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Solid-phase synthesis of 2,3-diphenylpropionic acid library as VLA-4 antagonists

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Abstract—2,3-Diphenylpropionic acid library for VLA-4 antagonist was synthesized on solid-phase. Comparison of the two synthetic routes via an orthogonal generation of two aromatic amino functional groups are discussed. From this work, several compounds were identified as potent VLA-4 antagonists.

There are many well-known, drug-like (druggable) structural templates in medicinal chemistry. The phenylalanine skeleton is one of those important templates. The angiotensin converting enzyme inhibitor alacepril[1] and the insulin promoter nateglinide[2] are the two key examples based on this template (Fig. 1). Another structurally related chemical class is represented by the recently synthesized VLA-4 antagonists.[3]

The integrin, very late antigen-4 (VLA-4), is a hetero dimeric adhesion molecule ($\alpha_4\beta_1$) expressed on the surface of leukocytes, which binds vascular cell adhesion molecule-1 (VCAM-1) on endothelial cell surfaces.[4] Small molecule antagonists for VLA-4 should be useful in the treatment of chronic inflammatory diseases such as asthma,[5] multiple sclerosis,[6] and rheumatoid arthritis.[7] In our research on VLA-4, we have reported the discovery of a potent and orally bioavailable antagonist **1** (Fig. 1), which is based on the 2,3-diphenylpropionic acid template.[8] This template was successfully developed as a bioisostere of phenylalanine derivatives, such as **2**,[9] during the effort to improve the bioavailability and pharmacokinetic properties of the earlier lead compounds. Moreover, the 2,3-diphenylpropionic acid template has the potential to replace other phenylalanine derivatives that require peptide backbone modification. In order to examine the potential ability of the 2,3-diphenylpropionic acid template, we chose to develop an efficient library synthesis of diverse substituted 2,3-diphenylpropionic acids. In this letter, we report a solid-phase synthesis of those derivatives as VLA-4 potential antagonists.

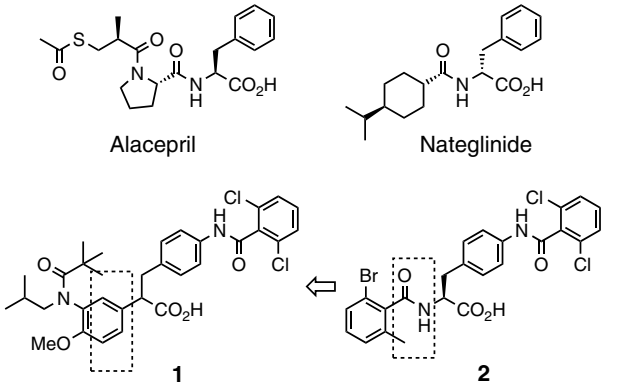

Figure 1. Chemical structure of phenylalanine derivatives as drugs (alacepril and nateglinide), the VLA-4 antagonist having 2,3-diphenylpropionic acid template **1**, and one of the potent VLA-4 antagonist having phenylalanine skeleton **2**.

Keywords: Solid-phase synthesis; 2,3-Diphenylpropionic acid; VLA-4 antagonist.
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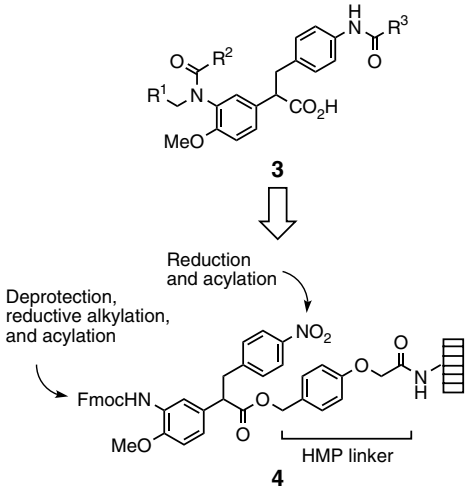
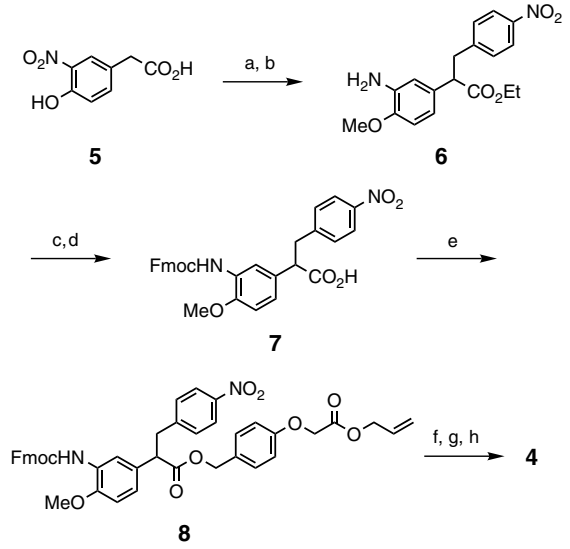
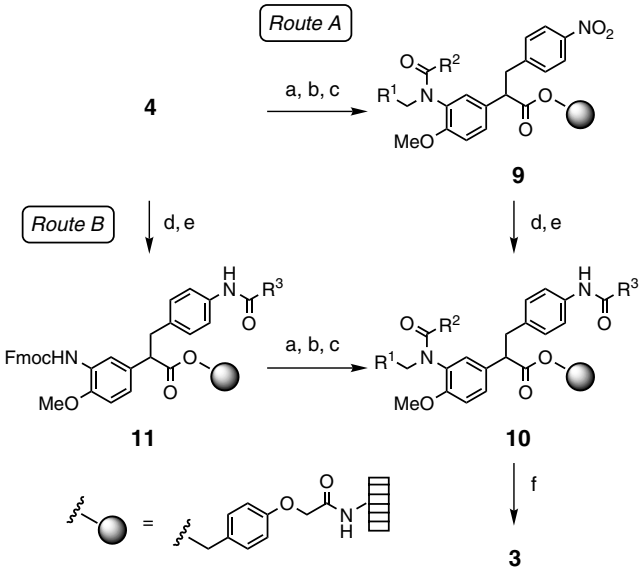


Figure 2. Synthetic strategy of 2,3-diphenylpropionic acid library on solid support.



Scheme 1. Reagents and conditions: (a) EtOH, H_2SO_4, 99%; (b) Ref. 8; (c) NaOH aq, EtOH, 95%; (d) FmocCl, acetone, H_2O, 98%; (e) allyl (4-hydroxymethyl)phenoxyacetate, EDCI, DMAP, CH_2Cl_2, quant.; (f) $Pd(PPh_3)_4$, Et_3SiH, AcOH, CH_2Cl_2, 94%; (g) SynPhase™ Lantern (aminomethylated), DIC, HOBt, DMF, CH_2Cl_2; (h) Ac_2O, DIEA, DMF.



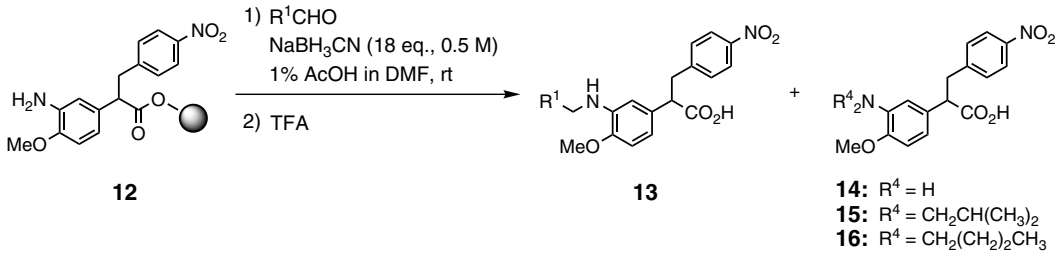
Scheme 2. Reagents and conditions: (a) 20% piperidine/DMF, 1 h; (b) R^1CHO (36 equiv, 1.0 M), $NaBH_3CN$ (18 equiv, 0.5 M), 1% AcOH/DMF, 14 h; (c) R^2COCl (28 equiv, 0.5 M), DIEA (56 equiv, 1.0 M), 20% DMF/CH_2Cl_2, 14 h; (d) $SnCl_2{\cdot}2H_2O$ (70 equiv, 2.0 M), 50% DMF/CH_2Cl_2, 4 h; (e) R^3COCl (17 equiv, 0.5 M), DIEA (34 equiv, 1.0 M), 20% DMF/CH_2Cl_2, 2 h; (f) TFA, 1 h.

The synthetic strategy is shown in Figure 2. We designed the immobilized compound **4** as an important key intermediate to provide the target compounds **3** with three diversity points (R^1, R^2, R^3). 2,3-Diphenylpropionic acid with orthogonally masked amino groups on each benzene ring was coupled onto SynPhase™ Lanterns[10] through 4-hydroxymethyl phenoxyacetic acid (HMP) linker.[11] The amine protected by 9-fluorenylmethoxycarbonyl (Fmoc) and the nitro group can be selectively converted to the required amino groups as required. For the purpose of creating a versatile VLA-4 antagonist library, the amino group at 3′-position on the 2-phenyl group would be converted to the *tert*-amide by reductive alkylation followed by acylation, and the other to the *sec*-amide by acylation. After full functionalization, trifluoroacetic acid (TFA) mediated cleavage would afford the target compound **3**.

The pivotal intermediate **4** was prepared as shown in Scheme 1.[8,12] After the hydrolysis of the ethyl ester **6**, the amino group was protected by Fmoc to give **7**. Carboxylic acid **7** was reacted with an HMP linker segment to yield the corresponding ester **8**. The allyl ester group of **8** was selectively removed by a palladium-catalyzed reaction. The resulting acid was attached onto SynPhase™ Lantern (aminomethylated) by amide bond formation to afford **4**. The loading of **4** was determined as 29.6 μmol/Lantern by quantitative Fmoc determination.

Since the two amino groups of **4** can be generated orthogonally, there are two possible synthetic routes, A and B, to give **3** as outlined in Scheme 2. We assessed both routes. First, the Fmoc group of **4** was removed with piperidine/DMF solution to follow route A. The amino group was reductively alkylated with isobutyraldehyde under various conditions to afford the desired mono-alkylated amine. As shown in Table 1, the target mono-isobutylated compound **13** could be obtained in excellent HPLC purity[13] (entry 3). The di-isobutylated amine **15** was not detected at all. In addition, the reaction with *n*-butyraldehyde also yields mono-butylated amine in good selectivity. This selectivity is probably due to the steric hindrance caused by the methoxy group at 4′-position. This secondary amine was then reacted with 2,2-dimethylpropionic acid in various conditions such as DIC–HOBt–DMAP, TFFH–DIEA, or HATU–DIEA, but, the desirable amide **9** was not obtained. However, treatment of pivaloyl chloride in the presence of DIEA gave amide **9** in good conversion and purity. Then, to make it easy to confirm the

Table 1. Optimization of the reductive alkylation



12 **13**

14: $R^4 = H$
15: $R^4 = CH_2CH(CH_3)_2$
16: $R^4 = CH_2(CH_2)_2CH_3$

Entry	R^1CHO	Concentration (M)	Time (h)	HPLC area (%)[a]	
				13	**14**
1	$(CH_3)_2CHCHO$	0.2	3	72	22
2	$(CH_3)_2CHCHO$	0.5	3	88	5.6
3	$(CH_3)_2CHCHO$	1.0	14	94	0
4	$CH_3CH_2(CH_3)CHCHO$	1.0	14	94	0
5	$(CH_3)_3CCHO$	1.0	14	79	14
6	PhCHO	1.0	14	90	4.2
7	$4\text{-}MeOC_6H_4CHO$	1.0	14	50	43
8[b]	$CH_3(CH_2)_2CHO$	0.5	3	89	0

[a] HPLC peak areas % at 254 nm.
[b] **16** was detected, HPLC peak area = 2.4%.

structure, we synthesized our very standard compound **1** from this amide **9** in the first place. The nitro group of **9** was reduced with tin(II) chloride dihydrate followed by an acylation with 2,6-dichlorobenzoyl chloride to give amide **10**. Cleavage with TFA (100%) afforded the crude compound **1** in 89% HPLC purity, and 64% isolate yield after purification with preparative TLC. The spectral properties of **1** were identical with those of **1** prepared via a conventional liquid phase synthetic method. On the basis of these results, route A was confirmed practical enough for the VLA-4 library.

Route B was examined in the same manner, that is with the same reaction conditions for each step as optimized in route A. We chose **1** as the first compound to evaluate route B. The nitro group of **4** was reduced to the corresponding amine followed by the acylation with 2,6-dichlorobenzoyl chloride to give amide **11**. After the deprotection of Fmoc group, the reductive alkylation and subsequent acylation afforded amide **10**. Treatment of **10** with TFA gave **1** in 89% HPLC purity, and 70% isolate yield. These results indicated that there was no major difference in purity and yield between the routes A and B. By using either route, the lability characteristics of the R groups (i.e., to Fmoc deprotection or nitro reduction conditions) can be accommodated.

We mainly selected the building blocks in consideration of a diversity of R^3, since we were interested in exploring alternatives for 2,6-dichlorobenzoyl group (**L**) or 3,5-dichloroisonicotinoyl group (**K**). Two aldehydes for R^1, four acid chlorides for R^2 and 12 acid chlorides for R^3 were selected as shown in Figure 3. We synthesized 96 compounds via route A, by using a split and pool method with color tagged Lanterns. As a result, the target compounds were obtained in 81% average HPLC purity (Table 2). All compounds were characterized by LC–MS and selected members of the library gave excellent ^1H NMR spectra.[14]

Some of the selected unpurified compounds were assayed for their ability to inhibit the binding of VLA-4 expressing human leukemia cells (HL-60) to human VCAM-1 expressed on Chinese hamster ovary (CHO) cells.[15] As shown in Table 3, most compounds were found to be less potent than **1** (**iaL**). In most cases, the compounds that have the building block **L** retained the inhibition activities. Compound **iaK** has a similar potency to **1**.

In conclusion, we have developed a general solid-phase library synthesis of substituted 2,3-diphenylpropionic acids. The 96-member library was synthesized in good purity, and several library members showed potent activity as VLA-4 antagonist. We also demonstrated that there were two practical orthogonal routes from the key intermediate **4**. This synthetic method will be

Table 2. HPLC purity (peak area % at 254 nm) of the products

R^3COCl	R^1CHO/R^2COCl							
	ia	**ib**	**ic**	**id**	**iia**	**iib**	**iic**	**iid**
A	77	86	74	81	74	87	74	79
B	82	85	83	82	75	81	80	85
C	76	81	77	80	76	82	85	81
D	79	84	79	87	83	85	88	82
E	75	82	74	83	74	83	81	78
F	86	83	80	83	76	84	84	88
G	75	79	72	77	70	80	80	80
H	77	83	80	84	84	85	85	82
I	68	87	69	80	68	82	74	80
J	63	75	61	73	71	75	73	74
K	93	91	90	94	90	92	92	92
L	89	80	90	82	83	84	84	78

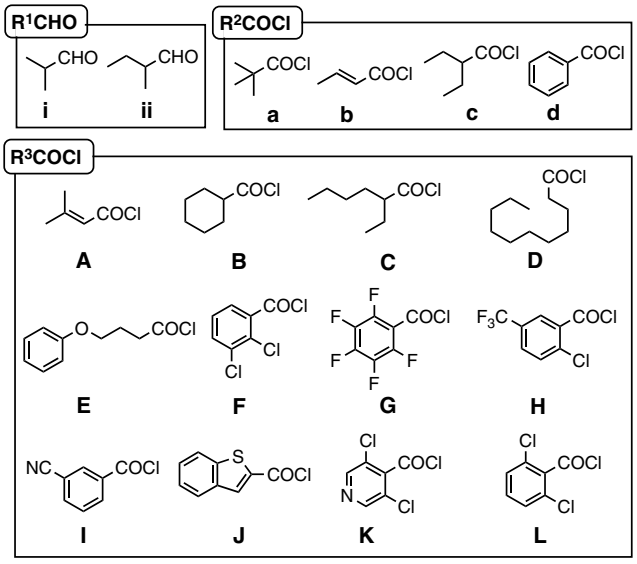


Figure 3. Building blocks.

Table 3. VCAM-1/VLA-4 inhibition activity of the selected compounds

Building blocks	IC_{50} (μM)
iaA	5.9
iaB	>10
iaC	2.0
iaD	>10
iaE	3.1
iaF	0.27
iaG	0.12
iaH	0.15
iaI	>10
iaJ	>10
iaK	0.0017
iaL	0.002
ibL	3.0
icL	0.0005
idL	0.27
iiaL	0.005
iibL	0.01–0.1
iicL	0.0045
iidL	0.039

applicable for a variety of propionic acid derivatives that have two aromatic amino groups. This synthetic route is useful for the discovery of new drug candidates derived from N-acylated phenylalanine derivatives.

Acknowledgments

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13. Reversed phase HPLC was performed on Hewlett Packard HP-1100 series system with a linear gradient of 10% of B (0–1 min), 10–100% of B (1–10 min), 100% of B (10–12 min) using 0.1% formic acid in water as solvent A, 0.1% formic acid in acetonitrile as solvent B (1.0 mL/min). The column was GL Sciences Inc. Inertsil ODS-3, 3 μm, 4.6 × 75 mm. Peak areas were integrated with 254 nm.
14. Selected data for **iaK**: 3-{4-[(3,5-dichloropyridine-4-carbonyl)amino]phenyl}-2-{3-[(2,2-dimethylpropionyl)isobutylamino]-4-methoxyphenyl}propionic acid: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.69–0.94 (15H, m), 1.38–1.67 (1H, m), 2.43–2.58 (1H, m), 2.80–3.04 (1H, m), 3.18–3.40 (1H, m), 3.77 (3H, s), 3.81–3.97 (2H, m), 6.96–7.08 (1H, m), 7.27 (1H, d, $J = 8.7$ Hz), 7.09–7.18 (2H, m), 7.26–7.36 (1H, m), 7.38–7.53 (2H, m), 8.78 (2H, s), 10.79 (1H, br s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.0, 20.2, 26.2, 28.4, 38.1, 51.2, 55.4, 56.5, 112.0, 119.3, 128.1, 128.7, 129.4, 131.1, 131.2, 135.1, 136.1, 142.5, 147.7, 154.3, 159.6, 174.4, 176.5; HRMS: calcd for $C_{31}H_{36}{}^{35}Cl_2N_3O_5$ $(M+H)^+$ 600.2032, found 600.2042.
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