Backbone Amide Linker (BAL) Strategy for Solid-Phase Synthesis of C-Terminal-Modified and Cyclic Peptides[1,2,3]

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Abstract: Peptide targets for synthesis are often desired with C-terminal end groups other than the more usual acid and amide functionalities. Relatively few routes exist for synthesis of C-terminal-modified peptides—including cyclic peptides—by either solution or solid-phase methods, and known routes are often limited in terms of ease and generality. We describe here a novel *B*ackbone *A*mide *L*inker (BAL) approach, whereby the growing peptide is anchored through a backbone nitrogen, thus allowing considerable flexibility in management of the termini. Initial efforts on BAL have adapted the chemistry of the tris(alkoxy)benzylamide system exploited previously with PAL anchors. Aldehyde precursors to PAL, e.g. 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid, were reductively coupled to the α-amine of the prospective C-terminal amino acid, which was blocked as a *tert*-butyl, allyl, or methyl ester, or to the appropriately protected C-terminal-modified amino acid derivative. These reductive aminations were carried out either in solution or on the solid phase, and occurred without racemization. The secondary amine intermediates resulting from solution amination were converted to the 9-fluorenylmethoxycarbonyl (Fmoc)-protected preformed handle derivatives, which were then attached to poly(ethylene glycol)−polystyrene (PEG-PS) graft or copoly(styrene−1% divinylbenzene) (PS) supports and used to assemble peptides by standard Fmoc solid-phase chemistry. Alternatively, BAL anchors formed by on-resin reductive amination were applied directly. Conditions were optimized to achieve near-quantitative acylation at the difficult step to introduce the penultimate residue, and a side reaction involving diketopiperazine formation under some circumstances was prevented by a modified protocol for N^{α}-protection of the second residue/introduction of the third residue. Examples are provided for the syntheses in high yields and purities of representative peptide acids, alcohols, *N,N*-dialkylamides, aldehydes, esters, and head-to-tail cyclic peptides. These methodologies avoid postsynthetic solution-phase transformations and are ripe for further extension.

Introduction

Peptides for biological investigations and pharmaceutical development are required primarily as their C-terminal acid or amide derivatives. Nevertheless, the myriad of naturally occurring peptides, and their biologically relevant synthetic analogues, includes numerous examples where the carboxy termini are modified to other functionalities, e.g. alcohols, ethers, esters, *N*-alkylamides, hydrazides, trifluoromethyl ketones, aldehydes, mercaptoalkylamides, thioesters, and thioamides.[6] Similarly, the importance of cyclic peptides is well established.[7] Modifications at the C-terminus, as well as the elimination of termini via cyclization, represent potential avenues to more effective therapeutic agents;[6,7] furthermore, some peptide segments (protected and/or activated as needed) that are designed to be intermediates in convergent syntheses have nonstandard C-termini.[8,9]

The present paper reports on a novel and general concept for solid-phase synthesis[8a,9a,10] that involves attachment of a backbone amide nitrogen to an appropriate handle[11]/support [i.e., with the general structures of Scheme 1, drawn as implemented

(1) Taken in part from the Ph.D. theses of M.F.S. (University of Minnesota, July, 1996) and J.A. (University of Barcelona, October, 1997).

(2) Portions of this work were reported in preliminary form: (a) Jensen, K. J.; Songster, M. F.; Vágner, J.; Alsina, J.; Albericio, F.; Barany, G. In *Peptides—Chemistry, Structure and Biology: Proceedings of the Fourteenth American Peptide Symposium*, Columbus, OH, June 18−23, 1995; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific: Kingswinford, U.K., 1996; pp 30−32. (b) Jensen, K. J.; Songster, M. F.; Vágner, J.; Alsina, J.; Albericio, F.; Barany, G. In *Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 1996, Collected Papers, Fourth International Symposium*, Edinburgh, Scotland, September 12−16, 1995; Epton, R., Ed.; Mayflower Scientific: Kingswinford, U.K., 1997; pp 187−190.

(3) After our preliminary reports on BAL (ref 2), we became aware of two conceptually related handles, neither of which was exploited to its full potential: (a) Rivier, J.; Porter, J.; Hoeger, C. In *Peptides: Chemistry, Biology, Interactions with Proteins*; Penke, B., Török, A., Eds.; Walter de Gruyter: Berlin, 1988; pp 75−78. (b) Renil, M.; Pillai, V. N. R. *Tetrahedron Lett.* **1994**, *35*, 3809−3812. In addition, the aldehyde precursor to PAL was subjected to reductive amination with amino acid methyl esters to achieve a linkage akin to BAL, in work that was submitted later than our disclosures: (c) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742−5743. (d) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240−1256. Finally, BAL chemistry is implicit in the anchoring of modified purines through an aniline side chain, as reported by (e) Gray, N. S.; Kwon, S.; Schultz, P. G. *Tetrahedron Lett.* **1997**, *38*, 1161−1164, and in the anchoring of *O*-protected hydroxylamines to access hydroxamates, see (f) Ngu, K.; Patel, D. V. *J. Org. Chem.* **1997**, *62*, 7088−7089.

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(6) Review: Songster, M. F.; Barany, G. *Methods Enzymol.* **1997**, *289*, 126−174.

(7) Reviews: (a) Ovchinnikov, Y. A.; Ivanov, V. T. In *The Proteins*; Neurath, H., Hill, R. L., Eds.; Academic: New York, 1982; pp 307−642. (b) Rizo, J.; Gierasch, L. M. *Annu. Rev. Biochem.* **1992**, *61*, 387−418. (c) Kates, S. A.; Solé, N. A.; Albericio, F.; Barany, G. In *Peptides: Design, Synthesis, and Biological Activity*; Basava, C., Anantharamaiah, G. M., Eds.; Birkhäuser: Boston, MA, 1994; pp 39−58.

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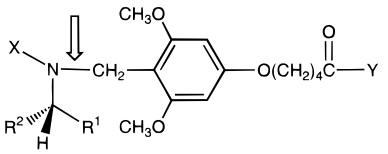
Scheme 1. Structures of Tris(alkoxy)benzylamide-Based BAL Handles, Supports, and Peptide-Resins[a]



X = Fmoc, Y = OH — Fmoc-(BAL-OH)NCHR^{1}R^2 (**1**) — (preformed handle)

X = H, Y = NH–Ⓡ — H-(BAL-NH-Resin)NCHR^{1}R^2 — (resin-bound handle)

X = peptidyl, Y = NH–Ⓡ — (peptide-BAL-resin)

R^1 = appropriately modified *C*-terminus [bracketed is structure after final cleavage]
- e.g., CO_2tBu [CO_2H, *peptide acids*]
- CO_2Allyl [access to *cyclic peptides*]
- CO_2R^3 [*peptide esters*]
- CH_2O*t*Bu [CH_2OH, *peptide alcohols*]
- $CH(OMe)_2$ [(C=O)H, *peptide aldehydes*]
- $(C{=}O)N(R^3)_2$ [*peptide dialkylamides*]

R^2 = appropriate side-chain functionality

[a] The arrow shows the site of attachment (via reductive amination) as well as cleavage (via acidolysis). For each R^1 cited, an experimental example is provided in this paper.

with the tris(alkoxy)benzylamide (PAL) system[12]]. This *B*ackbone *A*mide *L*inker (BAL) anchor is established by reductive amination with an amino acid residue (or an appropriately modified derivative); following *N*-acylation by an appropriately protected second amino acid residue, a dipeptidyl unit forms that is linked to the support through a backbone amide bond. Further chain growth is then expected to proceed normally. Since the C-terminal carboxyl is not involved as the point of attachment and initiation of solid-phase synthesis, this BAL anchoring approach offers a simple and direct way to prepare peptides having a variety of C-terminal functionalities (Scheme 1). Additionally, when used in conjunction with an orthogonally removable C-terminal carboxyl protecting group (e.g., allyl), it becomes possible to manipulate further the resin-bound peptide, for example to carry out head-to-tail cyclizations. The BAL approach involves a single cleavage step for side-chain deprotection and concomitant release of peptides from the support, and circumvents side reactions and drawbacks from methods involving nucleophilic cleavages of ester or oxime anchoring linkages.[6,8−10]

Results and Discussion

Backbone Amide Anchoring Concept. This work represents the evolution and convergence of several research themes: (i) mild orthogonal protection schemes with base-sensitive 9-fluorenylmethoxycarbonyl (Fmoc) for N^{α}-amino group protection, acid-labile *tert*-butyl (*t*Bu) and relatives for side-chain protection,[10c−e] and, as needed, allyl esters removable by treatment with palladium(0) in the presence of appropriate nucleophiles for an additional dimension of orthogonality;[7c,9a] (ii) reductive amination procedures[12c,d] that attach amine derivatives to aldehyde-containing handle precursors; (iii) acid-labile tris(alkoxy)benzylamide (PAL)[12a−c] anchoring to provide C-terminal peptide amides and corresponding *N*-alkyl[tris-(alkoxy)benzyl]amide [(R)PAL][12d] anchoring to provide C-terminal peptide *N*-alkylamides; (iv) side-chain anchoring[7c,13] as a way to access unusual C-termini including free end groups needed for cyclization (n.b., the aforementioned approaches all require appropriately protected trifunctional amino acid residues, a restriction that does not apply to the BAL approach); and (v) precedents due to Weygand, Sheppard, and others on protection of α-amide bonds.[14]

Implementation of BAL Concept Based on Tris(alkoxy)-benzylamide System. PALdehyde (**2**)[12b,15] was coupled through a reductive amination procedure to the amine of the prospective C-terminal residue, suitably protected as required (Scheme 2, first stage). Either the free amine or any of a variety of salts (hydrochloride, trifluoroacetate, tosylate) could be used. Our initial approach followed earlier precedents[12c,d] and involved reaction of the aldehyde and amine in methanol to form the corresponding imine, followed by addition of $NaBH_3CN$. When imine formation was carried out at reflux, high levels of racemization were observed, whereas at 25 °C, no racemization occurred. Systematic studies revealed that a separate imine formation step was not necessary at either 25 or 60 °C. Our preferred optimized procedure involves mixing the aldehyde, amine, and cyanoborohydride (1 equiv each) *simultaneously* in

(8) For reviews describing convergent approaches for syntheses of longer peptides, see: (a) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; Vol. 2, pp 1−284. (b) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065−11133. (c) Benz, H. *Synthesis* **1994**, 337−358. (d) Albericio, F.; Lloyd-Williams, P.; Giralt, E. *Methods Enzymol.* **1997**, *289*, 313−336.

(9) The use of C-terminal-modified peptides in convergent syntheses has been covered in several further reviews, see: (a) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC: Boca Raton, FL, 1997. (b) Muir, T. W.; Dawson, P. E.; Kent, S. B. H. *Methods Enzymol.* **1997**, *289*, 266−298. (c) Tam, J. P.; Spetzler, J. C. *Methods Enzymol.* **1997**, *289*, 612−637.

(10) (a) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical: Rockford, IL, 1984. (b) Merrifield, R. B. *Science* **1986**, *232*, 341−347. (c) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705−739. (d) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis: A Practical Approach*; IRL: Oxford, U.K., 1989. (e) Fields, G. B.; Tian, Z.; Barany, G. In *Synthetic Peptides: A User's Guide*; Grant, G. A., Ed.; W. H. Freeman: New York, 1992; pp 259−345.

(11) Handles are defined as bifunctional spacers which serve to attach the initial residue to the polymeric support in two discrete steps. One end of the handle incorporates features of a smoothly cleavable protecting group, and the other end allows facile coupling to a previously functionalized support. For reviews and extensive tabulations of handles, see refs 6, 8, and 10c,e.

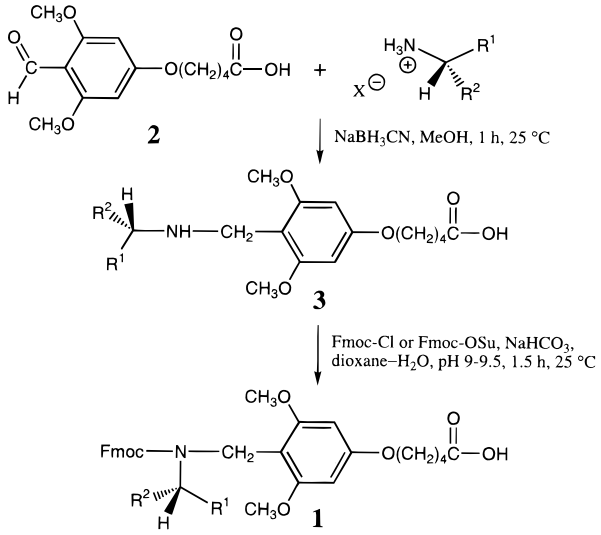
(12) (a) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1987**, *30*, 206−216. (b) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743. (c) Sharma, S. K.; Songster, M. F.; Colpitts, T. L.; Hegyes, P.; Barany, G.; Castellino, F. J. *J. Org. Chem.* **1993**, *58*, 4993−4996. (d) Songster, M. F.; Vágner, J.; Barany, G. *Lett. Pept. Sci.* **1996**, *2*, 265−270.

(13) (a) Breipohl, G.; Knolle, J.; Stüber, W. *Int. J. Pept. Protein Res.* **1990**, *35*, 281−283. (b) Albericio, F.; Van Abel, R.; Barany, G. *Int. J. Pept. Protein Res.* **1990**, *35*, 284−286. (c) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, 33, 4557−4560. (d) Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549−1552. (e) Lee, J.; Griffin, J. H.; Nicas, T. I. *J. Org. Chem.* **1996**, *61*, 3983−3986. (f) Alsina, J.; Chiva, C.; Ortiz, M.; Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 883−886.

(14) (a) Weygand, F.; Steglich, W.; Bjarnason, J. *Tetrahedron Lett.* **1966**, 3483−3487. (b) Narita, M.; Fukunaga, T.; Wakabayashi, A.; Ishikawa, K.; Nakano, H. *Int. J. Pept. Protein Res.* **1984**, *23*, 306−314. (c) Blaakmeer, J.; Tijsse-Klasen, T.; Tesser, G. I. *Int. J. Pept. Protein Res.* **1991**, *37*, 556−564. (d) Johnson, T.; Quibell, M.; Sheppard, R. C. *J. Pept. Sci.* **1995**, *1*, 11−25. (e) Quibell, M.; Packman, L. C.; Johnson, T. *J. Am. Chem. Soc.* **1995**, *117*, 11656−11668. (f) Simmonds, R. G. *Int. J. Pept. Protein Res.* **1996**, *47*, 36−41. (g) Clausen, N.; Goldammer, C.; Jauch, K.; Bayer, E. In *Peptides−Chemistry, Structure and Biology: Proceedings of the Fourteenth American Peptide Symposium;* Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific: Kingswinford, U.K., 1996; pp 71−72. (h) Nicolás, E.; Pujades, M.; Bacardit, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 2317−2320.

(15) For all structural formulas in this paper, PALdehyde and BAL handles are depicted as the isomer in which the aminomethyl group is para to the valeryl linking group (refs 12b,d). Some experiments described herein started instead with a mixture (1:2) of the ortho and para isomers of PALdehyde, i.e. *o,p*-PALdehyde (refs 12a−c); furthermore, the side-chain moiety had one fewer carbon (substitution of butyryl for valeryl). The latter compound is commercially available from the BioSearch division of PerSeptive Biosystems (Framingham, MA).

Scheme 2. Preparation of Tris(alkoxy)benzylamide-Based BAL Handles



a $R^1 = CO_2Me$, $R^2 = H$
b $R^1 = CO_2Allyl$, $R^2 = H$
c $R^1 = CH(OMe)_2$, $R^2 = H$
d $R^1 = CO_2tBu$, $R^2 = CH_3$
e $R^1 = CO_2tBu$, $R^2 = CH_2CH(CH_3)_2$
f $R^1 = CO_2Me$, $R^2 = CH_2C_6H_5$
g $R^1 = CO_2tBu$, $R^2 = CH_2C_6H_5$

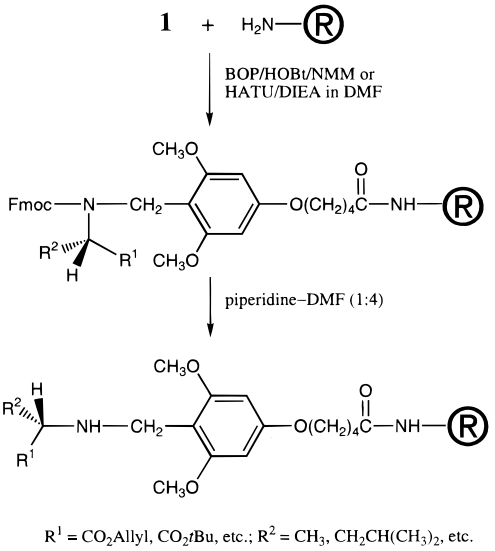
X = Cl, CF_3CO_2, or $CH_3C_6H_4SO_3$

methanol;[16] reactions at 25 °C for 1 h provided the products in high yields without any detectable racemization.

The secondary amine intermediates (**3**) thus obtained were converted *directly* to the Fmoc derivatives, hence providing the corresponding protected amino acid preformed handle derivatives **1** in 57−95% yields [Scheme 2, second stage; *N*-(9-fluorenylmethoxycarbonyloxy)succimide (Fmoc-OSu) was acceptable for sterically unhindered amino acid derivatives, e.g. H-Gly-OAllyl and $H_2NCH_2CH(OMe)_2$, but essentially complete derivatization of even slightly hindered derivatives, e.g. H-Phe-O*t*Bu and H-Leu-O*t*Bu, required the more reactive 9-fluorenylmethyl chloroformate (Fmoc-Cl)]. The preformed handles **1** were then attached essentially quantitatively to poly(ethylene glycol)−polystyrene graft (PEG-PS)[17] or polystyrene (PS) solid supports by standard couplings mediated by benzotriazol-1-yl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) or *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU), plus any needed additives and/or bases (Scheme 3). Finally, Fmoc removal gave BAL-anchored derivatives suitable for further steps.

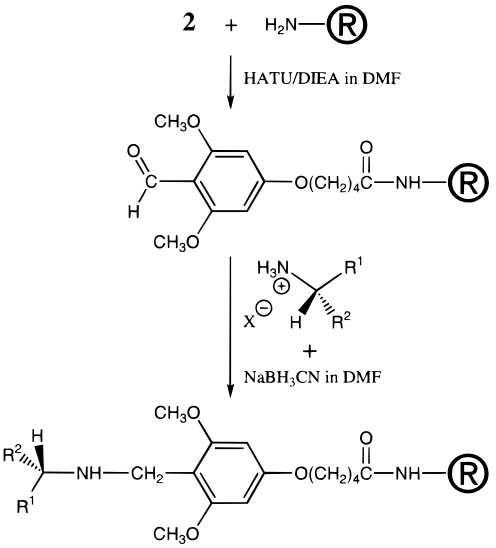
In an alternative procedure, PALdehyde (**2**)[12b,15] was coupled first to the support, followed by on-resin reductive amination using conditions similar to those developed by Sasaki and Coy.[18,19] This operationally simpler approach gave the desired

Scheme 3. Preparation of BAL Anchors with Preformed Handles



$R^1 = CO_2Allyl$, CO_2tBu, etc.; $R^2 = CH_3$, $CH_2CH(CH_3)_2$, etc.

Scheme 4. Preparation of BAL Anchors by On-Resin Reductive Amination



$R^1 = CO_2Allyl$, CO_2tBu, etc.; $R^2 = CH_3$, $CH_2CH(CH_3)_2$, etc.
X = Cl, CF_3CO_2, or $CH_3C_6H_4SO_3$

BAL anchors in good-to-excellent yields while obviating the need for an intermediate *N*-protection step (Scheme 4; Table 1 in the Experimental Section). Nearly quantitative incorporation [i.e., >95%, as judged by "internal reference" amino acids (IRAA's)[20]] was achieved with either MeOH or *N*,*N*-dimethylformamide (DMF) as solvents, and using the amine and

(16) Similar results were obtained using as solvent HOAc−MeOH (1:100), MeOH−THF (1:4), or allyl alcohol. Reaction was incomplete in *i*PrOH, while in DMF (neat) or HOAc−DMF (1:100), the desired secondary amines (**3**) were sometimes minor while the major products were the di-BAL-kylated tertiary amines, apparently formed by addition of a second equiv of **2** (see ref 12c for precedents).

(17) (a) Barany, G.; Albericio, F.; Solé, N. A.; Griffin, G. W.; Kates, S. A.; Hudson, D. In *Peptides 1992: Proceedings of the Twenty-Second European Peptide Symposium*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1993; pp 267−268. (b) Zalipsky, S.; Chang, J. L.; Albericio, F.; Barany, G. *React. Polym.* **1994**, *22*, 243−258. (c) Barany, G.; Albericio, F.; Kates, S. A.; Kempe, M. In *Chemistry and Biological Application of Polyethylene Glycol*; ACS Symposium Series 680; Harris, J. M., Zalipsky, S., Eds.; American Chemical Society Books: Washington, DC, 1997; pp 239−264.

(18) Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119−121.

(19) Solvents of choice were DMF, as reported by Sasaki and Coy in ref 18, or MeOH, as suggested by solution precedents (this work, covered previously). Given the tendency for dialkylation in solution with DMF as solvent (see footnote 16), the relative absence of such an unfavorable side reaction in the solid-phase case is taken as evidence for relative site isolation, as has been covered in numerous review articles (e.g., ref 8a). The success of on-resin monoreductive amination in DMF is also attributable to the considerable excess of amine, later removed readily by filtration and washing, that can be used in the reaction. In the solution case, use of excess amine leads to complications in the workup and purification of the Fmoc-protected preformed handle **1**.

(20) (a) Atherton, E.; Clive, D. L.; Sheppard, R. C. *J. Am. Chem. Soc.* **1975**, *97*, 6584−6585. (b) Matsueda, G. R.; Haber, E. *Anal. Biochem.* **1980**, *104*, 215−227. (c) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1993**, *41*, 307−312.

cyanoborohydride both in considerable excess (10 equiv each) over resin-bound aldehyde. In some cases, the reactions could be made to proceed effectively (e.g., 67−89% yields) with as little as 1−2 equiv of reactants. Our optimal protocols, when applied to amino acid derivatives, proceed without racemization; the key to this may be to avoid preequilibration and to ensure a neutral or slightly acidic reaction milieu.

Optimization of Conditions for Acylation of BAL-Anchored Amines by the Penultimate Amino Acid Residue. The next reaction required for stepwise synthesis, i.e. acylation of the secondary α-amino group attached to the handle, was found to be slower than comparable reactions of unsubstituted primary amines. A variety of standard coupling protocols were examined in order to define the most efficient method for acylating the sterically hindered *N*-substituted handle resins (Tables 2 and 3 in the Experimental Section). Commonly applied in situ coupling reagents[21,22] in DMF, for example, BOP, HATU, and *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), used in the equimolar presence of bases such as *N*-methylmorpholine (NMM) or *N*,*N*-diisopropylethylamine (DIEA), and/or additives such as 1-hydroxybenzotriazole (HOBt) or 3-hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridine [1-hydroxy-7-azabenzotriazole (HOAt)], were all inefficient in mediating the acylation. However, high yields for acylation of the secondary amine were obtained by applying the symmetrical anhydrides of Fmoc-amino acids; the optimal solvent was CH_2Cl_2 [plus whatever amount of DMF was needed for solubility reasons, e.g. CH_2Cl_2−DMF (9:1)], and no base was needed. Other reagents giving satisfactory results with CH_2Cl_2−DMF (9:1) as solvent [*always* preferred over neat DMF or similar solvents such as *N*-methyl-2-pyrrolidinone (NMP)] included HATU/DIEA (1:2), 1,1,3,3-tetramethyl-2-fluoroformamidinium hexafluorophosphate (TFFH)/DIEA (1:2), 7-azabenzotriazol-1-yl-*N*-oxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)/DIEA (1:2), and bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP)/DIEA (1:2). Preformed acid fluorides[23] were also effective, particularly in the presence of DIEA (1.1 equiv). It is of interest to compare these results with other examples of difficult acylations of secondary amines.[14d,21f,h,24]

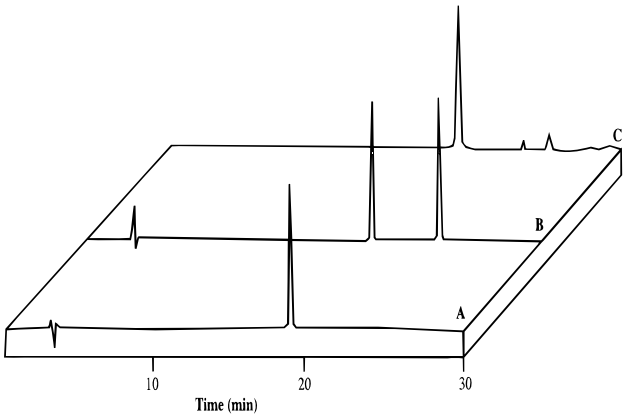


Figure 1. Analytical HPLC of crude H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-enkephalin), as obtained directly upon acidolytic release from BAL (A and B) and Wang (C) supports. Chromatography followed condition B as listed in the Experimental Section, General Procedures. Ordinate is absorbance at 220 nm. (A) Synthesis started with *p*-PALdehyde-Ile-PEG-PS resin (0.20 mmol/g), to which H-Leu-O*t*Bu·HCl was attached by on-resin reductive amination. Further details are given in the Experimental Section. (B) Synthesis started with preformed handle Fmoc-(BAL-OH)Leu-O*t*Bu (**1e**) that had been prepared in refluxing MeOH with a 1-h delayed addition of $NaBH_3CN$, conditions later recognized to lead to substantial racemization. The racemized handle was coupled onto H-Ile-PEG-PS (0.18 mmol/g), and after Fmoc removal, the resultant H-(BAL-Ile-PEG-PS)Leu-O*t*Bu resin was extended further exactly as in A. The later-eluting major peak was cochromatographed with authentic H-Tyr-Gly-Gly-Phe-D-Leu-OH, and shown to comigrate. (C) Synthesis started with *p*-alkoxybenzyl alcohol (Wang)-resin (100 mg, 0.49 mmol/g), to which the C-terminal Fmoc-Leu-OH (87 mg, 5 equiv) was esterified using DIPCDI (38 μL, 5 equiv), HOBt (33 mg, 5 equiv), and DMAP (1.2 mg, 0.2 equiv) in DMF−CH_2Cl_2 (1:1, 1 mL) at 25 °C for 15 h. Further residues were incorporated by DIPCDI/HOBt-mediated couplings in DMF (no special procedure needed for Phe, as was the case in BAL syntheses), and other aspects were exactly as in A and B [cleavage yield with TFA−phenol−H_2O (18:1:1), 1 h, 25 °C, >95%]. (D) Synthesis exactly as in A, except that the initial on-resin reductive amination was carried out in the presence of DIEA equimolar to H-Leu-O*t*Bu·HCl and a 1-h preincubation period preceded addition of $NaBH_3CN$. Chromatogram not shown because it is very similar to B.

Solid-Phase Synthesis on Tris(alkoxy)benzylamide-Derived BAL Anchors: Peptide Acids, Alcohols, *N*,*N*-Dialkylamides, and Aldehydes. With the C-terminal residue introduced as part of the BAL anchor and the penultimate residue incorporated successfully by the optimized acylation conditions just described, further stepwise chain elongation by addition of Fmoc-amino acids generally proceeded normally by any of a variety of peptide synthesis protocols. For example, starting with PALdehyde-resin and continuing with an on-resin reductive amination, the linear leucine enkephalin sequence was assembled as the following peptide-resin: Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-(BAL-Ile-PEG-PS)Leu-O*t*Bu. All residues were incorporated in integer ratios (with respect to each other and to the Ile IRAA), and final Fmoc removal followed by acidolytic cleavage for 1 h with trifluoroacetic acid (TFA)−H_2O (19:1) released the desired product as a single homogeneous component upon analytical HPLC (Figure 1A; 90% cleavage yield, >98% purity). Comparison to a standard of H-Tyr-Gly-Gly-Phe-D-Leu-OH led to the conclusion that no racemization had occurred at the C-terminal Leu residue. This result is significant because (i) prior to optimization of conditions for reductive amination

(21) The development and applications of the revelant coupling reagents are reported by the following: (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219−1222. (b) Dourtoglou, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269−1272. (c) Fournier, A.; Wang, C. T.; Felix, A. M. *Int. J. Pept. Protein Res.* **1988**, *31*, 86−97. (d) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617−624. (e) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1219−1222. (f) Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259−270. (g) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (h) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203. (i) Kates, S. A.; Minor, C. A.; Shroff, H.; Haaseth, R. C.; Triolo, S.; El-Faham, A.; Carpino, L. A.; Albericio, F. In *Peptides 1994: Proceedings of the Twenty-Third European Peptide Symposium*; Maia, H. L. S., Ed.; ESCOM: Leiden, The Netherlands, 1995; pp 248−249. (j) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401−5402. (k) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268−274. (l) Albericio, F.; Carpino, L. A. *Methods Enzymol.* **1997**, *289*, 104−126.

(22) Although initial reports (refs 21b,e,g,h) showed the structures of HBTU and HATU as uronium salts, it has been shown more recently that both compounds crystallize as aminium salts (guanidinium *N*-oxides). See: (a) Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57−67. (b) Henklein, P.; Costisella, B.; Wray, V.; Domke, T.; Carpino, L. A.; El-Faham, A.; Kates, S. A.; Abdelmoty, I.; Foxman, B. M. In *Peptides 1996: Proceedings of the Twenty-Fourth European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower Scientific; Kingswinford, England, 1998, in press.

(23) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651−9652.

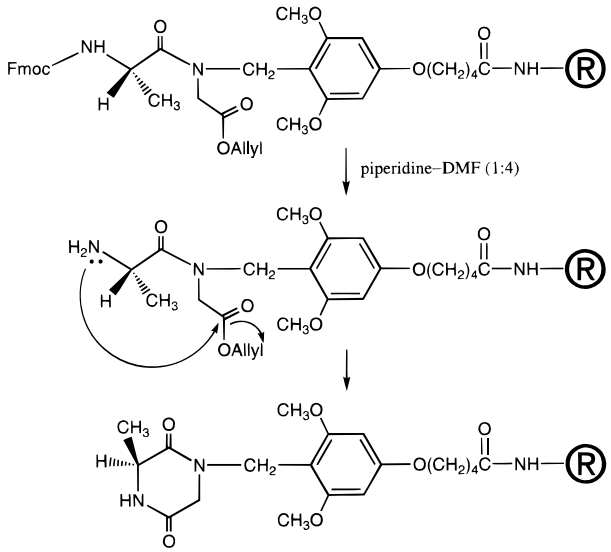
(24) (a) Angell, Y. M.; García-Echeverría, C.; Rich, D. H. *Tetrahedron Lett.* **1994**, *35*, 5981−5984. (b) Angell, Y. M.; Thomas, T. L.; Flentke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1995**, *117*, 7279−7280.

of Leu-O*t*Bu into BAL structures (see earlier discussion), racemization that had occurred during the preparation of preformed handles and/or handle-resin derivatives was carried over to result in substantially racemized final peptide products (e.g., Figure 1B and legend to Figure 1, section D), and (ii) when synthesis of the enkephalin pentapeptide started with *N*,*N*′-diisopropylcarbodiimide (DIPCDI)/*N*,*N*-dimethyl-4-aminopyridine (DMAP)-mediated acylation of a *p*-alkoxybenzyl alcohol (Wang)-resin with Fmoc-Leu-OH,[25] the final product was less pure and showed detectable (∼2.6% D-Leu) racemization (Figure 1C).

The methodology was extended for Fmoc solid-phase synthesis of C-terminal peptide alcohols, *N*,*N*-dimethylamides, and aldehydes, classes of compounds that were previously accessible only by relatively cumbersome routes.[26] Starting with the *tert*-butyl ether of phenylalaninol (H-Phe-o*t*Bu, **5**)[27] and carrying out on-resin reductive amination, the protected model peptide-resin Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-(BAL-Ala-MBHA-PS)-Phe-o*t*Bu was obtained. Fmoc removal followed by final acidolytic cleavage gave the desired peptide alcohol in >95% purity (Supporting Information Figure 1). Similarly, Fmoc-Tyr-(*t*Bu)-Gly-Gly-Phe-(BAL-Ile-PEG-PS)Leu-N(CH_3)$_2$ was prepared by starting with H-(BAL-Ile-PEG-PS)Leu-N(CH_3)$_2$ resin that was obtained by on-resin reductive amination with H-Leu-N(CH_3)$_2$·HCl (**6**). In this case, Fmoc removal followed by final acidolytic cleavage gave the desired leucine enkephalin dimethylamide in >85% purity (Supporting Information Figure 2). As a final illustration, the preformed BAL handle **1c**, prepared from glycinal dimethyl acetal, was coupled onto PEG-PS and extended to add six further amino acid residues. Treatment of the completed peptide-resin for 2 h with TFA−H_2O (19:1) cleaved the Boc side-chain protecting group and the acetal protecting the C-terminal aldehyde moiety, and concomitantly released the model peptide aldehyde H-Ala-Leu-Ala-Lys-Leu-Gly-Gly-H from the support (Supporting Information Figure 3; >70% purity).

Further Solid-Phase Synthesis on Tris(alkoxy)benzylamide-Derived BAL Anchors: Detection and Avoidance of Diketopiperazine Side Reaction, and Successful Protocols for Peptide Esters and Cyclic Peptides. Part of our original vision with BAL was to use allyl chemistry to introduce a third dimension of orthogonality and access cyclic peptides.[13c,d] However, we observed that, with BAL-anchored glycyl allyl esters, piperidine-promoted removal of Fmoc at the dipeptidyl level was accompanied by almost quantitative diketopiperazine formation (Scheme 5).[28] Such a process is favored by the allyl alcohol leaving group, the sterically unhindered Gly residue, and the BAL secondary amide, which allows the required cis transition state; as is implicit from the previous section, diketopiperazine formation was not observed with *t*Bu ester protection or with modified end groups at the C-terminus. On the basis of earlier precedents,[29] we expected that the level of diketopiperazine formation could be reduced substantially by using an acidolytically removable N^{α}-amino protecting group so that the amine end group of the BAL-anchored dipeptide would remain protonated until the time for coupling. Experimentally, this was accomplished by (i) incorporation of the penultimate residue as its N^{α}-trityl (Trt) derivative; (ii) *selective* detritylation with TFA−H_2O−CH_2Cl_2 (2:1:97), for 5 min, without cleavage of the BAL anchor (negligible premature loss of chains at this stage); and (iii) incorporation of the third residue as its N^{α}-Fmoc derivative under in situ neutralization/coupling conditions mediated by PyAOP/DIEA in DMF (Scheme 6) *or* (i′) use of the N^{α}-2-(3,5-dimethoxyphenyl)propyl[2]oxycarbonyl (Ddz) protected derivative [preferred over N^{α}-Trt because of superior coupling characteristics]; (ii′) removal of Ddz with TFA−H_2O−CH_2Cl_2 (3:1:96), for 6 min; and (iii′) same as (iii). These revised protocols allowed the preparation of allyl ester-protected peptides Fmoc-Pro-Tyr-Leu-Ala-Gly-OAllyl [Supporting Information Figure 4, started from preformed handle Fmoc-(BAL-OH)Gly-OAllyl (**1b**), final cleaved product >85% purity, 85% cleavage yield] and Fmoc-Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala-OAllyl [Supporting Information Figure 5, started from H-(BAL-Ile-PEG-PS)Ala-OAllyl prepared by on-resin reductive amination, final cleaved product >85% purity, 85% cleavage yield], as well as the linear peptide ester H-Tyr-Gly-Gly-Phe-Leu-OOctyl [Leu-enkephalin C_8 ester, Supporting Information Figure 6, started from H-(BAL-Ile-MBHA-PS)Leu-

Scheme 5. Diketopiperazine Formation Following Fmoc Deprotection with Piperidine



(25) (a) Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328−1333. (b) Atherton, E.; Benoiton, N. L.; Brown, E.; Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1981**, 336−337. (c) Wang, S. S.; Tam, J. P.; Wang, S. B.; Merrifield, R. B. *Int. J. Pept. Protein Res.* **1981**, *18*, 459−467.

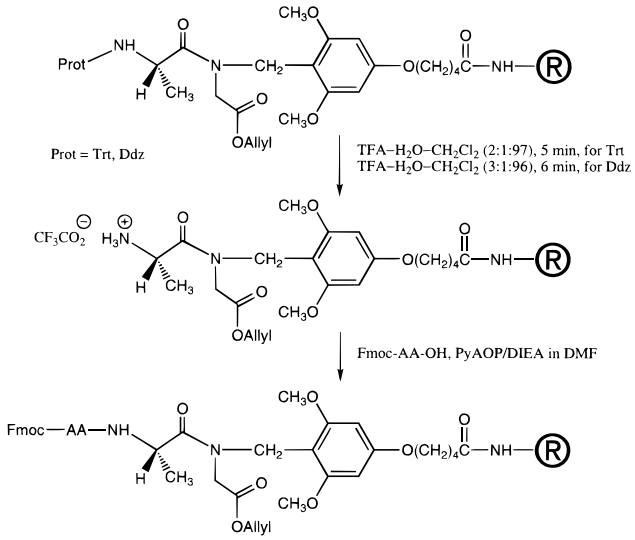
(26) (a) Neugebauer, W.; Escher, E. *Helv. Chim. Acta* **1989**, *72*, 1319−1323. (b) Swistok, J.; Tilley, J. W.; Danho, W.; Wagner, R.; Mulkerins, K. *Tetrahedron Lett.* **1989**, *30*, 5045−5048. (c) Mergler, M.; Nyfeler, R. In *Peptides 1992: Proceedings of the Twenty-Second European Peptide Symposium*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM: Leiden, The Netherlands, 1992; pp 177−178. (d) Murphy, A. M.; Dagnino, R.; Vallar, P. L.; Trippe, A. J.; Sherman, S. L.; Lumpkin, R. H.; Tamura, S. Y.; Webb, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 3156−3157. (e) Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 405−410. (f) Fehrentz, J. A.; Paris, M.; Heitz, A.; Velek, J.; Winternitz, F.; Martinez, J. *J. Org. Chem.* **1997**, *62*, 6792−6796.

(27) Earlier experiments directed at peptide alcohols were carried out with the simpler sequence Fmoc-Ala-Gly-Phe-oh and unprotected phenylalaninol. Analytical HPLC indicated the product mixture from those syntheses contained ∼50% of the desired material (MALDI-TOF MS, calcd for $C_{29}H_{31}N_3O_5$: 501.6. Found: *m*/*z* 502.0 $[M + H]^+$), admixed with several other impurities. One of these byproducts presumably corresponds to acylation of the alcohol by the growing peptide chain (MALDI-TOF MS, calcd for $C_{49}H_{49}N_5O_9$: 852.0. Found: *m*/*z* 852.2 $[M + H]^+$). Such branching did not occur in the text synthesis of H-Tyr-Gly-Gly-Phe-Leu-Phe-oh with *tert*-butyl protection of the alcohol.

(28) Experiments to exploit this observation for *intentional* preparation of diketopiperazines have been reported elsewhere: del Fresno, M.; Alsina, J.; Royo, M.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1998**, *39*, 2639−2642.

(29) (a) Gisin, B. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 3102−3106. (b) Khosla, M. C.; Smeby, R. R.; Bumpus, F. M. *J. Am. Chem. Soc.* **1972**, *94*, 4721−4724. (c) Giralt, E.; Eritja, R.; Pedroso, E. *Tetrahedron Lett.* **1981**, *22*, 3779−3782. (d) Alsina, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1996**, *37*, 4195−4198 and references therein.

Scheme 6. Use of Acid-Labile N^{α}-Amino Protection to Avoid Diketopiperazine Formation



OOctyl prepared by on-resin reductive amination, final cleaved product 98% pure,[30] 86% cleavage yield].

On-resin cyclization starting with Fmoc-Arg(Pmc)-D-Phe-Pro-Glu(O*t*Bu)-Asp(O*t*Bu)-Asn(Trt)-Tyr(*t*Bu)-Glu(O*t*Bu)-Ala-(BAL-Ile-PEG-PS)Ala-OAllyl (the fully protected resin-bound intermediate corresponding to peptide described in previous paragraph) was tested as follows: First, selective removal of the C-terminal allyl ester was achieved by treatment with $Pd(PPh_3)_4$ in DMSO−THF−0.5 N aqueous HCl−morpholine (2:2:1:0.1). Next, the Fmoc group was cleaved to give the BAL-anchored resin-bound peptide with its free C- and N-termini. Use of PyAOP/HOAt in the presence of DIEA in CH_2Cl_2 provided the expected ten-residue head-to-tail cyclic peptide[31] in >85% purity by analytical HPLC (Figure 2). The C-terminally epimerized cyclized peptide was formed in 12% of the amount of the desired species with L-Ala at the point of cyclization (determined by analytical HPLC; the diastereomers were isolated preparatively and shown to have the same molecular weight upon matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), as well as the same amino acid composition). Other experiments in which DIEA was replaced with 2,4,6-trimethylpyridine (collidine), and/or CH_2Cl_2 was replaced with DMF or $CHCl_3$−trifluoroethanol (7:3), gave rise to poorer cyclization yields and/or substantially higher levels of racemization.

Conclusions

We have devised and shown experimentally a novel and general backbone anchoring (BAL) strategy for solid-phase synthesis of peptides and peptide derivatives; this strategy promises to overcome many of the limitations inherent in C-terminal and side-chain anchoring. BAL anchoring has allowed the particularly straightforward preparation of linear peptides with a considerable range of C-terminal modifications, including alcohols, aldehydes, esters, and *N*,*N*-dialkylamides. In addition, the BAL approach fits well with three-dimensional orthogonal schemes for solid-phase synthesis of cyclic peptides.

(30) An earlier synthesis directed at the same peptide, using all-Fmoc chemistry as had been done successfully for Leu-enkephalin with BAL-anchored Leu-O*t*Bu, gave only 25% of the desired product along with 75% of the diketopiperazine cyclo(Phe-Leu).

(31) This peptide was described first by: McMurray, J. S. *Tetrahedron Lett.* **1991**, *32*, 7679−7682. See also Kates et al. (ref 13d) for syntheses using side-chain anchoring strategies, with varying yields and purities depending on the point of cyclization.

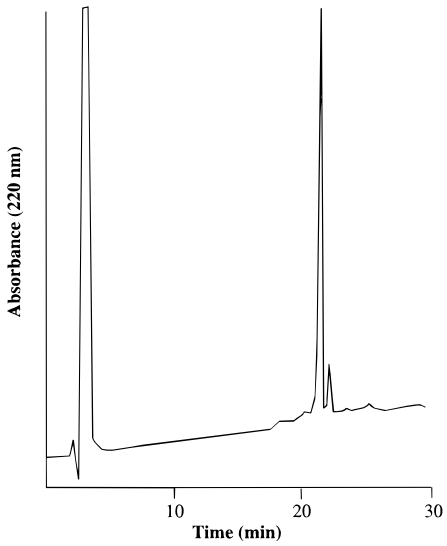


Figure 2. Analytical HPLC of crude peptide, cyclo(Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala), as obtained directly after on-resin cyclization and acidolytic cleavage of the BAL anchor. Further synthetic details are given in the Experimental Section; chromatography followed condition B as listed in the General Procedures.

As with other handles, BAL is compatible with a wide range of functionalized polymeric supports; for the present work, both polystyrene (PS) and poly(ethylene glycol)−polystyrene graft (PEG-PS) supports have been used. Our work to date has focused on illustrating these concepts in the tris(alkoxy)-benzylamide system that we described previously for the acid-labile PAL handle; extensions to numerous other handle systems and the corresponding cleavage principles are readily envisioned. A further important direction for generalization of BAL chemistry will be to apply optimized reductive amination procedures reported herein to essentially any nitrogen-containing building block in combination with any of a variety of aldehyde-functionalized polymeric supports. Such experiments will provide needed versatile anchoring as a starting point for solid-phase organic synthesis of non-peptide combinatorial libraries.[3c−f,28,32]

Experimental Section

General Procedures. Materials, solvents, instrumentation, and general methods were essentially as described in previous publications from our laboratories,[12,13b,d,17b,20c,29d] as summarized further in the Supporting Information. 5-(4-Formyl-3,5-dimethoxyphenoxy)valeric acid (**2**) (PALdehyde) was prepared according to ref 12b. PEG-PS resins[17] (0.18−0.24 mmol/g and containing Nle as the IRAA[33]) and 4-[(4 or 2)-formyl-3,5-dimethoxyphenoxy]butyric acid (ortho and para

(32) Reviews: (a) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401. (b) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17−42; *Angew. Chem.* **1996**, *108*, 19−44. (c) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (d) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527−4554. (e) Lam, K. S.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411−448. (f) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449−472. (g) *Combinatorial Chemistry, Synthesis and Application*; Wilson, S. R., Czarnik, A. W., Eds.; Wiley: New York, 1997. (h) *Annual Reports in Combinatorial Chemistry and Molecular Diversity*; Moos, W. H., Pavia, M. R., Ellington, A. D., Kay, B. K., Eds.; ESCOM: Leiden, Netherlands, 1997; Vol. 1. (i) Barany, G.; Kempe, M. In *A Practical Guide to Combinatorial Chemistry*; Czarnik, A. W., DeWitt, S. H., Eds.; American Chemical Society: Washington, DC, 1997; pp 51−97 and other chapters in this monograph.

(33) Commercial PEG-PS has a Nle IRAA *between* the PS and bifunctional PEG, the latter of which sometimes acts as a spacer and other times cross-links two Nle sites. Hence, ratios of Nle to incorporated amino acids of 2.5−4 represent quantitative yields.

mixture; *o,p*-PALdehyde[15]) were obtained from the BioSearch division of PerSeptive Biosystems (Framingham, MA). MBHA-PS resin (0.57 mmol/g) was from NovaBiochem (San Diego, CA) and *p*-alkoxybenzyl alcohol (Wang)-resin (0.49 mmol/g) was from Advanced ChemTech (Louisville, KY). NovaBiochem also supplied L-phenylalaninol; amino acid *tert*-butyl ester hydrochloride salts were from Advanced ChemTech or from Bachem Bioscience (Philadelphia, PA). Ddz-Phe-OH was also from Advanced ChemTech; Trt-Ala-OH was made according to Barlos,[34] and Fmoc-Phe-F was prepared by Dr. S. A. Kates following the procedure of Carpino et al.[23] To accurately determine anchoring, coupling, and cleavage yields in this work, resins were extended further with an "internal reference" amino acid[20] (IRAA; either Ile or Ala was used), introduced as its Fmoc derivative by standard coupling methods, at a point before introduction of the handle. Analytical HPLC was performed using either Vydac or Nucleosil C_{18} reversed-phase columns (0.46 × 25 cm), and linear gradients of 0.1% TFA in CH_3CN and 0.1% aqueous TFA were run at 1.0 mL/min flow rate from (condition A) 1:9 to 1:0 over 30 min and then 1:0 for 5 min; (condition B) from 1:9 to 2:3 over 30 min and then 1:0 for 5 min; or (condition C) from 1:9 to 2:3 over 30 min, then to 1:0 over the next 10 min, and finally 1:0 for 5 min.

Preparation of Preformed BAL Handles by Reductive Amination of 5-(4-Formyl-3,5-dimethoxyphenoxy)valeric Acid (Scheme 2): *N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)glycine Methyl Ester [Fmoc-(BAL-OH)Gly-OMe, 1a]. 5-(4-Formyl-3,5-dimethoxyphenoxy)valeric acid (**2**,[12b] 0.14 g, 0.5 mmol), H-Gly-OMe·HCl (63 mg, 0.5 mmol), and $NaBH_3CN$ (31 mg, 0.5 mmol) were suspended in MeOH (5 mL) and stirred at 25 °C for 60 min. The suspension was concentrated to dryness in vacuo, and the residual oil (secondary amine **3a**) was resuspended in dioxane−H_2O (1:1, 4 mL). Solid $NaHCO_3$ (0.13 g, 1.5 mmol) was added, the suspension was cooled in an ice-bath, and Fmoc-Cl (0.16 g, 0.6 mmol) dissolved in dioxane (1 mL) was added. [When Fmoc-OSu (0.25 g, 0.75 mmol) in dioxane (2 mL) was substituted for Fmoc-Cl at this point, the remaining procedure was essentially the same and the same final yield was obtained.] Stirring was continued for 90 min while cooling in an ice-bath and at 25 °C for another 90 min. The pH was then adjusted to approximately 9 by addition of solid $NaHCO_3$. The suspension was diluted with H_2O (20 mL) and washed with Et_2O (2 × 25 mL). Phase separations were slow, and the organic layer remained cloudy. The aqueous layer was acidified to pH 3 with 4 N aqueous HCl and extracted with EtOAc (2 × 25 mL). The combined EtOAc phases were concentrated to dryness in vacuo to provide 0.24 g (83%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)glycine Allyl Ester [Fmoc-(BAL-OH)Gly-OAllyl, 1b]** was prepared as described above for **1a**, but starting with H-Gly-OAllyl·TFA (**4**, 0.14 g, 0.6 mmol) and using Fmoc-OSu (0.22 g, 0.66 mmol), to provide 0.29 g (95%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-2,2-dimethoxyethylamine [Fmoc-(BAL-OH)-NCH$_2$CH(OMe)$_2$, 1c]** was prepared as described above for **1a**, but starting with 2,2-dimethoxyethylamine (54 μL, 0.5 mmol), to provide 0.26 g (88%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information. The synthesis was repeated on a 2.0-mmol scale, but using Fmoc-OSu (0.89 g, 2.6 mmol) in place of Fmoc-Cl; after crystallization of the crude oil from Et_2O−hexane at −20 °C, 0.5 g (42%) of the title product was obtained, mp 101−102.5 °C; elemental analysis data are given in the Supporting Information.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)alanine *tert*-Butyl Ester [Fmoc-(BAL-OH)Ala-O*t*Bu, 1d]** was prepared as described above for **1a**, but starting with H-Ala-O*t*Bu·HCl (91 mg, 0.5 mmol), to provide 0.25 g (78%) of the title product as an oil; ^{1}H NMR, FABMS, and elemental analysis data are given in the Supporting Information.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)leucine *tert*-Butyl Ester [Fmoc-(BAL-OH)Leu-O*t*Bu, 1e]** was prepared as described above for **1a**, but starting with H-Leu-O*t*Bu·HCl (0.12 g, 0.5 mmol), to provide 0.21 g (63%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)phenylalanine Methyl Ester [Fmoc-(BAL-OH)-Phe-OMe, 1f]** was prepared as described above for **1a**, but starting with H-Phe-OMe·HCl (0.11 g, 0.5 mmol), to provide 0.19 g (57%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information. The synthesis was repeated on a 2.0-mmol scale to provide 1.14 g (84%) of pure title product as an oil.

***N*$^{\alpha}$-[4-(Carboxylbutyloxy)-2,6-dimethoxybenzyl]-*N*$^{\alpha}$-(9-fluorenylmethoxycarbonyl)phenylalanine *tert*-Butyl Ester [Fmoc-(BAL-OH)-Phe-O*t*Bu, 1g]** was prepared as described for **1a**, but starting with H-Phe-O*t*Bu·HCl (0.24, 0.5 mmol), to provide 0.24 g (66%) of the title product as an oil; ^{1}H NMR and FABMS data are given in the Supporting Information.

Glycine Allyl Ester, Trifluoroacetate Salt (H-Gly-OAllyl·TFA, 4). Method A. Boc-Gly-OH (8.8 g, 50 mmol) was suspended in dioxane−H_2O (4:1, 100 mL), and a solution of $CsHCO_3$ (10.7 g, 55 mmol) in H_2O (20 mL) was added over 5 min. After 10 min, the suspension was concentrated in vacuo to give a foam, which was suspended in DMF (80 mL), stirred with allyl bromide (4.8 mL, 55 mmol) at 25 °C for 14 h, and then concentrated to dryness in vacuo. The resultant solid was suspended in EtOAc (300 mL) and extracted with 10% aqueous $NaHCO_3$ (3 × 150 mL). The combined aqueous phases were extracted with more EtOAc (100 mL), and the combined organic phases were concentrated in vacuo to an oil (10.6 g) which was purified by vacuum liquid chromatography over TLC-grade Silica Gel 60 G, using EtOAc−hexane (1:4) as the eluent. The resultant oil (9.3 g) was treated with TFA−CH_2Cl_2 (1:1, 50 mL) for 1 h to remove the Boc group and then concentrated in vacuo to give an oil, which upon washing with Et_2O (3 × 50 mL) provided off-white crystals (7.74 g, 68%), mp 95.5−96.5 °C; ^{1}H NMR and elemental analysis data are given in the Supporting Information. **Method B.** DIEA (5.8 mL, 34 mmol) was added to a solution of Boc-Gly-OH (3.0 g, 17 mmol) in CH_3CN−allyl bromide (2:3, 25 mL), and the mixture was heated at reflux, i.e. 75−80 °C, for 90 min, at which point TLC [$CHCl_3$−MeOH−HOAc (95:5:3)] indicated complete esterification. The reaction mixture was diluted with EtOAc (300 mL), washed with 0.1 N aqueous HCl (3 × 100 mL), 5% aqueous $NaHCO_3$ (3 × 100 mL), and brine (3 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resultant yellow oil was dissolved in TFA−CH_2Cl_2 (1:1, 50 mL) and stirred for 1 h, at which point TLC indicated complete Boc removal. The homogeneous reaction was concentrated in vacuo and chased with Et_2O (3 × 50 mL, followed by reconcentration). The residual solid was once again suspended in Et_2O (100 mL), filtered, and washed with additional Et_2O (50 mL) to give 3.3 g (84%) of NMR-pure title product as a white solid, mp 103−104 °C; ^{1}H NMR and CIMS data are given in the Supporting Information.

Phenylalaninol *tert*-Butyl Ether (H-Phe-o*t*Bu, 5). DIEA (2.8 mL, 16 mmol) was added, with stirring, to a solution of H-Phe-oh (2.0 g, 13 mmol) in dioxane (40 mL) at 25 °C. The mixture was cooled to 0 °C, and benzyl chloroformate (2.3 mL, 16 mmol) in dioxane (2 mL) was added dropwise over 15 min. After 30 min of reaction at 0 °C, TLC [EtOAc−hexane (7:3)] showed that H-Phe-oh had been consumed. Solvent was removed under reduced pressure, EtOAc (150 mL) was added, and the organic phase was washed with 5% aqueous $KHSO_4$ (25 mL), 5% aqueous $NaHCO_3$ (25 mL), and brine (1 × 25 mL), and dried ($MgSO_4$). Concentration in vacuo gave HPLC-pure (t_R 19.6 min, obtained using condition A) Z-Phe-oh as a white solid (3.6 g, 95%), mp 89−90 °C. Next, a portion of the Z-Phe-oh (1.3 g, 4.6 mmol) was dissolved in CH_2Cl_2 (25 mL), and a solution of *tert*-butyl 2,2,2-trichloroacetimidate (2.0 g, 9.1 mmol) in cyclohexane (20 mL) was added dropwise over 5 min at 25 °C. The required *tert*-butyl etherification reaction was initiated by addition of BF_3·Et_2O (0.18 mL, 1.4 mmol) and continued with stirring at 25 °C for 96 h, at which point TLC [EtOAc−hexane (4:1)] and HPLC (condition A) indicated completion. The reaction mixture was next concentrated in vacuo and the crude product purified by flash chromatography [silica gel, EtOAc−hexane (4:21)], to give HPLC-pure (t_R 26.9 min, obtained using condition A) Z-Phe-o*t*Bu as a colorless oil (1.2 g, 79%). Finally, the

(34) Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *J. Org. Chem.* **1982**, *47*, 1324−1326.

Table 1. On-Resin Reductive Amination of *o,p*-PALdehyde-Ile-Resins with Amino Acetals, Amino Alcohols, and Amino Acid Esters as a Function of Reagent Ratios, Solvent, and Time [a]

entry	amine	solvent	amine (equiv)	$NaBH_3CN$ (equiv)	time (h)	AA:Ile
1	$H_2N(CH_2)_3CH(OEt)_2$	MeOH	1	1	1	0.67:1.0
2	$H_2N(CH_2)_3CH(OEt)_2$	MeOH	2	2	1	0.89:1.0
3	$H_2N(CH_2)_3CH(OEt)_2$	MeOH	10	2	1	0.62:1.0
4	$H_2NCH_2CH(OMe)_2$	MeOH	10	2	1	0.72:1.0
5	$H_2N(CH_2)_3CH(OEt)_2$	MeOH	10	10	1	0.96:1.0
6	$H_2N(CH_2)_3CH(OEt)_2$	MeOH	10	10	24	1.04:1.0
7	$H_2N(CH_2)_3CH(OEt)_2$	DMF	10	2	1	0.48:1.0
8	$H_2N(CH_2)_3CH(OEt)_2$	DMF	10	10	24	0.90:1.0
9	H-Phe-oh	DMF	10	10	1	0.34:1.0
10	H-Phe-oh	DMF	10	10	19	0.86:1.0
11	H-Phe-oh	HOAc−DMF (1:99)	10	10	1	0.97:1.0
12	H-Leu-O*t*Bu·HCl	DMF	10	10	1	0.97:1.0
13	H-Leu-O*t*Bu·HCl	DMF	10	10	19	0.95:1.0
14	H-Gly-OAl·TFA	DMF	10	10	1	1.02:1.0
15	H-Leu-OBzl·*p*-tosylate	DMF	10	10	1	1.05:1.0

[a] Entries 1−8 were with *o,p*-PALdehyde-Ile-PEG-PS (0.24 mmol/g); entries 9−15 with *o,p*-PALdehyde-Ile-MBHA-PS (0.48 mmol/g). The AA is Leu for entries 12, 13, and 15, and Gly for remaining entries. With amino acetals and amino alcohols (entries 1−11), on-resin reductive amination under the described conditions was followed by acylation of the resultant secondary amine either with $(Fmoc\text{-}Gly)_2O$ in CH_2Cl_2−DMF (9:1) or with Fmoc-Gly-OH as mediated by PyAOP/DIEA in DMF. Fmoc removal, hydrolysis, and amino acid analysis gave the ratio Gly ("reporter"):Ile (IRAA), which was taken to indicate the overall yield of the reaction sequence. With amino acid esters (entries 12−15), hydrolysis and amino acid analysis immediately after reductive amination gave the yield directly.

Z-Phe-o*t*Bu (1.1 g, 3.3 mmol) was dissolved in MeOH (20 mL), and 10% Pd/C (113 mg) was introduced under N_2. The system was purged and charged with H_2, and the reaction was stirred at 25 °C for 40 min, at which point TLC [EtOAc−hexane (7:3)] indicated complete removal of the Z group. The reaction mixture was filtered through Celite and concentrated in vacuo to give the title product as a colorless oil (0.68 g, 99%); ^{1}H NMR and CIMS data for final product and both intermediates are given in the Supporting Information.

Leucine Dimethylamide, Hydrochloride Salt [H-Leu-N(CH$_3$)$_2$·HCl, 6]. In turn, *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride salt (3.9 g, 20.1 mmol), dimethylamine hydrochloride salt (1.6 g, 20.1 mmol), and DIEA (3.4 mL, 20.1 mmol) were added, with stirring, to a solution of Boc-Leu-OH (1 g, 4.0 mmol) in CH_2Cl_2 (25 mL). The heterogeneous reaction mixture was stirred for 16 h at 25 °C, following which it was concentrated in vacuo. The residue was suspended in EtOAc (150 mL), washed with 10% aqueous Na_2CO_3 (4 × 50 mL), 0.1 N aqueous HCl (4 × 50 mL), and brine (4 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The Boc-Leu-N(CH$_3$)$_2$ intermediate, a colorless oil, was dissolved in 4 N HCl−dioxane (20 mL) and stirred at 25 °C for 1 h, at which point TLC indicated complete Boc removal. The homogeneous reaction mixture was concentrated in vacuo and chased with Et_2O (4 × 50 mL, followed by reconcentration) to provide the title product as a white foam (0.57 g, 74%); ^{1}H NMR and CIMS data are given in the Supporting Information.

Alanine Allyl Ester, Hydrochloride Salt (H-Ala-OAllyl·HCl, 7). DIEA (5.4 mL, 32 mmol) was added to a solution of Boc-Ala-OH (3.0 g, 16 mmol) in CH_3CN−allyl bromide (1:1, 30 mL), and the mixture was heated to 70−75 °C for 2 h, at which point TLC [$CHCl_3$−MeOH−HOAc (95:5:3)] indicated complete esterification. The reaction mixture was diluted with EtOAc (300 mL), washed with 0.1 N aqueous HCl (4 × 100 mL), 5% aqueous $NaHCO_3$ (4 × 100 mL), and brine (4 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo. The resultant yellow oil was dissolved in 4 N HCl−dioxane (40 mL) and stirred at 25 °C for 3 h, at which point TLC indicated complete Boc removal. The homogeneous reaction mixture was concentrated in vacuo and chased with Et_2O (4 × 50 mL, followed by reconcentration). The title product was obtained as a yellow oil (2.45 g, 93%); ^{1}H NMR and CIMS data are given in the Supporting Information.

Leucine Octyl Ester, Hydrochloride Salt (H-Leu-OOctyl·HCl, 8). DIEA (1.4 mL, 8.0 mmol) and 1-bromooctane (5 mL, 29.0 mmol) were added successively to a solution of Boc-Leu-OH·H_2O (1.0 g, 4.0 mmol) in CH_3CN (5 mL), and the mixture was heated at 90−95 °C for 5 h, at which point TLC [$CHCl_3$−MeOH−HOAc (95:5:3)] indicated complete esterification. The reaction mixture was diluted with EtOAc (150 mL), washed with 0.1 N aqueous HCl (4 × 50 mL), 5% aqueous $NaHCO_3$ (4 × 50 mL), and brine (4 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The resultant colorless oil was dissolved in 4 N HCl−dioxane (25 mL) and stirred at 25 °C for 40 min, at which point TLC indicated complete Boc removal. The homogeneous solution was concentrated in vacuo and chased with Et_2O (4 × 50 mL, followed by reconcentration). The title product was obtained after recrystallization from Et_2O as a white solid (0.46 g, 41%); ^{1}H NMR and CIMS data are given in the Supporting Information.

Optimization of Solution-Phase Reductive Amination of *p*-PALdehyde with H-Leu-O*t*Bu·HCl. The unprotected BAL handle H-(BAL-OH)Leu-O*t*Bu **3e** was prepared as described above for **1e** (10-μmol scale; n.b. the Fmoc protection step was omitted) at either 25 or 60 °C; in addition, the delay time prior to addition of cyanoborohydride was varied from 0 min (no delay) to 60 min. The resultant secondary amines **3e** (∼5 mg) were cleaved with TFA−H_2O (19:1, 1 mL) for 1 h at 25 °C and concentrated under reduced pressure, and the residues were taken up in 0.1 M aqueous $NaHCO_3$ (0.4 mL). To determine the extent of racemization, the literature descriptions[35] for use of Marfey's reagent [N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide, FDAA] were followed: A 10 mM solution of FDAA in acetone (0.4 mL) was added, and derivatizations proceeded for 1 h at 40 °C, followed by quenching with 0.2 N aqueous HCl (0.2 mL). The diastereomeric amino acid−FDAA adducts were then analyzed by HPLC-PDA using a linear gradient of 0.1% TFA in CH_3CN and 0.1% aqueous TFA from 1:9 to 2:3 over 25 min, flow rate 1.2 mL/min. The retention times, determined from a similarly analyzed standard solution of H-Leu-OH (10 mM in 0.1 M aqueous $NaHCO_3$) with a known D:L ratio (2:3) were 16.2 min (L-Leu) and 18.1 min (D-Leu). In the title experiments, the ratio of D-Leu:L-Leu, ×100, ranged from 0.1 to 0.7, when reductive aminations were carried out at 25 °C; this was considered to be a statistically insignificant deviation from the amount of D-Leu, 0.22%, observed upon analysis of the starting material H-Leu-O*t*Bu·HCl under the same conditions. However, when reductive aminations were carried out at 60 °C after a 60-min delay, the ratio of D-Leu:L-Leu ×100 was 43.7, indicating a substantial level of racemization under those conditions.

Evaluation of Conditions for On-Resin Reductive Amination of *o,p*-PALdehyde-Ile-PEG-PS (Table 1, Entries 1−8). Fmoc-Ile-PEG-PS resin (2.0 g, 0.24 mmol/g) was washed with DMF (2 × 2 min) and CH_2Cl_2 (2 × 2 min) and then treated with piperidine−DMF (1:4, 2 × 2 min, 1 × 15 min), followed by washings with DMF (5 × 2 min) and CH_2Cl_2 (2 × 2 min). Solid 4-[(4 or 2)-formyl-3,5-dimethoxyphenoxy]-butyric acid (*o,p*-PALdehyde, **2**,[12a,b,15] 0.26 g, 2.0 equiv), BOP (0.43 g, 2 equiv), and HOBt (0.19 g, 3 equiv) were combined and dissolved

(35) (a) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596. (b) Adamson, J. G.; Hoand, T.; Crivici, A.; Lajoie, G. A. *Anal. Biochem.* **1992**, *202*, 210−214. (c) Branton, W. D.; Fields, C. G.; VanDrisse, V. L.; Fields, G. B. *Tetrahedron Lett.* **1993**, *34*, 4885−4888.

Table 2. Acylation of H-(BAL-PEG-PS)Phe-O*t*Bu with Various Fmoc-Amino Acids as a Function of Coupling Conditions [a]

	coupling conditions					coupling yields (%)		
entry	reagent[b]	additive[b]	base[b]	solvent	time (h)	Gly	Ala	Leu
1	BOP (3)	HOBt (3)	NMM (6)	DMF	1	21	15	14
2	BOP (3)	HOBt (3)	NMM (6)	DMF	2 × 1	27	17	13
3	BOP (4)	HOBt (4)	DIEA (8)	DMF	1			15
4	HBTU (4)	HOBt (4)	DIEA (8)	DMF	1	25	17	15
5	HATU (4)	HOAt (4)	DIEA (8)	DMF	1	77	23	18
6	PyAOP (5)		DIEA (10)	CH_2Cl_2−DMF[c]	1			10
7	TFFH (5)		DIEA (10)	CH_2Cl_2−DMF[c]	1			22
8	HATU (5)		DIEA (10)	CH_2Cl_2−DMF[c]	1			39
9	**HATU (5)**		**DIEA (10)**	**CH_2Cl_2−DMF[c]**	**2**			**61**
10	Sym anhy (5)[d]			DMF	1			15
11	Sym anhy (5)[d]			CH_2Cl_2−pyridine[c]	1			21
12	Sym anhy (5)[d]		DMAP (0.5)	CH_2Cl_2−pyridine[c]	1			30
13	Sym anhy (5)[d]			CH_2Cl_2	1			41
14	**Sym anhy (5)[d]**			**CH_2Cl_2**	**2 × 1**	**97**	**95**	**71**

[a] H-(BAL-PEG-PS)Phe-O*t*Bu (50 mg, 0.14 mmol/g) was acylated with the Fmoc derivatives of Gly, Ala, or Leu (net concentration ∼0.05 M; number of equivalents matching the number in parentheses in "reagent" column), as further activated in the indicated solvent by the indicated reagent/additive/base combination [for entries 1−9, solids dissolved and base added last, followed by 10-min preactivation period, then addition to resin; for entries 10−14, see footnote *d*, below] for the indicated times (double couplings, with intervening washes, for entries 2 and 14). Fmoc removal (including UV quantification), hydrolysis, and amino acid analysis gave a yield of AA:Phe:Nle (IRAA), from which coupling yields were calculated. Conditions that are considered "optimal", i.e. high coupling yields, are highlighted in bold. [b] Number of equivalents used, with respect to secondary amine on BAL support, are indicated in parentheses. [c] CH_2Cl_2−DMF mixtures were 9:1 (v/v); CH_2Cl_2−pyridine mixture was 3:2 (v/v). [d] Just before use, the Fmoc-amino acid (10 equiv) and DCC (8.8 mg, 5 equiv) were dissolved in CH_2Cl_2 (1 mL) and allowed to react at 25 °C for 10 min. The resultant insoluble *N*,*N*′-dicyclohexylurea was removed by filtration, the filtrate was concentrated in vacuo, and the residue, containing the desired symmetric anhydride, was taken up in the indicated solvent.

in DMF (5 mL), DIEA (0.25 mL, 3 equiv) was added, and after 5-min preactivation, this solution was added to the resin. Coupling was allowed to proceed for 15 h, at which time the resin was only slightly positive to the Kaiser ninhydrin test.[36] The resultant *o*,*p*-PALdehyde-Ile-PEG-PS resin was washed with DMF (2 × 2 min) and CH_2Cl_2 (2 × 2 min) and then treated with acetic anhydride−DMF (1:9, 20 min), washed with DMF (5 × 2 min), CH_2Cl_2 (2 × 2 min), and MeOH (2 × 2 min), and finally dried in vacuo; aliquots were taken to test reductive amination (Scheme 4, second stage) as described in the following paragraph.

The appropriate amino acetal (1, 2, or 10 equiv) and $NaBH_3CN$ (1, 2, or 10 equiv) were combined, dissolved in DMF or MeOH (1.5 mL), and added to *o*,*p*-PALdehyde-Ile-PEG-PS (208 mg, 46 μmol). Each reaction was allowed to proceed at 25 °C for 1 h, unless specified otherwise. The resin was then washed with MeOH (2 × 1.5 min), DMF (2 × 1 min), CH_2Cl_2 (3 × 1 min), and MeOH (2 × 1 min) and dried in vacuo. Fmoc-Gly-OH (0.45 g, 1.5 mmol) was dissolved in CH_2Cl_2−DMF (10:1, 5.5 mL), and a solution of DCC (0.16 g, 0.75 mmol) in CH_2Cl_2 (1 mL) was added. After 15 min, the white precipitate (*N*,*N*′-dicyclohexylurea) was removed by filtration, and *one-third* of the filtrate, which contained the symmetrical anhydride of Fmoc-Gly, was added to the resin in each of three experiments carried out concurrently. The acylation reactions (total volume 2 mL) were carried out for 1 h, after which each resin was washed with DMF (2 × 1 min), CH_2Cl_2 (6 × 1 min), and MeOH (3 × 1 min) and dried in vacuo. Each resin was deprotected by treatment with piperidine−DMF (1:4, 2 × 2 min, 1 × 15 min), and a sample (∼6 mg) was then hydrolyzed with 12 N aqueous HCl−propionic acid (1:1) at 160 °C for 1 h and evaluated further by amino acid analysis. The data obtained were used to determine the reductive amination yields.

Evaluation of Conditions for On-Resin Reductive Amination of *o*,*p*-PALdehyde-Ile-MBHA-PS (Table 1, Entries 9−15). By a procedure quite similar to the previous, each experiment started with Fmoc-Ile-MBHA-PS resin (50 mg, 0.48 mmol/g); this was deprotected and *o*,*p*-PALdehyde (26 mg, 4 equiv) was coupled for 2 h (Kaiser ninhydrin test[36] was negative after this time) with aid of HATU (36 mg, 4 equiv) and DIEA (33 μL, 8 equiv) in DMF (0.4 mL) (1-min preactivation before adding to resin). Subsequently, the appropriate amine (10 equiv; free amine or its hydrochloride, trifluoroacetate, or tosylate salt) and $NaBH_3CN$ (15 mg, 10 equiv) were combined, dissolved in DMF (0.4 mL) or in HOAc−DMF (1:99, 0.4 mL), and added to the resin, and reactions were allowed to proceed at 25 °C for 1 or 19 h. Each resin from experiments with amino acid esters was then washed with DMF (5 × 0.5 min), CH_2Cl_2 (5 × 0.5 min), DMF (3 × 0.5 min), piperidine−DMF (1:4, 3 × 1 min), DMF (5 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min) and dried in vacuo. Each resin in experiments with amino alcohols was washed in the same way, but in lieu of drying at that point, an acylation was carried out as follows: Fmoc-Gly-OH (71 mg, 10 equiv) and PyAOP (125 mg, 10 equiv) were combined and dissolved in DMF (0.4 mL), DIEA (82 μL, 20 equiv) was added, and after 5-min preactivation, this solution was added to the resin and 2-h coupling gave a negative Kaiser ninhydrin test.[36] These resins were then washed with DMF (5 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min), deprotected by treatment with piperidine−DMF (1:4, 3 × 1 min, 1 × 10 min), washed with DMF (5 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min), and dried in vacuo. In all cases, a resin sample (∼6 mg) was then hydrolyzed and evaluated by amino acid analysis.

Evaluation of Conditions for Acylation of H-(BAL-PEG-PS)Phe-O*t*Bu (Table 2). PEG-PS resin (1.0 g, 0.18 mmol/g) was washed with CH_2Cl_2 (5 × 1 min), neutralized with DIEA−CH_2Cl_2 (1:19, 5 × 2 min), and washed further with CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). Fmoc-(BAL-OH)Phe-O*t*Bu (**1g**, 0.38 g, 3 equiv), BOP (0.24 g, 3 equiv), and HOBt (84 mg, 3 equiv) were combined and dissolved in DMF (5 mL), following which NMM (0.12 mL, 6 equiv) was added; after a 5-min preactivation period, this solution was added to the resin. After 4 h of coupling, the resin was negative to the Kaiser ninhydrin test.[36] The resultant Fmoc-(BAL-PEG-PS)Phe-O*t*Bu resin was washed with DMF (2 × 2 min) and CH_2Cl_2 (2 × 2 min), and then dried in vacuo. A sample (11 mg) of the dried resin was swollen in DMF, treated with piperidine−DMF (1:4, 1 × 2 min + 2 × 10 min + 2 × 2 min), and analyzed on the basis of UV (301 nm, $\epsilon = 7800$ cm^{-1} M^{-1}) absorbance of the resultant piperidine−dibenzofulvene adducts. A second sample (6 mg) of resin was similarly deprotected and then hydrolyzed with 12 N aqueous HCl−propionic acid (1:1) at 160 °C for 1 h and evaluated by amino acid analysis. These complementary methods showed a final loading for Phe of 0.14 mmol/g (Nle^{33} IRAA = 0.43 mmol/g). Aliquots (50 mg) were taken, treated to remove Fmoc, washed with CH_2Cl_2 (5 × 2 min) and coupling solvent (5 × 2 min), and then used in tests of a variety of coupling procedures for introductions of the second Fmoc-amino acid (tested for Gly, Ala, and Leu; final reaction volumes during couplings = 1 mL). Upon completion of coupling (or double-coupling), resins were washed with DMF (5 × 2 min) and CH_2Cl_2 (5 × 2 min) and dried in vacuo for 15 h. A sample (5−10 mg) of each coupled resin was treated to remove

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Table 3. Acylation of H-(BAL-Ile-MBHA-PS)-Leu-O*t*Bu with Fmoc-Phe-OH as a Function of Coupling Conditions [a]

	coupling conditions			coupling yields (%)	
entry	reagent[b]	base[b]	solvent	2 h	2 x 2 h
1	PyBOP (10)	DIEA (20)	DMF	5	8
2	PyBOP (10)	DIEA (20)	CH_2Cl_2−DMF[c]	27	42
3	PyBroP (10)	DIEA (20)	DMF	58	72
4	PyBroP (10)	DIEA (20)	CH_2Cl_2−DMF[c]	71	84
5	PyAOP (10)	DIEA (20)	DMF	22	33
6	PyAOP (10)	DIEA (20)	NMP	12	24
7	**PyAOP (10)**	**DIEA (20)**	**CH_2Cl_2−DMF[c]**	**88**	**88**
8	TFFH (10)	DIEA (10)	DMF	17	28
9	TFFH (10)	DIEA (10)	CH_2Cl_2−DMF[c]	26	36
10	TFFH (10)	DIEA (20)	DMF	8	16
11	**TFFH (10)**	**DIEA (20)**	**CH_2Cl_2−DMF[c]**	**91**	**95**
12	Fmoc-Phe-F (10)[d]		CH_2Cl_2−DMF[c]	55	60
13	**Fmoc-Phe-F (10)[d]**	**DIEA (11)**	**CH_2Cl_2−DMF[c]**	**90**	**94**
14	HATU (10)	DIEA (20)	DMF	26	40
15	HATU (10)	DIEA (20)	NMP	15	28
16	**HATU (10)**	**DIEA (20)**	**CH_2Cl_2−DMF[c]**	**92**	**93**
17	**Sym anhy (5)[e]**		**CH_2Cl_2−DMF[c]**	**82**	**87**

[a] H-(BAL-Ile-MBHA-PS)-Leu-O*t*Bu (50 mg, 0.43 mmol/g) was acylated with Fmoc-Phe-OH (83 mg, 10 equiv, net concentration ~0.4 M in the indicated solvent), as activated further by the indicated reagent/base combination [for entries 1−11 and 14−16, Fmoc-Phe-OH was dissolved in 0.5 mL of solvent, followed by base, and then this solution was added to the resin, followed after 30 s of stirring by solid coupling reagent; for entries 12, 13, and 17, see footnotes *d* and *e*, below], for 2-h single (penultimate column) or double (far-right column) couplings. Fmoc removal, hydrolysis, and amino acid analysis gave a yield of Phe:Leu:Ile (IRAA), from which coupling yields were calculated. Conditions that are considered "optimal", i.e. high coupling yields, are highlighted in bold. [b] Number of equivalents used, with respect to secondary amine on BAL support, are indicated in parentheses. [c] CH_2Cl_2−DMF mixtures were 9:1 (v/v). [d] Fmoc-Phe-F, prepared according to Carpino et al. (ref 23), was dissolved in CH_2Cl_2−DMF (9:1) and added to the resin in the absence (entry 12) or presence (entry 13) of base. [e] Fmoc-Phe-OH (83 mg, 10 equiv) and DIPCDI (20 μL, 6 equiv) were dissolved in CH_2Cl_2−DMF (9:1, 0.5 mL) and, after a 5-min preactivation period, filtered and added to the resin.

Fmoc, analyzed by UV, hydrolyzed, and evaluated further by amino acid analysis, all as outlined earlier in this paragraph.

Evaluation of Conditions for Acylation of H-(BAL-Ile-MBHA-PS)Leu-O*t*Bu (Table 3). For each experiment, *o*,*p*-PALdehyde-Ile-MBHA-PS resin (50 mg, 0.48 mmol/g) was prepared as in the first sentence of "Evaluation of Conditions for On-Resin Reductive Amination of *o*,*p*-PALdehyde-Ile-MBHA-PS (Table 1, Entries 9−15)." Subsequently, reductive amination was carried out using H-Leu-O*t*Bu·HCl (54 mg, 10 equiv) and $NaBH_3CN$ (15 mg, 10 equiv) in DMF (0.4 mL), at 25 °C for 1 h. After the usual washes, the resultant H-(BAL-Ile-MBHA-PS)Leu-O*t*Bu (0.43 mmol/g) was available for evaluation of coupling conditions (Table 3) by a general procedure similar to what was used for Table 2 (final reaction volumes during couplings = 0.5 mL; yields calculated only by amino acid analysis).

H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enkephalin). For preparation of the handle-resin (100-mg scale), as well as for synthesis, wash volumes were 2 mL, and Fmoc removal was accomplished with piperidine−DMF (1:4; 3 × 1 min, 2 × 5 min, 2 × 1 min), followed by washes with DMF (5 × 0.5 min). Fmoc-Ile-PEG-PS (100 mg, 0.20 mmol/g) was deprotected, while 5-(4-formyl-3,5-dimethoxyphenoxy)-valeric acid (**2**,[12b] 22 mg, 4 equiv) and HATU (30 mg, 4 equiv) were dissolved in DMF (0.5 mL), DIEA (27 μL, 8 equiv) was added, and after 1-min preactivation, the entire solution was added to the resin. Coupling for 2 h gave *p*-PALdehyde-Ile-PEG-PS resin (negative to the Kaiser ninhydrin test[36]) which was washed with DMF (5 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min) and dried in vacuo. Next, H-Leu-O*t*Bu·HCl (45 mg, 10 equiv) and $NaBH_3CN$ (12 mg, 10 equiv), dissolved together in DMF (0.6 mL), were added to the resin, and reaction at 25 °C for 1 h gave H-(BAL-Ile-PEG-PS)Leu-O*t*Bu resin which was washed consecutively with DMF (5 × 0.5 min), CH_2Cl_2 (3 × 0.5 min), DMF (3 × 0.5 min), piperidine−DMF (1:4, 3 × 1 min), DMF (5 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min), dried in vacuo, and used as a starting point for manual chain assembly of the enkephalin sequence. Fmoc-Phe-OH (74 mg, 10 equiv) was dissolved in CH_2Cl_2−DMF (9:1, 0.5 mL), and DIPCDI (18 μL, 6 equiv) was added. After 5 min, the symmetrical anhydride solution was filtered and added to the BAL-resin, and coupling was allowed to proceed for 2 h. The resin was washed subsequently with CH_2Cl_2 (5 × 0.5 min), then DMF (5 × 0.5 min), and the coupling procedure was repeated (2 h). Peptide chain elongation proceeded normally for the remaining three residues (side-chain protection for Tyr was provided by *t*Bu), using Fmoc-AA-OH (5 equiv), DIPCDI (16 μL, 5 equiv), and HOBt (15 mg, 5 equiv) in DMF (0.5 mL) for 1 h per residue, to provide the completed peptide-resin. The amino acid composition of the hydrolyzed peptide-resin was as follows: Tyr, 0.85; Gly, 1.69; Phe, 0.85; Leu, 0.97; Ile, 1.00; Nle,[33] 3.04. A portion of the completed peptide-resin (50 mg) was treated first with piperidine−DMF (1:4) to remove Fmoc, followed by washings with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min), and then cleaved with TFA−H_2O (19:1) at 25 °C for 1 h. The filtrate from the cleavage reaction was collected, combined with TFA washes (1 mL) of the cleaved peptide-resin, and dried to give material that showed a single major component (90% cleavage yield) by analytical HPLC (t_R 18.7 min, >98% purity; negligible racemized peptide which from separate experiments is known to have t_R 22.9 min; see Figure 1A, obtained using condition B). FABMS, calcd $C_{28}H_{37}N_5O_7$: 555.6. Found: *m*/*z* 556.2 $[M + H]^+$. Alternative syntheses of the title peptide, showing other aspects of BAL chemistry, are sketched in the text and in the legend to Figure 1.

H-Tyr-Gly-Gly-Phe-Leu-Phe-oh (Leu-Enkephalin Phenylalaninol). The starting *o*,*p*-PALdehyde-Ala-MBHA-PS (50 mg, 0.48 mmol/g) was prepared essentially as the starting resin for the studies in Table 1, entries 9−15; to this was added H-Phe-o*t*Bu (**5**, 50 mg, 10 equiv) and $NaBH_3CN$ (15 mg, 10 equiv) dissolved in HOAc−DMF (1:99, 0.5 mL), and the reductive amination reaction was allowed to proceed for 18 h. This gave H-(BAL-Ala-MBHA-PS)Phe-o*t*Bu resin, which was washed consecutively with DMF (5 × 0.5 min), CH_2Cl_2 (3 × 0.5 min), DMF (3 × 0.5 min), piperidine−DMF (1:4, 3 × 1 min), DMF (5 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min), dried in vacuo, and used for manual chain assembly. Fmoc-Leu-OH (76 mg, 10 equiv) was dissolved in CH_2Cl_2−DMF (9:1, 0.5 mL), DIEA (73 μL, 20 equiv) was added, and this solution was added to the resin; after 30 s of stirring, solid HATU (82 mg, 10 equiv) was added to initiate coupling. After 2 h, the dipeptide-resin was washed with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min), and a second 2-h coupling by the same procedure was carried out. The remaining four amino acid residues were introduced just as for the Leu-enkephalin synthesis by DIPCDI/HOBt-mediated couplings in DMF. The amino acid composition of the hydrolyzed peptide-resin was as follows: Tyr, 0.87; Gly, 1.82; Phe, 0.86; Leu, 0.92; Ala, 1.00; the amino alcohol was not detected by amino acid analysis. Fmoc removal and cleavage with TFA−H_2O (19:1) for 75 min, followed by the same workup as for the Leu-enkephalin synthesis, gave the desired peptide alcohol (87% cleavage yield) which was characterized by analytical HPLC (t_R 29.2 min, >95% purity; see the Supporting Information Figure 1, obtained using condition C). ESMS, calcd for $C_{37}H_{48}N_6O_7$: 688.8. Found: *m*/*z* 689.9 $[M + H]^+$.

H-Tyr-Gly-Gly-Phe-Leu-N(CH$_3$)$_2$ (Leu-Enkephalin Dimethylamide). Essentially as for the Leu-enkephalin synthesis, but working with *o*,*p*-PALdehyde, using H-Leu-N$(CH_3)_2$·HCl (**6**, 39 mg, 10 equiv) instead of H-Leu-O*t*Bu·HCl, and carrying out on-resin reductive amination for 18 h, the starting H-(BAL-Ile-PEG-PS)Leu-N$(CH_3)_2$ resin (100 mg, 0.19 mmol/g) was obtained. To continue, Fmoc-Phe-OH (74 mg, 10 equiv) was dissolved in CH_2Cl_2−DMF (9:1, 0.5 mL), DIEA (65 μL, 20 equiv) was added, the overall solution was added to the resin, and coupling was initiated 30 s later by addition of solid PyAOP (99 mg, 10 equiv) [double-coupling, 2 h each, washings with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) between couplings]. The remaining three residues were added just as for the Leu-enkephalin synthesis by DIPCDI/HOBt-mediated couplings in DMF. The amino acid composition of the hydrolyzed peptide-resin was as follows: Tyr, 0.88; Gly, 1.83; Phe, 0.85; Leu, 0.62; Ile, 1.00; Nle,[33] 3.95. Fmoc removal and cleavage with TFA−H_2O (19:1) for 90 min, followed by the same workup as for the Leu-enkephalin synthesis, gave the desired

peptide dimethylamide (60% cleavage yield) which was characterized by analytical HPLC (t_R 24.2 min, >85% purity; see the Supporting Information Figure 2, obtained using condition C). MALDI-TOF MS, calcd for $C_{30}H_{42}N_6O_6$: 582.7. Found: *m*/*z* 584.0 $[M + H]^+$, 606.0 $[M + Na]^+$.

H-Ala-Leu-Ala-Lys-Leu-Gly-Gly-H (Model Peptide Aldehyde). The preformed handle Fmoc-(BAL-OH)$NCH_2CH(OMe)_2$ (**1c**, 214 mg, 0.36 mmol), BOP (160 mg, 1 equiv), and HOBt (49 mg, 1 equiv) were dissolved in DMF (3 mL), DIEA (78 μL, 2.5 equiv) was added, and after a 5-min preactivation period, the solution was added to neutralized PEG-PS resin (1.00 g, 0.18 mmol/g). After overnight coupling, the resultant Fmoc-(BAL-PEG-PS)$NHCH_2CH(OMe)_2$ resin was washed with DMF (5 × 2 min) and CH_2Cl_2 (5 × 2 min), capped for 1 h with acetic anhydride−CH_2Cl_2 (1:3, 4 mL), washed with CH_2Cl_2 (10 × 2 min), and dried in vacuo at 25 °C. A portion of this resin (200 mg, 0.13 mmol/g) was used for manual chain assembly of the title peptide (side-chain protection for Lys provided by Boc); wash volumes were 2 mL, and Fmoc removal was accomplished with piperidine−DMF (1:4; 5 min + 25 min), followed by washes with DMF (5 × 2 min). The second residue in the sequence was added as the symmetrical anhydride [double-coupling, 1 h each, washings with DMF (5 × 2 min) and CH_2Cl_2 (5 × 2 min) between couplings], which was prepared by taking Fmoc-Gly-OH (89 mg, 0.30 mmol) in CH_2Cl_2−DMF (5:1, 1.2 mL), adding a solution of DCC (31 mg, 0.15 mmol) in CH_2Cl_2 (0.5 mL), after 15 min removing the white precipitate (*N*,*N*′-dicyclohexylurea) by filtration, and adding the filtrate (total volume of 2 mL including rinses with CH_2Cl_2). The remaining five residues were added by standard Fmoc chemistry, using Fmoc-AA-OH (0.13 mmol, 5 equiv), BOP (43 mg, 0.096 mmol), HOBt (13 mg, 0.096 mmol), and DIEA (25 μL, 0.145 mmol) for 45-min couplings (after 5-min preactivation) in DMF (1.5 mL). The amino acid composition of the hydrolyzed [12 N aqueous HCl−propionic acid (1:1), at 160 °C for 1 h] peptide-resin was as follows: Ala, 1.98; Leu, 1.87; Lys, 0.91; Gly, 1.00; Nle,[33] 4.14. A portion (19 mg) of the completed peptide-resin was treated first with piperidine−DMF (1:4) to remove Fmoc, followed by washings with DMF (5 × 2 min) and CH_2Cl_2 (5 × 2 min), and then cleaved with TFA−H_2O (19:1) at 25 °C for 2 h. The filtrate from the cleavage reaction was collected and combined with TFA washes (3 × 2 mL) of the cleaved peptide-resin, and the resultant solution was concentrated under a stream of N_2. The crude cleaved peptide was precipitated with methyl *tert*-butyl ether (2 mL), washed with Et_2O (2 × 10 mL), and dried to give material that showed a single major component by analytical HPLC (t_R 13.0 min; >70% purity; see the Supporting Information Figure 3). ESMS, calcd for $C_{28}H_{52}N_8O_7$: 612.8. Found: *m*/*z* 613.4 $[M + H]^+$. The amino acid composition of the hydrolyzed crude cleaved peptide was as follows: Gly, 1.00; Ala, 2.06; Leu, 1.86; Lys, 0.85.

Near-Quantitative Resin-Bound Diketopiperazine Formation (Scheme 5) during Attempted Preparation of Fmoc-Pro-Tyr-Leu-Ala-Gly-OAllyl. The preformed handle, Fmoc-(BAL-OH)Gly-OAllyl (**1b**, 63 mg, 3 equiv), was dissolved along with HATU (40 mg, 3 equiv) and DIEA (38 μL, 6 equiv) in DMF (0.6 mL) and, after 1-min preactivation, coupled onto H-Ile-MBHA-PS resin (75 mg, 0.48 mmol/g) for 15 h. After the usual washes, a portion of the resultant Fmoc-(BAL-Ile-MBHA-PS)Gly-OAllyl resin (25 mg, 0.40 mmol/g) was elaborated further by Fmoc chemistry protocols described earlier for the Leu-enkephalin phenylalaninol synthesis. The synthesis was abandoned when incorporation of Leu proved to be low, as evidenced by the amino acid composition of the hydrolyzed peptide-resin at that stage: Leu, 0.03; Ala, 0.92; Gly, 0.91; Ile, 1.00. Identical results were obtained when Fmoc cleavage at the dipeptide-resin stage was substantially abbreviated (piperidine−DMF, 1:4, 3 × 1 min).

Fmoc-Pro-Tyr-Leu-Ala-Gly-OAllyl. Manual chain assembly was carried out, starting with Fmoc-(BAL-Ile-MBHA-PS)Gly-OAllyl resin (25 mg, 0.40 mmol/g), prepared exactly as described above, and removing the Fmoc group with piperidine−DMF (1:4, 3 × 1 min, 2 × 5 min, 2 × 1 min). Next, Trt-Ala-OH (17 mg, 5 equiv) was dissolved in DMF (0.2 mL), DIEA (17 μL, 10 equiv) was added, the solution was added to the resin, and coupling initiated by addition of solid PyAOP (26 mg, 5 equiv) was carried out for 2 h. The peptide-resin was then washed with DMF (5 × 0.5 min), and the coupling procedure (2 h) was repeated. Subsequent removal of the Trt group with TFA−H_2O−CH_2Cl_2 (2:1:97, 5 × 1 min) was followed by washing with CH_2Cl_2 (5 × 0.5 min). Next, Fmoc-Leu-OH (18 mg, 5 equiv) and PyAOP (26 mg, 5 equiv) were dissolved separately in DMF (0.2 mL total), combined, and added to the resin, and in situ neutralization/coupling initiated by the addition of DIEA (17 μL, 10 equiv) was carried out for 2 h (Kaiser ninhydrin test[36] negative after this time). The remaining two residues, Fmoc-Tyr(*t*Bu)-OH and Fmoc-Pro-OH, were introduced by Fmoc chemistry and PyAOP/DIEA-mediated single couplings. The amino acid composition of the hydrolyzed peptide-resin was as follows: Pro, 0.82; Tyr, 0.84; Leu, 0.90; Ala, 0.93; Gly, 0.93; Ile, 1.00. Fmoc removal and cleavage with TFA−H_2O (19:1) for 3 h gave the title peptide (85% cleavage yield) which was characterized by analytical HPLC (t_R 24.0 min, >85% purity; see the Supporting Information Figure 4, obtained using condition A). ESMS, calcd for $C_{43}H_{51}N_5O_9$: 781.9. Found: *m*/*z* 782.5 $[M + H]^+$, 804.6 $[M + Na]^+$.

Fmoc-Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala-OAllyl. Essentially as for the Leu-enkephalin synthesis, but working with *o*,*p*-PALdehyde, using H-Ala-OAllyl·HCl (**7**, 33 mg, 10 equiv) instead of H-Leu-O*t*Bu·HCl, and carrying out on-resin reductive amination for 18 h, the starting H-(BAL-Ile-PEG-PS)Ala-OAllyl resin (100 mg, 0.19 mmol/g) was obtained. Acidolysable side-chain protection was provided by Pmc (Arg), Trt (Asn), and *t*Bu (Asp, Glu, Tyr). To begin chain assembly, Trt-Ala-OH (32 mg, 5 equiv) was dissolved in CH_2Cl_2−DMF (9:1; 0.6 mL), DIEA (32 μL, 10 equiv) was added, the solution was added to the resin, and coupling initiated by addition of solid PyAOP (50 mg, 5 equiv) was carried out for 2 h. The peptide-resin was then washed with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min), and the coupling procedure (2 h) was repeated. Trityl removal with TFA−H_2O−CH_2Cl_2 (2:1:97, 5 × 1 min) was followed by washing with CH_2Cl_2 (5 × 0.5 min). Next, Fmoc-Glu(O*t*Bu)-OH (81 mg, 10 equiv) and PyAOP (99 mg, 10 equiv) were dissolved separately in DMF (0.6 mL total), combined, and added to the resin, and in situ neutralization/coupling initiated by the addition of DIEA (65 μL, 20 equiv) was carried out for 2 h (Kaiser ninhydrin test[36] negative after this time). The remaining seven amino acid residues were added in turn using Fmoc-AA-OH (5 equiv), PyAOP (5 equiv), and DIEA (10 equiv) in DMF (0.5 mL) for 1 h. The amino acid composition of the hydrolyzed peptide-resin was as follows: Arg, 1.05; Phe, 0.99; Pro, 1.04; Glu, 2.03; Asp, 2.05; Tyr, 0.90; Ala, 1.94; Ile, 1.01; Nle,[33] 3.71. The final peptide, retaining the N-terminal Fmoc and C-terminal allyl groups, was cleaved from the resin by treatment with TFA−Et_3SiH−H_2O (92:5:3) at 25 °C for 2 h (85% cleavage yield) and characterized by analytical HPLC (t_R 18.6 min, >85% purity; see the Supporting Information Figure 5, obtained using condition A). MALDI-TOF MS, calcd for $C_{71}H_{88}N_{14}O_{21}$: 1473.6. Found: *m*/*z* 1474.8 $[M + H]^+$.

H-Tyr-Gly-Gly-Phe-Leu-OOctyl (Leu-Enkephalin C_8 Ester). As described in "Evaluation of Conditions for Acylation of H-(BAL-Ile-MBHA-PS)Leu-O*t*Bu (Table 3)," but using H-Leu-OOct·HCl (**8**, 67 mg, 10 equiv) instead of H-Leu-O*t*Bu·HCl, the starting H-(BAL-Ile-MBHA-PS)Leu-OOctyl (50 mg, 0.42 mmol/g) was obtained. Next, Ddz-Phe-OH (81 mg, 10 equiv) was dissolved in CH_2Cl_2−DMF (9:1, 0.5 mL), DIEA (72 μL, 20 equiv) was added, the solution was added to the resin, and coupling initiated by addition of solid PyAOP (109 mg, 10 equiv) was carried out [triple coupling 2 h each, washings with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 mL) between couplings] to provide the dipeptide-resin which was checked by hydrolysis: Phe, 1.00; Leu, 0.98. The Ddz group was removed by treatment with TFA−H_2O−CH_2Cl_2 (3:1:96, 6 × 1 min), followed by washing with CH_2Cl_2 (5 × 0.5 min). Next, Fmoc-Gly-OH (63 mg, 10 equiv) and PyAOP (109 mg, 10 equiv) were dissolved separately in DMF (0.4 mL total), combined, and added to the resin, and in situ neutralization/coupling initiated by the addition of DIEA (72 μL, 20 equiv) was carried out for 2 h (Kaiser ninhydrin test[36] negative after this time). The remaining two residues, Fmoc-Gly-OH and Fmoc-Tyr(*t*Bu)-OH, were introduced by standard Fmoc chemistry and DIPCDI/HOBt-mediated couplings in DMF. The amino acid composition of the hydrolyzed peptide-resin was as follows: Tyr, 0.89; Gly, 1.86; Phe, 0.93; Leu, 1.02; Ile, 1.00. Fmoc removal and cleavage with TFA−H_2O (19:1, 1 mL) for 90 min gave the title peptide (86% cleavage yield) which was characterized

by analytical HPLC (t_R 40.1 min, 98% purity; see the Supporting Information Figure 6, obtained using condition C). MALDI-TOF, calcd for $C_{36}H_{53}N_5O_7$: 667.8. Found: *m*/*z* 668.6 $[M + H]^+$, 692.6 $[M + Na]^+$. For the synthesis outlined in footnote 30, which gave evidence for substantial diketopiperazine formation (t_R 26.4 min, obtained using condition C), the amino acid composition of the hydrolyzed peptide-resin was as follows: Tyr, 0.20; Gly, 0.42; Phe, 0.83; Leu, 0.97; Ile, 1.00.

Cyclo(Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala). Synthesis of the corresponding fully protected BAL-anchored peptide-resin has already been described. A portion (20 mg, 0.13 mmol/g) was washed with DMF (5 × 0.5 min), and the C-terminal allyl ester cleaved by treatment with $Pd(PPh_3)_4$ (15 mg, 5 equiv) in DMSO−THF−0.5 N aqueous HCl−morpholine (2:2:1:0.1, 1.5 mL) under argon at 25 °C for 3 h. The peptide-resin was then washed with THF (3 × 2 min), DMF (3 × 2 min), CH_2Cl_2 (3 × 2 min), DIEA−CH_2Cl_2 (1:19, 3 × 2 min), CH_2Cl_2 (3 × 2 min), sodium *N*,*N*-diethyldithiocarbamate (0.03 M in DMF, 3 × 15 min), DMF (5 × 2 min), CH_2Cl_2 (3 × 2 min), and DMF (3 × 1 min), and the Fmoc group was removed with piperidine−DMF (1:4, 3 × 1 min + 2 × 5 min + 2 × 1 min) followed by washings with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min). Next, a solution of PyAOP (6.8 mg, 5 equiv) plus HOAt (1.8 mg, 5 equiv) in CH_2Cl_2 (0.3 mL) was added, and cyclization was initiated by addition of DIEA (4.4 μL, 10 equiv). After 2 h at 25 °C, the peptide-resin was negative to the Kaiser ninhydrin test.[36] Treatment with TFA−Et_3SiH−H_2O (92:5:3) at 25 °C for 3 h gave the cyclic peptide (85% cleavage yield), which was characterized by analytical HPLC (t_R 22.5 min assigned to cyclic peptide with L-Ala at point of cyclization; t_R 23.4 min assigned to cyclic peptide with D-Ala at point of cyclization; ratio L:D = 88:12, overall purity for both peaks >95%; negligible linear peptide which from separate experiments is known to have t_R 20.5 min; see Figure 2, obtained using condition B). MALDI-TOF MS of cyclic peptide, calcd for $C_{53}H_{72}N_{14}O_{18}$: 1193.3. Found: *m*/*z* 1194.3 $[M + H]^+$. For the linear fully deprotected peptide, purity >85%. MALDI-TOF MS, calcd for $C_{53}H_{74}N_{14}O_{19}$: 1211.3. Found: *m*/*z* 1212.6 $[M + H]^+$.

Other cyclization conditions were tested with the following results: (a) solvent was DMF in place of CH_2Cl_2; complete cyclization; ratio L:D = 75:25; (b) base was 2,4,6-trimethylpyridine in place of DIEA; 26% uncyclized linear; of the cyclized material, ratio L:D = 76:24; (c) base was 2,4,6-trimethylpyridine and solvent was DMF; complete cyclization; ratio L:D = 50:50; and (d) base was 2,4,6-trimethylpyridine and solvent was $CHCl_3$−trifluoroethanol (7:3); 75% uncyclized linear (after 3 × 2 h treatments with activating agents); of the cyclized material, ratio L:D = 78:22.

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Supporting Information Available: Additional experimental information, including more details on general procedures, characterization of compounds **1a**−**g** and **4**−**8**, and Supporting Information Figures 1−6, which are analytical HPLC chromatograms of most of the peptides described in the text (12 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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