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Stereoselective Formation of Trisubstituted (*Z*)-Chloroalkenes Adjacent to a Tertiary Carbon Stereogenic Center by Organocuprate-Mediated Reduction/Alkylation

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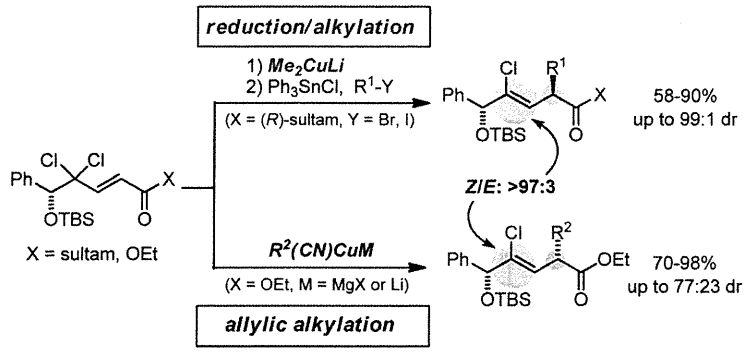
ABSTRACT



A robust and efficient method for the synthesis of trisubstituted (*Z*)-chloroalkenes is described. A one-pot reaction of γ,γ-dichloro-α,β-enoyl sultams involving organocuprate-mediated reduction/asymmetric alkylation affords α-chiral (*Z*)-chloroalkene derivatives in moderate to high yields with excellent diastereoselectivity, and allylic alkylation of internal allylic *gem*-dichlorides is also demonstrated. This study provides the first examples of the use of allylic *gem*-dichlorides adjacent to the chiral center for novel 1,4-asymmetric induction.

Stereoselective formation of functionalized alkenes is a challenging task in organic synthesis, and construction of halogenated alkenes while controlling the geometry of double bonds is of particular interest.[1] Among various halogenated alkenes, chloroalkenes have attracted considerable interest in recent years,[2–6] not only because of their potential as synthetically valuable intermediates[7] but also because of their importance as structural components of natural products.[8]

(1) (a) Guinchard, X.; Roulland, E. *Synlett* **2011**, *19*, 2779. (b) Shen, Y. *ACC. Chem. Res.* **1998**, *31*, 584.

(2) For selected reviews of chloroalkene syntheses, see: (a) *Comprehensive Organic Functional Group Transformations*; Katritzky, A. R., Meth-Cohn, O., Rees, C. W., Eds.; Pergamon Press: Oxford, 1995; Vol. 2, pp 606–619. (b) *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Semmelheck, M. F., Eds.; Pergamon Press: Oxford, 1991; Vol. 4, pp 272–278. (c) *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Schreiber, S. L., Eds.; Pergamon Press: Oxford, 1991; Vol. 1, pp 807–809.

(3) For some examples of trisubstituted (*E*)-chloroalkene syntheses, see: (a) Kigoshi, H.; Kita, M.; Ogawa, S.; Itoh, M.; Uemura, D. *Org. Lett.* **2003**, *5*, 957. (b) Trost, B. M.; Pinkerton, A. B. *J. Am. Chem. Soc.* **1999**, *121*, 1988.

(4) For examples of terminal (*Z*)-chloroalkene syntheses, see: (a) Giannerini, M.; Fañanás-Mastral, M.; Feringa, B. L. *J. Am. Chem. Soc.* **2012**, *134*, 4108. (b) Sashuk, V.; Samojłowicz, C.; Szadkowska, A.; Grela, K. *Chem. Commun.* **2008**, 2468. (c) Barluenga, J.; Moriel, P.; Aznar, F.; Valdés, C. *Adv. Synth. Catal.* **2006**, *348*, 347. (d) Baati, R.; Barma, D. K.; Krishna, U. M.; Mioskowski, C.; Falck, J. R. *Tetrahedron Lett.* **2002**, *43*, 959 and references cited therein.

(5) (a) Baati, R.; Barma, D. K.; Falck, J. R.; Mioskowski, C. *J. Am. Chem. Soc.* **2001**, *123*, 9196. (b) Falck, J. R.; Bandyopadhyay, A.; Barma, D. K.; Shin, D.-S.; Kundu, A.; Kishore, R. V. K. *Tetrahedron Lett.* **2004**, *45*, 3039. (c) Baati, R.; Barma, D. K.; Falck, J. R.; Mioskowski, C. *Tetrahedron Lett.* **2002**, *43*, 2183. (d) Su, W.; Jin, C. *Org. Lett.* **2007**, *9*, 993.

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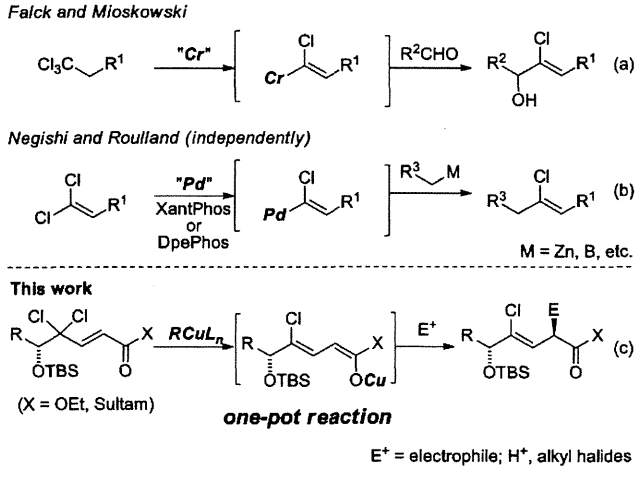
Despite the utility and importance of chloroalkenes, however, reactions leading to the stereoselective formation of trisubstituted (*Z*)-chloroalkenes are still limited.[5,6] Falck and Mioskowski reported that the reaction of $CrCl_2$ with 1,1,1-trichloroalkanes leads to the formation of (*E*)-chlorovinylidene chromium carbenoids, which can react with aldehydes to afford (*Z*)-chlorinated allylic alcohols (Scheme 1a).[5a] An alternative method is the Pd-catalyzed cross-coupling of 1,1-dichloro-1-alkenes with organometallic reagents.[6] In particular, Pd-catalyzed couplings with large bite angle bisphosphines such as Xantphos and DPEphos allow the selective formation of (*Z*)-chloroalkenes while avoiding the formation of bis-substituted products as has been described independently by Negishi[6a] and by Roulland[6b–d] (Scheme 1b). While these protocols have found widespread utility for the synthesis of these important structures, the development of efficient systems for stereoselective and divergent synthesis of trisubstituted (*Z*)-chloroalkenes bearing various functionalities remains challenging.

As part of a program aimed at development of novel approaches to chloroalkenes, we envisioned that the organocuprate-mediated reduction[9] of γ,γ-dichloro-α,β-unsaturated carbonyl compounds would permit an efficient access to (*Z*)-chlorinated dienolate intermediates, which can be trapped with an appropriate electrophile, providing trisubstituted (*Z*)-chloroalkenes (Scheme 1c).

Scheme 1. Synthesis of Trisubstituted (*Z*)-Chloroalkenes



In this paper, we describe the stereoselective formation of trisubstituted (*Z*)-chloroalkenes utilizing the organocuprate-mediated reduction/asymmetric alkylation of γ,γ-dichloro-α,β-enoyl sultam. This is a one-pot reaction which provides in high yield the synthetically valuable compounds containing a (*Z*)-chloroalkene flanking two stereogenic centers, the α-chiral-β,γ-unsaturated carbonyl motif, and a chiral allylic alcohol. In addition, we report the first allylic alkylation of *internal* allylic *gem*-dichlorides that provides an alternative method for the diastereoselective synthesis *via* 1,4-asymmetric induction of these important structural motifs.

We prepared sultam **1** and enoate **2** from chiral α,α-dichloro-β-hydroxyester,[10] reported by Imashiro and Kuroda, as suitable substrates for reaction development (Figure 1). At the onset of our studies, it was unclear if the reaction of those substrates with organocuprates would entail reduction, generating the dienolate intermediate. In order to estimate the electron-accepting ability, our investigation started with measurement of the reduction potentials (E_{Red}). The reduction potentials of sultam **1** and enoate **2** were -1.50 and -1.65 V, respectively. Based on these results and House's observation that α,β-unsaturated carbonyl compounds with reduction potentials between *ca.* -2.4 V and *ca.* -1.1 V can react with organocuprates such as Me_2CuLi to give the conjugate addition products,[11] these substrates were expected to promote both the single-electron transfer reduction and the allylic alkylation.

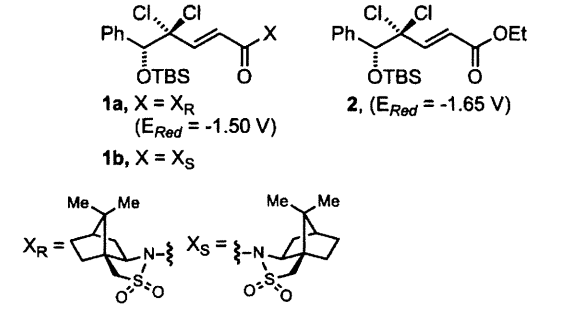


Figure 1. Substrates for organocuprate-mediated reduction and their reduction potentials (E_{Red}).

In order to control the reaction products, the reactivity of sultam **1a** with organocuprates was examined (Table 1),

(6) For selected examples of Pd-catalyzed cross-coupling, see: Cross-coupling with organozincs: (a) Tan, Z.; Negishi, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 762. Cross-coupling with organoborans: (b) Guinchard, X.; Bugaut, X.; Cook, C.; Roulland, E. *Chem.—Eur. J.* **2009**, *15*, 5793. (c) Roulland, E. *Angew Chem. Int. Ed.* **2008**, *47*, 3762. (d) Liron, F.; Fosse, C.; Pernolet, A.; Roulland, E. *J. Org. Chem.* **2007**, *72*, 2220. Cross-coupling with other organometallics, see ref 1a.

(7) (a) Geary, L. M.; Hultin, P. G. *J. Org. Chem.* **2010**, *75*, 6354. (b) Bell, M.; Poulsen, T. B.; Jørgensen, K. A. *J. Org. Chem.* **2007**, *72*, 3053. (c) Jones, G. B.; Wright, J. M.; Plourde, G. W., II; Hynd, G.; Huber, R. S.; Mathews, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 1937. (d) Alami, M.; Gueugnot, S.; Domingues, E.; Linstrumelle, G. *Tetrahedron* **1995**, *51*, 1209.

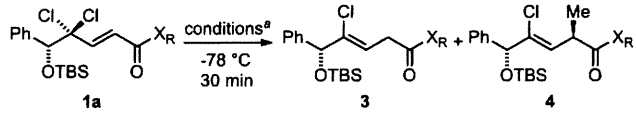
(8) For a recent example of the natural product bearing chloroalkene motif: Ando, H.; Ueoka, R.; Okada, S.; Fujita, T.; Iwashita, T.; Imai, T.; Yokoyama, T.; Matsumoto, Y.; van Soest, R. W. M.; Matsunaga, S. *J. Nat. Prod.* **2010**, *73*, 1947 and also ref 1a.

(9) For selected examples of organocuprate-mediated reduction, see: (a) Narumi, T.; Niida, A.; Tomita, K.; Oishi, S.; Otaka, A.; Ohno, H.; Fujii, N. *Chem. Commun.* **2006**, 4720. (b) Meyers, A. I.; Snyder, L. *J. Org. Chem.* **1992**, *57*, 3814. (c) Fujii, N.; Habashita, H.; Shigemori, N.; Otaka, A.; Ibuka, T.; Tanaka, M.; Yamamoto, Y. *Tetrahedron lett.* **1991**, *32*, 4969. (d) Takano, S.; Sekiguchi, Y.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* **1988**, 449 and references cited therein.

(10) Imashiro, R.; Kuroda, T. *J. Org. Chem.* **2003**, *68*, 974. For details of the preparation of sultam **1** and enoate **2**, see the Supporting Information.

(11) House, H. O.; Umen, M. J. *J. Org. Chem.* **1973**, *38*, 2417.

Table 1. Reactivity of Sultam **1a** with Organocuprates



entry	reagents	additives[b]	Z/E of 3[c]	3+4, yield (%)[d]	3/4[c]
1	Me_2CuLi	–	>97:3	81	77:23
2	Me_2CuLi[e]	–	>97:3	93	>97:3
3	n-Bu_2CuLi[e]	–	>97:3	99	90:10
4	MeCu(CN)Li	–	>97:3	99	61:39[f]
5	Me_2CuLi[e]	TMSCl	>97:3	83	83:17
6	Me_2CuLi[e]	$BF_3 \cdot OEt_2$	>97:3	91	31:69
7	Me_2CuLi[e]	HMPA	>97:3	76	>97:3
8	$Me_2Cu(CN)Li_2$	–	>97:3	99	>97:3

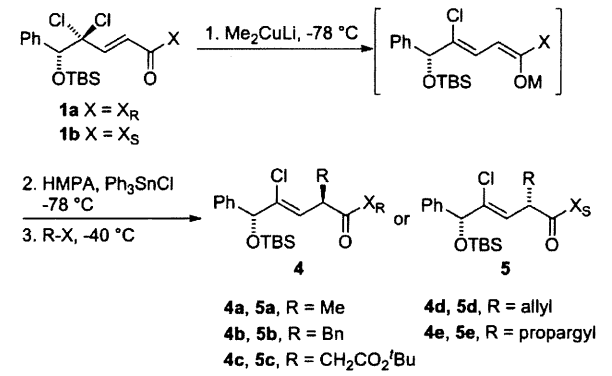
[a] All reactions were carried out on a 0.1 mmol scale with 4 equiv of organocuprates in the presence of Li salts. [b] 4 equiv. [c] Determined by ^1H NMR. [d] Yields of isolated products. [e] Higher order cuprates (*ca.* 0.4 equiv) were contained. [f] Diastereomeric ratio (dr) = 97:3.

and as expected, exposure of **1a** to Me_2CuLi followed by protic workup afforded a mixture of the reduced compound **3** and the α-alkylated product **4** in high yield (81%, entry 1). Significantly, excellent *Z*-selectivity was observed.[12] The use of a 2.4:1 MeLi·LiBr/CuI mixture enabled selective reduction, providing pure reduced compound **3** in excellent yield (93%, entry 2). Changing the methyl group of alkyl ligands to an *n*-butyl group resulted in decreased selectivity (entry 3). Although the reaction with lower order cyanocuprate or Me_2CuLi with TMSCl did not furnish better selectivity, addition of $BF_3 \cdot OEt_2$ led to the preferable formation of α-alkylated product **4** (entries 4–6). In contrast, the reaction with HMPA provided excellent selectivity to the reduction but a decreased yield (76%, entry 7). The best result was obtained with higher order cyanocuprate, derived from CuCN·2LiCl and 2 equiv of MeLi·LiBr, which gave **3** in excellent yield and selectivity (entry 8). Having identified higher order cyanocuprate as the preferred reducing agent, we selected the Gilman reagent (Me_2CuLi) as an optimal reducing agent because of the sufficient reactivity and selectivity to reduction.

The optimized reduction condition in Table 1 (entry 2) was applied to the one-pot reduction/asymmetric alkylation (Table 2). Previous studies have revealed that the transmetalation from Cu and/or Li dienolate intermediates to the more reactive Sn dienolate intermediates is critical for smooth alkylation.[9a] A variety of alkyl halides were allowed to react with the (*Z*)-chlorinated dienolate intermediate to provide chloroalkenes **4a**–**4e** flanking two stereogenic centers in moderate to high yield with >97% *Z*-selectivity. HPLC analysis showed that all the reactions

(12) A NOESY cross-peak was observed between the olefinic proton and the allylic stereogenic center, suggesting that the geometry of the double bond was defined as shown; see the Supporting Information.

Table 2. One-Pot Reduction/Asymmetric Alkylation of (*R*)-Sultam **1a** and (*S*)-Sultam **1b**[a]

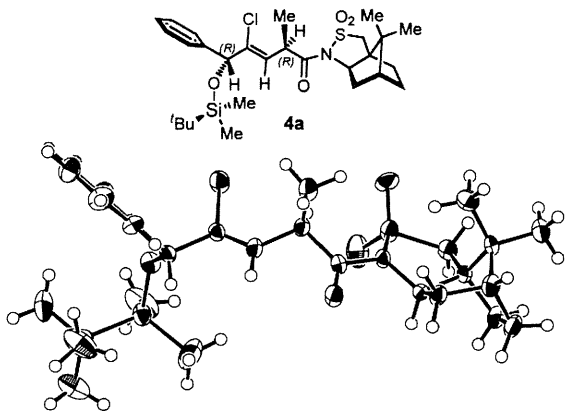


entry	substrate	R–X	4 or 5, yield (%)[b]	dr (%)[c]
1	**1a**	MeI	**4a**, 58	97:3
2[d]	**1a**	BnBr	**4b**, 83	99:1
3	**1a**	$BrCH_2CO_2{}^tBu$	**4c**, 90	97:3
4	**1a**	allylBr	**4d**, 81	97:3
5[d]	**1a**	propargylBr	**4e**, 82	>95:5[e]
6	**1b**	MeI	**5a**, 60	99:1
7	**1b**	BnBr	**5b**, 86	95:5
8	**1b**	$BrCH_2CO_2{}^tBu$	**5c**, 57	97:3
9	**1b**	allylBr	**5d**, 70	97:3
10[d]	**1b**	propargylBr	**5e**, 46	>95:5[e]

[a] All reactions were carried out with 4 equiv of organocuprates, 16 equiv of HMPA, 2 equiv of Ph_3SnCl, and 8 equiv of alkyl halide. [b] Yields of isolated products. [c] Determined by HPLC. [d] At −30 °C. [e] Determined by ^1H NMR.

proceeded with excellent diastereoselectivity. The reactions with methyl iodide and bezyl bromide provided the corresponding α-alkylated products **4a** and **4b** in 58% and 83% yields, respectively (entries 1 and 2), and the absolute configuration of **4a** was confirmed by single-crystal X-ray analysis (Figure 2). Importantly, this strategy is amenable to the introduction of functional groups such as ester, allyl, and propargyl groups suitable for further transformation. Treatment of dienolate with *tert*-butyl bromoacetate and allyl bromide afforded the desired (*Z*)-chloroalkenes **4c** and **4d** with ester and allyl functionality, in high yields (entries 3 and 4). Propargyl bromide also gave the corresponding (*Z*)-chloroalkene **4e** in moderate yield (entry 5). In addition, this one-pot strategy can be applied to (*S*)-sultam **1b**, providing the corresponding chloroalkenes **5a**–**5e** in moderate to high yields (entries 6–10).

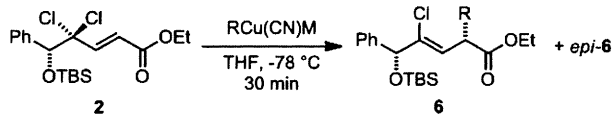
Finally, allylic alkylation of allylic *gem*-dichlorides was examined. Recently, Feringa reported that terminal allylic *gem*-dichlorides undergo Cu-catalyzed asymmetric allylic alkylation with Grignard reagents affording (*Z*)-chloroalkenes bearing an allylic stereogenic center with excellent regio- and enantioselectivity.[4a] Guided by this work, we attempted Cu-catalyzed S_N2'-type alkylation with γ,γ-dichloro-α,β-enoate **2**, but these conditions did not work for enoate **2**, possibly due to the lower reactivity of the



4a

Figure 2. ORTEP representation of **4a**.

Table 3. Diastereoselective Allylic Alkylation of γ,γ-Dichloro-α,β-enoate **2** by 1,4-Asymmetric Induction[a]



2 **6**

entry	RCu(CN)M	Z/E[b]	**6**, yield (%)[c]	dr[d]
1	MeCu(CN)Li	>97:3	**6a**, 98	74:26
2	EtCu(CN)MgBr	>97:3	**6b**, 96	66:34
3	BnCu(CN)MgCl	>97:3	**6c**, 70	77:23
4	^{i}BuCu(CN)MgCl	>97:3	**6d**, 95	69:31

[a] All reactions were carried out on a 0.2 mmol scale with 4 equiv of organocuprates in the presence of Li salts. [b] Determined by ^{1}H NMR. [c] Yields of isolated products. [d] Determined by HPLC.

internal allylic system (see Supporting Information). Attention was therefore turned to the organocuprate-mediated allylic alkylation. As presented in Table 1 (entry 4), the lower order cyanocuprate (MeCu(CN)Li) promotes the allylic alkylation preferably to provide the α-methylated product **4a** with excellent diastereoselectivity (dr = 97:3). Extensive experimentation with MeCu(CN)Li revealed that the electron transfer from organocuprates competes significantly with allylic alkylation of the sultam **1a**, and the exclusive formation of **4a** was not realized. During the course of our studies on the allylic alkylation, we considered that the chiral center at C5 adjacent to the allylic *gem*-dichloride might induce the diastereoselectivity without chiral auxiliaries.

This hypothesis was tested with the enoate **2**. As shown in Table 3, treatment of **2** with MeCu(CN)Li afforded the α-methylated β,γ-enoate **6a** in 98% yield as a 74:26 mixture of diastereomers with excellent *Z*-selectivity. The major isomer was the (2*S*)-isomer, identified by the correlation with the same compound **6a**, prepared from the corresponding (*S*)-sultam-derived compound **5a**.[13] Similar results were obtained using EtCu(CN)MgBr, BnCu(CN)-MgCl, and *i*BuCu(CN)MgCl affording the corresponding α-alkylated chloroalkenes **6b**–**d** in high yields with similar selectivities (entries 2–4). Although the observed diastereoselectivity has not been rationalized,[14] these results suggest that stereochemistry at C2 can be controlled by the chiral center at C5 *via* 1,4-asymmetric induction.

In conclusion, we have described a one-pot organocuprate-mediated reduction/asymmetric alkylation of γ,γ-dichloro-α,β-unsaturated carbonyl compounds. This protocol allows not only the exclusive formation of trisubstituted (*Z*)-chloroalkenes in high yields but also the construction of an α-stereogenic center with excellent diastereoselectivity. The resulting products are notable for their high functionality and can perform as a potentially useful intermediate for this important class of molecules. In addition, we have identified a unique reactivity of substrates containing an allylic *gem*-dichloride system with organocuprates. These findings have proven to be useful for the development of novel reactions based on these classes of molecules. Efforts to elucidate the origin of novel 1,4-asymmetric induction and to extend this work to the diastereoselective synthesis of peptidomimetics with a chloroalkene moiety are currently in progress.

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Supporting Information Available. Representative procedures, characterization data, cif file of compound **4a**, and copies of NMR and HPLC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

(13) See the Supporting Information for details.

(14) At the present stage of our understanding, the steric repulsions between the olefinic proton at C3 and the Ph group at C5 may destabilize the reactive conformer, which would lead to the (2*R*)-isomer. DFT calculations also suggest that the reactive conformer to the (2*S*)-isomer is favored by 4.42 kJ/mol over the conformer to the (2*R*)-isomer. See the Supporting Information.

The authors declare no competing financial interest.

—Review—

ケミカルバイオロジーを基盤とした抗 HIV 剤の創製

玉村啓和

Development of Anti-HIV Agents Based on Chemical Biology

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Recently, highly active anti-retroviral therapy (HAART), which involves a combinational use of reverse transcriptase inhibitors and HIV protease inhibitors, has brought us a great success in the clinical treatment of AIDS patients. However, HAART has several serious clinical problems. These drawbacks encouraged us to find novel drugs and increase repertoires of anti-HIV agents with various action mechanisms. The recent disclosing of the dynamic supramolecular mechanism in HIV-entry has provided potentials to find a new type of drugs. To date, we have synthesized HIV-entry inhibitors, especially coreceptor CXCR4 antagonists. In addition, CD4 mimics in consideration of synergic effects with other entry inhibitors or neutralizing antibodies have been developed. The development of the above anti-HIV agents is based on the concept of reverse chemical genomics, in which target molecules are fixed. On the other hand, based on the concept of forward chemical genomics, in which active compounds are searched according to the screening of random libraries, effective peptide leads such as integrase inhibitors derived from fragment peptides of HIV-1 Vpr have been discovered. As such, from a point of view on chemical biology, anti-HIV leads have been found utilizing reverse and forward chemical genomics. Furthermore, antibody-based therapy or AIDS vaccine is still thought to be a promising treatment. Thus, peptidic antigen molecules based on artificial remodeling of the dynamic structures of a surface protein gp41 in HIV fusion have been developed. The present chemical biology approaches would be essential for discovery of anti-HIV agents in consideration of cocktail therapy of AIDS.

Key words——anti-human immunodeficiency virus (HIV) agent; AIDS vaccine; chemical biology; CXCR4 antagonist; CD4 mimic; integrase inhibitor

1. はじめに

1980 年頃サンフランシスコやニューヨークにおいて，これまで知られていなかった進行性の呼吸器障害により死亡する例が相次いで報告された．患者らは体内の CD4 陽性リンパ球が急速に減少又は消失し，免疫不全状態を呈しており，この疾患は後天性免疫不全症候群（acquired immunodeficiency syndrome, AIDS）と名付けられた．この疾患はすぐに全世界に広がり，原因ウイルスであるヒト免疫不全ウイルス（human immunodeficiency virus, HIV）が単離された．HIV は空気感染ではなく主に性的感染，血液感染，母子感染の 3 つの経路により感染し，現在世界で 4000 万人以上の HIV 感染者がおり，多くの発展途上国においては今もなお感染者数が増加している．さらに，多くの先進国では感染者数が減少しているのに対し，日本では増加傾向にある．[1,2]

大きく分けて HIV には，HIV-1 及び HIV-2 が存在し，HIV-1 は西半球，ヨーロッパ，アジア，アフリカ中央部・南部・東部で多くみられ，HIV-2 はアフリカ西部で多くみられる．HIV-2 に比べ HIV-1 は感染例が多く，感染力も強いため，抗 HIV 薬やエイズワクチン開発は主に HIV-1 を標的として行われている．

HIV-1 は，直径約 110 nm の RNA 型エンベロープウイルス（レトロウイルス）で，約 9500 塩基からなる 2 コピーの RNA ゲノムや逆転写酵素，インテグラーゼなどを含む核（キャプシド，capsid）と，それを取り囲む球状エンベロープによって構成され

東京医科歯科大学生体材料工学研究所（〒101-0062 東京都千代田区神田駿河台 2-3-10）
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本総説は，日本薬学会第 131 年会シンポジウム S05 で発表したものを中心に記述したものである．

る．ウイルス粒子の外側を構成するエンベロープには，外に突き出している糖タンパク質 gp120 とその内側に存在する脂質二重膜を貫通する糖タンパク質 gp41 からなるスパイクがある．この gp120 と gp41 はヘテロダイマーを形成し，さらにヘテロダイマーを形成したタンパク質がホモ三量体を形成して HIV 膜上に十数個存在している［Fig. 1(A)］．これまでの研究により HIV-1 の宿主細胞への侵入から新ウイルスの出芽までの一連の複製サイクル（ライフサイクル）が詳細に解明されてきた．このサイクルは数ステップに分けることができ，ウイルスの宿主細胞への吸着・膜融合，RNA ゲノムの逆転写，ウイルス DNA の宿主 DNA への組込み（インテグレーション）によるプロウイルス DNA の形成と複製，ウイルス構成タンパク質のプロセッシング，ウイルス粒子の構築，出芽，ウイルスの成熟化の過程を経て増殖していく［Fig. 1(B)］.[3,4]

これまで，上述のような HIV のライフサイクルを各ステップで阻害するような薬剤の研究・開発が進められてきた．主に HIV 固有の酵素をターゲットとして，ウイルスのライフサイクルの異なる作用点に働くものがある．代表的な抗 HIV 薬として核酸系若しくは非核酸系逆転写酵素阻害剤，プロテアーゼ阻害剤，インテグラーゼ阻害剤が開発され，臨床において用いられている．また，侵入過程を阻害する膜融合阻害剤，CCR5 阻害剤も FDA で認可されている.[5] しかし，HIV-1 は変異を起こし易いため，単剤療法では薬剤耐性があらわれ薬剤の効果がなくなってしまう．そのため HIV 感染症の治療には，薬剤の耐性変異が重ならないように数種の薬剤を組み合わせた多剤併用療法を行っている．これは，highly active anti-retroviral therapy（HAART）と呼ばれ，主に逆転写酵素阻害剤，プロテアーゼ阻害剤，インテグラーゼ阻害剤の中から 2，3 剤の組み合わせで用いられる.[6] HAART は有効な治療法として成果を挙げているが，長期投与による高額な治療費，重篤な副作用，耐性ウイルスの出現などの問題点もある．そのため，これまでの薬剤とは異なる作用機序を持つ新たな薬剤の開発が望まれており，創薬化学者には実際に使用できる薬剤のレパートリーを増やすことが求められている．われわれは以前からコレセプター CXCR4 阻害剤を中心に抗 HIV 剤を創製しており，最近ではケミカルバイオ

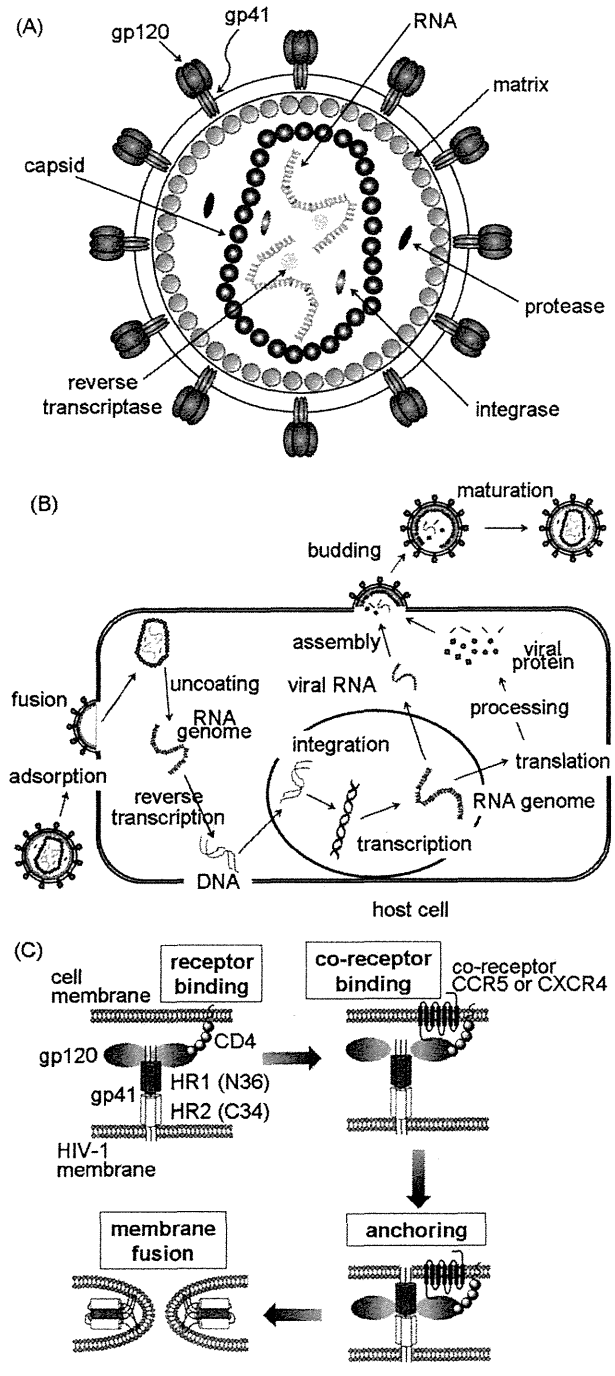

Fig. 1. (A) Structure of HIV Virion, (B) Replication Cycle of HIV, (C) Mechanism of HIV-1 Entry and Fusion

玉村啓和

1964 年生まれ．京都大学大学院薬学研究科修士課程在学中に助手に着任，博士（薬学）．京都大学大学院薬学研究科助教授を経て，2005 年より東京医科歯科大学生体材料工学研究所教授．1999–2000 年 米国国立衛生研究所/米国国立がん研究所へ留学．専門はペプチド・天然物を基盤とした創薬化学，ケミカルバイオロジー．

ロジー的手法を駆使して，吸着阻害剤 CD4 ミミック，膜融合阻害剤，インテグラーゼ阻害剤，HIV の侵入の膜融合機構をターゲットとした人工設計型抗原分子を創製している．本論文では，その研究内容について報告する．

2. コレセプター CXCR4 阻害剤

HIV の宿主細胞への吸着・膜融合に関して，まず，HIV エンベロープタンパク質 gp120 が細胞表面上の第一受容体である糖タンパク質 CD4 に結合し，gp120 のコンフォメーション変化の後に，gp120 は第二受容体（コレセプター）である CCR5 あるいは CXCR4 に結合する．[7,8] CCR5 と CXCR4 は 7 回膜貫通 G タンパク質共役型受容体（7TM-GPCR）に属するケモカイン受容体である．CCR5 は HIV 感染の初期に主流になるウイルス株マクロファージ指向性 HIV-1（R5-HIV-1）が主に使用するコレセプターであり，CXCR4 は HIV 感染の後期に主流になるウイルス株 T 細胞指向性 HIV-1（X4-HIV-1）が主に使用するコレセプターである．gp120 の CD4，CCR5 あるいは CXCR4 に対する結合より，gp120 と非共有結合的にヘテロダイマーを形成している gp41 の N 末端側が露出され，gp41 に存在する膜挿入ペプチドが標的細胞の細胞膜にアンカリングする．アンカリングの後，三量体の gp41 の N 末端側の helix 領域である HR1（NHR, N-region）領域（N36 配列を含む領域）と C 末端側 helix 領域である HR2（CHR, C-region）領域（C34 配列を含む領域）が逆平行に結合し，六量体を形成することで HIV の膜と標的細胞の膜が近づき膜融合を引き起こす［Fig. 1(C)］．[9]

まず，玉村の以前の所属機関である京都大学大学院薬学研究科の藤井信孝教授の下で，コレセプター CXCR4 阻害剤の創製に取り組んだ．コレセプター CXCR4 を抗エイズ薬のターゲットとすることは，もう 1 つの主要なコレプターである CCR5 の阻害剤 Maraviroc（Pfizer Inc.）[10]が臨床使用されたことより妥当であると考えられ，CXCR4 阻害剤も早急な開発と安全性の確認が期待される．HIV のコレセプター指向性を考えると，HIV 感染直後の前期からエイズを発症する後期に移行するに従って，CCR5 指向性の株から CXCR4 指向性の株が主流になっていく．このことから，CCR5 阻害剤だけでなく，片手落ちにならないように CXCR4 阻害剤も必要と思われる．1989 年頃から，カブトガニの血球由来の防御ペプチド polyphemusin の構造活性相関研究を精力的に行い，T22 という 18 残基からなる侵入阻害ペプチドを見い出した．[11]CXCR4 がコレセプターとして同定された後，T22 は CXCR4 アンタゴニストであることが証明され，[12] 構造最適化により 14 残基からなる強力な CXCR4 アンタゴニスト活性を示す T140 を見い出した．[13] その生体内安定性を向上させた T140 誘導体は現在，臨床試験（phase II）中である．[14,15] また，T140 のファルマコフォアのアミノ酸残基を基にした環状ペンタペプチドライブラリーを構築し，この中から T140 と同等のアンタゴニスト活性を有する誘導体 FC131 の創出に成功した［Fig. 2(A)］．[16]

これらのリード化合物を基にさらに低分子量のペプチドミミックも見い出しており，[17] さらに，非ペプチド性の CXCR4 アンタゴニストである二核亜鉛錯体[18]やその誘導体[19]を創出している［Fig. 2(B)］．CXCR4 が HIV のコレセプターであることが報告されてから数年後に，CXCR4 とその内因性リガンド CXCL12 の相互作用が，種々の固形がんの転移や血液がんの進行，関節リウマチの炎症等に大きく係わっていることが明らかにされた．[20] それに伴い，T140 誘導体等ががん転移阻害活性，白血病の進行の阻害活性，抗関節リウマチ作用を有することを明らかにした．[20-22] また，他の研究者からも AMD3100（Genzyme Corp.）や KRH-1636（Kureha Chemical & Daiichi Sankyo Co., Ltd.）等種々の CXCR4 アンタゴニストが報告されているが，誌面の関係上，総説を参照されたい．[23] 生理的には，CXCR4 は CXCL12 との相互作用により，胎生時の血管形成や心形成，造血，神経形成において progenitor cell の遊走や活性化等の重要な作用を示すことから，CXCR4 アンタゴニストの副作用を十分検討する必要がある．以上より CXCL12/CXCR4 情報伝達系の制御は，HIV 感染症，がん転移，白血病，関節リウマチ等の多くの疾病に対する創薬戦略として有望であり，言い方を変えれば，CXCR4 は multiple 創薬ターゲットとして魅力的である．現在，立体配座固定化テンプレートを活用し，より有用な創薬リードへ導くよう進めている．

3. 吸着阻害剤 CD4 ミミック

HIV は細胞侵入時に，エンベロープタンパク質

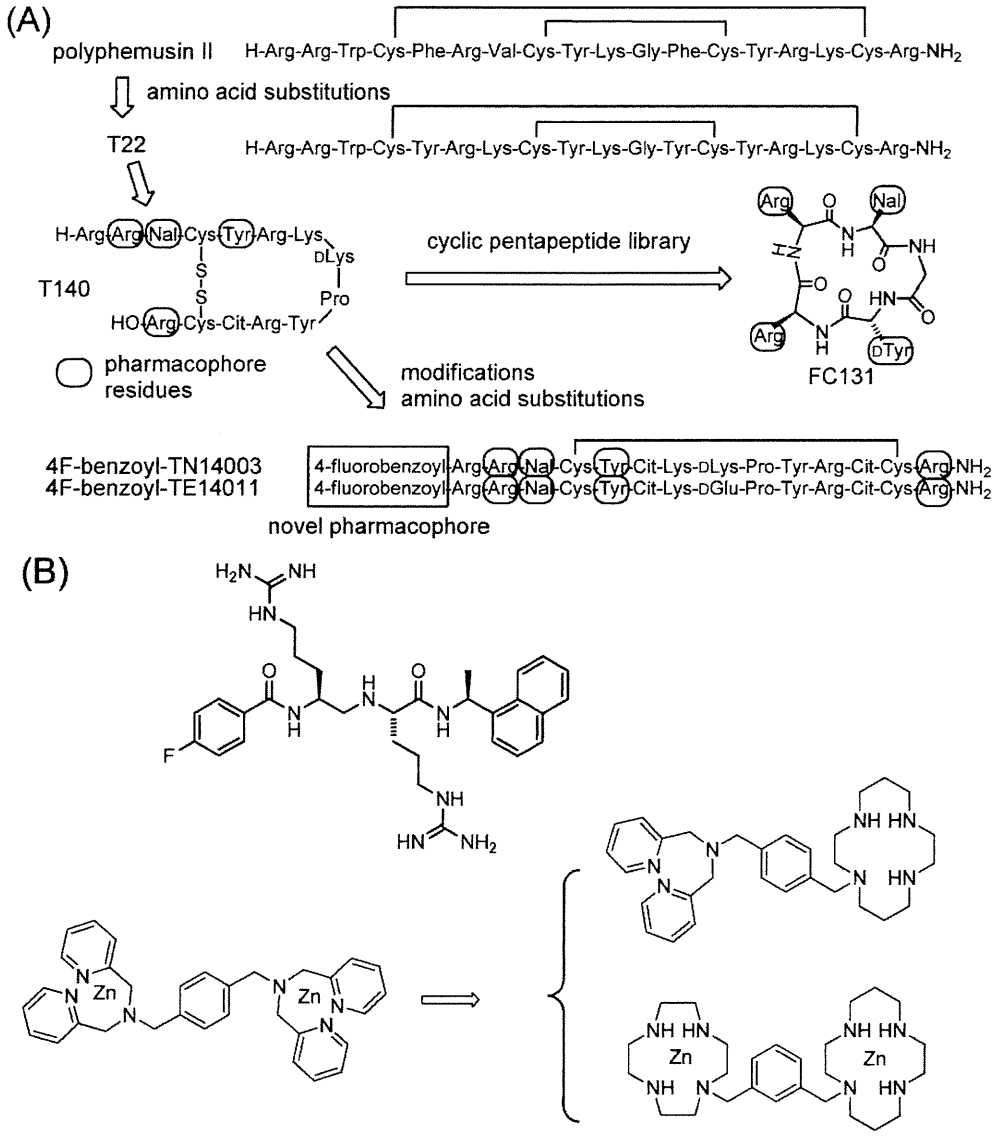

Fig. 2. (A) Development of CXCR4 Antagonists Based on Horseshoe Crab Peptides, Nal=L-3-(2-naphthyl)alanine, Cit=L-citrulline, (B) Structures of a Peptide Mimic CXCR4 Antagonist with Low Molecular Weight (upper), a Dipicolylamine (Dpa)-Zinc(II) Complex (lower left) and Its Derivatives (lower right)

gp120 が細胞表面上の第一受容体 CD4 に結合し，gp120 のコンフォメーション変化が生じ，gp120-CD4-コレセプター（非共有結合複合体）の形成，gp41 と宿主細胞膜の相互作用を経て膜融合する (Fig. 1)．NBD-556 は HIV-1 の複合体形成阻害スクリーニングにより見い出された HIV 侵入阻害剤である.[24] また，NBD-556 (Fig. 3) は可溶性 CD4 と同様に gp120 と相互作用することにより gp120 の構造変化を促すことから，低分子型 CD4 ミミックとして注目されている．これまでに可溶性 CD4 と gp120 の共結晶構造が明らかにされ，CD4 の Phe^{43} の側鎖が gp120 の特徴的な空洞 (Phe-43 cavity) に入り込む形で相互作用することが明らかにされている.[25] われわれは，可溶性 CD4 と gp120 の共結晶構造を基にした分子モデリング解析 (Flex-SIS module of SYBYL 7.1) を行い，Phe^{43} だけでなく Arg^{59} も gp120 と相互作用することを示し，[26] NBD-556 のアニリン部位が Phe^{43} の側鎖と，テトラメチルピペリジン環部位が Arg^{59} の側鎖と対応するように gp120 と相互作用することが示唆された (Fig. 3)．さらに，NBD-556 のクロロアニリン部位は CD4 に比べ，Phe-43 cavity に 6.5 Å 程度深く入り，gp120 の芳香族アミノ酸 (Trp^{447}, Phe^{382}, Trp^{112}) と，リンカーであるオキサミド構造は水素

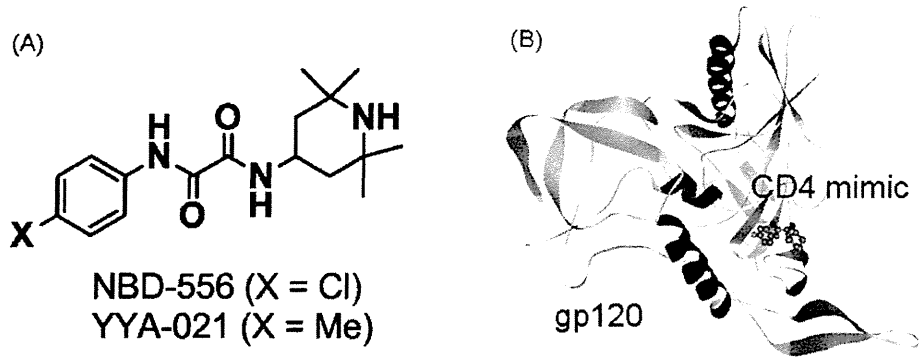

Fig. 3. (A) Structures of CD4 Mimics NBD-556 and YYA-021, (B) Docking Model of gp120 and NBD-556

結合供与体として gp120 と相互作用していることも示唆されている.[25] すなわち，この CD4 ミミックは適切なリンカーを用いることにより，CD4 のペプチド二本鎖上の 2 つの部位を含む広範囲な領域をミミックしている．われわれは Phe-43 cavity 周辺の構造的及び静電的要求を明らかにする目的で，NBD-556 の芳香環パラ位に関する構造活性相関研究を行った.[26] これにより，芳香環パラ位にある程度嵩高くかつそれほど電子供与性の強くない官能基を有する誘導体（YYA-021 等）が顕著な抗 HIV 活性及び gp120 の構造変化能を有することを明らかにした（Fig. 3）．また，この構造変化により CCR5/CXCR4 などのコレセプター結合領域や CD4-induced site を認識する中和抗体の結合能が上昇することも併せて明らかにしている.[27] さらに，これら CD4 ミミックは前章で述べた CXCR4 アンタゴニスト T140 と併用すると，顕著な相乗効果を示し,[26] CD4 ミミック-T140 誘導体のハイブリッド化合物の創製にも成功している.[28] このように CD ミミックは中和抗体やコレセプター阻害剤との併用により，さらに有用性が上がると思われる．

一般にタンパク質-タンパク質間の相互作用では，相互作用領域が連続した一次構造（アミノ酸配列）で存在するのではなく，高次構造の中で不連続に存在することが少なくない．この場合，相互作用領域に係わる官能基を適当なリンカーで架橋した低分子化合物により，分子量が数百倍という元の親タンパク質の機能を模倣することも可能である．この HIV 侵入阻害剤 CD4 ミミックはその一例であろう．

4. インテグラーゼ阻害剤

これまで臨床で使用された抗エイズ薬や上述のわれわれが創製した抗 HIV 剤はすべて標的分子設定型のリバースケミカルジェネティクス的手法により創出されたものである．例えば，逆転写酵素阻害剤やプロテアーゼ阻害剤を開発する場合は，それぞれの酵素（標的分子）に結合するように分子設計されている．CXCR4 アンタゴニストや CD4 ミミックを創製した際は，CXCR4 や gp120（標的分子）に結合するようにデザインした．次に，創薬候補品等有用なリード化合物を創出するケミカルバイオロジー的手法として，このようなリバースケミカルジェネティクス的手法とは方向性が正反対になるフォワードケミカルジェネティクス的手法に着目した．すなわち，ランダムライブラリーから抗 HIV 活性を指標にスクリーニングするというフォワードケミカルジェネティクス的手法を用い，有用な抗 HIV 剤のリード化合物を見い出そうとした．

まず，HIV 構成タンパク質の中に HIV 自身の複製を阻害するものが存在するだろうという仮説の下に，上述のランダムライブラリーのソースを HIV-1 の遺伝子産物であるタンパク質（Gag・Pol・Env・Vpu・Vpr・Rev・Tat）由来のアミノ酸配列を基にしたオーバーラッピングペプチドライブラリー（アミノ酸 10–17 残基）とした．このライブラリーを用いて，簡便に評価できるインテグラーゼ（IN）阻害活性（細胞内で阻害活性を評価する系ではない）のスクリーニングを行った結果，アクセサリータンパク質である Vpr 由来の 3 個の部分ペプチドに抗 IN 活性が見い出された．これらの部分ペプチドには共通して LQQLLF 配列が含まれていた（Fig. 4）.[29] LQQLLF 配列が阻害活性の発現に重要なモチーフであると考えられ，また，細胞内で活性を発現させるため，LQQLLF モチーフを中心に数種類のペプチドを合成し，細胞膜透過性モチーフで

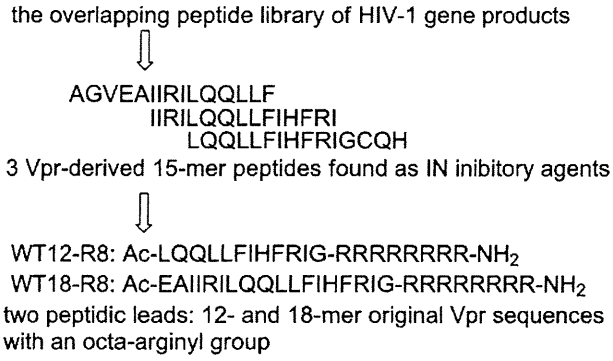

Fig. 4. Development of Vpr-derived IN Inhibitors

あるocta-Arg（RRRRRRRR）をC末端側に付与した．その結果，抗IN活性だけでなく細胞レベルでの抗HIV-1活性ともに強力なペプチドWT18-R8（Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2）及びWT12-R8 (Ac-LQQLLFIHFRIG-RRRRRRRR-NH_2）を同定した（Fig. 4）．LQQLLFモチーフはこのVpr中のαヘリックス構造部分に存在していることから，αヘリックス構造が阻害活性の発現に重要であると考えられる．[30]感染の前期過程において，VprもINも酵素や鋳型RNA及び様々な細胞内宿主因子を含むプレインテグレーション複合体（Pre-Integration Complex, PIC）と呼ばれる複合体の中に存在し，VprはINと相互作用することでHIV自身のオートインテグレーションを制御している（BAFのような作用）かもしれない．いずれにしても，HIV構成タンパク質の中にHIV自身の複製を阻害するものが存在する可能性がある．また，PIC中でVprは溶液中とは異なるコンフォメーションをとり，LQQLLFモチーフを含むαヘリックス構造部分が外側に提示され，INと相互作用して，INの作用をマスクしているのかもしれない．これは，大きなタンパク質の中に秘められた神秘的な活性が部分ペプチドに存在する，すなわち，親タンパク質の作用とは異なる作用が部分ペプチドに存在する，といういわゆるクリプタイド（＝cryptic peptide）の概念にも関係すると思われる．[31]このIN阻害ペプチドはさらに構造を最適化することにより，Raltegravir（Merck Sharp & Dohme Corp.）[32]とは違うアロステリック機構を持つ新たな抗IN剤の創出として期待できる．

現在同様に，ランダムライブラリーから抗HIV活性を指標にスクリーニングするというフォワードケミカルジェネティクス的手法を用い，HIV-1 matrix protein（MA）由来の部分ペプチドが抗HIV活性を示すことを見い出しており，本法を用いることにより，有用な抗HIV剤のリード化合物を見い出すことができる可能性がある．

5. 膜融合機構をターゲットとしたワクチンと膜融合阻害剤

これまで臨床で使用できる多くの抗HIV薬が開発され，多剤併用療法（HAART）は大きな効果を上げてきた．さらに，広範囲のウイルス株に対して中和活性を持つワクチンを開発することによって，特に最近感染者が増加しているアフリカ，アジア等の発展途上国でAIDS及びHIV感染症で苦しむ患者のQOLの改善や治療における選択肢の拡大に貢献できると考えられる．HIVワクチンの開発においてはこれまでの通常の感染症に対して有効である弱毒化ワクチン，生ワクチンといった方法はHIVの易変異性ゆえに危険視されたこともあり使うことができない．さらに，通常抗体誘導を行う際には，ウイルス粒子表面のタンパク質における部分配列を合成しその配列特異的な抗体を誘導しているが，誘導された抗体はそのアミノ酸配列に特異的に結合するものの，中和標的の立体構造に対しての特異性や結合活性は概して低い．変異の激しいHIVを標的とする場合，アミノ酸の一次配列だけではなく，立体構造を認識して結合する中和抗体を誘導することが望ましいと考えられ，これまでとは違ったワクチン開発が必要となっている．そこで，立体構造を保持した状態の抗原分子を用いることによって，立体構造に対しても特異的な抗体を誘導することができれば，HIVに対してもより高い中和活性を有する抗体が誘導できるのではないかと考えた．

HIVは先に述べたように，gp120-CD4-コレセプター（非共有結合複合体）の形成後，ホモ三量体のgp41が宿主細胞膜にアンカリングし，HR1領域とHR2領域の会合により6ヘリックスバンドル（六量体）を形成して宿主細胞膜と膜融合する（Fig. 1）．これまでにこの膜融合過程を標的とした創薬研究が広く行われ，Enfurvirtide（fuzeon / T20）（Roche/Trimeris）が膜融合阻害剤としてFDAから認可されている．[33] EnfurvirtideはHR2（CHR, C-region）領域の部分ペプチドであり，HR2ミメティックとしてHR1領域に相互作用することで，

6ヘリックスバンドル形成を阻害する．また，HR1及びHR2ヘリックス領域はHIV株間で高く保存されていることから，gp41のアミノ酸配列を認識する中和抗体（2F5及び4E10）が見い出されている．[34] さらに，近年ではgp41に対する特異性向上を目的として，その立体構造を模倣したgp41ミメティックの創製研究が行われ，HR1又はHR2ヘリックス領域を3価型テンプレートで束ねたgp41ミメティックが複数合成され，阻害剤や人工抗原分子として応用されている．[35-37] しかし，これらgp41ミメティックは非等価なテンプレートの構造上，ヘリックス領域の位相にずれが生じ，天然の三量体構造を再構築しているとは言いがたい．われわれは天然型のgp41が有する等価な三量体構造を模倣するために，ヘリックス領域を等価に配置可能なC3対称性テンプレートを新たにデザインし，3本のHR1（NHR, N-region）領域由来ペプチド（N36）をN端側から集積させたgp41ミメティック（N36三量体）を創製した（Fig. 5）．[38] 合成したN36三量体のCDスペクトル測定により，三量体は単量体に比べ高いαヘリックス性を有していることが明らかになり，C3対称性テンプレート上でのN36三量体構造の形成が示唆された．また，HR2（CHR, C-region）領域由来ペプチド（C34）と混合させるとαヘリックス性が上昇したことから，このgp41ミメティックは天然の構造を高く反映していると考えられる．合成したN36三量体をマウスに免疫した結果，得られた血清はN36単量体よりもN36三量体に対する抗体価が約30倍高く，三量体構造を特異的に認識する抗体が誘導されていることが示唆された．さらに，N36三量体の免疫によって得られた血清は，単量体の免疫に比べ，強い中和活性がみられたことから，三量体構造を特異的に認識する抗体の有用性が示唆された．われわれが合成したN36三量体ミミックはC3対称性テンプレートを用いることにより，天然の三量体構造を再構築することができ，これを抗原分子として使用することにより天然の高次構造を特異的に認識する抗体が誘導されたと考えられる．なお，このN36三量体ミミックは，阻害剤としてもN36の量論比に相当する抗HIV活性を有していた．また，HR1領域ペプチドの対になっているHR2領域ペプチド（C34）の三量体ミミックに関しても，別のC3対称性テンプレートを合成し，3本のC34をC端側から集積させたgp41ミメティック（C34三量体）を創製した．同様に，C34三量体ミミックは単量体よりも三量体

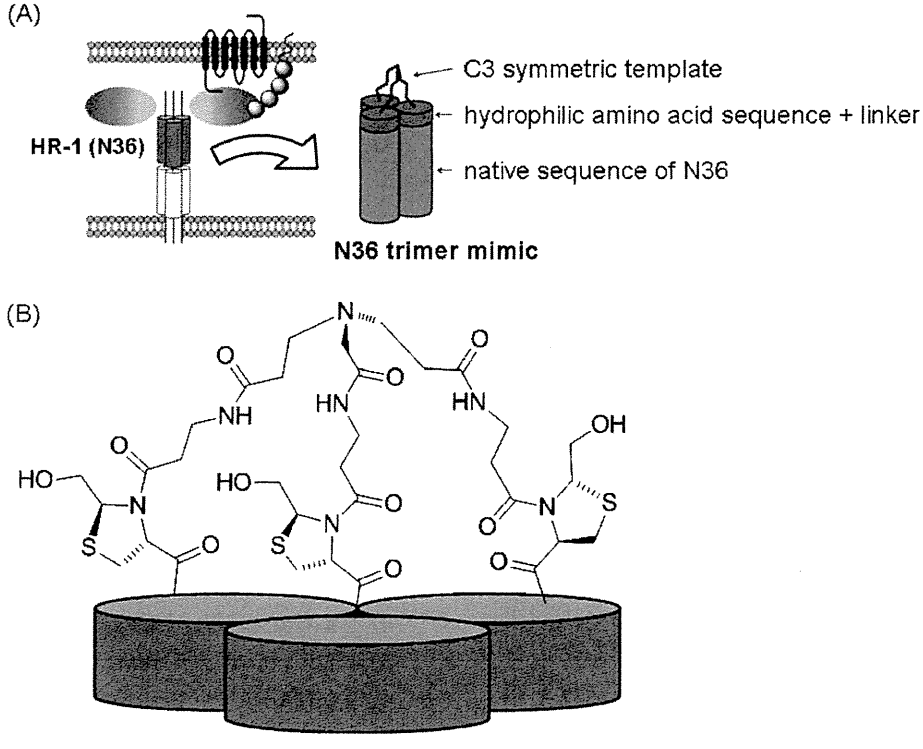

Fig. 5. (A) Development of an N36 Trimer Mimic Using a C3-Symmetric Template, (B) Structure of the C3-Symmetric Template

構造を特異的に認識する抗体の誘導，及び，中和抗体の誘導において優れていることが示唆された．なお，この C34 三量体ミミックは，阻害剤としては単量体よりも 100 倍強い抗 HIV 活性を有しており，現在，この理由を解明中である．

第 3 章の CD4 ミミックは，タンパク質-タンパク質間（gp120-CD4 間）の相互作用の領域に係わる官能基を適当なリンカーで架橋した低分子化合物であり，元の親タンパク質の機能を模倣したものである．本章の gp41 ミメティックは，タンパク質-タンパク質間の相互作用に係わる部分ペプチドを適当なテンプレート上に配置することで，タンパク質の高次構造そのものを再構築したものである．このケミカルバイオロジー的手法は規則的な二次構造の組み合わせで構成されるタンパク質を模倣する場合に有効である．

これらの gp41 三量体ミメティックは今後実験動物レベルを上げていくことにより，立体構造を保持した抗原分子の有効性を示すことができると考えられる．ワクチンに関しては，gp41 以外にも宿主細胞側のタンパク質 CXCR4 の細胞外領域を標的とした人工抗原分子を創製し，免疫により抗体誘導を確認している．宿主側のタンパク質を抗原分子とすることは，常識的ではないが，広範囲のウイルス株に対して有効性が期待でき，耐性出現の危険性も少ないという利点もある．

6. おわりに

毎年種々の抗 HIV 薬が開発され，薬を飲み続ければ AIDS は死なない病のようになってきた．しかし，一生投薬を続ける必要があり，いずれの抗 HIV 薬も根治に至るものではない．そのため，臨床で使用できる薬剤のレパートリーを増やすことが求められている．われわれは以前から HIV 感染のコレセプターである CXCR4 の阻害剤を中心に，標的分子設定型のリバースケミカルジェネティクス的手法により抗 HIV 剤を創製してきた．最近さらに抗 HIV 剤のターゲットを増やし，HIV 侵入の動的超分子機構をターゲットとした CD4 ミミックを創製した．この CD4 ミミックは CXCR4 阻害剤や中和抗体等との併用において，相乗効果を示した．さらに，ランダムライブラリーから抗 HIV 活性を指標にスクリーニングするというフォワードケミカルジェネティクス的手法を用い，リード化合物を見い出した．すなわち，インテグラーゼ阻害活性を有する Vpr の部分ペプチドを見い出した．このようにケミカルバイオロジー的方法も取り入れ，いろいろな観点からリード化合物を探索し，種々の抗 HIV 剤の創製を行っている．また，最近再度注目されてきたエイズワクチンに関しても，HIV 侵入の動的超分子機構をターゲットとしてテンプレート等ケミカルバイオロジーの概念を用い，人工設計型抗原分子を創製している．阻害剤及びワクチンの両方に力を入れており，カクテル療法を視野に入れた抗 AIDS 薬の創製を考えている．

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CD4 mimics as HIV entry inhibitors: Lead optimization studies of the aromatic substituents

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ABSTRACT

Several CD4 mimics have been reported as HIV-1 entry inhibitors that can intervene in the interaction between a viral envelope glycoprotein gp120 and a cell surface protein CD4. Our previous SAR studies led to a finding of a highly potent analogue **3** with bulky hydrophobic groups on a piperidine moiety. In the present study, the aromatic ring of **3** was modified systematically in an attempt to improve its antiviral activity and CD4 mimicry which induces the conformational changes in gp120 that can render the envelope more sensitive to neutralizing antibodies. Biological assays of the synthetic compounds revealed that the introduction of a fluorine group as a *meta*-substituent of the aromatic ring caused an increase of anti-HIV activity and an enhancement of a CD4 mimicry, and led to a novel compound **13a** that showed twice as potent anti-HIV activity compared to **3** and a substantial increase in a CD4 mimicry even at lower concentrations.

1. Introduction

The first step of HIV entry into host cells is the interaction of a viral envelope glycoprotein gp120 with the cell surface protein CD4.[1] Such a viral attachment process is an attractive target for the development of the drugs to prevent the HIV-1 infection of its target cells.[2] Several small molecules including BMS-806,[3] IC-9564[4] and NBDs[5] have been identified that inhibit the viral attachment process by binding to gp120. Recently, we and others have been exploring the potentials of NBDs-derived CD4 mimics as a novel class of HIV entry inhibitors (Fig. 1).[6–8]

Small molecular CD4 mimics identified by an HIV syncytium formation assay showed potent cell fusion and virus cell fusion inhibitory activity against several HIV-1 laboratory and primary isolates.[5] Furthermore, the interaction of CD4 mimics with a highly conserved and functionally important pocket on gp120, known as the 'Phe43 cavity', induces conformational changes in gp120,[9] a process which occurs with unfavorable binding entropy, leading to a favorable enthalpy change similar to those caused by binding of the soluble CD4 binding to gp120. These unique properties render CD4 mimics valuable not only for the development of entry inhibitors, but which also, when combined with neutralizing antibodies function as envelope protein openers-putatively, stimulants.[10]

The structure of the complex formed by NBD-556 (**1**) bound to the gp120 core from an HIV-1 clade C strain (C1086) was recently determined by X-ray analysis (PDB: 3TGS).[11] As expected with molecular modeling by us[8a] and others,[6a] NBD-556 binds with Phe43 cavity with its *p*-chlorophenyl ring inserted into the cavity, and in addition multiple contacts were observed, with Trp112, Val255, Phe382, Ile424, Asn425, Trp427, Gly473, and Val430 of gp120 were observed (Fig. 2). However, no obvious interaction with Arg59 of CD4 was observed, although the salt bridge formation between Arg59 of CD4 and Asp368 of gp120 is a critical interaction of the viral attachment.[12] Based on this binding model, several potent compounds were recently identified.[6c,7]

Prior to those studies, we performed structure–activity relationship (SAR) studies based on the modification of the piperidine moiety of CD4 mimics to interact with Val430 and/or Asp368. These resulted in the discovery of a potent compound **3** which has bulky hydrophobic groups on its piperidine ring, and shows significant anti-HIV activity and lower cytotoxicity than other known CD4 mimics.[8c] Our study of the docking of **3** into the Phe43 cavity of gp120 suggests that the cyclohexyl group of **3** can interact hydrophobically with the isopropyl group of Val430.

We hypothesized that the optimization of the aromatic ring of **3** would lead to an increase of antiviral activity and CD4 mimicry, the latter inducing the conformational changes in gp120. Here, we de-

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scribe the systematic modification of the aromatic ring of **3** for further optimization to evaluate substituent effects on anti-HIV activity, cytotoxicity and CD4 mimicry.

2. Results and discussion

The co-crystal structure of **1** with the gp120 core revealed that the aromatic group of **1** binds to gp120 by several aromatic–aromatic and hydrophobic interactions (Fig. 2). In particular, hydrophobic space surrounded by the hydrophobic amino acid residues Trp112, Val255, Phe382, and Ile424 is likely to be affected by substituents at the *meta*- and *para*-positions of the aromatic ring, and consequently we decided to investigate substituents at these positions (Fig. 3).

Initially, we selected a chlorine or a methyl group to serve as the *para*-substituent of the aromatic group because CD4 mimic compounds such as **1** (NBD-556) with a *p*-chloro substituent, and because **3** showed significant anti-HIV activity compared to other substituents. Further, CD4 mimic structures such as **2** with a *p*-methyl substituent also showed potent anti-HIV activity and exhibits lower cytotoxicity than those with the *p*-chlorophenyl derivatives.[8a] Next, we chose several halogens including F, Cl and Br, to be the *meta*-substituent on the aromatic group since previous SAR studies revealed that the introduction of an appropriate group with an electron-withdrawing ability at the *meta*-position leads to an increase of binding affinity and antiviral activity.[6a] Furthermore, to investigate whether electron withdrawal and hydrophobicity of the *meta*-position are appropriate, the CD4 mimics with a *meta*-methyl substituent, which has electron-donating properties and is similar in size to bromine, were also synthesized. Finally, two piperidine scaffolds (the 2,2,6,6-tetramethylpiperidine **A** and the dicyclohexylpiperidine **B**) were combined with these aromatics via the oxalamide linker.

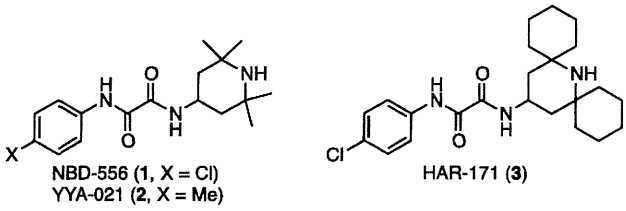


Figure 1. Structures of NBD-556 (**1**), YYA-021 (**2**) and HAR-171 (**3**).

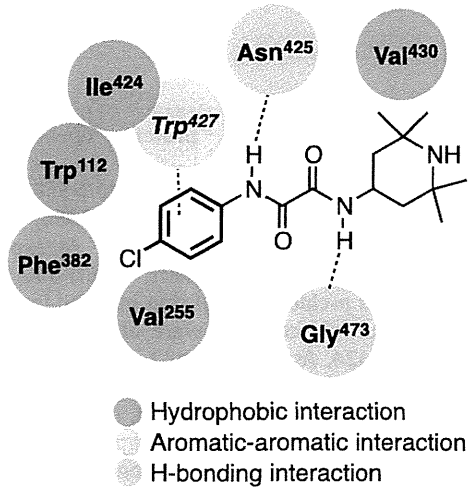


Figure 2. Major interactions between NBD-556 and Phe43 cavity of gp120.

2.1. Chemistry

The syntheses of novel compounds are depicted in Schemes 1 and 2. Starting from the appropriate aniline with *m*- and *p*-substituents, coupling with ethyl chloroglyoxylate in the presence of Et_3N gave the corresponding amidoesters **6a–c** and **7a–c**. Subsequently, microwave-assisted aminolysis[13] of **6a–c** and **7a–c** with commercially available 4-amino-2,2,6,6-tetramethylpiperidines afforded the desired compounds **8a–c** and **9a–c** (Scheme 1). A series of CD4 mimics with two cyclohexyl groups **13a–c** and **14a–c** were prepared from 2,2,6,6-tetramethylpiperidin-4-one **10** by the method previously reported,[8c] with slight modification (Scheme 2). Briefly, treatment of **10** with cyclohexanone in the presence of ammonium chloride gave a 2,6-substituted piperidin-4-one **11** via Grob fragmentation followed by intramolecular cyclization.[14] Reductive amination with *p*-methoxybenzyl amine, acidic treatment with TMSBr/TFA, and oxidative cleavage of *p*-methoxybenzyl group with cerium(IV) ammonium nitrates (CAN) furnished the corresponding 4-aminopiperidines (**12**) with higher yields and less burdensome purifications than the previous method. Finally, coupling of **12** with the corresponding esters **6a–c** and **7a–c** under microwave irradiation provided the desired compounds **13a–c** and **14a–c**.

2.2. Biological evaluation

The anti-HIV activity of the synthetic compounds was evaluated against an R5 primary isolate YTA strain. IC_{50} values were determined by the WST-8 method as the concentrations of the compounds that conferred 50% protection against HIV-1-induced cytopathogenicity in PM1/CCR5 cells. Cytotoxicity of the compounds based on the viability of mock-infected PM1/CCR5 cells was also evaluated using the WST-8 method. The assay results for compounds **8a–c** and **13a–c** with a *p*-chlorophenyl group are shown in Table 1. The parent compound **1** and compound **8a**,[6a] known as JRC-II-191, showed significant anti-HIV activities (IC_{50}

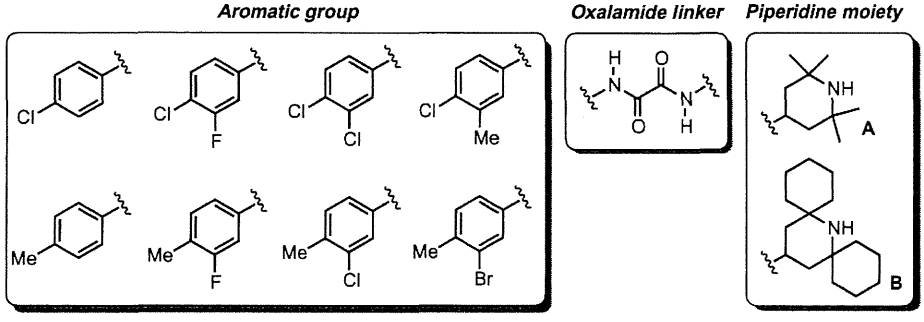
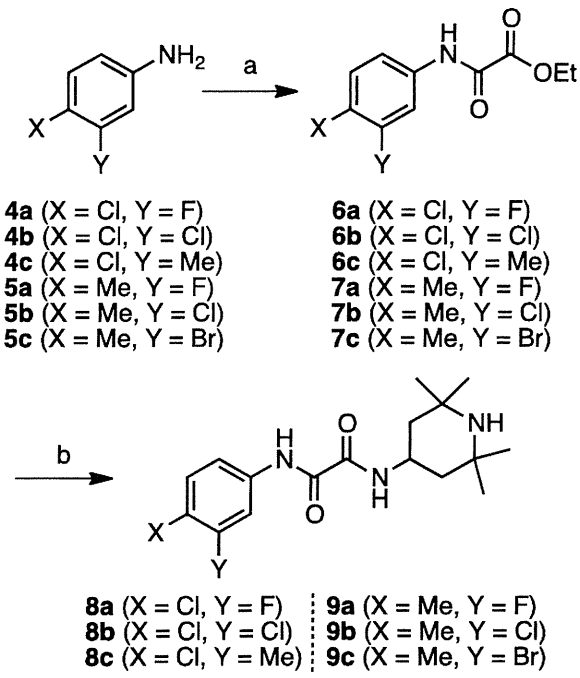
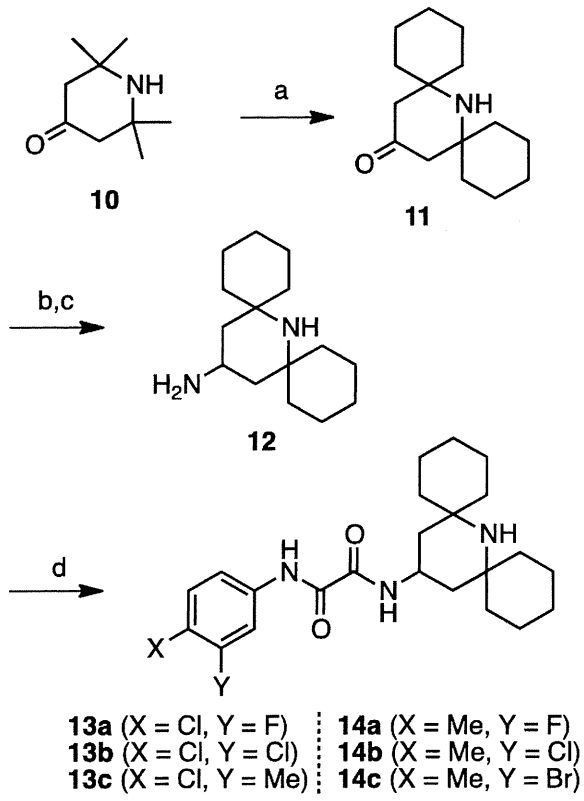


Figure 3. The structures of scaffolds in the design of novel CD4 mimics.



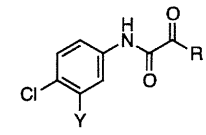
Scheme 1. Reagents and conditions: (a) ethyl chloroglyoxylate, Et_3N, THF; (b) 4-amino-2,2,6,6-tetramethylpiperidine, Et_3N, EtOH, 150 °C, microwave.

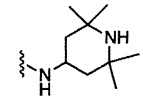
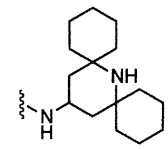


Scheme 2. Reagents and conditions: (a) cyclohexanone, NH_4Cl, DMSO, 60 °C; (b) *p*-methoxybenzylamine, $NaBH_3CN$, MeOH, then 1 M TMSBr in TFA; (c) CAN, CH_3CN/H_2O (v:v = 2:1); (d) **6** or **7**, Et_3N, EtOH, 150 °C, microwave.

of **1** = 0.61 μM and IC_{50} of **8a** = 0.32 μM). Compound **8b**[6a] having a *m,p*-dichlorophenyl group and compound **8c**[6a] (JRC-II-193) having a *p*-chloro-*m*-tolyl group showed moderate anti-HIV activity (IC_{50} of **8b** = 4.1 μM and IC_{50} of **8c** = 3.3 μM) but their potency was

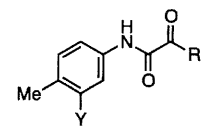
Table 1
Anti-HIV activity and cytotoxicity of compounds **8a–c** and **13a–c** containing a *p*-chlorophenyl group[a]

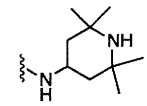
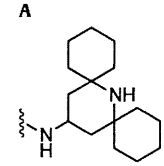


Compd	R	Y	IC_{50}[b] (μM) YTA48P	CC_{50}[c] (μM)
1		H	0.61	110
8a	**A**	F	0.32	94
8b	**A**	Cl	4.1	36
8c	**A**	Me	3.3	38
3		H	0.43	120
13a	**B**	F	0.23	11
13b	**B**	Cl	0.62	11
13c	**B**	Me	2.6	15

[a] All data are the mean values from three of more independent experiments.
[b] IC_{50} values of the multi-round assay are based on the inhibition of HIV-1-induced cytopathogenicity in PM1/CCR5 cells.
[c] CC_{50} values are based on the reduction of the viability of mock-infected PM1/CCR5 cells.

Table 2
Anti-HIV activity and cytotoxicity of compounds **9a–c** and **14a–c** containing a *p*-tolyl group[a]



Compd	R	Y	IC_{50}[b] (μM) YTA48P	CC_{50}[c] (μM)
2		H	9.0	260
9a	**A**	F	2.8	110
9b	**A**	Cl	3.2	62
9c	**A**	Br	>10	32
14a		F	0.54	91
14b	**B**	Cl	6.2	11
14c	**B**	Br	3.2	11

[a] All data are the mean values from three of more independent experiments.
[b] IC_{50} values of the multi-round assay are based on the inhibition of HIV-1-induced cytopathogenicity in PM1/CCR5 cells.
[c] CC_{50} values are based on the reduction of the viability of mock-infected PM1/CCR5 cells.

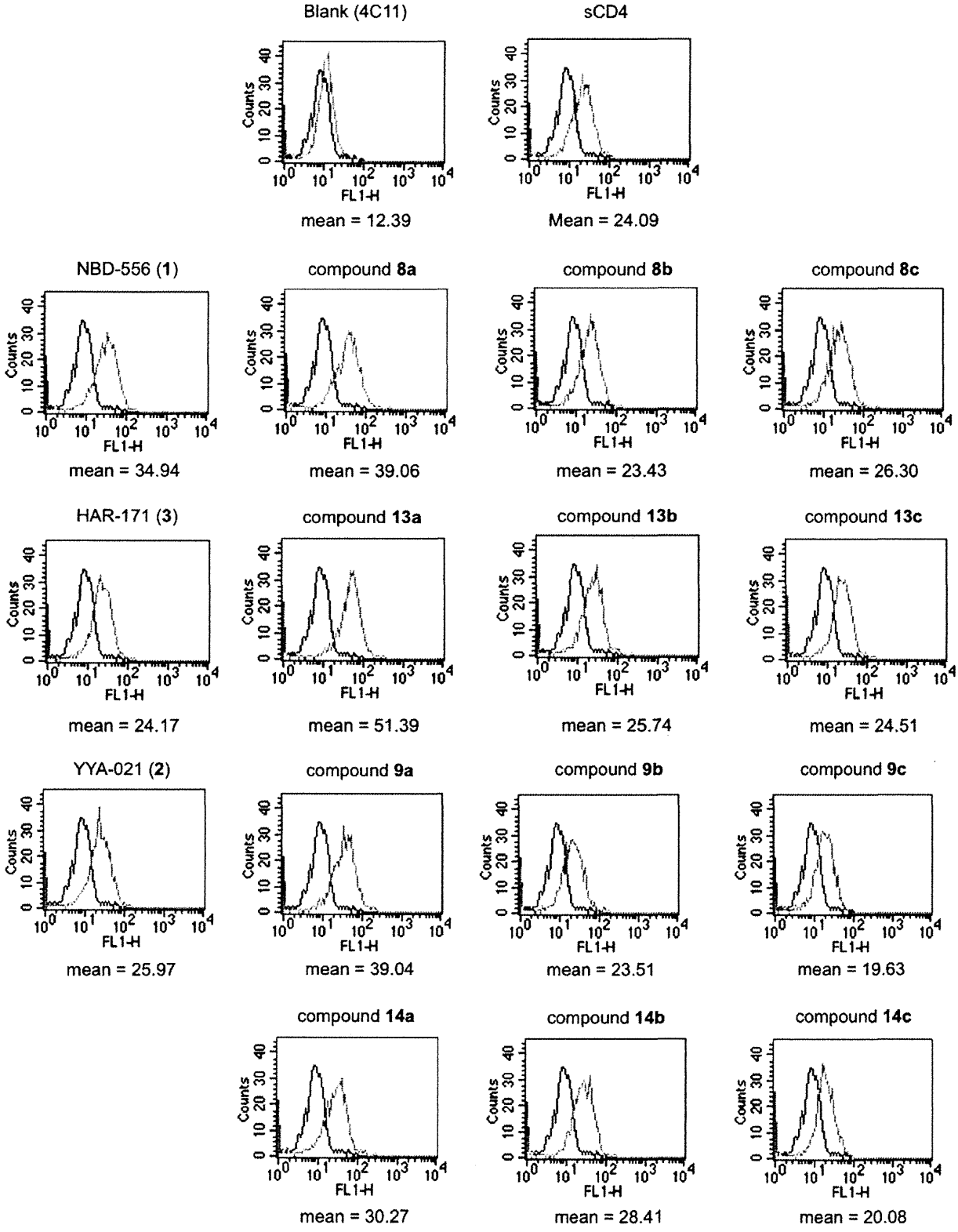


Figure 4. FACS analysis of synthetic compounds **8**, **9**, **13** and **14**.

approximately 10-fold lower than that of compound **8a**. The cytotoxicity of **8b** and **8c** is relatively stronger than that of **8a** (CC_{50} of **8b** = 36 μM and CC_{50} of **8c** = 38 μM). Compounds **13a–c** with hydrophobic cyclohexyl groups in the piperidine moiety showed more potent anti-HIV activity than the corresponding compounds **8a–c**, confirming the contribution of the bulky hydrophobic group(s) to an increase of antiviral activity. Our lead compound **3** showed significant anti-HIV activity comparable to that of compound **8a** (IC_{50} = 0.43 μM) but, consistent with previous results, exhibited lower cytotoxicity. In particular, compound **13a** with a *m*-fluoro-*p*-chlorophenyl group exhibited the highest anti-HIV activity. The IC_{50} value of **13a** was 0.23 μM, whose potency was

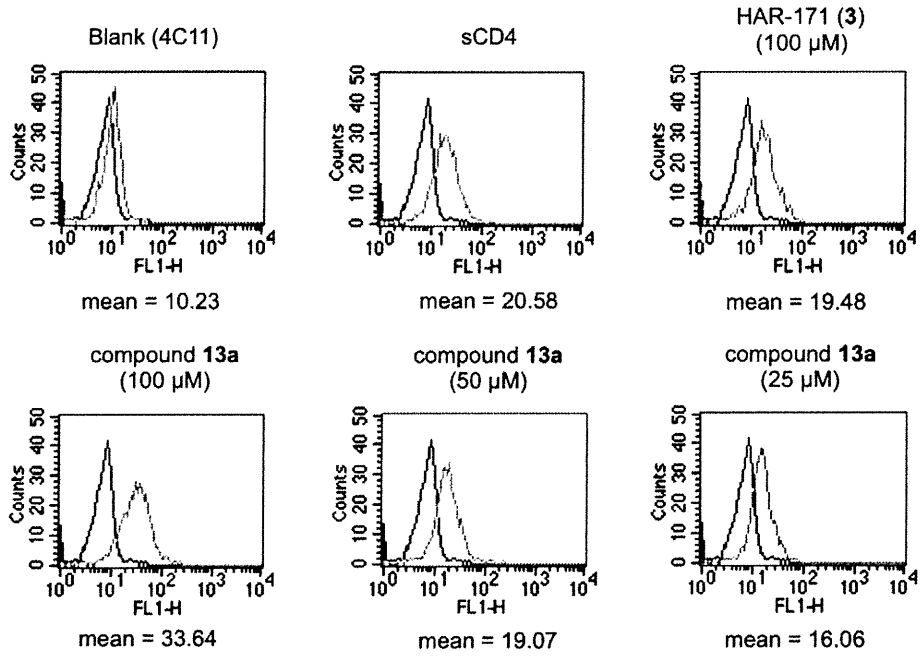


Figure 5. FACS analysis of **3** and **13a** in different concentrations.

approximately twice as high as that of compound **3**. Notably, compound **13b** with a *m,p*-dichlorophenyl group showed 7-fold more potent anti-HIV activity than the corresponding compound **8b**. Compound **13c**, which has a *p*-chloro-*m*-tolyl group, showed potent anti-HIV activity comparable to that of the corresponding compound **8c** and an increase of cytotoxicity (CC_{50} = 15 μM). We observed a tendency for compounds **13a–c** with both hydrophobic cyclohexyl groups and a *m,p*-disubstituted phenyl group to exhibit higher cytotoxicity than the corresponding tetramethyl-type compounds **8a–c**. No clear reason for an increase of cytotoxicity in the *m,p*-disubstituted phenyl group-containing compounds is apparent.

Assay results for the compounds **9a–c** and **14a–c** with a *p*-tolyl group are shown in Table 2. As expected, replacement of the *p*-chloro substituent with a *p*-methyl group resulted in somewhat reduction of anti-HIV activity. Compound **2**, YYA-021 has significant anti-HIV activity (IC_{50} = 9.0 μM) and exhibits the lowest cytotoxicity among all of the compounds tested (CC_{50} = 260 μM). These results are consistent with our previous SAR studies involving the aromatic ring. Introduction of a fluorine at the *meta*-position of the *p*-tolyl group, e.g. in compound **9a** and **14a**, improved the antiviral activity, as observed with **8a** and **13a** and a similar tendency was observed for compound **9b** with a *m*-chloro-*p*-tolyl group. In particular, compound **14a** with cyclohexyl groups and a *m*-fluoro-*p*-tolyl group showed slightly higher anti-HIV activity than the parent compound **1**. Among the compounds with *m*-bromo-*p*-tolyl groups, it was found that compound **9c**, with a 2,2,6,6-tetramethylpiperidine group, showed no anti-HIV activity at a concentration below 10 μM, whereas compound **14c** with hydrophobic cyclohexyl groups attached to the piperidine moiety, showed moderate activity (IC_{50} = 3.2 μM), indicating that the hydrophobic modification of piperidine ring can contribute to an increase in anti-HIV activity.

All the synthetic compounds were evaluated for their CD4 mimicry on the conformational changes in gp120 by fluorescence activated cell sorting (FACS) analysis, and the results are shown in Figure 4. The profile of binding of a CD4-induced (CD4i) monoclonal antibody (4C11) to the Env-expressing cell surface pretreated with the synthetic compounds was assessed in terms of the mean fluorescence intensity (MFI). The increase in binding affinity for 4C11 (by the pretreatment with synthetic compounds) suggests that those compounds can reflect the CD4 mimicry as a consequence of the conformational changes in gp120. Our previous studies disclosed that the profiles of the binding to the cell surface pretreated with **1**, **2**, or **3** were similar to those observed in pretreatment with soluble CD4, indicating that these compounds offer a significant enhancement of binding affinity for 4C11.[8] As shown in Figure 4, similar results were obtained with those compounds in this FACS analysis (MFI of **1**, **2**, and **3** = 34.94, 25.97, and 24.17, respectively). A notable increase in binding affinity for 4C11 was observed in essentially all the synthetic compounds. The compounds **8a**, **9a**, **13a** and **14a** with a *meta*-fluorine in the aromatic ring, showed significant anti-HIV activity, and produced a substantial increase in binding affinity for 4C11. These results suggested that the introduction of a fluorine group at the meta position of the aromatic ring is significant not only for the increase of anti-HIV activity, but also for the enhancement of a CD4 mimicry. In particular, a remarkable improvement in binding affinity for 4C11 was observed with **13a** (MFI = 51.39) which has twofold more potent anti-HIV activity than the lead compound **3** (HAR-171), and is the most active compound in terms of both anti-HIV activity and the CD4 mimicry resulting from the conformational change in gp120. The profiles of pretreatment of the cell surface with compounds **8b** and **13b** having a *m,p*-dichlorophenyl group, compounds **8c** and **13c** having a *p*-chloro-*m*-tolyl group, and compounds **9b** and **14b** with a *m*-chloro-*p*-tolyl group were similar to results obtained for **3**, suggesting that these compounds produced slightly lower enhancement compared to those of compounds **8a**, **9a**, **13a** and **14a** but significant levels of binding affinity for 4C11. On the other hand, pretreatment with compounds **9c**, which failed to show significant anti-HIV activity and **14c**, which had moderate anti-HIV activity resulted in a slight decrease of binding affinity for 4C11, suggesting that the introduction of a Br group at the *meta*-position of *p*-tolyl group is not advantageous to a CD4 mimicry, possibly due to the steric hindrance caused by the two bulky substituents. These results are consistent with previous observations that a limited size and electron-withdrawing ability of the aromatic substituents are required for potent anti-HIV activity and CD4 mimicry.[8a]

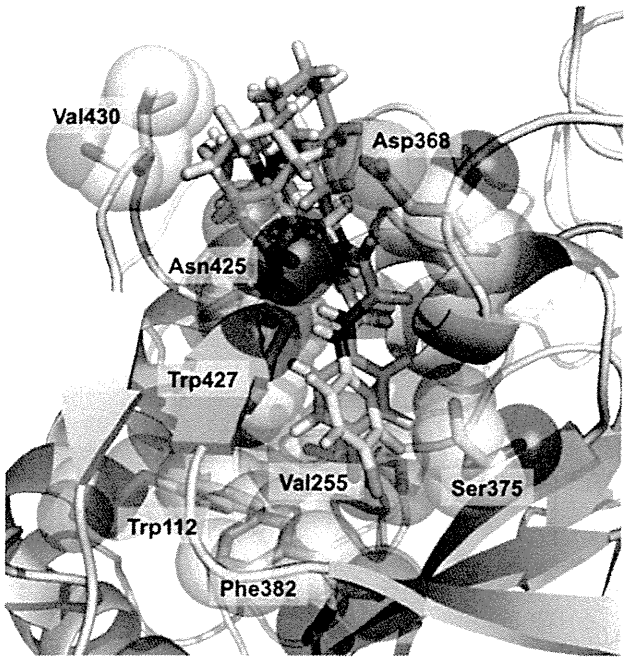


Figure 6. The modeled structure of **13a** (yellow carbon atoms) in the complex with the Phe43 cavity in gp120 (3TGS) overlaid with the modeled structure of **3** (green carbon atoms).

Since **13a** showed higher CD4 mimicry than the other compounds tested, the effect of the solution concentration of **13a** on the binding affinity for 4C11 was investigated. As shown in Figure 5, pretreatment of the cell surface with a 100 μM solution of **13a** produced a higher increase in the binding affinity for 4C11 than pretreatment with the same concentration of compound **3**. Interestingly, the profile pretreated with a 50 μM solution of **13a** was similar to that with a 100 μM of compound **3**, and even with a 25 μM solution of **13a** a potent enhancement of the binding affinity for 4C11 was observed: MFI of **13a** at concentrations of 50 μM and 25 μM = 19.07 and 16.06, respectively. This observation suggests that **13a** could serve as a novel lead compound for the development of envelope protein openers for the use combined with neutralizing antibodies because of its effectiveness at low concentrations.

The substantial increase in the CD4 mimicry of **13a** even at a low concentration is not easily explained because HAR-171 (**3**) and **13a** would be expected to form the similar binding modes with gp120. A probable contribution of **13a** is suggested by modeling studies docked into the Phe43 cavity in gp120 (3TGS) in which the depth and direction of the aromatic ring of **13a** is slightly different from those in compound **3** (Fig. 6), leading to the possible formation of appropriate interactions with the hydrophobic amino acid residues such as Val255 and Phe382, and therefore explaining the increased potency observed in the anti-HIV activity and CD4 mimicry of **13a**.

3. Conclusion

CD4 mimics are attractive agents not only for the development of a novel class of HIV entry inhibitors but also as possible cooperating agents for the neutralizing antibodies—that is, envelope protein openers. In the present study, a structure–activity relationship study of a series of CD4 mimic compounds was performed with a view to improving the biological activity of HAR-171 (**3**), which was identified in our previous studies as a promising lead compound with anti-HIV activity, cytotoxicity and CD4 mimicry resulting from the conformational change in gp120. Systematic modification of the *meta*- and *para*-substituents of the aromatic ring of **3** led to some potent compounds. In particular, **13a**, which has a bulky hydrophobic group on its piperidine ring and a *m*-fluoro-*p*-chlorophenyl group, demonstrated twofold more potent anti-HIV activity and much higher CD4 mimicry than **2** following the conformational changes in gp120, although the cytotoxicity of **13a** is relatively high. Further structural modification studies of the aromatic ring and the oxalamide linker to improve pharmaceutical profiles will be the subject of future reports.

4. Experimentals

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III spectrometer. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as internal standard. Low- and high-resolution mass spectra were recorded on a Bruker Daltonics microTOF focus in the positive and negative detection mode. For flash chromatography, silica gel 60 N (Kanto Chemical Co., Inc.) was employed. Microwave reactions were performed in Biotage Microwave Reaction Kit (sealed vials) in an Initiator™ (Biotage). The wattage was automatically adjusted to maintain the desired temperature for the desired period of time.

4.1. Chemistry

4.1.1. Ethyl 2-((4-chloro-3-fluorophenyl)amino)-2-oxoacetate (6a)

To a stirred solution of 3-fluoroaniline (1.11 g, 10.0 mmol) in $CHCl_3$ (30.0 mL) was added dropwise *N*-chlorosuccinimide (NCS) in $CHCl_3$ (20.0 mL) at 0 °C. The mixture was stirred at 0 °C for 42 h. After the reaction mixture was concentrated under reduced pressure, the residue was dissolved in Et_2O. The mixture was washed with water, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc/*n*-hexane gave 4-chloro-3-fluoroaniline (259.4 g, 18% yield) as crystalline solids. To a stirred solution of the above aniline (259.4 mg, 1.78 mmol) in THF (8.9 mL) were added at 0 °C ethyl chloroglyoxylate (237.3 μL, 2.14 mmol) and Et_3N (296.6 μL, 2.14 mmol). The mixture was stirred at room temperature for 12 h. After the precipitate was filtrated off, the filtrate solution was concentrated under reduced pressure. The residue was dissolved in EtOAc, and washed with 1.0 M HCl, saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Concentration under reduced pressure to provide the title compound **6a** (435.2 mg, 99% yield) as brown crystals, which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (t, J = 7.50 Hz, 3H), 4.43 (q, J = 7.50 Hz, 2H), 7.24–7.25 (m, 1H), 7.35–7.40 (m, 1H), 7.70–7.75 (m, 1H), 8.93 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.0, 64.1, 108.5 (d, J = 26.3 Hz), 115.9 (d, J = 3.75 Hz), 117.3 (d, J = 18.8 Hz), 130.9 (d, J = 10.0 Hz), 135.9, 153.9, 158.1 (d, J = 246.3 Hz), 160.5; HRMS (ESI), *m*/*z* calcd for $C_{10}H_{10}ClFNO_3$ (MH^-) 244.0182, found 244.0183.

4.1.2. Ethyl 2-((3,4-dichlorophenyl)amino)-2-oxoacetate (6b)

To a stirred solution of 3,4-dichloroaniline **4b** (1.94 g, 12.0 mmol) in THF (20.0 mL) were added ethyl chloroglyoxylate (1.11 mL, 10.0 mmol) and Et_3N (15.2 mL, 11.0 mmol) at 0 °C. The mixture was stirred at room temperature for 6 h. After the precipitate was filtrated off, the filtrate solution was concentrated under reduced pressure. The residue was dissolved in EtOAc, and washed with 1.0 M HCl, saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Concentration under reduced pressure to provide the title compound **6b** (1.58 g, 95% yield) as white powder, which was used without further purification.