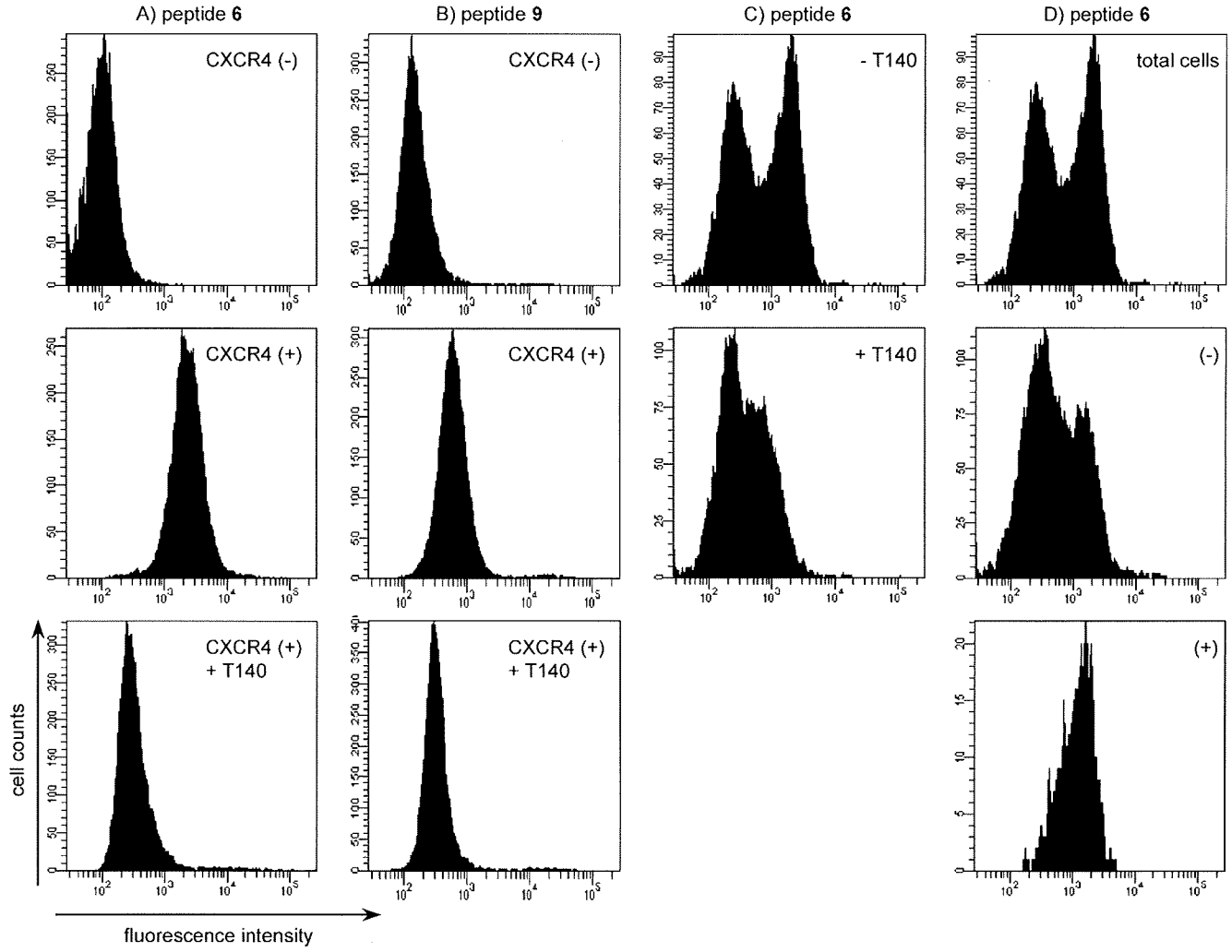

Figure 1. Application of fluorescent CXCR4 antagonists **6** and **9** to flow cytometry. CHO cells were incubated with labeled peptides (200 nM) A) **6**, and B) **9**. The top and middle panels show the results with cells that did not (–) and did express CXCR4 (+), respectively. Competitive binding was assessed with T140 (200 nM; lower panels). C) FACS (fluorescence-activated cell sorting) data for mouse spleen cells treated with peptide **6** (200 nM) in the presence (+) and absence (–) of T140 (200 nM). D) Chemotaxis experiment with mouse spleen cells (top panel). Cells from the total population that did not display chemotaxis are shown in the middle panel (–), and cells that migrated in response to a gradient of SDF-1 are shown in the lower panel (+).

(39 μL, 0.25 mmol) and HOBt (38 mg, 0.25 mmol) in DMF (2 mL). The resulting protected peptide resin (0.05 mmol) was treated with TFA/H_2O/EDT (95:2.5:2.5, 4 mL) for 2 h at room temperature. After removal of the resin by filtration, ice-cold dry Et_2O (100 mL) was added to the residue. The resulting powder was collected by centrifugation and then washed with ice-cold dry Et_2O (3×50 mL). The crude reduced peptide was dissolved in H_2O (100 mL), and the pH value was adjusted to 8.0 with NH_4OH. After oxidation by exposure to air for 1 day, the crude product in the solution was purified by preparative HPLC to afford the desired peptide as a white powder.

Removal of the Mtt protecting group: The resin (0.05 mmol) was treated with CH_2Cl_2/HFIP/TFE/TES (6.5:2:1:0.5, 10 mL) for 2 h at room temperature. It was then washed with the same mixture twice, treated with 10% iPr_2NEt in DMF, and used for the next coupling.

Conjugation of Alexa Fluor 488 succinimidyl ester with peptides: Lyophilized peptide (4.66 μmol) and iPr_2NEt (3.77 μL, 27.2 μmol) were added to a solution of Alexa Fluor 488 succinimidyl ester (2.50 mg, 3.88 μmol) in DMF (250 μL), and the resulting mixture was stirred in the dark for 12 h at room temperature. The crude mixture was then diluted with MeOH (100 μL) and purified by HPLC. Fractions containing Alexa Fluor 488 conjugates were collected and lyophilized to give **5** (3.3 mg, 27%), **9** (5.1 mg, 51% from **1**), or **10** (5.88 mg, 46% from **17**) as a red powder.

Acknowledgements

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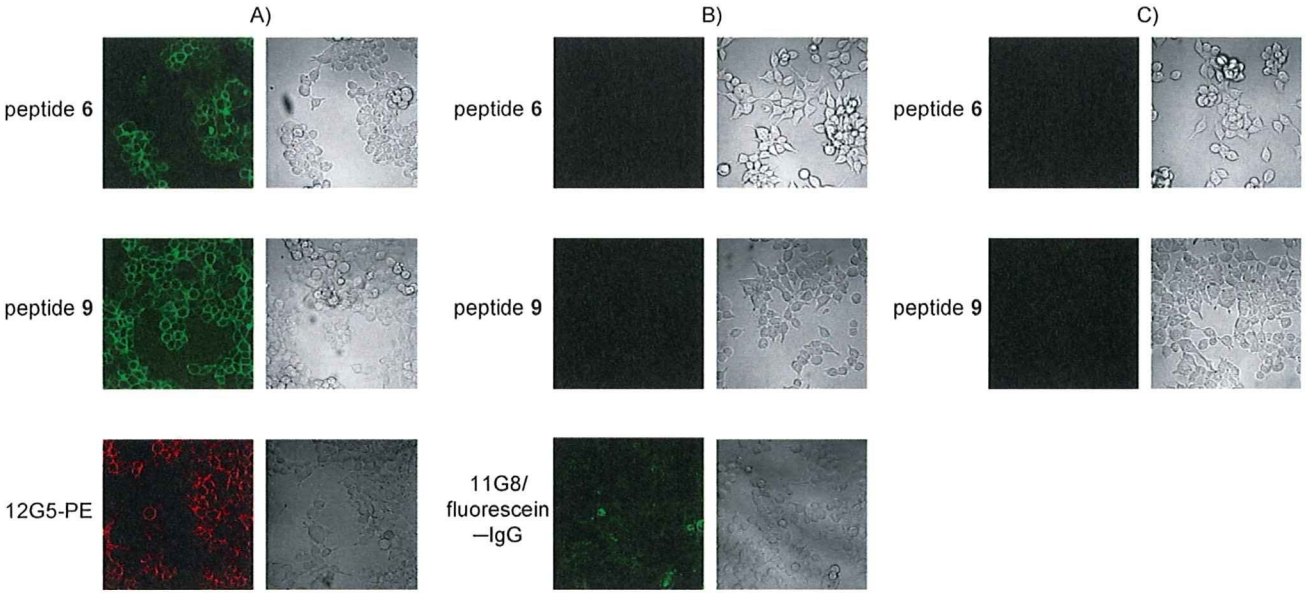

Figure 2. Confocal images of HEK293 cells stained with peptides **6** and **9** (100 nM): A) CXCR4-expressing cells, B) CXCR7-expressing cells, C) CXCR4-negative control cells. CXCR4- and CXCR7-receptor expression was verified by using the monoclonal antibodies 12G5 and 11G8.

Keywords: cell imaging · chemokine receptors · CXCR4 antagonists · fluorescent probes · peptides

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Facile synthesis of 3-(aminomethyl)isoquinolines by copper-catalysed domino four-component coupling and cyclisation†

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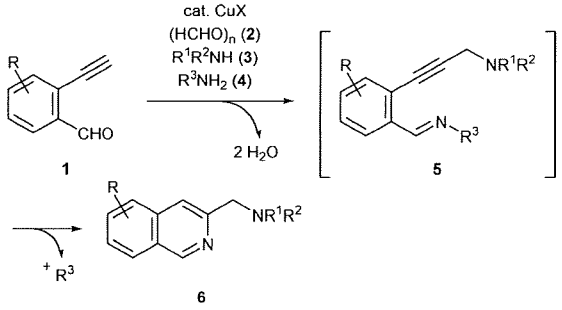
Copper(I)-catalysed domino four-component coupling–cyclisation using 2-ethynylbenzaldehydes, paraformaldehyde, secondary amine, and *t*-BuNH$_2$ in DMF leads to direct and efficient formation of 3-(aminomethyl)isoquinolines in good to high yields.

The isoquinoline scaffold can be found in a wide variety of biologically active natural and synthetic compounds.[1] Particularly, isoquinolines having an additional nitrogen atom tethered by one carbon at the 3-position, including such isoquinoline alkaloids as quinocarcin[2] and ecteinascidin 597 and 583,[3] and 3-(2-pyridinyl)isoquinolines,[4] constitute an important class of compounds with important biological activities. With a continuing interest in the development of environmentally-benign synthesis as well as multi-component reactions in modern synthetic chemistry,[5] we planned a novel diversity-oriented synthetic methodology for the construction of these molecules by the use of a domino multi-component coupling–cyclisation reaction.

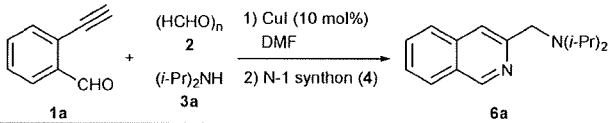
Recently, we have reported an efficient construction of 2-(aminomethyl)indoles by a copper-catalysed three-component coupling–cyclisation reaction.[6] This reaction proceeds through Mannich-type coupling followed by indole formation. On the basis of our indole synthesis, we expected that a four-component coupling reaction of 2-ethynylbenzaldehydes **1**, aldehyde **2**, secondary amine **3**, and an appropriate N-1 synthon **4**, followed by cyclisation of the alkyne intermediate **5**, having a nitrogen atom in proximity to the triple bond,[7,8] would provide a direct route to 3-(aminomethyl)isoquinolines **6** without wasting any salts (Scheme 1). Herein, we describe a copper-catalysed domino four-component coupling–cyclisation reaction for diversity-oriented synthesis of 3-(aminomethyl)isoquinolines. To the best of our knowledge, this is the first example of a four-component synthesis of an isoquinoline scaffold.[9]

In the initial investigation, we examined the effect of the N-1 synthon on the copper-catalysed four-component synthesis of 3-(aminomethyl)isoquinoline using 2-ethynylbenzaldehyde **1a** as a model substrate, paraformaldehyde **2** and diisopropylamine **3a** (Table 1). Since two nucleophilic reagents coexist with two aldehydes in the reaction system, progress of the nucleophilic reactions in the desired order might be hampered on one-portion reaction.[10] Accordingly, after the copper-catalysed three component reaction of **1a**, **2**, and **3a** in DMF was complete, being monitored by TLC, the N-1 synthon was added. Whereas ammonium nitrate **4a**, perchlorate **4b**, hydroxide **4c**, formate **4d**, chloride **4e**, and sulfate **4f** were ineffective (entries 1–6), the use of acetate **4g** and hydrogen carbonate **4h** gave, as expected, the desired isoquinoline **6a** in moderate yields (42–53%, entries 7 and 8).[11] More promising results were obtained with primary amines having a readily cleavable alkyl group such as 2,4,6-trimethoxybenzylamine hydrochloride **4i** and *tert*-butylamine **4j**,[7] leading to high yields of **6a**



Scheme 1 Construction of 3-(aminomethyl)isoquinolines by copper-catalysed four-component coupling–cyclisation.

Table 1 Optimisation of the N-1 synthon **4**[a]



Entry	N-1 synthon	Yield (%)[b]
1	NH_4NO_3 (**4a**)	Decomp.
2	NH_4ClO_4 (**4b**)	Decomp.
3	28% NH_4OH (**4c**)	Trace
4	$NH_4(HCO_2)$ (**4d**)	Trace
5	NH_4Cl (**4e**)	Trace
6	$(NH_4)_2SO_4$ (**4f**)	Trace
7	NH_4OAc (**4g**)	42
8	NH_4HCO_3 (**4h**)	53
9	2,4,6-$(MeO)_3C_6H_2CH_2NH_2 \cdot HCl$ (**4i**)	82
10	*t*-$BuNH_2$ (**4j**)	83

[a] After a mixture of 2-ethynylbenzaldehyde **1a**, paraformaldehyde **2** (2 equiv.), amine **3a** (2 equiv.) and CuI (10 mol%) in DMF was stirred at room temperature for 1 h, and N-1 synthon **4** (6 equiv.) was added. The resulting mixture was stirred for 5 h at room temperature and for an additional 45 min at 140 °C. [b] Isolated yield.

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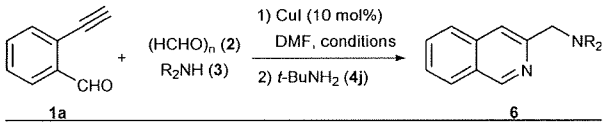
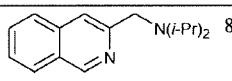
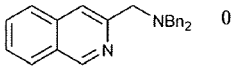
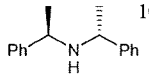
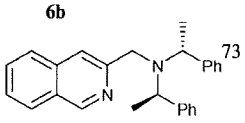
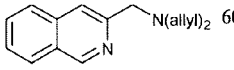
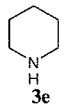
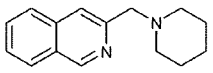
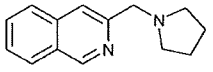
† Electronic supplementary information (ESI) available: Experimental details and NMR spectra. See DOI: 10.1039/b718201e

Table 2 Synthesis of 3-(aminomethyl)isoquinolines[a]



Entry	Amine	Conditions[b]	Product	Yield (%)[d]
1	i-Pr_2NH	rt, 1 h		83
	3a		**6a**	
2	Bn_2NH	100 °C, 1 h		0
	3b		**6b**	
3		100 °C, 1 h		73
	3c		**6c**	
4	$(allyl)_2NH$	rt, 1 h[c]		60
	3d		**6d**	
5		rt, 1 h[c]		88
	3e		**6e**	
6		rt, 1 h[c]		79
	3f		**6f**	

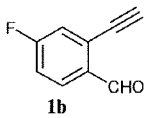
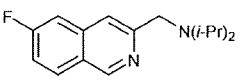
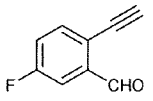
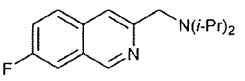
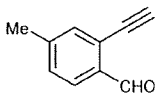
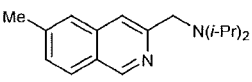
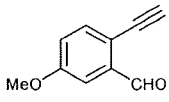
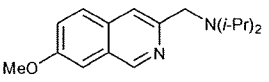
[a] After the three-component reaction of **1a**, **2** (2 equiv.), and **3** (2 equiv.) in the presence of CuI (10 mol%) in DMF was completed on TLC, t-$BuNH_2$ (**4j**, 6 equiv.) was added and the reaction mixture was stirred for 5 h at room temperature and for an additional 45 min at 140 °C. [b] Conditions for the three-component coupling. [c] Before **1a** was added, a mixture of **2**, **3** and CuI in DMF was stirred for 30 min at room temperature. [d] Isolated yield.

(entries 9 and 10).[12] Taking the atom economy of the reaction into consideration, we regarded **4j** as the most potent N-1 synthon.

Next, various secondary amines were employed to determine the scope of this reaction (Table 2). Although dibenzylamine **3b** showed lower reactivity toward Mannich-type coupling with **1a** and **2**, leading to recovery of the unchanged starting material (entry 2),[13] the reaction with more bulky bis(1-phenylethyl)amine **3c** led to successful conversion into the corresponding isoquinoline **6c** (73%, entry 3). Unfortunately, the initial Mannich type reaction with highly nucleophilic diallylamine, piperidine, or pyrrolidine was unsuccessful, producing a complex mixture, presumably due to the simultaneous presence of two aldehydes (2-ethynylbenzaldehyde **1a** and paraformaldehyde **2**) and a reactive amine. Extensive optimisation of the reaction conditions brought about addition of 2-ethynylaldehyde **1a** after the formation of iminium ions between secondary amines **3d–f** and paraformaldehyde **2**. As a result, the corresponding 3-(aminomethyl)isoquinolines **6d–f** were obtained in moderate to high yields (entries 4–6).

The copper-catalysed domino four-component syntheses of 3-(aminomethyl)isoquinolines with some substituted 2-ethynylbenzaldehydes were next investigated (Table 3). The use of 2-ethynyl-4-fluorobenzaldehyde **1b** in the presence of CuI (10 mol%) gave the desired 3-(aminomethyl)-6-fluoroisoquinoline derivative **7** in high yield (83%, entry 1). Benzaldehyde **1c**, which has a fluorine atom at the *meta*-position to the formyl group, afforded the corresponding isoquinoline **8** (79%, entry 2). Also, in the cases of 2-ethynylbenzaldehydes containing an electron-donating group such as a methyl or a methoxy group at the *para*- or *meta*-position to the formyl group (**1d** and **1e**, respectively), the copper-catalysed four-component isoquinoline formation proceeded smoothly (87 and 84% yield, respectively, entries 3 and 4). Thus, this isoquinoline formation was proven to be widely applicable to 2-ethynylbenzaldehydes having an electron-withdrawing and -donating group.

Table 3 Reactions with substituted 2-ethynylbenzaldehydes[a]

Entry	Substrate	Product	Yield (%)[b]
1			83
	1b	**7**	
2			79
	1c	**8**	
3			87
	1d	**9**	
4			84
	1e	**10**	

[a] After the three-component reaction of **1**, **2** (2 equiv.), and **3a** (2 equiv.) in the presence of CuI (10 mol%) in DMF was completed on TLC, t-$BuNH_2$ (**4j**, 6 equiv.) was added and the reaction mixture was stirred for 5 h at room temperature and for an additional 45 min at 140 °C. [b] Isolated yield.

In conclusion, we have developed a novel copper-catalysed domino four-component coupling–cyclisation reaction for the synthesis of 3-(aminomethyl)isoquinolines, which form one carbon–carbon and three carbon–nitrogen bonds. This methodology could be applied to the construction of a highly potent isoquinoline library in terms of diversity and biological activity.

Notes and references

1 For recent reviews, see: (*a*) J. D. Scott and R. M. Williams, *Chem. Rev.*, 2002, **102**, 1669–1730; (*b*) M. Chrzanowska and M. D. Rozwadowska, *Chem. Rev.*, 2004, **104**, 3341–3370. For recent examples, see: (*c*) A. Bermejo, I. Andreu, F. Suvire, S. Leonce, D. H. Caignard, P. Renard, A. Pierré, R. D. Enriz, E. Cortes and N. Cabedo, *J. Med. Chem.*, 2002, **45**, 5058–5068; (*d*) A. Morrel, S. Antony, G. Kohlhagen, Y. Pommier and M. Cushman, *J. Med. Chem.*, 2006, **49**, 7740–7753; (*e*) G. Bringmann, M. Dreyer, J. H. Faber, P. W. Dalsgaard, D. Stærk, J. W. Jaroszewski,

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10 Actually, one-portion addition of all the four components using **4j** gave a complex mixture of unidentified products without producing **6** (compare with Table 1, entry 10).

11 For isoquinoline formation with such ammonium salts as formate, carbonate, and ammonia, see ref. 8*c*.

12 In the reaction using **4i**, a hydrogen atom at the 4-position of **6a** would come from H_2O generated in imine formation.

13 At the present stage of our understanding, the reason for this unsatisfactory result is unclear.

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Direct Construction of Bicyclic Heterocycles by Palladium-Catalyzed Domino Cyclization of Propargyl Bromides

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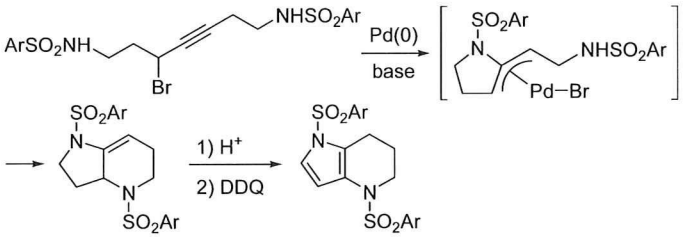
ABSTRACT



The palladium-catalyzed domino cyclization of propargyl bromides having two nucleophilic functional groups is described. Treatment of 1,7-diamino-5-bromohept-3-yne derivatives with catalytic $Pd(PPh_3)_4$ in the presence of NaH in MeOH gives the 2,7-diazabicyclo[4.3.0]non-5-enes in good yields. Interestingly, the regioselectivity of the reaction is completely controlled by the relative reactivity of the amine functional groups, irrespective of the position of the nucleophiles. The malonate derivative also undergoes domino cyclization to produce a hexahydroindole derivative.

Palladium-catalyzed reactions of propargylic compounds developed by Tsuji and co-workers are widely used as an efficient tool for the introduction of two nucleophiles into a substrate.[1,2] The reaction with dual nucleophiles such as acetoacetonates and diols forms cyclic products including furans and dioxanes.[1,3] Recent contributions in this area have revealed that a combination of nucleophilic attacks by an internal nucleophilic functional group and an appropriate external nucleophile can be a convenient approach to carbapenems,[4] furans,[5] indoles,[6] indenes,[7] and cyclic carbonates.[8] In contrast, there have been no reports of the direct

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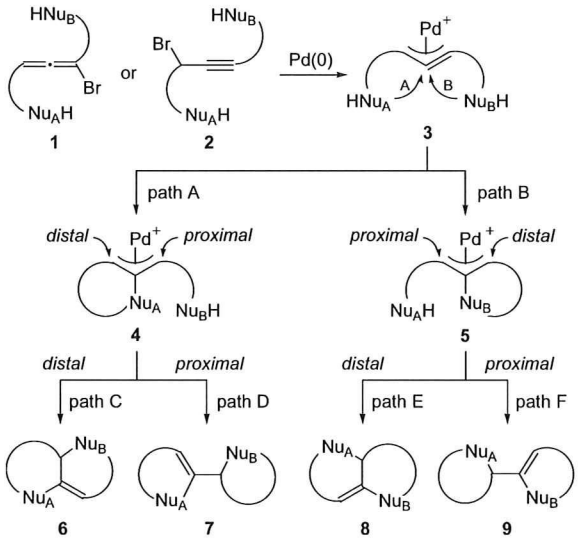
construction of bicyclic heterocycles by domino cyclization using propargylic substrates having two nucleophilic functional groups.

During the course of our studies directed toward elucidating efficient cyclization reactions of allenic compounds,[9] we found that bromoallenes can act as allyl dication equivalents in the presence of palladium(0) and alcohol. This reactivity has been shown to be extremely useful for the synthesis of medium-sized heterocycles[10] as well as bicyclic sulfamides[11] by successive bond formation. In light of this chemistry, we envisioned that the domino cyclization of bromoallenes **1** having two nucleophilic sites (Nu_A and Nu_B) might lead to bicyclic compounds by domino cyclization (Scheme 1).

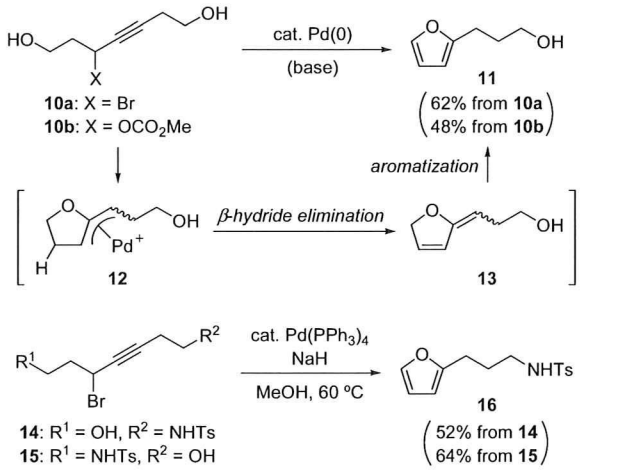
Scheme 1. Reaction Course of Palladium-Catalyzed Domino Cyclization of Allenyl/Propargyl Bromides **1** and **2**



However, since efficient chemoselective preparation of 1,3-disubstituted bromoallenes of the type **1** via conventional bromination methods has proven to be difficult,[12] we turned our attention to the reaction of propargyl bromides **2**, which can be considered as a synthetic equivalent of bromoallenes in the palladium-catalyzed reaction.[13] As shown in Scheme 1, this domino cyclization could afford four types of bicyclic products **6**–**9**, depending on (1) which nucleophilic site (Nu_A and Nu_B) would participate in the first cyclization (path A vs B) and (2) which carbon (distal or proximal) would be attacked on the second cyclization (path C vs D and E vs F). Herein we describe the domino cyclization of propargyl bromides **2** having two nitrogen functional groups to form fused azacycles of the type **6** and **8**. The remarkable effect of the nucleophilicity of N_A and N_B on the outcome as well as the stereochemical course of the reaction is also presented.

To avoid the regioselectivity issue during the first cyclization (path A vs B, Scheme 1), we investigated the reaction of propargyl bromide **10a** containing two hydroxy groups tethered by two carbon atoms (Scheme 2). Use of this starting material has the advantage of allowing the production of a highly symmetrical allenylpalladium intermediate. The bromide **10a** was readily prepared through the addition of the acetylide of a protected but-3-yn-1-ol to a hydroxypropanal derivative followed by bromination of the resulting protected propargyl alcohol with CBr_4 and PPh_3 in the presence of imidazole. Unfortunately, treatment of **10a** with $Pd(PPh_3)_4$ (5 mol %) in the presence of in situ generated NaOMe (standard conditions for cyclization of bromoallenes)[10,11] gave the furan derivative **11** in 62% yield. The reaction of the carbonate **10b** with $Pd_2(dba)_3$·$CHCl_3$ (2.5 mol %)/dppe (10 mol %) in dioxane also afforded **11** in 48% yield, without promoting the desired domino cyclization. Formation of the furan **11** can be rationalized through a β-hydride elimination from the π-allylpalladium intermediate **12** followed by aromatization of the diene **13**. Similar results were obtained with amino alcohol derivatives **14** and **15**, both leading to furan **16** in moderate yields. However, these unsuccessful results clearly show that the anion of the hydroxy group of **14** and **15** is more reactive than that of the tosylamide group, irrespective of their location (R^1 or R^2). Thus, both Nu_A and

Scheme 2. Formation of Furan Derivatives by Monocyclization[a]



[a] Reaction conditions: for **10a**, **14**, and **15**: $Pd(PPh_3)_4$ (5 mol %), NaH (2.5 equiv), MeOH, 60 °C; for **10b**: $Pd_2(dba)_3$·$CHCl_3$ (2.5 mol %), dppe (10 mol %), dioxane, 80 °C.

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(11) (a) Hamaguchi, H.; Kosaka, S.; Ohno, H.; Tanaka, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 1513–1517. (b) Hamaguchi, H.; Kosaka, S.; Ohno, H.; Fujii, N.; Tanaka, T. *Chem.–Eur. J.* **2007**, *13*, 1692–1708.

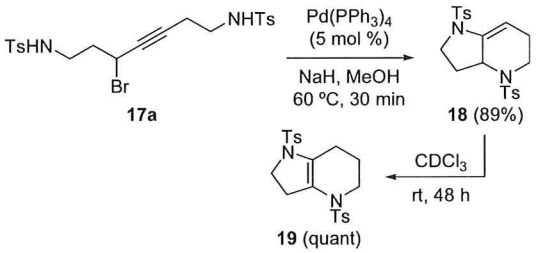
(12) For example, treatment of propargyl alcohols with CuBr·SMe_2 in the presence of LiBr gave a mixture of allenyl/propargyl bromides.

(13) The reactivities of allenic and propargylic compounds are not necessarily the same. For example, propargyl bromides and carbonates are more reactive than bromoallenes toward S_N2 reactions and alcoholysis, respectively.[10b]

Nu_B with appropriate carbon tethers in the palladium complex **3** (Scheme 1) can readily react with the central carbon of the propargylic moiety.

Next, the reaction of diamine derivative **17a** was investigated (Scheme 3). Fortunately, treatment of **17a** with 5 mol

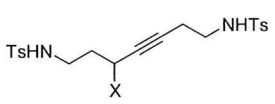
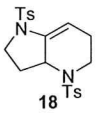
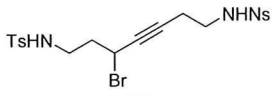
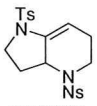
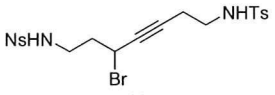
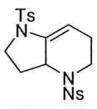
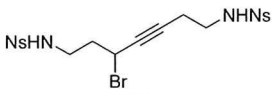
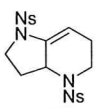
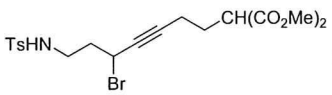
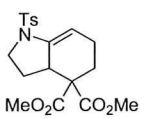
Scheme 3. Domino Cyclization of Diamine Derivatives **17a**



% of $Pd(PPh_3)_4$ in the presence of NaH (2.5 equiv) in MeOH afforded the desired bis-cyclized product **18** in 89% yield. This enamine is relatively unstable, and complete isomerization to the dihydropyrrole derivative **19** having an ethene diamide moiety proceeded in $CDCl_3$ within 48 h at room temperature.[14]

The results of the palladium-catalyzed domino cyclization using selected diamine derivatives are summarized in Table

Table 1. Domino Cyclization of Diamine and (Aminoalkyl)malonate Derivatives[a]

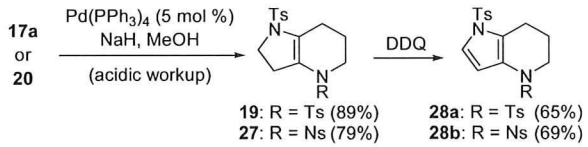
entry	substrate	time (min)	product (yield)[b]
			
1	**17a** (X = Br)	30	**18** (89%)
2	**17b** (X = OCO_2Me)	90[c]	(38%)
3	 **20**	5	 **21** (79%)
4	 **22**	5	 **21** (87%)
5	 **23**	5	 **24** (91%)
6	 **25**	30	 **26** (78%)

[a] Unless otherwise stated, the reactions were carried out with $Pd(PPh_3)_4$ (5 mol %), NaH (2.5 equiv) in MeOH at 60 °C. [b] Yields of isolated products. [c] The reaction was carried out with $Pd_2(dba)_3$·$CHCl_3$ (2.5 mol %)/dppe (10 mol %) in dioxane.

1. As shown in entries 1 and 2, the reaction of propargyl carbonate **17b** under aprotic conditions[15] gave a lower yield of **18** (38%) than the reaction of the bromide **17a** in MeOH. The reaction of the bromide **20** having tosyl- and nosylamide groups afforded the bicyclic product **21** in 79% yield (entry 3), in which the tosylamide group is incorporated into the five-membered ring.[16] Interestingly, the same product was obtained in 87% yield from the bromide **22** which has a nosylamide group on the carbon close to the bromine atom (entry 4). These results show that the tosylamide group participates in the first cyclization to form a five-membered ring, which is followed by the second cyclization by the nosylamide, irrespective of their location. When bis-nosylamide **23** was employed in the domino cyclization, the corresponding bicyclic product **24** was obtained in 91% yield (entry 5). The malonate derivative **25** was successfully converted to hexahydroindole dicarboxylate derivative **26** in 78% yield. This result can be partly attributed to steric hindrance of the malonate moiety which hampers the first cyclization.

To expand the synthetic utility of this domino cyclization, conversion to a fused pyrrole derivative was then investigated (Scheme 4). The domino cyclization of **17a** and **20** using

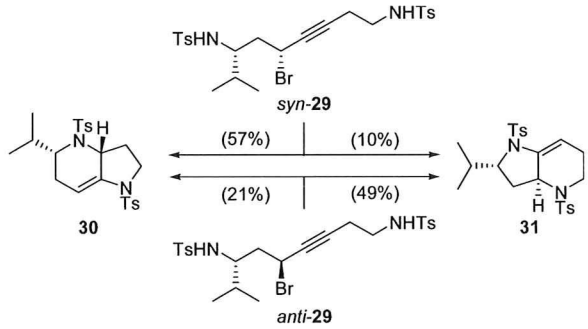
Scheme 4. Synthesis of Fused Pyrrole Derivatives



acidic workup (4% HCl) directly gave the dihydropyrrole-fused bicyclic compounds **19** and **27** both in good yields. DDQ-mediated oxidation of these compounds easily afforded the desired fused pyrroles **28**.

Finally, the stereochemical course of the domino cyclization was examined using *syn*- and *anti*-**29** derived from L-valine (Scheme 5). The reaction of *syn*-**29** under the

Scheme 5. Domino Cyclization of *syn*- and *anti*-**29**

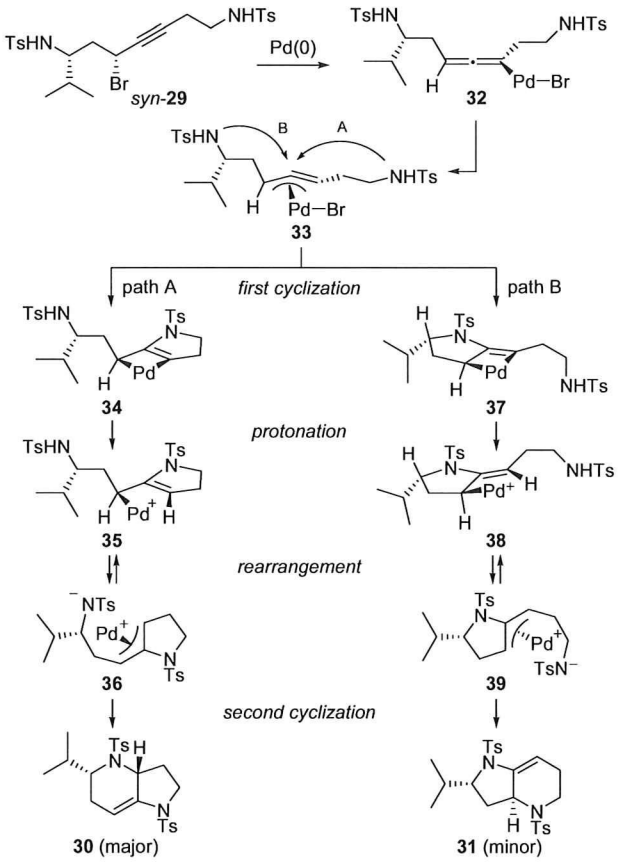


standard conditions using $Pd(PPh_3)_4$ (5 mol %) and in situ generated NaOMe in MeOH gave **30** (57%) and **31** (10%), both in a stereoselective manner.[17] Quite interestingly, the

isomeric *anti*-**29** afforded the same products, although the major isomer was **31** (**30**: 21%; **31**: 49%). These results suggest that the stereochemistry of the substrates is not reflected in that of the products, although it affects the regioselectivity, i.e., which nitrogen attacks the central carbon of the allenylpalladium intermediate in the first cyclization.

Formation of **30** and **31** from *syn*-**29** can be explained as follows (Scheme 6). Attack of palladium(0) to *syn*-**29** from

Scheme 6. Stereochemical Course of the Cyclization of *syn*-**29**



the opposite face of the bromine atom gives an allenylpalladium(II) intermediate **32**. After formation of the π-propargylpalladium complex **33**,[18] the first cyclization by the less-hindered nitrogen atom on the methylene carbon (path A) would form a fused palladacyclobutene intermediate **34**.[19] Protonation of **34** leads to the allylpalladium complex **35**, which is in a state of equilibrium with the π-allylpalladium intermediate **36**. The second *anti*-cyclization from the resulting π-allylpalladium intermediate **36** by the more hindered nitrogen atom would give the major isomer **30** in a stereoselective manner.[20] On the other hand, the domino cyclization in the opposite order through the first cyclization by the more hindered nitrogen atom (path B) will form the minor isomer **31** in a similar manner. Although the production of the same isomers **30** and **31** from *anti*-**29** should involve inversion of the configuration through the sequence of these steps which requires further consideration,[21] these stereochemical outcomes demonstrate an interesting aspect of the palladium-catalyzed domino cyclization of propargyl bromides.

In conclusion, we have developed a novel domino cyclization of propargyl bromides having two nucleophilic sites catalyzed by palladium(0). The regioselectivity of the reaction depends on the relative reactivities of the nucleophilic moieties, irrespective of their location. This domino cyclization provides convenient access to fused bicyclic compounds such as hexa- or tetrahydro-1*H*-pyrrolo[3,2-*b*]pyridine derivatives as well as hexahydroindole derivatives.

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Supporting Information Available: Representative experimental procedures, characterization data of all new compounds, and a crystallographic information file for **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800063D

(14) However, isolation and characterization of the bicyclic compounds of the type **18**, some of which are crystaline compounds, are possible.

(15) The reaction of **17b** under the standard conditions in MeOH promoted methanolysis to give propargyl alcohol in 77% yield.

(16) The structure of **21** was confirmed by acid-mediated isomerization, oxidation to **28b** (Scheme 4) followed by deprotection of the nosyl group. For more details, see the Supporting Information.

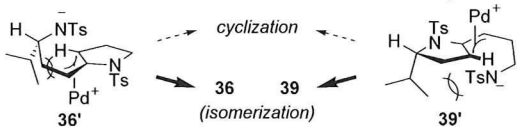
(17) The unambiguous structure assignment for **30** and **31** was made by X-ray analysis and NOE experiment (see the Supporting Information).

(18) (a) Ogoshi, S.; Tsutsumi, K.; Nishiguchi, S.; Kurosawa, H. *J. Organomet. Chem.* **1995**, *493*, C19−C21. (b) Tsutsumi, K.; Ogoshi, S.; Nishiguchi, S.; Kurosawa, H. *J. Am. Chem. Soc.* **1998**, *120*, 1938−1939. (c) Tsutsumi, K.; Kawase, T.; Kakiuchi, K.; Ogoshi, S.; Okada, Y.; Kurosawa, H. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2687−2692.

(19) (a) Casey, C. P.; Nash, J. R.; Yi, C. S.; Selmeczy, A. D.; Chung, S.; Powell, D. R.; Hayashi, R. K. *J. Am. Chem. Soc.* **1998**, *120*, 722−733. (b) Ogoshi, S.; Kurosawa, H. *J. Synth. Org. Chem. Jpn.* **2003**, *61*, 14−23. Formation of a palladacyclobutene intermediate in a related reaction has been well rationalized by DFT calculation. (c) Labrosse, J.-R.; Lhoste, P.; Delbecq, F.; Sinou, D. *Eur. J. Org. Chem.* **2003**, 2813−2822.

(20) For related chirality transfer in the reaction of the propargylic compounds via palladacyclobutene intermediates, see: Yoshida, M.; Fujita, M.; Ihara, M. *Org. Lett.* **2003**, *5*, 3325−3327.

(21) For example, unfavorable steric repulsions around the isopropyl group in **36′** and **39′** (respective epimers of **36** and **39**) might hamper the second cyclization (see the graphic below), which assist isomerization of these intermediates to **36** and **39** via an inversion of configuration.



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Diastereoselective synthesis of highly functionalized fluoroalkene dipeptide isosteres and its application to Fmoc-based solid-phase synthesis of a cyclic pentapeptide mimetic

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Abstract

A diastereoselective and divergent method for synthesis of a highly functionalized (*Z*)-fluoroalkene dipeptide isosteres has been developed. The key feature of this synthetic method is an efficient one-pot reaction involving reduction/asymmetric alkylation via transmetalation, which produces *trans*-amide type (*Z*)-fluoroalkenes flanking two stereogenic centers in high yields, with excellent (*Z*)-selectivity and diastereoselectivity. Practical Fmoc-based solid-phase synthesis of a specific CXCR4 antagonistic pseudopeptide **25** containing (*Z*)-fluoroalkene isostere is also described.

Keywords: Fluoroalkene; Peptide isosteres; Transmetalation; CXCR4 antagonist

1. Introduction

Replacement of native hydrolyzable peptide bonds with non-hydrolyzable mimetics is an established approach toward overcoming the major limitations of peptides, including poor bioavailability and short physiological half-life due to rapid proteolysis, which limit their use as therapeutic or chemical probes.[1] Furthermore, conformationally restricted analogs of biologically active peptides represent an attractive structural motif leading to the more effective agents.

Alkene-type dipeptide isosteres (ADIs),[2] whose design is based on the partial double-bond character of the peptide bond in its most stable conformation, have been thought to be ideal dipeptide mimetics. ADIs possessing an (*E*)- or (*Z*)-alkene unit[3] with increased lipophilicity relative to native dipeptides are resistant to enzymatic cleavage. Because of their fixed *cis*/*trans* conformation, ADIs can be used as chemical probes for determining the bioactive conformation of peptide bonds,[4a,b] which is difficult by other means such as NMR analysis or X-ray diffraction, and for the precise functional analysis of the role of each peptide bond[5] (Fig. 1).

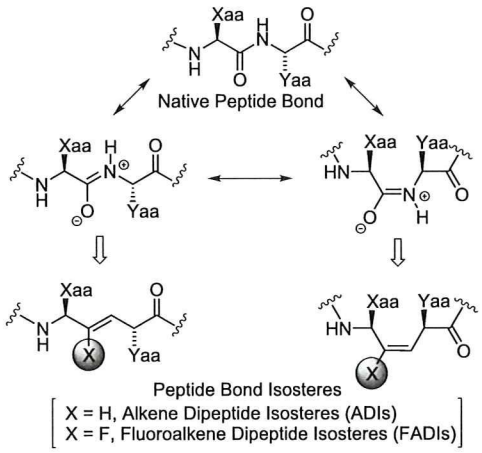

Figure 1. Native peptide bond and its corresponding isosteres.

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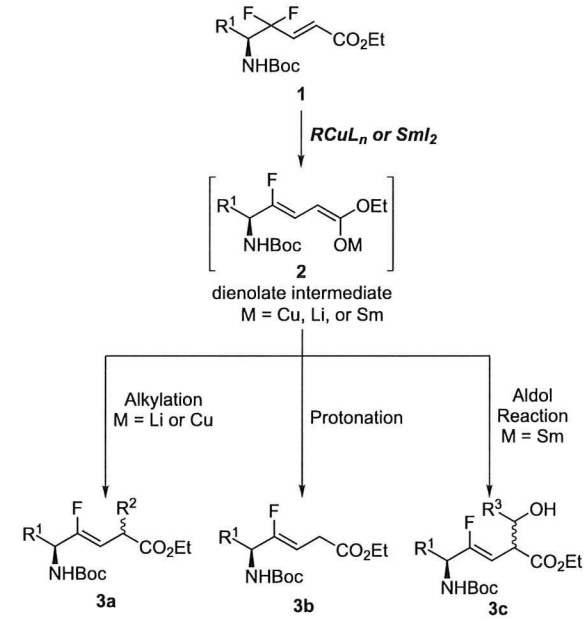
Over the past 15 years, we have engaged in the syntheses of several types of ADIs and have developed diastereoselective methods toward synthesis of (*E*)-Alkene Dipeptide Isosteres (EADIs),[2a] (*Z*)-Alkene Dipeptide Isosteres (ZADIs),[3] Trisubstituted Alkene Dipeptide Isosteres (TADIs),[4a,b] and Xaa–Pro isosteres[6] utilizing organocopper-mediated S_N2' alkylation as a key reaction. These methodologies have been applied to the preparation of biologically active peptidomimetics.[2c,3e,4a] The studies have revealed that a simple alkene unit is not always sufficient for replacement of peptide bond probably because of (1) the lack of a hydrogen-bonding site, (2) a smaller dipole moment [$\mu_{\text{peptide bond}}$=3.6 D vs μ_{alkene}=0.1 D], and (3) flexible ϕ- and ψ-angle rotations due to the lack of steric interactions between the carbonyl oxygen and the side chain on the β-carbon.

On the other hand, in 1986, Abraham et al. reported a theoretical and crystallographic study of fluoroalkene as peptide bond analog.[7a] They have shown that the fluoroalkene unit could be a more effective peptide bond analog than a simple alkene unit due to the presence of a highly electronegative fluorine substituent with a larger dipole moment [$\mu_{\text{F–alkene}}$= 1.4 D].[8] The van der Waals radii of the fluorine atom (1.47 Å) is sufficiently close to that of the oxygen atom (1.52 Å),[9] thus it favors the induction of the steric restriction of ϕ- and ψ-dihedral angles. A computational study with water molecules has revealed that the fluoroalkene moiety can be involved in hydrogen-bonding interactions.[10]

In spite of their potency as dipeptide mimetics, difficulties in the stereoselective and divergent synthesis of fluoroalkene isosteres have hampered their application to biologically active peptides.[7b,d,f,12e,g] As such, there is an upsurge of interest in the development of efficient methodologies to synthesize α-substituted fluoroalkene isosteres. For the synthesis of fluoroalkene isosteres, it is necessary to stereoselectively construct a (*Z*)- or (*E*)-fluoroalkene unit as well as to control the configuration of two stereogenic centers at the α- and δ-positions. It is more desirable to introduce a variety of 'functionalized' alkyl side chains, which play important roles in bioactivity.

Although numerous synthetic approaches to fluoroalkene isosteres have been developed including classical olefination reactions such as the Peterson reaction,[7c,e] the aldol condensation,[7d] and the Horner–Wadsworth–Emmons reaction,[7f] only a few examples of diastereoselective synthesis of FADIs have been reported to date. Recently, Pannecoucke's group have provided a practical route for synthesis of FADIs using a temperature-controlled Negishi-coupling reaction[11] and asymmetric reductive amination of α-fluoroenones[7g] as key transformations. This approach has significant advantages in synthesizing both (*E*)- and (*Z*)-isomer.

Alternatively, we[12] and Taguchi's group[13] have independently developed effective approaches to (*Z*)-fluoroalkene isosteres via a reductive defluorination reaction of α,β-enoates **1** possessing two fluorine atoms at the γ-positions, in which organocopper reagents derived from CuX (X=CN or I) and the MeLi·LiBr complex were utilized for carbon–fluorine bond cleavage via a single electron transfer mechanism[12d,14,15] (Scheme 1). In these reactions, the dienolate intermediates **2** can be trapped in situ by an alkyl halide, resulting in the formation of α-substituted fluoroalkene isosteres **3a**.[12b] The reaction with samarium diiodide (SmI_2) in the presence of *t*-BuOH easily provides fluoroalkene isosteres **3b**, and the replacement of *t*-BuOH with carbonyl compounds such as aldehydes or ketones provides α-substituted fluoroalkene isosteres **3c** via aldol reactions of Sm dienolates.[12d] Although this reductive defluorination is useful for the regio- and stereoselective formation of the (*Z*)-fluoroalkene unit, the method has not addressed the stereoselective construction of the α-side chain.



Scheme 1. Synthesis of fluoroalkene dipeptide isosteres by reductive defluorination/enolate trapping methodology.

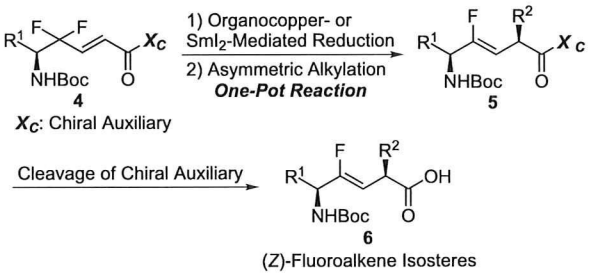
In this paper, we describe a diastereoselective, divergent, and practical approach for the synthesis of highly functionalized fluoroalkene isosteres utilizing an efficient one-pot reaction involving organocopper-mediated reduction/asymmetric alkylation via transmetalation. Its application to Fmoc-based solid-phase peptide synthesis (SPPS) of a fluoroalkene isostere-containing potential CXCR4 antagonist is also presented.

2. Results and discussion

2.1. Diastereoselective synthesis of L-Val–(D/L)-Xaa type (Z)-fluoroalkene dipeptide isosteres by one-pot reaction involving organocopper-mediated reduction/asymmetric alkylation via transmetalation

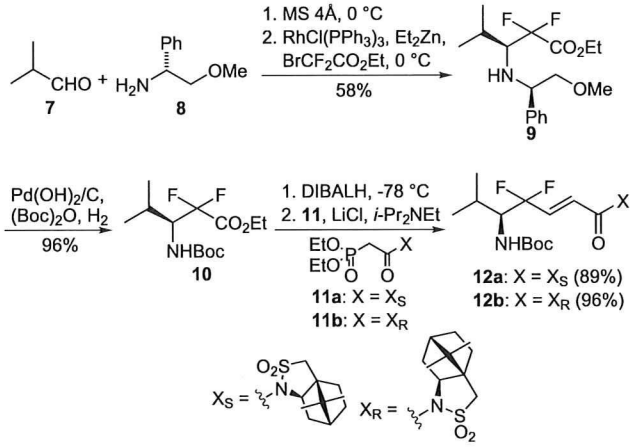
On the basis of our previous study on reductive defluorination shown in Scheme 1,[12b,d] we envisioned extending this approach to the diastereoselective synthesis of (*Z*)-fluoroalkene isosteres as depicted in Scheme 2. It was our expectation that formation of the fluoroalkene-containing dienolates carrying a chiral auxiliary by organocopper- or SmI_2-mediated reduction of γ,γ-difluoro-α,β-unsaturated carbonyl compounds **4**, followed by trapping with electrophiles, would result in

regio- and stereoselective construction of (*Z*)-fluoroalkene isosteres **5**. This synthetic strategy can be extended to diastereoselective syntheses of (L,L), (L,D), (D,L), and (D,D)-type isosteres by simply using an appropriate starting material and a chiral auxiliary. Introduction of various alkyl groups at the α-position in the final stage would allow diversity-oriented synthesis of (*Z*)-fluoroalkene isosteres.



Scheme 2. Planned diastereoselective synthesis of (*Z*)-fluoroalkene dipeptide isosteres.

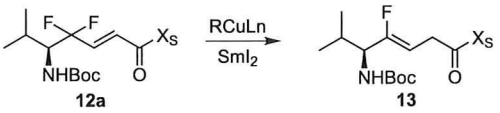
The synthetic sequence leading to the key *N*-enoyl sultam intermediates **12** is illustrated in Scheme 3. The known α,α-difluoro-β-amino ester **9**,[12d] prepared from isobutyl aldehyde **7** by a Rh-catalyzed Reformatsky–Honda reaction,[16] was subjected to hydrogenolysis with $Pd(OH)_2/C-H_2$ in the presence of $(Boc)_2O$, affording the Boc-protected ester **10**.[12d] The resulting ester **10** was converted to the desired *N*-enoyl sultam **12a** in 89% yield, by a sequence of reactions involving reduction by DIBALH and Horner–Wadsworth–Emmons coupling with (*S*)-*N*-[(diethoxyphosphono)acetyl]camphorsultam **11a**,[17] with exclusive *E*-selectivity. The diastereomer **12b** was also prepared using (*R*)-sultam derivative **11b** in 96% yield.



Scheme 3. Synthesis of key substrates **12** for one-pot reduction/asymmetric alkylation.

In order to achieve consecutive one-pot reduction/asymmetric alkylation, the following two-step sequence was needed: (1) the reduction of **12a** to give the Li or Cu dienolate intermediate; (2) trapping of the dienolate intermediate with an alkyl halide in a regio- and stereoselective fashion. However, to the best of our knowledge, there is no example of

Table 1
Reduction of γ,γ-difluoro-α,β-*N*-enoyl sultam **12a** with organocopper reagents and SmI_2[a]

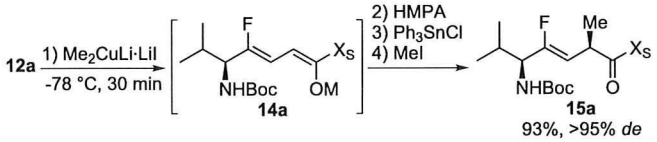


Entry	Reagents[b]	Additives (equiv)	Yield[d] (%)
1	SmI_2[c]	—	—[e]
2	$Me_3CuLi_2\cdot LiI$	—	52
3	$Me_2Cu(CN)Li_2$	—	74
4	$Me_2CuLi\cdot LiI$	—	95
5	$Me_2CuLi\cdot LiI$	HMPA (10)	85
6	$Me_2CuLi\cdot LiI$	TMSCl (4)	81
7	$Me_2CuLi\cdot LiI$	$BF_3\cdot OEt_2$ (4)	87

[a] Unless otherwise stated, the reactions were carried out with 4 equiv of the reagent at −78 °C for 30 min.
[b] Organocopper reagents include other Li salts (LiCl and/or LiBr).
[c] 0 °C, 60 min, with 6 equiv of SmI_2.
[d] Isolated yield.
[e] A mixture of unidentified compounds was obtained.

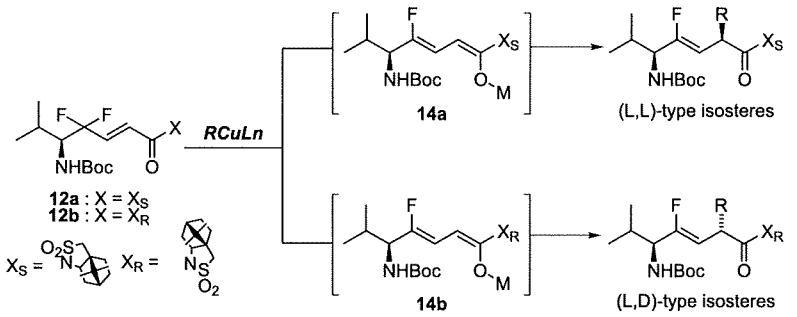
the reduction of *N*-enoyl sultam, which has a different electron density compared with the corresponding enoates with a single electron reductant. In this regard, we first examined the organocopper- or SmI_2-mediated reduction of **12a** without any alkyl halides (Table 1). We previously reported that the reaction of the enoates with SmI_2 gave better results than using organocopper reagents.[12d] The reduction of the enoyl sultam **12a** with SmI_2 only afforded a mixture of unidentified products (entry 1). In contrast, organocopper reagents were effective in producing the reduced product **13**[7h] (entries 2–7): the reaction with a higher order cuprate ($Me_3CuLi_2\cdot LiI\cdot 3LiBr$) produced **13** in moderate yield (entry 2).[18] The reactions using either the cyano Gilman reagent ($Me_2CuLi\cdot LiCN\cdot 2LiBr$) or the Gilman reagent ($Me_2CuLi\cdot LiI\cdot 2LiBr$), which shows lower electron-donating ability,[15] proceeded more smoothly to give **13** with yields of 74% and 95%, respectively (entries 3 and 4). We examined the introduction of additives such as HMPA, TMSCl, and $BF_3\cdot OEt_2$; however, no obvious differences in reactivity or in chemical yield were observed (entries 5–7). Based on these results, the Gilman reagent was chosen as a single electron reductant.[19]

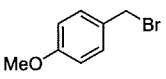
Next, trapping of the Cu or Li dienolate intermediate **14a**, generated from **12a** by organocopper-mediated reduction with methyl iodide to construct a stereogenic center at the α-position (Scheme 4) was investigated. Direct treatment of **14a** with methyl iodide yielded only a complex mixture. As this unsuccessful result might be attributed to the low reactivity of Cu or Li dienolate **14a** toward alkylation, the next step was to use the more reactive Sn dienolate.[20] After reduction of



Scheme 4. Diastereoselective synthesis of L-Val–L-Ala (*Z*)-fluoroalkene isostere by one-pot reduction/asymmetric alkylation via transmetalation.

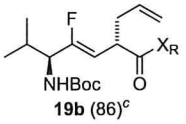
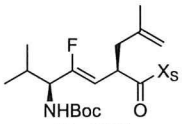
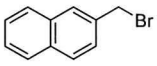
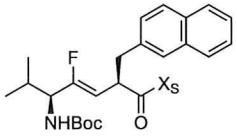
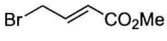
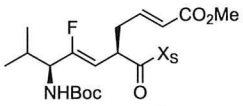
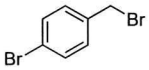
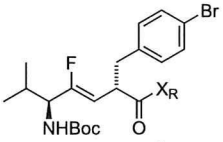
Table 2
Diastereoselective synthesis of (*Z*)-fluoroalkene isosteres by one-pot organocopper-mediated reduction/transmetalation/asymmetric alkylation



Entry	Electrophiles	Substrates	Products[a] (%)	% de[b]
1	MeI	**12a**	**15a** (93)[c]	>95
2	MeI	**12b**	**15b** (69)[c]	95
3	BnBr	**12a**	**16a** (93)[c]	95
4	BnBr	**12b**	**16b** (77)[c]	91
5	Br$\sim$$CO_2t$-Bu	**12a**	**17a** (80)[c]	>95
6	Br$\sim$$CO_2t$-Bu	**12b**	**17b** (75)[c]	92
7	Br$\sim\sim$$CO_2Me$	**12a**	—[d]	—
8		**12b**	**18b** (77)[c]	93
9		**12a**	**19a** (99)[c]	>95

(*continued on next page*)

Table 2 (*continued*)

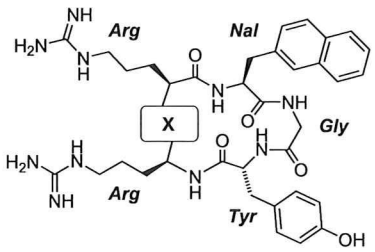
Entry	Electrophiles	Substrates	Products[a] (%)	% de[b]
10		**12b**	 **19b** (86)[c]	91
11		**12a**	 **20a** (99)[c]	>95
12		**12a**	 **21a** (99)[c]	91
13		**12a**	 **22a** (94)[c]	91
14		**12b**	 **23b** (71)[c]	92

[a] Isolated yield.
[b] Determined by RP-HPLC of purified products.
[c] A trace amount of either γ-alkylated products or *E*-isomer was detected by RP-HPLC.
[d] Reduced product **13** was obtained.

12a with $Me_2CuLi\cdot LiI\cdot 2LiBr$, sequential treatment with HMPA, triphenyltin chloride, and methyl iodide, regioselectively produced an α-methyl fluoroalkene isostere **15a** in 93% yield with exclusive *Z*-selectivity. RP-HPLC analysis showed that the α-methylation proceeded with >95% de.[21]

With these results in hand, the scope of this one-pot reduction/asymmetric alkylation via transmetalation with various alkyl halides was examined (Table 2). In all cases, good to excellent yields of α-alkylated fluoroalkene isosteres were obtained with excellent diastereo- and *Z*-selectivity. Successive treatments of *N*-enoyl sultam **12a** with the organocopper reagent, HMPA, triphenyltin chloride, and benzyl bromide proceeded smoothly to yield L-Val–L-Phe isostere **16a** in 93% yield and 95% de (entry 3). This one-pot strategy also showed comparable reactivity and selectivity to the corresponding (*R*)-sultam **12b** to provide L-Val–D-Phe isostere **16b** in 77% yield and 91% de (entry 4). The use of *tert*-butyl bromoacetate as an electrophile produced an L-Val–L-Asp(O*t*-Bu) isostere **17a** in 80% isolated yield and >95% de (entry 5); also (L,D)-type **17b** in 75% isolated yield and 92% de (entry 6). For the synthesis of the L-Val–D-Glu fluoroalkene isostere, however, use of methyl 3-bromopropionate gave reduced product **13** with no alkylated product (entry 7). Treatment of dienolate **14b** with *p*-methoxybenzyl bromide proceeded smoothly to give an L-Val–D-Tyr(Me) fluoroalkene isostere **18b** in 77% isolated yield and 93% de (entry 8). As expected, this one-pot strategy can also be applied to the synthesis of fluoroalkene isosteres of unnatural amino acids. Trapping of the dienolates **14a** and **14b** with allyl bromide afforded L-Val–L-Gly(allyl) isostere **19a** (99% yield, >95% de, entry 9) and (L,D) isostere **19b** (86% yield, 91% de, entry 10), respectively. In a similar manner, the reaction of dienolates **14a** with 1-bromo-2-methyl-2-propene (methallyl bromide) also yielded an L-Val–L-(γ′-dehydro)Leu isostere **20a** in 99% yield and >95% de (entry 11). The use of 2-(bromomethyl)naphthalene and methyl 4-bromocrotonate stereoselectively gave the corresponding α-alkylated fluoroalkene isosteres **21a** and **22a** (entries 12 and 13). Synthesis of a fluoroalkene isostere **23b** bearing an aryl bromide moiety is of significant synthetic value with respect to the further elaboration. For such purposes, we attempted to trap the dienolates **14b** with *p*-bromobenzyl bromide, chemoselectively affording the corresponding isostere **23b** in 71% yield and 92% de (entry 14).

The de of each product was determined by RP-HPLC. Fluoroolefinic geometries of all the products were established by ^1H NMR,[22] and the absolute configurations of the alkyl groups at the α-position were determined by circular dichroism



FC131 (**24**): X = -CO-NH-
FCN001 (**25**): X = -ψ[(Z)-CF=CH]-

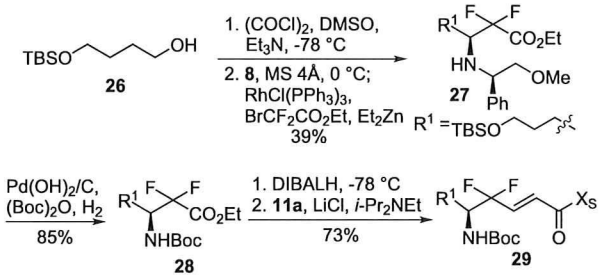
Figure 2. FC131 and its cyclic fluoroalkene pseudopeptide (FCN001). Nal= L-3-(2-naphthyl)alanine.

measurements with an empirical rule after converting to the corresponding methyl esters.[23]

2.2. Diastereoselective synthesis of L-Arg–L-Arg (Z)–fluoroalkene isosteres and its application to a cyclic pseudopeptide analog of specific CXCR4 antagonist, FC131

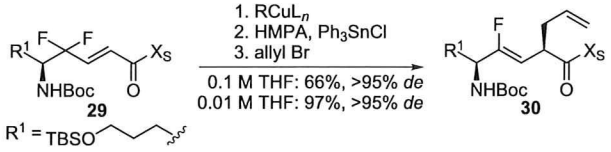
Efforts were then made to apply the developed methodology to synthesize a fluoroalkene-containing pseudopeptide. Previously, bioactivity investigations replacing the L-Arg–L-Nal and L-Nal–Gly peptide bonds of the specific CXCR4 antagonistic peptide, FC131 [*cyclo*(–D-Tyr-Arg-Arg-Nal-Gly–)] **24**,[24] were carried out.[2c] Replacement of the amide-bonds with (*E*)-alkene isosteres led to a significant loss of CXCR4 antagonistic activity. On the other hand, the importance of the amide bond between Arg and Arg has not been studied. To this end, FCN001 (**25**) was designed bearing an Arg-Arg fluoroalkene isostere, which is a mimetic of **24** (Fig. 2).

Our synthesis began with commercially available 4-(*tert*-butyldimethylsilyloxy)butan-1-ol **26** (Scheme 5). Swern oxidation of the alcohol **26**, and subsequent Reformatsky–Honda reaction gave ester **27** in a synthetically acceptable yield (39%). Cleavage of the chiral auxiliary of **27** by hydrogenation in the presence of $(Boc)_2O$, reduction of the resulting Boc-protected ester **28** with DIBALH, and coupling with (*S*)-sultam derivative **11a** produced the desired sultam **29**.



Scheme 5. Synthesis of *N*-enoyl sultam **29**.

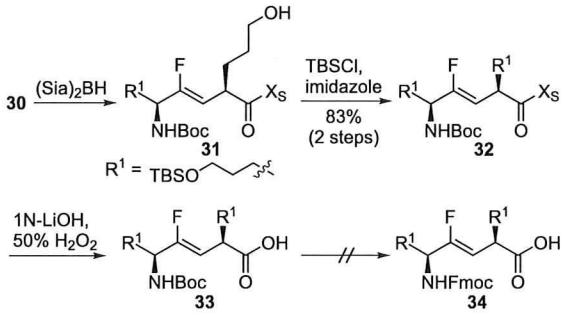
One-pot reaction of sultam **29** with allyl bromide in a larger scale (>1500 mg) yielded the desired α-allyl sultam **30** (Scheme 6). Despite its excellent diastereoselectivity (>95% de), the chemical yield of the one-pot reaction was relatively low compared with the results shown in Table 2. Initially,



Scheme 6. One-pot reaction of *N*-enoyl sultam **29**.

we speculated that the TBS group would be removed by the fluoride anion, which is generated by organocopper-mediated reduction. However, the addition of TMSCl to trap the fluorine anion was not effective for improvement of the yield. Considering that the good to high yields shown in Table 2 might be attributed to the relatively small scale of the reaction (<100 mg), we next attempted the reaction under more dilute conditions. Slow addition of sultam **29** (0.01 M in THF) to $Me_2CuLi\cdot LiI\cdot 2LiBr$, subsequent transmetalation, and asymmetric alkylation proceeded readily to give the α-allyl sultam **30** (97% yield, >95% de).

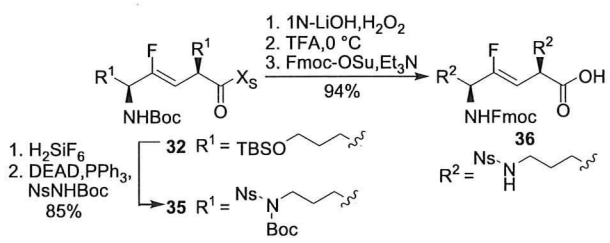
Next, synthesis of Fmoc-protected isostere **34** having a 3-hydroxypropyl group was investigated (Scheme 7). The α-allyl sultam **30** was subjected to disiamylborane $[(Sia)_2BH]$-mediated hydroboration, readily producing the corresponding alkyl borane compound. Mild oxidation conditions were required for conversion of the alkyl borane to the corresponding alcohol, since the chiral auxiliary would be cleaved easily by hydroperoxide anion. The use of aqueous 20% AcOK and 50% H_2O_2 in THF was entirely successful, chemoselectively effecting the desired conversion to give the desired alcohol **31** in 69% isolated yield on a small scale (<100 mg). However, upon increasing the reaction scale (2000 mg), the chiral auxiliary was cleaved to produce the corresponding hydroxy acid in 44% yield, along with the desired alcohol **31** in 30% yield. Under more dilute THF conditions, with slow addition of the minimum amount of aqueous 20% AcOK and 50% H_2O_2 provided **31** in good conversion. To employ Fmoc-based SPPS, the conversion of *N*-Boc to the *N*-Fmoc group was attempted. The alcohol **31** was protected with TBSCl in CH_2Cl_2 to yield the TBS ether **32**, which was converted to the corresponding acid under basic conditions without epimerization at the α-carbon. The resulting acid **33** was subjected to several conditions to remove the Boc group in the presence of TBS ethers; however, all attempted conditions [TFA, 0 °C;



Scheme 7. Conversion of α-allyl sultam **30** to Fmoc-protected fluoroalkene isostere.

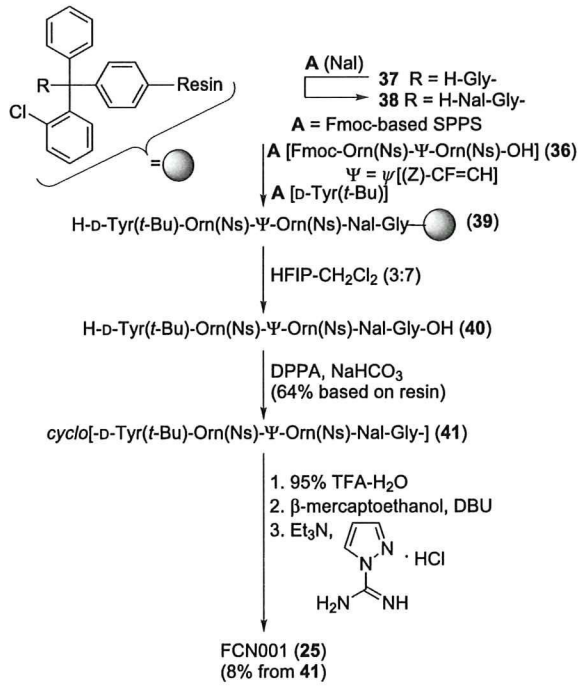
HCl/AcOEt, 0 °C;[25] $ZnBr_2$;[26] etc.] produced the desilylated products with no detectable amount of the desired amine.

Attention was next turned to synthesis of an L-Orn—L-Orn isostere **36** by introduction of two nitrogen functionalities (Scheme 8), which could be converted to guanidino groups using 1*H*-pyrazole-1-carboxamidine hydrochloride. Thus, bis-TBS ether **32** was cleaved readily by aqueous 3.28 N H_2SiF_6 to give the diol, which was subsequently subjected to the Mitsunobu reaction with NsNHBoc,[27] leading to the formation of bis(sulfonamide) **35** in 85% yield. The sulfonamide **35** was converted to the corresponding acid under basic conditions, which was subjected to cleavage of the three Boc groups with TFA followed by Fmoc protection to give desired isostere **36** with the L-Orn—L-Orn sequence (94% in 3 steps).



Scheme 8. Synthesis of Fmoc-protected Orn—Orn fluoroalkene isostere **36**.

Finally, synthesis of a cyclic pseudopeptide utilizing Fmoc-based SPPS and well-established cyclic peptide synthesis protocols[28] was investigated (Scheme 9). Fmoc-amino acid-containing fluoroalkene isostere **36** was coupled onto the resin **38**. A protected peptide resin **39** was treated with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)—CH_2Cl_2 (3:7) to afford the side chain-protected pseudopeptide **40**. Cyclization of linear peptide **40** with diphenylphosphoryl azide (DPPA)[29] and $NaHCO_3$ in DMF, led to the formation of protected cyclic pseudopeptide **41** with a 64% isolated yield based on the initial resin-loading. Completion of the synthesis of FCN001 thus required three further transformations: removal of the *t*-Bu group with aqueous 95% TFA, deprotection of bis-Ns groups with β-mercaptoethanol and DBU in DMF,[30] and guanidination of the resulting diamine. These functional group modifications thus provided the expected cyclic pseudopeptide FCN001 **25** bearing an L-Arg—L-Arg fluoroalkene isostere in 8% overall yield from **41**. During the synthesis of **25** via Fmoc-base SPPS, cyclization, deprotection, and guanidination, neither isomerization of double bonds nor epimerization of stereogenic centers was detected.



Scheme 9. Synthesis of FCN001 via Fmoc-based SPPS.

3. Conclusion

In conclusion, we have developed an effective one-pot methodology for the diastereoselective synthesis of (*Z*)-fluoroalkene dipeptide isosteres, which are potential *trans*-peptide bond mimetics. This synthetic procedure consists of three successive steps: (1) organocopper-mediated reduction of *N*-enoyl sultam bearing two fluorine atoms at the γ-position, (2) transmetalation of the Li or Cu dienolate intermediate to the more reactive tin dienolates, and (3) Oppolzer sultam-assisted stereoselective trapping of the dienolate intermediate with alkyl halides. Our methodology features smooth α-alkylation to produce fluoroalkene isosteres in good to excellent yields with high diastereoselectivity. Since a broad range of reactive alkyl halides can be used to trap the tin dienolate intermediates, many natural and unnatural amino acid-containing isosteres can be prepared. Moreover, cyclic pseudopeptide **25**, which contains the L-Arg—L-Arg isostere, was synthesized utilizing Fmoc-based SPPS with no detectable quantity of the epimerized product at the α-position. To the best of our knowledge, this is the first example successfully applying fluoroalkene isosteres with stereogenic centers at both the α- and the δ-position to Fmoc-based SPPS. A biological evaluation of FCN001 is currently underway and will be reported in due course.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded using a JEOL AL-400 and JEOL ECA-500 spectrometer. Chemical shifts are reported in δ (ppm) relative to TMS (in $CDCl_3$) or solvent peak (in D_2O, CD_3OD) as internal standard. ^{13}C NMR spectra were recorded using a JEOL AL-400 and JEOL ECA-500, and referenced to the residual $CHCl_3$ signal. ^{19}F NMR spectra were recorded using a JEOL AL-400 and JEOL ECA-500, and referenced to the residual $CFCl_3$ signal (δ_F 0.00 ppm). Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Optical rotations were measured with a JASCO sodium automatic polarimeter P-1020. Infrared (IR) spectra were obtained on a JASCO FT/IR-4100 FT-IR spectrometer with

JASCO ATR PRO410-S. For flash chromatography, Wakosil C-300, C-300E, and silica gel 60H (silica gel for thin-layer chromatography, Merck) were employed.

4.2. (5S,2E)-5-[N-(tert-Butoxycarbonyl)amino]-4,4-difluoro-6-methylhept-2-enoyl (S)-sultam (12a)

To a solution of the ester **10**[12d] (687 mg, 2.00 mmol) in CH_2Cl_2 (20 mL) at −78 °C under argon was added dropwise a solution of DIBALH in toluene (0.93 M, 3.44 mL, 3.20 mmol), and the mixture was stirred for 2 h at −78 °C. The reaction was quenched with aqueous 0.5 N Rochelle salt and extracted with Et_2O. The extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure gave the aldehyde as an oil, which was used immediately in the next step without purification. To a stirred solution of (*S*)-D-*N*-(diethoxyphosphonoacetyl)camphorsultam (970 mg, 2.46 mmol) in CH_3CN (15 mL) at 0 °C under argon were added LiCl (112 mg, 2.60 mmol) and *i*-Pr_2NEt (452 μL, 2.60 mmol). After stirring for 30 min, a solution of the above aldehyde in CH_3CN (5 mL) was added to the mixture at 0 °C, and the mixture was stirred for 2 h at 0 °C and for 12 h at room temperature. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O. The extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–AcOEt (5:1) gave the title compound **12a** (845 mg, 89% yield) as a colorless oil: $[\alpha]_D^{23}$ −73.6 (*c* 1.05, $CHCl_3$); IR (ATR): 3376 (NHCO), 1712 (CO), 1686 (CO), 1330 (NSO_2), 1164 (NSO_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.90–0.99 (m, 6H), 0.99 (s, 3H), 1.17 (s, 3H), 1.32–1.49 (m, 2H), 1.43 (s, 9H), 1.90–1.98 (m, 3H), 2.04–2.17 (m, 3H), 3.43–3.55 (m, 2H), 3.92–4.03 (m, 2H), 4.70 (d, *J*=10.5 Hz, 1H), 6.87 (dt, *J*=15.4, 11.5 Hz, 1H), 7.01 (d, *J*=15.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6, 19.8, 20.7, 20.8, 26.4, 27.3, 28.2 (3C), 32.9, 38.3, 44.7, 47.8, 48.7, 53.0, 58.2 (t, *J*=24.8 Hz), 65.1, 79.9, 120.1 (t, *J*=246.6 Hz), 124.9 (t, *J*=7.9 Hz), 138.4 (t, *J*=26.5 Hz), 155.7, 162.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −107.4 (dt, *J*=249.7, 11.9 Hz), −105.3 (dt, *J*=251.0, 13.4 Hz); HRMS (FAB), *m/z* calcd for $C_{23}H_{37}F_2N_2O_5S$ ($[M+H]^+$) 491.2391, found 491.2385.

4.3. (5S,2E)-5-[N-(tert-Butoxycarbonyl)amino]-4,4-difluoro-6-methylhept-2-enoyl (R)-sultam (12b)

By use of a procedure similar to that described for the preparation of (*S*)-sultam derivative **12a**, the ester **10** (687 mg, 2.00 mmol) was converted into the title compound **12b** (913 mg, 96% yield) as a colorless oil: $[\alpha]_D^{24}$ +44.7 (*c* 1.14, $CHCl_3$); IR (ATR): 3400 (NHCO), 1709 (CO), 1684 (CO), 1334 (NSO_2), 1165 (NSO_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92–1.00 (m, 6H), 0.98 (s, 3H), 1.17 (s, 3H), 1.35–1.47 (m, 2H), 1.43 (s, 9H), 1.86–1.98 (m, 3H), 2.07–2.18 (m, 3H), 3.43–3.55 (m, 2H), 3.91–4.03 (m, 2H), 4.68 (d, *J*=11.0 Hz, 1H), 6.89 (dt, *J*=15.4, 11.0 Hz, 1H), 7.01 (d, *J*=15.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 19.8, 20.7, 20.8, 26.4, 27.3, 28.2 (3C), 32.8, 38.3, 44.6, 47.8, 48.7, 53.0, 58.1 (t, *J*=25.7 Hz), 65.1, 80.0, 120.1 (t, *J*=246.2 Hz), 124.8 (t, *J*=8.3 Hz), 138.5 (t, *J*=26.1 Hz), 155.7, 162.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −107.5 (dt, *J*=249.7, 14.0 Hz), −105.5 (dt, *J*=252.4, 11.4 Hz); HRMS (FAB), *m/z* calcd for $C_{23}H_{37}F_2N_2O_5S$ ($[M+H]^+$) 491.2391, found 491.2398.

4.4. General procedure for one-pot reduction/asymmetric alkylation via transmetalation. synthesis of (2R,5S,3Z)-5-[N-(tert-butoxycarbonyl)amino]-4-fluoro-2,6-dimethylhept-3-enoyl (S)-sultam (15a)

To a suspension of CuI (64.0 mg, 0.335 mmol) in THF (1 mL) at −78 °C under argon was added dropwise a solution of MeLi·LiBr complex in Et_2O (1.5 M, 0.45 mL, 0.670 mmol), and the mixture was stirred for 10 min at 0 °C. To the solution of the above organocopper reagent at −78 °C was added dropwise a solution of the *N*-enoyl sultam **12a** (40.0 mg, 0.0815 mmol) in THF (1.5 mL). The mixture was stirred for 30 min at −78 °C and HMPA (233 μL, 1.34 mmol) was added dropwise to the mixture. After stirring for 30 min at −78 °C, a solution of triphenyltin chloride (64.6 mg, 0.168 mmol) in THF (1.5 mL) was added dropwise, and the mixture was then stirred for 30 min at −40 °C. Methyl iodide (95 μL, 0.670 mmol) was added dropwise and the mixture was stirred for 20 h at −40 °C. The reaction was quenched at −40 °C by addition of a 1:1 saturated NH_4Cl–28% NH_4OH solution (4 mL) and the mixture was stirred at room temperature for additional 30 min. The mixture was extracted with Et_2O and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–AcOEt (5:1) gave the title compound **15a** (36.9 mg, 93% yield) as a colorless oil: $[\alpha]_D^{24}$ −95.5 (*c* 0.945, $CHCl_3$); IR (ATR): 3370 (NHCO), 1698 (CO), 1333 (NSO_2), 1165 (NSO_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92–0.94 (m, 6H), 0.97 (s, 3H), 1.16 (s, 3H), 1.19–1.41 (m, 2H), 1.33 (d, *J*=7.1 Hz, 3H), 1.44 (s, 9H), 1.81–1.96 (m, 4H), 2.02–2.06 (m, 2H), 3.42–3.52 (m, 2H), 3.86–3.97 (m, 1H), 3.89 (t, *J*=6.1 Hz, 1H), 4.12–4.19 (m, 1H), 4.70 (d, *J*=9.8 Hz, 1H), 5.02 (dd, *J*=37.8, 9.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 19.3, 19.8, 20.8 (2C), 26.4, 28.3 (3C), 30.4, 32.8, 35.9, 38.3, 44.6, 47.7, 48.4, 53.0, 57.4 (d, *J*=26.5 Hz), 65.0, 79.6, 105.9 (d, *J*=11.6 Hz), 155.7, 157.4 (d, *J*=251.6 Hz), 173.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −119.63 (dd, *J*=37.8 22.8 Hz); HRMS (FAB), *m/z* calcd for $C_{24}H_{40}FN_2O_5S$ ($[M+H]^+$) 487.2642, found 487.2636.

4.5. (2S,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-2,6-dimethylhept-3-enoyl (R)-sultam (15b)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (40.0 mg, 0.0838 mmol) was converted into the title compound **15b** (28.0 mg, 69% yield): $[\alpha]_D^{24}$ +49.2 (*c* 1.13, $CHCl_3$); IR (ATR): 3383 (NHCO), 1703 (CO), 1335 (NSO_2), 1166 (NSO_2); ^1H NMR (400 MHz,

$CDCl_3$) δ 0.83–0.86 (m, 6H), 0.90 (s, 3H), 1.09 (s, 3H), 1.17–1.33 (m, 2H), 1.26 (d, J=7.1 Hz, 3H), 1.37 (s, 9H), 1.77–1.89 (m, 4H), 1.97–1.99 (m, 2H), 3.42–3.52 (m, 2H), 3.81 (t, J=6.3 Hz, 1H), 3.90–3.99 (m, 1H), 4.06–4.14 (m, 1H), 4.62 (d, J=9.3 Hz, 1H), 4.99 (dd, J=37.2, 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 19.2, 19.8, 19.9, 20.8, 26.4, 28.3 (3C), 30.2, 32.8, 35.9 (d, J=4.1 Hz), 38.3, 44.6, 47.7, 48.4, 53.0, 56.8 (d, J=27.3 Hz), 65.0, 79.5, 105.2 (d, J=11.6 Hz), 155.1, 157.7 (d, J=260.7 Hz), 173.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ −117.2 (dd, J=37.2, 18.6 Hz); HRMS (FAB), *m/z* calcd for $C_{24}H_{38}FN_2O_5S$ ($[M-H]^-$) 485.2491, found 485.2491.

4.6. (2R,5S,3Z)-2-Benzyl-5-[N-(tert-butoxycarbonyl)amino]-4-fluoro-6-methylhept-3-enoyl (S)-sultam (16a)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (40.0 mg, 0.0838 mmol) was converted into the title compound **16a** (43.6 mg, 93% yield) as a colorless oil: $[\alpha]_D^{25}$ −82.5 (*c* 1.51, $CHCl_3$); IR (ATR): 3370 (NHCO), 1698 (CO), 1333 (NSO_2), 1164 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70 (s, 3H), 0.86–0.90 (m, 9H), 1.13–1.36 (m, 2H), 1.45 (s, 9H), 1.69–2.04 (m, 6H), 2.79 (dd, J=13.2, 7.6 Hz, 1H), 3.11 (dd, J=12.9, 8.1 Hz, 1H), 3.34–3.42 (m, 2H), 3.77 (br, 1H), 3.89 (dt, J=23.3, 9.0 Hz, 1H), 4.45 (q, J=8.1 Hz, 1H), 4.64 (d, J=9.5 Hz, 1H), 4.97 (dd, J=35.6, 9.5 Hz, 1H), 7.14–7.26 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 19.1, 19.8, 20.4, 26.4, 28.3 (3C), 30.4, 32.7, 38.2, 40.4, 43.0, 44.6, 47.5, 48.1, 53.0, 57.6 (d, J=24.8 Hz), 65.0, 79.5, 104.3 (d, J=12.4 Hz), 126.6, 128.2 (2C), 129.5 (2C), 137.5, 155.1, 158.2 (d, J=261.5 Hz), 172.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −119.4 to −119.1 (br m); HRMS (FAB), *m/z* calcd for $C_{30}H_{44}FN_2O_5S$ ($[M+H]^+$) 563.2955, found 563.2965.

4.7. (2S,5S,3Z)-2-Benzyl-5-[N-(tert-butoxycarbonyl)amino]-4-fluoro-6-methylhept-3-enoyl (R)-sultam (16b)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (40.0 mg, 0.0838 mmol) was converted into the title compound **16b** (36.2 mg, 77% yield): $[\alpha]_D^{26}$ +21.9 (*c* 0.825, $CHCl_3$); IR (ATR): 3379 (NHCO), 1701 (CO), 1334 (NSO_2), 1165 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (s, 3H), 0.77–0.81 (m, 6H), 0.89 (s, 3H), 1.10–1.35 (m, 2H), 1.44 (s, 9H), 1.74–1.99 (m, 6H), 2.76–2.81 (m, 1H), 3.11–3.16 (m, 1H), 3.34–3.44 (m, 2H), 3.77 (br, 1H), 3.94–4.02 (m, 1H), 4.48 (q, J=8.3 Hz, 1H), 4.64 (d, J=9.8 Hz, 1H), 4.93 (dd, J=36.2, 9.2 Hz, 1H), 7.13–7.28 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 18.9, 19.8, 20.5, 26.4, 28.3 (3C), 30.2, 32.8, 38.2, 40.3, 42.9, 44.6, 47.6, 48.1, 53.0, 56.7 (d, J=29.0 Hz), 65.0, 79.5, 103.6 (d, J=12.4 Hz), 126.6, 128.2 (2C), 129.4 (2C), 137.5, 155.1, 158.7 (d, J=262.3 Hz), 172.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −115.88 (dd, J=36.2, 17.6 Hz); HRMS (FAB), *m/z* calcd for $C_{30}H_{42}FN_2O_5S$ ($[M-H]^-$) 561.2804, found 561.2797.

4.8. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-2-(2-tert-butoxy-2-oxoethyl)-4-fluoro-6-methylhept-3-enoyl (S)-sultam (17a)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (40.0 mg, 0.0838 mmol) was converted into the title compound **17a** (46.6 mg, 80% yield) as a colorless oil: $[\alpha]_D^{26}$ −81.7 (*c* 1.01, $CHCl_3$); IR (ATR): 3369 (NHCO), 1703 (CO), 1337 (NSO_2), 1164 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92–0.94 (m, 6H), 0.96 (s, 3H), 1.21 (s, 3H), 1.25–1.47 (m, 2H), 1.41 (s, 9H), 1.43 (s, 9H), 1.82–1.94 (m, 4H), 2.00–2.06 (m, 1H), 2.12–2.16 (m, 1H), 2.51 (dd, J=16.0, 5.7 Hz, 1H), 2.82 (dd, J=16.0, 8.2 Hz, 1H), 3.41–3.51 (m, 2H), 3.88–3.98 (m, 2H), 4.33–4.39 (m, 1H), 4.72 (d, J=9.8 Hz, 1H), 4.90 (dd, J=36.3, 9.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 19.2, 19.9, 20.6, 26.5, 28.0 (3C), 28.3 (3C), 30.5, 32.8, 37.8 (d, J=3.3 Hz), 37.9, 39.2, 44.6, 47.8, 48.5, 52.9, 57.4 (d, J=25.7 Hz), 65.1, 79.6, 81.0, 103.5 (d, J=12.4 Hz), 155.0, 158.5 (d, J=262.3 Hz), 169.8, 171.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −117.1 (br s); HRMS (FAB), *m/z* calcd for $C_{29}H_{46}FN_2O_7S$ ($[M-H]^-$) 585.3015, found 585.2998.

4.9. (2S,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-2-(2-tert-butoxy-2-oxoethyl)-4-fluoro-6-methylhept-3-enoyl (R)-sultam (17b)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (40.0 mg, 0.0838 mmol) was converted into the title compound **17b** (37.0 mg, 75% yield): $[\alpha]_D^{23}$ +39.5 (*c* 1.06, $CHCl_3$); IR (ATR): 3367 (NHCO), 1702 (CO), 1335 (NSO_2), 1162 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87–0.92 (m, 6H), 0.96 (s, 3H), 1.21 (s, 3H), 1.24–1.46 (m, 2H), 1.41 (s, 9H), 1.43 (s, 9H), 1.86–2.16 (m, 6H), 2.51–2.56 (m, 1H), 2.78–2.84 (m, 1H), 3.39–3.50 (m, 2H), 3.90–4.07 (m, 2H), 4.33–4.38 (m, 1H), 4.66 (d, J=9.8 Hz, 1H), 4.91 (dd, J=36.2, 9.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.6, 19.2, 19.9, 20.6, 26.5, 28.0 (3C), 28.3 (3C), 29.9, 32.8, 37.7 (d, J=3.3 Hz), 37.9, 39.1, 44.6, 47.8, 48.5, 52.9, 56.7 (d, J=29.0 Hz), 65.1, 79.6, 81.0, 102.8 (d, J=12.4 Hz), 155.1, 159.0 (d, J=264.0 Hz), 169.8, 171.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −114.7 (dd, J=36.2, 15.5 Hz); HRMS (FAB), *m/z* calcd for $C_{29}H_{46}FN_2O_7S$ ($[M-H]^-$) 585.3015, found 585.3016.

4.10. (2S,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-2-(4-methoxybenzyl)-6-methylhept-3-enoyl (R)-sultam (18b)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (50.0 mg, 0.102 mmol) was converted into the title compound **18b** (50.2 mg, 77% yield) as a colorless oil: $[\alpha]_D^{24}$ +24.6 (*c* 1.31, $CHCl_3$); IR (ATR): 1701 (CO), 1335 (NSO_2), 1165 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.74 (s, 3H), 0.89 (s, 3H), 0.79–0.83 (m, 6H), 1.26–1.35 (m,

2H), 1.44 (s, 9H), 1.75–1.98 (m, 6H), 2.73–2.77 (m, 1H), 3.03–3.08 (m, 1H), 3.34–3.42 (m, 2H), 3.75–3.76 (m, 4H), 3.96–4.03 (m, 1H), 4.41–4.45 (m, 1H), 4.66 (d, J=9.6 Hz, 1H), 4.92 (dd, J=35.2, 9.3 Hz, 1H), 6.77 (d, J=8.4 Hz, 2H), 7.13 (d, J=8.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.6, 19.0, 19.8, 20.4, 26.4, 28.3 (3C), 30.2, 32.8, 38.2, 39.6, 43.2, 44.6, 47.5, 48.1, 53.0, 55.2, 56.7 (d, J=29.4 Hz), 65.0, 79.5, 103.7 (d, J=12.6 Hz), 113.7 (2C), 129.6, 130.4 (2C), 155.1, 158.4, 158.6 (d, J=262.1 Hz), 172.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −116.01 (dd, J=35.2, 16.6 Hz); HRMS (FAB), m/z calcd for $C_{31}H_{44}FN_2O_6S$ ([M−H]$^-$) 591.2910, found 591.2905.

*4.11. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-6-methyl-2-(prop-2-enyl)hept-3-enoyl (S)-sultam (**19a**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (40.0 mg, 0.0838 mmol) was converted into the title compound **19a** (42.9 mg, 99% yield) as a colorless oil: $[\alpha]_D^{22}$ −81.0 (*c* 1.06, $CHCl_3$); IR (ATR): 3380 (NHCO), 1699 (CO), 1335 (NSO_2), 1166 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92–0.94 (m, 6H), 0.97 (s, 3H), 1.16 (s, 3H), 1.21–1.40 (m, 2H), 1.44 (s, 9H), 1.76–1.94 (m, 4H), 1.97–2.09 (m, 2H), 2.31–2.40 (m, 1H), 2.49–2.59 (m, 1H), 3.42–3.52 (m, 2H), 3.83–3.97 (m, 2H), 4.22 (q, J=9.8 Hz, 1H), 4.72 (d, J=9.8 Hz, 1H), 4.90–5.09 (m, 3H), 5.67–5.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5, 19.2, 19.8, 20.8, 26.4, 28.3 (3C), 30.3, 32.8, 38.3, 38.6, 40.7, 44.6, 47.7, 48.3, 53.0, 57.5 (d, J=26.5 Hz), 65.2, 79.5, 104.1 (d, J=11.6 Hz), 117.8, 134.0, 155.1, 158.0 (d, J=263.2 Hz), 172.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −119.0 (dd, J=36.2, 23.8 Hz); HRMS (FAB), m/z calcd for $C_{26}H_{40}FN_2O_5S$ ([M−H]$^-$) 511.2647, found 511.2629.

*4.12. (2S,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-6-methyl-2-(prop-2-enyl)hept-3-enoyl (R)-sultam (**19b**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (40.0 mg, 0.0838 mmol) was converted into the title compound **19b** (37.1 mg, 86% yield) as a colorless oil: $[\alpha]_D^{24}$ +34.3 (*c* 0.900, $CHCl_3$); IR (ATR): 3380 (NHCO), 1699 (CO), 1334 (NSO_2), 1165 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90–0.93 (6H), 0.97 (s, 3H), 1.16 (s, 3H), 1.25–1.40 (m, 2H), 1.44 (s, 9H), 1.85–2.08 (m, 6H), 2.34–2.41 (m, 1H), 2.51–2.58 (m, 1H), 3.41–3.52 (m, 2H), 3.86–3.97 (m, 1H), 3.99–4.07 (m, 1H), 4.23 (dt, J=9.0, 6.8 Hz, 1H), 4.69 (d, J=9.8 Hz, 1H), 4.91–5.09 (m, 3H), 5.70–5.81 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 19.1, 19.9, 20.8, 26.4, 28.3 (3C), 30.2, 32.8, 38.6, 38.7, 40.7, 44.6, 47.7, 48.3, 53.0, 56.8 (d, J=28.1 Hz), 65.2, 79.5, 103.5 (d, J=12.4 Hz), 117.8, 134.1, 155.1, 158.4 (d, J=262.3 Hz), 172.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −115.8 (dd, J=36.2, 17.6 Hz); HRMS (FAB), m/z calcd for $C_{26}H_{40}FN_2O_5S$ ([M−H]$^-$) 511.2647, found 511.2647.

*4.13. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-6-methyl-2-(2-methylprop-2-enyl)hept-3-enoyl (S)-sultam (**20a**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (78.2 mg, 0.159 mmol) was converted into the title compound **20a** (82.7 mg, 99% yield) as a colorless oil: $[\alpha]_D^{23}$ −89.7 (*c* 1.09, $CHCl_3$); IR (ATR): 3381 (NHCO), 1698 (CO), 1333 (NSO_2), 1163 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92–0.93 (m, 6H), 0.96 (s, 3H), 1.15 (s, 3H), 1.24–1.40 (m, 2H), 1.44 (s, 9H), 1.77–2.04 (m, 9H), 2.15–2.19 (m, 1H), 2.57–2.61 (m, 1H), 3.41–3.51 (m, 2H), 3.87–3.95 (m, 2H), 4.32–4.37 (m, 1H), 4.73 (s, 3H), 4.89 (dd, J=36.4, 8.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 19.2, 19.8, 20.6, 21.7, 26.4, 28.3 (3C), 30.3, 32.7, 38.3, 39.5, 42.6, 44.6, 47.7, 48.2, 53.0, 57.5 (d, J=25.8 Hz), 65.1, 79.4, 104.5 (d, J=12.0 Hz), 113.7, 141.7, 155.0, 158.0 (d, J=262.7 Hz), 172.7; ^{19}F NMR (470 MHz, $CDCl_3$) δ −119.4 to −119.33 (m); HRMS (FAB), m/z calcd for $C_{27}H_{42}FN_2O_5S$ ([M−H]$^-$) 525.2804, found 525.2809.

*4.14. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-6-methyl-2-(naphth-2-ylmethyl)hept-3-enoyl (S)-sultam (**21a**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (50.0 mg, 0.105 mmol) was converted into the title compound **21a** (64.0 mg, 99% yield) as a colorless oil: $[\alpha]_D^{24}$ −71.8 (*c* 1.41, $CHCl_3$); IR (ATR): 3368 (NHCO), 1710 (CO), 1678 (CO), 1341 (NSO_2), 1160 (NSO_2); ^{1}H NMR ($CDCl_3$) δ 0.21 (s, 3H), 0.74 (s, 3H), 0.85–0.89 (m, 6H), 1.19–1.30 (m, 2H), 1.44 (s, 9H), 1.55–1.61 (m, 2H), 1.73–1.92 (m, 4H), 2.97–3.01 (m, 1H), 3.24–3.31 (m, 3H), 3.72 (br s, 1H), 3.88–3.96 (m, 1H), 5.03 (dd, J=36.6, 8.9 Hz, 1H), 7.38–7.43 (m, 3H), 7.64 (s, 1H), 7.72–7.76 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.4, 19.1, 19.6, 19.7, 26.3, 28.3 (3C), 30.4, 32.7, 38.1, 40.7, 43.0 (d, J=3.0 Hz), 44.5, 47.3, 48.0, 52.9, 57.6 (d, J=25.8 Hz), 64.9, 79.5, 104.5 (d, J=13.8 Hz), 125.3, 125.7, 127.4, 127.5, 127.8, 127.9, 127.9, 132.5, 133.4, 135.0, 155.1, 158.2 (d, J=263.3 Hz), 172.3; ^{19}F NMR (470 MHz, $CDCl_3$) δ −118.9 (br s); HRMS (FAB), m/z calcd for $C_{34}H_{44}FN_2O_5S$ ([M−H]$^-$) 611.2960, found 611.2958.

*4.15. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-4-fluoro-2-(4-methoxy-4-oxobut-2-enyl)-6-methylhept-3-enoyl (S)-sultam (**22a**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **12a** (50.0 mg, 0.105 mmol) was converted into the title compound **22a** (56.3 mg, 94% yield) as a colorless oil: $[\alpha]_D^{23}$ −68.8 (*c* 1.30, $CHCl_3$); IR (ATR): 3371 (NHCO), 1703 (CO), 1335 (NSO_2), 1165 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90–0.94 (m, 6H), 0.96 (s, 3H), 1.11 (s, 3H), 1.31–1.43 (m, 2H), 1.44 (s, 9H), 1.85–2.07 (m, 6H),

2.44–2.50 (m, 1H), 2.66–2.72 (m, 1H), 3.42–3.52 (m, 2H), 3.70 (s, 3H), 3.86–3.98 (m, 2H), 4.29–4.33 (m, 1H), 4.70 (d, J=9.7 Hz, 1H), 4.96 (dd, J=36.2, 9.1 Hz, 1H), 5.86 (d, J=15.6 Hz, 1H), 6.82–6.88 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.4, 19.2, 19.8, 20.6, 26.4, 28.3 (3C), 30.3, 32.8, 36.8, 38.3, 40.1 (d, J=3.0 Hz), 44.6, 47.7, 48.4, 51.4, 53.0, 57.5 (d, J=25.8 Hz), 65.2, 79.6, 103.6 (d, J=12.6 Hz), 123.7, 144.0, 155.1, 158.6 (d, J=263.9 Hz), 166.2, 171.7; ^{19}F NMR (470 MHz, $CDCl_3$) δ −117.3 to −117.2. (m); HRMS (FAB), m/z calcd for $C_{28}H_{42}FN_2O_7S$ ([M−H]$^-$) 569.2702, found 569.2709.

4.16. (2S,5S,3Z)-2-(4-Bromobenzyl)-5-[N-(tert-butoxycarbonyl)amino]-4-fluoro-6-methylhept-3-enoyl (R)-sultam (23b)

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*R*)-sultam derivative **12b** (50.0 mg, 0.105 mmol) was converted into the title compound **23b** (47.8 mg, 71% yield) as a colorless oil: $[\alpha]_D^{24}$ +25.1 (c 1.12, $CHCl_3$); IR (ATR): 1703 (CO), 1337 (NSO_2), 1165 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.72 (s, 3H), 0.80–0.84 (m, 6H), 0.90 (s, 3H), 1.21–1.45 (m, 2H), 1.44 (s, 9H), 1.70–1.98 (m, 6H), 2.74–2.78 (m, 1H), 3.04–3.08 (m, 1H), 3.35–3.43 (m, 2H), 3.78 (br s, 1H), 3.97–4.03 (m, 1H), 4.42–4.47 (m, 1H), 4.65 (d, J=9.6 Hz, 1H), 4.92 (dd, J=35.9, 9.2 Hz, 1H), 7.10 (d, J=8.2 Hz, 2H), 7.35 (d, J=8.3 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.6, 19.0, 19.7, 20.2, 26.4, 28.3 (3C), 30.1, 32.8, 38.2, 39.7, 42.8, 44.6, 47.6, 48.2, 53.0, 56.7 (d, J=28.2 Hz), 65.1, 79.6, 103.3 (d, J=13.2 Hz), 120.6, 131.3 (2C), 131.3 (2C), 136.5, 155.1, 159.0 (d, J=263.9 Hz), 172.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −115.4 (dd, J=35.9, 15.5 Hz); HRMS (FAB), m/z calcd for $C_{30}H_{41}BrFN_2O_5S$ ([M−H]$^-$) 639.1909, found 639.1902.

4.17. Ethyl (3S)-6-(tert-butyldimethylsiloxy)-2,2-difluoro-3-{N-[(1R)-(2-methoxy-1-phenylethyl)]amino}hexanoate (27)

To a solution of oxalyl chloride (7.45 g, 58.7 mmol) in CH_2Cl_2 (120 mL) at −78 °C under argon was added dropwise a solution of DMSO (3.82 mL, 53.8 mmol) in CH_2Cl_2 (84 mL). After 10 min, 4-(*tert*-butyldimethylsilyloxy)-1-butanol **26** (10.2 g, 48.9 mmol) in CH_2Cl_2 (72 mL) was added dropwise. After 0.5 h, Et_3N (33.8 mL, 244.5 mmol) was added dropwise. After stirring at −78 °C for 1 h, the mixture was diluted with CH_2Cl_2 (200 mL). The diluted mixture was washed with saturated NH_4Cl and brine, and dried over $MgSO_4$. Concentration under reduced pressure gave an oily aldehyde, which was used immediately in the next step without purification. A solution of the above aldehyde and amine **8** in THF (263 mL) was stirred at 0 °C for 4 h under argon in the presence of activated molecular sieves 4 Å. To the mixture were successively added a suspension of $RhCl(PPh_3)_3$ (2.07 g, 2.24 mmol) in THF (20 mL), a solution of $BrCF_2CO_2Et$ (9.96 mL, 48.9 mmol) in THF (20 mL), and a solution of Et_2Zn in n-hexane (1.0 M, 195.6 mL, 195.6 mmol). After stirring at 0 °C for 1 h, the reaction was quenched with saturated $NaHCO_3$. The mixture was filtered over Celite and the filtrate was extracted with AcOEt. The extract was washed with saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexane–AcOEt (40:1) gave the title compound **27** (8.69 g, 39% yield) as a colorless oil: $[\alpha]_D^{24}$ −33.9 (c 1.28, $CHCl_3$); IR (ATR): 1770 (CO), 1758 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.10 (m, 6H), 0.77 (s, 9H), 1.33–1.15 (m, 5H), 1.48–1.42 (m, 1H), 1.68–1.60 (m, 1H), 3.28–3.25 (m, 4H), 3.39–3.31 (m, 3H), 4.25–4.16 (m, 3H), 7.28–7.15 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4 to −5.5 (2C), 13.8, 18.2, 25.8, 26.3, 28.9, 56.3 (t, J=22.5 Hz), 58.4, 59.7, 62.5 (d, J=6.6 Hz), 77.8, 117.7 (t, J=256.7 Hz), 127.7, 128.0, 128.3, 140.3, 164.1 (t, J=32.4 Hz); ^{19}F NMR (470 MHz, $CDCl_3$) δ −110.5 (dd, J=256.7, 10.2 Hz), −112.6 (dd, J=256.8, 12.6 Hz); HRMS (FAB), m/z calcd for $C_{23}H_{38}F_2NO_4Si$ ([M−H]$^-$) 458.2544, found 458.2566.

4.18. Ethyl (3S)-3-[N-(tert-butoxycarbonyl)amino]-6-(tert-butyldimethylsiloxy)-2,2-difluorohexanoate (28)

To a solution of the ester **27** (7.05 g, 15.2 mmol) in EtOH (50.0 mL) were added 20% $Pd(OH)_2$/C (1.11 g, 1.58 mmol) and $(Boc)_2O$ (7.26 g, 33.3 mmol), and the suspension was stirred for 12 h under H_2 at room temperature. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure, followed by flash chromatography over silica gel with n-hexane–AcOEt (40:1) to give the title compound **28** (5.47 g, 85% yield) as a colorless oil: $[\alpha]_D^{22}$ −13.9 (c 1.16, $CHCl_3$); IR (ATR): 3364 (NHCO), 1770 (CO), 1717 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 1.29 (t, J=7.2 Hz, 3H), 1.37–1.66 (m, 12H), 1.78–1.84 (m, 1H), 3.56–3.63 (m, 2H), 4.17–4.29 (m, 3H), 4.85 (d, J=10.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.7 (2C), 13.6, 18.0, 23.7, 25.6 (3C), 27.9 (3C), 28.2, 52.3 (dd, J=27.6, 23.4 Hz), 61.8, 62.6, 79.6, 114.4 (t, J=255.2 Hz), 155.0, 163.1 (dd, J=33.3, 30.9 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.6 (dd, J=256.6, 8.3 Hz), −119.8 (dd, J=256.6, 18.6 Hz); HRMS (FAB), m/z calcd for $C_{19}H_{36}F_2NO_5Si$ ([M−H]$^-$) 424.2336, found 424.2343.

4.19. (5S,2E)-5-[N-(tert-Butoxycarbonyl)amino]-8-(tert-butyldimethylsiloxy)-4,4-difluorooct-2-enoyl (S)-sultam (29)

By use of a procedure similar to that described for the preparation of the (*S*)-sultam derivative **12a**, the ester **28** (5.49 mg, 12.9 mmol) was converted into the title compound **29** (5.83 g, 73% yield) as a colorless oil: $[\alpha]_D^{20}$ −59.8 (c 1.33, $CHCl_3$); IR (ATR): 3370 (NHCO), 1713 (CO), 1692 (CO), 1332 (NSO_2), 1165 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.84 (s, 9H), 0.94 (s, 3H), 1.12 (s, 3H), 1.30–1.44 (m, 12H), 1.48–1.65 (m, 2H), 1.77–1.94 (m, 4H), 2.06–2.08 (m, 2H), 3.39–3.50 (m, 2H), 3.54–3.61 (m, 2H), 3.88–3.91 (m, 1H), 3.94–4.03 (m, 1H), 4.56 (d, J=10.2 Hz, 1H), 6.79–6.87 (m, 1H), 6.95 (d, J=15.3 Hz, 1H); ^{13}C NMR