Preliminary Communication

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Preparation of cysteine adducts by regioselective ring-opening reactions of phenyloxirane

Abstract: Regioselective ring-opening reactions of phenyloxirane by protected cysteine are described, which enable the synthesis of pure regioisomeric cysteine adducts required for bioanalytical studies on adducts of protein with styrene. The reaction catalyzed by tris(pentafluorophenyl) borane proceeds regioselectively and stereospecifically to give protected *S*-(2-hydroxy-1-phenylethyl)cysteine (α-adduct) with the inversion of configuration at the α-carbon. By contrast, when the same reaction is catalyzed by tetraalkylammonium fluorides, *S*-(2-hydroxy-2-phenylethyl)cysteine (β-adduct) is formed predominantly, and a compete racemization is observed.

Keywords: cysteine adducts; oxirane ring opening; protein adducts; styrene; styrene oxide.

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Adducts with blood proteins, mainly albumin and globin, are formed by reactions of numerous xenobiotics entering human body and/or their electrophilic metabolites. They found applications in biological monitoring of cumulative exposure to environmental and occupational mutagens and carcinogens [1, 2]. The main site of attack in both globin and albumin is the highly nucleophilic SH group of cysteine [3]. For bioanalytical studies on protein adducts, *S*-substituted cysteines are needed as analytical standards as well as other research tools. The nucleophilic cysteine SH group of glutathione also plays a fundamental role to prevent cell damage by various electrophiles as well as by free radicals [4, 5]. In connection with our ongoing research on protein adducts, we needed to prepare cysteine adducts of phenyloxirane (styrene oxide, **1**), a mutagenic and carcinogenic metabolite of styrene. The direct reaction of L-cysteine (**2**) with **1** in strongly alkaline solution (EtOH/EtONa) afforded a 1:2 mixture of *S*-(2-hydroxy-1-phenylethyl)cysteine (**3**) and *S*-(2-hydroxy-2-phenylethyl)cysteine (**4**) as reported by Cundari et al. [6] (Scheme 1). These authors also attempted to achieve the regioselectivity of this reaction by using unprotected 2-bromo-2-phenylethanol and 2-bromo-1-phenylethanol as synthetic equivalents of **1**. However, under the alkaline reaction conditions, these bromohydrines underwent dehydrobromination to give **1** so that mixtures **3** and **4** were always formed. This mixture was converted to (*t*-butoxycarbonyl)amino (*N*-BOC) derivatives **3a** and **4a** [6]. Small samples of **3** and **4** were previously obtained by the direct reaction of **1** with cysteine. This reaction gave a 2:1 mixture of **3** and **4**, each of them consisting of two diastereomers. Individual isomers were then separated by high-performance liquid chromatography. However, it was not possible to scale up this procedure to obtain sufficient quantities of the individual products [7]. As a rule, a direct attack by nucleophiles proceeds on the less substituted (β) carbon of the oxirane ring. By contrast, in an acidic environment, where the oxirane oxygen is either protonated or coordinated to a Lewis acid, the nuclephilic attack is redirected to the more substituted (α) carbon [8]. However, **1** shows a rather anomalous regioselectivity of ring-opening reactions. In reactions of **1** with thiols in slightly alkaline aqueous solutions [9, 10] or in ionic liquids [11, 12] mainly but not exclusively, the products of α-attack were observed. However, with a wide range of substituted oxiranes, the same reactions gave exclusively products of β-attack. This may be due to a combination of the resonance stabilization of α-carbocation by adjacent aromatic ring and of the electron withdrawing effect of the phenyl group. The resonance stabilization of the carbocation at α-position is expected to play a crucial role if the reaction proceeds by S_N1 mechanism, which should be preferred in acidic solutions. However, when **1** was allowed to react with **2** in acidic aqueous solutions at pH

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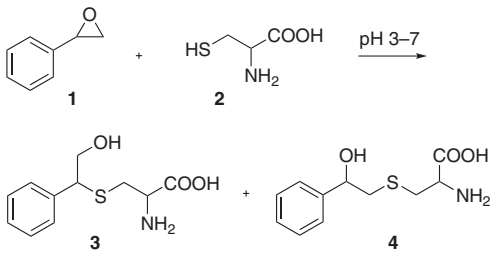


Scheme 1

ranging from 3 to 6, we observed the formation of both adducts **3** and **4** in the ratio ranging from 2:1 to 3:4. Less α-adduct was formed with decreasing pH but the ratio did not differ very much from that observed at pH 7. Apparently, at this pH range, the reaction does not proceed by S_N1 mechanism. The reaction is shown in Scheme 1.

Observed minor changes in regioselectivity may be attributed to decreasing ratio of S^-/SH in cysteine (Table 1). Ring-opening reactions of **1** were further tested with two protected cysteine derivatives, namely, *N*-*t*-butoxycarbonyl- and *N*-benzyloxycarbonylcysteine methyl esters, **2a** and **2b**, respectively. Ring opening proceeding in the presence of *N*-bromosuccinimide (5 mol%), which for various benzenethiols and alkanethiols was reported to give mainly products of α-attack [13], gave low yields of expected products with the protected cysteines **2a** and **2b**. These products were accompanied by the corresponding cysteine derivatives (Table 2).

In an attempt to improve regioselectivity of the reaction and/or yields of the products, we further tested several combinations of the activating agent and solvent, such as Et_3N, $LiClO_4$, or $ZnCl_2$ in dichloromethane or acetonitrile. Products of the α-attack always prevailed, and the yields were always low due to both low conversion and by-product formation. Efficient and regioselective cleavage of **1** via α-attack by benzenethiol, aniline, allyl alcohol, and propargyl alcohol was reported in the presence of tris(pentafluorophenyl)borane as a Lewis acid catalyst [14]. This process proved to be applicable for the

Table 1: Conversion of **2** and product ratio (**3:4**) after 3 h of reaction with **1** in neutral and acidic aqueous solutions as determined by LC/MS.

Acidity	Conversion of 2 (%)	Product ratio 3:4
pH 3	0.9	74:26
pH 4	1.0	73:27
pH 5	5.1	67:33
pH 6	41	66:34
pH 7[a]	100	70:30

[a]Reaction at pH 7 was performed with both *R*- and *S*-enantiomers of **1**.

Table 2: Reactions of racemic **1** with protected cysteines 2a and 2b.

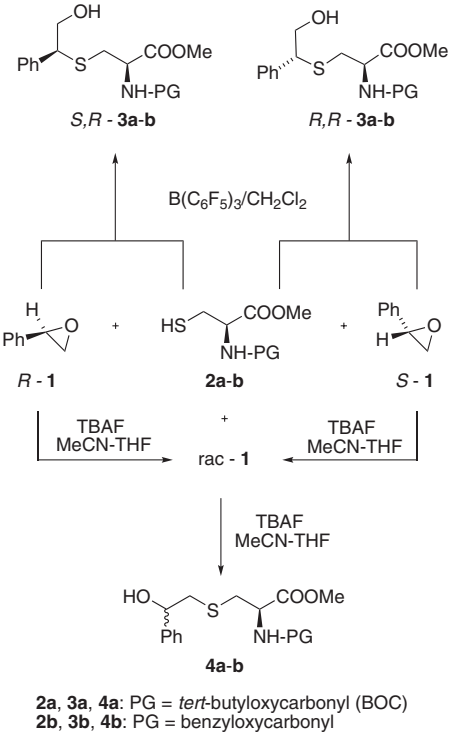
Cysteine derivative	Reagent	Conditions	Yield of protected 3+4[a]	Ratio of protected 3:4[b]
2a	NBS/Et_3N	CH_3CN/rt	10%	**3a** only
2b	NBS/Et_3N	CH_3CN/rt	20%	**3b** only
2a	$B(C_6F_5)_3$	CH_2Cl_2/rt	20%	**3a** only[c]
2b	$B(C_6F_5)_3$	CH_2Cl_2/rt	40%	**3b** only[c]
2a	KF	CH_3CN/rt	traces	–
2a	TBAF	CH_3CN/rt	60%	1:10[c]
2b	TBAF	CH_3CN/rt	65%	1:10[c]
2a	TMAF	CH_3CN/rt	61%	1:9
2a	Et_3N	CH_3CN/rt	20%	1:3
2a	$ZnCl_2$	CH_3CN/rt	<5%	**3a** only
2b	NBS	CH_3CN/rt	<10%	**3b** only

[a]**3a+4a** or **3b+4b**.

[b]**3a:4a** or **3b:4b**.

[c]This reaction was performed with racemic **1** as well as with its pure enantiomers.

reaction with **2a** and **2b**, which afforded the corresponding protected isomer **3** exclusively (Scheme 2). However, the reaction proceeded slowly, and conversion was still incomplete after 3 days at room temperature (Table 2). Increasing the reaction temperature did not lead to any improvement of the yield but resulted in lower product purity. Reaction with **2b** afforded a better yield than that



Scheme 2

with **2a** (Table 2). Acid-catalyzed ring-opening reactions of substituted oxiranes generally proceed by S_N1 mechanism, resulting in the racemization of the products [8]. Nevertheless, we observed stereospecific ring opening with the inversion of configuration at the α-carbon, so that the reaction of pure (*R*)-**1** gave exclusively *S,R*- **3a** or *S,R*-**3b** from **2a** and **2b**, respectively. In a similar way, pure substrate (*S*)-**1** was transformed into the diastereomer *R,R*-**3a** or *R,R*-**3b** (Scheme 2). Absolute configuration of the products was confirmed by the comparison of ^{13}C NMR shifts with those of the analogous *N*-acetyl derivatives [15].

The inversion of configuration is characteristic for S_N2 reactions. Therefore, it can be concluded that, at least under the conditions used, oxirane ring opening catalyzed by $B(C_6F_5)_3$ proceeds by S_N2 mechanism, without formation of an intermediary carbocation but probably with a significant weakening of the C_α-O bond of **1** in the transition state due to the binding interaction of the Lewis acid with the oxirane oxygen.

Eventually, experiments with tetrabutylammonium fluoride (TBAF) as a base afforded predominantly products of β-attack. Thus, the reaction of **1** with **2a** in the presence of 10 mol% of TBAF led to a 10:1 mixture of protected products **4a** and **3a**. After chromatographic separation, pure protected isomer **4a** was obtained in a satisfactory yield (Table 2). In fact, the SH group of cysteine can be easily deprotonated (pK_a=8.2). Hence, the observed exceptional regioselectivity indicates that TBAF does not serve solely as a base because other bases direct the SH-attack mainly to the α-carbon. A commercially available solution of TBAF in THF used in these experiments contained approximately 5% of water so that the nucleophilic attack could be redirected to the β-carbon of **1** by the formation of complexes held together by hydrogen bonding. 'Anhydrous' TBAF was reported to contain about 0.3 equivalents of water [16]. Attempts to remove this residual water results in the degradation of TBAF due to Hofmann elimination [17]. Therefore, we also used tetramethylammonium fluoride (TMAF) as a catalyst that, unlike TBAF, can be obtained in an anhydrous form. The result, however, was very similar to that obtained with TBAF. Unlike the reaction catalyzed by Lewis acids, base-catalyzed reaction should be stereospecific so that a single diastereomer of **4a** and **4b** should be obtained from each enantiomer of **1**. However, we observed the formation of 1:1 diastereomer mixtures of **4a** and **4b** in the reaction with pure enantiomers (*R*)-**1** and (*S*)-**1** as well as with racemic **1**. Control experiments showed that in the presence of enantiomeric **1**, the products rapidly racemized in the presence of TBAF. The deprotection of **3a** and **4a** was conducted by treatment of trifluoroacetic acid (TFA) in dichloromethane for 4 h at room temperature. As expected, acid-catalyzed racemization was observed after the deprotection of pure diastereomers of **3a**. Attempts to remove the Z-protecting group of **3b** and **4b** by Pd-catalyzed hydrogenolysis were unsuccessful probably because of catalyst poisoning by sulfur-containing substrates. This protecting group can be removed by reaction with trimethylsilyl iodide (TMSI) [18]. With **3b**, this reaction followed by alkaline ester hydrolysis afforded a mixture of **3** and **4**. Pure diastereomers *R,R*- and *S,R*-**3b** gave predominantly but not exclusively *R,R*- and *S,R*-diastereomers, respectively, accompanied with a mixture of *R,R*- and *S,R*-diastereomer of **3**, as determined by LC/MS analysis comparing retention times and mass spectra with those of the products obtained by the reaction of pure enantiomers of **1** with **2**. Although this reaction was not a useful preparative route to pure **3**, it helped us to confirm the absolute configuration of *R,R*-**3** and *S,R*-**3**. The deprotection of **4b** by TMSI also yielded a product mixture.

In conclusion, simple regioselective ring-opening methods were developed, enabling the preparation of pure regioisomeric adducts **3** and **4** of phenyloxirane with cysteine. These products are useful tools in the research on protein adducts and in the biological monitoring of long-term cumulative exposure to styrene. Surprisingly, the oxirane ring opening of **1** catalyzed by tris(pentafluorophenyl) borane proceeds by S_N2 mechanism with full inversion of configuration. By contrast, the ring opening catalyzed by TBAF, which was expected to proceed stereospecifically, always led to the mixture of diastereomers because of full racemization of **1** enantiomers in the presence of TBAF.

Experimental details

Tris(pentafluorophenyl)borane, 97% pure, was obtained from Alfa Aesar. TBAF, 1 M solution in THF, TMAF, TMSI, racemic styrene oxide (**1**), and (*R*)- and (*S*)-enantiomers of **1** and L-cysteine were purchased from Sigma-Aldrich. All other chemicals were of analytical or synthetic grade and were used as received. SPE columns Strata X (1 g/12 mL) were purchased from Chromservis, precoated plates of silica gel 60 F254 were from Merck and silica gel 60, and 230–400 mesh for column chromatography was from Sigma-Aldrich.

NMR spectra were recorded on a Varian 400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. LC/MS analyses were conducted on a Thermo Scientific LXQ linear trap mass spectrometer in tandem with a Janeiro LC system consisting of two Rheos 2200 pumps and CTC PAL autosampler. Electrospray ionization in the positive ion mode was used. The collision gas was helium, the capillary temperature was set at 300°C, and the capillary voltage was 4.6 kV. High-resolution mass spectra (HRMS) were measured on a Thermo Fisher Scientific LTQ Orbitrap mass spectrometer using electrospray ionization in the positive mode. Specific optical rotation was measured on a polarimeter Perkin-Elmer 241.

Reaction of styrene oxide (1) with protected cysteines in the presence of Lewis acid

To a stirred solution of protected cysteine, **2a** [19] or **2b** [20] (0.94 mmol) and **1** (0.12 g, 0.95 mmol) in dry dichloromethane $B(C_6F_5)_3$ (25 mg, 5 mol%) was added, and stirring was continued for 3 days at room temperature. The mixture was concentrated, and the crude residue was purified by column chromatography using cyclohexane/acetone 5:1 as an eluent.

***N*-(*tert*-Butoxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (3a)** Colorless oil; 1:1 diastereomeric mixture; yield 67 mg (21%); R_f=0.22 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 1.43 and 1.45 (s, 9H), 2.24 (br s, 1H), 2.85 (m, 2H), 3.69 and 3.73 (s, 3H), 3.85 (m, 2H), 3.98 (m, 1H), 4.50 (m, 1H), 5.24 and 5.36 (br s, 1H), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 28.5, 33.9 and 34.5, 52.8, 53.6, 53.7, 65.9 and 66.1, 80.5, 128.1, 128.3, 129.0, 139.3 and 139.5, 155.3 and 155.6, 171.6; HRMS: Found m/z 378.1352 $[M+Na]^+$, calcd m/z 378.1346; ESI-MS: m/z 356 $[M+H]^+$.

(*1S*)-*N*-(*tert*-Butoxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (*S,R*-3a) Colorless oil; one diastereomer; yield 65 mg (20%); R_f=0.22 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 2.88 (m, 2H), 3.74 (s, 3H), 3.86 (m, 2H), 3.97 (m, 1H), 4.50 (m, 1H), 5.23 (d, 1H, J = 7.4 Hz), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 28.3, 33.7, 52.6, 53.4, 53.5, 65.6, 80.3, 127.9, 128.0, 128.8, 139.0, 155.1, 171.3; HRMS: Found m/z 378.1349 $[M+Na]^+$, calcd m/z 378.1346; ESI-MS: m/z 356 $[M+H]^+$; $[\alpha]_D^{20}$ +100.5° (c=1, $CHCl_3$).

(*1R*)-*N*-(*tert*-Butoxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (*R,R*-3a) Colorless oil; one diastereomer; yield 57 mg (17%); R_f=0.22 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H), 2.51 (br s, 1H), 2.84 (m, 2H), 3.69 (s, 3H), 3.84 (m, 2H), 4.01 (m, 1H), 4.54 (m, 1H), 5.36 (d, 1H, J = 7.4 Hz), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 28.3, 34.3, 52.6, 53.2, 53.5, 65.9, 80.3, 127.8, 128.0, 128.8, 139.2, 155.4, 171.3; HRMS: Found m/z 378.1349 $[M+Na]^+$, calcd m/z 378.1346; ESI-MS: m/z 356 $[M+H]^+$; $[\alpha]_D^{20}$ -103.5° (c=1, $CHCl_3$).

***N*-(Benzyloxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (3b)** Colorless oil; 1:1 diastereomeric mixture; yield 145 mg (40%); R_f=0.25 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 2.89 (m, 2H), 3.69 and 3.74 (s, 3H), 3.84 (m, 2H), 3.96 (m, 1H), 4.59 (m, 1H), 5.09 and 5.13 (s, 2H), 5.55 and 5.70 (d, 1H, J = 7.3 and 8.0 Hz), 7.32 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 33.6 and 34.1, 52.8, 53.5 and 53.6, 53.8, 65.7 and 65.9, 67.1 and 67.2, 127.9, 128.0, 128.1, 128.2, 128.5, 128.8, 136.0, 138.9 and 139.1, 155.7 and 155.9, 171.0; HRMS: Found m/z 412.1194 $[M+Na]^+$, calcd m/z 412.1189; ESI-MS: m/z 390 $[M+H]^+$.

(*1S*)-*N*-(Benzyloxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (*S,R*-3b) Colorless oil; one diastereomer; yield 128 mg (35%); R_f=0.25 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 2.91 (m, 2H), 3.73 (s, 3H), 3.83 (m, 2H), 3.94 (m, 1H), 4.55 (m, 1H), 5.09 (s, 2H), 5.60 (d, 1H, J = 7.8 Hz), 7.32 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 33.5, 52.7, 53.4, 53.8, 65.8, 67.1, 127.9, 128.0, 128.1, 128.2, 128.5, 128.8, 136.1, 139.0, 155.7, 171.0; HRMS: Found m/z 412.1187 $[M+Na]^+$, calcd m/z 412.1189; ESI-MS: m/z 390 $[M+H]^+$; $[\alpha]_D^{20}$ +137.1° (c=0.6, $CHCl_3$).

(*1R*)-*N*-(Benzyloxycarbonyl)-*S*-(2-hydroxy-1-phenylethyl)-L-cysteine methyl ester (*R,R*-3b) Colorless oil; one diastereomer; yield 139 mg (38%); R_f=0.25 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 2.85 (m, 2H), 3.69 (s, 3H), 3.82 (m, 2H), 3.99 (m, 1H), 4.60 (m, 1H), 5.13 (s, 2H), 5.73 (d, 1H, J = 8.2 Hz), 7.32 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 34.0, 52.7, 53.6, 53.8, 65.9, 67.2, 127.9, 128.0, 128.1, 128.3, 128.6, 128.8, 136.1, 139.1, 156.0, 171.0; HRMS: Found m/z 412.1185 $[M+Na]^+$, calcd m/z 412.1189; ESI-MS: m/z 390 $[M+H]^+$; $[\alpha]_D^{20}$ -243.5° (c=0.6, $CHCl_3$).

Reaction of styrene oxide (1) with protected cysteines in the presence of TBAF

A solution of protected cysteine **2a** or **2b** (0.68 mmol) and **1** (0.082 g, 0.68 mmol) in dry CH_3CN was treated with 1 M TBAF solution in THF (0.068 mL, 10 mol%). The solution was stirred until the reaction was completed as judged by thin layer chromatographic (TLC) analysis (approximately 5 h). The solvent was removed *in vacuo*, and the product was then isolated by column chromatography using cyclohexane/acetone, 5:1, as an eluent.

***N*-(*tert*-Butoxycarbonyl)-*S*-(2-hydroxy-2-phenylethyl)-L-cysteine methyl ester (4a)** Colorless oil; 1:1 diastereomeric mixture, yield 125 mg, (52%), R_f=0.32 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 2.76 (m, 1H), 2.97 (m, 3H), 3.75 and 3.76 (s, 3H), 4.57 (m, 1H), 4.76 (m, 1H), 5.43 and 5.55 (d, 1H, J = 7.6 and 7.6 Hz), 7.31 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 28.3, 35.2 and 35.3, 52.6, 53.6, 53.7, 72.3 and 72.6, 80.3, 125.7, 127.9, 128.5, 142.4, 155.3, 171.4; HRMS: Found m/z 378.1352 $[M+Na]^+$, calcd m/z 378.1346; ESI-MS: m/z 356 $[M+H]^+$.

***N*-(Benzyloxycarbonyl)-*S*-(2-hydroxy-2-phenylethyl)-L-cysteine methyl ester (4b)** Colorless oil; 1:1 diastereomeric mixture; yield 128 mg; (48%), R_f=0.30 (chloroform/ethyl acetate, 10/1); ^{1}H NMR ($CDCl_3$) δ 2.90 (m, 4H), 3.75 and 3.76 (s, 3H), 4.63 (m, 1H), 4.75 (m, 1H), 5.12 (s, 2H), 5.79 and 5.93 (br s, 1H, J = 7.3 Hz), 7.32 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 35.1, 42.3 and 42.5, 52.8, 53.9 and 54.1, 67.2, 72.5 and 72.7, 125.7, 127.9, 128.1, 128.3, 128.6, 136.1, 142.4, 155.9, 171.1; HRMS: Found m/z 412.1198 $[M+Na]^+$, calcd m/z 412.1189; ESI-MS: m/z 390 $[M+H]^+$.

Deprotection of Z-protected cysteine methyl ester adducts 3b and 4b

A solution of protected cysteine **3b** or **4b** (30 mg, 0.08 mmol) in dichloromethane was treated at 0°C with three equivalents of TMSI, and the mixture was stirred overnight. Then, the mixture was quenched with water, concentrated, extracted with ethyl acetate, and dried over $MgSO_4$. The solvent was evaporated, and the resulting *N*-deprotected methyl ester was hydrolyzed with aqueous NaOH (1 M, 0.1 mL) diluted with 1 mL of methanol. The solvent was evaporated, the residue was diluted with water, and the pH level of the solution was adjusted to 5–6 before analysis by LC/MS.

Deprotection of Boc-protected cysteine methyl ester adducts 3a and 4a

Protected compound **3a** or **4a** (30 mg, 0.09 mmol) was dissolved in a 1:1 mixture of TFA and dichloromethane. The solution was stirred at room temperature for 4 h then concentrated on a rotary evaporator.

The residue was dissolved in water (5 mL), and pH of the solution was adjusted to 5–6. The solution was poured on a Strata X SPE column, which was then washed with 10 mL of water and eluted with 10 mL of pure methanol.

***S*-(2-Hydroxy-1-phenylethyl)-L-cysteine [7]** This compound was obtained as white solid; yield 10 mg (50%); R_f=0.20 (chloroform/methanol/acetic acid, 7/2/1); ^{1}H NMR (D_2O): δ 2.87 (m, 2H), 3.56 and 3.71 (m, 1H), 3.81 (m, 2H), 4.01 (t, 1H, *J* = 6.7 Hz), 7.28 (m, 5H); ^{13}C NMR (D_2O): δ 31.2 and 31.4, 50.8 and 51.6, 53.6, 64.4 and 64.5, 128.1, 128.2, 128.9, 138.7 and 139.3, 172.4; ESI-MS: m/z 242 $[M+H]^+$; MS/MS: m/z 225 $[M+H - NH_3]^+$, 121 $[PhCHCH_2OH]^+$.

***S*-(2-Hydroxy-2-phenylethyl)-L-cysteine [7]** This compound was obtained as white solid; yield 32 mg (80%): R_f=0.20 (chloroform/methanol/acetic acid, 7/2/1); ^{1}H NMR (D_2O): δ 2.92 (m, 4H), 3.82 (m, 1H), 4.79 (t, 1H, *J* = 6.6 Hz), 7.32 (m, 5H); ^{13}C NMR (D_2O): δ 32.4 and 32.5, 39.5 and 39.6, 53.3, 72.4 and 72.5, 126.1, 128.2, 128.7, 141.9, 172.0; ESI-MS: m/z 242 $[M+H]^+$; MS/MS: m/z 242 $[M+H - H_2O]^+$.

Racemization of styrene oxide enantiomers in the presence of TBAF

Two solutions were used, one with (*R*)-**1** ($[\alpha]_D^{20}$ +5.3° (*c*=2.1, CH_3CN)) and 10 mol% TBAF in acetonitrile and the other without TBAF. In a few hours, the complete racemization of styrene oxide in the presence of TBAF was observed. However, there was no change in specific rotation of the solution without TBAF.

LC/MS analyses

Aliquot samples (10 μL) of the reaction mixture of **1** and **2** at various pH were injected onto a 150×2 mm Phenomenex Aqua C18 column, particle size 5μm, and eluted with 4% acetonitrile in 0.1% aqueous formic acid at a flow rate of 150 μL/min. The mass analyzer was set to monitor full-scan MS in the range of m/z 100–400 and collisionally activated MS/MS taken at m/z 242 for $(M+H)^+$ ion of **3** and **4**. These conditions enabled full separation of the diastereomers of **3** and **4**.

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