A practical synthesis of (3*R*,4*R*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyrrolidin-3-ol†

Keith Clinch,[a] Gary B. Evans,[a] Richard H. Furneaux,[a,b] Dirk H. Lenz,[a] Jennifer M. Mason,*[a] Simon P. H. Mee,[a] Peter C. Tyler[a] and Sarah J. Wilcox[b]

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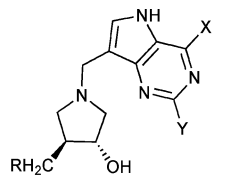
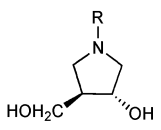
The title compound (+)-**5**, required for production of transition state analogue inhibitors of enzymes involved in T-cell-dependent disorders, was synthesized in five steps. A 1,3-dipolar cycloaddition of the nitrone formed from formaldehyde and *N*-benzylhydroxylamine to diethyl maleate gave the racemic *cis*-isoxazolidine (±)-**7**. Reduction of the N–O bond of this compound gave pyrrolidone (±)-**8** in excellent yield. A very efficient enzymic resolution of this racemic product led to the title enantiomer (+)-**5**. This route employs only one chromatographic purification.

Introduction

The nucleoside analogues **1–3**, 'DADMe-Immucillins', are highly potent transition state analogue inhibitors of *N*-ribosyl transferase enzymes. DADMe-Immucillins **1** and **2** inhibit purine nucleoside phosphorylase (PNP), a therapeutic target for the control of disorders involving proliferation of T-cells.[1,2] Compound **1** is currently in phase I clinical trials for treatment of T-cell-mediated autoimmune diseases.[3] Compound **3** inhibits 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN)[4] and 5′-methylthioadenosine phosphorylase (MTAP).[5] MTAN is a bacterial enzyme which has been identified and validated as a potential target for broad-spectrum antimicrobials.[4] MTAP functions solely in the polyamine pathway of mammals. Inhibition of this pathway is a validated anti-cancer target.[5]



1 R = OH, X = OH, Y = H
2 R = OH, X = OH, Y = NH_2
3 R = SMe, X = NH_2, Y = H



(+)-**4** R = H
(+)-**5** R = Boc

To supply our ongoing synthetic efforts in this area, we required an efficient route to kilogram quantities of the key intermediate (3*R*,4*R*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyrrolidin-3-ol [(+)-**5**]. The parent amine, 4-hydroxymethylpyrrolidin-3-ol, was first synthesised as a component of a racemic *cis*,*trans* mixture,[6] and later as part of a *trans* racemic mixture.[7] The (3*R*,4*R*) enantiomer [(+)-**4**] has been prepared enantio-pure from both D-glucose and D-xylose,[8,9] and in very high enantiomeric excess by a route involving opening of a Sharpless epoxide with cyanide,[10] and also by two routes involving incorporation of a chiral auxiliary and separation of diastereomers.[11,12] The key step of the more enantioselective of these last methods is a 1,3-dipolar cycloaddition of an azomethine ylide to a 3-benzyloxy-substituted alkenoylcamphorsultam,[12] and this procedure has been adapted to afford a large scale synthesis.[13] Most recently, we have obtained pyrrolidinol (+)-**4** by enantioselective acylation of racemic ethyl *trans*-*N*-benzyl-4-hydroxypyrrolidine-3-carboxylate catalysed by Novozyme 435 (an immobilized form of lipase B of *Candida antarctica*).[14] We now report a more efficient synthesis of pyrrolidine (+)-**5** that mostly avoids chromatography and is suitable for kilogram-scale preparations.

Results and discussion

Our route to pyrrolidinol (+)-**5** established the required *trans* orientation of the ring substituents with a 1,3-dipolar cycloaddition between diethyl maleate and the nitrone formed from formaldehyde and *N*-benzylhydroxylamine, which gave racemic *cis*-isoxazolidine (±)-**7** (97%) (Scheme 1). When the N–O bond of isoxazolidine (±)-**7** was cleaved with zinc in acetic acid the resulting aminodiester spontaneously formed the known[15] *trans*-lactam (±)-**8** in 94% yield. These two steps were carried out without any chromatographic purification. The purity of lactam (±)-**8** obtained this way, and used in the next step, was estimated by NMR to be 90–95%.

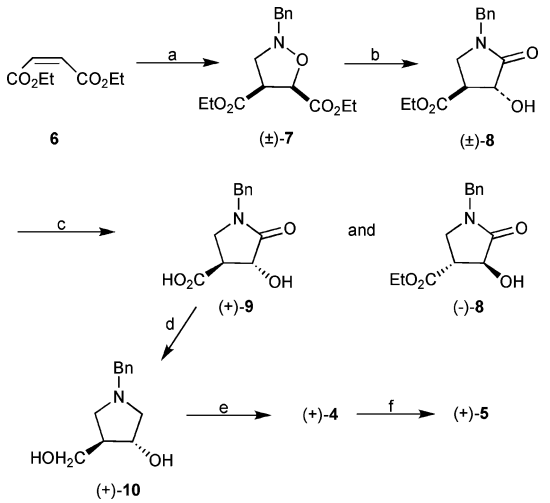
The hydrolytic resolution of racemic ester (±)-**8** using Novozyme 435‡ (10% w/w) in aqueous buffer at pH 7.5 was superbly selective ($E > 300$).[16] At the end of the reaction (50 ± 2% conversion)§ ester (−)-**8** and all non-acidic organic impurities were removed from the aqueous reaction medium by extraction with chloroform. The aqueous phase was then acidified and multiple extractions with ethyl acetate gave a moderate yield of crystalline acid (+)-**9** (32%, based on amount of (±)-**8**), pure by NMR and of

[a]*Carbohydrate Chemistry Team, Industrial Research Ltd, P.O. Box 31310, Lower Hutt, New Zealand. E-mail: j.mason@irl.cri.nz*
[b]*GlycoSyn[irl], P.O. Box 31310, Lower Hutt, New Zealand*

† Electronic supplementary information (ESI) available: NMR spectra of (±)-**7**, (±)-**8**, (−)-**8** and (+)-**10**. See DOI: 10.1039/b708796a

‡ A total of twenty-two commercially available lipases, proteases and esterases were screened for efficacy in this hydrolysis. Only Novozyme 435 and pig liver esterase were able to hydrolyse (±)-**8** efficiently, and the former was more selective.

§ Inhomogeniety of the reaction mixture prevents precise determination of degree of conversion.



Scheme 1 *Reagents and conditions*: (a) BnNOH, CH_2O, EtOH, reflux, 2.5 h; (b) Zn, AcOH; (c) Novozyme 435, pH 7.5, 27 °C, 5.7 h; (d) $BF_3{\cdot}Et_2O$, $NaBH_4$, THF, 72 h, then HCl; (e) H_2, Pd/C; (f) $(Boc)_2O$.

high enantiomeric purity (ee 95%). Reduction of (+)-**9** with borane gave *N*-benzyl amine (+)-**10** in excellent yield (91%) and greater than 97% purity (NMR) without chromatography. Benzyl amine (+)-**10** was hydrogenolysed to amine (+)-**4** which was converted to the *N*-*tert*-butoxycarbonyl- protected amine (+)-**5** in excellent yield (99%). Purified through a short column of silica gel, diol (+)-**5** was analytically pure and had ^1H and ^{13}C NMR spectra in accordance with literature data.[1]

Conclusion

In summary, this synthesis of (+)-**5** is short, efficient and robust. It provides the target compound in excellent chemical and enantiomeric purity in five steps and 26% overall yield from commercially available starting materials. It has been undertaken many times in our laboratories and has been used to manufacture compound (+)-**5** in kilogram quantities.

Experimental

General

All reagents, including anhydrous solvents, were used as supplied. Organic solutions were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. Flash column chromatography was performed on Scharlau or Merck silica gel 60 (40–60 μm) and the solvents used were distilled prior to use or were of AR grade. Melting points were recorded on a Reichert hot stage microscope and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm and are in units of 10^{-1}deg cm^2 g^{-1}; concentrations are in g per 100 ml. NMR spectra were recorded on a Bruker AC300E spectrometer in $CDCl_3$. ^1H spectra at 300 MHz are referenced to internal Me_4Si (δ 0) and ^{13}C spectra at 75.5 MHz are referenced to the centre line of residual $CHCl_3$ (δ 77.0). Coupling constants (J) are quoted in Hz. Positive ion electron impact (EI+) HRMS were measured on a VG 70SE instrument. HPLC analysis was preformed on a Chiralcel OD-H column connected to an HP Series 1100 chromatograph and eluted with 20% isopropanol in hexane. Microanalyses were carried out by the Campbell Microanalytical Laboratory, University of Otago.

(±)-*cis*-Diethyl 2-benzylisoxazolidine-4,5-dicarboxylate [(±)-7]. *N*-Benzylhydroxylamine hydrochloride (280.0 g, 1.75 mol) and anhydrous NaOAc (157.8 g, 1.92 mol) were stirred together in EtOH (1.2 L) at rt for 1 h, after which time aqueous formaldehyde (37%, 260 mL, 3.5 mol) was added and stirring was continued for 1 h. Diethyl maleate (**6**, 260 mL, 1.61 mol) was added and the mixture heated under reflux for 2.5 h. After cooling, the solvent was evaporated and the residue partitioned between EtOAc (1.5 L) and $NaHCO_3$ (sat. aq., 1.6 L). The organic layer was dried and concentrated to give the product (±)-**7** as a yellow oil (523 g, 97%). This material was pure by NMR and was used in the next step without further treatment. NMR δ_H 7.40–7.20 (5 H, m, Ar), 4.76 (1H, br d, H-5), 4.29–4.08 (5 H, m, 2 × CH_2CH$_3$ and CHPh), 4.02 (1H, br m, CHPh), 3.77 (1 H, q, J 8.7, H-4), 3.60–3.00 (2 H, m, H-3, H-3), 1.28 (3H, t, J 7.2, CH_3), 1.25 (3H, t, J 7.2, CH_3); δ_C 169.7 (*C*O), 169.1 (*C*O), 136.4 (Ar), 129.0 (ArH), 128.4 (ArH), 127.6 (ArH), 77.0 (C-5), 62.5 (Ph*C*H$_2$), 61.4 (*C*H$_2$CH$_3$), 61.3 (*C*H$_2$CH$_3$), 56.8 (C-3), 50.4 (C-4), 14.0 (2 × CH$_2$*C*H$_3$); HRMS (EI) m/z 307.1418, $C_{16}H_{21}NO_5$ (M$^+$) requires 307.1420.

(±)-*trans*-Ethyl 1-benzyl-4-hydroxy-5-oxopyrrolidine-3-carboxylate [(±)-8]. Powdered zinc (210 g, 3.2 mol) was added portionwise to a solution of crude isoxazolidine (±)-**7** (523 g, 1.7 mol) in AcOH (1.5 L) cooled with an ice–water bath. The mixture was stirred for 1 h and then filtered through Celite. The solvent was evaporated and the residue was taken up in CH_3Cl (1 L) and washed with $NaHCO_3$ (sat. aq., 2 × 2 L). The organic layer was dried and concentrated to give the product (±)-**8** as a yellowish waxy solid (442 g, 99%). This material was 90–95% pure by NMR and was used in the next step without further treatment. A small amount was purified by column chromatography, eluting with EtOAc–hexanes 1 : 1. Mp 65–66 °C (EtOAc–hexanes), lit. 62–63.5 °C;[15] NMR δ_H in agreement with literature data;[15] δ_C 172.9 (*C*O), 171.4 (*C*O), 135.1 (Ar), 128.9 (ArH), 128.2 (ArH), 128.0 (ArH), 72.3 (C-4), 61.5 (*C*H$_2$CH$_3$), 47.0 (Ph*C*H$_2$), 46.1 (C-3), 45.1 (C-2), 14.1 (CH$_2$*C*H$_3$); HRMS (EI) m/z 263.1154, $C_{14}H_{17}NO_4$ (M)$^+$ requires 263.1158.

(3*R*,4*S*)-Ethyl 1-benzyl-4-hydroxy-5-oxopyrrolidine-3-carboxylate [(−)-8] and (3*S*,4*R*)-1-Benzyl-4-hydroxy-5-oxopyrrolidine-3-carboxylic acid [(+)-9]. A suspension of crude ester (±)-**8** (345 g, 1.31 mol) in acetone (350 mL) and potassium phosphate buffer (0.5 M, pH 7.5, 4 L) was stirred over Novozyme 435 (34.5 g) for 5.7 h at 27 °C. The enzyme was removed by filtration. The filtrate was saturated with NaCl and extracted with $CHCl_3$ (4 × 600 mL). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give (−)-**8** (138 g, 40%) as a light brown oil. Column chromatography of a small sample eluting with EtOAc–hexanes (1 : 1) gave a pale yellow waxy solid. $[\alpha]_D^{21}$ −46.8 (*c* 1.1, EtOH); NMR spectra in agreement with those of the racemate; ee (HPLC) 98%. The aqueous phase was bought to pH 1 with HCl (6 M), resaturated with NaCl and extracted with EtOAc (7 × 0.8 L). The combined extracts were dried and concentrated to give a light brown solid that was purified by trituration with EtOAc to give (+)-**9** (109 g, 32%). This material was pure by NMR and was used in the next step without further treatment. Crystallised from water as colourless needles, it gave mp 146–147 °C; $[\alpha]_D^{21}$ +62.3

(*c* 1, EtOH); NMR δ_H 7.37–7.21 (5H, bm, Ar), 4.76 (3H, bs, O*H* and H_2O) 4.70 (1H, d, *J* 8.2, H-4), 4.49 (1H, d, *J* 14.6, C*H*HPh), 4.38 (1H, d, *J* 14.6, CH*H*Ph) 3.44 (2H, m, H-2, H-2), 3.19 (1H, m, H-3); δ_C 174.7 (*C*O), 173.8 (*C*O), 135.1 (Ar), 129.3 (ArH), 128.6 (ArH), 128.5 (ArH), 72.6 (C-4), 47.6(Ph*C*H_2), 46.1 (C-3), 45.5 (C-2); found C 61.36, H 5.56, N 5.92, $C_{12}H_{13}NO_4$ requires C 61.27, H 5.57, N 5.95%; a sample that was converted to the methyl ester using Me_3SiCHN_2 gave an ee of 95% by HPLC.

(3*R*,4*R*)-1-Benzyl-4-(hydroxymethyl)pyrrolidin-3-ol [(+)-10]. Freshly distilled $BF_3{\cdot}OEt_2$ (258 mL, 2.06 mol) was added to a suspension of acid (+)-**9** (96.6 g, 411 mmol) and $NaBH_4$ (62.5 g, 1.64 mol) in anhydrous THF (1.9 L) under argon at 0 °C, and stirring was continued at rt for 72 h. The reaction was then quenched with MeOH (100 mL) under ice-cooling and the solvent evaporated. The residue was stirred with aqueous HCl (6 M, 200 mL) for 10 min and concentrated. This residue was slurried with aqueous NaOH (15%, 200 mL) and again evaporated. The residue was suspended in $CHCl_3$ (1 L), filtered through Celite and a plug of silica gel, and evaporated to dryness to give (+)-**10** (77.8 g, 376 mmol, 91%) as a an oil that solidified on standing. This material was pure by NMR and was used in the next step without further treatment. A small quantity was recrystallised from EtOAc–hexanes. Mp 60–62 °C; $[\alpha]_D^{21}$ +36.0 (*c* 1.1, MeOH), lit.[14] $[\alpha]_D^{21}$ +33.0 (*c* 0.75, MeOH). ^{1}H and ^{13}C NMR spectra were identical to those reported in the literature.[14]

(3*R*,4*R*)-*N*-*tert*-Butoxycarbonyl-4-hydroxymethylpyrrolidin-3-ol [(+)-5]. Crude amine (+)-**10** (69.9 g, 338 mmol) was dissolved in MeOH (440 mL), Pd/C (10%, 6 g) was added and the mixture was stirred overnight under hydrogen. The catalyst was removed by filtration through Celite and di-*tert*-butyl dicarbonate (81 g, 371 mmol) was added to the solution of amine (+)-**4** at such a rate as to maintain the temperature below 40 °C. The mixture was stirred at rt for 1 h and the solvent was evaporated. The residue was chromatographed on a column eluted with CH_2Cl_2–MeOH (9 : 1) to give the title compound (+)-**5** (72.4 g, 333 mmol, 99%) as an oil that formed an amorphous solid on standing. ^{1}H and ^{13}C NMR spectra were identical to those reported in the literature.[1] $[\alpha]_D^{21}$ +16 (*c* 0.8, MeOH), lit.[14] $[\alpha]_D^{21}$ +15.9, +16.2 (*c* 0.8–1.1, MeOH); found C 55.53, H 8.84, N 6.34, $C_{10}H_{19}NO_4$ requires C 55.28, H 8.81, N 6.54%.

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