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Backbone amide linker (BAL) strategy for N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis of peptide aldehydes‡

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Abstract: A rapid and efficient strategy has been developed for the general synthesis of complex peptide aldehydes. N^{α}-Benzyloxycarbonylamino acids were converted to protected aldehyde building blocks for solid-phase synthesis in four steps and moderate overall yields. The aldehydes were protected as 1,3-dioxolanes except for one case where a dimethyl acetal was used. These protected amino aldehyde monomers were then incorporated onto 5-[(2 or 4)-formyl-3,5-dimethoxyphenoxy]butyryl-resin (BAL-PEG-PS) by reductive amination, following which the penultimate residue was introduced by HATU-mediated acylation. The resultant resin-bound dipeptide unit, anchored by a backbone amide linkage (BAL), was extended further by routine Fmoc chemistry procedures. Several model peptide aldehydes were prepared in good yields and purities. Some epimerization of the *C*-terminal residue occurred (10% to 25%), due to the intrinsic stereolability conferred by the aldehyde functional group, rather than any drawbacks to the synthesis procedure.

Keywords: peptide aldehydes; backbone amide linker (BAL); solid-phase synthesis; protease inhibitors; 1,3-dioxolanes; dimethyl acetals

INTRODUCTION

Proteolytic enzymes, in any of four major classes (serine, cysteine, aspartyl and metallo), play key roles in the regulation of a multitude of physiological processes including digestion, fertilization, growth, differentiation, cell signalling/migration, immunological defense, wound healing and apoptosis [3–7]. Uncontrolled proteolysis, however, is implicated in disease states such as AIDS, hypertension, stroke, inflammation, asthma, osteoporosis, the common cold, cancer, muscular dystrophy, arthritis and multiple sclerosis, among others [6]. The design of inhibitors as therapeutic agents to treat such disorders is an active area of medical research [8]. Ever since the discovery that two peptide aldehydes, leupeptin and antipain, isolated from a screening program, inhibit trypsin, plasmin, papain, and the cysteine proteases cathepsin A and B [9], the medicinal properties of peptide aldehydes have been a focus of much research.

The reversible inhibition of proteases by peptide aldehydes results from the formation of hemiacetal (serine proteases) or hemithioacetal (cysteine proteases) tetrahedral intermediates, mimicking the transition states of the enzymes [10–16]. It has also been suggested that peptide aldehyde inhibitors of aspartyl proteases act as transition state analogues, the hydrated form of the aldehyde being responsible for this activity [17]. Examples of naturally occurring and/or synthetic peptide aldehyde inhibitors, along with their respective enzyme targets, are shown in Table 1.

The need for simple and efficient methodology to access peptide aldehydes is due not only to their potential as protease inhibitors. For example, *N*-protected α-amino aldehydes are important starting materials in organic synthesis [18] and in the asymmetric synthesis of heterocyclic compounds [19]. Aldehydes and ketones are used in peptidomimetic design to form reduced peptide bonds, $\Psi[CH_2NH]$, in the hope of generating compounds with increased metabolic stability [20–22]. Aldehyde functionality is also used in preparing larger peptides through ligation strategies, including oxime [23,24] and hydrazone ligations [25], as well as a chemoselective ligation method that forms pseudoproline-containing peptides [26]. Peptides can be modified by α-oxo-aldehydes obtained by periodate oxidation of the side-chains of serine, threonine or

Abbreviations: BAL, 5-[(2 or 4)-formyl-3,5-dimethoxyphenoxy]butyryl handle, or 'backbone amide linker'; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; HPLC, high performance liquid chromatography; NMM, *N*-methylmorpholine; *o*,*p*-PALdehyde, 5-[(2 or 4)-formyl-3,5-dimethoxyphenoxy]butyric acid; PEG-PS, polyethylene glycol–polystyrene (graft support); TMOF, trimethyl orthoformate.

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‡ Taken in part from the Ph.D. thesis of J. C. Kappel, University of Minnesota, November 2003. Portions of this work were reported in preliminary form at the Sixteenth American Peptide Symposium, Minneapolis, Minnesota, USA, 26 June–1 July 1999 [1], and in a *Tetrahedron Letter* [2].

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Table 1 Peptide Aldehyde Protease Inhibitors

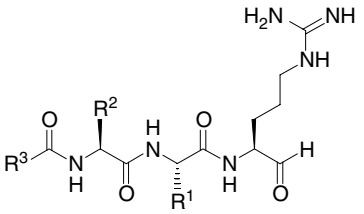
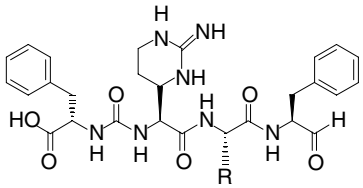
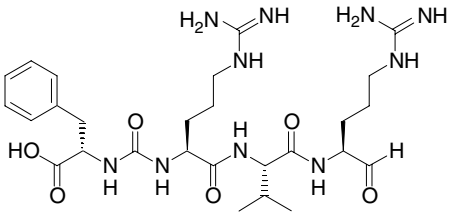
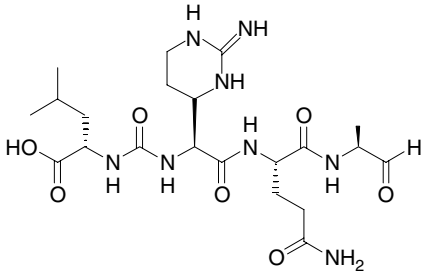
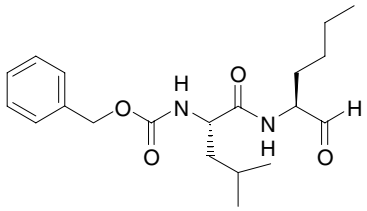
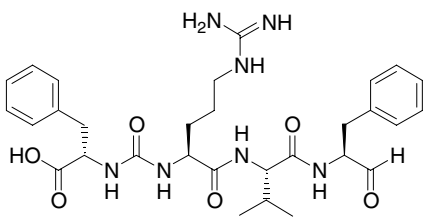
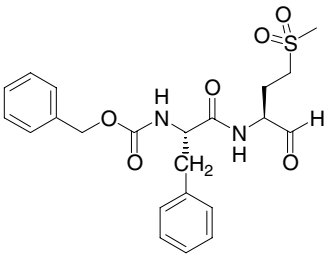
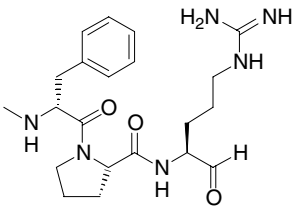
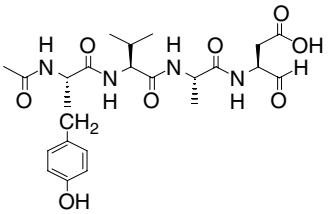
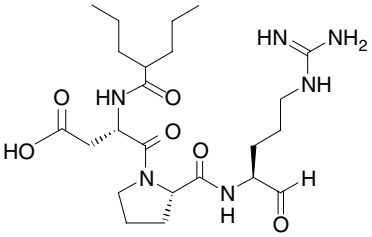
Peptide aldehydes and references	Structure	Protease	Enzyme class	Associated disease state(s)/therapeutic use(s)
Leupeptins [54–59]	 R^1, R^2 = *i*Pr, *i*Bu, *s*Bu; R^3 = Me, Et	Trypsin Plasmin Kallikrein Thrombin Cathepsin B	Serine Serine Serine Serine Cysteine	Pancreatis Tumor invasion, fertility control Inflammation Thrombosis Tumor metastasis, inflammation, bone resorption, myocardial infarction
Chymostatins [54,55,60,61]	 R = *i*Bu, *i*Pr, *s*Bu	Chymotrypsin	Serine	Pancreatis
Antipain [54,55,61–64]		Trypsin Plasmin Cathepsin B	Serine Serine Cysteine	Pancreatis Fertility control Tumor metastasis, inflammation, bone resorption, myocardial infarction
Elastatinal [54,55,65–69]		Elastase	Serine	Inflammation, emphysema, adult respiratory distress syndrome, rheumatoid arthritis
Calpeptin [70,71]		Calpain	Cysteine	Stroke, Alzheimer's disease, muscular dystrophy, cataracts, arthritis
α-MAPI [17,55,72]		Human immunodeficiency virus (HIV) protease	Aspartyl	AIDS

Table 1 (*Continued*)

Peptide aldehydes and references	Structure	Protease	Enzyme class	Associated disease state(s)/therapeutic use(s)
LY338387 [73]		Human rhinovirus 3C protease	Cysteine	Common cold
Efegatran (LY-294468) [74]		Thrombin	Serine	Thrombosis
L-709,049 [75]		Interleukin-1β converting enzyme (ICE)	Cysteine	Chronic and acute inflammatory disease
CVS-1123 [76,77]		Thrombin	Serine	Thrombosis

cysteine [27]; these react with *N*-terminal cysteine to form thiazolidine-ligated peptides [28] or with aminooxy groups to form oxime bonds [29,30].

Various solid-phase strategies have been developed for the synthesis of peptide aldehydes. A popular method utilizes a Weinreb amide [31] linking strategy [32,33]. Following linker loading and peptide assembly, the product is cleaved from the support with $LiAlH_4$. This strategy has been used successfully to synthesize aspartyl aldehyde peptides [34], dipeptide aldehyde inhibitors of β-amyloid production [35], aldehyde and ketone libraries [36], and amino and peptide aldehydes and ketones in concert with 'unnatural amino acid/peptide synthesis' (UPS) methodology [37]. A phenyl ester linker, cleavable upon hydride treatment, has also been used to prepare peptide aldehydes [33]. In another method, Wittig chemistry was used to assemble an olefin linker through which the peptide was bound; following chain elongation, ozonolysis gave the peptide aldehyde [38,39]. Hydrolysable linkers that lead to peptide aldehydes include oxazolidines [40], thiazolidines [41], dibenzosuberyl semicarbazides [42,43], semicarbazones [44], acetals [45] and thioacetals [46,47]. In addition, photolysable substituted anthraquinones have been reported [48].

Contrasting with essentially all previously described solid-phase methods, the present paper shows a way to access peptide aldehydes wherein the management of the aldehyde moiety is *separate* from the anchoring chemistry that first links, and eventually cleaves, the peptide from the support. Thus, the very general backbone amide linker (BAL) strategy [49–51] is

applied herein to appropriate building blocks in which the eventual aldehyde is masked by standard acetal protecting groups.

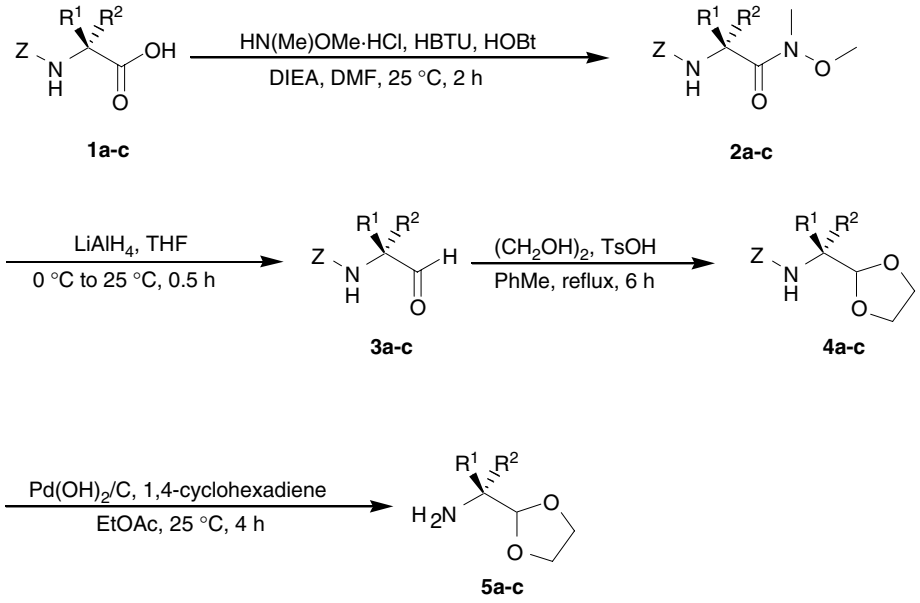
RESULTS AND DISCUSSION

Synthesis of Protected α-Amino Aldehydes

The eventual *C*-terminal aldehyde residues needed to be converted into appropriately protected monomers that could be loaded later onto the solid support. For *C*-terminal alaninals and phenylalaninals, the required 1,3-dioxolanes (**5a–c**) were prepared according to the four-step sequence shown in Scheme 1. Weinreb amides **2** were prepared from N^{α}-benzyloxycarbonylamino acids (**1**), and then reduced with $LiAlH_4$ to give the corresponding aldehydes **3**. Next, treatment with ethylene glycol in refluxing toluene, in the presence of a catalytic amount of *p*-toluenesulfonic acid (TsOH), generated the protected 1,3-dioxolanes **4**.

To optimize removal of the Z group from **4**, various hydrogenolytic conditions were examined (Table 2); the most effective of these was catalytic transfer hydrogenation with 1,4-cyclohexadiene [52] in the presence of Pearlman's catalyst (Table 2, Entry 5). The desired amino acetals **5a–c** were all prepared using these conditions, and obtained in overall yields (based on **1a–c**) of 44%, 51% and 35% respectively.

An alternative procedure [53] was used in the case of aspartic acid to accommodate the *tert*-butyl (O*t*Bu) ester side-chain protecting group (Scheme 2). Activation of Z-Asp(O*t*Bu)-OH as a mixed anhydride, followed by hydride reduction, generated the corresponding alcohol **6**. Swern oxidation gave protected aldehyde intermediate **3d**, which was treated with trimethyl orthoformate



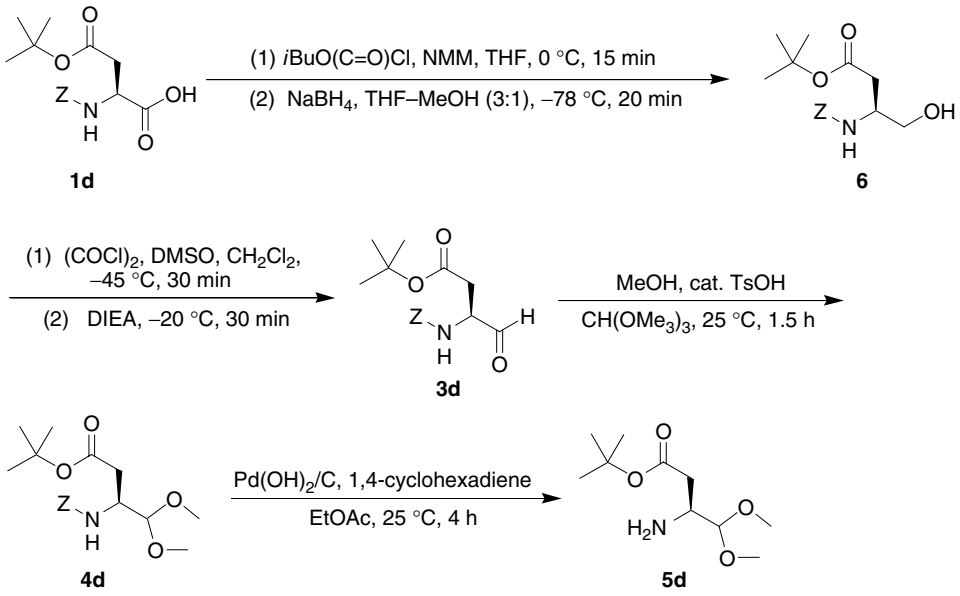
a: $R^1 = CH_3$, $R^2 = H$; **b:** $R^1 = CH_2Ph$, $R^2 = H$; **c:** $R^1 = H$, $R^2 = CH_2Ph$

Scheme 1 Preparation of protected amino aldehydes (**5a–c**).

Table 2 Optimization of Hydrogenation Conditions used for Transformation of **4a** to **5a**

Entry	Hydrogen source	Catalyst	Solvent	Time (h)	Yield (%)
1	H_2	Pd/C	EtOAc	12	22
2	H_2	Pd/C	MeOH	5	9
3	H_2	$Pd(OH)_2/C$	EtOAc	6	63
4	1,4-cyclohexadiene	Pd/C	EtOAc	5	37
5	**1,4-cyclohexadiene**	**$Pd(OH)_2/C$**	**EtOAc**	**2**	**87**

See Experimental section for detailed conditions. Entry 5 is in bold because these conditions give the highest yield.



Scheme 2 Preparation of H-Asp(O*t*Bu)-H dimethyl acetal (**5d**).

(TMOF)–MeOH–TsOH to generate dimethyl acetal **4d**. Removal of the Z group using the already described optimized conditions gave compound **5d** with a free amino group in an overall yield of 24% based on starting protected amino acid.

Solid-Phase Synthesis of Peptide Aldehydes Using the Backbone Amide Linker (BAL) Strategy

BAL-PEG-PS resin (**7**) was prepared according to previously described procedures [49]. The masked α-amino aldehydes **5a–d** were linked onto the resin by $NaBH_3CN$-promoted reductive aminations in DMF–HOAc (99 : 1) (Scheme 3). Peptide synthesis proceeded with acylation of the secondary BAL-linked amine, by using the incoming Fmoc-amino acid as activated *in situ* by HATU-DIEA in CH_2Cl_2–DMF (9 : 1). Past this point, chain elongation followed standard Fmoc procedures. Treatment of completed peptidyl-resins with trifluoroacetic acid (TFA)–H_2O (19 : 1) released the final products, with concomitant cleavage of the acetal moieties to free the *C*-terminal aldehyde functionalities (Scheme 3).

Model peptide aldehydes synthesized by the outlined method were analysed by reversed-phase HPLC and FABMS (Table 3). Cleavage yields were typically above 50%, and as high as 73%. Analytical HPLC (e.g. Figure 1) showed that the initial cleaved products were reasonably homogeneous (78% to 97%).

Racemization Studies

The C^{α}-proton adjacent to an aldehyde function, be it in the starting α-amino aldehyde or in the product *C*-terminal peptide aldehyde, is reported to be susceptible to epimerization under conditions of silica gel chromatography or reversed-phase HPLC [32,40]. To address the question of racemization of the α-amino aldehyde moiety, the route to Fmoc-Glu-Val-Val-Phe-H (Table 3, Entry 3), starting from Z-Phe-OH, was repeated starting from Z-D-Phe-OH (**1c**, Scheme 1) to provide a reference for the corresponding diastereomeric Fmoc-protected peptide aldehyde (Table 3, Entry 4). Separate HPLC analyses of the Fmoc-protected L and D peptides showed similar retention times, and when the compounds were co-injected, only one main peak was observed. Starting with the same protected peptide-resins, final Fmoc deprotection steps were carried out, followed by TFA cleavage, to provide the free peptides H-Glu-Val-Val-Phe-H and H-Glu-Val-Val-D-Phe-H. These free peptides were easily resolved

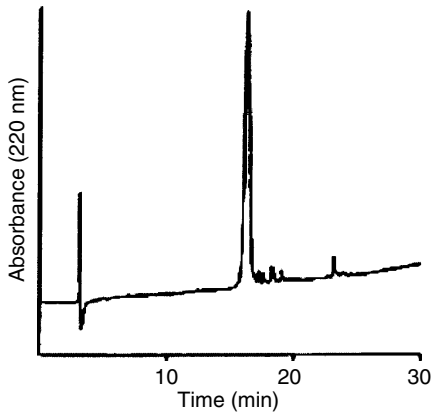
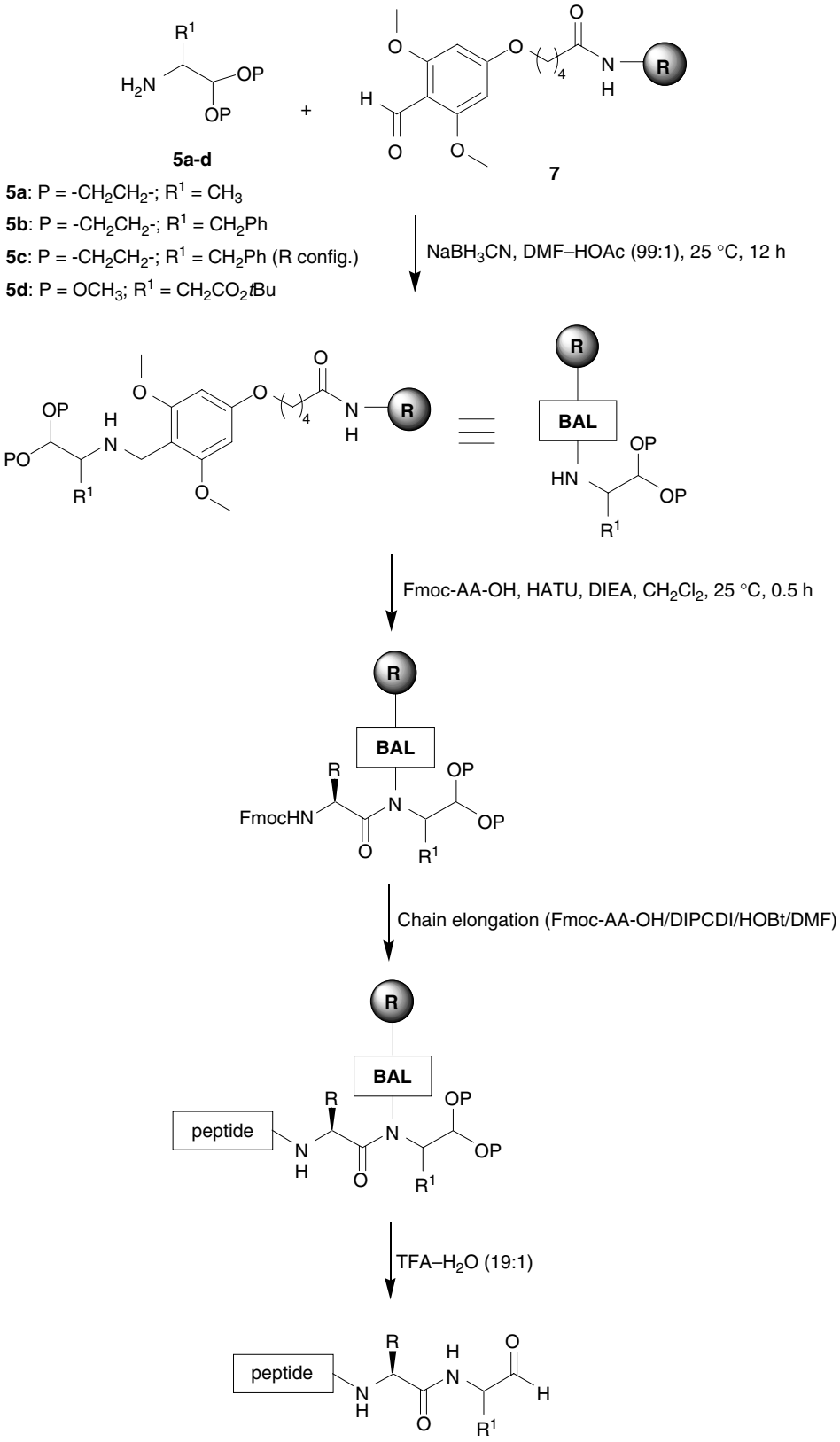


Figure 1 Analytical reversed phase-HPLC profile of Fmoc-Val-Ala-Asp-H (Table 3, Entry 5). Linear gradient of 0.1% aqueous TFA and 0.1% TFA in CH_3CN was run at 1.0 ml/min flow rate from 9 : 1 to 0 : 10 over 35 min, then to 0 : 10 over the next 5 min.



Scheme 3 Solid-phase synthesis of peptide aldehydes with BAL anchoring.

Table 3 Peptide Aldehydes Synthesized According to Scheme 3

Entry	Peptide aldehyde	HPLC analysis[a]	Cleavage yield (%)[b]
1	Fmoc-Asp-Phe-Val-Ala-H	17.4 (82)	56
2	Fmoc-Ala-Phe-H	22.4 (82)	37
3	Fmoc-Glu-Val-Val-Phe-H	18.9 (78)	50
4	Fmoc-Glu-Val-Val-D-Phe-H	18.6 (92)	N.D.
5	Fmoc-Val-Ala-Asp-H	16.3 (97)	73

[a] Retention time (min) and initial purity (%).
[b] Measured by amino acid analysis (N.D. = not determined)

Table 4 Enantiopurity of H-Val-Val-Phe-H as a Function of Time Following Acidic Cleavage

Time (h)	L (%)	D (%)
1	90.0	10.0
3	89.8	10.2
18	85.5	14.5

by HPLC: in the peptide with *C*-terminal L-Phe-H, 10% of the opposite diastereomer (D) was present, while in the peptide with the *C*-terminal D-Phe-H, 26% of L was present. After the peptide with the *C*-terminal L-Phe-H was left to stand in neat TFA for 18 h, 14.5% of the D-diastereomer was observed (Table 4).

CONCLUSIONS

In conclusion, an efficient strategy, based on BAL anchoring, has been developed for preparing *C*-terminal peptide aldehydes. Amino acid-derived acetals were synthesized in solution, and loaded onto the support by reductive amination. Acylation of the resulting secondary amines, followed by standard peptide synthesis protocols, was used to assemble protected resin-bound peptides. Treatment with TFA–H_2O (19 : 1) released the peptides from the supports, with concomitant cleavage of protecting groups, to give the free peptide aldehyde products. Racemization of aldehyde products was observed by HPLC when the peptides were fully deprotected, and proceeded slowly under the acidic cleavage conditions. The method reported here differs from literature procedures to prepare *C*-terminal peptide aldehydes because special linkers or non-standard cleavage conditions do not need to be applied. Therefore, this method is potentially of considerable generality and should be applicable to aldehydes more complex than those presented here.

EXPERIMENTAL SECTION

General

Materials, solvents, instrumentation and general methods were essentially as described in previous publications from our laboratory [49,50]. Organic transformations and washes were at 25 °C, unless indicated otherwise. Polymer-supported reactions were carried out using plastic syringes (3, 5 and 10 ml) fitted with polypropylene frits. PEG-PS · HCl resin and 5-[(2 or 4)-formyl-3,5-dimethoxyphenoxy]butyric acid (*o,p*-PALdehyde) were obtained from PE Biosystems (Framingham, MA). All solvents were reagent grade from Sigma-Aldrich Chemicals (Milwaukee, WI). CH_2Cl_2 was freshly distilled from anhydrous calcium hydride.

^{1}H NMR spectra were obtained at ambient temperature on Varian VI 300 or Varian VI 200 spectrophotometers. Fast atom bombardment mass spectroscopy (FABMS) was performed on a VG7070E-HF mass spectrometer and chemical ionization mass spectroscopy (CIMS) was performed on a Perkin Elmer Sciex API *III* triple quadrupole mass spectrometer equipped with ionspray interface. Analytical HPLC was performed using a Vydac C_{18} reversed-phase column (0.46 × 25 cm) on a Beckman instrument, configured with two 112 pumps and a 165 variable wavelength detector set at 220 nm and 280 nm. Linear gradients of 0.1% aqueous TFA and 0.1% TFA in CH_3CN were run at 1.0 ml/min flow rate from: 9 : 1 to 0 : 10 over 35 min, then to 0 : 10 over the next 5 min.

Amino-protected Weinreb amides

(S)-(1-(Methoxymethylcarbamoyl)ethyl)carbamic acid benzyl ester (Z-Ala-N(Me)OMe) (2a). N^{α}-Benzyloxycarbonyl-L-alanine (2.82 g, 12.6 mmol) was dissolved in DMF (40 ml), and then HBTU (5.27 g, 13.9 mmol), HOBt · H_2O (2.13 g, 13.9 mmol), DIEA (5.73 g, 32.9 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (3.08 g, 31.6 mmol) were added sequentially to the solution. After 2 h at 25 °C, the reaction mixture was diluted with EtOAc (400 ml), and was washed with 5% aqueous $NaHCO_3$ (3 × 100 ml), 10% aqueous citric acid (3 × 100 ml), and brine (3 × 100 ml), dried ($MgSO_4$), and concentrated *in vacuo* providing the title compound as a white solid (2.99 g, 89%); R_f 0.70 ($CHCl_3$–MeOH, 9 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.27–7.37 (m, 5H), 5.54 (d, J = 7.8 Hz, 1H), 5.08 (s, 2H), 4.73 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.34 (d, J = 6.8 Hz, 3H); FABMS calcd for $C_{13}H_{18}N_2O_4$ 266.3, found 267.2 $[M + H]^+$.

(S)-(1-(Methoxymethylcarbamoyl)phenylethyl)carbamic acid benzyl ester (Z-Phe-N(Me)OMe) (2b). Prepared as described for **2a**, but starting with N^{α}-benzyloxycarbonyl-L-phenylalanine (2.74 g, 9.2 mmol), providing the title compound as a colorless oil (2.82 g, 90%); R_f 0.57 (hexanes–EtOAc, 1 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.13–7.32 (m, 10H), 5.42 (d, J = 8.4 Hz, 1H), 4.98–5.13 (m, 3H), 3.68 (s, 3H), 3.17 (s, 3H), 2.86–3.13 (m, 2H); FABMS calcd for $C_{19}H_{22}N_2O_4$ 342.4, found 343.2 $[M + H]^+$.

(R)-(1-(Methoxymethylcarbamoyl)phenylethyl)carbamic acid benzyl ester (Z-D-Phe-N(Me)OMe) (2c). Prepared as described for **2b**, but starting with N^{α}-benzyloxycarbonyl-D-phenylalanine (1.53 g, 5.1 mmol), providing the title compound as a colorless oil (1.59 g, 91%); ^{1}H NMR same as **2b**.

Reduction of amino-protected Weinreb amides to generate the corresponding aldehydes

(S)-(1-formyl-2-ethyl)carbamic acid benzyl ester (Z-Ala-H) (3a). $LiAlH_4$ (1 M in THF; 10 ml) was added dropwise at 0 °C to a solution of **2a** (0.89 g, 3.4 mmol) in THF (25 ml) under N_2. The ice bath was then removed, and the reaction was allowed to proceed for 30 min at 25 °C. Next, the reaction mixture was diluted with Et_2O (100 ml) and quenched with 0.5 N aqueous HCl (20 ml). The aqueous phase was extracted with Et_2O (3 × 20 ml), and the combined organic layers were washed with 1.0 N aqueous HCl (3 × 20 ml), 5% aqueous $NaHCO_3$ (3 × 20 ml), brine (3 × 20 ml), dried (Na_2SO_4) and concentrated *in vacuo* to provide the title compound as a colorless oil (0.57 g, 83%); R_f 0.45 ($CHCl_3$–MeOH, 9 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.57 (s, 1H), 7.36 (s, 5H), 5.38 (s, 1H), 5.12 (s, 2H), 4.26–4.36 (m, 1H), 1.38 (d, J = 7.2 Hz, 3H).

(S)-(1-formyl-2-phenylethyl)carbamic acid benzyl ester (Z-Phe-H) (3b). Prepared as described for **3a**, but starting from **2b** (1.45 g, 4.2 mmol), providing the title compound as a waxy solid (1.02 g, 85%); R_f 0.52 (hexanes–EtOAc, 1 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.62 (s, 1H), 7.01–7.36 (m, 10H), 5.41 (d, J = 6.4 Hz, 1H), 5.12 (s, 2H), 4.37–4.60 (m, 1H), 3.13 (d, J = 6.6 Hz, 2H); FABMS calcd for $C_{17}H_{17}N_1O_3$ 283.3, found 284.1 $[M + H]^+$.

(R)-(1-formyl-2-phenylethyl)carbamic acid benzyl ester (Z-D-Phe-H) (3c). Prepared as described for **3b**, but starting from **2c** (1.66 g, 4.9 mmol), providing the title compound as a colorless oil (1.28 g, 93%); ^{1}H NMR same as **3b**.

3-Benzyloxycarbonylamino-4-oxobutyric acid tert-butyl ester (Z-Asp(OtBu)-H) (3d). Oxalyl chloride (3.3 ml, 6.5 mmol) was added dropwise, at −45 °C, to a solution of DMSO (0.85 ml, 12.0 mmol) in CH_2Cl_2 (15 ml) under N_2. After 5 min, Z-Asp(O*t*Bu)-ol (**6**) (1.91 g, 5.4 mmol) in CH_2Cl_2 (9 ml) was added dropwise, and the mixture was stirred for 30 min at −45 °C. DIEA (2.9 ml, 16.9 mmol) was added, and the reaction was allowed to warm to −20 °C following which it was stirred for 30 min. The reaction solution was diluted with CH_2Cl_2 (60 ml), then washed with H_2O (20 ml), 5% aqueous $NaHCO_3$ (20 ml), H_2O (3 × 20 ml), and dried (Na_2SO_4), and concentrated *in vacuo* to provide the title compound as a yellow oil (1.32 g, 75%); R_f 0.63 (CH_2Cl_2–MeOH, 20 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.64 (s, 1H), 7.36 (s, 5H), 5.90 (d, J = 8 Hz, 1H), 5.13 (s, 2H), 4.38 (ddd, J_1 = 8.4 Hz, J_2 = 4.8 Hz, J_3 = 4.6 Hz, 1H), 2.95 (dd, J_1 = 17 Hz, J_2 = 4.6 Hz, 1H), 2.75 (dd, J_1 = 17 Hz, J_2 = 4.8 Hz, 1H), 1.41 (s, 9H).

Ketal formation from amino protected α-amino aldehydes to generate the corresponding 1,3-dioxolanes

(S)-(1-(1,3)Dioxolan-2-yl-ethyl)carbamic acid benzyl ester (Z-Ala-H ethylene acetal) (4a). A solution of the protected α-amino aldehyde **3a** (1.17 g, 5.7 mmol) with ethylene glycol (3.2 ml, 57 mmol) and TsOH (0.14 g, 0.7 mmol) in toluene (90 ml) was refluxed for 6 h using a Dean-Stark trap to remove H_2O formed during the reaction. Following concentration *in vacuo*, the crude product mixture was taken up in EtOAc (60 ml), washed with H_2O (3 × 20 ml) and brine (3 × 20 ml) and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was then purified by silica gel chromatography (hexanes–EtOAc, 1 : 1), providing the title compound as a colorless oil (1.43 g, 68%); R_f 0.41 (hexanes–EtOAc, 1 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.34–7.38 (m, 5H), 5.11 (s, 2H); 4.90 (d, J = 7.2 Hz, 1H), 4.84 (d, J = 2.2 Hz, 1H), 3.88–3.98 (m, 5H), 1.16 (d, J = 6.8 Hz, 3H); FABMS calcd for $C_{13}H_{17}N_1O_4$ 251.3, found 252.1 $[M + H]^+$.

(S)-(1-(1,3)Dioxolan-2-yl-2-phenylethyl)carbamic acid benzyl ester (Z-Phe-H ethylene acetal) (4b). Prepared as described for **4a**, but starting from **3b** (1.03 g, 3.6 mmol), providing the title compound as a white solid (0.83 g, 70%); R_f 0.75 (hexanes–EtOAc, 1 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.20–7.36 (m, 10H), 5.04 (s, 2H), 4.92 (s, 1H), 4.85 (d, J = 2.0 Hz, 1H), 3.87–4.00 (m, 5H), 2.86 (t, 2H); FABMS calcd for $C_{19}H_{21}N_1O_4$ 327.4, found 328.2 $[M + H]^+$.

(R)-(1-(1,3)Dioxolan-2-yl-2-phenylethyl)carbamic acid benzyl ester (Z-D-Phe-H ethylene acetal) (4c). Prepared as described for **4b**, but starting from **3c** (1.47 g, 4.5 mmol), providing the title compound as a white solid (0.72 g, 49%); R_f 0.74 (hexanes–EtOAc, 1 : 1); ^{1}H NMR same as **4b**.

(S)-3-Benzyloxycarbonylamino-4,4-dimethoxybutyric acid tert-butyl ester (Z-Asp(OtBu)-H dimethyl acetal) (4d). A solution of Z-Asp(O*t*Bu)-H (**3d**) (1.32 g, 4.1 mmol) plus TsOH (20 mg, 0.1 mmol), in MeOH (6 ml) plus $CH(OMe)_3$ (2.2 ml, 20.3 mmol), was stirred at 25 °C for 1.5 h, and then concentrated. The residue was then diluted with CH_2Cl_2 (60 ml), washed with 5% aqueous $NaHCO_3$ (2 × 20 ml) and H_2O (2 × 20 ml), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was then purified by silica gel chromatography (hexanes–EtOAc, 4 : 1), providing the title compound as a yellow oil (0.66 g, 44%); R_f 0.71 (hexanes–EtOAc, 1 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33–7.37 (m, 5H), 5.14 (d, J = 12.3 Hz, 1H), 5.08 (d, J = 12.3 Hz, 1H), 4.35 (d, J_1 = 3.9 Hz, 1H), 4.16–4.25 (m, 1H), 3.42 (s, 6H), 2.54 (dd, J_1 = 5.7 Hz, J_2 = 15.7 Hz, 1H), 2.45 (dd, J_1 = 6.5 Hz, J_2 = 15.7 Hz, 1H), 1.43 (s, 9H); FABMS calcd for $C_{18}H_{27}NO_6$ 353.4, found 354.2 $[M + H]^+$.

Hydrogenation of amino-protected 1,3-dioxolane derivatives to selectively generate free α-amino groups

(S)-1-(1,3)Dioxolan-2-yl-ethylamine (H-Ala-H ethylene acetal) (5a). Palladium hydroxide [20 wt. % on carbon; Pearlman's catalyst] (0.22 g) was added to a N_2-purged solution of **4a** (0.22 g, 0.9 mmol) in EtOAc (3 ml). 1,4-Cyclohexadiene (0.84 ml, 8.9 mmol) was then added, and the reaction was allowed to proceed for 4 h at 25 °C. Next, the reaction mixture was filtered through Celite, and concentrated *in vacuo* to provide the title compound as a yellow oil (90 mg, 87% overall); R_f 0.19 (EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.61 (d, J = 3.8 Hz, 1H), 3.87–3.96 (m, 4H), 2.85–2.97 (m, 1H), 1.53 (s, 2H), 1.08 (d, J = 6.6 Hz, 3H); CIMS calcd for $C_5H_{11}N_1O_2$ 117.2, found 118.1 $[M + H]^+$.

(S)-1-(1,3)Dioxolan-2-yl-2-phenylethylamine (H-Phe-H ethylene acetal) (5b). Prepared as described for **5a** but starting from **4b** (0.66 g, 2.0 mmol), providing the title compound as a yellow oil (0.37 g, 95%); R_f 0.48 ($CHCl_3$–MeOH, 9 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.21–7.38 (m, 5H), 4.75 (d, J = 3.6 Hz, 1H), 3.91–4.05 (m, 4H), 3.12 (quintet, J = 4.4 Hz, 1H), 2.95 (dd, J_1 = 13.5 Hz, J_2 = 4.8 Hz, 1H), 2.62 (dd, J_1 = 9.2 Hz,

$J_2 = 4.5$ Hz, 1H), 1.34 (s, 2H); CIMS calcd for $C_{11}H_{15}N_1O_2$ 193.2, found 194.1 $[M + H]^+$.

(R)-1-(1,3)Dioxolan-2-yl-2-phenylethylamine (H-D-Phe-H ethylene acetal) (5c). Prepared as described for **5b** but starting from **4c** (0.31 g, 1.1 mmol), providing the title compound as a yellow oil (0.18 g, 85%); ^{1}H NMR same as **5b**.

(S)-3-Amino-4,4-dimethoxybutyric acid* tert*-butyl ester (H-Asp(OtBu)-H dimethylacetal) (5d). Palladium hydroxide [20 wt. % on carbon; Pearlman's catalyst] (0.33 g) was added to a N_2-purged solution of Z-protected dimethyl ketal **4d** (0.33 g, 0.9 mmol) in EtOAc (2.5 ml), following which the suspension was diluted with further EtOAc (2.5 ml) and then 1,4-cyclohexadiene (0.84 ml, 8.9 mmol) was added. The reaction was allowed to proceed for 24 h at 25 °C, following which the mixture was filtered through Celite and concentrated, providing the title compound as a yellow oil (0.16 g, 77%); R_f 0.49 ($CHCl_3$–MeOH, 9 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.12 (d, $J = 5.7$ Hz, 1H), 3.42 (s, 3H), 3.40 (s, 3H), 3.23–3.29 (m, 1H), 2.52 (dd, $J_1 = 16.2$ Hz, $J_2 = 3.9$ Hz, 1H), 2.22 (dd, $J_1 = 8.7$ Hz, $J_2 = 15.9$ Hz, 1H), 1.44 (s, 9H); CIMS calcd for $C_{10}H_{21}N_1O_4$ 219.3, found 220.1 $[M + H]^+$.

(S)-3-Benzyloxycarbonylamino-4-hydroxybutyric acid* tert*-butyl ester (6). *N*-Methylmorpholine (0.71 ml, 6.5 mmol) and isobutyl chloroformate (0.80 ml, 6.2 mmol) were added, at 0 °C, to a solution of Z-Asp(O*t*Bu)-OH (2.00 g, 5.86 mmol) in THF (35 ml). Activation continued for 15 min, following which the solution was added dropwise to a suspension of $NaBH_4$ (0.44 g, 11.7 mmol) in THF–MeOH (3 : 1, 50 ml) at −78 °C. Stirring continued for 20 min at −78 °C and then the reaction was quenched with HOAc–H_2O (1 : 9; 25 ml). The solvent was partially concentrated, and the residue was extracted with EtOAc (3 × 50 ml). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (3 × 50 ml) and H_2O (3 × 50 ml), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was then purified by silica gel chromatography (CH_2Cl_2–MeOH, 20 : 1), providing the title compound as a yellow oil (1.77 g, 93%); R_f 0.32 (CH_2Cl_2–MeOH, 20 : 1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.32 (s, 5H), 5.54 (d, $J = 6.2$ Hz, 1H), 5.07 (s, 2H), 4.00–4.04 (m, 1H), 3.65–3.73 (m, 2H), 2.66–2.76 (m, 1H), 2.52 (d, $J = 6.2$ Hz, 1H), 1.41 (s, 9H).

Solid-Phase Synthesis

BAL-Ile-PEG-PS resin (7) (49). PEG-PS · HCl resin (2 g, 0.20 mmol/g) was washed with CH_2Cl_2 (3 × 0.5 min), TFA–CH_2Cl_2 (2 : 3, 1 × 1 min, 1 × 20 min), CH_2Cl_2 (5 × 0.5 min), DIEA–CH_2Cl_2 (1 : 19, 4 × 1 min), CH_2Cl_2 (5 × 0.5 min) and DMF (5 × 0.5 min). Next, Fmoc-Ile-OH (0.68 g, 5 equiv) and HOBt · H_2O (0.26 g, 5 equiv) were dissolved separately in CH_2Cl_2–DMF (1 : 1, 5 ml total), combined, and added to the resin. DIPCDI (0.3 ml, 2.0 mmol, 5 equiv) was added next to the resin, and the reaction was agitated for 12 h. This was followed by washings with DMF (5 × 0.5 min), CH_2Cl_2 (5 × 0.5 min) and DMF (3 × 0.5 min). Fmoc removal was accomplished by treatment with piperidine–DMF (1 : 4, 3 × 1 min, 3 × 5 min), followed by washing with DMF (10 × 0.5 min). Solid *o,p*-PALdehyde (0.45 g, 1.6 mmol, 4 equiv) and HATU (0.61 g, 4 equiv) were combined and dissolved in DMF (3 ml); DIEA (0.56 ml, 8 equiv) was added, and, after 1 min preactivation, this solution was added to the resin. Coupling proceeded for 2 h, followed by resin washings with DMF (5 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min).

Fmoc-Asp-Phe-Val-Ala-H (Table 3, Entry 1). **5a** (17 mg, 15 equiv) and $NaBH_3CN$ (11 mg, 15 equiv) were dissolved together in DMF–HOAc (99 : 1, 0.6 ml), and added to BAL-Ile-PEG-PS resin (**7**) (50 mg, 0.20 mmol/g). Reaction for 12 h at 25 °C gave H-(BAL-Ile-PEG-PS)Ala-$(OCH_2)_2$, which was washed consecutively with DMF (5 × 0.5 min), CH_2Cl_2 (5 × 0.5 min), DMF (3 × 0.5 min), piperidine–DMF (1 : 4, 3 × 1 min), DMF (5 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). Subsequently, Fmoc-Val-OH (35 mg, 0.1 mmol, 10 equiv) was dissolved in CH_2Cl_2–DMF (9 : 1, 0.6 ml), DIEA (35 µl, 0.2 mmol, 20 equiv) was added, the solution was added to the resin, and coupling initiated by addition of solid HATU (38 mg, 0.1 mmol, 10 equiv) was carried out for 2 h. The peptide-resin was then washed with CH_2Cl_2 (5 × 0.5 min), DMF (5 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). The remaining two residues, Fmoc-Phe-OH and Fmoc-Asp-(O*t*Bu)-OH, were incorporated using standard Fmoc protocols [Fmoc-AA-OH (5 equiv), DIPCDI (8 µl, 5 equiv)/HOBt (8 mg, 5 equiv) in DMF] to provide the completed peptide-resin. An aliquot of resin was cleaved (56% cleavage yield) with TFA–H_2O (19 : 1) at 25 °C for 1 h, and the filtrate was collected, concentrated and analysed by analytical HPLC (t_R 17.4 min, 82% purity); FABMS calcd for $C_{36}H_{40}N_4O_8$ 656.3, found 657.3 $[M + H]^+$.

Fmoc-Ala-Phe-H (Table 3, Entry 2). Prepared as described above, but using **5b** to reductively aminate BAL-Ile-PEG-PS resin (**7**) and Fmoc-Ala-OH to acylate the resulting secondary amine. An aliquot of resin was cleaved (37% cleavage yield) with TFA–H_2O (19 : 1) at 25 °C for 1 h, and the filtrate was collected, concentrated and analysed by analytical HPLC (t_R 22.4 min, 82% purity). FABMS calcd for $C_{27}H_{26}N_2O_4$ 442.2, found 443.1 $[M + H]^+$.

Fmoc-Glu-Val-Val-Phe-H (Table 3, Entry 3). Prepared as described above, using **5b** to reductively aminate BAL-Ile-PEG-PS resin (**7**), and peptide elongation with Fmoc-Val-OH (×2) and Fmoc-Glu(O*t*Bu)-OH. An aliquot of resin was cleaved (50% cleavage yield) with TFA–H_2O (19 : 1) at 25 °C for 1 h, and the filtrate was collected, concentrated and analysed by analytical HPLC (t_R 18.9 min, 90% purity); FABMS calcd for $C_{39}H_{46}N_4O_8$ 698.3, found 699.2 $[M + H]^+$.

Fmoc-Glu-Val-Val-D-Phe-H (Table 3, Entry 4). Prepared as described above, but using **5c** to reductively aminate BAL-Ile-PEG-PS resin (**7**). An aliquot of resin was cleaved with TFA–H_2O (19 : 1) at 25 °C for 1 h, and the filtrate was collected, concentrated and analysed by analytical HPLC (t_R 18.6 min, 90% purity); FABMS calcd for $C_{39}H_{46}N_4O_8$ 698.3, found 699.4 $[M + H]^+$, 697.4 $[M - H]^-$.

Fmoc-Val-Ala-Asp-H (Table 3, Entry 5). Prepared as described above, but using **5d** to reductively aminate BAL-Ile-PEG-PS resin (**7**), and peptide elongation with Fmoc-Ala-OH and Fmoc-Val-OH. An aliquot of resin was cleaved (73% cleavage yield) with TFA–H_2O (19 : 1) at 25 °C for 1 h, and the filtrate was collected, concentrated and analysed by analytical HPLC (t_R 16.3 min, 97% purity); FABMS calcd for $C_{27}H_{31}N_3O_7$ 509.2, found 510.1 $[M + H]^+$, 508.1 $[M - H]^-$.

H-Glu-Val-Val-Phe-H. Fmoc-Glu-Val-Val-(BAL-Ile-PEG-PS) Phe-$(OCH_2)_2$ was assembled as already described (experimental corresponding to Table 3, entry 3), treated with piperidine–DMF (1 : 4, 3 × 1 ml, 3 × 5 ml), and then washed with DMF (10 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). An aliquot of resin was treated with TFA–H_2O (19 : 1, 1 ml) for 1.5 h, and the

filtrate was collected, concentrated and analysed by analytical HPLC [t_R 13.6 min (87%) and 17.5 min (13%)]; HPLC/ESIMS calcd for $C_{24}H_{36}N_4O_6$ 476.3, found 477.1 $[M + H]^+$ (13.3 min to 13.9 min) and 477.1 $[M + H]^+$ (16.9 min to 17.3 min).

H-Glu-Val-Val-D-Phe-H. Prepared as just described for H-Glu-Val-Val-Phe-H, but starting from Fmoc-Glu-Val-Val-(BAL-Ile-PEG-PS)D-Phe-$(OCH_2)_2$ (experimental corresponding to Table 3, entry), HPLC [t_R 13.5 min (26%) and 17.0 min (74%)].

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REFERENCES

1. Guillaumie F, Kelly NM, Kappel JC, Barany G, Jensen KJ. Solid-phase synthesis of peptide aldehydes by a backbone amide linker (BAL) strategy. In *Peptides for the New Millennium: Proceedings of the Sixteenth American Peptide Symposium*, Fields GB, Tam JP, Barany G (eds). Kluwer: Dordrecht, The Netherlands, 2000; 100–101.
2. Guillaumie F, Kappel JC, Kelly NM, Barany G, Jensen KJ. Solid-phase synthesis of *C*-terminal peptide aldehydes from amino acetals anchored to a backbone amide linker (BAL) handle. *Tetrahedron Lett.* 2000; **41**: 6131–6135.
3. James MNG, Sielecki AR. Aspartic proteinases and their catalytic pathway. In *Biological Macromolecules and Assemblies: Active Sites of Enzymes*, vol. 3, Jurnak FA, McPherson A (eds). John Wiley & Sons: New York, 1987; 413–482.
4. Babine RE, Bender SL. Molecular recognition of protein-ligand complexes: Applications to drug design. *Chem. Rev.* 1997; **97**: 1359–1472.
5. Otto HH, Schirmeister T. Cysteine proteases and their inhibitors. *Chem. Rev.* 1997; **97**: 133–171.
6. Leung D, Abbenante G, Fairlie DP. Protease inhibitors: Current status and future prospects. *J. Med. Chem.* 2000; **43**: 305–341.
7. Walker B, Lynas JF. Strategies for the inhibition of serine proteases. *Cell. Mol. Life Sci.* 2001; **58**: 596–624.
8. Craik MS, Debouck C. Proteases as therapeutic targets. In *Perspectives in Drug Discovery and Design*, vol. 2, James MNG (ed.). ESCOM: Leiden, 1995; 1–125.
9. Aoyagi T, Takeuchi T, Matsuzaki A, Kawamura K, Kondo SI, Hamada M, Maeda K, Umezawa H. Leupeptins, new proteinase inhibitors from actinomycetes. *J. Antibiot.* 1969; **22**: 283–286.
10. Wolfenden R. Analog approaches to the structure of the transition state in enzyme reactions. *Acc. Chem. Res.* 1972; **5**: 10–18.
11. Leinhard GE. Enzymatic catalysis and transition-state theory. *Science* 1973; **180**: 149–154.
12. Thompson RC. Use of peptide aldehydes to generate transition-state analogs of elastase. *Biochemistry* 1973; **12**: 47–51.
13. Schultz RM, Cheerva AC. The binding of a non-specific 'transition state analogue' to α-chymotrypsin. *FEBS Lett.* 1975; **50**: 47–49.
14. Lewis CA, Jr, Wolfenden R. Thiohemiacetal formation by inhibitory aldehydes at the active site of papain. *Biochemistry* 1977; **16**: 4890–4895.
15. Kennedy WP, Schultz RM. Mechanism of association of a specific aldehyde 'transition-state analogue' to the active site of α-chymotrypsin. *Biochemistry* 1979; **18**: 349–356.
16. Gamcsik MP, Malthouse PG, Primrose WU, Mackenzie NE, Boyd ASF, Russell RA, Scott AI. Structure and stereochemistry of tetrahedral inhibitor complexes of papain by direct NMR observation. *J. Am. Chem. Soc.* 1983; **105**: 6324–6325.
17. Sarubbi E, Seneci PF, Angelastro MR, Peet NP, Denaro M, Islam K. Peptide aldehydes as inhibitors of HIV protease. *FEBS Lett.* 1993; **319**: 253–256.
18. Jurczak J, Golebiowski A. Optically active N-protected α–amino aldehydes in organic synthesis. *Chem. Rev.* 1989; **89**: 149–164.
19. Sardina FJ, Rapoport H. Enantiospecific synthesis of heterocycles from α-amino acids. *Chem. Rev.* 1996; **96**: 1825–1872.
20. Jacobson KA, Marr-Leisy D, Rosenkranz RP, Verlander MS, Melmon KL, Goodman M. Conjugates of catecholamines. 1. N-alkyl-functionalized carboxylic acid congeners and amides related to isoproterenol. *J. Med. Chem.* 1983; **26**: 492–499.
21. Hocart SJ, Nekola MV, Coy DH. Effect of the CH_2NH and CH_2NAc peptide bond isosteres on the antagonistic and histamine releasing activities of a luteinizing hormone-releasing hormone analogue. *J. Med. Chem.* 1988; **31**: 1820–1824.
22. Greenlee WJ. Renin inhibitors. *J. Med. Res. Rev.* 1990; **10**: 173–236.
23. Rose K. Facile synthesis of homogeneous artificial proteins. *J. Am. Chem. Soc.* 1994; **116**: 30–33.
24. Canne LE, Ferré-D'Amaré AR, Burley SK, Kent SBH. Total chemical synthesis of a unique transcription factor-related protein: cMyc-Max. *J. Am. Chem. Soc.* 1995; **117**: 2998–3007.
25. King TP, Zhao SW, Lam T. Preparation of protein conjugates via intermolecular hydrazone linkage. *Biochemistry* 1986; **25**: 5774–5779.
26. Liu C-F, Rao C, Tam JP. Orthogonal ligation of unprotected peptide segments through pseudoproline formation for the synthesis of HIV-1 protease analogs. *J. Am. Chem. Soc.* 1996; **118**: 307–312.
27. Dixon HBF, Weitkamp LR. Conversion of the *N*-terminal serine residue of corticotrophin into glycine. *Biochem. J.* 1962; **84**: 462–468.
28. Zhang L, Torgerson TR, Liu X-Y, Timmons S, Colosia AD, Hawiger J, Tam JP. Preparation of functionally active cell-permeable peptides by single-step ligation of two peptide modules. *Proc. Natl Acad. Sci. USA* 1998; **95**: 9184–9189.
29. Pallin TD, Tam JP. Cyclization of totally unprotected peptides in aqueous solution by oxime formation. *J. Chem. Soc. Chem. Commun.* 1995; **19**: 2021–2022.
30. Carulla N, Woodward C, Barany G. Toward new designed proteins derived from bovine pancreatic trypsin inhibitor (BPTI): Covalent cross-linking of two 'core modules' by oxime-forming ligation. *Bioconjug. Chem.* 2001; **12**: 726–741.
31. Nahm S, Weinreb SM. N-methoxy-N-methylamides as effective acylating agents. *Tetrahedron Lett.* 1981; **22**: 3815–3818.
32. Fehrentz JA, Paris M, Heitz A, Velek J, Liu CF, Winternitz F, Martinez J. Improved solid phase synthesis of *C*-terminal peptide aldehydes. *Tetrahedron Lett.* 1995; **36**: 7871–7874.
33. Fehrentz JA, Paris M, Heitz A, Valek J, Winternitz F, Martinez J. Solid phase synthesis of *C*-terminal peptide aldehydes. *J. Org. Chem.* 1997; **62**: 6792–6796.
34. Tong X-H, Hong A. Solid phase synthesis of aspartyl peptide aldehydes. *Tetrahedron Lett.* 2000; **41**: 8857–8860.
35. Higaki JN, Chakravarty S, Bryant CM, Cowart LR, Harden P, Scardina JM, Mavunkel B, Luedtke GR, Cordell B. A combinatorial approach to the identification of dipeptide aldehyde inhibitors of β-amyloid production. *J. Med. Chem.* 1999; **42**: 3889–3898.
36. Salvino JM, Mervic M, Mason HJ, Kiesow T, Teager D, Airey J, Labaudiniere R. Parallel synthesis of aldehydes and ketone facilitated by a new solid-phase Weinreb amide. *J. Org. Chem.* 1999; **64**: 1823–1830.
37. O'Donnell MJ, Drew MD, Pottorf RS, Scott WL. UPS on Weinreb resin: A facile solid-phase route to aldehyde and ketone derivatives of 'unnatural' amino acids and peptides. *J. Comb. Chem.* 2000; **2**: 172–181.

38. Hall BJ, Sutherland JD. A practical method for the combinatorial synthesis of peptide aldehydes. *Tetrahedron Lett.* 1998; **39**: 6593–6596.
39. Paris M, Heitz A, Guerlavais V, Gristau M, Fehrentz JA, Martinez J. Synthesis of peptide aldehydes on solid support using ozonolysis. *Tetrahedron Lett.* 1998; **39**: 7287–7290.
40. Ede NJ, Eagle SN, Wickham G, Bray AM, Warne B, Shoemaker K, Rosenberg S. Solid phase synthesis of peptide aldehyde protease inhibitors. Probing the proteolytic sites of hepatitis C virus polyprotein. *J. Peptide Sci.* 2000; **6**: 11–18.
41. Galéotti N, Giraud M, Jouin P. Solid phase synthesis of peptidyl aldehydes from *C*-terminal thiazolidinyl peptides. *Lett. Pept. Sci.* 1997; **4**: 437–440.
42. Ramage R, Irving SL, McInnes C. Design of a versatile linker for solid phase peptide synthesis: Synthesis of *C*-terminal primary/secondary amides and hydrazides. *Tetrahedron Lett.* 1993; **34**: 6599–6602.
43. Patterson JA, Ramage R. Solid phase synthesis of peptide *C*-terminal semicarbazones and aldehydes. *Tetrahedron Lett.* 1999; **40**: 6121–6124.
44. Murphy AM, Dagnino R, Vallar PL, Trippe AL, Sherman SL, Lumpkin RH, Tamura SY, Webb TR. Automated synthesis of peptide *C*-terminal aldehydes. *J. Am. Chem. Soc.* 1992; **114**: 3156–3157.
45. Yao W, Xu HY. Solid-phase synthesis of peptide aldehydes directly on acetal resin. *Tetrahedron Lett.* 2001; **42**: 2549–2552.
46. Huwe CM, Kunzer H. A novel, thioacetal based linker for solid-phase synthesis. *Tetrahedron Lett.* 1999; **40**: 683–686.
47. Yokum TS, Barany G. Preparation and applications of a thioacetal handle for solid-phase synthesis. In *Peptides for the New Millennium: Proceedings of the Sixteenth American Peptide Symposium*, Fields GB, Tam JP, Barany G (eds). Kluwer Academic: Dordrecht, The Netherlands, 2000; 179–180.
48. Blankespoor RL, DeVries T, Hansen E, Kallemeyn JM, Klooster AM, Mulder JA, Smart RP, Griend DAV. Photochemical synthesis of aldehydes in the solid phase. *J. Org. Chem.* 2002; **67**: 2677–2681.
49. Jensen KJ, Alsina J, Songster MF, Vagner J, Albericio F, Barany G. Backbone amide linker (BAL) strategy for solid-phase synthesis of *C*-terminal-modified and cyclic peptides. *J. Am. Chem. Soc.* 1998; **120**: 5441–5452.
50. Alsina J, Yokum TS, Albericio F, Barany G. Backbone amide linker (BAL) strategy for N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis of unprotected peptide *p*-nitroanilides and thioesters. *J. Org. Chem.* 1999; **64**: 8761–8769.
51. Alsina J, Yokum TS, Albericio F, Barany G. A modified backbone amide linker (BAL) solid-phase peptide synthesis strategy accommodating prolyl, *N*-alkylamino acyl, or histidyl derivatives at the *C*-terminus. *Tetrahedron Lett.* 2000; **41**: 7277–7280.
52. Felix AM, Heimer EP, Lambros TJ, Tzougraki C, Meienhofer J. Rapid removal of protecting groups from peptides by catalytic transfer hydrogenation with 1,4-cyclohexadiene. *J. Org. Chem.* 1978; **43**: 4194–4196.
53. Mindt T, Michel U. Synthesis and evaluation of enantiomeric purity of protected α-amino and peptide aldehydes. *Helv. Chim. Acta* 1999; **82**: 1960–1968.
54. Frommer W, Junge B, Müller L, Schmidt D, Truscheit E. New enzyme inhibitors from microorganisms. *Planta Med.* 1979; **35**: 195–217.
55. Umezawa H, Aoyagi T. In *Proteinase Inhibitors: Medical and Biological Aspects*, Holzer H (ed.). Springer Verlag: Berlin, 1983; 3–15.
56. Schultz RM, Varma-Nelson P, Ortiz R, Kozlowsky KA, Orawsky AT, Pagast P, Frankfater A. Active and inactive forms of the transition-state analog protease inhibitor leupeptin: Explanation of the observed slow binding of leupeptin to cathepsin and papain. *J. Biol. Chem.* 1989; **264**: 1497–1507.
57. McConnell RM, Barnes GE, Hoyng CF, Gunn JM. New leupeptin analogues: Synthesis and inhibition data. *J. Med. Chem.* 1990; **33**: 86–93.
58. McConnell RM, York JL, Frizzel D, Ezell C. Inhibition studies of some serine and thiol proteinases by new leupeptin analogues. *J. Med. Chem.* 1993; **36**: 1084–1089.
59. Schroder E, Phillips C, Garmen E, Harlos K, Crawford C. X-ray crystallographic structure of a papain-leupeptin complex. *FEBS Lett.* 1993; **315**: 38–42.
60. Tatsuta K, Mikami N, Fujimoto K, Umezawa S, Umezawa H, Aoyagi T. The structure of chymostatin, a chymotrypsin inhibitor. *J. Antibiot.* 1973; **26**: 625–646.
61. Bullock TL, Breddam K, Remington SJ. Peptide aldehyde complexes with wheat serine carboxypeptidase II: Implications for the catalytic mechanism and substrate specificity. *J. Mol. Biol.* 1996; **255**: 714–725.
62. Suda H, Aoyagi T, Hamada M, Takeuchi T, Umezawa H. Antipain, a new proteinase inhibitor isolated from actinomycetes. *J. Antibiot.* 1972; **25**: 263–266.
63. Umezawa S, Tatsuta K, Fujikane K, Tsujinaka T, Umezawa H, Naganawa H. Structure of antipain, a new Sakaguchi-positive product of *Streptomyces*. *J. Antibiot.* 1972; **25**: 267–270.
64. Umezawa H. Structures and activities of protease inhibitors of microbial origin. *Methods Enzymol.* 1976; **45**: 678–695.
65. Umezawa H, Aoyagi T, Okura A, Marishima H, Takeuchi T, Okami Y. Elastatinal, a new elastase inhibitor produced by actinomycetes. *J. Antibiot.* 1973; **26**: 787–789.
66. Okura A, Morishima H, Takita T, Aoyagi T, Takeuchi T, Umezawa H. The structure of elastatinal, an elastase inhibitor of microbial origin. *J. Antibiot.* 1975; **28**: 337–339.
67. Feinstein G, Malemuc CJ, Janoff A. The inhibition of human leukocyte elastase and chymotrypsin-like proteinase by elastatinal and chymostatin. *Biochem. Biophys. Acta* 1976; **429**: 925–932.
68. Umezawa H, Aoyagi T. Activities of proteinase inhibitors of microbial origin. In *Proteinases in Mammalian Cells and Tissues*, Barrett A (ed.). Elsevier: Amsterdam, 1977; 637–662.
69. Zimmerman M, Ashe BM. Substrate specificity of the elastase and the chymotrypsin-like enzyme of the human granulocyte. *Biochem. Biophys. Acta* 1977; **480**: 241–245.
70. Mehdi S, Angelastro MR, Wiseman JS, Bey P. Inhibition of the proteolysis of rat erythrocyte membrane proteins by a synthetic inhibitor of calpain. *Biochem. Biophys. Res. Commun.* 1988; **157**: 1117–1123.
71. Ariyoshi H, Shiba E, Kambayashi J, Sakon M, Tsujinaka T, Uemura Y, Mori T. Characteristics of various synthetic peptide calpain inhibitors and their application for the analysis of platelet reaction. *Biochem. Int.* 1991; **23**: 1019–1033.
72. Page P, Bradley M. Solid-phase synthesis of tyrosine peptide aldehydes. Analogues of (S)-MAPI. *J. Org. Chem.* 1999; **64**: 794–799.
73. Shepherd TA, Cox GA, McKinney E, Tang J, Wakulchik M, Zimmerman RE, Villarreal EC. Small peptidic aldehyde inhibitors of human rhinovirus 3C protease. *Bioorg. Med. Chem. Lett.* 1996; **6**: 2893–2896.
74. Bajusz S, Szell E, Bagdy D, Barabas E, Horvath G, Dioszegi M, Fittler Z, Szabo G, Juhasz A, Tomori E, Szilagyi G. Highly active and selective anticoagulants: D-Phe-Pro-Arg-H, a free tripeptide aldehyde prone to spontaneous inactivation, and its stable *N*-methyl derivative, D-MePhe-Pro-Arg-H. *J. Med. Chem.* 1990; **33**: 1729–1735.
75. Chapman KT. Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* 1992; **2**: 613–618.
76. Cousins GR, Friedrichs GS, Sudo Y, Rebello SS, Rote WE, Vlasuk GP, Nolan TG, Mendoza C, Lucchesi BR. Orally effective CVS-1123 prevents coronary artery thrombosis in the conscious dog. *Circulation* 1996; **94**: 1705–1712.
77. Rebello SS, Miller BV, Basler GC, Lucchesi BR. CVS-1123, a direct thrombin inhibitor, prevents occlusive arterial and venous thrombosis in a canin model of vascular injury. *Cardiovasc. Pharmacol.* 1997; **29**: 240–249.