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N-Terminal Peptide Aldehydes as Electrophiles in Combinatorial Solid Phase Synthesis of Novel Peptide Isosteres[1,†]

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N-Terminal peptide aldehydes were synthesized on a solid support and utilized as electrophiles in nucleophilic reactions in order to furnish novel and diverse peptide isosteres. The aldehyde moiety of the peptide was synthesized by coupling a protected aldehyde building block to the peptide and deprotecting it quantitatively in less than 3 min. It was found that protection of the two succeeding amide nitrogens was necessary in order to avoid reaction between the aldehyde and backbone amides. The N-terminal peptide aldehydes were successfully reacted in the following way: (a) reductive amination with a large variety of amines, leading to *N*-alkyl-γ-aminobutyric peptide isosteres positioned centrally in the peptide; (b) reductive amination with amino esters, leading to N-terminal 2,5-diketopiperazine peptides; (c) Horner−Wadsworth−Emmons olefination, leading to unsaturated peptide isosteres positioned centrally in the peptide; and (d) Pictet−Spengler condensations, leading to tetrahydro-β-carbolines either positioned centrally in a peptide or fused with a diketopiperazine ring in the N-terminus of the peptide.

Introduction

Peptide isosteres and peptide mimetics are two classes of compounds which are interesting with respect to their proteolytic enzyme inhibition.[2,3] The appeal of enzymes as drug targets has increased in the past decade as a consequence of the continuous development of combinatorial chemistry as a tool to generate lead compounds in drug discovery.[4] Protease inhibitors of both peptidic and nonpeptidic natures may be synthesized by combinatorial methods on a solid support, and active compounds may be identified by on-bead assaying and single bead analysis.[5−7] For peptide isosteres, the replacement of the scissile amide bond with a chemically more inert type of bond may produce a compound which exhibits a high affinity toward a given protease and no or a very low degree of hydrolysis, thus being a potent inhibitor. Incorporation of a dipeptide isosteric moiety into a randomized peptide library has been shown to afford active enzyme inhibitors.[8] The dipeptide moiety was, in this case, synthesized in solution and successively used as a building block. Another strategy which is attractive is to produce the dipeptide isosteric moiety directly on the N-terminus of a peptide by first attaching the right-hand side of the dipeptide isostrere to the resin bound peptide and, second, coupling the left-hand side of the isostere. The advantage of this approach in library synthesis is the possibility of synthesizing the isosteric moiety *in a combinatorial fashion*, resulting in a much higher degree of diversity. The assembly of a dipeptidic isostere was designed to incorporate the right-hand side as an electrophile, namely an aldehyde, which then was coupled with a variety of nucleophiles (Scheme 1). The reaction between *n* differently substituted aldehydes and *m* different nucleophiles thus produces $n \times m$ isosteres, which thus will be positioned at the N-terminus of the peptide or centrally in the peptide if peptide synthesis can be continued after the nucleophilic reaction. The sheer number of reactions that involve aldehydes is tremendously large, and consequently only a fraction of these were considered. The types of nucleophilic reactions which were selected for transformation of the N-terminal peptide aldehydes were those which were expected to give high yields of the desired products as well as only minor amounts of side products. But more importantly, the resulting products should exhibit some sort of inhibitory/isosteric structure.

C-Terminal peptide aldehydes are interesting molecules for inhibition of serine and cysteine proteases due to their electrophilic nature and properties as transition state analouges.[9−11] Therefore, N-terminal aldehydes are not only interesting as reactive intermediates in peptide isostere synthesis but also as putative protease inhibitors themselves.

† **Abbreviations.** BTC: bis-trichloromethyl carbonate; DCE: 1,2-dichloroethane; DhbtOH: 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; DKP: 2,5-diketopiperazine; ESMS: electrospray mass spectrometry; HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; Hmb: 2-hydroxy-4-methoxybenzyl; HMBA: hydroxymethylbenzoic acid; HOAt: 1-hydroxy-7-azabenzotriazole; HWE: Horner−Wadsworth−Emmons; LCMS: liquid chromatography mass spectrometry; MALDI-TOF-MS: matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MSNT: 2,4,6-mesitylenesulfonyl-3-nitro-1,2,4-triazolide; NEM: *N*-ethyl morpholine; PEGA: poly(ethylene glycol)-poly(acyrl amide) copolymer; Pfp: pentafluorphenol; POEPOP: polyoxyethylene-polyoxypropylene copolymer; RP-HPLC: reversed phase high performance liquid chromatography; TBAF: tetra-*n*-butylammonium fluoride; TBTU: *O*-benzotriazo-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; Trt: trityl. One- and three-letter codes are used for amino acids according to IUPAC recommendations; see http://www.chem.qmw.ac.uk/iupac/AminoAcid/.

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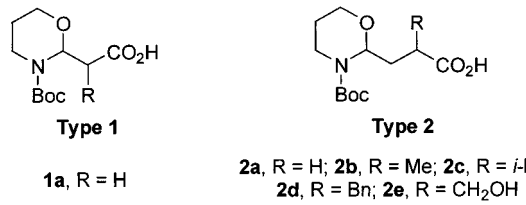
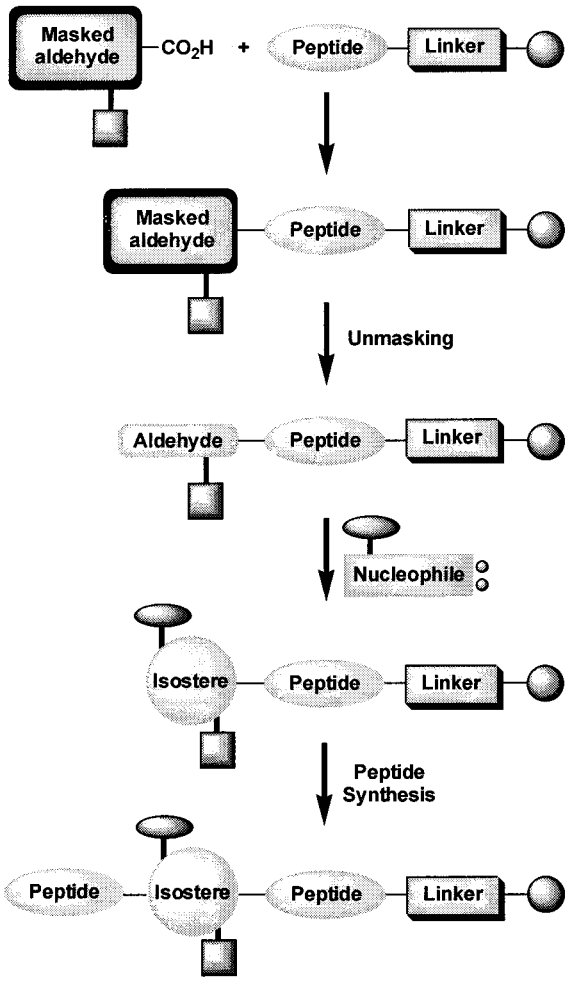


Figure 1. Aldehyde building blocks protected as acid labile *N*-Boc *N*,*O*-acetals.

Scheme 1. Strategy for Synthesizing Peptide Isosters on Solid Phase in a Combinatorial Fashion, via N-Terminal Aldehydes Which Are Incorporated as Building Blocks



Whereas C-terminal aldehydes are well known, N-terminal aldehydes are still quite novel. N-terminal glyoxalic peptide aldehydes have been investigated;[12] however, lack of a side chain proximate to the aldehyde limited the structural mimicry obtainable with this system. The desire to incorporate a side chain on the right-hand side of the isostere resulted in the synthesis of building blocks of type **1** and **2** (Figure 1).[13] These building blocks were designed to contain three essential constituents: (i) a carboxylic acid for attachment onto a peptide, (ii) a C-2 side chain that mimics the side chain of an amino acid, and (iii) a masked aldehyde. As a proof of concept of the usefulness of the *N*-Boc *N*,*O*-acetal aldehyde protection group, unmasking of the aldehyde of resin bound **2a** and successive chemical transformations of the aldehyde have been demonstrated.[13]

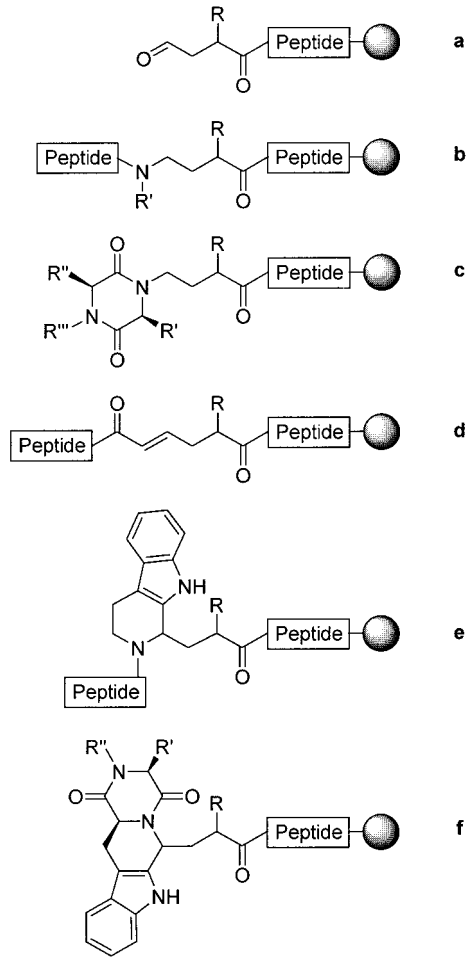


Figure 2. Accessible structures (**b**−**f**) from N-terminal peptide aldehydes (**a**).

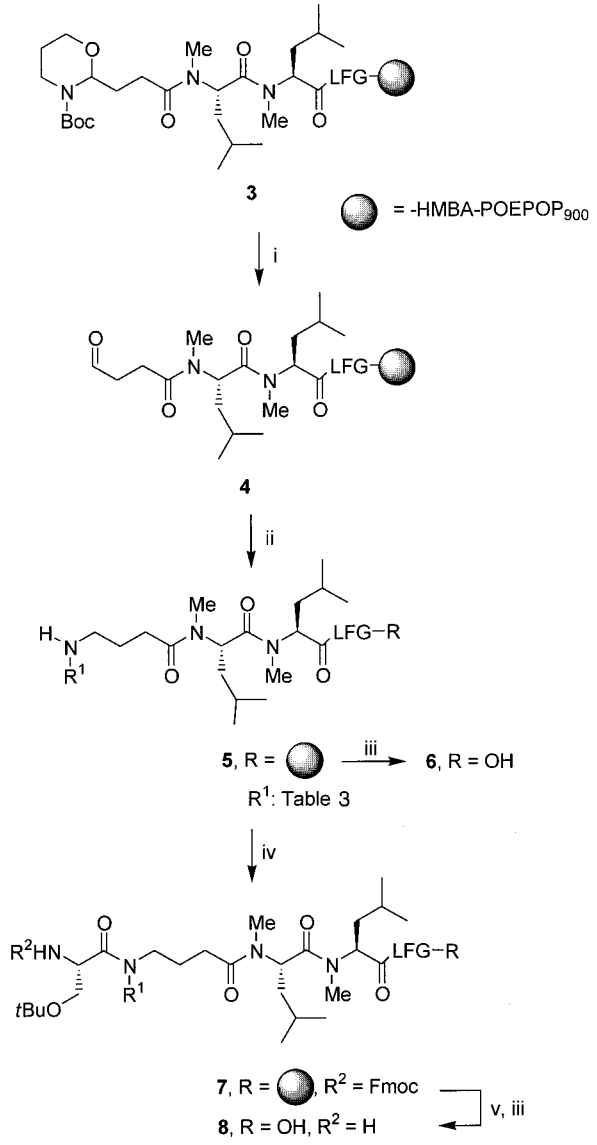
The present paper describes the use of these building blocks for the generation of stable N-terminal peptide aldehydes, the backbone protection necessary for achieving this goal, and their use in various reactions. With the methodologies presented here, structures of type **b**−**f** (Figure 2) are readily accessible from **a**, and the present work thus establishes the chemistry and conditions required for synthesis and on-bead screening of combinatorial libraries of these structural motifs.

Results and Discussion

The Solid Support and Linker. The methodologies established in the present work are aimed at generating combinatorial libraries for on-bead screening of protease inhibitor activity. For this reason, resins that are chemically inert as well as permeable to enzymes were selected as solid supports. In the bulk of the work presented here, the polar PEG-1500 based $POEPOP_{1500}$[14,15] was used, and in some cases the solid supports $POEPOP_{900}$, $SPOCC_{1500}$,[15,16] and $PEGA_{1900}$[17−19] were selected. The chemical structure of POEPOP and SPOCC resins are very similar, and no differences in the reactions described were observed. The properties of these resins have recently been thoroughly investigated.[20]

The hydroxymethyl benzoic acid (HMBA) linker was used throughout this work. This linker is stable to acid and cleaved

Scheme 2. Reductive Amination of Resin Bound N-Terminal Aldehyde **4** and Succeeding Amino Acid Coupling[a]

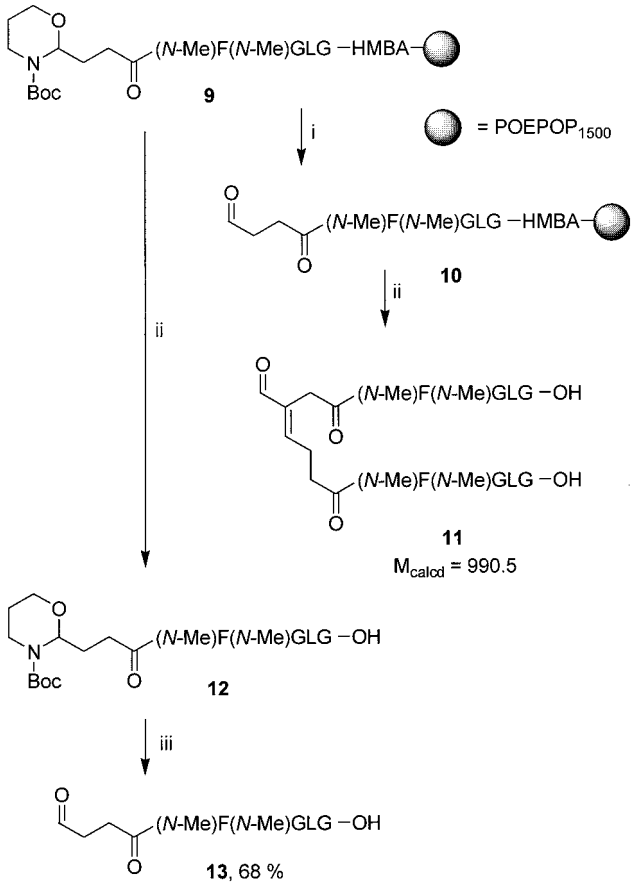


[a] Reagents and conditions: (i); (ii) R-NH_2, $NaBH(OAc)_3$, DMSO:CH_2Cl_2:AcOH (50:50:1); (iii) 0.1 M NaOH, 20 °C, 1−2 h; (iv) Fmoc-Ser(*t*Bu)-OPfp, HOAt, DMF, 50 °C, 2 h; (v) 20% piperidine in DMF.

with aqueous 0.1 M NaOH in 1 h. In a few instances, use of the linker was omitted.

Stable *N*-Terminal Peptide Aldehydes. Peptide **3** was assembled, where both the ultimate and the penultimate amide was N-methylated (Scheme 2). Protection of these amides is necessary for avoiding reaction between the N-terminal aldehyde and the backbone amides (vide infra). Upon treatment with TFA:water (95:5) (conditions under which the aldehyde is unmasked rapidly and cleanly[13]) the stable N-terminal peptide aldehyde **4** was formed. Since it is not feasible to cleave the resin bound base labile aldehyde off the solid support for analysis, the aldehyde was immediately submitted to reductive amination conditions (benzylamine, $NaBH_3CN$) in order to allow characterization of a product (**5**, R^1 = Bn) stable to the cleavage conditions. Using the HmbAc[21] group for protection of both of the two ultimate amides was examined. This group is stable to TFA and has the advantage over the methyl group that it can be removed

Scheme 3. Formation of Non Resin Bound Peptide Aldehyde[a]



[a] Reagents and conditions: (i) TFA:H_2O (95:5), 3 min; (ii) 0.1 M NaOH, 20 °C, 1−2 h; (iii) 1 M HCl, 20 °C 1−2 h.

by treatment with TFA after deacetylation with piperidine. However, when attempting to remove this group from a bis-sequentially Hmb protected peptide, a large range of side products were observed, even in the presence of scavengers (data not shown). Alternatively, the substitution of either one the methyl groups in compound **3** with an HmbAc group was successful. Using methyl groups for backbone protection is not optimal since they cannot be removed, and other amide protection strategies are currently being investigated. In the following, the use of bis-methylated peptides such as **3** as a model system is described.

Cleavage of the N-terminal peptide aldehyde **10** from the resin with 0.1 M NaOH yielded a product with a mass of 990.5 g/mol, corresponding to aldol product **11** (Scheme 3). Instead, demasking of the aldehyde was performed after cleavage of compound **12** from the solid support, yielding **13** in 68% yield after purification by preparative RP-HPLC. NMR as well as LCMS analysis of this product proved that the aldehyde was completely stable; the nonmethylated amides in the peptide did not react with the aldehyde. The reason for this is believed to be a result of the decreased flexibility of the N-methylated section of the backbone as well as the relatively large distance between the aldehyde and the nonmethylated amides.

^{1}H NMR spectra of **13** and other products containing a bis-methylated backbone are characterized by exhibiting signal splitting, apparently due to population of multiple cis/

Table 1. Coupling Percentages of Building Blocks **1a** and **2a**−**e** (3 equiv) onto Resin Bound Non-N-Alkylated Model Peptides Using TBTU (2.8 equiv) and NEM (3 equiv) in DMF at 20 °C for 1 and 24 h. ○ = $POEPOP_{1500}$

	peptide sequence							
	H-Gly-Phe-○		H-Phe-Phe-○		H-Ile-Phe-○		H-Pro-Phe-○	
building block	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
1a	100.0		100.0		100.0		100.0	
2a (R = H)	99.5	100.0					95.8	100.0
2b (R = Me)	99.5	100.0	99.4	100.0				
2e (R = CH_2OH)			99.3	99.5	99.4	100.0		
2c (R = *i*Bu)					82.5	99.5	46.8	97.7
2d (R = Bn)	99.5	100.0					63.5	99.7

Table 2. Coupling of Building Blocks **2a**−**e** to Resin Bound N-Alkylated Model Peptides

						coupling percentages (%)[a]			
entry	building block	activator	temp (°C)/solvent	equiv/concd (M)[b]	peptide[c]	1 h	5 h	24 h	48 h
1	**2a** (R = H)	HATU/HOAt	20/DMF	2/0.08	A		100[d]		
2	**2a** (R = H)	HATU/HOAt	50/DMF	2/0.07	B		100[d]		
3	**2a** (R = H)	HATU/HOAt	20/DMF	3/0.23	C	100			
4	**2a** (R = H)	HATU/HOAt	50/DMF	3/0.10	C	100			
5	**2b** (R = Me)	HATU/HOAt	50/DMF	3/0.10	C	62	93	99	
6	**2e** (R = CH_2OH)	HATU/HOAt	50/DMF	3/0.10	C	69	83	88	
7	**2c** (R = *i*Bu)	HATU/HOAt	50/DMF	3/0.10	C			15	
8	**2d** (R = Bn)	HATU/HOAt	50/DMF	3/0.10	C			26	
9	**2c** (R = *i*Bu)	DCC	50/DCE	3/0.10	C	0	0	3	
10	**2d** (R = Bn)	DCC	50/DCE	3/0.10	C	0	5	21	
11	**2e** (R = CH_2OH)	BTC	50/DCE	3/0.15	C			95	
12	**2c** (R = *i*Bu)	BTC	50/DCE	3/0.15	C			42	
13	**2c** (R = *i*Bu)	BTC	50/DCE	6/0.20	C		49		69
14	**2d** (R = Bn)	BTC	50/DCE	3/0.15	C			84	
15	**2d** (R = Bn)	BTC	50/DCE	6/0.20	C		83		87
16	**2c**-OPfp[e]	HOAt	50/DMF:DCE 1:1	3.9/0.2	C				65[f]

[a] Percentages of building block coupled peptide as determined by RP-HPLC and ESMS analysis at the indicated points of time, unless otherwise specified. [b] Equivalents/concentration of building block. [c] A = H-(*N*-Me)L(*N*-Me)LLFG-HMBA-$POEPOP_{900}$, B = H-(*N*-Me)F(*N*-Me)GLG-HMBA-$PEGA_{1900}$, C = H-(*N*-Me)F(*N*-Me)VLG-HMBA-$SPOCC_{1500}$. [d] Analyzed after 2 h. [e] Prepared by treatment of **2c** with 1.1 equiv of PfpOH and 1.1 equiv of DCC in dioxane. [f] Seven days.

trans conformations of the bis-methylated regions of the backbones. Full structure elucidation was accomplished by COSY experiments.

Coupling of Building Blocks 1a[13] and 2a−e[13] to Peptides. Non N-Alkylated Amines. The ease of coupling of building blocks **1a**[13] and **2a**−**e**[13] was first investigated by coupling with TBTU[22] onto model dipeptides, which were anchored on a solid support. Four different resin bound model dipeptides with different degrees of steric hindrance were selected for the coupling investigations and were examined in 14 representative coupling reactions (Table 1). The various degrees of coupling were determined by analytical RP-HPLC by integration of the peaks of unreacted starting peptide and products peaks. The identity of the product peaks were verified by LCMS. Since the building blocks were racemic, the RP-HPLC chromatograms of the coupling products ordinarily exhibited two or four peaks corresponding to different diastereomers formed.

The couplings between **1a** and the four dipeptides were in all cases complete within 1 h. Couplings of building blocks **2a**−**e** were dependent on the bulkiness of the C-2 substituents as well as the steric hindrance of the model peptides. For the two most sterically hindered building blocks, coupling to Pro required 24 h for essentially complete coupling, and as expected, the most sterically demanding building block (**2c**) displayed the lowest coupling rates. From these experiments it could therefore be concluded that coupling in a few cases could be relatively slow depending on the side chain of the building block and the steric hindrance of terminal amino acid.

N-Alkylated Amines. Coupling of **2a**−**e** to N-terminally alkylated peptides required much stronger activation than TBTU. As was indicated by the coupling experiments described above, the side chains of the building blocks are a highly determining factor for the rate of coupling, and this became even more pronounced for the coupling onto N-terminally alkylated peptides. Coupling of **2a** with HATU[23] onto four different N-alkylated model peptides proceeded without any difficulty regardless of the concentration and temperature (Table 2, entries 1−4). Coupling of **2b** with HATU proceeded somewhat slower, and thus required 24 h at 50 °C for almost complete coupling, whereas coupling of the even more bulky **2e** did not reach more than 88% completion within 24 h at this temperature. For the two most sterically hindered acids (**2c** and **2d**), degrees of coupling higher than 15% and 26%, respectively, were not achieved under these conditions (entries 7 and 8). Coupling of these building blocks with DCC in 1,2-dichloroethane at 50 °C did not prove satisfactory either (entries 9 and 10). The three most difficult couplings were therefore attempted with bis-trichloromethyl carbonate (BTC, or "trisphosgene")[24,25] in 1,2-dichloroethane at 50 °C, resulting in good coupling yields (entries 11, 12, and 14). BTC mediated couplings of **2c** and **2d** were repeated with 6 equiv of the acids and with

Table 3. Reductive Aminations of **4** and **10** with Various Amines in DMSO:CH_2Cl_2:AcOH (50:50:1) in the Presence of $NaBH(OAc)_3$ (unless otherwise specified)

			reductive amination product			acylated reductive amination product		
entry	peptide aldehyde	amine	purity[a] (%)	calcd mass $(MH)^+$	found mass[b] $(MH)^+$	serine coupling[c] (%)	calcd mass $(MH)^+$	found mass[b] $(MH)^+$
1	**4**	$MeNH_2$·HCl	90[d]	689.9	689.7	90	833.1	832.6
2	**4**	$EtNH_2$·HCl	90	703.9	703.6	95	847.1	846.5
3	**4**	*n*$PrNH_2$	100	717.5	718.8	75	860.6	860.9
4	**4**	*i*$BuNH_2$	100	732.0	731.6	95	875.2	874.7
5	**4**	*n*-hexylamine	100	760.0	759.7	100	903.2	902.6
6	**4**	allylamine	85	715.9	715.7	100	859.1	858.7
7	**4**	propargylamine	95	713.9	713.5	100	857.1	856.6
8	**4**	*c*$PrNH_2$	100	715.5	715.8	10	858.6	858.8
9	**4**	*cyclo*-hexylamine	100	758.0	757.6	100	901.2	900.6
10	**4**	$BnNH_2$	100[e]	766.0	765.6	95	909.2	908.6
11	**4**	2,4-dimethoxybenzylamine	100	825.5	825.8	100	968.6	969.8
12	**4**	3-picolylamine	100	766.5	766.5	100	909.7	909.6
13	**4**	$NC(CH_2)_2NH_2$	95	728.9	728.5	90	872.1	871.5
14	**4**	$HOCH_2CH_2NH_2$	100	719.9	719.6	80	863.1	862.5
15	**4**	$BocNH(CH_2)_2NH_2$	95	819.1	818.5	100	962.2	961.6
16	**4**	glycinamide·HCl	95	732.5	732.8	100	859.1[f]	858.8
17	**4**	*t*$BuOCOCH_2NH_2$[g]	95	733.9[h]	733.6	100	859.1[f]	858.5
18	**4**	(*p*-OH)$PhNH_2$	90	768.0	ND[i]	90	911.2	910.6
19	**10**	$BocNH(CH_2)_4NH_2$	100	677.8	677.2	95	821.0	820.4
20	**10**	$BocNH(CH_2)_6NH_2$·HCl	95	705.5	705.3	100	848.5	848.5
21	**10**	$(MeO)_2CHCH_2NH_2$	100	594.7	594.3	95	737.9	737.4
22	**10**	tryptamine·HCl	90	649.8	649.4	100	793.0	792.4

[a] Conversion of protected aldehyde to desired compound (±5%), determined by integration of analytical RP-HPLC peaks. [b] Mass of $(MH)^+$ determined by ESMS or MALDI-TOF. [c] After treatment with Fmoc-Ser(*t*Bu)-OPfp (20 equiv) in DMF at 50 °C for 4 h, determined by integration of analytical RP-HPLC peaks (±5%). [d] DMSO:CH_2Cl_2 (1:1), $NaCNBH_3$. [e] CH_2Cl_2, $NaCNBH_3$. [f] DKP formation. [g] Dibenzenesulfimid salt. [h] Free acid, not *t*Bu ester. [i] Not detected.

prolonged reaction time (entries 13 and 15). For **2d** this did not result in a significantly higher degree of coupling (compare entries 14 and 15); however, for **2c** the degree of coupling had improved to 69% (entry 13). Attempts to drive the more difficult couplings of the ones described above by conducting the reactions at temperatures ranging from 60 to 80 °C resulted in low purities of resin bound material and is therefore not advisable.

Finally, the Pfp ester of **2c** was prepared (**2c**, 1.1 of equiv DCC, 1.1 equiv of pentafluorphenol, dioxane) and used for acylation of the model peptide. After 7 days at 50 °C, only 65% coupled peptide had formed, and coupling via Pfp esters was therefore not pursued further.

Nucleophilic Reactions. In the nucleophilic reactions described in the following section, only the use of the simple building block **2a** is described. The C-2 substituted building blocks **2b**−**e** coupled to peptides and converted to the corresponding aldehydes were found to react as **2a** (data not shown); however, due to the racemic C-2 carbon of **2b**−**e**, the peptide aldehydes and products hereof were diastereomers. Analysis and reaction optimization was therefore found to be more facile when employing the nonchiral aldehyde precursor **2a**. Compound **1a** was not reactive (vide infra) and was therefore not investigated further.

(a) Reductive Amination Yielding *N*-Alkyl γ-Aminobutyryl-peptides. Reductive amination of the peptide aldehydes is an interesting reaction due to the large range of commercially available primary amines and, more importantly, due to the resulting structure (Scheme 2). The produced secondary amine (**5**) can be acylated, thus allowing the assembly of a peptide at this point. The resulting internal peptide isostere may confer endoprotease inhibitory activity to the compound due to the stability to hydrolysis. Both the alkyl group introduced by the amine and the side chains of the aldehyde building blocks **2b**−**e** may mimic amino acid side chains.

A broad range of amines were examined (Table 3) together with parameters such as solvent, drying agents, reducing agent, and pH in order to optimize the conditions for this reaction. In summary of these experiments, it was found that the number and amount of side products and impurities were determined by the following. (i) *Solvent:* "classic" reductive amination solvents such as DMF and methanol or mixtures thereof did not give satisfactory results; this was achieved by using a 1:1 mixture of dimethyl sulfoxide and dichloromethane. (ii) *Drying:* triethylorthoformate previously reported[12] as a drying agent had a negative effect on the reaction; considerable amounts of side products were observed in most cases. (iii) *Reducing agent:* $NaBH_3CN$, $NaBH_4$ and $NaBH(OAc)_3$ were investigated; the latter yielded a clean crude product, while borohydride and cyanoborohydride resulted in side products. This is in agreement with a previous study on reductive amination in solution.[26] In the case of methylamine and benzylamine, the choice of hydride was not crucial (entries 1 and 10). (iv) *pH:* no clear effect was observed on the purity of the crude reaction products when changing the pH by addition of acetic acid. However, because of the base labile linker, 1% acetic acid was added to the solvent; the pH of the amine solution thus was typically between 3 and 6. Furthermore, it has been reported that the

reactions proceed faster in the presence of acetic acid.[26] (v) *Procedure—stepwise vs direct reaction:*[26] although the reductive amination is a two-step process often performed by first forming the imine then drainage of the resin and reduction of the imine (stepwise reaction), it was found that this procedure gave rise to various side products. In contrast, when amine and reducing agent were mixed in the desired solvent and added to the resin bound aldehyde (direct reaction), a clean conversion was observed.

Reaction time was not thoroughly investigated; however, most reactions were found to be complete within hours. Nevertheless, reactions were typically left overnight, and no unreacted aldehyde was observed.

The optimized conditions were dimethyl sulfoxide to dichloromethane (1:1) + 1% acetic acid, 20−30 equiv of amine, 15−20 equiv of $NaBH(OAc)_3$. These were applied to a variety of different amines and aldehydes **4** and **10** (Table 3). The purities of the products were determined by RP-HPLC and were generally satisfying. Using ammonia or ammonium chloride did unexpectedly not give good yields. Treatment of the aldehyde with hydroxylamine gave the corresponding oxime in 70% purity, and the oxime was not reduced under the specified reductive amination conditions. With the optimized conditions, product purities between 90 and 100% were routinely observed regardless of the nature of amine (benzylic, cyclic, or acyclic aliphatic, or with other functionalities present). For amines added as hydrochlorides, a common side product was observed, e.g., in reductive aminations of **10** with hydrochloride amines a common minor peak at $R_t = 11.9$ min (see Figure 3d) was observed. The side product was stable to the conditions employed at later stages (e.g., acylation, TFA, piperidine) and therefore not of major concern. Furthermore, it could be avoided by freeing the amine of the hydrochloride prior to use.

To evaluate the various reductive amination products as peptide isostere precursors, coupling with Fmoc-Ser(*t*Bu)-OPfp (20 equiv) in DMF at 50 °C for 4 h was conducted (Scheme 2, Table 3). In most cases, the coupling proceeded without any difficulty; however, for R^1 = *n*Pr, *i*Pr, *c*Pr, $(CH_2)_2OH$, and PhOH, coupling degrees higher than 80% were not achieved under the employed conditions. In these reactions, more powerful activation or prolonged reaction time should therefore be employed.

The product derived from reductive amination of **10** with aminoacetaldehyde dimethylacetal (**14**) (Scheme 4) is particularly interesting with respect to inhibitors of serine and cysteine proteases since this isostere has an electrophilic aldehyde incorporated. After elongation of the isostere by coupling with serine and leucine, **15** was cleaved off the resin and treated with 1 M HCl. This yielded peptide isostere **16**, of which the left-hand side of the peptide strongly resembles the motif of a C-terminal aldehyde. By ESMS analysis of purified **16**, a signal with the expected mass (100% intensity) was observed as well as the mass of 18 mass units less, corresponding to loss of water (6% intensity). This mass is probably due to the aldehyde reacting irreversibly with either the leucine-serine amide or the leucine amine. Irreversible amide-aldehyde condensation (vide infra) does not seem to occur for C-terminal aldehydes, and should therefore not be a problem for compounds such as **16**.

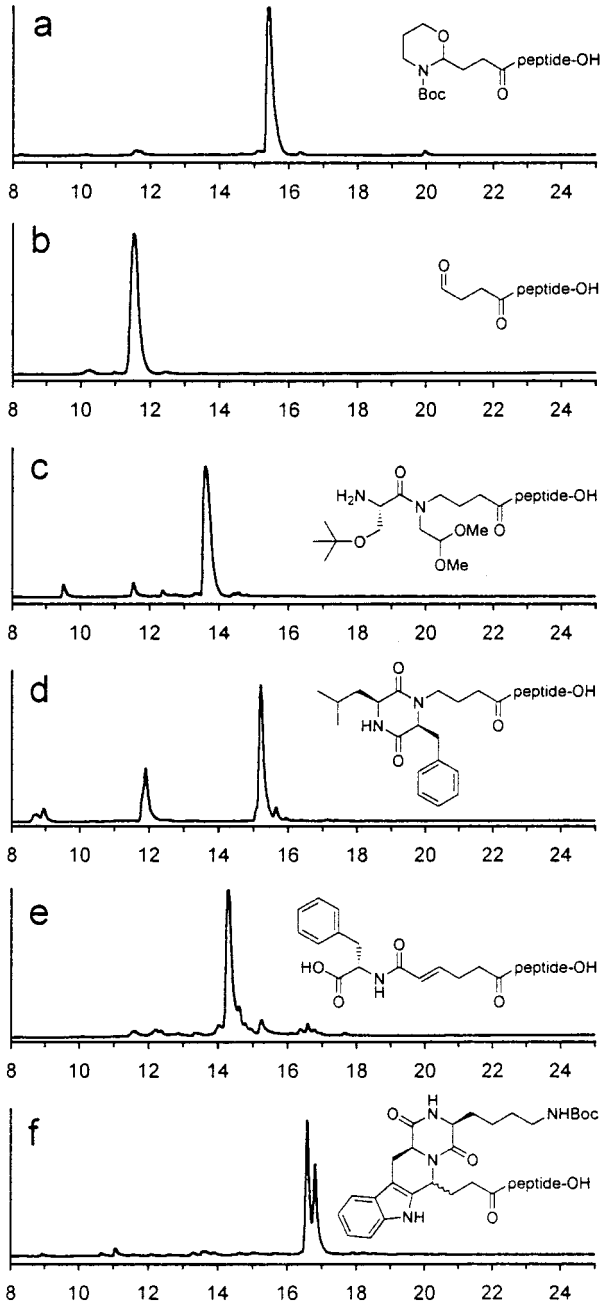
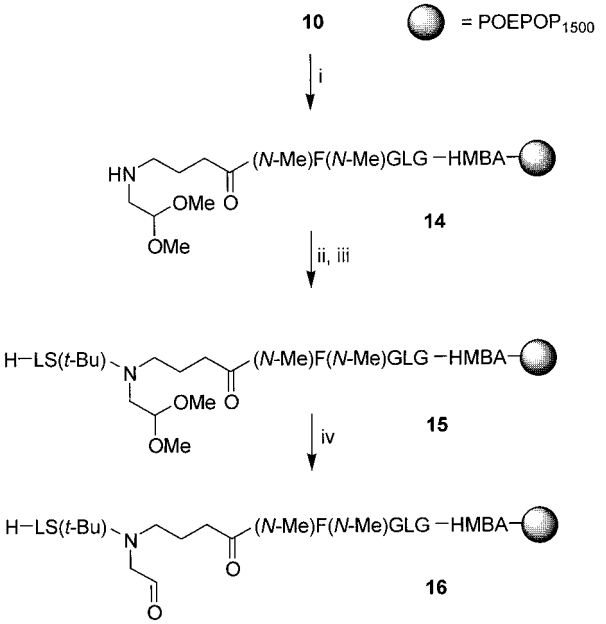


Figure 3. Representative RP-HPLC traces of crude products after cleavage from the solid support. -peptide- = -(*N*-Me)Phe-(*N*-Me)-Gly-Leu-Gly-. (a) Aldehyde precursor building block **2a** coupled to peptide. (b) Free N-terminal aldehyde **13**. (c) After reductive amination and amino acid coupling. (d) After (i) reductive amination with H-Phe-O*t*Bu, (ii) coupling of Fmoc-Leu, (iii) removal of Fmoc. (e) After (i) Horner−Wadsworth−Emmons reaction with an allyl phosphonate, (ii) cleavage of allyl ester with Pd(0), and (iii) elongation with H-Phe-O*t*Bu. (e) After (i) Pictet−Spengler condensation with H-Trp-OMe, (ii) coupling of Fmoc-Leu, (iii) removal of Fmoc (two diastereomers).

(b) Reductive Amination Yielding 2,5-Diketopiperazine-peptides. Libraries of 2,5-diketopiperazines (DKPs) have been prepared on a solid support by several groups[27−31] all with the common feature that the DKP ring is formed in a cyclization cleavage step. Therefore, the chemistry employed could not be used for on-bead screening of DKPs.

Scheme 4. Formation of Electrophilic Aldehyde Peptide Isostere **16**[a]



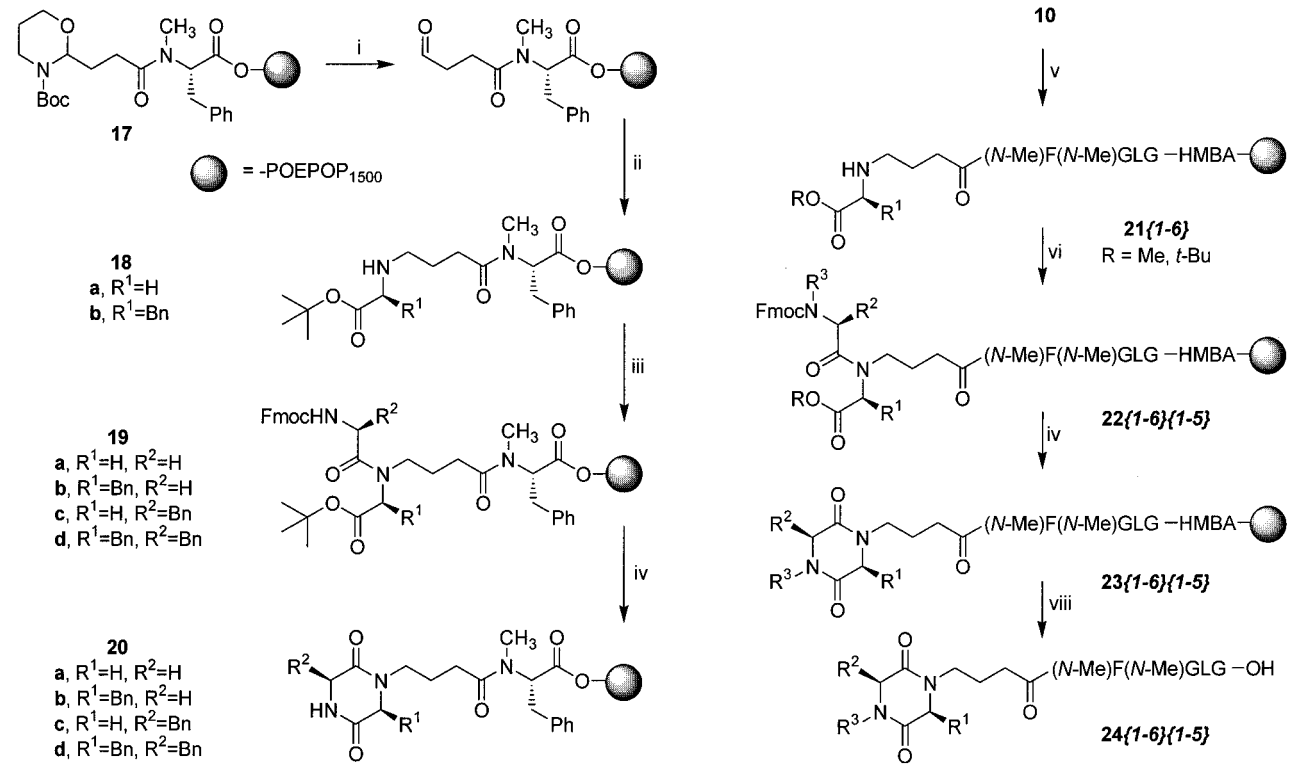
[a] Reagents and conditions: (i) $(MeO)_2CCH_2NH_2$, $NaBH(OAc)_3$, DMSO:CH_2Cl_2:AcOH (50:50:1); (ii) (a) Fmoc-Ser(*t*Bu)-OPfp, HOAt, DMF, 50 °C, 2 h, (b) 20% piperidine in DMF; (iii) (a) Fmoc-Leu-OPfp, HOAt, DMF, (b) 20% piperidine in DMF; (iv) TFA:H_2O (95:5), 5 min.

By anchoring the aldehyde to the resin, solid supported peptide DKPs can be synthesized in a stepwise manner, where the first half of the DKP ring is introduced by reductive amination with an amino acid ester. The second half of the ring is then introduced by coupling with a protected amino acid, and after deprotection the DKP ring is cyclized without cleavage from the resin. Cylizations of DKPs have previously been performed without simultaneous cleavage from the solid support; however, in these cases the DKP rings were anchored to the resin via the side chain of one of the amino acids, e.g., aspartic acid[32] or 4-hydroxyproline,[33] and this strategy is therefore not useful for general synthesis of DKP libraries.

The first attempts to generate DKPs via resin bound aldehydes consisted of functionalization of $POEPOP_{1500}$ resin with *N*-methylphenylalanine followed by coupling of building block **2a** to give compound **17** (Scheme 5). Compound **17** was converted into the corresponding aldehyde by treatment with TFA, and successively submitted to reductive amination with the *tert*-butyl esters of glycine and phenylalanine yielding secondary amines **18a** and **18b**. These were split into two portions which were treated with HATU activated Fmoc-Gly-OH and Fmoc-Phe-OH (20 °C, overnight), resulting in compounds **19a**−**c**. The reaction between **18b** and HATU activated Fmoc-Phe-OH to give **19d** was sluggish; repeating the coupling, raising the temperature, or using Pfp esters at elevated temperature was not efficient. Thus, **18b** was coupled with BTC activated Fmoc-Phe-OH, resulting in complete coupling after 30 min at 55 °C. After removal of the Fmoc protection group under standard conditions, the four different DKPs **20a**−**d** were formed in high purity (90−95%) as analyzed by RP-HPLC.

Using the optimized conditions, an array of $6 \times 5 = 30$ different peptide terminal DKPs was synthesized. Aldehyde **10** was reductively aminated with six different amino acid

Scheme 5. On-Bead Cyclization of Model DKPs **20a**−**d** and N-Terminal Peptide DKPs **24**[a]



[a] Reagents and conditions: (i) TFA:H_2O (95:5), 3 min; (ii) *t*BuOCOCHR[1]NH_2·HCl, $NaBH(OAc)_3$, DMSO:CH_2Cl_2:AcOH (50:50:1); (iii) Fmoc-Gly-OH or Fmoc-Phe-OH, HATU, HOAt, NEM, DMF; for **18b**→**18d**: Fmoc-Phe-OH, BTC, 2,4,6-collidine, DCE, 50−55 °C; (iv) 20% piperidine in DMF; (v) ROCOCHR[1]NH_2·HCl, $NaBH(OAc)_3$, DMSO:CH_2Cl_2:AcOH (50:50:1); (vi) Fmoc-AA-OH, BTC, 2,4,6-collidine, DCE, 50−55 °C; (vii) 0.1 M NaOH, 20 °C, 1 h.

*t*Bu (Gly, Ala, Val, Leu, and Pro) or Me (Ser) esters (Scheme 5, Table 4). Each new resin bound compound **21**{*1*−*6*} was then coupled with five different Fmoc protected amino acids (Gly, Leu, Pro, Lys(Boc), and Ser(*t*Bu)) with BTC at 55 °C for 4 h. After standard removal of the Fmoc group with piperidine in DMF, completion of the DKP cyclization was monitored with the Kaiser test; in cases were the cyclization was incomplete, the resin was heated to 55 °C for a few hours. The products (**24**) were then cleaved from the resins and analyzed by RP-HPLC and LCMS. Acylations of reductive amination products **21**{*1,2,4, and 5*} were essentially quantitative. In the case of the most sterically hindered amines **21**{*3*} (R^1 = *i*Pr) and **21**{*6*} (R^1 = CH_2-OH), acylation with BTC activated amino acids did not give quantitative conversions of the starting amine to DKP. However, the conditions employed were the same in all cases, and prolonged or repeated couplings on **21**{*3*} and **21**{*6*} to increase the yields was not attempted. On a preparative scale, the synthesis of **24**{*5*}{*2*} was accomplished in 36% yield of purified product.

(c) Horner−Wadsworth−Emmons Reaction Yielding Unsaturated Isosteres. Wittig reactions and modifications thereof are fundamental C−C bond forming transformations. The reactive species of the Horner−Wadsworth−Emmons (HWE) reaction, the carbanion of a phosphonoacetate, is more reactive than the conventional type of Wittig reagent, and the reaction can therefore be conducted under milder conditions.[34] Furthermore, HWE reagents are known to give higher trans selectivities than the phosphorane ylids. The solid support may have a large influence on the outcome of these reactions; in the present work POEPOP was used, although the HWE reaction successfully has been performed on PEGA also.

Aldehyde **10** was successfully converted into **25** with 1.1 equiv of triethyl phosphonoacetate and 1.1 equiv of butyllithium (THF, 0 °C 1 h, 4 °C overnight). However, it was found that equally good results could be obtained with the much milder and also more practical conditions introduced by Blanchette et al.,[35] optimized by Rathke and Nowak,[36] and adapted to solid phase synthesis by Johnson and Zhang[37] and Warrass et al.[38] In a typical HWE olefination of **10** (Scheme 6), the phosphonoacetate (30 equiv) was mixed with lithium bromide (25 equiv) and triethylamine (20 equiv) in acetonitrile, incubated for 5 min, and added to the resin at 20 °C. For R^1 = *t*Bu, the reaction was found to be 84% complete after 1 h, and 94% complete after 17 h. The reaction was attempted with three different phosphonoacetates (R^1 = Et, *t*Bu, allyl) and proceeded equally well.

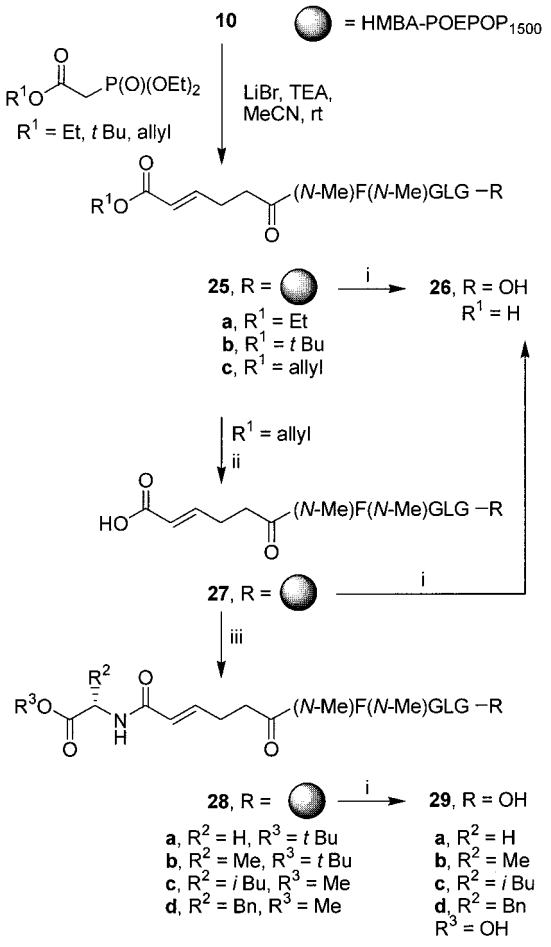
For further elongation of the resin bound material, the allyl ester **25c** was preferred. The ethyl ester could not be cleaved selectively over the ester bond of the linker by alkaline hydrolysis, and cleavage of the *tert*-butyl ester with, e.g., TFA often resulted in some decomposition of the peptide at the N-methylated amides. In contrast, the allyl group was cleanly removed using tetrakis ($Pd[PPh_3]_4$) (3 equiv, chloroform, 1 h). Elongation of the α,*β*-unsaturated acid **27** was achieved by treatment with HATU/HOAt/NEM for 5 min, followed by addition of the desired amine (H-Gly-O*t*Bu·HCl, H-Ala-O*t*Bu·HCl, H-Leu-OMe·HCl, or H-Phe-OMe·

Table 4. Combinatorial Formation of 2,5-Diketopiperazines **24**

amine for reductive amination	reductive amination products **21**[a]	2,5-diketopiperazines **24**									
		24{-}{*1*} Fmoc-Gly-OH $R^2 = R^3 = H$		**24**{-}{*2*} Fmoc-Leu-OH R^2 = *i*Bu, $R^3 = H$		**24**{-}{*3*} Fmoc-Pro-OH $R^2 - R^3$ = -$(CH_2)_3$-		**24**{-}{*4*} Fmoc-Lys(Boc)-OH $R^2 = (CH_2)_4NHBoc$, $R^3 = H$		**24**{-}{*5*} Fmoc-Ser(tBu)-OH $R^2 = CH_2OtBu$, $R^3 = H$	
		conv[b]	mass[c]	conv[b]	mass[c]	conv[b]	mass[c]	conv[b]	mass[c]	conv[b]	mass[c]
H-Gly-O*t*Bu $R^1 = H$	**21**{*1*} 619.3 (619.8)	100	602.3 (602.7)	100	658.3 (658.8)	100	642.3 (642.7)	100	773.5 (773.9)	100	688.6 (688.8)
H-Ala-O*t*Bu $R^1 = CH_3$	**21**{*2*} 633.3 (633.8)	100	616.2 (616.7)	100	672.5 (672.8)	100	656.3 (656.8)	100	787.5 (787.9)	100	702.5 (702.8)
H-Val-O*t*Bu $R^1 = CHMe_2$	**21**{*3*} 661.5 (661.8)	55	644.3 (644.8)	10	700.5 (700.9)	75	684.5 (684.7)	35	815.5 (816.0)	40	730.5 (730.9)
H-Leu-O*t*Bu R^1 = *i*Bu	**21**{*4*} 675.5 (675.8)	100	658.3 (658.8)	100	714.5 (714.9)	100	698.5 (698.9)	100	829.5 (830.0)	100	744.5 (744.9)
H-Phe-O*t*Bu R^1 = Bn	**21**{*5*} 709.5 (709.9)	100	692.3 (692.8)	95	748.5 (748.9)	100	732.3 (732.9)	100	863.5 (864.0)	100	778.5 (778.9)
H-Ser-OMe $R^1 = CH_2OH$	**21**{*6*} 593.3 (593.7)	90	632.2 (632.7)	75	688.5 (688.8)	85	672.3 (672.8)	65	803.5 (803.9)	85	718.5 (718.8)

[a] LCMS mass after subtraction of H^+ (for MH^+) or Na^+ (for MNa^+) of reductive amination products **21**. Figures in parentheses are calculated masses. [b] Conversion of amine to DKP (±5%), determined by integration of analytical RP-HPLC peaks. [c] LCMS mass after subtraction of H^+ (for MH^+) or Na^+ (for MNa^+) of DKPs **24**. Figures in parentheses are calculated masses.

Scheme 6. Synthesis of Unsaturated Peptide Isosteres via HWE Olefination and Successive Elongation[a]



[a] Reagents and conditions: (i) 0.1 M NaOH, 20 °C, 1−2 h; (ii) $Pd[PPh_3]_4$, $HCCl_3$:AcOH:NEM (37:2:1); (iii) $H_2NCHR^2CO_2tBu$, HATU, HOAt, NEM, DMF.

HCl). All amines coupled equally well, yielding one major peak in the RP-HPLC (Figure 3e). For the conversion of **27** to **28a**−**d**, *tert*-butyl and methyl esters of amino acid hydrochlorides were used, and consequently, further elongation on solid phase was not feasible. If further elongation is desired, it should be considered to use allyl esters of amino acids which may be deprotected by Pd(0) catalysis, or to use another inverse peptide strategy (e.g., as recently reported by Hallberg and co-workers[39]).

(d) Pictet−Spengler Reactions Yielding Tetrahydro-*β*-carbolines. The Pictet−Spengler reaction, which was discovered in 1911,[40] has been shown to be an important route toward indole and isoquinoline alkaloids,[41−43] and recently the reaction has been employed in the formation of combinatorial libraries by immobilizing tryptophan on a solid support.[44,45]

A report on the practical utilization of the Pictet−Spengler condensation claimed that the yields and purities obtainable for this class of reactions are highly dependent on the nature of the aldehyde, amine, solvent, pH, and temperature, and furthermore, that no obvious trends could be deduced.[42] This conversion therefore requires compound specific optimization.

Aldehyde **10** was submitted to a range of conditions with tryptophan and histidine including the methyl esters of these, tryptamine and phenylalanine. In Table 5 the results of the Pictet−Spengler condensations between various amines and resin bound aldehyde **10** (Scheme 7, steps i−iii) under various conditions have been evaluated on the basis of the RP-HPLC chromatograms of the products. Conversion of **10** to imidazole derivatives **30a**,**b** did not perform elegantly. Treatment of **10** with histidine yielded **30a** in 38% purity along with 17% unreacted starting material (converted to aldol product **11** during treatment with NaOH). Reaction between **10** and H-His-OMe·3TFA afforded **30b** as an impure mixture containing only 15% **30b** and 50% unreacted aldehyde. Condensations with tryptophan and the corresponding methyl ester were more rewarding; compounds **31a** and **31b** could be obtained in almost 100% purity within 20 h at 50 °C (Table 5). Likewise, condensations with tryptamine hydrochloride afforded an almost 100% pure product, namely carboline **32**.

Diketopiperazine Fused Tetrahydro-*β*-carbolines. A recent communication[30] has described the synthesis of DKP fused tetrahydro-*β*-carbolines, which not only is an interesting template for combinatorial chemistry but is also interesting since many biologically active natural products of this type have been discovered. Three routes toward DKP fused tetrahydro-*β*-carbolines **34a**−**f** were investigated (Scheme 7). First, **31a** was completely acylated with BTC activated Fmoc-Gly-OH and Fmoc-Lys(Boc)-OH followed by removal of the Fmoc group with piperidine yielding **33a** and **33b**, respectively. These compounds were treated with DCC/DMAP and monitored by the Kaiser test. After 5 h of treatment with a 0.2 M solution of DCC, the cyclization was found to be complete. For the formation of **34b**, however, compound **33b** required treatment with 10 equiv of DCC at 65 °C for 2 days followed by three additional days with a fresh portion of DCC.

Another attempt to obtain **34a** from **31a** by coupling glycine *tert*-butyl ester to the carboxylic acid of **31a** with HATU produced **35** which was expected to cyclize when heated in the presence of base. Surprisingly, heating **35** in 20% piperidine in DMF did not produce any trace of **34a**.

The third route utilized **31b** as the starting material for the synthesis of DKP fused tetrahydro-*β*-carbolines **34a**−**f**. Similar to the synthesis of DKPs **24** described above, **31b** was coupled with six different BTC activated Fmoc amino acids and treated with piperidine to remove the Fmoc group after which cyclization occurred either directly or after heating to 55 °C for a few hours. The purities of these compounds were by RP-HPLC shown to be high (typically above 93%), and for **34b**−**f** two diastereomers were observed (see Figure 3f) at the original aldehyde carbon.

Tetrahydro-*β*-carbolines as Internal Peptide Isosteres. Pictet−Spengler product **32** was quantitatively coupled with Pro and Leu, and **37b** was further elongated with another amino acid to give **38**, demonstrating the synthesis of peptides centrally incorporating tetrahydro-*β*-carbolines. On a preparative scale, **37a** was obtained in 76% yield.

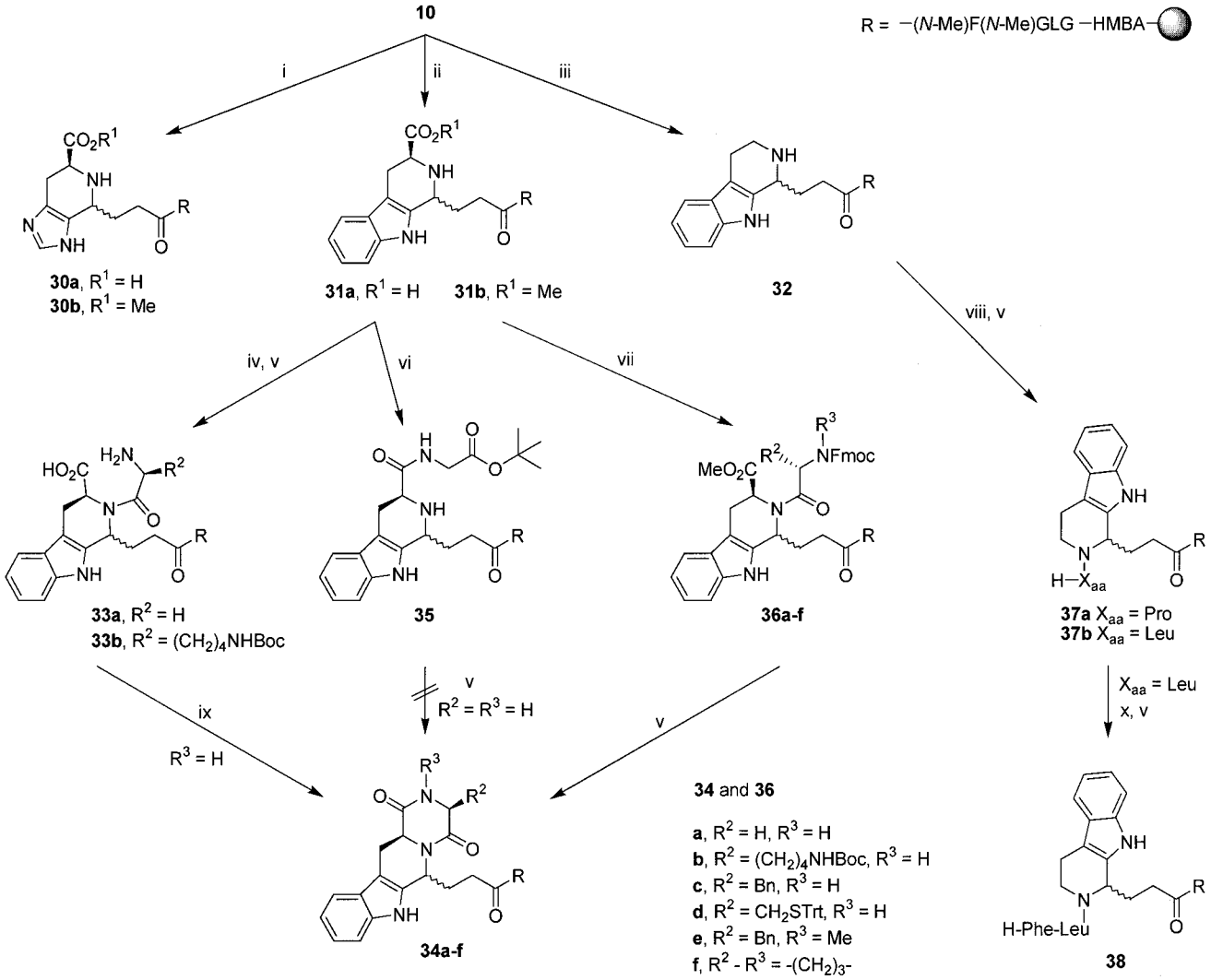
Amide-Aldehyde Condensations. Prior to obtaining stable peptide aldehydes by protection of the two ultimate amides, some interesting observations were made for non and partially protected peptide aldehydes.

Table 5. Evaluation of Pictet−Spengler Condensations between Resin Bound Peptide Aldehyde **10** and Various Amino Acids at 50 °C (unless otherwise specified)

	H-Trp-OH	H-Trp-OMe·HCl	H-His-OH	H-Phe-OH	tryptamine
water	- -		- -	- -	
aqueous 0.5 M HCl	+++	+++	- -		
CH_2Cl_2		- -[a]			
CH_2Cl_2:TFA (95:5)	+[a]/++	-[a]/- -	- -		
PhMe:DMSO (1:1)		+/- -[b]	+	- -/- -[c]	+++
PhMe:MeOH:AcOH (7:1:2)	+++		- -	-	
PhMe:DMSO:AcOH (2:2:1)	- -	+++	++		

Product purity evaluation marks: +++ excellent; ++ good; + moderate; - poor; - - very poor. [a] 20 °C. [b] H-Trp(Boc)-O*t*Bu·HCl. [c] H-Phe-O*t*Bu·HCl.

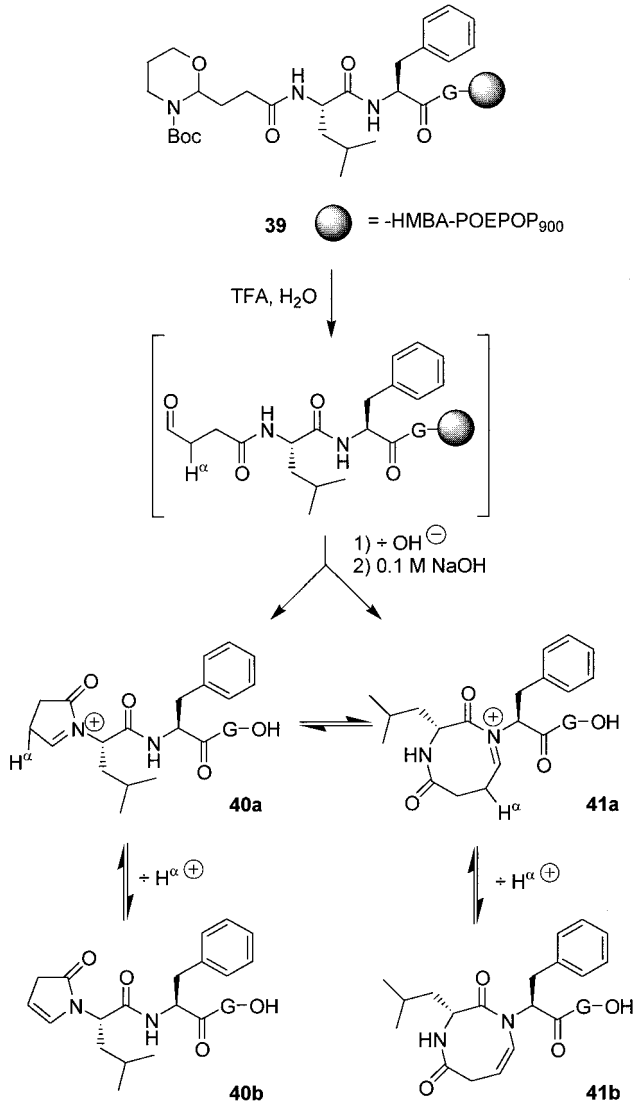
Scheme 7. Pictet−Spengler Condensations with Peptide Aldehyde **10**[a]



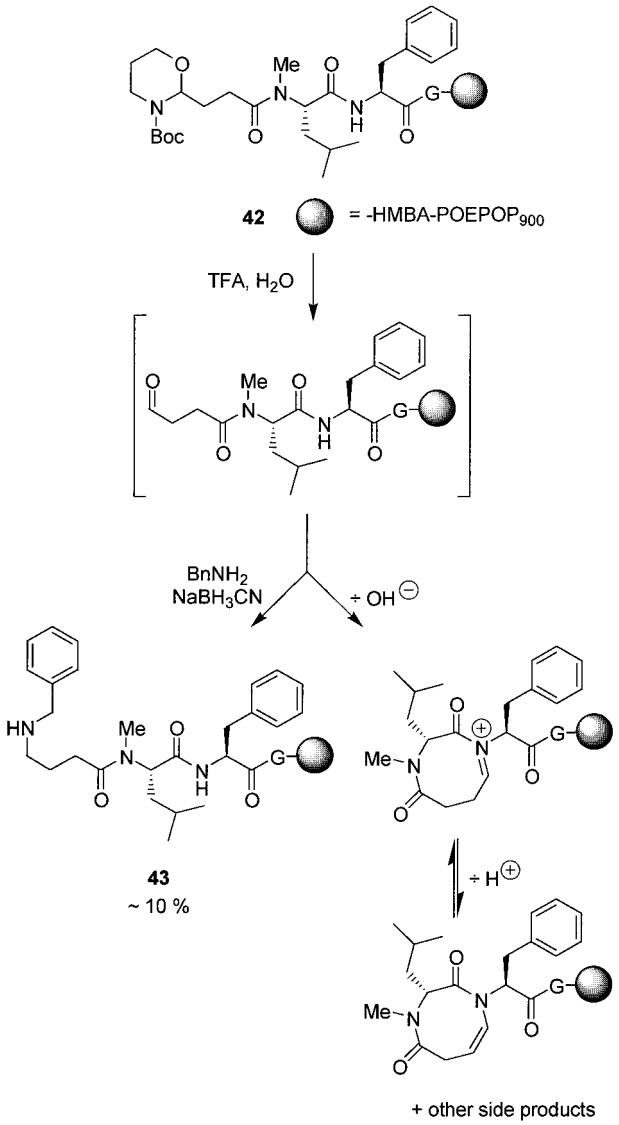
[a] Reagents and conditions: (i) 0.1 M H-His-OH in DMSO:toluene:AcOH (2:2:1), 60 °C, 7 days; (ii) R = H: 0.1 M H-Trp-OH in 0.5 M aq HCl, 60 °C, 20 h; R = Me: 0.1 M H-Trp-OMe·HCl in 0.5 M aq HCl or DMSO:toluene:AcOH (2:2:1), 60 °C, 20 h; (iii) 0.5 M tryptamine hydrochloride, DMSO:toluene (1:1), 50 °C, 3 days; (iv) Fmoc-Gly-OH or Fmoc-Lys(Boc)-OH, BTC, 2,4,6-collidine, DCE, 55 °C; (v) 20% piperidine in DMF; (vi) H-Gly-O*t*Bu, HATU, HOAt, NEM, DMF; (vii) as iv, also with Fmoc-Phe-OH, Fmoc-Cys(Trt)-OH, Fmoc-(*N*-Me)Phe-OH and Fmoc-Pro-OH; (viii) Fmoc-Pro-OH or Fmoc-Leu-OH, HATU, HOAt, NEM, DMF, 50 °C; (ix) R = H: DCC, DMAP, CH_2Cl_2, 20 °C, 1 h; R = $(CH_2)_4$NHBoc: DCC, DMAP, DCE, 65 °C, 5 days; (x) Fmoc-Phe-OPfp, HOAt, 20 °C.

Unprotected Backbone. Aldehyde precursor **2a** was attached to resin bound tripeptide LFG to give **39** which was treated with TFA and immediately submitted to reductive amination conditions (Scheme 8). RP-HPLC chromatograms and ESMS analysis of the resulting multiple peaks (two major, one minor) revealed products all having masses 18.0 mass units smaller than expected. Omitting the reductive amination yielded the same compounds. These facts suggested that a condensation had occurred between the aldehyde and the backbone amide nitrogens before the reductive amination was attempted (two possibilities are depicted in Scheme 8). Separation of the two major peaks (**40a/b** and **41a/b**) by RP-HPLC was attempted, but reinjection of either RP-HPLC-isolated peak resulted in an RP-HPLC diagram

Scheme 8. Amide-Aldehyde Side Products Formed in the Absence of Backbone Amide Protection



Scheme 9. Methyl Protection of the Ultimate Amide and Subsequent Reductive Amination

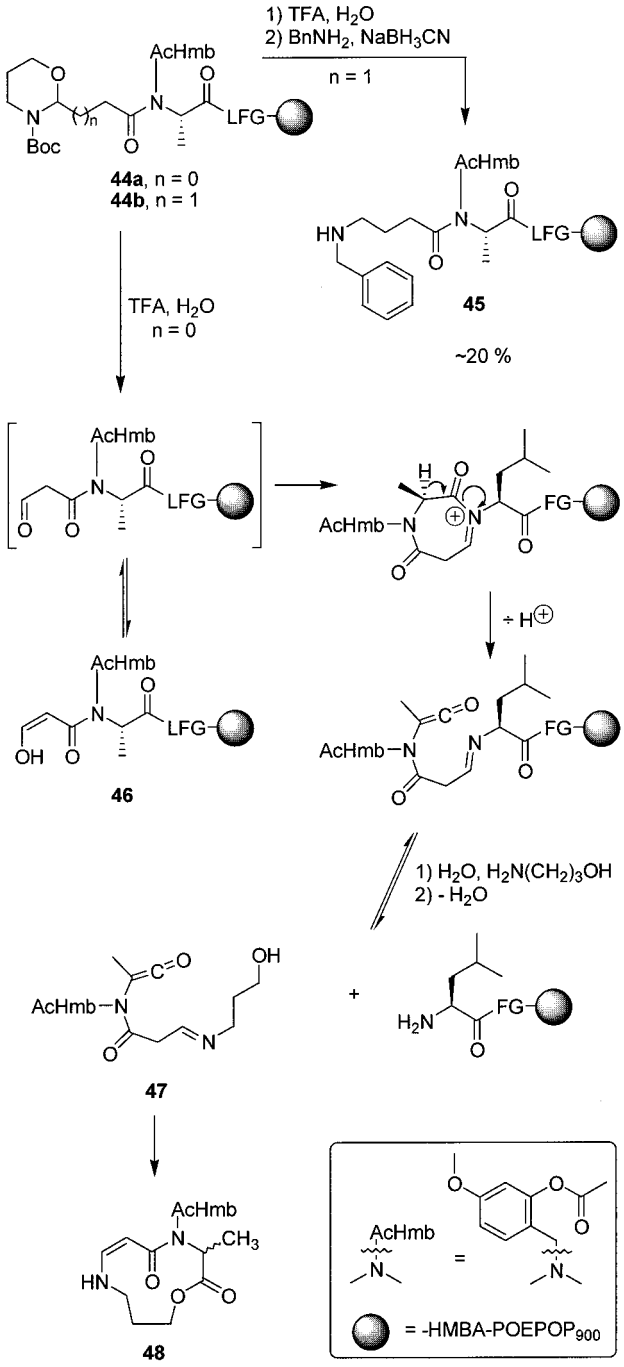


containing the original peaks, in the same ratios. This was interpreted as the presence of an equilibrium between **40a/b** and **41a/b**. In the NMR spectra of the equilibrium mixture compounds **40a**, **40b** and **41a** were detected, and in addition, traces of a forth compound was present.

Mono Protection with Methyl. Methylation of the ultimate amide was performed in attempts to decrease the flexibility of the backbone in order to avoid amide-aldehyde condensation. This improved the stability of the aldehyde to the extent that after immediate reductive amination the desired product **43** was detected, however, only in very low yield and purity (~10%) (Scheme 9).

Mono Protection with Hmb. Increased suppression of the amide-aldehyde condensation by applying the larger Hmb group was investigated (Scheme 10). Building blocks **1a** and **2a** were coupled to resin bound tetrapeptide (*N*-Hmb)ALFG and acetylated to give **44a** and **44b**, respectively. The latter was treated with aqueous TFA for 10 min and immediately submitted to a reductive amination. The desired product (**45**) was formed in low yield and purity (~20%). The result of exchanging the methyl group with the Hmb group apparently yielded an increase in aldehyde stability, however, still not to an acceptable extent. A small amount (~10%) of tripeptide LFG was detected, which initially was believed to be a result of acid-catalyzed hydrolysis of the amide bond on the C-terminal side of the N-alkylated alanine. Fragmentation of this sort by prolonged TFA treatment (e.g., 90 min) has been described for peptides containing an N-methylated amide,[46] and could therefore perhaps also occur in this case. However, when submitting **44a** to TFA treatment for 10 min to obtain the peptide aldehyde, a much higher degree of fragmentation (~40%) was observed, and complete fragmentation could be obtained by TFA treatment for 1 h. The nonfragmented material, compound **46**, was found to be inert to the employed reductive amination conditions, suggesting pronounced enolization. Analysis of the TFA solution used in the conversion of **44a** to **46** was by LCMS shown to contain a compound with a mass of 376.1 g/mol as the major component (besides 3-aminopropanol). Purification of the TFA solution by RP-HPLC yielded several compounds, one of which was found to be compound **48**. If acid catalyzed hydrolysis solely should account for the fragmentation, compound **48** should not be observed, nor would the rate of fragmentation be as rapid as observed (~40% in 10 min). A

Scheme 10. AcHmb Protection of the Ultimate Amide and Proposed Mechanism for the Observed Formation of Ketene **47** and Resin Bound Tripeptide LFG



proposed mechanism for the fragmentation is shown in Scheme 10 and involves the formation of ketene **47**.

It is reasonable to assume that the pronounced amide-aldehyde condensation observed for the unprotected peptide aldehydes presented above is facilitated by the flexibility of the aldehyde. This is supported by the fact that the N-terminal peptide aldehyde derived by the coupling between 4-carboxybenzaldehyde and POEPOP anchored dipeptide H-Phe-Leu does not exhibit this kind of behaivior,[47] presumably due to the more rigid nature of this system preventing the aldehyde and the backbone amides to come into contact. For *N*-glyoxalyl peptides which are also flexible, reaction between amide nitrogens and the aldehyde has been observed;[12] however, due to the lack of a proton α to the aldehyde, loss of water cannot occur and hence the reaction is reversible. Amide-aldehyde condensations in C-terminal peptides have not been reported, although a six-membered ring in theory could arise from such a reaction.

Utilization of the cyclic *N*-acyl iminium ion initially formed by condensations of the aldehyde and backbone amides as an electrophile reacting intramolecularly with nucleophilic amino acid side chains is currently being investigated.

Conclusions

It has been demonstrated that clean and rapid (3 min) formation of N-terminal peptide aldehydes can be accomplished by employing *N*-Boc-*N*,*O*-acetal building blocks in addition to protecting the peptide backbone. It was also shown that protection of the two ultimate amides in the peptide backbone is required to avoid an irreversible reaction between the aldehyde and the peptide amides. The rate of coupling of building blocks **2a**−**e** to peptides was very dependent on the C-2 substituent of the building block. In the case of coupling to N-terminally methylated peptides, activation with HATU/HOAt is sufficient for the three least bulky building blocks **2a**, **2b**, and **2e**, whereas **2c** and **2d** require activation by BTC at elevated temperatures.

The goal of using resin bound N-terminal peptide aldehydes for generating diverse peptide isosteres, mimetics and large heterocycles either centrally or at the N-terminus of a peptide has been demonstrated. Reductive amination was employed in order to obtain *N*-alkyl-γ-aminobutyric peptide isosteres as well as N-terminal 2,5-diketopiperazine peptides, Horner−Wadsworth−Emmons olefination led to unsaturated peptide isosteres positioned centrally in a peptide, and Pictet−Spengler condensations afforded tetrahydro-β-carbolines either centrally in a peptide or fused with a diketopiperazine ring in the N-terminus. The possibility of generating diverse structures from a common electrophilic intermediate is valuable for the generation of combinatorial libraries, and the methodologies presented here are currently being employed in library synthesis. The synthesis and screening of these libraries will reported separately. A strategy which allows splitting of a library at an intermediate stage in order to produce several distinct libraries is valuable for generating diversity, as well as saving time.

Experimental Section

General. All solvents were stored over molecular sieves. All starting materials and reagents were commercially available, except for building blocks **1a** and **2a**−**2e**,[13] and TBDMS−HMBA.[48] Solid phase reactions run at 20 °C were performed in flat-bottom polyethylene syringes equipped with sintered Teflon filters (50 μm pores), Teflon tubing, Teflon valves for flow control, and suction to drain the syringes from below.[49] Reactions at elevated temperatures were conducted in Reacti-Vials (Pierce, Rockford, IL) under argon or by placing the syringe packed with resin in a heated sand bath. Resin loadings were determined by Fmoc[50] cleavage and optical density measurements of the eluate at 290 nm and were calculated by employing a calibration curve.[51]

Removal of the Fmoc protection group was accomplished by treatment with 20% piperidine in DMF (3 + 17 min) followed by washing with DMF (8 × 1 min).

Reactions were evaluated by cleaving material off the resin (0.5−2 mg) with 50 μL of 0.1 M NaOH (1−2 h), adding 50 μL of MeCN and 10 μL of saturated aqueous NH_4Cl, performing analytical RP-HPLC followed by ESMS, LCMS, or MALDI-TOF analysis of the individual peaks.

Analytical RP-HPLC was performed on a Zorbax column (C-18, 300 Å, 0.45 mm × 50 mm) with a linear gradient of 100% A (0.1% TFA in water) to 100% B (0.1% TFA in 1:9 water:MeCN) in 25 min, 1 mL min^{-1}, with detection at 215 and 280 nm by a programmable multiwavelength detector (Waters 490E) or on a Millipore Delta Pak column (C-18, 15μm, 8 × 200 mm, 100% A to 100% B in 50 min). Unless otherwise specified, the Zorbax column was used. Preparative purifications were performed on a Millipore Delta Pak column (RP-18 semipreparative, 15 μm, 25 × 200 mm, 75% A for 2 min, then a linear gradient of 0.5% B per minute, 10 mL min^{-1}) with detection at 215 nm by a photodiode array detector (Waters M991). Reported retention times are for the Zorbax column.

^{1}H NMR and COSY spectra were recorded at 250 MHz (Bruker DPX) or 500 MHz (Varian Unity Inova). Shifts are reported relative to TMS (0.00 ppm) as internal standard.

General Coupling Procedures (A1−A5). Coupling reactions to primary amines were monitored by the Kaiser test,[52] couplings to secondary amines by the chloranil test.[53] After complete coupling, the resins were washed with DMF (3×). *Coupling with TBTU (general procedure A1):*[22] 3.0 equiv of acid, 2.8 equiv of TBTU, and 3.0 equiv of NEM was mixed in the appropriate amount of DMF, allowed to stand for 10 min, then added to the resin. *With HATU (general procedure A2):*[23] 3.0 equiv of acid, 2.8 equiv of HATU, 1.0 equiv of HOAt, and 3.0 equiv of NEM was mixed in the appropriate amount of DMF, allowed to stand for 2 min, then added to the resin. *With Pfp esters (general procedure A3):*[54] 3−5 equiv of Pfp ester and 0.1−0.5 equiv of HOAt or DhbtOH was dissolved in the appropriate amount of DMF and added to the resin. *With BTC (general procedure A4):*[25] 3 equiv of acid was mixed with 8 equiv of 2,4,6-collidine in half the volume of DCE solvent under argon, and added 1.0 equiv of BTC dissolved in the other half of DCE (final acid concentration 0.1−0.2 M). The mixture was thoroughly mixed, allowed to stand for 1 min, then added to the resin, and kept under argon. *Coupling to alcohols with MSNT (general procedure A5):*[55] The amino acid (3 equiv) was dissolved in CH_2Cl_2 and *N*-methyl imidazole (2.25 equiv; for nonchiral acids: 6.0 equiv), and MSNT (3 equiv) was added. Five minutes later the mixture was added to the preswollen resin and reacted for 45 min. The resin was drained, washed once with CH_2Cl_2, and the coupling was repeated with a fresh portion of reagents. The resin was washed with CH_2Cl_2 (3×), 5% DIPEA in CH_2Cl_2 (1 min), and CH_2Cl_2 (5×).

Fmoc-Gly-HMBA Functionalized Resins. Typical Procedure. POEPOP[14,20] or SPOCC[16] resins (0.3−0.6 mmol/g) were esterified with MSNT activated TBDMS-HMBA (general procedure A5). The resins were washed with CH_2Cl_2 (3×), THF (3×), and treated with TBAF (5 equiv, 1 M in THF) and AcOH (6 equiv) in THF (1.5 mL) for 2−3 h, washed with THF (3×), MeCN (3×), and CH_2Cl_2 (3×), and dried in vacuo. Attachment of Fmoc glycine to the resin was done with MSNT, after which the resin was dried in vacuo, and the loading was determined.

Resin Bound Fmoc Peptides. Typical Procedure. Fmoc-Gly-HMBA functionalized resin was treated with 20% piperidine, washed, and the next amino acid was attached by the TBTU method (general procedure A1) or the Pfp method (general procedure A3). Successive couplings were also performed in this manner. Couplings onto N-alkylated amino acids were typically performed by activation with HATU (general procedure A2) at 50 °C.

***N*-Boc *N,O*-Acetal Protected N-Terminal Peptide Aldehydes.** Starting materials for the following compounds were prepared using the typical procedures described above.

3: Fmoc-(*N*-Me)Leu-(*N*-Me)Leu-Phe-Gly-HMBA-POEPOP$_{900}$ (164 mg, 0.36 mmol/g, 59 μmol) was treated with 20% piperidine, washed, and treated with HATU activated **2a** (30.6 mg, 118 μmol, 2 equiv) for 2 h. Analysis after cleavage from resin: R_t = 44.2 min (Millipore), ESMS *m*/*z* calcd for $C_{43}H_{71}N_6O_{10}$ $(M + H)^+$: 832.1. Found: 831.5.

9: Fmoc-(*N*-Me)Phe-(*N*-Me)Gly-Leu-Gly-HMBA-POEPOP$_{1500}$ (1.10 g, 0.22 mmol/g, 0.24 mmol) was treated with 20% piperidine, washed, and treated with HATU activated **2a** (125 mg, 0.48 mmol, 2 equiv) for 2 h. Analysis after cleavage from resin: R_t = 15.4 min, ESMS *m*/*z* calcd for $C_{33}H_{52}N_5O_9$ $(M + H)^+$: 662.8. Found: 662.3.

27: POEPOP$_{1500}$ (0.50 g, 0.41 mmol/g, 0.21 mmol) was esterified with Fmoc-(*N*-Me)Phe-OH the MSNT (general procedure A5). Loading 0.24 mmol/g. Coupling of **2a** (1.5 equiv) after removal of the Fmoc group was performed with HATU for 4 h at 50 °C. Analysis after cleavage from resin: R_t = 39.1 min (Millipore).

39: Fmoc-Leu-Phe-Gly-HMBA-POEPOP$_{900}$ (46 mg, 0.53 mmol/g, 24 μmol) was treated with 20% piperidine, washed, and coupled with **2a** (10.2 mg, 39 μmol, 1.6 equiv) using TBTU (1.5 equiv) and NEM (1.6 equiv). Analysis after cleavage from resin: R_t = 38.1 min (Millipore), 15.3 and 15.5 min (Zorbax), ESMS *m*/*z* calcd for $C_{29}H_{45}N_4O_8$ $(M + H)^+$: 577.7. Found: 577.4.

42: Fmoc-(*N*-Me)Leu-Phe-Gly-HMBA-POEPOP$_{900}$ (14 mg, 0.46 mmol/g, 6.4 μmol) was treated with 20% piperidine, washed, and coupled with **2a** (2.5 mg, 9.7 μmol, 1.5 equiv) using TBTU (1.4 equiv) and NEM (1.5 equiv). Analysis after cleavage from resin: R_t = 39.2 min (Millipore), ESMS *m*/*z* calcd for $C_{30}H_{47}N_4O_8$ $(M + H)^+$: 591.7. Found: 591.4.

44a: Fmoc-(FmocHmb)Ala-Leu-Phe-Gly-HMBA-POEPOP$_{900}$ (296 mg, 0.37 mmol FmocNH/g, 108 μmol) was prepared from H-Leu-Phe-Gly-HMBA-POEPOP$_{900}$ and TBTU activated Fmoc-(FmocHmb)Ala-OH (2 equiv). The Fmoc group was removed, and the resin was treated with HATU activated **1a** (3 equiv) overnight, washed with DMF (3×), treated with 20% piperidine in DMF for 2 × 3 min, washed with DMF, treated with Ac_2O:pyridine (1:2) for 45 min, and washed with DMF (3×) and MeCN (8×). Analysis after cleavage from resin: R_t = 17.2 and 17.5 min, ESMS *m*/*z* calcd for $C_{39}H_{55}N_5O_{11}$ $(M + H)^+$: 770.9. Found: 770.6 (for both diastereomers).

44b: Prepared as described for **44a** using **2a** instead of **1a**. Analysis after cleavage from resin: R_t = 40.3 min (Millipore), ESMS *m*/*z* calcd for $C_{40}H_{58}N_5O_{11}$ $(M + H)^+$: 784.9. Found: 784.5.

Coupling of 1a and 2a−e to Model Peptides. POE-POP_{1500} resin (0.41 mmol/g) was functionalized with Fmoc-Phe-OH according to general procedure A5. Four 50−60 mg of portions of the resulting resin were then treated with piperidine to remove the Fmoc group, and each coupled with an Fmoc protected amino acid (Gly, Phe, Ile, or Pro) using TBTU (general procedure A1). The loadings of the dipeptide functionalized resins were 0.35 mmol/g (Fmoc-Gly-Phe-POEPOP) and 0.34 mmol/g (the other resins).

For each coupling experiment (cf. Table 1), approximately 10 mg of resin was used, and the Fmoc group was removed. For each portion of resin, 3.0 equiv of building block was dissolved in DMF containing 0.093 M TBTU and 0.10 M NEM, resulting in a 0.10 M solution of building block. The mixtures were incubated for 10 min and then added to the resins. A small sample of each resin (1−2 mg) was withdrawn from the reaction vessel after 1 and 24 h, washed with DMF (5×) and MeCN (3×), and analyzed by RP-HPLC. The identity of each product was verified by LCMS analysis (data not shown).

General Procedure B. Unmasking of *N*-Boc *N*,*O*-Acetal Protected Aldehyde. Resin bound *N*-Boc *N*,*O*-acetal protected aldehyde (**3** or **9**) was swollen in MeCN, drained, treated with TFA:H_2O (95:5) for 3 min, and washed with DMF (3×), MeCN (5×), and followed by the solvent for the subsequent reaction.

General Procedure C. Reductive Amination of Resin Bound *N*-Terminal Aldehydes. Typical reductive aminations were performed by treating 1−5 mg of freshly prepared peptide aldehyde **4** or **10** (general procedure B) with a mixture of the desired amine (20−30 equiv) and $NaBH(OAc)_3$ (15−20 equiv) in DMSO:CH_2Cl_2:AcOH (50:50:1, amine concentration 0.2−0.5 M) and allowed to react overnight. The resin was washed with DMF (3×), H_2O (3×), MeCN (3×), CH_2Cl_2 (3×), and MeCN (3×) and analyzed by RP-HPLC and ESMS or LCMS (see Table 3 for results). A specific example was scaled up as described below.

H-Phe-Leu-*N*-*cyclo*-Hexyl-4-aminobutyryl-(*N*-Me)Phe-(*N*-Me)Gly-Leu-Gly-OH. Resin **9** (152 mg, 33 μmol) was treated according to general procedure B and then treated with a mixture of *cyclo*-hexylamine (115 μL, 30 equiv) and $NaBH(OAc)_3$ (142 mg, 20 equiv) in DMSO:CH_2Cl_2:AcOH (50:50:1, 4 mL) and allowed to react overnight. The resin was washed and treated with Fmoc-Leu-OPfp (5 equiv) according to general procedure A3 at 50 °C overnight after which the chloranil test showed a trace of nonacylated material. The coupling was repeated with a fresh batch of reagents for 4 h, resulting in complete coupling. The Fmoc group was removed, and coupling with Fmoc-Phe-OPfp (3 equiv) was performed overnight. After removal of the Fmoc group and additional washing with MeCN (5×) and H_2O (3×), the resin was treated with 0.1 M NaOH (2 × 1.5 mL, 1 h each) and washed with H_2O (3×), 1,4-dioxane (3×), and MeCN (3×). The supernatant was neutralized with 1 M HCl, concentrated, and purified by preparative RP-HPLC yielding a white solid (8.8 mg, 31%). R_t = 15.8 min, ESMS *m*/*z* calcd for $C_{46}H_{70}N_7O_8$ $(M + H)^+$: 849.1. Found: 849.6. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.75 (m, 1H, *H*N-Leu), 8.3−8.1 (m, 1H, *H*N-Gly), 8.2 (m, 1H, *H*N-Phe), 8.1 (bs, 3H, H_3N^+-Leu), 7.3−7.1 (m, 10H, 2 Ph), 5.58 (m, 1H, (*N*-Me)Phe_α), 4.8 (m, 1H, Gly-*Leu*$_\alpha$), 4.3 (m, 1H, H-Leu_α), 4.2−3.9 (m, 1H, Phe_α), 4.18 + 3.65 (2 × m, 2H, Gly_α), 3.7 (m, 2H, (*N*-Me)Gly_α), 3.1−2.7 (m, 7H, Phe_β, (*N*-Me)Phe_β, *c*Hex H^1, NC*H*$_2$CH$_2$CH$_2$CO), 2.9−2.8 (4 s, 6H, 2 CH_3N), 2.3−2.0 (m, 2H, NCH$_2$CH$_2$C*H*$_2$CO), 2.0−1.0 (m, 18H, *c*Hex H^2-H,[6] 2 $Leu_{\beta+\gamma}$, NCH$_2$C*H*$_2$CH$_2$CO), 0.9 (m, 12H, 2 Leu_δ).

***N*-Terminal Peptide Aldehyde 13.** A total of 105.2 mg (23.1 μmol) of **9** was cleaved with 0.1 M NaOH (2 × 500 μL of each 1 h), and the resin was washed with water (5×) and MeCN (3×). The combined supernatant was acidified to pH 2 with 1 M HCl (2 mL) and lyophilized. The crude material was purified by preparative RP-HPLC to give **13** as a colorless syrup which was lyophilized to a fine white powder. Yield: 7.97 mg (15.8 μmol, 68%). R_t = 11.6 min, ESMS *m*/*z* calcd for $C_{25}H_{37}N_4O_7$ $(M + H)^+$: 505.6. Found: 505.2. ^{1}H NMR (250 MHz, $DCCl_3$): δ 9.76 + 9.66 (2 × s, 1H, CHO), 7.5−7.1 (m, 7H, Ph, *H*N-Gly, *H*N-Leu), 5.46 (t, 1H, *J* = 7.6, Phe_α), 4.5 (m, 1 H, Leu_α), 4.1 (m, 1H, Gly_α), 3.8 (m, 1H, Gly_α), 3.72 (m, 2H, (*N*-Me)Gly_α), 3.2−2.9 (m, 2H, Phe_β), 3.00 (s, 3H, CH_3N), 2.93 (s, 3H, CH_3N), 2.9−2.4 (m, 4H, C*H*$_2$C*H*$_2$CHO), 1.7−1.5 (m, 2H, $Leu_{\beta+\gamma}$), 0.9 (m, 6H, Leu_δ).

Internal Peptide Aldehyde 16. Compound **9** was treated with aminoacetaldehyde dimethylacetal according to general procedure C. The resin was then treated with Fmoc-Ser(*t*Bu)-OPfp (general procedure A3) at 50 °C and was 1 h later complete according to the chloranil test. The Fmoc group was removed, the resin was coupled with Fmoc-Leu-OPfp (general procedure A3) within 1 h, and the Fmoc group was removed. The resin was washed with MeCN (3×), treated with 0.1 M NaOH (50 μL) for 1 h, and 1 M HCl (50 μL) was added affording compound **16**. RP-HPLC analysis of the supernatant: R_t = 14.2 min, ESMS *m*/*z* calcd for $C_{40}H_{66}N_7O_{10}$ $(M + H)^+$: 805.0. Found: 804.4.

2,5-Diketopiperazines 20a−d: Two portions of **17** (of each 15 mg, 0.24 mmol/g) were treated according to general procedure B. One portion was treated as described in general procedure C with H-Gly-O*t*Bu dibenzenesulfimide salt (31 mg, 20 equiv), the other with HPhe-O*t*Bu·HCl (18.6 mg, 20 equiv). The resins were then each split into two and each portion was treated overnight with either Fmoc-Gly-OH or Fmoc-Phe-OH (10 equiv) activated by HATU (general procedure A2). Coupling of Fmoc-Phe-OH to **18b** was done with BTC as described in general procedure A4 and was completed within 30 min. Analysis after cleavage from resin: **20a**: R_t = 24.9 min (Millipore), ESMS *m*/*z* calcd for $C_{18}H_{24}N_3O_5$ $(M + H)^+$: 362.4. Found: 362.1. **20b**: R_t = 31.3 min (Millipore), ESMS *m*/*z* calcd for $C_{25}H_{30}N_3O_5$ $(M + H)^+$: 452.5. Found: 452.2. **20c**: R_t = 31.3 min (Millipore), ESMS *m*/*z* calcd for $C_{25}H_{30}N_3O_5$ $(M + H)^+$: 452.5. Found: 452.2. **20d**: R_t = 15.4 min (Zorbax), ESMS *m*/*z* calcd for $C_{32}H_{36}N_3O_5$ $(M + H)^+$: 542.6. Found: 542.3.

2,5-Diketopiperazines 24: Six portions of **9** (20−24 mg each) were converted into aldehyde **10** (general procedure

B) and reductively aminated according to general procedure C with H-Gly-O*t*Bu dibenzenesulfimide salt, H-Ala-O*t*Bu·HCl, H-Val-O*t*Bu·HCl, H-Leu-O*t*Bu·HCl, H-Phe-O*t*Bu·HCl, and H-Ser-OMe·HCl. Analysis by RP-HPLC and LCMS showed formation of the expected compounds (see Table 4) along with minor amounts of a common impurity at R_t = 11.9 min, LCMS: 748.5 $(M + H)^+$.

Resins **21**{*1*−*6*} were then divided into five portions of resin (2 mg each) and coupled with BTC activated Fmoc amino acid (Gly, Leu, Pro, Lys(Boc) and Ser(*t*Bu)) according to general procedure A4 (9 equiv of amino acid, 3 equiv of BTC, 24 equiv of collidine) for 4 h at 55 °C. After washing with DMF (3×), the Fmoc group was removed. The Kaiser test of a few beads from each portion of resin was used to monitor the completion on the DKP ring closure; in cases where this was not achieved, the resin was kept in DMF at 55 °C until accomplished (a few hours). Results of RP-HPLC and LCMS analysis are summarized in Table 4.

Preparative Synthesis of 24{*5*}{*2*}**.** Compound **9** (152 mg, 33 μmol) was hydrolyzed to **10** (general procedure B) and treated with H-Phe-O*t*Bu·HCl (259 mg, 30 equiv) according to general procedure C. The resin was treated with Fmoc-Leu-OH (106 mg, 9 equiv), BTC (30 mg, 3 equiv), and collidine (107 μL, 24 equiv) according to general procedure A4 at 55 °C overnight, after which the chloranil test was negative. The Fmoc group was removed, and the resin was washed with MeCN (5×) and H_2O (3×), treated with 0.1 M NaOH (2 × 1.5 mL, 1 h each), and washed with H_2O (3×), 1,4-dioxane (3×), and MeCN (3×). The supernatants were neutralized with 1 M HCl, concentrated, and purified by preparative RP-HPLC yielding **24**{*5*}{*2*} as a white solid (9.0 mg, 36%). R_t = 15.2 min, ESMS *m*/*z* calcd for $C_{46}H_{70}N_7O_8$ $(M + H)^+$: 749.9. Found: 749.5. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.45 (m, 1H, DKP *H*N-Leu), 8.43 (m, 1H, *H*N-Gly), 8.32 (m, 1H, Gly-*H*N-Leu), 7.5−7.2 (m, 10H, 2 Ph), 5.75 (m, 1H, (*N*-Me)Phe$_\alpha$), 4.53 (m, 1H, Gly-Leu$_\alpha$), 4.35 + 3.95 (m, 2H, Gly$_\alpha$), 4.34 (m, 2H, DKP Phe$_\beta$), 4.0−3.9 (m, 2H, (*N*-Me)Gly$_\alpha$), 3.95 (m, 1H, DKP Leu$_\alpha$), 3.3−2.9 (m, 2H, (*N*-Me)Phe$_\beta$), 3.29 (m, 1H, DKP Phe$_\alpha$), 3.2−3.0 (m, 2H, NC*H*$_2$CH$_2$CH$_2$CO), 3.1−2.9 (4 s, 6H, 2 CH$_3$N), 2.5−2.2 (m, 2H, NCH$_2$CH$_2$C*H*$_2$CO), 1.9−1.6 (m, 8H, Gly-*Leu*$_{\beta+\gamma}$, DKP Leu$_{\beta+\gamma}$, NCH$_2$C*H*$_2$CH$_2$CO), 1.06 (2 d, 6H, *J* = 6.2, DKP Leu$_\delta$), 0.81 (t, 6H, *J* = 6.5, Gly-*Leu*$_\delta$).

Horner−Wadsworth−Emmons Reactions. Typical Procedure. Typical HWE reactions were performed by treating freshly prepared peptide aldehyde **10** overnight with a mixture (incubated 5 min) of ethyl-, *tert*-butyl-, or allyl-*P*,*P*-diethylphosphonoacetate (30 equiv), TEA (20 equiv, 0.5 M), and LiBr (25 equiv) in MeCN (~30 μL per μmol aldehyde). The resin was then washed with MeCN (6×). A specific example is described below.

HWE Olefinated Peptide (25c). Compound **9** (192.6 mg, 42 μmol) was hydrolyzed to **10** (general procedure B) and treated with allyl-*P*,*P*-diethylphosphonoacetate (267 μL, 30 equiv), TEA (118 μL, 20 equiv), and LiBr (92 mg, 25 equiv) in MeCN (1.32 mL) for 28 h, after which the resin was washed with MeCN (6×) and lyophilized. A total of 1 mg of resin was cleaved with 0.1 M NaOH affording **26** which by RP-HPLC was shown to be 95% pure. **26**: R_t = 12.2 min, LCMS *m*/*z* calcd for $C_{27}H_{39}N_4O_8$ $(M + H)^+$: 547.6. found: 547.2.

HWE Olefinated Peptide (27): Compound **25c** (162 mg, ~36μmol) was swollen in $HCCl_3$, drained, and treated with an argon bubbled solution of Pd[PPh_3]$_4$ (120 mg, 104 μmol) in $HCCl_3$:AcOH:NEM (37:2:1, 1.5 mL) for 1.25 h, washed with CH_2Cl_2 (3×), THF (3×), and DMF (3×) to yield **27**. Cleavage of some compound from 1 mg of resin afforded **26** with identical purity and analytical data (RP-HPLC, LCMS) as **26** in the above paragraph.

Unsaturated Peptide Isosteres (29a−d). These four compounds were prepared by the following general route: To 2 mg of resin (**27**) was added 50 μL of a DMF solution containing HATU (0.3 M), HOAt (0.1 M), and NEM (0.3 M). Ten minutes later was added 50 μL of a DMF solution containing H-Gly-O*t*Bu·HCl (**a**), H-Ala-O*t*Bu·HCl (**b**), H-Leu-O*t*Bu·HCl (**c**), or H-Phe-O*t*Bu•HCl (**d**) (0.5 M) and NEM (0.5 M) (the resin was not drained prior to amine addition). After incubation overnight, the resin was washed with DMF (6×) and MeCN (6×), and cleaved products were analyzed by RP-HPLC and LCMS. **29a**: R_t = 11.5 min, 91% purity, ESMS *m*/*z* calcd for $C_{29}H_{42}N_5O_9$ $(M + H)^+$: 604.7. Found: 604.2. **29b**: R_t = 12.2 min, 93% purity, ESMS *m*/*z* calcd for $C_{30}H_{44}N_5O_9$ $(M + H)^+$: 618.7. Found: 618.3. **29c**: R_t = 13.9 min, 89% purity, ESMS *m*/*z* calcd for $C_{33}H_{50}N_5O_9$ $(M + H)^+$: 660.8. Found: 660.3. **29d**: R_t = 14.3 min, 93% purity, ESMS *m*/*z* calcd for $C_{36}H_{48}N_5O_9$ $(M + H)^+$: 693.8. Found: 693.3.

The synthesis of **28d** was scaled up: **27** (~36 μmol) was treated with HATU (48 mg, 3 equiv), HOAt (5.8 mg, 1 equiv), and NEM (5.4 mg, 1 equiv) in DMF (500 μL), and 10 min later H-Phe-O*t*Bu·HCl (66 mg, 6 equiv) and NEM (33 μL, 6 equiv) in DMF (500 μL) were added overnight, washed with DMF (6×), MeCN (3×), and water (3×), and cleaved with 0.1 M NaOH (2 × 1 mL of each 1 h) after which the resin was washed with water (5×) and MeCN (3×). The combined supernatant was neutralized with 1 M HCl, lyophilized, and purified by preparative RP-HPLC to give **29d** as a white solid. Yield: 4.29 mg (6.18 μmol, 17%). R_t = 14.3 min, ESMS *m*/*z* calcd for $C_{36}H_{48}N_5O_9$ $(M + H)^+$: 694.8. Found: 694.8. ^{1}H NMR (500 MHz, $DCCl_3$): δ 7.43−7.12 (m, 1H, *H*N-Leu), 7.37 (m, 1H, *H*N-Gly), 7.33−7.12 (m, 10H, 2 Ph), 7.16 (m, 1H, *H*N-Phe), 6.81 + 6.70 (2 × dt, 1H, *J* = 15.4, 6.2, CH═C*H*CH$_2$), 5.92 + 5.79 (2 × d, 1H, *J* = 15.4, C*H*═CHCH$_2$), 5.63 + 5.52 (2 × t, 1H, *J* = 7.7, (*N*-Me)Phe$_\alpha$), 4.92−4.74 (m, 1H, Phe$_\alpha$-OH), 4.69 + 4.50 (2 × m, 1H, Leu$_\alpha$), 4.22 + 3.79 (2 × m, 2H, Gly$_\alpha$), 4.06 (d, 1H, *J* = 16.9, (*N*-Me)Gly$_\alpha$), 3.53 (d, 1H, *J* = 16.9, (*N*-Me)-Gly$_\alpha$), 3.23 (m, 1H, (*N*-Me)Phe$_\beta$), 3.19−3.11 (m, 2H, Phe$_\beta$-OH), 3.08−2.89 (4 × s, 6H, 2 CH$_3$N), 2.97 (dd, 1H, *J* = 7.7, (*N*-Me)Phe$_\beta$), 2.62−2.21 (m, 4H, C*H*$_2$C*H*$_2$CO), 1.78−1.51 (m, 3H, Leu$_{\beta+\gamma}$), 0.96 (m, 6H, Leu$_\delta$).

Pictet−Spengler Reactions. Typical Procedure. Pictet−Spengler condensations were performed by treating 1−5 mg of freshly prepared peptide aldehyde **10** with a 0.1 M solution of the desired amine/amino acid in the appropriate solvent at 50 °C for 1−3 days. Specific examples, some of which are scaled up, are described below.

Condensation of 10 with H-His-OH (30a). Compound **9** (10.0 mg, 2.2 μmol) was converted into **10** as described above and treated with 500 μL of a 0.1 M solution of H-His-OH in DMSO:toluene:AcOH (2:2:1) at 60 °C for 7 days and washed with water (5×) and MeCN (5×). A total of 1 mg of resin was cleaved with 0.1 M NaOH and analyzed by RP-HPLC. Two diastereomers of **30a** were present in 38% purity along with 17% aldol product **11**. **30a**: R_t = 9.8 and 9.9 min, ESMS *m*/*z* calcd for $C_{32}H_{43}N_7O_8$ $(M + H)^+$: 642.7. Found: 642.3 (for both diastereomers).

Condensation of 10 with H-His-OMe·3TFA (30b). Compound **9** (3 mg, 0.7 μmol) was converted into **10** as described above, treated with 100 μL of 0.5 M solution of H-His-OH·3TFA in DMSO:toluene:AcOH (2:2:1) at 50 °C for 3 days, and washed with DMF (3×), water (5×), DMF (3×), and MeCN (3×). A total of 1 mg of resin was cleaved with 0.1 M NaOH resulting in hydrolysis of **30b** to **30a** and analyzed by RP-HPLC. Two diastereomers of **30a** were present in approximately 15% purity along with 50% aldol product **11**. RP-HPLC retention times and ESMS masses were identical with those obtained for **30a** (see above).

Condensation of 10 with H-Trp-OH (31a). Compound **9** (210.4 mg, 46.3 μmol) was converted into **10** as described above, washed with water (5×), treated with 2 mL of 0.1 M H-Trp-OH·HCl in 0.5 M HCl at 60 °C for 20 h, and washed with water (5×), aqueous $NaHCO_3$ (1×), water (5×), and MeCN (5×). A total of 1 mg of resin was cleaved with 0.1 M NaOH and analyzed by RP-HPLC. The two diastereomers of **31a** were present in ∼100% purity. **31a**: R_t = 13.7 and 13.8 min, ESMS *m*/*z* calcd for $C_{36}H_{47}N_6O_8$ $(M + H)^+$: 691.8. Found: 691.3 (for both diastereomers).

Condensation of 10 with H-Trp-OMe (31b). Compound **9** (11.7 mg, 2.6 μmol) was converted into **10** as described above, treated with 500 μL of a 0.1 M solution of H-Trp-OMe·HCl in either DMSO:toluene:AcOH (2:2:1) or 0.5 M HCl at 60 °C for 20 h, and washed with water (5×) and MeCN (5×). A total of 1 mg of resin was cleaved with 0.1 M NaOH and analyzed by RP-HPLC, RP-HPLC, and ESMS, giving results identical to those of **31a** (see above).

Condensation of 10 with Tryptamine (32). Compound **9** (126.0 mg, 27.7 μmol) was converted into **10** as described above and treated with tryptamine hydrochloride (109 mg, 20 equiv) in DMSO:toluene (1:1, 1 mL) at 50 °C for 3 days, washed with DMF (3×), CH_2Cl_2 (3×), and DMF (5×). A total of 1 mg of resin was washed with MeCN (5×), cleaved with 0.1 M NaOH, and analyzed by RP-HPLC. The two diastereomers of **32** were present in ∼100% purity. **32**: R_t = 13.8 and 14.0 min, ESMS *m*/*z* calcd for $C_{35}H_{47}N_6O_6$ $(M + H)^+$: 647.8. Found: 647.4 (for both diastereomers).

DKP Fused Tetrahydro-*β*-carboline 34a via 31a: Compound **31a** (4 mg, 0.88 μmol) swollen in DCE was treated with a DCE solution of Fmoc-Gly-OH (0.1 M), BTC (0.033 M), and 2,4,6-collidine (0.27 M) at 55 °C overnight, washed with CH_2Cl_2 (3×) and DMF (3×), treated with 20% piperidine, washed with DMF (8×), MeCN (3×), and CH_2Cl_2 (5×), and treated with CH_2Cl_2 containing DCC (0.2 M) and DMAP (catalytic amount) for 5 h, after which the reaction was complete according to chloranil test. The resin was washed with DMF (3×) and MeCN (3×). Analysis after cleavage from resin: R_t = 14.4 min, ESMS *m*/*z* calcd for $C_{38}H_{48}N_7O_8$ $(M + H)^+$: 730.8. Found: 730.3.

DKP Fused Tetrahydro-*β*-carboline 34b via 31a: Compound **31a** (211 mg, 46 μmol) swollen in DCE was treated with Fmoc-Lys(Boc)-OH (217 mg, 10 equiv), BTC (46 mg, 3.3 equiv), and 2,4,6-collidine (164 μL, 27 equiv) in DCE (2 mL) (general procedure A4). After being kept at 55 °C for 1 h, chloranil test was negative, the resin was washed with CH_2Cl_2 (3×) and DMF (3×), the Fmoc group was removed, and the resin was washed with MeCN (3×) and CH_2Cl_2 (5×) and treated with DCC (96 mg, 10 equiv) and DMAP (28 mg, 5 equiv) in DCE (1.5 mL) at 60−65 °C for 2 days after which the chloranil test of some washed resin still showed unreacted starting material. The resin was drained, washed with DCE (5×), and treated with DCC and DMAP at 65 °C for 3 days after which no starting material could be detected. The resin was washed with CH_2Cl_2 (3×) and MeCN (3×), lyophilized, cleaved with 0.1 M NaOH (2 × 1 mL of each 1 h), and washed with water (5×), MeCN (3×), and 1,4-dioxane (3×). The combined extracts were neutralized with 1 M HCl and concentrated to a solid residue which was purified by preparative RP-HPLC to give a total of 15.6 mg (17.3 μmol, 37%) of the two diastereomers of **34b**. From the purification, 5.8 and 6.3 mg of pure diastereomers were isolated. R_t = 16.6 and 16.8 min, ESMS *m*/*z* calcd for $C_{38}H_{48}N_7O_8$ $(M + H)^+$: 902.1. Found: 901.5 (both diastereomers). ^{1}H NMR (500 MHz, $DCCl_3$) of diastereomer R_t = 16.6 min: δ 7.69 (m, 1H, N*H*Boc), 7.47 (m, 1H, *H*N-Gly),7.36−6.99 (m, 10H, Ar, carboline NH), 7.28 (m, 1H, *H*N-Leu), 7.26 (m, 1H, *H*N-Lys), 5.56−5.46 (m, 1H, Phe_α), 4.85 (m, 1H, carboline CH_2CH), 4.68 (m, 2H, Lys_ϵ), 4.66 (m, 1H, Leu_α), 4.21 + 3.80 (2×m, 2H, Gly_α), 4.16 (m, 1H, Lys_α), 4.11 (d, 1H, J = 17.0, (*N*-Me)Gly_α), 4.01−3.95 (m, 1H, $CHCH_2CH_2CO$), 3.57 (d, 1H, J = 17.0, (*N*-Me)Gly_α), 3.24 (m, 2H, carboline CH_2CH), 3.23−3.17 (m, 2H, Phe_β), 2.94−2.86 (4 × s, 6H, 2 CH_3N), 2.42−2.27 (m, 2H, $CHCH_2CH_2CO$), 2.00 (m, 2H, Lys_β), 1.88−1.80 (m, 2H, $CHCH_2CH_2CO$), 1.88 (m, 2H, Lys_δ), 1.66−1.55 (m, 3H, $Leu_{\beta+\gamma}$), 1.46 (s, 9H, Boc), 1.37 (m, 2H, Lys_γ), 0.92 (m, 6H, Leu_δ).

DKP Fused Tetrahydro-*β*-carbolines 34a−f via 31b. Six portions of each 3 mg of **31b** were treated with a DCE solution of 0.15 M Fmoc-AA-OH (AA = Gly (**34a**), Lys(Boc) (**34b**), Phe (**34c**), Cys(Trt) (**34d**), (*N*-Me)Phe (**34e**), and Pro (**34f**)), 0.05 M BTC, and 0.40 M 2,4,6-collidine (general procedure A4) at 55−60 °C overnight after which all couplings were complete. The resins were washed with CH_2Cl_2 (3×) and DMF (3×), treated with 20% piperidine in DMF at 60 °C for 5 h, washed with DMF (3×) and MeCN (3×), and cleaved with aqueous NaOH. RP-HPLC and LCMS analyses were performed. **34a**: R_t = 14.4 min, (88% purity), ESMS *m*/*z* calcd for $C_{38}H_{48}N_7O_8$ $(M + H)^+$: 730.8. Found: 730.3. **34b**: R_t = 16.6 and 16.8 min, (96% purity), ESMS *m*/*z* calcd for $C_{47}H_{65}N_8O_{10}$ $(M + H)^+$: 902.1. Found: 901.5. **34c**: R_t = 15.9 and 16.1 min, (93% purity), ESMS *m*/*z* calcd for $C_{45}H_{54}N_7O_8$ $(M + H)^+$: 820.9. Found: 820.3. **34d**: R_t = 20.2 and 20.4 min + 14.9 and 15.2 min (**34d**−Trt), (combined: 82% purity), ESMS *m*/*z* calcd for $C_{58}H_{63}N_7O_8SNa$ $(M + Na)^+$: 1040.4. Found: 1040.3 and

759.2 $(M + Na - Trt)^+$. **34e**: $R_t = 16.6$ and 16.7 min, (94% purity), ESMS *m/z* calcd for $C_{46}H_{56}N_7O_8$ $(M + H)^+$: 835.0. Found: 834.3. **34f**: $R_t = 14.8$ and 15.1 min, (93% purity), ESMS *m/z* calcd for $C_{41}H_{51}N_7O_8$ $(M + H)^+$: 770.9. Found: 770.3.

Fmoc-Pro Acylation of 32 (37a): Compound **32** (28 μmol) was treated with Fmoc-Pro-OPfp (42 mg, 83 μmol, 3 equiv) and a catalytic amount of HOAt in DMF (300 μL) for 3 h, washed with DMF (3×), treated with 20% piperidine, washed with DMF (8×) and MeCN (5×), and cleaved with 0.1 M NaOH (2 × 1 mL of each 1 h). The resin was washed with water (5×) and MeCN (3×). The combined supernatants were neutralized with 1 M HCl, lyophilized, and purified by preparative RP-HPLC to give **37a** as a white solid. Yield: 15.6 mg (21.0 μmol, 76%). **37a**: $R_t = 14.7$ and 15.0 min, ESMS *m/z* calcd for $C_{40}H_{53}N_7O_7$ $(M + H)^+$: 744.9. Found: 744.3 (for both diastereomers).

Phe-Leu Acylation of 32 (47b): Resin **32** (4 mg, 0.9 μmol) was treated with HATU activated Fmoc-Leu-OH (10 equiv) (general procedure A2) for 4 h, the Fmoc group was removed, and the resin was washed with MeCN (3×) yielding **37b**. Analysis after cleavage from resin: $R_t = 15.6$ and 15.9 min, ESMS *m/z* calcd for $C_{41}H_{58}N_7O_7$ $(M + H)^+$: 760.9, found: 760.6.

Resin **37b** was treated with Fmoc-Phe-OPfp (5 equiv) (general procedure A3) overnight, treated with 20% piperidine, and washed with DMF (8×) and MeCN (3×) yielding **38**. Analysis after cleavage from resin: $R_t = 16.4$ and 16.6 min, ESMS *m/z* calcd for $C_{50}H_{67}N_8O_8$ $(M + H)^+$: 908.1. Found: 907.6.

Amide-Aldehyde Condensation Products (40a, 40b, and 41a): Compound **39** (380 mg, 0.20 mmol) was hydrolyzed according to general procedure B and washed with H_2O (3×). The resin was treated with 0.1 M NaOH (2 × 1 h) and washed with H_2O (3×), MeCN (3×), and 1,4-dioxane (3×). The supernatant was neutralized with 1 M HCl, freeze-dried, and purified by preparative RP-HPLC to give 42.0 mg of a white solid (105 μmol, 52%). Analytical RP-HPLC showed two inseparable peaks ($R_t = 11.1$ and 11.8 min) which were in equilibrium. NMR showed the presence of cyclic compounds **40a**, **40b**, and **41a** in approximately equal amounts along with traces of at least one more compound. ESMS *m/z* calcd for $C_{21}H_{28}N_3O_5$ $(M + H)^+$: 402.5. Found: 402.3. ^{1}H NMR (500 MHz, $DCCl_3$) **40a:** δ 7.88 (d, 1H, $J = 7.8$, *H*N−Phe), 7.4−7.1 (m, 5H, Ph), 7.33 (m, 1H, C*H* = N), 7.25 (m, 1H, *H*N-Gly), 6.4 (bs, 1H, CO_2H), 4.86 (m, 1H, Gly_α), 4.83 (m, 1H, Phe_α), 4.76 (m, 1H, Leu_α), 4.14 (m, 1H, C*H*HCH=N), 3.96 (m, 1H, C*H*HCH=N), 3.19 (m, 1H, Phe_β), 2.99 (m, 1H, Phe_β), 1.93 (m, 1H, C*H*HCH$_2$CH=N), 1.72−1.53 (m, 2H, Leu_β), 1.65 (m, 1H, C*H*HCH$_2$CH=N), 1.44−1.27 (m, 1H, Leu_γ), 0.94−0.82 (m, 6H, Leu_δ). **40b:** δ 7.61 (d, 1H, $J = 8.7$, *H*N−Phe), 7.4−7.1 (m, 5H, Ph), 7.25 (m, 1H, *H*N-Gly), 7.07 (d, 1H, $J = 5.9$, CH=C*H*N), 6.4 (bs, 1H, CO_2H), 6.18 (d, 1H, $J = 5.9$, C*H* = CHN), 4.96 (m, 1H, Phe_α), 4.86 (m, 1H, Gly_α), 4.76 (m, 1H, Leu_α), 3.81 (d, 1H, $J = 21$, C*H*HCH=CHN), 3.50 (d, 1H, $J = 21$, CH*H*CH=CHN), 3.20 (m, 1H, Phe_β), 2.89 (m, 1H, Phe_β), 1.72−1.53 (m, 2H, Leu_β), 1.44−1.27 (m, 1H, Leu_γ), 0.94−0.82 (m, 6H, Leu_δ). **41a:** δ 8.01 (d, 1H, $J = 7.8$, *H*N-Leu), 7.4−7.1 (m, 5H, Ph), 7.25 (m, 1H, *H*N-Gly), 6.4 (bs, 1H, CO_2H), 5.38 (d, 1H, $J = 5.2$, CH=N), 4.96 + 4.85 (2 × m, 1H, Phe_α), 4.86 (m, 1H, Gly_α), 4.85 (m, 1H, Leu_α), 3.24 (m, 1H, Phe_β), 3.21 (m, 1H, Phe_β), 2.70 (m, 1H, C*H*HCH=N), 2.34 (m, 1H, C*H*HCH$_2$CH=N), 2.15 (m, 1H, CH*H*CH=N), 2.03 (m, 1H, CH*H*CH$_2$CH=N), 1.75−1.63 (m, 2H, Leu_β), 1.44−1.27 (m, 1H, Leu_γ), 0.94−0.82 (m, 6H, Leu_δ).

Peptide Aldehyde Fragmentation Product 48. Compound **44a** (108 μmol) was treated twice with TFA:H_2O (95:5) for 10 min, drained, treated with aqueous TFA for 40 min, washed with MeCN (3×) with H_2O (3×), and the combined extracts were lyophilized to a yellow oil which was purified by preparative RP-HPLC. Of the six fractions which were collected and analyzed by NMR and LCMS, only one exhibited agreeable NMR spectral properties, namely compound **48** (6.7 mg, 16%). ESMS *m/z* calcd for $C_{19}H_{24}N_2O_6$ $(M + H)^+$: 377.4. Found: 377.1. ^{1}H NMR (500 MHz, $DCCl_3$): δ 9.38 (m, 1H, C*H*=CHNH), 7.68 (dd, 1H, $J = 14.1$, 10.4, CH=CHN*H*), 7.21 (dd, 1H, $J = 8.5$, 1.6, Hmb H^6), 6.75 (dd, 1H, $J = 8.5$, 2.5, Hmb H^5), 6.60 (d, 1H, $J = 2.5$, Hmb H^3), 5.10 + 5.00 (2 × d, 1H, $J = 15.1$, Ar-C*H*H), 3.94 + 3.93 (2 × d, 1H, $J = 15.1$, Ar-CH*H*), 3.78 (m, 5H, MeO, CH_2OCO), 3.66−3.45 (m, 4H, Ala_α, NC*H*$_2$-CH$_2$CH$_2$O, CH=C*H*NH), 2.31 + 2.30 (2 × s, 3H, Ac), 1.87 (p, 2H, $J = 6.0$, NCH$_2$C*H*$_2$CH$_2$O), 1.26 (2 × d, 3H, $J = 6.9$, Ala_β).

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Supporting Information Available. Spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting

Two errors were inadvertently introduced during the final correction phase and appear in the version released ASAP on 10/28/00. The caption to Figure 3 should read "(b) Free N-terminal aldehyde **13**" instead of "...aldehyde[13]". Also, in the Conclusions section, second paragraph, the 13th line should read "intermediate is" and not "is intermediate". These changes have been incorporated in this corrected version.

References and Notes

(1) Groth, T.; Meldal, M.; Bock, K. Backbone Amide Protection in Solid-Phase Synthesis of Peptide Isosters Derived from *N*-Terminal Aldehydes. In *Peptides for the New Millennium, Proc. 16th Am. Pept. Symp.*; Fields, G., Tam, J. P., Barany, G., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000, pp 146−147.

(2) Gante, J. Peptidomimetics − Tailored Enzyme Inhibitors. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699−1720.

(3) Giannis, A.; Kolter, T. Peptidomimetics for Receptor Ligands − Discovery, Development, and Medical Perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.

(4) Dolle, R. E. Comprehensive survey of chemical libraries yielding enzyme inhibitors, receptor agonists and antagonists, and other biologically active agents: 1992 through 1997. *Mol. Diversity* **1998**, *3*, 199−233.
(5) Meldal, M.; Svendsen, I. Direct visualization of enzyme inhibitors using a portion mixing inhibitor library containing a quenched fluorogenic peptide substrate. Part 1. Inhibitors for substilisin Carlsberg. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1591−1596.
(6) Meldal, M. Intramolecular Fluoresence-Quenched Substrate Libraries. In *Methods in Molecular Biology*, Vol. 87: *Combinatorial Library Protocols*; Cabilly, S., Eds.; Humana Press Inc.: Totowa, NJ, 1998; pp 65−74.
(7) Meldal, M. The Solid Phase Enzyme Inhibitor Library Assay. In *Methods in Molecular Biology*, Vol. 87: *Combinatorial Library Protocols*; Cabilly, S., Eds.; Humana Press Inc.: Totowa, NJ, 1998; pp 75−82.
(8) Buchardt, J.; Schiødt, C. B.; Krog-Jensen, C.; Delaissé, J.-M.; Foged, N. T.; Meldal, M. Solid Phase Combinatorial Library of Phosphinic Peptides for Discovery of Matrix Metalloproteinase Inhibitors. *J. Comb. Chem.* **2000**, *2*, 624−638.
(9) Fehrentz, J. A.; Paris, M.; Heitz, A.; Velek, J.; Winternitz, F.; Martinez, J. Solid Phase Synthesis of C-Terminal Peptide Aldehydes. *J. Org. Chem.* **1997**, *62*, 6792−6796.
(10) Page, P.; Bradley, M. Solid-Phase Synthesis of Tyrosine Peptide Aldehydes. Analogues of (*S*)-MAPI. *J. Org. Chem.* **1999**, *64*, 794−799.
(11) Ede, N. J.; Eagle, S. N.; Wickham, G.; Bray, A. M.; Warne, B.; Shoemaker, K.; Rosenberg, S. Solid Phase Synthesis of Peptide Aldehyde Protease Inhibitors. Probing the Proteolytic Sites of Hepatitis C Virus Polyprotein. *J. Pept. Sci.* **2000**, *6*, 11−18.
(12) Rademann, J.; Meldal, M.; Bock, K. Solid-Phase Synthesis of Peptide Isosters by Nucleophilic Reactions with N-Terminal Peptide Aldehydes on a Polar Support Tailored for Solid-Phase Organic Chemistry. *Chem. Eur. J.* **1999**, *5*, 1218−1225.
(13) Groth, T.; Meldal, M. Synthesis of Aldehyde Building Blocks Protected as Acid Labile *N*-Boc *N*,*O*-Acetals: Toward Combinatorial Solid Phase Synthesis of Novel Peptide Isosteres. *J. Comb. Chem.* **2001**, *3*, 34−44.
(14) Renil, M.; Meldal, M. POEPOP and POEPS: Inert Polyethylene Glycol Cross-linked Polymeric Supports for Solid Synthesis. *Tetrahedron Lett.* **1996**, *37*, 6185−6188.
(15) Grøtli, M.; Rademann, J.; Groth, T.; Lubell, W. D.; Miranda, L. P.; Meldal, M. Surfactant Mediated Cationic and Anionic Suspension Polymerization of PEG-based Resins in Silicon Oil; Beaded SPOCC 1500 and POEPOP 1500. *J. Comb. Chem.* **2001**, *3*, 28−33.
(16) Rademann, J.; Grøtli, M.; Meldal, M.; Bock, K. SPOCC: A Resin for Solid-Phase Organic Chemistry and Enzymatic Reactions on Solid Phase. *J. Am. Chem. Soc.* **1999**, *121*, 5459−5466.
(17) Meldal, M. PEGA: A flow stable polyethylene glycol dimethyl acrylamide copolymer for solid phase synthesis. *Tetrahedron Lett.* **1992**, *33*, 3077−3080.
(18) Meldal, M.; Auzanneau, F.-I.; Bock, K. PEGA, Characterization and application of a new type of resin for peptide and glycopeptide synthesis. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Eds.; Mayflower Worldwide Ltd.: Kingswinford, 1994; pp 259−266.
(19) Renil, M.; Meldal, M. Synthesis and Application of a PEGA Polymeric Support for High Capacity Continuous Flow Solid-Phase Peptide Synthesis. *Tetrahedron Lett.* **1995**, *36*, 4647−4650.
(20) Grøtli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. Ø.; Meldal, M. Physical Properties of Poly(ethylene glycol) (PEG)-Based Resins for Combinatorial Solid Phase Organic Chemistry: A Comparison of PEG-Cross-Linked and PEG-Grafted Resins. *J. Comb. Chem.* **2000**, *2*, 108−119.
(21) Johnson, T.; Quibell, M.; Owen, D.; Sheppard, R. C. A Reversible Protecting Group for the Amide Bond in Peptides. Use in Synthesis of "Difficult Sequences". *J. Chem. Soc., Chem. Commun.* **1993**, 369−372.
(22) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. New Coupling Reagents in Peptide Synthesis. *Tetrahedron Lett.* **1989**, *30*, 1927−1930.
(23) Carpino, L. A. 1-Hydroxy-7-azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(24) Cotarca, L.; Delogu, P.; Nardelli, A.; Šunjić, V. Bis-(trichloromethyl) Carbonate in Organic Synthesis. *Synthesis* **1996**, 553−576.
(25) Falb, E.; Yechezkel, T.; Salitra, Y.; Gilon, C. *In situ* generation of Fmoc-amino acid chlorides using bis-(trichloromethyl)carbonate and its utilization for difficult couplings in solid-phase peptide synthesis. *J. Pept. Res.* **1999**, *53*, 507−517.
(26) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxybotohydride. Studies on Direct and Indirect Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849−3862.
(27) Kowalski, J.; Lipton, M. A. Solid Phase Synthesis of a Diketopiperazine Catalyst Containing the Unnatural Amino Acid (*S*)-Norargenine. *Tetrahedron Lett.* **1996**, *37*, 5839−5840.
(28) Szardenings, A. K.; Antonenko, V.; Cambell, D. A.; DeFrancisco, N.; Ida, S.; Shi, L.; Sharkow, N.; Tien, D.; Wang, Y.; Navre, M. Identification of Highly Selective Inhibitors of Collagenase-1 from Combinatorial Libraries of Diketopiperazines. *J. Med. Chem.* **1999**, *42*, 1348−1357.
(29) Gordon, D. W.; Steele, J. Reductive Alkylation on a Solid Phase: Synthesis of a Piperazinedione Combinatorial Library. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47−50.
(30) Wang, H.; Ganesan, A. The *N*-Acyliminium Pictet−Spengler Condensation as a Multicomponent Combinatorial Reaction on Solid Phase and Its Application to the Synthesis of Demethoxyfumitremorgin C Analogues. *Org. Lett.* **1999**, *1*, 1647−1649.
(31) Scott, B. O.; Siegmund, A. C.; Marlowe, C. K.; Pei, Y.; Spear, K. L. Solid Phase Organic Synthesis (SPOS): A novel route to diketopiperazines and diketomorpholines. *Mol. Diversity* **1995**, *1*, 125−134.
(32) Krchňák, V.; Weichsel, A. S.; Cabel, D.; Lebl, M. Structurally Homogeneous and Heterogeneous Libraries: Scaffold-Based Libraries and Libraries Built Using Bifunctional Building Blocks. In *Molecular Diversity and Combinatorial Chemistry: Libraries and Drug Discovery*; Chaiken, I. M., Janda, K. D., Eds.; American Chemical Society: Washington, DC, 1996; pp 99−115.
(33) Bianco, A.; Sonksen, C. P.; Roepstorff, P.; Briand, J.-P. Solid-Phase Synthesis and Structural Characterization of Highly Substituted Hydroxyproline-Based 2,5-Diketopiperazines. *J. Org. Chem.* **2000**, *65*, 2179−2187.
(34) Boutagy, J.; Thomas, R. Olefin Synthesis with Organic Phosphonate Carbanions. *Chem. Rev.* **1974**, *74*, 87−99.
(35) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. Horner-Wadsworth-Emmons Reaction: Use of Lithium Chloride and an Amine for Base-sensitive Compounds. *Tetrahedron Lett.* **1984**, *25*, 2183−2186.

(36) Rathke, M. W.; Nowak, M. The Horner-Wadsworth-Emmons Modification of the Wittig Reaction Using Triethylamine and Lithium or Magnesium Salts. *J. Org. Chem.* **1985**, *50*, 2624−2626.
(37) Johnson, C. R.; Zhang, B. Solid Phase Synthesis of Alkenes Using The Horner-Wadsworth-Emmons Reaction and Monitoring by Gel Phase ^{31}P NMR. *Tetrahedron Lett.* **1995**, *36*, 9253−9256.
(38) Warrass, R.; Gras-Masse, H.; Lippens, G.; Melnyk, O. Introducing Conformational Contraints into a Peptide Chain by Chemical Ligation. In *Peptides 1998, Proceedings of the Twenty-Fifth European Peptide Symposium*; Bajusz, S., Hudecz, F., Eds.; Akadémiai Kiadó: Budapest, 1999; pp 188−189.
(39) Johansson, A.; Åkerblom, E.; Ersmark, K.; Linderberg, G.; Hallberg, A. An Improved Procedure for N- to C-Directed (Inverse) Solid-Phase Peptide Synthesis. *J. Comb. Chem.* **2000**, *2*, 496−507.
(40) Pictet, A.; Spengler, T. On the formation of isochinoline derivatives through the reaction of formaldehyde with phenylethylamine, phenylalanine, and tyrosine. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 2030−2036.
(41) Cox, E. D.; Cook, J. M. The Pictet−Spengler Condensation: A New Direction for an Old Reaction. *Chem. Rev.* **1995**, *95*, 1797−1842.
(42) Whaley, W. M.; Govindachari, T. R. The Pictet−Spengler Synthesis of Tetrahydroisoquinolines and Related Compounds. In Organic Reactions, Vol. 6; Adkins, H., Blatt A. H., Cope A., McGrew, F. C., Niemann, C., Snyder, H. R., Eds.; John Wiley & Sons: New York, 1951; pp 151−190.
(43) Abramovitch, R. A.; Spenser, I. D. The Carbolines. In *Advances in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1964; pp 79−207.
(44) Mohan, R.; Chou, Y.-L.; Morrissey, M. M. Pictet−Spengler Reaction on Solid Support: Synthesis of 1,2,3,4-Tetrahydro-β-Carboline Libraries. *Tetrahedron Lett.* **1996**, *37*, 3936−3966.
(45) Cheng, C. C.; Chu, Y.-H. Characterization of Spatially Adressable Libraries: Stereoisomer Analysis of Tetrahydro-β-carbolines as an Example. *J. Comb. Chem.* **1999**, *1*, 461−466.
(46) Urban, J.; Vaisar, T.; Shen, R.; Lee, M. S. Lability of *N*-alkylated peptides towards TFA cleavage. *Int. J. Pept. Res.* **1996**, *47*, 182−189.
(47) Graven, A.; Grøtli, M.; Meldal, M. Towards peptide isostere libraries: aqueous aldol reactions on hydrophilic solid supports. *J. Chem. Soc., Perkin Trans. 1* **2000**, 955−962.
(48) Kita, Y.; Akai, S.; Ajimura, N.; Yoshigi, M.; Tsugoshi, T.; Yasuda, H.; Tamura, Y. Facile and Efficient Syntheses of Carboxylic Anhydrides and Amides Using (Trimethylsilyl)-ethoxyacetylene. *J. Org. Chem.* **1986**, *51*, 4150−4158.
(49) Christiansen-Brams, I.; Meldal, M.; Bock, K. Protected-mode Synthesis of *N*-Linked Glycopeptides: Single-step Preparation of Building Blocks as Peracetyl Glycosylated N^{α} Fmoc Asparagine OPfp Esters. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1461−1471.
(50) Atherton, E.; Fox, H.; Harkiss, D.; Logan, C. J.; Sheppard, R. C.; Williams, B. J. A Mild Procedure for Solid Phase Peptide Synthesis: Use of Fluorenylmethoxycarbonylamino-acids. *J. Chem. Soc., Chem. Commun.* **1978**, 537−539.
(51) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis − A Practical Approach;* IRL Press: Oxford, 1989.
(52) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem.* **1970**, *34*, 596−598.
(53) Vojkovsky, T. Detection of Secondary Amines on Solid Phase. *Pept. Res.* **1995**, *8*, 236−237.
(54) Kisfaludy, L.; Schön, I. Preparation and Applications of Pentafluorophenyl Esters of 9-Fluorenylmethyloxycarbonyl Amino Acids for Peptide Synthesis. *Synthesis* **1983**, 325−327.
(55) Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. An Efficient Method for Anchoring Fmoc-Amino Acids to Hydroxyl-Functionalised Solid Supports. *Tetrahedron Lett.* **1990**, *31*, 1701−1704.

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