A General Asymmetric Synthesis of *syn*- and *anti*-β-Substituted Cysteine and Serine Derivatives

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Abstract: A stereodivergent synthetic route has been developed to make the optically pure *anti*- and *syn*-β-substituted cysteine and serine derivatives. In this approach, the key intermediates, >94% enantiomerically pure cyclic sulfates **3** and aziridines **7**, were prepared from α,β-unsaturated esters **1**, employing the Sharpless asymmetric dihydroxylation. The high regio- and stereoselective ring-opening reactions of cyclic sulfates and aziridines provided enantiomerically pure β-substituted cysteine and serine derivatives.

A complete understanding of the stereochemical requirements of side chain groups important in peptide ligand−receptor/acceptor interactions plays a crucial role in the rational design of bioactive peptides and their nonpeptide mimetics. This approach can be realized by incorporation of conformationally constrained novel amino acids into a peptide or nonpeptide template.[1] Among novel amino acids, β-substituted cysteines and serines can play a unique function in peptide conformational constraints. β-Substituted cysteines, when introduced into the peptide chain, can constrain the backbone conformation through the formation of a disulfide bridge, as well as preserve the respective side chains, which are important for molecular recognition.[2] β-Substituted cysteines and serines also can be used as building blocks for dipeptide β-turn mimetics (Figure 1).[3]

As part of our α-MSH (melanocyte-stimulating hormones) program, we have identified the core sequence of α-MSH peptides His-(D/L)Phe-Arg-Trp and found a β-turn that includes the Phe and Arg residues.[4] A conformationally constrained bicyclic dipeptide mimetic scaffold (Figure 1) can exist as up to 32 different isomers with different backbone geometries and side chain orientations, which can provide specific insights into the bioactive conformation. Furthermore, cysteine and its β-substituted derivative residues are present in many peptide/protein sequences having important bioactivities, including DPDPE, a cyclic enkephalin analogue (H-Tyr-c[D-Pen-Gly-Phe-D-Pen]-OH),[5a] and melanotropin analogues.[5b] Introduction of appropriate β-substituted cysteines into a peptide sequence will preserve the appropriate side chain orientation and restrict the C−S−S−C dihedral angle. Hence, there is a need for efficient, stereospecific synthetic approaches toward these molecules.

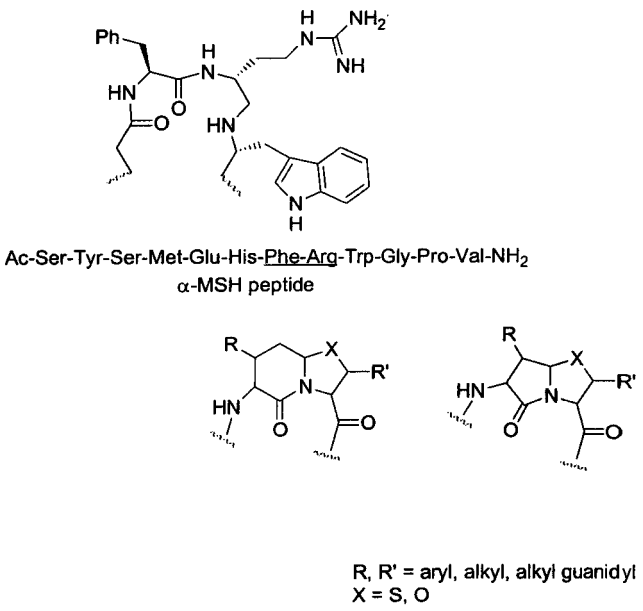

Figure 1. β-Turn in α-MSH peptide and proposed β-turn mimetics.

Although the synthesis of β-hydroxy amino acids has been well documented,[6] there appears to be no general stereospecific methodology directed at the synthesis of β-substituted cysteines. Goodman and co-workers reported the synthesis of α,β-dimethylcysteines and serines,[7] but only the *anti* isomers were accessible with high ee. Recently, we reported the enantioselective synthesis of β-phenylcysteine, β-phenyltryptophan, and β-phenylserine through the ring-opening reaction of 3-phenylaziridine-2-carboxylic ester.[8] We now have further developed this strategy in the first general asymmetric synthesis of all four isomers of β-substituted cysteines and serines.

Substituted α,β-unsaturated benzyl esters were the starting points of the synthesis. α,β-Unsaturated benzyl esters **1** (Scheme 1) were subjected to Sharpless asymmetric dihydroxylation in the presence of $(DHQ)_2PHAL$ (AD-mix-α) and methanesulfonamide. The reaction pro-

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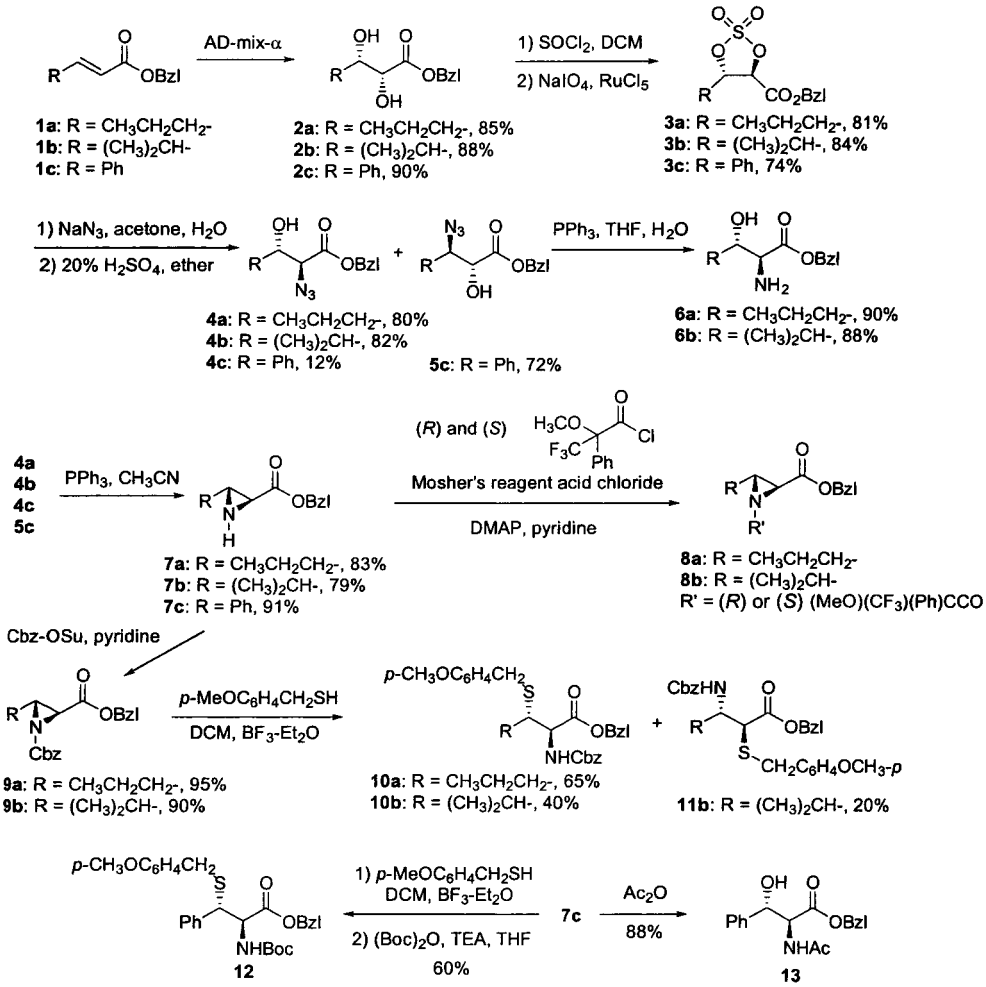
Scheme 1. Synthesis of *anti*-β-Substituted Serine and Cysteine Derivatives



ceeded smoothly to yield (2*R*,3*S*)-diol **2** in high yield and excellent optical purity (>94% ee).[9] The vicinal diol **2** was then converted to the cyclic sulfite as a mixture of diastereomers in a 1:1 ratio,[10] and the cyclic sulfite was further oxidized to the cyclic sulfate **3** with RuO_4 ($NaIO_4$/catalytic $RuCl_3$) in acetonitrile and water. Ring-opening reaction of cyclic sulfate **3** in an S_N2 fashion with N_3^- was generally carried out for 2 h at room temperature. Acidic hydrolysis provided azido esters **4** and **5**. In the case of **3a** and **3b**, nucleophilic substitution by NaN_3 happened exclusively at the α-position of cyclic sulfite **3** with clean inversion of chirality. Since there is competition of reaction sites between the benzylic and the α-position in the case of **3c**, nucleophilic substitution by NaN_3 gave regioisomers **5c** and **4c** in a 6:1 β/α ratio,[11] as determined by ^1H NMR. Reductions of azido esters **4a** and **4b** were accomplished under neutral conditions with PPh_3 in THF and water to provide the (2*R*,3*S*)-β-alkyl-substituted serine derivatives **6a** and **6b**. By switching the Sharpless chiral catalytic ligand to $(DHQD)_2PHAL$ (AD-mix-β), the other *anti* isomer could be made available by the same methodologies.

Subsequent aziridine formations were accomplished by the Staudinger reaction.[12] Regioisomers **4c** and **5c** lead to the same chiral product **7c**, since the hydroxy group serves as a leaving group in the aziridine formation. Treatment of 3-phenylaziridine-2-carboxylic ester **7c** with 4-methoxybenzylthiol in dichloromethane in the presence of $BF_3 \cdot Et_2O$ followed by amino group protection resulted in (3*S*,3*S*)-N^{α}-B*oc*-protected β-phenyl cysteine derivative **12** in 67% yield. This ring-opening process occurred without activating the aziridine ring and was a stereospecific S_N2 reaction at the benzylic C-3 position. The β-phenyl serine derivative was obtained when substrate **7c** was stirred at 70 °C with acetic acid for 2 h. The ring opening proceeded with complete inversion of configuration at C-3 position to give the (2*S*,3*S*)-product **13** in 88% yield.[8] The dominant influence of the phenyl group was demonstrated by the fact that both sulfur and oxygen reagents gave exclusively C-3 attacks to provide the α-amino acid derivatives (Scheme 1).

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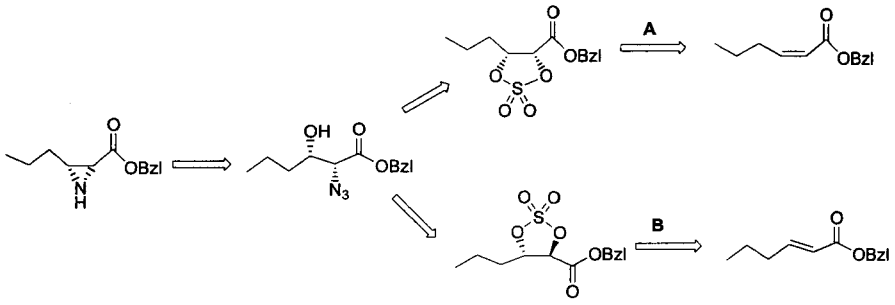
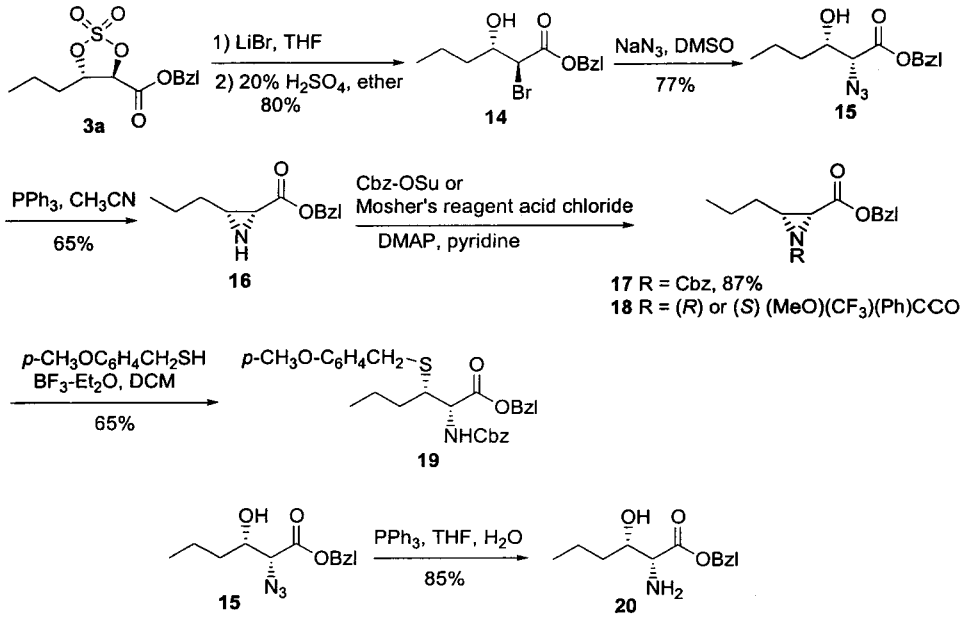
Scheme 2. Retrosynthesis of *syn* Products



Scheme 3. Preparation of *syn*-*β*-Propyl Cysteine and Serine Derivatives



Although the regioselective ring opening of aziridines with sulfur nucleophiles in the case of *β*-unsubstituted or α,*β*-dimethyl aziridine-2-carboxylate has been documented,[7,13] there are only limited examples of results on more general C-3-substituted aziridine 2-carboxylates.

Since nonactivated alkyl-substituted aziridines did not react using previously reported ring-opening reaction conditions,[14] aziridines **9** bearing electron-withdrawing groups such as Cbz (benzyloxycarbonyl) on the nitrogen were used. In the case of **9a**, a sulfur nucleophile with the aid of boron trifluoride etherate gave an exclusive C-3 attack to provide the *β*-propyl cysteine **10a** in 65% yield. The regiochemistry was confirmed by ^{1}H NMR analysis: the signal for the proton α to the ester group was a doublet and doublet; thus, the amido group was in the α-position. However, the 3-isopropyl aziridine 2-carboxylate **9b** provided a 2:1 mixture of products of C-3 and C-2 attack. It was obvious that the steric bulk presented by 3-substitution has great influence on the regioselectivity of the aziridine ring-opening reaction. The behavior of related *β*,*β*-disubstituted aziridine-2-carboxylates is under investigation.

There are several possibilities to consider for asymmetric synthesis of the *syn* isomers of *β*-substituted serine and cysteine derivatives (Scheme 2). However, a fundamental weakness of the Sharpless AD reaction was revealed when route A was examined: we found that this reaction is not sufficiently stereoselective to be synthetically useful when (*Z*)-alkenes are used as substrates. The highest ee so far observed is ca. 80% with (*Z*)-alkenes.[15] In route B, manipulating the configuration of the α-position seemed to be a practical way to provide the *syn* isomer through the formation of a *syn*-azido alcohol and a *syn*-aziridine.

To make the *syn* product, we first used LiBr to ring open the sulfate **3a**, followed by a second S_N2 nucleophilic substitution at the α-position with NaN_3 in acetone and water or with NaN_3 and 50 mol % *n*-$Bu_4^+HSO_4^-$ in CH_2Cl_2 to obtain the desired stereochemistry at the α-position. However, the NMR spectrum of the crude product showed it to be a mixture of the epoxide and C-2 diastereomers. We solved this problem by adding NaN_3 (2.0 equiv) in DMSO at room temperature to the bromide **14**, and this reaction condition led to smooth S_N2 displacement of the bromide by the azide to provide the *syn*-azido alcohol **15** in good yield (Scheme 3). The stereochemistry of the product was confirmed by comparing ^{1}H NMR spectra with that of the *anti* product. In general, any system that has a potentially nucleophilic substituent group situated properly for backside displacement of

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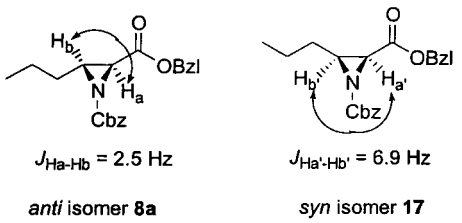

Figure 2. Coupling constants of *syn*- and *anti*-aziridines **9a** and **17** in ^{1}H NMR.

a leaving group at another carbon atom of the molecule can be expected to display neighboring-group participation. There will always be competition between direct S_N2 displacement by an external nucleophile and neighboring-group participation of an internal nucleophile. The extent of the rate enhancement will depend on how effectively the groups act as nucleophiles in different solvents and the ease with which the molecular geometry required for participation can be achieved.[7] Alcohol **15** was subjected to the reaction conditions previously described to provide the *syn*-*β*-propylserine ester **20** or *syn*-*β*-propyl aziridine **16**. Figure 2 shows the coupling pattern in the ^{1}H NMR spectra of *trans*-aziridine **9a** and *cis*-aziridine **17**. The coupling constants between H_a and H_b and those between H'_a and H'_b confirmed that the desired stereochemistry was formed.[16] Aziridine **16** was converted to the *syn*-*β*-propylcysteine derivative **19** (Scheme 3) using reaction conditions described in Scheme 1 for the preparation of **10a**.

The configurational integrity of both the *syn*- and *anti*-alkylsubstituted aziridine series **7a**, **7b**, and **16** was evaluated after conversion to the corresponding diastereomeric amide (1**R*)- and (1**S*)-**8a**, **8b**, and **18** as shown in Schemes 1 and 3. Aziridines **7a**, **7b**, and **16** were coupled with (*R*)- and (*S*)-Mosher's agents, respectively, to give amides, which were directly examined in ^{19}F NMR spectroscopy using $CFCl_3$ as the internal reference (−178.00 ppm). Measurements of the diastereomeric trifluoromethyl singlets at −70.32 and −71.12 ppm for **8a**, −71.30 and −70.23 ppm for **8b**, and −71.33 and −71.07 ppm for **18** demonstrated amides **8a**, **8b**, and **18** to be of >94%, >97%, and >94% diasteromeric excesses, respectively. The high diasteromeric purity of amides **8a**, **8b**, and **18** indicated that enantiopure materials were produced from the Sharpless asymmetric dihydroxylation and in subsequent transformations. Hence, aziridine **7a**, serine derivative **6a**, and cysteine derivative **10a** are presumed to be of >94% enantiomeric purity; aziridine **7b**, serine derivative **6b**, and cysteine derivatives **10b** and **11b** are presumed to be of >97% enantiomeric purity, and aziridine **16**, serine derivative **20**, and cysteine derivative **19** are presumed to be of >94% enantiomeric purity.

The transformation of phenyl-substituted aziridine **7c** to the Mosher amide failed because of the concomitant ring-opening reaction. The enantiomeric purity of aziridine **7c** was determined to be >96% by evaluation of **12**, which is the ring-opening reaction product of **7c**.[17]

In summary, a general and practical new pathway to the synthesis of both *anti*- and *syn*-*β*-substituted serine and cysteine derivatives has been developed. A key step involving regio- and stereoselective ring-opening reactions of cyclic sulfate **3** and aziridines **9** and **17** has been thoroughly investigated. Incorporation of the amino acids into biologically active α-MSH peptides and peptidomimetics, biological evaluation, and structure−biological activity relationship studies of the bioactive peptides and peptidomimetics are in progress.

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Note Added After ASAP Posting. All experimental details were removed from the version posted March 22, 2002, and transferred to Supporting Information. The new version was posted April 16, 2002.

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra and experimental details for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Specifically, the deprotection of Boc in **12** and its enantiomer gave a free amine, which was coupled with (S)-(−)-α-methoxyl-α-trifluoromethyl phenylacetic chloride (Mosher's agent) to afford the amides. Measurement of the diastereomeric methyl singlets at 3.35 and 3.28 ppm in proton NMR spectroscopy in $CDCl_3$ demonstrated the amides to be of >96% diastereomeric excess (the limits of detection were determined by measurement of the signal-to-noise ratio in NMR spectra; in each case, the signal-to-noise ratio was 90:1 and the minor product peak was not observed). Hence, amino acid **12** and its enantiomer are all presumed to be of a >96% enantiomeric excess.