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Stereoselective synthesis of a thiazolane amide using molecular recognition in the triazolyl-activated ester intermediate

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Abstract—An amide derived from penicillin V and racemic (*R*/*S*)-2-aminobutanol was prepared with 83% de and shows significantly higher toxicity than the pure diastereomers prepared from homochiral 2-aminobutanol. This has been attributed to conformational changes in the resolved product brought about through hydrogen-bonded self-assembly in the intermediate.

Efforts to combat the replication of the HIV[1,2] virus have led to the development of a number of inhibitors of the HIV-1 protease enzyme based on readily available penicillin G[3,4] as the chiral building block. Derivatisation of penicillin G has resulted in a range of materials that contain several amide motifs within the low molecular weight structures.[3] These are associated with the N-substituted β-lactam ring, the carboxy function on the thiazolane ring and also the amide formed on nucleophilic opening of the β-lactam ring using a primary amine. Computer assisted molecular simulation and crystallographic studies[4,5] have shown these to be essential for binding with lysine and aspartic acid residues at the active site of the HIV-1 protease enzyme.

In the course of our investigations into new HIV-1 protease inhibitors we have extended the penicillin-based methodology to include the previously unstudied derivatives of penicillin V. The final step in the synthetic procedure is amide formation at the thiazolane carboxy group of a benzylamine ring-opened penicillin V using TBTU [2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate] as the coupling agent[6] in the presence of an organic amine base. TBTU gives the 1-oxybenzotriazolyl-activated ester that can be readily converted to an amide by reaction with an appropriate amine. This has been performed using each of the pure enantiomers of 2-aminobutanol as well as the racemic mixture with surprising results. While both pure enantiomers react as expected to give the homochiral amide product, reaction of the acid with racemic 2-aminobutanol leads to the isolation of an enantiomerically enriched amide, the results of which will be reported here. As a comparison, the same reactions were performed using the analogous penicillin G intermediate, however, no resolution of enantiomers was observed. A similar effect has been observed by Birch and co-workers[7] in the synthesis of peptides using 1-hydroxybenzotriazole and DCC on racemic mixtures of *N*-phthaloyl and *N*,*N*-dimethylamido protected amino acids. This has been shown to occur through competitive reaction of the two enantiomers in a kinetically controlled reaction. However, we will show by comparison that our resolution is somewhat more complex, involving a proposed self-organisation of the ester intermediate, and that even with prolonged reaction times the racemic amide is not formed to any great extent, as would be expected if the resolution mechanism was purely kinetically controlled.

The synthetic procedures[3] for both the penicillin G (R^1 = Ph) and V (R^1 = PhO) derivatives were the same and are summarised in Scheme 1. The amides of chiral [**5**-(*R*) **5**-(*S*) R^1 = Ph, **6**-(*R*) **6**-(*S*) R^1 = OPh] and racemic 2-aminobutanol (**5**-(*R*/*S*) R^1 = Ph, and in theory **6**-(*R*/*S*) R^1 = OPh) were obtained using a peptide-coupling method employing TBTU as the activating agent with DIPEA (*N*,*N*-diisopropylethylamine) as base.[8] The target polyamides were isolated by flash chromatography over silica gel using step gradient 1–10% MeOH in

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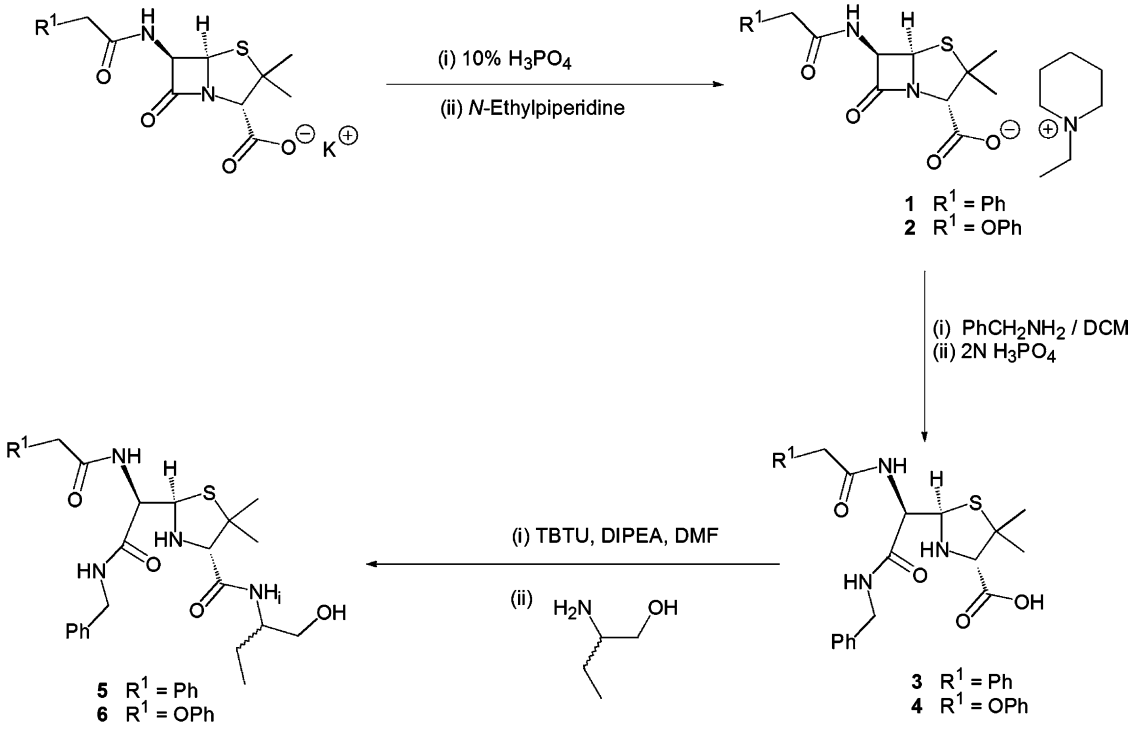


Scheme 1. General scheme for the synthesis of the target amides.

DCM as an eluent. The yields reported are isolated yields and the purity of the final products was confirmed using ^{1}H NMR, GC, IR and elemental analysis.

The carboxylate anions of **3** and **4** react with TBTU to form an intermediate **X** with the elimination of tetramethylurea. This activates the acid carbonyl group towards nucleophilic attack by an amine, as *O*-benzotriazole ($^-$OBt) is a good leaving group. The intermediate, obtained from penicillin V ($\mathbf{X}_V$) differs from that obtained from penicillin G ($\mathbf{X}_G$) by a single oxygen atom in the side chain [R^1 = Ph (G) and PhO (V)]. Each penicillin derivative was reacted with racemic (*R*/*S*)-2-aminobutanol, and enantiomerically pure (*R*)- and (*S*)-2-aminobutanol. The amides formed from penicillin G with the aminoalcohols did not show any unusual features in the ^{1}H NMR spectra using DMSO-d_6 as a solvent. The new amide proton, assigned as H_i (Scheme 1), appeared as a single proton doublet for the homochiral derivatives and as two equivalent 'half proton' doublets for the diastereomeric mixture. In the case of the homochiral amides derived from penicillin V (**6**-(*R*) and **6**-(*S*)) the spectra observed showed single doublets for H_i integrating to single protons at δ_H 7.54 and 7.76 ppm, respectively. The anomaly occurred when intermediate $\mathbf{X}_V$ was reacted with racemic (*R*/*S*)-2-aminobutanol to give in theory **6**-(*R*/*S*). Rather than the two equivalent doublets expected for H_i, two non-equivalent doublets were observed at δ_H 7.54 and 7.64 ppm with relative integrations of 91.5:8.5, respectively. Therefore, it can be concluded that the (*R*)-amide is forming stereoselectively relative to the (*S*)-amide. In fact from the relative integration we arrive at a de for the (*R*)-amide of 83%. As the only difference between the penicillin G and V derivatives is the single sp^3 hybridised chain oxygen, we propose that there must be an interaction between the aminoalcohol 'substrate' and $\mathbf{X}_V$ that leads to this stereoselectivity. In order to explain this effect intermediate $\mathbf{X}_V$ was constructed in CAChe Worksystem for Windows and the MM3[9,10] force field implemented to generate a minimum energy structure. Comprehensive conformational analysis calculations ensured that we arrived at the global minimum rather than a local intermediate energy minimum. The model was then studied in the presence of (*R*)-2-aminobutanol and (*S*)-2-aminobutanol. By evoking the hydrogen bond monitor within CAChe the inter-molecular interactions between each chiral substrate and $\mathbf{X}_V$ were probed. Both (*R*)- and (*S*)-2-aminobutanol showed hydrogen-bonded

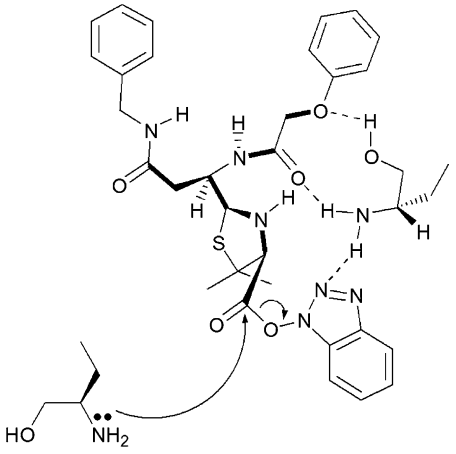


Figure 1. Nucleophilic attack of (*R*)-2-aminobutanol at the activated ester of the self-assembled intermediate showing 'bound' (*S*)-2-aminobutanol.

Compound	LC_{50} (μg/ml)	LC_{90} (μg/ml)
5-(*R*/*S*)	87 ± 10	112 ± 8
6-(*R*/*S*)	45 ± 6	86 ± 10
5-(*R*)	78 ± 10	134 ± 21
6-(*R*)	102 ± 20	184 ± 31
5-(*S*)	101 ± 12	150 ± 20
6-(*S*)	136 ± 22	192 ± 20

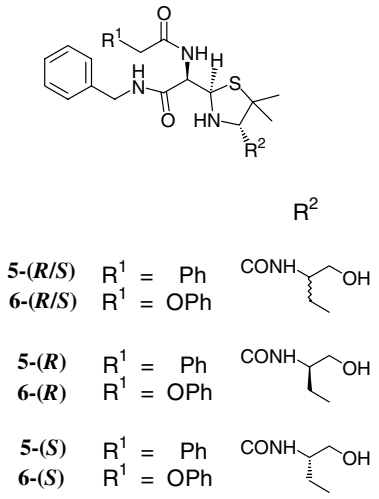
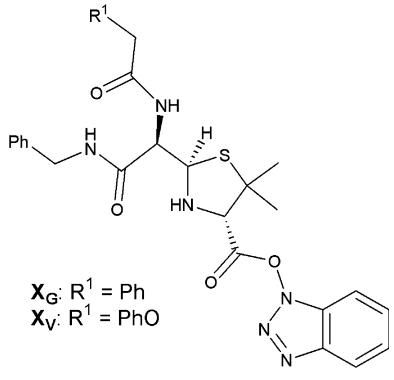


Figure 2. Toxicology data for the amides derived from penicillin V and G.

interactions with $\mathbf{X_V}$, the amino group being associated with the side chain amide of the penicillin, the phenyl oxygen of the side chain and interestingly the nitrogens of the triazole ring. Furthermore, it was noted that the benzotriazole motif had reoriented through space, compared to the free intermediate, to give the hydrogen bonds. The role of the phenyl oxygen also became apparent as a strong hydrogen bond was observed with the hydroxyl motif of the chiral substrate. This is not possible in the penicillin G derivative. Stereoselection can be explained by the steric crowding when the rotomers obtained by varying the conformation of the ethyl substituent at the chiral centre of the isomers of 2-aminobutanol associated with the intermediate $\mathbf{X_V}$ are considered.



For (*S*)-2-aminobutanol, the substrate sits in the polar 'cleft' with the ethyl group pointing away form the bulk of the molecule (Fig. 1). This may be considered to be a favourable form. The (*R*)-2-aminobutanol on the other hand fits less well with severe steric interactions between the ethyl group on the substrate and the bulky benzotriazole, and the phenoxy and benzylamine groups on $\mathbf{X_V}$. Therefore, binding of the (*S*)-isomer predominates and (*R*)-2-aminobutanol is free in excess to react with the activated ester group, which because of the conformational changes brought about by the rotation of the benzotriazole group is now exposed towards nucleophilic attack. Therefore, the (*R*)-amide forms in excess.

Toxicological studies[11–13] were performed using colonic epithelial cells in Eagle's medium supplemented by Foetal Calf serum at 37 °C.[†] All assays were performed in triplicate. The 100% and 0% controls were used to convert the other absorbances to a percentage growth value. From these, the percentage inhibition of growth was calculated and this was plotted against $\log_{10}$ drug concentration. The concentrations giving 50% (LC_{50}) and 90% inhibition (LC_{90}) were calculated and the values are shown in Figure 2. The data generally indicated that a minimum concentration of 68 μg/ml was required to impart a toxicological effect to 50% of the cultured cell, or 104 μg/ml for 90% of the cultured cell. This gave a positive implication stating that the above final compounds were relatively 'safe' and should proceed to the next stage of testing to determine their anti-viral potential.

The only exception to this was the stereoenriched mixture **6**-(*R*/*S*) that had shown the anomaly in the ^{1}H NMR spectrum. In comparison, **6**-(*R*/*S*) had much lower LC_{50} and LC_{90} values. NMR showed that this mixture

[†] A colonic epithelial cell line HT29 was cultured in Eagle's medium supplemented with 10% v/v Foetal Calf serum. For the assay, cells (≈500 per well) were seeded into a 96-well multiwell micro titre plate (Nunc) and 200 μl of medium was added. The test compounds were added into the wells dissolved in DMSO; the maximum amount of DMSO added to each well was 1% v/v. The plates were then incubated at 37 °C for 48 h in an atmosphere of 5% CO_2 and O_2 then the medium in each well was removed and replaced with fresh medium (100 μl) containing 0.1% MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; thiazole blue, Sigma]. The plate was then replaced into the incubator and left for 2 h. After this period, 100 μl of Sorensen's medium (pH 10) containing DMSO (1% v/v) was added to each well and the absorbance in each well read in a plate reader at 570 nm. For each plate several controls were used, which were cells grown in the absence of drug (100% control) and cells incubated with 1% v/v DMSO (solvent control). One uninnoculated well was used to determine the zero value.

possessed a stereoexcess of **6**-(*R*) and this suggested that while being chemically identical to **6**-(*R*) obtained from the pure enantiomer of 2-aminobutanol (albeit with some contamination from the other diastereoisomer), it had a different conformational structure. It is proposed that the conformation in the product is fixed by that in the self-assembled intermediate, and is a structure with low local minimum energy rather than the global minimum formed using the homochiral reagent. The change in conformation would then lead to a different bioactivity, most likely through the formation of a new polymorphic crystal form.[14] This is reflected in the significantly different result in the toxicity test. The difference is most striking when comparisons are made between the products formed using the homochiral substrates and the racemic substrates. If we compare the LC_{50} results for **5** we see that the value obtained for the racemic product (87±10 μg/ml) is intermediate between those of the pure (*S*)-(101±12 μg/ml) and pure (*R*)-(78±10 μg/ml) products. It is clear from the ^{1}H NMR spectrum of **6** obtained using the racemic substrate that it has a de of 83%, enriched with the (*R*)-substrate. Therefore, the LC_{50} results for **6**-(*R*/*S*) (45±6 μg/ml) should closely match **6**-(*R*) (102±20 μg/ml), which is clearly not the case.

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8. In a typical procedure: To a solution of the free penicillic V acid **4** (5.23 mmol) in DMF (50 cm^3) were added sequentially DIPEA (5.34 mmol), (*R*/*S*)-2-aminobutanol (5.38 mmol) and TBTU (5.36 mmol). The resulting solution was stirred at room temperature overnight, before partitioning between EtOAc (ca. 150 cm^3) and water (50 cm^3). The organic phase was removed and the aqueous phase was extracted with EtOAc (2 × 50 cm^3). The combined organic phases were washed with 2 N HCl (50 cm^3), saturated aqueous $NaHCO_3$ (50 cm^3) and brine (50 cm^3) before drying ($MgSO_4$). The solvent was removed in vacuo, and the resulting residue was purified by flash column chromatography (silica gel, eluent system: 1–10% MeOH in CH_2Cl_2 gradually increasing the polarity) to afford **6** as a white solid. Yield = 23%; mp 85.1 °C. $[\alpha]_D^{22}$ +56.20 (*c* 0.01192, $CDCl_3$). Elemental analysis: Found: C, 59.97; H, 7.03; N, 10.28%. Calcd for $C_{27}H_{36}O_5N_4S\cdot 0.5H_2O$ (FW 537.66): C, 60.32; H, 6.93; N, 10.42%. IR (KBr disc): ν_{max}/cm^{-1} 3700–3140 (br, OH); 3080, 3040 (–CONH–); 2900–2800 (saturated C–H); 1650 (C=O stretch, amide I band); 1530 (amide II); 1250 (C–O); 760, 700 (monosubstituted benzene ring).
^{1}H NMR (400 MHz, DMSO): δ_H 0.83 (3H, t, *J* 7.6 and 7.3, CH_2CH_3); 1.14 (3H, s, terminal CH_3); 1.33 (1H, m, $H_{l'}$); 1.50 (3H, s, terminal CH_3); 1.55 (1H, m, H_l); 3.27 (1H, m, H_j); 3.35 (1H, m, $H_{j'}$); 3.46 (1H, d, *J* 12.2, H_a); 3.66 (1H, m, H_k); 3.89 (1H, dd, *J* 12.2 and 8.3, H_h); 4.26, 4.28 (2H, dABq, *J* 13.6 and 2.9, H_g, $H_{g'}$); 4.40 (1H, t, *J* 8.3 and 7.8, H_b); 4.54 (2H, ABq, *J* 14.9 and 14.7, H_f, $H_{f'}$); 4.64 (1H, t, *J* 5.4 and 5.1, OH); 4.96 (1H, t, *J* 8.0 and 7.8, H_c); 6.94 (3H, m, aromatic protons *ortho*/*para* to phenoxygen); 7.18–7.32 (7H, m, aromatic ring protons); 7.54 (0.915H, d, *J* 8.6, H_i, diastereomer); 7.64 (0.085H, d, *J* 8.8, H_i, diastereomer); 8.15 (1H, t, *J* 8.1 and 8.8, H_d); 8.57 (1H, t, *J* 6.1 and 5.8, H_e). MS (EI): *m*/*z* 528 $[M]^+$; 497 $[M-CH_2CH_3]^+$; 260, 231, 91 $[PhCH_2]^+$.
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