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Formal Total Synthesis of (+)-Gephyrotoxin

Marco Santarem, Corinne Vanucci-Bacqué,* and Gérard Lhommet*

UPMC Univ Paris 06, CNRS, UMR 7611, Laboratoire de Chimie Organique, Equipe Chimie des Hétérocycles, 4 Place Jussieu, F-75005 Paris, France

corinne.bacque@upmc.fr; gerard.lhommet@upmc.fr

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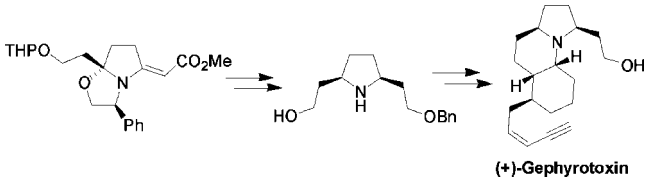
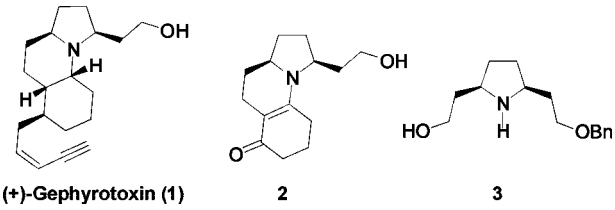


An efficient formal total synthesis of (+)-gephyrotoxin is described. The key step of our strategy relies on the diastereoselective reduction of a chiral pyrrolidine β-enamino ester obtained by condensation of (*S*)-phenylglycinol on a protected 8-hydroxy-3,6-dioxooctanoate.

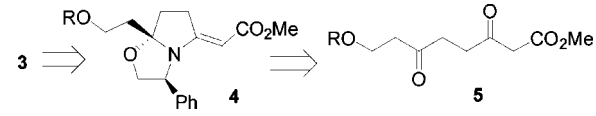
Gephyrotoxin is a naturally occurring alkaloid first isolated and characterized by Daly and co-workers in 1977 from the skin secretion of tropical frogs *Dendrobates histrionicus*.[1] This compound is relatively nontoxic and originally exhibited weak activity as a muscarinic antagonist.[2] More recent studies have shown it to display an interesting array of neurological activities.[3] As a result of these biological activities and the extreme scarcity of this natural product, the synthesis of gephyrotoxin and analogues has attracted much interest from several laboratories.[4,5] Among the different approaches, only two enantioselective syntheses of (+)-gephyrotoxin (**1**) have been reported both involving a common enantiopure tricyclic intermediate **2**. The first and only total synthesis has been reported by Kishi and co-workers,[6] who prepared intermediate derivative **2** in 18 steps from L-pyroglutamic acid. More recently, a formal synthesis of this alkaloid performed by Hsung and co-workers[7] described a shorter access to Kishi's intermediate **2** (10 steps) but included a poorly diastereoselective step.



In his strategy,[6] Kishi synthesized derivative **2** via the enantiopure *cis*-2,5-disubstituted pyrrolidine **3** that was obtained in 15 steps from L-pyroglutamic acid. We have recently reported the preparation of enantiopure *cis*-2,5-disubstituted pyrrolidine β-amino esters[8] by the diastereoselective reduction of chiral β-enamino esters, which in turn were obtained by condensation of a chiral amine on an ω-oxo β-ketoester.[9] We envisioned that we could apply our methodology to access to pyrrolidine **3**. We report herein the results of this work that afforded an efficient formal synthesis of (+)-gephyrotoxin (**1**).

Retrosynthetically, we anticipated that compound **3** could stem from the diastereocontrolled reduction of the appropriate chiral bicyclic pyrrolidine β-enamino ester **4**. The latter could be obtained by condensation of (*S*)-2-phenylglycinol on adequately protected 8-hydroxy-3,6-dioxooctanoate **5** (Scheme 1).

SCHEME 1



The most obvious access to our starting compound **5** involves the alkylation of the Weiler dianion of methyl acetoacetate **6**[10] by suitably functionalized derivatives. However, this approach turned out to lead to some disappointing results when we first attempted the condensation of the dianion of **6** with different compounds such as benzyloxy epoxide **7**,[11] variously protected 1,3-butanediol iodides **8** (R′ = Ac,[12] TBS, $SiEt_3$), and the cyclic sulfate ester **9**[13] (Scheme 2). In the case of success, these reactions would have led to the expected compounds **5** (R= Bn) after an oxidation step or a deprotection−oxidation sequence. Unfortunately, in all cases, only starting materials were surprisingly recovered.[14]

Funk and co-workers[15] reported recently the condensation of various β-keto ester dianions on 6-bromomethyl-4*H*-1,3-

(1) Daly, J. W.; Witkop, B.; Tokuyama, T.; Nishikawa, T.; Karle, I. L. *Helv. Chem. Acta* **1977**, *60*, 1128–1140.
(2) Mensah-Dwumah, M.; Daly, J. W. *Toxicon* **1978**, *16*, 189–194.
(3) Daly, J. W.; Spande, T. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier S. W. Ed.; John Wiley and Sons: New York, 1986; Vol. 4, pp 95−122.
(4) For previous total syntheses of racemic gephyrotoxin, see: (a) Fujimoto, R.; Kishi, Y.; Blount, J. F. *J. Am. Chem. Soc.* **1980**, *102*, 7154–7156. (b) Hart, D. J.; Kanai, K. *J. Am. Chem. Soc.* **1983**, *105*, 1255–1263. (c) Overman, L. E.; Lesuisse, D.; Hashimoto, M. J. *Am. Chem. Soc.* **1983**, *105*, 5373–5379.
(5) For previous formal syntheses of racemic gephyrotoxin, see: (a) Ito, Y.; Nakajo, E.; Nakatsuka, M.; Saegusa, T. *Tetrahedron Lett.* **1983**, *24*, 2881–2884. (b) Pearson, W. H.; Fang, W.-k. *J. Org. Chem.* **2000**, *65*, 7158–7174.
(6) Fujimoto, R.; Kishi, Y. *Tetrahedron Lett.* **1981**, *42*, 4197–4198.
(7) Wei, L.-L.; Hsung, R. P.; Sklenicka, H. M.; Gerasyuto, A. I. *Angew. Chem., Int. Ed.* **2001**, *40*, 1516–1518.
(8) Calvet-Vitale, S.; Vanucci-Bacqué, C.; Fargeau-Bellassoued, M.-C.; Lhommet, G. *Tetrahedron* **2005**, *61*, 7774–7782.
(9) David, O.; Calvet, S.; Chau, F.; Vanucci-Bacqué, C.; Fargeau-Bellassoued, M.-C.; Lhommet, G. *J. Org. Chem.* **2004**, *69*, 2888–2891.
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(11) Lygo, B. *Tetrahedron* **1988**, *44*, 6889–6896.
(12) Kwon, D. W.; Kim, Y. H.; Lee, K. *J. Org. Chem.* **2002**, *67*, 9488–9491.
(13) Bennett, C. E.; Figueroa, R.; Hart, D. J.; Yang, D. *Heterocycles* **2006**, *70*, 119–128.
(14) This lack of reactivity in particular with epoxide **7** was unexpected as the ring opening of this compound with the dianion of methyl 2-methylacetoacetate has been previously reported.[11]
(15) Greshock, T. J.; Funk, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 754–755.

10.1021/jo801150e CCC: $40.75
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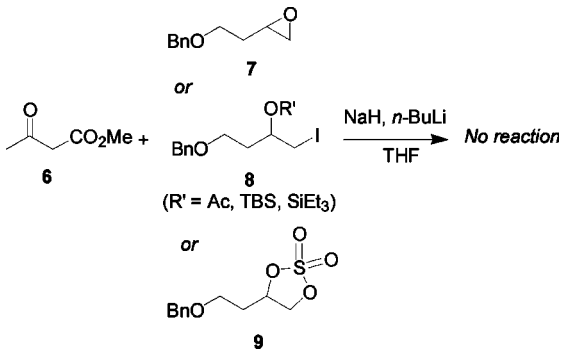
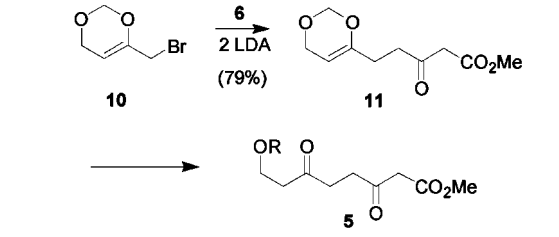
SCHEME 2



SCHEME 3



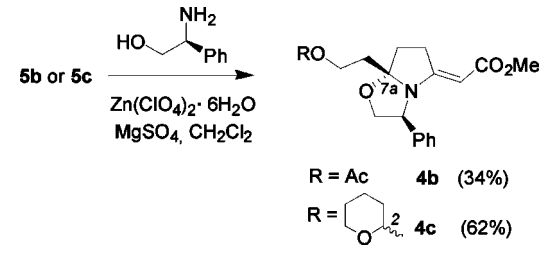
Reaction conditions	R	5 (Yields)[a]
p-TsOH (0.3 equiv), H_2O/CH_2Cl_2 (1:3)	H	**5a** (37%)
p-TsOH (0.1 equiv), Ac_2O (2 equiv), CH_2Cl_2	Ac	**5b** (52%)
p-TsOH (0.1 equiv), DHP(2 equiv), CH_2Cl_2	THP	**5c** (83%)

[a] isolated yields

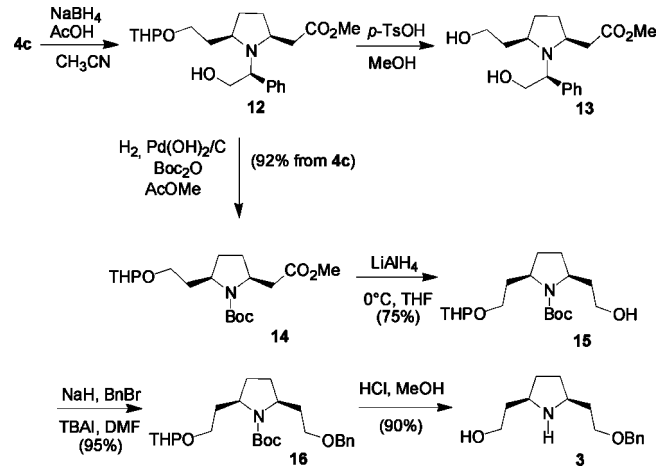
dioxin **10** as an equivalent of bromomethyl vinyl ketone. As for us, we envisioned compound **10** as a protected β-hydroxy ketone moiety. To our delight, reaction of the dianion of **6** with compound **10** afforded the expected β-keto ester **11** in 79% isolated yield (Scheme 3). While mild thermal retrocyclization of the 1,3-dioxin would give the corresponding vinyl ketone β-keto ester,[15] we found that acidic hydrolysis of the 1,3-dioxin moiety in the presence of *p*-TsOH and water afforded, as anticipated, β-hydroxy keto derivative **5a** (R = H) in 37% isolated yield (Scheme 3). At this stage, we envisaged to improve the isolated yield by in situ protection of the alcohol function. When the above deprotection step was performed in the presence of TBSCl, a mixture of starting material, alcohol **5a**, and unidentifiable products was obtained. On the other hand, reaction in the presence of acetic anhydride gave rise to acetate **5b** in 52% yield. The best result was obtained in the presence of dihydropyran providing tetrahydropyranyl ether **5c** in 83% yield (Scheme 3).

With the required protected ω-oxo β-keto esters **5b** and **5c** in hand, we turned our attention to their diastereoselective condensation with (*S*)-2-phenylglycinol to give the corresponding bicyclic chiral pyrrolidine β-enamino ester **4**[9] (Scheme 4). The first attempt to react **5b** in the presence of *p*-TsOH in benzene as previously described for analogous compounds[9] gave rise to the expected compound **4b** (R = Ac) as a single isomer in a disappointing 10% yield. A better result was obtained using catalytic amount of $Zn(ClO_4)_2.6H_2O$ and $MgSO_4$[16] to give oxazolidine **4b** in a still modest 34% yield. Its absolute configuration at C-7a was assigned as (*S*) based on previously obtained results for analogous compounds.[9] Condensation of

SCHEME 4



SCHEME 5

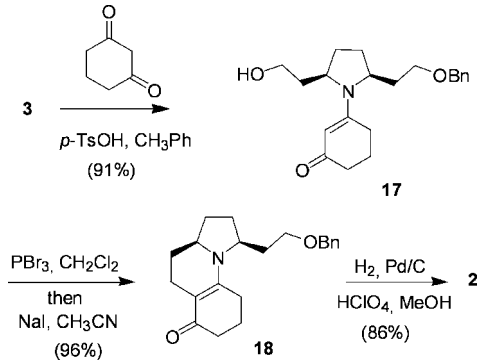


compound **5c** with (*S*)-2-phenylglycinol under the latter reaction conditions provided the expected oxazolidine **4c** (R = THP) in 62% isolated yield along with 25% recovery of **5c**. At this stage, compound **4c** appeared as an inseparable 1:1 mixture of diastereoisomers according to the NMR spectra. As we assumed that the condensation was diastereocontrolled at the C-7a center,[9] we assigned the epimeric carbon to be localized at C-2 of the THP group. This assumption was verified by the ultimate removal of the THP group as shown below. Considering the latter result, we chose to carry on the synthesis of our target compound from bicyclic β-enamino ester **4c**.

The key step of our strategy to prepare enantiopure compound **3** relied on the diastereoselective reduction of **4c** to give a *cis*-2,5-disubstituted pyrrolidine. As we have shown recently,[8] reduction using in situ generated sodium triacetoxyborohydride in acetic acid was considered an adequate method to obtain the target compound. Indeed, under these reaction conditions, the epimeric mixture of **4c** was diastereoselectively reduced to pyrrolidine **12** as two epimers in the same equimolar ratio (Scheme 5). In order to confirm the diastereoselective outcome of this reduction step, compound **12** was submitted to deprotection of the alcohol moiety in the presence of *p*-TsOH to give amino alcohol **13** as a single diastereoisomer according to GC and NMR (de > 95%). On the basis of previously obtained results,[8] the absolute configuration of **13** (and thus that of **12**) was assigned as to be (2*S*,5*R*). This stereochemistry was ultimately determined by the synthesis of compound **2**. Subsequent hydroxymethylbenzyl group removal of compound **12** (H_2, $Pd(OH)_2/C$) followed by in situ protection in the presence of Boc_2O gave rise to derivative **14** in 92% isolated overall yield from compound **4c** (two steps). Treatment with lithium aluminum hydride at 0 °C led to the chemoselective reduction of the ester moiety to give alcohol **15** in 75% isolated yield. The latter was subsequently *O*-benzylated (NaH, BnBr, DMF) to give **16**

(16) Noël, R.; Vanucci-Bacqué, C.; Fargeau-Bellassoued, M.-C.; Lhommet, G. *J. Org. Chem.* **2005**, *70*, 9044–9047.

SCHEME 6



in 95% isolated yield. Finally, simultaneous tetrahydropyranyl and Boc deprotections (HCl, MeOH) afforded the target pyrrolidine **3** in 90% isolated yield. Overall, this compound was obtained in eight steps from compound **10** in 31% overall yield to afford a new formal total synthesis of (+)-gephyrotoxin.

However, as compound **3** was not completely described in the literature,[6] we converted it into tricyclic derivative **2**, whose spectroscopic and physical data are known,[4a,5a,6] in order to secure its absolute configuration (Scheme 6). This was accomplished following Kishi's route[4a] by initial condensation with cyclohexane-1,3-dione (*p*-TsOH, $PhCH_3$) to give enamine **17** in 91% isolated yield. Access to tricyclic derivative **18** was carried out using a slightly modified procedure. The expected compound **18** was obtained in 96% overall yield by intramolecular cyclization in refluxing acetonitrile in the presence of NaI using the nonisolated bromide intermediate, which was in turn directly obtained by reacting alcohol **17** with PBr_3 in CH_2Cl_2.[17] Final hydrogenolysis of **18** (H_2, Pd/C, $HClO_4$, MeOH) gave rise to Kishi's intermediate **2** in 86% isolated yield. The spectroscopic data, the physical properties and the optical rotation of this compound [$[\alpha]^{20}_D$ +537 (*c* 2.0, EtOH)] corresponded to those reported in the literature[6] for the (1*S*, 3a*S*)-**2** diastereomer [$[\alpha]^{20}_D$ +538 (*c* 1.4, EtOH)], thus validating our configurational assignments.

In conclusion, we have completed an efficient total formal synthesis of (+)-gephyrotoxin. Our strategy was based on the obtention of an enantiopure *cis*-2,5-disubstituted pyrrolidine by the diastereoselective reduction of a chiral phenylglycinol-derived oxazolopiperidine enamino ester. Contrary to the other reported enantioselective syntheses which both involved one poorly diastereoselective step, our approach allowed the simultaneous creation of the two stereogenic centers of the target compound in a totally stereocontrolled manner and therefore compares very favorably to previous approaches.

Experimental Section

(3*S*,7a*S*)-[3-Phenyl-7a-[2-(tetrahydro-pyran-2-yloxy)ethyl]tetrahydropyrrolo[2,1-*b*]oxazol-5-ylidene]acetic Acid Methyl Ester (4c). To a solution of *β*-keto ester **5c** (3 g, 10.4 mmol) in CH_2Cl_2 (100 mL) was added (*S*)-phenylglycinol (1.44 g, 31.4 mol) followed by $ZnClO_4 \cdot 6H_2O$ (0.39 g, 1 mmol) and $MgSO_4$ (0.38 g, 3.1 mmol). The mixture was stirred at rt for 24 h. The resulting mixture was filtered and concentrated. Purification by silica gel column chromatography (ethyl acetate−cyclohexane, 3:7) afforded the isolation of **4c** (1:1 mixture of isomers) as a yellow oil (2.5 g, 62%) along with some recovered starting material (0.75 g, 25%): IR (neat) 1695, 1613 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.53−1.82 (m, 6H), 2.00−2.15 (m, 3H), 2.46−2.57 (m, 1H), 2.97−3.12 (m, 1H), 3.46−3.56 (m, 2 H), 3.59 (s, 3H), 3.74−3.99 (m, 4H), 4.55−4.73 (m, 4H), 7.21−7.38 (m, 5 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.5, 19.7, 25.4, 30.7, 32.7, 34.3, 34.5, 35.9, 50.4, 62.3, 62.6, 63.1, 63.4, 63.5, 74.9, 85.1, 98.9, 99.2, 105.4, 105.5, 125.4, 127.7, 129.0, 139.4, 166.6, 166.7, 169.0; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{29}NaNO_5$ (MNa^+) 410.1937, found 410.1933.

(2*S*,5*R*)-[1-(2-Hydroxy-1(*S*)-phenylethyl)-5-[2-(tetrahydropyran-2-yloxy)ethyl]pyrrolidin-2-yl]acetic Acid Methyl Ester (12). A solution of $NaBH(OAc)_3$ was prepared by portionwise addition of $NaBH_4$ (0.2 g, 5.3 mmol) to glacial acetic acid (3 mL, 53 mmol) at 0 °C. After the hydrogen evolution ceased (30 min), a solution of **4c** (0.41 g, 1.05 mmol) in acetonitrile (10 mL) was added. After being stirred for 48 h at rt, the resulting mixture was quenched with saturated aqueous $NaHCO_3$ until pH = 9 and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed with water and brine, dried with Na_2SO_4, and evaporated under vacuum to give crude **12** (0.42 g, 1:1 mixture of isomers) as a yellow oil which was used in the next step without further purification. An analytical sample was obtained by silica gel column chromatography (ethyl acetate−cyclohexane, 6:4): IR (neat) 3445, 1731 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.18−1.26 (m, 1H), 1.42−1.78 (m, 11H), 2.06−2.11 (m, 1H), 2.33−2.37 (m, 1H), 2.45−2.51 (m, 1H), 3.20−3.28 (m, 1H), 3.43−3.56 (m, 3H), 3.55−3.80 (m, 1H), 3.72 (s, 3H), 3.82−3.94 (m, 4H), 4.58−4.63 (m, 1 H), 7.25−7.35 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.7, 19.8, 25.6, 30.2, 30.3, 30.7, 30.8, 30.9, 36.4, 43.2, 51.7, 54.3, 54.4, 60.8, 61.0, 62.1, 62.5, 62.6, 64.9, 65.0, 65.1, 65.4, 99.0, 99.3, 128.0, 128.5, 129.0, 137.0, 172.8; HRMS (ESI) *m*/*z* calcd for $C_{22}H_{34}NO_5$ (MH^+) 392.2431, found 392.2428.

(2*S*,5*R*)-2-Methoxycarbonylmethyl-5-[2-(tetrahydropyran-2-yloxy)ethyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (14). A solution of crude **12** (0.28 g, 0.71 mmol) in methyl acetate (25 mL) was subjected to hydrogenation (1 atm) in the presence of $Pd(OH)_2/C$ (84 mg) and Boc_2O (0.17 g, 0.79 mmol) at rt for 12 h. The reaction mixture was filtered through Celite and the solvent removed under vacuum. Purification by silica gel column chromatography (ethyl acetate−cyclohexane, 3:7) afforded **14** (1:1 mixture of isomers) as a colorless oil (0.24 g, 92% over two steps from **4c**): IR (neat)1737, 1691 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.39−1.43 (m, 11H), 1.50−1.73 (m, 8H), 1.93−2.11 (m, 3H), 2.28 (dd, *J* = 15.0, 10.0 Hz, 1H), 3.35−3.49 (m, 2H), 3.63 (s, 3H), 3.75−3.91 (m, 3H), 4.03−4.09 (m, 1H), 4.48−4.53 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.6, 25.5, 27.0, 29.6, 29.9, 30.8, 35.7, 35.9, 37.4, 40.3, 51.6, 54.1, 55.2, 56.5, 56.7, 62.3, 65.1, 65.4, 79.6, 99.0, 154.6, 172.1; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{33}NO_6Na$ (MNa^+) 394.220, found 394.2200.

(2*S*,5*R*)-2-(2-Hydroxyethyl)-5-[2-(tetrahydropyran-2-yloxy)ethyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (15). To a solution of **14** (1.3 g, 3.66 mmol) in THF (35 mL) at 0 °C was added $LiAlH_4$ (0.34 g, 9.10 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous Na_2SO_4. Then the reaction mixture was filtered through Celite and the solvent removed under vacuum. Purification by silica gel column chromatography (ethyl acetate−cyclohexane, 6:4) afforded alcohol **15** (1:1 mixture of isomers) as a colorless oil (0.9 g, 75%): IR (neat) 3441, 1689, 1666 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.40−1.85 (m, 11H), 1.90−2.30 (m, 3H), 3.34−3.59 (m, 4H), 3.71−3.84 (m, 3H), 4.10−4.25 (m, 1H), 4.40−4.50 (m, 1H), 4.52−4.57 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.6, 25.5, 28.5, 30.5, 30.8, 36.9, 38.8, 54.8, 56.4, 56.7, 59.1, 62.3, 64.9, 65.1, 80.2, 98.7, 99.0, 156.6; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{33}NO_5Na$ (MNa^+) 366.2250, found 366.2247.

(2*S*,5*R*)-2-(2-Benzyloxyethyl)-5-[2-(tetrahydropyran-2-yloxy)ethyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (16). To a solution of **15** (0.16 g, 0.46 mmol) in DMF (15 mL) was added at 0 °C sodium hydride (74 mg, 1.86 mmol). The mixture was stirred at 0 °C for 1 h. Then, benzyl bromide (62 *μ*L, 0.51 mmol) and

(17) Kishi prepared compound **18** by cyclisation in DMF of the nonisolated bromide intermediate, which was obtained after mesylation of alcohol **17**.[6]

tetrabutylammonium iodide (17 mg, 0.046 mmol) were added. After being stirred for 36 h at rt, the mixture was diluted with ethyl acetate (10 mL), washed successively with saturated aqueous NH_4Cl (10 mL), water (10 mL), and saturated aqueous NaCl (10 mL), dried with Na_2SO_4, and concentrated under vacuum. Purification by silica gel column chromatography (ethyl acetate−cyclohexane, 3:7) provided **16** (1:1 mixture of isomers) as a colorless oil (0.19 g, 95%): IR (neat) 1683 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.50−1.80 (m, 10H), 1.85−2.00 (m, 2H), 2.05−2.15 (m, 2H), 3.38−3.55 (m, 4H), 3.76−3.88 (m, 4H), 4.49−4.50 (m, 2 H), 4.55−4.60 (m, 1H), 7.26−7.34 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.7, 25.6, 28.6, 30.2, 30.8, 30.7, 36.1, 56.3, 56.5, 62.2, 62.3, 65.2, 65.5, 68.3, 73.0, 79.2, 98.7, 99.0, 127.6, 127.7, 128.4, 138.6, 154.9; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{39}NO_5Na$ (MNa^+) 456.2720, found 456.2719.

(2*R*,5*S*)-2-[5-(2-Benzyloxyethyl)pyrrolidin-2-yl]ethanol (3). To a solution of **16** (0.23 g, 0.53 mmol) in methanol (6 mL) at 0 °C was added dropwise acetyl chloride (1.6 mL, 1.6 mmol). The mixture was stirred at rt for 2 h. The mixture was quenched with water. Methanol was partially evaporated. The crude mixture was diluted with CH_2Cl_2 (10 mL) and washed successively with a 2 M NaOH aqueous solution (2 mL) and saturated aqueous NaCl (5 mL). The combined extracts were dried (Na_2SO_4) and concentrated under vacuum. Purification by silica gel column chromatography (methanol−7 N ammonia in MeOH, 9:1) provided **3** as a colorless oil (0.12 g, 90%): IR (neat) 3319 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.45−1.62 (m, 4H), 1.74−1.90 (m, 4H), 3.22−3.35 (m, 1H), 3.36−3.40 (m, 1H), 3.54 (t, J = 6.2 Hz, 2H), 3.66−3.74 (m, 1H), 3.78−3.85 (m, 1H), 4.44−4.55 (m, 4H), 7.28−7.34 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 30.2, 31.2, 36.4, 36.5, 56.4, 57.8, 61.1, 68.3, 72.8, 127.5, 127.6, 128.3, 138.4; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{24}NO_2$ (MH^+) 250.1801, found 250.1797; $[\alpha]^{20}_D$ +8 (*c* 2.00, $CHCl_3$).

Supporting Information Available: Experimental procedures and characterization data for compounds **11**, **5a**−**c**, **4b**, **13**, **17**, **18**, and **2**. Copies of ^1H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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