Intrinsic Reactivities in the Alkylations of Protected Amino Acids by (*R*)- and (*S*)-Methyloxirane

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A comparative study is described of the reactions between protected amino acids [either *N*-acetyl-L-cysteine methyl ester (**2a**), L-valine methyl ester (**3a**), or *N*-benzoyl-L-histidine methyl ester (**5a**)] and the enantiomeric methyloxiranes [*R*-(**1a**) or *S*-(**1b**)]. At 45 °C in methanol the relative rates of the reactions with either (**1a**) or (**1b**) are (**5a**) ~ (**3a**) > (**2a**). The reaction of (**1a**)/(**1b**) with (**2a**) is dramatically accelerated (≥ 100-fold) by triethylamine, because of the conversion of (**2a**) into its thiolate. The products from (**1a**)/(**1b**) + (**2a**) thiolate are (*R*,α*R*)- and (*S*,α*R*)-*N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine methyl ester [(**2b**) and (**2c**), respectively]. These products undergo a slow further reaction with methyloxirane leading *via* a β-elimination to *N*-acetyldehydroalanine methyl ester and bis-(2-hydroxypropyl) sulphide. The products from (**1a**)/(**1b**) + (**3a**) are (*R*,α*S*)- and (*S*,α*S*)-*N*-(2-hydroxypropyl)-L-valine methyl ester [(**3b**) and (**3c**), respectively]. These products also undergo a slow further reaction with methyloxirane leading to an *NN*-bis-(2-hydroxypropyl)valine methyl ester and derived morpholone [*e.g.* excess of (**1a**) + (**3a**)→(*R*,*R*,α*S*)-*NN*-bis-(2-hydroxypropyl)valine methyl ester (**3f**) and δ-lactone (**3h**)]. The reactions of (**1a**) or (**1b**) with (**5a**) give, *via* the products of mono-*N*-alkylation in the imidazole ring [*e.g.* (*S*,α*S*)-N^{α}-benzoyl-N^{τ}-(2-hydroxypropyl)-L-histidine methyl ester (**5d**) and its N^{π}-isomer (**5e**) from (**1b**) + (**5a**)], a mixture [*e.g.* (*S*,*S*,α*R*/α*S*)-N^{α}-benzoyl-$N^{\pi}N^{\tau}$-bis-(2-hydroxypropyl)-L-histidinylimidazolium carboxylates (**6d**) + (**6e**) from excess of (**1b**) + (**5a**)]. The generation of methoxide during the reactions described is responsible for the β-elimination leading to a dehydroalanine derivative and for the epimerisation during the formation of (**6d**) + (**6e**). Kinetic studies show that for none of the substrates (**2a**), (**3a**), or (**5a**) is there significant enantioselectivity in their reactions with the enantiomers of methyloxirane. The relevance of the present study to the toxicology of methyloxiranes is discussed.

Methyloxirane (propylene oxide, epoxypropane) is an established alkylating agent which has widespread uses in chemical and related industries.[1] Although there is no epidemiological evidence to date correlating human exposure to methyloxirane with cancer in man, reports have indicated that it is carcinogenic in mice and rats.[2,3] Recent studies have also shown that it is capable of modifying DNA *in vitro*[4,5] and that it is mutagenic in yeasts,[6] bacteria,[7] and mammalian cells[8] *in vitro*. Ehrenberg *et al.*[9–11] have demonstrated that levels of human exposure to such alkylating agents can be monitored by quantitating the reaction products of these reagents with haemoglobin. The advantages of using haemoglobin as a dose monitor for mutagens and carcinogens have been noted by several authors.[12] More recent publications have described a mass spectrometric method for monitoring amino acid adducts in haemoglobin from rats exposed to methyloxirane.[13,14]

Studies to determine whether the absolute configuration of the administered methyloxirane has any toxicological significance have not been reported. The reactions of native double-stranded DNA with certain chiral reagents, for example benzo[*a*]pyrene-7,8-diol 9,10-epoxides, show high stereospecificity and enantioselectivity.[15]

Following exposure of a protein to methyloxirane there are four reaction parameters that could be investigated: (i) the relative extents of alkylation at different nucleophilic sites; (ii) the relative extents of mono- and di-alkylation at a particular site; (iii) the enantioselectivity of a particular site for (*R*)- and (*S*)-methyloxirane [(**1a**) and (**1b**), respectively]; (iv) the regioselectivity of a particular site in its attack on methyloxirane; in principle, (*R*)- and (*S*)-methyloxirane could exhibit different regioselectivities.

In connection with our studies of the reactions of electrophilic reagents with cellular macromolecules, we have investigated the above parameters using the protected amino acids (**2a**), (**3a**), and (**5a**) corresponding to the three likely sites of covalent modification in haemoglobin: cysteine SH, terminal valine NH_2, and histidine N (of the imidazole). It is of interest to determine the intrinsic reactivities of these functional groups in model compounds, although relative reactivities may change in the protein. Data about the relative rates of reactions of (*R*)- and (*S*)-methyloxirane with the functional groups of the model compounds and the amino acid adducts obtained will provide useful references for comparison with *in vivo* systems.

Results

Reactions between (R)-N-*Acetylcysteine Methyl Ester* (**2a**) *and the Enantiomers of Methyloxirane.*—Cysteine in aqueous solution reacts with simple epoxides to give an *S*-2-hydroxyalkylcysteine.[16–19] Kinetic studies over a range of pH showed that it is the thiolate which reacts with the epoxide.[20,21] It was difficult to obtain pure *S*-2-hydroxyalkylcysteines because these products were very soluble in water and alcohol.[16b] To avoid this problem we used *N*-acetylcysteine methyl ester (**2a**) which gave *S*-hydroxypropyl adducts having favourable physical properties. Furthermore, there are no complications from competing alkylations at a functional group other than the thiol (*cf.* ref. 16*b*).

Both (*R*)- and (*S*)-methyloxirane reacted with *N*-acetylcysteine methyl ester (**2a**) in methanol containing triethylamine. For a 0.125M solution of the thiol with 1 mol. equiv. triethylamine and 5 mol. equiv. methyloxirane, the reaction was

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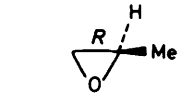
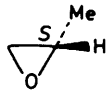
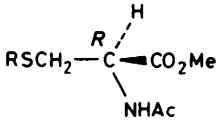


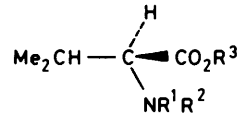
(1a)



(1b)

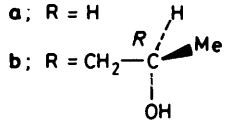


(2)

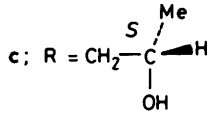


(3)

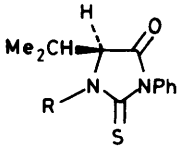
a; R = H



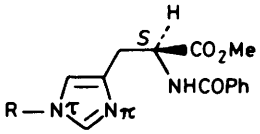
b; R = CH_2–C(R-Me)(OH)



c; R = CH_2–C(S-Me)(OH)



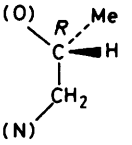
(4a) R as in (2c)
(4b) R as in (2b)



(5)

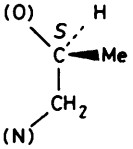
a; R = H
b; R as in (2b)
c; $N_{(\pi)}$-isomer of (5b)
d; R as in (2c)
e; $N_{(\pi)}$-isomer of (5d)

a; $R^1 = R^2 = H$, R^3 = Me
b; R^1 as in (2b), $R^2 = H$, R^3 = Me
c; R^1 as in (2c), $R^2 = H$, R^3 = Me
d; R^1 as in (2b), $R^2 = R^3 = H$
e; R^1 as in (2c), $R^2 = R^3 = H$
f; $R^1 = R^2$ as in (2b), R^3 = Me
g; $R^1 = R^2$ as in (2c), R^3 = Me
h; R^1 as in (2b), $R^2 R^3$ = (O)

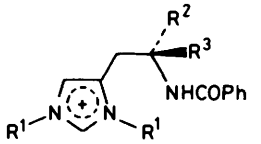


(N)

i; R^1 as in (2c), $R^2 R^3$ = (O)



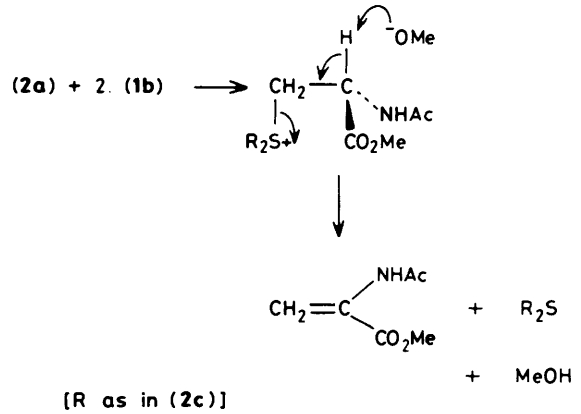
(N)



(6)

a; R^1 as R in (2b), $R^2 = H$, $R^3 = CO_2^-$
b; R^1 as R in (2b), $R^2 = CO_2^-$, $R^3 = H$
c; R^1 as R in (2b), $R^2 = H$, $R^3 = CO_2Me$
d; R^1 as R in (2c), $R^2 = H$, $R^3 = CO_2^-$
e; R^1 as R in (2c), $R^2 = CO_2^-$, $R^3 = H$

complete after 4 h at 0 °C. As expected for a mono alkyl-substituted epoxide,[22] the principal product from (*R*)-methyloxirane, characterised by its spectroscopic properties, was (*R*,α*R*)-*N*-acetyl-*S*-(2-hydroxypropyl)cysteine methyl ester (**2b**). In particular, its 360 MHz n.m.r. spectrum showed no evidence for an isomeric impurity derived by thiolate attack on the methine carbon of methyloxirane. This conclusion was supported by h.p.l.c. analysis, which showed a single peak. Similarly, the (*S*,α*R*)-diastereoisomer (**2c**), having distinctive spectroscopic properties from those of the (*R*,α*R*)-isomer (**2b**), was obtained from (*S*)-methyloxirane. The ^{1}H n.m.r. spectra of the diastereoisomers (**2b**) and (**2c**) gave different patterns for the diastereoisotopic protons of their methylene groups. The behaviour of (**1a**) contrasts with that of phenyloxirane, for which products from attack by glutathione at both carbon



Scheme 1. Reaction between ester (**2a**) and an excess of methyloxirane leading to *N*-acetyldehydroalanine and bis-(2-hydroxypropyl)sulphide

atoms of the oxiran ring have been observed[23,24] ($RSCH_2$-CHOHPh:$RSCHPhCH_2OH$ ~ 2:3 where RSH = glutathione).

The relative rates of the reactions of the enantiomeric methyloxiranes with ester (**2a**) were determined by using h.p.l.c. to measure product formation as a function of time. With a 10-fold excess of epoxide, good first-order plots for the disappearance of ester (**2a**) were obtained, from which k_R = 3.52 ± 0.10 × 10^{-4} s^{-1}, k_S = 4.12 ± 0.10 × 10^{-4} s^{-1}, and k_R/k_S = 0.85 ± 0.05 for triethylamine-catalysed reactions in methanol. This result was confirmed by carrying out a competitive reaction between ester (**2a**) and an excess of racemic methyloxirane in methanol containing triethylamine. The effect of varying the concentration of triethylamine was determined for reactions between ester (**2a**) and an excess of each methyloxirane. Without triethylamine, the extent of reaction during 4 h at 0 °C was negligible.

When a reaction lacking triethylamine was allowed to proceed for an extended period (several days at 45 °C), monitoring by ^{1}H n.m.r. spectroscopy showed the formation of *N*-acetyldehydroalanine methyl ester and (*S*,*S*)-bis-(2-hydroxypropyl) sulphide. A mechanism of formation of these substances *via* an intermediate sulphonium species is shown in Scheme 1. The formation of the sulphonium species generates base which induces ester exchange (observed by the disappearance of the OMe resonance between *ca.* 30 and 42 h), and the β-elimination shown in Scheme 1.

Reactions between L-*Valine Methyl Ester and the Enantiomers of Methyloxirane.*—The reaction between an alkyl-substituted epoxide and primary amine occurs with high regioselectivity at the primary carbon of the epoxide to give a mono-(2-hydroxyalkyl)amine.[25] This reaction is favoured by protic solvents and a 'push-pull' mechanism has been proposed.[26]

The reactions between L-valine methyl ester and (*R*)- and (*S*)-methyloxirane, respectively, proceed smoothly in methanol at 45 °C to give products of monoalkylation: (**3b**) from (*R*)- and (**3c**) from (*S*)-methyloxirane. Monitoring these reactions by ^{1}H n.m.r. spectroscopy showed that further alkylation of both (**3b**) and (**3c**) was much slower than their rate of formation (see below). These compounds were oils and so were hydrolysed to the *N*-(2-hydroxypropyl)valines (**3d**) and (**3e**), which were obtained as pure crystalline materials and were fully characterised. The ^{1}H n.m.r. spectra of esters (**3b**) and (**3c**), and the derived amino acids (**3d**) and (**3e**), respectively, proved that attack on methyloxirane by valine methyl ester occurs with high regioselectivity at the oxirane CH_2 group. Reaction of (**3c**) with

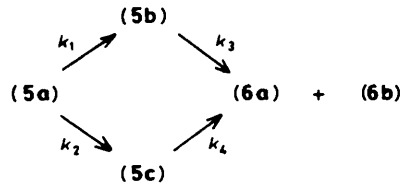
isothiocyanatobenzene[27] gave the crystalline hydantoin (**4a**), whereas (**3b**) gave an oily hydantoin (**4b**), which epimerised at C-5 when stored in ethanolic solution or when heated at 80 °C.

The relative rates of reactions of L-valine methyl esters with the enantiomers of methyloxirane were determined by monitoring the reaction between an excess of each enantiomer of methyloxirane and L-valine methyl ester in [$^2H_4$]methanol by ^1H n.m.r. spectroscopy. Good first-order kinetic plots were obtained from which k [formation of (**3b**)] = 6.40 ± 0.10 × 10^{-5} s^{-1} and k [(**3c**)] = 6.68 ± 0.12 × 10^{-5} s^{-1}. Hence, k(**3b**)/k(**3c**) = 0.96 ± 0.03. Although these reactions occur at a nucleophilic site adjacent to a chiral centre, this is insufficient to give rise to a significant degree of enantioselectivity. This was confirmed by reacting L-valine methyl ester with an excess of (±)-methyloxirane in [$^2H_4$]methanol, which gave approximately equal amounts of the products (**3b**) and (**3c**).

When L-valine methyl ester was allowed to react with an excess of (*R*)-methyloxirane in methanol at 45 °C for 3 weeks, a mixture of two products of dialkylation was obtained: (*R,R,αS*)-*NN*-bis-(2-hydroxypropyl)valine methyl ester (**3f**) (*ca.* 1 part) and the derived morpholone (**3h**) (*ca.* 3 parts). The morpholone was obtained in pure form by chromatography, whereas the ester could not be obtained completely pure because of its ready conversion into the morpholone. In a similar fashion, (*S*)-methyloxirane and L-valine methyl ester gave (*S,S,αS*)-*NN*-bis-(2-hydroxypropyl)valine methyl ester (**3g**) and morpholone (**3i**). *N*-(2-Hydroxyethyl)amino acid esters have been prepared by reacting amino acid esters with oxirane.[28] These compounds were shown to react further with oxirane in methanol to give *N*-(2-hydroxyethyl)morpholin-2-ones.[28]

Reactions between N^{α}*-Benzoyl-L-histidine Methyl Ester and the Enantiomers of Methyloxirane.*—Campbell[29] has synthesised N^{τ}-(2-hydroxypropyl)-L-histidine as a mixture of diastereoisomers *via* reduction of N^{α}-benzyloxycarbonyl-N^{τ}-(2-oxopropyl)histidine methyl ester. He required N^{τ}-(2-hydroxypropyl)histidines as standards for analysis by g.l.c. of the products of alkylation of haemoglobin by methyloxirane. We have studied the alkylation of N^{α}-benzoyl-L-histidine methyl ester (**5a**) by the enantiomers of methyloxirane and have isolated discrete diastereoisomeric products.

Dissolution of ester (**5a**) in [$^2H_4$]methanol and observation by ^1H n.m.r. spectroscopy showed that, over 4 days at 45 °C, both transesterification and exchange of the histidine 2-H occurred. In methanol solvent containing 10 mol. equiv. (*R*)-methyloxirane per mol ester (**5a**), observation by ^1H n.m.r. spectroscopy of the region δ 6.7—7.5 was possible and showed the formation at 45 °C of at least three products (new signals for histidine 5-H at δ 6.76, 6.93, and 7.34). Analysis of the reaction mixture by t.l.c. showed three new spots. From a reaction allowed to proceed for 10 h at 45 °C, the products of higher R_F were separated chromatographically and their structures were assigned as the monoalkylated histidines (**5b**) and (**5c**), respectively. It was assumed that (**5b**) is the product of alkylation at the less hindered N^{τ} of ester (**5a**), because it predominated over (**5c**) derived from alkylation at N^{π} [ratio of (**5b**):(**5c**) = *ca.* 2:1]. There is literature precedent to justify this assumption.[30] At longer reaction times, the amounts of (**5b**) and (**5c**) declined whilst a very polar product increased. From a reaction allowed to proceed for 42 h at 45 °C, a crystalline, sharp melting substance was isolated. The ^1H n.m.r. spectrum and analysis by h.p.l.c. of this 'substance' showed it to be a mixture of two histidinylimidazolium carboxylates, assigned structures (**6a**) and (**6b**). Formation of (**6a**) and (**6b**) is suggested to occur *via* the dialkylated intermediate (**6c**). Conversion of (**5b**) or (**5c**) into (**6c**) produces methoxide, which causes epimerisation at the α-CH. The carboxylate group of (**6a**) and (**6b**) probably arises by the adventitious presence of sufficient hydroxide. A comparable



Scheme 2. Consecutive, parallel reactions used for the analysis of reactions between ester (**5a**) and methyloxirane

set of results was obtained from studies of the reaction between ester (**5a**) and (*S*)-methyloxirane. The monoalkylated histidines [(**5d**) and (**5e**)] corresponding to (**5b**) and (**5c**) were obtained, as well as a crystalline mixture of dialkylated histidinylimidazolium carboxylates (**6d**) and (**6e**), which are the enantiomers of (**6a**) and (**6b**). The specific rotation of mixture (**6d**)–(**6e**) was of similar magnitude, but of opposite sign to that of the mixture of (**6a**) and (**6b**). The stereochemical designations for compounds (**6b**)—(**6e**) are (*R,R,αS*), (*R,R,αR*), (*S,S,αS*), and (*S,S,αR*), respectively.

Jones and Hysert[30] studied reactions between ester (**5a**) and alkyl halides (*e.g.* allyl bromide) and obtained products analogous to those reported here. They did not report optical rotations for their histidinylimidazolium carboxylates and their assignment of L-configuration to these products must be regarded as suspect.

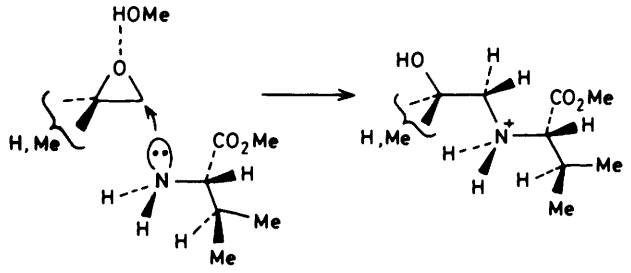
The kinetics of formation of compounds (**5b**)—(**5e**), (**6a**), (**6b**), (**6d**), and (**6e**) were determined by monitoring reactions between ester (**5a**) and an excess of an enantiomeric methyloxirane in methanol at 45 °C by ^1H n.m.r. spectroscopy. The results were analysed according to Scheme 2 and gave for (*R*)-methyloxirane, k_1 = 1.46 ± 0.04 × 10^{-5} s^{-1}, k_2 = 7.18 ± 0.46 × 10^{-6} s^{-1}, k_3 = 1.05 ± 0.06 × 10^{-5} s^{-1}, k_4 = 4.95 ± 0.54 × 10^{-5} s^{-1}; and for (*S*)-methyloxirane, k_1 = 1.41 ± 0.04 × 10^{-5} s^{-1}, k_2 = 6.93 ± 0.46 × 10^{-6} s^{-1}, k_3 = 1.00 ± 0.07 × 10^{-5} s^{-1}, k_4 = 4.77 ± 0.56 × 10^{-5} s^{-1}. Comparison of these sets of values show that the alkylation of each imidazole nitrogen shows no detectable enantioselectivity, as expected for reactions relatively distant from the chiral centre of histidine.

Discussion

From studies of the reactions of optically pure samples of (*R*)- and (*S*)-methyloxirane with amino acids (**2a**), (**3a**), and (**5a**) a series of well characterised adducts (products of monoalkylation have been isolated) [(**2a**) + (**1a**)→(**2b**); (**2a**) + (**1b**)→(**2c**); (**3a**) + (**1a**)→(**3b**); (**3a**) + (**1b**)→(**3c**); (**5a**) + (**1a**)→(**5b**) + (**5c**); (**5a**) + (**1b**)→(**5d**) + (**5e**)].

It has been shown that prolonged reactions of (**2a**) and (**3a**) with methyloxirane give products of dialkylation [*e.g.* (**2a**) + 2 (**1a**)→ see Scheme 1; (**3a**) + 2 (**1a**)→(**3f**) + (**3h**)]. The rate of further reaction of adducts (**5b**) and (**5c**) with methyloxirane to give imidazolium carboxylates (**6a**) + (**6b**) is comparable to the rate of formation of these adducts from ester (**5a**). The reactivities observed in methanol can probably be extrapolated to aqueous media. Reactions between epoxides and amines occur very much faster in protic compared with aprotic media, because of proton transfer to oxirane oxygen at the transition state for ring opening.[26]

For the amino acids (**2a**), (**3a**), and (**5a**) the relative rates of their reactions with methyloxirane in methanol at 45 °C are (**3a**) ~ (**5a**) > (**2a**). Conversion of ester (**2a**) into its thiolate anion by addition of a stoicheiometric quantity of triethylamine increases the rate of reaction with methyloxirane by at least



Scheme 3. Transition state for the reaction between ester (**3a**) and methyloxirane [either (*R*) or (*S*)]

100-fold. For (**2a**) [thiolate] and (**3a**), the products of monoalkylation with methyloxirane, *e.g.* (**2b**) from (**2a**), (**3b**) from (**3a**), are further alkylated much slower than their rate of formation. With ester (**2a**), this is because further alkylation takes place on the dialkyl sulphide (**2b**), whereas the initial alkylation involves the thiolate of (**2a**). Monoalkylation of ester (**3a**) is faster than dialkylation [*i.e.* alkylation of *e.g.* (**3b**)] probably for steric reasons. The rate of alkylation of amines is highly susceptible to the size of alkyl groups present in the amine.[31] With ester (**5a**), there are two sites of monoalkylation and the preferred one is N^{τ}, the least sterically hindered site (k^{τ}/k^{π} *ca.* 2). This ratio implies that alkylation of *e.g.* (**5c**) to give (**6a**) + (**6b**) should be significant on the timescale of formation of (**5c**), as is observed.

Two consequences of dialkylation of an amino acid by methyloxirane were observed. With ester (**2a**), the production of a sulphonium species, followed by its β-elimination, leads to a derivative of dehydroalanine (*cf.* Scheme 1). This process would cause 'sulphur-stripping' from the cysteine group(s) of a protein (*cf.* ref. 32). Alkylation of histidine N in either (**5b**) or (**5c**) generates methoxide, which causes epimerisation at the α-CH of *e.g.* (**6c**). The analogous dialkylation of a histidine of a protein by methyloxirane would generate hydroxide, which would probably be neutralised by an acidic group of the protein.

Industrially used chiral epoxides are racemates. When these enantiomers react with a protein or nucleic acid, the rates of reaction will differ. Two diastereoisomeric products of regiospecific monoalkylation will be possible. A stereochemical distinction might be made between alkylation of a biological nucleophile by an epoxide, according to whether one (metabolically generated epoxide)[33] or two (environmentally generated epoxide) diastereoisomeric product(s) of monoalkylation is (are) formed.

The kinetic data obtained for reactions of esters (**2a**), (**3a**), and (**5a**) with methyloxirane show that for each case the degree of enantioselectivity (k_R/k_S) is for (**2a**): 0.85 ± 0.05, for (**3a**): 0.96 ± 0.03 and for (**5a**) [→(**5b**) or (**5d**)]: 1.04 ± 0.05. This is not surprising for the reactions of esters (**2a**) and (**5a**), because the nucleophilic attack on the oxirane ring probably occurs relatively distant from the α-chiral centre. For ester (**3a**) the nucleophilic amino group is attached to the chiral centre. The lack of enantioselectivity observed in this reaction suggests a transition state for attack on the oxirane ring as shown in Scheme 3.* We have found that when cob(I)alamin reacts with an excess of racemic methyloxirane to give diastereoisomeric 2-hydroxypropylcobalamins, the (*R*)-isomer reacts about threefold faster than the (*S*)-isomer.[34] The chiral, reacting β-face of cob(I)alamin has 'sentinel' groups that interact significantly with methyloxirane at the transition state for the reaction.

When (±)-methyloxirane is presented to a protein, the degree of enantioselectivity will depend on the environment of the reacting amino acid residue, with a functionality deeply buried within the protein structure probably showing more discrimination than one exposed to the bulk solvent. In a physiological milieu the intrinsically greater reactivity of a particular functional group of a protein for one of the enantiomers of methyloxirane can only be expressed as the predominant formation of one diastereoisomeric adduct, if the molar ratio of (±)-methyloxirane to protein is >2. Previous studies of reactions between epoxides and proteins[35] or nucleic acids[36] have not discussed the importance of the above stereochemical considerations.

In the reaction between a protein and methyloxirane the regioselectivity could differ from that observed ($k_{CH_2}/k_{CH} \geq 20$) with esters (**2a**), (**3a**), and (**5a**). There could be an increased extent of attack at oxirane CH either because of protonation of the oxirane O prior to nucleophilic attack, or because the oxirane binds to the protein in such a way that sterically attack at CH is favoured.

Experimental

Solvents for preparative work were either AnalaR grade or redistilled laboratory reagents. For h.p.l.c., solvents from Rathburn Chemicals were used.

M.p.s were determined using either a Kofler block or an electrothermal apparatus and are uncorrected. Optical rotations were measured with a Bendix NPL automatic polarimeter 143D. Unless stated otherwise, ^{1}H n.m.r. spectra were recorded at 220 MHz with a Perkin-Elmer R34 instrument; tetramethylsilane was used as an internal standard in organic solvents and 3-trimethylsilylpropionic acid sodium salt in D_2O. I.r. spectra were obtained with a Perkin-Elmer 257 instruement. H.p.l.c. analyses were performed using a Waters instrument (equipped with variable-wavelength u.v. detector) and a Partisil 5 C8 (reverse phase) column (100 mm × 9.4 mm i.d.), eluting with methanol–water (1:4). Electron-impact mass spectra were obtained with a Kratos MS 80 instrument.

N-Acetyl-L-cysteine methyl ester (**2a**) was prepared by esterification of *N*-acetyl-L-cysteine with 0.2M-HCl in methanol. It was purified by recrystallisation from ether, followed by sublimation, m.p. 80—81 °C (lit.,[37] 79—80 °C); $\delta_H(D_2O)$ 2.06 (s, MeCO), 2.98 (d, CH_2), 3.79 (s, MeO), and 4.68 (t, CH).

N^{α}-Benzoyl-L-histidine methyl ester was prepared as described.[38]

The optically active methyloxiranes were prepared as described.[39]

(S,αR)-N-*Acetyl*-S-(2-*hydroxypropyl*)-L-*cysteine Methyl Ester* (**2c**).—To a stirred solution of *N*-acetyl-L-cysteine methyl ester (0.1 g, 6 × 10^{-4} mol) in dry methanol (5 cm^3) under nitrogen was added triethylamine (0.064 g, 6.4 × 10^{-4} mol). A pH change from 6.49 to 10.25 was observed. After 30 min the solution was cooled to 0 °C and (−)-(*S*)-methyloxirane (0.18 g, 3.1 × 10^{-3} mol) was added. T.l.c. analysis (silica gel; CH_2Cl_2–CH_3OH, 4:1) showed the reaction to be complete within *ca.* 4 h. The volatile components of the reaction were evaporated under reduced pressure giving an oil (0.119 g, 89%). This was pure (*S*,α*R*)-*N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine methyl ester (**2c**) according to its ^{1}H and ^{13}C n.m.r. spectra. An analytical sample of the ester (**2c**) was obtained by Kugelröhr distillation: oil, b.p. 90 °C at 0.01 mmHg; $\delta_H(CDCl_3)$ 1.21 (d, *Me*CH), 2.04 (s, MeCO), 2.50 (dd, *J* 13.8 and 7.2 Hz, H of cys CH_2), 2.70 (dd, *J* 13.8 and 4.2 Hz, H of cys CH_2), 3.0 (d, CH_2CHOH), 3.76 (s, MeO), 3.87 (m, CHOH), and 4.83 (m,

* *Note added in proof:* We have recently found that ester (**3a**) reacts with excess of t-butyloxirane to give a 3:2 ratio of diastereoisomeric monoadducts.

CHNH); δ_C($CDCl_3$) 22.26 (*Me*CH), 23.23 (*Me*CO), 35.18 and 42.40 (2 × CH_2), 52.29 (MeO), 52.72 (CHNH), 66.07 (CHOH), 169.63 and 170.7 p.p.m. (2 × CO); $\nu_{max.}$(film) 3 300m, 2 940w, 1 745s, 1 660s, 1 550s, and 1 440m cm^{-1}; M^+, 217.0776 ($C_9H_{15}NO_3S$ requires *M*, 217.0772); $[\alpha]_D^{27}$ −20.1° (*c* 2.98 in MeOH).

(R,αR)-N-*Acetyl*-S-(2-*hydroxypropyl*)-L-*cysteine Methyl Ester* (**2b**).—This was prepared from *N*-acetyl-L-cysteine methyl ester and (*R*)-methyloxirane in the manner described for ester (**2c**) to give essentially pure ester (**2b**) as an oil (98%). An analytical sample was obtained by Kugelröhr distillation: oil, b.p. 90 °C at 0.01 mmHg; δ_H($CDCl_3$) 1.23 (d, *Me*CH), 2.05 (s, MeCO), 2.46 (dd, *J* 13.8 and 8.8 Hz, H of cys CH_2S), 2.76 (dd, *J* 13.8 and 3.2 Hz, H of cys CH_2S), 2.96 (dd, *J* 14.0 and 5.8 Hz, H of CH_2CHOH), 3.04 (dd, *J* 14.0 and 4.6 Hz, H of CH_2CHOH), 3.77 (s, MeO), 3.85 (m, CHOH), and 4.86 (m, CHNH); δ_C($CDCl_3$) 22.32 (*Me*CH), 23.23 (*Me*CO), 35.24 and 42.40 (2 × CH_2), 52.35 (MeO), 52.78 (CHNH), 66.37 (CHOH), 169.63 and 170.78 p.p.m. (2 × CO); $\nu_{max.}$(film) 3 300m, 2 940w, 1 745s, 1 660s, 1 550s, and 1 440m cm^{-1}; M^+, 217.0784 ($C_9H_{15}NO_3S$ requires *M*, 217.0772); $[\alpha]_D^{25}$ −21.8° (*c* 2.94 in MeOH).

Prolonged Reaction of N-*Acetyl*-L-*cysteine Methyl Ester with* (S)-*Methyloxirane: Evidence for the Formation of* N-*Acetyldehydroalanine Methyl Ester and* (S,S)-*Bis*-(2-*hydroxypropyl*) *Sulphide*.—*N*-Acetyl-L-cysteine methyl ester was reacted with a five-fold excess of (*S*)-methyloxirane in [$^2H_4$]methanol at 45 °C, monitoring by ^1H n.m.r. spectroscopy. The solvent was evaporated and the resulting oil was redissolved in $CDCl_3$. Singlets at δ 2.13 ($COCH_3$), 5.89, and 6.59 (H_2C=C) were attributed to *N*-acetyldehydroalanine methyl ester.[40] Reactions performed in dry methanol also showed the methyl ester resonance at δ 3.85. Resonances at δ 1.25 (d, 2 × CH_3), 2.51 (dd, 2 × CH_2), 2.77 (dd, 2 × CH_2), and 3.89 (m, 2 × CHOH) were assigned to (*S,S*)-bis-(2-hydroxypropyl) sulphide.

(S,αS)-N-(2-*Hydroxypropyl*)-L-*valine Methyl Ester* (**3c**).—L-Valine methyl ester [prepared by neutralising its hydrochloride (0.638 g, 3.81 × 10^{-3} mol) with sodium methoxide] in dry methanol (5 cm^3) under nitrogen was treated with (*S*)-methyloxirane (0.22 g, 257 μl, 3.81 × 10^{-3} mol). The reaction was incubated at 45 °C for 108 h, when t.l.c. analysis showed it to be complete. Evaporation gave *ca.* 95% pure ester (**3c**) as a pale yellow oil (0.461 g, 64%), δ_H($CDCl_3$) 0.94 (d, Me), 0.96 (d, Me), 1.14 (d, *Me*CH), 1.94 (m, Me_2CH), 2.11 (dd, *J* 11.65 and 9.88 Hz, H of CH_2), 2.83 (dd, *J* 11.65 and 2.77 Hz, H of CH_2), 2.98 (d, CHNH), 3.67 (m, CHOH), and 3.72 (s, MeO). This compound was characterised by conversion into the hydantoin (**4a**) and by hydrolysis to (*S*,α*S*)-*N*-(2-hydroxypropyl)-L-valine (**3e**) [see below].

(R,αS)-N-(2-*Hydroxypropyl*)-L-*valine Methyl Ester* (**3b**).—This was prepared from L-valine methyl ester and (*R*)-methyloxirane in the manner described for ester (**3c**) to give *ca.* 95% pure ester (**3b**) as a pale yellow oil (64%): δ_H 0.97 (d, Me_2), 1.14 (d, *Me*CH), 1.95 (m, Me_2CH), 2.46 (d, CH_2, *J* 6.0 Hz), 3.04 (d, CHNH), 3.74 (s, MeO), and 3.75 (m, CHOH). This compound was characterised by hydrolysis to (*R*,α*S*)-*N*-(2-hydroxypropyl)valine (**3d**) [see below]. It gave an oily hydantoin (**4b**) on treatment with isothiocyanatobenzene in ethanol, δ_H($CDCl_3$) 0.96 (d, *Me*CH), 1.20 (d, CH_3), 1.23 (d, CH_3), 2.47 (m, Me_2CH), 3.25 (dd, *J* 14.6 and 9.8 Hz, H of CH_2), 4.28 (m, CHOH), 4.31 (dd, *J* 14.6 and 2.4 Hz, H of CH_2), 4.48 (d, CHNH) and ArH resonances; M^+, 292.1240 ($C_{15}H_{20}N_2O_2S$ requires *M*, 292.1245) [see data for crystalline hydantoin (**6a**) below].

1-[(S)-2-*Hydroxypropyl*]-5-(S)-*isopropyl*-3-*phenyl*-2-*thiohydantoin* (**4a**).—(*S*,α*S*)-*N*-(2-Hydroxypropyl)-L-valine methyl ester (**3c**) (0.1 g, 5.29 × 10^{-4} mol) in dry ethanol (1 cm^3) was treated with isothiocyanatobenzene (0.143 g, 1.05 × 10^{-3} mol). The mixture was stirred at room temperature for 1 h and then heated at 40 °C for 15 min. On cooling, crystallisation of hydantoin (**4a**) occurred. An analytical sample of the title compound was obtained by recrystallisation from ethanol: needles (36%), m.p. 146—148 °C; R_F 0.61 (silica gel F_{254}; CH_2Cl_2–MeOH, 10:1); $[\alpha]_D^{27}$ −1.4° (*c* 2.0 in MeOH); δ_H($CDCl_3$) 0.96 (d, *Me*CH), 1.26 (d, CH_3), 1.29 (d, CH_3), 2.25 (s, OH), 2.48 (m, Me_2CH), 3.38 (dd, *J* 14.4 and 3.0 Hz, H of CH_2), 4.19 (m, CHOH), 4.26 (d, CHNH), 4.57 (dd, *J* 14.4 and 7.8 Hz, H of CH_2), and ArH resonances; $\nu_{max.}$(Nujol) 3 495s, 3 440m, 3 050m, 1 725s, 1 600w, 1 425m, 1 295s, 878w, 750s, and 694m cm^{-1}; M^+ 292 (Found: C, 61.45; H, 6.9; N, 9.5; S, 11.0; $C_{12}H_{15}N_2OS$ requires C, 61.6; H, 6.9; N, 9.6; S, 10.95%).

(S,αS)-N-(2-*Hydroxypropyl*)-L-*valine* (**3e**).—This was obtained by hydrolysis of ester (**3c**) in water at room temperature for 6 days. Evaporation and recrystallisation of the residue from aqueous acetone gave the title compound as rectangular crystals: sublimes at 165—166 °C, m.p. 231—232 °C (in closed capillary); δ_H (360 MHz; D_2O) 1.01 (d, CH_3), 1.05 (d, CH_3),1.22 (d, *Me*CH), 2.24 (m, Me_2CH), 2.92 (dd, *J* 12.8 and 9.9 Hz, H of CH_2), 3.15 (dd, *J* 12.8 and 2.7 Hz, H of CH_2), 3.52 (d, *J* 5.0 Hz, CHND) and 4.10 (m, CHOD); δ_C(D_2O) 18.1 and 18.7 (Me_2), 20.5 (*Me*CH), 54.3 (CH_2), 63.4 (CHND), 68.7 (CHOD), and 173.3 p.p.m. (CO); $\nu_{max.}$(Nujol) 3 450s, 3 320s, 2 700—2 200br, 1 610s, 1 560s, 1 390s, 1 334s, and 1 030s cm^{-1}; *m/z* (FAB) 214 (*M* + K)$^+$, 198 (*M* + Na)$^+$, and 176 (*M* + H)$^+$; $[\alpha]_D^{27}$ −0.32° (*c* 1.55 in MeOH) (Found: C, 54.9; H, 9.7; N, 7.95. $C_8H_{17}NO_3$ requires C, 54.85; H, 9.75; N, 8.0%).

(R,αS)-N-(2-*Hydroxypropyl*)-L-*valine* (**3d**).—This was obtained by hydrolysis of ester (**3b**) and was recrystallised from aqueous acetone: sublimes at 178—179 °C, m.p. 237—238 °C (in closed capillary); δ_H (360 MHz; D_2O) 1.01 (d, CH_3), 1.05 (d, CH_3), 1.22 (d, *Me*CH), 2.24 (m, Me_2CH), 2.92 (dd, *J* 12.7 and 10.0 Hz, H of CH_2), 3.15 (dd, *J* 12.7 and 2.7 Hz, H of CH_2), 3.52 (d, *J* 4.9 Hz, CHND), and 4.10 (m, CHOD); δ_C(D_2O) 18.09 and 18.71 (Me_2), 20.5 (*Me*CH), 54.3 (CH_2), 63.4 (CHND), 68.7 (CHOD), and 173.2 p.p.m. (CO); $\nu_{max.}$(Nujol) 3 450s, 3 320s, 2 700—2 200br, 1 610s, 1 560s, 1 390s, 1 334s, and 1 030s cm^{-1}; *m/z* (FAB) 198 (*M* + Na)$^+$ and 176 (*M* + H)$^+$; $[\alpha]_D^{26}$ −9.0° (*c* 1 in MeOH) (Found: C, 54.8; H, 9.8; N, 7.8. $C_8H_{17}NO_3$ requires C, 54.85; H, 9.75; N, 8.0%).

(R,R,αS)-NN-*Bis*-(2-*hydroxypropyl*)*valine Methyl Ester and* δ-*Lactone* [*Ester* (**3f**) *and Morpholone* (**3h**) *respectively*].—L-Valine methyl ester was reacted with a 10-fold excess of (*R*)-methyloxirane in methanol for 3 weeks at 45 °C. The product mixture was separated by preparative t.l.c. [silica gel; elution with CH_2Cl_2–methanol (10:1)] to give (i) (*R,R*,α*S*)-*NN*-bis-(2-hydroxypropyl)valine methyl ester (**3f**) as an oil (40%), b.p. *ca.* 100 °C at 0.1 mmHg; δ_H($CDCl_3$) 0.87 and 1.06 (2 × d, Me_2CH), 1.14 (d, 2 × *Me*CHOH), 2.04 (m, Me_2CH), 2.48 (m, 2 × $CHOHCH_2$), 2.88 (d, α-CH), 3.71 (s, OMe), and 3.86 (m, 2 × CHOH); the c.i. mass spectrum (reagent ammonia) of the trideuteriomethyl ester of (**3f**) obtained from a reaction run in [$^2H_4$]methanol showed MH^+ at 251 (most abundant peak of mass > 18).This product was contaminated with morpholone (**3h**). There followed (ii) (*R,R*,α*S*)-*NN*-bis-(2-hydroxypropyl)-valine δ-lactone (**3h**) (60%), b.p. *ca.* 100 °C at 0.1 mmHg; δ_H($CDCl_3$) 1.01 and 1.14 or 1.16 (2 × d, Me_2CH), 1.16 or 1.14 (d, *Me*CHOH), 1.33 (d, *Me*CHOCO), 2.0 (m, Me_2CH), 2.50 (m, $CHOHCH_2$ and one of ring CH_2), 3.14 (m, one of ring CH_2 and α-CH), 3.88 (m, CHOH), and 4.60 (m, MeCHOCO); δ_C($CDCl_3$) 17.48, 18.79, 20.01, 20.82, 32.39, 56.65, 65.22, 65.87, 70.89, 74.04, and 169.92 p.p.m.; $\nu_{max.}$(film) 3 442br,

2 965s, 2 935s, 2 878s, 2 820m, 1 725s, 1 460s, 1 369s, 1 275s, 1 223s, 1 150s, 1 052s, 990m, and 950m cm^{-1}; *m/z* (e.i.) 215.1530 (M^+. $C_{11}H_{21}NO_3$ requires *M*, 215.1521); $[\alpha]_D^{27}$ +60° (*c* 0.7 in methanol).

(S,S,αS)-NN-*Bis*-(2-*hydroxypropyl*)*valine Methyl Ester and* δ-*Lactone* [*Ester* (**3g**) *and Morpholone* (**3i**), *respectively*].—L-Valine methyl ester was reacted with (*S*)-methyloxirane, following the procedure given in the previous section, to give (**3g**) and (**3i**): (**3g**), oil, b.p. *ca.* 70 °C at 0.04 mmHg; $\delta_H(CDCl_3)$ 0.88 and 1.10 (2 × d, Me_2CH), 1.13 (d, 2 × *Me*CHOH), 2.09 (m, Me_2CH), 2.45 (dd, 2 × one of $CHOHCH_2$, *J* 9.9 and 13.8 Hz), 2.89 (dd, 2 × one of $CHOHCH_2$, *J* 3.3 and 13.8 Hz), 3.72 (s, OMe), 3.84 (m, 2 × *CH*OH), and 4.75 (m, α-CH); (**3i**), oil (72%), b.p. *ca.* 70 °C at 0.04 mmHg; $\delta_H(CDCl_3)$ 1.05 and 1.09 (2 × d, Me_2CH), 1.18 (d, *Me*CHOH), 1.39 (d, *Me*CHOCO), 2.20 (m, Me_2CH), 2.38 (dd, one of $CHOHCH_2$, *J* 10.0 and 12.9 Hz), 2.76 (dd, one of $CHOHCH_2$, *J* 3.2 and 12.9 Hz), 2.83 (m, ring CH_2), 3.0 (m, α-CH), 3.82 (m, *CH*OH), and 4.74 (m, MeC*H*OCO); $\delta_C(CDCl_3)$ 18.20, 18.86, 19.70, 20.10 (4 × Me), 30.7 ($CHMe_2$), 50.1, 60.52 (CH_2CHOH and ring CH_2), 63.2 (α-CH), 69.9, 72.2 (CHOH and ring *C*HMe), and 170 p.p.m. (CO); $\nu_{max.}$(film) 3 440br, 2 970s, 2 935s, 2 878s, 2 825m, 1 725s, 1 457s, 1 372s, 1 275s, 1 220s, 989m, and 945m cm^{-1}; *m/z* (e.i.) 215.1521; $[\alpha]_D^{24}$ −10.77° (*c* 0.7 in methanol).

(S,S,αR/αS)-N$^{\alpha}$-*Benzoyl*-N$^{\pi}$N$^{\tau}$-*bis*-(2-*hydroxypropyl*)-L-*histidinylimidazolium Carboxylate* [(**6d**) + (**6e**)].—*N*-Benzoyl-L-histidine methyl ester (0.5 g, 1.82 × 10^{-3} mol) in dry methanol (5 cm^3) was treated with (*S*)-methyloxirane (0.53 g, 9.1 × 10^{-3} mol). The mixture was incubated at 45 °C for 4 days. Removal of the solvent gave a pale yellow oil that was crystallised from acetone. Recrystallisation from methanol–ether gave the imidazolium carboxylates [(**6d**) + (**6e**)] (0.418 g, 61%) as crystals, m.p. 146—147 °C; δ_H (360 MHz; D_2O) 1.086 (d, *Me*CH), 1.113 (d, *Me*CH), 1.26 (d, *Me*CH), 1.28 (d, *Me*CH), 3.21 (dd, H of his CH_2), 3.45 (2 × t, H of his CH_2), 3.94—4.04 [m, $CH(OH)CH_2$], 4.70 (m, CHND), 7.34 (s, 5-H), 8.77 (s, 2-H), and Ar resonances; $\delta_C(D_2O)$ 19.55 and 19.9 (Me), 27.1 (his CH_2), 53.8 and 56.3 (CH_2), 54.4 (CHND), 66.3 and 66.5 (CHOD), 121.9 (C-5), 127.9 (ArC_{meta}), 129.6 (ArC_{ortho}), 132.7 (C-4), 133.2 (ArC_{para}), 133.7 (C-2), 137.3 (ArC-1), 170.7 (Ph*C*O), and 176.7 p.p.m. (CO_2^-); $\nu_{max.}$(Nujol) 3 350m, 3 240m, 3 090m, 2 845s, 1 680m, 1 595s, 1 540m, 1 490m, 775m, and 690m cm^{-1}; *m/z* (e.i.) 375.1799 (M^+. $C_{19}H_{25}N_3O_5$ requires *M*, 375.1794); $[\alpha]_D^{17}$ +30.8° (*c* 2.4 in MeOH).

(R,R,αR/αS)-N$^{\alpha}$-*Benzoyl*-N$^{\pi}$N$^{\tau}$-*Bis*-(2-*hydroxypropyl*)-L-*histidinylimidazolium Carboxylate* [(**6a**) + (**6b**)].—This mixture was prepared in 66% yield from *N*-benzoyl-L-histidine methyl ester and (*R*)-methyloxirane in the manner described for imidazolium carboxylate mixture [(**6a**) + (**6b**)], m.p. 145.5—146.5 °C; δ_H, δ_C, $\nu_{max.}$ values identical to those reported for [(**6d**) + (**6e**)]; $[\alpha]_D^{15}$ −27.7° (*c* 2.4 in MeOH); *m/z* (FAB) 398 $(M + Na)^+$, 376 $(M + H)^+$, 332 $(MH - CO_2)^+$, 330 $(MH - HCO_2H)^+$, 274 ($MH - CO_2$ − MeCOHCH$_2$), 226, 199, and 105 $(PhCO)^+$.

(S,αS)-N$^{\alpha}$-*Benzoyl*-N$^{\tau}$-(2-*hydroxypropyl*)-L-*histidine Methyl Ester* (**5d**) *and its* N$^{\pi}$-*Isomer* (**5e**).—These compounds were obtained from reactions between N$^{\alpha}$-benzoyl-L-histidine methyl ester and (*S*)-methyloxirane in methanol at 45 °C, which were monitored by t.l.c. and stopped before an appreciable quantity of the imidazolium carboxylate mixture [(**6d**) + (**6e**)] had formed. They were separated by p.l.c. (silica gel PF_{254}; multiple development with CH_2Cl_2–MeOH, 16:1) to give esters (**5d**) and (**5e**) as oils: (**5d**), δ_H (400 MHz; D_2O) 1.05 (d, *Me*CH), 3.13 (dd, *J* 14.6 and 8.6 Hz, H of his CH_2), 3.22 (dd, *J* 14.6 and 5.8 Hz, H of his CH_2), 3.78 (s, MeO), 3.87 (dd, *J* 14.4 and 6.6 Hz, H of CH_2), 3.97 (dd, *J* 14.4 and 3.8 Hz, H of CH_2), 4.02 (m, *CH*OD), 4.87 (dd, *J* 8.6 and 5.8 Hz, CHND), 6.99 (s, 5-H), 7.58 (s, 2-H), and ArH resonances; $\nu_{max.}$(film) 3 330m, 2 935m, 1 740s, 1 650s, 1 604s, 1 580s, 1 219m, and 1 178m; *m/z* (e.i.) 331 (M^+, 41.6%), 272 (24.4), 226 (15.4), 210 (29.6), 139 (42.0), 121 (13.6), 105 (100), and 77 (62.8); (**5e**), δ_H (400 MHz; D_2O) 1.20 (d, *Me*CH), 3.24 (dd, *J* 15.6 and 9.8 Hz, H of his CH_2), 3.41 (dd, *J* 15.6 and 5.0 Hz, H of his CH_2), 3.79 (s, OMe), 3.94 (dd of CH_2), 4.10 (m, *CH*OD), 4.11 (dd, H of CH_2), 4.94 (dd, *J* 9.8 and 5.0 Hz, CHND), 6.93 (s, 5-H), and ArH resonances; $\nu_{max.}$(film) 3 330s, 3 360m, 2 925m, 1 740s, 1 650s, 1 605s, 1 580s, 1 224m, 719m, and 698 cm^{-1}; *m/z* (e.i.) 331 (M^+, 1.6%), 299 (3.5), 272 (1.4), 210 (20.0), 139 (34.8), 121 (12.8), 105 (100), and 77 (68.0).

Kinetic Studies of Reactions between Enantiomeric Methyloxiranes and Esters (**2a**), (**3a**), *and* (**5a**).—Reactions were studied quantitatively in dry methanol at 0 ± 0.2 °C [for (**2a**)] and 45 ± 0.1 °C [for (**3a**) and (**5a**)]. The initial concentrations of esters were 0.1M (**2a**), 0.25M (**3a**), and 0.13M (**5a**). For all reactions the initial concentration of epoxide was 10 times that of the ester. Triethylamine (0.11M) was present in reactions of ester (**2a**). For the reactions of esters (**3a**) and (**5a**) ^1H n.m.r. spectroscopy was used to monitor the reactions in regions of the spectrum where there was no interference from solvent or epoxide absorptions. For reactions of ester (**2a**) analysis by h.p.l.c. was used. With esters (**2a**), (**3a**), and (**5a**), pseudo-first-order rate constants were obtained by least-squares analysis of the data by a desk computer. The reactions of ester (**5a**) with the enantiomeric methyloxiranes were analysed as consecutive, parallel, pseudo-first-order reactions (*cf.* Scheme 3), measuring the rate of change of the species (**5a**) and products.[41,*]

By the procedure described, qualitative information about dialkylation of esters (**2a**) and (**3a**) was obtained and is discussed in the text.

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