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SYNTHESIS OF NEODESMOSINE, A CROSSLINKING PYRIDINIUM AMINO ACID OF ELASTIN, VIA A NEGISHI CROSS-COUPLING

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Abstract – Neodesmosine, isolated from the hydrolysate of bovine ligamentum nuchae, is a crosslinking pyridinium amino acid of elastin. In this study, the first total synthesis of neodesmosine is reported via a Negishi cross-coupling reaction between 3,5-dihalogenated pyridines and the corresponding iodo amino acid.

INTRODUCTION

Elastic fibers exist in vertebrate tissues and organs such as the lung, skin, blood vessels, and heart, and play an important role in providing their elasticity and stretch properties.[1] These unique functions are mainly derived from their self-assembling nature and crosslinked structure. Elastin, the main component of elastic fibers, is an extremely insoluble extracellular matrix protein and consists of soluble precursor tropoelastin monomers connected in a three-dimensional crosslinked network by amino acids. The elastin crosslinkers are thus significant amino acids with respect to their elasticity and stretch properties.

As one of the crosslinking pyridinium amino acids of elastin, neodesmosine (**1**, Figure 1) was isolated from the acid hydrolysate of bovine ligamentum nuchae.[2] Desmosine (**2**) and isodesmosine (**3**), known as attractive biomarkers for the diagnosis of COPD (chronic obstructive pulmonary disease),[3] have also been identified as major crosslinking pyridinium amino acids (Figure 1).[4] It has been known that the formation of the crosslinking amino acids occurs spontaneously after oxidative transformation of the lysine residues of elastin by lysyl oxidase.[5] Although a model structure for elastin has been proposed,[6] the three-dimensional structure of elastin including the crosslinking networks remains unknown due to its insoluble nature. Therefore, the chemical synthesis of these crosslinking amino acids would aid in the elucidation of the entire structure.

Recently, the first total synthesis of desmosine **2** starting from 4-hydroxypyridine or 3,5-dibromopyridine was achieved in our laboratory.[7] The synthesis relied on palladium-catalyzed cross-coupling reactions

between the pyridine cores and the corresponding segments as key steps. Herein, the first total synthesis of neodesmosine is described via a Negishi cross-coupling reaction[8] between 3,5-dihalogenated pyridines and the corresponding iodo amino acid.

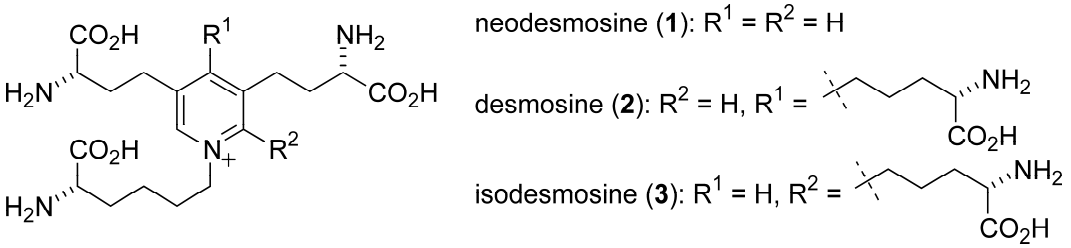


Figure 1. Structures of neodesmosine (**1**), desmosine (**2**), and isodesmosine (**3**)

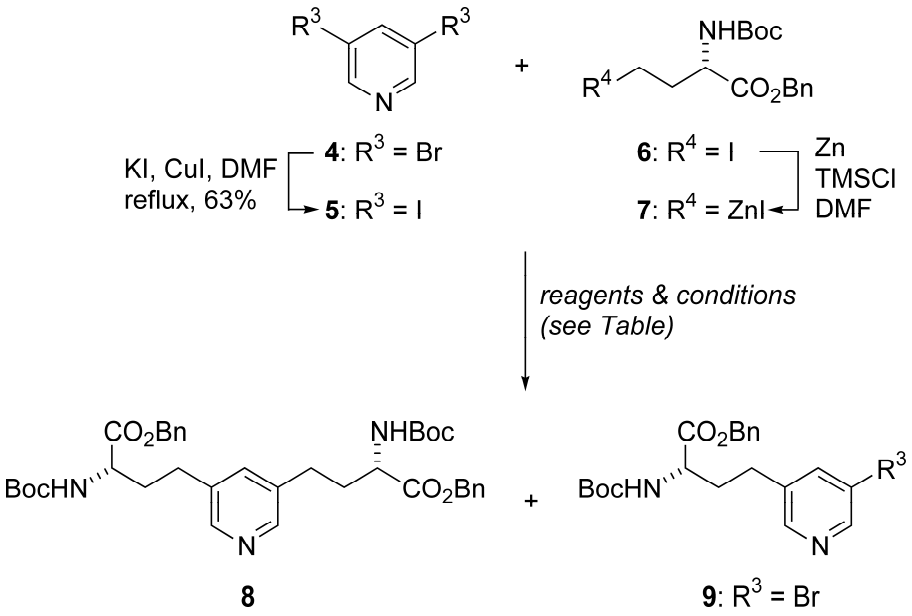
RESULTS AND DISCUSSION

The target molecule **1** consists of a pyridine core and two types of amino acids. Toward the total synthesis, our route commenced with optimization of the Negishi cross-coupling reaction of 3,5-dihalogenated pyridines **4**/**5** and protected γ-iodoalkylated L-glycine **6**, which can be derived from commercially available 4-benzoxyl-(*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-oxobutanoic acid in two steps (Table 1).[9] Firstly, 3,5-diiodopyridine **5** was prepared from commercially available 3,5-dibromopyridine **4** using an excess amount of KI in the presence of CuI.[10] The reaction was run for a week in order to complete the conversion of the bromide to the iodide and provide pure product **5**, because the polarity of the desired product was the same as that of the starting material **4**. Thus, the desired **5** was obtained in 63% yield.

The Negishi cross-coupling reactions were then investigated (Table 1, entries 1-6). For the insertion of zinc into protected γ-iodoalkylated L-glycine **6**, a modified protocol was applied, in which the extra zinc from the organozinc reagent can be removed via centrifugation.[11] The reaction between 3,5-dibromopyridne **4** and the pure organozinc reagent, protected γ-iodozinc-alkylated L-glycine **7**, was then carried out using 10 mol% $Pd_2(dba)_3$ with 40 mol% P(2-furyl)$_3$[12] at 50 °C for 24 h in order to afford the desired dicoupled product **8** in 39% yield (entry 1). When the reaction was run with 20 mol% Pd-PEPPSI-IPr, which was developed by Organ and co-workers,[13] the dicoupled product **8** and monocoupled byproduct **9** were obtained in 59% and 30% yield, respectively (entry 2). However, the yield of **8** was decreased in the presence of LiBr and LiCl as an additive in order to activate the organozinc reagents (entries 3 and 4). In the case of 3,5-diiodopyridine **5**, the Negishi cross-coupling reaction with 20 mol% Pd-PEPPSI-IPr gave the product **8** in only 32% yield (entry 5). However, the reaction run with 10 mol% $Pd_2(dba)_3$ and 40 mol% P(2-furyl)$_3$ afforded **8** in 76% yield (entry 6). An air

and moisture stable NHC-catalyst, Pd-PEPPSI-IPr can be useful for sp^3-sp^3 bond formation, and thus is known as a stronger catalyst than those used under normal Negishi conditions. Therefore, in the case of 3,5-diiodopyridine, the conditions with Pd-PEPPSI-IPr might be hard on the substrate, resulting in decomposition.

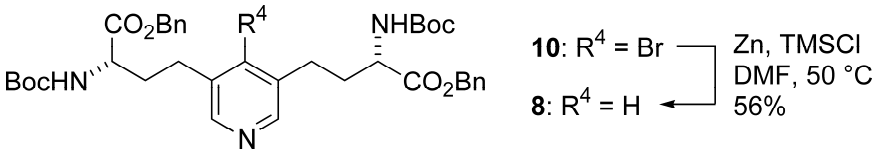
Table 1. Negishi cross-coupling reaction of **4**/**5** and **7**



entry[a]	R^3	Pd cat	ligand or additive	yield (**8**, %)[b]	yield (**9**, %)[b]
1	Br	$Pd_2(dba)_3$, 10[c]	P(2-furyl)$_3$, 40[c]	39	n.d.[e]
2	Br	Pd-PEPPSI-IPr, 20[c]	-	59	30
3	Br	Pd-PEPPSI-IPr, 20[c]	LiBr, 2[d]	24	0
4	Br	Pd-PEPPSI-IPr, 20[c]	LiCl, 2[d]	26	0
5	I	Pd-PEPPSI-IPr, 20[c]	-	32	n.d.
6	I	$Pd_2(dba)_3$, 10[c]	P(2-furyl)$_3$, 40[c]	76	n.d.

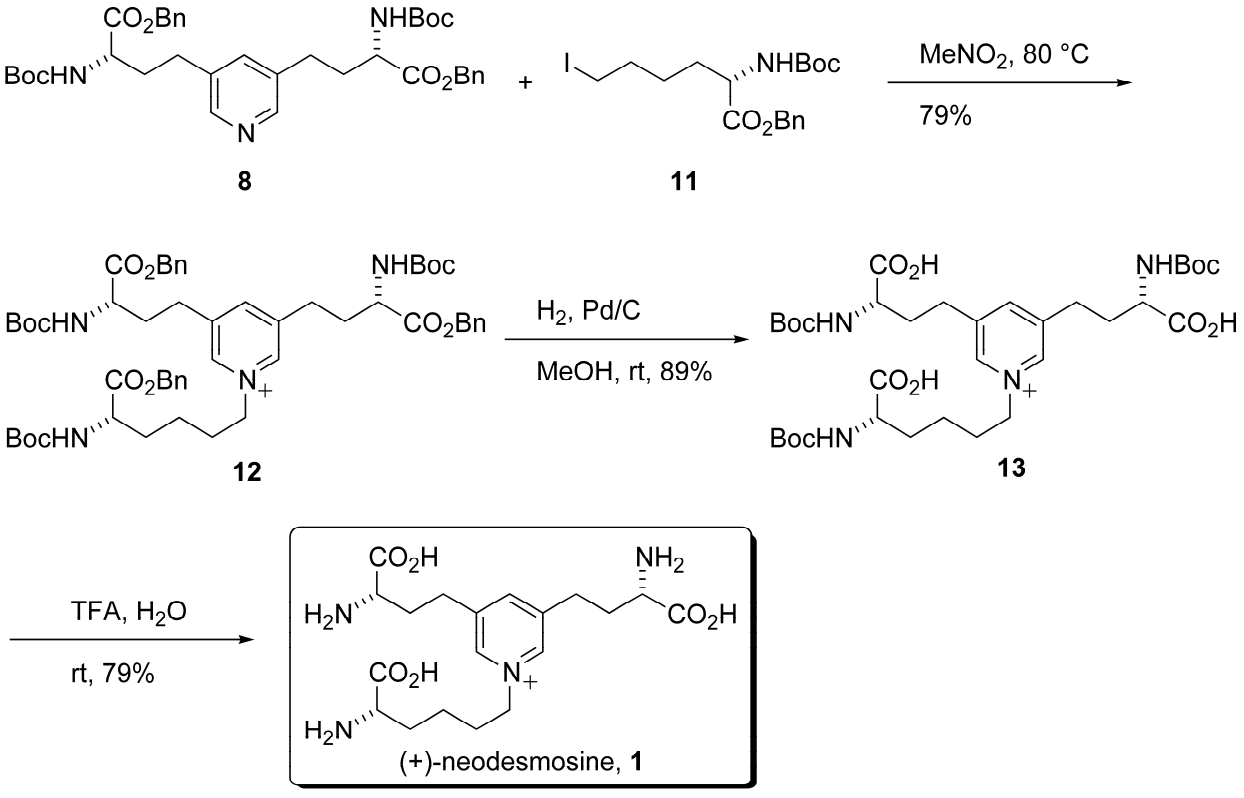
[a] Reaction was run in DMF at 50 °C for 24 h. [b] Isolated yield. [c] mol% [d] equivalent [e] n.d. = no data

Dicoupled product **8** can also be prepared from 3,5-dialkyl-4-bromopyridine **10**, which was obtained in our previous synthetic study of desmosine **2**.[7b] Hydrodehalogenation (hydrodebromination) of **10** was thus carried out using zinc in DMF to give **8** in 56% yield (Scheme 1).



Scheme 1. Hydrodebromination of **10**

Formation of the pyridinium salt of the obtained **8** with protected ω-iodoalkylated L-glycine **11**, which can be obtained from commercially available 5-benzoxyl-(*S*)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid in seven steps,[9] was then conducted in $MeNO_2$ at 80 °C to produce **12** in 79% yield (Scheme 2).[14] After reduction of the three benzyl groups with H_2 and Pd/C to give the tricarboxylic acid **13** in 89% yield, the three *t*-butoxycarbonyl protecting groups were successfully removed using TFA to provide the crude product. Purification by reversed-phase HPLC [column: Cosmosil $5C_{18}$-Ar-II (10 × 250 mm); mobile phase: MeOH/H_2O (1/9, linear gradient); flow rate: 1.5 mL/min; detection: 270 nm; temperature: 40 °C; R_t (**1**) = 7.3-8.5 min] afforded pure neodesmosine **1** in 79% yield. Spectroscopic data obtained for **1**, including that from ^{1}H NMR, MS, and UV analyses, were in good agreement with those reported for natural **1**.[2]



Scheme 2. Total synthesis of neodesmosine **1**

In summary, the total synthesis of neodesmosine **1**, a crosslinking pyridinium amino acid of elastin, has been achieved for the first time. The synthesis was conducted in 33% yield over four steps starting from 3,5-dibromopyridine **4** with a Negishi cross-coupling reaction as the key transformation. The synthesis described above is currently being applied to the preparation of other crosslinking amino acids[5] in order to elucidate the three dimensional structure of elastin fibers.

EXPERIMENTAL

All non-aqueous reactions were conducted under an atmosphere of nitrogen with magnetic stirring using dry solvents unless otherwise indicated. Dimethylformamide (DMF) was dried by distillation and stored over activated molecular sieves. Trimethylsilyl chloride (TMSCl), diisopropylethylamine (iPr$_2$NEt), and nitromethane ($MeNO_2$) were dried by distillation. Dehydrated methanol (MeOH) for the reactions was purchased from Kanto Chemicals (Tokyo, Japan). All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Centrifugation was performed by LMS Mini Centrifuge MCF-2360 (6,600 rpm). Analytical thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates produced by Merck. Column chromatography was performed with acidic Silica gel 60 (spherical, 40-50 μm) or neutral Silica gel 60N (spherical, 40-50 μm) produced by Kanto Chemicals.

Melting points were measured by an AS one ATM-01 apparatus. Optical rotations were measured on a JASCO P-2200 digital polarimeter at the sodium lamp (λ = 589 nm) D line and are reported as follows: $[\alpha]_D^T$ (c g/100 mL, solvent). UV spectra were recorded on a JASCO V-560 UV/VIS spectrophotometer and are reported in wavelengths (nm). Infrared (IR) spectra were recorded on a JASCO FT-IR 4100 spectrometer and are reported in wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EXC 300 spectrometer (300 MHz) or on a JEOL JNM-ECA 500 spectrometer (500 MHz). ^{1}H NMR data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J) in Hz, assignments. ^{13}C NMR data are reported in terms of chemical shift (δ, ppm). EI-MS spectra were recorded on a Shimadzu GCMS QP-5050 instrument. ESI-MS spectra were recorded on a JEOL JMS-T100LC instrument. Mass spectroscopic data were reported in m/z. JASCO HPLC systems PU-2085, MD-2010, and CO-2060 were used for the purification of neodesmosine **1**.

The carbon numbering on ^{1}H NMR of all compounds is corresponding with **1**.

3,5-Diiodopyridine 5: The mixture of 3,5-dibromopyridine **4** (500 mg, 2.1 mmol), KI (11.5 g, 69.1 mmol) and CuI (1.05 g, 55.1 mmol) was placed in a 100 mL two-necked flask. DMF (45 mL) was added, and the flask was heated at 180 °C under N_2. After 1 week, the flask was allowed to cool to room temperature and the solution was poured onto ice water. The precipitate was filtered, and dissolved in

CH_2Cl_2/pyridine (5/1) solution. Saturated Na-EDTA solution was added to remove excess Cu component from the organic layer, and the mixture was stirred overnight. After removal of the organic layer, the aqueous layer was then extracted with ethyl acetate (EtOAc). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product as a green solid. Purification by flash column chromatography (hexane/EtOAc = 10/1) afforded the pure **5** (438.3 mg, 1.32 mmol, 63%) as a colorless solid. The remaining colorless solid was recrystallized from benzene to give a colorless solid; R_f 0.57 (hexane/EtOAc = 5/1); mp 165-168 °C; IR (KBr, cm^{-1}) 3067, 2999, 1527, 1398, 1107, 1068, 999, 876, 723, 688, 636; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (2H, s, H2/6), 8.36 (1H, t, J = 2.1 Hz, H4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 151.6; EI-MS (*m/z*) calcd for $C_5H_3I_2N$ $[M]^+$ 330.84, found 330.70.

(*2S,2'S*)-Benzyl 4,4'-(pyridine-3,5-diyl)bis(2-(*tert*-butoxycarbonylamino)butanoate) 8 and *2S*-benzyl-4-(5-bromopyridin-3-yl)-(2-*tert*-butoxycarbonylamino)butanoate 9: Zinc dust (200 mg, 3.0 mmol) was placed in a nitrogen-purged 1.5 mL Eppendorf microtube. Dry DMF (150 μL) and TMSCl (60.0 μL, 0.47 mmol) were added to the microtube, and the resulting mixture was stirred vigorously for 15 min at room temperature. After stirring, the solution was removed by microsyringe. The remaining solid was dried using a hot air gun at reduced pressure. The activated zinc was then cool to room temperature. A solution of benzyl 2-(*S*)-((*tert*-butoxycarbonyl)amino)-3-iodobutanoate **6**[9] (210 mg, 0.5 mmol) in dry DMF (150 μL and washed with 100 μL DMF) was added to the activated zinc. The reaction mixture was stirred at room temperature for 1 h. The insertion of zinc to give the organozinc reagent **7** was monitored by TLC analysis (hexane/EtOAc = 5/1). After completion of the insertion, the zinc solution was allowed to settle using a centrifuge for 1 min at room temperature.

The solution of organozinc reagent **7** was removed via microsyringe with 200 μL DMF and added to a 10 mL flask containing Pd-PEPPSI-IPr (13.6 mg, 20 mol%) and the 3,5-dibromopyridine **4** (23.9 mg, 0.10 mmol, 1.0 eq). After stirring for 24 h at 50 °C, the reaction mixture was diluted with EtOAc and quenched with a saturated aqueous NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product as a yellow oil. Purification by flash column chromatography (hexane/EtOAc = 1/1 → 3/1) afforded the pure dicoupled product **8** (39.4 mg, 59.5 μmol, 59%) as a yellow oil and byproduct monocoupled **9** (13.5 mg, 30.0 μmol, 30%) as a yellow powder, respectively. **8**: R_f 0.52 (hexane/EtOAc = 1/2); $[\alpha]_D^{20}$ +10.3 (*c* 0.1, $CHCl_3$); IR (neat, cm^{-1}) 3354, 2976, 2932, 1713, 1500, 1455, 1366, 1252, 1166, 1050, 1026, 916, 865, 751, 699, 604, 461; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (2H, s, H2/6), 7.35 (10H, s, Bn), 5.23-5.10 (6H, m, Bn, NH), 4.36 (2H, d, J =5.4 Hz, H15/15'), 2.66-2.58 (4H, m, H13/13'), 2.17-2.07 (2H, m, H14/14'), 1.94-1.86 (2H, m, H14/14'), 1.44 (18H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3,

155.4, 147.7, 136.2, 135.4, 135.3, 128.8, 128.7, 128.6, 80.2, 67.3, 53.3, 34.2, 28.7, 28.4; ESI-HRMS (*m/z*) calcd for $C_{37}H_{47}N_3NaO_8$ $[M+Na]^+$ 684.3260, found 684.3256. **9**: R_f 0.39 (hexane/EtOAc = 2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (1H, s, H2/6), 8.27 (1H, s, H2/6), 7.56 (1H, s, H4), 7.37 (5H, s, Bn), 5.25-5.10 (3H, m, Bn, NH), 4.40 (1H, m, H15), 2.68-2.47 (2H, m, H13), 2.13 (1H, m, H14), 1.92 (1H, m, H14), 1.45 (18H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 148.9, 148.1, 138.6, 135.3, 128.9, 80.4, 67.5, 53.2, 34.1, 28.4; EI-MS (*m/z*) calcd for $C_{13}H_{18}BrN_2O_2$ $[M-CO_2Bn]^+$ 313.06, found 313.05, calcd for $C_{13}H_{18}BrN_2O_2$ $[M-CO_2Bn]^+$ 313.06, found 313.05; $C_{17}H_{16}BrN_2O_3$ $[M-tBu]^+$ 375.03, found 375.05.

(*2S,2'S*)-Benzyl 4,4'-(pyridine-3,5-diyl)bis(2-(*tert*-butoxycarbonylamino)butanoate) 8: Zinc dust (200 mg, 3.0 mmol) was placed in a 10 mL flask. Dry DMF (150 μL) and TMSCl (60.0 μL, 0.47 mmol) were added to the flask, and the mixture was stirred vigorously for 15 min at room temperature. After stirring, the solution was removed by microsyringe. A solution of (*2S,2'S*)-benzyl 4,4'-(4-bromopyridine-3,5-diyl)bis(2-(*tert*-butoxycarbonylamino)butanoate) **10**[7b] (21.1 mg, 28.5 μmol) in dry DMF (100 μL and washed with 100 μL DMF) was added to the activated zinc. After stirring for 24 h at 50 °C, the reaction mixture was diluted with EtOAc and quenched with a saturated aqueous NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product as a yellow oil. Purification by flash column chromatography (hexane/EtOAc = 1/1 → 3/1) afforded the pure dicoupled product **8** (10.6 mg, 16.0 μmol, 56%) as a yellow oil.

3,5-Bis((*S*)-4-(benzyloxy)-3-(*tert*-butoxycarbonylamino)-4-oxobutyl)-1-((*S*)-6-(benzyloxy)-5-(*tert*-butoxycarbonylamino)-6-oxohexyl)pyridinium 12: A mixture of **8** (41.9 mg, 63.3 μmol) and benzyl 2-(*S*)-((*tert*-butoxycarbonyl)amino)-6-iodohexanoate **11**[9] (56.6 mg, 126.6 μmol) in $MeNO_2$ (1.5 mL) was heated at 80 °C for 24 h. The reaction mixture was concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 1/1 → CH_2Cl_2/MeOH = 10/1) afforded **12** (55.6 mg, 50.1 μmol, 79%) as a yellow solid; R_f 0.58 (CH_2Cl_2/MeOH = 10/1); $[\alpha]_D^{20}$ +3.2 (*c* 0.1, $CHCl_3$); IR (KBr, cm^{-1}) 3371, 2976, 2932, 1710, 1499, 1455, 1366, 1256, 1166, 1051, 1026, 864, 752, 699, 581; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (2H, s, H2/6), 7.48-7.30 (20H, m, Bn), 6.05 (1H, s, 16NH), 5.63 (2H, m, 20NH/20'NH), 5.24-5.12 (4H, m, Bn), 4.72-4.70 (2H, m, H7), 4.58 (1H, m, H16), 4.35-4.27 (3H, m, H11/20/20'), 3.16 (2H, t, *J* = 5.1 Hz, H15), 2.87 (4H, m, H18/18'), 2.22-2.02 (6H, m, H8/19/19'), 1.76-1.62 (4H, m, H9/10), 1.42-1.45 (36H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 171.7, 155.7, 145.6, 142.1, 142.0, 135.4, 135.2, 128.7, 128.6, 128.5, 80.3, 67.5, 67.2, 61.5, 52.6, 32.8, 31.9, 30.8, 28.8, 28.4, 22.2; ESI-HRMS (*m/z*) calcd for $C_{55}H_{73}N_4O_{12}$ $[M]^+$ 981.5225, found 981.5218.

3,5-Bis((*S*)-3-(*tert*-butoxycarbonylamino)-3-carboxypropyl)-1-((*S*)-5-(*tert*-butoxycarbonylamino)-5-carboxypentyl)pyridinium 13: A solution of **12** (50.1 mg, 45.2 μmol) in MeOH (1.0 mL) was treated

with 10% Pd/C (271.1 mg, 254.7 μmol) and hydrogenated at balloon pressure at room temperature. After stirring for 24 h at room temperature, the insoluble was separated by filtration through a Celite pad on neutral silica gel eluting with MeOH. The filtrate was then concentrated *in vacuo*. Concentration of the filtrate yielded tetracarboxylic acid **13** (33.7 mg, 40.2 μmol, 89%) as a yellow solid. The product was used to the next reaction without further purification; ^{1}H NMR (300 MHz, CD_3OD) δ 8.73 (2H, s, H2/6), 8.37 (1H, s, H4), 4.56 (2H, m, H7), 3.93 (3H, m, H11/15/15'), 2.90 (4H, m, H13/13'), 2.16-2.05 (6H, m, H8/14/14'), 1.77 (2H, m, H10), 1.41 (38H, m, *t*Bu/H9); ESI-HRMS (*m/z*) calcd for $C_{34}H_{55}N_4O_{12}$ $[M]^+$ 711.3816, found 711.3816.

(+)-Neodesmosine 1; 3,5-bis((*S*)-3-amino-3-carboxypropyl)-1-((*S*)-5-amino-5-carboxypentyl)-pyridinium: A mixture of trifluoroacetic acid (TFA) and distilled water (3.0 mL, 95/5 ratio) was added to the crude tricarboxylic acid **13** (14.7 mg 17.5 μmol) at room temperature and stirred for 2 h. The solvent was concentrated *in vacuo*. Purification on C18 silica gel column chromatography (0.1% TFA in distilled water) afforded the crude product as a yellow oil (27.7 mg). The crude product was then purified by reversed phase HPLC system. The conditions were as follows: column, Cosmosil 5C_{18}-AR-II (10 × 250 mm, Nacalai tesque, Kyoto); solvent, linear gradient of 10% MeOH and 90% H_2O; flow rate, 1.5 mL/min; detection, 270 nm; temperature, 40 °C; R_t = 7.3-8.5 min (neodesmosine **1**). As a result, 7.3 mg of pure neodesmosine **1** was obtained as a colorless oil in 79%. R_f 0.32 [MeOH (0.1% TFA)/H_2O (0.1% TFA) = 1/9]; $[\alpha]_D^{20}$ +8.7 (*c* 0.10, H_2O); UV λ_{max}: 270 nm in H_2O (lit. value: λ_{max}: 270 nm in 0.1 M HCl)[3]; ^{1}H NMR (500 MHz, D_2O) δ 8.65 (2H, s, H2/6), 8.37 (1H, s, H4), 4.58 (2H, t, *J* = 7.0 Hz, H7), 3.78 (2H, t, *J* = 6.0 Hz, H15/15'), 3.73 (2H, t, *J* = 6.0 Hz, H11/11'), 2.97 (4H, m, H13/13'), 2.23 (4H, m, H14/14'), 2.06 (2H, t, *J* = 7.4 Hz, H8), 1.93-1.88 (2H, m, H10), 1.52-1.36 (2H, m, H9); ^{13}C NMR (75 MHz, D_2O) δ 175.2, 174.8 (C12/16), 146.2 (C4), 142.5 (C2/6 or 3/5), 142.2 (C2/6 or 3/5), 61.8 (C7), 55.0 (C11), 54.7 (C15), 31.7 (C14/14'), 30.7 (C8 or 10), 30.4 (C8 or 10), 28.4 (C13/13'), 21.7 (C9); ESI-LRMS (*m/z*) calcd for $C_{19}H_{31}N_4O_6$ $[M]^+$ 411.22, found 411.24. ESI-HRMS (*m/z*) calcd for $C_{19}H_{31}N_4O_6$ $[M]^+$ 411.2244, found 411.2244. These data were good agreement with natural **1**.[2b]

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