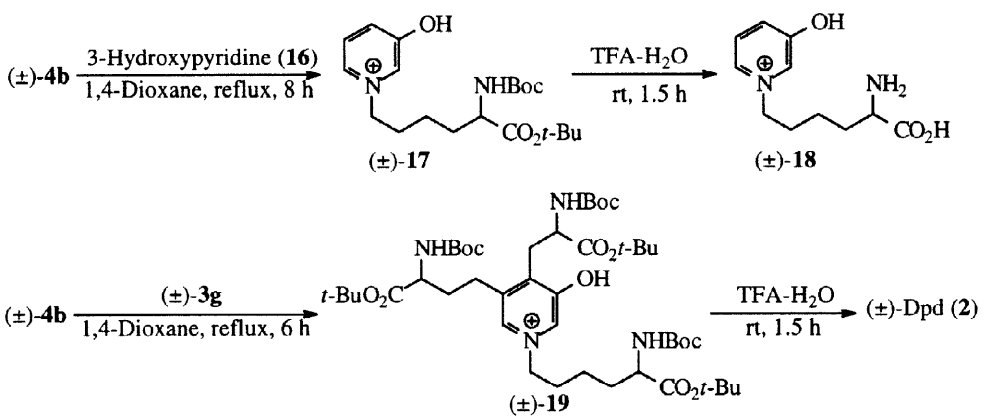
desired iodide (±)-**4b** by treatment with iodine and triphenylphosphine in THF[21] followed by silica gel column chromatography 52% yield.

Scheme 4



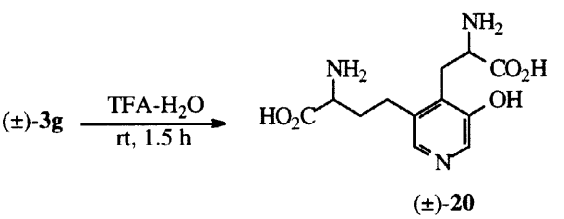
The iodo compound (±)-**4b** was first reacted (Scheme 5) with 3-hydroxypyridine (**16**) in order to study the quaternization reaction as well as to prepare a simple pyridinium analogue (±)-**18**. Thus, heating the mixture of iodide (±)-**4b** and 3-hydroxypyridine (**16**) in anhydrous 1,4-dioxane at 110 °C for 4 h and purification of the crude product by preparative HPLC afforded the pyridinium compound (±)-**17** in 40% yield. Hydrolysis of Boc and *tert*-butyl ester groups using trifluoroacetic acid-water at room temperature and purification of the crude product by preparative HPLC afforded the simplified analogue (±)-**18** in 81% yield as its trifluoroacetic acid salt. The analogue (±)-**18** was designed to evaluate the role of 4 and 5 substituents on the binding properties of Dpd (**2**) to the developed *anti*-Dpd antibodies. Similarly, the mixture of (±)-**3g** and (±)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) in anhydrous 1,4-dioxane was refluxed for 6 h to afford pyridinium compound (±)-**19** in 46% yield after preparative HPLC purification. Finally, hydrolysis

Scheme 5



of both Boc and *tert*-butyl ester groups using trifluoroacetic acid-water at room temperature followed by preparative RP HPLC purification afforded the racemic deoxypyridinoline [(±)-Dpd, **2**] as its TFA salt in 73% yield. We also prepared (Scheme 6) a pyridine analogue (±)-**20** which is lacking the lysine side chain at the

Scheme 6

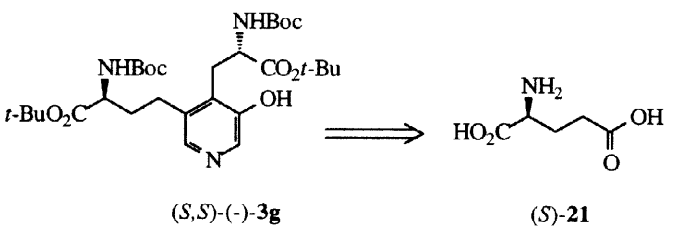


pyridine nitrogen. Thus hydrolysis of (±)-**3g** with trifluoroacetic acid-water gave the TFA salt of (±)-**20** in 73% yield. The analogue (±)-**20** is critical in defining the role of lysine chain in binding of Dpd (**2**) to the developed *anti*-Dpd antibodies, as well as in determining the specificity of *anti*-Dpd antibodies.

Chiral Synthesis of Collagen Cross-links (1,2)

Since Pyd (**1**) and Dpd (**2**) are formed from the natural lysine[1] and hydroxylysine[22] present in collagen, the stereochemistry of both Pyd (**1**) and Dpd (**2**) was therefore predicted to be (*S*) for all three α-amino acid stereocenters in both cross-links (**1,2**) and (*R*) for the lone secondary hydroxyl group in **1** [1-(2*R*)-OH]. However, the absolute stereochemistry of both Pyd (**1**) and Dpd (**2**) has not yet been independently established and therefore, the chiral synthesis was undertaken for cross-links (**1,2**) with the above proposed stereochemistry. Thus, based on the method developed for racemic Dpd [(±)-**2**, Figure 1, Scheme 1,5], the chiral synthesis of pyridinium cross-links (**1,2**) involves the construction (Figure 2, Scheme 7) of key chiral intermediate, (-)-*tert*-butyl-(2*S*)-4-(4-{(2*S*)-3-(*tert*-butoxy)-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-5-hydroxy-3-pyridinyl)-2-[(*tert*-butoxycarbonyl)amino]butanoate (**3g**). The 3-hydroxypyridine derivative, (*S,S*)-(-)-**3g** was envisioned from a chiral pool starting material *L*-glutamic acid (**21**).

Figure 2



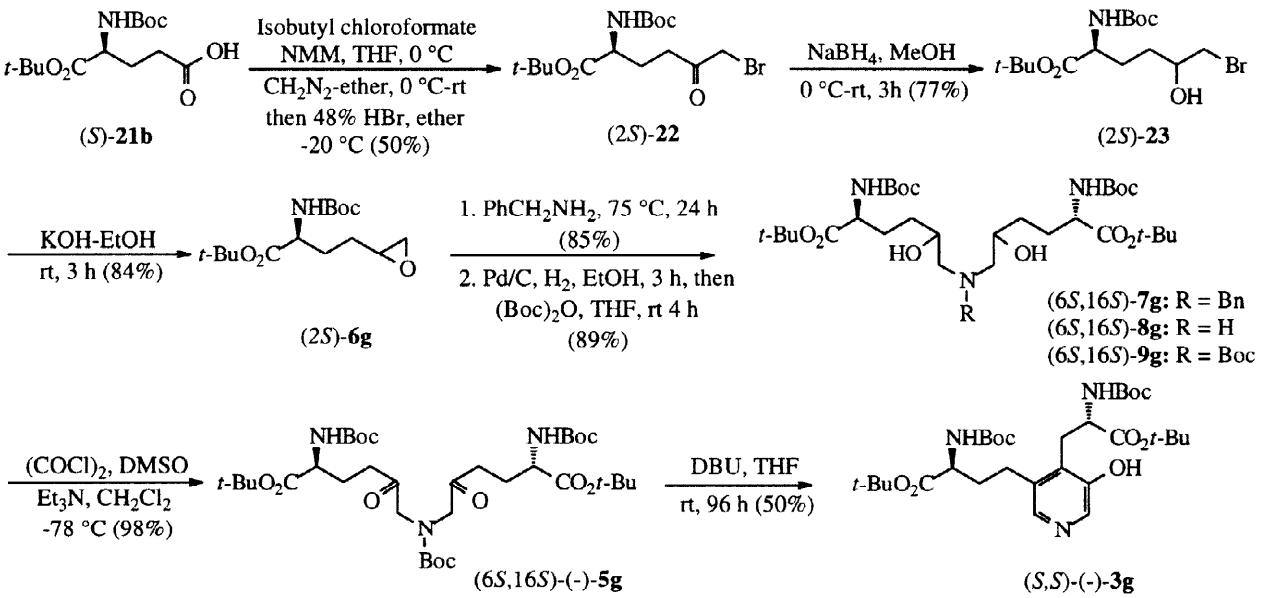
(*S,S*)-(-)-**3g** (*S*)-**21**

Chiral Synthesis of 3-Hydroxy-4,5-substituted pyridine derivative (*S,S*)-(-)-3g

Synthesis of the key synthon (*S,S*)-(-)-**3g** began from a commercially available glutamic acid (**21**) derivative, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**21b**), (Scheme 7). Thus, **21b** was treated with isobutyl chloroformate[23] in the presence of 4-methylmorpholine in THF and the resulting mixed anhydride was successively reacted with ethereal-diazomethane followed by 48% aq. HBr in ether at -20 °C. The (2*S*)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-bromo-5-oxohexanoate (**22**) was isolated by silica gel column chromatography in 50% yield. The keto functionality in (*S*)-**22** was reduced with $NaBH_4$ in methanol to afford the corresponding alcohol (2*S*)-**23** as a mixture of 1:1 diastereomers. The diastereomeric mixture of hydroxy-bromo compounds (2*S*)-**23** could not be separated either by silica gel column chromatography or HPLC under a variety of conditions. Since the hydroxyl group in (2*S*)-**23** will be eventually oxidized to the corresponding keto functionality in the synthesis of (*S,S*)-(-)-**3g**, the diastereomeric mixture (2*S*)-**23** was carried to the next step. Thus, treatment of the diastereomeric mixture of (2*S*)-**23** with ethanolic KOH at room temperature gave the desired epoxide (2*S*)-**6g** in 87% yield, a key synthon for the preparation of (*S,S*)-(-)-**3g**. The epoxide (2*S*)-**6g** was reacted with benzylamine as described for the preparation of racemic **3** (Scheme 2) to afford the *N*-benzyl compound (6*S*,16*S*)-**7g** in 85% yield. The *N*-benzyl group in (6*S*,16*S*)-**7g** was cleaved by hydrogenolysis and the crude amine (6*S*,16*S*)-**8g** was treated with di-*tert*-butyldicarbonate in dry THF to afford

(6*S*,16*S*)-**9g** in 89% yield. Swern oxidation of (6*S*,16*S*)-**9g** and purification by silica gel column chromatography afforded the aza-diketo compound (6*S*,16*S*)-(-)-**5g** in excellent yield (98%). Finally, the DBU promoted cyclization-aromatization reaction of (6*S*,16*S*)-(-)-**5g** in THF at room temperature followed by silica gel column chromatography afforded the chiral (*S*,*S*)-(-)-**3g** in 50% yield as a pale yellow crystalline product.

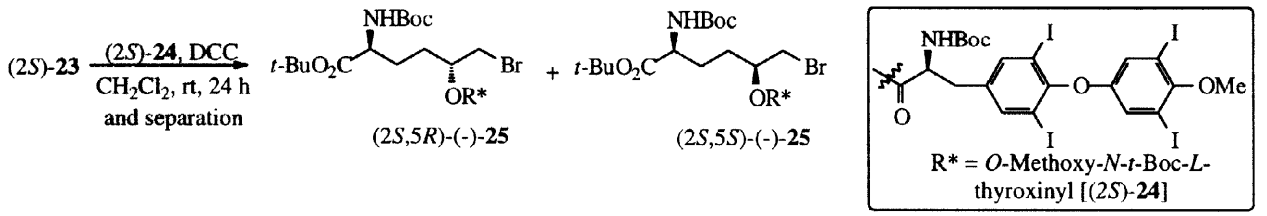
Scheme 7



Synthesis of (2*S*,5*R*)-(+)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (4a)

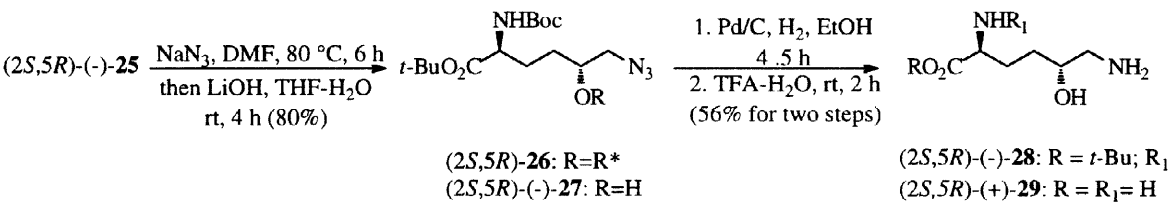
The next step in the synthesis of Pyd (**1**) was to prepare the iodide (2*S*,5*R*)-(+)-**4a** which is required for quaternization of (*S*,*S*-(-)-**3g**. The iodide (2*S*,5*R*)-(+)-**4a** and its diastereomer (2*S*,5*S*)-(+)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (**4a**) were prepared from the diastereomeric mixture of hydroxy compound (2*S*)-**23** via separation as shown in Scheme 8. Since our attempts to separate the hydroxy-bromo compound (2*S*)-**23**, which was obtained as 1:1 diastereomeric mixture (Scheme 7) failed, alternatively, we decided to derivatize the mixture (2*S*)-**23** for separation. Initially, the Mosher's acid [(*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid][24] seemed to be the obvious choice for derivetization of (2*S*)-**23**. Thus, the diastereomeric mixture of (2*S*)-**23** was esterified with (*R*)-(+)-Mosher's acid using DCC in CH_2Cl_2 to give the mixture of esters [**25**: R* = (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl] in 81% yield. Surprisingly, our efforts to separate the ester mixture [**25**: R* = (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl] either by crystallization or chromatography including HPLC (Waters μ Bondapak RCM C18 10μ (40 x 100 mm column) were unsuccessful. Therefore, we envisioned that a bulky hydrophobic chiral resolving agent was needed for separation of (2*S*)-**23**. Our experience with thyroxine[25] led us to explore the use of *O*-methoxy-*N*-(*tert*-butoxylcarbonyl)-*L*-thyroxine [(*S*)-**24**],[25b] for separation of (2*S*)-**23**. This chiral reagent (*S*)-**24**, which has already protected amino and phenolic groups as Boc and methyl ether, respectively, can be prepared easily and economically in gram quantities.[25b] Thus, (2*S*)-**23** was esterified with *O*-methoxy-*N*-(*tert*-butoxylcarbonyl)-*L*-thyroxine [(*S*)-**24**],[25b] using DCC in CH_2Cl_2 at room temperature. To our delight, the ester **25** was separated by

Scheme 8



preparative HPLC to give (-)-(2*S*,5*R*)-**25** (ee: >97%) and (-)-(2*S*,5*S*)-**25** (ee: >97%) in 28 and 34% yield respectively. Thus, to the best of our knowledge, this is the first report of the use of a thyroxine derivative [ex. (*S*)-**24**] for the separation of stereoisomers. The absolute stereochemistry of (2*S*,5*R*)-(-)-**25** was determined by correlation to the natural hydroxylysine (2*S*,5*R*)-(+)-**29** as shown in Scheme 9. Accordingly, (2*S*,5*R*)-(-)-**25** was treated with sodium azide in DMF and the resulting crude azide (2*S*,5*R*)-**26** (R=R* = *O*-methoxy-*N*-(*tert*-butoxycarbonyl-*L*-thyroxinyl) was hydrolyzed using lithium hydroxide in THF-water to give the hydroxy compound (2*S*,5*R*)-(-)-**27** in 80% yield. The azide functionality in (2*S*,5*R*)-(-)-**27** was reduced by hydrogenation using 10% Pd/C in ethanol and the resulting crude amine (2*S*,5*R*)-(-)-**28** was hydrolyzed using TFA-water to give hydroxylysine (2*S*,5*R*)-(+)-**29** in 56% yield. The properties of synthetic hydroxylysine (2*S*,5*R*)-(+)-**29** {$[\alpha]^{20}_D$ +14.9} were in agreement with natural hydroxylysine (2*S*,5*R*)-(+)-**29** {$[\alpha]^{20}_D$ +14.5} including spectra.[26]

Scheme 9

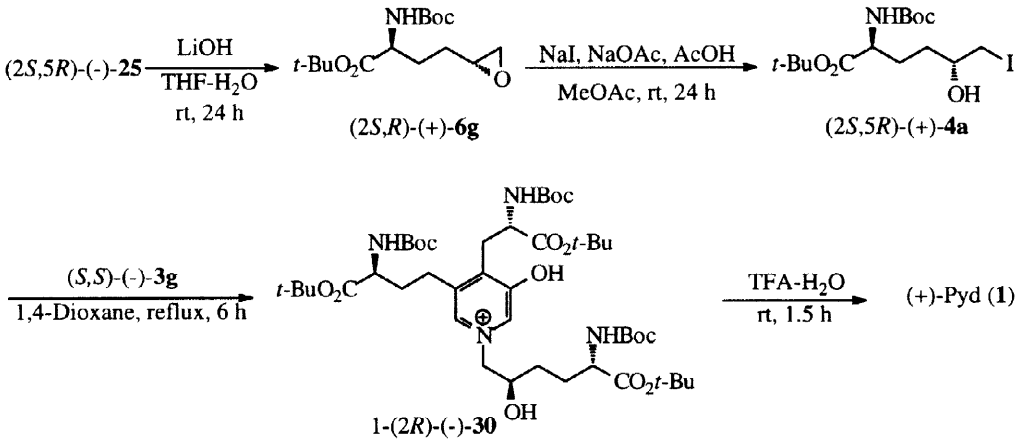


Synthesis of Natural (+)-Pyridinoline (1)

The ester (2*S*,5*R*)-(-)**25** (Scheme 10) upon treatment with LiOH in THF-H_2O at room temperature under went hydrolysis of the thyroxine ester and was subsequently converted to the epoxide (+)-*tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2*R*)-oxiranyl]butanoate (**6g**) in 69% yield. The epoxide (2*S*,*R*)-(+)-**6g** upon treatment with NaI in the presence of anhydrous sodium acetate and acetic acid in methyl acetate at room temperature,[27] afforded the desired iodo compound (2*S*,5*R*)-(+)-**4a,** as a sole product in excellent yield (94%). The quaternization of 3-hydroxy pyridine derivative (*S*,*S*)-(-)-**3g** with iodo compound (2*S*,5*R*)-(+)-**4a** (2.0 equiv.) was carried out by heating the mixture in anhydrous 1,4-dioxane for 6 h under nitrogen. The pyridinium product 1-(2*R*)-(-)-**30** was isolated by preparative HPLC followed by lyophilization in 42% yield. Hydrolysis of Boc and *tert*-butyl ester groups in 1-(2*R*)-(-)-**30** using TFA-water and purification of the crude product by preparative HPLC followed by lyophilization afforded the natural (+)-pyridinoline (**1**) in 83% yield as its TFA salt. The synthetic (+)-Pyd (**1**) {$[\alpha]^{20}_D$ +39.17 (c 0.24, MeOH)} was compared by analytical reverse phase HPLC to the natural Pyd (**1**) which was obtained by isolation from bone[12] and found to be identical. Thus, the

first total synthesis of natural (+)-pyridinoline (**1**) was achieved starting from a commercially available enantiopure chiral pool starting material, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**21b**).

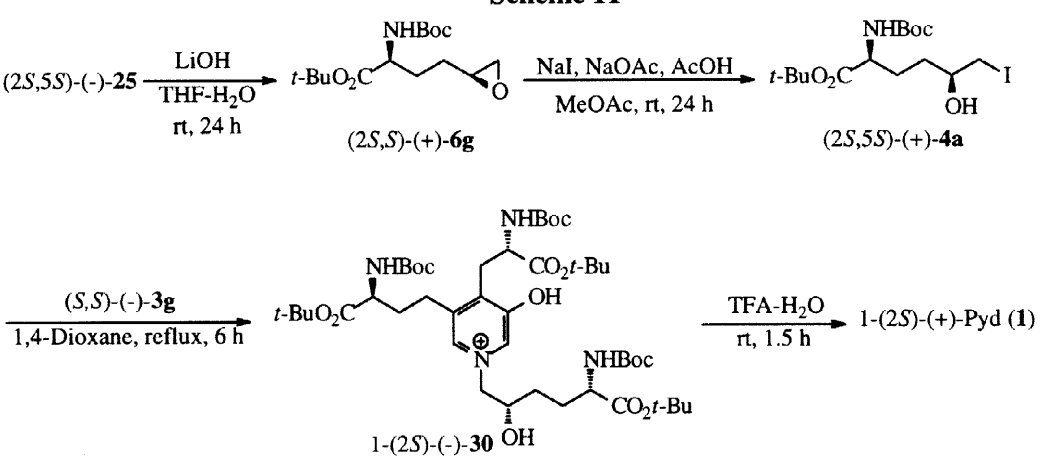
Scheme 10



Synthesis of 1-(2*S*)-(+)-Pyridinoline (1)

Similarly, treatment of isomeric ester (2*S*,5*S*)-(-)**25** (Scheme 11) with lithium hydroxide in THF-H_2O at room temperature, afforded (+)-*tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2*S*)-oxiranyl]butanoate (**6g**) in 81% yield. The epoxide (2*S*,*S*)-(+)-**6g** upon treatment with sodium iodide in methyl acetate gave the iodo compound (2*S*,5*S*)-(+)-**4a** in 93% yield. The reaction of iodo compound (2*S*,5*S*)-(+)-**4a** (2.0 equiv.) with (*S*,*S*)-(-)-**3g** was carried in anhydrous 1,4-dioxane for 6 h, which afforded 1-(2*S*)-(-)-**30** in 43% yield after preparative reverse phase HPLC purification and lyophilization. The hydrolysis of Boc and *tert*-butyl ester groups in 1-(2*S*)-(-)-**30** using trifluoroacetic acid-water and purification of the crude product by preparative reverse phase HPLC afforded the 1-(2*S*)-(+)-pyridinoline (**1**) in 95% yield as its TFA salt {$[\alpha]^{20}_D$ +43.23 (c 0.26, MeOH)} with an opposite stereochemistry for the hydroxyl group.

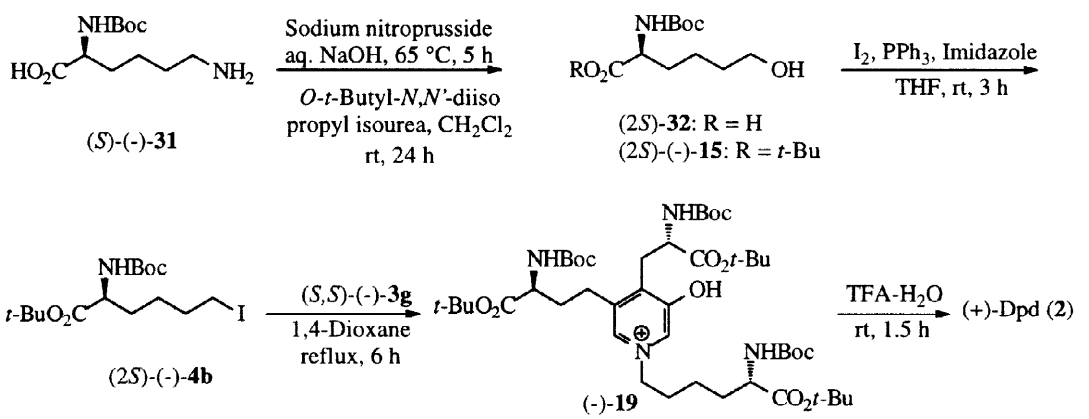
Scheme 11



Synthesis of Natural (+)-Deoxypyridinoline (2)

The iodide (2*S*)-**4b** required for chiral synthesis of deoxypyridinoline.(**2**) was prepared (Scheme 12) from a commercially available *L*-lysine derivative (**31**) in three steps. Thus, (2*S*)-6-amino-2-[(*tert*-butoxy carbonyl)amino]hexanoic acid (**31**) upon heating with sodium nitroprusside[28] in aqueous NaOH for 6 h at pH. 9.5 afforded the corresponding hydroxy compound (*S*)-**32**. Treatment of the crude (*S*)-**32** with *O*-*tert*-butyl-*N*,*N*'-diisopropylisourea in CH_2Cl_2[29] and purification by silica gel column chromatography afforded the corresponding *tert*-butyl ester (2*S*)-(-)-**15** in 45% yield for two steps. The chiral ester (*S*)-(-)-**15** was converted to the iodo compound (2*S*)-(-)-**4b** by treatment with iodine and triphenylphosphine in THF in 61% yield by following the procedure developed for racemic (±)-**4b** (Scheme 3). Thus the present synthesis of iodide (2*S*)-(-)-**4b** is convenient and efficient in comparison to the eleven step synthesis of (2*S*)-**4b** utilizing (2*S*)-2,5-dihydro-3,6-diethoxy-2-isopropylpyrazine with an undefined stereoselectivity reported previously by Waelchli *et al.*[14] The iodo compound (2*S*)-(-)-**4b** (2.0 equiv.) was reacted with (*S*,*S*)-(-)-**3g** in anhydrous 1,4-dioxane at reflux for 6 h to give the chiral pyridinium compound (-)-**19** in 40% yield after purification by preparative HPLC. Finally, hydrolysis of Boc and *tert*-butyl ester groups in (-)-**19** using trifluoroacetic acid-water at room temperature and purification by preparative HPLC afforded the (+)-Dpd (**2**) in 80% yield as its TFA salt. The synthetic (+)-Dyd (**2**) {$[\alpha]^{20}_D$ +31.6 (c 0.25, MeOH)} was compared by analytical HPLC to the natural Dpd (**2**) which was isolated from bone[12] and found to be identical.

Scheme 12



In summary, a variety of 3-hydroxy-3,4-substituted pyridines (**3a-g**) were prepared via a base (NaH or DBU) promoted cyclization-aromatization reaction of aza-diketones (**5a-g**) in up to 62% yield. The synthesis of racemic deoxypyridinoline [Dpd, (±)-**2**] and the preparation of its analogues (**18**, **20**) was also presented. The first synthesis of natural (+)-pyridinoline (Pyd, **1**) was achieved from a chiral pool starting material, (*S*)-**21b**. In the process of the preparation of iodide (2*S*,4*R*)-(+)-**4a**, a useful novel resolving agent, *O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine (**24**), was introduced. The synthesis of 1-(2*S*)-(+)-Pyd (**1**) was also achieved by utilizing the isomeric iodide (2*S*,4*S*)-(+)-**4a**. An efficient three step synthesis was developed for the iodide (2*S*)-(-)-**4b** from a commercially available (2*S*)-(-)-6-amino-2-[(*tert*-butoxycarbonyl)amino]hexanoic acid (**31**) and the natural (+)-deoxypyridinoline [Dpd, (+)-**2**] was synthesized in 5.6 % overall yield. The synthetic (+)-Pyd (**1**), (+)-Dpd (**2**) as well as the (±)-Dpd (**2**) together with various analogues (**3a-g**, **18**, **20**) presented here are important for development of assays for osteoporosis and to study bone metabolism.

Experimental

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 in ppm relative to TMS and coupling constants (*J*) were reported in Hz. IR spectra were recorded on Perkin-Elmer 1600 spectrometer using sodium chloride plates for liquids and potassium bromide disks for solids. Electrospray ionization mass spectrometry (ESI/MS) were carried on a Perkin-Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing Turbo Ionspray ion source and HRMS were obtained on a Nermang 3010 MS-50, JEOL SX102-A mass spectrometers. 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 $KMnO_4$ solution [$KMnO_4$ (1.0 g), NaOH (8.0 g) in water (200 mL)] or phosphomolybdic acid reagent (20 wt% solution 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. 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 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 x 100 mm) column (solvents ratio v/v reported) unless otherwise stated. Preparative reverse phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (40 x 100 mm) column (solvents ratio v/v reported) unless otherwise stated. Optical rotations were measured on Autopol III polarimeter from Rudolph Research, Flanders, NJ. Melting points were recorded in open capillary tubes on a Electrothermal Melting Point Apparatus and were uncorrected. The epoxides (**6b-g**) were prepared as follows: **6b** from 5-bromo-1-pentene [1. PhLi, HMPA, THF, -78 °C, 1.5 h (45%); 2. *m*-CPBA, CH_2Cl_2, rt 16 h (57%)]; **6c** from 5-hexene-1-ol [1. DHP, CH_2Cl_2, rt, 2 h (93%); 2. *m*-CPBA, CH_2Cl_2, rt 17 h (50%)]; **6d** from 9-decene-1-ol [1. Ms-Cl, CH_2Cl_2, 0 °C-rt, 4 h then KCN, DMSO, 100 °C, 2 h (93%); 2. *m*-CPBA, CH_2Cl_2, rt 16 h (71%)]; **6e** from 6-[(*tert*-butoxycarbonyl)amino]-hexanoic acid [1. $Cu(OAc)_2$ H_2O, $Pb(OAc)_4$, benzene, reflux,[30] 6.5 h (32%); 2. *m*-CPBA, CH_2Cl_2, rt 16 h (96%)]; **6f** from *tert*-butyl acetate [1. *n*-BuLi, HMPA, 4-bromo-1-butene, THF, -78 °C to -20 °C, 5 h (49%); 2. *m*-CPBA, CH_2Cl_2, rt 17 h (53%)]; and (±)-**6g** from *N*-[(*tert*-butoxycarbonyl)amino]glycine-*tert*-butyl ester [1. *n*-BuLi, HMPA, 4-bromo-1-butene, THF, -78 °C to -20 °C, 7.5 h (86%); 2. *m*-CPBA, CH_2Cl_2, rt 17 h (91%)]. The *O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine (**24**) was prepared from *L*-thyroxine sodium salt according to our previously described procedure[25b] on a 5.0 g scale.

General procedure for Preparation of *N*-benzylaza compounds (7), ex., 1-[Benzyl(2-hydroxy pentyl)amino]-2-pentanol (7a): Benzylamine (2.72 mL, 25.0 mmol, 0.5 equiv.) was added to (±)-2-(*n*-propyl)oxirane (4.3 g, 50 mmol) under nitrogen and the reaction mixture was heated at 75 -100°C for 20 h. The mixture was cooled to room temperature and purified by silica gel column chromatography (30%-40% EtOAc in hexanes) to afford 3.57 g of 1-[benzyl(2-hydroxy pentyl)amino]-2-pentanol (**7a**) in 51% yield. R_f: 0.38 (40% EtOAc in hexane); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/35:65, 2.0 mL/min at 225 nm; R_t: 5.14 min, 96%; ^{1}H NMR ($CDCl_3$): δ 7.36 – 7.26 (m, 5 H), 3.91 –3.45 (m, 4 H), 2.64 – 2.43 (m, 4 H), 1.48 – 1.24 (m, 8 H), 0.92 – 0.86 (m, 6 H); ESI/MS (m/z): 280 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{17}H_{30}NO_2$, 280.2277 $(M + H)^+$; observed, 280.2281.

1-[Benzyl(2-hydroxy-5-phenylpentyl)amino]-5-phenyl-2-pentanol (7b): Yield: 58%; Rf: 0.22 (35% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.34 – 7.14 (m, 15 H), 3.88 – 3.42 (m, 4 H), 2.62 – 2.42 (m, 8 H), 1.84 – 1.56 (m, 4 H), 1.43 –1.34 (m, 4 H); ESI/MS (m/z): 432 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{30}H_{38}NO_2$, 432.3003 $(M + H)^+$; observed, 432.3021.

1-{Benzyl[2-hydroxy-6-(tetrahydro-2H-pyran-2-yloxy)hexyl]amino}-6-(tetrahydro-2H-pyran-2-yloxy)-2-hexanol (7c): Yield: 89%; Rf: 0.32 (70% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.33 – 7.25 (m, 5 H), 4.60 (br. s, 2 H), 3.95 – 3.32 (m, 12 H), 2.65 – 2.43 (m, 4 H), 1.88 –1.32 (m, 24 H); ESI/MS (m/z): 508 $(M + H)^+$, 530 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{30}H_{50}NO_6$, 508.3638 $(M + H)^+$; observed, 508.3637.

11-[benzyl(10-cyano-2-hydroxydecyl)amino]-10-hydroxyundecanenitrile (7d): Yield: 84%; Rf: 0.46 (50% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.36 (m, 5 H), 3.91 – 3.45 (m, 4 H), 2.64 – 2.43 (m, 4 H), 2.33 (t, 4 H, J = 6.9 Hz), 1.69 – 1.59 (m, 4 H), 1.45 – 1.23 (m, 24 H); ESI/MS (m/z): 470 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{30}H_{48}N_3O_2$, $(M + H)^+$, 470.3747, observed, 470.3766.

***tert*-Butyl-5-(benzyl{5-[(*tert*-butoxycarbonyl)amino]-2-hydroxypentyl}amino)-4-hydroxypentylcarbamate (7e):** Yield: 52%; Rf: 0.22 (80% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.36 – 7.26 (m, 5 H), 4.63 (br. s, 2 H), 3.69 – 3.45 (m, 4 H), 3.16 – 3.04 (m, 4 H), 2.62 – 2.44 (m, 4 H), 1.64 – 1.22 (m, 8 H), 1.42 (s, 18 H); ESI/MS (m/z): 510 ($M + H)^+$, 532 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{48}N_3O_6$, 510.3543 $(M + H)^+$; observed, 510.3541.

1,1-Dihydroxyethyl-6-{benzyl[6-(*tert*-butoxy)-2-hydroxy-6-oxohexyl]amino}-5-hydroxyhexanoate (7f): Yield: 89%; Rf: 0.39 (50% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.35 – 7.25 (m, 5 H), 3.89 – 3.45 (m, 4 H), 2.64 – 2.43 (m, 4 H), 2.27 – 2.17 (m, 4 H), 1.78 – 1.56 (m, 4 H), 1.43 (s, 18 H), 1.40 – 1.30 (m, 4 H); ESI/MS (m/z): 480 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{46}NO_6$, 480.3325 $(M + H)^+$; observed, 480.3328.

(±)-Di(*tert*-butyl)-11-benzyl-9,13-dihydroxy-2,2,20,20-tetramethyl-4,18-dioxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate (7g): Yield: 83%; Rf: 0.20 (50% EtOAc in hexanes); Analytical HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 7.87 min, 99%; ^{1}H NMR ($CDCl_3$): δ 7.35 – 7.25 (m, 5 H), 5.18 – 5.02 (m, 2 H), 4.22 – 4.12 (m, 2 H), 3.89 – 3.46 (m, 4 H), 2.65 – 2.42 (m, 4 H), 1.98 – 1.38 (m, 8 H), 1.44 (s, 18 H), 1.43 (s, 18 H); ESI/MS (m/z): 710 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{37}H_{64}N_3O_{10}$: 710.4592 $(M + H)^+$; observed, 710.4604.

General procedure for the preparation of (*tert*-butoxycarbonyl)aza compounds (9), ex., *tert*-Butyl-bis(2-hydroxypentyl)carbamate (9a): To a solution of **7a** (3.5 g, 12.5 mmol) in ethanol (35 mL), 10% Pd/C (0.35 g, 10% w/w) was added and hydrogenated at 15-35 psi for 5 h. The mixture was filtered through celite powder, washed with ethanol (10 mL) and the filtrate was concentrated on a rotary evaporator. The crude amine (**8a**, 2.24 g) was dissolved in THF (27 mL) and di-*tert*-butyldicarbonate [$(Boc)_2O$, 5.45 g, 25.0 mmol, 2.0 equiv.] was added at room temperature under nitrogen. After stirring the mixture for 4 h, the solvent was removed on a rotary evaporator and the crude product was dissolved in EtOAc (75 mL) and water (30 mL). The aqueous layer was separated and extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$) and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 3.42 g of **9a** in 94% yield for

two steps as a colorless thick oil. Rf: 0.50 (45% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 4.02 – 2.80 (m, 8 H), 1.50 – 1.33 (m, 8 H), 1.45 (s, 9 H), 0.93 (t, 6 H, J = 6.3 Hz); ESI/MS (m/z): 290 $(M + H)^+$, 579 $(2 \times M + H)^+$.

***tert*-Butyl-bis(2-hydroxy-5-phenylpentyl)carbamate (9b):** Yield: 78%; Rf: 0.38 (60% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 7.30 – 7.15 (m, 10 H), 4.02 – 2.80 (m, 8 H), 2.68 – 2.59 (m, 4 H), 1.86 – 1.58 (m, 8 H), 1.44 (s, 9 H); ESI/MS (m/z): 442 $(M + H)^+$, 464 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{40}NO_4$, 442.3057 $(M + H)^+$; observed, 442.3060.

***tert*-Butyl-bis[2-hydroxy-6-(tetrahydro-2H-pyran-2-yloxy)hexyl]carbamate (9c):** Yield: 73%; Rf: 0.34 (70% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 4.56 (br s, 2 H), 3.95 – 2.95 (m, 14 H), 1.90 – 1.40 (m, 24 H), 1.45 (s, 9 H); ESI/MS (m/z): 518 $(M + H)^+$, 535 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{52}NO_8$, 518.3693 $(M + H)^+$; observed, 518.3698.

***tert*-Butyl-bis(10-cyano-2-hydroxydecyl)carbamate (9d):** Yield: 42%; Rf: 0.22 (40% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 3.95 – 2.95 (m, 8 H), 2.33 (t, 4 H, J = 7.2 Hz), 1.70 –1.60 (m, 4 H), 1.46 (s, 9 H), 1.44 – 1.26 (m, 24 H); ESI/MS (m/z): 480 $(M + H)^+$, 497 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{50}N_3O_4$, 480.3801 $(M + H)^+$; observed, 480.3805.

***tert*-Butyl-bis{5-[(*tert*-butoxycarbonyl)amino]-2-hydroxypentyl}carbamate (9e):** Yield: 35%; Rf: 0.18 (60% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 4.70 (br. s, 2 H), 4.21 – 3.80 (m, 4 H), 3.70 – 2.80 (m, 8 H), 1.80 – 1.25 (m, 8 H), 1.44 (s, 9 H), 1.43 (s, 18 H); ESI/MS (m/z): 520 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{25}H_{50}N_3O_8$, 520.3598 $(M + H)^+$; observed, 520.3601.

1,1-Dihydroxyethyl-6-{(*tert*-butoxycarbonyl)[6-(*tert*-butoxy)-2-hydroxy-6-oxohexyl]amino}-5-hydroxyhexanoate (9f): Yield: 96%; Rf: 0.33 (40% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 4.04 – 2.80 (m, 8 H), 2.30 – 2.22 (m, 4 H), 1.90 – 1.65 (m, 8 H) 1.45 (s, 9 H), 1.44 (s, 18 H); ESI/MS (m/z): 490 $(M + H)^+$, 512 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{25}H_{48}NO_8$, 490.3380 $(M + H)^+$; observed, 490.3374.

(±)-Di(*tert*-butyl)-11-(*tert*-butoxycarbonyl)-9,13-dihydroxy-2,2,20,20-tetramethyl-4,18-dioxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate (9g): Yield: 86%; Rf: 0.32 (50% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 17.07 min, 95%; ^{1}H NMR ($CDCl_3$): δ 5.22 – 5.10 (m, 2 H), 4.28 – 4.10 (m, 2 H), 4.08 – 2.80 (m, 8 H), 2.00 1.60 (m, 4 H), 1.58 – 1.36 (m, 4 H), 1.45 (s, 27 H), 1.43 (s, 18 H); ESI/MS (m/z): 720 $(M+ H)^+$; HRMS (FAB, m/z): calcd for $C_{37}H_{66}N_3O_{12}$: 720.4647 $(M + H)^+$; observed, 720.4622.

General procedure for the preparation of aza-diketo compounds (5), ex., *tert*-Butyl-bis(2-oxopentyl)carbamate (5a): Dimethylsulfoxide (4.19 mL, 59.17 mmol, 5.0 equiv.) in CH_2Cl_2 (30 mL) was added slowly via double ended needle to a cooled solution of oxalyl chloride (2.0 M soln in CH_2Cl_2, 14.7 mL, 30.57 mmol, 2.5 equiv.) in CH_2Cl_2 (30 mL) at -78 °C under nitrogen. After stirring the mixture for 30 min, a solution of **9a** (3.42 g, 11.83 mmol) in CH_2Cl_2 (40 mL) was added via double ended needle at -78 °C. The mixture was stirred for 3.5 h and allowed to warm to -60 °C slowly. Et_3N (15.0 mL, 118.0 mmol, 10.0 equiv.) was added at -60 °C and stirred for 15 min. The reaction was quenched with water (25 mL), the aqueous layer was separated and extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were washed with brine (25 mL), dried ($MgSO_4$) and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (20-40% EtOAc in hexanes) to afford 2.94 g of **5a** in 87% yield. Rf: 0.26 (20% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/ 60:40; 2.0 mL/min at

225 nm, Rt: 4.71 min, 97.1%; ^{1}H NMR ($CDCl_3$): δ 4.11 (s, 2 H), 3.99 (s, 2 H), 2.36 (q, 4 H, J = 15.6 Hz), 1.65 – 1.57 (m, 4 H), 1.41 (s, 9 H), 0.92 (t, 3 H, J = 7.2 Hz), 0.91 (t, 3 H, J = 7.2 Hz); ESI/MS (m/z): 286 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{15}H_{28}NO_4$, 286.2018 $(M + H)^+$; observed, 286.2002.

***tert*-Butyl-bis(2-oxo-5-phenylpentyl)carbamate (5b):** Yield: 98%; Rf: 0.41 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 7.30 – 7.15 (m, 10 H), 4.03 (s, 2 H), 3.91 (s, 2 H), 2.66 – 2.59 (m, 4 H), 2.37 (q, 4 H, J = 15.9 Hz), 1.97 – 1.87 (m, 4 H), 1.38 (s, 9 H); ESI/MS (m/z): 438 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{36}NO_4$, 438.2644 $(M + H)^+$; observed, 438.2659.

***tert*-Butyl-bis[2-oxo-6-(tetrahydro-2H-pyran-2-yloxy)hexyl]carbamate (5c):** Yield: 93%; Rf: 0.63 (60% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 4.54 (br. s, 2 H), 4.10 (s, 2 H), 3.99 (s, 2 H), 3.88 – 3.79 (m, 2 H), 3.78 – 3.69 (m, 2 H), 3.65 – 3.45 (m, 2 H), 3.42 –3.33 (m, 2 H), 2.47 – 2.38 (m, 4 H), 1.86 – 1.46 (m, 20 H), 1.40 (s, 9 H); ESI/MS (m/z): 531 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{48}NO_8$, 514.3380 $(M + H)^+$; observed, 514.3391.

***tert*-Butyl-bis(10-cyano-2-oxodecyl)carbamate (5d):** Yield: 63%; Rf: 0.3 (40% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 4.09 (s, 2 H), 3.99 (s, 2 H), 2.42 – 2.30 (m, 8 H), 1.66 – 1.44 (m, 8 H), 1.41 (s, 9 H), 1.44 – 1.30 (m, 16 H); ESI/MS (m/z): 476 $(M + H)^+$, 493 $(M + NH_4)^+$; HRMS (FAB, m/z) calcd for $C_{27}H_{46}N_3O_4$, 476.3488 $(M + H)^+$; observed, 476.3483.

***tert*-Butyl-bis{5-[(*tert*-butoxycarbonyl)amino]-2-oxopentyl}carbamate (5e):** Yield: 89%; Rf: 0.40 (60% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$): δ 4.65 (br. s, 2 H), 4.10 (s, 2 H), 4.02 (s, 2 H), 3.18 – 3.06 (m, 4 H), 2.44 (q, 4 H, J = 14.4 Hz), 1.82 – 1.72 (m, 4 H), 1.42 (s, 9 H), 1.41 (s, 18 H); ESI/MS (m/z): 538 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{25}H_{46}N_3O_8$, 516.3285 $(M + H)^+$; observed, 516.3303.

1,1-Dihydroxyethyl-6-{(*tert*-butoxycarbonyl)[6-(*tert*-butoxy)-2,6-dioxohexyl]amino}-5-oxohexano ate (5f): Yield: 95%; Rf: 0.18 (25% EtOAc in hexane); ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 2 H), 3.99 (s, 2 H), 2.44 (q, 4 H, J = 15.3 Hz), 2.27 – 2.21 (m, 4 H), 1.89 – 1.80 (m, 4 H), 1.43 (s, 9 H), 1.42 (s, 9 H), 1.41 (s, 9 H); ESI/MS (m/z): 486 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{25}H_{44}NO_8$, 486.3067 $(M + H)^+$; observed, 486.3076.

(±)-Di(*tert*-butyl)-11-(*tert*-butoxycarbonyl)-2,2,20,20-tetramethyl-4,9,13,18-tetraoxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate hydrate (5g): Yield: 89%; Rf: 0.53 (40% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/50:50, 2.0 mL/min at 225 nm, R_t: 6.44 min, 97.6%; ^{1}H NMR ($CDCl_3$): δ 5.12 – 5.02 (m, 2 H), 4.32 – 3.90 (m, 6 H), 2.60 – 2.32 (m, 4 H), 2.22 – 2.08 (m, 2 H), 1.84 – 1.68 (m, 2 H), 1.46 (s, 9 H), 1.45 (s, 9 H), 1.42 (s, 9 H, 1.41 (s, 9 H), 1.40 (s, 9 H); ESI/MS (m/z): 733 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{35}H_{62}N_3O_{12}$, 716.4333 $(M + H)^+$; observed, 716.4344.

General procedure for the preparation of 3-hydroxy-4,5-substituted pyridines using NaH (3a-e): In an oven dried round bottom flask, NaH (50%, 0.058 g, 1.36 mmol, 4.0 equiv.) was washed with hexane (3 x 1.0 mL) and dry THF (2.0 mL) was added under nitrogen. The suspension was cooled in an ice bath and a solution of aza-diketones (**5a-e**, 0.34 mmol) in THF (1.0 mL) was added. After 30 min, the cooling bath was removed and the reaction mixture was stirred at room temperature for an additional 24 h. The mixture was then cooled with an ice bath and quenched with methanol (5.0 mL). The solvent was removed on a rotary evaporator and the residue was dissolved in EtOAc (50 mL) and water (10 mL). The aqueous layer was separated and extracted with EtOAc (25 mL). The combined organic layers were washed with brine (10 mL) and dried

($MgSO_4$). The solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to afford 3-hydroxy-4,5-substituted pyridines (**3a-e**) in 36-49% yield.

4-Ethyl-5-propyl-3-pyridinol (3a): Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/30:70, 1.0 mL/min at 225 nm, R_t, 8.05 min, >99%; ^1H NMR ($CDCl_3$): δ 8.15 (s, 1 H), 7.88 (s, 1 H), 2.72 (q, 2 H, J = 7.2 Hz), 2.59 (t, 2 H, J = 7.8 Hz), 1.68 – 1.56 (m, 2 H), 1.19 (t, 3 H, J = 7.5 Hz), 0.97 (t, 3 H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 153.9, 140.5, 139.5, 137.6, 132.9, 32.0, 24.2, 19.1, 14.0, 13.2; ESI/MS (m/z): 166 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{10}H_{15}NO$, 166.1232 $(M + H)^+$; observed, 166.1235.

4-Phenethyl-5-(3-phenylpropyl)-3-pyridinol (3b): Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/60:40, 1.0 mL/min at 225 nm, R_t, 7.61 min, 94%; ^1H NMR ($CDCl_3$): δ 8.22 (s, 1 H), 7.90 (s, 1 H), 7.34 – 7.33 (m, 10 H), 3.00 – 2.82 (m, 4 H), 2.69 (t, 2 H, J = 7.5 Hz), 2.53 (t, 2 H, J = 7.5 Hz), 1.95 – 1.84 (m, 2 H); ^{13}C NMR ($CDCl_3$): δ 154.2, 141.9, 141.7, 139.2, 138.2, 137.9, 132.9, 128.6, 128.5, 128.4, 126.1, 126.0, 35.7, 34.7, 32.4, 30.4, 28.3; ESI/MS (m/z): 318 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{22}H_{33}NO$, 318.1858 $(M + H)^+$; observed, 318.1852.

5-[4-(Tetrahydro-2H-pyran-2-yloxy)butyl]-4-[3-(tetrahydro-2H-pyran-2-yloxy)propyl]-3-pyridinol (3c): Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 1.0 mL/min at 225 nm, R_t, 10.5 min, >99%; ^1H NMR ($CDCl_3$): δ 8.34 (s, 1 H), 8.33 (s, 1 H), 4.65 – 4.62 (m, 1 H), 4.58 – 4.56 (m, 1 H), 3.95 – 3.74 (m, 4 H), 3.56 – 3.38 (m, 4 H), 2.81 (t, 2 H, J = 7.2 Hz), 2.70 – 2.64 (m, 2 H), 1.95 – 1.48 (m, 18 H); ^{13}C NMR ($CDCl_3$): δ 153.5, 140.0, 137.6, 133.8, 98.9, 67.1, 66.8, 62.4, 62.3, 30.6, 30.5, 30.0, 30.8, 28.7, 27.8, 25.3, 25.2, 22.2, 19.5; ESI/MS (m/z): 394 $(M + H)^+$; HRMS (FAB): calcd for $C_{22}H_{36}NO_5$, 394.2593 $(M + H)^+$; observed, 394.2587.

9-[4-(7-Cyanoheptyl)-hydroxy-3-pyridinyl]nonanenitrile (3d): Analytical RP HPLC: MeCN:0.05% aqueous trifluoroacetic acid/50:50, 1.0 mL/min at 225 nm, R_t, 9.85 min, 98%; ^1H NMR ($CDCl_3$): δ 8.13 (s, 1 H), 7.89 (s, 1 H), 2.68 (t, 2 H, J = 7.2 Hz), 2.59 (t, 2 H, J = 7.5 Hz), 2.34 (t, 2 H, J = 7.2 Hz), 2.33 (t, 2 H, J = 6.9 Hz), 1.70 – 1.25 (m, 22 H); ^{13}C NMR ($CDCl_3$): δ 153.8, 139.3, 138.9, 137.9, 132.7, 119.9, 31.0, 30.0, 30.6, 30.3, 30.0, 28.7, 28.6, 28.5, 28.4, 25.8, 25.2, 17.0; ESI/MS (m/z): 356 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{22}H_{34}N_3O$, 356.2702 $(M + H)^+$; observed, 356.2703.

***tert*-Butyl-3-(4-{2-[(*tert*-butoxycarbonyl)amino]ethyl}-5-hydroxy-3-pyridinyl)propylcarbamate (3e):** Analytical RP HPLC: MeCN:0.05% aqueous trifluoroacetic acid/50:50, 1.0 mL/min at 225 nm, R_t, 5.61 min, >99; ^1H NMR ($CDCl_3$): δ 8.11 (s, 1 H), 7.88 (s, 1 H), 5.10 (br s, 2 H), 3.39 – 3.30 (m, 2 H), 3.25 – 3.15 (m, 2 H), 2.91 (t, 2 H, J = 6.6 Hz), 2.67 (t, 2 H, J = 6.3 Hz), 1.84 – 1.72 (m 2 H), 1.43 (s, 9 H), 1.42 (s, 9 H); ESI/MS (m/z): 397 $(M+ H)^+$; HRMS (FAB, m/z): calcd for $C_{20}H_{34}N_3O_5$, 396.2498; observed, 396.2502

General procedure for the preparation of 3-hydroxy-4,5-substituted pyridines using DBU (3f-g): ex., *tert*-Butyl-4-{4-[3-(*tert*-butoxy)-3-oxopropyl]-5-hydroxy-3-pyridinyl}butanoate (3f): 1,8-Diazabicyclo [5.4. 0]-undec-7-ene (DBU, 11.6 mL, 78.34 mmol, 6.4 equiv.) was added to a solution of **5f** (5.94 g, 12.24 mmol) in THF (120 mL) at room temperature under nitrogen. The pale yellow reaction mixture was stirred for 3.5 days, an additional 6 h in an open air atmosphere. The resulting dark pink reaction mixture was quenched with water (25) and the solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (100 mL) and water (30 mL). The aqueous layer was separated and extracted with EtOAc (2 x 50 mL) and the

combined organic layers were washed with brine (30 mL) and dried ($MgSO_4$). The solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (5% MeOH in EtOAc) to afford 2.45 g of **3f** in 55% yield. The faster eluting compounds (non-polar fractions) from column chromatography were concentrated to give 1.85 g of material which was dissolved in THF (25 mL). To this mixture, DBU (3.19 mL) was added at room temperature and the above procedure was followed to give an additional 0.325 g of **3f** in 7% yield (total yield 62%). R_f: 0.23 (90% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/35:65, 2.0 mL/min at 225 nm, R_t: 3.96 min, 97.8%; ^1H NMR ($CDCl_3$): δ 8.14 (s, 1 H), 8.11 (s, 1 H), 2.93 (t, 2 H, J = 6.6 Hz), 2.6 – 2.58 (m, 4 H), 2.30 (t, 2 H, J = 7.5 Hz), 1.90 – 1.80 (m, 2 H), 1.45 (s, 9 H), 1.43 (s, 9 H); ESI/MS (m/z): 366 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{20}H_{32}NO_5$, 366.2280 $(M + H)^+$; observed, 366.2289.

(±)-*tert*-Butyl-4-(4-{3-(*tert*-butoxy)-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-5-hydroxy-3-pyridinyl)-2-[(*tert*-butoxycarbonyl)amino]butanoate (3g): Yield: 58%; R_f: 0.19 (EtOAc); mp: 86-89 °C; Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 6.45 min, 98.6%; ^1H NMR ($CDCl_3$): δ 8.13 (s, 1 H), 7.92 (d, 1 H, J = 3.0 Hz), 5.65 (br. s, 1 H), 5.40 – 5.40 (m, 1 H), 4.44 – 4.18 (m, 2 H), 3.15 – 3.02 (m, 2 H), 2.75 – 2.61 (m, 2 H), 1.91 – 1.78 (m, 2 H), 1.48 (s, 9 H), 1.45 (s, 9 H), 1.40 (s, 9 H), 1.37 (s, 9 H); ESI/MS (m/z): 596 $(M + H)^+$; HRMS (FAB, m/z: calcd for $C_{30}H_{50}N_3O_9$, 596.3547 $(M + H)^+$; observed, 596.3542.

(±)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (15): (±)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hexenoate [**14** (prepared in the synthesis of (±)-**6g**) 2.85 g, 10.0 mmol) was dissolved in THF (50 mL), cooled to 0 °C and a solution of borane-THF complex (1 M soln in THF, 12.5 mL, 12.5 mmol, 1.25 equiv.) was added under nitrogen. The cooling bath was removed, the mixture allowed to warm to room temperature and stirred for 14 h. The reaction was cooled to 0 °C and 1N aq. NaOH (15.0 mL, 15.0 mmol, 1.5 equiv.) and 30% H_2O_2 (12 mL) were added sequentially and stirred for 30 min. The mixture was diluted with water (25 mL) and the solvent was removed on rotary evaporator. The residue was dissolved in EtOAc (75 mL) and water (25 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine (25 mL) and dried ($MgSO_4$). The solvent was removed on a rotary evaporator and crude product was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 1.39 g of (±)-**15** in 45% yield as a colorless oil. R_f: 0.23 (40% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60; 2.0 mL/min at 225 nm, R_t: 3.2 min, 98%; ^1H NMR ($CDCl_3$): δ 5.05 (d, 1 H, J = 8.1 Hz), 4.18 (q, 1 H, J = 12.3, 6.3 Hz) - 4.09 (m, 1 H), 3.61 (dist. t, 2 H),1.82 - 1.60 (m, 6 H), 1.46 (s, 9 H), 1.43 (s, 9 H); ESI/MS (m/z): 304 $(M + H)^+$.

(±)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (4b): Triphenylphosphine (1.75 g, 6.6 mmol, 1.5 equiv.), imidazole (0.49 g, 7.2 mmol, 1.6 equiv.) and iodine (0.86 g, 6.75 mmol, 1.5 equiv.) were added sequentially to a solution of (±)-**15** (1.35 g, 4.45 mmol) in THF (15 mL) at room temperature under nitrogen. After stirring the mixture for 5.5 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 0.95 g of (±)-**4b** in 52% yield as a colorless oil. R_f: 0.40 (20% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60; 2.0 mL/min at 225 nm R_t: 11.78 min, 95.6%; ^1H NMR ($CDCl_3$): δ 5.05 (d, 1

H, J = 8.1 Hz), 4.15 (q, 1 H, J = 13.5, 7.2 Hz), 3.17 (t, 2 H, J = 6.9 Hz), 1.96- 1.60 (m, 6 H), 1.47 (s, 9 H), 1.44 (s, 9 H); ESI/MS (m/z): 414 $(M + H)^+$.

(±)-Pyridinium compound (17): 3-Hydroxypyridine (**16**, 0.095 g, 1.0 mmol, 2.0 equiv.) was added to a solution of (±)-**4b** (0.207 g, 0.5 mmol) dissolved in anhydrous 1,4-dioxane (5.0 mL) under nitrogen. After refluxing the mixture for 10 h, the solvent was removed on a rotary evaporator to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/25:75, 45mL/min at 225 nm). The solvent was removed on a rotary evaporator to afford 0.183 g (±)-**17** in 95% yield as a colorless gummy material. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/25:75, 2.0 mL/min at 225 nm R_t: 6.98 min, 99.4%; ^1H NMR (CD_3OD): δ 8.53 -8.42 (m, 2 H), 7.96 - 7.86 (m, 2 H), 4.53 (t, 2 H, J = 7.8 Hz), 3.98 - 3.92 (m, 1 H), 2.08 - 1.40 (m, 6 H), (1.45 (s, 9 H), 1.43 (s, 9 H); ESI/MS (m/z): 381 $(M)^+$.

(±)-1-(5-Ammonio-5-carboxypentyl)-3-hydroxypyridinium di(2,2,2-trifluoroacetate) (18): A mixture of TFA (9.5 mL) and water (0.5 mL) was added to (±)-**17** (0.170 g, 0.446 mmol) at room temperature and stirred for 1.5 h. The solvent was removed to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/5:95, 45mL/min at 225 nm). The solvent was removed on a rotary evaporator to afford 0.041 g of (±)-**18** in 40% yield as a colorless gummy material. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/5:95, 2.0 mL/min at 225 nm, R_t: 2.39 min, 99.2%; ^1H NMR (CD_3OD): δ 8.53 - 8.50 (m, 1 H), 8.46 - 8.40 (m, 1 H), 7.97 - 7.86 (m, 2 H), 4.57 (t, 2 H, J = 7.2 Hz), 3.98 (t, 1 H, J = 6.3 Hz), 2.12 - 1.88 (m, 4 H), 1.64 - 1.44 (m, 2 H); ESI/MS (m/z): 225 $(M)^+$.

Racemic pyridinium compound [(±)-19]: A mixture of (±)-**3g** (0.065 g, 0.109 mmol) and iodide (±)-**4b** (0.207 g, 0.5 mmol)] in anhydrous 1,4-dioxane (2.5 mL) was gently refluxed for 6 h under nitrogen. The solvent was removed to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/46:54; 45mL/min at 225 nm). Lyophilization of the product afforded 0.044 g of (±)-**19** in 46% yield. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/50:50;2.0 mL/min at 225 nm, R_t: 6.30 min, 92%; ^1H NMR (CD_3OD): δ 8.37 (s, 50/100 H), 8.35 (s, 50/100 H), 8.17 (s, 1 H),), 4.52 - 4.42 (m, 3 H), 4.00 - 3.92 (m, 2 H), 3.42 - 3.12 (m, 2 H), 3.06 - 2.82 (m, 2 H), 2.18 - 1.22 (m, 8 H), 1.46 (s, 18 H), 1.45 (s, 9 H), 1.44 (s, 9 H), 1.43 (s, 9 H), 1.36 (s, 9 H); ESI/MS (m/z): 881 $(M)^+$.

Racemic Deoxypyridinoline [(±)-2]: A mixture of TFA (9.5 mL) and water (0.5 mL) was added to the racemic pyridinium compound (±)-**19** (0.042 g, 0.048 mmol) at room temperature and stirred for 1.5 h. The solvent was removed to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/1:99, 45mL/min at 225 nm). The solvent was removed on a rotary evaporator to afford 0.0145 g of (±)-**2** TFA salt in 73% yield as a colorless gummy material. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/5:95, 2.0 mL/min at 225 nm, R_t: 2.39 min, 99%; ^1H NMR (CD_3OD): δ 8.47 (s, 1 H), 8.38 (s, 1 H), 4.53 (t, 2 H, J = 7.2 Hz), 4.43 - 4.36 (m, 1 H), 4.11 - 4.06 (m, 1 H), 3.97 (t, 1 H, J = 6.6 Hz), 3.62 - 3.52 (m, 1 H), 3.47 - 3.38 (m, 1 H), 3.20 - 3.00 (m, 2 H), 2.38 - 2.14 (m, 2 H), 2.12 - 1.88 (m, 4 H), 1.68 - 1.48 (m, 2 H); ^{13}C NMR (CD_3OD): δ 171.7, 171.5, 170.9, 157.9, 142.9, 142.8, 142.2, 142.1, 137.2, 130.3, 62.3, 53.6, 53.3, 53.2, 52.2, 31.7, 31.5, 31.4, 30.9, 28.6, 27.2, 27.1, 22.9; ESI/MS (m/z): 413 $(M)^+$.

(±)-2-Amino-4-[4-(2-amino-2-carboxyethyl)-5-hydroxy-3-pyridinyl]butanoic acid (20): A mixture of TFA (9.5 mL) and water (0.5 mL) was added to (±)-**3g** (0.042 g, 0.071 mmol) at room temperature and stirred for 1.5 h. The solvent was removed to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/1:99; 45mL/min at 225 nm). The solvent was removed on a rotary evaporator to afford 0.016 g of (±)-**20**-TFA salt in 76% yield as a colorless gummy material. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/1:99, 2.0 mL/min at 225 nm R_t: 1.95 min, 99%; ^1H NMR (CD_3OD): δ 8.26 (s, 1 H), 8.18 (s, 1 H), 4.39 - 4.33 (m, 1 H), 4.09 - 4.03 (m, 1 H), 3.57 -3.38 (m, 2 H), 3.18 - 2.88 (m, 2 H), 2.36 - 2.10 (m, 2 H); ESI/MS (m/z): 284 $(M + H)^+$.

(2*S*)-*tert*-Butyl-6-bromo-2-[(*tert*-butoxycarbonyl)amino]-5-oxohexanoate (22): Isobutylchloroform ate (3.9 mL, 30.0 mmol, 1.0 equiv.) was added to a 0 °C cooled mixture of (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**21b**, 9.09 g, 30.0 mmol) and 4-*N*-methylmorpholine (NMM, 3.6 mL, 33.0 mmol, 1.1 equiv.) in THF (120 mL) under nitrogen. The mixture was stirred for 25 min and quickly filtered through a celite bed (about 5 mm thickness). The filtrate was added to a freshly generated ethereal-diazomethane [generated from *N*-nitroso-*N*-methylurea (30.9 g, 300.0 mmol, 10.0 equiv.) and KOH (67.2 g, 1.2 mol, 40.0 equiv.) in ether (120 mL)] via a double ended needle at 0 °C over 10 min period. The mixture was then stirred for 3 h and a slow stream of nitrogen was bubbled through the mixture for 5 min to remove the excess diazomethane. The solvent was then carefully removed on a rotary evaporator (<35 °C) and dried on a vacuum pump. The resulting red residue was dissolved in ether (120 mL), cooled to -20 °C and 48% aq. HBr (5.04 mL, 30.0 mmol, 1.0 equiv.) added. After stirring the reaction mixture for 25 min at -20 °C, it was then diluted with ether (250 mL) and washed successively with water (3 x 75 mL). The solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (10-20% EtOAc in hexanes) to afford 5.76 g of (*S*)-**22** in 50% yield. R_f: 0.67 (30% EtOAc in hexanes); ^1H NMR ($CDCl_3$): δ 5.07 (dist. d, 1 H), 4.20 - 4.18 (m, 1 H), 3.91 (s, 3 H), 2.82 - 2.64 (m, 2 H), 2.24 - 1.80 (m, 2 H), 1.47 (s, 9 H), 1.44 (s, 9 H); ESI/MS (m/z): 380 $(M + H)^+$, 397 $(M + NH_4)^+$; HRMS (FAB, m/z); calcd for $C_{15}H_{27}BrNO_5$, 380.1073 $(M + H)^+$; observed, 380.1078.

(2*S*)-*tert*-Butyl-6-bromo-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoate (23): $NaBH_4$ (0.905 g, 23.8 mmol) was added to a 0 °C cooled solution of (*S*)-**22** (9.1 g, 23.8 mmol) in MeOH (95 mL) under nitrogen. After stirring for 3 h, the mixture was quenched with water (30 mL) and diluted with EtOAc (150 mL). The aqueous layer was separated and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$) and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (30% EtOAc in hexanes) to afford 7.0 g of (2*S*)-**23** in 77% yield as a 1:1 diastereomeric mixture. R_f: 0.38 (30% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous acetic acid/30:70, 1.0 mL/min at 225 nm, R_t: 6.45 min, 98.5%; ^1H NMR ($CDCl_3$): δ 5.19 (d, 50/100 H, J = 7.8 Hz), 5.10 (d, 50/100 H, J = 7.5 Hz), 4.30 - 4.16 (m, 1 H), 3.86 - 3.78 (m, 1 H), 3.54 - 3.46 (m, 1 H), 3.42 -3.35 (m, 1 H), 2.91 (d, 50/100 H, J = 6.3 Hz), 2.33 (d, 50/100 H, J = 5.1 Hz), 2.00 - 1.50 (m, 4 H), 1.47 (s, 9 H), 1.44 (s, 9 H); ESI/MS (m/z): 382 $(M + H)^+$.

(2*S*)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)butanoate (6g): Ethanolic KOH (0.5 M soln., 40.2 mL, 20.15 mmol, 1.1 equiv.) was added to the diastereomeric mixture of (2*S*)-**23** (7.0 g, 18.3 mmol]

dissolved in ethanol (55 mL) at room temperature under nitrogen. After stirring the reaction mixture for 2.5 h, it was diluted with water (40 mL) and EtOAc (150 mL). The aqueous layer was separated and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 25 mL) and dried ($MgSO_4$). The solvent was removed on a rotary evaporator and crude product was purified by silica gel column chromatography (30% EtOAc in hexanes) to afford 5.5 g of (2*S*)-**6g** in 84% yield as a mixture of diastereomers in 1:1 ratio, which solidified on standing at room temperature. R_f: 0.55 (30% EtOAc in hexanes); mp: 34-36 °C; Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 3.24 min, 99.7%; IR (KBr): 3348, 3079, 1730, 1711, 1523, 1357, 1158 cm^{-1}, ^1H NMR ($CDCl_3$): δ 5.12 - 5.00 (m, 1 H), 4.26 - 4.16 (m, 1 H), 2.96 - 2.90 (m, 1 H), 2.78 - 2.74 (m, 1 H), 2.50 - 2.46 (m, 1 H), 2.02 - 1.62 (m, 4 H), 1.46 (s, 9 H), 1.44 (s, 9 H); ESI/MS (m/z): 302 $(M + H)^+$, 319 $(M + NH_4)^+$; 324 $(M + Na)^+$, 620 $(2 \times M + NH_4)^+$.

(6*S*,16*S*)-Di(*tert*-butyl)-11-benzyl-9,13-dihydroxy-2,2,20,20-tetramethyl-4,18-dioxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate (7g): A mixture of (2*S*)-**6g** (5.45 g, 18.1 mmol)] and benzylamine (0.99 mL, 9.05 mmol, 0.5 equiv.) was heated at 75-80 °C with stirring for 24 h under nitrogen. The mixture was cooled to room temperature and purified by silica gel column chromatography (50% EtOAc in hexanes) to afford 5.31 g of (6*S*,16*S*)-**7g** in 85% yield as a colorless glassy material. R_f: 0.40 (50% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 5.12 min, 97%; IR (KBr): 3373, 3077, 1715, 1498, 1392, 1367, 1250, 1155 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35 – 7.25 (m, 5 H), 5.18 – 5.02 (m, 2 H), 4.22 – 4.12 (m, 2 H), 3.89 – 3.46 (m, 4 H), 2.65 – 2.42 (m, 4 H), 1.98 – 1.38 (m, 8 H), 1.44 (s, 18 H), 1.43 (s, 18 H); ESI/MS (m/z): 710 $(M + H)^+$, 732 $(M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{37}H_{64}N_3O_{10}$, 710.4592 $(M + H)^+$; observed, 710.4604.

(6*S*,16*S*)-Di(*tert*-butyl)-11-(*tert*-butoxycarbonyl)-9,13-dihydroxy-2,2,20,20-tetramethyl-4,18-dioxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate (9g): 10% Pd/C (0.528 g) was added to a solution of (6*S*,16*S*)-**7g** (5.28 g, 7.64 mmol) in ethanol (115 mL) and hydrogenated at 15 psi pressure for 4.5 h. The catalyst was filtered, washed with ethanol (30 mL) and the filtrate was concentrated. The resulting crude amine (6*S*,16*S*)-**8f** (4.45 g) was dissolved in THF (45 mL), $(Boc)_2O$ (2.49 g, 11.46 mmol, 1.5 equiv.) was added and the procedure described for **9a** was followed. The crude product was purified by silica gel column chromatography (50-60% EtOAc in hexanes) to afford 4.88 g of (6*S*,16*S*)-**9g** in 89% yield for two steps as colorless glassy material. R_f: 0.32 (50% EtOAc in hexane); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 9.30 min, 97%; IR (KBr): 3367, 3077, 1715, 1505, 1367, 1249, 1157 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.22 – 5.10 (m, 2 H), 4.28 – 4.10 (m, 2 H), 4.08 – 2.80 (m, 8 H), 2.00 - 1.60 (m, 4 H), 1.58 – 1.36 (m, 4 H), 1.45 (s, 27 H), 1.43 (s, 18 H); ESI/MS (m/z): 720 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{37}H_{66}N_3O_{12}$, 720.4647 $(M + H)^+$; observed, 720.4622.

(6*S*,16*S*)-(-)-Di(*tert*-butyl)-11-(*tert*-butoxycarbonyl)-2,2,20,20-tetramethyl-4,9,13,18-tetraoxo-3,19-dioxa-5,11,17-triazahenicosane-6,16-dicarboxylate (5g): DMSO (2.39 mL, 33.73 mmol, 5.0 equiv.) in CH_2Cl_2 (17 mL) was added to a solution of oxalyl chloride (8.43 mL of 2.0 M soln in CH_2Cl_2, 16.88 mmol, 2.5 equiv.) in CH_2Cl_2 (17 mL) at -78 °C. After stirring the mixture for 30 min, a solution of (6*S*,16*S*)-**9g** (4.85 g, 6.75 mmol) in CH_2Cl_2 (34 mL) was added at -78 °C. The mixture was stirred for 3 h, Et_3N (9.4 mL, 67.5 mmol, 10.0 equiv.) was added at -60 °C and the procedure described for **5a** was followed. The crude product

was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 4.74 g of (6*S*,16*S*)-(-)-**5g** in 98% yield as a colorless glassy material. R_f: 0.53 (40% EtOAc in hexanes); mp: 54-57 °C; $[\alpha]^{20}_D$ -21.35 (c 1.18, MeOH); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 14.66 min, 99%; IR (KBr): 3366, 3078, 1708, 1506, 1455, 1392, 1367, 1251, 1156, 1053 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.12 – 5.02 (m, 2H), 4.32 – 3.90 (m, 6 H), 2.60 – 2.32 (m, 4 H), 2.22 – 2.08 (m, 2 H), 1.84 – 1.68 (m, 2 H), 1.46 (s, 9 H), 1.45 (s, 9 H), 1.42 (s, 9 H, 1.41 (s, 9 H), 1.40 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.6, 155.6, 155.4, 82.2, 82.1, 80.9, 79.8, 57.1, 56.6, 53.1, 52.9, 35.2, 35.1, 28.2, 28.1, 27.9, 26.7; ESI/MS (m/z): 750 $(M + Cl)^-$; HRMS (FAB, m/z): calcd for $C_{35}H_{62}N_3O_{12}$, 716.4333, observed, 716.4344.

(-)-*tert*-Butyl-(2*S*)-4-(4-{(2*S*)-3-(*tert*-butoxy)-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-5-hydroxy-3-pyridinyl)-2-[(*tert*-butoxycarbonyl)amino]butanoate (3g): DBU (6.2 mL, 41.17 mmol, 6.4 equiv.) was added to a solution (6*S*,16*S*)-(-)-**5g** (4.6 g, 6.43 mmol) in THF (65 mL) at room temperature and the procedure described for **3f** was followed. The crude product was purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to afford 1.63 g (*S,S*)-(-)-**3g** in 42% yield. The faster eluting compounds (non-polar fractions) from column chromatography were concentrated to give 1.13 g of material which was dissolved in THF (20 mL), DBU (1.5 mL) added, and above conditions were followed to give an additional 0.32 g of (*S,S*)-(-)-**3g** in 8% yield (total yield 50%). R_f: 0.45 (5% MeOH in EtOAc); mp: 89-91 °C; $[\alpha]^{20}_D$ –11.84 (c 0.62, MeOH), Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 4.23 min, 97.6%; IR (KBr): 3360, 3078, 1717, 1505, 1392, 1367, 1250, 1155, 1048, 1022 cm^{-1};^1H NMR ($CDCl_3$): δ 8.13 (s, 1 H), 7.93 (s, 1 H), 5.65 (br. s, 1 H), 5.23 (dist d, 1 H), 4.44 – 4.30 (m, 2 H), 3.25 – 3.05 (m, 2 H), 2.71 (t, 2 H, J = 8.4 Hz), 2.25 – 1.78 (m, 2 H), 1.48 (s, 9 H), 1.48 (s, 9 H), 1.45 (s, 9 H), 1.40 (s, 9 H), 1.37 (s, 9 H); ESI/MS (m/z): 596 $(M + H)^+$; HRMS (FAB, m/z: calcd for $C_{30}H_{50}N_3O_9$, 596.3547 $(M + H)^+$; observed, 596.3542.

(2*S*,5*R*)-(-)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-bromo-5-[*O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxinyl]-hexanoate (25): To a solution of (2*S*)-**23** (1.127 g, 2.95 mmol) dissolved in CH_2Cl_2 (40 mL), *O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine[25b] (**24**, 2.628 g, 2.95 mmol, 1.0 equiv.), 1,3-dicyclohexyl carbodiimde (DCC, 1.22 g, 5.9 mmol, 2.0 equiv.) and 4-dimethylaminopyridine (DMAP, 0.181 g) were added sequentially at room temperature under nitrogen. After stirring the mixture for 16 h, it was filtered and the filtrate was concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 2.995 g of the diastereomeric mixture of **25**. The mixture was separated on a preparative RP HPLC (Waters, C18, 7μm, Symmetry, 40 x 100 mm column, MeCN:01% aqueous acetic acid/90:10, 25 mL/min at 225 nm) to afford 1.044 g of (2*S*,5*R*)-(-)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-bromo-5-[*O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxinyl]-hexanoate (**25**) in 28% yield. Analytical RP HPLC: (Waters, C18, 7μm, Symmetry 8 x 100 mm), MeCN:01% aqueous acetic acid/90:10, 1.0 mL/min at 225 nm, R_t: 31.3 min, 99%; $[\alpha]^{20}_D$ –5.53 (c 1.15, MeOH); ^1H NMR ($CDCl_3$): δ 7.69 (s, 2 H), 7.16 (s, 2 H), 5.18 - 5.02 (m, 2 H), 4.60 - 4.54 (m, 1 H), 4.22 - 4.18 (m, 1 H), 3.84 (s, 3 H), 3.54 - 3.42 (m, 2 H), 3.14 (dd, 1 H, J = 14.4, 5.7 Hz), 2.94 (dd, 1 H, J = 14.2, 8.1 Hz), 1.84 - 1.60 (m, 4 H), 1.48 (s, 9 H), 1.44 (s, 9 H), 1.43 (s, 9 H); ESI/MS (m/z): 1255 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{36}H_{47}N_2O_{10}BrI_4Na$, 1276.8505 $(M + Na)^+$; observed:1276.8485

Also, 1.27 g of (2*S*,5*S*)-(-)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-bromo-5-[*O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxinyl]-hexanoate (**25**) was isolated in 34% yield. Analytical RP HPLC: (Waters C18 7μm, Symmetry, 8 x 100 mm column), MeCN:01% aqueous acetic acid/90:10, 1.0 mL/min at 225 nm, R_t: 33.43 min, 99%; $[\alpha]^{20}_D$ –10.71 (c 1.13, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 2 H), 7.16 (s, 2 H), 5.16 -5,12 (br s, 1 H), 4.58 - 4.52 (m, 1 H), 4.22 - 4.18 (m, 1 H), 3.83 (s, 3 H), 3.56 - 3.41 (m, 2 H), 3.21 (dd, 1 H, *J* = 14.4, 5.7 Hz), 2.93 (dd, 1 H, *J* = 14.1, 6.3 Hz), 1.84 - 1.60 (m, 4 H), 1.47 (s, 9 H), 1.44 (s, 18 H); ESI/MS (m/z): 1255 (M + H)$^+$; HRMS (FAB, m/z): calcd for $C_{36}H_{47}N_2O_{10}BrI_4Na$, 1276.8505 (M + Na)$^+$; observed:1276.8485.

(+)-*tert*-Butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2*R*)-oxiranyl]butanoate (6g): The ester (2*S*,5*S*)-(-)-**25** (0.580 g, 0.462 mmol) was dissolved in THF (6.0 mL) and LiOH (0.058 g, 1.386 mmol, 3.0 equiv.) added, followed by water (2.0 mL) at room temperature. After stirring the mixture for 20 h, it was diluted with water (15 mL) and EtOAc (75 mL). The aqueous layer was separated and extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and the solvent was removed on a rotary evaporator. Purification of the crude product by silica gel column chromatography (25% EtOAc in hexanes) afforded 0.096 g of (2*S*,*R*)-(+)-**6g** in 69% yield as a colorless oil. R_f: 0.55 (30% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/40:60, 2.0 mL/min at 225 nm, R_t: 3.05 min, 97.4%; mp: 45-47 °C; $[\alpha]^{20}_D$ +19.25 (c 1.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.08 (d, 1 H, *J* = 8.4 Hz), 4.20 (q, 1 H, *J* = 12.6, 7.5 Hz), 2.97 - 2.90 (m, 1 H), 2.76 (t, 1 H, *J* = 4.5 Hz), 2.49 (dd, 1 H, *J* = 5.1, 2.7 Hz), 2.04 - 1.91 (m, 1 H), 1.78 - 1.56 (m, 3 H), 1.46 (s, 9 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.7, 155.5, 82.1, 79.7, 53.6, 51.6, 47.0, 30.2, 28.3, 28.2, 27.9; ESI/MS (m/z): 302 (M + H)$^+$, 319 (M + NH_4)$^+$; HRMS (FAB, m/z): calcd for $C_{15}H_{28}NO_5$, 302.1967 (M + H)$^+$; observed, 302.1971.

(2*S*,5*R*)-(+)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (4a): To a solution of epoxide (2*S*,*R*)-(+)-**6g** (0.091 g, 0.302 mmol) in MeOAc (4.2 mL), NaI (0.059 g, 0.393 mmol, 1.3 equiv.), anhydrous sodium acetate (0.027 g, 0.332 mmol, 1.1 equiv.) and acetic acid (0.019 mL, 0.332 mmol, 1.1 equiv.) were added sequentially at room temperature under nitrogen. After stirring the mixture for 20 h, the mixture was diluted with water (15 mL) and EtOAc (100 mL). The aqueous layer was separated and the EtOAc layer was washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (25% EtOAc in hexanes) afforded 0.121 g of (2*S*,5*R*)-(+)-**4a** in 94% yield as a colorless gummy material. R_f: 0.35 (30% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/35:65, 2.0 mL/min at 225 nm, R_t: 4.49 min, 98%; $[\alpha]^{20}_D$ +15.47° (c 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.20 (d, 1 H, *J* = 7.5 Hz), 4.30-4.22 (m, 1 H), 3.62 - 3.52 (m, 1 H), 3.36 - 3,20 (m, 2 H), 2.90 (d. 1 H, *J* = 5.4 Hz), 2.00 - 1.60 (m, 4 H), 1.47 (s, 9 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.8, 155.8, 82.3, 80.0, 70.7, 53.3, 31.8, 30.9, 28.2, 27.9, 15.7; ESI/MS (m/z): 452 (M + Na)$^+$.

1-(2*R*)-(-)-Pyridinium compound (30): A mixture of (*S*,*S*)-(-)-**3g** (0.080 g, 0.135 mmol) and iodide (2S,5*R*)-(+)-**4a** (0.116 g, 0.27 mmol, 2.0 equiv.) in anhydrous 1,4-dioxane (2.6 mL) was gently refluxed for 6 h under nitrogen. The solvent was removed on a rotary evaporator to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/45:55, 45mL/min at 225 nm). Lyophilization of the product afforded 0.051 g of 1-(2*R*)-(-)-**30** in 42% yield as a pale yellow powder. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/45:55, 2.0 mL/min at 225 nm, R_t: 7.15 min,

99%; $[\alpha]^{20}_D$ -25.35 (c 0.28, MeOH); ^{1}H NMR (CD_3OD): δ 8.30 (s, 1 H), 8.14 9s, 1 H), 4,59 (dist. d, 1 H, *J* = 11.4 Hz), 4.46 (dd, 1 H, *J* = 9.3, 6.0 Hz), 4.25 (dd, 1 H, *J* = 12.6, 9.0 Hz), 4.00 - 3.87 (m, 3 H), 3.42 - 3.12 (m, 2 H), 2.98 - 2.90 (m, 2 H), 2.14 - 1.40 (m, 6 H), 1.46 (s, 27 H, 1.44 (s, 18 H), 1.37 (s, 9 H); ESI/MS (m/z): 897 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{45}H_{77}N_4O_{14}$, 897.5426 $(M)^+$; observed, 897.5431.

(+)-Pyridinoline (Pyd, 1): A mixture of TFA (9.5 mL) and water (0.5 mL) was added to the pyridinium compound 1-(2*R*)-(-)-**30** (0.038 g, 0.0423 mmol)] at room temperature and the reaction was stirred for 1.5 h. The solvent was removed to dryness on a rotary evaporator and the crude product was purified by preparative RP HPLC (MeCN:0.05% aqueous trifluoroacetic acid/2:98, 25 mL/min at 225 nm). Lyophilization of the product afforded 0.015 g of (+)-pyridinoline (**1**) as its TFA salt in 83% yield. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/2:98, 1.0 mL/min at 225 nm, R_t: 3.90 min, >99%; $[\alpha]^{20}_D$ +39.17 (c 0.24, MeOH); ^{1}H NMR (D_2O):[4b] δ 8.10 (s, 1 H), 8.07 (s, 1 H), 4.49 (dd, 1 H, *J* = 13.5, 2.4 Hz), 4.20 - 4.07 (m, 2 H), 3.92 - 3.85 (m, 3 H), 3.28 - 3.22 (m, 2 H), 2.94 - 2.70 (m, 2 H), 2.12 - 1.93 (m, 3 H), 1.92 - 1.79 (m, 1 H), 1.66 - 1.42 (m, 2 H); ^{13}C NMR (D_2O + 2.0 μL of MeOH):[31] δ 173.2, 172.9, 172.5, 155.9, 142.1, 141.5, 137.2, 130.1, 70.2, 66.7, 53.6, 53.4, 52.4, 30.7, 29.4, 28.1, 26.8, 26.8, 21.1; ESI-MS (m/z): 429 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{18}H_{29}N_4O_8$, 429.1985 $(M)^+$; observed, 429.1989. Analytical RP HPLC of natural pyridinoline (**1**)[12] which was obtained by isolation from bone: MeCN:0.1% aqueous trifluoroacetic acid/2:98, 1.0 mL/min at 225 nm, R_t: 3.89 min, 99.2%.

(+)-*tert*-Butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2*S*)-oxiranyl]butanoate (6g): The ester (2*S*,5*S*)-(-)-**25** (0.490 g, 0.4 mmol) was dissolved in THF (6.0 mL) and LiOH (0.050 g, 1.2 mmol, 3.0 equiv.) added, followed by water (2.0 mL), at room temperature. After stirring the mixture for 20 h, it was diluted with water (15 mL) and EtOAc (75 mL). The aqueous layer was separated and re-extracted with EtOAc (2 x 25 mL). The combined EtOAc layers were washed with brine (15 mL), dried ($MgSO_4$) and the solvent was removed on a rotary evaporator. Purification of the crude product by silica gel column chromatography (25% EtOAc in hexanes) afforded 0.081 g of (2*S*,*S*)-(+)-**6g** in 68% yield as a thick oil. R_f: 0.55 (30% EtOAc in hexanes); Analyti cal RP HPLC: MeCN:0.1% aqueous acetic acid/40:60; 2.0 mL/min at 225 nm, R_t: 3.24 min, >99%; $[\alpha]^{20}_D$ +3.65 (c 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.04 (d, 1 H, *J* = 8.4 Hz), 4.20 (q, 1 H, *J* = 12.6, 7.5 Hz), 2.97 - 2.90 (m, 1 H), 2.76 (t, 1 H, *J* = 5.1 Hz), 2.48 (dd, 1 H, *J* = 4.8, 2.7 Hz), 2.05 - 1.89 (m, 1 H), 1.83 - 1.40 (m, 3 H), 1.46 (s, 9 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.8, 155.5, 82.1, 79.7, 53.4, 51.5, 47.1, 30.1, 28.2, 27.9; ESI/MS (m/z): 302 $(M + H)^+$; HRMS (FAB, m/z): calcd for $C_{15}H_{28}NO_5$, 302.1967 $(M + H)^+$; observed, 302.1954.

(2*S*,5*S*)-(+)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (4a): To a solution of epoxide (2*S*,*S*)-(+)-**6g** (0.077 g, 0.256 mmol) in MeOAc (3.5 mL), NaI (0.50 g, 0.333 mmol, 1.3 equiv.), anhydrous sodium acetate (0.023 g, 0.282 mmol, 1.1 equiv.) and acetic acid (0.016 mL, 0.282 mmol, 1.1 equiv.) were added sequentially at room temperature under nitrogen. After stirring the mixture for 20 h, the mixture was diluted with water (15 mL) and EtOAc (100 mL). The aqueous layer was separated and the EtOAc layer was washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed on a rotary evaporator and the crude product was purified by silica gel column chromatography (25% EtOAc in hexanes) afforded 0.102 g of (2*S*,5*S*)-(+)-**4a** in 93% yield as a gummy material. R_f: 0.35 (30% EtOAc in hexanes); Analytical RP HPLC:

MeCN:0.1% aqueous acetic acid/35:65; 2.0 mL/min at 225 nm, R_t: 4.67min, 98%; $[\alpha]^{20}_D$ +15.5 (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.09 (d, 1 H), 4.14 (q, 1 H, *J* = 12.9, 6.9 Hz), 3.62 - 3.52 (m, 1 H), 3.36 (dd, 1 H, *J* = 10.2, 3.9 Hz), 3.22 (dd, 1 H, *J* = 10.2, 6.9 Hz), 2.18 (dist. d, 1 H), 1.92 - 1.40 (m, 4 H), 1.47 (s, 9 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.8, 155.6, 82.1, 79.8, 70.3, 53.5, 32.0, 30.1, 28.2, 27.9, 15.9; ESI/MS (m/z): 430 $(M + H)^+$, 447 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{15}H_{29}NIO_5$, 430.1091 $(M + H)^+$; observed, 430.1090.

1-(2*S*)-(-)-Pyridinium compound (30): A mixture of (*S*,*S*)-(-)-**3g** (0.028 g, 0.0466 mmol) and iodide (2*S*,5*S*)-(+)-**4a**, (0.040 g, 0.0932 mmol, 2.0 equiv.) in anhydrous 1,4-dioxane (1.5 mL) was gently refluxed for 6 h under nitrogen. The solvent was removed on a rotary evaporator to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.1% aqueous trifluoroacetic acid/45:55, 45 mL/min at 225 nm). Lyophilization of the product afforded 0.018 g of 1-(2*S*)-(-)-**30** in 43% yield as a pale yellow powder. Analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid/45:55; 2.0 mL/min at 225 nm, R_t: 7.36 min, 97.7%; mp: 79-84 °C; $[\alpha]^{20}_D$ -25.22 (c 0.23, MeOH); ^1H NMR (CD_3OD): δ 8.31 (s, 1 H), 8.13 (s, 1 H), 4.57 (d, 1 H, *J* = 13.2 Hz), 4.46 (dd, 1 H, *J* = 9.3, 6.0 Hz), 4.25 (t, 1 H, *J* = 9.0 Hz), 4.04 - 3.90 (m, 3 H), 3.54 - 3.12 (m, 2 H), 2.98 - 2.88 (m, 2 H), 2.16 - 1.34 (m, 6 H), 1.46 (s, 18 H), 1.45 (s, 27 H), 1.37 (s, 9 H); ^{13}C NMR (CD_3OD) δ 173.5, 173.1, 171.8, 158.4, 157.9, 157.2, 144.9, 143.2, 137.8, 130.1, 83.5, 83.2, 82.9, 80.9, 80.7, 70.8, 67.6, 55.3, 55.2, 53.9, 33.1, 31.8, 30.3, 28.7, 28.6, 28.2, 27.9; ESI/MS (m/z): 897 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{45}H_{77}N_4O_{14}$, 897.5425 $(M)^+$; observed, 897.5431.

1-(2*S*)-(+)-Pyridinoline (1): A mixture of TFA (9.5 mL) and water (0.5 mL) was added to the pyridinium compound [1-(2*S*)-(-)-**30**, 0.0128 g, 0.0134 mmol] at room temperature and the reaction was stirred for 1.5 h. The solvent was removed and the crude product was purified by preparative RP HPLC (MeCN/0.1% aqueous trifluoroacetic acid/2:98; 45 mL/min at 225 nm). Lyophilization of the product afforded 0.0058 g of 1-(2*S*)-(+)-pyridinoline (**1**) as its TFA salt in 95% yield. Analytical RP HPLC: MeCN:0.1% aqueous trifluoro acetic acid/2:98, 1.0 mL/min at 225 nm, R_t: 3.85 min, >99%; $[\alpha]^{20}_D$ +43.23 (c 0.26, MeOH); ^1H NMR (D_2O):[4b] δ 8.11 (s, 1 H), 8.07 (s, 1 H), 4.52 (dd, 1 H, *J* = 13.2, 2.7 Hz), 4.18 (dd, 1 H, *J* = 13.2, 9.0 Hz), 4.03 (dd, 1 H, *J* = 8.7, 6.3 Hz), 4.04 - 3.88 (m, 3 H), 3.44 - 3.32 (m, 2 H), 3.14 - 2.90 (m, 2 H), 2.34 - 1.98 (m, 4 H), 1.92 -1.78 (m, 1 H), 1.66 - 1.52 (m, 1 H); ^{13}C NMR (D_2O + 1.0 μL of MeOH):[31] δ 173.9, 173.7, 173.0, 155.9, 142.4, 141.6, 137.0, 130.1, 70.3, 66.6, 54.2, 53.9, 52.8, 30.9, 30.7, 28.3, 27.0, 26.1; ESI/MS (m/z): 429 $(M)^+$.

(2*S*)-(-)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (15): (2*S*)-(-)-6-Amino-2-[(*tert*-butoxycarbonyl)amino]hexanoic acid (**31**, 3.69 g, 15.0 mmol) was dissolved in water (50 mL) and the pH adjusted to 9.5 using 4M aq. NaOH. The mixture was heated to 60 °C, sodium nitroprusside (7.07 g, 23.7 mmol, 1.6 equiv.) was added portion wise over 30 min and the reaction heated for an additional 5 h. The pH of the reaction mixture was maintained between 9-10 with addition of 4M aq. NaOH during and after the addition of sodium nitroprusside. The reaction mixture was cooled to room temperature and filtered through celite powder. The pH of the filtrate was adjusted to 3.5 using 6M HCl and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (40 mL), dried (Na_2SO_4) and the solvent was removed on a rotary evaporator. The resulting crude hydroxy-acid (2*S*)-**32** (2.40 g) was dried using a vacuum pump and carried to the next reaction without purification.

The above prepared crude hydroxy-acid (2*S*)-**32** (1.13 g, 4.57 mmol) was dissolved in CH_2Cl_2 (60 mL) and a solution of *O*-*tert*-butyl-*N,N'*-diisopropylisourea[29] (2.74 g, 13.7 mmol, 3.0 equiv.) in CH_2Cl_2 was added at 0 °C under nitrogen. The reaction mixture was allowed to warm to room temperature, stirred for 6 h. and an additional amount of *O*-*tert*-butyl-*N,N'*-diisopropylisourea (1.83 g, 9.14 mmol, 2.0 equiv.) was added. The resulting mixture was stirred for 16 h at room temperature and filtered. The filtrate was concentrated on a rotary evaporator and the crude product was purified by silica gel column chromatography (50% EtOAc in hexanes) to afford 0.623 g of (2*S*)-(-)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (**15**) in 43% yield for two steps. R_f: 0.23 (40% EtOAc in hexane); Analytical RP HPLC: MeCN:0.1% aqueous acetic acid/50:50, 1.0 mL/min at 220 nm, R_t: 10.9 min, 97.5%; $[\alpha]^{20}_D$ -27.5 (c 1.28, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.05 (d, 1 H, *J* = 8.1 Hz), 4.20 - 4.09 (m, 1 H), 3.62 (q, 2 H, *J* = 11.4, 5.1 Hz), 1.80 - 1.60 (m, 6 H), 1.45 (s, 9 H), 1.43 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 172.1, 155.6, 81.7, 79.6, 62.4, 53.7,32.6, 32.0, 28.2, 27.9, 21.2; ESI/MS (m/z): 303 $(M)^+$, 321 $(M + NH_4)^+$.

(2*S*)-(-)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (4b): Triphenylphosphine (0.682 g, 2.6 mmol, 1.5 equiv.), imidazole (0.189 g, 2.77 mmol, 1.6 equiv.) and iodine (0.66 g, 2.60 mmol, 1.5 equiv.) were added sequentially to a solution of (2*S*)-(-)-**15** (0.526 g, 1.73 mmol) dissolved in THF (20 mL) at room temperature under nitrogen. After stirring the mixture for 3 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 0.559 g of (2*S*)-(-)-**4b** in 78% yield as colorless thick oil. R_f: 0.40 (20% EtOAc in hexanes); Analytical RP HPLC: MeCN:0.1% aqueous acetic acid/20:80, 1.0 mL/min at 225 nm, R_t: 9.85 min, >99%; $[\alpha]^{20}_D$ -13.7 (c 1.22, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.04 (d, 1 H, *J* = 8.1 Hz), 4.16 (q, 1 H, *J* = 13.5, 7.2 Hz), 3.17 (t, 2 H, *J* = 6.9 Hz), 1.96- 1.60 (m, 6 H), 1.46 (s, 9 H), 1.43 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.9, 155.4, 81.9, 79.6, 53.6,32.8, 31.8, 28.2, 27.9, 25.9; ESI/MS (m/z): 414 $(M + H)^+$, 431 $(M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{15}H_{30}N_4O_7I$, 414.1141 $(M + H)^+$; observed, 414.1137.

(-)-Pyridinium compound (19): A mixture of (*S,S*)-(-)-**3g** (0.163 g, 0.274 mmol) and iodide (2*S*)-(-)-**4b** (0.170 g, 0.411 mmol, 1.5 equiv.)] in anhydrous 1,4-dioxane (5.0 mL) was gently refluxed for 3 h under nitrogen. The solvent was removed on a rotary evaporator to dryness and the crude product was purified by preparative RP HPLC (MeCN:0.05% aqueous trifluoroacetic acid/80:20, 25 mL/min at 225 nm). The solvent was removed on a rotary evaporator and finally azeotroped with a mixture of MeCN-toluene (ratio: 1:1, 3 x 20 mL) to afford 0.070 g of (-)-**19** in 29% yield as a pale yellow solid. Analytical RP HPLC: MeCN:0.05% aqueous trifluoroacetic acid/80:20, 1.0 mL/min at 225 nm, R_t: 7.04 min, 96.8%; mp: 63-65 °C; $[\alpha]^{20}_D$ -22.1 (c 1.28, MeOH); ^{1}H NMR (CD_3OD): δ 8.37 (s, 1 H), 8.14 (s, 1 H), 4.56 - 4.44 (m, 3 H), 4.00 - 3.92 (m, 2 H), 3.40 - 3.10 (m, 2 H), 3.00 - 2.90 (m, 2 H), 2.18 - 1.22 (m, 8 H), 1.46 (s, 18 H), 1.45 (s, 18 H), 1.43 (s, 9 H), 1.36 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 171.6, 170.9, 169.8, 157.9, 155.7, 155.5, 142.5, 141.4, 133.0, 130.6, 83.0, 82.3, 80.1, 79.9, 61.4, 53.1, 32.6, 32.1, 30.5, 30.6, 30.4, 28.2, 28.0, 27.8, 27.7, 27.7, 25.9, 21.9; ESI/MS (m/z): 881 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{45}H_{77}N_4O_{13}$, 881.5487 $(M)^+$; observed, 881.5472.

(+)-Deoxypyridinoline (Dpd, 2): A mixture of TFA (9.5 mL) and water (0.5 mL) was added to the pyridinium compound (-)-**19** (0.056 g, 0.063 mmol)] at room temperature and stirred for 1 h. The solvent was removed to dryness and the crude product was purified by preparative RP HPLC [Waters μBondapak C18 10μ

(25 x 100 mm) reverse phase column, MeCN:0.05% aqueous trifluoroacetic acid/2:98, 25 mL/min at 225 nm]. The solvent was removed on a rotary evaporator and azeotroped with a mixture of MeOH-toluene (1:1 ratio, 3 x 10 mL) to afford 0.024 g of (+)-deoxypyridinoline-TFA salt (**2**) in 92% yield as pale yellow solid. Analytical RP HPLC: MeCN:0.05% aqueous trifluoroacetic acid/2:98; 1.0 mL/min at 225 nm, R_t: 4.03 min, >99%; $[\alpha]^{20}_D$ +31.6 (c 0.25, MeOH); ^{1}H NMR (D_2O + 2.0 μL MeOH)):[4b, 31] δ 8.12 (s, 1 H), 8.05 (s, 1 H), 4.32 (t, 2 H, J = 7.2 Hz), 3.97 (dd, 1 H, J = 8.4, 6.6 Hz), 3.77 (t, 1 H, J = 5.7 Hz), 3.66 (t, 1 H, J = 6.0 Hz), 3.26 - 3.12 (m, 2 H), 2.88 - 2.64 (m, 2 H), 2.18- 1.98 (m, 2 H), 1.94 - 1.80 (m, 2 H), 1.80 - 1.68 (m, 2 H), 1.38 - 1.16 (m, 2 H); ^{13}C NMR (D_2O + 2.0 μL MeOH)):[31] δ 174.4, 174.0, 173.1, 156.2, 142.0, 141.9, 136.5, 130.5, 61.7, 54.5, 53.0, 31.0, 30.5, 30.1, 28.3, 26.1, 21.6. ESI/MS (m/z): 413 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{18}H_{30}N_4O_7$, 413.2036 $(M)^+$; observed, 413.2032. Analytical RP HPLC of natural deoxypyridinoline (**2**)[12] which was obtained by isolation from bone: MeCN:0.05% aqueous trifluoroacetic acid/2:98; 1.0 mL/min at 225 nm; R_t: 4.0 min, 84%.

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