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SYNTHESIS OF *TRANS*-2,6-PIPERIDINEDICARBOXAMIDE USING THE UGI REACTION. A PLAUSIBLE MODEL FOR THE BIOSYNTHESIS OF HALICHONADIN P

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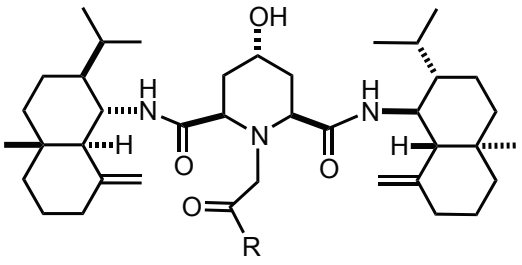
Abstract – The Ugi reaction of isocyanides, glutaraldehyde and glycine methyl ester is found to produce *trans*-2,6-piperidinedicarboxamides. It is proposed that this multi-component reaction is a plausible model for the biosynthesis of the marine natural product, halichonadin P.

INTRODUCTION

In 2012, Kobayashi and his co-workers at Hokkaido University reported the isolation and structural elucidation of the two sesquiterpenoids, halichonadins K (**1a**) and L (**1b**), from marine sponges collected at Unten Port in Okinawa Island (Figure 1).[1] The structures of these marine natural products are unique in that they are comprised of two homo-sesquiterpenes connected through two *cis*-disposed amide bonds to the 2,6-positions of a 4-hydroxy-piperidine ring.

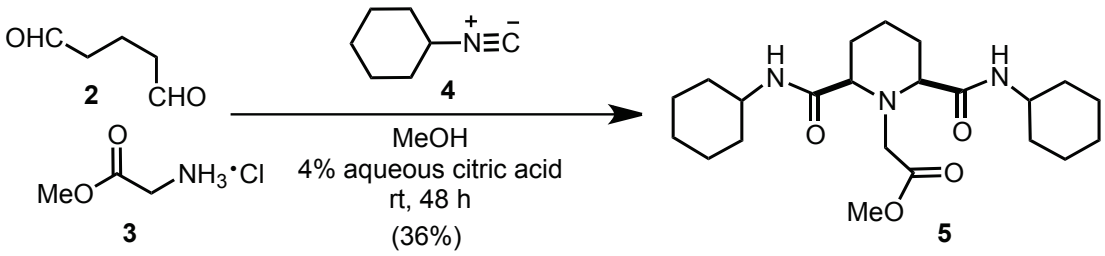


halichonadin K (**1a**): R = OMe
halichonadin L (**1b**): R = $NHCH_2CH_2Ph$

Figure 1. Structures of halichonadins K and L

Kobayashi and his co-workers proposed a possible pathway for the biosynthesis of these terpenoids, which involves reaction of the terpene isocyanide, halichonadin C with an iminium ion intermediate

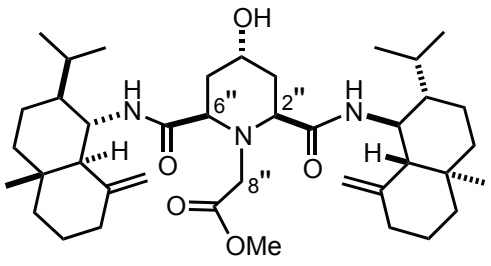
derived from glyoxylic acid and lysine. Poupon and his co-workers explored this hypothetical biosynthesis in studies of a one-pot cascade assembly of the central core of halichonadins K and L (Scheme 1).[2] The model Ugi-type coupling reaction between glutaraldehyde (**2**), glycine methyl ester (**3**) and cyclohexyl isocyanide (**4**), was reported to produce the *cis*-2,6-piperidinedicarboxamide **5** in a 36% yield.



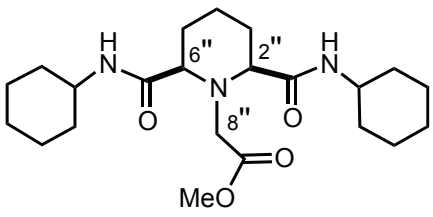
Scheme 1. One-pot three-component reaction reported by Poupon

The structure and stereochemistry of the Ugi product **5** was assigned using mainly NMR spectroscopic methods. Key ^{1}H NMR data of halichonadin K (**1a**) and the substance **5** synthesized by Poupon are summarized in Table 1.

Table 1. ^{1}H NMR data for halichonadin K (**1a**) and Poupon's product **5** measured in C_5D_5N



halichonadin K (**1a**)



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	halichonadin K	Poupon's product
H-2''	4.48 (dd, *J* = 10.9, 3.2 Hz)	4.15 (2H, m)
H-6''	4.49 (dd, *J* = 9.2, 4.1 Hz)	
H-8''	3.98 (1H, d, *J* = 18.1 Hz) 3.85 (1H, d, *J* = 18.1 Hz)	3.85 (1H, d, *J* = 17.2 Hz) 3.68 (1H, d, *J* = 17.2 Hz)

* NMR data of **1a** and **5** are reproduced from the respective ref. 1 and 2.

The chemical shifts and coupling constants for geminal protons at C-8'' (halichonadin numbering) in halichonadin K (**1a**) are similar to those of the corresponding methylene protons in Poupon's product. Specifically, although the central *cis*-piperidine moiety in halichonadin K (**1a**) is achiral, its geminal protons at C-8'' have a diastereotopic relationship due to the presence of stereogenic centers in the two

homo-sesquiterpene units. As a result of this relationship, the geminal protons at C-8'' in halichonadin K (**1a**) have different chemical shifts and couple to one another producing the AB pattern. In contrast, the *cis*-structure **5** proposed by Poupon possesses a plane of symmetry, and consequently its geminal protons at C-8'' are enantiotopic and should resonate as a singlet in the ^{1}H NMR. Contrary to expectations, the methylene protons at C-8'' in Poupon's product display the AB quartet pattern. These analyses led us to the conclusion that Poupon's assignment of **5** as the meso-diastereomer with *cis*-stereochemistry is incorrect and that the structure of Poupon's product is *trans*-isomer **6** as represented in Figure 2.[3] As a result of these considerations, we launched a more detailed investigation of the Ugi reaction of isocyanides, glutaraldehyde and glycine methyl ester and demonstrated that this process produces *trans*- not *cis*-2,6-piperidinedicarboxamides.

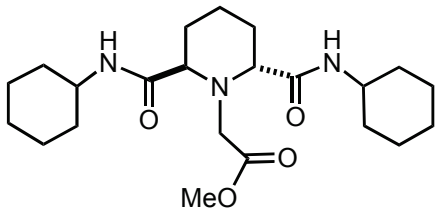

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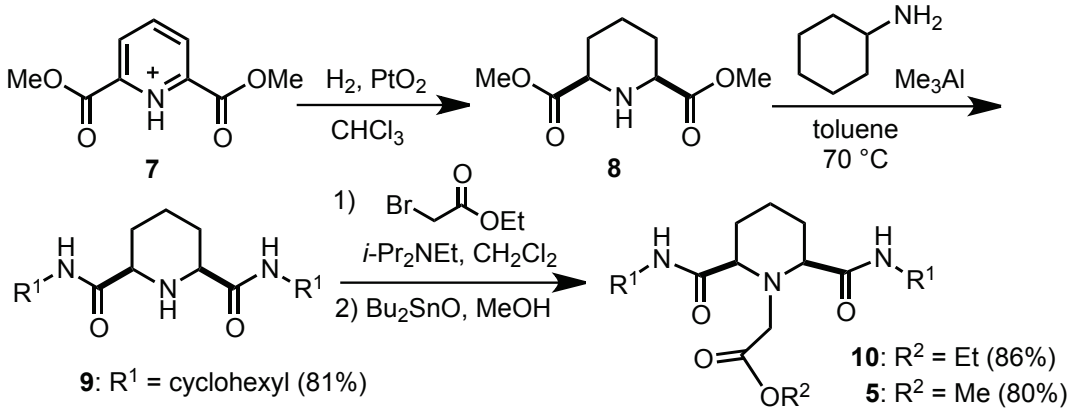
Figure 2. A newly proposed structure of the Poupon's product

RESULTS AND DISCUSSION

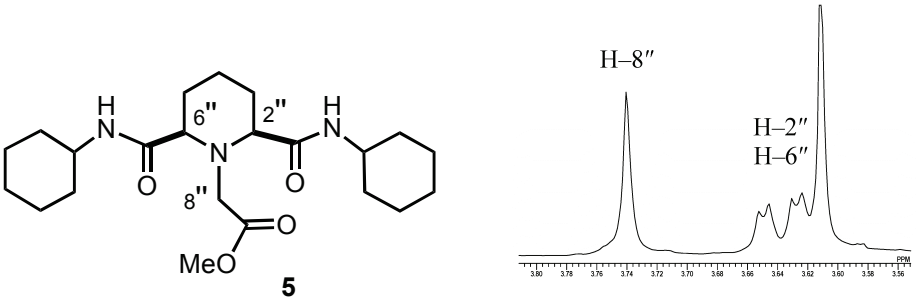
In order to determine if the conclusion presented above is correct, we first carried out a stereochemically unambiguous synthesis of *cis*-2,6-piperidinedicarboxamide **5** (Scheme 2). The known *cis*-dimethyl ester **8** was prepared by catalytic hydrogenation of the sulfate salt of **7**.[4] Conversion of dimethyl ester **8** to dicyclohexylamide **9** was achieved by using the Weinreb's method with cyclohexylamine and trimethylaluminum in toluene in 81% yield.[5] Alkylation of **9** with ethyl α-bromoacetate followed by tin-mediated transesterification of **10** furnished the methyl ester **5** in 70% yield over two steps.[6]



7 **8**

Scheme 2. Synthesis of *cis*-2,6-piperidinedicarboxamide **5**

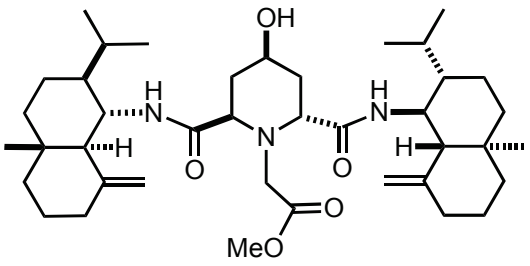
Selected regions of the ^{1}H NMR spectrum and data for synthetic *cis*-2,6-piperidinedicarboxamide **5** are displayed in Figure 3. As expected, the enantiotopic geminal protons at C-8'' in the synthetic meso isomer **5** resonate as a singlet. In addition, the coupling constants of the chemical-shift equivalent protons at C-2'' and C-6'' are 10.7 and 3.5 Hz, suggesting that these two protons are axially disposed and that the two cyclohexylamide moieties occupy equatorial positions. Moreover, these coupling constants observed in **5** are similar to those of the respective diastereotopic protons at C-2'' (dd, J = 10.9, 3.2 Hz) and C-6'' (dd, J = 9.2, 4.1 Hz) found in the spectrum of halichonadin K (**1a**) (Table 1). The current results demonstrate conclusively that the 2,6-piperidinedicarboxamide derivative described earlier by Poupon has *trans*-stereochemistry (Figure 2).



^{1}H NMR (C_5D_5N)	
H-2'' and H-6''	3.64 (2H, dd, J = 10.7, 3.5 Hz)
H-8''	3.74 (2H, s)

Figure 3. ^{1}H NMR spectra and data for the *cis*-2,6-piperidinedicarboxamide **5**

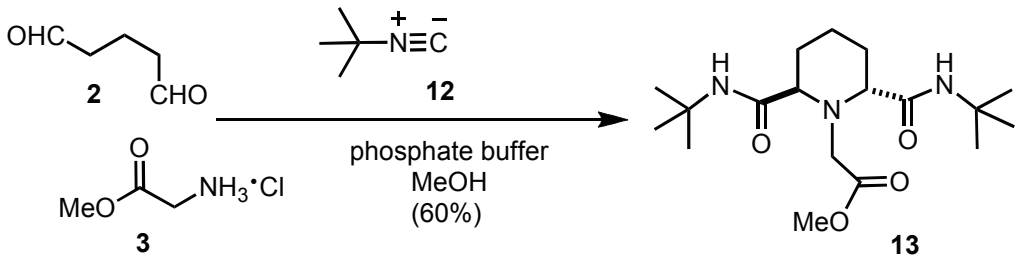
The fact that the Ugi reaction between cyclohexyl isocyanide, glutaraldehyde and glycine methyl ester produces a *trans*-2,6-piperidinedicarboxamide is interesting in light of the structure and stereochemistry of halichonadin P (**11**) (Figure 4), a marine natural product reported by the Kobayashi group in 2015.[7] The central core of *trans*-2,6-piperidinedicarboxamide found in halichonadin P suggests that the Ugi reaction might be involved in the biosynthesis of halichonadin P.



halichonadin P (**11**)

Figure 4. Structure of halichonadin P possessing *trans*-2,6-piperidinedicarboxamide moeity

As a model of a plausible biosynthesis of halichonadin P, three component reaction of *t*-butyl isocyanide (**12**), glutaraldehyde (**2**) and glycine methyl ester (**3**) was explored (Scheme 3). Unfortunately, when the reaction was carried out using the conditions reported by Poupon (MeOH and 4% aq. citric acid at room temperature), the piperidine bis-amide **13** was generated in only a 10% yield. Since this Ugi reaction is related to the Robinson–Schöpf synthesis, we applied the reaction conditions reported in the synthesis of pseudopelletierine to this Ugi reaction.[8] To our delight, the reaction of *t*-butyl isocyanide (**12**), glutaraldehyde (**2**) and glycine methyl ester (**3**), carried out in phosphate buffer for a prolonged reaction time of one week, led to the formation of **13** in 60% yield.



Scheme 3. One-pot synthesis of the central core of halichonadin P

Recrystallization of the Ugi product **13** from a mixture of dichloromethane and hexane produced crystals that are suitable for X-ray analysis (Figure 5). An ORTEP plot of the crystallographic data along with a partial ^{1}H NMR spectrum of **13** are displayed in Figure 5. Analysis of the plot shows that the two N-*t*-butyl carboxamide groups in **13** are *trans*-disposed. Moreover, in the ^{1}H NMR spectrum, the H-8'' methylene protons appear as an AB quartet (3.24 and 3.40 ppm, 1H each, J = 17.8 Hz). In addition, the broad signal at 3.22 ppm (2H) is likely associated with H-2'' and H-6'' which undergo slow pseudo-axial to pseudo-equatorial interconversion. This conformational behavior would explain the degeneracy of the carbon absorption in the definitive nine-line ^{13}C NMR spectrum of **13**.

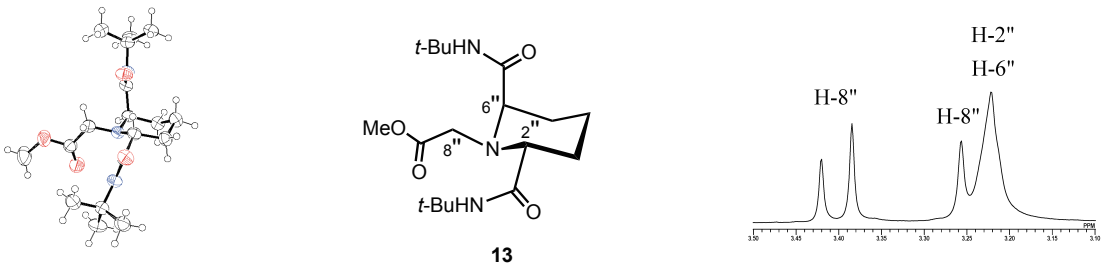
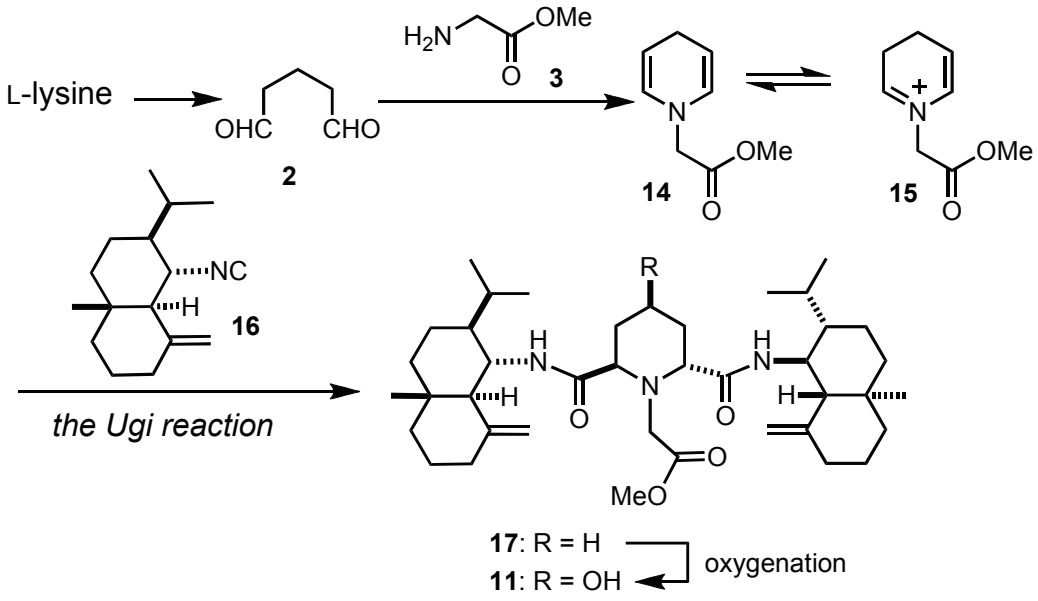


Figure 5. ORTEP plot of the X-ray data and selected ^{1}H NMR ($CDCl_3$) of **13**

In the investigation developed here, we demonstrated that the Ugi reaction using isocynanides, glutaraldehyde and glycine methyl ester generate *trans*- and not *cis*-2,6-piperidinedicarboxamides. The

stereochemical outcome of this Ugi reaction leading to the *trans*-2,6-disubstitued piperidine would be explained by the allylic $A^{1,2}$ strain and stereoelectronic control from the axial direction.[9] Moreover, we envisaged that the process is a plausible model for the biosynthesis of halichonadin P (Scheme 4). Specifically, following proposals made by Kobayashi and Poupon, we suggest that the route for halichonadin P biosynthesis is initiated by condensation of glutaraldehyde (**2**), derived by bis-deamination of lysine, and glycine methyl ester (**3**) to produce the 1,4-dihydropyridine **14**. Rapid tautomerization of **14** generates iminium intermediate **15**, which is then captured by two moles of halichonadin C (**16**) to form the piperidine bis-amide **17**. Importantly, halichonadin C (**16**) is a terpene isocyanide isolated from the the same marine sponge *Halichondria* sp. as is halichonadin P.[10] The product of the Ugi reaction is further transformed to halichonadin P (**11**) through an oxygenase-catalyzed hydroxylation reaction.



Scheme 4. Plausible pathway for the biosynthesis of halichonadin P

EXPERIMENTAL

***N*^{2},*N*^{6}-Dicyclohexylpiperidine-2,6-dicarboxamide (9).** To a solution of **8** (700 mg, 3.48 mmol) and cyclohexylamine (4.0 mL, 34.8 mmol) in toluene (30.0 mL) cooled to 0 °C under argon atomosphere was added a solution of trimethylaluminum (1.0 M solution in hexane, 14.7 mL, 14.7 mmol). The solution was warmed up to room temperature and then heated at 70 °C for 6 h. The reaction mixture was cooled to 0 °C, quenced by the addtion of MeOH (0.50 mL), and transferred to Erlenmyer flask. AcOEt (80 mL), water (200 mL) and sodium potassium tartrate (29.2 g, 103 mmol) were added, and the mixture was vigorously stirred. The white precipitates were filtered to give **9** as white crystals (948 mg, 81%); mp 190 °C (decomp.) (recrystallized from MeOH/CH_2Cl_2); IR (KBr) ν_{max} 3297, 3082, 2935, 2854, 1645,

1557, 1449 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.08–1.53 (m, 12H), 1.61 (brd, *J* = 13.5 Hz, 2H), 1.70 (brd, *J* = 13.5 Hz, 4H), 1.82–2.20 (m, 9H), 3.21 (brd, *J* = 9.5 Hz, 2H), 3.76 (m, 2H), 6.28 (brd, *J* = 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.0, 24.8, 25.4, 29.5, 32.9, 33.0, 47.7, 60.2, 172.0. HRMS (ESI): *m/z* calcd for $C_{19}H_{34}N_3O_2$ $[M+H]^+$ 336.2646, found 336.2641.

Ethyl 2-(2,6-bis(cyclohexylcarbamoyl)piperidin-1-yl)ethanoate (10). To a solution of **9** (848 mg, 2.53 mmol) and diisopropylethylamine (4.40 mL, 25.3 mmol) in CH_2Cl_2 (85.0 mL) under argon atomosphere was added ethyl bromoacetate (2.80 mL, 25.3 mmol). The solution was stirred at room temperature for 44 h, and then diluted with AcOEt (100 mL), water (100 mL) and satureated aqueous $NaHCO_3$ (100 mL). The separated aqueous layer was extracted with AcOEt (× 2), and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford white solids. Recrystallization from CH_2Cl_2 furnished **10** as white crystals (920 mg, 86%); mp 173–175 °C (recrystallized from CH_2Cl_2); IR (KBr) ν_{max} 3300, 3076, 2932, 2853, 1736, 1651, 1547 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.09–1.51 (m, 14H), 1.19 (t, *J* = 7.0 Hz, 3H), 1.57 (brd, *J* = 13.0 Hz, 2H), 1.61–1.78 (m, 6H), 1.83 (brd, *J* = 11.0 Hz, 2H), 1.98 (brd, *J* = 13.0 Hz, 2H), 3.02 (brd, *J* = 11.0 Hz, 2H), 3.18 (s, 2H), 3.65 (m, 2H), 4.09 (q, *J* = 7.0 Hz, 2H), 6.86 (brd, *J* = 8.0 Hz, 2H) ; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0, 22.0, 24.9, 25.4, 30.4, 32.4, 32.8, 47.9, 57.7, 61.3, 68.9, 171.5, 173.0. HRMS (ESI): *m/z* calcd for $C_{23}H_{40}N_3O_4$ $[M+H]^+$ 422.3013, found 422.3010.

Methyl 2-(2,6-bis(cyclohexylcarbamoyl)piperidin-1-yl)ethanoate (5). A solution of **10** (600 mg, 1.42 mmol) and dibutyltin oxide (35 mg, 0.14 mmol) in MeOH (15.0 mL) was heated at reflux for 84 h. The reaction mixture was diluted with water, saturated aqueous $NaHCO_3$ and AcOEt. The separated aqueous layer was extracted with AcOEt (× 3), and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford white solids (576 mg). Recrystallization from a mixture of CH_2Cl_2 and hexane (1:1) afforded **5** as white crystals (278 mg, 48%). The filtrate was concentrated and the resulting solids were crystallized from a mixture of CH_2Cl_2 and AcOEt (2:1) to give the second crops of **5** (100 mg, 17%). The filtrate was concentrated and the resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 20:1) to afford **5** (84 mg, total yield 80%) as white solids; mp 180–181 °C (recrystallized from CH_2Cl_2/hexane); IR (KBr) ν_{max} 3299, 2930, 2854, 1738, 1650, 1550, 1443 cm^{-1}; H NMR ($CDCl_3$, 500 MHz) δ 1.02–1.56 (m, 14H), 1.83–1.92 (m, 10H), 2.02 (brd, *J* = 12.5 Hz, 2H), 3.07 (brd, *J* = 9.5 Hz, 2H), 3.24 (s, 2H), 3.69 (s, 3H), 3.65–3.75 (m, 2H), 6.83 (brs, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.1, 24.9, 25.4, 30.4, 32.4, 32.8, 47.9, 52.3, 57.4, 69.0, 171.4, 173.4; ^1H NMR (C_5D_5N, 500 MHz) δ 0.92–1.32 (m, 10H), 1.43 (brd, *J* = 12.5 Hz, 2H), 1.52–1.69 (m, 4H), 1.76–2.05 (m, 10H), 3.61 (s, 3H), 3.64 (dd, *J* = 10.8, 3.5 Hz, 2H), 3.74 (s, 2H), 3.90–4.03 (m, 2H), 8.08 (brd, *J* = 8.0 Hz, 2H); ^{13}C NMR (C_5D_5N, 125 MHz) δ 22.4, 25.4, 25.8, 30.0, 33.0, 33.1, 48.4, 51.6, 54.5, 66.2, 172.38,

172.43. HRMS (ESI): *m/z* calcd for $C_{22}H_{38}N_3O_4$ $[M+H]^+$ 408.2857, found 408.2858.

Methyl 2-(2,6-*trans*-bis(*tert*-butylcarbamoyl)piperidin-1-yl)ethanoate (13). To a solution of glycine methyl ester hydrochloride (**3**) (78 mg, 0.62 mmol) in a mixture of phosphate buffer (100 μL) and MeOH (10.0 mL) was added *t*-butyl isocyanide (**12**) (0.14 mL, 1.25 mmol) and 25% aqueous glutaraldehyde (**2**) (0.75 mL, 1.31 mmol). The reaction mixture was stirred at room temperature for one week, and concentrated under reduced pressure. The resulting residue was dissolved in AcOEt and water. The separated aqueous layer was extracted with AcOEt (× 3). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The resulting residue (399 mg) was purified by silica gel chromatography (AcOEt/hexane 1:2) to afford **13** as white crystals (133 mg, 60%); mp 183–184 °C (recrystallized from CH_2Cl_2/hexane); IR (KBr) ν_{max} 3326, 3303, 2963, 2942, 1749, 1656, 1547, 1525 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.34 (s, 18H), 1.59–1.76 (m, 6H), 3.20–3.24 (m, 2H), 3.24 (d, 1H, *J* = 17.8 Hz), 3.40 (d, 1H, *J* = 17.8 Hz), 3.77 (s, 3H), 6.89 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 21.0, 28.6, 50.5, 51.3, 52.2, 62.0, 170.4, 173.2. HRMS (ESI): *m/z* calcd for $C_{18}H_{34}N_3O_4$ $[M+H]^+$ 356.2544, found 356.2539.

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