TABLE 3. Susceptibilities of HIV-1 IN recombinant molecular clones[a]

Molecular clone(s)	Mean EC_{50} (nM) ± SD (fold resistance compared to wild type)				
	AZT	EVG	L-870,810	S-1360	L-731,988
$HIV\text{-}1_{WT}$	32	1.1	5.8	1,239	736
EVG mutation (expt 1)[b]					
T66I[c]	43 ± 11 (1.3)	41 ± 14 (37)	4.7 ± 2.9 (0.8)	6,403 ± 2,349 (5.2)	7,234 ± 1,210 (9.8)
Q95K	34 ± 6 (1.1)	2.9 ± 0.4 (2.6)	18 ± 2 (3.1)	ND	ND
E138K	33 ± 8 (1.0)	1.1 ± 0.4 (1.0)	3.9 ± 0.4 (0.7)	ND	ND
Q146P	26 ± 2 (0.8)	12 ± 3 (11)	5.1 ± 0.4 (0.9)	ND	ND
S147G[d]	41 ± 5 (1.3)	12 ± 5 (11)	23 ± 6 (4.0)	ND	ND
T66I/Q146P	22 ± 2 (0.7)	131 ± 12 (119)	18 ± 5 (3.1)	ND	ND
T66I/Q146P/S147G	19 ± 5 (0.6)	453 ± 62 (412)	127 ± 37 (22)	ND	ND
T66I/Q95K/Q146P/S147G	31 ± 12 (1.0)	>1,000	303 ± 76 (52)	ND	ND
T66I/Q95K/E138K/Q146P/S147G	41 ± 7 (1.3)	>1,000	306 ± 76 (53)	>10,000	>50,000
EVG mutation (expt 2)[b]					
H51Y	34 ± 8 (1.1)	4.0 ± 0.6 (3.6)	3.3 ± 0.7 (0.6)	ND	ND
E92Q	32 ± 4 (1.0)	40 ± 12 (36)	63 ± 39 (11)	ND	ND
E157Q	34 ± 8 (1.1)	6.9 ± 1.4 (6.3)	52 ± 20 (9.0)	ND	ND
E92Q/S147G	39 ± 9 (1.2)	392 ± 133 (356)	587 ± 64 (101)	ND	ND
H51Y/E92Q/S147G	54 ± 6 (1.7)	769 ± 88 (699)	374 ± 100 (64)	>10,000	22,175 ± 1,299 (30)
H51Y/E92Q/S147G/E157Q	21 ± 2 (0.7)	>1,000	340 ± 26 (59)	>10,000	18,652 ± 4,575 (25)
L-870,810 mutation					
V72I	17 ± 1 (0.5)	4.3 ± 1.1 (3.9)	9.1 ± 2.5 (1.6)	ND	ND
L74M[c]	20 ± 3 (0.6)	3.3 ± 1.1 (3.0)	4.4 ± 1.7 (0.8)	1,500 ± 302 (1.2)	4,471 ± 942 (6.1)
F121Y	15 ± 1 (0.5)	28 ± 11 (25)	51 ± 23 (8.8)	ND	ND
T125K	17 ± 3 (0.5)	2.3 ± 1.1 (2.1)	9.9 ± 3.7 (1.7)	ND	ND
V151I	21 ± 4 (0.7)	11 ± 3 (10)	104 ± 29 (18)	ND	ND
G163R	22 ± 7 (0.7)	0.8 ± 0.2 (0.7)	6.5 ± 2.6 (1.1)	ND	ND
F121Y/G163R	36 ± 5 (1.1)	60 ± 20 (55)	219 ± 20 (38)	ND	ND
F121Y/T125K	38 ± 12 (1.2)	195 ± 73 (177)	393 ± 82 (68)	ND	ND
V72I/F121Y/T125K	33 ± 7 (1.0)	143 ± 25 (130)	886 ± 79 (153)	ND	ND
V72I/F121Y/T125K/V151I	64 ± 9 (2.0)	>1,000	>1,000	>10,000	>50,000
DKA mutation					
T66I/L74M	46 ± 11 (1.4)	49 ± 5 (45)	41 ± 10 (7.1)	>10,000	23,043 ± 4,886 (31)
T66I/S153Y	26 ± 8 (0.8)	285 ± 63 (259)	29 ± 9 (5.0)	>10,000	8,478 ± 1,267 (12)

[a] Antiviral activity was determined using the MAGI assay. Data shown are means and standard deviations obtained from at least three independent experiments, and resistance (*n*-fold) of the EC_{50} of the IN recombinant molecular clone compared to that of parental $HIV\text{-}1_{WT}$ is shown in parentheses. ND, not determined.
[b] EVG selection was performed in two independent experiments, and observed mutations are separately represented.
[c] Also observed in the DKA selected mutation.
[d] Observed in two independent EVG-selected experiments.

that seen for other antiretroviral drugs such as PIs; i.e., multiple mutations are introduced in a stepwise fashion and are required for high-level resistance to the selecting inhibitors (10, 50).

Strand transfer assay. To further characterize the effect of EVG-selected resistance mutations on IN function, the effect of mutations on the enzymatic activity of recombinant IN was evaluated in an in vitro strand transfer assay (Fig. 4). IN enzymes carrying the individual mutations H51Y, S147G, and E157Q had reduced strand transfer activity relative to that of the wild type (57%, 36%, and 79% of wild-type levels, respectively). Strand transfer activities of E92Q, E92Q/S147G, and H51Y/E92Q/S147G IN enzymes decreased with the accumulation of mutations from 57% to 29 and 22% of the wild type, respectively. However, the introduction of E157Q to H51Y/E92Q/S147Q partially restored strand transfer activity to 46% of wild-type activity, suggesting that E157Q may play a role in compensating for the loss of strand transfer activity resulting from the emergence of EVG resistance mutations.

The effect of EVG-selected mutations on the inhibition of strand transfer by EVG and L-870,810 was also determined (Fig. 4). Recombinant IN enzymes carrying the individual H51Y, S147G, and E157Q substitutions remained susceptible to both EVG and L-870,810 (0.7- to 2.1-fold reduced susceptibility). E92Q IN demonstrated only moderate resistance to both IN inhibitors in the strand transfer assay (4.3-fold reduced for both inhibitors). The combination of E92Q and S147G enhanced resistance to both EVG and L-870,810 (7.6- and 8.5-fold reduced susceptibility, respectively). However, unlike the IN recombinant viruses in the antiviral assay, neither the H51Y/E92Q/S147G nor the H51Y/E92Q/S147G/E157Q IN enzymes showed further enhancement of resistance in the strand transfer assay. This difference in results from the strand transfer assay versus those from the antiviral assay may reflect differences in the recombinant IN enzyme versus the viral IN enzyme in situ. Indeed, structure-activity relationship experiments described in a previous report (43) revealed that antiviral activity and in vitro enzyme inhibition were well correlated. Nevertheless, this biochemical analysis confirmed that the E92Q IN mutation confers significantly reduced suscepti-

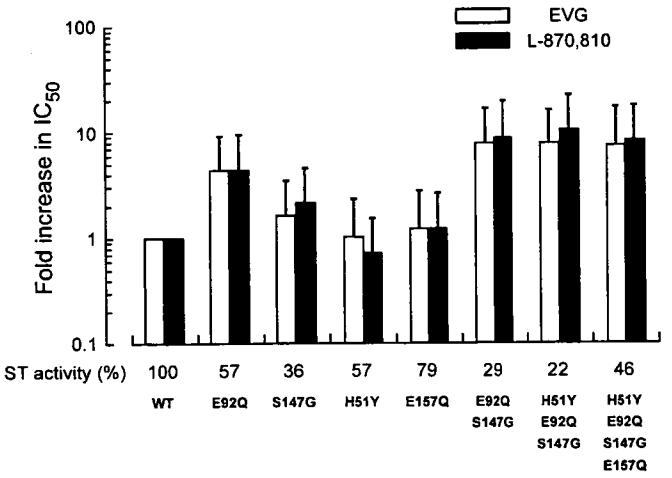

FIG. 4. Effect of EVG-selected mutations on IN strand transfer activity and on the inhibition of strand transfer by IN inhibitors. The strand transfer activities of recombinant IN enzymes carrying EVG-selected mutations were determined using an oligonucleotide-based strand transfer assay. Strand transfer (ST) activity of IN mutants was compared to that of the wild type (WT); results are shown as percentages of wild-type activity. The effect of IN inhibitors on strand transfer was also determined for wild-type and mutant IN enzymes; results are expressed as the increase (*n*-fold) in IC_{50} values of inhibitors relative to those of the wild type.

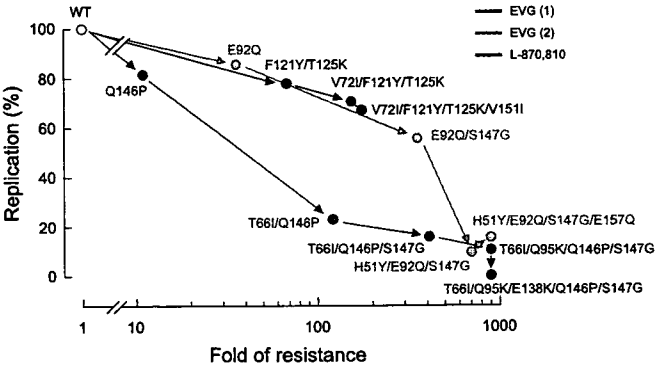

FIG. 5. Replication kinetics of EVG- and L-870,810-resistant viral variants. The replication kinetics of wild-type and IN inhibitor-resistant viral variants were determined by p24 ELISA. The relationship of replication capacity and change (*n*-fold) in susceptibility (shown in Table 3) is depicted. Variants are plotted according to the observed order of their emergence during selection experiments in vitro. Replication kinetics of EVG-selected mutants derived from the two independent selection experiments (shown in Fig. 3) are plotted in different colors. WT, wild type.

bility to EVG at the level of inhibition of strand transfer, consistent with its identification as a primary EVG resistance mutation in the virological analyses.

Replication kinetics of IN inhibitor-resistant variants. The effects of IN mutations on the replication kinetics of HIV-1 variants were assessed by comparing their levels of p24 production in culture supernatants to that of wild-type virus (Fig. 5). At day 5 postinfection, levels of p24 production by the HIV-1_{E92Q} and HIV-1_{Q146P} variants were 86% and 82% of HIV-1_{WT} levels, respectively. These variants showed high-level (36-fold) or moderate (11-fold) resistance to EVG (Table 3), whereas the replication levels of both were similar to those of the wild type. However, the introduction of additional EVG resistance mutations further decreased p24 production, which is indicative of a decline in the levels of viral replication. In particular, HIV-$1_{T66I/Q146P/S147G}$, HIV-$1_{T66I/Q95K/Q146P/S147G}$, HIV-$1_{T66I/Q95K/E138K/Q146P/S147G}$, HIV-$1_{H51Y/E92Q/S147G}$, and HIV-$1_{H51Y/E92Q/S147G/E157Q}$ all showed significantly reduced levels of p24 production (less than 20% of wild-type levels by day 5 in all cases). Thus, there was an inverse correlation between the levels of EVG resistance and the viral replication capacity; that is, as resistance to EVG increased, viral replication decreased. Interestingly, viral variants carrying L-870,810-selected mutations had more moderate reductions in replication capacity, even in the case of the HIV-$1_{V72I/F121Y/T125K/V151I}$ variant that had high-level resistance to both L-870,810 and EVG (68% of wild-type levels). These results indicate that mutations associated with resistance to IN inhibitors can have various effects on viral replication capacity. The reduced replication capacity of EVG-resistant variants was not rescued in the presence of the inhibitor (data not shown), as was observed previously for NFV-resistant variants in the presence of NFV (35). Thus, the reduced replication capacity of IN inhibitor-resistant variants may present a barrier to their emergence in vivo.

Antiviral effect of IN inhibitors on retroviruses. The antiviral activity of EVG against other retroviruses, including MLV and SIV, was assessed. EVG and L-870,810 inhibited the integration of the HIV-based vector used as a positive control for the luciferase assay (EC_{50} values of 0.8 and 5.0 nM, respectively), as observed in the MAGI assay with HIV-1_{IIIB} (Fig. 6). EVG and L-870,810 suppressed the replication of MLV infection (EC_{50} values of 5.8 and 22 nM, respectively) as well as that of the primate retrovirus SIV (0.5 and 3.2 nM, respectively), indicating that IN inhibitors have antiviral activity against a broad range of retroviruses.

DISCUSSION

The data described here show that EVG inhibits HIV replication by specifically blocking the strand transfer reaction mediated by IN, as demonstrated by the intracellular accumulation of 2-LTR DNA products, a signature of nonproductive integration. Furthermore, EVG directly blocked the production of strand transfer products in an in vitro strand transfer assay. Confirming that EVG is a bona fide IN inhibitor, we selected EVG-resistant viral variants in vitro and demonstrated that the resulting viral variants had acquired multiple mutations in the IN coding region and had simultaneously acquired reduced phenotypic susceptibility to EVG. HIV-1 molecular clones carrying the EVG-selected IN mutations had an EVG-resistant phenotype and in many cases also had reduced susceptibility to another IN inhibitor, L-870,810. These data provide formal proof that the observed IN mutations are indeed EVG resistance mutations and that EVG is an IN inhibitor.

Among the IN mutations observed to be selected by EVG, two mutations, T66I and E92Q, appeared to provide the major contribution to EVG resistance. Both of these individual mutations resulted in >30-fold reduced susceptibility to EVG. The T66I mutation conferred cross-resistance to S-1360 and

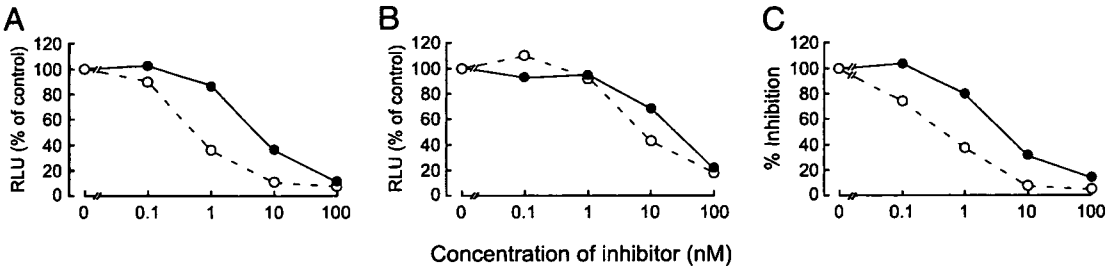

FIG. 6. Effect of IN inhibitors on retroviruses. Antiviral activities of EVG (open circles with dashed lines) and L-870,810 (closed circles with solid lines) against HIV-based (A) or MLV-based (B) vectors harboring the luciferase gene were determined by measuring luciferase activity at 48 h posttransduction. Results are expressed as percentages of relative luciferase units (RLU) compared to those of the no-inhibitor control. (C) Anti-SIV activity was determined using the MAGI assay. These results shown are one representative assay from three independent experiments.

L-731,988 (Table 3) and was also previously observed in an independent EVG selection by Jones et al. (26). The E92Q mutation, when introduced into a recombinant IN enzyme, also reduced the susceptibility of the resulting mutant IN enzyme to EVG, as measured by the reduced EVG inhibition of the in vitro strand transfer assay (Fig. 4). The other IN mutations identified, including H51Y, Q95K, E138K, Q146P, S147G, and E157Q, individually resulted in lower changes (*n*-fold) in EVG susceptibility (1.0- to 11.0-fold) but, when added to either the T66I or the E92Q mutation, further increased resistance to EVG to various degrees relative to either mutation alone. Interestingly, the accumulation of these EVG-selected IN mutations resulted in a significant attenuation of viral replication kinetics. Thus, the emergence of resistance to IN inhibitors may be associated with reductions in viral fitness, which may provide a barrier to the emergence of these mutations in vivo or be associated with lower viral loads if they do emerge.

Of the three HIV enzymes PR, RT, and IN, the structure and mechanism of IN are the least well understood, and despite extensive efforts, the structure of the complete IN enzyme remains to be determined. Only partial two-domain crystal structures of the IN apoenzyme are available, and no structure showing full-length IN bound to its viral cDNA substrate has been published. During integration in vivo, IN functions in the preintegration complex, which also includes RT and the viral DNA (2, 3). Some limited evidence suggests that RT interacts with the active site of IN (39). IN has also been proposed to function with several cellular factors including IN interactor 1 (Ini1) (27) and lens-epithelium-derived growth factor (LEDGF/p75) (7). In the context of these associated cellular factors, IN may retain a different conformation compared to that of the recombinant enzyme alone. This may be one of the reasons that only moderate EVG resistance was observed in the oligonucleotide-based strand transfer assay compared to a cell-based antiviral assay.

Alignment of several IN CCD structures deposited in the Protein Data Bank indicates that there are two regions with poorly defined or disordered structures, including residues 47 to 56 and 140 to 152 (Fig. 7; see Fig. S1 in the supplemental material). Of these two disordered regions, residues 140 to 152 have been implicated as a flexible loop involved in viral cDNA binding (20, 21, 53). Although the precise structural details are unknown, the flexible loop has been proposed to adopt different conformations in the presence or absence of the viral cDNA (12). Notably, several of the EVG-selected mutations that we observed are located on or adjacent to this proposed flexible loop, including E138K, Q146P, and S147G. The flexible loop is important for the catalytic activity of IN (21, 32), and as shown in Fig. 4, the introduction of mutations in these residues, especially S147G, drastically reduced the catalytic activity of IN. Previously published data also demonstrated that another mutation at codon 147 (S147I) resulted in HIV-1 that was highly replication defective, including effects on viral DNA synthesis (47). Indeed, S147 is highly conserved among various retroviruses (see Fig. S2 in the supplemental material), highlighting the importance of the loop for IN function. It is possible that IN inhibitor resistance mutants may have additional pleiotropic effects on processes in viral replication other

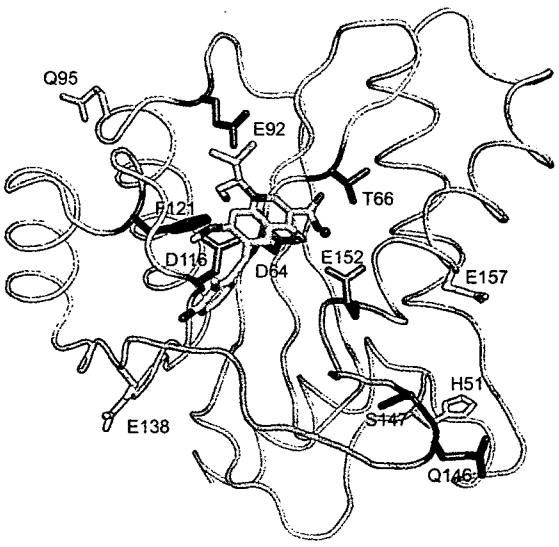

FIG. 7. Location of IN mutations associated with resistance to EVG. EVG in complex with the HIV-1 IN CCD is shown along with the catalytic triad residues (D64, D116, and E152) (green) and a magnesium ion (magenta). Amino acid residues conferring resistance to EVG as primary mutations (T66, E92, F121, Q146, and S147) or as secondary mutations (H51, Q95, E138, and E157) are shown in red and cyan, respectively. The flexible loop (residues 140 to 152) is shown in pink.

than integration; in particular, RT and IN were previously suggested to interact functionally (25).

Recently, an in silico docking simulation of HIV IN with several IN inhibitors including EVG was reported (44). Notably, that author showed that in the best-fit model for EVG docked to IN, the isobutyl substituent on the quionolone moiety of EVG orients directly towards IN residue E92. Interestingly, the hydroxyl component of the isobutyl on the quinolone replaces a water molecule that is coordinated by residue E92 between the two catalytic residues D64 and E152. This docking structure may provide insight into the mechanism of IN inhibition by EVG and provides a starting point for understanding the mechanism of EVG resistance mediated by the E92Q substitution. However, it is uncertain whether this docking simulation represents the precise binding mode of EVG with IN in vivo. Therefore, to accurately assess the binding mode of IN inhibitors with IN, available structural data need to be supplemented by a variety of other approaches. In this study, a virological approach and an enzymatic approach were integrated to characterize the mechanism of action, antiviral activity, and resistance profile of EVG in vitro.

As shown in Fig. 7, primary EVG resistance mutations are located around the catalytic triad of the CCD of IN and are surrounded by the secondary mutations. Among the residues affected by primary mutations, E92 and F121 are located close to EVG on the model and might interact with the IN inhibitor. However, the mechanism by which these mutations interact with the IN inhibitor or with the viral cDNA to mediate resistance is currently unclear. Recently, clinical isolate data from patients experiencing virologic failure in ongoing phase III studies of another IN inhibitor, raltegravir, were reported; E92Q was among the mutations noted to develop in these raltegravir failure patients, usually in combination with another IN mutation, N155H (11, 48). These preliminary clinical data and the data presented here with L-870,810, indicate that the E92Q mutation may be able to mediate resistance and potential cross-resistance to multiple IN inhibitors including EVG and raltegravir. Consistent with the data described here, site-directed mutant HIV carrying the E92Q mutation has been confirmed to show resistance to EVG and to have low-level (approximately sixfold) reduced susceptibility to raltegravir (26).

Several of the IN residues affected by primary mutations observed in EVG-selected variants including T66, E92, and S147 are absolutely conserved among the retroviruses tested (HIV-1, HIV-2, SIV, and MLV) and in retroviruses from multiple mammalian species (see Fig. S2 in the supplemental material). The significant conservation of mammalian retroviral IN CCDs at both the level of sequence homology and structure of the active site was demonstrated by the ability of EVG to inhibit HIV, SIV, and MLV IN activity. This suggests that EVG, and probably other IN inhibitors, binds to a conformationally conserved region of all retroviral INs; the binding of EVG and other IN inhibitors to IN is also likely to involve the catalytic magnesium ion. Taken together, these results suggest that several distinct mechanisms may contribute to IN inhibitor resistance, including conformational changes in the structure of IN that affect the binding of the IN inhibitor, charge effects, steric hindrance, loss of stabilizing binding interactions, or, possibly, alterations in magnesium binding.

A similar reduction in viral replication capacity as a result of drug resistance mutations was previously reported for NRTI resistance mutations (K65R, L74V, and M184V) (45, 55) and for PI resistance mutations (D30N) (49). Mutations that act to compensate for some of the loss of viral replication resulting from drug resistance, for example, GAG processing mutants, have also been described (18, 36, 52). At least one of the EVG secondary mutations, E157Q, may have an analogous role, as it partially restored strand transfer activity that was attenuated by other EVG-selected mutations and also further enhanced resistance to EVG (Fig. 4). Some secondary IN mutations might act to compensate for the altered conformation of IN resulting from the structural effects of primary resistance mutations. The E138K mutation may be such an example, as on its own, it showed no effect on susceptibility to either EVG or L-870,810. The clinical implications of the reduction in fitness resulting from the selection of EVG-resistant mutations are not yet understood.

In conclusion, EVG is a potent inhibitor of the HIV IN enzyme that acts by blocking the strand transfer reaction and is effective not only against HIV but also against other retroviruses. Moreover, the emergence of viral variants that were highly resistant to EVG was associated with significant reductions in viral replication in vitro. These results indicate that EVG should be highly effective for the treatment of HIV-1-infected patients, including those who have had virologic failure of their highly active antiretroviral therapy due to the emergence of HIV-1 drug resistance to approved antiretroviral drugs.

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Development of copper-mediated allylation of γ-activated-α,β-unsaturated lactam toward peptide mimetic synthesis

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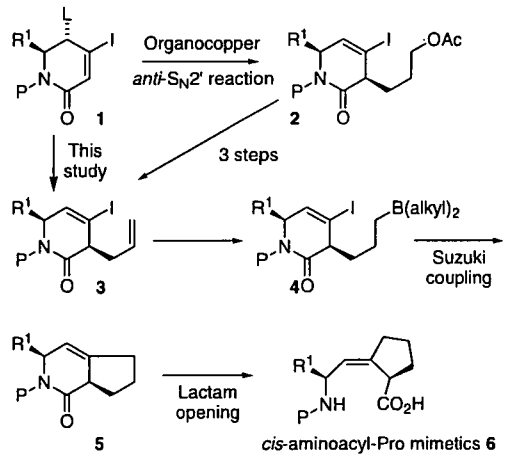
Abstract—Reactions of γ-activated-α,β-unsaturated lactams with allylboronate in the presence of LiO*i*-Pr and CuX (stoichiometric or catalytic amount) proceed in an *anti*-S_N2' manner to yield α-allylated compounds that serve as a potential synthetic intermediate for *cis*-aminoacyl-Pro mimetics.

The introduction of allyl units into an organic functional group serves as a versatile transformation, which allows a molecule to be subjected to further synthetic manipulations.[1] Numerous studies on allylations of aldehydes,[2] ketones[3] or imines[4] with allylic metal reagents, including catalytic and asymmetric versions,[5] have appeared in the literature. Although 1,4-[6] or S_N2'-sense reactions[7] of allylic metals in the presence of copper salts also constitute an indispensable part of allylation, many controversial issues regarding the regioselectivity of the reaction (1,2- vs 1,4-addition or S_N2 vs S_N2') and nature of the reagent (σ-allyl vs π-allyl) remain.[8]

Recently, we prepared configuration-fixed *cis*-aminoacyl-Pro dipeptide mimetics **6**,[9] which is a useful bioprobe for evaluating the structure–function relationships of Pro-containing peptides/proteins,[10] where (*Z*)-alkenes are substituted for the cis-peptide bond that is in equilibrium with the corresponding trans-peptide bond[11] (Scheme 1). A key transformation in our synthesis is the construction of a five-membered ring, which corresponds to the Pro moiety on unsaturated lactam **1**. Such five-membered ring formation involves the incorporation of a C3 unit at the α-position of **1**, followed by intramolecular Suzuki coupling, to give bicyclic lactam **5** as a crucial precursor of the (*Z*)-Pro mimetic. The '$CH_2CH_2CH_2OAc$' group as the C3 unit is incorporated at the α-position in regio- and diastereoselective manners with the aid of zinc–copper reagents[12] (e.g., $(IZn)_2Cu(CN)[(CH_2)_3OAc]_2{\cdot}2LiCl$) and is subsequently converted to the corresponding C3-borane moiety via an allyl group (Scheme 1, **1** to **4** via **2** and **3**). Directly incorporating the allyl group into **1** could decrease the synthetic steps; however, attempted reaction using allyl Grignard reagents in the presence of a copper



Scheme 1. Outline for the synthesis of *cis*-aminoacyl-Pro mimetics.

Keywords: Allylation; Copper-mediated *anti*-S_N2'; Dipeptide mimetic; Proline.
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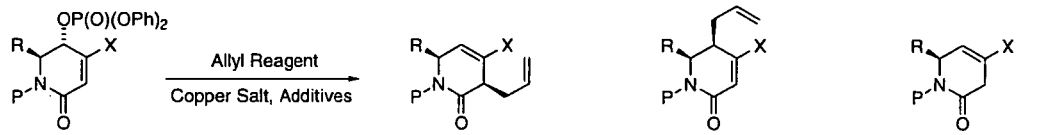
salt met with failure to give desired product in 9% yield with concomitant formation of various compounds including a reductive product. This situation prompted us to reconsider the feasibility of copper-mediated S_N2'-type allylation, which is applicable to the Pro-mimetic synthesis in the light of recent progress in the allylation reaction.

γ-Phosphoryloxy-α,β-unsaturated lactams[13] **7a–c** were selected as substrates for the examined reactions. Although non-β-halogenated substrate **7a** does not have potential as a precursor for mimetic synthesis, it is readily available. Using these substrates, we explored suitable reaction conditions (Table 1). Treatment of **7a** and **7b** with the allyl Grignard reagent in the presence of CuCN·2LiCl following methods described in the literature[7b–d] gave desired products **8a** and **8b**, respectively, in unacceptable yields accompanied by a non-negligible amount of reduced compound **10a** or **10b** (Table 1, entries 1 and 2). The addition of $ZnCl_2$ to the above reaction of **7a** improved the reaction outcomes, whereas the same tuning was unsatisfactory for the reaction of **7b** (Table 1, entries 3 and 4). Therefore, we next examined other allylmetal reagents as alternatives to the Grignard reagent.

Recently, Shibasaki's group has disclosed the synthetic utility of allylsilanes or allylboronates in the presence of copper salts and chiral ligands in the allylation of carbonyl compounds.[5] Inspired by their reports, we explored the synthetic applicability of a combination of allylsilanes (or allylboronates) and copper salts to the S_N2'-conversions. Treatment of **7a** with a reagent that consisted of allyltrimethoxysilane, tetrabutylammonium difluorotriphenylsilicate (TBAT), and CuCN in THF gave a mixture, which contained *anti*-S_N2' product **8a** (44%) and S_N2 product **9a** (23%) (Table 1, entry 5). In our previous study on the *anti*-S_N2' reaction on unsaturated lactams,[11b] the addition of lithium salts into the reaction mixture suppressed the formation of S_N2 products. However, the presence of lithium salts in the allylsilane–TBAT system inhibited the reaction, which was also the case for the allylboronate–TBAT system as discussed later (Table 1, entries 6 and 8). Our extensive search for suitable reaction conditions using allylsilanes was fruitless.

Table 1. Examination of *anti*-S_N2' allylation with various allylmetal reagents in the presence of copper salts



Substrates
7a (X = H, R = Me, P = Bzl)
7b (X = I, R = Me, P = DMB)
7c (X = I, R = CH_2OBzl, P = DMB)

anti-S_N2' products
8a
8b
8c

S_N2 products
9a
9b
9c

Reduction products
10a
10b

Entry	Sub.	Allylmetal reagent[a] (equiv)	Cu salt (equiv)	Additive(s) (equiv)	Conditions	Solvent(s)	Products (isolated yield %)
1	**7a**	Allyl Grignard (4)	CuCN (2)	LiCl (4)	−78 °C, 30 min	THF	**8a** (43), **10a** (27)
2	**7b**	Allyl Grignard (2)	CuCN (2)	LiCl (4)	−78 °C, 30 min	THF	**8b** (9), **10b** (12)
3	**7a**	Allyl Grignard (4)	CuCN (2)	$ZnCl_2$ (4), LiCl (4)	0 °C, 30 min	THF	**8a** (81), **9a** (6), **10a** (4)
4	**7b**	Allyl Grignard (4)	CuCN (2)	$ZnCl_2$ (4), LiCl (4)	0 °C, 30 min	THF	**8b** (22), **9b** (5), **10b** (24)
5	**7a**	Allylsilane (4)	CuCN (2)	TBAT (4)	0 °C, 1 h	THF	**8a** (44), **9a** (23)
6	**7a**	Allylsilane (4)	CuCN (2)	TBAT (4), LiCl (4)	0 °C, 1 h	THF	—[b]
7	**7a**	Allylboronate (4)	CuCN (2)	TBAT (4)	0 °C, 1 h	THF	**8a** (72), **9a** (16)
8	**7a**	Allylboronate (4)	CuCN (2)	TBAT (4), LiCl (4)	0 °C, 1 h	THF	—[b]
9	**7a**	Allylboronate (4)	CuCl (2)	TBAT (4)	0 °C, 1 h	THF	**8a** (72), **9a** (15)[c]
10	**7a**	Allylboronate (4)	CuBr (2)	TBAT (4)	0 °C, 1 h	THF	**8a** (68), **9a** (19)[c]
11	**7a**	Allylboronate (4)	CuSCN (2)	TBAT (4)	0 °C, 1 h	THF	**8a** (64), **9a** (8)[c]
12	**7b**	Allylboronate (4)	CuCN (2)	TBAT (4)	0 °C, 1 h	THF	**8b** (15), **9b** (39)
13	**7b**	Allylboronate (4)	CuCN (2)	TBAT (4)	0 °C, 1 h	DMF	**8b** (28), **9b** (42)
14	**7c**	Allylboronate (4)	CuCN (2)	TBAT (4)	0 °C, 1 h	DMF	**8c** (67), **9c** (20)
15	**7c**	Allylboronate (4)	CuCl (2)	TBAT (4)	0 °C, 1 h	DMF	**8c** (56), **9c** (28)
16	**7c**	Allylboronate (4)	CuBr (2)	TBAT (4)	0 °C, 1 h	DMF	**8c** (40), **9c** (25)
17	**7a**	Allylboronate (4)	CuSCN (2)	TBAF (4)	0 °C, 1 h	THF	**8a** (72), **9a** (4)
18	**7b**	Allylboronate (4)	CuSCN (2)	TBAF (4)	0 °C, 1 h	DMF–THF	**8b** (61), **9b** (21)
19	**7c**	Allylboronate (4)	CuSCN (2)	TBAF (4)	0 °C, 1 h	DMF–THF	**8c** (73), **9c** (11)
20	**7a**	Allylboronate (4)	CuCN (2)	LiO*i*-Pr (4)	0 °C, 1 h	THF	**8a** (81), **9a** (1)
21	**7b**	Allylboronate (4)	CuCN (2)	LiO*i*-Pr (4)	rt, 6 h[d]	THF	**8b** (73), **9b** (24), **7b** (3)
22	**7c**	Allylboronate (4)	CuCN (2)	LiO*i*-Pr (4)	rt, 6 h[d]	THF	**8c** (72), **9c** (4), **7c** (17)
23	**7b**	Allylboronate (4)	CuCN (0.1)	LiO*i*-Pr (4)	rt, 2 h[d]	THF	**8b** (92), **9b** (4)
24	**7c**	Allylboronate (4)	CuCN (0.1)	LiO*i*-Pr (4)	rt, 2 h[d]	THF	**8c** (84), **9c** (4), **7c** (10)

[a] Allyl magnesium chloride (allyl Grignard), allyltrimethoxysilane (allylsilane), or pinacol 2-propenylboronic ester (allylboronate) was used.
[b] Starting material was recovered.
[c] α-Phenylated material (ca. 5% (CuCl and CuBr) and 21% (CuSCN)) was detected.
[d] Because the starting materials were observed after 1 h, the reaction times were increased. However, the reactions are yet to be optimized.

Next, we examined the synthetic applicability of pinacol 2-propenylboronic ester as an allylboronate to the *anti*-S_N2' reaction. Reactions of **7a** with the allylboronate in the presence of various copper salts (CuCN, CuCl, CuBr, or CuSCN) and TBAT gave desired **8a** in moderate isolated yields (64–72%) with the accompanying S_N2 product (8–19%) (Table 1, entries 7, 9–11). In these reactions, an α-phenyl product was also formed. Especially, the use of CuSCN gave the phenyl product in 21% isolated yield, but a good regioselectivity was observed.[14] Encouraged by the fact that the desired product was obtained in moderate yield, we attempted reactions of **7b** and **7c** with CuX (X = Cl, Br or CN)–TBAT–allylboronate mixtures to prepare proline mimetics. Reaction of **7b** (Ala-Pro type) gave the *anti*-S_N2'/S_N2 mixture, but the S_N2 product was preferentially formed (Table 1, entries 12 and 13). Although treatment of **7c** (Ser-Pro type) afforded the *anti*-S_N2' product as the main compound, the conversion efficiency and *anti*-S_N2'/S_N2 selectivity remained unsatisfactory (Table 1, entries 14–16).

Hence, we reconsidered the reaction conditions in connection with the formation of the α-phenyl product. We speculated that the reaction of allylboronate with TBAT gave a mixture of the borate and silicate, and the remaining silicate formed of the α-phenyl product via a phenyl copper reagent (Fig. 1). Therefore, we initially examined the use of tetrabutylammonium fluoride (TBAF) as a non-silicate type fluoride source with the aid of CuSCN. Reactions of **7a–c** with the allylboronate–CuSCN–TBAF proceeded with moderate regioselectivities to afford the corresponding *anti*-S_N2' product **8a–c**,[15] respectively, without the accompanying α-phenylated product (Table 1, entries 17–19). Furthermore, to improve the regioselectivity, we employed an alkoxide (LiO*i*-Pr), which has been reported to effectively convert the borane to the corresponding borate.[5c] Fortunately, the reaction of **7a** with allylboronate–CuCN in the presence of LiO*i*-Pr in THF proceeded with almost perfect selectivity to furnish **8a** in 81% isolated yield (*anti*-S_N2':S_N2 = 81:1) (Table 1, entry 20). Applying this system to the reaction of **7b** and **7c** improved the products distribution, albeit some of the starting material remained (Table 1, entries 21 and 22).[16]

In order to demonstrate the synthetic usefulness of this system, we planned to use a catalytic amount of CuCN (10 mol %). It should be noted that the attempted reaction of **7b** with allylboronate (4 equiv) and LiO*i*-Pr (4 equiv) in the presence of 10 mol % CuCN yielded **8b** in 92% isolated yield with concomitant formation of **9b** (4%) (Table 1, entry 23). Application of this catalytic system to the conversion of **7c** also gave satisfactory results to give the desired **8c** in 84% isolated yield (S_N2'/S_N2 = 21:1 in Table 1, entry 24).

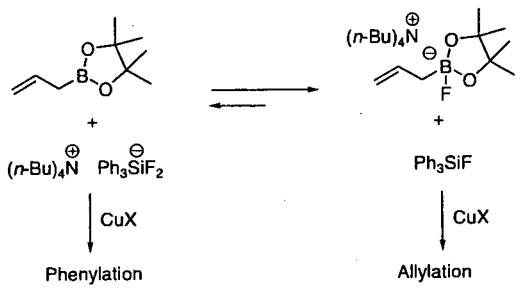
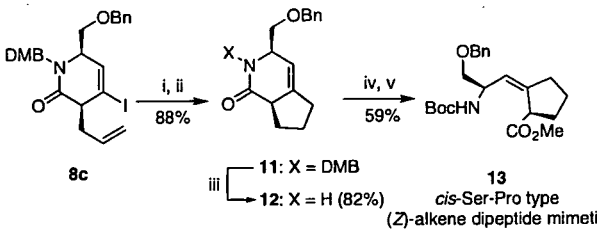


Figure 1. Plausible explanation for the formation of the α-phenylated product.



Scheme 2. Conversion of **8c** to *cis*-Ser-Pro mimetic **13**. Reagents and conditions: (i) 9-BBN–H (6 equiv) in THF at room temperature for 7 h; (ii) CsF (6 equiv) and $PdCl_2$(dppf) (10 mol %) in DMF at 50 °C for 3.5 h; (iii) TFA at 0 °C for 2 h then at room temperature for 4 h; (iv) $Me_3O{\cdot}BF_4$ (3 equiv) and 2,6-di-*t*-butylpyridine (1.1 equiv) in CH_2Cl_2 at room temperature for 5 h; (v) 0.1 M HCl in CH_2Cl_2–THF–MeOH–H_2O at 0 °C to room temperature for 12 h then Boc_2O (5.4 equiv) and Et_3N (5.0 equiv) for 5 h.

At this stage, origin of the improvement of the *anti*-S_N2'/S_N2 ratio in the use of allylboronate–CuCN–LiO*i*-Pr remains to be disclosed. Analysis of reagent formed in the reaction mixture by spectroscopic measurements would give some insight to clarify the factors responsible for the reaction outcomes.

Finally, the conversion of **8c** to the Ser-Pro type alkene dipeptide mimetic was conducted according to our previous synthetic protocol[9b] for the *cis*-Ala-Pro mimetic (Scheme 2).

In summary, we have developed a reliable *anti*-S_N2' allylation of γ-activated-α,β-unsaturated lactams using allylboronate and LiO*i*-Pr in the presence of a stoichiometric or catalytic amount of copper salt. Although the reason for the observed high regioselectivity has yet to be elucidated, the developed reactions have shortened the route to *cis*-Pro mimetics. Finally, our protocol may provide valuable insight into the development of copper-mediated allylation protocols on a wide variety of substrates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.017.

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13. Allylic phosphonates are a crucial substrate for copper-mediated *anti*-S_N2' reactions, see: Refs. 7b–d, 11a,b and (a) Calaza, M. I.; Hupe, E.; Knochel, P. *Org. Lett.* **2003**, *5*, 1059; (b) Soorukram, D.; Knochel, P. *Org. Lett.* **2004**, *6*, 2409.
14. In terms of the product distribution (*anti*-S_N2' vs S_N2), the use of CuSCN as a copper salt gave the best result among the examined conditions, which shows sharp contrast to the experimental results in Ref. 7d.
15. Relative configuration of **8b** was established by the comparison with the authentic sample in Ref. 9b. Reduction of the halogen in **8b** afforded **8b′** (*N*-DMB). On the basis of both NMR analyses of **8b** and **8b′** and the empirical rule mentioned below, cis-configuration was established. To our knowledge, copper-mediated S_N2'-reaction to the allyl phosphate proceeds in *anti*-manner with no exceptions.[7b,11a,b,13a,b] Therefore, relative configuration of **8c** was also tentatively assigned as cis.
16. The use of CuCN is critical in the allylboronate–LiO*i*-Pr system: reaction of **7b** with CuSCN resulted in the decrease in regioselectivity (**8b**/**9b** = 2.4:1); reaction with CuCl did not complete the reaction (**7b** (25%) was recovered).

One-pot synthesis of carbazoles by palladium-catalyzed *N*-arylation and oxidative coupling†

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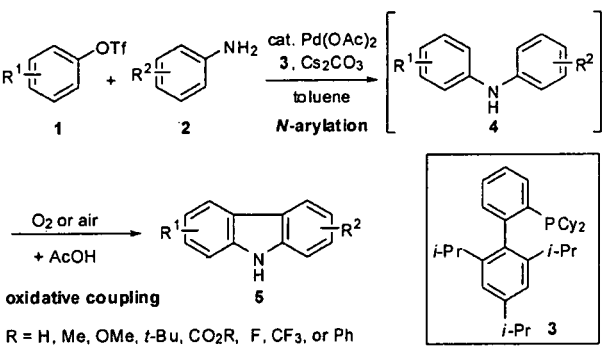
One-pot *N*-arylation and oxidative coupling can be promoted by a common palladium catalyst in the presence of appropriate additives: palladium-catalyzed *N*-arylation of anilines with aryl triflates under the standard conditions followed by addition of acetic acid under oxygen or air atmosphere afforded various types of functionalized carbazoles in good to excellent yields.

Palladium-catalyzed C–H activation of an aromatic group using aryl halides or triflates has received considerable attention in recent years, due to the wide-ranging need for the construction of fused aromatic ring systems using nonfunctionalized aryl groups.[1–3] A palladium(II)-mediated oxidative biaryl coupling reaction that activates two C–H bonds is more attractive in that a carbon–carbon bond can be directly formed from two nonfunctionalized aromatic carbon atoms with high atom economy. Formation of heterocycles through this type of intramolecular dehydrogenative coupling was first reported by Yoshimoto *et al.*[4] and Åkermark *et al.*,[5] by use of a stoichiometric amount of palladium(II) acetate.[6,7] Recent contributions to the catalytic version of this reaction in the presence of an appropriate co-oxidant such as cupric acetate,[8] *tert*-butyl hydroperoxide,[9] catalytic $Sn(OAc)_2$–oxygen,[10] or oxygen[10] significantly improved the potential synthetic utility of this type of transformation, although some catalytic reactions suffer from low yields.

We envisioned that one-pot Buchwald–Hartwig *N*-arylation[11,12] and oxidative biaryl coupling reaction in the presence of a common palladium catalyst[13] would serve as an attractive synthetic route to highly-functionalized carbazoles, which constitute an important class of compounds that exist in many biologically-active natural products.[14,15] Although a related carbazole synthesis by the reaction of haloanilines and halobenzenes through sequential palladium-catalyzed *N*-arylation and aromatic C–H activation has already been reported,[16] there are no precedents for a direct carbazole synthesis *via* oxidative biaryl coupling reaction that activates two C–H bonds. Herein we present the first direct construction of carbazoles **5** by coupling of readily available aryl triflates **1** and anilines **2**, through one-pot palladium-catalyzed *N*-arylation and oxidative coupling in the presence of molecular oxygen or air (Scheme 1).

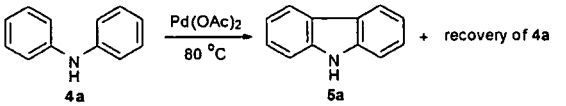
We first optimized reaction conditions in the oxidative coupling step (Table 1). In good accordance with Åkermark's observation,[10] oxygen proved to be effective to give **5a** in 91% yield (entry 1). We found that air, one of the ideal oxidants in practical use, appropriately promotes the coupling reaction, giving rise to a comparable result (90% yield, entry 2). By lowering the catalyst loading from 10 mol% to 5 mol%, the yield of the desired carbazole was slightly decreased (85%; see ESI†). Although the palladium-catalyzed *N*-arylation generally requires less polar solvents, solvents such as toluene were found to be ineffective for the oxidative carbazole formation (entry 3). In view of the addition of acetic acid to the reaction mixture after *N*-arylation, we investigated the carbazole formation in a mixed solvent including toluene and found that toluene–acetic acid (1 : 4) works well for this transformation under either oxygen or air (entries 5 and 6).



Scheme 1 Direct synthesis of carbazoles by one-pot *N*-arylation and oxidative coupling.

Based on the results of the oxidative coupling, we next investigated the one-pot carbazole formation, through Buchwald–Hartwig *N*-arylation[11,12] and oxidative coupling reaction, using

Table 1 Optimization of reaction conditions[a]



Entry	Oxidant (1 atm)	Solvent (0.1 M)	Time (h)	Yield (%)[b] 5a	4a
1	O_2	AcOH	24	91	—
2	Air	AcOH	24	90	—
3	O_2	toluene	24	15	62
4	O_2	toluene–AcOH (1 : 1)	24	12	84
5	O_2	toluene–AcOH (1 : 4)	36	80	—
6	Air	toluene–AcOH (1 : 4)	36	64	—

[a] Reactions carried out with 10 mol% of $Pd(OAc)_2$. [b] HPLC yield (absolute calibration curve method).

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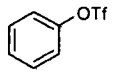
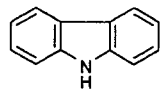
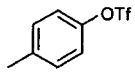
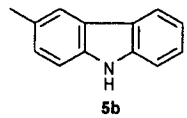
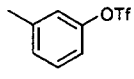
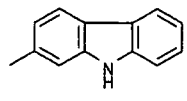
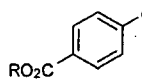
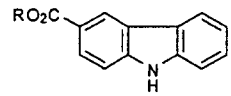
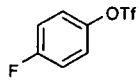
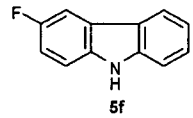
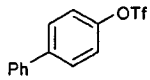
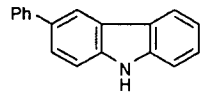
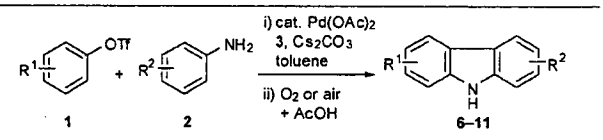
† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b707899d

Table 2 Reaction of aryl triflates with aniline[a]

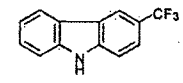
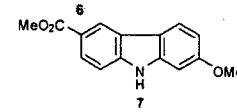
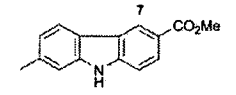
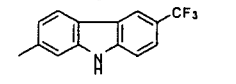
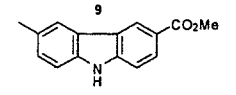
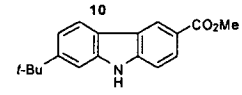
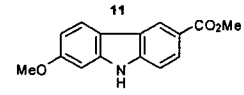
Entry	Triflate	T (°C)	Time (h)	Product	Yield (%)[b]
1	 **1a**	100[c]	10	 **5a**	69
2[d]		100	18		67
3	 **1b**	100	8	 **5b**	46
4	 **1c**	100	7.5	 **5c**	62
5[d]		100	24		82[e]
6	 **1d** (R = Me) **1e** (R = Bn)	120	30	 **5d** (R = Me) **5e** (R = Bn)	64[f]
7		120	8		63
8	 **1f**	100	38	 **5f**	57
9	 **1g**	120	10	 **5g**	65

[a] Reaction conditions: aryl triflate **1** (1.0 equiv.), aniline **2a** (1.1 equiv.), $Pd(OAc)_2$ (10 mol%), **3** (15 mol%), Cs_2CO_3 (1.2 equiv.), toluene (0.5 M), 100 °C, then O_2 (1 atm), AcOH (0.125 M). [b] Yields of isolated products. [c] When the reaction was performed at 80 °C, **5a** and **4a** were obtained in 55% and 15% yields, respectively. [d] Air was used in place of O_2. [e] Average isolated yield from two experiments. [f] *N*-arylation product, methyl 4-(phenylamino)benzoate, was obtained in 28% yield.

Table 3 Reaction of aryl triflates with substituted anilines[a]



1 **2** **6–11**

Entry	Triflate (R^1)	Aniline (R^2)	T (°C)	Time (h)	Product	Yield (%)[b]
1	**1a** (H)	**2b** (4-CF_3)	100	6	 **6**	72[c]
2	**1d** (4-CO_2Me)	**2c** (3-OMe)	100	17	 **7**	68
3	**1c** (3-Me)	**2d** (4-CO_2Me)	100	11	 **8**	>99
4[d]			100	24		93
5	**1c** (3-Me)	**2b** (4-CF_3)	100	6	 **9**	78
6	**1b** (4-Me)	**2d** (4-CO_2Me)	80	24	 **10**	78
7	**1h** (3-*t*-Bu)	**2d** (4-CO_2Me)	100	12	 **11**	95
8[d]			100	24		88
9	**1i** (3-OMe)	**2d** (4-CO_2Me)	100	9	 **7**	80

[a] Reaction conditions: aryl triflate **1** (1.0 equiv.), aniline **2** (1.1 equiv.), $Pd(OAc)_2$ (10 mol%), **3** (15 mol%), Cs_2CO_3 (1.2 equiv.), toluene (0.5 M), 100 °C, then O_2 (1 atm), AcOH (0.125 M). [b] Yields of isolated products. [c] *N*-arylation product, *N*-(4-trifluoromethylphenyl)aniline was obtained in 23% yield. [d] Air was used in place of O_2.

various aryl triflates **1a–g** and aniline **2a**. The results are summarized in Table 2. Typically, *N*-arylation of aniline **2a** with an aryl triflate **1a** was conducted in toluene by use of the standard procedure using palladium acetate (10 mol%) and phosphine ligand **3** (15 mol%).[17] After completion of the *N*-arylation determined by TLC, acetic acid was added and an oxygen balloon was connected to the reaction flask (oxygen conditions) or subjected to air by an open system (air conditions). As we expected, the one-pot reaction of phenyl triflate **1a** with aniline **2a** using the oxygen conditions gave the desired carbazole **5a** in 69% yield (entry 1). Moreover, under the air conditions, **5a** was obtained in 67% yield, although a slightly longer reaction time was required (entry 2). 3-Methylated triflate **1c** (entry 4) was more reactive than the 4-substituted one **1b** (entry 3) to afford carbazole **5c** regioselectively under the oxygen conditions. Of note, the reaction of **1c** under the air conditions furnished an even better yield in a prolonged reaction time (82%, entry 5) than that under the oxygen conditions (62%, entry 4). Triflates **1d–g** bearing an electron-withdrawing substituent also gave moderate yields of 3-substituted carbazoles **5d–g** (entries 6–9). It should be clearly noted that the use of aryl halides interfered with the oxidative coupling by concomitantly generating halide anion in *N*-arylation, as described by Åkermark and co-workers.[10a,18]

The one-pot reaction of various aryl triflates with substituted anilines was then investigated (Table 3). The reaction of phenyl triflate **1a** with electron-deficient 4-(trifluoromethyl)aniline **2b** (entry 1) gave a comparable result to that obtained by the reaction of electron-deficient triflates **1d–g** with aniline **2a** (Table 2, entries 6–9). The reaction of electron-deficient triflate **1d** with electron-rich aniline **2c** gave the desired carbazole **7** in 68% yield (entry 2). The combination of 3-methylated triflate **1c** with 4-(methoxycarbonyl)aniline **2d** gave the desired carbazole **8** in quantitative yield (entry 3). Similarly, good results were obtained using electron-rich triflates and electron-deficient anilines (entries 4–9). It was proven that the desired carbazoles can be obtained in high yields by the reaction of aryl triflates substituted by an electron-donating group at the 3-position such as **1c**, **1h** or **1i**, especially with anilines substituted by an electron-withdrawing group such as **2b** and **2d** (entries 3, 5, 7, and 9). Also in these reactions, air oxidation appropriately promoted the desired coupling reaction (entries 4 and 8). It is noteworthy that, although 3-substituted aryl triflates could give two regioisomers, the C–H

activation exclusively proceeded at the sterically less-hindered aromatic carbon of the triflate to afford carbazoles as the single isomer. In many cases, *N*-arylation proceeded almost quantitatively, so the yield of carbazoles was mainly dependent on the reactivity of the resulting diarylamines in the C–H activation step.

In conclusion, we have developed an efficient atom-economic synthetic route from aryl triflates and anilines to functionalized carbazoles in a one-flask reaction. This study first demonstrated that a common palladium catalyst with different additives promotes sequential *N*-arylation and oxidative C–H activation, besides careful examination of interesting substituent effects on the reactivity. Application of this method to the total synthesis of biologically-active natural products and an investigation of the reaction mechanism of C–H activation are now in progress.

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Notes and references

1 For pioneering work, see: (*a*) D. E. Ames and D. Bull, *Tetrahedron*, 1982, **38**, 383; (*b*) D. E. Ames and A. Opalko, *Tetrahedron*, 1984, **40**, 1919.

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17 Phosphine ligand **3** was oxidized under the oxidative coupling conditions (see ESI†).

18 Indeed, the treatment of diarylamine **4** in AcOH under oxygen atmosphere with palladium dichloride or palladium acetate–tetrabutylammonium bromide led to recovery of the starting material.

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Gold-Catalyzed Hydroarylation of Allenes: A Highly Regioselective Carbon–Carbon Bond Formation Producing Six-Membered Rings

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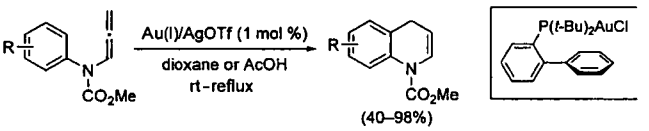
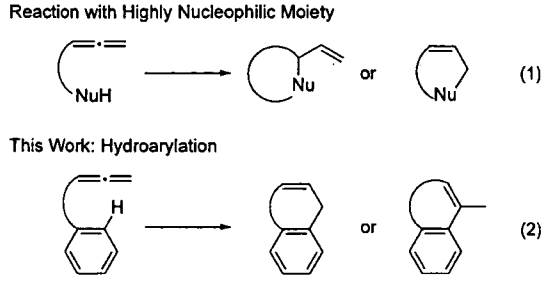
ABSTRACT



Gold-catalyzed intramolecular hydroarylation of allenic anilines and phenols offers an efficient route to dihydroquinoline and chromene derivatives under mild reaction conditions. The hydroarylation takes place at the terminal or central allenic carbon depending on the substrate structure, leading to a highly selective formation of six-membered rings.

Transition-metal-catalyzed cycloisomerization of allenes has received considerable attention as an atom-economical transformation.[1] Compared to the well-documented cyclization reactions with a highly nucleophilic functionality such as nitrogen, oxygen, or active methylene (Scheme 1, eq 1), cycloisomerization through functionalization of an aromatic C−H bond (hydroarylation; eq 2) has scarcely been investigated. Nagao and co-workers reported endo-mode hydroarylation of allenic ketones promoted by Lewis acids such as $BF_3{\cdot}OEt_2$ or $TiCl_4$.[2] Cycloisomerization of aryl allenic ethers mediated by a stoichiometric amount of mercury(II) trifluoroacetate to form regioisomeric six-membered rings has also been shown.[3−5]

Scheme 1. Transition-Metal-Catalyzed Reaction of Allenic Compounds



As a part of a program directed toward the development of novel methods for cyclization of allenic compounds,[6] we

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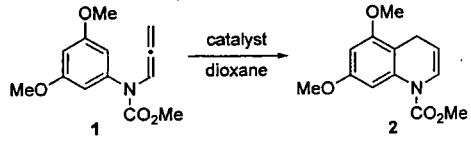
planned to investigate the catalytic hydroarylation of allenes in the presence of a gold salt. Gold-catalyzed reaction is of current interest for various transformations[7] including nucleophilic cyclization of allenes shown in eq 1.[8-10] Although 6-*exo* cyclization of allenes with a highly nucleophilic indole[11a] or pyrrole ring[11b] and 5-*endo* indene formation from acetoxy-substituted allenes[12] have been already reported, there have been no precedents for gold-catalyzed 6-*endo* hydroarylation of allenes that can be applied to a variety of allenes and aromatic rings.[13] Herein we report hydroarylation of allenes derived from anilines and phenols, leading to dihydroquinolines and chromenes, which are widely found as core structures of natural products and other biologically active compounds.[14,15]

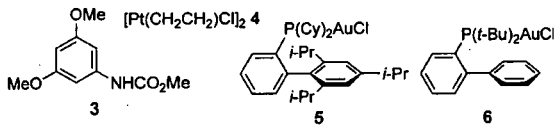
First, screening of transition-metal catalysts for the hydroarylation of allenes was performed by use of *N*-allenylaniline **1**, which was easily prepared by propargylation of *N*-protected 3,5-dimethoxyaniline followed by *t*-BuOK-mediated isomerization.[16,17] Results are summarized in Table

Table 1. Screening of Transition-Metal Catalysts[a]



entry	catalyst (mol %)	temp (°C)	time (min)	yield[b] 2	yield[b] 3
1	$Pd(OAc)_2$ (5)	80	360		14
2	$CuBr_2$ (5)	80	60		12
3	AgOTf (5)	25	720	trace	
4	AuCl (5)	80	90	29[d]	
5	$AuCl_3$ (5)	25	30	58	
6	$PtCl_2$ (5)	80	360	77	
7	**4**/P(*p*-$CF_3C_6H_4)_3$ (5)	25	5	57	
8	(Ph_3P)AuCl/AgOTf (5)	25	10	56	
9	**5**/AgOTf (5)	25	10	86	
10	**6**/AgOTf (5)	25	5	98	
11	**6**/AgOTf (1)	25	5	96	

[a] Reactions were carried out in dioxane at room temperature. [b] Yields based on ^{1}H NMR. [c] 13% of **1** was recovered.



1 (for more details, see Supporting Information). Whereas $Pd(OAc)_2$, $CuBr_2$, and AgOTf afforded a mixture of undesired products such as **3** as well as the recovered starting material (entries 1−3), AuCl, $AuCl_3$, and $PtCl_2$ gave the desired cyclization product **2** in low to good yields (29−77%, entries 4−6). The reaction with a platinum complex **4**/*p*-$(CF_3C_6H_4)_3P$[11a] completed in only 5 min, affording **2** in 57% yield (entry 7). While the reaction with (Ph_3P)AuCl/AgOTf gave **2** in moderate yield (56%, entry 8), a gold complex **5**[18] in the presence of AgOTf gave a more promising result (86% yield, entry 9). Among the catalysts investigated, gold complex **6**[18] with AgOTf was most effective in producing the desired product **2** in 98% yields (entry 10). By lowering the catalyst loading to 1 mol %, a comparable result was obtained (96% yield, entry 11). Considering that combination of the gold and silver salts is important (compare entries 3 and 11), a cationic gold complex would be the reactive species for this transformation.

We next investigated the cyclization of various *N*-allenylaniline derivatives based on the optimized conditions (Table 1, entry 11) for the allenylaniline **1**. Results are summarized in Table 2. Disubstituted electron-rich aniline derivatives **7** and **9** gave the desired products **8** and **10**, respectively (both in 88% yield after hydrogenation; entries 2 and 3). While the reaction of monomethoxyaniline derivative **11** provided **12** as the sole product (90% yield after

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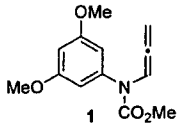
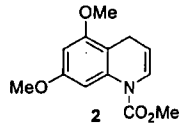
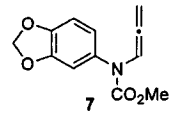
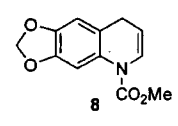
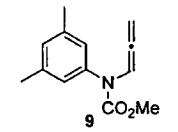
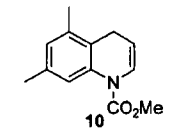
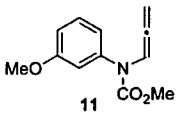
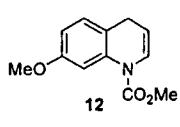
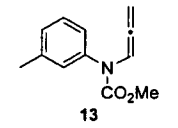
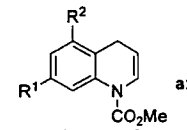
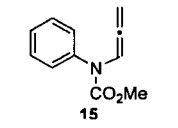
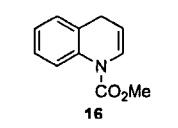
(17) Unfortunately, synthesis of internal allenes by this approach was difficult.

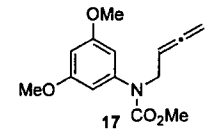
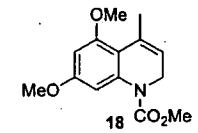
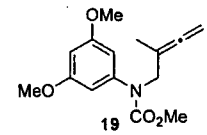
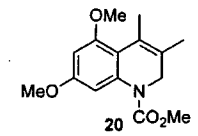
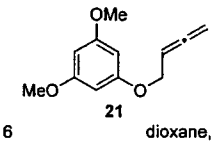
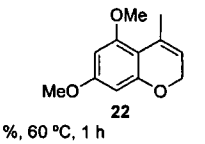
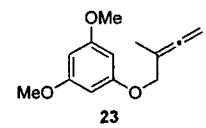
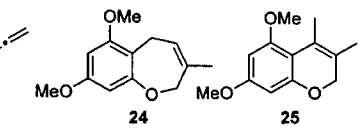
(18) Complexes **5** and **6** were prepared according to Lopez, S.; Nieto-Oberhuber, C.; Echavarren, A. M. *J. Am. Chem. Soc.* **2005**, *127*, 6178.

Table 2. Au-Catalyzed Cyclization of *N*-Allenylaniline Derivatives[a]

entry	substrate	conditions	product	yield(%)[b]
1		25 °C, 5 min		92
2		60 °C, 1 h		88
3		60 °C, 1 h		88
4		100 °C, 1 h		90
5		reflux, 3 h		72, a:b = 61:39[c]
			14a (R^1 = Me, R^2 = H) **14b** (R^1 = H, R^2 = Me)	
6		reflux, 3 h[d]		40

[a] Conditions: 1 mol % of **6**/AgOTf in dioxane. [b] Isolated yields after hydrogenation since most of the cyclized products with a 1,4-dihydroquinoline moiety were relatively unstable and gradually decomposed during isolation. [c] Ratio was determined by ^{1}H NMR. [d] 3 mol % of **6**/AgOTf was used.

Table 3. Au-Catalyzed Cyclization of *N*-(Buta-2,3-dienyl)aniline Derivatives and Their Phenol Analogues

entry	substrate	conditions[a]	product	yield (%)[b]
				
	17		**18**	
1		dioxane, 3 mol %, 60 °C, 1.5 h		63
2		AcOH, 3 mol %, 25 °C, 1 h		82
3		AcOH, 1 mol %, 60 °C, 1 h		85
				
	19		**20**	
4		AcOH, 3 mol %, 25 °C, 4 h		75
5		AcOH, 1 mol %, 60 °C, 1 h		74
				
	21		**22**	
6		dioxane, 1 mol %, 60 °C, 1 h		98[c]
				
	23		**24** **25**	
7		dioxane, 1 mol %, 60 °C, 4 h		68 (24:25 = 59:41)[d]
8		dioxane/AcOH (4:1), 1 mol %, 60 °C, 3.5 h		99 (24:25 = 48:52)[d]

[a] Solvent, loading of **6**/AgOTf, reaction temperature, and reaction time. [b] Isolated yield. [c] Isolated yield after hydrogenation. [d] Ratios were determined by ^{1}H NMR.

hydrogenation) by regioselective cyclization at the less hindered aromatic carbon (entry 4), 3-methylaniline derivative **13** afforded **14** as a mixture of regioisomers (**14a**:**14b** = 61:39, entry 5). As anticipated, unsubstituted derivative **15** showed lower reactivity; however, ca. 40% yield of the desired product **16** was obtained by refluxing in dioxane with 3 mol % of the catalyst. In all cases examined, reaction at the terminal allenic carbon resulted solely in formation of a six-membered ring.

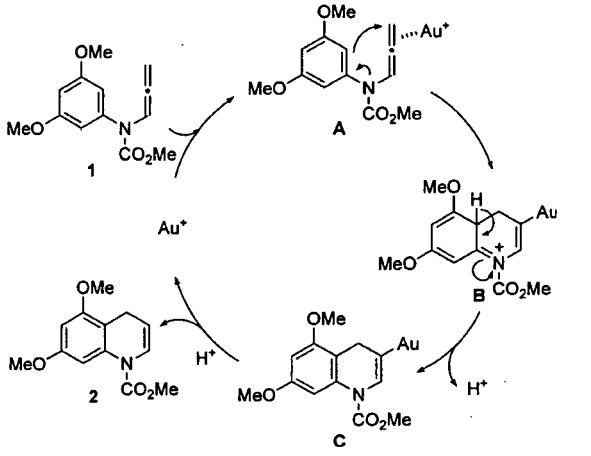
We proceeded to explore the scope of this cyclization (Table 3). Although the reactivity of *N*-(buta-2,3-dienyl)-aniline derivative **17** was relatively low even with 3 mol % of the catalyst at 60 °C (entry 1), use of AcOH[8d] instead of dioxane dramatically enhanced its reactivity to afford **18** in 82% yield at room temperature (entry 2).[19] When the reaction was performed in AcOH at 60 °C, 85% yield of **18** was obtained with 1 mol % of the catalyst (entry 3). A similar result was obtained with disubstituted allene derivative **19** (entries 4 and 5). Phenol derivative **21** has a sufficient reactivity in dioxane, affording **22** in 98% yield (after hydrogenation) with 1 mol % of the catalyst (entry 6). These are remarkable examples of transition-metal-catalyzed hydroarylation of unactivated allenes at the central carbon.[20] Interestingly, the reaction of phenol-derived methylated allene **23** in dioxane slightly favors seven-membered ring formation to give dihydrobenzo[*b*]oxepine derivative **24**, produced by hydroarylation at the terminal allenic carbon, as well as the six-membered ring **25** (**24**:**25** = 59:41, entry 7). Although the reaction of **23** in AcOH as the sole solvent gave a mixture of unidentified products, addition of a small amount of AcOH to dioxane gave **24** and **25** in 99% combined yield (entry 8).

A proposed mechanism for the hydroarylation of allenes is shown in Scheme 2. The allene is activated by coordination to cationic gold and undergoes electrophilic aromatic substitution with the electron-rich arene to give vinyl-gold complex **B**, which is deprotonated to produce the neutral vinyl-gold intermediate **C**. Cleavage of the gold–carbon

(19) The reaction did not proceed in AcOH without using the gold catalyst.

(20) Such reactions with Hg(II) reagent have already been reported; see ref 3. For the reactions of activated allenes, see refs 2 and 4b.

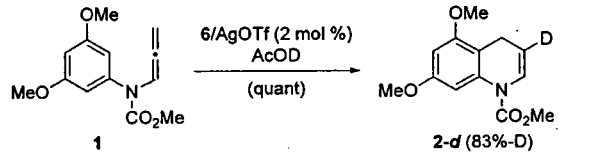
Scheme 2. Proposed Mechanism



bond by the proton generated in the previous step affords **2** and regenerates the cationic gold catalyst.

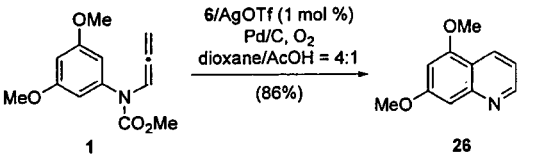
In order to reveal the role of AcOH as the activator of hydroarylation,[8d] we conducted deuterium experiments using AcOD (Scheme 3). As expected, the reaction of *N*-allenylaniline **1** in AcOD gave **2-*d*** (83%-d) in quantitative yield (Scheme 3). This result strongly suggests that the high reactivity in AcOH is attributed to acceleration of the protonation step from **C** (Scheme 2).

Scheme 3. Deuterium Experiment Using AcOD



Finally, one-pot synthesis of quinoline was investigated (Scheme 4).[21] When the reaction of the allenylaniline **1** was conducted in the presence of a catalytic amount of Pd/C under O_2, the desired quinoline **26** was obtained in 86% yield. This one-pot reaction clearly demonstrates the utility of the present hydroarylation of allenes as a convenient tool for construction of heterocycles.

Scheme 4. Synthesis of Quinoline by One-Pot Reaction



In conclusion, we have developed a novel efficient route from allenic compounds to dihydroquinolines and chromenes by means of gold-catalyzed intramolecular hydroarylation. We are now exploring ways to broaden the scope of the reaction, for example, to include various arenes.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Encouragement of Young Scientists (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, 21st Century COE Program "Knowledge Information Infrastructure for Genome Science", and the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

Supporting Information Available: Representative experimental procedure, as well as ^1H and ^{13}C NMR spectra for the novel compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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(21) For a related reaction, see: Bennasar, M. L.; Roca, T.; Monerris, M.; García-Díaz, D. *J. Org. Chem.* **2006**, *71*, 7028.

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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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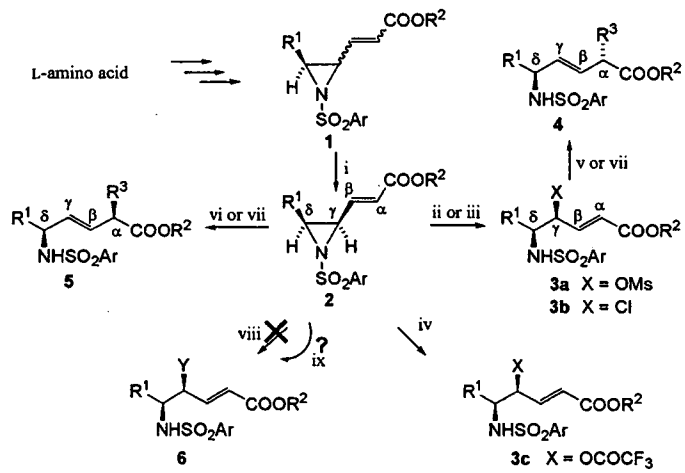
Abstract—Treatment of *N*-arylsulfonylaziridines bearing α,β-unsaturated esters with alcohols, thiols or weak acids such as AcOH in the presence of catalytic amount of Lewis acids affords regio- and stereoselectively ring-opened products, such as δ-aminated γ-alkoxy-(alkylthio or acetoxy)-α,β-enoates. 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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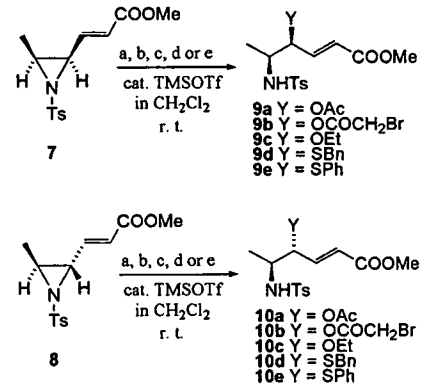
(*E*)-alkene dipeptide isosteres (EADIs) **4** via organocopper (or organozinc-copper)-mediated *anti*-S_N2' reactions.[3] On the other hand, organocopper (or organozinc-copper)-mediated *anti*-S_N2' reactions of *cis*-(*E*) isomers **2** exclusively provide (L,L)-type EADIs **5**. The utility of EADIs 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.



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

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

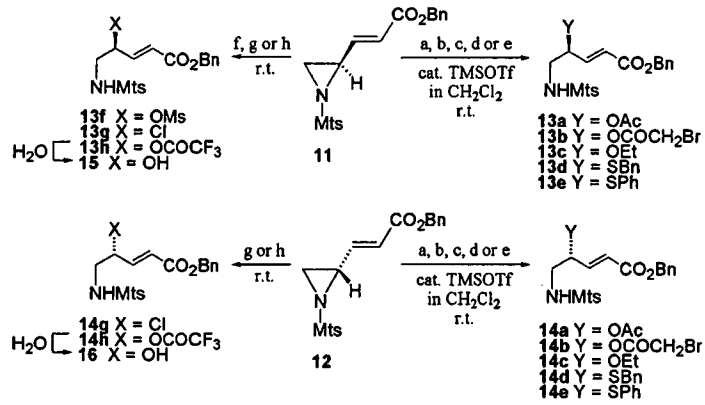
Substrate	YH	YH/TMSOTf (equiv)	Time (h)	Product	Yield (%)
7	AcOH	20/0.1	15	**9a**	90
7	$BrCH_2CO_2H$	10/0.1	15	**9b**	82
7	EtOH	3/0.3	7	**9c**	98
7	BnSH	10/0.1	1	**9d**	95
7	PhSH	10/0.1	1	**9e**	96
8	AcOH	20/0.1	15	**10a**	98
8	$BrCH_2CO_2H$	10/0.1	15	**10b**	86
8	EtOH	3/0.3	7	**10c**	99
8	BnSH	10/0.1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

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

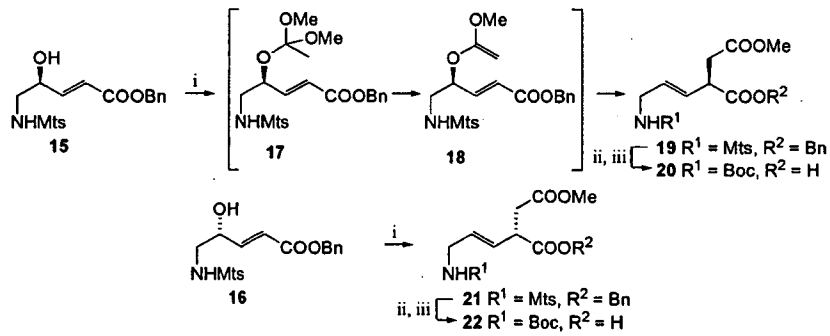


Scheme 3. Mts=2,4,6-trimethylphenysulfonyl; reagents: (a) AcOH; (b) $BrCH_2COOH$; (c) EtOH; (d) BnSH; (e) PhSH; (f) $MeSO_3H$ in $CHCl_3$; (g) HCl in 1,4-dioxane and (h) TFA.

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