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Diastereoselective Synthesis, Activity and Chiral Stability of Cyclic Alkoxyketone Inhibitors of Cathepsin K

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Abstract—The diastereoselective synthesis of a novel class of cathepsin K inhibitors together with their cathepsin K affinity and stability towards aqueous buffer is reported. © 2001 Published by Elsevier Science Ltd.

Cathepsin K (EC 3.4.22.38), a cysteine protease of the papain superfamily, is selectively expressed in osteoclasts and has been implicated in the process of bone resorption.[1] Selective inhibitors of cathepsin K therefore could be promising therapeutic agents for the treatment of diseases characterised by excessive bone loss, such as osteoporosis.

Recently we reported the design[2] and solid-phase synthesis[3] of a new class of cyclic alkoxyketones **1** as selective reversible inhibitors of cathepsin K (Fig. 1).

To enable the investigation of both the activity and chiral stability of these novel inhibitors, we required a diastereoselective synthesis, as all attempts to separate the individual diastereomers employing preparative chromatography proved unsuccessful.

Our initial approach towards the preparation of the single diastereomers of these cyclic ketones involved the preparation of the key enantiomerically enriched amino alcohols **3**. Desymmetrisation of *meso*-epoxide **4** with (*S*)-(−)-α-methylbenzylamine[4] provided the corresponding amino:alcohols **5** as a 1:1 mixture. Formation of the hydrochloride salt and fractional crystallisation from ethanol provided a single diastereomer of unknown stereochemistry (Scheme 1). Attempts to obtain an X-ray structure, enabling the absolute chirality of the ring stereochemistry to be established, proved unsuccessful.

Although we had a route towards the single diastereomers of the cyclic alkoxyketones we were interested in developing a synthesis to provide either diastereomer with known absolute stereochemistry. To this end, desymmetrisation of *meso*-epoxide **4** employing the (*R*,*R*)-salen catalyst **6**[5] and TMS-N_3 in ether at 25 °C provided the (3*S*,4*R*)-azido silyl alcohol **7** in 98% ee.[6] Employing the (1*S*,2*S*)-salen catalyst likewise provided the corresponding (3*R*,4*S*)-azido alcohol, again in 90% yield and 97% ee. Removal of the silyl group with camphor sulfonic acid in methanol at room temperature and subsequent reduction of the azido moiety with hydrogen in the presence of Pd/C afforded the amino-alcohol **8**. Selective amide bond formation with the mixed anhydride of Cbz-Leu-OH and subsequent hydrogenolysis of the Cbz group afforded the amine **9**. Amide bond formation and subsequent Dess–Martin oxidation of the secondary alcohol provided the 4*S* diastereomers **10** (Scheme 2).

The cathepsin K activity for the individual diastereomers showed that activity resided in the 4*S* isomer with the 4*R* isomer typically showing up to a 40-fold reduction in potency (Table 1).

The cyclic alkoxyketones were also examined for their stability towards epimerisation in a series of aqueous buffers (hepes, phosphate and acetate). In these studies, compound concentration was 1 μM and the final solution contained 20% THF to aid solubility. Analysis of samples was carried out after extraction into dichloromethane, using chiral HPLC.[8] Significant epimerisation over a period of several hours was evident in both diastereomers.

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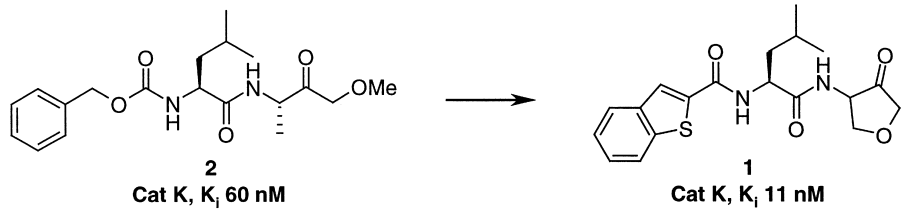
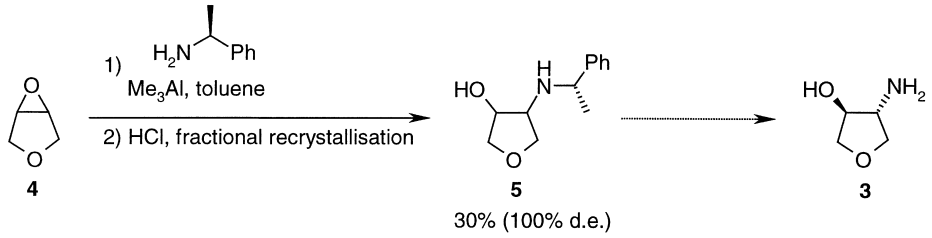
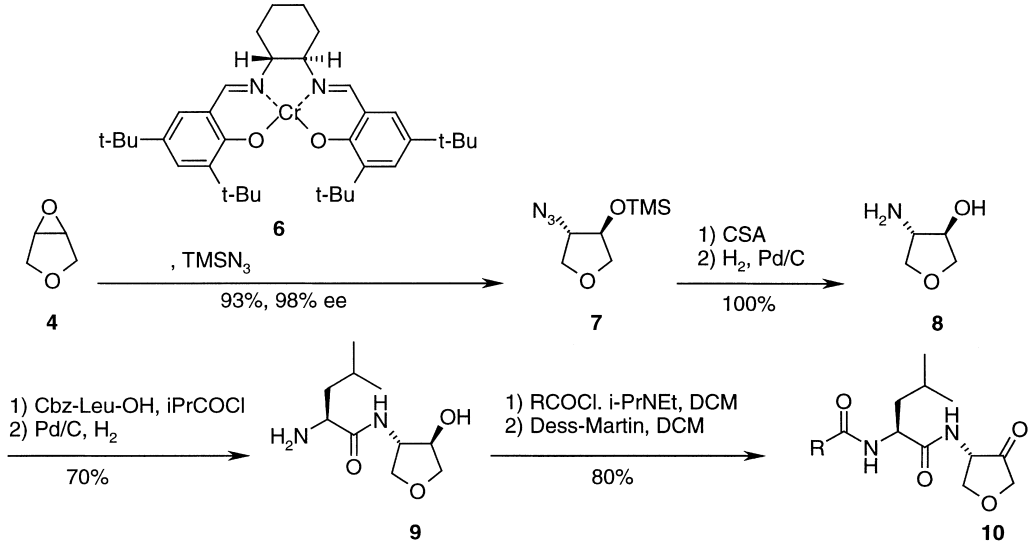


Figure 1. The evolution of cyclic alkoxyketones.

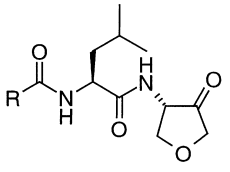


Scheme 1. Desymmetrisation employing (*S*)-(−)-α-methyl benzylamine.



Scheme 2. Diastereoselective synthesis of five membered cyclic alkoxyketones.

Table 1. Diastereomeric activity of tetrahydrofuranone inhibitors



Entry	R	*S* isomer, K_i (nM)[7]	*R* isomer, K_i (nM)
10a	2-Benzo[*b*]thiophenyl	7	68
10b	2-Naphthyl	14	470
10c	2-Quinolyl	15	590

However, the rate of epimerisation appears different in the various buffers, being fastest in hepes pH 6.8 buffer and slowest in acetate pH 5.5, indicating the epimerisation is catalysed by hydroxide anion (Fig. 2).

Having measured the chiral stability and activity for the five membered cyclic alkoxyketones, we wished to assess the stability of the related six membered cyclic alkoxyketones which also emerged as potent cathepsin K inhibitors.[9] Again, attempted separation of the individual diastereomers employing chiral HPLC proved unsuccessful. However, column chromatography of the related ketal analogues **11** followed by deketalisation afforded the diastereomers **12** in typically 95% d.e., albeit of unknown stereochemistry (Scheme 3).

Again, cathepsin K activity of the ketones **12** resided in predominately one, stereochemically undefined, diastereomer (Table 2).

Attempts to quantify epimerisation employing chiral HPLC analysis proved unsuccessful due to incomplete baseline separation. We were able, however, to employ ^{1}H NMR analysis for this purpose. Using the same protocol as for the five membered ketones, we observed

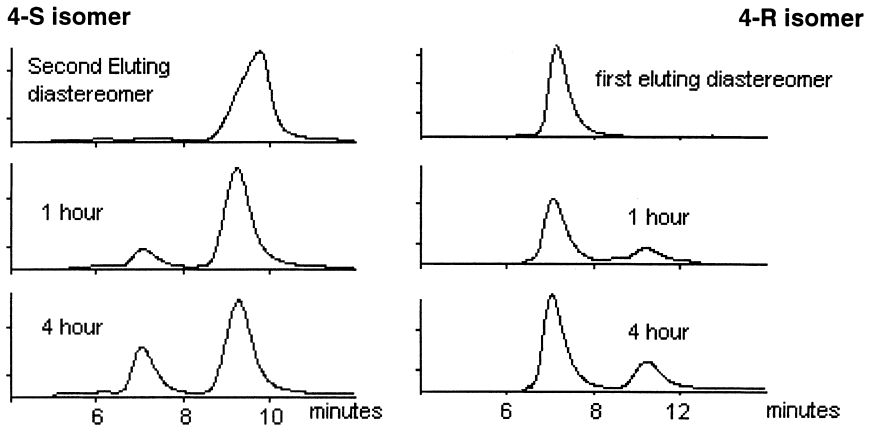
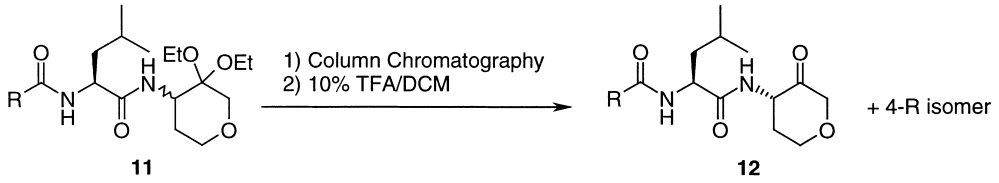


Figure 2. Diastereomeric epimerisation of **10b** in the acetate buffer, pH 5.5.



Scheme 3. Diastereoselective synthesis of six membered cyclic alkoxyketones.

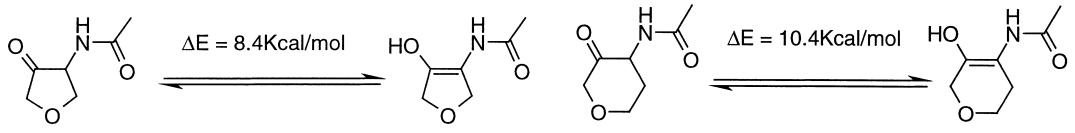
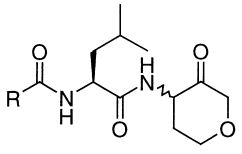


Figure 3. Ab initio calculations of cyclic alkoxyketones.

Table 2. Diastereomeric activity of tetrahydropyranone inhibitors



Entry	R	Diastereomer-1, K_i (nM)	Diastereomer-2, K_i (nM)
12a	2-Benzo[*b*]thiophenyl	8	70
12b	2-Naphthyl	32	380
12c	3,4-Dimethoxybenzyl	10	770

no significant levels of epimerisation in a series of aqueous buffers (hepes, phosphate and acetate). We hypothesised that the epimerisation differences observed between the five and six membered cyclic alkoxyketones could be due to the energy barrier between the ketol and enolic forms. Indeed, ab initio calculations in SPARTAN using 3-21G(*) indicate a larger energy difference (ΔE) between the two forms for the 6-ring system relative to the 5-ring (Fig. 3).

In summary we have completed the diastereoselective synthesis of a novel class of cathepsin K inhibitors and examined their stability to a range of buffer systems. Whilst the more potent diastereomer of the five and six membered cyclic alkoxyketones show comparable potency, the six membered ketones may offer significant development advantages due to their enhanced chiral stability.

Acknowledgements

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6. Optical rotations of **7** and **8** were identical to those reported by Jacobsen.[5]

7. Enzyme inhibition was evaluated as previously described: Votta, B.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Can, T.; Connor, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. *J. Bone Miner. Res.* **1997**, *12*, 1396.

8. Separation was achieved using a Chiralpak AD column (250 mm×4.6 mm) using a mobile phase of 50:50 hexane: IPA.

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