Aromatic β-Amino Acids as Asp−Phg Mimics in LDV Derived VLA-4 Antagonists

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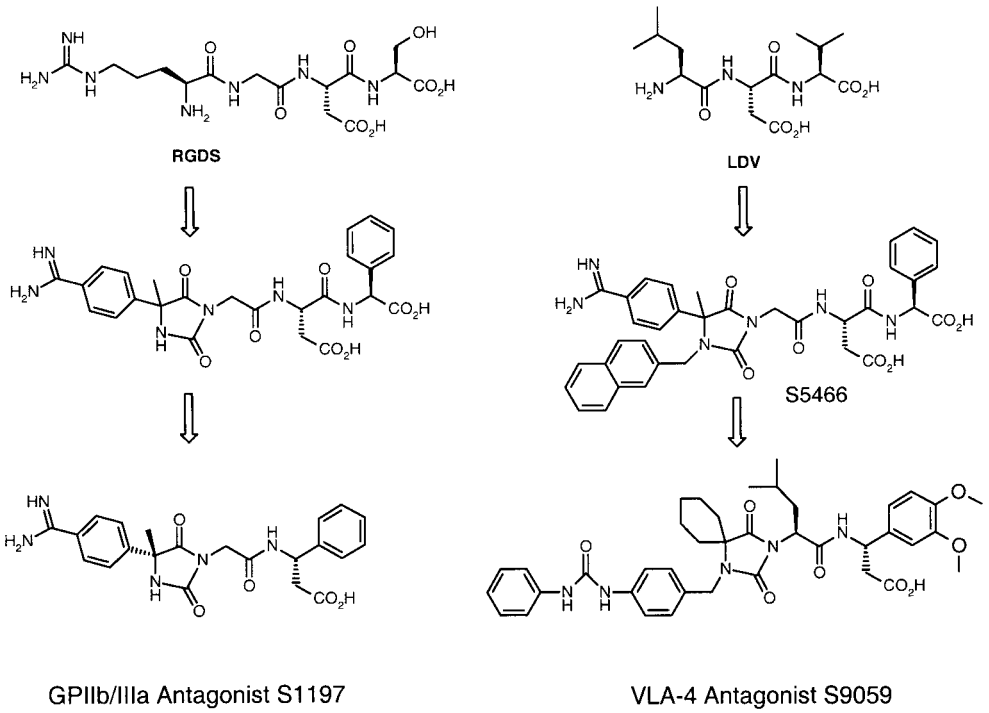
Dedicated to Professor Dieter Seebach on the occasion of his 65th birthday.

Abstract: Aromatic β-amino acid esters **2a**−**h** were prepared in racemic and enantiomerically pure form by the Radionow reaction or based on the method described by Davis and used as mimics of the Asp–Phg C-terminus in LDV derived VLA-4 antagonists. As a promising β-amino acid ester, **11** was identified and used for the synthesis of the highly potent VLA-4 antagonist **S9059** with an IC_{50} of 1.6 nM in a cell attachment assay.

Key words: amino acids, esters, asymmetric synthesis, chiral auxiliaries, VLA-4 antagonists

In the past years β-amino acids have become of great interest as building blocks for the synthesis of β-peptides due to their enhanced stability (compared to α-amino acids) towards peptidases,[1a–c] their interesting structural features[1b,2] and biological functions.[1b,2,3] Cyclo-β-tripeptides have been evaluated as antiproliferatives against human cancer cell lines[4] and as mimics of the human peptide hormone somatostatin.[3] β-Amino-acids have also been incorporated in pharmacologically active compounds.[2]

In pharmaceutical research, β-amino acids have been used as carboxy termini of blood platelet fibrinogen receptor (GPIIb/IIIa) antagonists, which were expected to be a promising new class of antithrombotic agents.[5] These compounds were derived from the RGDS tetrapeptide recognition motif, which is used by fibrinogen to bind to its receptor. In GPIIb/IIIa antagonists based on the hydantoin scaffold, the C-terminal aspartic acid–phenylglycine (Asp–Phg) could be replaced by β-amino acids leading to orally bioavailable compounds.[5] In the present paper, we demonstrate that β-amino acids could not only be used to replace the C-terminus in RGDS derived peptidomimetics



Scheme 1 β-Amino acids as C-termini of RGDS derived GPIIb/IIIa antagonists and LDV derived VLA-4 antagonists.

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but are also valuable C-termini in LDV derived antagonists of the leukocyte receptor VLA-4 (very late activating antigen 4), which are of interest as antiinflammatory agents.[6] In this case, the Asp–Phg residue could also be replaced by β-amino acids (see Scheme 1). Interestingly, VLA-4 antagonists with β-amino acid C-termini have been reported by other research groups as well.[7a–c]

There are several methods for the synthesis of racemic and enantiomerically pure β-amino acids described in the literature. The oldest and most commonly used method for the synthesis of aromatic β-amino acids was published by Radionow and coworkers. In the Radionow reaction, an aromatic aldehyde and malonic acid are condensed in the presence of ammonium acetate, which serves as a base and the source of the amino group.[8a,b] The β-amino acid precipitates from the solution and can be isolated by a simple filtration. Other methods used are the addition of ammonia to acrylic acid derivatives or the Reformatsky reaction with imines.[9a–d]

Enantiomerically pure (ee >95%) β-aryl-β-amino acids were prepared via penicillin acylase catalyzed hydrolysis of the corresponding *N*-phenylacetyl derivatives[10] which were prepared by the Radionow reaction[8a,b] and subsequent acylation. The addition of lithium-(*R*)-(α-methylbenzyl) benzylamide or lithium-(*R*)-*N*-benzyl-*N*-α-methyl-4-methoxybenzylamide as a homochiral ammonia equivalent to (*E*)-*t*-butyl crotonate or substituted (*E*)-cinnamic acid *t*-butyl esters and subsequent hydrogenolytic or oxidative cleavage of the chiral auxilary allows the synthesis of chiral β-amino acids with high enantiomeric purity (>95% ee).[11a,b]

A very general approach for the preparation of Fmoc or Boc protected enantiomerically pure β-amino acids from the corresponding α-amino acids was described by Seebach and coworkers. Using the Arndt–Eistert homologation sequence, enantiomerically pure β-amino acids with aliphatic, aromatic or functionalized side chains were prepared.[2,12a–c] The enantioselective synthesis of α-substituted β-amino acids[13]and the preparation of geminally disubstituted β-amino acids has been described as well.[14]

In the lead optimization process of VLA-4 antagonists, we found that the Leu–Asp–Val (LDV) binding motif of one of its natural ligands (fibronectin) could be replaced by a Gly–Asp–Phg C-terminus. Incorporation of the Gly–Asp–Phg C-terminus into a hydantoin scaffold led to the potent VLA-4 antagonist **S5466** with an IC_{50} of 0.5 μM in a cell attachment assay using the human VLA-4 expressing U937 cell line and one of the natural ligands of VLA-4, VCAM-1 (= vascular cell adhesion molecule 1) as substrate.

In order to improve the oral bioavailability of the compounds, we evaluated the replacement of the Asp–Phg terminus by aromatic β-amino acids, which proved successfull for RGDS derived GPIIb/IIIa antagonists.[5] For this purpose, a set of racemic aromatic β-amino acid ethylesters **2a**–**h** was prepared by the Radionow reaction (for the preparation of the β-amino acids) and subsequent esterification using a saturated solution of HCl in ethanol (Scheme 2, reaction conditions for the esterification and total yields see Table 1). The low yield for **2h** (23%) may be due to the partial cleavage of the acetal group (OCH_2O) under the acidic conditions of ester formation.

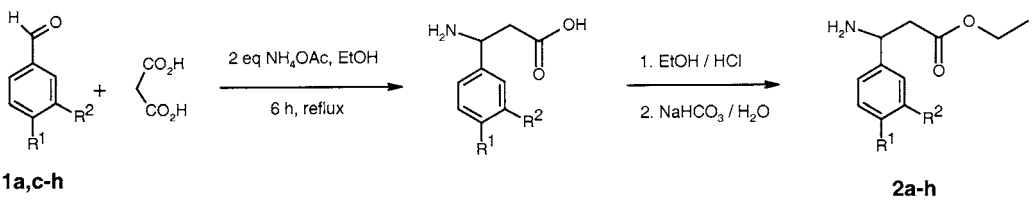
Table 1 Aromatic β-Amino Acid Ethyl Esters **2a**–**h** Prepared According to Scheme 2

Compound	R^1	R^2	Reaction conditions (esterification)	Total yield[a]
2a	F	H	7 h, reflux	33 %
2b[b]	Cl	H	7 h, reflux	84 %
2c	*t*-Bu	H	5 h, reflux	45 %
2d	*i*-Bu	H	7 h, reflux	49 %
2e	OMe	H	7 h, reflux	32 %
2f	OMe	OMe	7 h, reflux	32 %
2g	3,4-OCH_2CH_2O		7 h, reflux	39 %
2h	3,4-OCH_2O		22 h, r.t.	23 %[c]

[a] Compounds **2b**,**c**,**d**,**h** were isolated as free base.
[b] 3-Amino-3-(4-chlorophenyl) propionic acid was purchased from Maybridge.
[c] Work up procedure: The reaction mixture was filtered, the filtrate concentrated in vacuo, the residue distributed between H_2O and EtOAc, the H_2O phase washed with EtOAc and the combined organic phases dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the residue purified by chromatography over silica gel (70–200 μm) using CH_2Cl_2–MeOH, 9:1.

The β-amino acid ethyl esters prepared according Scheme 2 were coupled to the racemic hydantoin derivative **8**, which was synthesized as shown in Scheme 3.

4-Acetylbenzonitrile was reacted with potassium cyanide and ammonium carbonate in a water–ethanol mixture to give the hydantoin **4** as described.[5] Treatment of **4** with chloroacetic acid methylester in the presence of potassium



Scheme 2 Synthesis of racemic β-amino acid ethyl esters **2a**–**h**.

iodide and sodium methoxide in methanol provided intermediate **5**[5] which was alkylated with 2-bromomethylnaphthalene in *N*-methylpyrrolidone using potassium carbonate. The resulting hydantoin **6** was transformed to the hydroxamidino derivative **7** by addition of hydroxylamine. Hydrogenolytic cleavage followed by acidic hydrolysis of the methylester gave **8** which was coupled with the β-amino acid ethylesters **2a**–**h** using DCC/HOBt. Cleavage of the ethyl esters with lithium hydroxide in methanol led to the VLA-4 antagonists **3a**–**h** (Table 2) as mixtures of 2 diastereomers (ratio about 1:1, Table 5 and Table 6) as determined by ^{13}C NMR (Table 6). The 2 isomers were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry (HRMS) (Table 4, Table 5 and Table 6). 2D-NMR experiments (DQF-COSY, HMQC, HMBC) allowed the assignment of all hydrogen and carbon atoms. ^{1}H–^{1}H coupling constants for the 2 diastereomers could not be determined due to overlapping signals. The yield over the last 2 steps (coupling of **2a**–**h** to **8**, ester cleavage) varied between 12–46% depending on the β-amino acid ester used (Table 2)

Compounds **3a**–**h** were tested in a cell attachment assay using VLA-4 expressing human U937 cells which belong to the leukocyte family and one of the ligands of VLA-4, human VCAM-1 (vascular cell adhesion molecule 1) as substrate, which was fixed on a 96-well microtiter plate (results see Table 2).

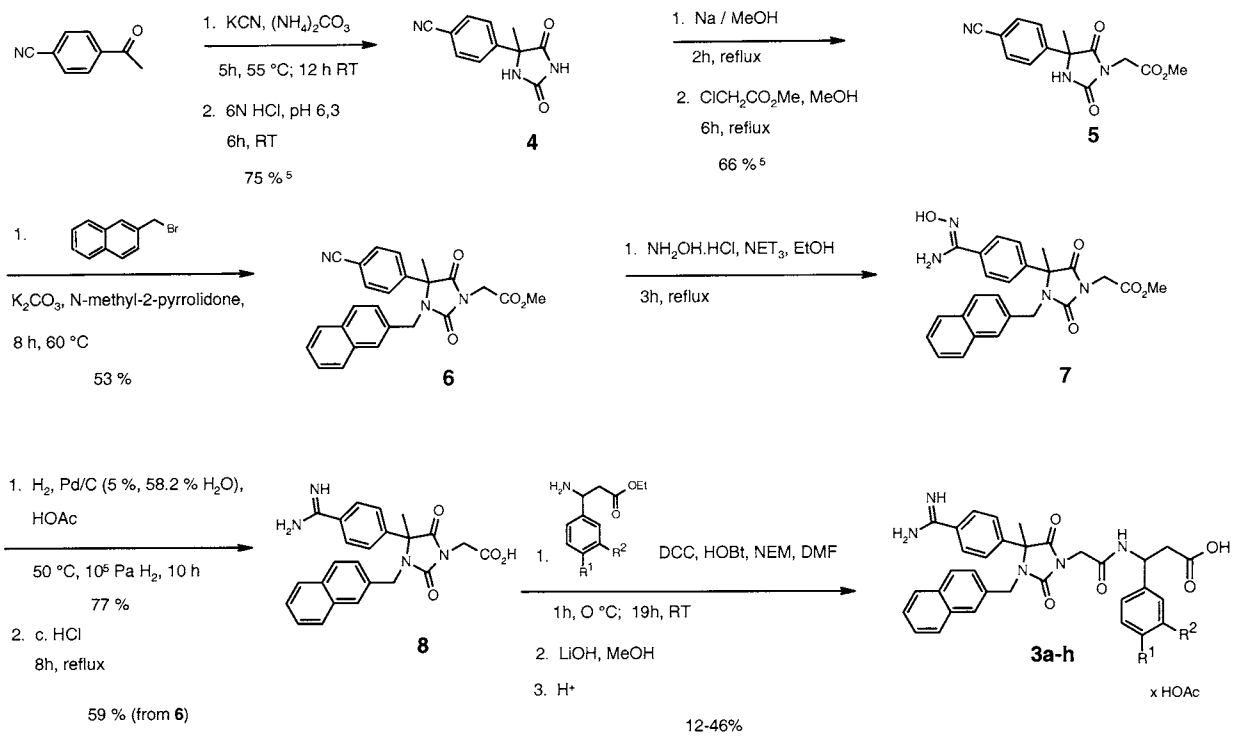
The intention of this lead optimization step was to identify the best replacement of the Asp–Phg C-terminus in **S5466**. The 2 diastereomers were therefore not separated but tested directly. **2f** (leading to compound **3f**, IC_{50} = 9.4 μM) was found to be the most suitable replacement for Asp–Phg. Considering that one could expect an improvement of potency by a factor of about 2 for the active diastereomer, only a factor of about 9 in potency was lost in **3f** compared to **S5466**.

Table 2 VLA-4 Antagonists **3a**–**h** Prepared According to Scheme 3 (Mixture of Two Diastereomers)

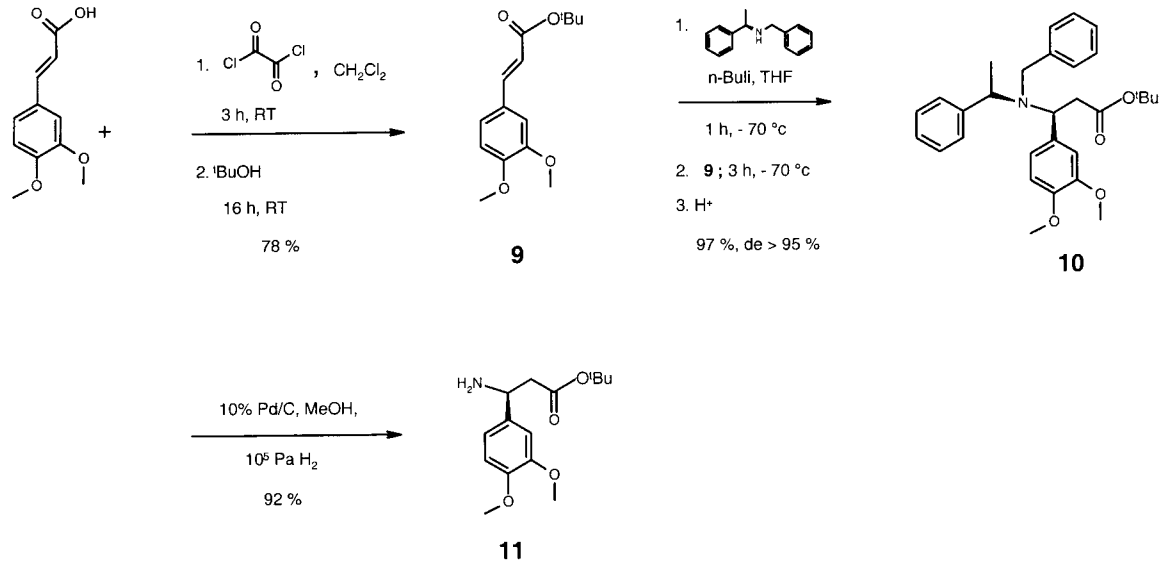
Compound	R^1	R^2	Yield[a]	IC_{50}[μM] U937(VLA-4)/ VCAM-1 cell attachment assay
3a	F	H	46%	31.6
3b	Cl	H	37%	46.0
3c	*t*-Bu	H	12%	88.7
3d	*i*-Bu	H	32%	75.2
3e	OMe	H	46%	17.7
3f	OMe	OMe	18%	9.4
3g	3,4-OCH_2CH_2O		30%	22.1
3h	3,4-OCH_2O		45%	19.6
S5466				0.5

[a] Yield over 2 steps (coupling and ester cleavage, **8** to **3a**–**h**).

In the next step of the lead optimization process, it was planned to prepare 3-amino-3-(3,4-dimethoxy)phenylpropionic acid (or a corresponding ester) in enantiomerically pure form and to connect it to the optimized hydantoin derivative **17** (see Scheme 5). During this work we



Scheme 3 Synthesis of VLA-4 antagonists **3a**–**h**.



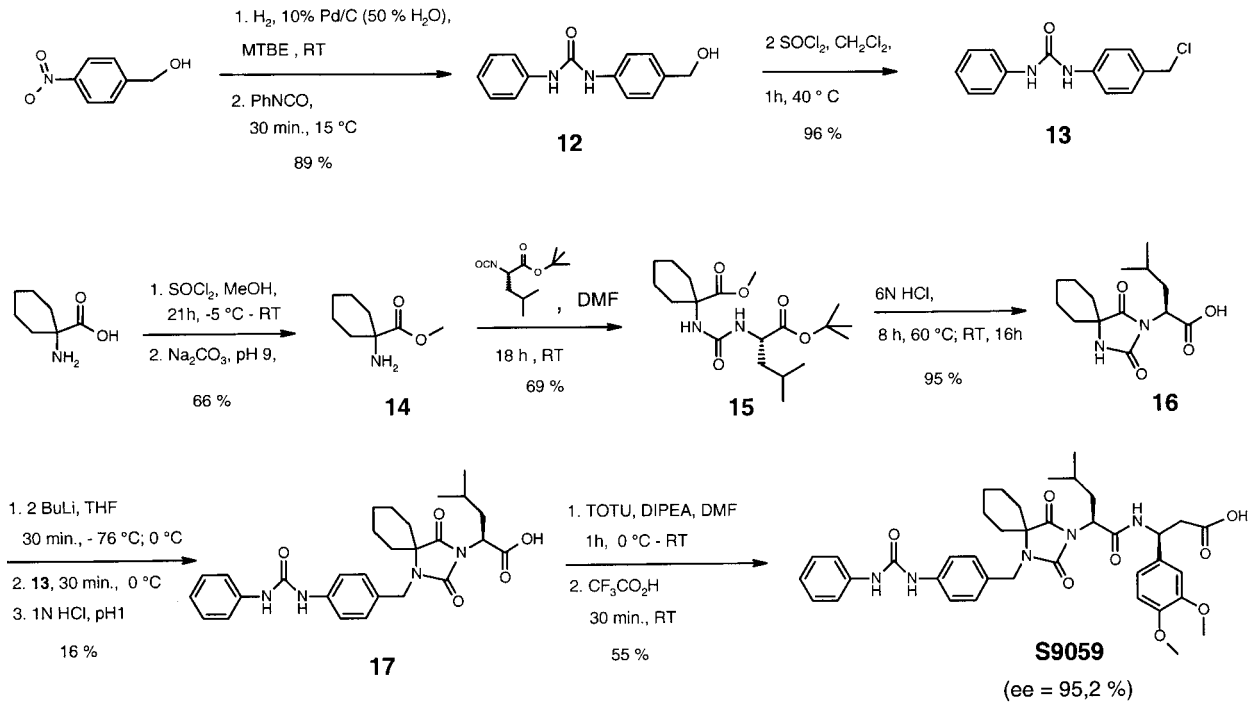
Scheme 4 Synthesis of (*S*)-3-Amino-3-(3,4-dimethoxy)-phenyl-propionic acid *t*-butylester **11**.

found that in the case of commercially available (*S*)- and (*R*)-3-amino-3-phenyl-propionic acid the (*S*)-enantiomer led to more potent compounds. Therefore only (3*S*)-amino-3-(3,4-dimethoxy)phenyl-propionic acid was prepared by the method described by Davis and coworkers.[11] It was found that the procedure for the debenzylation/cleavage of the chiral auxillary (see Scheme 4 and experimental section) could be performed under 10^5 Pa (= 1atm) H_2 pressure using 10% Pd/C in methanol instead of the 4×10^5 Pa using 30% Pd/C reported previously.[11] The ^{1}H NMR spectra (300 MHz, DMSO-d_6) of the adduct **10** showed only one set of signals indicating a diastereomeric purity of >95% de. Hydrogenolytic cleavage of the chiral auxilary and debenzylation, which did not influence the enantiomeric excess of the β-amino acid as shown by Davis,[11] gave **11** in 70% overall yield.

In parallel to the optimization of the C-terminus of the VLA-4 antagonists, the substitution pattern at the hydantoin scaffold was optimized. We found that spiro cyclohexyl hydantoin derivatives with a biphenyl urea substituent at N^3 led (after coupling of **11**) to highly potent VLA-4 antagonist **S9059**. In order to mimic the leucine side chain of the LDV binding motif, an isobutyl group was introduced next to the hydantoin as shown in Scheme 5. The IC_{50} of **S9059** in the cell attachment assay (see above) was 1.6 nM, a 313 fold increase in potency compared to **S5466**. The biphenyl urea substituent in **S9059** was mainly responsible for the marked increase in potency. Similar results have also been obtained by other groups working on LDV derived VLA-4 antagonists.[6]

S9059 was prepared according to Scheme 5. 4-Nitrophenyl-benzylalcohol was transformed to the biphenyl urea chloride **13** by catalytic reduction of the nitro group, addition of phenylisocyanate and reaction of the biphenyl alcohol intermediate with $SOCl_2$ in 85% yield over 3 steps. The hydantoin building block **16** was prepared starting from commercially available 1-amino-cyclohexane-carboxylic acid, which was transformed to the methylester **14** with $SOCl_2$ in MeOH. The isocyanate of L-leucine *t*-butylester (prepared as described by Nowick and coworkers[15]) was coupled with **14** in DMF followed by acidic cyclization of the adduct **15** to the hydantoin carboxylic acid **16**. Compound **16** was deprotonated at the CO_2H function and at N^3 by 2 equivalents of BuLi at –76 °C. The dianion was reacted with the biphenyl urea chloride **13** at 0 °C and the mixture was adjusted to pH 1 by the addition of 1 N HCl. The hydantoin **17** was isolated after chromatographic purification by preparative HPLC in 16% yield (not optimized). For the synthesis of **S9059**, the β-amino acid ester **11** was coupled to **17** using TOTU {*O*-[cyano(ethoxycarbonyl)methylene]amino}-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate) and the *t*-butylester of the adduct was cleaved with CF_3CO_2H. The enantiomeric purity of **17** was determined to be 97.6% (ee 95.2%) by HPLC on chiral phase (see experimental section).

In conclusion, we have shown that β-amino acids could be used as replacements of the pharmacokinetically less favourable Asp–Phg C-terminus in LDV derived hydantoin based antagonists of the leukocyte receptor VLA-4. (3*S*)-Amino-3-(3,4-dimethoxy-phenyl)-propionic acid was identified as a promising C-terminus. Parallel optimization of the substitution pattern at the hydantoin led to the **S9059**, a VLA-4 antagonist with an excellent in vitro potency (IC_{50} = 1.6 nM, cell attachment assay).



Scheme 5 Synthesis of VLA-4 antagonist S9059.

Table 3 Spectroscopic Data of β-Amino Acid Ethylesters **2a**–**h**

Compound	^{1}H NMR (DMSO-d_6), δ, *J* (Hz)	ESI-MS, *m/z* (%)[a]
2a	1.08 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 3.00 (A of ABX, *J* = 9.0, 16.5 Hz, 1 H, CH_AH), 3.20 (B of ABX, *J* = 5.5, 16.5 Hz, 1 H, CHH_B), 3.97 (q, 2 H, *J* = 7.0 Hz, CH_2CH_3), 4.60 (X of ABX, 1 H, *J* = 5.5, 9.0 Hz, CH), 7.20–7.32 (m, 2 H, ArH), 7.55–7.70 (m, 2 H, ArH), 8.75 (br s, 3 H, NH_3^+)	212.0 (87) $[M + H]^+$, 195.0 (100), 152.9 (34)
2b	1.12 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 2.01 (br s, 2 H, NH_2), 2.50–2.60 (m, 2 H, CH_2), 4.00 (q, 2 H, *J* = 7.0 Hz, CH_2CH_3), 4.20 (t, 1 H, $CHNH_2$, *J* = 7.5 Hz), 7.28–7.43 (m, 4 H, ArH)	228.2 (100) $[M + H]^+$, 211.1 (15), 140.0 (56)
2c	1.08 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 1.28 (s, 9 H, *t*- Bu), 2.97 (A of ABX, *J* = 9.0, 15.5 Hz, 1 H, CH_AH), 3.20 (B of ABX, *J* = 5.5, 15.5 Hz, 1 H, CHH_B), 4.00 (q, 2 H, *J* = 7.0 Hz, CH_2CH_3), 4.54 (X of ABX, 1 H, *J* = 5.5, 9.0 Hz, CH), 7.40–7.50 (m, 4 H, ArH), 8.68 (br s, 3 H, NH_3^+)	250.3 (16) $[M + H]^+$, 233.3 (32), 162.1 (100)
2d	0.85 [d, 6 H, *J* = 6.0 Hz, $C(CH_3)_3$], 1.10 (t, 3 H, *J* = 6.5 Hz, CH_2CH_3),1.80 [sept, 1 H, *J* = 6.0 Hz, $C(CH_3)_3$], 1.96 (br s, 2 H, NH_2), 2.40 [d, 2 H, *J* = 6.0 Hz, $C(CH_3)_3$], 2.46–2.58 (m, 2 H, CH_2), 4.00 (q, 2 H, *J* = 6.0 Hz, CH_2CH_3), 4.16 (t, 1 H, *J* = 6.5 Hz, CH), 7.08 (d, *J* = 8.0 Hz, 2 H, ArH), 7.25 (d, *J* = 8.0 Hz, 2 H, ArH)	250.3 (100) $[M + H]^+$, 233.2 (30), 162.1 (88)
2e	1.09 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 2.94 (A of ABX, *J* = 9.0, 16.0 Hz, 1 H, CH_AH), 3.16 (B of ABX, *J* = 5.5, 16.0 Hz, 1 H, CHH_B), 3.74 (s, 3 H, OCH_3), 3.99 (q, 2 H, *J* = 7.0 Hz, CH_2CH_3), 4.52 (X of ABX, 1 H, *J* = 5.5, 9.0 Hz, CH), 6.97 (d, *J* = 8.5 Hz, 2 H, ArH), 7.45 (d, *J* = 8.5 Hz, 2 H, ArH), 8.56 (br s, 3 H, NH_3^+)	224.0 (12) $[M + H]^+$, 207.0 (100), 165.0 (12)
2f	1.05 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 2.96 (A of ABX, *J* = 9.0, 16.0 Hz, 1 H, CH_AH), 3.17 (B of ABX, *J* = 5.5, 16.0 Hz, 1 H, CHH_B), 3.74 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 4.01 (q, 2 H, *J* = 7.0 Hz, CH_2CH_3), 4.42–4.58 (X of ABX, m,1 H, CH), 6.97–7.07 (m, 2 H, ArH), 7.28 (s, 1 H, ArH), 8.67 (br s, 3 H, NH_3^+)	254.2 (5) $[M + H]^+$, 253.2 (15), 237.2 (100), 166.0 (36)
2g	1.10 (t, 3 H, *J* = 6.5 Hz, CH_2CH_3), 2.90 (A of ABX, *J* = 9.0, 16.0 Hz, 1 H, CH_AH), 3.12 (B of ABX, *J* = 5.5, 16.0 Hz, 1 H, CHH_B), 4.02 (q, 2 H, *J* = 6.5 Hz, CH_2CH_3), 4.23 (s, 4 H, OCH_2CH_2O), 4.48 (X of ABX, 1 H, *J* = 5.5, 9.0 Hz, CH), 6.82–6.99 (m, 2 H, ArH), 7.09 (d, 1 H, *J* = 1.0 Hz, ArH), 8.55 (br s, 3 H, NH_3^+)	252.1 (16) $[M + H]^+$, 235.1 (100), 193.0 (3)
2h[b]	-	310.2 (30),. 238.1 (42) $[M + H]^+$, 221.1 (100), 150.0 (30)

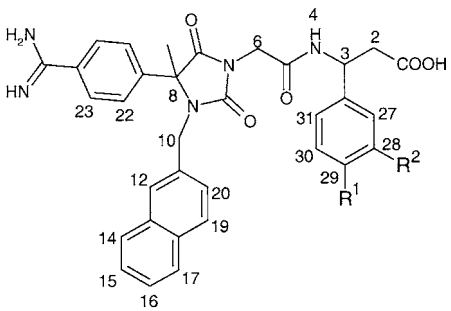
[a] DCI-MS in case of compound **2f**.
[b] Compound was characterized by MS and directly used for the synthesis of **3h**.

Table 4 HRMS Data of compounds **3a**–**h**

Compound	**3aa**, **ab**	**3ba**, **bb**	**3ca**, **cb**	**3da**, **db**
Diastereomeric Ratio	(1.0/1.0)[a]	(1.0/1.0)[a]	(1.2/1.0)[a]	(1.0/1.0)[a]
m/z = calcd for $[M + H]^+$	$C_{33}H_{31}N_5O_5F$	$C_{33}H_{31}N_5O_5Cl$	$C_{37}H_{40}N_5O_5$	$C_{37}H_{40}N_5O_5$
Calcd	596.22963	612.20022	634.30158	634.30158
Found	596.23037	612.20082	634.30240	634.30240
Compound	**3ea**, **eb**	**3fa**, **fb**	**3ga**, **gb**	**3ha**, **hb**
Diastereomeric Ratio	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
m/z = calcd for $[M + H]^+$	$C_{34}H_{34}N_5O_6$	$C_{35}H_{36}N_5O_7$	$C_{35}H_{34}N_5O_7$	$C_{34}H_{32}N_5O_7$
Calcd	608.24964	638.26001	636.24434	622.22885
Found	608.25036	638.26093	636.24527	622.22962

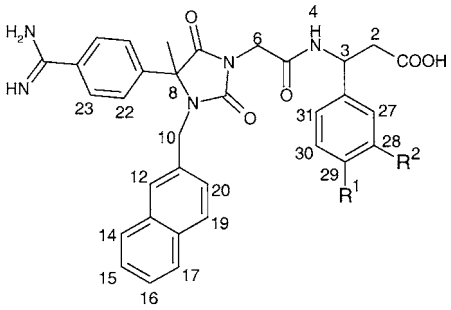
[a] Determined by ^{13}C NMR (125 MHz, DMSO-d_6), main diastereomer = **a**, minor diastereomer = **b** (e.g. **3aa, 3ab**).

Table 5 ^1H NMR Data of Compounds **3a**–**h**[a,b] (500 MHz, DMSO-d_6, δ)



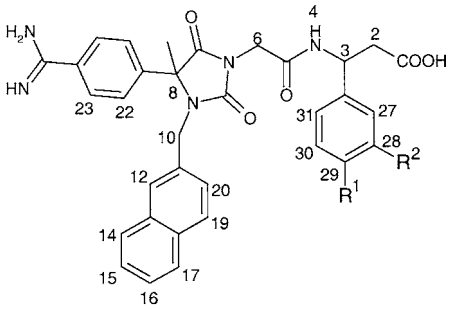
Compound Number	**3aa**	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[c]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
H-2	2.52	2.51	2.50	2.52	2.51	2.51	2.49	2.48
	2.52	2.51	2.50	2.52	2.51	2.51	2.49	2.48
3	5.19	5.17	5.16	5.19	5.14	5.16	5.08	5.11
	5.19	5.17	5.16	5.19	5.14	5.16	5.08	5.11
4	8.87	8.90	8.88	8.82	8.78	8.79	8.77	8.80
	8.87	8.90	8.87	8.82	8.78	8.79	8.76	8.79
6	4.20	4.20	4.17	4.20	4.17	4.20	4.16	4.18
	4.20	4.20	4.17	4.20	4.17	4.20	4.16	4.18
8	1.70	1.69	1.70	1.70	1.70	1.70	1.70	1.70
	1.69	1.69	1.69	1.69	1.69	1.70	1.69	1.69
10a	4.11	4.11	4.11	4.11	4.11	4.09	4.11	4.12
	4.09	4.08	4.08	4.08	4.08	4.12	4.08	4.08
10b	4.86	4.85	4.85	4.85	4.86	4.83	4.86	4.86
	4.84	4.84	4.84	4.84	4.84	4.86	4.84	4.84

Table 5 ^{1}H NMR Data of Compounds **3a**–**h**[a,b] (500 MHz, DMSO-d_6, δ) (continued)



Compound Number	**3aa**	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[c]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
H-12	7.63	7.62	7.64	7.64	7.63	7.64	7.63	7.63
	7.63	7.62	7.65	7.64	7.63	7.64	7.63	7.63
14	7.76	7.76	7.78	7.78	7.77	7.76	7.77	7.76
	7.76	7.76	7.78	7.78	7.77	7.76	7.77	7.76
15	7.46	7.46	7.47	7.46	7.46	7.46	7.46	7.46
	7.46	7.46	7.47	7.46	7.46	7.46	7.46	7.46
16	7.46	7.46	7.46	7.45	7.46	7.46	7.45	7.46
	7.46	7.46	7.46	7.45	7.46	7.46	7.45	7.46
17	7.84	7.85	7.85	7.84	7.84	7.84	7.84	7.84
	7.84	7.85	7.85	7.84	7.84	7.84	7.84	7.84
19	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80
	7.80	7.80	7.80	7.80	7.80	7.80	7.80	7.80
20	7.34	7.34	7.34	7.34	7.34	7.34	7.34	7.34
	7.34	7.34	7.34	7.34	7.34	7.34	7.34	7.34
22	7.70	7.69	7.68	7.70	7.70	7.71	7.70	7.70
	7.67	7.66	7.71	7.67	7.67	7.67	7.67	7.67
23	7.79	7.79	7.79	7.79	7.80	7.79	7.80	7.79
	7.78	7.76	7.80	7.78	7.78	7.78	7.78	7.78
27	7.36	7.35	7.24	7.22	7.25	6.95	6.80	6.90
	7.36	7.35	7.24	7.24	7.24	6.93	6.83	6.89
28	7.12	7.35	7.30	7.05	6.87	-	-	-
	7.12	7.35	7.30	7.08	6.84			
30	-	-	-	-	-	-	6.77	6.83
	6.77	6.83						
31	-	-	-	-	-	-	6.77	6.80
	6.77	6.80						
t-Bu	-	-	1.84 1.84	-	-	-	-	-

Table 5 ^{1}H NMR Data of Compounds **3a**–**h**[a,b] (500 MHz, DMSO-d_6, δ) (continued)



Compound Number	**3aa**	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[c]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
CH_2(*i*-Bu)	-	-	-	2.39 2.37	-	-	-	-
CH(*i*-Bu)	-	-	-	1.80 1.77	-	-	-	-
2 CH_3(*i*-Bu)	-	-	-	0.85 0.83	-	-	-	-
OCH_3	-	-	-	-	3.72 3.69	3.71 3.69	-	-
OCH_3	-	-	-	-	-	3.69 3.74	-	-
OCH_2CH_2O	-	-	-	-	-	-	4.17, 4.20 4.17, 4.20	-
OCH_2O	-	-	-	-	-	-	-	5.94, 5.97 5.94, 5.97

[a] Due to signal overlapping, coupling constants could not be determined.
[b] C(=NH)NH_2 and CO_2H protons appear between 8−12 ppm as a very broad signal.
[c] Determined by ^{13}C NMR (125 MHz, DMSO-d_6), main diastereomer = **a**, minor diastereomer = **b** (e.g. **3aa, 3ab**).

Table 6 ^{13}C NMR Data of Compounds **3a**–**h** (125 MHz, DMSO-d_6, δ)

Compound Number	**3aa**[c]	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab[c]	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[a,b]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
C-1	174.33	174.28	174.49	174.52	174.42	174.69	174.27	174.39
	174.33	174.28	174.49	174.52	174.45	174.69	174.30	174.43
2	43.69	43.59	43.69	43.59	43.72	43.78	43.59	43.79
	43.59	43.59	43.90	43.59	43.59	43.97	43.59	43.91
3	50.11	50.25	50.36	50.37	50.12	50.42	50.09	50.52
	50.11	50.25	50.42	50.37	50.12	50.37	50.09	50.52
5	164.77	164.85	164.55	164.65	164.56	164.66	164.55	164.60
	164.77	164.85	164.55	164.65	164.56	164.66	164.55	164.60
6	40.81	40.82	40.82	40.83	40.80	40.78	40.77	40.82
	40.73	40.73	40.73	40.74	40.73	40.87	40.70	40.73

Table 6 ^{13}C NMR Data of Compounds **3a**–**h** (125 MHz, DMSO-d_6, δ) (continued)

Compound Number	**3aa**[c] **3ab**[c]	**3ba** **3bb**	**3ca** **3cb**	**3da** **3db**	**3ea** **3eb**	**3fa** **3fb**	**3ga** **3gb**	**3ha** **3hb**
Diastereomeric Ratio[a,b]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
C-7	155.47	155.46	155.49	155.49	155.49	155.46	155.49	155.49
	155.43	155.42	155.45	155.46	155.46	155.51	155.46	155.46
8	67.01	67.01	66.98	66.98	66.98	66.99	66.99	67.00
	66.98	66.99	66.98	66.98	66.98	66.99	66.97	66.98
8-Me	20.11	20.11	20.07	20.10	20.10	20.11	20.10	20.11
	20.05	20.06	20.03	20.06	20.06	20.03	20.06	20.06
9	173.89	173.88	173.90	173.91	173.91	173.97	173.90	173.90
	173.93	173.92	173.93	173.93	173.94	173.93	173.94	173.94
10	43.59	43.59	43.58	43.59	43.59	43.58	43.59	43.59
	43.59	43.59	43.58	43.59	43.59	43.58	43.59	43.59
11	134.89	134.87	134.89	134.89	134.89	134.90	134.89	134.88
	134.87	134.89	134.89	134.91	134.91	134.90	134.89	134.90
12	126.09	126.09	126.09	126.09	126.08	126.08	126.09	126.09
	126.09	126.09	126.09	126.09	126.08	126.08	126.09	126.09
13	132.63	132.63	132.64	132.64	132.64	132.64	132.64	132.64
	132.63	132.63	132.64	132.64	132.64	132.64	132.64	132.64
14	127.55	127.55	127.56	127.55	127.56	127.55	127.56	127.55
	127.55	127.55	127.56	127.55	127.56	127.55	127.56	127.55
15	126.15	126.15	126.09	126.17	126.15	126.15	126.16	126.15
	126.15	126.15	126.09	126.17	126.15	126.15	126.16	126.15
16	125.88	125.87	125.85	125.87	125.87	125.87	125.88	125.87
	125.88	125.87	125.85	125.87	125.87	125.87	125.88	125.87
17	127.42	127.42	127.40	127.41	127.42	127.42	127.42	127.42
	127.42	127.42	127.40	127.41	127.42	127.42	127.42	127.42
18	132.08	132.08	132.07	132.08	132.08	132.08	132.08	132.08
	132.08	132.08	132.07	132.08	132.08	132.08	132.08	132.08
19	127.88	127.87	127.86	127.87	127.88	127.87	127.89	127.87
	127.88	127.87	127.86	127.87	127.88	127.87	127.89	127.87
20	125.76	125.76	125.75	125.76	125.76	125.76	125.77	125.76
	125.76	125.76	125.57	125.76	125.76	125.76	125.77	125.76
21	142.02	141.99	141.92	141.99	142.00	142.04	142.01	141.98
	142.04	142.03	141.95	142.01	142.03	142.01	142.03	142.01
22	127.42	127.42	127.40	127.41	127.42	127.42	127.42	127.42
	127.42	127.42	127.40	127.41	127.42	127.42	127.42	127.42

Table 6 ^{13}C NMR Data of Compounds **3a**–**h** (125 MHz, DMSO-d_6, δ) (continued)

Compound Number	**3aa**[c]	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab[c]	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[a,b]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
C-23	128.06	128.03	127.99	128.03	128.03	128.03	128.05	128.03
	128.06	128.03	127.99	128.03	128.03	128.03	128.05	128.03
24	129.18	129.21	129.33	129.24	129.24	129.26	129.20	129.25
	129.21	129.21	129.37	129.24	129.26	129.24	129.23	129.25
25	165.34	165.37	165.37	165.35	165.36	165.41	165.34	165.36
	165.38	165.39	165.43	165.38	165.39	165.38	165.38	165.40
26	139.46	142.30	140.26	140.38	135.11	135.90	136.25	137.23
	139.35	142.41	140.43	140.52	135.24	135.76	136.37	137.36
27	128.34 (8 Hz)	128.33	126.09	126.17	127.56	110.49	115.07	106.98
	128.34 (8 Hz)	128.33	126.09	126.17	127.56	110.49	115.08	106.98
28	114.80 (21 Hz)	128.05	124.81	128.64	113.49	148.47	142.89	145.84
	114.78 (21 Hz)	128.05	124.84	128.66	113.52	148.43	142.91	145.85
29	160.99 (242 Hz)	131.17	148.77	139.28	158.00	147.55	142.11	147.03
	161.01 (242 Hz)	131.17	148.77	139.28	158.02	147.53	142.13	147.06
30	-	-	-	-	-	-	116.57	107.79
							116.59	107.82
31	-	-	-	-	-	-	119.26	119.55
							119.26	119.57
$C(CH_3)_3$	-	-	34.05	-	-	-	-	-
			34.05					
$C(CH_3)_3$	-	-	31.12	-	-	-	-	-
			31.12					
$CH_2CH(CH_3)_2$	-	-	-	44.23	-	-	-	-
				44.23				
$CH_2CH(CH_3)_2$	-	-	-	29.50	-	-	-	-
				29.53				
$CH_2CH(CH_3)_2$	-	-	-	22.18	-	-	-	-
				22.18				
29-OCH_3	-	-	-	-	54.96	55.48	-	-
					54.99	55.53		
28-OCH_3	-	-	-	-	-	55.33	-	-
						55.27		
OCH_2CH_2O	-	-	-	-	-	-	64.00	-

Table 6 ^{13}C NMR Data of Compounds **3a**–**h** (125 MHz, DMSO-d_6, δ) (continued)

Compound Number	**3aa**[c]	**3ba**	**3ca**	**3da**	**3ea**	**3fa**	**3ga**	**3ha**
	3ab[c]	**3bb**	**3cb**	**3db**	**3eb**	**3fb**	**3gb**	**3hb**
Diastereomeric Ratio[a,b]	(1.0/1.0)	(1.0/1.0)	(1.2/1.0)	(1.0/1.0)	(1.0/1.0)	(1.1/1.0)	(1.1/1.0)	(1.0/1.0)
OCH_2CH_2O	-	-	-	-	-	-	64.03 63.90 63.93	-
OCH_2O	-	-	-	-	-	-	-	100.71 100.72

[a] Determined by ^{13}C NMR (125 MHz, DMSO-d_6).
[b] Main diastereomer = **a**, minor diastereomer = **b** (e.g. **3aa**, **3ab**).
[c] Coupling constants shown for C27-C29 in **3aa, 3ab** refer to J_{C-F}.

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) or a Bruker DRX 500 (500 MHz) spectrometer. ^{13}C spectra were recorded on a Bruker DRX 500 (125 MHz). The assignment of ^1H and ^{13}C chemical shifts in **3aa**–**hb** is based on the analysis of 2D-NMR experiments (DQF-COSY, HMQC and HMBC), δ values in ppm relative to TMS are given. DQF-COSY experiments were performed with a spectral width of 10 ppm. Spectra were recorded with 512 increments in t_1 and 2048 complex data points in t_2, with 8 transients averaged for each t_1 value. For the HMQC spectra, 512 increments (8 scans) with 2048 complex data points in t_2 were collected using a sweep width of 10 ppm in the proton and 160 ppm in the carbon dimension. The HMBC spectra were acquired with a sweep width of 10 ppm in the proton and 200 ppm in the carbon dimension. A total of 48 transients were averaged for each of 512 increments in t_1, and 2048 complex points in t_2 were recorded. A delay of 70 msec was used for the development of long range correlations. Low-mass spectra were recorded with positive electrospray ionization (+ESI): VG BIO-Q or dissociation chemical ionization (DCI): Kratos MS80 or VG Trio 2000. High-resolution spectra were recorded on a VG ZAB SEQ (+FAB). Optical rotations were determined on a Perkin-Elmer 241 polarimeter. The specific rotation has not been corrected. Preparative HPLC separations were performed using a Hibar column (LiChrosper 100 RP-18e, 5 μm, cat. 1.50004,708, r.t. 250–25, Merck, Germany). As eluent H_2O–CH_3CN with 0.1% CF_3CO_2H was used. Determination of the enantiomeric purity of **S9059** was performed by HPLC on a Chiral Pack AD column using heptane–EtOH–*i*-PrOH, 5:1:1 + 0.1% diethylamine and 0.1% CF_3CO_2H as eluent. The 3-amino-3-(4-chlorophenyl) propionic acid was purchased from Maybridge.

β-Amino Acid Ethylesters 2a−h;[8] General Procedure

A mixture of the substituted benzaldehyde **1a**,**c**−**h** (0.10 mol), ammonium acetate (15.4 g 0.20 mol) and malonic acid (10.4 g 0.10 mmol) in EtOH (25 mL) were heated under reflux for 6 h. After cooling to r.t., the β-amino acid was isolated by filtration, washed with EtOH and dried over P_2O_5.

For the preparation of the ethyl ester the β-amino acid was added to a sat. soln of HCl in EtOH at 0 °C, stirred at r.t. for 1 h and then under reflux (see Table 1). The solvent was removed in vacuo and the residue washed with Et_2O, filtered and dried in vacuo.

In the cases were the β-amino acid ethylesters were isolated as the free base (see Table 1), the solvent was removed and the residue distributed between H_2O and EtOAc. The two phases were neutralized with $NaHCO_3$ under stirring and separated. The organic phase was washed with H_2O and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo. The β-amino acid esters were characterized by ^1H NMR and MS (**2a**−**g**) or MS (**2h**) (Table 3) and directly used for the synthesis of the VLA-4 antagonists **3a**−**h**.

[4-(4-Cyano-phenyl)-4-methyl-3-naphthalene-2-ylmethyl-2,5-dioxo-imidazolidin-1-yl]-acetic Acid Methyl Ester (6)

To a soln of **5** (125 g, 435 mmol) and milled K_2CO_3 (66.1 g, 480 mmol) in *N*-methyl-2-pyrrolidone (1 L), 2-bromo-methylnaphthalene (96.4 g, 435 mmol) was added and the reaction mixture stirred at 60 °C for 8 h. After standing overnight, the mixture was filtered over charcoal, H_2O (2 L) was added and the product extracted with CH_2Cl_2 (2 × 500 mL). The combined organic phases were washed with sat. NaCl soln (2 × 250 mL) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the remaining oil was crystallized from *i*-PrOH. Compound **6** (98.5 g, 53%) was isolated as a colorless solid, Fp 104−106 °C and directly transformed to **7**.

{4-[4-(*N*-Hydroxycarbamimidoyl)-phenyl]-4-methyl-3-naphthalen-2-ylmethyl-2,5-dioxo- imidazolidin-1-yl}-acetic Acid Methyl Ester (7)

Under a nitrogen atm, a soln of **6** (160 g, 374 mmol), hydroxylamine-hydrochloride (52.4 g, 748 mmol) and Et_3N (103.3 mL, 748 mmol) in EtOH (1600 mL) was heated unter reflux for 3 h. EtOH (1200 mL) was partly removed in vacuo, ice H_2O (800 mL) was added and the soln extracted with EtOAc (3 × 300 mL). The combined organic phases were washed with ice H_2O (2 × 200 mL) and dried over Na_2SO_4. After filtration the solvent was removed in vacuo and the residue stirred with MTBE (400 mL). After filtration, **7** (181.2 g, 105%) was isolated as a colorless solid (containing traces of MTBE) which was used without further purification.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.62 (s, 3 H, CH_3), 3.80 (s, 3 H, OCH_3), 4.05 (A of AB, *J* = 15.0 Hz, 1 H, CHH_A), 4.40 (s, 2 H, $CH_2CO_2CH_3$), 4.78 (br s, 2 H, NH_2), 5.05 (B of AB, *J* = 15.0 Hz, 1 H, CHH_B), 7.85−7.15 (m, 12 H, ArH, OH).

ESI-MS: *m/z* (%) = 461.3 (100) $[M + H]^+$.

[4-(4-Carbamimidoyl-phenyl)-4-methyl-3-naphthalen-2-ylmethyl-2,5-dioxo-imidazolidin-1-yl]-acetic Acid (8)

Under a nitrogen atmosphere 5% Pd/C (24 g, 58.2% H_2O, Engelhard) was added to a soln of **7** (220 g, 478 mmol) in HOAc (1 L) and the mixture hydrogenated under an 10^5 Pa H_2 atm at 50 °C for 10 h. The catalyst was filtered, the solvent removed in vacuo and the residue stirred with MTBE (400 mL). After filtration, [4-(4-carbamimidoyl-phenyl)-4-methyl-3-naphthalen-2-ylmethyl-2,5-dioxo-imidazolidin-1-yl]- acetic acid methylester (185 g, 77%) was isolated

as a colorless solid, characterized by MS and directly transformed to **8**.

ESI-MS: *m*/*z* (%) = 445.3 $[M + H]^+$.

The solid (140 g, 278 mmol) was heated in concd HCl (2 L) under reflux for 8 h. The mixture was concentrated in vacuo and the residue distributed between H_2O (1 L) and EtOAc (1 L). The pH was adjusted to 4–5 by adding concd NaOH. After 30–45 min the carboxylic acid **9** crystallized. The compound was isolated by filtration and washed with H_2O (100 mL) and EtOAc (100 mL). The residue was dissolved in concd HCl (1 L), filtered over charcoal, the soln concentrated in vacuo and the residue distributed between H_2O–EtOAc, 1:1 (800 mL). The pH was adjusted to 4 and **8** precipitated as a colorless crystalline solid (70.5 g, 59%, Fp 258–260 °C) which was isolated by filtration.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.68 (s, 3 H, CH_3), 4.05–3.85 (AB, 2 H, $CH_2CO_2CH_3$), 4.06 (A of AB, *J* = 16.0 Hz, 1 H, CHH_A), 4.85 (B of AB, *J* = 16.0 Hz, 1 H, CHH_B), 7.30–7.94 (m, 11 H, ArH), 9.03 (br s, 2 H, NH_2), 11.75 (br s, 2 H, NH_2^+).

MS (FAB): *m*/*z* (%) = 431.2 (100) $[M + H]^+$, 386.2 (13).

VLA-4 Antagonists 3a–h; General Procedure

To a soln of the hydantoin derivative **8** (861 mg, 2.00 mmol), the β-amino acid **2a**–**h** (2.00 mmol), HOBt (270 mg, 2.00 mmol) in DMF (20 mL), *N*-ethyl morpholine (0.26 mL 2.00 mmol) and DCC (440 mg, 2.14 mmol) were added. The mixture was stirred 1 h at 0 °C and 4 h at r.t. After standing overnight, dicyclohexylurea was filtered off and the filtrate concentrated in vacuo. The residue was dissolved in pentanol, washed with sat. $NaHCO_3$ soln and sat. NaCl soln. The organic phase was dried over Na_2SO_4. After filtration the solvent was removed in vacuo and the residue triturated with Et_2O. After filtration the crude product was dissolved in MeOH (30 mL), 1 N LiOH soln (10 mL) was added at 0 °C and the mixture stirred for 4 h at r.t. After standing overnight at 4 °C, CF_3CO_2H was added to neutralize the reaction mixture. The mixture was filtered, the filtrate concentrated in vacuo, the residue triturated with H_2O, isolated by filtration and purified over sephadex LH20 using 10% HOAc in H_2O. The fractions containing the product were combined, the soln concentrated in vacuo and the products **3a**–**h** isolated after lyophilization as colorless amorphous solids (yields see Table 2, spectroscopic data see Table 4, Table 5 and Table 6).

3-(3,4-Dimethoxy-phenyl)-acrylic Acid *t*-Butyl Ester (9)

To a suspension of 3-(3,4-dimethoxy-phenyl)-acrylic acid (40 g, 192 mmol) in anhyd CH_2Cl_2 (400 mL), oxalic acid chloride (24.7 mL, 288 mmol) was added tropwise and the mixture stirred for 3 h at r.t. and a clear soln was formed. *t*-BuOH (300 mL) was added and the reaction mixture, after standing overnight at r.t., concentrated in vacuo. The residue was dissolved in EtOAc (450 mL) and the organic phase washed with sat. $NaHCO_3$ soln (3 × 250 mL). The phases were separated and the organic phase dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the residue purified by chromatography over silica gel (35–70 μm) using heptane–EtOAc (4:1) as solvent. The product fractions were combined to yield **9** (39.44 g, 78%) as an oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.50 (s, 9 H, *t*-Bu), 3.79 (s, 3 H, OCH_3),3.81 (s, 3 H,OCH_3), 6.44 (d, *J* = 15.0 Hz, 1 H, CH), 6.97 (d, *J* = 8.3 Hz, 1 H, ArH), 7.20 (dd, *J* = 0.8 Hz, *J* = 8.3 Hz, 1 H, ArH), 7.32 (d, *J* = 0.8 Hz, 1 H, ArH), 7.48 (d, *J* = 15.0 Hz, 1 H, CH).

(3*S*)-[(*R*)-Benzyl-1-phenyl-ethyl)-amino]-3-(3,4-dimethoxy-phenyl)-propionic Acid *t*-Butyl Ester (10)

To a soln of (*R*)-Benzyl-1-phenyl-ethylamine (17.6 g, 83.2 mmol) in anhyd THF (300 mL), BuLi (32.6 mL, 2.5 M in hexane, 81.4 mmol) were added over 1 h at –70 °C under an argon atm and the mixture stirred for 1 h at this temperature. A soln of **9** (20 g, 75.7 mmol) in anhyd THF (100 mL) was added dropwise over 1 h at –70 °C and the mixture was stirred for 2 h at –70 °C. The reaction mixture was quenched with citric acid (150 mL, 5% in H_2O), warmed up to r.t. and stirred at r.t. for 1 h. EtOAc (500 mL) was added, the phases separated and the H_2O phase was extracted with EtOAc (200 mL). The combined organic phases were washed with sat. $NaHCO_3$ soln (300 mL) and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the residue purified by chromatography over silicagel (35–70 μm) using heptane–EtOAc as eluent to yield **10** (34.7 g, 97%) as an yellow oil.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.09 (d, *J* = 6.0 Hz, 3 H, Me), 1.22 (s, 6 H, *t*-Bu), 2.53 (A of ABX, *J* = 6.8, 10.5 Hz, 1 H, H_A), 2.66 (B of ABX, *J* = 3.8, 10.5 Hz, 1 H, H_B), 3.60, 3.66 (AB system, *J* = 11.3, 2 H, NCH_2), 3.72 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 4.02 (q, *J* = 6.0 Hz, 1 H, CH), 4.12 (X of ABX, *J* = 3.8, 6.8 Hz, 1 H, H_X), 6.83–6.95 (m, 3 H, ArH), 7.12–7.48 (m, 10 H, ArH) (only 1 diastereomer detectable).

DCI-MS: *m*/*z* (%) = 276.4 (10) $[M + H]^+$, 360.3 (22), 265.3 (100), 212.3 (53), 105.1 (52).

(3*S*)-Amino-3-(3,4-dimethoxy-phenyl)-propionic Acid *t*-Butyl Ester (11)

A soln of **10** (34 g, 71.75 mmol) in MeOH (300 mL) was hydrogenated over 10% Pd/C (12 g) for 3 h. The catalyst was filtered and the soln concentrated in vacuo to yield **11** (18.5 g, 92%) as a yellow oil.

$[\alpha]_D^{27}$ +8.0 (*c* 1, MeOH).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.34 (s, 9 H, *t*-Bu), 1.95 (s, 2 H, NH_2), 2.43 (A of ABX, *J* = 6.0, 11.3 Hz, 1 H, H_A), 2.50 (B of ABX, *J* = 6.8, 11.3 Hz, 1 H, H_B), 3.72 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 4.10 (X of ABX, *J* = 6.0, 6.8 Hz, 1 H, H_X), 6.84 (s, 2 H, ArH), 6.98 (s, 1 H, ArH).

DCI-MS: *m*/*z* (%) = 282.4 (35) $[M + H]^+$, 265.3 (100), 224.3 (12), 180.3 (18), 166.2 (93).

1-(4-Hydroxymethyl-phenyl)-3-phenyl-urea (12)

4-Nitrobenzyl alcohol (30.6 g, 200 mmol) was hydrogenated over 10% Pd/C (1 g, 50% H_2O) in MTBE (500 mL). After the absorption of hydrogen was complete, the catalyst was filtered. Phenyl isocyanate (24 g, 200 mmol) was added to the filtrate at 15 °C with stirring in the course of 30 min. The precipitated solid was filtered with suction and washed with MTBE. Yield: (43 g, 89%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.43 (s, 2 H, CH_2), 5.05 (br s, 1 H, OH), 6.95 (t, *J* = 7.5 Hz, 1 H, ArH), 7.16–7.50 (m, 8 H, ArH), 8.60 (s, 1 H, NH), 8.63 (s, 1 H, NH)

DCI-MS: *m*/*z* (%) = 242.9 (60) $[M + H]^+$, 226.8 (32), 123.0 (52), 119.0 (100), 106.1 (38), 94.1 (53), 93.0 (95), 91.0 (40), 61.1 (41).

1-(4-Chloromethyl-phenyl)-3-phenyl-urea (13)

Thionyl chloride (42 g, 354 mmol) was added dropwise at 30 °C to a suspension of **12** (42.8 g, 177 mmol) in CH_2Cl_2 (500 mL). The mixture was subsequently stirred at 40 °C for 1 h. After completion of the evolution of gas, the mixture was allowed to cool to r.t. The precipitated product was filtered with suction and washed with CH_2Cl_2. Yield: (44.26 g, 96%) which was used in the next step after characterization by NMR spectroscopy.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 4.72 (s, 2 H, CH_2), 6.97 (t, *J* = 7.5 Hz,1 H, ArH), 7.23–7.50 (m, 8 H, ArH), 8.70 (s, 1 H, NH), 8.78 (s, 1 H, NH).

1-Amino-cyclohexanecarboxylic Acid Methyl Ester (14)

Thionyl chloride (51 mL) was added in portions at –5 °C to 1-aminocyclohexane-1-carboxylic acid (50 g, 350 mmol) in MeOH (1.25

L). The reaction mixture was subsequently stirred at r.t. for 5 h and allowed to stand overnight at r.t. MeOH was removed in vacuo, the residue was mixed with H_2O, and the aq soln was adjusted to pH 9 using sat. Na_2CO_3 soln and extracted with CH_2Cl_2 (2 × 350 mL). The phases were separated, the organic phase was dried over Na_2SO_4 and, after filtration, the solvent was removed in vacuo. Yield: (36.35 g, 66%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.20–1.85 (m, 12 H, cycl H, NH_2), 3.60 (s, 3 H, OCH_3)

DCI-MS: *m*/*z* (%) = 158.3 (100) $[M + H]^+$, 98.2 (56).

1-[3-(1-*t*-Butoxycarbonyl-3-methyl-butyl)-ureido]-cyclohexanecarboxylic Acid Methyl Ester (15)

A soln of **14** (28 g,179 mmol) was added to a soln of L-leucine *t*-butyl ester isocyanate (38 g,179 mmol)[15] in anhyd DMF (300 mL). After stirring at r.t. for 2 h, the reaction mixture was allowed to stand overnight. The solvent was removed in vacuo, the residue was treated with heptane (400 mL) and the mixture was stirred at r.t. for 2 h. The precipitate was filtered with suction and washed with heptane. Yield: (45.94 g, 69%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 0.87 (d, *J* = 7.5 Hz, 3 H, CH_3), 0.93 (d, *J* = 7.5 Hz, 3 H, CH_3),1.40 (s, 9 H, *t*-Bu), 1.13–1.99 (m, 13 H, cycl H, CH_2, CH), 3.53 (s, 3 H, OCH_3), 3.98 (dt, *J* = 6.5, 9.0 Hz, 1 H, NCH), 6.11 (d, *J* = 9.0 Hz, 1 H, NH), 6.31 (s,1 H, NH).

DCI-MS: *m*/*z* (%) = 371.4 (100) $[M + H]^+$, 315.3 (31).

(2*S*)-(2,4-Dioxo-1,3-diaza-spiro[4.5]dec-3-yl)-4-methyl-pentanoic Acid (16)

15 (11.4 g, 30.8 mmol) was heated at 60 °C for 8 h in 6 N HCl (200 mL), the mixture was allowed to stand at r.t. overnight and then extracted with Et_2O (400 mL). The combined extracts were concentrated in vacuo, the residue was dissolved in CH_3CN–H_2O (500 mL), and freeze-dried. Yield: (8.28 g, 95%).

$[\alpha]_D^{23}$ –47.8 (*c* 1, DMF).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 0.86 (d, *J* = 6,5 Hz, 3 H, CH_3), 0.92 (d, *J* = 6.5 Hz, 3 H, CH_3), 1.16–1.84 (m, 12 H, cycl H, CH_2), 2.00–2.19 (m, 1 H, CH), 4.48 (dd, *J* = 4.5 Hz, 11.0 Hz, 1 H, NCH), 8.67 (s,1 H, NH), 12.93 (br s, 1 H, COOH).

DCI-MS: *m*/*z* (%) = 283.5 (52) $[M + H]^+$, 169.2 (100), 117.2 (42).

(2*S*)-{2,4-Dioxo-1-[4-(3-phenyl-ureido)-benzyl]-1,3-diaza-spiro[4.5]dec-3-yl}-4-methyl- pentanoic Acid 17

A soln of BuLi (21 mL, 2.5 M in hexane) was added at –76 °C under argon to a soln of **16** (7.4 g, 26.24 mmol) in anhyd THF (150 mL). After stirring at –76 °C for 30 min, the reaction mixture was allowed to warm to 0 °C and **13** (6.82 g, 26.24 mmol) was added in portions. After stirring at 0 °C for 30 min, the mixture was adjusted to pH 1 by addition of 1 N HCl, diluted with H_2O (100 mL) and extracted with EtOAc (100 mL). After separating the phases, the organic phase was dried over Na_2SO_4 and, after filtration, the solvent was removed in vacuo. The residue thus obtained was purified by preparative HPLC. Yield: (2.18 g, 16%).

$[\alpha]_D^{23}$ –30.8 (*c* 1, DMF).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 0.89 (d, *J* = 6.0 Hz, 3 H, CH_3), 0.91 (d, *J* = 6.0 Hz, 3 H, CH_3), 1.03–1.97 (m, 12 H, cycl H, CH_2), 2.03–2.22 (m, 1 H, CH), 4.47 (s, 2 H, NCH_2), 4.58 (dd, *J* = 4.0, 10.5 Hz, 1 H, NCH), 6.95 (t, 1 H, *J* = 7.0 Hz, ArH), 7.17–7.50 (m, 8 H, Ar-H), 8.65 (s, 2 H, 2 × NH), 13.00 (br s, 1 H, COOH).

ESI-MS: *m*/*z* (%) = 507.3 (100) $[M + H]^+$, 285.9 (14), 114.4 (23), 93.9 (28).

(3*S*)-(3,4-Dimethoxy-phenyl)-3-[(2*S*)-{2,4-dioxo-1-[4-(3-phenyl-ureido)-benzyl]-1,3-diaza-spiro[4.5]dec-3-yl}-4-methyl-pentanoylamino]-propionic Acid S9059

TOTU (972 mg, 2.96 mmol) and DIPEA (478 μL, 2.81 mmol) were added successively with ice-cooling to a soln of **17** (1.50 g, 2.96 mmol) and **11** (833 mg, 2.96 mmol) in anhyd DMF (20 mL). After 1 h at r.t., the solvent was removed in vacuo, the residue was dissolved in EtOAc (100 mL) and the EtOAc soln was successively with an aq $KHSO_4/K_2SO_4$ soln (50 mL), a sat. $NaHCO_3$ soln (50 mL) and a sat. NaCl soln (50 mL). After separation of the phases and drying of the organic phase over Na_2SO_4 and filtering, the solvent was removed in vacuo and the residue was chromatographed on silica gel (35–70 μm) using EtOAc–heptane, (1:1). After concentrating the product fractions, the residue was dissolved in CF_3CO_2H (20 mL) and stirred for 30 min. at r.t. The mixture was concentrated in vacuo, the residue was dissolved in CH_3CN–H_2O (200 mL) and freeze dried. Yield (1.17 g, 55%); ee 95.2% (HPLC).

HRMS: *m*/*z* calcd for $C_{39}H_{47}N_5O_8$ $[M + H]^+$, 714.3499; found, 714.3497.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.90 (d, *J* = 6.6 Hz, 6 H, 2 CH_3), 1.12–1.84 (m, 10 H,cycl H), 1.42 (m, 1 H, CH), 2.26, 1.80 (m, 2 H, CH_2), 2.70 (m, 2 H, C*H*$_2$CO$_2$H), 3.72 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 4.41, 4.50 (d, *J* = 16.4 Hz, 2 H, NCH_2), 4.54 (dd, *J* = 4.6, 12.1 Hz, 1 H, NCHCO), 5.17 (m, 1 H, NCH), 6.81 (dd, *J* = 2.0, 8.4 Hz, 1 H, ArH), 6.89 (d, *J* = 8.4 Hz, 1 H, ArH), 6.92 (d, *J* = 2.0 Hz, 1 H, ArH), 6.96 (m, 1 H, ArH), 7.20 (d, *J* = 8.6 Hz, 2 H, ArH), 7.27 (m, 2 H, ArH), 7.39 (d, *J* = 8.6 Hz, 2 H, ArH), 7.44 (m, 2 H, ArH), 8.30 (d, *J* = 8.0 Hz, 1 H, NH), 8.64 (s, 1 H, NH), 8.66 (s, 1 H, NH), 12.3 (br s, 1 H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 20.62 (CH_3), 23.20 (CH_3), 20.86, 20.92, 23.74, 31.42, 31.54 (5 cycl C), 25.10 (CH), 35.99 (CH_2), 40.60 (CH_2CO_2H), 40.86 (NCH_2), 49.36 (NCH), 51.97 (NCHCO), 55.34 (OMe), 55.50 (OMe), 62.15 (cycl C), 110,41, 111.61, 118.13, 118.13, 118.13, 121,76, 127.44, 128.73 (12 ArC), 132.00 ($ArCCH_2$), 134.73 (ArCHNH), 138.52 (HNCAr), 139.66 (ArCNH), 147.77 (ArC–OCH_3), 148.52 (ArC–OCH_3), 152.49 (NH-CONH), 155.20 (NCON), 167.55 (CONH), 171.81 (CO_2H), 175.57 (NCO).

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