Solid-Phase Synthesis with Tris(alkoxy)benzyl Backbone Amide Linkage (BAL)[±]

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Abstract: Solid-phase synthesis has been applied successfully for the preparation of peptides, small proteins, oligonucleotides, and small organic molecules. A crucial part of the overall synthesis plan is the choice of an appropriate "handle" (linker) for attachment to the support. Here we describe a novel and general concept for solid-phase synthesis that involves attachment of a backbone amide nitrogen to an appropriate handle. This **b**ackbone **a**mide **l**inker (BAL) approach allows for the preparation of C-terminal-modified and cyclic peptides, small organic molecules, and modified amino sugars, as well as combinatorial synthesis applications.

Keywords: carbohydrates · combinatorial chemistry · handles · libraries · linkers · peptides · solid-phase synthesis

Introduction

In a seminal 1963 paper, R. Bruce Merrifield reported the concept and initial implementation of solid-phase peptide synthesis (SPPS).[1] Since then, SPPS has evolved into a highly efficient set of techniques for the preparation of numerous peptides and even small proteins.[2] Solid-phase synthesis has also been developed successfully for the preparation of oligonucleotides;[3] this technique has become the linchpin for much of modern molecular biology. In recent years, the advent of combinatorial chemistry has created an ever-growing interest in new methods for solid-phase synthesis of small organic molecules.[4]

Strategies for SPPS need to balance transient N^{α}-amino protecting groups, semi-permanent side-chain protecting groups, coupling methods, and procedures for attachment of the growing peptide chain to a solid support. A widely favored strategy for SPPS relies on the 9-fluorenylmethyloxycarbonyl (Fmoc) group for N^{α}-protection, in combination with trifluoroacetic acid (TFA)-labile side-chain protecting groups, such as *tert*-butyl esters, ethers, and carbamates, and release of the final peptide with suitable TFA/scavenger cocktails.

A crucial part of the overall synthesis plan is the choice of an appropriate "handle" (linker) for attachment. *Handles* are defined as bifunctional spacers that serve to attach the initial residue to the solid support in two discrete steps.[2a] 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. Compared with the alternative anchoring strategy in which functionalization of a support provides directly a resin-bound analogue to a C^{α}-carboxyl protecting group, the handle approach has a number of advantages.[5] These include: i) substitution levels (i.e., loadings) can be controlled better by using handles; ii) *any*

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[±] Key abbreviations: BAL, **b**ackbone **a**mide **l**inker, depending on context either the general concept described in this paper, or the specific implementation in the tris(alkoxy)benzylamide system as documented in the various schemes; DIEA: diisopropylethylamine; DMF *N*,*N*-dimethylformamide; HAL, 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy)-valeric acid (**hy**persensitive **a**cid-labile **l**inker); HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAc, acetic acid; HOAt, 1-hydroxy-7-azabenzotriazole; NMM, *N*-methylmorpholine; PAL, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid handle or tris(alkoxy)benzylamide anchor (**p**eptide **a**mide **l**inker); PALdehyde, 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid; *o*,*p*-PALdehyde, mixture of 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid and 4-(2-formyl-3,5-dimethoxyphenoxy)butyric acid; PEG-PS, polyethylene glycol-polystyrene (graft resin support); PS, copoly(styrene-1%-divinylbenzene) support; PyAOP, [(7-azabenzotriazol-1-yloxy)tris(pyrrolidino)]-phosphonium hexafluorophosphate; PyBroP, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; (R)PAL, 5-[4-(*N*-alkyl)amino-methyl-3,5-dimethoxyphenoxy]valeric acid handle or *N*-alkyl[tris-(alkoxy)benzyl]amide anchor (*N*-**alkyl**-**p**eptide **a**mide **l**inker); TFFH, tetramethylfluoroformamidium hexafluorophosphate.

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resin support or material that has been previously functionalized can be used as a parent for synthesis; and iii) handles are used readily in conjunction with "internal reference" amino acids (IRAAs), which facilitate monitoring of yields of the various steps by amino acid analysis of resin-bound peptides.

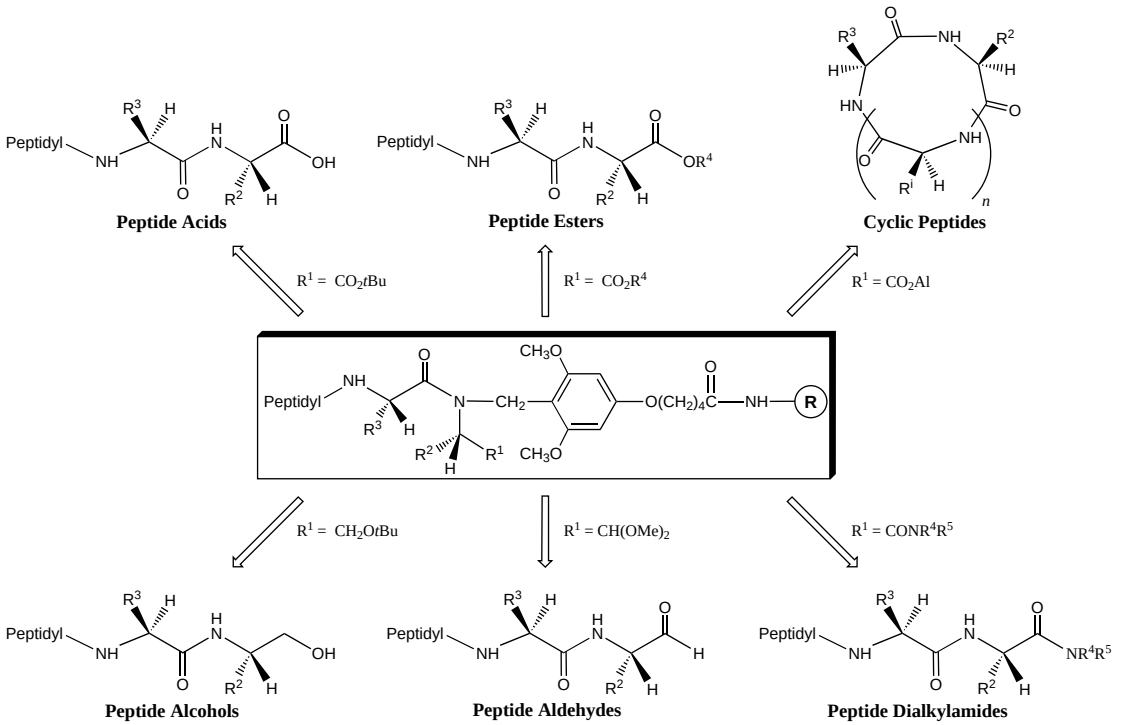
Although many different mechanisms, for example, photolysis, fluoridolysis, and base-catalyzed β-elimination, have been exploited for handle cleavage, the majority of handles rely on acidolysis for release of the final peptide from the support. By far, most handles anchor the growing peptide chain through the C^{α}-carboxyl as either an ester or an amide derivative. The three main classes of acid-labile handles are based on substituted benzyl, benzhydryl, and trityl derivatives. Introduction of alkoxy substituents at *para* and *ortho* positions increases the acid lability, and is used to fine-tune the properties of the handle.[5] For example, *para*-alkylbenzyl ester handles require HF to release the peptide acid, whereas analogous *para*-alkoxybenzyl handles release the peptide upon treatment with TFA.

The myriad of naturally occurring peptides, and their biologically relevant synthetic analogues, includes not only linear peptide acids and amides, but also numerous examples in which the C-termini are modified to other functionalities, for example, alcohols, ethers, esters, *N*-alkylamides, *N*,*N*-dialkylamides, hydrazides, trifluoromethyl ketones, aldehydes, mercaptoalkylamides, thioesters, and thioamides. Similarly, the importance of cyclic peptides is well established. C-terminal-modified peptides are important in enzymology for use as substrates to study active sites and catalytic mechanisms, to provide sensitive assays, and as inhibitors and analogues with specifically designed activities. Modifications at the C terminus, as well as elimination of termini through cyclization, represent avenues to more effective therapeutic agents, since they allow alteration of bioavailability of a peptide by protecting it from enzymatic degradation, by improving its ability to cross various biological barriers, by increasing its solubility, or by increasing its receptor-binding and substrate specificity. In addition to this biological importance, some C-terminal modifications are useful with peptide segments (protected and/or activated as needed) that are designed to be intermediates in convergent syntheses.

The present review describes a novel and general concept for solid-phase synthesis that involves attachment of a backbone amide nitrogen to an appropriate handle [i.e., with the general structure in the center box of Scheme 1, drawn as implemented with the tris(alkoxy)benzylamide (PAL) system].[6, 7] This **b**ackbone **a**mide **l**inker (BAL) approach allows for the preparation of peptides with a variety of C-terminal functionalities, for example, not only acids, but also alcohols, *N*,*N*-dialkylamides, aldehydes, and esters. Most of the other moieties discussed earlier are also accessible, in principle, and, importantly, BAL offers a completely general way to prepare cyclic peptides in the solid-phase mode without requiring a suitable side-chain functionality for anchoring. In addition, BAL anchoring can be extended readily to other nitrogen-containing organic molecules, allowing for significant applications to solid-phase organic synthesis and combinatorial chemistry.

Tris(alkoxy)benzyl Antecedents to BAL: PAL, (R)PAL, and HAL

In the mid-1980s, two of the authors developed the 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid (**p**eptide



Scheme 1. Backbone amide linker (BAL) concept as implemented for the tris(alkoxy)benzyl system: access to various C-terminal-modified and cyclic peptides. Modified from ref. [7].

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amide **l**inker ≡ PAL) handle, which provides a tris(alkoxy)-benzylamide anchoring linkage that cleaves upon mild acid treatment to provide C-terminal peptide amides.[8] A central intermediate in the preparation of PAL was the aldehyde, 5-(4-formyl-3,5-dimethoxyphenoxy)valeric acid, **1**, which we currently term "PALdehyde".[9] To synthesize PAL, a Vilsmeier formylation of commercially available 3,5-dimethoxyphenol gave the *para* isomer, 4-hydroxy-2,6-dimethoxybenzaldehyde, as the predominant regioisomer.[8, 10] Alkylation of the aforementioned benzaldehyde derivative with ethyl 5-bromovalerate, followed by saponification, gave PALdehyde, which was converted to the corresponding oxime, subjected to catalytic hydrogenolysis to generate the amine, and blocked with Fmoc. The Fmoc-PAL "universal" handle was coupled successfully to various amino-functionalized solid (polymeric) supports. Acidolytic cleavage yields were better for peptides made starting with the PAL handle, by comparison to those made with of benzhydrylamine-type handles, for example, Rink or Linker-AM. PAL-supports have been used widely to prepare many peptide amides in the authors' laboratories, and in numerous academic and industrial research programs.

More recently, one of us demonstrated that the critical conversion of the hindered electron-rich carbonyl in **1** to an aminomethyl group could be carried out by reductive amination with tritylamine (Trt-NH_2) in the presence of sodium cyanoborohydride in methanol; removal of the Trt moiety by catalytic hydrogenolysis provided PAL.[11] This theme was developed further in the preparation of the 5-(4-(*N*-alkyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid [(R)PAL] handles, which incorporate an R moiety, such as methyl, ethyl, and phenethyl.[12] Reductive aminations of **1** with the appropriate alkylamines R-NH_2 gave the desired (R)PAL handles, which upon Fmoc protection, coupling to PEG-PS, and chain-elongation were transformed further to peptide *N*-alkylamides.

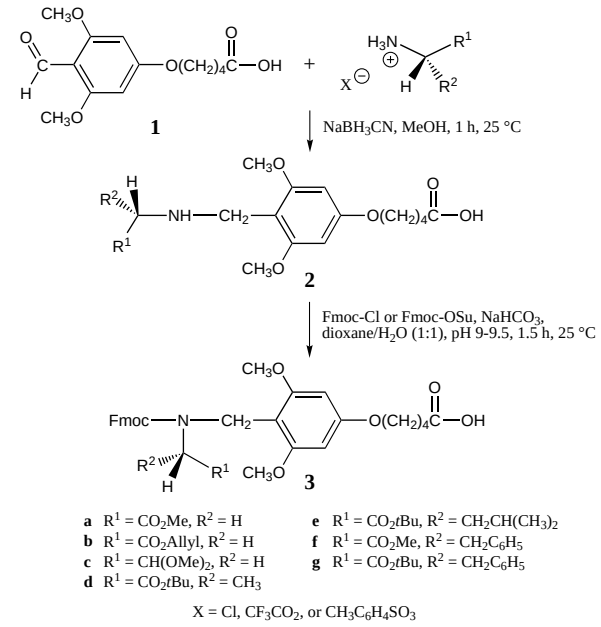
The reduction of **1** was also pursued to provide a very acid-labile handle for preparation of peptide *acids*. Reduction of **1** with sodium borohydride (instead of with sodium cyanoborohydride), and in the *absence* of an amine, gave 5-(4-hydroxymethyl-3,5-dimethoxyphenoxy)valeric acid. This **h**ypersensitive **a**cid-labile **l**inker (HAL) could be coupled to various amino-functionalized supports.[13] Upon esterification with the first Fmoc-amino acid and chain assembly, a peptidyl-HAL-resin was obtained that released the *fully protected* peptide acid under very mild conditions.

BAL Handle Concept

With optimal conditions for preparation of (R)PAL handles in hand, we reasoned that similar reductive amination reactions might also be used to attach amino acid esters through the N^{α}-amine. Because acylation of the amine would then convert the point of attachment to an amide, the envisaged approach would give a resin-bound analogue to backbone amide protection, as described by Weygand, Sheppard, and others.[14] **B**ackbone **a**mide **l**inking (BAL) does *not* rely on the C^{α}-carboxyl for attachment, and should thus allow a general strategy for the synthesis of C-terminal-modified and cyclic peptides. The previously developed **1**, readily available in our laboratories, was used for the initial implementation of the BAL handle concept (Scheme 1; see next section).

Implementation of BAL Handle Concept in Tris(alkoxy)benzyl System

PALdehyde **1** 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, and tosylate) could be used. Our initial



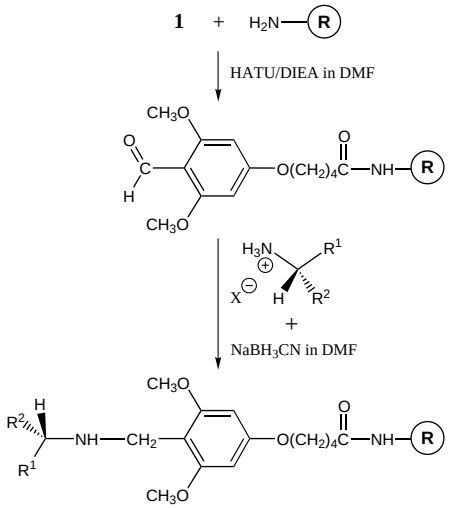
Scheme 2. Preparation of tris(alkoxy)benzylamine-based BAL handles. Taken from ref. [7].

experiments followed earlier precedents for preparation of (R)PAL handles, and involved reactions of **1** with amines in methanol to form the corresponding imines, 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 aldehyde **1**, amine, and cyanoborohydride (one equivalent of each) *simultaneously* in methanol;[15] reactions at 25 °C for 1 h provide the products in high yields without any detectable racemization.

The secondary amine intermediates **2** obtained from the reductive amination step were protected *directly* as Fmoc derivatives, hence providing the corresponding protected amino acid preformed handle derivatives **3**. Essentially complete derivatization of even slightly hindered derivatives, for example, H-Phe-O*t*Bu and H-Leu-O*t*Bu, required the reactive 9-fluorenylmethyl chloroformate (Fmoc-Cl) rather than *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-

OSu). The preformed handles **3** were then attached essentially quantitatively to polyethylene glycol-polystyrene graft (PEG-PS)[16] or polystyrene (PS) solid supports by standard couplings. Finally, Fmoc removal gave BAL-anchored derivatives suitable for further steps.

In an alternative procedure, PALdehyde **1** was coupled first to the support, followed by on-resin reductive amination with conditions similar to those developed by Sasaki and Coy.[17, 18] This operationally simpler approach gave the desired BAL anchors in good-to-excellent yields, while obviating the need for an intermediate *N*-protection step (Scheme 3). Nearly



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

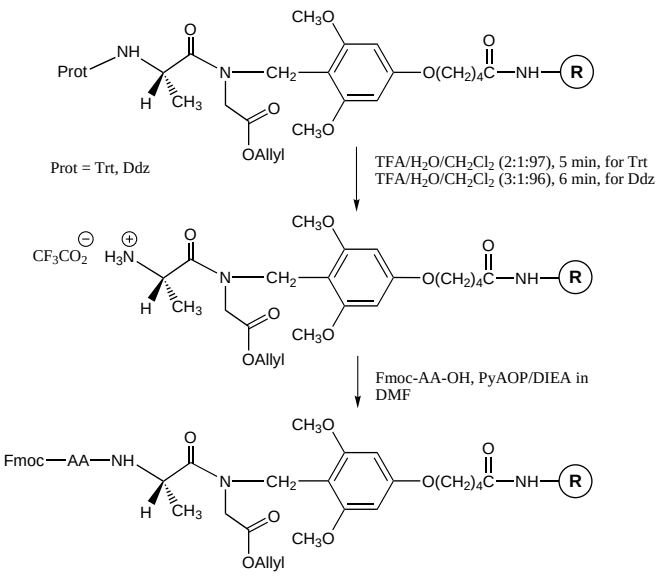
Scheme 3. Preparation of BAL anchors by on-resin reductive amination. Taken from ref. [7].

quantitative incorporation was achieved with either MeOH or *N*,*N*-dimethylformamide (DMF) as solvents, and by using the amine and cyanoborohydride both in considerable excess (10 equiv of each) over resin-bound aldehyde. Our optimal protocols when applied to amino acid derivatives proceed without racemization; key to this may be to avoid pre-equilibration, and to ensure a neutral or slightly acidic reaction milieu.

As may have been expected, acylations of the secondary α-amino group attached to the BAL handle by the *second* amino acid moiety (usually, introduced as an activated Fmoc-amino acid) proved to be slower than comparable acylations of unsubstituted primary amines. A variety of coupling protocols were examined in order to define the most efficient method for this difficult coupling. Symmetrical anhydrides of Fmoc-amino acids in CH_2Cl_2 [plus whatever amount of DMF was needed for solubility reasons, e.g., CH_2Cl_2/DMF (9:1)] in the *absence* of bases gave high yields in acylation of the hindered amines. Other reagents giving satisfactory results with CH_2Cl_2/DMF (9:1) as solvent included HATU/DIEA, TFFH/DIEA, PyAOP/DIEA, and PyBroP/DIEA. Preformed acid fluorides were also effective. In contrast, acylations in neat DMF gave only poor yields; thus CH_2Cl_2 is critical for successful acylation of the substituted secondary amine.

With the key initial steps, that is, reductive amination to introduce the first residue and acylation to introduce the second residue, accomplished successfully, further stepwise chain assembly proceeded normally by any of a variety of peptide synthesis protocols. Our first comprehensive publication[7] provided several detailed examples to show the scope of the method. BAL-anchored Leu *N*,*N*-dimethylamide gave, after chain elongation and cleavage, the C-terminal-modified Leu-enkephalin derivative H-Tyr-Gly-Gly-Phe-Leu-N$(CH_3)_2$. Similarly, BAL-anchored Leu *tert*-butyl ester was the starting point for synthesis of Leu-enkephalin, H-Tyr-Gly-Gly-Phe-Leu-OH (removal of *tert*-butyl protecting groups with simultaneous BAL cleavage). BAL-anchored Phe-ol-*tert*-butyl ether led to the peptide alcohol, H-Tyr-Gly-Gly-Phe-Leu-Phe-ol, and BAL-anchored 2,2-dimethoxyethylamine eventually gave the peptide aldehyde, H-Ala-Leu-Ala-Lys-Leu-Gly-Gly-H (dimethyl acetal cleavage during acid cleavage step).

During solid-phase synthesis of peptides using the BAL approach, we observed that when amino acid allyl and *n*-alkyl esters were used as the first building block, almost quantitative diketopiperazine (DKP) formation occurred at the dipeptidyl level after the Fmoc group was removed.[6, 7] The DKPs thus formed remain attached covalently to the solid support, but can be released later by TFA. DKP formation as a side reaction to the BAL methodology can be circumvented (Scheme 4) by i) incorporation of the second residue as its N^{α}-Trt or Ddz derivative; ii) selective removal of Trt or Ddz with



Scheme 4. Use of acid-labile N^{α}-amino protection to avoid diketopiperazine formation. Taken from ref. [7].

dilute TFA solutions; and iii) incorporation of the third residue as its N^{α}-Fmoc derivative under *in situ* neutralization/coupling conditions mediated by PyAOP in DMF, in the presence of DIEA.[19]

The BAL strategy has also been applied for the SPS of "head-to-tail" cyclic peptides according to the following general features: i) reductive amination to anchor the

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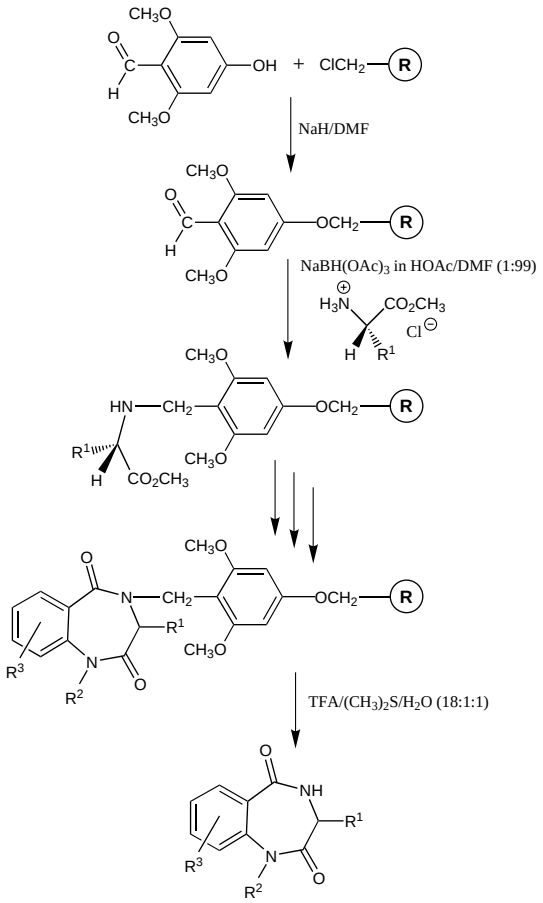
C-terminal amino acid residue as an allyl ester; ii) stepwise assembly of the linear sequence (including precautions to minimize DKP formation, as outlined in the above paragraph); iii) *orthogonal* removal of the allyl group to liberate selectively the free C^{α}-carboxyl group for the subsequent cyclization step; iv) efficient activation of the C^{α}-carboxyl group and its condensation with a free N^{α}-amino group to close the desired ring, taking advantage of the *pseudo-dilution* phenomenon which favors intramolecular resin-bound reactions; and v) final deprotection and cleavage to release the required free cyclic peptide into solution. A specific illustration is the sequence cyclo-(Arg-D-Phe-Pro-Glu-Asp-Asn-Tyr-Glu-Ala-Ala); cyclization was carried out by activation of a C-terminal Ala residue with PyAOP/HOAt in the presence of DIEA, with CH_2Cl_2 as solvent. In this procedure, C-terminally epimerized cyclized peptide was formed in only 12% of the amount of the desired species.

Our observations about DKP formation as a side reaction (see above) were adapted to devise an efficient method (Scheme 5) to intentionally prepare resin-bound DKPs, which can then be used in the construction of libraries.[20] For this purpose, commercially available amino acid methyl esters were used in the reductive amination step. Cyclization of the BAL-anchored Fmoc-dipeptidyl methyl ester occurred upon treatment with piperidine/DMF (1:4), to give a resin-bound DKP. Incorporation of amino acids, such as Lys or Orn, which each contain a second amino function in their side-chain, provided a DKP with two points for creating diversity; the most effective applications are when these two side-chain amino functions are blocked with orthogonally removable protecting groups. More diversity can be introduced by alkylation of the DKP amide bond (Scheme 5, last step).

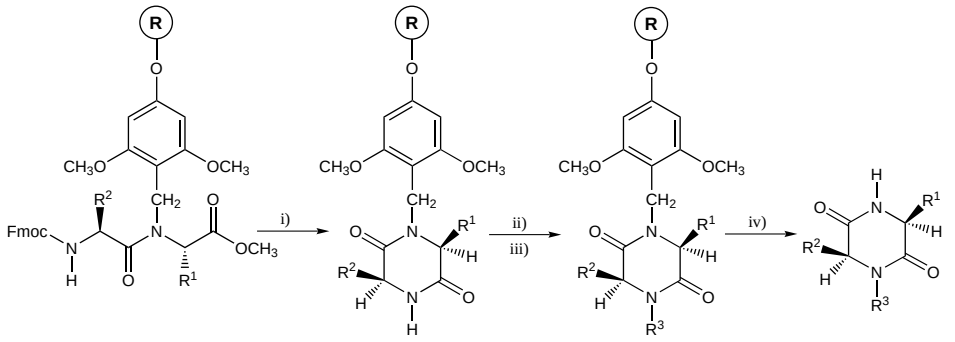
PALdehyde **1**, and its phenol precursor, 4-hydroxy-2,6-dimethoxybenzaldehyde, have also been used in the solid-phase synthesis of nonpeptidic small organic molecules and carbohydrate derivatives. Following coupling of the sodium salt of the phenol precursor to chloromethyl-PS resin, Ellman and co-workers subjected the resin to $NaBH(OAc)_3$-mediated reductive amination with amino acid methyl esters in HOAc/DMF (1:99), to achieve a linkage akin to BAL.[21] After acylation with anthranilic acid derivatives and base-promoted lactamization and *N*-alkylation, resin-bound 1,4-benzodiazepine-2,5-diones were obtained. Cleavage with TFA/$(CH_3)_2S$/H_2O (18:1:1) for 36 h gave the final benzodiazepine library (Scheme 6). Opposite to our findings, Ellman suggests the use of 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide · HCl in *N*-methylpyrrolidone for the difficult acylation of the resin-



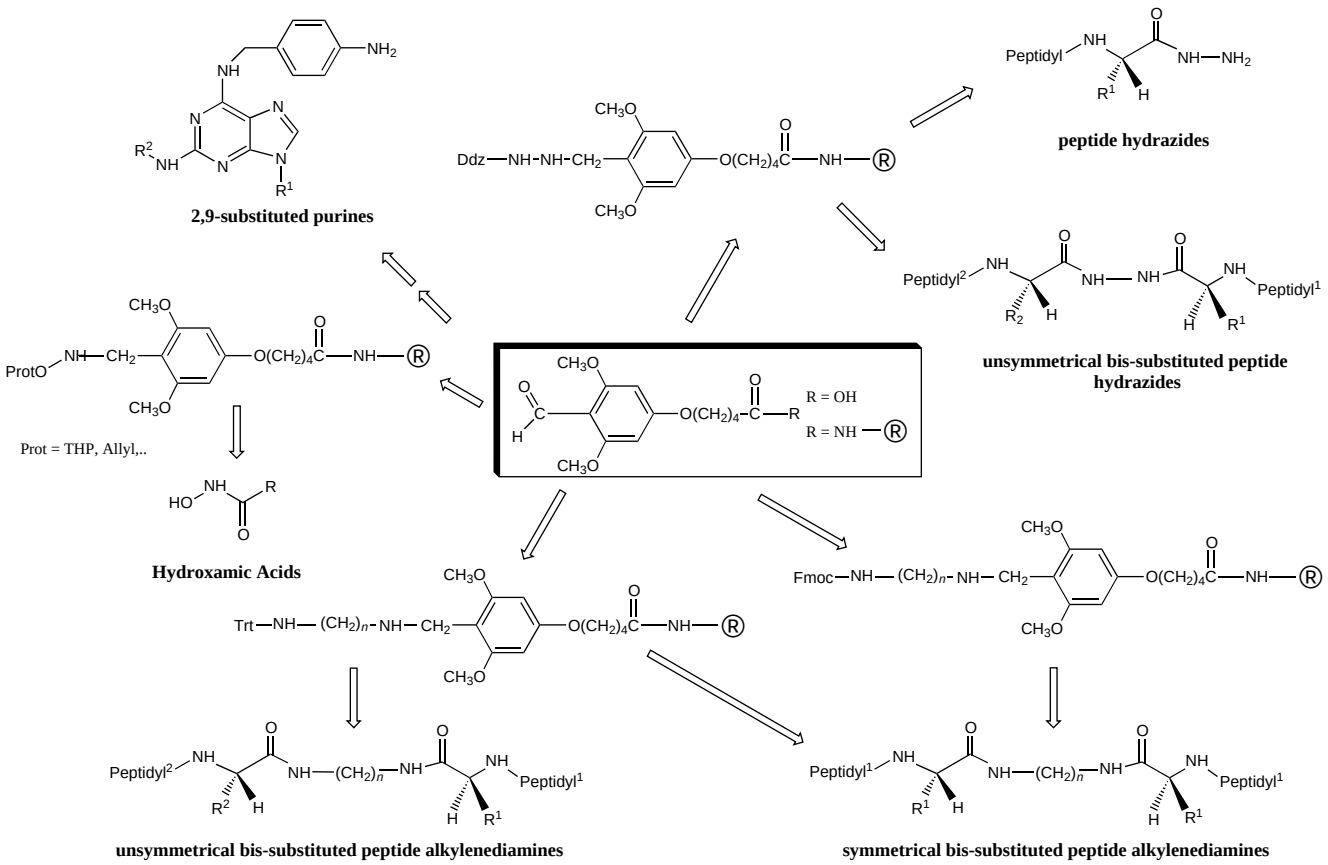
Scheme 6. Preparation of 1,4-benzodiazepine-2,5-diones. Modified from ref. [21].

bound secondary amine. In another example, Gray et al. coupled **1** to amine derivatized Synphase crowns (Chiron Mimeotopes), followed by $NaBH(OAc)_3$-mediated reductive amination with 2-fluoro-6-(4-aminobenzylamino)purine in HOAc/DMF (1:99) (Scheme 7).[22] After further manipulation of the purine core, final products were cleaved off the crowns with TFA/$(CH_3)_2S$/H_2O (19:1:1).

Ngu and Patel subjected commercial PALdehyde **1** to $NaBH_3CN$-mediated reductive amination with *O*-allyl- and *O*-tetrahydropyran-protected hydroxylamines in HOAc solution for 18 h to yield alkoxyamine acids.[23] These derivatives were *N*-protected by Fmoc, and then coupled to amino-functionalized TentaGel to yield *O*-protected alkoxyamine BAL-resins, which were used as starting materials for SPS of various hydroxamic acids (Scheme 7).



Scheme 5. Intentional diketopiperazine formation with three diversity elements; i) piperidine/DMF (1:4), 25 °C, 3 × 1 min, 3 × 5 min; ii) Evans oxazolidinone auxilliary, THF, −70 °C, 90 min; iii) R_3X, THF/DMF (7:3), 25 °C, 5 h; iv) TFA/H_2O (9:1), 25 °C, 2 h. Taken from ref. [20a].



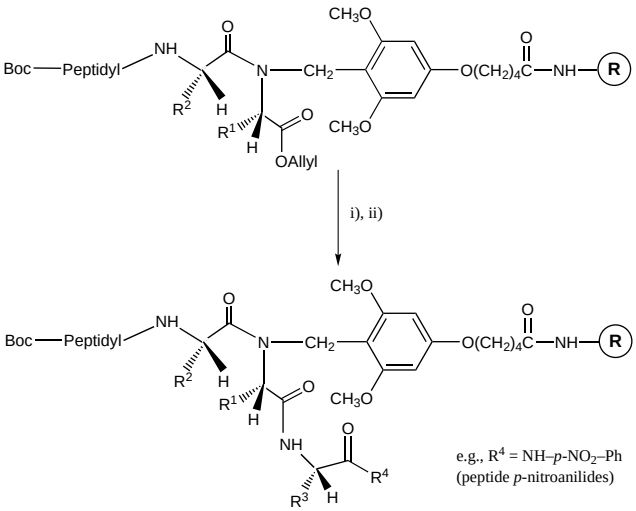
Scheme 7. Recent examples of applications of tris(alkoxy)benzyl (BAL) system to provide access to a diverse array of nitrogen-containing products. Modified from refs. [20d], [22], and [23].

N-acylation was followed by removal of the *O*-protecting group and cleavage with TFA/CH_2Cl_2/H_2O (50:49:1).

Bui et al. compared resin-bound **1** with 4-hydroxy-2,6-dimethoxybenzaldehyde attached directly to chloromethyl resin through an ether bond.[24] These workers found that reductive amination with benzylamine proceeded faster on **1** resins and crowns than on resins lacking a spacer. After acylation with N^{β}-Fmoc-βAla-OH and cleavage with TFA/CH_2Cl_2 (1:1) for 2 h at 25 °C, the handle incorporating a spacer gave higher yields. Furthermore, BAL and its dialkoxy analogue gave comparable yields.

The BAL handle has also been used for preparation of peptide hydrazides.[20d] To achieve this, Ddz mono-protected hydrazine was anchored through the unprotected nitrogen, and chain elongation continued from this position (Scheme 7). Cleavage with TFA/H_2O (19:1) provided the expected peptide hydrazide. Similarly, reductive amination with Trt- or Fmoc-monoprotected alkylenediamines provided resins for the preparation of compounds with two points of diversity, separated by a methylene chain (Scheme 7).[20d]

In recent work, we have extended the BAL methodology to accommodate C-terminal modifications that are labile to bases, for example, piperidine (as used in Fmoc chemistry), or to circumvent other synthetic challenges.[25] To do this, BAL anchoring was applied to the *penultimate* residue of the target peptide. An orthogonally removable allyl ester made it possible to selectively liberate, and then activate, a carboxyl group for coupling with the C-terminal species (Scheme 8). During the latter step, a readily epimerizable oxazolonium ion may form by attack of the oxygen from the BAL-amide function onto the activated carboxyl; despite this risk, we



Scheme 8. BAL methodology to accomodate C-terminal modifications that are labile to bases, for example, piperidine, or to circumvent other synthetic challenges; i) $Pd(PPh_3)_4$ (5 equiv) in $CHCl_3$/HOAc/NMM (37:2:1) under Ar, 25 °C, 3 h; ii) HCl·H-AA-R^4 (10 equiv)/HATU (10 equiv)/DIEA (20 equiv) in CH_2Cl_2 (30 min). Modified from ref. [25].

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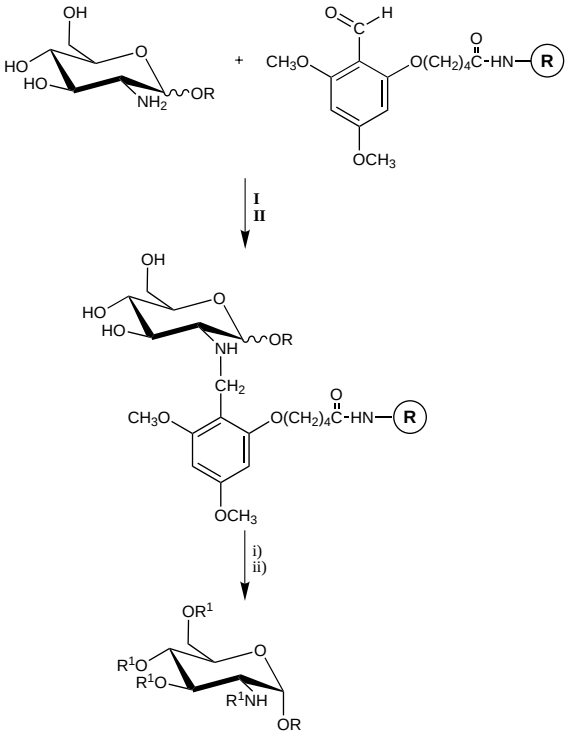
were able to develop effective protocols, which are empirically found to proceed with minimal racemization. In more detail, the modified BAL strategy comprises: i) initial anchoring of the penultimate residue, with its carboxyl group orthogonally protected, through the backbone nitrogen; ii) continuation with standard protocols for peptide chain elongation in the C→N direction; iii) selective orthogonal removal of the C^{α}-carboxyl protecting group; iv) solid-phase coupling to introduce the C-terminal residue; and v) final cleavage/deprotection to release the free peptide product into solution. To illustrate the approach, several peptide *p*-nitroanilides were prepared in excellent yields and purities, with minimal racemization.

As part of a strategy for solid-phase oligosaccharide synthesis, one of the authors has recently applied the BAL handle approach to anchoring D-glucosamine and corresponding glycosides to solid supports.[26] Methyl 2-amino-2-deoxy-α-D-glucopyranoside was coupled directly to *o*-PALdehyde-polystyrene (PS) resin by $NaBH_3CN$-mediated reductive amination through the 2-amino group, carried out in HOAc/DMF (1:49) (Scheme 9). Gravimetric assessment indicated 75% incorporation after 16 h of reaction. Following treatment



Ia. R, R^1: Ac; **Ib**. R, R^1: Bz
IIa. R: CH_3 (α), R^1: Bz

Scheme 9. Solid-phase carbohydrate chemistry using BAL handles; **I**: R = H (hydrochloride), $NaBH_3CN$ in DMF; **II**: R = CH_3 (α), $NaBH_3CN$ in DMF/HOAc (49:1); i) **I** a) acetylation; b) benzoylation, **II** benzoylation; ii) TFA/H_2O (19:1). Taken from ref. [26].

with benzoyl chloride in pyridine, the carbohydrate resin was cleaved with TFA/H_2O (19:1) to release the desired perbenzoylated derivative. Similarly, the peracetylated methyl 2-amino-2-deoxy-α-D-glucopyranoside was obtained. Importantly, the presence of three *O*- and one *N*-acyl groups in the final products indicate that anchoring occurred through reductive amination, as expected, and not by formation of a putative *N*,*O*-acetal. To minimize the number of chemical steps prior to anchoring, *underivatized* D-glucosamine was coupled *directly* to *o*-PALdehyde-PS resin by chemoselective reductive amination through the 2-amino group, in DMF with $NaBH_3CN$ (Scheme 9). Treatment with benzoyl chloride in pyridine, followed by release from the solid support with TFA/H_2O (19:1), gave the α-benzoate.

Practical Guidelines for Tris(alkoxy)benzyl BAL Chemistry

The aforementioned applications of BAL handles, as implemented in the tris(alkoxy)benzyl system to peptides, aminosugars, and small nitrogen-containing organic molecules, permit formulation of a few general guidelines:

1) All other things being equal, PALdehyde **1** should be attached to the resin by a spacer rather than by direct alkylation of 4-hydroxy-2,6-dimethoxybenzaldehyde with chloromethyl resin.
2) If economically possible, use ten equivalents each of amine and $NaBH_3CN$. In some cases, as little as 1–2 equivalents of amine will give efficient incorporation. $NaBH(OAc)_3$ can be used instead of $NaBH_3CN$. As a rule, reactions should be performed at 25 °C.
3) Methanol is well suited as solvent for reductive aminations carried out either in solution or on-resin, so long as hydrophilic PEG-PS and related (e.g., TentaGel, ArgoGel) supports are used. DMF is also an excellent solvent for on-resin reductive aminations and must be used when the support is PS. However, DMF is incompatible with corresponding reactions in solution.
4) When starting with an amine salt, no additional acid is required for successful reductive amination. When starting with a free amine, 1% HOAc is required for efficient incorporation.
5) When incorporating a chiral amino acid derivative, a separate imine-forming step should be avoided.
6) For acylation of a resin-bound secondary amine, the preferred solvent is CH_2Cl_2 or CH_2Cl_2/DMF (9:1).
7) Cleavage of final products can be accomplished with TFA/H_2O (19:1), TFA/$(CH_3)_2S$/H_2O (18:1:1), or TFA/H_2O/CH_2Cl_2 (10:5:85) for 2 h.

Conclusions and Future Perspectives

The novel and general backbone amide linker (BAL) strategy has allowed the particularly straightforward synthesis of linear C-terminal-modified and cyclic peptides. BAL is compatible with a range of functionalized polymeric supports, for example PS, PEG-PS, and Synphase crowns. The BAL handle concept has been extended to the solid-phase synthesis of nitrogen-containing small organic molecules and of monosaccharide derivatives. Outstanding features of the BAL handle strategy include easy attachment of amines by reductive amination, stability of the BAL anchor to numerous

chemical manipulations, and mild final cleavage conditions. The focus of work done to date in the authors' laboratories has been on the acidolyzable tris(alkoxy)benzylamide system. Extensions to other handles, both acidolyzable (fine-tuning with electron-donating or -withdrawing groups)[27, 28, 29] as well as exploiting other cleavage principles (e.g., photolysis and fluoridolysis), are readily envisaged and are under active current investigation.

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