The first stereospecific synthesis of L-tetrahydrodipicolinic acid †; a key intermediate of diaminopimelate metabolism

Jennifer F. Caplan, Andrew Sutherland and John C. Vederas *

Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada.
E-mail: john.vederas@ualberta.ca

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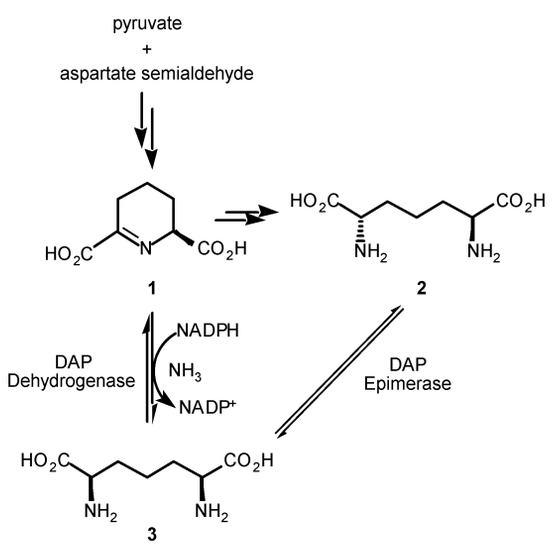
L-Tetrahydrodipicolinic acid **1**, a key intermediate of diaminopimelate metabolism has been prepared in 6 steps and 23% overall yield from L-allylglycine **4**. The key step during this synthesis involves a base mediated cyclisation of dimethyl (2*RS*,6*S*)-2-[*N*-(benzyloxycarbonyl)-*N*-(*p*-tolylsulfonyl)amino]-6-[*N*-(benzyloxycarbonyl)amino]heptane-1,7-dioate **8** to give (2*S*)-*N*-(benzyloxycarbonyl)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid **9**. ^{1}H NMR studies show this one pot transformation involves four steps; base elimination of toluene-*p*-sulfinic acid, intramolecular nucleophilic attack of the 6-benzyloxycarbonylamino group on the resulting imine, followed by hydrolysis of the esters and elimination of benzyl carbamate under acidic work-up to give the cyclic enamine **9**.

Introduction

The recurring emergence of bacterial resistance to current antibiotics continues to stimulate strategies to disrupt microbial cell wall biosynthesis.[1,2] The peptidoglycan cell wall layer in bacterial cells provides numerous targets for further antibiotic development.[3] Crucially most bacteria require either lysine, or its biosynthetic precursor, *meso*-diaminopimelic acid (DAP), as the key cross-linking amino acid of the peptidoglycan cell wall layer. Since mammals lack the biosynthetic pathway to DAP and require L-lysine in their diet, there has been substantial interest in the specific inhibition of enzymes involved in this pathway.[4]

One of the key intermediates in the DAP biosynthetic pathway is L-tetrahydrodipicolinic acid (L-THDP) **1** which is formed in two steps from aspartate semialdehyde and pyruvate (Scheme 1). After formation of L-THDP **1** the pathway then splits into two main routes, both of which have been identified in different bacterial species.[5] The main route proceeds *via* acylation of L-THDP **1**, followed by transamination and deacylation to give LL-DAP **2** which is then transformed to *meso*-DAP **3** using DAP epimerase. In the less common pathway, *meso*-DAP **3** is produced directly from L-THDP **1** by DAP dehydrogenase.



Scheme 1

Although L-THDP **1** is a key intermediate in the DAP pathway, the only reliable source of this compound to date, is the oxidative deamination of *meso*-DAP **3** using DAP dehydrogenase.[6] Two synthetic methods have been reported for the synthesis of racemic THDP,[7,8] but there is as yet no reported stereoselective or stereospecific synthesis of L-THDP **1**.

In the present study we report the first stereospecific synthesis of L-THDP **1** from L-allylglycine **4** in 6 steps and 23% overall yield. One of the key reactions during this synthesis involves an unusual one pot, four step formation of a tetrahydropyridine-2,6-dicarboxylate derivative. Examination of this reaction mechanism by ^{1}H NMR spectroscopy is also described.

Results and discussion

The first stage of the synthesis required the efficient preparation of a fully protected diaminopimelic acid derivative. We sought to achieve this using an intramolecular ene reaction between a protected allylglycine and methyl glyoxylate,[9] followed by a Mitsunobu reaction using a nitrogen nucleophile.[10] Thus, commercially available L-allylglycine **4** was treated with chlorotrimethylsilane and methanol followed by triethylamine and benzyl chloroformate in a one pot process to give the protected derivative **5** in 91% yield (Scheme 2). Reaction of **5** with freshly distilled methyl glyoxylate (prepared by oxidation of dimethyl tartrate[11]) in the presence of tin tetrachloride at −78 °C afforded the ene adduct **6** as a 1 : 1 mixture of diastereomers in 79% yield.

Rhodium metal has been shown to catalyse the hydrogenation of double bonds without the hydrogenolysis observed with palladium.[12] Thus, ene adduct **6** was hydrogenated in the presence of a catalytic amount of 5% rhodium on carbon in ethyl acetate to give the saturated compound **7** in 86% yield (Scheme 2). Treatment of the saturated alcohol **7** with *N*-(benzyloxycarbonyl)toluene-*p*-sulfonamide[13] under standard Mitsunobu conditions gave the protected diaminopimelic acid derivative **8** in 75% yield.

The base mediated cyclisation of DAP derivative **8** was initially attempted using non-hydrolytic bases in an effort to

† The IUPAC name for tetrahydrodipicolinic acid is 2,3,4,5-tetrahydropyridine-2,6-dicarboxylic acid.

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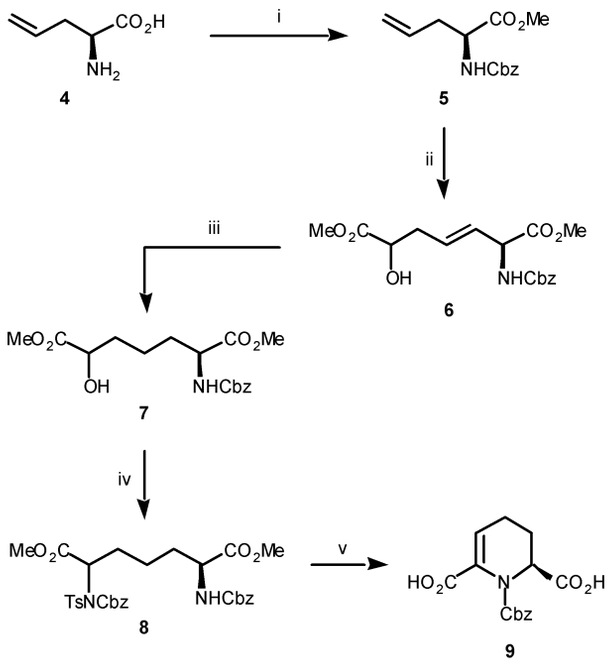
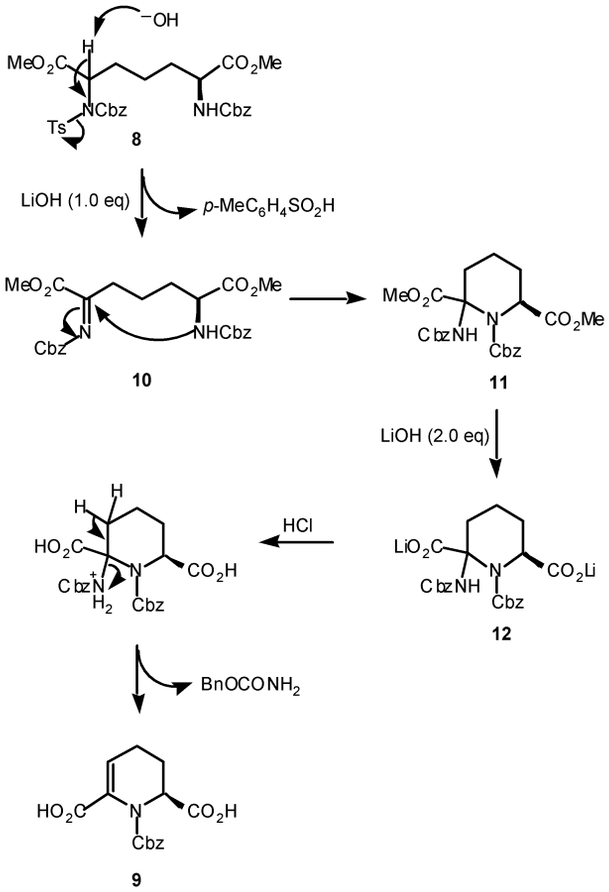


Scheme 2 *Reagents and conditions*: i, a) TMSCl, MeOH, b) BnOCOCl, Et_3N; ii, MeO_2CCHO, $SnCl_4$, CH_2Cl_2; iii, H_2, Rh/C, EtOAc; iv, TsNHCbz, DEAD, PPh_3, THF; v, LiOH, MeCN, H_2O.

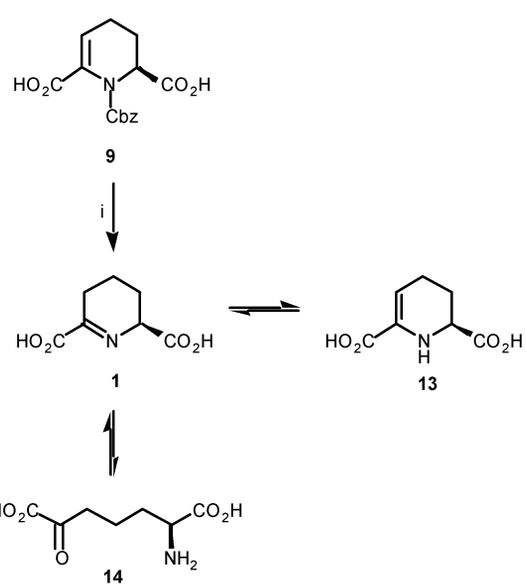
prepare the di-methyl ester analogue of **9**. It seemed that purification of such a compound might be more readily achieved than that of the corresponding di-acid derivative. However, attempted cyclisation using either diethylamine or sodium bis-(trimethylsilyl)amide returned only starting material. Thus, DAP derivative **8** was treated with three equivalents of lithium hydroxide in aqueous acetonitrile. Purification of the reaction mixture by reversed phase HPLC gave the tetrahydropyridine-2,6-dicarboxylic acid derivative **9** in 76% yield (Scheme 2). The structure of this compound was confirmed by a HMQC NMR experiment, which showed coupling of the alkene proton signals (H-5) at 6.14 ppm to a carbon signal (C-5) at 123.9 ppm.

The success of this cyclisation reaction prompted an investigation of the mechanism by ^1H NMR spectroscopy. One equivalent of lithium hydroxide in D_2O was added to a solution of the acyclic DAP derivative **8** in CD_3CN and the reaction was monitored at specific intervals. After 1 hour, loss of the H-2 proton signals and the appearance of signals corresponding to toluene-*p*-sulfinic acid were observed. This suggested that the first step involved deprotonation of the H-2 proton by hydroxide followed by elimination of the tosyl group to give imine **10** (Scheme 3). This unstable *N*-acyl imine is then readily attacked by the 6-benzyloxycarbonylamino group to give the cyclic compound **11**. After 17 hours no further change was noted, hence a second equivalent of lithium hydroxide was added. This led to only partial hydrolysis of both esters and so the third equivalent of lithium hydroxide was added to yield the di-acid **12** after a further 6 hours. Subsequent exposure of the reaction mixture to a 20% solution of DCl in D_2O resulted in the formation of benzyl carbamate and the cyclic enamine **9**. However, signals corresponding to the alkene proton (H-5) were not observed. This was attributed to deuterium exchange of the H-5 proton in the presence of the deuterated solvents. This was confirmed by repeating the reaction as described above, except that the deuterated solvents were removed before acidic work-up. As expected, the resulting ^1H NMR spectrum showed the presence of the H-5 signals at 6.14 ppm, demonstrating that elimination of benzyl carbamate to yield the cyclic enamine **9** is acid catalysed (Scheme 3).

The synthesis of L-THDP **1** was completed using a two step process as shown in Scheme 4. The di-lithium salt of cyclic enamine **9** was prepared using lithium hydroxide, which was



Scheme 3



Scheme 4 *Reagents and conditions*: i, a) LiOH, MeOH, H_2O, b) Li, NH_3 (l).

followed by reaction with lithium and liquid ammonia to give the target compound as an equilibrium mixture of tautomers in 66% yield after flash chromatography. The ^1H NMR spectrum showed the presence of L-THDP **1** along with its two equilibrium products,[8] enamine **13** and the acyclic keto acid **14** in a 1 : 1 : 1 mixture. The proton and carbon NMR signals of all three compounds were assigned using a combination of COSY, TOCSY, HMQC and HMBC NMR experiments.

The structure and stereochemistry of L-THDP **1** was further confirmed by incubation of the compound with NADPH and DAP dehydrogenase, which was isolated and purified from

the organism *Bacillus sphaericus* IFO 3525 as previously reported.[6,14] Monitoring of the reaction using a continuous spectrophotometric assay at 340 nm[14] showed consumption of the co-factor thus confirming that, as expected, the synthetic L-THDP **1** is a substrate for the enzyme and has the correct stereochemistry.

In summary, we have described the first stereospecific synthesis of L-THDP **1** in six steps from L-allylglycine **4**. This synthesis involves three key steps; an ene reaction using tin tetrachloride as the Lewis acid, a Mitsunobu reaction to insert the second nitrogen functionality and a lithium hydroxide mediated cyclisation. Deprotection of the resulting tetrahydropyridine-2,6-dicarboxylic acid **9**, gives the target compound **1** which is shown by ^{1}H NMR spectroscopy to be in equilibrium with the cyclic enamine **13** and the acyclic keto acid **14** as described by Robins and co-workers during their synthesis of racemic THDP.[8] The structure of the final product is confirmed by turnover of the DAP dehydrogenase enzyme using a UV spectrophotometric assay. Studies on the synthesis and interaction of other DAP analogues with enzymes in the pathway to L-lysine are currently in progress.

Experimental

All reactions were done under dry Ar. All solvents were purified and distilled according to Perrin *et al*.[15] Progress of reactions was monitored by TLC on commercial silica gel plates (Merck 60F-254) using UV fluorescence, ninhydrin or potassium permanganate for visualization. Flash chromatography employed silica gel 60 (Silicycle, 230–420 mesh) and was performed according to the Still procedure.[16] HPLC separations were performed on a Beckman System equipped with a 166 variable wavelength UV detector and an Altex 210A injector. HPLC separations were monitored at a wavelength of 219 nm. Mps were determined on a Büchi apparatus using open-end capillary tubes and are uncorrected. NMR spectra were recorded on Inova Varian 300 and 500 MHz instruments. IR spectra were determined with a Nicolet Magna 750 FT-IR spectrometer. Mass spectra (MS) were recorded with either a Micromass ZabSpec Hybrid Sector-TOF instrument (electrospray ionization (ES)) or a MS-50 high resolution (HR) mass spectrometer (chemical ionization (CI) NH_3). Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 26 °C with a micro cell (100 mm; 0.9 cm^3) or a standard cell (100 mm, 8 cm^3) respectively. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Microanalyses were completed at the University of Alberta Microanalytical Laboratory. All literature compounds had IR, ^{1}H NMR and mass spectra consistent with assigned structures. L-Allylglycine was purchased from Sigma-Aldrich and methyl glyoxylate was generated by standard literature procedures.[11]

Methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]pent-4-enoate 5

To a stirred suspension of L-allylglycine **4** (0.51 g, 4.35 mmol) in methanol (10 cm^3) at 0 °C was added chlorotrimethylsilane (1.7 cm^3, 13.05 mmol). After 2 h, the reaction mixture was warmed to room temperature and left stirring overnight. The reaction mixture was then cooled to 0 °C and triethylamine (2.5 cm^3, 17.40 mmol) and benzyl chloroformate (0.91 g, 5.22 mmol) were added. After 3 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in 2 M hydrochloric acid (50 cm^3) and extracted with ethyl acetate (2 × 40 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to leave a colourless oil. Purification by flash column chromatography, eluting with 30% ethyl acetate in hexane gave methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]pent-4-enoate **5** (1.06 g, 91%) as a colourless oil; $[\alpha]_D$ +16.4 (*c* 4.0, $CHCl_3$) [lit.[14] $[\alpha]_D$ +15.3 (*c* 1.6, $CHCl_3$)]; $\nu_{max}(CHCl_3)/cm^{-1}$ 3340 (NH), 1723 (ester C=O), 1525, 739, 698; δ_H (300 MHz, $CDCl_3$) 2.38–2.62 (2H, m, 3-H_2), 3.68 (3H, s, OMe), 4.45 (1H, dd, *J* 7.4 and 6.1 Hz, 2-H), 5.07–5.18 (4H, m, 5-H_2 and PhCH_2), 5.32 (1H, d, *J* 8.0 Hz, NH), 5.67 (1H, m, 4-H), 7.22–7.39 (5H, m, Ph); δ_C (75.5 MHz, $CDCl_3$) 36.6 (C-3), 52.2 (OMe), 53.3 (C-2), 66.9 (Ph*C*H$_2$), 119.2 (C-5), 127.9, 128.1, 128.4 (aromatics), 132.0 (C-4), 136.2 (quaternary aromatic C), 155.7 (OCON), 172.1 (*C*O$_2$Me); *m*/*z* (EI) 263.1155 (M$^+$). $C_{14}H_{17}NO_4$ requires 263.1157.

Dimethyl (2*RS*,6*S*)-2-hydroxy-6-[*N*-(benzyloxycarbonyl)-amino]hept-4-ene-1,7-dioate 6

Tin tetrachloride (24.9 cm^3, 212 mmol) was added to a solution of freshly distilled methyl glyoxylate (4.23 g, 47.9 mmol) in dichloromethane (65 cm^3) at −55 °C. After 10 min, the reaction mixture was cooled to −78 °C and a solution of methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]pent-4-enoate **5** (5.23 g, 19.9 mmol) in dichloromethane (25 cm^3) was added dropwise. The resulting white suspension was warmed to −25 °C and stirred for 5 h. The reaction mixture was then poured into 1 M hydrochloric acid (250 cm^3) and extracted with dichloromethane (4 × 200 cm^3). The combined organic layers were dried ($NaSO_4$) and concentrated *in vacuo*. The resulting yellow oil was purified by flash column chromatography (40% ethyl acetate in hexane) to give the title compound **6** (5.5 g, 79%) as a colourless oil (Found: C, 57.75; H, 6.05; N, 3.85. $C_{17}H_{21}NO_7$ requires C, 58.1; H, 6.0; N, 4.0%); ν_{max} ($CHCl_3$)/cm^{-1} 3355 (NH), 2953 (CH), 1738 (ester C=O), 1520, 1266; δ_H(300 MHz, $CDCl_3$) 2.34–2.59 (2H, m, 3-H_2), 3.72 (6H, s, 2 × OMe), 4.23 (1H, m, 2-H), 4.85 (1H, m, 6-H), 5.10 (2H, s, PhCH_2), 5.60–5.65 (2H, m, NH and 5-H), 5.72–5.82 (1H, m, 4-H), 7.29–7.39 (5H, m, Ph); δ_C(75.5 MHz, $CDCl_3$) 36.9 (C-3), 52.5 (OMe), 52.7 (OMe), 55.5 (C-6), 67.0 (Ph*C*H$_2$), 69.8 (C-2), 128.0, 128.1, 128.5, 128.6 (C-4, C-5 and aromatics), 136.2 (quaternary aromatic C), 155.5 (OCON), 171.1, 174.4 (2 × *C*O$_2$Me); *m*/*z* (EI) 351.1319 (M$^+$). $C_{17}H_{21}NO_7$ requires 351.1318.

Dimethyl (2*RS*,6*S*)-2-hydroxy-6-[*N*-(benzyloxycarbonyl)-amino]heptane-1,7-dioate 7

A solution of dimethyl (2*RS*,6*S*)-2-hydroxy-6-[*N*-(benzyloxycarbonyl)amino]hept-4-ene-1,7-dioate **6** (2.48 g, 7.07 mmol) in ethyl acetate (20 cm^3) was treated with 5% rhodium on carbon (0.2 g) and stirred under hydrogen at atmospheric pressure for 2 d. The reaction mixture was filtered through a pad of Celite and concentrated *in vacuo*. Purification by flash column chromatography eluting with 40% ethyl acetate in hexane gave dimethyl (2*RS*,6*S*)-2-hydroxy-6-[*N*-(benzyloxycarbonyl)-amino]heptane-1,7-dioate **7** (2.15 g, 86%) as a colourless oil (Found: C, 57.8; H, 6.7; N, 3.95. $C_{17}H_{23}NO_7$ requires C, 57.8; H, 6.6; N, 4.0%); ν_{max} ($CHCl_3$)/cm^{-1} 3354 (NH), 2953 (CH), 1737 (ester C=O), 1525, 740, 698; δ_H(300 MHz, $CDCl_3$) 1.40–1.95 (6H, m, 3-H_2, 4-H_2 and 5-H_2), 2.75 (1H, br s, OH), 3.75 (3H, s, OMe), 3.76 (3H, s, OMe), 4.16 (1H, m, 2-H), 4.37 (1H, m, 6-H), 5.10 (2H, s, PhCH_2), 5.36 (1H, br s, NH), 7.29–7.39 (5H, m, Ph); δ_C(75.5 MHz, $CDCl_3$) 20.6 (C-4), 32.3 (C-5), 33.6 (C-3), 52.4 (OMe), 52.6 (OMe), 53.7 (C-6), 67.1 (Ph*C*H$_2$), 70.1 (C-2), 128.1, 128.2, 128.6 (aromatics), 136.3 (quaternary aromatic C), 155.9 (OCON), 172.8, 175.4 (2 × *C*O$_2$Me); *m*/*z* (EI) 353.1478 (MH$^+$).$C_{17}H_{23}NO_7$ requires 353.1474.

Dimethyl (2*RS*,6*S*)-2-[*N*-(benzyloxycarbonyl)-*N*-(*p*-tolyl-sulfonyl)amino]-6-[*N*-(benzyloxycarbonyl)amino]heptane-1,7-dioate 8

A solution of dimethyl (2*RS*,6*S*)-2-hydroxy-6-[*N*-(benzyloxycarbonyl)amino]heptane-1,7-dioate **7** (2.17 g, 6.15 mmol) in THF (15 cm^3) was added to a solution of triphenylphosphine (2.66 g, 10.1 mmol) and *N*-(benzyloxycarbonyl)toluene-*p*-sulfonamide (2.87 g, 9.4 mmol) in THF (25 cm^3) at 0 °C. Diethyl azodicarboxylate (1.64 g, 9.4 mmol) was then added dropwise. After 5 h at room temperature, the reaction mixture was

concentrated *in vacuo*. The resulting residue was purified by flash column chromatography eluting with 25% ethyl acetate in hexane to give dimethyl (2*RS*,6*S*)-2-[*N*-(benzyloxycarbonyl)-*N*-(*p*-tolylsulfonyl)amino]-6-[*N*-(benzyloxycarbonyl)amino]heptane-1,7-dioate **8** (2.95 g, 75%) as a colourless oil (Found: C, 59.6; H, 5.6; N, 4.3. $C_{32}H_{36}N_2O_{10}S$ requires C, 60.0; H, 5.7; N, 4.4%); ν_{max} ($CHCl_3$)/cm^{-1} 3360 (NH), 2952 (CH), 1735 (ester C=O), 1596, 1520, 1455, 1218; δ_H(300 MHz, $CDCl_3$) 1.54 (2H, m, 4-H_2), 1.82 (2H, m, 5-H_2), 2.12 (2H, m, 3-H_2), 2.35 (3H, s, CH_3), 3.56 (3H, s, OMe), 3.74 (3H, s, OMe), 4.38 (1H, q, *J* 6.3 Hz, 6-H), 5.00–5.15 (5H, m, 2 × PhCH_2O and 2-H), 5.40 (1H, br d, *J* 6.3 Hz, NH), 7.06–7.36 (12H, m, 2 × OCH_2Ph, 3-CH and 3′-CH), 7.77 (2H, d, *J* 9.3 Hz, 2-CH and 2′-CH); δ_C(75.5 MHz, $CDCl_3$) 21.6 (CH_3), 22.1 (C-4), 29.3 (C-5), 31.8 (C-3), 52.3 (OMe), 52.4 (OMe), 53.6 (C-6), 59.3 (C-2), 66.9, 69.1 (2 × O*C*H_2Ph), 128.0, 128.4, 128.5, 128.9, 129.0 (aromatics), 134.2, 135.7, 136.3, 144.6 (4 × quaternary aromatic C), 151.3, 155.9 (2 × OCON), 169.9, 172.8 (2 × *C*O_2Me); *m*/*z* (ES) 663.1993 (MNa^+). $C_{32}H_{36}N_2O_{10}SNa$ requires 663.1988.

(2*S*)-*N*-(Benzyloxycarbonyl)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid 9

A solution of lithium hydroxide monohydrate (0.21 g, 4.99 mmol) in water (5 cm^3) was added to a solution of dimethyl (2*RS*,6*S*)-2-[*N*-(benzyloxycarbonyl)-*N*-(*p*-toluenesulfonyl)-amino]-6-[*N*-(benzyloxycarbonyl)amino]heptane-1,7-dioate **8** (0.51 g, 1.61 mmol) in acetonitrile (10 cm^3). After stirring for 8 h at room temperature, the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in water (10 cm^3), extracted with ethyl acetate (2 × 15 cm^3). The aqueous layer was acidified to pH 2 with 2 M hydrochloric acid (10 cm^3) and extracted with ethyl acetate (2 × 15 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by reversed phase HPLC (C_{18} Bondpak, gradient elution: 0–100% MeCN in water over 20 min) to afford (2*S*)-*N*-(benzyloxycarbonyl)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylate **9** (0.18 g, 76%) as a white solid; mp 90–92 °C (from methanol); $[a]_D$ −66.7 (*c* 1.0, MeOH); ν_{max} (MeOH)/cm^{-1} 3415 (OH), 2952 (CH), 1732 (C=O), 1716 (C=O), 1695 (C=C), 1455; δ_H (300 MHz, CD_3OD) 1.88–2.40 (4H, m, 3-H_2 and 4-H_2), 5.00 (1H, dd, *J* 5.3, 2.7 Hz, 2-H), 5.11 (2H, 2 × d, *J* 12.8 Hz, PhCH_2), 6.14 (1H, t, *J* 4.0 Hz, 5-H), 7.26–7.38 (5H, m, Ph); δ_C (75.5 MHz, CD_3OD) 21.4 (C-3), 27.2 (C-4), 58.1 (C-2), 69.1 (Ph*C*H_2), 123.9 (C-5), 128.7, 128.9, 129.0, 129.4 (aromatics), 135.1 (C-6), 137.3 (quaternary aromatic C), 155.9 (OCON), 170.0, 176.8 (CO_2H); *m*/*z* (ES) 328.0793 (MNa^+). $C_{15}H_{15}NO_6Na$ requires 328.0797.

(2*S*)-2,3,4,5-Tetrahydrodipicolinic acid 1

(2*S*)-*N*-(Benzyloxycarbonyl)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid **9** (0.088 g, 0.29 mmol) was dissolved in methanol (5 cm^3) and a solution of lithium hydroxide monohydrate (0.024 g, 0.58 mmol) in water (1 cm^3) was added. After 5 min, the reaction mixture was concentrated *in vacuo*. To the resulting residue was added predistilled liquid ammonia (15 cm^3). The reaction mixture was then cooled to −78 °C and freshly cut lithium metal (0.025 g) was added until a blue colour persisted. After 15 min, water (3 cm^3) was added and the reaction mixture was warmed to room temperature. The reaction mixture was then acidified to pH 7 by the addition of 0.1 M hydrochloric acid (2 cm^3) and concentrated *in vacuo*. The residue was then purified by flash column chromatography to remove trace impurities by eluting with 15% ammonia in isopropanol (propan-2-ol) to give the title compound **1** (0.033 g, 66%) as a white solid; ν_{max} (Nujol mull)/cm^{-1} 3432 (NH), 2924 (CH), 2852 (OH), 1735 (C=O), 1641 (C=C), 1454, 1377, 1209; δ_H (500 MHz, D_2O) 1.70–1.88 (6H, m, 3-H_2 of imine, 4-H_2 of enamine, 4-H_2 of keto), 2.00–2.26 (6H, m, 4-H_2 of imine, 3-H_2 of enamine, 3-H_2 of keto), 2.42 (2H, m, 5-H_2 of imine), 2.55 (2H, t, *J* 7.4 Hz, 5-H_2 of keto), 4.10 (1H, dd, *J* 11.7, 3.1 Hz, 2-H of imine), 4.17 (1H, t, *J* 6.6 Hz, 2-H of keto), 4.46 (1H, dd, *J* 7.4, 5.1 Hz, 2-H of enamine); δ_C (125 MHz, D_2O) 21.6 (C-4 of enamine), 22.5 (C-3 of imine), 22.6 (C-4 of keto), 24.7 (C-4 of imine), 27.6 (C-3 of enamine), 27.8 (C-5 of imine), 31.9 (C-3 of keto), 35.8 (C-5 of keto), 55.6 (C-2 of keto), 57.5 (C-2 of enamine), 59.9 (C-2 of imine), 131.5 (C-5 of enamine), 149.3 (C-6 of enamine), 155.9 (C-6 of imine), 166.9, 169.3, 171.1, 174.2, 174.8, 180.5 (C=O), 204.1 (C-6 of keto); *m*/*z* (ES) 170.04467 (M − H). $C_7H_8NO_4$ requires 170.04478.

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