Stereoselective Synthesis of the Epoxysuccinyl Peptide E-64c

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Abstract: A highly diastereoselective PTC epoxidation is employed in the synthesis of the potent cysteine protease inhibitor E-64c.

Key words: epoxysuccinates, asymmetric epoxidation, quaternary ammonium salts, phase-transfer catalysis

Epoxysuccinyl peptides have received much attention due to their propensity to act as potent and selective inhibitors of cysteine proteases.[1] In many instances these compounds lead to irreversible inhibition via reaction of the epoxysuccinyl moiety with the nucleophilic thiol present in the enzyme active site.[2] Since this mechanism is specific to cysteine proteases, epoxysuccinyl peptides generally show low reactivity towards other types of protease.

The naturally-occurring epoxysuccinyl peptide E-64, and the closely related compounds E-64c (**1**) and E-64d (**2**, loxistatin) are typical examples of this class of enzyme inhibitor.[3] They exhibit high activity towards a range of cysteine proteases and E-64 is widely employed as a means of characterising cysteine protease activity.

Most synthetic approaches to epoxysuccinyl peptides of this type employ the coupling of an intact epoxysuccinate derivative to the peptide fragment.[2–5] This is a highly effective strategy, but has limitations in terms of the functionality that can be incorporated at the carboxy terminus of the succinate.[2] In this paper we report the development of a novel stereoselective approach to epoxysuccinyl peptide derivatives that also allows straightforward access to keto-epoxide analogues. The utility of this chemistry is demonstrated in the synthesis of E-64c (**1**) and loxistatin (**2**).

We have recently been involved in the development of asymmetric phase-transfer-catalyzed (PTC) Weitz–Scheffer epoxidations.[6,7] This chemistry employs quaternary ammonium salts in conjunction with aqueous NaOCl to effect the epoxidation of electron deficient alkenes and we envisaged that it might offer an alternative route into epoxysuccinyl peptides whilst also allowing access to a range of epoxy-ketone analogues. To test this hypothesis we decided to investigate whether compounds such as **3** (Figure 1) could serve as intermediates in the synthesis of targets **1** and **2**.

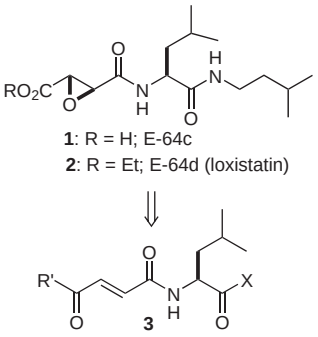
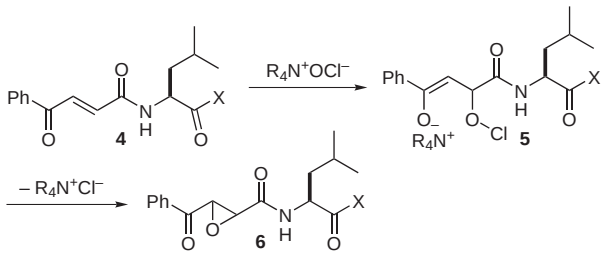


Figure 1

For the purposes of this study we opted to investigate the diastereoselective epoxidation of benzoylacrylamides **4**. These compounds were chosen for study because the direct precursors are commercially available, and because the γ-keto group of the acrylamide should activate the substrate towards Weitz–Scheffer epoxidation. The γ-keto group should also ensure that initial addition of hypochlorite occurs at the α-carbon (Scheme 1). An added advantage of this type of substrate is that the phenyl ketone can serve as a masked carboxylic acid. This avoids the problem of needing to differentiate between two carboxyl derivatives when applying this chemistry to targets such as **1**.



Scheme 1

Compounds **8a** and **8b** were prepared via coupling of (*E*)-3-benzoylacrylic acid with the appropriate leucine ester (Scheme 2). Epoxidation of these compounds was then investigated employing aqueous NaOCl in conjunction with a quaternary ammonium phase-transfer catalyst. These are conditions that we have shown to be effective for a wide range of enones,[6] however, as far as we are aware, they have never previously been applied to peptide substrates. A particular issue in applying this chemistry to these substrates lies in the fact that aqueous NaOCl is known to effect the N-chlorination of amides.[8] Thus if this

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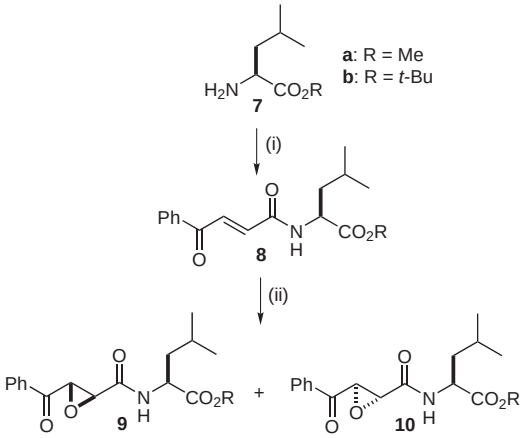
chemistry is to be successful, we need to identify conditions that favor epoxidation over this alternative process.

Initial studies into the PTC epoxidation of substrates **8** using conditions that were optimized for the epoxidation of simple chalcones (1 mol% catalyst, 4 equiv 15% aq NaOCl, 25 °C)[6] met with limited success. The epoxidations were fast ($t_{1/2}$ = 20 min compared with $t_{1/2}$ = 90 min for chalcone under the same conditions), but significant amounts of N-chlorination were observed. Control experiments established that N-chlorination also occurred in the absence of phase-transfer catalyst. Since epoxidation did not occur without the catalyst we speculated that HOCl rather than NaOCl might be responsible for this side reaction. Since the equilibrium concentration of HOCl should reduce as the pH of the aqueous phase increases, we decided to examine the effect of adding KOH to maintain a pH >12 in the aqueous phase. It was found that this suppressed the N-chlorination, and by combining this modification with a modest increase in catalyst loading (to 5–10 mol%), high selectivity for the desired epoxidation could be achieved.



Scheme 2 *Reagents and conditions*: (i) PhCOC=CCO$_2$H, *i*-BuO$_2$CCl, NMM (**8a**; 95%, **8b**; 85%); (ii) 15% aq NaOCl (3 equiv), pH >12, PhMe, PTC, 25 °C (for further details see Table 1).

When the epoxidation was performed on enone **8a** using the achiral phase-transfer catalyst *n*-Bu$_4$NBr, the corresponding products **9a** and **10a** were obtained as a 1:1 mixture (Scheme 2 and Table 1, entry 1). It is worth noting that little or no ester hydrolysis was observed despite the highly basic nature of the aqueous NaOCl solution. The corresponding *tert*-butyl ester **8b** gave a 2:3 mixture of diastereoisomers with *n*-Bu$_4$NBr (Table 1, entry 4), and again the products **9b** and **10b** could be isolated in good yield.

Both reactions involving *n*-Bu$_4$NBr resulted in little or no diastereoselectivity indicating that the pre-existing stereogenic center in substrates **8a** and **8b** has little influence over the stereochemical outcome of this epoxidation. Consequently, in an effort to develop a diastereoselective epoxidation we turned our attention to the use of chiral phase-transfer catalysts.

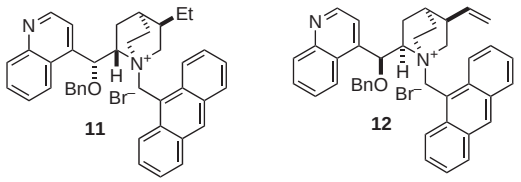


Figure 2

Table 1 Asymmetric PTC Epoxidation of **8a** and **8b**

Entry	Substrate	Catalyst	Yield (%)[a]	Ratio **9**:**10**[b]
1	**8a**	*n*-Bu$_4$NBr[c]	64	1:1
2		**11**[d]	70	5:1
3		**12**[d]	69	2:5
4	**8b**	*n*-Bu$_4$NBr[c]	58	2:3
5		**11**[d]	72	3:1
6		**12**[d]	72	1:5

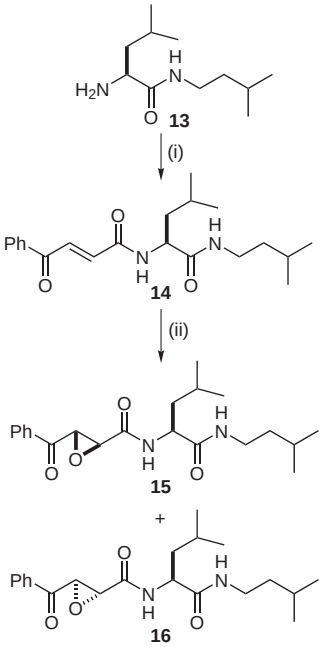
[a] Yield after purification, see representative experimental procedure[10] for further details.
[b] Estimated from the ^{1}H NMR spectra of the crude products.
[c] 10 mol% catalyst.
[d] 5 mol% catalyst.

Our earlier studies on the asymmetric epoxidation of chalcones demonstrated that the cinchona alkaloid-derived quaternary ammonium salts **11** and **12** (Figure 2) induce high levels of stereocontrol. These two catalysts usually give roughly equal but opposite levels of stereoselectivity with a given substrate.[6,9] Thus it was anticipated that they should provide a means of achieving stereoselective access to diastereoisomers **9** and **10**.

The results obtained using these catalysts are given in Table 1 (entries 2, 3, 5, 6). As expected they produced opposite diastereoisomers, but for a given substrate the magnitude of the diastereoselectivity differed somewhat. For diastereoisomer **9**, the methyl ester gave highest selectivity (5:1), whereas for diastereoisomer **10**, the *tert*-butyl ester gave the best selectivity (again 5:1). As the nature of the amino acid ester appeared to have a significant influence on the level of diastereoselectivity we decided to extend this study to include the amide substrate **14**.

Enone **14** was prepared via coupling of (*E*)-3-benzoylacrylic acid with leucine amide **13** (Scheme 3).[11] Epoxidation of **14** was then investigated as before. It was found that all three catalysts resulted in successful epoxidation, however, the levels of diastereoselectivity obtained using the chiral catalysts **11** and **12** (Table 2) were significantly lower than those obtained for the ester substrates **8**.

These results reinforce the earlier observation that the nature of the carboxyl terminus has a significant influence on the levels of diastereoselectivity when the chiral catalysts are employed. These results suggest that substrate **8a**



Scheme 3 *Reagents and conditions*: (i) $PhCOC{=}CCO_2H$, *i*-BuO_2CCl, NMM (84%); (ii) 15% aq NaOCl (3 equiv), pH >12, PhMe, PTC, 25 °C (for further details see Table 2).

Table 2 Asymmetric PTC Epoxidation of **14**

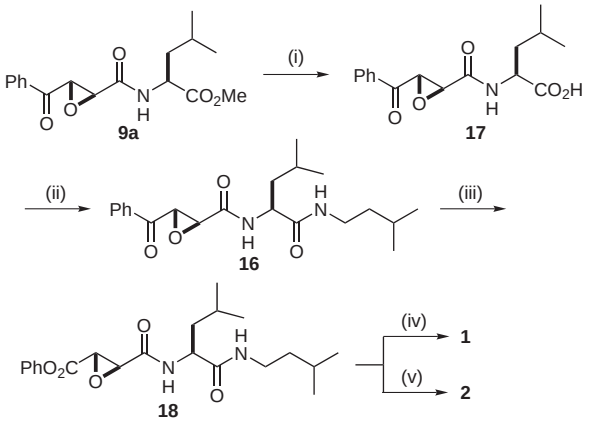
Entry	Catalyst	Yield (%)[a]	Ratio **15**:**16**[b]
1	*n*-Bu_4NBr[c]	67	1:1
2	**11**[d]	72	2:1
3	**12**[d]	64	2:3

[a] Yield after purification.
[b] Estimated from the ^{1}H NMR spectra of the crude products.
[c] 10 mol% catalyst.
[d] 5 mol% catalyst.

is the best precursor for targets such as E-64c (**1**), and crucially, this establishes that compound **9a** can be obtained in high yield and with good diastereoselectivity using this approach.

This brief study suggests that the asymmetric PTC epoxidation of substrates such as **8** is a useful way of accessing both diastereoisomers of epoxy ketones such as **9** and **10**. To further probe the utility of this approach we next examined conversion of **9a** into E-64c (**1**).

It was found that this could be achieved via the four-step sequence shown in Scheme 4. Thus, ester hydrolysis followed by carbodiimide-mediated coupling to 3-methylbutylamine gave amide **16** in good overall yield. We found that the EDCI coupling was best performed in dichloromethane rather than DMF, the latter solvent leading to significant amounts of epoxide-opening.[12] Baeyer–Villiger oxidation of the phenyl ketone then gave active ester intermediate **18**, which on exposure to ethanolic KOH provided direct access to loxistatin (**2**). Simply by adding water and extending the reaction time to two hours, E-64c (**1**) could be obtained. The ^{1}H NMR spectra of loxisatin (**2**) and E-64c (**1**) prepared by the methods described here were in agreement with those previously reported for these materials.[2,3]



Scheme 4 *Reagents and conditions*: (i) LiOH, H_2O, THF, MeOH, 0 °C (76%); (ii) $H_2N(CH_2)_2CH(CH_3)_2$, EDCI, HOBt, NMM, CH_2Cl_2 (76%); (iii) MCPBA, $CHCl_3$, 50 °C, (70%); (iv) EtOH, KOH, 0 °C, 30 min, (53%); (v) EtOH, aq KOH, 0 °C, 2 h, (65%).

This study has demonstrated that the asymmetric phase-transfer epoxidation of substrates such as **8** is an effective means of preparing epoxysuccinyl peptides derivatives. The chemistry described should allow access to a wide range of E-64c analogues and application of this methodology in the search for new cysteine protease inhibitors is currently underway in our laboratories.

Acknowledgment

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References and Notes

(1) (a) Leung-Toung, R.; Zhao, Y. Q.; Li, W. R.; Tam, T. F.; Karimian, K.; Spino, M. *Curr. Med. Chem.* **2006**, *13*, 547. (b) Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639.
(2) Meara, J. P.; Rich, D. H. *J. Med. Chem.* **1996**, *39*, 3357.
(3) (a) Barrett, A. J.; Kembhavi, A. A.; Brown, M. A.; Kirschke, H.; Knight, C. G.; Tamai, M.; Hanada, K. *Biochem. J.* **1982**, *201*, 189. (b) Tamai, M.; Hanada, K.; Adachi, T.; Oguma, K.; Kashiwagi, K.; Omura, S.; Ohzeki, M. *J. Biochem.* **1981**, *90*, 255. (c) Hanada, K.; Tamai, M.; Ohmura, S.; Sawada, J.; Seki, T.; Tanaka, I. *Agric. Biol. Chem.* **1978**, *42*, 529.
(4) See for example: (a) Verhelst, S. H. L.; Bogyo, M. *ChemBioChem* **2005**, *6*, 824. (b) Chehade, K. A. H.; Baruch, A.; Verhelst, S. H. L.; Bogyo, M. *Synthesis* **2005**, 240. (c) James, K. E.; Asgian, J. L.; Li, Z. Z.; Ekici, O. D.; Rubin, J. R.; Mikolajczyk, J.; Salvesen, G. S.; Powers, J. C. *J. Med. Chem.* **2004**, *47*, 1553. (d) Detterbeck, R.; Hesse, M. *Helv. Chim. Acta* **2003**, *86*, 222. (e) Roush, W. R.; Hernandez, A. A.; McKerrow, J. H.; Selzer, P. M.; Hansell,

E.; Engel, J. C. *Tetrahedron* **2000**, *56*, 9747. (f) Goursalin, B. J.; Lachance, P.; Bonneau, P. R.; Storer, A. C.; Kirschke, H.; Broemme, D. *Bioorg. Chem.* **1994**, *22*, 227. (g) Giordano, C.; Calabretta, R.; Gallina, C.; Consalvi, V.; Scandurra, R.; Noya, F. C.; Franchini, C. *Eur. J. Med. Chem.* **1993**, *28*, 917. (h) Huang, Z. Y.; McGowan, E. B.; Detwiler, T. C. *J. Med. Chem.* **1992**, *35*, 2048.

(5) For an alternative approach see: Sarabia, F.; Sanchez-Ruiz, A.; Chammaa, S. *Bioorg. Med. Chem.* **2005**, *13*, 1691.

(6) (a) Lygo, B.; To, D. C. M. *Chem. Commun.* **2002**, 2360. (b) Lygo, B.; To, D. C. M. *Tetrahedron Lett.* **2001**, *42*, 1343. (c) Lygo, B.; Wainwright, P. G. *Tetrahedron* **1999**, *55*, 6289. (d) Lygo, B.; Wainwright, P. G. *Tetrahedron Lett.* **1998**, *39*, 1599.

(7) For a recent review on epoxidation via asymmetric catalysis, see: Xia, Q.-H.; Ge, H.-Q.; Ye, C.-P.; Liu, Z.-M.; Su, K.-X. *Chem. Rev.* **2005**, *105*, 1603.

(8) See for example: (a) Jensen, J. S.; Lam, Y.-F.; Helz, G. R. *Environ. Sci. Technol.* **1999**, *33*, 3568. (b) Orton, K. J. P.; Bradfield, A. E. *J. Chem. Soc.* **1927**, 986.

(9) Lygo, B.; Andrews, B. I. *Metal-Catalysed Carbon–Carbon Bond-Forming Reactions*, In *Catalysts for Fine Chemical Synthesis*, Vol. 3; Roberts, S. M.; Whittall, J.; Mather, P.; McCormack, P., Eds.; J. Wiley and Sons, Ltd.: Chichester, **2004**, 27–33.

(10) **General Procedure for Asymmetric Epoxidation.**
A mixture of 15% aq NaOCl (9.0 mmol) and 12 M aq KOH (1 mL) was added dropwise to a solution of enone (3.0 mmol) and the appropriate catalyst (0.15 mmol) in PhMe (70 ml). The resulting mixture was stirred vigorously (1000 rpm) at r.t. for 30 min, then a second portion of 15% aq NaOCl (9.0 mmol) was added and stirring continued for 4–16 h. Then, H_2O (100 mL) was added and layers separated. The aqueous was extracted with EtOAc (2 × 100 mL) and the combined organics were dried (Na_2SO_4), then concentrated under reduced pressure. The residue was then purified by chromatography on silica gel.
Selected NMR Data.
Compound **9a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99–7.95 (2 H, m, ArH), 7.64–7.46 (3 H, m, ArH), 6.60 (1 H, br d, *J* = 8.5 Hz, NH), 4.72–4.64 (1 H, m, NHCH), 4.27 (1 H, d, *J* = 2.0 Hz, NHCOCH), 3.78 (3 H, s, OMe), 3.72 (1 H, d, *J* = 2.0 Hz, ArCOCH), 1.78–1.58 [3 H, m, CH_2, $CH(CH_3)_2$], 1.01 (3 H, d, *J* = 6.5 Hz, CH_3), 0.99 (3 H, d, *J* = 6.5 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 191.5 (C), 172.5 (C), 166.2 (C), 134.9 (C), 134.4 (CH), 129.0 (CH), 128.5 (CH), 56.2 (CH), 54.9 (CH), 52.5 (CH_3), 50.4 (CH), 41.3 (CH_2), 25.0 (CH), 22.7 (CH_3), 22.0 (CH_3).
Compound **10a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99–7.95 (2 H, m, ArH), 7.64–7.46 (3 H, m, ArH), 6.50 (1 H, br d, *J* = 8.5 Hz, NH), 4.72–4.64 (1 H, m, NHCH), 4.41 (1 H, d, *J* = 2.0 Hz, NHCOCH), 3.78 (3 H, s, OMe), 3.73 (1 H, d, *J* = 2.0 Hz, ArCOCH), 1.78–1.58 [3 H, m, CH_2, $CH(CH_3)_2$], 1.01 (3 H, d, *J* = 6.5 Hz, CH_3), 0.99 (3 H, d, *J* = 6.5 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 191.3 (C), 172.9 (C), 166.4 (C), 134.9 (C), 134.4 (CH), 129.0 (CH), 128.5 (CH), 55.8 (CH), 54.9 (CH), 52.5 (CH_3), 50.2 (CH), 41.0 (CH_2), 24.8 (CH), 22.8 (CH_3), 21.7 (CH_3).

(11) Tamai, M.; Yokoo, C.; Murata, M.; Oguma, K.; Sota, K.; Sato, E.; Kanaoka, Y. *Chem. Pharm. Bull.* **1987**, *35*, 1098.

(12) For further discussion of this, see: Roush, W. R.; Hernandez, A. A.; Zepeda, G. *Synthesis* **1999**, 1500.