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Use of hydrolases for the synthesis of cyclic amino acids

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Abstract—The synthesis of several cyclic amino acids that have all the necessary structural features to make them ideal scaffolds for use in medicinal chemistry is described. A key step in each synthesis is the use of hydrolase enzymes to define a chiral centre. In order to elaborate these building blocks into more complex molecules, crystallization and asymmetric hydrogenation were used to define further stereocentres.

1. Introduction

One can analyse the mainstream chemical literature to determine the habits of practicing chemists in their application of enzymes for (predominantly) chiral synthetic methodology. In doing this, one is immediately drawn to the conclusion that hydrolases dominate this field; they did in 1990 and continue to do so today. Scanning a few sample years of *Tetrahedron: Asymmetry* for use of different enzymes illustrates this point clearly, as shown in Figure 1 below. Since the mid-1990's hydrolases have been used at over twice the rate of non-hydrolases.

In particular, it is the lipase and esterase family of enzymes that dominate the hydrolases, and a more detailed snapshot of enzyme usage in 2002 is given in Figure 2.

The reasons for the popularity of these enzymes are well documented, as for example in 'Hydrolases in Organic Synthesis' by Uwe Bornscheuer and Romas Kazlauskas.[1] Some of these reasons include their relative commercial availability, the reasonable number that are available for screening, a lack of need for expensive or demanding cofactors, versatility for synthetic or hydrolytic usage, and the ability to perform reactions in aqueous or solvent based media. From an industrial perspective they often admirably meet the essential criteria for 'industrial readiness', such as availability, performance (turnover, selectivity, volume efficiency) and freedom from intellectual property issues. However, the main area of use of hydrolase enzymes has been in kinetic resolution chemistry, and a drawback of this approach is the maximum 50% theoretical yield obtained of the desired enantiomer. In recent years, approaches have

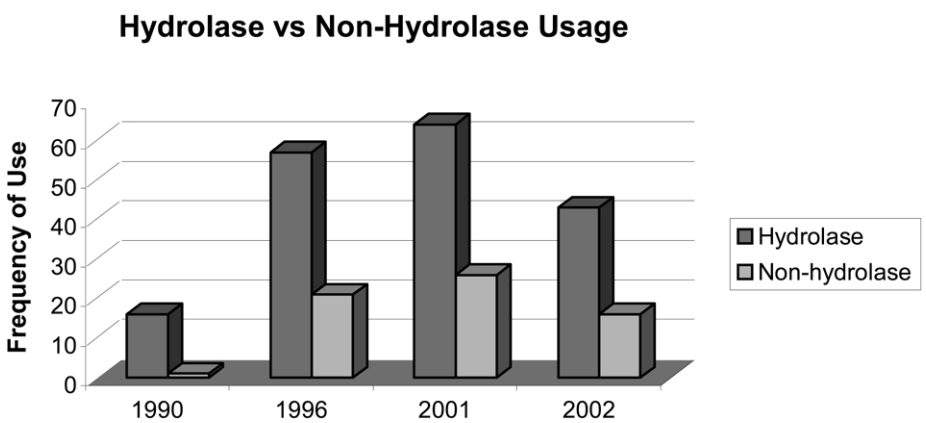

Figure 1. Reported use of hydrolase enzymes versus non-hydrolase enzymes. (Source: papers published in *Tetrahedron: Asymmetry* in years specified).

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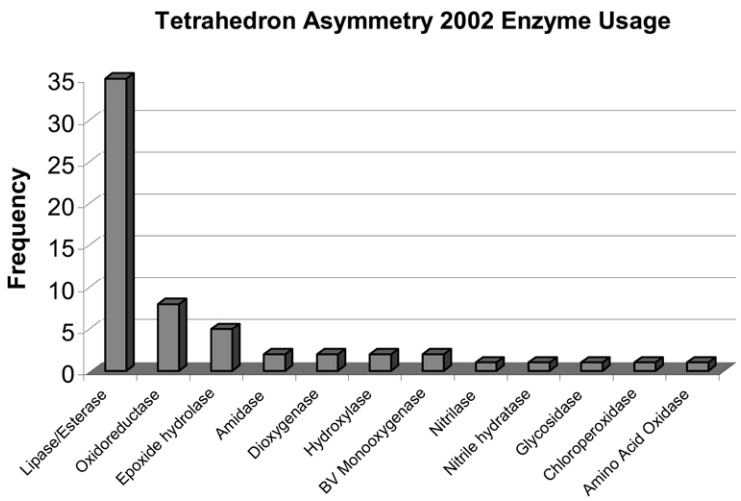


Figure 2. Breakdown of biocatalysis papers published in *Tetrahedron: Asymmetry* by enzyme class in 2002.

been made to overcome this, and hydrolases have now been employed in dynamic kinetic resolutions[2] in conjunction with racemase enzymes,[3] organometallic hydrogen transfer catalysts[4] or a more judicious selection of substrate.[5]

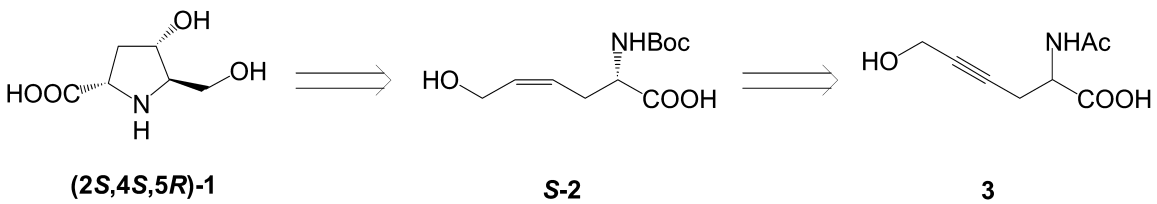
It is against this background that we have continued to develop and exploit hydrolases generally for chiral synthetic routes to more complex, conformationally rigid molecules, where these molecules are expected to usefully contribute in medicinal chemistry. Although Lipinski's rules[6] do not mention rigidity as a desirable function in drug-like molecules, recent studies have indicated that the number of rotatable bonds is a crucial parameter that should be considered.[7] However, putting extra chiral complexity into molecules means that one must call upon additional skills and approaches, such as discovery and development of new hydrolase enzymes, and the practice of complementing biocatalysis with other chiral technologies such as crystallisation and chemocatalysis. In this paper we describe some of our work that has allowed us to elevate the use of hydrolases for more sophisticated molecules.

2. Resolution of α-amino acids using acylases and subsequent elaboration to more complex molecules

2.1. Bulgecinine

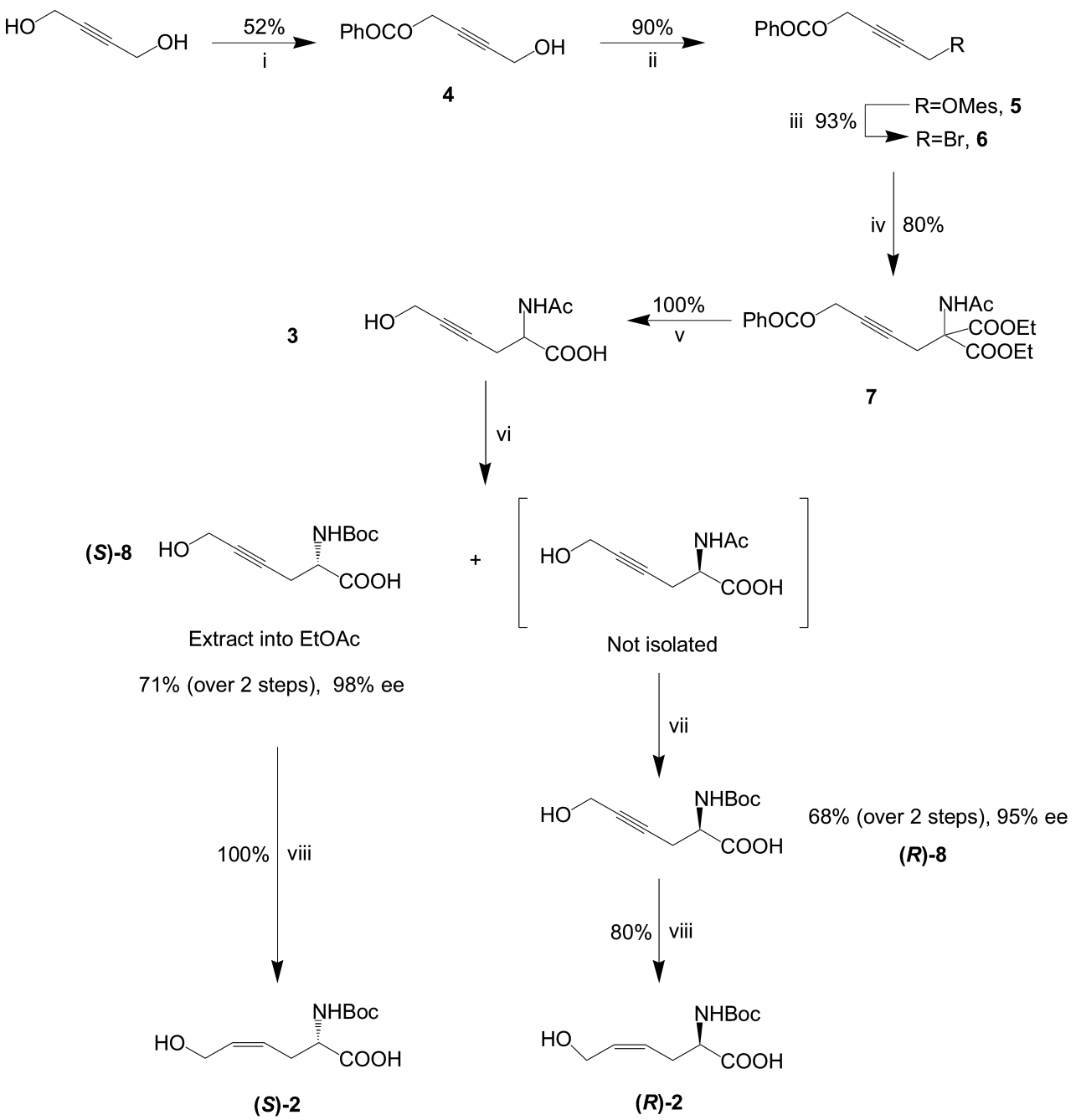
Acylases are commonly used for the synthesis of enantiopure amino acids.[8] We have successfully used proprietary *Thermococcus litoralis* L-aminoacylase[9] (extremophile) and *Alcaligenes* sp. D-aminoacylase[10] enzymes for the synthesis of a wide range of unnatural α-amino acids. When approaching the synthesis of cyclic amino acids, however, this approach has not been so successful. (−)-Bulgecinine, (2*S*,4*S*,5*R*)-**1**, is a cyclic amino acid with three stereocentres isolated in various glycosylated forms from *Pseudomonas acidophila* and *Pseudomonas mesoacidophila*.[11] Its rigid structure and multiple functionality make it an ideal candidate for use as a scaffold around which compound libraries may be designed: when used in conjunction with β-lactam antibiotics the natural glycosylated forms (bulgecins) enhance the antibiotic effect. Previous workers identified the *cis*-allylic alcohol *S*-**2** (Scheme 1) as a vital intermediate for the synthesis of **1**.[12] However, the functionality on the side-chain of *S*-**2** makes it a challenging synthetic target that has previously only been accessed using chemistry that we did not think would be scaleable. We targeted the racemic *N*-acetyl amino acid **3** as the key intermediate. It was anticipated that **3** would be a suitable substrate for resolution using acylases, which would provide a mild environment to introduce the chirality whilst protecting the functionality of the molecule. Following resolution, the key intermediate *S*-**2** would then be readily available, and the route could be carried out on 100 g scale.[13]

The *N*-acetyl amino acid **3** was prepared from the readily available 2-butyne-1,4-diol as shown in Scheme 2. Sequential use of L-acylase followed by D-acylase allowed both enantiomers of the *N*-Boc amino acid **8** to be prepared in a one pot procedure. Lindlar hydrogenation[14] of **8** was used to access both enantiomers of **2**. Halolactonisation of *S*-**2** gave the lactone (2*S*,4*S*,5*S*)-**9** with good diastereoselectivity,[12] and the modified conditions of Oppolzer[15] were then used to convert lactone (2*S*,4*S*,5*S*)-**9** to *N*-Boc-(−)-bulgecinine, *N*-Boc-(2*S*,4*S*,5*R*)-**1** (Scheme 3). Elaboration of *R*-**2** in a similar fashion gave the *N*-Boc-(2*R*,4*R*,5*S*) diastereomer. It can be seen that the two additional chiral centres introduced by cyclisation are both induced by the stereochemistry of

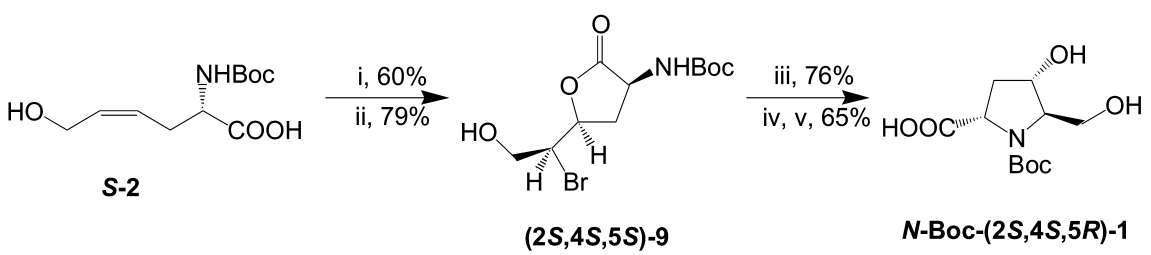


(2*S*,4*S*,5*R*)-1 **S-2** **3**

Scheme 1.



Scheme 2. *Reagents and conditions*: (i) PhCOCl (1 equiv.), Py (1 equiv.), DCM, 10 °C to RT, o/n; (ii) Et_3N (1.1 equiv.), DMAP (0.025 equiv.), MesCl (1.1 equiv.), DCM, 0–5 °C, 2 h; (iii) LiBr (2 equiv.), acetone, 10–15 °C, 1.5 h; (iv) Diethyl acetamidomalonate (1.4 equiv.), KO^{t}Bu (1.4 equiv.), THF, reflux, 22 h; (v) NaOH (3 equiv.), MeOH, reflux, 3.5 h, then acidify to pH 3.5 with conc. HCl, reflux, 22 h; (vi) L-acylase (200 U/g substrate), 30 mM KH_2PO_4, pH 7, 65 °C, 2.5 h, then Boc_2O (0.5 equiv. cf. racemic *N*-Ac acid), THF, 5 M NaOH to maintain pH at 10. RT, 3.5 h; (vii) D-Acylase (40 U/g substrate), aqueous NaOH to adjust to pH 8, RT, 21 h, then Boc_2O (0.5 equiv. cf. racemic *N*-Ac acid), THF, 5 M NaOH to maintain pH at 10, RT, o/n; (viii) 10 wt% Lindlar catalyst (5 wt% Pd on $CaCO_3$ poisoned with Pb), MeOH, 1 bar hydrogen, 20 °C, 1.5 h.



Scheme 3. *Reagents and conditions*: (i) NBS (1.1 equiv.), THF, −10–0 °C, 20 min; (ii) 40% EtOAc/60% heptane slurry, RT, 15 min–0.5 h; (iii) *p*TsOH·H_2O (2 equiv.), EtOAc, RT, 1 h; (iv) H_2O, 1 M LiOH to pH 9, RT, 16 h; (v) Boc_2O (1 equiv.), THF, 1 M LiOH to maintain pH 9, RT, 4.5 h.

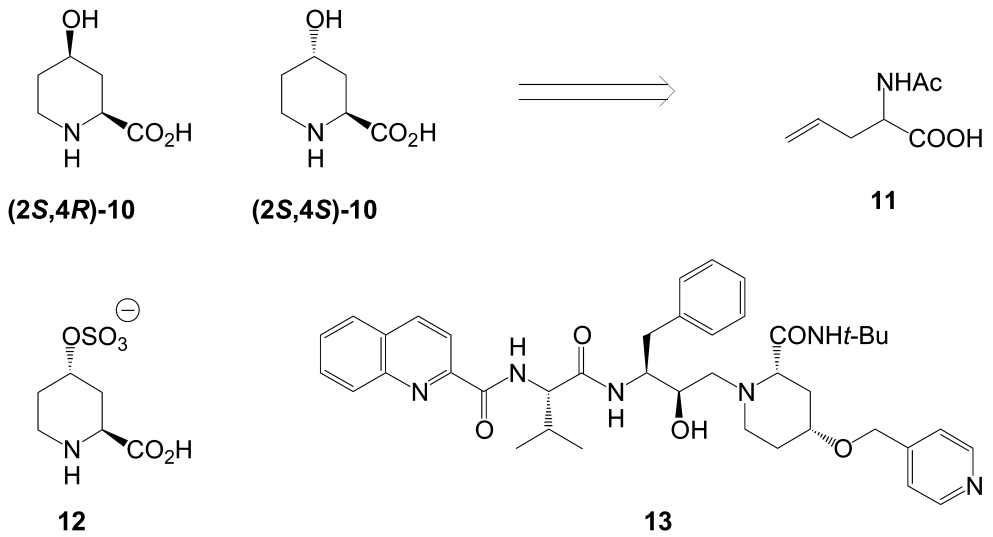


Figure 3. Source and use of 4-hydroxypipecolic acids.

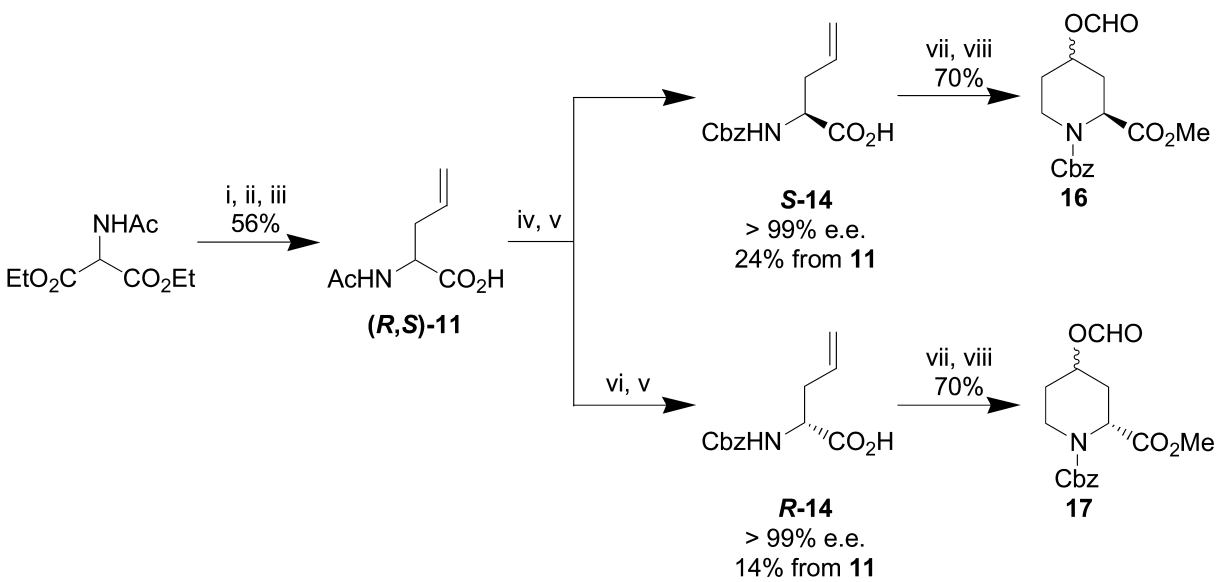
the amino acid **2**, which was, in turn, introduced to the synthesis by the choice of acylase enzyme.

2.2. 4-Hydroxypipecolic acids

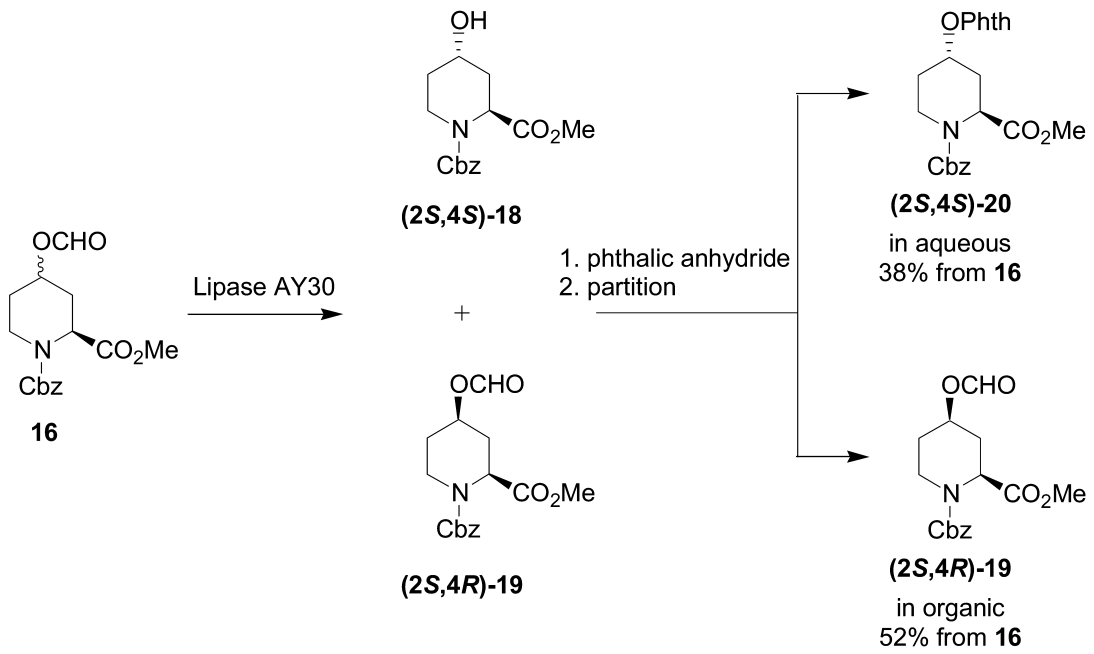
A similar approach was applied to the synthesis of all four diastereoisomers of 4-hydroxypipecolic acid **10**.[16] The (2*S*,4*RS*)-diastereomers are naturally occurring non-proteinogenic amino acids that have been isolated from the leaves of *Calliandra pittieri*, *Strophantus scandeus* and *Acacia oswaldii*.[17] Much like isomers of bulgecinine, these compounds possess all the necessary criteria for use as scaffolds in medicinal chemistry programmes. Indeed, molecules derived from both (2*S*,4*S*)-**10** and (2*S*,4*R*)-**10** have been demonstrated to possess biological activity: the naturally occurring sulphate **12**[18] is a NMDA receptor agonist[19] and (2*S*,4*R*)-**10** is a constituent of the synthetic HIV protease inhibitor palinavir **13** (Fig. 3).[20]

Previous work had identified the enantiomers of allylglycine, an ideal acylase substrate, as starting materials for the synthesis of the pipecolic acid core.[21] The racemic starting material, *N*-acetylallylglycine **11** (Fig. 3) was prepared from diethylacetamidomalonate and allyl bromide,[22] and proved to be an excellent substrate for both of our proprietary L- and D-acylase enzymes allowing for efficient resolution of both enantiomers (Scheme 4). The next key step in the synthesis, the acyliminium ion cyclisation of the methyl ester of **14**,[23] gave the 1:1 mixtures of diastereomers at C-4, **16** (from *S*-**14**) and **17** (from *R*-**14**), which had previously only been separated by chromatography, which we deemed unsatisfactory for a scaleable synthesis.

Selective hydrolysis of one of the formate esters in the mixtures **16** and **17** using lipases allowed the differentiation of the diastereomers in such a way they could then be



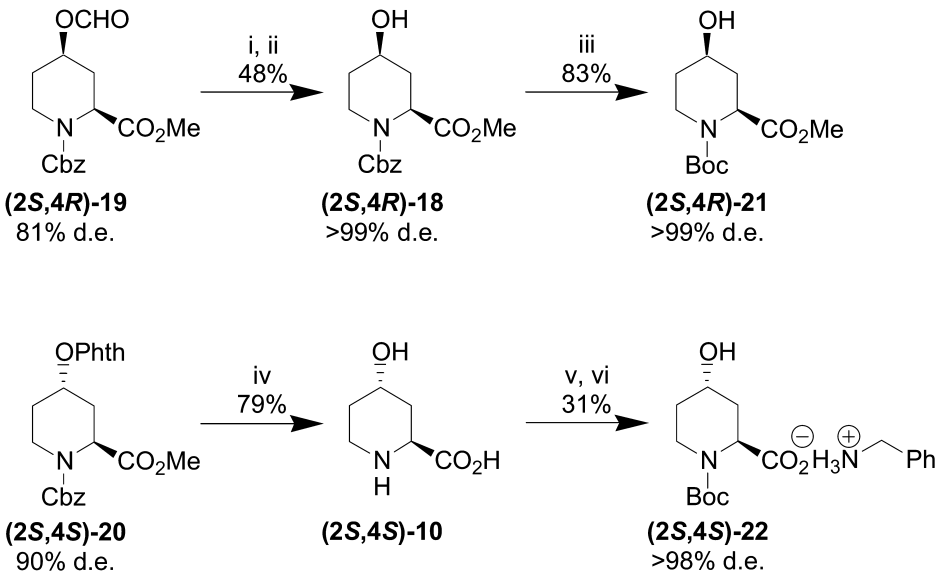
Scheme 4. *Reagents and conditions* (i) *t*-BuOK, allyl bromide (ii) NaOH (iii) HCl (iv) L-acylase, pH 8.0, 65 °C (v) Cbz-Cl, pH 8.0 (vi) D-acylase, pH 8.0 (vii) $SOCl_2$, MeOH (viii) paraformaldehyde, HCO_2H.



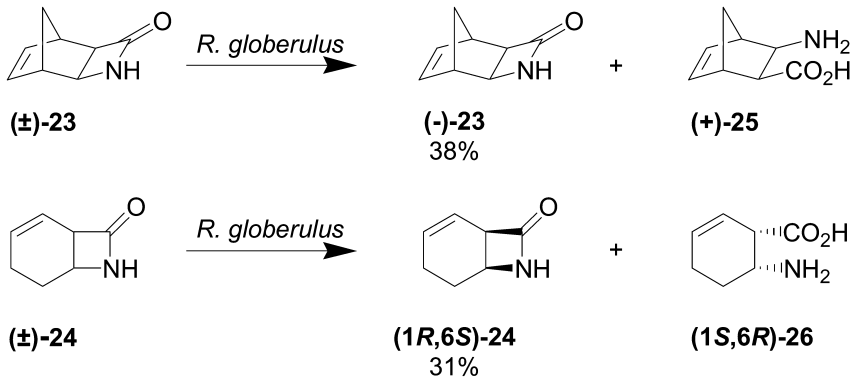
Scheme 5.

separated.[16] Lipase AY30 (*Candida rugosa*, Amano) selectively hydrolysed the formate ester of the (2*S*,4*S*)-diastereomer of mixture **16** (Scheme 5) to yield the 4-hydroxypipecolic acid methyl ester (2*S*,4*S*)-**18** (90% d.e.) and the 4-formyloxypipecolic acid methyl ester (2*S*,4*R*)-**19** (81% d.e.). The components of this mixture were separated by in situ derivatisation of (2*S*,4*S*)-**18** to the hemiphthalate ester derivative (2*S*,4*S*)-**20**, and partitioning between toluene and saturated ammonium carbonate solution.[24] Concentration of the toluene layer gave (2*S*,4*R*)-**19**. The hemiphthalate ester (2*S*,4*S*)-**20** was recovered by acidification of the aqueous layer and extraction into toluene. A similar approach was used for the separation of mixture **17**, using Chirazyme L9 (*Mucor miehei*, Roche) in the biotransformation, which preferentially hydrolysed the formate ester of the (2*R*,4*S*)-diastereomer of the mixture.

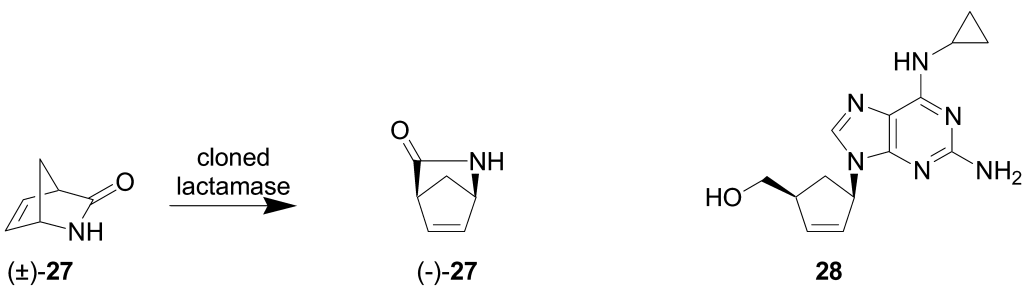
Although the lipases were able to separate the diastereomers, the products still lacked the required optical purity, which was gained using crystallisation. Optically pure *cis*-4-hydroxypipecolate (2*S*,4*R*)-**21** was obtained via (2*S*,4*R*)-**18** (Scheme 6), a known crystalline intermediate.[25] In the preparation of *trans*-4-hydroxypipecolic acid (2*S*,4*S*)-**10**, no suitable crystalline intermediate could be found, and thus was crystallised to diastereomeric purity as the *N*-Boc benzylamine salt (2*S*,4*S*)-**22**. Identical routes were used to prepare the enantiomeric compounds (2*R*,4*S*)-**21** and (2*R*,4*R*)-**22** from (2*R*,4*S*)-**19** and (2*R*,4*R*)-**20**, respectively. The syntheses of all four compounds demonstrate the usefulness of crystallisation as a dovetailing technology that greatly augments the power of the previously used chemistry. In this example, neither biocatalysis nor crystallisation alone was sufficient to separate the pairs of



Scheme 6. *Reagents and conditions* (i) K_2CO_3, MeOH (ii) recrystallise (iii) H_2, 10% Pd/C, Boc_2O (iv) 2 M HCl, then Amberlite IRA-93 (v) Boc_2O, Et_3N (vi) $BnNH_2$, recrystallise.



Scheme 7.

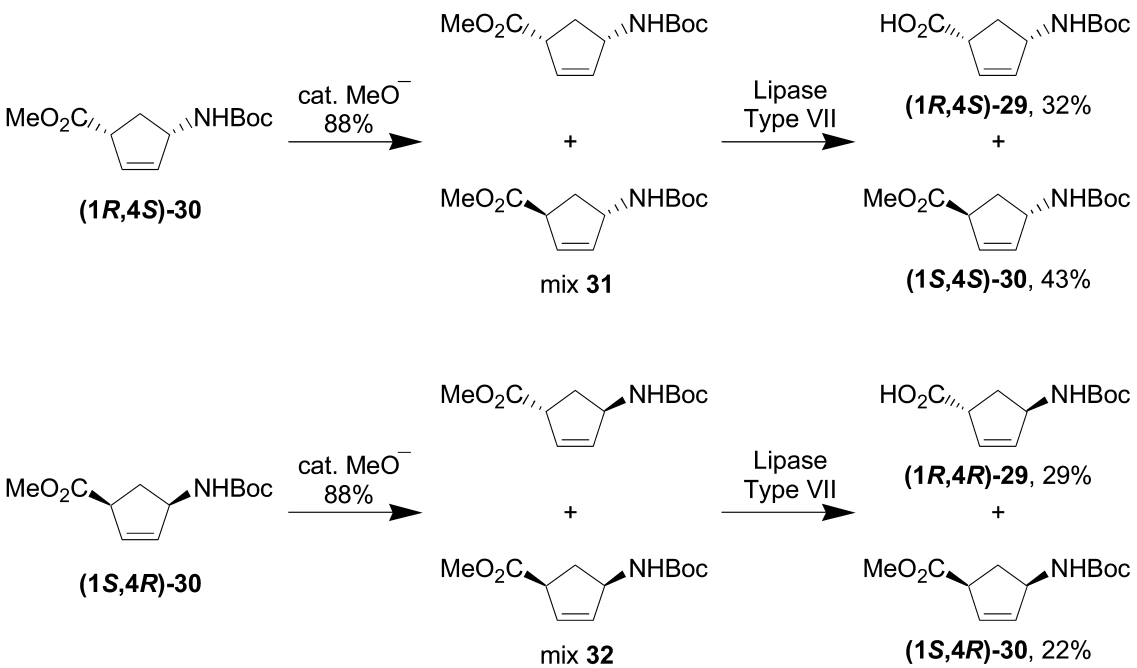


Scheme 8.

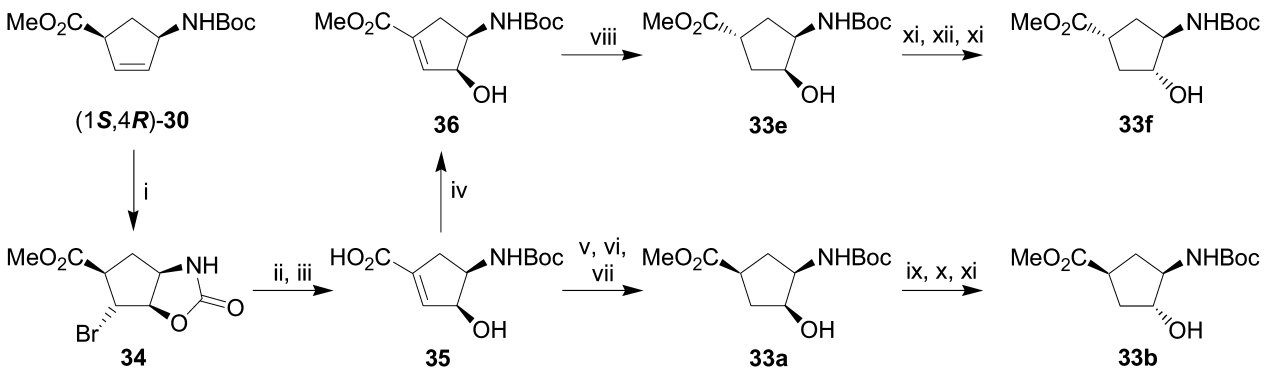
diastereomers; however, used in tandem they have provided an effective solution to this problem.

3. Use of lactamases for the preparation of β-and γ-amino acids

Many lactams possess biological activity, most notably the β-lactam class of antibiotics.[26] In addition to their own medicinal potential, lactams are a very useful source of amino acids. For example, a number of enantiopure cyclic β-amino acids have been prepared using lipase-catalysed kinetic resolutions of suitably protected β-lactams.[27] When condensed to form β-peptides, their properties differ greatly from α-peptides, especially when the $C_\alpha - C_\beta$ bond is constrained in a small ring.[28] In addition to this, β-peptide bonds appear far more stable than α-peptide bonds in a physiological environment as they are more resistant to protease enzymes.[29] We prepared 3-aza-tricyclo[4.2.1.$0^{2,5}$]-non-7-en-4-one **23** and 7-aza-bicyclo[4.2.0]oct-4-en-8-one



Scheme 9.



Scheme 10. *Reagents and conditions*: i, NBS, THF–H_2O (10:1), RT, 18 h, 95%; ii, KOH, MeOH–H_2O (1:1), 90 °C, 3 days; iii, Boc_2O, H_2O–THF (5:1), pH 10.5, 5 °C to RT, 18 h, 84% (overall for steps ii and iii); iv, MeOCOCl, Et_3N, MeOH, 5 °C then RT, 18 h, 81%; v, H_2 (35 psi), Pd/C, MeOH, RT, 18 h, (de 76%, 98%); vi, *tert*-butylamine, MTBE, recrystallise; vii, MeOCOCl, Et_3N, MeOH, 5 °C then RT, 18 h, 86%; viii, H_2 (75 psi), [(*R*,*R*)-Me-DuPHOS)-Rh(COD)]BF_4, MeOH, RT, (de >97%, 100%); ix, $MeSO_2Cl$, Et_3N, DMAP, CH_2Cl_2, 5 °C, 3 h, 97%; x, KOAc, DMF, 60 °C, 48 h, 85%; xi, NaOMe, MeOH, 5 °C, 7 h, 98%; xii, KOAc, DMF, 60 °C, 8 days, 88%.

24 as racemates from chlorosulphonylisocyanate and either 2,5-norbornadiene or 1,3-cyclohexadiene, respectively.[30] These were successfully resolved using a lactamase present in the whole cells of *Rhodococcus globerulus* (NCIMB 41042). The resolutions were carried out in phosphate buffer at pH 7 for 24 h, yielding (−)-**23** of >98% e.e. and (1*R*,6*S*)-**24** of >95% e.e. (Scheme 7). This stereochemistry was assigned by comparison of the optical rotation with that of its enantiomer.[27b]

We have previously reported the resolution of (±)-2-azabicyclo[2.2.1]hept-5-en-3-one **27** using a cloned lactamase[31] in a process that has been used to produce tonne quantities of (−)-**27**, an intermediate for the industrial synthesis of carbocyclic nucleosides, for example abacavir, **28** (Scheme 8).

Derivatives of the products of these resolutions of **27** can be used in the synthesis of a range of compounds with biological activity. An example of this is the synthesis of both enantiomers of *trans*-4-aminocyclopent-2-ene-1-carboxylic acid, (1*S*,4*S*)-**29** (as the methyl ester (1*S*,4*S*)-**30**) and (1*R*,4*R*)-**29**. It was previously shown that the racemic amino acid inhibited the uptake of GABA in rat brain (Scheme 9).[32]

Treatment of the Boc-protected *cis*-amino acid methyl ester (1*R*,4*S*)-**30** with catalytic sodium methoxide in methanol at 0 °C led to an epimerisation at C-1 to give the 1:1 mixture of diastereomers **31**. Although separation of these compounds was not possible by crystallisation, a screen of **31** against **5** commercially available lipases revealed Lipase Type VII (*Candida rugosa*, Sigma) as a highly competent differentiating agent that preferentially hydrolysed the ester of the *cis*-isomer. Interestingly, during the synthesis of the opposite enantiomer, (1*R*,4*R*)-**29**, we found that Lipase Type VII was again the enzyme of choice for the separation of the diastereomer mix **32**, but this time preferentially hydrolysing the *trans*-isomer. This demonstrates the very high selectivity the enzyme displays for the C-1 chiral centre: in each case it is the *R*-centre that is hydrolysed, in spite of the steric constraints placed on the molecule by both the change in relative stereochemisty and the steric bulk of the other pendant functional group, the *N*-Boc.

The products of the bioresolution of (±)-**27** have also been used as precursors of the cyclopentane series of scaffolds **33a**–**33h** shown in Figure 4[33] Starting from the *N*-Boc protected amino acid methyl ester (1*S*,4*R*)-**30**, treatment with NBS yielded the oxazolidinone **34**, which upon treatment with base, eliminated and rearranged to give the allylic alcohol **35** in excellent yield (Scheme 10). Palladium on charcoal hydrogenation of **35** gave an all *cis*-compound in 76% d.e. which could be crystallised to diastereomeric purity as the *tert*-butylamine salt. Cracking of this salt followed by esterification gave **33a**. If, however, **35** was esterified, it became an excellent substrate for hydrogenation using the [(*R*,*R*)-Me-DuPHOS)-Rh(COD)]BF_4 catalyst, which gave **33e** directly (>97% d.e.). A mesylation-acetate inversion sequence on **33a** and **33e** provided the diastereomers **33b** and **33f**, respectively.

The other four diastereomers were obtained from the enantiomeric starting material (1*R*,4*S*)-**30**. The only

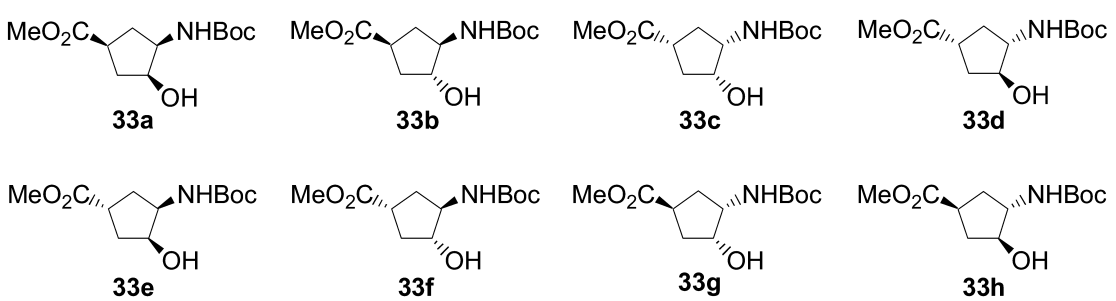


Figure 4. The eight diastereomers of 3-*tert*-butoxycarbonylamino-4-hydroxy-cyclopentanecarboxylic acid methyl ester.

difference in the sequence was that to obtain **33g**, the [(*S*,*S*)-Me-DuPHOS)-Rh(COD)]BF_4 catalyst was used for the hydrogenation. These scaffolds can be used for the synthesis of molecules with potential biological activity, for example in structures that are analogues of natural products.[34,35]

4. Conclusion

In the examples discussed in this paper, hydrolase enzymes have been used to resolve enantiomers and differentiate diastereomers such that they can be easily separated and provide a mild environment to enable manipulation of highly functionalised molecules. The nature of such chemistry allows for use on large scale. This broad spectrum of use clearly demonstrates why they are so widely used in synthetic chemistry today. However, to elaborate simple compounds into the more complex, rigid compounds described has emphasised the necessity for combining chiral technologies. In most of the syntheses described, although the biocatalytic step has been crucial for introducing at least one of the chiral centres in the target compound, others have been introduced by a compatible technology.

5. Experimental

5.1. General

All reagents and solvents were from commercially available sources and used as received. Proton NMR spectra were obtained on a Bruker Avance 400 spectrometer operating at 400 MHz for proton (^{1}H) and 100 MHz for carbon (^{13}C). Chemical shifts are reported in ppm using Me_4Si or residual nondeuterated solvent as reference. Coupling constants (*J*) are measured in Hz. GC-MS data were obtained using a HP 5890 Series 2 GC fitted with a J and W Scientific DB5 column, attached to a HP 5972 series Mass Selective Detector using electron impact ionisation. GC d.e. and e.e. data of final compounds were obtained using a Perkin Elmer Autosystem Gas Chromatograph fitted with a Varian Chirasil-Dex CB column (25 m×0.25 mm). HPLC data were obtained using a Gilson HPLC system fitted with a Luna Phenylhexyl column. HPLC e.e. data were recorded using a Phenomenex D-penicillamine (150×4.6 mm) column. Optical rotation data were obtained using a Perkin Elmer Polarimeter 341 instrument.

5.1.1. 4-Benzoyloxybut-2-yne-1-ol 4.[36] 2-Butyne-1,4-diol (500 g, 5.81 mol) was dissolved in CH_2Cl_2 (1 L) and pyridine (470 mL, 5.81 mol) and cooled to 5 °C under N_2. Benzoyl chloride (608 mL, 5.25 mol) in CH_2Cl_2 (1 L) was added over a 3 h period, keeping the reaction temperature below 10 °C. The resultant yellow suspension was allowed to warm to room temperature and stirred overnight. The reaction mixture was washed with 1 M H_2SO_4 (3×600 mL) and H_2O (2×600 mL) and organic phase concentrated to 900 mL volume. EtOH (750 mL) was added, and the solution placed in a freezer overnight. Any precipitate was removed by filtration and washed with EtOH (100 mL). The filtrate was concentrated under reduced pressure to give **4**, an orange oil (519 g, 52%). ^{1}H NMR ($CDCl_3$) 8.06 (2H, m), 7.57 (1H, m), 7.44 (2H, m), 4.96 (2H, t, *J*=2 Hz), 4.34 (2H, t, *J*=2 Hz), 2.97 (1H, br s). NMR also showed presence of ~8 mol% dibenzoate ester. ^{13}C NMR ($CDCl_3$) 166.4, 134.2, 130.9, 130.2, 128.8, 85.4, 81.4, 53.1, 51.5. *m*/*z* 190 (M^+), 173, 105, 77, 51.

5.1.2. 4-Benzoyloxybut-2-yne-1-ol, methanesulphonate ester 5.[37] **4** (278 g, 1.46 mol) in CH_2Cl_2 (2.1 L) was cooled to 0 °C under N_2 Triethylamine (224 mL, 1.61 mol) and DMAP (4.45 g, 36 mmol) were added, followed by methanesulphonyl chloride (125 mL, 1.61 mol) in CH_2Cl_2 (100 mL) dropwise over 30 min period, keeping the reaction temperature below 5 °C. The reaction was stirred at 0 °C for 2 h, when H_2O (1.5 L) was added. After stirring for 5 min, the layers were separated, and the organic layer washed with sat. $NaHCO_3$ (1.5 L) and 1 M HCl (1.5 L), dried ($MgSO_4$) and concentrated under reduced pressure to give **5**, a brown oil (350 g, 90%). ^{1}H NMR ($CDCl_3$) 8.04 (2H, m), 7.57 (1H, m), 7.45 (2H, m), 4.96 (2H, m), 4.89 (2H, t, *J*=2 Hz), 3.12 (3H, s). ^{13}C NMR ($CDCl_3$) 166.1, 134.1, 130.6, 130.1, 128.8, 84.5, 79.3, 57.9, 53.0, 39.5. *m*/*z* No M^+, 173, 105, 77, 51.

5.1.3. 1-Bromo-4-benzoyloxybut-2-yne 6.[37] A solution of **5** (345 g, 1.29 mol) in acetone (1.7 L) was cooled to 10 °C under N_2. LiBr (223 g, 2.57 mol) was added portionwise, keeping the temperature below 15 °C, and the reaction stirred at this temperature for 90 min. The mixture was filtered through celite, and the filtrate concentrated under reduced pressure. EtOAc (2 L) was added to the residue, and this solution washed with H_2O (2×1 L), dried ($MgSO_4$) and concentrated under reduced pressure to yield **6**, a brown oil (303 g, 90%). ^{1}H NMR ($CDCl_3$) 8.07 (2H, m), 7.57 (1H, m), 7.46 (2H, m), 4.99 (2H, m), 3.96 (2H, m). ^{13}C NMR ($CDCl_3$) 166.2, 133.8, 130.2, 129.9, 128.7, 82.2, 81.1, 53.1. *m*/*z* No M^+, 173, 105, 77, 51.

5.1.4. 1,1-Dicarboethoxy-1-acetamido-5-benzoyloxy-pent-3-yne 7. A suspension of diethylacetamidomalonate (258 g, 1.19 mol) and KO^{t}Bu (146 g, 1.30 mol) in THF (3 L) was heated to reflux for 1 h. To this refluxing mixture, a stream of **6** (300 g, 1.19 mol) in THF (500 mL) was added over a 10 min period. After 2.5 h, the reaction had not gone to completion, so a further 100 g (0.47 mol) diethyl-acetamidomalonate and KO^{t}Bu (58 g, 0.47 mol) were added and the mixture refluxed overnight. The reaction mixture was cooled and filtered through celite, and the filtrate concentrated under reduced pressure to a volume of 1 L. EtOAc (2 L) was added and the solution washed with 0.1 M HCl (1 L) and H_2O (1 L). The organic phase was concentrated under reduced pressure to yield **7**, a brown oil (365 g, 79%). ^{1}H NMR ($CDCl_3$) 8.05 (2H, m), 7.58 (1H, m), 7.46 (2H, m), 6.96 (1H, br s), 4.85 (2H, t, *J*=2 Hz), 4.25 (4H, m), 3.33 (2H, t, *J*=2 Hz), 2.04 (3H, s), 1.24 (6H, m). ^{13}C NMR (d_6-DMSO) 170.0, 166.6, 165.3, 134.0, 129.6, 129.5, 129.2, 81.8, 77.8, 65.6, 62.4, 53.0, 22.4, 14.1. *m*/*z* 389 (M^+), 345, 316, 274, 194, 174, 152, 124, 105, 77, 51.

5.1.5. *N*-Acetyl-(4-hydroxybut-2-ynyl)glycine 3. To a stirred solution of **7** (300 g, 0.77 mol) in EtOH (1.5 L) and H_2O (1 L) NaOH (92.5 g, 2.31 mol) was added in H_2O (500 mL) as a thin stream. The mixture was heated to reflux for 4 h, cooled and the EtOH removed under reduced pressure. The resultant aqueous solution was washed with

EtOAc (1 L), and then carefully acidified to pH 3.5 using conc. HCl, and heated to reflux for 24 h. After cooling, the solution was acidified to pH 2.5 with conc. HCl and extracted with EtOAc (1.5 L). The pH of the aqueous phase was then adjusted to 6.0 with 46–48% NaOH solution and concentrated under reduced pressure. The residue was slurried in MeOH (500 mL) and filtered through celite, after which decolourising charcoal (25 g) was added to the filtrate and this mixture heated to 50 °C for 20 min. After filtration through celite, the filtrate was concentrated under reduced pressure to give **3**, an orange oil (149 g, 100% crude yield). ^{1}H NMR (D_2O) 4.27 (1H, t, J=6 Hz), 4.15 (2H, t, J=2 Hz), 2.67 (2H, m), 2.01 (3H, s). ^{13}C NMR (d_6-DMSO) 172.5, 168.6, 82.7, 80.9, 53.3, 49.6, 23.3, 22.8.

5.1.6. Resolution of (±)-3. 3 (125 g, 0.67 mol) in 30 mM KH_2PO_4 (1.7 L) at pH 7 was treated with L-acylase solution (200 U/g substrate) and the solution stirred at 65 °C and pH 7 until ^{1}H NMR of an evaporated sample showed >40% conversion (44 h). Boc_2O (47 g, 0.27 mol) in THF (200 mL) was then added at RT, and the reaction maintained at pH 10 until the pH was static. The mixture was extracted with MTBE (500 mL), then the remaining aqueous acidified to pH 3 with $KHSO_4$ and extracted with EtOAc (3×1 L). The EtOAc portions were combined, dried ($MgSO_4$), filtered and evaporated in vacuo to give (*S*)-*N*-Boc-(4-hydroxybut-2-ynyl)glycine *S*-**8**, a pale yellow oil (44 g, 36%, 98% e.e.). ^{1}H NMR (d_6-DMSO) 12.6 (1H, br s), 7.05 (d, 1H, J=8 Hz), 5.07 (1H, br), 4.03 (2H, m), 2.55 (2H, m), 1.37 (9H, s). ^{13}C NMR (d_6-DMSO) 172.6, 155.6, 80.7, 78.6, 53.1, 54.0, 49.4, 28.5, 21.8. The remaining aqueous solution from above was then adjusted to pH 8 with 46/48% NaOH, and D-acylase solution added (40 U/g substrate). The reaction mixture was stirred at RT and pH maintained at pH 8 until the reaction was complete by ^{1}H NMR (21 h). The pH was then adjusted to 10 and the mixture treated with Boc_2O (54 g, 0.31 mol) and worked up as above to give (*R*)-*N*-Boc-(4-hydroxybut-2-ynyl)glycine *R*-**8**, an orange oil (48 g, 34%, 95% e.e.). ^{1}H NMR (d_6-DMSO): 12.6 (1H, br s), 7.05 (d, 1H, J=8 Hz), 5.07 (1H, br), 4.03 (2H, m), 2.55 (2H, m), 1.37 (9H, s). ^{13}C NMR (d_6-DMSO) 172.6, 155.6, 80.7, 78.6, 53.1, 54.0, 49.4, 28.5, 21.8.

5.1.7. *S*-*N*-Boc-(*Z*-4-hydroxybut-2-enyl)glycine *S*-2.[38] Lindlar's catalyst (3.0 g) was added to a degassed solution of *S*-**8** (30 g) in MeOH (600 mL), and the resultant mixture sealed in a 2 L Parr pressure vessel. After purging with N_2 and H_2, the vessel was charged to 1 bar with H_2 and the reaction mixture stirred at 20 °C until the H_2 pressure above remained constant. The remaining H_2 was released, and the vessel purged with N_2. The reaction mixture was filtered through celite and concentrated under reduced pressure to give *S*-**2** (30 g, 100%). This material can be purified by flash chromatography (eluent 5:4:1 EtOAc/heptane/AcOH) if required. ^{1}H NMR (d_6-DMSO): 7.02 (1H, d, J=8 Hz), 5.55 (1H, m), 5.34 (1H, m), 3.94 (2H, d, J=6), 3.86 (1H, m), 2.35 (2H, m), 1.36 (9H, s).

5.1.8. Resolution of 3-aza-tricyclo [4.2.1.0^{2,5}] non-7-en-4-one (±)-23. (±)-**23** (2 g, 15 mmol) and a whole cell preparation of *Rhodococcus globerulus* NCIMB 41042 (2 g, 1 wt equiv.) were suspended in 50 mM KH_2PO_4, pH 7 (40 mL). The resulting mixture was stirred at 25 °C for 24 h. The cells were then spun off by centrifuge (10 min, 3400 rpm) and the pellet was washed with H_2O (20 mL) and respun. The combined supernatants were extracted with EtOAc (3×50 mL) and the organic extracts dried over $MgSO_4$. Removal of solvent under reduced pressure yielded (−)-**23** (770 mg, 38%, e.e. (GC) >98%), a white solid. ^{1}H NMR ($CDCl_3$) 6.19–6.30 (1H, m), 6.00–6.18 (2H, m), 3.44–3.53 (1H, m), 2.98–3.10 (1H, m), 2.91–2.96 (1H, br s), 2.82–2.91(1H, br s), 1.81 (1H, d, J=10 Hz), 1.65 (1H, d, J=10 Hz). ^{13}C NMR ($CDCl_3$): 171.0, 138.6, 136.3, 58.6, 53.5, 44.0, 41.1, 39.1. *m*/*z* No M$^+$, 107, 91, 70, 66. $[\alpha]_D^{20}$ (*c* 0.01, MeOH) −91.

5.1.9. Resolution of racemic 7-aza-bicyclo [4.2.0]oct-4-en-8-one 24. 24 (1.49 g, 12 mmol) and a whole cell preparation of *Rhodococcus globerulus* NCIMB 41042 (1.4 g, 1 wt equiv.) were suspended in 50 mM KH_2PO_4, pH 7 (40 mL). The resulting mixture was stirred at 30 °C for 22 h. The cells were then spun off by centrifuge (10 min, 3400 rpm) and the pellet was washed with H_2O and respun. The combined supernatants were extracted with EtOAc (3×50 mL) and the organic extracts dried over $MgSO_4$. Removal of solvent under reduced pressure yielded (1*R*,6*S*)-**24**, (460 mg, 31%, e.e. (GC) >95%), an off-white solid. ^{1}H NMR ($CDCl_3$): 6.19–6.10 (1H, m), 6.07 (1H, br s), 5.98–5.90 (1H, m), 4.03 (1H, t, J=5 Hz), 3.55–3.48 (1H, m), 2.16–2.02 (3H, m), 1.68–1.55 (1H, m). ^{13}C NMR ($CDCl_3$) 172.3, 135.0, 126.3, 50.2, 44.7, 22.2, 22.1. *m*/*z* no M$^+$, 103, 80, 79. $[\alpha]_D^{25}$ (*c* 0.13, $CHCl_3$) −154.

5.1.10. Epimerisation (1*R*,4*S*)-*N*-Boc-4-aminocyclopent-2-ene-1-carboxylic acid, methyl ester, (1*R*,4*S*)-30. A solution of (1*R*,4*S*)-**30** (1.82 g, 7.55 mmol) in MeOH (30 mL) was cooled to 0 °C and NaOMe (25 wt% in MeOH, 2 mL, pre-cooled to 0 °C) was added. The resulting mixture was stirred for 120 min, maintaining the temperature at 0 °C throughout. After this time, it was quenched using glacial acetic acid (4 mL). The solvent was removed under reduced pressure to yield a yellow oil (1.6 g) which solidified on standing. GC–MS analysis indicates that the solid consists of a 1:1 mixture of (1*R*,4*S*) (*cis*) and (1*S*,4*S*) (*trans*) epimers (mixture **31**) in addition to a small amount (<10%) of the conjugated adduct. GC–MS: Retention time 16.59 min (*cis*-epimer, *m*/*z*: 185, 168, 141, 126, 82), 17.16 min (*trans*-epimer *m*/*z*: 185, 168, 141, 126, 82), 17.91 min (conjugated adduct).

5.2. Screening for selective hydrolysis of mixtures 31 and 32

50 mg of substrate (either **31** or **32**) was placed into a scintillation vial with 50 mM KH_2PO_4, pH 7 (5 mL) and enzyme (20 mg) and the vial agitated at 26 °C in a water bath/shaker After 18 h the reaction was extracted with EtOAc (5 mL). The organic extracts were dried over $MgSO_4$ and analysed by GC–MS. Enzymes used: Porcine pancreatic lipase (Sigma), Lipase Type VII (Sigma), Lipase PS (Amano), Chirazyme L9 (Roche) and Chirazyme L2 (Roche). Analysis revealed that Lipase Type VII gave the greatest selectivity for both mixtures, hydrolysing the *cis*-epimer of **31** and the *trans*-epimer of **32**.

5.2.1. Scale up of the separation of mixture 31. 31 (0.6 g,

2.5 mmol) was suspended in 50 mM phosphate buffer, pH 7 (60 mL) and Lipase type VII (250 mg) added The mixture was left to shake in a water bath/shaker at 26 °C for 24 h and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield (1*S*,4*S*)-**30**, an off-white solid (260 mg, 43%). GC-MS confirmed the structure of the recovered material. To recover the *cis*-epimer, the aqueous phase was acidified with 1.2 M HCl and extracted with EtOAc (3×50 mL). The combined extracts were dried over $MgSO_4$ and concentrated to yield a colourless oil (180 mg, 32%) that solidified on standing. ^{1}H NMR (CD_3OD) major (*cis*) diastereomer[39] 5.81 (1H, m), 5.71 (1H, m), 4.53 (1H, br s), 3.40 (1H, m), 2.42 (1H, dt, *J*=14, 8 Hz), 1.70 (1H, m), 1.34 (9H, s). ^{13}C NMR (CD_3OD) 177.9, 158.0, 135.5, 132.9, 80.6, 57.7, 50.8, 35.8, 29.2; minor (*trans*) diastereomer 5.81 (1H, m), 5.71 (1H, m), 4.63 (1H, br m), 3.56 (1H, m), 2.42 (1H, m), 1.70 (1H, m), 1.34 (9H, s). ^{13}C NMR (CD_3OD) 177.5, 157.9, 135.1, 132.9, 80.1, 57.6, 50.8, 35.7, 28.8. Comparison of relative integrations of dispersed resonances show ratio of *cis*:-*trans*-diastereomers to be 4:1.

5.2.2. Scale up of the separation of mixture 32. 32 (1.0 g, 4.15 mmol) was suspended in 50 mM phosphate buffer, pH 7 (100 mL) and Lipase type VII (400 mg) added. The mixture was left to shake in a water bath/shaker at 26 °C for 24 h and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield (1*S*,4*R*)-**30**, an off-white solid (220 mg, 22%). GC-MS confirmed the structure of the recovered material. To recover the *trans*-epimer, the aqueous phase was acidified with 1.2 M HCl and extracted with EtOAc (3×50 mL). The combined extracts were dried over $MgSO_4$ and concentrated to yield a white solid (270 mg, 29%) that solidified on standing. ^{1}H NMR (CD_3OD) major (*trans*) diastereomer 5.81 (1H, m), 5.71 (1H, m), 4.63 (1H, br m), 3.56 (1H, m), 2.42 (1H, m), 1.70 (1H, m), 1.34 (9H, s). ^{13}C NMR (CD_3OD) 177.5, 157.9, 135.1, 132.9, 80.1, 57.6, 50.8, 35.7, 28.8; minor (*cis*) diastereomer 5.81 (1H, m), 5.71 (1H, m), 4.53 (1H, br s), 3.40 (1H, m), 2.42 (1H, dt, *J*=14, 8 Hz), 1.70 (1H, m), 1.34 (9H, s). ^{13}C NMR (CD_3OD) 177.9, 158.0, 135.5, 132.9, 80.6, 57.7, 50.8, 35.8, 29.2. Comparison of relative integrations of dispersed resonances show ratio of *trans*:-*cis*-diastereomers to be 3:1.

5.2.3. (3α*R*,5*R*,6*R*,6α*R*)-6-Bromo-2-oxo-hexahydro-cyclopentaoxazole-5-carboxylic acid methyl ester 34. *N*-Bromosuccinimide (164 g, 0.92 mol) was added in portions to a solution of (1*S*,4*R*)-**30** (200 g, 0.83 mol) in THF (670 mL) and water (67 mL). The *N*-Bromosuccinimide was seen to completely dissolve over a 3 h period during which a mild exotherm was evident. The reaction was stirred for a further 14 h after which time it was concentrated to dryness under vacuum. The residue was redissolved in dichloromethane (1.1 L) and washed sequentially with 1 M HCl (aq.) (500 mL), saturated Na_2SO_3 (aq.) (500 mL) and brine (500 mL) before drying over $MgSO_4$. Following filtration, concentration of the organic under vacuum yielded 238 g of a light brown solid. Recrystallisation from EtOAc/heptane yielded **34** as white crystals (110 g, 50%). A further crop of crystals was obtained from the liquors though it was contaminated with a small amount of succinimide (49 g, 23%). ^{1}H NMR ($CDCl_3$) 5.79 (1H, br s,), 5.15 (1H, dd, *J*=7, 2 Hz), 4.79 (1H, m), 4.43 (1H, app dt, *J*=7, 2 Hz), 3.75 (3H, s), 3.23 (1H, app quintet, *J*=7 Hz), 2.52 (1H, dt, *J*=14, 7 Hz), 2.42 (1H, dm, *J*=14 Hz). ^{13}C NMR ($CDCl_3$) 171.2, 158.1, 87.8, 56.1, 53.3, 52.5, 51.5, 35.2. *m/z* 265, 263 (1:1, M^+), 184, 156, 140, 124, 115, 80.

5.2.4. (3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-cyclopent-1-enecarboxylic acid 35. KOH (43 g, 0.76 mol) in H_2O (150 mL) was added to a solution of **34** (36 g, 0.19 mol) in MeOH (150 mL). The mixture was heated under reflux at 90 °C for 2 days and allowed to cool. MeOH was removed under vacuum, the mixture diluted with H_2O (100 mL) and the pH of the reaction was adjusted to pH 10.5 on addition of 1 M HCl (aq.). The solution was cooled to 10 °C and a solution of Boc_2O (42 g, 0.19 mmol) in THF (60 mL) was added dropwise and the mixture allowed to warm to room temperature. The reaction was then stirred for 14 h, the layer of THF removed and the aqueous solution adjusted to pH 3 with 6 M HCl (aq). The acidic solution was extracted with EtOAc (3×200 mL) and the combined organic washings dried over $MgSO_4$. Filtration then concentration under vacuum gave **35** as a white solid (40 g, 86%). ^{1}H NMR (d_6-DMSO) 6.58 (1H, m), 6.40 (1H, d, *J*=7 Hz), 4.57 (1H, m), 4.05 (1H, m), 3.41 (1H, br s), 2.65 (1H, app dd, *J*=16, 7 Hz), 2.41 (1H, ddt, *J*=16, 6, 1 Hz), 1.46 (9H, s). ^{13}C NMR ($CDCl_3$) 168.5, 158.5, 142.8, 139.8, 80.7, 75.3, 54.2, 37.3, 29.1.

5.2.5. (3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-cyclopent-1-enecarboxylic acid methyl ester 36. Methyl chloroformate (29 mL, 0.37 mol) was added dropwise to a cooled solution (0 °C) of **35** (83 g, 0.34 mol) and triethylamine (52 mL, 0.37 mol) in MeOH (600 mL). The reaction was stirred for 1 h at 0 °C and then allowed to warm to room temperature. After stirring for 14 h it was apparent that the reaction was incomplete. After cooling to 0 °C, triethylamine (30 mL, 0.22 mol) was added then methyl chloroformate (15 mL, 0.19 mol) added dropwise. The reaction was complete after a further 2 h. The reaction mixture was concentrated under vacuum and the residue redissolved in CH_2Cl_2 (400 mL). The organic phase was washed sequentially with 1 M $KHSO_4$ (aq.) (2×200 mL), sat. $NaHCO_3$ (2×200 mL) and brine (200 mL) before drying over $MgSO_4$. Following filtration, the solvent was removed to yield an orange oil which was redissolved in MeOH (300 mL) and cooled to 5 °C. NaOMe (0.25 mL, 25 wt% solution in MeOH) was then added and the reaction mixture cooled and stirred for 4 h. The reaction was quenched with glacial AcOH, concentrated under vacuum and the residue redissolved in CH_2Cl_2 (300 mL). The organic phase was washed with sat. $NaHCO_3$ (100 mL) then brine (100 mL) and dried over $MgSO_4$. Following filtration, the solvent was removed under vacuum to yield **36** as an orange oil (76 g, 87%). ^{1}H NMR ($CDCl_3$) 6.71 (1H, m), 5.26 (1H, d, *J*=7 Hz), 4.77 (1H, br s), 4.25 (1H, br s), 3.77 (3H, s), 3.04 (1H, br s), 2.92 (1H, dd, *J*=17, 7 Hz), 2.50 (1H, m), 1.46 (9H, s). ^{13}C NMR ($CDCl_3$) 165.1, 156.1, 141.0, 137.6, 79.5, 74.7, 52.5, 51.8, 36.5, 28.3. *m/z* No M^+, 201, 183, 151, 137, 125, 106, 96, 78, 57.

5.2.6. (1*S*,3*R*,4*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-cyclopentanecarboxylic acid methyl ester 33a. 10% Pd/C (1 g) was added under N_2 to a solution of **35** (150 g, 0.29 mol) in MeOH (300 mL) The reaction mixture was transferred to a bomb and after purging with N_2 and then H_2, a H_2 pressure of 2 bar was applied and the reaction stirred for 18 h. (Periodically, additional hydrogen was added to the reaction to re-establish the initial reaction pressure.) The pressure was released and the bomb purged with N_2. The reaction mixture was cautiously filtered through celite and then concentrated. The residue was dissolved in EtOAc (1.6 L) and *tert*-butylamine (77 mL, 0.73 mol) added dropwise. After stirring for 2.5 h, the precipitate was recovered by filtration and washed with MTBE (200 mL). This material was recrystallised from 1:3 MeOH/MTBE (700 mL) to give the *tert*-butylamine salt as a white solid (91 g). This salt was dissolved in H_2O (350 mL), and the pH adjusted to 3 with 6 M HCl. The solution was stirred for 1 h and extracted with CH_2Cl_2 (3×350 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to give a free acid as a white solid (64 g). This was dissolved in MeOH (350 mL) and cooled to 5 °C. Et_3N (44 mL, 0.31 mol) was added followed by methyl chloroformate (25 mL, 0.31 mol) dropwise. The reaction mixture was stirred at 5 °C for 1 h, then allowed to warm to room temperature overnight. The solvent was removed in vacuo, and the residue taken up in CH_2Cl_2 (300 mL). This solution was washed with 1 M KHSO4 (2×200 mL), sat. $NaHCO_3$ (200 mL) and sat. brine (200 mL) and dried over MgSO4. Concentration of this material yielded **33a**, an orange oil (59 g, >98% d.e., 38% from **35**). ^{1}H NMR ($CDCl_3$) 5.17 (1H, br s), 4.09 (1H, m), 3.92 (1H, br s), 3.71 (3H, s), 2.91 (2H, m), 2.39 (1H, app dq, J=17 Hz), 2.12 (1H, m), 1.98 (1H, app dq, J=14 Hz), 1.74 (1H, app dq, J=17 Hz), 1.42 (9H, s). ^{13}C NMR ($CDCl_3$) 179.1, 156.2, 79.9, 73.4, 55.9, 52.8, 39.6, 36.0, 33.5, 28.8. *m/z* 241 (M^+), 202, 185, 172, 154, 140, 126, 116. $[\alpha]_D^{25}$ (*c* 1.0, MeOH) +3.3.

5.2.7. (1*S*,3*R*,4*R*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-cyclopentanecarboxylic acid methyl ester 33b. Methanesulfonyl chloride (17.0 mL, 0.22 mol) was added dropwise to a solution of **33a** (43.9 g, 0.18 mol), triethylamine (50 mL, 0.36 mol) and DMAP (1.1 g, 9 mmol) in CH_2Cl_2 (500 mL) at 0 °C. After 45 min the reaction was washed with 1 M citric acid (2x200 mL), sat. $NaHCO_3$ (200 mL), H_2O (200 mL) and brine (100 mL) and then dried over $MgSO_4$. After filtration, concentration of the organic under vacuum gave the mesylate as an off-white solid (55.6 g, 96% crude), which was immediately dissolved in DMF (250 mL) and KOAc (105 g, 1.05 mol) added. The stirred mixture was heated at 60 °C for 3 days, cooled and diluted with CH_2Cl_2 (700 mL). The mixture was washed with H_2O (1 L), sat. $NaHCO_3$ (500 mL), H_2O (500 mL) and brine (300 mL) before drying over $MgSO_4$. Following filtration, concentration under vacuum gave the acetate as a brown solid which was recrystallised from MTBE and hexanes. NaOMe (0.7 mL, 25 wt% solution in MeOH) was added to a solution of the recrystallised acetate (29.2 g, 0.10 mol) in MeOH (300 mL) at 0 °C. After 7 h the reaction was quenched on addition of glacial AcOH and the solvent removed under vacuum. The residue was redissolved in CH_2Cl_2 (500 mL) and washed with H_2O (200 mL), sat. $NaHCO_3$ (200 mL), H_2O (100 mL) and brine (200 mL) before drying over $MgSO_4$. Following filtration, concentration under vacuum yielded a brown solid which was taken up in hot MTBE (80 mL) and stirred with decolourising charcoal for 30 min. After filtration, heptane was added to yield **33b** as white crystals (18.6 g, 71%). ^{1}H NMR ($CDCl_3$) 5.26 (1H, d, J=5 Hz), 4.13 (1H, m), 3.96 (1H, br s), 3.79 (1H, br s), 3.70 (3H, s), 3.07 (1H, m), 2.40 (1H, dt, J=13, 8 Hz), 2.14 (1H, m), 1.99 (1H, m), 1.71 (1H, m), 1.44 (9H, s). ^{13}C NMR ($CDCl_3$) 177.4, 156.7, 80.3, 78.6, 60.2, 52.4, 40.2, 36.5, 33.7, 28.7. *m/z* 241 (M^+), 207, 185, 154, 140, 126, 116, 100, 83, 53. $[\alpha]_D^{25}$ (*c* 1.0, MeOH) −25.9.

5.2.8. (1*R*,3*R*,4*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-cyclopentanecarboxylic acid methyl ester 33e. ((1,2-Bis(2*R*,5*R*)-2,5-dimethylphospholano)benzene)(cyclooctadiene)rhodium(I) tetrafluoroborate (23 mg, 1 mol%) was added to a degassed solution of **36** (1 g, 3.9 mmol) in MeOH (5 mL). The reaction mixture was transferred to a bomb and after purging with N_2 and then H_2, an H_2 pressure of 5 bar was applied and the reaction stirred for 14 h. The pressure was released and the bomb purged with N_2. Concentration of the reaction mixture gave a residue which was redissolved in CH_2Cl_2 (5 mL). Addition of silica (0.5 g) with stirring removed the catalyst from the reaction and filtration and concentration of the organic gave **33e** as an off-white solid of 98% d.e. in quantitative yield. ^{1}H NMR ($CDCl_3$) 4.91 (1H, d, J=7 Hz), 4.27 (1H, m), 3.99 (1H, br s), 3.67 (3H, s), 3.10 (1H, m), 2.26 (1H, m), 2.05 (2H, m), 1.85 (1H, dt, J=13, 10 Hz), 1.43 (9H, s). ^{13}C NMR ($CDCl_3$) 176.8, 156.2, 80.0, 73.0, 55.0, 52.3, 39.5, 36.7, 32.9, 28.8. *m/z* No M^+, 202, 185, 172, 154, 140, 126, 116, 99, 87, 57. $[\alpha]_D^{25}$ (*c* 1.0, MeOH) +36.4.

5.2.9. (1*R*,3*R*,4*R*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-cyclopentanecarboxylic acid methyl ester 33f. Prepared in an identical manner to **33b**. ^{1}H NMR ($CDCl_3$) 4.67 (1H, br), 4.23 (1H, br s), 4.00 (1H, m), 3.76 (1H, m), 3.70 (3H, s), 2.91(1H, m), 2.43 (1H, m), 2.34 (1H, m), 1.91 (1H, m), 1.68 (1H, m), 1.45 (9H, s). ^{13}C NMR ($CDCl_3$) 176.2, 157.3, 80.6, 79.1, 59.3, 52.5, 39.3, 36.4, 33.2, 28.7. *m/z* 241 (M^+), 202, 185, 172, 154, 140, 126, 116, 83, 57. $[\alpha]_D^{25}$ (*c* 1.0, MeOH) −9.7.

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