Mechanistic studies on the synthesis of bicalutamide†

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Bicalutamide, a therapeutically important anti-androgen used in the treatment of hormone-sensitive cancers, may be synthesised from the appropriate halohydrin or epoxide. We report here studies aimed at demonstrating unambiguously that preparation of bicalutamide and its thioether analogue from the chlorohydrin under basic conditions proceeds *via* opening of an intermediate epoxide by the appropriate sulfinate or thiolate nucleophile, that the analogous anionic sulfur nucleophiles react under the same conditions and that the S_N2 pathway involving direct displacement of chloride by the nucleophile does not operate. The proposed mechanism is confirmed by the quantitative fitting of sequential reaction kinetics, taking into account the competing dimerisation of the thiolate nucleophile that occurs under basic conditions. The *O*-methyl analogue of the chlorohydrin is unreactive towards thiolate under the same conditions, although a slower cyclisation to the β-lactam was observed. The implications of these observations for the analogous preparation of thioethers and sulfones are discussed.

Introduction

The synthetic utility of epoxides has long been exploited, with much research effort focussing on stereocontrol, the impact of molecular rearrangements and the effects of neighbouring groups.[1] Our interest in the synthesis of the chemotherapeutic agent bicalutamide[2] (**1**) led us to consider the *in situ* reaction of the parent chlorohydrin (**2**) to form an intermediate epoxide (**3**) which is opened by a sulfur nucleophile (**4** or **5**)to give bicalutamide directly or after oxidation of an intermediate thioether (**6**) (Scheme 1).

In principle, it would be possible for a chlorohydrin to react directly with a nucleophile in an S_N2 displacement of chloride that would not involve the epoxide intermediate. Therefore in addition to characterising the reaction as set out above, our analysis also aimed to assess to what extent, if any, this alternative mechanism operated.

The formation of an epoxide from a chlorohydrin is essentially quantitative[3] and has been applied routinely in chemical synthesis,[1] including in organic syntheses employing water as the solvent.[4] In aqueous solution, the kinetics of the reaction exhibit specific base catalysis, consistent with a pre-equilibrium deprotonation of the alcohol followed by ring-closure.[5] In theory, the rates of such pre-equilibrium processes are expected to reach a pH-independent plateau as the alcohol becomes fully deprotonated at high pH, however the low acidity of the alcohol (the pK_a of 2-chloroethanol is 14.3[6]) is such that the pH-independent reaction is generally not observed in aqueous solution. Epoxides are also commonly formed *via* the displacement of other halides or pseudohalides by a neighbouring alcohol.[7]

Numerous mechanistic studies have shown that in general (and especially under neutral or basic conditions) terminal epoxides are opened at the less-hindered carbon.[8] Product mixtures are often obtained from internal epoxides and also notably styrene oxide,[8,9] with the latter being attributed to pronounced carbocation character in the developing transition state of nucleophilic attack.[10] Epoxides are reactive towards thiols,[11] sulfites,[12] sulfinates[13] and thiosulfates,[14] giving hydroxythioethers or their *S*-oxidised analogues, but unreactive towards sulfates.[15] This is consistent with the soft-nucleophilic sulfur-centred anions reacting with the epoxides.[15,16] As would be expected from their reduced nucleophilicity, sulfinates are much less reactive towards epoxides than are thiolates.[17] This potential limitation to their synthetic utility is exacerbated by the poor solubility of sulfinates in organic solvents, which has lead some researchers to employ solubilising amphiphiles.[18] Under basic conditions, the epoxide-opening reaction is second-order overall, depending upon both the concentration of the nucleophile and that of the epoxide.[5,19] It should be noted that the anionic nucleophile (thiolate or sulfinate) is the reactive species,[20] hence weakly acidic thiols might be poorly reactive under conditions where they are not deprotonated. The pK_a of 4-fluorobenzenethiol is 6.3,[21] hence it may be assumed to be fully deprotonated even under weakly alkaline conditions. Specific acid,[22] general acid[23] and Lewis acid[24] catalysis of epoxide-opening

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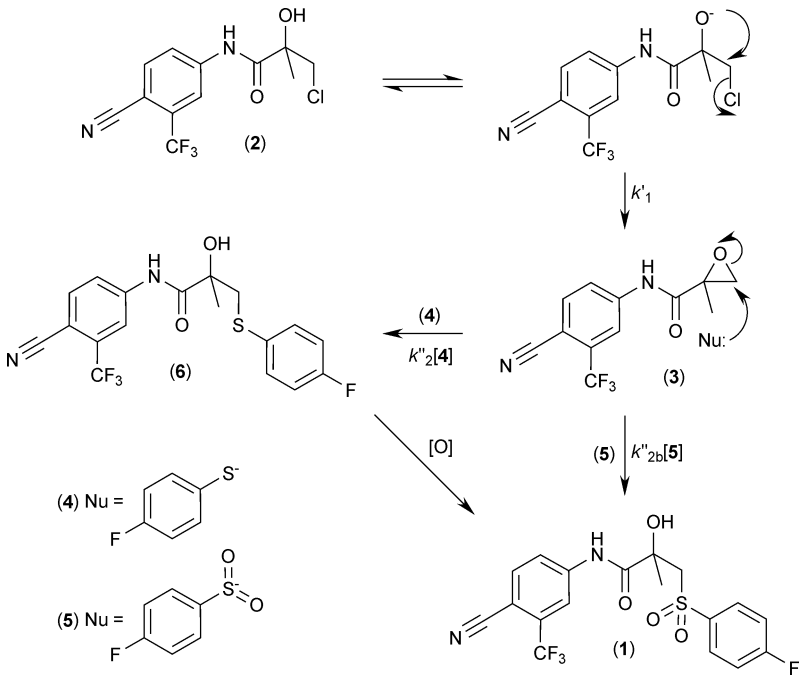
† Electronic supplementary information (ESI) available: Chromatograms of the chlorohydrin (**2**) and the epoxide (**3**), tables of rate data for the initial kinetic studies, serial chromatograms showing (a) the reaction of the chlorohydrin (**2**) to the epoxide and the diol (**7**) at pH 11 (b) the reaction of the epoxide (**3**) with both sulfur nucleophiles separately and in a "one-pot" experiment, the concentration–time profile for the "one-pot" experiment, concentration–time profiles showing improved fitting of the sequential kinetics when dimerisation of the thiolate (**4**) is taken into account, concentrations of the reactants and products in the sequential experiment derived from the determination of the relative extinction coefficient of the thioether (**6**), HPLC peak area integral-time profile showing dimerisation of the thiolate (**4**) occurring in parallel to the desired reactions, HPLC peak area integral-time profile for the formation of bicalutamide (**1**) from the chlorohydrin (**2**) reacting *via* the epoxide (**3**) with 4-fluorobenzenesulfinate (**5**), reaction scheme for synthesis of the *O*-methyl analogue (**8**) and β-lactam (**9**), LCMS trace and high resolution MS for *O*-methyl analogue (**8**), MS fragmentation pattern and high resolution MS for β-lactam (**9**). See DOI: 10.1039/b815894k



Scheme 1 Formation of bicalutamide (**1**) from the chlorohydrin (**2**).

have been reported, implying that the opening of an epoxide by an anionic sulfur nucleophile would be a suitable system for the type of bifunctional catalysis described by Kirby.[25] Epoxide-opening, especially under basic conditions, is also essentially quantitative; many papers that report the need for extended reaction times or significantly sub-stoichiometric yields exemplify 1:1 mixtures of the two reactants or only a slight excess of thiol.[26] Under such conditions, a second-order reaction would be expected to exhibit 'tailing' of the rate towards completion as the concentrations upon which the rate depends diminish.[27] Such reactions are commonly driven towards completion by using a significant excess of one reagent.

Being a hydroxysulfone, bicalutamide (**1**) may be prepared directly from opening the appropriate epoxide with an aryl sulfinate, or in two steps with a thiolate to give a hydroxythioether which may be oxidised subsequently to the sulfone.[28] Conceptually, these approaches differ only in whether oxidation of sulfur occurs prior to or after the epoxide-opening step, however the reduced nucleophilicity of the sulfinate relative to the thiolate clearly has practical implications. The aim of our studies was to demonstrate that the synthesis of bicalutamide (**1**) from the chlorohydrin (**2**) occurs *via* an intermediate epoxide (**3**) and that the anionic sulfur nucleophiles 4-fluorobenzenethiolate (**4**) and 4-fluorobenzenesulfinate (**5**) react under the same conditions *via* the same mechanism. Whilst confirmation of the expected sequential pathway, as opposed to a direct S_N2-displacement of chloride by the anionic sulfur nucleophiles, could be inferred from the presence and consumption of the intermediate epoxide in a "one-pot" reaction, we thought it prudent additionally to prepare and study the *O*-methyl analogue (**8**) of the chlorohydrin in order to verify that it did not form the *O*-methyl analogue of bicalutamide.

Results and discussion

Formation of the epoxide

Base-catalysed elimination of HCl from the chlorohydrin (**2**) to form the epoxide (**3**) under alkaline conditions was followed by HPLC-MS. In order to quantify the dependence of the reaction upon pH, samples of the chlorohydrin (**2**) were incubated at 30 °C in buffer solutions ranging from pH 9 to pH 11. Similarly, in order to assess the dependence of the reaction upon temperature samples were incubated in buffer solution at pH 9 at temperatures ranging from 35 °C to 55 °C. Rate constants were determined from changes in the HPLC peak area integrals of the relevant species over time and are derived in at least duplicate from a minimum of five serial chromatograms. Where the reaction proceeded too rapidly to be followed conveniently by serial HPLC sampling in real time, a quenching method was used. In all cases, the concentration–time profile of the reacting species gave good exponential fits indicating (pseudo)first-order kinetics. The two dependences are shown graphically below (Fig. 1).

The logarithm of the rate constant for epoxide formation was found to vary linearly with pH. The gradient of the plot is 1.06, consistent with pre-equilibrium deprotonation of the alcohol followed by cyclisation expelling chloride (theoretical gradient 1.0). The gradient of the plot of the logarithm of the rate constant for epoxide formation against reciprocal temperature is −6825 K, which corresponds to a ~5-fold rate acceleration per 10 °C increase in temperature over the range of temperatures studied.

Formation of the epoxide (**3**) from the chlorohydrin (**2**) proceeded cleanly and quantitatively in all the above experiments. Further hydrolysis of the epoxide (**3**) to the diol (**7**) only became significant at pH 11 (Scheme 2). The half-life for the latter reaction

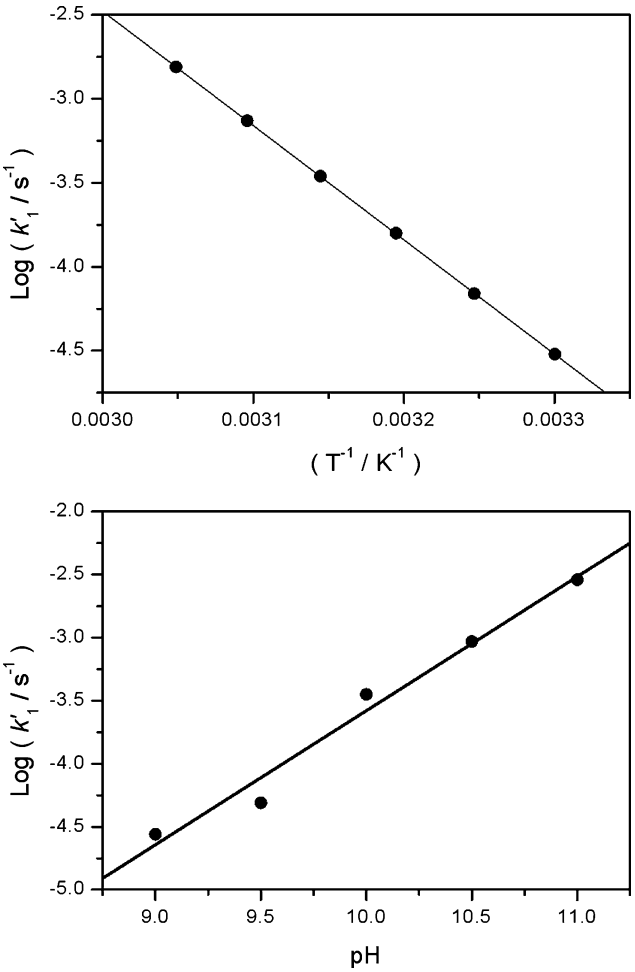
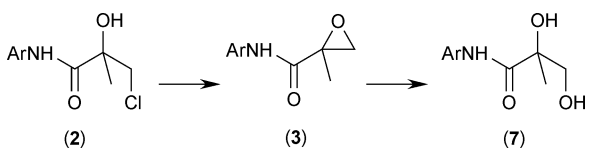


Fig. 1 Plots showing the dependence of the rate of epoxide-formation upon temperature (above) and upon pH (below).



Scheme 2 Further reaction of the epoxide (**3**) to form the diol (**7**) upon prolonged incubation at pH 11.

at pH 11 and 30 °C is 280 min, compared to 4 min for the formation of the epoxide (**3**).

It is therefore apparent that the chlorohydrin (**2**) reacts quantitatively and rapidly under mildly basic conditions to give the epoxide (**3**).

Epoxide-opening reactions

A similar HPLC-MS approach was used in order to study the opening of the epoxide (**3**) by anionic sulfur nucleophiles at 50 °C in pH 10 buffer solution. Incubation of 0.02 mM epoxide (**3**) with 0.2 mM 4-fluorobenzenethiolate (**4**) and with 500 mM 4-fluorobenzesulfinate (**5**) allowed the second-order rate constants of the two reactions to be determined as 1.3 M^{-1} s^{-1} and 0.00019 M^{-1} s^{-1} respectively. The ratio of these two rate constants gives an estimate that the thiolate is ~6800-fold more reactive (*i.e.* more nucleophilic) than the sulfinate.

In order to demonstrate that the two reactions were analogous, a "one-pot" competition experiment was set-up whereby 0.02 mM of the epoxide (**3**) was incubated with a mixture of 0.156 mM 4-fluorobenzenethiolate (**4**) (*i.e.* 7.8 equivalents) and 1000 mM 4-fluorobenzesulfinate (**5**) (*i.e.* 50000 equivalents), these concentrations being selected such that both reactions would proceed at approximately equal rates. Serial chromatograms for the parallel reactions of the epoxide (**3**) forming the thioether (**6**) and sulfone (**1**) products are shown below (Fig. 2).

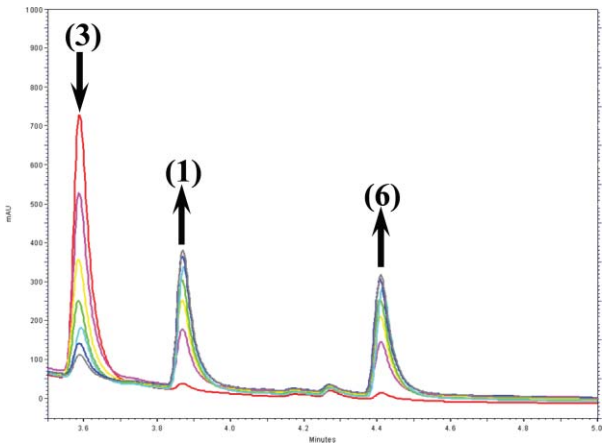


Fig. 2 HPLC traces showing the "one-pot" reaction of the epoxide (**3**) giving rise to bicalutamide (**1**) and the thioether (**6**) (red, t = 0; magenta, t = 16 min; yellow, t = 32 min; green, t = 48 min; cyan, t = 64 min; blue, t = 80 min; grey, t = 96 min).

The parallel reactions do indeed proceed at comparable rates and it is therefore clear that both nucleophiles are capable of reacting under the same conditions to give analogous products.

Mechanistic consequences

Since the rates of the epoxide-opening reactions at pH 10 and 50 °C are significantly slower than the rate at which the epoxide would be expected to form from the chlorohydrin, a mixture of either of the sulfur nucleophiles and the chlorohydrin (**2**) would be expected to give rise to the final product *via* the epoxide intermediate (**3**) at such a rate that the epoxide intermediate (**3**) would be detectable.

Failure of the expected sequential reactions to take place in the manner described could result if the components or by-products of one of the individual steps interfered with the other. Provided that their addition did not significantly perturb the pH of the reaction mixture, the presence of the sulfur nucleophiles would not be expected to impact the epoxide-forming reaction. Similarly, the liberation of HCl from the epoxide-forming reaction would not be expected to impact the nucleophilic addition unless it led to protonation of the sulfur nucleophile. The latter is also unlikely since completion of the first step relies on there being sufficient base available to deprotonate the chlorohydrin, whereas the solution would need to become acidic in order for the sulfur nucleophile to become protonated.

Another possibility meriting consideration is that the sulfur nucleophile could react directly with the chlorohydrin (**2**) to form the final product (or an undesired side-product). The direct S_N2 displacement of chloride would need to generate final product at a rate faster than that of epoxide-formation in order for the S_N2 mechanism to take over from the sequential process *via* the epoxide intermediate (**3**). If that were the case then the epoxide (**3**) would not be detectable in the reaction mixture. It should be noted that failure to observe the epoxide (**3**) would not constitute proof that the S_N2 mechanism were operating since the detection of a reactive intermediate depends both upon the extent to which it forms during the reaction and the methodology available for its quantification. The former line of reasoning is therefore the stronger: detection of the epoxide during the sequential reaction is evidence of it being an intermediate; formation of final product at a rate consistent with the reaction going through the epoxide only is evidence that the S_N2 pathway essentially does not operate. Conversely, formation of the final product at a rate that cannot be accounted for by the sequential pathway would be evidence for at least partial reaction *via* an alternative mechanism.

It is worth noting also that the reaction *via* an epoxide is formally a double-inversion at the chlorine-bearing carbon. A direct S_N2 pathway would give rise to a single inversion. The chlorohydrin (**2**) has a terminal methylene chloride, which is not therefore an asymmetric carbon centre, hence it is not possible to draw mechanistic conclusions from stereochemical analysis of the product.

Several alternative approaches to elucidating the mechanism also have merit: a similar line of reasoning to that set out above leads to the assertion that detection of the final product under conditions in which the epoxide intermediate is not formed would be evidence for an alternative mechanism operating under those conditions. By extension, the study of an analogous compound that is incapable of forming an epoxide could be informative. An *O*-alkyl analogue of the parent chlorohydrin would be such a compound. Since that compound could not ring-close to epoxide,[29] its reaction to the analogous final product at a similar rate to the chlorohydrin reaction would give credence to the direct S_N2 pathway. Similarly, substitution of the terminal methylene group to give an asymmetric carbon centre and analysis of the stereochemical outcome of the reaction of the homochiral chlorohydrin analogue would give unambiguous mechanistic information. The latter two approaches however both involve substantial perturbations around the reacting centre of the chlorohydrin. A judgement as to whether those perturbations were sufficiently small as to allow extrapolation of any conclusions to the original system would become necessary. The most direct evidence therefore is that available from the study of the sequential reaction kinetics.

Sequential reaction kinetics

A "one-pot" reaction to generate the thioether (**6**) was therefore carried out by incubating 0.02 mM of the chlorohydrin (**2**) with 0.156 mM 4-fluorobenzenethiolate (**4**) at 50 °C in buffer solution at pH 8.5. The epoxide (**3**) was indeed observed as an intermediate that subsequently reacted to give the thioether product (**6**). The variations in concentration of the three species over time are illustrated below (Fig. 3, data points).

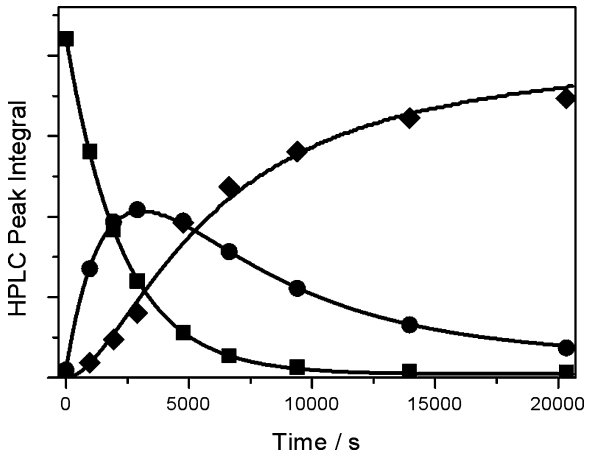


Fig. 3 Changes over time in the concentrations of the chlorohydrin (**2**, ■), the epoxide (**3**, ●) and the thioether product (**6**, ◆). Data points are experimental HPLC peak area integrals; fits are calculated from the proposed mechanism and derived rate constants.

As with previous experiments, the concentration of the chlorohydrin (**2**) decayed exponentially with time giving rise to the epoxide (**3**). The rate constant for the first step (k'_1) was 0.000438 s^{-1}, marginally larger than would be expected from extrapolating the above dependences upon pH and temperature.

Having determined the rate constant (k'_1) for formation of the epoxide (**3**), it is conventional to derive the rate constant for the subsequent thioether-generating reaction, noting that it may be derived as a pseudofirst-order rate constant provided that the concentration of the nucleophile does not change significantly during the reaction. The pseudofirst-order rate constant for the generation of the thioether is in fact the product of a second-order rate constant and the concentration of the nucleophile (k''_2.[**4**]). By definition therefore, the concentrations of both the epoxide intermediate (**3**) and the thioether final product (**6**) are defined by k'_1 and k''_2.[**4**]. The assumptions implicit in this approach are that the chlorohydrin (**2**) reacts exclusively to give the epoxide intermediate (**3**), that the latter species is only consumed by reaction with the nucleophile to give the final product (**6**) and (optionally) that the concentration of the nucleophile (**4**) changes negligibly during the reaction.

In principle, the use of 7.8 equivalents of nucleophile would introduce a systematic error associated with the latter assumption since 12.8% of the nucleophile would be consumed if the reaction were followed to completion. The pseudofirst-order rate constant for the second step would diminish proportionately as the reaction progressed. Furthermore, since only the initial epoxide-forming reaction exhibits exponential kinetics, it is necessary to establish the concentrations of the relevant species in the reaction mixture. This was conveniently achieved by relating the concentrations of the epoxide intermediate (**3**) and final product (**6**) back to the known initial concentration of the chlorohydrin (**2**) *via* their respective UV-vis absorptions and HPLC peak area integrals. In practice, the extinction co-efficient of the epoxide (**3**) was indistinguishable from that of the chlorohydrin (**2**), hence the relative extinction co-efficient of the final product (**6**) was calculated by assuming mass-balance of these three species and performing a one-variable minimisation on the sum of the weighted HPLC peak area integrals at eight different time-points.

In attempting to fit the sequential kinetics, neither assuming that the concentration of the nucleophile was constant during the reaction, nor taking into account its consumption to form thioether product gave satisfactory results. It was only when the competing dimerisation of the thiolate nucleophile[30] (**4**) (Scheme 3) was taken into account that a satisfactory fit to the concentration–time profile was achieved.

The HPLC peak area integral of the thiolate (**4**) was quantified in the same way as the other species and found to undergo a second-order decay over the course of the reaction. The concentration of the thiolate (**4**), initially 0.156 mM, was observed to drop to 0.050 mM by the last time point, with only 0.018 mM of that 0.106 mM decline attributable to reaction with the epoxide. Determination of the second-order rate constant for this decay (k''_{dim}) allowed the variation in concentration of the nucleophile during the sequential reaction, and hence the time dependence of k''_2[**4**], to be defined. Whilst the rigorously-defined rate of consumption of the thiolate (**4**) is given by $\{k''_{dim}[\mathbf{4}]^2 + k''_2[\mathbf{4}][\mathbf{3}]\}$, since the dimerisation reaction dominates, we judged it appropriate to consider only the dimerisation in defining [**4**] for determination of kinetics. The alternative approach would be to solve the consumption of thiolate (**4**) simultaneously with the determination of k''_2, which suffers the drawback of fitting multiple rate constants that may be mutually compensatory. As defined here, k''_{dim} is therefore a second-order approximation for the consumption of thiolate by both processes.

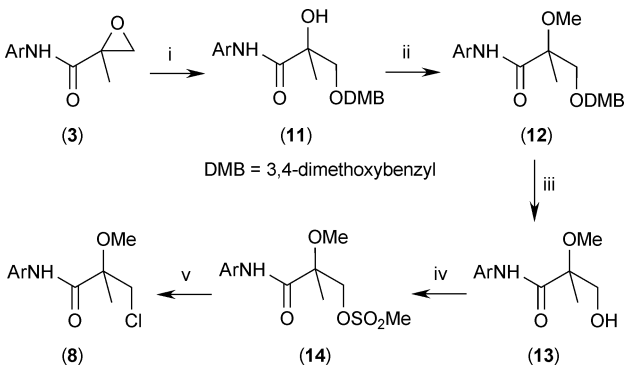
Our approach therefore was to derive: k'_1 from the exponential decay of the chlorohydrin (9 HPLC peak integrals), k''_{dim} from the concentration of the thiolate (9 HPLC peak integrals), the relative extinction co-efficient of the product (ε_6) from the mass balance of the chlorohydrin, epoxide and product (24 HPLC peak integrals), k''_2 from the above and the concentrations of the epoxide and product (17 HPLC peak integrals).

In this way, the second-order rate constant (k''_{dim}) for the dimerisation of 4-fluorobenzenethiolate (**4**) was found to be 39.7 M^{-1} s^{-1} and the second-order rate constant (k''_2) for the reaction of the epoxide (**3**) with 4-fluorobenzenethiolate (**4**) was found to be 2.14 M^{-1} s^{-1}. Using the three rate constants and the rate laws associated with the proposed reaction scheme, it is possible to calculate the expected concentration–time profile for the sequential reactions (Fig. 3, lines). The mean RMS difference between the calculated and experimental concentrations of the chlorohydrin (**2**), the epoxide (**3**), the thioether (**6**) and 4-fluorobenzenethiolate (**4**) are 1.5%, 1.5%, 7.4% and 3.1% respectively. The somewhat larger differences associated with the thiolate and thioether are presumably attributable at least in part to using only the dimerisation reaction in order to define the calculated thiolate concentration. It is therefore possible to conclude that the reaction of the chlorohydrin (**2**) with 4-fluorobenzenethiolate (**4**) to give the thioether (**6**) proceeds almost entirely *via* the intermediate epoxide (**3**). A similar sequential reaction using 4-fluorobenzesulfinate (**5**) as the nucleophile gave an analogous reaction profile (not shown).

We are not aware of any previous synthetic study making a quantitative link between the parallel competing dimerisation of a reactant thiolate and the failure of a reaction to complete, although clearly the dimerisation of thiols and the need for an excess of one reactant in order to drive a second-order process to completion are both well-precedented.

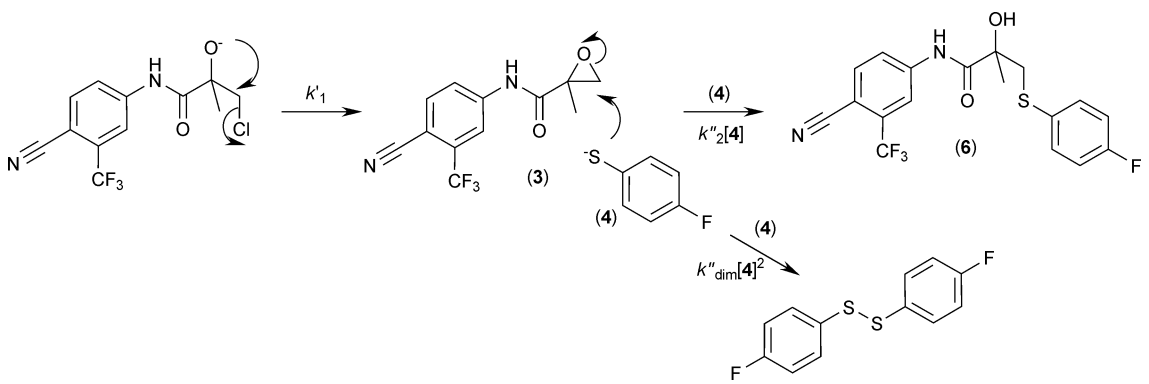
Studies on the *O*-methyl analogue

The *O*-methyl chlorohydrin analogue (**8**) was prepared from the epoxide (**3**) by methylation of the selectively-protected diol, deprotection and chlorination (Scheme 4).



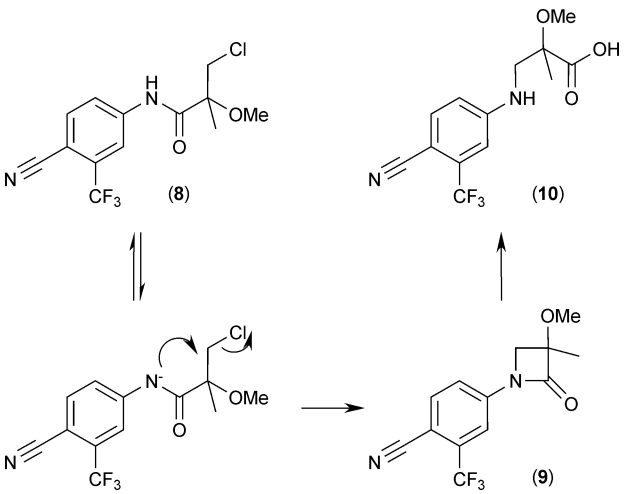
Scheme 4 Synthetic route to *O*-methyl chlorohydrin analogue (**8**). Conditions: (i) ^{n}BuLi, DMBOH, THF, –78 °C (ii) NaH, MeI, DMF (iii) DDQ, CH_2Cl_2 (iv) $MeSO_2Cl$, DIPEA, CH_2Cl_2, 0 °C, (v) LiCl, 150 °C.

Incubation of 0.02 mM of the *O*-methyl chlorohydrin analogue (**8**) at 30 °C in buffer solution at pH 8.5 and pH 10 in the presence of a large excess (1.56 mM) of 4-fluorobenzenethiolate (**4**) gave



Scheme 3 Competing dimerisation of the thiolate (**4**) impacts the rate of formation of the thioether product (**6**).

no thioester product. In the absence of the sulfur nucleophile, the *O*-methyl chlorohydrin analogue (**8**) is stable for more than 12 hours at 30 °C in buffer solution at pH 8.5, whereas partial decomposition of the starting material was observed in buffer solution at pH 10. The half-life for the latter decomposition was found to be 728 min, compared to 32.6 min for the formation of the epoxide (**3**) from the chlorohydrin (**2**). The initial product reacted further to give a hydrolysis product consistent with acid (**10**), leading us to postulate that the *O*-methyl chlorohydrin analogue (**8**) initially reacts to form the β-lactam (**9**) (Scheme 5). It is noteworthy that this reaction pathway is also available to the chlorohydrin (**2**) but with closure to the 4-membered ring being 22-fold slower than to the 3-membered epoxide, it is not observed.



Scheme 5 Ring-closure of the *O*-methyl analogue (**8**) to the β-lactam (**9**) and subsequent hydrolysis to the acid (**10**).

The identity of the postulated β-lactam product (**9**) was confirmed by a preparative-scale reaction. A related bromohydrin has previously been reported to ring-close to the β-lactam *via* *N*-deprotonation upon treatment with base in toluene but to the epoxide *via* *O*-deprotonation upon treatment with base in acetonitrile.[31]

Conclusions

Mechanistic analysis of the preparation of bicalutamide (**1**) from the chlorohydrin (**2**) and a thiolate (**4**) or sulfinate (**5**) nucleophile demonstrates unambiguously that both reactions proceed *via* an intermediate epoxide (**3**). Under weakly alkaline conditions, the rate of the epoxide-forming step is linear with hydroxide concentration and there is a ~5-fold rate enhancement upon increasing the temperature by 10 °C over the range of temperatures studied. The thiolate nucleophile (**4**) was found to be ~6800-fold more reactive than the analogous sulfinate (**5**), consistent with its much enhanced nucleophilicity. Consequently, if the sulfinate were to be used synthetically in place of the thiolate, the reaction time would need to be extended or the concentration of nucleophile would need to be increased some 6800-fold in order to achieve the same outcome.

Whilst both reactions are feasible, the more efficient route to the desired sulfone product would therefore appear to be *via* opening of the epoxide with thiolate followed by oxidation of the resultant thioether. Whilst it would clearly be prudent to use a significant excess of thiolate in order to drive the (second-order) epoxide-opening reaction to completion, we have further demonstrated quantitatively that the rate of epoxide-opening by thiolate is further diminished by the competing dimerisation of the thiolate to form the disulfide. Hence for this reaction and more generally where thiols are employed under basic conditions, the extent of competing dimerisation and its impact upon the desired chemistry must be considered when defining reaction conditions.

Our results show that the alternative S_N2 process involving direct displacement of chloride from the chlorohydrin (**2**) by the sulfur nucleophiles does not operate under basic conditions. Such a reaction would be in competition with the more-favourable formation of the intermediate epoxide (**3**).

The *O*-methyl chlorohydrin analogue (**8**) was synthesised and studied. This compound does not form an analogous epoxide intermediate, but does undergo a much slower ring-closure to the β-lactam (**9**). Neither compound was reactive towards thiolate, further confirming that the S_N2 displacement of chloride is not a route to bicalutamide (**1**) for the parent chlorohydrin under weakly basic conditions.

Experimental

General procedures

All organic solvents were HPLC grade, obtained from commercial sources and used as supplied. Aqueous incubation buffers were Fluka BioChemika HPCE grade and used as supplied. Other reagents were obtained from commercial sources and used as supplied.

HPLC experiments were run on a Waters 2795 fitted with a Phenomenex Synergi 4 μ MAX-RP 80A column (30 × 2.0 mm). Chromatograms were obtained at a flow rate of 0.7 ml min^{-1} using a water-methanol or water-acetonitrile gradient; the aqueous eluant being a 10 mM ammonium acetate buffer.

The incubation temperature for kinetic runs was controlled using a thermostatted sample tray and monitored with a thermocouple to be within ±0.2 °C of that stated.

Incubation buffers were pre-heated for 10 min prior to initiation of the reaction, which was achieved by injecting appropriate volumes of a concentrated DMSO stock solution of the compound being studied (generally a total of 0.02 ml DMSO was added, making the final aqueous solution 1.3% DMSO). 4-Fluorobenzenethiol was added as a DMSO stock solution, generating the thiolate (**4**) *in situ*, whereas the appropriate concentration of 4-fluorobenzenethiolate (**5**) was achieved through addition of the appropriate quantity of its sodium salt to the buffer solution prior to incubation. HPLC peak areas were quantified using Chromquest software v4.1 (Thermo Electron 2003).

Where the half-life of a reaction was greater than 20 min, its rate was determined by repeat sampling of the incubation in "real time". Where the half-life of a reaction was less than 20 min, its rate was determined by quenching of aliquots removed from the incubation at given time intervals. All reactions were followed to beyond 70% completion and rates are derived from a minimum of two experiments of at least five serial chromatograms each.

First-order rate constants were determined by exponential fitting of the concentration–time data; sequential reaction and second-order rate constants were determined numerically by minimisation of the RMS difference between the experimental concentration–time data and the appropriate rate laws derived from the mechanistic scheme.

The identities of bicalutamide (**1**), the chlorohydrin (**2**) and the epoxide (**3**) were confirmed by mass spectrometry and comparison to authentic samples from the AstraZeneca compound collection. Other species in the kinetic runs were identified by mass spectrometry.

Example procedure for the kinetic study of the formation of the epoxide (3) from the chlorohydrin (2). A stock solution of the chlorohydrin (**2**) was prepared at a concentration of 0.46 mg ml^{-1} in DMSO. For "real time" experiments, 0.02 ml of this stock solution was injected into 1.5 ml of aqueous buffer, the solution was quickly removed from the thermostatted sample tray, shaken and returned to the sample tray. Serial chromatograms were then collected using the water-methanol HPLC gradient. For "quenching" experiments, 0.1 ml of the DMSO stock solution was injected into 1.5 ml of aqueous buffer, the solution was quickly removed from the sample tray, shaken and returned to the sample tray. Subsequently, at appropriate time intervals, a 0.05 ml aliquot was removed from the incubation and quenched immediately into 0.45 ml of pH 6.5 buffer solution in order to hold the reaction for analysis using the same HPLC gradient as for the "real time" studies.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-hydroxy-2-methylpropanamide (11).** Butyllithium (18.50 ml, 46.26 mmol) was added dropwise to (3,4-dimethoxyphenyl)methanol (7.47 g, 44.41 mmol) in THF (74.0 ml) at −78 °C. The resulting solution was stirred for 20 min at −78 °C. To this was added *N*-(4-cyano-3-(trifluoromethyl)-phenyl)-2-methyloxirane-2-carboxamide (10 g, 37.01 mmol) and the reaction was allowed to warm to room temperature over 30 min. The reaction mixture was quenched with saturated NH_4Cl (10 ml), extracted with EtOAc (400 ml), the organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography (isocratic 2% MeOH in dichloromethane). Pure fractions were evaporated to dryness to afford *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-hydroxy-2-methylpropanamide (5.92 g, 36.5%) as an orange glassy foam. HPLC, ms detection: *m/z* (ESI^-) $(M - H)^- = 437.40$; HPLC t_R = 2.36 min. ^1H NMR (300.132 MHz, DMSO) δ 1.32 (3H, s), 3.17 (1H, d, J = 5.2 Hz), 3.44 (1H, d, J = 9.6 Hz), 3.63 (3H, s), 3.71 (3H, s), 4.40 (1H, d, J = 12.0 Hz), 4.47 (1H, d, J = 12.0 Hz), 5.95 (1H, s), 6.78 (1H, dd, J = 8.2, 1.7 Hz), 6.83–6.87 (2H, m), 8.08 (1H, d, J = 8.6 Hz), 8.28 (1H, dd, J = 8.6, 2.0 Hz), 8.55 (1H, d, J = 2.0 Hz), 10.44 (1H, s) ppm.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-methoxy-2-methylpropanamide (12).** Sodium hydride (60% in mineral oil) (0.532 g, 13.31 mmol) was added to *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-hydroxy-2-methylpropanamide (2.653 g, 6.05 mmol) in DMF (12.10 ml). The reaction was stirred for 20 min until gas evolution ceased. Iodomethane (0.565 ml, 9.08 mmol) was added in a single portion and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (200 ml), and washed sequentially with saturated NH_4Cl (100 ml), water (100 ml), and saturated brine (100 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 2% MeOH in dichloromethane. Pure fractions were evaporated to dryness to afford *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-methoxy-2-methylpropanamide (0.724 g, 26.4%) as a pale yellow oil. HPLC, ms detection: *m/z* (ESI^-) $(M - H)^- = 451.47$; HPLC t_R = 2.63 min. ^1H NMR (300.132 MHz, $CDCl_3$) δ 1.30 (3H, s), 3.32 (3H, s), 3.54–3.61 (2H, m), 3.72 (3H, s), 3.79 (3H, s), 4.37 (1H, d, J = 11.9 Hz), 4.43 (1H, d, J = 11.9 Hz), 6.72–6.72 (3H, m), 7.70 (1H, d, J = 8.5 Hz), 7.82 (1H, dd, J = 8.5, 2.1 Hz), 8.03 (1H, d, J = 2.1 Hz), 8.96 (1H, s) ppm.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-3-hydroxy-2-methoxy-2-methylpropanamide (13).** 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile (577 mg, 2.54 mmol) was added to *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-(3,4-dimethoxybenzyloxy)-2-methoxy-2-methylpropanamide (767 mg, 1.70 mmol) in dichloromethane (10 ml) at room temperature. The resulting solution was stirred at room temperature for 1 hour. The reaction mixture was diluted with dichloromethane (100 ml), and washed sequentially with saturated $NaHCO_3$ (50 ml) and saturated brine (100 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 2% MeOH in dichloromethane. Pure fractions were evaporated to dryness to afford *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-hydroxy-2-methoxy-2-methylpropanamide (405 mg, 79%) as a brown oil which solidified on standing. HPLC, MS detection: *m/z* (ESI^-) $(M - H)^- = 301.39$; HPLC t_R = 1.93 min. ^1H NMR (300.132 MHz, $CDCl_3$) δ 1.42 (3H, s), 2.12 (1H, dd, J = 7.8, 4.6 Hz), 3.46 (3H, s), 3.80 (1H, dd, J = 11.8, 4.5 Hz), 3.89 (1H, dd, J = 11.9, 7.8 Hz), 7.78 (1H, d, J = 8.5 Hz), 7.95 (1H, dd, J = 8.5, 2.1 Hz), 8.08 (1H, d, J = 2.1 Hz), 9.00 (1H, s) ppm.

3-(4-Cyano-3-(trifluoromethyl)phenylamino)-2-methoxy-2-methyl-3-oxopropyl methanesulfonate (14). Methanesulfonyl chloride (96 μl, 1.24 mmol) was added to *N*-(4-cyano-3-(trifluoromethyl)phenyl)-3-hydroxy-2-methoxy-2-methylpropanamide (300 mg, 0.99 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (347 μl, 1.99 mmol) in dichloromethane (3970 μl) and stirred under nitrogen at 0°C for 2 hours. The solution was allowed to warm to room temperature overnight. The reaction mixture was diluted with dichloromethane (100 ml), and washed sequentially with 2 M HCl (50 ml), water (50 ml), and saturated brine (50 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 2% MeOH in dichloromethane. Pure fractions were evaporated to dryness to afford 3-(4-cyano-3-(trifluoromethyl)phenylamino)-2-methoxy-2-methyl-3-oxopropyl methanesulfonate (340 mg, 90%) as a pale yellow oil. HPLC, MS detection: *m/z* (ESI^-) $(M - H)^- = 379.36$; HPLC t_R = 2.32 min. ^1H NMR (300.13 MHz, $CDCl_3$) δ 1.49 (3H, s), 3.00 (3H, s), 3.50 (3H, s), 4.41 (1H, d, J = 11.1 Hz), 4.49 (1H, d, J = 11.1 Hz), 7.80 (1H, d, J = 8.5 Hz), 7.94 (1H, dd, J = 8.5, 2.1 Hz), 8.09 (1H, d, J = 2.1 Hz), 8.99 (1H, s) ppm.

3-Chloro-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-methoxy-2-methylpropanamide (8). 3-(4-Cyano-3-(trifluoromethyl)phenylamino)-2-methoxy-2-methyl-3-oxopropyl methanesulfonate (500 mg, 1.31 mmol) and lithium chloride (557 mg, 13.15 mmol) were suspended in DMF (10 ml)and sealed in a microwave tube. The reaction was heated to 150 °C for 60 min in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with dichloromethane (200 ml), and washed sequentially with water (400 ml) and saturated brine (400 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 2% MeOH in dichloromethane. Pure fractions were evaporated to dryness to afford 3-chloro-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-methoxy-2-methylpropanamide (150 mg, 35.6%) as a cream solid. Elemental Analysis Found: C, 48.5; H, 3.7; N, 8.7 ($C_{13}H_{11}N_2O_2ClF_3$ requires: C, 48.7; H, 3.8; N, 8.7%). HPLC, MS detection: m/z (ESI$^-$) $(M - H)^- = 319.04666$; HPLC t_R = 2.57 min. ^{1}H NMR (300.132 MHz, $CDCl_3$) δ 1.45 (3H, s), 3.39 (3H, s), 3.66 (1H, d, $J = 12.2$ Hz), 3.88 (1H, d, $J = 12.2$ Hz), 7.73 (1H, d, $J = 8.5$ Hz), 7.90 (1H, dd, $J = 8.5$, 2.1 Hz), 8.00 (1H, d, $J = 2.1$ Hz), 8.88 (1H, s) ppm.

4-(3-Methoxy-3-methyl-2-oxoazetidin-1-yl)-2-(trifluoromethyl)-benzonitrile (9). 3-chloro-*N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-methoxy-2-methylpropanamide (80 mg, 0.25 mmol) was added to sodium carbonate buffer (pH 10) (12.5 ml) and DMSO (12.5 ml) at 30 °C. The resulting solution was stirred at 30 °C for 24 hours. The reaction mixture was concentrated and filtered to afford a DMSO solution of the product. The crude solution was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluants. Fractions containing the desired compound were evaporated to dryness to afford 4-(3-methoxy-3-methyl-2-oxoazetidin-1-yl)-2-(trifluoromethyl)benzonitrile (26.0 mg, 36.6%) as a white solid. Elemental Analysis Found: C, 54.7; H, 3.9; N, 9.53 ($C_{13}H_{11}N_2O_2F_3$ requires: C, 54.9; H, 3.9; N, 9.8%). HPLC, MS detection: m/z (ESI+) $(M^{\bullet})^+ = 284.0776$; HPLC t_R = 2.31 min. ^{1}H NMR (300.132 MHz, DMSO) δ 1.53 (3H, s), 3.35 (3H,s), 3.80 (1H, d, $J = 7.1$ Hz), 4.11 (1H, d, $J = 7.1$ Hz), 7.77 (1H, dd, $J = 8.6$, 1.9 Hz), 7.87 (1H, d, $J = 2.1$ Hz), 8.19 (1H, d, $J = 8.5$ Hz) ppm.

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