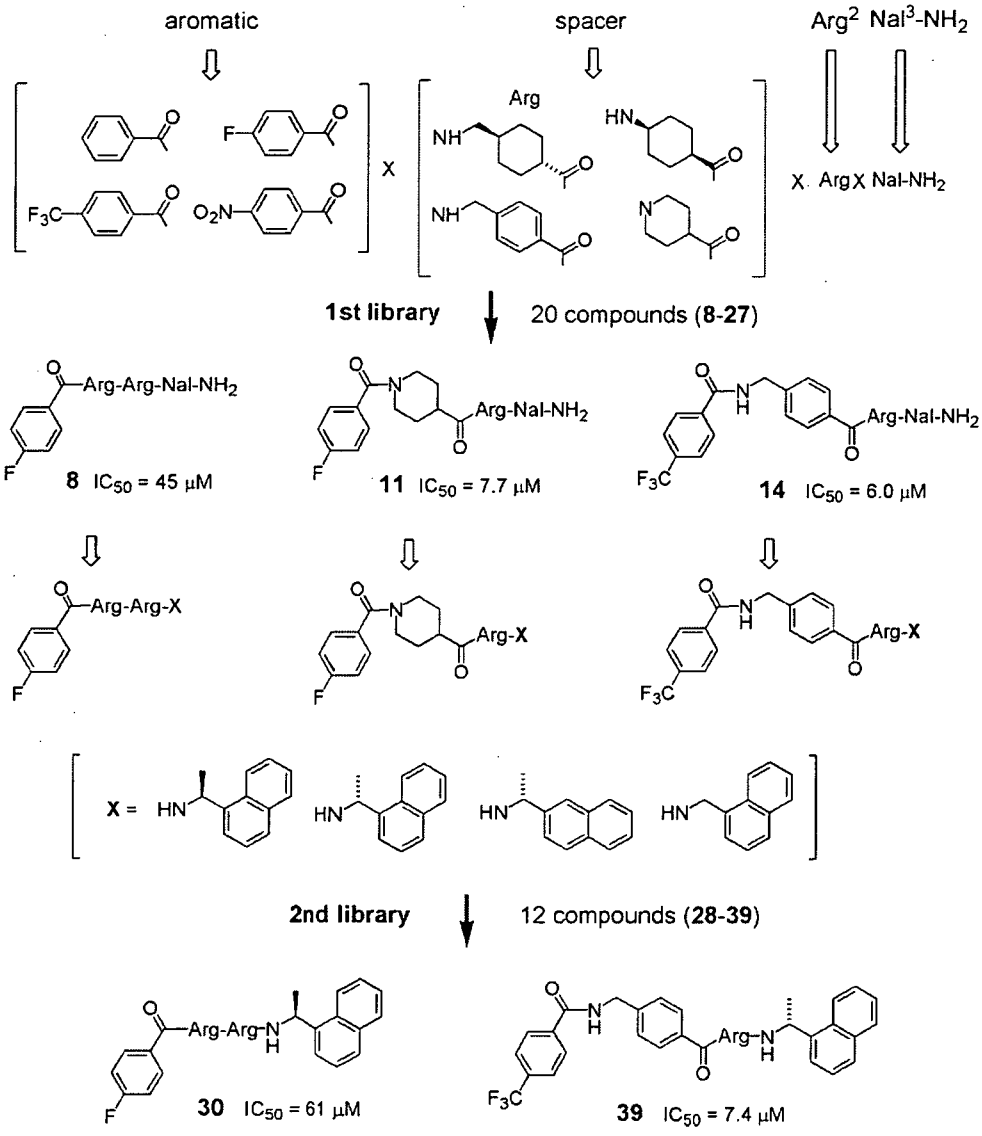

FIGURE 6 Design of two libraries involving three pharmacophores (aromatic ring, Arg^2, and Nal^3) and development of new leads. IC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells.

amide bond-forming condensation and reductive amination reactions.[69] Second, we synthesized two tetrapeptide mimetics, where a Tyr residue was added in the N-terminus based on the sequence of the FC131 sequence. These compounds showed anti-HIV activity, indicating that an N-terminal addition of a Tyr residue is effective for an increase in anti-HIV activity. Third, we synthesized *p*-fluorobenzoylated tripeptide mimetics based on the N-terminal sequence of 4F-benzoyl-TN14003 and 4F-benzoyl-TE14011. As a result, *p*-fluorobenzoylation caused an increase in anti-HIV activity. These results suggest that introduction of a reduced amide bond between two Arg residues is more suitable than that between Arg and naphthalenyl-ethylamine.

According to these results, two types of libraries based on the N-terminal region of 4F-benzoyl-TN14003 and 4F-benzoyl-TE14011 were constructed to find effective lead compounds (Figure 6). Linear-type low molecular weight compounds obtained in this study are thought to be useful leads.

Anthracene derivatives bearing two sets of zinc(II)-2,2′-dipicolylamine complex were previously identified as the first chemosensors that can selectively bind and sense phosphorylated peptide surfaces.[70] Several low molecular weight nonpeptide compounds having the dipicolylamine zinc(II) complex structure were also identified as potent and selective CXCR4 antagonists (Figure 7).[71]

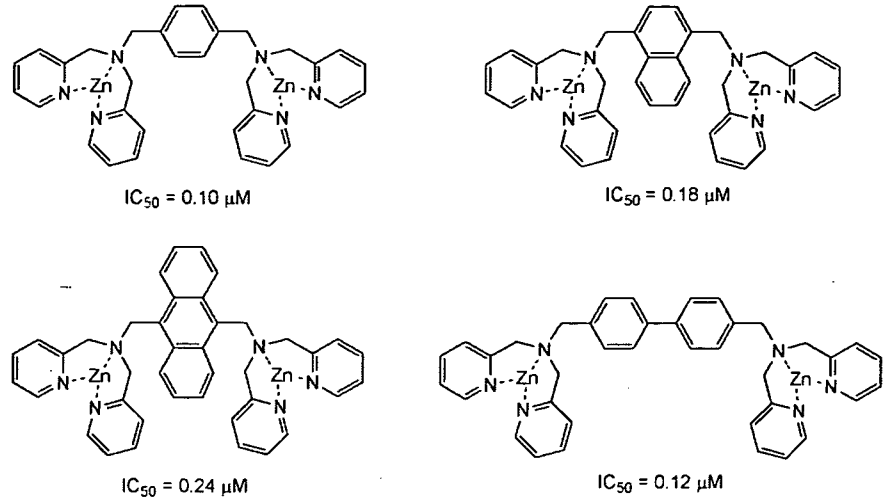

FIGURE 7 CXCR4 antagonists bearing the dipicolylamine-zinc(II) complex structure. IC_{50} values are based on the inhibition against Ca^{2+} mobilization induced by CXCL12 stimulation through CXCR4.

To date, several non-T140-related CXCR4 antagonists have been reported.[72,73] These include AMD3100 with two cyclam groups (AnorMED),[74] an *N*-pyridinylmethylene cyclam (monocyclam) AMD3465 (AnorMED),[75] AMD8665 without a cyclam group (AnorMED),[76] AMD070 (AnorMED),[77] ALX40-4C (Ac-[D-Arg]$_9$-NH_2; NPS Allelix),[78] CGP64222, R3G, NeoR,[79–81] a distamycin analog, NSC651016,[82] and a flavonoid compound, ampelopsin.[83] AMD3100 and galactosylceramide (GalCer) analogue conjugates have also been reported as doubly functionalized drugs.[84] KRH-1636 (Kureha Chemical and Sankyo) is an orally bioavailable agent containing *N*-pyridinylmethylene, Arg, and naphthalene moieties.[85]

CONCLUSION

The authors have developed selective antagonists against CXCR4, which is an important chemokine receptor in terms of physiology as well as pathology. T22 and its downsized analogue T140 bind specifically to the coreceptor CXCR4 and thus inhibit entry of X4-HIV-1 to T-cells. T140 and its analogues also show effective activity against cancer metastasis, leukemia, and RA. The T140 analogues were modified by structural matching, downsizing, and reduction of peptide character in attempt to develop new low-molecular weight CXCR4 antagonists. These antagonists could be promising agents for clinical chemotherapy of HIV infection, cancer metastasis, leukemia progression, and RA. However, we must recognize that blocking of the CXCL12-CXCR4 axis is a risky procedure in human subjects because CXCR4 plays a critical role in embryogenesis, homeostasis, and inflammation in the fetus, especially, in the embryonic development of hemopoietic, cardiovascular, and central nervous systems. Use of CXCR4 antagonists combined with CCR5 antagonists/fusion inhibitors might lead to an encouraging candidate for clinical chemotherapy of HIV infection and AIDS. Further development of CXCR4 antagonists with investigation of different administration routes might become important in the chemotherapy of multiple diseases involving CXCR4.

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Direct Assessment of CXCR4 Mutant Conformations Reveals Complex Link between Receptor Structure and Gα_i Activation*

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Ligand binding to G protein-coupled receptors (GPCRs) is thought to induce changes in receptor conformation that translate into activation of downstream effectors. The link between receptor conformation and activity is still insufficiently understood, as current models of GPCR activation fail to take an increasing amount of experimental data into account. To elucidate structure-function relationships in GPCR activation, we used bioluminescence resonance energy transfer to directly assess the conformation of mutants of the chemokine receptor CXCR4. We analyzed substitutions in the arginine cage DRY motif and in the conserved asparagine N(3.35)119, which are pivotal molecular switches for receptor conformation and activation. Gα_i activation of the mutants was either similar to wild-type CXCR4 (D133N, Y135A, and N119D) or resulted in loss of activity (R134A and N119K). Mutant N119S was constitutively active but further activated by agonist. Bioluminescence resonance energy transfer analysis suggested no simple correlation between conformational changes in response to ligand binding and activation of Gα_i by the mutants. Different conformations of active receptors were detected (for wild-type CXCR4, D133N, and N119S), suggesting that different receptor conformations are able to trigger Gα_i activity. Several conformations were also found for inactive mutants. These data provide biophysical evidence for different receptor conformations being active with respect to a single readout. They support models of GPCR structure-activity relationships that take this conformational flexibility of active receptors into account.

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GPCR[2] activation is commonly seen as the consequence of conformational rearrangements in the receptor upon ligand binding. The ternary complex model of GPCR activation and its derivatives postulates a limited number of defined receptor conformations, representing inactive, active, and some intermediate states of activation. However, the link between receptor conformation and activity is not entirely clear, and limitations of this model are beginning to emerge (1).

Receptor mutants have often been used to derive conformational models of receptor activation indirectly from pharmacological and biochemical data. Energy transfer techniques have significantly advanced our capacity to directly assess receptor conformation and conformational responses to ligand binding in live cells (2, 3). However, to date, only few reports directly measured mutant GPCR conformations (4, 5).

CXCR4 is a chemokine receptor involved in human immunodeficiency virus infection, spreading of tumors, and inflammatory diseases. Its sole natural agonist is the chemokine CXCL12. CXCR4/CXCL12 are essential during development, hematopoiesis, and immune system organization. The potential importance of CXCR4 also in the adult organism, where the receptor is expressed on a plethora of different cell types (6), calls for more subtle therapeutic interventions than simple inhibition of the receptor by antagonists. This may be particularly important in long term treatments, such as those required for antiretroviral therapy. Better understanding of the link between CXCR4 conformation and activation is therefore warranted.

We set out to directly measure the conformations of CXCR4 mutants bearing substitutions at positions that potentially affect receptor function. For this purpose, we used a bioluminescence resonance energy transfer (BRET) conformational sensor in live cells based on CXCR4 homodimers. Chemokine receptor homodimers form constitutively and remain associated upon ligand binding, as shown previously by ourselves and others (3, 7–9). Of note, our CXCR4 system uses C-terminal BRET donor (RLuc) and acceptor (yellow fluorescent protein, YFP) fusions of the receptors that preserve receptor activity and do not require receptor overexpression above physiological levels (3).

MATERIALS AND METHODS

Plasmids—The mutations were introduced into CXCR4 by the Kunkel method and subcloned into CXCR4-YFP and CXCR4-RLuc (described in Ref. 7).

Reagents—Recombinant CXCL12 was from R&D systems, and synthetic CXCL12 was a gift from Dr. F. Baleux, Institut Pasteur, Paris, France. AMD3100 was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. TC14012, a T140 analogue with similar biological properties, was synthesized as described previously (10).

Cell Culture and Transfection—HEK293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with

[2] The abbreviations used are: GPCR, G-protein-coupled receptor; BRET, bioluminescence resonance energy transfer; YFP, yellow fluorescent protein; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate.

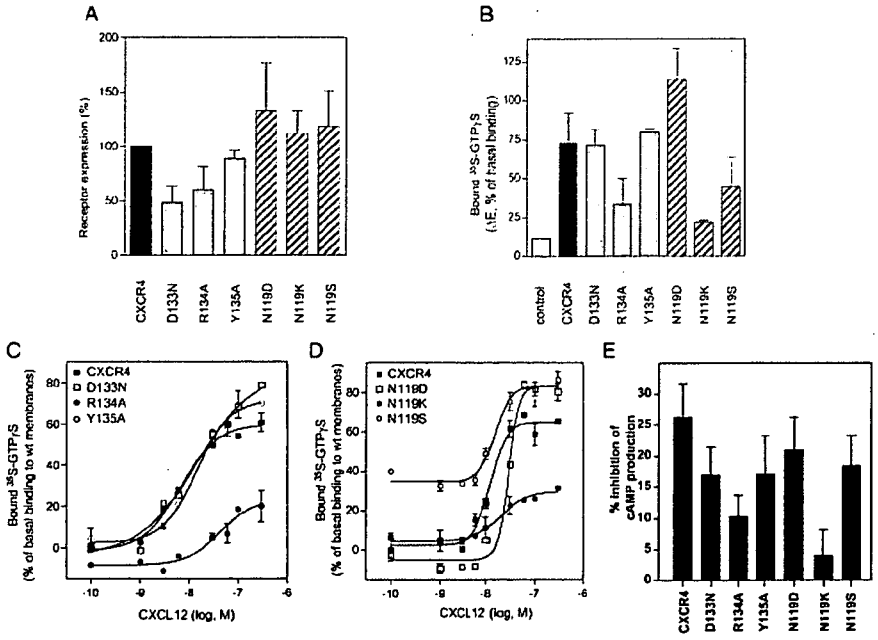

FIGURE 1. **Mutant expression and $G\alpha_i$ activation.** *A*, cell surface expression levels of CXCR4 mutants. *B*, ΔE, that is, maximal activation *versus* basal binding of GTPγS. Results are the percentage of basal binding to membranes from wild-type CXCR4-expressing cells and are representative of 4–5 independent experiments performed in duplicate. *C* and *D*, CXCL12-induced [^{35}S]GTPγS binding to membranes from cells expressing wild-type CXCR4 or receptors mutated in the DRY motif (*C*) or with substitutions of the Asn-119 residue (*D*). *E*, inhibition of cAMP production by CXCR4 mutants. Results are expressed as the percentage of adenylyl cyclase inhibition; background was subtracted from the data. Data are mean ± S.E. from 3 independent experiments.

10% fetal bovine serum (Wisent), 100 units/ml penicillin and streptomycin, 2 mM L-glutamine (Invitrogen). Transfections were performed in 6-well dishes using the polyethylenimine method (11). Receptor expression was tested by flow cytometry using the anti-CXCR4 monoclonal antibody 12G5 as described (3).

GTPγS Binding—[^{35}S]GTPγS binding experiments were carried out on crude membrane preparations from transfected cells, as described previously (12). 10 μg of membrane proteins were incubated in 96-well microplates for 15 min at 30 °C in assay buffer (20 mM Hepes, pH 7.4, containing 100 mM NaCl, 10 μg/ml saponin, 3 mM $MgCl_2$, 1 μM GDP) in the presence of the indicated concentrations of CXCL12. [^{35}S]GTPγS (Amersham Biosciences) at 0.1 nM was added, and membranes were further incubated for 30 min at 30 °C. After centrifugation at 4 °C and removal of supernatants, the plates were counted in a Wallac 1450 MicroBeta TriLux.

Adenylyl Cyclase Activity—cAMP was determined by radioimmunoassay using the Amersham Biosciences cAMP assays system following the manufacturer's instructions.

BRET Measurements—Usually 0.01–0.2 μg of RLuc constructs were cotransfected with increasing quantities of the corresponding YFP-tagged construct, completed to 2 μg with empty vector. Transfected cells were seeded in 96-well white clear bottom plates treated with poly-D-lysine and left in culture for 24 h. Cells were then washed once with phosphate-buffered saline and coelenterazine H (Nanolight Technology) added to a final concentration of 5 μM in phosphate-buffered saline. Readings were collected using a Mithras LB 940 reader (Berthold) by sequential integration of the signals detected in the 480 ± 20 (for luciferase) and 530 ± 20 nm (for YFP) windows. The BRET signal is the ratio of the receptor-YFP over the receptor-RLuc emission. The values were corrected to net BRET by subtracting the background BRET detected in cells expressing the RLuc constructs alone. Ligands were incubated in the presence of 0.1% bovine serum albumin (Sigma) with the cells at 37 °C for 5 min before the addition of coelenterazine H and BRET reading. In BRET titration experiments, net BRET ratios were expressed as a function of the [acceptor]/[donor] ratio (13). Total fluorescence (excitation filter at 485 nm and emission filter at 535 nm) and luminescence (measured 10 min after the addition of coelenterazine in the absence of emission filter) were used as a relative measure of total acceptor and donor protein expression. Ligand-induced BRET changes were monitored at maximal BRET ($BRET_{max}$), and we ensured that $BRET_{max}$ did not depend on the quantity of transfected Rluc or YFP fusion plasmids.

Data Analysis—Data were analyzed using GraphPad Prism 4.0 software. GTPγS binding was analyzed using nonlinear regressions applied to a sigmoidal dose-response model (variable slope). The statistical significance of the net BRET differences between the different mutants and conditions was calculated using one-way analysis of variance with Tukey's post-test for p value less than 0.05

RESULTS AND DISCUSSION

We constructed mutants D133N, R134A, and Y135A in the conserved DRY motif (residues (3.49), (3.50), and (3.51) following the assignment of Ballesteros and Weinstein (14)), a pivotal conformational switch for ligand-induced rearrangements leading to receptor activation (15). In addition, mutants N119D, N119K, and N119S were introduced in position asparagine 119 (Asn-119, or (3.35)), a key residue in which replacements change receptor activity as a function of the size of their lateral chain (16–18). Surface expression of mutants D133N and R134A was somewhat reduced, whereas the remaining substitutions showed similar surface expression as wild-type CXCR4 (Fig. 1*A*).

To analyze activation properties of the mutants, we measured G protein activation using a GTPγS binding assay (Fig. 1, *B–D*). D133N and Y135A displayed no apparent reduction of agonist-induced G protein activation, whereas R134A activity was strongly reduced. Replacement of the Asn-119 (N3.35) residue by lysine, serine, or aspartic acid resulted in the phenotypes previously reported by Zhang *et al.* (16). The N119D substitution resembled wild-type CXCR4, and N119K leads to loss of activity, whereas constitutive activity is seen with the N119S mutant. GTPγS binding of the N119S mutant is further increased in the presence of agonist to maximal activity identi-

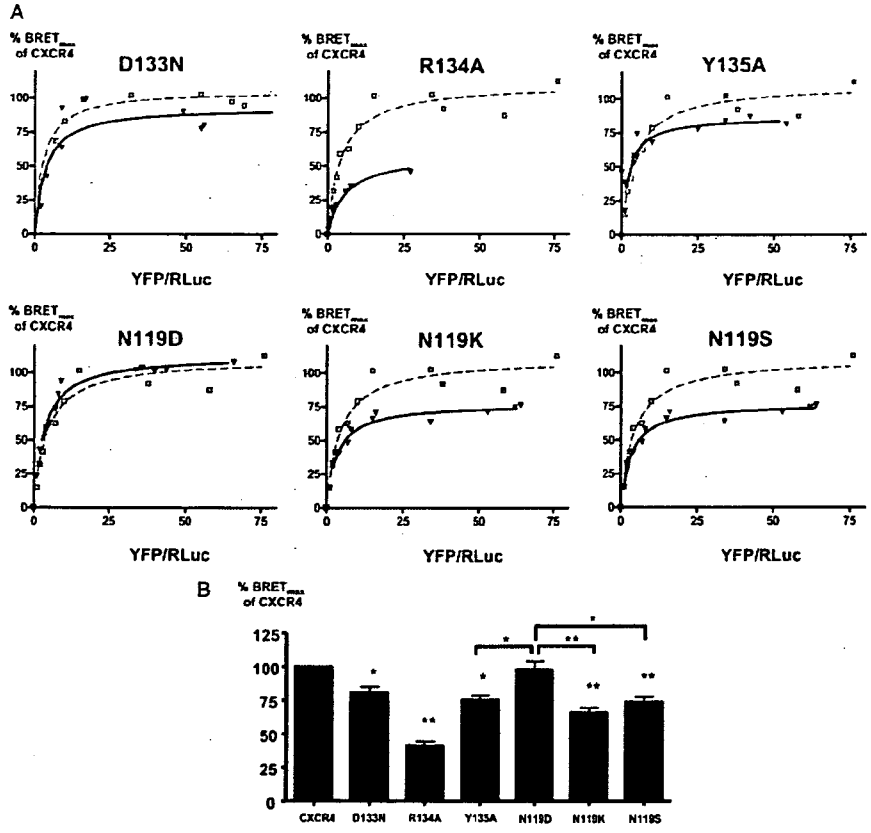

FIGURE 2. **[Acceptor]/[donor] titrations of wild-type and mutant CXCR4.** *A*, [acceptor]/[donor] titration curves of CXCR4 mutants (*black straight line*) in comparison with wild-type CXCR4 (*gray dotted line*). One representative experiment out of at least three per mutant is shown; the data except for D133N derive from the same experiment. ($BRET_{50}$ values for CXCR4, 2.83 ± 0.28; for D133N, 3.08 ± 0.50; for R134A, 4.91 ± 0.28; for Y135A, 2.321 ± 0.15; for N119D, 4.013 ± 0.47; for N119K, 2.57 ± 0.29; for N119S, 3.54 ± 1.09.) *B*, $BRET_{max}$ values for wild-type and mutant CXCR4. Data are means ± S.E. from 6–8 independent experiments per mutant. *p* values: *, $p < 0.05$, **, $p < 0.001$. Differences of R134A with all mutants were significant ($p < 0.001$).

cal to wild-type CXCR4. These observations were substantiated by measurement of $G\alpha_i$-mediated inhibition of forskolin-induced adenylyl cyclase activity at a single maximal dose of 200 nM CXCL12 (Fig. 1*E*), which is close to the maximum activity achievable with wild-type CXCR4 (3) and which corresponds to the concentrations used in the BRET assays (below). The results of both assays are in good agreement, suggesting that GTPγS binding is indeed due to $G\alpha_i$ activation. Taken together, our mutant panel comprises receptors that display a constitutive G protein activation that can be further activated by agonist (N119S), activity similar to that of wild-type CXCR4 (Y135A, D133N, N119D), or relative inactivity (N119K and R134A). The loss of activity of N119K and R134A is not due to loss of CXCL12 binding since the chemokine outcompetes binding of the monoclonal antibody 12G5 to all mutants (data not shown).

We then monitored the conformation of the CXCR4 mutant homodimers. We used a BRET sensor system that measures the relative distance and orientation of an energy donor (*Renilla* luciferase, RLuc) and acceptor (YFP) fused to the C terminus of CXCR4. Coexpression of the two constructs leads to the constitutive formation of homodimers and permits measurement of conformational changes that alter orientation/distance of the fused fluorophores, such as those brought about by ligand binding to the dimeric receptor (3).

Energy acceptor over donor titrations demonstrated that most mutants retained $BRET_{50}$ values (the YFP/RLuc ratio at which half-maximal BRET is obtained) in the same range as wild-type CXCR4 (Fig. 2*A*), and the observed differences are not statistically significant. $BRET_{50}$ has been interpreted as the propensity of a receptor to dimerize (13, 19). The maximal BRET varied between wild-type and mutant CXCR4 with the exception of N119D. These $BRET_{max}$ differences were significant, as were the differences between several mutants (Fig. 2*B*). Lower BRET results from the greater distance between the energy donor and acceptor or from altered orientation, which is likewise indicative of conformational change. This conclusion is in line with evidence presented by Zhang *et al.* (16) as a result of receptor modeling, which suggested conformational differences between wild-type CXCR4 and the N119S mutant. Of note, it is improbable that the mutations directly modify the orientation of the fused fluorophores since they are in TM3 and adjacent residues of the receptor. We therefore conclude that the observed differences in $BRET_{max}$ are due to overall conformational changes within the receptor.

We next measured BRET in the presence of the natural CXCR4 agonist CXCL12, the weak agonist AMD3100, or the inverse agonist TC14012. We have previously shown that these ligands induce distinct conformational changes in CXCR4 homodimers without affecting $BRET_{50}$ values (3). Acceptor over donor titration analyses performed with the constitutively active mutant N119S and the inactive mutant N119K in the absence and presence of ligands revealed no significant changes of $BRET_{50}$, suggesting that ligand binding also to mutant CXCR4 does not change their propensity to dimerize (N119S at basal condition, 4.01 ± 1.31; in the presence of CXCL12, 7.93 ± 4.32; in the presence of TC14012, 5.99 ± 2.43; and N119K at basal condition, 4.85 ± 1.73; in the presence of CXCL12, 3.33 ± 1.36; in the presence of TC14012, 5.57 ± 2.46, $n = 4$). Fig. 3*A* shows the observed ligand-induced $BRET_{max}$ changes of the CXCR4 mutants, expressed as the percentage of change relative to the respective $BRET_{max}$ in the absence of ligand. The relative responses to the agonist CXCL12 of the mutants were different. Although the differences between wild-type CXCR4 and the mutants did not reach statistical significance, the differences between mutant Y135A and mutants D133N, R134A, N119K, and N119S were significant ($p < 0.05$). The relative BRET

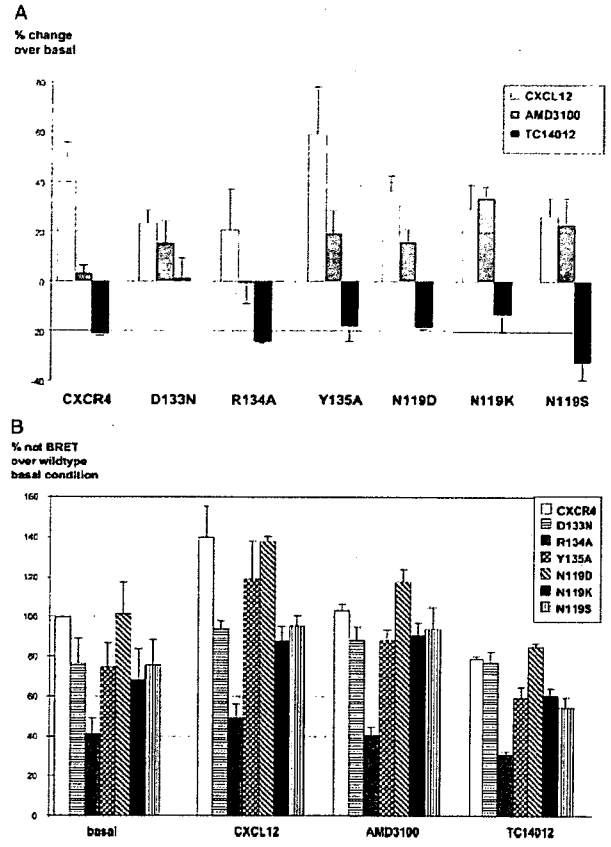

FIGURE 3. **Ligand-induced BRET changes.** *A*, relative BRET changes induced by the CXCR4 agonist CXCL12 (200 nM) ($n = 4$), the weak agonist AMD3100 (1 μg/ml) ($n = 4$), and the inverse agonist TC14012 (1 μg/ml) ($n = 2$), with respect to basal BRET of each mutant. Statistical significance: $p < 0.05$ for CXCL12 responses of D133N, R134A, N119K, and N119S with respect to Y135A; $p < 0.05$ for AMD3100 responses between CXCR4 and mutants N119K and N119S, D133A and N119K, R134A and Y135A, N119D, N119K, and N119S, and N119D and N119K; $p < 0.05$ for TC14012 responses between wild-type CXCR4 and D133N and D133N and mutants Y135A and N119S. *B*, the same values expressed on a scale relative to wild-type CXCR4 BRET in the absence of ligands (set as 100%). The *error bars* represent the mean ± S.E. of 4 independent experiments (except for TC14012, where 2 independent experiments were performed). One-way analysis of variance with Tukey's multiple comparison test of all data found for 115 out of 378 comparisons shows significant differences ($p < 0.05$), the most appealing ones being between inactive wild-type CXCR4/basal and R134A/CXCL12, between active wild-type CXCR4/CXCL12 and N119S/basal, D133N/CXCL12 and N119S/CXCL12, and between active N119D/CXCL12 and N119S/CXCL12. Comparison of selected inactive receptors found significant differences between R134A/basal and N119K/basal and between R134A basal and wild-type CXCR4/TC14012 is shown.

increase observed in response to AMD3100 is higher in the mutants than the very weak response of wild-type CXCR4, with the notable exception of R134A. In addition, there are significant differences among the mutant responses, the N119K response being more important than that of D133N and N119D. The differences between wild-type and mutant CXCR4 responses to TC14012 do not reach statistical significance, with the exception of D133N, which does not respond.

The differences in ligand-induced BRET changes yield information about relative differences between basal and ligand-bound receptor conformations of the mutants. For example, the weak BRET response of wild-type CXCR4 to AMD3100 indicates either that the wild-type receptor does not undergo

TABLE 1

Overview, mutant $G\alpha_i$ activation and $BRET_{max}$ in absence and presence of SDF-1

Receptor	$G\alpha_i$ activation	basal $BRET_{max}$ (relative to wildtype CXCR4)	$BRET_{max}$ with CXCL12 (relative to basal wildtype CXCR4)
CXCR4	yes	100	139 ± 15
D133N	yes	76 ± 12	94 ± 3
R134A	no	41 ± 7	49 ± 7
Y135A	yes	74 ± 12	119 ± 19
N119D	yes	101 ± 15	137 ± 3
N119K	no	68 ± 16	87 ± 8
N119S	constitutive, further activated by agonist	75 ± 13	95 ± 5

important conformational changes or that these changes do not translate into important BRET changes. In turn, the higher BRET responses of most mutants to AMD3100 suggest that the conformational changes that these mutants undergo upon AMD3100 binding are dissimilar from those of the wild-type receptor. This, in principle, might result from their different basal conformations. To directly compare the ligand-bound conformations of the mutants, $BRET_{max}$ values of the mutants in the presence of ligands were plotted as the percentage of wild-type CXCR4 $BRET_{max}$ under basal conditions (Fig. 3*B*, and for a summary, Table 1). This direct comparison reveals that the mutants also have altered conformations in complex with ligand. In the presence of CXCL12, only mutants Y135A and N119D have $BRET_{max}$ values similar to the wild type, indicating that agonist-bound receptor conformations of the other mutants are different, including mutants D133N and N119S ($p < 0.05$), which nevertheless stimulate $G\alpha_i$ activity in the presence of CXCL12. Differences in inactive conformations are suggested by the differences of R134A, N119K, and CXCR4 in the presence of inverse agonist ($p < 0.05$). Interestingly, despite the obvious functional differences between mutants N119K and N119S, almost identical BRET in the presence and absence of ligands was measured. This suggests that despite the recorded differences among many of the mutants, the technical limits of our method may not permit appreciation of subtle but functionally paramount conformational differences.

$BRET_{max}$ values are average values of a receptor population; it can therefore not be categorically ruled out that different $BRET_{max}$ values represent different states of equilibrium between only two distinct conformations (for example, one inactive and one active conformation). However, we find no linear link between $BRET_{max}$ and G protein activation (for example, R134A with CXCL12 has lower $BRET_{max}$ and higher activity than N119K with or without CXCL12, $p < 0.05$). Moreover, inactive and active receptors can have similar $BRET_{max}$ (such as mutants N119K and N119S in the presence of CXCL12). We therefore conclude that different $BRET_{max}$ corresponds to genuinely different receptor conformations rather than to changes in equilibrium between active and inactive receptors.

We have shown that mutations of the CXCR4 sequence can alter both the basal conformation and the conformational rearrangements induced by ligand binding. Confor-

mational rearrangements of a given mutant in response to agonist are not predictive of G protein activation (for example, mutant N119K conformationally responds to CXCL12 yet fails to activate G proteins). It remains to be investigated whether conformational responses could be predictive for receptor activities other than G protein activation; we do, however, not believe that conformational response *per se* must be followed by functional responses (*i.e.* the inverse agonist TC14012 induces conformational response but not activity). In turn, conformational differences between mutants in the presence of CXCL12 do not necessarily correlate with gross differences in G protein activation. For instance, the N119S mutant and wild-type CXCR4 have different conformations in the presence of CXCL12, yet both activate G proteins. Although it remains to be seen whether such mutants are functionally different with respect to other readouts, such differences would not affect our conclusion that distinct receptor conformations can mediate the same activity. Taken together, we detect important differences on both the functional and the conformational level in our mutant panel, but the link between function and conformation eludes simplification.

Theoretical models that describe GPCR activation have been repeatedly amended to accommodate new experimental data (20). The dominant ternary complex model and its derivatives assume the existence of a limited number of receptor conformations that derive from one another by intermediates between the extremes of one active and one inactive conformation. This assumption is increasingly difficult to reconcile with accumulating pharmacological evidence that different receptor ligands can stabilize different receptor conformations, as deduced from different functional consequences, and further revision of current models may be warranted (1). In this context, direct measurement of receptor conformation offers an additional perspective, contributing independent evidence to the elucidation of structure-function relationships in GPCRs. To date, few studies using these methods have employed mutant receptors in live cells (4, 5).

Our data derived from a set of CXCR4 mutants provide biophysical evidence that active receptor conformations can have a degree of conformational diversity that was previously unappreciated and that is consistent with multiple receptor conformations being able to perform the same activation step. This fits models that take conformational heterogeneity of receptors into account. Such models were initially forwarded to better describe allosterism and suppose that collections of receptor conformations make up functionally similar receptor subpopulations (21–23). Although it might be rightly argued that recombinant systems expressing receptor mutants in cell lines do not reflect what wild-type receptors actually perform *in vivo*, such artificial test systems highlight aspects of the conformational plasticity of the active receptor, as they have previously permitted the accumulation of functional information.

In conclusion, we find that heterogeneous CXCR4 conformations can lead to similar G protein activation, implying a flexibility of active receptors that is unaccounted for by the ternary complex model and its derivatives. Our findings might also help meet the conceptual challenges inherent to therapeutic targeting of CXCR4. The use of competitive inhibitors of CXCL12 binding to CXCR4 results in side effects that make the drug unsuitable for long term antiretroviral treatment in human immunodeficiency virus infection. Our data suggest that noncompetitive allosteric compounds, which modulate the receptor conformation rather than blocking ligand binding and thereby preserve some of the functions of the receptor, may be compatible with the maintenance of vital CXCR4 signaling but impinge on other properties such as the mediation of viral entry.

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Versatile use of acid-catalyzed ring-opening of β-aziridinyl-α,β-enoates to stereoselective synthesis of peptidomimetics

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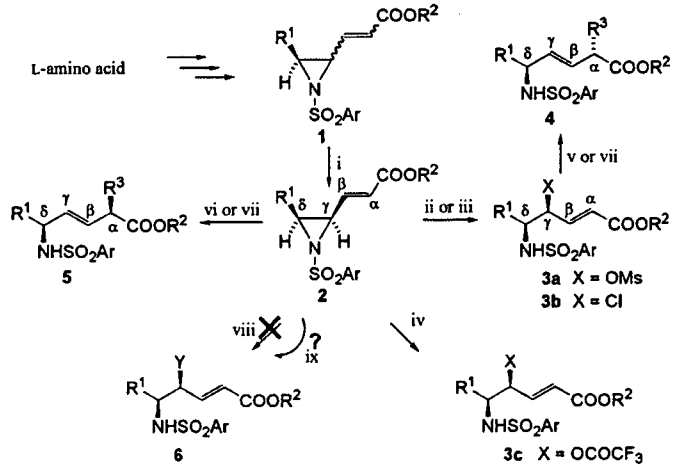
Abstract—Treatment of *N*-arylsulfonylaziridines bearing α,β-unsaturated esters with alcohols, thiols or weak acids such as AcOH in the presence of catalytic amount of Lewis acids affords regio- and stereoselectively ring-opened products, such as δ-aminated γ-alkoxy-(alkylthio or acetoxy)-α,β-enoates. In addition, the regio- and stereoselective ring-opening reactions can be performed on solid supports and applied to stereoselective synthesis of (*E*)-alkene dipeptide isosteres.

1. Introduction

Ring-opening reactions of *N*-activated aziridines have been widely used for the synthesis of various biological compounds such as β-lactams, alkaloids, dipeptide isosteres and sphingosines. Ample precedents, in which nucleophilic reagents, including acids such as HCl, AcOH, TFA and TsOH, attack either of the two carbon atoms of simple aziridines to afford the corresponding ring-opened products, have been documented to date.[1] The regiospecific ring-opening reactions of *N*-2,4,6-trimethylphenylsulfonyl (Mts)-protected (and activated) aziridines possessing α,β-unsaturated esters by strong acids, such as methanesulfonic acid (MSA), TFA or HCl (Scheme 1) have been reported by us.[2] The MSA (or HCl)-mediated ring-opening reactions of *N*-Mts-γ,δ-*cis*-γ,δ-epimino-(*E*)-α,β-enoates ((*cis*-(*E*)) **2** yield δ-aminated γ-mesyloxy (or -chloro)-α,β-enoates **3**, which can be converted into (L-amino acid, D-amino acid)-type



Scheme 1. R^1, R^2, R^3=alkyl; Ar=4-methylphenyl or 2,4,6-trimethylphenyl, Ms=methanesulfonyl; reagents: (i) $Pd(PPh_3)_4$; (ii) $MeSO_3H$ in $CHCl_3$; (iii) HCl in 1,4-dioxane; (iv) TFA; (v) $R^3Cu(CN)MgCl.BF_3$; (vi) $R^3Cu(CN)MgCl\cdot 2LiCl$; (vii) $R^3Cu(CN)ZnI\cdot 2LiCl$; (viii) YH (weak acids, alcohols or thiols) and (ix)YH, TMSOTf.

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(*E*)-alkene dipeptide isosteres (EADIs) **4** via organocopper (or organozinc-copper)-mediated *anti*-S_N2' reactions.[3] On the other hand, organocopper (or organozinc-copper)-mediated *anti*-S_N2' reactions of *cis*-(*E*) isomers **2** exclusively provide (L,L)-type EADIs **5**. The utility of EADIs as potential biomimics of amide bonds in peptides has been intensively investigated.[4] The above ring-opening reactions are proven to be useful for the stereoselective synthesis of a set of two diastereomeric EADIs starting from an L-amino acid, in the combination with the convergently transforming reactions from four stereoisomeric γ,δ-epimino-α,β-enoates **1** into the single *cis*-(*E*) isomer **2** by a Pd(0)-catalyst.[5] However, treatment of these β-aziridinyl-α,β-enoates **2** with weak acids such as AcOH, alcohols or thiols does not yield the corresponding ring-opened products **6**. It might be due to insufficient activation of *N*-arylsulfonylaziridines. Thus, in the present study, we investigated whether the catalytic amount of Lewis acids such as TMSOTf has an effect on the above ring-opening reactions of β-aziridinyl-α,β-enoates with weak acids, alcohols or thiols. In addition, the feasibility of the ring-opening reactions of β-aziridinyl-α,β-enoates bearing no side-chain group at the δ-position was examined. Furthermore, we investigated the ring-opening reactions using solid supports and their application to stereoselective synthesis of EADIs.

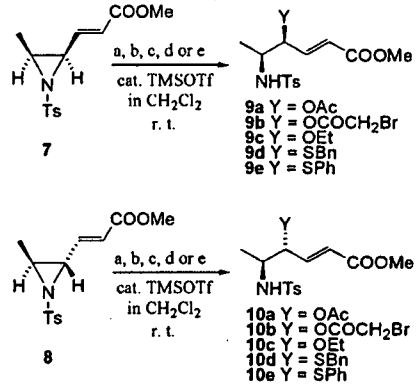
2. Results and discussion

2.1. Treatment of *N*-(4-methylphenylsulfonyl) (Ts)-γ,δ-epimino-(*E*)-α,β-enoates with weak acids, alcohols or thiols in the presence of Lewis acids

β-Aziridinyl-α,β-enoates, *cis*-(*E*)-enoate **7** and *trans*-(*E*)-enoate **8**, were prepared from Thr and D-*allo*-threonine, respectively, as previously reported by us.[6] These β-aziridinyl-α,β-enoates **7** and **8** did not react with weak acids such as AcOH, alcohols or thiols. Thus, examined was the effect of the addition of catalytic amount of Lewis acids such as TMSOTf on the ring-opening reactions with weak acids, alcohols or thiols. Treatment of **7** or **8** with AcOH, $BrCH_2COOH$, EtOH, BnSH or PhSH in the presence of catalytic amount of TMSOTf yielded the corresponding δ-aminated-γ-acyloxy (alkoxy or alkylthio)-α,β-enoates, **9a–e** or **10a–e**, exclusively and quantitatively, via the regioselective S_N2 ring-opening reaction at the γ-carbon position (Scheme 2, Table 1). Regiochemical assignments for products **9a–e** and **10a–e** were readily made by ^1H NMR (^1H–^1H COSY). The γ,δ-*syn* stereochemistry of **9a–e** and the γ,δ-*anti* stereochemistry of **10a–e** were based on X-ray analysis of **9a**. As a result, the addition of catalytic TMSOTf was proven to be efficient for the regio- and stereoselective ring-opening reactions with weak acids, alcohols or thiols as nucleophiles.

Table 1. Ring-opening reactions of β-aziridinyl-α,β-unsaturated esters by various nucleophiles in the presence of cat. TMSOTf

Substrate	YH	YH/TMSOTf (equiv)	Time (h)	Product	Yield (%)
7	AcOH	20/0.1	15	**9a**	90
7	$BrCH_2CO_2H$	10/0.1	15	**9b**	82
7	EtOH	3/0.3	7	**9c**	98
7	BnSH	10/0.1	1	**9d**	95
7	PhSH	10/0.1	1	**9e**	96
8	AcOH	20/0.1	15	**10a**	98
8	$BrCH_2CO_2H$	10/0.1	15	**10b**	86
8	EtOH	3/0.3	7	**10c**	99
8	BnSH	10/0.1	1	**10d**	90
8	PhSH	10/0.1	1	**10e**	99



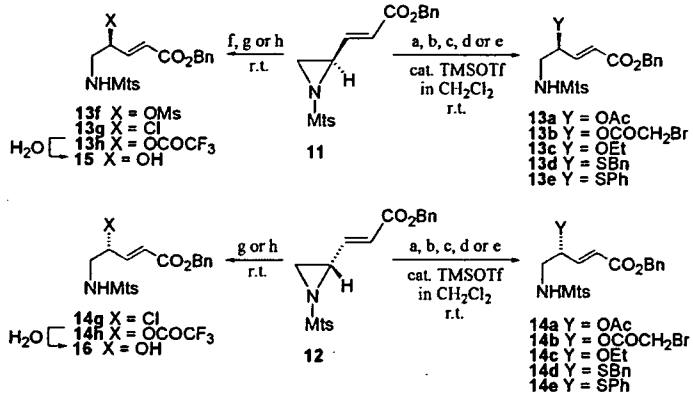
Scheme 2. Ts=4-methylphenysulfonyl; reagents: (a) AcOH; (b) $BrCH_2COOH$; (c) EtOH; (d) BnSH and (e) PhSH.

2.2. Ring-opening reactions of *N*-Mts-γ,δ-epimino-(*E*)-α,β-enoates having no side-chain group at the δ-position

Next, the feasibility of the regioselective ring-opening reactions of β-aziridinyl-α,β-enoates having no side-chain group at the δ-position was investivated. β-Aziridinyl-α,β-enoates, (4*R*,2*E*)-enoate **11** and (4*S*,2*E*)-enoate **12**, were prepared from Ser and D-Ser, respectively, according to our reported procedures. As shown in Scheme 3, exposure of **11** or **12** to several reactants afforded exclusively the corresponding δ-aminated-γ-acyloxy (alkoxy, alkylthio, mesyloxy or chloro,)-α,β-enoates, **13a–h** or **14a–h** in high yields, via the regioselective S_N2 ring-opening reaction at the γ-carbon position. Regiochemical assignments for products **13a–h** and **14a–h** were readily made by ^1H NMR. The stereochemistry at the γ-carbon position of **13a–h** and **14a–h** was based on X-ray analysis of **14g** and the analysis of **15** and **16** by the modified Mosher method.[7] As a result, the regio- and stereoselective ring-opening reactions of β-aziridinyl-α,β-enoates having no side-chain group at the δ-position were achieved by strong acids or by weak acids, alcohols or thiols in the addition of catalytic amount of TMSOTf (Table 2).

2.3. Synthesis of (Xaa, L-Asp)-type and (Xaa, D-Asp)-type EADIs

The stereoselective synthesis of a couple of diastereomeric EADIs from a single substrate of β-aziridinyl-α,β-enoate has been established as described in Section 1. One potential limitation to the use of these procedures for the synthesis of peptide mimetics is the introduction of various functional groups into the side chain (R^3) at the α-position. The stereoselective synthesis of (Xaa, L-Glu)-type and (Xaa, D-Glu)-type EADIs has been established by treatment of β-aziridinyl-α,β-enoates **2** and γ-chloro-α,β-enoates **3**, respectively, with organozinc-copper reagents (Scheme 1).[3] Next, we attempted to synthesize (Xaa, L-Asp)-type and (Xaa, D-Asp)-type EADIs. As shown in Scheme 4, orthoesterification of allylic alcohol **15**, which was obtained

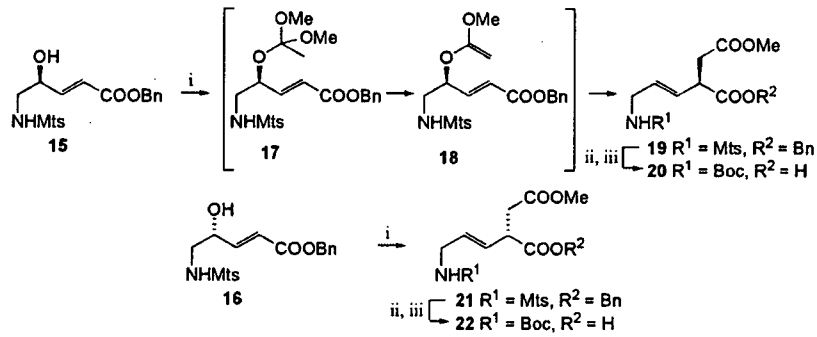


Scheme 3. Mts=2,4,6-trimethylphenysulfonyl; reagents: (a) AcOH; (b) $BrCH_2COOH$; (c) EtOH; (d) BnSH; (e) PhSH; (f) $MeSO_3H$ in $CHCl_3$; (g) HCl in 1,4-dioxane and (h) TFA.

Table 2. Ring-opening reactions of β-aziridinyl-α,β-unsaturated esters having no side-chain groups at the δ-position by various nucleophiles

Substrate	YH or XH	Solvent	YH or XH/TMSOTf (equiv)	Time	Product	Yield (%)
11	AcOH	CH_2Cl_2	20/0.1	15 h	**13a**	84
11	$BrCH_2CO_2H$	$CHCl_3$	10/0.1	15 h	**13b**	62
11	EtOH	CH_2Cl_2	3/0.3	7 h	**13c**	89
11	BnSH	CH_2Cl_2	10/0.1	1 h	**13d**	73
11	PhSH	CH_2Cl_2	10/0.1	1 h	**13e**	87
11	$MeSO_3H$	$CHCl_3$	10/—	10 min	**13f**	99
11	HCl	1,4-Dioxane	10/—	10 min	**13g**	87
11	CF_3CO_2H	—	>20/—	15 h	**13h**	72[a]
12	AcOH	CH_2Cl_2	20/0.1	15 h	**14a**	69
12	$BrCH_2CO_2H$	CH_2Cl_2	10/0.1	15 h	**14b**	69
12	EtOH	CH_2Cl_2	3/0.3	7 h	**14c**	77
12	BnSH	CH_2Cl_2	10/0.1	1 h	**14d**	69
12	PhSH	CH_2Cl_2	10/0.1	1 h	**14e**	81
12	HCl	1,4-Dioxane	10/—	10 min	**14g**	96
12	CF_3CO_2H	—	>20/—	15 h	**14h**	63[a]

[a] Isolated yield of **15** or **16**.

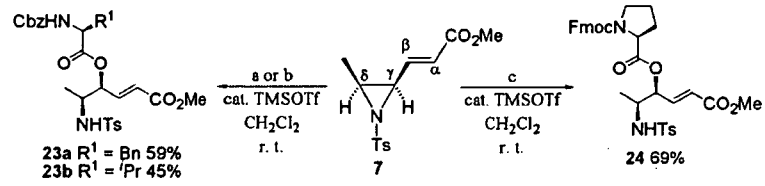


Scheme 4. Reagents: (i) $MeC(OMe)_3$, cat. PhCOOH, MS4Å, *o*-xylene; (ii) 1 M TMSBr-thioanisole/TFA; (iii) $(Boc)_2O$, Et_3N, THF.

by hydrolysis of γ-trifluoroacetate **13h** in Scheme 3, and the subsequent Claisen rearrangement[8] afforded an EADI, Mts–Gly–ψ[(*E*)-CH=CH]–L-Asp(OMe)–OBn, **19** in 34% yield. The enantiomeric EADI, Mts–Gly–ψ[(*E*)-CH=CH]–D-Asp(OMe)–OBn, **21** was also obtained from **16** in 19% yield in a similar way. The optical purities of **19** and **21** were found to be relatively low based on their HPLC analysis on chiral column: ee of **19**=33%; ee of **21**=43% on the contrary to our expectation. This might be attributable to instability of chair-like transition states. The improvement of these reactions in yields and optical purities is under investigation.

2.4. Ring-opening reactions of γ,δ-epimino-(*E*)-α,β-enoates by N^{α}-protected amino acids

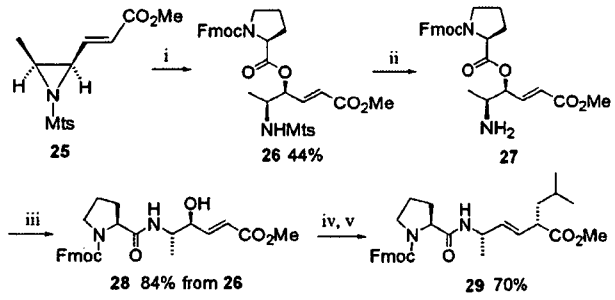
The feasibility of ring-opening reactions of γ,δ-epimino-(*E*)-α,β-enoates by N^{α}-protected amino acids was investigated, since N^{α}-protected amino acids are also weak carboxylic acids. It is thought that introduction of α-amino acids in the step of opening reactions of aziridine rings might lead to efficient synthesis of EADI-containing peptidomimetics. Treatment of aziridine **7** by N^{α}-Cbz-protected amino acids, N^{α}-Cbz-phenylalanine and N^{α}-Cbz-valine, in the



Scheme 5. Reagents: (a) Cbz–Phe–OH; (b) Cbz–Val–OH and (c) Fmoc–Pro–OH.

presence of catalytic amount of TMSOTf yielded the corresponding ring-opened products, **23a** and **b**, respectively (Scheme 5). Ring-opening reaction of **7** with N^{α}-Fmoc-proline in the presence of TMSOTf obtained **24**.

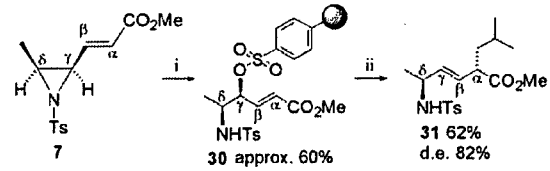
Next, this reaction was applied to the synthesis of Fmoc-Pro–Ala–ψ[(*E*)-CH=CH]–D-Leu–OMe **29** in combination with the *O*,*N*-acyl transfer reaction (Scheme 6). An *O*-acylated compound **26**, which was obtained by treatment of aziridine **25** with N^{α}-Fmoc-proline in the presence of TMSOTf, was subjected to deprotection of the N^{α}-Mts group using 1 M TMSBr-thioanisole/TFA[9] to yield **27**. Subsequent treatment of **27** with neutral phosphate buffer gave an *N*-acylated compound **28** based on the intramolecular *O*,*N*-acyl transfer.[10] *O*-Mesylation and *anti*-S_N2' type alkylation mediated by organocopper led to the stereoselective synthesis of a tripeptide mimetic **29**. The stereochemistry at the α-carbon position of **29** was determined by X-ray analysis.



Scheme 6. Reagents: (i) Fmoc–Pro–OH, cat. TMSOTf, CH_2Cl_2; (ii) 1 M TMSBr-thioanisole/TFA; (iii) pH 7.3 phosphate buffer, MeCN; (iv) MsCl, Et_3N, CH_2Cl_2 and (v) ^{t}BuCu(CN)MgCl·BF_3, THF.

2.5. Synthesis of EADIs from γ,δ-epimino-(*E*)-α,β-enoates using solid-phase techniques

To develop a convenient procedure for preparation of EADIs, simplification of isolation/purification of synthetic intermediate γ-sulfonates might be desirable and critical. Thus, ring-opening reactions of *N*-arylsulfonyl-γ,δ-epimino-(*E*)-α,β-enoates mediated by resin-bound sulfonic acid were applied to the synthesis of EADIs using solid-phase techniques. Treatment of β-aziridinyl-α,β-enoate **7** with toluenesulfonic acid resin (MP–TsOH, Argonaut Technologies) yielded a resin-bound γ-tosylate **30**, which was converted into an EADI **31** [Ts–Ala–ψ[(*E*)-CH=CH]–D-Leu–OMe] by organocopper reagents in 37% yield (Scheme 7). In this procedure, the resin-bound γ-tosylate **30** can be purified only by washing with solvents, suggesting that the present solid-phase techniques have the advantage of manipulation. However, the stereoselectivity of this reaction is not sufficiently high, compared to exceedingly high stereoselectivity in usual liquid techniques. Due to low reactivity of resin-bound γ-tosylates or basicity of organocopper reagents, **30** might partially return to the aziridine **7** via ring-closing, followed by organocopper-mediated alkylation to produce a diastereomer of **31** [Ts–Ala–ψ[(*E*)-CH=CH]–L-Leu–OMe]. The adjustment of loading amount of toluenesulfonic acid on resins and the improvement of linker/spacer units in these reactions might be required for the development of a convenient procedure for the synthesis of EADIs.



Scheme 7. Reagents: (i) MP–Ts–OH, CH_2Cl_2 and (ii) ^{t}BuCu(CN)MgCl·BF_3, THF.

3. Conclusion

In summary, the ring-opening reactions of β-aziridinyl-α,β-enoates with several nucleophiles involving alcohols, thiols and weak acids such as AcOH and N^{α}-protected amino acids in the presence of catalytic amount of Lewis acids such as TMSOTf have been fully investigated. The regio- and stereoselective S_N2' ring-opening at the γ-carbon position was observed. The combination of the ring-opening reactions with the Claisen rearrangement, the *O*,*N*-acyl transfer reaction and the organocopper-mediated *anti*-S_N2' type alkylation was efficiently applied to the synthesis of EADI-containing peptidomimetics. In addition, the ring-opening reactions of β-aziridinyl-α,β-enoates using solid-phase techniques were applied to the synthesis of EADIs.

4. Experimental

4.1. General

Melting points are uncorrected. ^{1}H NMR spectra were recorded using a JEOL EX-270, a Bruker AC 300, a JEOL AL-400 or a Bruker AM 600 spectrometer at 270, 300, 400 or 600 MHz ^{1}H frequency in $CDCl_3$, respectively. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Optical rotations were measured in $CHCl_3$ or H_2O with a JASCO DIP-360 digital polarimeter (Tokyo, Japan) or a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). The

X-ray analysis was carried out on a Rigaku AFC5R-RU200 Fourcircle diffractometer or a Rigaku RAXIS-RAPID Imaging Plate diffractometer. For flash column chromatography, silica gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed.

4.1.1. Methyl (2*E*,4*S*,5*S*)-4-acetyloxy-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 9a. To a stirred solution of the (*E*)-enoate **7** (50 mg, 0.169 mmol) in CH_2Cl_2 (0.5 cm^3) were added dropwise CH_3COOH (0.194 cm^3, 3.38 mmol) and CF_3SO_3TMS (0.00306 cm^3, 16.9 μmol) at rt, and the stirring was continued for 15 h. The mixture was purified by flash column chromatography over silica gel with *n*-hexane–EtOAc (3:1) to yield 54.2 mg (0.152 mmol, 90%) of compound **9a**, as colourless crystals, mp 108–110 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 53.95; H, 5.94; N, 3.76. $C_{16}H_{21}NO_6S$ requires C, 54.07; H, 5.96; N, 3.94%); $[\alpha]_D^{28}$ −22.1 (*c* 1.220 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.09 (3H, d, *J* 6.8, CMe), 2.05 (3H, s, CMe), 2.43 (3H, s, CMe), 3.59 (1H, m, 5-H), 3.72 (3H, s, OMe), 4.81 (1H, d, *J* 8.8, NH), 5.33 (1H, m, 4-H), 5.90 (1H, dd, *J* 15.8 and 1.6, CH=), 6.68 (1H, dd, *J* 15.8 and 5.5, CH=), 7.29 (2H, d, *J* 7.9, ArH), 7.74 (2H, d, *J* 8.3, ArH).

4.1.2. Methyl (2*E*,4*S*,5*S*)-4-(2-bromoacetyloxy)-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 9b. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-bromoacetyloxy-α,β-enoate **9b** (60.2 mg, 0.139 mmol, 82% yield) by treatment with $BrCH_2COOH$ (235 mg, 1.69 mmol) and CF_3SO_3TMS (0.00306 cm^3, 16.9 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 15 h.

Compound **9b**, colourless oil [Found (FAB): $(M+H)^+$, 434.0278. $C_{16}H_{21}BrNO_6S$ requires *M*+H, 434.0273]; $[\alpha]_D^{25}$ −42.7 (*c* 0.445 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.12 (3H, d, *J* 6.8, CMe), 2.43 (3H, s, CMe), 3.60–3.67 (1H, m, 5-H), 3.74 (3H, s, OMe), 3.79 (2H, s, CCH_2Br), 4.59 (1H, d, *J* 8.8, NH), 5.37 (1H, m, 4-H), 5.98 (1H, dd, *J* 15.8 and 1.5, CH=), 6.70 (1H, dd, *J* 15.7 and 5.6, CH=), 7.31 (2H, d, *J* 7.9, ArH), 7.74 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 436, 434 (MH^+, base peak), 391, 296, 259, 198, 167, 149 and 136.

4.1.3. Methyl (2*E*,4*S*,5*S*)-4-ethoxy-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 9c. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-ethoxy-α,β-enoate **9c** (56.4 mg, 0.165 mmol, 98% yield) by treatment with EtOH (0.0296 cm^3, 0.508 mmol) and CF_3SO_3TMS (0.00919 cm^3, 50.8 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 7 h.

Compound **9c**, colourless oil [Found (FAB): $(M+H)^+$, 342.1384. $C_{16}H_{24}NO_5S$ requires *M*+H, 342.1375]; $[\alpha]_D^{25}$ −20.8 (*c* 2.662 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.10–1.17 (6H, m, 2×CMe), 2.42 (3H, s, CMe), 3.26–3.41 (1H, m, 5-H and OC*H*HMe), 3.43–3.57 (1H, m, OCH*H*Me), 3.72 (3H, s, OMe), 3.77 (1H, m, 4-H), 4.87 (1H, d, *J* 7.5, NH), 5.93 (1H, dd, *J* 15.8 and 1.3, CH=), 6.64 (1H, dd, *J* 15.8 and 6.2, CH=), 7.28 (2H, d, *J* 7.9, ArH), 7.72 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 342 (MH^+), 296 (base peak), 279, 198, 184, 155 and 154.

4.1.4. Methyl (2*E*,4*S*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-4-(phenylmethylthio)hex-2-enoate 9d. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-phenylmethylthio-α,β-enoate **9d** (68.0 mg, 0.162 mmol, 96% yield) by treatment with BnSH (0.198 cm^3, 1.69 mmol) and CF_3SO_3TMS (0.00306 cm^3, 16.9 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 1 h.

Compound **9d**, colourless crystals, mp 100 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 60.05; H, 6.10; N, 3.32. $C_{21}H_{25}NO_4S_2$ requires C, 60.12; H, 6.01; N, 3.34%); $[\alpha]_D^{27}$ +73.2 (*c* 3.045 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.08 (3H, d, *J* 6.8, CMe), 2.42 (3H, s, CMe), 3.21 (1H, ddd, *J* 10.0, 5.2 and 0.5, 4-H), 3.47–3.58 (1H, m, 5-H), 3.49–3.68 (2H, m, SCH_2Ph), 3.74 (3H, s, OMe), 4.67 (1H, d, *J* 7.7, NH), 5.62 (1H, dd, *J* 15.5 and 0.7, CH=), 6.67 (1H, dd, *J* 15.5 and 10.0, CH=), 7.22–7.28 (7H, m, ArH and Ph), 7.66 (2H, d, *J* 8.3, ArH).

4.1.5. Methyl (2*E*,4*S*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-4-phenylthiohex-2-enoate 9e. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-phenylthio-α,β-enoate **9e** (66.0 mg, 0.163 mmol, 96%) by treatment with PhSH (0.174 cm^3, 1.69 mmol) and CF_3SO_3TMS (0.00306 cm^3, 16.9 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 1 h.

Compound **9e**, colourless crystals, mp 99–101 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 59.07; H, 5.49; N, 3.17. $C_{20}H_{23}NO_4S_2$ requires C, 59.23; H, 5.72; N, 3.45%); $[\alpha]_D^{28}$ +1.38 (*c* 3.610 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.16 (3H, d, *J* 6.8, CMe), 2.42 (3H, s, CMe), 3.58–3.68 (1H, m, 5-H), 3.70 (3H, s, OMe), 3.74 (1H, ddd, *J* 10.3, 5.6 and 0.8, 4-H), 4.80 (1H, d, *J* 7.8, NH), 5.63 (1H, dd, *J* 15.5 and 0.8, CH=), 6.75 (1H, dd, *J* 15.5 and 9.5, CH=), 7.26–7.32 (7H, m, ArH and Ph), 7.68 (2H, d, *J* 8.3, ArH).

4.1.6. Methyl (2*E*,4*R*,5*S*)-4-acetyloxy-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 10a. By use of a procedure identical with that described for the preparation of **9a** from **7**, the (*E*)-enoate **8** (50 mg, 0.169 mmol) was converted into the γ-acetyloxy-α,β-enoate **10a** (58.8 mg, 0.165 mmol, 98% yield).

Compound **10a**, colourless crystals, mp 99–101 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 54.03; H, 5.87; N, 3.85. $C_{16}H_{21}NO_6S$ requires C, 54.07; H, 5.96; N, 3.94%); $[\alpha]_D^{29}$ −8.80 (*c* 1.590 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.06 (3H, d, *J* 6.9, CMe), 2.02 (3H, s, CMe), 2.43 (3H, s, CMe), 3.61–3.67 (1H, m, 5-H), 3.73 (3H, s, OMe), 5.11 (1H, d, *J* 8.9, NH), 5.26 (1H, m, 4-H), 5.93 (1H, dd, *J* 15.8 and 1.7, CH=), 6.74 (1H, dd, *J* 15.8 and 5.1, CH=), 7.29–7.32 (2H, d, *J* 8.0, ArH), 7.74 (2H, d, *J* 8.4, ArH).

4.1.7. Methyl (2*E*,4*R*,5*S*)-4-(2-bromoacetyloxy)-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 10b. By use of a procedure identical with that described for the preparation of **9b** from **7**, the (*E*)-enoate **8** (50 mg, 0.169 mmol) was converted into the γ-bromoacetyloxy-α,β-enoate **10b** (63.6 mg, 0.146 mmol, 87% yield).

Compound **10b**, colourless oil [Found (FAB): $(M+H)^+$, 434.0262. $C_{16}H_{21}BrNO_6S$ requires *M*+H, 434.0273]; $[\alpha]_D^{26}$ −18.5 (*c* 3.085 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.06 (3H, d, *J* 6.9, CMe), 2.44 (3H, s, CMe), 3.64–3.70 (1H, m, 5-H), 3.73 (3H, s, OMe), 3.81 (2H, d, *J* 2.6, CCH_2Br), 5.27 (1H, d, *J* 9.0, NH), 5.32 (1H, m, 4-H), 6.02 (1H, dd, *J* 15.8 and 1.7, CH=), 6.75 (1H, dd, *J* 15.8 and 5.1, CH=), 7.32 (2H, d, *J* 7.9, ArH), 7.74 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 436, 434 (MH^+), 391, 296, 264, 250 (base peak), 198, 167, 155 and 110.

4.1.8. Methyl (2*E*,4*R*,5*S*)-4-ethoxy-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 10c. By use of a procedure identical with that described for the preparation of **9c** from **7**, the (*E*)-enoate **8** (50 mg, 0.169 mmol) was converted into the γ-ethoxy-α,β-enoate **10c** (57.4 mg, 0.168 mmol, 99% yield).

Compound **10c**, colourless oil [Found (FAB): $(M+H)^+$, 342.1367. $C_{16}H_{24}NO_5S$ requires *M*+H, 342.1375]; $[\alpha]_D^{23}$ −24.2 (*c* 2.768 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 0.98 (3H, d, *J* 6.8, CMe), 1.13 (3H, t, *J* 7.0, CMe), 2.43 (3H, s, CMe), 3.19–3.29 (1H, m, OC*H*HMe), 3.39–3.52 (2H, m, OCH*H*Me and 5-H), 3.74 (3H, s, OMe), 3.85 (1H, m, 4-H), 4.91 (1H, d, *J* 8.9, NH), 5.95 (1H, dd, *J* 15.8 and 1.5, CH=), 6.70 (1H, dd, *J* 15.8 and 5.6, CH=), 7.30 (2H, d, *J* 7.9, ArH), 7.76 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 342 (MH^+), 310, 296, 282, 264, 256 (base peak), 198, 186, 155, 144 and 110.

4.1.9. Methyl (2*E*,4*R*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-4-(phenylmethylthio)hex-2-enoate 10d. By use of a procedure identical with that described for the preparation of **9d** from **7**, the (*E*)-enoate **8** (50 mg, 0.169 mmol) was converted into the γ-phenylmethylthio-α,β-enoate **10d** (64.1 mg, 0.153 mmol, 90% yield).

Compound **10d**, colourless oil [Found (FAB): $(M+H)^+$, 420.1298. $C_{21}H_{26}NO_4S_2$ requires *M*+H, 420.1303]; $[\alpha]_D^{27}$ −144.2 (*c* 3.210 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.06 (3H, d, *J* 6.7, CMe), 2.42 (3H, s, CMe), 3.13 (1H, ddd, *J* 9.4, 4.3 and 0.5, 4-H), 3.44–3.67 (2H, m, SCH_2Ph), 3.47–3.54 (1H, m, 5-H), 3.73 (3H, s, OMe), 4.90 (1H, d, *J* 9.0, NH), 5.68 (1H, dd, *J* 15.4 and 0.9, CH=), 6.65 (1H, dd, *J* 15.4 and 9.4, CH=), 7.21–7.34 (7H, m, ArH and Ph), 7.66 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 420 (MH^+), 391, 249, 222 (base peak), 198, 155, 149 and 109.

4.1.10. Methyl (2*E*,4*R*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-4-phenylthiohex-2-enoate 10e. By use of a procedure identical with that described for the preparation of **9e** from **7**, the (*E*)-enoate **8** (50 mg, 0.169 mmol) was converted into the γ-phenylthio-α,β-enoate **10e** (68.6 mg, 0.169 mmol, 99%).

Compound **10e**, colourless oil [Found (FAB): $(M+H)^+$, 406.1133. $C_{20}H_{24}NO_4S_2$ requires *M*+H, 406.1147]; $[\alpha]_D^{28}$ −117.7 (*c* 3.730 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.15 (3H, d, *J* 6.8, CMe), 2.41 (3H, s, CMe), 3.59 (1H, ddd, *J* 8.8, 4.0 and 1.0, 4-H), 3.66–3.77 (1H, m, 5-H), 3.70 (3H, s, OMe), 5.07 (1H, d, *J* 9.2, NH), 5.66 (1H, dd, *J* 15.4 and 1.1, CH=), 6.76 (1H, dd, *J* 15.4 and 8.8, CH=), 7.19–7.27 (7H, m, ArH and Ph), 7.73 (2H, d, *J* 8.3, ArH); *m/z* (FABLRMS) 406 (MH^+), 391 (base peak), 374, 296, 235, 198, 175, 155 and 149.

4.1.11. Phenylmethyl (2*E*,4*S*)-4-acetyloxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13a. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **11** (50 mg, 0.130 mmol) was converted into the γ-acetyloxy-α,β-enoate **13a** (48.3 mg, 0.108 mmol, 84%) by treatment with CH_3COOH (0.149 cm^3, 2.60 mmol) and CF_3SO_3TMS (0.00235 cm^3, 13.0 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 15 h.

Compound **13a**, colourless crystals, mp 106 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 62.14; H, 6.10; N, 2.84. $C_{23}H_{27}NO_6S$ requires C, 62.00; H, 6.11; N, 3.14%); $[\alpha]_D^{26}$ +1.65 (*c* 1.208 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.03 (3H, s, CMe), 2.29 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 3.08–3.17 (1H, m, C*H*H), 3.19–3.28 (1H, m, CH*H*), 4.75 (1H, t, *J* 6.4, NH), 5.17 (2H, s, OCH_2Ph), 5.33–5.39 (1H, m, 4-H), 5.96 (1H, dd, *J* 15.8 and 1.6, CH=), 6.72 (1H, dd, *J* 15.8 and 5.1, CH=), 6.94 (2H, s, ArH), 7.33–7.39 (5H, m, Ph).

4.1.12. Phenylmethyl (2*E*,4*S*)-4-(2-bromoacetyloxy)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13b. By use of a procedure similar to that described for the preparation of **9b** from **7**, the (*E*)-enoate **11** (200 mg, 0.519 mmol) was converted into the γ-bromoacetyloxy-α,β-enoate **13b** (167.7 mg, 0.320 mmol, 62%) by treatment with $BrCH_2COOH$ (1.44 g, 10.4 mmol) and CF_3SO_3TMS (0.00940 cm^3, 51.9 μmol) in $CHCl_3$ (5 cm^3) at rt for 15 h.

Compound **13b**, colourless crystals, mp 91–93 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 52.70; H, 5.07; N, 2.69. $C_{23}H_{26}BrNO_6S$ requires C, 52.68; H, 5.00; N, 2.67%); $[\alpha]_D^{29}$ +3.29 (*c* 4.250 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 3.13–3.22 (1H, m, C*H*H), 3.26–3.34 (1H, m, CH*H*), 3.78 (2H, s, CCH_2Br), 4.91 (1H, t, *J* 6.6, NH), 5.18 (2H, s, OCH_2Ph), 5.39–5.45 (1H, m, 4-H), 6.04 (1H, dd, *J* 15.8 and 1.6, CH=), 6.73 (1H, dd, *J* 15.8 and 5.2, CH=), 6.95 (2H, s, ArH), 7.36–7.38 (5H, m, Ph).

4.1.13. Phenylmethyl (2*E*,4*S*)-4-ethoxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13c. By use of a procedure similar to that described for the preparation of **9c** from **7**, the (*E*)-enoate **11** (50 mg, 0.130 mmol) was converted into the γ-ethoxy-α,β-enoate **13c** (49.9 mg, 0.117 mmol, 89%) by treatment with EtOH (0.0227 cm^3, 0.390 mmol) and CF_3SO_3TMS (0.00235 cm^3, 13.0 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 7 h.

Compound **13c**, colourless oil [Found (FAB): $(M+H)^+$, 432.1859. $C_{23}H_{30}NO_5S$ requires *M*+H, 432.1844]; $[\alpha]_D^{25}$ +14.66 (*c* 1.705 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.16 (3H, t, *J* 7.0, CMe), 2.29 (3H, s, CMe), 2.62 (6H, s, 2×CMe), 2.74–2.83 (1H, m, C*H*H), 3.07–3.16 (1H, m, CH*H*), 3.21–3.31 (1H, m, OC*H*HMe), 3.44–3.54 (1H, m, OCH*H*Me), 3.91 (1H, m, 4-H), 4.95 (1H, br, NH), 5.16 (2H, dd, *J* 13.7 and 12.4, OCH_2Ph), 6.00 (1H, dd, *J* 15.8 and 1.3, CH=), 6.69 (1H, dd, *J* 15.8 and 6.0, CH=), 6.94 (2H, s, ArH), 7.31–7.38 (5H, m, Ph); *m/z* (FABLRMS) 432 (MH^+), 324 (base peak), 302, 261, 212, 183, 149 and 119.

4.1.14. Phenylmethyl (2*E*,4*S*)-4-(phenylmethylthio)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13d. By use of a procedure similar to that described for the preparation of **9d** from **7**, the (*E*)-enoate **11** (50 mg, 0.130 mmol) was converted into the γ-phenylmethylthio-α,β-enoate **13d** (48.0 mg, 0.0942 mmol, 73%) by treatment with BnSH (0.152 cm^3, 1.30 mmol) and CF_3SO_3TMS (0.00235 cm^3, 13.0 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 1 h.

Compound **13d**, colourless oil [Found (FAB): $(M+H)^+$, 510.1765. $C_{28}H_{32}NO_4S_2$ requires *M*+H, 510.1772]; $[\alpha]_D^{25}$ +81.8 (*c* 1.198 in $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 2.28 (3H, s, CMe), 2.55 (6H, s, 2×CMe), 3.00–3.13 (2H, m, CH_2), 3.23 (1H, br, 4-H), 3.53 (1H, d, *J* 13.6, SC*H*HPh), 3.53 (1H, d, *J* 13.5, SCH*H*Ph), 4.83 (1H, t, *J* 6.3, NH), 5.17 (2H, dd, *J* 15.2 and 12.3, OCH_2Ph), 5.63 (1H, d, *J* 15.5, CH=), 6.64 (1H, dd, *J* 15.5 and 9.0, CH=), 6.90 (2H, s, ArH), 7.20–7.30 (5H, m, Ph), 7.34–7.40 (5H, m, Ph); *m/z* (FABLRMS) 531, 510 (MH^+), 402, 298, 282, 256 (base peak), 207, 183, 154 and 119.

4.1.15. Phenylmethyl (2*E*,4*S*)-4-phenylthio-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13e. By use of a procedure similar to that described for the preparation of **9e** from **7**, the (*E*)-enoate **11** (50 mg, 0.130 mmol) was converted into the γ-phenylthio-α,β-enoate **13e** (55.7 mg, 0.112 mmol, 87%) by treatment with PhSH (0.133 cm^3, 1.30 mmol) and CF_3SO_3TMS (0.00235 cm^3, 13.0 μmol) in CH_2Cl_2 (0.5 cm^3) at rt for 1 h.

Compound **13e**, colourless crystals, mp 96–97 °C [from *n*-hexane–Et_2O (3:1)] [Found (FAB): $(M+H)^+$, 496.1629. $C_{27}H_{30}NO_4S_2$ requires *M*+H, 496.1616]; $[\alpha]_D^{25}$ +42.8 (*c* 1.495 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.28 (3H, s, CMe), 2.59 (6H, s, 2×CMe), 3.06–3.24 (2H, m, CH_2), 3.59–3.66 (1H, m, 4-H), 5.08 (1H, t, *J* 6.4, NH), 5.56 (2H, s, OCH_2Ph), 5.58 (1H, dd, *J* 15.6 and 1.0, CH=), 6.71 (1H, dd, *J* 15.6 and 8.7, CH=), 6.92 (2H, s, ArH), 7.22–7.29 (5H, m, Ph), 7.29–7.39 (5H, m, Ph); *m/z* (FABLRMS) 496 (MH^+), 444, 388, 386, 330, 296 (base peak), 284, 256, 207, 183, 149 and 119.

4.1.16. Reaction of phenylmethyl (2*E*,4*R*)-3-(2-((2,4,6-trimethylphenyl)sulfonyl)-2-aziridinyl)prop-2-enoate 11 with MSA in $CHCl_3$.

4.1.16.1. Phenylmethyl (2*E*,4*S*)-4-(methylsulfonyloxy)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13f. To a stirred solution of (*E*)-enoate **9** (7 mg, 0.0182 mmol) in $CHCl_3$ (0.182 cm^3) was added dropwise MSA (0.0118 cm^3, 0.182 mmol) at rt, and the stirring was continued for 10 min. The mixture was extracted with EtOAc and the extract was washed successively with aq 5% citric acid, brine, aq 5% $NaHCO_3$, brine, and dried over $MgSO_4$. Concentration under reduced pressure gave the crude mesyl compound **13f**, as a colourless oil (crude), δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.61 (6H, s, 2×CMe), 3.07 (3H, s, SMe), 3.13–3.30 (2H, m, CH_2), 5.04 (1H, t, *J* 6.7, NH), 5.18 (2H, s, OCH_2Ph), 5.22–5.30 (1H, m, 4-H), 6.13 (1H, dd, *J* 15.7 and 1.5, CH=), 6.78 (1H, dd, *J* 15.7 and 5.7, CH=), 6.95 (2H, s, ArH), 7.33–7.39 (5H, m, Ph); *m/z* (FABLRMS) 482 (MH^+), 391, 363, 296 (base peak), 279, 261, 212, 167 and 149.

4.1.17. Reaction of phenylmethyl (2*E*,4*R*)-3-(2-((2,4,6-trimethylphenyl)sulfonyl)-2-aziridinyl)prop-2-enoate 11 with HCl-1,4-dioxane.

4.1.17.1. Phenylmethyl (2*E*,4*S*)-4-chloro-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 13g. The (*E*)-enoate **11** (50 mg, 0.130 mmol) was dissolved in 4 M HCl-1,4-dioxane (0.325 cm^3, 1.30 mmol) at rt, and the solution was stirred for 10 min followed by extraction with EtOAc. The extract was washed successively with aq 5% citric acid, brine, aq 5% $NaHCO_3$, brine and dried over $MgSO_4$. Concentration under reduced pressure gave a crystalline residue, which was purified by chromatography over silica gel with *n*-hexane–EtOAc (3:1) to yield 47.5 mg (0.113 mmol, 87%) of compound **13g** as colourless crystals, mp 78–79 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 59.60; H, 5.92; N, 3.21. $C_{13}H_{17}NO_4S$ requires C, 59.78; H, 5.73; N, 3.32%); $[\alpha]_D^{25}$ −26.2 (*c* 1.185 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.62 (6H, s, 2×CMe), 3.14–3.23 (1H, m, C*H*H), 3.30–3.38 (1H, m, CH*H*), 4.45–4.52 (1H, m, 4-H), 4.97 (1H, t, *J* 5.8, NH), 5.18 (2H, s, OCH_2Ph), 6.04 (1H, dd, *J* 15.4 and 1.2, CH=), 6.77 (1H, dd, *J* 15.4 and 7.4, CH=), 6.95 (2H, s, ArH), 7.33–7.39 (5H, m, Ph).

4.1.18. Reaction of phenylmethyl (2*E*,4*R*)-3-(2-((2,4,6-trimethylphenyl)sulfonyl)-2-aziridinyl)prop-2-enoate 11 with TFA.

4.1.18.1. Phenylmethyl (2*E*,4*S*)-4-hydroxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 15. The (*E*)-enoate **11** (1 g, 2.60 mmol) was dissolved in TFA (10 cm^3) at rt, and the solution was stirred for 15 h. Concentration under reduced pressure gave a crude product **13h** as an oil. Hydrolysis and purification by flash chromatography over silica gel with *n*-hexane–EtOAc (4:1) afforded the hydrolyzate **15** (752 mg, 1.86 mmol, 72% yield based on **11**) as an oil.

Compound **13h**, colourless oil (crude), δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.59 (6H, s, 2×CMe), 3.26–3.32 (2H, br, CH_2), 5.14 (1H, t, *J* 6.7, NH), 5.17 (2H, s, OCH_2Ph), 5.47–5.53 (1H, m, 4-H), 6.03 (1H, dd, *J* 15.8 and 1.5, CH=), 6.74 (1H, dd, *J* 15.8 and 5.8, CH=), 6.95 (2H, s, ArH), 7.31–7.38 (5H, m, Ph); *m/z* (FABLRMS) 500 (MH^+), 404, 302 (base peak), 212, 183, 137 and 119.

Compound **15**, colourless oil [Found (FAB): $(M+H)^+$, 404.1527. $C_{21}H_{26}NO_5S$ requires *M*+H, 404.1532]; $[\alpha]_D^{22}$ −2.59 (*c* 3.855 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.28 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 2.83 (1H, m, C*H*H), 3.13 (1H, m, CH*H*), 4.12 (1H, m, 4-H), 5.16 (2H, s, OCH_2Ph), 5.22 (1H, t, *J* 5.8, NH), 6.13 (1H, dd, *J* 15.7 and 1.8, CH=), 6.82 (1H, dd, *J* 15.6 and 4.4, CH=), 6.94 (2H, s, ArH), 7.31–7.36 (5H, m, Ph); *m/z* (FABLRMS) 404 (MH^+), 302, 212, 183, 167, 149 (base peak) and 119.

4.1.19. Phenylmethyl (2*E*,4*R*)-4-acetyloxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14a. By use of a procedure identical with that described for the preparation of **13a** from **11**, the (*E*)-enoate **12** (50 mg, 0.130 mmol) was converted into the γ-acetyloxy-α,β-enoate **14a** (39.9 mg, 0.0896 mmol, 69%).

Compound **14a**, colourless crystals, mp 84–86 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 61.73; H, 6.05; N, 2.95.

$C_{23}H_{27}NO_6S$ requires C, 62.00; H, 6.11; N, 3.14%); $[\alpha]_D^{26}$ −2.00 (*c* 0.998 in $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 2.03 (3H, s, CMe), 2.29 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 3.10–3.23 (2H, m, CH_2), 4.92 (1H, m, NH), 5.16 (2H, s, CH_2), 5.35 (1H, m, 4-H), 5.96 (1H, dd, *J* 15.8 and 1.7, CH=), 6.72 (1H, dd, *J* 15.8 and 5.3, CH=), 6.94 (2H, s, ArH), 7.37 (5H, m, ArH).

4.1.20. Phenylmethyl (2*E*,4*R*)-4-(2-bromoacetyloxy)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14b. By use of a procedure similar to that described for the preparation of **13b** from **11**, the (*E*)-enoate **12** (100 mg, 0.260 mmol) was converted into the γ-bromoacetyloxy-α,β-enoate **14b** (93.6 mg, 0.178 mmol, 69%) by treatment with $BrCH_2COOH$ (721 mg, 5.19 mmol) and CF_3SO_3TMS (0.00470 cm^3, 26.0 μmol) in CH_2Cl_2 (1 cm^3) at rt for 15 h.

Compound **14b**, colourless crystals, mp 87–88 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 52.70; H, 5.26; N, 2.75. $C_{23}H_{26}BrNO_6S$ requires C, 52.68; H, 5.00; N, 2.67%); $[\alpha]_D^{28}$ −2.56 (*c* 3.905 in $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 3.16–3.28 (2H, m, CH_2), 3.79 (2H, s, CCH_2Br), 5.17 (2H, s, OCH_2Ph), 5.29 (1H, br, NH), 5.43 (1H, m, 4-H), 6.04 (1H, dd, *J* 15.8 and 1.7, CH=), 6.74 (1H, dd, *J* 15.8 and 5.3, CH=), 6.94 (2H, s, ArH), 7.36 (5H, m, Ph); *m/z* (FAB-LRMS) 432 (MH^+), 324, 302, 250 (base peak), 212, 183, 149 and 119.

4.1.21. Phenylmethyl (2*E*,4*R*)-4-ethoxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14c. By use of a procedure identical with that described for the preparation of **13c** from **11**, the (*E*)-enoate **12** (50 mg, 0.130 mmol) was converted into the γ-ethoxy-α,β-enoate **14c** (43.1 mg, 0.0999 mmol, 79%).

Compound **14c**, colourless oil [Found (FAB): $(M+H)^+$, 432.1856. $C_{23}H_{29}NO_5S$ requires *M*+H, 431.1766]; $[\alpha]_D^{25}$ −14.51 (*c* 2.205 in $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 1.16 (3H, t, *J* 6.9, CMe), 2.29 (3H, s, CMe), 2.62 (6H, s, 2×CMe), 2.77 (1H, m, C*H*H), 3.11 (1H, m, CH*H*), 3.22–3.28 (1H, m, OC*H*HMe), 3.46–3.52 (1H, m, OCH*H*Me), 3.91 (1H, m, 4-H), 4.98 (1H, m, NH), 5.16 (2H, s, OCH_2Ph), 6.00 (1H, dd, *J* 15.8 and 1.3, CH=), 6.69 (1H, dd, *J* 15.8 and 6.3, CH=), 6.95 (2H, s, ArH), 7.37 (5H, m, Ph); *m/z* (FABLRMS) 432 (MH^+), 324, 302, 250 (base peak), 212, 183, 149 and 119.

4.1.22. Phenylmethyl (2*E*,4*R*)-4-(phenylmethylthio)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14d. By use of a procedure similar to that described for the preparation of **13d** from **11**, the (*E*)-enoate **12** (50 mg, 0.130 mmol) was converted into the γ-phenylmethylthio-α,β-enoate **14d** (45.9 mg, 0.0901 mmol, 69%).

Compound **14d**, colourless oil [Found (FAB): $(M+H)^+$, 510.1760. $C_{28}H_{32}NO_4S_2$ requires *M*+H, 510.1772]; $[\alpha]_D^{25}$ −74.2 (*c* 1.145 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 2.28 (3H, s, CMe), 2.55 (6H, s, 2×CMe), 3.01–3.12 (2H, m, CH_2), 3.20–3.26 (1H, m, 4-H), 3.52–3.67 (2H, m, SCH_2Ph), 4.84 (1H, t, *J* 6.3, NH), 5.18 (2H, s, OCH_2Ph), 5.63 (1H, d, *J* 15.4, CH=), 6.64 (1H, dd, *J* 15.6 and 8.8, CH=), 6.90 (2H, s, ArH), 7.21–7.29 (5H, m, Ph) 7.38 (5H, m, Ph); *m/z* (FABLRMS) 510 (MH^+), 408, 402 (base peak), 311, 302, 221, 207, 183, 149 and 119.

4.1.23. Phenylmethyl (2*E*,4*R*)-4-phenylthio-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14e. By use of a procedure similar to that described for the preparation of **13e** from **11**, the (*E*)-enoate **12** (50 mg, 0.130 mmol) was converted into the γ-phenylthio-α,β-enoate **14e** (55.7 mg, 0.112 mmol, 87%).

Compound **14e**, colourless crystals, mp 99 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 65.19; H, 5.71; N, 2.82. $C_{27}H_{29}NO_4S_2$ requires C, 65.43; H, 5.90; N, 2.83%); $[\alpha]_D^{26}$ −41.2 (*c* 2.575 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.60 (6H, s, 2×CMe), 3.08–3.20 (2H, m, CH_2), 3.62 (1H, m, 4-H), 5.02 (1H, t, *J* 6.4, NH), 5.14 (2H, s, OCH_2Ph), 5.59 (1H, dd, *J* 15.5 and 1.0, CH=), 6.71 (1H, dd, *J* 15.4 and 8.8, CH=), 6.93 (2H, s, ArH), 7.24–7.27 (5H, m, Ph), 7.35 (5H, m, Ph).

4.1.24. Reaction of phenylmethyl (2*E*,4*S*)-3-(2-((2,4,6-trimethylphenyl)sulfonyl)-2-aziridinyl)prop-2-enoate 12 with HCl-1,4-dioxane.

4.1.24.1. Phenylmethyl (2*E*,4*R*)-4-chloro-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 14g. By use of a procedure identical with that described for the preparation of **13g** from **11**, the (*E*)-enoate **12** (50 mg, 0.130 mmol) was converted into the γ-chloro-α,β-enoate **14g** (52.7 mg, 0.125 mmol, 96%).

Compound **14g**, colourless crystals, mp 80–81 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 59.53; H, 5.73; N, 3.40. $C_{13}H_{17}NO_4S$ requires C, 59.78; H, 5.73; N, 3.32%); $[\alpha]_D^{25}$ +25.9 (*c* 1.390 in $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 2.30 (3H, s, CMe), 2.62 (6H, s, 2×CMe), 3.13–3.23 (1H, m, C*H*H), 3.28–3.38 (1H, m, CH*H*), 4.46 (1H, m, 4-H), 5.03 (1H, t, *J* 7.3, NH), 5.18 (2H, s, CH_2), 6.04 (1H, dd, *J* 15.5 and 1.0, CH=), 6.78 (1H, dd, *J* 15.5 and 7.6, CH=), 6.95 (2H, s, ArH), 7.37 (5H, m, ArH).

4.1.25. Reaction of phenylmethyl (2*E*,4*S*)-3-(2-((2,4,6-trimethylphenyl)sulfonyl)-2-aziridinyl)prop-2-enoate 12 with TFA.

4.1.25.1. Phenylmethyl (2*E*,4*R*)-4-hydroxy-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)pent-2-enoate 16. By use of a procedure identical with that described for the preparation of **13h** from **11**, the (*E*)-enoate **12** (200 mg, 0.519 mmol) was converted into the hydrolyzate **16** (131 mg, 0.325 mmol, 63% yield based on **12**) via the γ-trifluoroacetoxy-α,β-enoate **14h**.

Compound **14h**, colourless oil (crude), δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.59 (6H, s, 2×CMe), 3.20–3.38 (2H, m, CH_2), 4.99 (1H, t, *J* 6.7, NH), 5.18 (2H, s, CH_2), 5.46–5.52 (1H, m, 4-H), 6.03 (1H, dd, *J* 15.8 and 1.5, CH=), 6.73 (1H, dd, *J* 15.8 and 5.8, CH=), 6.94 (2H, s, ArH), 7.32–7.40 (5H, m, ArH); *m/z* (FABLRMS) 500 (MH^+), 404, 398, 302, 273 (base peak), 212, 183, 167 and 119.

Compound **16**, colourless oil [Found (FAB): $(M+H)^+$, 404.1521. $C_{21}H_{26}NO_5S$ requires *M*+H, 404.1532]; $[\alpha]_D^{22}$ +2.56 (*c* 2.340 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.62 (6H, s, 2×CMe), 2.85 (1H, m, C*H*H), 3.16 (1H, m, CH*H*), 4.42 (1H, m, 4-H), 4.98 (1H, s, NH), 5.17 (2H, s, CH_2), 6.13 (1H, dd, *J* 15.7 and 1.7, CH=),

6.82 (1H, dd, *J* 15.6 and 4.5, CH=), 6.95 (2H, s, ArH), 7.32–7.38 (5H, m, ArH); *m/z* (FABLRMS) 426, 404 (MH^+), 391, 302, 222 (base peak), 212, 183, 149 and 119.

4.1.26. Mts-Gly-ψ[(*E*)-CH=CH]-L-Asp(OMe)-OBn [methyl phenylmethyl (1*E*,2*R*)-2-(3-(((2,4,6-trimethylphenyl)sulfonyl)amino)prop-1-enyl)butane-1,4-dioate] 19. Allylic acetate **15** (2.30 g, 5.69 mmol), trimethyl orthoacetate (7.25 cm^3, 56.9 mmol), benzoic acid (139 mg, 1.14 mmol), and dried molecular sieves (4 Å, powder, 2.85 g) were mixed in 75 cm^3 *o*-xylene and then refluxed for 3 days. The mixture was cooled to rt and purified by chromatography over silica gel with *n*-hexane–EtOAc (3:1) to give 896 mg (1.95 mmol, 34%) of the mixture of Mts-Gly-ψ[(*E*)-CH=CH]-L-Asp(OMe)-OBn **19** and its enantiomer **21** (66.5: 33.5) as a colourless oil [Found (FAB): $(M+H)^+$, 460.1802. $C_{24}H_{30}NO_6S$ requires *M*+H, 460.1794]; δ_H (400 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.37–2.43 (1H, dd, *J* 16.6 and 5.9, C*H*H), 2.60 (6H, s, 2×CMe), 2.63–2.76 (1H, dd, *J* 16.6 and 8.5, CH*H*), 3.45–3.51 (3H, m, CH and CH_2), 3.62 (3H, s, OMe), 4.44 (1H, t, *J* 6.1, NH), 5.11 (2H, s, OCH_2Ph), 5.45–5.50 (1H, m, CH=), 5.57–5.63 (1H, dd, *J* 15.6 and 7.6, CH=), 6.94 (2H, s, ArH), 7.33 (5H, m, Ph); *m/z* (FABLRMS), 460 (MH^+), 352 (base peak), 183, 136, 119.

4.1.27. Mts-Gly-ψ[(*E*)-CH=CH]-D-Asp(OMe)-OBn [methyl phenylmethyl (1*E*,2*S*)-2-(3-(((2,4,6-trimethylphenyl)sulfonyl)amino)prop-1-enyl)butane-1,4-dioate] 21. By use of a procedure identical with that described for the preparation of **19** from **15**, the allylic acetate **16** (1.88 g, 4.64 mmol) was converted into the mixture (398 mg, 0.866 mmol, 19%) of Mts-Gly-ψ[(*E*)-CH=CH]-D-Asp-(OMe)-OBn **21** and its enantiomer **19** (71.5: 28.5) as a colourless oil [Found (FAB): $(M+H)^+$, 460.1801. $C_{24}H_{30}NO_6S$ requires *M*+H, 460.1794]; δ_H (300 MHz; $CDCl_3$) 2.29 (3H, s, CMe), 2.41 (1H, dd, *J* 16.6 and 5.8, C*H*H), 2.60 (6H, s, 2×CMe), 2.73 (1H, dd, *J* 16.7 and 8.7, CH*H*), 3.43–3.47 (1H, m, CH), 3.48–3.52 (2H, t, *J* 6.3, CH_2), 3.62 (3H, s, OMe), 4.41 (1H, t, *J* 6.4, NH), 5.12 (2H, s, OCH_2Ph), 5.42–5.51 (1H, m, CH=), 5.61 (1H, dd, *J* 15.5 and 7.6, CH=), 6.95 (2H, s, ArH), 7.29–7.39 (5H, m, Ph); *m/z* (FABLRMS), 460 (MH^+, base peak), 307, 289, 243, 154, 136.

4.1.28. Boc-Gly-ψ[(*E*)-CH=CH]-L-Asp(OMe)-OH [(3*E*,2*R*)-5-((*tert*-butoxy)carbonylamino)-2-((methoxycarbonyl)methyl)pent-3-enoic acid] 20. Mts-Gly-ψ[(*E*)-CH=CH]-L-Asp(OMe)-OBn **19** (48.7 mg, 0.106 mmol, the enantiomixture with **21**, ee=33%) was treated with 1 M TMSBr-thioanisole/TFA (2.5 cm^3) in the presence of *m*-cresol (0.122 cm^3, 1.17 mmol) and 1,2-ethanedithiol (0.050 cm^3, 0.595 mmol) at 0 °C with warming to rt for 15 h. After concentration with N_2 gas, ice-cold Et_2O was added. The resulting precipitate was collected by centrifugation, and the precipitate was washed three times with Et_2O, and dissolved with H_2O (0.150 cm^3). The solution was treated with 3 M $(Boc)_2O$ in THF (0.050 cm^3) in the presence of Et_3N (0.0334 cm^3, 0.240 mmol) at 0 °C with warming to rt for 15 h. The mixture was extracted with EtOAc, and the extract was washed with saturated aq citric acid, brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with CH_3Cl–MeOH (9:1) gave 14.0 mg (0.0487 mmol, 46%) of Boc-Gly-ψ[(*E*)-CH=CH]-L-Asp(OMe)-OH **20** accompanied with its enantiomer **22** as a colourless oil [Found (CI): $(M+H)^+$, 288.1453. $C_{13}H_{22}NO_6$ requires *M*+H, 288.1447]; δ_H (600 MHz; $CDCl_3$) 1.27 (9H, s, 3×CMe), 2.54–2.58 (1H, dd, *J*=16.6 and 5.2, C*H*H), 2.82–2.86 (dd, *J*=16.7 and 8.2, CH*H*), 3.55 (1H, m, 2-H), 3.69 (3H, s, OMe), 3.70 (2H, br, CH_2), 4.63 (1H, br, NH), 5.63–5.67 (2H, m, 2×CH=); *m/z* (CILRMS), 288 (MH^+, base peak), 260, 242, 232, 214, 188, 171.

4.1.29. Boc-Gly-ψ[(*E*)-CH=CH]-D-Asp(OMe)-OH [(3*E*,2*S*)-5-((*tert*-butoxy)carbonylamino)-2-((methoxycarbonyl)methyl)pent-3-enoic acid] 22. By use of a procedure identical with that described for the preparation of **20** from **19**, the allylic dioate **21** (58.1 mg, 0.126 mmol, the enantiomixture with **19**, ee=43%) was converted into Boc-Gly-ψ[(*E*)-CH=CH]-D-Asp(OMe)-OH **22** accompanied with its enantiomer **20** (16.0 mg, 0.0557 mmol, 44%) as a colourless oil [Found (CI): $(M+H)^+$, 288.1442. $C_{13}H_{22}NO_6$ requires *M*+H, 288.1447]; δ_H (270 MHz; $CDCl_3$) 1.45 (9H, s, 3×CMe), 2.52–2.60 (1H, dd, *J*=16.8 and 5.9, C*H*H), 2.80–2.89 (1H, dd, *J*=16.8 and 8.2, CH*H*), 3.53 (1H, m, 2-H), 3.69 (3H, s, OMe), 3.74 (2H, br, CH_2), 4.63 (1H, br, NH), 5.65–5.67 (2H, m, 2×CH=); *m/z* (CILRMS), 288 (MH^+, base peak), 272, 260, 242, 232, 214, 188, 171.

4.1.30. Methyl (2*E*,2*S*,4*S*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-4-(3-phenyl-2-((phenylmethoxy)carbonylamino)propanoyloxy)hex-2-enoate 23a. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-acyloxy-α,β-enoate **23a** (59.7 mg, 0.100 mmol, 59%) by treatment with Cbz-L-Phe-OH (507 mg, 1.69 mmol) and CF_3SO_3TMS (0.00920 cm^3, 50.8 μmol) in CH_2Cl_2 at rt for 15 h.

Compound **23a**, colourless crystals, mp 60–62 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 62.33; H, 5.77; N, 4.50. $C_{31}H_{34}N_2O_8S$ requires C, 62.61; H, 5.76; N, 4.71%); $[\alpha]_D^{29}$ +17.9 (*c* 0.335, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 0.87–0.89 (3H, m, CMe), 2.41 (3H, s, CMe), 3.09 (2H, d, *J* 6.4, CCH_2Ph), 3.47 (1H, br, 5-H), 3.71 (3H, s, OMe), 4.63 (1H, q, *J* 7.2, 2-H), 4.72 (1H, d, *J* 8.7, NH), 5.06–5.12 (2H, m, OCH_2Ph), 5.25 (1H, d, *J* 8.0, NH), 5.33 (1H, br, 4-H), 5.84 (1H, d, *J* 15.8, CH=), 6.62 (1H, dd, *J* 15.8 and 5.5, CH=), 7.22–7.35 (12H, m, ArH and 2×Ph), 7.71 (2H, d, *J* 8.2, ArH).

4.1.31. Methyl (2*E*,2*S*,4*S*,5*S*)-4-(3-methyl-2-((phenylmethoxy)carbonylamino)butanoyloxy)-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 23b. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (100 mg, 0.339 mmol) was converted into the γ-acyloxy-α,β-enoate **23b** (83.2 mg, 0.152 mmol, 45%) by treatment with Cbz-L-Val-OH (852 mg, 3.39 mmol) and CF_3SO_3TMS (0.0184 cm^3, 0.102 mmol) in CH_2Cl_2 at rt for 15 h.

Compound **23b**, colourless crystals, mp 51–52 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 59.52; H, 6.44; N, 4.85. $C_{27}H_{34}N_2O_8S$ requires C, 59.32; H, 6.27; N, 5.12%); $[\alpha]_D^{29}$ −49.0 (*c* 0.490, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 0.89 (3H,

d, *J* 6.6, CMe), 1.00 (3H, d, *J* 6.8, CMe), 1.05 (3H, d, *J* 6.7, CMe), 2.17–2.24 (1H, m, 3-H), 2.42 (3H, s, CMe), 3.61 (1H, m, 5-H), 3.72 (3H, s, OMe), 4.30 (1H, dd, *J* 8.6 and 4.6, 2-H), 4.67 (1H, d, *J* 8.7, NH), 5.09–5.16 (2H, m, OCH_2Ph), 5.19 (1H, m, NH), 5.37 (1H, br, 4-H), 5.92 (1H, d, *J* 15.7, CH=), 6.66 (1H, dd, *J* 15.7 and 5.5, CH=), 7.29 (2H, d, *J* 8.1, ArH), 7.32–7.36 (5H, m, Ph), 7.73 (2H, d, *J* 8.2, ArH).

4.1.32. Methyl (2*E*,2*S*,4*S*,5*S*)-4-(1-((fluoren-9-ylmethyl)-oxycarbonyl)pyrrolidin-2-ylcarbonyloxy)-5-(((4-methylphenyl)sulfonyl)amino)hex-2-enoate 24. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **7** (50 mg, 0.169 mmol) was converted into the γ-acyloxy-α,β-enoate **24** (73.9 mg, 0.117 mmol, 69%) by treatment with Fmoc–L-Pro–OH (852 mg, 3.39 mmol) and CF_3SO_3TMS (0.0184 cm^3, 0.102 mmol) in CH_2Cl_2 at rt for 6 h.

Compound **24**, colourless amorphous semisolid [Found (FAB): $(M+H)^+$, 633.2261. $C_{34}H_{37}N_2O_8S$ requires *M*+H, 633.2270]; $[\alpha]_D^{20}$ −38.6 (*c* 1.346, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 1.03 (3H, d, *J* 6.8, CMe), 1.93–2.32 (4H, m, 2×CH_2), 2.39 (3H, s, CMe), 3.51–3.66 (2H, m, CH_2), 3.59–3.66 (1H, m, 5-H), 3.72 (3H, s, OMe), 4.29–4.38 (2H, m, CH_2), 4.46 (1H, dd, *J* 8.6 and 3.9, 2-H), 4.58 (1H, dd, *J* 10.4 and 6.5, 9-H), 5.04 (1H, d, *J* 9.1, NH), 5.42 (1H, m, 4-H), 5.96 (1H, dd, *J* 15.7 and 1.4, CH=), 6.72 (1H, dd, *J* 15.8 and 5.2, CH=), 7.20–7.43 (6H, m, ArH), 7.63–7.78 (6H, m, ArH); *m/z* (FABLRMS), 633 (MH^+), 411, 296, 292 (base peak), 225, 179, 178 and 91.

4.1.33. Methyl (2*E*,2*S*,4*S*,5*S*)-4-(1-((fluoren-9-ylmethyl)-oxycarbonyl)pyrrolidin-2-ylcarbonyloxy)-5-(((2,4,6-trimethylphenyl)sulfonyl)amino)hex-2-enoate 26. By use of a procedure similar to that described for the preparation of **9a** from **7**, the (*E*)-enoate **25** (723 mg, 2.24 mmol) was converted into the γ-acyloxy-α,β-enoate **26** (657 mg, 0.994 mmol, 44%) by treatment with Fmoc–L-Pro–OH (5.20 g, 15.5 mmol) and CF_3SO_3TMS (0.121 cm^3, 0.669 mmol) in CH_2Cl_2 at rt for 15 h.

Compound **26**, colourless crystals, mp 77–79 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 63.80; H, 6.11; N, 4.20. $C_{36}H_{40}N_2O_8S\cdot H_2O$ requires C, 63.70; H, 6.24; N, 4.13%); $[\alpha]_D^{22}$ +14.88 (*c* 0.739, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 1.08 (3H, d, *J* 6.8, CMe), 1.92–2.17 (4H, m, 2×CH_2), 2.27 (3H, s, CMe), 2.61 (6H, s, 2×CMe), 3.50–3.57 (2H, m, C*H*H and 5-H), 3.61–3.67 (1H, m, CH*H*), 3.70 (3H, s, OMe), 4.27–4.56 (4H, m, CH_2, 2-H and 9-H), 5.08 (1H, d, *J* 9.3, NH), 5.42 (1H, m, 4-H), 5.91 (1H, dd, *J* 15.7 and 1.5, CH=), 6.64 (1H, dd, *J* 15.8 and 5.0, CH=), 6.89 (2H, s, ArH), 7.30–7.33 (2H, m, ArH), 7.36–7.42 (2H, m, ArH), 7.65 (2H, t, *J* 7.2, ArH), 7.73–7.78 (2H, m, ArH).

4.1.34. Methyl (2*E*,2*S*,4*S*,5*S*)-5-((1-((fluoren-9-ylmethyl)-oxycarbonyl)pyrrolidin-2-yl)carbonylamino)-4-hydroxyhex-2-enoate 28. The γ-acyloxy-α,β-enoate **26** (250 mg, 0.378 mmol) was treated with 1 M TMSBr-thioanisole/TFA (12.5 cm^3) in the presence of *m*-cresol (0.610 cm^3, 5.83 mmol) at 0 °C with warming to rt for 15 h. After thorough concentration under reduced pressure, the residue **27** was dissolved with CH_3CN (20 cm^3). To the solution was added dropwise PBS (20 cm^3) and saturated aq Na_2HPO_4 (3.4 cm^3) at 0 °C, and the mixture was allowed to warm to rt for 30 min. Concentration under reduced pressure gave an oily residue, which was purified by chromatography over silica gel with *n*-hexane–EtOAc (1:3) to yield 152 mg (0.318 mmol, 84%) of compound **28** as colourless crystals, mp 85–87 °C [from *n*-hexane–Et_2O (3:1)] (Found: C, 67.50; H, 6.25; N, 5.67. $C_{27}H_{30}N_2O_6$ requires C, 67.77; H, 6.32; N, 5.85%); $[\alpha]_D^{23}$ −21.9 (*c* 1.097, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 1.25–1.27 (3H, m, CMe), 1.91–1.96 (3H, br, CH_2 and C*H*H), 2.28 (1H, br, CH*H*), 3.32 (2H, m, CH_2), 3.70 (3H, s, OMe), 3.87 (1H, br, 5-H), 4.25 (3H, br, 2-H, 4-H and 9-H), 4.33 (2H, br, CH_2), 6.12 (1H, d, *J* 11.0, CH=), 6.82 (1H, br, NH), 6.90 (1H, d, *J* 12.7, CH=), 7.30–7.34 (2H, m, ArH), 7.41 (2H, t, *J* 5.7, ArH), 7.60 (2H, d, *J* 7.0, ArH), 7.77 (2H, d, *J* 7.5, ArH).

4.1.35. Fmoc–L-Pro–L-Ala–ψ[(*E*)-CH=CH]–D-Leu–OMe 29. To a stirred solution of the γ-hydroxy-α,β-enoate **28** (53.1 mg, 0.111 mmol) in CH_2Cl_2 (2 cm^3) were added dropwise MsCl (0.0859 cm^3, 1.11 mmol) and Et_3N (0.153 cm^3, 1.11 mmol) at 0 °C, and the mixture was stirred at this temperature for 3 h. To ice-cold saturated aq citric acid was added the mixture followed by stirring for 10 min. The mixture was extracted with EtOAc, and the extract was washed successively with aq 5% citric acid and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue of the crude γ-mesyloxy-α,β-enoate, which was utilized for the next reaction without purification.

To a stirred slurry of CuCN (79.9 mg, 0.888 mmol) in THF (1 cm^3) was added a solution of ^{i}BuMgCl in THF (1.3 M, 0.683 cm^3, 0.888 mmol) at −78 °C under argon, and the mixture was stirred at 0 °C for 15 min. $BF_3\cdot Et_2O$ (0.109 cm^3, 0.888 mmol) was added to the above mixture at −78 °C. After 10 min of stirring at −78 °C, a solution of the crude γ-mesyloxy-α,β-enoate in dry THF (2 cm^3) was added to the above mixture at −78 °C under argon. The stirring was continued at −78 °C for 30 min followed by quenching with saturated aq NH_4Cl at 0 °C. The mixture was extracted with Et_2O, and the extract was washed with water and dried over $MgSO_4$. Concentration under reduced pressure gave a colourless oil, which was purified by chromatography over silica gel with *n*-hexane–EtOAc (1:1) to yield 40.2 mg (0.0775 mmol, 70%) of **29** as colourless crystals, mp 158–159 °C [from EtOAc] (Found: C, 71.53; H, 7.38; N, 5.33. $C_{31}H_{38}N_2O_5$ requires C, 71.79; H, 7.16; N, 5.40%); $[\alpha]_D^{23}$ −22.8 (*c* 0.832, $CHCl_3$); δ_H (600 MHz; $CDCl_3$) 0.87–0.89 (6H, br, 2×CMe), 1.18 (3H, d, *J* 5.8, CMe), 1.36 (1H, br, CH), 1.50 (1H, br, C*H*H), 1.60 (1H, br, CH*H*), 1.92 (2H, br, CH_2), 2.17 (2H, m, CH_2), 3.07 (1H, br, 2-H), 3.43 (1H, br, C*H*H), 3.54 (1H, br, CH*H*), 3.64 (3H, s, OMe), 4.22–4.45 (4H, br, CH_2, 2-H and 9-H), 4.52 (1H, br, 5-H), 5.45–5.54 (2H, br, 2×CH=), 6.57 (1H, br, NH), 7.30–7.33 (2H, m, ArH), 7.40 (2H, t, *J* 7.4, ArH), 7.59 (2H, d, *J* 3.8, ArH), 7.76 (2H, d, *J* 7.4, ArH).

4.1.36. Methyl (2*E*,4*S*,5*S*)-5-(((4-methylphenyl)sulfonyl)-amino)-4-(phenylsulfonyloxy)hex-2-enoate resin 30. The (*E*)-enoate **7** (1.05 g, 3.56 mmol) was treated with MP–Ts–OH resin (Argonaut Technologies, California, U.S.A., 1.27 mmol/g, 933 mg, 1.19 mmol) in CH_2Cl_2 (12 cm^3) at rt, and the mixture was stirred for 15 h. The resin was filtered

and washed with dried THF (1 $cm^3 \times 7$) to give resin-bound enoate **30** (1.26 g). The filtrate was concentrated under reduced pressure and chromatographed by flash column over silica gel with *n*-hexane–EtOAc (4:1) to recover excess of **7** (0.84 g, 2.84 mmol).

4.1.37. Ts–L-Ala–ψ[(*E*)-CH=CH]-D-Leu–OMe [methyl (2*E*,2*S*,5*S*)-5-(((4-methylphenyl)sulfonyl)amino)-2-(2-methylpropyl)hex-3-enoate] 31. To a stirred slurry of CuCN (122 mg, 1.35 mmol) in THF (3 cm^3) was added a solution of ^{i}BuMgCl in THF (1.2 M, 1.13 cm^3, 1.35 mmol) at −78 °C under argon, and the mixture was stirred at 0 °C for 15 min. $BF_3 \cdot Et_2O$ (0.167 cm^3, 1.35 mmol) was added to the above mixture at −78 °C. After 10 min of stirring at −78 °C, the dried resin-bound enoate **30** (180 mg) was added to the above mixture at −78 °C. The stirring was continued at −78 °C for 30 min and then at 0 °C for 15 h followed by quenching with 2 cm^3 of saturated aq NH_4Cl-aq 28% NH_4OH (1:1 (v/v)). The mixture was extracted with Et_2O, and the extract was washed with water and dried over $MgSO_4$. Concentration under reduced pressure gave a colourless oil, which was purified by chromatography over silica gel with *n*-hexane–EtOAc (5:1) to yield 22.4 mg (0.0634 mmol, 37% based on **7**) of **31** accompanied with its 2*R*-isomer.

Compound **31**, colourless oil [Found (FAB): $(M+H)^+$, 354.1746. $C_{18}H_{28}NO_4S$ requires *M*+H, 354.1739]; $[\alpha]_D^{27}$ −7.14 (*c* 1.680, $CHCl_3$); Δε+2.833 (227 nm, isooctane); δ_H (600 MHz; $CDCl_3$) 0.81–0.86 (6H, m, 2×CMe), 1.17 (3H, d, *J* 6.8, CMe), 1.19–1.24 (1H, m, C*H*H), 1.37–1.42 (1H, m, CH), 1.48–1.55 (1H, m, CH*H*), 2.43 (3H, s, CMe), 2.95 (1H, q, *J* 7.9, 2-H), 3.65 (3H, s, OMe), 3.85–3.95 (1H, m, 5-H), 4.40 (1H, d, *J* 7.6, NH), 5.33–5.37 (1H, m, CH=), 5.40–5.45 (1H, m, CH=), 7.23–7.30 (2H, m, ArH), 7.72–7.74 (2H, m, ArH); *m/z* (FABLRMS), 354 (MH^+, base peak), 352, 338, 322, 294, 198, 183, 155, 123.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.06.029.

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