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Synthetic studies on *d*-biotin. Part 7:† A practical asymmetric total synthesis of *d*-biotin via enantioselective reduction of *meso*-cyclic imide catalyzed by oxazborolidine

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Abstract—A novel and convenient method for the stereoselective synthesis of *d*-biotin **1** starting from the commercially available *cis*-1,3-dibenzyl-2-imidazolidone-4,5-dicarboxylic acid **2** has been developed. The key features of this synthesis include the enantioselective reduction of a *meso*-cyclic imide, mediated by a chiral oxazborolidine catalyst, derived from (1*S*,2*S*)-(+)-*threo*-1-(4-nitrophenyl)-2-amino-1,3-propanediol and the direct introduction of a C_5 side chain to the (3*aS*,6*aR*)-thiolactone through a modified di-Grignard reaction. Enantioselectivities of 98% in the oxazborolidine-catalyzed asymmetric reduction process have been achieved.

1. Introduction

There has been considerable interest in the development of efficient routes for the synthesis of *d*-biotin **1**, a member of the Vitamin B complex group, because of its importance in human nutrition and animal health.[2] Although a large number of approaches involving different ingenious strategies for the control of the three adjacent stereogenic centers have been documented,[3] the Sternbach process, utilizing (3*aS*,6*aR*)-thiolactone **6**, developed by the Goldberg and Sternbach at Hoffmann–La Roche in 1949, is still the most reliable source of this essential vitamin with the overall synthesis scheme having hardly changed over the past 53 years. The unsurpassed efficiency and practicality of this Sternbach strategy results from its ability to make good use of a readily available fumaric acid with a symmetrically bifunctional structure as the starting material for the efficient construction of (3*aS*,6*aR*)-lactone **5**, and subsequently, to capitalize on the sterically well-differentiated two faces of the *cis*-fused bicycle ring in the (3*aS*,6*aR*)-thiolactone **6** to realize the controlled reaction of the third stereogenic center during the introduction of the side chain to give **1**.[4] However, there are few approaches based on this promising Sternbach strategy that overcome drawbacks such as the need for an intermediate resolution with possible recycling in the preparation of a key chiral building block, (3*aS*,6*aR*)-lactone **5**,[5] and the six-step conversion to **1** from (3*aS*,6*aR*)-thiolactone **6**. Therefore, the development of an efficient desymmetrization of dicarboxylic acid **2** to form **5** and one-step introduction of the carboxybutyl side chain to **6** is far from over. Herein we report a new and efficient method for the asymmetric synthesis of **1** based on the enantioselective oxazaborolidine-catalyzed reduction of *meso*-cyclic imide **3**.

2. Results and discussion

The total synthesis of **1** is presented in Scheme 1, using commercially available *cis*-1,3-dibenzyl-2-imidazolidione-4,5-dicarboxylic acid **2** as the starting material. Treatment of dicarboxylic acid **2** with benzylamine in xylene with a catalytic amount of pyridine and subsequent azeotropic removal of water, under reflux for 8 h afforded the *meso*-cyclic imide **3** in a 90% yield. It is worth mentioning that the reaction time was longer (15 h) and the yield of **3** decreased to 65% when no pyridine was used. Enantioselective differentiation of the prochiral functional groups in symmetrical bifunctional compounds such as *meso* compounds is an

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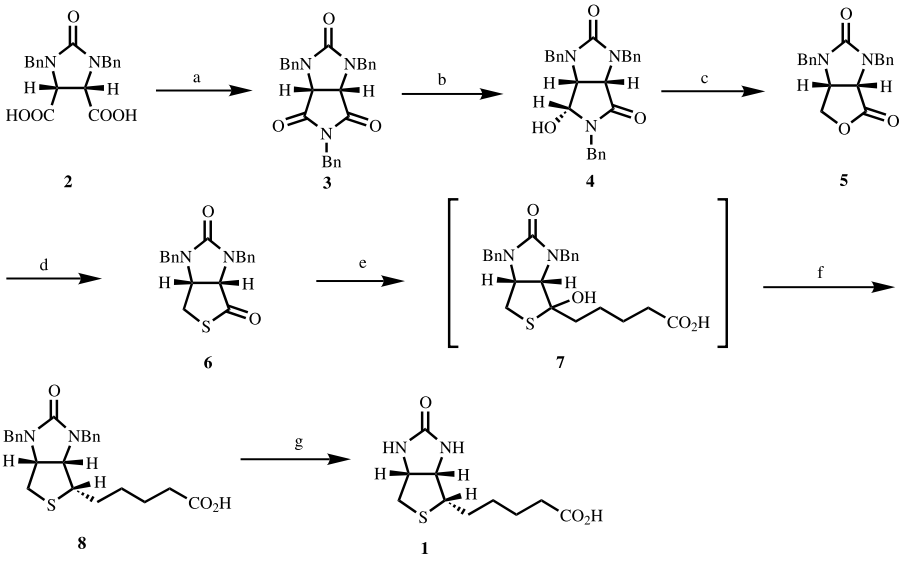


Scheme 1. *Reagents and conditions*: (a) $BnNH_2$, pyridine, xylene, reflux, 8 h 90%; (b) LiH, $BF_3{\cdot}Et_2O$, **12**, THF, reflux, 8 h; (c) KBH_4, $CaCl_2$, EtOH, rt 4 h, then 1 M aq HCl, rt, 92%; (d) $CH_3SC(S)SK$, DMA, 120°C 6 h, 88%; (e) (i) $BrMg(CH_2)_4MgBr$, THF, −30 to −25°C, 3 h; (ii) CO_2, −30 to −25°C, 3 h; (iii) 1 M aq NH_4Cl; (f) H_2, 5% Pd/C, $ZnCl_2$, toluene, 40 atm, 110°C, 6 h, 75% (from **6** to **8**); (g) H_2SO_4–HOAc–H_2O (1.5:1.5:1), reflux, 12 h, 75%.

important strategy for creating new stereogenic centers. A single, stereoselective transformation, namely the reduction of one of the carbonyls leads to a product with three contiguous stereocentres. Among various asymmetric reductions, the oxazaborolidine–mediated borane reduction is one of the most useful tools for the reduction of *meso*-cyclic imides into chiral hydroxylactams, which were eventually converted to the corresponding chiral lactones via further reduction and acid hydrolysis.[6] Shimizu recently reported the enantioselective reduction of *meso N*-benzyl imide **3** into the corresponding chiral hydroxylactam **4** with borane as the reducing agent in the presence of an oxazaborolidine catalyst derived from L-threonine. In spite of its brevity and high enantioselectivity (98%),[7] the yield of **4** was only moderate (65%). Due to borane and its complexes such as $BH_3{\cdot}THF$ or $BH_3{\cdot}Me_2S$ being expensive and the reagents, which often did not store well, requiring a lot of precautions because of their toxicity, this procedure appeared less attractive for a large-scale production of (3*aS*,6*R*,6*aR*)-hydroxylactam **4**.

Our attention was drawn to the development of an efficient, enantioselective reduction of *meso*-cyclic imide **3** into (3*aS*,6*R*,6*aR*)-hydroxylactam via the use of a chiral oxazaborolidine catalyst, derived from (1*S*,2*S*)-(+)-*threo*-1-(4-nitrophenyl)-2-amino-1,3-propanediol with in situ generated borane from cheap, safe and convenient lithium hydride and boron trifluoride etherate. The *meso*-cyclic imide **3** was subjected to enantioselective reduction upon treatment with LiH and $BF_3{\cdot}Et_2O$ in the presence of (1*S*,2*S*)-(+)-*threo*-1-(4-nitrophenyl)-2-amino-3-triphenylmethoxypropanol **12** under reflux in THF to give, after addition of satd. HCl/Et_2O and filtration, **4** in 85% yield and 98% enantiomeric excess (determined by chiral HPLC analysis using a chiral stationary phase), and recovered (1*S*,2*S*)-(−)-*threo*-1-(4-nitrophenyl)-2-amino-3-triphenylmethoxypropanol as its crystalline hydrochloride salt. The assignment of the stereochemistry of **4** was based on single crystal X-ray crystallography (Fig. 1) and ^1H NMR experiments. The coupling constant ($J = 5.5$ Hz) between C_6–H_{exo} and C_{6a}–H clearly indicates that C_6–H_{exo} and C_{6a}–H are *syn*-disposed.

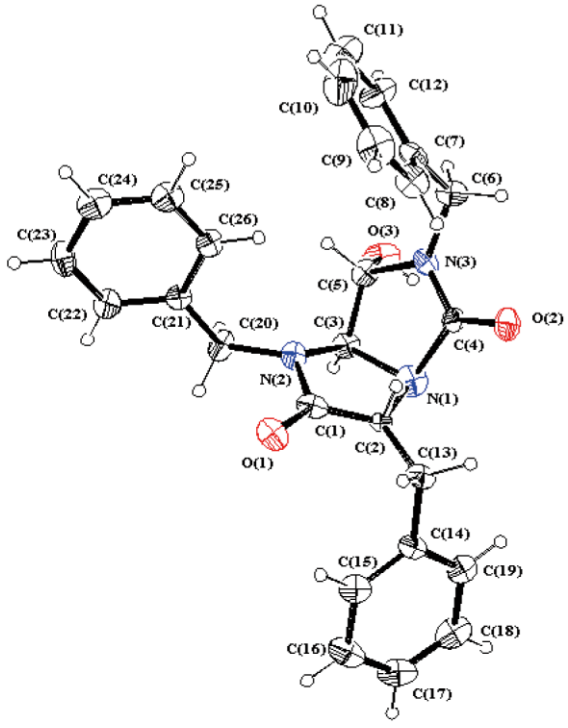


Figure 1. X-Ray determined structure of **4**.

Ligand **12** was readily prepared via a known procedure[9] from commercially available (1*S*,2*S*)-(+)-*thero*-1-(4-nitrophenyl)-2-amino-1,3-propanediol **9**. The (1*S*,2*S*)-amine **9** was regioselectively protected using di-*tert*-butyldicarbonate in methanol to give the derivative **10** (90%), which was *O*-alkylated with chlorotriphenylmethane in pyridine to afford compound **11** in 89% yield. Hydrolysis of **11** gave ligand **12** in 80% yield (Scheme 2).

The reduction of **4** with KBH_4 in the presence of anhydrous $CaCl_2$ in EtOH at rt followed by treatment with 1 M aq HCl gave the (3*aS*, 6*aR*)-lactone **5** in 92% yield. The enantiomeric purity of **5** was determined to be 96% by comparison of its specific rotation $[\alpha]_D^{20} = +56.8$ (*c* 2, $CHCl_3$) with that reported {lit.[9] $[\alpha]_D^{20} = +59.2$ (*c* 2, $CHCl_3$)} which was then increased to 99% ee by recrystallization from EtOH. The thiolactonization of 5 with potassium methylthioxanthogenate(MeSC (S)SK) in DMA at 120°C for 6 h to afford the (3*aS*, 6*aR*)-thiolactone **6** in 88% yield.

The carboxylbutyl side chain of **1** was assembled by the reaction of **6** with a di-Grignard reagent, derived from 1,4-dibromobutane with subsequent treatment of carbon dioxide to give the hydroxy acid **7**, without purification, which upon catalytic hydrogenesis under 40 atm of hydrogen using 5% palladium–carbon in the presence of anhydrous $ZnCl_2$ in toluene at 110°C provided *N*,*N*-dibenzylbiotin **8** stereoselectively in 75% yield. It is important to note that the reaction had to be maintained between −30 to −25°C during the addition of a solution of **6** in THF to the di-Grignard reagent and then afterwards during the carbon dioxide addition forming the carboxylic acid group. A decrease in yield (51%) was observed when the reaction was performed between −10 to −5°C for 2 h in an attempt to make the reaction go to completion.

Finally, the *N*,*N*-dibenzylbiotin **8** was efficiently converted to *d*-biotin **1** in 75% yield through deprotection with H_2SO_4–HOAc–H_2O (1.5:1.5:1) under reflux for 12 h.

3. Conclusion

In conclusion, we have completed an efficient synthesis of *d*-biotin **1**, proceeding in seven steps with 34.8% overall yield from **2**. The high overall yield, short route, simple operation and use of readily accessible reagents could allow a practical large-scale preparation of **1**.

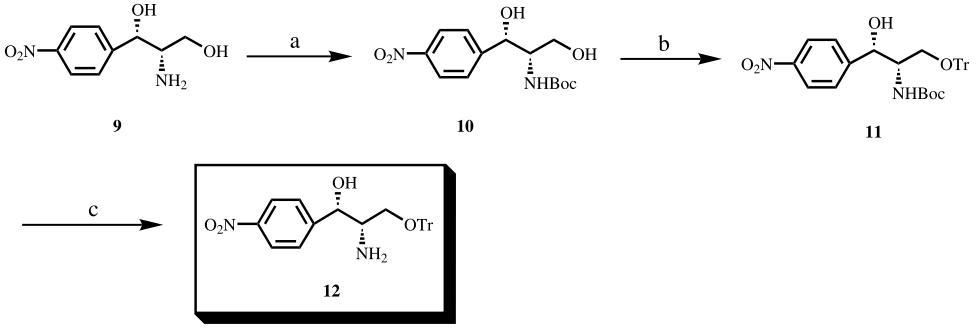
4. Experimental

4.1. General

Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were monitored by TLC on silica gel 60 F254 (0.25 mm, E. Merck). Spots were detected under UV light. Optical rotations were measured at 20±3°C on a WZZ-2S digital automatic polarimeter. Melting points are determined on a WRS-1 digital melting point apparatus and are uncorrected. The chiral HPLC analyses were performed on a Shimadzu LC010AT instrument fitted with a Chiralcel OD column (250×4.6 mm). IR spectra were recorded on a Nicolet FI-IR 360 spectrometer. ^1H NMR spectra were obtained on a Bruker AMX×300 spectrometer. Chemical shifts are reported relative to internal TMS. Mass spectra were measured on a HP-5988A spectrometer by direct inlet at 70 eV. HRMS were measured with EI techniques. Elemental analysis was carried on a Carlo Erba 1106 elemental analyzer. Atomic coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. CCDC number: 218562

4.2. *N*-Benzyl-*cis*-1,3-dibenzyl-2-imidazolidone-4,5-dicarboximide, 3

A mixture of dicarboxylic acid **2** (35.4 g, 0.10 mol), benzylamine (11.2 g, 0.105 mol), pyridine (12.5 mL, 0.16 mol) and xylene (200 mL) was stirred and refluxed under a Dean–Stark trap for 8 h. The reaction mixture was cooled to room temperature, whereupon H_2O (100



Scheme 2. *Reagents and conditions*: (a) $(Boc)_2O$, MeOH, reflux, 20 min, 90%; (b) TrCl, pyridine, CH_2Cl_2, 90°C, 30 min, 89%; (c) 5.4 M aq HCl, CH_2Cl_2, 50°C, 2 h, 80%.

mL) was added. The organic phase was separated, the aqueous phase extracted with xylene (3×35 mL) and the combined organic phases washed successively with 1 M aq HCl (3×25 mL), satd. aq NaCl (3×35 mL) and H_2O (3×40 mL), dried over Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was then recrystallized from toluene to form the pure **3** (38.3 g, 90%) as a white solid. Mp 115–117°C; IR (KBr, cm^{-1}): 3432, 1709, 1979, 1643; ^1H NMR ($CDCl_3$): δ 4.25 (*s*, 2H, C_{3a}–H and C_{6a}–H), 4.26, 4.29, 4.72, 4.78 (4×d, 4H, $J=15.38$, 15.42, 2×$ArCH_2$), 4.55 (*s*, 2H, $ArCH_2N$), 7.19–7.37 (*m*, 15H, 3×ArH); MS *m*/*z* (% ret. Int.): 425 (M^+, 28), 334 (5), 237 (6), 132 (12), 91 (100). Anal. calcd for $C_{26}H_{23}N_3O_3$ requires: C, 73.39; H, 5.45; N, 9.88. Found: C, 73.20; H, 5.38; N, 9.61.

4.3. (3*aS*,6*R*,6*aR*)-1,3,5-Tribenzyl-6-hydroxy-tetrahedro-4*H*-pyrolo [3,4-*d*] imidazole-2,4(1*H*)-dione, 4

To a stirred suspension of LiH (1.0 g, 125 mmol) in dry THF (25 mL) was added $BF_3{\cdot}Et_2O$ (23.8 mL, 187 mmol). Once the vigorous reaction had subsided, the reaction mixture was stirred at room temperature for 30 min. The chiral ligand **12** (5.7 g, 12.5 mmol) was then added and the reaction mixture heated at reflux for a further 30 min. Then a solution of *meso*-cyclic imide **3** (21.3 g, 50 mmol) in dry THF (150 mL) was added dropwise over a period of 4 h. The reaction mixture was stirred under reflux for an additional 30 min. After cooling to room temperature, the reaction mixture was quenched with CH_3OH (75 mL). To the resulting solution was added sat. HCl/Et_2O (80 mL) with stirring and ice bath cooling over 10 min. After 30 min, the solvent was removed under reduced pressure. To the residue was added AcOEt (250 mL) and the mixture cooled to 0–5°C. Colorless crystals of (1*S*,2*S*)-(+)-*threo*-1-(4-nitrophenyl)-2-amino-3-triphenylmethoxypropanol **12** hydrochloride were collected by filtration and converted to the amino alcohol **12** (recovery 5.46 g, 95.8%). The filtrate was washed successively with H_2O (3×45 mL), satd. aq $NaHCO_3$ (3×30 mL) and sat. aq NaCl (3×25 mL), dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the resulting residue by flash chromatography on silica gel (2:1, hexane /EtOAc) afforded pure **4** (18.2 g, 85%) as a white solid. Mp 128–131°C; $[\alpha]_D^{20}=+68.9$ (*c* 0.1, CH_2Cl_2). 98% ee [HPLC conditions: $\lambda=254$ nm; eluent: *n*-hexane: 2-propanol (6:4); flow rate, 0.6 mL/min]. IR (KBr, cm^{-1}): 3313, 2933, 1700, 1452, 1236, 1080, 740, 701; ^1H NMR (DMSO-d_6):δ 3.77–3.79 (*m*, 2H, C_{3a}–H and C_{6a}–H), 4.91 (2×*d*, 1H, $J=5.5$ Hz, CHOH), 4.21, 4.41, 4.90, 4.94, 5.04, 5.14 (6×*d*, 6H, 3×$ArCH_2$), 7.20–7.41 (*m*, 15H, 3×ArH); MS *m*/*z* (% ret. Int.): 427 (M^+, 13), 264 (22), 106 (5), 91 (100). Anal. calcd for $C_{26}H_{25}N_3O_3$ required C, 73.05; H, 5.89; N, 9.83. Found: C, 72.89; H, 5.65; N, 9.69; HRMS (EI) calcd for $C_{26}H_{25}N_3O_3$: 427.5018, found 427.4989.

4.4. (3*aS*,6*aR*)-1,3-Dibenzyl-tetrahydro-4*H*-furo[3,4-*d*]-imidazole-2.4(1*H*)-dione, 5

To a stirred mixture of KBH_4 (26.9 g, 0.50 mol) and anhydrous EtOH (75 mL) was added dropwise a solution of hydroxylactam **4** (128.25 g, 0.30 mol) in anhydrous EtOH (550 mL) at 0–5°C, followed by a solution of anhydrous $CaCl_2$ (27.75 g, 0.25 mol) at the same temperature. The reaction mixture was warmed gradually to room temperature and stirred for 4 h. Then 1 M aq HCl (145 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred at 55–60°C for 45 min. After cooling to room temperature, the reaction mixture was extracted with EtOAc (4×75 mL). The combined organic phases were washed successively with satd. aq NaCl (3×45 mL) and H_2O (3×45 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product, which was recrystallized from EtOH to afford the pure **5** (88.87 g, 92%) as a white solid. Mp 119–120°C $[\alpha]_D^{20}=+58.6$ (*c* 2, $CHCl_3$) {lit.[8] mp115–116°C, $[\alpha]_D^{20}=+59.2$ (*c* 2, $CHCl_3$)}; 96% ee. [HPLC conditions: $\lambda=254$ nm; eluent: *n*-hexane:2-propanol (9:1); flow rate, 0.8 mL/min]. Recrystallization from EtOH gave 82.11 g (85%, 99% ee) of **5**, mp 120–121°C, $[\alpha]_D^{20}=+61.5$ (*c* 2, $CHCl_3$). IR (KBr, cm^{-1}): 1776, 1705, 1210, 1185, 1028, 970; ^1H NMR ($CDCl_3$): δ 3.25 (*dd*, 1H, $J=2.3$, 12.8 Hz, CH_{endo} S), 3.35 (*dd*, 1H, $J=5.6$, 12.8 Hz, CH_{exo} S), 3.96 (*m*, 1H, $J=8.8$ Hz, C_{3a}-H), 4.20 (3×*d*, 1H, $J=2.3$, 5.4, 8.0 Hz, C_{6a}-H), 4.24, 4.32, 4.46, 4.95 (4×*d*, 4H, $J=14$ Hz, 2×$ArCH_2$), 7.27–7.38 (*m*, 10H, ArH); MS *m*/*z* (% ret. Int.): 322 (M^+, 25), 265 (58), 245 (78), 187 (62), 91 (100); HRMS (EI) calcd for $C_{19}H_{18}N_2O_3$ 322.3466, found: 322.3487. Anal. calcd for $C_{19}H_{18}N_2O_6$ required: C, 70.81; H, 5.59; N, 8.70. Found: C, 70.58; H, 5.42; N, 8.53.

4.5. (3*aS*,6*aR*)-1,3-Dibenzyl-tetrahydro-4*H*-thieno[3.4-*d*]imidazole-2.4(1*H*)-dione, 6

To a stirred solution of **5** (32.2 g, 0.10 mol) in DMA (150 mL) was added potassium methylthioxanthogenate (16.2 g, 0.1 mol). The reaction mixture was stirred at 120°C under N_2 atm for 6 h. After cooling to room temperature, H_2O (145 mL) was added to the reaction mixture. The reaction mixture was then extracted with toluene (4×40 mL). The combined organic phases were washed with satd. aq NaCl (3×35 mL) and H_2O (3×35 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give the crude product, which was then purified by recrystallization with EtOAc to afford pure **6** (29.74 g, 88%) as a crystalline powder. Mp 124–125°C $[\alpha]_D^{20}=+90.4$ (*c* 1, $CHCl_3$) {lit.[10] mp 125–127°C, $[\alpha]_D^{20}=+90.8$ (*c* 1, $CHCl_3$)}; 99.1% ee [HPLC conditions: $\lambda=254$ nm; eluent: *n*-hexane:AcOEt (2:1); flow rate, 0.4 mL/min]. IR (KBr, cm^{-1}): 1705, 1695, 1424, 1225; ^1H NMR ($CDCl_3$): δ 3.24 (*dd*, 1H, $J=2.2$, 12.8 Hz, CH_{endo}S), 3.35 (*dd*, 1H, $J=5.5$, 12.8 Hz, CH_{exo}S), 3.82 (*d*, 1H, $J=8.0$ Hz, C_{3a}-H), 4.14 (3×*d*, 1H, $J=2.2$, 5.5, 8.0 Hz, C_{6a}-H), 4.35, 4.38, 4.67, 5.00 (4×*d*, 4H, $J=15.2$ Hz, 2×$ArCH_2$), 7.27–7.35 (*m*, 10H, ArH); MS *m*/*z* (% ret. Int.): 338 (M^+, 5), 310 (25), 277 (8), 264 (66), 91 (100). Anal. calcd for $C_{19}H_{18}N_2OS$ required: C, 67.46; H, 5.33; N, 8.28; S, 9.47. Found: C, 67.23; H, 5.23; N, 8.12; S, 9.31.

4.6. (3*aS*,4*S*,6*aR*)-1,3-Dibenzyl-tetrahydro-1*H*-thieno[3,4-*d*]imidazole-2(3*H*)-one-4-ylpentanoic acid, 8

To a stirred suspension of Mg turnings (11 g, 0.45 mol) in dry THF (150 mL) was added dropwise 1,4-dibromo-

butane (21.6 g, 0.10 mol) at 30–35°C under nitrogen. When the reaction started, the remaining 1,4-dibromobutane (74.52 g, 345 mmol) in dry THF (320 mL) was added dropwise at 30–35°C over a period of 1 h. Stirring continued at the same temperature for 45 min, followed by cooling to −30 to −25°C, and dropwise addition of a solution of thiolactone **6** (50.7 g, 0.15 mol) in dry THF (250 mL) at this temperature. The reaction mixture was stirred for an additional 3 h at −30 to −25°C, and the carbon dioxide was led in at −30 to −25°C for 3 h. Then, the reaction mixture was quenched with satd. aq NH_4Cl (200 mL) and extracted with toluene (4×150 mL). The combined organic phases were washed successively with satd. aq NaCl (3×50 mL) and H_2O (3×40 mL) and dried over Na_2SO_4. The solvent was concentrated under reduced pressure to approximately a volume of 300 mL to give a solution of hydroxy acid **7** in toluene. To this stirred solution of hydroxy acid **7** was added $ZnCl_2$ (5.0 g, 37 mmol) and 5% Pd/C (5.0 g). The reaction mixture was hydrogenated under a hydrogen pressure of 40 atm at 110°C for 6 h. After cooling to room temperature, H_2O (40 mL) was added. Stirring continued at room temperature for a further 15 min. The catalyst was removed by filtration and the filtrate evaporated under reduced pressure to give the crude product 8 as a pale yellow oil, which was left to stand overnight in an refrigerator to be crystallized and recrystallized from *iso*-PrOH/hexane (2:1) to afford pure **8** (47.8 g, 75%) as a white solid. Mp 90–92°C; $[\alpha]_D^{23}=-26.7$ (*c* 1, CH_3OH) {lit.[11] mp 91–92°C; $[\alpha]_D^{23}=-26.8$ (*c* 1, CH_3OH)}; 98.9% ee [HPLC conditions: $\lambda=254$ nm; eluent: *n*-hexane:AcOEt (9:2); flow rate, 0.8 mL/min]. ^1H NMR ($CDCl_3$): δ 1.38–1.69 (*m*, 6H, 3×CH_2), 2.19 (*t*, 2H, $J=6.56$ Hz, CH_2COOH), 2.71 (4×d, 2H, $J=2.34$, 4.39 Hz, $C_{6\alpha}$–H and $C_{6\beta}$–H), 3.06 (*m*, 1H, $C_{4\beta}$–H), 3.89 (*dd*, 1H, $J=5.56$ Hz, C_{3a}–H), 3.96 (*m*, 1H, C_{6a}–H), 4.03, 4.15, 4.75, 5.00 (4×d, 4H, $J=15.1$, 15 Hz, 2×$CH_2C_6H_5$), 7.23–7.37 (*m*, 10H, 2× ArH). HRMS (EI) calcd for $C_{24}H_{28}N_2O_3S$ 424.5628, found: 424.5639. Anal. calcd for $C_{24}H_{28}N_2O_3S$ required: C, 67.90; H, 6.65; N, 6.60; S, 7.55. Found: C, 67.69; H, 6.49; N, 6.48; S, 7.36.

4.7. *d*-Biotin, 1

To a stirred mixture of *N*,*N*-dibenzylbiotin (42.4 g, 0.10 mol), glacial acetic acid (150 mL) and H_2O, (150 mL) was added dropwise 98% H_2SO_4 (150 mL) at room temperature, over a period of 25 min. The reaction mixture was stirred under reflux for 12 h. After cooling to room temperature, H_2O (200 mL) was added. The reaction mixture was extracted with toluene (4×65 mL). The aqueous phase was concentrated under reduced pressure to approximately a volume of 120 mL and kept in a refrigerator overnight. The precipitated crystals were collected by filtration and recrystallized from H_2O to give pure **1** (18.38 g, 75%) as a white crystalline powder. Mp 230–232°C, $[\alpha]_D^{22}=+91.1$ (*c* 1, 0.1 M NaOH) {lit.[12] mp 232–233°C, $[\alpha]_D^{22}=+91.2$ (*c* 1, 0.1 M NaOH)}; IR (KBr, cm^{-1}): 3311, 2933, 1705, 1665; ^1H NMR ($CDCl_3$): δ 1.30–1.61 (*m*, 6H, 3×CH_2), 2.17 (*t*, 2H, $J=7.3$ Hz, CH_2CO_2H), 2.58 (*dd*, 1H, $J=1.7$, 12.5 Hz, $CH_{endo}S$), 2.80 (*dd*, 1H, $J=4.7$, 12.5 Hz, $CH_{exo}S$), 3.14 (*m*, 1H, $C_{4\beta}$-H), 4.17 (*m*, 1H, C_{3a}-H), 4.36 (*m*, 1H, C_{6a}–H), 6.37 (*s*, 1H, NH), 6.47 (s, 1H, NH), 11.98 (br s, 1H, CO_2H). MS *m*/*z* (% rel. int.) 245 (M^++1, 15), 227 (9), 184 (25), 112 (26), 97 (100), 85 (66). Anal. calcd for $C_{10}H_{16}N_2O_3S$ required: C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 49.01; H, 6.49; N, 11.50; S, 13.40.

4.8. X-Ray structure analysis of 4

Crystals of $C_{26}H_{25}N_3O_3$ (427.50) suitable for X-ray analysis were obtained from EtOAc. A colorless monoclinic crystal of dimensions 0.40×0.30×0.30 mm was mounted on a Rigaku AFC7R diffractometer. Determination of the cell parameters was performed by least squares refinement of 25 reflections. The compound crystallizes in the monoclinic system, space group $P2_1,2_1,2_1$ with $a=10.910$ (6), $b=9.189$ (6), $c=10.949$ (4) Å; $\beta=104.87$ (4)° $Z=2$; $V=1060.9$ (10) Å^3; μ(Mo-Kα)$=0.089$ mm^{-1}; $D_{calcd}=1.338$ g cm^{-3}; $F(000)=452$ reflections were collected in the range of $1.92°<\theta<25.17°$ using Mo-Kα radiation (graphite monochromator, $\lambda=0.71073$ Å), ω–2θ scan mode. The structure was solved by direct methods and expanded using difference Fourier techniques and refined by full-matrix, least-square to $R=0.0811$, $R=0.1905$ with $\omega=1/[\sigma^2(F_o)+(0.1035\ P^2+1.3711\ P)\ R$ (where $P=(F_o^2+2F_c^2)/3$) by using the 1232 observed reflections having $I>2.00\sigma(I)$ for 284 parameters refined. All non-hydrogen atoms were refined anisotropically.

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